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EXERCISE VERSUS VASODILATOR STRESS LIMB PERFUSION IMAGING FOR THE ASSESSMENT OF PERIPHERAL ARTERY DISEASE

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Abstract

Purpose—Our aim was to determine whether pharmacologic vasodilation is an alternative to exercise stress during limb perfusion imaging for PAD.

Methods—Quantitative contrast-enhanced ultrasound (CEU) perfusion imaging of the bilateral anterior thigh and calf was performed in 9 control subjects and 9 patients with moderate to severe PAD at rest and during vasodilator stress with dipyridamole. For those who were able, CEU of the calf was then performed during modest plantar flexion exercise (20 Watts). CEU time-intensity data were analyzed to quantify microvascular blood flow (MBF) and its parametric components of microvascular blood volume and flux rate.

Results—Thigh and calf skeletal muscle MBF at rest was similar between control and PAD patients. During dipyridamole, MBF increased minimally (<2-fold) for all groups and there were only non-significant trends for a reduction in calf MBF in those with PAD (13.5 ± 6.9 , 10.0 ± 4.7 , and 8.2 ± 6.1 IU/s, for controls, moderate and severe PAD, respectively; $p=0.11$). In contrast, MBF during modest planar flexion exercise increased markedly in controls but not PAD patients (87.9 ± 79.9 vs. 15.2 ± 12.9 IU/s, $P<.05$). In three moderate PAD patients restudied after undergoing surgical revascularization, MBF during dipyridamole did not change whereas exercise MBF increased by an average of 7-fold.

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Conclusions—Resting limb skeletal muscle MBF in patients with moderate to severe PAD is similar to that in normal subjects. However, differences in hyperemic flow during contractile exercise but not during dipyridamole allows evaluation of the degree of flow impairment from PAD and the degree of improvement with revascularization.

The principal problem in symptomatic atherosclerotic peripheral artery disease (PAD) is insufficient limb blood flow either during exercise for those with intermittent claudication (IC) or at rest in most patients with critical limb ischemia (CLI). Perfusion deficits can result from the combined effects of arterial inflow stenosis, microvascular dysfunction, and inadequacy of collateral circuits.[1, 2] There is currently an unmet need for non-invasive methods able to directly measure tissue perfusion in PAD at rest or during stress in order to assess disease severity or response to therapy.

Contrast-enhanced ultrasound (CEU) perfusion imaging with a variety of tracer kinetic models has been used to quantify limb perfusion in patients with IC.[3–5] This technique has been shown to be more predictive of symptoms than conventional methods that rely on pressure gradients or pulse volume recordings.[6–8] Studies evaluating flow reserve with CEU have generally applied contractile exercise which in normal subjects produce an 8- to 12-fold increase in calf muscle perfusion.[9] Vasodilator stress perfusion testing has not been explored but has the potential advantage of providing more uniform hyperemic response by avoiding variations in the contribution of different muscle groups to the same form of exercise. Vasodilator stress can also potentially be used in those who cannot exercise due to the severity of symptoms or frailty. However, preclinical pharmacology studies suggest that peripheral vascular response to vasodilators that act through adenosine receptor signaling or phosphodiesterase inhibition may be small compared to that which occurs in the coronary vascular bed.[10–12] The aim of this study was to evaluate limb perfusion responses to pharmacologic vasodilation in healthy subjects and patients with a spectrum of PAD, and also to compare vasodilator response to that achieved with modest contractile exercise.

METHODS

Study Population

The study was approved by the Human Investigational Review Board at Oregon Health & Science University. We studied 18 consecutively recruited subjects including 9 patients with moderate to severe symptomatic PAD (Rutherford class 3) and 9 control volunteers within a target age range. All participants gave written informed consent. Control subjects without any symptoms of lower extremity weakness or claudication were recruited based on a targeted age and sex matching criteria. Control subjects were screened for normal bilateral pulses and ABIs prior to enrollment. Exclusion criteria for control or PAD subjects included pregnancy, lactation, evidence for right-to-left shunt, more than mild left ventricular dysfunction, threatened urgent limb amputation, muscular or neuromuscular disease, or any contraindication to receiving dipyridamole.

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designing the study; collecting the data; analyzing and interpreting the data; and writing the manuscript. All authors verified the accuracy and completeness of the data. The study was registered on *clinicaltrials.gov* (ID code: NCT01377649) before the start of patient enrollment.

Clinical and laboratory variables

Clinical characteristics of PAD patients and healthy control subjects are listed in Table 1. Patients with PAD were slightly older, had more co-morbidities including diabetes (present in 89% of PAD subjects), and were on more cardiovascular medications. Average hemoglobin was also slightly reduced in patients with PAD. Heart rate, systolic blood pressure and echocardiographic data on systolic function were similar between groups. In the PAD group, there were 5 patients with moderate disease (Rutherford class 3 or 4), and 4 patients with severe disease defined as minor tissue loss or ulceration/gangrene (Rutherford class 5 or 6).

Study Design and Protocol

Subjects underwent an initial screening including brief medical history and focused physical examination including blood pressure measurement, cardiopulmonary exam, and peripheral vascular exam. Limited echocardiography was performed to evaluate for exclusion criteria. Subjects completed the Vascular Quality of Life Questionnaire (VascuQoL-25) quality of life instrument[13] and had ankle-brachial index (ABI) measured. CEU perfusion imaging of the anterior thigh and calf was performed at rest. CEU perfusion imaging of the calf bilaterally was repeated during vasodilator stress with dipyridamole. After a waiting period, perfusion imaging of the calf was also performed during modest plantar flexion exercise using only the most symptomatic limb for those with PAD. All participants were able to complete the dipyridamole portion of the study whereas 3 of the subjects with severe PAD refused or could not perform exercise.

Ankle-Brachial Index (ABI)

For ABI measurements, systolic blood pressures for the brachial, dorsalis pedis, and posterior tibialis arteries were measured using a Doppler probe. ABI was calculated by dividing the systolic blood pressure at the ankle by the highest systolic blood pressures in the arm. For each measurement, the highest calculated ABIs from either the dorsalis pedis or posterior tibialis were reported.

Contrast Enhanced Ultrasound Perfusion Imaging

CEU perfusion imaging was performed with a phased-array transducer (S5-1, IE33, Philips Ultrasound) using a multipulse contrast-specific algorithm imaging at 1.3 MHz and a MI of 0.9–1.0. Gain settings were optimized and held constant. At rest, imaging was performed bilaterally using trans-axial imaging planes for the calf and the anterior thigh in order to image the gastrocnemius and rectus femoris muscles, respectively. Contrast enhancement was achieved using albumin-shelled octafluoropropane microbubbles (Optison, GE Healthcare) diluted 1:10 (v:v) in normal saline and infused intravenously at a rate of 2 ml/min. Images were acquired during continuous real-time imaging, followed by end-diastolic

images which were acquired during sequential prolongation of the time between high-power pulses to every 15 cardiac cycles. After acquisition of resting images, vasodilator stress was performed using dipyridamole (0.56 mg/kg, maximum 60 mg) infused over three minutes. Calf CEU imaging was performed 2 min after completing the infusion. After a waiting period, CEU imaging was repeated unilaterally (using the most symptomatic leg for those with PAD) during plantar flexion exercise for those who were able. Plantar flexion was performed with a work level of approximately 20 watts every 5 secs for a total duration of 2 min. In three subjects with IC, the imaging protocol was repeated within 2 months of lower extremity revascularization.

Contrast Ultrasound Image Analysis

Video intensity (VI) was measured from regions-of-interest placed over the muscle of interest. Several end-diastolic frames obtained during real-time imaging (20 Hz) were averaged and digitally subtracted from averaged frames obtained at each pulsing interval (every 1, 2, 3, 5, 8, 10, 12, and 15 cardiac cycles) in order to eliminate signal from tissue and large intramuscular vessels. Time versus video intensity data were fit to the function:

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

where y is VI at pulsing interval t , A is the plateau VI reflecting microvascular blood volume, and β is the rate of microbubble replenishment reflecting microvascular blood flux rate. Microvascular blood flow was quantified by the product of A and β . [14, 15] Flow reserve was calculated by the ratio of microvascular blood flow during hyperemic challenge (dipyridamole or exercise) to that at rest. [16]

Statistical Methods

Data are expressed as mean ($\pm$ standard deviation) unless otherwise stated. Clinical variables were compared between control and PAD subjects using the unpaired Student t-test (two-sided). Comparisons of all continuous imaging variables measured at baseline and during exercise or dipyridamole vasodilation were performed with the Mann-Whitney test for PAD versus control comparisons; or Kruskal-Wallis test using the Dunn's procedure to control for multiple comparisons when considering differences between PAD patients with moderate versus severe disease. Differences were considered significant at $P<0.05$.

RESULTS

Skeletal Muscle Blood Flow

Calf and anterior thigh skeletal muscle perfusion at rest was similar between control and PAD patients, irrespective of whether or not the limbs for the PAD patients were subdivided into moderate or severe PAD (Figure 1). Administration of intravenous dipyridamole at doses conventionally used for myocardial vasodilator stress perfusion imaging produced a minimal increase in perfusion in the thigh and calf skeletal muscle in the control group, and essentially no change perfusion in the PAD cohort. In contrast, modest brief contractile exercise of the calf muscle group resulted in a marked increase in perfusion in controls and

minimal increase in the PAD patients who were able to exercise (n=6), resulting in a much better discrimination between patient groups (Figure 1 and 2). When expressed as the degree of flow reserve, hyperemia was much greater for exercise than dipyridamole for control subjects whereas flow reserve was similar for exercise and dipyridamole for PAD subjects (Figure 3).

Parametric analysis of CEU perfusion imaging was performed to investigate the microvascular responses underlying group-related flow disparities (Figure 4). During dipyridamole, the small increase in blood flow in the control subjects was attributable to the small and not statistically significant increase in the microvascular flux rate (β -value). Modest contractile exercise produced an increase in the microvascular blood volume (A-value) consistent with functional recruitment of microvascular units, the degree of which was only slightly greater in control compared with PAD patients. In contrast, microvascular flux rate (β) during exercise was much greater in control subjects than in PAD patients and was the primary factor for the group-wise differences in flow reserve.

Although not a part of the primary study aim, three patients with severe IC underwent revascularization within 1 month of their initial CEU study and had repeat perfusion imaging performed within 2 months after revascularization. All three underwent revascularization with iliofemoral stenting or aorto-femoral bypass surgery and all had significant symptomatic relief. Calf CEU perfusion data from these three patients prior to revascularization was similar to the PAD group in general with no significant augmentation in blood flow in response to either dipyridamole or exercise (Figure 5). After revascularization resting perfusion did not change but flow during exercise increased markedly, although the low number of subjects studied after revascularization precluded statistical analysis.

DISCUSSION

The ability to measure regional perfusion at rest and during either contractile exercise or vasodilator stress has become a cornerstone in the diagnosis and management of patients with coronary artery disease (CAD). The application of perfusion imaging in a similar fashion for patients with suspected or known PAD has not been realized but could be useful for the early detection of disease; and for a complete assessment of the total impact of large vessel obstructive disease, microvascular dysfunction, and adequacy of collateral perfusion. Perfusion imaging could also prove useful for monitoring the response to therapies, particularly those targeted to deficits in the microvasculature.

There are several unique challenges to applying perfusion imaging in patients with PAD, aside from the need for an inexpensive and rapid method that can be incorporated into the workflow of a vascular laboratory. One challenge is that perfusion imaging for PAD must be quantitative, which is not necessarily the case for CAD where the evaluation of regional heterogeneity of flow between coronary beds provides good diagnostic performance. Another major technical challenge is the need to be able to assess the very low amount of skeletal muscle perfusion that is present at rest. In this study, we applied CEU which is a rapid, portable and sensitive technique for assessing limb skeletal muscle perfusion. We

employed intermittent high power ($MI > 0.8$) “destructive” imaging in order to increase contrast signal intensity. Conventional myocardial contrast enhanced ultrasound perfusion imaging with destruction replenishment analysis during contrast-specific low-power (mechanical index $[MI] < 0.2$) imaging results in very low contrast signal in resting skeletal muscle, because the blood volume and blood flow is much lower than that found in the myocardium, and is not reproducible between patients. Although we were able to measure resting blood flow with CEU, the technique was not able to differentiate PAD from controls, which is consistent with prior studies in patients with moderate PAD.[9, 17] There are several factors responsible for this finding. First and foremost, because resting MBF is very low even in normal individuals, there are extremely small absolute differences in MBF between controls and patients with even severe PAD. These very small differences are likely to be difficult to detect with current techniques with statistical certainty. Second, spatial heterogeneity in the distribution of MBF is likely to produce microdomains of severe ischemia in subjects with PAD.

Based on the considerations above, we investigated two different approaches for stress perfusion imaging: exercise and vasodilator stress. On CEU imaging, exercise stress produces an increase in microvascular blood flow in normal subjects that is attributable to increases in both the microvascular flux rate and functional microvascular blood volume from recruitment of microvascular units. We used a brief modest level of calf plantar flexion exercise that could be tolerated by all patients with moderate symptomatic and a few with severe PAD. Whereas control subjects had on average an 8- to 9-fold increase in perfusion, patients with PAD had almost no increase in perfusion despite the same degree of exercise. The inability to augment flow in response to stress appeared to occur due more to a deficit in microvascular flux rate than blood volume, which is similar to the pattern seen in CAD. The inability to augment blood flow in response to metabolic demand is thought to be the basis for the clinical manifestations of the disease, as suggested by a previous study which showed that flow reserve best correlated with the severity of claudication.[9]

Because many patients may not be able to exercise due to the severity of PAD, frailty, or other neurologic or musculoskeletal comorbidities, we investigated whether vasodilator stress could be used as an alternative method to uncover abnormal flow reserve. Studies investigating magnetic resonance perfusion imaging in PAD have used limb inflow occlusion and post-ischemic hyperemia to evaluate abnormalities in flow reserve.[18, 19] Mediators of post-ischemic hyperemia are complex, involving endothelial-dependent and independent factors that are released or activated in response to hypoxia, pH, shear, etc.[20] Adenosine is one endogenous factor which acts both through smooth muscle and endothelial cell adenosine receptors, and is used extensively for myocardial stress-rest perfusion imaging. Dipyridamole is also widely used and produces vasodilation primarily by blocking adenosine re-uptake, thereby increasing extracellular adenosine concentrations; although it can also act by increasing smooth muscle cell cyclic GMP.[21]

In this study, we failed to detect a significant increase in muscle blood flow with dipyridamole, even in control subjects. These findings indicate that this agent is not an adequate alternative to exercise stress. Studies examining adenosine or adenosine analogues have demonstrated less of an effect on peripheral compared to coronary vascular resistance.

[10, 12, 22] Thus, although dipyridamole can cause vasodilation in large-conduit arteries, its effects on the distal peripheral microvascular resistance are small.[23] The differences in the coronary and peripheral microvascular effects could be directly attributable to differences in signaling pathways involving the smooth muscle cell high affinity adenosine A2A (coronary vasodilation) versus low affinity endothelial A2B (peripheral vasodilation) pathways.[24–27] The A2B pathway is thought to work in part through eNOS phosphorylation and NO signaling which are known to be abnormal in atherosclerotic disease.[28]

Studies of other exogenously administered agents to pharmacologically simulate exercise in skeletal muscle have identified several vasodilatory pathways that contribute to flow augmentation but a considerable portion of the hyperemic response remains unexplained. Exogenously administered vasodilatory agents (adenosine, NO, and prostaglandins) in resting muscle cause vasodilation in large-conduit arteries such as the femoral or brachial arteries. However, this dilation is not functionally significant as a substantial part of the flow increase in the whole limb blood flow is conducted through the physiological non-nutritive shunts in muscle and/or also through tissues of the limb other than muscle. This was observed in our study in that dipyridamole increased the blood transit rate in large vessels but did not result in the microvascular flow enhancement that was prominent during exercise and differentiated control subjects from patients with PAD. Stimulation of Beta-2 receptors with agents such as dobutamine or dopamine have demonstrated conflicting results on skeletal muscle blood flow in animals and the flow enhancements observed were modest compared to exercise.[29, 30] To date, intra-arterially administered adenosine triphosphate (ATP) is the only exogenous vasodilator that can evoke levels of vasodilation seen in human limbs during heavy exercise.[31] This effect was blunted but not fully reversed by inhibitors of NO and prostaglandins either or alone or in combination, while adenosine inhibition had no effect.[32] Intravenous ATP infusion however, did not significantly enhance skeletal muscle blood flow, limiting its use in routine clinical diagnostic testing.

Although only a few patients with claudication were studied after revascularization, we observed an increase in the blood flow response to exercise and not dipyridamole. This augmentation in exercise perfusion was characterized both by increases in microvascular flux rate and functional blood volume. Future studies involving greater numbers of subjects and characterization of the temporal response to revascularization will be required to understand these preliminary observations.

There are several limitations of this study. The number of subjects was small and the PAD cohort was skewed towards subjects with more advanced severe disease. Our focus on these patients was intentional as these are the patients most likely to require methods other than exercise to elicit increases in skeletal muscle blood flow. While one could argue that ABI performed well for PAD diagnosis in this population, the ability to measure tissue blood flow during hyperemia is important to understand microvascular physiology in patients in whom aging and diabetes are likely to influence flow status. In our study, we tested only a single vasodilatory agent which was selected based on its widespread availability, duration of vasodilatory effect, ease of use, and extensive safety experience. Although infusions of adenosine or existing adenosine receptor agonists could potentially produce greater large

vessel vasodilatory effects, we are unsure whether this approach would be successful given the differences in purinergic signaling in the coronary versus peripheral beds.

In conclusion, in this study we applied CEU imaging of skeletal muscle microvascular blood flow in normal healthy controls and patients with moderate to severe PAD at rest, during exercise, and with pharmacologic vasodilatory stress to demonstrate the following: 1) Resting MBF by CEU perfusion imaging is similar between normal subjects and PAD patients; 2) modest exercise produces a significant increase in microvascular blood flow in the calf muscles of normal subjects but almost no augmentation in blood flow in subjects with moderate to severe PAD; and 3) vasodilator stress using dipyridamole does not significantly increase microvascular blood flow in either normal controls or PAD patients, thereby making this a non-feasible approach for detection of abnormal skeletal muscle blood flow reserve in PAD.

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ABBREVIATIONS

CEU	contrast-enhanced ultrasound
PAD	peripheral artery disease
MBF	microvascular blood flow
CLI	critical limb ischemia
ABI	ankle-brachial index

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Figure 1.

Mean ($\pm$ SEM) microvascular blood flow ($Ax\beta$) in the (A) thigh and the (B) calf at rest, during dipyridamole (DP) stress, and during plantar flexion exercise (Ex) (measured in calf alone). Data for PAD subjects are subdivided into those with moderate and severe symptoms and exercise data. * $P < .05$ vs. rest; † $P < .05$ vs. PAD

Figure 2.

Examples of background-subtracted color-coded CEU images from the calf of a control subject at rest, during dipyrindamole (DP), and during plantar flexion exercise (Ex); and corresponding time (pulsing interval) versus intensity curves. The images and data illustrate much greater flow reserve with exercise than dipyrindamole, and that increased flow during exercise was attributable to increases in both microvascular blood volume (A-value or plateau intensity) and flux rate (β -value or rate constant of the curve). BG= background image obtained immediately after microbubble destruction; b= interval number of hearts beats after destruction.

Figure 3.

Mean (±SEM) microvascular blood flow reserve from the different cohorts derived from the ratio of hyperemic (from dipyridamole [DP] or exercise) to resting MBF. * $P < .05$ versus PAD exercise and versus DP in controls.

Figure 4. Parametric CEU data (mean $\pm$ SEM) on (A) microvascular blood volume (A-value), and (B) microvascular blood flux rate (β -value) from the calf at rest, during dipyridamole (DP) stress, and during plantar flexion exercise (Ex). Data for PAD subjects are subdivided into those with moderate and severe symptoms and exercise data. * $P < .05$ vs. rest; † $P < .05$ vs. PAD.

Figure 5. Mean ($\pm$ SEM) microvascular blood flow ($Ax\beta$) in the calf at rest, during dipyridamole (DP) stress, and during plantar flexion exercise pre- and post-revascularization for the three PAD patients undergoing surgical revascularization. Examples of CEU images from the calf only during exercise are also shown for a single subject undergoing revascularization. Statistical analysis was not performed due to the low number of subjects undergoing revascularization ($n=3$). BG= background image obtained immediately after microbubble destruction; b= interval number of heart beats after destruction.

Table I

Clinical Characteristics

	Controls (n=9)	PAD (n=9)
Age (median years)	53 ± 14.5	68 ± 11.6 *
Male gender, n (%)	5 (56)	2 (22)
Body mass index (kg/m ²)	25.5 ± 3.6	28.6 ± 5.3
HR (beats per min)	66 ± 11	71 ± 11
SBP (mmHg)	125 ± 19	128 ± 19
DBP (mmHg)	76 ± 12	56 ± 9 *
ABI- most symptomatic limb (MS)	1.1 ± 0.2	0.57 ± 0.12 *
ABI- least symptomatic limb (LS)	1.1 ± 0.2	0.73 ± 0.23 *
ABI- non-compressible or absent pulse, n (%)	0 (0)	3 (33)
Prior Revascularization, n (%)	0 (0)	7 (78) *
VascuQoL Score	6.8 ± 0.1	3.3 ± 0.6 *
Rutherford Category		
1- Asymptomatic, n (%)	9 (100)	0 (0)
2- Mild Claudication, n (%)	0 (0)	0 (0)
3- Moderate Claudication, n (%)	0 (0)	3(33) *
4- Ischemic rest pain, n (%)	0 (0)	2 (22) *
5- Minor tissue loss, n (%)	0 (0)	1 (11) *
6- Ulceration and gangrene, n (%)	0 (0)	3 (33) *
Co-morbidities		
Coronary artery disease, n (%)	1 (11)	9 (100) *
Hypertension, n (%)	2 (22)	9 (100) *
Hyperlipidemia, n (%)	2 (22)	8 (89) *
Diabetes, n (%)	0 (0)	8 (89) *
Hx of tobacco use, n (%)	2 (22)	4 (44) *
Medications		
Aspirin, n (%)	1 (11)	9 (100) *
Clopidogrel	0 (0)	3 (33) *
Beta blocker, n (%)	1 (11)	7 (78) *
Statin, n (%)	2 (22)	8 (89) *
Insulin, n (%)	0 (0)	6 (67) *
Laboratory studies		
Hemoglobin (g/dl)	13.8 ± 1.5	11.3 ± 2.3 *
Hemoglobin A1c (mg/dl)	5.3 ± 0.4	6.2 ± 1.8

	Controls (n=9)	PAD (n=9)
Creatinine (mg/dl)	0.8 ± 0.4	1.2 ± 0.8
Echocardiography		
Ejection Fraction (%)	59 ± 4	55 ± 12
Stroke Volume (ml)	72 ± 15	70 ± 12

*
P < .05 compared to Control