Peripheral Arterial Disease

Multiparametric Assessment of Vascular Function in Peripheral Artery Disease

Dynamic Measurement of Skeletal Muscle Perfusion, Blood-Oxygen-Level Dependent Signal, and Venous Oxygen Saturation

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Background—Endothelial dysfunction present in patients with peripheral artery disease may be better understood by measuring the temporal dynamics of blood flow and oxygen saturation during reactive hyperemia than by conventional static measurements.

Methods and Results—Perfusion, Intravascular Venous Oxygen saturation, and T2* (PIVOT), a recently developed MRI technique, was used to measure the response to an ischemia–reperfusion paradigm in 96 patients with peripheral artery disease of varying severity and 10 healthy controls. Perfusion, venous oxygen saturation SvO2, and T2* were each quantified in the calf at 2-s temporal resolution, yielding a dynamic time course for each variable. Compared with healthy controls, patients had a blunted and delayed hyperemic response. Moreover, patients with lower ankle–brachial index had (1) a more delayed reactive hyperemia response time, manifesting as an increase in time to peak perfusion in the gastrocnemius, soleus, and peroneus muscles, and in the anterior compartment, (2) an increase in the time to peak T2* measured in the soleus muscle, and (3) a prolongation of the posterior tibial vein SvO2 washout time. Intrasession and intersession repeatability were also assessed. Results indicated that time to peak perfusion and time to peak T2* were the most reliable extracted time course metrics.

Conclusions—Perfusion, dynamic SvO2, and T2* response times after induced ischemia are highly correlated with peripheral artery disease severity. Combined imaging of peripheral microvascular blood flow and dynamics of oxygen saturation with Perfusion, intravascular SvO2, and T2* may be a useful tool to investigate the pathophysiology of peripheral artery disease. (Circ Cardiovasc Imaging. 2015;8:e002673. DOI: 10.1161/CIRCIMAGING.114.002673.)

Key Words: magnetic resonance imaging • perfusion • peripheral artery disease

Peripheral artery disease (PAD) is most commonly a manifestation of atherosclerosis in vessels supplying the lower limbs and causes significant morbidity and mortality in the United States.1–4 In PAD, atherosclerotic plaque encroaches on the peripheral artery lumen, decreasing blood flow and vascular reactivity of the large arteries.5,6 Collateral arteries vasodilate to meet the baseline metabolic demand of skeletal muscle; however, the collateral vasculature may be inadequate to quickly and adaptively accommodate increases in blood flow demand, such as those that occur during exercise.5,7 This effect can result in an oxygen supply–demand mismatch, causing patients to experience claudication.4

Interventions, such as cilostazol11 or exercise rehabilitation,12 lessen claudication symptomatology; however, they are not necessarily associated with a clinically significant improvement in the ABI. In addition, the ABI is not sensitive to primary microvascular impairment, endothelial dysfunction, or alterations in vascular reactivity that may coexist with the macrovascular lesions.13 Thus, there is a compelling need for the development of diagnostic tools that would allow for the interrogation of the contribution of vascular dysfunction to PAD.

Peripheral vascular function can be evaluated by monitoring the dynamics of blood flow and oxygenation in response to a stressor, analogous to cardiac stress testing. This can be accomplished using an ischemia–reperfusion paradigm, which induces reactive hyperemia. In such a paradigm, proximal arterial occlusion is sustained for several minutes using a blood pressure cuff secured around the subject’s thigh. During the period of arterial occlusion, blood flow in the arteries, veins, and capillaries is halted; however oxygen extraction continues in the stagnant blood of the capillaries. Because the venous blood is static, there is no change in venous oxygen saturation (SvO2) in the large

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The presence of flow-limiting stenoses can be quickly and noninvasively detected by measuring the ankle-brachial index (ABI), the ratio of systolic blood pressures in the ankle and brachial artery.4,5 An ABI of <0.9 confirms the diagnosis of PAD, and an ABI of <0.4 is suggestive of critical limb ischemia.4,10
draining veins. However, after cuff release, hyperemia ensues with an increase in arterial flow through the large arteries and collateral arteries because of endothelium-mediated vasodilation primarily from the release of nitric oxide.14 The oxygenated arterial blood travels to the capillary bed, causing tissue oxygenation to recover as well. At the onset of hyperemia, SvO₂ sharply decreases as the deoxygenated blood from the capillary bed travels to the large draining veins.15 Hyperperfusion of oxygenated arterial blood then causes SvO₂ to rise and surpass the baseline value during the period when blood velocity exceeds the oxygen extraction rate. The kinetics of the hyperemic response provide information about vascular reactivity and endothelial function.16

Several MRI techniques can noninvasively evaluate the dynamic changes that occur in blood flow and oxygenation during the hyperemic response. Specifically, previous studies have investigated perfusion using arterial spin labeling (ASL),17–18 dynamics of SvO₂ using MR susceptometry–based oximetry,19,20 and changes in the T₂* signal (commonly known as blood-oxygen-level dependent [BOLD] response).21–23 which provides a relative measure of muscle capillary bed oxygenation.24,25 In response to induced ischemia, the kinetics of each of these variables is associated with the presence of PAD. Compared with healthy controls, patients exhibit a blunted and delayed reperfusion26 and a reduced rate of recovery of tissue oxygenation, as evidenced by both direct measurement of SvO₂27 and of relative changes in T₂*.21

More recently, we developed a technique termed Perfusion, Intravascular Venous Oxygen saturation, and T₂* (PIVOT) that allows for dynamic and simultaneous quantification of perfusion, SvO₂, and skeletal muscle T₂*.28 The purpose of this work was to evaluate the hyperemic response in patients with PAD and healthy controls using PIVOT. We hypothesize that PIVOT will be able to detect PAD severity–dependent changes in vascular function and that the relationship between these impairments will help us to better understand the pathophysiologic processes that underlie the disease.

Methods

Subjects

Ninety-six patients with intermittent claudication and a diagnosis of PAD and 10 healthy controls were recruited to participate in this study. On enrollment, each subject was classified into a disease severity group based on ABI, where healthy subjects had an ABI of >0.90 (10 subjects; 3 men), mild disease corresponded to an ABI range of 0.7 to 0.89 (28 patients; 18 men), moderate disease to an ABI range of 0.50 to 0.69 (45 patients; 28 men), and severe disease to an ABI of <0.50 (17 patients; 13 men). Additional characteristics of the study participants are shown in Table 1.

Experimental Protocol

Before participation in the study, each subject provided written informed consent. The Institutional Review Board of the University of Pennsylvania approved all aspects of this study.

PAD Patient Protocol

Each patient reported to the testing center for 2 separate visits no >1 month apart. Some subjects were selected to return for a third visit, >3 months after the second visit. On the first visit, screening and medical history questionnaires were completed, and the ABI was measured bilaterally using Doppler sonography according to the current standards.29 Patients were instructed to refrain from strenuous exercise for 3 days before the second visit and to avoid alcohol and caffeine for 24 hours before. On reporting for the second visit, patients underwent a vascular function MRI scanning session. The MRI protocol consisted of dynamic imaging of blood flow and oxygenation of the midcalf using PIVOT throughout an ischemia–reperfusion paradigm. If selected to return, the third visit was identical to the second.

Healthy Subject Protocol

Healthy subjects reported to the testing center for 2 identical visits, separated by 1 day to 1 week. Each MRI session included 2 identical PIVOT scans throughout periods of ischemia–reperfusion.

MRI Scan Protocol

All imaging was performed on a 3T MR imaging system (Siemens, Erlanger, Germany). For every patient, the leg with the lower ABI was scanned, and for each healthy control, the right leg was scanned. The maximum girth of the calf was centered in an 8-channel transmit-receive knee coil (In vivo, Inc, Gainesville, FL). PIVOT data were continuously collected throughout 1 minute of baseline, 5 minutes of proximal arterial occlusion, and 6 minutes after cuff deflation. Proximal arterial occlusion was achieved using a pneumatic tourniquet system (Hokanson, Inc, Bellevue, WA) with a cuff placed on the midthigh. After 1 minute of baseline scanning, the cuff was rapidly inflated to 75 mm Hg above the measured systolic blood pressure, or 250 mm Hg, whichever was lower. For all healthy subjects, the ischemia–reperfusion paradigm was repeated within the same scan session to assess intra-session repeatability. As described previously,18 PIVOT allows for continuous, simultaneous measurement of perfusion, intravascular SvO₂, and skeletal muscle T₂* using an interleaved dual-slice pulsed ASL (PASL) and multiecho gradient-recalled echo (GRE) sequence. Briefly, perfusion quantification is accomplished using saturation inversion recovery,18

### Table 1. Characteristics of Study Participants

<table>
<thead>
<tr>
<th>Subject Group</th>
<th>Total</th>
<th>Repeat visit</th>
<th>Sex (male/female)</th>
<th>Age</th>
<th>BMI</th>
<th>Systolic BP</th>
<th>Diabetes mellitus</th>
<th>Smoking status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy ABI &gt;0.9</td>
<td>10</td>
<td>9</td>
<td>7/3</td>
<td>59.5 (3.9)*</td>
<td>26.2 (4.9)</td>
<td>123 (21)*</td>
<td>0</td>
<td>5/50*</td>
</tr>
<tr>
<td>Healthy ABI 0.7–0.89</td>
<td>28</td>
<td>11</td>
<td>18/10</td>
<td>66.8 (6.3)</td>
<td>27.6 (3.9)</td>
<td>142 (18)</td>
<td>10</td>
<td>2/19/7</td>
</tr>
<tr>
<td>Moderate ABI 0.5–0.69</td>
<td>49</td>
<td>23</td>
<td>29/20</td>
<td>69.4 (8.1)</td>
<td>27.4 (4.0)</td>
<td>141 (19)</td>
<td>15</td>
<td>4/32/13</td>
</tr>
<tr>
<td>Severe ABI &lt;0.5</td>
<td>19</td>
<td>8</td>
<td>15/4</td>
<td>71.3 (8.6)</td>
<td>27.5 (4.0)</td>
<td>137 (17)</td>
<td>6</td>
<td>2/7/10</td>
</tr>
<tr>
<td>All patients ABI &lt;0.9</td>
<td>96</td>
<td>42</td>
<td>62/34</td>
<td>69.0 (7.8)</td>
<td>27.5 (3.9)</td>
<td>141 (18)</td>
<td>31</td>
<td>8/58/30</td>
</tr>
</tbody>
</table>

*Mean (SD) or counts are shown. ABI indicates ankle-brachial index; BP, blood pressure; BMI, body mass index; PAD, peripheral artery disease.

*A significant difference between healthy subjects and all patients with PAD.
a flow-alternating inversion recovery ASL variant, in which slice-selective and nonselective adiabatic inversion pulses are used to achieve tag and control conditions, respectively. Image acquisition follows the postlabeling delay (PLD), which is immediately followed by a slice-selective saturation pulse to reset the magnetization. In PIVOT, a keyhole radiofrequency- spoiled multiecho GRE sequence acquires data at a slice located downstream from the PASL slice during the PLD. Only multiecho GRE data acquired during the PLD after the slice-selective inversion are used for SvO₂ and T₂* analysis; however, the multiecho GRE interleave was run during every PLD to control for potential magnetization transfer effects. From these data, SvO₂ is derived using susceptibility-based oximetry, and T₂* is calculated by fitting the signal magnitude to a monoeXponential function.

For the PASL interleave, images were acquired with the following parameters: partial-Fourier GRE-echo-planar imaging readout; field of view=250x250 mm²; acquired matrix=80x50, reconstructed to 80x80; slice thickness=1 cm; slice location=isocenter; repetition time (TR)/echo time (TE)=1 s/8.05 ms; and PLD=952 ms. For the multiecho GRE interleave, images were acquired with: field of view=96x96 mm²; keyhole acquired matrix=96x24, reconstructed to 96x96 pixels using outer k-space data from a fully sampled reference image obtained immediately after the dynamic PIVOT acquisition; only dynamic data were used for T₂* analysis. Each variable was quantified at 2-s temporal resolution.

Image Analysis
Image analysis was performed using MATLAB (The MathWorks, Inc., Natick, MA). Before data processing, the time series of images were motion corrected with rigid-body transformations using National Institutes of Health ImageJ software (developed by Wayne Rasband; National Institutes of Health, Bethesda, MD). Perfusion was quantified in the regions of interest (ROIs) in the gastrocnemius, soleus, and peroneus muscles, as well as in the anterior compartment, which includes the tibialis anterior and extensor digitorum longus muscles (Figure 1A). For the assessment of repeatability, a whole-leg ROI was used, which comprised of all 4 muscle groups. As described previously, an ROI was delineated in the muscle of interest using a high-resolution scout image as a reference for muscle boundaries. The average signal intensity in the defined ROI after slice-selective (MSS) or nonselective (MMSS) inversion was determined. Using the model described by Raynaud et al., perfusion (f, in mL/min per 100 g) was quantified for each tag-control pair as:

\[
f = -\lambda \ln \left(\frac{M_{SS} - M_{NS}}{M_{SS} + M_{NS}} \left(1 - e^{-\frac{T}{\gamma \Delta T}}\right) + 1\right)
\]

(1)

where λ is the tissue-partition coefficient (λ=0.9 mL/g), T is the PLD, Tp is the longitudinal relaxation time of blood, which in this model is assumed to be equivalent to that of tissue (Tp=1420 ms+12×T₁wa). The perfusion offset was corrected by subtracting the average perfusion during the period of cuff occlusion from each time point, as perfusion is assumed to be zero during the period of proximal arterial occlusion. For each muscle, the peak perfusion and time to peak perfusion (TTPₚₒ) were recorded from the dynamic time course data (Figure 1C).

Using the first 2 echoes of the multiecho GRE, SvO₂ was quantified in the larger posterior tibial vein (Figure 1B). As described previously, SvO₂ was calculated by measuring the difference in phase accrual (Δϕ) between echoes spaced apart by ΔTE in the blood surrounding reference tissue as:

\[
\%SvO_2 = 1 - \frac{2\Delta\phi / \Delta T}{\gamma \Delta \chi_{pip} \cdot Hct \cdot B_0 \left(\cos^2 \theta - \frac{1}{3}\right)} \times 100
\]

(2)

where γ is the proton gyromagnetic ratio, Δχₘ₀ is the susceptibility difference between fully oxygenated and deoxygenated blood (Δχₘ₀=4πx0.27 ppm), Hct is the hematocrit measured by venipuncture, B₀ is the main magnetic field strength, and θ is the angle of the vessel with respect to B₀ (measured from axial scout images). The washout time, equal to the time between cuff release and the minimum SvO₂ and the upslope, calculated as the maximum slope during recovery, were recorded (Figure 1D).

T₂* was measured within an ROI in the soleus muscle (Figure 1B) by fitting multiecho GRE data from echoes 2 to 5 to a monoeXponential function. Signal intensity in the ROI was first averaged for each echo; then the data were fit to a monoeXponential function and T₂* was computed at each time point. The T₂* time course data were normalized to the average baseline value, and relative T₂* was time to peak T₂* (TTP₂*) were recorded from the time course (Figure 1E).

Statistical Analysis
All statistical analyses were performed with JMP software (JMP; Version 11; SAS Institute Inc, Cary, NC). Data normality was evaluated using the Shapiro–Wilks test. For data from the normal or log-normal distribution, 2-sample Student t-tests with equal variance were used to determine whether differences existed between healthy subjects (ABI>0.9) and patients with PAD (ABI<0.9). Nonparametric Wilcoxon signed-rank tests were used to assess differences of non-normally distributed variables. In patients with PAD, correlations between the ABI and each time course metric and between pairs of time course metrics were calculated using Pearson correlation coefficient (if data were normally distributed) or Spearman rank correlation coefficient (if data were not normally distributed). Holm adjustment for multiple comparisons was applied to all tests and correlations to maintain the family-wise
error rate of 0.05. Thus, for all tests, $P_{\text{Holm}} < 0.05$ was considered to be significant.

In healthy controls, intrasession and intersession (1 day to 1 week between scans) repeatability were assessed. Intersession repeatability was also assessed in a subset of patients with PAD ($\approx$ 3 months between scans). In all cases, the intraclass correlation coefficients (ICC) and mean within-subject coefficients of variation ($CV_w$) were calculated to assess measurement repeatability.

## Results

In 6 subjects, a cuff pressure of 75 mmHg above systolic blood pressure did not result in a full occlusion, likely due to calcification of the feeding arteries. Insufficient occlusion was determined based on the appearance of venous pooling in the perfusion images and $T_2^*$ time course. In the remaining 100 subjects, several subjects’ MRI images were unanalyzable because of motion contamination, partial volume effects, low signal/noise ratio, or insufficient vein size. Table 2 shows the number of patients for each variable that were of sufficient quality for analysis. Notably, for all subjects in whom a full occlusion was achieved, at least 1 variable was fit for analysis.

For visualization purposes, average time courses were generated for all healthy subjects and patients with mild, moderate, and severe PAD disease burden for perfusion in the gastrocnemius muscle (Figure 2A), posterior tibial vein $SvO_2$ (Figure 2B), and relative $T_2^*$ in the soleus muscle (Figure 2C). The typical ischemia–hyperemia response is seen in each variable’s time course. During the period of arterial occlusion, $T_2^*$ decreases as the stagnant blood in the capillary bed becomes increasingly desaturated. After cuff release, $SvO_2$ initially drops as the deoxygenated blood from the capillary bed advances into the large draining veins in the measurement slice. Concurrently, perfusion increases to repay the oxygen debt incurred during the ischemic period. This hyperperfusion of oxygenated blood causes $T_2^*$ and $SvO_2$ to recover and surpass their baseline values. As blood flow and $SvO_2$ normalize, so too does $T_2^*$. From the average time courses, a striking difference is seen between healthy subjects and patients with PAD, and furthermore, $TTP_{\text{Perf}}$, $TTP_{\text{Perf}}$, and $TTP_{\text{Perf}}$ are increasingly prolonged as disease severity worsens.

The group-wise average (SD) of each time course metric for the measured variables is summarized in Table 3. $TTP_{\text{Perf}}$, in the peroneus, $T_2^*_{\text{max}}$, and $TTP_{\text{Perf}}$ were not from the normal distribution, and all other variables were from the log-normal distribution. Each variable, with the exception of $SvO_2$ upslope, was significantly different between healthy controls and patients with PAD. With the presence of PAD, there was a significant prolongation of the hyperemic response time and a reduction in the magnitude of the hyperemic response. $SvO_2$ washout time increased from 11 s in healthy subjects to 39 s in patients with PAD. $TTP_{\text{Perf}}$ was delayed as well, averaged over all muscle groups, and it increased from 24 s to 72 s in patients with disease. Peak perfusion decreased from

### Table 2. Synopsis of Number of Subjects With Analyzable Images for Each Measured Variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy ABI $&gt;0.9$</th>
<th>Mild ABI 0.7–0.89</th>
<th>Moderate ABI 0.5–0.69</th>
<th>Severe ABI $&lt;0.5$</th>
<th>All Study Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>10</td>
<td>27</td>
<td>45</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>Gastrocnemius perfusion</td>
<td>10</td>
<td>26</td>
<td>40</td>
<td>15</td>
<td>91</td>
</tr>
<tr>
<td>Soleus perfusion</td>
<td>10</td>
<td>26</td>
<td>41</td>
<td>14</td>
<td>91</td>
</tr>
<tr>
<td>Peroneus perfusion</td>
<td>10</td>
<td>25</td>
<td>40</td>
<td>9</td>
<td>84</td>
</tr>
<tr>
<td>AC perfusion</td>
<td>10</td>
<td>23</td>
<td>36</td>
<td>13</td>
<td>82</td>
</tr>
<tr>
<td>$SvO_2$</td>
<td>10</td>
<td>20</td>
<td>34</td>
<td>13</td>
<td>77</td>
</tr>
<tr>
<td>Relative $T_2^*$</td>
<td>10</td>
<td>27</td>
<td>44</td>
<td>18</td>
<td>99</td>
</tr>
</tbody>
</table>

ABI indicates ankle-brachial index; AC, anterior compartment; and $SvO_2$, venous oxygen saturation.
T2*max decreased from 110% to 106%. Averaged (SD) over all between healthy subjects and patients with PAD, and relative
significant correlation was detected between ABI and peak perfusion measured in the gastrocnemius and soleus (r = 0.53; P < 0.0001) or anterior compartment (r = 0.40; P < 0.001). The other significant correlation detected was between relative T2*max and TTPPerf in the peroneus (r = 0.43; P < 0.005).

In healthy subjects, both intrasession and intersession repeatability were assessed (Tables 4 and 5). The intrasession ICC was >0.7 for all measured variables, with the exception of TTPPerf (r = 0.61). One healthy subject was lost to follow-up; thus, the number of healthy subjects in whom intersession repeatability was assessed was 9. Although lower than intrasession repeatability, the intersession repeatability in the healthy subjects was still high for peak perfusion (r = 0.84) and TTPPerf (r = 0.74). The ICC, however, was lower for SvO2 upslope and washout time, T2*max, and TTPPerf. In all cases, the mean within-subject CV in healthy subjects was <25%. In patients with PAD, the ICCs of the perfusion time course metrics and TTPPerf were approximately equal to or better than the ICC of the ABI measurement (r = 0.53). The ICC for the SvO2 washout time and upslope, and T2*max, however, was low (r < 0.35). The mean within-subject

Table 3. Associations of the Presence of PAD With PIVOT
Time Course Metrics

<table>
<thead>
<tr>
<th></th>
<th>Healthy ABI &gt; 0.9, n = 10</th>
<th>Patients With PAD ABI &lt; 0.9, n = 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABI</td>
<td>1.11 (0.10)</td>
<td>0.60 (0.14)*</td>
</tr>
<tr>
<td>Gastrocnemius peak perfusion, mL/min per 100 g</td>
<td>54.0 (14.5)</td>
<td>28.3 (12.9)*</td>
</tr>
<tr>
<td>Gastrocnemius TTPPerf, s</td>
<td>29 (10)</td>
<td>77 (53)*</td>
</tr>
<tr>
<td>Soleus peak perfusion, mL/min per 100 g</td>
<td>74.9 (33.0)</td>
<td>28.9 (13.9)*</td>
</tr>
<tr>
<td>Soleus TTPPerf, s</td>
<td>25 (13)</td>
<td>72 (49)*</td>
</tr>
<tr>
<td>Peroneus peak perfusion, mL/min per 100 g</td>
<td>41.2 (11.3)</td>
<td>20.8 (12.4)*</td>
</tr>
<tr>
<td>Peroneus TTPPerf, s</td>
<td>24 (9)</td>
<td>74 (47)*</td>
</tr>
<tr>
<td>AC peak perfusion, mL/min per 100 g</td>
<td>43.4 (16.0)</td>
<td>24.5 (14.1)*</td>
</tr>
<tr>
<td>Washout time, s</td>
<td>11 (4)</td>
<td>39 (23)*</td>
</tr>
<tr>
<td>Upslope, %HbO2/s</td>
<td>0.91 (0.40)</td>
<td>0.51 (0.22)</td>
</tr>
<tr>
<td>T2*max, %</td>
<td>111 (4)</td>
<td>106 (3)*</td>
</tr>
<tr>
<td>TTPPerf, s</td>
<td>35 (12)</td>
<td>91 (55)*</td>
</tr>
</tbody>
</table>

ABI indicates ankle-brachial index; AC, anterior compartment; PAD, peripheral artery disease; PIVOT, perfusion, intravascular venous oxygen saturation, and T2*; and TTP, time to peak. *P < 0.001; †P < 0.0001. 

52.6 mL/min per 100 g to 25.6 mL/min per 100 g with disease. TTPPerf in the soleus muscle increased from 33 s to 91 s between healthy subjects and patients with PAD, and relative T2*max decreased from 110% to 106%. Averaged (SD) over all subjects, baseline T2* was 19 (2) ms, and averaged across all muscle groups, the perfusion offset during ischemia was 7.6 (2.7) mL/min per 100 g.

Intravascular correlations were assessed in patients with PAD only. The correlations between ABI and time course metrics reinforce some of the findings from the group-wise analysis. A significant correlation was found between the ABI and response time after cuff release (Figure 3A, 3C, and 3E). As the ABI decreases, an increase in the TTPPerf in the gastrocnemius muscle increased, so too did TTPPerf in the other muscles (averaged over all muscle ROIs: r = 0.71; P < 0.0001), TTPPerf in the soleus (r = 0.57; P < 0.0001), and SvO2 washout time (r = 0.53; P < 0.0001). The single exception was the lack of a correlation between the peroneous TTPPerf and SvO2 washout time (r = 0.26). Positive correlations were also detected between peak perfusion measured in the gastrocnemius and soleus (r = 0.53; P < 0.0001) or anterior compartment (r = 0.40; P < 0.001). The other significant correlation detected was between relative T2*max and TTPPerf in the peroneus (r = 0.43; P < 0.005).

In healthy subjects, both intrasession and intersession repeatability were assessed (Tables 4 and 5). The intrasession ICC was >0.7 for all measured variables, with the exception of TTPPerf (r = 0.61). One healthy subject was lost to follow-up; thus, the number of healthy subjects in whom intersession repeatability was assessed was 9. Although lower than intrasession repeatability, the intersession repeatability in the healthy subjects was still high for peak perfusion (r = 0.84) and TTPPerf (r = 0.74). The ICC, however, was lower for SvO2 upslope and washout time, T2*max, and TTPPerf. In all cases, the mean within-subject CV in healthy subjects was <25%. In patients with PAD, the ICCs of the perfusion time course metrics and TTPPerf were approximately equal to or better than the ICC of the ABI measurement (r = 0.53). The ICC for the SvO2 washout time and upslope, and T2*max, however, was low (r < 0.35). The mean within-subject

Figure 3. Correlation plots for ankle-brachial index (ABI) versus gastrocnemius muscle time to peak perfusion (TTPPerf; A) and peak perfusion (B), venous oxygen saturation washout time (C) and upslope (D), and time to peak T2* (E) and relative T2*max (F) measured in the soleus. As disease severity worsens, the reactive hyperemia response time is progressively prolonged. However, no correlation was observed between the magnitude of the hyperemic response and the ABI.
CV in patients with PAD was ≈10% greater than that in healthy subjects for all measured variables.

### Discussion

In this study, peripheral vascular function was investigated in patients with PAD and healthy controls using PIVOT, a recently developed MRI technique, allowing for simultaneous measurement of the dynamics of blood flow and oxygen saturation throughout an ischemia–reperfusion paradigm. Compared with healthy controls, patients with PAD exhibited a blunted and delayed hyperemic response, as measured by PIVOT. Moreover, the results showed a significant correlation between PAD disease severity and the temporal dynamics of recovery of blood flow and oxygenation after induced ischemia. With decreasing ABI, the time required to accommodate the hyperperfusion of oxygen-rich blood to the ischemic muscle was prolonged; there was an increase in the time needed for desaturated blood to flow out of the capillary bed into the large draining veins; and a delay in the TTP<sub>Perf</sub>, indicating a decrease in the rate of recovery of oxygen saturation in the capillary bed. These data are thus suggestive of disease severity–dependent impairment of vascular function in PAD.

Previous studies have used an ischemia–reperfusion model to investigate peripheral vascular function in patients with PAD compared with healthy controls; however, these studies were limited to the measurement of a single variable—perfusion or T<sub>2*</sub> or 2 variables—SvO<sub>2</sub> and bulk arterial flow. Separately measuring all of the variables in a single study would require multiple scans. However, with PIVOT, perfusion, SvO<sub>2</sub>, and T<sub>2*</sub> are measured simultaneously, allowing for the direct investigation of the temporal relationships between the variables at no additional scan time or patient discomfort. This interleaved method was previously evaluated by comparing results from PIVOT to those obtained with the traditional PASL or multiecho GRE sequences in a cohort of young healthy subjects. No bias was detected between PIVOT and the individual measurement methods, suggesting that the simultaneous measurement of perfusion, SvO<sub>2</sub>, and T<sub>2*</sub> at high temporal resolution is possible with PIVOT.

Data in this study show that the temporal dynamics during reactive hyperemia are associated with the presence of PAD and are tightly correlated with disease severity; thus, high temporal resolution sampling of the response is extremely important. PIVOT provides 2-s temporal resolution of each of the measured variables, higher sampling rate than previous patient studies investigating perfusion alone (16-s temporal resolution), or SvO<sub>2</sub> and bulk arterial flow (5-s temporal resolution of SvO<sub>2</sub>). Temporal resolution of 1 s was used in previous studies investigating only T<sub>2*</sub> in skeletal muscle. Unlike perfusion and SvO<sub>2</sub>, the origin of the BOLD signal is less physiologically straightforward. BOLD signal depends on blood oxygenation in the capillary bed, as well as microvascular flow, blood volume, cellular pH, and vessel diameter and orientation. So, although relative T<sub>2*</sub> can be quickly acquired, and in this study was the most reliable variable to measure (relative T<sub>2*</sub> was analyzable in 99/100 subjects), the multifactorial contrast mechanism and the fact that the measured response is relative, rather than in physiological units, makes the interpretation of the BOLD signal more complicated. To reduce potential confounds because of blood volume on the measured T<sub>2*</sub>, care was taken to ensure that the patient’s leg was at heart height, and each patient was in the supine position for ≈15 minutes before the onset of ischemia. In addition, arterial occlusion was induced using a pneumatic tourniquet system capable of fully inflating in ≈0.3 s, thus occluding venous and arterial flow nearly simultaneously.

Hyperemia can also be induced via exercise rather than induced ischemia. Exercise is more physiologically relevant to PAD; however, muscles may be stressed to varying degrees, depending on which muscle group is activated (eg, plantar flexion versus dorsiflexion), and total work is subject effort.

### Table 4. Assessment of Intrasession Repeatability in Healthy Subjects and Patients With PAD

<table>
<thead>
<tr>
<th>Subject</th>
<th>Scan A</th>
<th>Scan B</th>
<th>ICC</th>
<th>CV&lt;sub&gt;4&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leg peak perfusion, mL/min per 100 g</td>
<td>51.5 (13.5)</td>
<td>51.6 (14.3)</td>
<td>0.95</td>
<td>4.1%</td>
</tr>
<tr>
<td>Leg TTP&lt;sub&gt;Perf&lt;/sub&gt;, s</td>
<td>23 (8)</td>
<td>21 (7)</td>
<td>0.76</td>
<td>11.0%</td>
</tr>
<tr>
<td>Washout time, s</td>
<td>11 (4)</td>
<td>11 (5)</td>
<td>0.90</td>
<td>8.8%</td>
</tr>
<tr>
<td>Upslope, %HbO&lt;sub&gt;2&lt;/sub&gt;/s</td>
<td>0.9 (0.4)</td>
<td>0.9 (0.5)</td>
<td>0.79</td>
<td>18.5%</td>
</tr>
<tr>
<td>T&lt;sub&gt;2*&lt;/sub&gt; max, %</td>
<td>111 (4)</td>
<td>112 (4)</td>
<td>0.78</td>
<td>1.1%</td>
</tr>
<tr>
<td>TTP&lt;sub&gt;Perf&lt;/sub&gt;, s</td>
<td>35 (12)</td>
<td>35 (11)</td>
<td>0.61</td>
<td>15.3%</td>
</tr>
</tbody>
</table>

ABI indicates ankle-brachial index; and TTP, time to peak.

### Table 5. Assessment of Intersession Repeatability in Healthy Subjects and Patients With PAD

<table>
<thead>
<tr>
<th>Subject</th>
<th>Session 1</th>
<th>Session 2</th>
<th>ICC</th>
<th>CV&lt;sub&gt;4&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leg peak perfusion, mL/min per 100 g</td>
<td>52.1 (14.2)</td>
<td>50.3 (17.1)</td>
<td>0.78</td>
<td>11.8%</td>
</tr>
<tr>
<td>Leg TTP&lt;sub&gt;Perf&lt;/sub&gt;, s</td>
<td>23 (8)</td>
<td>21 (7)</td>
<td>0.71</td>
<td>10.9%</td>
</tr>
<tr>
<td>Washout time, s</td>
<td>12 (4)</td>
<td>14 (9)</td>
<td>0.61</td>
<td>23.4%</td>
</tr>
<tr>
<td>Upslope, %HbO&lt;sub&gt;2&lt;/sub&gt;/s</td>
<td>0.9 (0.5)</td>
<td>0.8 (0.4)</td>
<td>0.57</td>
<td>24.4%</td>
</tr>
<tr>
<td>T&lt;sub&gt;2*&lt;/sub&gt; max, %</td>
<td>111 (4)</td>
<td>110 (3)</td>
<td>0.58</td>
<td>2.0%</td>
</tr>
<tr>
<td>TTP&lt;sub&gt;Perf&lt;/sub&gt;, s</td>
<td>37 (12)</td>
<td>36 (12)</td>
<td>0.58</td>
<td>16.5%</td>
</tr>
</tbody>
</table>

Healthy subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Session 1</th>
<th>Session 2</th>
<th>ICC</th>
<th>CV&lt;sub&gt;4&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABI 0.59 (1.5)</td>
<td>0.59 (1.7)</td>
<td>0.53</td>
<td>11.8%</td>
<td></td>
</tr>
<tr>
<td>Leg peak perfusion, mL/min per 100 g</td>
<td>23.3 (8.9)</td>
<td>35.5 (9.5)</td>
<td>0.52</td>
<td>20.3%</td>
</tr>
<tr>
<td>Leg TTP&lt;sub&gt;Perf&lt;/sub&gt;, s</td>
<td>68 (42)</td>
<td>72 (44)</td>
<td>0.78</td>
<td>22.0%</td>
</tr>
<tr>
<td>Washout time, s</td>
<td>34 (17)</td>
<td>30 (16)</td>
<td>0.13</td>
<td>31.9%</td>
</tr>
<tr>
<td>Upslope, %HbO&lt;sub&gt;2&lt;/sub&gt;/s</td>
<td>0.5 (0.2)</td>
<td>0.7 (0.4)</td>
<td>0.08</td>
<td>36.7%</td>
</tr>
<tr>
<td>T&lt;sub&gt;2*&lt;/sub&gt; max, %</td>
<td>106 (3)</td>
<td>106 (3)</td>
<td>0.33</td>
<td>1.9%</td>
</tr>
<tr>
<td>TTP&lt;sub&gt;Perf&lt;/sub&gt;, s</td>
<td>90 (49)</td>
<td>84 (39)</td>
<td>0.57</td>
<td>23.4%</td>
</tr>
</tbody>
</table>

ABI indicates ankle-brachial index; PAD, peripheral artery disease; and TTP, time to peak.
dependent. Conversely, tourniquet-induced ischemia is a global stimulus, more uniformly affecting all muscle groups downstream from the site of inflation and is more consistent between subjects.

A previous study by Wu et al\textsuperscript{26} measured posts ischemic skeletal muscle perfusion with a continuous ASL technique in patients with PAD and healthy controls. Compared with the results from this study, there is good agreement in the trend of timing of the hyperemic response and disease severity, with the presence of PAD, and with increasing disease severity, the TTP\textsubscript{perf} is progressively prolonged.\textsuperscript{26} Wu et al\textsuperscript{26} also found an association between the peak perfusion and disease severity, whereas in this study, an association between peak perfusion and disease presence but not severity was detected. Continuous ASL provides a higher signal/noise ratio of the perfusion signal at the cost of lower temporal resolution (16 s with continuous ASL\textsuperscript{26} compared with 2 s in this study). As such, relative to this study, much higher peak perfusions were observed in the investigation by Wu et al\textsuperscript{26} for all disease severity subgroups. For instance, in the gastrocnemius muscle, a peak perfusion of \(60\) mL/min per 100 g was not reported in patients with PAD, compared with \(28.3\) mL/min per 100 g reported in this study. Using an invasive but highly precise method, Lassen et al\textsuperscript{17} investigated the clearance rate of radioactive xenon-133 to determine perfusion in patients and healthy controls. In that study, the reported peak perfusion and TTP\textsubscript{perf} are in agreement with the results of this study, \(51.8\) mL/min per 100 g versus \(17.1\) mL/min per 100 g, and \(22\) s versus \(138\) s for healthy subjects versus patients, respectively. Previous studies investigating perfusion in young healthy subjects using the same PASL variant, either saturation inversion recovery alone\textsuperscript{14,36} or using the combined PIVOT technique,\textsuperscript{28} found peak perfusion during reactive hyperemia to be similar to those values reported in this work.

Results of intravascular SvO\textsubscript{2} measured in the posterior tibial vein are in relative agreement with the work of Langham et al\textsuperscript{27} investigating SvO\textsubscript{2} in the femoral vein of patients with PAD compared with young healthy subjects and age-matched healthy controls. As in the results of this study, Langham et al\textsuperscript{27} reported a prolonged washout time in patients with PAD compared with their healthy peers. In this study, a correlation between the ABI and washout time in the posterior tibial vein was additionally detected. Langham et al\textsuperscript{27} also observed a reduction in upslope in patients compared with controls, which was not observed in this study. This deviation could be attributed to the different vessels investigated by the 2 studies, posterior tibial vein in this study, compared with the larger and more superior femoral vein in the work by Langham et al.\textsuperscript{27} A larger vein would result in a higher signal/noise ratio of the measured SvO\textsubscript{2}, thereby increasing measurement precision. In this study, it was necessary to acquire SvO\textsubscript{2} data downstream from the perfusion measurement location so as to not disturb the magnetization of inflowing blood.

Finally, the present results are in general agreement with previous BOLD studies in the leg.\textsuperscript{21,35} The average \(T_2^*\text{max}\) of patients with PAD of 106% is slightly lower than the relative \(T_2^*\text{max}\) of 110.5% reported by Ledermann et al.\textsuperscript{21} However, these authors normalized \(T_2^*\) to the average \(T_2^*\) value of the first 3 s after cuff release, whereas for this study, \(T_2^*\) was normalized to the baseline average \(T_2^*\). \(T_2^*\) decreases during the ischemic period; therefore, normalizing the data to the posts ischemic period would increase the \(T_2^*\) range and thus increase the relative \(T_2^*\text{max}\). In agreement with the study by Ledermann et al,\textsuperscript{21} this study showed that with decreasing ABI, there is an increase in TTP\textsubscript{TP*}.

The intervariable correlations were investigated to better understand the relationship between blood flow and oxygenation in the capillary bed and large draining veins. Strong positive correlations were detected between the various timing metrics; however, no significant correlations were observed between the response time and response magnitude (eg, peak perfusion in the gastrocnemius muscle was not significantly correlated with its TTP\textsubscript{perf}).

In addition to the benefit of assessing intervariable relationships, the acquisition of simultaneous perfusion, SvO\textsubscript{2}, and \(T_2^*\text{max}\) measurements with PIVOT increases the likelihood that at least one of the variables is of sufficient quality for analysis. For example, if a patient has a small posterior tibial vein, the measure of SvO\textsubscript{2} would not be possible. However, perfusion and \(T_2^*\text{max}\) data could still be used to investigate the hyperemic response. Alternatively, in some patients, perfusion was noisy because of partial volume effects from unperfused intramuscular fibrous inclusions. Yet the \(T_2^*\text{max}\) and SvO\textsubscript{2} data were still able to provide insight into vascular function. In this study, although only 71 subjects had analyzable images for all 3 variables, in all 100 subjects in whom a complete cuff occlusion was achieved, at least one of the variables was fit for analysis.

The dynamics of the hyperemic response are a function of upstream arterial occlusions, diffuse atherosclerosis, and endothelium-mediated vascular reactivity. Thus, the assessment of the response with several variables provides complementary rather than independent information. The combination of results from perfusion and SvO\textsubscript{2} could, for example, be used to investigate the relative metabolic response. Furthermore, by modeling the combined PIVOT data, there may be added sensitivity to detect a response to a therapeutic intervention.

The intrasession repeatability in healthy subjects shows that the measured time course metrics have excellent precision in the short term. The hyperemic response measured for relative \(T_2^*\text{max}\) had a broad peak, unlike the sharp peak of the perfusion measurement (as shown in Figure 2). The TTP\textsubscript{TP*}, was, therefore, more sensitive to time course noise than TTP perfusion, resulting in a reduction in repeatability. This pattern was observed for both intrasession and intersession repeatability. Compared with intrasession repeatability, intersession repeatability was slightly lower. However, it is likely that there are true differences in the reactive hyperemia response because of normal physiological variation. Care was taken to minimize known factors that affect vascular reactivity—all subjects were instructed to refrain from caffeine intake and vigorous exercise before scanning, and scans were conducted at the same time of day. The measured intrasession differences are therefore a combination of true physiological variation, measurement error (as is present for intrasession repeatability), as well as the potential effect of slight changes in slice location. Generally, the within-subject, CV was still small and similar to the reported results.
in a previous investigation of intersession and intrasession repeatability of PIVOT-derived time course metrics in young healthy subjects.24

The intersession repeatability in patients with PAD was acceptable for some variables and poor for others (SVo2, washout time and upslope, and T2* max). Overall, the variables were similarly repeatable in healthy subjects and patients with PAD. Given that the ABI measurement differed between the 2 scan sessions, there may be true pathophysiologic progression or regression of disease in some patients. The ICCs of peak perfusion, TTP perfusion, and TTP‡ were on the order of the ICC of the ABI measurement. The relatively low within-subject CVp suggests that although the measured response may differ between subjects, the span over which they differ is small compared with the range of values expected in healthy subjects versus patients with PAD. TTPperf and TTP‡ both had relatively good repeatability and were sensitive to disease presence and severity, making these metrics the most reliable for detection of alterations in vascular function.

There are several limitations to this study. Some variables have intersession ICCs of ≤0.6, suggesting that the measurement of vascular function by PIVOT during reactive hyperemia is not suitable for clinical monitoring of a single patient. However, the present method could be well suited to investigate the response to therapy in a cohort of patients with PAD.

The moderate disease severity group was much larger than either the mild or severe disease severity groups, although this is less of a limitation as correlations were used to investigate the relationship between disease severity and the MRI-measured variables. In addition, patients recruited for this study were not restricted to either aortoiliac or femoropopliteal artery PAD. Angiographic data were not available for these patients; thus, the site of obstruction is not known.

Furthermore, all correlations of the MRI data to disease severity were based on the ABI. Even though the ABI is the clinical standard for diagnosis of PAD, it has limitations. The ABI may be falsely elevated in patients with severely calcified arteries or in situations of undiagnosed brachial or subclavian artery stenosis.39 Direct measurement of a patient’s functional capacity via exercise testing, including the 100-foot walking time or the claudication onset time and peak walking time of the Gardner protocol,40 may provide more physiologically relevant information than the measurement of the ABI alone. However, these physiological measures are subjective based on an individual’s pain tolerance or effort. There have been mixed results in previous studies as to whether the physiological measures of disease severity correlate with the ABI. McDermott et al41 showed that maximum treadmill walking time did correlate with the ABI; however, Szuba et al42 found no correlation. Assessment of the correlation between the MRI time course metrics and physiological testing results would be of great interest.

The variables measured indicate impaired vascular function, related to both macrovascular lesions and microvascular dysfunction. Additional measurement of bulk arterial blood flow during the ischemia–reperfusion paradigm would allow for assessment of temporal dynamics of blood flow in the macrovasculature and microvasculature. Comparison of the temporal dynamics in the large artery and capillary bed could help to separate the macrovascular and microvascular responses. This can be accomplished by velocity encoding the multiecho GRE interleave of PIVOT, although the temporal resolution of the sequence must be decreased to accommodate double the number of phase-encoding segments of the multiecho GRE.31 In addition, the MRI data may be complemented with the measurement of hemoglobin oxygen saturation using continuous-wave near infrared spectroscopy.44,45

In conclusion, previous studies investigating peripheral vascular function in patients with PAD were limited to the investigation of only 1 or 2 variables. Using PIVOT, simultaneous measurement of perfusion, SVo2, and T2* can be achieved. Results indicate that increasing PAD disease severity is associated with a prolongation of response time after induced ischemia. TTPperf and TTP‡ were both repeatable measures and were sensitive to the presence and severity of PAD. In the future, PIVOT could be used as a means to monitor disease progression and evaluate treatment response in patients with PAD.

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Disclosures

None.

References


34. McDermott MM, Ferrucci L, Guralnik JM, Dyer AR, Liu K, Pearce WH, Clark E, Liao Y, Criqui MH. The ankle-brachial index is associated with...


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CLINICAL PERSPECTIVE

This study shows that vascular dysfunction present in patients with peripheral artery disease can be detected through assessment of the reactive hyperemia response by temporally resolving perfusion, venous oxygen saturation, and skeletal muscle blood-oxygen-level dependent signal. For the first time in patients with peripheral artery disease, these parameters were simultaneously measured in a single study using a new MRI technique, PIVOT. Patients with peripheral artery disease exhibited a blunted and delayed recovery after tourniquet-induced ischemia compared with their healthy peers. As disease severity worsens, there was an additional prolongation of the reactive hyperemia response time measured by perfusion, venous oxygen saturation, and skeletal muscle blood-oxygenation-level dependent signal. Assessment of vascular function with PIVOT was tightly correlated with the ankle-brachial index. The reactive hyperemia response kinetics are a function of diffuse macrovascular atherosclerosis and endothelium-mediated vascular reactivity. Thus, the assessment of dynamic vascular function provides more relevant information to peripheral artery disease pathophysiology than the ABI alone. The ability to noninvasively assess vascular function in patients with peripheral artery disease may provide a tool to assess disease progression and response to intervention.
Multiparametric Assessment of Vascular Function in Peripheral Artery Disease: Dynamic Measurement of Skeletal Muscle Perfusion, Blood-Oxygen-Level Dependent Signal, and Venous Oxygen Saturation

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