Aging per se is an independent risk factor for cholesterol gallstone formation in gallstone susceptible mice

David Q-H. Wang[1]

Department of Medicine, Gastroenterology Division, Beth Israel Deaconess Medical Center, Harvard Medical School and Harvard Digestive Diseases Center, Boston, MA

Abstract **Cholesterol gallstones occur rarely in childhood and adolescence and increase linearly with age in both genders. To explore whether aging per se increases cholesterol saturation of bile and gallstone prevalence, and to investigate age-related changes in hepatic and biliary lipid metabolism, we studied gallstone-susceptible C57L mice and resistant AKR mice of both genders fed 8 weeks with a lithogenic diet containing 1% cholesterol, 0.5% cholic acid, and 15% butter fat starting at (young adult) 8, (older adult) 36, and (aged) 50-weeks-of-age. After the 8-week feeding, gallstone prevalence, gallbladder size, biliary lipid secretion rate, and HMG-CoA reductase activity were significantly greater but cholesterol 7α-hydroxylase activity was lower in C57L mice of both genders compared with AKR mice. Increasing age augmented biliary secretion and intestinal absorption of cholesterol, reduced hepatic synthesis and biliary secretion of bile salts, and decreased gallbladder contractility, all of which increased susceptibility to cholesterol cholelithiasis in C57L mice. We conclude that aging per se is an independent risk factor for cholesterol gallstone formation. Because aging increases significantly biliary cholesterol hypersecretion and gallstone prevalence in C57L mice carrying *Lith* genes, it is highly like that *Longevity* (aging) genes can enhance lithogenesis of *Lith* (gallstone) genes.**—Wang, D. Q-H. **Aging per se is an independent risk factor for cholesterol gallstone formation in gallstone susceptible mice. *J. Lipid Res.* 2002.** 43: **1950–1959.**

Supplementary key words bile salt • bile flow • phospholipid • gallbladder size • mucin gel • HMG-CoA reductase • cholesterol 7α-hydroxylase

Cholesterol gallstone disease is influenced by a complex interaction of genetic and environmental factors (1) that determine the hypersecretion of biliary cholesterol into hepatic bile (2) and subsequently the precipitation of cholesterol crystals in gallbladder bile (3). Epidemiological and clinical investigations (4–8) have shown that cholesterol gallstones occur infrequently in childhood and adolescence, and the prevalence of cholesterol gallstone disease increases linearly with age in both genders and approaches 50% at age 70 in females. Furthermore, elderly individuals are at high risk for developing complications of gallstones and mortality from surgery is often unacceptably high in patients older than 65 (9–11). Although cholesterol saturation of bile is significantly higher in elderly Swedes (12) and Chilean women (13) and age correlates positively with increased hepatic cholesterol secretion rate (12), it has not been established how aging per se influences hepatic and biliary cholesterol metabolism. Furthermore, numerous studies on age-related hepatic cholesterol and bile salt metabolism (14–20), biliary cholesterol secretion and composition (12, 13, 21, 22), gallbladder contraction function (23–26), and intestinal cholesterol absorption (27, 28) have been performed in humans and in different animal species; however, effects of aging on these parameters varied tremendously, being either normal, diminished, or elevated compared to young subjects. In particular, little is known whether aging per se influences cholesterol gallstone formation. In this study, using a unique genetically gallstone-susceptible C57L mouse model with *Lith* genes (2, 3, 29) compared to resistant AKR mice, we investigated age-related hepatic cholesterol and bile salt metabolism, biliary lipid secretion rate, gallbladder bile lipid composition, gallbladder motility function, and intestinal cholesterol absorption, as well as gallstone prevalence rate. Our results showed that aging per se increases cholesterol gallstone formation through enhancing biliary secretion and intestinal absorption of cholesterol, decreasing hepatic synthesis and secretion of bile salts, and reducing gallbladder contractility in C57L mice.

MATERIALS AND METHODS

Chemicals

Medium-chain triglyceride was purchased from Mead Johnson (Evansville, IN), and Intralipid (20%, w/v) was from Pharmacia

Manuscript received 15 February 2002 and in revised form 28 June 2002.
Published, JLR Papers in Press, August 16, 2002.
DOI 10.1194/jlr.M200078-JLR200

Abbreviations: CCK-8, sulfated cholecystokinin octapeptide; CSI, cholesterol saturation index; HMG-CoA, hydroxy-3-methylglutaryl coenzyme A.

[1] To whom correspondence should be addressed.
e-mail: dqwang@caregroup.harvard.edu

(Clayton, NC). Sulfated cholecystokinin octapeptide (CCK-8) was obtained from Sigma Chemical (St. Louis, MO). Radioisotopes [1,2-^{3}H]cholesterol, [4-^{14}C]cholesterol, DL-[5-^{3}H]mevalonolactone, and DL-[3-^{14}C]hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) were purchased from NEN Life Science Products, (Boston, MA), and [5,6-^{3}H]sitostanol was from American Radiolabeled Chemicals (St. Louis, MO). For HPLC analyses of bile salt species and cholesterol, all reagents were HPLC grade and obtained from Fisher Scientific (Fair Lawn, NJ).

Animals and diets

Inbred C57L/J and AKR/J mice of both genders, 6-weeks-old, were purchased from The Jackson Laboratory, Bar Harbor, ME. C57L strain is homozygous for susceptible *Lith* alleles and AKR strain is homozygous for resistant *Lith* alleles (2, 3, 29). All animals were maintained in a temperature-controlled room (22 ± 1°C) with a 12-h day cycle (6 AM–6 PM), and were provided free access to water and normal mouse chow containing trace cholesterol (<0.02%) (The Mouse Diet 1401, St. Louis, MO). In general, total life expectancy of C57L mice is ~80 weeks, and AKR mice ~60 weeks (30, 31). Based upon measurements of physical growth and sexual maturation, the different age groups of mice were considered to be in the following stages of maturation: 8-weeks, young adult; 36-weeks, older adult; and 50-weeks, aged (30, 31). To explore whether aging per se increases cholesterol saturation of bile and gallstone prevalence in C57L and AKR mice of both genders, we studied three groups of mice at different ages, i.e., 8-, 36-, and 50-weeks-old (n = 20 per group). Moreover, we have observed that when feeding the lithogenic diet (32) containing 1% cholesterol, 0.5% cholic acid, and 15% butter fat starts at the age of 8-weeks-old, 27% of male C57L mice form gallstones at 4-weeks of feeding, 80% at 8-weeks (3), and 100% at 52-weeks (32), suggesting that time consuming the lithogenic diet is a risk factor. Therefore, all animals were fed 8-weeks of the lithogenic diet starting at 8-, 36-, and 50-weeks-of-age, respectively. Also, the biliary and gallstone phenotypes were examined at 8 weeks of feeding the lithogenic diet. 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.

Collection of gallbladder biles and gallstones and microscopic studies

After the 8-week lithogenic diet feeding, non-fasted animals were weighed and anesthetized with an intraperitoneal injection of 35 mg/kg pentobarbital. After cholecystectomy was performed, gallbladder volume was measured by weighing the whole gallbladder and equating gallbladder weight (including stones) with gallbladder volume. Five microliters of fresh gallbladder bile were examined for mucin gel, solid and liquid crystals, and gallstones, which were defined according to previously established criteria (3). After pooled gallbladder biles were ultracentrifuged at 100,000 *g* for 30 min at 37°C and filtered through a preheated (37°C) Swinnex-GS filter (0.22 μm) assembly (Millipore Products Division, Bedford, MA), samples were frozen and stored at −20°C for further lipid analyses (see below).

Cannulation of the common bile duct and collection of hepatic biles

Additional groups of mice (n = 5 per group) of different ages, which were fed the lithogenic diet for 8 weeks, were used for biliary lipid secretion studies (2). In brief, after cholecystectomy was performed, the common bile duct was cannulated and hepatic bile was collected by gravity. The first hour collection of hepatic biles was used to study biliary lipid outputs. To determine the circulating bile salt pool sizes, 8-h biliary "washout" studies were performed according to previous method (2). After fresh hepatic biles were examined by polarizing light microscopy and their volumes were determined by weighing, all samples were frozen and stored at −20°C for further lipid analyses (see below). During surgery and hepatic bile collection, mouse body temperature was maintained at 37 ± 0.5°C with a heating lamp and monitored with a thermometer.

Gallbladder contraction study

To explore whether gallbladder emptying in response to exogenously administered CCK changed with age, we studied three different age groups of C57L and AKR mice (n = 5 per group) at 8-weeks of feeding the lithogenic diet. In brief, non-fasted mice were anesthetized lightly with pentobarbital. An 0.4 cm incision was made on the right or left side of the neck and the jugular vein was cannulated. Then, the incision was closed tightly with 3-0 silk sutures. Following this procedure, a cholecystectomy was performed and gallbladder volume was measured by weighing (see above), which was considered as control groups. Additional groups of mice were injected intravenously through the jugular vein with exactly 17 nmol/kg body weight of sulfated CCK octapeptide (CCK-8) dissolved in 100 μl of phosphate buffered saline (PBS) solution, or 100 μl of only PBS solution. This procedure was carried out over 1 min to prevent cardiac arrest. In the mouse, CCK-8 is a full agonist at both subtypes of CCK-A and -B receptors (33). Exactly 15 min after the injection, a cholecystectomy was made and gallbladder size was determined by weighing. Gallbladder contractile function was determined by comparing gallbladder volumes of the CCK-8 groups with those of the control and the PBS groups.

Measurement of intestinal cholesterol absorption

Cholesterol absorption was determined by the plasma dual isotope ratio method (32, 34) in chow-fed C57L and AKR mice (n = 10 per group) of both genders. In brief, exactly 2.5 μCi of [^{3}H]cholesterol in 100 μl of Intralipid was injected intravenously (iv) into a jugular vein. Immediately, the animal was given by gavage an intragastric (ig) dose of 1 μCi of [^{14}C]cholesterol in 150 μl of medium-chain triglyceride. After dosing, mice were returned to individual cages with wire mesh bottoms, where they were free to eat chow for an additional 3 days. Mice were then anesthetized and bled from the heart into heparinized microtubes. Exactly 100 μl of plasma aliquots and the original dosing mixture, respectively, were mixed with 10 ml of EcoLite (ICN Biomedicals, Costa Mesa, CA). The vials were counted in a liquid scintillation spectrometer (Beckman Instruments, San Ramon, CA). The ratio of the two radiolabels in plasma was used for calculating the percent of cholesterol absorption:

$$\%\ \text{Cholesterol absorption} = \left(\frac{\text{Percent of IG dose [}^{14}\text{C]cholesterol per ml plasma}}{\text{Percent of IV dose [}^{3}\text{H]cholesterol per ml plasma}}\right) \times 100 \qquad \textit{(Eq. 1)}$$

Measurement of small intestinal transit time

Intestinal transit time was measured in three age groups of chow-fed C57L and AKR mice (n = 5 per group) according to a previously published method (34). Nonabsorbable [^{3}H]sitostanol is used as a reference marker (34). In brief, 2 μCi of [^{3}H]sitostanol in 100 μl of medium-chain triglyceride were instilled into the small intestine via a duodenal catheter. Exactly 30 min after instilling, mice were anesthetized with pentobarbital. The abdomen was opened quickly, and the stomach, small and large intestines, and cecum were removed carefully. The small in-

testine was cut into 20 segments equally with a scalpel blade. The individual segments were mixed with 10 ml of $CHCl_3$-CH_3OH (2:1, v/v), and stored at 4°C for 48 h. One milliliter of aliquots was transferred into counting vials and the solvent was then evaporated under nitrogen. Seven milliliters of Cytoscint (ICN Biomedicals, Costa Mesa, CA) were added and the radioactivity was determined by liquid scintillation spectrometry. Samples of stomach, cecum, and large intestine were also analyzed, but none ever showed appreciable radioactivity. Intestinal transit time was evaluated by a geometric center method (34). The geometric center of the distribution of radioactivity within the small intestine was the center of gravity for the distribution of the reference marker and was calculated using the following equation:

$$\text{Geometric center} = \sum(\text{fraction of } [^3\text{H}]\text{sitostanol per segment} \times \text{segment number}) \quad \textit{(Eq. 2)}$$

Determination of activities of hepatic HMG-CoA reductase and cholesterol 7α-hydroxylase

Liver samples were collected from non-fasted mice (n = 5 per group) after the 8-week lithogenic diet feeding. To minimize diurnal variations of hepatic enzyme activities, all procedures (35) were performed between 9:00 and 10:00 AM. Microsomal activities of HMG-CoA reductase were determined by measuring the conversion rate of [^{14}C]HMG-CoA to [^{14}C]mevalonic acid using a radiochemical assay (36). Products were quantified by liquid scintillation counting with [^{3}H]mevalonolactone as internal standard. Hepatic activities of cholesterol 7α-hydroxylase were determined by the HPLC-based assay system of Hylemon et al. (37). Protein concentration was determined by the assay of Bradford (38).

Lipid analyses

Total and individual bile salt concentrations were measured by HPLC according to the method of Rossi, Converse and Hofmann (39). Biliary phospholipids were determined as inorganic phosphorus by the method of Bartlett (40). Bile cholesterol as well as cholesterol content in the lithogenic diet, gallstones, and liver were determined by HPLC (2). Cholesterol saturation indexes (CSI) in gallbladder and hepatic biles were calculated from the critical tables (41). Hydrophobicity indexes of hepatic bile were calculated according to Heuman's method (42).

Statistical methods

All data are expressed as means ± SD. Statistically significant differences among groups of mice were assessed by Student's *t*-test or Chi-square tests. Analyses were performed with a *SuperANOVA* software (Abacus Concepts, Berkeley, CA). Statistical significance was defined as a two-tailed probability of less than 0.05.

RESULTS

Influence of aging on gallstone prevalence and lipid compositions of gallbladder biles

As expected for healthy rodents, mice of each strain displayed progressive weight gain from 28–35 g to 32–37 g from 8- to 50-weeks-of-age. AKR mice were 3–18% heavier than C57L mice, and males were 3–22% heavier than females in both strains. All these strains of mice ate similar amounts of the lithogenic diet (4.0–4.3 g/day), and displayed average dietary cholesterol intake of ~40–43 mg/day[2]. The fecal outputs (1.2–1.4 g/day dry weight) and the weight (1.5–1.7 g) and length (39–42 cm) of the small intestine were very similar in all mice of different ages. All animals appeared healthy with the exception of 10 male (50%) and 13 female (65%) aged AKR mice. These developed evidence of listlessness, lethargy, anorexia, sleepiness, and weight loss, dying eventually between 45 and 57 weeks. At autopsy, no special causes of death relevant to the hepato-biliary system were found. These aged AKR mice were not used for the present study.

Figure 1 demonstrates that at week 8 of feeding the lithogenic diet, gallstone prevalence rates in C57L mice of both genders were significantly ($P < 0.001$) greater compared with AKR mice of the same ages. Of note is that in young adult groups, gallstone prevalence rates were 2-fold higher ($P < 0.01$) in C57L males than females, but no gender differences were found in AKR mice. Moreover, gallstone prevalence rates were slightly but not significantly higher with increasing age in C57L mice of both genders; however this did not occur in AKR mice. With an increase in age, the gender difference in gallstone prevalence for C57L mice became smaller. The sterols extracted from the stones of each mouse strain contained only cholesterol, which constituted >99% of stone weight. In general, the size of gallstones was markedly greater in C57L mice than in AKR mice of various ages (**Fig. 2**). On average, the size of gallstones ranged from 0.28 to 0.38 mm in AKR mice with no gender or age differences. In contrast, the size of gallstones was 0.54 ± 0.28 mm in C57L males and was significantly ($P < 0.01$) bigger than females (0.31 ± 0.13 mm). Also, gallstone diameters in aged C57L mice of both genders became bigger compared with their young adults, but did not reach significant differences. Furthermore, **Fig. 3** shows that the number of gallstones in C57L mice of both genders fell between 7 and 9, whereas in AKR mice the corresponding values were 4 and 6. Of note is that gallbladders of AKR mice exhibited much lower gallstone numbers than in C57L mice, and 75–90% of AKR mice were gallstone free.

Table 1 lists biliary lipid compositions of pooled gallbladder biles. After the 8-week lithogenic diet feeding, all mice had supersaturated gallbladder biles (CSI = 1.30–1.77) in three different age groups of AKR and C57L mice. In general, the CSI values became higher gradually with increasing age. However, total lipid concentrations of gallbladder biles decreased (Table 1) and showed a reverse correlation with the age, suggesting that gallbladder concentration function is diminished with advancing age. Moreover, the pooled gallbladder biles of C57L mice displayed markedly higher CSI values but lower total lipid concentrations compared with AKR mice.

Effect of aging on gallbladder volumes and emptying

Figure 4 shows that after the 8 weeks of lithogenic diet feeding, gallbladder sizes in young adult C57L mice were

[2] The HPLC analyses (see Materials and Methods) verified that the lithogenic diet contains ~1% cholesterol. Because individual mouse ingests ~4.0–4.3 g of food daily, average dietary cholesterol intake from the lithogenic diet is ~40–43 mg/day.

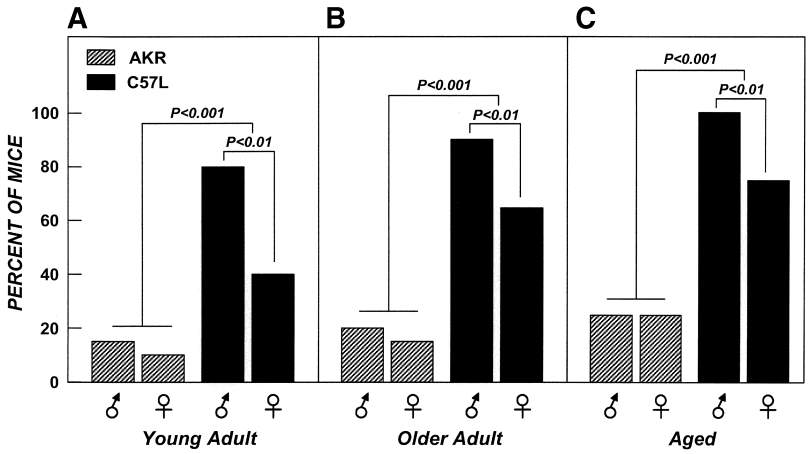

Fig. 1. Gallstone-susceptible C57L mice and resistant AKR mice of both genders (n = 20 per group) were fed 8 weeks with the lithogenic diet containing 1% cholesterol, 0.5% cholic acid, and 15% butter fat starting at (young adult) 8-, (older adult) 36-, and 50-weeks-of-age (aged). After the 8-week feeding, gallstone prevalence rates were significantly ($P < 0.01$) greater in male C57L mice than in females, and was independent of gender in AKR mice. Also, prevalence was significantly ($P < 0.001$) higher in C57L mice than in AKR mice, and increased slightly (P = NS) with age in both strains.

25 ± 8 μl (males) and 21 ± 6 μl (females), being significantly ($P < 0.001$) greater than those in AKR mice (10 ± 4 μl in males and 9 ± 4 μl in females). Furthermore, the size of gallbladders in C57L mice of both genders was significantly ($P < 0.001$) increased with advancing age from 21–25 μl in the young adult, to 57–50 μl in the older adult, to 90–95 μl in the aged, but this did not occur in AKR mice. **Figure 5** shows that after the 8-week feeding, the CCK-8 treatment induced significantly ($P < 0.05$) gallbladder emptying in three different age groups of male and female AKR mice. However, CCK-8-mediated gallbladder emptying was diminished in C57L mice of both genders and of various ages. Furthermore, the PBS administration did not influence gallbladder size in the two strains of mice.

Aging-related effects on bile flow and biliary lipid secretion rates

We observed changes in bile flow rates (**Table 2**) for the first hour following interruption of the enterohepatic circulation in different age groups of AKR and C57L mice fed the lithogenic diet for 8 weeks. Bile flow rates in C57L mice (range 97–117 μl/min/kg) of both genders were 2-fold higher than those in AKR mice (range 41–49 μl/

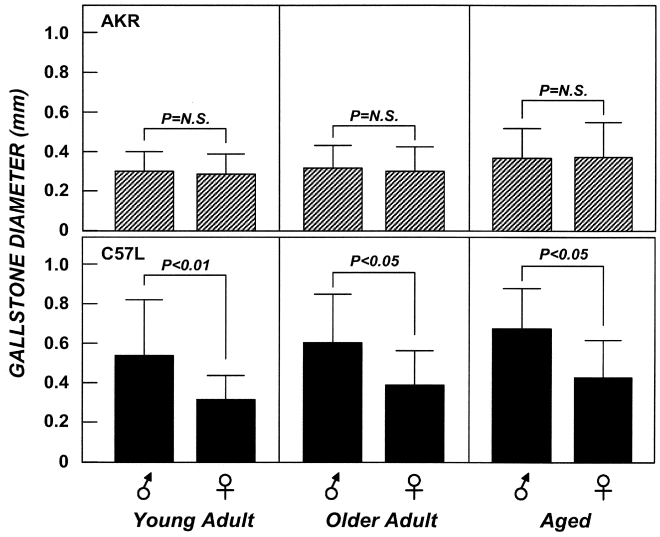

Fig. 2. Gallstone characteristics at 8 weeks of lithogenic diet feeding (n = 20 per group). Gallstone size was identical between AKR males and females, but was significantly ($P < 0.05$) bigger in male C57L mice than females.

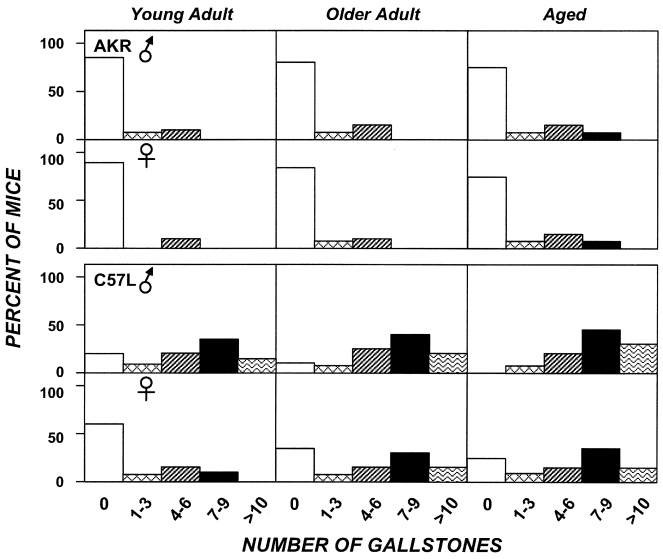

Fig. 3. After the 8-week lithogenic diet feeding, gallbladders of male and female C57L mice of various ages (n = 20 per group) showed significantly higher gallstone numbers than in AKR mice. Most C57L mice contained 7–9 gallstones, whereas AKR mice contained 4–6 stones.

TABLE 1. Biliary lipid compositions of pooled gallbladder biles in inbred mice of different ages[a]

	Males						Females					
Age[b]	Ch	PL	BS	PL/(PL+BS)	[TL]	CSI[c]	Ch	PL	BS	PL/(PL+BS)	[TL]	CSI
		mole%			*g/dl*			*mole%*			*g/dl*	
AKR Mice												
8-weeks	9.40	19.71	70.88	0.22	14.08	1.31	9.24	19.26	71.50	0.21	12.53	1.33
36-weeks	9.53	19.33	71.14	0.21	13.20	1.35	9.24	20.40	70.36	0.23	11.61	1.30
50-weeks	10.17	20.29	69.55	0.23	11.71	1.43	10.82	19.42	69.75	0.22	10.65	1.56
C57L mice												
8-weeks	9.58	19.11	71.32	0.21	8.87	1.48	9.21	18.44	72.35	0.20	8.64	1.47
36-weeks	9.89	18.21	71.90	0.20	7.38	1.63	10.38	18.28	71.34	0.20	7.95	1.67
50-weeks	12.41	18.95	68.64	0.22	7.35	1.95	10.96	18.64	70.39	0.21	7.27	1.77

Ch, cholesterol; PL, phospholipid; BS, bile salt; [TL], total lipid concentration; CSI, cholesterol saturation index.

[a] Values were determined from the pooled gallbladder biles (n = 20 per group).

[b] Based upon measurements of physical growth and sexual maturation, the different age groups of mice were considered to be in the following stages of maturation: 8-weeks, young adult; 36-weeks, older adult; and 50-weeks, aged (30, 31).

[c] These values represent the mean CSIs of the pooled gallbladder biles calculated from the critical tables (41).

min/kg) of the same gender and of the same ages, and increased significantly ($P < 0.05$) with age in both strains. However, we did not find any gender differences in bile flow for C57L and AKR mice of various ages.

Table 2 also shows biliary cholesterol, phospholipid, and bile salt secretion rates during the first hour of biliary washout in mice fed the lithogenic diet for 8 weeks. In general, outputs of all three biliary lipids were significantly ($P < 0.05$) higher in C57L mice compared with AKR mice of the same ages. The most marked changes were in cholesterol and phospholipid outputs in C57L mice (cholesterol = 38.6–38.8 μmol/h/kg and phospholipid = 68.2–68.9 μmol/h/kg) of both genders at 50-weeks-of-age, being significantly ($P < 0.05$) higher compared to mice at 8-weeks-of-age (cholesterol = 29.4–29.7 μmol/h/kg and phospholipid = 52.6–53.5 μmol/h/kg). However, biliary bile salt outputs decreased significantly ($P < 0.05$) from 192–211 μmol/h/kg at 8-weeks-of-age to 145–157 μmol/h/kg at 50-weeks-of-age. Biliary cholesterol and phospholipid outputs increased and bile salt outputs decreased slightly in AKR mice of both genders with age; however, they did not reach statistically significant differences. The pool sizes were significantly ($P < 0.05$) larger in C57L mice (2.6–3.6 μmol) than in AKR mice (2.1–2.2 μmol), and there were no gender differences in the circulating bile salt pool sizes (Table 2) in both mouse strains. Also, the circulating bile salt pool sizes decreased significantly ($P < 0.05$) with age in C57L mice of both genders, but this did not occur in AKR mice. Compared with AKR mice, the total bile salt pool sizes, i.e., the circulating bile salt pool size plus the bile salt pool in the gallbladder, were 1.6–2.7-fold higher in C57L mice, and it is likely due to the enlarged gallbladder size with increasing age in the latter mouse strain. Moreover, because aged C57L mice displayed larger gallbladder size than their young adults, there were age differences in the total bile salt pool sizes in C57L mice, with the aged (11.0–11.4 μmol) being ~2-fold higher than in the young adult (5.8–6.4 μmol). In contrast, aged AKR mice (4.1–4.3 μmol) displayed essentially similar total bile salt pool sizes compared with their young adults (3.6–4.0 μmol).

After the 8-week lithogenic diet feeding, young adult C57L mice of both genders displayed that the predominant bile salts were taurocholate (57–64%), taurodeoxycholate (12–23%), and taurochenodeoxycholate (11–14%). Present in smaller concentrations were tauro-β-muricholate (6–8%), tauroursodeoxycholate (1–2%) and tauro-ω-muricholate (1%). Furthermore, young adult AKR mice of both genders showed that the major bile salts were taurocholate (60–67%) and tauro-β-muricholate

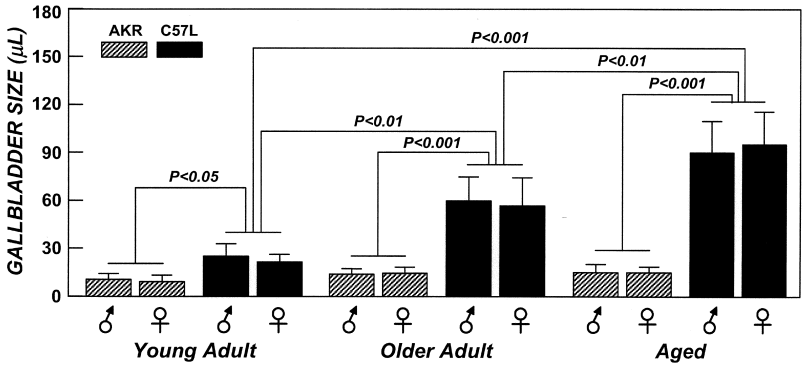

Fig. 4. Gallbladder sizes were significantly larger in C57L mice of both genders and of various ages (n = 20 per group) than in AKR mice. Aged C57L mice displayed ~3-fold increases ($P < 0.001$) in gallbladder volumes compared with their young adults, whereas AKR mice showed unchanged gallbladder sizes with age.

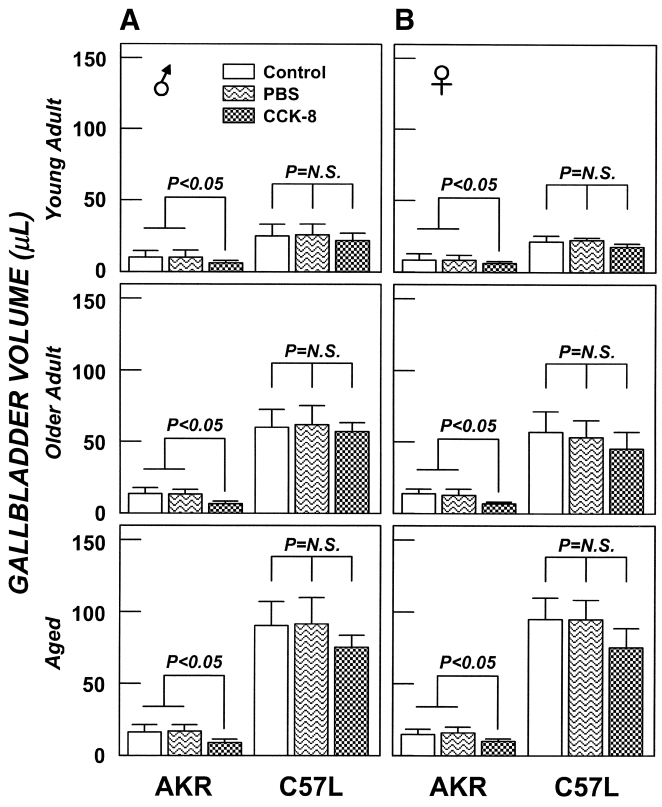

Fig. 5. The sulfated cholecystokinin octapeptide (CCK-8) treatment induced significant ($P < 0.05$) gallbladder emptying in male (A) and female (B) AKR mice of various ages (young adult, top panels; older adult, middle panels; and aged, bottom panels), but did not decrease gallbladder volumes in three age groups of male and female C57L mice. The phosphate buffered saline (PBS) solution administration did not influence gallbladder sizes in both strains. Of note is that in the control groups, C57L mice displayed significantly larger gallbladders than AKR mice.

(22–23%), and the minor bile salts were tauroursodeoxycholate (2–5%), taurodeoxycholate (1–5%), tauro-ω-muricholate (3–4%), and taurochenodeoxycholate (1–2%). So, in young adult groups, biliary hydrophobicity indexes in C57L mice (+0.06 to +0.11) were significantly ($P < 0.001$) higher than those in AKR mice (−0.20 to −0.21). With increasing age, the distributions of bile salt compositions and the hydrophobicity indexes remained unchanged in the two mouse strains.

Influence of aging on intestinal cholesterol absorption and small intestinal transit time

Figure 6 shows that over ages, percent cholesterol absorption was significantly ($P < 0.001$) higher in C57L mice of both genders (range 37–56%) compared to AKR mice (24–37%). Furthermore, intestinal cholesterol absorption increased significantly with age in the two strains of mice, especially in C57L mice. There were gender differences in cholesterol absorption in AKR mice of various ages, with 21–28% higher in females than in males ($P < 0.05$), but this did not occur in C57L mice.

We also found that the distribution of radioactivity in the small intestine was essentially similar between young adult groups of C57L mice and AKR mice of both genders, with peaks between segments 8 and 15. The geometric center of the distribution profile of radioisotope in the small intestine was 11.0 ± 1.0 in AKR mice and 11.1 ± 0.8 in C57L mice. Furthermore, small intestinal transit times in older adult (geometric center = 11.7 ± 1.2) and aged (geometric center = 12.1 ± 1.1) C57L mice were slightly slower compared to their young adults, but did not reach statistically significant differences. In contrast, in AKR mice of various ages, there were no age differences in small intestinal transit times, ranging from 11.0 (young adult) to 11.3 (old adult) to 11.5 (aged) for the geometric centers.

Aging-related changes in hepatic cholesterol and bile salt synthesis

Table 3 shows that under the chow conditions, the activities of hepatic HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis, were similar between AKR and C57L mice of both genders at 8-weeks-of-age. Increasing age reduced significantly ($P < 0.01$) the activity of HMG-CoA reductase in male and female AKR mice, however, such alterations did not occur in C57L mice. Furthermore, the 8-week feeding of the lithogenic diet decreased significantly ($P < 0.01$) this enzymatic activity in AKR mice of various ages (14 to 25 pmol/min/mg), but C57L mice still displayed high HMG-CoA reductase activities (54 to 62 pmol/min/mg).

Table 3 also demonstrates that in a chow-fed state, the activities of hepatic cholesterol 7α-hydroxylase were similar in the two strains of mice, and decreased significantly ($P < 0.05$) with advancing age. Moreover, there were no significant gender differences in the activities of cholesterol 7α-hydroxylase in AKR and C57L mice of various ages. At week-8 of feeding the lithogenic diet, although the activities of cholesterol 7α-hydroxylase were decreased significantly ($P < 0.05$) in both strains of mice, this enzymatic activity was significantly ($P < 0.05$) greater in three age groups of AKR mice (4 to 10 pmol/min/mg) compared with C57L mice (2 to 4 pmol/min/mg).

DISCUSSION

With improved life expectancy, the population older than the age of 65 years, which was 35 million in the United States in 2000, is expected to reach 78 million by 2050. More impressive is that cholesterol gallstone disease is more common in people over the age of 50, and 50% of females at age 70–75 and of males at age 80–85 has gallstones (7, 8). Why does the prevalence of gallstones increase with aging? To answer this question, we investigated gallstone prevalence, hepatic and biliary lipid metabolism, gallbladder contraction function, and intestinal cholesterol absorption in three different age groups (i.e., 8-, 36-, and 50-week-old) of gallstone-susceptible C57L mice and resistant AKR mice of both genders fed the lithogenic diet for 8 weeks. The most important find-

TABLE 2. Bile flow rates, biliary lipid outputs, and bile salt pool sizes in inbred mice of different ages[a]

Parameter	Males			Females		
	8-Weeks[b]	36-Weeks	50-Weeks	8-Weeks	36-Weeks	50-Weeks
AKR mice						
Bile flow (μl/min/kg body weight)	41.4 ± 4.6	44.0 ± 7.3	49.3 ± 4.8[e]	41.1 ± 5.5	42.3 ± 6.6	48.4 ± 7.7
Biliary output						
Cholesterol (μmol/h/kg body weight)	8.3 ± 2.1	8.5 ± 2.6	10.4 ± 2.5	8.4 ± 1.3	9.2 ± 1.1	8.8 ± 0.9
Phospholipid (μmol/h/kg body weight)	33.1 ± 8.7	35.2 ± 9.4	37.9 ± 7.3	34.8 ± 7.8	35.8 ± 5.8	39.7 ± 5.8
Bile salt (μmol/h/kg body weight)	95.8 ± 18.3	92.3 ± 21.3	91.1 ± 12.6	96.8 ± 19.4	92.4 ± 17.1	89.7 ± 9.9
Circulating bile salt pool size (μmol)	2.2 ± 0.4	2.2 ± 0.5	2.1 ± 0.2	2.2 ± 0.5	2.1 ± 0.4	2.1 ± 0.3
Total bile salt pool size (μmol)[c]	4	4.4	4.3	3.6	4.1	4.1
C57L mice[d]						
Bile flow (μl/min/kg body weight)	90.7 ± 4.7	92.7 ± 6.4	114.8 ± 12.3[f]	92.6 ± 7.7	94.7 ± 10.6	116.7 ± 19.3[g]
Biliary output						
Cholesterol (μmol/h/kg body weight)	29.4 ± 6.9	31.0 ± 5.1	38.6 ± 4.4[g,h]	29.7 ± 6.0	33.0 ± 8.0	38.8 ± 5.4[g]
Phospholipid (μmol/h/kg body weight)	53.5 ± 7.4	59.1 ± 17.5	68.2 ± 11.0[g]	52.6 ± 9.4	58.7 ± 6.8	68.9 ± 10.7[g]
Bile salt (μmol/h/kg body weight)	211.2 ± 22.7	171.2 ± 52.9	157.0 ± 28.6[g]	192.7 ± 14.1	166.4 ± 39.1	145.4 ± 28.9[g]
Circulating bile salt pool size (μmol)	3.6 ± 0.6	2.9 ± 0.5	2.8 ± 0.3[g]	3.4 ± 0.5	2.8 ± 0.4	2.6 ± 0.4[g]
Total bile salt pool size (μmol)	6.4	8.6	11.0	5.8	8.6	11.4

[a] Values represent means ± SD of five animals per group at 8-weeks of feeding the lithogenic diet containing 1% cholesterol, 0.5% cholic acid and 15% butter fat.

[b] Based upon measurements of physical growth and sexual maturation, the different age groups of mice were considered to be in the following stages of maturation: 8-weeks, young adult; 36-weeks, older adult; and 50-weeks, aged (30, 31).

[c] See Results for details.

[d] $P < 0.05$ compared with AKR mice of the same gender and of the same ages for bile flow, biliary lipid outputs, and circulating bile salt pool sizes.

[e] $P < 0.05$ compared with AKR mice of 8-weeks-old.

[f] $P < 0.01$ compared with C57L mice of 8-weeks-old.

[g] $P < 0.05$ compared with C57L mice of 8-weeks-old.

[h] $P < 0.05$ compared with C57L mice of 36-weeks-old.

ings in the present study were that aging per se increased biliary cholesterol hypersecretion and intestinal cholesterol absorption, and diminished hepatic bile salt synthesis and secretion, and induced an enlarged gallbladder with impaired contractility function, all of which increase susceptibility to cholesterol gallstone formation in C57L mice.

Our study showed that after the 8-week lithogenic diet feeding, secretion rates of biliary cholesterol and phospholipid were increased but secretion rates and circulating pool sizes of biliary bile salts (Table 2) were diminished with the progress of age in C57L mice. This supports the concept (2, 43) that in gallstone-susceptible mice, *Lith* genes induce both relative and absolute biliary cholesterol hypersecretion disproportionately to phospholipid and bile salt outputs thereby inducing lithogenic bile formation. Furthermore, gallstone prevalence rates favored males 2 to 1 (Fig. 1) in C57L mice of various ages, and were slightly but not significantly higher in aged C57L mice of both genders compared to their young adults. However, there were no age or gender differences in AKR mice. As C57L mice carry more than two *Lith* genes (29), it is highly probable that *Longevity* (aging) genes (44–47) influence *Lith* (gallstone) genes to augment cholelithogenesis. Our findings were in agreement with the results of Einarsson et al. (12) in Scandinavian subjects of both genders, but were different from those of Valdivieso et al. (13) in Chilean women. The latter research group observed that aging does not modify hepatic synthesis, biliary pool size, or turnover of bile salts, although there is an increment in the lithogenic index of gallbladder biles with increasing age. Nevertheless, although there may be ethnic differences in these human studies (12, 13), the available evidence (12, 13) indicated that increased biliary cholesterol secretion with age is a major risk factor in the pathogenesis of cholesterol gallstone formation.

Additionally, aged C57L mice showed enlarged gallbladders with poor emptying (Figs. 4 and 5), which may contribute to cholesterol gallstone formation by causing gallbladder stasis (43). We also found that exogenous CCK can induce significant gallbladder emptying in AKR mice of various ages. However, C57L mice showed enlarged gallbladders (Fig. 4) and a significant decrease in gallbladder emptying rate (Fig. 5) in response to CCK, suggesting that gallstone-susceptible mice displayed an impaired gallbladder contractility function, particularly in the aged mice. Our findings were consistent with the results of Poston et al. (26) in the study on different age groups of guinea pigs. Furthermore, it was found that duodenal concentration of CCK increases with age in guinea pigs (25) and in humans (48, 49). Therefore, our results support the hypothesis that aging may reduce expression of CCK-A receptor in the gallbladder (25), sensitivity of the gallbladder to CCK (24, 26), or both. In a pilot study with a small number of mice, we found that expression of CCK-A receptor mRNA in the gallbladder of aged C57L mice was significantly lower compared with aged AKR mice (unpublished observations). Of note is that gallbladder hypomotility may also result from absorption of cholesterol from supersaturated bile by the gallbladder epithelial cell (50, 51). Moreover, cholesterol molecules

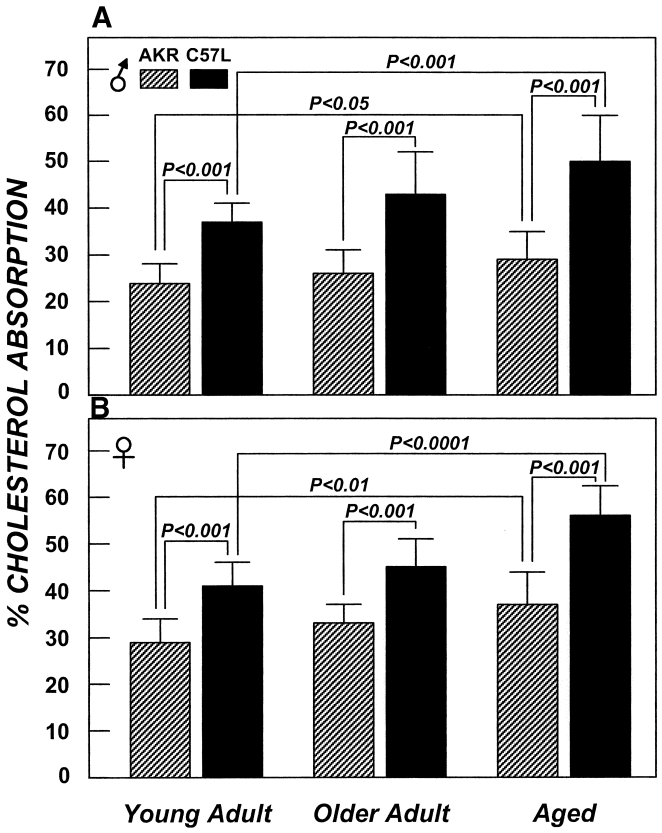

Fig. 6. Percent cholesterol absorption in chow-fed male (A) and female (bottom panel) AKR and C57L mice of various ages (n = 10 per group) was determined by the plasma dual isotope ratio method (32, 34). Three age groups of male and female C57L mice displayed significantly higher ($P < 0.001$) percent cholesterol absorption compared with AKR mice. Percentage of cholesterol absorption increased significantly ($P < 0.001$) in aged C57L mice of both genders and ($P < 0.05$) in aged AKR mice compared with their young adults, respectively.

from saturated bile could pass across the epithelium and may be incorporated into the sarcolemma of the gallbladder smooth muscle cells (52), which inhibits signal transduction when CCK bonds to CCK-A receptors in the gallbladder (53–55) and impairs gallbladder contractile function. In addition, the prolonged presence of gallbladder sludge and stones may be responsible for enlarged gallbladders with poor emptying in C57L mice. These alternations may be an important etiological factor for increased gallstone prevalence rate as well as gallstone size and number (Figs. 1–3), especially in aged C57L mice.

It has been reported (56) that high efficiency of intestinal cholesterol absorption correlates significantly and positively with high prevalence of cholesterol gallstone formation in mice. As aged C57L mice displayed significantly higher cholesterol absorption efficiency compared with their young adults, it is highly probable that dietary cholesterol could contribute more cholesterol to biliary secretion through chylomicron remnant pathway (57) in the aged than in the young adult. Hollander and Morgan (27) also showed that the absorption rate of cholesterol by the small intestine increased linearly with aging, with 2-fold increase in intestinal cholesterol absorption in aged rats (42-months-old) compared with young adult rats (1-month-old). Furthermore, Traber and Ostwald (58) found considerably slower intestinal transit time in aged guinea pigs, which may be a factor for increasing cholesterol absorption. In the present study, we observed that although there are slightly slower small intestinal transit rates in aged C57L mice compared with their young adults, it is likely that such alterations may not contribute to increased intestinal cholesterol absorption in the aged mice.

Since the liver has a critical role in cholesterol metabolism, studies on the influence of aging on activities of he-

TABLE 3. Activities of hepatic lipid regulatory enzymes in inbred mice of different ages[a]

	AKR				C57L			
	Males		Females		Males		Females	
Age[b]	Hmgr	C7h	Hmgr	C7h	Hmgr	C7h	Hmgr	C7h
Chow diet								
8-weeks	61 ± 12	18 ± 3	55 ± 11	16 ± 3	55 ± 10	14 ± 4	52 ± 10	12 ± 4
36-weeks	36 ± 10[c]	12 ± 3[d]	33 ± 8[c]	10 ± 4[d]	50 ± 11	8 ± 3[d]	45 ± 8	9 ± 3
50-weeks	29 ± 9[c]	9 ± 2[d]	27 ± 8[c]	7 ± 2[c]	47 ± 10	6 ± 2[c]	42 ± 9	7 ± 3[d]
Lithogenic diet								
8-weeks	25 ± 5[e]	10 ± 2[f]	27 ± 7[f]	8 ± 3[f]	55 ± 5[h]	3 ± 1[e,i]	42 ± 8[k]	4 ± 2[f,k]
36-weeks	19 ± 5[f]	8 ± 2[g]	20 ± 6[g]	6 ± 3	45 ± 7[d,h]	2 ± 1[f,i]	39 ± 9[j]	3 ± 1[f]
50-weeks	13 ± 4[c,f]	6 ± 2[g]	15 ± 5[d,g]	4 ± 2[d]	43 ± 10[d,i]	2 ± 1[f,j]	37 ± 10[j]	2 ± 1[f]

Hmgr, hydroxy-3-methylglutaryl coenzyme A reductase; C7h, cholesterol 7α-hydroxylase.

[a] Values were expressed in pmol/min/mg microsomal protein (n = 5 per group).

[b] Based upon measurements of physical growth and sexual maturation, the different age groups of mice were considered to be in the following stages of maturation: 8-weeks, young adult; 36-weeks, older adult; and 50-weeks, aged (30, 31).

[c] $P < 0.01$, compared with mice at 8-weeks-of-age on the same diet.

[d] $P < 0.05$, compared with mice at 8-weeks-of-age on the same diet.

[e] $P < 0.001$, compared with chow-fed mice of the same age and of the same gender.

[f] $P < 0.01$, compared with chow-fed mice of the same age and of the same gender.

[g] $P < 0.05$, compared with chow-fed mice of the same age and of the same gender.

[h] $P < 0.0001$, compared with AKR mice of the same age and of the same gender on the same diet.

[i] $P < 0.001$, compared with AKR mice of the same age and of the same gender on the same diet.

[j] $P < 0.01$, compared with AKR mice of the same age and of the same gender on the same diet.

[k] $P < 0.05$, compared with AKR mice of the same age and of the same gender on the same diet.

patic lipid regulatory enzymes in humans (12–15) and in animals (16–20) have been intensively performed. However, there is no consensus how aging per se influences these enzymatic activities during cholesterol gallstone formation. We found that in a chow-fed state, the activity of HMG-CoA reductase decreased by 47–49% from 8- to 50-weeks-of-age in AKR mice of both genders, but remained unchanged with increasing age in C57L mice. Further, the high dietary cholesterol reduced the activities of hepatic HMG-CoA reductase in AKR mice. The HMG-CoA reductase activity remained unchanged in C57L mice in the face of advancing age, increased intestinal cholesterol absorption, and the lithogenic diet feeding, suggesting that high cholesterol synthesis in the liver could contribute, in part, to biliary cholesterol hypersecretion. One explanation would be a mutation or influenced by *Lith* genes (32, 35). Furthermore, the activity of hepatic cholesterol 7α-hydroxylase gradually decreased from 8- to 50-weeks-of-age by 44–50% in male and female AKR mice and by 43–58% in C57L mice. Our data are consistent with the results of Choi et al. (59), who reported a significant decrease in the activity of cholesterol 7α-hydroxylase in the rat from 5- to 32-weeks-of-age. Under the lithogenic diet conditions, three age groups of C57L mice displayed significantly ($P < 0.05$) lower activities of cholesterol 7α-hydroxylase compared with AKR mice of the same ages. Therefore, it suggests that markedly lower cholesterol 7α-hydroxylase activities, which reflects a decreased capacity to metabolize cholesterol to bile salts, may contribute to the age-related decrease in biliary bile salt secretion in C57L mice (Table 2).

In summary, our study shows that gallstone prevalence rates increase with age as a consequence of enhanced biliary secretion and intestinal absorption of cholesterol, declined hepatic synthesis and secretion of bile salts, and decreased gallbladder contractility in gallstone-susceptible C57L mice. We conclude that aging per se is an independent risk factor for cholesterol gallstone formation. Furthermore, cholesterol gallstones are a multifactorial disease, which involves the interaction of multiple *Lith* genes as inferred from the mouse studies (2, 3, 29), with environmental conditions such as aging and the lithogenic diet. This work therefore provides a basic framework for further investigating molecular mechanisms of how *Longevity* (aging) genes influence *Lith* (gallstone) genes to enhance cholelithogenesis.

The author is greatly indebted to Dr. Martin C. Carey (Gastroenterology Division, Brigham and Women's Hospital, Boston, MA) for his support and encouragement of this study as well as for helpful discussions. The author also thanks Helen H-F. Wang and Drs. Akira Ohashi, Victor M. Morales, and Li-Ping Duan (Beth Israel Deaconess Medical Center) for excellent technical assistance. 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 a grant from the Ellison Medical Foundation and a Grant DK54012 from the National Institutes of Health.

REFERENCES

1. Paigen, B., and M. C. Carey. 2002. Gallstones. *In* The Genetic Basis of Common Diseases. 2nd edition. R. A. King, J. I. Rotter, and A. G. Motulsky, editors. Oxford University Press, New York, NY. 298–335.
2. Wang, D. Q-H., F. Lammert, B. Paigen, and M. C. Carey. 1999. Phenotypic characterization of *Lith* genes that determine susceptibility to cholesterol cholelithiasis in inbred mice: pathophysiology of biliary lipid secretion. *J. Lipid Res.* **40:** 2066–2079.
3. Wang, D. Q-H., B. Paigen, and M. C. Carey. 1997. Phenotypic characterization of *Lith* genes that determine susceptibility to cholesterol cholelithiasis in inbred mice: physical-chemistry of gallbladder bile. *J. Lipid Res.* **38:** 1395–1411.
4. Lieber, M. M. 1952. The incidence of gallstones and their correlation with other diseases. *Ann. Surg.* **135:** 394–405.
5. Torvik, A., and B. Hoivik. 1950. Gallstones in an autopsy series. Incidence, complications and correlations with carcinoma of the gallbladder. *Acta Chir. Scand.* **120:** 168–174.
6. Friedman, G. D., W. B. Kannel, and T. R. Dawber. 1966. The epidemiology of gallbladder disease: observations in the Framingham study. *J. Chronic Dis.* **19:** 273–281.
7. Sama, C., A. M. M. Labate, F. Taroni, and L. Barbara. 1990. Epidemiology and natural history of gallstone disease. *Semin. Liver Dis.* **10:** 149–158.
8. Diehl, A. K. 1991. Epidemiology and natural history of gallstone disease. *Gastroenterol. Clin. North Am.* **20:** 1–19.
9. Rosenthal, R. A., and D. K. Andersen. 1993. Surgery in the elderly: observations on the pathophysiology and treatment of cholelithiasis. *Exp. Gerontol.* **28:** 459–472.
10. Kahng, K. U., and J. J. Roslyn. 1994. Surgical issues for the elderly patient with hepatobiliary disease. *Surg. Clin. North Am.* **74:** 345–373.
11. Ido, K., T. Suzuki, K. Kimura, Y. Taniguchi, C. Kawamoto, N. Isoda, N. Nagamine, T. Ioka, and M. Kumagai. 1995. Laparoscopic cholecystectomy in the elderly: analysis of pre-operative risk factors and postoperative complications. *J. Gastroenterol. Hepatol.* **10:** 517–522.
12. Einarsson, K., K. Nilsell, B. Leijd, and B. Angelin. 1985. Influence of age on secretion of cholesterol and synthesis of bile acids by the liver. *N. Engl. J. Med.* **313:** 277–282.
13. Valdivieso, V., R. Palma, R. Wünkhaus, C. Antezana, C. Severín, and A. Contreras. 1978. Effect of aging on biliary lipid composition and bile acid metabolism in normal Chilean women. *Gastroenterology.* **74:** 871–874.
14. Van der Werf, S. D., A. W. Huijbregts, H. L. Lamers, G. P. van Berge Henegouwen, and J. H. vanTongeren. 1981. Age dependent differences in human bile acid metabolism and 7α-dehydroxylation. *Eur. J. Clin. Invest.* **11:** 425-431.
15. Bertolotti, M., N. Abate, S. Bertolotti, P. Loria, M. Concari, R. Messora, F. Carubbi, A. Pinetti, and N. Carulli. 1993. Effect of aging on cholesterol 7α-hydroxylation in humans. *J. Lipid Res.* **34:** 1001–1007.
16. Uchida, K., Y. Nomura, M. Kadowaki, H. Takase, K. Takano, and N. Takeuchi. 1978. Age-related changes in cholesterol and bile acid metabolism in rats. *J. Lipid Res.* **19:** 544–552.
17. Stahlberg, D., B. Angelin, and K. Einarsson. 1991. Age-related changes in the metabolism of cholesterol in rat liver microsomes. *Lipids.* **26:** 349–352.
18. Uchida, K., T. Satoh, T. Chikai, H. Takase, Y. Nomura, H. Nakao, and N. Takeuchi. 1996. Influence of cholesterol feeding on bile acid metabolism in young and aged germ-free rats. *Jpn. J. Pharmacol.* **71:** 113–118.
19. Spady, D. K., S. D. Turley, and J. M. Dietschy. 1983. Dissociation of hepatic cholesterol synthesis from hepatic low-density lipoprotein uptake and biliary cholesterol saturation in female and male hamsters of different ages. *Biochim. Biophys. Acta.* **753:** 381–392.
20. Yamamoto, M., and Y. Yamamura. 1971. Changes of cholesterol metabolism in the ageing rat. *Atherosclerosis.* **13:** 365–374.
21. Fujiyama, M., G. Kajiyama, A. Maruhashi, T. Mizuno, K. Yamada, T. Kawamoto, S. Kubota, H. Sasaki, K. Oyamada, S. Nakao, and A. Miyoshi. 1979. Change in lipid composition of bile with age in normal subjects and patients with gallstones. *Hiroshima J. Med. Sci.* **28:** 23–29.
22. Scobey, M. W., M. S. Wolfe, and L. L. Rudel. 1992. Age- and dietary fat-related effects on biliary lipids and cholesterol gallstone formation in African green monkeys. *J. Nutr.* **122:** 917–923.
23. Palasciano, G., G. Serio, P. Portincasa, V. Palmieri, M. Fanelli, A.

Velardi, B. Calo'Gabrieli, and V. Vinciguerra. 1992. Gallbladder volume in adults, and relationship to age, sex, body mass index, and gallstones: a sonographic population study. *Am. J. Gastroenterol.* **87:** 493-497.
24. Keane, P., D. Colwell, H. P. Baer, A. S. Clanachan, and G. W. Scott. 1986. Effects of age, gender and female sex hormones upon contractility of the human gallbladder in vitro. *Surg. Gynecol. Obstet.* **163:** 555–560.
25. Poston, G. J., P. Singh, D. G. Maclellan, C. Z. Yao, T. Uchida, C. M. Townsend, Jr., and J. C. Thompson. 1988. Age-related changes in gallbladder contractility and gallbladder cholecystokinin receptor population in the guinea pig. *Mech. Ageing Dev.* **46:** 225–236.
26. Poston, G. J., E. J. Draviam, C. Z. Yao, C. M. Townsend, Jr., and J. C. Thompson. 1990. Effect of age and sensitivity to cholecystokinin on gallstone formation in the guinea pig. *Gastroenterology.* **98:** 993–999.
27. Hollander, D., and D. Morgan. 1979. Increase in cholesterol intestinal absorption with aging in the rat. *Exp. Gerontol.* **14:** 201–204.
28. Pilotto, A., M. Franceschi, G. Del Favero, R. Fabrello, F. Di Mario, and G. Valerio. 1995. The effect of aging on oro-cecal transit time in normal subjects and patients with gallstone disease. *Aging.* **7:** 234–237.
29. Khanuja, B., Y-C. Cheah, M. Hunt, P. M. Nishina, D. Q-H. Wang, H. W. Chen, J. T. Billheimer, M. C. Carey, and B. Paigen. 1995. *Lith1*, a major gene affecting cholesterol gallstone formation among inbred strains of mice. *Proc. Natl. Acad. Sci. USA.* **92:** 7729–7733.
30. Zurcher, C., M. J. van Zwieten, H. A. Solleveld, and C. F. Hollander. 1982. Aging research. *In* The Mouse in Biomedical Research. Vol. 4. 1st edition. H. L. Foster, D. Small Jr., and J. G. Fox, editors. Academic Press, New York, NY. 11–35.
31. Fox, R. R., and B. A. Witham. 1997. Handbook on Genetically Standardized JAX Mice. 5th edition. The Jackson Laboratory, Bar Harbor, ME. 9–49.
32. Wang, D. Q-H., F. Lammert, D. E. Cohen, B. Paigen, and M. C. Carey. 1999. Cholic acid aids absorption, biliary secretion, and phase transitions of cholesterol in murine cholelithogenesis. *Am. J. Physiol.* **276:** G751–G750.
33. Wank, S. A. 1995. Cholecystokinin receptors. *Am. J. Physiol.* **269:** G628–G646.
34. Wang, D. Q-H., B. Paigen, and M. C. Carey. 2001. Genetic factors at the enterocyte level account for variations in intestinal cholesterol absorption efficiency among inbred strains of mice. *J. Lipid Res.* **42:** 1820–1830.
35. Lammert, F., D. Q-H. Wang, B. Paigen, and M. C. Carey. 1999. Phenotypic characterization of *Lith* genes that determine susceptibility to cholesterol cholelithiasis in inbred mice: Integrated activities of hepatic lipid regulatory enzymes. *J. Lipid Res.* **40:** 2080–2090.
36. Doerner, K. C., E. C. Gurley, Z. R. Vlahcevic, and P. B. Hylemon. 1995. Regulation of cholesterol 7α-hydroxylase expression by sterols in primary rat hepatocyte cultures. *J. Lipid Res.* **36:** 1168–1177.
37. Hylemon, P. B., E. J. Studer, W. M. Pandak, D. M. Heuman, Z. R. Vlahcevic, and J. Y. L. Chiang. 1989. Simultaneous measurement of cholesterol 7α-hydroxylase activity by reverse-phase high-performance liquid chromatography using both endogenous and exogenous [4-^{14}C]cholesterol as substrate. *Anal. Biochem.* **182:** 212–216.
38. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–254.
39. Rossi, S. S., J. L. Converse, and A. F. Hofmann. 1987. High pressure liquid chromatographic analysis of conjugated bile acids in human bile: Simultaneous resolution of sulfated and unsulfated lithocholyl amidates and the common conjugated bile acids. *J. Lipid Res.* **28:** 589–595.
40. Bartlett, G. R. 1959. Phosphorous assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
41. Carey, M. C. 1978. Critical tables for calculating the cholesterol saturation of native bile. *J. Lipid Res.* **19:** 945–955.
42. Heuman, D. M. 1989. Quantitative estimation of the hydrophilic-hydrophobic balance of mixed bile salt solutions. *J. Lipid Res.* **30:** 719–730.
43. Carey, M. C., and J. T. LaMont. 1992. Cholesterol gallstone formation. 1. Physical-chemistry of bile and biliary lipid secretion. *Prog. Liver Dis.* **10:** 139–163.
44. Vijg, J., and N. van Orsouw. 2002. Searching for genetic determinants of human aging and longevity: opportunities and challenges. *Mech. Ageing Dev.* **123:** 195–205.
45. Puca, A. A., M. J. Daly, S. J. Brewster, T. C. Matise, J. Barrett, M. Shea-Drinkwater, S. Kang, E. Joyce, J. Nicoli, E. Benson, L. M. Kunkel, and T. Perls. 2001. A genome-wide scan for linkage to human exceptional longevity identifies a locus on chromosome 4. *Proc. Natl. Acad. Sci. USA.* **98:** 10505–10508.
46. Perls, T., M. Shea-Drinkwater, J. Bowen-Flynn, S. B. Ridge, S. Kang, E. Joyce, M. Daly, S. J. Brewster, L. Kunkel, and A. A. Puca. 2000. Exceptional familial clustering for extreme longevity in humans. *J. Am. Geriatr. Soc.* **48:** 1483–1485.
47. Flurkey, K., J. Papaconstantinou, R. A. Miller, and D. E. Harrison. 2001. Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. *Proc. Natl. Acad. Sci. USA.* **98:** 6736–6741.
48. Khalil, T., J. P. Walker, I. Wiener, C. J. Fagan, C. M. Townsend, Jr., G. H. Greeley, Jr., and J. C. Thompson. 1985. Effect of aging on gallbladder contraction and release of cholecystokinin-33 in humans. *Surgery.* **98:** 423–429.
49. Upp, J. R., Jr., W. H. Nealon, P. Singh, C. J. Fagan, A. S. Jonas, G. H. Greeley, Jr., and J. C. Thompson. 1987. Correlation of cholecystokinin receptors with gallbladder contractility in patients with gallstones. *Ann. Surg.* **205:** 641–648.
50. Neiderhiser, D. H., C. K. Harmon, and H. P. Roth. 1976. Absorption of cholesterol by the gallbladder. *J. Lipid Res.* **17:** 117–124.
51. Corradini, S. G., W. Elisei, L. Giovannelli, C. Ripani, P. Della Guardia, A. Corsi, A. Cantafora, L. Capocaccia, V. Ziparo, V. Stipa, P. Chirletti, R. Caronna, D. Lomanto, and A. F. Attili. 2000. Impaired human gallbladder lipid absorption in cholesterol gallstone disease and its effect on cholesterol solubility in bile. *Gastroenterology.* **118:** 912–920.
52. Chen, Q., J. Amaral, P. Biancani, and J. Behar. 1999. Excess membrane cholesterol alters human gallbladder muscle contractility and membrane fluidity. *Gastroenterology.* **116:** 678–685.
53. Yu, P., Q. Chen, K. M. Harnett, J. Amaral, P. Biancani, and J. Behar. 1995. Direct G protein activation reverses impaired CCK signaling in human gallbladders with cholesterol stones. *Am. J. Physiol.* **269:** G659–G665.
54. Xiao, Z. L., Q. Chen, J. Amaral, P. Biancani, R. T. Jensen, and J. Behar. 1999. CCK receptor dysfunction in muscle membranes from human gallbladders with cholesterol stones. *Am. J. Physiol.* **276:** G1401–G1407.
55. Yu, P., Q. Chen, P. Biancani, and J. Behar. 1996. Membrane cholesterol alters gallbladder muscle contractility in prairie dogs. *Am. J. Physiol.* **271:** G56–G61.
56. Wang, D. Q-H., and L-N. Zhang. 2000. Dietary cholesterol through chylomicron pathway enhances murine cholelithogenesis. *Gastroenterology.* **118:** A715.
57. Wang, D. Q-H., B. Paigen, and M. C. Carey. 1998. Genetic variations in cholesterol absorption efficiency are associated with cholesterol gallstone formation in inbred mice. *Hepatology.* **28:** 163A.
58. Traber, M. G., and R. Ostwald. 1978. Cholesterol absorption and steroid excretion in cholesterol-fed guinea pigs. *J. Lipid Res.* **19:** 448–456.
59. Choi, Y. S., T. Ide, and M. Sugano. 1987. Age-related changes in the regulation of cholesterol metabolism in rats. *Exp. Gerontol.* **22:** 339–349.