Vasa Vasorum in Atherosclerotic Coronary Arteries: Responses to Vasoactive Stimuli and Regression of Atherosclerosis

J. Koudy Williams, Mark L. Armstrong, and Donald D. Heistad

The goals of this study were to determine whether vasa vasorum in atherosclerotic coronary arteries respond to vasoactive stimuli and to examine effects of regression of atherosclerosis on blood flow through vasa vasorum in coronary arteries. We studied three groups of monkeys: normal, atherosclerotic, and regression. Blood flow to vasa vasorum was measured with microspheres. Blood flow to intima-media (ml/min x 100 g) was 5 ± 1 (mean ± SEM) in normal and 47 ± 7 in atherosclerotic monkeys (p < 0.05). Infusion of phenylephrine or serotonin did not alter flow through vasa in normal monkeys. In atherosclerotic monkeys, phenylephrine decreased flow through vasa vasorum in intima-media of coronary arteries to 24 ± 4 (p < 0.05), and serotonin decreased flow to 27 ± 5 (p < 0.05).

In regression monkeys, blood flow to intima-media was sixfold less (7 ± 2 ml/min x 100 g) than in atherosclerotic monkeys (p < 0.05). During infusion of adenosine, blood flow to vasa increased fourfold greater than in atherosclerotic monkeys, but after regression of atherosclerosis. This finding suggests that atherosclerosis in regression monkeys (p < 0.05). During infusion of adenosine, blood flow to vasa increased fourfold greater than in atherosclerotic monkeys, but after regression of atherosclerosis. We conclude that vasa vasorum in atherosclerotic coronary arteries respond to vasoconstrictor stimuli and that there is loss of vasa vasorum and a large decrease in blood flow through vasa to intima-media of coronary arteries after regression of atherosclerosis. (Circulation Research 1988;62:515-523)

In the second part of the present study, the first measurements of blood flow through vasa vasorum in coronary arteries after regression of atherosclerosis were obtained. During regression of atherosclerosis, there is reduction of lesion mass, resorption of lipid from the vessel wall, and reduction of intimal thickness by about 40%. Vasa might respond to regression of atherosclerosis in several ways. First, regression might reduce growth factors and lead to loss of vasa. Second, regression might reduce vascular metabolism, with resultant reduction in blood flow through vasa without loss of vasa. Third, wall thickness is an important determinant of growth of vasa, and because the vessel wall remains thickened after regression of atherosclerosis, regression might not lead to loss of vasa. Our goal was to determine whether regression of atherosclerotic lesions in coronary arteries decreases blood flow through vasa vasorum.

Materials and Methods

Three groups of adult male cynomolgus monkeys were studied. Normal monkeys (n = 12) were fed Purina monkey chow (Ralston-Purina, Richmond, Indiana) that contains approximately 4% fat and is virtually free of cholesterol. Atherosclerosis was induced by feeding another group of monkeys (n = 12) a diet with 41% fat and 0.8% cholesterol for 17.5 ± 1 (mean ± SEM) months. A third group, regression monkeys (n = 12), were fed atherogenic diet for 18 months followed by normal diet for 17 ± 1 months. Normal monkeys weighed 4.9 ± 0.3 kg, atherosclerotic weighed 5.4 ± 0.5 kg, and regression weighed 5.0 ± 0.4 kg. Venous blood samples were drawn at 2-month intervals after the monkeys had been sedated.
with ketamine HCl 12 mg/kg i.m. Total plasma cholesterol and triglycerides were determined by the methods of the Lipid Research Clinics protocol.46

Measuring Blood Flow Through Vasa Vasorum

Monkeys were sedated with ketamine HCl (12 mg/kg i.m.) and anesthetized with chloralose (75 mg/kg i.v.). They were intubated and ventilated with room air that was supplemented with oxygen.

Catheters were inserted into a brachial artery for measurement of arterial pressure, a brachial and common carotid artery for withdrawal of reference blood samples, and a femoral vein for infusion of drugs. A thoracotomy was performed, and a catheter was placed in the left atrium for injection of microspheres. Blood gases and pH were measured frequently and maintained at normal levels. In normal, atherosclerotic, and regression monkeys, arterial blood Po2 was approximately 100–110 mm Hg, Pco2 was 33–35 mm Hg, and pH was 7.35–7.40.

Microspheres with a mean diameter of 15 or 50 μm were injected into the left atrium to measure blood flow through vasa vasorum, using the method described previously.37 Reference arterial blood samples were withdrawn at 1.03 ml/min starting 20 seconds before injection of microspheres and continuing for two minutes after injection. We injected 2–4 million 15-μm diameter spheres labeled with different isotopes to measure blood flow under control conditions and during infusion of adenosine, serotonin, and phenylephrine. The order of interventions and isotopes used for each intervention was randomized.

In four normal and four atherosclerotic monkeys, microspheres 15-μm diameter (2 million) and microspheres 50-μm diameter (0.4 million) were injected simultaneously during infusion of adenosine to determine whether there was arteriovenous shunting of 15-μm spheres.

The first goal of the present study was to examine responsiveness of vasa vasorum in atherosclerotic coronary arteries to vasoactive stimuli. Blood flow during infusion of serotonin (40 μg/kg/min in the left atrium) and infusion of phenylephrine (25 μg/kg/min i.v.) was measured. Serotonin was infused into the left atrium instead of intravenously because, during intravenous infusion, much of the serotonin is removed in the lungs.17

During control conditions, mean arterial pressure (in mm Hg) was 79 ± 3 in normal monkeys, 79 ± 5 in atherosclerotic monkeys, and 88 ± 4 in regression monkeys. Because changes in arterial pressure affect conductance of vasa,2 mean arterial pressure was maintained at control levels during infusion of serotonin and phenylephrine by removing blood.

The second goal of the present study was to compare blood flow through vasa vasorum in coronary arteries of normal, atherosclerotic, and regression monkeys. Blood flow was measured under control conditions and during infusion of adenosine at a dose (5 μM/kg/min i.v.) that appears to produce maximal dilatation of vasa vasorum.13

The monkeys were killed with intravenous potassium chloride at the end of each experiment. The proximal 2–3 cm of left anterior descending, circumflex, and right coronary arteries were removed and placed in 10% buffered formalin for 2 days. The intima and media were stripped from the adventitia with the aid of a dissecting microscope, using the method of Wolinsky and Daly.40 Histological examination of the intima-media indicated that tissue samples of intima-media had minimal contamination by adventitia.

Samples of intima-media and adventitia were weighed and counted in a gamma counter, together with the reference blood samples, as described previously.3,7 Samples of intima-media weighed approximately 0.05 g in normal and regression monkeys and 0.25 g in atherosclerotic monkeys. Samples of adventitia weighed approximately 0.15 g in each group. Blood flow through vasa vasorum was calculated from the equation: flow (ml/min/100 g vessel) = (counts/g coronary artery × 100 × rate of withdrawal of reference arterial blood sample in ml/min)/(total counts in reference arterial blood). An average value for counts in the two blood samples was calculated; samples of small bowel also were obtained. Conductance of vasa was calculated from the equation: conductance = blood flow (ml/min/g vessel)/(mean arterial pressure).

Values in normal, atherosclerotic, and regression monkeys were compared using unpaired t test. Bonferroni correction was used for multiple comparisons.19

Morphological Examination

The coronary arteries were examined grossly for the extent of atherosclerosis. Sections of formalin-fixed coronary arteries were examined with light microscopy. Intima-media area was determined using methods previously described.11-12 To visualize vasa vasorum in coronary arteries, colloidal carbon was infused into two normal, three atherosclerotic, and two regression monkeys. After the monkeys were killed, the proximal aorta was isolated by ligation of the ascending aorta and by inflation of a balloon in the left ventricle. Approximately 30 ml colloidal carbon was infused into the proximal aorta. The coronary arteries were removed, fixed in formalin, dehydrated by graded alcohols, and made translucent by clearing with methyl salicylate.20 Sections were cut approximately 1 mm in thickness and examined microscopically.

In two atherosclerotic monkeys, vasa vasorum in the intima-media of coronary arteries were examined using transmission electron microscopy. After the monkeys were killed, the proximal left anterior descending artery was cannulated and perfuse-fixed with 7% glutaraldehyde solution at 80-mm-Hg perfusion pressure. After fixation, the vessels were postfixed with osmium tetroxide, dehydrated with alcohol, and embedded in Epon 812. The vessels were stained en bloc with 2% uranyl acetate and 60-nm sections, which were obtained on a LKB ultratome III, were counterstained with alkaline lead.
Results

Plasma Lipids

Total plasma cholesterol concentration increased fivefold to sixfold within 2 months after feeding monkeys the atherogenic diet and changed little thereafter. Total plasma triglyceride concentrations were not elevated by the atherogenic diet. In regression monkeys, total plasma cholesterol concentration returned to near normal levels within 2 months after changing from an atherogenic diet to a normal diet. At the terminal measurement, the total cholesterol concentration was 93 ± 11 mg/dl in normal monkeys and 588 ± 25 mg/dl in atherosclerotic monkeys. In regression monkeys, total plasma cholesterol concentration was 542 ± 21 mg/dl while they were fed the atherogenic diet and 120 ± 5 mg/dl while they were fed the normal diet. The increase in cholesterol during atherogenic diet and the decrease during dietary treatment of atherosclerosis are produced primarily by a change in concentration of low-density lipoproteins.12,21

Morphology

In normal monkeys, the coronary arteries were thin-walled and were without gross or microscopic evidence of atherosclerotic lesions. In monkeys that were fed the atherogenic diet, the epicardial coronary arteries were thickened and elongated. Microscopic examination showed diffuse lesions throughout the epicardial arteries. The lesions ranged from fatty streaks to fibrofatty plaques.

There was a pronounced increase in cross-sectional area of the intima in the left circumflex and left anterior descending arteries. In normal monkeys, area of intima was <0.01 mm² in the circumflex and left anterior descending artery. Thus, area of intima was 0.37 ± 0.07 mm² in the left circumflex artery and 0.33 ± 0.05 mm² in the left anterior descending artery. In regression monkeys, area of intima was 0.37 ± 0.07 mm² in the left circumflex artery and 0.68 ± 0.07 mm² in the left anterior descending artery. In regression monkeys, there was loss of fatty material in the intima and reduction of lesional mass in epicardial arteries, but fibrous material was increased. In regression monkeys, area of intima was 0.37 ± 0.07 mm² in the left circumflex artery and 0.33 ± 0.05 mm² in the left anterior descending artery. Thus, area of intima in coronary arteries of regression monkeys decreased 50–60% from atherosclerotic coronary arteries but remained much greater than that of normal monkeys.

Calculation of wall thickness demonstrated pronounced thickening of coronary arteries in atherosclerotic monkeys (Table 1). Wall thickness of coronary arteries from regression monkeys decreased 30–40% from atherosclerotic coronary arteries but remained twofold to threefold thicker than normal coronary arteries. Lumen diameter was not reduced in atherosclerotic monkeys, despite intimal proliferation, and lumen diameter tended to be greater in regression monkeys than in normal monkeys.

In sections of coronary arteries that were prepared for examination of vasa vasorum by injection of colloidal carbon, vasa were observed in the adventitia of normal, atherosclerotic, and regression monkeys. In the present study, as in previous studies, it was not possible to quantitate the number of vasa on morphological examination. No vasa were seen in the media of coronary arteries from normal or regression monkeys. In atherosclerotic arteries, vasa were seen branching from adventitial vessels and penetrating into the intima-media. Vasa that originate from the lumen were not seen.

Atherosclerotic coronary arteries from two monkeys were examined using transmission electron microscopy. In each monkey, approximately 10 medial vasa vasorum that ranged in diameter from 20 to 100 μm were examined. Smooth muscle cells were seen surrounding endothelial cells of vasa vasorum (Figure 1). Smooth muscle cells were oriented around the vasa and not in relation to the coronary artery.

Blood Flow Through Vasa Vasorum: 15-μm Versus 50-μm Diameter Spheres

When 15- and 50-μm diameter spheres were injected simultaneously, values for blood flow to the adventitia obtained with 50-μm spheres were very high, and values for flow to the media were minimal, as observed previously in normal vessels.14 This finding suggests that 50-μm diameter spheres may be trapped in the adventitia and do not reach the media. Total blood flow to the wall of coronary arteries (adventitia plus intima-media) in normal and atherosclerotic monkeys was similar when 15-μm and 50-μm diameter spheres were injected simultaneously during infusion of adenosine. In normal monkeys, blood flow (in ml/min/100 g) to the wall of coronary arteries was 15 ± 4 measured with 15-μm diameter spheres and 18 ± 6 measured with 50-μm spheres. In atherosclerotic monkeys, blood flow to the wall of coronary arteries was 43 ± 11 measured with 15-μm diameter spheres and 50 ± 15 measured with 50-μm spheres. This finding suggests

<table>
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<th>Table 1. Morphometry of Coronary Arteries</th>
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<tr>
<td>Circumflex</td>
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<tr>
<td>Lumen diameter (mm)</td>
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<tr>
<td>Normal (n=12)</td>
</tr>
<tr>
<td>0.54 ± 0.03</td>
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<tr>
<td>Wall thickness (mm)</td>
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<tr>
<td>Normal (n=12)</td>
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<tr>
<td>0.08 ± 0.006</td>
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<tr>
<td>Intima (mm)</td>
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<tr>
<td>Normal (n=12)</td>
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<td>&lt;0.01</td>
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<tr>
<td>Media (mm)</td>
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<tr>
<td>Normal (n=12)</td>
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<tr>
<td>0.08 ± 0.005</td>
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Values are mean ± SEM.  
*p < 0.05 versus normal, †p < 0.05 versus atherosclerotic, using analysis of variance and Student-Newman-Keuls test.
that arteriovenous shunting of 15-μm spheres did not occur in vasa of atherosclerotic coronary arteries.

**Responsiveness of Vasa Vasorum**

In normal monkeys, blood flow through vasa vasorum in adventitia and intima-media of coronary arteries was not altered by infusion of phenylephrine or serotonin (Figure 2). Infusion of phenylephrine was efficacious as a vasoconstrictor: Blood flow to the jejunum was decreased from 60 ± 9 ml/min/100 g during control conditions to 30 ± 5 ml/min/100 g during infusion of phenylephrine in normal monkeys (p<0.05). Serotonin did not reduce blood flow to the jejunum in normal monkeys.

In atherosclerotic monkeys, blood flow through adventitial vasa of coronary arteries was not altered by
infusion of phenylephrine or serotonin (Figure 3). In contrast, blood flow through vasa vasorum in intima-media was decreased about 50% during infusion of phenylephrine and serotonin (Figure 3). In atherosclerotic monkeys, phenylephrine reduced flow to jejunum from 60 ± 9 to 32 ± 5 ml/min/100 g (p < 0.05), and serotonin reduced flow to jejunum from 60 ± 9 to 17 ± 4 ml/min/100 g (p < 0.05).

**Effect of Regression of Atherosclerosis: Blood Flow During Control Conditions**

In normal monkeys, there was minimal blood flow and conductance through vasa vasorum to the intima-media of coronary arteries (Figure 4). There were substantial levels of flow to the adventitia (Figure 5). Thus, in normal monkeys, the media of coronary arteries must receive little nourishment from medial vasa and must be nourished primarily by diffusion from the lumen of the artery and from adventitial vasa. Blood flow and conductance of vasa vasorum in the intima-media were increased eightfold in atherosclerotic coronary arteries (Figure 4). Blood flow and conductance of adventitial vasa in coronary arteries were not altered by atherosclerosis (Figure 5).

Regression of atherosclerosis reduced blood flow and conductance of vasa vasorum in the intima-media of coronary arteries toward normal levels (Figure 4). Regression of atherosclerosis did not alter blood flow or conductance of adventitial vasa in coronary arteries (Figure 5).

**Effect of Regression of Atherosclerosis: Blood Flow During Adenosine Infusion**

During infusion of adenosine, mean arterial pressure (in mm Hg) decreased from 79 ± 3 to 47 ± 5 in normal monkeys, 79 ± 5 to 57 ± 2 in atherosclerotic monkeys, and 88 ± 4 to 41 ± 3 in regression monkeys.

During infusion of adenosine, blood flow and conductance of vasa vasorum in the intima-media were sixfold to eightfold greater in atherosclerotic coronary arteries than in normal coronary arteries, and they decreased toward normal in regression monkeys (Figure 6). Blood flow and conductance of vasa in adventitia were similar in normal, atherosclerotic, and regression monkeys during infusion of adenosine (Figure 7).

**Discussion**

The major new findings of the present study are that vasa vasorum in intima-media of atherosclerotic coronary arteries contain smooth muscle and respond to vasoconstrictor stimuli and that blood flow returned to virtually normal levels in the intima-media of coronary arteries after regression of atherosclerosis. During infusion of adenosine, values for conductance of vasa vasorum in intima-media were fourfold less in coronary arteries of regression monkeys than atherosclerotic monkeys. This finding suggests that loss of vessels, not constriction of existing vessels, accounts for the decrease in blood flow through vasa in coronary arteries after regression of atherosclerosis.
erosclerotic coronary arteries respond to constrictor stimuli with surprising consistency with the ability of the vasa to respond to constrictor stimuli. The finding that vasa in the intima-media of atherosclerotic coronary arteries have a layer of smooth muscle is consistent with the ability of the vasa to respond to constrictor stimuli.

The concentration of serotonin that was achieved during its infusion in these experiments was estimated and compared with concentrations in blood that have been observed in vivo. We assumed that 90% of serotonin is cleared in one passage through the pulmonary circulation,17 that the circulation time in monkeys is 14 seconds, and that the space of distribution is limited to plasma. We thereby estimated that infusion of 40 μg/kg/min serotonin into the left atrium produces plasma concentrations of 215 ng/ml. For comparison, partial occlusion of a coronary artery with a thrombus increases the level of serotonin in blood distal to the occlusion from 9 to 213 ng/ml.24 Thus, the concentration of serotonin in blood that was achieved in the present study may be similar to the concentrations that occur in pathophysiological states. Furthermore, the concentration of serotonin in arterial segments under a thrombus has been reported to increase to over 800 ng/g of vessel.24

It is more difficult to compare the dose of phenylephrine that was used in the present study to endogenous α-adrenergic stimuli. Very high concentrations of norepinephrine can be achieved in the neuromuscular cleft of sympathetic nerves. It is difficult to estimate, however, the concentration of circulating or neuronal α-adrenergic agonists that may reach vasa vasorum.

Atherosclerosis augments vasoconstrictor responses to serotonin in the hind limb and cerebral vessels of monkeys.25,26 The constrictor response of vasa vasorum to serotonin and phenylephrine may reduce blood flow through vasa, despite pronounced constriction of atherosclerotic coronary arteries. Therefore, blood flow to the intima-media of atherosclerotic coronary arteries may be compromised at a time when there is increased metabolic demand on the artery.

Morphometry

In normal monkeys, the luminal diameter of the coronary arteries was approximately 0.5 mm. In atherosclerotic monkeys, the thickness of the intima increased from <0.01 to approximately 0.3 mm. If the intima had proliferated inward in atherosclerotic arteries, the lumen would have been virtually obliterated. Thus, it is remarkable that there was no reduction in luminal diameter in atherosclerotic arteries. Similar observations have been made previously in primates and humans.27,28 Although humans eventually develop obstructive lesions, compensatory mechanisms appa-
ently allow outward displacement of moderately severe lesions with relative preservation of the arterial lumen.

After regression of atherosclerosis, thickness of the arterial wall was reduced, in comparison with atherosclerotic monkeys, but thickness of the vessel wall remained substantially greater than normal. Despite the increased thickness of the wall in regression monkeys, the luminal diameter was greater than in normal monkeys. Thus, outward displacement of the wall, which is characteristic of atherosclerotic arteries, is not reversed by regression of atherosclerosis. In regression monkeys, as a result of outward displacement of the vessel wall by atherosclerosis and reduction of the intimal thickness toward normal during regression, luminal diameter becomes larger than normal.

**Loss of Vasa Vasorum During Regression of Atherosclerosis**

Morphological studies of coronary arteries have demonstrated proliferation of vasa vasorum in intima-media during atherosclerosis. In normal and regression monkeys, few vasa were observed in the intima-media of coronary arteries. No methods are available to quantitate vasa vasorum using morphological approaches. Therefore, changes in blood flow, during maximal vasodilatation, were used to estimate changes in density of vasa in coronary arteries.

Adenosine was infused to determine whether blood flow was decreased in the intima-media of coronary arteries in regression monkeys by constriction of vessels or loss of vessels. We reasoned that if resting flow is decreased in vasa to coronary arteries of regression monkeys as a result of constriction of vasa, values for conductance during infusion of adenosine in regression monkeys should be similar to those observed in atherosclerotic monkeys. We found, however, that blood flow and conductance of vasa during infusion of adenosine remained much lower in coronary arteries from regression monkeys than from atherosclerotic monkeys. This finding indicates that decreased blood flow in the intima-media of coronary arteries from regression monkeys is not produced primarily by constriction of vasa and must reflect loss of vessels that proliferate in the intima-media of atherosclerotic coronary arteries.

**Stimuli for Loss of Vasa**

We have speculated previously that several factors may contribute to formation of new vessels in intima-media of atherosclerotic coronary arteries. These factors include increases in vascular oxygen consumption, increased diffusion distance of nutrients due to intimal proliferation, alterations in radial stress, and synthesis of growth factors.

Loss of vasa vasorum in intima-media of coronary arteries after regression of atherosclerosis may be due to several factors. During regression of atherosclerosis, lesional mass is reduced and most of the lipid is removed from the thickened intima. Vessel wall thickness is decreased to approximately 60% of the thickness of atherosclerotic coronary arteries. Loss of lesional mass by removal of lipids, with a decrease in vessel wall thickness, decreases the diffusion distance of nutrients. Therefore, we speculate that loss of vasa in intima-media after regression of atherosclerosis occurs in part because thickness of the vessel wall decreases and diffusion of nutrients from adventitia and the lumen of the coronary artery are adequate to supply nutrients to the vessel wall.

Inflammation in the vessel wall is reduced during regression of atherosclerosis. Inflammatory products associated with wound healing have been reported to stimulate angiogenesis, and lipoxygenase products have been reported to be mitogenic. We speculate that reduction of inflammation during regression of atherosclerosis may reduce production of mitogenic factors and lead to loss of vasa.

Several growth factors have been identified that are associated with atherosclerotic lesions. Platelet-derived growth factor is a mitogenic factor that stimulates proliferation of smooth muscle cells in atherosclerotic lesion. Macrophages and fibroblasts also produce factors that stimulate angiogenesis in vitro. We speculate that locally released growth factors may stimulate growth of vessels in atherosclerotic coronary arteries. Loss of macrophages in the vessel wall during regression of atherosclerosis has been reported to affect regression of atherosclerotic lesions. Thus, loss of vasa during regression of atherosclerosis may be due in part to decreased release...
of growth factors. The present study, however, does not address specific factors that may account for growth and loss of vasa.

Implications of Findings

It has been suggested that new vasa may be fragile and prone to rupture because their structure differs from that of normal vasa and that rupture of vasa may produce intimal hemorrhage and coronary occlusion. Although the present study does not address this question directly, we found that vasa in atherosclerotic arteries contain vascular muscle and respond to constrictor stimuli. We speculate that vasa in atherosclerotic arteries may not be especially fragile and may actively regulate blood flow in the intima-media of atherosclerotic coronary arteries. The pronounced increase in blood flow to the intima-media in atherosclerotic coronary arteries suggests that vasa in intima-media may play an important role in nourishing these vessels. Loss of vasa varorum in the intima-media of coronary arteries during regression of atherosclerosis may indicate a decreased need for nourishment of the coronary artery. It has been proposed that new vasa in atherosclerotic coronary arteries may deliver increased amounts of vasoactive substances to the wall of the coronary arteries and thereby predispose to vascular spasm. We speculate that loss of vasa during regression of atherosclerosis may reduce delivery of vasoconstrictor substances to the coronary arteries and thus reduce susceptibility to coronary vasospasm.

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References


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