# Dietary oxidized fatty acids may enhance intestinal apolipoprotein A-I production

**Rong Rong, Sumathi Ramachandran, Meera Penumetcha, Nadya Khan, and Sampath Parthasarathy**[1]

Department of Gynecology and Obstetrics and Nutrition and Health Sciences Program, Emory University School of Medicine, Atlanta, GA 30322

**Abstract Apolipoprotein (apo)A-I, the major protein component of HDL, is synthesized principally in the small intestine and liver. Recently we observed an increase in plasma apoA-I level in humans who were on an oxidized fat diet. To test whether oxidized fatty acids could affect apoA-I synthesis, we incubated day 4 (undifferentiated) and day 14 (differentiated) Caco-2 cells with varying concentrations of oxidized linoleic acid (ox-linoleic acid) (5, 10, and 25 μM) and unoxidized linoleic acid for 24 h. Ox-linoleic acid caused a dose-dependent increase in the levels of apoA-I protein in both differentiated and undifferentiated Caco-2 cells as assessed by ELISA and Western blot analysis. Whereas apoB production was not increased by ox-linoleic acid in both day 4 and day 14 Caco-2 cells. The mRNA expression for apoA-I paralleled the protein expression when measured by RT-PCR. We also found that both day 4 and day 14 Caco-2 cells did express peroxisomal proliferator-activated receptor-γ (PPAR-γ). mRNA and PPAR-γ ligand could increase apoA-I secretion in these cells. Therefore we propose that the mechanism for the induction of apoA-I might include PPAR-γ for which oxidized fatty acid is a ligand.**—Rong, R., S. Ramachandran, M. Penumetcha, N. Khan, and S. Parthasarathy. **Dietary oxidized fatty acids may enhance intestinal apolipoprotein A-I production.** ***J. Lipid Res.*** **2002.** 43: **557–564.**

**Supplementary key words** atherosclerosis • oxidized linoleic acid • Caco-2 cells • high density lipoprotein • oxidative stress • brush border • 13-HPODE • 13-HODE • antioxidant defense • oxidized low density lipoprotein • apolipoprotein B • PPAR-γ

Apolipoprotein (apo)A-I is a major protein component of HDL. Its level is positively correlated with HDL-cholesterol (HDL-C) and negatively correlated with atherosclerotic cardiovascular disease (1). The cardio-protective effects of HDL have been largely attributed to the ability of apoA-I to initiate cholesterol efflux and thereby facilitate the removal of excess cholesterol from peripheral tissues, and deliver it to the liver for degradation through reverse cholesterol transport pathways (2, 3). Using transgenic animal model systems, recent studies have demonstrated that the cholesterol-fed transgenic mice engineered to produce high concentrations of apoA-I developed reduced levels of atherosclerotic lesions (4). Additional studies have indicated that increased apoA-I gene expression in apoE deficient mice markedly suppressed atherosclerosis, further supporting a protective role for apoA-I (5). ApoA-I has been shown to enhance fibrinolysis and to inhibit platelet activity (6, 7) suggesting a possible protective role of apoA-I in thrombotic processes associated with coronary disease. In addition to these beneficial effects, apoA-I and HDL are implicated in preventing the oxidative modification of LDL and in the removal of lipid peroxides or oxidatively modified lipids from minimally modified LDL (8–10).

Mammalian apoA-I is synthesized principally in the small intestine and liver (11, 12). It has been shown that in fat-fed rats, the intestine contributes 56% of plasma apoA-I (13), and unsaturated fatty acids can stimulate apoA-I secretion from newborn swine intestinal epithelial cells (14). Significant amounts of oxidized lipids are found in processed foods (15, 16). During our studies on the absorption of oxidized lipids, we noted elevated levels of plasma apoA-I in humans (unpublished observations). In vivo studies conducted in animals have clearly demonstrated that dietary oxidized lipids are absorbed by the intestine and released into the circulation within triacylglycerol-rich lipoproteins (17–20). Our previous studies have shown that oxidized free fatty acids (ox-FFA) are efficiently taken up by Caco-2 cells, and the uptake was dependent on the presence of brush border structure (21). However, it is unknown whether ox-FFA will have any effect on intestinal apoA-I synthesis and secretion. Therefore it would be important to understand the function of oxidized and unoxidized fatty acids in regulating intestinal apoA-I synthesis or secretion.

Peroxisome proliferator-activated receptor-gamma (PPAR-γ) is a ligand-dependent nuclear receptor that is activated by a range of synthetic and naturally occurring substances in-

Abbreviations: 13-HPODE, 13-hydroperoxylinoleic acid; ox-FFA, oxidized free fatty acids; ox-linoleic acid or ox-linoleate, oxidized linoleic acid; unox-linoleic acid or unox-linoleate, unoxidized linoleic acid; PPAR-γ, peroxisome proliferator-activated receptor-gamma; 15d-$PGJ_2$, 15-deoxy-$^{\Delta 12,14}$-prostaglandin $J_2$.

[1] To whom correspondence should be addressed.
e-mail: spartha@emory.edu

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cluding 15-deoxy-$^{\Delta 12,14}$-prostaglandin $J_2$ (15d-PG$J_2$) (22) and oxidized linoleic acid (ox-linoleic acid) (23, 24). Studies performed years ago have shown that fibrates might increase HDL and apoA-I production by way of an unknown mechanism (25–29). One of our concerns is whether the response observed on addition of ox-FFA to Caco-2 cells is via PPAP-γ activation.

Caco-2 cells, derived from a human colonic adenocarcinoma, can be induced to develop enterocytic features under appropriate conditions in cell culture and secrete associated apolipoproteins (30–39). Linoleic acid is the most predominant fatty acid among the dietary PUFA. There is evidence that in addition to dietary fatty acids, the intestine can also convert linoleic acid to 15-lipoxygenase products (40). In this study, using Caco-2 cells as an in vitro model system, we compared the effects of oxidized linoleic acid [ox-linoleic acid, 13-hydroperoxylinoleic acid (13-HPODE), and 13-hydroxylinoleic acid (13-HODE)] and unoxidized linoleic acid (unox-linoleic acid) on apoA-I secretion and steady-state mRNA expression.

## MATERIALS AND METHODS

### Materials

All cell culture materials were purchased from Cellgro Mediatech (Herndon, VA) with exception of Eagle's minimum essential medium (EMEM) (Bio Whittaker). Fatty acids, soybean lipoxidase, alkaline phosphotase-conjugated secondary antibody (anti-goat IgG), peroxidase-conjugated secondary antibodies (anti-goat and anti-rabbit IgG), *N*-ethylmaleimide, *N*-acetyl-*L*-leucinyl-*L*-leucinyl-*L*-norleucinal (ALLN), and TRI Reagent were purchased from Sigma (St. Louis, MO). Authentic hydroperoxyoctadecadienoic acid [13(s)-HPODE] and 15d-PG$J_2$ were purchased from Cayman Chemicals (Ann Arbor, MI). Pure human apoA-I was obtained from Academy Biomedical Company (Houston, TX). Goat anti-human apoA-I polyclonal antibody was obtained from Rockland Immunochemicals (Gilbertsville, PA). Monoclonal antibody to human apoB and peroxidase-conjugated goat anti-human apoB-48/100 were purchased from Biodesign (Saco, Maine). GW9662 was synethesized by the Medicinal Chemistry Department at Glaxo Wellcome Research and Development and was a generous gift from Dr. T. M. Willson of the same institute (Glaxo Wellcome Co., Researeh Triangle Park, NC). RT-PCR kit was from PerkinElmer Life Sciences (Boston, MA). Caco-2 cells were purchased from American Type Culture Collection (Rockville, MD). All other chemicals used were of molecular biology grade.

### Oxidation of linoleic acid

Stock solution of linoleic acid was prepared in absolute ethanol, and then diluted in PBS. The linoleic acid (18:2, v/v, 50 μM) solution was oxidized with soybean lipoxidase (30–100 U/100 nmol, 2 h at 37°C) to produce ox-linoleic acid (13-HPODE and 13-HODE) (41). The formation of the conjugate diene (13-HPODE and 13-HODE) was monitored spectrophotometrically by scanning the absorption between 200 and 300 nm (model DB-3500, SLM-Aminco, Urbana, IL), using PBS as the reference. Under these conditions, the conversion of the linoleic acid into oxidized lipid is observed as an increase in absorbance at an optical density of 234 nm. Usually, about a 90% conversion of linoleic acid to 13-HPODE and 13-HODE was achieved. In addition, the formation of 13-HPODE was determined by leucomethylene blue assay (42).

### Cell culture

Caco-2 cells were plated in 60 mm cell culture dish at $5 \times 10^5$ cells per dish in 5 ml EMEM with nonessential amino acids and supplemented with FBS (10%), 1 mM glutamine, and 5% penicillin-streptomycin. Experiments were carried out on days 4 and 14. For experiments with cells on day 4, cells were seeded at a higher density to ascertain confluency. On the day of the experiments, the cells were washed and incubated in serum-free medium. After 6–8 h, fresh serum-free media was added to cells, together with 25 μM unox-linoleic acid, 5μM, 10 μM, or 25 μM ox-linoleic acid, 5 μM 15d-PG$J_2$ (PPAR-γ agonist), and/or 5 μM GW9662 (PPAR-γ antagonist). After 20 h, culture medium from each dish was collected for apoA-I measurement, and the cell monolayer washed with PBS, solubilized in cell lysis buffer, and used for cellular protein measurement and Western blot analysis. Our previous study indicated that the presence or absence of albumin had no effect on the uptake of fatty acids (21). Furthermore, the intestinal lumen does not contain any albumin and fatty acids in the free form.

### Secretion of apoA-I by Caco-2 cells

ELISA using goat anti-human apoA-I polyclonal antibody was used to measure the apoA-I content of cell conditioned media. About 100 μl of culture medium was added to the wells of the ELISA plate, and incubated overnight at 37°C. Human plasma apoA-I was used as standard antigen. The next day, plates were washed three times with PBS, and blocked with 1% BSA (radioimmunoassay grade) at 37°C for 1 h. Wells were washed three times with PBS, incubated for 2 h at 37°C with 100 μl goat anti-human apoA-I polyclonal antibody at a dilution of 1:5,000 (v/v). Washed twice and secondary antibody (anti-goat IgG conjugated with alkaline phosphatase) was added at a dilution of 1:10,000 (v/v) and incubated at 37°C for 2 h. The wells were washed twice, and the substrate ρ-nitrophenyl phosphate was added. The optical density was measured at 405 nm after 30 min. All samples were run in duplicate, and variability between duplicates was $<5\%$. The concentration of apoA-I was expressed as micrograms per milligram of cellular protein.

### Western blot analysis

Cell lysates were prepared by lysis and sonication in a hypotonic buffer [50 mM Tris (pH 8), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 5 mM EDTA containing 10 μg/ml aprotinin, 10 μg/ml leupeptin and 0.1 mM phenylmethylsulfonyl fluoride]. Samples were centrifuged at 13,000 *g* for 10 min at 4°C. Protein concentration was measured in the cell lysate using bicinchoninic acid kit, according to the procedure of Smith et al. (43). Protein extract (80–100 μg/well) was separated on 15% SDS-PAGE. The gel was transblotted onto a nitrocellulose membrane, blocked with 10% milk powder in TBS-T [Tris-buffered saline (pH 7.4), 0.1% Tween 20] overnight, and then incubated with goat polyclonal anti-human apoA-I antibody (1:1,000, v/v dilution) for 120 min, washed with TBS-T, and incubated with secondary antibody (anti-goat IgG conjugated to horseradish peroxidase, 1:4,000, v/v dilution) for 1 h. After five washes, the signal was detected with a chemiluminescence kit (ECL kit, Amersham, Arlington Heights, IL). The membrane was then stripped in 62.5 mM Tris (pH 6.7), 2% SDS, and 0.75% β-mercaptoethanol for 30 min at 50°C. After three washes in TBS-T, the membrane was reprobed with anti-β-actin antibody (1:500, v/v dilution). β-actin was used to justify equal protein loading. Films were analyzed by densitometry (Bio-Rad model GS-700). The optical density (OD) of apoA-I-OD of β-actin ratio was calculated, and the fold increase is reported in each figure, considering untreated control as 1.



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## RT-PCR analysis

The RT-PCR technique was used to ascertain levels of apoA-I mRNA in the Caco-2 cells after subjecting to various treatments. Total RNA was isolated from Caco-2 cells using TRI Reagent according to the manufacturer's instructions. Quality of the RNA was checked, and about 4.5 μg of RNA was reverse transcribed. The reaction was run at 42°C for 20 min, and the resulting cDNA samples were PCR amplified. After denaturation at 95°C for 10 s, PCR amplification of apoA-I and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNAs were conducted for 32 cycles consisting of denaturation (95°C for 10 s), primer annealing (60°C for 15 s), and extension (72°C for 15 s). This procedure was followed by a final extension at 72°C for 30 s. Initially, the quantity of total RNA for RT-PCR and the number of cycles were determined to ensure that PCR amplification of target cDNAs (apoA-I and GAPDH) remained in the exponential range and that saturation had not occurred. In order to investigate the status of PPAR-γ, we also measured the PPAR-γ mRNA expression in day 4 and day 14 Caco-2 cells. The following primers were used: apoA-I, 5′-TGGGATCGAGTGAAGGACCT-3′ & 5′-CTCCTCCTGCCACTTCTTCTG-3′; GAPDH, 5′-ACCACAGTCCAT-GCCATCAC-3′ & 5′-TCCACCACCCTGTTGCTGTA-3′; PPAR-γ, 5′-TCTCTCCGTAATGGAAGACC-3′ & 5′-GCATTATGAGACATCCCCAC-3′. The final PCR products (20 μl) were electrophoresed on a 0.8% agarose gel and visualized by ethidium bromide staining under UV light. The amplified DNA fragments obtained were of the appropriate base-pair sizes: 312 bp (apoA-I), 500 bp (GAPDH), and 474 bp (PPAR-γ). The relative intensities of the bands were quantified by densitometric analysis. The apoA-I mRNA levels were corrected for the corresponding GAPDH band densities.

## ApoB mass

Caco-2 cells were cultured as above. On the day of the experiments, the cells were washed and incubated in serum-free medium. After 6–8 h, fresh serum-free media was added to cells, together with 25 μM unox-linoleic acid and 5 μM, 10 μM, or 25 μM ox-linoleic acid. After 20 h, the culture medium was harvested, and the cells were lysed in 2% sodium cholate in HEPES-buffered saline (50 mM HEPES, 200 mM NaCl, pH 7.4) containing 1 mM phenylmethylsufonyl fluoride, 0.1 mM ALLN, 5 mM *N*-ethylmaleimide, and complete protease inhibitors. Insoluble material in the cell lysate (44) was removed by centrifugation at 10,000 × *g* for 10 min. All samples were stored at −80°C until analysis (within 48–72 h). ApoB mass was estimated by sandwich ELISA (34). Ninety-six-well ELISA plates were precoated with 1 μg monoclonal antibody to apoB in 200 μl 0.05 mM $NaHCO_3$ buffer, pH 8. The plates were then covered with Saran Wrap and incubated for 18 h at 4°C. The wells were washed three times with PBS containing 0.05% Tween-20. The unbound sites on the wells were blocked by adding to the wells 200 μl 3% BSA in PBS and incubated for 1 h at room temperature. The wells were then washed. ApoB standard was diluted with buffer A (PBS containing 0.05% Tween-20 and 0.5% BSA), and aliquots of 200 μl containing between 0 and 50 ng apoB were added per well in triplicate. Samples of the cell and basolateral media were added to the wells in triplicate, and the plates were wrapped in Saran Wrap and incubated for 18 h at room temperature. The wells were then washed and blotted dry. Two hundred microliters of buffer A containing 10 ng anti-apoB immunopurified sheep polyclonal antibody conjugated to horseradish peroxidase was added to each well. The plates were again wrapped and incubated for 4 h at room temperature. The wells were then washed three times with PBS containing 3% BSA followed by three washes in PBS and blotted dry. The activity of horseradish peroxidase was estimated by adding 100 μl 3,3′,5,5′-tetramethylbenzidine and hydrogen peroxide (1:1, v/v). After 10 min, the reaction was stopped with the addition of 100 μl 1 N HCl and the absorbance was read at 450 nm. The concentration of apoB was expressed as μg/mg of cellular protein.

## Statistical analysis

The data in the manuscript are expressed as mean ± SE. Comparisons of data were made by paired Student's *t*-test. A $P < 0.05$ was considered statistically significant. Statistical significance is represented as *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ respectively.

# RESULTS

Incubation of Caco-2 cells with different concentrations of ox-linoleic acid for 24 h resulted in a dose-dependent increase in apoA-I concentration in the medium of both day 4 and 14 cells (**Fig. 1A**). For day 4 cells, ox-linoleic acid at the concentrations of 5, 10, and 25 μmol/l

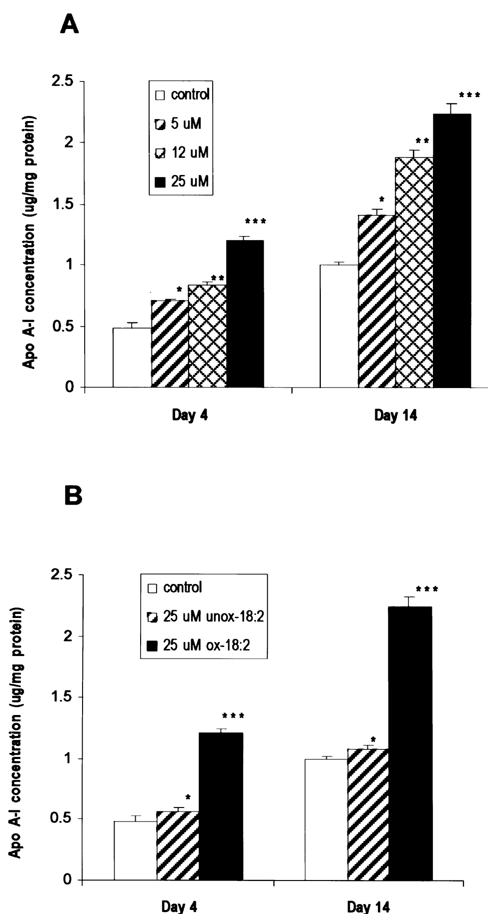


**Fig. 1.** Effect of unoxidized and oxidized linoleic acid (unox- and ox-linoleic acid) on the secretion of apoA-I. Day 4 and day 14 Caco-2 cells were incubated for 20 h with 25 μM unox-linoleic acid (unox-18:2) and 5 μM, 10 μM, and 25 μM ox-linoleic acid (ox-18:2) (control cells treated with PBS). At the end of the incubation, culture medium was assayed for apoA-I protein levels by ELISA and expressed in terms of total cellular protein. A: Shows the effect of ox-linoleic acids. B: Compares the effect of unox-linoleic acid with ox-linoleic acid. Statistical comparisons were made with reference to control. Mean ± SE of five experiments are shown in the figure. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

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resulted in a significant increase in apoA-I secretion by 45%, 76%, and 150% respectively. For day 14 cells, apoA-I secretion increased by 42%, 90%, and 125% respectively.

Figure 1B shows secretion of apoA-I into the medium by day 4 and 14 Caco-2 cells after incubation for 24 h with unox-linoleic and ox-linoleic acids. Ox-linoleic acid increased apoA-I concentration in day 4 and 14 Caco-2 cells as measured by ELISA assay, and day 14 cells secreted more apoA-I as compared with day 4 cells. In contrast, unox-linoleic acid induced far lower level of apoA-I than ox-linoleic acid, and it is possible that unox-linoleic acid was oxidized during the incubation because it is sensitive to oxidition. The apoA-I concentration, in day 4 or day 14 Caco-2 cells exposed to 25 μM unox-linoleic acid was 1.21- or 1.08-fold greater as compared with untreated cells (n = 5, $P < 0.001$ or $P < 0.05$). Interestingly, in the day 4 and 14 cells exposed to 25 μM ox-linoleic acid, the apoA-I concentration was 2.50- or 2.25-fold greater when compared with untreated cells (n = 5, $P < 0.001$), and 2.07- or 2.09-fold greater when compared with 25 μM unox-linoleic acid treated cells (n = 5, $P < 0.001$) respectively (Fig. 1B). Incubation of Caco-2 cells with fatty acids did not alter either the morphology or viability of the cells (data not shown).

Western blot analysis showed that cell-associated apoA-I protein content (both day 4 and 14 cells) was also increased by oxidized fatty acids. As seen in **Fig. 2A** and **B**, treatment of Caco-2 cells with 25 μM unox-linoleic acid for 24 h induced a 1.2-fold increase in apoA-I protein levels in both day 4 and 14 cells. Treatment with ox-linoleic acid at various concentrations was able to induce a dose-dependent increase in apoA-I content (1.8- to 4.5-fold increase) in both day 4 and 14 Caco-2 cells. Interestingly, day 4 Caco-2 cells seemed to be more sensitive to ox-linoleic acid than day 14 cells.

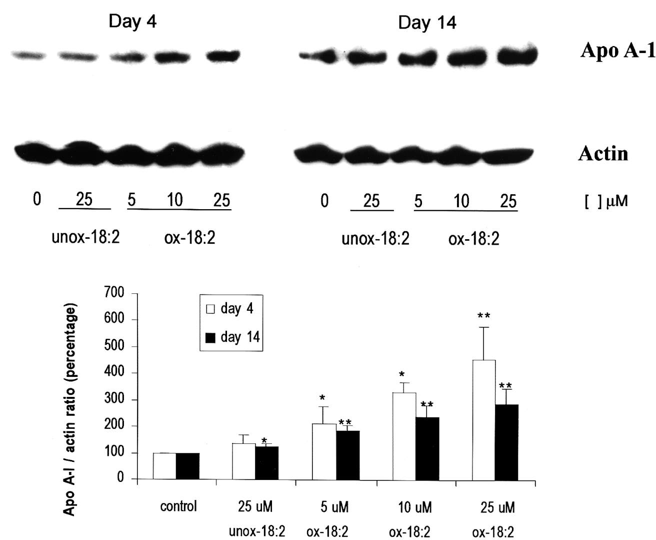


**Fig. 2.** ApoA-I protein level in day 4 and day 14 Caco-2 cells. Caco-2 cells were placed in serum-free medium for 6–8 h, and then stimulated with unox- (unox-18:2) and ox-linoleic acids (ox-18:2) for 20 h. Western blot analysis was performed with 80–100 μg of total protein from cell lysates. After densitometric analysis, the optical density (OD) of apoA-I/OD of β-actin ratio was considered as 100% in untreated cells. A: A representative polyacrylamide gel. B: The average results from three separate experiments are shown as mean ± SE. *$P < 0.05$, **$P < 0.01$.

To determine the effect of fatty acids on apoA-I mRNA, we performed RT-PCR analysis using total RNA isolated from cells. The mRNA expression paralleled the protein expression (**Fig. 3A** and **B**). Both unox-linoleic acid and ox-linoleic induced apoA-I mRNA expression in both day 4 and 14 Caco-2 cells, and ox-linoleic acid treatment caused a dose-dependent increase in apoA-I mRNA expression. These results suggest that the upregulation of apoA-I by fatty acids is at least in part due to transcriptional activation and/or increased apoA-I mRNA stability.

We also show that both day 4 and day 14 Caco-2 cells have PPAR-γ mRNA expression (**Fig. 4A**). PPAR-γ agonist (15d-$PGJ_2$) increased apoA-I secretion by both day 4 and day 14 Caco-2 cells (Fig. 4 B and C); whereas PPAR-γ antagonist (GW9662) had no significant activity on apoA-I secretion itself, it reduced both ox-linoleic acid- and 15d-$PGJ_2$-mediated induction in day 4 (by 34% and 22%, respectively) and day 14 (44% and 38%, respectively) Caco-2 cells.

In order to determine if the apoA-I induction in Caco-2 cells is a general effect of ox-linoleic acid, we determined apoB mass from both day 4 and day 14 Caco-2 cells after incubation with unox- or ox-linoleic acid. Our data shows that the total apoB mass in the medium and the cells did not change during the 20 h incubation with unox- or ox-linoleic acid (**Fig. 5A** and **B**).

## DISCUSSION

This is the first report of an induction of apoA-1 synthesis by oxidized fatty acids. Yet another observation is that the apoB production did not change during the incubation with unox- or ox-linoleic acids. While the mechanism(s) of such induction is not yet fully understood, the implications of our findings are intriguing and enormous. First, the diet contains unsaturated lipids and, depending on the source and the processing and storage of food, there is no doubt that diet may contain varying amounts of oxidized lipids. These are broken down to FFAs during the absorption process.

A plethora of studies have suggested that heated fat that contains oxidized fatty acids in the diet might be taken up by the intestinal cells (17–20). A previous study from our laboratory showed that oxidized fatty acids are efficiently taken up by Caco-2 cells in a dose-dependent pattern, and that the difference in the uptake of fatty acids between day 4 and day 14 cells may be because of the absence of brush border membrane in the day 4 cells (21).

In the current study, we show that the induction of apoA-1 by ox-linoleic acid is dose-dependent, and differentiated (day 14) Caco-2 cells produce more apoA-I as compared with undifferentiated (day 4) cells. Thus, the



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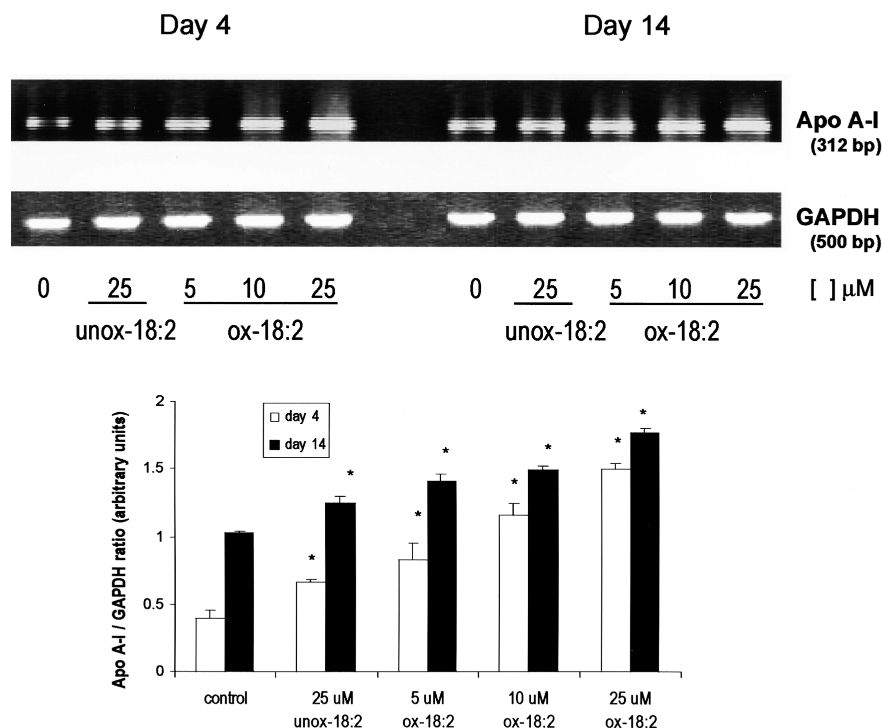


**Fig. 3.** Effect of unox- and ox-linoleic acids on the steady state mRNA levels of apoA-I. Caco-2 cells were placed in serum-free medium for 6–8 h, and then stimulated with unox- and ox-linoleic acids for 20 h. RNA was harvested and analyzed. A: A representative agarose gel. B: The graphic representation of the average results from three separate experiments after correction using glyceraldehyde 3-phosphate dehydrogenase. Data are mean ± SE of three separate experiments. *$P < 0.05$.

uptake of oxidized fatty acids by Caco-2 cells might be an important step in stimulating production of apoA-I. The increase in apoA-I mRNA transcript stability and/or increase in the mRNA transcription of the apoA-I gene are possible. However, considering that the difference in uptake was over 10-fold between the undifferentiated and differentiated cells (21) and the induction of mRNA was greater in the undifferentiated cells (Fig. 3), it is also possible that specific cellular signaling mechanisms other than simple uptake-mediated induction are important.

Recently, differentiation dependent expression and localization of class B type 1 scavenger receptor was demonstrated in the Caco2 cells (45). We considered the possibility that oxidized fatty acids might have triggered rapid differentiation of day 4 cells thus accounting for the increased apoA-1 synthesis. However, the activity of alkaline phosphatase, a marker for brush border (21, 46) did not change during the treatment with ox-linoleic acid, nor did the uptake of ox-linoleic acid increase significantly after exposure to ox-fatty acid (results not shown).

The cellular mechanisms involved in the regulation of apoA-I are not known at present. Oxidized fatty acids have been suggested to be a ligand for PPAR-γ (23, 24), a potent mediator of apoA-I synthesis in the liver (47, 48). Our study shows that both differentiated and undifferentiated Caco-2 cells had PPAR-γ gene expression, and PPAR-γ activation could increase apoA-I secretion by both differentiated and undifferentiated Caco-2 cells. Thus, the induction of apoA-1 as seen in the current study might simply reflect induction of PPAR-γ mediated signaling mechanisms.

Navab et al. have demonstrated that apoA-I can interact and remove oxidized lipids from ox-LDL and render LDL resistant to oxidation by human artery wall cells (10). Our previous studies also showed that oxidized fatty acids induced cellular antioxidant potential gene (nitric oxide synthase and catalase) expression (49, 50). Others have also observed the induction of heme oxygenase and manganese-containing superoxide dismutase (MnSOD) by oxidized lipids (51, 52). Thus, the induction of apoA-I gene expression might reflect a general trend in inducing antioxidant defense mechanisms.

As mentioned previously, our unpublished observation that oral administration of oxidized lipids resulted in increased plasma HDL levels and the observation by Sutherland et al. that feeding of heated oil increased plasma apoA-1 levels (53) might lead to the conclusion that in the absence of hypercholesterolemia, tissue response to oxidized lipids might be generally favorable and tend to induce differentiation strategies.

Our results might explain the paradoxical HDL lowering effect of antioxidants in recent clinical trials (54). Unfortunately, no dietary details (whether the subjects consumed any heated or fried food) or when the antioxidant was supplemented are given. Considering that probucol, a well known antioxidant, lowered HDL-C (55–57), it might



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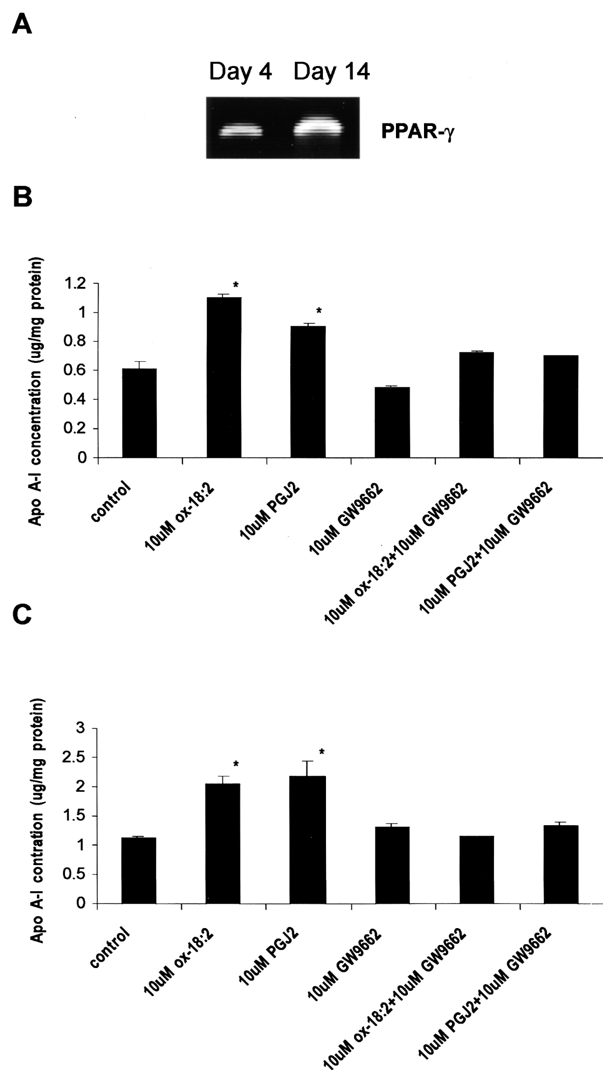


**Fig. 4.** Effect of peroxisome proliferator-activated receptor-gamma (PPAR-γ) activation on the secretion of apoA-I. Day 4 and day 14 Caco-2 cells were incubated for 20 h with 10 μM ox-lineic acid (ox-18:2), 10 μM 15d-$PGJ_2$ in either the absence or presence 10 μM GW9662 (control cells treated with 0.2% DMSO). At the end of the incubation, culture medium was assayed for apoA-I protein levels by ELISA and expressed in terms of total cellular protein. A: PPAR-γ mRNA expression in day 4 and day 14 Caco-2 cells. Caco-2 cells were cultured for 4 or 14 days. RNA was harvested and analyzed by RT-PCR. B: ApoA-I secretion by day 4 Caco-2 cells. C: ApoA-I secretion by day 14 Caco-2 cells. Statistical comparisons were made with reference to control. Mean ± SE of three experiments are shown in the figure. *$P < 0.05$.

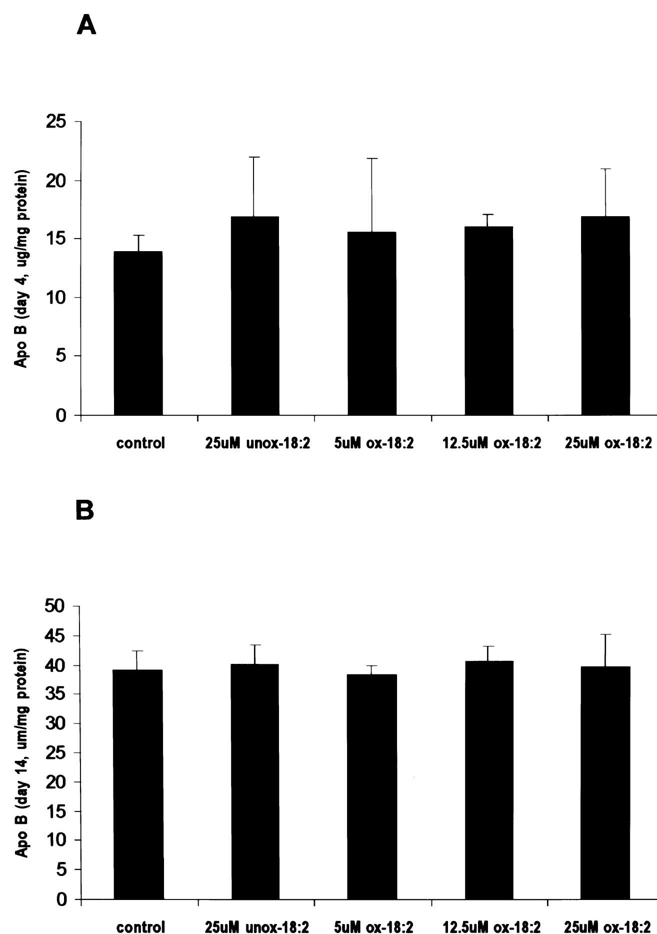


**Fig. 5.** Effect of unox- and ox-linoleic acid on apoB mass. Day 4 and day 14 Caco-2 cells were incubated for 20 h with 25 μM unox-linoleic acid (unox-18:2) and 5 μM, 10 μM, and 25 μM ox-linoleic acid (ox-18:2) (control cells treated with PBS). At the end of the incubation, apoB in the culture medium and in the cell lysate were measured by sandwich ELISA and expressed in terms of total cellular protein. A: ApoB in the medium and cell lysate of day 4 Caco-2 cells. B: ApoB in the medium and cell lysate of day 14 Caco-2 cells. Mean ± SE of three experiments are shown in the figure.

suggest that dietary oxidized lipids (particularly in the absence of cholesterol) might help to maintain high HDL levels and antioxidant supplementation might be considered counter productive. In this regard, dietary ox-FFA might act like exercise and help to maintain high levels of tissue antioxidant enzymes (58–60) but might also induce HDL synthesis.

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