Endothelial Dysfunction of the Coronary Microvasculature Is Associated With Impaired Coronary Blood Flow Regulation in Patients With Early Atherosclerosis

Andreas M. Zeiher, MD; Helmut Drexler, MD; Helmut Wollsläger, MD; and Hänjörg Just, MD

Background. The vascular endothelium is capable of regulating tissue perfusion by the release of endothelium-derived relaxing factor to modulate vasomotor tone of the resistance vasculature.

Methods and Results. To test whether atherosclerosis is associated with a functional abnormality of endothelium-mediated microvascular relaxation affecting coronary blood flow regulation, we compared coronary blood flow responses with cold pressor testing with the response of the coronary vasculature to acetylcholine (an endothelium-dependent vasodilator) and to papaverin (a direct dilator of vascular smooth muscle) in 12 normal control patients and in 19 patients with non-flow-limiting epicardial atherosclerosis (CAD). The drugs were subselectively infused into the left anterior descending coronary artery via a Doppler catheter, and the response in coronary blood flow was assessed by measuring intracoronary blood flow velocity and cross-sectional vascular area (quantitative angiography). Coronary vascular resistance decreased in all normal control patients by -24.1±5.5% (mean±SD) during the cold pressor test, whereas the CAD patients demonstrated a variable coronary vascular resistance response to cold pressor testing despite comparable changes in the rate-pressure product. The slopes of the acetylcholine dose-blood flow response (percent change in coronary blood flow/dosage of acetylcholine) were significantly reduced in the CAD patients with 38.5±24.8 compared with the normal patients (80.8±28.1; p < 0.001). Although coronary blood flow responses to papaverin were slightly but significantly (p < 0.05) reduced in the CAD patients, the response to the endothelium-dependent dilator acetylcholine was considerably out of proportion to the papaverin response in these patients compared with the normal patients. The capacity of the coronary system to increase blood flow in response to acetylcholine expressed as relative proportion of the maximal papaverin response was 52.5±18.2% in the normal control patients but only 33.6±23.6% in the CAD patients (p < 0.025 versus normals). There was a significant negative correlation (r = -0.69; p < 0.0001) between cold pressor test-induced changes in coronary vascular resistance and the capacity of the coronary system to increase blood flow in response to acetylcholine.

Conclusions. Early stages of epicardial atherosclerosis are associated with an impairment in endothelium-dependent dilation of the coronary microvasculature, indicating that the pathophysiological consequences of atherosclerosis may extend into the human coronary microcirculation. The correlation between cold pressor test-induced coronary vascular resistance changes and the extent of endothelial dysfunction suggests a relation between endothelial function of the microvasculature and coronary blood flow regulation during sympathetic stimulation associated with increased myocardial work. (Circulation 1991;84:1984–1992)

Furchgott and Zawadzki first discovered that the endothelial cell lining was essential for the vasodialator action of acetylcholine on simple strips of artery in an organ bath. Subsequently, a variety of additional receptor-dependent agonists have been identified to mediate endothelium-dependent relaxation by triggering the release of endothelium-derived relaxing factor (EDRF), which...
induces smooth-muscle relaxation by the stimulation of guanylate cyclase to form intracellular cyclic guanosine monophosphate. Palmer et al. finally demonstrated that one such relaxing factor is nitric oxide or a related compound synthesized from L-arginine. Recently, the potential physiological role of EDRF was emphasized by the results of studies in which an inhibitor of the formation of EDRF, $N^G$-monomethyl-L-arginine, was infused into the intact circulation of experimental animals. The intravenous infusion of $N^G$-monomethyl-L-arginine caused an immediate and substantial rise in blood pressure that could be reversed by L-arginine. These results indicated that there is a continuous basal release of EDRF, which keeps the vasculature in a dilated state and thereby regulates tissue perfusion.

The potential clinical implications of the role of EDRF activity were highlighted by the demonstration that the forearm vessels of healthy humans are continuously diluted by nitric oxide released from endothelial cells and that patients with essential hypertension exhibit an impaired vasodilator response of their forearm vasculature to the endothelium-dependent agonist acetylcholine. With respect to the human coronary circulation, a number of studies have shown that the vasodilator responses to acetylcholine in normal coronary arteries are reversed to constrictor responses in patients with evidence of coronary artery disease, thus suggesting a disturbance of endothelial function in the atherosclerotic large epicardial vessels of these patients. While possibly important in the genesis of vascular spasm, this abnormality of atherosclerotic conduit artery function probably contributes little to the regulation of tissue perfusion in the absence of vascular spasm because myocardial perfusion is regulated predominantly by resistance arteries less than 200 μm in diameter. However, recent experimental data demonstrated an abnormal endothelium-dependent dilation in the coronary microvascularature of atherosclerotic animals, thereby suggesting that the functional consequences of atherosclerosis may extend into the coronary microcirculation despite the absence of gross atherosclerotic lesions in these vessels.

It was the aim of the present study to assess whether endothelium-mediated modulation of the microvasculature plays a role for blood flow regulation in the intact human coronary circulation. Our study was designed to test the hypothesis that patients with non-flow-limiting epicardial atherosclerosis have a functional abnormality of endothelium-mediated microvascular relaxation that affects coronary blood flow regulation by the resistance vasculature during sympathetic stimulation. An endothelium-dependent, functional abnormality at the level of the resistance vessels might play an important role in the pathogenesis of myocardial ischemia caused by an impaired regulation of myocardial perfusion in response to neurohumoral stimulation associated with increased myocardial work.

**Methods**

**Study Population**

Thirty-one patients undergoing routine diagnostic cardiac catheterization were studied. Written informed consent was obtained from all patients before the study. The study protocol was approved by the Ethical Committee of the University of Freiburg.

**Normal control patients.** Twelve patients with angiographically normal coronary arteries and without a history of arterial hypertension (defined as chronically elevated blood pressure 150/95 mm Hg or higher), diabetes mellitus, or hypercholesterolemia (total cholesterol serum level higher than 210 mg%) served as the normal control group. The age of these patients ranged from 37 to 60 years. All subjects had angiographically normal, smooth coronary arteries without luminal irregularities and no evidence of segmental wall motion abnormalities on their left ventricular cineangiograms. All patients underwent diagnostic coronary angiography for evaluation of atypical chest pain. Patients with valvular heart disease were excluded.

**Patients with minimal disease of the left anterior descending coronary artery.** Nineteen patients were studied who had angiographically visible luminal irregularities of the left anterior descending artery (the vessel under study) but no more than 30% luminal narrowing. Seven of these patients had greater than 50% luminal narrowing of the right coronary artery, one patient had a hemodynamically significant stenosis of the left circumflex artery, and 11 patients had only luminal irregularities of the proximal left anterior descending artery. They ranged in age from 36 to 75 years (mean, 55.1 years).

None of these patients had a history of myocardial infarction within the territory of the left anterior descending artery. All patients had normal left ventricular contraction patterns in the anterior and septal left ventricular wall and a normal global ejection fraction as assessed by biplane cineventriculography. One patient with an occluded right coronary artery demonstrated a regional wall motion abnormality confined to the diaphragmal segment of the left ventricle but still had a normal global ejection fraction. Left ventricular end-diastolic pressure was 12 mm Hg or less in all patients. Six of the 19 patients had a history of arterial hypertension requiring antihypertensive therapy. Eight patients were cigarette smokers. No patient had a clinical history suggestive of variant angina.

Patients with hypercholesterolemia (serum cholesterol level higher than 210 mg%) at the time of the study were excluded because recent experimental studies suggest that lipoproteins may induce a nonspecific inhibition of endothelium-dependent relaxation by interfering with the agonist-induced release of EDRF.

**Study Protocol**

Vasoactive medications including calcium channel blockers, angiotensin converting enzyme inhibitors, and long-acting nitrates were withheld at least 24 hours before cardiac catheterization. No patient received $\beta$-adrenergic blockers within 48 hours before the study. Diagnostic left heart catheterization and coronary angiography were performed by a standard
percutaneous femoral approach. After completion of the diagnostic catheterization, an additional 5,000 units of heparin were given intravenously and an 8F guiding catheter (Schneider, Zurich, Switzerland) was introduced into the left main coronary artery. A 3F Monorail-Doppler catheter (Schneider) with a 20-MHz pulsed Doppler crystal was advanced into the left anterior descending artery via a 0.014-in. guide wire. The Doppler catheter was carefully positioned to obtain a stable flow velocity signal. Before introducing the Doppler catheter into the guiding catheter, the flow velocity recordings were referenced to zero and calibrated. A 5F bipolar pacing catheter was placed in the right ventricular apex and set in demand mode to prevent the heart rate from slowing below 40 beats per minute. At least 40 minutes elapsed between the completion of diagnostic catheterization and the beginning of the study.

Five minutes after the control angiogram, cold pressor testing was performed by immersion of the patient's hand and forearm in ice water for 90 seconds.

Ten minutes after cold pressor testing, 7 mg papaverin was subselectively injected into the left anterior descending artery via the Doppler catheter to assess endothelium-dependent coronary flow reserve in the territory of the left anterior descending artery. Previous studies have demonstrated that the dose of 7 mg papaverin, subselectively infused into the left anterior descending artery, elicits a maximal increase in coronary blood flow without affecting global hemodynamic parameters.

Ten minutes after papaverin infusion, acetylcholine was selectively infused into the left anterior descending artery via the Doppler catheter to assess endothelium-dependent increases in coronary blood flow. Increasing dosages of acetylcholine (0.036 μg/ml, 0.36 μg/ml, and 3.6 μg/ml) were infused at an infusion rate of 2 ml/min, lasting 3 minutes for each concentration. The lowest dose of 0.036 μg acetylcholine/ml corresponds to an estimated blood concentration in the coronary bed of 10^{-8} M assuming a blood flow of 80 ml/min. Stepwise acetylcholine infusions were terminated either when vessel occlusion occurred or when the largest dose (3.6 μg/ml) was reached.

Throughout the study, phasic and mean intracoronary blood flow velocity, heart rate, and aortic pressure (via the guiding catheter) were continuously measured. Serial hand injections of nonionic contrast material (Ultravist, Schering AG, Berlin) were performed during control, at the end of cold pressor testing, at re-control after the cold pressor test, at the end of each acetylcholine infusion period, at recontrol after acetylcholine infusion, and after subselective infusion of papaverin.

Quantitative Coronary Angiography

Coronary angiography was performed using a simultaneous biplane multidirectional isocentric x-ray system (Siemens Bicor, Erlangen, FRG). The coronary arteries under study were positioned near the isocenter, and special care was taken to avoid overlapping of coronary segments. Biplane cineangiograms were recorded at a frame rate of 25 frames per second. For quantitative analysis, end-diastolic cine frames were videodigitized and stored in the image analysis system (Mipron I, Kontron Electronics, Eching, FRG) in a 512×512 matrix with an eight-bit gray scale. Using the 12-cm field of view, the resulting pixel density was 7.3 pixels/mm. The geometrical resolution of the x-ray imaging chain is more than 4 line pairs/mm. Quantitative coronary angiography by automatic contour detection was performed by a previously described and validated method using a geometric edge differentiation technique. Calculation of the exact radiological magnification factor of the measured segment was used to scale the data from pixels to millimeters as previously described. The accuracy and precision of this technique as well as the reproducibility of serial measurements under routine clinical conditions have been established in previous studies.

Quantitative angiography of the epicardial artery was performed for two reasons: first, to determine cross-sectional area of the artery immediately distal to the radiopaque tip of the Doppler catheter to convert the Doppler-derived flow velocity to an estimate of coronary arterial flow, and second, to exclude limitations of coronary artery flow caused by epicardial coronary artery constriction in response to acetylcholine by measuring the most constricting epicardial artery segment distal to the tip of the Doppler catheter as previously suggested by Treasure et al.

To determine cross-sectional area of the artery, a 5–7-mm segment was measured immediately distal to the tip of the Doppler catheter. A series of diameter measurements were obtained for each scan line for the length of the arterial segment and displayed in graph form showing diameter versus segment length, and the mean diameter value was calculated. Whenever possible, measurements were performed in both views of the biplane images using the radiopaque tip of the Doppler catheter for identification of corresponding vessel segments, and the vessel's cross-sectional area was calculated from both views assuming an elliptical shape. Only single-plane analysis was performed for those coronary segments demonstrating overlapping with other parts of the coronary tree in one view; in those cases (seven of 31 patients; 23%), vessel cross-sectional area was calculated assuming a circular shape. Measurement of the most constricting artery segment was performed in a similar fashion. However, instead of calculating the mean diameter value, the minimal absolute diameter of the analyzed segment was identified in both views and minimal cross-sectional area was calculated.

Flow-limiting constriction was defined as greater than 50% cross-sectional area reduction compared with pre-acetylcholine cross-sectional area of the identical segment.

Data Analysis

For estimation of directional changes in coronary blood flow, a coronary flow index was calculated by
multiplying the mean Doppler-derived blood flow velocity with the computed cross-sectional area of the vessel segment immediately distal to the tip of the Doppler catheter. Because the injection of contrast material into the coronary circulation resulted in the typical biphasic response of coronary blood flow velocity with an initial decrease followed by an increase in flow velocity caused by the hyperemic effects of the contrast material, the mean blood flow velocity immediately before the contrast injection was used for estimation of coronary blood flow. To correct for the fact that a few patients did not receive all three doses of acetylcholine and that severe vasoconstriction with greater than 50% cross-sectional area reduction of the most constricting segment precluded the assessment of acetylcholine-induced increases in coronary blood flow, the coronary blood flow responses to acetylcholine were evaluated by calculating the dose–response relation to acetylcholine. Using linear regression, the slope of the acetylcholine dose–response relation (percent change in coronary blood flow index/dosage of acetylcholine) was calculated from the available doses for each patient. In three of the normal patients, the 0.036 μg/ml dose was omitted to limit the angiographic contrast load. In six of the patients with coronary atherosclerosis, the 3.6 μg/ml dose of acetylcholine induced greater than 50% cross-sectional area reduction in the most constricting segment of the left anterior descending artery, which precluded the assessment of acetylcholine-induced increases in coronary blood flow because of potential limitation of coronary arterial flow caused by epicardial artery constriction. In the dose range of acetylcholine used in this study, a linear relation occurred between acetylcholine dose and the percent change in coronary blood flow index in each individual patient. The mean correlation coefficient was 0.90±0.07, ranging from 0.74 to 0.99, indicating good fit for the calculated regression lines used for the slope calculation.

Mean aortic pressure was calculated by adding one third of the pulse pressure to the diastolic pressure. An index of coronary vascular resistance was calculated by the ratio of the mean aortic pressure divided by the coronary flow index.

**Statistical Analysis**

All data are expressed as mean±SD unless otherwise stated. Statistical comparisons were made by analysis of variance followed by the Bonferroni modified t test. Linear regression analysis was used to compare cold pressor test–induced changes in local and systemic hemodynamic parameters. Statistical significance was assumed if a null hypothesis could be rejected at the 0.05 probability level.

**Results**

**Responses to Cold Pressor Testing**

Table 1 summarizes the baseline characteristics as well as the cold pressor test–induced changes in the

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normals (n=12)</th>
<th>CAD (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.3±6.7</td>
<td>55.1±6.9*</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>3/9</td>
<td>5/14</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>0/12</td>
<td>6/19</td>
</tr>
<tr>
<td>Cigarette smokers</td>
<td>4/12</td>
<td>8/19</td>
</tr>
<tr>
<td>Plasma cholesterol (mg/dl)</td>
<td>182±17</td>
<td>191±24</td>
</tr>
<tr>
<td>Heart rate (min⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before CPT</td>
<td>73.8±9.8</td>
<td>70.4±12.7</td>
</tr>
<tr>
<td>End of CPT</td>
<td>82.3±12.4</td>
<td>80.7±13.8</td>
</tr>
<tr>
<td>Change</td>
<td>11.5±8.5%</td>
<td>14.8±4.5%</td>
</tr>
<tr>
<td>Mean aortic pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before CPT</td>
<td>96.9±16.2</td>
<td>95.4±10.5</td>
</tr>
<tr>
<td>End of CPT</td>
<td>116.1±18.1</td>
<td>114.8±14.5</td>
</tr>
<tr>
<td>Change</td>
<td>20.3±7.5%</td>
<td>20.4±10.3%</td>
</tr>
<tr>
<td>Rate–pressure product (mm Hg×min⁻¹×10⁶)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before CPT</td>
<td>9.5±2.2</td>
<td>9.2±1.8</td>
</tr>
<tr>
<td>End of CPT</td>
<td>12.9±2.6</td>
<td>12.6±2.3</td>
</tr>
<tr>
<td>Change</td>
<td>37.5±9.2%</td>
<td>37.3±12.6%</td>
</tr>
<tr>
<td>Epicardial artery cross-sectional area (mm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before CPT</td>
<td>7.8±3.3</td>
<td>6.1±2.2</td>
</tr>
<tr>
<td>End of CPT</td>
<td>9.1±4.0</td>
<td>5.2±2.0†</td>
</tr>
<tr>
<td>Change</td>
<td>17.2±5.5%</td>
<td>−15.5±8.1% ‡</td>
</tr>
<tr>
<td>Coronary blood flow index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before CPT</td>
<td>47.3±37.5</td>
<td>47.5±36.9</td>
</tr>
<tr>
<td>End of CPT</td>
<td>74.4±56.2</td>
<td>59.7±47.9</td>
</tr>
<tr>
<td>Change</td>
<td>59.5±15.6</td>
<td>26.3±30.8†</td>
</tr>
<tr>
<td>Coronary vascular resistance index (mm Hg/kHz×mm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before CPT</td>
<td>2.86±1.44</td>
<td>3.29±2.3</td>
</tr>
<tr>
<td>End of CPT</td>
<td>2.17±1.11</td>
<td>3.20±2.4</td>
</tr>
<tr>
<td>Change</td>
<td>−2.41±5.5</td>
<td>−0.6±20.5‡</td>
</tr>
</tbody>
</table>

Values are mean±SD. CAD, patients with coronary artery disease; CPT, cold pressor test.

*p<0.05 vs. normals.

†p<0.01 vs. normals.

‡p<0.001 vs. normals.

hemodynamic variables for the normal patient group and for the group of patients with early atherosclerosis (CAD). The patients with atherosclerosis were slightly but significantly (p<0.05) older than the normal patients. There were no statistically significant differences between the two groups of patients with respect to the systemic hemodynamic variables at baseline and at the end of cold pressor testing. Moreover, epicardial artery cross-sectional area, coronary blood flow index, and coronary vascular resistance index did not significantly differ between both groups at baseline.

In all normal patients, epicardial artery cross-sectional area increased by 17.2±5.5% during cold pressor testing. In contrast, in the patients with CAD, epicardial artery cross-sectional area decreased by −15.5±8.1% (p<0.001 versus normal control pa-
patients). Figure 1 illustrates an original recording during cold pressor testing in a patient of the CAD group demonstrating an increase in Doppler-derived intracoronary flow velocity with increased heart rate and aortic pressure. Despite opposite epicardial artery vasomotor responses during cold pressor testing, increases in Doppler-derived coronary blood flow velocity were similar in the normal patients (35.8±9.0%) and in the CAD patients (49.1±29.7%, NS, versus normals). Because of the blunted increase in coronary flow velocity despite epicardial artery vasoconstriction, cold pressor test–induced increases in coronary blood flow indexes were significantly lower (p<0.002) in the patients of the CAD group compared with the normal control patients (Table 1). Thus, given the comparable cold pressor test–induced changes in mean aortic pressure in both groups, mean coronary vascular resistance responses did significantly (p<0.001) differ between the normal and the CAD patients (Table 1). Figure 2 illustrates the coronary vascular resistance indexes for each patient before and at the end of cold pressor testing. Coronary vascular resistance decreased in all normal control patients during cold pressor testing. In contrast, the patients with CAD exhibited a variable coronary vascular resistance response to cold pressor testing. Whereas 10 patients demonstrated a decrease in coronary vascular resistance, a paradoxical increase in coronary vascular resistance in response to cold pressor testing was observed in nine patients.

The heterogeneous coronary blood flow response to the increased myocardial demand during cold pressor testing in the patients of the CAD group was also evident when the data were plotted so that increases in calculated coronary blood flow indexes were compared with increases in the rate–pressure product. Figure 3 illustrates that there was a statistically significant positive correlation for the normal patients (p<0.005, r=0.77) but there was no relation for the patients of the CAD group.

Response to Acetylcholine and Papaverin

No significant changes in mean aortic pressure or heart rate occurred during subselective infusion of either papaverin or acetylcholine.

Table 2 summarizes the epicardial artery cross-sectional areas for the two groups during the infusions of acetylcholine and papaverin, respectively. Intracoronary infusion of acetylcholine induced dilation of the epicardial arteries in all normal patients with an increase in luminal area of 22.7±12.9% at

![Figure 1](image1.png)  
**Figure 1.** Original recording during cold pressor testing (CPT) demonstrating an 80% increase in Doppler-derived coronary blood flow velocity (CBFV, expressed in kHz) with increases in heart rate (HR) and mean aortic pressure (Ao). Contrast injection (CM-Inj) revealed an almost completely exhausted contrast material–induced flow velocity reserve at the end of cold pressor testing. ECG, electrocardiogram; PAo, aortic pressure measured via guiding catheter.

![Figure 2](image2.png)  
**Figure 2.** Graph shows coronary vascular resistance index before (pre) and at the end (post) of cold pressor testing (CPT) in normal patients and in patients with epicardial atherosclerosis (CAD).
Table 2. Absolute Epicardial Artery Cross-sectional Areas in Response to Papaverin and Acetylcholine

<table>
<thead>
<tr>
<th></th>
<th>Normals (n=12)*</th>
<th>CAD (n=19)†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epicardial artery cross-sectional area (mm²)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before papaverin</td>
<td>7.8±4.3</td>
<td>6.2±3.0</td>
</tr>
<tr>
<td>After papaverin</td>
<td>9.7±5.1</td>
<td>8.3±3.3</td>
</tr>
<tr>
<td><strong>Epicardial artery cross-sectional area (mm²)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before acetylcholine</td>
<td>8.0±5.7</td>
<td>6.1±2.9</td>
</tr>
<tr>
<td>After acetylcholine</td>
<td>8.2±6.5</td>
<td>4.4±2.4♀</td>
</tr>
<tr>
<td>0.036 µg/ml</td>
<td>8.1±4.8</td>
<td>5.7±2.7</td>
</tr>
<tr>
<td>0.36 µg/ml</td>
<td>8.8±5.9</td>
<td>4.7±2.3♀</td>
</tr>
<tr>
<td>3.6 µg/ml</td>
<td>9.8±6.4</td>
<td>4.4±2.4♀</td>
</tr>
</tbody>
</table>

CAD, patients with coronary artery disease. *Values at 0.036 µg/ml acetylcholine are mean±SD of nine patients. †Values at 3.6 µg/ml acetylcholine are mean±SD of 13 patients. ♀p<0.05 vs. normals. §p<0.01 vs. normals.

The highest dose. In contrast, in all patients with early atherosclerosis, epicardial artery luminal area decreased by −30.5±7.8% (p<0.001 versus normals) at the highest dose of acetylcholine.

The changes in coronary blood flow in response to papaverin and acetylcholine are summarized in Table 3. Coronary blood flow responses to intracoronary papaverin were slightly but significantly (p<0.05) reduced in the CAD patients compared with the normal control group. Figure 4 illustrates that the slope of the acetylcholine dose–response relation (percent change in coronary blood flow/dosage of acetylcholine) was significantly (p<0.001) lower with 38.5±24.8 in the patients of the CAD group compared with the normal control patients (80.8±28.1), mainly resulting from a blunted increase in coronary blood flow at the highest dose of acetylcholine (see Table 3). There was no relation between the dose–response effects of acetylcholine on epicardial cross-sectional areas and coronary blood flow indexes (r=0.26, p=0.3), indicating that epicardial artery constriction was not the major determinant of the blunted increase in coronary blood flow in response to acetylcholine in the CAD patients.

The finding of an impaired coronary blood flow response to acetylcholine in the patients of the CAD group was further substantiated when each patient’s acetylcholine dose–response was related to papaverin response. The proportion of acetylcholine-induced increases in coronary blood flow relative to papaverin-induced increases in blood flow was 52.5±18.2% in the normal control patients but significantly reduced to 33.6±23.6% in the patients of the CAD group (p<0.025 versus normals). Similar results were obtained when each patient’s maximal coronary blood flow response to acetylcholine was related to papaverin response (53.3±19.9% in the normal patient group versus 31.5±21.1% in the CAD patient group; p<0.01). Thus, the patients with CAD demonstrated an impaired capacity of their coronary system to increase blood flow in response to acetylcholine. When the patients of the CAD group were separately analyzed according to the presence or absence of a history of hypertension, no significant differences were
noted: The proportion of acetylcholine-induced increases in blood flow relative to papaverin-induced increases in blood flow was 40.9±28.4% in the 13 patients without hypertension compared with 21.6±24.8% in the six patients with a history of hypertension (p=0.3).

To evaluate any potential relation between acetylcholine-induced vasodilator capacity and coronary vascular resistance response during cold pressor testing, the capacity to increase coronary blood flow by acetylcholine was compared with the change in coronary vascular resistance during the cold pressor test. Figure 5 illustrates that there was a significant negative correlation between cold pressor test–induced changes in coronary vascular resistance and the capacity of the coronary system to increase blood flow in response to acetylcholine. When the patients with a history of hypertension were excluded, the correlation coefficient actually improved to r=−0.72 (p<0.0001, n=25), indicating that the relation was not primarily determined by the coronary vascular response of the patients with arterial hypertension. Thus, a reduction in the capacity of endothelium-dependent coronary vasodilation was associated with a reduced dilator capacity of the coronary resistance vasculature during cold pressor testing.

**Discussion**

The present investigation demonstrates that, in addition to the paradoxical constrictor response of epicardial conductance vessels, early stages of coronary atherosclerosis (as assessed by angiography) can be associated with an impairment in endothelium-dependent dilation of the microvasculature. These results suggest that the pathophysiological consequences of early atherosclerosis may extend into the human coronary microcirculation. The impairment of endothelium-dependent coronary vasodilation is associated with a reduced dilator capacity of the coronary resistance vasculature during cold pressor testing.

Previous studies assessing endothelium-dependent modulation of vascular tone in the human coronary circulation have primarily focused on the epicardial conduit vessels, which are the major target of coronary atherosclerosis, whereas gross atherosclerotic lesions do not develop in the coronary microcirculation. A number of studies have demonstrated that endothelial functioning is abnormal in atherosclerotic human epicardial arteries.11–14 Moreover, we have recently shown that hypercholesterolemia not only affects endothelial functioning of epicardial conductance vessels but also profoundly impairs endothelium-dependent relaxation of the human coronary microcirculation in vivo.24 Similar results have been obtained in atherosclerotic animals fed a hypercholesterolemic diet.16,17,25 The present investigation extends these findings by demonstrating that the pathophysiological manifestations of atherosclerosis may also extend into the coronary microcirculation of patients without elevated serum cholesterol levels. The patients with evidence of epicardial atherosclerosis exhibited a profoundly impaired capacity to increase blood flow in response to the endothelium-dependent dilator acetylcholine. Although the coronary blood flow response to the smooth muscle relaxant papaverin was also slightly reduced in these patients, the acetylcholine response was considerably out of proportion to the papaverin response. The impaired relaxation to acetylcholine suggests a role for endothelial dysfunction in the coronary microvasculature of these patients. An impaired endothelium-dependent dilator response of the coronary microvasculature implicates that the pathophysiological consequences of early atherosclerosis do extend into the coronary microcirculation.

These findings might have important implications regarding regulation of myocardial perfusion in the
setting of early atherosclerosis even in the absence of hemodynamically significant epicardial artery lesions. Indeed, an additional important finding of the present study was that the coronary vascular resistance response to cold pressor testing was related to the capacity of the microvasculature to dilate in response to the endothelium-dependent dilator acetylcholine. Those patients who exhibited a blunted cold pressor test-induced increase in coronary blood flow despite augmented metabolic demands leading to a paradoxical increase in coronary vascular resistance during cold pressor testing also demonstrated a strikingly abnormal blood flow response to the endothelium-dependent dilator acetylcholine. These results indicate that early atherosclerosis may be associated with an abnormal coronary blood flow regulation during cold pressor testing and suggest a relation between endothelial function of the microvasculature and coronary blood flow regulation during sympathetic stimulation associated with increased myocardial demands.

The cause of endothelial dysfunction of the coronary microvessels in patients with early atherosclerosis in this study remains to be defined. The mechanisms underlying abnormal endothelium-dependent vascular relaxation in atherosclerosis may include decreased or abnormal production and/or release of EDRF, destruction of EDRF, and the concomitant release of constricting factors. With respect to blood flow regulation during cold pressor testing, the impaired dilator response of the microvasculature might represent a generalized altered sensitivity to coronary vasoconstrictor stimuli in the presence of a dysfunctional endothelium. Recent studies demonstrated that atherosclerosis considerably potentiated vascular constriction to serotonin in the microcirculation of nonhuman primates as well as in the human coronary circulation, which exhibited a particularly intense constriction in small distal and collateral vessels. We have recently shown that intracoronary platelet aggregation causes profound constriction of atherosclerotic epicardial arteries in humans in vivo. The potent coronary constrictors neuropeptide Y and endothelin have been found to constrict distal vessels rather than large coronary arteries. It is conceivable that, in the presence of a dysfunctional endothelium, the release of such a constrictor factor or pressure-induced constriction of the microvessels caused by unopposed myogenic constriction may account for the increase in coronary vascular resistance despite augmented metabolic demands.

Nonselective impairment of coronary vasodilator reserve was excluded in all patients examined in the present study and thus cannot account for the observed differences in endothelium-mediated dilation of the microvasculature. Patients with hypercholesterolemia, which we have shown profoundly impairs endothelium-dependent dilation of the human coronary resistance vasculature, were excluded from the present study. Moreover, the present data failed to demonstrate that hypertension might independently alter endothelial function of the coronary microvasculature or affect the relation between cold pressor test-induced coronary blood flow regulation and endothelial function of the microvasculature. Thus, endothelial dysfunction of the coronary microvessels in the patients of the present study cannot be attributed to the presence of associated diseases that promote the development of atherosclerosis. It should be noted, however, that the patients with a disorder of the coronary microvasculature described as "microvascular" angina demonstrate a dynamic vascular abnormality that leads to abnormal pacing-induced increases in coronary resistance, which are considerably amplified after ergonovine administration. Recent experimental studies demonstrated that the endothelium inhibits the contractions evoked by ergonovine, and thereby endothelial dysfunction can contribute to the predominant vasoconstrictor response to ergonovine. In addition, patients with dilated cardiomyopathy have recently been shown to exhibit a selective impairment in endothelium-dependent dilation of the coronary microvasculature. Thus, there is considerable scope to explore the role of endothelial function for the mechanisms that control the tone of the coronary microvasculature in gaining insight into the pathophysiology of human heart disease.

The most important limitation of the present study is that the data do not prove a cause-and-effect relation between endothelial dysfunction of the coronary microvasculature and the altered response to cold pressor testing. The only way to prove such a cause-and-effect relation would be to demonstrate an altered cold pressor test–induced coronary blood flow regulation after the administration of a selective inhibitor of endothelium-dependent vascular relaxation like NO-arginine. Although NO-arginine has been infused into the human forearm circulation, its selective intracoronary administration might expose the patient to a potentially hazardous risk. However, the results of the present study demonstrate that an abnormal coronary blood flow response to sympathetic stimulation occurred in those patients who also exhibited an impairment in endothelium-dependent dilation of the coronary microvasculature. Thus, although the response to cold pressor testing might be controlled by a separate mechanism apart from endothelial regulation, the relation between endothelial function of the microvasculature and coronary blood flow regulation during sympathetic stimulation suggests that endothelial dysfunction contributes to the impaired coronary blood flow regulation either by direct interference or by allowing separate mechanisms to become operative. Another limitation of the present study refers to the methodology used to assess coronary vascular resistance, which provides no direct data to identify the specific site of the impaired dilator response of the coronary vessels. Thus, although flow-limiting vasoconstriction of epicardial conductance vessels was excluded, the precise site of coronary vascular abnormalities downstream from
the epicardial vessels cannot be assessed in the intact coronary circulation.

Summary

Early stages of epicardial atherosclerosis can be associated with an impairment in endothelium-dependent dilation of the coronary microvasculature, indicating that the pathophysiological consequences of early atherosclerosis may extend into the human coronary microcirculation. Endothelial dysfunction of the coronary microvasculature is associated with an impaired coronary blood flow regulation during sympathetic stimulation associated with increased myocardial work and might thereby contribute to the pathogenesis of myocardial ischemia.

References


Key Words: coronary blood flow • endothelium • coronary artery disease • cold pressor test • acetylcholine • coronary microvasculature • sympathetic stimulation • atherosclerosis
Endothelial dysfunction of the coronary microvasculature is associated with coronary blood flow regulation in patients with early atherosclerosis.
A M Zeiher, H Drexler, H Wollschläger and H Just

doi: 10.1161/01.CIR.84.5.1984

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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/84/5/1984

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/