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Long-Term Follow-Up of Patients With Mild Coronary Artery Disease and Endothelial Dysfunction

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Background—Coronary endothelial dysfunction is characterized by vasoconstrictive response to the endothelium-dependent vasodilator acetylcholine. Although endothelial dysfunction is considered an early phase of coronary atherosclerosis, there is a paucity of information regarding the outcome of these patients. Thus, this study was designed to evaluate the outcome of patients with mild coronary artery disease on the basis of their endothelial function.

Methods and Results—Follow-up was obtained in 157 patients with mildly diseased coronary arteries who had undergone coronary vascular reactivity evaluation by graded administration of intracoronary acetylcholine, adenosine, and nitroglycerin and intracoronary ultrasound at the time of diagnostic study. Patients were divided on the basis of their response to acetylcholine into 3 groups: group 1 (n=83), patients with normal endothelial function; group 2 (n=32), patients with mild endothelial dysfunction; and group 3 (n=42), patients with severe endothelial dysfunction. Over an average 28-month follow-up (range, 11 to 52 months), none of the patients from group 1 or 2 had cardiac events. However, 6 (14%) with severe endothelial dysfunction had 10 cardiac events ($P<0.05$ versus groups 1 and 2). Cardiac events included myocardial infarction, percutaneous or surgical coronary revascularization, and/or cardiac death.

Conclusions—Severe endothelial dysfunction in the absence of obstructive coronary artery disease is associated with increased cardiac events. This study supports the concept that coronary endothelial dysfunction may play a role in the progression of coronary atherosclerosis. (*Circulation*. 2000;101:948-954.)

Key Words: atherosclerosis ■ acetylcholine ■ endothelium ■ coronary disease

Coronary heart disease is the leading cause of morbidity and mortality in most industrialized societies.¹ Cardiac risk factors were shown to cause impairment of endothelial vasodilator function of both the epicardial conductance vessels and coronary resistance arteries,²⁻⁶ which is considered an important phase in atherogenesis.⁷⁻⁹ We and others^{10,11} have demonstrated that coronary endothelial dysfunction in humans may be associated with myocardial ischemia. Furthermore, modifications of cardiovascular risk factors that contribute to endothelial dysfunction improve patient outcomes disproportionately to the improvement in coronary atherosclerosis,¹² thus implying that these beneficial effects may be mediated in part through improvement in endothelial function. Despite the general concept that endothelial dysfunction may be the earliest stage of coronary atherosclerosis, follow-up study in patients with endothelial dysfunction is unavailable. Thus, the purpose of this study was to evaluate the outcome of patients with mildly diseased coronary arteries and endothelial dysfunction.

Methods

Study Population

Between January 1993 and June 1997, 168 consecutive patients who had been referred for coronary atherosclerosis were studied prospec-

tively. Patients were included in this study if they had angiographically coronary artery lesions <40% lumen diameter stenosis without evidence of coronary spasm. Exclusion criteria included history of myocardial infarction, percutaneous coronary revascularization, CABG, unstable angina pectoris, history of variant angina, uncontrolled hypertension, peripheral vascular disease, ejection fraction $\leq 50\%$, and valvular heart disease, and/or significant endocrine, hepatic, renal, or inflammatory disease. Long-acting nitrates or calcium channel blocking agents were withheld for 36 to 48 hours before study to allow assessment of baseline coronary physiology. Patient demographics and laboratory data, including fasting lipid profile and serum glucose, were obtained at baseline. The Mayo Clinic Institution Review Board approved the study, and informed consent was obtained from all patients.

Study Protocol

Diagnostic coronary angiography and determination of endothelium-dependent and endothelium-independent flow reserve were performed as previously described.¹⁰ A Doppler guide wire (0.014-in diameter, FloWire, Endosonics Incorporated) within a 2.2F coronary infusion catheter (Ultrafuse, SciMed Life System) was advanced and positioned in the middle portion of the left anterior descending coronary artery (LAD). Intracoronary bolus injections of incremental doses (18 to 36 μg) of adenosine (Fujisawa), an endothelium-independent vasodilator primarily of the microcirculation,¹³ were administered into the guiding catheter until maximal hyperemia was achieved.

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TABLE 1. Clinical Characteristics, Fasting Serum Lipids, and Blood Glucose

| | Group 1 (n=83; 53%) | Group 2 (n=32; 20%) | Group 3 (n=42; 27%) |
|-------------------------------|------------------------|------------------------|------------------------|
| Age (range), y | 52 (23–72) | 52 (30–78) | 55 (17–72) |
| Male, n (%) | 30 (36) | 11 (34) | 12 (29) |
| Female, n (%) | 53 (64) | 21 (66) | 30 (71) |
| Hypertension, n (%) | 23 (28) | 10 (31) | 17 (40) |
| Diabetes, n (%) | 3 (3) | 2 (6) | 0 (0) |
| Smoking, n (%) | 12 (14) | 3 (9) | 4 (10) |
| Hypercholesterolemia, n (%) | 38 (46) | 12 (38) | 17 (40) |
| Postmenopausal, n (%) | 35 (66) | 18 (86) | 21 (70) |
| Family history, n (%) | 50 (60) | 17 (53) | 19 (45) |
| Exercise test, n (%) | 68 (81) | 22 (69) | 32 (76) |
| Abnormal exercise test, n (%) | 25 (37) | 9 (41) | 12 (36) |
| EF, % | 65.4±1.3 | 65.5±1.7 | 66.5±1.5 |
| Total cholesterol, mmol/L | 5.6±0.1 | 5.4±0.2 | 5.8±0.2 |
| LDL cholesterol, mmol/L | 3.4±0.1 | 3.1±0.2 | 3.6±0.2 |
| HDL cholesterol, mmol/L | 1.4±0.05 | 1.3±0.09 | 1.4±0.06 |
| Triglycerides, mmol/L | 1.8±0.1 | 1.9±0.2 | 1.8±0.1 |
| Fasting blood glucose, mmol/L | 5.2±0.2 | 5.9±0.3 | 5.3±0.2 |

EF denotes left ventricular ejection fraction. Values are mean±SE.

Assessment of the endothelium-dependent coronary flow reserve was performed by selective infusion of acetylcholine into the LAD. Acetylcholine (Iolab Pharmaceuticals) 0.182, 1.82, and 18.2 $\mu\text{g}/\text{mL}$ (10^{-6} , 10^{-5} , and 10^{-4} mol/L, respectively) was infused at 1 mL/min for 3 minutes.^{10,14} Hemodynamic data (heart rate and mean arterial pressure), Doppler measurements, and coronary angiography were obtained after each infusion. The infusion was terminated when the largest molar concentration of acetylcholine (10^{-4} mol/L) was reached. Nitroglycerin (200 μg , Abbott Laboratories) was then injected as an intracoronary bolus.¹⁵

Quantitative Coronary Angiography

Artery diameter was analyzed from cine films with use of a modification of a previously described technique from this institution.^{10,16} Measurements were made in the segment 5 mm distal to the tip of the Doppler wire by 2 independent investigators.

Intravascular Ultrasound Examination

Intravascular ultrasound (IVUS) systems were used in this study to assess changes in early atherosclerosis in 86 of the patients studied. Details of these systems have been described elsewhere.^{17,18} After optimization of the ultrasound image and continuous real-time images were recorded, 4 to 5 LAD segments were identified.

Assessment of Coronary Blood Flow

Doppler flow velocity spectra were analyzed online to determine the time-averaged peak velocity. Volumetric coronary blood flow (CBF) was determined from the following relation: $\text{CBF} = \text{cross-sectional area} \times \text{average peak velocity} \times 0.5$.¹⁹ Endothelium-dependent coronary flow reserve was calculated as percent change in CBF in response to acetylcholine as previously described. The endothelium-independent coronary flow reserve ratio was calculated by dividing the average peak velocity after adenosine injection by the baseline average peak velocity.¹⁰

Ultrasound Image Analysis

An offline computer-interactive analysis system was used to digitize the IVUS video images. Measurements of lumen, plaque plus media, and vessel areas were made at each specific segments of the artery as previously described.^{16–18} Percent area stenosis was calculated as the

ratio of plaque plus media area to vessel area. Morphological plaque features were classified as previously described.^{16–18} These measurements were done without knowledge of the results of endothelial function.

Definition of Endothelial Function and Coronary Flow Reserve

A normal coronary endothelium-dependent function was defined as an increase in CBF of >50%, ie, a ratio of >1.5 in response to acetylcholine, calculated by dividing the CBF after 10^{-4} mol/L acetylcholine (18.2 $\mu\text{g}/\text{mL}$) by the baseline. Endothelial dysfunction was classified as mild (a percent change in CBF between 0% to 50%) or severe (percent change in CBF <0%). The decision to divide patients into these groups was based on the association between the changes in CBF in response to acetylcholine and perfusion defects we previously reported.¹⁰ Moreover, endothelial dysfunction was also evaluated according to the epicardial coronary artery diameter response to acetylcholine [% ΔCAD (Ach)]. Patients were divided into 3 groups: group 1, normal endothelial function [% ΔCAD (Ach) >20%, n=20]; group 2, mild endothelial dysfunction [% ΔCAD (Ach) 20% to -20%, n=89]; and group 3, severe endothelial dysfunction [% ΔCAD (Ach) <-20%, n=48], and their outcome at follow-up was evaluated. Impaired coronary endothelium-independent function was defined as a ratio of flow velocity to adenosine of ≤ 2.5 .²⁰

Follow-Up

All patients received questionnaires concerning the occurrence of cardiac events (myocardial infarction, heart failure, and surgical or percutaneous coronary revascularization). For those who were not followed up at our institution, attempts were made to contact patients or their relatives. In addition, hospital records were reviewed. Cardiac events were defined as myocardial infarction, percutaneous or surgical revascularization, and cardiac death. All cardiac events were confirmed by a review of hospital records.

Interobserver and Intraobserver Variabilities

Two ultrasound sites from 10% of the patients studied were randomly selected and measured by the same observer at 2 separate occasions and by a second observer. These measurements were then

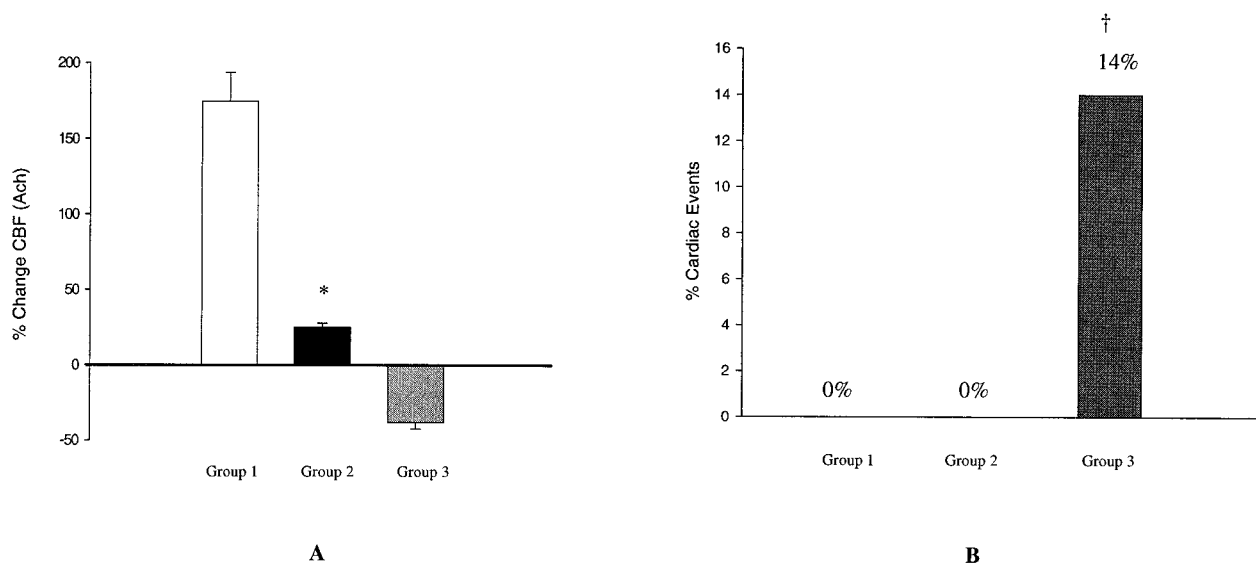


Figure 1. A, Mean percent change in CBF in response to acetylcholine (ACh) among 3 groups. * $P < 0.0001$ vs groups 1 and 3. B, Cardiac events (myocardial infarction percutaneous revascularizations, CABG, and/or cardiac death). † $P < 0.05$ vs groups 1 and 2.

used to evaluate intraobserver and interobserver variabilities at the 2 separate occasions and the second observer. From 20% of the patients, 2 coronary flow reserve measurements also were selected to assess intraobserver variability. These were expressed as linear regression between the 2 observations and as percent error, derived as the absolute difference between observations.

Statistical Analysis

Values are expressed as mean \pm SE. Comparisons of the baseline cardiovascular risk variables between the 3 groups were done with Pearson's χ^2 test. Comparisons of left ventricular ejection fraction, fasting serum lipids, and blood glucose between the study groups were done with 1-way ANOVA. Logistic regression analysis was performed to determine independent predictors of cardiac events. Statistical significance was accepted when $P < 0.05$.

Results

We studied 168 patients. Eleven patients were excluded because follow-up could not be obtained (4 with normal endothelial function and 4 with mild and 3 with severe endothelial dysfunction). Follow-up was obtained in 157 (93%) of the initial patients studied, and they constituted the study group. There were 104 women (67%) and 53 men (33%). The mean age of the study population was 52 years (range, 17 to 78 years). Most patients (92%) had ≥ 1 risk factor for coronary artery disease.

Patients were divided into 3 groups according to their response to acetylcholine. Group 1 consisted of 83 patients who had normal endothelium-dependent coronary flow reserve; group 2 consisted of 32 patients with mildly abnormal endothelium-dependent coronary flow reserve; group 3 consisted of 42 patients with severely abnormal endothelium-dependent coronary flow reserve.

Patient Characteristics

The clinical characteristics of the patients according to their response to acetylcholine are shown in Table 1. Distribution of sex, age, and other cardiovascular risk factors was similar between the study groups. Furthermore, there were no significant differences between the 3 groups in frequency of use of

cardiac medications, including β -blockers, ACE inhibitors, or lipid-lowering agents.

Changes in CBF

The acetylcholine-induced percent changes in CBF in the 3 groups are shown in Figure 1. There were significant differences between group 3 ($-38.2 \pm 4.3\%$) compared with groups 1 and 2 ($174.5 \pm 18.8\%$ and $24.8 \pm 2.8\%$ in groups 1 and 2, respectively). There were also significant differences between groups 1 and 2. The acetylcholine-induced percent changes in coronary artery diameter also revealed significant differences between the 3 groups ($5.7 \pm 2.8\%$, $-13.7 \pm 2.8\%$, and $-35.5 \pm 3.7\%$ in groups 1 through 3). The epicardial vasoconstrictor response to acetylcholine in these patients was diffuse rather than focal without complete epicardial constriction. Noninvasive functional studies, including treadmill exercise test, exercise thallium, or exercise echoes, were performed in 78% of the patients studied. There were no significant differences in the prevalence of positive noninvasive functional studies between study groups. The coronary flow reserve to adenosine was significantly lower in group 3 (2.6 ± 0.1) compared with groups 1 and 2. In addition, there were no significant differences in systemic hemodynamic parameters (mean arterial pressure and heart rate) between the 3 study groups.

IVUS Data

Thirteen segments with technical problems in their video images were excluded from analysis. Thus, 295 segments were analyzed (Table 2). There were no significant differences between study groups in vessel, lumen, and plaque plus media areas in absolute terms or when indexed to body surface area or maximal plaque thickness. Furthermore, there were no significant differences between groups in plaque morphology and no significant correlation between coronary artery disease assessed with IVUS and endothelium-dependent or -independent coronary flow reserve abnormalities assessed with acetylcholine and adenosine.

TABLE 2. Characteristics of Plaque and Coronary Hemodynamics

| | Group 1 (n=83; 53%) | Group 2 (n=32; 20%) | Group 3 (n=42; 27%) |
|---|------------------------|------------------------|------------------------|
| Segments, n | 130 | 80 | 85 |
| Quantitative coronary ultrasound | | | |
| Vessel area index, mm ² | 6.12±.20 | 5.96±.29 | 5.87±.29 |
| Lumen area index, mm ² | 4.30±.17 | 4.46±.25 | 4.08±.24 |
| Plaque plus media area index, mm ² | 1.82±.11 | 1.50±.12 | 1.80±.15 |
| Area stenosis, % | 29.1±1.5 | 25.4±1.5 | 29.8±1.9 |
| Maximal plaque thickness, mm | 0.57±.04 | 0.51±.04 | 0.59±.05 |
| ΔCBF (Ach), % | 174.5±18.8 | 24.8±2.8* | -38.2±4.3 |
| ΔCAD (Ach), % | 5.7±2.8 | -13.7±2.8† | -35.5±3.7 |
| CFR | 2.9±.1 | 3.0±.1 | 2.6±.1‡ |

ΔCBF (Ach) indicates change in CBF in response to acetylcholine; ΔCAD (Ach), change in coronary artery diameter in response to acetylcholine; and CFR, coronary flow reserve to adenosine.

*P<0.0001 vs groups 1 and 3; †P<0.01 vs groups 1 and 3; ‡P<0.05 vs groups 1 and 2.

The interobserver variability was 0.4±2.4% and 1.06±4.3% and the intraobserver variability was 0.8±1.9% and 1.5±3.3% for the coronary diameter and area measurements, respectively. The intraobserver variability for flow velocity measurement was 2.0±2.4%.

Follow-Up

Patients were followed up for a mean of 28 months (range, 11 to 52 months). During follow-up, none of the patients in group 1 or 2 had cardiac events. However, in patients with severe impairment of endothelium-dependent coronary flow reserve (group 3), 6 patients (14%) developed cardiac events (Table 3 and Figure 1). This 14% incidence of cardiac events in group 3 represents the number of patients affected or the incidence of the most severe cardiac event in the group rather than the total number of events (n=10). Further analysis of the incidence of cardiac events according to the percent change in coronary artery diameter in response to acetylcholine was made. Patients were divided into 3 groups: group 1, normal endothelial function (n=20); group 2, mild endothelial dysfunction (n=89); and group 3, severe endothelial dysfunction (n=48). This division produced results identical to the original classification in that none of the patients in groups 1 and 2 had cardiac events and all 6 patients who had cardiac events were in group 3 (13%). The mean age, sex,

cardiovascular risk factors, and left ventricular ejection fraction of these 6 patients were comparable to the various groups studied. Noninvasive functional studies were performed in 4 patients at the time of initial evaluation and were abnormal in 2 patients. The prevalence of use of ACE inhibitors and lipid-lowering agents was also comparable between groups. Furthermore, although the percent change in CBF to acetylcholine was even more severely reduced (-47±14.0% in this subgroup), endothelium-independent coronary flow reserve in these 6 patients was not significantly different from that in patients with normal endothelial function or mild endothelial dysfunction (2.8±0.5). Cardiac events included myocardial infarction (Figure 2) and percutaneous coronary revascularization (Figure 3). CABG was performed in 2 patients because of the development of multivessel obstructive coronary artery disease. Of these 6 patients, 2 subsequently developed congestive heart failure caused by systolic dysfunction and 2 died of cardiac causes, 1 secondary to congestive heart failure and the other a sudden death. Compared with groups 1 and 2, endothelial dysfunction was the only predictor of increased cardiac events.

Discussion

The present study demonstrates for the first time that patients with nonobstructive coronary artery disease and severe endothelial dysfunction are at increased risk for cardiac events.

Several studies have demonstrated that treatment of cardiovascular risk factors known to lead to endothelial dysfunction is associated with a decrease in cardiac events in both primary and secondary prevention studies,^{21,22} underscoring the concept that the reduction in cardiac events in this patient population may be at least in part secondary to improvement in coronary endothelial function. The mechanism by which endothelial dysfunction leads to cardiac events may be multifactorial. One possible mechanism is myocardial ischemia secondary to endothelial dysfunction even in the absence of obstructive coronary artery disease.^{10,11} Indeed, we have recently demonstrated that the reduction in CBF response to acetylcholine resulting from coronary endothelial dysfunction

TABLE 3. Cardiac Events at Follow-Up

| | Group 1 (n=83; 53%), n | Group 2 (n=32; 20%), n | Group 3 (n=42; 27%), n |
|-----------------------|------------------------------|------------------------------|------------------------------|
| Cardiac death | 0 | 0 | 2 |
| MI | 0 | 0 | 1 |
| CHF | 0 | 0 | 2 |
| CABG | 0 | 0 | 2 |
| PCI | 0 | 0 | 3 |
| Cardiac events, n (%) | 0 | 0 | 6 (14.0)*† |

MI indicates myocardial infarction; CHF, congestive heart failure; and PCI, percutaneous coronary intervention.

*P<0.05 vs group 1 or 2.

†Total of 10 cardiac events occurred in 6 patients.



Figure 2. A, ECG of 58-year-old patient at time of endothelial function evaluation (September 7, 1995). Mean percent change in CBF in response to acetylcholine was -35% . B, ECG when patient presented with 3 hours of typical anginal pain and elevated creatine kinase to 800 U (July 6, 1997), revealing new T-wave inversion in anterolateral leads.

was associated with myocardial perfusion defects. However, the lack of any correlation between the frequency of abnormal noninvasive tests among the 3 groups may suggest that endothelial dysfunction in the absence of obstructive coronary artery disease may not cause myocardial ischemia that can be detected noninvasively. Another possible mechanism by which coronary endothelial dysfunction may contribute to cardiac events is through acceleration of coronary atherosclerosis, as evidenced by the development of obstructive coronary artery disease. This is also supported by the observation in cardiac transplant patients that coronary endothelial dysfunction precedes the development of coronary atherosclerosis.²³ It may be hypothesized that endothelial dysfunction represents the stage of rapid progression of atherosclerosis, which may be secondary to the loss of various protective physiological roles of endothelial cells. The abnormal response to the endothelium-dependent vasodilator acetylcholine may represent a reduction in nitric oxide (NO) bioavailability.^{9,24,25} We have previously reported that in this patient population the second messenger of NO is reduced in the coronary circulation,¹⁴ implying a decrease in NO activity. NO plays a pivotal role in antiatherogenesis; in addition to being a vasodilator, it inhibits platelet adherence and aggregation, smooth muscle proliferation, and endothelial cell-leukocyte interaction, all of which are key events in atherogenesis.²⁶ Pathophysiological states associated with a decrease in NO bioavailability and endothelial adhesion molecules for monocytes are upregulated.²⁷ This could enhance local inflammation of the vessel wall, which may play

a critical role in plaque rupture.²⁸ This hypothesis is supported by the observation that L-arginine supplementation, the precursor of NO, improves endothelium-dependent vasorelaxation²⁹ and attenuates the progression of atherosclerosis in an experimental rabbit hypercholesterolemia model.²⁶ Indeed, medical intervention that increases NO bioavailability was shown to improve patient outcome.^{21,22} The possibility that experimenting on the LAD caused accelerated iatrogenic atherosclerosis can be ruled out because all patients underwent similar procedures; moreover, cardiac events were not restricted to the LAD territory.

IVUS parameters in our study, including plaque plus media area, maximal plaque thickness, and plaque morphology, were not helpful in predicting cardiac events. This finding is consistent with previous studies demonstrating the dissociation between the vasoreactive response to acetylcholine and coronary atherosclerosis as assessed with IVUS.¹⁶ The lack of significant differences in cardiovascular risk factors between the various groups may be related to the duration of risk factors between the various groups in that patients with severe endothelial dysfunction possibly had longer exposure to these risk factors. Another potential explanation may be the presence of significant differences in other unmeasured cardiovascular risk factors such as oxidative stress and asymmetric dimethylarginine that have recently been associated with endothelial dysfunction.³⁰

Study Limitations

One of the main limitations of this study is the invasive method of coronary angiography, which is a potential risk

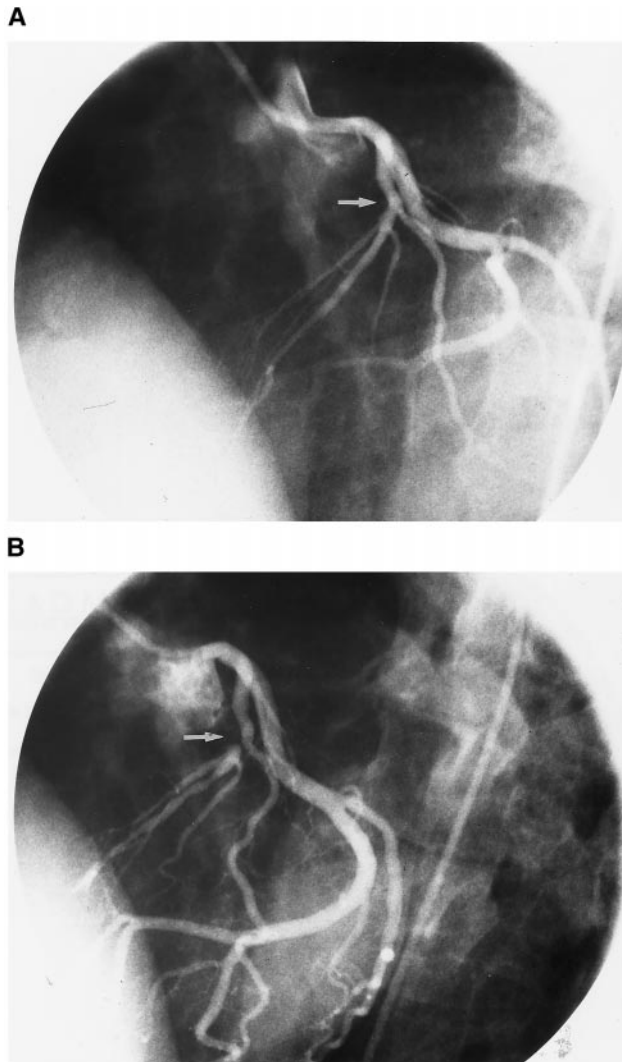


Figure 3. A, Coronary angiogram (left coronary artery in left cranial view) of 51-year-old patient at time of endothelial function evaluation (January 11, 1996), demonstrating 20% diameter stenosis in mid-LAD (arrow). Mean percent change in CBF in response to acetylcholine was -50% . B, Patient who presented on August 1, 1997, with progressive exertional angina and dyspnea. Exercise sestamibi revealed large, reversible anterolateral perfusion defect, and repeated coronary angiography revealed 95% diameter stenosis in mid-LAD (arrow). Patient successfully underwent percutaneous coronary angioplasty and stent placement with resolution of symptoms.

for the patient and requires special expertise to perform. Thus, this method cannot be used in asymptomatic patients as a screening procedure to define their risk for future cardiovascular events. Future studies may be needed to develop a more clinically applicable methodology for wider clinical use.

Clinical Implications

This study extends previous observations that early coronary atherosclerosis is associated with endothelial dysfunction and demonstrates for the first time that severe endothelial dysfunction in patients with nonobstructive coronary artery disease is associated with increased cardiac events.

Acknowledgments

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