Cholesterol 7α-hydroxylase influences the expression of hepatic apoA-I in two inbred mouse strains displaying different susceptibilities to atherosclerosis and in hepatoma cells

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Abstract C57BL/6 mice are susceptible to diet-induced atherosclerosis, whereas BALB/c mice are resistant. The susceptibility of C57BL/6 mice has been linked to decreased plasma HDL cholesterol in response to a diet containing fat, cholesterol, and cholic acid. Feeding C57BL/6 mice a diet consisting of fat and cholesterol, but no cholic acid, increased plasma high density lipoprotein (HDL) cholesterol. The increase in HDL was associated with increases in both plasma apolipoprotein (apo)A-I and hepatic apoA-I mRNA. Supplementation of the cholesterol-rich diet with cholic acid inhibited the stimulatory effect of cholesterol on hepatic apoA-I mRNA expression, resulting in similar hepatic apoA-I mRNA levels compared to chow-fed mice. Atherosclerosis-resistant BALB/c mice were also resistant to diet-induced changes in plasma HDL, apoA-I, and hepatic apoA-I mRNA levels. Previous studies showed that the diets changed both the activity and mRNA encoding the liver specific enzyme 7α-hydroxylase (1993. *J. Lipid Res.* **34:** 923–931). In both strains of mice, hepatic expression of apoA-I and 7α-hydroxylase mRNA varied in parallel. Whereas susceptible C57BL/6 mice also showed a significant correlation between HDL cholesterol and expression of 7α-hydroxylase, no such correlation was observed in BALB/c mice, suggesting that genetic differences in HDL metabolism, not hepatic apoA-I synthesis, are responsible for the strain specific differences in plasma HDL levels. The finding that lecithin:cholesterol acyltransferase (LCAT) activity was significantly decreased in C57BL/6 mice, but not in BALB/c mice fed the atherogenic diet, further supports this conclusion. Additional studies show that McArdle hepatoma cells stably expressing plasmid-derived rat 7α-hydroxylase recapitulated the parallel linear relationship between 7α-hydroxylase and apoA-I mRNA expression observed in both strains of mice. These data link hepatic apoA-I mRNA expression to hepatic cholesterol/bile acid metabolism.—**Dueland, S., D. France, S-L. Wang, J. D. Trawick, and R. A. Davis.** Cholesterol 7α-hydroxylase influences the expression of hepatic apoA-I in two inbred mouse strains displaying different susceptibilities to atherosclerosis and in hepatoma cells. *J. Lipid Res.* 1997. **38:** 1445–1453.

Supplementary key words HDL • LCAT • atherosclerosis • 7α-hydroxylase

Different strains of mice show variable responses to diet-induced atheroslerosis. C57BL/6 mice are susceptible to lesion formation whereas BALB/c mice are relatively resistant (1–3). Both cholesterol and cholic acid supplementation of a high fat diet are required to induce lesion formation in C57BL/6 mice. Susceptibility to diet-induced atherosclerosis is related to decreased plasma high density lipoprotein (HDL) levels. Genetic crosses between the susceptible strain C57BL/6 and the resistant strain BALB/c have shown that lesion development and HDL levels co-segregate as a major genetic locus on mouse chromosome 1 (designated *Ath-1*) (3), although additional genes also appear to contribute to the lesion phenotypes among certain strains (4–6). The *Ath-1* gene has been located in close proximity to the apoA-I gene (3). Other evidence indicating that HDL cholesterol levels are of crucial importance for lesion formation has been obtained in transgenic C57BL/6 mice over-expressing human apoA-I (7). These transgenic mice have high HDL cholesterol levels on atherogenic diets and are relatively resistant to lesion formation compared to control mice (7). While these studies show the importance of maintaining

Abbreviations: HDL, high density lipoproteins; apoA-I, apolipoprotein A-I; 7α-hydroxylase, cholesterol 7α-hydroxylase; NADPH, oxygen oxido reductase (EC 1.14.13.17); FPLC, fast protein liquid chromatography; MOPS, 3-(N-morpholino)-propaneusulfonic acid; SSC, 1.5 M NaCl, 0.15 M Na citrate, pH 7.0; LCAT, lecithin:cholesterol acyltransferase; CMV, cytomegalovirus; LDL, low density lipoproteins.

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normal HDL levels for resistance to dietary-induced atherosclerosis, they do not indicate that susceptibility is caused by a defect in apoA-I synthesis. Previous studies show that decreased hepatic expression of apoA-I mRNA cannot account for the increased susceptibility of C57BL/6 mice to dietary-induced atherosclerosis(8).

A unique characteristic of the susceptibility of C57BL/6 mice to atherosclerosis is that cholic acid is required as a component of the "high fat" diet (9). We have examined the basis for this and found that while cholic acid did not increase cholesterol absorption, it was required to inhibit the activity of 7α-hydroxylase, the first and rate-limiting enzyme in bile acid synthesis (10). In the present study we examined the effect of cholesterol-rich diets (with and without cholic acid) on strain-specific differences in HDL metabolism which may contribute to differences in their susceptibility to atherosclerosis.

The function and metabolism of HDL is not well characterized although it has been proposed that HDL in connection with LCAT (lecithin:cholesterol acyltransferase) function in the transport of excess cholesterol from the peripheral tissues to the liver (reverse-cholesterol transport) for subsequent excretion in bile (11). Biliary excretion of cholesterol after conversion to bile acids or as unmodified cholesterol accounts for up to 90% of cholesterol eliminated from the body (12). Kinetic analysis of HDL free cholesterol turnover has led to the concept that it facilitates the delivery of cholesterol to the liver for bile acid synthesis (13). Hepatic uptake of HDL cholesteryl ester may occur by a selective uptake of core cholesterol esters (14, 15), perhaps via the SR-B1 receptor of the "acetyl-LDL receptor" family (16). In cultured rat hepatocytes, cholesterol associated with lipoproteins containing high affinity ligands for the LDL receptor (i.e., apoB-100 and apoE) increased bile acid synthesis (17–19). Thus, while HDL may help to target cholesterol to the hepatic bile acid synthetic pathway, other lipoproteins are capable of performing this function. Clearly, the liver is both the most important quantitative recipient of lipoprotein cholesterol and the most important quantitative site of cholesterol metabolism and excretion (20, 21). The balance between hepatic lipoprotein uptake and the bile acid synthetic and excretory pathway has a profound influence on plasma levels of lipoproteins. While this balance is well established as a major determinant of plasma LDL levels (21, 22), its relationship to plasma HDL levels is less clear. The research presented in this report is directed toward examining the relationship between the bile acid synthetic pathway and HDL metabolism.

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 were fed 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% cholic acid (high cholesterol + cholic acid diet). Mice were maintained on the above diets for 3 weeks.

Serum lipid determinations

Lipoprotein cholesterol was measured using enzymatic cholesterol reagent (Sigma) and commercially available calibration standards (Gilson). Fractionation of serum lipoproteins by Superose-6 (Pharmacia) gel permeation chromatography was performed using a modification of the methodology described by Clifton et al. (23) with a robotic FPLC system (10). Column buffer consisted of 50 mM Tris (pH 7.4) containing 0.15 M NaCl with 0.01% sodium azide. Mouse serum (100 μl) was applied to the column, forty 0.5-ml fractions were collected, and the fractions containing HDL were assayed for cholesterol and apoA-I content, as described (24). LCAT activity in plasma samples was determined as described (25).

McArdle 7777 rat hepatoma cells

A plasmid encoding rat 7α-hydroxylase was prepared using the CMV driven pcDNA 3 plasmid which also encodes a neomycin resistance gene product (Invitrogen). The coding region of rat 7α-hydroxylase was placed in the linker region using EcoR1. The plasmid was shown to contain the expected insert in the appropriate orientation (data not shown). McArdle 7777 rat hepatoma cells, a gift from Dr. Thomas Innerarity, were transfected with this plasmid and screened for resistance to G418. Individual G418 (400 μg/ml) resistant colonies were picked using a sterile cotton swab containing trypsin and single cell cloned. Individual single cell clones were grown to 85% confluence. Cells were harvested and mRNA was isolated as described below.

Isolation of RNA

Liver samples (0.5 g) were flash frozen in liquid nitrogen immediately after excision and then homogenized in guanidinium isothiocyanate (10). RNA was extracted using acid guanidinium isothiocyanate–phenol–chloroform (26) and further purified by oligo-dT, as described (27,28).

Quantitation of mRNA for apoA-I, A-II, A-IV, 7α-hydroxylase and β-actin

RNA samples (1,2,5, and 10 μg from each animal) were loaded on a slot-blot apparatus, as described (10). Each blot was hybridized with ^{32}P-labeled cDNA probes consisting of a 550 bp (apoA-I), 500 bp (apoA-II), and 1450 bp (apoA-IV) fragments of cDNAs of the different mouse apoA's, a 1300 bp EcoR1 fragment of a cDNA clone for rat 7α-hydroxylase, and a 2000 bp fragment of β-actin, as described (10). The autoradiograms were scanned by a densitometer and the relative abundance of the different mRNAs was determined as described (10). The results of slot-blot studies were confirmed by Northern blot analysis, which showed that the level of nonspecific hybridization with each of the five probes was minimal.

Statistical analysis

Results are given as mean ± SEM. Values of $P \leq 0.05$ determined by Student's t test were considered to be significant.

RESULTS

Cholic acid is required for diet-reduced HDL cholesterol

We examined the effect of cholic acid as a component of the cholesterol-rich atherogenic diet on plasma HDL cholesterol. The cholesterol-rich diet (without cholic acid) increased HDL cholesterol levels by 30% in susceptible C57BL/6 mice (**Fig 1A**). However, adding cholic acid to this diet decreased HDL levels by 50% (Fig. 1) compared to the levels obtained on the same diet without cholic acid. Atherosclerosis-resistant BALB/c mice also showed a resistance of HDL cholesterol levels to change in response to diet (Fig. 1A).

Plasma levels of HDL apoA-I were not as responsive to diet as HDL cholesterol levels (Fig. 1B). C57BL/6 mice showed a 50% increase in HDL apoA-I on the cholesterol-rich diet, compared to the levels observed when mice were fed chow only. When mice were fed the cholesterol-rich diet containing cholic acid, HDL apoA-I levels decreased compared to those observed when C57BL/6 mice were fed the chow diet (Fig. 1B). In atherosclerosis-resistant BALB/c mice, HDL apoA-I levels were unresponsive to diet.

Hepatic expression of apoA-I mRNA is sensitive to the cholesterol-rich diet in C57BL/6 mice, but not in BALB/c mice

In C57BL/6 mice, the cholesterol-rich diet without cholic acid increased hepatic apoA-I mRNA levels by 75%, compared to the levels observed when mice were fed chow only (**Fig. 2**). Adding cholic acid to this diet decreased hepatic apoA-I mRNA levels compared to those observed when mice were fed chow only (Fig. 2). In contrast, in resistant BALB/c mice, diet did not significantly affect hepatic apoA-I mRNA levels (Fig. 2). As shown below, these strain differences in hepatic apoA-I mRNA levels cannot account for the strain differences in plasma HDL levels. In both strains, hepatic expression of apoA-I and apoA-IV was not significantly affected by diet (data not shown).

In C57BL/6 mice, the levels of hepatic apoA-I mRNA and plasma apoA-I varied as a significant direct linear correlation (**Fig. 3A**, $r = 0.61$, $P < 0.05$). In contrast, in resistant BALB/c mice there was no correlation between plasma apoA-I levels and apoA-I mRNA (Fig. 3B, $r = -0.43$, $P =$ ns). Furthermore, in C57BL/6 mice, but not in BALB/c mice, a direct significant linear correlation was observed between plasma HDL cholesterol and hepatic apoA-I mRNA levels ($r = 0.58$, $P < 0.01$) (Figs. 3C and 3D). These data suggest that the two strains of mice have different factors controlling HDL levels. In C57BL/6 mice, the supply of apoA-I from hepatic synthesis appears to influence HDL levels (Fig. 3A). In contrast, in BALB/c mice, hepatic apoA-I production does not appear to be a significant factor in determining HDL levels.

Relationship between hepatic apoA-I and 7α-hydroxylase mRNA levels

We examined the relationship between the expression of 7α-hydroxylase mRNA and the expression of apoA-I mRNA in each individual mouse (**Figs. 4A and 4B**). Previous studies show that both inbred strains of mice display similar diet-induced changes in the mRNA expression and activity of hepatic 7α-hydroxylase (10). The high-fat diet containing cholesterol but not cholic acid increases, whereas adding cholate to this diet decreases both the mRNA and activity of 7α-hydroxylase (10). While the diet-induced changes in both apoA-I and 7α-hydroxylase mRNA were less in BALB/c mice, compared to C57BL/6 mice, in both strains the expression of apoA-I mRNA correlated with the expression of 7α-hydroxylase mRNA (C57BL/6: $r = 0.54$, $P < 0.05$; BALB/c: $r = 0.55$, $P < 0.025$).

Additional analysis showed that in C57BL/6 mice 7α-hydroxylase mRNA levels correlated with HDL cholesterol concentrations (Fig. 4C, $r = 0.85$, $P < 0.001$). In contrast, no such correlation was evident in resistant BALB/c mice (Fig. 4D).

Cholic acid-containing atherogenic diet impairs LCAT activity in C57BL/6 mice

Without cholic acid in the diet, the activity of LCAT in plasma was similar between the two strains of mice

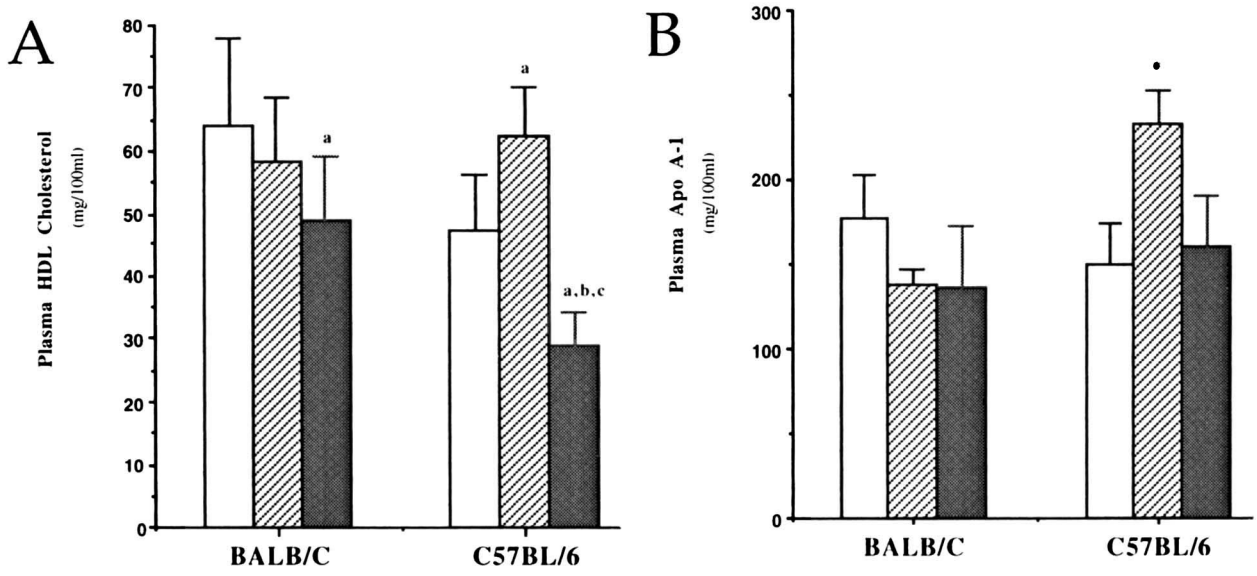

Fig. 1. Effect of diets on HDL cholesterol (A) and apoA-I (B) levels. BALB/c and C57BL/6 mice were fed chow (open bar), chow plus 20% olive oil and 2% cholesterol with (stippled bar) and without (solid bar) 0.5% cholic acid for 3 weeks. Mice were killed after an overnight fast. HDL was isolated from plasma by FPLC and the amount of cholesterol (A) and apoA-I (B) was quantitated. Each value represents the mean ±SEM. [a]Significant difference from controls. [b]Significant difference from the olive oil, cholesterol diet without cholic acid. [c]Significant difference from BALB/c. *Significant difference between the chow-fed and cholesterol without cholic acid-fed groups of C57BL/6 mice. In the C57BL/6 group, there were 6 mice fed chow, 5 fed the high-fat diet without cholic acid, and 5 fed the high-fat diet with cholic acid. In the BALB/c group, there were 5 mice fed chow, 5 fed the high-fat diet without cholic acid, and 5 fed the high-fat diet with cholic acid.

(**Fig. 5**). However, when fed the cholic acid-containing atherogenic diet, the activity of LCAT in C57BL/6 mice was decreased by 60%, whereas it was unchanged in BALB/c mice. As a result, when fed the atherogenic cholic acid-containing diet, LCAT activity was significantly less in C57BL/6 mice compared to BALB/c mice ($P < 0.01$).

Recapitulation of a relationship between 7α-hydroxylase and apoA-I mRNA expression in hepatoma cells

The data showing a significant correlation between the hepatic expression of 7α-hydroxylase and apoA-I do not, by themselves, indicate any cause and effect relationship. To further examine the mechanism responsible for the linear relationship between 7α-hydroxylase and apoA-I mRNA expression exhibited by both strains of mice, McArdle hepatoma cells were transfected with a plasmid encoding 7α-hydroxylase and a contiguous neomycin-resistance gene. Seven individual single cell clones stably expressing 7α-hydroxylase were obtained by screening for neomycin resistance. While there was no detectable expression of 7α-hydroxylase mRNA in wild-type McArdle cells, a single mRNA that hybridized to the ^{32}P-labeled 7α-hydroxylase cDNA probe having a size predicted by the plasmid, but distinct from the endogenous (rat) 7α-hydroxylase mRNA (27, 28), was present (**Fig. 6A**). Analysis of the seven individual single cell clones stably expressing 7α-hydroxylase showed a variable expression of 7α-hydroxylase mRNA (Fig. 6C). The level of expression of 7α-hydroxylase mRNA was estimated to be from 0.1 to 5 times that expressed in mouse liver in vivo. This large variation in expression of 7α-hydroxylase mRNA exhibited by the individual single cell clones provided an opportunity to examine the influence of 7α-hydroxylase on the expression of apoA-I. The data obtained from these single cell clones recapitulated the parallel linear relationship between 7α-hydroxylase and apoA-I mRNA expression observed in both strains of mice (Fig. 6C).

DISCUSSION

Our results show that compared to atherosclerosis-resistant BALB/c mice, atherosclerosis-susceptible C57BL/6 mice display a greater responsiveness of HDL cholesterol and apoA-I as well as the hepatic expression (production) of apoA-I to a high fat diet that contained cholesterol with and without cholic acid. Furthermore, in both strains of mice, hepatic expression of apoA-I mRNA correlates with 7α-hydroxylase expression, suggesting for the first time that there is a link between hepatic bile acid production and apoA-I gene expression. This relationship was further demonstrated in

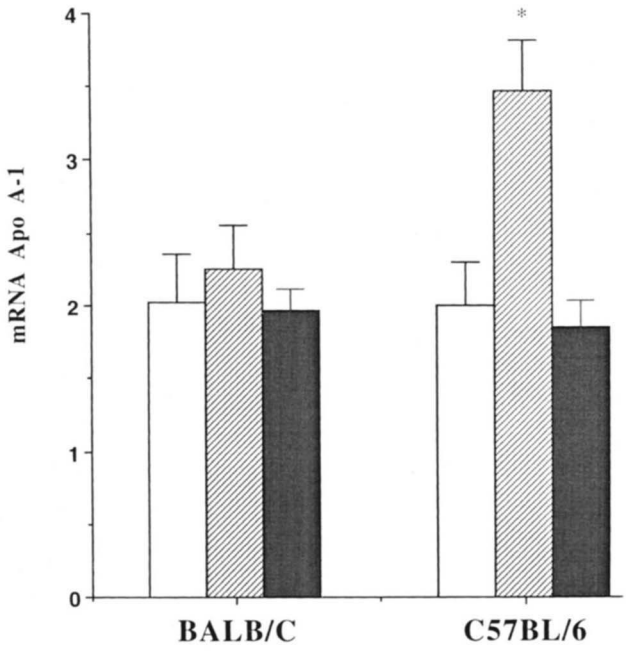

Fig. 2. Effect of diets on hepatic apoA-I mRNA levels. BALB/c and C57BL/6 mice were fed chow (open bar), chow plus 20% olive oil and 2% cholesterol with (stippled bar) and without (solid bar) 0.5% cholic acid for 3 weeks. Mice were killed after an overnight fast. PolyA-mRNA was isolated and the abundance of apoA-I mRNA relative to β-actin was determined by slot-blot analysis. Each value represents the mean ±SEM. *Denotes a significant difference between C57BL/6 and BALB/c mice fed the cholesterol-rich diet without cholic acid. In the C57BL/6 group, there were 6 mice fed chow, 5 fed the high-fat diet without cholic acid, and 5 fed the high-fat diet with cholic acid. In the BALB/c group, there were 5 mice fed chow, 5 fed the high-fat diet without cholic acid, and 5 fed the high-fat diet with cholic acid.

McArdle hepatoma cells in which variable plasmid-driven expression of 7α-hydroxylase altered the expression of apoA-I mRNA in parallel. However, our findings show that decreased hepatic expression of apoA-I mRNA cannot account for the decreased HDL levels observed in C57BL/6 mice fed the cholic acid-containing atherogenic diet. Rather, it is likely that decreased plasma LCAT activity in C57BL/6 mice fed the cholic acid-containing atherogenic diet contributes to this strain's decrease in HDL levels.

Cholic acid is required to reduce HDL levels in C57BL/6 mice

The strain-specific reduction in HDL levels was uncovered by examining how dietary cholesterol and cholate interact to produce changes in HDL and hepatic apoA-I mRNA expression. When fed the cholesterol-rich diet without cholic acid, C57BL/6 mice, but not BALB/c mice, showed significant increases in both plasma HDL cholesterol (Fig. 1A) and hepatic apoA-I mRNA (Fig. 1B). Moreover, adding cholate to the cholesterol-rich diet significantly decreased both HDL cholesterol and apoA-I levels. These data establish that cholate is required as a component of the cholesterol-rich atherogenic diet to reduce HDL levels in C57BL/6 mice.

The mechanism underlying the greater sensitivity of plasma HDL levels in C57BL/6 mice to the cholic acid-containing atherogenic diet was shown by the significant relationship between plasma HDL (cholesterol and apoA-I) and hepatic apoA-I mRNA expression (Figs. 2 and 3). In C57BL/6 mice, but not BALB/c mice, plasma levels of both apoA-I and HDL cholesterol correlated with hepatic apoA-I mRNA. These data suggest that in C57BL/6 mice hepatic production of apoA-I may be an important determinant of plasma HDL levels. In contrast, in resistant BALB/c mice, while apoA-I mRNA may also determine HDL levels, other factors appear to have a greater influence.

Impaired LCAT activity is responsible for strain differences in diet-induced reduction of plasma HDL

The finding that plasma LCAT activity is selectively decreased by the cholic acid-containing diet in C57BL/6 mice, but not in BALB/c mice (Fig. 5), provides evidence that a diet-induced decrease of this enzyme may contribute to the decreased HDL levels. The mechanism responsible for the decreased LCAT activity in C57BL/6 mice fed the atherogenic diet is not caused by decreased hepatic LCAT mRNA expression (29). Based on the findings that HDL structure is altered in C57BL/6 mice fed the atherogenic cholate-containing diet (1–5, 8, 29), it is reasonable to propose that these structural changes may impair the enzymatic activity of LCAT.

LCAT plays an essential role in HDL metabolism and reverse cholesterol transport (30). The finding that transgenic expression of LCAT increases HDL levels in mice (31,32) is consistent with the proposal that its decreased activity in C57BL/6 mice contributes to the decreased HDL levels caused by the cholic acid-containing atherogenic diet. Decreased HDL half-life due to impaired LCAT activity in C57BL/6 mice can explain: *1*) why HDL is dependent upon apoA-I mRNA in C57BL/6 mice, but not BALB/c mice and *2*) why hepatic apoA-I mRNA levels are similar in both strains of mice, but only C57BL/6 mice show reduced HDL. Increased HDL turnover due to impaired LCAT activity in C57BL/6 mice would make HDL levels more dependent upon production (apoA-I mRNA levels; Fig. 4C) than in BALB/c mice (Fig. 4D) having slower rates of HDL turnover. Increased HDL turnover due to impaired LCAT activity in C57BL/6 mice would also lead to reduced plasma HDL levels (Fig. 1A) even though production rates as indicated by apoA-I mRNA levels (Fig. 1B) were similar.

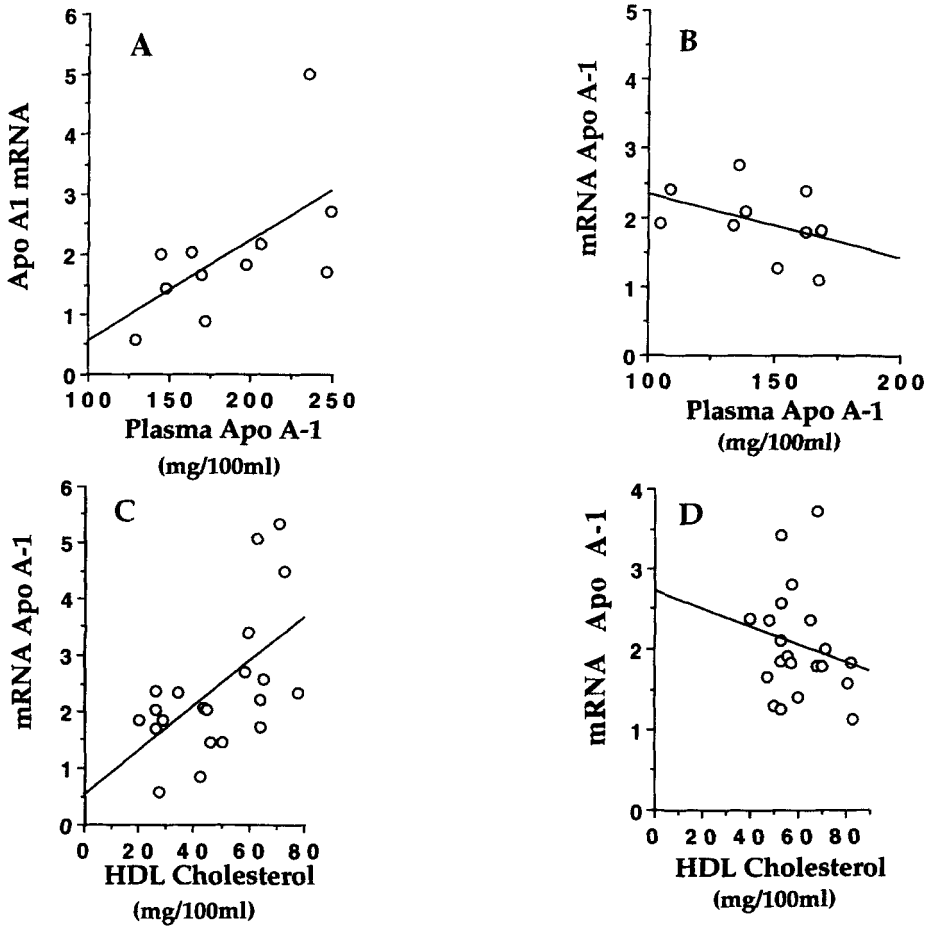

Fig. 3. Relationships between hepatic apoA-I mRNA levels and plasma HDL apoA-I levels (A and B) and plasma HDL cholesterol levels (C and D) in C57BL/6 and BALB/c mice. For each individual mouse, apoA-I mRNA levels were related to plasma HDL apoA-I levels. A: C57BL/6 mice: 4 fed chow, 4 fed the high-fat diet without cholic acid, and 3 fed the high-fat diet with cholic acid; B: BALB/c mice: 4 fed chow, 3 fed the high-fat diet without cholic acid, and 3 fed the high-fat diet with cholic acid. For each individual mouse, apoA-I mRNA levels were related to plasma HDL cholesterol levels. C: C57BL/6 mice: 7 fed chow, 7 fed the high-fat diet without cholic acid, and 7 fed the high-fat diet with cholic acid; D: BALB/c mice: 7 fed chow, 7 fed high-fat diet without cholic acid, and 7 fed the high-fat diet with cholic acid. Each correlation was statistically analyzed by linear least squares analysis. Statistical analysis using Student's t test showed that the relationships between apoA-I mRNA levels and HDL apoA-I levels (A) ($r = 0.61$, $P < 0.02$) and HDL cholesterol levels (C) ($r = 0.58$, $P < 0.01$) in C57BL/6 mice were significant. The other linear relationships in BALB/c mice (B; $r = -0.43$, and D; $r = -0.19$) were not statistically significant.

In McArdle rat hepatoma cells, expression of apoA-I is influenced by the expression of 7α-hydroxylase

To examine the possibility that 7α-hydroxylase may influence the expression of apoA-I mRNA, single cell clones of McArdle hepatoma cells stably expressing 7α-hydroxylase at variable levels were obtained. Variable levels of expression of a plasmid-derived mRNA are a common occurrence in stably transfected cells and are likely due to differences in the number or plasmids that are integrated into the genome, as well as influences caused by the site of integration. The variable and stable expression of the exogenous plasmid gene construct in these cells provided an opportunity to observe a significant linear relationship between expression of 7α-hydroxylase and apoA-I mRNA (Fig. 6C). Thus, the hepatoma cell studies recapitulated the relationship observed in vivo in both strains of mice (Figs. 4A and 4B). The mechanism through which expression of 7α-hydroxylase influences the expression of apoA-I mRNA is not established. However, it is interesting to note that phenobarbital induces the expression of apoA-I mRNA in rats (33). Phenobarbital is a potent inducer of cytochrome P450s, and in some rats, it induces 7α-hydroxylase (34). It is plausible that hepatic apoA-I gene expression is linked to the expression of one or more cytochrome P450 genes or that a common factor regulates these genes.

It is important to emphasize that in the two inbred

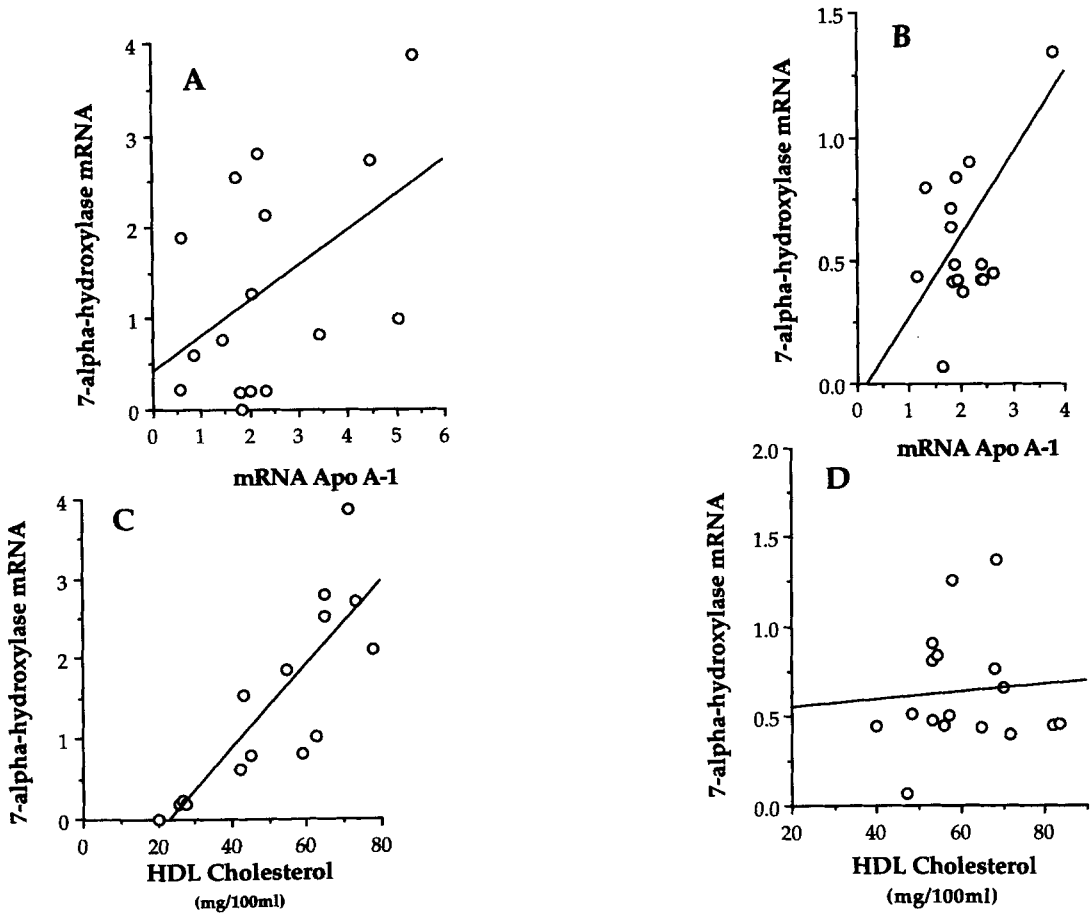

Fig. 4. Relationships between hepatic 7α-hydroxylase mRNA levels and apoA-I mRNA levels and HDL cholesterol levels in C57BL/6 and BALB/c mice. For each individual mouse, hepatic levels of 7α-hydroxylase mRNA were related to apoA-I mRNA levels. A: C57BL/6 mice: 6 fed chow, 5 fed the high-fat diet without cholic acid, and 5 fed the high-fat diet with cholic acid; B: BALB/c mice: 5 fed chow, 5 fed the high-fat diet without cholic acid, and 5 fed the high-fat diet with cholic acid. For each individual mouse, hepatic levels of 7α-hydroxylase mRNA were related to HDL cholesterol levels. C: C57BL/6 mice: 5 fed chow, 5 fed the high-fat diet without cholic acid, and 4 fed the high-fat diet with cholic acid: D: BALB/c mice: 6 fed chow, 6 fed the high-fat diet without cholic acid, and 5 fed the high-fat diet with cholic acid. The linear relationships between 7α-hydroxylase mRNA and apoA-I mRNA levels were statistically significant: A; $r = 0.54$, $P < 0.05$, B; $r = 0.55$, $P < 0.025$. While the relationship between 7α-hydroxylase mRNA and HDL levels in C57BL/6 mice was statistically significant (C) $r = 0.85$, $P < 0.001$ in BALB/c mice this relationship was not significant, (D) $r = 0.03$, $P =$ ns.

strains of mice, the only parameter that was observed to be correlated consistently with 7α-hydroxylase was hepatic apoA-I mRNA levels. While both inbred strains displayed statistically significant correlations between 7α-hydroxylase and hepatic apoA-I mRNA levels, the changes in apoA-I mRNA levels were clearly less in BALB/c mice compared to C57BL/6 mice. Furthermore, HDL levels were linked to 7α-hydroxylase only in the C57BL/6 mice. These data suggest that genetic background determines both the magnitude in response of hepatic apoA-I mRNA to changes in 7α-hydroxylase and whether the individual mouse strain displays a change in HDL levels. The hepatoma cell studies recapitulated the relationship between 7α-hydroxylase and apoA-I mRNA. Based on these data we would expect that factors that influence 7α-hydroxylase expression would likely alter the hepatic expression of apoA-I mRNA, not necessarily HDL levels. Studies in rats support these predictions. Treatment of rats with cholestyramine, which is known to induce 7α-hydroxylase (35, 36), increases the hepatic expression of apoA-I, with no significant change in HDL levels (37). The strain of rats used for these studies (37) behave in a manner similar to BALB/c mice used in our studies. The genes that are responsible for the heterogenous response of HDL levels to the different diets are not known. However, recent studies clearly establish a unexpected genetic link between plasma HDL cholesterol levels in C57BL/6 mice and 7α-hydroxylase. Three of the genetic loci determining the decrease in HDL-cholesterol in C57BL/6 mice are associated with differences in expression of 7α-hydroxylase (A. J. Lusis, personal communication).

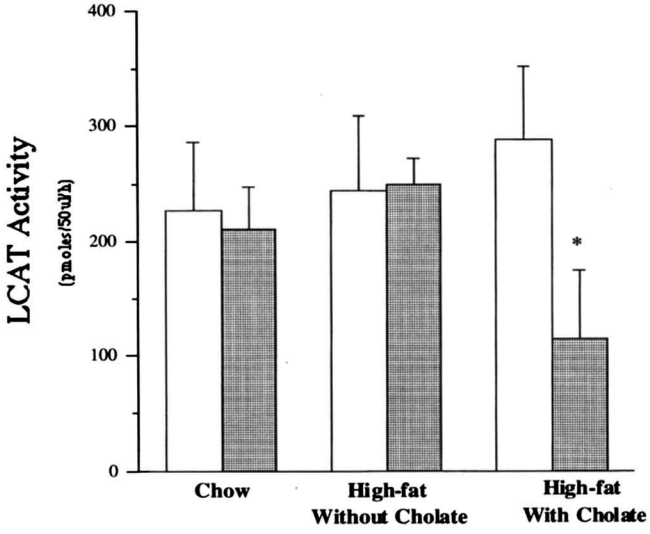

Fig. 5. Effect of diet on plasma LCAT activity in C57BL/6 and BALB/c mice. BALB/c (open bars) and C57BL/6 mice (shaded bars) were fed chow, chow plus 20% olive oil and 2% cholesterol with and without 0.5% cholate for 3 weeks. Mice were killed after an overnight fast. Plasma LCAT activities were determined (25). *Significant difference between both strains of mice fed the high-fat diet containing cholate, $P < 0.01$. In the C57BL/6 group, there were 4 mice fed chow, 4 fed and high-fat diet without cholic acid, and 3 fed the high-fat diet with cholic acid. In the BALB/c group, there were 4 mice fed chow, 3 fed the high-fat diet without cholic acid, and 3 fed the high-fat diet with cholic acid.

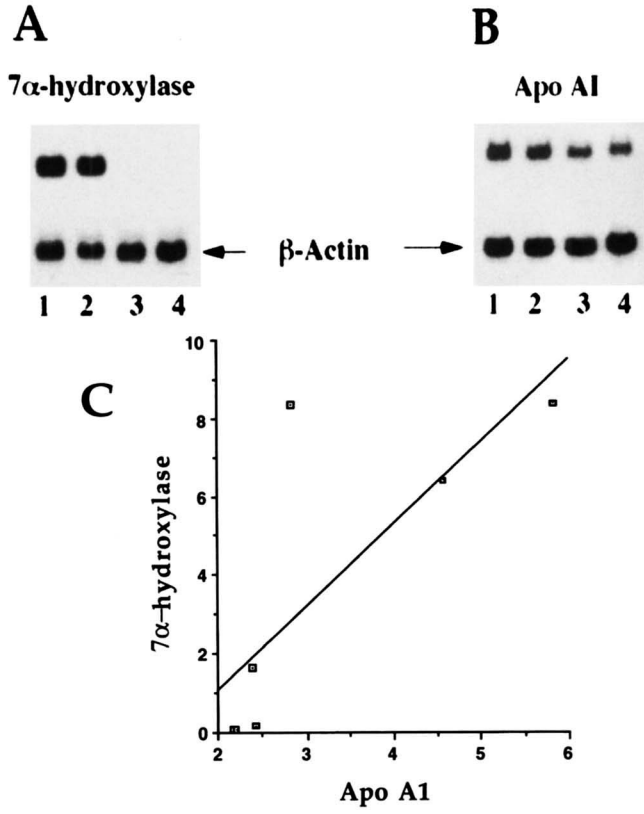

Fig. 6. Expression of 7α-hydroxylase mRNA (A), apoA-I mRNA (B), and the relationship between 7α-hydroxylase mRNA and apoA-I mRNA (C) in McArdle rat hepatoma cells. McArdle rat hepatoma cells (lanes 3 and 4, A and B) were transfected with a CMV driven plasmid encoding rat 7α-hydroxylase (lanes 1 and 2, A and B). Individual single cell clones were obtained by selection for neomycin resistance. The abundance of 7α-hydroxylase mRNA (A) and apoA-I mRNA (B) was determined relative to β-actin by densitometric analysis using a phosphorimager. The cellular content of apoA-I mRNA was correlated to the content of 7α-hydroxylase mRNA for each single cell clone (C). Each value is the mean of 3 separate determinations. There was a statistically significant positive correlation: $r = 0.590$, $P < 0.05$.

There are additional situations showing a functional linkage between 7α-hydroxylase and plasma HDL. It is well established that some patients treated with bile acid sequestrants (e.g., cholestyramine) show increased 7α-hydroxylase expression (35) and small, but detectable increases in plasma HDL levels (36). Furthermore, surgical removal of the ileum, which also increases hepatic 7α-hydroxylase expression (35), significantly raises HDL cholesterol levels (38). These findings suggest that depending upon the genetic background of individuals, hepatic 7α-hydroxylase expression may influence HDL metabolism in humans in a manner similar to what we have observed in inbred strains of mice displaying different susceptibility to atherosclerosis.

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