Limb Stress-Rest Perfusion Imaging With Contrast Ultrasound for the Assessment of Peripheral Arterial Disease Severity

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OBJECTIVES We hypothesized that stress-rest perfusion imaging of skeletal muscle in the lower extremity with contrast-enhanced ultrasound (CEU) could evaluate the severity of peripheral arterial disease (PAD).

BACKGROUND Perfusion imaging may provide valuable quantitative information on PAD, particularly in patients with diabetes in whom microvascular functional abnormalities are common.

METHODS Study subjects included 26 control subjects and 39 patients with symptomatic PAD, 19 of whom had type 2 diabetes mellitus. A modified treadmill exercise test was performed to determine exercise time to development of claudication. Multilevel pulse-volume recordings and ankle-brachial index (ABI) at rest and post-exercise ABI were measured in both extremities. Microvascular blood flow in the gastrocnemius and soleus muscles was measured at rest and after 2 min of calibrated plantar-flexion exercise.

RESULTS During exercise, claudication did not occur in normal subjects and occurred earlier in PAD patients with diabetes than without (median time 1.2 min [95% confidence interval (CI) 0.6 to 2.5] vs. 3.0 min [95% CI 2.1 to 6.0], p < 0.01). Compared to control subjects, patients with PAD had lower skeletal muscle blood flow during plantar-flexion exercise and lower flow reserve on CEU. After adjusting for diabetes, the only diagnostic tests that predicted severity of disease by claudication threshold were CEU exercise blood flow and flow reserve (odds ratios 0.67 [95% CI 0.51 to 0.88; p < 0.003] and 0.64 [95% CI 0.46 to 0.89, p = 0.008], respectively). A quasi-likelihood information analysis incorporating all non-invasive diagnostic tests indicated that the best models for predicting severity of disease were the combination of diabetes and either exercise blood flow or flow-reserve on CEU.

CONCLUSIONS Perfusion imaging of limb skeletal during exercise and measurement of absolute flow reserve can provide valuable information on the severity PAD. This strategy may be useful for evaluating the total impact of disease in patients with complex disease or those with coexisting functional abnormalities of flow regulation. (J Am Coll Cardiol Img 2008;1:343–50) © 2008 by the American College of Cardiology Foundation
Many noninvasive methods commonly used for diagnosing peripheral arterial disease (PAD) rely on the detection of pressure gradients, such as the ankle-brachial index (ABI) and Doppler-derived flow velocity, or on abnormal pulse volumes caused by stenosis. Although these methods have performed quite well for the detection of moderate-to-severe disease in symptomatic patients, they also have well-recognized limitations. Resting pressure gradients do not develop until inflow vessel stenosis becomes relatively severe (1,2). Current diagnostic methods are also poorly suited to evaluating the impact of diffuse or multilevel disease and the influence of collateral flow. They also provide little information on abnormal microvascular functional responses that can limit flow responses to exercise. A method for evaluating skeletal muscle perfusion and perfusion reserve during exercise would potentially be valuable for quantifying the total impact of the complex pathophysiologic processes in patients with limb ischemia, particularly in those with diabetes in whom distal arterial disease and abnormal microvascular functional responses are common (3,4). Perfusion assessment could also play a valuable role in the development and testing of therapies designed to increase tissue perfusion.

Noninvasive imaging techniques that commonly are used to evaluate myocardial perfusion in those with suspected coronary artery disease have not routinely been used in PAD largely because of practical considerations of cost and time. There is also the need for quantitative information because regional heterogeneity in tracer uptake cannot be used for diagnosis. Contrast-enhanced ultrasound (CEU) is a quantitative perfusion imaging technique that has recently been applied to study skeletal muscle flow responses to exercise, hyperinsulinemia, and exogenous growth factor therapy (5–7) and to quantify abnormal microvascular responses in patients with insulin resistance (8). In this study, we hypothesized that stress-rest perfusion imaging of leg skeletal muscle with CEU could be used to detect PAD and evaluate its severity, and would be of particular value in patients with diabetes mellitus (DM).

**METHODS**

**Study population.** The study was approved by the Human Investigation Committee. We studied 26 consecutively recruited control subjects and 39 patients with documented PAD, of whom 19 had type 2 DM. All participants gave written informed consent. Control subjects were excluded for a history of coronary artery disease, hypertension moderate or greater in severity (≥140/90 mm Hg), dyslipidemia, history of diabetes, or first-degree relatives with diabetes determined on a prestudy evaluation. Control subjects were also excluded if body weight was >10% over ideal. Patients with PAD were enrolled if they had classic symptoms of claudication and a history of at least one abnormal diagnostic test (ABI, pulse volume recording [PVR], Doppler ultrasound, or angiography). Exclusion criteria for PAD subjects were angina, congestive heart failure, ischemic ulcers of the lower extremity, or inability to perform walking exercise. Diabetes mellitus was defined by fasting blood glucose ≥126 mg/dl on 2 or more studies and microangiopathic complications were defined by either recent eye examination (performed within 1 year on all patients) and/or proteinuria.

**Protocol.** Blood was drawn in the fasting state and ABI measurement and PVRs were performed for both legs. A modified upright treadmill walking exercise test was performed to determine the time to development of claudication and claudication-limited exercise time. Exercise was performed at a walking speed of 2.0 mph with an initial gradient of 0% that increased by 2% every 3 min (9). Pulmonary oxygen consumption and minute ventilation were measured by a metabolic sensor system (VMax29, SensorMedics; Cardinal Health, Dublin, Ohio). Subjects were instructed to report the onset of claudication for each limb. Exercise was continued for 20 min unless terminated as the result of intolerable claudication or other limiting symptoms. Immediate postexercise ABIs were measured. Subjects underwent stress-rest CEU perfusion imaging of the calf plantar-flexor muscles on a separate day.

**ABI and PVR.** For ABI measurements, systolic blood pressure of the brachial, dorsalis pedis, and posterior tibialis arteries were measured bilaterally at rest and immediately after treadmill exercise in the supine position (10). For each leg, the lowest calculated ABI from either dorsalis pedis or posterior tibialis was used. Bilateral lower-extremity PVRs were measured in the supine position by pneumoplethysmography (1058-C Vascular Mini-
Angiography. Angiographic data were analyzed if performed without intervention within 3 months of study participation. Disease severity was assessed by an experienced vascular surgeon who was blinded to other study information. The severity of disease in the inflow and outflow vessels was scored as: 1 = normal, 2 = minor irregularity or focal stenosis, 3 = multiple nonhigh-grade or a discrete high-grade (>70%) stenosis, 4 = severe diffuse disease, and 5 = total occlusion.

CEU. Harmonic power-Doppler imaging (HDI-5000cv, Philips Ultrasound Systems, Bothell, Washington) was performed at a transmission frequency of 3.7 MHz with a linear-array transducer at a mechanical index of 1.1 to 1.2 and a pulse-repetition frequency of 2.5 KHz. The plantar-flexor muscles on both legs were imaged in the transaxial plane during an intravenous infusion of lipid-shelled octafluoropropane microbubbles (Definity, Bristol-Myers Squibb Medical Imaging, New York, New York) at 0.20 to 0.27 ml/min. Images were acquired during incremental prolongation of the pulsing intervals (time between destructive pulses from 0.05 to 15 s). Imaging was performed first at rest for both limbs, then during plantar-flexion exercise on a calibrated pedal ergometer with a 15-min separation period between limbs. For the stress portion, image acquisition was started 2 min into exercise and completed within a total duration of 4 min. Plantar flexion was performed every 5 s at a power (range 30 to 43 W) that was determined a priori by the maximal resistance that a 60° plantar-flexion arc and return could be performed in 1 s.

Video intensity (VI) was measured from a region-of-interest placed over the entire gastrocnemius and soleus muscles (localized by fundamental B-mode imaging) after subtracting averaged frames at a pulsing interval of 0.05 s to eliminate signal from tissue and large intramuscular vessels (5). Time versus VI data were fit to the function: \( y = A(1 - e^{-\beta t}) \), where \( y \) is VI at time \( t \), \( A \) is the plateau VI reflecting microvascular blood volume, and \( \beta \) is the rate constant of blood transfer that reflects microvascular blood velocity (5,11). Blood flow was determined by the product of \( A \) and \( \beta \) (11), whereas flow reserve was calculated by the ratio of exercise to rest blood flow. In a group of 5 normal healthy volunteers, average variability of blood flow measured on separate days was approximately 20% for resting values and 15% for 80% maximal exercise values, but flow reserve varied by <10% in all cases.

**RESULTS**

**Clinical and laboratory variables.** The clinical characteristics for control subjects, PAD patients without DM, and PAD patients with DM are provided in Table 1.

**Exercise data and indicators of PAD.** All control subjects completed the 20-min treadmill walking exercise protocol without symptoms of claudication or other limiting symptoms. Claudication occurred during treadmill exercise in all patients with PAD and was eventually severe enough to necessitate premature termination of exercise in all but 2 patients. There were significant differences between groups for total exercise duration, oxygen consumption at peak exercise, and exercise time until the onset of claudication (Table 2). More than one-half of each of the PAD patient groups experienced bilateral claudication before test termination.

Noninvasive test results (ABI, PVRs, and CEU) were significantly different for both PAD patient cohorts compared with control subjects (Table 3).
In symptomatic limbs of patients with PAD and DM, the ABI at rest was normal (≥0.90) in 25% despite the presence of claudication on treadmill exercise. The bilateral rest-stress CEU perfusion studies were able to be completed without premature termination in all study subjects. The median power achieved during plantar flexion exercise was identical power level for plantar flexion exercise (38 W), the patient with PAD had a much smaller increase in the peak VI (A-value, representing relative microvascular blood volume) and rate constant of the refill curves (β-value, representing microvascular blood velocity) in response to exercise, resulting in a much smaller degree of flow reserve.

Predictors of disease severity. Data on disease severity were censored for asymptomatic legs where symptoms in the contralateral leg caused early test termination. The CEU parameters of exercise blood flow and flow reserve were univariate predictors of disease severity defined by the claudication threshold for each leg (Fig. 2A). Stratified analysis assuming a different risk profile for PAD patients with DM revealed that the only predictors of PAD

### Table 1. Clinical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 26)</th>
<th>PAD (n = 20)</th>
<th>PAD + DM (n = 19)</th>
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<tbody>
<tr>
<td><strong>Age, yrs</strong></td>
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<tr>
<td>Median</td>
<td>47</td>
<td>52</td>
<td>56†</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>41–52</td>
<td>48–55</td>
<td>50–61</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>11/26 (42)</td>
<td>11/20 (55)</td>
<td>7/19 (37)</td>
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<tr>
<td>Current tobacco, n (%)</td>
<td>1/26 (4)</td>
<td>11/20 (55)*</td>
<td>9/19 (47)*</td>
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<tr>
<td>Dyslipidemia, n (%)</td>
<td>3/26 (12)</td>
<td>17/20 (85)*</td>
<td>19/19 (100)*</td>
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<tr>
<td>Hypertension, n (%)</td>
<td>2/26 (8)</td>
<td>13/20 (65)*</td>
<td>18/19 (95)*</td>
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<td>Body mass index, kg/m²</td>
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<tr>
<td>Median</td>
<td>23.5</td>
<td>27.2</td>
<td>31.3†</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>21.8–25.7</td>
<td>24.2–28.6</td>
<td>27.7–35.9</td>
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<tr>
<td><strong>Medications</strong></td>
<td></td>
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<tr>
<td>Aspirin or clopidogrel, n (%)</td>
<td>4/26 (15)</td>
<td>16/20 (80)*</td>
<td>16/19 (84)*</td>
</tr>
<tr>
<td>Beta blocker, n (%)</td>
<td>0/26 (0)</td>
<td>6/20 (30)*</td>
<td>8/19 (42)*</td>
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<tr>
<td>ACE-I or ARB, n (%)</td>
<td>1/26 (4)</td>
<td>9/20 (45)*</td>
<td>13/19 (68)*</td>
</tr>
<tr>
<td>Statin, n (%)</td>
<td>1/26 (4)</td>
<td>10/20 (50)*</td>
<td>17/19 (89)*</td>
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<tr>
<td>Fibrates, n (%)</td>
<td>0/26 (0)</td>
<td>2/20 (10)</td>
<td>2/19 (11)</td>
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**Table 2. Treadmill Exercise Variables**

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 26)</th>
<th>PAD (n = 20)</th>
<th>PAD + DM (n = 19)</th>
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<tbody>
<tr>
<td><strong>Exercise duration, min</strong></td>
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<tr>
<td>Median</td>
<td>20.0</td>
<td>9.8*</td>
<td>4.5†</td>
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<tr>
<td>Interquartile range</td>
<td>20–20</td>
<td>6.4–12.3</td>
<td>2.0–7.0</td>
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<tr>
<td><strong>Peak VO₂, ml/min/kg</strong></td>
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<tr>
<td>Median</td>
<td>16.3</td>
<td>13.2*</td>
<td>8.7†</td>
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<tr>
<td>Interquartile range</td>
<td>14.5–17.6</td>
<td>11.2–15.1</td>
<td>7.7–9.9</td>
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<tr>
<td><strong>Metabolic units, METS</strong></td>
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<td></td>
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<tr>
<td>Median</td>
<td>4.7</td>
<td>3.7*</td>
<td>2.6†</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>4.2–5.0</td>
<td>3.2–4.1</td>
<td>2.3–2.9</td>
</tr>
<tr>
<td><strong>Time to claudication, min</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>3.0</td>
<td>6.0</td>
<td>1.21</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>2.1 to 6.0</td>
<td>0.6 to 2.5</td>
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<td><strong>Bilateral claudication, n (%)</strong></td>
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<tr>
<td>Median</td>
<td>13/20 (65)</td>
<td>11/19 (58)</td>
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Bonferroni-corrected p value: *p < 0.01 versus control; †p < 0.01 versus control. DM = diabetes mellitus; PAD = peripheral arterial disease; VO₂ = oxygen consumption.
severity were the CEU measurements of exercise flow and flow reserve (Fig. 2B). On multivariate analysis, both of these parameters added incremental benefit to clinical and other test variables and the best predictive models for severity of disease were the presence of DM together with either exercise blood flow or flow reserve on CEU (Fig. 3).

Angiographic data was available in 19 patients with PAD. Combined angiographic severity score in patients without DM correlated moderately well with CEU data on exercise blood flow ($r = 0.70$, $SEE = 5.2$, $p = 0.003$) and flow reserve ($r = 0.56$, $SEE = 2.4$, $p = 0.047$). In PAD patients with DM, angiography did not correlate with either of the CEU parameters, nor did it correlate with time to claudication, ABI, or PVR score. Angiographic severity score for inflow vessels only did not correlate with any noninvasive test in any of the cohorts.

**DISCUSSION**

In this study, we have demonstrated that CEU imaging of skeletal muscle microvascular blood flow and flow reserve provides information on the presence and severity of PAD in symptomatic patients. It was particularly useful in the diabetic population in whom conventional non-invasive tests were less predictive of the severity of symptoms.

The application of perfusion imaging to evaluate PAD in this study was a response to several emerg-
of disease in these individuals is important not only for preventing disease progression, but also because it identifies patients with increased risk for adverse cardiovascular events, even when ABI values are normal or elevated (20).

There is also clinical and research utility for accurate quantification of disease severity. This information could help determine the origin of atypical symptoms that are common in PAD (21). Techniques that rely on measurement of pressure gradients or resting flow are limited in their ability to measure severity of disease when in the mild-to-moderate range because of the complex and non-linear relation of these parameters with stenosis severity (1,2). Although large population studies have demonstrated a relationship between ABI and symptoms, normal ABIs are commonly encountered in patients with symptomatic PAD (22).

Moreover, carefully performed studies examining lower-extremity functional capacity demonstrated that performance is not well differentiated in the relatively wide range of ABI values of 0.5 to 0.9 (23). The ability to accurately measure tissue blood flow may also be important for evaluating responses to conventional therapies or emerging pro-angiogenic therapies. Underscoring this issue, recent studies with autologous bone-marrow mononuclear cell transplantation in patients with severe PAD have indicated that symptomatic improvement in individual limbs often occurs independent of any significant change in the ABI (24,25). Moreover, in one of these studies the baseline ABI value was normal in one third of limbs despite the diagnosis of thromboangiitis obliterans (25).

It has been shown in a canine model of femoral artery stenosis that CEU can detect mild to moderate impairment in flow and flow reserve (26). These findings formed the basis for the current clinical study. Perfusion imaging also has the advantage of being able to evaluate functional abnormalities in microvascular flow in patients with DM, including potential loss of the ability to redistribute flow between limb tissues or between nutritive and non-nutritive circuits within muscle during exercise (27) or impaired neurogenic vascular responses further upstream (28). In this study, there was a potential selection bias toward conventional techniques because of the entry criteria of established diagnosis of PAD based on at least one abnormal diagnostic test. Yet, CEU provided additional diagnostic value for detecting the severity of symptomatic PAD. In particular, CEU added predictive information in patients with DM in whom
diagnostic tests that rely on pressure gradients are disadvantaged. It should be noted that other methods for skeletal muscle perfusion have been developed that are based on transit-rate analysis after bolus injection of ultrasound contrast material (29). With this technique, the time to peak contrast enhancement after an intravenous injection was able to distinguish differences in perfusion in patients with severe PAD (median ABI 0.5) from control individuals. This technique has advantages in its simplicity although, unlike the methods used in this study, differences in cardiac output, injection rate, or volume of distribution will affect time to peak values (30).

**Study limitations.** The major limitation of this study was the lack of a true gold standard for evaluating the impact of proximal and distal disease, and functional impairments. In this regard, it can only be considered an exploratory study without true validation against other methods such as positron emission tomography or blood oxygenation level-dependent magnetic resonance imaging that can be used to evaluate blood flow but may not be suited for routine screening purposes. Also, toe pressures were not measured in this study and may have provided better information on distal vascular impairment.

Angiographic data were available in a subset of patients but could not be considered a true gold standard because of the complex anatomic and functional determinants of capillary flow. This issue is exemplified by the poor relationship between angiography and all other predictors of disease severity in patients with DM. It should also be noted that entry criteria of known symptomatic PAD with classic claudication selected for patients with moderate disease in whom the test may have the most value. We had to exclude patients with severe disease because of failure to perform exercise. This exploratory study will need to be followed with future studies to evaluate the incremental diagnostic value of CEU in patients referred for initial diagnostic testing or screening asymptomatic-but-high-risk populations, or for determining relative value in those with severe disease where the diagnostic value of the test is likely to be less.

**CONCLUSIONS**

Stress-rest CEU provides important information on the presence and severity of PAD, and the total impact of complex disease on microvascular perfusion. The technique may be able to detect more modest disease than conventional techniques and, hence, could be valuable for adopting a strategy of primary preventive strategies in PAD. Tissue perfusion imaging by CEU also holds promise for determining the efficacy of conventional or experimental therapeutic interventions. The feasibility for using CEU in routine clinical practice or in clinical trials is supported by the practical advantages of the technique such as speed, low cost, and current availability of technology in most vascular labs.

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