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PRECLINICAL RESEARCH

Detection of Peripheral Vascular Stenosis by Assessing Skeletal Muscle Flow Reserve

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OBJECTIVES We sought to determine whether the severity of peripheral arterial disease (PAD) can be assessed by measuring blood flow reserve in limb skeletal muscle with contrast-enhanced ultrasound (CEU).

BACKGROUND Noninvasive imaging of distal limb perfusion could improve management of patients with PAD by evaluating the impact of large and small vessel disease, and collateral flow.

METHODS In 12 dogs, blood flow in the quadriceps femoris was measured by CEU at rest and during either electrostimulated contractile exercise or adenosine infusion. Femoral artery blood flow was measured by Doppler ultrasound. Studies were performed in the absence and presence of either moderate or severe stenosis (pressure gradient of 10 to 20 mm Hg and >20 mm Hg, respectively).

RESULTS Resting femoral artery blood flow progressively decreased with stenosis severity, while resting skeletal muscle flow was reduced only with severe stenosis (52 ± 21% of baseline, $p < 0.05$), indicating the presence of collateral flow. Skeletal muscle flow reserve during contractile exercise or adenosine decreased incrementally with increasing stenosis severity ($p < 0.01$). The stenotic pressure gradient correlated with skeletal muscle flow reserve for exercise and adenosine ($r = 0.70$ for both, $p < 0.01$).

CONCLUSIONS Contrast-enhanced ultrasound of limb skeletal muscle can be used to assess the severity of PAD by measuring muscle flow reserve during either contractile exercise or pharmacologic vasodilation. Unlike currently used methods, this technique may provide a measure of the physiologic effects of large- and small-vessel PAD, and the influence of collateral perfusion. (J Am Coll Cardiol 2005;45:780–5) © 2005 by the American College of Cardiology Foundation

Current methods that are routinely used to diagnose peripheral arterial disease (PAD) and evaluate its severity rely on imaging the degree of large vessel stenosis, measuring pressure gradients, or identifying abnormalities in pulse-volume recordings. These approaches do not measure nutrient blood flow and are, thereby, limited in their ability to evaluate diffuse or small vessel disease and the influence of collateral perfusion. A noninvasive method for assessing skeletal muscle perfusion and flow reserve could potentially improve the management of patients with PAD by providing information on the physiologic impact of the disease. Although noninvasive imaging methods such as positron emission tomography and contrast-enhanced magnetic resonance imaging can be used to evaluate limb perfusion (1,2), they do not meet many of the requirements needed for routine patient screening such as low cost, portability, and rapid protocol implementation necessary for high throughput.

In this study, we hypothesized that the severity of PAD can be quantified by measuring limb skeletal muscle blood flow reserve with contrast-enhanced ultrasound (CEU). This technique relies on the measurement of microvascular red blood cell velocity (V_{RBC}) and microvascular blood volume (3). It is uniquely suited for evaluating the physiologic impact of PAD because it directly assesses nutritive microvascular flow that can originate from multiple sources, including stem artery inflow, collateral vessel networks, or redistribution from other limb tissues and nonnutritive pathways (4,5). Contrast-enhanced ultrasound has recently been used in animal models and humans to study insulin-mediated changes in skeletal muscle nutritive blood flow that occur independent of changes in large vessel inflow (6–8). In the current study, limb skeletal muscle flow reserve was determined by performing CEU at rest and during stress produced by either moderate contractile exercise or vasodilator administration in a canine model of peripheral arterial stenosis.

METHODS

Animal preparation. The study was approved by the Animal Research Committee at the University of Virginia and conformed to the "American Heart Association Guidelines for the Use of Animals in Research." Twelve dogs (20 to 30

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Abbreviations and Acronyms

CEU = contrast-enhanced ultrasound
PAD = peripheral arterial disease
PI = pulsing interval
V_{RBC} = microvascular red blood cell velocity

kg) were anesthetized, mechanically ventilated, and warmed by a heating blanket. Arterial O_2 saturation and the electrocardiogram were continuously monitored. Catheters were placed in the femoral veins for administration of drugs, fluids, and microbubbles. A catheter was advanced to the aorta via a carotid artery for pressure measurement. An adjustable screw occluder was placed on the common femoral artery, and a catheter was placed in the proximal portion of the lateral circumflex femoral artery to measure pressure distal to the stenosis.

CEU. Imaging of the distal quadriceps femoris in the axial plane was performed with intermittent ultraharmonic imaging (Sonos 5500, Philips Ultrasound, Bothell Washington) at a transmission frequency 1.3 MHz, and a mechanical index of 1.0. The compression was set at maximal, and gains were optimized and held constant. For CEU, lipid-shelled decafluorobutane microbubbles were prepared by sonication of an aqueous dispersion of 1 $mg \cdot ml^{-1}$ polyethyleneglycol stearate, 2 $mg \cdot ml^{-1}$ distearoyl phosphatidylcholine, and decafluorobutane gas. Microbubble concentration was determined by electrozone sensing with a Coulter Multisizer IIe (Beckman-Coulter, Fullerton, California). Microbubbles were suspended in saline (4×10^6 ml^{-1}) and infused intravenously at 2.5 $ml \cdot min^{-1}$. Images were acquired digitally during continuous imaging at 30 Hz and at pulsing intervals (PI) ranging from 0.2 to 15 s.

Images were transferred off-line to a computer, and the backscatter for each pixel was transformed from a logarithmic to linear scale using known dynamic range scales in order to derive acoustic intensity values. Background frames acquired during continuous imaging were aligned, averaged, and digitally subtracted from averaged frames obtained at each PI in order to eliminate signal from tissue and from large intramuscular vessels (6). Background-subtracted acoustic intensity at each PI was measured from a region-of-interest placed over the quadriceps muscle; PI versus intensity data were fit to the function:

$$y = A(1 - e^{-\beta t})$$

where y is acoustic intensity at a PI of t; A is the plateau acoustic intensity reflecting relative microvascular blood volume, and β is the rate constant reflecting V_{RBC} (3).

Femoral artery blood flow. Femoral artery blood flow was determined by two-dimensional and Doppler ultrasound (HDI-5000, Philips Ultrasound) with a linear-array transducer. Vessel cross-sectional area was determined from videocaliper measurement of vessel diameter. The centerline time-averaged peak velocity was determined from arterial pulsed-wave Doppler and multiplied by 0.625 to correct for parabolic flow profile. Heel-toe angulation was performed to align the Doppler cursor along the long-axis of the vessel at an angle correction of 60°. Femoral artery blood flow was measured by the product of cross-sectional area and mean velocity.

Experimental protocols. Thirty minutes after surgical preparation, heart rate and hemodynamics were recorded, femoral artery blood flow was measured, and CEU of the proximal hindlimb skeletal muscle was performed. All measurements were then repeated during exercise or vasodilator stress. For contractile exercise, needle electrodes were placed in the proximal and distal portions of the quadriceps femoris and connected to a pulse generator (model 5345, Medtronic, Shoreview, Minnesota). Contractions were stimulated at 1 Hz using 10 mA monophasic square-wave pulses 1 ms in duration. Measurements were made 5 to 10 min after starting exercise. For vasodilator stress, adenosine was infused intravenously at 70 $\mu g \cdot kg^{-1} \cdot min^{-1}$. All measurements at rest and during stress were repeated after creation of a moderate or severe femoral artery stenosis, defined by pressure gradients of 10 to 20 or >20 mm Hg, respectively. These pressure gradients reflect an approximate area narrowing of 75% to 85% for moderate stenosis, and >85% for severe stenosis (4,9).

Statistical methods. Data were analyzed in RS/1 (Domain Manufacturing Corp., Burlington, Massachusetts). Comparisons between stages were made using repeated measures analysis of variance. Individual comparisons were performed using paired Student t test. Correlations were performed using least-squares fit regression analysis. The relationship between stenotic pressure gradient and flow reserve best fit to an exponential function using a least-squares fit. Linear regression analysis was used for the correlation between pressure gradient and femoral artery blood flow. Differences were considered significant at $p < 0.05$ (two-sided).

RESULTS

Resting heart rate and systemic blood pressure were similar between stages (Table 1). The pressure gradient increased, and both distal arterial pressure and femoral artery blood flow decreased incrementally with increasing stenosis severity (Table 1). The pressure gradient at rest correlated linearly with the percent reduction in femoral artery blood flow (Fig. 1). The mean pressure gradient across both moderate and severe stenoses increased with adenosine-mediated hyperemia (Table 2) due to augmentation in femoral artery blood flow. The pressure gradients did not significantly change with contractile exercise, primarily because contraction of a single muscle group produced little change in femoral artery blood flow.

Figure 2 illustrates examples of background-subtracted CEU images obtained at increasing PIs, and corresponding PI versus intensity data. These images demonstrate typical examples of normal and impaired flow reserve in response to

Table 1. Heart Rate, Hemodynamic, and Blood Flow Data at Rest

	No Stenosis	Moderate Stenosis	Severe Stenosis
Heart rate (min^{-1})	92 ± 20	89 ± 19	85 ± 16
Mean aortic pressure (mm Hg)	92 ± 18	94 ± 19	88 ± 17
Stenotic pressure gradient (mm Hg)	2 ± 2	12 ± 3*	28 ± 7†
Femoral artery blood flow ($ml \cdot min^{-1}$)	140 ± 46	98 ± 52*	72 ± 38†
Distal pressure (mm Hg)	92 ± 18	83 ± 19	61 ± 20*

*$p < 0.05$ compared with no stenosis; †$p < 0.05$ compared with no stenosis and moderate stenosis.

contractile exercise. In the absence of a stenosis, skeletal muscle blood flow increased approximately three-fold, which was attributable to both an increase in the microvascular blood volume (plateau intensity or A value) and V_{RBC} (rate constant or β). Severe stenosis resulted in a decrease in resting blood flow, indicated by a lower plateau intensity and V_{RBC} compared with no stenosis. These parameters did not increase substantially during contractile exercise, indicating complete loss of flow reserve.

Skeletal muscle blood flow data from CEU at rest and stress for all animals are shown in Figure 3. Unlike femoral artery blood flow, skeletal muscle blood flow was preserved with moderate stenosis but reduced significantly with severe stenosis. The reduction in resting muscle flow with severe stenosis was primarily due to a 39 ± 24% ($p < 0.001$) decrease in V_{RBC} with little change in microvascular blood volume. Muscle flow reserve in the absence of stenosis was between 2.5 and 3.5 for both moderate contractile exercise and adenosine (Fig. 3). Flow reserve in response to either exercise or adenosine was reduced with moderate and severe stenosis. The relation between muscle flow reserve and stenosis gradient was nonlinear due to near-total exhaustion of flow reserve with moderate stenosis (Fig. 4). Despite the presence of a severe stenosis that was flow-limiting at rest, a small amount of flow reserve was still present with contractile exercise but not with adenosine.

The percent changes in microvascular blood volume and in V_{RBC} are shown in Figures 5A and 5B, respectively. Exercise produced an increase in both V_{RBC} and microvascular blood volume, which was attenuated in the presence of stenosis. The effect of stenosis was greater for myocardial blood volume than V_{RBC}. In the absence of a stenosis, hyperemic response with adenosine was due solely to an increase in V_{RBC}, the degree of which was incrementally reduced with progressive stenosis severity.

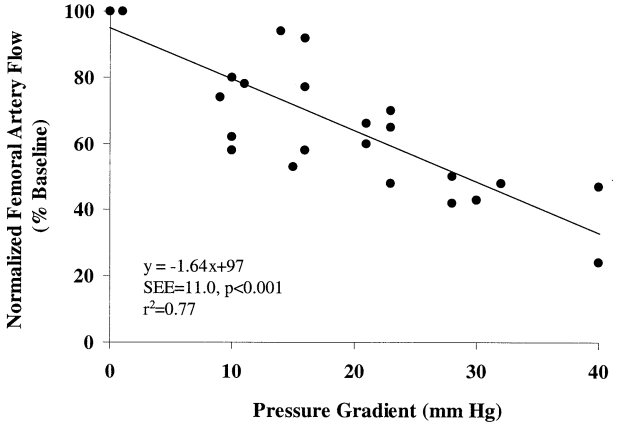

Figure 1. Relation between the stenotic pressure gradient at rest and femoral artery blood flow normalized to baseline values. Data points at a pressure gradient of 0 to 1 mm Hg represent baseline values in the absence of a stenosis.

DISCUSSION

In this study, we have demonstrated that noninvasive imaging of limb skeletal muscle flow reserve with CEU can be used to evaluate the physiological significance of peripheral arterial stenosis. We intentionally utilized a modest level of exercise that, in the absence of stenosis, produced a threefold increase in skeletal muscle flow rather than the ≥10-fold increase possible with maximal exercise (4,10) in order to reproduce the expected low exercise capacity in patients with claudication. The degree of flow reserve with exercise was similar to that achieved with intravenous administration of adenosine, which increases skeletal muscle flow primarily through activation of adenosine-A_1 and A_{2a} receptors (11). Progressive degrees of stenosis, which were designed to mimic moderate-to-severe symptomatic PAD in patients (9,12), produced an incremental reduction in skeletal muscle flow reserve.

Advantages of perfusion imaging for PAD. In the clinical setting, a technique for assessing skeletal muscle perfusion will likely provide incremental information not available with noninvasive diagnostic techniques currently used to evaluate PAD. Imaging the anatomic severity of stenosis or measuring the Doppler pressure gradient is often unreliable for quantifying disease severity in the presence of diffuse stenosis or multiple sequential lesions. Plethysmography and ankle brachial index measurements can provide information on the physiologic impact of diffuse large-vessel disease, but they are not sufficient for evaluating microvascular disease and can be inaccurate in patients with severely

Table 2. Mean Pressure Gradients (mm Hg) at Rest and Stress

	Contractile Exercise		Adenosine	
	Rest	Stress	Rest	Stress
No stenosis	2 ± 1	2 ± 1	1 ± 1	2 ± 2
Moderate stenosis	12 ± 4	15 ± 5	13 ± 4	27 ± 11*
Severe stenosis	30 ± 7	32 ± 8	24 ± 3	40 ± 15*

*$p < 0.05$ compared with corresponding rest data.

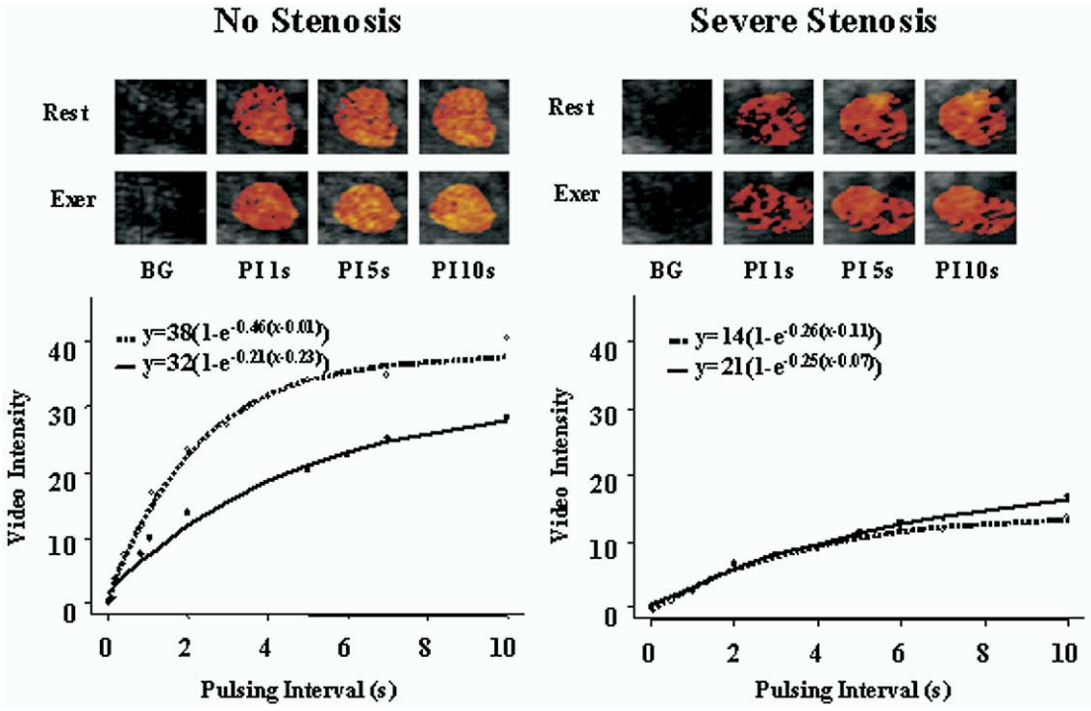

Figure 2. Examples of background (BG)-subtracted color-coded contrast-enhanced ultrasound images of the quadricep muscle group at increasing pulsing intervals (PI), and corresponding PI versus acoustic intensity curves in the absence of a stenosis and in the presence of a severe stenosis. Data are shown at rest **(solid lines)** and during contractile exercise **(dashed lines)**. Exer = contractile exercise.

reduced vascular compliance that occurs with aging and hypertension (13).

There are other reasons why direct assessment of nutrient perfusion may be more accurate for noninvasively assessing PAD than techniques that rely on pressure gradients or flow within the large vessels. As schematically illustrated in Figure 6, nutrient flow can derive from several sources other than the major limb inflow vessel. In the proximal extremities, there is an extensive and preexistent circuit of medium- and large-size arteries that are capable of providing collateral flow in the presence of stem-vessel stenosis (4). Muscle perfusion can also be augmented by redistribution of flow from other limb tissues and from nonnutritive channels that exist within the muscle (5). Flow redistribution occurs during both modest exercise and physiologic hyperinsulinemia where nutrient flow increases independent of changes in limb inflow (5–8). Consequently, perfusion imaging is likely to provide the most accurate information on the total physiologic impact of PAD by taking into account all sources of flow.

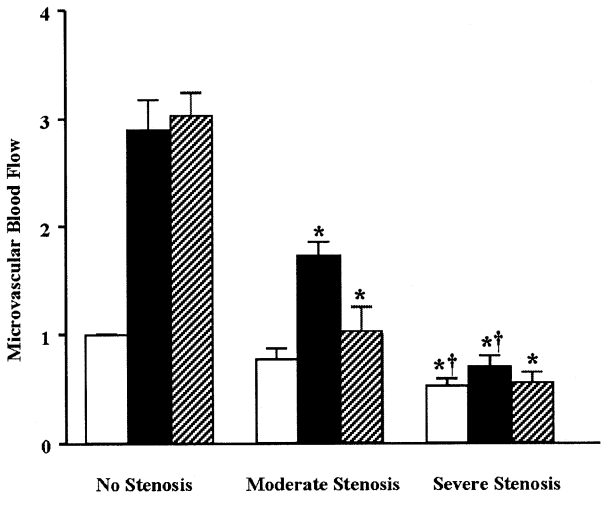

Figure 3. Skeletal muscle blood flow measured by contrast-enhanced ultrasound at rest, and during either contractile exercise or adenosine. Values are normalized to baseline values at rest. *$p < 0.05$ compared with no stenosis; †$p < 0.05$ compared with moderate stenosis. **Open bars** = rest; **solid bars** = contractile exercise; **ruled bars** = adenosine.

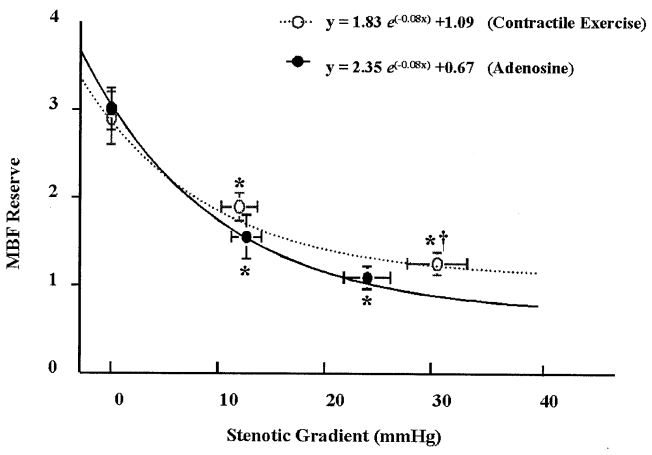

Figure 4. Relation between stenotic pressure gradient and mean flow reserve in skeletal muscle measured by contrast-enhanced ultrasound during contractile exercise **(open circles)** or adenosine **(solid circles)**. *$p < 0.05$ compared with no stenosis; †$p < 0.05$ compared with moderate stenosis.

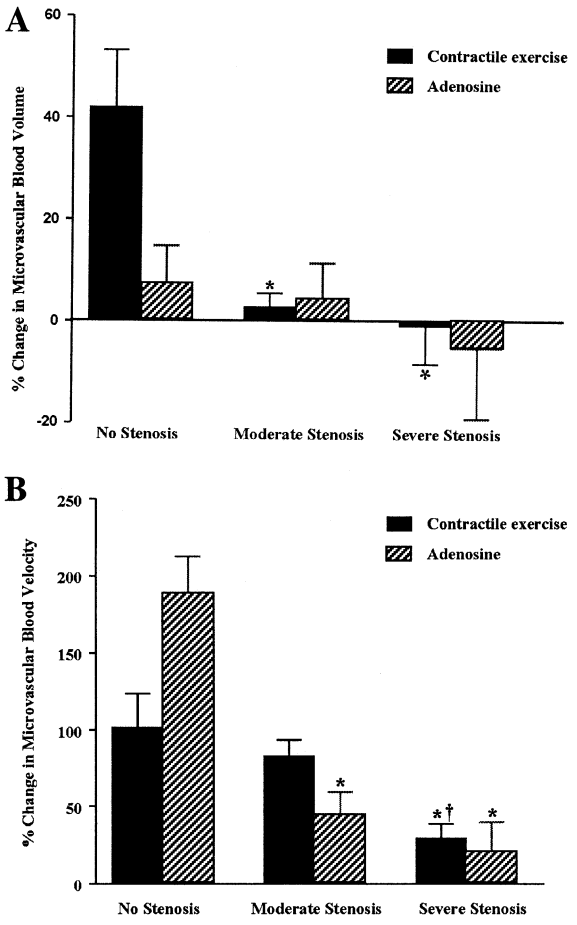

Figure 5. Percent change in **(A)** microvascular blood volume (A value) and **(B)** microvascular red blood cell velocity (V_{RBC}) (β value) compared with baseline for contractile exercise and intravenous adenosine. *$p < 0.05$ compared with no stenosis; †$p < 0.05$ compared with mild stenosis.

Techniques such as ankle-brachial index and Doppler that rely on the evaluation of pressure loss have inherent limitations in detecting stenoses and evaluating their severity. Unlike the coronary circulation, in major proximal limb vessels, a pressure drop is not detected until there is ≥75% to 80% stenosis, at which point resting arterial flow also decreases (4,9). One potential explanation for this finding is the extensive large-vessel collateral circuit that can maintain distal pressure (4,9). Although not reported in our results, we consistently found a distal pressure >40 mm Hg even during total vessel occlusion. An alternative explanation is that, based on hydrodynamic principles, energy loss in the form of pressure drop across a stenosis is determined by the relationship between the flow rate and vessel radius. Under resting conditions, these relationships are more favorable for producing pressure drops with lesser degrees of stenosis in coronary than peripheral arteries (9). Exercise or other stimuli that reduce distal vascular resistance can increase the pressure gradient in large-limb vessels when there is severe stenosis and can enhance diagnostic accuracy in symptomatic patients (14). However, pressure losses are often still minimal in the presence of moderate disease (4,9).

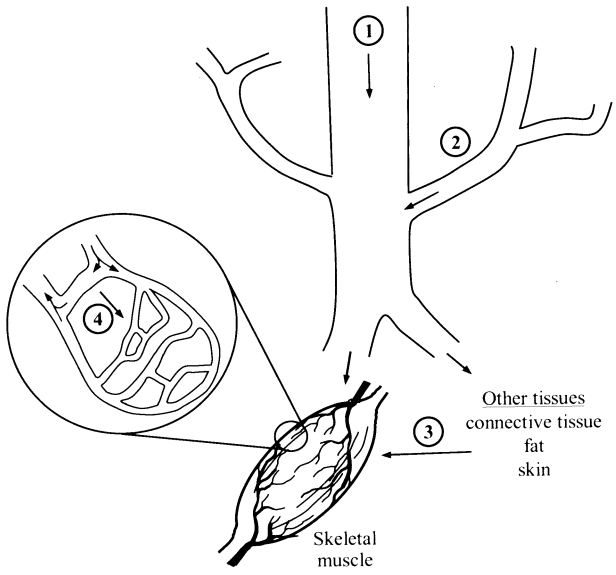

Figure 6. Schematic illustration depicting potential sources of limb skeletal muscle blood flow. Besides inflow from the major limb feeding artery **(1)**, muscle perfusion can be derived from proximal large vessel collateral circuits **(2)**, redistribution from other limb tissues **(3)**, or redistribution from nonnutritive sources that may be extramuscular or intramuscular **(4)**.

CEU for assessment of PAD. Because it measures nutritive perfusion, CEU is likely to be more accurate for assessing the severity of PAD and the combined effects of large- and small-vessel disease and collateral perfusion. In this study, we have demonstrated that peripheral stenosis can be detected by measuring alterations in skeletal muscle flow reserve. Stenoses were created that were classified as moderate or severe according to resting pressure gradients that reflect 75% to 85% and >85% stenosis, respectively (4,9). At each level of stenosis, resting arterial inflow was reduced to a much greater degree than skeletal muscle blood flow determined by CEU. The discrepancy between large arterial inflow and microvascular blood flow indicates that CEU can assess the contribution of the alternate sources of perfusion illustrated in Figure 6. The contribution of these alternative sources to flow reserve was also detected by CEU. With severe stenoses that reduced microvascular blood flow at rest, contractile exercise of the quadriceps consistently produced a small increase in tissue perfusion. We believe that this likely represented shunting from other limb tissue or from adjacent nonexercise muscle groups because femoral artery blood flow and distal pressure did not change.

Although the degree of muscle flow reserve was similar for exercise and adenosine, the microvascular changes responsible for the increase in flow were different. Contractile exercise, which increases muscle oxygen consumption, produced an increase in both microvascular blood volume and V_{RBC}, the degree of which was greater for the latter. These findings are in agreement with changes in capillary density and capillary RBC flux rate observed by intravital microscopy during contractile work (15,16). The presence of a

stenosis during exercise tended to blunt changes in microvascular blood volume more than V_{RBC}. Although the exact mechanism for this hierarchal response is not known, reduction in capillary blood volume during exercise could occur from an active derecruitment to preserve precapillary pressure and maintain red blood cell flux in remaining capillaries (17,18). During adenosine, augmentation in muscle flow was primarily attributable to an increase in V_{RBC}, similar to that described for the intact coronary circulation (19,20). The adenosine-mediated increase in V_{RBC} was reduced in proportion to stenosis severity. Unlike the coronary system, however, skeletal muscle microvascular blood volume was unchanged in the presence of a critical flow-limiting stenosis at rest, and during adenosine in the presence of stenosis. Although the reason for these differences is not known, it is possible that further adaptive reduction in capillary density did not occur because of equalization of precapillary pressure across all vascular beds or to direct flow from nonnutritive sources, which would preserve precapillary pressures. These adaptations would serve to maintain capillary blood volume in resting skeletal muscle, which is already very low under basal conditions.

Study limitations. We used a model of single large-vessel stenosis, and only one level of exercise. Further studies will be needed to examine the effect of different levels of exercise and whether the physiologic impact of diffuse multiple stenosis can be accurately determined. Validation of flow changes by an independent method was not performed. However, CEU-derived flow and flow-reserve measurements have previously been shown to correlate closely with radiolabeled microsphere measurements (3,21), and relative changes in microvascular blood volume by CEU have correlated with those made by assessing capillary endothelial xanthine oxidase availability (6). One limitation regarding the exercise protocol is that electrostimulation results in contractile exercise in one muscle group rather than an entire limb. Whether relative changes in V_{RBC} and microvascular blood volume produced by stenosis are influenced by the metabolic status of the entire limb will need to be determined in conscious animals or human studies. Finally, although CEU was able to detect the presence of alternate sources of nutritive flow at rest when femoral artery flow was reduced, it cannot differentiate the relative contributions from these potential sources.

Conclusions. In summary, we have demonstrated that the physiologic effect of PAD can be assessed by microvascular perfusion imaging with CEU. This technique is well-suited for this application because of its ability to assess nutritive perfusion, and has great potential for clinical implementation because of its portability, low cost, and requirement for equipment already in place in most vascular laboratories. Future studies are needed to characterize the incremental value of this technique for evaluating patients in whom disease pathophysiology is more complex than animal models of stenosis.

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