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Hyperemic flow heterogeneity within the calf, foot, and forearm measured with continuous arterial spin labeling MRI

Wen-Chau Wu^{1,6}, Jiongjiang Wang¹, John A. Detre^{1,2}, Felix W. Wehrli¹, Emile Mohler 3rd³, Sarah J. Ratcliffe⁴, and Thomas F. Floyd^{2,5}

¹Department of Radiology, The Hospital of University of Pennsylvania, Philadelphia, Pennsylvania

²Department of Neurology, The Hospital of University of Pennsylvania, Philadelphia, Pennsylvania

³Department of Medicine, The Hospital of University of Pennsylvania, Philadelphia, Pennsylvania

⁴Department of Biostatistics and Epidemiology, The Hospital of University of Pennsylvania, Philadelphia, Pennsylvania

⁵Department of Anesthesiology and Critical Care, The Hospital of University of Pennsylvania, Philadelphia, Pennsylvania

⁶Graduate Institute of Clinical Medicine, National Taiwan University, Taipei, Taiwan

Abstract

Arterial spin labeling (ASL) is a noninvasive magnetic resonance imaging (MRI) technique for microvascular blood flow measurement. We used a continuous ASL scheme (CASL) to investigate the hyperemic flow difference between major muscle groups in human extremities. Twenty-four healthy subjects with no evidence of vascular disease were recruited. MRI was conducted on a 3.0 Tesla Siemens Trio whole body system with a transmit/receive knee coil. A nonmagnetic orthopedic tourniquet system was used to create a 5-min period of ischemia followed by a period of hyperemic flow (occlusion pressure = 250 mmHg). CASL imaging, lasting from 2 min before cuff inflation to 3 min after cuff deflation, was performed on the midcalf, midfoot, and midforearm in separate sessions from which blood flow was quantified with an effective temporal resolution of 16 s. When muscles in the same anatomic location were compared, hyperemic flow was found to be significantly higher in the compartments containing muscles known to have relatively higher slow-twitch type I fiber compositions, such as the soleus muscle in the calf and the extensors in the forearm. In the foot, the plantar flexors exhibited a slightly delayed hyperemic response relative to that of the dorsal compartment, but no between-group flow difference was observed. These results demonstrate that CASL is sensitive to flow heterogeneity between diverse muscle groups and that nonuniform hyperemic flow patterns following an ischemic paradigm correlate with relative fiber-type predominance.

Keywords

skeletal muscle; magnetic resonance imaging

PREVIOUS STUDIES IN nondiseased skeletal muscle have shown that blood supply is not uniformly distributed between muscle groups during exercise (19,21,22). Variability in muscle fiber populations results in different metabolic (43) and microvascular profiles (14,16,46). These differences may impact muscle flow responses to ischemia and exercise. Muscles composed principally of highly oxidative red fibers are reported to have larger maximal flow responses after contraction than muscles with a relatively higher percentage of glycolytic white fibers (23,27). Consequently, flow measurements made for a physiological and diagnostic purpose using whole limb dilution techniques are unable to resolve physiological or pathophysiological effects in specific muscle groups.

Measures of whole extremity blood flow have been achieved with plethysmography (38), thermodilution (29,31), and Doppler ultrasound (42). An assessment of regional microvascular flow (or perfusion) is more complicated, however, and there remains no gold standard. Published methods include sestamibi (45), thallium (25), positron emission tomography (6,32), contrast-enhanced magnetic resonance (MR) imaging (MRI) (26), Doppler ultrasound (5,11), and optical methods (34). The ability to interrogate microvascular function and integrity may yield important physiological and clinical diagnostic information in various disease states including diabetes, atherosclerosis, myopathies, muscular dystrophies, and skeletal muscle tumors.

The measurement of skeletal muscle blood flow at rest is challenging because the resting flow rate is very low ($<5 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$) (41) and below the threshold of detection for most measurement approaches. Furthermore, resting blood flow appears to be well preserved even in the presence of disease (6). Blood flow after exercise or following a period of ischemia, the hyperemic flow response, is severalfold higher than resting flow and is more easily measurable in both physiological and pathophysiological investigations (26,35).

Arterial spin labeling (ASL) (9) is a noninvasive MRI technique for perfusion measurement and has been widely used in the brain. ASL makes use of the protons in arterial blood as endogenous tracers, which are magnetically labeled by radio frequency pulses' yielding quantitative measurements with temporal and spatial resolution on the order of seconds and millimeters, respectively. Few groups have adapted this technique to study the blood flow in skeletal muscle, and to date, studies investigating the spatial flow difference between muscle groups have been very limited (4,12,24). Hyperemic flow paradigms, when combined with ultrasound or the measurement of systolic pressure indexes, have been used extensively in the diagnosis of peripheral vascular disease in conduit vessels. The same paradigm combined with ASL may enable the diagnosis of early microvascular disease within muscle, such as occurs in diabetes mellitus. To further such work, it is important to characterize differences in microvascular hyperemic flow between muscle groups in a disease-free population.

In the present work, we tested the hypothesis that heterogeneity in hyperemic flow exists between large muscles or between functional muscle groups exposed to an ischemic stimulus using a continuous scheme for ASL (CASL). Specifically, we investigated the hyperemic flow difference between two well-studied calf muscles, the soleus (Sol) and gastrocnemius, considering that the former is composed of a relatively higher proportion of slow-oxidative red fibers, whereas the latter is populated with a greater fraction of fast-glycolytic white fibers (10,13). We then used CASL to systematically study hyperemic perfusion and its differences between major muscle groups in the calf, foot, and forearm.

MATERIALS AND METHODS

MR experiments

Twenty-four healthy subjects (ages 25–49 yr; 11 women and 13 men) were scanned after Institutional Review Board approval and appropriate consent were obtained. None of the subjects had evidence of vascular disease as assessed by medical history and noninvasive vascular systolic pressure indexes or the San Diego Claudication Questionnaire. The subjects were instructed to avoid intense exercise 2 h before the experiment, and none had endurance or resistance training within 6 mo before the recruitment. Studies were conducted on a 3.0 Tesla Siemens Trio whole body MR system (Erlangen, Germany) with a custom-designed dual-tuned proton/phosphorous transmit/receive knee coil (Nova Medical, Wakefield, MA).

Imaging was performed on the midcalf ($n = 19$), midfoot ($n = 9$), and midforearm ($n = 15$) in separate sessions. The subjects were placed supine with the knee slightly flexed for studies of the midcalf and transmetatarsal. The subjects were placed prone in a swimming position for the midforearm imaging. Extremities were positioned within the coil and fixed in position with a VacFix vacuum-cushion bead bag (PAR Scientific A/S, Houston, TX). The midcalf, midforearm, and midfoot or transmetatarsal cross sections were the anatomic targets. For each anatomic location, the target slice was determined in two steps. Following a scout scan composed of three orthogonal image slices, a second scout scan was performed to obtain five axial slices. Among these, the image that best distinguished different muscle groups was chosen for functional perfusion imaging. Perfusion-weighted images were obtained using a single-slice version of the CASL sequence (2) with a single-shot gradient-echo echoplanar readout [field of view (FOV), 22 cm; in-plane matrix size, 64×64 ; slice thickness, 1 cm; repetition time (TR), 4 s; echo time (TE), 17 ms; and flip angle, 90°]. The labeling plane was 6 cm proximal to the imaging slice. The tagging duration equaled 2 s, and the postlabeling delay (PLD) equaled 1,900 ms. For the control scan, the inversion plane was placed 6 cm distal to the imaging slice to compensate for the magnetization transfer effect (2).

Considering that exercise may preferentially stimulate certain muscle groups and complicate flow comparisons between them, we elected to employ a brief ischemic-hyperemic paradigm to more uniformly challenge all muscle groups. This approach is used extensively in the diagnosis of vascular disease since it may also be applied to nonambulatory patients. Temporary occlusion was achieved with a Zimmer 1000 (Warsaw, IN) nonmagnetic orthopedic tourniquet system, with the cuff placed on the thigh or upper arm to create a 5-min period of ischemia followed by a period of hyperemic flow. The occlusion pressure was 250 mmHg. CASL imaging commenced 2 min before cuff inflation and ended 3 min after cuff deflation. A two-dimensional spoiled gradient-echo sequence (TR/TE, 50/3.4 ms; FOV, 220 mm; in-plane matrix size, 256×256 ; and flip angle, 50° ; 4 averages) was then used to acquire a high-resolution anatomic image of the slice where CASL imaging was performed.

Data processing and analysis

Subject motion during the scan was retrospectively corrected using the built-in motion correction function of the scanner. Complex data were then reconstructed to yield magnitude images, which were exported to a workstation for analysis using Voxbo (<http://www.voxbo.org/>) and custom-programmed scripts in IDL (RSI, Boulder, CO). Signals for CASL were generated by the pairwise subtraction of tag and control images. After subtraction, two adjacent data points in time were averaged due to gradient cycling, resulting in an effective temporal resolution of 16 s. The CASL signals were then converted

to quantitative flow following the model in reference (44), assuming $T_1 = 1.6$ s and $T_2^* = 100$ ms for arterial blood at 3.0 Tesla.

The high-resolution spoiled gradient-echo anatomic image was used to hand draw regions of interest (ROIs) for six muscle groups in the calf and to define flexor and extensor ROIs within the forearm and foot (Fig. 1). ROIs in the calf included muscles within the anterior, lateral, deep posterior, and superficial posterior compartments. The anterior compartment (AC) contains extensor muscles including the tibialis anterior, extensor digitorum longus, extensor hallucis longus, and peroneus muscles. The lateral compartment (LC) contains the peroneus longus and peroneus brevis muscles. The deep posterior compartment (DPC) contains the flexor digitorum longus, tibialis posterior, flexor hallucis longus, and popliteus. The posterior compartment contains the Sol and medial and lateral gastrocnemius muscles. Muscles within each compartment of the leg were grouped together in the analysis except within the posterior compartment, where the Sol, medial (Gstrc-M), and lateral (Gstrc-L) gastrocnemius muscles could be easily discerned. Within the forearm, the forearm flexors (F-Flx) were analyzed as a group and included the flexor carpi radialis, flexor carpi ulnaris, flexor digitorum (superficial and profundus), flexor pollicis longus, and palmaris longus muscles. The forearm extensors (F-Ext) were also analyzed as a group and included the extensor carpi radialis, extensor carpi ulnaris, extensor digitorum, extensor digit minimi, and brachioradialis and abductor extensor pollicis and pollicis longus muscles. In the foot, the dorsal group (D-Ext) analyzed consisted primarily of the interosseus muscles. The plantar flexor (P-Flx) group analyzed, also in the foot, included the flexor digitorum brevis, abductor hallucis, abductor digiti minimi, flexor digiti minimi, flexor hallucis, quadratus plantae, and lumbricale muscles. Any vessels visually discernible on the anatomic image were excluded from analysis.

For each muscle group, five indexes were computed, and their definitions are described as follows: 1) hyperemic period (in s): after cuff deflation, the period measured from the time when flow increases above the baseline (t_{beg}) to the time when flow returns to the baseline (t_{end}). Here the baseline refers to the average flow before cuff inflation; 2) peak hyperemic flow (in $\text{ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$): the peak flow observed during hyperemia; 3) mean hyperemic flow (in $\text{ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$): the mean flow during the hyperemic period; 4) hyper-emic flow volume (in $\text{ml}/100 \text{ g}$): the integrated flow over the hyperemic period; and 5) time to peak (in s): measured from t_{beg} to the time when hyperemic flow peaks.

For flow calculation, we adopted a single-compartment model, assuming that the tagged spins arriving in the capillary bed did not diffuse into interstitial space by the time of data acquisition. To estimate the error from this approximation requires a priori knowledge of exchange time (T_{ex} , the water molecules stay in the capillary bed before entering interstitial space), which is usually unknown. Previous studies have shown that water exchange between intra- and extravascular compartments accounts for a minor error relative to other factors such as transit delay and slice profile (7). The error originating from the different relaxation times in the two compartments has been reported with detailed numerical simulation and is beyond the scope of this study. Yet, to estimate the effect of different relaxation times between muscle groups on flow quantification, we conducted the calculation described below. The tagged water diffuses into extravascular space with a delay time after entering the capillary bed with a finite exchange rate. Therefore, a fraction of tags may reside in the vessel during image acquisition, whereas the rest exit the vascular and enter the tissue compartment. For simplicity, we assume that there are two discrete populations of tags; those exiting the vessel do so immediately after having entered the capillary bed, i.e., $T_{\text{ex}} = 0$, yielding for the difference in magnetization between tag and control:

$$\Delta M_{\text{ex}} = 2\alpha \frac{M_{0b}}{\lambda} f \exp\left(\frac{-\Delta t}{T_{1b}}\right) T_{1t} \left[\exp\left(\frac{-\text{PLD} + \Delta t}{T_{1t}}\right) - \exp\left(\frac{-\text{PLD} + \Delta t - \tau}{T_{1t}}\right) \right] \quad (1)$$

where ΔM_{ex} is the ASL signal from the extravascular compartment, M_{0b} is the longitudinal magnetization of arterial blood at thermal equilibrium, λ is the blood/tissue partition coefficient, α is the tagging efficiency, T_{1b} and T_{1t} are the longitudinal relaxation time of arterial blood and tissue, respectively. τ is the tagging duration, and Δt is the transit delay from the tagging region to the imaging region.

For the second population of tags, $T_{\text{ex}} = \infty$, i.e., they never leave the vascular compartment:

$$\Delta M_{\text{in}} = 2\alpha \frac{M_{0b}}{\lambda} f T_{1b} \left[\exp\left(-\frac{\text{PLD}}{T_{1b}}\right) - \exp\left(-\frac{\text{PLD} + \tau}{T_{1b}}\right) \right] \quad (2)$$

where ΔM_{in} is the ASL signal from the intravascular compartment. The overall ASL signal (ΔM) is the weighted sum of the signals from the two fractions, i.e.,

$$\Delta M = (1 - \xi) \Delta M_{\text{in}} + \xi \Delta M_{\text{ex}} \quad (3)$$

where $0 \leq \xi \leq 1$. For a derivation of Eqs. 1 and 2, see Appendix.

For a given flow (f_{th}), ΔM can be generated by Eqs. 1-3 and then used to calculate the flow based on the single compartment model (f_{cal}). To evaluate the quantitative flow variability originating from the model and varying tissue T_1 relaxation times (T_{1t}), we conducted the calculation for the midcalf for a range of T_{1t} (1,430–1,490 ms; according to our unpublished data, $T_{1t} = 1,492, 1,445$, and $1,434$ ms for the deep flexor, Sol muscle, and gastrocnemius, respectively) and reported the results as apparent flow (f_{app}):

$$f_{\text{app}} = \frac{f_{\text{cal}}}{f_{\text{th}}} \quad (4)$$

PLD = 2 s and $\tau = 2$ s. We assumed that $\Delta t = 1$ s, $f_{\text{th}} = 60 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$, and $T_{1b} = 1.6$ s.

Statistical analysis

Flow measures between the extensor and flexor groups within the forearm and within the foot were tested for significant differences using repeated-measures ANOVA, where muscle type was used to define the repeated measurements for each subject. In the calf, a linear mixed-effects regression model (17) was conducted to test the heterogeneity between the six muscle groups. This method is similar to a repeated-measures ANOVA, with adjustment for the subject-dependent correlation between multiple measurements (random effects); however, all available measurements from each subject can be utilized in the analysis, not just the subjects with complete measurements on all muscle groups. The mixed-effects model was used to test the dependence of each flow parameter (peak flow, mean flow, etc.) on a muscle group (fixed effect), having adjusted for the subject-dependent correlation. Utilizing the repeated-measures design of this study for within-subject ROI comparisons afforded a more statistically powerful comparison of population ROI means than that obtained by standard ANOVA. Lastly, given the fact that muscle fiber populations have

been well described for the gastrocnemius muscle, the regression model was constructed so that for each muscle group comparisons were made between that group and the gastrocnemius muscle reference group.

RESULTS

Figure 1 depicts the mean hyperemic flow image (Fig. 1D) with ROIs for the midcalf shown in color (Fig. 1C). The relatively greater flow within the Sol is easily discernable. The flow measurements and results of the linear mixed model analysis are summarized in Tables 1 and 2, respectively. The muscle group was found to have a significant effect on the dependent variable peak flow ($P < 0.0001$), mean hyperemic flow ($P < 0.0001$), and hyperemic flow volume ($P < 0.0001$) but was not important in determining the time-to-peak ($P = 0.76$) or hyperemic ($P = 0.74$) period (not listed in Table 2). When individual muscle groups were addressed, DPC, Sol, and LC were consistently and significantly different from Gstrc-L in peak flow, mean hyperemic flow, and hyperemic flow volume measurements. The Sol and DPC regions had flow measurements considerably in excess of all other groups studied. Gstrc-M and Gstrc-L were not statistically different across any of the flow indexes measured. The flow measurements and results of repeated-measures ANOVA in the foot and forearm are summarized in Table 3. For the foot, only the time to peak was significantly different between muscle groups. In the forearm, there was a marked difference in flow between F-Flx and F-Ext muscle groups in all flow measurements except for time to peak.

Figure 2 shows the temporal evolution of flow in the forearm and foot. In the forearm, peak flow, mean hyperemic flow, and hyperemic flow volume are significantly larger in the F-Ext. In the foot, the P-Flx exhibited a delayed hyperemic response compared with that of the D-Ext. Figure 3 shows the flow-time curves obtained from the more superficial muscles in the midcalf. A high similarity is found in the temporal evolution between the Gstrc-L and Gstrc-M and between the AC and LC regions. By comparison, deep muscles exhibit significantly higher peak hyperemic flow at $\sim 100 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ in the DPC and Sol muscle groups, as opposed to $< 80 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ in the more superficial muscles (Fig. 4). It is also noted that the DPC has higher resting flow than other muscles and a slightly delayed hyperemic response. Slightly different peak flow values are shown in the figures and in Table 1 because time to peak varies between subjects.

Figure 5 shows the numerical estimates of the variability due to T_1 differences between muscle groups and the uncertainty in the degree of water extraction. By the use of a single compartment model (assuming blood to remain in the vascular compartment), flow is underestimated when the tagged water molecules are allowed to diffuse into the extravascular space. The T_1 -related quantitative variability increases with increased water extraction, reaching 5% in the most extreme case.

DISCUSSION

Few investigations have employed ASL to study skeletal muscle perfusion (4,12,24), and despite evidence suggesting nonuniformity of blood supply (37,43), little attention has been paid to the heterogeneity between muscle groups, especially following an ischemic paradigm. The results of this study suggest that there is significant heterogeneity between muscle groups in the hyperemic response to limb ischemia as measured by ASL.

The accuracy of ASL-based perfusion measurements principally relies on two factors. One is the PLD, the time between spin labeling and data acquisition. If the PLD is too short, the label remaining in large vessels leads to regional flow overestimation, whereas the signal-to-noise ratio can be compromised if the PLD is too long. Our previous study suggested that

arterial transit times in the skeletal muscle may be prolonged and that venous outflow of unextracted label during hyperemia occurs (48). These confounds can be minimized by using a relatively long PLD (1,900 ms) to maximize inflow along with a relatively long TR (4 s) to allow unextracted label to fully decay. These parameters were employed in the present study.

The calculated flow rates further depend on the relaxation times (T_1 and T_2^*) used in the quantitative model. To distinguish the flow heterogeneity between muscle groups, the quantitative variability, as a consequence of T_1 and T_2^* discrepancy, must be smaller than the actual flow variability between muscles. To estimate whether this condition is met, we conducted computer simulations to assess the maximum T_1 -related flow variability. As shown in Fig. 5, T_1 variations in muscle groups are responsible for 5% variability if all tagged spins diffuse into the extravascular space. On the other hand, T_2^* variations ranging from 25 to 33 ms can cause errors as large as 10% (unpublished data). With the consideration that the vascular-extravascular exchange rate in skeletal muscle lies between 1 and 3 s⁻¹ (33), it is reasonable to expect the T_1/T_2^* related quantitative flow variability to be <10%. As a result, the observed differences in hyperemic flow rates between Sol, deep flexor, and superficial muscles as shown in Fig. 4 are unlikely to be due to differences in relaxation parameters between muscle groups. The description, however, does assume that the water extraction rate does not vary among muscle groups (Fig. 5), which is probably reasonable. Under these conditions, even though the single compartment model can underestimate flow by as much as 30%, such an error would not affect the relative flow differences between muscle groups.

Previous studies have suggested that the capillary supply to skeletal muscle is determined by its metabolic profile, oxidative capacity, and fiber size (40,43) and that feeding artery reactivity may be greater with type I than type II muscle (47,49), although the difference in capillary supply to slow- and fast-twitch fibers is less distinct in humans than in other mammals (36). Our data in the midcalf show that the Sol muscle has a significantly higher hyperemic flow response to a 5-min occlusion than the more superficial muscles, including the gastrocnemius. This observation is in agreement with another recent MR study comparing Sol and gastrocnemius muscles (26) and can be attributed to the fact that the Sol is composed of ~70–80% slow-twitch type I fibers with a higher capillarity and oxidative capacity, whereas the gastrocnemius is more evenly composed of type I (50–60%) and type II fibers (10,13). The flow pattern in the gastrocnemius muscle is less distinct from AC and LC than from the Sol muscle. Gregory et al. (15) reported that the percentage of type I fibers is similar in the anterior tibialis and Sol, whereas the succinic dehydrogenase activity is significantly higher in the Sol, which suggests that muscle oxidative capacity may also contribute to the flow difference between muscles. The DPC in the midcalf also exhibits a peak hyperemic flow comparable with that in the Sol muscle and a resting flow significantly higher than the value in other muscle groups (~40 ml·100 ml⁻¹·min⁻¹ vs. undetectable). We have not found documentation in the literature of the fiber type distribution and physiological/metabolic characteristics of the deep flexor. Similar perfusion patterns of high resting flow and high exercise hyperemia were reported in the deep muscles in rats (22), although a direct comparison with our measurements is not possible since the muscle fiber is extremely stratified in rats (8), whereas in humans muscle fibers exhibit a distribution between muscles. In a study of miniature pigs with a fiber distribution similar to that in humans, high post-exercise flow was found in muscles with a high composition of oxidative slow-twitch fibers (3). In Fig. 1, one notices more large vessels passing through the deep flexor. These vessels have been carefully excluded from our data analysis, although partial volume effects could be responsible for some residual intravascular contamination. Overall, our data suggest that the fiber composition in the DPC group may be more similar to that in the Sol muscle.

In the forearm, the F-Ext was found to have a significantly larger hyperemic response than that of the F-Flx. Data from the literature regarding the differences in fiber type between muscles in the forearm are scant. A primate study documented a slightly higher proportion of type I fibers in the extensor communis, brachioradialis, extensor carpi ulnaris, and flexor carpi radialis muscles (28). Although the ulnar head of the flexor carpi ulnaris is composed of predominantly type II fibers (71% vs. 27% type I fibers), the humeral head contains a larger proportion of type I fibers (58% vs. 40% type II fibers). In the foot, no significant flow difference between the D-Ext and the P-Flx muscle groups was observed, and we have found little supportive fiber composition data that would allow us to differentiate these muscle groups. Nevertheless, the substantial difference in the onset and recovery of postischemic hyperemia between D-Ext and P-Flx suggests that the differential involvement of these muscles might be observed in patients with small vessel disease such as diabetes.

The peak hyperemic flow measured in our study ($80\text{--}140\text{ ml}\cdot 100\text{ g}^{-1}\cdot \text{min}^{-1}$ at large) is in good agreement with the measurements ($123 \pm 42\text{ ml}\cdot 100\text{ g}^{-1}\cdot \text{min}^{-1}$) of Lebon et al. (24) using a similar paradigm. Furthermore, we were able to measure the hyperemic flow across multiple muscle groups and demonstrated differences in hyperemic flow responses, which appear to correlate with existing data on differences in fiber composition between these groups. In contrast, Frank et al. (12) reported no clear pattern of flow heterogeneity among muscle groups in their study comprising only four subjects. The discrepancy is likely due to the employment of a cuff occlusion in our work versus a plantar-flexion exercise paradigm in the design employed by Frank et al. (12). As mentioned previously, exercise is likely to stimulate specific muscle groups, which may introduce several confounds such as the different relationship between O_2 supply and muscle oxidative capacity in trained and untrained individuals (20,30).

One concern regarding the present study is the temporal evolution of relaxation times and flow-dependent water extraction. Although the mechanism is not fully understood, T_2 (T_2^*) changes have been shown to correlate with variability in the water exchange between compartments, presumably driven by the increased metabolism and the consequent increase in tissue osmolarity and intracellular water. In the study of Frank et al. (12), ASL was used to measure the hyperemia in the midcalf after rigorous exercise. The authors suggested that the errors in the estimates of T_1 , T_2 , and T_{ex} of as much as 20% tend to cancel each other out, yielding a worst case estimated error of 12% (12). Also of interest, radioactive tracer studies suggest that a significant fraction of labeled water is actually shunted by vessels and unavailable for extraction (18,39). The degree to which this may be exacerbated by a hyperemic paradigm is not known. To improve the accuracy of ASL measurement in skeletal muscle, further investigation of the dynamics of water extraction and T_{ex} is necessary. Another factor that could potentially confound data interpretation in this study is the lack of histological correlation, since muscle biopsy was not included in our protocol. Although the distribution of muscle fibers may vary between individuals, previous studies (1) showed that training could cause a temporary alteration between type IIa and type IIb fibers, whereas the percentage of type I and type II fibers remains unchanged. The latter effect should be absent or minor because we only recruited subjects who did not undergo training within 6 mo. Individual variability is expected to reduce the statistical significance without altering the results and conclusions since the composition ratio between type I and type II fibers is very unlikely to change significantly.

In summary, we have shown that CASL can be employed to noninvasively measure flow heterogeneity between diverse muscle groups. Furthermore, we have demonstrated that flow patterns following an ischemic paradigm correlate with relative fiber-type dominance. The application of this technique and these findings may be of value in further physiological and pathophysiological studies.

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APPENDIX

The signal difference between control and tag images, ΔM , can be expressed as

$$\Delta M(t) = 2\alpha \frac{M_{0b}}{\lambda} f [C(t) \otimes R(t)] \quad (\text{A1})$$

where α is the tagging efficiency, M_{0b} is the longitudinal magnetization of arterial blood at thermal equilibrium, λ is the blood/tissue partition coefficient, f is blood flow, and $\otimes$ denotes convolution. $C(t)$ is the normalized arterial concentration of magnetization arriving at the voxel at time t . $R(t)$ is the residue function that describes the fraction of tagged water remaining in the voxel at a time t after the arrival. For CASL,

$$C(t) = \begin{cases} \exp\left(-\frac{\Delta t}{T_{1b}}\right) & \Delta t \leq t \leq \Delta t + \tau \\ 0 & \text{otherwise} \end{cases} \quad (\text{A2})$$

where Δt is the transit delay, T_{1b} is the longitudinal relaxation time of arterial blood, and τ is the tagging duration. For tags that enter the interstitial space immediately after entering the capillary bed, the longitudinal relaxation of tags is dictated by the T_1 of tissue (T_{1t}), i.e.,

$$R(t) = \exp\left(-\frac{t}{T_{1t}}\right) \quad (\text{A3})$$

By applying Eqs. A2 and A3 to Eq. A1, we get ΔM for the extravascular component of ASL signal (ΔM_{ex}) after a PLD:

$$\Delta M_{\text{ex}} = 2\alpha \frac{M_{0b}}{\lambda} f \exp\left(-\frac{\Delta t}{T_{1b}}\right) T_{1t} \left[\exp\left(-\frac{\text{PLD} + \Delta t}{T_{1t}}\right) - \exp\left(-\frac{\text{PLD} + \Delta t - \tau}{T_{1t}}\right) \right] \quad (\text{A4})$$

Similarly, the intravascular component of the ASL signal (ΔM_{in}) can be obtained by replacing Eq. A3 by Eq. A5

$$R(t) = \exp\left(-\frac{t}{T_{1b}}\right) \quad (\text{A5})$$

in which the longitudinal relaxation of tags follows T_{1b} , leading to

$$\Delta M_{\text{in}} = 2\alpha \frac{M_{0b}}{\lambda} f T_{1b} \left[\exp\left(-\frac{\text{PLD}}{T_{1b}}\right) - \exp\left(-\frac{\text{PLD} + \tau}{T_{1b}}\right) \right] \quad (\text{A6})$$

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Fig. 1.

The 3 anatomic sites and regions of interest (ROIs) analyzed. The colored ROIs are superimposed upon high-resolution gradient echo images (axial slices). *A*: right midforearm: extensor (F-Ext; red) and flexor (F-Flx; purple). *B*: right transmetatarsal foot: plantar flexors (P-Flx; red) and dorsal extensors (D-Ext; purple). *C*: left midcalf: medial gastrocnemius (Gstrc-M; red) and lateral gastrocnemius (Gstrc-L; pink), soleus (Sol; blue), deep posterior compartment (DPC; yellow), and anterior (AC; light blue) and lateral (LC; green) compartments. *D*: the mean hyperemic flow image obtained at the slice shown in *C*.

Fig. 2.

Flow-time curves obtained from forearm (black, F-Ext; and gray, F-Flx; $n = 15$; A) and foot (black, D-Ext; and gray, P-Flx; $n = 9$; B). Error bars indicate the standard error of the subjects included. Thick black lines indicate the occlusion period. TR, repetition time.

Fig. 3. Flow-time series obtained from midcalf ($n = 19$). *A*: gastrocnemius (black, Gstrc-M; and gray, Gstrc-L). *B*: superficial extensors (black, AC; and gray, LC). Error bars indicate the standard error across subjects. Thick black lines indicate the occlusion period.

Fig. 4. Flow-time series comparison between 4 muscle groups in the calf (black, Gstrc-L; green, AC; blue, Sol; and red, DPC). The horizontal black bar indicates the occlusion period.

Fig. 5.
Simulated effects of heterogeneous longitudinal relaxation time (T_1) between muscle groups on flow calculation.

Table 1

Calf Muscle Group	n	Peak Flow, ml · 100 g ⁻¹ · min ⁻¹	Mean Hyperemic Flow, ml · 100 g ⁻¹ · min ⁻¹	Hyperemic Flow Volume, ml/100 g	Hyperemic Period, s	Time To Peak, s
DPC	19	127 ± 49	69 ± 20	96 ± 33	77 ± 27	69 ± 11
Sol	18	116 ± 57	67 ± 39	106 ± 66	84 ± 29	66 ± 9
Gstre-L	18	87 ± 32	49 ± 20	75 ± 40	87 ± 23	68 ± 13
Gstre-M	19	85 ± 43	49 ± 25	75 ± 48	80 ± 29	68 ± 12
AC	19	86 ± 33	53 ± 22	72 ± 33	76 ± 25	66 ± 12
LC	17	75 ± 47	43 ± 25	64 ± 49	79 ± 32	66 ± 11

Values are means ± SD; n, number of subjects. Flow measurements in the midcalf. Six regions of interest were analyzed: deep posterior compartment (DPC), soleus (Sol), lateral (Gstre-L) and medial (Gstre-M) gastrocnemius, and anterior (AC) and lateral compartment (LC).

Table 2

Muscle Group	Peak Flow	Mean Hyperemic Flow	Hyperemic Flow Volume
Gstc-L	Reference	Reference	Reference
DPC	0.0001	<0.0001	0.0075
Sol	0.0006	0.0007	<0.0001
Gstc-M	0.11	0.073	0.27
AC	0.29	0.039	0.048
LC	0.0014	0.001	0.0062

Summary of statistical analysis in the midcalf muscles. Six regions of interest were analyzed: DPC, Sol, Gstc-L and Gstc-M, and AC and LC. A significant dependence of flow measures on muscle group was found. We also tested the difference between individual muscle groups and elected Gstc-L to be the reference. Muscle groups, $P < 0.0001$.

Table 3

Muscle Group	n	Peak Flow, ml · 100 g ⁻¹ · min ⁻¹	Mean Hyperemic Flow, ml · 100 g ⁻¹ · min ⁻¹	Hyperemic Flow Volume, ml/100 g	Hyperemic Period, s	Time To Peak, s
Foot						
D-Ext	9	117 ± 44	66 ± 23	94 ± 39	85 ± 15	62 ± 13
P-Flx	9	105 ± 46 (0.31)	57 ± 20 (0.17)	79 ± 49 (0.34)	78 ± 23 (0.43)	78 ± 5 (0.0085)
Forearm						
F-Flx	15	72 ± 22	46 ± 33	56 ± 41	75 ± 10	49 ± 11
F-Ext	15	103 ± 37 (0.0013)	81 ± 43 (0.0020)	91 ± 58 (0.020)	66 ± 12 (0.015)	49 ± 17 (1.000)

Values are means ± SD; n, number of subjects. Values in parentheses are P values from repeated-measures ANOVA. Flow measurements in the foot and forearm. D-Ext, dorsal group; P-Flx, plantar flexor group; F-Flx, forearm flexors; F-Ext, forearm extensors.