Effect of dietary cholesterol and taurocholate on cholesterol 7α-hydroxylase and hepatic LDL receptors in inbred mice

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Abstract Compared to BALB/c mice, inbred C57BL/6 mice are more susceptible to developing fatty streak atherosclerotic lesions when fed a cholesterol-rich diet containing taurocholate. We examined the metabolic basis for the taurocholate requirement. In contrast to widely accepted assumptions, taurocholate did not increase cholesterol absorption in either strain of mouse. However, in susceptible C57BL/6 mice, taurocholate was required to increase plasma concentrations of apoB. In both strains, the cholesterol-rich diet increased both the activity and mRNA for 7α-hydroxylase, a compensatory response to maintain cholesterol homeostasis. In both strains, adding taurocholate to the diet suppressed both the activity and mRNA for 7α-hydroxylase, thus blocking this important compensatory response. The cholesterol-rich diet (without taurocholate) significantly increased hepatic cholesterol content in both strains of mice, but repressed low density lipoprotein (LDL) receptor mRNA only in BALB/c mice (not in C57BL/6 mice). However, adding taurocholate to the cholesterol-rich diet did decrease LDL receptor mRNA in C57BL/6 mice. In C57BL/6, but not in BALB/c mice, there was a linear parallel relationship between 7α-hydroxylase mRNA and LDL receptor mRNA. These data show the existence of strain-specific differences in the effects of dietary cholesterol and taurocholate on 7α-hydroxylase and LDL receptor expression. The combined data suggest that genetic factors determine how the expression of hepatic LDL receptors responds to dietary cholesterol and taurocholate. **—Dueland, S., J. Drisko, L. Graf, D. Machleder, A. J. Lusis, and R. A. Davis.** Effect of dietary cholesterol and taurocholate on cholesterol 7α-hydroxylase and hepatic LDL receptors in inbred mice. *J. Lipid Res.* 1993. **34:** 923–931.

Supplementary key words apoB • atherosclerosis • BALB/c mice • c57BL/6 mice • mRNA • cholesterol absorption

Inbred strains of mice have been identified as being susceptible (C57BL/6) or resistant (BALB/c) to the development of early atherosclerotic "fatty streak" lesions when fed a diet containing fat, cholesterol, and taurocholate (1–3). In C57BL/6 mice, lesion formation is the result of increased apoB-containing lipoproteins (i.e., VLDL, IDL, and LDL) and decreases in HDL apoA-I (4). Without dietary taurocholate, the susceptible C57BL/6 mice do not develop early atherosclerosis. It has been assumed (but not proven) that taurocholate was required in order to increase the absorption of cholesterol (5).

Dietary taurocholate may potentiate the atherosclerotic diet by repressing 7α-hydroxylase, a liver specific cytochrome P 450 enzyme responsible for regulating bile acid synthesis (6). It is well established that dietary taurocholic acid dramatically decreases the activity (6, 7) and relative abundance of mRNA (8–11) for 7α-hydroxylase. Bile acid synthesis is the major pathway responsible for regulating whole body cholesterol homeostasis. In the rat, as much as 90% of the cholesterol is removed from the body in the form of biliary bile acids (12). The bile acid synthetic pathway is readily adaptable to changes in whole body cholesterol pools, providing a mechanism to maintain whole body cholesterol homeostasis (6–13). Feeding rats a cholesterol rich diet leads to a rapid increase in bile acid synthesis (6–8). As a result, rats are unusually resistant to dietary cholesterol-induced hypercholesterolemia.

The major goal of this study was to determine whether there is a difference in the response of inbred strains of mice to dietary cholesterol and taurocholate. Specifically, we examined whether there is a strain-related difference in the manner in which taurocholate affects dietary cholesterol absorption. We also examined possible strain-related differences in the effects of dietary cholesterol and taurocholate on the expression of 7α-hydroxylase and hepatic LDL receptors.

Abbreviations: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; GLC, gas-liquid chromatography.

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METHODS

Animals and diets

Female BALB/c and C57BL/6 mice, 10–12 weeks old, were obtained from Jackson Laboratory, Bar Harbor, ME. The mice, housed in a room with a reverse-light cycle (light from 7 PM to 7 AM), were fed for 3 weeks either: *1)* normal Purina breeder chow, *2)* chow supplemented with 20% olive oil, 2% cholesterol (high cholesterol diet) or, *3)* chow supplemented with 20% olive oil, 2% cholesterol, 0.5% taurocholate (high cholesterol + taurocholate diet). There was no difference in the amount of food consumed or body weight gain throughout the study. At 9 AM, mice were anesthetized with pentobarbital and killed by exsanguination.

Cholesterol 7α-hydroxylase activity in isolated mouse liver microsomes

Mouse liver microsomes were isolated as described (7). The assay involved incubating microsomes (0.5 mg protein) for 30 min at 37°C in the presence of an NADPH generating system, as described in detail (7). The incubation was terminated by the addition of chloroform–methanol 2:1 and 1 μg [7β-^{2}H]7α-hydroxycholesterol. The samples were reacted with trimethylchlorosilane and analyzed using a Hewlett-Packard gas chromatograph with a selective mass detector. The mass of ions 457 (deuterium) and 456 (endogenous) 7α-hydroxycholesterol formed was calculated from the ratio of mass ion 457/456.

Serum apoB determinations

Serum apoB was quantitated using a competitive solid phase ELISA assay. Mouse LDL (0.3 μg/μl) was added to plastic 96-well microtiter dishes in a solution of 0.1 M potassium carbonate, pH 9.6, and incubated overnight. The plates were washed 3 times with a solution of 0.1% BSA, 150 mM sodium chloride, and 50 mM sodium phosphate, and then blocked with 1% BSA, 100 mM sodium chloride, and 50 mM sodium phosphate for 2 h at room temperature. An aliquot of a solution containing the serum or lipoprotein fraction in a buffer containing 0.1% BSA, 150 mM sodium chloride, 50 mM sodium phosphate, 0.8% SDS, 0.8% sarcosyl, and 0.8% Triton X-100 was incubated with a polyclonal rabbit anti-serum (kindly provided by Dr. John Elovson). After an overnight incubation, the mixture was added to the 96-well plate (previously coated with antigen) and incubated overnight. The plate was washed and then incubated with a biotinylated goat anti-rabbit IgG and washed. Horseradish peroxidase conjugated with streptavidin was added, the plate was washed, and *o*-phenylenediamine dihydrochloride was added and the plate was incubated. The activity of horseradish peroxidase was determined from the absorbance at 450 nm using a Bio-Rad Microplate reader. The assay gave a 10% coefficient of variation and the amount of apoB was determined using delipidated apoB as the standard. The proportion of apoB-100/apoB-48 was determined by densitometric scanning of the Coomassie blue-stained SDS/PAGE gels of serum VLDL, IDL, and LDL.

Isolation of RNA

The livers of mice were placed on aluminum foil on ice, weighed, and then homogenized in guanidinium isothiocyanate using a tissue homogenizer. RNA was isolated after extraction with phenol–chloroform and precipitated with ethanol, as described (14).

Quantitation of mRNA for cholesterol 7α-hydroxylase, LDL receptor, and β-actin

Relative abundance of specific mRNAs was determined by slot-blot analysis using methods previously described in detail (14). RNA was denatured in a solution containing 50% deionized formamide, 6% formaldehyde, and 20 mM MOPS buffer, pH 7.0, and then applied to a nylon membrane (Zetaprobe-Bio-Rad). The RNA was cross-linked by UV light to the nylon membrane and baked for 30 min at 80°C. The blots were hybridized sequentially first with a ^{32}P-labeled 1300 bp EcoR1 fragment of a cDNA clone for rat 7α-hydroxylase (8), a 2100 bp fragment of rat LDL receptor (15), or 2000 bp fragment of β-actin (16). Blots were first probed with the 7α-hydroxylase cDNA, stripped in a low salt buffer containing 1 mM EDTA, 1 mM Tris-HCl (pH 8.0), and 0.1% Denhardt's reagent for 2 h at 75°C and then reprobed with the LDL receptor cDNA. The blot was then stripped again and reprobed with the β-actin probe. Hybridization was performed in a buffer containing 10% dextran sulfate, 5 × SSPE, 50% formamide, 5 × Denhardt's, 0.2% SDS, and 0.2 mg/ml salmon sperm DNA at 42°C. The blots were washed in 2 × SSC, 0.1% SDS at 55°C for 5 min and then in 0.1 × SSC, 0.1% SDS at 55°C for 5 min and subjected to autoradiography. The autoradiograms were scanned using a densitometer and the slope of the relationship between density of the band and amount of RNA applied was determined by linear least squares analysis. The correlation coefficient for each autoradiogram was >0.9. The relative abundance of the different mRNAs was determined by the ratio of the slope of the linear function obtained from the desired cDNA to the slope of the linear function obtained from β-actin. Each value represents the mean ± SEM of the indicated number of mRNA samples (each from a separate mouse).

Determination of cholesterol absorption

Cholesterol absorption was determined both by the "dual isotope plasma ratio" method and the "single isotopic meal feeding" method, as described in detail (17). Prior to the absorption studies the mice received the various nonradioactive diets for 1 week. In the dual isotope plasma ratio method, after an overnight fast, mice were

fed [4-^{14}C]cholesterol (3.6 μCi) added to the diet. [2,3-^{3}H]cholesterol (6.25 μCi dissolved in 200 μl saline containing 2.5% ethanol) was administered by intravenous injection. Plasma was obtained 48 h later.

In the single isotopic meal feeding method, mice were housed in individual wire-mesh cages and fed the indicated diets for 6 d. [2,3-^{3}H]cholesterol (50 μCi) and [4-^{14}C]β-sitosterol (100 μCi) were mixed with olive oil and added to the indicated powdered diet. After an overnight fast, the diets containing the radioisotopes were fed to the mice. After the entire radioactive diet was consumed by each mouse, the same diet (without radioactive isotopes) was made available and the mice were fed this diet ad libitum for the remainder of the study (6 days). Feces were collected throughout the experiment, extracted with chloroform–methanol 2:1, and the water phase was re-extracted with chloroform. The combined chloroform extracts were dried under N_2 and resuspended in 10 ml hexane, and an aliquot was transferred to counting vials, dried under vacuum, and scintillation fluid was added. The samples were counted in a scintillation counter with correction for quenching by an external standard.

Bile collection and composition

After an overnight fast, mice were anesthetized with pentobarbital. Gallbladder bile was obtained by aspiration using a syringe with a 25-gauge needle. The gallbladders were sliced open and the contents were rinsed into a microfuge tube. The presence or absence of sandy gallstones was noted. In three separate experiments the gallstones were washed with ice-cold phosphate-buffered saline, completely dried in a vacuum oven, weighed, and the content of cholesterol was determined by GLC, as described (7). The bile acid composition and concentration was determined by GLC, as described (18); cholesterol was quantitated by GLC, as described (18); and phospholipids in bile were extracted with chloroform–methanol 2:1 (v/v) and quantitated by an inorganic phosphate assay, as described (18). In a third study, mice were fed the diets for 6 weeks, after which the total contents of bile were rinsed into microfuge tubes using phosphate-buffered saline at 4°C. The tubes were centrifuged at maximum speed and the supernatant was drawn off by suction. The precipitate was washed 2 more times and then dissolved in hexane and the content of cholesterol was determined by GLC, as described (7).

Statistical analysis

All data are reported as the mean ± SEM. Differences between groups were compared by Wilcoxen non-parametric test. The significance of each linear correlation comparison was determined by the Pearson correlation test. Values of $P \leq 0.05$ were considered to be significant.

RESULTS

Taurocholate does not augment intestinal cholesterol absorption

We examined the possibility that taurocholate augments cholesterol absorption. Mice were fed the high fat, cholesterol-rich diet with and without 0.5% taurocholate and cholesterol absorption was measured by both the dual isotope plasma ratio method and the single meal feeding method. The dual ratio method showed that both C57BL/6 and BALB/c mice absorbed equal amounts of cholesterol from the diet lacking taurocholate: 32 ± 5% and 34 ± 6%, in C57BL/6 and BALB/c mice, respectively (**Table 1**). Adding taurocholate to the cholesterol-rich diet did not increase cholesterol absorption in either strain: 31 ± 3% and 24 ± 4%, in C57BL/6 and BALB/c mice, respectively. Similar results were obtained with the single meal feeding method (Table 1).

Bile composition

In addition to being more susceptible to atherosclerosis, C57BL/6 mice also show a greater propensity to develop gallstones when fed the diet containing cholesterol and taurocholate (19). We examined the bile composition in the two strains of mice. Mice were fed the chow diet or the chow diet containing cholesterol and sodium taurocholate for 6 weeks. In all 6 C57BL/6 mice examined, the gallbladder was distended and showed the presence of stones that resembled sand. In contrast, the sand-like stones were observed in only two of 6 BALB/c mice. In three separate experiments, the cholesterol content of the stones was analyzed after vacuum desiccation, gravimetric analysis, and solubilization in hexane. Unesterified cholesterol accounted for >95% of the material in the sand-like stones. To obtain a more quantitative analysis of the difference in precipitated cholesterol in the gallbladders of the mice, the gallbladder contents were

TABLE 1. Effect of taurocholate on cholesterol absorption

	Percent of Dose			
Method and Diet	C57BL/6	n	BALB/c	n
Dual isotope method				
Chow + cholesterol	32 ± 2	5	34 ± 3	5
Chow + cholesterol + taurocholate	31 ± 3	6	24 ± 4	6
Meal feeding method				
Chow + cholesterol	39 ± 5	5	31 ± 3	5
Chow + cholesterol + taurocholate	30 ± 5	3	25 ± 4	3

Mice were fed the indicated diet for 1 week, after which cholesterol absorption was measured using the indicated method, as described in detail (17). Each value represents the mean ± SEM for the indicated number of mice (n).

TABLE 2. Effect of atherogenic diet on bile and bile acid composition

Diet	n	Bile Composition[a]				Biliary Bile Acid Composition[b]			
		Bile Acid	Phospholipid	Cholesterol	Lithogenic Index	Deoxycholic	Chenodeoxycholic	Cholic	Muricholic
		μmol/ml				*% of total bile acids*			
Chow									
C57BL/6	5	204 ± 15	36 ± 2	5.0 ± 0.4	0.42 ± 0.03	6.3 ± 0.8	11.1 ± 0.9	75.7 ± 3.3	7.0 ± 4.0
BALB/c	3	177 ± 5	26 ± 2[c]	3 ± 0	0.30 ± 0.0	3.6 ± 0.3[c]	5.0 ± 0.7[c]	72.0 ± 4.2	19.4 ± 4.6[c]
Atherogenic									
C57BL/6	5	162 ± 27	35 ± 4	17 ± 2[d]	1.43 ± 0.8[d]	6.9 ± 1.0	1.6 ± 0.9	91.6 ± 0.5	ND
BALB/c	3	163 ± 6	26 ± 4[c]	13 ± 2[d]	1.30 ± 0.13[d]	12.2 ± 1.0[c]	ND	87.8 ± 1.0	ND

[a]Mice were fed the indicated diets for 6 weeks. Gallbladder bile was collected after an overnight fast and the composition of bile was determined as described (18). Each value represents the mean ± SEM for the indicated number of mice (n).
[b]Mice were fed the indicated diets for 6 weeks. Gallbladder bile was collected and the bile acid composition of the bile was determined by GLC. Values represent mean ± SEM for three individual determinations; ND, not detected.
[c]Significantly different from C57BL/6, $P < 0.05$.
[d]Significantly different from chow diet, $P < 0.05$.
[e]Statistically significant differences between C57BL/6 and BALB/c mice at $P < 0.05$.

rinsed into microfuge tubes, washed with ice-cold phosphate-buffered saline and the cholesterol content was quantitated by GLC. C57BL/6 mice had significantly ($P < 0.05$) more precipitated cholesterol: 4.6 ± 2.8 mg (C57BL/6 mice, n = 6) compared to 1.0 ± 0.8 (BALB/c mice, n = 6).

In both strains, the cholesterol-rich diet containing taurocholate increased the concentration of biliary cholesterol by 3-fold, while not affecting the concentration of either bile acids or phospholipids (**Table 2**). As a result of the increased biliary cholesterol, the lithogenic index increased 3-fold in both strains. The only difference between the two strains of mice in bile composition was a higher content of phospholipids in bile from C57BL/6 mice fed both the chow and the cholesterol-rich taurocholic acid diets (Table 2). Thus, while the C57BL/6 mice displayed cholesterol gallstones and the BALB/c mice did not, the lithogenic index was similar in both groups.

The relative amount of the primary bile acid β-muricholate was dramatically reduced in both strains fed the cholesterol-rich diet containing taurocholate, suggesting that bile acid synthesis was reduced. Feeding the cholesterol-rich diet containing taurocholate increased by 3-fold the relative amount of deoxycholic acid in the bile obtained from C57BL/6 mice (Table 2). It is interesting to note that bile obtained from C57BL/6 mice fed the chow diet, contained more deoxycholic acid and less β-muricholic acid compared to bile obtained from BALB/c mice, whereas on the cholesterol-rich, taurocholate-containing diet the relative amount of deoxycholic acid was significantly greater in the bile of BALB/c mice compared to the bile obtained from C57BL/6 mice (Table 2).

TABLE 3. Apolipoprotein B concentrations

Strain	Chow Diet	Cholesterol-rich + Taurocholate Diet	Cholesterol-rich − Taurocholate Diet
	mg/dl		
BALB/c	5.8 ± 1	5.2 ± 0.4	7.8 ± 1.9
C57BL/6	3.4 ± 0.5	4.3 ± 0.5	8.6 ± 1.0[a]

Mice were fed the indicated diets for 3 weeks, after which plasma apoB levels were determined by ELISA. Values represent mean ± SEM of three individual mice.
[a]Significant difference from chow diet ($P < 0.05$).

Taurocholate is required to increase plasma apoB levels

We examined the effect of the high fat, cholesterol diet with and without taurocholate on plasma concentrations of apoB. In both groups of mice, the cholesterol-rich diet (without taurocholate) did not significantly affect plasma concentrations of apoB (**Table 3**). However, when taurocholate was added to this diet, the plasma concentration of apoB tended to increase in both strains of mice, although the increase was significant only in C57BL/6 mice (Table 3). The increase in apoB was due solely to an increase in apoB-48 (in both strains on both diets apoB-100 contributed less than 10% to the total pool of apoB). These results are in agreement with those published previously (4). Thus, taurocholate was required to increase plasma apoB levels.

Response of 7α-hydroxylase to dietary cholesterol and taurocholate

We examined the effect of dietary cholesterol with and without taurocholate on the activity and mRNA abundance of 7α-hydroxylase. C57BL/6 mice fed the chow diet expressed a significantly higher 7α-hydroxylase activity than did BALB/c mice (**Fig. 1**). When fed the high fat, cholesterol-rich diet (without taurocholate) the activity of 7α-hydroxylase increased significantly in both strains. Adding taurocholate to this diet dramatically decreased

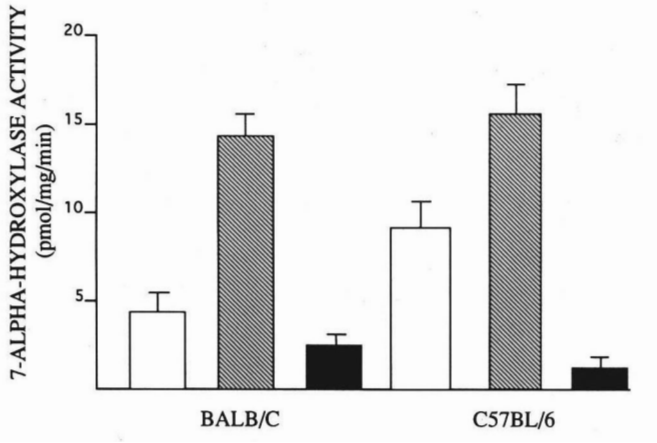

Fig. 1. Effects of diets on 7α-hydroxylase activity. BALB/c and C57BL/6 mice were fed diets containing chow (open bars), chow plus 20% olive oil and 2% cholesterol (hatched bars), and chow plus 20% olive oil, 2% cholesterol, and 0.5% cholic acid (solid bars) for 3 weeks in a reverse light cycle room. Three h after the initiation of the dark cycle, microsomes were prepared from livers and the amount of 7α-hydroxycholesterol formed was determined by GLC–mass spectrometry. Each point represents the mean ± SEM of six individual mice in each group. In all groups, the activity of 7α-hydroxylase was significantly higher in mice fed chow plus 20% olive oil and 2% cholesterol than in those fed chow ($P < 0.01$). Adding taurocholate to this diet also significantly decreased the activity of 7α-hydroxylase ($P < 0.01$). On the taurocholate diet, the activity of 7α-hydroxylase was significantly less in C57BL/6 mice than in BALB/c mice ($P < 0.05$).

the activity of 7α-hydroxylase in both strains. In C57BL/6 mice fed the taurocholate-containing diet, the activity of 7α-hydroxylase was significantly less (i.e., only 37% of the activity expressed in BALB/c mice, $P < 0.05$).

Dietary-induced changes in 7α-hydroxylase activity were accompanied by parallel changes in mRNA levels (**Fig. 2**). C57BL/6 mice fed the chow diet expressed higher mRNA levels compared to BALB/c mice. When fed the cholesterol-rich diet (without taurocholate) the relative abundance of 7α-hydroxylase mRNA was increased in both strains. In both strains, adding taurocholate to this diet dramatically decreased 7α-hydroxylase mRNA levels. C57BL/6 mice fed the taurocholic acid-containing diet expressed significantly ($P < 0.01$) less 7α-hydroxylase mRNA compared to BALB/c mice (Fig. 2).

Effect of diets on hepatic LDL receptor expression

Feeding BALB/c mice a high fat, cholesterol-rich diet (without taurocholate) decreased LDL receptor mRNA by 50% (**Fig. 3**). Surprisingly, feeding C57BL/6 mice the cholesterol-rich diet (without taurocholate) did not reduce the relative abundance of LDL receptor mRNA levels. However, adding taurocholate to the cholesterol-rich diet caused a dramatic 3-fold decrease in hepatic LDL receptor mRNA levels in C57BL/6 mice (Fig. 3). There was no significant difference in the relative abundance of LDL receptor mRNA between the two strains of mice on the cholesterol-rich, taurocholic acid-containing diet.

The cholesterol-rich diet was sufficient to maximally increase hepatic cholesterol concentrations (**Table 4**). Adding taurocholate to this diet did not cause further increases in hepatic cholesterol concentrations. Therefore, the inability of the cholesterol-rich diet (without taurocholate) to repress LDL receptor mRNA in C57BL/6 mice was not due to an inability to cause hepatic cholesterol accumulation.

Relationship between expression of 7α-hydroxylase and the LDL receptor

In C57BL/6 mice, LDL receptor mRNA varied in parallel with 7α-hydroxylase mRNA (**Fig. 4A**, $r = 0.74$, $P < 0.001$). In contrast, no such relationship was observed in BALB/c mice (Fig. 4B). These data clearly show a difference between the two strains of mice in the metabolic relationship between 7α-hydroxylase and LDL receptor mRNA.

DISCUSSION

Inbred C57BL/6 mice showing a reproducible susceptibility to dietary-induced atherosclerosis have provided a valuable model to define how genetic differences lead to changes in cholesterol and lipoproteins associated with atherogenesis (1–3, 20). One particular advantage to this model is uncovering genetic loci and their resulting

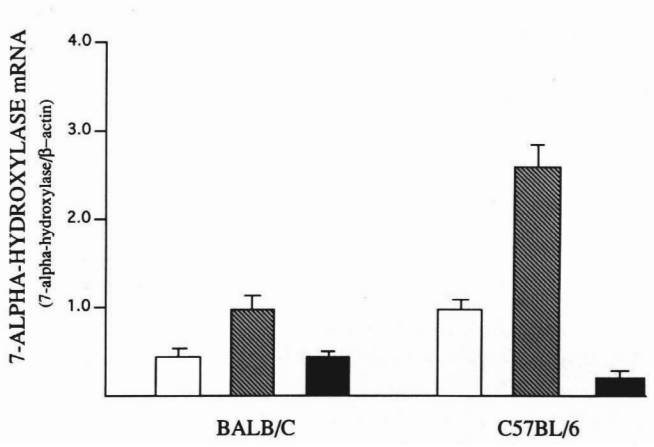

Fig. 2. Effects of diets on 7α-hydroxylase mRNA. BALB/c and C57BL/6 mice were fed diets containing chow (open bars), chow plus 20% olive oil and 2% cholesterol (hatched bars), and chow plus 20% olive oil, 2% cholesterol, and 0.5% cholic acid (solid bars) for 3 weeks in a reverse light cycle room. Three h after the initiation of the dark cycle, total RNA was obtained from livers. The relative amount of 7α-hydroxycholesterol mRNA was quantitated relative to β-actin using slot-blot analysis. Each point represents the mean ± SEM of six individual mice in each group. In all groups, the relative abundance of 7α-hydroxylase mRNA was significantly higher in mice fed chow plus 20% olive oil and 2% cholesterol than in those fed chow ($P < 0.01$). Adding taurocholate to this diet also significantly decreased the relative abundance of 7α-hydroxylase mRNA ($P < 0.01$). On the taurocholate diet, the relative abundance of 7α-hydroxylase mRNA was significantly less in C57BL/6 mice than in BALB/c mice ($P < 0.05$).

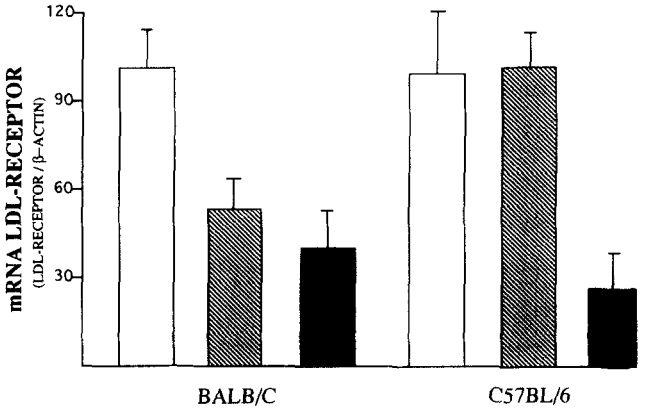

Fig. 3. Effects of diets on LDL receptor mRNA. BALB/c and C57BL/6 mice were fed diets containing chow (open bars), chow plus 20% olive oil and 2% cholesterol (hatched bars), and chow plus 20% olive oil, 2% cholesterol, and 0.5% cholic acid (solid bars) for 3 weeks in a reverse light cycle room. Three h after the initiation of the dark cycle, total RNA was obtained from livers. The relative amount of LDL receptor mRNA was quantitated relative to β-actin using slot-blot analysis. Each point represents the mean ± SEM of six individual mice in each group. Compared to the chow diet, BALB/c mice fed the cholesterol-rich diet without taurocholate had significantly less LDL receptor mRNA. In contrast, in C57BL/6 mice, the same cholesterol-rich diet (without taurocholate) did not significantly change the relative abundance of LDL receptor mRNA. In C57BL/6, but not in BALB/c mice, adding taurocholate to the cholesterol-rich diet significantly decreased the relative abundance of LDL receptor mRNA, ($P < 0.05$).

phenotypes that act on the atherogenic process. One gene locus (*Ath-1*) that appears to be responsible for the susceptibility of C57BL/6 mice to dietary-induced atherosclerosis is located on chromosome 1 and controls HDL levels (3). Complementing C57BL/6 mice with the human apoA-I trans-gene blocks both the dietary-induced decrease in plasma HDL and the susceptibility to atherosclerosis, providing compelling evidence that low HDL apoA-I is responsible for the dietary-induced atherosclerosis (21). Additional studies show that in addition to the *Ath-1* locus, several as yet unidentified genes contribute to the susceptibility of C57BL/6 mice to atherosclerosis (22).

The major goal of this research was to examine potential differences in the responses of inbred mice that are either susceptible or resistant to dietary-induced atherosclerosis to the effects of dietary cholesterol and taurocholic acid. The results show that the two strains of mice displayed unexpected differences in their response to both dietary cholesterol and taurocholate.

Contrary to expectations, we found that inclusion of taurocholate in the cholesterol-enriched diet did not augment intestinal cholesterol absorption in either strain (Table 1). These studies are the first to establish that the taurocholate requirement for the development of atherosclerosis in mice is not based on its augmentation of cholesterol absorption. In another study in mice fed a chow diet (without cholesterol), taurocholate also did not increase cholesterol absorption (23).

While taurocholic acid did not augment dietary cholesterol absorption, it completely blocked the ability of both strains to induce 7α-hydroxylase in response to increased dietary cholesterol. Similar to the response observed in rats (7–11), both strains of mice were able to respond to the cholesterol-rich diet by increasing the activity and mRNA of 7α-hydroxylase (Figs. 1 and 2). However, when taurocholate was added to the cholesterol-rich diet, both the activity and mRNA of 7α-hydroxylase of both strains of mice were significantly reduced (Figs. 1 and 2). The combined data show that, in mice, the repressive effect of taurocholic acid overcomes the inductive effect of cholesterol on the activity and mRNA for 7α-hydroxylase. This response in mice is in contradistinction to that observed in rats which show an induction of 7α-hydroxylase when they consume a diet containing cholesterol and taurocholic acid (9–11). Apparently, while both mice and rats show similar induction of 7α-hydroxylase in response to dietary cholesterol, mice are more sensitive to taurocholate repression of 7α-hydroxylase than are rats. This difference may explain why rats are much more resistant to dietary-induced atherogenesis than are mice.

Our studies show remarkable strain-specific differences in the regulation of the LDL receptor (Fig. 3). The cholesterol-rich diet (without taurocholate) significantly

TABLE 4. Hepatic cholesterol concentrations

Diet	Free Cholesterol				Cholesteryl Ester			
	BALB/c	n	C57BL/6	n	BALB/c	n	C57BL/6	n
	μg/mg				*μg/mg*			
Chow	1.80 ± 0.05	4	1.62 ± 0.01	5	0.6 ± 0.1	4	0.4 ± 0.1	5
Cholesterol-rich	2.60 ± 0.04	5	2.76 ± 0.05	4	10.0 ± 0.6	4	28 ± 5[a]	4
Cholesterol-rich + taurocholate	2.4 ± 0.1	3	3.1 ± 0.1	5	14.7 ± 0.7	3	13 ± 1	5

Mice were fed the indicated diets for 3 weeks, after which livers were obtained and analyzed for cholesterol and cholesteryl ester content by GLC. Each value represents the mean ± SEM of indicated number (n) of animals in each group.

[a]Denotes significant difference between BALB/c and C57BL/6 mice at $P < 0.05$.

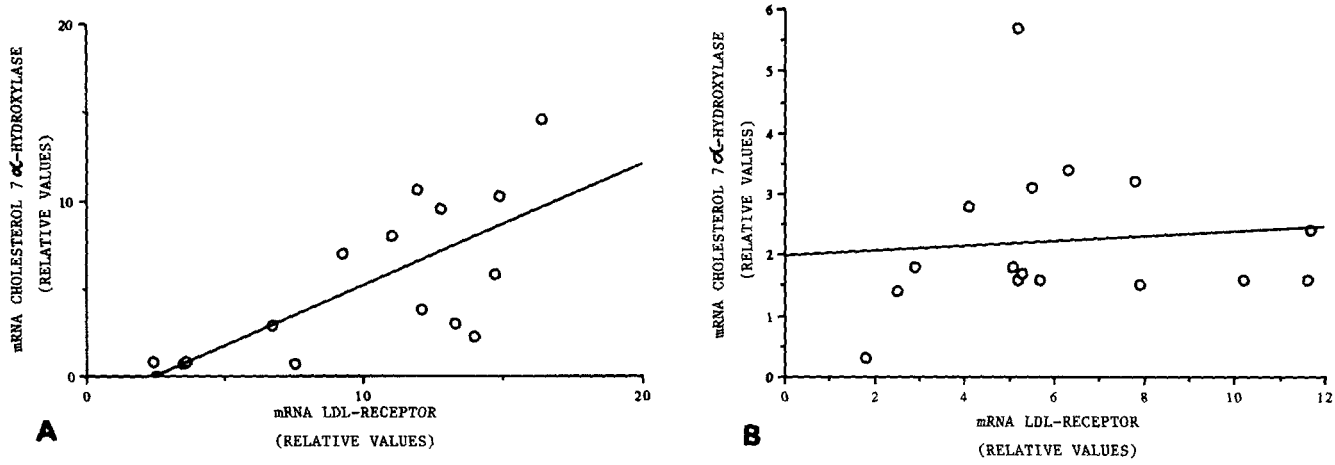

Fig. 4. Relationship between 7α-hydroxylase and LDL-receptor mRNA. The relative abundance of 7α-hydroxylase and LDL receptor mRNA, determined as described in Figs. 2 and 3, were analyzed by linear least squares analysis. For each mouse fed one of the three diets, the relative abundance of 7α-hydroxylase mRNA was plotted as the ordinate and the relative abundance of the LDL receptor mRNA was plotted as the abscissa. The correlation coefficient obtained from the C57BL/6 mice data (A) is $r = 0.74$ and was determined to be significant, $P < 0.001$. In contrast, the correlation coefficient obtained from the BALB/c mice data (B) was $r = 0.10$, not a significant relationship.

repressed LDL receptor mRNA in BALB/c mice, but not in C57BL/6 mice (Fig. 3). However, adding taurocholate to the cholesterol-rich diet did decrease LDL receptor mRNA in C57BL/6 mice. Because the cholesterol-rich diet (without taurocholate) significantly increased hepatic cholesterol concentrations in both strains, the inability of the cholesterol-rich diet (without cholic acid) to repress LDL receptor mRNA in C57BL/6 cannot be explained by differences in hepatic cholesterol concentrations. Increased expression of hepatic LDL receptors is associated with decreased atherosclerosis (24). Therefore, the resistance of C57BL/6 mice to dietary cholesterol repression of the LDL receptor is unlikely to contribute to this strain's susceptibility to atherosclerosis.

Of particular interest were the differences between the two strains of mice in the relationship between the LDL receptor mRNA and 7α-hydroxylase mRNA. In C57BL/6 mice fed the three different diets, there was a linear parallel relationship between 7α-hydroxylase mRNA and LDL receptor mRNA. But in BALB/c mice this relationship was not evident (Fig. 4). The finding that in C57BL/6 mice 7α-hydroxylase mRNA varies in parallel with LDL receptor mRNA (Fig. 4A) suggests that either both mRNAs are regulated by a common factor or that the expression of one gene somehow influences the expression of the other. Expression of the LDL receptor shows a precise transcriptional regulation (25–27). In nonhepatic cells, when cellular cholesterol is increased, the transcription of the LDL receptor gene is decreased, providing a mechanism to regulate intracellular cholesterol homeostasis (28). Additional studies show that a hydroxylated derivative of cholesterol (i.e., an oxysterol) is required for cholesterol to repress LDL receptor expression (29). In contrast to nonhepatic cells, expression of the LDL receptor in liver cells shows a resistance to repression by cellular cholesterol (30–32). Recent experiments in which 7α-hydroxylase was expressed in nonhepatic cells showed that functional expression of 7α-hydroxylase resulted in a resistance to repression by cellular cholesterol of the LDL receptor gene, reminiscent of the hepatocyte (33). Moreover, the mechanism responsible for the indirect effect of 7α-hydroxylase on expression of the LDL receptor appears to involve metabolism (i.e., inactivation) of oxysterol repressors (33). These results are consistent with the ability of the liver in vivo to convert the oxysterols 25-hydroxycholesterol (34) and 26-hydroxycholesterol (35) to bile acids. While in C57BL/6 mice the relationship between 7α-hydroxylase and the LDL receptor is consistent with the proposal that 7α-hydroxylase indirectly regulates the LDL receptor, BALB/c mice clearly behave differently. It is unlikely that the existence of this relationship in C57BL/6 mice and its absence in BALB/c mice can explain the strain-specific differences in susceptibility to dietary-induced atherosclerosis. In this study we found that apoB-48 accounted for essentially all of the apoB that accumulates in the mice fed the cholesterol-rich taurocholate diet (Table 3). These data agree with those of a previous report showing that in both strains of mice the cholesterol-rich taurocholate-containing diet only increased apoB-48 levels and did not significantly affect apoE levels, suggesting that the hypercholesterolemia is not due to a defect in the removal of either chylomicron remnants or LDL (4).

A parallel relationship in the expression of 7α-hydroxylase and the LDL receptor has been observed in several studies. Feeding bile acid binding resins, which in most studies increases 7α-hydroxylase activity (6), to rabbits increases the clearance of LDL presumably by LDL re-

ceptor (36). In contrast, feeding dogs taurocholate, which in most studies decreases 7α-hydroxylase activity (6), decreases the expression of hepatic LDL receptors (37). Perhaps one of the most compelling experiments linking 7α-hydroxylase to the functional expression of hepatic LDL receptors was performed in hamsters (38). Hamsters fed a diet containing chenodeoxycholic acid, which decreased bile acid synthesis, presumably by inhibiting the activity of 7α-hydroxylase, reduced the clearance of plasma LDL by hepatic LDL receptors (38). However, hamsters fed ursodeoxycholic acid, which does not inhibit bile acid synthesis, did not exhibit decreased removal of LDL by hepatic LDL receptors.

Our studies do not delineate the metabolic differences that can account for the strain-specific susceptibility to dietary-induced atherosclerosis. The data suggest that differences in cholesterol absorption, expression of hepatic LDL receptors and 7α-hydroxylase, and plasma concentrations of apoB cannot account for the differences between the two strains in their susceptibility to dietary cholesterol-induced atherosclerosis. Moreover, the results of this study support the conclusion that the strain-specific difference in plasma HDL levels is the primary difference responsible for the strain-specific susceptibility to atherosclerosis.

The 3-fold increase in biliary cholesterol in both groups of mice fed the taurocholic acid-containing diet compared to the chow diet (Table 2) indicates that biliary excretion of cholesterol can contribute to the maintenance of cholesterol homeostasis in both strains of mice. In agreement with the results reported previously (19), we observed that the taurocholate-containing diet caused an enlargement of the gallbladder and cholesterol gallstone formation in the C57BL/6 mice, but not in the BALB/c mice. As the concentration of deoxycholic acid was actually greater in the bile of BALB/c mice (Table 2), the formation of cholesterol gallstones in C57BL/6 mice cannot be ascribed to this lithogenic bile acid. These combined data suggest that a factor in bile other than the lipids used to calculate the lithogenic index is responsible for the differences between the two strains of mice in their susceptibility to cholesterol gallstone formation. This mouse model may provide new insights into the mechanism of cholelithsiasis.

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REFERENCES

1. Lusis, A. J., B. A. Taylor, R. W. Wangenstein, and R. L. LeBoeuf. 1983. Genetic control of lipid transport in mice. II. Genes controlling structure of high density lipoproteins. *J. Biol. Chem.* **258:** 5071–5078.
2. LeBoeuf, R. C., D. L. Puppione, V. N. Schumaker, and A. J. Lusis. 1983. Genetic control of lipid transport in mice. I. Structural properties and polymorphisms of plasma lipoproteins. *J. Biol. Chem.* **258:** 5063–5070.
3. Paigen, B., D. Mitchell, K. Reue, A. Morrow, A. J. Lusis, and R. C. LeBoeuf. 1987. Ath-1, a gene determining atherosclerosis susceptibility and high density lipoprotein levels in mice. *Proc. Natl. Acad. Sci. USA.* **84:** 3763–3767.
4. Lusis, A. J., B. A. Taylor, D. Quon, S. Zollman, and R. C. LeBoeuf. 1987. Genetic factors controlling structure and expression of apolipoproteins B and E in mice. *J. Biol. Chem.* **262:** 7594–7604.
5. Thomas, W. A., and W. S. Hartroft. 1959. Myocardial infarction in rats fed diets containing high fat, cholesterol, thiouracil and sodium cholate. *Circulation.* **19:** 65–74.
6. Myant, N. B., and K. A. Mitropoulis. 1977. Cholesterol 7α-hydroxylase. *J. Lipid Res.* **18:** 135–153.
7. Straka, M. S., L. H. Junker, L. Zaccaro, D. I. Zogg, S. Dueland, G. T. Everson, and R. A. Davis. 1990. Substrate stimulation of 7α-hydroxylase, an enzyme located in the cholesterol-poor endoplasmic reticulum. *J. Biol. Chem.* **265:** 7145–7149.
8. Jelinek, D. F., S. Andersson, C. A. Slaughter, and D. W. Russell. 1990. Cloning and regulation of cholesterol 7α-hydroxylase, the rate-limiting enzyme in bile acid biosynthesis. *J. Biol. Chem.* **265:** 8190–8197.
9. Pandak, W. M., Y. C. Li, J. Y. Chiang, E. J. Studer, E. C. Gurley, D. M. Heuman, Z. R. Vlahcevic, and P. B. Hylemon. 1991. Regulation of cholesterol 7α-hydroxylase mRNA and transcriptional activity by taurocholate and cholesterol in the chronic biliary diverted rat. *J. Biol. Chem.* **266:** 3416–3421.
10. Shefer, S., L. B. Nguyen, G. Salen, G. C. Ness, G. S. Tint, A. K. Batta, S. Hauser, and I. Rani. 1991. Regulation of cholesterol 7α-hydroxylase by hepatic 7α-hydroxylated bile acid flux and newly synthesized cholesterol supply. *J. Biol. Chem.* **266:** 2693–2696.
11. Spady, D. K., and J. A. Cuthbert. 1992. Regulation of hepatic sterol metabolism in the rat. Parallel regulation of activity and mRNA for 7α-hydroxylase but not 3-hydroxy-3-methylglutaryl-coenzyme A reductase or low density lipoprotein receptor. *J. Biol. Chem.* **267:** 5584–5591.
12. Siperstein, M. D., M. E. Jayko, I. L. Charkoff, and W. E. Dauben. 1952. Nature of the metabolic products of ^{14}C-cholesterol excreted in bile and feces. *Proc. Soc. Exp. Biol. Med.* **81:** 720–724.
13. Davis, R. A., P. M. Hyde, J. C. Kuan, M. M. Malone, and S. J. Archambault. 1983. Bile acid secretion by cultured rat hepatocytes. Regulation by cholesterol availability. *J. Biol. Chem.* **258:** 3661–3667.
14. Leighton, J. K., J. Joyner, J. Zamarripa, M. Deines, and R. A. Davis. 1990. Fasting decreases apolipoprotein B mRNA editing and the secretion of small molecular weight apoB by rat hepatocytes: evidence that the total amount of apoB secreted is regulated post-transcriptionally. *J. Lipid Res.* **31:** 1663–1668.
15. Lee, L. Y., W. A. Mohler, B. L. Schafer, J. S. Freudenberger, C. N. Byrne, K. B. Eager, S. T. Mosley, J. K. Leighton, R. N. Thrift, R. A. Davis, and R. D. Tanaka.

1989. Nucleotide sequence of the rat low density lipoprotein receptor cDNA. *Nucleic Acids Res.* **17:** 1259–1260.
16. Gunning, P., P. Ponte, H. Okayama, J. Engel, H. Blau, and L. Kedes. 1983. Isolation and characterization of full-length cDNA clones for human α-, β- and γ-actin mRNAs: skeletal but cytoplasmic actins have an amino-terminal cysteine that is subsequently removed. *Mol. Cell Biol.* **3:** 787–795.
17. Zilversmit, D. B., and L. B. Hughes. 1974. Validation of a dual-isotope plasma method for measurement of cholesterol absorption in rats. *J. Lipid Res.* **15:** 465–473.
18. Dueland, S., J. Reichen, G. T. Everson, and R. A. Davis. 1991. Regulation of bile acid homeostasis in bile-obstructed rats. *Biochem. J.* **280:** 373–377.
19. Nishina, P. M., J. Verstuyft, and B. Paigen. 1990. Synthetic low and high fat diets for the study of atherosclerosis in the mouse. *J. Lipid Res.* **31:** 859–869.
20. Lusis, A. 1988. Genetic factors affecting blood lipoproteins: the candidate gene approach. *J. Lipid Res.* **29:** 397–430.
21. Ruben, E. M., R. M. Krauss, E. A. Spangler, J. G. Verstuyft, and S. M. Clift. 1991. Inhibition of early atherogenesis in transgenic mice by human apolipoprotein A-I. *Nature.* **353:** 265–267.
22. Paigen, B., M. N. Nesbitt, D. Mitchell, D. Albee, and R. C. LeBoeuf. 1989. Ath-2, a second gene determining atherosclerosis susceptibility and high density lipoprotein levels in mice. *Genetics.* **122:** 163–168.
23. Reynier, M. O., J. C. Montet, A. Gerolami, C. Marteau, C. Crotte, A. M. Montet, and S. Mathieu. 1981. Comparative effects of cholic, chenodeoxycholic, and ursodeoxycholic acids on micellar solubilization and intestinal absorption of cholesterol. *J. Lipid Res.* **22:** 467–473.
24. Brown, M. S., and J. L. Goldstein. 1984. How LDL receptors influence cholesterol and atherosclerosis. *Sci. Am.* **251:** 58–66.
25. Sudhof, T. C., J. L. Goldstein, M. S. Brown, and D. W. Russell. 1985. The LDL receptor gene: a mosaic of exons shared with different proteins. *Science.* **228:** 815–822.
26. Brown, M. S., and J. L. Goldstein. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science.* **232:** 34–47.
27. Dawson, P. A., S. L. Hofmann, D. R. van der Westhuyzen, M. S. Brown, and J. L. Goldstein. 1988. Sterol-dependent repression of low density lipoprotein receptor promotor mediated by 16-base pair sequence adjacent to binding site for transcription factor Sp1. *J. Biol. Chem.* **263:** 3372–3379.
28. Brown, M. S., and J. L. Goldstein. 1985. The LDL receptor and HMG-CoA regulate cholesterol homeostasis. *Curr. Top. Cell. Regul.* **26:** 3–15.
29. Takagi, K., J. G. Alvarez, M. F. Favata, J. M. Trzaskos, and J. F. Strauss. 1989. Control of low density lipoprotein receptor gene promoter activity. Ketoconazole inhibits serum lipoprotein but not oxysterol suppression of gene transcription. *J. Biol. Chem.* **264:** 12352–12357.
30. Pangburn, S. H., R. S. Newton, C-M. Chang, D. B. Weinstein, and D. Steinberg. 1981. Receptor-mediated catabolism of homologous low density lipoproteins in cultured pig hepatocytes. *J. Biol. Chem.* **256:** 3340–3347.
31. Edge, S. B., J. M. Hoeg, T. Triche, P. D. Schneider, and H. B. Brewer. 1986. Cultured human hepatocytes. Evidence for metabolism of low density lipoproteins by a pathway independent of the classical low density lipoprotein receptor. *J. Biol. Chem.* **261:** 3800–3806.
32. Havekes, L. M., H. Verboom, E. De Wit, S. H. Yap, and H. M. G. Princen. 1986. Regulation of low density lipoprotein receptor activity in primary cultures of human hepatoblastoma cell line. *Hepatology.* **6:** 1356–1360.
33. Dueland, S., J. D. Trawick, M. S. Nenseter, A. A. MacPhee, and R. A. Davis. 1992. Expression of 7α-hydroxylase in non-hepatic cells results in liver phenotypic resistance of the low density lipoprotein receptor to cholesterol repression. *J. Biol. Chem.* **267:** 22695–22698.
34. Swell, L., C. C. Schwartz, J. Gustafsson, H. Danielsson, and Z. R. Vlahcevic. 1981. A quantitative evaluation of the conversion of 25-hydroxycholesterol to bile acids in man. *Biochim. Biophys. Acta.* **663:** 163–168.
35. Ayaki, Y., E. Kok, and N. B. Javitt. 1989. Cholic acid synthesis from 26-hydroxycholesterol and 3-hydroxy-5-cholestenoic acid in the rabbit. *J. Biol. Chem.* **264:** 3818–3821.
36. Slater, H. R., C. J. Packard, S. Bicker, and J. Shepard. 1980. Effects of cholestyramine on receptor-mediated plasma clearance and tissue uptake of human low density lipoproteins in the rabbit. *J. Biol. Chem.* **255:** 10210–10213.
37. Angelin, B., C. A. Raviola, T. L. Innerarity, and R. W. Mahley. 1983. Regulation of hepatic lipoprotein receptors in the dog. Rapid regulation of apolipoprotein B,E receptors, but not of apolipoprotein E receptors, by intestinal lipoproteins and bile acids. *J. Clin. Invest.* **71:** 816–831.
38. Malavolti, M., H. Fromm, S. Ceryak, and I. M. Roberts. 1987. Modulation of low density lipoprotein receptor activity by bile acids: differential effects of chenodeoxycholic and ursodeoxycholic acids in the hamster. *J. Lipid Res.* **28:** 1281–1295.