Dietary fats modulate the regulatory potential of dietary cholesterol on cholesterol 7α-hydroxylase gene expression

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Abstract Cholesterol 7α-hydroxylase (cyp7) is the rate-limiting enzyme in bile acid biosynthesis. Previously, dietary cholesterol was shown to induce cyp7 gene expression. However, recent studies have produced data that are inconsistent with this observation, suggesting the possibility that other factors in the diet are also important in the regulation of cyp7 by dietary cholesterol. The effect of dietary fats on the ability of dietary cholesterol to regulate cyp7 activity and mRNA abundance was assessed. High fat diets composed primarily of polyunsaturated (PUFA), monounsaturated (MUFA), or saturated (SFA) fatty acids induced hypercholesterolemia regardless of whether cholesterol was present or not. However, the effects of each diet on bile composition and hepatic cholesterol content were variable. Microsomal fatty acid profiles reflected the fatty acid composition of the diets. Addition of cholesterol to the PUFA diet increased cyp7 mRNA abundance and activity, analogous with the results observed in mice fed a chow plus cholesterol diet. On the other hand, addition of cholesterol to diets high in MUFA or SFA caused a significant reduction of cyp7 mRNA abundance and activity. Addition of cholesterol to all the diets caused the expected changes in low density lipoprotein receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA abundance but was not correlated with the changes in cyp7 mRNA abundance. No relationship between cyp7 mRNA abundance and hepatic total cholesterol content or hepatic microsomal cholesterol content was evident, suggesting that cholesterol status does not necessarily determine cyp7 mRNA abundance. The results of this study illustrate that the type of dietary fat is important in elaborating the regulatory potential of dietary cholesterol on cyp7 gene expression and suggest that the regulation of cyp7 gene expression does not involve the classical sterol-mediated pathway.—**Cheema, S. K., D. Cikaluk, and L. B. Agellon.** Dietary fats modulate the regulatory potential of dietary cholesterol on cholesterol 7α-hydroxylase gene expression. *J. Lipid Res.* 1997. **38:** 315–323.

Supplementary key words hypercholesterolemia • bile acids • fatty acids

Whole body cholesterol homeostasis is controlled by supply and removal pathways. The liver plays a central role in this process as it is the major site for cholesterol removal. Cholesterol can be either excreted into bile directly or after conversion into bile acids. Bile acid synthesis occurs exclusively in the liver and cholesterol 7α-hydroxylase (cyp7) is the first and rate-limiting enzyme of the pathway (1). Conversion of cholesterol into bile acids is an irreversible and terminal process of cholesterol catabolism. Thus, the activity of cyp7 can have a major impact on the overall catabolism of excess cholesterol.

Several studies have demonstrated that cyp7 activity and mRNA abundance are induced by dietary cholesterol (reviewed in ref. 1). However, recent studies have provided data that are inconsistent with this concept. In non-human primates, a high fat and cholesterol diet caused the reduction of both cyp7 activity and mRNA abundance (2). Similarly, a reduction in cyp7 mRNA levels was also observed in an inbred strain of rabbits fed cholesterol (3, 4). Thus, these studies show that dietary cholesterol may not always have a stimulatory effect on cyp7. Whether or not cholesterol is directly involved in regulating cyp7 activity and gene expression is not yet known.

In order to explain this discrepancy, we determined whether the other components of the diet modify the regulatory potential of dietary cholesterol. Fats are important constituents of the diet and are known to alter plasma and hepatic cholesterol levels. Addition of cholesterol to high fat diets typically modifies the impact of the diet on cholesterol homeostasis. Thus, in the

Abbreviations: cyp7, cholesterol 7α-hydroxylase; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low density lipoprotein: MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

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present study we assessed the role of polyunsaturated, monounsaturated, and saturated fats on the ability of dietary cholesterol to modulate cyp7 activity and mRNA abundance.

MATERIALS AND METHODS

Animals and diets

Female mice (C57BL/6J, 8 wk old) were obtained from Jackson laboratory. The mice were housed in a room with reverse light cycle (lights from 1700 h to 0500 h) and fed a chow diet for 1 week prior to the initiation of the controlled diet study. Mice (n = 5 per group) were fed for 3 weeks with either a chow (Purina #5001) diet or a semipurified diet (Teklad #84172, nutritionally adequate) containing 20% (w/w) fat from either safflower oil (polyunsaturated fatty acids; PUFA), olive oil (monounsaturated fatty acids; MUFA), or beef tallow (saturated fatty acids; SFA), in the presence or absence of 1% cholesterol. Linseed oil (1% w/w of the dietary fat) was also added to all the semipurified diets to ensure an adequate source of n–3 fatty acids (5). The fatty acid composition of the sources of dietary fats were determined by gas–liquid chromatography and is given in **Table 1.** Mice were given free access to food and water. Plasma, bile, and liver samples were collected 3 weeks later and sampling was done according to a consistent schedule (at 0900 h). The mice fed high fat diets showed no difference in the amount of food consumption or body weight gain throughout the duration of the study (**Table 2**). This suggests that the fatty acid intake and caloric consumption in the different dietary groups were comparable throughout the experimental period.

TABLE 1. Fatty acid composition of the fat sources

Fatty Acids	Safflower Oil	Olive Oil	Beef Tallow
		% w/w	
14:0	0.2	0.1	2.9
16:0	7.0	15.5	27.9
18:0	2.5	2.6	64.3
18:1	14.2	66.5	1.0
18:2 n–6	74.5	10.9	1.0
18:3 n–3	1.2	1.3	1.0
ΣSFA	9.7	18.2	95.1
ΣMUFA	14.2	66.5	1.0
ΣPUFA	75.7	12.2	2.0

Abbreviations used: ΣSFA, sum of saturated fatty acids; ΣMUFA, sum of monounsaturated fatty acids; ΣPUFA, sum of polyunsaturated fatty acids.

TABLE 2. Food intake and weight gain

Diet	Food Intake	Total Weight Gain
	g/day	*g*
PUFA	2.3 ± 0.1	3.0 ± 0.4
PUFA + chol	2.3 ± 0.3	3.0 ± 0.5
MUFA	2.3 ± 0.1	2.7 ± 0.2
MUFA + chol	2.4 ± 0.1	2.7 ± 0.3
SFA	2.2 ± 0.1	2.7 ± 0.2
SFA + chol	2.2 ± 0.1	2.6 ± 0.1

Mice were fed the indicated diets (n = 5 per group) for 3 weeks. Food consumption of all the groups was monitored weekly. The total weight gain represents the difference in weight of individual mice at day 0 and day 21 of the study. Chol represents 1% cholesterol.

Biochemical analyses

Fasting blood samples were collected in tubes containing EDTA. Plasma was separated and assayed for total cholesterol using enzymatic methods (Sigma diagnostic kit #352). Gallbladder bile was collected by aspiration and assayed for cholesterol as above. Biliary bile acids were measured using an enzymatic kit (Sigma diagnostic kit #450). Biliary phospholipid content was measured by lipid phosphorous determination (6). Lipids were extracted from liver samples (50 mg) as described previously (7) and the mass of total cholesterol and free cholesterol was determined by enzymatic methods (Sigma diagnostic kit #352 for total cholesterol and Boehringer Mannheim kit #139050 for free cholesterol).

Cholesterol 7α-hydroxylase assay

Samples of frozen livers (100–500 mg) were minced in 1 ml of ice-cold buffer containing 0.3 M sucrose, 1 mM EDTA, 50 mM KF, 50 mM KCl, 5 mM DTT, 0.1 M K_2HPO_4 (pH 7.4), and then disrupted in a 2-ml Potter-Elvehjem homogenizer. Homogenates were centrifuged for 20 min at 10,000 rpm at 4°C. The supernatant was recentrifuged for 70 min at 35,000 rpm in a SW-60 rotor (Beckman Instruments) at 4°C. The microsomal pellet from the second spin was resuspended in 1 ml of buffer containing 0.1 M K_2HPO_4 (pH 7.4), 1 mM EDTA, 50 mM KF, 5 mM DTT and 50 mM KCl. Aliquots of liver microsomes were then rapidly frozen in liquid nitrogen and stored at −80°C until assayed. Microsomal free cholesterol was determined by an enzymatic method (Boehringer Mannheim kit #139050) and the protein content was determined using the Bio-Rad protein assay.

The cyp7 activity was measured using a modification of a previously described HPLC method (8). Liver microsomes (1 mg) were incubated in 1 ml of buffer containing 0.1 M K_2HPO_4 (pH 7.4), 1 mM EDTA, 50 mM KF, 5 mM DTT and 0.015% CHAPS. The mixture was preincubated for 5 min at 37°C and the reaction was initiated by addition of 100 μl of 10 mM NADPH. The

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samples were incubated for 30 min at 37°C and the reaction was terminated by adding 30 μl of 20% sodium cholate. Cholesterol oxidase (from *Nocardia erthropolis,* 45 U/mg; Boehringer Mannheim) was then added to oxidize the products of the reaction. After 15 min, the assay mixtures were extracted twice with 4 ml of petroleum ether. Extracts were stored under nitrogen until analysis.

The residue was resuspended in 100 μl of acetonitrile–methanol 70:30 and a 20-μl aliquot was analyzed by HPLC (Beckman System Gold) on a 4.6 × 250 mm Ultrasphere (Beckman) column with running solvent (acetonitrile–methanol, 70:30) at a flow rate of 0.8 ml/min. The absorbance of the sample was measured at 240 nm. The 20α; 7α-, and 7β-hydroxy-4-cholesten-3-one derivatives eluted at about 8, 12, and 15 min, respectively.

Microsomal fatty acid analysis

Lipids from liver microsomal preparations were extracted with chloroform–methanol 2:1 (v/v) (7). For fatty acid analysis, total lipids were saponified and then methylated using 14% (w/w) BF_3–methanol reagent at 110°C for 1 h. Fatty acid methyl esters were extracted with hexane and analyzed by automated gas-liquid chromatography. Fatty acid methyl ester peaks were identified by injecting standard mixtures of fatty acid methyl esters.

Determination of mRNA abundance

Total RNA from mouse livers was purified according to standard procedures (9). Hepatic cyp7 mRNA levels were determined by a ribonuclease protection assay. Total RNA (20 μg) was hybridized with ^{32}P-labeled antisense probes for mouse cyp7 (71 nt from intron 2 and 228 nt from exon 3 of the mouse cyp7 gene) and mouse glyceraldehyde-3-phosphate dehydrogenase (G3PDH) synthesized from pTRI-G3PDH (Ambion), at 55°C overnight. Unhybridized probes were removed by treatment with RNAse One (Promega Biotech) (4 U/μg RNA) for 1 h at 25°C. The protected mRNA fragments were separated on 5% polyacrylamide sequencing gels. The radioactivity in each band was quantitated by phosphorautoradiography using Fuji-X BAS 1000 plate imager. The G3PDH mRNA abundance was not affected by any of the diets and was used as the internal standard to quantitate the cyp7 mRNA content. LDL receptor and HMG-CoA reductase mRNA levels were determined by reverse transcription and multiplex in vitro DNA amplification. Complementary DNA was synthesized from total liver RNA (1 μg) using Superscript reverse transcriptase (Life Technologies, Inc.) and then used as templates for in vitro DNA amplification reactions. G3PDH, LDL receptor, and HMG-CoA reductase mRNA sequences were simultaneously amplified using specific primers. No amplification products were detectable in the absence of reverse transcriptase. Amplification products were quantitated by dot blot hybridization and the radioactivity in each dot was determined by phosphorautoradiography as described above. The amounts of LDL receptor and HMG-CoA reductase mRNA were normalized to G3PDH mRNA content.

Statistical analyses

Data were analyzed using Student's *t* test or analysis of variance (Statistical Analysis Software package) where appropriate. Differences between groups were considered significant when $P < 0.05$.

RESULTS

Plasma cholesterol and gallbladder bile composition

Changes in the plasma cholesterol content under various dietary conditions are shown in **Fig. 1.** Feeding the semipurified diet supplemented with 20% safflower oil, olive oil, or beef tallow (predominantly PUFA, MUFA, and SFA, respectively) induced hypercholesterolemia in mice. Addition of cholesterol to all three diets showed a small but further increase in the plasma cholesterol levels ($P < 0.05$, for PUFA and MUFA diets), except for the SFA-fed mice where the increase was not significant. Feeding a diet composed predominantly of MUFA stimulated the greatest induction of hypercholesterolemia compared to the other dietary fats. Although olive oil is generally regarded to have a hypocholesterolemic ef-

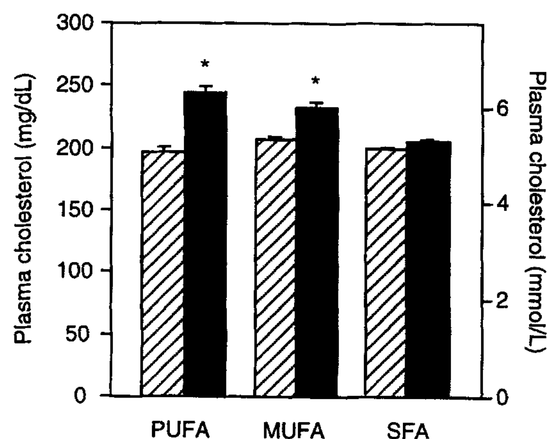

Fig. 1. Total plasma cholesterol levels of C57BL/6J mice. Mice were fed a high fat diet in the presence or absence of 1% cholesterol as described in Materials and Methods. Values are mean ± SE (n = 5). The difference between the groups consuming the same fat diet in the presence (solid bars) and absence (hatched bars) of cholesterol was tested using Student's *t* test: $^*P < 0.05$.

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TABLE 3. Effect of dietary fat on gallbladder bile composition

Diet	Bile Acids	Cholesterol	Phospholipids
		mmol/L	
PUFA	206 ± 3.0^{d}	11.73 ± 0.20^{c}	13.0 ± 0.5^{c}
PUFA + chol	$229 \pm 5.0^{c*}$	$20.78 \pm 0.30^{b**}$	14.0 ± 0.5^{c}
MUFA	279 ± 7.0^{b}	13.31 ± 0.50^{d}	27.0 ± 3.0^{b}
MUFA + chol	270 ± 14.0^{b}	$20.21 \pm 0.23^{b***}$	$33.0 \pm 1.0^{a*}$
SFA	316 ± 24.0^{a}	21.91 ± 0.40^{a}	27.0 ± 2.0^{b}
SFA + chol	305 ± 12.0^{a}	$19.25 \pm 0.34^{c*}$	26.0 ± 1.0^{b}

Gallbladder bile from mice fed the various diets was analyzed as described in Materials and Methods. Values shown are mean ± SE (n = 5). Differences among the groups were evaluated by analysis of variance. Values without a common superscript are significantly different ($P < 0.05$). The difference between the groups consuming the same fat diet in the presence and absence of 1% cholesterol (chol) was tested using Student's t test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 4. Hepatic cholesterol content

Diet	Total Cholesterol	Free Cholesterol
	mmol/g	*mmol/g*
PUFA	0.17 ± 0.02^{fg}	0.07 ± 0.01^{cde}
PUFA + chol	$0.33 \pm 0.03^{ab**}$	$0.12 \pm 0.02^{b*}$
MUFA	0.25 ± 0.02^{ef}	0.11 ± 0.01^{bc}
MUFA + chol	$0.41 \pm 0.02^{bc**}$	0.11 ± 0.01^{bc}
SFA	0.17 ± 0.02^{fg}	0.09 ± 0.01^{def}
SFA + chol	$0.27 \pm 0.04^{cde**}$	0.11 ± 0.01^{bcde}

Mice were fed the indicated diets for 3 weeks. Liver lipids were extracted and analyzed as described in Materials and Methods. All values shown are mean ± SE (n = 5) per gram of tissue. Differences among the groups were evaluated by analysis of variance. Values without a common superscript are significantly different ($P < 0.05$). The difference between groups consuming the same fat diet in the presence and absence of 1% cholesterol (chol) was tested using Student's t test: * $P < 0.05$; ** $P < 0.01$.

fect in humans (10–12), our data are consistent with observations showing that plasma cholesterol is increased by olive oil in animal species (13, 14). The SFA-fed mice showed a slightly lower level of plasma cholesterol as compared to the MUFA-fed mice. The high 18:0 fatty acid content (65%) of the beef tallow was selected in the present study to maintain a comparable chain length among the major fatty acids species (see Table 1).

The liver maintains whole body cholesterol homeostasis either by secreting cholesterol directly into the bile or after conversion of cholesterol to bile acids. We studied the composition of gallbladder bile in C57BL/6J mice under various dietary conditions (**Table 3**). Feeding the SFA diet revealed the highest level of bile cholesterol, followed by the MUFA and the PUFA diets. In general, feeding cholesterol increased the bile cholesterol levels except for the SFA diet where a slight but significant decrease was observed ($P < 0.05$). Mice fed the SFA diet with or without cholesterol showed the highest level of bile acids as compared to the other diets. Addition of cholesterol to the PUFA diet showed an increase in the bile acid content ($P < 0.05$). The phospholipid content of gallbladder bile was significantly higher in mice fed the MUFA and the SFA diets, with or without cholesterol compared to the PUFA-fed mice. Addition of cholesterol to the MUFA diet showed an increase in the phospholipid content of gallbladder bile ($P < 0.05$).

Hepatic cholesterol content

We examined the effects of dietary fats and cholesterol on the major pathways that determine the net sterol balance across the liver. **Table 4** shows the hepatic total and free cholesterol content of C57BL/6J mice under the various dietary conditions. Addition of cholesterol to all the diets increased the hepatic total cholesterol ($P < 0.01$). Interestingly, the hepatic free cholesterol content was not significantly increased by addition of cholesterol to the MUFA and SFA diets. The MUFA diet showed the highest level of hepatic total cholesterol and cholesteryl esters in the presence or absence of cholesterol as compared to the other dietary fats. The preferred fatty acid substrate for cholesterol esterification by acyl coenzyme A:cholesterol acyltransferase is 18:1 fatty acids (15, 16), which may explain this finding. The liver microsomes of mice fed the different fat diets had similar free cholesterol content (**Table 5**). Addition of cholesterol to the fat diets increased the microsomal free cholesterol levels, and the absolute levels were comparable in all three groups.

Microsomal fatty acid content

Table 6 shows the fatty acid profiles of the liver microsomes under various dietary conditions. The microsomal fatty acid profile was indicative of the fatty acid composition of the diets. Feeding the MUFA diet showed higher levels of 18:1 whereas the PUFA diet showed higher levels of 18:2 fatty acids in liver micro-

TABLE 5. Hepatic microsomal cholesterol content

Diet	Free Cholesterol
	μmol/mg protein
PUFA	0.72 ± 0.03
PUFA + chol	0.91 ± 0.02***
MUFA	0.74 ± 0.04
MUFA + chol	0.91 ± 0.02*
SFA	0.72 ± 0.04
SFA + chol	0.92 ± 0.04**

Microsomes were prepared from pooled mouse liver tissue (n = 5, 50 mg per liver sample). The values shown are mean ± SD (n = 3 determinations) per mg microsomal protein. Differences between diet groups in the presence and absence of cholesterol were evaluated by Student's t test: * $P < 0.005$; ** $P < 0.001$; *** $P < 0.0005$.

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TABLE 6. Fatty acid composition of the total lipids from liver microsomes

Diet	Fatty Acid					
	16:0	18:0	18:1	18:2 n–6	20:4 n–6	Others
	% *w/w*					
PUFA	18.3 ± 0.42^{d}	18.8 ± 0.56^{b}	6.0 ± 0.28^{e}	37.0 ± 0.08^{a}	18.0 ± 0.29^{c}	2.8 ± 0.50^{a}
PUFA + chol	17.0 ± 0.08^{e}*	18.8 ± 0.28^{b}	9.3 ± 0.42^{f}**	31.0 ± 1.69^{b}**	19.8 ± 0.29^{b}**	3.0 ± 0.30^{a}
MUFA	22.0 ± 0.29^{c}	19.3 ± 0.42^{b}	18.8 ± 0.57^{c}	15.0 ± 0.08^{e}	21.7 ± 0.43^{a}	3.0 ± 0.29^{a}
MUFA + chol	22.0 ± 0.29^{c}	14.6 ± 0.42^{c}**	24.6 ± 0.42^{a}**	17.3 ± 0.40^{d}**	17.8 ± 0.08^{c}**	3.0 ± 0.29^{a}
SFA	24.3 ± 0.41^{b}	21.0 ± 0.08^{a}	17.6 ± 0.84^{d}	17.0 ± 0.08^{d}	16.0 ± 0.28^{d}	3.4 ± 0.57^{a}
SFA + chol	26.4 ± 0.57^{a}*	15.3 ± 0.42^{c}***	22.3 ± 0.42^{b}**	18.5 ± 0.40^{c}**	13.4 ± 0.50^{e}**	3.5 ± 0.41^{a}

Mice were fed the indicated diets (n = 5 per group) for 3 weeks. Microsomal lipids were extracted and analyzed for fatty acid composition as described in Materials and Methods. Differences between groups were evaluated by analysis of variance. Values without a common superscript are significantly different ($P < 0.05$). The difference between groups consuming the same fat diet in the presence and absence of 1% cholesterol (chol) was tested using Student's *t* test: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

somes, consistent with the respective dietary fat composition. Addition of cholesterol to these diets showed further alterations in the microsomal fatty acid profiles. Cholesterol supplementation to the PUFA diet decreased the levels of 16:0 and 18:2 fatty acids ($P < 0.05$ and $P < 0.01$, respectively), while the levels of 18:1 and 20:4 fatty acids increased ($P < 0.01$). On the other hand, addition of cholesterol to the MUFA and the SFA diets decreased the levels of 18:0 and 20:4 fatty acids, whereas the levels of 18:1 and 18:2 fatty acids increased ($P < 0.01$). These results are in agreement with previous observations where cholesterol feeding to the beef tallow and linseed oil diet was found to inhibit Δ6-desaturase enzyme activity, thereby decreasing the conversion of 18:2 to 20:4 fatty acid (17, 18). It is likely that the decrease in the levels of 18:2 and increase in the levels of 20:4 fatty acids induced by the PUFA + cholesterol diet is due to an increase in the Δ6-desaturase activity.

Response of cyp7 to dietary fat and cholesterol

Cyp7 was studied because this enzyme is involved in the catabolism of cholesterol through bile acid synthesis. At least four different cyp7 mRNA isoforms are detected by blot analysis of liver RNA from mice fed cholesterol or cholestyramine diets (L. B. Agellon and S. K. Cheema, unpublished observations). Thus, we developed a ribonuclease protection assay to accurately measure the total changes in cyp7 mRNA abundance under the various dietary conditions.

Detection of the cyp7 and G3PDH mRNAs is shown in **Fig. 2.** Feeding mice the chow diet with cholesterol increased the cyp7 mRNA abundance, in agreement with our previous study (19). The effect of the various diets on cyp7 mRNA abundance is shown in **Fig. 3** (top panel). Cyp7 mRNA abundance was found to be significantly higher in mice fed the MUFA diet compared to the PUFA and SFA fed mice. Interestingly, varying effects were observed when cholesterol was added to the fat diets. The addition of cholesterol to the PUFA diet increased the cyp7 mRNA abundance relative to mice fed the PUFA diet alone ($P < 0.01$). However, when cholesterol was added to the MUFA or SFA diets, the cyp7 mRNA abundance decreased significantly compared to mice fed the MUFA or SFA diets alone ($P < 0.001$ and $P < 0.01$, respectively). Correlation of cyp7 mRNA abundance with total plasma cholesterol, hepatic total and cholesteryl ester content did not reveal any significant relationship.

The pattern of change in hepatic cyp7 enzyme activity (Fig. 3, bottom panel) that was induced by the diet was identical to the change in cyp7 mRNA abundance. Addition of cholesterol to the PUFA diet increased cyp7 activity ($P < 0.01$) whereas addition of cholesterol to the MUFA and the SFA diets decreased cyp7 activity (P

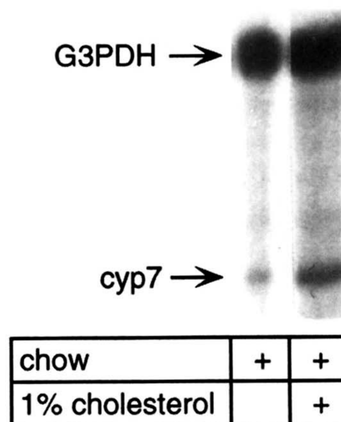

Fig. 2. Detection of cyp7 mRNA by a ribonuclease protection assay. The figure illustrates the detection of cyp7 mRNA in total RNA isolated from livers of C57BL/6J mice fed chow diet with or without 1% cholesterol for 3 weeks. Abundance of the cyp7 mRNA (bottom arrow) is expressed relative to the abundance of the G3PDH mRNA (top arrow).

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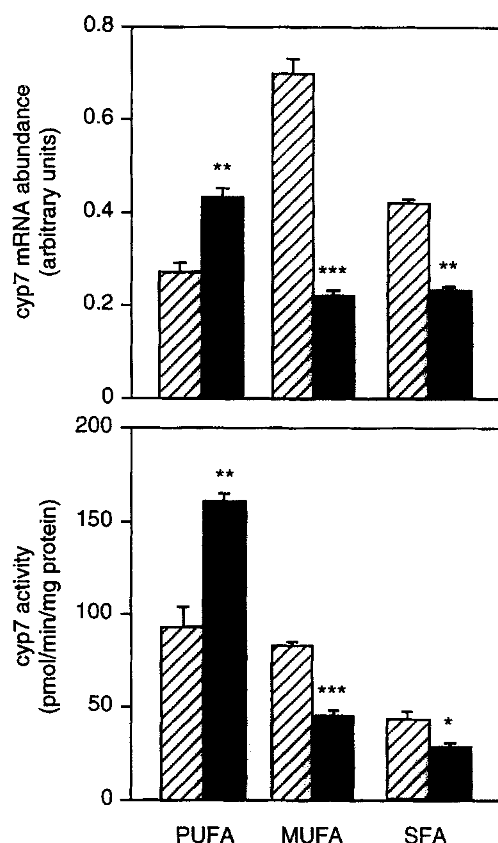

Fig. 3. Quantitation of cyp7 mRNA abundance and activity. Cyp7 mRNA abundance (top panel) and cyp7 activity (bottom panel) were quantitated as described in Materials and Methods. The cyp7 mRNA (mean ± SE, n = 5) abundance is shown relative to the abundance of the G3PDH mRNA. The difference between the groups consuming the same fat diet in the presence (solid bars) and absence (hatched bars) of cholesterol was tested using Student's t test: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

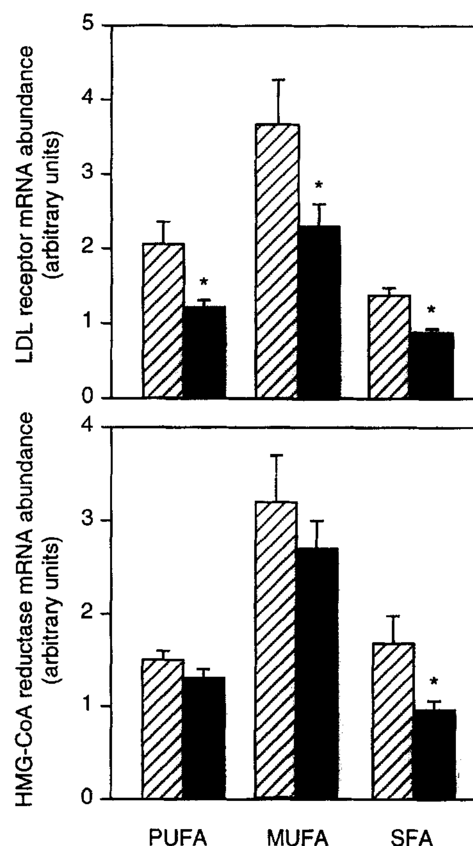

Fig. 4. Quantitation of LDL receptor and HMG-CoA reductase mRNA abundance. LDL receptor (top panel) and HMG-CoA reductase (bottom panel) mRNA abundance were quantitated as described in Materials and Methods. The figure depicts the abundance of each mRNA (mean ± SE, n = 5) normalized to G3PDH mRNA content. The difference between the groups consuming the same fat diet in the presence (solid bars) and absence (hatched bars) of cholesterol was tested using Student's t test: $^{*}P < 0.05$.

< 0.001 and $P < 0.05$, respectively). The identical pattern of changes was obtained when cyp7 activity was determined using an excess of exogenous radiolabeled cholesterol as a substrate in a conventional cyp7 activity assay (data not shown).

Hepatic LDL receptor and HMG-CoA reductase mRNA abundance

In response to changes in cholesterol levels, the liver maintains cholesterol homeostasis by regulating internalization of lipoproteins and de novo cholesterol synthesis. Previously, Fox et al. (20) showed that feeding cholesterol with saturated or unsaturated fat decreased the hepatic LDL receptor mRNA level in baboons. Thus, we studied the LDL receptor and HMG-CoA reductase mRNA abundance to verify the impact of the cholesterol-containing fat diets on sterol balance. Addition of cholesterol to the dietary fats decreased LDL receptor mRNA abundance ($P < 0.05$) (**Fig. 4,** top panel). HMG-CoA reductase mRNA abundance was also decreased by the addition of dietary cholesterol, although the extent of reduction did not reach statistical significance (Fig. 4, bottom panel). Nonetheless, these results indicate that both genes are responding to the increased cellular cholesterol status in the predicted manner.

DISCUSSION

The degree of tolerance to increased dietary cholesterol and the response to a given intake of cholesterol varies remarkably among different species and even among individuals of the same species (21, 22). Presumably, this is due to a wide variety of factors including

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absorption of dietary cholesterol, metabolism of plasma cholesterol, de novo cholesterol synthesis, cellular uptake of cholesterol, excretion of cholesterol into bile and the conversion of cholesterol to bile acids. Cyp7 is the key regulatory enzyme in the conversion of cholesterol to bile acids. Our current understanding of cyp7 gene regulation is derived from studies that demonstrate that dietary cholesterol and bile acids induce and repress cyp7 gene expression, respectively (23–29). These studies also demonstrate a positive correlation between cyp7 mRNA abundance and cyp7 enzyme activity. Thus, it appears that regulation at the level of gene transcription and/or mRNA degradation is the primary determinant of cyp7 activity. In this and a previous study (19) we demonstrated that cyp7 mRNA abundance is increased when the chow diet is supplemented with cholesterol, consistent with previous studies (27, 30, and reviewed in 1). However, recent investigations have also shown that inclusion of cholesterol in the diet does not always produce the expected stimulatory effect on cyp7 (2–4). This suggests that other components of the diet might also be important in the regulation of cyp7.

Dietary fats are known to modify cholesterol metabolism thereby affecting the whole body cholesterol homeostasis (31). As dietary fats constitute a major part of a typical western diet, it was important to study the regulation of cyp7 by cholesterol in the presence of dietary fats. We also assessed the impact of dietary fats on the ability of dietary cholesterol to regulate cyp7 gene expression in the C3H mouse strain (an atherosclerosis-resistant strain) and the results obtained were consistent with those observed in C57BL/6J (an atherosclerosis-sensitive strain).

The results of the present investigation indicate that dietary fats increase the cyp7 mRNA abundance and enzyme activity compared to the chow diet independent of dietary cholesterol (data not shown). However, addition of cholesterol to the semipurified diets that are high in fats did not always result in a further stimulation of cyp7 enzyme activity and mRNA abundance. Whereas an additional increase in cyp7 activity and mRNA abundance was induced by the cholesterol-supplemented PUFA diet, supplementation of the MUFA and SFA diets with cholesterol resulted in the significant reduction of both cyp7 activity and mRNA abundance. It should be noted that the majority of the studies documenting the induction of cyp7 mRNA abundance in response to dietary cholesterol used chow as the basal diet. For example, the relative abundance of cyp7 mRNA was increased in mice fed a chow diet supplemented with 20% olive oil and 2% cholesterol (32). In the present study, a semipurified diet containing 20% olive oil and no cholesterol induced the highest level of cyp7 mRNA. When the olive oil diet was supplemented with 1% cholesterol, the cyp7 mRNA abundance decreased by 69%. In a recent study, hypercholesterolemia was produced in an inbred strain of rabbits by feeding a semi-synthetic low fat cholesterol-free diet that contained casein as the sole protein source (3). The cyp7 mRNA abundance in these rabbits was significantly reduced compared to the same strain of rabbits fed a chow diet. These observations suggest that the amount of fat (or specific type of fat) in the semipurified diet or other factors in the chow diet alter the stimulatory effect of dietary cholesterol on cyp7 gene expression.

Bile acids are known to be necessary for the absorption of dietary fat and cholesterol absorption (33). It is assumed that both dietary and biliary cholesterol contribute to the total cholesterol pool that can be absorbed from the intestines. Changes in bile acid synthesis may alter bile acid availability in the intestines causing variations in cholesterol absorption efficiency and ultimately the amount of dietary cholesterol delivered to the liver by chylomicron remnants. Fatty acids modify bile composition by altering bile flow, and biliary bile acid, phospholipid and cholesterol content (34–37). The composition of bile from mice fed the different diets differed (see Table 3), but, based on the changes observed, the amount of bile acids available for release into the intestines under all the dietary conditions is not limiting.

Fatty acids have been shown to alter the activity of acyl coenzyme A:cholesterol acyltransferase (38–41). Thus, fatty acids can induce alterations in the cellular cholesterol pools. Feeding mice the different fat diets in the absence of cholesterol caused variable changes in the hepatic cholesterol pools. However, when the fat diets were fed together with cholesterol, there was a consistent increase in total hepatic cholesterol due to the accumulation of cholesteryl esters. The microsomal free cholesterol content also increased whenever cholesterol was present in the diets and the resulting absolute levels were similar among the diet groups. However, cyp7 activity or mRNA abundance did not always increase when cholesterol was added to the fat diets. There was no relationship between cyp7 mRNA abundance and hepatic total cholesterol, hepatic cholesteryl ester, or microsomal free cholesterol content. Previously, Björkhem and Akerlund (42) showed that microsomal free cholesterol concentration was not a direct regulator of cyp7 activity. The findings taken together can now be extended to suggest that the overall cholesterol status of the liver, or the free cholesterol content of hepatic microsomes, does not directly regulate cyp7 gene expression.

The LDL receptor and HMG-CoA reductase mRNA abundance decreased as expected when the cholesterol

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status of the cell increased. Although sequences that bear some similarity to sterol response elements (SRE) (43, 44) exist in the 5′ upstream region of the hamster cyp7 gene (45), the functionality of these sequences remains to be demonstrated. However, given that changes in cyp7 mRNA abundance do not correlate (positively or negatively) with the changes in LDL receptor and HMG-CoA reductase mRNA abundance, it is unlikely that the SRE binding protein is involved in the regulation of the cyp7 gene. Oxysterols have been shown to be important in suppressing LDL receptor and HMG-CoA reductase gene expression (46, 47). However, the involvement of oxysterols in the regulation of cyp7 gene expression cannot be excluded in this study. Certain species of fatty acids (notably polyunsaturated fatty acids) have been shown to specifically regulate the expression of several genes (48, 49). In light of the present observations, fatty acids may play a direct role in regulating cyp7 gene expression.

Our laboratory recently showed that feeding cholesterol to mice decreased ileal sodium bile acid transporter gene expression (19). This finding likely accounts for the efficient bile acid excretion observed when mice and rats are fed a high cholesterol diet. The involvement of bile acids notwithstanding, it appears that dietary cholesterol itself does not have a dominant singular effect on cyp7 gene expression.

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