Atherosclerosis or Lipoprotein-Induced Endothelial Dysfunction
Potential Mechanisms Underlying Reduction in EDRF/Nitric Oxide Activity

N.A. Flavahan, PhD

Activation of endothelial cells by physical, chemical, and hormonal stimuli can result in the release of a number of vasoactive mediators.1 Under physiological conditions, mediator release appears to be balanced in favor of inhibitory factors such as endothelium-derived relaxing factor (EDRF, identified as nitric oxide) and prostacyclin (PGI2) (Figure 1).1-3 EDRF-NO and PG12 have important protective actions in the vascular wall: They are potent inhibitors of smooth muscle contraction and smooth muscle proliferation,4-6 and they also inhibit platelet aggregation, stimulate platelet disaggregation, and inhibit platelet or monocyte adhesion to the endothelial surface.2,5,6 The endothelial cells located at sites that are prone to atherosclerosis appear to be morphologically and functionally different from normal endothelial cells.7-10 These endothelial cells have a diminished protective role in the blood vessel wall and may actively promote the atherosclerotic process.9 Numerous studies have demonstrated that a dysfunction in the release of EDRF-NO appears to occur at an early stage in the atherosclerotic process in animals and humans.11-23 This impairment in EDRF-NO release may be associated with an increased release of endothelium-derived contracting factors (EDCFs),18,24 which are functional and chemical antagonists of EDRF-NO1,25-29 (Figure 1). The shift in the balance of endothelial mediators might contribute in part to the diminution in the protective role of the endothelium and could predispose the blood vessel to vasospasm or to a further progression of the disease process.

Previous studies have demonstrated that the impairment in endothelial dilator activity occurs in atherosclerotic or hypercholesterolemic blood vessels reflects an impairment in the ability of the cells to respond to specific stimuli. Thus, in a number of different arterial preparations (Table 1), hypercholesterolemia did not affect the endothelium-dependent relaxations evoked by certain stimuli, e.g., A23187, but caused marked impairment of responses evoked by other agonists, e.g., serotonin or acetylcholine.17-19,21-23,30 (Table 1). The endothelium-dependent relaxation evoked by each of the stimuli, whether or not they were impaired during hypercholesterolemia, appears to be mediated by EDRF-NO. For example, in rabbit aorta, the endothelium-dependent relaxations evoked by A23187 or acetylcholine were unaffected by inhibition of cyclooxygenase but were both highly sensitive to inhibition by hemoglobin, which binds EDRF-NO; by methylene blue, which inhibits guanyl cyclase; or by l-arginine analogues, which inhibit the synthesis of EDRF-NO.30,31 The endothelium-dependent relaxations evoked by acetylcholine and A23187 in human coronary arteries and in rabbit aorta were each associated with increases in cyclic GMP in the vascular smooth muscle, mediated presumably by EDRF-NO.17 Although hypercholesterolemia reduced the increase in cyclic GMP and the endothelium-dependent relaxation evoked by acetylcholine, there was no change in the response produced by A2318717 (Table 1). The impairment in endothelial responses was not accompanied by any reduction in the ability of the vascular smooth muscle to respond to nitrovasodilators, which may be considered as exogenous mimics of EDRF-NO (Table 1). Therefore, these results would suggest that the endothelial dysfunction does not result from an inability of the smooth muscle to respond to EDRF-NO but reflects an impaired release of the mediator from the endothelial cells. This has been confirmed using bioassay techniques.18,30,32 Moreover, the selective nature of the dysfunction would suggest that it is not caused by a nonspecific impairment in the ability of the cells to synthesize/release EDRF-NO.

In contrast to the results obtained in rabbit aorta and human coronary arteries, different endothelial stimuli may cause the release of distinct endothelium-derived dilators in porcine coronary arteries. Richard et al13 have proposed that the endothelium-dependent relaxations evoked by serotonin in this blood vessel are mediated by EDRF-NO, whereas those produced by bradykinin are mediated by a different factor. If this occurred, then the selective impairment in endothelium-dependent responses caused by hypercholesterolemia in this blood vessel (Table 1) could result from a nonspecific reduction in the synthesis/release of EDRF-NO. The proposal by Richard et al was based on the apparent inability of inhibitors of EDRF-NO to reduce...
the endothelium-dependent relaxations evoked by bradykinin. However, the endothelium-dependent relaxations evoked by equipotent concentrations (producing 80% relaxation) of bradykinin, serotonin, UK 14,304, thrombin, ADP, or A23187 were virtually abolished by hemoglobin or by methylene blue34 (Figure 2). These results suggest that the primary mediator that is released after activation of porcine coronary endothelial cells is EDRF-NO. High concentrations of certain agonists including A23187, bradykinin, or thrombin may activate the endothelium to release a secondary dilator that is distinct from EDRF-NO.33,34 Certainly, the endothelium-dependent relaxations evoked by high concentrations of these agonists were resistant to inhibitors of EDRF-NO34 and may be associated with hyperpolarization of vascular smooth muscle rather than activation of guanyl cyclase.35,36 Thus, this secondary, nonprostanoid mediator may be EDHF.

Therefore, the primary mediator that is released by each of the endothelial stimuli, including those not affected by the hypercholesterolemic process, is EDRF-NO. This indicates that the endothelial dysfunction caused by hypercholesterolemia does not result from a nonspecific impairment in the synthesis or release of EDRF-NO. In each blood vessel (Table 1), hypercholesterolemia inhibited endothelium-dependent relaxations evoked by receptor-dependent stimuli but did not affect the responses evoked by the receptor-independent stimulus A23187. Bossaller et al17 suggested that hypercholesterolemia might act selectively to impair endothelial muscarinic receptor function. More recent studies suggest that the dysfunction may be expressed at a site distal to endothelial receptors, perhaps at the level of endothelial G proteins.

**Pertussis Toxin–Sensitive G Proteins and Atherosclerosis-Induced Endothelial Dysfunction**

The signal transduction processes of a large number of membrane-bound receptors involve G proteins that couple the receptors to a variety of subcellular effector systems.37-40 A number of distinct G proteins have been identified, including Gs protein, which inhibits adenyl cyclase and activates K+ channels; Gi protein, which can activate adenyl cyclase and calcium channels; and Gq protein, which activates phospholipase C. Certain G proteins can be ADP-ribosylated by pertussis toxin, resulting in an irreversible inhibition of their function41 (Figure 3).

Direct activation of endothelial G proteins by fluoride ions causes the release of EDRF-NO and initiates endothelium-dependent relaxation.42-44 This response was inhibited partly by pertussis toxin, indicating that at least two types of G protein may be involved, one (or more) of which is sensitive to pertussis toxin.42,43 The pertussis toxin–sensitive G protein(s) couple certain endothelial receptors to the release of EDRF, including those activated by serotonin (exogenous or derived from...
aggregating platelets), \(\alpha\)-adrenergic stimuli, thrombin, and leukotriene C\(_4\).\(^{34,43}\) This endothelial pertussis toxin-sensitive G protein has been identified as a \(G_i\) protein.\(^{45}\) Although activation of this \(G_i\) protein inhibits adenylyl cyclase in endothelial cells,\(^{45}\) it is not known whether this action initiates the release of endothelial mediators. The \(G_i\) protein could also activate the endothelial cells by a direct action on \(K^+\) channels to cause hyperpolarization and a subsequent increase in calcium influx\(^{46}\) (Figure 3). Other endothelial receptors, including \(\beta_2\)-bradykinin receptors, are coupled to a G protein (\(G_q\) protein) that is insensitive to pertussis toxin and activates phospholipase C\(^{34,37,43,47}\) (Figure 3).

In porcine coronary arteries, endothelium-dependent relaxations are depressed during hypercholesterolemia\(^{18,23}\) (Table 1). Although the endothelium-dependent relaxations to bradykinin or A23187 were similar in coronary arteries from control and hypercholesterolemic pigs, endothelium-dependent relaxations evoked by agents that activate the pertussis toxin–sensitive G protein (e.g., serotonin, UK 14,304, aggregating platelets, thrombin) were all reduced in blood vessels from hypercholesterolemic animals\(^{23}\) (Figures 4 and 5). Because of the similarity in the inhibitory effects of pertussis toxin and hypercholesterolemia, it was considered that hypercholesterolemia might act by inhibiting the \(G_i\) protein–dependent signal transduction pathway. Indeed, although pertussis toxin inhibited the endothelium-dependent relaxations to UK 14,304, serotonin, aggregating platelets, and thrombin in normal arteries, the residual responses to these agents that remained in arteries from hypercholesterolemic animals were not affected greatly by the toxin\(^{23}\) (Figures 4 and 5). Therefore, the activity of the pertussis toxin–sensitive, \(G_i\) protein–dependent pathway appears to be reduced in hypercholesterolemic endothelial cells. Indeed, the magnitude of the \(G_i\) protein–dependent component of the response to serotonin was reduced by hypercholesterolemia, whereas the \(G_i\) protein–independent component was unchanged (Figure 6). After inactivation of the \(G_i\) protein by pertussis toxin, there were no longer any significant differences in endothelium-dependent relaxations to these stimuli in arteries from control or hypercholesterolemic animals\(^{23}\) (Figures 4 and 5). Thus, the impairment in endothelium-dependent relaxations to these stimuli caused by hypercholesterolemia can be accounted for entirely by an impairment in the \(G_i\) protein–dependent pathway in the endothelium.

The nature and the cause of the dysfunction are unknown. Because the impairment occurred to a similar extent in several receptor systems and occurred only in the pertussis toxin–sensitive component of the responses to serotonin, UK 14,304, aggregating platelets, and thrombin\(^{23}\) (Figures 4, 5, and 6), it is likely that the site of the impairment is beyond the level of the receptor. Moreover, because the endothelium-dependent relaxations evoked by a number of agonists were not affected by hypercholesterolemia, the endothelial cells were still able to generate EDRF-NO in response to other stimuli. The dysfunction may therefore occur at

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Unless stated otherwise, experiments were performed on animals fed a high-cholesterol diet.
*Data for human coronary arteries represent atherosclerotic blood vessels.
†Data from Shimokawa et al.,\(^{18}\) 1989, were obtained on arteries with “regenerated” endothelium.
the level of the G protein(s) or its subcellular effector system(s). Dysfunction of a G protein–dependent pathway would also explain the selective endothelial dysfunction that occurs in other hypercholesterolemic blood vessels (Table 1).

**G{i} Protein–Independent Mechanisms Underlying Endothelial Dysfunction**

In the early stages of the atherosclerotic process, the endothelial dysfunction appears to be limited to the pertussis toxin–sensitive, G{i} protein–dependent pathway. Later in the atherosclerotic process, dysfunction also occurs in endothelial mechanisms distinct from the G{i} protein–dependent pathway. For example, although hypercholesterolemia selectively inhibited the pertussis toxin–sensitive component of the response to serotonin in porcine coronary arteries, when a more advanced atherosclerotic lesion was imposed, the G{i} protein–independent component was also inhibited23 (Figure 6). As the disease process develops after endothelial regeneration in porcine coronary arteries, the endothelial cells initially demonstrate a dysfunction in the G{i} protein–dependent pathway.24 The endothelial cells next display a diminished reactivity to thrombin and ADP, followed later by a diminished reactivity to bradykinin.24 However, the endothelial cells still responded in a normal fashion to the receptor-independent stimulus A23187. Therefore, endothelial dysfunction associated with the early atherosclerotic process may be mediated by a progressive loss in distinct G proteins or signal transduction processes. In pigs, it is only when the disease process is well established (combination of endothelial regeneration and hypercholesterolemia) that there is any reduction in the response evoked by A23187.23 Progressive endothelial dysfunction also appears to occur in other animal models of atherosclerosis. For example, in rabbits fed a high-cholesterol diet (0.3–1% cholesterol) for 2–12 weeks, there was a marked impairment in endothelium-dependent relaxations evoked by acetylcholine, whereas endothelial responses produced by A23187 were well maintained17,22,30 (Table 1). When cholesterol feeding was continued (16 weeks to 6 months), resulting in pronounced atherosclerotic lesions, endothelium-dependent relaxations to A23187 were also impaired.30,48 However, even during advanced stages of the disease, the vascular smooth muscle still responded in a normal fashion to exogenous mimics of EDRF-NO.23 Thus, the endothelial dysfunction that occurs later in the disease process may be mediated by a nonspecific perturbation of the synthesis/release of EDRF-NO.

The synthesis of EDRF-NO involves the oxidation of a guanidino nitrogen on l-arginine, a reaction that is catalyzed by a calcium/calmodulin–sensitive enzyme NO synthase.49–52 Cooke et al53 have proposed that the decreased ability of hypercholesterolemic endothelial cells to synthesize EDRF-NO results from a diminished availability of the substrate l-arginine. This type of dysfunction would be expected to cause nonspecific impairment in endothelium-dependent responses. Cooke et al demonstrated that in vivo administration of l-arginine reversed the depression caused by hypercholesterolemia in the endothelium-dependent response to acetylcholine in rabbit isolated aorta. In this blood vessel, the endothelium-dependent relaxations evoked by acetylcholine and A23187 are both mediated by EDRF-NO, and both responses are equally sensitive to inhibition by l-arginine analogues (see above). Therefore, a nonspecific reduction in l-arginine availability would be expected to inhibit the endothelium-dependent relaxation evoked by A23187 and acetylcholine. However, in this blood vessel, hypercholesterolemia caused a selective impairment in the endothelial response to acetylcholine and did not inhibit the endothelium-dependent relaxation or the increase in cyclic
FIGURE 3. Schematic diagram of receptor signal transduction processes in the endothelial cell. AC, adenylyl cyclase; K\textsuperscript{+} ch, K\textsuperscript{+} channels; PLC, phospholipase C; \(\alpha_\text{2}\text{AR}, \text{SHT}_{1}\text{D}R, B_2\text{R}, P_2\text{YR};\) membrane-bound receptors for UK 14,304, serotonin, bradykinin, and ADP, respectively; EDRF-NO, endothelium-derived relaxing factor-nitric oxide.

GMP evoked by A23187\textsuperscript{17,22,30} Cooke et al did not analyze the influence of hypercholesterolemia on responses to A23187. However, they imposed a severe cholesterol feeding regimen (2% cholesterol) compared with previous studies using this model (1% cholesterol)\textsuperscript{17,22,30} and the resulting endothelial dysfunction may have progressed from an impairment in signal transduction to a nonspecific impairment involving a generalized reduction in EDRF-NO release. If that occurred, then one might have expected L-arginine administration to cause only a partial recovery of the endothelial dysfunction. Although this may have occurred in some animals (Figure 2 in Reference 53), the majority of animals appear to have regained a normal ability to respond to muscarinic stimulation.

The L-arginine reversal of hypercholesterolemic dysfunction may not simply reflect a restoration of the substrate for endothelium-derived nitric oxide. Indeed, so far, the reversal has been achieved only in vivo\textsuperscript{54} and has not been demonstrated in other blood vessels, including human coronary arteries.\textsuperscript{55} Furthermore, although L-arginine treatment inhibited the vascular relaxation evoked by sodium nitroprusside in control animals (threelfold decrease in sensitivity), in hypercholesterolemic animals, L-arginine treatment actually tended to increase relaxations to the nitrodiator (2.5-fold increase in sensitivity).\textsuperscript{53} This might suggest that the facilitation of endothelium-dependent relaxation caused by L-arginine in hypercholesterolemia was mediated by a nonendothelial action of the amino acid. NO synthase isozymes can be present in a variety of cell types, including vascular smooth muscle, platelets, and macrophages,\textsuperscript{52} and induction of the enzyme may occur during hypercholesterolemia. The production of NO from L-arginine by these enzymes might act to sensitize guanylyl cyclase in the smooth muscle cells, or it might act to scavenge superoxide anion released from monocytes/macrophages.

The nonspecific reduction in endothelial dilator activity that occurs late in the atherosclerotic process may result from accelerated degradation of EDRF-NO. Minor et al\textsuperscript{30} observed that hypercholesterolemia or atherosclerosis increased the release of nitrosylated compounds detected by a chemiluminescent assay from the rabbit aorta. These authors proposed that the disease process caused an upregulation of NO synthase leading to an increased production of EDRF-NO that was offset by an increased breakdown of the mediator.\textsuperscript{30} Because the synthesis of EDRF-NO was thought to be increased, Minor et al dismissed the possibility that there was any disruption of signal transduction processes or reduction in the availability of L-arginine. The assay system used by Minor et al detected nitrosylated compounds irrespective of their biological activity and was designed to measure EDRF-NO and its inactive degradative products. However, the assay system used by Minor et al does not detect simple degradative products such as NO\textsubscript{3}\textsuperscript{-}, which is formed after the interaction of nitric oxide and superoxide anion. Therefore, the increase in nitrosylated compounds that was detected in hypercholesterolemic blood vessels may not represent an upregulation of NO synthase but could have resulted from an alteration in the metabolism of EDRF-NO in hypercholesterolemic or atherosclerotic blood vessels. Therefore, their conclusions regarding endothelial receptor signal transduction processes may be invalid. Indeed, when the authors used a bioassay system to detect EDRF-NO, they observed that hypercholesterolemia inhibited the acetylcholine-induced release of EDRF-NO but did not affect the release of EDRF-NO in response to A23187. These data are consistent with an impairment in receptor signal transduction caused by hypercholesterolemia.
The release of EDRF-NO evoked by either agonist was impaired during advanced atherosclerosis.\textsuperscript{30}

**Lipoproteins and Endothelial Dysfunction**

Hypercholesterolemia is generally associated with an increase in low density lipoproteins (LDL), which are the major carriers of cholesterol in the blood. Elevated levels of LDL have been identified as a major risk factor for atherosclerosis, and the exposure of vascular cells to high concentrations of lipoproteins may initiate or accentuate the atherosclerotic process.\textsuperscript{7,9,56} Endothelial cells can modify LDL to an oxidized form (oxidized LDL) that may be more atherogenic than native LDL.\textsuperscript{57,58} Several studies have attempted to analyze the influence of LDL and oxidized LDL on the release of EDRF-NO by analyzing the influence of the lipoproteins on endothelium-dependent relaxations. However, the lipoproteins appear to have multiple effects on endothelial-smooth muscle cell interactions that complicate such physiological studies.

**Mechanisms Underlying Lipoprotein-Induced Endothelial Dysfunction**

Native lipoproteins and inhibition of endothelial mediators. Although not reported by all investigators, native LDL appears to be able to inhibit endothelium-dependent relaxation by a rapid and reversible mechanism that may result from a direct interaction between LDL and EDRF-NO.\textsuperscript{59,60} By using a bioassay system, Galle et al.\textsuperscript{61} demonstrated directly that LDL inactivates EDRF-NO. These authors proposed that EDRF-NO may be sequestered and inactivated within the hydrophobic core of the lipoprotein molecule. Conversely, several studies have failed to demonstrate any effect of native LDL on endothelium-dependent relaxations.\textsuperscript{62-64} This may reflect differences in the ability of LDL to penetrate the endothelial layer. Indeed, Jacobs et al.\textsuperscript{59} demonstrated that an inhibitory effect of LDL could be observed only if the arterial rings were exposed to norepinephrine or serotonin at concentrations that did not affect contractile tone. These two agents may act on the endothelial cells to increase the transfer of lipoproteins across the endothelial cell layer.

Endothelial cells release superoxide anions (Figure 1), which may initiate or accelerate the oxidative modification of LDL.\textsuperscript{57,58,63} Under physiological conditions, the level of superoxide anion may be low because of its interaction with EDRF-NO.\textsuperscript{66,67} However, inactivation of EDRF-NO by high concentrations of LDL will decrease the superoxide scavenging effect of EDRF-NO. This in turn may enable superoxide to initiate oxidation of LDL. Oxidized LDL could then act directly on vascular cells, including endothelial cells, to initiate or accelerate the atherosclerotic process.

Oxidized or cell-modified lipoproteins and inhibition of endothelial function. Most studies have demonstrated that oxidized LDL but not native LDL can cause slowly developing, apparently irreversible inhibition of endo-
endothelial-dependent relaxation. This inhibitory effect of oxidized LDL appears to result from an action of the lipoprotein on the endothelial cell to reduce the release of EDRF-NO. Although native LDL has also been reported to cause irreversible inhibition of endothelium-dependent responses, this action may result from the conversion of LDL to oxidized LDL by the highly oxygenated environment used in these physiological studies (endothelial cells and 95% oxygen).

As with hypercholesterolemia-induced endothelial dysfunction, the mechanism underlying the inhibitory effect of oxidized LDL on endothelial cells may involve disruption of endothelial receptor signal transduction processes. At low concentrations (<50 μg protein/ml), oxidized LDL inhibited endothelium-dependent relaxations evoked by receptor-dependent (acetylcholine) but not receptor-independent stimuli (A23187) in rabbit aorta. However, at higher concentrations (>50 μg/ml), oxidized LDL caused nonspecific inhibition of endothelium-dependent relaxations and inhibited receptor-independent responses (A23187). Therefore, in an analogous fashion to the endothelial dysfunction caused by atherosclerosis, oxidized LDL may induce endothelial dysfunction by selectively interrupting endothelial receptor signal transduction, but as the concentration of the lipoprotein is increased, the lipoprotein may then induce a more nonspecific type of dysfunction.

Tanner et al. sought to evaluate whether oxidized LDL might inhibit endothelial function in porcine coronary arteries by inhibiting the pertussis toxin–sensitive Gi protein. The concentration range analyzed by these authors was 30–300 μg/ml (it was not stated whether this referred to protein or cholesterol content). Based on their data, it would appear that the inhibitory effect of oxidized LDL was most potent against the endothelium-dependent relaxations evoked by serotonin and platelets (which are highly dependent on the pertussis toxin–sensitive Gi protein), less potent against thrombin (which is less dependent on the Gi protein), and apparently ineffective against A23187 and bradykinin (which act independently of the Gi protein). Therefore, the ability of oxidized LDL to inhibit endothelium-dependent relaxations appeared to be dependent on whether the stimuli activated the pertussis toxin–sensitive Gi protein. These results might suggest that inhibition of the Gi protein–dependent pathway might contribute at least in part to the inhibitory effects of the lipoprotein. Tanner et al. observed that oxidized LDL (100 μg/ml) was a more potent inhibitor of responses to serotonin than was pertussis toxin, and the authors concluded that the lipoprotein was not acting by inhibiting the Gi protein–dependent pathway. However, the only conclusion that can be drawn from their experiment is that oxidized LDL (100 μg/ml) is more potent than pertussis toxin at inhibiting responses evoked by serotonin.

If inhibition of the Gi protein–dependent pathway were contributing to the inhibitory effects of oxidized LDL, then one would expect the lipoprotein to markedly inhibit the magnitude of the pertussis toxin–sensitive response. However, Tanner et al. did not determine whether the Gi protein–dependent pathway was
still functional after exposure of the endothelium to the lipoprotein. In the series of experiments with pertussis toxin, Tanner et al analyzed the influence of the toxin or of the toxin plus oxidized LDL on the endothelium-dependent relaxations evoked by serotonin. To assess the functional status of the $G_i$ protein–dependent pathway, one would also need to know the effect of oxidized LDL on the control response to serotonin. The effect of oxidized LDL appears to be variable between different experiments, and it is therefore difficult to use data obtained in one set of experiments to predict the inhibitory potency of oxidized LDL in the experiments with pertussis toxin; however, if one uses the data obtained in their Figure 1, then oxidized LDL (100 μg/ml) markedly inhibited the pertussis toxin–sensitive component of the response to serotonin (by 92%, determined by analyzing the area under the concentration–effect curve for the toxin-sensitive component) (Figure 7). Therefore, these results would suggest that the pertussis toxin–sensitive pathway is highly sensitive to the inhibitory effects of oxidized LDL and would explain why the $G_i$ protein–dependent endothelial responses appear to be inhibited more by oxidized LDL.

At this concentration, oxidized LDL also inhibited the pertussis toxin–insensitive component of the response (by 57%, determined by analyzing the area under the concentration–effect curve for the toxin-insensitive component). Considering the concentration of oxidized LDL that was used, this may not be too surprising. Although Kugiyama et al. demonstrated that only low concentrations of oxidized LDL (≤50 μg/ml) were selective for receptor-dependent relaxations, Tanner et al. used a relatively high concentration of oxidized LDL (100 μg/ml). At this concentration, oxidized LDL may be inhibiting signal transduction pathways in addition to the $G_i$ protein–dependent pathway, and it is possible that lower concentrations of oxidized LDL might be more selective at inhibiting the $G_i$ protein–dependent pathway.

The inhibitory effect of oxidized LDL on endothelium-dependent relaxation is associated with the lipid fraction of the molecule. During the oxidative modification of LDL, lecithin (phosphatidylcholine) is converted to lysolecithin (lysophosphatidylcholine), causing lysolecithin to be present in high concentrations on oxidized but not on native LDL. Fractionation of the individual lipids revealed that lysolecithin derived from oxidized LDL inhibited endothelium-dependent relaxation in rabbit aorta, whereas the remaining lipids including oxidized free fatty acids, sphingomyelin, and other phospholipids had no effect. Moreover, the effects of oxidized LDL could be mimicked by using

**Figure 6.** Graphs show effects of hypercholesterolemia (hyperchol.) or atherosclerosis (atheroscl.) on the magnitude of the pertussis toxin–sensitive component (upper panel) and the toxin-insensitive component (lower panel) of the response to serotonin in porcine coronary arteries. At each concentration of serotonin, the toxin-sensitive component of the response was calculated by subtracting the response obtained in toxin-treated arterial rings from that obtained in untreated rings. In a similar manner, the toxin-insensitive component reflects the response obtained to serotonin after treatment of the arterial rings with pertussis toxin. Atherosclerosis was induced by a combination of high cholesterol diet and "endothelial regeneration" (data derived from Reference 23).
The inhibitory effect of oxidized LDL in l-arginine-treated blood vessels cannot be compared with its action in untreated tissues because the authors did not present the effects of l-arginine treatment on the control response to serotonin. Only a small potentiating effect of l-arginine on control responses to serotonin would be needed to offset the observed effect of l-arginine on tissues treated with oxidized LDL. Furthermore, the authors did not rule out additional effects of these interventions by determining whether the combination of l-arginine and oxidized LDL affected the sensitivity of the vascular segments to other endothelium-dependent or independent dilators.68 As with hypercholesterolemia, oxidized LDL may induce a NO synthase enzyme that could convert arginine to NO.

**Cholesterol, Lysolipids, and G Protein Signaling**

The disruption of receptor signal transduction processes caused by hypercholesterolemia may result from an increased incorporation of lipids into endothelial cells. Lipoprotein-derived lipids, e.g., lyssolecithin or cholesterol, may alter the physical characteristics of the plasma membrane (e.g., microviscosity) and so disrupt interactions between signal-transducing proteins.71,72 Membrane-bound receptors as well as many G protein effector systems (e.g., ion channels, adenylyl cyclase) are integral proteins that span the plasma membrane and whose activity may be altered by changes in membrane lipids.73–77 Although G proteins lack hydrophobic, membrane-spanning regions, they undergo post-translation modification with lipid moieties that enable them to associate with the membrane.77 Indeed, G protein γ-subunits, which are required for receptor–G protein coupling, are modified by attachment of a farnesyl or geranylgeranyl moiety, both of which are products of cholesterol biosynthesis.77,78 The availability of these moieties may therefore be reduced in hypercholesterolemic endothelial cells because of a reduction in cholesterol biosynthesis caused by lipoprotein-derived cholesterol.78 A reduction in lipid modification of G protein subunits would decrease their association with the membrane and inhibit G protein–dependent responses (e.g., Reference 79). The inhibitory effect of lyssolecithin on the G_{i}-protein–dependent pathway suggests that endothelial receptor signal transduction processes can also be modified independently of cellular cholesterol levels. Lyssolecithin may be metabolized to diacylglycerol, causing activation of protein kinase C.80 This enzyme can phosphorylate the G_{i} protein, causing inhibition of its function.81,82 In endothelial cells, activation of protein kinase C by phorbol esters selectively inhibited the pertussis toxin–sensitive, G_{i} protein–dependent pathway.83

**Atherosclerosis, Lipoproteins, and Endothelial Dysfunction**

The endothelial dysfunction associated with hypercholesterolemia or atherosclerosis and that produced by oxidized LDL appear to be similar. Previous studies have suggested that an alteration in endothelial G proteins or G protein–dependent pathways may explain the dysfunction associated with atherosclerosis23,24,45 and also that induced by oxidized lipoproteins or lipoprotein-derived lipids.62,69 In contrast, a decreased
availability of L-arginine has now been proposed to explain the inhibitory influence of lipoproteins and of atherosclerosis on endothelium-dependent relaxation. Endothelial dysfunction appears to occur in distinct phases, and the underlying mechanisms may be determined by the stage of the disease process or the concentration of oxidized LDL. Early in the disease process or after low concentrations of oxidized LDL, endothelial dysfunction may be caused by a selective impairment in certain G proteins or G protein-dependent pathways (Figure 8). As the disease process progresses or as the concentration of oxidized LDL is increased, the dysfunction may spread to other signal transduction processes in the endothelial cell and may eventually inhibit the release of EDRF-NO by a nonspecific action, e.g., to decrease the availability of L-arginine (Figure 8). This latter type of nonspecific inhibitory effect could also result from a reduced sensitivity of the vascular smooth muscle to EDRF-NO or from an increased breakdown of EDRF-NO.

References


Figure 8. Schematic diagram of proposed mechanisms of endothelial dysfunction associated with atherosclerosis and with oxidized low density lipoprotein (LDL).


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N A Flavahan

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