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The Effect of Long Chain Saturated Fatty Acids (12 : 0, 14 : 0, 16 : 0, 18 : 0) and Dietary Cholesterol Levels on Plasma and Hepatic Cholesterol Concentrations in the Mongolian Gerbil

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ABSTRACT

In order to independently examine the effects of long-chain saturated fatty acids and dietary cholesterol levels on plasma and hepatic cholesterol concentrations, six different diets were fed to male Mongolian gerbils (14 gerbils pes group) for an 8-week period. Purified diets contained 36% energy as fat (each saturated fatty acid tested comprised about 20% of the total fat energy) and 0.06% (w/w) cholesterol, corresponding to typical human consumption patterns in Western diets. Fat blends were formulated with natural fat sources. To determine the effects of different saturated fatty acids on plasma and liver cholesterol levels, four of the six diets contained constant levels of all nutrients except for the amounts of lauric acid (12 : 0), myristic acid (14 : 0), palmitic acid (16 : 0), and stearic acid (18 : 0). Dietary cholesterol effects were tested using 16 : 0-enriched diets containing 0, 0.006, and 0.06% (w/w) cholesterol. None of the plasma lipids were influenced by fatty acid treatment, including triglycerides, plasma total−, VLDL+LDL−, and HDL-cholesterol. However, hepatic esterified cholesterol concentrations were increased in the palmitic and stearic acid diet groups compared to the lauric and myristic acid diet group. The molar ratios of hepatic EC/FC were the highest in the palmitic acid diet (12.2 ± 0.6) and the lowest in the myristic acid diet (6.4 ± 0.2). Dietary cholesterol significantly ($p < 0.001$) increased the plasma total cholesterol which was due to the increase of both HDL− and VLDL+LDL-cholesterol. In the absence of dietary cholesterol and compared to other species, the gerbil exhibited a high molar ratio of hepatic EC/FC, which was further elevated by dietary cholesterol feeding (0.06%). The results from this study indicate that hepatic cholesterol concentrations are sensitive to both low levels of dietary cholesterol and saturated fatty acid chain length and also, that plasma cholesterol concentrations are sensitive to low levels of dietary cholesterol.

KEY WORDS : saturated fatty acids (SFA) · dietary cholesterol · plasma cholesterol · hepatic cholesterol · Mongolian gerbils.

INTRODUCTION

Dietary saturated fatty acids and cholesterol have been implicated as important factors in the development of hypercholesterolemia leading to coronary artery disease. However, the plasma lipid responses to saturated fatty acids vary between individual human subjects as well as between animal species.[1,2] The saturated fatty acids found in the food supply do not exert an equivalent cholesterolemic effect. The saturated fatty acids that most consistently increase plasma cholesterol levels compared with isocaloric amounts of carbohydrates are lauric (12 : 0), myristic (14 : 0), and palmitic (16 : 0) acids.[3,4] Stearic acid (18 : 0) doesn't seem to increase plasma total[5,6] or low density lipoprotein (LDL) cholesterol levels[7] relative to the other long-chain saturated fatty acids. Medium-chain saturated fatty acids (6 : 0, 8 : 0, 10 : 0) were reported not to raise plasma cholesterol concentrations.[8] Although there is a general agreement that lauric, myristic, and palmitic acids are hypercholesterolemic.[3,4,7,9] there is some controversy about the relative effects of these saturated fatty acids on plasma cholesterol concentrations. Keys *et al.*[3] suggested that these three saturated fatty acids have roughly equivalent effects, while Hegsted *et al.*[4] indicated that myristic acid was quantitatively most important, that palmitic acid had a less effect and that lauric acid had little measurable effect. More recent data indicate that a liquid formula diet high in lauric acid (in a synthesized test fat) elevates plasma total and LDL-cholesterol levels relative to a liquid formula diet high in oleic acid, but to a lesses extnet than palmitic acid.[9] Other studies suggest that palmitic acid significantly decreased plasma total and LDL-cholesterol levels relative to lauric plus myristic acids.[10-13] In general, previous study designs may have been limited in at least two important ways. First, in most studies comparing fatty acid effects using mixtures of natural fat sources, myristic acid was combined with lauric acid.[12] Under these conditions, it is difficult to determine the independent effect of myristic acid on plasma cholesterol levels without con-

founding by lauric acid. Second, in several cases, modified or semisynthetic fats were used to manipulate the saturated fatty acid involved.[5][9][13][14] These modified or semisynthetic fats contain randomized triglycerides, in which one-third of the total amount of each fatty acid was esterified in each of three possible positions on the glycerol backbone. However, in natural fats, most fatty acids are not randomly located ; stearic and palmitic acids are esterified almost exclusively in the 1- or 3-positions, oleic acid mainly in the 2-position.[15] It is known that the position of the fatty acid in the triglyceride influences its metabolism.[16]

In the present study, dietary saturated fatty acids (lauric, myristic, palmitic, and stearic acids) were systematically studied using controlled experimental diets which maintained constant levels of all nutrients except for the fatty acids of interest. Each of the test fatty acids was fed at 6-8% of kcal by blending commercially available natural fat sources. We used the Mongolian gerbil since this animal is known to be very sensitive to cholesterolemic response to dietary fatty acids fed under conditions of zero cholesterol or very small amounts of cholesterol among rodents.[17] Further, gerbils can respond rapidly and proportionately to relatively low levels of dietary cholesterol.[18] They also bear a similarity to humans regarding relative proportions of plasma free versus esterified cholesterol (gerbil : 75%, humans : 75-80%), and the type of cholesteryl esters (major type is cholesteryl linoleate).[19]

The purposes of this present study were : 1) to explore the effects of individual saturated fatty acids (lauric, myristic, palmitic, stearic acid) at a dose level comparable, based on energy equivalence, to human consumption ; and 2) to examine the effects of dietary cholesterol levels on plasma and hepatic cholesterol concentrations in the Mongolian gerbil.

MATERIALS AND METHODS

1. Animals

Male Mongolian gerbils (*Meriones unguiculatus*, Tumblebrook Farm Inc., West Brookfield, MA), initially weighing 60-70 g were housed two to three per screen-bottomed cage in a room with controlled temperature (25℃) and light cycle (06 : 00-18 : 00). Animals were maintained on rodent chow (Rodent Laboratory Chow 5001, Purina Labs) for 5 days prior to being started on their dietary treatments. Eighty-four gerbils were equally and randomly divided into six treatment groups. Purified experimental diets, differing only in saturated fatty acid profiles and levels of cholesterol, were fed in pelleted form for 8 weeks. Animals were allowed free access to food and water. Body weight and food intake were recorded weekly and biweekly, respectively. At the end of 8 weeks, animals were fasted for 12 hours (07 : 00-19 : 00), anesthetized by chloral hydrate injection, and exsanguinated via jugular vein or/and heart puncture. Whole blood was collected into tubes coated with EDTA (1 mg/ml blood). Livers were excised, weighed and stored at -80℃ for further analysis. Animals were housed and used in compliance with the University of Minnesota policy on animal care and use.

2. Preparation of experimental diets

Experimental diets were formulated (Table 1) based on the average intake values derived from over 6,000 participants of the Minnesota Heart Health Program (MHHP). Using a variety of naturally occurring oils and butters, it was possible to formulate fatty acid profiles that varied the levels of lauric, myristic, palmitic, and stearic acids as shown in this context (Table 2). Diet 3 represents a typical Western diet in terms of cholesterol content (300 mg/day), fatty acid profile (high palmitic acid, 7.7% kcal), and energy contributions from carbohydrate (46%), protein (18%), and fat (36%). Vegetable fat sources were high in either lauric (palm kernel oil) or myristic acids (nutmeg butter) as well as high in palmitic (palm oil) or stearic acids (shea butter). Levels of oleic acid were manipulated by adjusting the amount of high oleic safflower oil and levels of linoleic acid were set at 4.5% of total energy us-

Table 1. Composition of the experimental diets[1]

Ingredient	g/100 g
Casein	17.9
DL-Methionine	0.3
Corn starch	34.0
Sucrose	13.4
Fat[2]	17.1
Cellulose	4.5
Mineral mix[3]	5.3
Vitamin mix	2.2
Wheat bran[4]	5.0
myo-Inositol	0.1
Choline bitartrate	0.2
Vitamin K	1.33 mg/kg
Cholesterol (varied with study)	0, 0.006, or 0.06

1) Diets were fed as pellet form
2) Diets contained different fat blends as detailed in the Table 2
3) The levels of mineral mixture and vitamin mixture were based on NRC20 nutrient requirement of the gerbil
4) Wheat bran contributed 0.7% en protein, 2.4% en carbohydrate, and 0.3% en fat to the total diet. Fat is composed of C16 : 0 (17.8%), C18 : 1 (15.9%), and C18 : 2 (66.3%). Fatty acid contents were reflected on fatty acid profiles in Table 2

ing high linoleic safflower oil based on the concept of Hayes and Khosla.[21] Total amounts of saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), and poly-unsaturated fatty acids (PUFA) were relatively constant among all six diet groups resulting in a correspondingly constant polyunsaturate to saturate ratio (P/S ratio). Dietary cholesterol was added (Diets 1 through 4) at the level of 0.06% (0.14 mg/kcal) to approximate average human consumption. Diets 3, 5, and 6 served to examine the effects of dietary cholesterol, each containing 0.06%, 0%, and 0.006% cholesterol (w/w), respectively (Table 2).

Table 2. Fatty acid profiles and fat formulation of dietary fats

	Diet 1	Diet 2	Diet 3, 5, 6	Diet 4
	Lauric acid	Myristic acid	Palmitic acid	Stearic acid
Fatty acid		%kcal		
<C10 : 0	1.1	0.0	0.2	0.2
C12 : 0	6.3	0.8	0.9	1.0
C14 : 0	2.1	7.3	2.1	2.1
C16 : 0	2.1	2.3	7.7	2.2
C18 : 0	4.1	4.1	4.1	7.7
total SFA[1]	15.7	14.3	15.0	13.2
total MUFA[2]	15.7	17.4	16.6	18.4
total PUFA[3]	4.5	4.5	4.5	4.5
total Fat	35.9	36.1	36.1	36.1
P/S ratio[4]	0.3	0.3	0.3	0.3
Fat		g/100 g		
Palm kernel	38.0	3.0	4.8	5.5
Shea butter	25.0	26.0	–	51.0
HO safflower[5]	33.2	43.9	29.9	30.9
HL safflower[6]	3.0	2.5	2.6	6.0
Nutmeg butter	–	23.8	5.5	3.3
Cocoa butter	–	–	25.2	2.5
Corn oil	–	–	–	3.3
Beef tallow[7]	–	–	3.8	2.5
Palm oil	–	–	27.3	–

Diet 1, 2, 3, and 4 contained 0.06% level of dietary cholesterol
Diet 5 and 6 contained 0 and 0.006% level of dietary cholesterol, respectively
1) SFA=Saturated fatty acids, 2) MUFA=Monounsaturated fatty acids
3) PUFA=Polyunsaturated fatty acids
4) Calculated as total % of PUFA divided by total % of SFA
5) HO=high oleic, 6) HL=high linoleic
7) Cholesterol-stripped beef tallow

Fatty acid profiles of fat sources and final mixed diets were analyzed via gas chromatography (injector temp.= 200℃ ; detector temp.=300℃ ; oven temp. : initial=125 ℃, final=260℃ increased at 6℃/minute ; capillary column) using a Hewlett Packard Model 5890A gas chromatography following the procedure of Einig.[22] Hydrogen and nitrogen gases were used as the carrier and make-up gases, respectively. Methylated fatty acid standards were used to establish retention times and working standards were used to determine detector response factors prior to injection of samples. Relative mass amounts were determined by quantification of peak areas.

Nutmeg butter was derived from nutmeg oleo resin by extraction in hot ethanol and freezing this mixture until the nutmeg butter had solidified. The nutmeg butter was collected in a Buchner funnel and washed with cold ethanol. Residual ethanol was removed using a rotoevaporator.

3. Lipid analyses

Small aliquots of plasma from each animal were taken for total- and HDL-cholesterol (Sigma Diagnostics) and triglyceride (Boehringer Mannheim Diagnostics) analysis. VLDL+LDL-cholesterol values were obtained by subtracting the HDL-cholesterol from the total cholesterol for each animal.

Hepatic lipids were extracted by the method of Folch *et al.*[23] and analyzed enzymatically for total (Sigma Diagnostics) and free cholesterol.[24] Esterified cholesterol concentration was calculated by subtracting the free cholesterol from the total cholesterol measured for each animal. The fatty acid abundance in three major lipid classes was determined after triglycerols, phospholipids, and cholesteryl esters were first separated on thin layer chromatography plates. Silica Gel G (Whatman Ltd., Kent, England) plates were developed in hexane/diethyl ether/glacial acetic acid (80 : 20 : 2, v/v/v). Areas containing lipid classes were cut and analyzed by gas chromatography following methyl esterification.[22]

Table 3. Effects of long chain saturated fatty acids and dietary cholesterol levels on body weight gain, food intake, and liver weight

	Diet 1 Lauric acid 0.06% chol	Diet 2 Myristic acid 0.06% chol	Diet 3 Palmitic acid 0.06% chol	Diet 4 Stearic acid 0.06% chol	Diet 5 Palmitic acid 0% chol	Diet 6 Palmitic acid 0.006% chol
Body weight			g			
Final	98.6±0.6	95.2±2.1	97.3±1.5	97.4±2.0	94.6±1.0	97.6±1.7
Gain	29.4±0.5[b]	28.2±2.2[ab]	30.4±1.2[b]	27.6±0.6[ab]	25.5±0.4[a]	28.5±1.4[ab]
Food intake			g/day			
	5.0±0.0	5.0±0.0	5.0±0.1	5.0±0.0	4.9±0.1	4.9±0.1
Liver weight			g/100g body weight			
	3.6±0.1	3.8±0.2	3.7±0.2	3.6±0.1	3.5±0.1	3.6±0.1

Values are mean±SEM Values in a row not sharing a common superscript are significantly different ($p<0.05$, $n=14$)

4. Statistical analysis

Values were analyzed by General linear model and LS-MEANS in SAS.[25] All results were presented as mean± standard error. A one-way analysis of variance was used to test the effects of dietary fat and cholesterol levels, and all pairwise multiple comparisons were evaluated by Student-Newman-Keuls method. Differences among means were considered to be statistically significant at $p<0.05$.

RESULTS

The body weight, food intake, and liver weight of gerbils fed different diets are shown in Table 3. Treatment of dietary saturated fatty acids (diet 1, 2, 3, and 4) did not influence the final weight and the weight gain. However, significant ($p<0.005$) differences in body weight gains were noted between groups fed 0.06% vs. 0% cholesterol (30.4±1.2 vs. 25.5±0.4 g). There was no difference in food consumption among dietary groups, as each gerbil consumed about 5 g of feed per day. Liver weights (g/100 g body weight) were similar in all the diet groups.

None of the plasma lipids were influenced by fatty acid treatment, including triglycerides, plasma total−, VLDL+LDL−, and HDL-cholesterol (Table 4). However,

Table 4. Effects of long chain saturated fatty acids and dietary cholesterol levels on the concentrations of plasma and liver lipids in gerbils

	Diet 1 Lauric acid 0.06% chol	Diet 2 Myristic acid 0.06% chol	Diet 3 Palmitic acid 0.06% chol	Diet 4 Stearic acid 0.06% chol	Diet 5 Palmitic acid 0% chol	Diet 6 Palmitic acid 0.006% chol
Plasma lipids			mg/dl			
Total cholesterol	202.9±12.1^{b}	194.9±18.2^{b}	190.4±13.5^{b}	195.7±14.0^{b}	129.4± 6.6^{a}	141.1±10.4^{a}
HDL-cholesterol	111.8± 7.3^{b}	114.2± 8.8^{b}	107.4± 7.6^{b}	104.5± 8.4^{b}	83.1± 4.8^{a}	79.4± 6.9^{a}
V+LDL-cholesterol	91.1± 7.7^{c}	80.7±15.3^{bc}	82.9±10.1^{bc}	91.2± 7.7^{c}	46.3± 2.9^{a}	61.8± 5.2^{a}
Triglycerides	457.8±38.3^{b}	428.8±50.9^{ab}	396.9±31.8^{ab}	471.0±52.3^{b}	319.4±28.3^{a}	363.5±26.1
Liver cholesterol			mg/g liver			
Free cholesterol	3.2±0.1^{bc}	3.5±0.1^{c}	2.8±0.2^{ab}	4.1±0.2^{d}	2.4±0.1^{a}	2.4±0.1^{a}
Esterified cholesterol	24.0±1.1^{a}	22.5±1.0^{a}	33.6±2.2^{b}	29.9±2.0^{b}	21.1±0.7^{a}	23.5±0.9^{a}
EC/FC molar ratio	7.5±0.3^{b}	6.4±0.2^{a}	12.2±0.6	7.4±0.3^{ab}	8.8±0.4^{c}	9.8±0.3^{c}

Values are mean±SEM. Values in a row not sharing a common superscript are significantly different ($p<0.05$, $n=14$)
EC=esterified cholesterol, FC=free cholesterol, V+LDL-cholesterol=VLDL+LDL-cholesterol

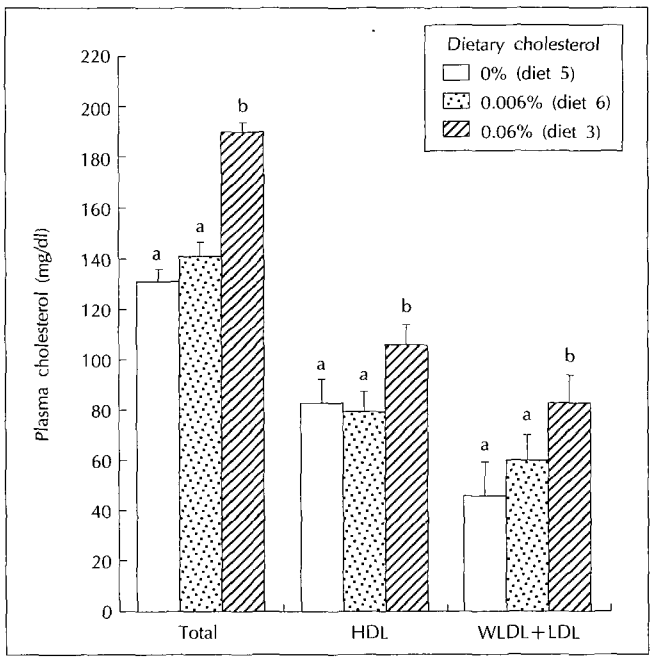

Fig. 1. Effect of dietary cholesterol levels on plasma cholesterol concentrations in the Mongolian gerbil. Letters indicate comparisons among diet groups. Group not sharing a common letter are significantly different ($p<0.05$, $n=14$). Diet 3, 5, and 6 contained similar amounts of dietary fatty acids with different levels of dietary cholesterol, 0.06, 0, and 0.006%, respectively.

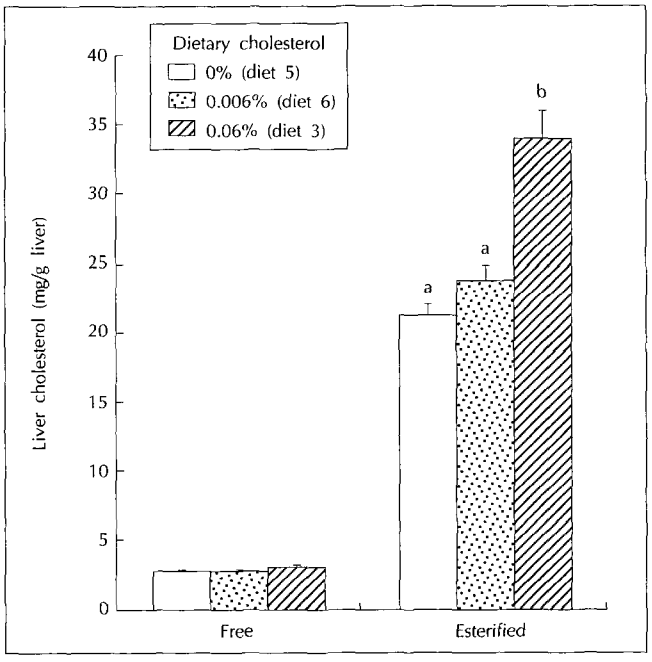

Fig. 2. Effect of dietary cholesterol levels on hepatic cholesterol concentrations in the Mongolian gerbil. Letters indicate comparisons among diet groups. Group not sharing a common letter are significantly different ($p<0.05$, $n=14$). Diet 3, 5, and 6 contained similar amounts of dietary fatty acids with different levels of dietary cholesterol, 0.06, 0, and 0.006%, respectively.

hepatic cholesterol contents were responsive to dietary fatty acids. The level of hepatic free cholesterol was significantly ($p<0.009$) higher in gerbils fed the stearic acid diet (4.1±0.2 mg/g liver) than the other three saturated fatty acid diets, while hepatic free cholesterol was the lowest in animals fed the palmitic acid diet ($p<0.0009$) among the four diet groups (diet 1, 2, 3, and 4). There were no differences in hepatic free cholesterol levels between lauric and myristic acid diets, and between lauric and palmitic acid diets. Both palmitic and stearic acid diets resulted in a significantly greater level of hepatic esterified cholesterol (33.6±2.2 and 29.9±2.0 mg/g liver, respectively) compared with either the lauric or myristic acid diets (24.0±1.1 and 22.5±1.0 mg/g liver, respectively). When the ratio of hepatic esterified cholesterol to free cholesterol was expressed as a molar ratio (EC/FC), the palmitic acid diet showed the highest ratio (12.2±0.6), which is significantly different from the other three diets ($p=0.0001$) and the myristic acid diet showed the lowest (6.4±0.2), which is significantly different from the lauric acid diet ($p<0.03$).

Effects of dietary cholesterol on plasma lipids can be examined by comparing diets 3, 5, and 6 in Table 4 and Fig. 1. The 0.06% cholesterol diet raised plasma total−, HDL− and VLDL+LDL-cholesterol concentrations relative to the cholesterol-free and 0.006% cholesterol diets. However, no differences were observed between the 0.006% cholesterol diet and the cholesterol-free diet with respect to any plasma or liver cholesterol levels. As the levels of dietary cholesterol increased, plasma triglyceride concentrations tended to increase, these increases were not statistically significant (see Table 4). Dietary cholesterol did not influence hepatic free cholesterol concentrations. Feeding the 0.06% cholesterol diet increased hepatic esterified cholesterol concentration (33.6±2.2 mg/g liver) compared the with cholesterol-free (21.1±0.7 mg/g liver) or 0.006% cholesterol (23.5±0.9 mg/g liver) diets ($p=0.0001$)(Table 4, Fig. 2). Also, the 0.06% cholesterol diet (12.2±0.6) significantly ($p=0.0001$) increased the hepatic EC/FC molar ratio compared with the cholesterol-free (8.8±0.4) or 0.006% (9.8±0.3) cholesterol groups (Table 4).

To determine the distribution of specific fatty acids in the liver, small aliquots of liver were removed and total hepatic lipids were extracted and separated into phospholipids, triglycerides, and cholesteryl esters. As shown in Table 5, the major fatty acid in all three hepatic lipid classes was oleic acid (18 : 1), comprising approximately 60−70% of the total fatty acid mass. Feeding of lauric and palmitic acids significantly increased the mean levels of their respective abundance in hepatic triglycerides and cholesteryl esters fractions but not in the phospholipids fraction. Feeding of myristic acid significantly increased its abundance in all three lipid classes. Only the hepatic

Table 5. Fatty acid composition of phospholipid, triglycerides and cholesteryl esters isolated from the total lipid extracts of liver in gerbils

Dietary group	Relative abundance of each fatty acid (% of total mass)						
	12 : 0	14 : 0	16 : 0	16 : 1	18 : 0	18 : 1	18 : 2
Phospholipids							
Diet 1 (12 : 0)	<0.1^{a}	0.7±0.2^{a}	14.4±0.4^{a}	1.2±0.3^{b}	9.0±1.6	64.0±2.8^{ab}	10.1±1.0^{ab}
Diet 2 (14 : 0)	0.8±0.3^{b}	1.6±0.3^{b}	15.1±0.5^{ab}	n. d.	14.9±3.2	53.5±4.7	12.3±1.2^{b}
Diet 3 (16 : 0)	<0.1^{a}	0.3±0.1^{a}	16.4±0.6^{b}	0.7±0.2^{ab}	7.7±1.9	67.7±1.9^{b}	8.3±0.5^{a}
Diet 4 (18 : 0)	n. d.	0.7±0.2^{a}	14.5±1.3^{a}	0.4±0.2^{ab}	8.0±2.2	67.4±3.3^{b}	8.6±0.9^{ab}
Diet 5 (16 : 0)	n. d.	0.1±0.1^{a}	18.1±0.8	0.5±0.2^{ab}	11.8±1.0	60.8±0.9^{ab}	8.9±1.1^{ab}
Diet 6 (16 : 0)	n. d.	0.3±0.1^{a}	17.1±0.5	0.8±0.2^{ab}	11.3±1.4	59.3±2.1^{ab}	9.9±0.8^{ab}
Triglycerides							
Diet 1 (12 : 0)	1.1±0.1^{b}	2.3±0.1^{b}	16.4±0.4^{b}	2.2±0.0^{c}	5.7±0.3^{b}	62.8±1.0^{a}	8.9±0.3
Diet 2 (14 : 0)	0.1±0.0^{a}	3.0±0.2^{bc}	14.6±0.3^{a}	1.9±0.1^{b}	4.0±0.1^{a}	66.9±1.0^{b}	8.7±0.5
Diet 3 (16 : 0)	0.2±0.0^{a}	1.4±0.1^{a}	19.4±0.7^{c}	2.1±0.1^{c}	4.4±0.3^{a}	63.1±1.7^{ab}	9.1±0.7
Diet 4 (18 : 0)	0.1±0.0^{a}	1.2±0.1^{a}	13.6±0.4^{a}	1.5±0.1^{a}	4.4±0.2^{a}	72.0±0.6^{c}	7.2±0.3
Diet 5 (16 : 0)	0.1±0.0^{a}	1.1±0.0^{a}	20.0±0.3^{c}	1.9±0.0^{b}	3.7±0.1^{a}	65.5±0.6^{ab}	8.0±0.4
Diet 6 (16 : 0)	0.1±0.0^{a}	1.0±0.0^{a}	20.0±0.2^{c}	1.9±0.1^{b}	3.6±0.2^{a}	65.8±0.5^{ab}	7.8±0.3
Cholesteryl esters							
Diet 1 (12 : 0)	0.3±0.0^{b}	1.1±0.1^{b}	11.3±0.7^{a}	2.9±0.2^{b}	4.1±0.2^{abc}	75.2±0.8^{c}	5.5±0.1
Diet 2 (14 : 0)	<0.1^{a}	1.9±0.2^{c}	10.9±0.7^{a}	2.1±0.2^{a}	3.8±0.2^{ab}	74.9±0.7^{c}	6.2±0.4
Diet 3 (16 : 0)	<0.1^{a}	0.8±0.1^{a}	16.0±0.7^{b}	2.0±0.1^{a}	3.6±0.1^{a}	71.0±0.7^{b}	6.1±0.3
Diet 4 (18 : 0)	<0.1^{a}	0.7±0.1^{a}	9.9±0.7^{a}	1.9±0.1^{a}	4.5±0.2^{bcd}	76.8±0.8^{c}	5.6±0.1
Diet 5 (16 : 0)	<0.1^{a}	0.7±0.0^{a}	17.2±0.7^{b}	2.3±0.1^{a}	5.0±0.3^{d}	67.9±0.3^{a}	6.2±0.2
Diet 6 (16 : 0)	<0.1a	0.6±0.0^{a}	16.7±0.5	2.1±0.1^{a}	4.8±0.2^{cd}	70.1±0.6^{b}	5.8±0.2

Values are mean±SEM. Values in a row of each lipid class not sharing a common superscript are significantly different ($p<0.05$, $n=14$). Diet 1, 2, 3 and 4 contained 0.06% (w/w) dietary cholesterol. Diet 5 and 6 contained 0% and 0.006% (w/w) dietary cholesterol, respectively. n.d.=not detected

cholesteryl esters fraction, however, became enriched with stearic acid (18 : 0) when this fatty acid was fed. Generally, the abundance of different fatty acids in the cholesteryl esters of the liver was relatively sensitive to the concentration of these fatty acids in the diet. As the dietary cholesterol levels increased, the abundance of stearic acid in the esterified cholesterol decreased, but the abundance of oleic acid (18 : 1) increased.

DISCUSSION

The present study confirms the gerbil response to relatively modest doses of dietary cholesterol typifying human consumption (0.06%)(Figs. 1 and 2). At this level, gerbils exhibited a markedly increased pool of hepatic esterified cholesterol relative to free cholesterol (Fig. 2). It has been shown that dietary cholesterol increases hepatic acyl-CoA : cholesterol acyltransferase (ACAT) activity.[26][27] It appears, therefore, that a portion of dietary cholesterol in gerbils may have been routed into storage as cholesteryl ester in the liver by esterification, primarily with oleic acid which is the preferred fatty acid for ACAT (Table 5).

Gerbils appear more sensitive in increasing hepatic cholesteryl ester content in response to dietary cholesterol levels than rats. A study by Temmerman *et al.*[28] compared dietary cholesterol (0.006% or 0.2%) response to plasma and hepatic cholesterol levels among gerbils and rats. After feeding 0.006% dietary cholesterol, gerbils showed increased serum and hepatic total cholesterol compared to rats. At 0.2% dietary cholesterol, both serum and hepatic total cholesterol were markedly higher in gerbils than in rats. These authors also showed a higher percentage of hepatic cholesterol in the esterified form in gerbils compared to rats. Total sterol excretion rates in gerbils were half of those in rats at either 0.006 or 0.2% dietary cholesterol ; most sterol excretion was in the form of bile acids.[28] The authors concluded that the elevated hepatic esterified cholesterol levels in gerbils fed cholesterol may be due to the inability of gerbils to increase fecal neutral sterol excretion sufficiently compared to rats.[28] Therefore, it is plausible that a limited capacity to increase neutral sterol excretion may result in higher levels of hepatic cholesteryl ester in gerbils fed a cholesterol-free diet. Another possible hypothesis is that a low activity of cholesteryl ester hydrolysis in liver may lead to high levels of hepatic cholesteryl ester in gerbils fed a cholesterol-free diet relative to other animals.[29]

The increase in plasma total cholesterol in response to cholesterol feeding observed here agrees with the findings from previous short-term and long-term gerbil studies.[30][31] Temmerman *et al.*[31] used long-term experimental periods ; in a 6-month trial, gerbils fed 0.05% of dietary cholesterol showed significantly higher serum cholesterol concentrations than those fed 0.005% of dietary cholesterol. Anderson and Holub,[30] using a three-week experimental period, reported similar findings.

The increase in both HDL and VLDL+LDL cholesterol in response to dietary cholesterol feeding contrasts with previous findings from other gerbil studies. Feeding 0.5% cholesterol in a purified diet to gerbils resulted in a redistribution of cholesterol among the lipoproteins ; VLDL- and LDL-cholesterol levels were markedly increased and became the major cholesterol carrier and HDL carried proportionally less cholesterol.[32] Other investigators have reported that inclusion of 0.5% cholesterol in the diets of gerbils resulted in a three- to five-fold decrease in HDL-cholesterol.[33] However, the present study showed that HDL was the major carrier of plasma cholesterol. This discrepancy is unlikely to be due to differences in the basal diet, as those studies reporting large treatment changes in VLDL- and LDL-cholesterol used a semi-purified diet as we did in the present study. In the present study, however, dietary cholesterol was fed at levels of 0.006% and 0.06% by weight as compared to a level of 0.5% in previous studies. Perhaps the redistribution of cholesterol among lipoproteins occurs as a function of the dietary cholesterol concentration.

Palmitic and stearic acid diets increased hepatic esterified cholesterol concentrations with no change in plasma cholesterol concentrations compared to lauric and myristic acid diets. These results suggest that dietary long-chain saturated fatty acids may influence hepatic cholesterol metabolism with no apparent alteration in plasma cholesterol concentrations. Similar responses were observed in dietary mono- or polyunsaturated fatty acids in rats.[34][35] In contrast to the present study, stearic acid resulted in lower hepatic cholesterol concentrations without changing plasma cholesterol concentrations in hamsters[14] or rats[34] fed synthetic fats. In these studies, rats and hamsters fed stearic acid increased fecal neutral sterol excretion compared to those fed other fatty acids. Therefore, this discrepancy in hepatic cholesterol response to stearic acid may be due to the difference in neutral sterol excretion among animal species.

The effects of different dietary fatty acids on hepatic cholesteryl ester levels and LDL receptor activity were studied previously in hamsters.[36][37] In these studies, lauric,

myristic, and palmitic acids, but not stearic acid, lowered the hepatic cholesteryl ester concentration, depressed hepatic LDL receptor activity, and elevated the plasma LDL-cholesterol concentration. The authors speculated that these effects could be explained by a redistribution of cholesterol within the hepatocyte between storage and putative regulatory pools. Whatever the mechanisms, the regulatory effects of dietary fat in these studies were observed under conditions without any apparent change in net cholesterol delivery to the liver.

In the present study, gerbils fed different saturated fatty acids did not show differences in plasma total−, VLDL+LDL−, and HDL-cholesterol concentrations. Pronczuk *et al.*,[17] however, demonstrated that gerbils respond to changes in dietary saturated fatty acids. They conducted multiple regression analysis of plasma total cholesterol (TC) response to dietary fatty acids based on gerbils fed 21 diets supplying about 40% energy as fat from single or blended fat sources. They developed a multiple regression equation including coefficients for the dietary concentration (% energy : E) of myristic, palmitic, and linoleic acids as well as dietary cholesterol (C)(TC=135+5.2 E14 : 0+1.5 E16 : 0−73 log E18 : 2+0.18C). Application of this regression equation to our diet formulations (Table 2) predicts differences in plasma total cholesterol concentrations among saturated fatty acid treated groups as follows : predicted values for lauric, myristic, palmitic, and stearic acids are 126.6, 153.9, 135.0, and 126.7 mg/dl, respectively. These predicted values are smaller than those in our study. These discrepancies may be due to the large dietary fatty acid changes (7−26% energy) and the moderate response to cholesterol feeding in their study relative to our present study (dietary fatty acid changes ; 6.3−7.7% energy).

The pattern of cholesterol metabolism in the liver is complex. It involves cholesterol uptake from circulating lipoproteins, *de novo* synthesis, formation and secretion of lipoproteins, esterification to form cholesteryl esters, hydrolysis of cholesteryl esters, and utilization of cholesterol for the synthesis of bile acids and biliary cholesterol excretion. Therefore, studies measuring those parameters in gerbils fed long chain saturated fatty acid are needed to understand the mechanism behind these results.

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