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Simultaneous measurement of macro- and microvascular blood flow and oxygen saturation for quantification of muscle oxygen consumption

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Abstract

Purpose—To investigate the relationship between blood flow and oxygen consumption in skeletal muscle, a technique termed “Velocity and Perfusion, Intravascular Venous Oxygen saturation and T_2^* ” (ν PIVOT) is presented. ν PIVOT allows quantification of feeding artery blood flow velocity, perfusion, draining vein oxygen saturation, and muscle T_2^* , all at four-second temporal resolution. Together, the measurement of blood flow and oxygen extraction can yield muscle oxygen consumption ($\dot{V}O_2$) via the Fick principle.

Methods—In five subjects, ν PIVOT-derived results were compared to those obtained from stand-alone sequences during separate ischemia-reperfusion paradigms to investigate the presence of measurement bias. Subsequently, in ten subjects, ν PIVOT was applied to assess muscle hemodynamics and $\dot{V}O_2$ following a bout of dynamic plantar flexion contractions.

Results—From the ischemia-reperfusion paradigm, no significant differences were observed between data from ν PIVOT and comparison sequences. After exercise, the macrovascular flow response reached a maximum 8 ± 3 s after relaxation, however perfusion in the gastrocnemius muscle continued to rise for 101 ± 53 s. Peak $\dot{V}O_2$ calculated based on mass-normalized arterial blood flow or perfusion was 15.2 ± 6.7 mL O_2 /min/100g or 6.0 ± 1.9 mL O_2 /min/100g, respectively.

Conclusion— ν PIVOT is a new method to measure blood flow and oxygen saturation, and therefore to quantify muscle oxygen consumption.

Keywords

muscle; oxygen consumption; exercise; ischemia; perfusion

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Disclosures

None.

INTRODUCTION

Peripheral vascular reactivity and the dynamics of muscle metabolism can be interrogated by measuring the temporal relationship between blood flow and oxygen saturation in the micro- and macrovasculature. Magnetic resonance imaging (MRI) methods have previously been developed and applied to assess the response of blood flow in the large arteries and veins (1), microvascular perfusion in skeletal muscle (2,3), intravascular venous oxygen saturation (SvO_2) (1,2), and T_2^* , also known as the blood-oxygen level dependent (BOLD) response (4,5). There are several technical and physiologic parameters that will affect the measurement of T_2^* including magnetic field inhomogeneity, blood oxygenation, perfusion, cellular pH and vessel orientation (6–9), thus the BOLD response is generally reported as a relative measure (% change from baseline), providing a surrogate measure of tissue activation. During and following exercise, the measurement of blood flow and oxygen saturation together can provide oxygen consumption ($\dot{V}O_2$, in units of mL O_2 /min/100g tissue) in skeletal muscle through the Fick principle:

$$\dot{V}O_2 = C_aO_2 \cdot \dot{Q} \cdot (SaO_2 - SvO_2) \quad [1]$$

where C_aO_2 is the oxygen carrying capacity of blood ($C_aO_2 = C_a \cdot \text{Hgb}$, $C_a = 1.34$ mL O_2 /g hemoglobin, Hgb in units of g Hgb/dL blood), $\dot{Q}$ is the volumetric blood flow rate in units of mL blood /min/100g tissue, and SaO_2 and SvO_2 represent the oxygen saturation of arterial and venous blood, respectively, in units of percent oxygenated Hgb (%Hb O_2).

Measurements of blood flow and oxygen extraction can be obtained locally at the level of the skeletal muscle capillary bed, or globally in the large feeding arteries and draining veins. Methods to measure $\dot{V}O_2$ locally are based on perfusion obtained with arterial spin labeling (ASL) MRI (10) combined with a measure of blood oxygen level in the capillary bed based on either an empirically derived (11) or theoretical (12) relationship between %Hb O_2 and the RF-reversible transverse relaxation rate (R_2'), where R_2' is determined as the difference between R_2^* and R_2 . Due to the relatively long image acquisition, separate periods of exercise are required to measure perfusion and oxygen extraction. Alternatively, the aggregate oxygen consumption of the limb can be computed by measuring the blood flow and oxygen saturation in the feeding arteries and draining veins (13), similar to methods for whole-brain cerebral metabolic rate of oxygen consumption (14,15). While this approach provides higher temporal resolution and can be conducted with only one period of exercise, the measurement is restricted to quantification of average oxygen consumption of the whole limb, and may not be reflective of the microvascular dynamics.

Results from previous studies have employed various measurement methods and yielded disparate results for peak $\dot{V}O_2$ responses. The discrepancy could be due to the different methods, exercise paradigms (isometric plantar flexion (12), dorsiflexion (11), or dynamic contractions (13)), activated muscles (gastrocnemius (12), tibialis anterior (11), or quadriceps muscles (13)), measurement timing (during contraction (11,12), or after relaxation (11,13)), or based on local (11,12) versus global (13) flow and oxygen saturation measurements. Therefore, a method to simultaneously measure macro- and microvascular

blood flow and oxygen saturation would be desirable and could provide new insight into the relationship between blood flow in the micro- and macro-vasculature, and may address questions on the optimal method for assessing muscle oxygen consumption.

Previously, an interleaved pulsed ASL (PASL) and multi-echo gradient recalled echo (GRE) sequence, termed “Perfusion, Intravascular Venous Oxygen saturation, and T_2^* ” (PIVOT) was introduced that allows for the simultaneous acquisition of perfusion, draining vein SvO_2 , and the skeletal muscle BOLD response (16). However, so far no single technique has been reported that additionally measures arterial blood flow. Concurrent acquisition of micro- and macrovascular blood flow alongside SvO_2 and T_2^* would permit functional assessment of skeletal muscle, and results from such an approach could be used in aggregate to calculate muscle oxygen consumption via the Fick principle (equation [1]).

To address this unmet need, a 3-slice interleaved PASL, multi-echo GRE, and dual-echo phase contrast sequence is presented, termed “Velocity and PIVOT” (ν PIVOT). We hypothesize that ν PIVOT allows unbiased measurement of each of the individual parameters, and that ν PIVOT data will permit quantification of $\dot{V}O_2$ following a bout of exercise based on draining vein oxygen saturation and either mass-normalized large-vessel flow or local tissue perfusion.

METHODS

Sequence overview

The original PIVOT sequence comprises a dual-slice acquisition scheme in which a multi-echo GRE sequence acquires data at a slice located distal to the perfusion acquisition during the PASL post-labeling delay (PLD) (16). From this dual-slice PIVOT sequence, perfusion is quantified from the difference between alternately acquired label and control data as described by Raynaud, et al. (17). Venous oxygen saturation is computed from the multi-echo GRE data through susceptibility-based oximetry (SBO) which relates the incremental field offset inside the vein relative to surrounding muscle tissue to oxygen saturation in physiologic units of %HbO₂ (18). Post-processing of the multi-echo GRE data also yields T_2^* (a measure of relative muscle activation/oxygenation) by fitting the magnitude signal intensities to an exponential decay.

Here, the framework of PIVOT was expanded to include an upstream (proximal), velocity-encoded, dual-echo GRE acquisition following the PASL EPI readout. The proposed 3-slice interleaved ν PIVOT sequence is shown in Figure 1. With ν PIVOT, in addition to the previously mentioned PIVOT measures, bulk arterial and venous blood flow can be computed using phase contrast (19) and SvO_2 can be quantified in a large draining vein via SBO (18,20) (analogous to the downstream slice). A similar sequence combining phase contrast and SBO was previously proposed for the measurement of oxygen metabolism in the brain (14,21) and was termed OxFlow (22).

Experimental protocol

In order to investigate the presence of potential bias created via the combined sequence approach, ν PIVOT-derived time-course metrics of the reactive hyperemia response were

compared to independently acquired PIVOT or velocity-encoded dual-echo GRE (OxFlow) data during separate ischemia-reperfusion paradigms. Subsequently, the exercise hyperemia response following a bout of plantar flexion contractions of the calf was assessed with ν PIVOT. The dynamics of the macro- and microvascular flow responses were compared, and $\dot{V}O_2$ in the gastrocnemius muscle was calculated.

The Institutional Review Board of the University of Pennsylvania approved all aspects of the study. A total of ten healthy subjects (31.7 ± 5.0 years old, 7 males) were recruited to participate after providing written, informed consent. All imaging was performed at 3 T (Siemens Magnetom Tim Trio (VB17), Siemens Medical Equipment; Erlangen, Germany) with an eight-channel transmit/receive knee coil (InVivo, Inc; Gainesville, FL) with longitudinal sensitivity ≈ 200 mm. Subjects were positioned supine and the maximum cross-section of the calf was centered in the coil. Experimental imaging sequences were written in SequenceTree (23). Data analysis was performed with in-house-written software using MATLAB, Version 7.12.0 (MathWorks, Natick, MA), and statistical analyses were executed with JMP, Version 11 (SAS Institute, Cary, NC, 1989–2007). For reactive hyperemia experiments, arterial occlusion was achieved by application of supra-systolic pressure (at least 200 mmHg) in a cuff secured around the thigh, inflated by a pneumatic tourniquet system (D.E. Hokanson, Inc; Bellevue, WA). For exercise hyperemia experiments, exercise was performed against a custom-built, MRI-compatible, pneumatic plantar flexion ergometer described previously (24).

Sequence parameters—PIVOT and OxFlow sequences were adapted to match the sequence parameters employed in ν PIVOT. In ν PIVOT, PASL images were acquired with a partial Fourier GRE-EPI readout with the following parameters: FOV = 250×250 mm²; acquisition matrix = 80×50 , reconstruction matrix = 80×80 ; slice thickness = 1 cm; location = isocenter (positioned as the maximum girth of the calf, central slice Figure 1); TR/TE = 2 s/8.05 ms; PLD = 952 ms. The distal slice location multi-echo GRE, located downstream from the PASL slice location (distal slice location in Figure 1), used the following parameters: FOV = 96×96 mm²; acquisition matrix = 96×24 , (for SvO₂ data analysis, reconstruction matrix = 96×96 via keyhole (25) using fully sampled reference images; only dynamic data were used for T₂* analysis); slice thickness = 1 cm; location = 3 cm inferior to isocenter; TR/TE1/TE2/TE3/TE4/TE5 = 38.1/3.8/7.1/10.4/17.4/24.4 ms, $\alpha = 15^\circ$. In ν PIVOT, the superiorly-located velocity-encoded dual-echo GRE (proximal slice location in Figure 1) was executed with: FOV = 96×96 mm²; acquisition matrix = 96×24 , (SvO₂ and velocity had reconstruction matrix = 96×96 via keyhole (25) using fully sampled reference images); VENC = 120 cm/s; slice thickness = 0.5 cm; location = 3–4 cm superior to isocenter; TR/TE1/TE2 = 20.0/6.0/9.7 ms, $\alpha = 10^\circ$. From the ν PIVOT data, perfusion, bulk flow, SvO₂, and T₂* each were quantified with four-second temporal resolution.

Parameters of the comparison sequences were modified to match those of ν PIVOT. In PIVOT, the overall TR was increased from 1 s in its original implementation to 2 s. The acquisition parameters for the PASL interleave were identical, and those of the distally located multi-echo GRE were also equivalent with the exception of TE3, TE4, and TE5 which were 12.4 ms, 19.4 ms, and 26.4 ms, respectively. OxFlow used the same pulse sequence parameters as the proximally-positioned GRE interleave of ν PIVOT, but acquired

full images rather than employing a keyhole acquisition strategy (25) (FOV = 96×96 mm²; acquisition matrix = reconstruction matrix = 96×96). Thus one OxFLOW image was acquired every 3.84 s.

Reactive hyperemia protocol

To assess whether ν PIVOT was biased in its measurement of each parameter relative to the corresponding stand-alone sequences, ν PIVOT-derived perfusion, peroneal vein SvO₂, and T₂* were compared to results obtained with PIVOT, and tibioperoneal vein SvO₂ and large-vessel blood flow were compared between ν PIVOT and OxFLOW. In five healthy subjects (4 male, 32.4±6.4 years old), data were acquired with each of the three sequences throughout separate ischemia-reperfusion paradigms, each with 2 minutes of baseline, 3 minutes of ischemia, and 4 minutes of recovery. The order of the scans was OxFLOW, ν PIVOT, and PIVOT. Two to three minutes of additional recovery time was allotted between scans to allow each subject to return to his or her relative baseline state.

For perfusion quantification, average signal intensity was determined for label and control data at each time point in regions of interest (ROIs) in the gastrocnemius, soleus and peroneus muscles, and in the anterior compartment comprising the tibialis anterior and extensor digitorum longus muscles. Perfusion was quantified from the label and temporally-matched control data using the model proposed by Raynaud, et al. (17). The baseline perfusion offset was corrected by subtracting the average perfusion during the period of arterial occlusion from each time point (26). From the perfusion time courses, the time to peak perfusion and peak perfusion were determined. Maps were generated for visualization purposes by computing perfusion on a voxel-wise basis, then smoothing, and averaging the data over a period of 20 s.

The superiorly located GRE acquisition was positioned such that it intersected the tibioperoneal trunk, a short arterial segment distal to the branch point of the anterior tibial artery, before the bifurcation of the posterior tibial and peroneal arteries (see Figure 1). ν PIVOT-derived artery and vein velocities were compared to OxFLOW-derived measures from the same slice location. Velocity was integrated over the vessel ROI to compute flow rates.

Using the SBO model (11), SvO₂ was quantified for both the proximal and distal GRE data in ν PIVOT and results were compared to OxFLOW and PIVOT, respectively. Vessel tilt angle was measured from axial scout images. Hematocrit was assumed to be 0.45 for male and 0.40 for female subjects. In the distal slice, SvO₂ was quantified in a peroneal vein and results were compared to those from PIVOT. In the proximal slice, positive and negative VENC acquisitions were averaged and SvO₂ was quantified in the tibioperoneal vein and results were compared to those from OxFLOW. In all cases, the average baseline SvO₂ and the washout time (time to minimum SvO₂) were recorded from the dynamic time course.

From the inferiorly located multi-echo GRE data in both ν PIVOT and PIVOT acquisitions, T₂* was quantified in a ROI in the soleus muscle by fitting the magnitude signal intensities from echoes 2–5 to a mono-exponential function. T₂* was then normalized to the average baseline value, and the maximum relative T₂* (T₂*_{max}) and time to peak T₂* were

determined. Additionally, for visualization purposes, voxelwise T_2^* was computed, smoothed, and averaged over 20 s.

Exercise protocol

The five subjects involved in the reactive hyperemia evaluation plus an additional five subjects, thus a total of ten subjects, (7 male, 31.7 ± 5.0 years old) were scanned during a dynamic exercise protocol. For subjects involved in both the reactive and exercise hyperemia exams, imaging occurred on the same day (with the exception of one subject whose exercise evaluation occurred on a later date). ν PIVOT data were acquired continuously throughout 2 minutes baseline, 2 minutes of resisted dynamic plantar flexion contractions, and 8 minutes recovery. Contractions were performed against the foot pedal of a custom-built, MRI-compatible ergometer (24). Subjects were coached to perform one full contraction and relaxation cycle every second against a pressure of 10 psi. Based on specifications of the ergometer setup, this corresponded to a power of approximately 6 W.

Data analysis for each parameter was the same as for the reactive hyperemia protocol. Of note, for subjects involved only in the exercise study (and for the subject whose imaging was performed on separate days), ν PIVOT data were acquired during a scan consisting of 2 mins baseline and 2 mins of arterial occlusion to determine the baseline perfusion offset described above. In subjects who participated in both the ischemia and exercise protocols, the baseline offset calculated from the ischemia data were applied to the exercise data.

In all subjects, gastrocnemius muscle volume was quantified via manual segmentation of a 3D SSFP-echo acquisition, which provides contrast between muscle tissue and facial planes and blood, yielding good conspicuity of muscle boundaries. 3D SSFP-echo data were acquired with the following parameters: FOV = $160 \times 160 \times 416$ mm³; acquisition matrix = $128 \times 128 \times 104$; $\alpha = 14^\circ$; TR/TE = 7.59/3.02 ms; acquisition time = 2 min 6 s per station, and 2 stations were acquired to cover the length of the gastrocnemius muscle. Muscle volume was converted to mass using the density of muscle (1.06 g/mL).

$\dot{V}O_2$ was calculated via the Fick principle (equation [1]) using flow measured either from microvascular perfusion ($\dot{V}O_{2,perf}$) or gastrocnemius muscle mass-normalized large vessel arterial flow ($\dot{V}O_{2,flow}$). In both cases, SaO_2 was assumed to be 100% and thus arteriovenous oxygen difference was estimated as $(1 - SvO_2)$. Peak $\dot{V}O_2$, time to peak $\dot{V}O_2$, and net $\dot{V}O_2$ (total integrated post-exercise area) were recorded for $\dot{V}O_{2,perf}$ and $\dot{V}O_{2,flow}$.

Statistical analyses

Bland-Altman analyses (27) were conducted on time-course data to assess agreement between ν PIVOT and comparison sequences, and the intraclass correlation coefficient (ICC) was computed. Due to the relatively small sample size, normal distribution cannot be assumed, thus non-parametric Wilcoxon signed rank tests were used to assess whether significant differences existed between ν PIVOT-measured responses and PIVOT or OxFlow measures from the reactive hyperemia evaluation. For the exercise evaluation, Wilcoxon-signed rank tests were also used to assess whether significant differences existed between

the time to peak perfusion and time to peak arterial and venous blood flow, and whether differences existed between $\dot{V}O_{2,flow}$ and $\dot{V}O_{2,perf}$ peak, time to peak, or net responses.

RESULTS

Post-ischemia reactive hyperemia evaluation

Figure 2 shows images obtained with the ν PIVOT sequence in a representative subject during the reactive hyperemia portion of the ischemia-reperfusion protocol. Figure 3 shows the time courses for the reactive hyperemia experiments from ν PIVOT and comparison sequences. Table 1 shows the results of the comparison between the full time-course data of ν PIVOT and OxFLOW and PIVOT, and Table 2 lists the results derived from the time courses. Overall, the reactive hyperemia response was not observed to differ significantly when measured with ν PIVOT and PIVOT or OxFLOW.

For the large-vessel flow time-course results, significant differences were not detected between ν PIVOT and OxFLOW. Time to peak arterial flow was 6 ± 2 s or 6 ± 3 s for ν PIVOT and OxFLOW, respectively and peak arterial flow was 706 ± 442 mL/min for ν PIVOT and 701 ± 445 mL/min when quantified from OxFLOW data. One subject had particularly high flow values as measured by both sequences. Removing the outlying subject's data, flow was 527 ± 220 mL/min and 513 ± 172 mL/min for ν PIVOT and OxFLOW, respectively ($p=1$). Similarly, significant differences were not observed in venous blood flow metrics between ν PIVOT and OxFLOW data.

The Wilcoxon signed rank tests were not significant for differences between ν PIVOT and PIVOT perfusion magnitude or timing. In the gastrocnemius muscle, peak perfusion was 50 ± 13 mL/min/100 g and 52 ± 13 mL/min/100g when measured with ν PIVOT or PIVOT, respectively. Time to peak in the gastrocnemius muscle was 20 ± 6 s and 18 ± 9 s with ν PIVOT and PIVOT, respectively. In the soleus muscle, peak perfusion was 42 ± 17 mL/min/100g and 44 ± 19 mL/min/100g, and time to peak was 14 ± 6 s and 15 ± 7 s when measured with ν PIVOT and PIVOT. The perfusion offset averaged over the leg during the period of arterial occlusion was 6.5 ± 4.1 mL/min/100g for ν PIVOT, and 6.5 ± 3.8 mL/min/100g for PIVOT.

SvO₂ dynamics were not significantly different between ν PIVOT and PIVOT in the distal slice location, nor between ν PIVOT and OxFLOW in the proximal slice location. In two subjects, SvO₂ could not be measured in the distal slice location with PIVOT or ν PIVOT because the peroneal veins were too small. In the remaining three subjects, mean washout time was 16 ± 7 s and 23 ± 15 s. SvO₂ was measured in the proximal slice location in four subjects, washout time was 8 ± 0 s and 8 ± 3 s for ν PIVOT and OxFLOW, respectively. In one subject (other than the two mentioned previously), no change in SvO₂ was observed in the post-ischemic hyperemia state, thus washout time could not be determined. There was no difference in baseline SvO₂ between the sequences at either slice location. However, baseline SvO₂ was not compared between the proximal and distal slice locations as complete datasets were obtained in only two subjects.

The average baseline T₂* was 24 ± 2 ms as quantified by both ν PIVOT and PIVOT sequences. The BOLD response in the soleus muscle was the most delayed in reaching its

peak magnitude for both ν PIVOT and PIVOT ($TTP_{T2^*} = 30 \pm 7$ s or 30 ± 8 s, respectively). Relative $T2^*$ changes were successfully measured in each of the five subjects, and significant differences between ν PIVOT and PIVOT data were not detected. Relative $T2^*_{\max}$ was $107 \pm 3\%$ for both sequences.

Exercise hyperemia investigation

Although hyperemia was induced by the exercise in all subjects, two datasets were removed from the final analysis: one because the subject was unable to maintain the 1-s contraction rate, and the other due to substantial motion during the recovery period following exercise.

Figure 4 shows perfusion maps calculated during the exercise protocol in one of the subjects in whom reactive hyperemia had been measured previously (Figure 2). Figure 5 shows time courses of the average perfusion response in each of the calf muscles. Note that the hyperemic response is dominant in the gastrocnemius muscle, the muscle activated in plantar flexion contraction. Table 3 summarizes the results from the exercise protocol, and Figure 6 shows the time courses of the ν PIVOT measures. The timing of the arterial and venous flow peaks is similar in exercise and reactive hyperemia, however there is a substantial delay in the time to peak perfusion following exercise. Both arterial and venous flow rates peaked 8 ± 3 s after cessation of exercise, while gastrocnemius muscle perfusion did not reach its maximum until 101 ± 53 s ($p < 0.005$).

SvO_2 was computed for both the proximal and distal slice locations and results were averaged when used to quantify muscle oxygen consumption. Average gastrocnemius muscle mass was 349 ± 75 g. Figure 7 shows the calculated $\dot{V}O_{2,flow}$ and $\dot{V}O_{2,perf}$ responses. Similar to the relationship between blood flow and perfusion responses alone, $\dot{V}O_{2,flow}$ peaks and recovers quickly, while the $\dot{V}O_{2,perf}$ response is delayed and prolonged. Peak $\dot{V}O_2$ was significantly different when measured with mass-normalized flow compared to perfusion: 15.2 ± 6.7 versus 6.0 ± 1.9 mL O_2 /min/100g ($p < 0.01$) for macroscopic flow and perfusion, respectively. Baseline $\dot{V}O_2$ differed between large-vessel flow and perfusion-derived values: 1.2 ± 0.4 mL O_2 /min/100g versus 0.6 ± 0.3 mL O_2 /min/100g ($p < 0.01$), respectively. The time to peak also differed: 12 ± 7 and 102 ± 37 s ($p < 0.01$) for macroscopic flow and perfusion, respectively. However, net $\dot{V}O_2$ was not significantly different for the two measures: 20.6 ± 9.7 mL/100g and 22.3 ± 13.5 mL/100g ($p > 0.05$) for large-vessel flow and perfusion derived $\dot{V}O_2$ values, respectively.

DISCUSSION

Overall, ν PIVOT is able to measure the dynamics of the reactive hyperemia and exercise hyperemia responses. In the distal peripheral circulation, SvO_2 is the most challenging of the parameters to measure due to the small size of the vessels and the potential for venous compression from the weight of the calf (2). Measurement of SvO_2 at two different slice locations improves the possibility of encountering a vein with sufficient size for analysis. Dynamic measurement of the exercise hyperemia response following plantar flexion exercise was demonstrated, providing insight into the regulation of blood flow and oxygen consumption in skeletal muscle, and permitting direct comparison between macrovascular flow- and microvascular perfusion-based oxygen consumption.

vPIVOT sequence evaluation

To evaluate vPIVOT, post-ischemic reactive hyperemia was selected as the functional paradigm since relatively good within-session repeatability has been reported (16). The ischemia-reperfusion protocol is independent of subject effort, and a recent report by Lopez et al. suggests that reactive hyperemia is the paradigm of choice for the assessment of vascular reactivity (28). However $\dot{V}O_2$ cannot be quantified from the reactive hyperemia data, as the measured oxygen saturation dynamics in the large draining vein do not reflect the concurrent oxygen consumption occurring at the level of the capillary bed. Rather, the SvO₂ response measured in the vein is the aggregate of oxygen consumption in the capillary bed from the prior period of ischemia.

If the combined sequence structure were to introduce a systematic bias, it would most likely be in the quantification of perfusion. The sinc-shaped RF excitation pulses applied proximal to the PASL EPI readout perturb the spin magnetization of the inflowing blood. For this reason the superior GRE data were acquired after the PASL image acquisition. However, the superior GRE interleave will still cause a reduction in the longitudinal magnetization prior to inversion, potentially reducing the labeling efficiency and leading to an underestimation of the measured perfusion. In vPIVOT, the excitation flip angle and slice thickness were reduced for the superior GRE acquisition to minimize this potential error source. No significant differences were observed, however, in the perfusion measurements between vPIVOT and PIVOT data. Furthermore, Bland-Altman analysis verified that there was no statistically significant bias between vPIVOT and PIVOT data, and the ICC showed that vPIVOT and PIVOT time-course data were in excellent mutual agreement.

Furthermore, reactive hyperemia perfusion results are in good agreement with prior studies (16,17,29). For instance, peak perfusion in the soleus muscle was 42 ± 17 mL/min/100g in the present study, compared to 37.0 ± 6.1 mL/min/100g from a previous study using PIVOT (16), and 50 ± 6 mL/min/100g from a previous study using pseudo-continuous ASL (29). However, all of these results are much lower than the peak perfusion in the soleus muscle of 116 ± 57 mL/min/100g reported by Wu, et al. using a continuous ASL technique (30).

In general, the results presented in this study are also in agreement with prior reports of arterial blood flow in the peripheral circulation (1,31), SvO₂ dynamics (1,16,32), and the relative response of T₂* (4,16).

Exercise hyperemia evaluation

In all subjects, exercise hyperemia was stimulated from the bout of resisted plantar flexion contractions. Subjects reported that the exercise was moderately intense. The average perfusion response was greatest in the gastrocnemius muscle, although some subjects also had an increase in perfusion in the soleus muscle, another muscle involved in plantar flexion contraction. The exercise paradigm provoked almost no sustained hyperemic response in the anterior compartment, which is primarily involved in dorsiflexion (Figure 5).

The combined measurement of SvO₂ and relative T₂* response may inform on the relationship between the oxygen saturation in the draining vein and that in the capillary bed. As mentioned previously, T₂* depends on blood oxygen saturation in the capillary bed as

well as perfusion, vessel orientation, and intracellular pH (6–9). The only two of those factors that are expected to change following moderate exercise are oxygen saturation and perfusion. Here, it is observed that SvO_2 and T_2^* initially decrease upon relaxation (Figure 6) and concurrently perfusion begins to increase, thus suggesting that the draining vein SvO_2 response (quantified in physiologic units and yielding oxygen extraction) may be used as a marker of capillary bed oxygen saturation. However, in future work, a direct comparison of capillary bed oxygen saturation and SvO_2 in the draining vein could provide more definitive insight into this question.

The discrepancy between $\dot{V}O_{2,flow}$ and $\dot{V}O_{2,perf}$, both at baseline and peak, can in part be explained by the normalization of tibioperoneal trunk blood flow. Bulk flow was normalized to the gastrocnemius muscle mass, yet the artery supplies other muscles of the calf and foot in addition to non-muscle structures of the leg (e.g. tendons, bone, skin). Based on that alone, $\dot{V}O_{2,flow}$ will overestimate the true oxygen consumption in the gastrocnemius muscle. However, here it is additionally observed that the temporal dynamics of the macrovascular- and microvascular-derived $\dot{V}O_2$ responses differ from each other during the hyperemic period (Figure 7). If there were a constant relationship between blood flow in the large vessel and capillary bed, the ratio between $\dot{V}O_{2,flow}$ and $\dot{V}O_{2,perf}$ would be temporally stable. This disparity between the instantaneous measures of $\dot{V}O_2$, based on large-vessel flow and perfusion may due to non-nutritive blood flow (33). This interpretation is supported by the fact that macrovascular blood flow in the arteries and veins peak simultaneously, well before the perfusion time to peak, suggesting that blood may be shunted from arterioles to venules upon relaxation.

In recent work by Mathewson, et al. (13) $\dot{V}O_2$ was quantified from a combined measure of femoral vein bulk blood flow through projection phase-contrast (34), and femoral vein intravascular oxygen saturation by SBO (18,20). Mathewson et al.'s results showed that $\dot{V}O_2$ in the quadriceps muscles following resisted knee extension exercise increased by a factor of approximately 13, reaching a peak of 9.6 ± 1.8 mL/min/100g. Similar to the results of $\dot{V}O_{2,flow}$ presented in this study, the peak response occurred almost immediately after cessation of exercise (13).

Two recent studies have employed local measurement methods to quantify $\dot{V}O_2$, combining ASL-based perfusion with R_2' -based oximetry (11,12). Zheng, et al. reported $\dot{V}O_2$ of 3.8 ± 2.5 mL/min/100g and 6.1 ± 2.9 mL/min/100g during two different workloads of sustained isometric plantar flexion contraction in the calf (12). However due to limited temporal resolution, only the physiologic response during the steady-state of a sustained contraction could be quantified, thus dynamic changes during the transition between exercise and rest were not measured. A more recent method proposed by Buck, et al. (11) employed a rapid spin and gradient echo (SAGE) technique to measure R_2' and used an empirically derived relationship between R_2' and %HbO₂ (35). With temporal resolution of 2.5 s, the investigators assessed the dynamic changes in oxygen consumption during the transition between exercise (in the form of a sustained isometric dorsiflexion contraction) and rest. Results showed that oxygen consumption following isometric contraction in the tibialis anterior muscle increased by a factor of 3–4 from baseline to the peak post-exercise response (11), however only the relative $\dot{V}O_2$ response was reported.

Intravascular blood oxygen measurements through T₂-based oximetry (36) were previously combined with an independent acquisition of perfusion through ASL by Decorte, et al. (37). However, their study aimed at investigating the response to hyperoxia, rather than the dynamic response following exercise. The baseline $\dot{V}O_2$ of 0.6 ± 0.2 mL O₂/min/100g (37) was in agreement with the baseline $\dot{V}O_{2,perf}$ presented herein of 0.6 ± 0.3 mL O₂/min/100g.

Study limitations

There are several limitations to the present study. At the limited sample size investigated for this study, no differences between ν PIVOT and comparison sequences could be detected even if they were to exist. The ν PIVOT data acquired during reactive hyperemia paradigms were exclusively used to assess the ability of the sequence to quantify individual measurement parameters. The order of the scans was fixed for the sequence evaluation to allow comparisons to be made between neighboring acquisitions since ν PIVOT data were collected between the OxFLOW and PIVOT scans. With this experimental design, a training effect due to the repetition of induced ischemia could introduce a systematic error, however significant differences between quantified measures were not observed in this study.

Blood flow in the tibioperoneal trunk does not exclusively supply the gastrocnemius muscle, but also supplies the soleus and peroneus muscles. Both of these muscles can be involved in plantar flexion contraction, and the peroneus muscle also plays a role in eversion of the foot. In addition, blood in the tibioperoneal trunk supplies some muscles of the foot, and skin, tendons, and other connective tissues of the calf and foot. Therefore, baseline and maximum $\dot{V}O_{2,flow}$ may be overestimated since it was normalized to only the mass of the gastrocnemius muscle. Quantification of blood flow in arteries distal to the popliteal trifurcation would allow for better isolation of inflow, but would also necessitate higher spatial resolution.

Oxygen saturation was quantified in the large draining veins rather than locally in the capillary bed. A direct comparison between $\dot{V}O_2$ measured with either macrovascular flow or microvascular perfusion, and with either draining vein SvO₂ or R₂'-based capillary bed oxygen saturation would be of great interest. However, dynamic quantification of capillary bed oxygen saturation in physiologic units of %HbO₂ remains a difficult problem.

The most accurate measure of local oxygen consumption would be based on microvascular blood flow and oxygen saturation in the post-capillary venules, however current methods are not capable of quantifying dynamic changes in signal isolated from venules.

Conclusions

In summary, the feasibility of quantification of arterial and venous bulk flow, perfusion, draining vein SvO₂, and skeletal muscle T₂^{*}, as part of a single integrated procedure, has been demonstrated in healthy volunteers. The relationship between macro- and microvascular blood flow was explored following induced ischemia and dynamic exercise, demonstrating the disparate behavior in microvascular response between the two paradigms. The measurement of the relationship between macro- and microvascular blood flows may be clinically relevant in patient populations such as diabetics, or patients with peripheral artery

disease. Finally, oxygen consumption was calculated based on the aggregate measures of perfusion or bulk arterial flow and draining vein SvO₂, allowing for the dynamic measurement of the muscle's metabolic response following exercise.

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Figure 1.

vPIVOT pulse sequence diagram, showing PIVOT and OxFlow components as well. Similar to PIVOT, multi-echo GRE data are acquired distal to the PASL slice location during the PLD. In addition, velocity-encoded GRE data are acquired after the perfusion EPI acquisition at a location superior to the PASL slice location. Perfusion is quantified through ASL (data used are shown in light red), SvO₂ through susceptibility-based oximetry (data used are shown in blue), blood velocity through phase contrast (data used are shown in dark red), and T₂^{*} by fitting the magnitude signal to a mono-exponential function (data used are shown in green). Temporal resolution for all quantified parameters is 4 s.

Figure 2.

Example images from vPIVOT data in a representative subject during post-ischemia reactive hyperemia. Proximal slice location: velocity (A), and field map for SvO₂ quantification (B); central slice location: perfusion (C); distal slice location: T₂* (D) and field map (E). Perfusion map shows aberrantly high values centrally due to enhancement from large vessels. Note that perfusion and T₂* maps are generated for visualization purposes only. Time-course data were derived from the average raw signal intensities inside muscle ROIs.

Figure 3.

Averaged across all five subjects, vPIVOT reactive hyperemia data are compared to OxFlow-derived flow and SvO₂, and PIVOT-derived perfusion and T₂^{*}. Grey box indicates period of arterial occlusion and error bars indicate standard error. A significant difference was not observed between vPIVOT and OxFlow or PIVOT for any of the quantified time-course metrics.

Figure 4.

Perfusion maps from the exercise protocol shown (A) at baseline, (B) immediately after exercise, and (C) 40 s after exercise in the same subject as shown in Figure 2. Maps are displayed for visualization purposes only, whereas the time courses were derived from ROI-averaged raw image signal intensities. Here, perfusion was computed on a voxel-wise basis, smoothed, and averaged over 20 s of data. The hyperemic response is sustained in the posterior compartment, particularly in the medial head of the gastrocnemius muscle, at 40 s after exercise.

Figure 5.

Average exercise vPIVOT-derived perfusion data in individual muscles. Error bars represent standard error. Grey box indicates the period of exercise, during which motion artifacts prohibited quantification of perfusion and other vPIVOT responses. Hyperperfusion is sustained for several minutes in the gastrocnemius muscle, whereas the response normalizes much sooner in the soleus and peroneus muscles and in the muscles of the anterior compartment (AC).

Figure 6.

ν PIVOT measures in response to the dynamic exercise protocol (time courses averaged over the eight subjects, error bars indicate standard error). The grey box indicates the period of exercise. Arterial and venous blood flow peaked and recovered rather quickly, while perfusion continued to increase for more than one minute after exercise. The SvO_2 and T_2^* responses both drop upon relaxation then begin to recover at a similar rate.

Figure 7.

Oxygen consumption calculated via the Fick principle using perfusion or mass-normalized average arterial flow (average from eight subjects, with error bars indicating standard error). Exercise occurred during the grey box from 2 minutes to 4 minutes. Although the peak amplitude and time to peak of the $\dot{V}O_{2,flow}$ and $\dot{V}O_{2,perf}$ are significantly different, the total integrated post-exercise response is similar (see Table 3).

Table 1

Intraclass correlation coefficient (ICC) and Bland-Altman analyses comparing ν PIVOT time-course data to OxFlow and PIVOT time-course data from the reactive hyperemia experiments. Bland-Altman analysis yielded the average difference between ν PIVOT and comparison sequences (bias) and lower and upper limits of agreement (LOA) computed as $\text{mean} \pm 1.96 \times \text{SD}$. Note that for SvO₂ time courses, data were not compared during the period of arterial occlusion and not all subjects were included in the analysis (n = 4 for tibioperoneal trunk data, and n = 3 for peroneal vein data).

	ICC	Bias	Lower LOA	Upper LOA
<i>νPIVOT versus OxFlow</i>				
Venous blood flow (mL/min)	0.86	-8.4	-93.6	76.7
Arterial blood flow (mL/min)	0.94	11.3	-97.6	120.3
Tibioperoneal vein SvO ₂ (%HbO ₂)	0.85	-1.3	-11.9	9.3
<i>νPIVOT versus PIVOT</i>				
Average perfusion (mL/min/100g)	0.97	-0.7	-5.5	4.2
Peroneal vein SvO ₂ (%HbO ₂)	0.92	0.5	-5.2	6.3
Relative T ₂ * (%)	0.98	0.1	-1.1	1.3

Table 2

ν PIVOT derived time-course metrics from the post-ischemic hyperemia experiment compared to those obtained with OxFlow or PIVOT, reported as mean (standard deviation). *P* value is reported for comparison between ν PIVOT and OxFlow or PIVOT. Note that for SvO₂ data, not all subjects were included in the analysis (n=4 for tibioperoneal trunk data, and n=3 for peroneal vein data).

	OxFlow	ν PIVOT	PIVOT	<i>P</i> value
Large-vessel blood flow				
Peak venous flow (mL/min)	405 (429)	399 (467)		0.81
TTP venous flow (s)	7 (3)	6 (4)		0.38
Peak arterial flow (mL/min)	701 (445)	706 (442)		0.81
TTP arterial flow (s)	6 (3)	6 (2)		1
Perfusion				
Gastroc peak perfusion (mL/min/100g)		50 (13)	52 (13)	0.81
Gastroc TTP perfusion (s)		20 (6)	18 (9)	0.5
Soleus peak perfusion (mL/min/100g)		42 (17)	44 (19)	0.31
Soleus TTP perfusion (s)		14 (6)	15 (7)	1
Whole-leg peak perfusion (mL/min/100g)		40 (12)	41 (12)	0.81
Whole-leg TTP perfusion (s)		13 (7)	16 (7)	0.25
SvO₂				
Tibioperoneal trunk baseline SvO ₂ (%HbO ₂)	71 (9)	72 (14)		1
Tibioperoneal trunk SvO ₂ washout time (s)	8 (3)	8(0)		0.63
Peroneal vein baseline SvO ₂ (%HbO ₂)		63 (7)	65 (7)	0.25
Peroneal vein SvO ₂ washout time (s)		16 (7)	23 (15)	0.25
T₂[*]				
Soleus muscle relative T ₂ [*] _{max} (%)		107 (3)	107 (3)	0.44
Soleus muscle TTP T ₂ [*] (s)		30 (7)	30 (8)	1

Table 3

Synopsis of ν PIVOT-measured responses during the dynamic exercise protocol, reported as mean (standard deviation).

	Macrovascular	Microvascular	<i>P</i> value
Baseline $\dot{V}O_2$ (mL O_2 /min/100g)	1.2 (0.4)	0.6 (0.3) *	< 0.05
Maximum flow (mL/min/100g)	185 (72)	88 (33) *	< 0.01
TTP flow (s)	8 (3)	101 (53) *	< 0.01
Peak $\dot{V}O_2$ (mL O_2 /min/100g)	15.2 (6.7)	6.0 (1.9) *	< 0.01
TTP $\dot{V}O_2$ (s)	12 (7)	102 (37) *	< 0.01
Net $\dot{V}O_2$ (mL O_2 /100g)	20.6 (9.7)	22.3 (13.5)	0.84

* indicates presence of a significant difference ($P < 0.05$) between macrovascular (arterial blood flow) and microvascular (perfusion) responses.