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Strong Relationship Between Vascular Function in the Coronary and Brachial Arteries: A Clinical Coming of Age for the Updated Flow-Mediated Dilation Test?

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Strong Relationship Between Vascular Function in the Coronary and Brachial Arteries: A Clinical Coming of Age for the Updated Flow-Mediated Dilation Test?


Abstract

Early detection of coronary artery dysfunction is of paramount cardiovascular clinical importance, but a noninvasive assessment is lacking. Indeed, the brachial artery flow-mediated dilation test only weakly correlated with acetylcholine-induced coronary artery function ($r=0.36$). However, brachial artery flow-mediated dilation methodologies have, over time, substantially improved. This study sought to determine if updates to this technique have improved the relationship with coronary artery function and the noninvasive indication of coronary artery dysfunction. Coronary artery and brachial artery function were assessed in 28 patients referred for cardiac catheterization (61±11 years). Coronary artery function was determined by the change in artery diameter with a 1.82 μg/min intracoronary acetylcholine infusion. Based on the change in vessel diameter, patients were characterized as having dysfunctional coronary arteries (>5% vasoconstriction) or relatively functional coronary arteries (<5% vasoconstriction). Brachial artery function was determined by flow-mediated dilation, adhering to current guidelines. The acetylcholine-induced change in vessel diameter was smaller in patients with dysfunctional compared with relatively functional coronary arteries (−11.8±4.6% versus 5.8±9.8%, $P<0.001$). Consistent with this, brachial artery flow-mediated dilation was attenuated in patients with dysfunctional compared with relatively functional coronaries (2.9±1.9% versus 6.2±4.2%, $P=0.007$). Brachial artery flow-mediated dilation was strongly correlated with the acetylcholine-induced change in coronary artery diameter ($r=0.77$, $P<0.0001$) and was a strong indicator of coronary artery dysfunction.
Coronary artery dysfunction precedes the clinical manifestation of cardiovascular disease and is an independent predictor of cardiovascular disease risk and cardiac events. 1–5 As such, the early detection of coronary artery dysfunction is of paramount cardiovascular clinical importance. However, the assessment of coronary artery function remains costly, time-consuming, and invasive, highlighting the need for alternative approaches capable of noninvasively assessing coronary artery function. A seminal study by Anderson et al6 documented a weak relationship between brachial artery (BA) flow-mediated dilation (FMD) and the acetylcholine-induced coronary artery response (r=0.36), which was subsequently confirmed by Teragawa et al7 (r=0.31). Anderson et al6 also demonstrated that BA FMD was lower in patients responding to acetylcholine with >5% coronary artery vasoconstriction than patients with <5% coronary artery vasoconstriction. These findings have been used to support BA FMD as a noninvasive surrogate of coronary artery function and were a primary impetus for BA FMD becoming a cornerstone in clinical cardiovascular

Key Words: acetylcholine, brachial artery, catheterization, endothelium, vasodilation

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(receiver operator characteristic=78%). The current data support that updates to the brachial artery flow-mediated dilation technique have strengthened the relationship with coronary artery function, which may now provide a clinically meaningful indication of coronary artery dysfunction.
research. However, BA FMD has not been adopted in clinical practice because of the considerable variability in these relationships ($r=0.3$) and previous methodological issues with BA FMD.

Over the decades since the publications by Anderson et al$^6$ and Teragawa et al,$^7$ the methodological guidelines and technology now available for the BA FMD technique have improved substantially. Specifically, new guidelines were established in an attempt to improve the reliability and clinical utility of BA FMD,$^8,9$ after the recognition that the technical aspects of the FMD measurements account for the majority of the variability in this assessment.$^{10}$ It was recently demonstrated that the level of adherence to the new guidelines is directly related to the reproducibility of BA FMD.$^{11}$ The most notable refinements include enhanced image quality and resolution, continuous acquisition of diameter measurements, improved diameter analyses, and standardizing the measurement of diameter to be proximal to the occlusion cuff.$^8,9$ An outcome of these refinements, however, is that any inference about coronary artery function, based on BA FMD, is now being made with markedly different techniques from these original studies. Thus, it is imperative both in terms of re-search and, potentially, clinical assessment, to determine if the methodological refinements to the BA FMD technique have improved the relationship with coronary artery function. Such an improvement in this relationship would add credence to BA FMD as a noninvasive assessment of coronary artery function which has, to this date, been lacking.

Consequently, this study was designed to determine the relationship between coronary artery function and BA function determined according to the updated BA FMD guidelines. It was hypothesized that BA FMD would be directly related to the acetylcholine-induced coronary artery response. Furthermore, it was anticipated that the strength of this relationship would be improved from the previously documented relationships.$^{6,7}$ Additionally, coronary artery dysfunction was discerned by the acetylcholine-induced change in left anterior descending (LAD) artery diameter, characterizing patients as having either dysfunctional coronary arteries (>5% coronary artery vasoconstriction) or relatively functional coronary arteries (<5% coronary artery vasoconstriction). It was hypothesized that this patient classification would be clearly reflected in the BA FMD assessment.
Methods

Patients

The data that support the findings of this study are available from the corresponding author on request. Twenty-eight patients, referred for cardiac catheterization, volunteered to have their coronary artery and BA function assessed. All study procedures were approved by the Institutional Review Boards of the University of Utah and the Salt Lake City Veterans Affairs Medical Center, and conformed to the Declaration of Helsinki, except for registration in a database. Written informed consent was attained after patients were informed of the experimental protocol and the potential risks of participation. Patient characteristics and medications are presented in Table 1. Patients were excluded based on documented coronary artery disease, recent myocardial infarction, >50% stenosis in the LAD artery, valvular disease, prior stent or coronary artery bypass grafting, and severe kidney dysfunction. Similarly to the study of Anderson et al,6 the majority of patients in the current study were medicated, and medication status was not altered for testing. A minimum of 10 days was allotted after the angiographic procedures, to allow for recovery, and follow-up testing was typically completed within 3 weeks thereafter. All testing was typically conducted in the morning in a temperature controlled environment with patients instructed to have abstained from food and caffeine for at least 4 hours and from vigorous exercise the day before. All coronary artery and BA function procedures were conducted by trained investigators and, for each patient, image analyses were performed by 1 of 2 trained investigators.

Coronary Artery Function

After diagnostic coronary angiography in the cardiac catheterization laboratory, a 6F guide-catheter was positioned in the left main coronary artery and heparin administered to achieve an activated clotting time of ≈250 seconds. An infusion balloon was then placed directly into the proximal LAD using a workhorse coronary wire. Baseline conditions were then established and serial 3 minutes intracoronary acetylcholine infusions, through the balloon, were conducted at 0.182, 1.82, and 18.2 μg/min.12,13 After reestablishing baseline conditions, a 50 μg intracoronary bolus of sodium nitroprusside (SNP) was administered (n=22). Quantitative angiographic images
were taken for each condition using a single monoplane angiographic view and a nonionic contrast medium injected into the left main coronary artery to achieve optimal opacity of the LAD. Absolute diameter measurements of the LAD were made with a computer-based image analysis system, using the guide catheter as the calibration standard (IMPAX cardiovascular review station, Agfa HealthCare, Mortsel, Belgium). The proximal, middle, and distal segments of the LAD were delineated and 5 to 10 serial diameter measurements were made across each segment during diastole, with a perpendicular angle to the long axis of the artery. Absolute and relative changes in LAD diameter from baseline were determined for each condition using the average response of the segmental measurements to account for potential segmental heterogeneity. The diameter changes across all 3 segments were in agreement, as previously documented. In line with Anderson et al, patients were categorized as having either dysfunctional coronary arteries (>5% coronary artery vasoconstriction) or relatively functional coronary arteries (<5% coronary artery vasoconstriction) based on the vasomotor response to the acetylcholine infusion.

**BA Function**

After arriving at the vascular research laboratory, patients rested in the supine posture for ≈20 minutes before testing. BA FMD was performed in accordance with current recommendations. Briefly, a rapid inflating cuff (D.E. Hokanson Inc, Bellevue, WA) was placed around the right forearm, immediately distal to the epicondyles. After 30 seconds of baseline data acquisition, the cuff was inflated to 250 mm Hg for 5 minutes, then the cuff was rapidly deflated and data continuously acquired for 2 minutes. In the same posture, data were continuously acquired for 30 seconds before and 5 minutes following 0.8 mg sublingual nitroglycerin (NTG) administration (n=23). Continuous BA diameter and blood velocity measurements were simultaneously obtained during all data acquisition periods with Doppler ultrasound (Logiq e9, GE Healthcare, Chicago, IL). Absolute diameter measurements of the BA were made perpendicular to the long axis of the artery during diastole using a computer-based image analysis system (Medical Imaging Applications LLC, Coralville, IA). FMD was quantified as the maximal percent change in BA diameter after cuff release. Angle-
corrected, intensity weighted area under the curve (AUC) mean blood velocity ($V_{\text{mean}}$) was measured with an insonation angle of 60° and the sample volume maximized to the vessel size (Logiq e9). Shear rate was calculated as follows: shear rate ($s^{-1}$) = $8V/diameter$.

Table 1. Patient Characteristics and Medications

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>All Patients</th>
<th>&gt;5% Coronary Artery Vasoconstriction</th>
<th>&lt;5% Coronary Artery Vasoconstriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (men/women)</td>
<td>17/11</td>
<td>14/3*</td>
<td>3/8</td>
</tr>
<tr>
<td>Age, y</td>
<td>61±11</td>
<td>58±9</td>
<td>66±14</td>
</tr>
<tr>
<td>Stature, cm</td>
<td>171±10</td>
<td>174±11†</td>
<td>167±8</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>91±20</td>
<td>95±21</td>
<td>84±18</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>31.2±6.8</td>
<td>31.6±7.3</td>
<td>30.5±6.3</td>
</tr>
<tr>
<td>History of smoking, n (%)</td>
<td>11 (39%)</td>
<td>9 (53%)</td>
<td>2 (18%)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>17 (61%)</td>
<td>8 (47%)</td>
<td>9 (82%)</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>13 (46%)</td>
<td>7 (41%)</td>
<td>6 (55%)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>3 (11%)</td>
<td>0 (0%)</td>
<td>3 (27%)</td>
</tr>
<tr>
<td>COPD or emphysema, n (%)</td>
<td>5 (18%)</td>
<td>4 (24%)</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>Heart failure, n (%)</td>
<td>2 (7%)</td>
<td>1 (6%)</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>Pulmonary hypertension, n (%)</td>
<td>1 (4%)</td>
<td>1 (6%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>97.5±26.7</td>
<td>96.5±27.5</td>
<td>99.0±26.6</td>
</tr>
<tr>
<td>Sodium, mmol/L</td>
<td>139.4±1.7</td>
<td>139.2±2.0</td>
<td>139.6±1.3</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>3.9±0.3</td>
<td>3.9±0.3</td>
<td>3.8±0.3</td>
</tr>
<tr>
<td>Chloride, mmol/L</td>
<td>105.3±2.6</td>
<td>105.9±2.7</td>
<td>104.4±2.1</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>0.9±0.2</td>
<td>1.0±0.2</td>
<td>0.9±0.2</td>
</tr>
<tr>
<td>Calcium, mg/dL</td>
<td>9.4±0.5</td>
<td>9.2±0.3†</td>
<td>9.7±0.5</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>164±47</td>
<td>151±42</td>
<td>184±48</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>104±37</td>
<td>93±35</td>
<td>120±35</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>50±13</td>
<td>47±13</td>
<td>55±13</td>
</tr>
<tr>
<td>LDL, mg/dL</td>
<td>96±36</td>
<td>90±37</td>
<td>104±39</td>
</tr>
<tr>
<td>WBC, K/μL</td>
<td>6.6±2.2</td>
<td>6.9±2.1</td>
<td>6.1±2.5</td>
</tr>
<tr>
<td>RBC, M/μL</td>
<td>4.9±0.5</td>
<td>5.1±0.5†</td>
<td>4.7±0.4</td>
</tr>
<tr>
<td>Medication, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antihypertension drugs</td>
<td>17 (61%)</td>
<td>8 (47%)</td>
<td>9 (82%)</td>
</tr>
<tr>
<td>Statins</td>
<td>16 (57%)</td>
<td>9 (53%)</td>
<td>7 (64%)</td>
</tr>
<tr>
<td>Bronchodilator</td>
<td>5 (18%)</td>
<td>4 (24%)</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>Metformin</td>
<td>4 (14%)</td>
<td>2 (12%)</td>
<td>2 (18%)</td>
</tr>
</tbody>
</table>

Mean±SD. BMI indicates body mass index; COPD, chronic obstructive pulmonary disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RBC, red blood cell; and WBC, white blood cell.

*Significantly different number of men and women (P<0.05).
†Significantly different from <5% coronary artery vasoconstriction (unpaired t test, P<0.05).

Statistics

Differences in coronary artery and BA function between the >5% coronary artery vasoconstriction and <5% coronary artery vasoconstriction groups were assessed using
unpaired \( t \) tests. The capacity for BA FMD to differentiate between >5% coronary artery vasoconstriction and <5% coronary artery vasoconstriction groups was assessed using a receiver operator characteristic curve analysis to determine the AUC, as well as the sensitivity and specificity for the most clearly defined cut score. The relationship between BA FMD and the acetylcholine-induced coronary artery response was assessed with a Pearson correlation coefficient. Statistical analyses were performed with the use of commercially available software (SigmaPlot 11.0, Systat Software, Point Richmond, CA and SPSS, IBM Corporation, Armonk, NY). Statistical significance was defined as \( P<0.05 \), and the data are presented as mean\( \pm \)SD unless otherwise noted.

Table 2. Baseline and Absolute Change in Coronary and Brachial Artery Diameters in Response to ACh and SNP and FMD and Nitroglycerin, Respectively, in Patients Exhibiting >5% Coronary Artery Vasoconstriction or <5% Coronary Artery Vasoconstriction

<table>
<thead>
<tr>
<th></th>
<th>&gt;5% Coronary Artery Vasoconstriction</th>
<th>&lt; 5% Coronary Artery Vasoconstriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary Artery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline diameter, mm</td>
<td>3.19±0.47</td>
<td>3.20±0.65</td>
</tr>
<tr>
<td>Peak change in diameter, mm</td>
<td>−0.36±0.15*</td>
<td>0.18±0.28</td>
</tr>
<tr>
<td>SNP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline diameter, mm</td>
<td>2.83±0.49</td>
<td>2.96±0.55</td>
</tr>
<tr>
<td>Peak change in diameter, mm</td>
<td>0.33±0.20</td>
<td>0.37±0.25</td>
</tr>
<tr>
<td>Brachial artery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FMD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline diameter, mm</td>
<td>4.65±0.86</td>
<td>4.07±0.70</td>
</tr>
<tr>
<td>Peak change in diameter, mm</td>
<td>0.13±0.08*</td>
<td>0.24±0.12</td>
</tr>
<tr>
<td>Nitroglycerin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline diameter, mm</td>
<td>4.80±0.52*</td>
<td>4.05±0.74</td>
</tr>
<tr>
<td>Peak change in diameter, mm</td>
<td>0.67±0.24</td>
<td>0.65±0.12</td>
</tr>
</tbody>
</table>

Mean\( \pm \)SD. ACh indicates acetylcholine; FMD, flow-mediated dilation; and SNP, sodium nitroprusside. *Significantly different from <5% coronary artery vasoconstriction (unpaired test, \( P<0.05 \)).

**Results**

**Coronary Artery Function**

There were no complications as a result of the intracoronary acetylcholine or SNP administration. In characterizing coronary artery function across acetylcholine doses, the 1.82 \( \mu \)g/min dose elicited the most evenly distributed vasomotor responses and the greatest magnitude of change in patients demonstrating vasodilation. As such, this acetylcholine dose was used for all data analyses, with 17 patients categorized as having dysfunctional coronary arteries (>5% coronary artery vasoconstriction) and 11 patients categorized as having relatively functional coronary arteries (<5% coronary
artery vasoconstriction). There were significant differences between groups in terms of sex, stature, red blood cell count, and plasma calcium concentration. In terms of patient characteristics, no other between-group differences were evident (Table 1). Before the acetylcholine infusion, LAD baseline diameters were not significantly different between groups. The absolute change in diameter, because of the acetylcholine infusion, was significantly smaller in the >5% coronary artery vasoconstriction group than the <5% coronary artery vasoconstriction group (Table 2). The acetylcholine-induced percent change in LAD diameter was also smaller for the >5% coronary artery vasoconstriction group than the <5% coronary artery vasoconstriction group (−11.8±4.6% versus 5.8±9.8%, P<0.001; Figure 1). LAD baseline diameters before SNP administration, and the absolute change in LAD diameter with SNP administration, were not significantly different between groups (Table 2). The SNP-induced percent change in LAD diameter was also not different between the >5% coronary artery vasoconstriction and the <5% coronary artery vasoconstriction groups (12.7±8.1% versus 12.9±5.7%, P=0.94; Figure 1).
Figure 1. Coronary artery function. The percent change in the left anterior descending (LAD) artery diameter with the intracoronary administration of acetylcholine (ACh) or sodium nitroprusside (SNP) in patients exhibiting >5% coronary artery vasoconstriction or <5% coronary artery vasoconstriction. Data are presented as mean±SE. †Significantly different from <5% coronary artery vasoconstriction group for the ACh condition (unpaired t test, P<0.001).

BA Function

Before the FMD, BA baseline diameters were not significantly different between groups. The absolute change in BA diameter was significantly smaller for the >5% coronary artery vasoconstriction group than the <5% coronary artery vasoconstriction group (Table 2). The FMD percent change in BA diameter was smaller for the >5% coronary artery vasoconstriction group than the <5% coronary artery vasoconstriction group (2.9±1.9% versus 6.2±4.2%, P=0.007; Figure 2). Additionally, the total sum of shear was not different between the >5% coronary artery vasoconstriction group and the <5% coronary artery vasoconstriction group (54 662±19 871 versus 58 259±26 023 AU, P=0.68). The AUC of the receiver operator characteristic curve generated to identify patients with >5% coronary artery vasoconstriction was 78%. For patients in the current

Figure 2. Brachial artery function. The percent change in brachial artery diameter with flow-mediated dilation (FMD) and sublingual nitroglycerin (NTG) in patients demonstrating >5% coronary artery vasoconstriction or <5% coronary artery vasoconstriction. Data are presented as mean±SE. † Significantly different from <5% coronary artery vasoconstriction group for the FMD (unpaired t test,
study, the BA FMD cut score was 2.9% with a sensitivity of 0.59, a specificity of 0.91, and an accuracy of 71%. The NTG BA baseline diameter was significantly smaller for the >5% coronary artery vasoconstriction group than the <5% coronary artery vasoconstriction group. The absolute change in BA diameter with NTG was not significantly different between the 2 groups (Table 2). The NTG-induced percent change in BA diameter was not different between the >5% coronary artery vasoconstriction and the <5% coronary artery vasoconstriction groups (14.0±5.3% versus 16.5±4.5%, P=0.26; Figure 2).

![Figure 3](image)

**Figure 3.** Relationship between coronary and brachial artery function. Pearson correlation between the percent change in left anterior descending (LAD) artery diameter with acetylcholine (ACh) and the percent change in brachial artery diameter with flow-mediated dilation (FMD).

**Relationship Between Coronary and BA Function** There was a strong, positive relationship between FMD-induced percent change in BA diameter and the acetylcholine-induced percent change in LAD diameter (r=0.77, P<0.0001; Figure 3). There was no significant relationship between the NTG-induced percent change in BA diameter and the SNP-induced percent change in LAD diameter (r=0.20, P=0.40).
**Discussion**

The purpose of this study was to assess the relationship between coronary artery and BA function, incorporating the updated methodological guidelines for the BA FMD technique. Adhering to these guidelines, BA FMD was strongly positively related to the acetylcholine-induced coronary artery response. Importantly, the strength of this relationship was enhanced substantially from the 2 previous seminal studies using older BA FMD protocols. It was also demonstrated that BA FMD, using current guidelines, was lower in patients with dysfunctional coronary arteries than those patients with relatively normal coronary arteries and was a strong indicator of coronary artery dysfunction (receiver operator characteristic AUC=78%). These findings suggest that the methodological refinements to the BA FMD technique have strengthened the relationship with coronary artery function. Thus, the noninvasive BA FMD technique, using current methodological guidelines, may now provide clinically meaningful insight into coronary artery dysfunction.

**Relationship Between Coronary Artery and BA Function**

A relationship between BA FMD and the acetylcholine-induced coronary artery response was previously documented in 2 studies.\(^6,7\) Although significant, the strength of these relationships was weak \((r=0.3)\) and, as recognized by the authors, insufficient for the accurate prediction of coronary artery function using BA FMD. Interestingly, Takase et al.\(^16\) reported a stronger relationship between BA FMD and coronary artery FMD \((r=0.78)\). The explanation for this much stronger relationship was that the vasodilation assessed in the coronary arteries was in response to the blood flow increase induced by ATP infusion. Thus, the stimuli for the BA and coronary arteries were equated on the basis of shear forces. However, it is unclear whether there was a direct effect of the ATP infusion at the site of measurement, as ATP is a potent vasodilator. Regardless, over the decades since these studies, substantial methodological and technical improvements have been made to the BA FMD technique in an attempt to improve reliability and clinical utility.\(^8,9\) An outcome of these refinements, however, is that any inference about coronary artery function, based on the
BA FMD, is now being made with markedly different techniques from the original validation studies. Importantly, the findings of the current study suggest that the methodological refinements to the BA FMD technique have substantially strengthened the relationship with the acetylcholine-induced coronary artery response (Figure 3). Indeed, this new relationship between coronary artery and BA function is more than twice as strong as previously documented\textsuperscript{6,7} and is now in agreement with the relationship between BA FMD and ATP-mediated coronary artery FMD.\textsuperscript{16} Although one atypical data point for this group is positioned to leverage the correlation, this data point was in line and within the 95\% prediction interval of the correlation with this data point omitted, which did not alter statistical significance ($r=0.60$, $P=0.0009$). Overall, the findings of the current study document a strong positive relationship between BA FMD and the acetylcholine- induced coronary artery response when the new methodological guidelines for FMD are used.

**Delineating Between Dysfunctional and Relatively Functional Coronaries With BA FMD**

In the current study, patients with coronary artery dysfunction exhibited a markedly lower BA FMD than patients with relatively functional coronary arteries (Figure 2). These differences, in both coronary artery and BA function, were likely not because of alterations in smooth muscle function, as vasodilation in response to SNP and NTG was not different between groups. The findings of the current study agree with those from Anderson et al,\textsuperscript{6} demonstrating that the methodological refinements to the BA FMD technique have not only retained but also improved the capacity to detect systemic differences in vascular function based on coronary artery function. In addition to strengthening the relationship between coronary artery and BA function, the updated BA FMD test now provides an assessment of BA function that is a strong indicator of coronary artery dysfunction (receiver operator characteristic AUC=78\%). In the current patients, a BA FMD cut score of 2.9\% demonstrated high specificity and was 71\% accurate in differentiating patients with coronary artery dysfunction and relatively functional coronary arteries. These AUC and specificity findings demonstrate that poor FMD-assessed BA function is likely indicative of coronary artery dysfunction (ie, >5\% coronary
artery vasoconstriction). Importantly, the BA FMD technique, with updated guidelines, may now be a clinically meaningful noninvasive screening test for predicting coronary artery dysfunction.

**Role of Nitric Oxide in the Link Between Coronary and BA Function**

BA FMD and the acetylcholine-induced coronary artery response have been the most widely used assessments of nitric oxide (NO)-mediated vascular function in the systemic and coronary vasculature, respectively. Support for the NO-mediated nature of FMD comes from early studies where the inhibition of NO synthase abolished the FMD response in the radial and BAs. However, FMD protocols across these studies were inconsistent and are now outdated. Using current guidelines, as in this study, the contribution of NO to FMD has more recently been determined to be between 0% and 20% in the radial and BAs. Indeed, Wray et al demonstrated that inhibition of NO synthase had no effect on BA FMD, when accounting for alterations in the shear stress stimulus. Support for the NO-mediated nature of the acetylcholine-induced coronary artery response also comes from studies demonstrating that NO synthase inhibition abolished the coronary artery vasodilation to acetylcholine.

However, it is now widely recognized that acetylcholine can induce coronary artery vasoconstriction, even in apparently healthy coronary arteries, via smooth muscle muscarinic receptor activation. Mounting evidence supports the contention that the acetylcholine-induced change in coronary artery diameter and the BA FMD response is not always predominantly NO-mediated and that the presence of cardiovascular risk factors, as in the current patients, is inversely related to the contribution of NO. Furthermore, the interaction between NO and endothelin plays a prominent role in coronary and peripheral vascular function. Lerman et al elegantly demonstrated that patients who responded to acetylcholine infusion with coronary artery vasoconstriction had elevated baseline concentrations of coronary and circulating endothelin. In these patients, coronary artery acetylcholine infusion resulted in an increase in coronary endothelin concentration with no change in cGMP, the downstream target of NO, while acetylcholine infusion resulted in no change in endothelin with an increase in cGMP in the patients that responded with coronary artery vasodilation. The
interaction between endothelin and NO also plays an important role in the peripheral vascular function.\textsuperscript{32–34} Additionally, oxidative stress can diminish the acetylcholine-induced change in coronary artery diameter and the BA FMD response, as vitamin C infusion has been demonstrated to improve dilation in response to both of these stimuli.\textsuperscript{35,36} Thus, there is growing evidence that both the BA FMD and the acetylcholine-induced coronary artery response may not be as NO-mediated as previously thought.

Interestingly, in this and 2 previous studies,\textsuperscript{6,7} because of a degree of unavoidable subject selection bias resulting from the need for an angiography for inclusion, the majority of patients demonstrated coronary artery vasoconstriction in response to acetylcholine infusion (Table 1; Figure 3). Nevertheless, coronary artery function was strongly related to, and indicated by, BA function. This unveils the interesting paradox that systemic vasodilation is actually related to coronary artery vasoconstriction. Furthermore, it is important to note that coronary artery vasoconstriction to acetylcholine infusion is not a NO-mediated response, per se, but rather a consequence of smooth muscle muscarinic receptor activation.\textsuperscript{12,13,24–29} Although it may be that lower levels of NO allow for vasoconstriction to occur, the relationship between BA and coronary artery function suggests that the mechanisms underpinning BA FMD and the acetylcholine-induced coronary artery response are likely not NO-mediated. Importantly, this does not contradict the substantial amount of evidence supporting both BA FMD and acetylcholine- induced coronary artery dilation as valid assessments of cardiovascular disease risk and progression, but, rather, suggests that the mechanisms mediating the responses may be reliant on mechanisms other than NO.

**Experimental Considerations**

For consistency with previous work,\textsuperscript{6} patients in the current study were delineated using 5% coronary artery vasoconstriction to acetylcholine. Importantly, the statistical significance of the primary outcome measures remained when patients were delineated based purely on coronary artery vasodilation or vasoconstriction in response
to acetylcholine. The current study was not designed to conduct multivariate analyses to determine which, if any, patient characteristics are related to the differential coronary artery and BA function, but future large-scale investigations may provide further insight into determinants or predictors of systemic vascular function. While advances in coronary angiography may have contributed to the improved relationship between coronary artery and BA function in the current study compared with the study by Anderson et al,\textsuperscript{6} it seems likely that this improvement is primarily attributable to refinements in BA FMD. The ability to measure acetylcholine-induced changes in coronary artery diameter is likely conserved across these studies, as the vessel wall was able to be consistently identified. Furthermore, the improvements in BA FMD (eg, proximal versus distal cuff location) have substantially altered the physiological mechanisms mediated the FMD response, while the improvements in coronary angiography have not altered the physiological effect of acetylcholine. The measurement of peak dilation has also been substantially improved by the continuous acquisition of BA diameter following cuff release\textsuperscript{37}, compared with a single fixed time point of 1 minute used in the Anderson et al\textsuperscript{6} study. Importantly, the markedly improved relationship between coronary artery and BA function, regardless of the exact contribution from improved coronary angiography techniques, has clinically meaningful implications.

**Clinical Implications**

The findings of the current study reinforce, and strengthen, the notion that BA FMD may be a meaningful indicator of coronary artery dysfunction. Specifically, refinements in the BA FMD technique, over the last quarter of a century, have enhanced the relationship with coronary artery function, as well as the capacity to indicate coronary artery dysfunction. This is now in agreement with the association between BA FMD and cardiovascular disease risk as well as cardiovascular events, providing independent predictive information beyond traditional risk factors.\textsuperscript{38–46} The current data, documenting an improved relationship between BA FMD and coronary artery function, suggest that the new guidelines have significantly improved the BA FMD assessment. Thus, the noninvasive assessment of BA FMD, with the new
methodological guidelines, may provide a clinically meaningful indication of coronary artery dysfunction.

Conclusions
Advancing the early detection of coronary artery dysfunction relies on a valid, noninvasive assessment that could, relatively easily, be administered in the clinical setting. Using updated BA FMD guidelines, this study documents that a strong relationship between, the noninvasively, assessed BA FMD and the acetylcholine-induced coronary artery response. Importantly, the strength of this relationship was enhanced substantially from the original investigations using older BA FMD techniques. In addition to strengthening this relationship, the updated BA FMD test provides an assessment of BA function that is a strong indicator of coronary artery dysfunction. Thus, the noninvasive BA FMD technique, using current methodological guidelines, may now provide clinically meaningful insight into coronary artery dysfunction.

Perspectives
Previously, the noninvasive BA FMD test has been documented to weakly correlate with acetylcholine-induced coronary artery function. Since then, FMD testing and analyses have substantially improved, however, the influence of these advancements on the relationship with coronary artery function remains unknown. The current study documents that these updates to the BA FMD technique have strengthened the relationship with coronary artery function. Thus, the noninvasive BA FMD technique, adhering to current guidelines, may now provide clinically meaningful insight into coronary artery dysfunction and may enhance the early detection of coronary artery dysfunction that precedes or underlies most cardiovascular diseases.

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Disclosures
None.

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Novelty and Significance

What Is New?
- The noninvasive brachial artery (BA) flow-mediated dilation (FMD) technique, adhering to current guidelines, may now provide noninvasive clinically meaningful insight into coronary artery dysfunction.

What Is Relevant?
- Early detection of coronary artery dysfunction is of paramount cardiovascular clinical importance, which the updated noninvasive BA FMD technique may now provide.

Summary
The noninvasive BA FMD test has been documented to weakly correlate with acetylcholine-induced coronary artery function. Since then, FMD testing and analyses have substantially improved, however, the influence of this on the relationship with coronary artery function remains unknown. The current study documents that these updates to the BA FMD technique have strengthened the relationship with coronary
artery function. Thus, the noninvasive BA FMD technique, adhering to current guidelines, may now provide clinically meaningful insight into coronary artery dysfunction and may enhance the early detection of coronary artery dysfunction that precedes or underlies most cardiovascular diseases.