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Oxygen consumption in cycling: The difference between the whole body and the local muscles

BEV3901 Master Thesis

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Abstract

Introduction: Oxygen consumption during exercise has been extensively studied with the focus primarily on whole body oxygen consumption (tVO_2). The purpose of this thesis was to use Near-infrared spectroscopy (NIRS) to compare tVO_2 to oxygen consumption in the local muscles (mVO_2) at increasing work rate. **Method:** 18 male cyclists performed an incremental cycling test until exhaustion. tVO_2 was measured through pulmonary gas exchange and mVO_2 was measured using NIRS in combination with arterial occlusion (AO). mVO_2 was measured in the vastus medialis (VM) and vastus lateralis (VL) muscles. **Results:** tVO_2 showed an linear increase with increasing work rate. However, tVO_2 showed an initially faster increase followed by a slower increase with increased work rate when compared to tVO_2. No increase in cadence was seen with increasing work rate. **Discussion:** The main finding was a significant different effect of work rate on mVO_2 and tVO2. The steep increase in mVO_2 during low intensity exercise found in the present study indicates that the VM and VL muscles are activated at an early stage during increasing intensity. The results from the present study indicate that there are differences between what happens in the local muscle and what is observed when looking at the whole body. The increase in work rate with no observed increase in cadence may indicate increased intramuscular pressure which may occlude blood flow in the muscle and thus be part of the reason for the decrease in mVO_2 seen at high intensity. **Conclusion:** This study shows that care should be taken with results from tVO_2 for practical application because the mechanisms at the local level are more complex and deviate substantially from that what you can derive from whole body measurements.

Key words: Near-infrared spectroscopy, cycling, local muscle VO_2, mVO_2, whole body VO_2, tVO_2, vastus lateralis, vastus medialis.

Contents

1. Introduction

Energy consumption in the human body during exercise has been studied extensively for many years. Human skeletal muscle is highly dependent on oxidative metabolism and the oxygen consumption in human skeletal muscle may increase up to 50 fold during exercise (Hamaoka et al., 2007). This makes oxygen consumption during exercise, and thus, oxidative metabolism an important and interesting field of research. In addition, the external work produced is also an interesting and widely studied variable. Cycling is often a preferred mode of exercise in these studies due to the possibility to easily and accurately measure and control the external work produced and energy consumption in cycling has also been studied extensively (Ettema and Lorås, 2009).

Whole body oxygen consumption (tVO_2) has been shown to increase linearly as a function of work rate during cycling (Ettema and Lorås, 2009; Leirdal and Ettema, 2009). In addition, tVO_2 is often used to say something about local work, which, based on what is seen in the literature, not necessarily yields valid answers. Studies of muscle activity at the local level, using several different methods (e.g. MRI, EMG, PET and joint moments), show significant heterogeneity with regards to muscle activity during exercise at different intensities (Ericson et al., 1986; Reid et al., 2001; Sanderson and Black, 2003; Endo et al., 2007; Dorel et al., 2009; Boisen-Møller et al., 2010). The very linear and consistent oxygen consumption to work rate relationship in the whole body, despite the large heterogeneity in local muscle activity, is a very interesting phenomenon.

Problem exist with the abovementioned methods for local muscle measurements in that they are either invasive, non continuous, cannot be done during exercise or there is disagreement in regards to interpreting the results (Endo et al., 2007; Hug and Dorel, 2009). Nevertheless, the study of oxygen consumption in the local muscles can provide a more direct approach to gaining additional knowledge regarding the contributions of individual muscles during exercise. Oxygen consumption cannot be measured directly in the local muscles in a similar way as for the whole body (e.g. through gas exchange), but several other methods are available today that can indirectly measure mVO_2. The Fick equation (O_2 consumption = blood flow · arteriovenous O_2 difference) defines the gold standard for measuring peripheral oxygen consumption as the combination of measuring blood flow (e.g. by strain gauge plethysmography) and the arteriovenous O_2 difference (blood samples). Several problems exist with this method however, in that it is invasive and non-continuous. Another problem is that it only provides a regional value for oxygen consumption and is unable to differentiate

between individual muscles, something that has been shown with the use of near-infrared spectroscopy (Van Beekvelt et al., 2001a).

Near-infrared spectroscopy (NIRS), is an optical method for measuring changes in local muscle blood volume and oxygenation that is both continuous, non-invasive and can be used during exercise (Van Beekvelt et al., 2001b). NIRS has been used in the study of exercising muscle since 1992 (Chance et al., 1992) and has since then developed into a very useful tool for studying the metabolism of human skeletal muscle in vivo (Hamaoka et al., 2007). NIRS has been used to study the physiological responses, at rest and during exercise, of several muscles in healthy subjects (e.g. in VM (Neary et al., 2001, Neary et al., 2005), VL (Chance et al., 1992; Takaishi et al., 2002; Kime et al., 2005; Kennedy et al., 2006; Nagasawa, 2007), gastrocnemius (Kubo et al., 2008), rectus femoris (Chance et al., 1992)), but also in several pathological states (e.g. in patients with heart failure (Mancini et al., 1994a, Matsui et al., 1995, Wilson et al., 1989), peripheral vascular disease (Kooijman et al., 1997, McCully et al., 1994), and metabolic myopathies (Abe et al., 1997, Van Beekvelt et al., 1999, Van Beekvelt et al., 2002a)).

NIRS uses light in the near-infrared spectrum (650-900 nm) using specific wavelengths that are mainly absorbed by hemoglobin and myoglobin. By using a modified Lambert-Beer law, the NIRS signal can be used to study relative changes in oxyhaemoglobin (O_2Hb), deoxyhaemoglobin (HHb), and total haemoglobin (tHb) (summation of O_2Hb and HHb). Since NIRS only measures relative changes in blood flow and oxygenation, a direct measurement of oxygen consumption is not possible. In order to obtain a quantitative value for muscle blood flow and oxygen consumption, a physiological intervention (e.g. an arterial occlusion (AO) or a venous occlusion) must be used to control inflow and outflow of blood to the limb (Van Beekvelt et al., 2001b, Hamaoka et al., 2007). Most of previous studies using AO have been done on the forearm (Van beekvelt et al., 2001b) and very few studies have used arterial occlusion on large muscles during dynamic exercise (e.g. cycling). The few studies that have used leg muscles have applied AO directly after exercise instead of during exercise (Nagasawa, 2007) or only investigated blood flow (Kime et al., 2005).

The purpose of this study was to investigate the relationship between oxygen consumption measured in the local muscle and that measured for the whole body. In order to do so we simultaneously used NIRS to measure local muscle oxygen consumption, and compared it with whole body oxygen consumption measured through pulmonary gas exchange measurements. We measured used an incremental cycling exercise protocol to investigate the relationship using several various workloads.

2. Methods and materials

2.1 Subjects

The subjects for the study were 18 healthy, well trained male cyclists recruited through several cycling clubs in Norway. The mean age (SD, min – max) was 26 years (± 7, 18 – 44), weight was 75.9 kg (± 5.3 kg, 65 – 86) and height was 178.8 cm (± 5.0 cm, 170.5 – 190). The mean amount of training was 10.7 (± 4.3) hours and ranged from 4 to 18 hour per week and the mean amount of specific cycling training was 7.4 (± 4.0) hours and ranged from 4 to 14 hours per week. The study was assessed by the regional medical ethical committee and all subjects signed an informed consent prior to participating in the study.

2.1 Experimental design

All subjects came to the lab for one cycle test. Before the start of the test, the weight and height of subjects were measured and information regarding training status was obtained. During the test, heart rate was measured continuously with a heart rate monitor (Polar RS800, Polar Electro OY, Kempele, Finland). All cycling was performed on an electronically braked cycle ergometer (Velotron, Racemate inc, Washington, USA). Cadence was measured continuously with a sampling frequency of 33.3 Hz throughout the test and work rate was changed by means of the velotron software package. tVO_2 was measured by pulmonary gas exchange (Oxycon Pro, Jaeger GmbH, Hoechberg, Germany) with a sample rate of 0.1Hz. The gas exchange equipment was calibrated at least on each day of testing or more often if laboratory conditions required it (e.g. increasing temperature during the day) and has been previously validated (Foss and Hallen, 2005). Blood lactate measurements were derived from blood samples taken from the tip of the ring finger on the right hand (Lactate Pro LT-1710, ArkRay Inc, Kyoto, Japan) at rest, after the warm up phase and immediately after each workload. Before taking a blood sample, the finger was wiped clean from sweat. When lactate measurements were done during cycling, subjects were instructed to rest their hand on the handlebars to minimize movement in order to make sure that the measuring unit was only touching the blood drop and not the skin. After the test, skinfold thickness was measured by skinfold caliper measurements (Holtain Tanner/Whitehouse skinfold caliper, Holtain Ltd, Crymych, Wales) at the sites of NIRS optode placement. Three caliper measurements were taken at each measurement site and the average of the three, in millimeters, divided by two, was taken as the skin fold thickness. Thigh circumference was also measured after the test at the height of the NIRS optodes. All data were stored for offline analysis.

2.3 Experimental protocol

A schematic representation of the protocol is shown in figure 1. A blood lactate value during rest, taken prior to three AOs with the subjects seated in a chair in a relaxed position. This was followed by a ten minute cycling warm-up at a work rate of 75 or 100 W. The subjects used a freely chosen cadence for both the warm up and the actual test. After the warm up, blood lactate was measured again and another three AOs were applied while the subject remained on the bike and with the right leg in the non-weight-baring position with an approximate angle of 90 degrees in hip and knee. After finishing the test procedures at rest and after warm up, the gas exchange equipment was attached and the incremental cycle test was started at a work rate of 100 W. Each work rate lasted 5 minutes, after which, work rate was increased with 50 W. During the last 20 seconds of each work rate, an AO was applied. Immediately after the end of the AO, the resistance was increased with 50 W increments. Blood lactate measurements were performed immediately after the increase in workload, thus reflecting blood lactate values at the end of the previous workload. The work rate increments were repeated until the subjects failed to complete a full, five minute, work period.

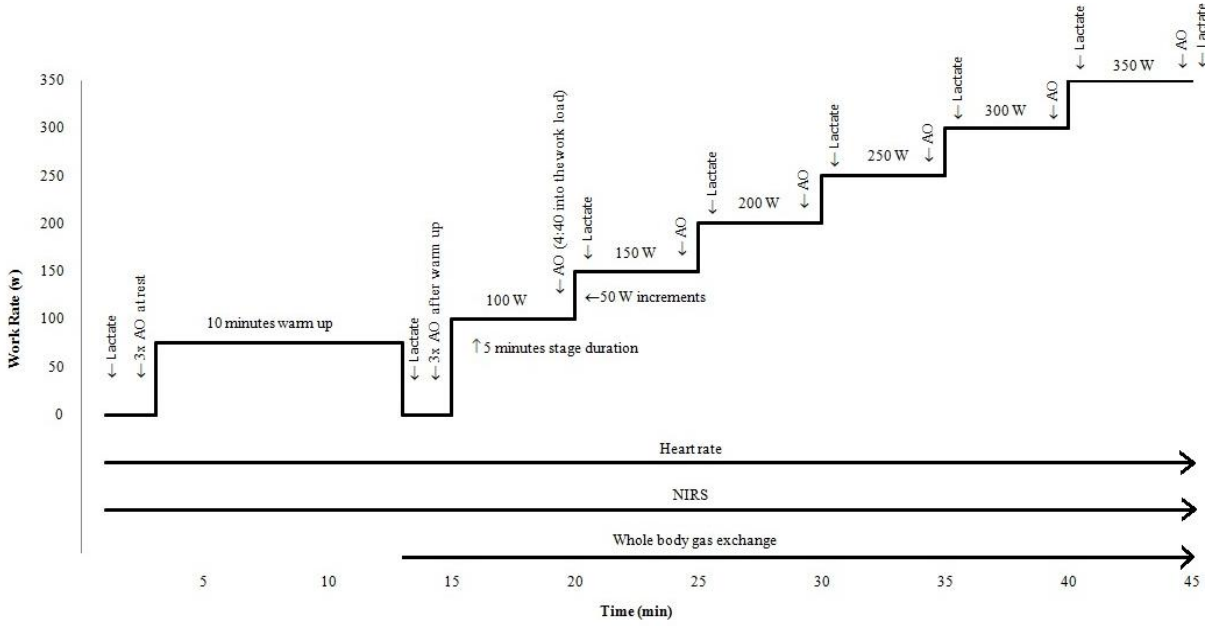

Figure 1: Schematic representation of the complete test protocol. Heart rate, NIRS and pulmonary gas exchange were measured continuously throughout the test. AO = arterial occlusion. The test started with a lactate measurement and three AOs were performed prior to and after a 10 minute warm up. A starting workload of 5 minute at 100 W with an AO applied after 4 minutes and 40 seconds by a pneumatic cuff around the thigh, rapidly inflated to 260mmHg and rapidly deflated after 20 seconds. This was followed by 50 W increment and a lactate measurement. This was repeated until the subjects could not complete the entire 5 minute workload.

2.4 Near-infrared spectroscopy

In the present study, local changes in muscle oxygenation were measured by continuous near-infrared spectroscopy (Oxymon MKIII, Artinis Medical Systems, the Netherlands) using wavelengths of 766 and 856 nanometer. These particular wavelengths are used due to their ability to penetrate biological tissue because they are mainly absorbed by hemoglobin, myoglobin and, to a lesser extent, cytochrome oxidase. Compared to hemoglobin and myoglobin, absobtion by cytochrome oxidase is very small and can be neglected (Van Beekvelt, 2002). Due to identical absorption spectra, it is not possible to differ between myoglobin and hemoglobin. However, since we only used NIRS to investigate mVO_2 in the present study, this does not affect our results. Oxyhaemoglobin (O_2Hb) and deoxyhaemoglobin (HHb) do have different absorption spectra and this makes it possible, by using a modified Lambert-Beer law, to study relative changes in O_2Hb and HHb.

Quantitative values for mVO_2 were derived using the AO method. Although calculations using the AO method rely on the assumption that tHb stays constant during the AO (De Blasi et al 1997), the AO method for measuring mVO_2 has been shown to be reliable in previous studies (Van Beekvelt et al., 2001b, Sako et al., 2001; Hamaoka et al., 2007, Gerovasili et al., 2010). A pneumatic cuff was used to apply the AO to the right leg. The AO was applied by an automatic inflation system (Hokanson E20 Rapid Cuff Inflator + Hokanson AG-101 Air Source, Marcom Medical ApS, Denmark) set to a pressure of 260 mmHg. The cuff was also rapidly deflated after the AO. Rapid inflation and deflation is necessary to keep blood volume in the leg as constant as possible during AO, thus, an automatic system is preferable. When calculating mVO_2 from the NIRS signal during occlusion, the difference between O_2Hb and HHb (Hb_{diff}) was used. Concentration changes were expressed in $\mu M \cdot s^{-1}$ and converted to $ml O_2 \cdot min^{-1} \cdot 100g^{-1}$ using a value of $1.04 kg \cdot L^{-1}$ for muscle density, 4 molecules of O_2 per hemoglobin and 22.4 L as the molar volume of gas. We corrected for scattering using a DPF of 4.0.

NIRS optodes were fastened to the bulk of the muscle on the vastus medialis (VM) and vastus lateralis (VL) muscles of both legs. The VL and VM muscles were chosen for their known involvement in cycling exercise and previous appearances in the literature (Chance et al., 1992; Takaishi et al., 2002; Kime et al., 2005; Kennedy et al., 2006; Nagasawa, 2007 (VL), Neary et al., 2001, Neary et al., 2005 (VM)). Distance between the light source and detector was 40 mm and data were sampled at 50 Hz.

2.5 Data analysis

In order to remove the artifacts resulting from the rhythmic contractions and relaxations of the muscles during cycling, a Butterworth filter was used on the raw NIRS signals. The time in which the decrease in concentration of O_2Hb and Hb_{diff} stays linear during application of AO, decreases with increasing work rate due to an accelerated depletion of oxygen in the tissue. This means that the time period over which the slope is calculated, cannot be of a standardized duration. The time periods used for calculating mVO_2 during AO were set manually and R^2 values were used to check the linearity of the regressions

Whole body measurements of tVO_2 and RER (i.e. the ratio of carbon dioxide produced to oxygen consumed) were derived from gas exchange measurements and were averaged over the last minute of each workload. The whole body metabolic rate in terms of watt was calculated using the amount of liters of O_2 per minute taken up by the lungs and RER values, both measured by gas exchange. The average last minute heart rate is presented as % of the maximal value obtained during the cycling test. tVO_{2peak} was defined as the highest value obtained during the cycling test.

2.6 Statistics

Results are presented as means ± SD. Students paired t-test was used to test for differences in ATT between the sites of the VL and the VM. A one-way ANOVA was used to assess possible differences between the participants that completed a maximum work rate of 300 and those that completed a maximum work rate of 350w. An ANOVA for repeated-measures was used to assess the effect of work rate on mVO_2, tVO_2, cadence, blood lactate and heart rate. When significant differences were found, within-subject contrasts were used to assess differences between work rates. A mixed design multivariate ANOVA for repeated measures was used to assess differences in how local and total VO_2 were affected by work rate. Again, within-subject contrasts were used to assess differences between work rates. If significant differences were found, they were further analyzed using within-subjects contrasts. When the assumption of sphericity was violated, significance was adjusted using the Greenhouse-Geisser method. The significance level was set at 0.05. Data analysis, filtering and statistics were performed with Matlab, Excel and SPSS (18.0.0) for Windows.

3. Results

In total, 18 subjects performed the cycling test, of which, 12 completed a maximal workload of 300 W and 6 completed a maximum workload of 350 W. The subject characteristics are presented in table 1. No differences between subjects were found when comparing the subjects that completed a maximum workload of 300 W and the subjects that completed a maximum workload of 350 W, apart from height ($p = 0.012$) (tested variables: weight, height, age, tVO_{2peak} , thigh circumference, skinfold thickness and maximum lactate values).

Table 1: Subject characteristics.

	Mean	SD	Min	Max
Height (cm)	178.8	5.1	170.5	190
Weight (kg)	76.0	5.3	65.0	86.2
Age (years)	26.8	7.9	18	44
HR_{max} (bpm)	199	7.7	185	212
tVO_{2Peak}(ml/min/kg)	59.7	4.0	52.1	66.8
Maximum Lactate value (mmol/l)	12.4	2.2	9.1	16.7
ATT (Right VM) (mm)	4.9	0.9	3.8	7.3
ATT (Right VL) (mm)	5.1	1.0	3.6	7.3

ATT = adipose tissue thickness, VM =vastus medialis, VL = vastus lateralis, HR = heart rate and tVO_{2Peak} = maximum whole body oxygen consumption obtained during the test.

3.1 General response

Figure 2 shows the general response for one of the subjects in heart rate, tVO_2 and NIRS signals during the cycling test. As work rate increased during the test, both heart rate and tVO2 increased. This indicates that the protocol we used produced results in accordance with what is to be expected on the basis of basic physiology.

With regards to the NIRS signal, tHb increases throughout the test and stabilizes at hi intencity. O_2Hb and HHb start to deviate from each other especially at higher work rate. This indicates decreased muscle oxygenation and increased blood volume following increased exercise intensity. The marked changes prior to each increase in work rate are results of the AO being applied for the last 20 seconds of each workload.

Figure 3 shows the effect of work rate on gross efficiency and whole body metabolic rate. As can be seen in figure 3, both gross efficiency and whole body metabolic rate increases with work rate.

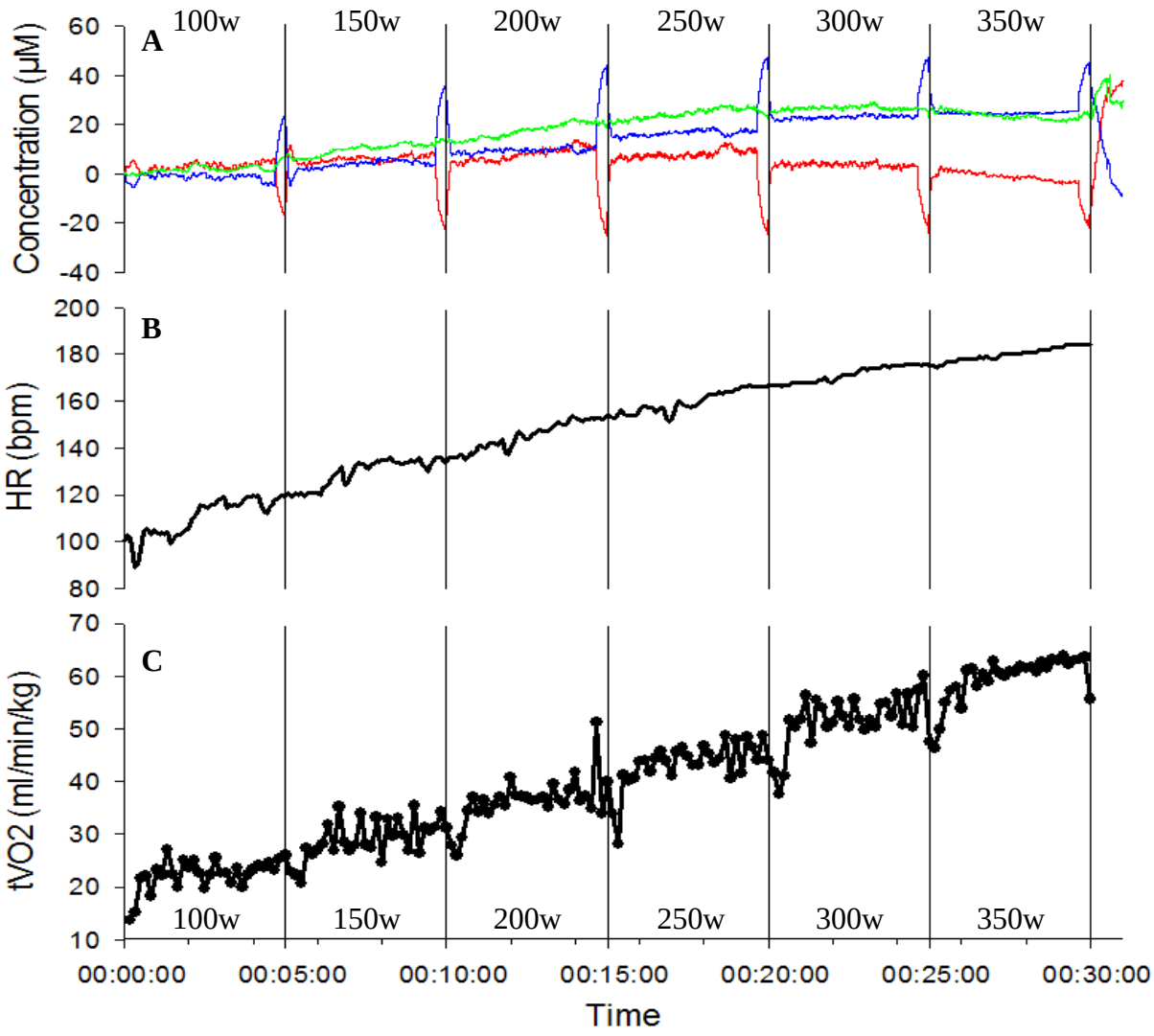

Figure 2: General response for NIRS (A)(O_2Hb = red line, HHb = blue line, and tHb = green line), heart rate (HR) (B) and tVO_2 (C) during the incremental cycling test with work rate increasing every 5 minutes immediately following a 20-second AO. Vertical lines indicate a 50 W increase in work rate.

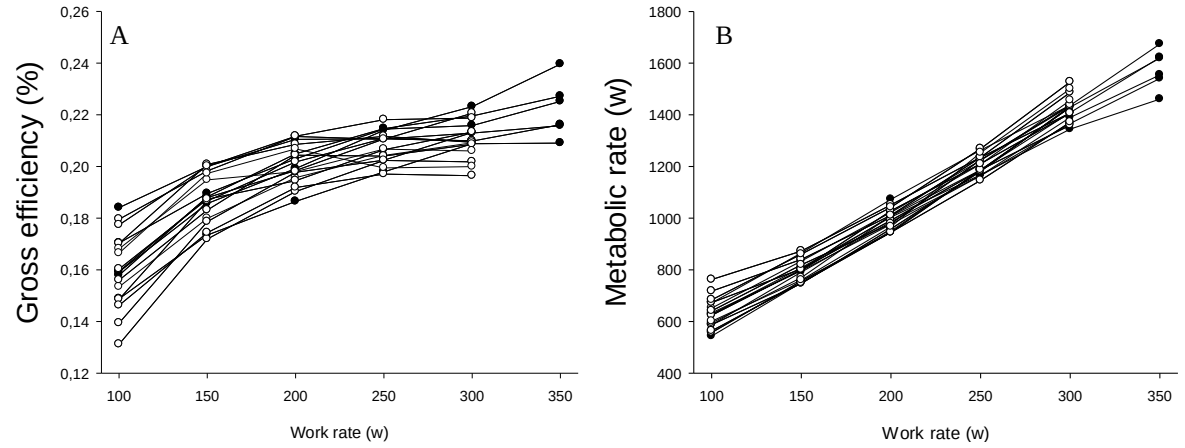

Figure 3: Gross efficiency (A) and total body metabolic rate (B) as a function of work rate. Subjects that completed 300 W are shown with open circles and those that completed 350 W are shown with filled circles.

3.2 Near-infrared spectroscopy

The filtered and unfiltered data from AOs at three different work rates from a single subject is presented in figure 4. As can be seen in figure 4, a steeper decrease in the Hb_{diff} occurred when the work rate increases. A steeper decrease in the Hb_{diff} indicates higher mVO_2 with increasing work rate. Apart from that, it can also be seen that the time in which the decrease in O_2Hb and the Hb_{diff} stays linear, decreased with increasing work rate. No major change can be observed in tHb during the AO, indicating that blood flow is sufficiently occluded.

The lowest R^2 values used was 0.91 (VM) and 0.95 (VL) and the mean values were 0.98 (VM) and 0.99 (VL). This indicates that the regressions done in order to calculate the steepness of the slope are valid despite the fact that they were set manually.

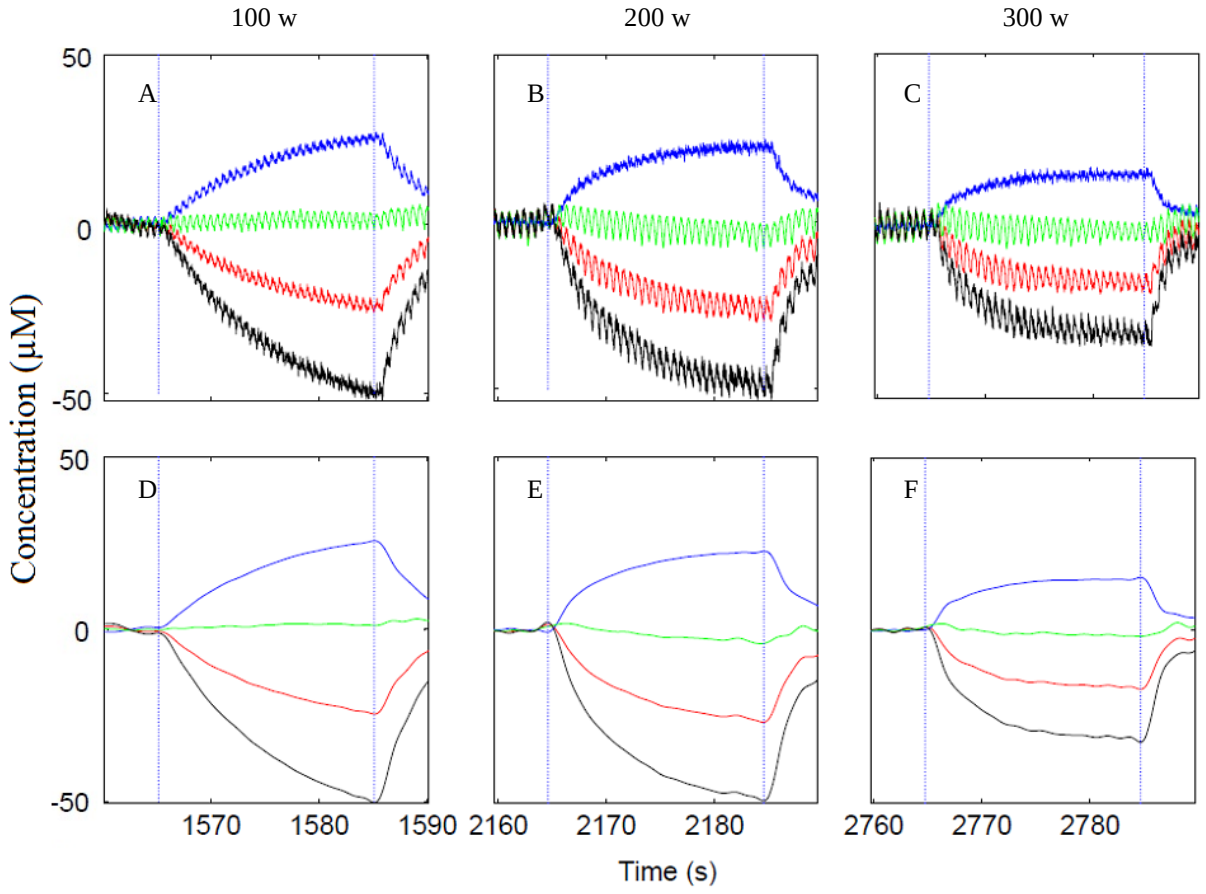

Figure 4: Unfiltered (A,B,C) and filtered (D,E,F) NIRS signals for AO at 100 (A,D), 200 (B,E), and 300 (C,F) watts. Lines indicate concentration of O2Hb (red line), HHb (blue), tHb (green) and Hb_{diff} (black). Vertical lines indicate start and stop of AO.

3.3 Effect of occlusion

Since it is more common to apply AO immediately after exercise, instead of during exercise, we have looked at the specific effect of AO on all our variables. No clear effects were seen

apart from those on cadence and tVO_2. As shown in figure 5, we found significant higher cadences when comparing the mean cadence derived from the final 20 seconds of each work rate (e.g. the period during AO) with the mean cadence derived from the entire work rate prior to AO (100 W, $p < 0.001$, 150 W, $p < 0.001$, 200 W, $p < 0.001$, 250 W, $p \leq 0.001$) for all but the two last workloads (300 W, $p = 0.447$, 350 W, $p = 0.669$) (figure 5). The increase in cadence was present in most subjects (11 of the 18 subjects). For tVO_2, no change was seen during AO, but a marked decrease was seen shortly after the occlusion. This is also barely visible in figure 2 which includes only one subject. Although not present in all the subjects, roughly 13 of the 18 subjects showed signs of tVO_2 decrease. When comparing the mean tVO_2 over the last minute with the mean derived from the first four measurements (10 second intervals) after the occlusion, a significant lower value was found for the 150 ($p = 0.024$), 200 ($p = 0.001$), 250($p < 0.001$), and 300 ($p = 0.014$) W workloads. For the 100 W work rate, the difference was close to significant ($p = 0.064$). This indicates that the application of the AO leads to a decrease in tVO_2. Since the gas exchange measurements were stopped immediately after the completion of the last workload, it was not possible to assess any change after the final workload of 350 W.

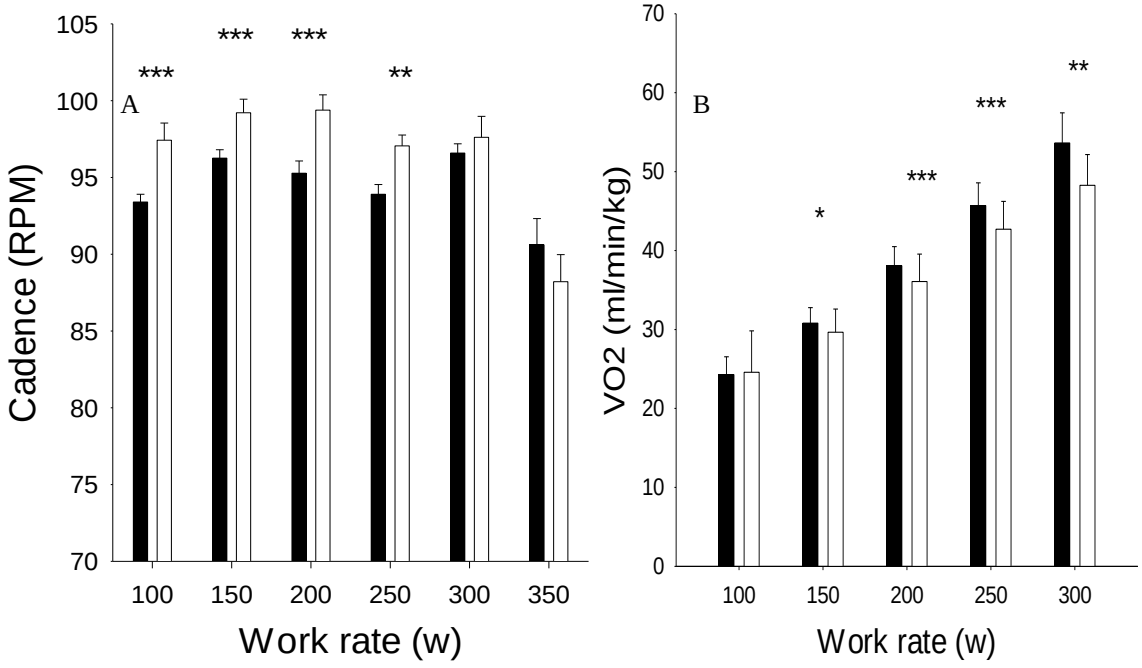

Figure 5: A: Mean (± SD) cadence for the complete duration of various workloads compared to mean cadence during the occlusions. indicates significant differences between entire workload and the last 20 seconds (during occlusion) (** = p <0.01, *** = p < 0.001). B: tVO_2 during the last minute compared to tVO_2 derived from the first four measurements after the AO. * indicates significant differences between values (* = p < 0.05, ** = p < 0.01, *** = p < 0.001).*

3.4 Effect of work rate

The effect of work rate on the various measured variables is shown in table 2. Workload had no significant effect on cadence ($p = 0.135$). Neither was there any effect of cadence when the subjects that completed 350 W were analyzed separately ($p = 0.842$). Work rate had a significant effect on heart rate ($F(1.253, 21.293) = 461.312$, $p < 0.001$)) and tVO2 ($F(1.462, 24.859) = 1236.786$, $p < 0.001$). Contrast revealed significant differences between all work rates up to 300w indicating that both heart rate and tVO_2 increased with increasing work rate. Again, separate analysis of the six subjects that completed the 350 W work rate revealed similar results for both heart and tVO_2 (both $p < 0.001$).

A significant effect of work rate was also found for blood lactate values ($F(1.43, 24.311) = 88.325$, $p < 0.001$), indicating that increasing workload leads to higher blood lactate values. Contrasts analysis showed significant differences between all work rates. When the six subjects that completed the 350 W work rate were tested separately, a similar effect of work rate was observed ($F(1.155, 5.777) = 169.948$, $p < 0.001$).

Table 2: Mean (±SD) values for the various measured variables at rest and at various work rates.

Work Rate	Rest	100 W	150 W	200 W	250 W	300 W	350 W
Lactate (mmol/l)	2.3 (±1.3)	1.4** (±0.5)	1.7*** (±0.5)	2.5*** (±0.9)	4.4*** (±1.7)	9.8*** (±3.6)	12.4† (±1.8)
Cadence (RPM)	Na	93.4 (±0.5)	96.3 (±0.5)	95.3 (±0.8)	93.9 (±0.6)	96.6 (±0.6)	90.6 (±1.7)
Heartrate (% of HRmax)	Na	61.7*** (±5.9)	69.4*** (±5.4)	78.7*** (±4.3)	87.5*** (±3.7)	94.5*** (±3.0)	97.4†† (±0.7)
Total VO_2 (ml/min/kg)	Na	24.3*** (±3.5)	30.8*** (±3.0)	38.1*** (±3.7)	45.7*** (±3.7)	53.5*** (±4.6)	58.5††† (±5.6)
mVO_2 (VL)	0.05 (±0.01)	0.95*** (±0.42)	1.39*** (±0.64)	1.62*** (±0.65)	1.66 (±0.69)	1.50** (±0.59)	1.67† (±0.53)
mVO_2 (VM)	0.05 (±0.02)	1.21*** (±0.46)	1.72*** (±0.64)	1.93** (±0.64)	2.06* (±0.64)	2.00 (±0.61)	1.95 (±0.53)
n	18	18	18	18	18	18	6

Asterisks indicate a significant difference from previous work rate (= $p < 0.05$, **= $p < 0.01$, *** = $p < 0.001$). Significant differences from previous workload within the subgroup of the 6 subjects that completed 350w are indicated by † = $p < 0.05$, †† = $p < 0.01$, ††† = $p < 0.001$.*

3.5 Local muscle oxygen consumption

The results for mVO_2, measured by NIRS for the VM and VL muscle at various work rates are presented in figure 6. A significant effect of work rate on mVO_2, measured by NIRS, was found for both the VM ($F(1.875, 31.869) = 134.907$ $p < 0.001$) and the VL muscle ($F(1.627, 27.651) = 27.651$ $p < 0.001$), indicating that oxygen consumption in the local muscles increase as a result of the increased work rate. A similar result was found when the six subjects that completed 350 W were assessed separately ($p < 0.001$ for both muscles).

For the VM muscle, contrasts revealed a significant increase in mVO_2 between rest and post warm-up ($p = 0.001$), post warm-up and 100 W ($p < 0.001$), 100 W and 150 W ($p < 0.001$), 150 W and 200 W ($p = 0.001$) and 200 W and 250 W ($p = 0.028$) but not between 250 W and 300 W ($p = 0.438$), indicating that the increase in mVO_2 seemed to plateau at the highest work rates of 250 and 300 W. For the VL muscle, contrasts revealed a significant increase in mVO_2 between 100 W and 150 W ($p < 0.001$) and 150 W and 200 W ($p < 0.001$) but not between 200 W and 250 W ($p = 0.395$). This indicates a similar pattern in mVO_2 as in the VM muscle, but the plateau occurs at an earlier stage. Between 250 W and 300 W ($p = 0.003$) a significant decrease in mVO_2 was seen. This indicates that the VL muscle decreases its mVO_2 at the highest work rates.

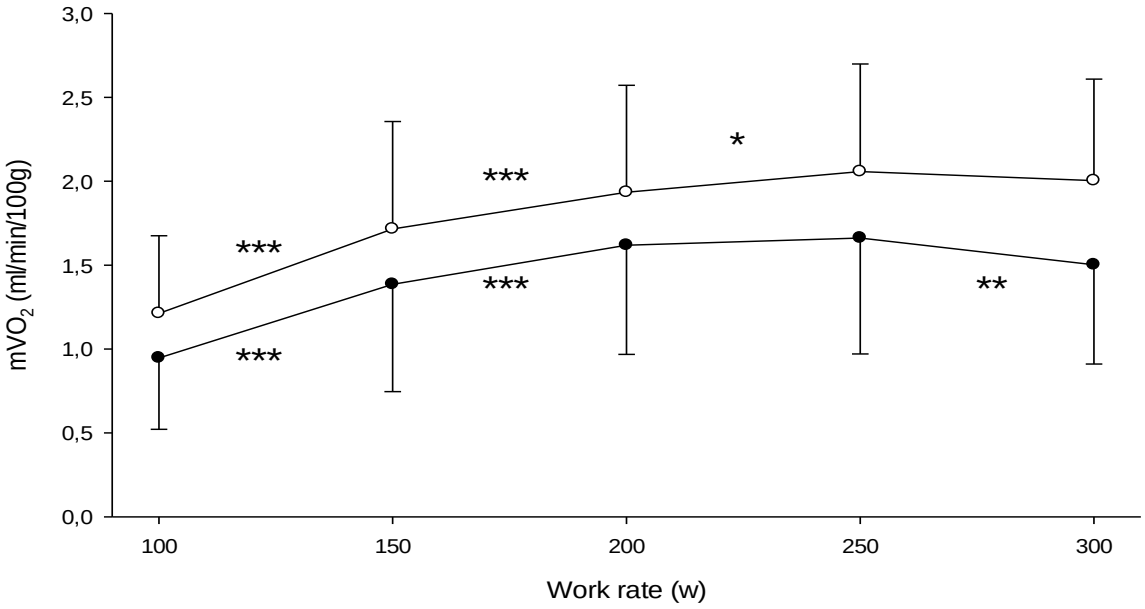

Figure 6: Local muscle oxygen consumption measured by NIRS in the vastus lateralis (filled) and vastus medialis (open) muscle as a function of work rate. Asterisks indicate a significant effect of work rate between subsequent work rates (= p < 0.05, ** = p < 0.01, *** = p < 0.001).*

3.6 Differences between total and local oxygen consumption

In order to assess differences between total and local oxygen consumption, total and local values for oxygen consumption were normalized to their respective maximum value obtained during the test. With respect to the mVO_2 values, the average of VL and VM was taken as the value for local oxygen consumption for each work rate due to the very similar results seen in figure 6.

In order to assess the differences between tVO_2 and mVO_2, under aerobic conditions, we excluded measurements with RER values above 1 and/or lactate values above 5. Twelve subjects were included in the analysis. Six subjects were excluded from analysis because they had less than four different work rates after the exclusion of RER and lactate values. These results are presented in figure 7. A significant contrast was found between tVO_2 and mVO_2 for all work rate increments (100 W to 150 W, $p < 0.001$, 150 W to 200 W, $p = 0.033$ and 200 W to 250 W, $p = 0.013$). This indicates that the response is different for tVO_2 and mVO_2 over the full range of work rates. Looking at figure 7, we see that the relative increase in oxygen consumption from 100w to 150w is faster in the local muscles compared to the whole body. We also see that the increase in oxygen consumption is slower in the local muscles from 150w to 200w and from 200w to 250w when compared to the whole body.

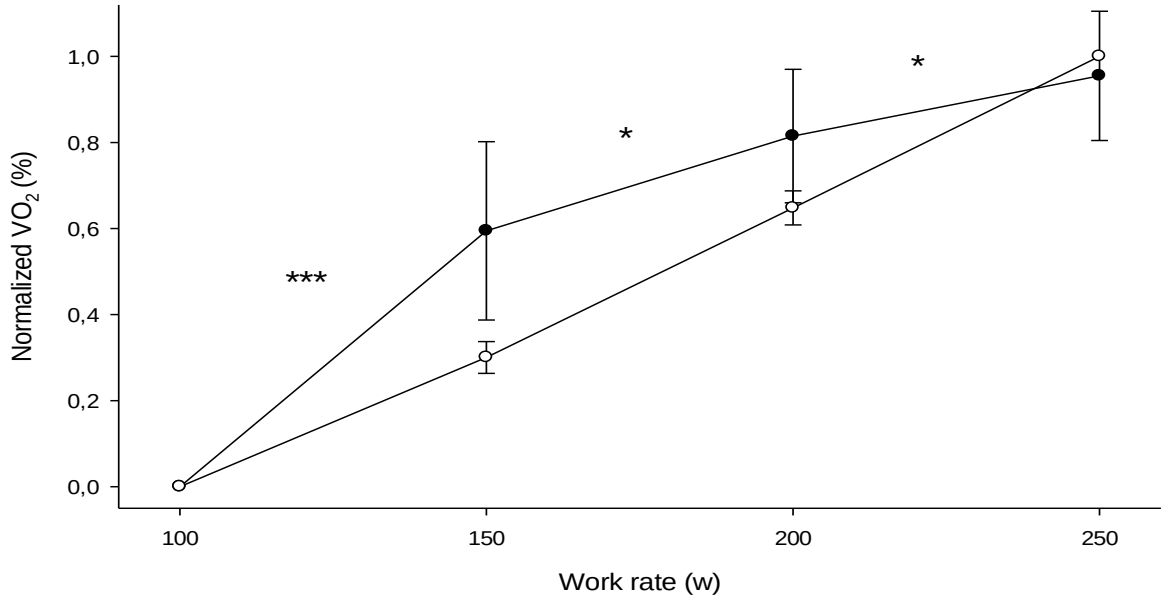

Figure 7: Local muscle oxygen consumption measured by NIRS for the (filled) and whole body oxygen consumption (open), normalized for maximum values, as a function of work rate. Measurements with RER values >1 and/or lactate values > 5 are excluded. Asterisks indicate significant difference in how local and whole body oxygen consumption is affected by a change in work rate (= p < 0.05, *** = p < 0.001). N=12.*

When including all 18 subjects up to the 300w work rate, a significant difference between mVO_2 and tVO_2 was also found (F (2.315, 39.353) = 29.114 ($p < 0.001$). These results are presented in figure 8. Contrast revealed a significant difference in mVO_2 and tVO_2 from the first (100 W) to the second workload (150 W) ($p < 0.001$), but not from the second to third (200 W) (F (1, 17) =1.029, p = 0.325). This indicates the same as was seen in figure 7 (an initially steeper increase in mVO_2 compared to tVO_2 followed by a plateau). Significant differences were again seen from the third to the fourth (250 W) (F (1, 17) = 7.388, p = 0.015), thus indicating a plateau in mVO_2. From the fourth to the fifth workload, a significant contrast was again found (F (1, 17) =38.502, $p < 0.001$) and a decrease in mVO_2 can be seen. When the 6 subjects that completed the 350 W workload were analyzed separately, a similar trend was seen (data not shown).

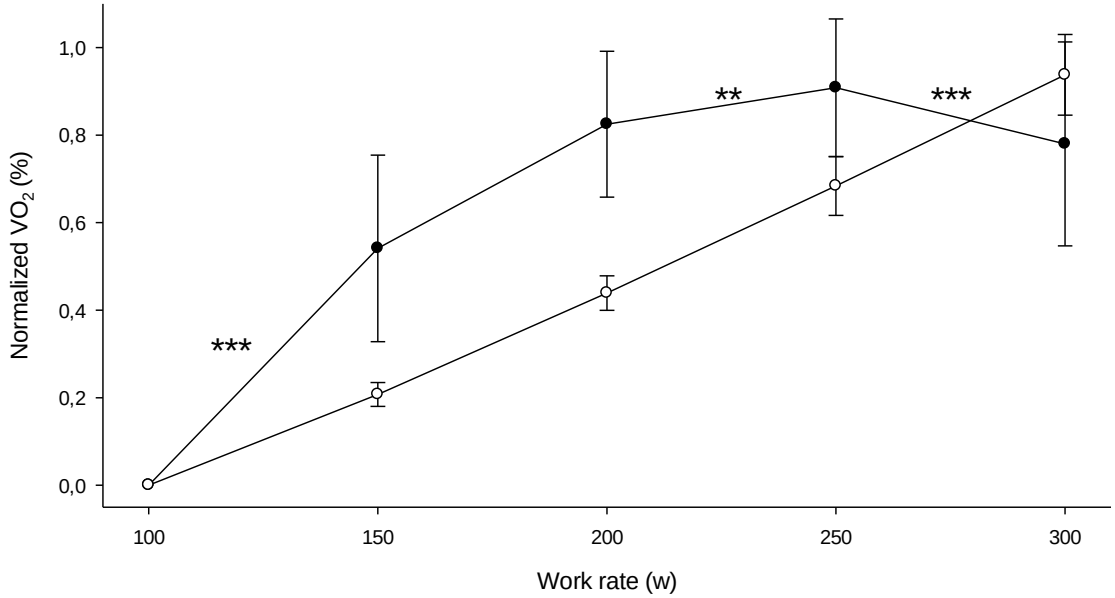

*Figure 8: Oxygen consumption measured by NIRS for the local muscle (filled) and whole body (open) muscle, normalized for maximum values, as a function of work rate. Asterisks indicate significant difference in how local and whole body oxygen consumption is affected by a change in work rate (** p < 0.01, *** p < 0.001). N = 18.*

4. Discussion

In this study we used NIRS to investigate the relationship between mVO_2 and tVO_2 during cycling exercise at different work rates. The main finding of the present study was a significantly different response of mVO_2, compared to tVO_2, with increased work rate. Whereas tVO_2 showed a continued increase with increasing work rate, mVO_2 shows a plateau at high work rates in and even a decrease in mVO_2 is seen in the VL muscle.

4.1 General response

The increase in work rate led to an increase in heart rate, tVO_2 and blood lactate as can be expected based on basic physiology. At submaximal work rate, heart rate and tVO_2 increased linearly with work rate. When calculating metabolic rate from tVO_2, it showed a linear increase with increased work rate. This is in agreement with previous studies, where a linear relationship between whole body metabolic rate and work rate has been shown during cycling exercise (Ettema and Lorås, 2009; Leirdal and Ettema, 2009). This was originally shown in isolated muscle by Fenn (1922) and is known as the Fenn effect. Gross efficiency (i.e. the ratio of metabolic rate to work rate) showed an increase with increasing work rate, but with the distinct curve resulting from the decreasing relative contribution of the offset (i.e. the resting metabolic rate which can be seen as the y-intercept (if extrapolated) in figure 3 B). This is also in agreement with previous studies (Ettema and Lorås, 2009).

Most of the previous measurements of mVO_2 have been done on the arm probably due to a smaller ATT. Van Beekvelt (2002) reported a resting mean mVO_2 value of 0.11(± 0.05) $ml \cdot min^{-1} \cdot 100g^{-1}$, with a range of 0.04 to 0.21 ($ml \cdot min^{-1} \cdot 100g^{-1}$) when reviewing 24 previous studies using NIRS with AO or venous occlusion. Resting mVO_2 in the present study is thus within this range, however, none of the included studies measured mVO_2 in the VL or VM muscles. In the present study, the mVO_2 obtained, was higher for the VM than the VL muscle at rest and all work rates (table 2). One of the possible reasons for this could have been differences in ATT between the VL and VM. However we found no difference in ATT between the VL and the VM in our subjects (table 1). Another possible explanation for the difference in mVO_2 may be a difference in the fiber type distribution within both muscles. Johnson et al. (1973) found a larger relative percentage of type I muscle fibers in the VM compared to the VL. This indicates that the oxidative capacity of the VM might be higher than that of the VL and thus contribute to the observed difference in mVO_2 between the VL and VM. Based on the literature, this is probably a factor contributing to the observed difference.

4.2 Near-infrared spectroscopy

Although one of the advantages of NIRS is the possibility for measurement during exercise, measurement of mVO_2 during dynamic exercise is not common. This might have probably been due to the lower sampling frequencies of some of the NIRS equipment. However, in the present study we had the possibility for a sampling frequency of 50Hz which enabled mVO_2 measurements during exercise. Because the use of NIRS and AO during exercise is uncommon, it is interesting to investigate how the AO affects the data collection, analysis and cycling performance. As shown in figure 2, a relative increase in HHb and tHb and a decrease in O_2Hb were seen during the whole incremental exercise test. This is indicating an increase in blood volume and an increase in deoxygenation in the exercising limb with increased work rate. This general response in the NIRS signals is in agreement with previous studies (Kime et al., 2005, Kennedy et al., 2006). One of the consequences of using NIRS and AO during exercise are the observed oscillations in the NIRS signal (figure 3) as a result of the rhythmic contraction and relaxation of the muscle. Due to the contractions, blood will be forced out of the muscles and this, coupled with the subsequent relaxations, leads to the observed oscillations. In order to accurately set the time periods for mVO_2 calculations, we filtered out the oscillations. As mentioned earlier, the time period in which mVO_2 is calculated cannot be standardized. This is due to a leveling off in the rate of change in O_2Hb and HHb, which typically occurs faster at higher work rates (figure 4). Optimally, you want to use as long a period as possible, but if you choose a period which is too long, you will underestimate the steepness of the slope, and thus, mVO_2. All R^2 values used in this study were higher than 0.91, with a mean R^2 value of 0.98 (± 0.02) for the VM and 0.99 (± 0.01) for the VL. This indicates a small error in the calculation of the slope, which again is used to calculate mVO_2.

One interesting result is the significant decrease in tVO_2 that was seen directly after the AO (figure 5). The AO cuts off (in a sense), roughly half of the most active muscle mass from the rest of the body. This means that a smaller amount of muscle needs to be supplied with oxygen and thus a smaller cardiac output (heart rate · stroke volume) is needed. Grassi et al. (1996) found that increased tVO_2 was seen within the first 15 seconds after an increase in workload. This indicates that the body is capable to adjust tVO_2 very rapidly. Cardiac output can be decreased by a decrease in heart rate or a decrease in stroke volume. Thus, if this decrease in tVO_2 was the result of a decreased cardiac output, a decrease in heart rate or stroke volume might be expected. Although no decrease was seen in heart rate, we have no information on stroke volume in the present study. Since blood is trapped the occluded leg, a decrease in venous return (due to decrease blood volume) may decrease the stroke volume

through decreased cardiac filling. Although interesting, the effect of an AO on tVO_2 during continuous exercise needs to be further investigated in order to make any justified conclusions or interpretations regarding why it occurred and weather it affects our results.

Another interesting effect of AO that we saw in this study was a significant increase in cadence, averaged for all subjects, during the AO in all but the last two workloads. An increase in cadence was seen in most of the subjects (11 of the 18 subjects). A possible reason for the increase in cadence may have been a strategy used by the participants in order to cope with the AO. However, since the affects of the occlusion was not a primary purpose of this study, we can not say for certain. With respect to the effect of this increase, the whole body energy cost of cycling has been shown to increase with increasing cadence (Foss and Hallen, 2004; Ettema and Lorås, 2009). Since tVO_2 have been shown to increase as early as in the first 10-15 seconds after an increase in workload (Grassi et al. 1996), this may lead to a slight overestimation of tVO_2 in the present study, thereby affecting mVO_2 as well. The difference decreased and was not significant for the last two workloads. This may contribute to the plateau seen in the mVO_2 because of a decrease in the degree of overestimation. However, since the time used for calculating mVO_2 is very short and the increase in cadence, although significant, is only a few rounds per minute, the influence is probably minimal. The effect of the AO was not the primary purpose of this study, and, to the best of my knowledge, no previous studies have reported on the subject. Therefore we can only conclude that more research is needed to investigate whether the observed increase in cadence is coping strategy related to the AO and if it affects the results of the present study.

4.3 Total vs. local oxygen consumption

The main finding in the present study was a plateau in mVO_2 at high work rates which occurred despite a continued increase seen in the tVO_2 (figures 7 and 8). Little research regarding mVO_2 during high intensity cycling exercise has been published, but Nagasawa (2007) found no significant increase in mVO_2 of the VL immediately after 20 min cycling at 50 and 70 percent of VO_{2max} when looking at mVO_2 directly after exercise. This is consistent with the results from the present study where no difference is seen in mVO_2 of the VL between 200 W and 250 W work rates. The reasons for the observed plateau can be many but one influential factor, and also a methodological consideration, may be due to regional differences within the single muscle.

The relative small area of the NIRS measurement (2-6 cm^3) (Ferrari et al., 1997) makes the results in this, and other studies using NIRS, vulnerable to such intramuscular

regional differences. This is something that must be taken into account when interpreting results from NIRS measurements. Regional differences within the muscle have been shown to exist through the study of muscle oxygenation using NIRS (Kennedy et al., 2006 and Kime et al., 2005). Although muscle oxygenation does not directly reflect mVO_2, it does reflect the balance between oxygen supply and mVO_2 (Koga et al., 2007) and can detect regional differences within a single muscle. Kennedy et al (2006) and Kime et al. (2005) used NIRS to show regional differences in muscle oxygenation during cycling exercise using several NIRS optodes on the VL muscle. At high intensity, Kime et al (2005) found reduced heterogeneity of muscle oxygenation and inter-individual differences. Also Kennedy et al., (2006) found no significant regional differences at 75% and 100% of maximum intensity. The decreasing regional differences in oxygenation within the muscle with increasing work rate can partly explain the plateau in mVO_2 observed in the present study (e.g. if mVO_2 increases more rapidly at an earlier stage in the part of the muscle measured in the present study). However, we measured very similar results in both the VM and the VL muscle. This is in support of that, although heterogeneity within a muscle may exist, it will probably not have a large impact on measurements of mVO_2. This does however, show the need for more than one optode when measuring mVO_2 during exercise. More than one optode is needed both for estimating the potential error in mVO_2 measurements and possibly revealing additional interesting information. Regional differences have also been found in local muscle blood flow. Kime et al. (2005) found increased muscle reoxygenation time, which is thought to reflect decreased blood flow, in distal compared to proximal parts of the VL muscle. Mizuno et al (2003) used PET to show decreased blood flow in the distal region compared to the proximal region of the VL muscle. Mizuno et al. (2003) attributed their results to increased intramuscular pressure, resulting from muscle contractions compressing blood vessels and squeezing blood out of the tissue, in the distal part of the muscle, previously reported by Ameredes and Provenzano (1997). Increase and decrease in intramuscular pressure are thought to result in the observed oscillations in the NIRS signals in the present study.

In addition to underlining the need for the inclusion of several NIRS optodes on the same muscle in future studies, the results of Mizuno et al. (2003) and Kime et al. (2005) reveal increasing intramuscular pressure, due to more forceful contractions, as a response to increased exercise intensity that needs to be taken into account when interpreting the results in the present study. Increased intramuscular pressure has been shown to prevent blood flow in the muscle with contractions as low as 30% of maximal voluntary contraction (Barcroft and Millen, 1939). Hagberg (1981) reported a rapid decrease in endurance time for isometric and

dynamic contraction above 15-20% of MVC, levels which have been associated with cycling at high power outputs and low cadence (Vercruyssen and Brisswalter, 2008). No increase in cadence was seen with increasing work rate in our study, although subjects used a freely chosen cadence. An increase in work rate with no increase in cadence will result in larger force requirements locally in the muscle per cycle, meaning the muscle must produce a force closer to its maximum. This will lead to an increase in intramuscular pressure which may contribute to decreased blood flow in the muscle at high intensity. Takaishi et al. (1996) suggested that improved blood flow and venous return was a result of the high cadence used by cyclists due to decreased force applied to the cranks and shortened contraction time. In agreement with this, Takaishi et al. (2002) found decreased occlusion of blood flow at higher cadences and constant workload for non-cyclists and tri-athletes as compared to lower cadences. We cannot quantify the degree of occluded blood flow due to increased intramuscular pressure in the present study, but since no increase in cadence was seen, it may have had an influence on our results. Foss and Hallen (2004) reported that the most economical cadence increased with increasing work rate. Leirdal and Ettema (2009) reported increasing freely chosen cadence with increasing work rate when cycling on a roller, but not when cycling on a cycle ergometer. The results in the present study are consistent with findings of Leirdal and Ettema (2009) and taken together with the results from Foss and Hallen (2004), it raises the question of whether the same pattern of mVO_2 would be seen if cadence would have increased with increasing work rate. If the increasing intramuscular pressure due to higher forces exerted by the local muscles is a significant factor, you would expect a less clear plateau in mVO_2 or a plateau at a higher work rate if cadence would have increased with increasing work rate. This is due to the possibility of the local muscle to increase its work rate via an increase in contraction rate and not in contraction force, thus maintaining blood flow at higher exercise intensity. The decrease in mVO_2 observed in the VL muscle in the present study may be attributed to decreased blood flow resulting from increased intramuscular pressure.

Changing the work rate in individual muscles may be a strategy to minimize the problem of increasing intramuscular pressure. In regards to this it should be kept in mind that although the results for mVO_2 in the present study may seem to contradict the previously mentioned Fenn effect, this may not be the case. We measured whole body work rate and not local work rate which, though difficult to measure in vivo, would be a more valid measurement to compare with mVO_2. Altered relative contributions of different muscles with

increasing exercise intensity might be an important factor leading to the observed plateau in mVO_2 in the present study.

So, apart from the variations within active muscles, variation between various muscles may be of importance during exercise with increasing intensity. Several methods have previously been used to investigate muscle contributions during exercise. Using EMG, Dorel et al. (2009), found large increases in gluteus maximus and biceps femoris activity, when comparing initial and end values, during an exhaustive cycling exercise, with no similar increase in VL or VM activity. They proposed that the increased EMG was not due to fatigue in the gluteus maximus and biceps femoris muscles, but rather a way of preventing or compensating for fatigue in the quadriceps muscles by a change in pedaling strategy (e.g. by increasing the force in the lower push phase and when pulling the pedal back). Although this study focused on fatigue during constant load exercise, it is not unreasonable to think that a similar change in muscle use can happen due to increased work rate in specific muscles (Dorel et al., 2009). This would be in agreement with results from the present study where a plateau in mVO_2 would indicate an inability to increase the local work rate of that particular muscle further, without an increased contribution of anaerobic processes. Also Ericson et al. (1986) showed lower relative activation in gluteus maximus during submaximal cycling exercise compared to the VM. This would indicate that the VM is relatively more active during lower exercise intensity and relatively less active during high intensity exercise when compared to the gluteus maximus. In agreement with these previous studies, which indicate activity in the VL and VM early and during low intensity exercise, the results from the present study indicate that mVO_2 in the VL and VM, increase faster than the whole body in the early phase (lower intensities). The continued increase in tVO_2 at the higher intensities is likely due to the increased contribution of other muscles such as gluteus and hamstring muscles.

The use of functional MRI and the measurement of $_1H$ transverse relaxation time have been suggested as a useful, complementary tool to EMG in the measurement of specific muscle activity during exercise (Meyer and Prior, 2000). Using functional MRI, Endo et al. (2007) found that VM was the only muscle to show increased muscle activity compared to rest during moderate intensity cycling exercise. At high and very high (above lactate threshold) intensity exercise, vastus intermedius and gracilis and adductor magnus also showed significant increase compared to resting values. At very high work rate also other muscles in the study (gracilis, adductor magnus, sartorius, rectus femoris, semitendonosus and gluteus maximus) showed signs of increased activity. Reid et al. (2001) found that activation in the VL, VM, vastus intermedius, and sartorius was increased when cycling at 50% of

VO_{2max}. When cycling at 90% of VO_{2max}, they found increased activity in rectus femoris, adductor magnus, gracilis and semitendinosus in addition to the VL, VM, and vastus intermedius. This is again supportive of non-uniform recruitment of muscles during different intensities in cycling exercise.

Using kinematic and pedal-force data, Sanderson and Black, (2003) found increasing moments in the hip extensors, but no significant change in knee moments during an exhaustive cycling exercise. The use of joint moments cannot differentiate between individual muscles but this is indicative of an increased use of hip muscles during exhaustive exercise. During double poling exercise, Boisen-Møller et al. (2010) used PET to show increased use of muscles spanning the spine, hip and knee joints with increased exercise intensity. The study by Boisen-Møller et al. (2010) shows similar results as the present study with regards to differences in muscle activity at different exercise intensities and taken together we see that these intensity related differences are not constrained to a specific mode of exercise.

Taken together there is significant evidence of altered muscle use during increasing intensity cycling exercise. This implies that the work rate of a specific muscle does not necessarily increase linearly with whole body work rate. This shows the need to realize that the processes in the local muscle deviate substantially from what is seen for the whole body. Although all of the above mentioned methods have limitations, the use of NIRS might provide a unique noninvasive and direct way of studying local oxygenation patterns in muscle and provide an indication of the contributions of various muscles during cycle exercise. The need for future investigations of several muscles simultaneously is apparent and, probably to a lesser account, the heterogeneity within the muscle must be taken into account as well. Future studies investigating the effect of cadence on mVO_2 might clarify this and might also reveal additional information regarding the high cadences used by competitive cyclists (Lucia et al., 2001).

5. Conclusions

This is the first study to measure mVO_2 during dynamic cycling exercise. The main finding of the present study is a significantly different effect of work rate on mVO_2 and tVO_2. mVO_2 increased faster at low intensity and reached a plateau at higher intensities when compared with tVO_2 which increased linearly with increased work rate. On the basis of altered muscle use during cycling with increasing intensity, the present findings indicate that the VL and the VM reached their maximum potential for oxygen consumption at lower intensities than the whole body during incremental cycling exercise. A further increase in work rate may, to a larger extent, be met by work production in other muscles (e.g. gluteus and hamstring muscles). The increased intramuscular pressure associated with high work rate may also contribute to the observed plateau due to restricted blood flow. Several previous studies using several methods show results in support of this conclusion and the use of NIRS can provide a new, more direct way to study what is happening in the local muscle and muscle contribution during exercise through mVO_2. The need for future studies investigating several muscles in addition to the VL and VM is apparent. Investigating the effect of cadence and other cycling related variables may also help to gain a better understanding of both mVO_2 and muscle use in cycling. This study also underlines the need for knowledge of more than the whole body response when moving from research to practice.

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