Genetic factors at the enterocyte level account for variations in intestinal cholesterol absorption efficiency among inbred strains of mice[1]

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Abstract **Interindividual and interstrain variations in cholesterol absorption efficiency occur in humans and animals. We investigated physiological biliary and small intestinal factors that might determine variations in cholesterol absorption efficiency among inbred mouse strains. We found that there were significant differences in cholesterol absorption efficiency measured by plasma, fecal, and lymphatic methods: <25% in AKR/J, C3H/J, and A/J strains; 25–30% in SJL/J, DBA/2J, BALB/cJ, SWR/J, and SM/J strains; and 31–40% in C57L/J, C57BL/6J, FVB/J, and 129/SvJ strains. In (AKR×C57L)F_1 mice, the cholesterol absorption efficiency (31 ± 6%) mimicked that of the C57L parent (37 ± 5%) and was significantly higher than in AKR mice (24 ± 4%). Although biliary bile salt compositions and small intestinal transit times were similar, C57L mice displayed significantly greater bile salt secretion rates and pool sizes than AKR mice. In examining lymphatic cholesterol transport in the setting of a chronic biliary fistula, C57L mice displayed significantly higher cholesterol absorption rates compared with AKR mice. Because biliary and intestinal transit factors were accounted for, we conclude that genetic variations at the enterocyte level determine differences in murine cholesterol absorption efficiency, with high cholesterol absorption likely to be a dominant trait. This study provides baseline information for identifying candidate genes that regulate intestinal cholesterol absorption at the cellular level.**—Wang, D. Q-H., B. Paigen, and M. C. Carey. **Genetic factors at the enterocyte level account for variations in intestinal cholesterol absorption efficiency among inbred strains of mice.** ***J. Lipid Res.*** **2001.** 42: **1820–1830.**

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Because biliary cholesterol (Ch) hypersecretion is an important prerequisite for Ch gallstone formation (1) and elevated plasma Ch is an independent risk factor for cardiovascular disease (2), considerable interest has been focused on identifying physical-chemical, biochemical, and genetic determinants of intestinal Ch absorption (3–5). Furthermore, understanding the sequential steps in intestinal Ch absorption may lead to novel approaches to the treatment of these diseases that affect millions in Westernized societies. "Absorption of Ch" is most accurately defined as the transfer of intralumenal Ch into intestinal or thoracic duct lymph (6). "Uptake of Ch" refers to entry of Ch into intestinal absorptive cells (6). According to these definitions, Ch absorption is a multistep process (3–6): *i*) hydrolysis of cholesteryl esters in the lumen, *ii*) solubilization of the free sterol in mixed micelles, *iii*) diffusive and possibly facilitated transport of Ch into enterocytes, *iv*) partial efflux of Ch from the enterocyte back into the lumen, *v*) its intracellular re-esterification, and *vi*) assembly into chylomicrons followed by their secretion into intestinal lymph. Any factor that changes the transportation of Ch from the intestinal lumen to the lymph may influence intestinal Ch absorption efficiency. **Table 1** summarizes dietary factors (7–16), including administered therapeutic agents, biliary factors (17–21), cellular factors (22–42), and lumenal factors (43–45) that could influence intestinal Ch absorption. Despite these findings, 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. The differences in Ch absorption efficiency among individuals or strains have been observed in primates (46, 47), including humans (48–51), as well as in inbred strains of rabbits (52, 53), rats (54), and mice (55–58). However, the factors that determine variations in Ch absorption efficiency have not been evaluated systematically. In the

Abbreviations: ABC, ATP-binding cassette (transporter); BS, bile salt; Ch, cholesterol; *Mdr2*, multidrug resistance gene 2.

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TABLE 1. Possible factors influencing intestinal cholesterol absorption

Factors	Effect on Percent Ch Absorption and Type of Study	References
Dietary factors		
↑ Ch[a]	↓ Human and animal feeding studies	7
Fat		
↑ Monounsaturated	↓ African green monkey feeding studies	8
↑ ω-3 polyunsaturated	↓ African green monkey feeding studies	8
↑ Fish oils	↓ Rat lymphatic transport studies	9
↑ Fiber	↓ Human and animal feeding studies	10, 11
↑ Plant sterols	↓ Human and animal feeding studies	12, 13
↑ Hydrophilic bile acids	↓ Human and animal feeding studies	14, 15
↑ Sphingomyelin	↓ Animal feeding studies	16
Biliary factors		
↓ Biliary BS output	↓ *Ch 7α-hydroxylase* (−/−) mice	17
↓ Size of biliary BS pool	↓ *Ch 7α-hydroxylase* (−/−) mice	17
↓ Biliary PL output	↓ *Mdr2*(−/−) mice	18, 19
↑ Biliary Ch output	↑ Diabetic mice	20
↑ Ch content of bile	↑ Diabetic mice	20
↑ HI of biliary BS pool	↑ Diabetic mice	21
Cellular factors		
↓ ACAT2	↓ ACAT2 inhibitors	22, 23
↓ HMG-CoA reductase	↓ HMG-CoA reductase inhibitors	24–26
↓ ABCA1	↓↑*Abca1* (−/−) mice[b]	27–29
ABCG5	To be identified	30, 31
ABCG8	To be identified	30
Human 2p21	To be identified	32
Ch transporter	To be identified	33–35
SR-BI	↓ *Sr-b1* transgenic mice, and ↑ *Sr-b1* (−/−) mice	36, 37
Caveolin	To be identified	38
MTP	To be identified	39, 40
↓ ApoB-48	↓ *ApoB-48* (−/−) mice	41
ApoA-I, ApoA-IV, ApoC-III	To be identified	42
Lumenal factors		
↑ Small intestinal transit time	↑ *Cck-A receptor* (−/−) mice	43
↓ Carboxyl ester lipase	— *Carboxyl ester lipase* (−/−) mice	44
Sphingomyelinase	To be identified	45

Abbreviations: Ch, cholesterol; BS, bile salt; PL, phospholipid; *Mdr2*, multidrug resistance gene 2; HI, hydrophobicity index; ACAT2, acyl-CoA:cholesterol acyltransferase, isoform 2; HMG, 3-hydroxy-3-methyglutaryl; ABC, ATP-binding cassette (transporter); SR-BI, scavenger receptor class B type I; MTP, microsomal triglyceride transfer protein; apo, apolipoprotein; CCK, cholecystokinin.

[a] ↑, increase; ↓, decrease; —, no effect.

[b] Contradictory results were reported by three groups (27–29).

present study, we investigated by three independent methods whether differences in intestinal Ch absorption efficiency exist among inbred strains of mice, and have tested the principal biliary and intestinal factors contributing to intestinal Ch absorption. Our results showed that there were marked differences among 12 mouse strains with results that ranged from 22% to 39%. Of note is that when dietary factors were controlled by feeding chow (<0.02% Ch), the C57L mice absorbed more Ch from the intestine than AKR mice, a result due only in part to higher biliary bile salt (BS) outputs and pool sizes, but not to molecular compositions of the BS pool or intestinal transit times. As inferred from lymphatic transport of Ch in AKR mice and C57L mice with total biliary fistulae, we found that the absorption and lymphatic transport of Ch remained significantly higher in C57L mice than in AKR mice. Therefore, we conclude that genetic factors at the enterocyte level play major roles in determining variations of intestinal Ch absorption efficiency in different strains of inbred mice.

MATERIALS AND METHODS

Chemicals

Intralipid (20%, w/v) was obtained from Pharmacia (Clayton, NC), and medium-chain triglyceride oil was from Mead Johnson & Co. (Evansville, IN). Radioisotopes [1,2-^{3}H]Ch and [4-^{14}C]Ch were purchased from NEN Life Science Products (Boston, MA), and [5,6-^{3}H]sitostanol was from American Radiolabeled Chemicals (St. Louis, MO). The purities of all radiochemicals were >98% as determined by HPLC and thin-layer chromatographic analyses. For HPLC analyses of BS species and Ch, all reagents were HPLC grade and obtained from Fisher Scientific (Fair Lawn, NJ). BS standards were obtained from Sigma (St. Louis, MO) and CalBiochem-Behring (San Diego, CA), with the exception of the taurine conjugates of β- and ω-muricholates (3α,6β,7β-trihydroxy-5β-cholanoate and 3α,6α,7β-trihydroxy-5β-cholanoate), which were provided generously by Tokyo Tanabe (Tokyo, Japan) (courtesy of H. Sugata). Purity of individual BS was >98% by HPLC (59). Grade I egg yolk lecithin (Lipid Products, South Nutfield, Surrey, UK) was >99% pure by thin-layer chromatography ($CHCl_3$–CH_3OH–H_2O 65:25:4, v/v/v) (59). All other chemicals and solvents were

American Chemical Society or reagent-grade quality (Fisher Scientific, Medford, MA).

Animals and diets

Male inbred mice, 6–8 weeks old, were obtained from the Jackson Laboratory (Bar Harbor, ME). Strains studied were A/J (A), AKR/J (AKR), BALB/cJ (BALB), C57BL/6J (C57BL), C3H/J (C3H), C57L/J (C57L), DBA/2J (DBA), FVB/J (FVB), SJL/J (SJL), SM/J (SM), SWR/J (SWR), and 129/SvJ (129) mice. We crossed AKR with C57L to breed heterozygous (AKR× C57L)F_1 mice (60, 61). All animals were maintained in a temperature-controlled room (22 ± 1°C) with 12-h light cycles (6 AM–6 PM). Mice were allowed to adapt to the environment for at least 2 weeks before the Ch absorption studies, and were provided free access to water and food throughout the experiments. Mice were fed Purina (St. Louis, MO) laboratory chow (mouse diet 1401), which contains trace Ch (<0.02%) (60–62). All experiments were executed according to accepted criteria for the care and experimental use of laboratory animals and euthanasia was consistent with recommendations of the American Veterinary Medical Association. All protocols were approved by the Institutional Animal Care and Use Committees of Harvard University and The Jackson Laboratory.

Determination of Ch absorption by plasma dual isotope ratio method

For measurement of Ch absorption by the plasma dual isotope ratio method (62), all strains of mice (n = 10–35 per strain) were fed chow. Nonfasted mice were anesthetized lightly by intraperitoneal injection of pentobarbital (35 mg/kg). An incision of 0.4 cm was made on the neck, and the jugular vein was exposed. Exactly 2.5 μCi of [^{3}H]Ch in 100 μl of Intralipid was injected with a 100-μl Hamilton syringe fitted with a 30-gauge needle. The incision was closed with 3-0 silk sutures. A feeding needle with round tip (18 gauge, 50 mm in length) was then inserted into the stomach of the mouse, and each animal was given an intragastric bolus of 1 μCi of [^{14}C]Ch in 150 μl of medium-chain triglyceride oil by gavage. After dosing, mice were returned to individual cages with wire mesh bottoms, where they were free to eat chow for an additional 3 days. Animals were then anesthetized, and were bled from the heart into heparinized microtubes. Plasma was obtained by centrifugation at 10,000 *g* for 30 min at room temperature. To determine the proportions of [^{14}C]Ch and [^{3}H]Ch doses remaining in plasma at 3 days, 10 ml of EcoLite (ICN Biomedicals, Costa Mesa, CA) was added to 100-μl portions of plasma and the original dosing mixture, respectively. The vials were shaken vigorously for 10 min, and counted in a liquid scintillation spectrometer (Beckman Instruments, San Ramon, CA). The plasma ratio of the two radiolabels was used for calculating the percent Ch absorption:

$$\% \text{ Ch absorption} = \left(\frac{\text{percent of intragastric dose } [^{14}\text{C}]\text{Ch / ml plasma}}{\text{percent of intravenous dose } [^{3}\text{H}]\text{Ch / ml plasma}}\right) \times 100$$

Determination of Ch absorption by fecal dual isotope ratio method

On the basis of the results of the plasma dual isotope ratio method, we examined Ch absorption further by the fecal dual isotope ratio method in groups of the lower-absorbing AKR and C3H strains, the middle-absorbing BALB and SWR strains, and the higher-absorbing FVB and C57L strains fed chow (n = 10 per group). In brief, nonfasted and nonanesthetized mice were given intragastrically by gavage 150 μl of medium-chain triglyceride containing a mixture of 1 μCi of [^{14}C]Ch and 2 μCi of [^{3}H]sitostanol. Mice were then transferred to individual cages with wire mesh bottoms, where they continued on the chow diet for the next 4 days. During this period, mouse feces were collected daily and kept frozen until analyzed as a pooled sample. After the 4-day combined feces were dried in a 60°C oven under reduced pressure for 48 h, they were ground to a fine powder, using a mortar and pestle. A 1.2-g aliquot of the powder and triplicate 100-μl aliquots of the original dosing mixture were added to 12 ml of $CHCl_3$–CH_3OH (2:1, v/v) (63). The samples were boiled in a water bath for 3 min, and then stirred vigorously and filtered into 50-ml volumetric flasks. A 5-ml aliquot was transferred to a 34-ml glass tube, and the solvent was evaporated under reduced pressure at 70°C overnight. The residue was "saponified" (64) in 10 ml of 2 N NaOH/CH_3OH (1:1, v/v) and incubated at 60°C for 1 h, after which the labeled sterols were extracted into 10 ml of petroleum ether. After duplicate 1-ml aliquots of the organic phase were transferred into counting vials and dried in a vacuum oven at 70°C overnight, 10 ml of Cytoscint (ICN Biomedicals) was added. The vials were then shaken vigorously for 10 min, and counted in a liquid scintillation spectrometer. The ratio of two radiolabels in the fecal extracts and the dosing mixture was used for calculating the percent Ch absorption:

$$\% \text{ Ch absorption} = \left(\frac{^{14}\text{C}/^{3}\text{H dosing mixture} - ^{14}\text{C}/^{3}\text{H feces}}{^{14}\text{C}/^{3}\text{H dosing mixture}}\right) \times 100$$

Measurement of biliary lipid output and BS pool size

We investigated bile flow, biliary lipid secretion, and BS pool size in additional groups of AKR, C3H, BALB, SWR, FVB, and C57L mice (n = 5 per strain) according to our earlier methods (61, 62). In brief, the common bile duct was cannulated below the entrance of the cystic duct via a PE-10 polyethylene catheter, with an outer diameter of 0.61 mm (Becton Dickinson, Sparks, MD), and a cholecystectomy was performed. Hepatic bile was collected by gravity every hour for 8 h, with the first-hour samples being used to study biliary lipid outputs and molecular composition of the BS pool, and the 8-h collection for the measurement of pool sizes. After hepatic bile volumes were determined by weighing (61, 62), all samples were frozen and stored at −20°C for further lipid analyses (see below). During surgery and bile collection, mouse body temperature was maintained at 37 ± 0.5°C with a heating lamp and monitored with a thermometer.

Influence of biliary lipid secretion on absorption and lymphatic transport of Ch

To quantify the effects of bile on intestinal Ch absorption, we established a mouse model with a chronic biliary fistula and investigated whether there were any differences in Ch absorption compared with intact biliary lipid secretion. We studied the absorption and lymphatic transport of Ch in the lower-absorbing AKR and the higher-absorbing C57L strains.

First experiment. To establish a chronic biliary fistula, AKR and C57L mice (n = 5 each) were fasted overnight, and then anesthetized with pentobarbital. The jugular vein was cannulated (62) with a PE-10 catheter that connected with an infusion pump (Kent Scientific, Litchfield, CT). For maintenance of hydration, animals were infused intravenously with 0.9% NaCl at 200 μl/h. Laparotomy was performed under sterile conditions through an upper midline incision. After cannulation of the common bile duct, a cholecystectomy was performed (60) and hepatic bile was drained externally (61, 62). After 4-h bile drainage, a PE-10 catheter was inserted into the duodenum 5 mm be-

low the pylorus and secured with 6-0 sutures and cyanoacrylate adhesive (Loctite, Rocky Hill, CT). The duodenal catheter was externalized through the abdominal wall and connected with a second infusion pump. Fresh hepatic biles (percent molar ratio of Ch:lecithin:BS = 6.5:15.0:78.5 and total lipid concentration of ~2.5 g/dl) (61), which were collected from C57L mice, were infused continuously at 250 μl/h through the duodenal catheter into the small intestine of both AKR and C57L mice for 2 h.

Second, we performed cannulation of the mesenteric lymph duct in the same mice. To better expose the mesenteric duct (65, 66), each animal was dorsally arch-bridged over a 3-ml syringe (outer diameter, 12 mm). The inferior vena cava was separated from underlying tissue with a microforceps. The beveled end of the fistula catheter was then threaded under the inferior vena cava and the right kidney to place it parallel to the lymphatic duct. After a thin layer of membrane that covered the lymphatic duct was removed, the PE-10 catheter was inserted into the mesenteric lymph duct with magnification provided by a zoom stereomicroscope (Olympus America, Melville, NY). Several drops of adhesive were placed at the junction of the lymph duct and catheter, and on both sides of the inferior vena cava, to secure the catheter firmly. Small accessory lymphatic vessels, when present, were disrupted intentionally and sealed off with a few drops of adhesive. The catheter was externalized through the abdominal wall and connected with heparinized microcentrifuge tubes, and the abdominal incision was closed with 4-0 sutures. The lymph was collected by gravity for 6 h.

After 2-h duodenal infusion of hepatic bile, exactly 2.5 μCi of [^{14}C]Ch dissolved in 100 μl of medium-chain triglyceride containing 0.5% taurocholate and 0.2% egg yolk lecithin were instilled into the small intestine through the duodenal catheter. To maintain intestinal lymph flow, we performed continuous intraduodenal infusion of 0.5% taurocholate and 0.2% egg yolk lecithin in medium-chain triglyceride at 300 μl/h. Fresh lymph was collected hourly into heparinized microtubes for a total of 6 h after instillation of radiolabeled Ch. 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 (61) was maintained with i.p. injections of 17 mg/kg pentobarbital every 2 h.

Second experiment. To study absorption and lymphatic transport of Ch in additional groups of AKR and C57L mice (n = 5 each) with intact biliary lipid secretion, all surgical and experimental procedures were identical to those as described above, except that 4-h bile drainage and 2-h duodenal infusion of hepatic bile were not carried out before the lymphatic Ch transport study. After successful cannulation of the mesenteric lymphatic duct, we initiated measurement of absorption and lymphatic transport of Ch collected in heparinized microtubes. Exactly 2.5 μCi of [^{14}C]Ch dissolved in 100 μl of medium-chain triglyceride oil containing 0.5% taurocholate was instilled through the duodenal catheter. To maintain lymph flow, a continuous intraduodenal infusion of medium-chain triglyceride containing 0.5% taurocholate was performed at 300 μl/h for 6 h.

Measurement of small intestinal transit times

Small intestinal transit times in AKR, SWR, and C57L strains (n = 10 each) were measured by the method of Miller, Galligan, and Burks (67) with major modifications for a radiolabeled marker and the administration of a lipid volume to each mouse. [^{3}H]sitostanol (a nonabsorbable marker) was substituted for $Na_2{}^{51}CrO_4$ (67) as the reference compound, because in a preliminary study we found that sitostanol is essentially nonabsorbed in mice. In brief, mice were weighed and anesthetized with an intraperitoneal injection of pentobarbital (35 mg/kg). Laparotomy was performed through an upper midline incision (0.8 cm in length), and a PE-10 catheter was inserted into the duodenum 5 mm beyond the pylorus and secured with 6-0 sutures and adhesive. The duodenal catheter was externalized through the incision and implanted under the skin, and the abdominal incision was closed with 4-0 sutures. Mice were returned to cages with wire mesh bottoms, and allowed a 24-h recovery period. After fasting for 18 h, 2 μCi of [^{3}H]sitostanol dissolved in 100 μl of medium-chain triglyceride was instilled into the small intestine of mice via the re-exposed duodenal catheter, and the animals were transferred to individual cages. Exactly 30 min after dosing, mice were anesthetized by intraperitoneal injection of pentobarbital (35 mg/kg). The abdomen was opened, and ligatures were placed around the gastroesophageal, gastroduodenal, and ileocecal junctions. The stomach, small and large intestines, and cecum were removed, and the small intestine was frozen immediately with liquid nitrogen. The frozen intestine was placed on a 50-cm ruled template and cut into 20 equal segments with a scalpel blade. The individual segments were placed in tubes containing 10 ml of $CHCl_3$–CH_3OH (2:1, v/v) (63), and stored at 4°C for 48 h. After tissues were homogenized and centrifuged at 10,000 *g* for 30 min in the organic solution, 1-ml aliquots were pipetted into counting vials and the solvent was evaporated under reduced pressure at 30°C overnight. After addition of 7 ml of EcoLite, radioactivity was determined by liquid scintillation spectrometry. Intestinal transit times were quantified mathematically by two methods (67): *i*) the percentage of total radioactivity in the small intestine was determined for each of the 20 segments and these data were transformed to cumulative percentage of radioactivity passing each segment; and *ii*) the geometric center, with respect to the distribution of radioactivity within the small intestine, was calculated as the sum of the fraction of [^{3}H]sitostanol per segment times the segment number.

Lipid analyses

Total and individual BS concentrations were measured by HPLC according to the methods of Rossi, Converse, and Hofmann (68). Bile Ch as well as Ch content in chow and gallstones were determined by HPLC (60). Biliary phospholipids were determined as inorganic phosphorus by the method of Bartlett (69). Ch saturation indexes in hepatic biles were calculated from critical tables (70). Hydrophobicity indexes of hepatic bile were calculated according to the method of Heuman (71).

Statistical methods

Data were expressed as means ± SD. Differences among groups of mouse strains were assessed for statistical significances by Student's *t*-test. Statistical significance was defined as a two-tailed probability of less than 0.05.

RESULTS

Variations in intestinal Ch absorption efficiency

Figure 1 summaries the percent Ch absorption by the plasma dual isotope ratio method determined 3 days after dosing (62) in male inbred mice fed chow. There were appreciable differences with respect to intestinal Ch absorption efficiency, varying from C3H (22 ± 5%), AKR (24 ± 4%), and A (24 ± 5%) strains, which displayed the lowest percent Ch absorption, to the highest in 129 (31 ± 4%), FVB (34 ± 5%), C57L (37 ± 5%), and C57BL (39 ± 5%) strains, with SJL (26 ± 3%), DBA (27 ± 3%), BALB (27 ± 4%), SWR (28 ± 4%), and SM (29 ± 4%) strains giving intermediate values. Of note is that the level of Ch absorption was significantly higher ($P < 0.001$) in 129, FVB,

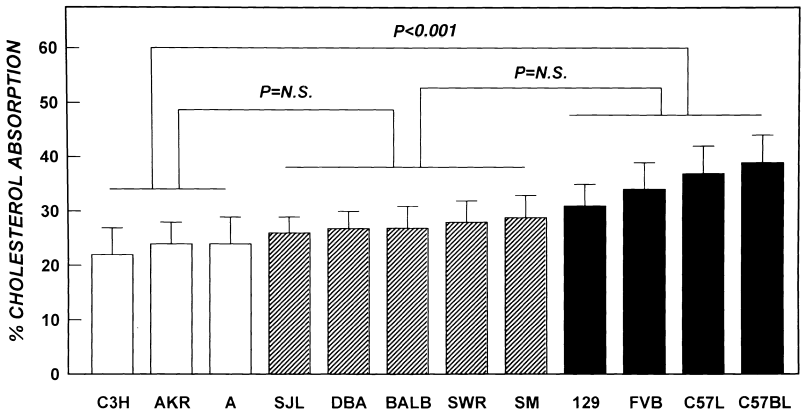

Fig. 1. Percent Ch absorption (means ± SD) was determined by the plasma dual isotope ratio method (62) in 12 strains of inbred male mice (n = 10–35 per strain) fed normal mouse chow containing trace amounts of Ch (<0.02%). There are marked differences among mouse strains with respect to intestinal Ch absorption efficiency: C3H, AKR, and A strains display the lowest (<25%) Ch absorption; SJL, DBA, BALB, SWR, and SM strains give intermediate (25–30%) values; and 129, FVB, C57L, and C57BL strains show the highest (31–40%) values for Ch absorption.

C57L, and C57BL strains compared with C3H, AKR, and A strains. Furthermore, percent Ch absorption (31 ± 6%) in (AKR×C57L)F_1 mice (**Fig. 2**) mimicked the higher-absorbing C57L parent, being significantly ($P < 0.05$) higher than in the AKR parent, suggesting a dominant trait.

Table 2 lists biological characteristics of strains displaying the high (FVB and C57L mice), intermediate (BALB and SWR mice), and low (AKR and C3H mice) percent intestinal Ch absorption. All strains of mice showed similar body weights (28–32 g), ate similar amounts of chow (4.0–4.3 g/day), and exhibited an average dietary Ch intake[3] of ~0.8–0.9 mg/day. The fecal outputs (1.3–1.4 g/day dry weight), and weight (1.4–1.5 g) and length (40–42 cm) of the small intestine, were similar in all six strains. However, the intestinal Ch absorption levels were significantly ($P < 0.01$) higher in C57L and FVB mice (34–42%) compared with AKR and C3H mice (22–29%), with SWR and BALB mice having intermediate levels of Ch absorption (Fig. 1).

Biliary lipid secretion rates and lipid compositions of hepatic biles

Table 3 summaries the biliary Ch, phospholipid, and BS outputs during the first hour of washout, that is, in the earlier phase of interruption of the enterohepatic circulation. In general, biliary Ch, phospholipid, and BS outputs in C57L and FVB mice are significantly greater ($P < 0.05$) than in AKR and C3H mice, with BALB and SWR mice displaying intermediate values (NS). Furthermore, we observed that bile flow during the first hour of bile collection in FVB and C57L mice (61–90 μl/min/kg) was somewhat higher than in BALB and SWR mice (46–56 μl/min/kg), and significantly higher ($P < 0.01$) than in AKR and C3H mice (41–42 μl/min/kg).

Using the "washout" technique with continuous biliary drainage for 8 h (61), we measured the circulating BS pool (see Materials and Methods). Table 3 shows that the circulating BS pool sizes in C57L (3.1 ± 0.4 μmol) and FVB mice (2.4 ± 0.2 μmol) mice were similar to those in BALB (2.0 ± 0.2 μmol) and SWR mice (2.2 ± 0.3 μmol), but were significantly larger ($P < 0.05$) than those in AKR (1.7 ± 0.3 μmol) and C3H mice (1.8 ± 0.3 μmol). Furthermore, the total BS pool sizes calculated from the circulating BS pool size plus the BS pool in the gallbladder (61) showed that C57L mice (4.7 μmol) had markedly larger total BS pool sizes than SWR mice (3.1 μmol) or AKR mice (2.7 μmol).

Table 4 shows biliary lipid compositions of hepatic biles from six strains of mice during the first hour of interrupted enterohepatic circulation. Mean Ch saturation indexes of hepatic biles were >1 and total lipid concentrations varied from 2.1 to 3.1 g/dl in all strains of inbred mice. Moreover, Ch saturation indexes in the higher-absorbing C57L and FVB mice (1.58–1.61) were significantly ($P < 0.05$) higher than in the lower-absorbing AKR and C3H mice (1.10–1.11), and somewhat higher than in the middle-absorbing BALB and SWR mice (1.29–1.32). As we found in previous studies (61), relative lipid compo-

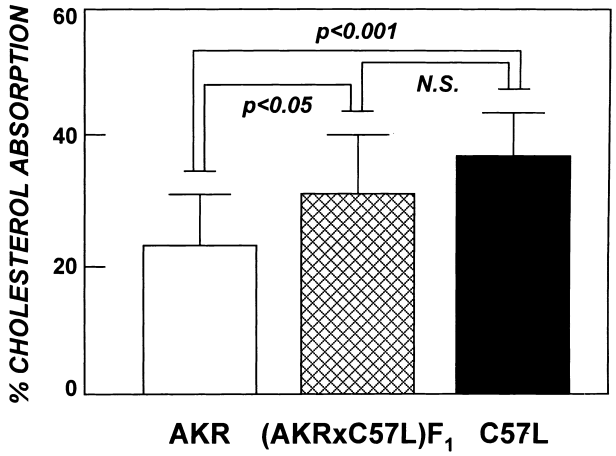

Fig. 2. Comparison of intestinal Ch absorption among AKR, C57L, and (AKR×C57L)F_1 male mice (n = 10 per strain) as determined by the plasma dual isotope ratio method (62). Percent Ch absorption (31 ± 5%) in the progeny (AKR×C57L)F_1 mice mimicks the higher-absorbing C57L parent (37 ± 5%) rather than the AKR strain (21 ± 4%) ($P < 0.05$).

[3] We found by HPLC (see Materials and Methods) that Purina (St. Louis, MO) laboratory chow (mouse diet 1401) contains trace Ch (~0.02%). Because each mouse ingests ~4.0–4.3 g of chow daily, average dietary Ch intake from chow is ~0.8–0.9 mg/day.

TABLE 2. Biological characteristics of inbred mouse strains putatively related to intestinal cholesterol absorption[a]

Parameter	AKR	C3H	BALB	SWR	FVB	C57L
Body weight (g)	32.1 ± 1.8	29.1 ± 1.3	28.2 ± 1.5	28.3 ± 1.2	29.6 ± 1.4	28.2 ± 1.3
Food intake (g/day)	4.3 ± 0.6	4.2 ± 0.6	4.0 ± 0.8	4.3 ± 0.7	4.2 ± 0.6	4.1 ± 0.7
Feces (dry weight) (g/day)	1.4 ± 0.3	1.3 ± 0.2	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.3
Small intestine weight (g)	1.5 ± 0.2	1.4 ± 0.4	1.5 ± 0.3	1.4 ± 0.3	1.5 ± 0.3	1.5 ± 0.3
Small intestine length (cm)	42.2 ± 2.3	39.5 ± 2.6	41.5 ± 1.8	40.6 ± 2.1	41.3 ± 1.9	42.0 ± 2.2
Intestinal cholesterol absorption						
Plasma dual isotope ratio	24 ± 4%[b]	22 ± 5%[b]	27 ± 4%	28 ± 4%	34 ± 5%	37 ± 5%
Fecal dual isotope ratio	29 ± 5%[b]	28 ± 4%[b]	33 ± 5%	32 ± 5%	39 ± 8%	42 ± 7%

[a] Values represent means ± SD of 10 animals per strain. All inbred strains of mice were 8 weeks old, and fed Purina laboratory chow containing trace cholesterol (<0.02%).
[b] $P < 0.01$ compared with FVB and C57L strains.

sitions of all strains of inbred mice plotted in a crystallization pathway denoted region E (59) for dilute hepatic biles, where at theoretical equilibrium the systems would be composed of liquid crystals and saturated micelles but not solid crystals (59).

Molecular species of BS in hepatic biles

Table 5 lists the percent distribution of BS species in hepatic biles during the first hour of biliary secretion in all 12 strains of inbred mice fed chow. HPLC analysis of the individual BS revealed that they were all taurine conjugated, and all strains of inbred mice exhibited similar BS compositions (Table 5). The predominant molecular species were taurocholate (range, 46.8–50.9%), and tauro-β-muricholate (range, 42.3–46.1%), with taurochenodeoxycholate (0.4–1.7%), tauro-ω-muricholate (0.9–2.7%), tauroursodeoxycholate (1.5–4.7%), and taurodeoxycholate (1.2–4.0%) being present in much smaller concentrations. Furthermore, for all strains of inbred mice (Table 5), we found that the hydrophilicity indexes of biliary BS pool were essentially identical (−0.33 to −0.37).

Intestinal transit time

Figure 3 illustrates the distribution of radioactivity 30 min after intraduodenal dosing of medium-chain triglyceride containing [^{3}H]sitostanol along the small intestine of the lower Ch-absorbing AKR, the middle Ch-absorbing SWR, and the higher Ch-absorbing C57L mice. The radioactivity distributions were similar among the three strains of mice, and showed a broad peak between segments 8 and 15. The geometric center of the [^{3}H]sitostanol distribution profiles in the small intestine was 10.8 ± 0.9 in AKR, 11.1 ± 0.8 in SWR, and 11.0 ± 1.0 in C57L strains. Neither the distribution of radioactivity nor the calculation of the geometric center revealed statistically significant differences among the strains. It is reasonable to infer from these results that AKR, SWR, and C57L strains have identical small intestinal transit times. Furthermore, samples of stomach, cecum, and large intestine were also analyzed, but none showed significant radioactivity.

Effects of a chronic biliary fistula on absorption and lymphatic transport of Ch

We observed differences in bile flow and biliary lipid secretion rates as well as Ch content of bile and BS pool sizes between AKR and C57L mice (Table 3). We found that in animals with intact biliary lipid secretion (**Fig. 4**, left), cumulative radioactivities 6 h after instillation of [^{14}C]Ch were 24 ± 3% in the C57L strain, significantly ($P <$ 0.01) higher than in the AKR strain (14 ± 3%). This might be due to C57L mice showing significantly ($P <$ 0.01) higher biliary BS outputs and bigger BS pool sizes than AKR mice. To exclude these effects on intestinal Ch absorption, we used a chronic biliary fistula mouse model (see Materials and Methods), and our results (Fig. 4, right) showed that cumulative radioactivities 6 h after instillation of the lipid mixture containing [^{14}C]Ch were 22 ± 3% in C57L mice, which was significantly ($P < 0.01$) greater than in AKR mice (13 ± 3%) with a similar fistula. The absorption and lymphatic transport of Ch in mice with

TABLE 3. Bile flow, biliary lipid outputs, and bile salt pool sizes in inbred mice[a]

Parameter	AKR	C3H	BALB	SWR	FVB	C57L
Bile flow (μl/min/kg body weight)	41 ± 3[b]	42 ± 4[b]	46 ± 4[c]	56 ± 3	61 ± 5	90 ± 9
Biliary lipid output						
Cholesterol (μmol/h/kg body weight)	5.0 ± 1.3[b]	5.2 ± 1.5[b]	6.1 ± 2.2[c]	6.6 ± 1.7	7.9 ± 2.5	10.2 ± 4.0
Phospholipid (μmol/h/kg body weight)	16.9 ± 5.7[c]	17.2 ± 4.3[c]	19.6 ± 5.1	20.5 ± 4.2	19.7 ± 4.6	25.6 ± 9.3
Bile salt (μmol/h/kg body weight)	97.1 ± 21.7[c]	98.6 ± 23.8[c]	106.8 ± 29.3	112.7 ± 28.6	118.6 ± 31.2	146.7 ± 52.6
Circulating bile salt pool size (μmol)	1.7 ± 0.3[c]	1.8 ± 0.3[c]	2.0 ± 0.2[c]	2.2 ± 0.3	2.4 ± 0.2	3.1 ± 0.4
Total bile salt pool size (μmol)[d]	2.7	—	—	3.1	—	4.7

[a] Values represent means ± SD of five animals per strain. All strains of inbred mice were 8 weeks old and fed Purina laboratory chow containing trace cholesterol (<0.02%).
[b] $P < 0.01$ compared with FVB and C57L strains.
[c] $P < 0.05$ compared with FVB and C57L strains.
[d] See Results for details.

TABLE 4. Biliary lipid compositions of hepatic biles in inbred mice[a]

Mouse Strain	Ch	L	BS	L/(L + BS)	[TL]	CSI[b]
	mol%	*mol%*	*mol%*		*g/dl*	
AKR	3.77 ± 0.34[c]	11.87 ± 1.97	84.36 ± 1.81	0.12 ± 0.02	2.55 ± 0.74	1.10 ± 0.20[d]
C3H	3.84 ± 0.32[c]	12.12 ± 1.66	84.04 ± 3.40	0.13 ± 0.02	2.35 ± 0.54	1.11 ± 0.27[d]
BALB	4.86 ± 1.20	12.75 ± 1.35	82.39 ± 4.47	0.13 ± 0.02	3.02 ± 0.39	1.32 ± 0.44
SWR	4.93 ± 0.84	13.71 ± 2.01	81.36 ± 4.95	0.14 ± 0.03	2.42 ± 0.28	1.29 ± 0.30
FVB	6.02 ± 1.23	12.45 ± 1.53	81.53 ± 6.12	0.13 ± 0.02	3.08 ± 0.60	1.58 ± 0.43
C57L	6.36 ± 1.95	14.71 ± 3.64	78.93 ± 5.31	0.16 ± 0.04	2.11 ± 0.37	1.61 ± 0.37

Abbreviations: Ch, cholesterol; L, lecithin; BS, bile salt; [TL], total lipid concentration; CSI, cholesterol saturation index.

[a] Values were determined from five hepatic biles (the first hour of biliary secretion) per mouse strain.

[b] The cholesterol saturation index values of five hepatic biles were calculated from the critical tables (70), and thus they are estimates based on taurocholate and not on an equimolar taurocholate plus tauro-β-muricholate mixture (59) (see Table 5).

[c] $P < 0.01$ compared with FVB and C57L strains.

[d] $P < 0.05$ compared with FVB and C57L strains.

chronic biliary fistulae were similar to those in mice with intact biliary lipid secretion because the lipid mixture contained 0.2% (w/w) egg yolk lecithin and 0.5% taurocholate. The differences (~2–3%) at 6 h between Fig. 4A and B are the result of the differential biliary lipid secretion rates between AKR and C57L mice. Therefore, it suggests that genetic factors at the enterocyte level are major determinants in determining differences in intestinal Ch absorption efficiency between AKR and C57L mice.

DISCUSSION

Variations in murine intestinal Ch absorption efficiency

Beynen, Katan, and Van Zutphen (72) stressed that with similar dietary Ch intake, interindividual and interstrain variations in intestinal Ch absorption efficiency exist in primates (46, 47), including humans (48–51), as well as in inbred strains of rabbits (52, 53), rats (54), and mice (55–58). This strongly suggests that intestinal Ch absorption is regulated by multiple genes because diet, a key environmental factor, was controlled in these studies. Therefore, it seemed crucial that factors influencing Ch absorption should be evaluated systematically in relevant strains of inbred mice. In the present study, we showed that among 12 strains of inbred mice, there are variations in Ch absorption efficiency as measured by the plasma dual isotope ratio method (Fig. 1), the fecal dual isotope ratio method (Table 2), and the lymphatic transport of Ch (Fig. 4). It is to be noted that our measured efficiencies of Ch absorption vary from 22% to 39%, substantially lower than those observed by Sehayek et al. (36) in individual C57BL mice (80%) and in strain surveys by Kirk et al. (55) (74–87% in C3H, C57BL, DBA, and 129) and Carter, Howles, and Hui (56) (65–75% in AKR, BALB, C3H, C57BL, C57L, DBA, and 129). Although the putative explanation is not addressed here, we have investigated the reasons for these discordances (D. Q-H. Wang and M. C. Carey, 2001, unpublished observations). Furthermore, we observed that two key biliary factors, BS secretion rates and pool sizes, regulate intestinal Ch absorption, as evidenced by our present studies of AKR and C57L mice. Of note is that neither molecular compositions of BS pool nor small intestinal transit times varied significantly among these mouse strains. In the face of a chronic biliary fistula, absorption and lymphatic transport of Ch remained significantly greater in C57L mice compared with AKR mice. Interestingly, Ch absorption in (AKR×C57L)F_1 mice mimicked

TABLE 5. Percent bile salt species in hepatic biles of 12 strains of inbred mice fed chow[a]

Mouse	TC	T-β-MC	TCDC	T-ω-MC	TUDC	TDC	HI[b]
A	47.0 ± 5.8	44.3 ± 5.6	1.7 ± 0.8	1.1 ± 0.8	1.9 ± 0.9	4.0 ± 1.7	−0.33 ± 0.04
AKR	46.8 ± 4.8	43.3 ± 3.0	0.4 ± 0.2	2.7 ± 1.4	4.7 ± 2.8	2.1 ± 1.4	−0.37 ± 0.05
BALB	48.9 ± 9.8	42.8 ± 3.8	0.8 ± 0.5	2.1 ± 1.3	2.3 ± 1.6	3.1 ± 1.2	−0.34 ± 0.03
C3H	50.2 ± 9.4	44.5 ± 5.1	0.9 ± 0.6	1.6 ± 0.9	1.5 ± 0.9	1.3 ± 0.8	−0.36 ± 0.05
C57BL	47.9 ± 5.5	45.2 ± 6.5	0.6 ± 0.4	0.9 ± 0.5	1.7 ± 0.9	3.7 ± 1.2	−0.34 ± 0.02
C57L	49.7 ± 6.7	42.3 ± 5.7	0.6 ± 0.3	1.4 ± 0.6	2.6 ± 1.6	3.4 ± 1.3	−0.33 ± 0.05
DBA	47.8 ± 6.6	43.1 ± 6.2	1.2 ± 0.6	1.2 ± 0.9	3.5 ± 1.7	3.2 ± 1.5	−0.34 ± 0.03
FVB	48.2 ± 6.2	44.6 ± 4.3	1.0 ± 0.5	1.9 ± 0.8	1.6 ± 1.3	2.7 ± 0.7	−0.35 ± 0.02
SJL	49.7 ± 8.6	42.6 ± 4.9	0.7 ± 0.4	1.9 ± 1.1	2.2 ± 1.3	2.9 ± 0.8	−0.34 ± 0.04
SM	47.0 ± 5.8	44.3 ± 5.6	1.7 ± 0.8	1.1 ± 0.8	1.9 ± 0.9	4.0 ± 1.7	−0.33 ± 0.03
SWR	47.3 ± 6.3	46.1 ± 6.9	0.6 ± 0.4	1.6 ± 0.6	1.7 ± 1.0	2.7 ± 0.6	−0.36 ± 0.04
129	50.9 ± 7.5	43.4 ± 7.1	0.9 ± 0.6	1.8 ± 0.8	1.8 ± 1.2	1.2 ± 0.7	−0.35 ± 0.04

Abbreviations: TC, taurocholate; T-β-MC, tauro-β-muricholate; TCDC, taurochenodeoxycholate; T-ω-MC, tauro-ω-muricholate; TUDC, tauroursodeoxycholate; TDC, taurodeoxycholate; HI, hydrophobicity index.

[a] Vaues represent means ± SD determined from hepatic biles (n = 5 per strain) obtained during the first hour of biliary fistulation.

[b] The hydrophobicity index values of five hepatic biles were calculated (71).

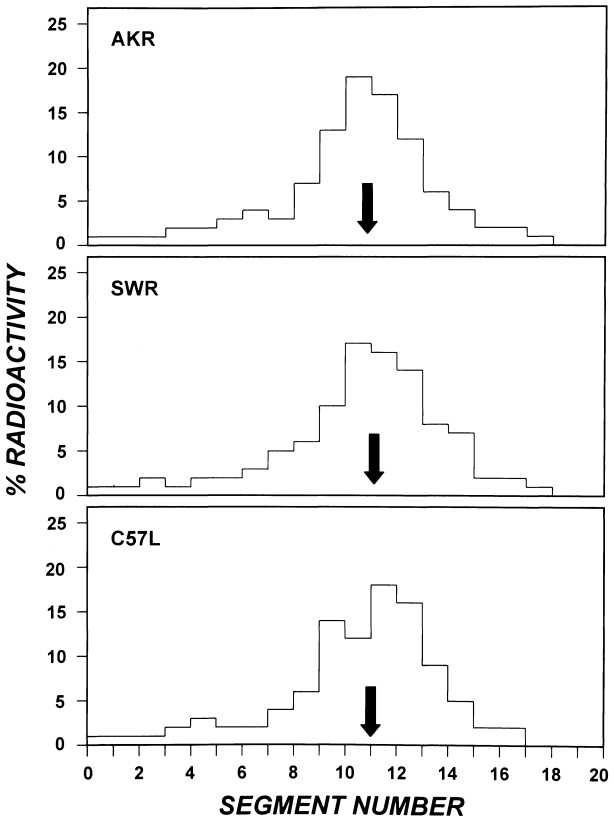

Fig. 3. Measurement of small intestinal transit time of inbred mice in the fasted state. Shown is the distribution of small intestinal radioactivity 30 min after intraduodenal instillation of medium-chain triglyceride containing [^{3}H]sitostanol in AKR (top), SWR (middle), and C57L (bottom) mice. Each column is the mean percentage of radioactivity in each segment for 10 mice per strain (see Materials and Methods). Segments 1 and 20 represent the most proximal to the distal parts of the small intestine. Arrows indicate the geometric center, which is similar between the AKR strain (geometric center, 10.84), SWR strain (geometric center, 11.12), and C57L strain (geometric center, 11.04).

the higher-absorbing C57L parent. Our findings therefore suggest that genetic factors at the enterocyte level are crucial in determining the variations of intestinal Ch absorption efficiency observed, and that high Ch absorption is a dominant trait in mice.

Effects of biliary lipid outputs, Ch content of bile, and BS pool sizes

In this study we controlled the dietary conditions by feeding mice chow (Ch < 0.02%) so that total Ch mass consumed by individual mice showed small variations.[4] Under these conditions, biliary factors such as secretion rates of biliary lipids (BS, Ch, and phospholipids), Ch and phospholipid content of bile, as well as sizes, molecular compositions, and hydrophilic-hydrophobic balance of the BS pool, could together exert major influences on the efficiency of intestinal Ch absorption. Therefore, all of these could explain the differences in Ch absorption efficiency between the higher Ch-absorbing and lower Ch-absorbing strains of inbred mice with intact biliary lipid secretion.

The knockout of multidrug resistance gene 2 (*Mdr2*) inhibits biliary secretion of phospholipids and curtails intestinal Ch absorption efficiency markedly (18, 19). Studies of homozygous and heterozygous *Mdr2*-deficient mice therefore suggest that physiological phospholipid outputs are necessary for normal intestinal Ch absorption. This was not a factor in our mice because the phospholipid:(phospholipid + BS) ratios were similar (~0.12–0.16) (Table 4). In cholesterol 7α-hydroxylase knockout mice (17), biliary BS pool sizes and biliary BS outputs are reduced notably and the animals absorb only trace amounts of Ch because of BS deficiency. However, Ch absorption is reversed readily by feeding a diet containing 0.2% cholic acid (17). This confirms that biliary BS pool sizes and biliary BS outputs play crucial roles in Ch absorption via intralumenal BS micellar concentrations. Changes in the hydrophilic-hydrophobic balance of the BS pool also influence Ch absorption. Akiyoshi et al. (21) found that Ch absorption was increased by 50–60% in diabetic mice compared with healthy controls because taurocholate (78%) became the major BS in the BS pool with a concomitant marked decrease (to 6%) in tauro-β-muricholate. In the present study we observed that the inbred mice displayed identical BS patterns and hydrophobicity indexes of the basal BS pool (Table 5), such that we concluded these factors were not responsible for the observed variations of intestinal Ch absorption.

Nevertheless, we observed that the higher Ch-absorbing mice exhibit significantly higher secretion rates of all three major biliary lipids (BS, Ch, and phospholipids) and ele-

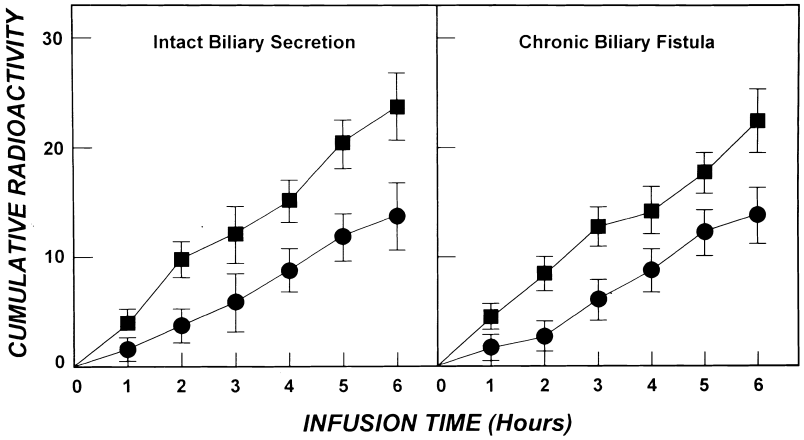

Fig. 4. Comparison of the absorption and lymphatic transport of Ch between AKR and C57L mice (n = 5 per group) with intact biliary lipid secretion (left) and with chronic biliary fistula (right). These results show that in C57L mice with intact biliary lipid secretion, cumulative radioactivities at 6 h after instilling [^{14}C]Ch are significantly ($P < 0.01$) greater compared with AKR mice with intact biliary lipid secretion. There are no significant differences in the absorption and lymphatic transport of Ch in mice with chronic bile fistula (right) but in the setting of infusion of 0.5% (w/w) taurocholate and 0.2% egg yolk lecithin compared with those with intact biliary lipid secretion (left). Male C57L mice (solid squares), male AKR mice (solid circles).

vated Ch content of bile compared with the lower Ch-absorbing mice. Our observations are in agreement with the results of Ishikawa, Uchida, and Akiyoshi (20) in diabetic mice. They found a positive relationship between high intestinal Ch absorption (21) and augmented biliary Ch outputs (20), as well as high gallstone prevalence rates (21), suggesting that the effect on bile is secondary to increased intestinal Ch absorption. Our findings differ from the results of Sehayek et al. (36), who showed that biliary Ch concentrations were inversely correlated with percent Ch absorption. Of note is that these authors (36) examined only the Ch concentrations in gallbladder biles and not secretion rates.

Lumenal factors and intestinal Ch absorption

Several studies of animals and humans suggest that rapid intestinal transit times decrease Ch absorption. Traber and Ostwald (73) found that in guinea pigs resistant to the plasma effects of dietary Ch, intestinal transit times were more rapid than in guinea pigs with hypercholesterolemia. Also, Ponz de Leon et al. (74) produced evidence in humans by pharmacological intervention that acceleration of small intestine transit time was consistently associated with decreased Ch absorption. We found elsewhere (43) that cholecystokinin-A receptor-deficient mice display significantly higher intestinal Ch absorption rates, which correlate with slow small intestinal transit rates (43); this in turn induced biliary Ch hypersecretion and Ch gallstone formation. We were surprised to find in the present work that small intestinal transit times (Fig. 3) and the length and weight of small intestine (Table 2) among low, middle, and high Ch-absorbing strains were essential identical. Taken together, these results reveal that lumenal factors were not responsible for the differences in intestinal Ch absorption efficiency in these diverse mouse strains.

Enterocyte factors play crucial roles in intestinal Ch absorption efficiency

When our studies were repeated in mice with chronic biliary fistulae, we found that the marked differences in Ch absorption efficiency (Fig. 4) persisted between AKR and C57L strains. The question arises, therefore, as to which cellular step(s) in the absorption of Ch might be inherently different. The discovery of potent and specific compounds that inhibit Ch absorption implies that there is a Ch transporter (33–35) critical to the uptake of Ch across the brush border of the enterocyte. The fact that Ch is absorbed, but structurally similar phytosterols (75) may not be, suggests that a Ch transporter is localized in the brush border membrane that facilitates selective Ch but not phytosterol uptake by the enterocyte. Although candidate proteins have been proposed for the role of Ch transporters (33–35), the exact identity of such a protein remains elusive. It has been suggested recently that several members of the ATP-binding cassette (ABC) transporter family, possibly ABCA1 in mice (27–29) and ABCG5 and ABCG8 in humans (30, 31), mediate partial efflux of Ch and nearly complete efflux of phytosterols from the enterocyte into the intestinal lumen. On entering the enterocytes, approximately half the Ch molecules move to the endoplasmic reticulum, where they are esterified by ACAT before incorporation into nascent chylomicron particles. It was observed that transmucosal transport of Ch was reduced in rats with inhibition of mucosal ACAT (22, 23). Also, the inhibition of intestinal 3-hydroxy-3-methylglutaryl-CoA reductase by pharmacological intervention with "statins" decreases intestinal Ch absorption in animals (24, 25) and humans (26). Moreover, Young et al. (41) found that Ch absorption was significantly inhibited in apolipoprotein B-48-deficient mice because of a failure in the assembly and/or secretion of chylomicrons.

In summary, our data show that there are obvious differences with respect to intestinal Ch absorption efficiency in inbred strains of mice. Although variations in BS secretion rates and pool sizes were minor and intestinal motility was identical, the major differences in Ch absorption still persist between AKR and C57L mice. Our results demonstrate, therefore, that there must be a strong genetic basis regulating the amount of Ch absorbed from the small intestine among diverse strains of inbred mice. This study therefore provides a framework for investigating molecular mechanisms of action of ABCA1, ABCG5, and ABCG8, and perhaps other ABCs, on intestinal Ch absorption. These mouse models can be used to search for new genetic determinants that regulate intestinal Ch absorption from the ABC transporter family (76) as well as to identify other genetic modifiers of Ch influx and efflux at the enterocyte level. In particular, the well-defined homology between the human and mouse genomes (77) makes the mouse an ideal surrogate species for the identification of genes involved in controlling Ch absorption from the human small intestine.

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