Cholesterol absorption is mainly regulated by the jejunal and ileal ATP-binding cassette sterol efflux transporters Abcg5 and Abcg8 in mice

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Abbreviations: ABC, ATP-binding cassette (transporter); LXR, liver X receptor; NPC1L1, Niemann-Pick C1 Like 1 (protein); RXR, retinoid X receptor.
Abstract In the present study, we investigated whether intestinal sterol efflux transporters Abcg5 and Abcg8 play a major role in determining variations in cholesterol absorption efficiency, and compared the physiological functions of the duodenal, jejunal, and ileal Abcg5 and Abcg8 on the absorption of cholesterol and sitostanol in inbred mice challenged with various amounts of cholesterol, sitostanol, hydrophilic or hydrophobic bile acids. We found that Abcg5 and Abcg8 in the jejunum and ileum, but not in the duodenum, were main determinants in determining, in part, variations in cholesterol absorption efficiency. The jejunal and ileal Abcg5 and Abcg8 played a major regulatory role in response to high dietary cholesterol and were more sensitive in the regulation of cholesterol absorption compared with sitostanol absorption. These results, combined with different sterol uptake rates, suggest that the absorption efficiency of cholesterol and sitostanol is determined by the net results between influx and efflux of intraluminal cholesterol and sitostanol molecules cross the apical membrane of the enterocyte. Hydrophilic and hydrophobic bile acids influenced cholesterol absorption through mediating cholesterol solubilization and its physical-chemical state within the small intestinal lumen. We conclude that cholesterol absorption is mainly regulated by the jejunal and ileal Abcg5 and Abcg8 in mice.

Key words: micelle; lymph; chylomicron; bile salt; phospholipid; intestinal lipid uptake; nutrition; genetics.
INTRODUCTION

The small intestine is a unique organ providing dietary and re-absorbed biliary cholesterol to the body, and plays a critical role in the regulation of whole body cholesterol balance (1,2). Because elevated plasma cholesterol concentrations is an important risk factor for cardiovascular diseases, intensive studies have been carried out to search for physical-chemical, biochemical and genetic determinants of intestinal cholesterol absorption. Recently, two independent groups (3,4) used a microarray analysis of mouse intestine and liver genes upregulated by a liver X receptor (LXR) agonist as well as a positional cloning approach to identify mutations in sitosterolemia patients in either member of an adjacent pair of genes, \textit{ABCG5} and \textit{ABCG8}, encoding ATP-binding cassette (ABC) transporters expressed in the liver and intestine. By studying their function and expression in some “manufactured” mouse strains treated with or without LXR agonists (5-9), it has been proposed that ABCG5 and ABCG8 could promote efflux of cholesterol and sitosterol (plant sterol) from the enterocyte back into the intestinal lumen for elimination, and mediate biliary sterol secretion in the human. Although a very large number of polymorphic variants in both \textit{Abcg5} and \textit{Abcg8} among 20 strains of inbred mice have been identified (10), none of these mice showed any detectable levels of plasma sitosterol under chow (containing ~0.01% sitosterol) diet conditions. Thus whether the polymorphisms at the \textit{Abcg5} and \textit{Abcg8} genes and their expression levels in the intestine are an important determinant of intestinal cholesterol absorption efficiency in healthy inbred mice remains to be investigated. Furthermore, the autosomal recessive disorder sitosterolemia (11-13) is mainly characterized by hyperabsorption of cholesterol and sitosterol, and reduced secretion of these sterols
into bile. In patients with sitosterolemia (14-17), the intestinal absorption of cholesterol is increased by ~30% (from ~46% to ~60%); however, the intestinal absorption of sitosterol is greatly increased by ~800% (from <5% to ~45%). This suggests that there may be different mechanisms in the regulation of intestinal cholesterol and sitosterol absorption under normal physiological conditions.

Both LXR\(\alpha\) and LXR\(\beta\) are members of the nuclear receptor superfamily and are involved in regulation of cholesterol and lipid metabolism (18,19). LXRs bind to DNA as obligate heterodimers with retinoid X receptors (RXR) and are activated by oxysterols. The decreased fractional absorption of dietary cholesterol associated with RXR agonist treatment was attributed initially to the action of Abca1 (20), because levels of Abca1 mRNA increased markedly in the small intestine of mice given a highly specific RXR ligand. Subsequent characterization of mice expressing no Abca1 (Abca1\(^{-/-}\) mice) (21), and of the Wisconsin hypoalpha mutant (WHAM) chicken with spontaneously occurring ABCA1 dysfunction (22,23) revealed no impairment in either percent cholesterol absorption, fecal neutral steroid excretion, or biliary cholesterol secretion, even under the treatment of the synthetic LXR agonist. Furthermore, using in situ hybridization techniques, Abca1 is found predominately in cells present in the lamina propria in mice (24) and occasionally in the enterocyte in the primate (25). Recent data showed that ABCA1 is localized in the basolateral membrane of the enterocyte in chicken (22,23). Accordingly, it is most likely that other intestinal sterol transporters may mediate a LXR agonist-associated increase in fecal excretion of cholesterol and reduction in cholesterol absorption.
It has been reported that there are significant variations in cholesterol absorption efficiency, and genetic factors at enterocyte level may play a major role in determining intestinal cholesterol absorption efficiency in inbred mice (26-28). Despite it has been found that some dietary factors, biliary factors, enterocyte factors, and luminal factors could influence cholesterol absorption (1,2), it remains poorly understood which step(s) in the absorption process differ inherently among individuals in any population to explain variations in intestinal cholesterol absorption efficiency. In the present study, we studied (i) whether intestinal sterol efflux transporters Abcg5 and Abcg8 play a major role in determining variations in cholesterol absorption efficiency in inbred mice; (ii) compared the physiological functions of the duodenal, jejunal, and ileal Abcg5 and Abcg8 on the absorption of cholesterol and sitostanol; and (iii) investigated whether both transporters influence intestinal cholesterol absorption in mice challenged with various amounts of high dietary cholesterol with or without sitostanol, as well as to hydrophilic or hydrophobic bile acids.
MATERIALS AND METHODS

Chemicals. Radioisotope [4⁻¹⁴C]cholesterol was purchased from NEN Life Science Products (Boston, MA), and [5,6⁻³H]sitostanol was from American Radiolabeled Chemicals (St. Louis, MO). Cholesterol, sitostanol, chenodeoxycholic acid, cholic acid, 22(R)-hydroxycholesterol, and 22(S)-hydroxycholesterol were purchased from Sigma (St. Louis, MO). The synthetic LXR agonist T0901317 (N-(2,2,2-trifluoro-ethyl)-N-[4-(2,2,2-trifluoro-1-hydroxy-1-trifluoromethyl-ethyl)phenyl]benzenesulfonamide) was purchased from Cayman Chemical (Ann Arbor, MI). Ursodeoxycholic and deoxycholic acids were purchased from FalkGmbH (Freiburg, Germany). Dehydrocholic, hyocholic, hyodeoxycholic, and ursodeoxycholic (3α,7β,12α-trihydroxy-5β-cholan-24-oic) acids were purchased from Calbiochem-Behring (San Diego, CA), and α-, β-, and ω-muricholic acids were from Tokyo Tanabe (Tokyo, Japan). Grade I egg yolk lecithin was purchased from Lipid Products (Surry, UK). Medium-chain triglyceride was purchased from Mead Johnson (Evansville, IN), and Intralipid (20%, wt/vol) was from Pharmacia (Clayton, NC).

Animals and diets. Male strains of inbred A/J (A), AKR/J (AKR), DBA/2J (DBA), C57BL/6J (C57BL), C57L/J (C57L), and SWR/J (SWR) mice (6-8 wk old) were purchased from The Jackson Laboratory (Bar Harbor, ME). All animals were maintained in a temperature-controlled room (22±1°C) with a 12-h day cycle (6:00 AM-6:00 PM). Mice were fed with normal rodent chow (Harlan Teklad F6 Rodent Diet 8664, Madison, WI) containing trace (<0.02%) amounts of cholesterol. All procedures were in accordance with current National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee of Harvard University.
Measurement of absorption and lymphatic transport of cholesterol and sitostanol.

After AKR and C57L mice (n=5 per group) were anesthetized with pentobarbital, laparotomy was performed under sterile conditions through an upper midline incision. To exclude the effects of bile on intestinal absorption of cholesterol and sitostanol, a mouse model with a chronic biliary fistula was established according to published methods (29). To better expose the mesenteric lymphatic duct, the animal was dorsally arch-bridged over a 3-ml syringe. A PE-10 catheter was inserted into the mesenteric lymph duct with magnification provided by a zoom stereomicroscope (Olympus America, Melville, NY). The catheter was externalized through the abdominal wall and connected with a heparinized tube. The abdominal incision was closed tightly with 5-0 sutures. Exactly 2.5 µCi of [14C]cholesterol and 5.0 µCi of [3H]sitostanol dissolved in 100 µl of medium-chain triglyceride containing 0.5% (wt/wt) taurocholate and 0.2% egg yolk lecithin were instilled into the small intestine through a duodenal catheter. Measurement of absorption and lymphatic transport of cholesterol and sitosterol was initiated, and fresh lymph was collected hourly into a heparinized tube for a total of 12 h (30). The two radioactive isotopes in the lymph were extracted and counted. To maintain lymph flow, a continuous intraduodenal infusion of 0.5% taurocholate and 0.2% egg yolk lecithin in medium-chain triglyceride was performed at 300 µl/h for 12 h. During surgery and lymph collection, mouse body temperature was maintained at 37±0.5°C with a heating lamp and monitored with a thermometer. Continuous anesthesia was maintained with an intraperitoneal injection of 25 mg/kg pentobarbital every 2 h.

Measurement of cholesterol absorption by fecal dual-isotope ratio method. Non-fasted and non-anesthetized mice of inbred A, AKR, DBA, C57BL, C57L, and SWR
strains (n=5 per group) were given intragastrically by gavage 1 µCi of [14C]cholesterol and 2 µCi of [3H]sitostanol in 150 µl of medium-chain triglyceride. Mice were then transferred to individual metabolic cages with wire mesh bottoms where they continued to ingest the chow diet for the next 4 days. During this period, mouse feces were collected daily. The radioactive isotopes from 4-day pooled fecal samples were saponified, extracted, and counted. The ratio of the two radionuclides in the fecal extracts and the dosing mixture was used for calculation of percent cholesterol absorption (30).

**Cholesterol balance analysis.** Because high dietary cholesterol exerts the effect of the radioisotope dilution on specific activity of cholesterol in the upper small intestine, we used cholesterol balance analysis (30) to determine cholesterol absorption efficiency in mice under high dietary sterol loads. Mice housed in individual metabolic cages with wire mesh bottoms were allowed to adapt to the environment for 2 wk. When body weight, food ingestion, and fecal excretion were constant, i.e., an apparent metabolic steady state, food intake was measured and feces were collected daily for continuous 7 days for the balance study. AKR and C57L mice (n=5 per group) were fed 7 days with chow, or chow supplemented with 0.5% (by weight), 1%, or 2% cholesterol. Additional groups of AKR and C57L mice (n=5 per group) were fed 7 days with chow, or chow supplemented with 0%, 0.5%, 1%, or 2% sitostanol plus 1% cholesterol. Following this procedure, animals were anesthetized with pentobarbital. After cholecystectomy, the common bile duct was cannulated with a PE-10 catheter and hepatic bile was collected for the first hour of biliary secretion. Bile cholesterol and cholesterol content in the diet were measured by HPLC. Fecal neutral steroids were saponified and extracted,
as well as being measured by HPLC (30). Percent cholesterol absorption was calculated according to published methods (30).

**Measurement of intestinal sterol uptake.** After anesthesia, the duodenum was cannulated with a PE-10 catheter, and the catheter was externalized through the incision and implanted subcutaneously (26). The abdominal incision was closed tightly with 5-0 sutures. After 24-h recovery from the surgery and another 12 h fasting (but with water), exactly 2 µCi of [14C]cholesterol and 2 µCi of [3H]sitostanol in 100 µl of medium-chain triglyceride were injected into the non-anesthetized AKR and C57L mice (n=5 per group) via the duodenal catheter. After the injection, the mice were allowed to move freely in the cage. Exactly 45 min after instillation, the animals were anesthetized with pentobarbital. At laparotomy, the entire small intestine was removed and flushed with taurocholate buffer. After being wet-weighed, the small intestine was cut into three segments with length ratios of 1:3:2 (duodenum:jejunum:ileum). The two radiolabeled sterols were extracted and counted, as well as the radioactivity was used to calculate intestinal sterol uptake *in vivo*, which is expressed as dpm/g tissue/45 min.

**Quantitative real-time PCR assay.** To observe the role of intestinal Abcg5, Abcg8, and Lxrα in cholesterol absorption, AKR and C57L mice (n=4 per group) were fed (i) chow, or chow supplemented with 0.5% (by weight) of each, cholic, chenodeoxycholic, dehydrocholic, deoxycholic, hyocholic, hyodeoxycholic, α-muricholic, β-muricholic, ω-muricholic, ursodeoxycholic, or ursocholic acids; (ii) various amounts (0.02%, 0.5%, 1%, and 2%) of cholesterol or sitostanol; as well as (iii) administrated intragastrically by gavage daily with a vehicle control in propylene glycol/Tween 80 (4:1, vol/vol) formulation, 22(R)-hydroxycholesterol or 22(S)-hydroxycholesterol at a dose of
1 mg/day/kg body weight, or the synthetic LXR agonist T0901317 at a dose of 10 mg/day/kg body weight. Because the magnitude of gene regulation by orally administered agents may vary as a function of time following dosing, we fed the mice with the agents at 9:00 AM daily and harvested the intestine tissues for the extraction of total RNA at 10:00 AM. After the 7-day feeding, the mice were anesthetized with pentobarbital. The small intestine was removed, flushed with ice-cold saline solution, and cut into three segments with length ratios of 1:3:2 (duodenum:jejunum:ileum). In the middle of each intestinal segment, 1.5 cm of the duodenal, jejunal and ileal tissues were cut out, and the tissues from 4 mice per group were pooled. Total RNA was extracted from the intestine using RNeasy Midi (Qiagen, Valencia, CA). Reverse-transcription reaction was performed using the SuperScript II First-Strand Synthesis System (Invitrogen, Carlsbad, CA) with 5 µg of total RNA and random hexamers to generate cDNA. Primers and probes (Table 1) for Abcg5, Abcg8, Lxrα, and Lxrβ were designed using Primer Express Software (Applied Biosystems, Foster City, CA) based on sequence data available from GenBank. Real-time PCR assays (31) were performed in triplicate on a GeneAmp 5700 Sequence Detection System (Applied Biosystems). Relative mRNA levels were calculated using the threshold cycle (CT) of an unknown sample against a standard curve with known copy numbers. To obtain a normalized target value, the target amount was divided by the endogenous reference rodent glyceraldehyde-3-phosphate dehydrogenase (Gapdh) as the invariant control (Part No. 4308313, Applied Biosystems, Foster City, CA).

Statistical methods. All data are expressed as means±SD. Statistically significant differences among groups of mice were assessed by Student’s t-test or Mann-Whitney
U tests. If the F-value was significant, comparison among groups of mice was further analyzed by a multiple comparison test. Analyses were performed with SuperANOVA software (Abacus Concepts, Berkeley, CA). Statistical significance was defined as a two-tailed probability of less than 0.05.
RESULTS

Absorption and lymphatic transport of cholesterol and sitostanol. To examine whether there are differences in intestinal absorption between cholesterol and sitostanol, we studied the absorption and lymphatic transport of cholesterol and sitostanol (a direct measurement of sterol absorption) in the higher-absorbing C57L mice compared with the lower-absorbing AKR mice. Because differences in biliary lipid outputs and bile salt pool sizes between AKR and C57L mice have some effects on cholesterol absorption (26), we investigated mice with chronic biliary fistula but in the setting of infusion of 0.5% taurocholate and 0.2% egg yolk lecithin. As expected (30), the intraduodenal infusion of the lipid emulsion produced a steady and continuous lymph flow in both AKR and C57L mice for longer periods of time. Figure 1A shows that under these experimental conditions, lymph flow rates (260-290 µl/h) were constant and similar in both mouse strains over the 12 h period. As shown in Figure 1B, cumulative radioactivities in lymph at 12 h after instillation of [14C]cholesterol are 36±4% in C57L strain, which is significantly (P<0.001) greater than those in AKR strain (22±3%), consistent with previous results (30). Furthermore, cumulative radioactivities (12 h) of sitostanol in the lymph of C57L mice (3.7±0.5%) were significantly (P<0.05) greater compared with those in AKR (2.1±0.5%). In comparison, absorption and lymphatic transport of cholesterol was significantly greater than that of sitostanol.

Cholesterol absorption efficiency and gene expression of intestinal sterol efflux transporters. Figure 2A shows cholesterol absorption efficiency determined by the fecal dual-isotope ratio method in six strains of inbred male mice on chow. In agreement with previous study (26) as measured by the plasma dual-isotope ratio method, we observed
that there were marked differences among mouse strains with respect to intestinal cholesterol absorption efficiency: AKR (27±3%) and A (28±3%) strains showed the lowest cholesterol absorption; DBA (33±4%) and SWR (34±3%) strains gave intermediate values; and C57L (40±4%) and C57BL (42±4%) strains displayed the highest values for cholesterol absorption. Figure 2(B and C) exhibits that expression levels of Abcg5 and Abcg8 in the jejunum and ileum are the highest in AKR and A mice. In contrast, expression levels of the jejunal and ileal Abcg5 and Abcg8 were the lowest in C57L and C57BL mice, with DBA and SWR mice showing intermediate values for Abcg5 and Abcg8 mRNA levels in the jejunum and ileum. We noted that the relative mRNA levels for the duodenal Abcg5 and Abcg8 were essentially similar in these mouse strains with different cholesterol absorption efficiency. Furthermore, our results demonstrate that there is a remarkably negative correlation between percent cholesterol absorption and expression levels of the jejunal and ileal Abcg5 and Abcg8 in chow-fed mice; however, they do not reach statistically significant differences.

*Sterol uptake by the small intestine.* To observe whether there are differences in uptake of cholesterol and sitostanol by the enterocyte, we compared their uptake rates in AKR and C57L mice. Our results (Figure 3) show that at 45 min after dosing, the radiolabeled cholesterol and sitostanol uptake by the enterocytes of C57L mice was 1.5-fold higher (P<0.05) compared with AKR mice. The small intestine absorbed significantly (P<0.001) greater amounts of [14C]cholesterol compared with [3H]sitostanol. Together, these results suggest that there may be a selective uptake mechanism between cholesterol and sitostanol in mice.
Responses of intestinal sterol efflux transporters to high dietary cholesterol and sitostanol. Table 2 summaries the data for daily cholesterol intake, daily biliary cholesterol output, daily fecal total neutral steroid excretion, daily absorbed cholesterol, and percent cholesterol absorption in AKR and C57L mice in the metabolic steady state. We found that the lower-absorbing AKR mice and the higher-absorbing C57L mice ate basically similar amounts of food, whatever chow or high dietary cholesterol was fed. However, on the chow diet (0.02% cholesterol), daily biliary cholesterol outputs in C57L mice (2.11±0.08 mg/day) were significantly (P<0.0001) higher compared with AKR mice (1.14±0.08 mg/day) because C57L mice are a gallstone-susceptible strain (32). Because of higher biliary cholesterol secretion, daily fecal total neutral steroid excretion was significantly (P<0.0001) greater in C57L mice (2.62±0.08 mg/day) than in AKR mice (1.75±0.11 mg/day). Nevertheless, an input-output analysis found that absorbed mass of cholesterol daily in C57L mice (0.31±0.02 mg/day) was significantly (P<0.001) higher compared with AKR mice (0.21±0.03 mg/day). The calculated percent cholesterol absorption in C57L mice was 38±3%, being significantly (P<0.01) greater than that in AKR mice (26±4%). Under the higher (≥0.5%) dietary cholesterol feeding conditions, C57L mice produced significantly (P<0.01) higher biliary cholesterol outputs than AKR mice. Furthermore, cholesterol mass absorbed from the small intestine was significantly (P<0.01) greater in C57L mice than in AKR mice because the latter excreted more fecal neutral steroids compared with the former. Of note is that compared with the basal (0.02% cholesterol) diet, cholesterol absorption efficiency measured by the mass balance method remains unchanged in mice fed various amounts of high dietary cholesterol.
cholesterol. Taken together, these results suggest that C57L mice absorb more cholesterol than AKR mice, whatever chow or high dietary cholesterol is fed.

Because plant sterols have been used as cholesterol-lowering agents (33-35), we examined their effects on intestinal cholesterol absorption. Our results (Table 3) show that AKR and C57L mice ate identical amounts of cholesterol although various amounts of sitostanol were fed. Sitostanol (≤1%) feeding did not cause significant changes in intestinal cholesterol absorption (percentage and total mass), biliary cholesterol outputs, and fecal steroid excretion. In contrast, increasing dietary sitostanol to 2% induced a significant increase in fecal total neutral steroid excretion (P<0.05), as well as significant decreases in biliary cholesterol outputs (P<0.001), absorbed cholesterol mass (P<0.01), and percent cholesterol absorption (P<0.05) in both strains of mice, favoring AKR mice more than C57L mice.

To further examine responses of intestinal sterol efflux transporters to high dietary cholesterol and sitostanol, we studied expression levels of Abcg5 and Abcg8 in AKR and C57L mice fed various amounts of cholesterol or sitostanol. Compared with the basal (0.02% cholesterol) diet, feeding 0.5% or higher dietary cholesterol significantly (P<0.01) increased expression levels of Abcg5 and Abcg8 in the jejunum and ileum, but not in the duodenum (Figure 4A), which is associated with increased fecal neutral steroid excretion (Table 2). Our results suggest that in response to high (≥0.5%) dietary cholesterol, the jejunal and ileal Abcg5 and Abcg8 may efflux more cholesterol from the enterocyte back into the lumen and reduce its fractional absorption. Furthermore, this kind of response was not observed in mice fed sitostanol until its concentration in the diet was increased to 2% (Figure 4B). This suggests that the
intestinal Abcg5 and Abcg8 may be more sensitive in regulating cholesterol absorption compared with sitostanol absorption.

*Regulation of intestinal sterol efflux transporters by nuclear receptor LXR.* To explore whether there is an “oxysterol-LXR-ABCG5/G8” pathway for regulating intestinal cholesterol absorption, we fed AKR and C57L mice with diets under conditions in which the *Lxr* gene is stimulated by the naturally occurring oxysterols or the synthetic LXR agonist T0901317. Figure 5 depicts that feeding T0901317 at a dose of 10 mg/day/kg significantly (P<0.01) increases expression levels of *Lxrα* (Figure 5A), *Abcg5* (Figure 5B), and *Abcg8* (Figure 5C) in the jejunum and ileum and to a lesser extent in the duodenum. The relative mRNA levels for *Lxrβ* (data not shown) were very low in the mouse small intestine. Furthermore, the sterol balance studies revealed that fecal total neutral steroid excretion (Figure 5D) was significantly (P<0.05) increased and percent cholesterol absorption (Figure 5E) was significantly (P<0.05) reduced by the synthetic LXR agonist feeding. This strongly suggests that the intestinal transporters Abcg5 and Abcg8 may serve to efflux cholesterol from the enterocyte into the intestinal lumen for elimination. Several *in vitro* experiments (36-39) have showed that trace amounts of the naturally occurring oxysterols could influence the sterol efflux functions of ABC transporters such as ABCA1 and ABC8 by activating LXR. However, we observed that feeding 22(R)-hydroxycholesterol and its isoform 22(S)-hydroxycholesterol (as a control) at a dose of 1 mg/day/kg did not influence the relative mRNA levels for *Lxrα*, *Lxrβ*, *Abcg5* and *Abcg8* (data not shown) in the small intestines of both AKR and C57L mice, fecal total neutral steroid excretion, or percent cholesterol absorption.
Effect of bile acids on expression of intestinal sterol efflux transporters. Figure 6 shows expression levels of the Abcg5 and Abcg8 genes in the duodenum, jejunum, and ileum of AKR and C57L mice fed chow, or chow supplemented with 0.5% (by weight) bile acids for 7 days. Compared with the chow feeding, expression levels of Abcg5 and Abcg8 in the jejunum and ileum were essentially similar in mice fed the hydrophilic bile acids, but were increased significantly (P<0.001) by cholic acid feeding and to a lesser extent by deoxycholic and dehydrocholic acid feeding. In contrast, the relative mRNA levels for Abcg5 and Abcg8 in the duodenum were not influenced, whatever hydrophilic or hydrophobic bile acids were fed.
DISCUSSION

In the present study, the most important findings are that (i) sterol efflux transporters Abcg5 and Abcg8 in the jejunum and ileum, but not in the duodenum, are major determinants in determining, in part, variations in cholesterol absorption efficiency in inbred mice, as well as the jejunal and ileal Abcg5 and Abcg8 play a main regulatory role in response to high dietary cholesterol and sitostanol; (ii) The absorption efficiency of cholesterol and sitostanol in the small intestine is predominantly determined by the net results of a complex series between influx and efflux of intraluminal cholesterol and sitostanol molecules cross the apical membrane of the enterocyte; (iii) The intestinal Abcg5 and Abcg8 are involved in LXR agonist-associated regulation of cholesterol absorption; and (iv) Hydrophilic and hydrophobic bile acids influence cholesterol absorption by mediating cholesterol solubilization and its physical-chemical state within the small intestinal lumen.

Sitostanol is a saturated form of sitosterol, and both are structurally similar to cholesterol but differ in their side chain configuration (41-43). In this study, using lymphatic sterol transport approaches in animals with a chronic biliary fistula but in the setting of infusion of the lipid emulsion, we observed that the higher-absorbing C57L mice displayed significantly higher cholesterol and sitostanol absorption rates than the lower-absorbing AKR mice. Our study also produced direct evidence demonstrating that at 12 h after instilling radiolabeled cholesterol and sitostanol, cumulative radioactivities of sitostanol (2-4%) in the lymph were significantly lower compared with those of cholesterol (22-36%) in both mouse strains. Furthermore, compared with the basal (0.02% cholesterol) diet, higher (≥0.5%) dietary cholesterol significantly increased
Abcg5 and Abcg8 mRNAs levels in the jejunum and ileum, but not in the duodenum, favoring AKR mice more than C57L mice. Of note is that compared with C57L mice, AKR mice showed a significantly higher fecal total neutral steroid excretion (Table 2). These data suggest that in response to high dietary cholesterol, the jejunal and ileal sterol transporters Abcg5 and Abcg8 may efflux more cholesterol from the enterocyte back into the lumen and reduce its fractional absorption in AKR mice than in C57L mice. However, this kind of response was not observed in both strains of mice fed sitostanol until its concentration in the diet was increased to 2%, suggesting that there may be a selective regulatory mechanism between cholesterol and sitostanol absorption and the intestinal Abcg5 and Abcg8 may be more sensitive in the regulation of cholesterol absorption compared with sitostanol absorption. Our findings suggest that intestinal sterol efflux transporters Abcg5 and Abcg8 may provide a barrier to cholesterol and sitostanol accumulation by promoting partial efflux of cholesterol and nearly complete efflux of sitostanol from the enterocyte into the intestinal lumen for elimination.

The differences in cholesterol absorption efficiency among strains or individuals have been observed in animals (26-28,44-50) and humans (51-54). Recent studies (26-28) suggest that such variability could result from the interaction of multiple genes in the enterocyte. With inbred mouse models, we studied whether intestinal Abcg5 and Abcg8 influence the absorption of cholesterol under normal physiological conditions. We found that the relative mRNA levels for Abcg5 and Abcg8 in the jejunum and ileum were the highest in AKR and A strains with the lowest cholesterol absorption efficiency. In contrast, expression levels of Abcg5 and Abcg8 mRNAs in the jejunum and ileum were the lowest in C57L and C57BL strains with the highest cholesterol absorption efficiency.
Moreover, DBA and SWR strains displayed intermediate mRNA levels for Abcg5 and Abcg8 in the jejunum and ileum as well as intermediate values for cholesterol absorption efficiency. Our results demonstrate that expression levels of Abcg5 and Abcg8 in the jejunum and ileum are reduced with increasing cholesterol absorption efficiency, suggesting a strikingly reverse relationship between expression levels of the jejunal and ileal Abcg5 and Abcg8 and percent cholesterol absorption in chow-fed mice; however, they do not reach statistically significant differences. Nevertheless, our results suggest that the jejunal and ileal sterol efflux transporters Abcg5 and Abcg8 may determine, in part, variations in cholesterol absorption efficiency among inbred mice. Moreover, expression levels of the duodenal Abcg5 and Abcg8 were essentially similar among these inbred mice with diverse cholesterol absorption efficiency, and were not influenced in mice challenged with various amounts of cholesterol, sitostanol, hydrophilic or hydrophobic bile acids. Especially, the sterol uptake studies showed that the duodenum and jejunum accumulated the most radioactivities following the administration of radiolabeled cholesterol and sitostanol, which is 1.5-fold higher in the higher-absorbing C57L mice than in the lower-absorbing AKR mice. More recently, Altmann and colleagues (55) found that disruption of the NPC1L1 gene encoding the Niemann-Pick C1 Like 1 protein induces a significant decrease in intestinal cholesterol absorption in mice. They (55) also observed that ezetimibe lowered cholesterol absorption by ~70% in the wild-type mice, and this level of cholesterol absorption was similar to that seen in NPC1L1 knockout mice not treated with ezetimibe. Thus, NPC1L1 was proposed to be a sterol influx transporter in the small intestine, which may be critical for intestinal uptake of sterols. Furthermore, the expression levels of NPC1L1
mRNA and protein are relatively higher in the proximal small intestine of rats and relatively lower in the distal small intestine (55). Therefore, our results support the concept that the main sites of cholesterol absorption are the duodenum and proximal jejunum (56-59). It is important to note that in the chow-fed mouse, the expression levels of \textit{Abcg5} and \textit{Abcg8} in the duodenum and jejunum are somewhat greater compared with those in the ileum as measured by Northern blot analysis (3,5,24). In this study, the relative mRNA levels for \textit{Abcg5} and \textit{Abcg8} are slightly higher in the jejunum and ileum than in the duodenum as assayed by our highly precise quantitative real-time PCR methods. A possible explanation for these differences is that other research groups (3,5,24) extracted total RNA from the entire intestinal mucosa of three segments with length ratios of 1:1:1 (duodenum:jejunum:ileum). We cut the small intestines into three segments with length ratios of 1:3:2 (duodenum:jejunum:ileum). Then, the middle 1.5 cm of intestinal tissue in each segment from 4 mice per group was pooled, which was used for total RNA extraction. Another reason is possibly due to different murine genetic backgrounds.

“Uptake of cholesterol” refers to entry of cholesterol into intestinal absorption cells (1,2). To investigate whether there are differences in intestinal uptake between cholesterol and sitostanol, we compared their uptake rates in AKR and C57L mice. Our results show that at 45 min after dosing, radiolabeled cholesterol and sitostanol uptake by the enterocytes of C57L mice was 1.5-fold higher compared with AKR mice. Furthermore, the fact that cholesterol is absorbed more than structurally similar sitostanol in both strains of mice, suggests that there may be a selective uptake mechanism between cholesterol and sitostanol, and a cholesterol transporter(s) (55,60-
64) may be localized at the apical membrane that facilitates selective cholesterol but not sitostanol uptake by the enterocyte. It is important to note that the techniques used in the present study cannot determine the amounts of sterols that are re-secreted into the intestinal lumen by the Abcg5 and Abcg8 pathway. However, in the previous study of small intestinal transit in mice (26), we found that 30 min after the installation of radiolabeled sitostanol into the small intestine through the duodenal catheter, the radioactivity can be detected at the end of small intestine, i.e., in the ileum, but not in the cecum or large intestine. Accordingly, we arbitrarily define that within 45 min, the intestinal sterol uptake is a predominant event, and there is no or less efflux of sterols from the enterocyte back to the lumen. Furthermore, it is imperative to investigate whether the newly identified intestinal sterol influx transporter NPC1L1 (55) plays a critical role in determining cholesterol absorption efficiency and in regulating cholesterol and sitostanol absorption in healthy inbred mice.

To explore the role of “oxysterol-LXRα-ABCG5/G8 pathway” in the regulation of cholesterol absorption, we challenged mice with diets under conditions in which the Lxrα gene is stimulated by the naturally occurring oxysterols or the synthetic LXR ligand. Several in vitro experiments (36-39) have showed that trace amounts of 22(R)-hydroxycholesterol but not its isoform 22(S)-hydroxycholesterol could influence the lipid efflux functions of ABC transporters such as ABCA1 and ABC8 by activating LXR. We observed that feeding these two oxysterols did not influence expression levels of the intestinal Lxrα, Abcg5, or Abcg8 in mice. Most likely, under chow feeding conditions, mouse small intestine contains large amounts of hydrophilic bile salts secreted by the liver, which does not favor their absorption by the enterocyte (31,65). In contrast,
feeding the synthetic LXR ligand T0901317 up-regulated expression levels of the intestinal $Lxr\alpha$, $Abcg5$, and $Abcg8$, increased fecal neutral steroid excretion, and reduced fractional absorption of cholesterol. Clearly, additional in vivo experiments are required to evaluate the role of the naturally occurring oxysterols in the regulation of cholesterol absorption.

Consistent with previous findings (31), we found that the relative mRNA levels for the duodenal $Abcg5$ and $Abcg8$ were not influenced by hydrophilic or hydrophobic bile acid feeding. Furthermore, compared with the chow diet, expression levels of the jejunal and ileal $Abcg5$ and $Abcg8$ mRNAs were not influenced by the hydrophilic bile acid feeding, but were increased significantly by cholic acid feeding and slightly by deoxycholic and dehydrocholic acids. In an experiment with small number of mice (L.-P. Duan, H. H. Wang, and D. Q.-H. Wang, unpublished observations), we found that cholic acid-fed C57L mice display markedly higher cholesterol absorption efficiency compared with AKR mice treated with deoxycholic or dehydrocholic acids although the relative mRNA levels for $Abcg5$ and $Abcg8$ in the jejunum and ileum of AKR mice fed deoxycholic and dehydrocholic acids are similar to those in C57L mice fed cholic acid. Taken together, this response is most likely an indirect effect because gene expression of these two sterol efflux transporters is highly sensitive to the larger amounts of absorbed cholesterol that is augmented mainly by fed cholic acid. Another possible explanation is that cholic acid may promote intestinal absorption of biliary (and dietary) oxysterols (40) that bind and activate the oxysterol receptor LXR, a transcriptional regulator of the two sterol efflux transporter genes (24). Our results did not exclude the possibility that cholic acid per se may have a specific enhancing effect on the
expression of the *Abcg5* and *Abcg8* genes. As inferred from the gene expression studies, our data support the notion that hydrophilic and hydrophobic bile acids may influence cholesterol absorption mainly by mediating intraluminal micellar cholesterol solubilization and its physical-chemical state (31).

In summary, our results show that under normal physiological conditions, the absorption of cholesterol and sitostanol may be mainly regulated by the jejunal and ileal *Abcg5* and *Abcg8* in mice challenged with high dietary cholesterol and sitostanol. Furthermore, our studies suggest that the absorption efficiency of cholesterol and sitostanol in the small intestine of mice is most likely to be determined principally by the net results between influx and efflux of intraluminal cholesterol and sitostanol molecules cross the brush border of the enterocyte (Figure 7). More importantly, understanding the molecular, genetic, and biochemical regulation of intestinal cholesterol absorption may lead to novel targets and strategies, through inhibiting intestinal cholesterol absorption, for the prevention of these cholesterol-related diseases such as atherosclerosis and cholesterol gallstones that affect millions in Westernized societies.
ACKNOWLEDGMENTS

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sitosterol absorption, decreased removal, and expanded body pools compensate for reduced cholesterol synthesis in sitosterolemia with xanthomatosis. *J. Lipid Res.* **30**: 1319-1330.


cholelithiasis in inbred mice: Pathophysiology of biliary lipid secretion. *J. Lipid Res.* **40**: 2066-2079.


FIGURE LEGENDS

Figure 1. Comparison of the absorption and lymphatic transport of cholesterol and sitostanol between AKR and C57L mice (n=5 per group) with chronic biliary fistula but in the setting of infusion of 0.5% (wt/wt) taurocholate and 0.2% egg yolk lecithin. (A) The lymph flow rates (260-290 µl/h) are constant and similar in both AKR and C57L mice over the 12 h period. (B) By 12 h, 36±4% of the instilled [14C]cholesterol dose is recovered in lymph of C57L mice and 22±3% in AKR mice (P<0.001). The absorption and lymphatic transport of [3H]sitostanol in C57L mice (3.7±0.5%) is significantly (P<0.05) greater than that in AKR mice (2.1±0.5%). Furthermore, cumulative radioactivities (12 h) of sitostanol in the lymph of mice were significantly (P<0.00001) lower compared with those of cholesterol.

Figure 2. Relationship between cholesterol absorption efficiency and expression levels of Abcg5 and Abcg8 in the duodenum, jejunum, and ileum of six strains of male inbred AKR, A, DBA, SWR, C57L, and C57BL mice on the chow diet. (A) The cholesterol absorption efficiency for each strain (n=5 per group) was determined by the fecal dual-isotope ratio method (30). Consistent with previous study (26) as measured by the plasma dual-isotope ratio method, cholesterol absorption efficiency varies from low to high among these inbred mice. Gene expression of (B) Abcg5 and (C) Abcg8 in the duodenum is essentially similar in these mouse strains. The relative mRNA levels for the jejunal and ileal Abcg5 and Abcg8 are the highest in AKR and A strains. In contrast, expression levels of the transporter genes in the jejunum and ileum are the lowest in C57L and C57BL mice. DBA and SWR mice show intermediate values for Abcg5 and
Abcg8 mRNA levels in the jejunum and ileum. Our results demonstrate that there is a remarkably negative correlation between percent cholesterol absorption and expression levels of the jejunal and ileal Abcg5 and Abcg8 in chow-fed mice; however, they do not reach statistically significant differences. Of note is that relative mRNA levels are calculated using the threshold cycle of an unknown sample against a standard curve with known copy numbers. To obtain a normalized target value, the target amount is divided by the endogenous reference rodent Gapdh as the invariant control. Furthermore, total RNA was extracted from the pooled intestine tissues (n=4 per group) and real-time PCR assays were performed in triplicate from the same sample. As a result, the error bars depict the technique variance rather than the biological variance.

**Figure 3.** Sterol uptake by the small intestine. To observe whether there are differences in intestinal uptake between cholesterol and sitostanol, we compared their uptake rates in AKR and C57L mice. At 45 min after dosing, the radiolabeled cholesterol and sitostanol uptake by the enterocytes of C57L mice are 1.5-fold higher (P<0.05) compared with those in AKR mice. The small intestine absorbs significantly (P<0.001) greater amounts of [14C]cholesterol than those of [3H]sitostanol, suggesting that there may be a selective uptake mechanism between cholesterol and sitostanol.

**Figure 4.** To examine responses of intestinal sterol efflux transporters to high dietary cholesterol and sitostanol, we studied expression levels of Abcg5 and Abcg8 in AKR and C57L mice fed various amounts of cholesterol and sitostanol. (A) Compared with the basal (0.02% cholesterol) diet, feeding 0.5% or higher dietary cholesterol
significantly (P<0.01) increases expression levels of Abcg5 and Abcg8 in the jejunum and ileum, but not in the duodenum, which is associated with increased fecal neutral steroid excretion (Table 2). These data suggest that the jejunal and ileal Abcg5 and Abcg8 may play a major regulatory role in cholesterol absorption. (B) However, this kind of response is not observed in mice fed sitostanol until its concentration in the diet is increased to 2%, suggesting that Abcg5 and Abcg8 may be more sensitive in regulating cholesterol absorption than sitostanol absorption. Because real-time PCR assays were carried out in triplicate from the same sample of the pooled intestine tissues (n=4 per group), the error bars represent the technique variance rather than the biological variance.

Figure 5. Regulation of intestinal Abcg5 and Abcg8 by nuclear receptor LXR. Compared with the vehicle control, feeding the synthetic LXR agonist T0901317 at a dose of 10 mg/day/kg significantly (P<0.01) increases expression levels of (A) Lxrα, (B) Abcg5, and (C) Abcg8 in the jejunum and ileum, and to a lesser extent in the duodenum. Of note is that because real-time PCR assays were carried out in triplicate from the same sample of the pooled intestine tissues (n=4 per group), the error bars represent the technique variance rather than the biological variance. Furthermore, the sterol balance analysis (30) reveals that (D) fecal neutral steroid excretion is significantly increased, but (E) percent cholesterol absorption is significantly reduced by the synthetic LXR agonist, suggesting that Abcg5 and Abcg8 may serve to efflux cholesterol from the enterocyte into the intestinal lumen and reduce its fractional absorption. Asterisks indicate P<0.05.
Figure 6. The relative mRNA levels for Abcg5 and Abcg8 in the duodenum, jejunum, and ileum of AKR and C57L mice fed chow, or chow supplemented with 0.5% (by weight) bile acids. Top arrows show increasing hydrophobicity indices of the biliary bile salt pools in mice fed the corresponding bile acids (31). Because of distinct hepatic metabolism of some of the congener series, the hydrophobicity indices of the bile salt pools are changed markedly as a result. Therefore, hydrophobicity indices of specific bile acids do not parallel those of the secreted bile salts in the corresponding hepatic biles (31). Please note that expression levels of the two transporter genes in the duodenum are not influenced by bile acids feeding. Furthermore, compared with the chow feeding, the relative mRNA levels for Abcg5 and Abcg8 in the jejunum and ileum are similar in mice fed hydrophilic bile acids, but are increased significantly (P<0.001) by cholic acid feeding and somewhat by deoxycholic and dehydrocholic acids. This response may be an indirect effect because intestinal Abcg5 and Abcg8 are very sensitive, through the oxysterol reporter LXR, to the mass of absorbed cholesterol and probably biliary (and dietary) oxysterols (40) induced by cholic acid. Again, because real-time PCR assays were carried out in triplicate from the same sample of the pooled intestine tissues (n=4 per group), the error bars represent the technique variance rather than the biological variance. Abbreviations: CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; DHCA, dehydrocholic acid; HCA, hyocholic acid; HDCA, hyodeoxycholic acid; MCA, muricholic acid; UCA, ursocholic acid; and UDCA, ursodeoxycholic acid.
Figure 7. Intestinal cholesterol absorption is a multistep process that is regulated by multiple genes. The Niemann-Pick C1 Like 1 protein (NPC1L1), a newly identified sterol influx transporter, is located at the apical membrane of the enterocyte (55), which may actively facilitate the uptake of cholesterol. ABCG5 and ABCG8 promote partial efflux of cholesterol and nearly complete efflux of plant sterols from the enterocyte into the intestinal lumen. NPC1L1, ABCG5, and ABCG8 may play a critical role in modulating the amount of cholesterol that reaches the lymph from the intestinal lumen. Also, several proteins involved in other steps in the absorption process, e.g., LXRα, acyl-CoA:cholesterol acyltransferase isoform 2 (ACAT2), apolipoprotein B48 (APO-B48), and microsomal triglyceride transfer protein (MTP) in the enterocyte, may exert major influences on cholesterol absorption. Furthermore, the absorption efficiency of cholesterol and sitostanol in the duodenum, jejunum, and ileum may be different, which may be mainly determined by the net results of a complex series between influx and efflux of intraluminal cholesterol and sitostanol molecules cross the apical brush board membrane of the enterocyte.
Table 1. Primer and probe sequences used in mRNA quantification by real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession No.</th>
<th>Forward</th>
<th>Reverse</th>
<th>Probe</th>
</tr>
</thead>
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<tr>
<td>Abcg5</td>
<td>AF312713</td>
<td>5'-CCTGCAGAGC</td>
<td>5'-GCATCGCTG</td>
<td>5'-AGCAGGCTCAC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GACGTTTTTC-3'</td>
<td>TGTATCGCAAC-3'</td>
<td>TGTGCCGAGA-3'</td>
</tr>
<tr>
<td>Abcg8</td>
<td>AF324495</td>
<td>5'-TGGATAGTG</td>
<td>5'-AATTGAATCTG</td>
<td>5'-CAAGCTGCGTT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CTGCATGGATC-3'</td>
<td>CATCAGGCCC-3'</td>
<td>CCTCCCGGGTG-3'</td>
</tr>
<tr>
<td>Lxrα</td>
<td>NM_013839</td>
<td>5'-CGACAGAGCTT</td>
<td>5'-ACAGCTCGTT</td>
<td>5'-CGGAAAAAGGCG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CGTCCACAA-3'</td>
<td>CCCAGCAT-3'</td>
<td>CCAGGCC-3'</td>
</tr>
<tr>
<td>Lxrβ</td>
<td>NM_009473</td>
<td>5'-TTGCGACTCCAG</td>
<td>5'-CTACTCGTGCA</td>
<td>5'-CTGCCGCCCTT</td>
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<td></td>
<td></td>
<td>GACAAGAA-3'</td>
<td>CATCCAGATC-3'</td>
<td>GCTGTCCG-3'</td>
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</tbody>
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Table 2. Cholesterol balance data in cholesterol-fed AKR and C57L mice during metabolic steady state conditions

<table>
<thead>
<tr>
<th>Diet</th>
<th>Cholesterol intake (mg/day)</th>
<th>Biliary cholesterol (mg/day)</th>
<th>Steroid excretion (mg/day)</th>
<th>Absorbed cholesterol&lt;sup&gt;a&lt;/sup&gt; (mg/day)</th>
<th>Cholesterol absorption&lt;sup&gt;b&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKR mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.02%</td>
<td>0.82±0.01</td>
<td>1.14±0.08</td>
<td>1.75±0.11</td>
<td>0.21±0.03</td>
<td>26±4</td>
</tr>
<tr>
<td>0.5%</td>
<td>21.00±0.50&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1.75±0.17&lt;sup&gt;h&lt;/sup&gt;</td>
<td>13.71±0.90&lt;sup&gt;g&lt;/sup&gt;</td>
<td>5.54±0.49&lt;sup&gt;g&lt;/sup&gt;</td>
<td>26±3</td>
</tr>
<tr>
<td>1%</td>
<td>41.40±1.14&lt;sup&gt;g&lt;/sup&gt;</td>
<td>2.32±0.22&lt;sup&gt;g&lt;/sup&gt;</td>
<td>28.88±0.77&lt;sup&gt;g&lt;/sup&gt;</td>
<td>10.19±0.87&lt;sup&gt;g&lt;/sup&gt;</td>
<td>25±3</td>
</tr>
<tr>
<td>2%</td>
<td>81.60±2.97&lt;sup&gt;g&lt;/sup&gt;</td>
<td>3.41±0.26&lt;sup&gt;g&lt;/sup&gt;</td>
<td>56.69±3.92&lt;sup&gt;g&lt;/sup&gt;</td>
<td>21.50±1.67&lt;sup&gt;g&lt;/sup&gt;</td>
<td>26±3</td>
</tr>
<tr>
<td>C57L mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.02%</td>
<td>0.82±0.02</td>
<td>2.11±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.62±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.31±0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>38±3&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5%</td>
<td>21.10±0.96&lt;sup&gt;i&lt;/sup&gt;</td>
<td>3.07±0.13&lt;sup&gt;d,i&lt;/sup&gt;</td>
<td>10.20±1.08&lt;sup&gt;f,i&lt;/sup&gt;</td>
<td>7.83±0.48&lt;sup&gt;e,i&lt;/sup&gt;</td>
<td>37±3&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>1%</td>
<td>41.20±0.84&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4.49±0.50&lt;sup&gt;e,i&lt;/sup&gt;</td>
<td>20.88±1.26&lt;sup&gt;c,i&lt;/sup&gt;</td>
<td>15.84±1.54&lt;sup&gt;e,i&lt;/sup&gt;</td>
<td>38±3&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>2%</td>
<td>82.80±3.03&lt;sup&gt;f&lt;/sup&gt;</td>
<td>6.44±0.73&lt;sup&gt;e,i&lt;/sup&gt;</td>
<td>46.28±4.96&lt;sup&gt;f,i&lt;/sup&gt;</td>
<td>30.08±1.97&lt;sup&gt;e,i&lt;/sup&gt;</td>
<td>36±3&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Absorbed cholesterol was determined by subtracting the daily fecal neutral steroid output from the daily cholesterol intake and the daily biliary cholesterol output as measured by the HPLC methods (30).

<sup>b</sup>The percent cholesterol absorption was determined by the cholesterol balance analysis according to published methods (30).

<sup>c</sup>P<0.0001 compared with AKR mice fed the same amount of cholesterol.

<sup>d</sup>P<0.001 compared with AKR mice fed the same amount of cholesterol.

<sup>e</sup>P<0.01 compared with AKR mice fed the same amount of cholesterol.

<sup>f</sup>P<0.01 compared with AKR mice fed the same amount of cholesterol.

<sup>g</sup>P<0.0001 compared with 0.02% cholesterol feeding in AKR mice.

<sup>h</sup>P<0.01 compared with 0.02% cholesterol feeding in AKR mice.

<sup>i</sup>P<0.0001 compared with 0.02% cholesterol feeding in C57L mice.

<sup>j</sup>P<0.001 compared with 0.02% cholesterol feeding in C57L mice.
Table 3. Effects of sitostanol on intestinal cholesterol absorption in AKR and C57L mice in the metabolic steady state

<table>
<thead>
<tr>
<th>Diet</th>
<th>Ch intake (mg/day)</th>
<th>Biliary Ch (mg/day)</th>
<th>Steroid excretion (mg/day)</th>
<th>Absorbed Ch (^a) (mg/day)</th>
<th>Ch absorption (^b) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AKR mice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1%Ch alone</td>
<td>41.40±1.14</td>
<td>2.32±0.22</td>
<td>28.88±0.77</td>
<td>10.19±0.87</td>
<td>25±3</td>
</tr>
<tr>
<td>0.5%S+1%Ch</td>
<td>40.20±1.92</td>
<td>2.25±0.23</td>
<td>27.32±1.52</td>
<td>10.63±1.33</td>
<td>26±3</td>
</tr>
<tr>
<td>1%S+1%Ch</td>
<td>42.00±1.00</td>
<td>1.90±0.35</td>
<td>31.11±1.24</td>
<td>9.00±1.53</td>
<td>21±3</td>
</tr>
<tr>
<td>2%S+1%Ch</td>
<td>39.00±3.24</td>
<td>1.14±0.15(^g)</td>
<td>32.00±2.75(^i)</td>
<td>5.85±0.91(^h)</td>
<td>15±2(^l)</td>
</tr>
<tr>
<td><strong>C57L mice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1%Ch alone</td>
<td>41.20±0.84</td>
<td>4.49±0.50(^e)</td>
<td>20.88±1.26(^c)</td>
<td>15.84±1.54(^e)</td>
<td>38±3(^e)</td>
</tr>
<tr>
<td>0.5%S+1%Ch</td>
<td>39.60±1.14</td>
<td>4.35±0.33(^c)</td>
<td>20.44±1.24(^c)</td>
<td>14.81±0.92(^e)</td>
<td>37±3(^e)</td>
</tr>
<tr>
<td>1%S+1%Ch</td>
<td>42.20±1.92</td>
<td>3.80±0.36(^d)</td>
<td>25.51±2.84(^e)</td>
<td>12.89±1.41</td>
<td>31±4(^f)</td>
</tr>
<tr>
<td>2%S+1%Ch</td>
<td>39.20±2.68</td>
<td>2.13±0.16(^c, l)</td>
<td>28.96±2.15(^k)</td>
<td>8.11±1.66(^f,m)</td>
<td>21±4(^f,n)</td>
</tr>
</tbody>
</table>

Abbreviations: S, sitostanol; Ch, cholesterol.

\(^a\)Absorbed cholesterol was determined by subtracting the daily fecal neutral steroid output from the daily cholesterol intake and the daily biliary cholesterol output as measured by the HPLC methods (30).

\(^b\)The percent cholesterol absorption was determined by the cholesterol balance analysis according to published methods (30).

\(^c\)P<0.0001 compared with AKR mice fed the same amounts of cholesterol and sitostanol.

\(^d\)P<0.001 compared with AKR mice fed the same amounts of cholesterol and sitostanol.

\(^e\)P<0.01 compared with AKR mice fed the same amounts of cholesterol and sitostanol.

\(^f\)P<0.05 compared with AKR mice fed the same amounts of cholesterol and sitostanol.

\(^g\)P<0.0001 compared with 1% cholesterol feeding in AKR mice.

\(^h\)P<0.001 compared with 1% cholesterol feeding in AKR mice.

\(^i\)P<0.01 compared with 1% cholesterol feeding in AKR mice.

\(^j\)P<0.05 compared with 1% cholesterol feeding in AKR mice.

\(^k\)P<0.0001 compared with 1% cholesterol feeding in C57L mice.

\(^l\)P<0.001 compared with 1% cholesterol feeding in C57L mice.

\(^m\)P<0.01 compared with 1% cholesterol feeding in C57L mice.

\(^n\)P<0.05 compared with 1% cholesterol feeding in C57L mice.
Fig. 1

A

Cumulative Radioactivities in Lymph

Infusion Time (Hours)

C57L

AKR

B

Cumulative Radioactivities in Lymph

Infusion Time (Hours)

C57L

AKR

Cholesterol

Sitostanol
Fig. 3

**A**

**Uptake Rates (x10³ dpm/g tissue/45 min)**

**Sitostanol**

- Duodenum
  - P < 0.05

- Jejunum
  - P < 0.05
  - P < 0.01

- Ileum
  - P < 0.01

**Cholesterol**

- AKR
  - P < 0.01

- C57L
  - P < 0.001
  - P < 0.01
**A**

- **Relative mRNA Levels**
- **Abcg5, Abcg8**

**Dietary Cholesterol Content**

- AKR
- C57L

**Dietary Sitostanol Content**

- AKR
- C57L

Fig. 4
Fig. 5
LUMEN ENTEROCYTE LYMPH

NPC1L1

ACAT2

MTP

APO-B48

Chylomicrons

ABCG5/G8

LXRα

Fig. 7