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BLOOD FLOW AFTER CONTRACTION AND CUFF OCCLUSION IS REDUCED IN SUBJECTS WITH MUSCLE SORENESS AFTER ECCENTRIC EXERCISE

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ABSTRACT

Purpose: Delayed onset muscle soreness (DOMS) occur within 1-2 days after eccentric exercise but the mechanism mediating hypersensitivity is unclear. This study hypothesized that eccentric exercise reduces the blood flow response following muscle contractions and cuff occlusion, which may result in accumulated algesic substances being a part of the sensitization in DOMS.

Methods: Twelve healthy subjects (5 women) performed dorsiflexion exercise (5 sets of 10 repeated eccentric contractions) in one leg, while the contralateral leg was the control. The maximal voluntary contraction (MVC) of the tibialis anterior muscle was recorded. Blood flow was assessed by ultrasound Doppler on the anterior tibialis artery (ATA) and within the anterior tibialis muscle tissue before and immediately after 1-s MVC, 5-s MVC, and 5-min thigh cuff occlusion. Pressure pain thresholds (PPTs) were recorded on the tibialis anterior muscle. All measures were done bilaterally at day-0 (pre-exercise), day-2 and day-6 (post-exercise). Subjects scored the muscle soreness on a Likert scale for 6 days.

Results: Eccentric exercise increased Likert scores at day-1 and day-2 compared with day-0 ($P<0.001$). Compared with pre-exercise (day-0), reduced PPT (~25%, $P<0.002$), MVC (~22%, $P<0.002$), ATA diameter (~8%, $P<0.002$), ATA post-contraction/occlusion blood flow (~16%, $P<0.04$), and intramuscular peak blood flow (~23%, $P<0.03$) were found in the DOMS leg on day-2 but not in the control leg.

Perspectives: These results showed that eccentric contractions decreased vessel diameter, impaired the blood flow response and promoted hyperalgesia. Thus, the results suggest that the blood flow reduction may be involved in the increased pain response after eccentric exercise.

New and noteworthy

Delayed onset muscle soreness (DOMS) follow eccentric exercise but the specific mechanism leading to increased pain sensitivity is unclear. This study tested the hypothesis that eccentric exercise reduces the blood flow response to muscle contractions and cuff occlusion. The results showed that eccentric contractions decreased vessel diameter, reduced blood flow, and promoted hyperalgesia. A failure in drain of algesic substances induced by blood flow reduction may increase the pain response after eccentric exercise.

INTRODUCTION

Delayed onset muscle soreness (DOMS) is a kind of muscle strain injury that includes increased pain sensitivity to palpation and/or stiffness during movement (Karoline et al; 2003; Gibson et al., 2006; Hayashi et al. 2017). The DOMS is usually induced after performing unaccustomed vigorous muscular work and precipitated by eccentric actions (Clarkson et al., 1992; Nosaka and Clarkson 1995; Hayashi et al. 2017). The appearance of DOMS occurs 24 hours after eccentric exercise and remains up to 72 hours (Gibson et al., 2006; Larsen et al., 2015). One involved factor is the oblique arrangement of muscle fibers shortly before the myotendinous junction reducing its ability to withstand high tensile forces and the contractile element of the muscle fibers in the myotendinous junction (Clarkson et al., 1992).

Skeletal muscles undergo changes in blood flow and oxygen consumption as part of their normal physiological function, which may be altered by pathology (Towse et al., 2011). Simons and Mense (1998) suggested that failure in muscle blood flow perfusion may be related with accumulation of algesic substances in damaged tissue and consequently muscle pain. In normal conditions there is an immediate increase in blood flow response within

seconds after the release of muscle contraction or cuff occlusion, and this increase is related to the metabolic demand of the tissue (Clifford 2007; Clifford 2011; Green et al., 2014). However, the most studies on blood flow response after brief contraction are done in forearm in humans (Berry et al., 2000; Brock et al., 1998). The underlying mechanism for the post-contractile and cuff occlusion blood flow increase is not fully understood, but probably results from a combination of factors including the muscle pump, the myogenic effect, and a host of vasodilators, including potassium and nitric oxide (Clifford 2011; Green et al., 2014). The structure and function of muscular arteries and capillaries are affected following eccentric muscle work (Barnes et al., 2010; Kano et al., 2004). Specifically, eccentric exercise results in a disruption in the capillary geometry for up to 7 days in rats (Kano et al., 2004) and change the hemodynamics response in humans 24 hours after eccentric exercise (Barnes et al., 2010). Moreover, the microvascular regulation in muscle 1-2 days after eccentric exercise (Larsen et al., 2015) may impede rapid adjustments in muscle blood. If the post contraction/occlusion blood flow increase is impaired due to eccentric exercise, accumulation of inflammatory and algesic substances (e.g. IL-1, IL-6, glutamate, prostaglandin E2, substance P, bradykinin and nerve growth factor (NGF) is likely being a part of the sensitization of muscular nociceptors in DOMS (Cannon et al., 1989; Jonsdottir et al., 2000; Tegeder et al., 2002, Murase et al., 2010).

This study aimed to assess the pressure pain sensitivity and blood flow following brief muscle contractions and reactive hyperemia induced by cuff occlusion before and several days after eccentric contractions. It was hypothesized that eccentric exercise would reduce the post contraction/occlusion muscle blood flow response 2 days after eccentric exercise and this reduction is part of increased pain sensitivity.

METHODS

Subjects

The study included 12 subjects (5 women; age: 27.2 ± 1.3 years; weight 68.7 ± 2.5 kg; height: 173.7 ± 2.7 cm) without symptoms of musculoskeletal pain. The sample size was based on PPT change expectative value after eccentric exercise (Gibson et al., 2006, Larsen et al., 2015). None of the subjects used any medication known to influence pain or vascular responses. The physical activity level of subjects was determined by the short IPAQ questionnaire (www.ipaq.ki.se) (Booth et al., 2000) where scores were converted to Metabolic Equivalent Task minutes per week (MET-min week). The physical activity level of subjects was moderate (2271 ± 478 Met-min/week) (Craig et al., 2003). The study was approved by the local ethical committee (N-20130029). All participants read and signed an informed consent prior to enrollment and the study was performed according with the Declaration of Helsinki.

Experimental design

The study was conducted on 3 different days in legs with the same experimental protocol performed bilaterally except for the eccentric exercise in one leg included at the end of the day-0 session. The maximum voluntary contractions (MVCs) of the dorsiflexor muscles was performed where the subjects did two 1-s MVCs and two 5-s MVCs followed by 1 and 3 min of rest, respectively. The pressure pain thresholds were assessed by pressure algometry on the tibialis anterior (TA) muscle. Before and after the MVCs, blood flow in the anterior tibial artery (ATA) was recorded by ultrasound Doppler. Similarly, blood flow in the ATA was evaluated before and after 5 min of cuff occlusion applied proximal to the knee joint. Subsequently the blood flow of the tibialis anterior (TA) muscle was recorded by ultrasound Doppler during the same contraction/occlusion protocol as used for ATA assessments. Half

of the subjects were randomized to start the experimental procedures with the control (no exercise) leg while the other half started the procedures with the exercise leg. The second and third experimental sessions were held two (day-2) and six (day-6) days after the first session. Subjects scored the muscle soreness in the lower limbs on a Likert scale for all 6 days.

Muscle pain evoked by eccentric exercise

DOMS was evoked by the eccentric exercise of the dorsiflexor muscles. Previous work using this protocol (Gibson et al., 2006; 2009; Larsen et al., 2015) suggests that DOMS peaks 2 days post-exercise and has returned to baseline levels at day 6. The subjects stood on a metal platform (height 13 cm and 50 cm away from the wall). Subjects were instructed to keep the foot of the experimental leg on the border of the platform and support with the non-experimental leg foot on the platform. The subjects then raised the non-experimental leg off the platform by bending at the hip and knee, transferring weight to the experimental leg. Subsequently they carried out a slow plantar flexion of the foot and ankle. This move allows the toes to touch a soft foam pad (2 cm thick) placed underneath the platform. Also, this movement requires eccentric controlled stretching of the tibialis anterior muscle. At this point, the control leg was extended to transfer the weight to be used to assist the subject's return to the initial starting position. Participants repeated this protocol ten times per set. Five sets of ten repeated contractions were separated by 20-s of rest. The effectiveness of eccentric activity was manifested by difficulty walking and the poor performance in this task was assumed to be due to impairment induced by eccentric activity. The eccentric protocol was performed in the non-dominant limb to minimize interference of pain in carrying out daily activities.

Assessment of muscle soreness

Participants were asked to evaluate the intensity of pain in the experimental leg for 6 consecutive days, during the same time of day that the first session was completed. The pain intensity was scored on a Likert scale defined as: [0] Complete absence of muscle pain; [1] a light soreness in the muscle felt only when touched/a vague ache; [2] a moderate soreness felt only when touched/a slight persistent ache; [3] a light muscle soreness when walking up and down stairs; [4] a light muscle soreness when walking on flat surface; [5] a moderate muscle soreness, stiffness or weakness when walking; [6] severe muscle pain with stiffness or weakness that limits the ability of movements (Gibson et al., 2006). Two subjects were excluded from further analysis if they did not develop DOMS (Likert scores = 0) on day 1 and 2.

Pressure algometry

A handheld pressure algometer (Type II algometer, Somedic AB, Sweden) was used to measure the pressure pain threshold (PPT). Based on our previous work using this particular protocol (Gibson et al., 2009), maximal sensitivity was localized to the belly of the muscle. The origin and insertion of the TA muscle was located by palpation on both legs. The probe (1 cm^2) was placed perpendicular to the skin on the belly of TA muscle. The pressure stimulation was gradually increased (30 kPa/s) until the subjects identified the pressure exerted defined as pain and pressed a button. The PPT was evaluated three times with 1 min interval and the average was used in the statistical analysis. The pressure algometry protocol show normal inter-tester reliability on tibialis anterior muscle (Bisset et al., 2015).

MVC and force recordings

The MVC of the dorsiflexor muscles was determined using a custom build footplate (Larsen et al., 2015). 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 subjects were positioned supine with the knee and the ankle at 120° angle. The force data acquired during the MVCs were analyzed to determine peak force (N) and Force-time integral (N×s) using custom written MatLab program (The Mathworks, Natick, MA, USA version 2015a). The average peak force for each contraction was used as a measure of dorsiflexor muscle strength during the 1-s and 5-s contractions, respectively.

Reactive hyperemia induced by cuff occlusion

The cuff mounted around the leg, proximal to the knee, was automatically inflated (270 mmHg) for 5 min (Nocitech, Aalborg). The cuff occlusion promotes a reduction or blockage of blood flow (Scholbach et al., 2014) and 5 min of cuff occlusion have been considered a model to assess flow-mediated dilation or vascular reactivity (Green et al., 2014).

Ultrasound assessment of blood flow

It is unclear whether the vessel compression time affects the vessel diameter and consequently the blood flow response (Clifford et al., 2006). Thus, the maximum voluntary contractions (MVCs) were performed by 1-s MVCs and 5-s MVCs followed by 1 and 3 min of rest, respectively. Changes in blood flow before and after MVCs/occlusion was monitored by ultrasound Doppler (Logiq S7 Expert / Pro, GE, USA) with a 5.0 MHz (9L-D, GE, USA) or 10 MHz (ML6-15, GE, USA) probe.

For ATA blood flow assessment the 5.0 MHz probe was fixed with an adjustable mechanical arm positioned 2-3 cm below the head of the fibula. In the image B-mode, the sample of spectral Doppler (1 mm diameter) was positioned inside the ATA and the insonation angle was adjusted to $60°$. The average blood flow velocity (BFV, cm/s) was recorded in epochs of 1-s for a total of 60-s. The first 12-s was recorded before contraction or before cuff release and the remaining time was recorded to analyze BFV after MVC contraction and cuff occlusion. An additional record of 12 seconds before the cuff occlusion was performed as control. The last 10-s prior contraction was analyzed to avoid any difference in the time between the subjects. The BFV typically returns to normal values 35-s after MVC and cuff release. Thus, the time course data of average BFV was initially extracted in 1-s epochs (Figure 2) and subsequently as average values in the 10-s before and 35-s after MVC and cuff occlusion. In a subset of subjects, the ATA diameter was extracted from 5 images equally distributed during the 10-s pre-recording and 5 images during 35-s after MVC and cuff release. In these subjects, the resting ATA diameter was not significantly different from the post-contraction diameter. Thus, for all subjects the average of the ATA diameter from 5 images recorded at pre-contraction (resting) was used to calculate blood flow post-contractions and the difference in ATA diameter between days before and after eccentric exercise. The blood flow (BF) in milliliters per minute was calculated by multiplying the ATA cross-sectional area (CSA) by the mean blood velocity (cm/s) (time average mean, ATAMEAN-velocity): BF (ml/min) = $3.14 \cdot$ ATA-radius^2 (cm^2) $\cdot$ BFV (cm/s) $\cdot$ 60 (s). Thus, the time course of blood flow was used to determine time to peak and peak blood flow.

The intramuscular blood flow was assessed in the TA muscle. The origin and insertion of the TA muscle was located by palpation and the 10 MHz probe (ML6-15) was fixed 4-6 cm laterally to anterior border of the tibia with adjustable mechanical arm perpendicular to the skin along of the belly of TA muscle to assess the intramuscular BF. In the B-mode

image, sample of color Doppler (rectangular area: 4.7 cm^2) was positioned on the TA muscle belly to record immediately below the fat/fascia tissue. Recent work showed that the blood perfusion can be assessed by the color Doppler technique and changes in pixel number with color activities was interpreted as changes in perfusion of the microcirculation (Scholbach et al., 2014; Lomonte et al., 2015). Thus, the number of pixels with color activities within the TA muscle was recorded continuously (6 frames/s) for 60-s. The number of pixels with color activity in the sample Doppler area was extracted in each image (6 per second). The color activity data was extracted using custom written MatLab program (The Mathworks, Natick, MA, USA, version 2015a). The average number of pixels with color activity in the 6 frames was used to define the pixel color activity per second and data are expressed as pixel color activity (PCA). The first 10 seconds was recorded before MVC contraction and the remaining time were recorded to analyze pixel color activities within the TA muscle after contraction (1-s and 5-s). The change in pixel number with color activities over time was interpreted as variation in blood flow perfusion in the TA muscle. Figure 3 illustrates the 8-s before and 40-s after MVC. The time course of color activity was used to determine peak blood flow and time to peak blood flow in TA muscle after muscle contractions. The frames identified with displacement of the probe (40% change of the PCA from the previous recording, not immediately after the MVC) were certified like outliers in video records and excluded from the analysis.

Statistics

Descriptive statistics were used for the analyses of subject characteristics using Graphpad (version 5.0 www.graphpad.com) or Statistica (version 10.0). All parameters are presented as means ± standard deviation (SD) with statistical significance being accepted when $P<0.05$. The ATA peak flow and TA muscle peak color activity were normalized and analyzed as the

difference between the peak flow or peak color activity relative to the average baseline (resting time) value. Data were analyzed using two-way repeated-measure analysis of variance (RM-ANOVA). The PPTs were analyzed with factors *legs* (exercise and control leg) and *days* (0, 2, 6). For force and intramuscular color activity, data were analyzed in exercise and control legs separately with factors *days* and *contraction time* (1-s and 5-s). For ATA diameter and ATA blood flow, data were analyzed with factors *days* and contraction/occlusion time (1-s, 5-s and cuff occlusion 5 min). Muscle soreness (Likert scale) was analyzed using Kruskal-Wallis (KW) test with days (0-6) as factor. The Newman-Keuls (NK) post-hoc test was used when factors and interactions were significant. Associations between muscle soreness and physical activity level or muscle soreness and blood flow were assessed by Spearman´s rank correlation coefficient. The between session test-retest reliability was calculated for each test measure in the control leg to 1-s MVC, 5-s MVC, and cuff occlusion. The reliability of criterion measures (PPT, MVC, ATA diameter, ATA blood flow) across days 0, 2 and 6 were analyzed in the control leg and assessed using intraclass correlation coefficient (ICC) (the respective 95% confidence intervals-CI are reported for ICC) (Atkinson and Nevill, 1998). Interpretation of ICC was 1.00-0.76 (good to excellent), 0.75-0.41 (fair to good), and 0.40-0.00 (poor) (Fleiss, 1986).

RESULTS

Muscle soreness after eccentric exercise

The Likert scores showed that the eccentric exercise protocol induced muscle soreness in the exercised leg compared with day-0 with a peak score at day-1 and day-2 (KW:P<0.01) which returned to baseline levels after 6 days (Figure 1A). A negative correlation was found between Likert score of muscle soreness at day-2 in the exercise leg and physical activity level (r = -0.75, P<0.005).

The recruitment of participants accounted for non-responders, which was supported by our results showing a significant decrease in PPT. Thus, the ANOVA of PPT in the TA muscle showed an interaction between factors legs (exercise and control) and days (0, 2, 6) (RM-ANOVA: $F_{(2,18)}=4.08$ P<0.03). In the exercised leg, the PPT was reduced at day-2 compared with day-0 (NK: P<0.002) and compared with the control leg (NK: P<0.001; Figure 1B). The reliability of PPT in the control leg during days was ICC: 0.59; CI: 0.23-0.86. Two subjects with the higher level of physical activity (5988 and 3144 Met-min/week) did not develop DOMS (Likert scores were 0) on day 1 and 2 and were excluded from further analysis.

Maximum contraction force after eccentric exercise

The ICCs of the Force-time integral and peak force in the control leg during days were 0.94 (0.89-0.97) and 0.89 (0.8-0.95), respectively. The ANOVAs of the Force-time integral in both exercise and control legs demonstrated a main effect of contraction time (Table 1; RM-ANOVA: $F_{(1,9)}>68.0$, P<0.001; NK: P<0.001) with higher values for 5-s MVC compared with 1-s MVC. No difference was found in peak force between 1-s and 5-s MVC in exercise and control leg (RM-ANOVA: $F_{(1, 9)}=2.71$, P=0.13; $F_{(1,9)}=4.85$ P=0.055).

The Force-time integral and peak force of 1-s and 5-s contractions, respectively, were reduced on day-2 compared with day-0 and day-6 (RM-ANOVA: $F_{(2,18)}= 12,49$ P<0.001; NK: P<0.001; $F_{(2,18)}=8.26$, P<0.002; NK: P<0.003) in the exercise leg, with no differences in Force-time integral and peak force on days in the control leg (RM-ANOVA: $F_{(2,18)}<0.13$, P>0.7; $F_{(2,18)}<0.72$, P>0.49).

Artery diameter after eccentric exercise

The reliability of ATA diameter in the control leg during days was ICC: 0.83; CI: 0.72-0.91. The ANOVA of the ATA diameter at rest (before contraction/occlusion) in the exercise leg

demonstrated a main effect of days (Table 2; RM-ANOVA: $F_{(2,18)}=8.29$, $P<0.002$) with the ATA diameter being reduced before all contractions/occlusions on day-2 compared with day-0 and-6 (NK: $P<0.003$) and no effect was found on contraction times or occlusion (RM-ANOVA: $F_{(2,18)}=1.78$ $P<0.19$). No differences in ATA diameter was found in the control leg on days (RM-ANOVA: $F_{(2,18)}=0.89$, $P<0.42$) or on contraction/occlusion time (RM-ANOVA: $F_{(2,18)}=0.52$, $P<0.59$).

Anterior tibial artery blood flow

The average ATA blood flow before and after experimental protocols in the exercise and control legs on day-0, day-2 and day-6 are illustrated in Figure 2. No difference was found in resting blood flow prior to contractions or cuff occlusion across days, in exercise and control leg (RM-ANOVA: $F_{(2,18)}>0.19$, $P>0.64$). In both exercise and control legs at all days, the ATA peak blood flow was higher after cuff occlusion compared with 1-s MVC and 5-s MVC (Table 3; RM-ANOVA: $F_{(2,18)}>33.0$, $P<0.001$; NK: $P<0.04$). Also, the ATA peak blood flow in both legs and across days was higher after 5-s MVC compared with 1-s MVC (NK:$P<0.04$) suggesting that the increase in peak blood flow is associated with the occlusion time.

When analyzing the peak ATA blood flow an interaction between days and contraction/occlusion time was found in the exercise leg (RM-ANOVA: $F_{(4,36)}=2.83$, $P<0.03$). The peak blood flow was reduced after 1-s MVC (~30%, NK: $P<0.04$), 5-s MVC (~30%, NK: $P<0.001$), and cuff occlusion (~16%, NK: $P<0.007$) on day-2 compared with day-0 and day-6 in the exercise leg. In the control leg no difference was observed between days in the peak blood flow to contractions and cuff occlusion (RM-ANOVA: $F_{(2,18)}=0.98$, $P<0.39$). Also, no interaction was observed between factors (RM-ANOVA: $F_{(4,36)}=0.55$, $P<0.69$). In both exercise and control legs no difference was found between days in time to

peak blood flow for MVC (1-s and 5-s) or cuff occlusion (Table 3; RM-ANOVA: $F_{(2,18)} < 0.76$) $P > 0.47$). However, in both exercise and control legs, and for all days, time to peak blood flow was faster after 1-s MVC compared with 5-s MVC and cuff occlusion (RM-ANOVA: $F_{(2,18)} > 4.62$, $P < 0.02$; NK: $P < 0.01$). The reliability of ATA blood flow in the control leg during days was ICC: 0.76; CI: 0.61-0.86.

Intramuscular blood flow

In two subjects the PCA data for intramuscular tissue (exercise and control leg) was missing due to technical problems, and PCA data for intramuscular tissue were therefore analyzed for 8 subjects. Also, approximately 9% of frames analyzed were excluded due to probe displacement. The average PCA data over time before and after MVC in the exercise and control legs on day-0, day-2 and day-6 is illustrated in Figure 3.. No significant difference was observed in intramuscular color activity at rest, between days, for 1-s and 5-s contractions in exercise and control leg (RM-ANOVA: $F_{(2,6)} > 0.44$, $P > 0.28$). The intramuscular peak PCA was higher after the 5-s MVC compared with 1-s MVC in both exercise and control leg (RM-ANOVA: $F_{(1,4)} > 13.02$, $P < 0.03$). In the exercise leg, peak PCA was reduced after 1-s (~32%) and 5-s (~23%) on day-2 and main effects was observed between session days (RM-ANOVA: $F_{(2,6)} = 6.93$, $P < 0.027$; NK: $P < 0.03$). No interaction was observed between factors (RM-ANOVA: $F_{(2,6)} = 0.57$, $P = 0.58$). In the control leg no difference was observed on days (RM-ANOVA: $F_{(2,8)} = 0.53$, $P < 0.6$) and no interaction was observed between factors (RM-ANOVA: $F_{(2,8)} = 3.28$, $P < 0.09$). Finally, no difference was observed in time to peak PCA for 1-s and 5-s MVC between days, in both exercise and control leg (RM-ANOVA: $F_{(2,6)} < 0.93$, $P > 0.44$). The reliability of intramuscular blood flow in the control leg during days was ICC: 0.46; CI: 0.11-0.78

Associations between blood flow and pain sensitivity

There were no significant correlations between markers of muscle damage (Likert scores, PPTs and MVC-reductions) and changes in parameters of ATA blood flow and ATA diameter 2 days after eccentric exercise ($0.04 < r < 0.53$, $0.11 < p < 0.89$).

DISCUSSION

The present study demonstrated reduced peak blood flow response in both feed artery and muscular tissue after brief maximal contractions and cuff occlusion during muscle soreness 2 days after unaccustomed eccentric exercise. Moreover, two days post-exercise reduced ATA diameter and maximal voluntary force level were found. These results suggest that eccentric exercise may change the ability to regulate the blood flow response required to match the metabolic demand which may consequently accumulate algesic mediators contributing to DOMS and impaired muscle function.

Impaired blood flow after eccentric exercise

It is unclear whether there is difference in the response to eccentric exercise between genders (Stupka et al., 2001; Larsen et al., 2015). On the other hand, the blood flow response can be influenced by force level (Hamann et al., 2004b; Clifford 2007) and Van Teeffelen and Segal (2006) demonstrated that the magnitude of vasodilation of arterioles following a single muscle contraction is proportional to the number of active motor units. About all motor units in TA are recruited between 60-70% MVC (Erim et al., 1996). The results showed that the intraclass reliability measures in Force-time integral (ICC: 095) and peak force (ICC: 0.89) during days were good to excellent. These data suggest that single maximal contractions (1-s and 5-s) used to evoke blood flow response were reproducible over days and recruited all TA motor units, before and after eccentric exercise. Consistent with our results after eccentric

exercise, other studies have shown impaired hemodynamic flow after continuous and brief muscle activity (Kano et al., 2005; Larsen et al., 2015). The present work extends these studies and showed that lower peak blood flow response after eccentric exercise is also observed following cuff occlusion.

In an animal model, the eccentric exercise (300 electrically induced eccentric contractions) resulted in a marked degeneration-regeneration response (~50 % damaged muscle fibers) and decrements in twitch peak torque (~16 % of baseline) after eccentric protocols (Kano et al., 2004; 2008). Such degrees of fiber damage and decrements in muscle function are almost never seen in human studies, except in occasional high-responders (Paulsen et al., 2012). Due to possible differences between species (rodents vs humans) and models of eccentric exercise (maximal electrically induced contractions vs submaximal voluntary exercise), the findings from studies reporting capillary changes in animal models (Kano et al., 2004) need to be investigated in human models of voluntary eccentric exercise. The exercise protocol used by Friden and colleagues (1986) resulted in large degree of fiber swelling (23-27% on average) and an increase in intramuscular pressure (10 mmHg) at 48 h post exercise, which was interpreted not to compromise microvascular circulation. As the protocol used in the present study has shown to result in a modest (7%) increase in muscle cross-sectional area in MRI (Larsen et al., 2015), intramuscular pressure was likely not elevated to an extent that would limit blood flow through the microvasculature.

Obtaining biopsies would have added information about possible structural changes of the microvasculature, however recent work suggests that injury from mechanical forces on vessels after eccentric exercise can compromise an adequate blood supply to muscle tissue (Barnes et al., 2010; Larsen et al., 2015). Also, MRI data (transverse relaxation time, T2, as a proxy for damage/swelling and BOLD images to reflect the hyperemic response to brief contractions) acquired 48 h after eccentric contractions do not show large variations in T2 or

hyperemic response within the dorsiflexor muscle compartment, suggesting an overall decline in vessel function (in contrast to regions with collapse or mechanical hindrance) (Larsen et al., 2015). . Augmented local and central blood pressure responses and increased heart rate have been reported up to three days after eccentric exercise (Miles et al., 1997; Barnes et al., 2010), suggesting arterial stiffening and increased sympathetic activity during DOMS. Therefore, the present results can indicate that the increased vascular tone induced by eccentric exercise may induce changes in blood flow response that possibly contribute to reduced ability to rapidly augment blood flow to match O_2 delivery to the metabolic demand in the exercising muscle during DOMS, which underscores the physiological and clinical relevance of these results. It is well documented that the second bout of eccentric exercise performed within several weeks induces less DOMS than the first exercise bout. Thus, the potential 'repeated bout effect' of eccentric exercise on blood flow measures can help as a direction for further investigation.

Blood flow in feed artery

The ATA blood flow time course was analyzed using the first 35-s following release of muscle contraction or cuff occlusion and the range of reliability for ATA blood flow and ATA diameter during days (ICC: 0.76 and 0.83) show good to excellent reproducibility of ATA blood flow and ATA diameter analyses. In a subset of subjects no difference was observed in the ATA diameter at rest and after release of muscle contraction or cuff occlusion. While most studies on blood flow response after brief contraction are done in forearm in humans, the characteristic of the blood flow response is similar in legs (Credeur et al., 2015). In muscle, the majority of the total resistance is provided by the arterioles (Tschakovsky et al., 1996; Brock et al., 1998; Tschakovsky and Sheriff 2004). Arteriolar dilation precedes changes in blood flow in the feed arteries which are located external to the

muscle and do not dilate immediately in response to brief muscle contractions (Towse et al., 2011). In line, the diameter of the femoral and tibialis anterior arteries did not change immediately after brief muscle contraction involved with its blood supply (Radegran, 1997; Towse et al., 2011) and the peak vasodilation on feed artery can be expected to occur later (45-80 seconds) following cuff release (Harris et al., 2010). Also, using the same muscle compression intensity the diameter of the soleus feed artery in rat did not vary between 1-s and 5-s (Clifford et al., 2006). However, in line with previous studies of the femoral artery and ATA (Radegran, 1997; Hamman et al., 2004b; Towse et al., 2011), a consistent and immediate increase in ATA peak blood flow was observed around 4-s after blood flow release following muscle contraction. Further, peak blood flow was greater after 5-s contraction compared with 1-s contraction. The release of contraction and cuff is accompanied by release of vasodilators locally, which results in an immediate (up until 15 s) and transient increase in blood flow response detected in feed artery (Harris et al., 2010, Towse et al., 2011). The fact that the largest decreases in blood flow occurred within the first 10 s suggest that eccentric exercise had the greatest impact on vasodilation and peak blood flow.

In the present study the reduced ATA diameter 2 days after eccentric exercise did not change resting blood flow in the presence of DOMS which could suggest that the decreased diameter had insufficient impact on the accumulation of algesic substances. However, many activities are characterized by brief and repetitive actions of muscle contractions. The impaired blood flow response after muscle contraction during DOMS may therefore contribute to increased algesic substance concentrations in muscle tissue. Notably, our correlation analyses do not provide a direct link between changes in blood flow and measures of muscle soreness. Indeed, there is an intricate and complex interaction between muscle sensitization and vascular function. In addition, modest changes in these variables, combined

with a small sample size, limit the ability to establish significant correlations. Therefore, larger studies are warranted to explore correlations between changes in blood flow and muscle sensitization.

Muscle tissue blood flow

The relative change in blood flow response after 1-s and 5-s muscle contractions was similar between TA feed artery and TA muscle, with fair to good reliability of blood flow assessment in the TA muscle. The resting flow, flow distribution, and increased perfusion due to increased demand can be controlled by changes in vascular tone. The mechanism for rapid blood flow increase after muscle contraction is still poorly understood (Clifford 2011; Credeur et al., 2015). However, vasodilation in microcirculation within the muscle tissue is obligatory to observe this hyperemic phenomenon (Hamann et al., 2004a) and several vasodilatory mechanisms may contribute to the muscle vascular response (Credeur et al., 2015). The color Doppler technique utilized in this experimental protocol is commonly used to assess blood flow in vascular bed (Lomonte et al., 2015) and recently have been used to assess blood perfusion capacity in the microcirculation (Scholbach et al., 2014). The color pixel counts when using the color Doppler technique in the TA muscle was higher after 5-s MVC compared with 1-s MVC suggesting a greater perfusion of the microcirculation with longer contraction time. Moreover, the reduced color pixel count after muscle contractions during DOMS may indicate impaired blood perfusion of the eccentrically exercised tissue.

Muscle pain and blood flow

Usually eccentric exercise is accompanied by hyperalgesia (Gibson et al., 2006; Larsen et al., 2015). The second session of the study was conducted 48 hours after the first session because DOMS usually is established in this period (Gibson et al., 2006). The Likert scale score

showed that subjects developed typical muscle soreness with peak response between 24-72 h after eccentric exercise and this was inversely correlated with physical activity levels of the subjects. Consistent with our results, high level of physical activity prevents the onset of muscle soreness induced by eccentric exercise (Newton et al., 2008). The reliability for PPT during days (ICC: 0.59) show fair to good reproducibility of PPT analyses. Thus, the reduced pressure pain threshold observed 2 days after eccentric exercise is consistent with previous work reporting hyperalgesia after eccentric stimulus (Karoline et al., 2003; Gibson et al., 2006). Also, the soreness ratings from the TA muscle, displayed a typical temporal profile matching the time points for measurements of impaired vascular reaction function using Doppler ultrasound. Zainuddin and collaborators (2006) showed that light concentric contractions reduced pain levels during DOMS probably by the contraction-evoked blood flow increase. Thus, the blood flow decrease found in the present study may be involved in the mechanism of DOMS. The DOMS is not necessarily associated with myofiber damage, but more likely associated with increased sensitivity of extracellular matrix (ECM) and/or myofascia due to damage/inflammation (Paulsen et al., 2010; Lau et al., 2015). The recognized inflammatory substances (ex. IL-1, IL-6) are observed in muscle tissue after eccentric exercise during DOMS and have association with the sensitivity of nociceptors (Cannon et al., 1989; Jonsdottir et al., 2000). In addition, another study shows that bradykinin and NGF play roles in muscular mechanical hyperalgesia after eccentric exercise in rat model of muscle soreness (Murase et al., 2010). The protocol to induce DOMS (2 sets of 50 concentric/eccentric contractions (Tegeder et al., 2002) was more severe than the protocol used in present study (5 sets of 10 eccentric contractions) but the intensity of DOMS after eccentric exercise was similar in both studies. Also, during a similar profile matching the time points of muscle soreness, the sequence of dynamic muscular contractions (2 sets of 10 repetitions) was sufficient to increase algesic substances as lactate, PGE2, glutamate and

substance P in muscle tissue in the DOMS leg compared with control leg for at least 20 min of collection using microdialysis (Tegeder et al., 2002). Taken together, these results suggest that accumulation of algesic substances may facilitate sensitivity in muscle tissue after eccentric exercise. In our experimental protocol, the blood flow response analyses and consequently impaired vascular response was not used prior to 48 h after the eccentric exercise. However, the failure to rapid adjustment of blood flow after muscle contraction was observed 24 and 48 h after eccentric exercise in DOMS leg in human (Larsen et al., 2015). Therefore, the failure on vascular control may contribute to hinder leakage and promote the buildup of these substances in muscle tissue, which possibly may increase muscle nociceptor sensitivity and account for the hyperalgesia in DOMS.

Perspectives

The present study showed consistent results on the blood flow response after different times of occlusion in both the TA artery and TA muscle. We report no change in diameter despite large changes in flow, suggesting that flow at least initially is not regulated by vasodilation in feed artery. However, the increased vascular tone (probably due to increased sympathetic activity) may limit peak flow under these conditions. Therefore, the failure of the vascular system may impair the recover process after eccentric exercise, increase the concentration of algesic substances and contribute to nociceptors sensitization during DOMS.

Acknowledgements

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TABLES

| Contraction time | Session | Peak force (N) | | Force-time integral (N·s) | |
		Exercise Leg	Control Leg	Exercise Leg	Control Leg
1-s MVC	day-0	159.8 ± 70.3	161.8 ± 61.7	*221.6 ± 85.4	*216.7 ± 102.9
	day-2	#130.4 ± 53.9	165.7 ± 79.6	*#183.3 ± 80.0	*216.7 ± 111.7
	day-6	176.5 ± 58.9	152.9 ± 58.7	*237.3 ± 73.6	*229.4 ± 84.8
5-s MVC	day-0	232.4 ± 81.5	227.5 ± 108.4	1018.9 ± 361.2	1016 ± 485.5
	day-2	#183.3± 86.1	225.5 ± 92.3	#769.8 ± 349.4	979.7 ± 420.1
	day-6	244.1 ± 77.3	239.2 ± 81.3	1069.9 ± 336.4	1022.8 ± 309.0

Table 1: Mean (± SD), N=10) peak force (N) and Force-time integral (N·s) for 1-s and 5-s maximal voluntary contractions (MVCs) before (day-0) and after eccentric exercise (day-2 and day-6). Significantly lower after 1-s MVC compared with 5-s MVC (*, $P<0.001$) and on Day 2 compared with Day-0 and Day-6 (#, $P<0.002$).

		ATA diameter (cm)	
Contraction / occlusion	Session	Exercise leg	Control leg
1-s MVC	day-0	0.255 ± 0.042	0.266 ± 0.044
	day-2	#0.236 ± 0.037	0.254 ± 0.039
	day-6	0.252 ± 0.04	0.259 ± 0.047
5-s MVC	day-0	0.254 ± 0.041	0.273 ± 0.05
	day-2	#0.236 ± 0.039	0.270 ± 0.054
	day-6	0.254 ± 0.041	0.256 ± 0.052
5-min cuff occlusion	day-0	0.286 ± 0.046	0.277 ± 0.039
	day-2	#0.268 ± 0.044	0.272 ± 0.039
	day-6	0.279 ± 0.044	0.285 ± 0.047

Table 2: Mean (± SD), N=10) anterior tibial artery (ATA) diameter assessed by ultrasound imaging in the exercise and control leg before the maximal voluntary contractions (MVCs, 1-s and 5-s durations) and before cuff occlusion (5 min). Significantly reduced at day-2 compared with day-0 and day-6 (#, P <0.002).

		Baseline (ml/min)		Peak (ml/min)		Time peak (s)	
Contraction / occlusion	Session	Exercise leg	Control leg	Exercise leg	Control Leg	Exercise leg	Control Leg
1-s MVC	day-0	10.5 ± 5.1	9.1 ± 3.8	39.1 ± 13.6	43.0 ± 11.8	4.2 ± 1.87	4.6 ± 1.8
	day-2	9.7 ± 6.6	10.8 ± 7.2	#27.0 ± 12.7	38.1 ± 10.9	5.4 ± 2.36	3.4 ± 2.0
	day-6	9.6 ± 5.1	12.1 ± 8.5	40.6 ± 13.1	41.3 ± 15.0	4.9 ± 2.07	5.5 ± 1.5
5-s MVC	day-0	10.3 ± 4.3	9.0 ± 4.3	*63.8 ± 22.6	*72 ± 21.2	*9.0 ± 1.94	*10.2 ± 3.3
	day-2	10.5 ± 5	11.2 ± 7.2	#*40.8 ± 16.7	*61.9 ± 17.3	*7.8 ± 3.9	*8.6 ± 3.3
	day-6	9.3 ± 5.2	11.8 ± 12.1	*58.4 ± 19.6	*60.2 ± 14.4	*9.2 ± 3.8	*8.2 ± 2.8
5-min cuff occlusion	day-0	10.2 ± 4.6	11.3 ± 3.7	¤123.8 ± 32.1	¤125.3 ± 32.9	7.7 ± 6.55	6.4 ± 5.7
	day-2	10.7 ± 5.4	9.4 ± 3.8	#¤86.5 ± 29.6	¤112.5 ± 54.5	5.7 ± 4.99	10.5 ± 5.6
	day-6	10.8 ± 7.7	12.8 ± 11.3	¤103.3 ± 48.3	¤126.8 ± 42.1	5.8 ± 5.99	5.1 ± 6.5

Table 3: Mean (± SD), N=10) anterior tibial artery blood flow before hyperemia (baseline), peak flow, and time for peak flow following the maximal voluntary contractions (MVCs, 1-s and 5-s) and cuff occlusion (5 min). Significantly increased compared with 1-s MVC (*, P<0.02)=0.0001; P=0.0002; P=0.02 P=0.001) or compared with the 5-s MVC (¤, P<0.001). Moreover, significantly reduced compared with day-0 and day-6 within that contraction/occlusion type (#, P<0.04).

Contraction	Session	Baseline PCA		Peak PCA		Time (s) PCA peak	
		Exercise leg	Control leg	Exercise leg	Control Leg	Exercise leg	Control Leg
1-s MVC	day-0	697± 709	358± 332	2141± 2138	1854 ± 1359	5.4± 4.8	6.3± 2.5
	day-2	634± 1009	490 ± 347	#1444 ± 1709	2482 ± 986	8.8± 5.5	8.2± 3
	day-6	357± 210	648± 549	2195± 1430	1948± 1389	6.2± 2.1	6.7± 5.4
5-s MVC	day-0	747± 820	401± 341	*3230± 3319	*3175± 2148	11.4 ± 3.3	14.1± 9.2
	day-2	191± 185	214± 185	*#2463± 1939	*2912± 1535	14.1 ± 9.2	14.1± 5.8
	day-6	420± 225	506± 376	*3213± 1620	*3031± 1214	9.7± 4.0	12.6± 5.9

Table 4: Mean (± SD), N=8) intramuscular blood flow (PCA) before hyperemia (baseline), peak flow, and time for peak flow following the maximal voluntary contractions (MVCs, 1-s and 5-s). Significantly increased compared with 1-s MVC (*, P<0.03). Moreover, significantly reduced compared with day-0 and day-6 within that contraction (#, P<0.03). PCA: pixel color activity.
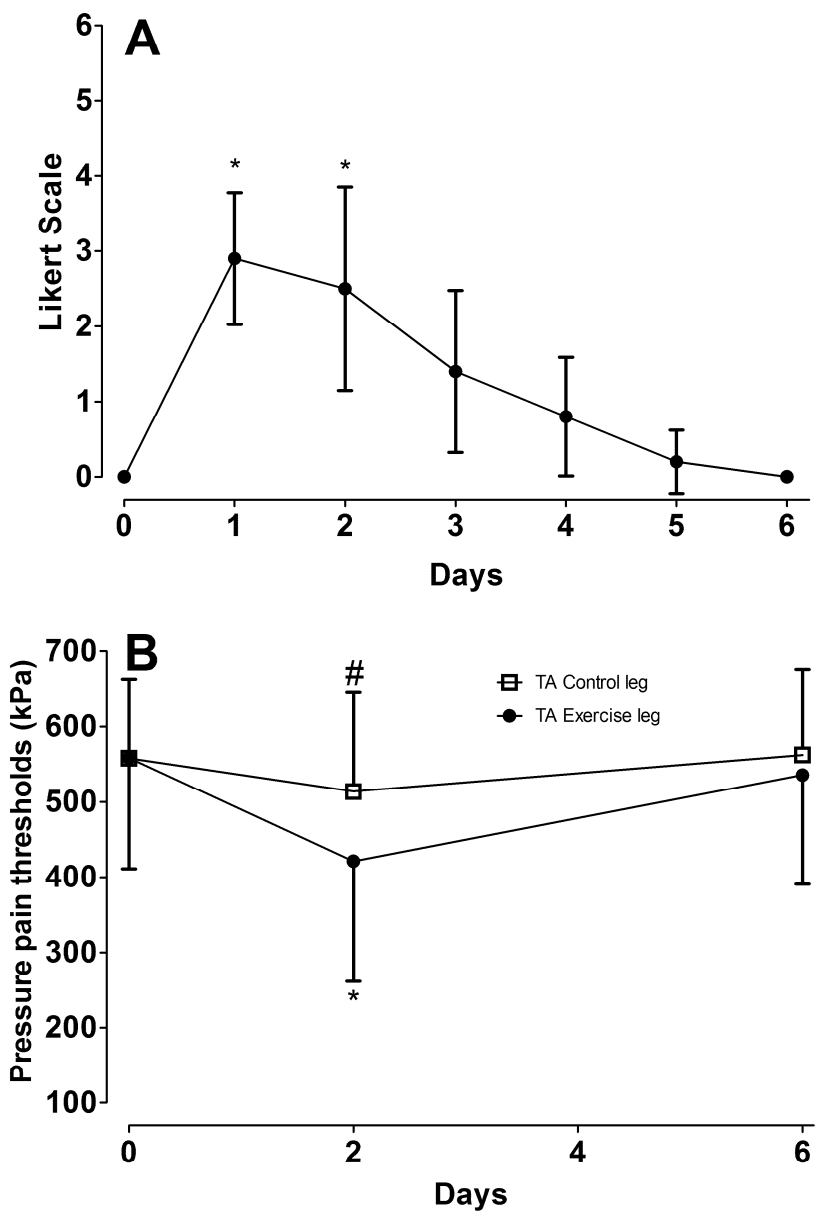
FIGURE LEGENDS

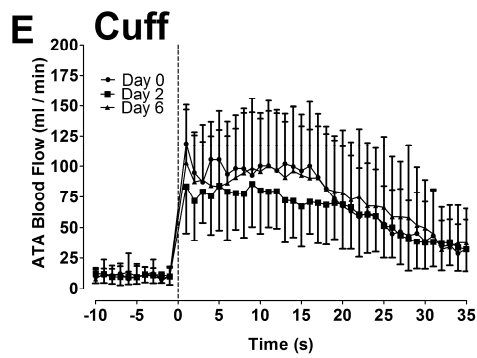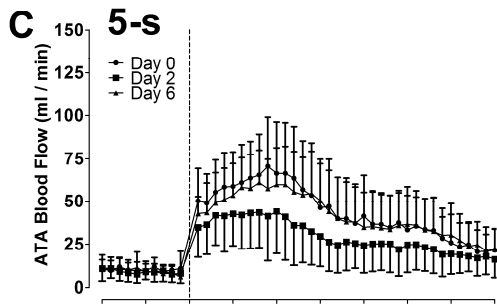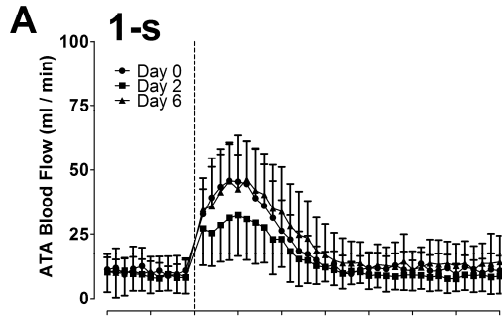Fig. 1**:** Delayed onset muscle soreness after eccentric exercise (DOMS). Mean (±SD, N=10). (A) Likert Scale scores over time for the tibialis anterior muscle in exercise leg (B) pressure pain thresholds (PPT) recorded from the tibialis anterior (TA) muscle. Significant differences from baseline (day-0, *, $P<0.001$) and between exercise and control leg (#, $P<0.001$).

Fig. 2**:** Mean (± SD, N=10) anterior tibial artery blood flow before hyperemia (baseline, -10 to 0 s) and blood flow following (0-35 s) the maximal voluntary contractions (MVCs, 1-s [A,B] and 5-s [C,D]) and cuff occlusion (5 min [E,F]) in the exercise and control leg on day-0, day-2, and day-6.

Fig. 3: Mean (± SD, N=8) intramuscular blood flow (pixel color activity-PCA) before hyperemia (baseline, -8 to 0 s) and blood flow following (0-40 s) the maximal voluntary contractions (MVCs, 1-s [A,B] and 5-s [C,D]) in the exercise and control leg on day-0, day-2, and day-6.

Exercise Leg   **Control leg**

A 1-s

B

C 5-s

D

E Cuff

F