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Enhanced Uptake of Modified Low-Density Lipoprotein by Eicosapentaenoic Acid-Treated THP-1 Macrophages

Young-Hee Kang[1§], Sung-Hee Park[1], Jung Sook Kang[2] and Jung Han Yoon Park[1]

[1]*Division of Life Sciences and Institute of Environment and Life Science, Hallym University, Chuncheon 200-702, Korea*
[2]*Department of Food and Nutrition, Cheju University, Cheju 690-756, Korea*

ABSTRACT

Animal and clinical studies as well as epidemiological data have provided convincing evidence that n-3 polyunsaturated fatty acids can protect against atherosclerosis. However, the effects of the fatty acids on atherogenesis are contradictory. This discrepancy could derive from great susceptibility of the fatty acids to oxidation. We investigated the effect of eicosapentaenoic acid (EPA) on cellular atherogenesis via the scavenger receptor of THP-1 derived macrophages. THP-1 cells were fully differentiated into macrophages by incubating with phorbol 12-myristate 13-acetate for seven days. Atherogenic features of EPA were compared by substituting for linoleic acid (LA). Macrophages were also incubated without treatment of the fatty acids as controls. EPA (5 ~ 50 nmol/mL) was not cytotoxic and did not measurably induce cellular oxidation compared to bovine serum albumin (BSA) vehicle or identical doses of LA. EPA increased macrophage uptake and degradation of acetylated LDL (AcLDL) up to 14% and 88%, respectively. EPA increased markedly total cellular sterol synthesis and heparin-releasable lipoprotein lipase activity of macrophages, indicating that EPA may enhance accumulation of cellular cholesteryl ester and possibly facilitate formation of foam cells. These results demonstrate that EPA promotes the modified LDL-triggered atherosclerotic process by the modulation of the scavenger receptor and the activation of LPL in macrophages.

KEY WORDS: eicosapentaenoic acid, linoleic acid, acetylated low-density lipoprotein, cellular sterol, lipoprotein lipase, macrophages.

INTRODUCTION

It has been demonstrated that fish oil n-3 polyunsaturated fatty acids may protect against the development of atherosclerosis in animal studies with non-human primates,[1][2] as reported in epidemiological studies on Greenland Eskimos.[3] Chronic fish oil feeding in rats exhibited the hypocholesterolemic features.[4] In addition, fish oil supplementation has resulted in hypotriglyceridemia and a reduction in low-density lipoprotein (LDL) cholesterol levels when saturated fat intake is partially replaced by fish oil.[5] However, depending on the species and lipidemic status, the effects of fish oil feeding on plasma cholesterol level are contradictory.[6-10] It has been reported that fish oil feeding enhances cholesterol-induced atherosclerosis in rabbits.[11] Fish oil fatty acids enhance the susceptibility of LDL to copper-induced and macrophage-mediated oxidation.[12]

Circulating monocyte-derived macrophages, which accumulate large amounts of cholesteryl ester, are known to become lipid-laden foam cells in early atherosclerotic and inflammatory lesions.[13-16] Foam cells are associated with the uptake of modified LDL such as oxidized or acetylated LDL (AcLDL), which is mediated by the scavenger receptor expressed on the membrane surface of macrophages.[15][17-19] The scavenger receptor-mediated macrophage endocytosis of modified LDL is not down-regulated by increased intracellular cholesterol, unlike the LDL receptor regulation.[20] Although the mediation mechanisms are not well defined, there are numerous reports demonstrating that the macrophage scavenger receptor is down-regulated by extrinsic platelet and intrinsic monocyte/macrophage activators including transforming growth factor-b and interferon-g,[21][22] In addition, the receptor appears to be up-regulated by the differentiation of monocytes into macrophages. Therefore, it is conceivable that the expression and activity of the scavenger receptor on macrophages may be amenable to interventions targeting various cellular mechanisms. Dietary fish oil has been documented to modify lipoprotein metabolism, platelet function, cytokine production, inflammation and immune response.[23-28]

To test the hypothesis that fish oil fatty acid modulates the scavenger receptor, we examined the effects of *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA) on the degradation and internalization of modified LDL in THP-1 differentiated macrophages. Cells were also incubated with a vehi-

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[§]To whom correspondence should be addressed.

cle bovine serum albumin (BSA) or n-6 polyunsaturated fatty acid linoleic acid (LA) as controls. Measuring cellular sterol synthesis and lipoprotein lipase (LPL) activity assessed the modified LDL metabolism in models employing monocytes/macrophages supplemented with dietary fish oil fatty acid EPA.

MATERIALS AND METHODS

1. Cell culture

Human monocytic cell line THP-1 cells were obtained from the American Type Tissue Culture Collection and grown in HEPES-buffered RPMI-1640 containing 10% fetal bovine serum (FBS), 2 mM glutamine, 100 U/mL penicillin and 100 mg/mL streptomycin. Cultures were maintained at 37℃ in humidified atmosphere of 5% CO_2 in air, and cells were passaged weekly 1 : 4. To differentiate THP-1 cells into macrophages, cells were plated at a density of 5×10^5/mL, and incubated in RPMI-1640 containing 10% FBS for 24 hrs in the presence of 100 ng/mL phorbol 12-myristate 13-acetate (PMA, Sigma Co, St. Louis, MO). After 24 hr incubation, cells were rinsed with phosphate buffer saline (PBS) and cultured in RPMI-1640 plus 10% FBS in the absence of PMA during indicated periods, as described elsewhere.[29] Unless indicated, THP-1 derived macrophages were incubated in RPMI-1640 containing 10% FBS for 7 days. Cells were then incubated for an additional 24 hrs with either LA (Sigma Co.) or EPA (Sigma Co.) at various concentrations in fresh RPMI-1640 containing 0.4% fatty acid free-BSA.

2. Preparation of fatty acid-albumin complex

Fatty acids LA and EPA were prepared by mixing with BSA.[30] LA and EPA of 20 mmoles sodium salts were dissolved in 0.4 mL of 95% ethanol containing 0.0004% butylated hydorxytoluene. The ethanol mixture was evaporated completely using N_2 gas. The pellet was dissolved in 5 mL hot deionized-distilled water, and the resulting solution was added to 5 mL of 2 mM fatty acid-free BSA. The mixture solution was gently stirred for 4 hrs at 4℃ and sterilized by a 0.45 μm filter and the aliquots were kept frozen at −20℃ until use. The fatty acid concentration of the fatty acid-BSA complex was 2 mM and the molar ratio of fatty acid to BSA was 2 : 1.

3. Preparation of lipoproteins

Human normolipidemic plasma LDL ($d = 1.020 - 1.063$ g/mL) was prepared by discontinuous density gradient ultracentrifugation as previously described.[31][32] Briefly, human plasma was adjusted to $d = 1.063$ g/mL with NaCl and centrifuged at 150,000 × g, 10℃ for 24 hrs. The plasma fractions of chylomicron plus VLDL and LDL were separated by centrifugation at 150,000 × g for 24 hrs after density adjustment to 1.014 g/mL. The LDL fraction was solely isolated by repetitive centrifugation at $d = 1.063$ g/mL. Isolated LDL was dialyzed overnight against 2 L buffer (pH 7.4) of 0.154 M NaCl and 0.01% EDTA.

AcLDL was prepared by a reaction with acetic anhydride as previously described.[31] Prepared AcLDL was labeled with $Na^{125}I$ (NEN Life Science, Boston, MA) by the iodine monochloride method.[33] In addition, incorporation of fluorescent dye, 1,1-dioctadecyl-1-3,3,3,3-tetramethylindocarbocyanine perchlorate (DiI, Molecular Probe Inc., Eugene, OR) dissolved in dimethylsulfoxide, into AcLDL was accomplished as previously described.[34] Labeled ^{125}I-AcLDL was 95% trichloroacetic acid-precipitable and 95% chloroform-non-extractable and its specific activity was 100 − 300 cpm/ng. Charge modification of each prepared lipoprotein was confirmed by agarose gel electrophoresis[35] and by 8 − 25% SDS gradient phastgel (Pharmacia LKB Biotech., Uppsala, Sweden). Protein content was determined using the method of Lowry *et al.*[36] Contents of triglyceride, total cholesterol and phospolipids were measured using diagnostic kits (Asan Pharmaceutical Co., Korea). All lipoproteins were used within four weeks after labeling.

4. Release of thiobarbituric acid reactive substances

To examine the extent of cellular oxidation by incubation of fatty acids, the production of thiobarbituric acid reactive substances (TBARS) was measured according to the method of Niehaus and Samuelsson.[37] Cells were incubated on culture dishes in RPMI-1640 plus 0.4% BSA in the absence and presence of either LA or EPA for the time periods indicated and the incubation medium collected. The cells were rinsed twice with PBS containing 2 mg/mL BSA and three times with PBS alone, and extracted in a lysis buffer of 0.1 N NaOH and 1 mg/mL SDS. The collected medium was briefly centrifuged to remove cell debris. The medium supernatant was subjected to the thiobarbituric acid assay. The concentrations of TBARS were expressed as nmol malondialdehyde/mg cell protein.

5. Degradation and internalization of AcLDL

DiI-labeled AcLDL is used to visualize the uptake of LDL by macrophages and endothelial cells.[34][38] After in-

cubation of cells with the fatty acids used, cells were washed and incubated with 10 μg/mL DiI-AcLDL in RPMI-1640 containing 0.4% BSA for four hrs in the absence and presence of 25-fold excess unlabeled AcLDL to determine specific uptake of AcLDL. At the end of incubation, the cells were rinsed with PBS, fixed in 4% cold formaldehyde and mounted in McIlvaines buffer/glycerol (Sigma Co.). Cellular distribution of DiI was analyzed using a fluorescent microscope (Olympus BX50, Olympus Optical Co., Tokyo, Japan) and rhodamine filter set.

The degradation and internalization of ^{125}I-labeled AcLDL were assessed using a method described elsewhere.[15][39] After pretreatment with LA or EPA for 24 hrs, cells were incubated in RPMI-1640 containing 0.4% BSA with 10 μg/mL ^{125}I-labeled AcLDL for four hrs in the absence and presence of 250 μg/mL unlabeled AcLDL. The trichloroacetic acid-soluble radioactivity of the collected incubation medium was measured using an automatic gamma-counter (Pharmacia LKB Biotech). The cells were washed twice with PBS containing 2 mg/mL BSA and twice with PBS, and incubated in a lysis buffer for one hr. Radioactivity data were normalized by cellular protein contents. Specific degradation and internalization were calculated by subtracting the non-specific radioactivity measured in excess unlabeled AcLDL from total radioactivity.

6. Cellular sterol synthesis

Cells were grown on culture dishes in RPMI-1640 containing 0.4% BSA and the fatty acids of LA or EPA for 24 hrs, followed by an additional four hr incubation in the absence and presence of 10 μg/mL AcLDL. After being rinsed twice with PBS, cells were incubated in fresh medium containing 0.8 μCi of ^{14}C acetate (Amersham Inc., Buckinghamshire, UK) and unlabeled carrier sodium acetate (0.2 μmol/mL, specific activity of 4 μCi/mmol) for two hrs.[40] Cells were then washed and lysed in 2 mL of 0.1 N NaOH plus 1 mg/mL SDS. A portion of cell lysate (1 mL) was used for the measurements of the radioactivity and cell protein content, and the remaining 1 mL lysate was further processed. In a screw-capped glass vial containing 1 mL of 100% ethanol and 0.2 mL of 90% KOH, 1 mL lysate was added, and the mixture was saponified at 85 - 90℃ for three hrs and cooled on ice. To extract non-saponifiable lipid, 1.5 mL distilled water and 2.5 mL hexane were added to the mixture. After centrifugation, the hexane phase was collected in a new screw-capped glass vial and the extraction procedure was repeated. After washing with 2 mL of 0.1 M sodium acetate, the hexane phase was collected and evaporated. The radioactivity was determined with a liquid scintillation counter. Incorporation of ^{14}C acetate into sterols is expressed as nmol acetate incorporated into non-saponifiable lipids per mg cell protein.

7. Heparin-releasable LPL activity

Activity of heparin-releasable LPL was assayed by a modification of previously described methods using an emulsion of radiolabeled triolein.[41][42] Cells were incubated on culture dishes in RPMI-1640 plus 0.4% BSA with LA or EPA for 24 hrs and were treated with 10 μg/mL AcLDL for four hrs. The incubation medium was collected five min after the addition of 50 U/mL of heparin (Sigma Co.). The cell layer was rinsed and extracted in a lysis buffer. The incubation medium was briefly centrifuged to remove cell debris. The lipolytic activity of the medium was measured by adding the final assay substrate mixture of 0.625 μCi glycerol tri-^{14}C oleate (specific activity of 0.037 μCi/mmol, Amersham) emulsified with intralipid (Kabi Pharmacia, Eriangen, Germary) in a Tris-NaCl buffer (pH 8.2) with 0.075 U/mL heparin and by activating with 5% (vol/vol) pooled human serum. The radioactivity was determined with a liquid scintillation counter. The heparin-releasable LPL activity was expressed in nmol of released free fatty acids per min per mL of medium.

8. Data analysis

All values are presented as means ± SEM of independent experiments. Differences between groups were evaluated by two-way analysis of variance followed by the Tukey correction (sas Institute Inc., Cary, NC). Statistical significance was set at $p < 0.05$. .

RESULTS

1. Cellular oxidation by fatty acids

The medium TBARS production by incubation with LA or EPA is shown in Fig. 1. To compare the effects of EPA on cellular oxidation, THP-1-derived macrophages were treated with BSA alone or with LA as controls. The macrophages did not measurably produce medium TBARS in untreated cells; only a very limited amount of TBARS was found. In addition, there were no apparent effects by LA and EPA on medium TBARS production within 24 hrs. It should be noted that LA and EPA within a range of given doses did not modulate cell growth (data not shown). Thus, cellular oxidation that could be possibly el-

icited due to incubation with the highly unsaturated fatty acid was not likely involved in the present cell system.

2. Effects of EPA on uptake of AcLDL

DiI-AcLDL taken up by macrophages was rapidly degraded in the lysosomes and the DiI accumulates in the lysosomal membranes.[34,35] Cellular distribution and fluore-scent intensity of DiI in THP-1 derived macrophages pre-treated with either LA or EPA were determined. A form of the perinuclear DiI fluorescence was observed in macr-ophages incubated with 10 μg/mL DiI-AcLDL for four hrs (Fig. 2, panels A – D). EPA appeared to intensify DiI staining, compared with those of BSA alone and LA (pa-nel C vs. panels A and D). When macrophages were incubated with DiI-AcLDL in the presence of 250 μg/mL unlabeled AcLDL, the fluorescent intensity visibly decreased (panel B).

Specific fluorescent intensity of DiI is the result of cells incubated with DiI-AcLDL in the presence of excess unlabeled AcLDL. Specific uptake of AcLDL influenced by 25 nmol/mL LA or EPA pre-treated prior to exposure of 10 μg/mL DiI-AcLDL in the presence of 250 μg/mL unlabeled AcLDL was determined (Table 1). EPA increased specific fluorescent intensity of DiI by 14% and 6% respectively, when compared to BSA alone or the same dose of LA. Thus, EPA increased specific uptake of AcLDL in macrophages, compared to those observed in untreated cells and in LA-treated cells. It is reasonable to believe that EPA activated the scavenger receptor that leads to intracellular cholesterol accumulation. The results clearly demonstrate that EPA substantially enhanced the uptake of modified LDL in THP-1 differentiated macrophages. In contrast, eq-

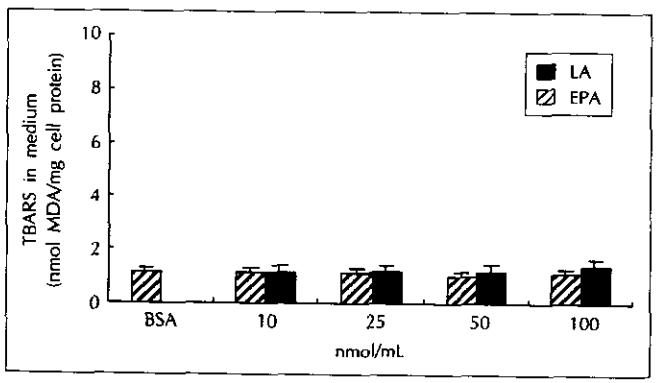

Fig. 1. Effects of linoleic acid (LA) and eicosapentaenoic acid (EPA) on medium thiobarbituric acid reactive substances (TBARS) production. THP-1 monocytes were differentiated into macrophages with 100 ng/mL phorbol 12-myristate 13-acetate for seven days. After additional 24 hr incubation in RPMI-1640 containing 0.4% bovine serum albumin (BSA) with and without various concentrations of LA or EPA, medium TBARS was measured using a thiobarbituric acid assay and expressed as nmol malondialdehyde (MDA)/mg cell protein. Values represent means ± SEM of four replicate experiments.

Table 1. Specific uptake of AcLDL by THP-1 derived macrophages supplemented with fatty acids

Treatments (nmol/mL)	Specific uptake of DiI-AcLDL (μg/mg cell protein)
BSA	2.65 ± 0.17
LA	2.84 ± 0.21
EPA	3.02 ± 0.23*

THP-1 cells were differentiated into macrophages with 100 ng/mL phorbol 12-myristate 13-acetate for seven days. Macrophages were incubated for additional 24 hrs in RPMI-1640 containing 0.4% bovine serum albumin (BSA) and supplemented with 25 nmol/mL linoleic acid (LA) or eicosapentaenoic acid (EPA). The THP-1 derived macrophages were incubated with 10 μg/mL DiI-AcLDL for four hrs in the absence and presence of 250 μg/mL unlabeled AcLDL. Fluorescent intensity of DiI was measured using a microplate reader and specific fluorescent intensity was determined by subtracting mean fluorescent intensity of unlabeled cells from that of DiI-AcLDL incubated cells. Values represent means ± SEM of four replicate dishes. *: $p < 0.05$, relative to BSA controls.

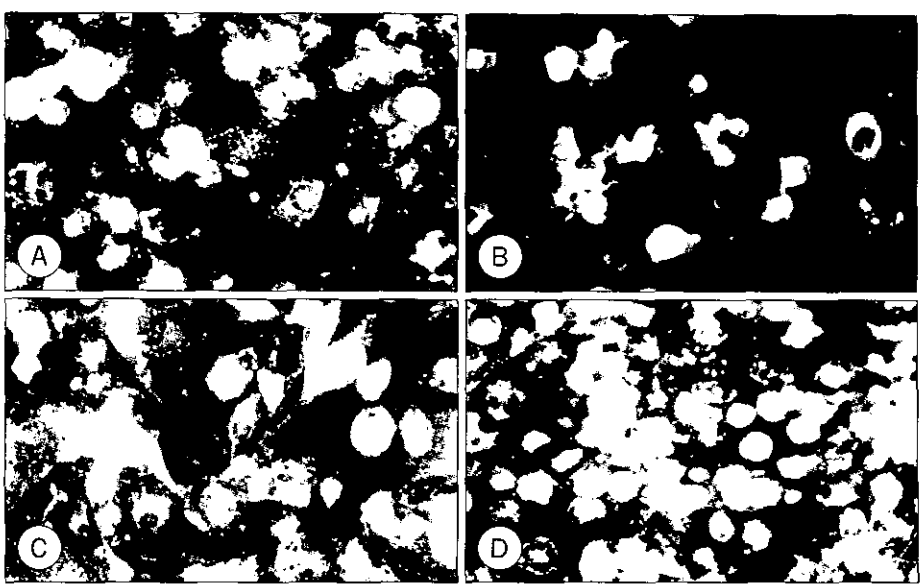

Fig. 2. Representative fluorescent microphotographs showing the uptake of 10 μg/mL DiI-AcLDL by THP-1 derived macrophages. The cells were grown for 24 hrs in RPMI-1640 containing 0.4% bovine serum albumin (BSA) and supplemented with 25 nmol/mL linoleic acid (LA, panel C) and eicosapentaenoic acid (EPA, panel D) for four hrs. Control culture was done by incubating 10 μg/mL DiI-AcLDL in RPMI-1640 medium containing 0.4% BSA for four hrs in the absence of fatty acids (panel A). When incubating macrophages with DiI-AcLDL in the presence of excess unlabeled AcLDL, the fluorescent intensity apparently decreased (panel B).

uimicromolar LA appeared to reduce their uptake.

3. Specific degradation of AcLDL in EPA-treated macrophages

Phorbol ester-treated THP-1 cells exhibit a temporal increase in the degradation of ^{125}I-labeled AcLDL that is inhibited by unlabeled AcLDL but not by unlabeled LDL, suggesting that the degradation of AcLDL by THP-1 macrophages be mainly mediated via a mechanism specific for AcLDL.[29] To determine whether the fatty acids influence the specific macrophage degradation of AcLDL in the presence of 25-fold excess unlabeled AcLDL, cells pre-treated with 25 and 50 nmol/mL LA or EPA were incubated for four hrs with ^{125}I-labeled AcLDL. Table 2 shows the effect of EPA on the specific degradation of ^{125}I-AcLDL. Treatment with 25 and 50 nmol/mL EPA enhanced the degradation of ^{125}I-AcLDL by 58% and 88%, respectively, compared to that of control untreated cells. LA was less adverse than equimicromolar EPA in increasing the specific degradation of ^{125}I-AcLDL in a concentration-dependent manner (Table 2): however, non-specific degradation of AcLDL observed in the presence of excess unlabeled AcLDL was similar (data not shown).

4. Effects of EPA on cellular sterol synthesis

Continuous uptake of modified LDL results in intracellular cholesterol accumulation and transformation of the macrophages into foam cells.[13-16] When macrophages were exposed to 10 μg/mL AcLDL in a medium containing 10% FBS, 5 nmol total cellular sterol/mg cell protein was synthesized, whereas macrophages incubated in 10% lipoprotein-deficient serum increased synthesis of cellular sterol by 300% (unpublished data). In addition, THP-1 derived macrophages contained 1.05 ± 0.18 nmol sterol/mg cell protein when cultured in a AcLDL-free medium with 0.4% fatty-free BSA (data not shown). Table 3 summarizes the effects of LA and EPA on total cellular sterol synthesis. LA and EPA at 5 – 50 nmol/mL increased the formation of cellular sterol in a dose-dependent manner by 4 – 62% and 11 – 94%, respectively. Cellular cholesterol is largely present as cholesteryl ester droplets when modified LDL is internalized via the ophage scavenger receptor and then intracellular cholesterol accumulated.[15][19][20][42] Thus, these results indicate that EPA may enhance the formation of cellular cholesteryl ester.

5. Effects of fatty acids on lipoprotein lipase activity

It has been demonstrated that THP-1 cells are used as a model to study the quantitative and temporal expression of LPL during differentiation.[41][42] Differentiation of THP-1 cells into macrophages is accompanied by activation and extracellular accumulation of LPL. Unstimulated THP-1 cells lack intracellular LPL and minimally secrete LPL.[41][42] The ability of THP-1 derived macrophages to secrete the enzyme into the medium with the addition of fatty acids was examined. Table 4 shows the activity of heparin-releasable extracellular LPL of macrophages cultured with LA and EPA. Induction of LPL in THP-1 macrophages was dependent on the types and concentrations of the fatty acids added. The activity of heparin-releasable LPL increased by 18 – 112% in macrophages

Table 2. Characterization of fatty acid effect on AcLDL degradation by THP-1 derived macrophages

Treatments (nmol/mL)		Specific degradation of ^{125}I-AcLDL (μg/mg cell protein)
BSA		0.60 ± 0.19
LA	25	0.73 ± 0.11
	50	0.89 ± 0.05*
EPA	25	0.95 ± 0.18*,†
	50	1.13 ± 0.13*,†

Macrophages were incubated for 24 hrs in RPMI-1640 containing 0.4% bovine serum albumin (BSA) and supplemented with 25 or 50 nmol/mL linoleic acid (LA) and eicosapentaenoic acid (EPA). THP-1 derived macrophages were incubated with 10 μg/mL ^{125}I-AcLDL in RPMI-1640 medium containing 0.4% BSA for four hrs in the presence of 250 μg/mL unlabeled AcLDL. ^{125}I radioactivity data for the AcLDL degradation were normalized by cellular protein contents. Specific degradation was calculated by subtracting the non-specific radioactivity measured with excess unlabeled AcLDL from total radioactivity. Values represent means ± SEM of triplicate dishes.

*: $p < 0.05$, relative to BSA controls.

†: $p < 0.05$, relative to respective dose of LA.

Table 3. Total cellular sterol synthesis in THP-1 derived macrophages supplemented with fatty acids

Treatments (nmol/mL)		Total cellular sterol (nmol acetate/mg cell protein)
BSA		21.0 ± 3.6
LA	5	21.8 ± 4.0
	25	24.8 ± 4.0
	50	34.0 ± 2.6*
EPA	5	23.4 ± 4.4
	25	27.2 ± 5.4*
	50	40.8 ± 10.6*,†

Macrophages were incubated for 24 hrs in RPMI-1640 containing 0.4% bovine serum albumin (BSA) and supplemented with linoleic acid (LA) or eicosapentaenoic acid (EPA) at various concentrations, followed by four hr incubation in the absence and presence of 10 μg/mL AcLDL. The radioactivity was determined (see MATERIALS AND METHODS), and incorporation of acetate into sterols is expressed as nmol acetate incorporated into non-saponifiable lipids per mg cell protein. Values represent means ± SEM of four replicate dishes.

*: $p < 0.05$, relative to BSA controls.

†: $p < 0.05$, relative to respective dose of LA.

Table 4. Effects of fatty acid supplementation on activity of heparin-releasable extracellular lipoprotein lipase in THP-1 derived macrophages

Treatments (nmol/mL)		Activity of heparin-releasable lipoprotein lipase (nmol of free fatty acid/mL/min)
BSA		11.6 ± 2.2
LA	25	13.7 ± 6.3
	50	24.6 ± 4.9*
EPA	25	16.2 ± 3.6
	50	31.4 ± 1.1*,†

Macrophages were incubated for additional 24 hrs in RPMI-1640 containing 0.4% bovine serum albumin (BSA) and supplemented with 25 or 50 nmol/mL linoleic acid (LA) or eicosapentaenoic acid (EPA). The lipolytic activity in medium was measured (see MATERIALS AND METHODS). The radioactivity was determined and LPL activity expressed in nmol of released free fatty acids per mL of medium per min. Values represents means ± SEM of triplicate dishes.
*: $p < 0.05$, compared to BSA controls.
†: $p < 0.05$, compared to respective dose of LA.

treated with 25 – 50 nmol/mL of the fatty acids. EPA at 25 nmol/mL and 50 nmol/mL enhanced the activity of LPL by 40% and 171%, respectively. The effect of EPA on the secretion of LPL was significantly greater than that of LA (Table 2). The results suggest that the highly polyunsaturated fatty acid EPA may facilitate the formation of foam cells by activating LPL.

DISCUSSION

The main findings of this study are that 1) highly unsaturated fatty acid EPA does not cause cellular oxidation possibly modulating the metabolism of modified LDL; 2) Micromolar EPA can enhance substantially specific degradation and internalization of modified LDL in macrophages via the activated macrophage scavenger receptor; 3) EPA increases the intracellular sterol synthesis of macrophages and may accumulate cellular cholesteryl ester; 4) EPA may facilitate the formation of foam cells by activating and secreting LPL. These findings highlight atherogenic properties of the highly unsaturated fatty acid EPA. It is tempting to speculate that fish oil feeding might play a pathogenic role in atherosclerosis *in vivo*.

Phorbol ester-treated THP-1 cells are time-dependently differentiated into macrophages. The phenotypes of macrophages and the capacity degrading modified LDL and non-specific esterase activity was first detected in THP-1 cells treated with phorbol ester for four days.[29] Circulating monocyte derived macrophages accumulating large amounts of cholesteryl ester are known to become lipid-laden foam cells in early atherosclerotic and inflammatory lesions via LDL internalization followed by the stimulation of the intracellular cholesterol esterification.[13,16] LDL internalization involves the uptake of modified LDL that is mediated via a protein termed as the scavenger receptor and expressed on the surface of macrophages.[15,17-19] Differentiation of monocytes into macrophages induces and activates the scavenger receptor. It is, therefore, conceivable that the expression and activation of the macrophage scavenger receptor may be amenable to interventions targeting a variety of cellular mechanisms suspected to activate macrophages. This study investigated whether the expression and activation of the scavenger receptor in THP-1 derived macrophages can be modulated by fish oil fatty acid EPA.

Dietary fish oil modifies lipoprotein metabolism, platelet function, cytokine production, inflammation and immune response.[24-28] It has been demonstrated that fish oil n-3 polyunsaturated fatty acids including EPA protect against the development of atherosclerosis.[1,3] Chronic fish oil feeding in rats exhibited the hypocholesterolemic effect.[4] However, the atherogenic features of fish oil fatty acids are conflicting, depending on species and lipidemic status.[6,10] It has been reported that fish oil feeding enhances cholesterol-induced atherosclerosis in rabbits.[11] In addition, fish oil fatty acids adversely enhance the susceptibility of LDL to copper-induced and macrophage-mediated oxidation,[12] which could be due to the highly polyunsaturated structure. In this study, THP-1 macrophages minimally produced TBARS within 24 hrs in the presence of either LA or EPA up to 50 nmol/mL. Our results suggest that cellular oxidation may not be a factor influencing the scavenger receptor and modulating cellular sterol synthesis of THP-1 macrophages during incubation with LA or EPA.

Increasing concentrations of EPA enhanced specific uptake of AcLDL in macrophages compared to those in macrophages incubated with BSA alone or same doses of LA. The enhanced uptake of modified LDL by EPA may be associated with a possible alteration in the scavenger receptor. It should be noted that the non-specific degradation of AcLDL by macrophages treated with fatty acids was similar to that of macrophages treated with BSA alone. Fluorescent DiI-labeled AcLDL taken up by macrophages is rapidly degraded in the lysosomes and the DiI accumulates in the lysosomal membranes.[34,35] A form of the perinuclear DiI fluorescence was observed in macrophages incubated with DiI-AcLDL. The distribution of fluorescent DiI and the non-specific degradation of AcLDL were similar to those observed in cells treated with BSA alone or LA. The AcLDL binding is shown to be saturable and inhibited by adding excess unlabeled AcLDL, indicating that AcLDL binds to a limited number of high

affinity receptors.[19,42] Thus, it is possible to speculate that EPA increases saturable binding of AcLDL without a significant effect on the non-specific binding.

Cellular cholesterol is accumulated as cholesteryl ester droplets, when modified LDL is internalized via macrophage scavenger receptor.[15,19,20,42] Testing was done to determine whether fatty acids promote total cellular sterol synthesis of macrophages including membranous free cholesterol. EPA increased concentration-dependently cellular sterol formation. It has been suggested that free cholesterol present in the cellular membrane could be less affected by interventions targeting cellular cholesterol metabolism.[20,42] Accordingly, one can assume that EPA may enhance the formation of cellular cholesteryl ester rather than membranous free cholesterol and hence promote atherogenicity with forming foam cells.

THP-1 cells lack intracellular LPL and the differentiation of THP-1 cells into macrophages activates and secretes LPL.[41,42] The ability of THP-1 derived macrophages to secrete the enzyme upon addition of fatty acids was examined. Heparin-releasable LPL of THP-1 macrophages was greatly induced by adding fatty acids. The effect of EPA on the LPL activity was significantly greater than that induced by identical doses of LA. The results suggest that highly polyunsaturated fatty acids may facilitate macrophage degradation of modified LDL and formation of foam cells by activating LPL and secreting it into the extracellular space. This finding is consistent with results with regard to the AcLDL uptake enhanced by EPA.

In summary, EPA increased the uptake and degradation of modified LDL, possibly via induction of the scavenger receptor and via activation of LPL in THP-1 derived macrophages. Furthermore, EPA facilitated cellular sterol synthesis and hence foam cell formation of the macrophages. Therefore, this highly polyunsaturated structure of EPA raises the possibility that it may now be feasible to promote the oxidized or modified LDL-triggered atherosclerotic process.

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