Functional Improvement Precedes Structural Regression of Atherosclerosis

Keith H. Benzuly, MD; Richard C. Padgett, MD; Sanjay Kaul, MD; Donald J. Piegors, BS; Mark L. Armstrong, MD; Donald D. Heistad, MD

**Background** Vasocostrictor responses to serotonin are augmented in monkeys with diet-induced atherosclerosis and improve after 18 months of normal diet. We tested the hypothesis that functional improvement may occur early during regression, before evidence of structural improvement.

**Methods and Results** Responses of the iliac artery to serotonin were measured by quantitative angiography and a Doppler flow probe in several groups of monkeys: (1) normal monkeys, (2) monkeys fed an atherogenic diet for 2 years (atherosclerotic), and (3) monkeys fed an atherogenic diet for 2 years (preregression) followed by a normal diet for 4, 8, or 12 months (regression). In normal monkeys, serotonin produced minimal constriction of the iliac artery, and blood flow to the legs increased. In atherosclerotic monkeys, there was pronounced constriction of the iliac artery, and blood flow to the legs decreased markedly. After 4 months of regression diet, four of eight monkeys demonstrated marked reduction in hyperresponsiveness to serotonin angiographically, and by 8 months, six of eight monkeys had significant improvement. After regression, serotonin produced minimal changes in flow. There was no reduction in intimal area (ie, atherosclerotic lesion) in iliac arteries from regression monkeys compared with atherosclerotic monkeys, but there was a marked reduction in cholesteryl ester in arteries from regression monkeys.

**Conclusions** Abnormal vasocostrictor responses to serotonin usually return to or toward normal within a few months during regression of atherosclerosis. Functional improvement occurs in conjunction with early resorption of lipid from the arterial wall and occurs before detectable changes in mass of the atherosclerotic lesion.

**Key Words** serotonin • atherosclerosis • angiography • vasocostriction
Angiograms

Animals were sedated with ketamine hydrochloride (10 mg/kg IM initially and supplemental doses as needed) and acepromazine maleate (1 mg/kg IM). Studies were performed under sterile conditions in an animal catheterization laboratory. Lidocaine (1%) was infiltrated locally, and a polyethylene catheter with multiple side holes and a 60° directional Doppler ultrasound transducer was inserted via an arteriotomy into the right femoral artery. The catheter was passed retrogradely under fluoroscopic visualization into the distal abdominal aorta immediately below the level of the renal arteries. Mean and phasic arterial pressure and Doppler frequency were recorded continuously. A Jelco 24-gauge intravenous catheter was inserted into the left dorsal pedal artery, and pressure was recorded continuously. Cineangiograms of the distal descending aorta and the left iliac arterial tree were obtained in an anteroposterior projection by use of power injections of nonionic contrast (Iohexol, Sanofi-Winthrop Pharmaceuticals, New York, NY) at a rate of 15 mL/s through the catheter in the distal abdominal aorta.

Two angiograms were obtained: at baseline and after injection of 100 μg serotonin IA. Pressures and velocity of blood flow were recorded at baseline and after injections of serotonin (30 and 100 μg IA). An angiogram was not obtained after injection of 30 μg of serotonin so as to avoid excessive injection of angiographic contrast. A dose-response relation to serotonin was demonstrated with measurement of flow velocity: the reductions in velocity of flow in atherosclerotic monkeys in response to 30 and 100 μg of serotonin were 2.1±0.4 and 3.9±0.7 kHz, respectively.

Arteriomy sites were sutured, and the animals received an injection of penicillin. A lethal dose of KCl was given intravenously to normal and atherosclerotic animals while the monkeys were anesthetized about 2 weeks after angiography. Hypercholesterolemic monkeys underwent two angiograms: first, while they were fed a normal diet, and then after 1 to 2 months of atherogenic diet. Regression animals underwent angiograms after 23±0.6 months of atherogenic diet. They were then placed on a normal diet, and angiography was repeated in 4.3±0.3 months. Regression monkeys were killed when there was significant reduction in hyperresponsiveness to serotonin, defined as <20% constriction of the iliac artery in response to serotonin and <50% of the response to serotonin that was observed when the monkeys were atherosclerotic. Regression animals without significant reduction in constrictor response continued on a normal diet, and angiography was repeated at 7.7±0.2 months. If marked vasoconstrictor responses to serotonin were still present at that time, the monkey remained on a normal diet, and a final angiogram was performed at 12.2±0.0 months. AS NC monkeys underwent a first angiogram after 34±2.2 months of atherogenic diet and a second angiogram after 1.5±0.1 months on a normal diet.

Angiographic data were analyzed quantitatively with computerized edge detection software (Cardiovascular Angiography Analysis System, Pie Data Medical, Maastricht, the Netherlands). This process is described in detail elsewhere. Briefly, a cine frame was selected for image focus and for uniform, complete opacification of the left common iliac artery. The image was digitized and magnified, and a segment of the iliac artery immediately proximal or distal to the origin of the hypogastric artery was selected for analysis. Criteria for selection of segments were that the section of artery was straight and without branch, overlying structures, or crossing vessels. An approximate centerline was defined by the operator. Edge detection and smoothing were performed by the software package, with correction by the operator only for obvious deviation that was usually a result of vessel branching. Mean arterial diameter was measured over the length of iliac artery, and focal diameter was taken as a minimum caliber of six to eight computer-defined subsegments of the same segment of artery. Diameter of the distal aorta was measured with a similar approach. The same segment of artery was identified on serial angiograms for each animal, using the origin of the hypogastric artery as a reference point.

Two angiograms were performed in four monkeys on atherogenic diet at intervals of 5 days to 5 months to examine reproducibility of measurements. Measurements for 16 angiograms were performed twice by the same operator to assess intraobserver variability. A second, independent operator also analyzed 14 angiograms to characterize interobserver variability.

Total blood flow in the distal aorta (primarily to the legs) was calculated from Doppler frequency shift and aortic mean diameter. Lower-body conductance was estimated by Q/MAP, and large artery conductance was estimated as Q/(MAP−DP), where MAP is mean aortic pressure, DP is dorsal pedal arterial pressure, and Q is aortic flow.

Morphological Studies and Arterial Lipid Analysis

After the monkeys were killed, iliac arteries were removed and fixed in Formalin. Specimens from the angiographic site (left common iliac artery at the level of the origin of the hypogastric artery) were embedded in paraffin and stained with hematoxylin and eosin and Verhoeff-van Gieson’s stain. Intimal, medial, and luminal areas were measured as described previously. Length of the internal elastic lamina was used to correct luminal area for absence of pressure.

Segments of artery were obtained from the site that corresponded to the angiographic measurements. Loose adventitia was removed from the vessel, and the artery was blotted dry, weighed, and homogenized. Free cholesterol and cholesteryl esters were extracted twice with chloroform and methanol (2:1 vol/vol) for at least 2 hours. Free cholesterol content in the chloroform phase was then determined by gas-liquid chromatography (Hewlett-Packard 5840 A gas chromatograph with a 3-ft glass column and 3% SP-2100 on 100/120 Supelcoport, Supelco Inc, Bellefonte, Pa) with cholestane as an internal standard. Free and esterified cholesterol in the remaining chloroform samples were then separated by thin-layer chromatography with a solvent system of hexane, diethyl ether, and acetic acid (80:20:1 vol/vol). Lipids were visualized with iodine vapor. In this experiment, cholesteryl ester comigrates with the esterified cholesterol. The cholesteryl ester band was scraped and saponified with 0.5N NaOH in acetone, methanol, and water (1:1:0.1 vol/vol) at 60°C for 1 hour. Cholesterol and cholestane were then extracted twice with hexane, and cholesteryl was determined by gas-liquid chromatography as described above.

Statistical Analysis

Results are expressed as mean±SEM. Mean values between groups were compared by a repeated-measures ANOVA with Bonferroni’s correction. A logarithmic or square-root transform was used in some analyses because variance of the normal group was less than that for the other groups. Mean values within the regression group were analyzed with a paired t test. Statistical significance was assumed at P<.05.

Results

Results will be described first for the normal, atherosclerotic, and regression monkeys, which were studied concurrently. Results will be described separately for the hypercholesterolemic monkeys and for AS NC (ie, 6 weeks regression) monkeys.

Plasma Lipids

Total plasma cholesterol was 130±9 mg/dL in the normal group and 614±36 mg/dL in the atherosclerotic group. Plasma cholesterol in regression monkeys was
Baseline Data and Response to Serotonin in Normal, Atherosclerotic, and Regression Monkeys

<table>
<thead>
<tr>
<th></th>
<th>Normal (n=6)</th>
<th>Atherosclerotic (n=7)</th>
<th>Preregression (n=8)</th>
<th>Regression (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline Value</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter, mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aorta</td>
<td>4.5±0.3</td>
<td>3.6±0.2*</td>
<td>4.1±0.3</td>
<td>4.2±0.2</td>
</tr>
<tr>
<td>Iliac</td>
<td>2.9±0.2</td>
<td>2.2±0.1*</td>
<td>2.8±0.2</td>
<td>3.0±0.2†</td>
</tr>
<tr>
<td>Blood flow to leg, L/min</td>
<td>0.52±0.11</td>
<td>0.35±0.04</td>
<td>0.45±0.04</td>
<td>0.50±0.06</td>
</tr>
<tr>
<td>Flow velocity, kHz</td>
<td>6.6±0.2</td>
<td>7.6±0.7</td>
<td>7.7±0.7</td>
<td>7.7±0.7</td>
</tr>
<tr>
<td>Pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic arterial</td>
<td>69±2</td>
<td>73±2</td>
<td>74±2</td>
<td>72±2</td>
</tr>
<tr>
<td>Dorsal pedal</td>
<td>64±2</td>
<td>58±2</td>
<td>64±2</td>
<td>62±2</td>
</tr>
<tr>
<td>Conductance, mL·min⁻¹·mm Hg⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total leg</td>
<td>7.6±1.4</td>
<td>4.9±0.6</td>
<td>6.1±0.5</td>
<td>7.1±0.9</td>
</tr>
<tr>
<td>Large artery</td>
<td>119±36</td>
<td>26.0±5*</td>
<td>57±10</td>
<td>141±95</td>
</tr>
<tr>
<td><strong>Value during maximum response to serotonin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter, mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aorta</td>
<td>4.5±0.3</td>
<td>3.4±0.2</td>
<td>3.7±0.3</td>
<td>4.0±0.2</td>
</tr>
<tr>
<td>Iliac</td>
<td>2.8±0.2</td>
<td>1.5±0.1*</td>
<td>1.6±0.3*</td>
<td>2.6±0.3†</td>
</tr>
<tr>
<td>Blood flow to leg, L/min</td>
<td>0.60±0.11</td>
<td>0.22±0.05*</td>
<td>0.22±0.05*</td>
<td>0.50±0.08†</td>
</tr>
<tr>
<td>Flow velocity, kHz</td>
<td>7.5±0.7</td>
<td>4.8±0.8</td>
<td>3.8±0.8*</td>
<td>8.0±0.6†</td>
</tr>
<tr>
<td>Pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic arterial</td>
<td>62±2</td>
<td>82±4*</td>
<td>78±6</td>
<td>65±4†</td>
</tr>
<tr>
<td>Dorsal pedal</td>
<td>53±2</td>
<td>18±4*</td>
<td>29±6*</td>
<td>30±6</td>
</tr>
<tr>
<td>Conductance, mL·min⁻¹·mm Hg⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total leg</td>
<td>9.6±1.6</td>
<td>2.6±0.6*</td>
<td>2.9±0.8*</td>
<td>8.2±1.6†</td>
</tr>
<tr>
<td>Large artery</td>
<td>80±18</td>
<td>3.8±1.2*</td>
<td>5.9±1.9*</td>
<td>47±29†</td>
</tr>
</tbody>
</table>

Values are for three groups of monkeys: Normal, Atherosclerotic, and Regression. "Preregression" shows values obtained when the Regression group was fed an atherogenic diet for 23±4 months, and "Regression" shows values for the same group after they were fed a normal diet for 4 to 12 months. Values are expressed as mean±SEM. *P<.05 vs Normal; †P<.05 vs Preregression.

649±43 mg/dL while on the atherogenic diet and 158±10 mg/dL after 4 to 7 weeks of normal diet.

Angiographic Studies and Blood Flow

**Baseline Values**

Aortic diameter was smaller, but calculated values for baseline blood flow to the leg were not different, in atherosclerotic compared with normal and regression monkeys (Table). Iliac artery diameter also was smaller in atherosclerotic animals than in normal monkeys. Large-artery conductance was lower in atherosclerotic monkeys than in normal or regression animals. Some differences in baseline values between normal and atherosclerotic animals were not consistent. For example, although aortic and iliac diameters were smaller in atherosclerotic than in normal monkeys, the preregression group represents a second cohort of atherosclerotic animals, and baseline aortic diameter, iliac diameter, and blood flow in this group do not differ from normal values.

**Reproducibility**

Four animals underwent two angiograms. There was no difference in baseline aortic or iliac diameter, and there was no difference in vasoconstrictor responses to serotonin between the first and second angiograms. Variability in angiographic measurements between two independent observers was determined for 14 angiographic segments. The interclass correlation was r=.990 (95% CI, 0.969 to 0.997). Within-observer variability was determined for 16 angiographic segments, based on two separate measurements of the same segment. The intraclass correlation was r=.989 (95% CI, 0.970 to 0.996).

**Response to Serotonin**

Serotonin produced minimal reduction in mean diameter of the iliac artery in normal monkeys (Fig 1). In contrast, there was a marked vasoconstrictor response to serotonin in both atherosclerotic monkeys and the regression group while they were fed atherogenic diet (Figs 1 and 2) (P<.05 versus normal). In regression monkeys, the constrictor response to serotonin was significantly less after regression of atherosclerosis (Figs 1 and 3) (P<.05). Values for the regression group (Fig 1) were taken from the final angiographic study for each animal; values were obtained 7.2±1.3 months (range, 4 to 12 months) after preregression (ie, atherosclerotic) monkeys started to receive a normal diet.

Focal changes in diameter (in contrast to changes in mean diameter, described above and below) of the iliac
arteries demonstrated the same pattern of responses to serotonin: there was little response to serotonin in normal animals; there was augmented vasoconstriction in atherosclerotic and preregression monkeys; and responses were significantly smaller in regression monkeys. Focal changes in diameter are very similar to changes in mean diameter and therefore are not presented.

Serotonin (100 µg IA) produced an increase in blood flow (P<.05) to the legs in normal monkeys (Fig 4). In atherosclerotic monkeys, serotonin produced a marked decrease in flow (P<.05 versus normal). After regression, serotonin did not alter flow (Figs 4 and 5) (P<.05 versus preregression).

Serotonin produced a decrease in large-artery conductance in normal monkeys (Fig 6, left). The vasoconstrictor response to serotonin was significantly greater in atherosclerotic monkeys than in normal monkeys (P<.01). After regression, serotonin reduced conductance less than in atherosclerotic and preregression monkeys (P<.05). Serotonin increased total leg conductance in normal animals (Fig 6, right) but produced a marked decrease in conductance in the atherosclerotic monkeys (P<.05 versus normal). The change in total leg conductance in response to serotonin returned to normal in regression monkeys (P<.05 versus preregression).

Angiographic data for individual monkeys during regression of atherosclerosis are presented in Fig 7. Baseline iliac diameter (left) increased slightly during the regression period (preregression, 2.8±0.2 mm vs terminal study, 3.0±0.2 mm, P<.05). In contrast, in four of eight regression monkeys, augmented vasoconstrictor responses to serotonin were markedly reduced (to <20%) at 4 months (Fig 7). After 8 months on a normal diet, two of the remaining four regression monkeys also had reduction of hyperresponsiveness to serotonin. Two regression monkeys had little or no improvement after 12 months of normal diet.

**Effects of Hypercholesterolemia**

In a separate group of five monkeys, total plasma cholesterol was 112±13 mg/dL while the monkeys were fed a normal diet. After the monkeys were fed an atherogenic diet for approximately 6 weeks, cholesterol was 571±86 mg/dL. Baseline iliac artery diameter and blood flow to the leg were similar when the monkeys were normocholesterolemic and hypercholesterolemic. Serotonin (100 µg IA) decreased diameter of the iliac artery by 6.3±2.2% in normocholesterolemic and by 11±5.8% in hypercholesterolemic monkeys (NS). Serotonin did not change blood flow to the leg (+2.8±3.7% and +2.1±6.1%) in normocholesterolemic or hypercholesterolemic monkeys (NS). Thus, vasoconstrictor responses to serotonin were not altered by hypercholesterolemia.

**Effect of Acute Reduction of Plasma Cholesterol**

In another group of four atherosclerotic monkeys, total plasma cholesterol was 649±40 mg/dL while the monkeys were fed an atherogenic diet. After approximately 6 weeks of a normal diet, total plasma cholesterol was 119±14. Baseline iliac artery diameter and
blood flow to the leg were similar when the atherosclerotic monkeys were hypercholesterolemic (AS HC) and when plasma cholesterol of the same group was normal (AS NC). Serotonin (100 μg IA) decreased diameter of the iliac artery by 33±5.3% in AS HC and by 36±5.9% in AS NC (NS versus AS HC). Serotonin decreased blood flow by 31±8.9% in AS HC and by 26±6.7% in AS NC (NS versus AS HC). Thus, the vasoconstrictor response to serotonin in atherosclerotic monkeys was not reduced significantly by reduction of plasma cholesterol to normal for 6 weeks.

**Arterial Wall Lipids**

Free cholesterol content of the arterial wall was significantly higher in atherosclerotic than in normal animals (Fig 8) (*P <.05*). Free cholesterol content was lower (*P <.05*) in the regression group than in atherosclerotic animals.

Cholesteryl ester of the iliac arterial wall at the site of angiographic measurement was not detectable in normal monkeys (Fig 8). There was marked accumulation of cholesteryl ester in the arterial wall of atherosclerotic monkeys. Cholesteryl ester content was significantly lower in regression animals than in atherosclerotic monkeys (*P <.05*). Thus, reabsorption of >80% of esterified cholesterol occurs within 4 to 12 months during dietary treatment of atherosclerosis. There appears to be preferential depletion of vascular wall cholesteryl ester compared with free cholesterol early in regression.

Atherosclerotic monkeys that were fed normal diet for approximately 6 weeks (to study effects of acute reduction of plasma cholesterol) still had high levels of free cholesterol in the arterial wall (2.55±0.52 mg/g). In monkeys that were fed atherogenic diet for 6 weeks (to study effects of hypercholesterolemia), free cholesterol level in the arterial wall was 0.6±0.04 mg/g, which is similar to normal animals (Fig 8).

Tissue cholesteryl ester was 0.80±0.23 mg/g in atherosclerotic monkeys that were fed normal diet for 6 weeks and 0.04±0.02 mg/g in hypercholesterolemic monkeys that were fed atherogenic diet for 6 weeks.

**Morphological Studies**

Atherosclerotic animals had dense fibrofatty intimal thickening and focal intimal necrosis of the iliac arteries as described previously.1,17 There was a significant increase in intimal area in atherosclerotic monkeys, with no reduction in regression monkeys (Figs 9 and 10, *P <.05* normal versus atherosclerotic and regression). As reported previously,17,18 luminal area did not decrease in atherosclerotic monkeys despite a marked increase in intimal area, since vascular “remodeling” preserved the lumen. The most prominent morphological change in atherosclerotic monkeys was intimal thickening, and this change persisted in regression animals.

In atherosclerotic monkeys that were fed normal diet for 6 weeks, there was persistent intimal thickening, with intimal area of 1.16±0.68 mm² (compare with Fig 10). Hypercholesterolemia for 6 weeks did not result in intimal thickening (intimal area, 0.01±0.01 mm²).

**Discussion**

The major new finding in this study is that marked functional improvement, with decreased hyperresponsiveness to serotonin, occurs in most monkeys during the first few months of regression of atherosclerosis. Marked improvement in functional responses occurred
Considerations and Limitations

FIG 5. Graphs showing response to serotonin (5-HT) (100 μg IA) in an atherosclerotic monkey (preregression) and the same animal after 8 months of regression. When the monkey was atherosclerotic, serotonin produced a transient increase in flow followed by a sustained decrease in flow. After 8 months of regression, serotonin produced a sustained increase in flow. Blood flow was calculated from velocity of flow in the distal aorta and diameter of the aorta. Serotonin produced a sustained decrease in pressure in the dorsal pedal artery when the monkey was atherosclerotic as the proximal artery constricted.

in the absence of consistent reversal of structural changes. Hence, the functional and structural changes associated with atherosclerosis appear to change at different rates during regression.

Methodological Considerations and Limitations

Eccentric lesions may result in overestimation or underestimation of arterial stenosis by angiography. Because we did not obtain biplanar angiograms, errors from eccentric lesions would be expected to be exaggerated. In this model of atherosclerosis, however, lesions are very predominantly concentric rather than eccentric.

We examined angiographic responses in only one segment of the iliac arterial tree. Atherosclerosis may produce both focal and diffuse arterial changes, but we assume that changes in constrictor responses in one segment reflect a diffuse alteration. Parallel changes in mean and focal constrictor responses are consistent with this assumption. In addition, changes in total limb and large-artery conductance, which are not compromised by focal variations in constriction, were generally similar to angiographic responses.

Vascular Responses in Atherosclerosis and Regression

Several studies in humans have used angiography to examine effects of treatment of atherosclerosis.4-7 The studies examined primarily regression of focal stenoses and did not evaluate functional consequences of regression. Assessments of focal stenoses during regression generally have demonstrated progression of some lesions and regression of others within the same individual. In groups treated for hypercholesterolemia, the overall mean scores show a tendency or a significant change toward regression or nonprogression compared with untreated patients, but the magnitude of improvement is modest. Furthermore, most studies of regression in humans have required at least 2 years to demonstrate significant structural improvement.

Recently, endothelium-dependent vasodilatation has been reported to improve after reduction of plasma cholesterol for 6 months in hyperlipidemic patients.13 Our results also demonstrate improvement of vascular function but have additional implications. First, we documented morphometrically that improvement of vascular responses precedes reduction in mass of atherosclerotic lesions. Second, serotonin has been implicated in the pathophysiology of clinical ischemic syndromes.19-23 The finding that hyperresponsiveness to serotonin subsides rapidly during regression of atherosclerosis implies that susceptibility to vasospasm also may subside rapidly. Moreover, vasospasm may be clinically important despite preservation of endothelium-dependent vasodilatation.24 Third, experiments in hypercholesterolemic monkeys and AS NC monkeys provide evidence that elevated lipid levels alone do not account for augmented vasoconstriction to serotonin.

Regression of atherosclerotic lesions has been demonstrated histologically and with morphometry in animal models of atherosclerosis.1 In the present study, we found no reduction in intimal area (ie, structural improvement) during this early period of regression of atherosclerosis. Angiographically detectable obstructions in humans usually form over many years, and marked structural regression in lesions may also require many years if, indeed, large changes can occur. Similarly, morphometric evidence of regression in animal models has required longer periods of time than those in the present study.

Although it is possible that regression of obstructive arterial plaques may be accomplished with aggressive therapy over a long period, we have not been able to demonstrate in primates that maximal vasodilator responses improve consistently during regression.15 Morphological regression of lipid deposits is probably accompanied by fibrosis of the arterial wall,25 leading to impairment of vasodilator reserve.

Fig 6. Bar graphs showing effects of serotonin (100 μg IA) on large-artery conductance (left) and total leg conductance (right) in normal (NL) monkeys, atherosclerotic (AS) monkeys, and before (PRE-R) and after (R) regression. Values are mean±SEM. *P<.05 vs NL; **P<.05 vs PRE-R.
Atherosclerotic arteries are characterized by exaggerated vasoconstrictor responses to serotonin.\textsuperscript{26,27} Because serotonin is a major product that is released by platelets, an augmented response to serotonin may be important in the pathophysiology of clinical ischemic syndromes.\textsuperscript{19,23,28} Dietary treatment of experimental atherosclerosis in primates results in improvement of hyperresponsiveness to serotonin and recovery of vasodilatation to ADP in vivo.\textsuperscript{5,9,11} Studies in vitro also demonstrate that abnormal endothelium-dependent relaxation in atherosclerosis improves during regression.\textsuperscript{12} In all of these studies, vascular responses were examined after at least 18 months of regression. Previously, it has been difficult to follow the course of vascular responses chronologically because the techniques required terminal studies. In the present study, we used a technique that allows us to examine vascular responses several times over 4 to 12 months to demonstrate early resolution of one of the functional abnormalities of atherosclerosis.

Functional changes in atherosclerosis and regression may occur at different rates and to different degrees in different parts of the vascular bed. Iliac artery diameter and large-artery conductance reflect responses of large arteries to serotonin. In contrast, values for limb blood flow and conductance reflect both large and small arteries. We found that atherosclerosis alters responses of iliac arteries to serotonin, but effects of atherosclerosis on changes in blood flow after serotonin are even more dramatic. Moreover, hyperresponsiveness of large arteries to serotonin is reduced during regression, but improvement in limb blood flow is even more marked. Thus, abnormalities in arterial function may regress more rapidly or more completely in small resistance vessels than in large arteries, and blood flow may be a more sensitive measure of functional improvement than large-artery diameter.

**Mechanisms**

Augmented vasoconstriction in response to serotonin occurs in atherosclerosis\textsuperscript{26,27} and normalizes during regression,\textsuperscript{9} but mechanisms that account for these changes are not entirely clear.

Several mechanisms may contribute to the functional abnormalities in atherosclerosis. First, atherosclerosis produces endothelial dysfunction.\textsuperscript{26,29,30} This may reflect impaired synthesis or release of endothelium-derived relaxing factor (EDRF).\textsuperscript{31} It appears that L-arginine can attenuate endothelial dysfunction in atherosclerosis.\textsuperscript{31-34} This finding suggests that a relative deficiency of L-arginine may contribute to abnormal vascular responses in atherosclerosis or that the abnormality can be improved by augmented synthesis of EDRF. Second, EDRF synthesis may be normal or even increased\textsuperscript{35} in atherosclerosis, and enhanced degradation of EDRF may result in impaired vasodilation. Third, smooth muscle of atherosclerotic arteries may be less sensitive to EDRF and thereby contribute to augmented constriction.\textsuperscript{36}

In Watanabe heritable hyperlipidemic rabbits, augmented vasoconstriction to serotonin can be demonstrated in vitro before atherosclerotic lesions are seen.\textsuperscript{37} Previous studies in vivo, however, have suggested that hypercholesterolemia in the absence of atherosclerotic lesions does not account for augmented constrictor responses to serotonin.\textsuperscript{38} Our findings in monkeys that received atherogenic diet for about 6 weeks support this conclusion. Although plasma cholesterol was elevated, vasoconstrictor responses to serotonin were not altered. This brief duration of hypercholesterolemia was not sufficient to produce significant intimal thickening. We also studied effects of plasma cholesterol in atherosclerotic monkeys. After approximately 6 weeks of normal diet, plasma cholesterol levels were normal, but constrictor responses to serotonin were not reduced in these atherosclerotic monkeys. These studies support the conclusion that augmented responses to serotonin in atherosclerotic monkeys are not simply a response to hypercholesterolemia.

**Speculation and Implications**

The present study demonstrates that normalization of function, as reflected by vascular responses to serotonin,
FIG 10. Sections of iliac artery in normal vs (R, n=8) monkeys. regression markedly and drastically in decrease the number of leukocytes and smooth muscle cells. Because leukocytes release oxygen radicals and may degrade EDRF, the decrease in cholesteryl ester may reflect a decrease in the number of leukocytes and improvement in vasodilatation. Alternatively, resorption of cholesteryl ester may produce biochemical changes in lipid-laden leukocytes and perhaps decrease free radical production. Thus, vasoconstrictor responses to serotonin improve markedly and rapidly during regression of atherosclerosis in most monkeys, before morphometric evidence of improvement. Because release of serotonin in platelets precedes detectable morphological changes. The finding that cholesteryl ester was preferentially resorbed coincidentally with these functional changes may or may not be important. Because cholesterol is esterified intracellularly, changes in cholesteryl ester may reflect changes in cellular components of the arterial wall, including leukocytes and smooth muscle cells. Because leukocytes release oxygen radicals and may degrade EDRF, the decrease in cholesteryl ester may reflect a decrease in the number of leukocytes and improvement in vasodilatation. Alternatively, resorption of cholesteryl ester may produce biochemical changes in lipid-laden leukocytes and perhaps decrease free radical production. Thus, vasoconstrictor responses to serotonin improve markedly and rapidly during regression of atherosclerosis in most monkeys, before morphometric evidence of improvement. Because release of serotonin in platelets has been implicated in vasospasm and unstable ischemic syndromes, we speculate that treatment of hypercholesterolemia and atherosclerosis may have salutary effects before regression of fixed stenoses. Moreover, we suggest that vasoconstrictor responses may be more sensitive than measurement of baseline arterial diameter as an index of regression of atherosclerosis.

Acknowledgments

We wish to thank Pam Tompkins for her technical assistance with morphometric measurements, Dr James Rossen for assistance with quantitative angiography, and Dr M. Bridget Zimmerman for assistance with statistical analysis. This study was supported by National Institutes of Health grants HL-14388, HL-16066, NS-24621, and AG-10269 and research funds from the Veterans Administration.

References


Functional improvement precedes structural regression of atherosclerosis.
K H Benzuly, R C Padgett, S Kaul, D J Piegors, M L Armstrong and D D Heistad

Circulation. 1994;89:1810-1818
doi: 10.1161/01.CIR.89.4.1810

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/89/4/1810

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/