Fatty Acids Regulate Endothelial Lipase and Inflammatory Markers in Macrophages and in Mouse Aorta

A Role for PPARγ

Un Ju Jung, Claudia Torrejon, Chuchun L. Chang, Hiroko Hamai, Tilla S. Worgall, Richard J. Deckelbaum

Objective—Macrophage endothelial lipase (EL) is associated with increased atherosclerosis and inflammation. Because of their anti-inflammatory properties we hypothesized that n-3 fatty acids, in contrast to saturated fatty acids, would lower macrophages and arterial EL and inflammatory markers.

Methods and Results—Murine J774 and peritoneal macrophages were incubated with eicosapentaenoic acid or palmitic acid in the presence or absence of lipopolysaccaride (LPS). LPS increased EL mRNA and protein. Palmitic acid alone or with LPS dose-dependently increased EL mRNA and protein. In contrast, eicosapentaenoic acid dose-dependently abrogated effects of LPS or palmitic acid on increasing EL expression. EL expression closely linked to peroxisome proliferator activated receptor (PPAR)γ expression. Eicosapentaenoic acid blocked rosiglitazone (a PPARγ agonist)-mediated EL activation and GW9662 (a PPARγ antagonist)-blocked palmitic acid-mediated EL stimulation. Eicosapentaenoic acid alone or with LPS blunted LPS-mediated stimulation of macrophage proinflammatory interleukin-6, interleukin-12p40, and toll-like receptor-4 mRNA and increased anti-inflammatory interleukin-10 and mannose receptor mRNA. In vivo studies in low density lipoprotein receptor knockout mice showed that high saturated fat rich diets, but not n-3 diets, increased arterial EL, PPARγ, and proinflammatory cytokine mRNA.

Conclusion—n-3 fatty acids, in contrast to saturated fatty acids, decrease EL in parallel with modulating pro- and anti-inflammatory markers, and these effects on EL link to PPARγ. (Arterioscler Thromb Vasc Biol. 2012;32:2929-2937.)

Key Words: atherosclerosis ■ endothelial lipase ■ inflammation ■ n-3 fatty acids ■ PPARγ

Inflammation adversely affects arterial wall biology.12 Much evidence supports a proatherogenic and proinflammatory effect of saturated fatty acids (FA).3,4 In contrast, protective actions with respect to the arterial wall have been attributed to n-3 FA.3–5 n-3 FA delivered from dietary fish oil are incorporated into atherosclerotic plaques, enhancing stability, whereas n-6 FA do not have these effects.5 Recent reviews indicate that increased consumption of long-chain n-3 FA, eicosapentaenoic acid (EPA), and docosahexaenoic acid, but not of α-linolenic acid (their n-3 essential FA precursor), reduced the rates of all-cause mortality and cardiac and sudden death.6 n-3 FA have been shown to reduce the macrophage infiltration into the vessel wall and secretion of proatherogenic and proinflammatory growth factors and cytokines by monocytes and macrophages.7

Macrophages play a pivotal role in the development and progression of atherosclerosis. Endothelial lipase (EL) is 1 of several lipases synthesized and secreted by macrophages. High levels of EL expression have been observed in macrophages present in human atherosclerotic plaques.8 EL deficiency is associated with a ≈70% decrease in atherosclerotic lesions in apoE KO mice.9 A proatherogenic effect of EL in macrophages has also been ascribed to bridging functions, which plays a role in the uptake of lipoproteins10 or recruitment of monocytes by blood vessel walls.11 Moreover, EL expression is upregulated in macrophages by proinflammatory cytokines.10 Upregulation of macrophage EL by toll-like receptor (TLR) 4 and 3 negatively modulates interleukin (IL)-10 and positively modulates IL-12 production, potentially influencing atherosclerosis.12 Accordingly, EL might be considered to be an attractive pharmacological target in the prevention of atherosclerosis.

Peroxisome proliferator activated receptor (PPAR)γ also has been implicated in atherogenesis. PPARγ is highly expressed in macrophages-derived foam cells in atherosclerotic lesions13 and activation of PPARγ has been shown to induce macrophage lipid accumulation by increasing the expression of the oxidized low density lipoprotein (LDL) scavenger receptor CD36 and lipoprotein lipase (LpL).14–16 In contrast to the proposed potentially proatherogenic effects of PPARγ, other limited evidence suggests PPARγ may mediate anti-inflammatory effects by negatively regulating proinflammatory cytokine expression.16 Also, macrophage PPARγ deficiency increases atherosclerosis in C57BL/6 and LDL receptor knockout (LDL-R KO) mice.17
indicating an antiatherogenic role for PPARγ. However, at present, no definitive studies support the premise that PPARγ is required for anti-inflammatory effects in macrophages. In fact, recent clinical trials have raised concerns on increased risk of myocardial infarction and cardiovascular death in diabetic patients treated with rosiglitazone. A strong PPARγ agonist.

We previously demonstrated that high saturated fat (SAT) diets increased contributions of LDL selective uptake to total arterial LDL-cholesteryl ester deposition and that increased selective uptake parallels increased LpL levels and distribution in the arterial wall. In contrast, n-3-rich diets decreased arterial total LDL delivery and abrogated LDL selective uptake in parallel with changing arterial wall LpL. We now questioned whether FA would regulate EL expression as different dietary FA modulated arterial LpL levels and distribution as described in our previous reports. Specifically, we asked whether n-3 FA, EPA, in contrast to a saturated FA, palmitic acid (PA), would decrease expression of EL and, if so, would these changes correlate with changes in inflammatory markers and in PPARs, which may also modulate lipid metabolism and inflammatory responses.

Our results demonstrate that PA increases EL expression and decreases anti-inflammatory IL-10 expression in macrophages. In contrast, EPA decreases EL expression, in parallel with decreasing proinflammatory markers and increasing anti-inflammatory markers. The changes in macrophage EL by FA were strongly related to the regulation of PPARγ. Moreover, LDL-R KO mice fed SAT diets, but not n-3 diets, showed the increases in EL, PPARγ, and proinflammatory responses in the arterial wall.

Materials and Methods

Methods are described in more detail in the online-only Data Supplement.

Cell Culture

Murine macrophage-like cells, J774 (A2), were grown in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum (vol/vol), 1% glutamine (vol/vol), and 1% penicillin/streptomycin (vol/vol). Thioglycolate-elicited macrophages were obtained from C57BL/6 mice (12–14 weeks old) by peritoneal lavage with phosphate-buffered saline at 4 days after injection of 1 mL of 3.8% thioglycollate broth (Sigma-Aldrich). Cells were suspended in Dulbecco’s RPMI 1640 supplemented with 10% fetal bovine serum (vol/vol), 1% glutamine (vol/vol), and 1% penicillin/streptomycin (vol/vol), and incubated at 37°C for 3 hours. For all experiments, the cells were washed twice with phosphate-buffered saline and the FA-containing media were added at different doses for 3 hours, whereas the control cells received only bovine serum albumin medium. Then, cells were incubated with lipopolysaccharide (LPS; 1 μg/mL), PPARγ agonist, or antagonist for 4 hours (for mRNA) or 21 hours (for protein).

For experiments requiring PPARγ mRNA knock-down, J774 cells (50–70% confluence) were transfected with optimized concentrations of either mouse PPARγ short hairpin RNA (shRNA) plasmid (Santa Cruz Biotechnology, Inc), or control nonsense shRNA plasmid using shRNA transfection reagent (sc-29528), according to the manufacturer’s instructions. Twenty-four hours after transfection, cells were treated with specific FA and processed for real-time polymerase chain reaction analyses as described below.

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was isolated with TRIzol reagent (Invitrogen) and quantitative real-time polymerase chain reaction was carried out on an iCycler real-time machine (BioRad) using the SYBR Green polymerase chain reaction master kit (Applied Biosystems). Values were normalized to GAPDH levels.

Western Blot

EL protein expression was analyzed by Western blot normalized to β-actin protein.

Animals and Diets

Eight-week-old male LDL-R KO mice were purchased from Jackson Laboratory. After 1 week acclimatization, mice were fed a semipurified, normal chow (total 5% fat, 0.02% cholesterol, wt/wt) or a high-fat, semipurified diet (total 19% fat, 0.2% cholesterol, wt/wt) enriched in either n-3 (9% menhaden fish oil and 9% corn oil; Harlan Teklad; TD. 07500) or SAT (78% SAT from coconut oil, 13% monounsaturated fat from olive oil, and 9% polysaturated fat from corn oil; Harlan Teklad; TD. 08081) for 12 weeks similar to our previous report. The aorta was dissected and measured for mRNA of EL, PPARγ, and specific inflammatory markers. All procedures were approved by the Institutional Animal Care and Use Committee of Columbia University.

Statistical Analyses

Statistical analyses were determined by 1-way ANOVA (for comparing FA), 2-tailed Student’s t-test, or Pearson correlation coefficients. Data are expressed as means±SE.

Results

LPS Increases EL Expression in Macrophages

We first examined the effect of LPS, a potent endotoxin, on EL expression in macrophages. J774 cells were incubated with increasing concentration of LPS (0.25–10 μg/mL). LPS increased EL mRNA by a maximum of 2.5-fold at 1 μg/mL (Figure 1A in the online-only Data Supplement). Similarly, LPS dose-dependently increased protein levels of EL with a maximum increase also at 1 μg/mL (Figure 1B in the online-only Data Supplement). We thus chose concentration of LPS of 1 μg/mL for the experiments below.

EPA Inhibits the Increase of EL mRNA and Protein Induced by LPS More Than Other FA

To determine the effects of different FA on macrophage EL mRNA expression, J774 cells were cultured in the presence or absence of LPS, 1 μg/mL, for 4 hours after preincubation with 150 μmol/L of unsaturated FA (EPA; arachidonic acid; linoleic acid), monounsaturated FA (oleic acid, OA), and saturated FA (PA) for 3 hours. Exposure of cells to linoleic acid and PA alone significantly increased macrophage EL mRNA levels compared with non-FA control by 2.2-fold and 2.6-fold, respectively (Figure 1A). PA together with LPS induced 1.4-fold higher levels of EL mRNA compared with LPS alone. In contrast, EPA, arachidonic acid, and oleic acid inhibited LPS-induced EL mRNA levels by 64, 37, and 39%, respectively. Among FA tested, PA induced the strongest response on increasing EL expression, whereas EPA was most potent at inhibiting EL expression induced by LPS.

As EPA and PA seemed to show the greatest differences on EL mRNA expression, we chose EPA and PA for dose-response experiments. J774 cells were cultured in the presence or absence of LPS, 1 μg/mL, for 4 to 21 hours after preincubation with 50, 150, 300 μmol/L of EPA or PA for 3 hours. PA alone as well as together with LPS dose-dependently decreased EL mRNA (Figure 1B and 1C). In contrast, EPA alone had no effect on EL mRNA but completely abrogated effects of LPS...
on increasing EL mRNA. Consistent with mRNA expression, EPA had no significant effect on EL protein expression but inhibited LPS-mediated increase of EL protein (Figure 1D and 1E). PA alone as well as with LPS increased EL protein levels in a dose-dependent manner.

**PA and LPS Have Similar Effects on EL Expression in Peritoneal Macrophages as in J774 Cells and EPA Inhibits LPS-Mediated Increase of EL Expression**

To determine whether EPA and PA also regulate EL expression in primary macrophages, we treated EPA and PA with or without LPS in peritoneal macrophages from C57BL/6 mice. Similar to J774 cells, LPS and PA alone significantly increased EL mRNA compared with control by 2.0- and 3.0-fold, respectively, whereas EPA significantly inhibited LPS-induced EL mRNA by ≈30% (Figure 1F). EL protein levels were also increased by LPS and PA, but EPA significantly abrogated effects of LPS on increasing EL protein (Figure 1G). Thus, EPA can mitigate the effects of PA-mediated increases of EL in different macrophage lines.

**EPA Inhibits PA-Mediated Increases of EL**

To determine effects of EPA together with PA on macrophage EL, we incubated cells with different ratios of EPA and PA (Figure 2). EL mRNA was lower in J774 cells treated with the combinations of PA plus EPA compared with the group treated only with PA; these differences became significant when EPA accounted for one third or more of total FA (Figure 2A). EL protein levels showed similar responses to incubations of PA versus PA and EPA (Figure 2B).
To determine how changes in PPARγ might affect macrophage EL expression, we treated cells with increasing concentrations of rosiglitazone or GW9662 in the presence or absence of EPA or PA. Rosiglitazone is a known PPARγ agonist and GW9662 is a PPARγ antagonist. Rosiglitazone (0.1–50 µmol/L) dose-dependently increased EL protein and mRNA, whereas GW9662 markedly blocked rosiglitazone-mediated increases of EL protein and mRNA in J774 cells (Figure II in the online-only Data Supplement; Figure 4A and 4B). Moreover, GW9662 significantly inhibited PA-induced EL mRNA and protein. Of note, EPA abrogated rosiglitazone-mediated increases of EL mRNA and protein in J774 cells (Figure 4C). PPARγ mRNA expression was dose-dependently increased by rosiglitazone, whereas GW9662 and EPA abrogate effects of rosiglitazone on increasing PPARγ mRNA in J774 cells (Figure III in the online-only Data Supplement). We also found that EPA inhibited the increase of mRNA levels of LpL and CD36, well known PPARγ target genes,15,16 after incubation with rosiglitazone (Figure IV in the online-only Data Supplement).

To determine whether the PPARγ agonist and antagonist also regulate EL expression in primary macrophages, we treated peritoneal macrophage with rosiglitazone and GW9662 in the presence of FA. Similar to J774 cells, rosiglitazone increased EL mRNA and protein by 70% and 60%, respectively, and EPA significantly blocked rosiglitazone-induced EL mRNA expression by 86% (Figure 4D). Also, GW9662 markedly blocked the PA and rosiglitazone-mediated increases of EL mRNA by 96% and 78%, respectively, and inhibited the increase in EL protein induced by PA and rosiglitazone (Figure 4D and 4E). Thus, EL in cultured cells in vitro closely linked to PPARγ and was regulated by FA, in part, through modifying macrophage PPARγ expression.

To further support the above association between PPARγ and EL expression, we performed experiments where we used shRNA mediated knock-down of PPARγ expression in J774 cells. We achieved a knock-down efficiency of >80% using PPARγ shRNA as compared with control shRNA controls (n=6; P<0.05). We then compared the effects of PA on increasing EL mRNA in these PPARγ knock-down J774 cells and found a mean 63% decrease in EL mRNA expression after incubation with PA compared with control levels (n=6, P=0.04). Thus, decreases in PPARγ blunt the ability of PA to increase EL expression in macrophages, indicating that EL is modulated, in part, by PPARγ-dependent pathways.

**Figure 2.** Effects of different ratios of eicosapentaenoic acid (EPA) and palmitic acid (PA) on endothelial lipase (EL). J774 macrophages were incubated with the indicated concentrations (EPA) and palmitic acid (PA) on endothelial lipase (EL). J774 cells, but not peritoneal macrophages, EPA alone or with LPS tended to increase PPARγ mRNA, whereas PA had no effect (data not shown). In contrast, PA alone as well as with LPS significantly increased PPARγ mRNA expression (Figure 3A and 3B). In addition, PPARγ mRNA positively correlated with EL mRNA in both macrophages, respectively (r=0.34, P<0.05; r=0.43, P<0.01).

EPA and PA Have Different Effects on Regulation of PPARγ Expression

Because PPARs are linked to a number of processes important to atherogenesis,14,28 we examined potential associations of PPARγ and PPARα with EL expression. PPARγ expression was more affected by FA than PPARα expression. In J774 cells, but not peritoneal macrophages, EPA alone or with LPS tended to increase PPARα mRNA, whereas PA had no effect (data not shown). In contrast, PA alone as well as with LPS significantly increased PPARγ mRNA expression (Figure 3A and 3B). In addition, PPARγ mRNA positively correlated with EL mRNA in both macrophages, respectively (r=0.34, P<0.05; r=0.43, P<0.01).

**Figure 3.** Effects of fatty acids (FA) on macrophage peroxisome proliferator activated receptor (PPARγ) mRNA expression. J774 (A) or peritoneal macrophages (B) were incubated with 150 µmol/L of eicosapentaenoic acid (EPA) or palmitic acid (PA) as previously described in Figure 1. *Means with unlike letters are significantly different at P<0.05 (1-way ANOVA). LPS indicates lipopolysaccharide; BSA, bovine serum albumin.
EPA Decreases Proinflammatory Markers, but Increases Anti-Inflammatory Markers in Peritoneal Macrophages

LPS is a bacterial endotoxin that is commonly used to stimulate inflammatory responses. Wang et al. reported that induction of macrophage EL by LPS can modulate macrophage inflammatory responses. To explore the relationships of EL and inflammatory markers, we compared well-defined pro- and anti-inflammatory markers in peritoneal macrophages incubated with EPA and PA. LPS significantly increased proinflammatory cytokines IL-6 and IL-12p40 (Figure 5A). However, EPA alone or with LPS blunted the stimulating effects of LPS on IL-6 and IL-12p40 mRNA by 62% and 60%, respectively. Also, EPA markedly attenuated TLR4 mRNA. We also found that in J774 cells, LPS increased IL-6 and IL-12p40 mRNA by 17- and 12-fold, respectively (P<0.001), and that these effects were diminished by EPA (data not shown). PA and EPA effects on tumor necrosis factor (TNF)-α expression were not similar to other proinflammatory markers; PA alone increased TNF-α mRNA level similar to LPS (LPS versus bovine serum albumin, 56%; PA versus bovine serum albumin, 55%), whereas EPA alone or with LPS had no effect. In contrast, anti-inflammatory IL-10 and mannose receptor were increased in EPA-treated cells by 2.1- and 1.5-fold, respectively (Figure 5B). PA had little effect on proinflammatory markers but decreased IL-10 mRNA (Figure 5A and 5B). Interestingly, EL mRNA showed positive correlations with increasing mRNA levels of pro-inflammatory markers, such as IL-6, IL-12p40, TLR4, and vascular cell adhesion molecule-1 (Figure V in the online-only Data Supplement). In contrast, there were negative correlations between EL and anti-inflammatory markers, IL-10 and mannose receptor, respectively. PPARγ mRNA was also positively correlated with proinflammatory IL-6, IL-12p40, TLR4, and vascular cell adhesion molecule-1 mRNA, whereas it was negatively correlated with anti-inflammatory IL-10 mRNA (Figure V in the online-only Data Supplement). There were no significant correlations between PPARα and inflammatory markers (data not shown).
Dietary Saturated Versus n-3 Diet Changes Arterial EL, PPARγ, and Inflammatory Marker Expression in LDL-R KO Mice In Vivo

We next investigated the potential effects of 12-week feeding of n-3- and SAT-rich diets on arterial EL, PPARγ, and inflammatory gene expression in aorta of LDL-R KO mice, which are susceptible to atherosclerosis. SAT diets led to 10-fold greater arterial EL mRNA compared with chow (Figure 6A). There was no significant difference in arterial EL mRNA levels between n-3 and chow diets. SAT diets were associated with markedly increased arterial PPARγ mRNA compared with chow diets, whereas the n-3 diets showed a 26% decrease in PPARγ mRNA (Figure 6B). Similar to in vitro data in macrophages, SAT diets increased arterial IL-6 and IL-12p40 mRNA 2.6- and 5.8-fold compared with chow, respectively, whereas arterial IL-10 mRNA was lowered in SAT-fed mice compared with chow-fed mice by 22% (Figure 6C–6E). In contrast, n-3 diets reduced both proinflammatory cytokine mRNA levels in aorta of LDL-R KO mice compared with chow by 74 and 50%, respectively, but increased IL-10 mRNA compared with SAT diet by 69%. Thus, in vivo effects of diets rich in SAT versus n-3 FA on arterial expression of EL and inflammatory markers paralleled effects observed in cultured macrophages in vitro.

Discussion

Macrophage-derived EL in the arterial wall is associated with increased atherosclerosis and arterial inflammatory markers in mice.9,10,12 Because of their anti-inflammatory properties we hypothesized that n-3 FA, in contrast to saturated FA, would lower expression of EL in vitro and in vivo. Our results show that a saturated FA, PA, increases macrophage EL expression and decreases anti-inflammatory IL-10 expression. In contrast, an n-3 FA, EPA, inhibits the increase of EL expression induced by LPS and PA, and this is accompanied by decreases in proinflammatory markers and increases in anti-inflammatory markers in cultured macrophages. Moreover, regulation of macrophage EL in response to FA is closely linked to changes in PPARγ activation. In vivo studies also show that SAT diets, but not n-3 diets, increase EL, PPARγ, and proinflammatory cytokine expression but decrease anti-inflammatory cytokine mRNA in aorta of LDL-R KO mice, suggesting that changes in EL by FA have important regulatory roles on atherosclerosis and inflammation in vivo as well as in vitro.

LPS, a major inflammatory stimulus, can play an important role in lipoprotein metabolism and atherosclerosis.29 Yasuda et al10 reported that EL expression was increased by LPS. In our current study, LPS also markedly increased EL protein as well as mRNA expression in J774 and peritoneal macrophages. Interestingly, saturated PA alone and with LPS increased EL mRNA and protein levels in both macrophages. In contrast, n-3 EPA had little effect on EL mRNA and protein but markedly inhibited the increase of macrophage EL expression induced by LPS. Furthermore, EL expression was significantly lower in cells treated with a combination of PA plus EPA compared with PA alone. Based on these results, EPA suppresses the increase of macrophage EL expression induced by EL activators PA and LPS.
PPARγ is a nuclear transcription factor that regulates numerous genes involved in lipoprotein metabolism and is highly expressed in macrophages, including foam cells of atherosclerotic lesions.14 PPARγ activation increased macrophage LpL mRNA and protein expression13 and promoted uptake of oxidized LDL through induction of macrophage CD36 expression,13,14 which suggest the potential role of PPARγ in the pathogenesis of atherosclerosis. Furthermore, PPARγ agonists, rosiglitazone and pioglitazone, enhance macrophage apoptosis via a PPARγ-independent mechanism, and pioglitazone promotes advanced plaque progression in LDL-R KO mice through enhancement of advanced lesion macrophage apoptosis.30 Our data indicate that EL might also contribute to atherogenesis and that PPARγ activation is associated with this regulation of EL.

In the current study, PPARγ activation using rosiglitazone as well as PA was related to increased EL mRNA and protein, and GW9662, a PPARγ antagonist, EPA and PPARγ-knock-down cells abrogated stimulation by rosiglitazone or PA. Rosiglitazone significantly increased PPARγ mRNA in a dose-dependent manner, whereas pioglitazone, an ~10 times less potent an activator of PPARγ than rosiglitazone,31 had little effects on PPARγ as well as on EL in our experiments in macrophages (data not shown). Also, GW9662 and EPA blocked rosiglitazone-induced PPARγ mRNA expression. Similar to our results with EPA, n-3 FA-docosahexaenoic acid suppressed CD36 expression induced by PPARγ agonist through the inhibition of transcriptional activity of PPARγ in human monocytes and colon tumor cells,32 and EPA and docosahexaenoic acid reduced the PPARγ response element reporter activity in colon cancer cells. Edwards et al13 proposed that n-3 FA may directly, or after being metabolized, activate extracellular signal-regulated kinase or other pathways that counteracts PPARγ signaling. However, PA significantly increased PPARγ mRNA or protein expression in several cell types, such as cardiomyocytes,34,35 and a high-fat diet enriched in PA enhanced PPARγ expression in macrophages.36 PA also stimulated the activity of the PPARγ response element in primary human adipocytes, suggesting activation of this nuclear signaling cascade.37 Taken together, our findings suggest that macrophage EL expression is partially mediated by the upregulation of PPARγ and that saturated versus n-3 FA affect the expression of EL, at least in part, by regulating PPARγ. It is possible that the transcriptional induction of EL gene might be mediated through binding PPAR-RXR heterodimer, as it is reported that CD36 and the LpL promoter is a direct target of PPAR-RXR heterodimer.14,38 Others have also shown different effects of saturated versus n-3 FA on PPARγ signaling and these differences are also related to the specific tissue or cell analyzed.32–37 To clearly understand whether regulation of macrophage EL is most likely mediated through PPARγ-dependent mechanism, further experiments using ligand binding assays are needed to be performed.

A PPARγ-related mechanism for regulation of arterial wall EL by FA is supported by our in vivo findings. SAT diets, but not n-3 diets, increased arterial EL and PPARγ mRNA in LDL-R KO mice, and EL was positively correlated with PPARγ (P<0.01). Ishida et al9 reported that EL protein was increased in aorta from apoE KO mice and this was accentuated by a high-fat diet (0.15% cholesterol, 21% milk fat).10 Also, there was a decrease in atherosclerotic lesions in animals lacking both EL and apoE compared with apoE KO alone.10 Moreover, EL protein39 and PPARγ mRNA40 were increased in the aorta, especially the atherosclerotic lesions in high-cholesterol diet fed animals. Herein we show that n-3 diets do not share the stimulatory effects of SAT diets in vivo.

We also found that, in parallel with the changes in macrophage EL and PPARγ, EPA alone or plus LPS reduced IL-6 and IL-12p40 mRNA in macrophages but increased the mRNA of IL-10 and mannose receptor which stimulates anti-inflammatory cytokines production including IL-10.41 Proinflammatory cytokines, such as IL-6 and IL-12, promote the development of atherosclerotic lesions,42,43 whereas anti-inflammatory IL-10 have antiatherogenic effects,44 EL as well as PPARγ positively correlated with proinflammatory markers but negatively correlated with anti-inflammatory markers. Moreover, similar changes in arterial inflammatory markers were found in LDL-R KO mice, suggesting that FA-regulated inflammatory responses could also occur in vivo. In contrast to other inflammatory cytokines mRNA expression, there was no significant effect of EPA on TNF-α mRNA expression in macrophages. Preliminary data (not shown) on LDL-R KO mice also show that feeding of n-3 diets for 12 weeks did not affect TNF-α mRNA expression in aorta compared with chow or SAT diets. Renier et al45 reported that macrophages derived from mice fed n-3 diets showed a significant decrease in TNF-α mRNA after 15 weeks, but not 6 weeks, suggesting that the maximum effect of n-3 FA might require a relatively longer observation period than that used in our study.
Several studies have consistently reported the proinflammatory effect of EL in macrophages. However, there have been contradictory reports on the potency of PPARγ activation on macrophage inflammatory responses. PPARγ agonists reduced proinflammatory cytokine production, including TNF-α and IL-6, in human monocytes, whereas Thieringer et al. failed to obtain an inhibitory effect of PPARγ agonists on TNF-α and IL-6 production in human monocytes or macrophages. Furthermore, PPARγ activation seemed to increase plasma cytokine levels in mice after LPS administration. Consistent with this finding, rosiglitazone increased LPS-induced TNF-α production in rat peritoneal macrophages. In fact, a PPARγ activator exerted anti-inflammatory effects in a PPARγ-independent mechanism via inhibition of nuclear factor-κB–dependent transcription, and PPAR agonists inhibited cytokine production in PPARγ-deficient macrophages, indicating that anti-inflammatory effects may also be mediated by other biological pathways.

Another possible mechanism by which FA affect macrophage-derived EL may be regulation of TLR4 and nuclear factor-κB, a transcription factor involved in TLR activation. EL expression was induced by inflammatory cytokines and LPS in endothelial cells and macrophages through nuclear factor-κB activation. In vitro treatment with n-3 FA, such as EPA, diminished TLR4 and nuclear factor-κB signaling, whereas saturated FA enhanced these. Consistent with these findings, we found that EPA attenuated TLR4 mRNA, and this correlated with lower EL expression. Thus, it seems possible that FA modulation of EL expression may also occur through interference with TLR4 pathways in macrophages. Indeed, Rader’s group has shown that EL is increased with increased TLR4 expression. Experiments in TLR4 knock-down cells would be informative in determining whether TLR4 is also directly linked to effects of FA on EL.

Our findings describe mechanisms whereby decreases in EL associated with dietary n-3 FA might be associated not only with higher plasma high-density lipid protein levels, as described by others, but also decreases in inflammatory pathways contributory to atherosclerosis. EL was associated with PPARγ expression, and the macrophage-derived lipase could be modified by specific FA, in part, through regulating macrophage PPARγ expression. We suggest that similar changes of the EL and inflammation could occur in vivo. The ability of EL to anchor low-density lipoprotein in macrophages could also contribute to atherogenesis. We hypothesize that diets rich in n-3 FA (eg, EPA), in contrast to saturated FA (eg, PA) decrease progression of atherosclerosis in humans, in part, by downregulating inflammatory markers and PPARγ, together with decreasing macrophage EL in the arterial wall.

Acknowledgments

U.J.J., C.T., C.L.C., and H.H. performed experiments and RJD drafted the outline of the manuscript with U.J.J. U.J.J. wrote the original article and R.J.D., C.L.C., and T.S.W. performed substantial editing. We also thank Drs Silke Vogel and Ira Goldberg, Columbia University, for their critical reviews of this article.

Sources of Funding

This work was supported by National Institutes of Health grant HL 40404 (to R.J.D.), T32 DK007647 (to C.L.C.), T32 HL007343 (to C.L.C.), and 5K08 AG028833 (to T.S.W.), and a fellowship from the International Nutrition Foundation/Ellison Medical Foundation (to C.T.).

Disclosures

Richard J. Deckelbaum received an honorarium from the American Society for Nutrition in 2011 for helping organize, chair, and speak at a symposium on omega-3 fatty acids titled “Heart Healthy Omega-3s for Food: Stearidonic Acid (SDA) as a Sustainable Choice” at the Experimental Biology 2011 meeting. No other authors had any conflict of interest.

References


50. Ajuwon KM, Spurlock ME. Palmitate activates the NF-kappaB tran.

Fatty Acids Regulate Endothelial Lipase and Inflammatory Markers in Macrophages and in Mouse Aorta: A Role for PPAR γ

Un Ju Jung, Claudia Torrejon, Chuchun L. Chang, Hiroko Hamai, Tilla S. Worgall and Richard J. Deckelbaum

Arterioscler Thromb Vasc Biol. 2012;32:2929-2937; originally published online October 4, 2012;
doi: 10.1161/ATVBAHA.112.300188
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/32/12/2929

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2012/10/04/ATVBAHA.112.300188.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/