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

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

Diet-induced atherosclerosis in primates impairs vasodilator responses and greatly potentiates vasoconstrictor responses to serotonin. Serotonin may play an important role in the pathogenesis of vasospasm. In diet-induced regression of atherosclerosis, intimal lesions are reduced, but maximal vasodilator responses do not improve, perhaps because of vascular fibrosis. Our goal was to determine whether dietary treatment of atherosclerosis reverses the augmented vasoconstrictor responses to serotonin and thus might reduce susceptibility to vasospasm. Normal cynomolgus monkeys, atherosclerotic monkeys, and atherosclerotic monkeys that were given a normal (regression) diet for 18 months were studied. Morphometric studies indicated that the regression diet reduced lesions in the iliac and femoral artery since intimal area was reduced by about 50%. In the hind limb perfused at constant flow, residual resistance during maximal vasodilatation produced by infusion of adenosine tended to be greater in atherosclerotic monkeys than in normals and failed to improve in regression monkeys. In contrast, vasoconstrictor responses to serotonin were greatly potentiated in atherosclerotic monkeys and were restored to normal in regression monkeys. Serotonin (20 μg i.a.) decreased hind limb resistance (in mm Hg/ml/min) 0.34 ± 0.06 (mean ± SE) in normal monkeys, increased resistance 0.58 ± 0.17 in atherosclerotic monkeys (p < 0.05 vs. normal), and decreased resistance 0.70 ± 0.15 in regression monkeys (p < 0.05 vs. atherosclerotic). Thus, dietary treatment of atherosclerosis abolishes augmented vasoconstrictor responses to serotonin. It is proposed that treatment of atherosclerosis may be beneficial, even when vasodilator responses fail to improve, by reducing susceptibility to serotonin-induced vasospasm. (Circulation Research 1987;61:346-351)
Hemodynamic Studies

The monkeys were sedated with ketamine hydrochlo-
ride (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 per-
formed, 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 ab-
dominal 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 ante-
gradely 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 athero-
sclerotic and regression monkeys, initial blood flow to
the limb was considerably lower than in normal
monkeys. Blood flow to the limb was then increased,
which tended to raise iliac perfusion pressure above the
level observed in normal monkeys.

Maximal perfusion pressure and pressure in the
dorsal pedal artery were measured during bolus injec-
tions 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.4

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 midssegment of the iliac artery
and proximal and midssegment of the femoral artery as
defined previously.5 Specimens were prepared for
microscopy, and paraffin sections were taken for
histologic study.

Morphometric comparisons were made using meth-
ods and calculations described previously. The spec-
imens 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.2,4,8 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>
<thead>
<tr>
<th>Table 1. Arterial Morphometric Values</th>
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<tbody>
<tr>
<td>Normal</td>
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<tr>
<td>Proximal iliac</td>
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<tr>
<td>Intima</td>
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<tr>
<td>Media</td>
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<tr>
<td>Midiliac</td>
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<td>Intima</td>
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<tr>
<td>Media</td>
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<tr>
<td>Proximal femoral</td>
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<tr>
<td>Media</td>
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<tr>
<td>Midfemoral</td>
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<tr>
<td>Intima</td>
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<tr>
<td>Media</td>
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Values for intimal and media 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.
monkeys than in atherosclerotic monkeys in midiliac and midfemoral arteries. Medial area was not significantly different in normal and regression monkeys although medial area tended to be increased in atherosclerotic arteries.

**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).

*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.*

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**Table 2. Baseline Hemodynamic Values**

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Atherosclerotic</th>
<th>Regression</th>
</tr>
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<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>80 ± 4</td>
<td>74 ± 5</td>
<td>79 ± 6</td>
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<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 (mm Hg/ml/min)</td>
<td>1.44 ± 0.15</td>
<td>1.99 ± 0.24</td>
<td>1.96 ± 0.18</td>
</tr>
<tr>
<td>Large artery pressure gradient (iliac-dorsal pedal pressure, mm Hg)</td>
<td>26 ± 4</td>
<td>36 ± 7</td>
<td>25 ± 3</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>
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**Figure 1. Vascular resistance during infusion of adenosine in perfused hind limb of normal, atherosclerotic (AS), and regression monkeys. Values (mean ± SEM) are resistance in total hind limb (left) and in large artery segment from iliac artery to dorsal pedal artery (right). Values are not significantly different (p > 0.05).**

**Figure 2. Responses to intra-arterial injections of serotonin in perfused hind limb. Values (mean ± SEM) are changes in total hind limb (or iliac) perfusion pressure. Increases in perfusion pressure indicate vasoconstriction, and decreases indicate vasodilatation. Responses to serotonin were different in atherosclerotic monkeys compared with normal and regression groups (* = p < 0.05).**
Vascular Effects of Atherosclerosis and Regression

Experimental atherosclerosis in primates, as well as human atherosclerosis, is associated with marked proliferation of intima. A surprising new concept, which was proposed by Bond et al and which is supported by several studies in primates and man, 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, probably because of vessel growth or remodeling.

Maximal vasodilator responses are impaired in the limb and other vascular beds in atherosclerotic primates. Impairment of the maximal vasodilator response was demonstrated in the limb by a shift in the pressure-flow relation. 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, despite marked intimal proliferation, must be related to the relative preservation of the vascular lumen.

Several studies in the limb and other vascular beds have demonstrated that intimal area is reduced during regression of atherosclerosis. 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. 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, 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. Vasospasm occurs spontaneously and in susceptible patients may be induced by ergonovine. Potentiation of vasoconstrictor responses to ergonovine appears to be mediated largely by serotoninergic receptors.

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.
Serotonin is an attractive candidate for the role of possible mediator of vasospasm. Platelets contain large amounts of serotonin and may aggregate at the site of atherosclerotic lesions and release serotonin. Serotonin normally produces only modest constriction of large arteries, but atherosclerosis greatly potentiates serotonin-mediated vasoconstrictor responses in vitro and in vivo. 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, 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. These 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, 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 serotonergic and adrenergic receptors is increased in atherosclerotic aortas. 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, including exogenous serotonin and serotonin that is released by platelets. Several recent studies indicate that atherosclerosis impairs endothelium-dependent vascular responses. A preliminary report 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, 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.

Acknowledgments

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