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Post-contrastile BOLD contrast in skeletal muscle at 7 tesla reveals inter-individual heterogeneity in the physiological responses to muscle contraction

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Abstract

Muscle BOLD contrast at 7T is greater in magnitude and potentially more influenced by extravascular BOLD mechanisms than it is at lower field strengths. Muscle BOLD-imaging of muscle contractions at 7T could, therefore, provide greater or different contrast than at 3T. The purpose of this study was to evaluate the feasibility of using BOLD imaging at 7T to assess the physiological responses to *in vivo* muscle contractions. Thirteen subjects (4 females) performed a series of isometric contractions of the calf muscles while being scanned in a Philips Achieva 7T human imager. Following 2 s maximal isometric plantarflexion contractions, BOLD signal transients ranging from 0.3–7.0% of the pre-contraction signal intensity were observed in the soleus muscle. We observed considerable inter-subject variability in both the magnitude and time course of the muscle BOLD signal. A subset of subjects (n=7) repeated the contraction protocol at two different repetition times (*TR*'s; 1000 and 2500 ms) to determine the potential of T_1 -related inflow effects on the magnitude of the post-contrastile BOLD response. Consistent with previous reports, there was no difference in the magnitude of the responses for the two *TR*'s (3.8 ± 0.9 vs. $4.0 \pm 0.6\%$ for *TR*=1000 and 2500 ms, respectively; mean $\pm$ standard error). These results demonstrate that studies of the muscle BOLD responses to contractions are feasible at 7T. Compared to studies at lower field strengths, post-contrastile 7T muscle BOLD contrast may afford greater insight into microvascular function and dysfunction.

Keywords

magnetic resonance imaging; skeletal muscle functional MRI; hyperemia

INTRODUCTION

The post-contraction muscle blood oxygenation-level dependent (BOLD) response has been described primarily as a small positive change in T_2^* -weighted signal intensity (SI) following a brief isometric contraction (1). The physiologic influences on this response include blood volume, blood flow, and muscle oxygen consumption. These processes impact the magnitude and time course of the response (2). Post-contraction BOLD contrast has been used to characterize skeletal muscle function in healthy, sedentary, and active populations (1–3). Structural and functional changes in the microvasculature play a pivotal role in the pathogenesis of cardiovascular disease, hypertension, and diabetes (4,5). Therefore, muscle BOLD may be a powerful tool for measuring peripheral vascular function in individuals with peripheral arterial occlusive disease (6), obesity (7), and diabetes (7,8). Despite these advances, muscle BOLD imaging still suffers from relatively low signal-to-noise ratio (SNR) and an incomplete understanding of its biophysical basis.

Muscle BOLD contrast results primarily from changes in hemoglobin content and oxygen saturation, acting through extravascular and intravascular relaxation mechanisms (9). The relative contributions of extravascular and intravascular contrast depend on the microvascular architecture. Unlike the case in the brain, blood vessels in skeletal muscle are primarily oriented parallel to each other and to the muscle fibers (5), imparting an orientation-dependence to the extravascular BOLD effect. When muscle fibers are aligned parallel to B_0 , as is the case in many MRI experiments, the extravascular contribution to the BOLD effect at field strengths of 3T or lower is negligible (1,7,10). Conversely, at higher field strengths such as 7T, the T_2^* of blood (~9 ms; (11)) is several times shorter than at 3T (12). Therefore, intravascular muscle BOLD contrast is likely to be small at 7T, while extravascular contrast is expected to dominate. If this is indeed the case, muscle BOLD studies at ultra-high field strengths may provide contrast that is also sensitive to structural features of the microvasculature.

Translating muscle BOLD studies to ultra-high field strengths such as 7T would expand our understanding of the biophysical basis of muscle BOLD and increase the utility of this technique in biomedical imaging. In addition to providing sensitivity to novel contrast mechanisms, muscle BOLD studies at ultra-high field strengths will benefit from increased SNR due to greater spin polarization and increased contrast-to-noise ratio (CNR; (13,14)). The increased SNR and CNR may improve the diagnostic capabilities of muscle BOLD imaging and could be used to improve both the spatial and temporal resolution of imaging studies. Collectively, the known and potential advantages of imaging at 7T provide compelling reasons to expand muscle BOLD to 7T.

Although promising, muscle BOLD imaging at ultra-high field strengths is challenging. At 7T, there can be large variation in both B_0 and B_1 as well as greater RF power requirements (15). B_0 inhomogeneities can lead to distorted images and unwanted signals from

incomplete suppression of blood and/or fat. Image-based shimming routines and dynamic shimming can mitigate some of the B_0 inhomogeneities; however, the problems caused by the different resonant frequencies of water and fat can be difficult to overcome (17). B_1 inhomogeneities result in nonuniform absorption of RF energy, creating “hot spots” of high signal in some areas and areas of low signal elsewhere. Novel coil designs and tailored RF pulses can overcome these issues (18), however, they have had limited application outside of brain fMRI. If the challenges of muscle BOLD imaging at ultra-high fields can be overcome, as preliminary studies suggest (19,20), muscle BOLD imaging at 7T may provide information regarding peripheral vascular health not available at lower field strengths. Therefore, the purpose of this study was to expand the use of postcontractile muscle BOLD imaging to 7T. The results of this study demonstrate not just the feasibility of this technique in general, but also its ability to reveal previously undescribed inter-individual differences in the physiological responses to muscle contraction.

METHODS

Thirteen subjects (four females, mean±standard deviation; age 28.3±6.8 years, height 175.3±4.8 cm, and body mass 76.0±4.8 kg) participated in the study. Prior to participation, all subjects gave written informed consent in accordance with the local institutional review board. All subjects were free from physician-diagnosed chronic disease. Subjects reported to the imaging center for a consenting and habituation session and an experimental testing session. On the initial visit, subjects read and signed the informed consent document and completed a health-history questionnaire and a magnetic materials safety screening form. Subjects were familiarized with the exercise procedures, their maximum voluntary isometric plantarflexor contraction (MVC) force was measured, and they practiced the contraction protocol. Criteria for an acceptable contraction included reaching peak force rapidly, maintaining a consistent force for 2 s, using proper technique including minimal involvement of accessory muscles, and returning to baseline rapidly. A contraction was considered maximal when the force varied by less than 5% for two subsequent contractions, did not increase with increased coaching or verbal encouragement, and was similar in force to that expected for a healthy individual with no known neuromuscular abnormalities.

To limit the influence of diet and exercise on study outcome variables, subjects were asked to refrain from the following: (i) vigorous exercise for at least 24 h prior to the testing, (ii) consuming caffeinated food or beverages for at least 6 h prior to testing, and (iii) taking any over-the-counter medication 24 h prior to the testing session. Each subject completed a pre-test questionnaire to assess compliance with pre-test conditions. Any subject not in compliance was rescheduled for testing at a later date.

MRI procedures:

Imaging was performed on a Philips Achieva 7T imager/spectrometer (Philips Healthcare, Best, The Netherlands) using a 20 cm, single-channel quadrature, transmit/receive volume coil (Nova Medical, Wilmington, MA, USA). The subject was positioned supine in the scanner with approximately the largest portion of the calf muscle of their dominant leg positioned in the center of the coil. The subject's foot was secured to a custom-built MR-

compatible isometric contraction device capable of measuring plantarflexion force (3,7,21). In addition, subjects were fitted with a custom harness, which, when secured to the patient table, limited translational motion in the foot-head direction during the contractions.

Three-plane localizers and high-resolution anatomical images were acquired to allow consistent planning of the functional images and to guide the placement of regions-of-interest during analysis of the functional images. The posterior compartment of the leg was shimmed using the pencil-beam volume shimming routine.

Functional images were acquired continuously for 14 min [single-slice, single shot, turbo field echo, turbo field echo, echo-planar imaging, TR/TE 1000/18 ms, 18 cm field of view (FOV), 64×64 acquisition matrix, 10 mm slice thickness] while the subjects performed seven, 2 s maximal isometric plantarflexor contractions (one contraction every 2 min). Force data were acquired continuously during the contraction protocol and each subject received verbal feedback after each contraction regarding the level of contraction force. To determine if inflow effects influenced the magnitude of the post-contraction BOLD response, seven subjects repeated the contraction protocol at a TR of 2500 ms. Subjects remained in the scanner without repositioning during the 5 min. rest period between the two contraction protocols.

The longitudinal relaxation time constant (T_1) was measured in four additional subjects using an inversion recovery sequence with a turbo field echo readout. Single-slice data were acquired at approximately the largest cross-sectional area of calf with the following imaging parameters: inversion times (TI) logarithmically spaced between 10 ms and 10 s (16 values), B_1/B_0 insensitive inversion pulse duration = 8 ms, echo time (TE) = 1.10 ms, FOV = 180×180 mm², in-plane resolution = 3×3 mm², slice thickness = 10 mm. To evaluate the effect of TR on the estimates for T_1 , data were acquired at two different pre-delay times (TD) = 2500 and 10,000 ms, where TD is the time from the end of the readout to the next inversion pulse (22).

Data Analysis:

Images were analyzed in MATLAB version 2015a (The MathWorks®, Natick, MA) using custom-written code. Three regions-of-interest (each 2–3 cm² in size) were drawn within the lateral, central, and medial regions of the soleus muscle. An example anatomical image is shown in Figure 1A. Example functional images are shown in Figures 1B,C, with regions-of-interest shown in Figure 1B. Regions-of-interest were specified to avoid visible vessels and connective tissue. The average SI of the three regions-of-interest was measured as a function of time in the functional images and the weighted average was calculated (Figure 1D). Each postcontractile transient was fitted to a ninth order polynomial using MATLAB's *polyfit* function, similar to the procedure described in Reference (23). The peak signal was obtained from the line of best fit and the time-to-peak signal was determined by identifying the inflection in the derivative of the line of best fit. For each post-contraction transient, the baseline was taken as the average SI in the 5 s prior to the contraction. Peak SI was calculated as the highest percentage increase from baseline, excluding the movement artifact (Figure 1D). The post-contraction response was only analyzed if the force of the contraction was within 5% of the previously measured MVC; up to four contractions were analyzed per

subject. Muscle force was calculated as the highest force achieved during each contraction. The coefficient of variation (CV%) in the peak SI was calculated to assess the between- and within-subject dispersion in the peak BOLD response. The T_1 data were fitted in a nonlinear least squares manner to an inversion-recovery with reduced TD model. Unless otherwise stated, data are presented as mean $\pm$ standard error (SE) or coefficient of variation (CV %). Data were analyzed in MATLAB version 2015a. Muscle BOLD responses and contraction forces at the two TR values were compared using a paired Student's t -test and a Wilcoxon signed-rank test was used to compare the T_1 estimates from the two TD values. Statistical significance was set at $p < 0.05$.

RESULTS

For the $TR=1000$ ms imaging sequence, post-contraction muscle BOLD responses ranging from 0.3–7.0% were detected in the soleus muscles of all subjects. For the full sample of 11 subjects, the mean peak bold response was $4.3\pm0.6\%$. The muscle BOLD transients peaked between 8 and 13 s after the contraction with a mean value of 9 ± 1 s. In many cases, the responses also returned to near baseline within 30 s. Figure 1 shows sample anatomical (A), functional (B, C), post-contraction muscle BOLD responses (D) and muscle force data (E) during the isometric muscle contractions. In panel D, the large spikes in SI greater are T_1 -related motion artifacts caused by movement of unsaturated spins into and out of the imaging plane during and after the contraction (1). As can be seen by comparing panels D and E, the saturation artifacts are temporally aligned with muscle force.

The magnitude of the postcontraction muscle BOLD response was not influenced by T_1 -related inflow effects, as evidenced by the similar magnitude of the response at two different TR values (3.8 ± 0.9 vs. $4.0\pm0.6\%$, $TR = 1000$ or 2500 ms, respectively; $n=7$, $p = 0.72$); see sample data in Figure 2. Figure 2 also illustrates sample polynomial fitting results and the location of the peak SI and time-to-peak SI measurements. The muscle force during the two contraction protocols did not differ significantly (647.6 ± 48.7 vs. 664.4 ± 41.3 N for $TR=1000$ and 2500 ms, respectively; $p = 0.47$).

Between-subject variability in the magnitude and time course of the muscle BOLD response is highlighted in Figure 3. Data are shown for the $TR=1000$ ms sequence. The subject in panel A displays a rapid response that peaks within 6–12 s and returns to baseline within 30 s after the contraction. Panel B shows an example of a subject with a small positive phase, followed by a prolonged post-contraction undershoot that reaches a nadir at 60 s post-contraction. The subject in panel C shows similar kinetics to the subject in panel A, but a lower magnitude of response. Like the subject in panel B, the subject depicted panel D also has a minimal peak BOLD response and a prolonged post-contraction undershoot. Although there is significant between-subject variability in the peak BOLD response (CV=50.2%), the response is repeatable within-subjects when the target force is achieved (CV=34.2%; see also Figures 1, panel D and Figure 3, panels E and F).

Figure 4 shows sample inversion recovery plots and images from a single subject acquired at two different TR values. The estimated T_1 s were 1884 ± 7 ms and 1848 ± 17 ms, for a TR of 2500 and 10,000 ms, respectively ($p = 0.125$). Comparable inversion ratios were achieved at

the different TR values (signal fractions = 60.1 ± 1.4 vs. 62.4 ± 0.4 % for 2,500 and 10,000 ms, respectively; $n = 4$). These data suggest T_1 can be readily measured at shorter TR values and this should prove useful when optimizing sequences at 7T.

DISCUSSION

This study demonstrates the feasibility of 7T, muscle BOLD-based studies of the physiological responses to brief isometric contractions. Consistent with previous reports (1,21), this study has also indicated that T_1 -related inflow effects do not directly influence the magnitude of the post-contraction muscle BOLD response. Lastly, this study has revealed considerable inter-individual heterogeneity in the temporal profile of these responses. All subjects had an initial positive muscle BOLD response that, in some subjects, was followed by a substantial negative muscle BOLD response. Given this feasibility, the greater magnitude of BOLD effects at 7T than at 3T (20), and the expected major dependence of 7T BOLD contrast on extravascular BOLD phenomena, 7T muscle BOLD imaging may provide insight into the microvascular and metabolic aspects of muscle contraction.

Post-Contraction muscle BOLD imaging is feasible at 7T

Using an MR compatible ergometer and a standard gradient-echo sequence, we detected muscle BOLD transients between 0.3 and 7.0% in the soleus muscle following 2 s maximal isometric plantarflexion contractions. Hennig *et al.* originally reported a BOLD-like contrast in muscles of the leg following 3 s muscle contractions (24). Subsequent studies confirmed the postcontractile BOLD response (25) and attributed it to intravascular changes in hemoglobin saturation and blood volume (2,21). The findings of the present study are consistent with these and other previous reports (1,3,21,24) showing transient changes in muscle BOLD contrast of up to 8%, peaking ~10–15 s after the muscle contraction. Direct comparison between the present study and these previous studies should be made with caution however, as differences in B_0 field, MRI acquisition parameters (21), and exercise protocols may contribute to differences in the peak change and the time course of post-contraction muscle BOLD signal changes.

The technical challenges of ultra-high field MRI studies have been described previously (26,27). In musculoskeletal imaging, B_0 and B_1 inhomogeneities are particularly problematic due to heterogeneity in the chemical shift and magnetic susceptibility of the tissues in the imaging plane. In this study, we used localized B_0 shimming routines over the posterior compartment of the leg to improve the field homogeneity in this anatomy of interest. The general pattern of lengthening of T_1 , shortening of T_2 , and disproportionate shortening of T_2^* provided additional challenges common at ultra-high fields.

Biophysical aspects of 7T muscle BOLD imaging

Quantitative models (1,2) and experimental data (28) suggest a negligible contribution from extravascular BOLD contrast in muscle at magnetic field strength of 3T or less.

Extravascular BOLD contrast relies heavily on two properties of tissue; 1) the rate of diffusion of water in the tissue, and 2) the geometry of the microvasculature, including the density of vessels and their orientation relative to B_0 (29). In skeletal muscle, the diffusion

of water is rapid ($\sim 1.7 \times 10^{-5} \text{ cm}^2/\text{s}$ (30,31), nearly twice that of brain water (32)). Furthermore, the vasculature in skeletal muscle, which comprises approximately 3% of the tissue volume, is dominated by capillaries with a mean diameter of $\sim 5 \text{ }\mu\text{m}$. The rapid diffusion of water around small vessels effectively averages out the effect of vessel-induced inhomogeneities (1). These features of muscle are present regardless of field strength. However, the magnetic susceptibility difference between the blood and the tissue is proportional to the square of the magnetic field strength, increasing the magnitude of extravascular BOLD effects at ultra-high fields (33,34). Combined with the much shorter T_2^* of blood than of muscle at 7T (~ 9 versus 16 ms, respectively (11,20)), the contribution from intravascular BOLD contrast mechanisms is expected to be small at 7T. Rather, extravascular BOLD mechanisms are expected to dominate.

Several other factors are expected to affect the balance of intravascular and extravascular BOLD contrast in muscle. First, extravascular BOLD contrast is also affected by vessel density and orientation (29,35). Thus, individual differences in vascular anatomy, such as the up-to 30% greater vessel density in chronically endurance trained individuals (36), may cause extravascular BOLD contrast to vary as well. Conversely, in an animal model of diabetes, skeletal muscle capillaries are both smaller and less tortuous than control animals (5). A second factor is the muscle length, when expressed relative to the optimum length for force generation. In mammalian skeletal muscle, the capillaries' geometries depend on muscle length (37). In muscles at resting length or greater, the capillaries are oriented parallel to the long axis of the fibers. At shortened muscle lengths, however, there is significant tortuosity in the capillaries. This interpretation does not negate previous findings of negligible extravascular BOLD contrast in muscle BOLD studies at field strengths of 3T or less (2,28). However, it does suggest that extravascular BOLD contrast may vary with the relative muscle length, and at some muscle lengths extravascular BOLD effects may have an even greater contribution to muscle BOLD contrast at ultra-high field strengths.

In the present study we also evaluated the possible contribution of inflow effects, the apparent T_1 shortening caused by the introduction of unsaturated spins to the slice plane by way of flow, to the post-contraction BOLD signal changes. Although studies at 3T have conducted similar analyses (1,21), the present study should not be viewed as merely confirmatory. Rather, the T_1 values at 7T for blood (38) and muscle (present study) and at 3T for blood (39) and muscle (31) suggest a tissue-specific field strength dependence of T_1 . This merits a specific evaluation of this potential contrast mechanism at 7T. The present findings indicate that for TR value in the range of 1000–2500 ms, inflow effects do not contribute significantly to the post-contraction BOLD signal changes.

Physiological aspects of 7T muscle BOLD imaging

Extravascular BOLD contrast mechanisms are the most likely direct, biophysical influence on the post-contraction muscle BOLD response. However, the primary physiological influence on post-contraction muscle BOLD contrast is likely to be contraction-induced hyperemia. The exact mechanism(s) for exercise hyperemia are not fully understood, and it appears that no single vasodilator is obligatory (40–42). Moreover, the contribution of specific vasodilators to exercise hyperemia may vary depending on the exercise task (43)

and the branch of the vascular tree studied (44). For brief contractions such as these, the hyperemic response is a consequence of many factors. The myogenic effect and the muscle pump contribute to the early phase of exercise hyperemia and result from the contraction-induced increase in intramuscular pressure (45) to values as high as 570 Torr (46). This increase in intramuscular pressure reduces transmural pressure and elicits relaxation of the vascular smooth muscle cells through mechanically-coupled membrane channels (47,48). The increase in intramuscular pressure also empties the venous vasculature and blood flow increases due to the widening of the arterial-venous pressure gradients (49,50).

Other contributors to exercise hyperemia include K^+ (51–54), a yet to be identified endothelium-derived hyperpolarizing factor (55–57), and nitric oxide. It is likely that the hyperemic response to exercise is controlled by a variety of factors whose relative impacts are governed by the contraction duration and intensity. Importantly, myogenic tone, endothelium-derived hyperpolarizing factor, and nitric oxide – three vasodilators prominent in exercise hyperemia – have been implicated in the pathogenesis of obesity and diabetes (55,58,59).

There was considerable between-subject variability in the magnitude and time course post-contraction muscle BOLD response. The peak increase in post-contraction muscle BOLD-weighted SI however was similar to those reported previously despite differences in the duration of contractions, field strength, and the muscle exercised (1,3,7,21). In this study we opted for a muscle contraction of 2 s in order to elicit a larger hyperemic response at a still-low metabolic demand. Previously, we demonstrated that a single 1 s contraction can elicit a 2 to 11-fold increase in blood flow above rest, with the highest flows occurring in chronically active individuals (2). Although not independently verified, many subjects in this study reported some level of physical activity, including an avid distance runner and a college soccer player. Based on these anecdotal reports of the subjects' activity patterns and our previous study (2), it is likely that differences in chronic physical activity levels contributed to the between-subject variability.

In this protocol we allowed for 2 min of recovery between each contraction. In some, but not all subjects, this was sufficient to allow full recovery (for example, Figures 1 and 2/ panels A, C, E and F). In individuals with low muscle oxidative capacity and a time constant for PCr recovery of 45–50 s, the post-contraction response may persist well beyond 2 min. However individuals with faster PCr recovery kinetics/mitochondria on-kinetics would be expected to have a lower peak BOLD and potentially a larger post-contraction undershoot (2). The magnitude of the peak muscle BOLD response aside, the time course of the muscle BOLD response looks strikingly similar across subjects for up to 30 s after the contraction, with peak responses occurring between 8 and 13 s after contraction. However, between 30 and 120 s post-contraction, there is considerable variability in the response. In three of the subjects there was a prominent post-contraction undershoot similar to that seen Figure 2B, D. This large and prolonged post-contraction undershoot makes calculating and interpreting the recovery characteristics difficult using simple measures of signal elevation and suggests that alternative data analysis strategies may be required.

Previous studies either used a shorter rest period between contractions or did not display all of the individual subject data, and so there is no way of knowing if this inter-individual heterogeneity in the post-contraction muscle BOLD signal time course is present in other studies or our unique to ours. Regardless, these findings suggest considerable value in analyzing the kinetics of the response in addition to the peak magnitude. Given the physiologic dependences just described and the predictions of modeling studies about the physiologic determinants of the post-contraction muscle BOLD time course (2), the variability in the kinetics and magnitude of the post-contraction muscle BOLD response at 7T may represent subject-specific differences in microvascular structure and function and/or mitochondrial oxidative capacity. Analyzing the kinetics and amplitudes of the post-contraction positive and negative muscle BOLD responses may provide insight into vascular vs. metabolic dysfunction in diabetes and the metabolic syndrome. Indeed, contraction-induced muscle BOLD imaging has been used to study pathological conditions including obesity and diabetes (7,8).

Summary and conclusions

In summary, muscle BOLD imaging is feasible at 7T and reveals considerable inter-individual variability in the physiological responses to muscle contraction. A sufficient understanding of the contributions from extravascular and intravascular BOLD contrast mechanisms at 7T and how factors such as oxygen supply and demand influence post-contraction muscle BOLD contrast, new insight may be gained into vascular and metabolic aspects of chronic diseases.

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Abbreviations used:

BOLD	blood oxygenation-level dependent
RF	radiofrequency
SI	signal intensity
CNR	contrast-to-noise ratio

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Figure 1.

Sample anatomical imaging, functional imaging, and force production data. A.

Representative anatomical image. B. and C. Functional images acquired at mid-calf from pre-contraction and peak-post contraction phases. In B, the location of the three regions of interest are indicated. D. Weighted average post-contraction signal time course and E.

corresponding muscle force for each contraction. The large spikes in normalized SI are T_1 -related motion artifacts caused by movement of unsaturated spins into and out of the imaging plane during and after the muscle contraction. These data acquired during contraction were not included for analysis of the post-contraction time courses.

Figure 2.

Representative postcontractile muscle BOLD responses for a single subject acquired at two different TR values. The data points are the observed signals and the lines represent the best fit to a ninth order polynomial function. The red point indicates the peak postcontractile BOLD response. There was not a significant difference between the magnitude of postcontractile BOLD response (3.8 ± 0.9 vs. 4.0 ± 0.6 %; $n=7$; $p = 0.72$) when acquired at a TR of 1000 and 2500 ms, respectively.

Figure 3:

Postcontractile BOLD responses for four individual subjects (panels A-D) and three consecutive responses from two other subjects (panels E and F). Panel A-D highlight the between-subjects variations in the muscle BOLD responses, which are likely due to differences in the magnitude and timing of the post-contraction increase in blood flow, the on-kinetics of mitochondrial respiration and or the architecture of the microvasculature in the muscle.

Figure 4:

Inversion recovery curves acquired at two different TR values from the leg at mid-calf and the corresponding images. The estimated TIs were 1884 ± 7 vs 1848.3 ± 2 ms ($n = 4$, $p = 0.125$) and signal fractions were 60.1 ± 1.4 vs. 62.4 ± 0.4 % ($n = 4$) for the 2,500 and 10,000 ms acquisitions respectively.