Saturated fat-induced changes in S_f 60–400 particle composition reduces uptake of LDL by HepG2 cells

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Abstract **The ability of human postprandial triacylglycerol-rich lipoproteins (TRLs), isolated after meals enriched in saturated fatty acids (SFAs), n-6 PUFAs, and MUFAs, to inhibit the uptake of ^{125}I-labeled LDL by the LDL receptor was investigated in HepG2 cells. Addition of TRLs resulted in a dose-dependent inhibition of heparin-releasable binding, cell-associated radioactivity, and degradation products of ^{125}I-labeled LDL ($P < 0.001$). SFA-rich Svedberg flotation rate (S_f) 60–400 resulted in significantly greater inhibition of cell-associated radioactivity than PUFA-rich particles ($P = 0.016$) and total uptake of ^{125}I-labeled LDL compared with PUFA- and MUFA-rich particles ($P < 0.02$). Normalization of the apolipoprotein (apo)E but not apoC-III content of the TRLs removed the effect of meal fatty acid composition, and addition of an anti-apoE antibody reversed the inhibitory effect of TRLs on the total uptake of ^{125}I-labeled LDL. Real time RT-PCR showed that the SFA-rich S_f 60–400 increased the expression of genes involved in hepatic lipid synthesis ($P < 0.05$) and decreased the expression of the LDL receptor-related protein 1 compared with MUFAs ($P = 0.008$).** **In conclusion, these findings suggest an alternative or additional mechanism whereby acute fat ingestion can influence LDL clearance via competitive apoE-dependent effects of TRL on the LDL receptor.**—Jackson, K. G., V. Maitin, D. S. Leake, P. Yaqoob, and C. M. Williams. **Saturated fat-induced changes in S_f 60–400 particle composition reduces uptake of LDL by HepG2 cells.** ***J. Lipid Res.*** **2006.** 47: **393–403.**

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The magnitude and duration of the postprandial triacylglycerol (TAG) response has been shown to play a role in the progression of coronary heart disease (1). The metabolic handling of triacylglycerol-rich lipoproteins (TRLs) is influenced by their apolipoprotein (apo) composition, in particular their apoE and apoC-III contents. ApoE plays a crucial role in mediating the hepatic recognition and receptor-mediated uptake of TRLs by the liver (2), whereas increased amounts of apoC-III on TRLs has been shown to impede their metabolism in the circulation (3). We have shown marked differences in the apolipoprotein composition of particles within two TRL fractions, Svedberg flotation rate (S_f) > 400 and S_f 60–400, in response to meals of different fatty acid composition. In particular, greater enrichment of TRLs with apoC-III and apoE was observed after a single meal containing predominantly saturated fatty acids (SFAs) compared with meals containing predominantly unsaturated fatty acids (4). Given the importance of apoC-III and apoE in the uptake and removal of these lipoproteins by the liver, the physiological significance of these diet-induced differences in determining the interaction of TRLs with the liver requires further investigation.

There have been some studies of the binding of radiolabeled TRLs (in particular fasting VLDL) to receptors on HepG2 cells (5–9) and more recently of their ability to compete with ^{125}I-labeled LDL for binding to LDL receptors (8, 10). A small number of studies have determined the hepatic uptake of LDL isolated after feeding of diets rich in walnuts (11) or fish oil (12, 13) by HepG2 cells, whereas the majority have used incubations of individual fatty acids/albumin complexes to determine the impact of different fatty acids on hepatic LDL receptor function (14–16). There is a paucity of data regarding the interaction of postprandial TRLs isolated after meals enriched in different fatty acids with HepG2 cells, despite the fact that these particles provide a major route for the uptake of dietary fatty acids by the liver.

In this investigation, we compared the ability of TRLs (S_f 60–400 and S_f 20–60), isolated from human plasma

Abbreviations: apo, apolipoprotein; FAOX, fatty acyl-coenzyme A oxidase; LRP1, low density lipoprotein receptor-related protein 1; MTP, microsomal triacylglycerol transfer protein; PGC-1β, peroxisome proliferator-activated receptor γ coactivator-1β; S1P, site 1 protease; SCAP, sterol-regulatory element binding protein cleavage-activating protein; S_f, Svedberg flotation rate; SFA, saturated fatty acid; SOAT, sterol *O*-acyltransferase; SREBP, sterol-regulatory element binding protein; TAG, triacylglycerol; TRL, triacylglycerol-rich lipoprotein.

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300–480 min after single meals enriched in SFAs, n-6 PUFAs, or MUFAs, to inhibit the binding and uptake of ^{125}I-labeled LDL to the LDL receptor. We also studied the impact of these human postprandial TRLs, coincubated with LDL, on the expression of genes involved in hepatic cholesterol homeostasis and lipid metabolism. The human hepatoma-derived cell line HepG2 was chosen in the present study because these cells have been reported to retain many normal hepatic metabolic functions, including expression of normal LDL receptors and internalization and metabolism of circulating lipoproteins (17). The findings support the view that competitive inhibition of the LDL receptor by TRLs is an apoE-dependent mechanism, which is accelerated in the presence of SFA-enriched TRLs.

MATERIALS AND METHODS

Subjects and plasma separation

The subject group, test meal composition, and postprandial study day are described in detail in a previous publication (4). Briefly, 10 normolipidemic middle-aged men [age (mean ± SD), 48 ± 9 years; body mass index (in kg/m^2), 25 ± 3; fasting TAG, 1.3 ± 0.4 mmol/l; cholesterol, 5.2 ± 0.7 mmol/l; and glucose, 5.7 ± 0.4 mmol/l] attended an investigation unit on three separate occasions. Blood samples were collected at fasting and 60, 180, 300, 360, and 480 min after test meals enriched in either SFAs (a mixture of palm oil and cocoa butter), n-6 PUFAs (safflower oil), or n-9 MUFAs (olive oil) were fed, and TRL fractions (S_f> 400, S_f 60–400, and S_f 20–60) were prepared by density gradient ultracentrifugation (18). The TAG, cholesterol, apoC-II, apoC-III, and apoE contents of the TRL in each lipoprotein fraction were characterized, and differences were observed among the SFA-, PUFA- and MUFA-enriched particles after the test meals (4).

In our in vitro investigations, the TRL fractions S_f 60–400 and S_f 20–60 were prepared from duplicate postprandial plasma samples collected 300–480 min after consumption of the test meals. These time points were chosen because we had observed a greater enrichment of the SFA-rich S_f 60–400 with apoE compared with the PUFA- and MUFA-rich particles in these later postprandial samples (4). In addition, to ensure a sufficient yield of apoB-containing lipoproteins in the S_f 60–400 and S_f 20–60 fractions, the 300, 360, and 480 min plasma samples were pooled before ultracentrifugation was performed. To protect the apolipoproteins in the TRL fractions from proteolytic cleavage, a preservative cocktail was added to all of the lipoprotein fractions to give a final concentration of 5% (v/v) (19). Before addition to cell cultures, lipoproteins were passed through PD-10 desalting columns (Amersham Biosciences, Chalfont St. Giles, UK) and further concentrated using Vivaspin concentrators (Sartorius Ltd., Epsom, UK). ApoB, apoC-III, and apoE were measured in the concentrated lipoprotein fractions using an ILAB 600 clinical chemistry analyzer (Instrumentation Laboratory, Warrington, UK) by turbidimetric immunoassay using kits supplied by Alpha Laboratories (Eastleigh, UK). ApoB-48 and apoB-100 were analyzed using specific ELISAs (4). TAG and cholesterol were measured using enzyme-based colorimetric kits supplied by Instrumentation Laboratory.

Cell culture and ^{125}I-labeled LDL uptake studies

HepG2 cells (European Collection of Cell Culture, Centre for Applied Microbiology and Research, Salisbury, UK) were maintained in complete medium: MEM (Sigma, Poole, UK) supplemented with 10% (v/v) FBS, 2 mM L-glutamine, 1% nonessential amino acids, penicillin (100 U/ml), and streptomycin (100 μg/ml) (Autogen Bioclear, Calne, UK).

Total LDL uptake, which is the sum of cell surface bound and internalized ^{125}I-labeled LDL and degradation products in the medium, was determined as described by Goldstein, Basu, and Brown (20) with a few modifications. Briefly, cells were grown to 80% confluence in 24-well plates, washed twice with MEM, and incubated in lipid-depleted medium in which the FBS was replaced by 10% (v/v) charcoal-stripped lipoprotein-deficient serum (First Link UK, Birmingham, UK) for 48 h to upregulate LDL receptors. LDL (1.019–1.063 g/ml) was isolated from fasting plasma and radiolabeled with ^{125}I using iodine monochloride as described previously (21). ^{125}I-labeled LDL, at a final concentration of 10 μg protein/ml, was added to the cell culture medium alone and simultaneously with a range of unlabeled S_f 60–400 and S_f 20–60 (2.5, 10, and 20 μg apoB/ml) for 5 h at 37°C. To investigate the effects of particle composition, initial experiments were controlled for apoB content to normalize for particle number. **Figure 1** shows typical results for *a*) unlabeled LDL (0–200 μg apoB/ml) and *b*) unlabeled S_f 60–400, S_f 20–60, and LDL (2.5, 10, and 20 μg apoB/ml) competing against ^{125}I-labeled LDL for binding and uptake by HepG2 cells. After the 5 h incubation, the medium was removed for measurement of the radioactive noniodide TCA-soluble degradation products, which are released into the medium after the lysosomal degradation of ^{125}I-labeled LDL (21). The cells were then washed three times with 1 ml of ice-cold PBS containing BSA (2 mg/ml) followed by two washes with 1 ml of PBS. The cells were then incubated with 1 ml of ice-cold buffer containing 50 mM NaCl, 10 mM Hepes (pH 7.4), and heparin (10 mg/ml) for 90 min at 4°C. The buffer solution was collected and a portion (0.9 ml) was counted for the amount of ^{125}I-labeled LDL released from the cell surface (heparin-releasable binding). The cells were washed with 1 ml of ice-cold PBS before the cells were lysed in 1.5 ml of 0.2 M NaOH by incubation at room temperature for 10 min. An aliquot of cell suspension (0.9 ml) was counted to determine the amount of ^{125}I-labeled LDL internalized by the cells (cell-associated radioactivity) and was analyzed for cell protein content by the modified Lowry procedure (22). All cell incubations were performed in triplicate with cell and cell-free wells, and all values were corrected for nonspecific heparin-releasable binding, cell-associated radioactivity, and degradation products in the cell-free wells.

The monoclonal antibody 1D7, which specifically blocks apoE-mediated binding to the LDL receptor (23), was obtained from the Ottawa Heart Institute Research Corporation (University of Ottawa, Canada). This antibody was incubated with the S_f 60–400 and S_f 20–60 fractions (1.75 μg antibody/μg lipoprotein apoB) for 16 h at 4°C before addition to the cells.

RNA extraction and cDNA synthesis

Trizol (Life Technologies, Paisley, UK) was used to isolate total RNA from HepG2 cells incubated with LDL (10 μg protein/ml) alone and simultaneously with S_f 60–400 or S_f 20–60 (20 μg apoB/ml) for 5 h at 37°C. cDNA was generated from 2.5 μg samples of total RNA at 42°C for 50 min (reaction volume, 20 μl) using oligo(dT) (Life Technologies) and RT (Superscript II; Life Technologies) using protocols recommended by the manufacturer.

Real-time RT-PCR

Specific primers for each gene (**Table 1**) were designed across an exon-exon junction from the published full-length mRNA sequences (www.ncbi.nlm.nih.gov) using Primer Express software

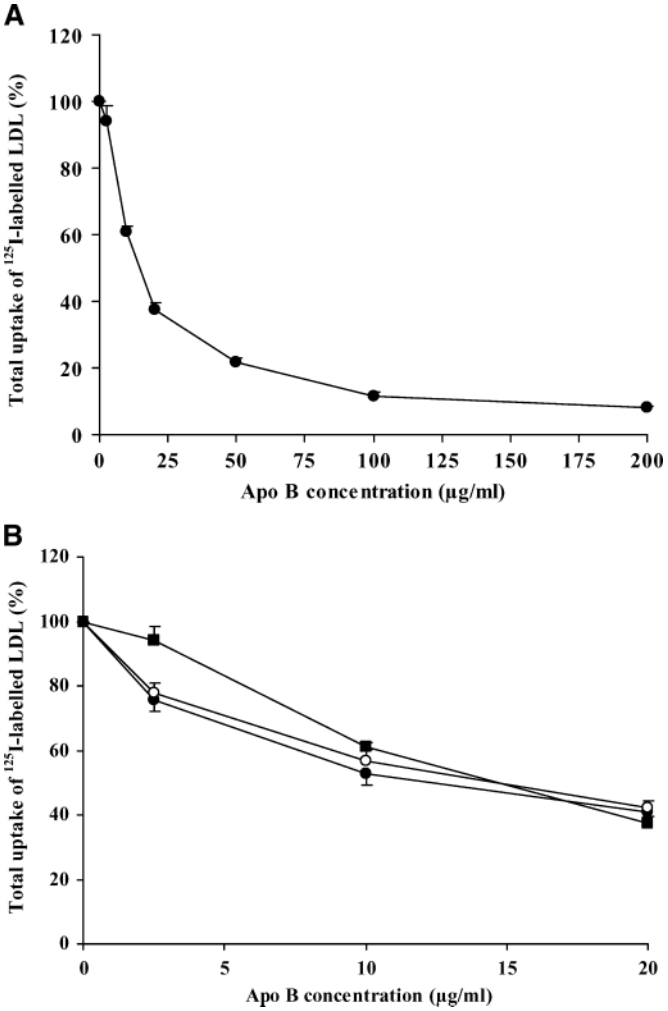

Fig. 1. Competition of unlabeled LDL [0–200 μg apolipoprotein B (apoB)/ml] (A) and unlabeled Svedberg flotation rate (S_f) 60–400 (closed circles), S_f 20–60 (open circles), and LDL (closed squares) (2.5, 10, and 20 μg apoB/ml) with ^{125}I-labeled LDL for binding and uptake by HepG2 cells (B). Cells were grown and LDL receptors were upregulated by incubation with lipid-depleted serum as described in Materials and Methods. S_f 60–400 and S_f 20–60 triacylglycerol-rich lipoprotein (TRL) samples were prepared from pooled postprandial samples collected after consumption of saturated, polyunsaturated, and monounsaturated fatty acid-rich meals. Lipoproteins were added to the cells in triplicate at the apoB concentrations shown, together with 10 μg protein/ml ^{125}I-labeled LDL, and incubated for 5 h at 37°C. Results are means of triplicate measurements ± SEM. The absolute value for the total uptake of the ^{125}I-labeled LDL in the absence of TRLs was 981 ± 46 ng/mg cell protein. Unlabeled S_f 60–400 and S_f 20–60 were shown to compete more effectively with ^{125}I-labeled LDL at 2.5 μg apoB/ml ($P < 0.03$) than did unlabeled LDL, but differences were not observed between the lipoproteins at 10 and 20 μg apoB/ml.

version 2 (Applied Biosystems, Warrington, Cheshire, UK). Real-time RT-PCR was performed using Absolute QPCR SYBR Green ROX Mix (Abgene, Epsom, UK) using a GeneAmp 5700 thermocycler (Applied Biosystems). The PCR was run for 15 min at 95°C followed by 40 cycles of 30 s at 94°C, 60 s at 55°C, and 30 s at 72°C. After amplification, melting curve analysis was performed to verify the specificity of the reactions. The end point used in the real-time RT-PCR quantification, Ct, was defined as the PCR cycle number at which each assay target passes the threshold, and the relative levels of gene expression were determined from the real-time PCR efficiencies using the Pfaffl method (24). The expression of each target gene was normalized to β-actin expression, and the data represent the fold change in mRNA expression relative to the control (HepG2 cells incubated with LDL alone), which is arbitrarily defined as 1.

Statistical analysis

Data were analyzed using SPSS version 11 (SPSS, Inc., Chicago, IL). Results are presented in the text and figures as means ± SEM. For the ^{125}I-labeled LDL uptake studies, data are expressed in **Fig. 2** as changes in heparin-releasable binding, cell-associated radioactivity, degradation products, and total uptake of ^{125}I-labeled LDL (ng/mg cell protein) in the absence of TRLs compared with the presence of increasing concentrations of S_f 60–400 and S_f 20–60. For incubation of the lipoprotein fractions with the anti-apoE monoclonal antibody, data are presented in **Fig. 3** as percentages of the total uptake of ^{125}I-labeled LDL in the absence of TRLs. The changes in the heparin-releasable binding, cell-associated radioactivity, degradation products, and total uptake of ^{125}I-labeled LDL determined over the concentration ranges of unlabeled TRLs were analyzed using a mixed-factor ANOVA, with apoB concentration as a within-subjects factor and meal composition as a between-subjects factor. A Tukey post hoc test was used for the detection of significant pairwise differences. The apoB-48 and apoB-100 concentrations, composition of the TRLs, and fold changes in mRNA gene expression were analyzed using a one-way ANOVA. The data were checked for normality and log-transformed where necessary to render their distribution normal before statistical analysis. Values of $P < 0.05$ were taken as significant.

RESULTS

Lipoprotein composition

The apoB-48 and apoB-100 concentrations of the pooled TRL samples isolated after consumption of the test meals are presented in **Table 2**. ApoB-48 concentrations in the S_f 60–400 fraction were higher after MUFA than after the SFA and PUFA meals ($P < 0.03$), with no differences observed in the apoB-100 concentrations between the meals. In the S_f 20–60 fraction, the apoB-100 concentrations were lower after PUFA than after the SFA and MUFA meals ($P < 0.02$). Similar apoB-48 concentrations were observed in this fraction between the meals.

Because each of the TRL fractions contains a mixture of apoB-48- and apoB-100-containing lipoproteins, the molarity of the apolipoproteins (apoC-III and apoE) and lipids (TAG and cholesterol) was divided by the molarity of total apoB in each lipoprotein fraction (apoB-48 and apoB-100 concentrations combined). Calculation of the lipid and apolipoprotein composition of the postprandial S_f 60–400 particles revealed the SFA-enriched particles to contain significantly more TAG, apoC-III, and apoE compared with MUFAs ($P < 0.006$) and significantly more apoE than PUFA-enriched TRLs ($P = 0.001$). In the S_f 20–60 fraction, the particles present in the circulation at 300–480 min after the SFA meal contained significantly more apoE than after the MUFA and PUFA meals ($P < 0.03$;

TABLE 1. Primer sequences of genes used for the relative quantification of mRNAs by real-time RT-PCR

Category	Gene	Accession Number	Sequences of the Forward and Reverse Primers (5′–3′)	Product Size
				bp
Housekeeping gene	β-Actin	X00351	CTACGTCGCCCTGGACTTCGAGC GATGGAGCCGCCGATCCACACGG	385
SREBP pathway	SREBP1	BC057388	CGACATCGAAGACATGCTTCAG GGAAGGCTTCAAGAGAGGAGC	180
	SREBP2	U02031	CAAGATGCACAAGTCTGGCG GCTTCAGCACCATGTTCTCCTG	101
	SCAP	BC027207	GCCATCCAGGAGTTCTGTCTC CATCCGGCGAATGTCAATG	105
	Site 1 protease	BC026330	CGTGTTAAACCTCAGCATCGG CCATAAAGAGGTCCGTCATTGC	126
	HMG-CoA reductase	BC033692	CTGTCATTCCAGCCAAGGTTG GTCCACAGGCAATGTAGATGG	169
	SOAT	BC028940	GCAAGGCGCTCTCTCTTAGATG GAACTCAAGCACCAGCCTTC	145
Cholesterol synthesis and uptake	LDL receptor	AY114155	TGGCTGCGTTAATGTGACACTC AGCCGATCTTAAGGTCATTGC	202
	LRP1	X13916	GAAGTAGCAGGACCAGAGGG CAGTACCCAGGCAGTTATGC	301
	LXRα	U22662	GATCGAGGTGATGCTTCTGGAG CCCTGCTTTGGCAAAGTCTTC	109
Fatty acid and TAG synthesis	FAOX	U07866	CCAGTCTGAAATCAAGCCAGG CACAAACTGGAAGGCATAGGC	109
	FAS	U26644	GACATCGTCCATTCGTTTGTG CGGATCACCTTCTTGAGCTCC	465
	GPAT	BC030783	CTGCAACTGAGACGAATTTGCC TCCGTCCATCTGGTGTTTCATC	216
	DGAT2	AF384161	CTGGAACACACCCAAGAAAGG GCAGGTTGTGTGTCTTCACC	113
	MCAD	AF251043	GAGGAGCCATTGATGTGTGC CCTCCGTTGGTTATCCACATC	137
VLDL synthesis and assembly	ApoB-100	J02610	AAGGACAAGGCCCAGAATCTGTACC GAATATACCTGGGACAGTACCGTCC	266
	MTP	X75500	CGAGTTCTGAAGGAAATGGTCG TAACCACCTGGCTACCGTGAAG	214
	PLTP	L26232	CTGTCCAGCATGACTATGGAC GACTCCAGTGCAGAATGGTTGG	122

ApoB-100, apolipoprotein B-100; DGAT2, diacylglycerol acyltransferase 2; FAOX, fatty acyl-coenzyme A oxidase; GPAT, glucose-3-phosphate acyltransferase; LRP1, low density lipoprotein receptor-related protein 1; LXRα, liver X receptor α; MCAD, medium chain acyl-coenzyme A dehydrogenase; MTP, microsomal triacylglycerol transfer protein; PLTP, phospholipid transfer protein; SCAP, SREBP cleavage activating protein; SOAT, sterol *O*-acyltransferase (also called ACAT); SREBP, sterol-regulatory element binding protein; TAG, triacylglycerol.

Table 2). TAG, cholesterol, and apoC-III contents were similar in the S_f 20–60 TRLs after the three test meals.

^{125}I-labeled LDL uptake studies

S_f 60–400 and S_f 20–60 TRL particles caused a dose-dependent reduction in heparin-releasable binding, cell-associated radioactivity, degradation products, and total uptake of ^{125}I-labeled LDL ($P < 0.001$; Fig. 2). There were significant apoB concentration-meal fat interactions for the cell-associated radioactivity ($P = 0.04$) and total uptake of ^{125}I-labeled LDL ($P = 0.02$) in the presence of the S_f 60–400 particles. The S_f 60–400 particles isolated after the SFA meal gave a significantly greater reduction in cell-associated radioactivity than the PUFA S_f 60–400 particles ($P = 0.016$; Fig. 2B) and reduced the total uptake of ^{125}I-labeled LDL significantly more than did PUFA and MUFA S_f 60–400 particles ($P < 0.02$; Fig. 2C). Although there were similar meal-induced trends for the S_f 20–60 fraction, none were significantly different from SFA.

These experiments were conducted with the addition of TRLs standardized for their apoB content (and therefore particle number). However, SFA-enriched TRLs contain higher amounts of apoE and apoC-III per particle, which may be responsible for the greater inhibition of ^{125}I-labeled LDL uptake. Therefore, the ability of TRLs to inhibit the uptake of ^{125}I-labeled LDL according to their *a*) apoB concentration (20 μg/ml), *b*) apoC-III concentration (2.2 μg/ml), and *c*) apoE concentration (0.6 μg/ml) was examined. Differences in total uptake of ^{125}I-labeled LDL according to meal fat composition were retained when the S_f 60–400 and S_f 20–60 particles were standardized for their apoB and apoC-III concentrations (Fig. 3). However, the meal fat-dependent differences between the S_f 60–400 and S_f 20–60 particles were no longer apparent when they were normalized for their apoE concentration (Fig. 3).

Addition of an anti-apoE monoclonal antibody to the incubates containing S_f 60–400 and S_f 20–60 fractions, matched for apoE concentration, resulted in a reversal

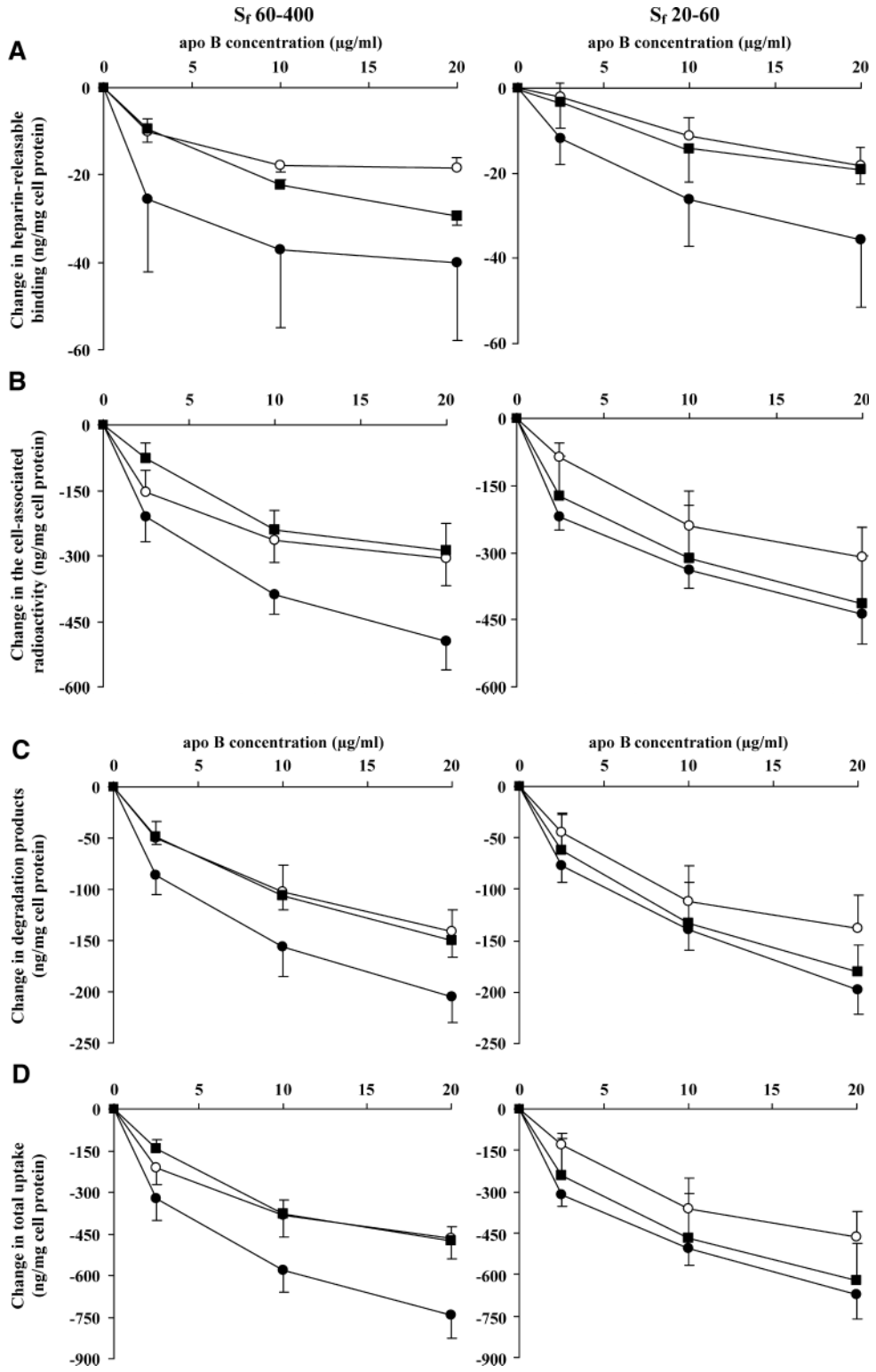

Fig. 2. Absolute changes in the heparin-releasable binding (A), cell-associated radioactivity (B), degradation products (C), and total uptake of ^{125}I-labeled LDL (D) during 5 h in the presence of S_f 60–400 (left panels) and S_f 20–60 (right panels) fractions after test meals enriched in saturated fatty acids (SFAs; closed circles), PUFAs (open circles), and MUFAs (closed squares). S_f 60–400 and S_f 20–60 fractions were added to the cells at 0–20 μg apoB/ml in lipid-depleted medium. Each experiment was performed in triplicate, and the results shown are means of five independent experiments ± SEM. The absolute values for the heparin-releasable binding, cell-associated radioactivity, degradation products, and total uptake of ^{125}I-labeled LDL in the absence of TRLs were 55.0 ± 9.2, 601.7 ± 47.3, 291.7 ± 25.4, and 951.1 ± 62.7 ng/mg cell protein, respectively. In the S_f 60–400 fraction, there were significant apoB concentration-meal fat type interactions for cell-associated radioactivity ($P = 0.04$) and total uptake of ^{125}I-labeled LDL ($P = 0.02$).

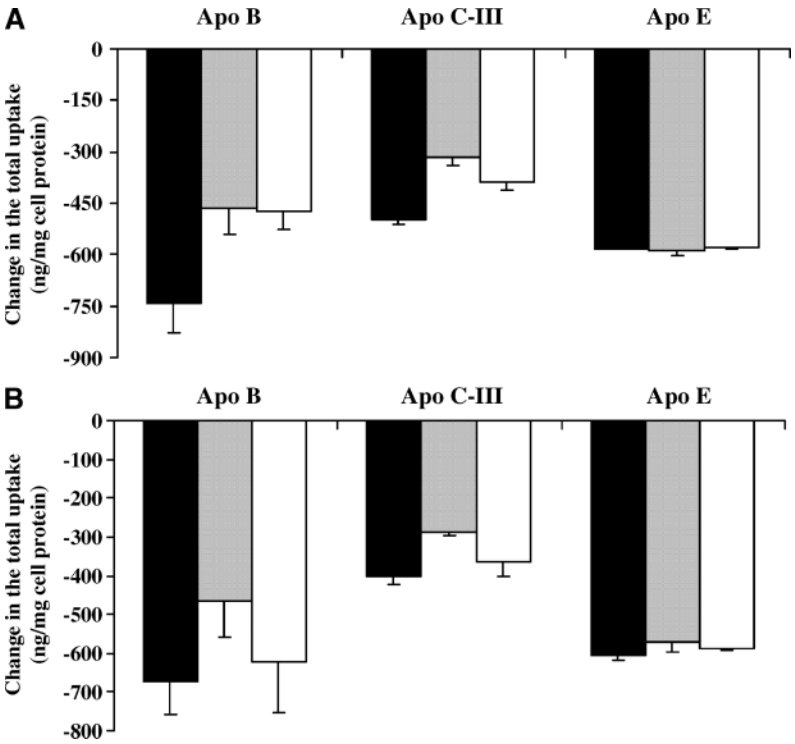

Fig. 3. Change in the total uptake of ^{125}I-labeled LDL compared with no TRL addition when the S_f 60–400 (A) and S_f 20–60 (B) fractions isolated after the SFAs (black bars), PUFAs (gray bars), and MUFAs (white bars) were added to the cells according to their apoB concentration (20 μg/ml), apoC-III concentration (2.2 μg/ml), and apoE concentration (0.6 μg/ml) in lipid-depleted medium. Each experiment was performed in triplicate; values for apoB represent means ± SEM for five independent experiments, and values for apoC-III and apoE represent one experiment. The absolute value for the total uptake of ^{125}I-labeled LDL in the absence of TRLs was 951.1 ± 55 ng/mg cell protein.

of the inhibition of the total uptake of ^{125}I-labeled LDL by TRLs, which was almost complete for S_f 60–400 and 45–75% for S_f 20–60 (**Fig. 4**).

Real-time RT-PCR

Figure 5 and **Table 3** show the fold changes of various mRNAs in HepG2 cells in the presence of LDL and postprandial TRLs (S_f 60–400 and S_f 20–60) isolated after meals enriched in SFAs, PUFAs, and MUFAs compared with LDL alone (control), which is designated as 1.

In the S_f 60–400 fraction, there was a consistent fatty acid-specific response pattern on the mRNA expression of sterol-regulatory element binding protein (SREBP) 1, sterol-regulatory element binding protein cleavage-activating protein (SCAP), site 1 protease (S1P), and sterol *O*-acyltransferase (SOAT; also known as ACAT), with greater mRNA expression in the presence of SFA-rich particles and to a lesser extent PUFA-rich particles compared with MUFA-rich particles (Fig. 5). The level of mRNA expression of SREBP1 ($P = 0.014$), SCAP ($P = 0.008$), and S1P ($P = 0.018$) was significantly different between the SFA- and MUFA-rich particles, and the expression of SOAT was different after the MUFA-rich particles compared with the SFA-rich ($P = 0.001$) and PUFA-rich ($P = 0.034$) particles. The mRNA expression of SOAT after SFA was also shown to be greater than after PUFA, although this difference did not reach statistical significance ($P = 0.08$). In addition, the expression of mRNA for the LDL receptor was significantly

TABLE 2. ApoB concentrations and lipid and apolipoprotein composition of the pooled S_f 60–400 and S_f 20–60 TAG-rich lipoproteins isolated 300–480 min after the SFA, PUFA, and MUFA meals

	Concentration		Molecules of Lipid and Apolipoprotein per apoB Particle			
Fraction	ApoB-48	ApoB-100	TAG	Cholesterol	ApoC-III	ApoE
	nmol/l					
S_f 60–400						
SFA	14.1 ± 2.0	41.8 ± 10.5	12,226 ± 818	3,953 ± 332	34.9 ± 3.9	3.0 ± 0.3
PUFA	14.2 ± 2.5	26.0 ± 4.5	10,704 ± 1177	3,300 ± 382	33.0 ± 4.5	2.0 ± 0.3[a]
MUFA	31.0 ± 6.0[b]	34.5 ± 3.8	8,961 ± 883[a]	3,098 ± 226	27.9 ± 3.9[c]	1.7 ± 0.2[a]
S_f 20–60						
SFA	13.2 ± 1.4	38.6 ± 5.4	4,765 ± 339	3,701 ± 292	14.7 ± 1.0	1.7 ± 0.2[d]
PUFA	15.1 ± 2.6	29.2 ± 3.9[e]	4,511 ± 374	3,410 ± 290	13.5 ± 1.7	1.4 ± 0.2
MUFA	16.5 ± 1.3	36.5 ± 3.6	4,318 ± 292	3,358 ± 180	13.7 ± 1.2	1.4 ± 0.1

S_f, Svedberg flotation rate; SFA, saturated fatty acid. Values are means ± SEM, n = 10. The statistically significant differences between the three test meals were calculated by one-way ANOVA and Tukey post hoc test.

[a] Compared with SFA ($P = 0.001$).
[b] Compared with SFA and PUFA ($P < 0.03$).
[c] Compared with SFA ($P = 0.005$).
[d] Compared with PUFA and MUFA ($P < 0.03$).
[e] Compared with SFA and MUFA ($P < 0.02$).

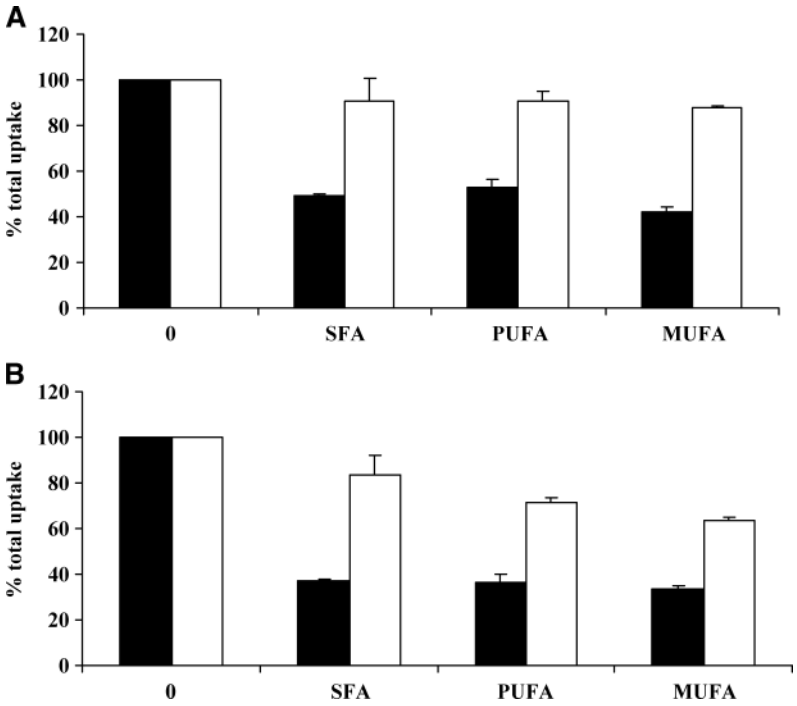

Fig. 4. Percentage total uptake of ^{125}I-labeled LDL when S_f 60–400 (0.65 μg apoE/ml) (A) and S_f 20–60 (0.75 μg apoE/ml) (B) fractions were incubated with the absence (black bars) and presence (white bars) of an anti-apoE monoclonal antibody before addition to the cells. Each experiment was performed in triplicate, and values represent means ± SEM for one experiment. The absolute value for the total uptake of the ^{125}I-labeled LDL in the absence of TRLs was 1,000 ± 48 ng/mg cell protein.

different in the presence of PUFA-rich than MUFA-rich TRLs ($P = 0.01$); relative to the control, the PUFA-rich particles showed a 43% increase and the MUFA-rich particles showed a 25% decrease in LDL receptor gene expression (Fig. 5). The mRNA expression for FAS was also significantly greater in the presence of PUFA-rich particles than SFA-rich ($P = 0.005$) and MUFA-rich ($P = 0.046$) particles. Although not statistically significant, the mRNA expression for apoB-100, SREBP2, HMG-CoA reductase, and microsomal triacylglycerol transfer protein (MTP) was in the order of SFA > PUFA > MUFA, and that for fatty acyl-coenzyme A oxidase (FAOX), glucose-3-phosphate acyltransferase, and diacylglycerol acyltransferase 2 was PUFA > SFA = MUFA (Table 3). There was a reduction in the mRNA expression of the low density lipoprotein receptor-related protein 1 (LRP1) after incubation of LDLs in the presence of each of the TRLs, which was significantly greater in the presence of SFA-rich than MUFA-rich particles ($P = 0.008$) (Fig. 5).

In the S_f 20–60 fraction, there were no significant effects of meal fatty acids on mRNA expression for genes involved in hepatic lipid metabolism (Fig. 5, Table 3). However, there was a tendency for the expression of SREBP2, SCAP, SOAT, and medium chain acyl-coenzyme A dehydrogenase to be SFA > PUFA and MUFA-rich TRLs. In contrast, mRNA expression of apoB-100, SREBP1, S1P, HMG-CoA reductase, LDL receptor, MTP, and FAOX showed a tendency to be lower in the presence of PUFA than MUFA-rich and SFA-rich S_f 20–60 particles (Fig. 5, Table 3).

DISCUSSION

This study has shown that consumption of a single SFA-rich meal influences the particle composition of the S_f 60–400 TRL, resulting in a greater inhibition of the total uptake of ^{125}I-labeled LDL (by ~70% at 20 μg apoB/ml) than particles isolated 300–480 min after PUFA or MUFA meals. The greater capacity of TRLs enriched with apoE to inhibit LDL binding and LDL uptake provides a potential mechanism by which the chronic consumption of dietary SFAs could contribute to greater increases in LDL cholesterol. That this is an apoE-dependent process is supported by the observation that matching TRL particle addition for the apoE concentration of the S_f 60–400 and S_f 20–60 particles removes the differences in LDL uptake according to TRL meal fatty acid. Addition of an anti-apoE monoclonal antibody also removes the differences in uptake according to TRL meal fatty acid and increases ^{125}I-labeled LDL uptake from ~45% to 75% of that in the absence of TRLs. These results demonstrate the importance of the presence and concentration of apoE on the surface of TRLs, in particular S_f 60–400 particles, in mediating their binding to the LDL receptor, supporting previous findings in the literature. In the case of the MUFA meal, the apoB content of the S_f 60–400 fraction contained significantly greater apoB-48 than the SFA and PUFA meals, and this suggests that at least some of the effects of dietary fats on circulating apoE concentrations could be attributable to alterations in the relative proportions of apoB-48 and apoB-100 particles. However, studies we performed to separate apoB-48- and apoB-100-containing lipoproteins (25) showed the apoE content of both the apoB-48- and apoB-100-containing lipoproteins to be in the order SFA > PUFA > MUFA. Although the impact of meal fatty acid composition on particle apoE content was greatest for the apoB-48-containing particles, the same trend (SFA > PUFA > MUFA) was observed for the apoB-100 particles. Overall, we concluded that for the PUFA meal, the reduction in

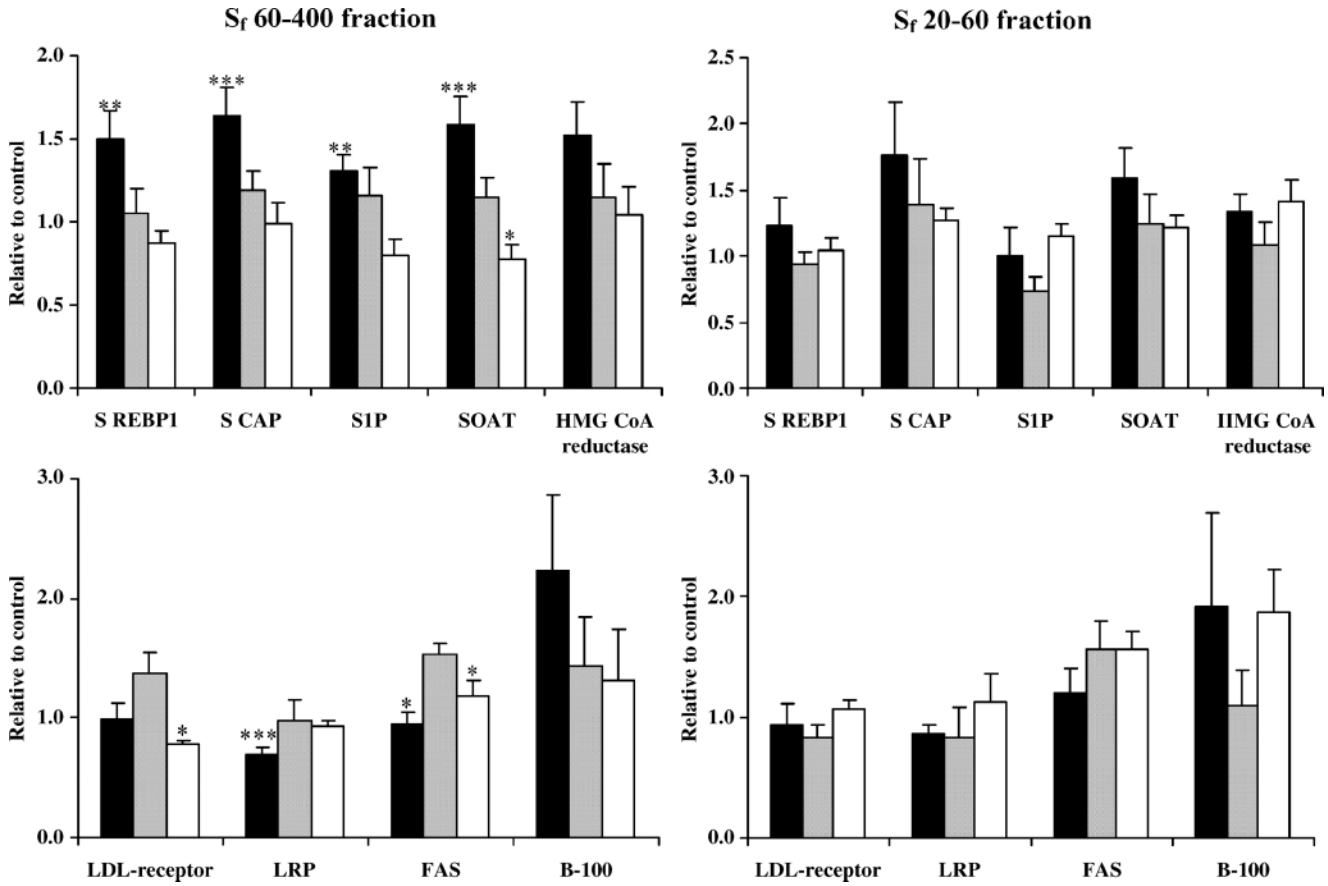

Fig. 5. Relative amounts of various mRNAs (normalized for β-actin) from HepG2 cells incubated with LDL (10 μg protein/ml) in the presence of S_f 60–400 (left panels) or S_f 20–60 (right panels) (both 20 μg apoB/ml) isolated after meals enriched in SFAs (black bars), PUFAs (gray bars), and MUFAs (white bars). Each value represents the amount of mRNA relative to the control (HepG2 cells incubated with LDL alone), which is arbitrarily set at 1. Each experiment was performed in triplicate, and values represent means ± SEM for five independent experiments. $^*P < 0.05$ compared with PUFA; $^{**}P < 0.05$, $^{***}P \leq 0.01$ compared with MUFA. LRP, low density lipoprotein receptor-related protein; S1P, site 1 protease; SCAP, sterol-regulatory element binding protein cleavage-activating protein; SOAT, sterol *O*-acyltransferase; SREBP1, sterol-regulatory element binding protein-1.

apoE largely reflected reduced particle content, whereas for MUFA, the reduction in apoE reflected both reduced particle content and a greater number of apoB-48-containing particles.

The majority of studies examining the binding and uptake of VLDL to the LDL receptor have been conducted using fibroblasts (26–30) and to a lesser extent HepG2 cells (5, 7, 9, 10) and HeLa cells (31). The general consensus is that apoE, and not apoB-100, mediates the binding of S_f 60–400 VLDL to the LDL receptor, with the greater degree of binding in the presence of hyperlipidemic than normolipidemic VLDL being attributable to the greater enrichment and correct conformation of apoE on the surface of these particles for hyperlipidemic subjects (26, 27). Addition of at least 1 mol of intact apoE to fasting normolipidemic VLDL and reconstituted TRL facilitated their binding to the LDL receptor in fibroblasts (26, 27, 32) and HeLa cells (31), respectively. In this study, postprandial SFA-rich S_f 60–400 particles were shown to be more enriched with apoE (3 mol) compared with the PUFA- and MUFA-rich particles (~2 mol). We have shown previously the postprandial apoE content of S_f 60–400 SFA-rich TRLs to increase 1.5-fold from fasting, whereas PUFA- and MUFA-rich particles do not show an increase in apoE content during the postprandial state (4). The greater inhibition of the total uptake of ^{125}I-labeled LDL in the presence of the SFA-rich S_f 60–400 suggests that the LDL receptor may play a more important role in the uptake of these TRLs compared with unsaturated fatty acid particles, an observation not previously reported in the literature. In the case of the S_f 20–60 fraction, the competition with ^{125}I-labeled LDL for specific binding to the surface of HepG2 cells (heparin-releasable binding) at 37°C appeared to be greatest in the presence of the SFA than the unsaturated S_f 20–60 particles, but there was no effect of TRL meal fatty acid on the total uptake of ^{125}I-labeled LDL by this fraction. It is possible that uptake of these smaller TRLs by the LDL receptor may be mostly dependent upon apoB-100 and not apoE, because in these smaller particles, apoB-100 is folded into the appropriate conformation that exposes the LDL receptor binding domain (33).

Although there is agreement between reports demonstrating the binding of VLDL to LDL receptors on fibro-

TABLE 3. Relative amounts of mRNAs from HepG2 cells incubated with ^{125}I-labeled LDL in the presence of S_f 60–400 and S_f 20–60 compared with values from ^{125}I-labeled LDL alone

Gene	SFA	PUFA	MUFA
S_f 60–400 fraction			
SREBP2	1.28 ± 0.07	1.13 ± 0.26	1.05 ± 0.15
LXRα	1.04 ± 0.17	1.06 ± 0.17	0.72 ± 0.05
FAOX	1.19 ± 0.19	1.35 ± 0.13	1.13 ± 0.26
GPAT	1.07 ± 0.13	1.17 ± 0.17	1.07 ± 0.13
DGAT2	1.01 ± 0.14	1.08 ± 0.09	0.84 ± 0.04
MCAD	1.12 ± 0.26	1.07 ± 0.05	1.14 ± 0.09
MTP	1.27 ± 0.07	1.22 ± 0.06	1.15 ± 0.13
PLTP	1.25 ± 0.39	0.90 ± 0.12	0.78 ± 0.13
S_f 20–60 fraction			
SREBP2	1.51 ± 0.20	1.21 ± 0.25	1.23 ± 0.17
LXRα	0.97 ± 0.18	0.74 ± 0.17	0.99 ± 0.05
FAOX	1.06 ± 0.11	1.04 ± 0.17	1.21 ± 0.11
GPAT	1.18 ± 0.10	1.14 ± 0.23	1.23 ± 0.09
DGAT2	1.02 ± 0.10	1.10 ± 0.18	0.98 ± 0.12
MCAD	1.40 ± 0.20	1.05 ± 0.04	1.07 ± 0.14
MTP	1.45 ± 0.15	1.34 ± 0.20	1.48 ± 0.13
PLTP	1.13 ± 0.26	1.12 ± 0.20	1.25 ± 0.17

Values represent amounts of mRNA (normalized for β-actin) relative to the control, which is arbitrarily defined as 1. Results are means of triplicate measurements ± SEM for five independent experiments.

blasts and HepG2 cells, there have been conflicting reports regarding the subsequent uptake of these particles by the receptor (34). LDL receptors have been shown to be small (molecular weight 115,000) relative to VLDL (molecular weight 8.9–24 × 10^6) and are clustered in clathrin-coated pits that constitute <2% of the surface area (29). It has been proposed that TRLs enriched in multiple copies of apoE could bind to more than one molecule of LDL receptor, leading to a significant reduction in the uptake of LDL (35). Björkegren and coworkers (30) have proposed that the greatest inhibition of LDL uptake by large VLDLs may be attributable to apoE binding to proteoglycans on the surface of fibroblasts, thereby blocking access, rather than binding directly to the LDL receptor itself. In this study, the SFA-rich S_f 60–400 particles were shown to be larger (i.e., greater TAG content) and to contain more apoE, supporting the possibility that these particles may bind to multiple LDL receptors and/or heparin sulfate proteoglycans, blocking the uptake of LDL by HepG2 cells. Although the present study demonstrates a clear apoE-dependent inhibition of LDL uptake by postprandial TRLs, we did not measure the uptake of the S_f 60–400 and S_f 20–60 particles themselves. Therefore, it cannot be conclusively determined that this competition results in greater uptake of competing TRLs into the hepatocytes, although the findings for the differential effects of TRLs on hepatic gene expression provide some support for this conclusion.

To determine whether there was any influence of TRL particle composition on the genes involved in hepatic lipoprotein metabolism, RNA was extracted from HepG2 cells after coincubation of LDLs with TRLs. The SFA-enriched S_f 60–400 particles significantly increased the expression of genes involved in the SREBP pathway (SREBP1, SCAP, and S1P) and cholesterol homeostasis (SOAT), more than in the presence of the MUFA-rich particles. Similar trends were observed for SREBP2, apoB-100, HMG-CoA reductase, and MTP. SREBP1 is a member of the SREBP family that regulates the transcription of genes involved in cholesterol metabolism and fatty acid synthesis. SREBPs are synthesized in a precursor form bound to the endoplasmic reticulum and are associated with SCAP, which senses the level of sterols (e.g., cholesterol) within the cell. In response to a decrease in cellular cholesterol content, specific proteases (e.g., S1P) cleave SREBP to release the N-terminal portion, which moves to the nucleus, where it binds to sterol response elements in the promoter region of genes involved in lipid synthesis. SREBP1, unlike SREBP2, is thought to increase the synthesis of fatty acids by enhancing the transcription of genes concerned with fat storage (e.g., FAOX and FAS), whereas SREBP2 stimulates genes involved with cholesterol homeostasis (e.g., LDL receptor and HMG-CoA reductase). However, minor involvement of SREBP1 in cholesterol metabolism has not been discounted (36).

Overall, the gene expression data show an upregulation of the SREBP pathway and SOAT in the presence of SFA-rich TRLs relative to the LDL control cells, with little effect on gene expression with PUFA- and MUFA-enriched TRLs. There are a number of possible explanations for these findings. One possibility suggested by the work of Björkegren and coworkers (30) is that apoE-enriched TRLs may bind to proteoglycans and block LDL uptake without promoting the uptake of TRLs, leading to a net depletion of intracellular cholesterol and upregulation of SREBP pathways. Greater upregulation of SREBP in cells incubated with SFA-rich TRLs may be attributable to the greater inhibition of LDL binding and uptake compared with unsaturated-rich TRLs. Alternatively, there may be hepatic uptake of TRLs via the LDL receptor, with subsequent effects on gene expression of the SREBP pathway reflecting the direct effects of the fatty acids released from TRLs after their uptake into the liver. Evidence from cell culture and animal studies has shown that fatty acids may regulate SREBPs differentially, with both oleic acid and PUFAs (including fish oil) being shown to suppress the expression of SREBP1 (37–39). However, very recent evidence in mice has shown that diets rich in SFAs may mediate their hyperlipidemic effects via peroxisome proliferator-activated receptor γ coactivator-1β (PGC-1β) coactivation of SREBP. PGC-1β expression was also shown to be greatly increased in cultured rat primary hepatocytes treated with individual SFAs, in particular after incubation with palmitic acid (40). In the present investigation, a mixture of palm oil and cocoa butter was used as the fat source in the SFA meal, in which the predominant SFA was palmitic acid (4). Because apoB-48-containing TRLs are preferentially internalized via LRP1 (41), the possibility should also be kept in mind that some of the differences we observed in gene expression reflect the different proportions of apoB-48-containing lipoproteins and their internalization via this receptor rather than the LDL receptor after the three meals.

Interestingly, of all the genes examined, only LRP1 mRNA expression was lower than that of the LDL control (i.e., downregulated) after incubation with SFA-rich TRL particles. The apoE-dependent receptor-mediated path-

ways on the liver include the LDL receptor and a second pathway that involves two cell surface receptors, the LRP1 and heparin sulfate proteoglycans, and by heparin sulfate proteoglycans alone (42). The S_f 60–400 TRLs are not a homogeneous fraction but contain a mixture of large VLDLs, small chylomicrons, and chylomicron remnants (43), and LRP1 is thought to play a significant role in the uptake of chylomicron remnants (41). A recent animal study that observed a slower uptake of chylomicron remnants enriched in SFAs by isolated rat hepatocytes compared with n-6 and n-3 PUFAs proposed that this was attributable to the influence of remnant fatty acid composition on the expression and/or activity of the LDL and LRP1 receptors (44).

In conclusion, although there are clearly influences of fat composition on hepatic metabolism and the expression of genes involved in cholesterol homeostasis that operate during chronic exposure (45, 46), very little is known about the regulation of these pathways after acute fat ingestion. A recent study has demonstrated the appearance of ingested fatty acids in an immunopurified VLDL fraction within 90 min of meal consumption (47), illustrating the potential for meal fatty acids to influence the composition and concentration of hepatically derived lipoproteins. This study provides support for acute effects of meal fatty acids on hepatic lipoprotein metabolism mediated via differential effects of TRLs on LDL receptor uptake, on gene expression pathways involved in hepatic cholesterol synthesis, and potentially on VLDL synthesis and secretion. In particular, this study demonstrates the metabolic implications of the postprandial enrichment of TRLs with apoE, which appears to be dependent on the meal fatty acid composition.

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