Dietary oxidized fatty acids: an atherogenic risk?

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Abstract **Previous studies have suggested that heated fat that contains oxidized fatty acids in the diet might contribute to the presence of oxidized components in circulating lipoproteins. On the other hand, studies in our laboratory showed that cultured cells such as smooth muscle cells take up oxidized fatty acids poorly. Because intestinal cells are morphologically quite distinct, we studied the uptake of oxidized linoleic acid by Caco-2 and smooth muscle cells (control). When 16-day-old Caco-2 cells were incubated with oxidized linoleic acid (ox-linoleic acid), its uptake was comparable to that of unoxidized linoleic acid (unox-linoleic acid) or that of oleic acid (40–58, 70, and 55%, respectively). In contrast, the uptake of ox-linoleate by smooth muscle cells was about 3%. To determine whether the brush border structure of Caco-2 cells was responsible for increased uptake of oxidized fatty acids, we compared uptake in 4- and 16-day-old cells. The uptake of unox-linoleate and oleic acid (18:1) was comparable for the 4- and 16-day cells. In addition, saturation and competition experiments showed that the uptake of ox-linoleate by Caco-2 cells is not saturable even at 150 μM and that this uptake is diluted in the presence of unox-linoleate. In esterification experiments utilizing rat intestinal microsomes, we show that both ox- and unox-linoleate are esterified equally well. In summary, dietary oxidized fatty acids can be absorbed by the intestine and incorporated into lipoproteins and could potentially impose an oxidative stress and exacerbate atherogenesis.—** Penumetcha, M., N. Khan, and S. Parthasarathy. **Dietary oxidized fatty acids: an atherogenic risk? *J. Lipid Res.* 2000.** 41: **1473–1480.**

Supplementary key words brush border • smooth muscle cells • lipid absorption • chylomicron • oxidized low-density lipoprotein • atherosclerosis

A plethora of studies have suggested that oxidized lipids may have atherogenic properties (1–3). Oxidized free fatty acids (ox-FFA) affect cell proliferation, signaling mechanisms, chemotaxis, and cell survival (apoptosis) (4–18), presumed components of cardiovascular dysfunction in atherosclerosis. Lipids that contain ox-FFA as esterified components, such as oxidized phospholipids, also have been shown to elicit various responses from cells (19–28). Studies by Cushing et al. (28) have described the biological effects of peroxidized phospholipids on vascular cells. Cells are constantly exposed to ox-FFA (secreted lipoxygenase and cyclooxygenase products, products of lipolysis of oxidized esterified lipids, extracellular actions of secreted enzymes such as phospholipases on peroxidized phospholipids). Of the cell types in the body, the intestinal enterocytes with microvilli are exposed to abundant levels of dietary oxidized fatty acids during absorption (**Scheme 1**).

Evidence suggests that peroxidized lipids in the diet might contribute to the atherogenicity of lipoproteins (29–31). Studies by Staprans and co-workers (29–31) suggest that in males, dietary peroxidized lipids can be secreted into chylomicrons and that these chylomicrons are more suseptible to in vitro oxidation. Another study showed that rabbits given a diet high in oxidized lipids had more atherosclerotic lesions compared with rabbits fed a diet low in oxidized lipids. In these studies heated oil was used as the source of peroxidized lipids and resulted in the appearance of oxidized lipids in the blood lipoproteins and enhanced the ex vivo oxidizability of chylomicrons. However, the extent of absorption of the oxidized lipids cannot be determined from these studies. The presence of only a minimal amount of the administered oxidized oil in the plasma lipoproteins is not only reflective of the absorption but also of flux between the different compartments.

Studies from our laboratory have demonstrated that cultured cells such as endothelial cells, smooth muscle cells, and macrophages take up oxidized fatty acids poorly as compared with unoxidized fatty acids (32). On the basis of uptake and competition studies, we proposed that the uptake of oxidized fatty acids by cells might involve specific cellular binding, if not a specific receptor. We also provided evidence to suggest that oxidized fatty acids might be poorly utilized for the resynthesis of complex lipids and instead might undergo extensive peroxisomal degradation resulting in the generation of hydrogen peroxide (H_2O_2).

The absorption of lipids by the intestinal enterocyte is

Abbreviations: alk-phos, alkaline phosphatase; FFA, free fatty acid; 13-HPODE, 13-hydroperoxylinoleic acid; 13-HODE, 13-hydroxylinoleic acid; LysoPtdCho, lysophosphatidylcholine; NL, neutral lipid; ox-FFA, oxidized free fatty acids; ox-linoleic acid or ox-linoleate, oxidized linoleic acid; PtdCho, phosphatidylcholine; SMC, smooth muscle cells; unox-linoleic acid or unox-linoleate, unoxidized linoleic acid.

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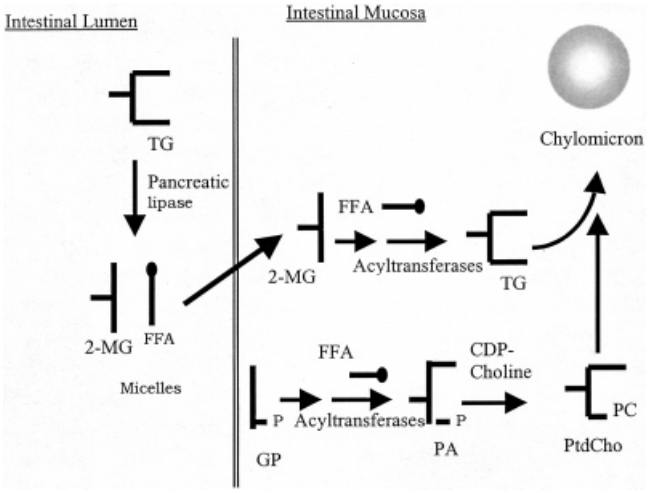

Scheme 1. Intestinal absorption of triglycerides. The triglycerides consist of a glycerol backbone with three esterified fatty acids. During intestinal absorption, the triglycerides are hydrolyzed to monoglyceride and free fatty acids. This is catalyzed by the pancreatic lipase in the presence of bile salts. The products form mixed micelles with bile salts and are absorbed into the enterocytes via the microvilli or brush border. Inside the cell, the monoglycerides and free fatty acids are reesterified to generate triglyceride via the intermediate generation of diglycerides. TG, triglycerides; MG, monoglycerides; FFA, free fatty acids; PtdCho, phosphatidylcholine; GP, glycerophosphate; PA, phosphatidic acid.

quite different from the uptake of lipids by other cells. During the digestion of lipids (e.g., triglycerides), there is extensive pancreatic lipase-catalyzed degradation resulting in the formation of monoacylglycerol and free fatty acids (33–36). These, together with bile salts (and lysophospholipids from the hydrolysis of dietary phospholipids by pancreatic phospholipase) form mixed micelles that are readily absorbed by passive diffusion mechanism(s) via the microvilli (brush border structure). In other words, compounds that readily enter the micellar phase (drugs, carcinogens, sterols, fat-soluble vitamins, etc.) are expected to be more efficiently absorbed by intestinal villus cells. More importantly, villus cells reassemble the absorbed fatty acids and other partially hydrolyzed components into lipids that constitute the chylomicron. Efficient acyltransferases are involved in the reesterification process, suggesting that if oxidized fatty acids are absorbed, they are also likely to be esterified.

In this study, we compared the uptake of oxidized fatty acids (13-hydroperoxylinoleic acid [13-HPODE] and 13-hydroxylinoleic acid [13-HODE]) by Caco-2 intestinal epithelial cells with that of smooth muscle cells (SMC). The Caco-2 cells provide an unique opportunity to study the role of microvilli because their differentiation during prolonged culture can be easily monitored by the presence of the marker enzyme, alkaline phosphatase (alk-phos). We chose the oxidized forms of linoleic acid as this is the most abundant dietary polyunsaturated fatty acid.

Our results show that oxidized fatty acids are efficiently taken up by Caco-2 cells and that the uptake was dependent on the presence of brush border structure. On the basis of our results, we propose that oxidized fatty acids may pose additional, externally derived oxidative stress and exacerbate the atherogenic process in subjects with inadequate antioxidant defense.

EXPERIMENTAL PROCEDURES

Fatty acids and soybean lipoxidase were purchased from Sigma (St. Louis, MO). [1-^{14}C]Linoleic acid (196×10^7/mmol) and [1-^{14}C]oleic acid (215×10^7/mmol) were obtained from New England Nuclear (Boston, MA). Authentic hydroperoxyoctadecadienoic acid [13(*S*)-HPODE] was purchased from Cayman Chemicals (Ann Arbor, MI).

Caco-2 cells were cultured in six-well plates for experiments and in 100-mm stock plates in Earle's minimum essential medium (EMEM) with nonessential amino acids (NEAA) and supplemented with fetal bovine serum (FBS; 10%), 1 mM glutamine, and 5% penicillin-streptomycin. SMC were used as controls and were cultured in EMEM with 10% FBS, 2 mM glutamine, and 5% penicillin. Cells were maintained at a pH of 7.4 at 37°C with a 95% airflow and 5% CO_2.

Oxidation of linoleic acid

Stock solutions of radioactive linoleic and oleic acids (41.67 Bq/nmol) were prepared in absolute ethanol and then diluted in phosphate-buffered saline (PBS). The linoleic acid (18:2; 50 μM) solution was oxidized with soybean lipoxidase (30–120 U/100 nmol, 2 h at 37°C) to produce oxidized linoleic acid (hydroperoxyoctadecadienoic acid [HPODE] and hydroxyoctadecadienoic acid [HODE])(37). The formation of the conjugated diene (HPODE and 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 the oxidized lipid is observed as an increase in absorbance at the optical density of 234 nm. Usually, about 90% conversion of linoleic acid to HPODE and HODE is achieved. In addition, the formation of HPODE was determined by leucomethylene blue (LMB)(38). Briefly, 40 μl of the ox-linoleate is added to 100 μl of LMB reagent in a microtiter plate and incubated at room temperature for 10 min. The sample is then read in an Anthos htll microtiter reader (Labtech International, East Sussex, UK). The amount of peroxide generated is quantitated against a standard curve generated by authentic 13-HPODE.

Caco-2 cells were seeded at an initial density of 2×10^4 and SMC at a density of 4×10^4 cells per well, in six-well plates. Experiments were carried out on days 4 and 15 or 16 for Caco-2 cells and on day 4 for SMC. For experiments with cells on day 4, cells were seeded at a higher density to ascertain confluency. On the day of the experiment, the medium was removed and the cells were washed three times with serum-free medium. Labeled fatty acid containing serum-free medium (41.67 Bq/nmol) was applied to cells in triplicate and incubated for the indicated time at 37°C. Preliminary results indicated that the presence or absence of albumin had no effect on the uptake. Furthermore, the intestinal lumen does not contain any albumin and fatty acids are present in the free form. An aliquot of the labeled fatty acid was added to EcoLume (ICN Biomedicals, Aurora, OH) to determine radioactivity. After incubation, the medium was collected and the cells were washed twice with PBS. An aliquot of the medium and the PBS used for washing were each added to EcoLume to count the label remaining in the medium and the wash, respectively. The cells were then solubilized in 2.0 ml of

deoxycholic acid solution (0.5 mg/ml) and the radioactivity was determined in 100 μl of the cell lysate. After acidification, by adding 20 μl of 6 N HCl, 4 ml of chloroform–methanol 1:1 (v/v) was added to the cell lysate to extract cellular lipids by the method of Bligh and Dyer (39). After centrifugation (5 min, 2,000 rpm), 500 μl of the upper phase was gently dried (37°C, under nitrogen) and the radioactivity was determined. The lower chloroform phase was collected and 100 μl was gently dried (37°C, under nitrogen), and the radioactivity associated with cellular lipids was determined. The remaining chloroform phase was gently dried (37°C, under nitrogen) and stored at −80°C for further analyses.

Thin-layer chromatography

The dried chloroform phase was dissolved in 100 μl of chloroform and then the neutral lipids were separated by thin-layer chromatography (TLC) on silica gel G60 analytical plates, using a solvent system containing chloroform–methanol–acetic acid–water (65:25:0.5:3). The neutral lipids (NL) and the phospholipids were identified by using iodine in the presence of standards. The lipid spots corresponding to NL and phospholipids were scraped off the plate and suspended in EcoLume, and the radioactivity was determined.

Assay for alkaline phosphatase

Caco-2 cells were grown in 100-mm plates to assay for alkaline phosphatase, a marker of cellular differentiation. On days 4 and 16, cells were harvested with cold saline and phenylmethylsulfonyl fluoride (PMSF, 40 μg/5 ml). After centrifugation at 700 rpm in a tabletop centrifuge, the saline was removed and the cells were frozen at −80°C for further analysis. On the day of the assay, the cells were resuspended in a 2 mM Tris, 50 mM mannitol buffer containing PMSF (40 μg/5 ml). The cells were homogenized for 5 sec and sonicated for a total of 15 sec on ice. The cell suspension was then centrifuged at 1,000 *g* for 10 min at 4°C to remove the nuclear membrane. The supernatant was centrifuged in an ultracentrifuge at 100,000 *g* for 1 h at 4°C. The supernatant was then removed and the cell pellet was suspended in 1 ml of saline with deoxycholate (0.01%). To assay for alkaline phosphatase (40), the cell membrane suspension (50 μl) was incubated with a 500-μl volume of 7 mM *p*-nitrophenyl phosphate in 0.1 M sodium bicarbonate–5 mM magnesium chloride buffer for 1 h at 37°C. This reaction was quenched with 1 ml of 0.1-M sodium hydroxide and read by a spectrophotometer at 410 nm. Protein content was determined by the method of Bradford.

Isolation of microsomes from rat intestine

Males Sprague-Dawley rats weighing 250–300 g were killed by CO_2 inhalation prior to isolating the microsomes (41). Each rat was placed on its back and a midline incision was made in the abdomen. The duodenum was visualized under the liver and the intestine was cut at the duodenum and was gently lifted out by clearing the mesenteric tissue. The intestine was then cut proximally to the ileocecal valve and then immediately washed with ice-cold saline. It was weighed and placed on a cold glass plate and the intestinal mucosa was gently scraped with a glass slide. The mucosal tissue was weighed and placed in cold 0.2 5M sucrose solution (pH 7.4) in a Potter-Elevehjem homogenizer and homogenized on ice at 3,000 rpm for 12–15 strokes. The homogenate was centrifuged at 3,000 rpm for 15 min at 4°C to remove the nuclear membrane. The supernatant was collected and centrifuged at 12,000 rpm for 15 min at 4°C. The pellet was discarded and the supernatant was subjected to a final centrifugation at 100,000 *g* for 1 h at 4°C. The supernatant was discarded and the pellet was resuspended in 0.25 M sucrose and homogenized at 2,000 rpm for 5 strokes. Protein was determined by the method of Bradford. The microsome suspension was frozen at −80°C.

Microsomal acyltransferase activity

Microsomal acyltransferase activity was performed in a final volume of 800 μL of Tris-HCl (0.1 mM, pH 7.4) as described by Parthasarathy, El-Rahman, and Baumann (42). Briefly, 100 μg of rat liver or intestinal microsomal protein was incubated in the presence of 50 μM [1-^{14}C]oleic, [1-^{14}C]linoleic, or [1-^{14}C]oxidized linoleic acid (41.67 Bq/nmol) as substrate, 100 μM lysophosphatidylcholine (LysoPtdCho), 200 μM ATP, 200 μM magnesium chloride, and 1 μM coenzyme A for 1 h at 37°C. After the incubation, lipids were extracted and the phospholipids were separated by TLC.

Preparation of rat everted intestinal sacs and incubation

Rat everted sacs were made according to the method of Wilson and Wiseman (43). Briefly, rats were killed by CO_2 asphyxiation, and 10-cm segments of the intestine were harvested and sacs were prepared. The sacs were incubated with 25 μM fatty acids in a 4-ml volume of serum-free EMEM. After incubation, the serosal contents were emptied and the radioactivity remaining in the medium was determined. The sac was washed with 1 ml of PBS, five times each. The mucosa was gently scraped into a test tube and the volume was adjusted to 1 ml by adding 0.25 M sucrose at pH 7.4. A 100-μl aliquot was placed in vial containing 4 ml of scintillation fluid and the radioactivity associated with the tissue was determined.

Scanning electron microscopy

Scanning electron microscopy (SEM) was performed on 4- and 16-day-old Caco-2 cells to look for the presence of microvilli. Caco-2 cells were grown in 24-well dishes on silicone chips in EMEM with NEAA supplemented with FBS (10%), 200 mM glutamine, and 5% penicillin-streptomycin. On the day of harvest the medium was removed and the cells were washed with 1 × PBS (room temperature) three times. After the wash cells were fixed in 500 μl of glutaraldehyde (room temperature). The cells were then prepared for SEM as described by Apkarian and Lund (44).

Statistical analysis

Differences between the values of the uptake of the three different fatty acids were assessed by one-way analysis of variance (one-way ANOVA). Differences between the days of culture and fatty acid uptake were assessed by two-way ANOVA. Statistical significance was set at $P < 0.05$. Values are expressed as means and standard error (SE).

RESULTS

Previous studies from our laboratory showed that cultured cells such as SMC, endothelial cells, and macrophages took up oxidized fatty acids poorly as compared with unoxidized linoleic acid or oleic acid (32). In contrast to unoxidized linoleic acid, the uptake of oxidized linoleate was saturable and was not competed for by unoxidized fatty acids. On the basis of this, we suggested the potential for a specific interaction of the ox-FFA with the cell membrane as opposed to diffusion across the cell membrane. As explained in the introduction, the intestinal enterocyte has a microvillar structure, which is suggested to

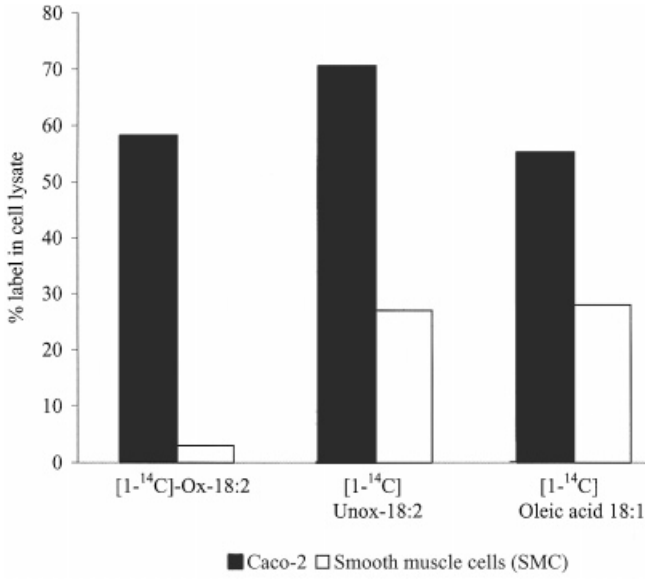

Fig. 1. Uptake of oxidized linoleic acid ([1-^{14}C]ox-18:2), unoxidized linoleic acid ([1-^{14}C]unox-18:2), and oleic acid ([1-^{14}C]oleic acid 18:1) by Caco-2 cells (solid columns) and smooth muscle cells (SMC, open columns). Caco-2 cells were seeded at 2×10^4 cells per well (4×10^4 for SMC) and were grown in EMEM as described in Experimental Procedures. On day 15, the Caco-2 cells (day 4 for SMC) were washed with serum-free medium three times and then were incubated for 30 min in serum-free medium containing 50 nmol of fatty acid. Afterward the incubation medium was removed and the cells were washed with PBS and then solubilized in 2 ml of deoxycholic acid (0.5 mg/ml). An aliquot of this cell lysate was added to EcoLume and the radioactivity (uptake) was quantitated. Each experiment was repeated four times, each time in triplicate. Data represent the mean uptake (as a percentage of total added) ± SE, $P < 0.001$. The error bars are too small to vizualize.

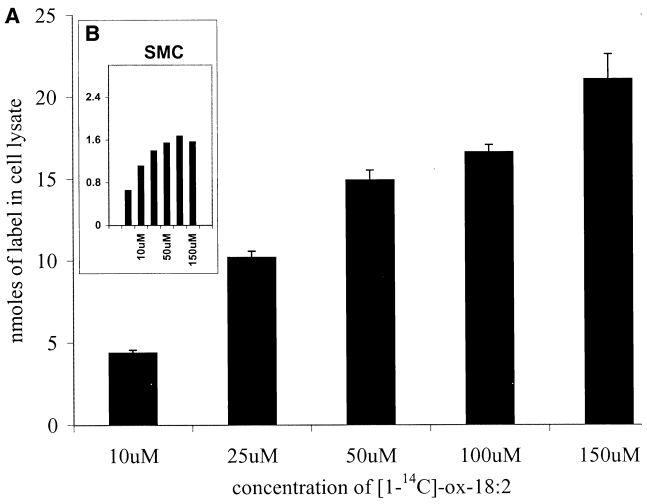

Fig. 2. (A) Saturation of Caco-2 cells on day 16 with 10, 25, 50, 100, and 150 μM oxidized linoleic acid ([1-^{14}C]ox-18:2). Caco-2 cells were treated with increasing concentrations of the oxidized linoleic acid for 30 min. After the incubation medium was removed and the cells were washed with PBS and then solubilized in 2 ml of deoxycholic acid (0.5 mg/ml). An aliquot of this cell lysate was added to EcoLume and the radioacitivity (uptake) was quantitated. Each experiment was repeated three times, each time in triplicate. Data represent the mean uptake (in nmol) ± SE, $P < 0.001$. (B) Saturation of SMC on day 4 with 5, 10, 25, 50, 100, and 150 μM oxidized linoleic acid ([1-^{14}C]ox-18:2). SMC were seeded at an initial density of 40,000 cells and were exposed to increasing concentrations of oxidized linoleic acid for 30 min. After incubation the cells were treated as in (A). Data represent mean values of triplicate samples.

play an important role in the uptake of mixed micelles that are generated in the lumen. Fatty acids constitute a major portion of lipids of the lumen as extensive lipolysis occurs of dietary triglycerides, phospholipids, and esterified cholesterol. We anticipate that ox-FFA might be readily absorbed from such a micelle.

Figure 1 illustrates the uptake of oxidized and unoxidized fatty acids by 15-day cultured Caco-2 cells and SMC. In contrast to the uptake of oxidized linoleic acid by SMC, the uptake by the 15-day-old Caco-2 cells was efficient and reached levels as high as 60%. Unoxidized linoleic and oleic acids were also readily taken up under these conditions and the level of uptake of all three fatty acids was similar. To determine whether there are differences in the mechanism of uptake between Caco-2 cells and smooth muscle cells we performed experiments utilizing increased concentrations of ox-linoleate. In these experiments linoleic acid oxidized to 90% was used. As seen in **Fig. 2A**, the uptake of ox-linoleate by Caco-2 cells steadily increased, not quite reaching saturation even at a concentration of 150 μM. In contrast, the uptake of ox-linoleate by SMC as shown in the Fig. 2B was only 3% and reached the saturating levels at about 25 μM. This also suggests that the uptake mechanism governing ox-linoleate is different between Caco-2 and SMC. To establish whether the uptake was indeed due to the presence of the brush border structure (differentiated state) in these cells, we used 4-day-old cultures (which presumably were deprived of brush border structures) and compared them with 16-day-old cultures (which are suggested to be rich in microvilli). We also measured the levels of alkaline phosphatase between the 4-day and 16-day cultured cells as an indicator of differentiation. The activity of alkaline phosphatase in 16-day-old cells was four times greater than that in 4-day-old cells (0.0485 ± 0.002 vs. 0.012 ± 0.006 units/μg of protein; $P < 0.001$). This suggested that the 16-day-old cells were well differentiated. We also confirmed the presence of microvilli in the 16-day-old cells by scanning electron microscopy (SEM). As shown in **Fig. 3B**, the 16-day-old cells show an abundant presence of microvilli compared with 4-day-old cells (Fig. 3A). However, there was no quantifiable relationship between the levels of alkaline phosphatase and the SEM appearance of the brush border. When we measured the uptake of ox-linoleate by 4- and 16-day-old cells as shown in **Fig. 4**, the uptake of ox-linoleate by 16-day cultures was about 50% of the amount added and the level of uptake of oleic and linoleic acids was about the same or a little higher (48 vs. 74%, respectively). In contrast, the uptake of unox-linoleate by the 4-day-old culture was 52% compared with the uptake of oxidized fatty acid at 10%. These results suggest again that ox-linoleic acid is dependent on the microvilli for its uptake.

The fatty acids that enter the cells readily undergo activa-

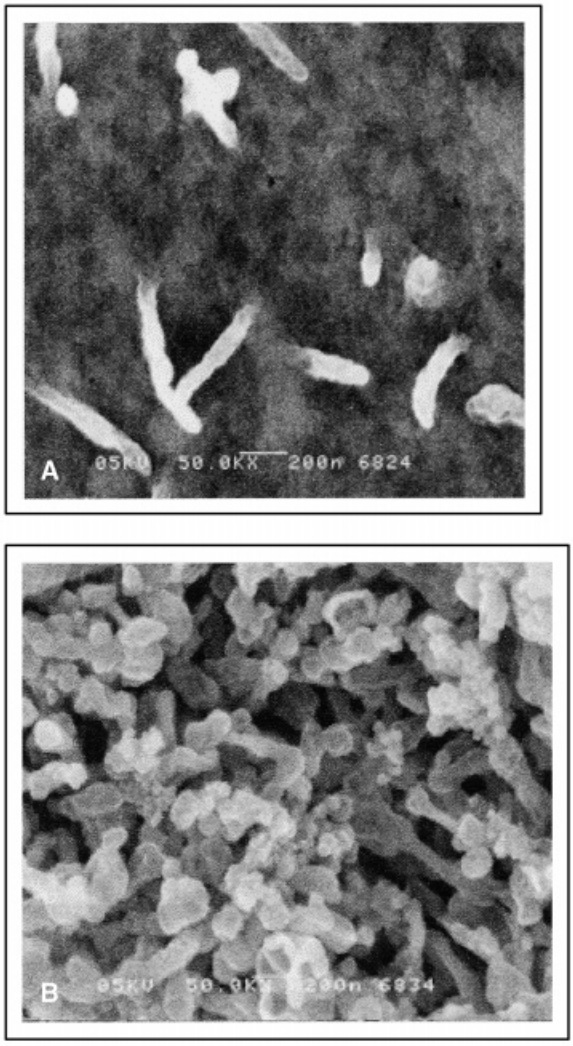

Fig. 3. Micrographs of 4- and 16-day-old Caco-2 cells by SEM. SEM is described in detail in Experimental Procedures. (A) Micrograph shows the presence of few microvilli on day 4. (B) Micrograph shows the abundant presence of microvilli on day 16. Original magnification: ×50,000.

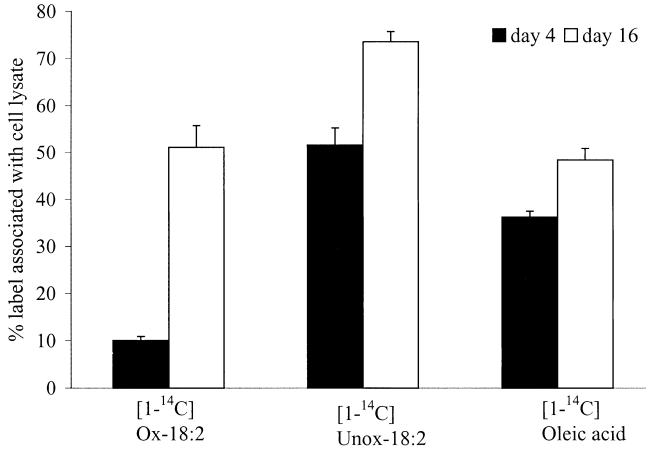

Fig. 4. Uptake of oxidized linoleic acid ([1-^{14}C]ox-18:2), unoxidized linoleic acid ([1-^{14}C]unox-18:2), and oleic acid ([1-^{14}C]oleic acid 18:1) by 4-day-old (solid columns) and 16-day-old (open columns) Caco-2 cells. Caco-2 cells were exposed to 50 nmol of the three types of fatty acids for 30 min. After the incubation medium was removed and the cells were washed with PBS and then solubilized in 2 ml of deoxycholic acid (0.5 mg/ml). An aliquot of this cell lysate was added to EcoLume and the radioactivity (uptake) was quantitated. Each experiment was repeated three times, in triplicate each time. Data represent means ± SE, $P < 0.001$.

tion and subsequent esterification to form complex lipids. Earlier studies in cultured cells demonstrated that fatty acid esterification might be limiting when oxidized fatty acids are used as substrate. We studied the esterification of LysoPtdCho by ox-linoleate, using microsomes from rat intestine. As shown in **Fig. 5A**, both ox- and unox-linoleate were esterified to LysoPtdCho to form PtdCho. The difference in esterification was not significant. This suggests that in the intestine, if oxidized fatty acids are readily absorbed, they can also be readily esterified. This is also illustrated in the formation of lipids when ox- or unox-linoleate was presented to 16-day cultured Caco-2 cells. As shown in Fig. 5B, 37 and 47% of the total cellular lipid was distributed between phospholipids and NL, respectively, as analyzed by TLC. Previous studies with cultured SMC also demonstrated that the uptake of oxidized fatty acid was not competed by unoxidized fatty acids. On the other hand, we show in this study that the presence of excess unlabeled, unoxidized fatty acid was readily able to dilute ox-linoleate (**Fig. 6A**) and that the labeled oxidized linoleic acid was truly competed by unlabeled ox-linoleic acid (Fig. 6B). We use the word "apparent competition" between oxidized and unoxidized fatty acid because true competition of a ligand can be expected only at its saturating concentration. We chose to use 5 μM labeled oxidized linoleic acid, which is far below the saturation concentration (150 μM from Fig. 2A) to allow the addition of large amounts (40-fold) of unlabeled unox-linoleic acid. The saturating concentration of the labeled ligand could not be used, as massive amounts of unlabeled linoleic acid need to be added as competitor and this could lead to cell toxicity. Overall, we believe that oxidized and unoxidized linoleic acids are absorbed by similar mechanisms. In a preliminary study utilizing rat intestinal everted sacs we show that the uptake of ox- and unox-linoleic acid are time dependent and similar (**Fig. 7**).

DISCUSSION

In this study we demonstrate that intestinal cells might take up large quantities of oxidized fatty acids. This is a striking contrast to other cell types, such as SMC and endothelial cells, that had limited capacity to take up oxidized fatty acids. Staprans and co-workers have suggested that heated oil contributes to the presence of peroxide in the plasma and could be repackaged and redistributed as lipoproteins. However, these studies do not address a number of questions, such as whether the presence of oxidized fatty acid in the lipoproteins truly reflected what was present in the diet, or resynthesized as a result of oxida-

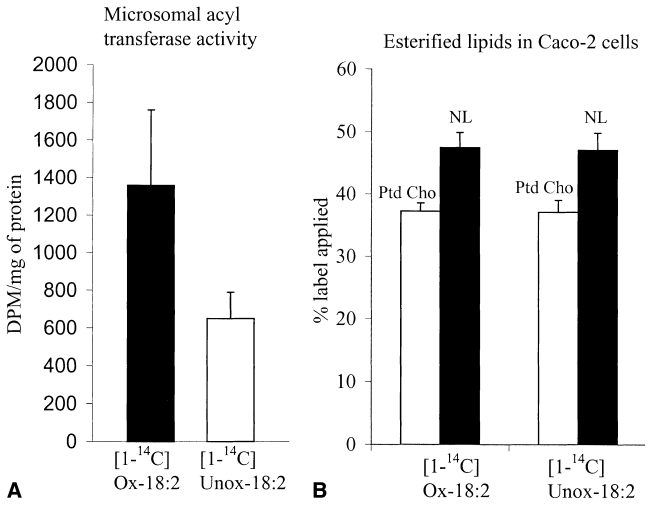

Fig. 5. (A) Acyltransferase activity in rat intestinal microsomes. Intestinal microsomes were isolated from rat intestine as described in Experimental Procedures. Acyltransferase activity was determined by incubating 100 μg of microsomal protein with 50 nmol of oxidized (solid column) or unoxidized (open column) linoleate in the presence of 100 μM LysoPtdCho, 200 μM ATP, 200 μM magnesium chloride, and 1 μM coenzyme A for 1 h at 37°C. Data represent means ± SE of three separate experiments done in duplicate each time; $P = 0.126$. (B) Radiolabel expressed as a percentage of total lipids in Caco-2 cells treated with 50 nmol of ([1-^{14}C]ox-18:2 or [1-^{14}C]unox-18:2 for 30 min. After acidification of cell lysates by 20 μl of 6 N HCl the lipids were extracted as described in Experimental Procedures. The extracted lipids were then separated by TLC, using chloroform–methanol–acetic acid–water 65:25:0.5:3. Each experiment was repeated three times, each time in triplicate. Data represent means ± SE; $P < 0.0$.

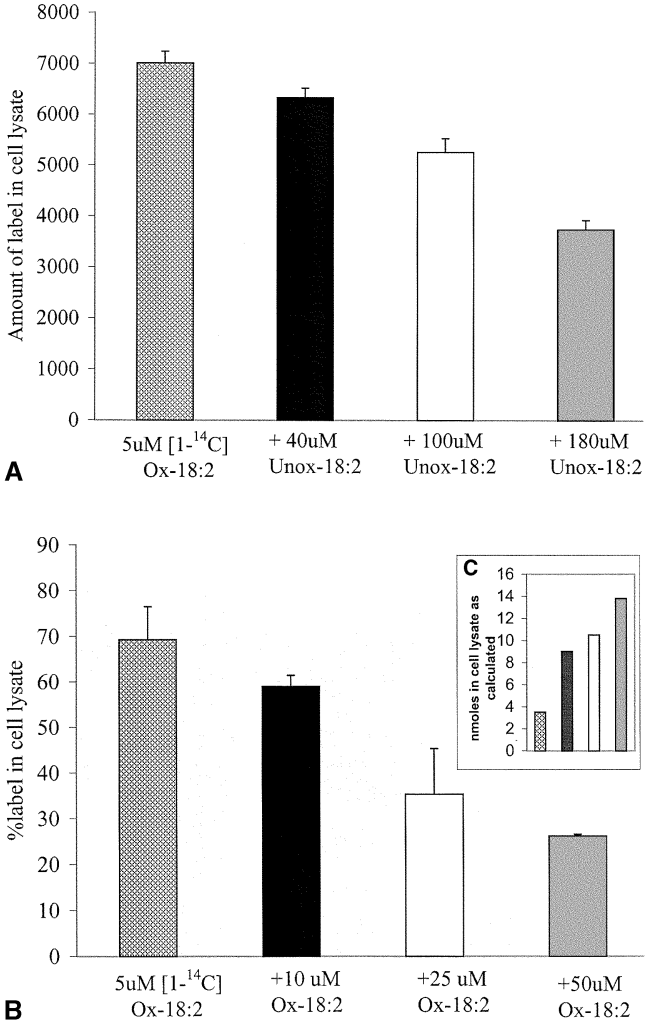

Fig. 6. (A) Apparent competition of oxidized [1-^{14}C]linoleic acid by unlabeled, unoxidized linoleic acid. Caco-2 cells were incubated with 5 μM oxidized [1-^{14}C]linoleic acid alone (cross-hatched column) or in the presence of 40 μM (solid column), 100 μM (open column), or 180 μM (gray column) unlabeled unoxidized linoleic acid. After the incubation medium was removed and the cells were washed with PBS and then solubilized in 2 ml of deoxycholic acid (0.5 mg/ml). An aliquot of this cell lysate was added to EcoLume and the radioactivity (uptake) was quantitated. Each experiment was repeated three times, in triplicate each time. Data represent means ± SE, $P < 0.001$. (B) Caco-2 cells were incubated with 5 μM oxidized [1-^{14}C]linoleic acid alone (cross-hatched column) or in the presence of 10 μM (solid column), 25 μM (open column), or 50 μM (gray column) unlabeled oxidized linoleic acid. After incubation the cells were treated as in (A) and the radioactivity was quantitated in cell lysate (n = 3). (C) Same experiment as in (B). The uptake of oxidized [1-^{14}C]linoleic acid is expressed in nanomoles as per calculations.

tion due to the presence of peroxidized lipids in the intestine; furthermore, the oxidized lipids in the diet were not characterized and their source of oxidized fat (heated oil) generates products such as aldehydes, which could also enhance oxidation. There was yet another study (45) demonstrating that dietary peroxides could also be broken down extensively in the stomach, resulting in other products of lipid oxidation (epoxy ketones of the fatty acid) with little release of oxidized fatty acid from the stomach. However, this contradicts previous data and the conclusion might be premature because the investigators did not look for the presence of oxidized lipids in the plasma. In addition, this study did not address the potential of oxidized lipids such as phospholipids crossing the stomach. When phospholipids or triglycerides are presented to the intestine they are extensively degraded by lipases as well as phospholipases to fatty acids, which are suggested to be in a mixed micellar phase in the presence of deoxycholic acid, monoglyceride, and LysoPtdCho. These mixed micelles are then absorbed by the way of the microvilli. We predicted that if such structures are important in the absorption of fatty acid there should not be any differences in the uptake of oxidized and unoxidized fatty acids. Our results not only support such a hypothesis but also provide evidence to suggest that the oxidized fatty acids that enter the intestine could be readily esterified to form complex lipids. We observed both the syntheses of phospholipids and triglycerides under those conditions. These two components are essential for the formation of chylomicrons. However, in these in vitro studies molecules such as monoglyceride or lysophosphatidylcholine were not presented and the presence of these compounds could change the distribution of esterified lipids in the intestine. Our results using microsomes strongly support the con-

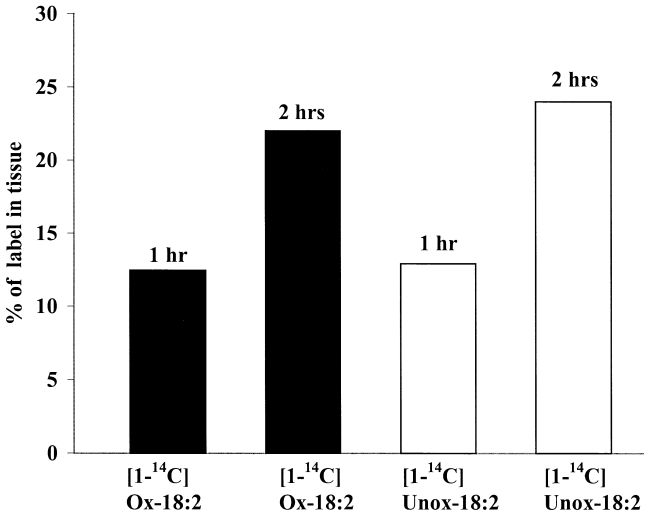

Fig. 7. Uptake of oxidized or unoxidized linoleate by rat everted intestinal sacs. Everted sacs were prepared as described in Experimental Procedures. Each sac was incubated in 4 ml of serum-free EMEM with 200 mM glutamine and 5% penicillin-streptomycin in the presence of 25 μM oxidized [1-^{14}C]linoleic acid (solid columns) or unoxidized (open columns) [1-^{14}C]linoleic acid for the indicated times at 37°C. Each bar represents the mean (n = 2).

tention that the intestine may have the capacity to take up large amounts of oxidized fatty acids and readily convert them into esterified lipids. Thus, these studies suggest that, if oxidation of lipoprotein is an important event in the pathogeneses of atherosclerosis, then the presence of oxidized fatty acid in the diet could contribute to atherosclerosis. Rapid or increased uptake of oxidized fatty acids by the intestine would also caution not only against the ability of dietary oxidized lipids to contribute to atherosclerosis, but also that their levels might be important. Thus not only the type but also the extent of oxidation of dietary lipids would be an important risk factor of atherosclerosis.

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