Coronary Endothelial Dysfunction Is Associated With an Increased Risk of Cerebrovascular Events

Paul V. Targonski, MD, PhD*; Piero O. Bonetti, MD*; Geralyn M. Pumper, RN; Stuart T. Higano, MD; David R. Holmes, Jr, MD; Amir Lerman, MD

Background—Stroke, mainly attributable to atherothrombotic disease, represents a leading cause of disability and death in the Western world. Endothelial dysfunction, which is considered a key factor in atherogenesis, is associated with an increased risk of cardiovascular events. However, the magnitude of the association between coronary endothelial dysfunction (CED) and cerebrovascular events is unknown. This study was performed to investigate the association between CED and cerebrovascular events.

Methods and Results—We studied 503 patients without obstructive coronary artery disease (CAD) who underwent coronary endothelial function testing by intracoronary acetylcholine infusion. Patients were divided according to the presence (n=305) or absence (n=198) of CED, and medical records were examined for the occurrence of ischemic or hemorrhagic stroke or transient ischemic attack either before (prevalent) or after (incident) coronary endothelial function testing. Among the study population, a total of 25 cerebrovascular events were documented, 22 in patients with CED (15 prevalent) and 3 in patients without (all prevalent) (P=0.008). Multivariable logistic regression, which included traditional cerebrovascular disease–related risk factors, identified the presence of CED as the single strongest factor associated with cerebrovascular events (OR, 4.32; 95% CI, 1.26 to 14.83). Kaplan-Meier analysis indicated that patients with CED had a significantly higher cumulative cerebrovascular event rate than those without (P=0.04).

Conclusions—Presence of CED in patients without obstructive CAD is independently associated with an increased risk of cerebrovascular events. Thus, detection of this early stage of atherosclerosis may provide important information to identify patients who benefit from aggressive preventive strategies. (Circulation. 2003;107:2805-2809.)

Key Words: atherosclerosis ● endothelium ● epidemiology ● risk factors

In the United States, an estimated 600 000 people suffer a new or recurrent stroke each year, and stroke represents the third leading cause of death behind heart disease and cancer. Although 50% to 70% of patients who survive a stroke regain functional independence, up to 30% remain permanently disabled.1

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Coronary artery disease (CAD) and most of its risk factors are associated with an increased risk of stroke.2 Endothelial dysfunction is considered a key early stage in the development of coronary atherosclerosis,3 and the detection of coronary endothelial dysfunction is associated with an increased risk for cardiovascular events.4–6 Moreover, the presence of endothelial dysfunction in the peripheral vasculature represents an independent predictor of future cardiovascular events in patients with cardiovascular disease or its risk factors.7–9 These results suggest that endothelial dysfunction is a systemic disorder that portends a poor cardiovascular prognosis.

However, despite the established relationship between endothelial dysfunction and cardiovascular events in general on the one hand and between CAD and stroke on the other hand, little is presently known about the magnitude of the association between coronary endothelial dysfunction and cerebrovascular events in particular. This study evaluated whether the presence of coronary endothelial dysfunction is associated with an increased prevalence or incidence of cerebrovascular events in middle-aged patients without obstructive CAD.

Methods

Patient Population

The present study was approved by the Mayo Clinic Institutional Review Board. Consecutive patients who were referred to the Mayo Clinic between January 1992 and April 2001 for cardiac catheterization for the evaluation of coronary artery disease and who were found to have no significant epicardial coronary stenoses (≥30% diameter) underwent coronary endothelial function testing and were included in the present study. Exclusion criteria included a history of
percutaneous coronary intervention, coronary artery bypass graft surgery, unstable angina pectoris, or variant angina, an ejection fraction ≤50%, valvular heart disease, peripheral vascular disease, uncontrolled arterial hypertension, or significant endocrine, hepatic, renal, or inflammatory disease.

**Assessment of Coronary Vasoreactivity**
After diagnostic angiography and exclusion of significant obstructive CAD, endothelium-dependent and endothelium-independent coronary vasoreactivity were assessed as previously described. In brief, a Doppler guidewire within a coronary-infusion catheter was positioned into the midportion of the left anterior descending coronary artery. Then intracoronary bolus injections of incremental doses (18 to 36 μg) of adenosine were administered until maximal hyperemia was achieved. Subsequently, acetylcholine at increasing concentrations (10−8 to 10−5mol/L) was selectively infused for 3 minutes at each concentration into the left anterior descending coronary artery. Hemodynamic data, Doppler measurements, and coronary angiograms were obtained after each infusion. Coronary angiograms and intracoronary Doppler data were analyzed according to our previous studies. Coronary artery diameter was measured by an independent investigator in the segment 5 mm distal to the tip of the Doppler wire using a computer-based image analysis system. Average peak velocity (APV) was derived from the Doppler flow velocity spectra, and coronary blood flow (CBF) was determined as π×(coronary artery diameter/2)²×(APV/2). Endothelium-dependent coronary flow reserve was calculated as percent change in CBF in response to acetylcholine, and endothelium-independent coronary flow reserve was calculated by dividing the APV after adenosine injection by the APV at baseline. According to our previous studies linking the presence of coronary endothelial dysfunction to myocardial perfusion defects and an increased rate of cardiac events, endothelial dysfunction was defined as an increase in CBF ≥50% or an increase in epicardial coronary artery diameter ≥20% in response to the maximum dose of acetylcholine.

**Patient History and Follow-Up**
Medical information about cerebrovascular events was obtained from the patient’s medical records using the Rochester Epidemiology Project medical records linkage system, which provides access to the patients’ past medical history and updated data for any health related visit to the Mayo Clinic. Events of interest were defined as ischemic or hemorrhagic stroke or transient ischemic attack (TIA) either before or after coronary endothelial function testing. All cerebrovascular events were confirmed by Mayo Clinic neurologists to meet the criteria for stroke or TIA through appropriate combination of medical history, examination, and neuroimaging.

**Statistical Analysis**
Student’s t test for continuous data and χ² test for categorical data were used to compare the distribution of baseline characteristics between patients with and without endothelial dysfunction. Dose-response relationship between quartiles of coronary response to acetylcholine and cerebrovascular accident was examined using a χ² test for trend, whereas univariate and multivariable logistic regression was used to quantify the risk for cerebrovascular accident associated with endothelial dysfunction and conventional stroke-related risk factors in this population. Multivariable models included the following variables: age, gender, hyperlipidemia, hypertension, history of smoking, diabetes, obesity, atrial fibrillation, history of myocardial infarction, and coronary endothelial dysfunction. Effect modification was explored in multivariable logistic regression analysis. Cumulative event rates in individuals without a history of cerebrovascular events preceding coronary endothelial function testing were estimated by Kaplan-Meier survival curves, and the log-rank test was used to examine differences in survival between patients with and without coronary endothelial dysfunction. P<0.05 was considered statistically significant.

## Table 1. Baseline Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Normal Coronary Endothelial Function (n=198)</th>
<th>Coronary Endothelial Dysfunction (n=305)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>48±12</td>
<td>51±12*</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>75 (38)</td>
<td>126 (41)</td>
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<tr>
<td>BMI, kg/m²</td>
<td>27.5±6.4</td>
<td>28.9±6.3*</td>
</tr>
<tr>
<td>Hypercholesterolemia, n (%)</td>
<td>66 (33)</td>
<td>133 (44)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>66 (33)</td>
<td>103 (34)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>14 (7)</td>
<td>25 (8)</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>32 (16)</td>
<td>53 (17)</td>
</tr>
<tr>
<td>Obesity, n (%)</td>
<td>43 (22)</td>
<td>79 (26)</td>
</tr>
<tr>
<td>Myocardial infarction, n (%)</td>
<td>15 (8)</td>
<td>29 (10)</td>
</tr>
<tr>
<td>Atrial fibrillation, n (%)</td>
<td>7 (4)</td>
<td>16 (5)</td>
</tr>
<tr>
<td>Aspirin, n (%)</td>
<td>68 (34)</td>
<td>130 (43)</td>
</tr>
<tr>
<td>Warfarin, n (%)</td>
<td>7 (4)</td>
<td>21 (7)</td>
</tr>
<tr>
<td>Statin, n (%)</td>
<td>26 (13)</td>
<td>37 (12)</td>
</tr>
<tr>
<td>ACE inhibitor, n (%)</td>
<td>11 (6)</td>
<td>22 (7)</td>
</tr>
</tbody>
</table>

Values are mean±SD or n (%). BMI indicates body mass index. *P<0.05 vs normal coronary endothelial function.

**Results**

**Patient Population**
A total of 503 patients without significant coronary stenoses were included in the present study. Of these, 198 (39%) had normal coronary endothelial function and 305 (61%) had evidence of coronary endothelial dysfunction. Average endothelium-independent coronary flow reserve in response to adenosine was normal in patients with and without coronary endothelial dysfunction (2.9±0.7 and 3.0±0.8, respectively). Coronary blood flow response to acetylcholine was significantly lower in the patients with coronary endothelial dysfunction compared with patients with normal coronary endothelial function (12±6% versus 13±8%, P<0.001) Baseline patient characteristics are presented in Table 1. After assessment of coronary endothelial function, patients were followed for a median of 16 months (range, 1 to 88 months), with no difference in follow-up duration between those with and without coronary endothelial dysfunction (Wilcoxon rank sum, P=0.10).

**Association Between Coronary Endothelial Dysfunction and Cerebrovascular Events**
In our study population, a total of 25 cerebrovascular events were documented either before coronary endothelial function testing or during follow-up, including 13 ischemic strokes, 2 hemorrhagic strokes, and 10 TIAs. Additional evaluation of patients with cerebrovascular events for potential cardiac or extracardiac causes of stroke or TIA revealed history of patent foramen ovale in 2 patients, without a difference between the groups. There was no evidence for history of carotid artery atherosclerosis/stenosis or atrial septal defect in the affected individuals.

Twenty-two of the cerebrovascular events occurred in patients with coronary endothelial dysfunction, and 3 occurred in patients with normal coronary endothelial function.
Thus, cerebrovascular events were significantly more frequent in patients with impaired endothelial function (7.2%; 95% CI, 4.6 to 10.7) than in individuals with normal endothelial function (1.5%; 95% CI, 0.0 to 4.4; P=0.008). Consistent with the increased frequency of cerebrovascular accident among patients with coronary endothelial dysfunction, response to acetylcholine was also significantly smaller among those with cerebrovascular accident compared with those without (percent response, mean±SD (n); 12.11±46.73 (25) versus 70.20±131.13 (457), respectively; Z=2.44, P=0.015). When prevalence of cerebrovascular accident was examined by quartile response to acetylcholine (Table 2), a statistically significant and inverse dose-response relationship was observed (χ²=7.10, P=0.008).

Univariate analysis identified the presence of coronary endothelial dysfunction (defined as a dichotomous variable) as the single greatest risk factor for stroke or TIA in the population studied (OR, 5.08; 95% CI, 1.50 to 17.16), followed by diabetes, history of myocardial infarction, and increasing age (Table 3).

Multivariable analysis was performed to identify an independent association between coronary endothelial dysfunction and cerebrovascular events. After adjustment for cerebrovascular disease–related risk factors, coronary endothelial dysfunction remained the strongest factor associated with stroke or TIA (OR, 4.32; 95% CI, 1.26 to 14.83) followed by diabetes, history of myocardial infarction, and increasing age (Table 4). No interaction was observed between independent variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, per-year increase</td>
<td>1.04 (1.01 to 1.08)</td>
</tr>
<tr>
<td>Male gender</td>
<td>0.84 (0.36 to 1.94)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>1.69 (0.76 to 3.79)</td>
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<tr>
<td>Hypertension</td>
<td>1.59 (0.70 to 3.57)</td>
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<td>History of smoking</td>
<td>0.65 (0.19 to 2.26)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3.26 (1.15 to 9.22)</td>
</tr>
<tr>
<td>BMI, per-unit increase</td>
<td>1.01 (0.94 to 1.08)</td>
</tr>
<tr>
<td>Obesity</td>
<td>1.87 (0.80 to 4.40)</td>
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<tr>
<td>Myocardial infarction</td>
<td>2.97 (1.05 to 8.38)</td>
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<tr>
<td>Atrial fibrillation</td>
<td>1.89 (0.42 to 8.54)</td>
</tr>
<tr>
<td>Coronary endothelial dysfunction</td>
<td>5.08 (1.50 to 17.16)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary endothelial dysfunction</td>
<td>4.32 (1.26 to 14.83)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3.38 (1.13 to 10.09)</td>
</tr>
<tr>
<td>Myocardial Infarction</td>
<td>3.08 (1.04 to 9.11)</td>
</tr>
<tr>
<td>Age</td>
<td>1.04 (1.00 to 1.07)</td>
</tr>
</tbody>
</table>

**TABLE 3. Univariate Logistic Regression Analysis of Risk Factors for Stroke or TIA**

**TABLE 4. Independent Risk Factors for Stroke or TIA**

Incidence of Cerebrovascular Events During Follow-Up

No procedural-induced events were observed during the first month after coronary endothelial function testing. However, during follow-up, a total of 7 cerebrovascular events were reported, all of which occurred in patients with endothelial dysfunction. By Kaplan-Meier analysis, event-free survival was significantly worse for patients with coronary endothelial dysfunction compared with those with normal endothelial function (P=0.04) (Figure). There were 12 myocardial infarctions at follow-up, 11 in the group with coronary endothelial dysfunction and 1 in the group with normal endothelial function (P<0.05).

**Discussion**

The present study demonstrates that coronary endothelial dysfunction is associated with an increased risk of stroke or TIA in middle-aged patients without obstructive CAD.

Several recent studies showed that the presence of endothelial dysfunction in the coronary or peripheral circulation is independently associated with an increased risk of cardiovascular events in patients with various stages of atherosclerosis or its risk factors. However, although most of these studies included stroke in their combined end point of cardiovascular events, the numbers of cerebrovascular events that occurred in these studies were too small to draw any conclusions about the magnitude of the association between endothelial dysfunction and cerebrovascular events. Thus, the results of the present study extend these previous observations by demonstrating that individuals with coronary endothelial dysfunction were more than 4 times as likely to have a...
prevalent or incident cerebrovascular event than those with normal coronary endothelial function, even after statistical adjustments for cerebrovascular disease–related risk factors. The results of the survival analysis, which showed a significantly higher cumulative event rate in patients with coronary endothelial dysfunction than in those without, additionally underscore the prognostic significance of coronary endothelial dysfunction.

Atherothrombosis represents the most common cause of stroke, and individuals with established atherosclerotic disease, including those with CAD, are at increased risk of experiencing such an event. 1,2 Endothelial dysfunction, which is associated with most if not all cardiovascular risk factors, may represent the very early stage of atherosclerosis. 3 In addition to preceding the physical presence of overt atherosclerotic lesions, endothelial dysfunction, which is characterized by a tendency toward vasoconstriction and a proinflammatory, proliferative, and procoagulatory milieu, also contributes to all stages of plaque formation and is additionally involved in the pathogenesis of thrombotic atherosclerotic complications. 12,13 Growing evidence additionally suggests that endothelial dysfunction is a systemic disorder affecting both conduit arteries and microvessels in various vascular beds. 14 Given the systemic nature of endothelial dysfunction, it may be speculated that the status of endothelial function in the coronary arteries mirrors that in the cerebral vasculature. The connection between endothelial dysfunction and ischemic cerebrovascular events may be related to the critical involvement of endothelial dysfunction in the development of thrombotic atherosclerotic complications. 12 The role of endothelial dysfunction in the pathogenesis of hemorrhagic stroke is less clear. On the one hand, it has been argued that cerebral hemorrhage may arise from previous ischemic damage to the walls of cerebral arterioles, which can be caused by prolonged vasospasm. 15 Hence, it is conceivable that endothelial dysfunction may favor hemorrhagic stroke by inducing cerebral arteriolar vasoconstriction with consecutive ischemic damage to the vascular wall. On the other hand, atherosclerotic manifestations may vary between different vascular beds, and, therefore, it may be speculated that patients with the very early stages of atherosclerotic disease in the coronary arteries (endothelial dysfunction) may have more advanced lesions in the cerebral vasculature, which may predispose these vessels to rupture. Finally, it is possible that vasoconstriction associated with cerebrovascular endothelial dysfunction may trigger physical rupture of diseased cerebral vessels. In this context, the results of the present study suggest that cerebrovascular endothelial dysfunction may be an important contributor to the pathogenesis of cerebrovascular events. This hypothesis is additionally supported by the observation that various strategies that decrease the risk of cerebrovascular events, including aspirin, statins, and angiotensin-converting enzyme inhibitors, have been shown to improve endothelial function. 16–18

The observed association between cerebrovascular events and endothelial dysfunction of epicardial coronary arteries but also coronary microvessels, which hardly ever develop atherosclerotic lesions, not only underscores the systemic nature of endothelial dysfunction but also points out the importance of microvascular disease for the pathogenesis of cerebrovascular events. In line with this concept, it was shown recently that otherwise healthy, middle-aged patients with manifestations of microvascular disease, such as retinal microvascular abnormalities or cerebral white matter lesions detected on MRI, are at increased risk of stroke. 19

Although older age and the presence of significant CAD or a history of myocardial infarction are established risk factors for stroke, 1,2 our results indicate that a significant increase in the risk of experiencing a cerebrovascular event extends to middle-aged individuals of both genders with the very early stages of CAD. The importance of this finding is underscored by the fact that 28% of all patients suffering a stroke in a given year are younger than 65 years of age. 1 Thus, our data suggest that detection of this early stage of atherosclerosis may provide important prognostic information that complements traditional risk factor assessment in middle-aged women and men. In the present study there were no statistically significant relationships between traditional risk factors for cerebrovascular events, such as hypertension, hyperlipidemia, or atrial fibrillation, and cerebrovascular outcomes. In all cases, the direction of association is consistent with expected outcomes, and the lack of statistical significance may reflect insufficient power secondary to small numbers in this study population. Moreover, given that only patients who were referred for coronary angiography were included, selection bias may represent another possible reason for the lack of an association between established stroke risk factors and events observed in the present study. Also, the cumulative exposure of this middle-aged population to these risk factors may have been below the threshold for triggering a cerebrovascular event. However, although the absence of these associations with cerebrovascular outcomes in the study population may reflect a limitation of the study, it also strengthens the role of endothelial dysfunction as an important independent factor involved in the pathogenesis of cerebrovascular events.

Because this study was performed retrospectively, confirmation by larger prospective investigations is required. Furthermore, the fact that the patients in the present study were referred for coronary angiography by an independent physician to rule out the presence of CAD may result in a referral bias and may prevent the generalization of our results. However, although the comparison is inexact with reference to disease definition, the 1.5% prevalence of cerebrovascular events observed in subjects with normal coronary endothelial function in the present study approximates the 1.3% prevalence of cerebrovascular disease among middle-aged United States citizens from the 1996 National Health Interview Survey. 20

In the present study, most cerebrovascular events occurred before endothelial function testing. Thus, incidence-prevalence bias may arise from the inclusion of patients with prevalent cerebrovascular events. However, survival analysis, which excluded patients with previous cerebrovascular events, corroborated prevalence findings in our study. This suggests a similar relationship between coronary endothelial dysfunction and both prevalent and incident cerebrovascular
events, and, hence, a major impact of an incidence-prevalence bias on the results of the present study is rather unlikely.

In summary, the results of the present study demonstrate that coronary endothelial dysfunction is independently associated with an increased risk of cerebrovascular events. These findings additionally support the concept that endothelial dysfunction is a systemic and prognostically relevant disorder and add to the results of previous studies suggesting that assessment of endothelial function may play a role as an additional strategy to identify patients who would benefit from aggressive preventive measures.

Acknowledgments
This study was supported by the National Institutes of Health (grant No. R01 HL-63911), the American Heart Association, and the Mayo Foundation.

References
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_Circulation_. 2003;107:2805-2809; originally published online May 27, 2003;
doi: 10.1161/01.CIR.0000072765.93106.EE
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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