



**Abstract:**

Delayed Onset Muscle Soreness (DOMS) is the sore and tender feeling that appears after severe or unaccustomed exercise, especially eccentric exercise (EE), which causes structural damage to the muscle fiber. Some of these structural damages are related to microvascular function and therefore there is suggested an effect on the microvascular function in the damaged muscle due to mechanisms such as alteration in activation patterns, myoglobin leakage and increased capillary luminal area. Near Infrared Spectroscopy (NIRS) is a non-invasive measuring tool, based on optical light, measuring oxygen saturation in tissue continuously that enables measurement of microvascular function. The aim of this study was to investigate the effect of DOMS caused by EE to the microvascular function, using NIRS as measuring tool. Fourteen (8 female, 6 male) subjects participated in the study. The study consisted of pre-test, EE and post-test. Pre-test measured indirect DOMS markers by registration of perceived pain ranged on a visual analog scale (VAS), a maximum voluntary contraction test (MVC), a pain pressure threshold test (PPT). Microvascular function was investigated with NIRS, measuring oxygen saturation in vastus lateralis (VL) and rectus femoris (RF) at both legs during a 10 min occlusion and a 5 min cycling period. The EE consisted of 6x10 repetitions of front lunges, with an external loading of 40% of body weight for women and 50% for men, performed with the dominant leg only, to induce one DOMS leg and one control leg. Post-test was identical to the pre-test and was performed 48h after EE. VAS increased significantly ( $P < 0.01$ ) with 2.7 cm from pre to post in DOMS leg, while no significant change in control leg with an increase of 0.7 cm. PPT showed no significant differences. MVC during extension decreased in DOMS leg ( $P = 0.01$ ), while not in control leg. MVC during flexion increased in DOMS leg ( $P = 0.02$ ) while not in control leg. NIRS results showed significant difference ( $P = 0.04$ ) in recovery rate (tau-SmO<sub>2</sub>%) in RF DOMS leg compared to control leg at post test, with 5.4 ( $\pm 5.2$ ) s faster recovery in DOMS leg after cycling. There were significant differences in VL control leg from pre to post ( $P = 0.01$ ) after cycling, with 3.6 ( $\pm 2.7$ ) s slower recovery rate at post-test. There were no significant differences in either desaturation, oxyhaemoglobin or total haemoglobin from either pre to post EE or DOMS to control in either occlusion or cycling test. Due to the lack of clear results for the DOMS markers the results of microvascular function is affected, and one has to evaluate the results with a limitation. However the minor significant results that were found in

changes in microvascular functions indicates that there might be changes in microvascular function. The study do not provide support to the findings of any of the earlier studies in the field. As there are suggested microvascular changes following muscle damage as a result of EE, the subject is interesting for further research to confirm or disprove these theories.

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## **1. Introduction**

Delayed Onset Muscle Soreness (DOMS) is a well-known phenomenon, as a perceived feeling of sore and tenderness and as a research topic. DOMS was first mentioned by Hough in 1905 and is described as pain or soreness felt when palpating or activating muscles that are damaged by unaccustomed and/or severe exercise (Nosaka 2008). DOMS is defined as a muscle injury (Cheung et al. 2003) and the feeling of DOMS can be experienced immediately after performing exercise and last up to 10 days following the exercise (Cleak et al. 1992). The pain and feeling of DOMS is increasing from time of exercise until 72h after, with a peak between 24h-72h post exercise, before decreasing (Cheung et al. 2003). The duration is dependent on the grade of damage, warm up and cool-down exercises performed prior and after DOMS inducing exercise (Olsen et al. 2012). Earlier studies show that eccentric exercise is effective in inducing DOMS, especially when one is unaccustomed to the specific exercise (Allen et al. 2001). Eccentric exercise means that the muscle is lengthening during contraction. Such a muscle contraction can produce more force than a concentric (shortening) contracting muscle, and the mechanical load may be much greater than on a concentric contracting muscle. As the mechanical load is greater the potential of damaging the muscle increases. In eccentric contractions the sarcomere can become overstretched and damaged (Umbel et al. 2009). Depending on load and repetitions, the damage may occur at different levels, as damage to a single muscle fiber, single sarcomere or over larger parts of the muscle fiber, as damage to multiple sarcomeres (Proske & Morgan 2012). Proske & Morgan (2012) presents the cause of the damage as uncontrolled sarcomere lengthening, until overstretch in the weakest sarcomere occurs and is followed by increased load on the second weakest sarcomere, and thus overload and overstretching occur in these sarcomeres as well, causing an increased damage in the muscle fiber. Furthermore Proske & Morgan (2012) report that as the eccentric contractions are repeated the damage to the sarcomeres increases, until a point is reached, when the membrane of muscle fiber and the excitation contraction-coupling (EC-coupling) is damaged. Damage to the membrane causes leakage of myoglobin (Mb) and creatine kinase (CK) (Roxin et al. 1986). In the muscle fibre there seems to be a rupture of the t-tubulus, causing an inactivation of the sarcomeres, this leads to a further loss of force producing muscle fibers and an increased load on the remaining active

sarcomeres (Proske & Morgan 2001). Post exercise, the muscle starts to swell, which seem to cause changes in capillary properties, such as increased luminal area (Kano et al. 2004), which causes alteration in O<sub>2</sub> flux from capillaries to the muscle cell (Davies et al. 2008, Yu et al. 2013). The damage to the t-tubulus causes an inactivation of the damaged sarcomeres, and thus cause alteration of the activation patterns (Vila-Cha et al 2012, Hedayatpour et al. 2014, Wakefield et al. 2011). The leakage of myoglobin may reduce the oxygen uptake in the muscle, and the membrane damage may also alternate the cells diffusion properties. These damages and their effects suggests an alteration to the microvascular function in the damaged muscle, as investigated by e.g Ahmadi et al. (2008a, 2008b), Davies et al. (2008) and Walsh et al. (2001). However there seem to be conflicting results in the literature as Ahmadi et al. (2008a) found faster saturation recovery kinetics in the affected muscle after DOMS induced by eccentric exercise, while Davies et al. (2008) found slower recovery kinetics. Walsh et al. (2001) concluded with no changes in microvascular function after damaging exercise. These studies use Near-Infrared Spectroscopy (NIRS) to measure microvascular functions. NIRS is a fairly new tool, based on optical light, measuring oxygen saturation in the local tissue non-invasive and continuously (Quaresima et al. 2003, Ferrari et al. 2011). NIRS can be applied during both rest and dynamic activity (Bhambhani 2004), thus making it a suitable method to investigate the changes in microvascular functions following DOMS as a result of eccentric exercise. The earlier studies have been using a symmetrical protocol, inducing DOMS to both legs, whereas this study aims to induce DOMS to one leg, keeping one leg unaffected as a control leg, similar to Ahmadi et al (2008b), who induced DOMS to one arm, leaving the other as a control arm. As the earlier studies investigating microvascular changes following DOMS caused by eccentric exercise seem to be inconclusive and conflicting, additional research is needed to further investigate the effects of muscle damage caused by eccentric exercise to microvascular function. Therefore the aim of this study is to investigate the effect of DOMS caused by eccentric exercise on microvascular function using NIRS as measuring tool by using an asymmetrical protocol inducing DOMS to one leg, keeping the other leg as a control leg.

## **2. Methods**

### **2.1 Subjects**

Fourteen healthy subjects (6 male, 8 female) voluntarily participated in the study, recruited through posters at the campus of the university. The study was approved by the regional medical ethical committee and participants signed a written informed consent approving their participation. The mean ( $\pm$ SD) age of the participants was 22.7 ( $\pm$ 3.2) yrs with a mean weight of 71.5 ( $\pm$ 13.1) kg and a height of 177.4 ( $\pm$ 9.7) cm. Mean BMI was 22.5 ( $\pm$ 2.7) kg/m<sup>2</sup> and mean percentage of body fat 20.3 ( $\pm$ 3.7) %. Subjects that were pregnant, had experienced leg injury the last 12 months, had reduced function in back, hips or legs, those who had been recommended to avoid physical activity by a physician, or those performing strength training regularly on the legs were excluded from the study.

### **2.2 Study Design**

The study was a within-subjects design, where each participant served as their own control at both pre-test and post-test. The experiments were conducted over three consecutive days. The pre-tests were done on day one and consisted of measurements of anthropometrics, DOMS markers (perceived pain, pain pressure threshold and force production) and measurement of microvascular function. Day one ended with a bout of eccentric exercise, consisting of front lunges performed with the dominant leg only in order to produce one DOMS leg and one control leg. Day two was a rest day. Day three consisted of post-test, measuring the effects of eccentric exercise on DOMS markers and microvascular function in both the DOMS and control leg following the same protocol as for the pre-test.

#### **2.2.1 Pre-test**

The pre-test consisted of collecting anthropometric data of gender and age, measurements of height, weight, and percentage of body fat, adipose tissue thickness, DOMS indicators and microvascular function. Measurements of DOMS markers included reporting perceived pain on a visual analog scale (VAS), a pain pressure threshold (PPT) test, and measuring maximum force production, done by a maximal voluntary contraction (MVC) test. Microvascular function during



rest was tested by a vascular occlusion test (VOT), measuring microvascular function during occlusion and recovery kinetics immediately after occlusion. The VOT test consisted of a 5 min rest period, followed by a 10 min occlusion, with a 5 min rest finishing the test. Following the VOT test was a cycling test, measuring muscle oxygenation changes during activity, and recovery kinetics immediately after activity. The cycling test consisted of a 5 min rest period, a 5 min work period with a work rate of 75% of max HR and cadence of 90 RPM, and a 5 min rest period. Microvascular function was measured in both legs.

### **2.2.2 Eccentric Exercise**

After the pre-test, one bout of eccentric exercise in the form of front lunges, was performed to induce DOMS. Subjects were instructed to only perform lunges with the dominant leg, in order to induce DOMS in one leg only, keeping the non-dominant leg as a control leg. Subjects completed 6 sets of 10 repetitions of lunges, with 30 s rest in between sets. Male subjects had an external load of 50% of body weight while the female subjects had an external load of 40% of body weight, added by a barbell bar with extra weight on their shoulders. Subjects were instructed to perform the lunges as explosive and fast as possible, and to execute the lunges so that their thigh were parallel with the floor, with a 90° angle in both the hip and knee joints. The exercise was intended to activate the anterior muscles of the thigh, and minimize activation in control leg. Subjects were encouraged and supervised during their exercise and instructed to rest as much as possible in between the pre-test and post-test, in order to prevent faster recovery and restitution by increased blood flow and muscle activity as reported by Cleary et al. (2002) and Cheung et al (2003).

### **2.2.3 Post-test**

The post-test were performed 48h ( $\pm 2$ ) after the pre-tests. The post-tests were similar as those performed during the pre-test and consisted of measurements of DOMS markers (registration of perceived pain on VAS, PPT test, MVC tests) followed by measurements of microvascular function during rest and work (VOT and Cycling). Protocol and data acquisitions were identical to the pre-tests.

## **2.3 Data Acquisition**

### **2.3.1 Anthropometrics**

Gender and age was reported and noted, height measured standing barefeet by the wall, measuring atop of head to floor. Weight was measured by a standard bathroom weight. Percentage of body fat was calculated through the equation presented by Peterson et al. (2003) using the measurements of skinfold thickness at triceps, iliac crest, subscapula, proximal thigh, mid-thigh, distal thigh and calf, measured with a skinfold caliper (Holtain Skinfold Caliper, Holtain Ltd, Crymych, UK), and measurements of circumference at mid arm, upper, mid and lower thigh and mid-calf. Adipose Tissue Thickness (ATT) underneath the NIRS probes was measured by skinfold thickness measurements at the site where the NIRS measurements were done, and the mean of two measurements were divided by two to determine ATT.

### **2.3.2 DOMS markers**

Measurements of perceived pain were based on the methods described by Hedayatpour et al. (2008) and Olsen et al. (2012). The pain perception of the subjects was tested by the subjects rating pain on a visual analog scale (VAS). The scale ranged from “no pain at all” (0 cm) to “worst pain imaginable” (10 cm) without markers for subjects, scaled from 0-10 cm for tester. Rating was done once for the control leg and once for the DOMS leg. Perceived pain was tested with a pain pressure threshold (PPT) test, using an algometer (Algometer, Sodomedic, Sweden). The tip of the algometer is 1cm<sup>2</sup> and it measures pressure in kiloPascal. The algometer was pressed against the muscle belly, with gradually increasing pressure. Subjects were to report when the feeling of pressure became painful. PPT was measured on top of the m.vastus lateralis ( $\frac{2}{3}$  of the length from anterior iliac superior to the lateral side of the patella) and m. rectus femoris ( $\frac{1}{2}$  of the distance from the anterior spina iliaca superior to the superior part of the patella). The different measurements points were measured three times, and the mean values of the measurements were used for further analysis. PPT measurements were performed by the same lab technician for all tests and for all subjects. MVC was tested for both extension and flexion of both legs. The extension test was performed seated in a chair, with a strap around the ankle, extending the leg towards the strap, which was attached to the force transducer. The

flexion test was done laying on the stomach, with a strap around the ankle, flexing the leg towards the strap. Force production was recorded with a force transducer (SM-2000N, Interface, Az. USA + Bagnoli Handheld EMG system, Delsys Inc., Ma. USA).

### **2.3.3 Microvascular function**

Microvascular function was measured by near-infrared spectroscopy (NIRS), which uses optical light in the near infrared wavelength spectra, transmitting light through the muscle. Light of 760 nm and 850 nm are absorbed by oxy- and deoxygenated blood (Rittweger et al. 2010). Using a modified Beer-Lambert Law, it is, with knowledge of absorbed light of different wavelengths, possible to calculate concentration changes of oxyhaemoglobin, deoxyhaemoglobin and oxymyoglobin and deoxymyoglobin. Due to an overlap in wavelength spectra in which haemoglobin (Hb) and myoglobin (Mb) absorption occur, it is not possible to distinguish Hb and Mb from each other (Boushel et al. 2001), and therefore the two will be presented as Hb. With knowledge of concentration changes of oxyhaemoglobin and deoxyhaemoglobin (Depley et al. 1988), one can use NIRS to measure microvascular function by applying an additional intervention, such as a vascular occlusion. In the present study four NIRS (Portamon, Artinis Medicine Systems, Nijmegen, The Netherlands) devices were used, and there were placed one on the vastus lateralis and one on rectus femoris, approximately 10 cm above the top of the patella, of both DOMS and control leg. Subjects were placed in a supine position with their upper body slightly elevated. Occlusion cuffs were placed proximal on the thigh of both legs. The VOT test started with a 5min rest period before a 10min occlusion and ended with another 5min rest. During the occlusion the cuffs were inflated to 295 mm Hg using a rapid cuff inflator (Hokanson E20 Rapid Cuff Inflator + Hokanson AG101 air source, Marcom Medical ApS, Denmark) at both legs simultaneously. NIRS measurements were collected continuously during the whole VOT test. After the VOT test the microvascular function during activity was investigated during a cycling exercise. The NIRS devices were kept in the same position as during the VOT. Subjects were placed on a cycle ergometer (Velotron, Racermate Inc., Washington, USA), resting the legs on a specialmade footrest, keeping the legs slightly bent at a height similar to the horizontal crank position of the ergometer, and the hands resting on the handlebar. The cycling

test consisted of a rest period of 5 min to stabilize saturation values, followed by a work period of cycling, at an intensity of 75% of max HR and a cadence of 90 RPM, followed by a recovery period of 5 min, to measure recovery kinetics. Immediately following the exercise the subjects were instructed to rest the feet on the footrest, and hands on handlebar, to maintain the body in the same position for the whole 5 min rest periods to prevent disturbances to the NIRS signal. The NIRS system was set to a sampling frequency of 10 Hz, with distances of 30, 35 and 40 mm between transmitter and receiver. NIRS data were collected through Artinis software Oxysoft, and displayed in real-time on screen and stored for offline analysis. NIRS placements at VL and RF were marked, in order to ensure exact same positioning at posttest as in pre-test.

## **2.4 Data Analysis**

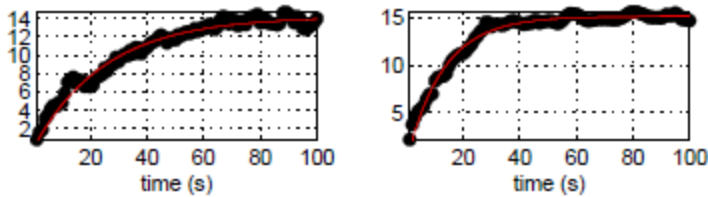
### **2.4.1 DOMS markers**

The PPT results were calculated as the mean of the two PPT measurements. PPT results were analyzed from pre to post eccentric exercise and between control and DOMS leg. Delta values of PPT were calculated as the difference from pre to post in the two legs and compared to each other. VAS results were analyzed by the difference from pre to post. MVC results were calculated by the mean of the three contraction peaks in each test, and analyzed from pre to post eccentric exercise and DOMS to control in extension and flexion. Delta values of MVC data was calculated as difference from pre to post eccentric exercise in each leg, in both flexion and extension, and further compared from DOMS leg to control leg.

### **2.4.2 Microvascular Function**

NIRS measurements returns data of oxygenated ( $O_2Hb$ ), deoxygenated ( $HHb$ ) and total haemoglobin ( $tHb$ ) in micromole ( $\mu M$ ), which is used to calculate muscle oxygen saturation ( $SmO_2\%$ ) ( $SmO_2\% = \frac{O_2Hb}{O_2Hb+HHb}$ ). Oxygenation changes were analyzed by calculating delta values of  $O_2Hb$ ,  $HHb$ ,  $tHb$ ,  $SmO_2\%$  and by calculating recovery rate of  $SmO_2\%$  ( $\tau-SmO_2\%$ ). Baseline values were set to “0” ( $\mu M$ ) at the start of events (occlusion and cycling), and the delta values were calculated as the difference from baseline to nadir values during the events. The recovery rates ( $\tau-SmO_2\%$ ) was calculated as the time from the end of the event to stable values

of oxygenation using a monoexponential curve fit (example in *fig.1*) calculated in Matlab. Delta values and recovery rates were compared from pre to post eccentric exercise, between DOMS and control leg and between the two muscles.



*Figure.1: Example of monoexponential curve fit used to calculate Tau-SmO<sub>2</sub> during the recovery phase following VOT test and cycling exercise.*

## **2.5 Statistics:**

VAS means of pre and post were calculated and analyzed through a paired sampled t-test, and further compared to match the significance level of Gallagher et al. (2001). All PPT, MVC and NIRS data were tested for normal distribution using the Shapiro-Wilk test. MVC, PPT and NIRS data were analyzed using a general linear model repeated measurements ANOVA. For the ANOVA analysis, the data was split into three factors, muscle (VL and RF), condition (DOMS and control) and time (pre and post). If the assumption of sphericity was not met, the results were interpreted by the Greenhaus-Geisser method. If there was a significant effect of interactions, post-hoc tests were performed by general linear model repeated measurements ANOVA, analysing the simple main effects of the factors included in the interaction analysis. The significance level was set to  $P < 0.05$ . Data was processed and filtered in Matlab (MATLAB 2012b, The MathWorks, Inc., Natick, Massachusetts, United States) and/or Microsoft Excel for Windows. Statistical analysis of all data was done through SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp).

### 3. Results

#### 3.1 General results

All 14 subjects performed all pre-tests, eccentric exercise and post-tests, and all subjects were included in the analysis. However, MVC data was missing for 3 subjects due to technical problems. All data of DOMS markers were normally distributed apart from PPT data of RF in DOMS leg at posttest ( $P=0.04$ ) and data of VL in DOMS leg at posttest ( $P=0.01$ ), while data of VL in control leg at post were close to normally distributed ( $P=0.044$ ). The delta values of PPT in VL and RF DOMS leg were not normally distributed ( $P<0.01$ ). NIRS data were normally distributed except for the data of recovery kinetics in VL control leg ( $P=0.01$ ) and RF DOMS leg ( $P=0.025$ ) at pretest, and VL control leg at posttest was close to normally distributed ( $P=0.046$ ). Statistical analysis of the data was also performed with exclusion of statistically outliers, to check for the effect of the non-normally distributed data. However there were minor changes following the exclusion of statistically outliers, and thus the results presented includes all data. ATT underneath NIRS probes showed small differences between the two legs with a difference at VL of 0.33mm ( $\pm 0.5$ ), while 0.06mm ( $\pm 0.9$ ) mean difference at RF.

#### 3.2 DOMS markers

There was a significant difference in VAS from pre to post in DOMS leg ( $P<0.01$ ), with a mean increase of 3.32 ( $\pm 2.3$ ) cm. The difference in control leg was not significant ( $P=0.98$ ), with a mean increase of 0.45 ( $\pm 0.5$ ) cm. The difference between DOMS and control VAS score at posttest was significant, with a difference in mean of 2.87 cm.

The results for the PPT measurements showed no significant effect of the interaction *muscle x condition x time* were found. When analyzing the delta values of PPT data of both DOMS and control leg, there were no significant effect of the individual factors, and no significant effects of interactions. Thus there were no significant differences in PPT when compared DOMS leg to control leg and pre to post eccentric exercise results. Results are presented in *fig. 2 A and B*.

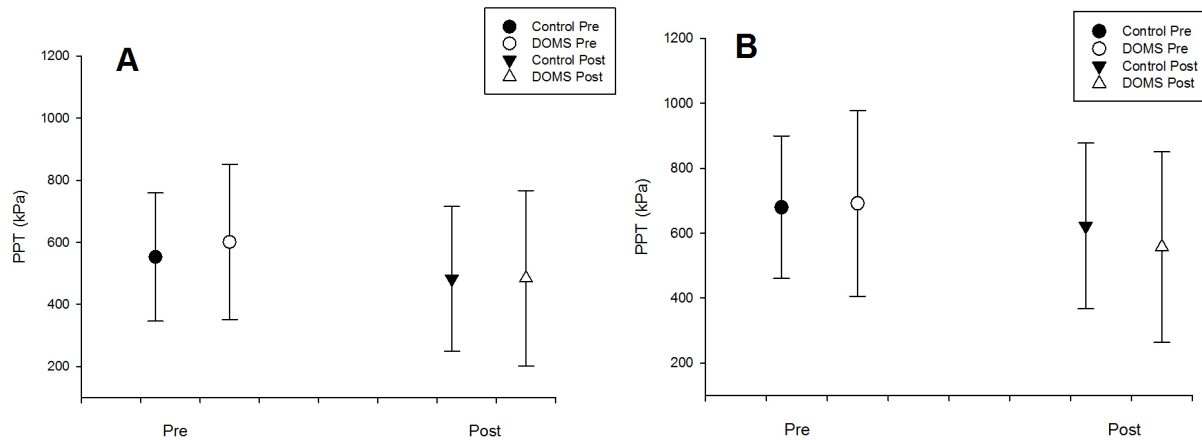


Figure 2. Group mean ( $\pm$ SD) changes in pain pressure threshold in kPa from pre to post eccentric exercise in A) vastus lateralis and B) rectus femoris of both DOMS and control leg.

The differences in MVC are presented in *fig. 3*. Repeated measurements ANOVA showed a decrease in the leg extension force after eccentric exercise, with a significant decrease in the DOMS leg ( $P < 0.01$ ), while there was no significant difference in force production in control leg. Opposite results were found in flexion force production, as there was a significant increase in flexion force in DOMS leg after eccentric exercise ( $P = 0.02$ ), this was not found in the control leg. Delta values for MVC showed significant differences from DOMS extension to control extension ( $P < 0.01$ ), as the DOMS leg had a decrease of 70.7 N compared to the increase of 80.7 N in control leg. There was no significant effect of delta values in flexion.

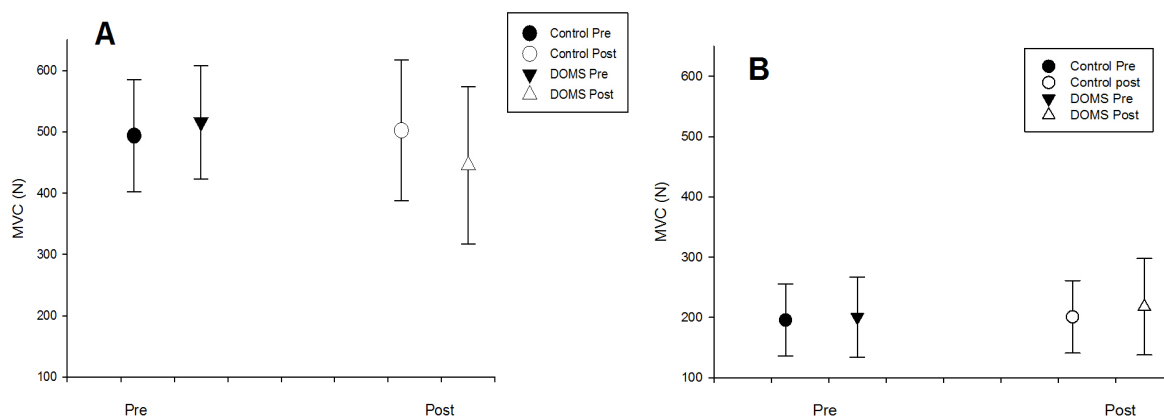


Figure 3. Group mean ( $\pm$ SD) changes in maximum voluntary contraction from pre to post eccentric exercise in (A) leg extension (B) leg flexion of both DOMS and control leg.

### 3.3 Microvascular function

#### 3.3.1 Recovery rates (tau-SmO<sub>2</sub>%)

The changes in recovery rate after VOT is presented in *fig. 4A*. The repeated measurements ANOVA showed no significant effects of interactions in recovery rate of SmO<sub>2</sub>% during VOT, and there were no significant effects of either muscle, condition or time.

The changes in recovery rate after cycling are presented in *fig. 4B*. Repeated measurements ANOVA testing the recovery rate after cycling showed significant effect in the interactions *muscle x condition* and *condition x time*. Analysis of the *muscle x condition* interaction presented significant differences in the RF muscle from DOMS to control at posttest ( $P=0.04$ ), while no significant difference at pre-test. This indicates a slower recovery rate in RF control, with 5.4 ( $\pm 5.2$ ) sec difference in recovery rate compared to DOMS at post test. There were no differences in VL muscle. The simple main effect analysis of the *condition x time* interaction showed a significant difference from pre to post in VL control ( $P=0.01$ ), while not in VL DOMS or in either of the legs at the RF muscle. VL control had a 3.64 ( $\pm 2.7$ ) sec slower recovery rate at post-test compared to pre-test. There was no significant effect of the interaction *muscle x condition x time*.

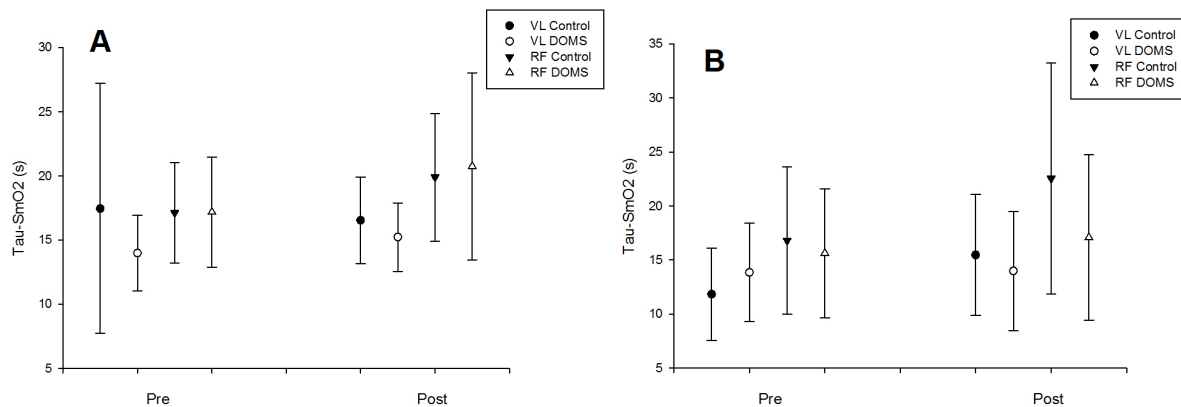


Figure 4. Changes in tau-SmO<sub>2</sub> (s) from pre to post eccentric exercise after A) vascular occlusion test and B) cycling test, in vastus lateralis and rectus femoris in DOMS and control leg.



### 3.3.2 Muscle desaturation (SmO<sub>2</sub>%)

No significant effect of interactions in muscle desaturation during VOT was found. A significant effect was found of the single factor *muscle* ( $P=0.02$ ) as it was a greater decrease in SmO<sub>2</sub>% in VL than in RF, with a mean( $\pm$ SD) % decrease in VL of 26.0 ( $\pm$ 4.3) %, while RF had a decrease of 23.1 ( $\pm$ 4.3) %. Differences are presented in *fig.5A*.

No significant interactions were found for desaturation during cycling, and thus the effects are similar in both VL and RF, in both control and DOMS leg, at pre and post-test. However, there was a significant effect of the single factor *muscle* ( $P=0.001$ ), meaning there was a greater SmO<sub>2</sub>% decrease in VL than in RF, as VL had a decrease of 12.7 ( $\pm$ 1.7)% compared to RF decrease of 7.4 ( $\pm$ 1.0)%. Differences are presented in *fig. 5B*.

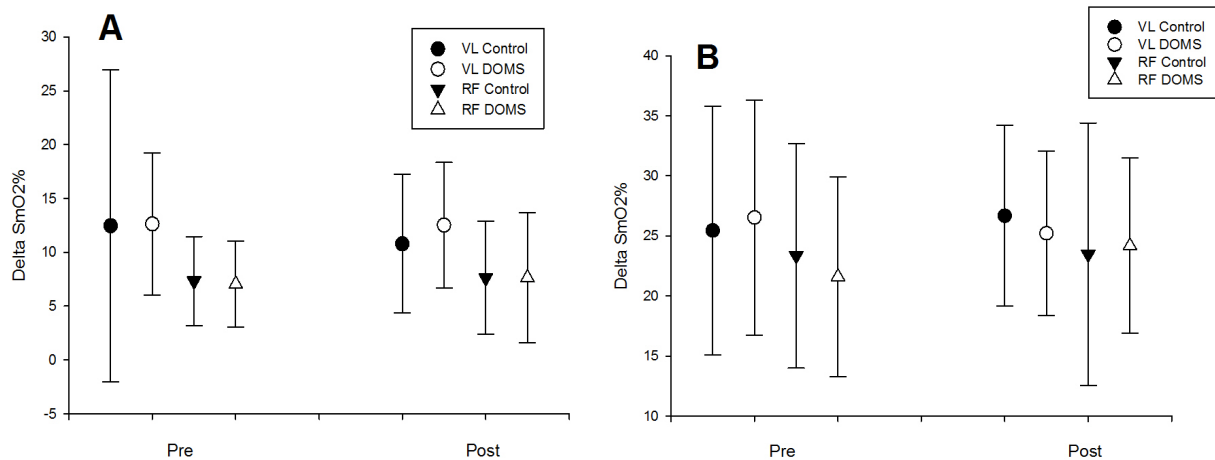


Figure. 5. Mean ( $\pm$ SD) changes in  $\Delta$ SmO<sub>2</sub>% from pre to post eccentric exercise during A) VOT and B) cycling test in both DOMS and control leg.

### 3.3.3 Oxyhaemoglobin (O<sub>2</sub>Hb) & total haemoglobin (tHb)

Analysis of concentration changes in oxyhaemoglobin and total haemoglobin results show roughly similar results as in recovery rate (O<sub>2</sub>Hb) and desaturation (tHb). There were no significant effects of the interaction of *muscle x condition x time* in either O<sub>2</sub>Hb or tHb concentration changes during VOT or cycling. However there were some significant changes in main factors. There was a significant effect of the factor *muscle* in O<sub>2</sub>Hb during VOT, as there was a greater decrease in VL with a mean decrease of 22.5 ( $\pm$ 4.1)  $\mu$ M, compared to a mean

decrease of 18.0 ( $\pm 3.4$ )  $\mu\text{M}$  in RF. There were no significant effects of either interactions or single factors in  $\text{O}_2\text{Hb}$  during cycling. Analysis of tHb showed similar results, as there were a significant effect of muscle ( $P < 0.01$ ) during cycling, in which VL had smaller decrease of tHb than RF in average, with mean ( $\pm \text{SD}$ ) decrease of 5.0 ( $\pm 1.8$ )  $\mu\text{M}$  in VL compared to a decrease of 9.49 ( $\pm 23.1$ )  $\mu\text{M}$  in RF. There were no other significant effects found in tHb during cycling or VOT.

#### **4. Discussion**

The present study aimed to measure changes in microvascular function affected by muscle damage caused by eccentric exercise. The main findings were that there were no significant differences in microvascular functions, as there were only minor differences from pre to post in DOMS leg, and only minor differences from DOMS to control leg in post-tests in both concentration changes and recovery kinetics. There were some significant differences in between muscles, however this difference was found in each leg and at both pre and post-tests.

As the results of the measurements of microvascular functions are dependent on the effect of the eccentric exercise, the results of DOMS markers are important in consideration of the evaluation of the results. To induce DOMS the eccentric exercise of front lunges was chosen based on previous studies (e.g. Olsen et al. 2012). In addition front lunges also allowed us to induce DOMS to one leg only, keeping one leg unaffected of muscle damage as a control leg. Further, the present study measures DOMS markers and compared to muscle biopsies and blood serum samples, investigating structural changes in the muscle cell, the present results cannot confirm, only indicate muscle damage, this has to be taken into account in evaluation of the results. The results showed limited effect of the eccentric exercise, as there were little to no differences from pre to post in either leg or from DOMS to control at post test in PPT. The VAS scores were matched to Gallagher et al. (2001), who suggests the level of changes in a VAS test has to be an average of 13mm to reach significant changes. In VAS there were differences from pre to post in the DOMS leg, however it was only 3.32 cm compared to Davies et al. (2008) difference of 7.1cm, indicating that Davies et al. managed to induce DOMS to a greater extent than the present study. There were differences in MVC, indicating a loss of force production in the affected leg

caused by DOMS, which were not found in the control leg. This indicates DOMS, however, combined with the results of VAS and PPT it is hard to confirm DOMS. The limited results may be an effect of the front lunges, as it is a difficult exercise. It is hard to 1) continue to perform lunges when exhausted, and 2) completely prevent activation of the control leg, especially when tired, thus potentially inducing some damage to the control leg as well. The limited differences in the DOMS markers results may also be caused by differences in the subjects and their level of fitness prior to the exercise. Even though we only recruited subjects that were unaccustomed to strength training on the legs, there were clearly differences in the level of fitness in between subjects causing different effect of the eccentric exercise. Further the PPT itself is a method to be discussed, as subjects seemed to endure very high pressure, and further affecting the results. As the present results shows limited differences in DOMS markers results one have to assume that DOMS was induced in a smaller extent than intended.

No major findings were found in the NIRS variables describing changes in microvascular functions after eccentric exercise, as DOMS were limited, to confirm or disprove previous studies. There were some differences between the two muscles, as there was a greater desaturation during occlusion and cycling in VL compared to RF. There were some differences in recovery rate, indicating a faster recovery in RF DOMS leg than in control leg after cycling in the post test, and faster recovery in VL control muscle at post-test compared to pre test after cycling. However the differences in RF DOMS were not found different from pre to post, and for VL control there were no differences from control to DOMS leg, thus making it impossible to assume changes in microvascular function. The present results are similar to the findings of Walsh et al. (2001), who found no differences from DOMS subjects to control subjects or from pre to post in microvascular function. Walsh et al. measured microvascular function with NIRS in the VL, and compared the half time of recovery rate. The present results are also in agreement with the results of Ahmadi et al. (2008a) who found significant decrease in desaturation in the biceps brachii during isometric contractions up to 6 days post eccentric exercise, suggesting higher rate of anabolic metabolism in the working muscle due to mitochondrial disturbances. However, they did not find any changes in resaturation post exercise, even though DOMS seemed to be present in both of the mentioned studies. Davies et al. (2008) and Ahmadi et al

(2008b) found interesting differences. Ahmadi et al. (2008b) found faster resaturation after downhill walking as DOMS inducing exercise, while Davies et al. found a slower mean response time, meaning the resaturation after activity had a greater delay than pre eccentric exercise, after eccentric exercise, non alike the present studies findings. Walsh et al. (2001) and Ahmadi et al. (2008b) used 30 min high intensity eccentric cycling and 40 min steep downhill walking to induce DOMS in contrary to the present study which used front lunges, which is more similar to the protocol of Davies et al. (2008) using 100 repetitions of squats to induce DOMS. The present study did measure two muscles in one DOMS and one control leg, compared to Davies et al.(2008) and Ahmadi et al (2008b) and Walsh et al. (2001) studies who induced DOMS to both legs and measured only VL, leaving out the possibility to measure within subjects control and detect eventual differences in effects of the eccentric exercise to the microvascular function. The only study who has performed a similar DOMS model is Ahmadi et al. (2008a) who induced DOMS to biceps brachii in one arm by eccentric contractions, leaving the other arm as control.

## **5. Conclusion**

The main findings of the study were no major changes in microvascular function. However, as DOMS markers results was unclear, it is unclear whether the absence of changes in microvascular function is an effect of DOMS not being induced to a great enough extent, or there in fact are no effect of DOMS on microvascular function. In conclusion the study did not find any changes in microvascular function following DOMS caused by eccentric exercise, and therefore the present study did not confirm or disprove earlier studies. As earlier studies confirms structural damages to the muscle and suggests changes to the microvascular function due to these damages, the subject is interesting for research for further knowledge about the DOMS phenomenon.

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