Oxidized Low Density Lipoproteins Inhibit Relaxations of Porcine Coronary Arteries
Role of Scavenger Receptor and Endothelium-Derived Nitric Oxide

Felix C. Tanner, MD; Georg Noll, MD; Chantal M. Boulanger, PhD; and Thomas F. Lüscher, MD

Background. We studied the effects of low density lipoprotein (LDL) on endothelium function. Methods and Results. Porcine epicardial and intramyocardial coronary arteries suspended in organ chambers for isometric tension recording were exposed to LDL for 2 hours and were then washed. In epicardial coronary arteries, oxidized LDL (30–300 μg/ml) but not native LDL or lyssolecithin inhibited endothelium-dependent relaxations to serotonin, thrombin, and aggregating platelets (5,000–75,000/μl). Endothelium-dependent relaxations to bradykinin and A23187 and endothelium-independent relaxations to SIN-1 were unaffected by oxidized LDL. In intramyocardial coronary arteries, oxidized LDL had no appreciable effect on relaxations to serotonin. The effect of oxidized LDL on the response to serotonin in epicardial coronary arteries was completely prevented by dextran sulfate (10 μg/ml). The inhibitory effect of oxidized LDL persisted in the presence of pertussis toxin. Similar to the lipoproteins, 1-N\textsuperscript{G\textsubscript{u}}-monomethyl arginine (1-NMMA) reduced relaxations to serotonin but not to bradykinin in epicardial coronary arteries. In the presence of 1-NMMA, oxidized LDL further reduced the response to serotonin. In arteries in which relaxations to serotonin were inhibited by oxidized LDL, l-arginine but not d-arginine induced a full relaxation. Pretreatment with l-arginine potentiated relaxations to serotonin in arteries exposed to oxidized LDL.

Conclusions. Thus, oxidized LDL activates the scavenger receptor on endothelial cells and inhibits the receptor-operated nitric oxide formation in epicardial but not in intramyocardial coronary arteries. The mechanism is not related to dysfunction of a G protein but is related to a reduced intracellular availability of l-arginine. The reduced nitric oxide formation at sites of early atherosclerotic lesions may favor platelet aggregation and vasospasm, both of which are known clinical events in patients with coronary artery disease. (Circulation 1991;83:2012–2020)

Low density lipoproteins (LDLs) are a major risk factor for coronary artery disease.\textsuperscript{1} Oxidized LDLs have a marked atherogenic potential: They are chemotactic for circulating monocytes, inhibit the motility of the generated macrophages, and are taken up by the latter, leading to the formation of foam cells.\textsuperscript{2} Monocytes,\textsuperscript{3} endothelial cells,\textsuperscript{4} and smooth muscle cells\textsuperscript{5} can oxidize LDLs; hence, they are present in atherosclerotic lesions.\textsuperscript{6}

Atherosclerotic arteries have decreased antithrombotic properties in their endothelial layer.\textsuperscript{7,8} An important antithrombotic feature of endothelial cells is the production of nitric oxide.\textsuperscript{9,10} Thus, a reduced synthesis or release of the endogenous nitrate may be involved. Indeed, LDLs reduce the basal and receptor-operated release of endothelium-derived relaxing factors from cultured endothelial cells.\textsuperscript{11,12} In the rabbit aorta, LDLs inhibit endothelium-dependent relaxations to acetylcholine\textsuperscript{13,14} and in the porcine coronary artery to serotonin.\textsuperscript{15} Therefore, LDL and, in particular, its oxidized form may specifically interact with endothelial cells and promote functional alterations.

Atherosclerosis does not uniformly affect all blood vessels. Indeed, although the disease is very common
in epicardial coronary arteries and in certain peripheral arteries,\textsuperscript{16} it rarely occurs in the microcirculation. Because all blood vessels are similarly exposed to circulating LDLs, this suggests a heterogeneity in the vascular susceptibility to the atherogenic substance.

This study was designed 1) to investigate the effects of native and oxidized LDLs on endothelial function of porcine coronary arteries, 2) to compare the responses of epicardial and intramyocardial coronary arteries, and 3) to delineate the cellular mechanisms.

**Methods**

**Preparation of Blood Vessels**

*Epicardial coronary arteries.* Farm pigs (weights 20±5 kg) were anesthetized with ketamine (50 mg/kg i.m.) followed by pentobarbital sodium (12.5 mg/kg i.v.). Blood (200 ml) was collected from the left atrium for the preparation of platelets. The heart was removed and immediately placed into cold, modified Krebs-Ringer bicarbonate solution (control solution) of the following composition (mM): 118.3 NaCl, 4.7 KCl, 2.5 CaCl\(_2\), 1.2 MgSO\(_4\), 1.2 KH\(_2\)PO\(_4\), 25.0 NaHCO\(_3\), 0.026 edetate calcium disodium, and 11.1 glucose. The proximal parts of the left anterior descending and the left circumflex coronary arteries were excised, cleaned of loose connective tissue, and cut into 3-mm rings. For some experiments, the endothelium was removed by gently rubbing the luminal surface with a cotton swab wetted with control solution. The rings were suspended horizontally between two stirrups in organ chambers filled with 25 ml of control solution (37°C, pH 7.4) and aerated with 95% O\(_2\)-5% CO\(_2\). One stirrup was connected to an anchor and the other to a force transducer (Scaime, Paris, France) for the recording of isometric tension. After a 30-minute equilibration period, the rings were progressively stretched until the contractile response to potassium chloride (20 mM) was maximal. The vessels were again allowed to equilibrate for 30 minutes before the beginning of the experiments.

*Intramyocardial coronary arteries.* Intramyocardial coronary arteries in the distribution of the left anterior descending coronary artery were dissected free under a dissection microscope (Wild-Leitz, Zurich, Switzerland) and cut into 2-mm rings with an internal diameter of 300 \(\mu\)m. The rings were mounted in a myograph system by passing two stiff tungsten wires through the lumen and fastening these wires to clamps attached to a force transducer for isometric tension recording (Rikadenki, Freiburg, FRG).\textsuperscript{17,18} The vessels were kept under the same conditions as the epicardial coronary arteries.

**Preparation of Platelets**

Autologous blood obtained from the left atrium was drawn into citrate anticoagulant to yield final concentrations of 9.3 mM sodium citrate, 0.7 mM citric acid, and 14 mM glucose. The blood was centrifuged for 40 minutes at 55g at room tempera-

ture, and the platelet-rich plasma was pipetted off. An equal volume of cold citrate anticoagulant solution (mM: sodium citrate 93, citric acid 7, glucose 105, and KCl 5; pH 6.5) was added to the platelet-rich plasma, and the mixture was centrifuged for 20 minutes at 570g. The supernatant was discarded, and the remaining platelet pellet was resuspended in a small volume of the second anticoagulant solution. A platelet count of this suspension was then obtained.\textsuperscript{19}

**Preparation of Low Density Lipoproteins**

LDLs were isolated from human plasma collected in 10\textsuperscript{-3} M EDTA and 10\textsuperscript{-5} M butylhydroxytoluene (BHT) by sequential ultracentrifugation with density adjustments by potassium bromide.\textsuperscript{20} The isolated LDLs were dialyzed against phosphate-buffered saline (PBS) in the presence of EDTA and BHT and were then sterilized by filtration (pore size, 0.45 \(\mu\)m; Gelman, Ann Arbor, Mich.). Protein concentration was determined as previously described with bovine serum albumin used as a standard.\textsuperscript{21} The integrity of LDL was tested by shape change of human platelets.\textsuperscript{22} LDL samples were stored at 4°C in the dark and used within 2 weeks. LDL samples prepared under these conditions are referred to as native LDL.

Before oxidation, LDLs were dialyzed against PBS to remove EDTA and BHT; then they were oxidized at a concentration of 200 \(\mu\)g/ml by exposure to 5 \(\mu\)M CuCl\(_2\) for 24 hours at 37°C.\textsuperscript{23} The extent of lipid peroxidation was estimated as thiobarbituric acid reactive substances.\textsuperscript{23,24} Tetramethoxypropane was used as a standard, and results are expressed as nanomoles of malondialdehyde equivalents per milliliter of the diluted LDL solution. The average degree of oxidation corresponded to 9.0±1.9 nmol malondialdehyde equivalents/ml.

**Protocols**

All protocols in epicardial coronary arteries were performed in parallel; one vessel was exposed to one of the atherogenic substances, and the other was used as control. In intramyocardial coronary arteries, one vessel was used as control and was then exposed to LDL. To compare epicardial and intramyocardial coronary arteries, we obtained both types of vessels from the same animal. Oxidized and native LDLs were added to the organ chambers for 2 hours and then removed by changing the control solution several times. The functional properties of the vessels were examined 10 minutes after washing. Lyssolecithin (10 \(\mu\)g/ml) was present in the organ chambers for 2 hours\textsuperscript{14} and was not removed to test the responses of the arteries.

The relaxations were examined during a contraction caused by the thromboxane analogue U46619 (2×10\textsuperscript{-9} M to 5×10\textsuperscript{-8} M). To obtain the same precaritions for endothelium-dependent relaxation of the treated vessel and the corresponding control, we matched the contractions to U46619 until they did not differ by more than 10%. In addition, these contractions were between 60% and 80% of the
maximal contraction obtained by exposure to potassium chloride (100 mM).

Relaxations to SIN-1 were performed in vessels without endothelium. The other experiments were performed in vessels with endothelium. The presence or absence of endothelial cells was verified before the incubation with one of the atherogenic substances by adding a concentration of bradykinin (10^{-7} M) that evoked maximal relaxation. All experiments were carried out in the absence of inhibitors of cyclooxygenase because both indomethacin and tranilcypromine do not affect endothelium-dependent relaxations in porcine coronary arteries. When determining relaxations to serotonin and aggregating platelets, the rings were incubated with ketanserin (10^{-6} M for 45 minutes) to inhibit the 5-hydroxytryptamine-mediated direct contractile effect of the monoamine on vascular smooth muscle cells. Monomethyl arginine (L-NMMA, 10^{-4} M for 45 minutes) was applied to prevent the production of nitric oxide from L-arginine. Pertussis toxin (100 ng/ml for 1 hour) was used to ribosylate irreversibly certain G proteins. Dextran sulfate (10 μg/ml for 2 hours) was applied to inhibit competitively the binding of oxidized LDL to the scavenger receptor. The polymer was added to the organ chambers at the same time as oxidized LDL.

Drugs

The following drugs were used: d-arginine, L-arginine, 2,6-di-tert-buty1-p cresol, copper chloride, dextran sulfate (MW=1,000,000), EDTA, thiobarbituric acid, dichloroacetic acid (Fluka, Buchs, Switzerland), bradykinin, A23187, thrombin (Sigma Chemical Co., St. Louis), L-N^{G}-monomethyl arginine, pertussis toxin (Calbiochem, Lucerne, Switzerland), 5-hydroxytryptamine creatinine sulfate (Serva, Heidelberg, FRG), ketanserin (Janssen Pharmaceutical, Beerse, Belgium), SIN-1 (Hoechst Pharmaceutica, Paris), tetramethoxyp propane (Aldrich, Steinheim, FRG), and U46619 (Cayman Chemical Co., Ann Arbor, Mich.). All drugs were prepared daily. Dextran sulfate was dissolved in the control solution; BHT and L-NMMA were dissolved in methanol, and A23187 was dissolved in dimethylsulfoxide. L-NMMA and A23187 were further diluted in distilled water. The other drugs were dissolved in distilled water.

Data Analysis

In all series of experiments, n equals the number of animals used. Results are expressed as mean±SEM. The concentration of an agonist causing half-maximal relaxation was calculated for each experiment and is expressed as negative log M (pD_{2}). The maximal relaxation is indicated as percentage of the contraction to U46619. The area under the curve is expressed in arbitrary units. Statistical evaluation of the data was performed by Student’s t test for unpaired observations. A two-tailed p value less than 0.05 was considered to indicate a statistically significant difference.

Results

Oxidized Low Density Lipoproteins

Endothelium-dependent relaxations. Epidermal coronary arteries. Oxidized LDL (30–300 μg/ml) inhibited relaxations of epicardial coronary arteries to serotonin in a dose-dependent manner (Figure 1 and Table 1). Relaxations to thrombin (Figure 2 and Table 1) and to aggregating platelets (Figure 3 and Table 1) were also inhibited by oxidized LDL (300 and 100 μg/ml, respectively). The sensitivity (pD_{2}) to these relaxing agonists remained the same in treated vessels and in controls. The endothelium-dependent relaxations to bradykinin (Figure 1 and Table 1) and A23187 (Figure 2 and Table 1) were unaffected by oxidized LDL at concentrations of 300 μg/ml.

Intramyocardial coronary arteries. In contrast to epicardial coronary arteries, endothelium-dependent relaxations of intramyocardial coronary arteries to serotonin were unaffected by oxidized LDL (100 μg/ml; n=5; p=NS; Figure 4) and were also not significantly inhibited by high concentrations of oxidized LDL (300 μg/ml).

Endothelium-independent relaxations. Relaxations of arteries without endothelium to the nitric oxide donor SIN-1 were not different in rings exposed to...
oxidized LDL (300 μg/ml) and in the corresponding controls (Table 1).

Contractions of vascular smooth muscle. Contraction to potassium chloride (100 mM) averaged 5.5±0.4 g in vessels exposed to oxidized LDL (300 μg/ml) and 5.7±0.4 g in controls (n=4; p=NS). Contractions of treated rings to U46619 (3×10⁻⁹ M) reached 3.6±0.7 g and were comparable to those in controls (4.0±1.0 g; n=4; p=NS).

Similarly, contractions to L-NMMA (10⁻⁴ M) in treated rings (1.3±0.2 g) were not different from those in controls (1.5±0.3 g; n=6; p=NS). Oxidized LDL did not cause contractions when present in the organ chambers.

Cellular mechanisms. Receptor. Vessels treated with oxidized LDL (30 μg/ml) in the presence of dextran sulfate (10 μg/ml) to block the scavenger receptor relaxed to serotonin to a maximum of 95±3% and, thus, did not differ from controls (88±6%; n=5; p=NS; Figure 5). In the absence of dextran sulfate, however, the maximal relaxation of treated vessels was inhibited (56±10%; p<0.05 versus control; n=5). Hence, dextran sulfate prevented the inhibiting effect of oxidized LDL on the relaxations to serotonin (p<0.01 versus vessels exposed to oxidized LDL in the absence of dextran sulfate).

G PROTEINS. Pertussis toxin (100 ng/ml) reduced the maximal relaxation to serotonin (54±10%) compared with that in control (88±3%; p<0.01; n=8; Figure 6). When oxidized LDL was added to vessels treated with pertussis toxin, the relaxation was further inhibited (30±5%; p<0.05 versus pertussis toxin alone; n=8).

L-ARGININE PATHWAY. The maximal relaxation to serotonin was reduced by L-NMMA (52±9%; control: 88±3%; p<0.01; n=6). In the presence of L-NMMA, oxidized LDL caused a further inhibition (22±4%; p<0.01 versus L-NMMA alone; n=6; Figure 6).

L-Arginine (10⁻⁴ M) but not D-arginine relaxed vessels (from 60±7% to 79±5%) exposed to oxidized LDL (100 μg/ml) when added after serotonin (p<0.05 versus treated rings before addition of L-arginine; n=6; Figure 7). L-Arginine (3×10⁻⁴ M) fur-

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**TABLE 1. Effect of Oxidized LDL (30–300 μg/ml) on Endothelium-Dependent and Endothelium-Independent Relaxations in Porcine Coronary Arteries**

<table>
<thead>
<tr>
<th>[LDL] (μg/ml)</th>
<th>Control</th>
<th>Oxidized LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pD₂</td>
<td>Max</td>
</tr>
<tr>
<td>Serotonin (n=6)</td>
<td>300</td>
<td>6.7±0.1</td>
</tr>
<tr>
<td>Serotonin (n=6)</td>
<td>100</td>
<td>7.1±0.1</td>
</tr>
<tr>
<td>Serotonin (n=6)</td>
<td>30</td>
<td>7.1±0.1</td>
</tr>
<tr>
<td>Thrombin (n=6)</td>
<td>300</td>
<td>1.6±0.1</td>
</tr>
<tr>
<td>Platelets (n=5)</td>
<td>100</td>
<td>...</td>
</tr>
<tr>
<td>Bradykinin (n=6)</td>
<td>300</td>
<td>8.7±0.2</td>
</tr>
<tr>
<td>A23187 (n=6)</td>
<td>300</td>
<td>7.7±0.3</td>
</tr>
<tr>
<td>SIN-1 (n=6)</td>
<td>300</td>
<td>7.2±0.2</td>
</tr>
</tbody>
</table>

pD₂ value calculated as the negative log M concentration of the agonist evoking a half-maximal relaxation; Max, maximal relaxation expressed as percentage of contraction to U46619.

*The area under the curve is expressed in arbitrary units.

†p<0.005 vs. control; †p<0.01 vs. control; $p<0.05 vs. control.

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**FIGURE 2. Plots of effect of oxidized low density lipoprotein (Ox LDL) on endothelium-dependent relaxations to thrombin (left panel) and A23187 (right panel).** Oxidized LDL (300 μg/ml) inhibited relaxations to thrombin (p<0.005 vs. controls). In contrast, relaxations to A23187 were not inhibited.
FIGURE 3. Plot of effect of oxidized low density lipoproteins (Ox LDL; 100 μg/ml) on endothelium-dependent relaxations to platelets in porcine coronary arteries. Relaxations were inhibited in arteries exposed to lipoprotein (p<0.005 vs. controls).

Further relaxed these vessels to 87±3% (p<0.005 versus treated rings before addition of L-arginine; n=6). After the addition of L-arginine (10⁻⁴ or 3×10⁻⁴ M), the relaxations induced by serotonin of vessels exposed to oxidized LDL were not significantly different from those of controls (88±3%; n=6; p=NS). When L-arginine (3×10⁻⁴ M) was added before serotonin, vessels treated with oxidized LDL (100 μg/ml) relaxed to serotonin to a maximum of 88±6% and, thus, did not differ from controls (93±4%; n=5; p=NS). In the absence of L-arginine, however, the maximal relaxation was inhibited (58±8%; p<0.005 versus control; n=5). Hence, L-arginine potentiated relaxations to serotonin in vessels exposed to oxidized LDL (p<0.05 versus treated vessels in the absence of L-arginine). L-Arginine did not affect relaxations of control vessels (n=5; NS).

LYSOLEICHTIN. Lysolecithin (10 μg/ml) had no effect on endothelium-dependent relaxations to serotonin (maximal relaxation of controls, 96±5%; treated vessels, 98±4%; n=5; p=NS).

Native Low Density Lipoproteins

Native LDL at a concentration of 300 μg/ml had no effect on endothelium-dependent relaxations to

FIGURE 4. Plots of effect of oxidized low density lipoproteins (Ox LDL) on endothelium-dependent relaxations to serotonin in epicardial and intramyocardial coronary arteries. Oxidized LDL (100 μg/ml) inhibited relaxations of epicardial coronary arteries (p<0.01 vs. controls). In contrast, relaxations of intramyocardial coronary arteries were not inhibited.
serotonin and bradykinin or on endothelium-independent relaxations to SIN-1 (Table 2).

**Discussion**

This study demonstrates that oxidized but not native LDL inhibited endothelium-dependent relaxations to serotonin, thrombin, and aggregating platelets in porcine epicardial coronary arteries, whereas the lipoproteins did not affect endothelium-dependent relaxations to serotonin in intramyocardial coronary arteries. Endothelium-dependent relaxations to bradykinin and to A23187 as well as endothelium-independent relaxations to SIN-1 were unaffected by oxidized LDL in epicardial coronary arteries.

The inhibitory effect of oxidized LDL on endothelium-dependent relaxations could be due to a decreased synthesis or release of endothelium-derived relaxing factors or could be related to a reduced vascular responsiveness to these factors. The latter possibility can be excluded because endothelium-independent relaxations to SIN-1 were not affected even by a high concentration of oxidized LDL. Because the potency of endothelium-dependent relaxations is influenced by the level of precontraction of the blood vessels, oxidized LDL could exert its inhibitory effect by increasing vascular contractility. This, however, can be excluded because the lipoproteins in porcine coronary arteries—in contrast to the rabbit femoral artery33—did not enhance contractions to potassium chloride or U46619 under our experimental conditions where oxidized LDL was washed before the experiment. Thus, oxidized LDL specifically interfered with endothelial function of porcine epicardial coronary arteries.

In contrast to the rabbit aorta,14 the inhibitory effect of oxidized LDL could not be mimicked by lyssolecithin. However, it was completely prevented by dextran sulfate, which is a competitive antagonist of acetylated LDL for the scavenger receptor.31,32 The latter is present on endothelial cells34–36 and binds oxidized LDL.4,5 Because the effects of oxidized LDL could be prevented by dextran sulfate and a direct interaction of oxidized LDL and the polymere has been excluded,31 oxidized LDL must interfere with endothelium function by activating the scavenger receptor. In line with that interpretation, native LDL—which interacts with its own receptor37—that affected endothelium-dependent nor endothelium-independent relaxations.

An interference of oxidized LDL with a specific endothelial receptor such as the serotonergic receptor can be excluded because relaxations to different receptor-operated agonists were inhibited by the lipoproteins. In coronary arteries with regenerated endothelium, endothelium-dependent relaxations to serotonin are reduced to the same extent as those of arteries exposed to pertussis toxin. Thus, functional alterations of regenerated endothelial cells have been attributed to the dysfunction of a pertussis toxin–sensitive G protein linked to serotonergic receptors.38 Oxidized LDL, however, is unlikely to exert its effects through inactivation of a pertussis toxin sensitive G protein because the inhibition persisted in the presence of the toxin and was more pronounced than that of the toxin.

In contrast to the endothelium-dependent relaxations to serotonin, thrombin and aggregating platelets, those to A23187 were not inhibited by oxidized...
LDL. This indicates that oxidized LDL interferes with receptor-operated signal transduction mechanisms linked to the formation of endothelium-derived relaxing factors upstream of the elevation of intracellular calcium. However, endothelium-dependent relaxations to bradykinin were also unaffected by oxidized LDL even though the kinin activates specific endothelial receptors. This discrepancy could be related to the release of different endothelial mediators by bradykinin and serotonin. Indeed, endothelium-dependent relaxations to bradykinin are only minimally inhibited by L-NMMA, methylene blue, and hemoglo-

**TABLE 2. Effect of Native LDL (30–300 μg/ml) on Relaxations to Serotonin, Bradykinin, and SIN-1 in Porcine Coronary Arteries**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Native LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pD2 Max Area*</td>
<td>pD2 Max Area*</td>
</tr>
<tr>
<td>Serotonin (n=6)</td>
<td>7.2±0.1 77±7 430±36</td>
<td>7.3±0.1 85±3 378±27</td>
</tr>
<tr>
<td>Bradykinin (n=6)</td>
<td>7.4±0.2 105±1 297±47</td>
<td>7.3±0.2 107±2 230±24</td>
</tr>
<tr>
<td>SIN-1 (n=6)</td>
<td>7.4±0.1 119±8 249±29</td>
<td>7.6±0.1 118±5 217±32</td>
</tr>
</tbody>
</table>

pD2, value calculated as the negative log M concentration of the agonist evoking a half-maximal relaxation; Max, maximal relaxation expressed as percentage of contraction to U46619.

*The area under the curve is expressed in arbitrary units.
The same pattern of action of oxidized LDL and the inhibitors of endothelium-derived nitric oxide suggests that the lipoproteins specifically interfere with the activity of the L-arginine pathway during stimulation with certain agonists. Oxidized LDL could interfere with the activity of the nitric oxide–forming enzyme, compete with L-arginine for the enzyme, or interfere with the receptor-mediated intracellular availability of L-arginine. Inhibition of the nitric oxide–forming enzyme can be excluded because L-arginine but not D-arginine fully relaxed vessels in which the relaxation to serotonin was inhibited by oxidized LDL, whereas the amino acid did not relax arteries when added after U46619 had caused contraction. Competitive inhibition is unlikely because the basal release— as estimated by the endothelium-dependent contraction to L-NMMA—was not affected by oxidized LDL and because the lipoproteins did not cause endothelium-dependent contractions by themselves. Thus, the most likely explanation for the effect of oxidized LDL is an interference with the receptor-mediated intracellular availability of L-arginine. Indeed, L-arginine added before serotonin potentiated relaxations to the monoamine in vessels exposed to oxidized LDL, whereas the response remained unaffected in controls. Oxidized LDL may interfere with the receptor-operated release of L-arginine from intracellular stores or the synthesis of the amino acid. In line with that interpretation, the effect of oxidized LDL persisted in the presence of L-NMMA. Moreover, the inhibition of endothelium-dependent relaxations by high concentrations of the lipoproteins was stronger than that by L-NMMA. If oxidized LDL indeed interferes with the intracellular availability of L-arginine, a further inhibition of the response in the presence of a competitive antagonist is expected.

The inhibitory effect of oxidized LDL on the production of endothelium-derived nitric oxide most probably is involved in the pathogenesis of coronary artery disease. The reduced endothelium-dependent relaxations of hypercholesterolemic porcine coronary arteries may be related to an accumulation of oxidized LDL in the vessel wall because the same inhibitory pattern of relaxations to serotonin and bradykinin was obtained in vessels exposed to oxidized LDL and in hypercholesterolemic arteries. The involvement of oxidized LDL is further supported by the fact that a decreased release of endothelium-derived relaxing factor accounts for the impaired endothelium-dependent relaxations in hypercholesterolemia. It is also consistent with the hypothesis that oxidized LDL plays an important role, particularly in early stages of atherogenesis. Indeed, when compared with hypercholesterolemia, established atherosclerosis further impairs endothelium-dependent relaxations to serotonin and also reduces those to bradykinin. Thus, at this stage of the disease, the changes in endothelial function cannot be explained only by the presence of oxidized LDL in the vessel wall.

The resistance of intramyocardial coronary arteries toward the effect of oxidized LDL may be related to a smaller number of scavenger receptors on endothelial cells compared with those on epicardial coronary arteries, to a different function of the receptor in small coronary arteries, or to the fact that the contribution of nitric oxide to endothelium-dependent relaxations is smaller in these microvessels. The lack of inhibition of endothelium-dependent relaxation in intramyocardial coronary arteries further supports the involvement of oxidized LDL in coronary artery disease. Indeed, the atherosclerotic process is restricted to epicardial coronary arteries. Similarly, vasospastic and thrombotic events occur almost exclusively in large coronary arteries. They may be related to the reduced nitric oxide formation at sites where oxidized LDL accumulated in the vessel wall. Thus, oxidized LDL may not only play an important role in atherogenesis, but also promote clinical events like vasospasm and thrombus formation, both of which are well known in patients with coronary artery disease.

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References

11. Boulanger C, Bühler FR, Lüscher TF: Low density lipoproteins impair the release of endothelium-derived relaxing factor
from cultured porcine endothelial cells (abstract). *Eur Heart J* 1989;10:331


42. Hecker M, Mitchell JA, Aenggard E, Vane JR: L-arginine synthesis is a prerequisite for the release of EDRF from cultured endothelial cells (abstract). *Arch Int Pharmacodyn 1990;305:252


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