Eccentric exercise slows in vivo microvascular reactivity during brief contractions in human skeletal muscle

Larsen, Ryan G.; Hirata, Rogerio P.; Madzak, Adnan; Frøkjær, Jens B.; Graven-Nielsen, Thomas

Published in:
Journal of Applied Physiology

DOI (link to publication from Publisher):
10.1152/japplphysiol.00563.2015

Publication date:
2015

Document Version
Accepted author manuscript, peer reviewed version

ECCENTRIC EXERCISE SLOWS IN VIVO MICROVASCULAR REACTIVITY DURING BRIEF CONTRACTIONS IN HUMAN SKELETAL MUSCLE

Ryan G. Larsen[1], Rogerio P. Hirata[2], Adnan Madzak[3], Jens B. Frøkjær[3,4], Thomas Graven-Nielsen[2]

[1] Physical Activity and Human Performance Group, SMI, Department of Health Science and Technology, Aalborg University, Denmark

[2] Center for Neuroplasticity and Pain (CNAP), SMI, Department of Health Science and Technology, Aalborg University, Denmark

[3] Department of Radiology, Aalborg University Hospital, Denmark

[4] Department of Clinical Medicine, Aalborg University, Denmark

Running title: Slowed microvascular reactivity after eccentric exercise

Keywords: blood oxygen level dependent (BOLD), hyperemia, muscle damage

Correspondence:

Ryan Godsk Larsen, Ph.D.

Department of Health Science and Technology, Aalborg University

Fredrik Bajers Vej 7, 9220 Aalborg Ø, Denmark

E-mail: rl@hst.aau.dk

Phone: +45 52508061

ABSTRACT

Unaccustomed exercise involving eccentric contractions results in muscle soreness and an overall decline in muscle function, however little is known about the effects of eccentric exercise on microvascular reactivity in human skeletal muscle. Fourteen healthy men and women performed eccentric contractions of the dorsiflexor muscles in one leg, while the contralateral leg served as control. At baseline, 24h and 48h after eccentric exercise the following were acquired bilaterally in the tibialis anterior muscle: 1) transverse relaxation time (T2)-weighted magnetic resonance images to determine muscle cross-sectional area (mCSA) and T2, 2) blood oxygen level dependent (BOLD) images during and following brief, maximal voluntary contractions (MVC) to monitor the hyperemic responses with participants positioned supine in a 3T magnet, 3) muscle strength, and 4) pain pressure threshold. Compared with the control leg, eccentric exercise resulted in soreness, decline in strength (~20%), increased mCSA (~7%), and prolonged T2 (~7%) 24h and 48h ($P<0.05$). The BOLD response to a brief MVC was altered 24h and 48h after eccentric exercise, such that time-to-peak (~35%, $P<0.05$) and time-to-half-recovery (~23%, $P<0.05$) were prolonged. The altered contraction-induced hyperemic response suggests slowed microvascular reactivity and altered matching of O_2 delivery to O_2 utilization within muscle tissue showing signs of muscle damage. These changes in microvascular regulation after eccentric exercise may impede rapid adjustments in muscle blood flow at exercise onset and during activities involving brief bursts of muscle activation, which may impair O_2 delivery and contribute to reduced muscle function after eccentric exercise.

Word Count: 244

INTRODUCTION

Physical activities that are unaccustomed and involve lengthening (i.e. eccentric) muscle contractions are known to induce structural changes in skeletal muscle, and result in a decline in force generating capacity as well as an overall reduction in muscle function (5). This phenomenon is referred to as exercise-induced muscle damage, and is often documented by elevated concentration of intracellular proteins (e.g., creatine kinase or myoglobin) in blood plasma (5). A limitation of this approach is that plasma protein levels do not provide information about which muscle group(s) is affected by the exercise. In contrast, measures of muscle soreness, muscle pain sensitivity and muscle strength comprise information regarding the effects on distinct muscle groups. In addition, eccentric exercise is accompanied by processes that ultimately result in muscle swelling and edema, which can be localized to specific muscles and quantified based on relative increases in muscle cross-sectional area (mCSA) and prolonged transverse relaxation time (T2) using magnetic resonance (MR) imaging techniques (5; 30; 35).

Overall muscle function is influenced by numerous factors, one being the ability to deliver and distribute blood flow (i.e., O_2 delivery) to match local metabolic demand in the muscle. Eccentric contractions of distinct muscle groups have been shown to result in stiffening of carotid arteries, suggesting an effect of local muscle damage on central vascular function (2). However the effect of muscle damage has been proposed to be greater on microvascular compared with macrovascular function (2). While studies have provided evidence of altered structure of the microvasculature after eccentric exercise (22; 23; 25; 26), it is unclear to what extent these structural changes translate into altered function of the microvasculature. Regulation of blood flow during continuous muscle activity is, indeed, complex and involves a variety of stimuli for hyperemia that may assist in maintaining blood flow via compensatory mechanisms

(4; 7). While the hyperemic response at the onset of exercise may be an important initiating event to exercise hyperemia, the mechanisms that initiate the hyperemic response to a single contraction may differ from those that maintain blood flow in skeletal muscle (4; 7; 9).

Blood flow increases rapidly in response to a brief, single contraction, and multiple mechanisms have been proposed to contribute to the hyperemic response (6; 7; 9; 38). Notably, examining the hyperemic response to a brief, single contraction eliminates confounding effects of continuous stimulus for hyperemia (due to repeated contractions) or significant changes in muscle oxidative metabolism, and thus provides a tool to evaluate vascular reactivity (3; 9; 27; 43). Traditionally, this approach has been used to assess the hyperemic response in large arteries (primarily the brachial artery) with the use of Doppler ultrasound (3; 6; 7; 9). However, the hyperemic response to brief contractions has recently been evaluated in the microvasculature of skeletal muscle using blood oxygen level dependent (BOLD) MR imaging (11; 33; 36; 37; 39; 41; 42).

The BOLD method relies on the principles that T2 of water protons inside or in the vicinity of small blood vessels is influenced by the oxygenation status of hemoglobin, as oxygenated and deoxygenated hemoglobin possesses different magnetic properties. Specifically, an increase in the microvascular ratio of oxyhemoglobin to deoxyhemoglobin results in an increase in BOLD signal intensity in T2-weighted images (11; 24; 41). A single contraction elicits a transient increase in BOLD signal intensity, which reflects that oxygen, spatially and temporally, is delivered in excess of the O_2 required to restore the small phosphocreatine (PCr) breakdown during the brief contraction (41; 42). As this phenomenon primarily results from an increase in oxygen saturation and blood volume in the smaller vessels, the time course of the BOLD response has been interpreted to reflect microvascular reactivity (39; 41).

Many activities involve brief bursts of muscle activity, which emphasizes the physiological significance of the ability to rapidly augment blood flow to match O_2 delivery to O_2 demand. So far, little is known about the effects of eccentric exercise on microvascular reactivity in human skeletal muscle. Therefore, the aim of this study was to examine in vivo microvascular function using BOLD MR imaging during brief maximal voluntary contractions of the dorsiflexor muscles before and after (24 h and 48 h) eccentric exercise. It was hypothesized that the eccentrically exercised tibialis anterior (TA) muscle would exhibit an altered hyperemic BOLD response, specifically 1) increased time-to-peak (TTP), mirroring slowed vasodilation, 2) lower peak magnitude, reflecting reduced total vasodilator capacity, and 3) prolonged time to half relaxation, indicative of altered matching of O_2 delivery to O_2 utilization in the exercised muscle tissue.

METHODS

Participants

Fourteen healthy, young men (n = 7) and women volunteered to participate in the study. A preliminary screening session was used to confirm that participants were non-smokers, and not taking any medications or dietary supplements known to affect metabolism or blood flow. All participants were recreationally active, but did not engage in strength training of the lower body. Regular physical activity did not exceed 3 h per week, which was verified by the short form of the International Physical Activity Questionnaire (8), where scores were converted to Metabolic Equivalent Task minutes per week (MET-min $week^{-1}$). Volunteers were screened for metal implants to ensure that they were able to undergo MR procedures. Experimental procedures and potential risks of the study were explained to all participants, who then provided informed

consent, as approved by the local ethical committee (N-20130029) and in accordance with the declaration of Helsinki.

Experimental Protocol

Participation in the study involved one habituation session and four experimental sessions. On the first visit (habituation), participants were resting in a chair for 10 min after which blood pressure was measured twice (Omron M4-I, Omron Matsusaka, Japan), separated by 1 min of rest, and the lowest reading was used for further analysis. Participants then practiced brief maximal voluntary contractions (MVCs) of the dorsiflexor muscles, of both legs, to ensure that these contractions could be performed consistently, as this was an integral part of assessing microvascular function during the MR sessions. The third session involved an exercise protocol consisting of lengthening contractions of the dorsiflexor muscles, previously shown to result in moderate degree of delayed onset muscle soreness (18; 19). The second (baseline, 24 h pre-eccentric), fourth (24 h post-eccentric) and fifth (48 h post-eccentric) sessions involved acquisition of MR images to determine mCSA, T2, and microvascular function of the TA muscles in both legs.

To standardize conditions, sessions 2 to 5 were performed at the same time of day for each participant. Further, participants were asked to avoid strenuous physical activity 24 h prior to the first MR session and for the remaining duration of the study (i.e., another 72 h). In addition, to avoid effects of food and caffeine on blood flow and muscle function, participants were instructed to be fasted for at least 4 h prior to sessions 2 to 5, and abstain from caffeine from the night before each of these sessions.

Muscle strength and force recordings

Participants were positioned supine with the foot secured to a custom-built footplate (fixed at a 120º angle) using a nylon strap with Velcro closures. The footplate was connected to a force transducer (SSM-AJ-1000, Interface, Scottsdale, AZ, USA) and the signal from the force transducer was amplified, filtered (Butterworth second order low pass filter), sampled at 500 Hz, and stored on a computer. The footplate device was built in non-magnetic materials allowing force measures to be obtained while the participants performed MVCs in the MR scanner.

Eccentric exercise protocol

The eccentric exercise protocol consisted of controlled lengthening contractions of the dorsiflexor muscles of the experimental leg (18; 19), which was randomized and counterbalanced, such that half of the participants performed contractions with the right leg, while the other half used the left leg. Participant stood on a 13 cm high platform placed 45 cm from a wall. The foot of the experimental leg was secured to a footplate that was attached to the platform via a hinge, allowing free dorsiflexion and plantarflexion. The palms of the participant's hands were placed flat on the wall at shoulder level for support during the contractions. The non-experimental leg (i.e., control leg) was lifted off the platform by flexing at the hip and knee, and thus transferring to single leg stance on the experimental leg side. Then, the participant performed a controlled plantarflexion of the foot and ankle (requiring lengthening of the TA muscle) until the footplate touched the floor. At this point, the control leg was extended until weight bearing and used to assist in returning the participant to the initial starting position. This process was repeated 10 times per set separated by 30 s of rest, and continued until

the participant was unable to perform controlled, lengthening contractions. As the efficacy of this protocol various across individuals (18; 19), participants performed between 3 and 5 sets.

Muscle soreness

To evaluate progression of soreness in the TA muscle of the experimental leg, participants filled out a modified Likert scale questionnaire (0: no soreness, 6: severe soreness) at baseline and every 12 h for the first 2 days after the eccentric protocol and then every 24 h for the following 4 days (18). In addition, a measure of pain pressure threshold (PPT) was obtained from the belly of the TA muscle using a handheld ergometer (Somedic, Horby, Sweden) with a 1-cm^2 probe. The ergometer was oriented perpendicular to the muscle and pressure stimulation gradually increased (30kPa s^{-1}) until PPT was reached. Via a push button, participants were instructed to indicate PPT when pressure sensation became painful. The PPT procedure was repeated twice at baseline, 24 h post and 48 h post. The averages of these measurements were used for further analyses.

Magnetic resonance imaging

All MR imaging acquisitions were performed using a standard, 14-cm diameter GE clinical extremity coil on a 3T Signa GE imaging system (GE Healthcare, Milwaukee, WI, USA). Participants were positioned supine in the MR scanner with the foot secured to the force recording apparatus, as described above. The extremity coil was positioned around the lower leg with the belly of the dorsiflexor muscles in the center of the coil. This position was marked with a pen on the participant's leg and used for identical positioning during subsequent sessions. To limit motion artifact and participant discomfort during the MR session, padding was placed

around the leg and knee. Prior to the first MR scan in each session, participants rested supine in the scanner for approximately 15 min.

At the start of each MR session, gradient echo images were acquired in 3 planes, and all subsequent images were acquired from the slice location corresponding to the center of the coil. T2-weighted axial images [TR = 1500 ms, TE = 24 ms, number of echoes = 4, FOV = 18 cm, Slice thickness = 10 mm, Slice gap = 1 mm, Acquisition matrix 320 x 224, NEX = 1] were acquired to determine CSA and T2 of the TA muscle using commercial software (AW server 2.0, GE Healthcare, Milwaukee, WI, USA). In brief, mCSA was determined by manually outlining the TA muscle compartment, while the transverse relaxation time was determined using T2 mapping in a large region-of-interest (ROI) within the TA compartment, excluding resolved vessels and subcutaneous fat. These analyses were done by the same investigator, who was blinded to the identity of the data.

Assessing microvascular function

Prior to assessing microvascular function using a MR BOLD protocol adapted from previous studies (33; 41), participants performed 3 MVCs of 3-5 s duration in the scanner to determine muscle strength of that day. To allow recovery, participants rested 1 min between each of these contractions. Then BOLD, one-shot, gradient echo images [TR = 1000 ms, TE = 40 ms, FOV = 18 cm, Slice thickness = 10 mm, Acquisition matrix 64 x 64, NEX = 1, Flip angle = 90º] were acquired continuously for 7.5 min (i.e., 450 images) during which time participants performed a brief MVC (a total of 7 contractions) every 60 s. Each participant received verbal cues and encouragement by the same investigator through all contractions to ensure consistent timing and maximal effort of the contractions. The order of MR procedures was balanced by leg, such that

half of the participants started the MR procedures with the control leg, while the other half started the MR procedures with the experimental leg.

The force data acquired during the brief contractions were analyzed to determine peak force, time-tension-integral (TTI), and duration of the contraction using custom written MatLab program (The Mathworks, Natick, MA, USA). Similarly, a custom-written MatLab program was used to analyze the BOLD images. Specifically, a manually drawn ROI that comprised the majority of the TA compartment and excluded bone, subcutaneous fat and resolved vessels was stored, and the signal intensity was extracted within the ROI for each of the 450 images. The data were temporally aligned and averaged across all 7 contractions to create an individual 60-s time course for BOLD signal intensity (Figure 1). This time course was used to determine peak change in BOLD signal intensity (relative to minimum signal intensity post contraction), as well as TTP (from end of contraction) and time-to-half recovery of the BOLD transient.

Statistics

Statistical analyses were done using SAS software (SAS Institute Inc., Cary, NC, USA). Three-way (sex, leg, session) repeated measures ANOVA were used to test gender-based differences in any markers of muscle damage. There were no significant effects of sex or any sex-by-leg or sex-by-session interactions. Therefore, data for subsequent analyses were collapsed across sex. Two-way (leg, session) repeated measures ANOVA models were used to examine the effects on eccentric exercise on measures of muscle strength, mCSA, T2, PPT, force data from the BOLD protocol, and measures of microvascular function in the experimental leg and control leg (leg), across the three (baseline, 24 h, 48 h) testing sessions (session). Significant interactions effects were further explored with post-hoc pairwise comparisons using Tukey's procedure correcting

for multiple comparisons. Muscle soreness (Likert scale) was analyzed using one-way repeated measures ANOVA with Tukey's post hoc tests. Associations between markers of muscle damage and changes in measures of microvascular function were assessed via Pearson's product-moment correlation coefficient. All data are presented as mean and standard deviation (SD) with statistical significance being accepted when $P < 0.05$. As measures of absolute reliability, coefficient of variation (CV) and intra class correlation coefficients (ICC) were calculated for all BOLD parameters in the control leg based on measurements acquired across all three testing days. Specifically, $ICC_{(3,1)}$ was calculated using a two-way mixed effects model in SPSS (IBM Corp., Armonk, New York).

RESULTS

One of the 14 volunteers did not develop soreness or signs of damage in the TA muscle after eccentric exercise, as indicated by Likert scores equal to 0 (i.e., no soreness) and confirmed by no apparent decline in muscle strength. Therefore, the data from this participant is not included in the analyses. Participant characteristics are presented in Table 1.

Muscle soreness and damage

Likert scores from the TA muscle of the experimental leg displayed a typical temporal profile for delayed onset muscle soreness with peak levels between 24 h and 48 h, and soreness values returning to baseline levels after 120 h (Figure 2). The ANOVA revealed significant main effects by leg ($P < 0.01$) and session ($P < 0.002$) but no interaction effect ($P < 0.89$) for PPT. Relative to baseline, PPT was reduced to 85.2 ± 20.6 % (24 h) and 86.9 ± 24.6 % (48 h) in the experimental leg, which was not different from changes in PPT in the control leg, 86.0 ± 15.2 % and 90.9 ±

22.3 %, respectively. There were significant main effects by leg ($P < 0.001$) and session ($P < 0.04$) and an interaction effect ($P < 0.02$) for muscle strength. Compared with baseline, muscle strength was reduced by approximately 20% at 24 h ($P < 0.01$) and 48 h ($P < 0.04$) in the experimental leg, while muscle strength was maintained in the control leg (Figure 3).

There were significant main effects by leg ($P < 0.001$) and session ($P < 0.01$) and an interaction effect ($P < 0.04$) for T2 (Figure 3). Compared with baseline, T2 was elevated at 24 h ($P < 0.01$) and 48 h ($P < 0.02$) in the experimental leg, with no changes in the control leg. Similarly, there were significant main effects by leg ($P < 0.001$) and session ($P < 0.01$) and an interaction effect ($P < 0.02$) for mCSA, such that mCSA in the experimental leg, but not in the control leg, was increased at 24 h ($P < 0.004$) and 48 h ($P < 0.02$) compared with baseline (Figure 3).

Contractions during the BOLD protocol

The ANOVA revealed no significant main effects or interaction effect on duration and or contraction level (% MVC) during the brief contractions used to evoke the BOLD responses (Table 2). However, there were main effects by leg ($P < 0.004$) and session ($P < 0.005$) and an interaction effect ($P < 0.008$) for TTI. Compared with baseline, TTI was unchanged in the control leg, but reduced in the experimental leg at 24 h ($P < 0.002$) and 48 h ($P < 0.004$), consistent with the overall decline in muscle strength at these time points.

Microvascular function

Figure 1 shows a representative example of the BOLD responses acquired during a sequence of seven brief, maximal contractions obtained from the TA muscle in the experimental leg at

baseline and 48 h after the eccentric exercise. Minimal BOLD responses precluded analyses of temporal hemodynamics in one participant, such that these analyses are based on data from 12 participants. In addition, BOLD data at 24 h are missing in one participant (control and experimental leg), due to technical problems. The ANOVA revealed main effects by leg ($P < 0.006$) and session ($P < 0.008$) and an interaction effect ($P < 0.02$) for TTP of the BOLD response (Figure 4). Compared with baseline, TTP was prolonged at 24 h (33%, $P < 0.01$) and 48 h (36%, $P < 0.006$) with no changes in the control leg. There were significant main effects by leg ($P < 0.001$) and session ($P < 0.02$) and an interaction effect ($P < 0.009$) for time-to-half recovery of the BOLD response (Figure 4). Specifically, compared with baseline, time-to-half recovery was prolonged at 24 h (25%, $P < 0.005$) and 48 h (21%, $P < 0.01$) in the experimental leg with no changes in the control leg. There were no significant main effects or interaction effects for peak magnitude of the BOLD response (Figure 4). The CV of peak magnitude, TTP and time-to-half recovery for the control leg across the three testing days was 18.2%, 11.4% and 7.0%, respectively. The $ICC_{(3,1)}$ of peak magnitude, TTP and time-to-half recovery for the control leg, across the three testing days, was 0.26, 0.29 and 0.20, respectively.

Associations between markers of muscle damage and changes in microvascular function

Changes in TTP were associated with declines in muscle strength ($r^2 = 0.41$, $P < 0.02$, $N = 12$ Figure 5), such that greater loss of muscle strength was associated with longer TTP, 48 h after eccentric exercise. There were no other significant associations between other markers of muscle damage and changes in parameters of microvascular function after eccentric exercise.

DISCUSSION

The primary result of this study is the altered contraction-induced hyperemic response of the microvasculature following unaccustomed, eccentric exercise. Specifically, despite no change in peak magnitude, time-to-peak and half recovery time of the BOLD response to brief, maximal contractions were prolonged at 24 h and 48 h after eccentric exercise. These results indicate that moderate degree of exercise induced muscle damage slows vasodilation of the microvasculature, and alters the matching of O_2 delivery to O_2 utilization in response to brief contractions. Thus, these findings suggest that eccentric exercise may reduce the ability to rapidly augment blood flow (to match O_2 delivery to O_2 demand) at exercise onset and during activities with intermittent muscle activity bursts, which ultimately may impair muscle function.

Prolonged TTP after eccentric exercise

Monitoring the hyperemic responses to brief contractions using MR BOLD imaging provided high temporal and spatial resolution, and allowed examining distinct components of the hyperemic response in the microvasculature of the eccentrically exercised muscle tissue. Further, this approach eliminated the influence of continuous stimulation of the vasculature and changes in muscle oxidative metabolism, and thus examined contraction-induced microvascular responsiveness specifically (3; 6; 9). Prior to the eccentric exercise, characteristics of the BOLD response were comparable to results from previous studies that have used a similar approach to examine microvascular function in the TA muscle of healthy, young adults (33; 39; 41; 42).

In the present study, prolonged TTP (~35%) in response to brief contractions was found at 24 h and 48 h after eccentric exercise. Consistent with our results showing prolonged TTP after eccentric exercise, lengthening contractions of the rat soleus muscle have been shown to attenuate dilation of microvessels in response to adenosine, a potent metabolic dilator (22). In

addition, Kano and colleagues (25) reported an accelerated fall in Pmv_{o2} (~40%) at the onset of electrically stimulated contractions in rat spinotrapezius muscle, 24 h and 72 h after downhill running, suggesting impaired microvascular hemodynamics. While Kano et al. (25) examined changes in microvascular hemodynamics in response to continuous, stimulated muscle activity, the present study extend these results by showing slowed microvascular reactivity in response to single, brief muscle contractions. The present study thus provides novel evidence of blunted contraction-induced microvascular reactivity after eccentric exercise in human skeletal muscle.

Slowed vascular reactivity has been demonstrated in patients with peripheral artery disease, diabetics and elderly, and interpreted as evidence of impaired vascular function (3; 15; 34; 44). Using BOLD imaging, slowed TTP after reactive hyperemia was reported in the TA muscle of patients with peripheral arterial occlusive disease compared with age-matched controls, supporting the notion that prolonged TTP of the BOLD response reflects microvascular dysfunction. While the results of the present study demonstrate slowed contraction-induced hyperemic response, the functional significance of a 35% decline in microvascular reactivity is unclear. Various vasodilatory signals may compensate for impaired microvascular responsiveness, and thus preserve blood flow during continuous muscle activity. However, many activities are characterized by short bursts of muscle activity, and the slowed hyperemic response after eccentric exercise may thus indicate reduced ability to rapidly augment blood flow in order to match O_2 delivery to the metabolic demand in the exercising muscle, which highlights the physiological and clinical relevance of this result.

Mechanisms for rapid vasodilation

The increase in blood flow in response to a single, brief contraction is thought to be due to local vasodilation. However, the mechanisms for the rapid vasodilation have not been fully elucidated, yet mechanical factors as well as release of vasodilators from contracting skeletal muscle cells and the endothelium have been proposed to contribute (4; 7; 10). Notably, most studies examining the underlying mechanisms of the hyperemic response to brief single contractions have been conducted in the upper extremity, which questions the extrapolations of findings in the arms to the vasculature in the lower leg (10). However, a recent study reported similarities between the hyperemic responses to single contractions in the brachial and femoral arteries, suggesting similar vascular control during brief contractions in the upper and lower extremities (10). In the present study, participants were positioned supine in the MR scanner while performing the brief contractions, which prevented an increase in hydrostatic pressure in the lower leg and thus diminished a possible muscle pump contribution to the hyperemic response (10). Collectively, these results suggest that the BOLD response to brief contractions, as used in the present study, primarily is influenced by factors related to function of the endothelium and smooth muscle in the microvasculature.

Inflammation and effects on vasodilation

Strenuous exercise involving eccentric muscle contractions is associated with inflammation and elevated oxidative stress (23; 40), which acutely can impair nitric oxide (NO)-mediated vasodilator function (21). There is evidence to suggest that even low levels of oxidative stress can contribute to altered vascular control in sedentary young adults (32). Specifically, scavenging of ROS via enhancing antioxidant capacity by intra-arterial infusion of ascorbic acid resulted in increased vascular responsiveness to acetylcholine and nitroprusside, endothelial and

vascular smooth muscle agonists, respectively (32). Thus, the inflammatory processes accompanying eccentric exercise may lower NO-bioavailability and impair endothelial function and vascular smooth muscle responsiveness, which possibly could contribute to delayed onset vasodilation of the microvasculature. Notably, the present study was not designed to investigate the contribution of various mechanisms to the altered BOLD response after eccentric exercise. Therefore, future studies are warranted to investigate the underlying mechanisms for blunted microvascular reactivity after eccentric exercise.

Prolonged time-to-half recovery of BOLD response

While peak magnitude of the BOLD response to brief, maximal contractions has been reported to be three-fold greater in trained compared with untrained young adults (42), the unaltered peak magnitude in the present study suggested no apparent decline in overall vasodilator capacity to a brief contraction after eccentric exercise. However, the time-to-half recovery of the BOLD response was prolonged (~23%) 24 h and 48 h after eccentric exercise. Considering that the BOLD signal is influenced by hemoglobin saturation levels and blood volume (41; 44), the prolonged hyperemic response to a brief contraction can occur as a consequence of various scenarios resulting in increased proportion of oxygenated hemoglobin and/or an increase blood volume post contraction (11; 41).

The prolonged BOLD response to a brief contraction in the present study is consistent with previous studies providing evidence of an increased ratio of O_2 delivery-to-O_2 utilization during continuous muscle activity after eccentric exercise (13; 29). Davies et al. (13) monitored the dynamic balance between O_2 delivery and O_2 utilization in human vastus lateralis muscle during rest-to-exercise transitions, before and 48 h after eccentric exercise. The kinetics of

deoxygenated hemoglobin, measured by near infrared spectroscopy, was slowed by ~30%, while the kinetics of pulmonary O_2 uptake (used as a proxy of muscle O_2 utilization) was maintained, suggesting an increased ratio of O_2 delivery-to-O_2 utilization in the exercised muscle. The authors interpreted these results to suggest that an increase in local muscle blood flow was required to maintain muscle O_2 kinetics after eccentric exercise (13). Similarly, Laaksonen et al. (29) reported an increase in local blood flow, measured by positron emission tomography, during submaximal exercise, despite no change in pulmonary O_2 consumption at 72 h after eccentric exercise. A possible explanation for the apparent increase in O_2 delivery-to-O_2 utilization could be diminished extraction of O_2 in muscle tissue affected by eccentric exercise (12; 25). In contrast, Ahmadi et al. (1) reported faster deoxygenation of hemoglobin during sustained, submaximal MVC after an exhaustive session of downhill walking. Assuming impeded blood flow during sustained isometric contractions, faster O_2 deoxygenation indicates greater muscle O_2 consumption after muscle damaging exercise..While muscle damage induced by neuromuscular electrostimulation has been shown to result in slowed PCr recovery, indicative of impaired mitochondrial function (17), other studies have reported preserved mitochondrial function after moderate degree of exercise induced muscle damage (12; 46), suggesting that eccentric exercise may not compromise muscle oxidative function per se. ***Experimental considerations***

An inevitable consequence of exercise induced muscle damage is a decline in muscle strength (5; 30). Consistent with this notion, the eccentric protocol used in the present study resulted in a modest (~20%) decline in muscle strength at 24 h and 48 h post exercise. Force output from the muscle is regulated by recruitment and rate coding of motor units, and studies in the TA muscle of young adults have suggested that all motor units are recruited by 60-70% MVC (14). These

results suggest that the brief, maximal contractions used to evoke BOLD responses in the present study recruited all TA motor units**,** both before and after eccentric exercise. This is important as the magnitude of muscle arteriole vasodilation in response to a single contraction is proportional to the number of recruited motor units (45). Consistent with the assumption of activation of all TA motor units, no change in peak magnitude of the BOLD response was found after eccentric exercise. Similarly, Wigmore and colleagues (47) monitored the hyperemic responses to a series of brief isometric contractions of the dorsiflexor muscles, ranging from 10 to 100% MVC, and showed a plateau in the post contraction hyperemic response at 60% MVC. Together, these results indicate that the modest decline in muscle strength at 24 h and 48 h post-exercise did not influence the stimulus for vasodilation to brief maximal contractions, and consequently the interpretation of the BOLD responses. Notably, Meyer and colleagues (33) showed lower peak BOLD during 50% MVC compared with MVC, providing some evidence that force production influences BOLD magnitude. Thus, the small sample size may have concealed a possible decline in peak BOLD in response to lower force production (20%) after eccentric exercise. However, if anything, lower force output during the brief contractions would result in shorter TTP (37) and shorter time-to-half recovery (3; 9) of the hyperemic response, which emphasize the physiological significance of our results.

Although no direct evidence of muscle damage (i.e., muscle biopsy) was obtained, compelling evidence indicate that the eccentric exercise protocol resulted in damage localized to the TA muscle. Specifically, in agreement with results from previous studies that have used a similar protocol, a decline in strength of the dorsiflexor muscles was found (19; 20). These findings were further extended by increased mCSA and prolonged T2 at 24 h and 48 h after exercise. Several studies have shown prolonged muscle T2 after eccentric exercise, and reported

correlations between T2 and ultrastructural changes as well as plasma levels of intracellular proteins (16; 30; 31; 35). Furthermore, soreness ratings from the TA muscle, evaluated by the Likert scale, displayed a typical temporal profile for delayed onset muscle soreness with peak soreness levels 24 h and 48 h post exercise, matching the time points for measurements of microvascular function using BOLD MR imaging. However, the decrease in PPT in the experimental leg was not different from the change in PPT in the control leg, suggesting that the protocol did not develop hyperalgesia in the eccentrically exercised muscle. The link between muscle damage and altered microvascular function was emphasized by a significant correlation between prolonged TTP and the decline in muscle strength at 48 h after eccentric exercise. In contrast, prolonged T2 and Likert scores were not significantly correlated with longer TTP, highlighting the intricate and complex interaction between indices of muscle damage and microvascular function. Notably, the eccentric protocol used in the present study elicited relatively small changes in markers of muscle damage, muscle pain sensitivity, and measures of microvascular function, which will limit the ability to establish significant associations, particularly in combination with a relatively small sample size. While gender differences in exercise induced muscle damage have previously been reported, no gender-based differences were found in any markers of muscle damage. It is possible, however, that potential differences between genders were masked due to the small sample size used in the present study. While CVs of BOLD parameters in the control leg, across testing days, were relatively good (7-18%), $ICCs_{(3,1)}$ showed poor absolute reliability (<0.40). Although, there was no effect of time on the BOLD response in the control leg, it is possible that systemic effects (due to the eccentric exercise) influenced measures of absolute reliability, as these measurements were acquired across all three testing days.

The MR BOLD technique used to evaluate microvascular function in the present study does not allow absolute quantification of perfusion or blood flow. However, a significant strength of this non-invasive methodology is the high temporal and spatial resolution of the acquired MR images. Specifically, the temporal resolution of the BOLD response captures distinct components (i.e., time-to-peak, peak magnitude, and time-to-half recovery) of the hyperemic response to brief contractions (33; 39; 41), which is essential when examining microvascular reactivity. In addition, the spatial resolution of the MR images allowed us to monitor the hyperemic response in a large, well-defined proportion of the TA muscle, and as such is representative of the majority of the muscle tissue involved in the eccentric exercise. These features of the BOLD method are critical in monitoring skeletal muscle hyperemia, as heterogeneity exists within muscle tissue, with respect to dynamics of oxygenation as well as distribution of damaged tissue (13; 28; 30).

Conclusion

These results show that eccentric exercise alters the hyperemic response of the microvasculature to brief muscle contractions. Specifically, time-to-peak and time-to-half recovery of the BOLD response were prolonged at 24 h and 48 h after eccentric exercise, suggesting slowed microvascular reactivity and altered matching of O_2 delivery to O_2 utilization in muscle tissue showing signs of moderate degree of damage. Such changes may hinder rapid adjustments in blood flow to support O_2 delivery at exercise onset and during brief bursts of muscle activity, which underlines the physiological and clinical relevance of these results.

Author contributions

R.G.L contributed to the conception and design of the experiments, collection, analysis and interpretation of the data, and writing of this article. R.P.H contributed to the analysis and assembly of the data, and critical revision of this article. A.M. contributed to the analysis and assembly of the data, and critical revision of this article. J.B.F contributed to the conception and design of the experiments, and critical revision of this article. T.G.N contributed to the conception and design of the experiments, interpretation of the data, and critical revision of this article. The experiments were performed in the Exercise Physiology Laboratory at Aalborg University and at the Magnetic Resonance Research Unit, Aalborg University Hospital.

Acknowledgement

The authors sincerely thank the volunteers for participating in this study. In addition, we thank Carsten W. Simonsen for technical assistance. TGN is a part of Center for Neuroplasticity and Pain (CNAP) which is supported by the Danish National Research Foundation (DNRF121).

Reference List

1. **Ahmadi S, Sinclair PJ and Davis GM**. Muscle oxygenation after downhill walking-induced muscle damage. *Clin Physiol Funct Imaging* 28: 55-63, 2008.

2. **Barnes JN, Trombold JR, Dhindsa M, Lin HF and Tanaka H**. Arterial stiffening following eccentric exercise-induced muscle damage. *J Appl Physiol* 109: 1102-1108, 2010.

3. **Carlson RE, Kirby BS, Voyles WF and Dinenno FA**. Evidence for impaired skeletal muscle contraction-induced rapid vasodilation in aging humans. *Am J Physiol Heart Circ Physiol* 294: H1963-H1970, 2008.

4. **Casey DP, Walker BG, Ranadive SM, Taylor JL and Joyner MJ**. Contribution of nitric oxide in the contraction-induced rapid vasodilation in young and older adults. *J Appl Physiol (1985)* 115: 446-455, 2013.

5. **Clarkson PM and Hubal MJ**. Exercise-induced muscle damage in humans. *Am J Phys Med Rehabil* 81: S52-S69, 2002.

6. **Clifford PS**. Skeletal muscle vasodilatation at the onset of exercise. *J Physiol* 583: 825-833, 2007.

7. **Clifford PS and Hellsten Y**. Vasodilatory mechanisms in contracting skeletal muscle. *J Appl Physiol* 97: 393-403, 2004.

8. **Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF and Oja P**. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 35: 1381-1395, 2003.

9. **Crecelius AR, Kirby BS, Luckasen GJ, Larson DG and Dinenno FA**. Mechanisms of rapid vasodilation after a brief contraction in human skeletal muscle. *Am J Physiol Heart Circ Physiol* 305: H29-H40, 2013.

10. **Credeur DP, Holwerda SW, Restaino RM, King PM, Crutcher KL, Laughlin MH, Padilla J and Fadel PJ**. Characterizing rapid-onset vasodilation to single muscle contractions in the human leg. *J Appl Physiol (1985)* 118: 455-464, 2015.

11. **Damon BM, Wadington MC, Hornberger JL and Lansdown DA**. Absolute and relative contributions of BOLD effects to the muscle functional MRI signal intensity time course: effect of exercise intensity. *Magn Reson Med* 58: 335-345, 2007.

12. **Davies RC, Eston RG, Fulford J, Rowlands AV and Jones AM**. Muscle damage alters the metabolic response to dynamic exercise in humans: a 31P-MRS study. *J Appl Physiol (1985)* 111: 782-790, 2011.

13. **Davies RC, Eston RG, Poole DC, Rowlands AV, DiMenna F, Wilkerson DP, Twist C and Jones AM**. Effect of eccentric exercise-induced muscle damage on the dynamics of muscle oxygenation and pulmonary oxygen uptake. *J Appl Physiol (1985)* 105: 1413-1421, 2008.

14. **Erim Z, De Luca CJ, Mineo K and Aoki T**. Rank-ordered regulation of motor units. *Muscle Nerve* 19: 563-573, 1996.

15. **Feng J, Liu Y, Chu LM, Singh AK, Dobrilovic N, Fingleton JG, Clements RT, Bianchi C and Sellke FW**. Changes in microvascular reactivity after cardiopulmonary bypass in patients with poorly controlled versus controlled diabetes. *Circulation* 126: S73-S80, 2012.

16. **Fleckenstein JL, Weatherall PT, Parkey RW, Payne JA and Peshock RM**. Sports-related muscle injuries: evaluation with MR imaging. *Radiology* 172: 793-798, 1989.

17. **Foure A, Wegrzyk J, Le Fur Y, Mattei JP, Boudinet H, Vilmen C, Bendahan D and Gondin J**. Impaired mitochondrial function and reduced energy cost as a result of muscle damage. *Med Sci Sports Exerc* 47: 1135-1144, 2015.

18. **Gibson W, Arendt-Nielsen L and Graven-Nielsen T**. Delayed onset muscle soreness at tendon-bone junction and muscle tissue is associated with facilitated referred pain. *Exp Brain Res* 174: 351-360, 2006.

19. **Gibson W, Arendt-Nielsen L, Taguchi T, Mizumura K and Graven-Nielsen T**. Increased pain from muscle fascia following eccentric exercise: animal and human findings. *Exp Brain Res* 194: 299-308, 2009.

20. **Gibson W, Arendt-Nielsen L, Taguchi T, Mizumura K and Graven-Nielsen T**. Increased pain from muscle fascia following eccentric exercise: animal and human findings. *Exp Brain Res* 194: 299-308, 2009.

21. **Harrison DG, Widder J, Grumbach I, Chen W, Weber M and Searles C**. Endothelial mechanotransduction, nitric oxide and vascular inflammation. *J Intern Med* 259: 351-363, 2006.

22. **Heap SJ, Fulgenzi GL and Hudlicka O**. Microcirculation in rat soleus muscle after eccentric exercise: the effect of nifedipine. *Eur J Appl Physiol* 97: 687-694, 2006.

23. **Hellsten Y, Frandsen U, Orthenblad N, Sjodin B and Richter EA**. Xanthine oxidase in human skeletal muscle following eccentric exercise: a role in inflammation. *J Physiol* 498 (Pt 1): 239-248, 1997.

24. **Jacobi B, Bongartz G, Partovi S, Schulte AC, Aschwanden M, Lumsden AB, Davies MG, Loebe M, Noon GP, Karimi S, Lyo JK, Staub D, Huegli RW and Bilecen D**. Skeletal muscle BOLD MRI: from underlying physiological concepts to its usefulness in clinical conditions. *J Magn Reson Imaging* 35: 1253-1265, 2012.

25. **Kano Y, Padilla DJ, Behnke BJ, Hageman KS, Musch TI and Poole DC**. Effects of eccentric exercise on microcirculation and microvascular oxygen pressures in rat spinotrapezius muscle. *J Appl Physiol* 99: 1516-1522, 2005.

26. **Kano Y, Sampei K and Matsudo H**. Time course of capillary structure changes in rat skeletal muscle following strenuous eccentric exercise. *Acta Physiol Scand* 180: 291-299, 2004.

27. **Kirby BS, Voyles WF, Simpson CB, Carlson RE, Schrage WG and Dinenno FA**. Endothelium-dependent vasodilatation and exercise hyperaemia in ageing humans: impact of acute ascorbic acid administration. *J Physiol* 587: 1989-2003, 2009.

28. **Koga S, Poole DC, Ferreira LF, Whipp BJ, Kondo N, Saitoh T, Ohmae E and Barstow TJ**. Spatial heterogeneity of quadriceps muscle deoxygenation kinetics during cycle exercise. *J Appl Physiol (1985)* 103: 2049-2056, 2007.

29. **Laaksonen MS, Kivela R, Kyrolainen H, Sipila S, Selanne H, Lautamaki R, Nuutila P, Knuuti J, Kalliokoski KK and Komi PV**. Effects of exhaustive stretch-shortening cycle exercise on muscle blood flow during exercise. *Acta Physiol (Oxf)* 186: 261-270, 2006.

30. **Larsen RG, Ringgaard S and Overgaard K**. Localization and quantification of muscle damage by magnetic resonance imaging following step exercise in young women. *Scand J Med Sci Sports* 17: 76-83, 2007.

31. **LeBlanc AD, Jaweed M and Evans H**. Evaluation of muscle injury using magnetic resonance imaging. *Clin J Sport Med* 3: 26-30, 1993.

32. **Limberg JK, Harrell JW, Johansson RE, Eldridge MW, Proctor LT, Sebranek JJ and Schrage WG**. Microvascular function in younger adults with obesity and metabolic syndrome: role of oxidative stress. *Am J Physiol Heart Circ Physiol* 305: H1230-H1237, 2013.

33. **Meyer RA, Towse TF, Reid RW, Jayaraman RC, Wiseman RW and McCully KK**. BOLD MRI mapping of transient hyperemia in skeletal muscle after single contractions. *NMR Biomed* 17: 392-398, 2004.

34. **Milio G, Novo G, Ferrara F, Raimondi F and Novo S**. Changes of arterial hemodynamics in patients with type 2 diabetes mellitus or impaired glucose tolerance. *Int Angiol* 25: 73-77, 2006.

35. **Nurenberg P, Giddings CJ, Stray-Gundersen J, Fleckenstein JL, Gonyea WJ and Peshock RM**. MR imaging-guided muscle biopsy for correlation of increased signal intensity with ultrastructural change and delayed-onset muscle soreness after exercise. *Radiology* 184: 865-869, 1992.

36. **Sanchez OA, Copenhaver EA, Chance MA, Fowler MJ, Towse TF, Kent-Braun JA and Damon BM**. Postmaximal contraction blood volume responses are blunted in obese and type 2 diabetic subjects in a muscle-specific manner. *Am J Physiol Heart Circ Physiol* 301: H418-H427, 2011.

37. **Sanchez OA, Louie EA, Copenhaver EA and Damon BM**. Repeatability of a dual gradient-recalled echo MRI method for monitoring post-isometric contraction blood volume and oxygenation changes. *NMR Biomed* 22: 753-761, 2009.

38. **Segal SS**. Regulation of blood flow in the microcirculation. *Microcirculation* 12: 33-45, 2005.

39. **Slade JM, Towse TF, Gossain VV and Meyer RA**. Peripheral microvascular response to muscle contraction is unaltered by early diabetes but decreases with age. *J Appl Physiol* 111: 1361-1371, 2011.

40. **Tidball JG**. Inflammatory processes in muscle injury and repair. *Am J Physiol Regul Integr Comp Physiol* 288: R345-R353, 2005.

41. **Towse TF, Slade JM, Ambrose JA, DeLano MC and Meyer RA**. Quantitative analysis of the postcontractile blood-oxygenation-level-dependent (BOLD) effect in skeletal muscle. *J Appl Physiol* 111: 27-39, 2011.

42. **Towse TF, Slade JM and Meyer RA**. Effect of physical activity on MRI-measured blood oxygen level-dependent transients in skeletal muscle after brief contractions. *J Appl Physiol (1985)* 99: 715-722, 2005.

43. **Tschakovsky ME and Sheriff DD**. Immediate exercise hyperemia: contributions of the muscle pump vs. rapid vasodilation. *J Appl Physiol (1985)* 97: 739-747, 2004.

44. **Utz W, Jordan J, Niendorf T, Stoffels M, Luft FC, Dietz R and Friedrich MG**. Blood oxygen level-dependent MRI of tissue oxygenation: relation to endothelium-dependent and endothelium-independent blood flow changes. *Arterioscler Thromb Vasc Biol* 25: 1408-1413, 2005.

45. **VanTeeffelen JW and Segal SS**. Effect of motor unit recruitment on functional vasodilatation in hamster retractor muscle. *J Physiol* 524 Pt 1: 267-278, 2000.

46. **Walsh B, Tonkonogi M, Malm C, Ekblom B and Sahlin K**. Effect of eccentric exercise on muscle oxidative metabolism in humans. *Med Sci Sports Exerc* 33: 436-441, 2001.

47. **Wigmore DM, Damon BM, Pober DM and Kent-Braun JA**. MRI measures of perfusion-related changes in human skeletal muscle during progressive contractions. *J Appl Physiol (1985)* 97: 2385-2394, 2004.

Figure legends

Figure 1. Time course of signal intensity changes in tibialis anterior muscle in one representative participant, reflecting the hyperemic response to brief, maximal contractions. The figure illustrates signal intensity changes for each of the 7 contractions at baseline (A), and 48 h after eccentric exercise (B), and the response averaged over all 7 contractions, at baseline (C) and 48 h after eccentric exercise (D). Spikes in signal intensity (every 60s) coincide with contractions, and are due to changes in signal saturation when the muscle contract and move in the imaged slice.

Figure 2. Likert scores over time as a measure of delayed onset muscle soreness in the tibialis anterior muscle of the experimental leg. Data are from 13 participants and presented as means ± SD. Post hoc (Tukey's procedure) significant differences ($P < 0.05$) from baseline (0 hours) are indicated by *.

Figure 3. Representative anatomic T2-weighted images acquired from the experimental leg and the control leg in one participant during the MRI protocol, 48 h after eccentric exercise. Markers of muscle damage obtained from the tibialis anterior muscle (outlined) in the experimental leg (eccentric, filled bars) and control leg (control, open bars). Muscle strength, muscle cross-sectional area, and T2 values are illustrated. Data are from 13 participants and presented as means ± SD. Post-hoc (Tukey's procedure) significant differences ($P < 0.05$) between baseline and 24 h or 48 h are indicated by *.

Figure 4. Peak, time-to-peak, and time-to-half recovery of the BOLD response are presented for the experimental leg (eccentric, filled bars) and control leg (control, open bars). Data are from 12 participants and presented as means ± SD. Post hoc (Tukey's procedure) significant differences ($P < 0.05$) between baseline and 24 h or 48 h after eccentric exercise are indicated by *.

Figure 5. Correlation between decline in muscle strength and increase in BOLD time-to-peak at 48 h post exercise ($r^2 = 0.41$, $p < 0.02$). Data are from 12 participants.

FIGURE 1

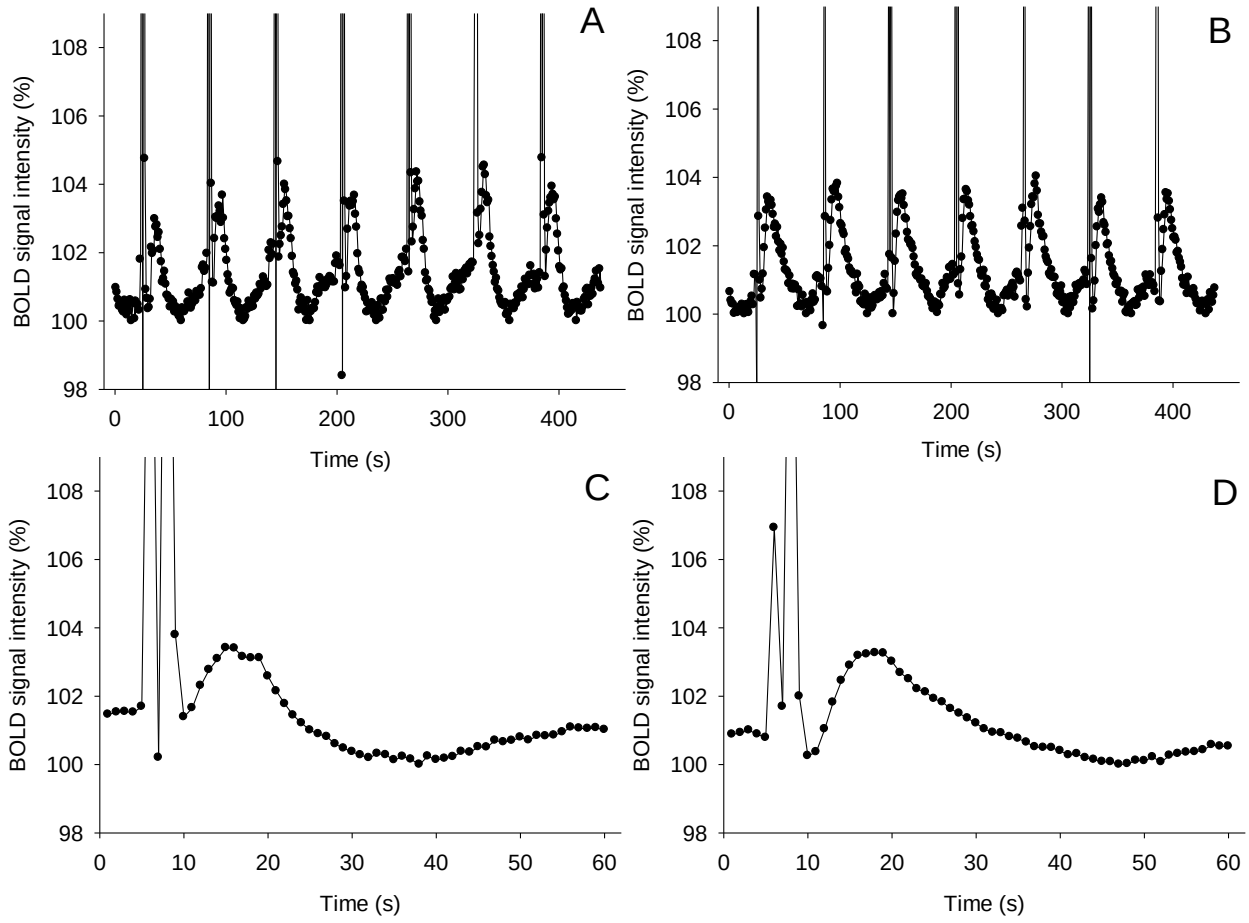
A
B
C
D
BOLD signal intensity (%)
Time (s)
98
100
102
104
106
108
0
100
200
300
400
10
20
30
40
50
60

FIGURE 2

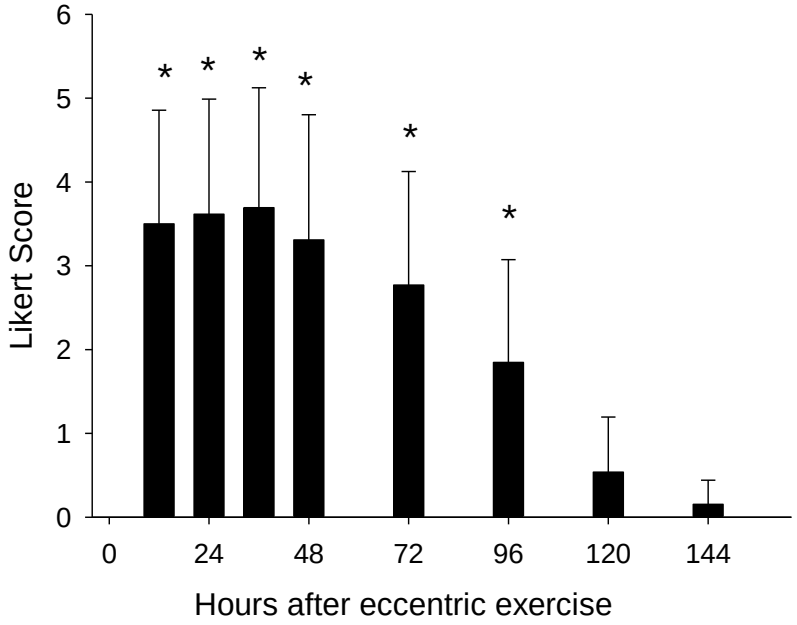
Likert Score
0
1
2
3
4
5
6
*
*
*
*
*
*
0
24
48
72
96
120
144
Hours after eccentric exercise

Figure 3

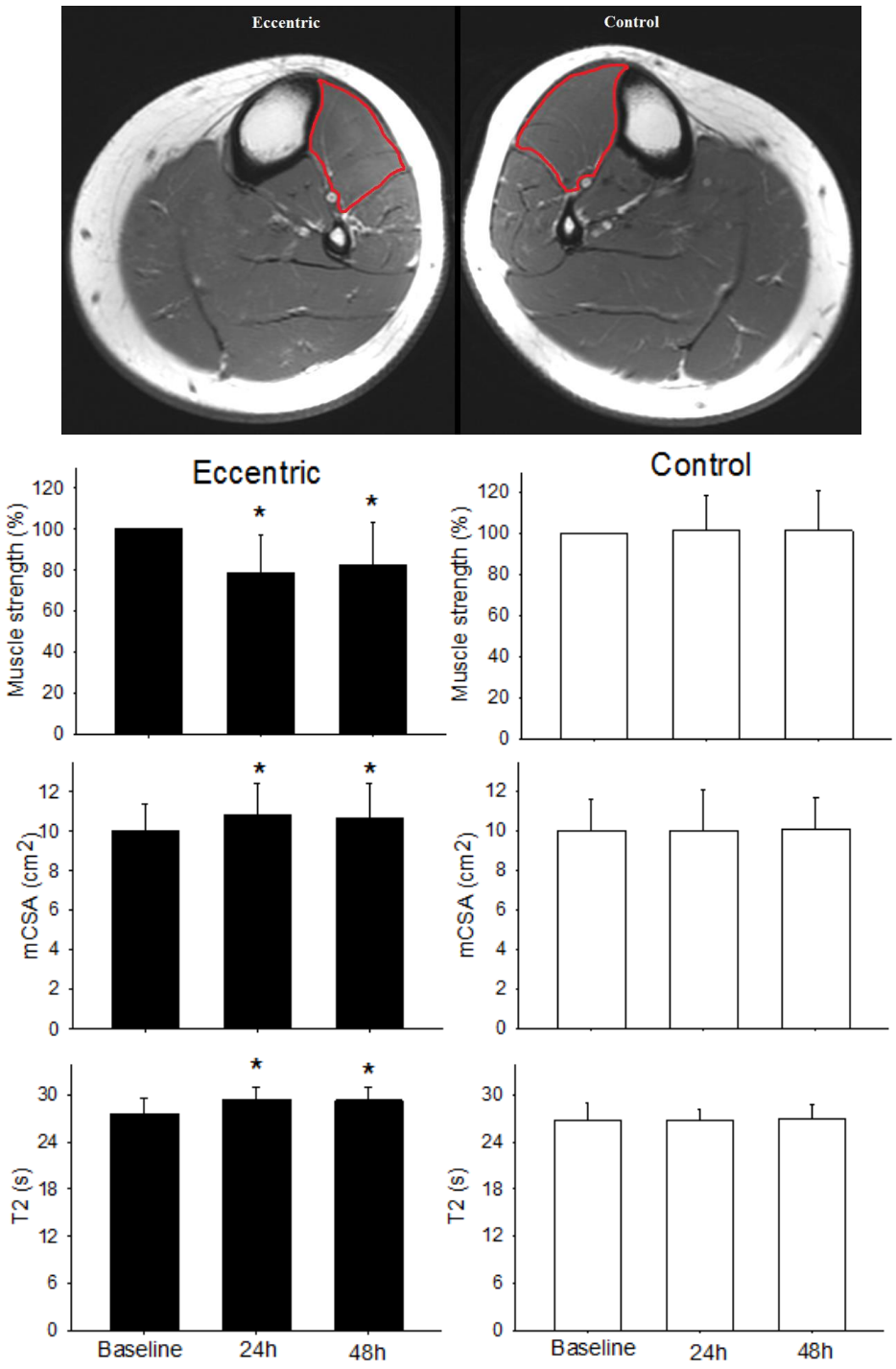
Eccentric
Control
Eccentric
Control
Muscle strength (%)
mCSA (cm2)
T2 (s)
Baseline
24h
48h

Figure 4

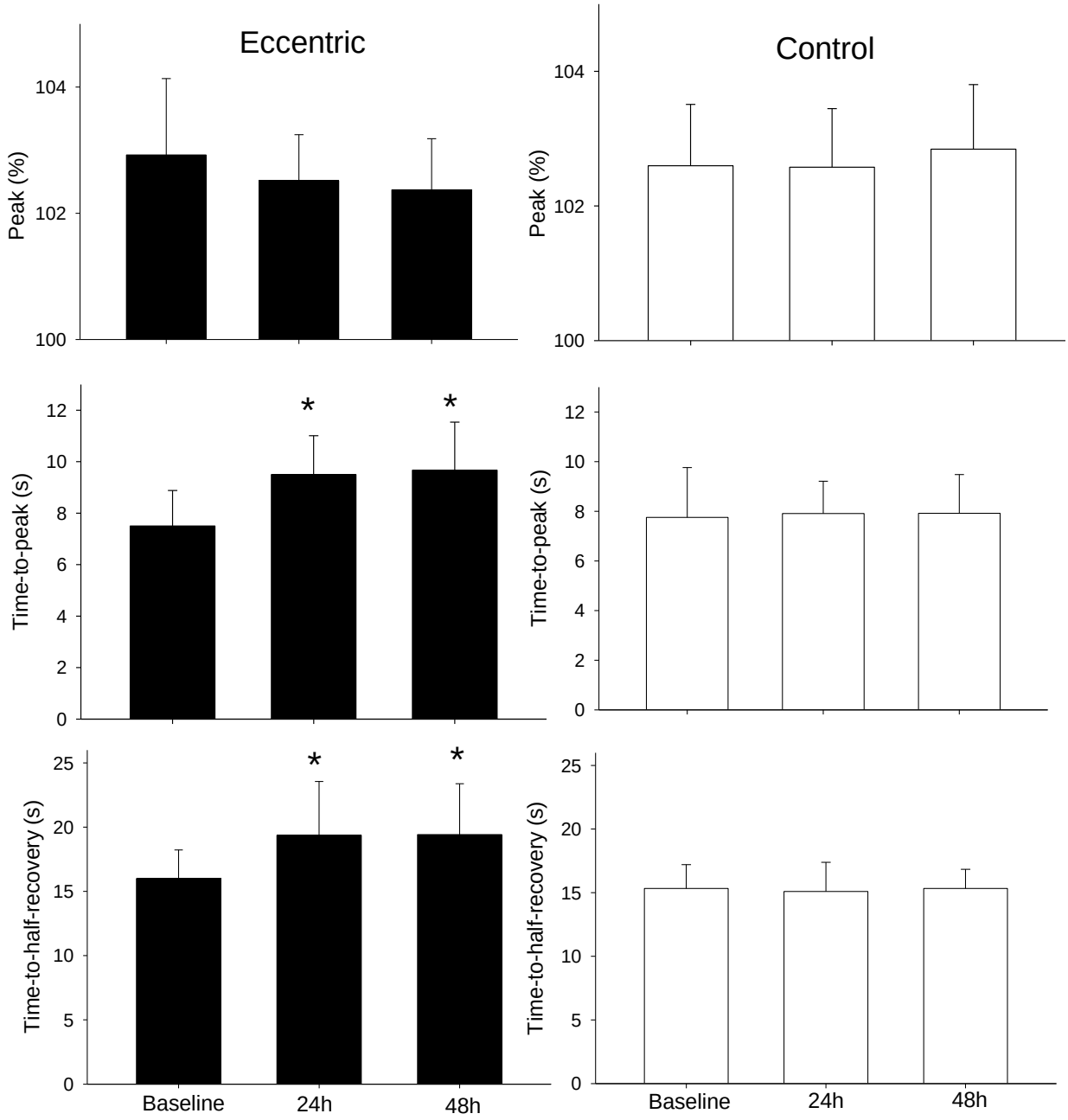
Eccentric
Control
Peak (%)
104
102
100
Time-to-peak (s)
12
10
8
6
4
2
0
*
*
Time-to-half-recovery (s)
25
20
15
10
5
0
*
*
Baseline
24h
48h

Figure 5

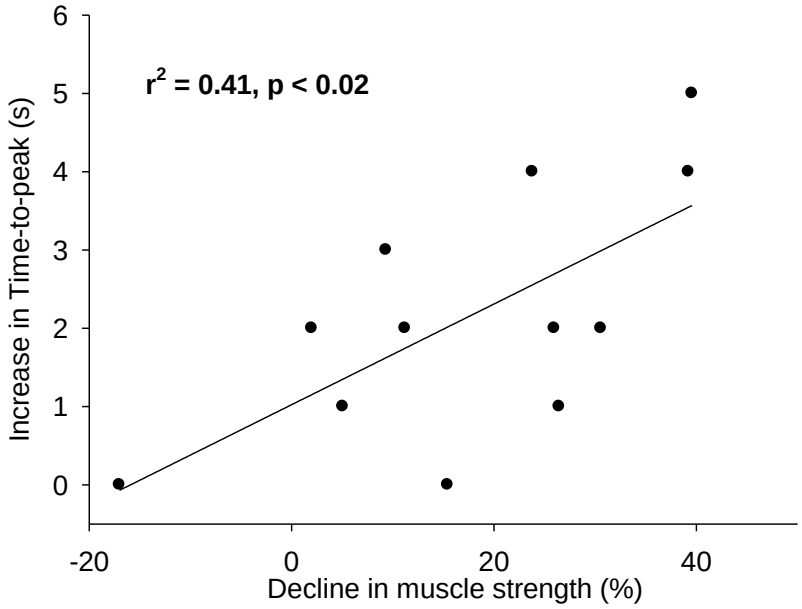
r^2 = 0.41, p < 0.02
Increase in Time-to-peak (s)
Decline in muscle strength (%)
0
1
2
3
4
5
6
-20
0
20
40

Table legends

Table 1. Data are from 13 participants and presented as means ± SD. MET, metabolic equivalent task.

Table 2. Data are from 13 participants and presented as means ± SD. Post hoc (Tukey's procedure) significant differences ($P < 0.05$) between baseline and 24 h or 48 h after eccentric exercise are indicated by *. BOLD, blood oxygen level dependent; TTI, time tension integral; MVC, maximal voluntary contraction.

Table 1: Participant characteristics at baseline

Age (years)	25.8 ± 4.9
Height (m)	1.74 ± 0.08
Body mass (kg)	65.6 ± 7.7
Body mass index (kg m^{-2})	21.7 ± 2.1
Systolic blood pressure (mmHg)	116.8 ± 8.7
Diastolic blood pressure (mmHg)	71.2 ± 6.5
Physical activity (MET-minutes $week^{-1}$)	1502.0 ± 1656.3

Table 2. Muscle contraction parameters from magnetic resonance BOLD protocol

		Baseline	24 h	48 h
Muscle strength, MVC (N)	Control	286.7 ± 109.9	289.3 ± 110.4	287.8 ± 112.1
	Eccentric	263.7 ± 100.4	203.4 ± 83.7*	211.7 ± 79.4*
Duration of contraction (s)	Control	1.93 ± 0.21	1.87 ± 0.12	1.90 ± 0.16
	Eccentric	1.92 ± 0.20	1.94 ± 0.17	1.90 ± 0.20
Peak force (% MVC)	Control	94.0 ± 4.7	92.1 ± 9.7	96.2 ± 8.9
	Eccentric	96.4 ± 8.4	93.2 ± 13.3	93.8 ± 9.5
TTI (N s)	Control	277.8 ± 102.3	271.5 ± 105.8	279.1 ± 92.3
	Eccentric	290.7 ± 116.6	206.9 ± 88.2*	212.6 ± 81.1*