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Repeatability of a Dual Gradient-Recalled Echo MRI Method for Monitoring Post-Isometric Contraction Blood Volume and Oxygenation Changes

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Abstract

The purpose of this study was to assess the repeatability of a dual gradient-recalled echo (GRE) muscle functional MRI technique. On two days, subjects ($n=8$) performed 10 s isometric dorsiflexion contractions under conditions of: 1) maximal voluntary contraction (MVC), 2) 50% MVC (50%MVC) or 3) 50% MVC with concurrent proximal arterial cuff occlusion (50%MVC_{cuff}). Functional MRI data were acquired using single-slice dual GRE (TR/TE=1000/6, 46 ms) EPI for 20 seconds before, during, and for 180 seconds after each contraction. The mean signal intensity (SI) time courses at each TE (SI₆ and SI₄₆, reflecting variations in blood volume and %HbO₂, respectively) from the tibialis anterior (TA) and extensor digitorum (EDL) muscles were characterized with the post-contraction change in SI and the time to peak SI (SI and TTP, respectively). SI₆ and SI₄₆ were 36% and 31% higher following an MVC than after a 50%MVC ($p = 0.05$ and $p = 0.07$ respectively). For SI₆ the highest intraclass correlation coefficients (ICC) were observed for the TA muscle at the 50%MVC and MVC condition, with values of 0.83 ($p = 0.01$) and 0.88 ($p = 0.005$), respectively. Bland-Altman plots revealed repeatability coefficients (RC) for the 50%MVC and MVC conditions in the TA muscle of 1.9 and 1.4, respectively. The most repeatable measures for SI₄₆ were obtained for the 50%MVC and MVC conditions in the EDL muscle ($p = 0.01$ and $p = 0.04$, respectively). Bland-Altman plots revealed RC's for 50%MVC and MVC conditions in the EDL muscle of 3.9 and 5.7, respectively.

SI₆ and SI₄₆ increased as a function of contraction intensity. The repeatability of the method depends on the muscle and contraction condition being evaluated, and in general, is higher following an MVC.

Keywords

repeatability; blood volume; %HbO₂; mfMRI; microvasculature; isometric contractions

Introduction

Regulating the amount and the distribution of blood flow through the peripheral microvasculature requires the presence of a functional endothelium (1,2). Unfortunately, the vascular endothelium is frequently impaired in sedentary individuals (3,4), chronic smokers (5), insulin resistant subjects and patients with type 2 diabetes (6-8), affecting the microvascular blood flow regulation and elevating the risk factors for cardiovascular disease (9-12). Because skeletal muscle is the principal site for glucose disposal (13), reduced or poorly regulated perfusion to skeletal muscle may contribute to the pathogenesis and development of complications in type 2 diabetes. In addition, the weak (or lack of) association between measurements of peripheral vascular reactivity and microvascular function (14) suggests microvascular and macrovascular function should be evaluated separately and that different physiological mechanisms regulate microvascular and macrovascular function. Therefore, developing a tool to evaluate skeletal muscle microvascular function is important for the early detection and prevention of cardiovascular complications in subjects at high risk of cardiovascular disease.

Methods to evaluate skeletal muscle microvascular function for clinical purposes should include the following features: 1) that it be minimally or non-invasive; 2) have high time resolution (1-2 seconds), because blood flow changes in response to a muscle contraction initiate within 1-2 s (15); 3) use tomographic imaging; 4) be simple and easy to implement; and 5) have a well understood physiologic basis. Also, they should not require administration of gadolinium-based contrast agents, because of high frequency of renal complications in diabetes and the risk of systemic nephrogenic fibrosis (16) and would ideally not use ionizing radiation. Finally, an ideal method should be reproducible, be observer independent, and add to the prediction of cardiovascular risk factors above and beyond current methods (17).

Unfortunately, no current method for in vivo, non-invasive assessment of skeletal muscle microvascular function meets all of these requirements. Optical methods are limited by the depth of light penetration to the skin: laser Doppler perfusion imaging is only able to probe the microvasculature of the skin (12) and near infrared spectroscopy (NIRS) can only evaluate the microvasculature of tissues at depths of 1-2 cm, limiting its use to persons with thin layers of subcutaneous fat (18). Positron emission tomography requires the administration of short-lived radioactive tracer isotopes, such as [^{15}O]H₂O, and does not have the resolution to distinguish between different muscles unless co-registration with CT or MRI is used (19,20). Venous plethysmography is not specific for the study of muscle vascular function since it includes changes in blood volume that occur in connective tissues and skin (21). Contrast enhanced ultrasound, although specific for microvasculature, uses projection imaging and is only able to assess superficial muscles (22). Dynamic contrast enhanced (23,24) and arterial spin labeling (25) MRI allow for direct evaluation of the microvasculature, but the former uses gadolinium-based contrast agents and the latter does not have the high time resolution require to evaluate hemodynamic changes following brief skeletal muscle contractions.

The recent development of an MRI method that can simultaneously detect variations in blood volume and oxygenation at the muscle tissue bed enables non-invasive assessment of vascular function and metabolic responses of human skeletal muscles to brief isometric contractions with high temporal resolution (1 s) (26). This method builds on previously described similar approaches (27-29) by implementing a dual gradient-recalled echo (GRE) MRI sequence. Following brief isometric contractions, the signal intensity (SI) values for echo times (TE) of 6 and 46 ms (SI_6 and SI_{46} , respectively) are transiently elevated. The responses can be characterized with the difference between baseline and peak SI (ΔSI) and time to peak SI (TTP) for each TE. This method was validated by comparing post-contraction SI_6 and SI_{46} changes of the tibialis anterior (TA) muscle to NIRS-measured variations in total hemoglobin (THb), and oxyhemoglobin saturation (%HbO₂) (26). By examining the different responses of THb and %HbO₂ to arterial occlusion, the different responses of %HbO₂ to contractions of different durations, and their correspondence to the SI_6 and SI_{46} data, Damon et al concluded that SI_6 and SI_{46} primarily reflect variations in blood volume and %HbO₂, respectively, in the muscle microvascular bed.

In addition to its well understood physiological basis and high temporal resolution, this method is also non-invasive, does not use ionizing radiation, and does not require exogenous contrast agents, and uses tomographic imaging. However, to differentiate between healthy and dysfunctional vasculature accurately and consistently, a method must have a low within subject between-day variation. In this study, we present the repeatability of this previously validated (26) dual GRE MRI sequence that can simultaneously measure changes in blood volume and oxygenation in the skeletal muscle vasculature at high temporal resolution.

Materials and Methods

Subjects

This study was approved by the Vanderbilt University institutional review board. Eight apparently healthy subjects (7 males), with (mean $\pm$ SD) 32.7 ± 5.7 years of age, body mass index (BMI) 26.2 ± 5.4 and an ankle brachial index (ABI) 1.00 ± 0.04 (indicating healthy peripheral vasculature) participated in this study. All subjects signed a written informed consent form.

Experimental protocol

Subjects reported to the lab for an orientation session and two testing sessions, spaced at least 48 hours apart. To control for factors that affect vascular function, subjects were instructed to refrain from moderate to vigorous physical activities, medications that could affect blood flow and alcohol for 24 hours before testing session and not to use caffeine or tobacco products for 6 hours before the testing sessions. During the orientation session, the subjects received a detailed and complete explanation of the procedures for the study and filled out a health and physical activity questionnaire. Height and weight were recorded and the blood pressure was measured by auscultation at the brachial and dorsalis pedis arteries to determine the ABI. The subjects then performed a series of isometric dorsiflexion contractions using their self-reported dominant foot. The contractions were performed on a custom-built, MRI-compatible isometric dorsiflexion exercise device. The subjects lay

supine on an exercise table with the leg flexed about the knee at an angle of $\sim 7.5^\circ$. The foot was tightly strapped to the exercise device using 3.8 cm nylon straps. The strap was placed at the level of the metatarsal bones and the foot was held in 15° of plantarflexion. Subjects performed two maximal voluntary contractions (MVC's) for 3 seconds and if the peak forces were within 5% of each other, the highest value was used to represent the MVC force. If the difference in force between contractions was larger than 5%, a third MVC was performed and the highest value was used. The subjects then practiced using a computer screen to obtain feedback about relative contraction intensity during submaximal contractions and were familiarized with cuff occlusion procedure (see below).

Contraction protocol

On each testing day, the subjects performed isometric dorsiflexion contractions under three different conditions: 1) maximal voluntary contraction (MVC), 2) 50% MVC (50%MVC) or 3) 50% MVC with concurrent 10 second proximal arterial cuff occlusion (50%MVC_{cuff}). The purpose for including the 50%MVC_{cuff} condition was to determine whether or not complete arterial occlusion superimposed on a 50% maximal effort would stimulate a greater vascular response. Each type of contraction was performed twice per day; the order was randomized on day 1 of testing and repeated on day 2 of testing. Four minutes of rest were allowed between each contraction in order to allow sufficient time to recover energy stores and prevent muscle fatigue (30). During the submaximal contractions, the subjects received visual feedback that allowed them to maintain the specified percentage of their MVC force. For 20 s before, during, and 180 s after the contractions, dynamic functional MRI data were recorded (see below). Subjects received strong verbal encouragement during the contractions and were instructed to remain as still as possible after the contraction ended to avoid motion artifacts of the MR images. For the 50%MVC_{cuff} protocol, the vascular cuff was placed around the distal portion of the thigh and inflated to 240 mmHg during the contraction period only, using a rapid cuff inflator/deflator (Hokanson Model E20, Bellevue, WA).

The force data acquisition and analysis procedures have been described previously (26). In brief, the dorsiflexion device consisted of a polycarbonate foot holder with an attached load cell (model SSM-AJ-500; Interface Force, Scottsdale AZ, USA). The signals from the load cell were amplified and conditioned using a bridge amplifier (Model DMD-465WB; Omega Engineering, INC. Stamford, CT) and connected using a serial connector box (model SCB-68; National Instruments, Austin TX, USA) to an analog-to-digital conversion card (model 6036E; National Instruments). Force data were acquired at 1 kHz using a custom-written software program in LabVIEW 7.1 (National Instruments), which provided real-time, 20 Hz visual feedback to the participant using a computer screen during the practice sessions outside the magnet and using a pair of MRI-compatible LCD goggles during the imaging sessions. The force data were low-pass filtered at 25 Hz using a fifth-order Butterworth filter. The mean relative (% baseline MVC) and absolute (N) force were calculated for each contraction to verify that subjects performed at target intensities.

MRI data acquisition

Functional MRI data were acquired on a 3T/60 cm horizontal bore Philips Intera Achieva MR Imager/Spectrometer with a gradient performance of 80 mT/m and 200T/m/s slew-rate. The MRI scanner operates using R2.1.3 software and Intel based PC's are used for the console and reconstructor computers running embedded Windows XP. Hardware efficiency and calibrations are performed in automated preparation steps for coil identification, noise measurement, power optimization, center frequency, and shim. Gradient waveform pre-emphasis was used for eddy current compensation.

The subjects lay supine with the foot strapped to the exercise device. The imager's body coil was used for transmission; a 2-element flexible surface coil was placed along the longitudinal length of the TA muscle and used for signal reception. Each element of the surface coil had dimensions of 14×17 cm. Second order shimming was performed on a voxel just thicker than the slice and including the imaged leg only. Imaging procedures were initiated with the acquisition of 3-plane GRE scout images to determine the maximal cross sectional area of the TA muscle. A single slice at that location was chosen for the rest of the imaging acquisitions. Following the scout images, a high resolution T₁-weighted anatomical image was acquired using a turbo spin-echo sequence and the following imaging parameters: slice thickness= 7.5 mm, field of view (FOV) = 18×18 cm, acquired matrix = 128×128 (reconstructed matrix = 256×256), repetition time (TR) = 500 ms, echo time (TE) = 16 ms, and number of excitations (N_{EX})= 2. Next, muscle functional MRI data were acquired using dual GRE EPI for 20 seconds before, during and for 180 seconds after each contraction. The functional images were acquired using the same geometric parameters as for the high resolution anatomical image, acquired matrix = 64×64 (reconstructed matrix = 128×128), TR = 1000 ms, and TE = 6 ms/46 ms.

Data analysis

Images were analyzed by using custom-written Matlab v. 7.0.1 (The Mathworks, Natick, MA, USA) routines. Using the high-resolution anatomical images, regions of interest (ROI's) were specified separately around the extensor digitorum longus (EDL) and the tibialis anterior (TA) muscles. Resolved connective tissues and blood vessels were excluded from the ROI's. The mean SI time courses from the functional images at TE=6 ms and TE=46 ms were calculated from each ROI and divided by the corresponding mean pre-contraction SI values. The minimum post-contraction, maximum post-contraction and minimum post-maximum SI values were identified initially by visual inspection (Figure 1A). The SI time course between the minimum post-contraction and the minimum post-maximum values was fitted to polynomial functions of progressively higher order, beginning with 3rd order and ending with 12th order. The curve-fitting procedure was stopped at the lowest polynomial order that provided for unstructured residuals, as determined by visual inspection and little or no further change in the autocorrelation of the residuals. The first derivative of the line of best fit was taken, solved for many values of time, and the times at which the slope of the tangent lines was zero were identified. From these points, the post-contraction minimum SI and the post-contraction maximum SI were determined (Figures 1A and 1B). To characterize the microvascular and metabolic responses to contraction, SI₆, SI₄₆, TTP₆ and TTP₄₆ were calculated. SI₆ and SI₄₆ were

interpreted as reflecting variations in blood volume and %HbO₂, respectively, in the muscle's microvasculature.

Statistical analysis

Statistical analysis was performed using SPSS v. 15.0. (SPSS Inc., Chicago IL, USA). The average SI and TTP within and between days were determined for each muscle and TE, for each of the three different experimental conditions (50%MVC, MVC and 50%MVC_{cuff}). Values are presented as mean ± SD. A one-sample t-test was used to determine if subjects attained target force values during the contractions. The mean SI and TTP from day 1 and day 2 for each condition were averaged and a general linear model (GLM) repeated measures analysis was performed to test for differences in SI and TTP by Condition, Muscle and Condition × Muscle interaction separately for each echo time. Bonferroni's *post hoc* pairwise comparison was applied when indicated. Linear regression statistics and Pearson correlation coefficients were calculated between TTP₆ and TTP₄₆.

For each variable a one sample *t*-test was performed to test whether or not the difference between days was equal to zero and then Bland-Altman plots were generated and repeatability coefficients (RC's) were calculated to determine the between-day variation in SI and TTP within subjects (31). The RC's were calculated according to:

$$RC = \left(\sqrt{\frac{\sum (x_1 - x_2)^2}{n}} \right) \times 2 \quad [1]$$

where x_1 and x_2 are the average values obtained for SI and TTP at each echo time for each subject on testing days 1 and 2, respectively, and n is number of subjects. RC is the 95% confidence interval of the differences between days for the method (31) and it should be lower than the expected differences between a population with a specific condition and a healthy population. The intraclass correlation coefficient (ICC) (32) was determined to assess the test-retest reliability in SI and TTP in the different conditions for both echo times. ICC measures the ratio of true variance to the variance due to measurement error; values closer to 1 indicate less difference due to measurement error. Statistical significance was set at $P < 0.05$.

Results

Force measurements

For isometric contractions at 50%MVC and 50%MVC_{cuff} the average relative force production values during the contractions ($55.1 \pm 4.1\%$ and $52.2 \pm 1.0\%$, respectively) were not significantly different from their target value of 50%; $p = 0.25$ and $p = 0.1$, respectively. However, the average relative force production value during the MVC isometric contractions ($87.5 \pm 5.1\%$) was significantly lower than its target value ($p = 0.04$).

Variations in SI_6 and SI_{46} as a function of condition

Pairwise comparison revealed that SI_6 was 36% higher following an MVC than after a 50%MVC isometric contraction; 2.6 ± 0.4 vs. 1.8 ± 0.4 ($p = 0.05$; Fig. 2A). The SI_6 following a 50%MVC_{cuff} was not significantly different from 50%MVC or MVC ($p = 0.93$ and $p = 0.88$, respectively; Fig. 2A). Changes in SI_6 were larger in the EDL than in the TA muscle (2.6 ± 0.4 vs. 1.8 ± 0.3 , respectively; $p = 0.02$). There was no Condition $\times$ Muscle interaction ($p = 0.45$). Pairwise comparison revealed that SI_{46} tended to be higher, by 31%, following MVC than after a 50%MVC (7.6 ± 0.7 vs. 4.9 ± 0.7) ($p = 0.07$; Figure 2B). The change in SI_{46} following a 50%MVC_{cuff} isometric contraction was not significantly different from those following 50%MVC or MVC ($p = 0.28$ and $p = 1.0$, respectively; Figure 2B). There was a trend toward significance for differences in SI_{46} between muscles ($p = 0.09$), but no Condition $\times$ Muscle interaction ($p = 0.19$).

Variations in TTP_6 and TTP_{46} as a function of condition

TTP_6 did not differ between conditions ($p = 0.39$) or between muscles ($p = 0.63$) and the Muscle $\times$ Condition interaction was not significant ($p = 0.32$; Figure 3A). For TTP_{46} there was a main effect for condition ($p = 0.05$). TTP_{46} following a 50%MVC isometric contraction was 18% longer than after an MVC ($p = 0.03$). There was no significant main effect for muscle or Muscle $\times$ Condition interaction for TTP_{46} ($p = 0.22$ and $p = 0.54$, respectively; Figure 3B).

Table 1 shows the linear regression analyses and Pearson correlation coefficients of TTP_{46} as a function of TTP_6 for the EDL and TA muscles. These analyses revealed a positive association between TTP_6 and TTP_{46} in the TA muscle for both 50%MVC and MVC conditions ($r = 0.93$, $p = 0.001$ and $r = 0.79$, $p = 0.02$, respectively) with intercepts that did not differ significantly from zero and slopes that did not differ significantly from one (Table 1 and Figure 4).

Between-day repeatability measurements for SI_6

Table 2 compares the mean SI_6 and SI_{46} values between days 1 and 2 and their respective ICC values. The mean SI_6 values differed between days for the TA muscle following an MVC only; $p = 0.05$. The ICC's for SI_6 ranged between 0.05 and 0.89 with the lowest value not reaching significance and belonging to SI_6 following a 50%MVC_{cuff} for the EDL muscle ($p = 0.48$). The mean ICC for the remaining conditions for both muscles was 0.82 ± 0.06 . The two highest ICC's were for the TA muscle under the 50%MVC and MVC conditions, with values of 0.83 ($p = 0.01$) and 0.88 ($p = 0.005$), respectively. Bland-Altman plots for all SI data are shown in Figures 5A-C and reveal RC's for the 50%MVC and MVC conditions in the TA muscle of 1.9 and 1.4, respectively (Figures 5A and 5C; Table 2).

The ICC values for SI_{46} ranged from 0.16 to 0.87, with the lowest value not reaching significance and belonging to SI_{46} following a 50%MVC_{cuff} for the EDL muscle ($p = 0.34$). The mean ICC for the 50%MVC and MVC conditions were 0.82 ± 0.07 ($p \leq 0.04$). For SI_{46} , the significant ICC's were for the 50%MVC and MVC conditions in the EDL muscle ($p = 0.01$ and $p = 0.04$, respectively); the ICC for the MVC condition in the TA approached significance ($p = 0.06$). Bland-Altman plots are shown in Figures 5D-5F and

reveal RC's for 50%MVC and MVC conditions in the EDL muscle of 3.9 and 5.7, respectively (Figures 5D and 5F; Table 2).

Between-day repeatability measurements for TTP_6 and TTP_{46}

Table 3 presents the mean values of TTP_6 and TTP_{46} for days 1 and 2 and their respective ICC's. The only significant between-day differences in the mean TTP_6 values were for the TA muscle following an MVC ($p = 0.03$). The corresponding Bland-Altman plots are shown in Figure 6. The two lowest RC values were of 18.8 in the EDL muscle for TTP_6 at 50%MVC_{cuff} and 27.6 in the TA muscle for TTP_{46} at 50%MVC, with ICC values of 0.67 and 0.68, respectively ($p = 0.08$ for both). No other ICC's for TTP_6 and TTP_{46} in the TA or EDL muscles were significant for any condition (Figures 6A-F; Table 3).

Discussion

The repeatability of a dual GRE MRI technique for assessing changes in blood volume and oxygenation in the microvasculature of the EDL and TA muscles following isometric contractions was determined. We found that the repeatability of this technique depends on the parameter being measured, the contraction condition and the muscle being evaluated.

SI_6 measurements were most repeatable following 50%MVC and MVC isometric contractions calculated over the TA muscle. For SI_{46} measurements, the most repeatable results were obtained from the EDL muscle following 50%MVC and MVC isometric contractions.

Changes in blood volume and %HbO₂ as a function of contraction intensity

In a previous report (26), Damon et al demonstrated that variations in proton density explained about 50% of the change in SI_6 (26). The increase in proton density was shown to reflect an increase in blood volume in all unresolved vessels of the imaging voxel, mainly those vessels less than 1 mm in diameter. The increased blood volume was most likely the result of vasodilatation of terminal arterioles and refilling of venous circulation. In this same study (26) it was also estimated that at least 90% of the SI_{46} reflected variations in %HbO₂ and (to a much lesser degree) changes in myoglobin oxygen saturation, with the remaining 10% reflecting variations in proton density. Moreover, it was shown experimentally that the different responses of THb and %HbO₂ to prolonged arterial occlusion and to isometric contractions of different durations (2 and 8 s) were similar to the different behaviors observed for SI_6 and SI_{46} , respectively. The similar behaviors of SI_6 and THb and of

SI_{46} and %HbO₂ under different contraction conditions, as well as some of the findings of this study (see *TTP_6 and TTP_{46} as a function of condition*, below), strongly supports the argument that the variations in SI_6 and SI_{46} primarily reflect changes in blood volume and %HbO₂, respectively. Therefore, we continue the discussion of the physiological interpretation of the data by using the terms blood volume and %HbO₂ rather than SI_6 and SI_{46} .

We observed that the changes in blood volume in the EDL and the TA muscles following an isometric contraction varied as a function of contraction intensity and also that there were larger differences observed in the EDL than in the TA muscle. The former finding agrees

with the result obtained in a study by Wigmore et al (28) in which SI following isometric contractions also increased as a function of contraction intensity. The conclusion that blood volume changes increase following contractions of high intensity is also consistent with those of previous *in vivo* animal studies. VanTeeffelen et al (33) showed in the hamster retractor muscle that peak muscle tension and dilation of terminal arterioles increased as a function of motor unit recruitment; in the hamster cremaster muscle, erythrocyte flow through capillaries and arteriolar diameter increased as a function of contraction frequency (34). The larger blood volume increase following more intense contractions may reflect the response of the vascular system to meet the increasing metabolic requirements of muscle cells (1,2,35).

Similar to the changes in blood volume, the change in %HbO₂ following isometric contractions increased as a function of contraction intensity, but with no difference in the change in %HbO₂ between muscles. Variations in %HbO₂ reflect a balance between the rates of oxygen delivery by the vasculature and the uptake by skeletal muscle. An increase in the change in %HbO₂ as a function of contraction intensity indicates that, at least in healthy individuals, a 10 s isometric dorsiflexion contraction is always followed by transient excess in oxygen delivery relative to its demand and this effect increases as a function of contraction intensity.

Adding a 10 second cuff occlusion during the contraction period did not modify the changes in blood volume or %HbO₂ following an isometric contraction. In a study aimed at evaluating the influence of mechanical compression on rapid vasodilation induced by muscle contraction or forearm cuff occlusion at different intensities and durations, it was shown that increases in intramuscular pressure imposed by a single two second cuff occlusion period enhanced vascular conductance, but only for cuff occlusions pressures between 25-100 mmHg (36). Beyond 100 mmHg of occlusion pressure, vascular conductance did not further increase until occlusion pressure reached 300 mmHg (36). In the present study we used an occlusion pressure of 240 mmHg. In the forearm muscles, a 50% MVC contraction generates an estimated intramuscular pressure of ~100 mmHg (36). In the vastus medialis muscle contracting at 50%MVC, the intramuscular pressure increased to ~150 mmHg (37). Assuming that the intramuscular pressure in the TA muscle at 50% MVC is intermediate to these values, then the cuff occlusion probably had no additional effect on vascular conductance. This provides one possible explanation for the absence of significant differences in the post-contraction blood volume changes between the 50%MVC and 50%MVC_{cuff} conditions.

TTP₆ and TTP₄₆ as a function of condition

TTP₆ did not differ between conditions or between the EDL and TA muscles. TTP₄₆ was 18% longer following an MVC than after a 50%MVC. The physiological factors affecting TTP₆ or TTP₄₆ following isometric contractions and its physiological and clinical significance have yet to be determined. However, similar to our findings, Kirby et al (36) observed an increase in TTP with increasing contraction intensities when measuring forearm vascular conductance during handgrip isometric exercises. In a study by Towse et al (29), %HbO₂-weighted signals (single GRE-EPI MRI with TR/TE = 1000/40 ms) following brief

(1 s) isometric ankle dorsiflexion contractions were compared between sedentary and active individuals. TTP was not significantly different between groups and was only 5.8 ± 0.3 s for sedentary individuals, about four-fold shorter than what we found in our study. The shorter TTP observed by Towse et al. than in the present study is probably related to the shorter duration of the isometric contraction protocol (1 s) used by Towse et al.

An important finding is the linear association between TTP_6 and TTP_{46} in the TA muscle following 50% MVC and MVC contractions (Figures 4A and 4B). The temporal association between these two physiological parameters is consistent with the coupling between oxygen delivery and metabolic requirements (34). As we have noted above, SI_6 and SI_{46} are not completely independent from each other; therefore, the lack of complete independence in SI_6 and SI_{46} may overestimate the statistical association between these two parameters. However, it is noteworthy that there was not a significant association between TTP_6 and TTP_{46} in the TA muscle for the 50%MVC_{cuff} condition or in the EDL muscle under any conditions; therefore, a close correlation is not automatic. Also, the absence of correlation under these conditions supports further the hypothesis that the SI_6 and SI_{46} time courses report of blood volume and %HbO₂ changes, respectively, in an independent fashion.

The close association between TTP_6 and TTP_{46} in the TA muscle that was observed in these healthy individuals may potentially be modified by disease. For example, in an individual with a decreased oxidative capacity and a normal microvascular endothelium, the SI_{46} response to an MVC would be expected to induce a higher SI_{46} than the response observed in individuals with normal oxidative capacity. In addition, this might lead to a shorter TTP_{46} with no change in TTP_6 . Thus a change in the temporal association between these two parameters might also be a useful means of assessing post-contraction muscle microvascular and metabolic function. The regression statistics provided in Table 1 provide normative values for future comparisons of this type.

Between-day repeatability of SI_6 and SI_{46}

For SI_6 , the most repeatable measurements were acquired in the TA following isometric contractions of 50%MVC and MVC. Bland-Altman plots revealed RC values for SI_6 following 50%MVC and MVC isometric contractions in the TA muscle of 1.9 and 1.4, respectively. The RC indicates the 95% confidence interval for the mean difference between study days (31). Studies evaluating forearm blood flow using cuff occlusion plethysmography in response to infusion of acetylcholine or reactive hyperemia by cuff occlusion have shown that peak blood flow is about 45% lower in diabetic patients when compared to healthy age matched individuals (6,7,21,38-41). If this difference in blood flow between diabetics and healthy individuals is also true for changes in blood volume, we would expect that diabetic individuals would have a change in blood volume following MVC isometric contractions of about 1.27. Using our RC measurements, this hypothetical value for change in blood volume in a diabetic individual following an MVC isometric contraction would be outside the between-day range of variations expected from measurement error alone, and so the observed between-day variability would be small enough to detect differences in the change in blood volume in response to isometric contractions between diabetic and healthy individuals.

For SI_{46} , the most repeatable measurements were acquired in the EDL following isometric contractions of 50%MVC and MVC. Bland-Altman plots revealed RC's for SI_{46} following 50%MVC and MVC isometric contractions in the TA muscle of 3.9 and 5.7, respectively. The mean value observed for SI_{46} following an MVC isometric contraction was 8.0. Since the RC for SI_{46} following an MVC was 5.7, the mean value of 8.0 could range on different days between 5.1 and 10.9. Therefore, to detect differences between a population with a defect in oxygen utilization and healthy individuals this population would have to have a change in %HbO₂ more than 35% different from the value for healthy subjects. Unfortunately the change in %HbO₂ following isometric contractions, challenge with acetylcholine or cuff occlusion has only been reported in healthy populations, so differences in the change in %HbO₂ following isometric contractions cannot be estimated between healthy and affected populations.

Regardless of whether SI_6 or SI_{46} was considered, the data for the 50%MVC_{cuff} condition were generally not repeatable. The reasons for this are unclear, but may relate to excess post-contraction movements to relieve the discomfort associated with the occlusion procedure. The lower repeatability may be another factor influencing the lack of significant differences between this condition and the 50%MVC and MVC conditions.

Repeatability of TTP

Under these experimental conditions, the measurement of TTP was highly variable and not reliable for any condition, muscle or TE. TTP was measured in one study comparing blood flow using blood occlusion plethysmography between diabetics, glucose intolerance and normal glucose tolerance individual. It was found that time to peak was significantly increased in both diabetic and glucose intolerance subjects when compared to normal glucose tolerance individuals (6). Further improvements to the signal-to-noise ratio or other aspects of the experimental protocol are required in order to assess this potentially significant parameter.

Summary

The dual GRE MRI method is able to detect changes in blood volume and %HbO₂ as a function of contraction intensity in the muscle's microvasculature. The repeatability of the method varies with the muscle, echo time being assessed, and type of contraction, and in general is most repeatable following MVC. This method is sensitive enough to study the physiological mechanisms implicated in the vascular response to isometric contractions and may also have the potential to detect differences between healthy individuals and subjects with dysfunctional skeletal muscle microvasculature.

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Abbreviations

GRE-EPI	gradient recalled echo-echo planar imaging
SI	signal intensity
ΔSI	difference between post-contraction maximum and minimum signal intensities
TTP	time to peak
TA	tibialis anterior
EDL	extensor digitorum longus
THb	total hemoglobin
%HbO₂	hemoglobin oxygen saturation
BMI	body mass index
ABI	ankle brachial index
MVC	maximal voluntary contraction
MVC_{cuff}	maximal voluntary contraction with concurrent cuff occlusion
RC	repeatability coefficient
ICC	intraclass correlation coefficient
GLM	general linear model

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Figure 1.

SI_6 time course with 8th order polynomial fit (A) and plot of the residual SI (B). The minimum, maximum, and minimum post-maximum points **a**, **b** and **c**, respectively, were identified by determining the points at which the slope of the tangent line equaled zero. SI was calculated as **b-a**. The time at point **b** was defined as time to peak. On the abscissa, Time refers to the time following contraction (s).

Figure 2.

A. Comparison of SI_6 by condition for the EDL and TA muscles. *Higher at MVC than at 50% MVC ($p = 0.05$). #Higher in the EDL than in the TA muscle ($p = 0.02$). B. Comparison of SI_{46} by condition for the EDL and TA muscles. *Higher at MVC than at 50% MVC (trend towards significance; $p = 0.07$). A trend towards a main effect for muscle was found, $p = 0.09$. No Condition $\times$ Muscle interaction was found, $p = 0.3$.

Figure 3.

A. Plot of TTP₆ as a function of condition. There were no main effects for muscle or condition and no Condition $\times$ Muscle interaction ($p = 0.39, 0.63$ and 0.32 , respectively). B. Plot of TTP₄₆ as a function of condition. A main effect for condition was found for TTP₄₆ ($p = 0.05$). *TTP₄₆ following a 50%MVC was 18% lower than after an MVC ($p = 0.03$). No main effect for muscle or Condition $\times$ Muscle interaction was found ($p = 0.22$ and $p = 0.54$, respectively).

Figure 4.

Linear regression and correlation analyses. TTP₄₆ in the TA muscle as a function of TTP₆ following a 50%MVC (A) and an MVC (B) isometric contraction. For 50% MVC, $r = 0.93$ and $p = 0.001$ and for MVC, $r = 0.79$ and $p = 0.02$. The regression statistics are presented in Table 1.

Figure 5.

Bland-Altman plots for SI_6 (A-C) and SI_{46} (D-F) for 50% MVC (A and D), for SI_6 and SI_{46} at 50% MVC_{cuff} (B and E) and for SI_6 and SI_{46} at MVC (C and F) for the TA and EDL muscles. The continuous top and bottom lines indicate the limits of agreement, reflecting the 95% confidence interval for the mean difference between days.

Figure 6.

Bland-Altman plots for TTP_6 and TTP_{46} for 50%MVC (A and D), for TTP_6 and TTP_{46} at 50%MVC_{cuff} (B and E) and for TTP_6 and TTP_{46} at MVC (C and F) for the TA and EDL muscles. The continuous top and bottom lines indicate the limits of agreement, reflecting the 95% confidence interval for the mean difference between days.

Table 1

Linear regression of TTP46 on TTP6 and the corresponding Pearson correlation coefficients, by contraction condition, for the EDL and TA muscles.

Condition	Muscle	Intercept	Slope (95% CI)	Correlation
50% MVC	EDL	23.1±9.9	-0.06± 0.4	-0.07
	TA	-2.1±4.7	1.2±0.2	0.93*
50% MVC _{cuff}	EDL	19.0±8.7	0.21±0.4	0.21
	TA	30.4±5.6	-0.13±0.2	0.60
MVC	EDL	22.4±9.1	0.85±0.7	0.47
	TA	4.8±8.9	0.99±0.3	0.79**

Mean ± SE is given

*
p<0.01

**
p<0.05

Table 2Comparison between day 1 and day 2 for SI₆ and SI₄₆ by condition and muscle

TE	Condition	Muscle	Day 1	Day 2	RC	ICC
SI ₆	50% MVC	EDL	2.3 ± 0.4	1.9 ± 0.4	2.1	0.83 ^{**}
		TA	1.6 ± 0.4	1.7 ± 0.6	1.9	0.86 ^{**}
	50% MVC _{cuff}	EDL	2.0 ± 0.5	3.2 ± 0.6	8.1	0.05
		TA	1.9 ± 0.3	1.7 ± 0.3	2.0	0.73 ^{**}
	MVC	EDL	3.3 ± 0.6	3.4 ± 0.5	2.7	0.79 ^{**}
		TA	2.1 ± 0.3	2.6 ^a ± 0.4	1.4	0.89 [*]
	50% MVC	EDL	4.6 ± 1.0	4.3 ± 1.2	3.9	0.87 [*]
		TA	6.0 ± 0.7	7.0 ± 1.3	4.2	0.63
	50% MVC _{cuff}	EDL	7.5 ± 1.1	7.3 ± 1.6	9.3	0.16
		TA	7.2 ± 1.0	7.1 ± 1.2	6.9	0.48
SI ₄₆	MVC	EDL	7.0 ± 1.3	7.6 ± 0.8	5.7	0.76 ^{**}
		TA	9.0 ± 0.8	8.5 ± 1.1	4.9	0.71 ⁺

Mean ± SE is given

*
p<0.01**
p<0.05+
p=0.06^aIndicates differences between days (p <0.05).

Table 3Comparison between day 1 and day 2 for TTP₆ and TTP₄₆ by condition and muscle

TE	Condition	Muscle	Day 1	Day 2	RC	ICC
SI ₆	50% MVC	EDL	25.0 ± 5.1	22.5 ± 3.0	26.7	0.50
		TA	23.5 ± 6.6	19.0 ± 3.0	27.3	0.47
	50% MVC _{cuff}	EDL	23.5 ± 2.8	18.0 ± 3.0	18.8	0.67 ⁺
		TA	21.4 ± 3.3	17.6 ± 2.3	41.4	0.19
	MVC	EDL	25.5 ± 3.8	24.4 ± 5.6	31.5	-1.94
		TA	34.8 ± 6.2	21.8 ^a ± 3.1	41.4	0.36
SI ₄₆	50% MVC	EDL	21.5 ± 5.9	21.6 ± 3.3	39.2	-0.17
		TA	26.3 ± 7.0	16.6 ± 2.8	27.6	0.68 ⁺
	50% MVC _{cuff}	EDL	25.9 ± 3.2	21.0 ± 5.5	40.2	-1.10
		TA	20.9 ± 3.7	23.0 ± 3.7	44.7	-3.00
	MVC	EDL	24.4 ± 5.6	24.4 ± 3.4	37.6	-0.40
		TA	33.5 ± 4.6	26.5 ± 4.3	32.5	0.53

Mean ± SE is given

⁺
p=0.08^a
Indicates differences between days (p < 0.05).