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Measurement of Skeletal Muscle Perfusion Dynamics With Pseudo-Continuous Arterial Spin Labeling (pCASL): Assessment of Relative Labeling Efficiency at Rest and During Hyperemia, and Comparison to Pulsed Arterial Spin Labeling (PASL)

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Abstract

Purpose—To compare calf skeletal muscle perfusion measured with pulsed arterial spin labeling (PASL) and pseudocontinuous arterial spin labeling (pCASL) methods, and to assess the variability of pCASL labeling efficiency in the popliteal artery throughout an ischemia-reperfusion paradigm.

Materials and Methods—At 3T, relative pCASL labeling efficiency was experimentally assessed in five subjects by measuring the signal intensity of blood in the popliteal artery just distal to the labeling plane immediately following pCASL labeling or control preparation pulses, or without any preparation pulses throughout separate ischemia-reperfusion paradigms. The relative label and control efficiencies were determined during baseline, hyperemia, and recovery. In a separate cohort of 10 subjects, pCASL and PASL sequences were used to measure reactive hyperemia perfusion dynamics.

Results—Calculated pCASL labeling and control efficiencies did not differ significantly between baseline and hyperemia or between hyperemia and recovery periods. Relative to the average baseline, pCASL label efficiency was $2 \pm 9\%$ lower during hyperemia. Perfusion dynamics measured with pCASL and PASL did not differ significantly ($P > 0.05$). Average leg muscle peak perfusion was 47 ± 20 mL/min/100g or 50 ± 12 mL/min/100g, and time to peak perfusion was 25 ± 3 seconds and 25 ± 7 seconds from pCASL and PASL data, respectively. Differences of further metrics parameterizing the perfusion time course were not significant between pCASL and PASL measurements ($P > 0.05$).

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Conclusion—No change in pCASL labeling efficiency was detected despite the almost 10-fold increase in average blood flow velocity in the popliteal artery. pCASL and PASL provide precise and consistent measurement of skeletal muscle reactive hyperemia perfusion dynamics.

Studies have shown that the dynamics of skeletal muscle perfusion can provide insight into endothelial function and vascular reactivity in normal physiology and in pathophysiologic states.^{1–4} Perfusion is a tightly regulated process, serving to provide oxygen and nutrients to active tissue, and to remove waste products. Because metabolic demand of resting skeletal muscle is quite low, skeletal muscle perfusion at baseline is generally maintained even in situations of disease such as peripheral artery disease (PAD).⁵ However, patients with PAD do manifest altered vascular reactivity.⁶ These functional deficits of the vasculature can be evaluated by monitoring the kinetics of the response to a situation of increased flow demand, akin to cardiac stress testing. In skeletal muscle, this can be accomplished by measuring the response during or following exercise (functional hyperemia),^{7–9} or following induced ischemia (reactive hyperemia).^{7,10,11} Functional vascular impairment manifests as a blunting and delay of reperfusion in response to the stressor.¹ Even though it is not physiologically analogous to the demands of daily living, reactive hyperemia provides a more reproducible and subject effort-independent stimulus compared to functional hyperemia, and thus has been purported as the preferred stressor for studying peripheral vascular function.¹²

Arterial spin labeling (ASL) permits the measurement of perfusion in physiologic units.^{13,14} While several ASL labeling schemes exist, they each work by taking the pairwise difference of two images—one with magnetically labeled blood (label) and one without (control).¹³ Tissue magnetization is measured with and without inversion of arterial blood water and after allowing enough time for the inverted or uninverted spins to perfuse the organ of interest. Subtraction between the label and control images cancels out the contribution from static tissue and yields a signal that is proportional to microvascular perfusion. This signal can be converted into perfusion in units of mL/min/100g through application of various models.^{7,11,15,16}

Models for perfusion quantification derive from the general solution to the Bloch equation for longitudinal magnetization including a term for inflow and outflow of blood, originally described by Detre et al¹³:

$$\frac{dM}{dt} = \frac{M_0 - M}{T_1} + f(M_a - M_v) \quad (1)$$

where M is the longitudinal magnetization of water in tissue, M_a is that of arterial blood

water, M_v is that of venous blood water ($M_v = \frac{M}{\lambda}$, where λ is the tissue partition coefficient), T_1 is the relaxation time of tissue, and f represents perfusion.

In pseudo-continuous ASL (pCASL), inflowing arterial blood is selectively inverted in the label condition through flow-driven adiabatic inversion, which uses small flip angle radiofrequency (RF) pulses along with a nonzero net gradient amplitude to drive spins moving perpendicularly through the labeling plane to the negative Z axis. In the control

condition, spins remain uninverted through application of similar RF pulses (but with 180° phase shift between adjacent pulses) and zero net gradient amplitude to control for magnetization transfer effects.¹⁷ Assuming a one-compartment model for blood (eg, blood water is delivered and accumulates in tissue), pCASL perfusion can be quantified as described by Alsop et al¹⁶ as:

$$f = \frac{\lambda \cdot (M_{\text{control}} - M_{\text{label}}) \cdot \frac{\text{PLD}}{e^{T_{1,\text{blood}}}}} {2 \cdot \alpha \cdot T_{1,\text{blood}} \cdot M_{\text{control}} \cdot \left(1 - e^{\frac{-\tau}{T_{1,\text{blood}}}}\right)} \quad (2)$$

where *PLD* represents the post labeling delay—the time between the end of the labeling period and image acquisition, $T_{1,\text{blood}}$ is the longitudinal relaxation time of blood, α is the labeling efficiency, and τ is the labeling duration.

An alternate form of this model can be used to quantify perfusion using a pulsed ASL (PASL) sequence. The PASL method employed for this study is a flow-alternating inversion recovery variant developed by Raynaud et al⁷ in which slice-selective and nonselective adiabatic inversion RF pulses are used for label and control conditions, respectively. Using a similar one-compartment model and assuming the T_1 of blood and tissue can be approximated as equal, perfusion can be quantified as:

$$f = \frac{\lambda}{\text{PLD}} \cdot \ln \left[\frac{M_{\text{label}} - M_{\text{control}}}{M_{\text{label}} + M_{\text{control}}} \cdot \left(1 - e^{\frac{\text{PLD}}{T_{1,\text{blood}}}}\right) + 1 \right] \quad (3)$$

Skeletal muscle perfusion during hyperemia has been quantified with PASL,⁷ continuous ASL (CASL),¹¹ or, more recently, pCASL.² Each of these methods has benefits and drawbacks. PASL uses a single RF pulse for nearly instantaneous and complete inversion,¹⁸ therefore perfusion can be measured at a temporal resolution of up to 2 seconds.⁷ On the other hand, pCASL and CASL utilize a series of pulses to achieve inversion, thereby reducing labeling efficiency¹⁷ and limiting temporal resolution to 6–8 seconds.^{1,2,11} CASL or pCASL methods do offer a theoretical increase in signal-to-noise ratio (SNR) compared to PASL as the labeled blood water spins accumulate in the tissue throughout the longer labeling duration.¹⁹

Both CASL and pCASL rely on flow-driven adiabatic inversion to label arterial blood.^{13,17} To achieve efficient flow-driven adiabatic inversion, blood flow velocity (v) must satisfy the

adiabatic condition: $\frac{1}{T_2} \ll \frac{Gv}{|B_1|} \ll \gamma|B_1|$, where T_2 is the transverse relaxation time of oxygenated blood, G is the average gradient amplitude, B_1 is the applied RF magnetic field, and γ is the proton gyromagnetic ratio. Flow-driven adiabatic inversion will invert spins that satisfy this condition. However, if blood flow velocity is too slow, transverse relaxation effects dominate, and blood will not be inverted. On the other hand, if flow is too fast, the effective frequency sweep rate will be too fast and the magnetization will not be inverted. Blood flow velocity, therefore, must remain in a specific range in order to achieve efficient

flow-driven adiabatic inversion.²⁰ As a specific example, given $T_2 = 122$ msec (for hematocrit of 0.44 and oxygen saturation of 99% at 3T²¹), $G = 1$ mT/m, and $B_1 = 1.7$ mT, arterial blood velocity would have to remain well within the range of 1.4 cm/s to 77.3 cm/s for efficient inversion.

In contrast to the arteries supplying the brain, the peripheral circulation has higher impedance, resulting in a distinctive flow waveform.^{22–25} Specifically in the popliteal artery, which supplies muscles in the calf, the blood flow waveform is triphasic at baseline with antegrade flow during systole followed by retrograde then antegrade flow during diastole.^{22–24} During reactive hyperemia, the flow waveform becomes entirely antegrade due to vasodilation of capillaries to accommodate increased blood flow demand downstream.²⁵ Beyond the changes in the waveform characteristics, average blood flow velocity differs substantially between baseline, ischemia, and reactive hyperemia. This variability may result in blood flow velocity-dependent changes in labeling efficiency for pCASL and CASL.

Theoretical explorations of the dependence of blood flow velocity on labeling efficiency show that with decreasing peak flow there is a decrease in labeling efficiency.^{14,17,20} These simulations have been corroborated by experimental investigations of tagging efficiency performed in the brain.²⁶ However, those results may not apply to muscle perfusion, due to the different characteristics of the blood flow waveform in the peripheral vasculature.

Prior studies investigating muscle perfusion dynamics with PASL and pCASL or CASL have yielded disparate results, wherein peak perfusion measured with a pCASL² or CASL¹ method was much higher than that measured by PASL.^{7,27} Current hardware limitations make implementation of CASL on clinical imaging systems difficult. Thus, the purpose of this study was to experimentally investigate pCASL label efficiency during an ischemia reperfusion paradigm in the peripheral vasculature, and to compare muscle perfusion dynamics measured using pCASL and PASL.

Materials and Methods

The Institutional Review Board of the University of Pennsylvania approved all aspects of the study. Fifteen healthy subjects were recruited to participate after providing written, informed consent. Five healthy subjects (33 ± 7 years old, four male) participated in the pCASL labeling efficiency experiments, and 10 healthy subjects (60 ± 4 years old, seven females) were scanned with both PASL and pCASL sequences. All subjects were normotensive, nondiabetic, and none had a history of claudication. For each subject, all experiments were performed on the same day, within the same scan session.

Imaging was performed at 3.0T (Siemens Magnetom Tim Trio, Siemens Medical Equipment, Erlangen, Germany) with an eight-channel transmit/receive knee coil (InVivo, Gainesville, FL). Experimental imaging sequences were written in SequenceTree.²⁸ Reactive hyperemia was induced for both sets of experiments using an ischemia-reperfusion paradigm consisting of 3–5 minutes of proximal arterial occlusion via a pneumatic cuff secured around the thigh. The cuff was rapidly inflated to 75 mmHg above each subject's systolic blood pressure using the Hokanson E20 AG101 Rapid Cuff Inflation System (D.E.

Hokanson, Bellevue, WA). Data analysis was performed with in-house-written software using MatLab (MathWorks, Natick, MA), and statistical analyses were executed with JMP (SAS Institute, Cary, NC, 1989–2007).

pCASL Labeling Efficiency During Reactive Hyperemia

First we aimed to assess whether pCASL labeling efficiency varied over the course of an ischemia reperfusion paradigm. By measuring the signal intensity in the artery following pCASL labeling period, the inversion efficiency can be experimentally estimated.^{29,30} For the purpose of this study, precise quantification of the inversion efficiency was of less interest than its variation over the course of the reactive hyperemia paradigm.

To determine relative inversion efficiency, data were acquired with a modified pCASL sequence in which the imaging readout, from an imaging plane located just distal to the labeling plane, immediately followed the labeling or control period (Fig. 1A). Rather than measuring signal intensity in the muscle tissue, magnitude signal intensity was measured in the blood contained within the popliteal artery. Following the imaging readout, additional time was allotted for signal recovery and inflow of fresh spins in the artery.

The reactive hyperemia response is relatively short-lived,^{25,31} therefore temporal sampling of popliteal artery blood signal intensity was maximized by conducting three separate experiments: with EPI data acquired following pCASL label; following pCASL control; or without label or control preparations (termed “Reference”) (Fig. 1B). The duration of the label and control preparations were selected to match Wu et al’s prior study using CASL for skeletal muscle perfusion quantification.¹¹ For each scan, data were acquired during 1 minute baseline, 3 minutes ischemia, and 2 minutes recovery. Each subject received additional time to recover back to his or her relative baseline state between scans.

The modified pCASL sequences used the following parameters: for pCASL label or control experiments, labeling duration = 2000 msec, PLD = 1.6 msec, Hanning window-shaped pulses with average $B_1 = 1.7 \mu\text{T}$, pulse interval = 1 msec, $G_{\text{max}}/G_{\text{avg}} = 9/1 \text{ mT/m}$. The unbalanced control condition utilized average gradient = 0 mT/m and 180° phase shift between adjacent RF pulses. The labeling and control plane was located 20 mm superior from the imaging plane. The reference experiment had identical temporal sampling as the pCASL label and control experiments, but neither pCASL label nor control module was applied before the EPI readout. For all scans, an identical GRE-EPI readout was used for image acquisition with flip angle = 90° , field of view (FOV) = $20 \times 20 \text{ cm}^2$, slice thickness = 10 mm, acquired matrix = 80×50 (partial Fourier, reconstructed to 80×80), TR/TE = 4000/10 msec.

To assess relative changes in the labeling and control efficiencies, the average signal intensity in the popliteal artery during label and control acquisitions was first divided by that of the reference acquisition to account for T_2^* modulation throughout the ischemia-reperfusion paradigm. The corrected label and control time series were then normalized to their average baseline value to allow for investigation of the relative changes of label and control signal intensity, which is related to the label or control efficiency.

A fourth scan was conducted during which projection phase-contrast data were continuously acquired in the labeling plane in order to characterize the arterial velocity waveform throughout the ischemia-reperfusion paradigm. Projection phase-contrast data were acquired continuously throughout the same ischemia-reperfusion paradigm with 1 minute of baseline, 3 minutes of proximal arterial occlusion, and 2 minutes of recovery. Although the beat-to-beat variation in arterial velocity will not match between this acquisition and the previously described modified pCASL scans, average arterial velocity and the velocity range throughout the reactive hyperemia paradigms are repeatable.³² Acquisition parameters for the projection phase-contrast scan were: FOV = 176×176 mm, slice thickness = 5 mm, acquired matrix = 208×1 , VENC = 80–120 cm/s, TR/TE 5 24/6.5 msec. Fully phase encoded reference images were acquired immediately before and after the projection data with identical imaging parameters and acquired matrix = 208×208 to allow for quantification of velocity as described in.²⁵

Briefly, to quantify velocity a static tissue k -space projection was computed by first masking out the artery in the reference images, then taking the inverse Fourier transform and isolating the center k -space line ($k_y = 0$). Unavoidable motion ensues during the reactive hyperemia paradigm due to cuff inflation and deflation, which will cause an error if there is no direct anatomic correspondence between the static tissue k -space projection and the dynamic projections. To address this problem, the reference image acquired prior to the dynamic acquisition was used to create the pre-cuff static tissue k -space projection, and similarly the reference image acquired following the dynamic acquisition is used to create the post-cuff static tissue k -space projection. This static tissue k -space projection was subtracted from every dynamically acquired velocity-encoded projection to isolate blood signal.²⁵ Then the phase difference between adjacent positive and negative velocity-encoded projections was computed and the average velocity across the popliteal artery was quantified with 24 msec temporal resolution.

The time-resolved velocity and maximum velocity were recorded. Projection velocity data were averaged over one cardiac cycle, and downsampled to match the temporal resolution of the pCASL labeling scans. The average arterial blood flow velocity data were used to estimate the transit time between the labeling location and the imaging slice, which will differ between the baseline and hyperemic states. The difference in transit time will result in different amounts of T_1 recovery of the inverted blood signal. Inversion was assumed to occur at the location of the labeling plane, and pCASL label data were then corrected for the T_1 recovery. The arterial velocity data were also used to define the period of hyperemia for the modified pCASL scans. The hyperemia period was defined as the time during which the average arterial velocity was more than twice the baseline velocity.

Perfusion Quantification with pCASL and PASL

Next we aimed to compare perfusion dynamics measured during an ischemia-reperfusion paradigm with PASL and pCASL to assess whether significant differences existed between results obtained with the two methods. The reactive hyperemia perfusion data quantified with PASL had been published previously.³ Each subject underwent three ischemia-reperfusion paradigms consisting of 1 minute of baseline, 5 minutes of ischemia induced via

proximal arterial occlusion, and 6 minutes of postischemia recovery during a 1-hour scan session. In the first two ischemia-reperfusion scans, perfusion was quantified using a PASL variant, and in the third scan a pCASL sequence was used to measure perfusion (Fig. 1C).

A standard pCASL sequence as described by Alsop et al¹⁶ was implemented with the following parameters: Single-slice GREPI: acquisition matrix = 80×50 (partial Fourier, reconstructed to 80×80), FOV = $25 \times 25 \text{ cm}^2$, slice thickness = 10 mm, TR/TE = 4000/8.1 msec. Labeling duration and postlabeling delay matched values used in Wu et al's prior CASL muscle perfusion investigation,¹ with labeling duration = 2000 msec, postlabeling delay (PLD) = 1900 msec, Hanning window-shaped pulses with average $B_1 = 1.7 \text{ } \mu\text{T}$, pulse interval = 1 msec, $G_{\text{max}}/G_{\text{avg}} = 9/1 \text{ mT/m}$. Unbalanced control condition utilized average gradient = 0 mT/m and 180° phase shift between adjacent RF pulses. The labeling plane was located 60 mm superior to the imaging slice. Perfusion was quantified with temporal resolution of 8 seconds.

PASL data were acquired with the perfusion, intravascular venous oxygen saturation, and T_2^* (PIVOT) sequence,²⁷ an interleaved dual-slice PASL and multiecho GRE sequence. PIVOT employs the saturation inversion recovery PASL variant described by Raynaud et al⁷ for perfusion quantification. Notably, evaluation of PIVOT compared to PASL showed that no error was introduced by interleaving the acquisition of multiecho GRE data during the PLD.²⁷ Imaging readout for the PASL acquisition was identical to that employed in the pCASL acquisition. Slice-selective or nonselective adiabatic inversion via hyperbolic secant pulse was used for label and control condition, respectively, with labeling duration = 8 msec, PLD = 940 msec, and TR = 1000 msec. Temporal resolution of perfusion quantified with PASL was 2 seconds.

For both PASL and pCASL datasets, rigid-body motion correction was applied to the time-series of label and control images using National Institutes of Health ImageJ software (developed by Wayne Rasband; NIH, Bethesda, MD). One observer (E.E., 8 years of experience in processing of musculoskeletal MRI) performed all image analysis. Regions of interest (ROIs) were manually drawn on the EPI images using anatomical images with resolution of 0.83 mm by 0.83 mm as a reference for muscle boundaries (Fig. 2A). After masking out the large arteries, signal intensity was averaged in the gastrocnemius, soleus, and peroneus muscles, and in the anterior compartment, composed of the tibialis anterior and extensor digitorum longus muscles for label and control time series data. These individual muscle ROIs were also combined to determine the average muscle perfusion across the entire cross-section of the calf, referred to as "whole-leg." The EPI readout used for both PASL and pCASL sequences is T_2^* -weighted, resulting in appreciable change in the signal intensity of the images throughout the ischemia-reperfusion paradigm. Thus, without any correction for the temporal offset, subtraction between adjacent label and control images yields a difference that is unrelated to perfusion. To account for the temporal offset between the control and label image series, adjacent control data were averaged to yield a temporally matched control series.³³ Perfusion was computed from the ROI-averaged signal intensity in pairs of label and temporally matched control data according to the appropriate models (Eqs. 2 and 3) for each ASL method as described in the introduction with PLD = 1900 msec in pCASL, 940 msec in PASL, $T_{1,\text{blood}} \approx T_{1,\text{tissue}} = 1420 \text{ msec}$.³⁴ In

the pCASL perfusion model, α is the labeling efficiency (85%),¹⁷ τ is the labeling duration ($\tau = 2000$ msec).

Cuff inflation and deflation resulted in slight motion of the calf, thus the pair of label and control images acquired immediately after inflation or deflation were excluded from the time series. The baseline perfusion offset was calculated by averaging perfusion during the period of cuff ischemia,¹¹ and the offset was then subtracted from each timepoint. PASL and pCASL perfusion time courses were smoothed to minimize nonphysiologic noise using a three-timepoint sliding-window average. The peak perfusion, time to peak perfusion, hyperemic flow volume, and the hyperemic duration were identified and recorded from the perfusion time courses for each muscle as shown schematically in Fig. 2B.

Statistical Analysis

The pCASL efficiency scans were divided into four distinct intervals: baseline, ischemia, hyperemia, and recovery. To assess the relative pCASL label and control efficiencies, paired-sample Wilcoxon signed rank tests were used to test whether the average label or control efficiency differed between baseline and hyperemia, or between hyperemia and recovery.

To assess whether PASL and pCASL measured perfusion time courses differed, paired-sample Wilcoxon signed rank tests were used to compare the perfusion measurements acquired at two different times. Holm adjustment for multiple comparisons was applied to maintain the familywise error rate of 0.05 and $P_{holms} < 0.05$ was considered significant.

Results

pCASL Labeling Efficiency During Reactive Hyperemia

Figure 3A shows the temporally resolved and average velocity in a single representative subject throughout the ischemia reperfusion paradigm as measured in the popliteal artery at the location of the pCASL labeling plane. Velocity during the baseline period (Fig. 3B) exhibited the expected triphasic waveform, with antegrade flow during systole, followed by slight retrograde and antegrade flow during diastole. During the hyperemic period blood flow became monophasic, as it was antegrade during both systole and diastole (Fig. 3C). From baseline to peak hyperemia, the average blood velocity increased by ~10-fold and the peak velocity during systole roughly doubled. Across all subjects, the average baseline blood flow was 3.9 ± 0.9 cm/s, and the diastolic to systolic range was -13.7 ± 2.1 to 46.3 ± 5.4 cm/s. The average peak hyperemic blood flow was 34.1 ± 12.5 cm/s, with a range of 14.0 ± 6.4 to 87.6 ± 33.6 cm/s.

The average size of the popliteal artery ROI was 30 ± 7 mm². Figure 4 shows the average label efficiency with and without correction for the T_1 recovery during the transit time between the labeling plane and image slice location and the control efficiency, as well as the average blood flow velocity during the ischemia reperfusion paradigm. During baseline, hyperemia, and recovery, significant changes in relative control efficiency or label efficiency were not detected (Table 1).

pCASL Versus PASL

One subject was excluded from the analyses because the cuff-occlusion paradigm did not result in a reactive hyperemia response in pCASL data. Averaged over the remaining nine subjects, significant differences between the temporal dynamics of perfusion measured with PASL and pCASL were not observed in any muscle groups. The average ROI size for each muscle was $14.5 \pm 4.1 \text{ cm}^2$, $15.5 \pm 4.0 \text{ cm}^2$, $4.3 \pm 0.8 \text{ cm}^2$, and $7.5 \pm 1.8 \text{ cm}^2$ for the gastrocnemius, soleus, and peroneus muscles, and the anterior compartment, respectively. The ROI size for the whole-leg was $41.8 \pm 9.9 \text{ cm}^2$. Table 2 shows the results of the parameterized time-course data. Differences between PASL and pCASL or between the two PASL acquisitions were not significant for any of the perfusion time course metrics ($P_{\text{holms}} > 0.05$ in all cases). Perfusion time courses were calculated for each scan and were averaged across all subjects (Fig. 5). Qualitatively, the measured whole-leg perfusion dynamics are in agreement between pCASL and PASL. For visualization purposes, a perfusion map from a representative subject is shown for pCASL and PASL acquisitions during baseline, ischemia, and hyperemia (Fig. 6).

Discussion

Previous studies have investigated the impact of temporal variation of blood velocity on the measured perfusion in the brain.^{20,26,35,36} In the brain, all blood delivered through the internal carotid and vertebral arteries traverses the brain via capillary microcirculation. A simple method to experimentally measure pCASL labeling efficiency was reported by Aslan et al²⁶ in which total cerebral blood flow was measured in both the internal carotid and vertebral arteries using phase contrast MRI and was compared to whole-brain averaged blood flow measured by pCASL. If all other assumptions in the perfusion model are correct, the difference between total cerebral blood flow measured by phase contrast and pCASL can be accounted for by the labeling efficiency.

The approach utilized by Aslan et al²⁶ cannot be employed in the peripheral circulation for several reasons. First, blood flow in the popliteal artery is not exclusively delivered to the calf muscles. The popliteal artery branches to supply muscles of the foot, and also delivers blood to the skin, intramuscular fibrous tissue, bone, and other structures; thus, a precise and specific measure of muscle blood flow in a large artery is difficult. Furthermore, even if a distinct measure of total muscle blood flow in the feeding artery were achieved, the PASL and pCASL sequences employed in this study provided single-slice measurements therefore do not yield total muscle blood flow.

The methodology employed herein was not directed to measure absolute labeling efficiency, although a similar approach has been used to measure labeling efficiency in a phantom experiment,³⁰ and in an animal model.²⁹ The more pertinent question was to determine whether the relative labeling or control efficiencies were impacted by the substantial changes in average arterial velocity between rest and reactive hyperemia in skeletal muscle. As expected, the control condition did not significantly impact signal in the feeding artery, and control efficiency remained relatively constant throughout the paradigm. No significant change was detected in labeling efficiency during the period of hyperemia compared to the baseline and recovery periods, which remained at approximately the average baseline value.

The correction of the label signal intensity for T_1 recovery is a necessary step as the transit time between the labeling location and the imaging slice location differs substantially between the baseline and hyperemic states. For example, at an average blood flow velocity of ~4 cm/s at baseline it would take 0.5 seconds for the blood to traverse the 20 mm separation between the labeling plane and image slice location. If the blood water spins were 100% inverted at the labeling plane, this would account for ~26% signal recovery. However, during hyperemia, arterial velocity increases by an order of magnitude, and the blood would traverse the distance between the labeling and imaging locations in only 0.05 seconds, resulting in much less time for T_1 recovery (only about 3% signal recovery). Following correction for this effect, the differences in relative labeling efficiency between the baseline and hyperemia, or between hyperemia and recovery states, were not significant ($P > 0.05$). Of note, the risk of unlabeled blood entering the measurement slice is negligible, as flow velocity would have to exceed 1250 cm/s to travel more than 20 mm in the 1.6 msec delay between labeling and image acquisition.

Partial-volume effects due to the low resolution of the EPI readout would cause an underestimation of the calculated labeling efficiency. Given that the purpose of this study was to evaluate label and control efficiency variation between physiologic states, we opted to not calculate the actual control or label efficiency. Furthermore, the ratio between control and reference, or between label and reference acquisitions, was used to determine the relative efficiency rather than the more standard formulation of Label efficiency =

$$\frac{\text{Reference} - \text{Label}}{2 \cdot \text{Reference}} \times 100.$$
 Suppose, for instance, that the signal intensity in the artery during the label acquisition was small with respect to the reference acquisition. Defining the label efficiency as described above and then normalizing to the average baseline value would conceal the changes in the label signal intensity. Normalizing the label and control data to the reference, however, is necessary to correct for the underlying T_2^* changes.

The finding of relative stability of labeling efficiency is surprising, as studies in the brain have shown a significant decrease in labeling efficiency in response to a stimulus that increases arterial velocity.²⁶ Potentially, the periods of retrograde and low velocity antegrade blood flow during diastole at baseline may not substantially contribute to the downstream delivery of blood, and therefore may not impact the overall labeling efficiency. Rather than blood water spins traveling forward at a constant velocity of 5 cm/s, blood in the artery spurts forward at speeds approaching 40 cm/s, but only for the short systolic period. This hypothesis is supported by 4D flow measurements of blood flow in the peripheral vasculature³⁷ in which streamline analysis shows fast forward flow during systole and near stagnation during diastole.

A subset of the data included in this study has been published previously. Specifically, the repeated PASL measurements were used to investigate intrasession repeatability of perfusion dynamics and to compare perfusion dynamics between healthy individuals and patients with peripheral artery disease.³ In the present work, we sought to examine the agreement between skeletal muscle perfusion dynamics measured with PASL and pCASL. One subject was excluded from the present analysis since no hyperemic response was detected with pCASL.

In the nine subjects in whom perfusion was measured with PASL and pCASL, the quantified perfusion time course metrics did not significantly differ for any muscle group. The average perfusion time course was very similar in appearance between the two PASL scans, implying that there was little difference between the successive reactive hyperemia responses, and between either PASL scan and the pCASL scan, suggesting that the different methods for measuring perfusion may not impact the measurement precision.

Despite the differences between the employed PASL and pCASL methods in labeling method, labeling duration, postlabeling delay, and perfusion quantification model, the differences between the PASL and pCASL quantified time course metrics were not statistically significant for any muscle group ($P > 0.05$). Quantified reactive hyperemia peak perfusion was in approximate agreement with prior studies using the SATIR PASL variant.^{7,10,27} However, compared to Wu et al's results from a CASL sequence,¹ Grozinger et al's results using pCASL,² or Lopez et al's results using Q2TIPS PASL,¹² peak perfusion in all muscles was lower in the present study. This bias could be attributed to different postprocessing methods as we excluded macrovessels from the ROI, which would increase apparent perfusion if included. It is important to note that the exact same muscle ROIs were used for quantification of both PASL- and pCASL-measured perfusion in this study.

The present study is the first that we are aware of to directly compare skeletal muscle perfusion dynamics with PASL and pCASL sequences. Limitations of the study include the small sample size both for the label efficiency calculation study as well as the comparison of PASL and pCASL. In general, the results failed to reject the null hypothesis, but due to the small sample size this conclusion is susceptible to Type II error. Furthermore, no clinical patients were included in this evaluation of the sequences. Patients with impaired arterial flow such as those with peripheral artery disease may have reduced pCASL labeling due to the low blood flow and dampened pulsatility. Finally, although internal consistency was found between PASL and pCASL results, there was no independent reference standard to which our results could be compared. A prior study compared the PASL method employed herein to venous occlusion plethysmography and found good agreement.⁷ Nevertheless, the present data lend credence to the quantified perfusion time courses measured with PASL and pCASL.

In conclusion, the pCASL labeling efficiency was not impacted by the variation between baseline and reactive hyperemia arterial velocity. Time course metrics were similar for both PASL scans, and between both PASL scans and the pCASL acquisition. The benefit of increased temporal resolution with PASL, particularly during periods of nonlinear changes in the T_2^* -weighted EPI time course may outweigh the theoretical benefit of the greater SNR achievable with pCASL. Overall, reactive hyperemia perfusion dynamics could be measured with PASL and pCASL sequences, and differences between the quantified pCASL and PASL perfusion time courses were not significant.

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FIGURE 1.

(A) Diagram of measurement locations for both the pCASL labeling experiments (blue slice, located 2 cm distal from labeling plane) and perfusion quantification experiments (purple slice, located 6 cm distal from labeling plane). For both experiments, the location of the pCASL labeling plane was approximately at the level of the popliteal artery (gray slice). For PASL, slice-selective and nonselective inversion was used for label and control conditions, respectively, and the perfusion measurement slice remained at the same location as in pCASL (purple slice). (B) Data to estimate stability of pCASL efficiency were acquired from repeated ischemia-reperfusion paradigms in which the imaging readout immediately followed the control or label period, and were normalized by an image in which no labeling was conducted (reference acquisition-shown in the top line of B). A fourth scan was conducted to measure blood flow in the popliteal artery during the ischemia-reperfusion paradigm at the level of the labeling plane. (C) Timing diagram for PASL and pCASL perfusion quantification sequences. C and L refer to control (nonselective inversion) and label (slice-selective inversion) conditions, respectively for the PASL scan.

FIGURE 2.

(A) Anatomical image showing muscle locations and boundaries. (B) A schematic of the perfusion time course highlighting the extracted metrics. Gray box indicates period of ischemia. Reactive hyperemia ensues following deflation of the cuff. Metrics of the dynamic reactive hyperemia response include the peak perfusion, time to peak perfusion (TTP), hyperemic flow volume (HFV), and the hyperemic duration.

FIGURE 3.

(A) Temporally resolved (light red) and averaged over ~3 cardiac cycles (dark red) blood flow velocity in the popliteal artery in a representative subject. Baseline VENC was 80 cm/s and was raised to 120 cm/s at ~150 seconds into the experiment to account for the higher flow velocities during hyperemia. (B) Temporally resolved velocity from the baseline period in (A) shows the typical and expected triphasic waveform. (C) During reactive hyperemia, the blood flow is entirely antegrade, with forward flow throughout the cardiac cycle.

FIGURE 4.

Baseline-normalized control/reference and label/reference signals are plotted over the course of the ischemia reperfusion paradigm (gray box indicates period of arterial occlusion). Label/reference data are plotted both with (black) and without (gray) correction for T_1 recovery during the transit time between the labeling plane and the imaging slice location. The transit time was calculated as the separation distance (20 mm) divided by the average velocity (shown in red). Data are averaged across all subjects and error bars indicate standard error. Blood velocity was used to define the hyperemic period (indicated by the red box) as the time during which average blood velocity was at least more than double the average baseline velocity. Differences were not significant between the baseline and hyperemia periods, or between the hyperemia and recovery periods for control or label data.

FIGURE 5.

Perfusion time course averaged across nine subjects quantified with pCASL (purple), and two identical PASL scans (gray and black). Gray box indicates period of arterial occlusion. Error bars indicate standard error. The reactive hyperemia perfusion time course was not observed to differ significantly between PASL and pCASL measurements.

FIGURE 6.

Parametric maps of perfusion are shown for pCASL and PASL acquisitions. (A) Structural image and (B) sample EPI image are shown for reference. Baseline, ischemia, and hyperemia perfusion maps are shown for pCASL (C–E) and PASL (F–H). The voxelwise perfusion map was computed, smoothed, and averaged over 16 seconds of acquisition (two repeats for pCASL, eight repeats for PASL). The three feeding arteries of the leg are located centrally, where aberrant perfusion values are seen.

TABLE 1

Average (Standard Deviation) of Baseline-Normalized Control and Label Relative Signal Intensities During the Average Hyperemia and Recovery Periods

	Hyperemia		Recovery	
	Average (SD)	<i>P</i> -value	Average (SD)	<i>P</i> -value
Control/Reference (%)	100 (5)	1	99 (7)	0.8125
Label/Reference (%)	98 (9)	0.8125	105 (29)	0.625

Uncorrected *P*-values are reported from baseline value of 100% and hyperemia, or between hyperemia and recovery periods.

TABLE 2

Average Perfusion Time Course Metrics Measured With pCASL or PASL

	pCASL	PASL (scan 1)	PASL (scan 2)	P_{holms}
Gastroc Peak Perfusion (mL/min/100g)	41 (9)	51 (11)	52 (13)	0.508
Soleus Peak Perfusion (mL/min/100g)	50 (26)	78 (33)	69 (28)	0.620
Peroneus Peak Perfusion (mL/min/100g)	50 (6)	41 (12)	45 (12)	0.825
AC Peak Perfusion (mL/min/100g)	47 (13)	44 (16)	45 (14)	0.620
Whole-Leg Peak Perfusion (mL/min/100g)	47 (20)	51 (12)	50 (12)	0.998
Gastroc TTP Perfusion (s)	23 (3)	33 (9)	30 (8)	1.000
Soleus TTP Perfusion (s)	28(8)	30 (12)	22 (7)	1.000
Peroneus TTP Perfusion (s)	26 (5)	27 (10)	24 (7)	1.000
AC TTP Perfusion (s)	23 (3)	20 (8)	18 (7)	1.000
Leg TTP Perfusion (s)	25 (3)	27 (7)	23 (7)	0.795
Gastroc HFV (mL/100g)	33 (10)	42 (13)	37 (14)	0.943
Soleus HFV (mL/100g)	39 (23)	70 (35)	52 (24)	1.000
Peroneus HFV (mL/100g)	29 (10)	23 (8)	20 (7)	0.973
AC HFV (mL/100g)	25 (11)	22 (10)	18 (5)	1.000
Leg HFV (mL/100g)	33 (15)	42 (12)	38 (13)	0.986
Gastroc HD (s)	82 (21)	88 (14)	90 (21)	0.458
Soleus HD (s)	80 (29)	101 (35)	102 (28)	0.458
Peroneus HD (s)	73 (22)	68 (17)	65 (26)	1.000
AC HD (s)	65 (45)	71 (25)	63 (20)	1.000
Whole-Leg HD (s)	77 (19)	99 (22)	105 (29)	0.913

Two identical PASL scans were conducted to assess repeatability of the measured hyperemic response. Results shown are average (standard deviation). Reported P -value is for comparison between PASL and pCASL results. Gastroc, gastrocnemius muscle; AC, anterior compartment; TTP, time to peak; HFV, hyperemic flow volume; HD, hyperemic duration.