Long-Chain Polyunsaturated Fatty Acids Upregulate LDL Receptor Protein Expression in Fibroblasts and HepG2 Cells

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ABSTRACT The objective of this study was to investigate the effect of individual PUFAs on LDL receptor (LDLr) expression in human fibroblasts and HepG2 cells, and to evaluate whether acyl CoA:cholesterol acyltransferase (ACAT) and sterol regulatory element-binding protein 1 (SREBP-1) were involved in the regulation of LDLr expression by fatty acids. When fibroblasts and HepG2 cells were cultured with serum-free defined medium for 48 h, there was a 3- to 5-fold ($P < 0.05$) increase in LDLr protein and mRNA levels. Incubation of fibroblasts and HepG2 cells in serum-free medium supplemented with 25-hydroxycholesterol (25OH-cholesterol, 5 mg/L) for 24 h decreased LDLr protein and mRNA levels by 50–90% ($P < 0.05$). Arachidonic acid [AA, 20:4(n-6)], EPA [20:5(n-3)], and DHA [22:6(n-3)] antagonized the depression of LDLr gene expression by 25OH-cholesterol and increased LDLr protein abundance 1- to 3-fold ($P < 0.05$), but had no significant effects on LDLr mRNA levels. Oleic (18:1), linoleic (18:2), and α-linolenic acids [18:3(n-3)] did not significantly affect LDLr expression. ACAT inhibitor (58–035, 1 mg/L) attenuated the regulatory effect of AA on LDLr protein abundance by ~40% ($P < 0.05$), but did not modify the regulatory effects of other unsaturated fatty acids in HepG2 cells. The present results suggest that AA, EPA, and DHA increase LDLr protein levels, and that ACAT plays a role in modulating the effects of AA on LDLr protein levels. Furthermore, the effects of the fatty acids appeared to be independent of any change in SREBP-1 protein. J. Nutr. 135: 2541–2545, 2005.

KEY WORDS: • *fatty acids* • *LDL receptor protein* • *LDL receptor mRNA*

Numerous studies showed that individual fatty acids have remarkably different cholesterolemic effects (1). However, the biological mechanisms by which these different fatty acids exert their diverse effects on plasma LDL cholesterol levels remain unclear. The findings from in vivo studies are controversial. For example, some animal studies showed that SFAs decrease LFL receptor (LDLr)[2] activity, LDLr protein, and mRNA abundance, whereas unsaturated fatty acids have an opposite effect (2–4). Other animal studies, however, showed that fatty acids did not alter LDLr protein and mRNA levels (5,6). In this context, studies with cultured cells offer an advantage in that the effects of specific fatty acids on LDLr gene expression can be evaluated in the presence or absence of cholesterol. Few studies examined the effects of individual fatty acids on LDLr gene expression in cell culture models (7–10). Similar to results from in vivo studies, the findings from cell culture studies also were inconsistent. An earlier study in our laboratory showed that conjugated linoleic acid upregulated LDLr gene expression, but linoleic acid did not (7). Two studies found that palmitate and oleate decreased (8) and increased (9) LDLr binding activity, respectively, without altering LDLr mRNA levels in HepG2 cells. Another study, however, reported that LDL enriched in n-3 fatty acids depressed LDLr activity and mRNA levels (10). Thus, our understanding of how individual fatty acids regulate plasma LDL cholesterol and LDLr expression remains unclear.

Animal studies demonstrated that dietary fatty acids and cholesterol regulate hepatic LDLr activity via a cholesteryl ester (CE) and the free cholesterol regulatory pool (8). This cholesterol regulatory pool is affected by acyl CoA:cholesterol acyltransferase (ACAT), which catalyzes esterification of CE to different fatty acids. It was suggested that cholesterol-raising SFAs suppress the CE pool and downregulate LDLr activity. In contrast, unsaturated fatty acids increase ACAT activity, enhance the esterification of free cholesterol to form CE, and stimulate LDLr activity (11). However, it is not clear whether and how ACAT and cholesterol are involved in the regulation of LDLr expression by fatty acids.

Although little is known about fatty acid regulatory effects on LDLr expression, it is well established that sterols exert their effects via a sterol regulatory element 1 (SRE-1) in the LDLr gene promoter. SRE-binding proteins (SREBP) bind to SRE to activate LDLr gene transcription in sterol-depleted cells (12). SREBP-1 is synthesized as 125-kDa precursor proteins that are cleaved by specific proteases to release a mature SREBP-1 (~68 kDa) when cells are depleted of sterols (13).

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[2] Abbreviations used: ACAT, acyl CoA:cholesterol acyltransferase; BSA, bovine serum albumin; CE, cholesteryl ester; Ctrl, control; FBS, fetal bovine serum; LDLr, LDL receptor; 25OH-cholesterol, 25-hydroxycholesterol; TC, total cholesterol.

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The mature SREBP-1 enters the nucleus and activates transcription of the LDLr gene. When sterols accumulate within cells, LDLr gene expression is repressed.

The present study was conducted to examine how the predominant individual unsaturated fatty acids in the diet affect LDLr gene expression in fibroblast and HepG2 cells. The mechanisms by which individual fatty acids exert their regulatory effects also were evaluated. Specifically, an ACAT inhibitor (58–035) was added to the culture medium to examine whether suppression of ACAT activity would change the cellular CE pool, thereby modulating the regulatory effects of cholesterol and fatty acids on LDLr protein and mRNA abundance. In addition, the effects of fatty acids on SREBP-1 abundance in cultured cells were examined to determine whether SREBP-1 is involved in the regulation of LDLr expression by fatty acids.

MATERIALS AND METHODS

Cell culture and treatments. Human skin fibroblast cells were maintained in DMEM containing 10% fetal bovine serum (FBS) under 10% CO_2 at 37°C in 10-cm dishes. HepG2 cells were maintained in DMEM plus 10% FBS supplemented with 0.1 mmol/L nonessential amino acids and 1 mmol/L sodium pyruvate under 10% CO_2 at 37°C in 10-cm dishes. When the cells were 60–70% confluent, the maintenance medium was removed and cells were treated with the defined DMEM [1.5% bovine serum albumin (BSA), 5 mg/L transferrin, 2 μg/L selenium, 10 μg/L α-tocopherol, and 1 nmol/L triiodothyronine] for 24 h to upregulate LDLr expression and then treated with 25-hydroxycholesterol (25OH-cholesterol; 2 or 5 mg/L) and/or fatty acids (0.4 mmol/L) for another 24 h.

Fatty acids were added to the culture medium as a fatty acid-BSA complex as previously described (7). Briefly, unsaturated fatty acids, e.g., 18:1, 18:2, α18:3, 20:4(n-6), 20:5(n-3), and 22:6(n-3) were dissolved in the serum-free defined DMEM containing 1.5% BSA. After fatty acids were dissolved, the solution was filtered using a 0.2-μm filter. Fatty acid concentration in DMEM was verified using GC analysis. The fatty acids were from Sigma Chemical.

Fatty acid-BSA complexes were added to culture dishes at a concentration of 0.4 mmol/L of fatty acid and 1.5% BSA (FA: BSA = 2:1 mol/L ratio) with or without 25OH-cholesterol (2 mg/L for fibroblasts and 5 mg/L for HepG2 cells) in 0.05% ethanol. Control cells were treated with the defined DMEM (Ctrl). ACAT inhibitor (1 mg/L, 58–035, kindly provided by Sandoz) was added to the medium to determine whether fatty acid regulation of LDLr gene expression was due to a change in the CE:free cholesterol pool. The ACAT inhibitor, 58–035, was used because it was shown to strongly inhibit ACAT activity (9). Cells were cultured for 24 h with test fatty acids with or without 25OH-cholesterol and with or without the ACAT inhibitor.

Western blot analysis of LDLr and SREBP-1. Western blot analysis was performed as previously described (7). Fibroblasts or HepG2 Cells were washed 3 times with PBS at 4°C and harvested. Cell lysates (25 μg) were separated on a 7.5% SDS-PAGE gel and then transferred to a nitrocellulose membrane. LDLr was detected with anti-LDLr IgG (generously provided by Dr. Allen Cooper, Stanford University, Stanford, CA) using the ECL-Western blotting protocol (Amersham Life Sciences). A polyclonal antibody against SREBP-1 (K10, 1:500, Santa Cruz Biotechnology) was used to detect precursor and mature SREBP-1. Signals were quantified using the ImageQuant Image Analysis Software (Amersham Biosciences).

Northern blot analysis of LDLr mRNA levels. LDLr mRNA expression was measured by Northern Blot analysis as described by Mustad et al. (4). Total RNA (15 μg) was separated on a 1% agarose gel and transferred onto a Genescreen membrane. The blot was probed with ^{32}P-labeled human LDLr cDNA (1.2 kb cDNA was generously provided by Dr. Allen Cooper) and then exposed to a Kodak X-Omat AR film at –70°C. To assess variation in loading or transfer of RNA, the blot was reprobed with a cDNA for 18S rRNA. The blots were scanned using the ImageQuant Image Analysis Software and normalized using the rat 18S rRNA to account for differences in loading.

Quantification of cellular CEs, total cholesterol (TC), and cellular fatty acids. After the treatment period, cells were washed 3 times with PBS and collected. Cell lipids were extracted overnight using hexane:isopropanol (3:2). β-Sitosterol (10 μg) was added as an internal standard. Samples were split into 2 equal fractions and dried for subsequent free cholesterol and TC measurements (9). Cellular cholesterol was analyzed using a Hewlett Packard 5890 gas chromatograph equipped with a Supelco SE-30 capillary column with helium carrier flow rate at 30 mL/min, hydrogen 30 mL/min, and air 300 mL/min. Cellular fatty acids were measured as previously described (7,14).

Statistical analysis. Data were analyzed using SAS statistical analysis computer program (version 6.12; SAS Institute) and expressed as means ± SEM. Three-way ANOVA was performed to determine treatment the effects of 25OH-cholesterol, fatty acids, and the ACAT inhibitor. When there was a significant main effect of treatment, a *t* test (least significant differences) was used for the mean comparisons (e.g., with and without 25OH-cholesterol or with and without ACAT inhibitor comparisons within fatty acids). Student's *t* test was used to determine the differences in fatty acid composition and CE:TC ratios between treatments. Differences were considered significant at $P < 0.05$.

RESULTS

Fatty acid profiles after treatments. To confirm that fatty acids in the culture medium were incorporated into cell lipids, we measured the fatty acid profiles in both fibroblasts and HepG2 cells after exposure to the respective fatty acid. The fatty acid profiles of fibroblasts and HepG2 cells are summarized in **Tables 1** and **2**. After each fatty acid treatment, the percentage of the target fatty acid increased ~30–40% ($P < 0.05$) in fibroblasts and 15–25% ($P < 0.05$) in HepG2 cells compared with the control. These results suggested that the targeted fatty acid was incorporated into cell lipids.

Regulation of LDLr abundance by fatty acids. We studied the effects of individual long-chain fatty acids on LDLr protein abundance in fibroblasts and HepG2 cells by Western blotting analysis. Compared with fibroblasts cultured with a growth medium containing 10% FBS, LDLr protein was increased 3- to 5-fold in control cells cultured in the defined medium (Ctrl, **Fig. 1***A*). LDLr protein abundance decreased by ~80% ($P < 0.05$) in fibroblasts treated with 25OH-cholesterol only (Fig. 1). All fatty acid plus 25OH-cholesterol treatments had lower LDLr protein levels than the respective fatty acid only treatments ($P < 0.05$). In the presence of 25OH-cholesterol, AA, EPA, and DHA, but not 18:1, 18:2, or 18:3 upregulated LDLr protein levels 1- to 3-fold ($P < 0.05$) in fibroblasts compared with control cells treated with 25OH-cholesterol only (Fig. 1*B*). We also examined the effects of long-chain unsaturated fatty acids on LDLr protein levels in HepG2 cells. The findings from HepG2 cells were similar to those observed for fibroblasts (data not shown). When HepG2 cells were cultured with AA, EPA, or DHA, there was a significant attenuation of the suppressive effects of 25OH-cholesterol on LDLr protein (i.e., LDLr protein increased 1- to 3-fold compared with 25OH-cholesterol only treatment).

The present study also showed that 25OH-cholesterol decreased the abundance of LDLr in a dose-responsive manner over a dose range of 0–5 mg/L in fibroblasts (**Fig. 2**). The addition of 18:2 did not modify the effect of 25OH-cholesterol on LDLr protein abundance, whereas 20:4(n-6) (AA) antagonized the suppressive effects of 25OH-cholesterol on LDLr levels (Fig. 2).

The addition of the ACAT inhibitor (58–035, 1 mg/L) reduced the stimulatory effect of AA (20:4n-6) on LDLr

TABLE 1

Fatty acid profiles in fibroblasts treated with the defined DMEM for 24 h and then treated with 25OH-cholesterol (2 mg/L) and/or fatty acids (0.4 mmol/L) for another 24 h[1]

	Treatment							
Fatty acid	Control	25OH-cholesterol	18:1	18:2	18:3	AA	EPA	DHA
	% of total fatty acids							
14:0	0.9 ± 0.1	1.0 ± 0.3	0.6 ± 0.2	0.9 ± 0.5	trace	0.5 ± 0.1	1.4 ± 0.4	—
16:0	27.9 ± 0.7	25.9 ± 1.1	12.1 ± 0.8	19.6 ± 2.1	15.4 ± 0.9	14.6 ± 0.8	22.9 ± 1.4	16.9 ± 1.7
18:0	29.4 ± 0.8	29.2 ± 1.1	15.0 ± 1.9	24.2 ± 2.7	21.2 ± 1.9	16.2 ± 0.4	25.6 ± 1.4	18.3 ± 1.3
18:1	24.7 ± 0.5	28.6 ± 1.3	64.8 ± 2.0*	13.5 ± 3.3	11.3 ± 1.3	11.1 ± 0.5	16.3 ± 0.3	12.8 ± 0.2
18:2(n-6)	0.7 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	36.3 ± 5.2*	0.6 ± 0.1	0.7 ± 0.1	trace	trace
18:3(n-3)	trace	trace	trace	trace	40.1 ± 1.6*	trace	trace	trace
20:4(n-6)	16.4 ± 0.2	14.9 ± 0.3	7.3 ± 0.4	5.5 ± 1.8	9.0 ± 0.8	57.0 ± 0.3*	1.7 ± 0.6	5.5 ± 0.6
20:5+22:5(n-3)	trace	trace	trace	trace	trace	trace	32.1 ± 1.9*	trace
22:6(n-3)	trace	trace	trace	trace	trace	trace	trace	46.4 ± 3.6*

[1] Values are means ± SEM, $n = 3$. * Different from control, $P < 0.05$.

protein abundance by ~40% ($P < 0.05$) in HepG2 cells (Fig. 2). However, the ACAT inhibitor did not significantly affect the regulation of LDLr protein by the other fatty acids examined.

Regulation of LDLr mRNA levels by fatty acids. Changes in LDLr mRNA abundance were not as large as the changes in LDLr protein after the long-chain fatty acid treatments. Compared with the control, 25OH-cholesterol decreased LDLr mRNA by 70% ($P < 0.05$) (**Fig. 3**). All fatty acid plus 25OH-cholesterol treatments had lower LDLr mRNA levels compared with the respective fatty acid only treatments ($P < 0.05$). The fatty acids examined in the present study (AA, EPA, DHA, 18:1, 18:2, and 18:3) did not significantly antagonize the suppressive effects of 25OH-cholesterol on LDLr mRNA abundance (Fig. 3). The LDLr mRNA levels did not differ between fatty acid plus 25OH-cholesterol treatments and 25OH-cholesterol only treatments.

CE:TC ratios after treatments. Because cholesterol status in cells affects LDLr expression, we measured TC and free cholesterol levels in HepG2 cells after fatty acid plus 25OH-cholesterol treatments supplemented or not with the ACAT inhibitor. We observed a decrease in the cellular CE:TC ratio in HepG2 cells treated with 18:1, AA, and EPA plus 25OH-cholesterol supplemented with the ACAT inhibitor compared with the corresponding fatty acid treatments without the ACAT inhibitor (**Table 3**, $P < 0.05$).

SREBP-1 abundance after treatments. SREBP-1 levels were quantified in both cell lines after treatment with both 25OH-cholesterol and fatty acids (**Fig. 4**). 25OH-cholesterol (5 mg/L) decreased SREBP-1 levels by 30–50% ($P < 0.05$) in fibroblasts and 20–40% ($P < 0.05$) in HepG2 cells compared with control cell cultures. None of the fatty acids evaluated affected precursor or mature SREBP-1 abundance.

DISCUSSION

Although some in vivo and in vitro studies addressed how fatty acids regulate LDLr expression, the effects of fatty acids

TABLE 2

Fatty acid profiles in HepG2 cells treated with the defined DMEM for 24 h and then treated with 25OH-cholesterol (2 mg/L) and/or fatty acids (0.4 mmol/L) another 24 h[1]

	Treatment							
Fatty acid	Control	25OH-cholesterol	18:1	18:2	18:3	AA	EPA	DHA
	% of total fatty acids							
14:0	2.5 ± 0.5	3.1 ± 0.4	2.4 ± 0.2	3.2 ± 0.4	2.9 ± 0.2	3.0 ± 0.1	2.8 ± 0.3	2.9 ± 0.1
16:0	20.9 ± 0.6	21.8 ± 0.6	17.1 ± 0.3	20.0 ± 0.4	18.3 ± 0.5	21.4 ± 0.6	19.5 ± 0.6	19.9 ± 0.6
16:1	11.2 ± 0.6	11.1 ± 0.1	7.5 ± 0.3	10.1 ± 0.2	8.6 ± 0.1	8.8 ± 0.6	8.6 ± 0.5	8.9 ± 0.1
18:0	7.8 ± 0.3	8.7 ± 0.3	6.2 ± 0.2	8.9 ± 0.2	7.1 ± 0.2	7.6 ± 0.2	7.2 ± 0.1	6.6 ± 0.2
18:1(n-9)	30.4 ± 0.2	28.9 ± 0.4	46.6 ± 0.8*	26.4 ± 0.3	19.6 ± 0.9	23.6 ± 1.5	23.0 ± 1.7	20.9 ± 0.3
18:1(n-6)	17.6 ± 0.1	16.9 ± 0.5	12.9 ± 0.4	13.1 ± 0.4	11.7 ± 0.01	12.7 ± 0.3	12.1 ± 0.4	12.3 ± 0.2
18:2(n-6)	2.8 ± 0.01	2.9 ± 0.1	2.1 ± 0.1	12.8 ± 0.8*	1.8 ± 0.02	1.9 ± 0.02	2.0 ± 0.1	2.0 ± 0.2
18:3(n-3)	trace	trace	trace	trace	23.0 ± 0.7*	trace	trace	trace
20:2(n-6)	3.4 ± 0.5	4.2 ± 0.5	3.7 ± 0.3	4.0 ± 0.4	3.0 ± 0.3	3.0 ± 0.1	2.7 ± 0.2	2.5 ± 0.1
20:4(n-6)	2.1 ± 0.4	1.5 ± 0.2	1.2 ± 0.1	1.5 ± 0.1	2.7 ± 0.2	18.1 ± 0.2*	1.0 ± 0.01	1.2 ± 0.0
20:5+22:5(n-3)	trace	trace	trace	trace	1.9 ± 0.2	trace	19.8 ± 0.4*	0.8 ± 0.0
22:6(n-3)	1.4 ± 0.3	2.4 ± 0.9	0.9 ± 0.0	0.9 ± 0.0	0.7 ± 0.01	trace	2.1 ± 0.2	23.0 ± 0.3*

[1] Values are means ± SEM, $n = 3$. * Different from control, $P < 0.05$.

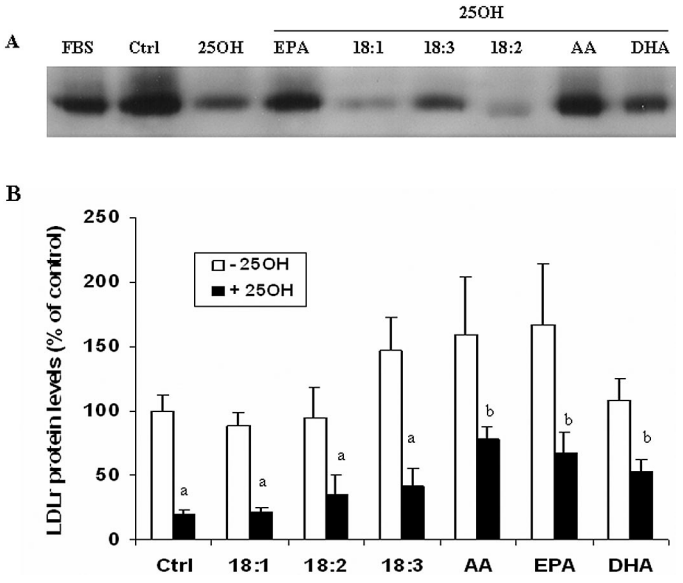

FIGURE 1 LDLr expression in fibroblasts treated with the defined DMEM for 24 h and then treated with fatty acids (0.4 mmol/L) with (+) or without (−) 25OH-cholesterol (2 mg/L) for another 24 h. (*A*) A representative Western blot. (*B*) LDLr protein levels are presented as a percentage of the control in the absence of 25OH-cholesterol. Values are means ± SEM, $n = 4$. Means without a common letter differ, $P < 0.05$.

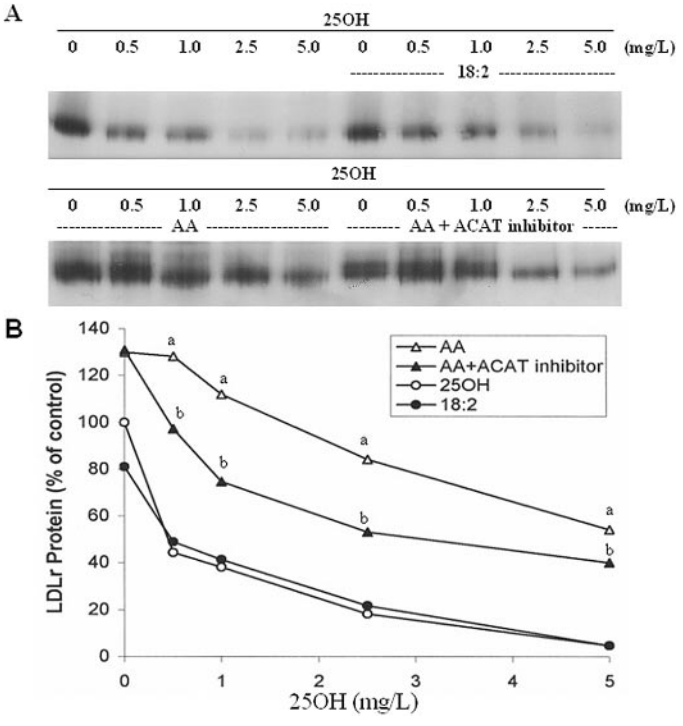

FIGURE 2 The effects of different doses of 25OH-cholesterol supplemented with 18:2 or AA on LDLr protein abundance in fibroblasts. Cells were treated with defined DMEM for 24 h and then treated with different doses (0–5 mg/L) of 25OH-cholesterol and/or fatty acids for another 24 h. (*A*) A representative Western blot. (*B*) LDLr protein levels are presented as a percentage of the control in the absence of 25OH-cholesterol. Values are the mean of 3 experiments. Means without a common letter at each dose level differ, $P < 0.05$.

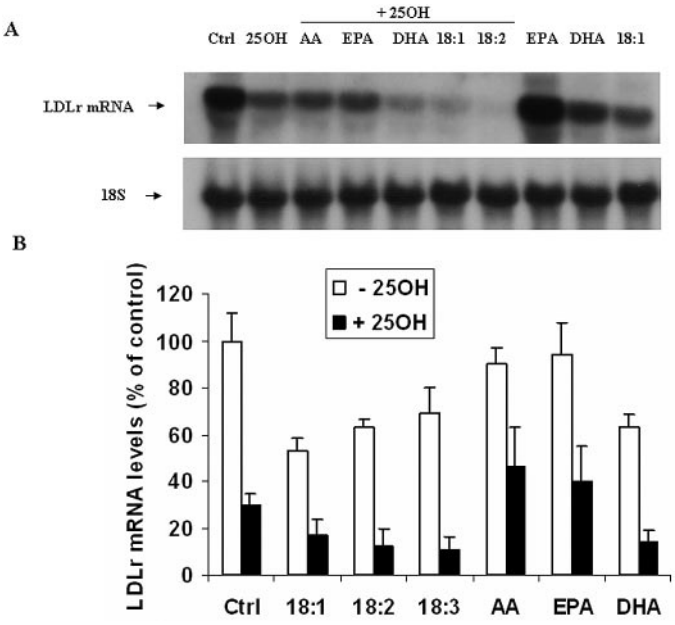

FIGURE 3 LDLr expression in fibroblasts treated with the defined DMEM for 24 h and then treated with fatty acids (0.4 mmol/L) with or without 25OH-cholesterol (2 mg/L) and with (+) or without (−) the ACAT inhibitor (1 mg/L) for another 24 h. (*A*) A representative Northern blot. (*B*) LDLr mRNA levels are presented as a percentage of the control in the absence of 25OH-cholesterol. Values are means ± SEM, $n = 4$.

on LDLr gene and the mechanisms by which fatty acids exert their regulatory effects remain unclear. The results from the present study showed that AA, EPA, and DHA increased LDLr protein abundance, but had no significant effects on LDLr mRNA levels, suggesting that regulation of LDLr expression by AA, EPA, and DHA may be at the translational or post-translational level. In the present study, 18:1, 18:2, and 18:3 had no significant effects on LDLr expression, which agrees with findings from earlier in vivo and in vitro studies (5–9). These results suggested that fatty acids may affect plasma cholesterol levels via other mechanisms in addition to LDLr gene expression, e.g., de novo cholesterol synthesis or apolipoprotein B secretion and LDL production.

Earlier studies demonstrated that the LDLr gene is highly regulated by cellular sterol content (15,16). When sterols accumulate in cells, transcription of the LDLr gene is suppressed. Sterols also stimulate ACAT activity, resulting in an increase in cellular CE. Cholesteryl esters have a neutral effect

TABLE 3

CE:TC ratios in HepG2 cells after fatty acid treatments with or without ACAT inhibitor (1 mg/L) for 24 h[1]

Fatty acid	CE:TC (no ACAT inhibitor)	CE:TC (+ ACAT inhibitor)	Difference in CE:TC ratio	*P*-value
18:1	0.321 ± 0.04	0.216 ± 0.01	−0.105	<0.05
18:2	0.215 ± 0.03	0.184 ± 0.01	−0.031	>0.05
18:3	0.286 ± 0.02	0.227 ± 0.02	−0.060	>0.05
AA	0.306 ± 0.02	0.180 ± 0.01	−0.126	<0.05
EPA	0.281 ± 0.01	0.198 ± 0.02	−0.083	>0.05
DHA	0.258 ± 0.01	0.212 ± 0.02	−0.046	>0.05

[1] Values are means ± SEM, $n = 3$.

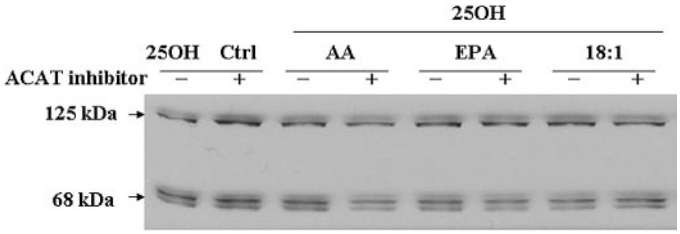

FIGURE 4 Western blotting analysis of SREBP-1 in HepG2 cells treated with the defined DMEM for 24 h and then treated with 25OH-cholesterol (5 mg/L) and/or fatty acids (0.4 mmol/L) for another 24 h with (+) or without (–) ACAT inhibitor (1 mg/L). A representative blot is shown. The upper bands are SREBP precursor with a molecular weight of 125 kDa. The lower bands are mature SREBP with a molecular weight of 68 kDa. The experiments were repeated 3 times.

on LDLr gene expression. Some in vitro studies showed that fatty acids such as 18:1, AA, and EPA increase cellular ACAT activity (9,17). In contrast, linoleic acid might inhibit ACAT activity (9,18). It was suggested that fatty acids affect LDLr activity via ACAT and the cellular cholesterol pool (11,12). In the present study, an ACAT inhibitor (58–035, 1 mg/L) was used to determine whether ACAT is involved in LDLr regulation by fatty acids. HepG2 cells were used because of their high ACAT activity (19). In agreement with earlier findings, the present study showed that AA and oleic acid increased cellular CE, whereas linoleic acid had little effect on cellular cholesterol distribution. EPA may also increase cellular CE content to some extent. When the ACAT inhibitor was added to the medium, the upregulatory effect of AA on LDLr protein was partially blocked. However, the stimulatory effects of EPA and DHA on LDLr protein levels were not affected significantly. Thus, ACAT may partially account for the effect of AA on the LDLr protein level; however, it does not appear to be involved in other fatty acid effects on LDLr protein levels. Our previous studies demonstrated that ACAT is also not involved in the stimulatory effect of CLA on LDLr expression (7). In the present study, oleic acid increased cellular CE, but had no effect on LDLr protein or mRNA abundance, suggesting that oleic acid may affect only LDLr activity instead of regulating LDLr gene expression (9).

One possible mechanism by which fatty acids elicit their regulatory effects is via sterol-mediated feedback repression of LDLr gene expression. Elevated levels of cellular sterols block the SREBP maturation process, thereby inhibiting LDLr expression (20). PUFAs were shown to reduce the hepatic precursor and mature SREBP-1 (21). However, another study indicated that fat intake suppressed fatty acid synthesis in hamster intestine independently of SREBP-1 expression. Dietary fatty acids did not change SREBP-1 abundance (22). Similarly, our previous study showed that conjugated linoleic acid stimulated LDLr expression independently of SREBP-1 (7). In agreement with the earlier findings, the present study did not observe any differences in the effects of the fatty acids examined on SREBP-1 abundance. Therefore, the long-chain fatty acids may regulate LDLr expression via a pathway that is independent of SREBP-1. Further studies are required to determine the mechanisms by which individual fatty acids regulate LDLr expression. The findings of the present study suggest that multiple mechanisms are involved in regulating the LDLr gene.

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