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# **Oxygen Consumption in Cycling:**

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The Relationship between Whole Body  
Pulmonary O<sub>2</sub> Consumption and Muscle  
Oxygenation in Different Muscles During  
Constant-Load Cycling

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## Abstract

**Introduction:** Oxygen consumption during prolonged cycling exercise has been extensively studied at different work rates and durations, but with the focus primarily on pulmonary oxygen consumption ( $pVO_2$ ). The purpose of this study was to use near-infrared spectroscopy (NIRS) to investigate the relationship between  $pVO_2$  and local oxygenation responses in six active leg muscles during prolonged constant-load cycling at different intensities. **Methods:** 26 recreational male cyclists performed a constant-load high-intensity cycling test at 75% maximal aerobic power (MAP) for 30 min duration or until exhaustion. Of the 26 subjects, 14 performed a constant-load low-intensity cycling test for the same duration as well, at 50% of the work rate found to elicit blood lactate levels of  $4 \text{ mmol}\cdot\text{l}^{-1}$  during incremental exercise. Pulmonary gas exchange ( $pVO_2$ , RER,  $V_E$ ), heart rate, and NIRS measurements of the muscles vastus lateralis (VL), vastus medialis (VM), biceps femoris (BF), gluteus maximus (GMax), gastrocnemius lateralis (GL), and tibialis anterior (TA) were obtained continuously through both tests, while blood lactate and RPE was measured at specific time intervals. **Results:** Local oxygenation measurement for all the muscles collectively behaved in a similar manner as  $pVO_2$  at both intensities with an increase in  $O_2$  utilization only found in the initial phase, and additionally showed a surprisingly homogenous response. However, differences were found between the muscle groups with heterogeneity in regard to the amount of desaturation at low- and high-intensity. **Discussion:** Although the local responses were similar to each other and that of  $pVO_2$ , differences were found between the muscles with heterogeneity in regard to the amount of saturation. The distal muscles TA and GL showed less difference in saturation between low-intensity and high-intensity than the more proximal muscles (VL, VM, BF, and GMax). Also the BF and GMax muscles were found to behave different with a lack of TSI steady-state during high-intensity. **Conclusion:** The use of NIRS might provide a noninvasive and direct way of measuring local oxygenation responses in muscles and provide an indication of the work contribution of various muscles during cycling exercise. Although local oxygenation responses across the muscles were in agreement with  $pVO_2$ , difference in amount of saturation was found between muscle groups in the present study. Also peripheral differences were found between the subjects able to complete 30-min constant-load high-intensity cycling and those who did not.

**Key words:** Near-infrared spectroscopy, NIRS, cycling, constant-load,  $VO_2$ , local oxygenation, tissue saturation,  $SmO_2$ , muscle, muscle groups.

## Preface

The data collection for this master thesis was part of a more comprehensive research project at the department of Human Movement Science NTNU, on the ongoing debate concerning efficiency of the whole body as opposed to the active skeletal muscle. A group of subjects ( $n = 40$ ) were to be followed through a specific training period of 4 months with pre- and post-testing of physiological measures both at a whole-body and local scale to investigate the possible effect of an assigned training program. Additional physiological and performance measurements will be included in the main research project, such as cycling efficiency, inverse dynamics, cadence, and  $m\dot{V}O_2$ . When the data for the present study was collected, during the pre-testing period, the cyclists were in off-season with little or none sport specific training during the last months.

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## List of Abbreviations

|         |   |                    |   |
|---------|---|--------------------|---|
| AO      | arterial occlusion  | [La <sup>-</sup> ] | blood lactate concentration                                       |
| ATT     | adipose tissue thickness  | LT                 | lactate threshold   |
| CL-LI   | constant-load low-intensity   |                    |   |
| CL-HI   | constant-load high-intensity  | MAP                | maximal aerobic power   |
| EMG     | electromyography  | MLSS               | maximal lactate steady-state                                      |
| FCC     | freely chosen cadence   | NIRS               | near-infrared spectroscopy  |
| Group 1 | group of subjects who cycled both the low- and the high-intensity constant-load cycling tests | OBLA               | onset of blood lactate accumulation                               |
| Group 2 | group of subjects who only cycled the high-intensity constant-load cycling test               | O <sub>2</sub> Hb  | oxyhemoglobin   |
| G30     | group of subjects who completed 30 minutes of constant-load high-intensity cycling            | pV̇O <sub>2</sub>  | pulmonary oxygen consumption                                      |
| G<30    | group of subjects who completed less than 30 minutes of constant-load high-intensity cycling  | RER                | respiratory exchange ratio  |
|         |   | RPE                | rating of perceived exertion                                      |
|         |   | SmO <sub>2</sub>   | muscle oxygen saturation  |
|         |   | tHb                | total hemoglobin  |
|         |   | TSI                | tissue saturation index   |
|         |   | TTF                | time-to-task-failure  |
|         |   | V <sub>E</sub>     | ventilation   |
| HHb     | deoxyhemoglobin   | WR                 | work rate   |
| HR      | heart rate  | WR <sub>OBLA</sub> | work rate to elicit blood lactate level of 4 mmol·l <sup>-1</sup> |

## 1. Introduction

Human skeletal muscle is highly dependent upon oxidative metabolism, and the oxygen consumption ( $\text{VO}_2$ ) may increase up to 50 fold during exercise (Hamaoka et al., 2007). The measurement of whole body  $\text{VO}_2$  by means of measuring pulmonary gas exchange has become an established method for studying energy expenditure in the field of both sports science and medicine, and has a long history in literature. During constant-load cycling,  $\text{pVO}_2$  is repeatedly found to achieve a steady-state condition within the first minutes of exercise, where the time to steady-state and rate of increase is dependent on the intensity (Turner et al., 2006). Although we have required extensive knowledge of pulmonary  $\text{O}_2$  consumption ( $\text{pVO}_2$ ) to various activities and intensities, less is known about the oxygen responses on a more local level. The known heterogeneity of muscle recruitment during cycling, both in terms to various intensities (Bini and Diefenthaler, 2010, Ericson, 1988) as well as with duration of constant-load cycling (Dorel et al., 2009, Sanderson and Black, 2003), coupled with the frequently reported  $\text{pVO}_2$  steady-state at sub-maximal intensities is an interesting topic.

The manner of how  $\text{pVO}_2$  develops during the early transition phase of constant-load cycling, and whether it reaches steady-state or not, is highly dependent on exercise intensity (Turner et al., 2006). Following the onset of exercise, or a work rate (WR) increment,  $\text{pVO}_2$  cannot increase immediately to the steady-state value. Consequently, the active muscles are forced to work with oxygen deficit during this transition period, and the energy demand has to be met partially from other sources (e.g.  $\text{O}_2$  stores in the body, stores of muscle phosphocreatine (PCr), and minor amounts converted from lactate production) (Xu and Rhodes, 1999). When the oxygen supply is insufficient, the generation of ATP has to rely on anaerobic glycolysis. This results in the formation of lactate, which seems to play a central role in the  $\text{pVO}_2$  response. At higher power outputs, typically above lactate threshold,  $\text{pVO}_2$  either reaches a delayed steady-state above that predicted from lower power outputs, or continues to increase until the end of exercise (Xu and Rhodes, 1999). This latter effect has been called the slow component of  $\text{VO}_2$  kinetics by Whipp and Wasserman (1972), and numerous studies have demonstrated it to be closely linked with blood lactate ( $[\text{La}^-]$ ) levels (Hagberg et al., 1978, Turner et al., 2006, Whipp and Wasserman, 1972, Xu and Rhodes, 1999).

When exercise is performed at work rates below the lactate threshold (LT) (i.e. onset of increased anaerobic glycolysis (Beaver et al., 1985), and thus a measurable increase of

blood lactate levels), steady-state  $pV_{O_2}$  levels are reached within 3 minutes (Pringle et al., 2003, Stringer et al., 1994, Turner et al., 2006). During heavier exercise, with WR above LT, the rate of lactate production initially exceeds the rate of clearance. However, as long as the WR is below the maximal lactate steady-state (MLSS) (i.e. the highest work rate that can be maintained over time without a continual accumulation of blood lactate (Billat et al., 2003)), the lactate level can once again be stabilized at a new but elevated level. This enables a steady-state  $pV_{O_2}$  during heavy exercise as well, but at a greater  $pV_{O_2}$  level than that predicated from the relationship between WR and  $pV_{O_2}$  during moderate exercise (Pringle et al., 2003, Turner et al., 2006, Xu and Rhodes, 1999). When WR is above MLSS,  $pV_{O_2}$  can no longer be stabilized, and continues to increase until the point of fatigue (Pringle et al., 2003, Turner et al., 2006, Xu and Rhodes, 1999). The  $pV_{O_2}$  responses to exercise intensity can then roughly be divided into three domains, which all can be related to the LT; (1) moderate exercise where  $WR < LT$ , (2) heavy exercise, i.e.  $LT < WR < MLSS$ , and (3) severe exercise (i.e.  $MLSS < WR$ ) (Xu and Rhodes, 1999).

Although numerous studies have demonstrated a high correlation between the magnitude of the  $pV_{O_2}$  slow component and blood lactate levels (Stringer et al., 1994, Turner et al., 2006), there is, however, no proof to indicate the cause and effect relationship between the two variables. Despite that  $VO_2$  kinetics have been extensively studied since the first report of the exponential nature of gas exchange responses during constant-load exercise almost a century ago, the question regarding the mechanism(s) controlling the rate of the  $pV_{O_2}$  responses remains unanswered (Xu and Rhodes, 1999, Zoladz and Korzeniewski, 2001). A number of possible factors have been proposed to play a role, such as increased muscle temperature (MacDougall et al., 1974, Koga et al., 1997), lactate and  $H^+$  accumulation (Stringer et al., 1994), and activation of additional muscle groups and recruitment of less efficient type II fibres (Krustrup et al., 2004, Pringle et al., 2003). In addition, it has been suggested that the increase in pulmonary oxygen uptake could simply reflect an increase in the cost of ventilation, cardiac output and posture relative to the external power delivered. However, Poole et al. (1991) demonstrated that 86% of the additional increase in  $pV_{O_2}$  of the slow component, seen between 3 and 21 minutes of constant-load high-intensity exercise, was attributable to the exercising legs. Hence, a lot of information that could help explain this phenomenon is likely located locally within the active muscles.

The response of  $pV_{O_2}$  during incremental exercise is believed to have a linear relationship with work rate up to near maximal aerobic power production (MAP) (Ettema et

al., 2009, Ettema and Lorås, 2009, Zoladz and Korzeniewski, 2001). Unpublished data from a recent study at the department of Human Movement Science NTNU found during an incremental cycling protocol that while  $pVO_2$  showed a linear relationship with WR, as expected, muscle oxygen consumption ( $mVO_2$ ) of the vastus lateralis and vastus medialis muscles did not. Both muscles showed an exponential rise of  $mVO_2$  as a function of WR with a fast initial increase at lower intensities followed by a plateau at the higher intensities. A nonlinear increase in  $mVO_2$  of the main muscles involved in cycling suggests increased involvement of additional muscles during higher intensity. This is supported by findings of other studies using MRI (Reid et al., 2001, Endo et al., 2004), EMG (Dorel et al., 2009, Ericson, 1988), PET (Bojsen-Moller et al., 2010), and joint moments (Bini and Diefenthaler, 2010) as well. Similar findings have also been reported during prolonged cycling as part of the fatiguing progress (Dorel et al., 2009, Sanderson and Black, 2003), suggesting that the same coping strategy exists during constant-load cycling. If the recruitment of additional muscle groups is, at least, partly responsible for the linear relationship during incremental exercise between  $pVO_2$  and WR at higher intensities, this could also be a major contributing factor to the  $pVO_2$  slow component at higher constant-load intensities in representing a coping strategy as fatigue occurs, to prevent fatigue from occurring in the main power producer muscles, or simply to add to the power production already delivered.

Even though local measurements related to local muscle activity (e.g. MRI, EMG, PET, and joint moments) have been available and applied in several studies, direct measurement of muscle oxygen consumption is complicated. Regional measurements of oxygen consumption is possible based on the Fick equation (i.e.  $VO_2 = \text{blood flow} \cdot (a-vO_2\text{diff})$ ). However, this method has been used less frequently due to its invasive nature (blood samples), and the fact that application during exercise is cumbersome. Near-infrared spectroscopy (NIRS) is a measuring method that allows us a non-invasive, continuous, and direct measurement of local oxygenation changes, and with the use of an arterial occlusion also can provide us with a quantitative value for local  $mVO_2$ . Specifically, in the field of sports science the advantage of providing a more local measurement together with the easy application of the method during exercise can be a major advantage to study muscular response to different activities and physiological stress.

Near-infrared spectroscopy (NIRS) is a non-invasive optical method that utilizes the capability of light in the near-infrared (NIR) region to penetrate skin and reach deeper structure such as muscle tissue. The NIR light, in the region of 650-to 950-nm (depending on



the equipment used) wavelength, is absorbed by chromophores such as hemoglobin, water, and lipids (Wolf et al., 2007). As the absorption of hemoglobin (Hb) differs between oxyhemoglobin (O<sub>2</sub>Hb) and deoxyhemoglobin (HHb), the use of two different but specifically chosen wavelengths enables us to differentiate between changes in optical density of O<sub>2</sub>Hb and HHb. With the application of a modified Lambert-Beer law, it is then possible to calculate the concentration changes in O<sub>2</sub>Hb, HHb, and total hemoglobin (tHb) (i.e. the sum of O<sub>2</sub>Hb and HHb) (Ferrari et al., 1997). By using two or three additional channels at small measuring distance from each other, it is also possible to get a continuous and quantitative measure of tissue saturation (Gerovasili et al., 2010). The use of a simple maneuver, arterial occlusion (AO), also enables the calculation of a quantitative value for mVO<sub>2</sub> (Boushel et al., 2001, Gerovasili et al., 2010). Inflating a pneumatic cuff around a limb above systolic pressure is thought to achieve a temporary blockade of blood flow (inflow and outflow) within the limb. With no blood flow, the linear decrease in O<sub>2</sub>Hb or Hb<sub>diff</sub> (i.e. the difference between O<sub>2</sub>Hb and HHb) can be used to calculate a quantitative measure of mVO<sub>2</sub> (in μM/min). This technique has been evaluated *in vivo* with good reproducibility (van Beekvelt et al., 2001b).

Although NIRS is not yet widely used in exercise physiology studies, the measurement method has been used to study the relationship between local oxygenation and the slow component of pVO<sub>2</sub>. Belardinelli et al. (1995b) found a high correlation between the magnitude of the slow component of pVO<sub>2</sub> and the amount of decrease in O<sub>2</sub>Hb saturation in the vastus lateralis muscle during several constant-load work rates. In addition, Grassi et al. (1999) found a significant correlation between the onset of lactate accumulation and onset of muscle deoxygenation in the vastus lateralis muscle during incremental cycling. To our knowledge, local oxygenation responses have yet to be investigated for several muscles simultaneously during prolonged constant-load cycling. While only one study has applied NIRS in investigating multiple muscles and muscle groups during incremental cycling (Takagi et al., 2013), studies applying NIRS during constant-load cycling (Grassi et al., 1999, Belardinelli et al., 1995b) have only investigated parts of the quadriceps muscle. In addition, the mentioned studies have merely presented saturation measurements without the raw signals, which may exclude valuable information about e.g. blood volume changes.

The purpose of this study was, therefore, to investigate the relationship between whole body pulmonary O<sub>2</sub> consumption and muscle oxygenation during prolonged constant-load cycling at low intensity (below LT) and at high intensity (well above LT). In addition, to study the possible effects of altered muscle recruitment during prolonged cycling exercise,

NIRS was used to simultaneously measure local oxygenation responses in six active leg muscles.

## 2. Methods

### 2.1 Subjects

26 male recreational cyclists participated in the study. All subjects were recruited through several cycling clubs in Norway, and had both cycling experience and competing in various recreational races as goal for the next bicycle season. The mean age (SD, range) was 38.6 years ( $\pm 7.0$ , 23 – 48), height 182.5 cm ( $\pm 5.6$ , 173.2 – 195.1), and weight at onset of the first day of testing 83.1 kg ( $\pm 6.0$ , 73.7 – 103.2). We were given permission to conduct the study by the local ethics committee and all subjects signed an informed written consent before participating in the study.

### 2.2 Study Design

All subjects performed two incremental tests and a constant-load cycling test at high-intensity. Of the 26 subjects, 14 did a second constant-load cycling test at low-intensity as well. Testing occurred on 2 or 3 separate days in an air-conditioned lab with room temperature of 20-22°C, with no more than seven days between the first and last day of testing. Information about height and age were measured on the first day of testing, while weight was measured at each testing day. All subjects were told to avoid hard ( $> 87\%$  maximum HR) and long exercise ( $>2\text{h}$ ) the last day before testing.

Day 1 consisted of two ramp protocols (Fig. 1) to determine the lactate threshold (LT) and maximal aerobic power (MAP), which was used to calculate the intensities for day 2 and 3. Blood lactate measurements ( $[\text{La}^-]$ ) were used to determine the LT during the first incremental test. Since our CL-LI test was to be of a WR well below LT, the individual's true LT was of less interest, and a WR eliciting a blood lactate level of  $4 \text{ mmol}\cdot\text{l}^{-1}$  (OBLA) was taken as  $\text{WR}_{\text{OBLA}}$  and used in the intensity calculation. Day 2 consisted of 30 min constant-load low-intensity cycling (CL-LI) at  $50\% \text{WR}_{\text{OBLA}}$ , while day 3 consisted of 30 min constant-load high-intensity cycling (CL-HI) at  $75\% \text{MAP}$ .

### 2.3 Experimental Protocol

A schematic representation of the protocols for the tests on day 1 and 2/3, including the various methods and the specific time points, is shown in respectively Fig. 1 and 2.

### 2.3.1 Incremental Cycling Tests (Day 1)

Day 1 consisted of two incremental tests with intermittent measurements of  $[La^-]$  and rating of perceived exertion (RPE), and continuous measurement of pulmonary oxygen uptake ( $pVO_2$ ) and heart rate (HR).

The first test started with a lactate measurement at rest, followed by 4 min unloaded cycling. After which, the WR was set at 100 W and increased by 25 W/4min until a lactate value above OBLA ( $4 \text{ mmol}\cdot\text{l}^{-1}$ ) was reached.  $[La^-]$  and RPE measurements were collected immediately after each increment. In between the tests, the subjects were allowed a maximum of 5 min cycling at freely chosen WR and a drink of water. The 2<sup>nd</sup> incremental test started at a WR 50 W lower than  $WR_{OBLA}$ , and increments of 25 W/min was given until the subject reached voluntarily exhaustion. MAP was taken as the highest work rate sustained for a full minute. If the subject sustained an additional work rate for 30 seconds, half the increment (i.e. 12.5 W) was added to the MAP.

RPE was collected together with a  $[La^-]$  measurement at the end of exercise to verify that the criteria for achieved maximum  $pVO_2$  ( $pVO_{2max}$ ) had been met. Both tests were performed at freely chosen cadence (FCC).

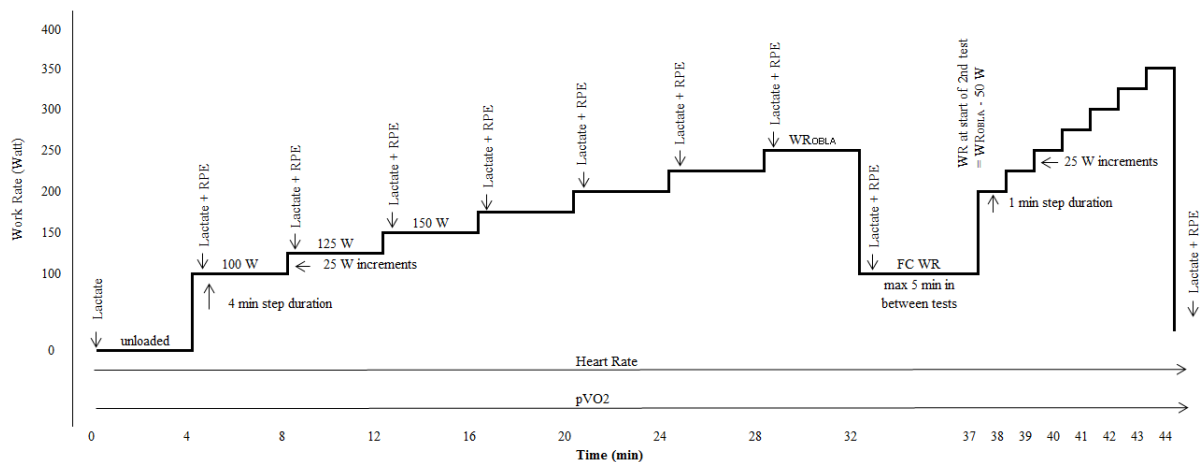


Figure 1: Schematic representation of both incremental tests. FC = freely chosen, RPE = rating of perceived exertion, WR = work rate,  $WR_{OBLA}$  = work rate that elicited blood lactate level of  $4 \text{ mmol}\cdot\text{l}^{-1}$ ,  $pVO_2$  = pulmonary oxygen uptake. Heart rate and  $pVO_2$  were measured continuously throughout the test. The test started with a lactate measurement, followed by 4 min unloaded pedaling. Next work rate (WR) was set at 100 W, and followed by 25 W/4min increments until a blood lactate level of  $>4 \text{ mmol}\cdot\text{l}^{-1}$  (OBLA) was reached. Lactate measurements were taken immediately after each increment. After  $<5$ min active rest, the WR was set at  $WR_{OBLA} - 50 \text{ W}$ , and increased by 25 W/min until voluntarily exhaustion.

### 2.3.2 Constant-Load Low-Intensity Cycling Test (Day 2)

Day 2 consisted of a 30-min constant-load cycling test at a WR of 50%  $WR_{OBLA}$  at FCC (Fig. 2). Because of the low intensity of the test itself, no warm-up was considered necessary.

Concentration changes of muscle oxygenation were collected continuously with the NIRS apparatus. In order to calculate quantitative measurements of  $mVO_2$  (in all the muscles except GMax) in analysis, the subjects were exposed to 10 arterial occlusions (AO) during exercise. The AOs were applied for 20 sec periods, at the end of every 1 min during the first 5 min, followed by every 5 min until the end of the test. Coupled with these occlusions, a blood sample was collected immediately after each AO, in addition to a baseline measurement prior to the test, and analyzed for  $[La^-]$  levels. Also, RPE was collected every 5 min throughout.

Gas exchange and HR measurements were measured continuously throughout the test, except from 1 min breaks every 5 min from the 6<sup>th</sup> minute where the subjects were allowed a drink of water.

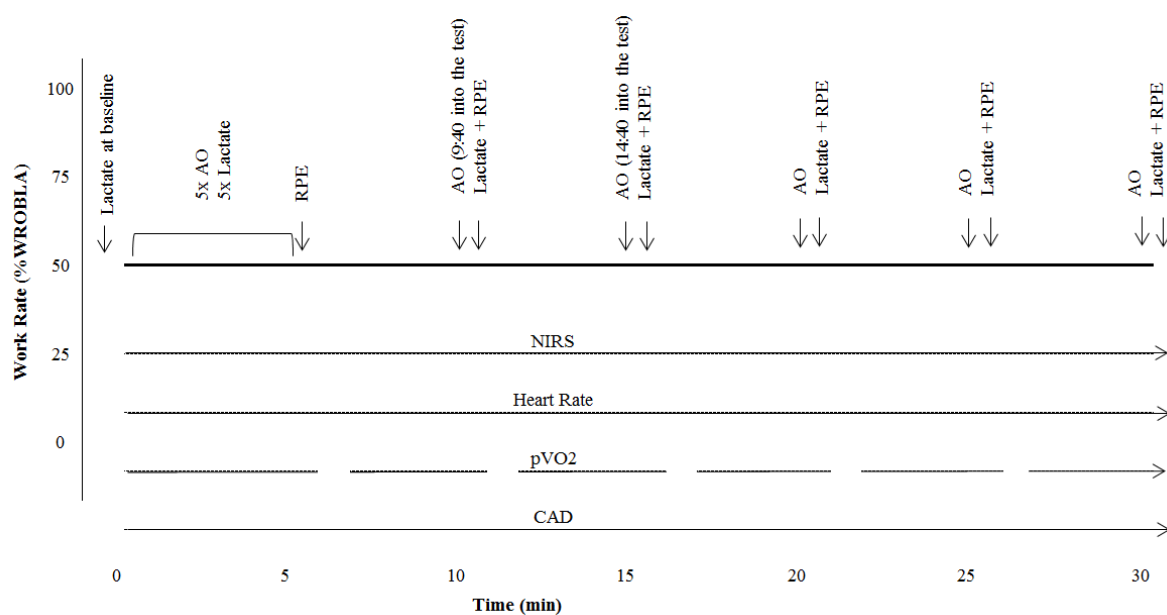


Figure 2: Schematic representation of the constant-load low-intensity (CL-LI) test. AO = arterial occlusion, RPE = ratings of perceived exertion,  $pVO_2$  = pulmonary oxygen uptake, NIRS = near-infrared spectroscopy, CAD = cadence,  $WR_{OBLA}$  = work rate eliciting blood lactate levels of  $4 \text{ mmol} \cdot \text{l}^{-1}$ . NIRS, HR,  $pVO_2$ , and CAD were measured continuously throughout the test. The test started with a lactate measurement at rest, and WR was then set at 50%  $WR_{OBLA}$  and remained constant through the entire test. An AO was applied every min during the first 5 min, with  $AO_1$  40 sec after start of test. Further 5 AOs was applied every 5 minutes thereafter, each AO lasting 20 sec. A lactate measurement was coupled with each AO, and taken immediately after deflation of the cuff. RPE was taken every 5 min throughout the test.

### 2.3.3 Constant-Load High-Intensity Cycling Test (Day 3)

Day 3 consisted of a 30-min constant-load cycling test at a WR of 75% MAP at FCC with identical protocol as Day 2 (Fig. 2) apart from the work rate and a 5-min warm-up period. The subjects performed 5-min warm-up cycling at 50%  $WR_{OBLA}$  (intensity from Day 2) where the WR was gradually increased during the last 20 seconds to enable a correct WR at the start of the high-intensity test. A lactate measurement was taken at the end of warm-up in addition to the measurements at baseline and during the test.

If the subjects were not able to complete a full 30-min cycling at 75% MAP before voluntarily exhaustion, the time-to-task-failure (TTF) was registered.

## 2.4 Measurements

All participants cycled on a cycle ergometer with a computer-controlled electro-magnetic brake mechanism (Velotron, Racermate inc, Washington, USA), which generates a constant power condition, independent of cadence. The seat and handlebar position were adjusted according to the subject's individual preference at the onset of each testing day. All subjects wore their own cycling shoes, and had to remain seated during cycling. All tests were done at FCC, with pedal rate measured continuously by the ergometer's recording system (sampling frequency of 33.3 Hz).

Gas exchange values were measured continuously by open-circuit indirect calorimetry using an Oxycon Pro apparatus (Jaeger GmbH, Hoechberg, Germany), with a sampling frequency of 0.1 Hz. At the beginning of each experiment day, and more often when needed, the  $VO_2$  and  $VCO_2$  gas analyzers were calibrated using high-precision gases (15.00%  $O_2$  and 5.85%  $CO_2$ , Riessner-Gase GmbH & Co, Liechtenfels, Germany). The flow meter was calibrated with a 3 L volume syringe (Hans Rudolph Inc., Kansas City, MO). Heart rate (HR) was measured continuously with a sampling rate of 0.2 Hz, using a heart rate monitor (Polar RS800, Polar Electro OY, Kempele, Finland).

Lactate measurements were measured using blood samples taken from the tip of the middle and/or ring finger of the left hand at baseline and during exercise. Using 20  $\mu$ l capillaries, blood was collected and analyzed immediately (Biosen Lactate, EKF Industrial Electronics, Magdeburg, Germany). The lactate measurement device was calibrated every 60 min using a 12  $mmol \cdot l^{-1}$  standard.

The RPE was measured with a revised Borg scale (see appendix I) during cycling together with lactate measurements, except during the first 5 minutes of constant-load cycling. The subjects were asked to rate their whole body stress during the last WR period on a scale from 6 (= resting on a bed) to 20 – “maximal” (Borg, 1982).

#### **2.4.1 Near-Infrared Spectroscopy (NIRS)**

To measure the local changes in muscle oxygenation and tissue saturation (TSI), continuous NIRS measurements were done on the muscles vastus lateralis (VL), vastus medialis (VM), gluteus maximus (GMax), gastrocnemius lateralis (GL), tibialis anterior (TA), and biceps femoris (BF) of the right leg. The specific muscles were chosen for their known involvement in cycling and previous appearances in literature. A continuous-wave near-infrared spectrophotometer (Oxymon MKIII, Artinis Medical Systems, the Netherlands) with a sampling frequency of 50 Hz, wavelengths of 766 and 856 nanometer, and a source-detector distance of 31, 35, and 39 mm was used on BF and VL. In addition, four wireless NIRS devices (Portamon, Artinis Medical Systems, the Netherlands) (sampling frequency of 10 Hz, wavelengths of 761 and 845 nm, source detector distance 30,35, and 40 mm) were used on the remaining muscles. Changes in oxygenation were measured continuously during all tests.

It is not possible to differentiate between hemoglobin (Hb) and myoglobin (Mb), as their absorption spectra overlap (Boushel et al., 2001, Ferrari et al., 2011, Hamaoka et al., 2007, van Beekvelt et al., 2001a). However, as the focus of this study is on oxygen consumption, and not whether it came from Hb or MB, this is less relevant and the sum of the two will be presented as Hb in this paper.

All of the NIRS optodes were positioned on the bulk of the muscle parallel to the muscle fibre length, according to the SENIAM guidelines for EMG sensor placement. Some exceptions were made for BF and VL. The pneumatic cuff was placed as proximal as possible on the right leg, but for some of the shorter subjects the cuff would be placed too far down for an ideal placement on BF, and in some cases even VL. In these cases, the optodes were placed perpendicular to the muscle length and as close to the bulk as possible. The subjects were shaved at the site of optode placements, and the placements were marked with a pen to control that the optodes had not moved during the testing, as well as to ensure the same optode placement in those subjects who cycled both constant-load tests.

Skinfold thickness was measured on all the subjects by skinfold caliper measurements (Holtain Tanner/Whitehouse skinfold caliper, Holtain Ltd, Crymch, Wales) at the sites of

NIRS optode placement, and compared in later analysis. The average of two caliper measurements divided by 2 was taken as the adipose tissue thickness (ATT). Thigh and calf circumference was also measured at height of the NIRS optodes.

In order to be able to calculate muscle oxygen consumption ( $mVO_2$ ), arterial occlusions were applied at regular time intervals (at minute 1-2-3-4-5-10-15-20-25-30) during both constant-load protocols, using a pneumatic cuff that was rapidly inflated/deflated with an automatic inflation system (Hokanson E20 Rapid Cuff inflator + Hokanson AG-101 Air Source, Marcom Medical ApS, Denmark) set to a pressure of 300 mmHg. The initial goal was to include the quantitative measurements of  $mVO_2$  as well in this thesis, but due to the limited time span,  $mVO_2$  will not be presented in this paper, and the focus will rather be on the muscle oxygenation.

## 2.5 Data Analysis

Data analysis, filtering, and statistics were carried out in Matlab (7.8.0.347, The MathWorks Inc.), Microsoft Excel 2010, and SPSS (20.0, IBM Corp) for Windows.

### 2.5.1 Maximum / Peak Values

Peak heart rate ( $HR_{peak}$ ),  $pVO_{2max}$ , and MAP were collected from the second incremental test on day 1.  $HR_{peak}$  and  $pVO_{2max}$  were calculated as the average of the last 30 seconds of the MAP test, where  $pVO_{2max}$  was reached when 4 out of 5 criteria for  $pVO_{2max}$  were met ( $HR_{peak} > 90\% [220 - \text{age (years)}]$ ,  $RPE > 18$ ,  $[La^-] > 8 \text{ mmol}\cdot\text{l}^{-1}$ ,  $RER > 1.10$ , plateau in  $pVO_2$  (i.e. no further increase in  $pVO_2$  despite increase in WR)). MAP was calculated as the highest completed WR over 60 seconds. If an additional WR was sustained for full 30 seconds,  $\frac{25}{2}$  W was added to the MAP value.

### 2.5.2 Mean Values

For analysis purposes, mean values of HR, pulmonary gas exchange, and NIRS cycling were calculated from the continuous measurements from the constant-load cycling tests to represent specific time points. For the first 5 minutes, averages for pulmonary and HR measurements were taken from the last 30 seconds in each minute while NIRS concentration changes were taken from the last 10 seconds just prior to each occlusion. For the remainder of the test, 60 seconds averages were calculated for pulmonary and NIRS from the last minute prior to occlusion. HR average was still calculated from 30 second periods, and from one minute earlier than  $pVO_2$  and NIRS to avoid any effect of the anticipation of an occlusion on the



heart rate data (subjects were alerted to the upcoming AO 30-40 seconds prior to the inflation).

In order to remove the artifacts resulting from the rhythmic contractions and relaxation of the muscles during cycling, all NIRS data were filtered using an eighth-order low-pass Butterworth filter (10 Hz, zero-lag). To normalize the concentration changes, all baseline concentrations measured for 30 seconds prior to the tests were subtracted from all the NIRS measurements (except TSI). Hence, baseline was set to zero and the values for the remainder of time points during constant-load cycling are relative changes to the baseline concentration.

## 2.6 Statistical Analysis

All statistical analyses were computed using IBM SPSS Statistics 20.0. A one-way ANOVA was used to assess possible differences in baseline measurements between the participants that completed both CL-LI and CL-HI (group 1) and those who completed only CL-HI (group 2), as well as between those who completed 30-min CL-HI (G30) and those who did not (G<30). The same analysis was used to assess possible differences in ATT between the sites of the muscles followed by post-hoc analysis (Dunnett's T3 for unequal variances) if a significant difference was found. When the assumption of normality was violated, the non-parametric Kruskal-Wallis test was used. To investigate whether the blood lactate level at the end of CL-HI was of similar level between G30 and G<30, a one-way ANOVA was also used on the  $\Delta[\text{La}^-]$  calculated from the end high-intensity lactate value and the maximum achieved lactate value from day 1.

A two-way ANOVA for repeated measures was used for group 1 to examine the effects of intensity (CL-LI vs. CL-HI) and time on the various variables  $p\text{VO}_2$ ,  $V_E$ , RER, blood lactate, HR, and the concentration changes of  $\text{O}_2\text{Hb}$ , HHb, tHb, and TSI for the 6 investigated muscles. The significance level was set at 0.05, and if the assumption of sphericity was violated, significance was adjusted using the Greenhouse-Geisser method. When significant differences were found, within-subject contrasts were used to assess differences between time points. Due to the fact that not all of the subjects were able to sustain more than 15 minutes of the CL-HI test, only the first 15 minutes of the constant-load tests were investigated in this analysis to include as many subjects as possible.

A three-way ANOVA was then carried out in the same way for the same variables to test whether the 0 – 15 min response found during CL-LI and CL-HI in group 1 was the same between G30 and G<30. When no significant difference between the two groups was found,

G30 was used in an additional two-way repeated-measures analysis including time points for the 20<sup>th</sup>, 25<sup>th</sup>, and 30<sup>th</sup> minute. This was done to investigate whether we could find any different response between the intensities in the second half of the test (15 – 30 min).

A final two-way ANOVA was used to test whether the response during CL-HI was similar in group 1 and 2. This way, it would be possible to increase the group size for CL-HI, and especially for analysis of the second half of the test (15 – 30 min).

### 3. Results

In total, 26 subjects ( $n = 26$ ) performed the CL-HI test, of which 14 subjects ( $n = 14$ ) performed CL-LI as well. All participants fulfilled 4 out of 5 criteria set for achieved  $pVO_{2max}$  during the MAP test on day 1. The subject characteristics are presented in table 1. There was no significant difference when comparing the subjects of group 1 and group 2 (tested variables: age, weight, height,  $pVO_{2max}$ ,  $HR_{peak}$ , maximum lactate value, MAP,  $WR_{OBLA}$ , thigh and calf circumference, and skinfold thickness).

All subjects in group 1 completed 30-min cycling at low-intensity ( $n = 14$ ), while only a total of 10 ( $n = 10$ ) from a pool of 26 subjects completed the full 30-min cycling at high-intensity. The number of subjects in each finishing category as well as the average finishing time is presented in table 2. Factors thought to affect endurance performance are presented for G30 and G<30 in table 3. No significant difference in the variables  $pVO_{2max}$ , maximum achieved lactate, MAP,  $WR_{OBLA}$ ,  $WR_{OBLA}$  in percentage of MAP, ATT, or circumference was found when comparing G30 and G<30 (neither for the entire pool of subjects nor for group 1 and 2 in separated analysis).

Significant difference in ATT was found between the muscle sites ( $p < 0.001$ ), where post-hoc analysis revealed the ATT of TA and GMax being significantly lower and higher (respectively) than all the remaining muscles ( $p = 0.000 - 0.001$ ), except from the difference between TA and VL ( $p = 0.065$ ).

Table 1: Subject characteristics

|   | LI (GR 1) | HI (GR 1)                   | HI (GR 2)                   |
|---|-----------|-----------------------------|-----------------------------|
| n   |           | 14                          | 12                          |
| Age (years)   |           | 40.0 (5.7, 32 – 48)         | 37.1 (8.0, 23 – 47)         |
| Weight (kg)   |           | 82.9 (7.6, 73.7 – 103.2)    | 83.2 (3.1, 78.1 – 89.2)     |
| Height (cm)   |           | 182.3 (4.6, 173.2 – 189.5)  | 182.8 (6.5, 174.3 – 195.1)  |
| pVO <sub>2max</sub> (ml·kg <sup>-1</sup> ·min <sup>-1</sup> ) |           | 52.6 (6.3, 35.9 – 60.3)     | 55.6 (4.9, 44.6 – 63.2)     |
| HR <sub>peak</sub> (bpm)                                      |           | 185.1 (10.0, 165 – 203)     | 184.3 (10.0, 172 – 202)     |
| Maximum Lactate Value (mmol·l <sup>-1</sup> )                 |           | 12.57 (2.32, 8.1 – 16.8)    | 12.61 (2.38, 8.7 – 15.8)    |
| MAP (W)   |           | 364.3 (30.9, 300.0 – 412.5) | 378.1 (19.2, 337.5 – 412.5) |
| WR <sub>OBLA</sub> (W)  |           | 230.5 (23.6, 196 – 270)     | 239.1 (31.8, 176 – 282)     |
| WR (W)  |           | 115.4 (11.9, 100 – 135)     | 283.3 (13.9, 255 – 310)     |
|   |           |                             |                             |
| ATT (Right VL) (mm)   |           | 5.1 (2.3, 3.3 – 11.5)       | 5.1 (1.6, 2.8 – 8.6)        |
| ATT (Right VM) (mm)   |           | 6.0 (2.0, 3.7 – 9.5)        | 5.9 (1.5, 3.0 – 8.6)        |
| ATT (Right BF) (mm)   |           | 3.4 (1.1, 2.0 – 6.3)        | 3.9 (1.7, 1.9 – 8.0)        |
| ATT (Right GMax) (mm)   |           | 9.7 (2.7, 4.8 – 15.3)       | 10.9 (3.5, 4.9 – 19.5)      |
| ATT (Right GL) (mm)   |           | 5.3 (1.7, 2.9 – 9.6)        | 6.0 (1.7, 3.9 – 9.0)        |
| ATT (Right TA) (mm)   |           | 3.8 (1.1, 2.3 – 5.5)        | 3.9 (1.0, 2.5 – 6.5)        |
| CF (Right thigh) (cm)   |           | 57.1 (2.4, 53.5 – 60.3)     | 57.1 (2.9, 54.0 – 64.0)     |
| CF (Right calf) (cm)  |           | 39.7 (5.3, 33.5 – 57.0)     | 38.6 (1.3, 35.7 – 41.0)     |

*Mean (SD, range) for subject characteristics. pVO<sub>2max</sub> = maximum whole body oxygen uptake, HR = heart rate, MAP = maximal aerobic power, WR<sub>OBLA</sub> = work rate eliciting blood lactate level of >4mmol/l obtained on day 1, WR=work rate, ATT = adipose tissue thickness, VL = vastus lateralis, VM = vastus medialis, BF = biceps femoris, GMax = gluteus maximus, GL = gastrocnemius lateralis, TA = tibialis anterior, CF = circumference*

Table 2: Finishing time

|           | 15min  | 20min  | 25min  | 30min  | TTF (min)  |
|-----------|--------|--------|--------|--------|------------|
| GR1 LI:   | n = 14 | n = 14 | n = 14 | n = 14 | 30.0 (0.0) |
| GR1 HI:   | n = 14 | n = 9  | n = 6  | n = 6  | 22.9 (6.5) |
| GR2 HI:   | n = 12 | n = 10 | n = 6  | n = 4  | 24.2 (4.9) |
| Total LI: | n = 14 | n = 14 | n = 14 | n = 14 | 30.0 (0.0) |
| Total HI: | n = 26 | n = 19 | n = 12 | n = 10 | 23.5 (5.8) |

*Number of subjects within each finishing group and mean (SD) TTF (=time-to-task-failure) for each subject group. GR1 = subjects who performed both low- and high-intensity tests, GR2 = subjects who performed only high-intensity test, LI = low intensity, HI = high intensity, TTF = time-to-task-failure.*

Table 3: Factors thought to affect performance

|   | <30-min      |              | 30-min (n = 10) |              |
|---|--------------|--------------|-----------------|--------------|
|   | Mean (SD)    | Min – Max    | Mean (SD)       | Min – Max    |
| pVO <sub>2max</sub> (ml·kg <sup>-1</sup> ·min <sup>-1</sup> ) | 53.4 (6.4)   | 35.9 – 60.3  | 55.0 (4.8)      | 44.9 – 63.2  |
| Max La (mmol·l <sup>-1</sup> )                                | 13.03 (2.29) | 8.7 – 16.8   | 11.79 (2.22)    | 8.1 – 15.8   |
| MAP (W)   | 372.9 (24.7) | 338 – 413    | 365.0 (32.7)    | 300 – 400    |
| WR <sub>OBLA</sub> (W)  | 231.4 (25.8) | 186 – 277    | 238.4 (33.3)    | 176 – 282    |
| WR <sub>OBLA</sub> (%MAP)                                     | 62.1 (5.4)   | 49.6 – 68.4  | 65.3 (7.0)      | 52.2 – 72.8  |
| ΔLa (mmol·l <sup>-1</sup> )                                   | 0.25 (2.15)  | -4.05 – 3.76 | 1.41 (2.58)     | -2.98 – 4.09 |

*Factors thought to affect performance within the two groups that did and did not complete 30-min of high-intensity cycling. ΔLa = difference in blood lactate level between end MAP and end high-intensity test. For abbreviations, see table 1.*

### 3.1 Whole Body (Systemic) Response

Individual pVO<sub>2</sub> and [La<sup>-</sup>] responses during CL-HI for both group 1 and 2 are presented in Fig. 4. After an initial increase in pVO<sub>2</sub>, no further increase after the 6<sup>th</sup> minute was seen in any of our subjects (n = 26), suggesting that the intensity of 75% MAP was below the MLSS threshold. This is, however, in contrast with the increase of blood lactate levels in some of the subjects. High levels and continuous increase of [La<sup>-</sup>] were present in several of the subjects unable to complete 30-min cycling, with the highest increase of lactate from the 10<sup>th</sup> minute to finish being 4.21 mmol·l<sup>-1</sup>. Nevertheless, the Δ[La<sup>-</sup>] (i.e. Δ[La<sup>-</sup>] = [La<sup>-</sup>]<sub>end MAP</sub> – [La<sup>-</sup>]<sub>end CL-HI</sub>)

was not significantly different ( $p = 0.229$ ) between G30 and G<30, nor was the  $p\text{VO}_2$  response different between the two groups, as can be seen in Fig. 4A. No significant difference was found in the response of neither blood lactate ( $p = 0.454$ ) nor  $p\text{VO}_2$  ( $p = 0.226$ ) between group 1 and 2.

Fig. 5 shows the average responses for the systemic variables (whole body measurements) measured in group 1 during both intensities. High-intensity cycling had a significant different effect on  $p\text{VO}_2$  ( $F(1.80, 19.82) = 29.17, p < 0.001$ ), HR ( $F(1.36, 17.61) = 33.32, p < 0.001$ ), RER ( $F(2.24, 24.69) = 44.11, p < 0.001$ ),  $[\text{La}^-]$  ( $F(1.21, 10.87) = 65.28, p < 0.001$ ), and RPE ( $F(2, 26) = 15.85, p < 0.001$ ) compared to low-intensity, with both higher overall values and a more pronounced increase during the initial phase of exercise. This indicates that the protocol we used produced results in accordance with what can be expected of exercise of two substantially different intensities.

The difference in effect of the two intensities on  $p\text{VO}_2$  response is mainly found in the first 10 minutes of exercise, where contrasts analysis showed significant differences between all time periods ( $p = 0.000 - 0.014$ ). At both CL-LI and CL-HI,  $p\text{VO}_2$  shows a steep increase from the 1<sup>st</sup> to 2<sup>nd</sup> min. However, while  $p\text{VO}_2$  at CL-LI achieves steady-state already at the 2<sup>nd</sup> minute,  $p\text{VO}_2$  at CL-HI continues to rise with a gentler curve until the 6<sup>th</sup> minute where steady-state is achieved (but not represented in Fig. 5 until the 10<sup>th</sup> minute). No further differences were found between min 10 and min 15. In addition, investigating G30 ( $n = 6$ ) revealed no difference in  $p\text{VO}_2$  response between the 15<sup>th</sup> min and the 30<sup>th</sup> ( $p = 0.385 - 0.730$ ), suggesting that the steady-state acquired during the course of the first 15 minutes was maintained throughout the last 15 minutes of the test as well.

Differences in the responses of RER can also be found in the same initial phase as for  $p\text{VO}_2$ , with neither a difference in response between CL-LI and CL-HI from the 10<sup>th</sup> to 15<sup>th</sup> minute ( $p = 0.378$ ), nor after the 15<sup>th</sup> minute in the sub analysis of the subjects in G30 ( $p = 0.500 - 0.836$ ), when the RER value was at steady-state level. However, while both  $p\text{VO}_2$  and RER reached steady-state at both intensities by the 10<sup>th</sup> minute,  $V_E$ , HR, and  $[\text{La}^-]$  continued to increase at CL-HI also after the 10<sup>th</sup> minute.

HR reaches steady-state early during CL-LI, while it continues to increase during CL-HI, producing a significant different response from the 1<sup>st</sup> to 20<sup>th</sup> minute ( $p = 0.000 - 0.015$ ), and close to significant between the 20<sup>th</sup> - 25<sup>th</sup> min ( $p = 0.064$ ). The response during CL-HI can be divided into two parts; (1) the initial response and (2) post 5 minutes of exercise, with

the difference being the steepness of the curve. The second response is most likely related to the known effect of prolonged exercise on HR measurements, known as cardiac drift.

Sub-analysis for 15 – 30 min was not possible for  $V_E$  and  $[La^-]$ , as the initial response (0 – 15 min) was significant different between G30 and G<30. For  $[La^-]$ , this difference displayed after the 5<sup>th</sup> minute, with a much steeper curve between 5 – 10 min and 10 – 15 min in the latter group (see Fig. 5D).  $V_E$  on the other hand, showed a more clearly different curve between the two groups, with the 6 subjects in G30 having a more concave curve with a steep increase in the first 3 min followed by a more gentle increase, while the response in G<30 was more linear during the full 15 min (see Fig. 5E). In other words, ventilation seems to match the lactate responses with an inability of reaching a plateau in the <30 min group.

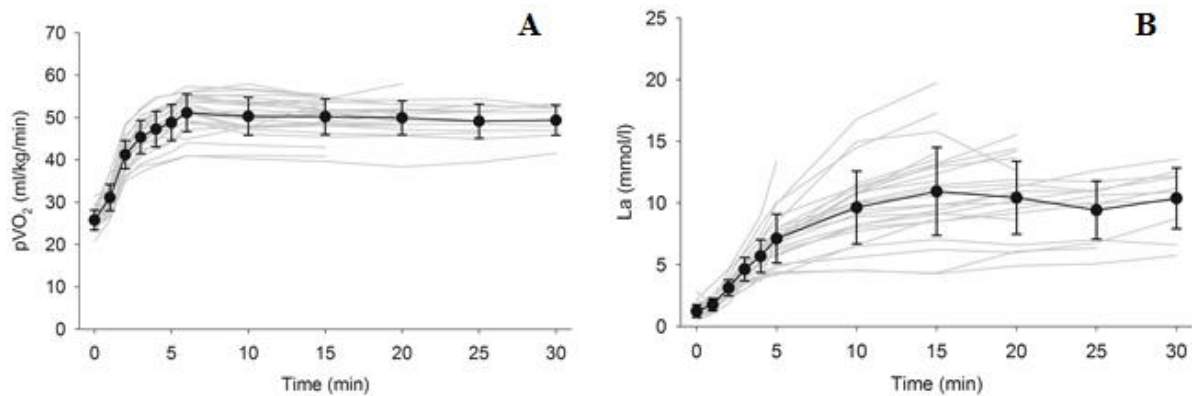


Figure 4: Group mean ( $\pm$  SD) and individual responses for (A) pulmonary oxygen uptake and (B) blood lactate levels for both groups ( $n = 26$ ) at constant-load high-intensity cycling. Lines represent individual responses (grey) and the average of the entire group (black).  $pVO_2$  = pulmonary oxygen uptake,  $La$  = blood lactate.

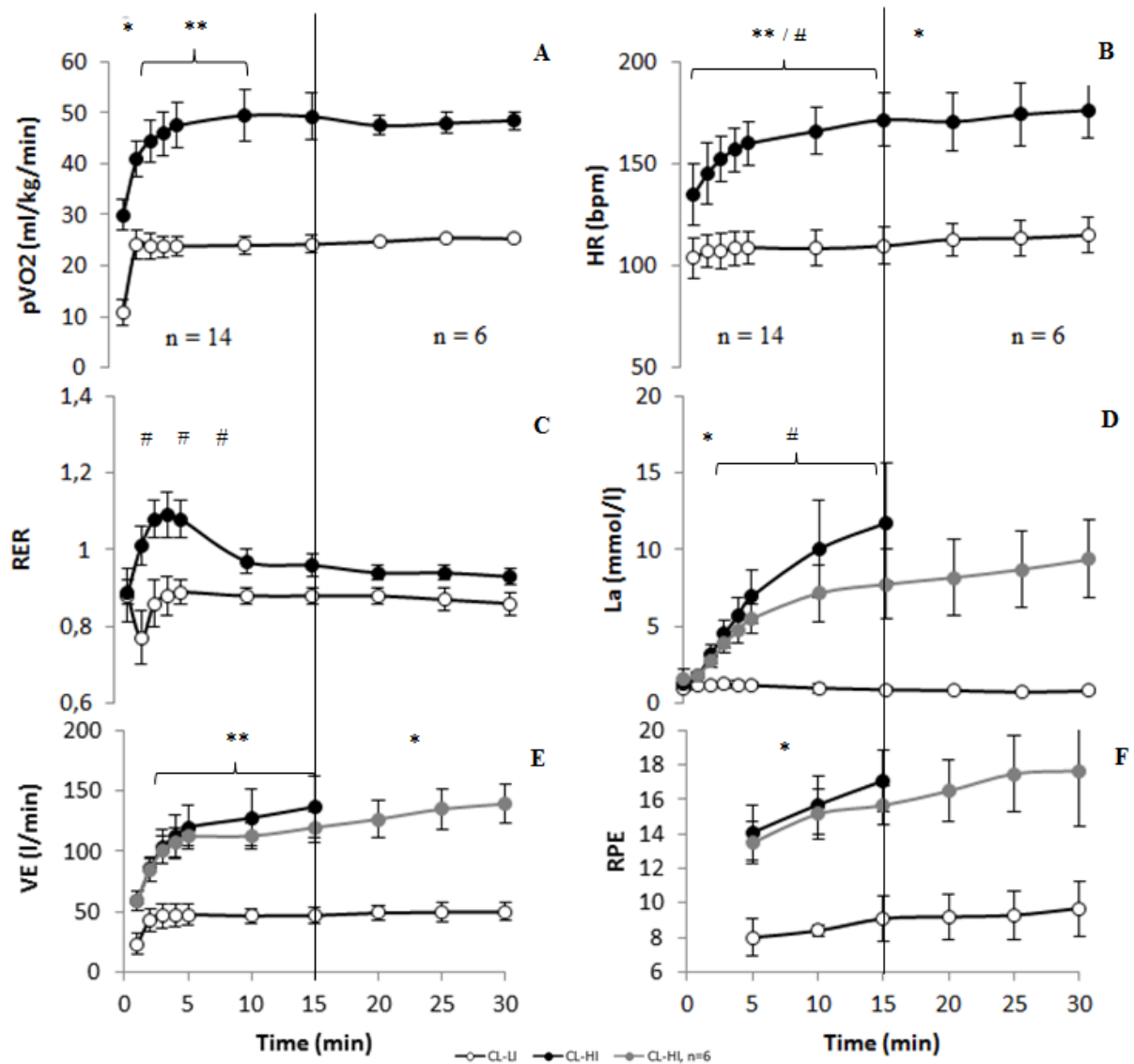


Figure 5: Mean ( $\pm$  SD) systemic responses for group 1 (i.e. subjects that cycled both low- and high-intensity) at low-(open) and high-intensity (filled). (A)  $pVO_2$  = pulmonary oxygen uptake, (B) HR = heart rate, (C) RER = respiratory exchange ratio, (D) La = blood lactate, (E)  $V_E$  = ventilation, (F) RPE = rating of perceived exertion, CL-LI = constant-load low-intensity, CL-HI = constant-load high-intensity. 14 subjects ( $n = 14$ ) were included to the left of the central vertical black line, while only the 6 subjects who completed 30-min CL-HI are represented to the right ( $n = 6$ ). For La (D),  $V_E$  (E), and RPE (F) the subjects who completed 30-min CL- HI are represented by a separate line (grey, filled) as they showed significant different response during 1 – 15 min than those who completed <30min. Asterisks and hash indicate the significant different response between CL-LI and CL-HI (\* =  $p < 0.05$ , \*\* =  $p < 0.01$ , # =  $p < 0.001$ ).

### 3.2 Local Muscle Oxygenation Response

The unfiltered continuous NIRS signal of VL for one subject during CL-LI and CL-HI is presented in Fig. 6. During high-intensity, HHb and  $O_2Hb$  deviate clearly in the initial phase after the warm-up and remains respectively above and below baseline throughout the test. The



spikes visible for the HHb/O<sub>2</sub>Hb signals are a result of the occlusions and can be applied in the calculation of mVO<sub>2</sub>. The mean concentration changes of all the measured muscles for group 1 are presented in Fig. 7. No difference in the response between the 8 subjects in G<30 and those 6 in G30 was found during the first 15 minutes of cycling in VL, VM, GMax, GL, and TA. However, a significant difference was found in the response of O<sub>2</sub>Hb and tHb in BF with an earlier and steeper reoxygenation in the latter group. Consequently, the average for these signals is separated between the two groups in the figure (Fig. 7).

During CL-LI, similar response of O<sub>2</sub>Hb and tHb can be found in all the muscles; a sharp decrease after the onset of exercise, followed by a steady increase throughout the exercise. This increase in blood volume (represented by the increase of tHb) to the exercising muscles enables muscle oxygenation levels during exercise to reach or exceed the baseline levels. Furthermore, the rate of O<sub>2</sub>Hb increase follows more or less that of tHb in all of the muscles, suggesting a steady-state condition in the balance between O<sub>2</sub> delivery and O<sub>2</sub> consumption. In other words, the demand is met by the supply. HHb, on the other hand, show some more heterogeneity between the muscles. In the VL, VM, and GL HHb increases after onset of exercise, but then decrease and stabilize (but still above baseline level) as tHb increases. For the BF, GMax, and TA however, the HHb decreases, and even at later stabilized levels remains under baseline level.

During CL-HI there is a much clearer deviation of HHb and O<sub>2</sub>Hb during exercise in all of the muscles. After the work rate is increased from the warm-up, HHb and O<sub>2</sub>Hb respectively increase and decrease rapidly during the first 2 minutes. By then, tHb is increasing in all of the muscles, and eventually leads to tHb concentration above baseline level (except from BF). The further initial development of O<sub>2</sub>Hb in the muscles after the 2<sup>nd</sup> minute of exercise differs between the proximal and distal muscles, where TA and GL seemed to reach their minimum sooner. However, despite any difference in the initial response, all of the muscles show an increase in O<sub>2</sub>Hb from the 5<sup>th</sup> – 10<sup>th</sup> minute as tHb continues to increase, followed by a stabilized level from the 10<sup>th</sup> – 15<sup>th</sup> minute. The increase in blood volume is, however, not enough to enable baseline oxygenation levels during exercise. No significant differences was found in the concentration responses during CL-HI between group 1 and 2 ( $p = 0.129 - 0.925$ ) indicating that the described responses for high-intensity were the same for 26 subjects ( $n = 26$ ).

Cycling during CL-HI had a significant different effect than CL-LI on the O<sub>2</sub>Hb, HHb, and tHb response in all of the muscles except from O<sub>2</sub>Hb in TA ( $p = 0.121$ ). Looking at within-subject contrasts for VL (the main power producer during cycling), the O<sub>2</sub>Hb response was not significant different between the intensities from baseline to the 1<sup>st</sup> minute ( $p = 0.548$ ), where there were a steep decrease at both intensities, and between the 10<sup>th</sup> and 15<sup>th</sup> minute of exercise ( $p = 0.291$ ), where stabilized concentration level was achieved at both intensities. Otherwise, there were significant differences between all time points ( $p = 0.000 - 0.006$ ), where O<sub>2</sub>Hb started increasing after the 1<sup>st</sup> minute of CL-LI and continued to decrease during CL-HI. The only significant difference for tHb response in VL was between baseline and the 1<sup>st</sup> minute ( $p < 0.05$ ) and 1<sup>st</sup> – 2<sup>nd</sup> minute ( $p < 0.01$ ), suggesting that an attempt to increase blood volume to the exercising muscle was present in similar degree at both intensities. However, for those 6 subjects in sub-analysis, tHb response to the intensities was significant different in VL from the 15<sup>th</sup> – 20<sup>th</sup> minute ( $p < 0.05$ ) and 20<sup>th</sup> – 25<sup>th</sup> min ( $p < 0.05$ ), with no further increase of blood volume during CL-HI while it continued to increase during CL-LI. As a result of this, significant difference was also found for O<sub>2</sub>Hb between the 15<sup>th</sup> – 20<sup>th</sup> min ( $p < 0.01$ ), and the difference between the 25<sup>th</sup> – 30<sup>th</sup> min being close to significant ( $p = 0.054$ ).

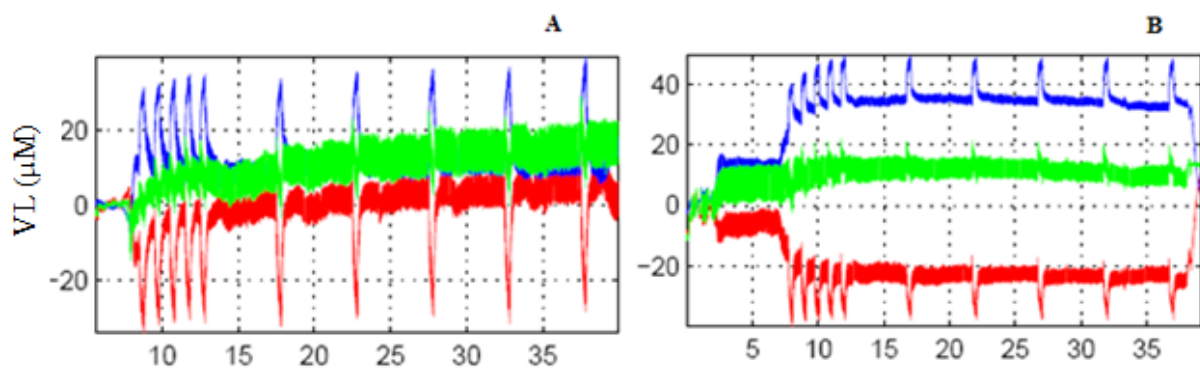


Figure 6: Unfiltered continuous NIRS signal of vastus lateralis (VL) during 30-min of constant-load low- (A) and high-intensity (B) cycling. Lines indicate concentration of O<sub>2</sub>Hb (red line), HHb (blue), and tHb (green). The spikes in the O<sub>2</sub>Hb and HHb signal is a result of arterial occlusions, which can be used to calculate oxygen consumption of the muscle ( $mVO_2$ ). However,  $mVO_2$  is not presented in the present study.

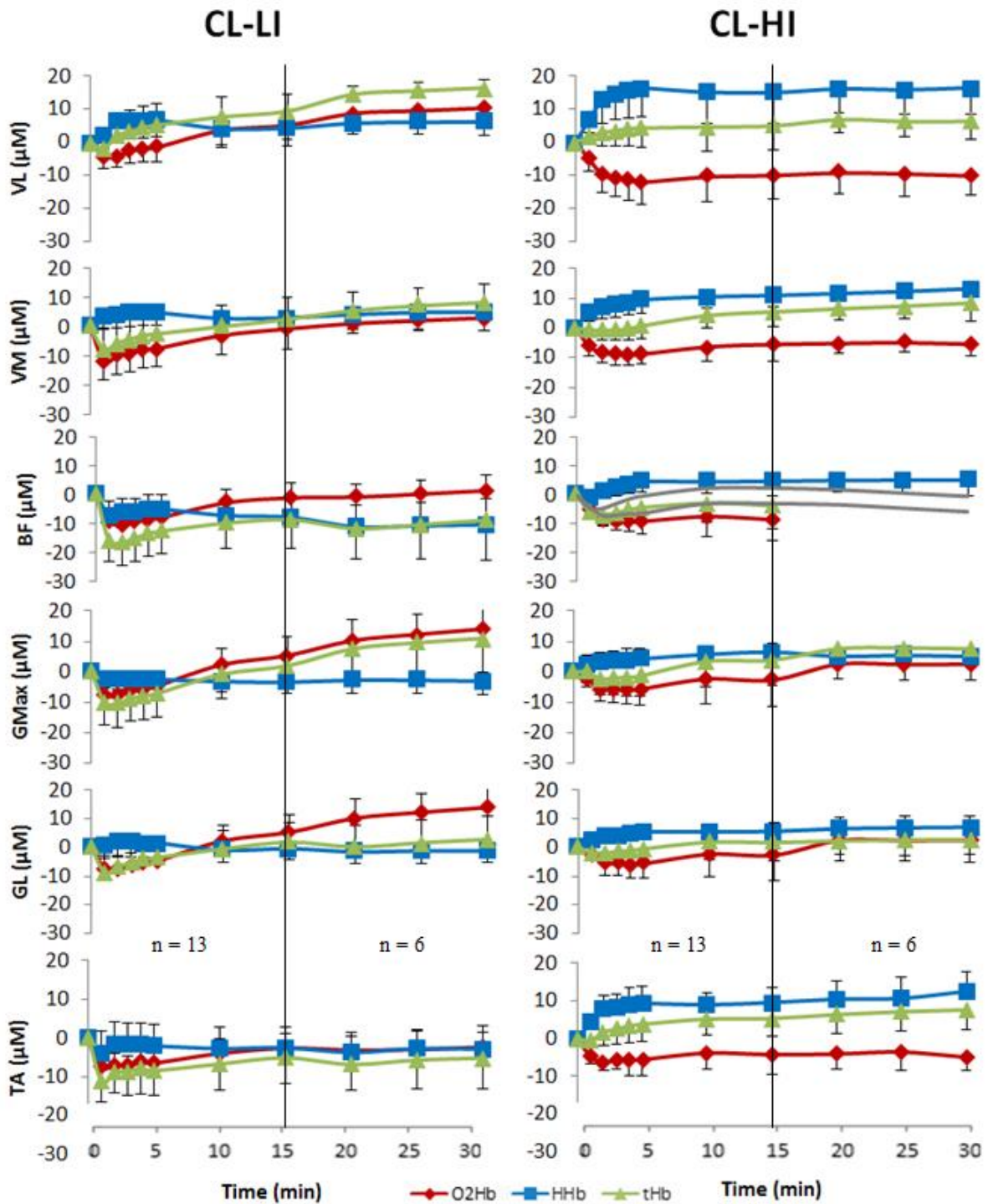


Figure 7: Muscle oxygenation responses in  $O_2Hb$  (red line),  $HHb$  (blue), and  $tHb$  (green) for group 1 (i.e. subjects that cycled both low- and high-intensity) during low- (left) (CL-LI) and high-intensity (right) (CL-HI) constant-load cycling. VL = vastus lateralis, VM = vastus medialis, BF = biceps femoris, GMax = gluteus maximus, GL = gastrocnemius lateralis, TA = tibialis anterior. 13 subjects ( $n = 13$ ) are included to the left of the black vertical lines, while only the 6 subjects who completed 30-min high-intensity are represented to the right ( $n = 6$ ). For BF at constant-load high-intensity,  $O_2Hb$  and  $tHb$  of the subjects who completed 30 min are represented by separate lines (grey) as they showed significant different response during 1 – 15 min than those who completed <30 min.

### 3.3 Tissue Saturation Index (TSI)

Tissue saturation index (TSI) of all the 6 muscles during CL-LI and CL-HI is presented in Fig. 8. The subjects in both G30 and G<30 showed a similar 0 – 15 min TSI response in all 6 muscles ( $p = 0.154 – 0.790$ ). Hence, the former group is used for presentation of TSI response after the 15<sup>th</sup> minute.

During CL-LI an initial steep desaturation between baseline and 1<sup>st</sup> minute of exercise was present in all of the muscles except the BF and TA, where TSI remained unchanged at baseline level throughout the exercise. Also the GMax and GL eventually recovered to baseline saturation levels as blood volume increased, and a steady-state was visible at both muscles from the 10<sup>th</sup> minute. The knee extensors VL and VM showed a resaturation as well, with a steady-state present from the 10<sup>th</sup> minute, but none were able to recover baseline values (although VM is close with a  $\Delta$ TSI from baseline to the 30<sup>th</sup> min of only  $\sim -3\%$ ). VL distinct itself further with being the only muscle with an additional desaturation from the 1<sup>st</sup> – 2<sup>nd</sup> minute.

CL-HI had a statistical significant different effect than CL-LI on TSI responses in all of the muscles ( $p = 0.000 – 0.002$ ). In general, none of the muscles are able to recover from the initial response during high intensity while all the desaturated muscles showed an increase after the initial decrease during CL-LI. Furthermore, the initial desaturation was also present for BF and TA. Although not able to recuperate to baseline levels, most of the muscles still acquire a steady-state also at CL-HI within 5 minutes of exercise. The exceptions were BF and GMax, where both showed a continuous (but gentle) decrease to the 15<sup>th</sup> minute. Even though VL, VM, GL, and TA all seem to acquire a TSI steady-state, the distal muscles GL and TA acquire this earlier (within 2 min) than VL and VM. The TSI response of VL during CL-HI is the one that resembles the  $p\text{VO}_2$  response the most, with an initial steep decrease during the first two minutes, followed by a gentler curve until the 5<sup>th</sup> minute, where steady-state is achieved. No differences were found in 1 – 15 min TSI response in any of the muscles between group 1 and 2 ( $p = 0.263 – 0.805$ ).

No difference in 0 – 15 min TSI response was found between G30 and G<30 ( $p = 0.263 – 0.805$ ). Within-subject contrasts on the subjects in G30 ( $n = 6$ ) revealed no significant differences in TSI response between low-intensity and high-intensity ( $p = 0.056 – 0.995$ ) after the 15<sup>th</sup> minute, except from in VM between 25<sup>th</sup> – 30<sup>th</sup> min ( $p < 0.05$ ), suggesting that the steady-state acquired in the muscles during the course of the first 15 minutes was maintained

throughout the last 15 minutes of the test as well. It may also suggest that the steady-state in BF and GMax was most likely eventually acquired in these subjects, but just at a later stage of constant-load exercise.

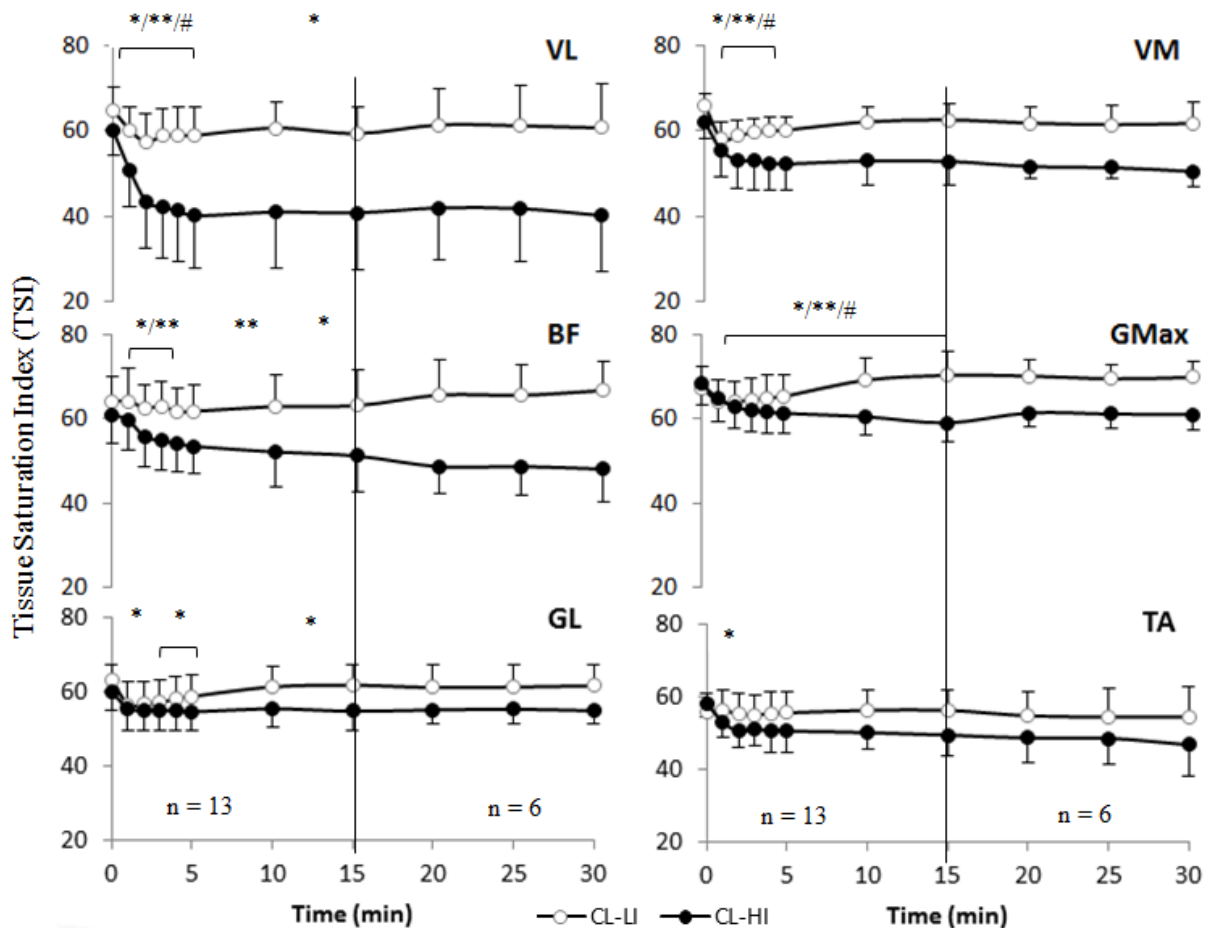


Figure 8: Mean (+/- SD) tissue saturation (TSI) responses for group 1 (i.e. subjects that cycled both low- and high-intensity) during low- (open) and high-intensity (filled) constant-load cycling. For abbreviations, see previous figure. 13 subjects ( $n = 13$ ) are included to the left of the central vertical black line, while only the 6 subjects who completed 30-min high-intensity are represented to the right ( $n = 6$ ). Asterisks and hash indicate the significant different response between constant-load low- and high-intensity ( $* = p < 0.05$ ,  $** = p < 0.01$ ,  $\# = p < 0.001$ ).

### 3.4 Difference between Finish Groups

The TSI response of the first 15 min CL-HI between G30 ( $n = 10$ ) and G<30 ( $n = 14$ ), including subjects from both group 1 and 2 ( $n = 24$ ), is presented in Fig. 9. Overall, the TSI response in all the muscles seems more or less identical, with the only difference being the baseline level and amount of desaturation in some of the muscles. This suggests that there were no differences in the balance of peripheral  $O_2$  delivery and  $O_2$  consumption between the finishing groups, even though some were unable to complete more than 15 minutes while

others were only halfway through the test. However, an exception can be spotted in GMax, where those close to voluntarily exhaustion show a close to significant difference from the 5<sup>th</sup> to the 10<sup>th</sup> minute ( $p = 0.064$ ) and a significant difference from 10<sup>th</sup> – 15<sup>th</sup> min ( $p < 0.001$ ) with a decrease in saturation. During the same period, the only difference in any of the other muscles can be found in BF from the 10<sup>th</sup> – 15<sup>th</sup> minute ( $p < 0.05$ ). Although the response was not found significant different, the amount of desaturation in VL and VM is also less in G<30 with the same TSI baseline at both groups.

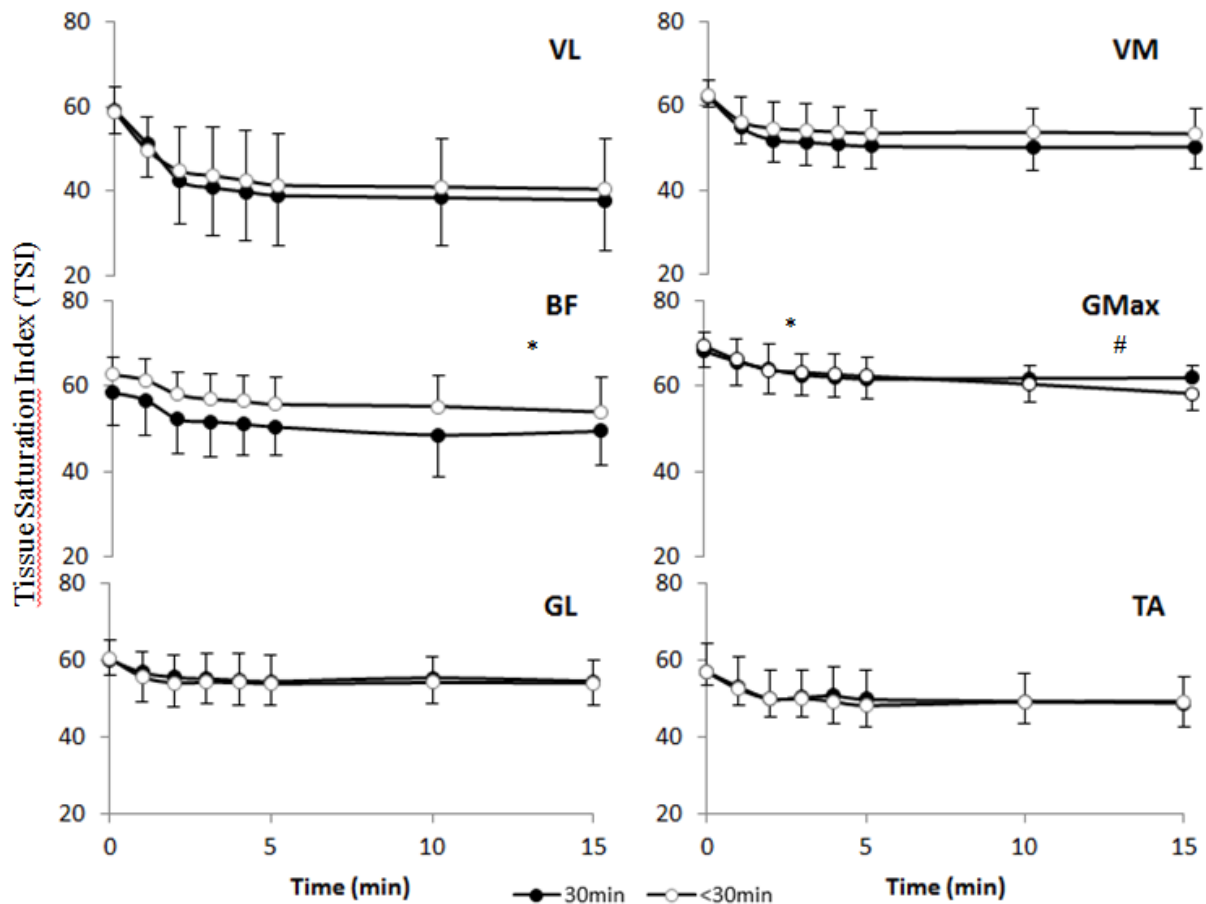


Figure 9: Mean (+/- SD) tissue saturation (TSI) responses during constant-load high-intensity cycling (1 – 15min) of the subjects who completed 30-min cycling (filled) and those who did not (open). For abbreviations, see previous figure. 24 subjects ( $n = 24$ ) from both group 1 and 2 are included, with a total of 10 ( $n = 10$ ) subjects in the 30-min group. Asterisks and hash indicate the significant different response between the two subgroups ( $* = p < 0.05$ ,  $\# = p < 0.001$ ).

## 4. Discussion

The main finding of the present study was the similar oxygenation pattern that was found for all 6 muscles in response to high-intensity and low-intensity, and achieving a steady-state condition during high-intensity within the same time period as  $pVO_2$ . Although the local responses were similar, differences were found between the muscles with heterogeneity in regard to the amount of deoxygenation and desaturation. Less difference in saturation was found between the intensities in the distal muscles (TA and GL) compared to the more proximal muscles (VL, VM, BF, and GMax), as well as a lack of TSI steady-state during high-intensity for the BF and GMax muscles. Investigating the TSI response of GMax during CL-HI further, we found a significant difference between the subjects in G30 and those in G<30. While the group of subjects in G30 maintained the desaturated level achieved by the 5<sup>th</sup> minute, G<30 showed a continuous desaturation throughout.

To our knowledge, no other studies have investigated multiple muscles during constant-load cycling. Takagi et al. (2013) is the only study that has applied NIRS in investigating multiple muscles and muscle groups simultaneously during cycling. However, they did so during incremental cycling exercise, and not during constant-load cycling. There have been other studies that have had more similar protocols to ours, but they have typically measured only one or two muscles. In addition, several of the more recent studies applying NIRS during cycling exercise have presented only TSI (in many studies referred to as  $SmO_2$ ) results and not the raw signal, e.g. the mentioned study of Takagi et al. (2013). TSI is a valuable variable to present in an effort to understand human skeletal muscle physiology as it reflects the balance between two important mechanisms in the vessels; the oxygen supply and oxygen consumption. Furthermore, it provides a more quantified measurement of saturation, and thus is easier to interpret and compare across studies. However, when leaving out the raw signals one loses important information about e.g. blood volume changes, which can be of great assistance in interpreting the TSI results. Also, in our study we found greater between-subjects variability in TSI than we did for the raw signals.

No data is available on the validity of the saturation percent, and how large the variability is within a group of subjects. However, a substantial variability is present in both Takagi et al. (2013) and our study, illustrating the need for further studies to address this issue. One way to possibly decrease this variability *in vivo* is by normalizing the TSI to each individual's functional 0 – 100% saturation by the use of a cuff ischemia response. This involves inflating a pneumatic cuff proximal of the probes to suprasystolic pressure until a

plateau in desaturation is reached. A hyperemic response will occur with the deflation of the cuff, and the minimum and maximum achieved saturation can be used to establish a quasi-quantitative scale through the range of 0 – 100% functional saturation. The desaturation measured during dynamic exercise can then be calculated to a percent of this 100% desaturation. However, the validity of both the measured and normalized TSI scale remains uncertain, and the inclusion of raw data is encouraged either way. With concern to the comparison of response as a product of time between the intensities, the lack of such a normalized scale does not affect the analysis or results of this study. Also, independent of the uncertainty of the validity of the quantitative percentage of TSI, all muscles showed lower saturation levels during high-intensity than low-intensity as expected in our study.

Another possibility to obtain a quantitative value from the NIRS signal is by using an arterial occlusion (AO) during (or immediately after) exercise. In the present study, we found significant different oxygenation responses between low-intensity and high-intensity exercise, but with a remarkable similar response across the muscles both in terms of intensity and duration. However, the concentration changes of O<sub>2</sub>Hb and tHb still only reflects the balance between O<sub>2</sub> delivery and O<sub>2</sub> consumption and not the actual oxygen consumption in the muscle (mVO<sub>2</sub>). In other words, although we find a steady-state condition in oxygenation, it is not to say that the mVO<sub>2</sub> remains the same throughout the test. In fact, based on previous literature on altered muscle coordination during prolonged cycling (Dorel et al., 2009, Sanderson and Black, 2003) there is evidence to suggest otherwise. With the application of AO during exercise, and thus controlling the blood flow (i.e. no inflow or outflow), the rate of deoxygenation may be used to provide us with a more quantitative measurement of mVO<sub>2</sub>. As our initial goal was to also investigate mVO<sub>2</sub> during prolonged cycling, the application of AO was part of both constant-load protocols, but the results have not yet been analyzed. However, we found the occlusion to influence the systemic variables such as pVO<sub>2</sub> and HR. Especially during the initial phase (5 min) this effect is apparent in our data in a less smooth initial increase in pVO<sub>2</sub> as to what might be expected based on regression analysis reported in VO<sub>2</sub> kinetics studies. The AO cuts off, in a sense, most of the right leg from the rest of the body. With roughly half of the most active muscle mass cut off, and thus a smaller amount of muscle mass with the need of oxygen supply, the technique is expected to have some impact on the whole-body measurements. We found the HR and pVO<sub>2</sub> to decrease respectively during and after the AO followed by a compensation period (i.e. increase and overshoot, before it again stabilized at pre-AO level). With less muscle mass to supply with oxygen



during the AO, a smaller cardiac output is needed, which will lead to a lower pVO<sub>2</sub>. The delayed effect on pVO<sub>2</sub> relative to HR may therefore be a systemic delay as cardiac output kinetics has been found to be faster than that of pVO<sub>2</sub> (Xu and Rhodes, 1999), and/or a delay caused by the difference in sampling frequency (0.1 vs. 0.2 Hz). However, as the effect of AO was not the primary purpose of this study, we can only conclude that further research is needed to make any justified conclusions or interpretations of the effect on pVO<sub>2</sub> and HR.

The response we saw for pVO<sub>2</sub> and HR to the AO has an effect on the analysis, as to where one should calculate the averages. For the latter 25 min of the tests, this could easily be accounted for by taking the average prior to the occlusion. However, for the first 5 minutes, with 20 seconds occlusion every minute, the systemic compensation might have had a greater influence on the data as they may either be over- or underestimated. In the present study, we chose to calculate the 30 sec averages for pulmonary and heart rate data from the 10 seconds prior to AO plus the 20 seconds of AO, as the greatest effect of the AO was found in the 30 seconds period after the occlusion. Nevertheless, despite any possible over- or underestimation in the initial phase, this effect might not be a problem for our purpose since the focus was not so much on the pVO<sub>2</sub> kinetics as on the overall difference in response between the measured muscles. Moreover, when comparing the same work rate with (0 – 5 min CL-LI) and without (0 – 5min warm-up phase CL-HI) AO, the average for pVO<sub>2</sub> was approximately the same for each whole minute.

#### **4.1 Near-Infrared Spectroscopy (NIRS)**

Because of the relatively small area of NIRS measurement (3 – 4 cm in our study), some considerations to regional differences within the single muscle must be made when interpreting the results. Both Kennedy et al. (2006) and Kime et al. (2005) have shown such differences to exist in the VL muscle at low intensity, but with less/none heterogeneity of muscle oxygenation and inter-individual differences during high intensity cycling exercise. Regional differences have also been found in blood flow, with a decreased blood flow in the distal region compared to the proximal region of the VL muscle (Kime et al., 2005, Mizuno et al., 2003).

Another issue is the possible difference in adipose tissue thickness (ATT) between subjects and muscles. In investigating the skeletal muscle, the NIRS light also has to penetrate subcutaneous tissue, which may vary considerably between subjects' ATT. Since the measurement depth of NIRS is limited (i.e. approximately half the measurement distance (van

Beekvelt et al., 2001a)), a thick skin or fat layer will decrease the amount of light passing through the muscle tissue of interest, thereby underestimate the measured muscle oxygenation (van Beekvelt et al., 2001a). A method to adjust for differences in ATT has recently been proposed by Koga et al. (2011), which may help to provide more comparable data between studies and subjects in the future. However, further studies are first needed to standardize this method and establish its validity and reliability before it can be widely applied.

No significant difference in ATT, in any of the muscles, was found when comparing the subjects in group 1 and 2. Nor were any difference found between G30 and G<30, thus enabling across group comparisons. However, a significant difference was found between the muscles, with especially GMax having a higher ATT (Table 1) than the other muscles. As a higher ATT underestimate the measured muscle oxygenation, the responses found in our study for GMax in comparison with the other muscles may therefore be underestimated as well. Also, the ATT average for TA and BF (Table 1) was lower than for the remaining muscles. Consequently, the difference found in our study between the TA/BF and the other muscles may only be greater.

## 4.2 Effect of Intensity

Systemic and local responses were investigated during two different constant-load intensities designed to elicit different systemic responses. In accordance with Xu & Rhodes (1999), we hypothesized that CL-LI ( $WR = 50\% WR_{OBLA}$ , i.e. below LT) would provide us with an early  $pVO_2$  steady-state within 3 minutes of exercise, coupled with no increase in  $[La^-]$  levels. As expected, subjects achieved steady-state levels in the systemic variables within few minutes of exercise. Furthermore,  $[La^-]$  remained well below LT (Fig. 5D) during the full 30 minutes of CL-LI cycling, thus providing evidence that the WR was in fact below the lactate threshold. Investigating the local oxygenation responses revealed that an early recovery and surpassing of baseline levels was also present in all 6 muscles, with an increase in blood volume enabling oxygenation above baseline levels.

The initial intention for CL-HI was to select an intensity above each subject's individual MLSS, thought to elicit a  $pVO_2$  slow component as well as representing an intensity where a possible altered muscle recruitment would surely take place, but still within the limit of 30-min tolerance. Ideally, one would then test for the MLSS work rate or the critical power (i.e. the highest power one can hold for a theoretically infinite amount of time (Miura et al., 2002)) of the subjects by using multiple tests at various work rates. However, as

the testing procedure for both variables is greatly time-consuming, it was not feasible for the present study. Consequently, the intensity was set to 75% MAP based on evaluation of literature with similar protocols and pilot studies in our lab. This relative work rate, without consideration for individual lactate thresholds, led to different finishing times ranging between 15 and 30 minutes. However, those subjects who completed 30-min rated their perceived exhaustion at the end of exercise no lower than those who completed merely 15 minutes, suggesting an equal level of overall fatigue.

Regardless of time-to-task-failure, the high-intensity exercise led to statistical significant differences compared to low-intensity in the response of all systemic variables. However, no further increase of  $pV_{O_2}$  after the 6<sup>th</sup> minute was present in any of the subjects (Fig. 4A), thus suggesting that the WR was collectively in the range of heavy exercise (i.e.  $LT < WR < MLSS$ ) rather than that of severe exercise (i.e.  $WR > MLSS$ ) (Xu and Rhodes, 1999). This is in contrast with the continuous  $[La^-]$  accumulation found in some of the subjects in  $G < 30$  (Fig. 4B) and the described relationship between these two variables (Jones et al., 2011, Xu and Rhodes, 1999). Nevertheless, all systemic variables showed responses in accordance with what can be expected of intensities above LT.

A clear effect of work intensity was also present in the local oxygenation response, where  $O_2Hb$  and  $HHb$  deviated to a greater extent early on during high-intensity in all 6 muscles (Fig. 7). Blood volume increases during high-intensity both intensities, but while the increase during low-intensity was enough to enable recovery of baseline oxygenation levels,  $O_2Hb$  and  $HHb$  remained respectively below and above baseline levels during high-intensity cycling. However, the oxygenation level stabilized in most of the muscles within 5 minutes, where also little or no further increase in  $tHb$  was present (except from VM). Depending on the different muscle groups, and their involvement in cycling (for a review, see Hug & Dorel (2009)), this plateau may suggest one of several things; (1) the work performed by the muscle does not demand further deoxygenation, (2) the muscle(s) has reached its minimum oxygenation level, or (3) an impaired blood flow hinders the increase of  $O_2Hb$  delivery. Redistribution of blood flow in the legs have been found to occur predominantly during mild to moderate exercise (Asanoi et al., 1992), while an increase in blood flow during severe exercise is thought to depend on an increase in cardiac output (i.e. heart rate x stroke volume). A continuous increase in heart rate is present during high-intensity, but without measurement of stroke volume we cannot be sure whether this increase is a result of an effort to increase blood flow or to the phenomenon cardiac drift (i.e. an increase in heart rate and a reduction in

stroke volume, while cardiac output remains the same), which is repeatedly reported in prolonged exercise studies (Cheatham et al., 2000). If an effort to increase blood flow is present, the blood flow to some of the working muscles (e.g. VL) may be impaired due to intramuscular pressure exceeding perfusion pressure (Sadamoto et al., 1983). That is, the requirement of force production during CL-HI exceeds the critical point for further increase in blood flow to the muscles. However, as this is out of the scope of this study, further research is necessary to provide more founded interpretations.

### **4.3 Pulmonary O<sub>2</sub> Consumption vs. Muscle Oxygenation**

The main finding of the present study was that local oxygenation measurement for all the muscles collectively behaved in a similar manner as pVO<sub>2</sub>, and additionally showed a surprisingly homogenous response. During CL-LI, an intensity that got ratings exclusively from the lower end of the Borg scale, the demand for O<sub>2</sub> delivery to produce the required work aerobically is easily met. The oxygenation level in all the measured muscles decreases in the initial phase, most likely because of the mechanical effect of contraction in squeezing the blood out of the muscle. However, as heart rate and pVO<sub>2</sub> increases, so does tHb, enabling oxygenation at or above baseline level in all of the muscles except TA.

During CL-HI, it takes longer time to reach pVO<sub>2</sub> steady-state, and O<sub>2</sub>Hb and HHb deviate to a larger extent. This deviation in O<sub>2</sub>Hb and HHb occurs after 5 min warm-up at the same intensity as the CL-LI test, thus clearly illustrating a higher need for oxygen in all the measured muscles. During this transition period, tHb starts to increase in all of the muscles rather rapidly (within approximately 1 – 3 min) after the initial immediate decrease while O<sub>2</sub>Hb levels remain either at the same level or continue to decrease before a resaturation period between 5 and 10 min of cycling (except from VL). After 10 min of exercise, there is a steady-state present in both local oxygenation and pVO<sub>2</sub>. The finding of VL not showing any resaturation may suggest that the muscle is working at its maximum capacity and has reached its plateau in deoxygenation, while an impaired blood flow hinders any possible resaturation.

The relatively delayed reoxygenation compared to the early increase in tHb is interesting. Chance et al. (1992) studied the resaturation of the quadriceps muscle (RF (i.e. rectus femoris), VL, and VM) at rest after several intermittent sub-maximal loads during cycling. They found the time of resaturation of hemoglobin in the muscles to be prolonged as the intensity increased, and that the blood volume returned to a resting level faster than the resaturation of hemoglobin. The authors contributed this finding to a repayment of oxygen

deficit at higher work rates. Whether the delayed resaturation relative to the increase in tHb in our study is a result of any such repayment in the muscle is not possible to conclude based on the available data. However, Chance et al. (1992) investigated resaturation after exercise, and their results are thereby in agreement with studies on O<sub>2</sub> kinetics on a pulmonary level, where any oxygen deficit acquired during the initial phase after onset of exercise is argued to be repayed after exercise (Xu and Rhodes, 1999, Whipp and Wasserman, 1972). Consequently, there is no evidence to suggest that any such repayment occurs during exercise. Thus the delayed resaturation we see during high-intensity may just as well represent a further increase in mVO<sub>2</sub> enabled by the early increase of tHb. Nonetheless, further research is needed to enlighten any possible underlying causes for the delayed reoxygenation during high-intensity cycling.

The steady-state found in pVO<sub>2</sub> and local oxygenation between the 10<sup>th</sup> and 15<sup>th</sup> minute was also found throughout the full 30-minutes for those 6 subjects who completed 30-minutes at both intensities. This suggests that the O<sub>2</sub> steady-state found on a pulmonary level at both intensities is also reflected by a steady-state balance between oxygen delivery and utilization found on a local level. The adjustments for improving the balance between O<sub>2</sub> delivery and utilization are in other words first and foremost found in the initial phase of constant-load exercise.

#### 4.4 Tissue Saturation Index (TSI)

Studies that have applied NIRS during dynamic exercise typically report the results in terms of oxygenation index (i.e.  $Hb_{diff} = \Delta[O_2Hb - HHb]$ ) or tissue saturation index (TSI), both reflecting the balance between oxygen supply and consumption in small vessels in the muscle. The measurement of TSI requires multiple measurement distances, but provides a more quantified measurement of saturation. Most previous studies have typically investigated parts of the quadriceps muscle, while Takagi et al. (2013) is the only study (to our knowledge) to investigate several muscle groups simultaneously, but did so during incremental cycling with 20 W/min increments and not using specifically trained cyclists.

From Fig. 8 it is clear that the amount of desaturation and the initial response is different between low-intensity and high-intensity in all of the muscles. The VL muscle is thought to be the most active leg muscle in cycling and has consequently been used frequently in NIRS literature. In our study, the VL is the muscle that resembles pVO<sub>2</sub> the most in terms of TSI response, with a rapid desaturation during the first two minutes of exercise at both

intensities, but followed by a TSI steady-state at low-intensity and a continued gentler desaturation at high-intensity. The desaturation during high-intensity continues until the 5<sup>th</sup> minute, before a steady-state is present at the 10<sup>th</sup> minute, also in agreement with pVO<sub>2</sub>. CL-LI and CL-HI were found to elicit an average pVO<sub>2</sub> steady-state of respectively ~45% and ~95% of pVO<sub>2max</sub>. Comparing the  $\Delta$ TSI from rest to TSI steady-state in VL to similar intensity levels in the study of Takagi et al. (2013) (40% and 100% pVO<sub>2peak</sub>), our results are of similar value for CL-LI (3% vs. 4%) but substantially higher for CL-HI (10% vs. 24%). This difference could be a result of different training status in the subjects, as Takagi et al. (2013) included generally healthy young men that had not participated in any type of endurance training program for the last 12 months, while we included specifically recreational cyclists that perhaps have the ability to achieve lower saturation level in VL during severe exercise. However, the difference could also, at least to some extent, be a consequence of the different protocols (i.e. incremental vs. constant-load cycling protocol).

Also Grassi et al. (1995) and Belardinelli et al. (1995a) investigated the saturation in VL (using Hb<sub>diff</sub>) during incremental exercise, and they found the pattern of change in saturation to depend on whether the exercise intensity was below or above the subject's LT. This is in accordance with the results of Takagi et al. (2013), where there is a steeper decrease in saturation after 60% pVO<sub>2peak</sub> (an intensity level where one can expect to find the LT in normal healthy subjects (Belardinelli et al., 1995a), as well as with the difference found between the intensities in our study. Belardinelli et al. (1995a) concluded this finding to be owed to the Bohr effect. That is, at low-intensity exercise the O<sub>2</sub> supply is sufficient to meet the aerobic demand for ATP resynthesis, while at intensities above LT the accumulation of muscle lactate ultimately shift the Hb dissociation curve to the right and enables greater O<sub>2</sub> extraction from the capillaries.

While a difference between low-intensity and high-intensity was most pronounced in VL and the other proximal muscles (BF, VM, and GMax), it was much less in the distal muscles (GL and TA). Both GL and TA showed an initial desaturation during high-intensity followed by an early stabilized level at similar saturation index as during low-intensity. However, while TA showed no initial desaturation during low-intensity, GL had a similar initial desaturation at both intensities, but increases gradually to a saturation level equal to that of baseline by the 10<sup>th</sup> min during low-intensity. This is similar to the results of Takagi et al. (2013), where the TSI of TA remains unaltered to the WR increments until the intensity exceeds 60% pVO<sub>2peak</sub>, while changes in GL TSI can be seen at all increments. The  $\Delta$ TSI

during CL-LI is also similar to the mentioned study (GL: 0% vs. 1%, TA: -1% vs. 0%), while a 4% higher and a 6% lower  $\Delta$ TSI was found for respectively GL and TA during CL-HI. This difference is most likely caused by the better trained cyclists in our study (Chapman et al., 2008). A possible explanation for the different response and  $\Delta$ TSI between these two muscles may be a result of mono- vs. bi-articular muscles and what is known about these muscles and their participation during cycling. While mono-articular muscles are generally believed to have the role as power producer muscles (Ryan and Gregor, 1992), and one would expect the power production for dorsiflexion to be low at lower intensities such as 50%  $WR_{OBLA}$  as well as at high intensity for experienced cyclists, bi-articular muscles are thought to be primarily active in the transfer of energy between joints and in controlling the direction of force production on the pedal (van Ingen Schenau et al., 1992). EMG studies on the gastrocnemius muscle have shown it to have a constant EMG activity level at lower intensities in the range below 70% MAP (Hug et al., 2004, Jorge and Hull, 1986), which is in accordance with the similar initial response for the muscle seen in our study between the intensities. The general low activity of GL during cycling compared to mono-articular muscles (Hug and Dorel, 2009), would then suggest that the increase in blood volume following onset of exercise is not impaired (i.e. less activation  $\rightarrow$  less force production  $\rightarrow$  less impairment), and thereby enough to recover baseline subtraction.

In the study of EMG activity level of several main lower limb muscles during constant-load exercises at different work rates, Ericson (1988) found increased EMG activity level in VL, VM, BF, and GMax (among others) as power output increased from 120 to 240 W. Furthermore, it was suggested that especially GMax activity is greatly influenced by the work rate level with a relative higher activity during high intensity exercise when compared to VM. This is in accordance with the results of the present study, where a significant difference in saturation response between the intensities is found for the same muscles. This response is also most distinctly different in GMax, with a more pronounced effect of intensity in the period after the initial start with a recovery of saturation during low-intensity and a further decrease during high-intensity despite an increase in tHb. In addition, because of the high ATT for the GMax muscle, this difference may be underestimated. Similar response was also found for BF during high-intensity. The unaltered saturation response in VL and VM coupled with the desaturation present in BF and GMax during high-intensity may suggest an altered muscle coordination during exhaustive exercise. There have been found a significant increase in the EMG activity level of VL and VM during constant-load exercises to exhaustion at high

intensity cycling (Housh et al., 2000, Petrofsky, 1979), suggesting that additional motor units of the muscles are recruited to compensate for the decrease in force in the fatigued muscle fibers (Dorel et al., 2009). Also the mechanical pattern at the end of prolonged exhaustive exercise has been shown to change (Sanderson and Black, 2003), strongly suggesting that the muscle coordination is affected by fatigue. Dorel et al. (2009) found in their study of constant-load exercise an increase in EMG activity level in both GMax (29%) and BF (15%). This supports that the desaturation apparent in GMax and BF in the present study during high-intensity but not low-intensity may reflect a coping strategy to counteract fatigue in VL and VM and/or an increase in force production by the knee flexors (Elmer et al., 2011) and hip extensors to add to the maximum capacity already delivered by the knee extensors.

#### 4.5 Finishing Groups

In the present study, a total of 24 subjects completed CL-HI at 75% MAP, and all subjects rated themselves within the same range on the Borg scale, suggesting an equal overall perception of fatigue. However, only 10 of the subjects sustained the full 30-min duration that was scheduled. When comparing baseline characteristics that were thought to be determining factors for performance ( $WR_{OBLA}$  in absolute (W) and percentage of MAP,  $VO_{2max}$ , MAP, and max achieved lactate), no statistical significant differences were found between the two groups (i.e. G30 and G<30). Nor did we find any difference in the initial response (0 – 15 min) for  $pVO_2$ , HR, and RER. The only difference found between the two groups on a whole-body scale were  $[La^-]$  and  $V_E$  responses during CL-HI. In G30, the initial fast increase in  $V_E$  flattened more as compared to G<30 where it continued to increase as in Hagberg et al.'s study (1978) with normal healthy adults (non-cyclists). In agreement with  $V_E$ ,  $[La^-]$  showed a much steeper increase in G<30. A general principle to explain coupling between blood lactate and ventilation is pulmonary buffering due to acidification under high intensity through an increase in  $pVCO_2$ , and thus the need for an increase in  $V_E$  (Perrey et al., 2003). In other words, the difference in  $V_E$  response between the groups may merely be a result of the difference found in the accumulation of blood lactate. When comparing the subjects' end results of CL-HI, no significant difference was found in  $\Delta[La^-]$  from the maximum achieved lactate during from day 1, nor were there a visible difference in end  $V_E$  level, thus the difference is found in the rate of accumulation and not end values. Our results are thereby in agreement with that the exercise intensities associated with the discontinuities in both blood lactate accumulation and ventilation are highly related to the exercise intensity that can be tolerated during sustained exercise (Billat et al., 2003, Stegmann and Kindermann, 1982).



Well trained cyclists are expected to have high absolute values of  $\text{VO}_{2\text{max}}$ , but they separate themselves perhaps first and foremost from the recreational level in the ability to work at a higher percentage of their aerobic capacity without high levels of lactate, and subsequently early fatigue (Lucia et al., 2001). In this line of thought, it is easy to suggest that the subjects in G30 were better trained cyclists who were able to sustain the same level of relative intensity (%MAP) for a longer duration, with less accumulation of  $[\text{La}^-]$ . If so, this is not clear from the difference in  $\text{WR}_{\text{OBLA}}$  as percentage of MAP between the two groups, with a mean difference of only 3.2% in favor of G30. One possible explanation for this is that OBLA is not the same as the individual lactate threshold, and the single reference at a blood lactate concentration of  $4 \text{ mmol}\cdot\text{l}^{-1}$  may be too simplistic (Beneke et al., 2000, Foxdal et al., 1996, Billat et al., 2003, van Schuylenbergh et al., 2004). However, a fixed lactate threshold of  $4 \text{ mmol}\cdot\text{l}^{-1}$  has been found to overestimate the individual threshold in more aerobic trained subjects (Billat et al., 2003, Stegmann et al., 1981). Hence, if the subjects in G30 in fact were better aerobically trained (i.e. typically better trained cyclists) the mean difference in individual lactate threshold may be less. Nonetheless, the blood lactate response of G30 resembles to a great deal what have been found in highly trained cyclists cycling at critical power for a  $t_{\text{lim}}$  of 30 minutes (Jenkins and Quigley, 1990), thus suggesting that some important factor for performance is overlooked in our whole-body measurements.

Although no difference in  $\text{pVO}_2$  response during CL-HI was found between G30 and G<30, physical training is known to cause adaptations to not only the cardiopulmonary system (typically measured by an increase in  $\text{pVO}_{2\text{max}}$ ) but also the peripheral system. This has also been supported in NIRS studies, with high correlation between peripheral effects of training and cycling performance (Costes et al., 2001, Neary et al., 2002). In investigating TSI response during 0 – 15min of CL-HI, we found a significant difference in the response of GMax between G30 and G<30. While G30 is able to sustain the desaturated level achieved by the 5<sup>th</sup> minute, G<30 show a continuous desaturation. This is in line with the earlier mentioned increased GMax activity found in EMG studies during exhaustive exercise, and may further be connected to the lesser amount of desaturation found in VL and VM for G<30. While the 30-min group were able to sustain further 15 minutes of exercise, 7 of the 16 subjects in G<30 had reached voluntarily exhaustion by 15 minutes. This may reflect a need for the GMax to contribute relatively more to the force production in the <30 group compared to G30. However, as TSI only reflects the balance between local  $\text{O}_2$  delivery and  $\text{O}_2$  consumption, we cannot conclude whether there is an increase in force production and/or

muscle activity of GMax. Nevertheless, a significant difference between the two groups that may have been a contributing factor to their performance was found on the peripheral level using NIRS, and the need for further studies coupling other measurement techniques with NIRS (e.g. inverse dynamics, EMG) on several muscles during exhaustive exercise is needed to help explain this further.

## 5. Conclusion

This is the first study to measure local oxygenation responses to constant-load cycling in several muscle groups simultaneously. The main finding of the present study was that local oxygenation measurement for all the muscles collectively behaved in a similar manner as  $pVO_2$  at both intensities, and additionally showed a surprisingly homogenous response. However, although the local responses were similar, differences were found between the muscles with heterogeneity in regard to the amount of desaturation. We found the distal muscles TA and GL to show less difference in saturation between the two intensities compared to the more proximal muscles (VL, VM, BF, and GMax), as well as a lack of TSI steady-state during CL-HI was found for the BF and GMax muscles. These findings are in agreement with previous EMG studies that have found GL to show steady activity during low intensity cycling (<70% MAP) while the activity of BF and GMax have showed to increase with increasing work rate and duration. Another interesting finding was found when comparing G30 and G<30. While none of the baseline characteristics were significant different between the two groups, and the only significant differences in whole-body measurements were found in  $[La^-]$  and  $V_E$  responses, we found peripheral differences that may have contributed to their performance. G30 showed less desaturation in VL and VM as well as a significant different response in GMax TSI. While G30 maintained the desaturated level in GMax achieved by the 5<sup>th</sup> minute, G<30 showed a continuous desaturation throughout. This may reflect a strategy to add to the maximum capacity already delivered by the knee extensors, which then hypothetically may have been less in the latter group. Changing the work rate in individual muscles may also be a strategy to counteract early fatigue in the first recruited muscles. However, as the measurement of TSI only reflects the balance between  $O_2$  delivery and  $O_2$  consumption, future studies investigating actual muscle oxygen consumption coupled with measurements of local work rate through inverse dynamics may help us to gain a better understanding of both  $mVO_2$  and muscle use during prolonged cycling. This study also underlines the need for knowledge of peripheral differences when assessing training status in an otherwise homogenous group, as well as illustrating how the use of NIRS can provide a new, more direct way to study what is happening in the local muscle during exercise.

## 6. References

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## Appendix

Appendix I. Revised version of the Borg scale for Rating of Perceived Exertion.

### RPE

|    |                      |
|----|----------------------|
| 6  |                      |
| 7  | Veldig, veldig lett  |
| 8  |                      |
| 9  | Veldig lett          |
| 10 |                      |
| 11 | Ganske lett          |
| 12 |                      |
| 13 | Noe tungt            |
| 14 |                      |
| 15 | Tungt                |
| 16 |                      |
| 17 | Veldig tungt         |
| 18 |                      |
| 19 | Veldig, veldig tungt |
| 20 | Maksimalt            |

Appendix II. ANOVA repeated measurement analysis of systemic variables 0-15 min.

**pVO2 (ml/kg/min)**

|                            | DF1  | DF2    | F      | sig.  |
|----------------------------|------|--------|--------|-------|
| Time                       | 2,56 | 145,04 | 363,58 | 0,000 |
| Intensity                  | 1    | 11     | 496,33 | 0,000 |
| Time*intensity             | 1,8  | 19,82  | 29,17  | 0,000 |
| <i>G30 vs. G&lt;30</i>     |      |        |        |       |
| Time*groups                | 2,67 | 26,65  | 1,80   | 0,177 |
| Intensity*groups           | 1    | 10     | 5,54   | 0,040 |
| Time*intensity*groups      | 2    | 20,02  | 2,22   | 0,135 |
| <i>Group 1 vs. Group 2</i> |      |        |        |       |
| Time                       | 3,03 | 72,73  | 523,96 | 0,000 |
| Time*groups                | 3,03 | 72,73  | 1,48   | 0,226 |

**HR (bpm)**

|                            | DF1  | DF2   | F      | sig.  |
|----------------------------|------|-------|--------|-------|
| Time                       | 1,5  | 19,49 | 59,82  | 0,000 |
| Intensity                  | 1    | 13    | 614,39 | 0,000 |
| Time*intensity             | 1,36 | 17,61 | 33,32  | 0,000 |
| <i>G30 vs. G&lt;30</i>     |      |       |        |       |
| Time*groups                | 1,57 | 18,84 | 2,14   | 0,153 |
| Intensity*groups           | 1    | 12    | 1,31   | 0,275 |
| Time*intensity*groups      | 1,41 | 16,96 | 2,44   | 0,129 |
| <i>Group 1 vs. Group 2</i> |      |       |        |       |
| Time                       | 1,32 | 31,65 | 141,06 | 0,000 |
| Time*groups                | 1,32 | 31,65 | 0,49   | 0,540 |

**VE (l/min)**

|                            | DF1  | DF2   | F      | sig.  |
|----------------------------|------|-------|--------|-------|
| Time                       | 1,94 | 21,3  | 94,36  | 0,000 |
| Intensity                  | 1    | 11    | 222,30 | 0,000 |
| Time*intensity             | 1,7  | 18,7  | 42,61  | 0,000 |
| <i>G30 vs. G&lt;30</i>     |      |       |        |       |
| Time*groups                | 1,93 | 19,3  | 6,02   | 0,010 |
| Intensity*groups           | 1    | 10    | 10,73  | 0,008 |
| Time*intensity*groups      | 2,14 | 21,44 | 6,61   | 0,005 |
| <i>Group 1 vs. Group 2</i> |      |       |        |       |
| Time                       | 1,97 | 47,17 | 227,54 | 0,000 |
| Time*groups                | 1,97 | 47,17 | 0,44   | 0,645 |

**RER**

|                            | DF1  | DF2   | F      | sig.  |
|----------------------------|------|-------|--------|-------|
| Time                       | 2,65 | 29,18 | 39,73  | 0,000 |
| Intensity                  | 1    | 11    | 128,30 | 0,000 |
| Time*intensity             | 2,24 | 24,69 | 44,11  | 0,000 |
| <i>G30 vs. G&lt;30</i>     |      |       |        |       |
| Time*groups                | 2,77 | 27,72 | 2,32   | 0,102 |
| Intensity*groups           | 1    | 10    | 9,69   | 0,011 |
| Time*intensity*groups      | 2,09 | 0,07  | 0,56   | 0,587 |
| <i>Group 1 vs. Group 2</i> |      |       |        |       |
| Time                       | 3    | 72,09 | 197,50 | 0,000 |
| Time*groups                | 3    | 72,09 | 0,66   | 0,577 |

**La (mmol/l)**

|                            | DF1  | DF2   | F      | sig.  |
|----------------------------|------|-------|--------|-------|
| Time                       | 1,14 | 10,21 | 59,63  | 0,000 |
| Intensity                  | 1    | 9     | 115,30 | 0,000 |
| Time*intensity             | 1,21 | 10,87 | 65,28  | 0,000 |
| <i>G30 vs. G&lt;30</i>     |      |       |        |       |
| Time*groups                | 1,17 | 9,33  | 4,74   | 0,052 |
| Intensity*groups           | 1    | 8     | 2,87   | 0,129 |
| Time*intensity*groups      | 1,29 | 10,34 | 5,07   | 0,040 |
| <i>Group 1 vs. Group 2</i> |      |       |        |       |
| Time                       | 1,17 | 24,46 | 130,37 | 0,000 |
| Time*groups                | 1,17 | 24,46 | 0,64   | 0,454 |

**Borg**

|                            | DF1 | DF2 | F      | sig.  |
|----------------------------|-----|-----|--------|-------|
| Time                       | 2   | 26  | 47,06  | 0,000 |
| Intensity                  | 1   | 13  | 342,36 | 0,000 |
| Time*intensity             | 2   | 26  | 15,85  | 0,000 |
| <i>G30 vs. G&lt;30</i>     |     |     |        |       |
| Time*groups                | 2   | 24  | 1,17   | 0,329 |
| Intensity*groups           | 1   | 12  | 0,01   | 0,933 |
| Time*intensity*groups      | 2   | 24  | 8,48   | 0,002 |
| <i>Group 1 vs. Group 2</i> |     |     |        |       |
| Time                       | 2   | 48  | 96,48  | 0,000 |
| Time*groups                | 2   | 48  | 0,63   | 0,536 |

Appendix III. ANOVA repeated measurement analysis of systemic variables 0-30 min.

**pVO2**

|                | DF1 | DF2 | F      | sig.  |
|----------------|-----|-----|--------|-------|
| Time           | 9   | 45  | 172,26 | 0,000 |
| Time*Intensity | 9   | 45  | 11,63  | 0,000 |

**HR**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 9   | 45  | 74,87 | 0,000 |
| Time*Intensity | 9   | 45  | 22,07 | 0,000 |

**VE**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 9   | 45  | 39,03 | 0,000 |
| Time*Intensity | 9   | 45  | 24,5  | 0,000 |

**RER**

|                | DF1 | DF2 | F    | sig.  |
|----------------|-----|-----|------|-------|
| Time           | 9   | 45  | 9,95 | 0,000 |
| Time*Intensity | 9   | 45  | 21,8 | 0,000 |

**La**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 10  | 30  | 44,5  | 0,000 |
| Time*Intensity | 10  | 30  | 62,89 | 0,000 |

**Borg**

|                | DF1  | DF2  | F    | sig.  |
|----------------|------|------|------|-------|
| Time           | 5    | 25   | 28,8 | 0,000 |
| Time*Intensity | 1,17 | 5,85 | 0,68 | 0,464 |

Appendix IV. ANOVA RM analysis of O<sub>2</sub>Hb concentration changes 0-15 min.

|                            | <b>vastus lateralis (VL)</b> |       |       |       | <b>vastus medialis (VM)</b> |       |       |       |
|----------------------------|------------------------------|-------|-------|-------|-----------------------------|-------|-------|-------|
|                            | DF1                          | DF2   | F     | sig.  | DF1                         | DF2   | F     | sig.  |
| Time                       | 1,98                         | 23,78 | 25,91 | 0,000 | 1,96                        | 23,57 | 37,67 | 0,000 |
| Intensity                  | 1                            | 12    | 25,41 | 0,000 | 1                           | 12    | 0,13  | 0,722 |
| Time*intensity             | 2,5                          | 30,02 | 32,85 | 0,000 | 2,37                        | 28,44 | 17,88 | 0,000 |
| <i>G30 vs. G&lt;30</i>     |                              |       |       |       |                             |       |       |       |
| Time*groups                | 1,92                         | 21,13 | 1,17  | 0,329 | 1,88                        | 20,67 | 0,34  | 0,704 |
| Intensity*groups           | 1                            | 11    | 0,34  | 0,572 | 1                           | 11    | 0,94  | 0,353 |
| Time*intensity*groups      | 2,39                         | 26,29 | 0,62  | 0,571 | 2,19                        | 24,05 | 1,80  | 0,184 |
| <i>Group 1 vs. Group 2</i> |                              |       |       |       |                             |       |       |       |
| Time                       | 1,9                          | 41,74 | 44,70 | 0,000 | 1,82                        | 40,05 | 38,12 | 0,000 |
| Time*groups                | 1,9                          | 41,74 | 0,62  | 0,537 | 1,82                        | 40,05 | 0,20  | 0,803 |

|                            | <b>biceps femoris (BF)</b> |       |       |       | <b>gluteus maximus (Gmax)</b> |       |       |       |
|----------------------------|----------------------------|-------|-------|-------|-------------------------------|-------|-------|-------|
|                            | DF1                        | DF2   | F     | sig.  | DF1                           | DF2   | F     | sig.  |
| Time                       | 1,43                       | 17,12 | 37,31 | 0,000 | 1,63                          | 19,61 | 29,85 | 0,000 |
| Intensity                  | 1                          | 12    | 0,54  | 0,477 | 1                             | 12    | 0,19  | 0,672 |
| Time*intensity             | 2,1                        | 25,16 | 11,04 | 0,000 | 1,96                          | 26,53 | 13,17 | 0,000 |
| <i>G30 vs. G&lt;30</i>     |                            |       |       |       |                               |       |       |       |
| Time*groups                | 1,5                        | 16,48 | 2,54  | 0,120 | 1,81                          | 19,91 | 3,59  | 0,051 |
| Intensity*groups           | 1                          | 11    | 3,09  | 0,107 | 1                             | 11    | 0,28  | 0,606 |
| Time*intensity*groups      | 2,56                       | 28,13 | 3,80  | 0,026 | 1,94                          | 21,35 | 1,42  | 0,264 |
| <i>Group 1 vs. Group 2</i> |                            |       |       |       |                               |       |       |       |
| Time                       | 1,61                       | 35,49 | 28,78 | 0,000 | 1,56                          | 34,33 | 14,79 | 0,000 |
| Time*groups                | 1,61                       | 35,49 | 0,48  | 0,580 | 1,56                          | 34,33 | 0,57  | 0,528 |

|                            | <b>gastrocnemius lateralis (GL)</b> |       |       |       | <b>tibialis anterior (TA)</b> |       |       |       |
|----------------------------|-------------------------------------|-------|-------|-------|-------------------------------|-------|-------|-------|
|                            | DF1                                 | DF2   | F     | sig.  | DF1                           | DF2   | F     | sig.  |
| Time                       | 1,96                                | 23,55 | 35,92 | 0,000 | 2,83                          | 33,95 | 28,30 | 0,000 |
| Intensity                  | 1                                   | 12    | 0,02  | 0,898 | 1                             | 12    | 0,40  | 0,537 |
| Time*intensity             | 2,35                                | 28,21 | 22,01 | 0,000 | 2,96                          | 35,48 | 2,08  | 0,121 |
| <i>G30 vs. G&lt;30</i>     |                                     |       |       |       |                               |       |       |       |
| Time*groups                | 2                                   | 21,95 | 1,17  | 0,330 | 2,89                          | 31,8  | 0,66  | 0,578 |
| Intensity*groups           | 1                                   | 11    | 0,58  | 0,462 | 1                             | 11    | 0,21  | 0,655 |
| Time*intensity*groups      | 2,36                                | 25,93 | 0,36  | 0,738 | 2,82                          | 30,96 | 0,61  | 0,602 |
| <i>Group 1 vs. Group 2</i> |                                     |       |       |       |                               |       |       |       |
| Time                       | 2,15                                | 47,29 | 22,60 | 0,000 | 2,79                          | 61,34 | 15,02 | 0,000 |
| Time*groups                | 2,15                                | 47,29 | 0,80  | 0,465 | 2,79                          | 61,34 | 0,74  | 0,524 |

Appendix V. ANOVA RM analysis of O<sub>2</sub>Hb concentration changes 0-30 min.

**vastus lateralis (VL)**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 10  | 50  | 12,46 | 0,000 |
| Time*Intensity | 10  | 50  | 29,88 | 0,000 |

**vastus medialis (VM)**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 10  | 50  | 37,14 | 0,000 |
| Time*Intensity | 10  | 50  | 14,84 | 0,000 |

**biceps femoris (BF)**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 10  | 50  | 16,73 | 0,000 |
| Time*Intensity | 10  | 50  | 4,84  | 0,000 |

**gluteus maximus (Gmax)**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 10  | 50  | 66,45 | 0,000 |
| Time*Intensity | 10  | 50  | 9,8   | 0,000 |

**gastrocnemius lateralis (GL)**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 10  | 50  | 16,56 | 0,000 |
| Time*Intensity | 10  | 50  | 9,81  | 0,000 |

**tibialis anterior (TA)**

|                | DF1 | DF2 | F    | sig.  |
|----------------|-----|-----|------|-------|
| Time           | 10  | 50  | 7,51 | 0,000 |
| Time*Intensity | 10  | 50  | 1,56 | 0,146 |

Appendix VI. ANOVA RM analysis of HHb concentration changes 0-15 min.

|                            | <b>vastus lateralis (VL)</b>        |       |       |       | <b>vastus medialis (VM)</b>   |       |       |       |
|----------------------------|-------------------------------------|-------|-------|-------|-------------------------------|-------|-------|-------|
|                            | DF1                                 | DF2   | F     | sig.  | DF1                           | DF2   | F     | sig.  |
| Time                       | 1,48                                | 17,78 | 34,97 | 0,000 | 1,51                          | 18,11 | 32,86 | 0,000 |
| Intensity                  | 1                                   | 12    | 26,79 | 0,000 | 1                             | 12    | 18,11 | 0,001 |
| Time*intensity             | 1,54                                | 18,51 | 16,14 | 0,000 | 2,55                          | 30,6  | 23,43 | 0,000 |
| <i>G30 vs. G&lt;30</i>     |                                     |       |       |       |                               |       |       |       |
| Time*groups                | 1,49                                | 16,42 | 0,33  | 0,662 | 1,4                           | 15,45 | 1,55  | 0,240 |
| Intensity*groups           | 1                                   | 11    | 24,70 | 0,828 | 1                             | 11    | 0,833 | 0,381 |
| Time*intensity*groups      | 1,51                                | 16,6  | 0,25  | 0,717 | 2,47                          | 27,21 | 0,49  | 0,656 |
| <i>Group 1 vs. Group 2</i> |                                     |       |       |       |                               |       |       |       |
| Time                       | 1,42                                | 31,33 | 69,01 | 0,000 | 1,72                          | 37,75 | 79,58 | 0,000 |
| Time*groups                | 1,42                                | 31,33 | 0,81  | 0,415 | 1,72                          | 37,75 | 0,57  | 0,542 |
|                            | <b>biceps femoris (BF)</b>          |       |       |       | <b>gluteus maximus (Gmax)</b> |       |       |       |
|                            | DF1                                 | DF2   | F     | sig.  | DF1                           | DF2   | F     | sig.  |
| Time                       | 1,55                                | 18,6  | 7,88  | 0,005 | 2,64                          | 31,67 | 4,34  | 0,014 |
| Intensity                  | 1                                   | 12    | 44,12 | 0,000 | 1                             | 12    | 30,42 | 0,000 |
| Time*intensity             | 2,24                                | 26,87 | 26,35 | 0,000 | 1,76                          | 21,09 | 29,60 | 0,000 |
| <i>G30 vs. G&lt;30</i>     |                                     |       |       |       |                               |       |       |       |
| Time*groups                | 1,55                                | 17,05 | 0,69  | 0,480 | 2,49                          | 27,42 | 0,91  | 0,433 |
| Intensity*groups           | 1                                   | 11    | 6,40  | 0,028 | 1                             | 11    | 0,49  | 0,497 |
| Time*intensity*groups      | 2,36                                | 25,94 | 2,46  | 0,097 | 1,73                          | 19,06 | 0,73  | 0,478 |
| <i>Group 1 vs. Group 2</i> |                                     |       |       |       |                               |       |       |       |
| Time                       | 1,53                                | 33,56 | 35,56 | 0,000 | 2,4                           | 52,86 | 50,21 | 0,000 |
| Time*groups                | 1,53                                | 33,56 | 0,46  | 0,000 | 2,4                           | 52,86 | 1,59  | 0,211 |
|                            | <b>gastrocnemius lateralis (GL)</b> |       |       |       | <b>tibialis anterior (TA)</b> |       |       |       |
|                            | DF1                                 | DF2   | F     | sig.  | DF1                           | DF2   | F     | sig.  |
| Time                       | 1,69                                | 20,22 | 9,31  | 0,002 | 3,2                           | 38,38 | 9,12  | 0,000 |
| Intensity                  | 1                                   | 12    | 7,20  | 0,020 | 1                             | 12    | 38,74 | 0,000 |
| Time*intensity             | 2,35                                | 28,14 | 10,23 | 0,000 | 2,68                          | 32,11 | 18,20 | 0,000 |
| <i>G30 vs. G&lt;30</i>     |                                     |       |       |       |                               |       |       |       |
| Time*groups                | 1,93                                | 21,2  | 3,35  | 0,056 | 3,11                          | 34,23 | 0,31  | 0,827 |
| Intensity*groups           | 1                                   | 11    | 1,03  | 0,331 | 1                             | 11    | 0,07  | 0,800 |
| Time*intensity*groups      | 2,37                                | 26,08 | 0,97  | 0,404 | 2,58                          | 28,4  | 0,40  | 0,728 |
| <i>Group 1 vs. Group 2</i> |                                     |       |       |       |                               |       |       |       |
| Time                       | 2,19                                | 48,07 | 36,14 | 0,000 | 2,97                          | 65,29 | 63,94 | 0,000 |
| Time*groups                | 2,19                                | 48,07 | 1,56  | 0,220 | 2,97                          | 65,29 | 1,96  | 0,129 |

Appendix VII. ANOVA RM analysis of HHb concentration changes 0-30 min.

**vastus lateralis (VL)**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 10  | 50  | 26,56 | 0,000 |
| Time*Intensity | 10  | 50  | 8,96  | 0,000 |

**vastus medialis (VM)**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 10  | 50  | 25,20 | 0,000 |
| Time*Intensity | 10  | 50  | 8,81  | 0,000 |

**biceps femoris (BF)**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 10  | 50  | 3,11  | 0,004 |
| Time*Intensity | 10  | 50  | 11,89 | 0,000 |

**gluteus maximus (Gmax)**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 10  | 50  | 0,95  | 0,496 |
| Time*Intensity | 10  | 50  | 10,49 | 0,000 |

**gastrocnemius lateralis (GL)**

|                | DF1 | DF2 | F    | sig.  |
|----------------|-----|-----|------|-------|
| Time           | 10  | 50  | 5,08 | 0,000 |
| Time*Intensity | 10  | 50  | 7,05 | 0,000 |

**tibialis anterior (TA)**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 10  | 50  | 3,14  | 0,003 |
| Time*Intensity | 10  | 50  | 11,50 | 0,000 |

Appendix VIII. ANOVA RM analysis of tHb concentration changes 0-15 min.

| <b>vastus lateralis (VL)</b> |      |       |       |       | <b>vastus medialis (VM)</b> |       |       |       |
|------------------------------|------|-------|-------|-------|-----------------------------|-------|-------|-------|
|                              | DF1  | DF2   | F     | sig.  | DF1                         | DF2   | F     | sig.  |
| Time                         | 2,04 | 24,44 | 14,80 | 0,000 | 2,43                        | 29,2  | 19,89 | 0,000 |
| Intensity                    | 1    | 12    | 0,23  | 0,640 | 1                           | 12    | 4,16  | 0,064 |
| Time*intensity               | 2,56 | 30,75 | 7,21  | 0,001 | 1,78                        | 21,34 | 5,98  | 0,011 |
| <i>G30 vs. G&lt;30</i>       |      |       |       |       |                             |       |       |       |
| Time*groups                  | 2,14 | 23,55 | 1,32  | 0,286 | 2,22                        | 24,4  | 1,68  | 0,206 |
| Intensity*groups             | 1    | 11    | 0,36  | 0,563 | 1                           | 11    | 1,92  | 0,149 |
| Time*intensity*groups        | 2,47 | 27,12 | 0,33  | 0,762 | 1,82                        | 20,06 | 2,44  | 0,117 |
| <i>Group 1 vs. Group 2</i>   |      |       |       |       |                             |       |       |       |
| Time                         | 1,78 | 39,13 | 8,99  | 0,001 | 2,1                         | 46,21 | 29,69 | 0,000 |
| Time*groups                  | 1,78 | 39,13 | 0,68  | 0,498 | 2,1                         | 46,21 | 0,54  | 0,593 |

| <b>biceps femoris (BF)</b> |      |       |       |       | <b>gluteus maximus (Gmax)</b> |       |       |       |
|----------------------------|------|-------|-------|-------|-------------------------------|-------|-------|-------|
|                            | DF1  | DF2   | F     | sig.  | DF1                           | DF2   | F     | sig.  |
| Time                       | 1,96 | 23,52 | 33,56 | 0,000 | 2,38                          | 28,5  | 32,47 | 0,000 |
| Intensity                  | 1    | 12    | 9,33  | 0,010 | 1                             | 12    | 4,02  | 0,068 |
| Time*intensity             | 2,18 | 26,11 | 4,37  | 0,021 | 1,78                          | 21,35 | 5,35  | 0,016 |
| <i>G30 vs. G&lt;30</i>     |      |       |       |       |                               |       |       |       |
| Time*groups                | 1,98 | 21,82 | 0,64  | 0,534 | 2,43                          | 26,67 | 1,74  | 0,190 |
| Intensity*groups           | 1    | 11    | 5,47  | 0,039 | 1                             | 11    | 0,01  | 0,947 |
| Time*intensity*groups      | 2,69 | 29,6  | 4,15  | 0,017 | 1,68                          | 18,45 | 0,53  | 0,566 |
| <i>Group 1 vs. Group 2</i> |      |       |       |       |                               |       |       |       |
| Time                       | 1,73 | 38,01 | 11,52 | 0,000 | 1,82                          | 40,05 | 16,42 | 0,000 |
| Time*groups                | 1,73 | 38,01 | 0,92  | 0,396 | 1,82                          | 40,05 | 1,44  | 0,248 |

| <b>gastrocnemius lateralis (GL)</b> |      |       |       |       | <b>tibialis anterior (TA)</b> |       |       |       |
|-------------------------------------|------|-------|-------|-------|-------------------------------|-------|-------|-------|
|                                     | DF1  | DF2   | F     | sig.  | DF1                           | DF2   | F     | sig.  |
| Time                                | 1,96 | 23,54 | 19,93 | 0,000 | 2,5                           | 30    | 12,02 | 0,000 |
| Intensity                           | 1    | 12    | 2,56  | 0,136 | 1                             | 12    | 33,86 | 0,000 |
| Time*intensity                      | 2,04 | 24,47 | 6,14  | 0,007 | 2,16                          | 25,94 | 17,87 | 0,000 |
| <i>G30 vs. G&lt;30</i>              |      |       |       |       |                               |       |       |       |
| Time*groups                         | 1,94 | 21,39 | 0,90  | 0,418 | 2,48                          | 27,24 | 0,87  | 0,451 |
| Intensity*groups                    | 1    | 11    | 1,47  | 0,251 | 1                             | 11    | 0,24  | 0,633 |
| Time*intensity*groups               | 2,09 | 22,99 | 1,21  | 0,319 | 2,08                          | 22,9  | 0,68  | 0,522 |
| <i>Group 1 vs. Group 2</i>          |      |       |       |       |                               |       |       |       |
| Time                                | 1,95 | 42,78 | 7,44  | 0,002 | 2,39                          | 52,61 | 18,73 | 0,000 |
| Time*groups                         | 1,95 | 42,78 | 0,07  | 0,925 | 2,39                          | 52,61 | 0,45  | 0,674 |



Appendix IX. ANOVA RM analysis of tHb concentration changes 0-30 min.

**vastus lateralis (VL)**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 10  | 50  | 25,24 | 0,000 |
| Time*Intensity | 10  | 50  | 19,22 | 0,001 |

**vastus medialis (VM)**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 10  | 50  | 28,49 | 0,000 |
| Time*Intensity | 10  | 50  | 1,89  | 0,068 |

**biceps femoris (BF)**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 10  | 50  | 12,43 | 0,000 |
| Time*Intensity | 10  | 50  | 3,30  | 0,002 |

**gluteus maximus (Gmax)**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 10  | 50  | 47,76 | 0,000 |
| Time*Intensity | 10  | 50  | 4,55  | 0,000 |

**gastrocnemius lateralis (GL)**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 10  | 50  | 17,44 | 0,000 |
| Time*Intensity | 10  | 50  | 2,76  | 0,009 |

**tibialis anterior (TA)**

|                | DF1 | DF2 | F    | sig.  |
|----------------|-----|-----|------|-------|
| Time           | 10  | 50  | 5,43 | 0,000 |
| Time*Intensity | 10  | 50  | 6,73 | 0,000 |

Appendix X. ANOVA RM analysis of TSI response 0-15 min.

|                            | <b>vastus lateralis (VL)</b> |       |       |       | <b>vastus medialis (VM)</b> |       |       |       |
|----------------------------|------------------------------|-------|-------|-------|-----------------------------|-------|-------|-------|
|                            | DF1                          | DF2   | F     | sig.  | DF1                         | DF2   | F     | sig.  |
| Time                       | 1,33                         | 14,58 | 28,58 | 0,000 | 1,8                         | 21,56 | 50,11 | 0,000 |
| Intensity                  | 1                            | 11    | 48,14 | 0,000 | 1                           | 12    | 33,53 | 0,000 |
| Time*intensity             | 1,8                          | 19,75 | 19,39 | 0,000 | 2,48                        | 29,77 | 17,87 | 0,000 |
| <i>G30 vs. G&lt;30</i>     |                              |       |       |       |                             |       |       |       |
| Time*groups                | 1,28                         | 12,8  | 0,19  | 0,727 | 1,73                        | 19,03 | 0,30  | 0,714 |
| Intensity*groups           | 1                            | 10    | 0,38  | 0,551 | 1                           | 11    | 0,03  | 0,863 |
| Time*intensity*groups      | 1,54                         | 15,44 | 0,97  | 0,379 | 2,42                        | 26,65 | 0,54  | 0,623 |
| <i>Group 1 vs. Group 2</i> |                              |       |       |       |                             |       |       |       |
| Time                       | 1,58                         | 33,08 | 61,41 | 0,000 | 1,92                        | 42,16 | 70,60 | 0,000 |
| Time*groups                | 1,58                         | 33,08 | 0,81  | 0,429 | 1,92                        | 42,16 | 1,17  | 0,323 |

|                            | <b>biceps femoris (BF)</b> |       |       |       | <b>gluteus maximus (Gmax)</b> |       |       |       |
|----------------------------|----------------------------|-------|-------|-------|-------------------------------|-------|-------|-------|
|                            | DF1                        | DF2   | F     | sig.  | DF1                           | DF2   | F     | sig.  |
| Time                       | 1,96                       | 23,56 | 11,96 | 0,000 | 1,95                          | 23,35 | 22,41 | 0,000 |
| Intensity                  | 1                          | 12    | 13,22 | 0,003 | 1                             | 12    | 12,01 | 0,005 |
| Time*intensity             | 1,87                       | 22,47 | 8,99  | 0,002 | 1,55                          | 18,62 | 44,57 | 0,000 |
| <i>G30 vs. G&lt;30</i>     |                            |       |       |       |                               |       |       |       |
| Time*groups                | 1,86                       | 20,45 | 0,41  | 0,653 | 1,98                          | 21,77 | 5,99  | 0,009 |
| Intensity*groups           | 1                          | 11    | 8,69  | 0,013 | 1                             | 11    | 0,64  | 0,441 |
| Time*intensity*groups      | 1,72                       | 18,9  | 0,80  | 0,447 | 1,5                           | 16,51 | 2,17  | 0,154 |
| <i>Group 1 vs. Group 2</i> |                            |       |       |       |                               |       |       |       |
| Time                       | 1,88                       | 41,27 | 42,41 | 0,000 | 1,99                          | 43,68 | 46,16 | 0,000 |
| Time*groups                | 1,88                       | 41,27 | 0,66  | 0,511 | 1,99                          | 43,68 | 0,31  | 0,947 |

|                            | <b>gastrocnemius lateralis (GL)</b> |       |       |       | <b>tibialis anterior (TA)</b> |       |       |       |
|----------------------------|-------------------------------------|-------|-------|-------|-------------------------------|-------|-------|-------|
|                            | DF1                                 | DF2   | F     | sig.  | DF1                           | DF2   | F     | sig.  |
| Time                       | 2,15                                | 25,8  | 21,56 | 0,000 | 3,27                          | 39,22 | 8,91  | 0,000 |
| Intensity                  | 1                                   | 12    | 13,67 | 0,003 | 1                             | 12    | 17,51 | 0,001 |
| Time*intensity             | 2,93                                | 35,18 | 11,44 | 0,000 | 3,17                          | 38,02 | 11,42 | 0,000 |
| <i>G30 vs. G&lt;30</i>     |                                     |       |       |       |                               |       |       |       |
| Time*groups                | 2,28                                | 25,1  | 2,23  | 0,122 | 3,26                          | 35,85 | 0,56  | 0,657 |
| Intensity*groups           | 1                                   | 11    | 2,69  | 0,129 | 1                             | 11    | 6,63  | 0,026 |
| Time*intensity*groups      | 2,89                                | 31,81 | 0,34  | 0,790 | 3,06                          | 33,66 | 0,48  | 0,699 |
| <i>Group 1 vs. Group 2</i> |                                     |       |       |       |                               |       |       |       |
| Time                       | 3,02                                | 66,33 | 27,36 | 0,000 | 2,87                          | 63,07 | 31,32 | 0,000 |
| Time*groups                | 3,02                                | 66,33 | 1,36  | 0,263 | 2,87                          | 63,07 | 1,12  | 0,355 |

Appendix XI. ANOVA RM analysis of TSI response 0-30 min.

**vastus lateralis (VL)**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 10  | 40  | 16,59 | 0,000 |
| Time*Intensity | 10  | 40  | 13,73 | 0,000 |

**vastus medialis (VM)**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 10  | 50  | 22,19 | 0,000 |
| Time*Intensity | 10  | 50  | 15,89 | 0,000 |

**biceps femoris (BF)**

|                | DF1 | DF2 | F    | sig.  |
|----------------|-----|-----|------|-------|
| Time           | 10  | 50  | 4,23 | 0,000 |
| Time*Intensity | 10  | 50  | 3,34 | 0,002 |

**gluteus maximus (Gmax)**

|                | DF1 | DF2 | F    | sig.  |
|----------------|-----|-----|------|-------|
| Time           | 10  | 50  | 7,20 | 0,000 |
| Time*Intensity | 10  | 50  | 7,97 | 0,000 |

**gastrocnemius lateralis (GL)**

|                | DF1 | DF2 | F     | sig.  |
|----------------|-----|-----|-------|-------|
| Time           | 10  | 50  | 4,69  | 0,000 |
| Time*Intensity | 10  | 50  | 10,13 | 0,000 |

**tibialis anterior (TA)**

|                | DF1 | DF2 | F    | sig.  |
|----------------|-----|-----|------|-------|
| Time           | 10  | 50  | 3,61 | 0,001 |
| Time*Intensity | 10  | 50  | 7,21 | 0,000 |