Dietary Treatment of Atherosclerosis Abolishes Hyperresponsiveness to Serotonin: Implications for Vasospasm

Donald D. Heistad, Allyn L. Mark, Melvin L. Marcus, Donald J. Piegos, and Mark L. Armstrong

Dietary treatment of atherosclerosis in primates results in morphologic evidence for regression of lesions with reduction in lesion size and a marked reduction in intimal lipids. Vasodilator responses remain markedly impaired, however, after dietary treatment of atherosclerosis, especially in the limb. It is likely that vascular fibrosis, which occurs during regression of atherosclerosis, limits hemodynamic improvement.

An important concept which has emerged in the last few years is that atherosclerosis predisposes to vasospasm in addition to limiting maximal vasodilator responses. Serotonin, which is present in substantial concentrations in platelets and may be released when platelets aggregate at atherosclerotic lesions, produces marked constriction in atherosclerotic vessels. Thus, serotonin has been implicated as a possible mediator in the pathogenesis of vasospasm. Increased cholesterol content of membranes, an increase in the number of serotonin receptors, and alteration of endothelium-dependent vascular responses are potential mechanisms by which atherosclerosis potentiates vasoconstrictor responses to serotonin.

The purpose of this study was to determine whether dietary treatment of atherosclerosis affects hyperresponsiveness to serotonin. It was hypothesized that because mechanisms by which atherosclerosis impairs vasodilatation and potentiates vasoconstrictor responses to serotonin probably differ, hemodynamic consequences of regression of atherosclerosis also may differ in relation to limitation of maximal vasodilator responses and hyperresponsiveness to serotonin. Thus, the present study examined the possibility that dietary treatment of atherosclerosis might reduce hyperresponsiveness to serotonin despite failure to improve maximal vasodilator responses.

Materials and Methods

Three groups of male Malaysian cynomolgus monkeys were studied: normal, atherosclerotic, and regression. Eight normal monkeys (weight 5.8 ± 0.2 kg, mean ± SEM) were fed commercial laboratory chow.
Hemodynamic Studies

The monkeys were sedated with ketamine hydrochloride (10 mg/kg i.m.) and then anesthetized with α-chloralose (100 mg/kg i.v.). Supplemental doses of chloralose were given as needed. Catheters (PE-90) were placed in a brachial vein for administration of drugs and in a brachial artery for monitoring blood pressure and blood gases. A tracheotomy was performed, and the animals were intubated and ventilated with room air and supplemental oxygen using a respirator. Gallamine triethiodide (4 mg/kg i.v.) was administered for skeletal muscle paralysis and heparin sodium (500 U/kg i.v.) for anticoagulation.

Through a laparotomy, the bifurcation of the abdominal aorta and the proximal left iliac artery was exposed. The left dorsal pedal artery was exposed, and a PE-50 catheter was inserted retrogradely to measure pressure. The left hypogastric artery was cannulated retrogradely with a PE-90 catheter to record pressure in the iliac artery. For perfusion of the hind limb, the abdominal aorta was cannulated retrogradely, and blood was pumped through a cannula placed anterogradely in the proximal left iliac artery. The limb was perfused with pulsatile pressure (about 30 mm Hg pulse pressure). Blood flow to the limb was adjusted prior to interventions so that iliac perfusion pressure was similar to systemic arterial pressure. In some atherosclerotic and regression monkeys, initial blood flow to the limb was considerably lower than in normal monkeys.

Maximal perfusion pressure and pressure in the dorsal pedal artery were measured during bolus injections of 5 and 20 μg of serotonin (5-hydroxytryptamine as creatine sulfate complex) and 5 and 20 μg of phenylephrine hydrochloride. Maximal vasodilator responses were studied by infusion of adenosine at 1.34 mg/min into the perfusion tubing. Infusion of higher doses of adenosine produced no greater vasodilatation.

Hind limb vascular resistance was calculated by dividing iliac perfusion pressure by blood flow through the perfused iliac artery. The difference between iliac pressure and dorsal pedal pressure indicates large artery resistance in the limb. The method has been described in detail previously. 14

Morphologic Studies

The monkeys were killed with KCl i.v. The iliac and femoral arteries were removed and fixed by immersion in formalin. Specimens were taken at standardized sites from the proximal and midsegment of the iliac artery and proximal and midssegment of the femoral artery as defined previously. 3 Specimens were prepared for microscopy, and paraffin sections were taken for histologic study.

Morphometric comparisons were made using methods and calculations described previously. 2 The specimens were projected, and cross-sectional areas of the intima and media were digitized.

Statistical Analysis

Mean values and variance were analyzed with a split-plot design, using repeated measures analysis with the Bonferroni multiple comparison test. Statistical significance was considered as p < 0.05.

Results

Plasma Lipids

Plasma total cholesterol was 132 ± 23 mg/dl in normal monkeys and 554 ± 19 mg/dl in atherosclerotic monkeys. Plasma cholesterol in regression monkeys was 565 ± 33 while they received atherogenic diet and 113 ± 8 when they received regression (normal) diet. Plasma triglycerides were < 40 mg/dl in all 3 groups.

Morphologic Changes

In atherosclerotic monkeys, morphologic changes were similar to those described previously. 2, 4, 5, 8 There was dense fibrofatty intimal thickening with focal intimal necrosis in the aorta and its major branches, including the iliac arteries and the proximal part of the femoral arteries. Intimal thickening in the midportion of the femoral arteries consisted largely of fatty streak lesions. Morphometry demonstrated increases in intimal area in the iliac and femoral arteries of atherosclerotic monkeys (Table 1). Intimal area was less in regression

Table 1. Arterial Morphometric Values

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Atherosclerotic</th>
<th>Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal iliac</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intima</td>
<td></td>
<td>0.05 ± 0.05</td>
<td>1.49 ± 0.23*</td>
</tr>
<tr>
<td>Media</td>
<td></td>
<td>1.01 ± 0.13</td>
<td>1.05 ± 0.07</td>
</tr>
<tr>
<td>Midiliac</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intima</td>
<td>0</td>
<td>0.68 ± 0.24*</td>
<td>0.26 ± 0.10†</td>
</tr>
<tr>
<td>Media</td>
<td>0.53 ± 0.10</td>
<td>0.78 ± 0.11</td>
<td>0.54 ± 0.08</td>
</tr>
<tr>
<td>Proximal femoral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intima</td>
<td>0</td>
<td>1.01 ± 0.31*</td>
<td>0.53 ± 0.22</td>
</tr>
<tr>
<td>Media</td>
<td>0.80 ± 0.09</td>
<td>1.01 ± 0.06</td>
<td>0.78 ± 0.04</td>
</tr>
<tr>
<td>Midfemoral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intima</td>
<td>0</td>
<td>0.24 ± 0.18</td>
<td>0</td>
</tr>
<tr>
<td>Media</td>
<td>0.51 ± 0.06</td>
<td>0.84 ± 0.24</td>
<td>0.48 ± 0.02</td>
</tr>
</tbody>
</table>

Values for intimal and medial area (in mm²) are expressed as mean ± SEM. Values that were <0.01 mm² are expressed as 0.

* p < 0.05 vs. normal. † p < 0.05 vs. atherosclerotic.
Table 2. Baseline Hemodynamic Values

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Atherosclerotic</th>
<th>Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>80 ± 4</td>
<td>74 ± 5</td>
<td>79 ± 6</td>
</tr>
<tr>
<td>Hind limb blood flow (ml/min)</td>
<td>65 ± 8</td>
<td>56 ± 6</td>
<td>57 ± 6</td>
</tr>
<tr>
<td>Iliac perfusion pressure (mm Hg)</td>
<td>87 ± 6</td>
<td>99 ± 4</td>
<td>106 ± 4*</td>
</tr>
<tr>
<td>Hind limb vascular resistance</td>
<td>1.44 ± 0.15</td>
<td>1.99 ± 0.24</td>
<td>1.96 ± 0.18</td>
</tr>
<tr>
<td>(mm Hg/ml/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large artery pressure gradient</td>
<td>26 ± 4</td>
<td>36 ± 7</td>
<td>25 ± 3</td>
</tr>
<tr>
<td>(iliac-dorsal pedal pressure, mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large artery resistance (mm Hg/ml/min)</td>
<td>0.41 ± 0.07</td>
<td>0.75 ± 0.21</td>
<td>0.45 ± 0.05</td>
</tr>
</tbody>
</table>

Values are mean ± SEM in 8 normal, 8 atherosclerotic, and 8 regression monkeys. *p < 0.05 vs. normal; other variables were not significantly different in normal, atherosclerotic, and regression monkeys.

Hemodynamic Studies

Baseline Values. Total hind limb vascular resistance and large artery resistance were not significantly different in normal and atherosclerotic monkeys (Table 2) although the values tended to be increased in atherosclerotic monkeys. Hind limb resistance was similar in atherosclerotic and regression monkeys.

During infusion of adenosine, vascular resistance in the total limb and in the large artery segment tended to be higher in atherosclerotic than normal monkeys, but differences did not achieve statistical significance (Figure 1). These values were similar in atherosclerotic and regression monkeys (Figure 1). Thus, atherosclerosis and regression did not produce consistent changes in baseline resistance or responses to adenosine.

Responses to Serotonin. Serotonin produced vasodilatation in the limb (reduction in iliac perfusion pressure) in normal monkeys (Figure 2). In contrast, serotonin produced vasoconstrictor responses in atherosclerotic monkeys (p < 0.05 vs. normal monkeys). Vascular responses to serotonin were restored to dilatation in regression monkeys (p < 0.05 vs. atherosclerotic monkeys) as shown in Figure 2. Vasodilator responses to serotonin tended to be greater (but p > 0.05) in regression monkeys than in normal monkeys, perhaps because baseline resistance in the hind limb tended to be higher in regression monkeys than in normal monkeys (Table 2).

Serotonin produced minimal constriction of the large artery segment in the limb of normal monkeys (Figure 3). This response to serotonin was greatly potentiated in atherosclerotic monkeys (p < 0.05 vs. normal monkeys). The response to serotonin was restored to normal levels in regression monkeys (p < 0.05 vs. atherosclerotic monkeys).

Responses to Phenylephrine. To test the specificity of altered vascular responses, effects of phenylephrine were examined. Vasoconstrictor responses to phenylephrine in the hind limb were similar in normal and atherosclerotic monkeys (Figure 4). Responses to 20 μg (but not 5 μg) phenylephrine were significantly augmented in regression monkeys (p < 0.05 vs. normal and atherosclerotic monkeys). It is possible that the augmented response to phenylephrine in regression monkeys is related in some way to elevation of baseline iliac perfusion pressure (Table 2). Phenylephrine produced modest constriction of the large artery segment in the limb of normal monkeys (Figure 5). This response was not significantly different in atherosclerotic or regression monkeys (p > 0.05).
probably 25 despite severe atherosclerotic lesions, 522 Thus, the vascular lumen may be preserved completely perfused hind limb of monkeys. Increases in pressure gradient (Δ perfusion pressure) of large artery segment indicate constriction of large arteries. Values are mean ± SEM. Responses to serotonin were greater in atherosclerotic monkeys than in normal or regression groups (* = p < 0.05).

Discussion
The striking new finding in this study is that vasoconstrictor responses to serotonin, which were greatly potentiated by atherosclerosis, were restored to normal by dietary treatment of atherosclerosis. The observation that regression of atherosclerosis did not alter maximal vasodilator responses but completely corrected the hyperresponsiveness to serotonin implies that different mechanisms account for these hemodynamic abnormalities.

Vascular Effects of Atherosclerosis and Regression
Experimental atherosclerosis in primates, as well as human atherosclerosis, is associated with marked proliferation of intima.1-3 A surprising new concept, which was proposed by Bond et al22 and which is supported by several studies in primates5-24 and man,25 is that marked intimal proliferation often is associated with little or no encroachment on the vascular lumen. Thus, the vascular lumen may be preserved completely despite severe atherosclerotic lesions,1-22-25 probably because of vessel growth or remodeling.

Maximal vasodilator responses are impaired in the limb and other vascular beds in atherosclerotic primates.5-24 Impairment of the maximal vasodilator response was demonstrated in the limb by a shift in the pressure-flow relation.5-24 In this study, in which one value for pressure and flow was obtained, minimum resistance tended to be increased by atherosclerosis although the difference did not achieve statistical significance. It is likely that determination of the pressure-flow relation, which is more sensitive than measurement of one value of pressure and flow, would have demonstrated a significant increase in resistance in atherosclerotic monkeys. The modest change in minimal resistance in the atherosclerotic limb,5-24 despite marked intimal proliferation, must be related to the relative preservation of the vascular lumen.5-22-24

Several studies in the limb and other vascular beds have demonstrated that intimal area is reduced during regression of atherosclerosis.1-9 These studies provide strong evidence that dietary treatment of atherosclerosis leads to morphologic improvement in atherosclerotic vessels. There is a tendency for intimal fibrosis to become more marked, however, during regression of atherosclerosis in cynomolgus monkeys.7-10 A previous study reported the disappointing finding that dietary treatment of atherosclerosis produced no change of maximal vasodilator responses in the limb or coronary circulation,7 presumably because intimal fibrosis prevented hemodynamic improvement. In this study also, no improvement of maximal vasodilator responses to adenosine was found during regression of atherosclerosis.

Pathogenesis of Vasospasm
Vasospasm is an important complication of atherosclerotic cardiovascular disease.11 Vasospasm occurs spontaneously11 and in susceptible patients may be induced by ergonovine.26 Potentiation of vasoconstrictor responses to ergonovine appears to be mediated largely by serotoninergic receptors.15

It is not yet clear which vasoactive substances are of primary importance in the pathogenesis of vasospasm. Serotonin, thromboxane, thrombin, adrenergic stimuli, and histamine are some potential mediators of vasospasm.15

Figure 3. Effects of intra-arterial injections of serotonin on large artery segment (iliac artery to dorsal pedal artery) in perfused hind limb of monkeys. Increases in pressure gradient (Δ perfusion pressure) of large artery segment indicate constriction of large arteries. Values are mean ± SEM. Responses to serotonin were greater in atherosclerotic monkeys than in normal or regression groups (* = p < 0.05).

Figure 4. Responses to intra-arterial injections of phenylephrine. Values (mean ± SEM) are changes in total hind limb (iliac) perfusion pressure. Responses to 20 µg phenylephrine were greater in regression monkeys than in normal or atherosclerotic groups (* = p < 0.05).

Figure 5. Effects of intra-arterial injections of phenylephrine on large artery segment. Values are mean ± SEM. Responses to phenylephrine were not significantly different in normal, atherosclerotic, and regression monkeys.
Serotonin is an attractive candidate for the role of possible mediator of vasospasm. Platelets contain large amounts of serotonin\(^\text{12}\) and may aggregate at the site of atherosclerotic lesions and release serotonin. Serotonin normally produces only modest constriction of large arteries,\(^\text{14,27,28}\) but atherosclerosis greatly potentiates serotoninergic vasoconstrictor responses in vitro\(^\text{13}\) and in vivo.\(^\text{14}\) Thus, it seems reasonable to suggest that serotonin may play an important role in the pathogenesis of vasospasm.

**Mechanisms of Altered Vascular Responses**

In normal animals, as described previously,\(^\text{14,27,28}\) serotonin constricted large arteries but dilated small vessels and thereby decreased total limb resistance in normal monkeys. Atherosclerosis potentiated responses to serotonin at the level of large arteries, and dietary treatment of atherosclerosis abolished hyperresponsiveness to serotonin in the large artery segment.

Several mechanisms may contribute to altered vascular responses during atherosclerosis and regression of atherosclerosis. First, increased cholesterol content of membranes in atherosclerosis may increase vascular responses,\(^\text{16,17}\) and regression of atherosclerosis might correct the abnormality. One might anticipate that a membrane abnormality would alter vasoconstrictor responses to several receptor-mediated agonists that are membrane-dependent. Responses to phenylephrine provide evidence against this possible mechanism. Vasoconstrictor responses to phenylephrine were not altered by atherosclerosis, and they appeared to increase with regression of atherosclerosis, in sharp contrast to the marked increase in vasoconstrictor responses to serotonin with atherosclerosis and decrease with regression.

Second, the number of serotoninergic and adrenergic receptors is increased in atherosclerotic aortas.\(^\text{18}\) There have been no studies concerning effects of regression of atherosclerosis on receptor binding. Thus, it is not clear whether changes in receptor number or affinity contribute to the altered vascular responses that were observed.

Third, changes in endothelial function may play an important role in alteration of vascular responses during atherosclerosis and regression. Vasoactive substances that are released by the endothelium modulate responses to many vasoactive substances,\(^\text{19-21}\) including exogenous serotonin and serotonin that is released by platelets.\(^\text{30}\) Several recent studies indicate that atherosclerosis impairs endothelium-dependent vascular responses.\(^\text{19-21}\) A preliminary report\(^\text{11}\) suggests that regression of atherosclerosis restores endothelium-dependent vascular responses to normal. Thus, it is speculated that hyperresponsiveness to serotonin may be abolished during dietary treatment of atherosclerosis in part by restoration of normal endothelium-dependent responses.

**Implications**

The findings in this study may have important implications for dietary treatment of atherosclerosis in humans, based on the assumption that atherosclerosis and regression of atherosclerosis in this primate model can be extrapolated to humans.

Ischemic pain in the limb (intermittent claudication) and myocardium (angina pectoris) during exercise in patients with atherosclerotic vascular disease is presumably related to limitation of maximal vasodilator responses. Thus, the finding that regression of atherosclerosis does not improve maximal vasodilator responses in the limb and myocardium, as reported in this study and previously,\(^\text{4}\) implies that intermittent claudication and exercise-induced angina may not improve consistently despite effective dietary treatment of atherosclerosis with reabsorption of vascular lipids.

In contrast, the finding that hyperresponsiveness to serotonin in atherosclerotic vessels is abolished by dietary treatment of atherosclerosis may have important implications for treatment of vasospasm. It is speculated that dietary treatment of atherosclerosis, even when it fails to improve maximal vasodilator responses, may be effective in reducing susceptibility to vasospasm.

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