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Dietary fatty acids differentially modulate messenger RNA abundance of low-density lipoprotein receptor, 3-hydroxy-3-methylglutaryl coenzyme A reductase, and microsomal triglyceride transfer protein in Golden-Syrian hamsters

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Abstract

Dietary fatty acids modulate plasma and intracellular cholesterol concentrations. Circulating non–high-density lipoprotein cholesterol (nHDL-C) concentration is determined by rates of hepatic very low-density lipoprotein assembly and secretion, and clearance of subsequent metabolic products. The effect of dietary fat (butter, traditional margarine, soybean oil, and canola oil) was assessed with respect to plasma lipids, hepatic lipid composition, and messenger RNA (mRNA) abundance of low-density lipoprotein (LDL) receptor, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, sterol regulatory element-binding protein (SREBP) 2, and microsomal triglyceride transfer protein (MTP) in the Golden-Syrian hamster (Charles River Laboratories, Wilmington, MA). Hamsters were fed with a nonpurified diet (6.25 fat g/100 g) with 0.1 g cholesterol/100 g (control diet) or control diet with an additional 10 g experimental fat/100 g for 12 weeks. Hamsters fed with the control diet, unsaturated fats (canola and soybean oils), and margarine, relative to butter, had significantly lower total cholesterol and nHDL-C and triglyceride concentrations. Additional dietary fat, regardless of fatty acid profile, resulted in higher hepatic cholesterol concentrations. In contrast, relative to the control diet-, butter-, or margarine-fed hamsters, these changes were associated with a 4- and 8-fold higher LDL receptor and 5- and 9-fold higher SREBP mRNA abundance, in hamsters fed with canola and soybean oils, respectively. MTP mRNA, a marker of very low-density lipoprotein particle formation, was higher in canola- and soybean oil–fed hamsters relative to the control diet–fed hamsters, although differences were modest. These results suggest that the substitution of canola and soybean oils for butter results in lower nHDL-C concentrations that may be related to increased mRNA abundance of the LDL receptor, SREBP-2, and MTP genes.

1. Introduction

Dietary fatty acids and cholesterol interact to modulate plasma and intracellular cholesterol homeostasis. The fatty acid profile of the dietary fat is a major determinant of circulating low-density lipoprotein cholesterol (LDL-C) concentrations [1]. In humans, saturated fatty acids (SFA) and *trans*-fatty acids result in higher LDL-C concentrations than monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) [2]. A consequence is that Western diets, high in both SFA and *trans*-fatty acids, are associated with high rates of cardiovascular disease [3,4].

The concentration of cholesterol transported in circulation by non–high-density lipoprotein (nHDL) particles (all apolipoprotein [apo] B–containing particles) is regulated by metabolic events in the liver, plasma, and peripheral tissues. These events include rates of hepatic very low-density lipoprotein (VLDL) particle assembly and secretion, plasma conversion of VLDL to intermediate-density lipoprotein (IDL) and LDL, and subsequent clearance of these nHDL particles. In both humans and hamsters, most LDL particles are taken up by the LDL receptor–mediated pathway [5]. One way dietary cholesterol and fatty acids modulate circulating nHDL concentrations is by altering LDL

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Table 1
Composition of the diets

	Nonpurified diet (control) (g/kg)	Experimental fat-enriched nonpurified diet (g/kg)
Protein	186.2	186.2
Fat[a]	62.5	162.5
Cholesterol[b]	1	1
Fiber	45.3	45.3
Nitrogen free extract	664	563
Vitamins[c]	2	2
Minerals[d]	40	40

[a] Nonpurified diet contained 62.5 g fat/kg. Experimental diets had an additional 100 g fat per kilogram with type varying among diets.

[b] Nonpurified and experimental diets contained 1 g cholesterol per kilogram.

[c] Vitamins provided in the diet: vitamin A, 2.4 μmol/L; vitamin D_3, 0.28 nmol/L; vitamin E, 10.4 μmol/L; choline, 1.89 mg/g; niacin (nicotinic acid), 86.61 mg/kg; pantothenic acid, 36.87 mg/kg; pyridoxine (vitamin B_6), 9.47 mg/kg; riboflavin, 7.40 mg/kg; thiamine, 76.31 mg/kg; vitamin K_3, 22.70 mg/kg; folic acid, 1.48 mg/kg; biotin, 0.29 mg/kg; vitamin B_{12}, 62.00 μg/kg.

[d] Minerals provided in the diet: calcium, 1.19%; phosphorus, 0.92%; sodium, 0.31%, chlorine, 0.48%; potassium, 0.63%; magnesium, 0.20%; iron, 336.2 mg/kg; manganese, 155.94 mg/kg; zinc, 48.22 mg/kg; copper, 13.22 mg/kg; iodine, 2.01 mg/kg; cobalt, 0.53 mg/kg; selenium, 0.30 mg/kg.

receptor messenger RNA (mRNA) expression [6-8]. This multifaceted regulatory process minimizes excessive LDL particle uptake by cells, especially in response to high plasma LDL-C concentrations.

In conjunction with changes in LDL receptor expression and activity, delivery of cholesterol to the liver and other tissues significantly alters the rate of cholesterol biosynthesis [9]. Intracellular free cholesterol concentrations modulate the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in this biosynthetic process. Similar to LDL receptor regulation, dietary SFA has been shown to down-regulate HMG-CoA reductase expression and rate of activity [9,10].

Circulating LDL particles are the final products of the progressive delipidation of VLDL and IDL and loss of apolipoproteins other than apoB-100. Assembly and secretion of nHDL particles, primarily VLDL, is dependent upon microsomal triglyceride transfer protein (MTP) activity. MTP facilitates proper apoB-100 folding during translation of the peptide chain and facilitates the intracellular transfer of triglycerides and cholesteryl esters to the lipid-binding domains within apoB-100 of the nascent lipoprotein particle [11].

The present study was conducted to examine the effect of diets differing in fatty acid profile with cholesterol on the expression of the LDL receptor, HMG-CoA reductase, sterol regulatory element-binding protein 2 (SREBP-2), and MTP, components involved in modulating nHDL cholesterol (nHDL-C) concentrations. Golden-Syrian hamsters (Charles River Laboratories, Wilmington, MA) were used as an animal model of diet-induced lipoprotein changes because of previously demonstrated responsiveness to dietary fatty acids and cholesterol.

2. Materials and methods

2.1. Animals and diets

Eight-week-old, male CR Golden Syrian hamsters were individually housed in stainless steel suspended rodent cages with free access to modified rodent sterilizable diet (Harlan-Teklad, Madison, WI) and water for a 2-week acclimation period. The hamsters were maintained in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, in an environmentally controlled atmosphere (temperature, 23°C; 45% relative humidity) with 15 air changes of 100% fresh HEPA-filtered air per hour and reverse 10:14 light-dark cycle [12]. The health status of the hamsters was monitored daily. After the acclimation period, each hamster was weighed, ear punched, and randomly assigned to 1 of 5 experimental diets (Tables 1 and 2). The hamsters were housed 4 hamsters per cage (28 × 11 × 7 in) and were maintained on the experimental diets for 12 weeks. Before initiating the experimental diet period and every 2 weeks thereafter, blood was collected from the retro-orbital sinus under isoflurane anesthetization after a 16- to 18-hour fast. Body weights were recorded every 2 weeks, beginning before the initiation of the experimental diet period. During the 12th week, hamsters were fasted for 16 to 18 hours, blood samples collected, and then the hamsters were killed by terminal exsanguinations from the abdominal aorta under isoflurane anesthetization. Livers were removed, flash frozen, and stored at −80°C. Hearts were then perfused in situ with phosphate-buffered saline for 2 minutes, removed with aortas attached from the body, and stored in 50-mL tubes containing phosphate-buffered saline.

This project was approved by the USDA Human Nutrition Research Center on Aging Animal Care and Use Committee.

Table 2
Fatty acid composition and cholesterol content of the diets

	Control	Butter	Stick margarine	Canola oil	Soybean oil
Total fat energy (g/100 g)	6.25	16.25	16.25	16.25	16.25
SFA	18.7	45.9	18.0	11.0	16.9
12:0	0	1.9	0	0	0
14:0	0.72	6.8	0	0	0
16:0	14	24.3	11.9	7.8	12.5
18:0	3.7	10.4	5.8	2.6	4.1
TFA	0.6	3.1	16.5	2.3	0.7
18:1t	0	2.8	15.6	0.2	0.1
18:2t	0.2	0.2	0.7	0.1	0.3
18:3t	0.4	0.1	0.1	1.9	0.3
MUFA	25.3	29.0	29.2	53.2	24.0
15:1	1.1	1.6	0	0	0
18:1	24	27.2	28.9	52.6	23.8
PUFA	55.4	21.5	33.8	33.6	58.3
18:2	50.3	19.7	30.5	28.4	52.6
18:3	5.1	1.8	3.3	5.2	5.7
20:4	0	0	0	0	0
Cholesterol (wt/wt)	0.1	0.1	0.1	0.1	0.1

Table 3
Body weights, serum lipid and lipoprotein concentrations, apoB-100, and aortic cholesteryl ester concentrations in Golden-Syrian hamsters fed with a nonpurified diet (6.25 g fat/100 g) enriched in 0.1 g cholesterol/100 g or nonpurified diet (6.25 g fat/100 g) enriched with 0.1 g cholesterol/100 g and an additional 10 g fat/100 g (butter, traditional stick margarine, canola oil, or soybean oil) for 12 weeks

	Control (n = 20)	Butter (n = 18)	Stick margarine (n = 20)	Canola oil (n = 20)	Soybean oil (n = 20)	*P*
Body weight (g)	168 ± 16[a]	173 ± 26[a]	180 ± 24[a]	177 ± 21[a]	170 ± 21[a]	.3926
Total cholesterol[1] (mg/dL)	184 ± 39[b]	291 ± 126[a]	228 ± 81[b]	171 ± 30[b]	196 ± 36[b]	<.0001
nHDL-C[2]	168 ± 16[b]	214 ± 139[a]	134 ± 99[b]	92 ± 34[b]	92 ± 27[b]	<.0001
HDL-cholesterol[1] (mg/dL)	94 ± 18[a]	77 ± 26[b]	95 ± 26[a]	80 ± 14[b]	104 ± 31[a]	.0001
Triglyceride[1] (mg/dL)	80 ± 17[b]	218 ± 117[a]	125 ± 111[b]	106 ± 44[b]	119 ± 42[b]	<.0001
Total cholesterol/ HDL-C	2.0 ± 0.3[b]	4.6 ± 3.5[a]	2.2 ± 0.6[b]	2.2 ± 0.7[b]	2.0 ± 0.4[b]	<.0001
ApoB-100/GAPDH[1]	14.2 ± 10.9[b]	15.2 ± 9.5[b]	19.7 ± 4.1[a]	5.9 ± 5.8[c]	10.1 ± 5.4[b]	<.0001
Aortic cholesteryl ester (μg/mg wet weight)	1.8 ± 3.5[a]	0.9 ± 0.6[a]	0.9 ± 0.9[a]	0.8 ± 0.8[a]	0.7 ± 0.3[a]	.9926

Values are expressed as mean ± SD. Means in a row without common superscripts differ ($P < .05$). To convert mean values for total cholesterol, nHDL-C, and HDL-C to millimoles per liter, divide by 38.65; to convert triglyceride to millimoles per liter, divide by 88.54.
[1] Values were log-transformed before statistical analysis.
[2] Calculated as the difference between total cholesterol and HDL-C.

2.2. Serum lipid and lipoprotein analysis

Serum was separated from red blood cells by centrifugation at 1100*g* at 4°C and assayed for total cholesterol and HDL-C, and triglyceride concentrations on a Hitachi 911 automated analyzer (Roche Diagnostics, Indianapolis, IN) using enzymatic reagents. The assays are standardized through the Lipid Standardization Program of the Centers for Disease Control, Atlanta, GA.

2.3. Western blot analyses of plasma apoB-100

Plasma apoB-100 was determined by immunoblotting using a polyclonal antibody specific to human apoB-100 (Calbiochem, San Diego, CA) that recognized hamster apoB-100 because of considerable consensus between primary amino acid sequences. Briefly, 20 μg of protein was separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis using a 5% separating gel. Proteins were transferred to polyvinylidene difluoride membranes using a wet transfer system at 4°C overnight. Membranes were then blocked in 5% nonfat milk Tris-buffered saline with Tween 20 and incubated for 2 hours with a 1:1000 dilution of rabbit anti–apoB-100 prepared in the same blocking solution followed by 1 hour with secondary antibody, 1:5000 (Bio-Rad, San Diego, CA). Bands were visualized with chemiluminescence (Bio-Rad), and apoB-100 was quantitated using densitometry (Quantity One, Bio-Rad).

2.4. Lipid composition of aorta and liver

Lipids were extracted from both the aorta and liver using the method of Folch and Stanley [13]. Total and free cholesterol were determined by the method described by Carr et al [14] and cholesteryl ester concentrations calculated by difference. Data were expressed as milligram per grams of wet weight tissue.

2.5. Real-time polymerase chain reaction analysis of LDL receptor, HMG-CoA reductase, MTP, and SREBP-2

Total RNA was extracted from liver using Qiagen Rneasy kits (Qiagen, Valencia, CA). Reverse transcription of total RNA into complementary DNA was performed using Pharmacia first-strand synthesis kit (Amersham Pharmacia Biotech, Piscataway, NJ). Messenger RNA expression levels were semiquantitatively determined on an ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). Briefly, for each sample, the C_t for the LDL

Table 4
Liver lipid composition in Golden-Syrian hamsters fed with nonpurified diet (6.25 g fat/100 g) enriched in 0.1 g cholesterol/100 g or nonpurified diet (6.25 g fat/100 g) enriched with 0.1 g cholesterol/100 g and an additional 10 g fat/100 g (butter, traditional stick margarine, canola oil or soybean oil) for 12 weeks[1]

	Control (n = 20)	Butter (n = 18)	Stick margarine (n = 20)	Canola oil (n = 20)	Soybean oil (n = 20)	*P*
Liver weight (g)	6.7 ± 1.0[b]	7.7 ± 1.3[ab]	8.2 ± 1.2[a]	7.5 ± 1.2[ab]	7.2 ± 0.8[ab]	.0059
Liver weight (% body weight)	4.0[b]	4.5[a]	4.6[a]	4.2[ab]	4.2[a b]	.0046
Total cholesterol[1] (mg/g)	6.7 ± 4.1[b]	9.1 ± 4.7[ab]	14.3 ± 9.0[a]	13.0 ± 7.2[a]	12.0 ± 7.0[a]	.0055
Free cholesterol[1] (mg/g)	1.8 ± 0.4[b]	2.2 ± 0.6[ab]	2.9 ± 1.1[a]	2.2 ± 0.6[ab]	2.4 ± 0.8[a]	.0010
Cholesteryl ester (mg/g)	4.9 ± 3.8[b]	6.9 ± 4.3[ab]	11.4 ± 8.1[a]	10.8 ± 6.7[a]	9.7 ± 6.6[ab]	.0115
Triglyceride (mg/g)	3.6 ± 1.0[a]	4.4 ± 1.5[a]	4.2 ± 2.2[a]	5.2 ± 6.4[a]	3.9 ± 1.3[a]	.7614
Phospholipid[1] (mg/g)	11.5 ± 2.1[a]	12.8 ± 2.6[a]	15.2 ± 6.2[a]	12.1 ± 2.7[a]	12.4 ± 2.5[a]	.0606

Values are expressed as means ± SD. Means in a row without common superscripts differ ($P < .05$).
[1] Values were log-transformed before statistical analysis.

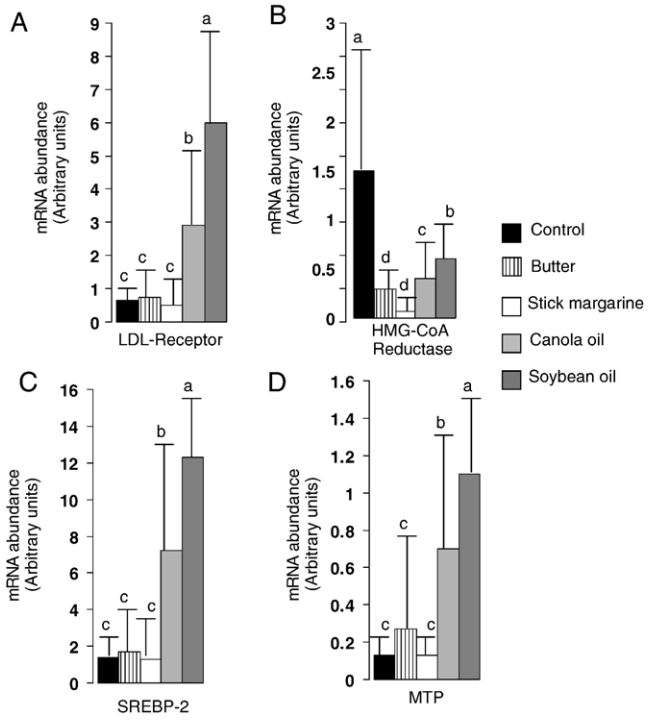

Fig. 1. Effect of butter, traditional stick margarine, canola oil, and soybean oil on hepatic LDL receptor, HMG-CoA reductase, SREBP- 2, and MTP mRNA abundance. A, LDL receptor mRNA. B, HMG-CoA reductase. C, SREBP-2. D, MTP. Hepatic mRNA abundance was quantified by real-time reverse transcriptase–polymerase chain reaction analysis. Data were log-transformed before statistical analyses. Bars represent means ± SD. Bars without a common letter differ ($P < .05$).

receptor, HMG-CoA reductase, MTP, and SREBP-2, and for the endogenous control, ribosomal subunit 18s, was determined to calculate $\Delta C_{t,sample}$ ($C_{t,target\ gene} - C_{t,18s}$), thus normalizing the data and correcting for the differences in amount and/or quality between the different RNA samples. Messenger RNA expression levels (mRNA abundance) are reported as arbitrary units.

2.6. Statistics

All data are reported as the mean ± SD. Differences among groups of hamsters fed with the control and experimental fat-enriched diets were determined by analysis of variance, (SAS, Cary, NC). Transformations were used to normalize the data when appropriate and are so indicated. The Student-Newman-Keuls test was used for post hoc analysis after analysis of variance. Differences were considered significant at $P \leq .05$.

3. Results

3.1. Dietary fatty acid effects on serum lipids and lipoproteins, plasma apoB-100 concentrations, and aortic cholesteryl ester content

Dietary fat feeding independent of fatty acid class resulted in similar body weights among each group throughout the study period (Table 3). The hamsters consuming the butter-enriched diet had significantly higher total cholesterol concentrations relative to the other diets ($P < .0001$) (Table 3). Total cholesterol concentrations were similar among the hamsters fed with cholesterol-enriched non-purified diet (control), margarine, canola oil, and soybean oil diets. A similar pattern was observed for triglyceride concentrations. Non–HDL-C concentrations were 21%, 37%, 57%, and 57% lower in hamsters fed with the control, margarine, canola oil, and soybean oil diets, respectively, relative to the group fed with the butter diet. In contrast, plasma apoB-100 concentrations were highest in the hamsters fed with the margarine diet, intermediate in the hamsters fed with the butter, soybean oil, and control diets, and lowest in hamsters fed with the canola oil diet ($P < .0001$). As estimated from the ratio of nHDL-C to apoB-100, the hamsters fed with the margarine diet had smaller, denser particles, whereas butter- and canola oil–fed hamsters had larger, less dense particles. High-density lipoprotein cholesterol concentrations did not follow a predictable pattern. They were higher in the hamsters fed with margarine and soybean oil diets relative to hamsters fed the butter and canola oil diets ($P = .0001$). Despite these differences in circulating plasma lipid and lipoprotein concentrations, atherosclerotic lesion formation was minimal and there were no significant difference among the diet groups (Table 3). The low level of cholesteryl ester accumulation in the aorta was striking [15].

3.2. Hepatic lipid composition

Liver weights of those hamsters fed with the butter- and margarine-enriched diets were heavier than hamsters fed with the control diet, both in actual terms or as a percentage of final body weight (Table 4). Unsaturated fatty acid–fed hamsters, including those with either *cis* or *trans* double-bond–containing fatty acids (canola and soybean oil, or margarine, respectively), had higher hepatic total cholesterol concentrations ($P = .0055$) than saturated fat fed hamsters; the biggest difference was in the cholesteryl ester fraction. All groups fed with the fat-enriched diets had higher hepatic free cholesterol and cholesteryl ester concentration than the control diet–fed group ($P = .0010$ and .0115, respectively). Hepatic triglyceride and phospholipid concentrations were similar among the experimental groups, suggesting that the dietary perturbation most likely did not adversely affect liver function (Table 4).

3.3. Hepatic LDL receptor, HMG-CoA reductase, SREBP-2, and MTP mRNA abundance

Diets high in *cis*-unsaturated fatty acids (canola and soybean oils) had 4- and 8-fold, respectively, higher LDL receptor mRNA abundance than hamsters fed with the control diet (Fig. 1A). Hepatic LDL receptor mRNA abundance was similar among hamsters fed with the control, butter, and margarine diets.

Supplementing the diet with cholesterol has been reported to decrease endogenous cholesterol synthesis

through reduced activity of HMG-CoA reductase [10]. In general, addition of fat, regardless of type, blunted this response (Fig. 1B). This blunting effect diminished with increased unsaturation of the dietary fat, with the exception of the fat high in *trans*-unsaturated fatty acid (margarine). Nevertheless, the magnitude of the differences was modest and would not be predicted to have a large biologic effect.

Both the LDL receptor and HMG-CoA reductase gene have a sterol regulatory element in their promoter regions and are regulated, in part, by SREBP-2 [16-19]. Therefore, the abundance of hepatic SREBP-2 mRNA was assessed. The response patterns observed were similar to the LDL receptor mRNA abundance. Relative to the control diet–fed hamsters, SREBP-2 message levels were 5- and 9-fold higher in hamsters fed with the diets high in *cis*-unsaturated fatty acids, canola and soybean oils, respectively (Fig. 1C). Message levels were similar among hamsters fed with the control, butter, and margarine diets.

The assembly of VLDL in the liver and ultimately the metabolic fates of both VLDL and IDL are regulated by dietary fat and cholesterol. MTP mRNA expression was measured as a marker for hepatic-derived nHDL particle formation. Hamsters fed with the *cis*-unsaturated fatty acid diets, canola and soybean oils, had 5- and 7-fold, respectively, higher MTP mRNA abundance. Differences in mRNA abundance in the control-, butter- and margarine-fed hamsters were small and similar among each other (Fig. 1D). Striking was the consistent pattern of response among LDL receptor, SREBP-2, and MTP mRNA abundance in response to diets high in *cis*-unsaturated fatty acids (canola and soybean oils), relative to the other dietary perturbations, suggesting enhanced regulation of genes involved in lipid metabolism by unsaturated fatty acids [19,20].

4. Discussion

The present study was designed to investigate potential mechanisms responsible for the differential effects of dietary fats representing a wide range of fatty acid profiles on nHDL-C metabolism in the Charles River Golden-Syrian hamster. The following aspects were studied: fatty acid saturation (SFA, MUFA, and PUFA) and hydrogenation (traditional soybean oil–based stick margarine [*trans*-fatty acids]).

One of the mechanisms by which the liver regulates plasma nHDL-C concentrations is through changes in intracellular sterol pools. The influx of cholesterol into hepatocytes causes an increase in the activity of acyl CoA:cholesterol acyltransferase, resulting in a decrease in the free cholesterol pool [21,22]. The addition of *cis*- and *trans*-unsaturated fatty acids (canola oil, soybean oil, and margarine) to the diet resulted in higher hepatic cholesteryl ester concentrations as compared with the saturated (butter) fatty acid–fed hamsters. The higher cholesteryl ester concentration may be a reflection of unsaturated fatty acids being preferred substrates for acyl CoA:cholesterol acyltransferase [23]. The reasons for the higher cholesteryl ester concentration in hamsters fed with the margarine diet are unclear at this time and may be related to differential rates of hepatic cholesteryl ester species being incorporated into the nascent VLDL particle [24].

In both humans and hamsters, most nHDL-C is taken up by the LDL receptor–mediated pathway [5]. In this study, dietary fatty acid type altered LDL receptor mRNA abundance. Hamsters fed with *cis*-unsaturated fatty acids, canola and soybean oils, had higher mRNA abundance than the other groups, suggesting higher rates of LDL clearance. The higher LDL receptor expression was likely a reflection of smaller free cholesterol pools within the liver of these hamsters. The difference would be expected to contribute to the lower nHDL-C concentrations observed in those hamsters. This observation is not without precedence in humans [7].

Ness and Gertz [9] observed a striking correlation between HMG-CoA reductase mRNA and protein abundance, and resistance to dietary cholesterol induced hypercholesterolemia among animal species. Animals with the highest HMG-CoA reductase mRNA and protein abundance, for example, rats, were the most resistant to dietary cholesterol relative to animals with lower abundance, for example, the hamster. This implies a cause-and-effect relationship between low HMG-CoA reductase mRNA abundance and high nHDL-C concentrations in response to butter.

The response seen in mRNA abundance of SREBP-2 to canola and soybean oil feeding is noteworthy. Similar to the pattern observed for LDL receptor mRNA abundance, SREBP-2 mRNA abundance was highest in hamsters fed with canola and soybean oils, relative to the other diets. Sterol regulatory element-binding protein 2, an activated transcription factor, is regulated at the transcriptional level by sterol depletion as well as at the posttranslational level by a proteolytic cleavage cascade [25]. Elevated concentrations of sterols block SREBP-2 maturation, thereby interrupting LDL receptor transcription. Dietary fat increased, rather than decreased, SREBP-2 mRNA expression as might have been expected, suggesting regulation at the posttranslational level. These data imply that the regulation of the LDL receptor by dietary fat is occurring independently of SREBP-2, as has been previously suggested [26]. Conversely, the modest rise in HMG-CoA reductase mRNA abundance may be a result of an increase in SREBP-2 transcription. It has previously been reported that cholesterol depletion up-regulates the cholesterol biosynthetic pathway [27].

ApoB-100, the apolipoprotein associated with hepatic-derived nHDL, is incorporated into nascent lipoprotein particles along with cholesterol and triglycerides in concert with MTP [28,29]. The apoB-100 protein concentrations were higher in response to the *trans*-fatty acid (margarine)–fed hamsters than SFA (butter), canola oil (MUFA), and soybean oil (PUFA) and were inconsistent with differences

in nHDL-C concentrations observed among the groups of hamsters. This may suggest the formation and secretion of smaller, denser nHDL particles, as observed by a smaller nHDL/apoB-100 ratio, in the hamsters fed with the margarine diet, yielding a more atherogenic particle as has been reported in humans in response to hydrogenated fat feeding [30-32].

In a separate study, Golden-Syrian hamsters have previously been shown to respond differently to *trans*-fatty acids than humans with respect to HDL-C metabolism [33]. Based on the current study, we have concluded that nHDL metabolism likewise differs. Despite a similar response in serum nHDL-C concentrations and hepatic lipid composition in *cis*-fatty acid (canola and soybean)–and *trans*-fatty acid (margarine)–fed hamsters, gene expression patterns follow a pattern more similar to the saturated (butter) fat than the unsaturated fat–fed hamsters. These data suggest alternative mechanisms may be contributing to these differences observed between the butter- and margarine-fed hamsters.

A limitation of this work is that although gene transcription as assessed by mRNA abundance can provide information on message levels, there are other mechanisms that ultimately determine activity such as protein concentration and enzyme activity. Teasing out the critical components by focusing on each factor will be necessary to further understand the nature of nHDL metabolism regulation by dietary fatty classes and cholesterol in a whole-animal model system.

In summary, the addition of different types of dietary fat, substituting *cis* unsaturated fats (canola or soybean oils) for saturated fats (butter), resulted in lower nHDL-C concentrations. This response was associated with an increase in mRNA abundance of LDL receptor, SREBP-2, and MTP. *trans*-Fatty acids, although having adverse effects on lipoprotein profiles in humans, appear to have an intermediate effect in hamsters, between the unsaturated fatty acids and SFA, suggesting that the hamster is not an appropriate model in which to investigate *trans*-fatty acid metabolism as it applies to humans.

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