Intestinal scavenger receptor class B type I as a novel regulator of chylomicron production in healthy and diet-induced obese states

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Lino M, Farr S, Baker C, Fuller M, Trigatti B, Adeli K. Intestinal scavenger receptor class B type I (SR-BI) has been found to be upregulated in animal models of insulin resistance. Here we characterized the potential importance of SR-BI in contributing to chylomicron production and postprandial hypertriglyceridemia in vivo. Postprandial triglyceride (TG)-rich lipoprotein (TRL) production was characterized in hamsters treated with the SR-BI inhibitor to block lipid transport-1 (BLT-1) under healthy conditions or conditions of diet-induced obesity and dyslipidemia. BLT-1 (1 mg/kg) or vehicle was administered acutely in chow-fed hamsters or gavaged twice daily over 10 days during high-fructose, high-fat, high-cholesterol (FFC) feeding. Effects of acute SR-BI inhibition by BLT-1 were confirmed in healthy fat-loaded rats. Finally, plasma lipid levels were compared between SR-BI−/− mice and their wild-type counterparts fed either chow or a 12-wk high-fat diet. Acute BLT-1 treatment reduced postprandial plasma and TRL TG levels in healthy hamsters and rats. Chronic BLT-1 treatment of FFC-fed hamsters blunted diet-induced weight gain and fasting hypertriglyceridemia, and lowered postprandial TRL-TG, -cholesterol, and -apoB48 levels. Finally, SR-BI−/− mice displayed lower plasma and TRL TG levels relative to wild type, and diet-induced weight gain and postprandial hypertriglyceridemia were hindered in SR-BI−/− mice. We conclude that intestinal SR-BI is a critical regulator of postprandial lipoprotein production, emphasizing its potential as a target for preventing diabetic dyslipidemia.

apoB48; insulin resistance

Lipoprotein metabolism is significantly altered in states of insulin resistance and type 2 diabetes, resulting in postprandial dyslipidemia where levels of circulating lipids are elevated after a meal (8). The small intestine is the primary contributor to postprandial diabetic dyslipidemia through the overproduction of apolipoprotein B48 (apoB48)-containing chylomicron particles following the absorption of dietary lipids (10). The process of intestinal lipid uptake involves various transporters such as fatty acid translocase/CD36 or fatty acid transport protein 4 for fatty acid handling, and Niemann-Pick C1 Like 1 for cholesterol uptake (19, 26, 9). More recently, the apically expressed protein scavenger receptor class B type I (SR-BI) has also been implicated in intestinal lipid handling, since its subcellular localization in enterocytes and Caco-2 cells changes in response to the presence of lipid micelles (12, 14). Interestingly, intestinal SR-BI expression is enhanced in an animal model of insulin resistance where chylomicrons are produced in excess (14).

SR-BI is a cell-surface glycoprotein expressed in various tissues (25). As reviewed by Saddar and colleagues, it binds to a range of ligands but has been shown to play a particularly important role in selective cholesterol uptake (25). In reverse cholesterol transport, cholesterol is carried by high-density lipoprotein (HDL) particles from peripheral tissues to the liver for incorporation into bile, entry into the small intestine, and eventual excretion from the body (25). Hepatic SR-BI has been referred to as the HDL receptor, important in mediating this transfer of cholesterol and cholesteryl esters from HDL to the liver (25). In this way, hepatic SR-BI facilitates the removal of cholesterol from the body, and is considered to be atheroprotective (25). Indeed, SR-BI−/− mice display hypercholesterolemia and reduced biliary cholesterol (18), whereas liver-specific SR-BI overexpression reduces circulating cholesterol levels and enhances selective uptake of cholesteryl esters from HDL into the liver (27).

In contrast to our understanding of hepatic SR-BI, there is a paucity of information on the role of SR-BI in the intestine. In vitro evidence suggests its involvement in the apical absorption of dietary cholesterol and cholesteryl esters from bile salt-containing micelles (13), and intestinal SR-BI overexpression has been shown to enhance dietary cholesterol absorption in mice (7). Yet SR-BI−/− mice display normal cholesterol absorption (3). Recently, our laboratory demonstrated that small-interfering RNA (siRNA)-induced silencing of SR-BI expression decreased apoB48/100 secretion while SR-BI overexpression increased apoB48/100 secretion from Caco-2 cells (14). However, the ability for intestinal SR-BI to regulate chylomicron production in an in vivo setting and modulate not only circulating cholesterol levels but also the postprandial hypertriglyceridemia associated with insulin resistance has never before been assessed.

In the present study, we aimed to elucidate the role of intestinal SR-BI in postprandial lipemia in hamsters, rats, and mice. Acute in vivo inhibition of SR-BI was achieved by the chemical inhibitor block lipid transport-1 (BLR-1) (20, 28), and postprandial plasma and triglyceride (TG)-rich lipoprotein (TRL) TG levels were subsequently measured. We also sought to determine the effects of prolonged SR-BI
inhibition in our established hamster model of diet-induced obesity and dyslipidemia (5). Finally, postprandial lipid handling was examined in SR-BI−/− mice that were either chow fed or placed on a high-fat diet (HFD). We demonstrate that intestinal SR-BI is a critical player in intestinal lipoprotein production and has an important role in modulating postprandial triglyceride levels in healthy states and under conditions promoting the development of obesity, dyslipidemia, and insulin resistance.

MATERIALS AND METHODS

Animals. Male Syrian golden hamsters (100–120 g) and Sprague Dawley rats (180–200 g) were purchased from Charles River (Montreal, PQ) and housed individually on a 12:12-h light-dark cycle with a 1-wk acclimatization period. Animals were fed a regular chow diet ad libitum or were given a high-fructose, high-fat, high-cholesterol (FFC) diet (40, 30, and 0.25%, respectively) (catalog no. 101711; Dyets, Bethlehem, PA) for 10 days to induce insulin resistance (5). Before the FFC diet commenced, hamsters were fasted for 5 h and anesthetized under isoflurane-induced general anesthesia (3% mixed with oxygen) (AErrane, catalog no. CA2L9108; Baxter, Mississauga, ON) for collection of baseline intraorbital bleeds. Hamsters were then randomized by weight and fasting plasma TG and cholesterol levels to receive either vehicle (PBS, catalog no. CA12001-676; VWR, Mississauga, ON) or 1 mg/kg BLT-1 (catalog no. 5234221; Chembridge) (selected based on a dose-response performed) by oral gavage twice daily for 10 days while on the FFC diet. BLT-1 and vehicle were given under isoflurane anesthetic to minimize stress or injury to the animals. Body weight was recorded daily, and food was weighed every morning to measure food consumption. At the end of the 10-day feeding and dosing period, hamsters were subject to assessment of fasting plasma lipid parameters and postprandial TRL production following a final BLT-1 gavage. Tissues were collected under isoflurane anesthesia and flash-frozen in liquid nitrogen, and fecal samples were also collected from the cages. The key advantage of the hamster model is that, similar to humans, apoB48-containing chylomicron particles are exclusively produced by the hamster while apoB100-containing very low density lipoprotein (VLDL) particles are produced by the liver.

C57B6/J WT and SR-BI−/− mice were maintained in Dr. Bernardo Trigatti’s laboratory at McMaster University. C57B6/J WT mice were originally ordered from Jackson Laboratories (Bar Harbor, ME) and bred in-house, and SR-BI−/− mice were backcrossed 10 generations on a C57B6/J background. The mice were housed in pairs on a 12:12-h light-dark cycle and were fed a regular chow diet ad libitum or a high-fat diet (11.5% coconut oil, 11.5% corn oil, 5% fructose, and 0.5% cholesterol) (Purina Rodent Chow no. 5001, catalog no. C11953; Research Diets, New Brunswick, NJ) for 12 wk to induce dyslipidemia (24). All procedures were carried out in compliance with the guidelines of the Hospital for Sick Children Animal Ethics Committee, the Canadian Council on Animal Care, and the Institutional Ethics Committee at McMaster University.

Assessment of postprandial TRL production in vivo in hamsters and rats. TRL production was determined as previously described (15). Briefly, animals were placed under isoflurane anesthetic for cannulation of the right jugular vein with silicone tubing (catalog no. 60985-708; VWR). The cannula contained heparinized saline (40 IU/ml) (heparin sodium injection, catalog no. C504701; PPC, Richmond Hill, ON) and was exteriorized through the back of the neck. After half a day of recovery and an overnight fast, conscious animals underwent baseline bleeds through the cannula, followed by an oral gavage of 1 mg/kg BLT-1 or PBS vehicle. A single dose of 1 mg/kg BLT-1 was administered by oral gavage 30 min before an oral fat load. Pluronic F-127 (Poloxamer 407, catalog no. P2443; Sigma-Aldrich) was injected intraperitoneally at a dose of 500 mg/kg to inhibit the catabolism and

![Graph A](image1.png)  
**Fig. 1.** Acute treatment of chow-fed hamsters with the scavenger receptor class B type I (SR-BI) inhibitor block lipid transport-1 (BLT-1) lowers postprandial plasma and triglyceride-rich lipoprotein (TRL) triglyceride (TG) levels. A single dose of 1 mg/kg BLT-1 was given IP to block lipolysis. Plasma was collected every 30 min for 2 h and analyzed for total plasma TG (**A**) and cholesterol (CHOL) (**B**). TRL fractions were isolated from the plasma and analyzed for TRL TG (**C**) and TRL CHOL (**D**). Results were analyzed by 2-way repeated-measures ANOVA with the Bonferroni posttest (*P* < 0.05 and **P** < 0.01); *n* = 4 experiments.
clearance of TRL particles, including chylomicrons and VLDL. Blood samples (400 μl) were collected at 30, 60, 90, and 120 min through the cannula after the fat load for TG and cholesterol measurements and TRL isolation. The jejunum was isolated at 120 min, flash-frozen in liquid nitrogen, and stored at −80°C. The jejunum was separated from the duodenum and the ileum by cuts at the ligament of Treitz and at 5 cm from the distal end of the small intestine. Jejunum, liver, and kidney segments were also cryopreserved in optimum cutting temperature (OCT) buffer (catalog no. 4583-S; Somagen, Edmonton, AB) or fixed in formalin.

Assessment of postprandial TRL production in vivo in mice. Following an overnight fast, baseline blood samples were collected by tail-vein bleeds. This was followed by a 200-μl oral gavage of olive oil, and, at 20 min postfat load, 20% Pluronic F-127 was injected intraperitoneally at a dose of 500 mg/kg. At 30, 60, and 90 min, 50 μl of blood were collected by tail bleeds in EDTA-coated blood collection tubes (catalog no. 16.444.100; Sarsedt, Montreal, PQ), and, at 120 min, a final blood sample (1 ml) was collected under isoflurane anesthesia by cardiac puncture. Excised jejunum was flash-frozen in liquid nitrogen and stored at −80°C.

TRL isolation. Blood was centrifuged for 10 min at 4°C and 2,300 g to isolate plasma. To obtain the TRL fraction of the plasma, 150 μl of plasma were laid under 4 ml potassium bromide solution (density 1.006 g/ml) and spun for 70 min at 15°C and 116,140 g using an ultracentrifuge (SW55Ti; Beckman Coulter, Mississauga, ON, Canada). The TRL fraction (top 300 μl) was then collected (Svedberg flotation rate >100).

Plasma measurements. Total plasma and TRL fractions were analyzed for TG and cholesterol content using colorimetric assay kits (catalog no. TR210 and CH200; Randox, Crumlin, UK). TRL apoB48 levels were determined by immunoblotting as previously described (26a) using a goat polyclonal anti-human antibody (anti-human apolipoprotein B serum, catalog no. 71301; Midland Bioproducts, Boone, IA). Plasma creatinine, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels were determined on the Ortho Vitros Fusion 5.1 automated analyzer.

Assessment of tissue lipid content. Flash-frozen tissues were homogenized in liquid nitrogen with a mortar and pestle on dry ice. Approximately 300 mg tissue homogenate were placed in 20 vol of 2:1 chloroform-methanol mixture for a 48-h lipid extraction. Samples were washed with 0.2 vol of 0.9% NaCl, the upper phase was suctioned off, and the chloroform and methanol were left to evaporate over several days. Remaining lipid was dissolved in 100% ethanol for measurements of TG and cholesterol using colorimetric assay kits.

Histological analysis. Tissues cryopreserved in OCT buffer were sectioned and mounted on microscope slides for Oil-Red O and hematoxylin and eosin (H&E) staining. Oil-Red O staining was performed as previously described (17) and visualized and quantified with Volocity software. Red pixels were expressed as a percentage of the total image pixels. H&E staining was visualized using Mirax Viewer software (Informer Technologies).

Statistical analysis. Results are presented as means ± SE. Student’s unpaired t-tests and two-way repeated-measures ANOVA with the Bonferroni posttest were performed using GraphPad Prism software (GraphPad Software, San Diego, CA).

RESULTS

Acute SR-BI inhibition reduces postprandial plasma and TRL TG accumulation in hamsters and rats. We first examined whether acute inhibition of SR-BI by BLT-1 administration had any effect on postprandial TRL production in chow-fed hamsters. Hamsters were orally administered a single dose of vehicle or 1 mg/kg BLT-1 before an oral fat load, and postprandial plasma lipids were examined over 120 min. Both plasma and TRL TG accumulation were lower in hamsters treated with BLT-1 (difference of 0.21 mM in TRL-TG at 120

Fig. 2. Acute treatment of chow-fed rats with the SR-BI inhibitor BLT-1 lowers postprandial plasma and TRL TG levels. A single dose of 1 mg/kg BLT-1 was administered by oral gavage 30 min before an oral fat load. Pluronic F-127 was given ip to block lipolysis. Plasma was collected every 30 min for 2 h and analyzed for total plasma TG (A) and CHOL (C). TRL fractions were isolated from the plasma and analyzed for TRL TG (B) and TRL CHOL (D). Results were analyzed by 2-way repeated-measures ANOVA with the Bonferroni posttest (*P < 0.05 and ***P < 0.001); n = 3–4.
min, \( P < 0.05 \) (Fig. 1, A and B). However, no changes were observed in total plasma or TRL cholesterol levels (Fig. 1, C and D). To confirm that these findings were not specific to the hamster model, Sprague Dawley rats were also gavaged with vehicle or 1 mg/kg BLT-1 and challenged with an oral fat load. As observed in hamsters, total plasma and TRL TG accumulation was significantly reduced in BLT-1-treated rats (difference of 0.49 mM in TRL TG at 120 min, \( P < 0.05 \)) (Fig. 2, A and B). No significant reductions in plasma or TRL cholesterol levels were detected (Fig. 2, C and D).

Prolonged SR-BI inhibition prevents weight gain and hypertriglyceridemia, and lowers chylomicron production in a hamster model of diet-induced obesity and dyslipidemia. Given that acute SR-BI inhibition prevented the postprandial rise in plasma TG levels, we questioned whether chronic SR-BI inhibition would protect against the dyslipidemia and chylomicron overproduction that is often observed under conditions of diet-induced obesity (8). Hamsters were used to study chylomicron production, since apoB48 is not incorporated into VLDL particles and is secreted solely from the intestine in these animals, allowing for chylomicron particle number to be directly assessed via apoB48 measurements. Hamsters were placed on an FFC diet for 10 days (5) during which time 1 mg/kg BLT-1 was orally administered two times daily. Over the 10-day period, there was a trend toward reduced weight gain in BLT-1-treated hamsters (Fig. 3A) that was significant at day 10 (\( P = 0.017 \) by Student’s unpaired t-test), despite no change in food consumption (Fig. 3B). The FFC diet significantly increased fasting plasma TG and cholesterol levels by day 10 of feeding (Fig. 3, C and D), and BLT-1 treatment prevented this fasting hypertriglyceridemia (difference of 4.0 mM compared with vehicle on day 10, \( P < 0.05 \)) (Fig. 3C), although fasting plasma cholesterol levels remained elevated (Fig. 3D). Hamsters were challenged with an oral fat load following a final gavage of BLT-1 or vehicle. While no change in plasma cholesterol levels was detected (Fig. 4C), BLT-1-treated hamsters displayed significantly reduced postprandial plasma TG and TRL-TG, -cholesterol, and -apoB48 excursions (difference of 0.36 mM in TRL-TG at 120 min, \( P < 0.05 \); difference of 0.16 mM in TRL-cholesterol at 120 min, \( P < 0.01 \); difference in TRL-apoB48 at 120 min, \( P < 0.05 \)) (Fig. 4, A–B and D–F). The reduction in apoB48 can be seen even at baseline on day 10, before the final BLT-1 treatment and oral fat load, demonstrating the effectiveness of chronic BLT-1 in a model where chylomicrons are overproduced (Fig. 4F). These important effects on apoB48 levels demonstrate that a lower number of chylomicron particles are secreted during intestinal SR-BI inhibition. To examine whether a reduction in plasma lipids could be accounted for by lipid accumulation in the intestinal mucosa or by malabsorption from the gut lumen, lipid was extracted from the jejunum and feces at 120 min postfat load. No significant differences were observed between BLT-1-treated and vehicle-treated hamsters in fecal TG or cholesterol content, and only a trend toward reduced jejunal TG and cholesterol content was observed with BLT-1 (Fig. 5, A and B). These observations were confirmed with jejunal Oil Red O staining, revealing no significant difference in neural lipid staining with BLT-1 treatment (Fig. 5, C and D). Finally, to
confirm that toxicity could not account for the observed effects of BLT-1, plasma creatinine, ALT, and AST levels were measured and found to be unchanged relative to vehicle-treated controls (Fig. 6, A–C), and tissue morphology of the jejunum, liver, and kidney was unaffected by chronic BLT-1 treatment (Fig. 6, D–I).

**SR-BI**−/− mice display reduced postprandial plasma and TRL TG levels. To validate our findings from the hamster model without the use of a chemical inhibitor, fat tolerance tests were performed in C57BL/6 WT and SR-BI−/− mice. In accordance with our observations in BLT-1-treated hamsters and rats, a significant reduction in plasma TG accumulation was observed at 90 and 120 min in SR-BI−/− mice compared with WT mice (Fig. 7A). SR-BI−/− mice also exhibited significantly elevated plasma cholesterol levels, which is not surprising given the involvement of hepatic SR-BI in reverse cholesterol transport (18) (Fig. 7C). Interestingly, despite elevated plasma cholesterol levels, significantly lower TRL TG levels and a trend toward lower TRL cholesterol levels were observed in SR-BI−/− mice (Fig. 7, B and D), suggesting a likely reduction in postprandial lipoprotein production.

**SR-BI**−/− mice display lower postprandial TG levels, even on a HFD. Finally, we examined whether SR-BI−/− mice would still exhibit reduced plasma TG levels even on a HFD, which promotes postprandial dyslipidemia (24). Following a 12-wk HFD feeding period, SR-BI−/− mice displayed a significantly lower body weight than their WT counterparts (difference of 3.525 g at week 12, P < 0.01) (Fig. 8A). SR-BI−/− mice had no significant difference in fasting plasma TG levels before or after the diet relative to WT mice (Fig. 8B); however, they did have significantly elevated plasma cholesterol levels at baseline and week 12 of feeding as expected (difference of 8.0 mM at week 12, P < 0.001) (Fig. 8C). Whereas fasting TG levels were unchanged, postprandial TG levels were significantly lower in SR-BI−/− mice (difference of 11.6 mM at 120 min, P < 0.001) (Fig. 8D), and postprandial cholesterol levels were heightened (difference of 5.0 mM at 120 min, P < 0.01) (Fig. 8E).

**DISCUSSION**

The function of intestinal SR-BI is a topic of uncertainty. Originally identified as the HDL receptor in the liver, SR-BI has a recognized role in cholesterol transport (1). However, despite the ability for SR-BI overexpression to increase cholesterol uptake in vitro (13) and in vivo (7), absorption studies performed in SR-BI−/− mice suggest that this scavenger receptor is not essential for intestinal cholesterol uptake (3). To gain insight into the role of intestinal SR-BI in gut lipid and lipoprotein metabolism, our laboratory previously examined the correlation between SR-BI expression and apoB secretion from Caco-2 cells. Overexpression of SR-BI led to an increase in apoB48/100 secretion, whereas inhibition of SR-BI by siRNA reduced apoB48/100 secretion (14). Furthermore, protein and mRNA expression of intestinal SR-BI was found to be upregulated during insulin resistance (14). The present study

Fig. 4. Chronic BLT-1 treatment of FFC-fed hamsters reduces postprandial plasma TG levels, and TRL-TG, -CHOL, -apolipoprotein B48 (apoB48) levels. After FFC feeding and chronic BLT-1 administration for 10 days, fat tolerance tests were performed. A single dose of 1 mg/kg BLT-1 was given orally 30 min before an oral fat load, Pluronic F-127 was given ip to block lipolysis, and plasma was collected every 30 min for 2 h. Plasma was measured for TG (A) and CHOL (C) levels. TRL fractions were isolated from the plasma and analyzed for TG (B), apoB48 (E) levels. Representative immunoblots demonstrating TRL-apoB48 accumulation are shown (F). Results were analyzed by 2-way repeated-measures ANOVA with the Bonferroni posttest (*P < 0.05 and **P < 0.01); n = 5.
sought to examine whether modulating SR-BI function in an in vivo setting could in fact modulate postprandial chylomicron secretion, and whether intestinal SR-BI plays a regulatory role in not only cholesterol but also triglyceride metabolism. Findings are summarized in Fig. 9.

First we examined the effect of acute intestinal SR-BI inhibition on postprandial plasma lipids. Acute treatment with the SR-BI inhibitor BLT-1 in hamsters and rats significantly reduced postprandial plasma and TRL TG levels but not cholesterol accumulation. This suggests that intestinal SR-BI may be more involved in postprandial TG handling than in cholesterol uptake, which was once considered its primary role. Consistent with our findings, another group observed that transgenic mice overexpressing SR-BI under an intestine-specific promoter had increased plasma TG and cholesterol levels (7). The increase in TG secretion was greater than that of cholesterol (7), suggesting increased chylomicron production since chylomicrons contain primarily TG. However, chylomicron production could not be directly assessed in this previous study, since the mouse liver produces apoB48 and apoB100, making it difficult to differentiate between VLDL and chylomicrons.

To directly assess the effects of intestinal SR-BI inhibition on chylomicron production in vivo, we examined apoB48 accumulation in our hamster model of diet-induced obesity and dyslipidemia (5). Unlike mice and rats, hamsters produce apoB48 solely from the intestine with one apoB48 secreted per chylomicron particle, making apoB48 a measure of chylomicron number in this model. The fasting hypertriglyceridemia and hypercholesterolemia observed following FFC feeding in the vehicle-treated hamsters was expected given similar findings from previous studies that employed this diet (5). Conversely, fasting plasma TG and apoB48 levels were significantly lower in hamsters that received prolonged BLT-1 treatment, suggesting that intestinal SR-BI may play a role in overall lipid metabolism and energy balance. Postprandially, BLT-1 treatment not only reduced plasma and TRL TG accumulation but also TRL apoB48 levels, demonstrating for the first time that intestinal SR-BI regulates chylomicron particle number in vivo. Total plasma cholesterol was not affected by BLT-1 treatment, consistent with a more prominent role of intestinal SR-BI in regulating enterocyte TG handling, as opposed to cholesterol absorption. Furthermore, the absence of a BLT-1 effect on cholesterol via hepatic SR-BI inhibition may have resulted from low oral bioavailability of BLT-1, preventing it from accessing the liver. Alternatively, BLT-1 may not have been sufficiently absorbed by 2 h to impair hepatic clearance of HDL-cholesterol, and the fat load did not contain dietary cholesterol to drive reverse cholesterol transport. However, TRL cholesterol was significantly reduced. This may be attributed to the significant reduction in chylomicron secretion, since chylomicrons contain some cholesterol. These findings of reduced TG and chylomicron accumulation are consistent with our acute BLT-1 studies and could offer an explanation for the reduced weight gain observed with BLT-1 treatment. It should be noted that this reduction in weight gain occurred despite no changes in food consumption, or in jejunal or fecal lipid content (steatorrhea), suggesting that intestinal lipid absorption was unimpaired by BLT-1. Interestingly, CD36, another member of the class B scavenger receptor family, has been implicated in promoting skeletal muscle fatty acid oxidation (16) and brown adipose tissue function (4). Whether SR-BI similarly modulates energy expenditure to lower circulating lipids, fat deposition, and body weight is an area for further study. Finally, our observations complement human studies showing a link between certain SR-BI polymorphisms and

![Fig. 5. Chronic BLT-1 treatment of FFC-fed hamsters has no effect on jejunal or fecal lipid content. Lipid was extracted from jejunal tissue and feces collected from FFC-fed hamsters treated for 10 days with 1 mg/kg BLT-1. Lipid extracts were analyzed by colorimetric assays for TG (A) and CHOL (B) content. Oil Red O staining was performed on the jejunal mucosa and quantified (C), with representative images shown at ×20 magnification (D). Results were analyzed by Student’s t-test; n = 3–6.](http://ajpgi.physiology.org/doi/10.220.33.6)
changes in insulin sensitivity, body mass index, and TG metabolism (23, 2).

A likely explanation for our observations of reduced chylomicron production with SR-BI inhibition is defective intracellular apoB48 and lipid trafficking. There are a number of studies that have implicated SR-BI as a “lipid sensor” to direct the intracellular movement of apoB and lipid. Culturing Caco-2/TC7 cells with lipid micelles has been shown to cause a subapical pool of apoB48 to relocalize to secretory domains like the ER in a MAP kinase-dependent manner. This processes of MAP kinase activation and apoB48 movement was found to be SR-BI dependent, since it could be prevented by SR-BI knockdown or BLT-1 treatment. Micelles were further shown to directly interact with SR-BI and induce SR-BI clustering at the apical brush-border membrane, specifically within lipid rafts where MAP kinases are located (6). As such, upon lipid sensing, SR-BI may relocalize to direct intracellular signaling pathways that will direct apoB48 trafficking toward the ER for chylomicron secretion. This indirect manner of modulating intracellular apoB48 movement is supported by findings from pig enterocytes, where apical SR-BI was found to relocalize to apoAI-negative cytosolic lipid droplets upon fat ingestion (12). The absence of an interaction with apoAI-containing lipid droplets suggests that SR-BI does not associate with nascent chylomicrons, and may participate more in directing the trafficking of stored lipids as opposed to directly interacting with chylomicron particles. Further supporting the link between SR-BI, MAP kinases, and chylomicron output, both intestinal SR-BI levels and basal ERK activation (downstream of MAP kinase) have been shown to be elevated in the fructose-fed hamster model of insulin resistance and chylomicron overproduction, and ERK inhibition can prevent apoB48 secretion from enterocytes (11). Finally, our laboratory has also shown that stimulation of Caco-2 cells with lipid micelles led to SR-BI relocalization to the plasma membrane and perinuclear region. The subcellular localization of SR-BI was insulin sensitive (14), suggesting that SR-BI trafficking may change during insulin resistance, potentially contributing to dyslipidemia. Therefore, it is possible that intestinal SR-BI senses dietary lipids at the apical membrane of enterocytes, activates intracellular signaling pathways, and moves to cytosolic lipid droplets to induce the movement of apoB48 and

Fig. 6. Chronic BLT-1 treatment of FFC-fed hamsters had no effect on plasma markers of drug toxicity [creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT)], or on jejunal, liver, or kidney tissue morphology. Fasting plasma was collected before and after the 10-day FFC feeding and 1 mg/kg BLT-1 dosing period, and was analyzed for creatinine (A), AST (B), and ALT (C) levels. Results were analyzed by Student’s unpaired t-test; n = 3–4. Tissue morphology was visualized by hematoxylin and eosin (H&E) staining of formalin-fixed tissues. ×10 magnification for jejunum (D and G), liver (E and H), and kidney (F and I) sections.
**Fig. 7.** SR-BI^−/−^ mice display reduced postprandial plasma and TRL TG levels and increased plasma CHOL levels relative to WT mice. Fat tolerance tests were performed in WT and SR-BI^−/−^ mice, Pluronic F-127 was used to block lipolysis, and plasma was collected every 30 min for up to 2 h. Plasma was analyzed for TG (A) and CHOL (C) levels. TRL fractions were isolated and analyzed for TG (B) and CHOL (D). Results were analyzed by 2-way repeated-measures ANOVA with the Bonferroni posttest (A and C) or Student’s unpaired t-test (B and D) (*P < 0.05, **P < 0.01, and ***P < 0.001); n = 3–4.

**Fig. 8.** High-fat-fed SR-BI^−/−^ mice have lower body weights and postprandial plasma TG levels, but higher fasting and postprandial plasma CHOL levels relative to high-fat-fed wild-type (WT) mice. SR-BI^−/−^ and WT mice were fed a HFD for 12 wk, and body weight (A) was recorded daily. Plasma collected in the fasting state at the start and end of the feeding period was analyzed for TG (B) and CHOL (C) levels. Fat tolerance tests were performed with Pluronic F-127 given ip to block lipolysis, and plasma was collected every 30 min for 2 h. Plasma was analyzed for TG (D) and CHOL (E) levels. Results were analyzed by 2-way repeated-measures ANOVA with the Bonferroni posttest (A–E) (*P < 0.05, **P < 0.01, and ***P < 0.001, C57BL6/J vs. SR-BI^−/−^). # # # P < 0.01 and ### P < 0.001, day 0 vs. day 84); n = 5–8.
lipid to the ER for chylomicron assembly. Our observations of reduced chylomicron production with SR-BI inhibition likely result in part from impaired intracellular lipid and apoB48 trafficking.

Following our studies with BLT-1, it was important to use SR-BI−/− mice as a second approach, confirming that the absence of intestinal SR-BI function truly impairs intestinal lipoprotein secretion. Previous studies performed using SR-BI−/− mice have largely focused on cholesterol metabolism, since these mice display marked elevations in plasma cholesterol and HDL-cholesterol levels, due to reduced hepatic reverse cholesterol transport (18). Similarly, we observed that postprandial plasma cholesterol levels were elevated in SR-BI−/− mice compared with the wild type (WT). However, few studies have characterized TG metabolism in these mice. Fat tolerance tests revealed a significant reduction in postprandial TG secretion in SR-BI−/− mice, which was also reflected in TRL TG levels. Despite the elevated total plasma cholesterol levels, a trend toward lower TRL cholesterol levels was observed relative to WT, which is likely because TRL fractions lack HDL and low-density lipoprotein particles, the primary carriers of cholesterol. These findings further allude to a role for intestinal SR-BI in chylomicron secretion. Aside from the observations presented here, one other study to date has alluded to a role for intestinal SR-BI in chylomicron metabolism in an in vivo setting. This result may result from impairments in intracellular trafficking of cytosolic lipid droplets (cLD) and apoB48, which have previously been shown to be modulated by SR-BI. Potential effects of BLT-1 on the liver are not shown, since the oral bioavailability of BLT-1 is unclear. In SR-BI−/− mice, similar effects on intestinal chylomicron output are likely to occur, resulting in lower postprandial plasma and TRL TG levels. Additionally, a lack of SR-BI in the liver prevents hepatic cholesterol uptake from HDL particles, raising circulating plasma cholesterol levels.

In conclusion, this study is the first to demonstrate a functional role for intestinal SR-BI in chylomicron metabolism in an in vivo setting. Using multiple animal models, we consistently observed reductions in postprandial TRL TG levels during SR-BI inhibition, and intestinal SR-BI was implicated in diet-induced weight gain, dyslipidemia, and apoB48 secretion. Whether prolonged administration of an intestinal SR-BI inhibitor could blunt the development of obesity and the onset of insulin resistance and the metabolic syndrome is an area for future study. Furthermore, lipid handling in the gut is a multistep process involving luminal absorption, resynthesis of TG in the ER, and subsequent lipidation, packaging, and secretion of apoB48-containing chylomicrons. Prospective studies will aim to elucidate which of these processes SR-BI modulates, as well as the effectiveness of BLT-1 and other potential inhibitors in an effort to develop treatments.

Fig. 9. Model of key findings from BLT-1 vs. SR-BI knockout studies. Oral delivery of the SR-BI inhibitor BLT-1 hinders chylomicron particle secretion from the small intestine (reflected in lower circulating apoB48 levels), resulting in lower postprandial plasma and TRL TG levels. This may result from impairments in intracellular trafficking of cytosolic lipid droplets (cLD) and apoB48, which have previously been shown to be modulated by SR-BI. Potential effects of BLT-1 on the liver are not shown, since the oral bioavailability of BLT-1 is unclear. In SR-BI−/− mice, similar effects on intestinal chylomicron output are likely to occur, resulting in lower postprandial plasma and TRL TG levels. Additionally, a lack of SR-BI in the liver prevents hepatic cholesterol uptake from HDL particles, raising circulating plasma cholesterol levels.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: M.L. and K.A. conception and design of research; M.L., C.B., and M.F. prepared experiments; M.L., S.F., and B.T. interpreted results of experiments; M.L. and S.F. prepared figures; M.L., S.F., C.B., and K.A. edited and revised manuscript; M.L., S.F., C.B., M.F., B.T., and K.A. approved final version of manuscript; S.F. drafted manuscript.

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