Docosahexaenoic acid suppresses apolipoprotein A-I gene expression through hepatocyte nuclear factor-3β1–3

Yu-Lin Kuang, K Eric Paulson, Alice H Lichtenstein, Nirupa R Matthan, and Stefania Lamon-Fava

ABSTRACT

Background: Dietary fish-oil supplementation has been shown in human kinetic studies to lower the production rate of apolipoprotein (apo) A-I, the major protein component of HDL. The underlying mechanism responsible for this effect is not fully understood.

Objective: We investigated the effect and the mechanism of action of the very-long-chain n–3 (omega-3) polyunsaturated fatty acid docosahexaenoic acid (DHA), relative to the saturated fatty acid palmitic acid (PA), on the hepatic expression of apo A-I in HepG2 cells.

Design: HepG2 cells were treated with different doses of DHA and PA (0–200 μmol/L). mRNA expression levels of apo A-I were assessed by real-time polymerase chain reaction, and apo A-I protein concentrations were measured by immunoassay. DHA dose-dependently suppressed apo A-I mRNA levels and also lowered apo A-I protein concentrations in the media, with maximum effects at 200 μmol/L. This concentration of fatty acids was used in all subsequent experiments.

Results: To elucidate the mechanism mediating the reduction in apo A-I expression by DHA, transfection experiments were conducted with plasmid constructs containing serial deletions of the apo A-I promoter. The DHA-responsive region was mapped to the −185 to −148 nucleotide region of the apo A-I promoter, which binds the hepatocyte nuclear factor (HNF)-3β. Nuclear extracts from cells treated with DHA or PA had a similar nucleotide abundance of HNF-3β. However, electrophoresis mobility shift assays showed less binding of HNF-3β to the −180 to −140 sequence of the apo A-I promoter than did PA-treated cells. As shown by chromatin immunoprecipitation analysis, less HNF-3β was recruited to the apo A-I promoter in DHA-treated cells than in PA-treated cells, which supports the concept of an interference of DHA with the binding of HNF-3β to the apo A-I promoter.

Conclusion: These findings suggest that, in human hepatoma HepG2 cells, DHA inhibits the binding of HNF-3β to the apo A-I promoter, resulting in the repression of apo A-I promoter transactivity and thus a reduction in apo A-I expression. Am J Clin Nutr 2011;94:594–600.

INTRODUCTION

Apolipoprotein (apo) A-I is the principal apolipoprotein in HDL, which plays an important role in the reverse cholesterol transport (RCT) pathway by facilitating the removal of excess cholesterol from peripheral tissues and its delivery back to the liver for utilization or elimination (1). An inverse relation between plasma HDL-cholesterol and apo A-I concentrations and the risk of coronary heart disease is well established (2). The antiatherogenic property of apo A-I has been clearly shown in animal studies: either the intravenous infusion of apo A-I in rabbits or the transgenic overexpression of human apo A-I in mice has resulted in a reduction in atherosclerosis (3, 4). Similarly, infusion of the human apo A-I precursor pro-apo A-I in patients with familial hypercholesterolemia has resulted in increased cholesterol fecal excretion, which implies a facilitative role of apo A-I in RCT (5). Injection of [3H]cholesterol-labeled macrophage foam cells into apo A-I-overexpressing mice has resulted in a greater rate of RCT from macrophages to the liver and feces than in control mice, which further supports the role of apo A-I in promoting increased efficiency of the RCT pathway (6). Additional properties of apo A-I that may contribute to its cardioprotective effects include antiinflammatory and antioxidant activities (7–9).

Habitual fish consumption has been associated with a reduction in coronary heart disease risk (10). The supplementation of 2 major very-long-chain n–3 polyunsaturated fatty acids (PUFAs) from fish, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has also been shown to be beneficial in the secondary prevention of coronary heart disease (11). Most intervention studies have shown no significant effect of fish-oil supplementation on plasma apo A-I concentrations (12–15). However, kinetic studies have shown that, within the context of unchanged plasma apo A-I protein concentrations, supplementation with high doses of very-long-chain n–3 PUFAs resulted in a significant decrease in both apo A-I production and clearance rates (14, 15). To date, the underlying mechanism of how very-long-chain n–3 PUFAs influence apo A-I production through gene expression regulation has not been defined.

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Apo A-I is synthesized and secreted primarily by the liver and the intestine (16). Some nutrients have been shown to regulate the expression of the apo A-I gene through transcription factors capable of binding to its promoter region (17). The proximal promoter region of the apo A-I gene is well characterized, with the −214 to −192 nucleotide region interacting with peroxisome proliferator–activated receptors (PPARs) (18), 9-cis-retinoic acid receptors (19), and hepatocyte nuclear factor (HNF)-4α (20) and the −169 to −146 nucleotide region binding to HNF-3β (21). Because very-long-chain n−3 PUFAs are known to modulate the activities of several transcription factors, including PPARs (22), HNF-4α (23), and retinoic acid receptor α (24), we hypothesized that very-long-chain n−3 PUFAs decrease apo A-I production at the transcriptional level by regulating the activities of transcription factors binding to the apo A-I promoter. The aim of this investigation was to determine the role of DHA in the regulation of hepatic apo A-I.

MATERIALS AND METHODS

Cell culture and fatty acid treatments

HepG2 cells (ATCC, Rockville, MD) were maintained in DMEM (Invitrogen, Carlsbad, CA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Hyclone, Logan, UT), 1% GlutaMax, 100 μmol nonessential amino acids/L, 100 U penicillin/mL, and 100 μg 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. Palmitic acid (PA, 16:0) (Nu-Chek, Elysian, MN) and DHA (22:6n−3) (Sigma-Aldrich, St Louis, MO), in the form of sodium salts, were dissolved in water and combined with fatty acid–free BSA (Sigma-Aldrich) dissolved in DMEM in a molar ratio of 2:1. BSA was used as a control. Cells were cultured in DMEM containing 10% LD-FBS for 24 h before fatty acid treatments and were then incubated for 24 h with BSA or BSA fatty acid complexes at the concentrations indicated in DMEM containing 10% LD-FBS. Cell viability after fatty acid treatments was monitored by trypan blue exclusion. No change in viability was observed up to a fatty acid concentration of 200 μmol/L, but increased cell death was observed at higher concentrations.

Fatty acid analysis

At the end of treatment with fatty acids, HepG2 cells were harvested. Cell lipid extraction and total fatty acid analysis were performed as previously described (25). Cellular fatty acid profile is expressed as molar percentage (mol%) of total fatty acids.

Quantitative real-time PCR

Total cellular RNA was extracted by using TRIzol reagent and reverse transcribed with random hexamers by using SuperScript III First Strand Synthesis Kit (Invitrogen, Carlsbad, CA) following the manufacturer’s protocol. The mRNA levels of apo A-I and of the housekeeping gene GAPDH were measured by using the Applied Biosystems 7300 Real-Time PCR system (V1.4 SDS software; Applied Biosystems, Carlsbad, CA) with power SYBR Green Master Mix (Applied Biosystems, Foster City, CA) by using the following primer sets designed by Primer Express Software V2.0: apo A-I forward 5′-GCC TTG GGA AAA CAG CTA AAC C-3′, apo A-I reverse 5′-TCC TGG GTC ACA GGG CC-3′, GAPDH forward 5′-CCT GTT CGA CAG TCA GCC G-3′, GAPDH reverse 5′-CGA CCA AAT CCG TTG ACT CC-3′. The relative quantification (Comparative C_T, ΔΔC_T) method was used to determine the expression of the target genes.

Enzyme-linked immunosorbent assay

The media of HepG2 cells were collected after 24 h incubation with fatty acids, and the concentration of apo A-I was measured with an enzyme-linked immunosorbent assay (ELISA) developed in our laboratory, as previously described (26). Apo A-I concentrations were normalized by the total cellular protein concentration.

Transient transfections

The luciferase reporter constructs (pGL2 basic vector; Promega, Madison, WI) containing serial deletions of the human apo A-I promoter were previously described (27). The plasmid containing 3 copies of a PPAR response element (PPRE) upstream of the tk promoter, 3xPPRE-tk-pGL3 was a generous gift from Dr MW Kilgore (28). Transient transfection experiments were carried out overnight in 12-well plates by using 0.4 μg of the test plasmid per well and 0.03 μg of the Renilla luciferase plasmid (Promega) per well as an internal control reporter and using the FuGENE 6 Transfection Reagent (Roche, Indianapolis, IN) in DMEM containing 10% LD-FBS. Transfected cells were then treated with 0.1% ethanol (vehicle, control), 1 μmol GW7647/L (PPARα activator; Cayman Chemicals, Ann Arbor, MI) in 0.1% ethanol, or 200 μmol PA/L and 200 μmol DHA/L in DMEM containing 10% LD-FBS for 24 h. The cell lysates were collected and stored at −70°C until luciferase activities were measured with the Dual-Luciferase Reporter Assay System (Promega).

Western blot

Nuclear and cytoplasmic protein extracts were prepared with NE-PER Reagents (Pierce Biotechnology, Rockford, IL) according to the manufacturer’s protocol. Nuclear protein extracts (15 μg) or cytoplasmic protein extracts (15 μg) were separated on 7.5% polyacrylamide gels, transferred to nitrocellulose membranes, and blotted with a specific antibody against HNF-3β, Histone H1, or GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA).

Electrophoretic mobility shift assay

The complementary oligonucleotides spanning the −180 to −3 nucleotide region of the human apo A-I promoter were end-labeled with the Biotin 3′ End DNA Labeling Kit (Pierce Biotechnology). The biotin-labeled double-strand probe was incubated with 5 μg nuclear protein extract, and the DNA–protein complexes were analyzed by 5% native polyacrylamide gel electrophoresis and transferred to a nylon membrane. Biotin-labeled DNA-protein complexes were then detected by using streptavidin conjugated with horseradish peroxidase and chemiluminescent substrate with the LightShift Chemiluminescent EMSA (electrophoretic mobility shift assay) Kit (Pierce Biotechnology). For supershift analysis, nuclear extracts were preincubated with an HNF-3β antibody (Santa Cruz Biotechnology) for 30 min at room temperature before the addition of the labeled probe. Competition studies were performed by adding 200-fold molar excess unlabeled probe to
the binding reaction. Results were quantified by using a GS-710 calibrated imaging densitometer with Quantity One software (Bio-Rad Laboratories, Hercules, CA).

Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (ChIP) assays were performed by using the SimpleChIP Enzymatic Chromatin IP Kit (Cell Signaling Technology, Danvers, MA) according to the manufacturer’s protocol. Briefly, fatty acid-treated HepG2 cells were fixed with 1% formaldehyde and then lysed. Nuclei were pelleted and chromatin was fragmented by nuclease digestion, and released from the nuclei with brief sonication. The cross-linked chromatin preparations were then subjected to immunoprecipitation at 4°C overnight with either the HNF-3β antibody (Santa Cruz Biotechnology) or the negative control Normal Rabbit IgG (Cell Signaling Technology) at a concentration of 4 μg/mL. For quantifications, ChIP-enriched DNA samples from 3 independent experiments were analyzed by real-time PCR with specific primers that amplify −214 to −78 of the apo A-I promoter region: forward 5′-CGG CCC TGT TGC TGC TCA CT-3′ and reverse 5′-ACT GAA CCC TTG ACC CCT GC-3′. Data were normalized to the input DNA.

Statistical analysis

Treatments were performed in duplicate, and mean values of ≥ 3 independent experiments are presented as means ± SDs. The statistical analysis was performed with the SAS 9.1 statistical package. Significant differences between treatments were assessed by ANOVA followed by Tukey’s post hoc test, 2-tailed Student’s t test, or linear regression (test for trend) as indicated. A P < 0.05 was considered significant.

RESULTS

Cell viability and fatty acid profile

Cell viability after both PA and DHA treatments at concentrations up to 200 μmol/L for 24 h was ≥90%. The incorporation of the treatment fatty acid into HepG2 cells was evident from the cellular fatty acid profile after treatments (Table 1). Cells treated with PA had a significantly higher concentration of PA (26.4 mol%) than did the cells treated with control or DHA (21.7 and 23.8 mol%, respectively), and DHA-treated cells had a significantly higher concentration of DHA (20.2 mol%) than did the control or PA-treated cells (1.2 and 1.0 mol%, respectively).

DHA suppresses apo A-I gene expression and protein secretion

The dose-response effects of PA and DHA on HepG2 apo A-I mRNA levels were analyzed by real-time PCR. Whereas there was a lack of dose effect for PA, incubation of HepG2 cells with DHA for 24 h dose-dependently down-regulated apo A-I mRNA levels with a maximum inhibitory effect at 200 μmol/L concentration (Figure 1). Compared with PA treatments at the same concentration, DHA treatment at 50 and 200 μmol/L significantly reduced apo A-I mRNA levels. Consistent with the decrease in apo A-I mRNA levels, apo A-I concentrations in the media of HepG2 cells treated for 24 h with 200 μmol DHA/L was significantly lower than those in cells treated with 200 μmol PA/L (Figure 2).

The DHA-responsive region is mapped to the −185 to −148 sequence of the apo A-I promoter region

To determine the mechanism of DHA-mediated suppression in apo A-I mRNA levels, we first investigated the involvement of PPAR, because a PPRE is located between nucleotides −214 and −192 of the apo A-I promoter (18), and DHA is known to activate PPAR (22). In HepG2 cells transfected with a PPRE reporter construct, GW7647 significantly increased PPARα activity (Figure 3). DHA also significantly up-regulated PPAR activity by 30% compared with PA treatment (Figure 3). This

### Table 1

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control</th>
<th>PA</th>
<th>DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFAs</td>
<td>mol%</td>
<td>mol%</td>
<td>mol%</td>
</tr>
<tr>
<td>16:0</td>
<td>32.52 ± 0.181</td>
<td>35.718 ± 0.471</td>
<td>35.563 ± 0.289</td>
</tr>
<tr>
<td>18:0</td>
<td>21.708 ± 0.107</td>
<td>26.390 ± 0.669</td>
<td>23.794 ± 0.206</td>
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<tr>
<td>MUFAs</td>
<td>58.452 ± 0.299</td>
<td>57.314 ± 0.130</td>
<td>39.428 ± 2.632</td>
</tr>
<tr>
<td>18:1n–9</td>
<td>21.538 ± 0.619</td>
<td>18.641 ± 0.896</td>
<td>15.877 ± 1.264</td>
</tr>
<tr>
<td>PFAs</td>
<td>9.023 ± 0.125</td>
<td>6.968 ± 0.395</td>
<td>25.014 ± 2.432</td>
</tr>
<tr>
<td>n−6 PFAs</td>
<td>7.605 ± 0.275</td>
<td>5.858 ± 0.454</td>
<td>3.897 ± 0.325</td>
</tr>
<tr>
<td>18:2n−6</td>
<td>0.700 ± 0.104</td>
<td>0.757 ± 0.089</td>
<td>0.180 ± 0.046</td>
</tr>
<tr>
<td>18:3n−6</td>
<td>0.044 ± 0.009</td>
<td>0.040 ± 0.010</td>
<td>0.053 ± 0.016</td>
</tr>
<tr>
<td>20:4n−6</td>
<td>1.951 ± 0.203</td>
<td>1.505 ± 0.094</td>
<td>1.415 ± 0.060</td>
</tr>
<tr>
<td>n−3 PFAs</td>
<td>1.418 ± 0.176</td>
<td>1.109 ± 0.062</td>
<td>21.117 ± 2.740</td>
</tr>
<tr>
<td>18:3n−3</td>
<td>0.085 ± 0.027</td>
<td>0.055 ± 0.019</td>
<td>0.104 ± 0.039</td>
</tr>
<tr>
<td>20:5n−3</td>
<td>0.131 ± 0.026</td>
<td>0.103 ± 0.040</td>
<td>0.796 ± 0.232</td>
</tr>
<tr>
<td>22:6n−3</td>
<td>1.202 ± 0.176</td>
<td>0.952 ± 0.072</td>
<td>20.217 ± 2.484</td>
</tr>
</tbody>
</table>

All values are means ± SDs from 3 independent experiments. Only individual fatty acids of interest are shown. PA, palmitic acid; DHA, docosahexaenoic acid; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PFAs, polyunsaturated fatty acids; mol%, molar percentage. Values in the same row with different superscript letters are significantly different, P < 0.05 (ANOVA followed by Tukey’s adjustment).

### Figure 1

Suppression of apolipoprotein (apo) A-I gene expression by docosahexaenoic acid (DHA). Apo A-I mRNA concentrations were measured in HepG2 cells treated with 50, 100, or 200 μmol palmitic acid (PA) or DHA/L for 24 h. The results were normalized to glyceraldehyde 3-phosphate dehydrogenase mRNA concentrations and expressed as fold changes relative to the values of cells treated with bovine serum albumin control. Values are means ± SDs from 3 independent experiments of duplicates. *P < 0.05, **P < 0.01. *P for trend < 0.05 for dose-dependent effect.
treated with bovine serum albumin control. Values are means ± 3 independent experiments of duplicates. *Significantly different from PA, P < 0.05 (2-tailed Student’s t test).

result indicates that DHA activates PPAR in HepG2 cells and thus would be predicted to enhance apo A-I promoter activity and increase apo A-I mRNA expression. Therefore, it is likely that other DHA-mediated mechanisms cause the overall reduction in apo A-I mRNA expression observed in the present experiment.

Next, the transcriptional activity of the apo A-I promoter in the presence of DHA or PA was tested in transient transfection experiments. With a plasmid construct containing the −256 to +396 region of the apo A-I gene, which includes all the regulatory elements necessary for expression of the apo A-I gene in hepatic cells, we observed a 30% suppression of the promoter activity by 200 μmol DHA/L, compared with 200 μmol PA/L (Figure 4). The DHA-mediated reduction in apo A-I promoter transcriptional activity agreed with the observed reduction in apo A-I mRNA expression. In contrast, cells transfected with a plasmid construct containing 2 copies of the apo A-I promoter-specific PPRE (A; Figure 4) showed a 48% increase in the apo A-I promoter activity with DHA treatment compared with PA treatment. Similarly, DHA treatment of cells transfected with the plasmid construct containing the −256 to −185 region of the apo A-I promoter resulted in a 23% increase in promoter activity compared with PA treatment (Figure 4). These results agree with the activation of PPAR by DHA shown in Figure 3.

To locate the region mediating the suppression of the apo A-I promoter activity by DHA and thus counteracting the effect of PPAR activation, cells were transfected with plasmid constructs containing serial deletions of the −256 to −41 region of the apo A-I gene and treated with 200 μmol/L of PA or DHA for 24 h. The suppressive effect of DHA was still maintained with a plasmid containing the −256 to −148 region of the promoter, but this effect was abolished with a plasmid containing the −256 to −185 region of the promoter (Figure 4). These results indicate that the region mediating the repression of DHA on apo A-I promoter activity is located between nucleotides −185 and −148, which causes the overall reduction in both apo A-I promoter activity and apo A-I mRNA levels.

DHA suppresses HNF-3β binding to apo A-I promoter

The DHA-responsive region identified by the promoter serial deletion experiments contains the binding site for HNF-3β (nucleotides −169 to −146). We performed EMSA to study whether DHA treatment affects the binding activity of HNF-3β to the apo A-I promoter. As shown in Figure 5A, nuclear protein extracts formed several complexes with the −180 to −140 region of the apo A-I promoter (lanes 1–3). The specificity of the interactions was evident from the competition experiment, in which 4 labeled complexes were displaced in the presence of excess unlabeled probe (lanes 4–6). To identify HNF-3β as one of the specific transcription factors binding to this region, a specific antibody against HNF-3β was used, which showed formation of a supershift band (lanes 7–9). The nuclear extracts from HepG2 cells treated with DHA showed less (mean reduction of 47% from 3 independent experiments) binding of HNF-3β to the apo A-I promoter than did the extracts from PA-treated cells, as assessed by quantification of the supershift bands (Figure 5A; lane 8 compared with lane 7). The concentration of HNF-3β in the nuclear and cytoplasmic extracts from cells treated with PA, DHA, or BSA (control) was determined by Western blot. DHA treatment did not significantly alter the nuclear abundance of HNF-3β compared with PA treatment (Figure 5B). We did not observe HNF-3β in the cytoplasmic fraction under any of our treatment conditions (Figure 5C). This suggests the possibility of interference of the binding of nuclear HNF-3β to the apo A-I promoter by DHA.

The interference of DHA with the HNF-3β binding to apo A-I promoter was further assessed by using the ChIP assay. The recruitment of HNF-3β to the apo A-I promoter was studied in HepG2 cells treated with PA, DHA, or BSA control. DNA co-precipitated with HNF-3β antibodies was analyzed by real-time PCR with specific primers amplifying the apo A-I promoter. The specificity of the immunoprecipitation was determined by using an unrelated antibody against normal rabbit IgG. Consistent with the EMSA findings, DHA treatment significantly reduced the association of HNF-3β with the apo A-I promoter (70%) compared with PA treatment (Figure 6).

DHA REPRESSION OF THE APO A-I PROMOTER THROUGH HNF-3β
DISCUSSION

It is well established that plasma apo A-I concentrations are inversely correlated with atherosclerosis (2). Dietary fish-oil supplementation was previously shown in human kinetic studies to decrease the production rate of apo A-I without changing its plasma concentration (14, 15). However, the mechanism of action by which very-long-chain n-3 PUFAs inhibit apo A-I expression is not yet well understood. In the current study, we focused on how DHA influences hepatic apo A-I gene regulation. PA was used as the reference fatty acid because it is the dominant saturated fatty acid in the typical US diet (29) and also in human plasma (30, 31). The cellular fatty acid profile indicated the incorporation of both PA and DHA into HepG2 cells. It also suggested a relatively low retroconversion rate of DHA to EPA within the 24-h DHA treatment, although cells treated with DHA had a significantly higher concentration of EPA than did cells treated with control or PA.

Our results show that DHA treatment decreases hepatic cellular apo A-I mRNA levels and medium apo A-I concentrations. This finding agrees with that of a previous study, in which supplementation with very-long-chain n-3 PUFAs (4 g fish oil/d for 6 wk) decreased the production rate of apo A-I in insulin-resistant obese men (14). Similarly, patients with diabetes treated with maxEPA (1.08 g EPA + 0.72 g DHA + 10.5 mg α-tocopherol acetate per day for 2 mo) also had a significantly lower apo A-I production rate (15). In rats fed isocaloric diets enriched with different dietary fatty acids, a significant decrease in liver apo A-I mRNA levels was also observed after fish-oil feeding compared with saturated fat (32). In contrast, n-3 PUFA supplementation did not alter the hepatic expression of apo A-I mRNA in another study in rats compared with saturated fatty acid, monounsaturated fatty acid, or n-26 PUFA supplementation (33). The physiologic significance of the observed reduction in apo A-I expression by n-3 PUFA is not clear.

In HepG2 cells, consistent with the reduction in mRNA levels, DHA decreased apo A-I promoter activity. A previous study showed decreased plasma apo A-I and liver apo A-I mRNA levels in wild-type but not in PPARα-deficient mice after fish-oil feeding, which suggests that PPARα may play a role in the effect of fish oil on apo A-I gene expression (34). In our study, however, PPAR did not directly contribute to the DHA-mediated reduction in apo A-I promoter activity, as suggested by an increased activity of the plasmid construct containing the −256 to −185 region or 2 copies of the −214 to −192 region after DHA supplementation.

FIGURE 4. Analysis of the human apolipoprotein (apo) A-I promoter. HepG2 cells were transiently cotransfected with serial deletion constructs of the apo A-I promoter and a Renilla luciferase plasmid, which was followed by treatment with 200 μmol of palmitic acid (PA) or docosahexaenoic acid (DHA)/L for 24 h. The effect of DHA on apo A-I promoter activity was measured by relative luciferase activities and expressed as the percentage change relative to PA treatment of the plasmid construct containing the −256 to +396 region of the apo A-I gene. Values are means ± SDs from ≥3 independent experiments conducted in triplicate. A, apo A-I promoter-specific peroxisome proliferator-activated receptor response element (−214 to −192 region of the apo A-I gene).

FIGURE 5. Interference of hepatocyte nuclear factor (HNF)-3β binding to apolipoprotein (apo) A-I promoter in vitro and modulation of HNF-3β nuclear abundance by docosahexaenoic acid (DHA). A: Five micrograms of nuclear protein extracts from HepG2 cells treated with 200 μmol palmitic acid (PA)/L, 200 μmol DHA/L, or bovine serum albumin (BSA) control were incubated with biotin-labeled apo A-I promoter region −180 to −140. Specific HNF-3β antibody or 200-molar excess of unlabeled apo A-I promoter was added to the reaction mixture where indicated. The specific DNA-protein complexes are indicated by arrows on the left. B: Fifteen micrograms of nuclear protein extracts. C: Fifteen micrograms of cytoplasmic protein extracts from HepG2 cells treated with 200 μmol PA/L, 200 μmol DHA/L, or BSA control were separated on 7.5% polyacrylamide gels. Amounts of HNF-3β, histone H1, and GAPDH (glyceraldehyde 3 phosphate dehydrogenase) were determined by Western blot with specific antibodies.
HNF-3β (also known as Foxa2) is a member of the forkhead transcription factor family and regulates many liver-specific genes (35, 36). HNF-3β was found to activate hepatic apo A-I expression through its interaction with the −169 to −146 region of the apo A-I promoter (21) and to function synergistically with HNF-4α and additional co-activators (37, 38). The disruption of the −214 to −192, −169 to −146, or −134 to −119 nucleotide regions of the apo A-I promoter, individually or in combination, all significantly reduced its transcriptional activity (21). This phenomenon was observed in our study, in which plasmid constructs containing serial deletions of the apo A-I promoter had lower basal luciferase activities than did the full promoter, which contained the −256 to +396 region of the apo A-I gene.

We showed that deleting the −185 to −148 region of the apo A-I promoter completely abolished the ability of DHA to inhibit apo A-I promoter activity, implying the requirement of this region for the DHA-mediated apo A-I suppression and a potential role for HNF-3β in the regulation of DHA on apo A-I gene expression. We observed a 43% decrease in the abundance of HNF-3β mRNA in HepG2 cells treated with 200 μmol DHA/L compared with cells treated with 200 μmol PA/L (data not shown). However, the nuclear HNF-3β protein concentration did not differ between treatment groups. The possible posttranscriptional or posttranslational modifications responsible for the differential effect of DHA on HNF-3β mRNA and protein need to be further confirmed and investigated. It has been suggested that nutrition states influence HNF-3β localization in a phosphorylation-dependent (Thr 156) manner (39). However, we did not find HNF-3β in the cytoplasmic fraction of cells treated with either PA or DHA. This is consistent with the lack of cytoplasmic HNF-3β under normal conditions (39). Taken together, our data suggest that DHA suppresses the interaction of HNF-3β with the apo A-I promoter mainly by interfering with the binding of HNF-3β to the apo A-I promoter, rather than decreasing the nuclear abundance of HNF-3β, and thus caused the reduction in apo A-I promoter activity and mRNA expression. Recently, it was reported that very-long-chain n−3 PUFAs up-regulate the activity of AMP-activated protein kinase, which functions as an energy sensor within the cell (40). The activation of AMP-activated protein kinase has been shown to induce expression of the small heterodimer partner (41), an orphan nuclear receptor that is highly expressed in the liver and represses the transcriptional activity of many nuclear receptors, including HNF-3β (42, 43). It was shown that the small heterodimer partner physically interacts with HNF-3β and inhibits DNA binding of HNF-3β without influencing its subcellular localization (43). These observations provide a possible molecular mechanism of HNF-3β repression by very-long-chain n−3 PUFAs, which need to be further elucidated. The transcription factor NFY has also been shown to bind to the −175 to −148 region of the apo A-I promoter (44, 45). However, adding a specific antibody against NFY to the EMSA binding reaction with labeled apo A-I promoter did not supershift any of the specific DNA-protein complexes (data not shown), which suggests that it is unlikely that NFY plays a direct role in DHA-mediated apo A-I promoter regulation.

In human kinetic studies, the reduction in the apo A-I production rate by very-long-chain n−3 PUFAs was coupled with a significant decrease in the apo A-I fractional catabolic rate; therefore, the plasma apo A-I concentration remained unchanged (14, 15). This lack of change in plasma apo A-I concentrations after very-long-chain n−3 PUFA supplementation has also been observed in other studies (12, 13). Thus, the suppressive effect of DHA on apo A-I production may not directly translate into an increased risk of coronary heart disease. However, significantly lower plasma concentrations of apo A-I were found in Cynomolgus monkeys fed an atherogenic diet containing fish oil (46) and in rats fed an n−3 PUFA–supplemented diet (33) than in animals fed saturated fat. Furthermore, the effect of very-long-chain n−3 PUFAs on plasma HDL-cholesterol concentrations is inconsistent between studies. This may be due the fact that HDL metabolism is very complex, with many other factors that influence HDL-cholesterol concentrations besides apo A-I expression. Fish oil with very-long-chain n−3 PUFAs is often prescribed for patients with hypertriglyceridemia because of its well-established triglyceride-lowering effect (47, 48). Lowering plasma triglyceride concentrations reduces cholesteryl ester transfer protein activity and therefore increases the cholesterol content in HDL. It has also been suggested that very-long-chain n−3 PUFAs alter the composition of phosphatidylcholine in HDL, which makes it a poor substrate for lecithin-cholesterol acyltransferase (46). The change in activity of these enzymes would also affect HDL metabolism. Hence, the mechanisms of action responsible for the effect of fish-oil very-long-chain n−3 PUFAs on HDL and apo A-I metabolism remain open to further investigations.

In summary, we showed that DHA inhibits the binding of HNF-3β to the apo A-I promoter, which results in the repression of apo A-I promoter transactivity and thus a reduction in apo A-I expression in human hepatoma cells. Our findings elucidate the mechanism for the decreased production of apo A-I with very-long-chain n−3 PUFA supplementation observed in human kinetic studies.

The authors’ responsibilities were as follows—Y-LK and SL-F: designed the overall research project; Y-LK: conducted the research and analyzed the data; Y-LK, KEP, AHL, and SL-F: contributed to the data interpretation; Y-LK: wrote the initial draft, which was modified after feedback from all of the coauthors; and Y-LK and SL-F: had primary responsibility for the final content. None of the authors had a conflict of interest.
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