Abstract

When exercise intensity increases, the oxygen demand of the working muscles also has to increase. After a while the musculature will become progressively deoxygenated, and with the use of near-infrared spectroscopy (NIRS) these mechanisms can be measured and give valuable information about how the musculature is affected by exercise intensity in the current exercise. This study investigated how different oxygenation patterns in several muscles is affected by exercise intensity. Eighteen healthy subjects performed an incremental exercise test (25watt/min, applied as 0.83 every 2 seconds) to voluntary exhaustion. The muscles (i.e., vastus lateralis and tibialis anterior bilaterally) were measured by the use of NIRS. The oxygen uptake was measured by using breath-by-breath. A two-way repeated anova showed significantly differences between the deoxygenated haemoglobin breakpoints in the muscles but no differences between legs. Breakpoints in vastus lateralis occurred at a higher exercise intensity than tibialis anterior. The study showed that the slopes in vastus lateralis and tibialis anterior acted differently, this was tested by using a paired sample t-test that revealed significantly differences between the slopes before and after the breakpoint occurred in all muscles, in both legs. The study showed that there are differences in how exercise intensity affects the different muscles during increasing exercise in cycling.

Sammendrag

Når treningsintensiteten øker vil kravet til oksygen for musklene øke, etter en stund vil muskulaturen gradvis bli deoksygenert og nærme seg sitt maksimale utbytte. For å måle oksygenering i muskulaturen kan man bruke Nær-infrarød spektroskopi (NIRS) som gir verdifull informasjon om hvordan muskulaturen påvirkes av treningsintensitet i den gitte aktiviteten. Denne studien så på hvordan oksygenering av forskjellige muskler (dvs., vastus lateralis og tibialis anterior bilateral) blir påvirket av treningsintensitet. Atten friske subjekter utførte en trinnvis treningstest (25 watt/ minutt, økt med 0.83 watt hvert 2 sekund) inntil frivillig utmattelse. Det ble gjort målinger av oksygenering i fire muskler (vastus lateralis og tibialis anterior bilateralt, høyre og venstre fot), ved bruk av NIRS. Oksygenopptaket ble målt ved å bruke breath-by-breath. En toveis repeterende anova viste at det var signifikante forskjeller mellom knekkpunktene i deoxygenert hemoglobin (Hhb) i de målte musklene, men ingen forskjell mellom sidene. Knekkpunktene i vastus lateralis kom litt senere enn hva de gjorde i tibialis anterior bilateralt. Det ble oppdaget at stigningstallet på linjene hos vastus lateralis og tibialis anterior muskelen var forskjellige. For stigningstallene viste en paret samlede t-test signifikante forskjeller mellom stigningstallet før og etter knekkpunktene i alle musklene. Denne studien viser at det er forskjeller på hvordan forskjellige muskler reagerer på en økende ytre belastning under sykling.

Table of contents

Introduction

The effect exercise has on the acute whole-body physiological responses as pulmonary gas exchange, cardiac output and arterio-venous O_2 differences can provide information on the function of the pulmonary, cardiovascular and metabolic system. The technological advances in the past three decades has given us the opportunity of studying the oxygenation patterns in the muscles (1).
The insight in acute physiological responses in muscles to changes in exercise intensity might give valuable information about the functional capacity and exercise tolerance/intolerance in healthy subjects. Additionally, an integration of the physiological systems determines the relationship between oxygen supply to demand oxygen uptake (VO2) and allows the individual to adjust to changes in metabolic demand(2). The concentration of oxygenated and deoxygenated haemoglobin and myoglobin responses gives the information needed for calculating the total amount of tissue haemoglobin and tissue saturation (1).

Oxygenated haemoglobin (O_2Hb) represents blood with oxygen(3), deoxygenated haemoglobin (Hhb) represents blood without oxygen and tissue saturation index represents the absolute amount of the O_2Hb(1). These measurements can be measured by the use of near-infrared spectroscopy (NIRS), that is a useful non-invasively measurement (4). Due to the fact that the movement ability of the measured limb is painless when nothing is penetrated through the skin, respectively, makes this equipment valuable to use when participants are going to push themselves to fatigue. NIRS can provide information in local oxygen consumption and delivery at muscle level by sending wavelengths in a region between 700-1300 mm and the near-infrared region absorption of both haemoglobin and myoglobin is less, and therefore, a significant amount of the infrared light can effectively travel through the tissue (5). NIRS can therefore measure the oxygenation inside the muscle during different exercise intensities, and show what happens when the musculature becomes progressively deoxygenated as O_2 delivery will decrease throughout the an exercise with progressive increase in intensity (6).

Total haemoglobin and myoglobin contributes to the information on the increase in haematocrit of the microcirculation with exercise (2). Furthermore, the oxygenation responses to incremental exercise have been measured for the last three decades and it started in the 1990s by Belardinelli et al (1995), Bhambhani et al (1997,1998) and Grassi et al (1999) (7-10). They mainly focused on the O_2Hb of the vastus lateralis (VL) during an incremental exercise cycling test (1). The most typically measured muscles in the lower limb, in cycling, are vastus lateralis, vastus medialis, gastrocnemius lateralis, gastrocnemius medialis (3), biceps femoris and rectus femoris (11). To the authors knowledge, there is some research papers that measures the tibialis anterior and the hamstring muscle, but there is fewer than for the other mentioned muscles in the lower limb.

As the local muscle oxygenation responses to exercises started getting tested in the early 1990s there has also been developed different methods to analyze and evaluate these responses to exercise. First we have the sigmoid model, a "S"-curve model that implies that the concentration of haemoglobin and myoglobin responses has a symmetrical lower and upper curvatures for which there is no physiological justification (1). There is also the double linear model that is more line-shaped and it is more able to show a

breakpoint. These models are complex and often very specific to the individual and the exercise protocol. Therefore, when investigating the differences in between muscles during an incremental exercise with a high increase in workload, one should use a model that can show what happens before and after the breakpoints occurs (12).

Boone et al (2016) found a strong correlation between the breakpoints in O_2Hb and Hhb related to the VO_{2max} as the responses from the function of metabolic rate or work rate is shifted to the right in subjects with a higher peak oxygen uptake (1). This is further by research that found a rightward shift of the O_2Hb and Hhb relative to the percent of peak oxygen uptake in a group of cyclists compared to a group of physically active students (4). However, the rightward shift in the muscle oxygenation may be more detectable for more trained individuals than for untrained individuals (13).

In the past decades NIRS has been used to understand the skeletal muscle physiology by investigating the upper-and lower-limb muscles (11). An investigation on the oxygen saturation heterogeneity on the left leg muscles (i.e., vastus lateralis, gastrocnemius medialis, gastrocnemius lateralis and tibialis anterior) found that the blood flow and metabolic demand differs between the four measured muscles in the left leg. It was also found that although the VL muscle contributes more during cycling, the other muscles were also enhanced as they were deoxygenated. It was proposed that possibly that the responses that differs may have been influenced by the anatomical differences and which role the different muscles has during cycling(14). There has been some investigating on the differences between the left and right leg, were they tested the dominant and nondominant leg in one-leg cycling. This research showed that the dominant leg had a greater power output than the other leg had (15). Iannetta (2019) looked at the difference between the vastus lateralis in the dominant and nondominant leg and found that the dominant leg was able to sustain a greater power output when comparing it to the nondominant leg. This result came when comparing the single-leg counterweighted cycling and is reflecting the critical intensity. When comparing the dominant and nondominant leg there were also found a strong correlation between Hhb and VO_{2peak} which was higher in the dominant leg versus the non-dominant leg.

Therefore, to the authors knowledge there has been no previous studies looking at the oxygenation responses in several muscles in both legs at the same time (i.e., vastus lateralis and tibialis anterior). The studying of the oxygenation patterns in different muscles during changes in exercise intensity is highly relevant to understanding the key aspects of metabolic and vascular control (1).

The purpose of this study is to examine how the oxygenation of different muscle in the thigh (vastus lateralis) and shank (tibialis anterior) in both legs are affected by exercise intensity. It is hypothesized that there will be a difference between vastus lateralis and tibialis anterior, mainly because of the anatomical differences and the different functions the muscles has during cycling.

Method

Participants

In total, 20 healthy subjects came to the lab and performed an incremental exercise test on an ergometer bicycle, due to some missing data only 18 subjects (body mass 82.5 ± 30.5 kg; height 172 ± 20 cm) were taken to further analysis. The subjects were informed before the study about the aim and that they at any time could drop out. They had to sign an informed consent approved by Norwegian center for research data (NSD) before the study could start. Participants were required to attend the laboratory in a rested state, they were advised to refrain from any training on the lower body 24 hours before the test trial.

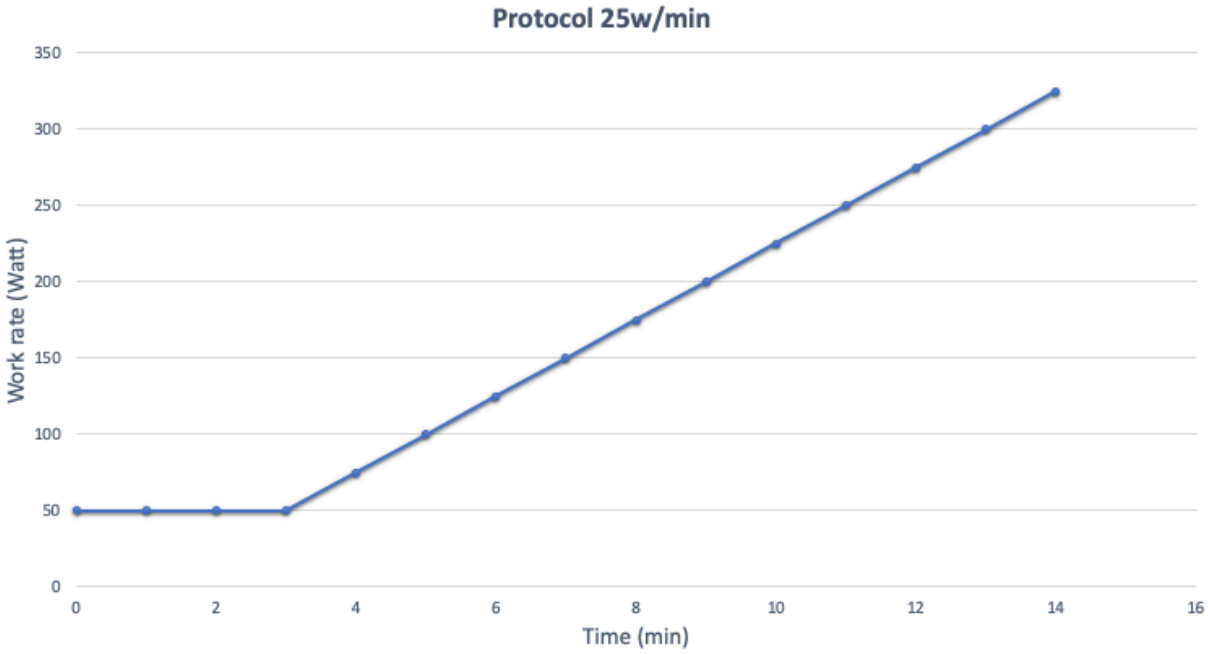
Protocol

The protocol is graphically presented in figure 1**.** An incremental exercise test until volitional exhaustion was performed on an electromagnetically braked cycle (Lode B. V., Groningen, Netherlands).

When participants came to the lab the body mass and height were measured, afterwards the participants adjusted the distance from seat to handlebar, handlebar height and the height of the seat at the ergometer cycle to make sure they were comfortable for their fit. Afterwards the NIRS equipment was fastened to the belly of VL and TA in both legs, then a five-minute rest for baseline measurements was preformed while the subject was seated on a chair. When the participants were ready, they got help for fastening their feet to the pedals with straps or they did it alone if they had sport-cycling shoes.

The protocol had a three-minute warm-up sequence at a workload of 50 watts (W) followed by a ramp incremental exercise protocol at 25 W/min (applied as 0.83W every 2 seconds). Throughout the test, subjects were asked to cycle at a pedal frequency over 60 rpm, if they dropped below 60 rpm despite strong verbal encouragement the test ended. The subjects were asked before the test, in which way they liked to be encouraged so they could perform their best. After the test there was performed a lactate measurement on the ring fingertip at the left hand, then when the NIRS equipment was taken off, the adipose tissue where the NIRS had been was measured.

Figure 1. Averaged Protocol



A graphic presentation on the average lasted time and watt achievements for all the subjects included in the study. It increases with 25W/min (0.83 W every 2 seconds) until the subjects dropped below 60 rpm. Every subject had a warm-up sequence for three-minutes at 50 watts before the workload began to increase.

Measurements

NIRS

Muscle oxygenated and deoxygenated haemoglobin was measured by four continuous wave NIRS (Portamon, Artinis Medical System, the Netherlands). The NIRS probe transmits wavelengths of 762 – 841 nm. These wavelengths are used due to their ability to penetrate biological tissue and are mainly absorbed by hemoglobin and myoglobin (5). The probe was positioned longitudinally on the muscle belly of vastus lateralis (VL) and tibialis anterior (TA) at the left and right leg. The VL muscle was chosen for the involvement it has during cycling exercise, TA muscle was chosen for the lack of published information and additionally for its role in cycling as it helps to control the foot during pedal stroke(16). The NIRS system was calibrated and the skin was carefully shaved. The NIRS was fasted on the participants leg by using an elastic bandage and covered with a black cloth in order to minimize the amount of ambient light detected by the optodes.

A skinfold caliber (Holtain Skinfold caliper, Holtain Ltd, Crymych, Wales) was used to measure the adipose tissue at VT and TA where NIRS probe had been. This was performed after the test and when the subject was seated.

NIRS has three transmitters that gives three different signals, called T1, T2 and T3 and are used for further analysis. These three transmitters sends signals into the skin, further to the muscle and then back to a receiver in a banana formed path. Figure 2 presents a painted NIRS device that shows that the transmitters sends the signal form T1, T2 and T3 through the muscle and to the receiver in a banana formed shape.

Figure 2. NIRS

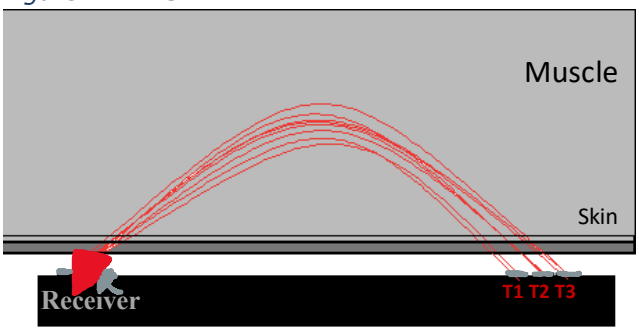


Figure 2. A graphical presentation of the behavior of NIRS. Presents the three transmitters, T1, T2 and T3 that sends infrared lights (presented as red threads) to the muscle (presented as a light grey box) and to the receiver. The infrared light goes through the skin (dark grey area in the box), muscle, skin and then to the transmitter in a form shaped as a banana.

Pulmonary oxygen consumption

Oxygen uptake was measured using Oxycon Pro™ (Oxycon, Jaeger GmbH, Hoechberg, Germany). The flow turbine (Triple V, Erich Jaeger) was calibrated before each test using a 3L calibration syringe (5530 series; Hans Rudolph, Kansas, MO, IL, United states). Calibration of the conditions, volume (the flow turbine) and calibration of the gas analyzer were performed by using a standardized gas concentration of 5% CO_2 and 15% O_2 before testing.

Lactate

When the test had ended, a lactate measurement was preformed within two minutes after the test was finished. A single blood sample was collected from the ring finger on the left hand, the fingertip was cleaned to remove sweat and dirt before sticking. The first blood drop was discarded with a clean paper and the second blood drop was used for measured the blood lactate concentration with a portable lactate measurement (Lactate Pro 2 Arkray). The goal was that everyone had a lactate concentration over 8mmol/l, due to one of the criteria to reach VO_{2max}.

Data analysis

Data from NIRS was filtered by using an eight-order butterworth lowpass filter with a cutoff frequency at 0.1 Hz and this was sampled at 10 Hz. The data was normalized in to 100 samples by taking 1 percent of the ramp test, excluding warm up, this was used for further analyses and it made it easier to compare different subjects with different time to exhaustion. For all NIRS signals, a linear regression was applied from the beginning of the signal to the end, the linear regression sets two lines throughout the data were the sum of the error and sum of square, this makes a regression value, the regression line that was closest to one was determined as a breakpoint and were used for further analysis.

All data was visually checked prior to data break point determination. Initially the breakpoint (NIRS) determination was done from 5% of the test duration and onward, but if the visual check determined it necessary due to clear artifacts, the start of the breakpoint determination was adjusted to 30(n=6), 40 (n=5) or 45 (n=1) of the test duration. The values were used for further analysis during statistics.

The obtained breath-by-breath values were averaged into 10^{th} second intervals and these values were used for further analysis. VO_{2max} was not accomplished by most of

the participants so instead there was used values from VO_{2peak}. VO_{2peak} was calculated in excel (version 16.35) by taking the mean of one-minute intervals for every 10'th sec from the measure v'o2 ml/min, afterwards we found the highest calculated mean value. The highest value was divided by the relative to body mass of the person gave the VO_{2peak} values.

Ventilatory threshold (VT) was calculated by using Matlab R2018b (MarhWorks inc. Natic, USA) by taking the relationship between VO_2 and oxygen carbon dioxide (VCO_2), for a valid VT the minute ventilation (VE)/VO_2 had to increase before an increase in VE/VCO_2, this was visually checked afterwards. The respiratory compensation point was calculated by taking the relationship between VE and VCO_2. VCO_2 was plotted against VE and two regression lines fit to the data were visually checked afterwards. A resample function in Matlab R2018b was also used to get the different datafiles into 100 dots, by making one point per percent of the ramp test, which made it easier to compare the different datafiles from the different subjects to each other.

Statistical analysis

The breakpoints from Hhb and TSI was tested by using a two-way ANOVA with setting muscle and leg against each other to test if there were any differences between them. If the assumption of shericity was violated, results were adjusted according to Greenhouse-Geisser. The breakpoints were evaluated in the different muscle and between the legs. This was done to evaluate the main effect of the muscle, main effect of the leg and if there was any interaction effect between muscle and leg. This was performed by a two-way ANOVA.

Data from first and second slope (before and after the breakpoints), were tested by using a paired sample t-test for testing the mean differences and if there were any significant differences between one muscle and between both muscles for each leg.

A correlation test between the different measurements was preformed to test if there was a positive significantly correlation between them. The results are presented as mean ± SD and all results were taken to further analysis.

Results

The test lasted on average 14 ± 5 minutes including three-minutes baseline warm-up cycling, with an average maximum load at 325 ± 125 W. The VO2peak values was on average at 46.5 ± 11.9, VT values were on average 57.96 ± 17.05 and RCP values were at average 64.07 ± 17.22.

Oxygenation responses

The two-way repeated ANOVA revealed a significant difference for deoxygenated haemoglobin (Hhb) between muscle ($p = 0.008$), but no significant difference between legs ($p=0.143$) or for the interaction effect between leg and muscle ($p=0.761$). There were no significant differences for tissue saturation index (TSI) between muscle ($p=0.766$), leg ($p=0.821$) or for the interaction effect between leg and muscle ($p=0.815$). Breakpoints and amplitudes for Hhb and TSI are presented in table 1, figure 3 presents the Hhb responses from one subject in percent and figure 4 presents the TSI responses from one subject in percent of the test were the breakpoints occurred for each muscle at each leg during the incremental exercise test.

Looking at the figures, especially figure 4 and figure 6, it showed that VL and TA in both deoxygenated and tissue saturation index had a different pattern before and especially after the breakpoint occurred. Whereas the VL flattens out and gets an "plateau" after the breakpoint as TA still increases after the breakpoint in Hhb and for TSI VL still flattens but TA keeps decreases for TSI, this is better showed in figure 4 where it is clear that TA keeps decrease and VL flattens out after the breakpoint occurs. The results from the paired sample t-test is represented in table 2, where the mean values from the first- and second slope for Hhb and TSI for all muscles are presented, all tested samples was significantly different from each other. The statistical test was preformed within the muscle but also muscle against muscle for comparing them. As an example, TA first slope versus VL first slope was checked if there were statistical alike or if there were any differences. This was also preformed within the muscle to test if there were differences in between first and second slope.

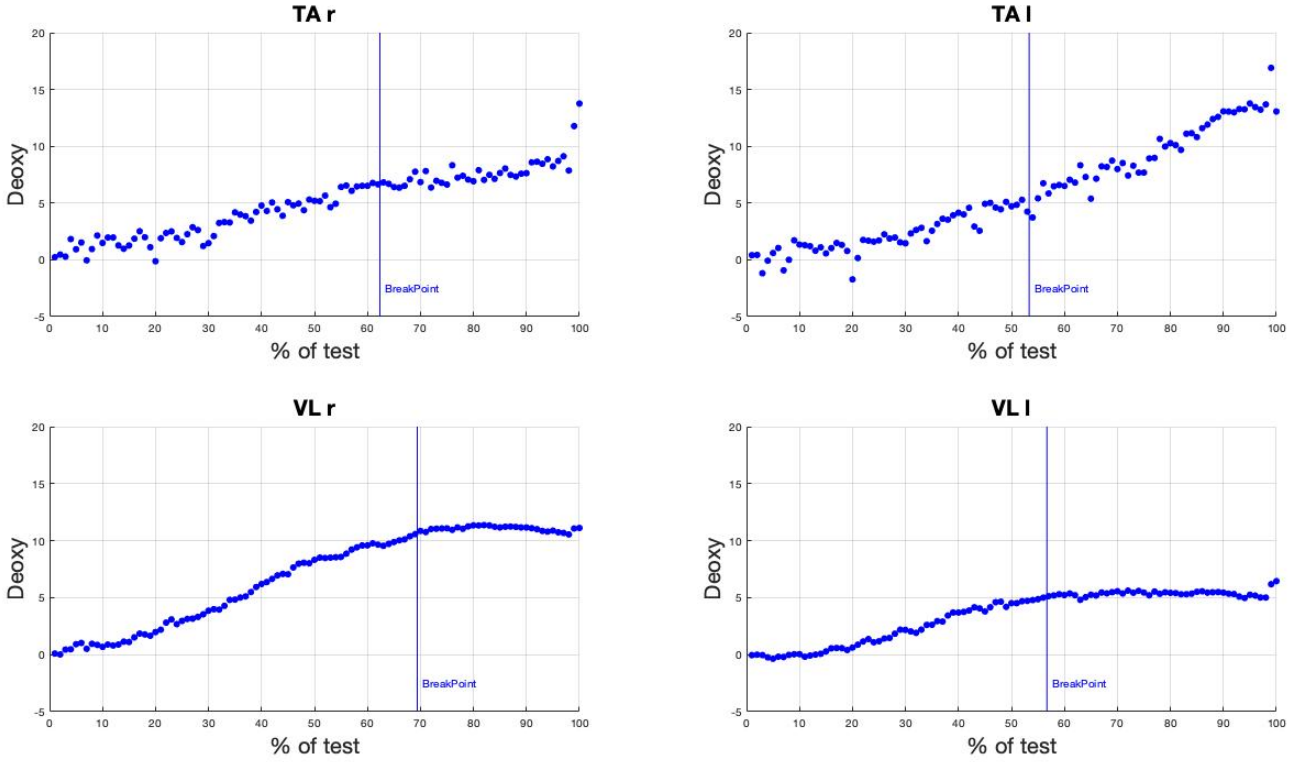
A pearson correlation analysis were preformed to test if there were any significantly correlations between the muscles. The results are presented in table 3, were Hhb is the only measurement that are presented, this is because the TSI had no correlation between the different muscles during this analysis.

Table 1: Break points and amplitudes in deoxyhaemoglobin and tissue saturation index

	Right		Left	
	Vastus Lateralis	**Tibialis Anterior**	**Vastus Lateralis**	**Tibialis Anterior**
BP_{HHb}	71.7 ± 9.4	65.3 ± 7.8*	68.3 ± 10.1	63.0 ± 16.4*
BP_{tsi}	67.3 ± 9.5	67.4 ± 9.1	67.3 ± 8.7	66.7 ± 11.9
Amp_{HHb}	7.2 ± 5.3	4.4 ± 4.1*	6.6 ± 5.2	3.1 ± 2.7*
Amp_{tsi}	63.6 ± 18.1	60.9 ± 4.5	68.9 ± 9.6	60.7 ± 3.15

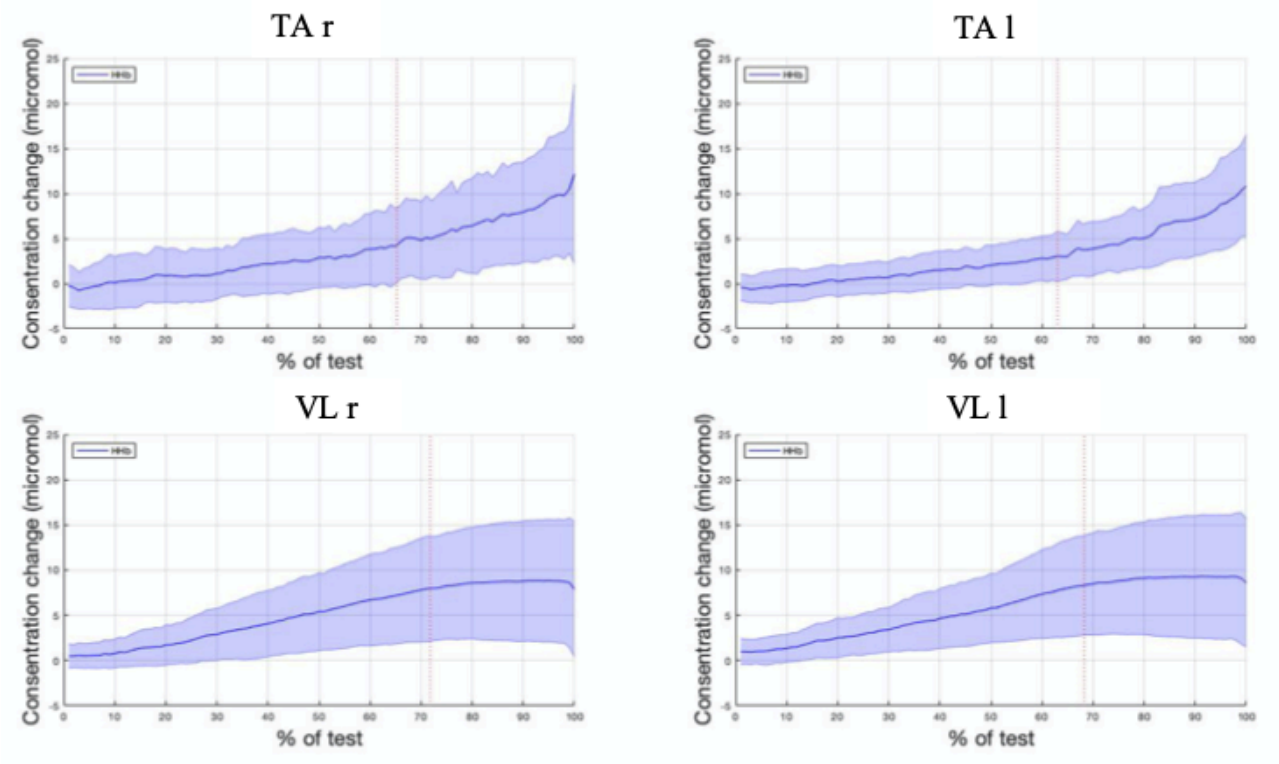
Mean and standard deviation results of breakpoints (BP) in deoxygenated haemoglobin (Hhb) and tissue saturation index (TSI) in both legs (right and left) and both muscles (vastus lateralis and tibialis anterior) for the first two rows. The results of the breakpoints are presented in percent of when during the test a breakpoint occurred. The two last rows represent the mean amplitudes (Amp) and standard deviation results for Hhb (presented as µmol) and TSI (presented in percent) for both muscles and legs. * indicated a significant difference from vastus lateralis ($p<0.05$).

Figure 3. Deoxygenated haemoglobin for one subject



Breakpoints for deoxygenated haemoglobin (HHb) in the tibialis anterior in the right leg (TA r), TA left leg, VL right leg, VL left leg as a function of the relative duration of the cycling ramp test for one subject. HHb is expressed in change in micromole from the average during the 3-minute warm up prior to the ramp test.

Figure 4. Deoxygenated haemoglobin for all subjects



Breakpoints for deoxygenated haemoglobin (HHb) in the tibialis anterior in the right leg (TA r), tibialis anterior left leg (TA l), vastus lateralis right leg (VL r), vastus lateralis left leg (VL l) as a function of the relative duration of the cycling ramp test for all subjects. HHb is expressed in change in micromole from the average during the 3-minute warm up prior to the ramp test

Table 2. Mean values from first and second slope for deoxyhaemoglobin and tissue saturation index

		Right				**Left**			
		Vastus Lateralis		**Tibialis Anterior**		**Vastus Lateralis**		**Tibialis Anterior**	
First	$Mean_{Hhb}$	0.14	± 0.12*	0.08	± 0.07*	0.13	± 0.09*	0.06	± 0.05*
	$Mean_{TSI}$	-0.18	± 0.13*	-0.09	± 0.09*	-0.19	± 0.09*	-0.06	± 0.06*
Second	$Mean_{Hhb}$	0.04	± 0.08*	0.16	± 0.10*	0.03	± 0.08*	0.22	± 0.14*
	$Mean_{TSI}$	-0.03	± 0.11*	-0.21	± 0.11*	-0.01	± 0.08*	-0.27	± 0.11*

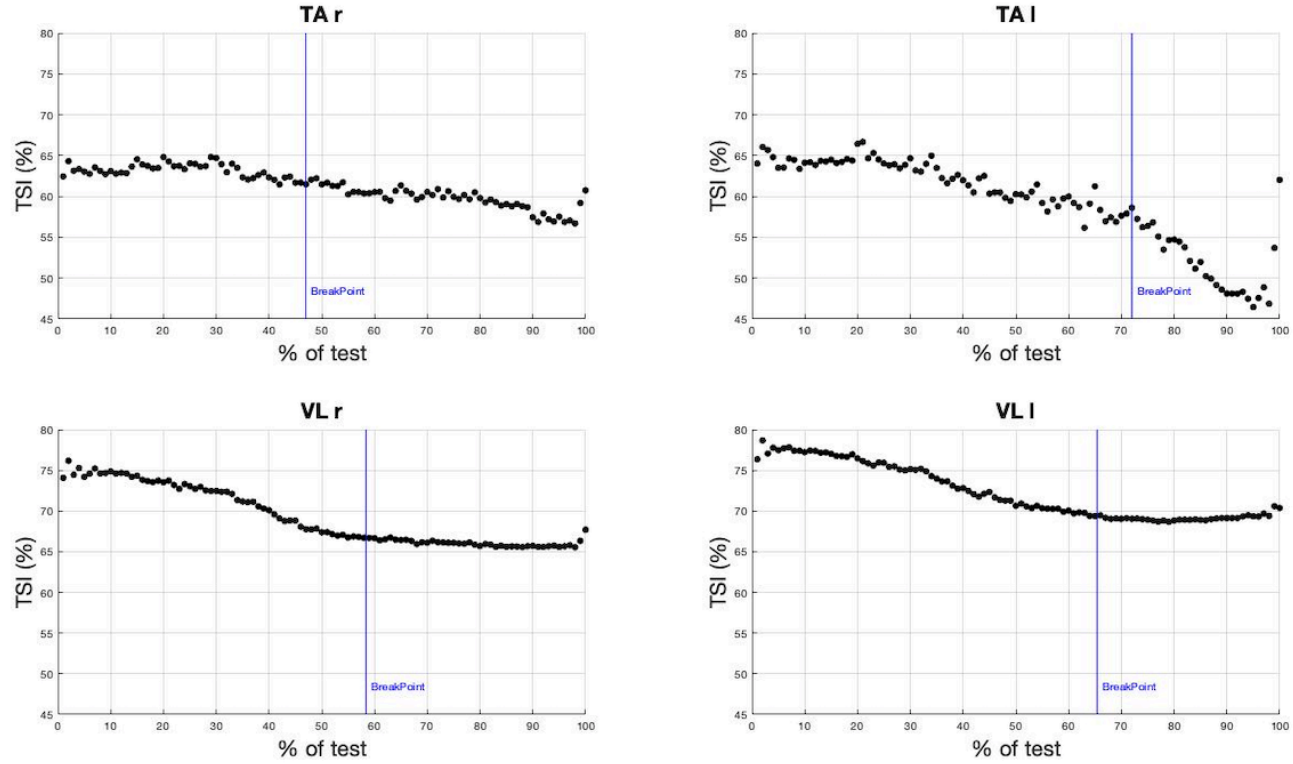
Table 1 presents the mean values for first and second slope, the slope before and after the breakpoint in both legs and muscles. * indicates a significant difference between the slopes ($p < 0.05$). These values represent the deoxygenated haemoglobin (Hhb) that are represented in µmol and tissue oxygen saturation (TSI) that are represented in percent slopes for the averaged prior to the ramp exercise test.

Table 3. Pearson correlation analysis, p-values

	Right		**Left**	
	Vastus Lateralis	**Tibialis Anterior**	**Vastus Lateralis**	**Tibialis Anterior**
VL R		0.341	0.429	0.581*
VL L	0.429	0.499*		0.557
TA R	0.341		0.499*	0.650*
TA L	0.587*	0.650*	0.557*	

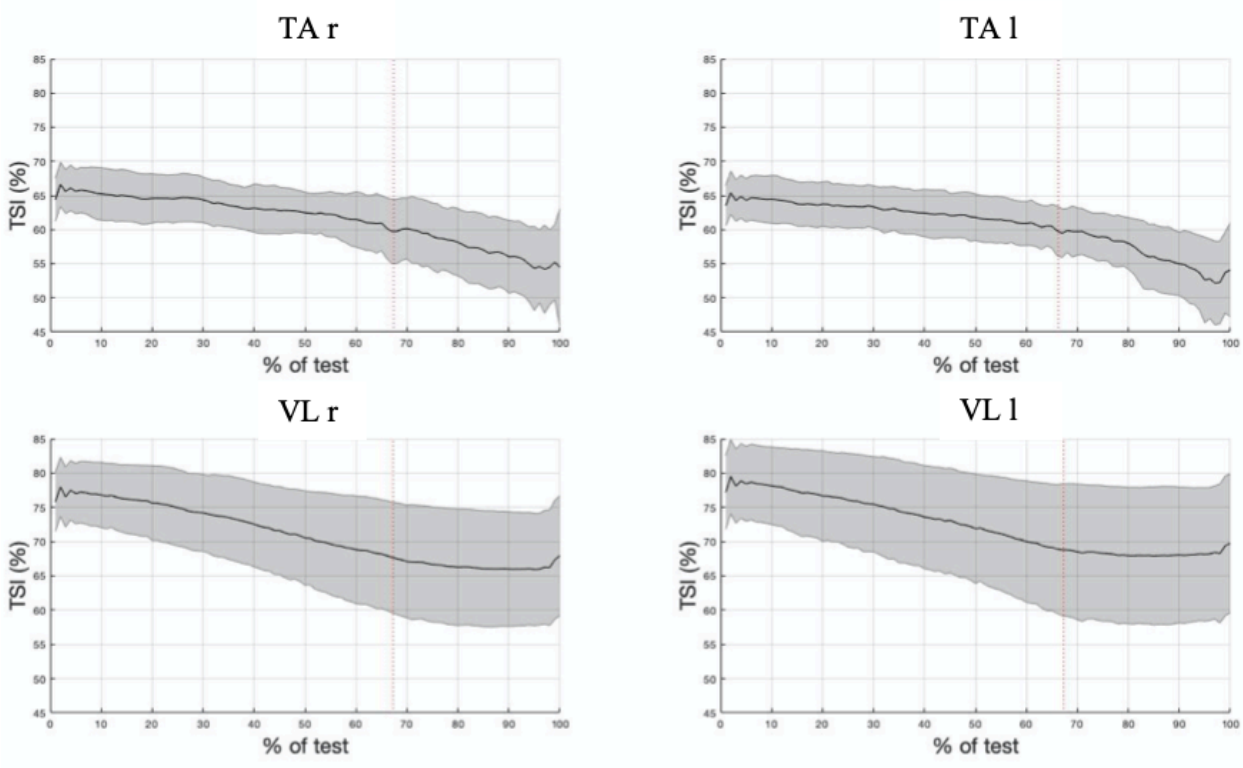
Values presents the R-value for deoxygenated haemoglobin (Hhb) from the Pearson correlation analyses between vastus lateralis (VL) and tibialis anterior (TA). * indicates a significant correlation between the muscles ($p < 0.05$). There was performed an correlation test on tissue saturation index as well, but there were no significantly correlations between the different muscles.

Figure 5. Tissue saturation index for one subject



Breakpoints for tissue saturation index (TSI) in the tibialis anterior in the right leg (TA r), tibialis anterior left leg (TA l), vastus lateralis right leg (VL r) and vastus lateralis left leg (VL l) as a function of the relative duration of the cycling ramp test for one subject. TSI is expressed as percent from the average during the 3-minute warm up prior to the ramp test

Figure 6. Tissue saturation index for all subjects



Breakpoints for tissue saturation index (TSI) in the tibialis anterior in the right leg (TA r), tibialis anterior left leg (TA l), vastus lateralis right leg (VL r) and vastus lateralis left leg (VL l) as a function of the relative duration of the cycling ramp test for all subjects. TSI is expressed in percent from the average during the 3-minute warm up prior to the ramp test

Discussion

This study investigated if there were any differences in the effect of exercise intensity in the thigh (vastus lateralis) and shank (tibialis anterior) muscles in both legs during incremental cycling exercise. The main finding in this study was that there were differences between the breakpoints in Hhb for both muscles, where the breakpoint in VL occurred later than the breakpoint in TA. It was no differences found between the legs during this study. The second finding was that there were differences in between the slopes before and after the breakpoint occured in the different muscles for both Hhb and TSI. Where it was clear that VL and TA were acting differently due to the exercise intensity. As an example, VL second slope Hhb had an increase of 0.03 and TA second slope Hhb had an increase at 0.22.

Oxygenation differences

This study showed significant differences between breakpoints in VL and TA, this may be due to the anatomical and functional differences. This study, hypothesized that there would be a difference between the muscle because of the anatomical and functional differences (14) and this is seen in other researches as well. The VL muscle is seen as one of the main working muscles during cycling (6), as for the TA muscle is thought to be contributing to transfer power to the crank and for controlling the foot during pedal

strokes (16). There is well known that different muscles have different tasks during the cycling exercise, due to this Wang et al (2012) studied the differences in breakpoints in VL and gastrocnemius lateralis (GL). The increase in intensity showed that breakpoints in VL comes earlier than GL and that it may therefore indicate that the oxygen supply-consumption balance in GL had a later breakpoint than VL during an incremental exercise (17). The representative study shows the opposite of what the study from Wang et al (2012) shows, as the breakpoint in VL comes a bit earlier than TA when visually see figure 4, 6, and the breakpoints values in table 1 where the percent of when the breakpoints are presented. The difference between GL and TA, as they both are shank muscles, are in which way they are contributing to the cycling exercise. As the GL contributes to the ankle stabiliser, respectively, and due to the small anatomical differences for these shank muscles there is also a difference for when the breakpoints occur during incremental exercise. Additionally, breakpoints in different muscles measured by electromyography (EMG) has shown that the breakpoints occurs after the RCP (18), this is the same as shown in this study for two of the muscles. VT muscles breakpoint (left leg: 68.27%, right leg: 71.75%) occurred in percent a bit after RCP (66.02%) but for the TA muscles (left leg: 63.04%, right leg: 65.26%), the breakpoint occurred before RCP but after VT (51.73%). There has been shown that pulmonary oxygen uptake with HHb breakpoints due to incremental exercise is strongly associated with RCP (19), and the study from Keir et al (2015) tested if RCP (and several more threshold) and Hhb breakpoint occurred at the same pulmonary oxygen uptake. Their results mainly demonstrated the relation between the metabolic and physiological responses and the pulmonary oxygen uptake values that was associated with different thresholds (i.e., RCP, Hhb, CP and maximal lactate steady state) suggesting that they may share a common underlying mechanistic link. This is way beyond the topic of this study, but an interesting view on a possible explaining on why RCP doesn't come after the breakpoints in VL. However, the breakpoint in TA may occur before VL due to the metabolic demand. As the work rate increases, the metabolic demand of VL will maybe be greater from the start of the exercise until almost exhaustion but for TA it will be more demanding to the end of the exercise. Previous discussions indicate (12, 13, 20) that based on observations of the total haemoglobin responses, it has been observed that this responses compared to Hhb had a lower ceiling at a lower intensity. There was uncertainty whether the muscle oxygen delivery can further increase at a high intensity. Boone et al (2016) keeps discussing this and shows that there are several studies that supports this, and that the respiratory muscles may contribute to limit blood flow to the locomotor muscles, and that based on this, the increase in pulmonary oxygen uptake above the breakpoint in Hhb can only contribute to an increased metabolic load of other muscles for supporting either the ventilation or the locomotion. This might be helpful to understand the differences found in this study, that TA is more supporting the VL so it can contribute at a higher level, but it might also have been a positive change for the body if it could "decide" to deoxygenated TA more so it had more power to use for VL at the end of the exercise. Therefore, it may be anatomical differences, movement differences or just the differences in muscle fibre type (21) that can explain physiologically why there are differences between the breakpoints in Hhb.

Differences between legs

There were no differences between the legs during this study, and in cycling were both leg attributes to generate power there is evidence that says that one leg contributes more to the exercise than the other. This was tested by Iannetta et al (2019) where they tested one-leg cycling and original two-leg cycling, they found a significantly differences

in the dominant leg versus the nondominant leg (15). Despite the fact that muscle activation patterns during cycling are reported unaffected by leg dominance, it may be the underpinned differences in musculoskeletal and motor control deficits on the nondominant-leg (22). There has been findings that shows that the dominant leg achieves a greater VO_{2peak} during single leg exercise, and that it was able to sustain a higher power output with stable metabolic responses when compared with the nondominant leg (15). There is important to have in mind when discussing this that the exact physiological reasons for these differences are difficult to establish (15). Additionally, the double leg cycling showed differences in the amplitude for the nondominant and dominant leg for Iannetta et al (2019) study, there are differences for the amplitudes for this study as well, but the differences may not be associate with nondominant and dominant leg. This is difficult to answer due to the fact that there were not taken any pre-test for testing which leg is dominant and which leg that are not dominant. Additionally, there seems to be lack of evidence that there are differences between legs during a double leg cycling exercise but when doing one-leg cycling exercise it can show differences.

Slope differences

During visual inspection of the figures representing different oxygen responses (figure 3,4,5 and 6) the observation was that the muscles were acting differently in the figures before and especially after the breakpoints for the slopes in both Hhb and TSI. Where VL flattens out and gets a "plateau" after the breakpoints occurs and TA actually keeps increasing after the breakpoint in Hhb, but in TSI, VL still flattens out and gets the "plateau" but TA keeps decreasing. The increase in Hhb and decrease in TSI are also a common behaviour for these measurements, as TSI decreases all the way from the beginning and Hhb should always increase, until exhaustion was there should be a flattening due to the oxygen level sinks, respectively. There were found differences between the slopes before and after the breakpoints in all of the tested muscles during this study. The flattening for the Hhb responses in VL may indicate that the fractional oxygen extraction reaches its limits (1), it also shows that there are differences between how the muscles are behaving during the cycling exercise. The differences in the increase for TA and flattening for VL after the breakpoint in Hhb occurs, and the differences between how the slopes behaves, may be due to the different recruitment of muscle fibers (21) or that the muscle force demand for TA is different from VL for the preformed task, in this study, cycling. There have been several different mechanisms that has been proposed to explain the differences in kinetics between muscular blood flow and oxygen uptake, and one of them were that there had been reported changes in muscle fibre recruitment as a consequence to the increase in work rate and that this may also contributed to a slowing effect on muscular blood flow relative to the oxygen uptake. This relationship was found in rats, were they found that O_2 delivery and O_2 extraction is dependent on fibre type, such that fast-twitch muscles are characterised as a greater O_2 extraction than to slow-twitch fibres (4). Another proposed that the mechanical effects of the muscle contraction contributes to a faster increase in muscular blood flow, this is following the onset of exercise without having attention metabolic recruitments, and therefore the different muscles gets less important during moderate to heavy intensity(4). Additionally, there has also been reports that has found that the increase in O_2 delivery for a given change in metabolic rate is similar for slow-twitch and fast-twitch muscle(23).

However, this research was based on animals (rats) and the results may not be the same if it was performed on humans. The differences between slow twitch and fast twitch muscle and the changes in muscle fibre recruitment may be different in heterogeneous skeletal muscles as VL and TA. However, the differences in between the slopes may just be due to the different O_2 demand of the working muscle. There could have been an advantage for the body if it could "decide" to deoxygenate other muscles (in this case, TA) more for saving the main muscles (in this case, VL) to later on in the exercise. The subjects during this study mentioned that the muscles were getting tired, then especially VL, before the lack of oxygen took. That can also explain why almost none of the subjects were reaching VO_{2max} during this study.

Correlation between muscles

A strong correlation was also observed between TA right leg, VL left leg and VT, there were also significantly positive correlation between TA left leg, VL left leg and VL right leg. There were no correlation between VO_{2peak} and the Hhb in the muscles, as some studies has reported earlier (1, 13), this studies mainly focused on one muscle (i.e., vastus lateralis) in one leg, respectively. This study measured four muscles at once and used physically active people and the subjects did not have cycling as their main sport. This may have consequences for the results, it could not be found any significant positive correlation between VO_{2peak} and the Hhb in VL and TA as some others has. However, there is a lot that is not known about the mechanisms behind these physiological parameters, but the results may be due to the fact there was not any active cyclists in this study.

Methodological consideration

During this study, there was measured four muscles at the same time. There is not a lot of studies, to the authors knowledge, that measure these many muscles at the same time. As this study also measures left and right leg for comparing if there are any differences between them. There were not preformed any pre-test to see if there were any differences between the legs. This was decided because it was not necessary for preforming or for discussing this paper, it might also have led to a longer time cap than 60 minutes for one subject and this could have leaded to more missing data or an earlier fatigue.

However, results from this study brings new information to the discussion in regard to what might happen in several muscles (vastus lateralis and tibialis anterior) at ones in both legs during cycling and it may give a more accurate interpretation of the results. There were not used professional cyclists in this study, that may not be a negatively thing because that using physically active people just gives the study a broader generalizable to other people as well. The result may also not have been any different except that the subjects may have reached VO_{2max} and that the mean protocol time-cap would have been longer, and that the Hhb responses may have come later in the test. If the study was going to be replicated, there may have been an advantage to perform a pre-test for deciding which leg was the and used a heart monitor to see if they were up to a maximum heartbeat per minute, this was left out under these tests.

Conclusion

This study showed that there were significantly differences between the breakpoints in vastus lateralis and tibialis anterior for Hhb, but no significantly differences between legs. There were also found differences in how the slopes are behaving during an incremental exercise test on an ergometer cycle for physically active people that does not have a lot of cycling experience. The different patterns in the slopes shows that there are differences in how different muscles (vastus lateralis and tibialis anterior) are reaction on increasing exercise intensity. There are metabolically differences that NIRS can measure and help to understand that the different muscles have different roles during different activities. This study shows that it is needed more research and investigation on several muscles at once for trying to understand the oxygenation patterns for several muscles during several activities, and for trying to understand the differences. There is also needed more investigation on the tibialis anterior muscle, and its behaviour and function during cycling.

References

1. Boone J, Vandekerckhove K, Coomans I, Prieur F, Bourgois JG. An integrated view on the oxygenation responses to incremental exercise at the brain, the locomotor and respiratory muscles. Eur J Appl Physiol. 2016;116(11-12):2085-102.
2. Boone J, Barstow TJ, Celie B, Prieur F, Bourgois J. The impact of pedal rate on muscle oxygenation, muscle activation and whole-body VO(2) during ramp exercise in healthy subjects. Eur J Appl Physiol. 2014;115(1):57-70.
3. Perrey S, Ferrari M. Muscle Oximetry in Sports Science: A Systematic Review. Sports Med. 2018;48(3):597-616.
4. Boone J, Koppo K, Barstow TJ, Bouckaert J. Pattern of deoxy[Hb+Mb] during ramp cycle exercise: influence of aerobic fitness status. Eur J Appl Physiol. 2009;105(6):851-9.
5. Beekvelt MCPv. Quantitative near-infrared spectroscopy in human skeletal muscle: methodological issues and clinical application: [Sl: sn]; 2002.
6. Raleigh C, Donne B, Fleming N. Association between different Non-Invasively Derived Thresholds with Lactate Threshold during graded incremental exercise. Int J Exerc Sci. 2018;11(4):391-403.
7. Belardinelli R, Barstow TJ, Porszasz J, Wasserman K. Changes in skeletal muscle oxygenation during incremental exercise measured with near infrared spectroscopy. Eur J Appl Physiol Occup Physiol. 1995;70(6):487-92.
8. Bhambhani Y, Maikala R, Buckley S. Muscle oxygenation during incremental arm and leg exercise in men and women. Eur J Appl Physiol Occup Physiol. 1998;78(5):422-31.
9. Bhambhani YN, Buckley SM, Susaki T. Detection of ventilatory threshold using near infrared spectroscopy in men and women. Med Sci Sports Exerc. 1997;29(3):402-9.
10. Grassi B, Quaresima V, Marconi C, Ferrari M, Cerretelli P. Blood lactate accumulation and muscle deoxygenation during incremental exercise. J Appl Physiol (1985). 1999;87(1):348-55.
11. Ferrari M, Muthalib M, Quaresima V. The use of near-infrared spectroscopy in understanding skeletal muscle physiology: recent developments. Philos Trans A Math Phys Eng Sci. 2011;369(1955):4577-90.
12. Spencer MD, Murias JM, Paterson DH. Characterizing the profile of muscle deoxygenation during ramp incremental exercise in young men. Eur J Appl Physiol. 2012;112(9):3349-60.
13. Boone J, Barstow TJ, Celie B, Prieur F, Bourgois J. The interrelationship between muscle oxygenation, muscle activation, and pulmonary oxygen uptake to incremental ramp exercise: influence of aerobic fitness. Appl Physiol Nutr Metab. 2015;41(1):55-62.
14. Takagi S, Kime R, Niwayama M, Murase N, Katsumura T. Muscle oxygen saturation heterogeneity among leg muscles during ramp exercise. Adv Exp Med Biol. 2013;765:273-8.
15. Iannetta D, Passfield L, Qahtani A, MacInnis MJ, Murias JM. Interlimb differences in parameters of aerobic function and local profiles of deoxygenation during double-leg and counterweighted single-leg cycling. Am J Physiol Regul Integr Comp Physiol. 2019;317(6):R840-r51.
16. Skovereng K, Ettema G, van Beekvelt M. The Effect of Cadence on Shank Muscle Oxygen Consumption and Deoxygenation in Relation to Joint Specific Power and Cycling Kinematics. PLoS One. 2017;12(1):e0169573.
17. Wang B, Xu G, Tian Q, Sun J, Sun B, Zhang L, et al. Differences between the Vastus Lateralis and Gastrocnemius Lateralis in the Assessment Ability of Breakpoints of Muscle

Oxygenation for Aerobic Capacity Indices During an Incremental Cycling Exercise. J Sports Sci Med. 2012;11(4):606-13.
18. Racinais S, Buchheit M, Girard O. Breakpoints in ventilation, cerebral and muscle oxygenation, and muscle activity during an incremental cycling exercise. Front Physiol. 2014;5:142.
19. Keir DA, Fontana FY, Robertson TC, Murias JM, Paterson DH, Kowalchuk JM, et al. Exercise Intensity Thresholds: Identifying the Boundaries of Sustainable Performance. Med Sci Sports Exerc. 2015;47(9):1932-40.
20. Murias JM, Keir DA, Spencer MD, Paterson DH. Sex-related differences in muscle deoxygenation during ramp incremental exercise. Respir Physiol Neurobiol. 2013;189(3):530-6.
21. Chin LM, Kowalchuk JM, Barstow TJ, Kondo N, Amano T, Shiojiri T, et al. The relationship between muscle deoxygenation and activation in different muscles of the quadriceps during cycle ramp exercise. J Appl Physiol (1985). 2011;111(5):1259-65.
22. Carpes FP, Diefenthaeler F, Bini RR, Stefanyshyn DJ, Faria IE, Mota CB. Influence of leg preference on bilateral muscle activation during cycling. J Sports Sci. 2011;29(2):151-9.
23. Ferreira LF, McDonough P, Behnke BJ, Musch TI, Poole DC. Blood flow and O2 extraction as a function of O2 uptake in muscles composed of different fiber types. Respir Physiol Neurobiol. 2006;153(3):237-49.