Restoration of Endothelium-dependent Relaxation by Dietary Treatment of Atherosclerosis

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Abstract

Atherosclerosis results in impaired relaxation to acetylcholine, thrombin, and the calcium ionophore A23187, all agents that require the presence of endothelium. We now report that dietary treatment of atherosclerosis in monkeys not only produces morphological improvement of the atherosclerotic lesion but restores endothelium-dependent vascular relaxation to normal. Because the intima remains thickened after regression of atherosclerosis, these studies suggest that intimal thickening which is present in both atherosclerotic vessels and after regression of atherosclerosis does not prevent the endothelium-derived relaxing factor from reaching the underlying vascular smooth muscle.

Introduction

Atherosclerosis produces vascular occlusion and also alters vascular responses to a variety of neurohumoral stimuli. Patients with atherosclerosis are prone to the development of coronary vasospasm, particularly at sites of coronary stenoses (1). In animals, cholesterol feeding produces augmented constriction to various neurohumoral agents both in vivo (2, 3) and in vitro (4).

Recent observations regarding the importance of the endothelium in modulating the reactivity of vascular smooth muscle have provided a potential mechanism that might explain in part why atherosclerotic vessels exhibit altered responses to various stimuli. In 1980, Furchgott and Zawadski found that acetylcholine produces vascular relaxation only if the endothelium is present (5). Subsequently, it has been shown that a variety of agents produce vascular relaxation by stimulating the release of an endothelium-derived relaxing factor (EDRF).[1] This factor diffuses to the underlying vascular smooth muscle and is thought to produce vascular relaxation by activating guanylate cyclase, increasing intracellular cyclic GMP concentrations, and decreasing cytoplasmic levels of calcium (6).

Recent studies in cholesterol-fed rabbits and in monkeys with diet-induced atherosclerosis have shown that responses to endothelium-dependent agents are abnormal (7–10). We have shown that vessels of monkeys with atherosclerosis demonstrate impaired responses to acetylcholine, thrombin, and the calcium ionophore A23187, but relax normally in response to the endothelium-independent agent nitroglycerin (10, 11).

In primate models of atherosclerosis, changing the dietary intake of cholesterol to normal levels results in regression of the atherosclerotic lesion (12, 13). Regression of atherosclerosis is associated with resorption of lipids from the vessel wall (14). This process results in a reduction in the intimal thickness by ~ 50% (13), but fibrosis develops within the intima (12). Inflammatory cells that are observed in the intima of atherosclerotic vessels are nearly absent after 18 mo of a regression diet. Despite the morphologic evidence of improvement after dietary treatment of atherosclerosis, the response to pharmacologic vasodilatation, which is impaired in the coronary and hindlimb beds of atherosclerotic animals, fails to improve, probably because of vascular fibrosis (13).

One explanation for the impairment of endothelium-dependent vascular relaxation observed in atherosclerotic vessels is that intimal thickening in atherosclerosis may provide a barrier to the diffusion of EDRF simply by increasing the distance between the endothelium and the underlying vascular smooth muscle. Such a barrier might exist by simply increasing the diffusion distance for EDRF. If this is so it might be expected that regression of atherosclerosis would be associated with only partial restoration of responses to acetylcholine because the intima remains substantially thickened after atherosclerosis regression. Alternatively, atherosclerosis may be associated with impairment of production of EDRF, or substances within the atherosclerotic intima may either inactivate EDRF or prevent its diffusion to underlying vascular smooth muscle. Under these latter circumstances, resorption of lipid and removal of inflammatory cells from the intima, two processes that are associated with regression, might restore endothelium-dependent vascular relaxation to normal. Thus, we have performed studies to test the hypothesis that dietary treatment of atherosclerosis improves endothelium-dependent vascular relaxation.

Methods

Iliac arteries from three groups of cynomolgus monkeys were studied. One group (n = 11) was fed standard monkey chow (normal monkeys).

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1. *Abbreviation used in this paper:* EDRF, endothelium-derived relaxing factor.

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Table I. Cholesterol Levels, Intimal Areas, and Vascular Responses in Normal, Atherosclerosis, and Regression Monkeys

Group	Cholesterol level	Intimal area	Ach responses		Thrombin responses		Nitroglycerin responses	
			Peak % relaxation	ED_{50}	0.1	1.0	Peak % relaxation	ED_{50}
	ng/dl	*mm²*		*μM*	*U/ml*	*U/ml*		
Control (n = 11)	96±6	< 0.1	72±8	0.4±0.1	12±4	38±7	99±1	22±10
Atherosclerotic (n = 10)	564±26*	1.8±0.3*	35±10	0.7±0.2	5±3*	12±7*	99±1	63±24
	599±39‡	0.7±0.2*,‖	77±9	0.3±0.1	18±7‖	30±9‖	97±1	17±7
Regression (n = 9)	119±7§							

Ach, Acetylcholine. * $P < 0.05$ vs. control. ‡ Cholesterol level on atherogenic diet. § Cholesterol level on regression diet. ‖ $P < 0.05$ atherosclerotic vs. regression.

The plasma cholesterol level in these animals averaged 96±6 (SE) mg/dl. A second group (n = 9) was fed an atherogenic diet containing 0.7% cholesterol and 40% fat for 18 mo (atherosclerotic monkeys). This diet increased the plasma cholesterol to 560±26 mg/dl. A third group was fed this atherogenic diet for 18 mo, during which time the plasma cholesterol averaged 599±39 mg/dl, and was subsequently fed standard monkey chow for 18–20 mo (regression group, n = 9). When the regression group was switched from the atherogenic diet to standard monkey chow, the serum cholesterol became normal within 1 mo and averaged 119±7 mg/dl for the duration of the experiment. Four animals in the atherosclerotic group and four in the normal group have been included in a previous report (10).

Ring segments of iliac arteries were studied in vitro at 38°C in a physiologic saline solution equilibrated with 95% oxygen and 5% carbon dioxide. All vessels were studied in the presence of 10^{-7} M propranolol to prevent activation of beta adrenergic receptors. The resting length of each segment was adjusted so that tension development to 100 mM KCl was optimized. After preconstriction with $PGF_{2\alpha}$ (1–3 μM), we examined responses to acetylcholine (10^{-9}–10^{-4} M), nitroglycerin (10^{-9}–10^{-6} M), and thrombin (0.1 and 1.0 U/ml).

After each study, the vessels were fixed in glutaraldehyde, and the presence of endothelium was confirmed by scanning electron microscopy. Paraffin-embedded transverse sections from the contralateral iliac artery were stained with Verhoeff–Van Gieson and projected at 60×. The transverse intimal area was digitized as described previously (13).

Statistical analysis. Data are presented as the mean±standard error of the mean. Comparisons of plasma cholesterol levels, vessel intimal areas, and vessel response to each agonist were made between normal, atherosclerotic, and regression groups using unpaired Student's t tests with a Bonferonni correction for multiple comparisons. A p value < 0.05 was considered significant.

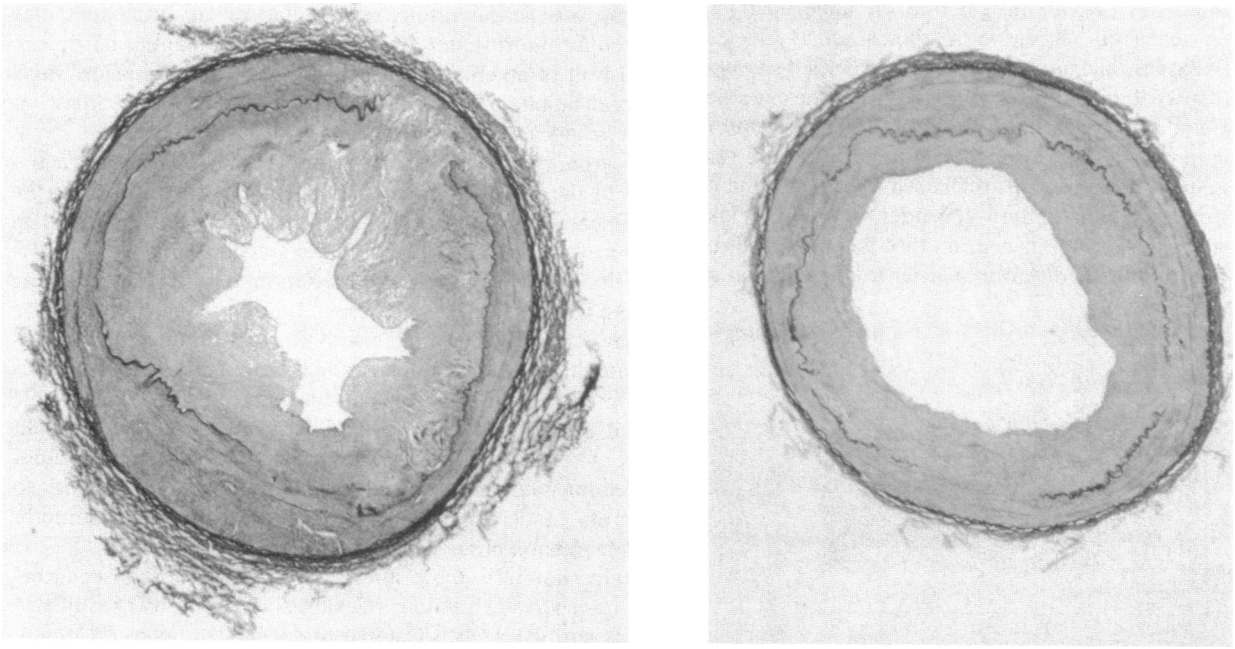

Figure 1. Histologic sections of atherosclerotic (*left*) and regression (*right*) iliac arteries stained with Verhoeff–Van Gieson (× 24). Atherosclerosis was produced by feeding cynomolgus monkeys a 0.7% cholesterol, 40% fat diet for 18 mo. Regression of atherosclerosis was accomplished by feeding the atherogenic diet for 18 mo and a standard monkey diet for the subsequent 18 mo. There is marked intimal thickening in the atherosclerotic vessel. The numerous vacuoles present in the intima of the atherosclerotic vessel are sites of lipid deposition within foam cells. The intima of the regression vessel contains substantial collagen, although foam cells and visible lipid are no longer present. The intimal area of the regression vessel is reduced compared with the atherosclerotic vessel but is greater than that of normal vessels.

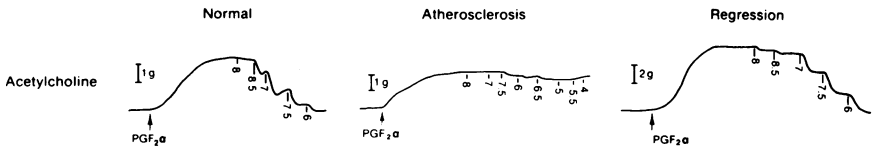

Figure 2. Responses to acetylcholine in iliac arteries obtained from normal monkeys, monkeys with diet-induced atherosclerosis, and monkeys after dietary treatment of atherosclerosis. Vessels were studied in the presence of propranolol (0.1 μM) and preconstricted with a ED_{30} to ED_{50} concentration of $PGF_{2\alpha}$. Acetylcholine was added in increasing concentrations (indicated as the negative log molar below each recording). Responses were allowed to stabilize before additional acetylcholine was added. Atherosclerotic vessels relaxed minimally to acetylcholine. After regression of atherosclerosis, responses to acetylcholine were restored to normal.

Results

The intimal area of normal vessels averaged < 0.1 mm^2. In atherosclerotic vessels the intimal area was markedly increased (Table I). Dietary treatment of atherosclerosis reduced the iliac intimal area by ~ 60% (Table I). Foam cells and inflammatory cells were prominent in atherosclerotic vessels but were not observed in regression vessels (Fig. 1).

Acetylcholine produced relaxation averaging 72±8% of the preconstricted tension of vessels from normal monkeys. In atherosclerotic vessels relaxation to acetylcholine was attenuated by more the one-half (Table I, Figs. 2 and 3). Relaxation to acetylcholine in vessels removed from regression animals was similar to that of normal vessels.

Responses to thrombin were greatly reduced in atherosclerotic vessels and were restored to normal after regression of atherosclerosis (see Fig. 4 and Table I).

Responses to nitroglycerin were not different between control, atherosclerotic, and regression animals (Table I).

Discussion

These findings may provide insight into the abnormality of endothelium-dependent relaxation in atherosclerosis. One explanation is that the thickened intima of atherosclerotic vessels provides a barrier to the diffusion of EDRF from the endothelium to the underlying vascular smooth muscle. A second is that production of EDRF is impaired in atherosclerotic vessels. Because the intima remains thickened after regression of atherosclerosis, yet endothelium-dependent vascular relaxation is restored to normal, it appears that the thickened intima does not provide a "distance" barrier to the diffusion of EDRF. It is conceivable that the lipids or oxygen-drived free radicals liberated from inflammatory cells present in the intima of atherosclerotic vessels prevent the diffusion of EDRF to adjacent smooth muscle by either binding or destroying this substance during transit (15). Thus, the intima of atherosclerotic vessels may not produce a distance barrier, but nonetheless could produce a functional barrier to diffusion of EDRF. In preliminary experiments, we have found that the production of EDRF is reduced in the thoracic aortas of rabbits with diet-induced atherosclerosis (16).

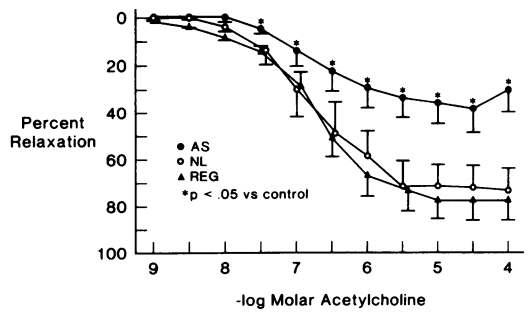

Figure 3. Average responses to acetylcholine of iliac arteries of normal (NL), atherosclerotic (AS), and regression (REG) monkeys. Values are mean±SE, * $P < 0.05$ vs. atherosclerotic. Responses to acetylcholine were reduced by approximately one-half in atherosclerotic vessels, and were restored to normal by dietary treatment of atherosclerosis.

Morphological evidence of regression of atherosclerosis has been documented in primates with diet-induced atherosclerosis (12, 13), but less frequently in other animal models of atherosclerosis or in humans (17). Regression in the monkey is most likely when lesions contain large quantities of foam cells and intracellular lipids. When the atherosclerotic lesion contains predominantly fibrotic plaque without a large amount of intracellular lipid and foam cells, unequivocal decreases in lesion size are less likely despite improvement or even correction of serum lipids (18).

In contrast to the concept that anatomic regression may be limited by the presence of fibrosis in the intima of atherosclerotic vessels, our results suggest that dietary treatment may produce improvement in functional abnormalities of the vessel wall in atherosclerosis. This "functional regression" may occur despite only a modest decrease in intimal thickness, or gross appearance of the lesion.

Abnormal modulation of vascular smooth muscle reactivity by the endothelium in atherosclerosis may contribute to the clinical syndrome of vascular spasm that is encountered in some patients. The concept that regression may restore endothelium-dependent vascular relaxation to normal by removing lipid from the vessel wall despite limited change in lesion size may explain why patients with coronary artery spasm associated with coronary stenoses may have striking spontaneous improvement in their anginal syndrome (19). It is conceivable that dietary changes or alterations in medical therapy might promote resorption of lipid from the vessel wall, restore endothelium modulation of vascular smooth muscle reactivity to normal, and result in resolution of the abnormal vasomotor phenomenon observed in these patients.

In summary, these studies show that abnormal endothelium-dependent vascular relaxation which is observed in atherosclerotic vessels is restored to normal by dietary treatment of atherosclerosis. This restoration of vascular responses was associated with marked resorption of lipids from the vessel wall, although the thickness of the intima remained substantially increased compared with normal vessels. These results may have important implications regarding the pathogenesis and treatment of abnormal vasoconstrictor phenomenon encountered in patients with atherosclerosis.

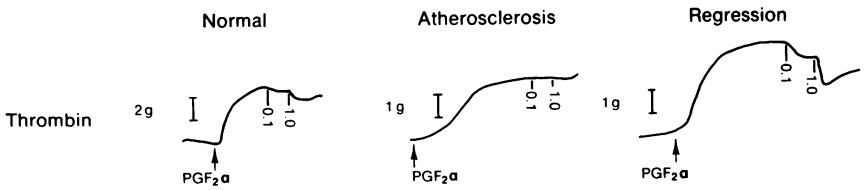

Figure 4. Responses to thrombin in iliac arteries of normal, atherosclerotic, and regression monkeys. After preconstriction with $PGF_{2\alpha}$, thrombin (0.1 and 1.0 U/ml) produced concentration-dependent relaxation in normal vessels. Removal of the endothelium abolished this response (data not shown). Responses to thrombin were markedly blunted in atherosclerotic vessels. After regression of atherosclerosis, responses to thrombin were restored to normal.

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