oxLDL specifically impairs endothelium-dependent, NO-mediated dilation of coronary arterioles

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Hein, Travis W., James C. Liao, and Lih Kuo. oxLDL specifically impairs endothelium-dependent NO-mediated dilation of coronary microvessels. Am. J. Physiol. Heart Circ. Physiol. 278: H175–H183, 2000.—Our previous studies implicated that oxidized low-density lipoprotein (oxLDL), a putative atherogenic agent, impairs endothelium-dependent, nitric oxide (NO)-mediated dilation of isolated coronary arterioles to pharmacological agonists. However, it is not known whether oxLDL specifically affects NO-mediated dilation or generally impairs endothelium-dependent function, including the release of hyperpolarizing factors. In this regard, we investigated the dilation of isolated porcine coronary arterioles (50- to 100-μm luminal diameter) in response to the activation of various endothelium-dependent pathways before and after intraluminal incubation of the vessels with oxLDL (0.5 mg protein/ml for 60 min). In the absence of oxLDL, all vessels developed basal tone and dilated in response to the activation of NO synthase (by flow and adenosine), cyclooxygenase (by arachidonic acid), cytochrome P-450 monoxygenase (by bradykinin), and endothelial membrane hyperpolarization (by sucrose-induced hyperosmolarity). Incubation of the vessels with oxLDL for 60 min did not alter basal tone but did inhibit the vasodilatory responses to increased flow and adenosine in a manner similar to that of the NO synthase inhibitor Nω-nitro-L-arginine methyl ester. Vasodilations in response to flow and adenosine were not affected by intraluminal incubation of the vessels with either a vehicle solution or the native LDL (0.5 mg protein/ml, 60 min). In contrast with the NO-mediated response, hyperosmotic vasodilations mediated by endothelial hyperpolarization was not affected by oxLDL. Endothelium-dependent dilations to the cyclooxygenase activator arachidonic acid and to the cytochrome P-450 monoxygenase activator bradykinin and endothelium-independent vasodilations to sodium nitroprusside were also not altered by oxLDL. Collectively, these results indicate that oxLDL has a selective effect on endothelium-dependent dilation with specific impairment of the NO-mediated response, whereas cyclooxygenase and cytochrome P-450 monoxygenase-mediated dilations are spared from this inhibitory effect. In addition, oxLDL does not appear to affect vasodilation mediated by hyperpolarization of the endothelium.

atherosclerosis; nitric oxide; potassium channels

THE VASCULAR ENDOTHELIUM under physiological conditions participates in the regulation of vascular tone by releasing vasodilative substances in response to various stimuli. Nitric oxide (NO), endothelium-derived hyperpolarizing factor (EDHF), and prostacyclin are endothelium-derived vasodilators that are synthesized by NO synthase (46), cytochrome P-450 monoxygenase (17), and cyclooxygenase (53), respectively. Several disease conditions, such as diabetes, hypertension, and atherosclerosis, have been identified with impaired endothelial function (12). One of the risk factors for the development of atherosclerosis is a high plasma level of low-density lipoproteins (LDLs). Recent clinical and experimental evidence suggests that an oxidized form of LDL (oxLDL) is primarily responsible for the deleterious effects of these lipoproteins (9). In fact, studies in large conduit arteries from animals (56, 57) and humans (18) indicate that the atherogenic agent oxLDL can impair endothelium-dependent relaxation of these vessels. Although the mechanism of this impairment is not well understood, a number of studies have shown that the NO synthase pathway and/or the released NO is influenced by oxLDL (8, 19, 23, 47, 57).

Although atherosclerotic lesions are generally confined to large arteries, functional consequences of this pathology have been shown to extend into the coronary microvessels (6, 33). This finding is important because microvessels (<150 μm in diameter) act as the primary controller of blood flow by adjusting their diameters in response to various physiological stimuli (7). We have previously shown that an ATP metabolite, adenosine (31), and an increased intraluminal flow (34) can elicit endothelium-dependent, NO-mediated dilation of coronary microvessels. In contrast, hyperosmotic stress elicits a dilation that is mediated by the endothelial hyperpolarization, which is independent of NO (27). Activation of cyclooxygenase (34) and monoxygenase (27) pathways in the endothelium also produces the dilation of coronary microvessels. Although dysfunction of NO-mediated relaxation of large conduit coronary vessels caused by oxLDL has been reported (56, 57), it remains unclear whether this modified lipoprotein specifically impairs vasodilation associated with the NO pathway or, rather, generally affects other endothelium-dependent vasodilatory mechanisms such as endothelial hyperpolarization, cyclooxygenase, and cytochrome P-450 monoxygenase pathways. Because microvessels are fundamentally important for coronary flow regula-
tion, in the present study we addressed this issue at the microcirculatory level. In this regard, we examined the endothelium-dependent vasodilation of isolated coronary arterioles (50- to 100-µm diameter) in response to the activation of various vasodilatory pathways including NO synthase (by adenosine and flow), endothelial hyperpolarization (by hyperosmotic stress), cytochrome P-450 monooxygenase (by bradykinin), and cyclooxygenase (by arachidonic acid) to determine whether oxLDL has a specific inhibitory effect on NO-mediated dilation of coronary microvessels.

MATERIALS AND METHODS

General preparation. Pigs (8–12 wk old, of either sex) were sedated with an intramuscular injection of Telazol (tiletamine and zolazepam, 1:1, 4.4 mg/kg) and xylazine (2.2 mg/kg) and then anesthetized and heparinized with an intravenous administration, via the marginal ear vein, of pentobarbital sodium (20 mg/kg) and heparin (1,000 U/kg). Pigs were intubated and ventilated with room air. After a left thoracotomy was performed, the heart was electrically fibrillated, excised, and immediately placed in cold (5°C) saline solution.

Isolation and cannulation of microvessels. The techniques for identification and isolation of porcine coronary microvessels were described previously (34). In brief, a mixture of India ink and gelatin in physiological salt solution (PSS) containing (in mM) 145.0 NaCl, 4.7 KCl, 2.0 CaCl2, 1.17 MgSO4, 1.2 NaH2PO4, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, and 3.0 MOPS was perfused into the left anterior descending artery (0.3 ml) and the circumflex artery (0.4 ml) to enable visualization of the coronary microvessels. Small subepicardial arterioles (0.6–1.0 mm in length and 50–100 µm in internal diameter, in situ) from the left anterior descending or circumflex arteries were selected and carefully dissected from the surrounding cardiac tissue under cold (5°C) PSS containing BSA (1% Amersham, Arlington Heights, IL) at pH 7.4. Each isolated arteriole was then transferred for cannulation to a Lucite vessel chamber containing PSS-albumin equilibrated with room air at ambient temperature. One end of the microvessel was cannulated with a glass micropipette (40 µm in tip diameter) filled with filtered PSS-albumin, and the outside of the microvessel was securely tied to the pipette with 11-0 ophthalmic suture (Alcon, Fort Worth, TX). The ink-gelatin solution inside the vessel was flushed out at a low perfusion pressure (<20 cmH2O). The other end of the vessel was then cannulated with a second micropipette and tied with a suture. Electrical resistances (measured by LCR Bridge Circuit, model LCR-740, Leader Electronics, Japan) of the micropipettes were matched (± 0.5%). In addition, we have previously shown (34) that the ink-gelatin solution has no detectable detrimental effect on either endothelial or vascular smooth muscle function. After cannulation of a blood vessel, the chamber was transferred to the stage of an inverted microscope (model IM35, Zeiss, Thornwood, NY) coupled to a charge-coupled device camera (KP-161, Hitachi) and video micrometer (Micrcirculation Research Institute, Texas A&M University Health Science Center). Internal diameters of the vessels were measured throughout the experiment using video microscopic techniques. The micropipettes were connected to independent reservoir systems, and intraluminal pressures were measured by micromanometric transducers (Statham P23 Db, Gould, Cleveland, OH). The isolated vessels were pressurized to 60 cmH2O luminal pressure without flow by setting both reservoirs at the same hydrostatic level. Leaks were detected by differences between reservoir pressure and intraluminal pressure. Preparations with leaks were excluded from further study.

Oxidation of LDLs. Human native LDL (nLDL; 5 mg protein/ml) was obtained from Sigma (St. Louis, MO). oxLDL was prepared as reported previously (23). Briefly, LDL was oxidized by exposure to 10 µM CuCl2 for 8–24 h at room temperature. The degree of LDL oxidation was measured spectrophotometrically (16) and with the thiobarbituric acid-reactive substances (TBARS) assay (5). TBARS data were expressed as nanomoles of malondialdehyde per milligram of LDL protein. nLDL and oxLDL were dialyzed separately against Dulbecco’s PBS for 24 h. The two forms of LDL were stored at 4°C and used within 2 wk. Before each experiment, nLDL and oxLDL were filtered with a 0.2-µm pore filter (Corning, Corning, NY) and then anesthetized and heparinized with an intravenous administration, via the marginal ear vein, of pentobarbital sodium (20 mg/kg) and heparin (1,000 U/kg). Pigs were intubated and ventilated with room air. After a left thoracotomy was performed, the heart was electrically fibrillated, excised, and immediately placed in cold (5°C) saline solution.

Coronary arteriolar dilation in response to activation of various endothelium-dependent pathways. The cannulated arterioles were bathed in PSS-albumin and equilibrated with room air; the temperature was maintained at 36–37°C by an external heat exchanger. The vessel was set to its in situ length and allowed to develop basal tone at 60 cmH2O intraluminal pressure without flow. This pressure was demonstrated in coronary arterioles of this size in vivo (7). Vasodilator response to increased flow was studied under constant intraluminal pressure using dual-reservoir techniques (34). In brief, the luminal flow was produced by simultaneously moving the pressure reservoirs in opposite directions of the same magnitude, which generates a pressure gradient (ΔP; range from 4 to 60 cmH2O) across the length of the vessel without changing intraluminal pressure (34). We have previously demonstrated that the luminal flow is increased linearly with increasing ΔP and the range of mean volumetric flows for ΔP between 0 and 60 cmH2O is 0–34.8 nl/s (34).

Hyperosmolar-induced dilation was elicited by incrementally adding sucrose (40–400 mM) to the vessel bath, which increased osmolarity from 300 to 340 mosM, as described in our previous study (27). Vasodilations in response to adenosine (0.1 nM to 10 µM), bradykinin (1 nM), and arachidonic acid (10 µM) were studied by adding agonists to the vessel bath. To determine the role of endothelium in these vascular responses, vasodilations were examined before and after endothelial denudation. The technique for endothelial removal has been described in detail in our previous study (26). Briefly, a nonionic detergent, 3-[[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (0.4%), was intraluminally perfused into the vessel for 1–2 min to remove endothelial cells. Only vessels that exhibited normal basal tone, showed no vasodilation to the endothelium-dependent vasodilator bradykinin (1 nM) (26), and showed unaltered vasodilation to sodium nitroprusside (1 nM to 100 µM) after endothelial removal were accepted for data analysis. The involvement of NO synthase and cyclooxygenase pathways in vasodilation was examined before and after incubation of the vessels with the specific inhibitors L-NAME, 10 µM (30 min) and indomethacin (10 µM, 30 min), respectively. The role of endothelial cytochrome P-450 monooxygenase pathway in vasodilation was examined by intraluminal incubation of the vessels with its specific inhibitor clotrimazole (30 µM, 30 min). The contribution of endothelial hyperpolarization to vasodilations was assessed by intraluminal incubation of the vessels with its specific inhibitor clotrimazole (30 µM, 30 min). The contribution of endothelial hyperpolarization to vasodilations was assessed by intraluminal incubation of the vessels with its specific inhibitor clotrimazole (30 µM, 30 min).
nal administration of the depolarizing agent KCl (80 mM, 30 min). The role of calcium-activated potassium (K_{Ca}) channels for vasodilation was assessed by incubation of the vessels with a selective K_{Ca} channel inhibitor, iberiotoxin (0.1 µM, 30 min).

Effect of oxLDL on endothelium-dependent vasodilations. Coronary arteriolar dilations were examined before and after the intraluminal solution was replaced with oxLDL (0.5 mg protein/ml) for 60 min. The vasodilations in the presence of oxLDL were further examined after extraluminal incubation with l-arginine (3 mM, 20 min) or d-arginine (3 mM, 20 min). To rule out time-dependent and nonspecific effects of oxLDL, the vasodilatory responses were also examined in another series of experiments after a 60-min incubation of the vessels with vehicle (PSS) and nLDL (0.5 mg protein/ml), respectively. To study the effect of oxLDL on endothelium-independent vasodilation, the concentration-dependent response of isolated vessels to sodium nitroprusside (1 nM to 100 µM) was studied before and after incubation of the vessels with oxLDL (0.5 mg protein/ml) for 60 min.

Chemicals. Drugs were obtained from Sigma, except as otherwise stated. Adenosine, bradykinin, d-arginine, l-arginine, l-NAME, iberiotoxin, and indomethacin were dissolved in a stock solution of ethanol, and subsequent concentrations were diluted in PSS. The final concentration of ethanol in the vessel bath was 0.1%, and vehicle control studies indicated that this concentration had no effect on the arteriolar function.

Data analysis. At the end of each experiment, the vessel was relaxed with sodium nitroprusside (100 µM) to obtain its maximal diameter at 60 cmH2O intraluminal pressure. We have previously shown (23) that this concentration of sodium nitroprusside produced maximal relaxation of isolated vessels because their diameters were not further increased by a calcium-free solution containing EDTA (1 mM). Therefore, all diameter changes in response to agonists were normalized to the vasodilation in response to 100 µM sodium nitroprusside and expressed as a percentage of maximal dilation. All data are presented as means ± SE. One or two vessels were used from each heart with each serving as its own control. Statistical comparisons of vasomotor responses under different treatments were performed with two-way ANOVA and tested with Fisher’s protected least significant difference multiple-range test. Differences in resting diameter before and after pharmacological interventions were compared by paired Student’s t-test. Significance was accepted at P < 0.05.

RESULTS

Effect of LDL on endothelium-dependent, NO-mediated vasodilation to flow. Before investigating the effect of oxLDL on flow-induced vasodilation, we first characterized various mechanisms involved, such as NO dependency, endothelial potassium channels, and membrane hyperpolarization. All isolated arterioles developed a similar level of basal tone (e.g., constricted to 69 ± 1% of their maximal diameter) within 40 min at a bath temperature of 36–37°C with 60 cmH2O intraluminal pressure. Figure 1 displays graded vasodilation of arterioles when the pressure gradient, and thus flow, was increased in a stepwise manner. Under control conditions, the vessels dilated to 70% of maximal diameter at the highest flow. Removal of the endothelium abolished vasodilation to flow, indicating that this vasodilatory response requires an intact endothelium (Fig. 1). The NO synthase inhibitor l-NAME (10 µM, extraluminal incubation) and intraluminal administration of depolarizing KCl solution (80 mM) also abolished flow-induced vasodilation (Fig. 1). The vasodilation was significantly inhibited by intraluminal administration of the K_{Ca}-channel blocker iberiotoxin (0.1 µM, Fig. 1) but was not affected by the ATP-sensitive potassium (K_{ATP})-channel blocker glibenclamide (data not shown). It should be noted that the resting vascular tone was not significantly affected by endothelial denudation, l-NAME, luminal KCI, or iberiotoxin. The vasodilatory response to increased flow in endothelium-intact vessels was not affected by a 60-min intraluminal incubation of arterioles with nLDL (0.5 mg protein/ml) but was significantly reduced to ~10% with oxLDL (0.5 mg protein/ml, Fig. 2). The vasodilation was restored toward normal by subsequent incubation of oxLDL-treated vessels with l-arginine (3 mM, extraluminal, 20 min, Fig. 2) but not with d-arginine (n = 2, data not shown). It is noted that the exposure of coronary arterioles to nLDL, oxLDL, or arginine did not alter resting vascular tone.

Effect of LDL on endothelium-dependent, NO-mediated vasodilation to adenosine. Adenosine (0.1 nM to 10 µM) produced concentration-dependent dilation of isolated coronary arterioles (Fig. 3). This vasodilatory response was significantly attenuated by l-NAME (10 µM, Fig. 3). In a similar manner, a 60-min intraluminal incubation with oxLDL (0.5 mg protein/ml), but not with nLDL (0.5 mg protein/ml), inhibited the vasodilation to adenosine. Subsequent administration of l-arginine (3 mM, 20 min) to these oxLDL-treated vessels completely restored the vasodilation in response to adenosine (Fig. 3). In contrast, the inhibited vasodilation was not reversed by treating the vessels with d-arginine (3 mM, 20 min; n = 4; data not shown).

Effect of oxLDL on endothelium-dependent vasodilation to hyperosmolality. As shown in Fig. 4, coronary arterioles dilated in a concentration-dependent man-
The increase in sucrose concentration from control (0 mM) to 10, 20, and 40 mM has been shown to produce corresponding increases in the osmolarity of vessel bath from 300 to 310, 320, and 340 mosM, respectively (27). This vasodilatory response was inhibited by removal of endothelium or by intraluminal KCl (80 mM), but not by L-NAME (10 µM) (Fig. 4). oxLDL (0.5 mg protein/ml, 60-min incubation) did not alter the arteriolar dilation to hyperosmolarity (Fig. 4); however, flow-induced dilation in these same vessels was abolished (n = 3, data not shown).

Effect of oxLDL on endothelium-dependent vasodilation to bradykinin and arachidonic acid. The dilation of isolated coronary arterioles to bradykinin and arachidonic acid is presented in Fig. 5. The vasodilation to bradykinin (1 nM) was not affected by L-NAME (10 µM) or indomethacin (10 µM, data not shown) but was blocked by denudation, indomethacin (10 µM), and iberiotoxin (0.1 µM) (Fig. 6).
Incubation of the vessels with oxLDL (0.5 mg protein/ml) for 60 min did not affect the vasodilation to arachidonic acid (Fig. 6). It is noted that these inhibitors did not alter resting vascular tone.

Effect of oxLDL on endothelium-independent vasodilation. Vascular smooth muscle function of isolated coronary arterioles was assessed by examining endothelium-independent vasodilation to sodium nitroprusside (1 nM to 100 µM) before and after incubation of the vessels with oxLDL (0.5 mg protein/ml) for 60 min. The concentration-response curves for sodium nitroprusside were identical before and after oxLDL treatment (Fig. 7).

DISCUSSION

The results of the present study demonstrate that the endothelium-dependent, NO-mediated dilation of isolated coronary arterioles in response to flow and to adenosine is inhibited by oxLDL. The impairment by oxLDL appears to be specific for the endothelial NO synthase pathway, because cytochrome P-450 monooxygenase- and cyclooxygenase-mediated dilations to bradykinin and arachidonic acid, respectively, are not affected by this lipoprotein. The hyperosmotic vasodilation mediated by the endothelial hyperpolarization is also not altered by oxLDL. Furthermore, the vasodilation in response to the NO donor sodium nitroprusside remains intact, indicating that the impairment of NO-mediated dilation by oxLDL is not at the vascular smooth muscle level.

The results of our study were derived from an isolated-vessel preparation that allowed us to directly examine the effect of oxLDL on microvascular function without the confounding influences from the interaction of vascular cells with either blood-borne substances or circulating cells. The intraluminal administration and incubation of oxLDL (60 min) might raise the possibility that the observed inhibitory effect of lipoproteins is a nonspecific phenomenon caused by time-dependent deterioration of NO-mediated function or related to an increase of protein in the lumen. However, a 60-min incubation with either vehicle solution or nLDL did not affect vasodilatory responses to adenosine and flow. Thus our present data support the view that the inhibited vasodilation observed in the present study is specifically a result of oxLDL. Although the plasma concentration of oxLDL in vivo is not known, it has been predicted to be 0.5–2 mg protein/ml in human atherosclerotic lesions (29). It appears that the concentration of oxLDL (0.5 mg protein/ml) used in the present study is within the pathophysiological range.

Dilation of coronary arterioles to an increased flow was previously characterized in our laboratory (32, 34). This vasodilatory mechanism is attributable to the activation of the NO synthase pathway in the endothelium because this dilation was abolished by either an NO synthase inhibitor or endothelial removal (32). Our present finding is consistent with the previous reports with respect to the involvement of endothelial NO, but the mechanism for the activation of NO pathway in the coronary microvessels remains unclear. Electrophysiological study of cultured endothelial cells implicated that flow/shear stress can activate potassium channels and lead to membrane hyperpolarization (28, 42, 45). However, whether these membrane potential changes lead to NO release is unknown. In the present study, we found that a high concentration of luminal KCl abolished flow-induced dilation, which is identical to that found in the vessels treated with NO inhibitor, suggesting that endothelial hyperpolarization is essential for the release of NO and the subsequent vasodilation. Activation of endothelial large-conductance K_{Ca} channels is likely to be responsible for the membrane hyperpolarization, because this vasodilatory response was effectively and specifically inhibited by iberiotoxin rather than by other potassium channel blockers (i.e., glibenclamide, apamin, 4-aminopyridine, and BaCl_{2}) as...
observed in our pilot studies (data not shown). In contrast, the study of bovine aortic endothelial cells grown on collagen-coated microcarrier beads showed that the increased NO release by flow is independent of the activation of potassium channels (43). Although the explanation for this discrepancy is unclear, the differences in species and/or endothelial environments, e.g., intact vessel versus cultured cells, might contribute to the inconsistent results. Nevertheless, the functional involvement of endothelial K<sub>Ca</sub> channels and hyperpolarization associated with NO-mediated dilation to flow in the coronary resistance vessels was characterized for the first time in the present study. Interestingly, a recent coronary microvascular study showed that flow-induced dilation is inhibited in the presence of tyrosine kinase inhibitors (38). Because K<sub>Ca</sub> channels can be regulated by tyrosine kinase (49) and inhibition of tyrosine kinase attenuates activity of an iberiotoxin-sensitive potassium channel (52), it is possible that activation of endothelial K<sub>Ca</sub> channels by tyrosine phosphorylation leads to flow-induced dilation of coronary arterioles.

In humans, angiographic studies show that coronary arteries free of atherosclerosis dilate to an increased blood flow, whereas arteries with early and more advanced atherosclerotic lesions fail to respond to increased flow (10, 14, 39). We previously (33) extended these findings to the microvascular level, showing that coronary arteriolar dilation to flow was abolished in hypercholesteremia-induced atherosclerotic pigs. However, a putative lipid component responsible for the impairment of flow-mediated vasodilation has not been established. In the present study, incubation of coronary arterioles with oxLDL, but not nLDL, was able to inhibit flow-induced vasodilation. The vasodilation was almost completely restored by incubation of oxLDL-treated vessels with L-arginine but not D-arginine, suggesting that the NO synthesis was impaired by oxLDL. It is worth noting that the concentration of L-arginine (3 mM) used in the present study had no effect on the vasodilation to increased flow in normal vessels (data not shown) as reported in our previous studies (33). Collectively, these results suggest that the microvascular dysfunction caused by oxLDL is related to the impairment of the NO-mediated vasodilatory pathway.

It is well documented that coronary blood flow is significantly influenced by interstitial levels of adenosine (3, 30, 55). Direct observation of coronary blood vessels in vivo (22) or in vitro (31) indicates that adenosine preferentially dilates microvessels <150 µm in diameter. This dilation is partially mediated by the endothelial release of NO because disruption of endothelium and administration of NO synthase inhibitor attenuates the dilation in an identical manner (31). Interestingly, inhibition of NO production by L-NAME abolished the vasodilation elicited by low concentrations of adenosine (≤10 nM), but the dilation to the higher concentrations of adenosine (≥1 µM) was only slightly affected (Fig. 3). These results indicate that the adenosine, at lower concentrations, primarily acts on endothelial cells to elicit vasodilation via released NO, whereas the contribution of NO to overall vasodilation is diminished with increasing adenosine concentration. It appears that the dilation of coronary arterioles to a high concentration of adenosine is primarily through the direct relaxation of vascular smooth muscle cells.

A recent in vitro study demonstrated that adenosine receptor-mediated dilation in porcine coronary arteries was inhibited by an oxidized form of LDL (1). However, the effect of LDL on vasodilation to the natural ligand adenosine was not examined. In the present study, coronary arteriolar dilation to adenosine was inhibited by oxLDL, but not by nLDL. The vasodilation was completely restored by incubating the vessels with the NO precursor L-arginine, suggesting that oxLDL impaired the NO synthesis pathway. Interestingly, coronary arteriolar dilation to adenosine was inhibited by oxLDL and NO synthase inhibitor in an identical manner, thus further supporting the idea that endothelium-dependent NO-mediated dilation, rather than smooth muscle function, is selectively impaired by oxLDL. This may explain the findings in previous in vivo studies that showed that coronary microvascular relaxation to adenosine, at a concentration (1–2.2 mg·kg<sup>-1</sup>·min<sup>-1</sup>) that produced near-maximal dilation (i.e., direct smooth muscle effect), was not altered in atherosclerotic monkeys (6) and in hypercholesterolemic humans (51), but the dilation to a lower concentration (0.14 mg·kg<sup>-1</sup>·min<sup>-1</sup>) of adenosine (e.g., NO-dependent effect) was significantly reduced in hypercholesterolemic humans (21, 25). Collectively, these results are consistent with the idea that the selective impairment of endothelium-dependent vasodilation by oxLDL may result from the deficiency of NO release.

In addition to adenosine, an increase in interstitial osmolarity also has been implicated in the metabolic control of coronary blood flow (2). In vivo (58) and in vitro (27) studies indicate that hyposmotic solutions elicit relaxation of the coronary microvasculature. However, in contrast with adenosine, hyposmolarity-induced dilation of isolated coronary arterioles was recently shown to be mediated by an endothelium-dependent mechanism that was independent of NO, prostaglandins, or cytochrome P-450 metabolites (27). In a consistent manner, our present findings demonstrated that this vasodilatory response was inhibited by intraluminal KCl but not by L-NAME, suggesting that endothelial hyperpolarization independent of NO production mediates coronary arteriolar dilation to hyposmolarity. In contrast with flow-mediated dilation, we (27) have previously shown that the endothelial hyperpolarization involved in hyposmolarity-induced dilation may be mediated by the opening of endothelial K<sub>ATP</sub> channels rather than K<sub>Ca</sub> channels. However, incubation of coronary arterioles with oxLDL for 60 min did not affect the vasodilatory response to hyposmolarity, which does not support the idea that oxLDL affects endothelial hyperpolarization. On the other hand, the findings suggest that oxLDL specifically impairs NO-mediated vasodilation because in
these same oxLDL-treated vessels flow-induced dilation was inhibited.

Two other pathways that are involved in mediating endothelium-dependent vasodilation are cytochrome P-450 monooxygenase and cyclooxygenase. A factor released on activation of cytochrome P-450 monooxygenase in endothelial cells has been considered an EDHF that subsequently diffuses to the underlying smooth muscle, producing hyperpolarization and thus vasorelaxation. However, the direct effect of oxLDL on this putative vasodilator has not been examined. In our study, coronary arteriolar dilation to bradykinin was not affected by the NO synthase inhibitor L-NAME but was inhibited by endothelial denudation and by cytochrome P-450 monooxygenase inhibitor clotrimazole, implicating a role for endothelial cytochrome P-450 monooxygenase. In contrast, oxLDL did not affect bradykinin-induced vasodilation, suggesting that this lipoprotein does not alter EDHF-mediated dilation of coronary arterioles. Interestingly, conflicting results exist in the literature for the effect of lysophosphatidylcholine, a component of oxLDL, on EDHF-mediated dilation of porcine coronary arterioles. Interestingly, conflicting results exist in the literature for the effect of lysophosphatidylcholine, a component of oxLDL, on EDHF-mediated dilation of porcine coronary arterioles. In fact, oxLDL did not affect bradykinin-induced vasodilation in opened ring preparations contracted with prostaglandin F2α (15, 35). This discrepancy may be related to tissue preparation or preconstrictor agents used to establish vessel tone. However, our preliminary observations with pressurized microvessels exhibiting spontaneous tone show that lysophosphatidylcholine does not affect coronary arteriolar dilation to bradykinin. Nonetheless, our results suggest that cytochrome P-450 monooxygenase-mediated dilation to bradykinin in porcine coronary arterioles is not influenced by oxLDL.

Arachidonic acid, a metabolite of cyclooxygenase activation, has been shown to mediate endothelium-dependent vasodilation in the coronary circulation (32, 53). However, the effect of oxLDL on the vasodilation mediated by the activation of cyclooxygenase pathway has not been investigated. In the present study, dilation of coronary arterioles to arachidonic acid was almost completely blocked by removal of endothelium and by indomethacin, suggesting that activation of endothelial cyclooxygenase mediates this vasodilatory response. The release of a cyclooxygenase metabolite from the endothelium may elicit coronary arteriolar dilation by opening smooth muscle K$_{Ca}$ channels because extraluminal incubation with iberiotoxin nearly abolished this vasodilation (Fig. 1). On the other hand, neither L-NAME nor oxLDL affected the arteriolar dilation to arachidonic acid, indicating that oxLDL does not affect this NO-independent vasodilation. These findings further support the contention that oxLDL specifically affects the NO-related vasoregulation in coronary microvessels.

The cellular mechanism responsible for the specific impairment of NO-mediated vasodilation remains unclear. However, the ability of L-arginine to restore the impaired NO-mediated vasodilation is consistent with results reported in our previous study (23) and suggests that a reduction in L-arginine availability for NO synthase is involved in the inhibitory effect of oxLDL. It is likely that the initial generation of superoxide anions after oxLDL insult is responsible for the reduction of intracellular L-arginine, as suggested in our previous study (23). These oxygen-derived free radicals inactivate NO (20) and contribute to the observed endothelium/NO dysfunction associated with hypercholesterolemia (44) and atherosclerosis (59). Furthermore, reduced levels of L-arginine have been shown to enhance the generation of superoxide anions from NO synthase by uncoupling the L-arginine/NO pathway (24, 48). This perturbation could further decrease functional levels of NO through direct inactivation of the synthesized NO by superoxide. In this regard, supplementation of exogenous L-arginine seems to be an effective way to restore impaired NO-mediated dilation associated with oxLDL, hyperlipidemia, and/or atherosclerosis as shown in our in vitro studies (23, 33) and in other intact animal preparations, including humans (4, 11, 13, 54).

Our direct assessment of the effect of oxLDL on coronary microvascular function might provide further insight to the dysfunction of coronary flow regulation during hypercholesterolemia or atherosclerosis. Specifically, previous in vivo studies suggest that impaired NO-mediated dilation of coronary resistance vessels under these pathophysiological conditions could alter the coupling between coronary blood flow and myocardial metabolic demands (50, 60). The present study supports this implication and extends it to propose that NO-mediated dilation of coronary arterioles is specifically inhibited by a putative risk factor for atherosclerosis, oxLDL. As a result, it is possible, under conditions of increased metabolic activity, that the coronary flow reserve may not be sufficient and thus impact myocardial dysfunction. On the contrary, it should be emphasized that other factors associated with an increase in metabolic activity may act as compensatory mechanisms during atherogenesis to maintain coronary blood flow. This consideration is evident by our data showing that NO-independent vasodilations to hyperosmolality, bradykinin, and arachidonic acid were not affected by oxLDL. Although it is unclear whether these NO-independent mechanisms can be affected by a higher concentration and/or longer incubation of oxLDL, our study suggests that the NO-mediated pathway is more susceptible to the insult of oxLDL. Interestingly, vasodilations mediated by hyperpolarizing mechanisms have been shown to be preserved in isolated coronary arterioles from patients with coronary risk factors, including hypercholesterolemia (37), and to be even enhanced in isolated carotid arteries from hypercholesterolemic rabbits (40, 41).

In summary, our present study documents that endothelium-dependent, NO-mediated dilation to both flow and adenosine in coronary microvessels is impaired by oxLDL. The impairment is specific for endothelial NO synthesis/release because vasodilations to the endothelium-independent vasodilator sodium nitroprusside and...
to activators of cytochrome P-450 monoxygenase (bradykinin) and cyclooxygenase (arachidonic acid) were not altered by oxLDL. In addition, it appears that oxLDL does not affect the vasodilation mediated by the endothelial hyperpolarization pathway.

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