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Linoleic acid-rich diet increases hepatic taurine and cholesterol 7α-hydroxylase activity in conjunction with altered bile acid composition and conjugation in gerbils

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Although dietary fatty acid saturation is an effective modulator of plasma cholesterol, its effects on bile acid metabolism are not well elucidated. In fact, it is possible that primary changes in bile acid metabolism account for the response in plasma lipids. Thus, the aim of this study was to compare the effect of a diet rich in specific saturated fatty acids [lauric and myristic acid (12:0 + 14:0)] with a diet containing a specific unsaturate, linoleic acid (18:2n6) on factors affecting bile acid metabolism in gerbils. Gerbils were fed a high-fat diet (40% of energy) based on either coconut oil (12:0 + 14:0-rich) or safflower oil (18:2n6-rich). After 4 weeks, plasma sterols, the gallbladder bile acid profile, and fecal bile acid excretion were analyzed along with hepatic concentrations of taurine and cholesterol. Hepatic activities of acyl-CoA cholesterol acyltransferase (ACAT) and cholesterol 7α-hydroxylase were also measured. Exchange of 29% energy as 18:2n6 for 12:0 + 14:0 increased the proportion of saturated and monounsaturated fatty acids at the expense of polyunsaturated fatty acids in plasma, hepatic microsomal membranes, and bile phospholipids. Concentrations of plasma cholesterol and 27-hydroxycholesterol were greatly increased by the 12:0 + 14:0-rich diet. This effect was coupled with significant decreases in hepatic taurine concentration and the activities of ACAT and cholesterol 7α-hydroxylase, as well as fecal bile acid excretion. Analysis of gallbladder bile acids revealed that the 12:0 + 14:0-rich diet decreased the cholate:chenodeoxycholate and primary:secondary bile acid ratios as well as the ratio of taurine:glycine conjugates. In essence, the exchange of dietary lauric and myristic acids for linoleic acid was associated with decreased production and excretion of bile acids concurrent with reduced hepatic taurine and taurine-conjugated bile acids. (J. Nutr. Biochem. 9:249–257, 1998) © *Elsevier Science Inc. 1998*

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Introduction

Numerous reports have shown that dietary fats rich in unsaturated fatty acids lower plasma lipids relative to diets rich in saturates (SATs)[1–3] and protect against cholesterol-induced gallstones.[4–6] However, the exact mechanism(s) whereby fatty acid saturation co-ordinately modifies plasma lipids, bile acid metabolism, and gallstone formation is not fully understood.[4,5] Dietary fatty acids impact bile acid metabolism at various levels, but to what degree polyunsaturated fatty acids (POLYs) may enhance bile acid and neutral sterol excretion to reduce plasma cholesterol remains unresolved. Initial studies in men[7,8] reported that polyunsaturated fat increased fecal bile acid output, but other investigations in men[9] and rats[10] did not observe this

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effect. On the other hand, bile acid composition and hydrophobicity can be altered by changes in dietary fat composition. For example, several animal species fed POLYs showed an increased cholate profile compared with animals fed saturated fat,[11,12] confirming similar conclusions in human patients.[13] Nonetheless, the relationship between dietary fatty acids and either the bile acid profile (including glycine-taurine conjugation) or the hydrophobicity index is still relatively undefined.

The latter point is important because hydrophobicity of bile acids, which appears directly related to gallstone formation in hamsters,[14] is inversely related to bile acid hydroxylation (i.e., chenodeoxycholate > cholate and secondary bile acids > primary bile acids) and is affected by conjugation with glycine or taurine.[14–16] Glycine-conjugated bile acids are more hydrophobic (lithogenic) than taurine conjugates and they enhance cholesterol transport by biliary micelles.[4,15,16] Thus, the balance between glycine and taurine conjugates, although less influential than the degree of hydroxylation, does affect biliary cholesterol solubilization and mobilization as well as intestinal cholesterol absorption. Hamsters fed a cholesterol-rich lithogenic diet to induce gallstones develop lower cholate:chenodeoxycholate and glycine:taurine ratios,[14] whereas dietary manipulations that prevent such gallstone formation, including supplementation with cholestyramine[14,17] or fiber,[14,18] increase both of these ratios. Kritchevsky[19] has noted that the glycine:taurine ratio of bile acids may influence the plasma cholesterol level because an inherently high ratio renders certain species vulnerable to diet-induced hypercholesterolemia.

In this study the gerbil was selected to evaluate dietary fat saturation on bile acid production and composition mainly because of its extreme cholesterolemic sensitivity to dietary fat saturation,[20] even in the absence of dietary cholesterol.[3,21] We compared the effects of purified diets containing either coconut oil (high 12:0 + 14:0) or safflower oil (high 18:2n6) on bile acid composition and excretion along with the changes in the activity of 7α-OHase and ACAT and the status of cholesterol and taurine in liver.

Methods

Animals and diets

Adult male Mongolian gerbils (*Meriones unguiculatus*), weighing 75 ± 5 g, were obtained from Tumblebrook Farms (West Brookfield, MA) and housed in individual cages in an air-conditioned room with a 12-hr reversed-light-dark cycle (light on at 18:00 hr). They were randomly assigned to two groups and were fed a cholesterol-free purified diet containing either a high proportion of linoleic acid (18:2n6-rich) or lauric and myristic acids (12:0 + 14:0-rich) for one month. Diets were fed to caloric requirement (9 g diet as prepared) to maintain constant body weight for the duration of the study. The basal composition of these diets (% of total dry weight) was as follow: casein, 22.2; cornstarch, 23.0; glucose, 13.3; cellulose, 15.0; fat, 20; mineral mix (Ausman-Hayes), 5.0; vitamin mix (Hayes-Cathcart), 1.2; and choline chloride: 0.3. Compositions of the mineral and vitamin mixes were previously detailed.[3] Fat provided approximately 40% of the total dietary energy and represented either a blend of 99% coconut oil and 1% safflower oil (12:0 + 14:0-rich) or 1% coconut and 99% safflower oil (18:2n6-rich). Accordingly, the fatty acid composition of both diets, analyzed by gas liquid chromatography, differed in their proportions of linoleic (18:2n6) and lauric (12:0) and myristic (14:0) acids. The proportion of all other fatty acids was similar between diets (*Table 1*).

Table 1 Fatty acid composition of purified gerbil diets

Fatty acid	12:0 + 14:0-rich (% of total fatty acids)	18:2n6-rich (% of total fatty acids)
<12:0	3.9 (1.6) [1]	—
12:0	48.7 (19.5)	0.5 (0.2)
14:0	20.3 (8.1)	0.2 (0.1)
16:0	11.2 (4.5)	9.2 (3.7)
16:1n7	trace [2]	trace
18:0	2.6 (1.0)	2.9 (1.2)
18:1n9	9.0 (3.6)	11.9 (4.8)
18:2n6	3.2 (1.3)	75.2 (30.1)
18:3n3	trace	trace
20:0	trace	trace
Total saturated	86.7 (33.7)	12.8 (5.2)
Total monounsaturated	9.0 (3.6)	11.9 (4.8)
Total polyunsaturated	3.2 (1.3)	75.2 (30.1)
P/S ratio	0.04	5.87

[1] Values in parenthesis represent percent energy contributed by individual fatty acid.
[2] Trace represent values < 0.1%.
Note: Dietary fatty acid profile was generated by gas liquid chromatography analysis as described in Materials and Methods.
P/S, polyunsaturated fatty acids/saturated fatty acids.

All experiments were performed after 4 weeks of diet consumption when the plasma cholesterol and triglycerides were stable indicating a new steady state. At the end of the 4 week feeding period, feces of each gerbil were collected for 3 days prior to an overnight fast, and blood was collected under carbon dioxide/oxygen anesthesia via cardiac puncture with a 30-gauge needle wetted with 10% EDTA. At necropsy the gallbladder bile was aspirated and the liver and an aliquot of perirenal adipose tissue were rapidly removed, weighed, and portions frozen for subsequent analysis.

Plasma lipid analysis

Total cholesterol and triglycerides were determined by enzymatic assay (Sigma kit nos. 352 and 336, respectively; Sigma Diagnostics, St. Louis, MO). Phospholipids and free cholesterol concentrations were determined by enzymatic assay (free cholesterol C and phospholipids B kits from Wako Chemicals, Richmond, VA). Cholesteryl ester was calculated as the difference between the free and total cholesterol concentration.

Bile lipid analysis

Lipids were isolated from 10 μl of bile by a modified Folch extraction[22] as previously described.[14] Free cholesterol and phospholipids were assayed enzymatically in the chloroform phase after evaporation and dissolution in isopropanol, as previously described.[14] Total bile acids were determined enzymatically in the methanol phase (Sigma kit no. 450). Individual bile acids were separated by a high performance liquid chromatography (HPLC) method[23] as previously described.[14] The bile acid hydrophobic index (HI) of each bile sample was calculated according to Heuman.[24]

Plasma 27-OH cholesterol analysis

The concentration of 27-hydroxycholesterol (27-OH cholesterol) in 0.5 to 1 ml of gerbil plasma was determined by HPLC after oxidation and analysis of α, β unsaturated ketones.[25] Highly purified 27-OH cholesterol (Research Plus, Bayonne, NJ) was used as a standard to produce an HPLC calibration curve. First, esterified 27-OH cholesterol was hydrolyzed enzymatically according to the procedure of Oda et al.[26] A mixture of cholesterol esterase (Sigma Chemical Co., St. Louis, MO) and sodium cholate, dissolved in 0.1 M of phosphate buffer (pH 7.4), was added to each sample to achieve a final concentration of 150 μmol of sodium cholate and 1 unit of cholesterol esterase per milliliter of sample. Then incubations were performed in a shaking water bath at 37°C for 45 min.[26] Sterols in plasma samples and the standard were then oxidized by adding cholesterol oxidase (Sigma Chemical Co.) to each tube prior to incubation for 20 min at 37°C to generate α, β unsaturated ketones. The reaction was stopped by adding 0.5 ml methanol followed by 0.5 ml KCl (1.12%). Sterols were extracted twice with 2 ml of hexane. The organic phase was dried under nitrogen. The residue was dissolved in isopropanol/dodecane (5/95, v/v), and an aliquot of 50 μl was injected into a normal phase HPLC spherisorb silica column (4.6 × 250 mm; Alltech Inc., Deerfield, IL). The isocratic mobile phase was hexane-isopropanol (95/5) and the flow rate was 1 ml/min. The peaks were detected at 240 nm and the 27-OH cholesterol was identified by comparing the retention time to the known standard. The concentration of 27-OH cholesterol in plasma samples was calculated from the standard calibration curve generated under identical circumstances. To assure the efficiency of this method, we performed preliminary experiments. To assure total hydrolysis of 27-OH cholesterol, we questioned whether the time of incubation with cholesterol esterase was long enough to achieve total hydrolysis of 27-OH cholesterol. In preliminary experiments, equivalent amounts of plasma (1 ml) were incubated with cholesterol esterase for 0, 10, 20, 30, 45, 60, and 120 min before adding cholesterol oxidase and incubating for an additional 20 min under the same conditions described above. This time course experiment revealed that 27-OH cholesterol increased rapidly after 10 min and reached a plateau after approximately 45 min of incubation with cholesterol esterase. Increasing the incubation time up to 2 hr gave similar results as 1 hr. Therefore, the incubation time of 60 min with cholesterol esterase followed by 20 min with both cholesterol esterase and cholesterol oxidase was long enough for total hydrolysis and oxidation of 27-OH cholesterol.

Taurine analysis

Liver and plasma taurine were analyzed by HPLC as previously published.[27] Briefly, taurine was extracted from 100 mg of homogenized liver by adding 100 μl of a 20% trichloroacetic acid solution (TCA). The liquid phase of three extractions was collected after centrifugation and combined. Plasma taurine was extracted from 300 μl with 30 μl of a 100% TCA solution. In order to remove amino acids that interfered with taurine separation on HPLC, an aliquot of 100 μl of liver and plasma extract was filtered through an ion exchange column prepared in a Pasteur pipette (0.5 cm i.d. × 150 mm) by layering 2 cm of anion AG 1-X resin (Dowex 200–400 mesh) under 2 cm of cation of AG-50W-X8 resin (200–400 mesh) as previously described.[26] The taurine eluant was collected by flushing 3 ml of water through the column. Taurine concentration was determined by HPLC after a precolumn derivatization with o-phthalaldhyde (OPA). The mobile phase consisted of sodium phosphate buffer (15 mmol/L, pH 6.0) and acetonitrile (80:20, v:v) and was pumped at 1 ml/min by using a Rainin Rabbit HP® (Rainin Instrument Co, Inc, Woburn, MA) solvent delivery system. The effluent was monitored with a fluorometer (SLM Amico Fluoro-Monitor II®, SLM Instruments, Inc, Urbana, IL) with 395 nm excitation and 455 nm emission filters and recorded with a CR3A Integrator® (Shimadzu Scientific Instruments, Inc, Columbia, MD).

Hepatic cholesteryl ester determination

Free and esterified cholesterol in the liver and plasma were determined by an HPLC method.[28] Hepatic cholesterol was extracted by grinding a piece of liver (200 mg) with anhydrous sodium sulfate to a fine powder and extracting lipids three times with 4 ml of chloroform:methanol (2:1, v:v). The combined extract was dried under nitrogen and redissolved in the mobile phase. A sample of 100 μl was injected into the HPLC and separated using Waters Radial-Pak® Resolve C18 cartridge column (8 mm × 10 cm, part no. 84720) eluted isocratically with acetonitrile:isopropanol (45/55, v/v) at 2.0 ml/min. The absorbance of the eluate was measured at a wavelength of 210 nm using an ultraviolet detector. Free and individual esters of cholesterol were determined by comparing the peak areas from samples with those obtained for the standards (Sigma Chemical Co.). To calculate the total esterified cholesterol the sum of cholesteryl esters was divided by 1.67 according to Wiztum et al.[29]

Microsomal enzyme activities

Microsomes were prepared immediately from fresh liver (approximately 1 g) according to the method of Chautan et al.[30] and frozen at −80°C until analyzed. Microsomal protein was determined by the Markwell assay.[31] Lipids in microsomes were extracted with chloroform:methanol (2:1, v:v). The lipid extract in the chloroform layer was dried under nitrogen and redissolved with an aliquot of isopropanol. Concentrations of free and total cholesterol and phospholipids were measured enzymatically as described earlier.[32] Both free cholesterol and phospholipid mass were linearly related to protein concentration in microsomes. Results were expressed as micrograms of free cholesterol or phospholipids per milligram of microsome protein. ACAT activity was performed by measuring the incorporation of [1-^{14}C]oleoyl-CoA into cholesteryl oleate.[30] A total volume of 250 μl of phosphate buffer (50 mM K_2HPO_4, 0.1 M sucrose, 30 mM EDTA, 70 mM KCl, 1 mM DTT), containing the microsomal suspension (0.25–0.5 mg of protein) and 67 μl of bovine serum albumin (20 mg/ml), was preincubated at 37°C for 5 min. Then 20 μl of [1-^{14}C]oleoyl-CoA (specific radioactivity 11,000 dpm/nmole) was added and the incubation continued for 3 minutes at 37°C with shaking. The reaction was stopped by adding 2.5 ml of isopropanol/heptane/sulfuric acid 1 N (77:19:4; v:v:v). Sterol extraction was performed by adding 1.5 ml of heptane and 1 ml of H_2O. Radioactive cholesteryl ester was separated from the nonreactive oleoyl-CoA by silica column chromatography performed in a Pasteur pipette,[30] and radioactivity was quantified in a Beckman liquid scintillation counter.

Cholesterol 7α-hydroxylase (7α-OHase) activity was assayed by the radioisotope method of Nicolau et al.[33] Briefly, microsomal proteins (0.5–1 mg) were diluted in 0.5 ml of phosphate buffer (75 mM, pH 7.4) containing EDTA (1 mM), DTT (2 mM), $MgCl_2$ (4 mM), and 0.54 μci of [4-^{14}C cholesterol] (specific radioactivity 12,000 dpm/nmoles) previously mixed in 0.15 ml of Tween 80. Samples were preincubated at 37°C for 5 min. The reaction was initiated by adding 0.15 ml of NADPH (10 mM) followed by incubation at 37°C with shaking. The reaction was stopped 20 min later by adding 5 ml of chloroform:ethanol (5:1, v:v) and 2.5 ml of H_2O. Sterols were extracted and separated by thin layer chromatography on silica gel, eluting with ethyl acetate:hexane (8:2, v:v). The region corresponding to cholesterol and 7α-hydroxycholesterol were scraped off and their radioactivity counted. For both reactions (ACAT and cholesterol 7α-OHase) samples were as-

Table 2 Plasma and liver lipids for gerbils fed 12:0 + 14:0-rich or 18:2n6-rich diets for four weeks

Tissue	12:0 + 14:0-rich	18:2n6-rich
Plasma [1]		
Total cholesterol, mmol/L	5.7 ± 0.2	1.9 ± 0.1[3]
Free cholesterol, mmol/L	1.6 ± 0.1	0.54 ± 0.05[3]
Esterified cholesterol, mmol/L	4.1 ± 0.3	1.32 ± 0.05[3]
Triglycerides, mmol/L	1.7 ± 0.1	0.71 ± 0.06[4]
27-hydroxycholesterol,[2] nmol/L	310 ± 20	120 ± 20[4]
Liver [1]		
Total cholesterol, μmol/g	19.4 ± 1.0	21.2 ± 0.9
Free cholesterol, μmol/g	16.0 ± 0.9	15.7 ± 0.7
Esterified cholesterol, μmol/g	g3.4 ± 0.4	5.5 ± 0.7[5]

Values are mean ± SEM.
[1] n = 13 per group.
[2] n = 5 per group.
[3] Significantly different from 12:0 + 14:0 at $P < 0.0001$.
[4] Significantly different from 12:0 + 14:0 at $P < 0.001$.
[5] Significantly different from 12:0 + 14:0 at $P < 0.05$.

sayed in duplicate, and a boiled microsomal incubation was used as blank for correction. Results are expressed as picomole formation of cholesteryl esters (ACAT) or 7α-hydroxycholesterol (cholesterol 7α-OHase) per minute and per milligram of microsomal protein.

Fatty acid analysis

Fatty acid composition of formulated diets and collected samples (plasma, adipose tissue, bile, liver microsomes) was determined by gas liquid chromatography according to a one-step transesterification procedure.[34] Fatty acid analysis was performed in a Shimadzu GC9-AM GLC using a 100-m fused capillary column (SP-2560, Supelco Inc.).

Fecal bile acids and cholesterol

Total bile acids and cholesterol were extracted from a 3-day fecal collection using a modified method[14] of Suckling et al.[35] Feces were dried and weighed, and a fine ground sample (0.1 g) was hydrolyzed with 0.2 ml of 10 M NaOH at 110°C overnight, and an aliquot of the hydrolysate was dried under nitrogen and extracted with 3 ml of chloroform:methanol (2:1) in the presence of 0.75 ml of a 1.12% of KCl solution. Bile acids were collected in the methanol/KCl phase, and their concentrations were determined by enzymatic kit (Sigma no. 450) as detailed earlier.[6] Cholesterol was determined in an aliquot of the chloroform layer using the Sigma enzymatic kit as described earlier.[6]

Statistics

Results are expressed as mean ± SEM and statistical differences between groups were performed by the Student *t*-test.

Results

Body and liver weights

Both groups of gerbils consumed similar amounts of purified diet, and no differences in terminal body (76 ± 2 and 77 ± 3 g) or liver weights (2.4 ± 0.1 and 2.5 ± 0.1 g) were observed between the 12:0 + 14:0-rich or 18:2n6-rich diet groups, respectively.

Plasma and liver lipids

After 4 weeks, the 12:0 + 14:0-rich diet caused a dramatic increase in both plasma cholesterol (+200%) and triglycerides (+140%) (*Table 2*). However, despite the difference in plasma cholesterol neither total nor free hepatic cholesterol were significantly affected by the change in fat composition. A significantly greater mass of hepatic cholesterol ester was associated with the 18:2n6-rich diet, but even this represented only one-third the free cholesterol mass (*Table 2*).

Plasma 27-OH cholesterol concentrations

The concentration of 27-OH cholesterol in the plasma of gerbils fed the 12:0 + 14:0-rich diet was significantly increased ($P < 0.01$) compared with those fed the 18:2n6-rich diet (*Table 2*).

Table 3 Fatty acid distribution (%) in lipids from plasma, adipose tissue, microsomes, and bile phospholipids of gerbils fed 12:0 + 14:0-rich or 18:2n6-rich for 4 weeks

Fatty acid	Plasma		Adipose tissue		Microsomal phospholipids		Bile phospholipids	
	12:0 + 14:0-rich	18:2n6-rich	12:0 + 14:0-rich	18:2n6-rich	12:0 + 14:0-rich	18:2n6-rich	12:0 + 14:0-rich	18:2n6-rich
12:0	3.1 ± 0.3	0.1 ± 0.1 [1]	20.0 ± 1.1	0.2 ± 0.0 [1]	1.1 ± 0.7	0.5 ± 0.1	0.22 ± 0.1	0.12 ± 0.1
14:0	4.2 ± 0.3	0.7 ± 0.1 [1]	18.4 ± 0.6	0.6 ± 0.0 [1]	3.8 ± 0.2	1.9 ± 0.8 [1]	2.6 ± 0.6	0.6 ± 0.3 [2]
16:0	22.9 ± 1.2	19.5 ± 1.0	19.8 ± 0.4	11.4 ± 0.3 [1]	26.7 ± 0.5	22.0 ± 0.5 [2]	31.7 ± 0.8	27.3 ± 0.3
16:1n7	1.4 ± 0.2	0.4 ± 0.1 [3]	2.0 ± 0.3	0.6 ± 0.3 [3]	1.2 ± 0.2	0.1 ± 0.1 [1]	1.2 ± 0.2	0.4 ± 0.1 [1]
18:0	12.1 ± 0.1	12.0 ± 0.6	4.0 ± 0.2	2.9 ± 0.1 [2]	20.6 ± 2.3	24.0 ± 0.7	12.4 ± 1.6	14.9 ± 3.4
18:1n9	21.9 ± 1.2	7.0 ± 0.5 [1]	28.8 ± 0.6	23.4 ± 2.0 [3]	23.9 ± 4.3	9.3 ± 1.7 [2]	21.5 ± 1.4	12.4 ± 2.8 [2]
18:2n6	27.4 ± 0.5	49.3 ± 0.5 [1]	6.5 ± 0.6	60.4 ± 2.4 [1]	12.9 ± 1.3	23.6 ± 3.4 [2]	22.5 ± 1.9	33.4 ± 3.4 [2]
18:3n3	0.10 ± 0.06	0.06 ± 0.01	0.20 ± 0.05	0.2 ± 0.07	0.2 ± 0.09	0.3 ± 0.1	0.4 ± 0.3	0.4 ± 0.3
20:3n6	1.1 ± 0.2	1.2 ± 0.8	0.14 ± 0.01	0.15 ± 0.1	1.9 ± 0.3	1.3 ± 0.5	2.1 ± 0.7	1.0 ± 0.3
20:4n6	3.7 ± 0.2	7.5 ± 0.5 [1]	0.07 ± 0.01	0.05 ± 0.02	3.3 ± 0.6	10.9 ± 0.9 [2]	3.3 ± 0.6	6.4 ± 1.0 [2]
22:6n3	1.7 ± 0.7	2.4 ± 0.4	ND	ND	4.3 ± 0.9	6.0 ± 0.8	2.0 ± 0.2	3.0 ± 0.6

Values are presented as mean ± SEM; n = 5 per group.
ND = not detected.
[1] Statistically significant at $P < 0.001$.
[2] Statistically significant at $P < 0.01$.
[3] Statistically significant at $P < 0.05$.

Table 4 Percent bile acid distribution (%) and concentration (mM) in the gallbladder of gerbils fed 12:0 + 14:0-rich or 18:2n6-rich purified diets for 4 weeks

Bile acid	12:0 + 14:0-rich	18:2n6-rich
Bile acid distribution (mM)		
TC [2]	57 ± 7 (37 ± 1) [1]	97 ± 11 [4] (72 ± 3) [3]
GC	77 ± 8 (51 ± 2)	28 ± 3 [3] (21 ± 8) [4]
TCDC	7 ± 1 (4.6 ± 0.3)	5 ± 1 (3.7 ± 0.3) [6]
GCDC	4.3 ± 0.6 (2.8 ± 0.3)	2 ± 0.4 [5] (1.5 ± 0.2) [5]
TDC	2.6 ± 0.5 (1.7 ± 0.3)	1 ± 0.1 [6] (0.7 ± 0.1) [6]
GDC	2.3 ± 0.4 (1.5 ± 0.2)	0.8 ± 0.2 [6] (0.6 ± 0.1) [6]
TLC	0.9 ± 0.1 (0.6 ± 0.1)	0.7 ± 0.2 (0.5 ± 0.1)
GLC	0.2 ± 0.1 (0.13 ± 0.01)	0.2 ± 0.1 (0.1 ± 0.01)
Ratio		
Σcholate:Σcheno	12 ± 1	18 ± 1 [3]
Σglycine:Σtaurine	1.2 ± 0.1	0.3 ± 0.1 [3]
Primary:secondary	24 ± 3	48 ± 12 [4]
Hydrophobicity index (HI)	0.10 ± 0.01	0.06 ± 0.01 [3]

Values are mean ± SEM.
n = 13 and 10, respectively, for 12:0 + 14:0- and 18:2n6-rich diets.
[1] Values in parentheses represent percent distribution of each bile acid.
[2] TC, taurocholate; GC, glycocholate; TCDC, taurochenodeoxycholate; GCDC, glychochenodeoxycholate; TDC, taurodeoxycholate; GDC, glycodeoxycholate; TLC, taurolithocholate; GLC, glycolithocholate.
[3] Significantly different from 12:0 + 14:0-rich diet at $P < 0.0001$.
[4] Significantly different from 12:0 + 14:0-rich diet at $P < 0.01$.
[5] Significantly different from 12:0 + 14:0-rich diet at $P < 0.05$.
[6] Significantly different from 12:0 + 14:0-rich diet at $P < 0.02$.

Fatty acid composition

The plasma and adipose tissue of gerbils fed the 12:0 + 14:0-rich diet were enriched in saturated (12:0, 14:0, 16:0) and monounsaturated (16:1n7, 18:1n9) fatty acids to the detriment of 18:2n6 (*Table 3*). In addition, the 18:2n6 and 20:4n6 in bile phospholipids were significantly decreased (approximately 30% and 50%, respectively). In liver microsomes from gerbils fed the 12:0 + 14:0-rich diet, 18:2n6 was 33% lower, essentially replaced by 18:1n9, while the proportion of 20:4n6 was not changed. The fatty acid composition in the adipose tissue resembled the fatty acid profile of the dietary fat.

Biliary lipid composition

Biliary lipids were not affected by the dietary shift in fatty acids; that is, concentrations (mmol/L) of cholesterol (6 ± 0.3), phospholipids (22 ± 2), and bile acids (148 ± 14) in the gallbladder bile of gerbils fed the 12:0 + 14:0-rich diet were similar to those of gerbils fed the 18:2n6-rich diet (5 ± 0.3, 24 ± 2, and 155 ± 15, respectively). The relative mol percent of biliary lipids was also similar between groups.

Bile acid profile in gallbladder bile

The bile acid profile of chow-fed gerbils was intermediate between the two purified diets, being mainly cholic acid (92%) with a small amount of chenodeoxycholic acid (4%), deoxycholic acid (2%), and lithocholic acid (approximately 1%) (data not shown). These bile acids were conjugated equally with taurine and glycine so that the glycine:taurine ratio was approximately 1.0. The concentration (mmol/L) and the mol percent of chenodeoxycholate was >40% greater in gerbils fed the 12:0 + 14:0-rich diet compared with the 18:2n6-rich diet (*Table 4*). Thus, the cholate:chenodeoxycholate ratio was 33% lower in gerbils fed the 12:0 + 14:0-rich diet, whereas the concentration and mol percent of deoxycholic acid (secondary bile acid) was >100% higher and the primary:secondary bile acid ratio approximately 50% lower in these gerbils. However, the 18:2n6-rich diet favored taurine-conjugated bile acids over glycine conjugates such that 75% of the cholate and chenodeoxycholate acids were conjugated with taurine in gerbils fed the 18:2n6-rich diet compared with only 40% to 45% for gerbils fed the 12:0 + 14:0-rich diet (*Table 4*). Conjugation of secondary bile acids (deoxycholic and lithocholic) to taurine or glycine was less affected by the dietary fatty acid exchange. The HI, which reflects the balance between the hydrophobic and hydrophilic bile acids, was 40% greater with the 12:0 + 14:0-rich diet (*Table 4*).

Hepatic and plasma taurine concentration

Plasma taurine was not affected by dietary fatty acids (133 ± 2 and 135 ± 10 μmoles/L for 12:0 + 14:0- and 18:2n6-rich diets, respectively). However, hepatic taurine was significantly ($P < 0.001$) decreased by 12:0 + 14:0 compared with 18:2n6 (3.6 ± 0.4 and 8.5 ± 0.6 μmoles/g wet weight, respectively). Liver taurine concentration was inversely correlated (r = 0.86) with the glycine:taurine ratio of gallbladder bile acids (*Figure 1A*). The lower taurine and increased glycine conjugates were limited to cholic acid, because the amino acid conjugations of chenodeoxycholate, deoxycholate, and lithocholate were unaffected by liver taurine. Accordingly, both the glycine:taurine ratio for bile acids and hepatic taurine concentration were directly related to the HI (*Figure 1B, 1C*).

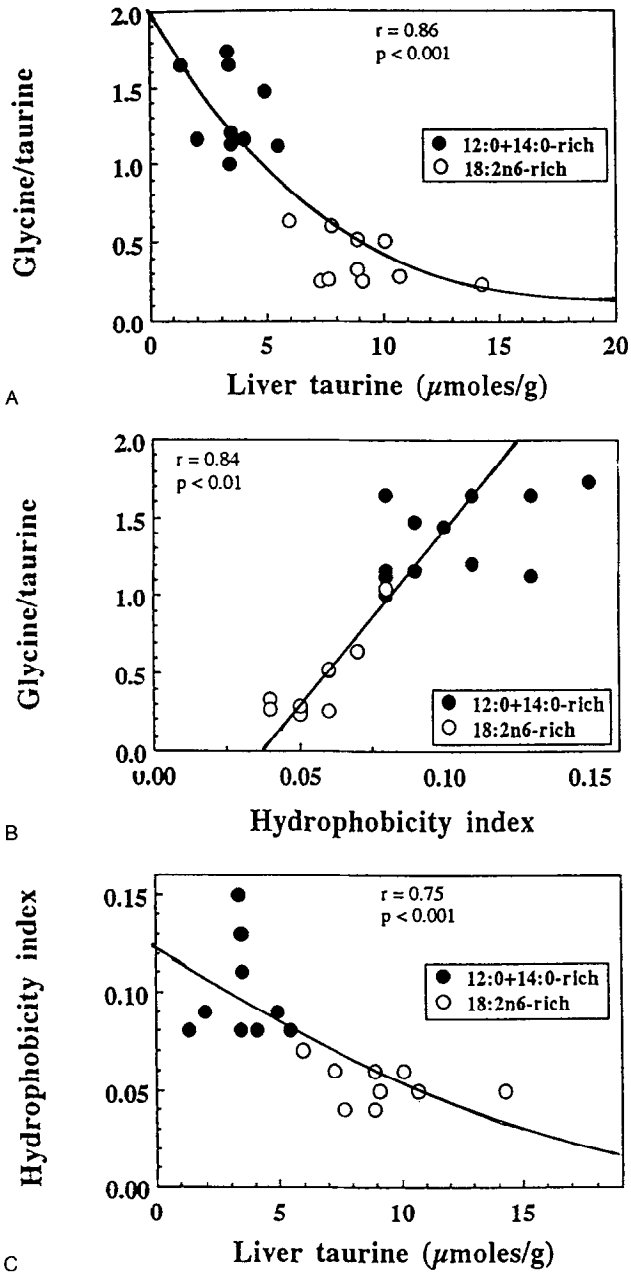

Figure 1 Correlations between (A) glycine:taurine bile acid ratio and liver taurine concentration; (B) hydrophobicity index (HI) gallbladder bile; and (C) HI of gallbladder bile and liver taurine concentration of individual gerbils fed 12:0 + 14:0-rich or 18:2n6-rich diets.

Fecal excretion of bile acids and cholesterol

Although total daily fecal volume was similar for both dietary groups, greater excretion of bile acids (+40%) and cholesterol (+50%) was observed in gerbils fed the 18:2n6-rich diet (*Table 5*).

Hepatic enzyme activities

The concentrations of free cholesterol and phospholipids as well as the free cholesterol:phospholipid ratio in liver microsomes were similar for both diets (*Table 6*). However, replacing 12:0 + 14:0 with 18:2n6 induced significant increases of 70% and 60% in ACAT and cholesterol 7α-OHase activity in liver microsomes (*Table 6*).

Table 5 Fecal cholesterol and bile acid output of gerbils fed 12:0 + 14:0-rich or 18:2n6-rich diets for 4 weeks

	12:0 + 14:0-rich	18:2n6-rich
Feces, g (3 days) [1]	7.0 ± 0.5	6.6 ± 0.2
Cholesterol		
μmol/g feces	2.8 ± 0.3	4.9 ± 0.6 [2]
μmol/day/100g bd wt	9.3 ± 1.0	14 ± 1 [3]
Bile acids		
μmol/g feces	56 ± 5	76 ± 5 [2]
μmol/day/100g bd wt	17 ± 1	24 ± 2 [3]

[1] Values are mean ± SEM.
n = 8 and 7, respectively, for 12:0 + 14:0-rich and 18:2n6-rich diets.
[2] Significantly different from 12:0 + 14:0 at $P < 0.01$.
[3] Significantly different from 12:0 + 14:0 at $P < 0.02$.

Discussion

As expected, the 18:2n6-rich diet lowered plasma cholesterol. This decline was associated with increases in the activity of cholesterol 7α-OHase and fecal output of bile acids. In addition, bile acid composition favored a high proportion of primary bile acids, mostly conjugated with taurine. Consequently, the HI of gallbladder bile was decreased, but not enough to affect the lithogenic index. Thus, the 18:2n6-rich diet appeared to enhance certain aspects of cholesterol disposition by the hepatocyte.

Cholesterol 7α-OHase, bile acid excretion, and fat saturation

High 18:2n6 intake enhanced the conversion of cholesterol to bile acids in the liver. Although the precise regulation of cholesterol 7α-OHase activity and bile acid output is not known, both the hepatic taurine level and the availability of

Table 6 Lipid concentrations and activities of acyl-CoA cholesterol acyltransferase (ACAT) and cholesterol 7α-hydroxylase in hepatic microsomes of gerbils fed 12:0 + 14:0-rich or 18:2n6-rich diets for 4 weeks

	12:0 + 14:0-rich	18:2n6-rich
Microsomal lipids (μmol/mg protein)		
Total cholesterol	1.35 ± 0.16	1.58 ± 0.36
Free cholesterol	1.32 ± 0.13	1.56 ± 0.31
Esterified cholesterol	0.03 ± 0.01	0.02 ± 0.01
Phospholipids	6.5 ± 0.5	6.3 ± 0.9
Phospholipids:cholesterol (mol:mol)	4.9 ± 0.8	4.0 ± 0.5
Enzyme activities (pmoles/min/mg protein)		
ACAT	11.2 ± 2.2	19.5 ± 1.7 [1]
Cholesterol 7α-hydroxylase	7.3 ± 1.0	11.7 ± 1.6 [1]

Values are mean ± SEM.
n = 5 per group.
[1] Significantly different from 12:0 + 14:0-rich diet at $P < 0.05$.

cholesterol may be involved. We previously reported that taurine and other sulfur amino acids stimulate bile acid synthesis in HepG2 cells.[36] Similarly, taurine added to the diet of rats[37] and hamsters[38] stimulated cholesterol 7α-OHase activity[37] and increased bile acid output.[38] Adding taurine to the liquid formula of preterm infants also increased bile acid synthesis and excretion.[39] The underlying mechanism may be that 7α-OHase gene expression can be stimulated by expanding the nuclear pool of sulfhydryl groups in metabolic or dietary situations in which that pool is limited.[40] Therefore, the enhanced activity of cholesterol 7α-OHase and bile acid output induced by the 18:2n6-rich diet could be related, in part, to the elevated level of hepatic taurine with its sparing effect on a casein-rich diet that somewhat limits sulfur amino acids (sulfhydryl-rich precursors).

Previous studies also suggest that availability of cholesterol in hepatocytes may modulate the activity of cholesterol 7α-OHase.[41,42] However, Bjorkhem and Akerlund[43] found that microsomal free cholesterol content was not a direct regulator of cholesterol 7α-OHase in rats. In our data, dietary fatty acids had no effect on microsomal free cholesterol in microsomes, even though cholesterol 7α-OHase activity was enhanced by 18:2n6. This further suggests that sulfhydryl group availability was the critical factor driving bile acid synthesis in our model.

The increased fecal output of bile acids and cholesterol induced by the extremely high 18:2n6 intake supports similar studies in humans fed high levels of POLYs,[7,8] but it begs the question of whether the increased excretion requires a certain intake of 18:2n6 (i.e., threshold) to induce the effect. On the other hand, from dietary designs such as these in which extremes in SATs and POLYs are compared, it is not possible to determine whether the rise in TC and reduced fecal sterol output during the 12:0 + 14:0-rich diet resulted from the high-SAT or low-POLY intake. Our diets contained no cholesterol, so the observed increase in fecal sterol output by the high-POLY diet necessarily represented a net increase in endogenous cholesterol synthesis and its subsequent excretion as bile acids (and cholesterol) through the bile. Because the biliary cholesterol concentration was unaltered, it would appear that accelerated bile flux may have been responsible for the increased sterol turnover. Surprisingly, a previous study in gerbils injected with ^3H_2O found that polyunsaturated fat depressed hepatic cholesterol synthesis,[20] whereas ^{14}C-acetate incorporation into cholesterol was increased by POLYs in monkeys.[44] Whole body cholesterol synthesis also increased during high polyunsaturated fat intake in humans.[45]

Cholate:chenodeoxycholate ratio and dietary fat unsaturation

The rise in chenodeoxycholate and decrease in the cholate:chenodeoxycholate ratio during the high-SAT, low-POLY diet, although not as pronounced as in cholesterol-fed hamsters,[14] are in keeping with the hypothesis that the chenodeoxycholate profile increases during hypercholesterolemia following 27-hydroxylation of excess plasma cholesterol by the liver and vascular endothelial cells.[46] This 27-OH cholesterol is putatively catabolized by the liver in a process that favors synthesis of chenodeoxycholic acid,[46] as opposed to the cholic acid profile that seems to prevail during normocholesterolemic situations. Feeding a diet high in saturated fat and cholesterol to baboons elevated plasma 27-OH cholesterol,[47] but our data indicate that increased fat saturation alone is sufficient to increase this hydroxysterol in plasma.

Similar to the current gerbil data, previous substitution of dietary corn oil for coconut oil in cebus and squirrel monkeys revealed a marked decrease in plasma cholesterol (esters) that was associated with a higher biliary cholate:chenodeoxycholate ratio.[12] Corn oil consumption by humans, which also lowers plasma cholesterol, can increase the cholate:chenodeoxycholate ratio as well.[14] In contrast to gerbils, monkeys[12] always maintained a considerably greater proportion of chenodeoxycholate. Based on these collective observations, it is conceivable that 27-OH cholesterol canotes a "detoxification pathway" for cholesterol linked to cholesterol ester (CE) accumulation in plasma and liver.

In this light, the shift to chenodeoxycholate was modest in gerbils fed the high-SAT diet—in which CEs increased in plasma but not in liver—compared with the shift observed in hypercholesterolemic hamsters fed cholesterol,[14]—in which a major shift to taurochemodeoxycholate (TCDC) and decrease in glycocholate (GC) was coupled with major increases in both plasma and liver CEs. By contrast, cholesterol-lowering regimens that enhance bile acid removal, such as certain dietary fibers or cholestyramine, dramatically restore the cholate:chenodeoxycholate ratio and reduce apoE-rich plasma lipoproteins and hepatic CEs in hamsters.[14] Thus, the bile acid excretion rate would seem to be positively related to the cholate:chenodeoxycholate ratio, which in turn may reflect alternate routes of cholesterol uptake by the liver.[4]

Hepatic taurine and bile acid profile

Because our experimental diets contained no taurine, the increased hepatic taurine concentration induced by the 18:2n6-rich diet presumably reflected increased hepatic synthesis or reduced taurine turnover. Numerous experiments have indicated a relationship between taurine and lipid metabolism.[39,48,49] Dietary supplementation with taurine can affect the concentration of hepatic lipids including phospholipids, cholesterol, and triacylglycerols.[48] Taurine also altered the cholesterol:phospholipid and lipid:protein ratios as well as the fatty acid composition of hepatic sinusoidal membranes[49] and plasma membranes[48] in animals. Because composition of membrane lipids modulates membrane fluidity and influences associated enzyme activity,[50,51] the 18:2n6-rich, SAT-poor diet may have increased activity of enzymes involved in taurine synthesis or enhanced the transport of taurine into hepatocytes. Hepatic taurine availability also positively impacts sulfur amino acid metabolism, including the sulfhydryl pool size in platelets.[52] As discussed above, because the nuclear pool of sulfhydryl groups modulates the production of cholesterol 7α-OHase,[40] it is conceivable that unsaturated fat enhancement of taurine synthesis indirectly increases cholesterol 7α-OHase activity and bile acid synthesis.

The relationship between hepatic and other tissue pools of taurine is poorly understood. Hardison[53] showed that in patients with biliary obstruction the muscle taurine pool did not parallel the increase in hepatic taurine. In those patients, the taurine:glycine ratio in bile was positively correlated with hepatic taurine but not with muscle taurine. In our study the hepatic concentration of taurine in both diet groups was unrelated to plasma taurine. Thus, taurine in various tissue pools does not appear to be readily equilibrated via transport and exchange with the plasma pool.

The modest shift to chenodeoxycholate during the 12:0 + 14:0 diet was contrasted by the striking shift to taurocholate (TC) from GC during high 18:2n6 consumption, which was associated with accelerated bile acid synthesis and excretion. The shift to TC during POLY intake presumably reflected the doubling of hepatic taurine, because taurine conjugation of bile acids is known to be directly related to the hepatic taurine pool.[53–55] Increased hepatic taurine synthesis is hypothesized because the greater bile acid excretion during 18:2n6 would be expected to increase the loss of taurine. It is noteworthy that taurine-supplemented cynomolgus monkeys (in which both glycine- and taurine-conjugated bile acids are similar to gerbils and dissimilar to the relatively exclusive taurine conjugation of New World monkeys), like gerbils, also responded to an increase in available taurine with a decrease in secondary bile acids, increase in TC, and decrease in chenodeoxycholate.[52] This may represent an hepatic "detoxification process" ascribed to taurine,[56] that inversely reflects the putative detoxification pathway for CEs, which seems to favor chenodeoxycholate synthesis.

Why hepatic taurine synthesis would be enhanced by dietary 18:2n6 (or depressed by 12:0 + 14:0) is unclear, but the link between the plasma lipoprotein profile, hepatic taurine, and bile acid output associated with changes in dietary fat saturation would appear to be based on more than conjecture and thus requires further definition. The major impact of these fatty acid exchanges on lipoproteins may indeed reflect a primary event in bile acid synthesis and excretion. Future studies must determine which comes first: altered bile acid metabolism, perturbation of hepatic taurine balance, or the striking alteration in plasma cholesterol.

In summary, these data support our empirical observations[4] and Javitt's hypothesis[46] that the two primary bile acids cholate and chenodeoxycholate may derive from different metabolic pathways, in this case elicited by extremes in dietary fatty acid unsaturation. Furthermore, the unique modulation of hepatic taurine and the bile acid profile by dietary fat saturation may directly or indirectly affect the plasma cholesterol concentration.

References

1 Spady, D.K., Woollett, L.A., and Dietschy, J.M. (1993). Regulation of plasma LDL-cholesterol levels by dietary cholesterol and fatty acids. *Ann. Rev. Nutr.* **13,** 355–381

2 Hayes, K.C. and Khosla, P. (1992). Dietary fatty acid thresholds and cholesterolemia. *FASEB J.* **6,** 2600–2706

3 Pronczuk, A., Khosla, P., and Hayes, K.C. (1994). Dietary myristic, palmitic, and linoleic acids modulate cholesterolemia in gerbils. *FASEB J.* **8,** 1191–1200

4 Hayes, K.C., Livingston, A., and Trautwein, E.A. (1994). Dietary impact on biliary lipids and gallstones. *Ann. Rev. Nutr.* **12,** 299–326

5 Cohen, B.I., Mosbach, E.H., Ayyad, N., Miki, S., and McSherry, C.K. (1992). Dietary fat and fatty acids modulate cholesterol cholelithiasis in the hamster. *Lipids* **27,** 526–532.

6 Jonnalagadda, S.S., Trautwein, E.A., and Hayes, K.C. (1995). Dietary fats rich in saturated fatty acids (12:0, 14:0, and 16:0) enhance gallstone formation relative to monounsaturated fat (18:1) in cholesterol-fed hamsters. *Lipids* **30,** 415–424

7 Connor, W.E., Witiak, D.T., Stone, D.B., and Armstrong, M.L. (1969). Cholesterol balance and fecal neutral steroid and bile acid excretion in normal humans fed dietary fats of different fatty acid composition. *J. Clin. Invest.* **48,** 1363–1375

8 Nestel, P.J., Havenstein, N., Homma, Y., Scott, T.W., and Cook, L.J. (1975). Increased sterol excretion with polyunsaturated-fat high-cholesterol diets. *Metabolism* **24,** 189–198

9 Grundy, S.M. and Ahrens, S.M. (1970). The effects of unsaturated dietary fat on absorption, excretion, synthesis, and distribution of cholesterol in man. *J. Clin. Invest.* **49,** 1135–1152

10 Ramesha, C., Paul, R., and Ganguly, J. (1980). Effect of dietary unsaturated oils on the biosynthesis of cholesterol, and on biliary and fecal excretion of cholesterol and bile acids in the rat. *J. Nutr.* **110,** 2149–2158

11 McGovern, R.F. and Quackenbush, F.W. (1973). Turnover of bile acids in hypercholesterolemic rats as influenced by saturation of dietary fat. *Lipids* **8,** 466–470

12 Armstrong, M.J., Stephan, Z., and Hayes, K.C. (1982). Biliary lipids in New World monkeys: dietary cholesterol, fat, and species interactions. *Am. J. Clin. Nutr.* **36,** 592–601

13 Hellstrom, K. and Linstedt, S. (1966). Studies on the formation of cholic acid in subjects given standardized diet with butter or corn oil as dietary fat. *Am. J. Clin. Invest.* **18,** 46–59

14 Trautwein, E., Siddiqui, A., and Hayes, K.C. (1993). Modeling plasma lipoprotein-bile lipid relationships: differential impact of psyllium and cholestyramine in hamsters fed a lithogenic diet. *Metabolism* **42,** 1531–1540

15 Armstrong, M.J. and Carey, M.C. (1982). The hydrophobic-hydrophilic balance salts: inverse correlation between reverse-phase high performance liquid chromatographic mobilities and micellar cholesterol-solubilizing capacities. *J. Lipid Res.* **23,** 70–80

16 Heuman, D.M., Hylemon, P.B., and Vlahcevic, Z.R. (1989). Regulation of bile acid synthesis. III. Correlation between biliary bile salt hydrophobicity index and the activities of enzymes regulating cholesterol and bile acid synthesis in the rat. *J. Lipid Res.* **30,** 1161–1171

17 Garbutt, J.T. and Kenney, T.J. (1972). Effect of cholestyramine on bile acid metabolism in normal man. *J. Clin. Invest.* **51,** 2781–2789

18 Ide, T. and Horii, M. (1989). Predominant conjugation with glycine of biliary and lumen bile acids in rats fed pectin. *Br. J. Nutr.* **61,** 545–557

19 Kritchevsky, D. (1991). Bile acids: biosynthesis and functions. *Europ. J. Cancer Prevent.* **1(Suppl.2),** 23–28

20 Mercer, J.H. and Holub, B.J. (1981). Measurement of hepatic sterol synthesis in the mongolian gerbil in vivo using [3H] water: diurnal variation and effect of type of dietary fat. *J. Lipid Res.* **22,** 792–799

21 Hajri, T., Khosla, P., Pronczuk, A., and Hayes, K.C. (1998). Myristic acid-rich fat raises plasma LDL by stimulating LDL production without affecting fractional clearance in gerbil fed a cholesterol-free diet. *J. Nutr.* **128,** 477–480

22 Folch, J., Lees, M., and Stanley, G.H.S. (1957). A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.* **226,** 497–509.

23 Rossi, S.S., Converse, J.L., and Hofman, A.F. (1987). High pressure liquid chromatographic analysis of conjugated bile acids in human bile: simultaneous resolution of sulfated and unsulfated lithocholyl amidates and the common conjugated bile acids. *J. Lipid Res.* **28,** 589–595

24 Heuman, D.M. (1989). Quantitative estimation of hydrophilic-hydrophobic balance of mixed bile salt solutions. *J. Lipid Res.* **30,** 719–730

25 Petrack, B. and Latario, B.J. (1993). Synthesis of 27-hydroxycholesterol in rat mithochondria: HPLC assay and marked activation by exogenous cholesterol. *J. Lipid Res.* **34,** 643–650

26 Oda, H., Yamashita, H., Kosahara, K., Kuroki, S., and Nakayama, F.

(1990). Esterified and total 7a-hydroxycholesterol in human serum as an indicator for hepatic bile acid synthesis. *J. Lipid Res.* **31,** 2209–2218

27 Trautwein, E.A. and Hayes, K.C. (1991). Evaluating taurine status: determination of plasma and whole blood taurine concentration. *J. Nutr. Biochem.* **2,** 571–576

28 Kim, J.C. and Chang, T.H. (1984). Direct determination of free cholesterol and individual cholesteryl esters in serum by high pressure liquid chromatography. *Korean J. Biochem.* **16,** 69–77

29 Witztum, J.L., Young, S.G., Elam, R.L., Carew, T.E., and Fisher, M. (1985). Cholestyramine-induced changes in low density lipoprotein composition and metabolism. I. Studies in guinea pig. *J. Lipid Res.* **26,** 92–103

30 Chautan, M., Termine, E., Nalbone, G., and Lafont, H. (1988). Acyl-coenzyme A:cholesterol acyltransferase: silica gel column separation of reaction products. *Anal. Biochem.* **173,** 436–439

31 Markwell, M.A.K., Hass, S.M., Bieber, L.L., and Tolbert, N.E. (1978). A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* **87,** 206–210

32 Ishida, F., Sato, A., Lisuka, K., Sawasaki, Y., and Kamei, T. (1989). Effects of MK-733 (simvastatin, an inhibitor of 3-hydroxy-3-methyl CoA reductase) on intestinal acylcoenzyme A: cholesterol acyltransferase activity in rabbits. *Biochim. Biophys. Acta.* **1004,** 117–123

33 Nicolau, G., Shefer, S., Salen, G., and Mosbach, E.H. (1974). Determination of hepatic cholesterol 7α-hydroxylase activity in man. *J. Lipid Res.* **15,** 146–151

34 Lepage, G. and Roy, C.C. (1986). Direct transesterification of all classes of lipids in one-step reaction. *J. Lipid Res.* **27,** 114–120

35 Suckling, K.E., Benson, G.M., Bond, B., Gee, A., Glen, A., Haynes, C., and Jackson, B. (1991). Cholesterol lowering and bile acid excretion in the hamster with cholestyramine treatment. *Atherosclerosis* **89,** 183–190

36 Stephan, Z., Lindsey, S., and Hayes, K.C. (1987). Taurine enhances low density lipoprotein binding, internalization, and degradation by cultured HepG2 cells. *J. Biol. Chem.* **262,** 6069–6075

37 Murakami, S., Nara, Y., and Yamori, Y. (1996). Taurine accelerates the regression of hypercholesterolemia in stroke-prone spontaneously hypertensive rats. *Life Science* **58,** 1643–1651

38 Bellantini, S., Pecorari, M., Cordoma, P., Marchegiano, P., Manenti, F., Bosisio, E., De Fabiani, E., and Galli, G. (1987). Taurine increases bile acid pool size and reduces bile saturation index in the hamster. *J. Lipid Res.* **28,** 1021–1027

39 Wasserhess, P., Becker, M., and Staab, D. (1993). Effects of taurine on synthesis of neutral and acidic sterols and fat absorption in preterm and full-term infants. *Amer. J. Clin. Nutr.* **58,** 349–353

40 Trawick, J.D., Wang, S.L., Bell, D., and Davis, R.A. (1997). Transcriptional induction of cholesterol 7 alpha-hydroxylase by dexamethasone in L35 hepatoma cells requires sulfhydryl reducing agents. *J. Biol. Chem.* **272,** 3099–3102

41 Jones, M.P., Pandak, W.M., Heuman, D.M., Chiang, J.Y.L., Hylemon, P.B., and Vlahcevic, Z.R. (1993). Cholesterol 7a-hydroxylase: evidence for transcriptional regulation by cholesterol or metabolic products of cholesterol in the rat. *J. Lipid Res.* **34,** 885–892

42 Myant, N.B. and Mitropoulos, K.A. (1977). Cholesterol 7α-hydroxylase. *J. Lipid Res.* **18,** 135–153

43 Bjorkhem, I. and Akerland, J.E. (1988). Studies on the link between HMG-COA reductase and cholesterol 7 alpha-hydroxylase in rat liver. *J. Lipid Res.* **29,** 136–143

44 Corey, J.E. and Hayes, K.C. (1974). Effects of diet on hepatic and intestinal lipogenesis in squirrel, cebus, and cynomologus monkey. *Atherosclerosis* **20,** 405–416

45 Jones, P.J.H., Lichtenstein, A.H., Schaefer, E.J., and Namchuck, G.L. (1994). Effects of dietary fat selection on plasma cholesterol synthesis in older moderately hypercholesterolmic humans. *Arterioscler. Thromb. Vasc. Biol.* **14,** 542–546

46 Javitt, N.B. (1994). Bile acid synthesis from cholesterol: regulatory and auxiliary pathways. *FASEB J.* **8,** 1308–1311

47 Kushwaha, R.S., Guntupalli, B., Rice, K.S., Dee Carey, K., and MacGill, Jr. H.C. (1995). Effects of dietary cholesterol and fat on the expression of hepatic sterol 27-hydroxylase and other hepatic cholesterol-response genes in baboons (papio species). *Arterioscler. Thromb. Vasc. Biol.* **15,** 1404–1411

48 Cho, C.Y., Bravo, E., and Cantafora, A. (1992). Effect of taurine levels on liver lipid metabolism: an in vivo study in the rat. *Proc. Soc. Exp. Biol. Med.* **202,** 88–96

49 Guertin, F., Roy, C.C., Lepage, G., Yousef, I., and Tuchweber, B. (1993). Liver membrane composition after short-term parenteral nutrition with and without taurine in guinea pigs: the effect of taurine. *Proc. Soc. Exp. Biol. Med.* **203,** 418–423

50 Sturman, J.A. (1993). Taurine in development. *Physiol. Revs.* **73,** 119–147

51 Spector, A.A. and Yorek, M. (1985). Membrane lipid composition and cellular function. *J. Lipid Res.* **26,** 1015–1035

52 Hayes, K.C., Pronczuk, A., Addesa, A.E., and Stephan, Z.F. (1989). Taurine modulates platelet aggregation in cats and humans. *Amer. J. Clin. Nutr.* **49,** 1211–1216

53 Hardison, W.G.M. (1978). Hepatic taurine concentration and dietary taurine as regulators of bile acid conjugation with taurine. *Gastroenterology* **75,** 71–75

54 Stephan, Z.F., Armstrong, M.J., and Hayes, K.C. (1981). Bile lipid alteration in taurine-depleted monkeys. *Amer. J. Clin. Nutr.* **34,** 204–210

55 Hardison, W.G.M. and Proffitt, J.H. (1977). Influence of hepatic taurine concentration on bile acid conjugation with taurine. *Am. J. Physiol.* **232,** E75–E79

56 Gaull, G.E. (1989). Taurine in pediatric nutrition: review and update. *Pediatrics* **83,** 433–442