Close Relation of Endothelial Function in the Human Coronary and Peripheral Circulations

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Objectives. The relation between endothelium-dependent vasodilator function in the brachial and coronary arteries was determined in the same subjects.

Background. Coronary artery endothelial dysfunction precedes the development of overt atherosclerosis and is important in its pathogenesis. A noninvasive assessment of endothelial function in a peripheral conduit vessel, the brachial artery, was recently described, but the relation between brachial artery function and coronary artery vasodilator function has not been explored.

Methods. In 50 patients referred to the catheterization laboratory for the evaluation of coronary artery disease (mean age ± SD 56 ± 10 years), the coronary vasomotor response to serial intracoronary infusions of the endothelium-dependent agonist acetylcholine (10^-8 to 10^-4 mol/liter), was studied. Endothelium-dependent vasodilation was also assessed in the brachial artery by measuring the change in brachial artery diameter in response to reactive hyperemia.

Results. Patients with coronary artery endothelial dysfunction manifested as vasoconstriction in response to acetylcholine had significantly impaired flow-mediated vasodilation in the brachial artery compared with that of patients with normal coronary endothelial function (4.8 ± 5.5% vs. 10.8 ± 7.6%, p < 0.01). Patients with coronary artery disease also had an attenuated brachial artery vasodilator response compared with that of patients with angiographically smooth coronary arteries (4.5 ± 4.6% vs. 9.7 ± 8.1%, p < 0.02). By multivariate analysis, the strongest predictors of reduced brachial dilator responses to flow were baseline brachial artery diameter (p = 0.007), coronary endothelial dysfunction (p = 0.003), the presence of coronary artery disease (p = 0.007) and cigarette smoking (p = 0.016). The brachial artery vasodilator response to sublingual nitroglycerin was independent of coronary endothelial responses or the presence of coronary artery disease. The positive predictive value of abnormal brachial dilation (<3%) in predicting coronary endothelial dysfunction is 95%.

Conclusions. This study demonstrated a close relation between coronary artery endothelium-dependent vasomotor responses to acetylcholine and flow-mediated vasodilation in the brachial artery. This noninvasive method may become a useful surrogate in assessing the predisposition to atherosclerosis in patients with cardiac risk factors.

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repeated or performed before coronary angiography becomes clinically necessary.

The vasodilator response in conduit arteries to an increase in blood flow is thought to be endothelium dependent (10–13). This flow-mediated dilation has been demonstrated in the epicardial coronary arteries and in the peripheral conduit vessels by intraarterial infusion of vasodilators. Recently, high resolution ultrasound has been used to assess brachial artery flow-mediated vasodilator responses noninvasively (14). This technique is both sensitive and reproducible. Preliminary data using this technique have demonstrated an impairment in the vasodilator response of the brachial artery response in patients with atherosclerosis or cardiac risk factors (14–16). Because the brachial artery rarely develops structural changes typical of coronary atherosclerosis, it was unclear whether this brachial artery vasodilator response is related to coronary endothelial function. The purpose of the present study was to compare the endothelium-dependent vasomotor function in the brachial and coronary arteries in the same patients with various degrees of coronary atherosclerosis.

Methods

Patients. The study group consisted of 50 consecutive patients referred to the cardiac catheterization laboratory who underwent coronary endothelial function testing and also agreed to a brachial ultrasound study. The patients ranged in age from 35 to 73 years (mean ± SD 56 ± 10), and 19 (38%) were female. Cardiac risk factors were present as follows: cigarette smoking in 7 patients (14%), hypertension in 22 (44%), hypercholesterolemia in 40 (80%), diabetes mellitus in 2 (4%) and a family history of premature atherosclerosis in 18 (36%). The mean number of risk factors was 1.3 ± 1.1. Coronary artery disease, defined as lumen irregularities or stenoses, was present in 32 patients (64%); the remaining 18 patients had entirely normal-appearing coronary arteries on angiography. Written informed consent was obtained from patients before the catheterization procedure in accordance with the guidelines established by the Committee for the Protection of Human Subjects at our institution.

Cholesterol-lowering drug therapy was used by 26 (52%) of the patients in the study. The total and low (LDL) and high (HDL) density lipoprotein cholesterol values were 173 ± 38, 103 ± 34 and 39 ± 11 mg/dl, respectively, at the time of the study.

Study protocol. Coronary endothelial function study. Patients' long-acting vasoactive medications including calcium channel blockers, beta-adrenergic blocking agents, nitrates and converting enzyme inhibitors were discontinued for ≥18 h before the catheterization. After diagnostic right and left heart catheterization, patients were eligible to receive vasoactive drugs if there were no stenoses >50% in a study vessel (either the left anterior descending or the left circumflex coronary artery).

After a 7F or 8F guiding catheter was positioned in the left main coronary artery and 10,000 IU of heparin was infused, a 2.5F infusion catheter was advanced through the guiding catheter over a 0.014-in. (0.036-cm) guide wire into the proximal portion of the left anterior descending or left circumflex coronary artery. Serial intracoronary infusions were made according to an established protocol: 1) control infusion (5% dextrose in water); 2) serial 2.5-min infusions of the endothelium-dependent vasodilator acetycholine (Miochol, Iolab Pharmaceuticals) in doses of 0.16, 1.6 and 16 μg/min, achieving final estimated intracoronary concentrations of 10^-8, 10^-7 and 10^-6 mol/liter; 3) 5-min recontrol infusion of 5% dextrose in water; 4) 3-min infusion of the endothelium-independent vasodilator nitroglycerin at 16 μg/min (3). During each infusion, the blood pressure, heart rate and electrocardiogram (ECG) were continuously monitored.

Quantitative coronary angiographic images were taken after each intervention by a previously validated method (3,17). A nonionic contrast medium (iohexol [Omnipaque], Winthrop Laboratories) was injected into the left coronary artery at 7 ml/s for a total of 9 ml with a power injector (Medrad) to opacify the coronary artery.

Brachial endothelial function study. The brachial artery protocol was completed within 24 h of the coronary artery study in 39 patients (78%). The remaining patients returned for study within 4 weeks. Vasoactive medications were held for ≥18 h before the study in all patients.

Brachial artery vasodilator responses were measured in the dominant arm of patients by a previously validated and reproducible technique (14,15,18). A 7.5-MHz linear phased array ultrasound transducer attached to a Toshiba ultrasound machine was used to image the brachial artery longitudinally just above the antecubital fossa. The intraserver and interserver variability for repeated measurements are 0 ± 0.07 mm and 0.05 ± 0.16 mm, respectively, in our laboratory. When reactive hyperemia studies are performed on 2 separate days, the mean difference in brachial vasodilator response in absolute terms is 1.0 ± 2.0%.

After baseline measurements of brachial artery diameter were recorded, a blood pressure cuff was inflated on the proximal portion of the arm to 200 mm Hg for 5 min, creating distal limb ischemia. After release of the cuff, reactive hyperemia occurs, that is, flow in the brachial artery increases to accommodate the dilated resistance vessels in the forearm. The brachial artery was imaged for the 1st 2 min of reactive hyperemia. The flow-mediated dilator response was used as a measure of endothelium-dependent vasodilation. The brachial artery diameter was allowed to return to baseline level (~5 min after cuff release). Then, 0.4 mg of nitroglycerin was given sublingually, and the brachial artery was imaged for the ensuing 3 min to measure peak diameter. The response to nitroglycerin is a measure of endothelium-independent vasodilation.

Analysis. Quantitative coronary angiography. Technically suitable single-plane angiograms were selected for computer analysis on the basis of a previously described method (3,17). An automated edge detection program was used to search densities and seek inflection points, thus measuring the seg-
ment diameter of the vessel along the length of the selected segment (ImageComm, Quantum IC software). Two segments 8 to 10 mm in length were selected for analysis prospectively on the basis of optimal regions for quantitative angiographic analysis. The vasomotor responses for the two segments in each patient were averaged to create a mean vasodilator response to acetylcholine and nitroglycerin. This average response was used to evaluate the relation between the magnitude of the coronary and brachial artery vasomotor responses in a continuous fashion.

For the purpose of classifying the coronary vasomotor response dichotomously, patients in whom either of the two coronary segments constricted significantly (>5% from baseline) were classified as having coronary endothelial dysfunction. All other patients were considered to have normal coronary endothelial function. The vasomotor response to acetylcholine was also compared with brachial responses in a continuous manner.

The coronary angiograms were coded for atherosclerosis after review by an observer who had no knowledge of the data on brachial artery vasodilator function. Only patients whose epicardial coronary arteries were completely smooth were classified as having no angiographic coronary disease.

**Brachial vasodilator responses.** Three sequential systolic frames (taken at the end of the T wave on the ECG) for each intervention (baseline, reactive hyperemia, repeat baseline and nitroglycerin) were digitized, with use of a Rasterops analog to digital converting board, into a Macintosh computer. Straight segments of the artery (10 mm in length) were chosen. The blood-intima interface was determined for the posterior portion of the artery and the blood-media border for the anterior surface (unless intima was clearly identified). A software algorithm automatically calculated the average diameter (15 to 20 points) over the selected segment. The three frames were then averaged for each intervention. Previous studies have shown that maximal dilation occurs 1 min after cuff deflation for reactive hyperemia and 3 min after sublingual nitroglycerin (18). Measurements were taken at these times. The end point was the percent diameter change of the brachial artery in response to reactive hyperemia or nitroglycerin. The results did not differ when they were also analyzed by using the absolute change in brachial diameter or brachial area. Only the data for percent diameter change are shown.

**Statistics.** Differences between clinical characteristics, and brachial artery vasodilator responses were evaluated and analyzed by unpaired t tests for two-group comparisons and one-way analysis of variance for multiple group comparisons. Post hoc testing was performed by using a Bonferroni correction. Linear regression analysis was used to compare the continuous relation between brachial artery and coronary artery responses. Predictors of brachial artery vasodilator responses to reactive hyperemia were obtained by forward stepwise multilinear regression analysis. Previous studies (14) have demonstrated that the percent brachial artery diameter change to reactive hyperemia is inversely correlated with the baseline diameter and, as such, this variable was included in the stepwise model. Statistical significance was defined as a two-sided p value < 0.05. The data are expressed as the mean value ± SD.

**Results**

**Coronary artery endothelial function.** Patients were separated into two groups on the basis of their coronary epicardial vasomotor response to acetylcholine. Normal coronary endothelial function was present in 13 patients (26%); the remaining 37 (74%) demonstrated endothelial dysfunction. Patients with preserved endothelial function demonstrated dilation by 9 ± 11% in response to the maximal dose of acetylcholine, whereas those with endothelial dysfunction demonstrated constriction by 17 ± 14%. The coronary vasodilator response to the endothelium-independent agonist nitroglycerin was not significantly different between the two groups (17 ± 10% vs. 22 ± 16%). There were more women in the group with normal coronary endothelial function. However, no other differences were present between the two groups in terms of clinical characteristics or cardiac risk factors (Table 1).

**Brachial artery endothelial function.** The change in brachial artery diameter in response to reactive hyperemia in all patients ranged from −2% to 25%. There was a significant inverse relation between the degree of dilation and the baseline vessel diameter, as has been previously reported (r = −0.47, p < 0.01).

The brachial artery vasodilator response to reactive hyperemia was significantly greater in the patients with normal coronary endothelial function than in those with coronary endothelial dysfunction (10.8 ± 7.6% vs. 4.8 ± 5.5%, p < 0.01). In contrast, the brachial artery response to nitroglycerin did not differ between these two groups (11.5 ± 5.9% vs. 10.6 ± 5.7%) (Fig. 1). There was no difference in the baseline brachial artery diameter to account for these differences (4.0 ± 0.7 mm vs. 3.9 ± 0.5 mm). If the normal brachial dilator response to reactive hyperemia was arbitrarily set at >3% (1 SD below the mean value in the normal endothelial function group), then 18 (95%) of 19 patients with an abnormal brachial

| Table 1. Clinical Characteristics of the Study Patients |
|-------------------------------------------|-------------------------------------------|
| Normal Coronary | Coronary Endothelial |
| Endothelial Function | Dysfunction (n = 37) |
| Age (yr) | 58 ± 8 | 55 ± 10 |
| Male/female (no.) | 4/5* | 27/10* |
| Total cholesterol (mg/dl) | 168 ± 38 | 175 ± 38 |
| LDL cholesterol (mg/dl) | 103 ± 36 | 102 ± 34 |
| HDL cholesterol (mg/dl) | 41 ± 13 | 38 ± 11 |
| Cigarette users | 4 (31%) | 3 (8%) |
| Hypertension | 6 (46%) | 16 (43%) |
| Diabetes mellitus | 1 (8%) | 1 (3%) |
| Risk factors (no.) | 1.4 ± 1.4 | 1.2 ± 0.9 |

*p < 0.05. Unless otherwise indicated, data presented are mean value ± SD or number (%) of patients. HDL = high density lipoprotein; LDL = low density lipoprotein.
response (<3%) had coronary endothelial dysfunction (positive predictive value). However, only 12 (39%) of 31 patients with brachial dilation >3% had normal coronary endothelial function (negative predictive value). The sensitivity of detecting patients with abnormal coronary endothelial function was 49%, and the specificity was 92% at this cutoff value.

Brachial artery dilator function was also compared in patients with and without angiographic evidence of coronary artery disease, irrespective of the coronary response to acetylcholine. (Fig. 2) Patients with no evidence of coronary artery disease had significantly more brachial artery vasodilation to reactive hyperemia than did patients with coronary artery disease (9.7 ± 8.1% vs. 4.5 ± 4.6%, p = 0.02). The baseline brachial artery diameter was not different in these two groups (4.0 ± 0.7 mm vs. 3.9 ± 0.6 mm); the brachial artery vasodilator response to nitroglycerin was also similar in these groups.

The interaction between coronary artery disease and coronary endothelial function was also explored. Patients with both coronary disease and coronary endothelial dysfunction had the smallest brachial artery vasodilator response (3.8 ± 5.8%). Patients with no evidence of coronary disease but with coronary endothelial dysfunction had an intermediate response (7.2 ± 5.6%). However, the patients with no angiographic evidence of coronary disease and normal coronary endothelial function had the greatest brachial artery vasodilator response (13.8 ± 5.8%). (Fig. 3) The latter group had a significantly better response than the group with both coronary disease and endothelial dysfunction (p < 0.001) and tended to have more arterial dilation than the group with coronary endothelial dysfunction without overt atherosclerosis (p = 0.08). Again, the baseline brachial artery diameter was the same in the three groups (4.0 ± 0.6, 4.0 ± 0.6 and 3.8 ± 0.6 mm, respectively).

The continuous relation between percent diameter change in the brachial artery in response to reactive hyperemia and the percent diameter change in the coronary artery in response to acetylcholine is shown in Figure 4. A significant relation was observed (p < 0.05), but considerable scatter of the data was present.

The response was compared in patients with a normal (<130 mg/dl) or an abnormal LDL cholesterol level. There was no difference in response in these two groups (6.8 ± 6.6% vs. 4.4 ± 6.0%).

A multivariate linear regression analysis was performed to determine the predictors of the brachial artery vasodilator response.
responses. The factors that predicted an attenuated brachial artery response were a larger baseline diameter, abnormal coronary endothelial function, coronary artery disease and cigarette smoking. Age, gender, cardiac risk factors other than smoking and treatment with cholesterol-lowering therapy were not significant predictors in the model (Table 2).

**Discussion**

Previous studies have demonstrated impaired vasodilator responses in the brachial arteries or coronary arteries of patients with cardiac risk factors or overt atherosclerosis but not concomitantly in the same patient. The important new finding in this study is that endothelial function in the coronary artery parallels that in a peripheral conduit vessel—the brachial artery in humans.

**Endothelial dysfunction in peripheral circulation.** The endothelium-dependent vasodilator response of limb resistance vessels is impaired in patients with risk factors for atherosclerosis (19). In patients with hypercholesterolemia or hypertension, for example, the flow response to infused cholinergic agonists is blunted compared with that of normal subjects (20,21). This response has also been shown (22) to be dependent on nitric oxide. Thus, even though there is good evidence that endothelium-derived nitric oxide is important in controlling vascular responsiveness in both conduit and resistance vessels in many vascular beds, studies have not been undertaken to study endothelial responses in more than one vascular bed in the same patient. Concordant responses in coronary and peripheral vasculature might allow for less invasive means of endothelial function testing in patients with suspected coronary endothelial dysfunction.

**Noninvasive assessment of endothelial function.** Recently, a noninvasive ultrasound technique has been described (14) for measuring the vasodilator responses of the brachial artery. In response to the increase in blood flow that occurs after 5 min of ischemia (upper arm occlusion), the normal brachial artery increases its diameter by up to 20%, with excellent reproducibility (14,15).

This flow-mediated dilation has been shown to be endothelium-dependent in vascular beds, in animal experiments, and this relation is likely to occur in humans as well (10-13). Preliminary evidence (23) suggests that the increase in shear stress produced by increased flow may release nitric oxide through activation of potassium ion channels. The increase in brachial artery diameter seen in response to reactive hyperemia can be attenuated by blocking nitric oxide synthase with N^G^-monomethyl-L-arginine (24).

**Present study.** This study demonstrated that, compared with patients without coronary artery disease, patients with angiographic evidence of coronary artery disease have a blunted brachial dilator response to reactive hyperemia but not to nitroglycerin. We (16) previously found in small numbers of patients that young persons with coronary disease (<40 years of age) had an attenuated brachial response compared with that of age-matched control subjects without coronary disease or risk factors. Coronary endothelial responses were not measured in those patients. Celermajer et al. (14) also reported that patients with coronary artery disease had less flow-mediated brachial artery dilation than did control subjects, although the patients with coronary disease were much older and had higher cholesterol levels than those of the control subjects. That study also demonstrated a blunted response to nitroglycerin in the patients with coronary artery disease compared with that of control subjects; however, this response was probably related to the larger baseline diameter brachial artery, which the investigators showed was also inversely related to the dilator response to nitroglycerin.
The new finding in the present study is that patients with coronary endothelial dysfunction had a blunted brachial artery vasodilator response to reactive hyperemia but not to nitroglycerin. This effect was independent of the influence of angiographic coronary artery disease. In a multivariate model, the presence of coronary endothelial dysfunction was the strongest predictor of an abnormal brachial artery vasodilator response after accounting for baseline brachial artery diameter. The normal brachial artery vasodilator response is intrinsically linked to the baseline coronary artery diameter (14).

The relation between the brachial and coronary vasodilator responses was significant, but not to the degree that the magnitude of one can be used to predict the magnitude of the other. If 3% dilation to flow induced by reactive hyperemia was chosen as an arbitrary normal cutoff value, then the majority of patients (18 of 19) with a lower level of brachial dilation had coronary endothelial dysfunction. However, the sensitivity for detecting coronary endothelial dysfunction was only 49%. The use of a single cutoff value to define normal is problematic because of the strong inverse relation between flow-mediated vasodilation and baseline brachial diameter. Subjects with small brachial arteries may have “normal” arterial dilation even in the presence of endothelial dysfunction, and patients with very large brachial arteries may appear to have abnormal dilation despite normal endothelial function. At this point, this technique appears to be better suited to the study of small groups instead of individual patients, matching groups for baseline brachial diameters.

In our study patients, the only cardiac risk factor associated with brachial endothelial dysfunction was cigarette smoking. Celermajer et al. (14,15) previously showed impaired brachial responses in cigarette smokers and children with hypercholesterolemia. Cholesterol levels were not predictive of brachial responses in cigarette smokers and children with hypercholesterolemia. However, previous work by our group (18) has shown that a decrease of this magnitude in peak flow will not significantly affect the flow-mediated vasodilation of the brachial artery. It is unlikely that the decreased dilator response is the result of a decreased flow stimulus.

The study group was recruited from patients who were referred to the catheterization laboratory for suspected coronary artery disease. As such there were no patients in the group without risk factors, and the majority had evidence of endothelial dysfunction. It would be important to determine the relation between the coronary and brachial responses in young patients who might be screened with this noninvasive technique. The youngest patient in our study was 35 years old. It is not known if young patients with brachial artery endothelial dysfunction, such as those reported by Celermajer et al. (14), have concomitant coronary endothelial dysfunction. However, because these patients do not present to the catheterization laboratory, it is unlikely that this information will become available. For now, we must assume that the good correlation observed in this study between coronary and brachial responses in older patients with risk factors with or without overt atherosclerosis is applicable to patients at an earlier stage of their disease.

Conclusions. Endothelial dysfunction is a generalized process and is not necessarily confined to vascular beds with clinically overt atherosclerosis. Using an ultrasound technique, we showed that the brachial vasodilator response to reactive hyperemia was closely related to the coronary vasodilator response to acetylcholine. This noninvasive technique could be a useful method of assessing arterial dysfunction before and during all stages in the development and treatment of atherosclerosis.

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