Simultaneous Measurement of Perfusion and $T_2^*$ in Calf Muscle at 7T with Submaximal Exercise using Radial Acquisition

Sultan Z. Mahmud1*, L. Bruce Gladden2, Andreas N. Kavazis2, Robert W. Motl3, Thomas S. Denney2 & Adil Bashir1

Impairments in oxygen delivery and consumption can lead to reduced muscle endurance and physical disability. Perfusion, a measure of microvascular blood flow, provides information on nutrient delivery. $T_2^*$ provides information about relative tissue oxygenation. Changes in these parameters following stress, such as exercise, can yield important information about imbalance between delivery and consumption. In this study, we implemented novel golden angle radial MRI acquisition technique to simultaneously quantify muscle perfusion and $T_2^*$ at 7T with improved temporal resolution, and demonstrated assessment of spatial and temporal changes in these parameters within calf muscles during recovery from plantar flexion exercise. Nine healthy subjects participated the studies. At rest, perfusion and $T_2^*$ in gastrocnemius muscle group within calf muscle were $5 \pm 2$ mL/100 g/min and $21.1 \pm 3$ ms respectively. Then the subjects performed plantar flexion exercise producing a torque of ~8ft-lb. Immediately after the exercise, perfusion was elevated to $79.3 \pm 9$ mL/100 g/min and $T_2^*$ was decreased by $6 \pm 3\%$. The time constants for 50% perfusion and $T_2^*$ recovery were $54.1 \pm 10$ s and $68.5 \pm 7$ s respectively. These results demonstrate successful simultaneous quantification of perfusion and $T_2^*$ in skeletal muscle using the developed technique.

Muscle weakness and fatigue are common manifestations of a variety of diseases that decrease mobility such as multiple sclerosis (MS), Parkinson’s disease (PD), and aging1–4. The underlying causes of muscle weakness and fatigue are not completely understood. It has been shown that impaired cellular energy production plays an important role in muscle fatigue5. Cells require adequate supplies of oxygen (O2) and nutrients for energy production, and deficiency in O2 supply and extraction may reduce cellular energy production required for muscle function. Accordingly, measurements of O2 delivery and microvascular oxygenation are of considerable interest and have the potential to provide useful understanding of the biochemical mechanisms of decreased mobility in disease and the assessment of treatment targets and responses.

Perfusion is the delivery of tissue mass normalized rate of blood, which also accommodates the supply of O2 and other nutrients to the tissue. Quantitative perfusion in skeletal muscle can be measured non-invasively using the arterial spin labeling (ASL) MRI technique. This technique has been used to study muscle perfusion in humans with ischemia- in reperfusion and exercise paradigms6–8. These studies have demonstrated that skeletal muscle perfusion dynamics are capable of providing insights into pathological and physiological functions6–8.

Blood oxygenation level-dependent (BOLD) contrast has been used to infer changes in blood oxygenation in skeletal muscle in response to various stimuli9. Muscle BOLD contrast results primarily from changes in the total amount of deoxyhemoglobin in the imaging volume10. The decrease in the deoxyhemoglobin content in a voxel causes a decrease in susceptibility hence increased signal intensity in the gradient echo BOLD MRI images. The BOLD signal largely depends on the changes in $T_2^*$. Quantitative $T_2^*$ maps may provide more specific physiological information about muscle oxygen extraction in response to stimuli.

Oxygen delivery and demand are tightly coupled in exercising muscle and this coupling might be compromised in pathologic state11. Combined measurement of dynamic perfusion and $T_2^*$ in skeletal muscle has the...
potential to provide important information about oxidative metabolism and functional deficits. Studies of combined quantification of perfusion and T2* or other markers of tissue oxygenation have been implemented for reactive hyperemia6,14,15, but there has been no demonstration of this technique for submaximal exercise (functional hyperemia) at 7T using radial acquisition. We chose functional hyperemia because it is more physiologically analogous to the demands of daily living than reactive hyperemia. High signal to noise advantage of 7T and low susceptibility to bulk motion due to radial acquisition will allow more robust determination of perfusion and T2* in dynamic studies with higher temporal resolution. Although several challenges, such as magnetic field inhomogeneity, need to be overcome at ultra-high filed studies, but improved SNR and improved contrast at ultra-high field benefits the dynamic measurement of ASL and T2*.

The main goal of this study is to develop and demonstrate the feasibility of a technique to simultaneously determine dynamic changes in perfusion and T2* in calf muscle at improved temporal resolution with an exercise stimulus. High temporal resolution is important for detection of significant changes in these metabolism related parameters following exercise. To accomplish this aim, we developed a novel interleaved golden angle radial MRI pulse sequence and quantified muscle perfusion and T2*. We built an MRI compatible plantar flexion exercise device and demonstrated the assessment of spatial and temporal changes in these parameters in calf muscle during recovery from plantar flexion exercise. Combined measurements can not only reduce experiment time but also several types of information can be obtained with same stimulus.

Methods

The study was approved by the Auburn University Institutional Review Board (IRB). All experiments were performed in accordance with the IRB guidelines and regulations. Informed consent was provided by each subject prior to participation in the study.

Recruitment. All experiments were done on a Siemens 7T Magnetom (Erlangen, Germany) using a surface coil. Nine healthy volunteers (six females), age = 28 ± 12 years, and weight = 67 ± 8 kg, participated in the study. Inclusion criteria was normal range of motion (>20°) for ankle joint flexion.

Imaging sequence. Flow alternating inversion recovery (FAIR) is an established ASL method in which slice-selective (SS) and non-selective (NS) inversion pulses are used to acquire tag and control image6. Saturation inversion recovery (SATIR) is a variant of FAIR with short recovery time acquisition, thus allowing for fast dynamic measurements17. The dead time between tagging and acquisition (post labeling delay (PLD)) during perfusion imaging can be used to interleave a gradient echo (GRE) imaging loop to acquire multi-echo images from a slice posterior to the perfusion imaging slice. These multi-echo GRE images are used to quantify T2*. This interleaved approach allows simultaneous measurement of perfusion and T2* with a single scan.

Schematic of pulse sequence is shown in Fig. 1. ASL sequence SATIR was implemented using adiabatic full passage hyperbolic secant inversion pulse for spin tagging with golden angle radial readout (Fig. 1- slice 1). The PLD was used for interleaved acquisition of T2* data for a slice located 3 cm distally from the perfusion slice (Fig. 1 - slice 2). A multi-echo GRE sequence with radial acquisition was used for T2* mapping. Temporal resolution for perfusion and T2* was approximately 1.3 seconds.
Phantom experiment. Initial experiments were done in phantoms to optimize radial image reconstruction at 7T and validate measurements from the new sequence.

Shift estimation and correction. In radial acquisition, the read-out orientation is changed to different angles for each k-space line to acquire a set of radial projections. Gradient imperfection and eddy current effects cause delays in gradient timing and have an angle dependent effect on the projections. This causes the center of acquired projections to shift yielding artifacts in reconstructed images. We characterized these gradient imperfections in phantoms by collecting projections for x-gradient along angles 0° and 180° and y-gradient along angles 90° and 270°. A delay in gradient timing causes a spatial shift in two projections acquired with opposite gradient polarity. This shift was corrected as described below for x-axis.

\[ \Delta k_x = \Delta k_x \cos^2(\theta) + \Delta k_x \cos^2(\theta) \] (1)

Agarose phantoms at 0.5, 1, 2, 3, 4 and 5% concentration were prepared in 50 mL vials to validate perfusion and \( T_2^* \) measurements. The phantoms were prepared by mixing Agarose powder with water, then heated until boiling. New interleaved pulse sequence was used to determine \( T_2^* \) and perfusion and the results were compared to the results from vendor provided multi-echo GRE pulse sequence.

In vivo study design. After scanner adjustments, perfusion and \( T_2^* \) data were acquired in the calves of the participants at rest. A \(^{3}H/^{31}P\) surface coil (RAPID Biomedical, Rimpar, Germany) with single channel compatible with Siemens Magnetom 7T system was used for the study. The maximum average transmit power of \(^{1}H\) coil was 11.5 W with quadrature RF polarization as per specification. The mean coil dimension of \(^{1}H\) double-loop was 140 mm × 225 mm.

A 6 ms long slice selective (SS) and non-selective (NS) hyperbolic secant adiabatic inversion pulse was used to acquire tag and control images in an interleaved fashion. The inversion efficiency was tested in phantoms as well as in vivo, and a voltage of 500 V was selected for satisfactory inversion slice profile (Figs. S.1 and S.2 in Supplementary Information). Specific common acquisition parameters for perfusion and \( T_2^* \) were: FOV = 192 mm, slice thickness = 5 mm, TR = 1.28 s, flip angle = 15°, bandwidth = 500 Hz/pixel and 64 radial projections. Perfusion PLD = 1 s. TE = 2.2 ms for perfusion and TE = 2.2, 5.0, 7.8, 10.6 and 13.4 ms for GRE sequence.

The subjects then performed 2 minutes of plantar flexion at 0.5 Hz using a home-built MRI compatible exercise device. The knee angle was 0° (aligned with the scanner table) during exercise and the leg was held in place with Velcro straps to prevent bulk motion. The paddle of the device was strapped to an adjustable support with Velcro straps to prevent bulk motion. The paddle of the device was fixed to an adjustable support with Velcro straps to prevent bulk motion.

It can be shown from SATIR signal derivation that

\[ M_{SS}(t) + M_{NS}(t) = M_0 \left( 2 - 2e^{-\frac{t}{T_1}} \right) \] (2)

where, \( M_{SS} \) and \( M_{NS} \) are slice selective and non-selective acquisitions respectively, \( M_0 \) is the tissue magnetization at equilibrium, \( T_1 \) is tissue spin-lattice relaxation time constant. As SATIR approach assumes constant \( T_1 \) for all perfusion values, Eq. (2) shows that, if the signals are corrected for the \( T_2^* \) weighting, the summation of SS and NS signal is approximately constant regardless of the perfusion value. NS value during recovery period can be estimated by subtracting the recovery SS value from the (SS+NS) value at rest. Not acquiring NS images during recovery from exercise results in improved temporal resolution. The recovery NS signal can be estimated using

\[ M_{NS,rec}(t) = (M_{SS,rest} + M_{NS,rec}) \left( \frac{1}{T_{1,rec}} - \frac{1}{T_{2,rec}} \right) - M_{SS,rec}(t) \] (3)

where \( M_{SS,rest} \) and \( M_{SS,rec} \) are NS and SS acquisitions respectively at rest, \( M_{NS,rec} \) and \( M_{SS,rec} \) are NS and SS signals respectively during recovery post-exercise, \( T_{1,rec} \) and \( T_{2,rec} \) are \( T_2^* \) values at rest and during recovery respectively.

Perfusion weighted images were generated from the differences between slice selective and non-selective images at rest. The muscle area activated with high perfusion due to exercise was identified from the perfusion.
weighted images immediately after exercise. From this muscle activation map a region of interest (ROI) was selected to calculate the perfusion using

$$f = -\frac{\lambda}{T} \ln \left[ \frac{M_{SS}(T) - M_{NS}(T)}{M_{SS}(T) + M_{NS}(T)} \left( 1 - e^{\frac{T}{T_1}} \right) + 1 \right]$$

(4)

where $f$ is the perfusion (mL/100 g/min), $\lambda$ is tissue partition coefficient (0.9 mL/g), $T$ is tagging time (or PLD), $T_1$ for tissue is 1.55 sec. at 7T. Dynamic change in perfusion was measured as a change from the resting perfusion during the recovery period.

$T_2^*$ was calculated by fitting a mono-exponential function to the magnitude signal intensity from multi-echo data using

$$A = A_0 e^{-\frac{TE}{T_2^*}}$$

(5)

$T_2^*$ was normalized to the resting $T_2^*$ values for each individual subject. This was done to account for differences in $T_2^*$ between subjects due to physiological variances or different shimming.

Results
Phantom experiment. Radial image reconstruction without correction of gradient delay errors showed artifacts (Fig. 2a). Two vials placed on the upper side of a box-shaped phantom were completely missing in the reconstructed image without delay correction. $0^\circ$ and $180^\circ$ calibration projections resulted in a sample shift of 0.595 along $k_x$ and $90^\circ$ and $270^\circ$ projections resulted in a sample shift of 0.696 along $k_y$.

In vivo calibration data ($\Delta k_x = 0.588$ and $\Delta k_y = 0.689$) was similar to that from phantom experiments. The artifacts in the reconstructed image were removed and the vials were visible after accounting for gradient delay errors (Fig. 2b). This same protocol was used for all imaging studies in vivo.

Phantom study. Figure 3 shows six agar phantoms with different concentrations. Perfusion image of the phantom shows noise indicating that the presence of second slice for $T_2^*$ has no spillover effect on the perfusion slice. Excellent correlation was found between the two $T_2^*$ values measured by standard multi-echo GRE sequence and new interleaved sequence with $r^2 = 0.98$. The Bland-Altman plot also shows very good agreement between two methods with mean difference $= -0.12 \pm 1.15$.

In vivo. Representative locations of perfusion (slice 1) and $T_2^*$ (slice 2) are shown in Fig. 4a. At rest, the gastrocnemius muscle has the highest perfusion $= 5 \pm 2$ mL/min/100 g. Sample SS signal time course from gastrocnemius muscle during recovery following plantar flexion exercise is shown in Fig. 5a. As SS, NS, $T_2^*$ signals were acquired at rest and only SS, $T_2^*$ were measured during recovery. Eq. (3) was used to estimate the NS signal during recovery period. Finally, Eq. (4) was used to calculate the perfusion. Figure 5b shows the sample perfusion time courses calculated with and without NS acquisition, which manifests that perfusion can be estimated with great accuracy even without the NS acquisition. Figure 4b,c show the perfusion results from plantar flexion exercise. Quantitative perfusion maps are overlaid on the anatomical images indicating the regions of highest change from rest (Fig. 4b). Immediately after exercise, the gastrocnemius muscle showed the highest increase in perfusion. A region of interest (ROI) corresponding to the activated muscle regions identified on the perfusion maps was manually traced on the images and the mean change in perfusion is shown in Fig. 4c. Immediately after exercise, the perfusion in the most activated area of the muscle increased to $79.3 \pm 9$ mL/min/100 g and decreased slowly to baseline after a relatively fast initial decline during post-exercise rest. Average time constant for 50% perfusion recovery was $54.1 \pm 10$ s. Figure 6a,b show the synopsis of change in perfusion in different
Figure 3. (a) Image of the phantom. (b) Perfusion map showing absence of perfusion in phantom. (c) Correlation plot showing excellent correlation between $T_2^*$ calculated from new sequence and GRE sequence. (d) Bland-Altman plot also showing very good agreement between two sequences.

Figure 4. Two slice locations for perfusion and $T_2^*$ on calf muscle (a). Slice 1 is perfusion slice and slice 2 is $T_2^*$ slice. Increased perfusion activated area on corresponding perfusion slice (b) and average perfusion with standard deviation of all the subjects during recovery period from selected ROI (green circle) (c). Decreased $T_2^*$ activated area on corresponding $T_2^*$ slice (d) and average relative $T_2^*$ with standard deviation of all subjects during recovery period from selected ROI (e).
Figure 5. Sample slice selective (SS) signal time course from gastrocnemius muscle during recovery following exercise (a) and corresponding perfusion calculated without the non-selective (NS) acquisition plotted together with a sample perfusion calculated with NS acquisition (b).

Figure 6. Different muscle groups in perfusion slice (a) and corresponding muscle perfusion (b) during recovery period. Same muscle groups in T2* slice (c) and corresponding muscle T2* (d) during recovery period.

muscle groups – gastrocnemius, soleus and peroneus muscles within calf muscle during recovery from exercise. The perfusion in soleus and peroneus muscles are not affected as much as gastrocnemius muscle due to plantar flexion exercise protocol used in this study.

Sample T2* curve fitting from one subject at rest is shown in Fig. S3 (Supplementary Information). Figure 4d shows overlay of change in T2* after plantar flexion exercise indicating regions of muscle activation. Change in T2* was determined from the difference between the first and last T2* map generated during the recovery period. A region of interest (ROI) was selected to observe the relative change of T2* (normalized by the resting T2*) and is shown in Fig. 6c. T2* decreased by 6 ± 3% immediately after exercise and recovered as the subjects rested. At the end of data acquisition time, T2* did not return to baseline (resting value) and was still off by 2 ± 0.7%. Average time constant for 50% recovery from minimum value to this peak value was 68.5 ± 7 s. Figure 6c,d show the synopsis of change in T2* in different muscle groups – gastrocnemius, soleus and peroneus muscles within calf muscle during recovery from exercise. It shows that T2* of soleus and peroneus muscles are not affected as much as gastrocnemius muscle and return approximately to baseline while gastrocnemius muscle T2* remains elevated.
by the end of acquisition time. Table 1 shows perfusion and $T_2^*$ values from the most activated muscle area due to exercise i.e. gastrocnemius muscle of all the subjects.

### Discussion

We have demonstrated the feasibility of simultaneous quantification of changes in perfusion and $T_2^*$ in lower leg following plantar flexion exercise. The sequence was tested and validated in phantoms before in vivo studies. Perfusion measurement without the NS acquisition was also compared with the one measured with the NS acquisition. The temporal resolution of the sequence allowed us to measure dynamic changes in perfusion and $T_2^*$ as the muscle recovered from the submaximal exercise. We used plantar flexion exercises because it is a simple exercise protocol and physiologically more relevant than reactive hyperemia. The changes in post exercise perfusion and $T_2^*$ values is in general agreement with values from previous reported studies. This pulse sequence and exercise protocol provides a unique ability to investigate different aspects of muscle metabolic function (i.e., regional perfusion and tissue blood oxygenation). Although some studies reported simultaneous measurement of perfusion and $T_2^*$ weighted signal at 7T, but none of them measured quantitative $T_2^*$ values. $T_2^*$ weighted signal, which can also be termed as BOLD signal, is dependent on the change in $T_2^*$ as well as the initial signal intensity, which is influenced by parameters such as inflow, baseline drifts and changes in $T_2^*$. Measurement of $T_2^*$ enables to separate the oxygenation related changes. Moreover, the advantage of our current technique compared to the other available techniques is higher temporal resolution, which is vital for measuring notable changes in these metabolism-related parameters following exercise.

Exercise increased the blood flow to the stimulated tissue. Perfusion measurement without the NS acquisition was compared with the one measured with NS acquisition from one subject in vivo during recovery following exercise, which shows that our current technique can measure perfusion with excellent accuracy. The muscle perfusion immediately at the end of exercise increased to 79.3 ± 60.7 mL/min/100 g from a resting value of 5 ± 2 mL/min/100 g. Prior studies with similar exercise protocols reported a peak perfusion in the range of 60 to 84 mL/min/100 g in gastrocnemius muscle, in close agreement with our results. The difference in peak perfusion could be due to different work rates during the exercise protocol and selection of ROI. Lower perfusion values have been reported following reactive hyperemia. This is expected because contraction elicits exercise dependent metabolic demand and increased flow directed to capillaries supplying the metabolically active tissue, whereas during reactive hyperemia the flow is elevated due to reduced vascular resistance caused by ischemia. Perfusion recovery is also in agreement with previous reports with similar exercise protocol.

Another study reported slower recovery of perfusion after exercise, similar to recovery reported following anaerobic exercise. It is difficult to speculate the difference in recovery rates between studies since exercise protocol, metabolic state of the tissue under examination or biological differences between study participants may affect the recovery rate.

$T_2^*$ at 7T is reported to be approximately 22 ms and our resting values are in agreement with previous studies. However magnetic field inhomogeneity and shim quality will affect the baseline $T_2^*$, as another previous study reported shorter $T_2^*$ of ~17 ms at 7T. We normalized the post exercise recovery $T_2^*$ in each subject with the baseline $T_2^*$ values to eliminate these scanner dependent variations and allow for comparison between subjects. A lower value of $T_2^*$ is observed immediately after exercise and indicates deoxygenation of activated muscle; i.e., a decrease in venous oxygen saturation. As $T_2^*$ starts to recover it goes to a peak value of approximately 2% higher than the baseline (resting value). Previous studies reported similar kind of $T_2^*$ response during recovery following exercise. This is also in agreement with BOLD studies in which the BOLD signal immediately decreased at the onset of exercise, stayed low during exercise and only recovered when the muscle was allowed to rest at the completion of exercise. A drop in $T_2^*$ weighted signal during exercise followed by a rise of the $T_2^*$ weighted signal before the end of the exercise in gastrocnemius muscle was reported in a previous study, which was probably caused by a falling pH. This can also be attributed to the fact that two competing factors, increase in flow and oxygen extraction to meet increased metabolic demand due to exercise, and rapid decrease in partial oxygen pressure in active muscle contribute to the $T_2^*$ weighted signal change. Also, activity level can

### Table 1.

| Subject | Perfusion (mL/min/100 g) | 50% Recovery time constant (s) | $T_2^*$ (ms) | Minimum relative $T_2^*$ | 50% Recovery time constant (s) |
|---|---|---|---|---|---|
| 1 | 83.1 | 44.5 | 20.49 | 0.96 | 67.2 |
| 2 | 88.6 | 66.1 | 21.23 | 0.99 | 58.6 |
| 3 | 78.3 | 41.9 | 23.27 | 0.94 | 64.3 |
| 4 | 84.2 | 49.3 | 24.58 | 0.91 | 71.1 |
| 5 | 72.6 | 45.6 | 19.73 | 0.96 | 73.7 |
| 6 | 91.1 | 71.1 | 22.25 | 0.88 | 81.6 |
| 7 | 67.8 | 51.2 | 18.31 | 0.97 | 65.4 |
| 8 | 77.1 | 56.2 | 17.92 | 0.95 | 69.4 |
| 9 | 76.3 | 60.7 | 23.4 | 0.90 | 70.8 |
| mean ± SD | 79.3 ± 9 | 54.1 ± 10 | 21.1 ± 3 | 0.94 ± 0.03 | 68.5 ± 7 |
impact $T_2^*$ weighted signal and $T_2^*$ weighted signal represents all potential contributions to signal loss, including $T_1$ effects, $T_2$ effects and magnetic field inhomogeneities. Following exercise, the perfusion in activated muscle is increased which also affects local blood volume, which is expected to have pronounced effect on BOLD signal. In future with carefully designed exercise paradigms it may be possible to separate the effect of $T_2^*$ changes due to perfusion and oxygen extraction.

There are a few limitations to the existing study. In order to calculate perfusion, the SATIR approach assumes that the muscle and blood $T_1$ are same. Measurement of blood and muscle $T_1$ at rest and during recovery post-exercise is not practical. Hence all previous reports using SATIR approach made the assumption that blood and tissue $T_1$ are equal. Different $T_1$ values for muscle ($T_1 = 1.55 \sec$) and blood ($T_1 = 2.08 \sec$) have been reported at 7T. Simulation using the reported $T_1$ values for muscle and blood shows that the error introduced in perfusion measurement is not expected to exceed 7%. Another issue of assuming constant $T_1$ throughout the recovery period post-exercise, as implemented by SATIR approach, may affect perfusion estimation as $T_1$ is expected to change due to the change in blood oxygenation levels. The maximum TE for multi echo GRE acquisition should be $\geq T_2^*$. Our maximum TE affected $T_2^*$ quantification with a maximum error <4%, which was validated in phantom experiment. Also, some recent works showed that muscle metabolic and oxidative capacity varies along the length of the muscle even within the same muscle group, which was also observed as a gradient in $T_2^*$ weighted signals between two distal slice locations following exercise. This suggests that even though the $T_2^*$ slice should have similar kind of $T_2^*$ response as the perfusion slice, but there might still be a gradient between the actual numbers. We did not quantify the actual work done during the exercise protocol. Metabolic demand for the tissue provides a feedback signal for increase in blood supply hence it is important to get a measure of work done by the muscles. We also used a surface coil for this study. Since there is no body coil in for 7T, we used the surface coil for both transmit/receive, which compromised the signal to noise from muscle groups at a distance from the coil. A knee coil or other volume coil placed around the calf would improve signal to noise and provide better homogeneous coverage. The plantar flexion device was not compatible with the knee coil due to size restrictions. In future, a new design of the plantar flexion device would allow the study to be conducted with the knee coil. We did not validate the perfusion measurements with an identical ASL sequence without multi-echo GRE loop. This would have required two separate exercise protocols. Difference in the exercise work rate during the two exercise protocols or fatigue can potentially make the comparison difficult. We did test in the phantom that the presence of the multi-echo loop did not affect the perfusion slice data (Fig. 3b). In the present study we evaluated the effect of perfusion loop on the $T_2^*$ measurements and did not find any evidence that perfusion slice affects the measurement of $T_2^*$ for at slice 3 cm distal location.

**Conclusion**

This study demonstrates the ability to simultaneously quantify skeletal muscle perfusion and $T_2^*$, both at rest and dynamically during post exercise recovery in calf muscle at 7T using radial acquisition at high temporal resolution. An interleaved golden angle radial acquisition pulse sequence was implemented for the study which helps reduce bulk motion artifacts. The temporal and spatial resolution of the protocol was sufficient to measure changes in metabolism-related parameters post exercise. Regulated plantar flexion exercise isolates the calf muscles and might provide valuable insight into pathophysiological processes independent of impaired heart function. Accordingly, the information provided by this technique may prove to be very valuable in understanding muscle metabolism in healthy subjects as well as patient populations who experience mobility disability such as MS, PD, or older adults. This later observation will be an important next step in this line of research.

Received: 14 December 2019; Accepted: 19 March 2020;
Published online: 14 April 2020

**References**

1. Finsterer, J. & Mahjoub, S. Z. Fatigue in Healthy and Diseased Individuals. *Am J Hosp Palliat Care* 31, 562–575, https://doi.org/10.1177/1081596514547849 (2014).
2. Kluger, B. M., Krupp, L. B. & Enoka, R. M. Fatigue and fatigability in neurologic illnesses Proposal for a unified taxonomy. *Neurology* 80, 409–416, https://doi.org/10.1212/WNL.0b013e318283a9e8 (2013).
3. Heesen, C. et al. Fatigue in multiple sclerosis: an example of cytokine mediated sickness behaviour? *J Neurol Neurosurg Psychiatry* 77, 34–39, https://doi.org/10.1136/jnnp.2005.065805 (2006).
4. Baksh, R. Fatigue associated with multiple sclerosis: diagnosis, impact and management. *Mult Scler* 9, 219–227, https://doi.org/10.1191/1352458503ms904oa (2003).
5. Enoka, R. M. & Duchateau, J. Muscle fatigue: what, why and how it influences muscle function. *J Physiol-London* 586, 11–23, https://doi.org/10.1113/jphysiol.2007.139477 (2008).
6. Dutel, S. et al. Influence of vascular filling and perfusion on BOLD contrast during reactive hyperemia in human skeletal muscle. *Magn Reson Med* 55, 450–454, https://doi.org/10.1002/mrm.20760 (2006).
7. Pollak, A. W. et al. Arterial Spin Labeling MR Imaging Reproducibly Measures Peak-Exercise Calf Muscle Perfusion A Study in Patients With Peripheral Arterial Disease and Healthy Volunteers. *JACC-Cardiovasc Imaging* 5, 1224–1230, https://doi.org/10.1016/j.jcmg.2012.03.022 (2012).
8. Grozinger, G. et al. Perfusion measurements of the calf in patients with peripheral arterial occlusive disease before and after percutaneous transluminal angioplasty using MR arterial spin labeling. *J Magn Reson Imaging* 40, 980–987, https://doi.org/10.1002/jmri.24463 (2014).
9. Wu, W. C. et al. Skeletal muscle microvascular flow in progressive peripheral artery disease: assessment with continuous arterial spin-labeling perfusion magnetic resonance imaging. *J Am Coll Cardiol* 53, 2372–2377, https://doi.org/10.1016/j.jacc.2009.03.033 (2009).
10. Englund, E. K. et al. Multiparametric assessment of vascular function in peripheral artery disease: dynamic measurement of skeletal muscle perfusion, blood-oxygen-level dependent signal, and venous oxygen saturation. *Circ Cardiovasc Imaging* 8, https://doi.org/10.1161/CIRCIMAGING.114.002673 (2015).
11. Elder, C. P., Cook, R. N., Chance, M. A., Copenhaever, E. A. & Damon, B. M. Image-Based Calculation of Perfusion and Oxyhemoglobin Saturation in Skeletal Muscle During Submaximal Isometric Contractions. *Magn Reson Med* **64**, 852–861, https://doi.org/10.1002/mrm.22475 (2010).

12. Donahue, K. M. et al. Simultaneous gradient-echo/spin-echo EPI of graded ischemia in human skeletal muscle. *J Magn Reson Imaging* **8**, 1106–1113, https://doi.org/10.1002/jmri.188000815 (1998).

13. Oliveira, M. F. et al. Does impaired O2-delivery during exercise accentuate central and peripheral fatigue in patients with coexistent COPD-CHF? Frontiers in Physiology **5**, ARTN 514, 10.3389/fphys.2014.00514 (2015).

14. Lebon, V., Carlier, P. G., Brillaut-Salvat, C. & Leroy-Willig, A. Simultaneous measurement of perfusion and oxygenation changes using a multiple gradient-echo sequence: application to human muscle study. *Magn Reson Imaging* **16**, 721–729, https://doi.org/10.1016/s0730-725x(98)00088-5 (1998).

15. Englund, E. K. et al. Combined measurement of perfusion, venous oxygen saturation, and skeletal muscle T2-* during reactive hyperemia in the leg. *J Cardiovasc Magn Res* **15**, ArtN 70 10.1186/1532-429X-15-70 (2013).

16. Kim, S. G. & Tsekos, N. V. Perfusion imaging by a flow-sensitive alternating inversion recovery (FAIR) technique: application to functional brain imaging. *Magn Reson Med* **37**, 425–435, https://doi.org/10.1002/mrm.1910370321 (1997).

17. Raynaud, J. S. et al. Determination of skeletal muscle perfusion using arterial spin labeling MRI: Validation by comparison with venous occlusion plethysmography. *Magn Reson Med* **46**, 305–311, https://doi.org/10.1002/mrm.1192 (2001).

18. Peters, D. C., Derbyshire, I. A. & McVeigh, E. R. Centering the projection reconstruction trajectory: reducing gradient delay errors. *Magn Reson Med* **50**, 1–6, https://doi.org/10.1002/mrm.10501 (2003).

19. Block, K. & Uecker, M. Simple method for adaptive gradient-delay compensation in radial MRI. In *Proceedings of 19th Annual Meeting of ISMRM, Quebec, Canada* (2011).

20. Armstrong, T. et al. Free-breathing liver fat quantification using a multiecho 3D stack-of-razial technique. *Magn Reson Med* **79**, 370–382, https://doi.org/10.1002/mrm.26693 (2018).

21. Jordan, C. D., Saranathan, M., Bangertler, N. K., Hargreaves, R. A. & Gold, G. E. Musculoskeletal MRI at 3.0 T and 7.0 T: A comparison of relaxation times and image contrast. *Eur J Radiol* **82**, 734–739, https://doi.org/10.1016/j.ejrad.2011.09.021 (2013).

22. Schwesow, K. et al. Dynamic ASL and T2-weighted MRI in exercising calf muscle at 7 T: A feasibility study. *Magn Reson Med* **73**, 1190–1195, https://doi.org/10.1002/mrm.23242 (2015).

23. Niess, F. et al. Interleaved P-31 MRS/F-1 ASL for analysis of metabolic and functional heterogeneity along human lower leg muscles at 7T. *Magn Reson Med*, https://doi.org/10.1002/mrm.28088 (2019).

24. Muller, M. D. et al. Muscle oxygenation during dynamic plantar flexion exercise: combining BOLD MRI with traditional physiological measurements. *Physiol Rep* **4**, https://doi.org/10.1484/PHY.1.20004 (2016).

25. Ledermann, H. P. et al. Blood oxygenation level-dependent magnetic resonance imaging of the skeletal muscle in patients with peripheral arterial occlusive disease. *Circulation* **113**, 2929–2935, https://doi.org/10.1161/Circulationaha.105.507717 (2006).

26. Schulte, A. C., Speck, O., Oesterle, C. & Hennig, J. Separation and quantification of perfusion and BOLD effects by simultaneous acquisition of functional I-0- and T-2*-parameter maps. *Magn Reson Med* **45**, 811–816, https://doi.org/10.1002/mrm.1109 (2001).

27. Speck, O. & Hennig, J. Functional imaging by I-0- and T-2*-parameter mapping using multi-image EPI. *Magn Reson Med* **40**, 243–248, https://doi.org/10.1002/mrm.1910400210 (1998).

28. Wray, D. W. et al. Multparametric NMR-Based Assessment of Skeletal Muscle Perfusion and Metabolism During Exercise in Elderly Persons: Preliminary Findings. *J Gerontol a-Biol Sci Med Sci* **64**, 968–974, https://doi.org/10.1093/gerona/gpl044 (2009).

29. Englund, E. K. et al. Simultaneous measurement of macro- and microvascular blood flow and oxygen saturation for quantification of muscle oxygen consumption. *Magn Reson Med* **79**, 846–855, https://doi.org/10.1002/mrm.26744 (2018).

30. Englund, E. K. et al. Combined measurement of perfusion, venous oxygen saturation, and skeletal muscle T2-* during reactive hyperemia in the leg. *J Cardiovasc Magn Res* **15**, 70, https://doi.org/10.1186/1532-429X-15-70 (2013).

31. Towse, T. F. et al. Comparison of muscle BOLD responses to arterial occlusion at 3 and 7 Tesla. *Magn Reson Med* **75**, 1333–1340, https://doi.org/10.1002/mrm.25562 (2016).

32. Cain, S. M. & Chapler, C. K. O-2 Extraction by Canine Hindlimb during Alpha–Adrenergic Blockade and Hypoxic Hypoxia. *J Appl Physiol* **48**, 630–635 (1980).

33. Kinganvivlak, C. E., Curtis, S. E., Mewburn, J. D., Cain, S. M. & Chapler, C. K. Role of Endothelial Factors in Active Hyperemic Responses in Contracting Canine Muscle. *J Appl Physiol* **79**, 107–112 (1995).

34. Schafrartzik, W. et al. Effect of Reduced Hemoglobin Concentration on Leg Oxygen-Uptake during Maximal Exercise in Humans. *J Appl Physiol* **75**, 491–498 (1993).

35. Poole, D. C. & Richardson, R. S. Determinants of oxygen uptake - Implications for exercise testing. *Sports Med* **24**, 310–322 (1997).

36. Li, Z. J. et al. Dynamic characteristics of T2-* weighted signal in calf muscles of peripheral artery disease during low-intensity exercise. *Journal of Magnetic Resonance Imaging* **46**, 40–48, https://doi.org/10.1002/jmri.25532 (2017).

37. Stacy, M. R. et al. Comparison of regional skeletal muscle tissue oxygenation in college athletes and sedentary control subjects using quantitative BOLD MR imaging. *Physiological Reports* **4**, UNSP e12903 10.14814/phy2.12903 (2016).

38. Zhang, X. et al. In vivo blood T1(1) measurements at 1.5 T, 3 T, and 7 T. *Magn Reson Med* **70**, 1082–1086, https://doi.org/10.1002/mrm.24550 (2013).

39. Boss, A. et al. Oxidative capacity varies along the length of healthy human tibialis anterior. *J Physiol-London* **596**, 1467–1483, https://doi.org/10.1113/jp275009 (2018).

Author contributions

Experiments, data analysis and manuscript preparation: S.Z.M.; Supervision of the study, experiments, edited the manuscript and conceived the study: A.B.; Edited the manuscript and conceived the study: T.S.D., R.W.M., L.B.G., A.N.K.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-63009-4.

Correspondence and requests for materials should be addressed to S.Z.M.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
