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.

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ENDOTHELIAL CELLS, OX-LDL, AND PGI₂
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CONTINUING TREATMENT WITH 2% NONIDET P-40.

25 /ug of LDL protein (0.5 mg/ml) was precipitated with 1 ml of
50 mM EDTA and 20 μM BHT. Oxidized LDL was obtained after dialysis with three changes (2 hours each)
and a fourth dialysis up to 24 to 48 hours against PB supplemented with 10 μM cupric acetate. After filter sterilization (0.22 μm, Millex-GV, Millipore Corporation, Bedford, MA), protein concentration was determined by the method of Lowry as modified by Markwell et al.25 with bovine serum albumin fraction V (Abbott Laboratories, South Pasadena, CA) as a standard. The protein purity of isolated LDL was examined by gradient (4% to 23%) sodium-dodecyl-sulfate polyacrylamide gel electrophoresis and only apolipoprotein (apo) B-100 was noted in control LDL. Before each medium reconstitution, agarose electrophoresis was used to check control LDL for typical β-mobility and Ox-LDL for increased anodic mobility as reported by others.26

Measure of EDTA Cytotoxicity

Cytotoxicity of EDTA in serum-free Dulbecco’s modified Eagle medium (DMEM) was assessed by measuring the specific release of 111In after 111In-oxine labeling of HSVEC monolayers. Metabolic labeling of the cells was performed as described by Pober et al.27 Confluent HSVEC were washed with Hank’s balanced salt solution (HBSS) and incubated with 4 μCi/ml 111In-oxine. After 30 minutes, the monolayers were washed with Tyrode’s balanced salt solution supplemented with 4% bovine albumin; the cells were then incubated in Tyrode-albumin solution at 37°C. After 90 minutes, the period of time necessary for spontaneous 111In release to reach a constant level (2% per 24 hours), the monolayers were washed with HBSS and incubated with DMEM containing EDTA (pH 7.5). After 24 hours, specific release was measured as the difference of EDTA-induced and spontaneously released 111In activity, normalized to the difference of total releasable and spontaneously released radioactivity. The total releasable 111In was assessed by measuring radioactivity in the medium after 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.28 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 trial 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 confluency (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 PGI₂ metabolite) or PGE₂ in the medium.29 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-IgG 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 antisera have been previously reported;30 however, Ox-LDL did react with the antisera (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 PGI₂ production was made by reversed-phase high performance liquid chromatography (RP-HPLC) analysis of the medium after ³H-ara-
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 to 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 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.

### Results

#### Incubation Conditions

Adult human venous endothelial cells were metabolically labeled with tritiated arachidonic acid (240 Ci/mmol, New England Nuclear, Boston, MA) and incubated with polypropylene under a stream of purified N2 and transferred to FBS by a 45-minute incubation at 37°C. After washing 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 prostanoids were adsorbed onto a octadeclaryl silica (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 re-dissolved 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 Tarrago’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 PGF2α) and on relative retention volume of the authentic standard 6,15-diketo-PGF1α.

#### Prostacyclin Production

Ox-LDL, compared to control LDL, caused a marked increase in 6-keto-PGF1α release from HSVEC. The increase in PGF1α 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 antiserum 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 Ox-LDL.

### Table 2. Cytotoxicity of EDTA on Human Saphenous Vein Endothelial Cells

<table>
<thead>
<tr>
<th>Concentration of EDTA in medium (mM)</th>
<th>0.1</th>
<th>0.5</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>111-inium specific release</td>
<td>0.1</td>
<td>2.3</td>
<td>18.1</td>
</tr>
</tbody>
</table>

Values are the means of triplicate incubations.

Confluent human saphenous vein endothelial cells were metabolically labeled with 111In-oxine and were incubated with Dulbecco’s modified Eagle’s medium 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.
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-PGF$_{1α}$ in the medium as described in the Methods section. Values are the means±SD of duplicate incubations with control LDL (A) 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-PGF$_{1α}$ (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 PGE$_2$ 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 PGE$_2$ 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. PGI$_2$ 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 PGF$_{2α}$, with some PGE$_2$ and little 6-keto-PGF$_{1α}$ (Figure 5). This prostanoid profile was identical to the profile reported by others in porcine aortic endothelial cells stimulated with A23187 after preincubation with $^{14}$C-arachidonate-labeled LDL.$^{34}$ In contrast, when HSVEC were incubated with Ox-LDL for 14 hours, the monolayers produced mainly PGI$_2$ metabolites as shown by the presence of 6-keto-PGF$_{1α}$ and another PGI$_2$ degradation product, which has a relative retention volume similar to the relative retention volume of 6,15-diketo-PGF$_{1α}$ (Figure 6). Using metabolic labeling and HPLC analysis, no PGI$_2$ 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 (B), 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 prostanoid 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 Forster³⁶ have reported that LDL decreases PGI₂ production in microsomal fractions of pig aorta. Szczeklik and Gryglewski³⁷,³⁸ 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.³⁹ have reported...
that LDL stimulates PGI₂ 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.36,37,38 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.7 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.7 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⁺.7 However, too much EDTA will cause the chelator to complex significantly with Ca²⁺ and Mg²⁺ (which have a lower affinity constant than transition metals).7 Using specific release of 111-indium as an index of cytolyisis, 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.40 The use of chloramphenicol during all steps of LDL purification protects against contamination with gram-negative bacterial endotoxins, which are known to induce PGI₂ production in endothelial cells.41 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.42

Ox-LDL can seriously affect radioimmunoassay of prostaglandins (Table 3). There are several possible explanations for this effect. One possibility is that Ox-LDL 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-PGF₁₅ (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$_{1\alpha}$ 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$_{1\alpha}$ to the cells or to the culture dishes. Also, the prostaglandin could bind to a cellular factor released in the medium. Since we did not find any evidence of 6-keto-PGF$_{1\alpha}$ 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$_{1\alpha}$ from the antiserum. Binding of PGI$_2$ to albumin and a Cohn fraction VI glycoprotein has been reported.43,44 Such findings raise the possibility that an endothelium-derived factor binds early metabolites of PGI$_2$, 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$_{1\alpha}$, a product of the cytoplastic enzyme 15-hydroxy-prostaglandin dehydrogenase found in bovine arteries and veins.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$_{1\alpha}$. Such a phenomenon is unlikely, because we estimate that less than 1% of Ox-LDL (100 µg/ml) is metabolized by HSVEC over 24 hours (calculations based on reported data about MDA- and acetylated-LDL catabolism by endothelial cells).46,47

The mechanisms by which Ox-LDL stimulates PGI$_2$ production in HSVEC are not clear. Endothelial cells express the LDL receptor and the scavenger receptor, which recognizes a more negatively charged apo B.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.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$_2$ release. Furthermore, a phospholipase A$_2$ activity has been associated with Ox-LDL,49,50 thus an increase in available arachidonic acid due to the lipoprotein could stimulate the cyclooxygenase pathway.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$_2$ after metabolic labeling of the monolayer (Figure 6). Also, lipid peroxides are known to stimulate or inhibit prostaglandin synthesis;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 apparition of lipid peroxides.

The in vivo significance of our observation that oxidized LDL stimulates PGI$_2$ release by HSVEC remains elusive, for one must first demonstrate the in vivo significance of Ox-LDL. Smith and Staples53 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,1,2,54 as well as PGI$_2$ 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 intimal presence of Ox-LDL is suggested by several lines of evidence. LDL isolated from human aorta is more electronegative than plasma LDL,55 a feature of oxidized LDL.7,8,28 Moreover, LDL isolated from human atherosclerotic lesions is degraded and induces cholesterol esterification in macrophages;56,57 both features are shared with Ox-LDL.10,11,12,42 Therefore, once Ox-LDL is present in the intima, it may trap macrophages, thereby inducing foam cell formation and thus initiating and amplifying the injury events of atherosclerosis.9,10,58 Remarkably, PGI$_2$ can antagonize these events by inhibiting leukocyte adhesion and cholesterol accumulation in aortic smooth muscle cells.59,60

These observations, interpreted with the injury hypothesis for the pathogenesis of atherosclerosis,58 suggest that oxidized LDL-induced production of PGI$_2$ by endothelial cells could be an in vivo mechanism of defense against the injury inflicted by modified lipoprotein.

Acknowledgments

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References


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


37. Szczeklik A, Gryglewski R. Low density lipoproteins (LDL) are carriers for lipid peroxides 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
51. Hemler ME, Cook HW, Lands WEM. Prostaglandin biosynthesis can be triggered by lipid peroxides. Arch Biochem Biophys 1979;193:340-345
52. Salmon JA, Smith DR, Flower RJ, Moncada S, Vane JR. Further studies on the enzymatic conversion of prostaglandin endoperoxide into prostacyclin by porcine aorta microsomes. Biochim Biophys Acta 1978;523:250-262

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