Oxidized Low Density Lipoprotein Stimulates Prostacyclin Production by Adult Human Vascular Endothelial Cells

J. Eric Triau, Simin Nibkin Meydani, and Ernst J. Schaefer

Interactions between vascular endothelium and low density lipoprotein (LDL) have been implicated in the development of atherosclerosis. The effect of normal and oxidized LDL (Ox-LDL) on prostaglandin release by cultured adult human saphenous vein endothelial cells was investigated. Ox-LDL induced a rapid release of prostacyclin (PGI2) to levels which were several-fold higher than those observed with control LDL. PGI2 release was concentration-dependent and was biphasic, with a first peak occurring within 30 minutes (followed by a decrease), and a second peak occurring after several hours of incubation. PGI2 production was inhibited by lipoprotein-depleted serum and by indomethacin, an antagonist of cyclooxygenase activity.

These cells produced mainly PGF2α, with some PGE2 and PGI2 when stimulated by the ionophore A23187 at confluency. However, among these prostanoids, mainly PGI2 was produced in response to Ox-LDL. The data indicate that Ox-LDL induces the production of PGI2 by human vascular endothelial cells. Since Ox-LDL is cytotoxic, this phenomenon may be a manifestation of an early response to injury.

(Arteriosclerosis 8:810–818, November/December 1988)

The interaction of low density lipoprotein (LDL) with endothelial cells is of significant interest to researchers in the field of atherosclerosis. LDL is toxic to endothelial cells cultured in serum-free medium.1-2 LDL cytotoxicity is due to oxidation of its lipid moiety, and oxidized LDL (Ox-LDL) cytotoxicity is prevented by the use of antioxidants in vitro.3-6 Endothelial cells, smooth muscle cells, and leukocytes oxidize LDL in vitro in a manner similar to the oxidation catalyzed by transition metals.5,7-9 Ox-LDL inhibits macrophage mobility, is metabolized via the scavenger receptor, and induces cholesterol ester accumulation in the macrophage.9-12 Ox-LDL may thus promote atherosclerosis.3-12

Prostacyclin (PGI2) is a potent vasodilator and decreases platelet aggregation.13 PGI2 is produced by endothelial cells, and increased levels of 2,3-dinor-6-keto-PGF1α, a major urinary metabolite of PGI2, have been observed in patients with atherosclerosis.14,15,16 When endothelial cells are subjected to various forms of injury such as balloon catheter trauma, radiation, shear stress, or exposure to mellitin, PGI2 is released.17-20

We tested the hypothesis that adult human vascular endothelial cells produce PGI2 in response to Ox-LDL. The data presented support this hypothesis.

From the USDA Human Nutrition Research Center on Aging at Tufts University, Boston, Massachusetts and Tufts University School of Nutrition, Medford, Massachusetts.

This work was supported by contract 53-3K06-5-10 from the U.S. Department of Agriculture Research Service.

This work was presented in part at the 70th Federation of American Society for Experimental Biology, St. Louis, Missouri, on April 14, 1986.

Address for correspondence: Ernst J. Schaefer, USDA Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111.

Received March 13, 1987; revision accepted June 7, 1988.
cantly was determined against a standard curve obtained with running water, and cleared by centrifugation. Fluorometric measurements were made at 553 nm with 515 nm excitation. The amount of malondialdehyde (MDA) equivalent was calculated using freshly diluted 1,1,3,3-tetramethoxypropane. The treatment of the cells with 2% Nonidet P-40.

**Quantitation of Lipid Oxidation**

Oxidation of LDL lipids was estimated by assaying for thiobarbituric acid reactive substances (TBARS) by using a modification of the fluorometric method of Yagi. Briefly, 50 μg of LDL protein (0.5 mg/ml) was precipitated with 1 ml of 20% trichloroacetic acid containing 0.1% BHT; 1 ml of a freshly dissolved solution (0.67%) of thiobarbituric acid was added and the mixture was boiled for 45 minutes, cooled with running water, and cleared by centrifugation. Fluorometric measurements were made at 553 nm with 515 nm excitation. The amount of malondialdehyde (MDA) equivalent was determined against a standard curve obtained using freshly diluted 1,1,3,3-tetramethoxypropane. The degree of LDL oxidation was expressed in nanomoles of MDA equivalent per milligram of LDL protein. Under these conditions, the TBARS values for control LDL preparations were typically 0 to 0.2 nmol/mg, while the values for Ox-LDL preparations were 3 to 18 nmol/mg, depending on the length of the fourth dialysis. Highly oxidized LDL (TBARS above 18 nmol/mg) was degraded, as evidenced by a trail of lipid staining (fat red 7B) after agarose electrophoresis. In our experiments, the maximum level of oxidation in Ox-LDL used had a TBARS value of 16 nmol/mg.

**Incubation of Low Density Lipoprotein with Cells**

To prevent oxidation of control LDL, incubation media were combined with concentrated DMEM just before incubation. Such preparations were found to have a level of gram-negative bacterial endotoxin below 20 pg/ml with the chromogenic limulus amebocyte lysate assay (MA Bioproducts, Walkersville, MD). Concentrations of nonlipoprotein species (including BHT, EDTA, and Cu2+) were identical in both control LDL and Ox-LDL preparations. HSVEC were grown on 2 cm² wells until confluence (defined as the absence of open space on the monolayer), were washed twice in HBSS, and were incubated once in 300 μl of medium. Before experimental incubation, each well was examined by inverted phase contrast microscopy, and any discontinuity in the monolayer resulted in the elimination of the well from the experiment. Human monocytes (3 × 10⁶) were incubated in 0.32 cm² wells containing 250 μl of LDL preparation. In each experiment, control LDL and Ox-LDL isolated from the same lipoprotein donor were used on the cells obtained from another single donor.

**Measurements of Prostaglandin Production**

Radioimmunoassay

Prostaglandin production was measured by radioimmunoassay (RIA) of 6-keto-PGF₁α (a stable PGF₁α metabolite) or PGE₂ in the medium. After each incubation, 200 μl of medium collected from HSVEC monolayers or from the centrifuged monocyte suspensions were frozen at −80°C until analysis. Rabbit antiserum specific for 6-keto-PGF₁α or PGE₂ was pre-precipitated overnight at 4°C with sheep anti-λG in the presence of nonimmune rabbit serum and PBS-EDTA. Serial dilutions of unlabeled prostaglandin standard were then incubated overnight at 4°C with tritiated standard and the antibodies. After decantation, the radioactivity was measured by liquid scintillation spectrometry, and quenching was monitored using an external source of ¹³⁷Cs. The sensitivity of the assay was 20 pg and a 50% displacement was obtained at about 300 pg. The specificities of our antiserum have been previously reported; however, Ox-LDL did react with the antiserum (see Results) and therefore, in all experiments, background values (obtained from RIA on parallel incubations of media in the absence of cells) were subtracted from total values.

**High Performance Liquid Chromatographic Analysis**

Further characterization of PG₁₂ production was made by reversed-phase high performance liquid chromatography (RP-HPLC) analysis of the medium after ³H-ara-

---

ENDOTHELIAL CELLS, OX-LDL, AND PG₁₂

Triau et al.
chidonic acid metabolic labeling. Tritiated arachidonic acid (240 Ci/mmol, New England Nuclear, Boston, MA) was adsorbed to polypropylene under a stream of purified N₂ and was transferred to FBS by a 45-minute incubation at 37°C. After washes with HBSS, subconfluent monolayers were incubated with 10 μCi/ml of ³H-arachidonic acid in fully supplemented M-199 medium. After 48 hours, approximately 80% of the radioactivity was associated with the cells; the monolayers were washed in HBSS-albumin (4%) and then HBSS and were incubated with the lipoprotein or the ionophore A23187. After incubation, the entire medium was collected and acidified. The prostanooids were adsorbed onto an octadecylsilica (C18) cartridge (Sep-pak, Millipore Corporation), were washed with a mixture of ethanol and water (15:85) and then with ether and were eluted with methyl formate, as described by Powell.31 Extraction efficiencies were greater than 95% in all cases. The solvent was evaporated under N₂, and the residue was redissolved in ethanol. The prostanoids were fractionated by RP-HPLC in a 4.6 mm×15 cm C18 column (3 μm, Adsorbosphere, Alltech Association, Incorporated, Deerfield, MA) guarded with a C18 precolumn (Brownlee Labs Incorporated, Santa Clara, MA). The mobile phase was Terragno's biphasic solvent (17.3 mM H₂PO₄/Ch₃CN, 7:3) used at a flow rate of 1.7 ml/min.32 Aliquots (1 ml) were collected and radioactivity was measured. Identification of the elution peaks was based on retention volume of authentic tritiated standards (6-keto-PGF₁α, PGE₂, and PGE₂) and on relative retention volume of the authentic standard 6,15-diketo-PGF₁α.

### Results

#### Incubation Conditions

Adult human venous endothelial cells require a complex culture medium for growth. However, confluent monolayers of these cells can be maintained for at least 24 hours in serum-free media such as DMEM or Eagle’s minimum essential medium (MEM). When LDL was incubated in either media without cells, no significant oxidation of LDL was observed (Table 1). However, in the presence of cells, the use of MEM induced several-fold more LDL oxidation than the use of DMEM over a 24-hour period. An increase of BHT concentration from 1.7 μM (average concentration after medium reconstitution) to 20 μM in DMEM did not significantly protect LDL against oxidation.

Since EDTA (0.01 to 0.05 mM) was present in the LDL preparations used for tissue culture, the extent of EDTA toxicity was assessed by specific ¹¹¹Indium release. When HSVEC were incubated in DMEM containing 1 mM EDTA, a significant specific release of ¹¹¹Indium was observed, indicating cytotoxicity (Table 2). When the cells were incubated with 0.1 mM EDTA, a concentration at least twice the concentration used during LDL incubation experiments, little toxicity was seen.

Confluent HSVEC can be maintained with control LDL in serum-free medium for at least 24 hours (Figure 1); under those conditions the monolayers displayed a cobblestone phenotype characteristic of quiescent endothelial cells. In contrast, when the monolayers were incubated with Ox-LDL, the cells displayed an “activated” phenotype; the presence of debris in the medium was an indication of cell injury.

### Prostacyclin Production

Ox-LDL, compared to control LDL, caused a marked increase in 6-keto-PGF₁α release from HSVEC. The increase in PGE₂, measured by RIA of the medium, was concentration-dependent (Figure 2). Ox-LDL induced a rapid rise in 6-keto-PGF₁α concentrations in the media of HSVEC, followed by a decrease, and a subsequent increase after several hours of incubations (Figure 3). The degree of LDL oxidation clearly affected this response, and the addition of LPDS decreased this response (Figure 3). Indomethacin inhibited the production of 6-keto-PGF₁α by HSVEC (Figure 4). Ox-LDL (500 μg/ml, TBARS 2.9 nmol/mg) induced a response similar to the response induced by the calcium ionophore A23187 (Figure 4).

The antisera for 6-keto-PGF₁α cross-reacted with LDL and especially with Ox-LDL at a given degree of oxidation. This cross-reactivity was linearly related to the amount of

#### Table 1. Oxidation of Low Density Lipoprotein in Culture Media Alone and in Presence of Cells

<table>
<thead>
<tr>
<th>Concentration of BHT in medium (μM)</th>
<th>Without cells</th>
<th>With cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEM (TBARS in nmol/mg)</td>
<td>0.4±0.18</td>
<td>0.5±0.09</td>
</tr>
<tr>
<td>DMEM (TBARS in nmol/mg)</td>
<td>0.3±0.14</td>
<td>0.5±0.04</td>
</tr>
</tbody>
</table>

Values are the means±SD of triplicate determinations on duplicate incubations.

Control low density lipoproteins (200 μg/ml) were incubated with or without human saphenous vein endothelial cells in either Eagle’s minimum essential medium (MEM) or Dulbecco’s modified Eagle’s medium (DMEM) containing 1.7 or 20 μM butylated hydroxytoluene (BHT). After 24 hours, the degree of oxidation (TBARS) in the media was measured as described in the Methods section.

<table>
<thead>
<tr>
<th>Concentration of EDTA in medium (mM)</th>
<th>Without EDTA</th>
<th>With EDTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.0±0.02</td>
<td>0.2±0.03</td>
</tr>
<tr>
<td>0.5</td>
<td>0.4±0.04</td>
<td>0.6±0.08</td>
</tr>
<tr>
<td>1</td>
<td>0.7±0.09</td>
<td>0.9±0.13</td>
</tr>
</tbody>
</table>

Values are the means±SD of triplicate incubations.

Confluent human saphenous vein endothelial cells were metabolically labeled with ¹¹¹Indium and were incubated with Dulbecco’s modified Eagle’s medium containing various concentrations of EDTA. After 24 hours, EDTA cytotoxicity was assessed by measuring the specific release of ¹¹¹Indium in the medium, as described in the Methods section.
ENDOTHELIAL CELLS, OX-LDL, AND PGI2  
Triau et al.

Figure 1. Inverted phase-contrast microscopic pictures of confluent human saphenous vein endothelial cells incubated for 24 hours with control (A) or oxidized low density lipoprotein (Ox-LDL) (B). Incubation media were prepared with serum-free Dulbecco's modified Eagle's medium as described in the Methods section. The level of LDL in A and B was 200 μg/ml; the degree of LDL oxidation in B was 12.6 nmol of malondialdehyde equivalent per milligram of LDL protein.

Figure 2. The effect of various levels of control and oxidized low density lipoprotein (Ox-LDL) incubated with human saphenous vein endothelial cells on prostacyclin production. Various protein concentrations of LDL and Ox-LDL (TBARS 5.4 nmol/mg) were incubated with confluent monolayers for 24 hours. Prostacyclin production was assessed by radioimmunoassay of 6-keto-PGF1α in the medium as described in the Methods section. Values are the means±SD of duplicate incubations with control LDL (●) or Ox-LDL (○).

Ox-LDL within a range of 3.5 to 105 μg of LDL protein (Table 3). Control or Ox-LDL, however, did not quench RIA detection of 6-keto-PGF1α (data not shown). The data indicate that parallel incubations of LDL without cells must be performed to correct for cross-reactivity.

In similar experiments with human monocytes, cross-reactivity of Ox-LDL (100 μg/ml, TBARS 10.3 nmol/mg) with PGE2 antiserum was observed (data not shown). In this system, the same kind of prostanoid response was noted, with Ox-LDL inducing a sixfold higher level of PGE2 in the media than did control LDL over a 20-hour incubation (579±29 pg/ml vs. 99±14 pg/ml).

Because of the cross-reactivity with the antisera, and our interest in examining the entire spectrum of prostanoids produced by HSVEC in response to LDL, we also used RP-HPLC methodology for assessing the presence of these constituents in the media after metabolic labeling. PGI2 is not produced uniformly by cells isolated from various vascular beds.33 To our knowledge, there is no available data on the HPLC profile of the prostanoids produced by adult human saphenous vein endothelial cells. Thus, we stimulated HSVEC with the ionophore A23187 to compare the prostanoid response to that induced by Ox-LDL. Confluent monolayers of HSVEC produced PGF2α with some PGE2 and little 6-keto-PGF1α (Figure 5). This prostanoid profile was identical to the profile reported by others in porcine aortic endothelial cells stimulated with A23187 after preincubation with 14C-arachidonate-labeled LDL.34 In contrast, when HSVEC were incubated with Ox-LDL for 14 hours, the monolayers produced mainly PGI2 metabolites as shown by the presence of 6-keto-PGF1α and another PGI2 degradation product, which has a relative retention volume similar to the relative retention volume of 6,15-diketo-PGF1α (Figure 6). Using metabolic labeling and HPLC analysis, no PGI2 metabolites were detected until after several hours of incubation. These data indicate that the initial release of this constituent detected by RIA was derived from a pool of unlabeled arachidonic acid, either intracellular or extra-
Figure 3. Time dependence of prostacyclin (PGI₂) production by human saphenous vein endothelial cells during incubation with control or oxidized low density lipoprotein (Ox-LDL) (A), and Ox-LDL or Ox-LDL with lipoprotein-depleted serum (LPDS) (B). In A, levels of LDL were 500 μg/ml (TBARS for Ox-LDL: 2.9 nmol/mg). In B, levels of Ox-LDL were 200 μg/ml (TBARS 6.8 nmol/mg), and 5% LPDS (1.25 mg protein/ml) was used. PGI₂ production was assessed by radioimmunoassay of 6-keto-PGF₁α in the medium, as described in the Methods section. The values are the means±SD of duplicate incubations with control LDL (A), Ox-LDL (○), Ox-LDL and LPDS (△).

Discussion

In characterized adult HSVEC, concentration and time-dependence experiments demonstrated that Ox-LDL versus control LDL increased PGI₂ synthesis (Figures 2 and 3). This observation was confirmed by using a cyclooxygenase antagonist, which inhibited production of PGI₂ (Figure 4). Further demonstration of this effect was provided by HPLC analysis after metabolic labeling, which showed that PGI₂ was the major prostanooid synthesized in response to Ox-LDL, despite the ability of these cells to also produce PGF₂α and PGE₂ when exposed to the ionophore A23187 (Figures 5 and 6). Although it was not possible to observe early cytopathological effects (within minutes) when the monolayers were incubated with Ox-LDL, cell morphology was drastically altered after several hours of incubation with Ox-LDL (Figure 1). These observations indicate a link between Ox-LDL cytotoxicity and late PGI₂ release, and suggest that early PGI₂ release may also be a manifestation of Ox-LDL-mediated cytotoxicity.

A number of investigators have examined the effects of LDL on PGI₂ production by vascular tissue. Beitz and Forster have reported that LDL decreases PGI₂ production in microsomal fractions of pig aorta. Szczeklik and Gryglewski have noted that Ox-LDL decreases PGI₂ production in rat aorta slices as compared to control LDL. In contrast to these data, Spector et al. have reported...
that LDL stimulates PG12 production by bovine aortic and human umbilical vein endothelial cells. In these latter studies, oxidation of LDL was not assessed. Our own experiments are not directly comparable with studies carried out in pig and rat aorta, since the preparations studied contained a variety of different cell types. However, in viable and well-characterized tissue cultures of endothelial cells, our results are consistent with those of Spector et al. We have extended these studies by examining the effects of Ox-LDL and control LDL.

Precise control of the experimental conditions under which incubations of LDL with endothelial cells are performed is critical. Endothelial cells can modify LDL in a manner similar to copper-induced oxidation. Certain media favor cell-induced LDL oxidation. Endothelial cells incubated in Ham’s F10 medium (which contains 10 nM Cu2+) induce rapid LDL oxidation, which can be prevented by BHT. BHT has similar antioxidant properties in DMEM. In our cell preparation and in the presence of BHT, more LDL oxidation was seen with MEM than with DMEM (Table 1). Such a difference may be due to the higher levels of cysteine (a primary antioxidant) and tryptophan (an oxygen radical quencher) in DMEM than in MEM (2- and 1.6-fold, respectively). The presence of EDTA in the medium has also been reported to inhibit LDL oxidation by endothelial cells, probably because of its high affinity for Fe2+, Cu2+, and Zn2+. However, too much EDTA will cause the chelator to complex significantly with Ca2+ and Mg2+ (which have a lower affinity constant than transition metals). Using specific release of 111-indium as an index of cytolysis, we have shown that up to 100 μM of EDTA can be tolerated by confluent HSVEC in DMEM over a 24-hour period (Table 2). In our experiments, the level of EDTA was always 100-fold higher than the level of Cu2+, but it never exceeded 100 μM. Such concentrations of EDTA (0.01 to 0.05 mM) with BHT in the media are not toxic but still prevent oxidation of LDL by endothelial cells. HEPES was excluded from the final serum-free media since this buffer has been reported to stimulate endothelial cells to produce reactive oxygen metabolites. The use of chloramphenicol during all steps of LDL purification protects against contamination with gram-negative bacterial endotoxins, which are known to induce PG12 production in endothelial cells. During LDL preparation, NaN3 was not used because this common bactericidal agent has been reported to promote LDL oxidation and subsequent apo B-100 degradation.

Ox-LDL can seriously affect radioimmunoassay of prostaglandins (Table 3). There are several possible explanations for this effect. One possibility is that Ox-LDL cross-reacts with the ligand-binding region of the pre-precipitated IgG and thus decreases the extent of competition between radiolabeled and unlabeled prostaglandins. A net decrease of recovered radioactivity would result in an increase of the calculated amount of prostaglandin. Another possibility is that Ox-LDL binds prostaglandins; this phenomenon is, however, unlikely since Ox-LDL does not quench RIA detection of 6-keto-PGF1α (data not shown). To our knowledge, such cross-reactivity, which can be considerable at elevated levels of highly oxidized LDL, has not been previously reported. Thus, particular care needs to be
taken when analyzing prostaglandins by RIA in media containing significant amounts of Ox-LDL.

The rapid initial increase of 6-keto-PGF\textsubscript{1a} in the medium followed by a longer period of disappearance (Figure 3) suggests that the epitope becomes shielded from the antiserum. A priori, such a phenomenon could be due to binding of 6-keto-PGF\textsubscript{1a} to the cells or to the culture dishes. Also, the prostanoid could bind to a cellular factor released in the medium. Since we did not find any evidence of 6-keto-PGF\textsubscript{1a} binding to the cells or to the culture dishes (data not shown), it is possible that a binding factor is released from the cells and sequesters 6-keto-PGF\textsubscript{1a} from the antiserum. Binding of PGI\textsubscript{2} to albumin and a Cohn fraction VI glycoprotein has been reported.\textsuperscript{43,44} Such findings raise the possibility that an endothelium-derived factor binds early metabolites of PGI\textsubscript{2}, perhaps to allow specific targeting to tissues equipped for further catabolism of these compounds. It is also possible that metabolizing enzymes are actively released and induce a modification of the epitope recognized by our antiserum. A third possibility is the leaking of metabolizing enzymes; this could occur at late stages of Ox-LDL cytotoxicity as indicated by the presence, after 14 hours of incubation, of 6,15-diketo-PGF\textsubscript{1a}, a product of the cytoplasmic enzyme 15-hydroxy-prostaglandin dehydrogenase found in bovine arteries and veins.\textsuperscript{45} Another explanation is that, as Ox-LDL is metabolized by the cells and disappears from the medium, the correction used for the RIA would then no longer be applicable and thus would reduce artefactually the final value of 6-keto-PGF\textsubscript{1a}.

Such a phenomenon is unlikely, because we estimate that less than 1% of Ox-LDL (100 \mu g/ml) is metabolized by HSVEC over 24 hours (calculations based on reported data about MDA- and acetylated-LDL catabolism by endothelial cells).\textsuperscript{46,47}

The mechanisms by which Ox-LDL stimulates PGI\textsubscript{2} production in HSVEC are not clear. Endothelial cells express the LDL receptor and the scavenger receptor, which recognizes a more negatively charged apo B.\textsuperscript{46,47,48} Ox-LDL is recognized exclusively by the scavenger receptor, and it has been shown that this receptor allows endothelial cells to catabolize twice as much MDA- or acetylated-LDL than the amount of LDL that can be catabolized via the LDL receptor.\textsuperscript{46,47} These observations indicate that the presence of Ox-LDL may increase delivery of the lipoprotein components to the cell; such components (either common to control and Ox-LDL or specific to Ox-LDL) could trigger PGI\textsubscript{2} release. Furthermore, a phospholipase A\textsubscript{2} activity has been associated with Ox-LDL,\textsuperscript{49} thus an increase in available arachidonic acid due to the lipoprotein could stimulate the cyclooxygenase pathway.\textsuperscript{34,50} The preferential use of arachidonic acid brought into the cells by the lipoprotein over endogenous arachidonate may be the reason for the late appearance of radiolabeled PGI\textsubscript{2} after metabolic labeling of the monolayer (Figure 6). Also, lipid peroxides are known to stimulate or inhibit prostaglandin synthesis;\textsuperscript{51,52} such compounds could be brought into the cells or formed from membranes by reactive oxygen metabolites associated with Ox-LDL. The biphasic response shown in Figure 3 could be due, in part, to an activation followed by an inhibition and another activation induced by the sequential appearance of lipid peroxides.

The in vivo significance of our observation that oxidized LDL stimulates PGI\textsubscript{2} release by HSVEC remains elusive, for one must first demonstrate the in vivo significance of Ox-LDL. Smith and Staples\textsuperscript{53} have shown that the relative distributions of LDL and proteins in plasma and intima are disparate: in normal aortic intima, these authors found a level of LDL twice the plasma level and a level of albumin a quarter of the plasma level. Serum inhibits oxidation and Ox-LDL-mediated injury,\textsuperscript{1,2,54} as well as PGI\textsubscript{2} stimulation in HSVEC (Figure 3). One can speculate that LDL in the intimal space is much more susceptible to oxidation because of the lack of serum antioxidants and the ability of endothelial cells to oxidize LDL. High plasma levels of LDL, presumably resulting in a larger LDL infiltration into the intima, would exacerbate this effect. The in vivo presence of Ox-LDL is suggested by several lines of evidence. LDL isolated from human aorta is more electronegative than plasma LDL.\textsuperscript{55} A feature of oxidized LDL is that oxidized LDL-induced production of PGI\textsubscript{2} by endothelial cells can be an in vivo mechanism of defense against the injury inflicted by modified lipoprotein.

Acknowledgments

We are indebted to Peter Libby of Tufts University for supplying endothelial cells and tissue culture facilities. We thank Jacqueline Dupont of Iowa State University for providing the prostaglandin antisera and John Pike and Frank Sun of the Upjohn Company for the gift of 6,15-diketo-PGF\textsubscript{1a}. We thank Maria Janicka, Patrice Barklund, Lisa Stocking, and Judith McNamara for technical assistance. The help of Cynthia Heim in typing the manuscript is gratefully acknowledged.

References

ENDOTHELIAL CELLS, OX-LDL, AND PGI₂
Triau et al.


31. Powell WS. Rapid extraction of oxygenated metabolites of arachidonic acid from biological samples using octadecylsilil silica. Prostaglandins 1980;20:947–957


37. Szczeklik A, Gryglewski R. Low density lipoproteins (LDL) are carriers for lipoperoxides and inhibit prostacyclin (PGI₂) biosynthesis in arteries. Artery 1980;7:486–495


in endothelial cell-induced modification of low density lipoprotein. Proc Natl Acad Sci USA 1985;82:3000–3004


Index Terms: endothelial cells • oxidized LDL • prostacyclin
Oxidized low density lipoprotein stimulates prostacyclin production by adult human vascular endothelial cells.

J E Triau, S N Meydani and E J Schaefer

Arterioscler Thromb Vasc Biol. 1988;8:810-818
doi: 10.1161/01.ATV.8.6.810

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/8/6/810