

Regulation of the expression of key genes involved in HDL metabolism by unsaturated fatty acids

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Abstract

The cardioprotective effects of HDL have been largely attributed to their role in the reverse cholesterol transport pathway, whose efficiency is affected by many proteins involved in the formation and remodelling of HDL. The aim of the present study was to determine the effects, and possible mechanisms of action, of unsaturated fatty acids on the expression of genes involved in HDL metabolism in HepG2 cells. The mRNA concentration of target genes was assessed by real-time PCR. Protein concentrations were determined by Western blot or immunoassays. PPAR and liver X receptor (LXR) activities were assessed in transfection experiments. Compared with the SFA palmitic acid (PA), the PUFA arachidonic acid (AA), EPA and DHA significantly decreased apoA-I, ATP-binding cassette A1 (ABCA1), lecithin-cholesterol acyltransferase (LCAT) and phospholipid transfer protein mRNA levels. EPA and DHA significantly lowered the protein concentration of apoA-I and LCAT in the media, as well as the cellular ABCA1 protein content. In addition, DHA repressed the apoA-I promoter activity. AA lowered only the protein concentration of LCAT in the media. The activity of PPAR was increased by DHA, while the activity of LXR was lowered by both DHA and AA, relative to PA. The regulation of these transcription factors by PUFA may explain some of the PUFA effects on gene expression. The observed *n*-3 PUFA-mediated changes in gene expression are predicted to reduce the rate of HDL particle formation and maturation.

Key words: Fatty acids; HDL; ApoA-I; ATP-binding cassette A1; HepG2

CHD remains one of the leading causes of morbidity and mortality in the USA⁽¹⁾. The inverse relationship between plasma levels of HDL-cholesterol (HDL-C) and the risk of CHD is well established⁽²⁾. Besides its antioxidant, anti-inflammatory and antithrombotic effects, the cardioprotective effects of HDL have been largely attributed to its role in the reverse cholesterol transport (RCT) pathway, in which excess cholesterol in peripheral tissues is returned to the liver for utilisation or elimination⁽³⁾. The efficiency of HDL in promoting RCT is related to the concentration, composition, shape and size of the HDL particles, which result from the continuous remodelling of HDL in the circulation by the action of numerous proteins⁽⁴⁾.

The initial step in HDL formation involves the synthesis and secretion of apoA-I by liver cells and, to a lesser extent, by

intestinal cells⁽⁵⁾. Then, the cell membrane transporter ATP-binding cassette A1 (ABCA1) mediates the transfer of cellular phospholipids and free cholesterol to lipid-poor apoA-I, leading to the formation of nascent HDL⁽⁶⁾. The interaction of hepatic ABCA1 with apoA-I is crucial for HDL maturation, as indicated by the dramatic reduction in plasma HDL levels in liver-specific ABCA1 knockout mice⁽⁷⁾. The free cholesterol in HDL is then esterified by lecithin-cholesterol acyltransferase (LCAT)⁽⁸⁾ to cholesteryl ester (CE). CE migrates into the core of the HDL particles, resulting in the formation of larger and spherical HDL particles. The different components of HDL are frequently exchanged with or transferred to other lipoproteins within the plasma compartment by the action of transfer proteins such as phospholipid transfer protein (PLTP) and CE transfer protein (CETP)⁽⁹⁾. The exchange of CE for TAG,

Abbreviations: AA, arachidonic acid; ABCA1, ATP-binding cassette A1; ALA, α -linolenic acid; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; HDL-C, HDL-cholesterol; HL, hepatic lipase; LA, linoleic acid; LCAT, lecithin-cholesterol acyltransferase; LD, lipoprotein-deficient; LXR, liver X receptor; LXRE, liver X receptor response element; OA, oleic acid; PA, palmitic acid; PLTP, phospholipid transfer protein; PPRE, PPAR response element; RCT, reverse cholesterol transport; SR-BI, scavenger receptor class B type I.

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mediated by CETP, results in HDL particles enriched with TAG. TAG and phospholipids in HDL are hydrolysed by hepatic lipase (HL)⁽¹⁰⁾, leading to the formation of HDL particles that are preferred ligands for the scavenger receptor class B type I (SR-BI)⁽¹¹⁾, which mediates the selective uptake of CE from HDL into hepatocytes⁽¹²⁾. These genes are all important players in HDL metabolism and are all expressed by human hepatocytes.

Both genetic and environmental factors regulate HDL metabolism and the RCT pathway. Among these factors, dietary fat composition affects both HDL-C concentrations and CHD risk. Dietary intervention studies have shown that an increased intake of PUFA, especially of the *n*-3 type, with or without reduced dietary SFA intake, is associated with a reduced risk of CHD^(13,14). In an attempt to define how individual unsaturated fatty acids regulate HDL metabolism, we determined the effect of the MUFA oleic acid (OA), the *n*-3 PUFA α -linolenic acid (ALA), EPA and DHA, and the *n*-6 PUFA linoleic acid (LA) and arachidonic acid (AA), relative to the SFA palmitic acid (PA), on *apoA-I*, *ABCA1*, *SR-BI*, *LCAT*, *PLTP*, *CETP* and *HL* gene expression in HepG2 cells.

Experimental methods

Cell culture and fatty acid treatments

HepG2 cells (ATCC HB-8065; ATCC) were maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Hyclone), 1% GlutaMax, 100 μ M non-essential amino acids, 100 U penicillin/ml and 100 μ g streptomycin/ml (Invitrogen) in an atmosphere of 5% CO₂ at 37°C. Lipoprotein-deficient (LD) FBS was obtained by ultracentrifugation of FBS at a density of 1.215 g/ml. PA (16:0), OA (18:1), LA (18:2, *n*-6), AA (20:4, *n*-6), ALA (18:3, *n*-3), EPA (20:5, *n*-3; Nu-Chek) and DHA (22:6, *n*-3; Sigma-Aldrich), in the form of Na salts, were dissolved in water and combined with fatty acid-free bovine serum albumin (Sigma-Aldrich) at a 2:1 molar ratio. Cells were cultured in DMEM containing 10% LD-FBS for 24 h before the fatty acid treatment and then incubated for 24 h with fatty acid-free bovine serum albumin (control cells) or with the bovine serum albumin–fatty acid complexes at the concentration indicated, in DMEM containing 10% LD-FBS. Cell viability was monitored by trypan blue exclusion and was \geq 90% after 24 h incubation with all fatty acids tested

at concentrations up to 200 μ M. Increased cell death was observed with long-chain PUFA (twenty or more carbons) treatments at concentrations > 200 μ M.

Quantitative real-time PCR

Total cellular RNA was extracted using TRIzol reagent and reverse transcribed with random hexamers using the SuperScript III First Strand Synthesis Kit (Invitrogen) following the manufacturer's protocol. The mRNA level of the genes of interest was determined using specific primers (Table 1), designed by Primer Express Software version 2.0, and the power SYBR Green Master Mix (Applied Biosystems) in the Applied Biosystems 7300 Real-Time PCR system (version 1.4 SDS software). The relative quantification ($\Delta\Delta C_T$) method was used to determine the expression of the target genes, using the glyceraldehyde-3-phosphate dehydrogenase gene as the internal control. Target gene mRNA levels were expressed relative to the PA treatment.

Western blots

Cells were lysed in RIPA buffer containing protease inhibitors. In brief, 30 μ g of cell lysates were separated by electrophoresis in 7.5% polyacrylamide gels, and transferred onto nitrocellulose membranes. Membranes were incubated for 2 h in blocking solution (1 \times Tris-buffered saline (TBS), 3% non-fat dry milk, 0.1% Tween 20) and then incubated overnight at 4°C with anti-ABCA1, anti-SR-BI (Novus Biologicals) or anti-glyceraldehyde-3-phosphate dehydrogenase (Santa Cruz Biotechnology) antibodies in blocking solution. Membranes were then washed three times with washing solution (1 \times TBS, 0.1% Tween 20), incubated with the horseradish peroxidase-conjugated secondary antibody at room temperature for 1 h, and washed again for three times. Signals were visualised by enhanced chemiluminescence (Amersham Biosciences) on X-ray films and quantified using a GS-710 calibrated imaging densitometer with Quantity One software (Bio-Rad Laboratories).

ELISA

HepG2 culture media were collected after 24 h of incubation with fatty acids, and the concentration of apoA-I was measured with an ELISA assay developed in our laboratory,

Table 1. Oligonucleotide primers for quantitative real-time PCR

Gene	Forward primer (5'–3')	Reverse primer (5'–3')	Amplicon (bp)	Accession no./reference
<i>ABCA1</i>	AGCCACAAGGCAGGTCATG	TCCGGACAAGGTCATTCTT	143	NM_005502
<i>apoA-I</i>	GCCTTGGGAAAACAGCTAAACC	TCCTGGGTCACAGGGCC	101	NM_000039
<i>CETP</i>	CGCATGCTGTACTTCTGGTTCT	GCCATCCTGGAAAGCTACCTT	69	Norata <i>et al.</i> ⁽⁶²⁾
<i>GAPDH</i>	CCTGTTTCGACAGTCAGCCG	CGACCAAATCCGTTGACTCC	101	NM_002046
<i>HL</i>	CACTACACCATCGCCGTCC	TTGAACAGATTCTCCAGCCA	84	NM_000236
<i>LCAT</i>	ACCGCTTTATTGATGGCTTCA	TCACCTGAGGCCAAGACCAG	82	NM_000229
<i>PLTP</i>	ACCATGCGGGATTCTCAC	GCAGGCCGGTTCTTCTCAA	82	NM_006227
<i>SR-BI</i>	GGCACACTCCTTGTTCCTGG	GTCCAATGCCTGCGACAGAT	107	NM_005505

ABCA1, ATP-binding cassette A1; *CETP*, cholesteryl ester transfer protein; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *HL*, hepatic lipase; *LCAT*, lecithin-cholesterol acyltransferase; *PLTP*, phospholipid transfer protein; *SR-BI*, scavenger receptor class B type I.

as described previously⁽¹⁵⁾. For LCAT measurement, conditioned media were concentrated twenty-five times with Centricon centrifugal filter devices (Millipore) and LCAT was quantified by an ELISA kit (ALPCO) according to the manufacturer's protocol. Both apoA-I and LCAT protein levels were normalised to total cellular protein concentrations.

Transient transfections

Transient transfection experiments were carried out in twelve-well dishes with 0.4 µg of the test plasmid and 0.03 µg of the *Renilla* luciferase plasmid (Promega) using the FuGENE 6 Transfection Reagent (Roche) in DMEM containing 10% LD-FBS for 24 h, or in serum-free DMEM for 6 h followed by 18 h incubation in DMEM containing 10% LD-FBS. The luciferase reporter construct (pGL2 basic vector; Promega) containing the -256 to +396 region of the human apoA-I promoter has been described previously⁽¹⁵⁾. The plasmid 3xPPRE-tk-pGL3, containing three copies of a PPAR response element (PPRE)⁽¹⁶⁾, and the plasmid tk-LXREx3-luc, containing three copies of an liver X receptor (LXR) response element (LXRE)⁽¹⁷⁾, were generous gifts from Dr Kilgore and Dr Mangelsdorf, respectively. Transfected cells were then incubated with 0.1% ethanol (vehicle, control), 1 µM-GW7647 (PPARα activator) or 1 µM-T0901317 (LXR activator; Cayman Chemicals) dissolved in 0.1% ethanol, or 200 µM-PA, -DHA and -AA, in DMEM containing 10% LD-FBS for 24 h. Cells were collected and stored at -70°C until luciferase activities were measured with the Dual-Luciferase Reporter Assay System (Promega).

Statistical analysis

Experiments were performed in duplicate and values of three independent experiments are presented as means and standard deviations. Statistical analysis was performed with the SAS 9.1 statistical package (SAS Institute Inc.). Significant differences among the treatments were assessed by one-way ANOVA followed by Dunnett's adjustment, two-tailed Student's *t* test or linear regression (test for trend) as indicated. $P < 0.05$ was considered to be statistically significant.

Results

The expression of the *apoA-I*, *ABCA1*, *SR-BI*, *LCAT*, *PLTP*, *CETP* and *HL* genes was analysed in HepG2 cells by real-time PCR. A dose-response curve with 0, 50, 100 and 200 µM of each fatty acid was developed for each target gene. A statistically significant dose-dependent down-regulation of apoA-I, LCAT and PLTP mRNA expression was observed with the DHA and AA treatments (P for trend < 0.05 ; data not shown). A similar dose-dependent repressive effect of EPA on LCAT mRNA expression was also found (P for trend < 0.05 ; data not shown). Compared with PA, the long-chain PUFA AA, EPA and DHA significantly decreased the mRNA level of apoA-I, ABCA1, LCAT and PLTP at the 200 µM concentration (Fig. 1). ALA, at the 200 µM concentration, also decreased apoA-I mRNA level,

but did not affect the expression of the other genes (Fig. 1). OA and LA did not affect the expression of any of the genes studied (Fig. 1). None of the fatty acids studied affected the mRNA levels of SR-BI, CETP or HL (Fig. 1).

The effects of the different fatty acids on the apoA-I, ABCA1, SR-BI and LCAT protein levels were assessed at the 200 µM concentration. After adjustment for multiple comparisons, the EPA treatment was associated with a significant reduction in apoA-I secretion (22%; Fig. 2(a)). DHA also reduced apoA-I protein level by 15%, which was significant ($P < 0.05$) when directly compared with PA. Consistent with the RNA data, the amount of LCAT protein secreted by HepG2 cells decreased upon the AA, EPA and DHA treatments, relative to the PA treatment (Fig. 2(b)). ABCA1 and SR-BI protein levels were quantified by Western blot, as shown in Fig. 2(c). At approximately 250 kDa, two ABCA1 bands were detected, representing the glycosylated and non-glycosylated forms. EPA and DHA significantly decreased total ABCA1 protein, relative to PA. Exposure of HepG2 cells to any of the unsaturated fatty acids did not significantly alter the amount of SR-BI protein, compared with PA (Fig. 2(c)).

Relative to PA, the DHA treatment of cells transfected with a reporter construct containing the full promoter region of the human *apoA-I* gene showed a 30% reduction in apoA-I promoter activity at 24 h (Fig. 3). A similar repression in apoA-I promoter activity was observed in one experiment comparing AA with PA (data not shown). GW7647, a PPARα activator, significantly induced PPAR activity (Fig. 4(a)). DHA also significantly increased PPAR activity by 30%, relative to PA. T0901317, an LXR activator, significantly increased the expression of the LXRE construct (Fig. 4(b)). However, LXR activity was significantly lowered by DHA or AA compared with PA.

Discussion

Fatty acids are able to regulate gene expression by influencing the activity or abundance of several transcription factors⁽¹⁸⁾. Unsaturated fatty acids have been shown in some, but not all, human and animal studies to alter the concentration or activity of proteins involved in HDL metabolism^(19–29). A more complete understanding of how different fatty acids influence HDL needs to be determined. Plasma concentrations of NEFA in normal healthy subjects have been reported to vary between 300 and 600 µM^(30,31), of which approximately 35% is OA, 17% are *n*-6 PUFA and 1.2–2.5% are *n*-3 PUFA^(32,33), with fish oil supplementation resulting in significant increases in plasma free EPA and DHA concentrations^(34,35). In the present study, PA was used as the reference fatty acid because it is the dominant SFA in the typical US diet⁽³⁶⁾ and also in human blood⁽³⁷⁾.

In two apoA-I protein kinetic studies conducted in insulin-resistant subjects, it has been reported that a high dose of *n*-3 PUFA (a mixture of EPA and DHA, 3.36 g/d for 6 weeks or 1.8 g/d for 8 weeks) decreased the production of HDL apoA-I^(25,29). However, a significant reduction in apoA-I clearance was also observed in these studies, so that no effects on plasma apoA-I concentrations were noted^(25,29). The present

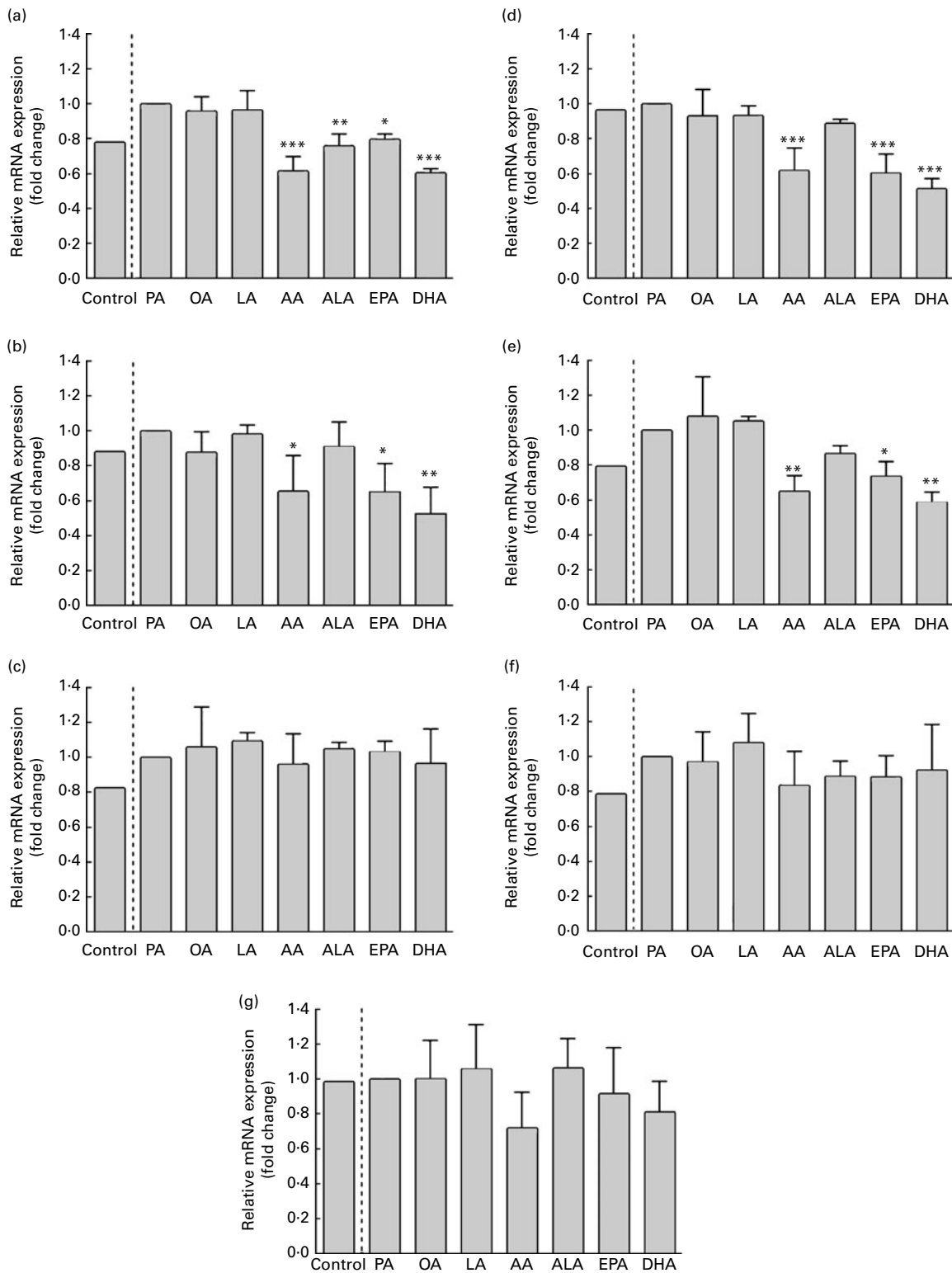


Fig. 1. Effects of fatty acids on gene expression. HepG2 cells were treated with 200 μ M of the indicated fatty acids or fatty acid-free bovine serum albumin (control) for 24 h, and mRNA levels of the (a) *apoA-I*, (b) ATP-binding cassette A1, (c) scavenger receptor class B type I, (d) lecithin-cholesterol acyltransferase, (e) phospholipid transfer protein, (f) cholesteryl ester transfer protein and (g) hepatic lipase genes were measured. Data are normalised to glyceraldehyde-3-phosphate dehydrogenase mRNA levels and are expressed as fold change compared with values of the cells treated with 200 μ M-palmitic acid (PA). Values are means from three independent experiments with each treatment in duplicate, with standard deviations represented by vertical bars. Mean values were significantly different from those of the PA treatment: * P <0.05, ** P <0.01, *** P <0.001; one-way ANOVA followed by Dunnett's adjustment. OA, oleic acid; LA, linoleic acid; AA, arachidonic acid; ALA, α -linolenic acid.

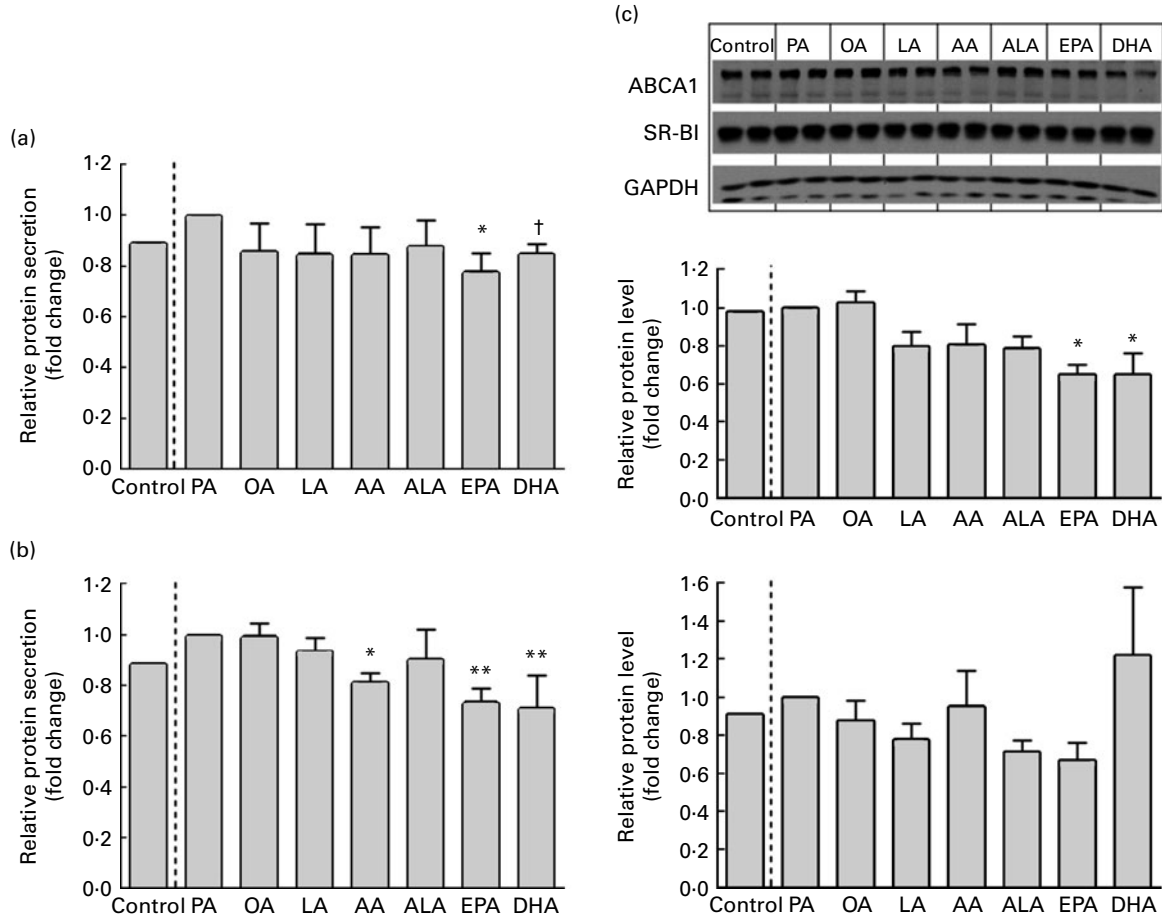


Fig. 2. Effects of fatty acids on protein levels. HepG2 cells were treated with 200 μM of the indicated fatty acids or fatty acid-free bovine serum albumin (control) for 24 h. (a) ApoA-I secreted in the culture media was determined by ELISA and normalised to total cellular protein. (b) Lecithin-cholesterol acyltransferase secreted in the culture media was determined by ELISA and normalised to total cellular protein. (c) Upper panel: Western blot of ATP-binding cassette A1 (ABCA1, 250 kDa) and scavenger receptor class B type I (SR-BI, 75 kDa). Lower panel: ABCA1 (upper graph) and SR-BI (lower graph) levels were normalised to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Results are expressed as fold change relative to values of the cells treated with 200 μM -palmitic acid (PA). Values are mean from three independent experiments conducted with duplicate treatments, with standard deviations represented by vertical bars. Mean values were significantly different from those of the PA treatment: * $P < 0.05$, ** $P < 0.01$; one-way ANOVA followed by Dunnett's adjustment. † Mean value was significantly different compared with the PA treatment ($P < 0.05$; two-tailed Student's t test). OA, oleic acid; LA, linoleic acid; AA, arachidonic acid; ALA, α -linolenic acid.

findings of decreased apoA-I expression with EPA and DHA treatments in HepG2 cells are consistent with the findings of reduced production of apoA-I with $n-3$ PUFA supplementations in these human kinetic studies. AA decreased apoA-I mRNA level but did not significantly decrease apoA-I protein secretion. It is possible that apoA-I production is regulated differently by $n-6$ and $n-3$ PUFA at the post-transcriptional level. This hypothesis requires further studies. LA did not significantly affect apoA-I mRNA or protein levels, which agrees with a study comparing the effect of soyabean oil (high LA content) with butter and showing no differences in HDL-C or apoA-I concentrations, clearance or production in hypercholesterolaemic women⁽³⁸⁾. In hepatic cells, the transcriptional activity of the apoA-I promoter is regulated by several transcription factors capable of binding to the apoA-I hepatic enhancer. A PPRE has been mapped to -214 to -192 region of the apoA-I promoter⁽³⁹⁾. Activation of PPAR α has been shown to increase hepatic human apoA-I mRNA levels as well as plasma human apoA-I concentrations in human apoA-I transgenic mice⁽⁴⁰⁾. Here, we have shown

that 200 μM -DHA, compared with PA, promotes PPAR activity in HepG2 cells. While this would be expected to up-regulate apoA-I gene expression, DHA suppressed both apoA-I promoter activity and apoA-I mRNA levels. We have recently shown that the DHA-associated suppression of the apoA-I promoter is mediated by the transcription factor HNF-3 β ⁽⁴¹⁾.

The effects of unsaturated fatty acids on ABCA1 expression have been studied predominantly in macrophages, with some studies also conducted in enterocytes and hepatocytes. In Caco-2/TC-7 human colon colorectal adenocarcinoma cells, exposure to 100 or 300 μM -AA and -EPA for 24 h decreased ABCA1 mRNA levels⁽⁴²⁾. EPA treatment at 10 or 100 μM for 18 h has also been shown to decrease ABCA1 mRNA levels in HepG2 cells⁽⁴³⁾. The suppressive effects of EPA became more evident when ABCA1 expression was induced with the LXR activator 22(R)-hydroxycholesterol or 9-*cis*-retinoic acid⁽⁴⁵⁾. The role of LXR in up-regulating ABCA1 gene expression is well established^(44,45). In macrophages, the suppressive effect of EPA on ABCA1 gene expression was shown to depend on the presence of an LXRE in the promoter⁽⁴⁶⁾.

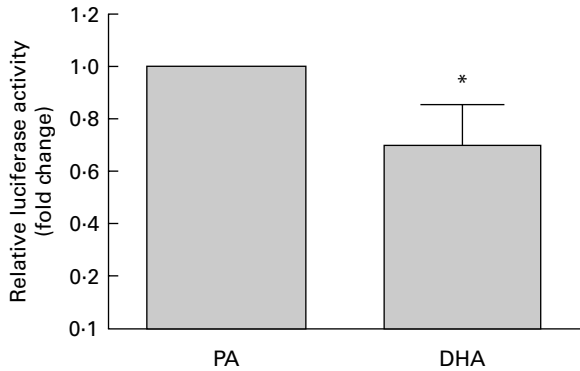


Fig. 3. Effects of DHA on apoA-I promoter activities. HepG2 cells were co-transfected with a reporter construct containing the human apoA-I promoter and the *Renilla* luciferase plasmid. After transfection, the cells were incubated with 200 μ M-palmitic acid (PA) or 200 μ M-DHA for 24 h. The activity of the apoA-I promoter was measured by relative luciferase activities and expressed as fold change relative to PA. Results are means from three independent experiments conducted in triplicate, with standard deviation represented by a vertical bar. * Mean value was significantly different compared with the PA treatment ($P < 0.001$; two-tailed Student's *t* test).

Ou *et al.*⁽⁴⁷⁾ have reported that unsaturated fatty acids antagonise oxysterol-induced LXR activity. Post-translational regulation of macrophage ABCA1 by unsaturated fatty acids, including OA, LA and AA, in the presence of a cyclic AMP analogue, has also been observed and involves the activation of protein kinase C δ through a phospholipase D2 pathway, leading to destabilisation of ABCA1 protein^(48–50). However, in the latter study, the suppressive effect of unsaturated fatty acids on ABCA1 mRNA levels was absent. Similarly, a recent report has shown that treatment of HepG2 cells with 125 μ M-OA or -LA for 16 h, relative to the non-treatment control, increased the degradation rate of ABCA1 protein without affecting its mRNA level⁽⁵¹⁾. In the present study, 200 μ M-AA, -EPA and -DHA, compared with PA, significantly suppressed ABCA1 mRNA levels while OA, LA and ALA did not. Since our transfection experiments indicated a DHA- and AA-dependent decrease in LXR activity, we speculate that the altered expression of hepatic ABCA1 mRNA by these fatty acids may be mediated by reduced LXR activity. EPA and DHA also lowered cellular ABCA1 protein content, compared with PA, and this lowering effect was similar to that observed for ABCA1 mRNA levels. Despite its lowering effect on ABCA1 mRNA level, AA did not significantly affect cellular ABCA1 protein level, suggesting the possibility of a differential mechanism between *n*-6 and *n*-3 PUFA on hepatic ABCA1 post-translational regulation. This need to be further investigated. In addition, it is not clear why there is discrepancy in results among studies. One possibility is that we did not induce ABCA1 expression by a cAMP analogue or LXR activators in our experiments. Alternatively, different experimental conditions may have led to different findings.

In human subjects, *n*-3 PUFA have been shown to reduce plasma LCAT activity in mildly hypercholesterolaemic men⁽²⁰⁾. In the present study, the long-chain PUFA AA, EPA and DHA significantly decreased both the mRNA and protein levels of LCAT, relative to PA. In agreement with the present findings, a previous study has reported a decrease in LCAT

secretion by primary rat hepatocytes treated with 1 mM-DHA⁽⁵²⁾. In the same study, however, an increased LCAT secretion was seen with 1 mM-OA and -LA treatments, which we did not see with 200 μ M concentrations. Our understanding of how LCAT is regulated at the transcriptional level is still very limited and the mechanism of PUFA regulation of LCAT expression is not clear.

It has previously been shown that LXR ligands induce the expression of PLTP both in HepG2 cells and in mouse liver cells *in vivo*. A high-affinity LXRE has been identified in the PLTP promoter⁽⁵³⁾. In addition, the promoter activity of the *PLTP* gene has been shown to be significantly reduced by fenofibrate, which indicates the potential involvement of

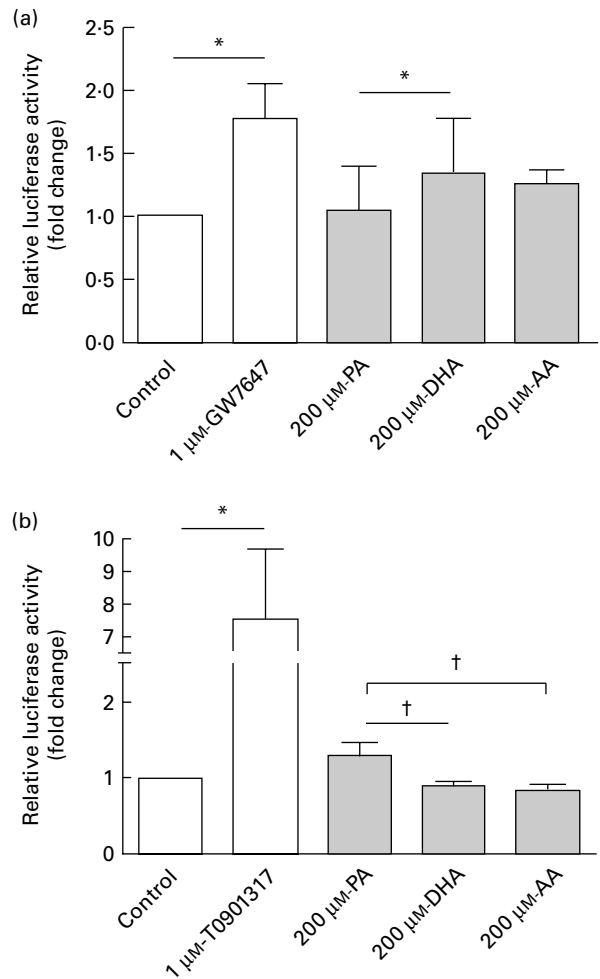


Fig. 4. Effects of DHA and arachidonic acid (AA) on PPAR and liver X receptor activities. HepG2 cells were co-transfected with either (a) the 3xPPRE-tk-pGL3 or (b) the tk-LXREx3-luc reporter construct and the *Renilla* luciferase plasmid. After transfection, the cells were incubated with (a) 1 μ M-PPAR α activator GW7647 or (b) 1 μ M-LXR activator T0901317, and vehicle control, or 200 μ M-palmitic acid (PA), 200 μ M-DHA and 200 μ M-AA for 24 h. The activation of PPAR or LXR was measured by relative luciferase activities and expressed as fold change relative to the control. Values are means from three independent experiments conducted in triplicate, with standard deviations represented by vertical bars. * Mean values were significantly different compared with the control and PA treatments ($P < 0.05$; two-tailed Student's *t* test). † Mean values were significantly different compared with the PA treatment ($P < 0.01$; one-way ANOVA followed by Dunnett's adjustment). PPRE, PPAR response element; LXRE, LXR response element.

PPAR α in the transcriptional regulation of PLTP⁽⁵⁴⁾. Taking into consideration that DHA enhances PPAR activity, and both DHA and AA reduce LXR activity in HepG2 cells, the observation of significantly reduced PLTP mRNA expression in cells treated with long-chain PUFA may be explained, at least in part, by the activation of PPAR and the suppression of LXR.

We did not observe a significant effect of unsaturated fatty acids compared with SFA on mRNA or protein levels of hepatic SR-BI. The *in vivo* observations are not conclusive: studies have indicated that PUFA increase the hepatic expression of SR-BI and the selective hepatic uptake of CE from HDL in mice and hamsters^(24,26,55), but not in rats⁽²⁸⁾.

In the present study, there were no significant differences in the mRNA expression of CETP or HL in cells treated with unsaturated fatty acids relative to PA at the concentrations tested. A previous report showed a 50% reduction in CETP mRNA levels with 500 μ M-AA, -EPA or -DHA, compared with the SFA stearic acid in HepG2 cells⁽⁵⁶⁾.

In summary, the present study shows that, compared with the SFA PA, the long-chain *n*-3 PUFA EPA and DHA decrease hepatic apoA-I, ABCA1 and LCAT mRNA and protein expression, as well as PLTP mRNA expression. While the AA treatment was as effective as the EPA and DHA treatments in lowering apoA-I and ABCA1 mRNA levels, it did not result in significant changes in the levels of these proteins. The AA treatment only affected LCAT protein secretion. Based on the roles of these proteins in HDL metabolism, our experiments suggest that EPA and DHA may affect both the rate of formation and maturation of HDL. Decreased secretion of lipid-poor apoA-I and reduced hepatic ABCA1 expression slow down the formation of nascent HDL particles and the lipidation of apoA-I⁽⁵⁷⁾. The formation of larger spherical HDL particles would also be reduced because of lower LCAT levels. Our observations also suggest that the impact of long-chain *n*-6 PUFA on HDL metabolism may be lower than that of *n*-3 PUFA. This is supported by clinical studies in which supplementation with high doses of fish oil, which is associated with a change in the AA:EPA + DHA ratio from approximately 5:1 to 2:1, caused a significant reduction in apoA-I production^(25,29). The present study could not address the effect of PUFA on HDL clearance. Human kinetic studies have clearly shown a reduction in HDL apoA-I clearance, compensating for the reduction in apoA-I production, following fish oil. There are a number of other factors by which PUFA could influence HDL-C concentrations and HDL function besides their effects on gene expression. It has been shown that *n*-3 PUFA lower plasma TAG *in vivo*⁽⁵⁸⁾, which in turn reduce CETP activity and increase the cholesterol content in HDL. In addition, a study conducted in mice has shown that RCT was enhanced in animals fed an *n*-3 PUFA diet compared with those fed an *n*-6 PUFA or saturated fat diet, despite having lower plasma HDL-C concentrations, suggesting a potential effect of *n*-3 PUFA on HDL function⁽⁵⁹⁾. In human subjects, while the effect of PUFA consumption on HDL-C concentration is modest^(60,61), PUFA may protect against CHD through several HDL- and non-HDL-dependent mechanisms.

In conclusion, long-chain *n*-3 PUFA have a greater effect on proteins involved in HDL metabolism and RCT than long-chain *n*-6 PUFA. Since the anti-atherogenic property of HDL is not simply determined by plasma HDL-C concentrations but depends also on the properties of HDL, the effects of these fatty acids on HDL functionality need to be investigated.

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