Functional and Structural Adaptations of Coronary Microvessels Distal to a Chronic Coronary Artery Stenosis


Abstract—Distal to a chronic coronary artery stenosis, structural remodeling of the microvasculature occurs. The microvascular functional changes distal to the stenosis have not been studied in detail. We tested the hypothesis that microvascular structural remodeling is accompanied by altered regulation of coronary vasomotor tone with increased responsiveness to endothelin-1. Vasomotor tone was studied in coronary microvessels from healthy control swine and from swine 3 to 4 months after implantation of an occluder that causes a progressive coronary narrowing, resulting in regional left ventricular dysfunction and blunted myocardial vasodilator reserve. Arterioles (≈200-μm passive inner diameter at 60 mm Hg) were isolated from regions perfused by the stenotic left anterior descending and normal left circumflex coronary arteries and studied in vitro. Passive pressure–diameter curves demonstrated reduced distensibility of subendocardial left anterior descending compared with subendocardial left circumflex or control arterioles, suggestive of structural remodeling. Myogenic responses were blunted in subendocardial left anterior descending compared with left circumflex arterioles, reflecting altered smooth muscle function. However, vasodilator responses to nitroprusside and bradykinin were not different in the endocardium, suggesting preserved endothelium and smooth muscle responsiveness. Finally, vasoconstrictor responses to endothelin-1 were enhanced in left anterior descending arterioles compared with left circumflex or control arterioles. Regional myocardial vascular conductance responses to bradykinin and endothelin in vivo confirmed the in vitro observations. In conclusion, inward remodeling of coronary microvessels distal to a stenosis is accompanied by exaggerated vasoconstrictor responses to endothelin-1. These structural and functional alterations may aggravate flow abnormalities distal to a chronic coronary artery stenosis. (Circ Res. 2008;102:795-803.)

Key Words: coronary artery disease ■ stenosis ■ microcirculation ■ remodeling ■ endothelin

Ischemic heart disease is often the result of progression of a stenosis in a major coronary artery. The resulting myocardial ischemia can lead to chronic ventricular dysfunction in the absence of infarction that can improve after revascularization.1,2 However, some patients show a delayed functional recovery of the ventricle despite revascularization,3 indicating that revascularization is not always sufficient for complete functional recovery. Moreover, data obtained in humans and animals indicate that progression of a stenosis results not only in functional but also in structural modifications of myocardial tissue, including an increased rate of apoptosis and fibrosis, some of which (apoptosis) even extend into remote ventricular regions with normal resting perfusion.4,5 In addition to these perturbations in the cardiomyocyte and extracellular compartments, there is evidence for microvascular abnormalities in chronically ischemic hearts.6,7 Animal studies suggest that vascular structural inward remodeling occurs in coronary resistance vessels situated downstream of a flow-limiting severe coronary stenosis (≈80% reduction in lumen diameter),8,9 which may explain the increase in minimal microvascular resistance distal to a stenosis in patients with a coronary artery stenosis.10 Furthermore, there are some indications of microvascular functional abnormalities in patients with coronary artery disease in vivo.11–14 However, the functional alterations within native microvessels embedded in dysfunctional myocardium distal to a chronic coronary stenosis have not been thoroughly investigated to date. In addition, it is not known whether these changes are restricted to the region distal to the stenosis or also extend into the remote myocardium.

In light of these considerations, we tested the hypothesis that microvascular functional abnormalities occur in chroni-
cally ischemic myocardium, as well as in the remote normally perfused myocardium. For this purpose, we investigated the presence of endothelial dysfunction and sensitivity to endothelin (ET)-1 in subendocardial and subepicardial arterioles in a swine model of chronic coronary artery stenosis.5,15,16

Materials and Methods

Animals

Studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996) and with approval of the Erasmus MC Animal Care Committee. Twenty-six, 2-month-old Yorkshire X Landrace swine (13 ± 1 kg at surgery; 66 ± 2 kg at euthanasia) of either sex entered the study. Five animals died prematurely, and 2 animals were excluded because of the presence of significant infarction in the left anterior descending (LAD) area. Thirteen animals, matched for weight (64 ± 2 kg at euthanasia) and sex were used as healthy controls.

Surgical Procedures

Swine were sedated with ketamine (30 mg/kg IM), anesthetized with thiopental (10 mg/kg IV), intubated, and ventilated with a mixture of O2 and N2O (1:2). Anesthesia was maintained with midazolam (2 mg/kg plus 1 mg/kg per hour IV) and fentanyl (10 μg/kg per hour IV).16-17 Under sterile conditions, the chest was opened via the third left intercostal space and an initial 10% reduction in lumen diameter was created by placing a plastic C-shaped occluder around the LAD coronary artery, immediately below the first side branch.5,15,16 Animals were allowed to recover from surgery and followed for the next 13 ± 0.3 weeks. Because the animals grow, the stenosis becomes progressively more obstructive.5,3,15

Experimental Protocols

In Vivo Hemodynamic Measurements

Pigs were sedated with ketamine (30 mg/kg IM) and midazolam (2 mg/kg IM), anesthetized with pentobarbital (20 mg/kg IV), and ventilated.18-19 Closed-chest angiograms of left and right coronary arteries were performed to assess stenosis severity and examine the extent of collateralization. Catheters were implanted for measurement of left ventricular and aortic blood pressure cardiac output (via thermodilution) and for blood sampling.17,18 Following thoracotomy, ultrasonic crystals were implanted in the LAD and left circumflex (LCx) perfusion areas for assessment of regional contractile function.19 In 10 animals with stenosis and 7 controls, an intracoronary fluid-filled catheter was inserted into the LAD distal to the occlusion to measure the poststenotic perfusion pressure of the distal microvasculature and the trans-stenosis pressure gradient. After completion of instrumentation, a 30-minute stabilization period was allowed, after which all hemodynamics and global and regional myocardial function measurements were obtained, and arterial blood samples were collected for ET plasma level determination.20,21 In 1 group of swine, hearts were arrested and immediately excised and used for dissection of coronary arterioles for in vitro studies. In a second group of animals, coronary function was studied in vivo.

Vascular Function In Vitro

After excision, the heart was placed in 4°C MOPS buffer. Resistance arteries of ~200 μm (see Table I in the online data supplement, available at http://circres.ahajournals.org) were isolated from both the subendocardium and subepicardium of the LAD and LCx region of stenosis animals (N = 13) and control animals (N = 9) and studied in a pressure myograph at a constant pressure of 60 mm Hg and no-flow conditions.22-24 Experiments were performed in 37°C Leibovitz culture medium. The intraluminal medium was supplemented with 10% heat-inactivated FCS to functionally preserve the endothelium. Because no differences in responses of vessels from LAD and LCx regions of control swine were recorded, data from these vessels were pooled.

After equilibration for 30 minutes at 60 mm Hg, a small number of subendocardial vessels from the LAD (N = 5) and LCx (N = 4) regions of the left ventricles of swine with stenosis developed spontaneous myogenic tone. In these vessels, the active pressure–diameter curve was recorded. After diameter stabilization at 60 mm Hg, pressure was changed in 20 mm Hg steps, between 20 and 120 mm Hg, and inner diameters were measured at each pressure.

In coronary arterioles isolated from 6 swine with stenosis and 9 controls, the dose–response relation for ET-1 (10-11 to 10-7 mol/L) was performed. Each concentration was maintained for 3 to 5 minutes, and responses were recorded during steady state. After 5 minutes of washout of ET-1, the concentration–response curve for bradykinin (10-10 to 10-7 mol/L) was recorded, using peak vasodilation at each concentration. Importantly, the vasodilator responses to bradykinin were not dependent on ET-1 pretreatment because similar vasodilator responses were observed when either U46619 or methacholine (a vasconstrictor in the porcine coronary circulation) was used as a preconstrictor (supplemental Figure I). Bradykinin was subsequently washed out for 15 minutes, and vascular smooth muscle reactivity to sodium-nitroprusside (SNP) was tested in a dose-dependent manner (10-10 to 5 × 10-7 mol/L). Finally, the passive pressure–diameter relations (between 10 to 120 mm Hg) were recorded in vessels fully dilated with 5 × 10-3 mol/L papaverine.25 Additional experiments demonstrated identical passive pressure–diameter curves with 5 × 10-3 mol/L papaverine during U46619 (supplemental Figure II), and identical passive pressure–diameter curves with 2 × 10-4 mol/L papaverine in Ca2+-free buffer (supplemental Figure III), indicating that ET-1 pretreatment did not interfere with assessment of the passive diameter. At full dilation and 60 mm Hg, wall cross-sectional areas and wall-to-lumen ratios were calculated from the vascular inner and outer diameters, as measured from digitized video images in subendocardial arterioles.

Subendocardial vessels were isolated from the LAD and LCx regions of 7 additional swine with stenosis, and concentration–response to ET-1 were recorded in control conditions and after 30 minutes of incubation with 10-8 mol/L BQ123 (ETα receptor antagonist), 10-7 mol/L BQ788 (ETβ receptor antagonist), or combined ETα and ETβ receptor blockade.26 Given the long-lasting vasoactive effect of ET-1, each protocol was performed on a separate vessel to prevent additive effects of successive ET treatments.

Vascular Function In Vivo

To study coronary function in vivo, changes in flow to various stimuli were assessed using neutron-activated microspheres (BioPAL) in 6 swine with stenosis and 4 control swine. Microspheres were injected when hemodynamics had stabilized (~6 to 8 minutes after initiation of each infusion). Flow reserve was assessed using adenosine (0.9 mg/kg per minute IV).3,4 After washout, endothelium-dependent vasodilation was assessed using bradykinin (0.3 and 1.0 μg/kg per minute IV).7 The α1- and α2-receptor agonist phenylephrine was confused during adenosine and bradykinin to maintain aortic pressure at ~100 mm Hg,28 while leaving coronary resistance vessel tone unperturbed.29 After washout, vasoconstrictor responses to ET (50 and 100 ng/kg per minute IV)20 were assessed. Subsequently, the heart was excised, LAD and LCx perfusion territories were separated, and each divided into 2 layers (subendocardium and subepicardium). Tissue was weighed, dried, and sent to BioPAL for analysis of microsphere flows. Coronary vascular conductance was calculated as the ratio of microsphere flow and mean aortic pressure. To allow comparison of these in vivo responses to those in isolated vessels, conductance was normalized to maximal coronary vascular conductance, as determined with adenosine. Because vascular conductance responses in LAD and LCx swine were virtually identical, data were pooled.

Morphometry

Subendocardial and subepicardial tissue samples from swine with LAD stenosis and control swine were obtained immediately after excision of the heart, fixated in 4% formaldehyde, and embedded in paraffin. Subsequently, 4- to 5-μm-thick slides were stained for histological analyses. Myocyte hypertrophy was quantified with a
### Table 1. Hemodynamic Effects of a Chronic LAD Stenosis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control Swine (N=7)</th>
<th>Swine With Stenosis (N=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hemodynamics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>106±6</td>
<td>118±5</td>
</tr>
<tr>
<td>Cardiac output (L/min)</td>
<td>5.4±0.4</td>
<td>5.9±0.3</td>
</tr>
<tr>
<td>Mean aortic pressure (mm Hg)</td>
<td>95±4</td>
<td>96±5</td>
</tr>
<tr>
<td>∆P stenosis (mm Hg)</td>
<td>3±2</td>
<td>19±7</td>
</tr>
<tr>
<td>Global LV function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV dP/dt0.01 (mm Hg/sec)</td>
<td>1811±144</td>
<td>1949±160</td>
</tr>
<tr>
<td>LV dP/dt0.90 (mm Hg/sec)</td>
<td>−2576±346</td>
<td>−2588±183</td>
</tr>
<tr>
<td>r (ms)</td>
<td>36±2</td>
<td>38±2</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>8±1</td>
<td>12±1</td>
</tr>
<tr>
<td>Local LV function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% SS LAD</td>
<td>14±1</td>
<td>7±2*</td>
</tr>
<tr>
<td>% SS LCx</td>
<td>13±2</td>
<td>11±2</td>
</tr>
<tr>
<td>Plasma [ET] (pg/ml)</td>
<td>1.08±0.20</td>
<td>1.78±0.08*</td>
</tr>
</tbody>
</table>

Data are means±SEM. ∆P stenosis indicates pressure gradient across the stenosis; LV dP/dt0.01/0.90, maximum and minimum rate of rise/decay of left ventricular pressure; LVEDP, left ventricular end diastolic pressure; r, time constant of exponential LV pressure decay; SS, regional systolic shortening. *P<0.05 vs control (unpaired t test).

### Results

#### Hemodynamic Data

The presence of the stenosis in the LAD did not result in significant changes in mean aortic pressure, heart rate, or cardiac output (Table 1). The reduction in vessel lumen area was 84±3%, as measured from the angiographic images. Perfusion pressure distal to the stenosis was 19±7 mm Hg lower than aortic pressure (P<0.05), whereas a difference of 3±2 mm Hg was recorded at the same location in control swine. Moreover, flow reserve as measured by infusion of adenosine was significantly reduced distal to the stenosis (Table 2). Global left ventricular function was essentially maintained, although there were trends toward diastolic dysfunction because left ventricular end-diastolic pressure tended to be higher in swine with stenosis compared with control animals, (Table 1, P=0.07). However, regional left ventricular dysfunction was seen in animals with LAD stenosis, as indicated by a significant reduction in systolic fractional shortening in the LAD region distal to the stenosis compared with the LCx region or corresponding regions in control animals. The regional left ventricular dysfunction was correlated with the severity of the stenosis (R²=0.80, P<0.05).

#### Plasma Levels of ET

The plasma levels of ET in swine with chronic LAD stenosis were significantly elevated compared with healthy animals (Table 1). The increase in ET plasma concentration was not correlated with the degree of ventricular dysfunction.

#### Morphometry

The TUNEL assay of subendocardial sections indicated an increased regional myocyte apoptosis rate in the area perfused by the stenotic LAD compared with the LCx region.

---

**Chemicals**

Leibovitz culture medium was obtained from Gibco (Paisley, UK); ET-1, nitroprusside, bradykinin, BQ788, and BQ123 were obtained from Sigma Chemicals (Zwijndrecht, The Netherlands).

**Data Analysis**

Microvascular diameter changes in response to bradykinin, SNP, and ET-1 were normalized to the value of the passive inner diameter measured at 60 mm Hg in the presence of 5×10⁻⁵ mol/L papaverine unless otherwise stated in the text. Data are presented as means±SEM. Statistical significance of microvessel responses was established by 2-way ANOVA for repeated measures, followed by Bonferroni post hoc tests when appropriate. A value of P<0.05 was considered statistically significant. Hemodynamics and ET plasma levels were analyzed using unpaired t test. Myocardial blood flow responses to adenosine were tested using 2-way ANOVA for repeated measures, followed by paired or unpaired t test as appropriate. Vascular conductance responses to bradykinin and ET were tested using 2-way ANOVA.
(Figure 1A and 1B), which was accompanied by a trend toward hypertrophy of the remaining myocytes (Figure 1C–1F) and increased collagen deposition (Figure 1G and 1H).

Vascular Functional and Structural Studies

Myogenic Responsiveness

Subendocardial vessels from both LAD and LCx regions of animals with stenosis developed myogenic tone at a constant pressure of 60 mm Hg. However, when pressure was increased stepwise from 20 to 120 mm Hg, LCx arterioles showed prominent myogenic responses, with diameters actually decreasing despite an increase in distension pressure, whereas in the LAD arterioles, the myogenic responses to pressure variation were blunted so that diameters were maintained (Figure 2 and supplemental Figure IV).

Vascular Responsiveness

Endothelial functional integrity in vessels isolated from the LAD and LCx region of swine with stenosis and control swine was assessed by recording the vascular responses to bradykinin. Following washout of ET-1, arterioles stabilized to diameters that were not significantly different between groups (supplemental Table I), although subepicardial control arterioles showed a tendency toward an increased level of preconstriction (P=NS by ANOVA). Bradykinin induced vasodilation in all vessels. In animals with LAD stenosis, subendocardial arterioles showed a similar response to bradykinin when compared with control arterioles (Figure 3A).

In contrast, subepicardial arterioles of both LAD and LCx regions isolated from animals with stenosis showed a blunted response to bradykinin. Importantly, the bradykinin-induced coronary vasodilation in vivo was not affected by the presence of a stenosis (Figure 4, left).
The reactivity of the smooth muscle cells to NO was tested in response to nitroprusside. Vessels in all groups had similar initial levels of tone (P>NS by ANOVA). Nitroprusside induced vasodilation in vessels from all groups. As seen in Figure 3C and 3D, there was no difference in nitroprusside-induced vasodilation between vessels irrespective of group (P>NS by ANOVA).

The concentration–response curve for ET-1 in arterioles isolated from the stenosed LAD region was shifted to the left compared with the LCx or control vessels, indicating an increased sensitivity to ET-1 of these arterioles (Figure 3E and 3F). This increase was not correlated to the ventricular dysfunction or the plasma ET concentrations. No difference in response was recorded between subendocardial and subepicardial arterioles from each region. Moreover, there was no statistically significant difference in responses to ET-1 between vessels from the LCx region of animals with ventricular dysfunction compared with control arterioles.

ET-1 Receptor Blockade
In a separate group of animals, we further investigated the mechanisms responsible for the ET-1-induced constriction in subendocardial arterioles. In vessels from both LAD and LCx regions of swine with stenosis, the ETA receptor was mostly responsible for the ET-1-induced vasoconstriction (Figure 5A and 5C). In vessels from the LAD region, blockade of the ETB receptor had no significant effect on the ET-induced vasoconstriction (Figure 5B). However, in the LCx arterioles, ETB receptor blockade induced a deeper vasoconstriction, indicating that in these arterioles, ET induces also vasodilation via ETB receptors (Figure 5D).

Passive Pressure–Diameter Relations
Vessels were maximally dilated with 5×10⁻⁵ mol/L papaverine. After increase in intraluminal pressure, vessels isolated from the subendocardial region of the hearts perfused by the stenotic LAD showed an increased stiffness, especially when pressure increased to >40 mm Hg, when compared with LCx or control arterioles (Figure 6A; P<0.05 by ANOVA).

---

**Figure 3.** Concentration–response curves to bradykinin (A and B), SNP (C and D), and ET-1 (E and F) in subendocardial (ENDO) and subepicardial (EPI) arterioles (a) isolated from the LAD and LCx areas of swine (s) with LAD stenosis and control animals. Data from LAD and LCx arterioles of control animals were pooled. *P<0.05, LAD versus LCx; #P<0.05, LAD vs control; †P<0.05, LCx vs control; ‡P<0.05 control subendocardium vs control subepicardium by ANOVA.

**Figure 4.** Vasomotor responses (change in vascular conductance normalized to maximal conductance determined with adenosine) to bradykinin and ET-1 in vivo in the LAD and LCx regions of swine with stenosis (BL), healthy controls (BK), and ET-1 induced constriction in subendocardial arterioles. In vessels from both LAD and LCx regions of swine with stenosis, the ETα receptor was mostly responsible for the ET-1-induced vasoconstriction (Figure 5A and 5C). In vessels from the LAD region, blockade of the ETB receptor had no significant effect on the ET-induced vasoconstriction (Figure 5B). However, in the LCx arterioles, ETB receptor blockade induced a deeper vasoconstriction, indicating that in these arterioles, ET induces also vasodilation via ETB receptors (Figure 5D).
ANOVA, LAD versus LCx or control). No such difference in stiffness was seen in subepicardial arterioles (Figure 6B). The increased stiffness in the subendocardial vessels originating from the stenotic LAD was consistent with vascular inward remodeling. There was no significant difference in wall cross-sectional areas between groups, suggesting similar amounts of wall material (Figure 6C). Wall/lumen diameter ratio tended to increase (P>0.05) in subendocardial LAD arterioles compared with remote LCx or control arterioles, consistent with slight inward remodeling (Figure 6D).

Discussion
Previous studies have shown that structural remodeling occurs in microvessels distal to a chronic coronary artery occlusion,8,9 which likely contributes to an increased minimal coronary vascular resistance.10 The present study is the first to report on specific alterations in regulation of microvascular tone. Our main finding is that arterioles embedded in the dysfunctional region of the left ventricle distal to a chronic coronary artery stenosis are altered in the microvasculature distal to a chronic coronary artery stenosis. First, the chronic stenosis resulted in impaired myogenic responsiveness in distal subendocardial arterioles compared with the remote area, so that the active pressure–diameter curve in the LAD arterioles was flat, indicating that the myogenic vasoconstriction at high pressures and vasodilation at low pressures was blunted. The latter may result in impaired arteriolar vasodilation in response to the lower perfusion pressures that are present distal to the stenosis. Nitroprusside-induced vasodilation was unperturbed, suggesting that vascular smooth muscle responsiveness was maintained and that the blunted myogenic response was not the result of generalized loss of vascular smooth muscle reactivity. Second, we observed both in vitro and in vivo that resistance vessels distal to the LAD stenosis showed an increased sensitivity to ET-1 compared with the vessels from the remote LCx region or from healthy hearts. In conjunction with higher concentrations of circulating plasma ET, which may be attributable to neurohumoral activation as a result of the left ventricular dysfunction,34,35 the increased ET-mediated vasoconstrictor influence could further limit myocardial perfusion. In support of this concept, endogenous ET was shown to limit blood flow to collateral-dependent myocardium in pigs with an ameroid occluder.36 The increased sensitivity to ET in resistance vessels from the LAD region in the present study appeared to be the result of a loss of net ETB receptor–mediated vasodilation. Thus, ETB receptor blockade produced vasoconstriction in vessels from the remote LCx region or from healthy controls (indicating a net vasodilator influence in these vessels), which is consistent with earlier observations in the porcine coronary microcirculation.21 In contrast, ETB blockade had no effect on ET-1–mediated vasoconstriction in the vessels from the LAD area. The latter could be explained by a loss of endothelial ETB receptor–mediated vasodilation, which is known to involve NO and prostaglandin I2.37 Alternatively, an increased vascular smooth muscle ETB-mediated vasoconstriction could also have contributed to the increased sensitivity to ET. This is likely in view of the observation that bradykinin-induced, endothelium-dependent vasodilation was maintained in the subendocardial resistance vessels in vivo and in vitro. Future studies are required to investigate the underlying molecular mechanism of the blunted ETB receptor–mediated coronary microvascular dilation in chronically ischemic hearts.

Figure 5. Concentration–response curves to ET-1 in the absence (control) and presence of the ETa receptor blocker BQ123 (A and C), ETB receptor blocker BQ788, or combined ETa and ETB receptor blockade (B and D) in subendocardial arterioles (a) isolated from the LAD and LCx areas of swine (s) with LAD stenosis. *P<0.05, ETa blockade vs control; †P<0.05, ETa blockade vs control; ‡P<0.05, ETa blockade vs ETB blockade; §P<0.1, ETB blockade vs control; P<0.1 effect of ETB blockade in LAD vs LCx arterioles.

Alterations in Control of Vascular Tone
Myriad vasomotor control mechanisms contribute to the regulation of coronary microvascular tone and structure in the healthy heart. We observed that several of these mechanisms are altered in the microvasculature distal to a chronic coronary stenosis. First, the chronic stenosis resulted in impaired myogenic responsiveness in distal subendocardial arterioles compared with the remote area, so that the active pressure–diameter curve in the LAD arterioles was flat, indicating that the myogenic vasoconstriction at high pressures and vasodilation at low pressures was blunted. The latter may result in impaired arteriolar vasodilation in response to the lower...
Alterations in Coronary Microvascular Structure

Subendocardial arterioles distal to the stenosis showed increased passive stiffness and a tendency toward an increased wall/lumen diameter ratio, suggestive of structural inward remodeling. Remodeling appears to be eutrophic because this increased wall/lumen ratio was not associated with an increase in wall cross-sectional area, suggesting a geometric reorganization of wall components around a smaller lumen. Previous animal studies have shown microvascular hypertrophic remodeling, which may be attributable to the severity of the flow-limiting stenosis in those studies, whereas the animals in the present study had only moderate stenoses (75 mm Hg perfusion pressure distal to the stenosis). Although the mechanisms underlying the structural remodeling cannot be determined in the present study, such remodeling could be attributable to the severity of the flow-limiting stenosis in those studies, whereas the animals in the present study had only moderate stenoses (75 mm Hg perfusion pressure distal to the stenosis).

Methodological Considerations

The development of a stenosis in 1 of the major coronary artery may not immediately result in flow limitation, leading to sustained ischemia and infarction, but may lead to episodes of local acute stunning during increased metabolic demand. Repetitive episodes of acute stunning can lead to cumulative and sustained contractile dysfunction distal to the occluded artery without a reduced resting flow, a situation defined as chronic stunning. As the stenosis increases in severity, resting flow will eventually be impaired in the dysfunctional part of the ventricle in the absence of infarction. Such a state of matched reduction in myocardial perfusion and function, which has been shown to be (partially) reversible on revascularization, has been termed myocardial hibernation.

In the present study, the chronic LAD stenosis had not yet resulted in reduced basal subendocardial flow (subendocardial flow was actually slightly elevated, possibly because of the higher basal heart rate in the stenosis group). However, subendocardial flow reserve was significantly impaired, which is associated with sustained regional myocardial hypofunction and increased levels of glycogen deposition in the dysfunctional area. The notion that our model may represent chronic stunning rather than true hibernation could also be
perceived by the observation that perfusion pressures distal to the LAD stenosis measured in the present study (\(=75\) mm Hg) were much higher than the pressures typically associated with decreased basal perfusion (\(<40\) mm Hg\(^9\)). Finally, in the LAD subendocardium, we observed increased numbers of apoptotic cells, together with a significant increase in connective tissue, but only a trend toward hypertrophy of the remaining myocytes. Taken together, these findings could be interpreted to suggest that our model represents chronic stunning,\(^5\) although it is important to recognize that, rather than being separate entities, chronic stunning and hibernation are a continuum in the pathophysiology of chronic ischemia produced by a progressively severe chronic coronary artery stenosis.\(^5\) This may explain why we observed negligible impairment of bradykinin-induced resistance vessel dilation distal to the stenosis, whereas in pigs with a complete coronary occlusion, bradykinin-mediated vasodilation of arterioles isolated from collateral-dependent myocardium is significantly blunted.\(^40\) These observations suggest that with increased severity of coronary artery obstruction, microvascular abnormalities are also likely to become more severe.

**Clinical Implications**

Although it is difficult to determine the independent roles of the stenosis and microcirculatory abnormalities on metabolic flow regulation and autoregulation in the awake state in vivo, the present study shows that microvasculature distal to a chronic coronary artery stenosis undergoes significant functional alterations, including altered myogenic activity and increased sensitivity to ET-1. These chronic functional alterations may result in a state of sustained microvascular vasconstriction and may reduce the vasodilator capacity of the microcirculation, potentially comprising myocardial perfusion at high metabolic demand. A sustained state of vasconstriction may contribute to the structural microvascular modifications reported in animals\(^8,9\) and increased minimal microvascular resistance and distal vasoconstriction reported in patients with coronary artery disease,\(^10,13,4\) further limiting myocardial perfusion and thereby enhancing the progressive loss of myocytes.\(^3\) Because myocardial viability is an important determinant of functional recovery after revascularization, the microvascular functional and structural alterations distal to a chronic stenosis should be considered as future targets for therapy to optimize perfusion in patients with coronary artery disease, particularly those patients that are not candidates for revascularization therapy.

**Acknowledgments**

We gratefully acknowledge Shahla Kazim for technical assistance. We, the authors, had full access to the data and take responsibility for its integrity. All of us have read and approved the manuscript as written.

**Sources of Funding**

This work was supported by Netherlands Heart Foundation grant 2000T038 (to D.J.D. and B.H.), 2000T042 (to D.M. and V.J.d.B.).

**Disclosures**

None.

**References**

Functional and Structural Adaptations of Coronary Microvessels Distal to a Chronic Coronary Artery Stenosis


Circ Res. 2008;102:795-803; originally published online February 21, 2008; doi: 10.1161/CIRCRESAHA.108.172528

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/102/7/795

Data Supplement (unedited) at:
http://circres.ahajournals.org/content/suppl/2008/02/26/CIRCRESAHA.108.172528.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research 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 Research is online at:
http://circres.ahajournals.org//subscriptions/
### Table S1: Vascular Inner Diameters

<table>
<thead>
<tr>
<th></th>
<th>LAD</th>
<th>LCx</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endo</td>
<td>Epi</td>
<td>Endo</td>
</tr>
<tr>
<td>Diameter before ET-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µm)</td>
<td>177±28</td>
<td>195±19</td>
<td>174±15</td>
</tr>
<tr>
<td>(%)</td>
<td>94±4</td>
<td>95±4</td>
<td>95±3</td>
</tr>
<tr>
<td>Diameter before bradykinin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µm)</td>
<td>52±12</td>
<td>58±17</td>
<td>78±13</td>
</tr>
<tr>
<td>(%)</td>
<td>28±7</td>
<td>26±6</td>
<td>37±6</td>
</tr>
<tr>
<td>Diameter before nitroprusside</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µm)</td>
<td>94±13</td>
<td>113±20</td>
<td>117±16</td>
</tr>
<tr>
<td>(%)</td>
<td>51±5</td>
<td>52±6</td>
<td>61±7</td>
</tr>
<tr>
<td>Passive diameter at 60 mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µm)</td>
<td>190±19</td>
<td>204±19</td>
<td>192±20</td>
</tr>
</tbody>
</table>

Data are mean±SEM. Diameters (µm and % of passive diameter) were determined at 60 mmHg and prior to the concentration-diameter curves for bradykinin, nitroprusside and endothelin-1 and at maximal vasodilation. There were no significant differences between vessels in LAD, LCx from chronically ischemic or control hearts.
Figure S1. Concentration-responses to bradykinin obtained in arterioles (a) isolated from control swine (s) and preconstricted by ET-1, 1µM U46619 or 1µM metacholine. In view of the differences in baseline vascular tone following preconstriction with each vasoconstrictor, data are normalized according to the formula: $(D-D_0)/(D_{papaverine}-D_0)$. Data are as mean±SEM. P=NS by two-way ANOVA.
Figure S2. Passive pressure-diameter curves constructed in the presence of 50 µM papaverine in sub-endocardial arterioles of control swine, upon preconstriction by ET-1 (following a concentration-response curve for ET-1), and 1µM U46619. Data are normalized to the diameter at 10 mmHg and presented as mean±SEM, (P=NS by two-way ANOVA).
Figure S3. Passive pressure-diameter curves obtained in sub-endocardial arterioles (a) of control swine (s), upon preconstriction by ET-1 (following a concentration-response curve for ET-1) in the presence of 50 µM papaverine and in the presence of 200 µM papaverine in Ca\(^{2+}\) free buffer. Data are normalized to the diameter at 10 mmHg and presented as mean±SEM, (\(P=NS\) by two-way ANOVA).
Figure S4. Example of a tracing of transient myogenic response in a subendocardial arteriole from the LAD region perfused by the stenotic LAD coronary artery. The example illustrates that although the myogenic response was still present it did not result in a diameter reduction in these vessels (in contrast to vessels from the LCx region, see Fig. 2 of the manuscript).