

# Impact of dietary fat type within the context of altered cholesterol homeostasis on cholesterol and lipoprotein metabolism in the F1B hamster

Jaime L. Lecker<sup>a</sup>, Nirupa R. Matthan<sup>a</sup>, Jeffrey T. Billheimer<sup>b</sup>,  
Daniel J. Rader<sup>b</sup>, Alice H. Lichtenstein<sup>a,b,\*</sup>

<sup>a</sup>Cardiovascular Nutrition Laboratory, Jean Mayer US Department of Agriculture, Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111, USA

<sup>b</sup>Cardiovascular Institute, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA

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## Abstract

Cholesterol status and dietary fat alter several metabolic pathways reflected in lipoprotein profiles. To assess plasma lipoprotein response and mechanisms by which cholesterol and dietary fat type regulate expression of genes involved in lipoprotein metabolism, we developed an experimental model system using F1B hamsters fed diets (12 weeks) enriched in 10% (wt/wt) coconut, olive, or safflower oil with either high cholesterol (0.1%; cholesterol supplemented) or low cholesterol coupled with cholesterol-lowering drugs 10 days before killing (0.01% cholesterol, 0.15% lovastatin, 2% cholestyramine; cholesterol depleted). Irrespective of dietary fat, cholesterol depletion, relative to supplementation, resulted in lower plasma non-high-density lipoprotein (non-HDL) and HDL cholesterol, and triglyceride concentrations (all  $P$ s < .05). In the liver, these differences were associated with higher sterol regulatory element binding protein-2, low-density lipoprotein receptor, 3-hydroxy-3-methylglutaryl coenzyme A reductase, and 7 $\alpha$ -hydroxylase messenger RNA (mRNA) levels; higher scavenger receptor B1 and apolipoprotein A-I mRNA and protein levels; lower apolipoprotein E protein levels; and in intestine, modestly lower sterol transporters adenosine triphosphate-binding cassette (ABC) A1, ABCG5, and ABCG8 mRNA levels. Irrespective of cholesterol status, coconut oil, relative to olive and safflower oils, resulted in higher non-HDL cholesterol and triglyceride concentrations (both  $P$ s < .05) and modestly higher sterol regulatory element binding protein-2 mRNA levels. These data suggest that, in F1B hamsters, differences in plasma lipoprotein profiles in response to cholesterol depletion are associated with changes in the expression of genes involved in cholesterol metabolism, whereas the effect of dietary fat type on gene expression was modest, which limits the usefulness of the experimental animal model.

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## 1. Introduction

Cholesterol homeostasis plays an important role in the regulation of whole-body cholesterol content by balancing absorption (intestinal and biliary) and synthesis (hepatic and extrahepatic), thereby preventing the net accumulation of cholesterol in circulation and tissues [1,2]. Dietary fatty

acid chain length and degree of saturation have been shown to alter several metabolic pathways involving cholesterol throughout the body, the combined effect of which is reflected in plasma lipid and lipoprotein profiles [3–5]. The availability of an intact and unmodified animal model would allow for the simultaneous assessment of dietary fat type-induced changes in multiple tissue systems within the context of altered cholesterol homeostasis and facilitate the development of a more complete understanding of the complex set of factors known to regulate lipid and lipoprotein metabolism and ultimately atherosclerotic lesion development.

To achieve this, we chose the hamster to develop an experimental animal model because it has a low rate of

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\* Corresponding author. JM USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111, USA. Tel.: +1 617 556 3127. E-mail address: [Alice.Lichtenstein@tufts.edu](mailto:Alice.Lichtenstein@tufts.edu) (A.H. Lichtenstein).

endogenous cholesterol synthesis and is one of the few rodents to have cholesteryl ester transfer protein activity and possess tissue-specific editing of apolipoprotein (apo) B messenger RNA (mRNA) [6–9]. Hamsters, like humans, take up approximately 80% of low-density lipoprotein (LDL) cholesterol via the LDL receptor pathway [7]. The F1B strain was chosen because it had been reported to respond to saturated fat and cholesterol by increasing the non-high-density lipoprotein (non-HDL) fraction to a greater extent than the HDL fraction [6,7,10–13]. Using the F1B hamster, whole-body cholesterol status was altered by feeding either a low-cholesterol diet followed by acute cholesterol depletion induced by plasma cholesterol-lowering drugs (3-hydroxy-3-methylglutaryl-coenzyme A [HMG-CoA] reductase inhibitor and bile acid sequestrant) or a high-cholesterol diet. This experimental approach has been used successfully in multiple animal species to address other experimental questions [12,14,15].

The liver plays a major role in lipoprotein metabolism. Hepatic cholesterol levels are controlled by a balance between cholesterol synthesis, uptake, and secretion, primarily through the activities of HMG-CoA reductase, LDL receptor, and 7 $\alpha$ -hydroxylase, respectively, and are important determinants of plasma lipoprotein profiles [6,16,17]. Sterol regulatory element binding protein (SREBP)–2, a member of the SREBP family of transcription factors, regulates cholesterol synthesis and uptake through alterations in the expression of HMG-CoA reductase and LDL receptor [1,18]. A sterol sensor in the endoplasmic reticulum modulates SREBP-2 transcriptional activity in response to changes in intracellular free cholesterol levels [1]. Fatty acid chain length and degree of saturation indirectly regulate SREBP-2 transcriptional activity by altering the free cholesterol regulatory pool [19]. The rate of acyl-CoA cholesterol acyl transferase (ACAT) activity alters intracellular free cholesterol levels and formation of cholesteryl ester for hepatic intracellular de novo lipoprotein synthesis [20]. The cell surface receptors adenosine triphosphate-binding cassette transporter (ABC) A1 and scavenger receptor class B type 1 (SR-B1) activities are determinants of hepatic substrate availability and circulating lipoproteins [21–24]. Additional factors regulating hepatic lipoprotein synthesis include the availability of triglyceride [6,25,26], apo B-100, and apo E [27,28], and microsomal triglyceride transfer protein (MTP) activity [29–31].

Intestinal cholesterol absorption also modulates lipoprotein metabolism, primarily through a family of ABC transporters: ABCA1, ABCG5, and ABCG8 [32]. These transporters control the trafficking of intestinal sterol balance by facilitating the efflux of sterols from the apical (ABCG5/8) [33] or basolateral (ABCA1) [34] membrane of the enterocyte. Niemann-Pick C1 Like1 (NPC1L1) facilitates intestinal sterol uptake on the apical side of the enterocyte [35].

Dietary fatty acid type and cholesterol have been shown to modulate the level and activity of transcription factors,

which regulate the expression of genes involved in cholesterol and lipoprotein metabolism [3]. Polyunsaturated fatty acids (PUFAs) and cholesterol metabolites are the ligands for transcription factors PPAR, LXR, and FXR, which play a role in regulating plasma lipoprotein profiles [3,36–38]. Dietary fatty acids and cholesterol regulate SREBP activity through altering levels and cellular location of the transcription factor [1,19,39,40].

To assess the effect of fatty acid chain length and degree of saturation, both cholesterol-supplemented and cholesterol-depleted F1B hamsters were fed diets enriched in fats high in saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), or PUFAs. Using this approach, our aim was to simultaneously assess the mechanisms by which whole-body cholesterol status and dietary fat type regulate the expression of genes involved in hepatic and intestinal cholesterol and lipoprotein metabolism.

## 2. Materials and methods

### 2.1. Animals and diets

Ninety-six 8-week-old male F1B hamsters (BioBreeders, Watertown, MA) were divided into 6 diet groups on the basis of body weight and housed in stainless steel suspended cages (4 hamsters per cage) with a reverse 12-hour:12-hour light/dark cycle. Hamsters were given free access to LM-485 mouse/rat diet (Harlan-Teklad, Madison, WI) and water during a 2-week acclimation period. Thereafter, the hamsters were switched to ad libitum semipurified diets containing 10% (wt/wt) coconut oil (SFAs), olive oil (MUFAs), or safflower oil (n-6 PUFAs), in combination with 0.1% (wt/wt) cholesterol or 0.01% (wt/wt) cholesterol for 12 weeks (Tables 1 and 2, diet composition and diet fatty acid profile, respectively). The analytical data were consistent

Table 1  
Composition of experimental diets

Ingredient	0.01% (wt/wt) –C diet <sup>a</sup>	0.1% (wt/wt) +C diet
	g/kg diet	
Casein	204	203
L-Methionine	4	4
Maltodextrin	102	102
Cornstarch	281	281
Sucrose	129	129
Cellulose	122	122
Coconut, olive, or safflower oil	100	100
Soybean oil	20	20
AIN93G mineral mix	28	28
AIN93 vitamin mix	8.0	8.0
Choline bitartrate	2.0	2.0
Cholesterol	0.1	1
tert-Butylhydroquinone	0.02	0.02

Semipurified diets were prepared by Research Diets, New Brunswick, NJ.

<sup>a</sup> Lovastatin (0.15% wt/wt) and cholestyramine (2.0% wt/wt) were added to the 0.01% (wt/wt) cholesterol diet the last 10 days of treatment.

Table 2  
Fatty acid and cholesterol composition of the diets

	Coconut oil	Olive oil	Safflower oil
	Percentage of total fatty acids		
Total SFA			
–C	78.3	19.4	14.4
+C	78.5	19.4	14.4
12:0			
–C	36.8	0.04	0.04
+C	37.2	0.04	0.04
14:0			
–C	15.9	0.1	0.2
+C	16.5	0.1	0.2
16:0			
–C	11.2	13.1	8.5
+C	11.6	13.1	8.6
18:0			
–C	6.7	6.1	5.5
+C	7.1	6.1	5.4
Total MUFA			
–C	10.1	62.4	16.8
+C	10.1	62.4	16.8
18:1			
–C	10.1	61.8	16.7
+C	10.1	61.8	16.8
Total PUFA			
–C	11.6	18.3	68.8
+C	11.4	18.3	68.7
18:2n-6			
–C	9.9	16.1	66.9
+C	9.7	16.1	66.8
18:3n-3			
–C	1.1	1.5	1.1
+C	0.9	1.5	1.1
20:5n-3			
–C	0.6	0.7	0.7
+C	0.8	0.7	0.8
Cholesterol	% wt/wt		
–C	0.01	0.01	0.01
+C	0.08	0.08	0.08

Values are averages of duplicate measurements of diet samples.

with the intended diet composition. To determine the effect of acute whole-body cholesterol depletion on gene expression and protein synthesis, during the last 10 days of the dietary period, 0.15% lovastatin (Merck, Rahway, NJ) and 2% cholestyramine (Bristol-Myers Squibb, Princeton, NJ) were added to the low-cholesterol diets. Hence, the 0.01% cholesterol plus lipid-lowering drug diets and 0.1% cholesterol were designed to deplete (–C) and supplement (+C) cholesterol, respectively, in the animals with the intent to alter cholesterol homeostasis (coconut –C, olive –C, safflower –C, coconut +C, olive +C, and safflower +C).

After 12 weeks, hamsters were fasted for 16 hours and killed by CO<sub>2</sub> inhalation. Livers were removed, rinsed with phosphate-buffered saline, and divided into segments. A portion was immediately used for nuclear and membrane protein extraction, and the remaining segments were frozen in liquid nitrogen and stored at –80°C until analysis. The small intestine was removed and flushed with phosphate-buffered saline, and the jejunum was placed in RNAlater

(Qiagen, Valencia, CA) and stored at –80°C. The animal protocol was approved by the Institutional Animal Care and Use Committee of the Jean Mayer Human Nutrition Research on Aging, Tufts University.

## 2.2. Plasma lipid and lipoprotein analysis

Retroorbital blood was collected into EDTA-coated tubes from fasted hamsters (16 hours) under isoflurane anesthesia at 0-, 6-, and 12-week time points. Plasma was separated from red blood cells by centrifugation at 1500g for 20 minutes at 4°C. Plasma total cholesterol, HDL cholesterol, and triglyceride concentrations were determined on a Cobas Mira automated analyzer using enzymatic reagents (Roche Diagnostics, Indianapolis, IN). Non-HDL cholesterol was calculated as the difference between total cholesterol and HDL cholesterol. In addition, 4 plasma pools per diet group were created by combining plasma from 4 animals to be used for fast protein liquid chromatography (FPLC) analysis using 2 Superose 6 columns (Amersham Biosciences, Piscataway, NJ) as previously described [41,42]. The total cholesterol concentration of the FPLC fractions was measured using enzymatic reagents (Wako, Richmond, VA).

## 2.3. Liver lipid composition

Liver lipids were extracted [43]; and total cholesterol, free cholesterol, and triglyceride were determined using enzymatic reagents (Wako and Roche Diagnostics) [44]. Esterified cholesterol was calculated as the difference between total and free cholesterol. Delipidated liver tissue was digested in 1 N NaOH for the determination of protein using the bicinchoninic acid assay (Pierce, Rockford, IL).

## 2.4. Fatty acid profiles

Red blood cell membranes were prepared by washing the cells 3 times with 0.9% NaCl (pH 7.4). Fatty acid profiles of red blood cell membranes and the experimental diets were determined by gas chromatography as previously described [45]. Peaks of interest were identified by comparison with authentic standards (Nu-Chek Prep, Elysian, MN) and expressed as mole percentage of total fatty acids.

## 2.5. Cholesterol content of experimental diets

Lipid was extracted from freeze-dried aliquots of diet overnight, and total cholesterol was determined by gas chromatography as previously described [46].

## 2.6. Quantitative real-time polymerase chain reaction

Total RNA was extracted from a portion of liver and small intestine (jejunum) using the Qiagen RNeasy Mini kit. Added to the protocol was an on-column DNase digestion step to eliminate contamination with genomic DNA. RNA was reverse transcribed using SuperScript II reverse transcriptase with random hexamers according to the manufacturer's instructions (Invitrogen, Carlsbad, CA).

Primers for ACAT-2, apo A-I, apo B-100,  $\beta$ -actin, 7 $\alpha$ -hydroxylase, HMG-CoA reductase, LDL receptor, MTP, PPAR- $\alpha$ , and SREBP-2 were designed using Primer Express software (Applied Biosystems, Foster City, CA); and primer specificity and amplification efficiency were verified before use. Real-time polymerase chain reaction (PCR) was conducted in an Applied Biosystems 7300 Sequence detection system using SYBR green reagents (Applied Biosystems) with the appropriate primers (Table 3). Reaction conditions were 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Values were normalized using  $\beta$ -actin as an endogenous control.

### 2.7. Immunoblotting analysis

Freshly excised liver tissue from 2 hamsters was pooled, and nuclear and membrane proteins were extracted as described [15]. All buffers contained 24  $\mu$ g/mL Pefabloc, 5  $\mu$ g/mL pepstatin A, 10  $\mu$ g/mL leupeptin, 2  $\mu$ g/mL aprotinin, and 50  $\mu$ g/mL *N*-acetyllecylnorleucinal (reagents from Roche Diagnostics). Liver cell lysates were prepared by homogenizing liver tissue in 5 vol of buffer (25 mmol/L HEPES [pH 7.5], 1.5 mmol/L  $MgCl_2$ , 300 mmol/L NaCl, 5 mmol/L DTT, 1 mmol/L EDTA, 10% [vol/vol] glycerol, 0.5% [vol/vol] Triton X-100, 5  $\mu$ g/mL  $Na_3VO_4$ , 5  $\mu$ g/mL NaF [reagents from Sigma, St Louis, MO] and protease inhibitors [Roche Diagnostics]) followed by 1 hour of agitation at 4°C. Homogenates were then centrifuged, and supernatants were collected. Protein concentration in the nuclear and membrane fractions, and cell lysate were determined using the bicinchoninic acid assay.

Proteins from nuclear and membrane fractions (50  $\mu$ g) and cell lysates (40  $\mu$ g) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes using a wet

transfer system. Proteins were detected as previously described [48]. Relative protein levels were normalized to the density of  $\beta$ -actin.

### 2.8. Statistical analysis

Before statistical analysis, data were checked for normality; and appropriate transformations were performed when necessary. Differences between cholesterol-supplemented (+C) and cholesterol-depleted (–C) hamsters within a dietary fat group were assessed using an unpaired Students *t* test; and those among dietary fat groups, using analysis of variance. Tukey honestly significant difference test was used for post hoc analysis. Differences were considered significant at *P* ≤ .05. All statistical analyses were performed using SAS (Version 9.1; SAS Institute, Cary, NC).

## 3. Results

### 3.1. Plasma lipid and lipoprotein profiles

At baseline, there were no significant differences in the plasma lipid and lipoprotein concentrations among the 6 diet groups (Supplementary Table 1). By design, after 12 weeks of diet treatment, the cholesterol-depleted hamsters had significantly lower plasma total, non-HDL, and HDL cholesterol, and triglyceride concentrations than cholesterol-supplemented hamsters, regardless of dietary fat type (Fig. 1). Plasma FPLC patterns indicated that, in the cholesterol-depleted hamsters, the majority of cholesterol was carried on the HDL fraction, whereas, in the cholesterol-supplemented hamsters, the majority of cholesterol was distributed between the very low-density lipoprotein (VLDL) and HDL fractions, again regardless of dietary fat type (Fig. 2). Independent of cholesterol status, hamsters fed the coconut oil diets had significantly higher total and non-

Table 3  
Primers for quantitative real-time PCR

Gene	Forward primer	Reverse primer	Accession no./reference
ABCA1 <sup>a</sup>	ATAGCAGGCTCCAACCCTGAC	GGTACTGAAGCATGTTTCGATGTT	[47]
ABCG5 <sup>a</sup>	TGATTGGCAGCTATAATTTTGGG	GTTGGGCTGCGATGGAAA	[47]
ABCG8 <sup>a</sup>	TGCTGGCCATCATAGGGAG	TCCTGATTTCATCTTGCCACC	[47]
NPC1L1 <sup>a</sup>	CCTGACCTTTATAGAACTCACCACAGA	GGGCCAAAATGCTCGTCAT	[47]
SREBP-2 <sup>b</sup>	GCAAGGTGTTCTGTCATGAA	TGGTGTCTGACTGGTACGCC	GU12330
LDL receptor <sup>b</sup>	GCAGTGTTCCTGTGGCTGACAC	GCCATGCACAGGGTCCA	M94387
HMG-CoA reductase <sup>b</sup>	GAGCTACATTTGTGCTTGCGC	TTCATTAGGCCGAGGCTCAC	L00173
SR-BI <sup>b</sup>	AAGCTGCAGGTCTATGAAGC	AGAAACCTTCATTGGGTGGGTA	[48]
ACAT-T <sup>c</sup>	GGTGGAATTATGTGGCCAAGA	CATGTTGGCAAAGACAGGGAC	NM_153728
7 $\alpha$ -Hydroxylase <sup>b</sup>	CACTCTGCACCTTGAGGATGG	GGGTCTGGGTAGATTGCAGG	L04690
MTP <sup>b</sup>	ACATGCTGACCTTTGTGCGA	ACGGTCATAATTGTGGGCAAC	U14995
Apo A-I <sup>b</sup>	GGCGGGAGATGAACAAGGA	GGCGGTAAAGAGCCACTTCC	AF046919
Apo B-100 <sup>b</sup>	TGATTATCTGAATGCATCTGACTGG	TCCTTGGCATTGGCTACTTGT	AF176576
$\beta$ -Actin <sup>b</sup>	TGCTGTCCCTGTATGCCTCTG	AGGGAGAGCGTAGCCCTCAT	AJ312092
PPAR- $\alpha$ <sup>b</sup>	GGCCAATGGCATCCAAAATA	CCTTGGCGAATTCTGTGAGC	AJ555631

<sup>a</sup> Mouse sequence.

<sup>b</sup> Hamster sequence.

<sup>c</sup> Rat sequence.



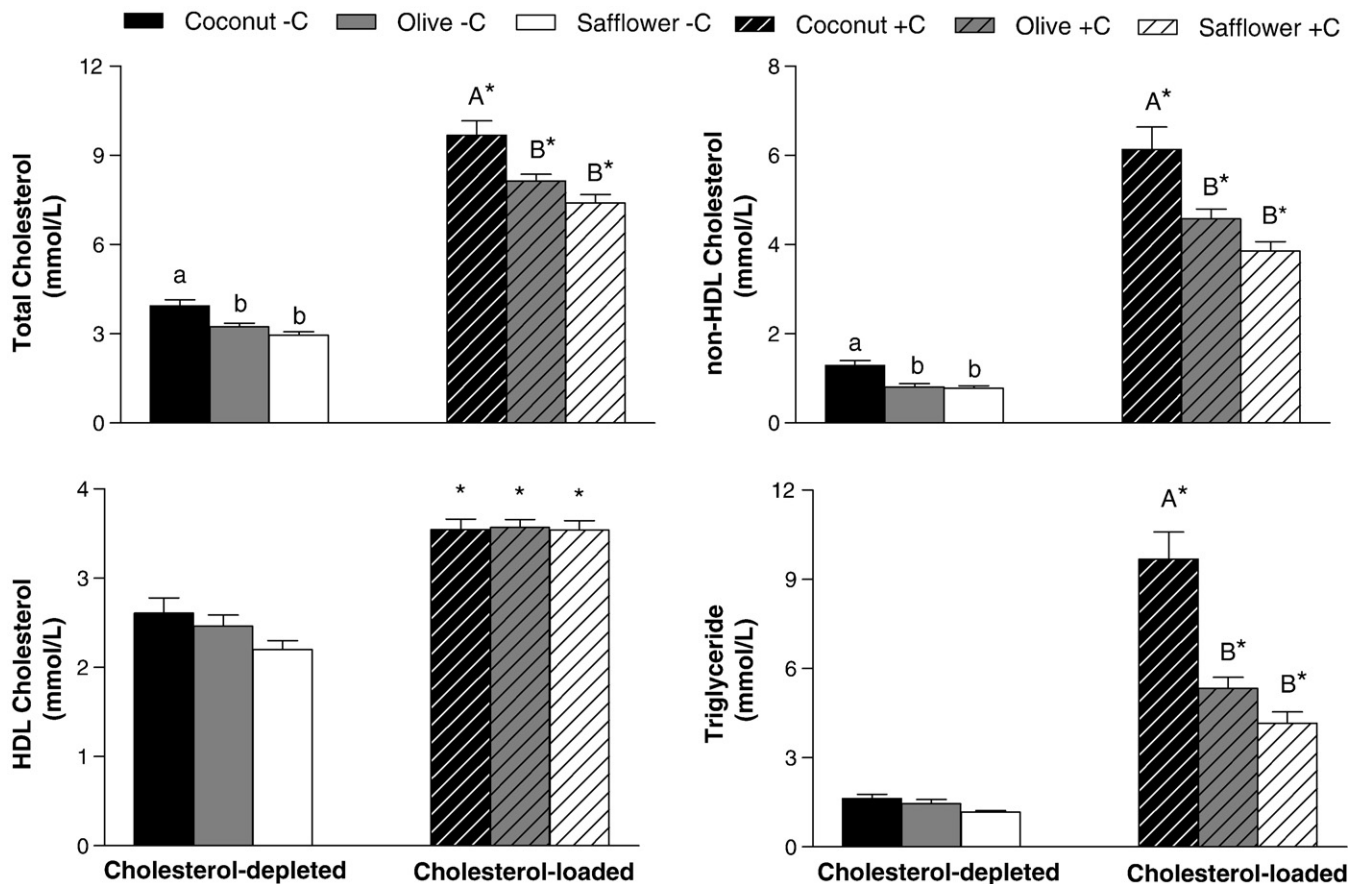


Fig. 1. Effect of cholesterol status and dietary fat type on fasting plasma lipid and lipoprotein cholesterol concentrations. Hamsters were fed diets enriched with coconut, olive, or safflower oil and 0.1% cholesterol (cholesterol supplemented, +C) or 0.01% cholesterol for 12 week plus 0.15% lovastatin and 2% cholestyramine 1 week before killing (cholesterol depleted, -C). Data represent means  $\pm$  SEM;  $n = 15$  to 16 animals per group. Appropriate transformations of the data (log HDL cholesterol; square root total cholesterol, non-HDL cholesterol; inverse triglyceride) were made before statistical analysis. Bars with different letters (lowercase for cholesterol depleted, uppercase for cholesterol supplemented) are significantly different,  $P \leq .05$ . Asterisks indicate significant differences between cholesterol-depleted and cholesterol-supplemented hamsters within a dietary fat treatment,  $P \leq .05$ .

HDL cholesterol concentrations compared with hamsters fed the olive and safflower oil diets.

### 3.2. Red blood cell fatty acid profile and hepatic lipid composition

Red blood cell membrane fatty acid profiles reflected the dietary fatty type (Supplementary Table 2). Differences among individual fatty acids on the basis of cholesterol status were modest and unlikely to be of biological significance. Hepatic liver weight, total cholesterol, and esterified cholesterol levels were significantly lower in cholesterol-depleted relative to cholesterol-supplemented hamsters among all dietary fat groups (Table 4). Interestingly, hepatic triglyceride levels were 2- to 2.8-fold higher in cholesterol-depleted relative to cholesterol-supplemented hamsters. Of note, in the cholesterol-supplemented but not cholesterol-depleted hamsters, hepatic esterified cholesterol levels were highest in the olive oil-fed, lowest in the coconut oil-fed, and intermediate in the safflower oil-fed

animals, with an almost 2-fold range of differences among dietary fat type groups.

### 3.3. Hepatic and intestinal mRNA levels

Regardless of dietary fat type, relative to cholesterol-supplemented hamsters, acute whole-body cholesterol depletion resulted in an up-regulation of hepatic mRNA levels of genes involved in cholesterol biosynthesis (HMG-CoA reductase, 34- to 65-fold), uptake (LDL receptor, 4.1- to 4.4-fold; SR-B1, 1.6- to 1.8-fold), metabolism ( $7\alpha$ -hydroxylase, 7.3- to 11.6-fold), and plasma transport (apo A-I, 1.7-fold) (Fig. 3A, B). Hepatic SREBP-2 mRNA levels were significantly higher (6- to 7.3-fold) in cholesterol-depleted relative to cholesterol-supplemented hamsters, consistent with its role in transcriptional regulation of the HMG-CoA reductase and LDL receptor genes. Cholesterol-depleted hamsters had modestly lower intestinal mRNA levels of the sterol transporters ABCA1 (1.5- to 2.6-fold), ABCG5 (1.4- to

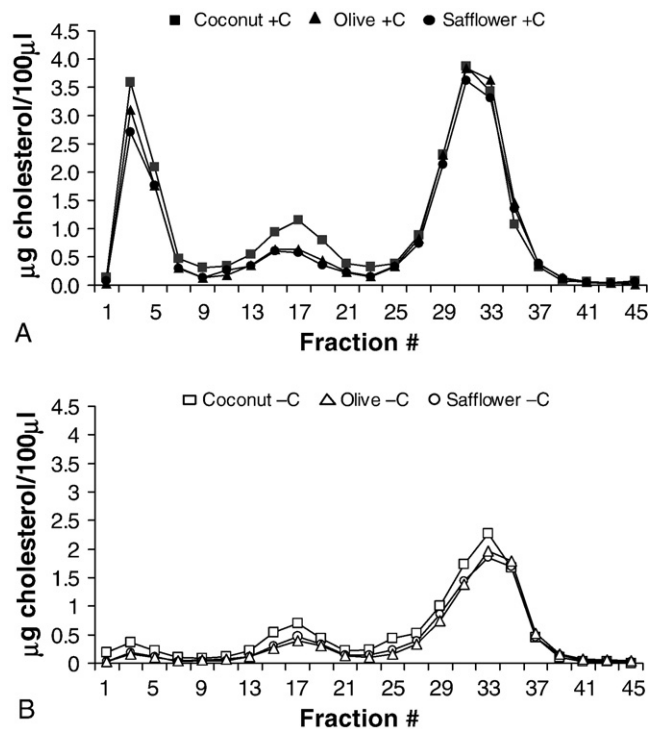


Fig. 2. Effect of cholesterol status and dietary fat type on plasma FPLC cholesterol profiles. Hamsters were fed diets enriched with coconut, olive, or safflower oil and 0.1% cholesterol (cholesterol supplemented, +C) (A) or 0.01% cholesterol for 12 weeks plus 0.15% lovastatin and 2% cholestyramine 1 week before killing (cholesterol depleted, -C) (B). Cholesterol concentrations were measured in odd-numbered fractions using standard enzymatic reagents. Data represent the mean of 4 plasma pools of 3 hamsters per pool.

2-fold) and ABCG8 (1.3- to 1.8-fold) relative to cholesterol-supplemented hamsters for all dietary fat types (Fig. 3C).

Consistent with this modest response of plasma lipid and lipoprotein concentrations to dietary fat type, there were only modest effects on message levels for the genes of interest. Cholesterol-supplemented hamsters fed coconut oil had significantly higher hepatic mRNA levels of hepatic SREBP-2 than hamsters fed olive or safflower oil (1.5- and 1.2-fold, respectively) (Fig. 3A). In contrast, cholesterol-depleted hamsters fed coconut oil had significantly higher hepatic mRNA levels of HMG-CoA reductase (2.2-fold) and lower levels of SR-B1 (1.4-fold) and ACAT-2 (1.3-fold) than hamsters fed safflower oil (Fig. 3A, B). Cholesterol-depleted hamsters fed coconut oil had modestly, albeit significantly, higher levels of SREBP-2 (1.4-fold) than both cholesterol-depleted hamsters fed olive and safflower oil. In addition, cholesterol-depleted hamsters fed safflower oil had modest but significantly higher levels of PPAR- $\alpha$  (1.7-fold) than cholesterol-depleted hamsters fed safflower oil. Intestinal NPC1L1 mRNA levels were similar on the basis of either cholesterol status or dietary fat type. Dietary fat type had no significant effect on intestinal mRNA levels of ABCA1, ABCG5, or ABCG8 (Fig. 3C).

### 3.4. Hepatic protein levels

Similar to the pattern observed for mRNA expression, differences in protein expression were primarily observed in response to cholesterol status rather than dietary fat type. Relative to cholesterol-supplemented hamsters, cholesterol-depleted hamsters had significantly higher hepatic levels of SR-B1 (2- to 5-fold) and lower levels of apo E (1.7- to 2-fold) (Fig. 4A, B and Supplementary Fig. 1). Cholesterol status had no significant effect on hepatic SREBP-2, PPAR- $\alpha$ , MTP, ACAT-2, apo B-100, or LDL receptor protein levels. Despite modest differences in both plasma lipid and lipoprotein concentrations, and hepatic lipid levels, in the animal model used for this work, there was no significant effect of dietary fat type on hepatic SREBP-2, PPAR- $\alpha$ , LDL receptor, SR-B1, apo A-I, apo B-100, apo E, MTP, or ACAT-2 protein levels (Fig. 4A, B).

## 4. Discussion

This work was designed to develop a novel experimental animal model for use to determine in multiple tissue systems, simultaneously, the mechanism(s) regulating circulating lipid and lipoprotein concentrations in response to changes in dietary fat type within the context of altered cholesterol status. The results indicate that FIB hamsters fed semipurified diets enriched with fats high in SFAs, MUFAs, and PUFAs were only modestly responsive to dietary fat type, despite dramatic differences in cholesterol status induced by a high-cholesterol diet or low-cholesterol

Table 4

Effects of cholesterol status and dietary fat type on liver lipid composition

	Coconut oil	Olive oil	Safflower oil
Liver weight	g		
-C	5.1 $\pm$ 0.2*	5.1 $\pm$ 0.1*	4.9 $\pm$ 0.1*
+C	7.3 $\pm$ 0.2	6.7 $\pm$ 0.3	7.1 $\pm$ 0.2
Total cholesterol	$\mu$ g/mg protein		
-C	23.8 $\pm$ 1.1*	27.4 $\pm$ 1.6*	24.3 $\pm$ 0.7*
+C	173.5 $\pm$ 10.0 <sup>C</sup>	326.3 $\pm$ 15.4 <sup>A</sup>	265.0 $\pm$ 24.4 <sup>B</sup>
Esterified cholesterol			
-C	1.8 $\pm$ 0.2*	3.7 $\pm$ 1.4*	2.6 $\pm$ 0.3*
+C	142.4 $\pm$ 8.9 <sup>C</sup>	267.0 $\pm$ 15.8 <sup>A</sup>	221.2 $\pm$ 24.2 <sup>B</sup>
Free cholesterol <sup>†</sup>			
-C	21.8 $\pm$ 1.3*	22.8 $\pm$ 1.9*	21.8 $\pm$ 0.6*
+C	33.3 $\pm$ 2.1 <sup>B</sup>	62.3 $\pm$ 6.5 <sup>A</sup>	43.8 $\pm$ 2.8 <sup>B</sup>
Triglyceride			
-C	126.3 $\pm$ 14.3*	140.9 $\pm$ 8.6*	107.5 $\pm$ 7.3*
+C	45.0 $\pm$ 3.5 <sup>B</sup>	68.8 $\pm$ 6.4 <sup>A</sup>	46.1 $\pm$ 3.1 <sup>B</sup>

Values are expressed as means  $\pm$  SEM; n = 15 to 16 animals per group. Means in a row without common letters (lowercase for -C [cholesterol depleted], uppercase for +C [cholesterol supplemented]) are significantly different,  $P \leq .05$ .

\* Significant differences between -C and +C hamsters within a dietary fat group,  $P \leq .05$ .

<sup>†</sup> Data were log-transformed before statistical analysis.

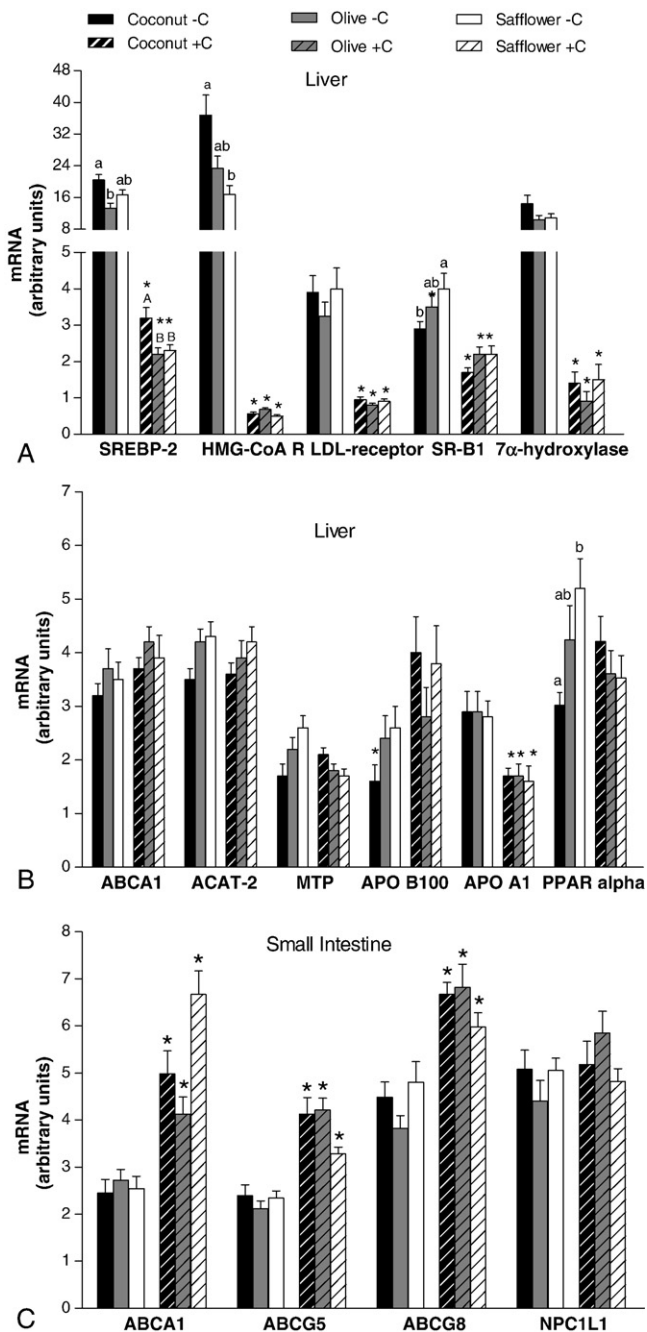


Fig. 3. Hepatic and small intestinal gene expression in response to alterations in cholesterol homeostasis and dietary fat type. Real-time PCR was used to measure gene expression in the liver (A and B) and small intestine (C). Values are expressed as mean  $\pm$  SEM;  $n = 14$  to 16 animals per group. Appropriate transformations of the data (log SREBP-2, CYP7A1, MTP, apo B-100, ABCA1, HMG-CoA R [HMG-CoA reductase]; square root SR-B1, SREBP-1c, apo A-I) were made before statistical analysis. Bars with different letters (lowercase for cholesterol depleted, -C; uppercase for cholesterol supplemented, +C) are significantly different,  $P \leq .05$ . Asterisks indicate significant differences between +C and -C hamsters within a dietary fat treatment,  $P \leq .05$ .

diet plus acute treatment with cholesterol-lowering drugs. Nonetheless, a number of findings shed light on the original intent of the work.

Part of the approach used to deplete whole-body cholesterol, acute treatment with cholestyramine, increased hepatic cholesterol demand for use in de novo bile acid synthesis. This was coupled with a drug that inhibited endogenous cholesterol synthesis. Elevated SR-B1 mRNA and protein levels facilitate the hepatic uptake of cholesterol from HDL, the preferred cholesterol source for bile acid biosynthesis [49,50]. Consistent with this sequence, we observed higher  $7\alpha$ -hydroxylase mRNA levels and lower circulating HDL cholesterol concentrations in the cholesterol-depleted hamsters. Both SR-B1 and  $7\alpha$ -hydroxylase are regulated by bile acids via pathways involving FXR, providing additional support for the coordinate regulation of SR-B1 activity and bile acid metabolism [51,52]. In

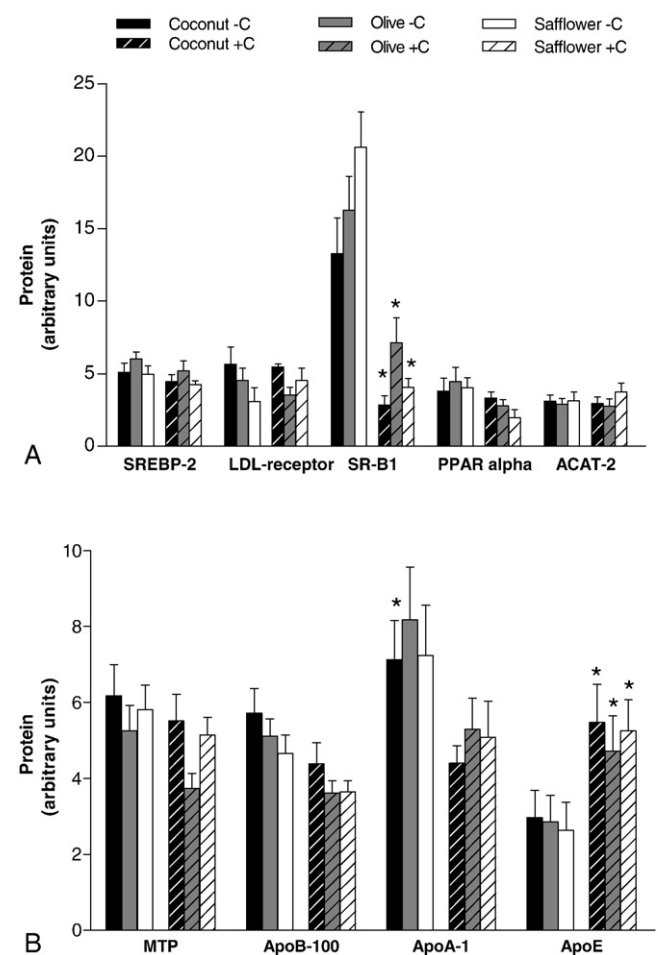


Fig. 4. Hepatic protein expression in response to alterations in cholesterol homeostasis and dietary fat type. Low-density lipoprotein receptor was detected in the membrane fraction; SREBP-2 was detected in the nuclear fraction; and SR-B1, ACAT-2, MTP, apo B-100, apo A-I, and apo E were detected in the cell lysate. Values are expressed as mean  $\pm$  SEM;  $n = 14$  to 16 animals per group. Appropriate transformations of the data (log apo A-I, apo E, LDL receptor, SREBP-1, SR-B1; square root apo B-100) were made before statistical analysis. Bars with different letters (lowercase for cholesterol depleted, -C; uppercase for cholesterol-supplemented, +C) are significantly different,  $P \leq .05$ . Asterisks indicate significant differences between -C and +C hamsters within a dietary fat group,  $P \leq .05$ .

contrast, hepatic MTP and ABCA1, also important regulators of hepatic intracellular cholesterol and lipoprotein metabolism, remained unaffected by cholesterol status of the hamsters.

Acute cholesterol depletion, compared with cholesterol supplementation, was also associated with higher hepatic apo A-I mRNA levels, yet lower plasma HDL cholesterol concentrations. Consistent with higher SR-B1 expression in the cholesterol-depleted hamsters, these data suggest an up-regulation of reverse cholesterol transport. Two-fold higher levels of hepatic apo A-I mRNA induced by simvastatin or cholestyramine have been reported in rats without a concomitant increase in plasma apo A-I concentrations [53]. Feeding bile acids to human apo A-I transgenic mice has also been shown to inhibit apo A-I expression via a pathway involving FXR [54]. Previous studies in F1B hamsters have shown an increase in HMG-CoA reductase activity in response to bile acid sequestrants, and this was associated with a decrease in hepatic cholesterol concentrations and increase in fecal bile acid excretion [55,56]. In addition, treatment with lovastatin has been shown to result in an up-regulation of HMG-CoA reductase mRNA levels in the F1B hamster [57].

Intestinal cholesterol absorption is one factor that modulates plasma cholesterol concentrations, and this mechanism has been exploited as a therapeutic target [33]. Intestinal ABCA1, ABCG5, and ABCG8 mRNA levels were significantly higher in cholesterol-supplemented compared with cholesterol-depleted hamsters, whereas NPC1L1 was unaffected. These data are consistent with those observed in other hamster studies [47]. ABCA1, ABCG5, and ABCG8 are regulated by the nuclear receptor LXR for which oxysterols are ligands [47,58–60]. Our work suggests that in vivo changes in hepatic cholesterol synthesis, uptake, and secretion induced by cholesterol status were more important modifiers of lipoprotein metabolism than changes in expression of intestinal cholesterol transporters.

Hepatic cholesterol levels are tightly regulated by the transcription factor SREBP-2. Consistent with this role, cholesterol depletion resulted in higher SREBP-2 mRNA levels relative to cholesterol supplementation. This in turn was associated with higher mRNA levels of 2 genes regulated by SREBP-2: HMG-CoA reductase and the LDL receptor [1]. A similar response has been reported in hamsters fed lovastatin and cholestyramine and is consistent with the cholesterol-depleted model used in our study [15,39]. Nonetheless, there was no significant effect of whole-body cholesterol status on nuclear SREBP-2 protein levels. This discrepancy may be attributable to the prandial state of the hamsters [61,62]. The ratio of nuclear to membrane SREBP-2 is indicative of proteolytic regulation [39]. However, because we were unable to detect membrane SREBP-2, we were unable to address this issue further.

Secondary to statin treatment, higher hepatic HMG-CoA reductase mRNA levels were observed in the cholesterol-depleted, relative to cholesterol-supplemented, hamsters, as

has been reported [63,64]. This is consistent with the transcriptional regulation of HMG-CoA reductase observed in hamsters in response to alterations in cholesterol homeostasis [65]. The modestly higher HMG-CoA reductase mRNA levels in cholesterol-depleted hamsters fed coconut oil, relative to olive or safflower oil, are likely the result of increased SREBP-2 transcription. These results suggest that, in this animal model, regulation of cholesterol synthesis is primarily at the level of transcription and enzyme activity [66]. Nonetheless, we cannot rule out the possibility that other pleiotropic effects on statin treatment influenced the outcomes observed.

The LDL receptor is the main route of LDL cholesterol uptake in both hamsters and humans and, thus, is a major determinant of plasma non-HDL cholesterol concentrations [6]. A 4-fold up-regulation of LDL receptor mRNA levels, but not protein levels, was observed in cholesterol-depleted, relative to the cholesterol-supplemented, hamsters. These differences were accompanied by lower plasma non-HDL cholesterol concentrations. A similar observation of increased LDL receptor mRNA levels and no difference in protein levels in response to cholesterol depletion has been reported in rats [63,65]. These data suggest an increased rate of receptor recycling [65].

The hamsters responded to different dietary fat types with modest alterations in circulating lipid and lipoprotein concentrations, and gene expression. Regardless of cholesterol status, coconut oil-fed hamsters had higher SREBP-2 mRNA levels than olive oil- or safflower oil-fed hamsters. Intracellular sterol levels regulate SREBP-2 expression and activity. Lower hepatic free cholesterol levels in hamsters fed coconut oil, relative to olive or safflower oil, may have been a reflection of these higher SREBP-2 mRNA levels [1]. This observation is consistent with the proposed mechanism by which SFAs increases SREBP-2 expression [19]. PPAR- $\gamma$  coactivator-1 $\beta$ , an activator of the SREBP family of transcription factors, is stimulated by SFAs and may have contributed to higher SREBP-2 mRNA levels observed in the coconut oil-fed hamsters [67].

Interestingly, cholesterol-supplemented hamsters fed the olive oil-enriched diet had the highest concentrations of hepatic esterified cholesterol levels. The ACAT-2 mRNA and protein levels were not altered by dietary fat type or cholesterol status. The preference of ACAT-2 for oleoyl-CoA may account for this observation [16,68–70]. These data are particularly striking in light of higher levels of hepatic esterified cholesterol, mainly cholesteryl oleate, that have been observed in African green monkeys and apo B-100 transgenic, LDLr $^{-/-}$  mice fed MUFA-enriched, relative to SFA- or n-6 PUFA-enriched, diets [70,71] and confirm the unanticipated effect of a dietary fat high in MUFAs.

Hepatic triglyceride levels were markedly higher and plasma triglyceride concentrations were lower in the cholesterol-depleted hamsters compared with cholesterol-supplemented hamsters. This unique finding in this animal model may be secondary due to insufficient hepatic



cholesterol, apo E, or apo B-100 levels to support VLDL formation and secretion [26,28,72–75].

Coconut oil was used as the experimental saturated fat rather than butter, a more common fat in the diet of humans, because it has been used extensively in prior hamster studies [76,77]. In the hamster, coconut oil has been reported to predominantly increase non-HDL cholesterol concentration, as was observed in the current study, and also has been demonstrated to induce a cytokine response [78–81]. Nonetheless, we observed only a modest response to changing the major dietary fat type with respect to plasma lipid and lipoprotein concentrations, less than anticipated [66,82–85].

In conclusion, the experimental hamster model system developed as part of this study to assess simultaneously in multiple tissue systems the mechanism(s) for differences in response to dietary fat type on circulating lipid and lipoprotein concentrations was not ideal because the hamsters responded only modestly to dietary fat type regardless of dramatic differences in cholesterol status. Nonetheless, this work demonstrated that lower plasma non-HDL cholesterol concentrations in cholesterol-depleted, relative to cholesterol-supplemented, hamsters was in part accounted for by increased expression of genes associated with hepatic cholesterol uptake (LDL receptor), metabolism ( $7\alpha$ -hydroxylase), and reverse cholesterol transport (SR-B1 and apo A-I). Lower hepatic apo E protein and cholesterol levels in cholesterol-depleted hamsters was associated with higher hepatic triglyceride levels and lower plasma non-HDL cholesterol and triglyceride concentrations, suggesting lower rates of de novo VLDL synthesis. Nonetheless, on the basis of the response to dietary fat type, the experimental animal model developed, in general, has limited value to study diet-induced atherosclerosis.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.metabol.2010.01.014](https://doi.org/10.1016/j.metabol.2010.01.014).

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