MRI measures of perfusion-related changes in human skeletal muscle during progressive contractions

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MRI measures of perfusion-related changes in human skeletal muscle during progressive contractions. J Appl Physiol 97: 2385–2394, 2004. First published August 6, 2004; doi:10.1152/japplphysiol.01390.2003.—Although skeletal muscle perfusion is fundamental to proper muscle function, in vivo measurements are typically limited to those of limb or arterial blood flow, rather than flow within the muscle bed itself. We present a noninvasive functional MRI (fMRI) technique for measuring perfusion-related signal intensity (SI) changes in human skeletal muscle during and after contractions and demonstrate its application to the question of occlusion during a range of contraction intensities. Eight healthy men (aged 20–31 yr) performed a series of isometric ankle dorsiflexor contractions from 10 to 100% maximal voluntary contraction. Axial gradient-echo planar images (repetition time = 500 ms, echo time = 18.6 ms) were acquired continuously before, during, and following each 10-s contraction, with 4.5-min rest between contractions. Average SI in the dorsiflexor muscles was calculated for all 240 images in each contraction series. Postcontraction hyperemia for each force level was determined as peak change in SI after contraction, which was then scaled to that obtained following a 5-min cuff occlusion of the thigh (i.e., maximal hyperemia). A subset of subjects (n = 4) performed parallel studies using venous occlusion plethysmography to measure limb blood flow. Hyperemia measured by fMRI and plethysmography demonstrated good agreement. Postcontraction hyperemia measured by fMRI scaled with contraction intensity up to ~60% maximal voluntary contraction. fMRI provides a noninvasive means of quantifying perfusion-related changes during and following skeletal muscle contractions in humans. Temporal changes in perfusion can be observed, as can the heterogeneity of perfusion across the muscle bed.

Ankle dorsiflexors; muscle activation; blood flow; occlusion

ADEQUATE PERFUSION, OR BLOOD flow across the tissue bed, is vital to the health and proper functioning of skeletal muscle. In healthy tissue, the metabolic demands of the muscle will largely determine the degree of its perfusion. While blood flow through the arteries is important in determining how much blood can reach the muscle, the amount of blood that enters the muscle bed via the microvasculature will determine the degree of gas and nutrient exchange.

Most often, in vivo human studies of muscle perfusion are limited to measures of limb (venous occlusion plethysmography) or arterial (Doppler ultrasound) blood flow. Whereas such measures provide information regarding the amount of blood flowing to a general area, they cannot be used to determine the relative volume of blood in the muscle bed or the distribution of blood within the muscle. Functional MRI (fMRI), however, may be a useful tool for evaluating perfusion within muscle, as it has several advantages over more commonly used techniques. In the general sense, fMRI is a noninvasive technique that can be used to track temporal changes in signal intensity (SI) within a tissue in response to some perturbation; depending on the acquisition parameters chosen, these changes may reflect a variety of physiological events in the tissue. To date, many studies have focused on fMRI applications related to activity-induced changes in transverse relaxation times (i.e., T2). As in the specific case addressed here, the fMRI technique can also be adapted to emphasize longitudinal relaxation time (T1) changes, thereby allowing measurement of perfusion-related changes within active muscle. This approach provides localized information about perfusion in the muscle bed, rather than in the artery or whole limb. An additional advantage of this approach is that the regional distribution of blood across the muscle can be evaluated, a feature that may be useful in evaluating how perfusion heterogeneity relates to motor unit recruitment patterns, motor unit reorganization subsequent to aging or disease, or the effects of advancing vascular disease. fMRI techniques have been used to measure perfusion in the brain (9, 22) and during cuff-induced reactive hyperemia in skeletal muscle (23, 30, 40). Our primary goal was to develop and apply an fMRI technique that can be used to measure perfusion-related signal changes in magnetic resonance (MR) images of human skeletal muscle before, during, and after voluntary isometric contractions.

Because the degree of hyperemia (i.e., increased flow) following a brief muscle contraction is inversely related to the degree of perfusion during the contraction (16, 32), we used postcontraction hyperemia as our primary index of muscle perfusion. Thus greater postcontraction hyperemia reflects reduced perfusion, or greater occlusion, during the contraction. To assess how perfusion-related changes measured by fMRI compare with blood flow measures made by a more traditional technique, we studied a subset of subjects with both fMRI and venous occlusion plethysmography (15).

A secondary objective was to apply this technique to the problem of blood flow occlusion during exercise. During a contraction, muscular force production is accompanied by an
increase in intramuscular pressure. Such increases in intramuscular pressure compress the arteries, and, when this pressure is sufficiently high, arteries may become completely occluded, thus cutting off the blood supply to the contracting muscle. The lack of blood and oxygen availability may impair muscle performance and hasten the onset of muscle fatigue (29). This argument has been applied as a potential mechanism for the effects of age and sex on muscle fatigue, whereby smaller muscles that generate less force may be relatively better perfused during exercise (14).

Intramuscular pressure, and consequently the degree of blood flow occlusion, increases linearly with increasing contraction intensity up to some level at which occlusion is complete (8, 34). The relationship between contraction intensity and limb blood flow has been examined in human calf, forearm, and quadriceps muscles by a variety of investigators (2, 3, 5, 16, 24, 37). However, there is no consensus regarding the force level at which occlusion occurs or whether complete occlusion occurs at a specific relative or absolute force level in a given muscle. We thus sought to apply our fMRI technique to the question of the relationship between skeletal muscle perfusion and contraction intensity.

METHODS

Subjects

Healthy, nonsmoking men (n = 8) between the ages of 20 and 31 yr were studied. To preclude variability due to gender effects, we included men only in this study. All subjects were screened by questionnaire for hypertension, diabetes, and cardiovascular and peripheral vascular disease. No subjects were taking medications that could affect blood flow (e.g., anti-hypertensives, anti-asthmatics, aspirin, and certain anti-depressants). Before enrollment in the study, all subjects were found to have ankle/brachial systolic blood pressure ratios >1.0, indicating healthy peripheral vasculature (26). Subjects with <2 h of organized or recreational activity per week were recruited for this study. Activity levels were confirmed by questionnaire (see below). All subjects provided written, informed consent before participation in the study, as approved by the human subjects review boards at the University of Massachusetts, Amherst, and Yale University School of Medicine.

Experimental Setup and Data Processing

fMRI studies. MRI studies were carried out at the Magnetic Resonance Imaging Center at Yale University School of Medicine in New Haven, CT. Subjects were positioned supine in a 1.5-T whole body Signa MR imager (GE Medical Systems, Milwaukee, WI). The subject’s leg was positioned inside a 17-cm-diameter GE volume coil, such that the maximum cross-sectional area of the ankle dorsiflexors was located in the center of the coil. To reduce motion artifact, foam braces were placed around the ankle. The subject was then positioned with the center of the coil at the magnet’s isocenter. The right foot was firmly strapped to a footplate with the ankle fixed at ~120°, measured as the angle between the dorsum of the foot and the tibia. Force was measured during isometric contractions of the dorsiflexors by using a nonmagnetic strain-gauge transducer (model SSM-AJ-250, Interface, Scottsdale, AZ). The signal from the strain gauge was amplified (model SGA, Interface) and transmitted to an analog-to-digital converter (DAQPAD-6020E, National Instruments, Austin, TX) linked to a laptop computer. During each contraction, the force signal was used to provide the subject with real-time feedback concerning relative contraction intensity, accomplished using a panel of light-emitting diodes. Force data were collected at a sampling rate of 500 Hz by using LabVIEW software (version 5.1, National Instruments, Austin, TX). A blood pressure cuff was secured around the midportion of the thigh and attached to an air source (D. E. Hokanson, Bellevue, WA) for rapid cuff inflation and deflation.

At the start of each proton MRI study, gradient-echo images were acquired in three planes and used to locate the largest cross section of the dorsiflexor muscles. All subsequent images were acquired at this slice location. Anatomic images were then acquired using single-slice T1-weighted spin-echo images (field of view = 20 cm, slice thickness = 10 mm). T1-weighted gradient-echo planar images were collected to measure SI changes related to muscle perfusion [repetition time (TR) = 500 ms, echo time = 18.6 ms, field of view = 20 cm, matrix = 64 × 64, slice thickness = 10 mm, bandwith = 62.5 kHz, flip angle = 90°]. Nine hundred and sixty images were acquired for the cuff occlusion series (60 before, 600 during, and 300 following occlusion) and 240 for each contraction series (60 before, 20 during, and 160 following contraction).

Matlab software (version 6.0) was used to analyze the functional images. A mask of the first image in each functional image series was placed over the reference anatomical image and shifted so that the functional and anatomical images were spatially registered. A polygonal region of interest (ROI) that encompassed the majority of the anterior compartment (primarily the tibialis anterior and extensor digitorum longus) and excluded bone and subcutaneous fat was drawn on the anatomical image (Fig. 1). The program then determined the SI of each voxel (3 × 3 × 10 mm) in the ROI for each image in the series. This procedure was repeated for all contraction (240 images) and cuff occlusion (960 images) series. To ascertain that the postcontraction hyperemia was localized to the contracting muscle, this analysis was also performed in a similarly sized ROI drawn in the gastrocnemius/soleus muscle complex with the use of data from a representative subject.

The data for each series were imported into a spreadsheet, where the mean SI for the entire ROI was calculated and displayed. To allow comparisons across contractions, the baseline (i.e., precontraction) SI was set to zero for each series. The postcontraction SI and peak SI during hyperemia were calculated from the time series plot (Fig. 2). For each contraction and the cuff occlusion, peak hyperemia was calculated as the difference between the peak SI during hyperemia (Fig. 2, b) and the postcontraction SI level (Fig. 2, a). Following the contraction, the point of highest SI was identified (b), and a four-data
maximal response, in this representative trace. During the same contraction. Note the movement artifacts, but lack of hyperemia from initiation and cessation of the isometric contraction. Hokanson). Signal from the plethysmograph was transmitted to an analog-to-digital converter and recorded by using LabVIEW at a sampling rate of 500 Hz. A second blood pressure cuff was placed around the subject’s ankle. Blood flow measurements were taken immediately after cessation of each contraction. The ankle cuff was inflated to ~200 mmHg (i.e., suprasystolic pressure) just before the start of the contraction and remained inflated until the measurement was finished. The thigh cuff was inflated to 50 mmHg as soon as the subject had fully released the contraction. Inflation of the ankle cuff causes the inflowing blood to remain in the lower leg, resulting in increased blood volume in this region, whereas inflation of the thigh cuff ensures that venous return does not affect the blood flow measure. The rate of change of leg blood volume equals the rate of inflowing arterial blood (15). These procedures followed standard practice for the venous occlusion plethysmography technique (15).

To calculate blood flow from the initial, linear portion of the venous occlusion plethysmography waveforms, linear regression analysis of the data encompassing the first and second heartbeats was performed. The slope of the regression line was used to estimate arterial inflow (%blood volume change/min), by using a conversion factor of 0.001 V/1% change in blood volume that was based on the calibration of the plethysmograph (15). Linear regression analysis was also used to examine the association between our fMRI measure of hyperemia and that measured by venous occlusion plethysmography. The agreement between the fMRI and plethysmography measures was assessed by using a Bland-Altman plot (4, 16).

Force measurements. A customized Matlab program was used to analyze all force data. To remove the MRI radio-frequency pulse artifact from the force signal, raw force data were filtered at 1 Hz by using a first-order low-pass Butterworth filter. The data were then converted from volts to Newtons (N), based on the linear calibration of the force transducer. Peak and average forces were calculated for each contraction. To determine the relative intensity of each contraction, the average force during the contraction was scaled to the peak force during the highest maximal voluntary contraction (MVC) for that day’s session for each subject.

Procedures

Habituation. At least 1 wk before the perfusion studies, subjects reported to the Muscle Physiology Laboratory for habituation. At this time, subjects provided written, informed consent and also completed a medical history form and a Physical Activity Readiness Questionnaire (35). Resting supine ankle and brachial systolic blood pressures were measured. The subject was familiarized with the experimental apparatus and protocol. After the volunteer was positioned in the experimental apparatus, the thigh cuff was inflated to 220 mmHg for 5 min, followed by 5 min of recovery. The subject then performed two 10-s isometric MVCs and five contractions at five different forces in random order (10, 30, 50, 70, and 90% MVC). The subject was given visual feedback via the light-emitting diode panel to assist in maintaining the appropriate force level. All contractions were 10 s long and separated by 2 min of rest.

Physical activity. Each subject was asked to keep a log of his physical activity for 7 days, starting the day following the habituation session. To quantify average physical activity level, subjects returned to the laboratory ~1 wk after habituation to be interviewed about their activity during those 7 days, using the Stanford Physical Activity Questionnaire (33). Activity data are reported as kilocalories per kilogram per day.

Maximal hyperemic response. At the beginning of all fMRI and venous occlusion plethysmography studies, the maximal hyperemic response to a 5-min cuff occlusion of the thigh was measured. Following ~10 min of supine rest, the thigh cuff was rapidly inflated to 220 mmHg and rapidly deflated 5 min later, at which time hyperemia (reflecting muscle perfusion for fMRI studies and leg blood flow for venous occlusion plethysmography studies) was measured. The subject was allowed 5 min to recover before the series of contractions began.

Fig. 2. Sample trace of signal intensity (SI) over time. Change in functional MRI (fMRI) SI before, during, and following a 10-s contraction at 90% MVC in one subject. A: in anterior compartment, a = immediate postcontraction SI, and b = peak SI following contraction; hyperemia = b – a. Large spikes are movement artifacts from initiation and cessation of the isometric contraction. B: SI changes in similar-sized ROI from the gastrocnemius/soleus muscles during the same contraction. Note the movement artifacts, but lack of hyperemic response, in this representative trace.

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Contraction protocol. The subject next performed two MVCs, each 10 s long and separated by 4.5 min of rest. The highest force achieved was taken as the subject’s maximal force-producing capability and was used to scale the submaximal contractions. To assess perfusion over a wide range of force levels, the subject then performed a series of 10 submaximal, isometric contractions in a randomized order. Subjects performed two contractions at each force level: 10, 30, 50, 70, and 90% MVC. Contractions were 10 s long and separated by 4.5 min of rest to allow recovery of perfusion between contractions. Visual feedback regarding target force level was given. To assess whether force recovery was complete between contractions, an MVC was performed 4.5 min after the last submaximal contraction and compared with the highest of the initial two MVCs.

Estimation of Force at Occlusion

Postcontraction hyperemia is expected to increase linearly with increasing contraction intensities until such time as complete occlusion occurs (24, 44). At this point, hyperemia should plateau. To examine the relationship between perfusion-related changes in fMRI signal and contraction intensity, hyperemia (% maximal) was plotted against absolute (N) and relative (% MVC) force for each subject. Because we expected hyperemia to plateau once occlusion occurred, we used a segmented regression model (i.e., with two lines) to fit the data. The NLIN procedure in SAS (version 8.02, SAS Institute, Cary, NC) was used to estimate the parameters in the following equation:

\[ y = a_0 - \left[ \text{id} \right] \left( a_1 - b_1 \right) + \left( \text{id} \right) \left( a_1 + b_1 \right) \]

where \( y \) is the estimated hyperemia value (% maximal hyperemia), \( a_0 \) is the intercept of the first line, \( a_1 \) is the slope of the first line, \( b_1 \) is the slope of the second line, \( \text{id} \) is the inflection point (i.e., where the two lines intersect), and force is the absolute or relative force value. If force < \( \text{id} \), then id = 1; in all other cases, id = 0. The id term determines whether the data point is part of the first line (force < \( \text{id} \)) or the second line (force > \( \text{id} \)). Thus the equation for the first line of the segmented regression is:

\[ y = a_0 - \left[ \text{id} \right] \left( a_1 - b_1 \right) \]

The equation for the second line is:

\[ y = a_0 + \left[ \text{id} \right] \left( a_1 + b_1 \right) \]

Based on the experimental data, the NLIN procedure uses a two-stage iterative fitting process to simultaneously determine the parameter estimates that minimize the error sum of squares for the model given above. A separate model was fit for each subject, and the individual parameter estimates were then used to provide descriptive statistics regarding the relationship between force and hyperemia. This method of averaging individual parameter estimates is considered to be valid in nonlinear regression problems (7). The mean SD and coefficient of variation (CV) for the estimate of the inflection point were determined for hyperemia vs. absolute force and hyperemia vs. relative force. The force measure (absolute or relative to MVC) that produced the smaller CV for the inflection point estimate was considered to be the better predictor of blood flow occlusion.

Repeatability of fMRI and Force Measures

A repeated-measures ANOVA was used to determine whether there were differences in force or hyperemia by fMRI between the two trials at each contraction intensity. Within-day repeatability of dorsiflexor strength (i.e., peak force generated during the MVC) was also assessed in this way. Intertrial repeatability was determined for these outcome measures by using intraclass correlation (42). Repeated-measures ANOVA was also used to determine whether hyperemia following each of the three MVCs changed during the course of the protocol and to determine whether hyperemia measured by fMRI and plethysmography was different. All data are presented as means ± SE, except where noted.

RESULTS

Eight subjects completed the fMRI protocol. Table 1 lists the descriptive characteristics for the total subject pool (\( n = 8 \)) and for the subset who performed both the MRI and venous occlusion plethysmography studies (\( n = 4 \)). For one subject, the fMRI data at the two lowest contraction intensities were not useable due to technical problems; thus this subject was not included in the NLIN fit analysis. However, the remainder of this subject’s data were used in the comparison of MRI and venous occlusion plethysmography techniques. Physical activity data could not be calculated for two subjects due to errors made by the investigator in the interview procedure. Height and weight were not obtained for one subject.

Reliability of Force and Perfusion-Related fMRI Measures

Within-day measures of maximal force were highly repeatable from trial 1 (198.5 ± 14.7 N) to trial 2 (195.2 ± 14.8 N; intraclass correlation coefficient = 0.97). When all intensities were used in the analysis, both mean force and hyperemia by fMRI were correlated from trial 1 to trial 2 (intraclass correlation coefficient = 0.98 and 0.68, respectively), with no systematic difference in successive measures (\( P = 0.82 \) and 0.24, respectively). However, hyperemia following a 10-s MVC was significantly higher for the MVC performed at the end of the protocol (63.3 ± 9.6% maximum) compared with that performed before the contraction series (39.3 ± 6.8% maximum; \( P < 0.01 \)), despite similar force between trials.

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Mass, kg</th>
<th>Dorsiflexor MVC, N</th>
<th>Postocclusion Hyperemia, Signal Intensity</th>
<th>Physical Activity, kcal/kg·day⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>23</td>
<td>175.7 (( n = 7 ))</td>
<td>72.7 (( n = 7 ))</td>
<td>201.3</td>
<td>642.1</td>
</tr>
<tr>
<td>SD</td>
<td>4</td>
<td>11.4</td>
<td>9.2</td>
<td>41.7</td>
<td>269.7</td>
</tr>
<tr>
<td>Range</td>
<td>21–31</td>
<td>158.8–182.9</td>
<td>55.9–82.3</td>
<td>119.4–256.0</td>
<td>293.2–1,100.3</td>
</tr>
<tr>
<td><strong>Subset</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>23</td>
<td>172.2</td>
<td>71.2</td>
<td>210.6</td>
<td>686.9</td>
</tr>
<tr>
<td>SD</td>
<td>5</td>
<td>9.3</td>
<td>11.4</td>
<td>26.3</td>
<td>282.2</td>
</tr>
<tr>
<td>Range</td>
<td>20–31</td>
<td>158.8–179.1</td>
<td>55.9–82.0</td>
<td>187.2–247.5</td>
<td>475.7–1,100.3</td>
</tr>
</tbody>
</table>

Data are for all subjects (\( n = 8 \), except where indicated) and the subset who performed both the functional MRI and venous occlusion plethysmography studies (\( n = 4 \), except where indicated). Maximal hyperemia is the raw signal intensity (baseline set to 0) obtained by functional MRI following the cuff occlusion. MVC, maximal voluntary contraction.
Perfusion-related fMRI Measures and Contraction Intensity

In our study group, the average increase in fMRI SI in response to the 5-min cuff occlusion was 4.0 ± 0.7% of the preocclusion SI. All subsequent postcontraction hyperemia data were expressed relative to each subject’s maximal value, obtained following occlusion.

Representative fMRI SI traces for each contraction intensity from one subject are shown in Fig. 3. The left panel of Fig. 3 shows the mean SI in the muscle ROI, whereas the right panel shows the SIs of each voxel in the ROI. Figure 3 illustrates the increase in postcontraction hyperemia as contraction intensity increases. Additionally, heterogeneity of the perfusion-related change in SI is apparent across the muscle, as seen by the large variability in SI of the individual voxels during and after each contraction. Analysis of the gastrocnemius/soleus region during all contractions in one subject indicated no measureable hyperemia in the noncontracting muscle; an example of this response is provided in Fig. 2.

Comparison of MRI and Venous Occlusion Plethysmography

The hyperemia data for each technique, grouped by contraction intensity, are shown in Fig. 4. All subjects were able to meet target force levels during the contractions, as indicated by the small error bars for force in Fig. 4, top. In the four subjects who completed both fMRI and venous occulsion plethysmography measures, relative hyperemia across the range of contraction intensities was similar by both techniques (P = 0.97, Fig. 4, top). Mean hyperemia by fMRI was associated with mean hyperemia by venous occulsion plethysmography (r = 0.99, Fig. 4, middle). Finally, an examination of the Bland-Altman plot (Fig. 4, bottom) shows that the errors (differences between fMRI and plethysmographic measurements) were approximately symmetrically distributed vertically around zero. This indicates that there was no systematic bias in the fMRI measurement compared with plethysmography.

Estimation of Force at Occlusion

To investigate the relationship between force and occlusion during contraction, we determined the inflection point between postcontraction hyperemia and both absolute and relative force levels by using the NLIN procedure. The inflection point was 116.9 ± 18.3 N (CV = 0.41) for hyperemia vs. absolute force and 59.2 ± 8.0% MVC (CV = 0.36) for hyperemia vs. relative force. Both relationships were better fit by the segmented (i.e., bilinear) regression model (mean r2 = 0.66 ± 0.09) than the linear regression model (mean r2 = 0.48 ± 0.12). Within each regression model (i.e., segmented and linear), the explained variance (r2) was the same to three decimal places, whether the analysis used absolute or relative force, because the sums of squares used to compute r2 were scaled linearly by the relative force analysis. Overall, the determination of an inflection point at 59% MVC agrees well with the plateau in hyperemia observed above this level in Fig. 4, top, and suggests that, above this level, perfusion was fully occluded during the 10-s contractions.

DISCUSSION

In this study, we present an fMRI protocol for measuring perfusion-related SI changes in the muscle bed during and following isometric contractions. We used this technique to evaluate postcontraction hyperemia for a series of isometric dorsiflexor contractions of varying intensity. Measurements made by fMRI were well-correlated with those made by venous occlusion plethysmography, indicating that fMRI produces hyperemia measures that are similar to those made by an established technique. We then applied the fMRI protocol to the question of the relationship between skeletal muscle perfusion and contraction intensity. In accordance with previous studies, postcontraction hyperemia increased with increasing contraction intensity up to the point of occlusion of blood flow (3, 16, 24).

Use of fMRI to Estimate Muscle Perfusion

A number of fMRI methods for measuring perfusion have been proposed and have been applied primarily to the brain; the application to skeletal muscle has so far been rather sparse. Toussaint and colleagues (40) calculated muscle blood flow based on changes in T1 during reactive hyperemia following cuff occlusion, a technique that requires the assumption of rapid exchange of water from the intravascular to extravascular space. They also adapted plethysmography methods to MRI to measure the rate of muscle volume changes with reactive hyperemia, but noted that this method may have underestimated blood flow due to the confounding effects of compliance changes in all tissues of the leg.

An alternative approach is arterial spin labeling (ASL), in which the magnetization of inflowing arterial spins is inverted, leading to SI differences between labeled and control images. ASL has recently been applied to human skeletal muscle by several groups (10, 30, 31), with one report of a direct validation using venous occlusion plethysmography (30). When other factors such as blood and tissue T1 and the blood-tissue partition coefficient are considered, ASL affords the potential for absolute quantitation of perfusion. However, as a difference technique, it is inherently limited by the signal-to-noise ratio, which will thus limit spatial and/or temporal resolution. Temporal resolution is further limited by the need to acquire two images separated by 6–10 s, depending on field strength. Finally, ASL requires a number of additional measurements or assumptions (e.g., T1 values, the relative amounts of blood and tissue parenchyma in the voxel, and so forth), which can result in error propagation.

The advantages and disadvantages of the technique that we have presented are essentially the converse of these: the signal-to-noise ratio is sufficient to quantify spatial variation in perfusion-dependent SI changes with good temporal resolution (0.5 s), but does not result in an absolute (ml·min⁻¹·ml⁻¹ tissue) measure of skeletal muscle perfusion. The SI changes that we observed in response to 5 min of cuff occlusion were 4% above the baseline (i.e., precuff) SI. After accounting for magnetic field strength effects, the changes that we measured are similar in magnitude to those reported by Toussaint et al. (40) and Lebon et al. (23) in their studies of leg ischemia, as well as to those of Meyer et al. (27) following brief MVCs. Lebon et al. (23) used the measured desaturation of myoglobin, as well as theoretical concerns, to show a temporal coincidence between a modeled hemoglobin desaturation time course and the loss of T2*-weighted SI during arterial occlusion. Toussaint et al. (40) reported a similar decrease in T2*-weighted SI, but
Fig. 3. Postcontraction hyperemia increases with increasing contraction intensity. Data are mean SI (left) and SI of individual voxels (right) in the ROI plotted over time for the 6 contraction intensities in 1 subject. The greater hyperemic response at higher contraction intensities reflects greater occlusion during the more intense contractions. Note the tendency for mean SI to decrease during each contraction as intensity increases (left). In contrast, the range of SI changes in the individual voxels (right) suggests that the heterogeneity of the response increases with increasing contraction intensity, presumably reflecting regional variation in perfusion within the muscle bed. MVC, maximal voluntary contraction.
attributed the effect to both oxygenation and vasodilation effects. To interpret fully the fMRI SI changes that we observed, we consider three potential contributors to these SI changes.

The first potential contributor is the reoxygenation of hemoglobin during postcontraction hyperemia, which could potentially increase the SI by affecting the MRI relaxation of intravascular and/or extravascular protons. This is commonly referred to as the “blood oxygen level-dependent” (BOLD) effect. We consider first the intravascular effects. During iso-osmotic exercise, hemoglobin deoxygenates in approximately linear proportion to exercise intensity (41) and with a time constant of roughly 8 s [estimated from data reported by Hicks et al. (13)]. This causes hemoglobin to become paramagnetic (28) and the blood’s inherent $T_2$ and effective $T^*_2$ transverse relaxation time constants to decrease (e.g., Ref. 38). Assuming a capillary hematocrit of 0.2 (21) and for an end-exercise fractional oxymyoglobin saturation of 0.5, the return of normal blood oxygenation during the postcontraction period would increase the blood $T^*_2$ from $\sim$90 to $\sim$145 ms (38). Assuming a $\sim$3% blood volume fraction [which follows from the measurements made by Kano et al. (19)], this process alone would be responsible for an SI increase of $<0.1%$.

We next consider the possible effect of blood oxygenation on the MR signal arising from the extravascular space. The increase in hemoglobin’s paramagnetism during blood deoxygenation results in a difference in magnetic susceptibility between the blood and the extravascular space (i.e., a magnetic field gradient). Water molecules diffusing through these magnetic field gradients experience a permanent loss of phase coherence and, therefore, MR signal. During the reoxygenation associated with hyperemia, however, these gradients would be diminished and the MR signal would tend to increase. The potential contribution of such changes can be predicted by using analytic models of the BOLD effect, such as that presented by Stables et al. (39). Assuming that the capillaries in skeletal muscle are a series of parallel cylinders [which is implied by data from Kindig et al. (21)], the magnitude of the magnetic field gradient will depend on mean capillary orientation to the magnetic field, relative blood volume, fractional oxymyoglobin saturation, hematocrit, and magnetic field strength (39). For a given gradient strength, the extravascular BOLD effect will further depend on the capillary radius and the diffusion coefficient for water perpendicular to the capillary axis (39). These latter two quantities combine to determine the diffusion correlation time, the inverse of which reflects how frequently a water molecule experiences each magnetic environment (i.e., intravascular and extravascular). Assuming reasonable values for each parameter [capillary and fiber orientation $= 10^\circ$ in the anterior tibialis at rest (25); hematocrit $= 0.20$ (21); capillary radius $= 2.7 \mu m$ (19, 21); transverse diffusion coefficient $= 0.88 \times 10^{-5}$ cm$^2$/s (6)] and the same end-contraction value of fractional oxymyoglobin saturation as that used above, the predicted impact of BOLD effects on the extravascular signal is also $\sim$0.1%. This rather small magnitude of effect arises because the capillaries in the anterior tibialis muscle are almost parallel to the magnetic field, a condition for which there will be no magnetic field gradient around the capillary. In addition, the diffusion correlation time, calculated as indicated by Kennan et al. (20), is such that extravascular BOLD effects in general are very small, with no practical difference between $T_2$ and $T^*_2$ effects. Parenthetically, we note that, by using gradient echo acquisitions, we were able to obtain a shorter echo time, lessening the image sensitivity to potential effects of exercise on the muscle $T_2$ (which are

Fig. 4. Comparable hyperemia measured by MRI and plethysmography. Hyperemia data (means $\pm$ SE) for the 4 subjects completing MRI and venous occlusion plethysmography protocols were grouped for each contraction intensity. Top: hyperemia for each contraction intensity, measured by IMRI (solid symbols) and plethysmography (open symbols). Note that error bars for force are mostly obscured by the symbols. For both techniques, complete occlusion occurs at $\sim$60% MVC in these subjects, as reflected by the lack of increase in hyperemia above that level, and in agreement with the estimated occlusion point provided by the NLIN procedure (see text for details). Middle: hyperemia measured by IMRI plotted against that measured by plethysmography for each of the 5 submaximal contraction intensities. Data show strong association between the 2 methods. Bottom: Bland-Altman plot of individual hyperemia measurements made by IMRI and plethysmography demonstrates that there was no systematic bias in hyperemia measures.
expected to be very small after a contraction duration of only 10 s, with no additional sensitivity of SI to blood oxygenation. Overall, given that the SI changes measured during maximal hyperemia following cuff occlusion were 4% over baseline, these theoretical treatments suggest that intra- and extravascular blood deoxygenation and reoxygenation had only a very small impact (<1%) on the SI changes that we measured.

The second potential contributor to our SI changes is the so-called “inflow effect,” which refers to the increase in MR signal due to blood flowing into the slice plane with full, rather than partial, longitudinal magnetization. The preliminary report by Meyer et al. (27) compared the preexercise vs. peak hyperemia SI change following 1-s dorsiflexor MVCs measured with TR = 1,000 ms to those observed with TR = 500 ms. If inflowing blood contributed to the SI change, the relative SI change would be greater at TR = 500 ms compared with TR = 1,000 ms. No difference in the relative SI change between measurements made with the two TRs was observed, implying that, when hyperemia was defined in this way, inflow effects did not contribute to their SI change. Our definition of the baseline differed from those of Meyer et al. (27), and so their data are not directly applicable to the present experiments; however, their results do suggest that any inflow effects that occurred in our study would have been small in magnitude.

The final (and probably major) contributor to our SI changes would thus be an increase in blood volume in the muscle brought about by increased flow through the capillary network, which would result in an increase in SI via an increase in the proton density in the tissue. It should be noted that this effect would necessarily have occurred in blood vessels that are smaller than the in-plane resolution of the image, as larger vessels were excluded from the ROI. Overall, the likelihood that our SI changes were due mainly to changes in blood volume agrees with those of Toussaint et al. (40), who made MRI-based plethysmography measurements, and is also supported by the good agreement between the MRI and venous occlusion plethysmography measures (Fig. 4 and DISCUSSION).

Skeletal Muscle fMRI and Venous Occlusion Plethysmography

Venous occlusion plethysmography provides a measure of blood flow to the limb. As blood flow to the limb increases, the girth of that limb will increase, causing a stretch on the strain gauge that alters its resistance and changes the signal on the plethysmograph (15). The rate of change of this signal reflects limb blood flow. The major limitation to this technique is that it measures the total flow to the limb and thus cannot differentiate between flow to active muscle vs. flow to inactive muscle, skin, or other tissues (36). This technique has been used to measure postcontraction hyperemia, with the assumption that changes in limb blood volume following contraction primarily reflect changes in blood flow to working muscle and not to other tissues. This makes venous occlusion plethysmography a simple, noninvasive way to measure blood flow, but one that has significant limitations for the study of regional differences in muscle perfusion during and after contractions.

Although the fMRI method measures perfusion-related changes in SI in the muscle bed, whereas venous occlusion plethysmography detects changes in limb volume, we expected changes in blood flow across a range of contraction intensities to be similar by the two techniques. For the subset of four subjects studied, the relationships between hyperemia (%max) and both absolute and relative force were similar for both techniques. Contractions at each contraction intensity produced similar hyperemia by both measures (Fig. 4). Although this comparison was made in only four subjects, and it is problematic to calculate a regression coefficient for mean data, the agreement between measurement techniques was quite good, indicating that fMRI yields hyperemia data similar to those produced by a more commonly used method. The random distribution of the errors between the two techniques (Fig. 4, bottom) also supports the concept that there was no systematic bias with either method.

Application of fMRI to the Role of Contraction Intensity in Blood Flow Occlusion

In addition to measuring postcontraction hyperemia, we also measured SI during each 10-s contraction (Figs. 2 and 3, between-movement artifacts). In general, average SI during the contractions decreased with increasing contraction intensity (Fig. 3, left), consistent with the likelihood of greater occlusion of blood flow as intensity increased. Although this response was variable, largely due to motion artifact and small modulations of force during the contraction, this observation suggests that fMRI may be a promising new tool for the investigation of muscle perfusion during contractions in skeletal muscle. Of note, our observation that there was an increase in hyperemia following the MVC performed at the end of the contraction series compared with that following the first MVC suggests that there may have been some cumulative warm-up effect due to the contractions. The design of future studies should include a standardized warm-up period.

A preliminary examination of SI across individual voxels in the ROI (Fig. 3, right) suggests that there was a large heterogeneity in perfusion across the muscle bed during each contraction. This heterogeneity may be related to differences in vascular recruitment (11) or intramuscular pressure (1) across the muscle bed during contraction or to effects of fiber-type heterogeneity in human muscle (18). It is interesting to note that, at all contraction intensities, there were voxels in which SI increased and voxels in which SI decreased (Fig. 3, right). At this point, it is difficult to know whether this heterogeneity is due to true physiological differences in perfusion across the muscle bed.

Previous investigators have shown that postcontraction hyperemia increases with increasing contraction intensity, indicating that blood flow occlusion during a contraction is directly proportional to the intensity of the contraction (3, 16, 24). It is unclear, however, which contraction intensity causes complete occlusion and whether that intensity occurs at a specific absolute or relative force in a given muscle. Furthermore, is “complete” occlusion the same in all regions of a muscle? These questions bear direct relevance to on-going discussions in the literature about the extent to which skeletal muscle fatigue is dependent on muscle mass, and, therefore, the intramuscular pressure and subsequent occlusion developed during contractions of the same relative intensity (12, 14, 17).

In the present study, we used fMRI to begin an investigation of the relationship between perfusion and isometric force in the dorsiflexor muscles. Based on the results of our NLIN analy-
ses, the CV for the inflection point estimates was similar whether hyperemia was described as a function of absolute (N) or relative (%MVC) force (0.41 and 0.36, respectively). In our subjects, the hyperemic response plateaued at 117 N or ~60% MVC in this muscle group, suggesting that occlusion was effectively complete at this level.

In addition to clarifying the role of contraction intensity in occluding muscle perfusion, the fMRI technique described in this study should prove useful in advancing our understanding of how blood flow impacts muscle energy metabolism and fatigue under a variety of conditions and in a range of populations. Furthermore, the usefulness of this approach for the study of conditions such as peripheral vascular disease as well as the response to therapeutic interventions designed to improve muscle perfusion is promising.

Summary and Conclusions

fMRI is a powerful, noninvasive tool that can be used to quantify perfusion-related signal changes in human skeletal muscle in response to cuff occlusion (23, 40) and now muscle contractions. In this study, we demonstrate the utility of fMRI for detecting perfusion-related changes in SI within the muscle during and after isometric contractions. Postcontraction hyperemia was scaled to maximal hyperemia, obtained following cuff occlusion. Hyperemia measurements made by fMRI agreed well with those made by venous occlusion plethysmography and showed that perfusion in the anterior compartment of the leg increased linearly up to ~60% MVC. This technique and advancements of it should prove quite valuable in future studies of the temporal and spatial changes in human skeletal muscle perfusion under a variety of conditions.

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