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Oxygen consumption during constant-load exercise

Heterogeneity of quadriceps muscle oxygen consumption during submaximal constant-load dynamic cycling

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Abstract

Introduction: There have been a lot of studies examining all aspects of whole body oxygen consumption (pVO2), but when it comes to what happens on a local level, there is still a lot that is unexplored. The aim of this study is to expand on the area that is heterogeneity of local muscle oxygen consumption (mVO2) using near-infrared spectroscopy (NIRS) as the measurement tool and a cycle ergometer as the exercise device. **Methods:** Using 15 trained cyclists to measure mVO2 at rest and at three different intensities in 3 different muscles, vastus lateralis, vastus medialis, and rectus femoris at once using an 8-channel NIRS setup. **Results:** Heterogeneity was found in various circumstances both between muscles and within muscles. Statistical tests showed that the participants were a homogeneous group. **Discussion:** Vastus lateralis and vastus medialis was heterogeneous at low intensity, but at this difference was lower at the highest intensity of 250 Watts. **Conclusion**: This is one of the first studies using an 8-channel NIRS setup to examine the oxygenation of quadriceps. There might be a displacement with NIRS on the rectus femoris muscle.

Keywords: pVO2, NIRS, mVO2, Vastus Lateralis, Vastus Medialis, Rectus Femoris, heterogeneity, cycle ergometer

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1. Introduction

Oxygen consumption (VO2) is an important indicator of energy expenditure (EE) in the human body since it is directly related to the availability of adenosine triphosphate (ATP) during exercise (Jéquier et al. 1987). EE is the ability to perform internal or external work, and it can be calculated by measuring VO2 using indirect calorimetry. Knowledge of oxidative metabolism is used for both physical activity and medical purposes. In order to measure VO2 accurately it is important to maintain steady state exercise for a prolonged period (Matarese 1997). Since cycle ergometers are very accurate when it comes to external work they are ideal to use for research on oxygen consumption during exercise. In addition pVO2 increases linearly with increasing workload during cycling (Ettema and Lorås, 2009). Skeletal muscle oxygen consumption during exercise can be up to 50 times higher compared to values at rest (Hamaoka et al. 2007). The ability to measure oxygen consumption accurately for the whole body has existed for over a century (Jéquier et al. 1987). The research done on pVO2 is extensive; however, what is less known is what happens to oxygenation on a more local level. The Fick equation can be used for regional oxygenation and blood flow but due to its nature it cannot distinguish between different muscles. There are several methods available for measuring muscle oxygen consumption (mVO2), but many of them have limitations such as being either expensive, large in size, invasive, hard to interpret the results, unable to measure during muscle contractions, or a combination of the limitations. These methods include biopsy analysis and magnetic resonance spectroscopy (Hamaoka et al. 2007). Near-infrared spectroscopy (NIRS) has been developed to overcome these limitations, and its validity has been explored (Van Beekfelt et al. 2001, Wolf et al. 2007, Hamaoka et al. 2007).

NIRS is a non-invasive optical method used to measure *in vivo* tissue oxygenation as well as blood flow. The method uses a transmitter to emit light through biological tissue. Some of the light is absorbed by haemoglobin and myoglobin in the blood, and with a receiver an estimation of oxygenation can be made (Bouschel et al. 2001, Van Beekvelt et al. 2001, Kennedy et al. 2006, Hamaoka et al. 2007). NIRS has been used for a numerous different applications such as muscle blood flow, muscle activation, muscle damage induced by exercise, role of the brain in muscle fatigue, and effect of exercise training. The

heterogeneity of muscle oxygenation has been shown to occur in the human body, but really few have examined it using a multichannel NIRS-setup Only about 20 (of around 600) different muscles in the human body have been examined using NIRS. In a four year period from 2007 to 2010 only 15 of 160 published studies used a multi-channel NIRS setup. (Ferrari et al. 2011) In order to measure oxygenation an intervention has to be applied, this is usually in the form of a vascular occlusion (Hamaoka et al. 2007). Studies have shown that not only was there a difference in oxygenation between the working muscles, but also within the same muscle. The heterogeneity of oxygen consumption in muscle tissue can be measured NIRS, which is the most common approach used in research (Kennedy et al. 2006, Takagi et al. 2013). The heterogeneity of muscle oxygenation can expand our knowledge about muscle physiology, both during rest and during exercise.

The aim of this study was to look at heterogeneity of quadriceps muscle oxygenation during submaximal constant-load dynamic exercise at different intensities. For this purpose an experimental cycling protocol was used.

2. Methods

2.1 Participants

Fifteen healthy trained male cyclists (age 35,7 ± 10,2 yrs; height 181,9 ± 7,8 cm; weight 80,7 ± 9,1 kg; body fat 18,7 ± 3,6 percent) participated in the study. The criteria for participation were no former history of cardiovascular disease, and no orthopedic injuries that could deteriorate further by participating in the study. Each subject signed an informed consent form after receiving written and oral information about possible health risks and the benefits of participating in the study. Permission to conduct the study was given by the Regional Committees for Medical and Health Research Ethics in Trondheim.

2.2 Procedures

All the participants performed the same protocol, and three tests were conducted on the same day. The first test was physiological measurements done while resting on a bed, the second test was constant-load cycling at submaximal intensity, and the last test was a maximal aerobic power (MAP) test. Height and weight were measured before the tests started. The first test was done to establish pVO_2, mVO_2 for the quadriceps, heart rate (HR), and blood lactate (BL) at rest. The second test was dynamic exercise on a cycle ergometer at three different intensities to measure the same parameters as in the first test. The MAP-test was done to establish the maximal effort values to be used in part of the statistical analysis. After the three tests skinfold measurements were conducted to calculate body fat percentage (%BF). To calculate %BF a method by Peterson et al. (2003) was used with measurements from 4 different sites; iliac crest, subscapular, triceps, and mid-thigh.

2.2.1 Resting values

The participants were placed in a comfortable horizontal position on a bed before the first test started with measuring resting HR and pVO2 for a 3 minute period. BL was also measured followed by a vascular occlusion test that was used to measure the resting values of quadriceps muscle oxygenation. The occlusion lasted for 10 minutes, but shorter occlusions had also been performed prior to this so that the participants would get used to the physical intervention of the occlusion. To conduct the measurements the participants were equipped with a cuff around the right thigh to occlude blood flow, an 8-channel NIRS-device on the right quadriceps, and a heart rate monitor. PVO2 was measured by gas exchange equipment, and an external air source was used to inflate the cuff.

2.2.2 Cycle ergometer tests

The second part of the protocol consisted of a warmup followed by 3 different intensities of constant-load exercise on a cycle ergometer. The participants were directly moved from the bed over to the cycle ergometer before starting the 10 minute warmup with freely chosen cadence and a work rate between 100 and 150 W. The intensities lasted for 7 minutes at submaximal effort, starting at 150 W work rate and increments of 50 W so the second and third intensity was done at 200 and 250 W. Immediately after the intensities an arterial occlusion was applied for 5 seconds and BL was also measured. The participants were instructed to maintain a cadence of ninety rounds per minute, to remain in a seated position, and to grip the handlebars on the hoods as standardization.. The participants were instructed to maintain the same position both during exercise and the resting period. During the 10 minute rest period a pedestal was placed under the right foot to standardize the pedal placement with the crank arm in a neutral positon horizontally. PvO_2 was not measured in the last 4 minutes of the resting period so the participants could drink or stand up if needed to get ready for the next intensity.

The third test was a maximal aerobic power test. A 5 minute break was allowed before starting the test, and the protocol started at 200 W, and the work rate was increased by 25 W every minute until a plateau was reached. During this test cadence was freely chosen. Blood lactate was taken as part of checking 4 criteria for a reached VO2max. The criteria were reaching a plateau in oxygen consumption, respiratory exchange ratio >1.1, high % of age-modified heart rate (220-age), and blood lactate < 8,0.

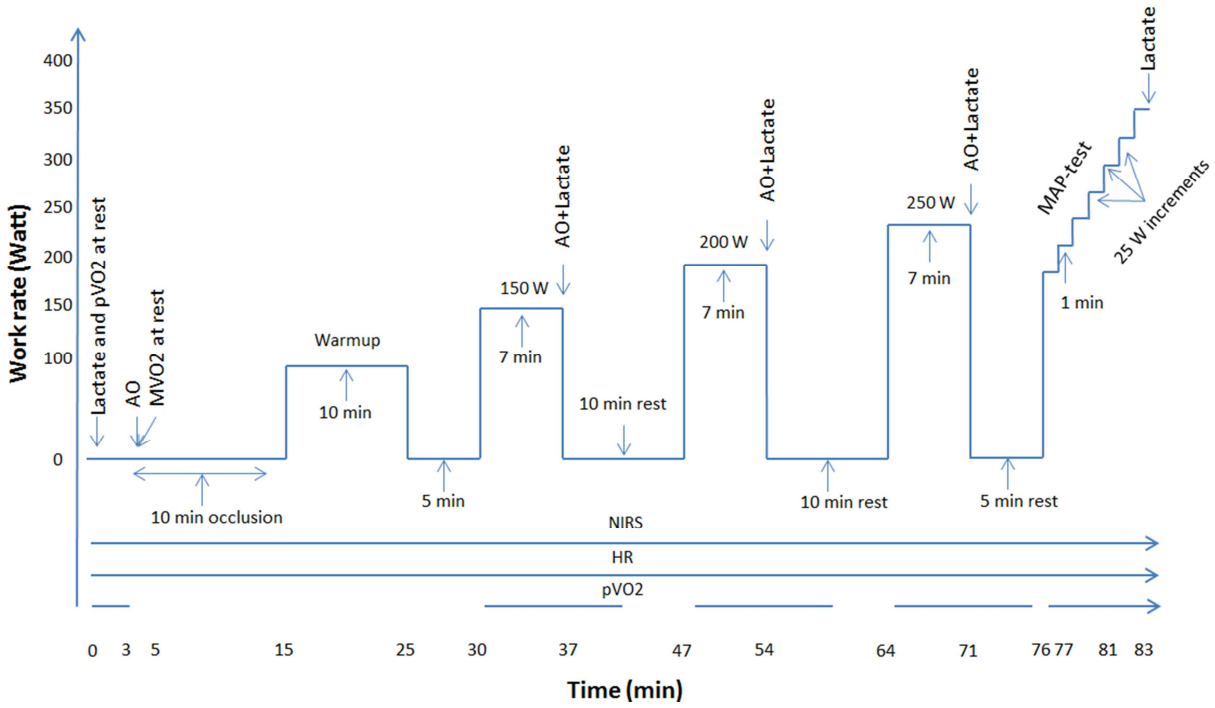


Figure 1: Schematic representation of three tests of the protocol. Near-infrared spectroscopy and heart rate was measured for the whole protocol while pVO2 was measured for 3 minutes at the start, for 12 minutes for the three submaximal intensities and for the whole MAP-test. AO=arterial occlusion

2.3 Measurements

Changes in muscle oxygenation were measured by an 8-channel NIRS-system (Oxymon MKIII, Artinis Medical Systems, Netherlands) continuously throughout all the three tests. NIRS is a non-invasive method used to measure the saturation of oxygenated and deoxygenated blood, and the concentration changes in biological tissue. With a modified Beer-Lambert law this can be calculated. The modified Beer-Lambert law states that the light is absorbed due to the concentration changes. The light is generated at 766 and 856 nm (Bouschel et al. 2001, Van Beekvelt et al. 2001, Kennedy et al. 2006, Hamaoka et al. 2007). The light also has a limited range making the measurements of deep tissue and muscles difficult (Hamaoka et al. 2007). The light is affected by adipose tissue thickness (ATT) and melatonin in hair follicles as well as muscle shape, blood volume, and tissue composition (Hamaoka et al. 2007, Reinstrup and Romner 2012, Van Beekvelt et al. 2001). An arterial occlusion was used to measure mVO2 as a change in O2Hb (mL/min/100g) as shown in figure 2.

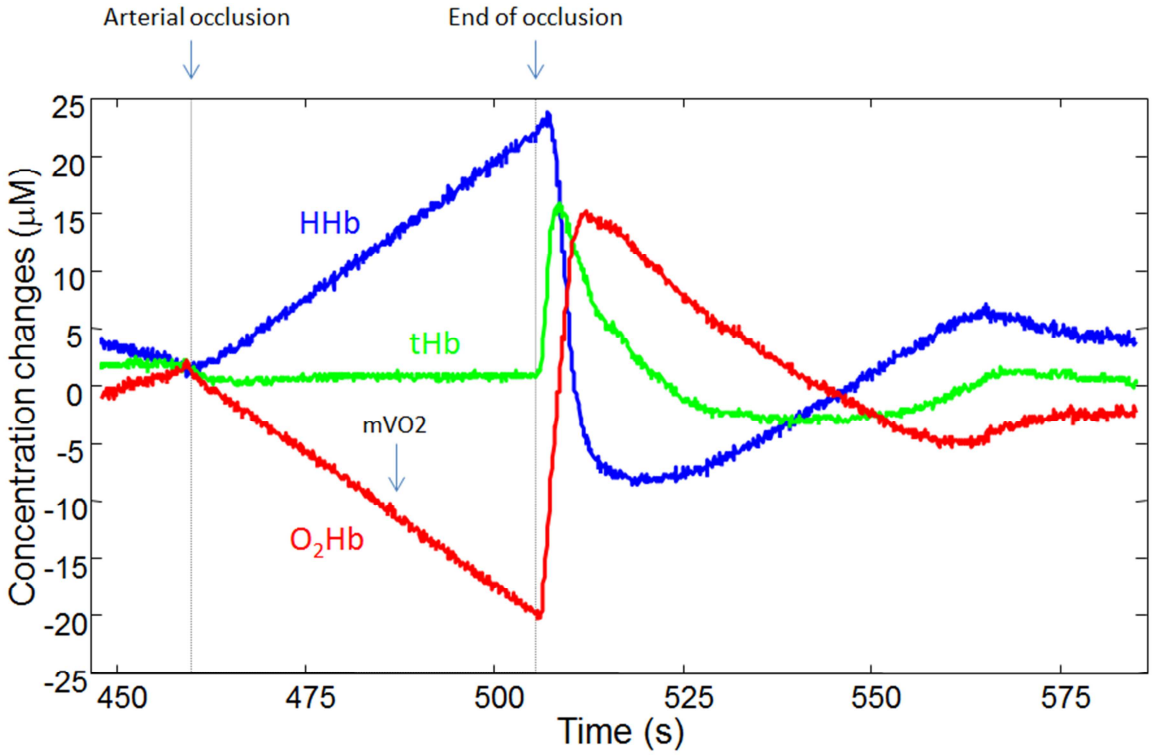


Figure 2: Example of signals from an arterial occlusion: HHb=deoxyhemoglobin, tHb=total hemoglobin, O2Hb=oxyhemoglobin.

A cuff (Hokanson SC12L; Marcom Medical ApS, Denmark) was used to constrict blood flow. This cuff was attached up to an inflator (Hokanson E20 Rapid Cuff Inflator, Marcom Medical ApS, Denmark). This was used to ensure rapid inflation and deflation of the cuff. The sensor has two light sources and six detectors making it capable of measuring at 8 sites simultaneously. The right quadriceps and the upper torso were shaved to make sure the sensors would properly stick to the area of placement. The placement of the NIRS-device was standardized 8 centimeters above the patella in a medial/lateral direction covering VL, VM, and RF. Elastic bands and sticky tape were used to keep the NIRS device in place and to counteract sweat. The sampling frequency of the sensor was set at 50 Hz.

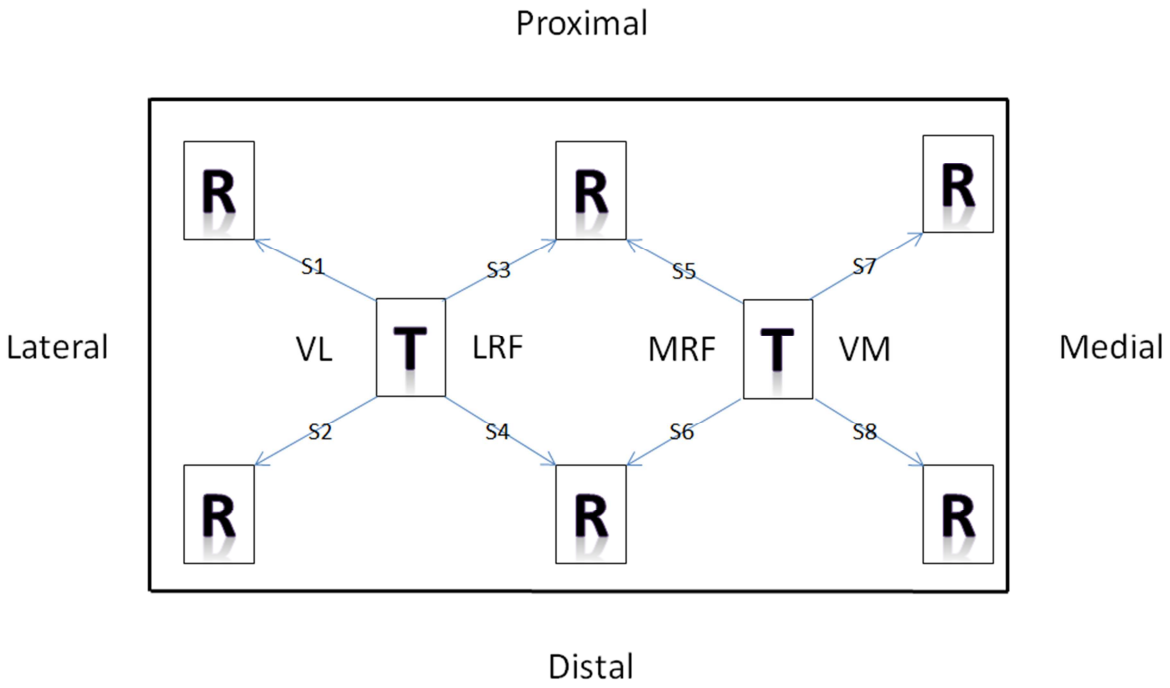


Figure 3: Schematic representation of the near-infrared spectroscopy probe: T= transmitters, R= recievers, VL=vastus lateralis, LRF=lateral part of rectus femoris, MRF=medial part of rectus femoris, VM=vastus medialis, S1-S8=sites of measurement.

Vastus lateralis (VL) is the main contributor during cycling exercise as well as the other quadriceps muscles (Takagi et al. 2013) For that reason VL, vastus medialis (VM) and rectus femoris(RF) was chosen for measurements, monitoring 3 different muscles at once and, both the proximal and the distal parts of the muscle. In addition rectus femoris was measured at both the lateral and medial part.

Pulmonary Vo2, respiratory minute volume (Ve), and respiratory exchange ratio (RER) were measured at rest and during the three cycling periods by a spirometer (Oxycon Pro, Jaeger GmbH, Hoechberg, Germany). It was calibrated before starting the testing and the sampling frequency was once every 10 seconds. Heart rate was also measured in the same time frame by a heart rate monitor (Polar RS800, Polar Electro OY, Kempele, Finland) using wireless transfer and a sampling frequency of once every 5 seconds. Blood lactate samples were taken a total number of 5 times with a blood lactate analyzer (Lactate Pro LT-1710, ArkRay Inc, Kyoto, Japan) first time was after the first test, then after each of the 3 submaximal intensities, and lastly after the Vo2max-test. A cycle ergometer (Velotron, RacerMate inc., Seattle, WA, USA) was used to electronically control the intensity accurately. For the anthropometric measurements a skin fold caliper (Holtain skinfold caliper, Holtain Ltd, Crymych, Wales) was used.

2.4 Data analysis

For this part Matlab (2013b, Mathworks Inc), MS Office (2010, Microsoft Corp), and SPSS (20.0, IBM Corp) were used.

PVO2 and HR examined were average values during a 2 minute steady state period in the middle of the intensities, from 2:40 minutes to 4:40 minutes. This applies to intensity 2, 3, and 4, while the numbers from intensity 1 is the last 2 minutes of the 3 minute period pVO2 was measured during rest. The values were changed to be a percentage of VO2max and HRmax of each participant. VO2max and HRmax numbers are from the MAP-test. VO2max is the average for the 2 highest measured 30 second periods, and HRmax is the highest number measured.

The occlusion markers were set manually, and the NIRS-data was run through a low pass filter. The NIRS-data from each of the 8 channels were separated into 3 variables; 1=Intensity, 2=Muscle, and 3= Proximal/Distal. Intensity was divided into 4 categories; 1=0 W/rest, 2=150 W, 3=200 W, and 4=250 W. Muscle was split into 4 categories; 1=Vastus Lateralis, 2=Lateral Rectus Femoris, 3=Medial Rectus Femoris, and 4=Vastus Medialis. Proximal/Distal was separated into 2 categories; 1=Proximal and 2=Distal.

2.5 Statistical analysis

SPSS (20.0, IBM Corp) was used for this part of the analysis.

The data used for the statistical analysis was mean ± SD. The normal distribution of NIRS (the 8 different channels), HR, and pVO_2 was examined, and in addition another analysis was done using transformed data using Log10. For NIRS, a third analysis of normal distribution was made with removing the first intensity (0 W/rest). The results from Shapiro-Wilk were used for this part of the analysis. An ANOVA repeated measures analysis using a linear model was performed to check if pVO2, HR, and lactate were influenced by intensity. An ANOVA repeated measures test using a linear model was performed to check how mVO2 was influenced by the 3 factors muscle (VL, LRF, MRF, and VM), position (proximal/distal), and intensity (0 W, 150 W, 200 W, and 250 W). The significance level was set at $p < 0,05$ for all tests. Where sphericity was violated, the result from Greenhouse-Geisser was used instead.

3. Results

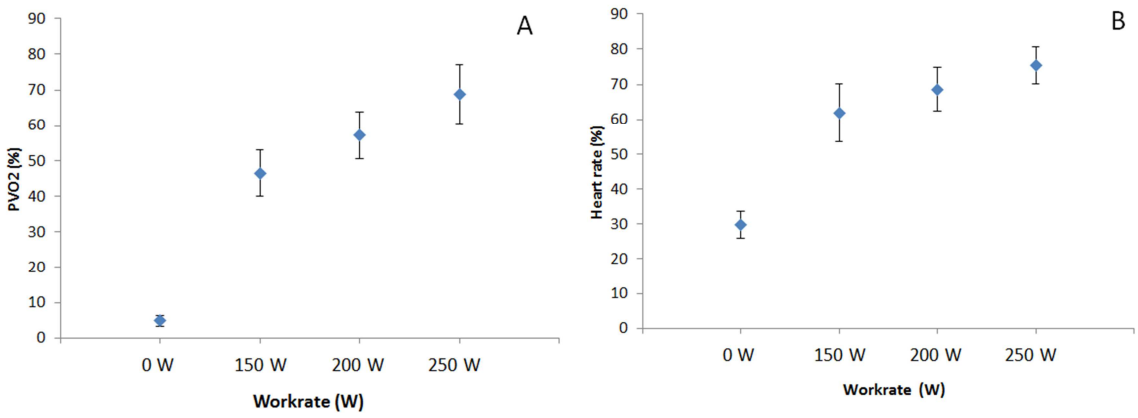
All 15 participants completed all tests, and all data was complete apart from heart rate data was missing from one subject. All the subjects had at least 3 of the 4 criteria necessary for an achieved VO2max.

Table 1: Physiological data of the subjects.

	Mean	SD±	Minimum	Maximum
Age (years)	35,7	10,2	22	50
Weight (Kg)	80,7	9,1	67,0	97,9
Body Fat (%)	18,7	3,6	11,3	23,6
VO2max (mL/kg/min)	70,0	8,2	55,3	84,6
HRmax (bpm)	182,3	12,3	163	202
Blood lactate maximum value (mmol/L)	11,0	1,7	7,3	12,9

3.1 Physiological responses

Normality was examined for the physiological variables pVo2, heart rate, and lactate. Each category contained 4 groups, and half of the groups were normally distributed (6/12), but after the data was transformed almost all of them were normally distributed (10/12). Only 150 W (p=0,023) and 250 W (p=0,026) for pVO2 were not normally distributed. When looking at the three variables pVo2, heart rate, and lactate compared to intensity they all showed a significant difference so they were all influenced by an increase of intensity. The only exception was in blood lactate between rest (0 W) and the first intensity (150 W) of exercise (p=0,148).



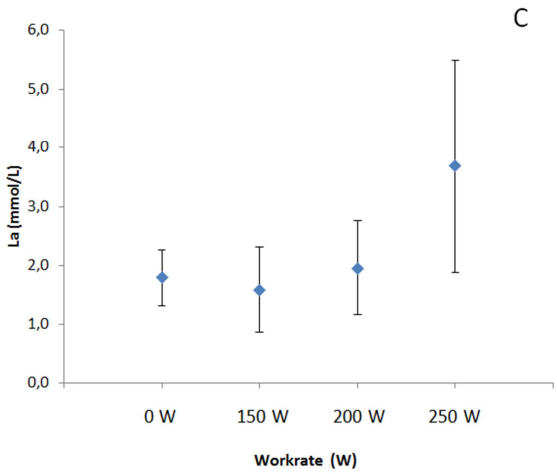
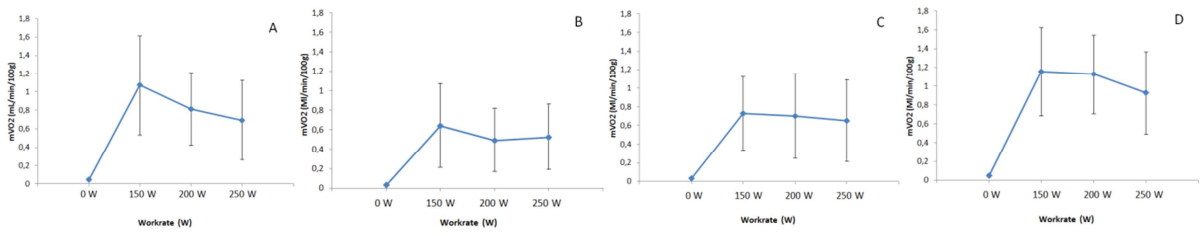


Figure 4: Physiological responses for pVO2 (A), HR (B), and (Blood lactate (C) as a function of work rate.

3.2 Local oxygen consumption in quadriceps

Normality was examined for mVO2 and the different quadriceps muscles. In total there were 32 groups since there were 8 channels and 4 different intensities. 24 of the 32 groups were normally distributed, and doing the same test with transformed data showed insignificant change (22/32). When running the test with the first intensity (0 W) removed for all channels, there was even less normal distribution (16 out of 24 groups). When running the ANOVA tests with transformed data and with the first intensity removed, there results were almost exactly the same with only a few minor adjustments. The tests comparing different muscles to one another showed that there is a significant difference between them all.



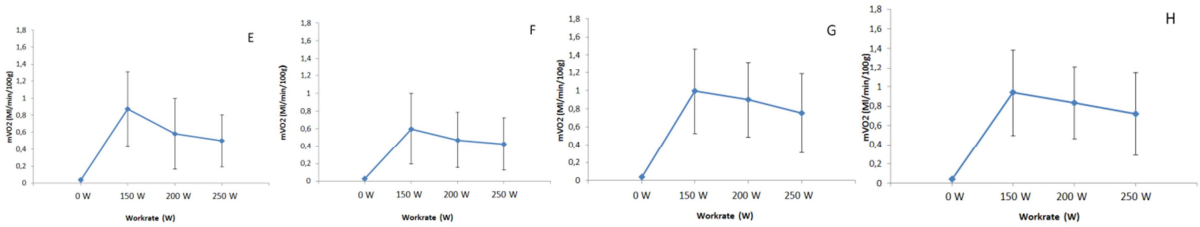


Figure 5: Measurements from NIRS showing the 8 channels and the effect of work rate.

A=proximal part of vastus lateralis, *B=proximal lateral part of rectus femoris*

C=proximal medial part of rectus femoris *D=proximal part of vastus medialis*

E=distal part of vastus lateralis *F=distal lateral part of rectus femoris*

G=distal medial part of rectus femoris *H=distal part of vastus medialis*

3.2.1 Effect of intensity on quadriceps muscle oxygenation

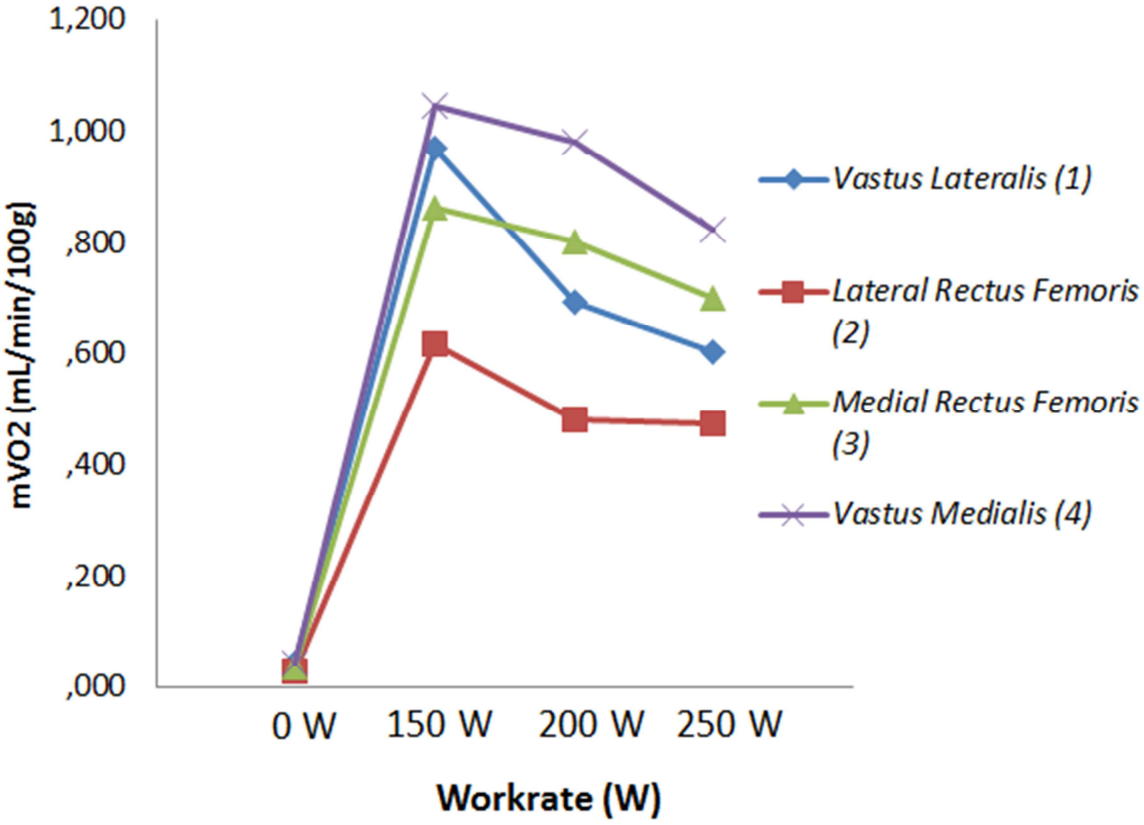


Figure 6: Shows how work rate impacts quadriceps muscle oxygenation

The general effect of intensity on the 4 muscles; rectus femoris had 4 sensors, it was divided into a lateral (LRF) and a medial (MRF) part, showed that there was a significant difference between them (p=0,004). However, when the individual muscles were compared to intensity there were only a few that were statistical significant. Those were VL and LRF (p=0,009), and MRF and VM (p=0,001) from 0 W to 150 W. MVO_2 values of VL and VM were initially the highest while LRF and MRF were the lowest but as the intensity increased the two medial muscles MRF and VM followed a similar decrease in values. VL decreased a lot from the second to the third intensity compared to the three other muscles. LRF had a plateau between the last two intensities, the only muscle to not have a significant decrease in muscle oxygenation.

3.2.2 Effect of intensity and quadriceps muscle position

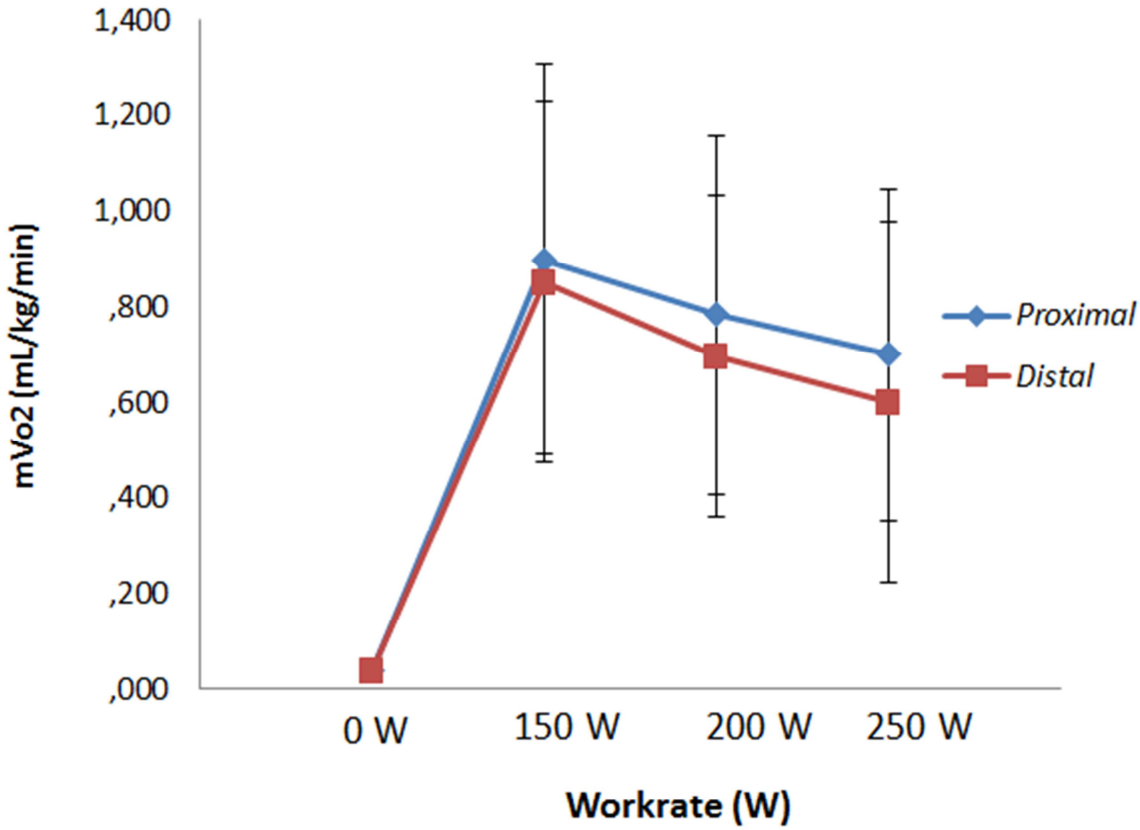


Figure 7: Shows how intensity impacts proximal/distal position in quadriceps muscles.

Position does not show a significant difference as they follow the same pattern. It is worth mentioning that the values of the proximal measurements are systematically higher, but not enough to be statistical significant.

3.2.3 Effect of muscle position on quadriceps muscle oxygenation

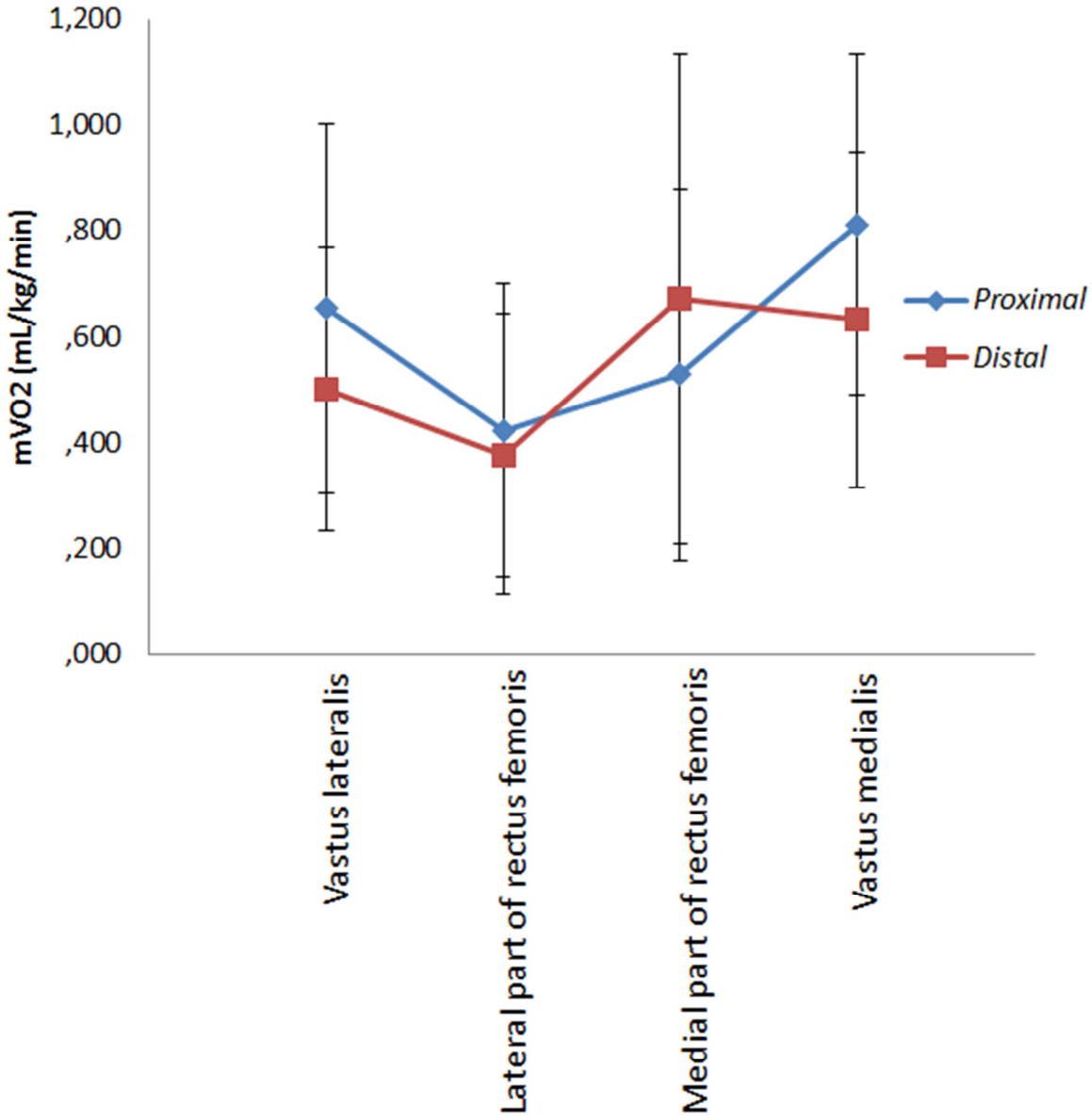


Figure 8: Shows how proximal/distal position impacts muscle oxygenation in different quadriceps muscles.

VL, LRF and VM seem to follow the same general effect where the proximal mVO_2 values were higher, and the distal values were visually lower, MRF however showed the opposite effect. The general effect showed a significant result (p=0,001)

4. Discussion

The main finding of this study was that during steady-state dynamic exercise muscle oxygenation is heterogeneous within vastus lateralis and vastus medialis. This difference was lower at increasing intensity.

4.1 Physiological responses

There was a linear increase in pVO2 and heart rate as the work rate increased. This is in line with what have been shown with cycling in the past (Ettema and Lorås, 2009). For blood lactate however the same linear increase is not shown. A possible explanation might be that only one participant measured BL above the onset of blood lactate accumulation (OBLA) of 4 mmol/L including the third intensity of 200 watt, thus the intensity was not high enough for a linear increase to occur. The added level of blood flow from the dynamic exercise at lower intensity (including warmup) would also further facilitate the oxidation of lactate in the mitochondria.

Since most of the physiological factors were normally distributed, it indicates that the participants are a homogeneous group, which also the general response data supports with very few possible outliers.

4.2 Local oxygen consumption in quadriceps

There is a high level of heterogeneity within VL and VM at the second intensity, but at 250 W the differences are smaller. This contradicts the findings of Kennedy et al. (2006) where there were no differences at higher intensity, and the findings of Takagi et al. (2013). LRF have no sign of heterogeneity within the muscle at all while MRF is heterogeneous at 150 W, but with increased work rate the differences are diminishing. The lower level of heterogeneity at high intensity might be explained by a higher oxygen demand overall or more use of fast twitch fibers.

4.2.1 Effect of intensity on quadriceps muscle oxygenation

VL and VM consume most oxygen at low intensity as expected, but as the intensity increases the oxygenation declines rapidly until it more or less flattens out at higher exertion. However, the heterogeneity is still substantial at 250 W. At the higher intensity the decrease in oxygenation flattens out for all the muscles.

4.2.2 Effect of intensity and quadriceps muscle position

The general effect indicates that mVO2 heterogeneity within the muscles increases in line with higher work rate, but since it is a general effect it does not show what happens on an individual level.

4.2.3 Effect of muscle position on quadriceps muscle oxygenation

MVO2 is a lot lower for the distal than the proximal site for VL; this is in line with previous findings (Takagi et al. 2013, Kennedy et al. 2006). A similar difference is visible in VM, while LRF show almost no difference and MRF show a reverse effect. Since MRF is so vastly different from the other muscles it might be because rectus femoris has a higher placement on the quadriceps, thus making the proximal channel actually measure distal on the muscle. This might also explain why the measurements for both the proximal and distal are quite similar for LRF in figure 5.

5. Conclusion

This is one of the first studies to use an 8-channel setup for examining quadriceps muscle oxygenation during dynamic exercise. Heterogeneity exists within VL and VM at 150 W, but the difference is smaller at 250 W. There are some strange results regarding RF, maybe caused by a misplacement of the probes on the muscle. There are few significant results when taking several factors in account at once.

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