Cholesterol absorption is mainly regulated by the jejunal and ileal ATP-binding cassette sterol efflux transporters Abcg5 and Abcg8 in mice[1]

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Abstract **In the present study, we investigated whether intestinal sterol efflux transporters Abcg5 and Abcg8 play a major role in determining variations in cholesterol (Ch) absorption efficiency, and we compared the physiological functions of the duodenal, jejunal, and ileal Abcg5 and Abcg8 on the absorption of Ch and sitostanol in inbred mice challenged with various amounts of Ch, sitostanol, hydrophilic, or hydrophobic bile acids. We found that Abcg5 and Abcg8 in the jejunum and ileum, but not in the duodenum, were main factors in determining, in part, variations in Ch 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 Ch absorption when compared with sitostanol absorption. These results, combined with different sterol uptake rates, suggest that the absorption efficiency of Ch and sitostanol is determined by the net results between influx and efflux of intraluminal Ch and sitostanol molecules crossing the apical membrane of the enterocyte. Hydrophilic and hydrophobic bile acids influenced Ch absorption through mediating Ch solubilization and its physical-chemical state within the small intestinal lumen. We conclude that Ch absorption is mainly regulated by the jejunal and ileal Abcg5 and Abcg8 in mice.**—Duan, L-P., H. H. Wang, and D. Q-H. Wang. **Cholesterol absorption is mainly regulated by the jejunal and ileal ATP-binding cassette sterol efflux transporters Abcg5 and Abcg8 in mice. *J. Lipid Res.* 2004.** 45: **1312–1323.**

Supplementary key words bile salt • chylomicron • genetics • intestinal lipid uptake • lymph • micelle • nutrition • phospholipid

The small intestine is a unique organ that provides dietary and reabsorbed biliary cholesterol (Ch) to the body and plays a critical role in the regulation of whole-body Ch balance (1, 2). Because elevated plasma Ch concentrations are important risk factors for cardiovascular diseases, intensive studies have been carried out to search for physical-chemical, biochemical, and genetic determinants of intestinal Ch 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, *ABCG5* and *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 Ch 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 *Abcg5* and *Abcg8* among 20 strains of inbred mice have been identified (10), none of these mice showed any detectable levels of plasma sitosterol under chow diet (containing ~0.01% sitosterol) conditions. Thus, whether the polymorphisms at the *Abcg5* and *Abcg8* genes and their expression levels in the intestine are an important determinant of intestinal Ch absorption efficiency in healthy inbred mice remains to be investigated. Furthermore, the autosomal recessive disorder sitosterolemia (11–13) is mainly characterized by hyperabsorption of Ch and sitosterol, and reduced secretion of these sterols into bile. In patients with sitosterolemia (14–17), the intestinal absorp-

Manuscript received 27 January 2004 and in revised form 29 March 2004.

Published, JLR Papers in Press, April 21, 2004.
DOI 10.1194/jlr.M400030-JLR200

Abbreviations: ABC, ATP-binding cassette; CA, cholic acid; Ch, cholesterol; DHCA, dehydrocholic acid; LXR, liver X receptor; NPC1L1, Niemann-Pick C1 Like 1 (protein); RXR, retinoid X receptor; T0901317, *N*-(2,2,2-trifluoro-ethyl)-*N*-[4-(2,2,2-trifluoro-1-hydroxy-1-trifluoromethyl-ethyl)phenyl]benzenesulfonamide.

[1] This paper was presented in part at the Annual Meeting of the American Gastroenterological Association, San Francisco, CA, in 2002 and published as an abstract (*Gastroenterology* 2002; 122: A403).

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tion of Ch 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 Ch and sitosterol absorption under normal physiological conditions.

Both LXRα and LXRβ are members of the nuclear receptor superfamily and are involved in regulation of Ch 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 Ch 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 chicken with spontaneously occurring ABCA1 dysfunction (22, 23), revealed no impairment in percent Ch absorption, fecal neutral steroid excretion, or biliary Ch 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 chickens (22, 23). Accordingly, it is most likely that other intestinal sterol transporters may mediate an LXR agonist-associated increase in fecal excretion of Ch and reduction in Ch absorption.

It has been reported that there are significant variations in Ch absorption efficiency, and genetic factors at enterocyte level may play a major role in determining intestinal Ch absorption efficiency in inbred mice (26–28). Even though it has been found that some dietary, biliary, enterocyte, and luminal factors could influence Ch 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 Ch absorption efficiency. In the present study, we *i)* studied whether the intestinal sterol efflux transporters Abcg5 and Abcg8 play a major role in determining variations in Ch absorption efficiency in inbred mice; *ii)* compared the physiological functions of the duodenal, jejunal, and ileal Abcg5 and Abcg8 on the absorption of Ch and sitostanol; and *iii)* investigated whether both transporters influence intestinal Ch absorption in mice challenged with various amounts of high dietary Ch with or without sitostanol, as well as hydrophilic or hydrophobic bile acids.

MATERIALS AND METHODS

Chemicals

Radioisotope [4-^{14}C]Ch was purchased from NEN Life Science Products (Boston, MA), and [5,6-^{3}H]sitostanol was from American Radiolabeled Chemicals (St. Louis, MO). Ch, sitostanol, chenodeoxycholic acid, cholic acid (CA), 22(R)-hydroxycholesterol, and 22(S)-hydroxycholesterol were purchased from Sigma (St. Louis, MO). The synthetic LXR agonist *N*-(2,2,2-trifluoro-ethyl)-*N*-[4-(2,2,2-trifluoro-1-hydroxy-1-trifluoromethyl-ethyl) phenyl]benzenesulfonamide (T0901317) was purchased from Cayman Chemical (Ann Arbor, MI). Ursodeoxycholic and deoxycholic acids were purchased from FalkGmbH (Freiburg, Germany). Dehydrocholic, hyocholic, hyodeoxycholic, and 3α,7β,12α-trihydroxy-5β-cholan-24-oic (ursocholic) 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, AKR/J (AKR), DBA/2J (DBA), C57BL/6J (C57BL), C57L/J (C57L), and SWR/J (SWR) mice (6–8 weeks 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 Ch. 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 Ch 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 Ch 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 [^{14}C]Ch and 5.0 μCi of [^{3}H]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 Ch 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 Ch absorption by fecal dual-isotope ratio method

Nonfasted and nonanesthetized mice of inbred A/J, AKR, DBA, C57BL, C57L, and SWR strains (n = 5 per group) were given 1 μCi of [^{14}C]Ch and 2 μCi of [^{3}H]sitostanol in 150 μl of medium-chain triglyceride intragastrically by gavage. 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 the 4-day pooled fecal samples were saponified, extracted, and counted. The ratio of the two radiolabels in the fecal extracts and the dosing mixture was used for calculation of percent Ch absorption (30).

Ch balance analysis

Because high dietary Ch exerts the effect of the radioisotope dilution on the specific activity of Ch in the upper small intestine, we used Ch balance analysis (30) to determine Ch 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 weeks. 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 a continuous 7 days for the balance study. AKR and C57L mice (n = 5 per group) were fed for 7 days with chow, or chow supplemented with 0.5% (by weight), 1%, or 2% Ch. Additional groups of AKR and C57L mice (n = 5 per group) were fed for 7 days with chow, or chow supplemented with 0%, 0.5%, 1%, or 2% sitostanol plus 1% Ch. Following this procedure, animals were anesthetized with pentobarbital. After cholecystectomy, the common bile duct was cannulated with a PE-10 cathether and hepatic bile was collected for the first hour of biliary secretion. Bile Ch and Ch content in the diet were measured by HPLC. Fecal neutral steroids were saponified and extracted, as well as being measured by HPLC (30). Percent Ch 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 a 24 h recovery from the surgery and another 12 h of fasting (with water), exactly 2 μCi of [^{14}C]Ch and 2 μCi of [^{3}H]sitostanol in 100 μl of medium-chain triglyceride were injected into the nonanesthetized 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, and 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 Ch absorption, AKR and C57L mice (n = 4 per group) were *i)* fed 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)* fed various amounts (0.02%, 0.5%, 1%, and 2%) of Ch or sitostanol; as well as *iii)* administrated intragastrically by gavage daily with a vehicle control in propylene glycol/Tween 80 (4:1, v/v) 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 four 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 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 as the invariant control (Part No. 4308313, Applied Biosystems).

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 test. 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 Ch and sitostanol

To examine whether there are differences in intestinal absorption between Ch and sitostanol, we studied the absorption and lymphatic transport of Ch 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

TABLE 1. Primer and probe sequences used in mRNA quantification by real-time PCR

Gene	Accession Number	Forward	Reverse	Probe
Abcg5	AF312713	5′-CCTGCAGAGCGACGTTTTTC-3′	5′-GCATCGCTGTGTATCGCAAC-3′	5′-AGCAGCCTCACTGTGCGCGAGA-3′
Abcg8	AF324495	5′-TGGATAGTGCCTGCATGGATC-3′	5′-AATTGAATCTGCATCAGCCCC-3′	5′-CAAGCTGTCGTTCCTCCGGTGGTG-3′
Lxrα	NM_013839	5′-CGACAGAGCTTCGTCCACAA-3′	5′-ACAGCTCGTTCCCCAGCAT-3′	5′-CGGAAAAAGGGCCCAGCCCC-3′
Lxrβ	NM_009473	5′-TTGCGACTCCAGGACAAGAA-3′	5′-CTACTCGTGCACATCCCAGATC-3′	5′-CTGCCGCCCTTGCTGTCCG-3′

LXR, liver X receptor.

some effects on Ch 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 Fig. 1B, cumulative radioactivity in lymph at 12 h after instillation of [^{14}C]Ch is 36 ± 4% in the C57L strain, which is significantly greater ($P < 0.001$) than those in the 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 greater ($P < 0.05$) compared with those in AKR (2.1 ± 0.5%). In comparison, absorption and lymphatic transport of Ch was significantly greater than that of sitostanol.

Ch absorption efficiency and gene expression of intestinal sterol efflux transporters

Figure 2A shows Ch absorption efficiency determined by the fecal dual-isotope ratio method in six strains of inbred male mice on chow. In agreement with the 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 Ch absorption efficiency: AKR (27 ± 3%) and A/J (28 ± 3%) strains showed the lowest Ch 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 Ch absorption. Figure 2B and C exhibits expression levels of *Abcg5* and *Abcg8* in the jejunum and ileum that are the highest in AKR and A/J mice. In contrast, expression levels of the jejunal and ileal *Abcg5*

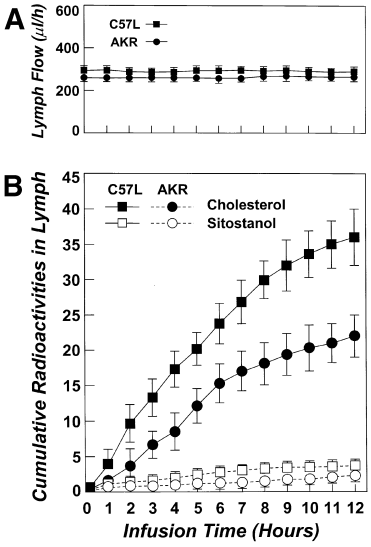

Fig. 1. Comparison of the absorption and lymphatic transport of cholesterol (Ch) and sitostanol between AKR and C57L mice (n = 5 per group) with chronic biliary fistula but in the setting of infusion of 0.5% (wt/wt) taurocholate and 0.2% egg yolk lecithin. A: The lymph flow rates (260–290 μl/h) are constant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

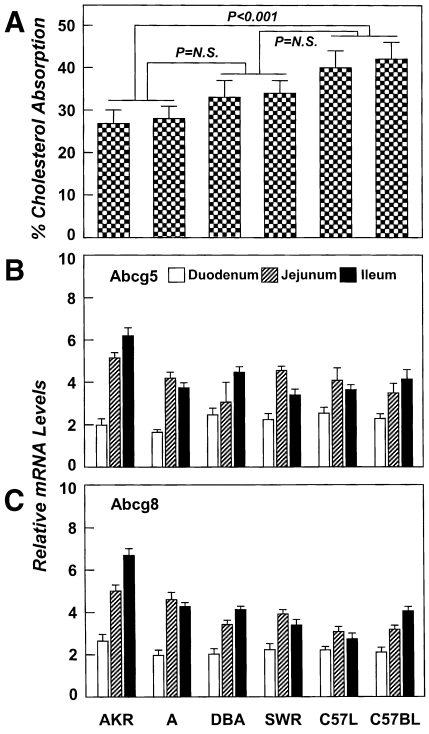

Fig. 2. Relationship between Ch absorption efficiency and expression levels of *Abcg5* and *Abcg8* in the duodenum, jejunum, and ileum of six strains of male inbred A/J, AKR/J (AKR), DBA/2J (DBA), C57BL/6J (C57BL), C57L/J (C57L), and SWR/J (SWR) mice on the chow diet. A: The Ch absorption efficiency for each strain (n = 5 per group) was determined by the fecal dual-isotope ratio method (30). Consistent with the previous study (26), as measured by the plasma dual-isotope ratio method, Ch absorption efficiency varies from low to high among these inbred mice. Gene expression of *Abcg5* (B) and *Abcg8* (C) 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/J 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 Ch 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 glyceraldehyde-3-phosphate dehydrogenase 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.

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 Ch absorption efficiency. Furthermore, our results demonstrate that there is a remarkably negative correlation between percent Ch 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 the uptake of Ch and sitostanol by the enterocyte, we compared their uptake rates in AKR and C57L mice. Our results (**Fig. 3**) show that at 45 min after dosing, the radiolabeled Ch and sitostanol uptake by the enterocytes of the C57L mice was 1.5-fold higher ($P < 0.05$) compared with the AKR mice. The small intestine absorbed significantly greater amounts of [^{14}C]Ch ($P < 0.001$) compared with [^{3}H]sitostanol. Together, these results suggest that there may be a selective uptake mechanism between Ch and sitostanol in mice.

Responses of intestinal sterol efflux transporters to high dietary Ch and sitostanol

Table 2 summarizes the data for daily Ch intake, daily biliary Ch output, daily fecal total neutral steroid excretion, daily absorbed Ch, and percent Ch 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 Ch was fed. However, on the chow diet (0.02% Ch), daily biliary Ch outputs in C57L mice (2.11 ± 0.08 mg/day) were significantly higher ($P < 0.0001$) compared with AKR mice (1.14 ± 0.08 mg/day) because C57L mice are a gallstone-susceptible strain (32). Because of higher biliary Ch secretion, daily fecal total neutral steroid excretion was significantly greater in C57L mice ($P < 0.0001$; 2.62 ± 0.08 mg/day) than in AKR mice (1.75 ± 0.11 mg/day). Nevertheless, an input-output analysis found that the absorbed mass of Ch daily in C57L mice (0.31 ± 0.02 mg/day) was significantly higher ($P < 0.001$) compared with AKR mice (0.21 ± 0.03 mg/day). The calculated percent Ch absorption in C57L mice was 38 ± 3%, being significantly greater ($P < 0.01$) than that in AKR mice (26 ± 4%). Under the higher dietary Ch feeding conditions (≥0.5%), C57L mice produced significantly higher biliary Ch outputs ($P < 0.01$) than AKR mice. Furthermore, Ch mass absorbed from the small intestine was significantly greater in C57L mice ($P < 0.01$) than in AKR mice, because the latter excreted more fecal neutral steroids compared with the former. Of note, compared with the basal (0.02% Ch) diet, Ch absorption efficiency measured by the mass balance method remains unchanged in mice fed various amounts of high dietary Ch. Taken together, these results suggest that C57L mice absorb more Ch than AKR mice, whatever chow or high dietary Ch is fed.

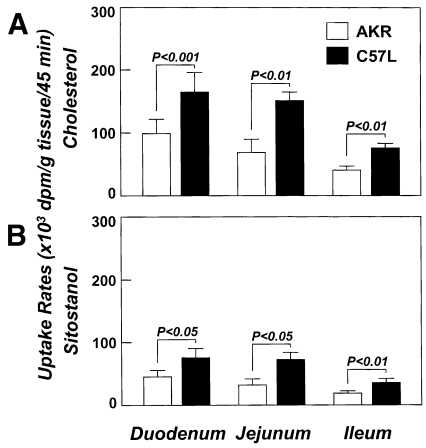

Fig. 3. Sterol uptake by the small intestine. To observe whether there are differences in intestinal uptake between Ch (A) and sitostanol (B), we compared their uptake rates in AKR and C57L mice. At 45 min after dosing, the radiolabeled Ch and sitostanol uptake by the enterocytes of C57L mice are 1.5-fold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

Because plant sterols have been used as Ch-lowering agents (33–35), we examined their effects on intestinal Ch absorption. Our results (**Table 3**) show that AKR and C57L mice ate identical amounts of Ch, although various amounts of sitostanol were fed. Sitostanol (≤1%) feeding did not cause significant changes in intestinal Ch absorption (percentage and total mass), biliary Ch 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 Ch outputs ($P < 0.001$), absorbed Ch mass ($P < 0.01$), and percent Ch 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 Ch and sitostanol, we studied expression levels of *Abcg5* and *Abcg8* in AKR and C57L mice fed various amounts of Ch or sitostanol. Compared with the basal (0.02% Ch) diet, feeding 0.5% or higher dietary Ch significantly increased expression levels of *Abcg5* and *Abcg8* in the jejunum and ileum ($P < 0.01$), but not in the duodenum (**Fig. 4A**), which is associated with increased fecal neutral steroid excretion (Table 2). Our results suggest that in response to high dietary Ch (≥0.5%), the jejunal and ileal Abcg5 and Abcg8 may efflux more Ch 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% (Fig. 4B). This suggests that the intestinal Abcg5 and Abcg8 may be more sensitive in regulating Ch 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 Ch absorption, we

TABLE 2. Ch balance data in Ch-fed AKR and C57L mice during metabolic steady state conditions

Diet	Ch Intake	Biliary Ch	Steroid Excretion	Absorbed Ch[a]	Ch Absorption[b]
			mg/day		%
AKR mice					
0.02%	0.82 ± 0.01	1.14 ± 0.08	1.75 ± 0.11	0.21 ± 0.03	26 ± 4
0.5%	21.00 ± 0.50[c]	1.75 ± 0.17[d]	13.71 ± 0.90[c]	5.54 ± 0.49[c]	26 ± 3
1.0%	41.40 ± 1.14[c]	2.32 ± 0.22[c]	28.88 ± 0.77[c]	10.19 ± 0.87[c]	25 ± 3
2.0%	81.60 ± 2.97[c]	3.41 ± 0.26[c]	56.69 ± 3.92[c]	21.50 ± 1.67[c]	26 ± 3
C57L mice					
0.02%	0.82 ± 0.02	2.11 ± 0.08[e]	2.62 ± 0.08[e]	0.31 ± 0.02[f]	38 ± 3[g]
0.5%	21.10 ± 0.96[h]	3.07 ± 0.13[f,h]	10.20 ± 1.08[i,h]	7.83 ± 0.48[g,h]	37 ± 3[g]
1.0%	41.20 ± 0.84[h]	4.49 ± 0.50[g,j]	20.88 ± 1.26[e,h]	15.84 ± 1.54[g,h]	38 ± 3[g]
2.0%	82.80 ± 3.03[h]	6.44 ± 0.73[g,j]	46.28 ± 4.96[i,h]	30.08 ± 1.97[g,h]	36 ± 3[g]

Ch, cholesterol.

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

[b] The percent Ch absorption was determined by the Ch balance analysis according to published methods (30).

[c] $P < 0.0001$ compared with 0.02% Ch feeding in AKR mice.

[d] $P < 0.01$ compared with 0.02% Ch feeding in AKR mice.

[e] $P < 0.0001$ compared with AKR mice fed the same amount of Ch.

[f] $P < 0.001$ compared with AKR mice fed the same amount of Ch.

[g] $P < 0.01$ compared with AKR mice fed the same amount of Ch.

[h] $P < 0.0001$ compared with 0.02% Ch feeding in C57L mice.

[i] $P < 0.05$ compared with AKR mice fed the same amount of Ch.

[j] $P < 0.001$ compared with 0.02% Ch feeding in C57L mice.

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 increases expression levels of *Lxrα* (Fig. 5A), *Abcg5* (Fig. 5B), and *Abcg8* (Fig. 5C) in the jejunum and ileum ($P < 0.01$) and, to a lesser extent, in the duodenum. The relative mRNA levels for *Lxrβ* were very low in the mouse small intestine (data not shown). Furthermore, the sterol balance studies revealed that fecal total neutral steroid excretion (Fig. 5D) was significantly increased ($P < 0.05$), and percent Ch absorption (Fig. 5E) was significantly reduced ($P < 0.05$) by the synthetic LXR agonist feeding. This strongly suggests that the intestinal transporters Abcg5 and Abcg8 may serve to efflux Ch from the enterocyte into the intestinal lumen for elimina-

TABLE 3. Effects of sitostanol on intestinal Ch absorption in AKR and C57L mice in the metabolic steady state

Diet	Ch Intake	Biliary Ch	Steroid Excretion	Absorbed Ch[a]	Ch Absorption[b]
			mg/day		%
AKR mice					
1.0% Ch alone	41.40 ± 1.14	2.32 ± 0.22	28.88 ± 0.77	10.19 ± 0.87	25 ± 3
0.5% S + 1.0% Ch	40.20 ± 1.92	2.25 ± 0.23	27.32 ± 1.52	10.63 ± 1.33	26 ± 3
1.0% S + 1.0% Ch	42.00 ± 1.00	1.90 ± 0.35	31.11 ± 1.24	9.00 ± 1.53	21 ± 3
2.0% S + 1.0% Ch	39.00 ± 3.24	1.14 ± 0.15[c]	32.00 ± 2.75[d]	5.85 ± 0.91[e]	15 ± 2[f]
C57L mice					
1.0% Ch alone	41.20 ± 0.84	4.49 ± 0.50[g]	20.88 ± 1.26[h]	15.84 ± 1.54[g]	38 ± 3[g]
0.5% S + 1.0% Ch	39.60 ± 1.14	4.35 ± 0.33[h]	20.44 ± 1.24[h]	14.81 ± 0.92[g]	37 ± 3[g]
1.0% S + 1.0% Ch	42.20 ± 1.92	3.80 ± 0.36[i]	25.51 ± 2.84[g]	12.89 ± 1.41	31 ± 4[j]
2.0% S + 1.0% Ch	39.20 ± 2.68	2.13 ± 0.16[h,k]	28.96 ± 2.15[l]	8.11 ± 1.66[j,m]	21 ± 4[j,n]

S, sitostanol.

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

[b] The percent Ch absorption was determined by the Ch balance analysis according to published methods (30).

[c] $P < 0.0001$ compared with 1% Ch feeding in AKR mice.

[d] $P < 0.05$ compared with 1% Ch feeding in AKR mice.

[e] $P < 0.001$ compared with 1% Ch feeding in AKR mice.

[f] $P < 0.01$ compared with 1% Ch feeding in AKR mice.

[g] $P < 0.01$ compared with AKR mice fed the same amounts of Ch and S.

[h] $P < 0.0001$ compared with AKR mice fed the same amounts of Ch and S.

[i] $P < 0.001$ compared with AKR mice fed the same amounts of Ch and S.

[j] $P < 0.05$ compared with AKR mice fed the same amounts of Ch and S.

[k] $P < 0.001$ compared with 1% Ch feeding in C57L mice.

[l] $P < 0.0001$ compared with 1% Ch feeding in C57L mice.

[m] $P < 0.01$ compared with 1% Ch feeding in C57L mice.

[n] $P < 0.05$ compared with 1% Ch feeding in C57L mice.

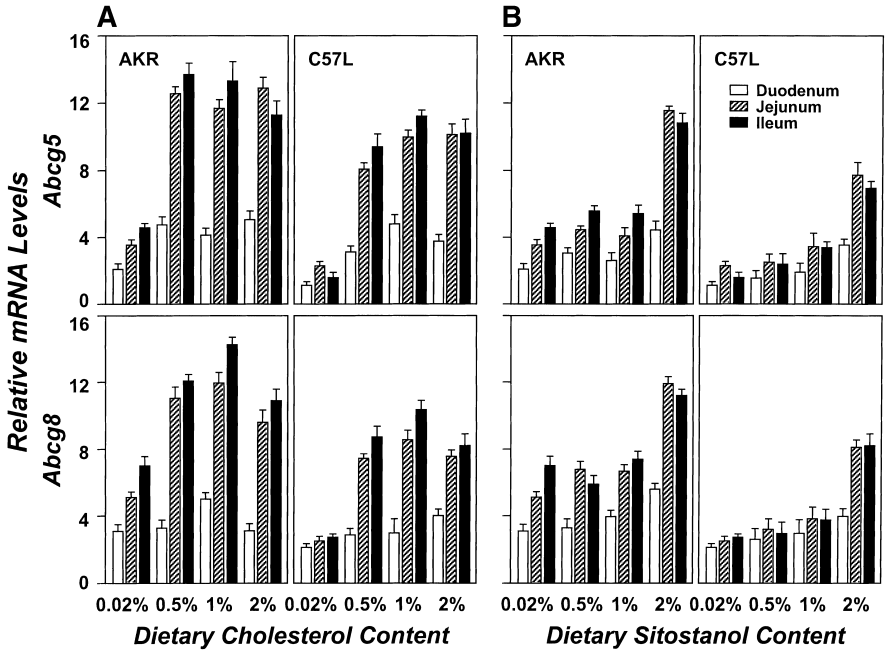

Fig. 4. To examine responses of intestinal sterol efflux transporters to high dietary Ch and sitostanol, we studied expression levels of *Abcg5* and *Abcg8* in AKR and C57L mice fed various amounts of Ch and sitostanol. A: Compared with the basal diet (0.02% Ch), feeding 0.5% or higher dietary Ch significantly increases expression levels of *Abcg5* and *Abcg8* in the jejunum and ileum ($P < 0.01$), 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 Ch 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 Ch 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.

tion. Several in vitro experiments (36–39) have shown 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* in the small intestines of both AKR and C57L mice, fecal total neutral steroid excretion, or percent Ch absorption (data not shown).

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 by CA feeding ($P < 0.001$), and to a lesser extent by deoxycholic and dehydrocholic acid (DHCA) 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 Ch absorption efficiency in inbred mice; *ii)* the jejunal and ileal Abcg5 and Abcg8 play a main regulatory role in response to high dietary Ch and sitostanol; *iii)* the absorption efficiency of Ch and sitostanol in the small intestine is predominantly determined by the net results of a complex series between influx and efflux of intraluminal Ch and sitostanol molecules crossing the apical membrane of the enterocyte; *iv)* the intestinal Abcg5 and Abcg8 are involved in LXR agonist-associated regulation of Ch absorption; and *v)* hydrophilic and hydrophobic bile acids influence Ch absorption by mediating Ch solubilization and its physical-chemical state within the small intestinal lumen.

Sitostanol is a saturated form of sitosterol, and both are structurally similar to Ch but differ in their side chain configuration (40–42). 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 Ch and sitostanol absorption rates than the lower-absorbing AKR mice. Our study also

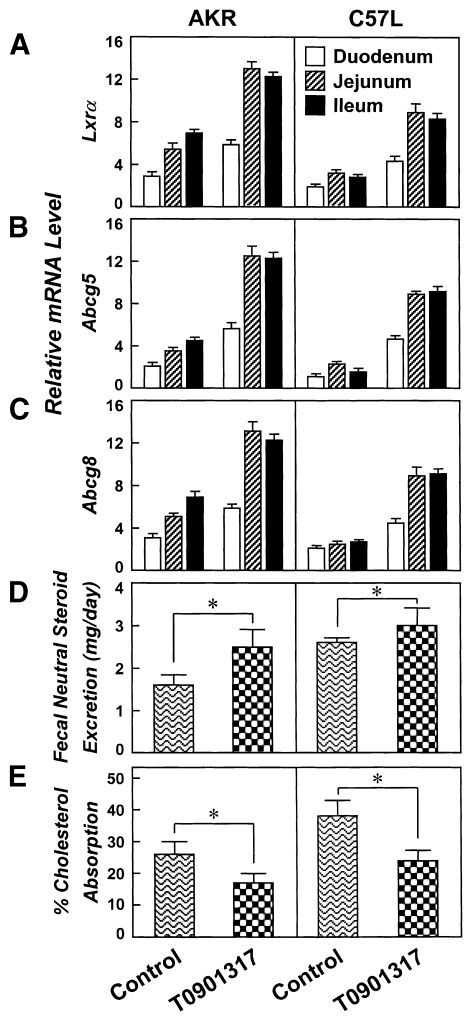

Fig. 5. Regulation of intestinal Abcg5 and Abcg8 by nuclear receptor lipid X receptor (LXR). Compared with the vehicle control, feeding the synthetic LXR agonist *N*-(2,2,2-trifluoro-ethyl)-*N*-[4-(2,2,2-trifluoro-1-hydroxy-1-trifluoromethyl-ethyl)phenyl]benzenesulfonamide (T0901317) at a dose of 10 mg/day/kg significantly increases expression levels of *Lxrα* (A), *Abcg5* (B), and *Abcg8* (C) in the jejunum and ileum ($P < 0.01$) 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 fecal neutral steroid excretion is significantly increased (D), but percent Ch absorption is significantly reduced by the synthetic LXR agonist (E), suggesting that Abcg5 and Abcg8 may serve to efflux Ch from the enterocyte into the intestinal lumen and reduce its fractional absorption. * $P <$ 0.05.

produced direct evidence demonstrating that at 12 h after instilling radiolabeled Ch and sitostanol, cumulative radioactivity of sitostanol (2–4%) in the lymph was significantly lower compared with that of Ch (22–36%) in both mouse strains. Furthermore, compared with the basal diet (0.02% Ch), higher dietary Ch (≥0.5%) 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 Ch, the jejunal and ileal sterol transporters Abcg5 and Abcg8 may efflux more Ch 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 Ch and sitostanol absorption, and the intestinal Abcg5 and Abcg8 may be more sensitive in the regulation of Ch absorption compared with sitostanol absorption. Our findings suggest that intestinal sterol efflux transporters Abcg5 and Abcg8 may provide a barrier to Ch and sitostanol accumulation by promoting partial efflux of Ch and nearly complete efflux of sitostanol from the enterocyte into the intestinal lumen for elimination.

The differences in Ch absorption efficiency among strains or individuals have been observed in animals (26–28, 43–49) and humans (50–53). 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 Ch 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/J strains with the lowest Ch 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 Ch 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 Ch absorption efficiency. Our results demonstrate that expression levels of *Abcg5* and *Abcg8* in the jejunum and ileum are reduced with increasing Ch absorption efficiency, suggesting a strikingly reverse relationship between expression levels of the jejunal and ileal *Abcg5* and *Abcg8* and percent Ch 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 Ch absorption efficiency among inbred mice. Moreover, expression levels of the duodenal *Abcg5* and *Abcg8* were essentially similar among these inbred mice with diverse Ch absorption efficiency and were not influenced in mice challenged with various amounts of Ch, sitostanol, hydrophilic bile acid, or hydrophobic bile acid. Especially, the sterol uptake studies showed that the duodenum and jejunum accumulated the most radioactivity following the administration of radiolabeled Ch and sitostanol, which is 1.5-fold higher in the higher-absorbing C57L mice than in the lower-absorbing AKR mice.

More recently, Altmann et al. (54) found that disruption of the *NPC1L1* gene encoding the Niemann-Pick C1 Like 1 protein (NPC1L1) induces a significant decrease in intestinal Ch absorption in mice. They (54) also observed that ezetimibe lowered Ch absorption by ~70% in the

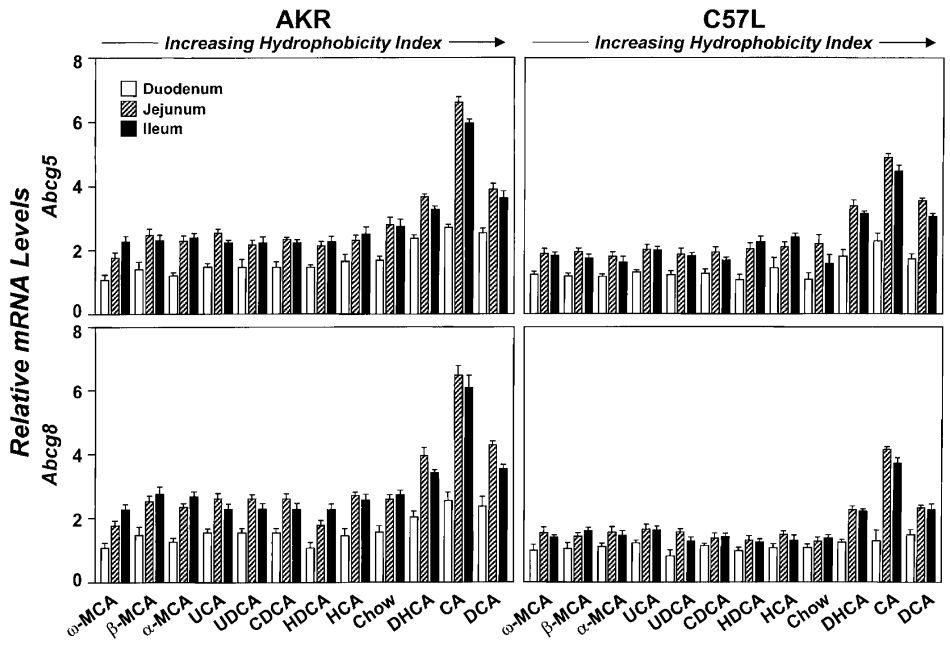

Fig. 6. The relative mRNA levels for *Abcg5* and *Abcg8* in the duodenum, jejunum, and ileum of AKR and C57L mice fed chow, or chow supplemented with 0.5% (by weight) bile acids. Top arrows show increasing hydrophobicity indices of the biliary bile salt pools in mice fed the corresponding bile acids (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 acid 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 by cholic acid (CA) feeding ($P < 0.001$) and somewhat by deoxycholic and dehydrocholic acids (DHCAs). 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 Ch and probably biliary (and dietary) oxysterols (65) induced by CA. Again, because real-time PCR assays were carried out in triplicate from the same sample of pooled intestine tissues (n = 4 per group), the error bars represent the technique variance rather than the biological variance. CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; HCA, hyocholic acid; HDCA, hyodeoxycholic acid; MCA, muricholic acid; UCA, ursocholic acid; UDCA, ursodeoxycholic acid.

wild-type mice, and this level of Ch 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 (54). Therefore, our results support the concept that the main sites of Ch absorption are the duodenum and proximal jejunum (55–58). It is important to note that in the chow-fed mouse, the expression levels of *Abcg5* and *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 *Abcg5* and *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 four 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 the entry of Ch into intestinal absorption cells (1, 2). To investigate whether there are differences in intestinal uptake between Ch and sitostanol, we compared their uptake rates in AKR and C57L mice. Our results show that at 45 min after dosing, radiolabeled Ch and sitostanol uptake by the enterocytes of C57L mice was 1.5-fold higher compared with AKR mice. Furthermore, the fact that Ch is absorbed more than the structurally similar sitostanol in both strains of mice suggests that there may be a selective uptake mechanism between Ch and sitostanol, and a Ch transporter(s) (54, 59–63) may be localized at the apical membrane that facilitates selective Ch 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 resecreted into the intestinal lumen by the

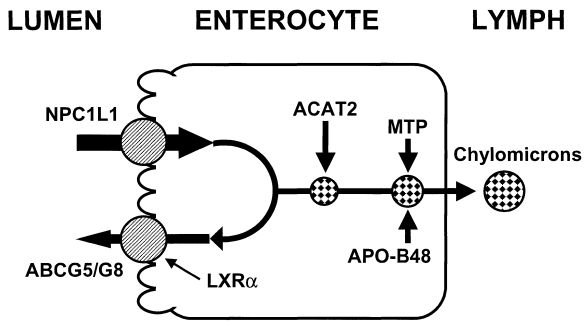

Fig. 7. Intestinal Ch absorption is a multistep process that is regulated by multiple genes. The Niemann-Pick C1 Like 1 protein (NPC1L1), a newly identified sterol influx transporter, is located at the apical membrane of the enterocyte (54), which may actively facilitate the uptake of Ch. ABCG5 and ABCG8 promote partial efflux of Ch 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 Ch that reaches the lymph from the intestinal lumen. Also, several proteins involved in other steps in the absorption process, e.g., LXRα, acyl-CoA:Ch acyltransferase isoform 2 (ACAT2), apolipoprotein B48 (APO-B48), and microsomal triglyceride transfer protein (MTP) in the enterocyte, may exert major influences on Ch absorption. Furthermore, the absorption efficiency of Ch 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 Ch and sitostanol molecules crossing the apical brush board membrane of the enterocyte.

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 more 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 (54) plays a critical role in determining Ch absorption efficiency and in regulating Ch and sitostanol absorption in healthy inbred mice.

To explore the role of "oxysterol-LXRα-ABCG5/G8 pathway" in the regulation of Ch 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 the 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, 64). In contrast, feeding the synthetic LXR ligand T0901317 upregulated expression levels of the intestinal *Lxrα*, *Abcg5*, and *Abcg8*, increased fecal neutral steroid excretion, and reduced fractional absorption of Ch. Clearly, additional in vivo experiments are required to evaluate the role of the naturally occurring oxysterols in the regulation of Ch 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 CA feeding and slightly by deoxycholic and DHCAs. In an experiment with a small number of mice (L-P. Duan, H. H. Wang, and D. Q.-H. Wang, unpublished observations), we found that CA-fed C57L mice display markedly higher Ch absorption efficiency compared with AKR mice treated with deoxycholic or DHCAs, although the relative mRNA levels for *Abcg5* and *Abcg8* in the jejunum and ileum of AKR mice fed deoxycholic and DHCAs are similar to those in C57L mice fed CA. 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 Ch that is augmented mainly by fed CA. Another possible explanation is that CA may promote intestinal absorption of biliary (and dietary) oxysterols (65) 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 CA 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 Ch absorption mainly by mediating intraluminal micellar Ch solubilization and its physical-chemical state (31).

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

D.Q-H.W. is a recipient of a New Scholar Award from the Ellison Medical Foundation (1999–2003). This work was supported in part by the Ellison Medical Foundation, as well as Research Grant DK-54012 from the National Institutes of Health (US Public Health Service).

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