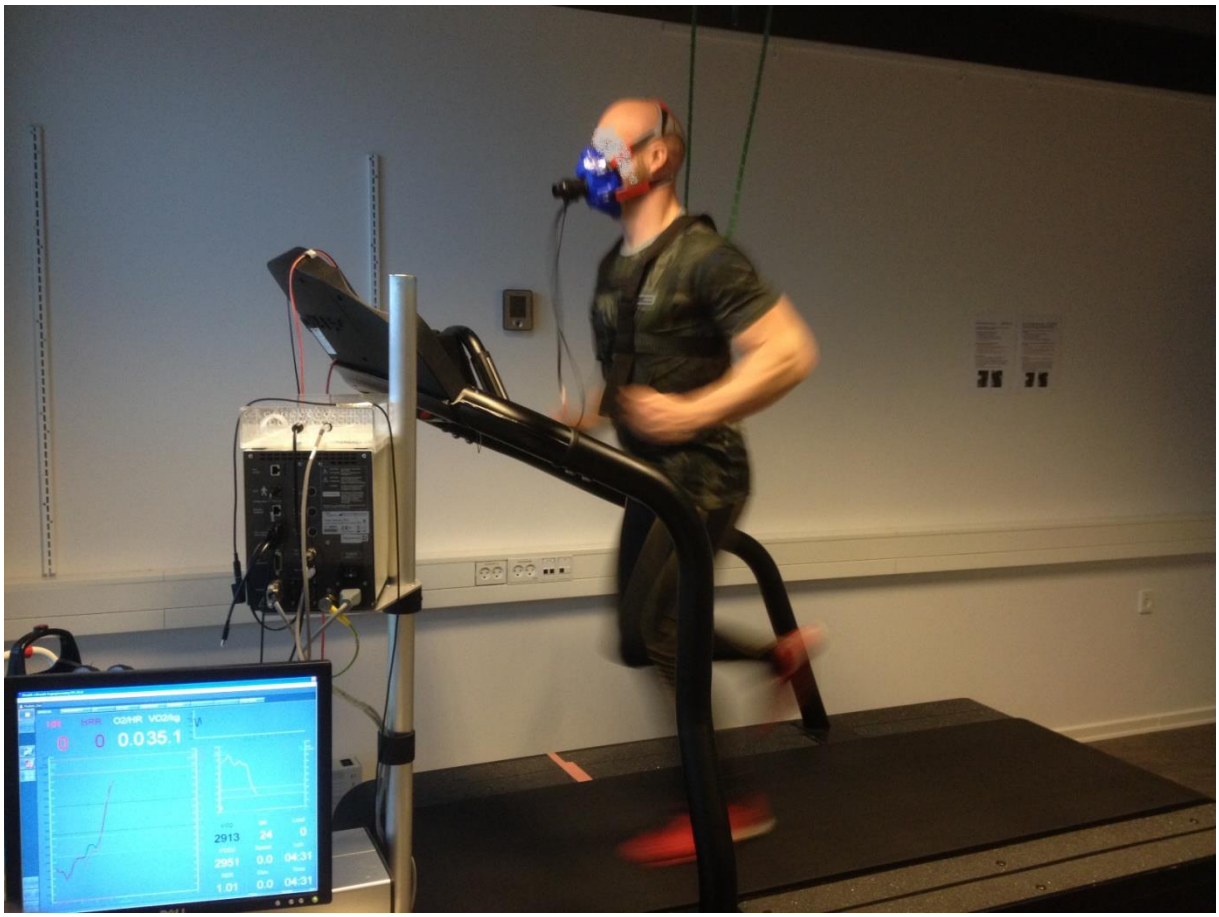
Comparisons of mitochondrial and microvascular function in three leg muscles, and the effects of two weeks of run sprint interval training on performance and performance markers in trained runners



10th semester Master's thesis, Sport Science, Aalborg University, Denmark, 2015

Group 1040

Lars Erik Haaber Bruun, Rasmus Thorø Thomsen & Anders Thomsen

Supervisor: Ryan Godsk Larsen

Abstract

We investigated the effects of a two-week run sprint interval training (SIT) intervention on 3000m performance, mitochondrial and microvascular function, maximal oxygen uptake (VO_{2max}) , oxygen uptake (VO_{2p}) kinetics, and running economy (RE) in trained runners. Further, at baseline mitochondrial and microvascular function were compared between the medial gastrocnemius (GM), tibialis anterior (TA), and vastus lateralis (VL) muscles. Twenty-four runners were recruited and assigned to either SIT (six sessions of four to six 30s sprints) or CON (maintain regular endurance training). The study found that at baseline, subjects had better mitochondrial function in the GM compared to the VL, while microvascular function was better in the GM and TA than in the VL. Post intervention, a non-significant improvement (2.8%, P = 0.10) in 3000m performance was observed in the SIT group, while no other changes were observed in either group. In trained runners, SIT may improve aerobic performance, independently of changes in VO_{2max}, VO_{2p} kinetics, RE, mitochondrial and microvascular function.

Preface

You are about to read the report of the study conducted by three students of a masters degree in sport science at Aalborg University. The full project time encompassed the 9th and 10th semester, making it an extended thesis. The study investigated the effects of two weeks of run sprint interval training on performance as well as markers of performance, including mitochondrial function and microvascular function, maximal oxygen uptake, oxygen uptake kinetics, and running economy in an already trained population. While collecting the variables at pre-test, comparisons of baseline values of these variables were done to study the characteristics of these in a trained population. Furthermore, correlation tests were performed to investigate the relationships of said variables.

Subjects were recruited in local running clubs. We would like to extend a big thank you to all participating subjects for their involvement – enthusiastic as it was, despite the grueling training and sometimes tedious stretches of waiting in the laboratory.

We also owe our gratitude to Aalborg Atletikklub, for use of facilities and assistance with recruitment of subjects.

For his invaluable assistance with handling, producing and interpreting at times very overwhelming data from the near-infrared spectroscopy, we also want to extend a courtesy to Ernest Nlandu Kamavuako, PhD, of Aalborg University.

During the course of the two semesters, our thesis supervisor, Ryan Godsk Larsen, PhD, has been an invaluable support in shepherding us through the scientific swamp as well as acquainting us with the measuring apparatuses used for the study. For his relentless guidance, we owe him a big thank you.

We hope you enjoy reading the thesis report,

Lars, Rasmus & Anders.

Contents

Introduction

It is well known that traditional endurance training (ET) encompassing continuous exercise for 40-120 minutes a day, 4-5 days a week, at 65-75% maximal oxygen uptake (VO_{2max}) leads to increases in performance in aerobic events (Gollnick et al. 1973, Jones, Carter 2000, Laursen, Jenkins 2002). In addition to this, markers of central and peripheral physiological functions such as, but not limited to, $\dot{V}O_{2max}$, pulmonary oxygen uptake kinetics and mitochondrial function also improve (Gollnick et al. 1973, Jones, Carter 2000, Laursen, Jenkins 2002). In recent years however, high-intensity interval training (HIT), has been shown to induce similar adaptations in performance and markers of physiological function, despite requiring a significantly smaller training volume. HIT has been broadly defined as short to moderate duration repeated bouts above anaerobic threshold lasting 10 seconds to 5 minutes (Laursen, Jenkins 2002). Bouts are generally interspersed by short periods of low intensity exercise or complete rest to allow for a partial or full recovery with work:rest ratios of ≤1:5 (Laursen, Jenkins 2002). In the more intense end of the HIT spectrum lies sprint interval training (SIT), which is defined as all-out effort sprints lasting 10-30s, performed for less than 12 bouts with a work:rest ratio of ≥1:5 (Sloth et al. 2013). SIT has been shown to improve aerobic exercise performance to the same extent as ET, in both moderately trained and untrained populations, despite requiring a greatly reduced time investment (Sloth et al. 2013, Macpherson, Weston 2015, Gist et al. 2014). Despite these similar performance improvements, SIT and ET appear to stimulate performance improvements via different physiological mechanisms. One study showed similar improvements in VO_{2max} following six weeks of either ET or SIT, however ET led to an increase in cardiac output via increased stroke volume, while SIT led to an increase in arterial-venous difference (Macpherson et al. 2011). This difference has been suggested to be due to improved local O_2 delivery (microvascular function) and/or improved mitochondrial function following SIT (Macpherson et al. 2011). This is in line with results showing increased muscle mitochondrial enzyme content following only two weeks of SIT (Burgomaster et al. 2005). Further, McKay et al. (2009) showed improved pulmonary oxygen uptake (VO_{2p}) kinetics following as little as two sessions of HIT. McKay and colleagues speculated this adaptation to be primarily caused by improved microvascular function. Altogether, these findings indicate that performance improvements following SIT can mainly be attributed to peripheral adaptations in both mitochondrial and microvascular function.

By measuring phosphocreatine (PCr) recovery and analyzing muscle biopsies, it has been well established that muscle mitochondrial function is improved in the vastus lateralis (VL) following SIT (Burgomaster et al. 2005, Larsen, Befroy & Kent-Braun 2013, Burgomaster et al. 2008, Burgomaster, Heigenhauser & Gibala 2006). As mentioned, it has been speculated that improved microvascular function is responsible for the rapid adaptations in VO_{2p} kinetics observed following SIT/HIT. Previous studies measuring rapid adaptations

in VO_{2p} kinetics have used near-infrared spectroscopy (NIRS) during exercise, to investigate local muscle deoxygenation and O_2 delivery (McKay, Paterson & Kowalchuk 2009, Williams, Paterson & Kowalchuk 2013, Bailey et al. 2009). Since no occlusion was applied on the exercising leg, this method did not distinguish between oxygen delivery and oxygen consumption. As such, any contribution to VO_{2p} kinetics adaptations from improved microvascular function could not be properly accounted for. Using near-infrared spectroscopy in conjunction with arterial occlusion creates a closed system in which any change in oxygenated hemoglobin has to come from a change in blood volume or oxygen consumption by mitochondria. When correcting for blood volume changes, the recovery of muscle oxygen consumption during repeated occlusions has been shown to correlate with mitochondrial function (Ryan et al. 2014, Ryan et al. 2013, Ryan et al. 2012). Furthermore reoxygenation, measured by NIRS, following arterial occlusion has been used as a marker of microvascular function (Bopp, Townsend & Barstow 2011). Combining these methods makes it possible to separately study adaptations in microvascular function and mitochondrial function. As such, this study aimed to investigate the effect of SIT on VO_{2p} kinetics, mitochondrial and microvascular function, in order to elucidate the mechanisms behind rapid VO_{2p} kinetics adaptations.

Mitochondrial function

Peripheral adaptations in mitochondrial function is a key adaptation stimulated by SIT, which results in improved a-vo2 difference and VO_{2max} (Macpherson et al. 2011). To the authors knowledge all studies looking at mitochondrial adaptations following SIT has examined the VL muscle (Burgomaster et al. 2005, Burgomaster et al. 2008, Burgomaster, Heigenhauser & Gibala 2006, Iaia et al. 2009, Barnett et al. 2004, MacDougall et al. 1998, Liljedahl et al. 1996, Gibala et al. 2006), and of these only one study intervened with running SIT (Iaia et al. 2009). Thus, little is known regarding differences in peripheral adaptations between muscles, and to which extent adaptations in different muscles may explain aerobic performance improvements. The use of running instead of cycling as training modality, presents the possibility of investigating and comparing adaptations in the muscles of the lower leg. One aim of this study was therefore to measure mitochondrial adaptations in three leg muscles, VL, medial gastrocnemius (GM) and tibialis anterior (TA) respectively, and correlate these findings with performance improvements in moderately trained runners.

During running the VL and GM work to create propulsion, while the TA works to stabilize the ankle joint upon ground contact, and dorsiflex the foot during the swing phase (Nicola, Jewison 2012). The TA performs primarily loaded eccentric contractions during running, while the GM and VL perform both concentric and eccentric contractions. This would in theory require a higher oxidative capacity of the GM

and VL, since concentric contractions are more metabolically demanding than eccentric contractions (Menard et al. 1991). The oxidative capacity of muscles has been shown to be primarily affected by usage and not fiber type composition (Larsen et al. 2009, Layec et al. 2013, Forbes et al. 2009), and as such, it was expected that the GM and VL had a higher baseline mitochondrial function than the TA.

Oxygen uptake kinetics

At the onset of exercise, VO_{2p} does not increase instantaneously, but increases exponentially towards a new steady state in which VO_{2p} reflects that of muscle O_2 utilization (Poole, Jones 2012). During the time it takes to reach steady state, the muscles have to rely on anaerobic resources resulting in an oxygen deficit. This is potentially limiting longer duration performance, since anaerobic resources (glycogen and creatine phosphate) are limited, and metabolites linked to muscle fatigue build up during the transition to a steady state level where oxygen uptake meets energy demand. The rate constant of the exponential rise in VO_{2p} reflects the speed of rise in VO_{2p} and has been shown to increase with ET, HIT and SIT, thus decreasing the time on which muscles must rely on anaerobic processes (McKay, Paterson & Kowalchuk 2009, Williams, Paterson & Kowalchuk 2013, Bailey et al. 2009, Da Boit et al. 2014). Improved VO_{2p} kinetics following SIT has been suggested to be due to improved microvascular function in the VL (McKay, Paterson & Kowalchuk 2009). An aim of the present study was to examine improvements in VO_{2p} kinetics following two weeks of running SIT, and investigate whether changes in VO_{2p} kinetics could be explained by improved microvascular function in the TA, VL and GM.

Running Economy

Running economy (RE) is defined as the energy expended when running at a given submaximal speed (Barnes, Kilding 2015). A better RE (lower energy requirements at a given speed) has been shown to be an important marker of endurance performance (Barnes, Kilding 2015). RE is influenced by many factors including ventilation, musculotendinous stiffness, neural and metabolic factors (Barnes, Kilding 2015). Currently ET, HIT and SIT has been shown to improve RE, possibly via different mechanisms (Laursen, Jenkins 2002, Iaia et al. 2009, Barnes, Kilding 2015). Iaia et al. (2009) showed an improvement of 5.7-7.6% in RE following 4 weeks of run SIT, however to the authors' knowledge, no studies have been carried out on the effects of running SIT for a period of less than four weeks on running economy. Thus an aim of this study was to examine the effects of two weeks of running SIT on running economy, to further clarify the time course of adaptations in RE.

Maximal Oxygen Consumption

There is a bulk of evidence in the scientific literature showing that 2-8 weeks of SIT-based training improves VO_{2max} in healthy sedentary and recreationally active adult men and women (Sloth et al. 2013, Gist et al. 2014). Concerning HIT it has been shown that the increase in VO_{2max} becomes smaller, or disappears, as training status of the subjects increases (Weston et al. 2014). For the sake of this study, maximal oxygen consumption was measured to compare and possibly correlate with other findings, specifically to examine any contribution from central adaptations to an increase in aerobic performance following running SIT.

Research question

In summary, the main aim of this study was to investigate the effects of two weeks of running SIT on local adaptations in oxidative capacity and microvascular function in the VL, GM, and TA in trained runners, and compare these adaptations with changes in 3000m running performance, $VO_{2max,}$ VO_{2p} kinetics and RE.

A second part of this study was to compare the oxidative capacity and microvascular function in the VL, GM and TA in a group of moderately trained runners and correlate these values with 3000m running performance, $VO_{2max,}$ VO_{2p} kinetics and RE.

Firstly, it was hypothesized that two weeks of running SIT would incur performance improvements in a 3000m time-trial, and that this would be associated with improvements in mitochondrial and microvascular function and VO_{2p} kinetics. Small or no changes in $VO_{2max,}$ and a small, possibly insignificant, improvement in RE were expected. Secondly, it was hypothesized that mitochondrial and vascular function would improve more in the VL and GM than in the TA.

Measurements of mitochondrial function

Since this study measures mitochondrial function changes in response to SIT, this section will briefly cover common methods to measure mitochondrial function with the primary focus being on near-infrared spectroscopy as used in this study.

There are two primary categories of measuring mitochondrial function: invasive and non-invasive. Invasive methods include biopsies to measure enzyme activity or isolated mitochondrial preparations or permeabilized muscle fiber preparations to measure respiration rate. Although very specific measurements these methods all come with the potential limitation of altering the tissue sample before or during measurements (Ryan et al. 2014).

The current gold standard non-invasive method used to study mitochondrial function is phosphorous magnetic resonance spectroscopy (P-MRS) (Hamaoka et al. 2011). With this method, recovery of phosphocreatine post exercise can be measured, and has been validated against in-vitro measurements of enzyme activity and high-resolution respirometry (Lanza et al. 2011). A downside to P-MRS, however, is the high cost and low availability of multinuclear MR-scanners, which has led researchers to look for other non-invasive measurements of mitochondrial function one of which is near-infrared spectroscopy (Ryan et al. 2014, Ryan et al. 2013, Kime et al. 2003).

Near-infrared spectroscopy

Near-infrared spectroscopy (NIRS) devices containing a transmitter and a receiver can shine near-infrared light (wavelengths 700-1300nm) through biological tissue and, based on tissue light absorption and reflection, provide data on oxygenated and deoxygenated hemoglobin or myoglobin (O_2Hb and HHb, respectively). The reason behind this is that the chromophore of the hemoglobin and myoglobin is influenced by different blood gases, including O_2 and CO_2, which also affects the chromophores ability to reflect light. Therefore, colors (wavelengths) reflect and absorb, depending on the saturation of hemoglobin with oxygen. Since diversities in the near-infrared part of the spectrum are difficult to identify, it is almost impossible to determine which data reflects from hemoglobin or myoglobin. In the present study NIRS was used to measure the oxygen saturation in the local muscle tissue, which also means that no distinction was made between hemoglobin and myoglobin (Hamaoka et al. 2011).

Muscle deoxygenation and oxygen consumption

NIRS has been used to assess muscle deoxygenation before and after a HIT intervention(McKay, Paterson & Kowalchuk 2009), however this approach does not distinguish between oxygen delivery and oxygen consumption. Recently, studies have investigated the reliability and validity of a new setup using NIRS to

measure mitochondrial function (Ryan et al. 2014, Ryan et al. 2013). With this setup, an arterial occlusion is applied proximal to the NIRS probe. Application of arterial occlusion creates a closed system. This allows for a measure of muscle oxygen consumption (mVO_2), since oxygen delivery via arteries is no longer present. mVO_2 is measured as the rate of change in oxygenated hemoglobin (O_2Hb) during occlusions and is a function of aerobic metabolism.

In resting muscle there are no differences between trained and untrained individuals in mVO_2, despite differences in mitochondrial function (Brizendine et al. 2013). However it has been shown that trained individuals exhibit a much larger mVO_2 following exercise, which in theory should help them recover phosphocreatine stores faster due to better mitochondrial function (Brizendine et al. 2013). In order to test this hypothesis, the recovery of mVO_2 back to baseline following exercise has been compared to other measurements of mitochondrial function, namely P-MRS and mitochondrial respiration in permeabilized muscle fibers (Ryan et al. 2014, Ryan et al. 2013). The recovery of mVO_2 shows strong to very strong correlations with mitochondrial respiration in permeabilized fibers (r = 0.61–0.74) (Ryan et al. 2014) and P-MRS (r = 0.88-0.95) (Ryan et al. 2013). Thus NIRS seems to be a valid non-invasive measure of mitochondrial function, which is more affordable and transportable than P-MRS (Ryan et al. 2013).

Interpreting NIRS data

When comparing NIRS measurements of mVO_2 between individuals, expressing the O_2Hb signal as a percentage of the individuals' maximal possible value of O_2Hb has been shown to remove the influence of adipose tissue thickness on measurements (Ryan et al. 2012). When applying an arterial occlusion for 3-5 minutes the TSI stabilizes at a low baseline, and when the occlusion is removed a hyperemic response occurs with the TSI rising to above resting values. The full physiological range is calculated as the difference between the peak value of TSI during the hyperemic response and the low baseline value during full occlusion.

In order to quantify recovery of mVO_2 exercise must be performed followed by a series of repeated arterial occlusions, as shown on Figure 1 from around minutes 13-16. On the same figure, mVO_2 is the slope of decrease in TSI (y-axis) occurring during each occlusion and recovery of the mVO_2 is the difference in slopes over time. Ryan et al. (2013) has shown that mVO_2 recovery measurements show good reproducibility between different exercise types such as voluntary contractions or electrically stimulated contractions. However, care should be taken not to decrease TSI below 30%, in order to avoid low oxygen tension, which may influence NIRS measurements. Several protocols of varying occlusion time have been used in different studies, however length of the occlusion time does not seem to influence mVO_2 measurements (Ryan et al. 2013, Ryan et al. 2012, Brizendine et al. 2013, Ryan, Brizendine & McCully 2013).

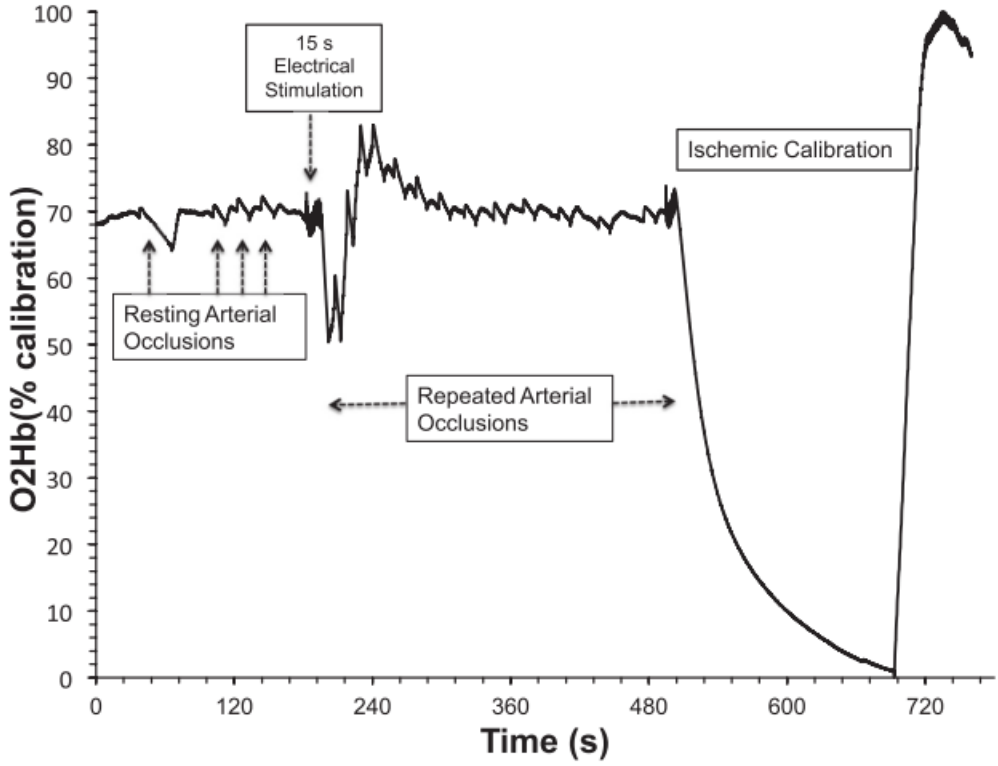


Figure 1. Full procedure of NIRS measurements. On the Y-axis O_2Hb, expressed in $\%*s^{-1}$, as a function of the ischemic calibration, is shown. On the x-axis is time. (Ryan et al. 2012, p. 176).

When fitting the change in mVO_2 over time to a monoexponential curve, the time constant of the curve is correlated to the mitochondrial function of the subject (Ryan et al. 2013). An example of such a curve for trained versus untrained subjects can be seen in Figure 2.

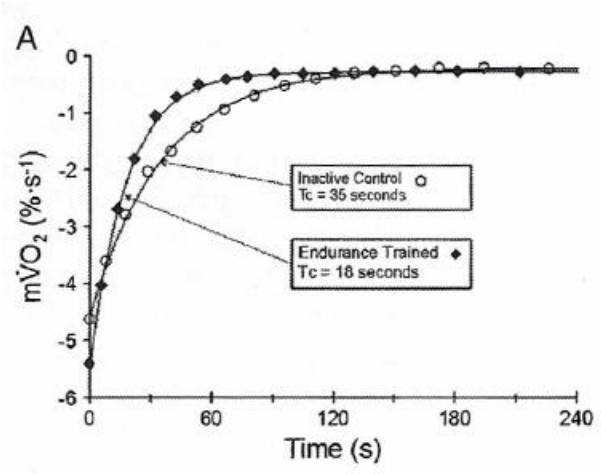


Figure 2. mVO_2 recovery over time following exercise. On the Y-axis mVO_2 is depicted as the percentage of the full physiological range of $Hb_{difference}$ decline per second, and on the X-axis is time. As shown mVO_2 returns to baseline over time faster in endurance trained athletes (full squares) compared to inactive controls (empty circles). (Brizendine et al. 2013, p. 872).

Correcting for blood volume

During occlusions, the total hemoglobin content (tHB) varies over time, which theoretically should not be possible in a closed system. This may be caused by a blood volume flux from the redistribution of heme between high pressure arteries/arterioles to low pressure veins/venules due to oxygen consumption in muscle (Ryan et al. 2012). This change in blood volume needs to be corrected for, in order to receive more accurate NIRS signaling with a constant tHB. Ryan et al. (2012) investigated a correction factor for blood volume changes, which ensures that changes in O_2Hb and HHb are inversely related and thus ensure a constant tHb. An example of the effect of the correction factor on NIRS data is shown in Figure 3.

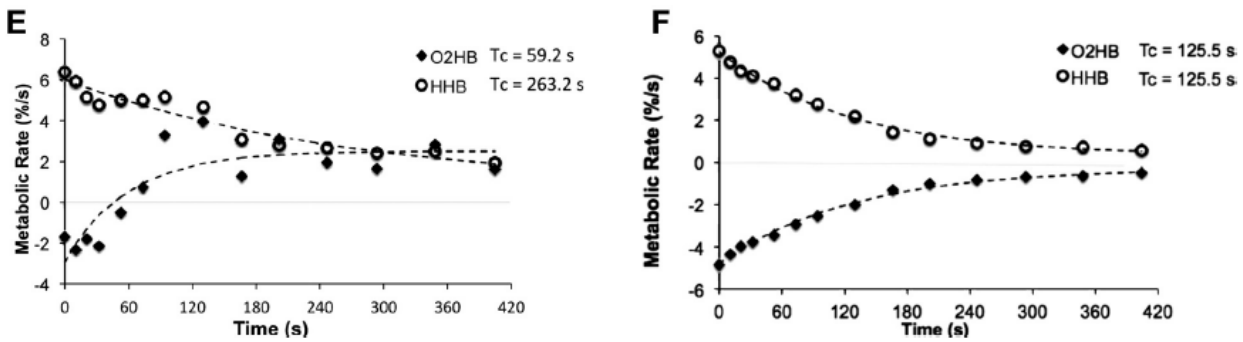


Figure 3. Effect of correction for blood volume on tHb, O_2Hb and HHb. Figure E on the left shows a disproportionate change in O_2Hb and HHb, due to no correction for blood volume changes. Figure F on the right shows that correcting for blood volume changes results in equal and inverse time constants for O_2Hb and HHb, which is a sign of a constant tHb. (Ryan et al. 2012, p. 178).

The authors noted variability of the correction factor between subjects, and as such recommend blood volume correction for each data set (Ryan et al. 2012)(Ryan et al. 2012). Furthermore, correcting each data point, rather than an average correction for the entire data set, produces more accurate results.

In summary NIRS data of recovery of mVO_2 following exercise, measured during repeated arterial occlusions, is a reliable and valid measure of mitochondrial function, even more so when correcting for blood volume changes.

NIRS and vascular function

Besides measuring mVO_2 during occlusion, the hyperemic response following occlusion has also been measured using NIRS, and used as an indicator of vascular function (Bopp, Townsend & Barstow 2011, Hamaoka et al. 2011). In this study, the hyperemic response following a five minute occlusion was analyzed. Furthermore, the rise in O_2Hb during free-flow periods between 15 repeated occlusions was analyzed and compared with the data for hyperemic responses. Analyzing change in rise in O_2Hb signal during free flow

periods with a monoexponential function has to the authors knowledge never been used in the scientific literature. As such, comparing this data to that of the hyperemic response could give insight into the validity of this method.

Methods and Materials

Study Design

The conduction of this study had two primary aims. Firstly, measurements at baseline of mitochondrial function and microvascular function for the TA, GM and VL were conducted, in order to investigate the training status in these muscles in trained runners. Further, these variables were compared with maximal oxygen consumption (VO_{2max}), running economy (RE), oxygen uptake kinetics (VO_{2p} kinetics), and aerobic performance. Secondly, a two-week intervention period was used to study the effects of SIT on the above mentioned variables. The tests utilized were near-infrared spectroscopy (NIRS) for mitochondrial function and microvascular function; treadmill pulmonary gas exchange testing for VO_{2max}, RE and VO_{2p} kinetics, and a running field test for performance. Based on 3000m performance, age, and gender, subjects were divided into intervention (SIT) or control (CON) group. An illustration of the study design can be seen in Figure 4.

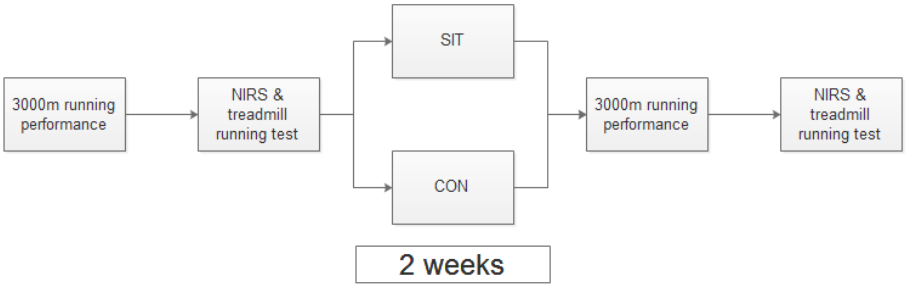


Figure 4. Illustration of the study design with two weeks intervention period, pre- and post-test, including 3000m performance test, NIRS and treadmill running test.

Subjects

A total of 24 subjects were recruited for this study. Subject characteristics are shown in Table 1. Subjects were recruited from local running clubs. Inclusion criteria for the subjects were: recreational runners involved in individual running programs encompassing 10 to 50 km*week^{-1}; non-smoker and declared injury free for at least six months prior to the study; executed ≤1 SIT session per month within the last six months. Information about experimental procedures and potential risks of the study were explained to the subjects, and informed written consents were obtained from all participating subjects. The study was approved by the local ethical committee of Northern Jutland (N-20140096). The study was conducted in accordance with the Declaration of Helsinki.

Table 1. *Characteristics of the 24 recruited subjects.*

Characteristics	SIT (*n* = 12)	CON (*n* = 12)	*P*-value
Age (y)	47 ± 8.4	44.3 ± 16	*P* = 0.61
Running distance (km*week^{-1})	29.2 ± 11	36 ± 12.6	*P* = 0.20
3000m performance test (s)	782.1 ± 88.5	808.3 ± 133.7	*P* = 0.58

Characteristics at baseline for all recruited subjects. Values are expressed as means ± standard deviation. Between group differences were tested using an independent samples t-test.

Experimental design

The duration of the intervention was two weeks, with pre- and post-tests lasting 7-10 days. Pre- and post-intervention, subjects performed three tests on separate days: (1) a 3000m running performance test; (2) a treadmill running test to determine running economy, oxygen uptake kinetics and VO_{2max}; and (3) near-Infrared Spectroscopy (NIRS) measurements to express mitochondrial function and microvascular function. The 3000m performance test was conducted first, and the treadmill and NIRS-tests were not ordered. When possible, pre- and post-training tests were conducted at the same time of day to avoid possible time-of-day effects (Racinais et al. 2005). In addition, treadmill and field post-tests for the intervention group were conducted no sooner than 72h after completed SIT protocol. Based on 3000m performance, age, and gender, subjects were divided into intervention (SIT) or control (CON) group. Subjects were asked to maintain their normal diet throughout the study. On testing days, subjects were asked to consume a light meal no later than 2h prior to testing and replicate this at post-testing. They were also asked not to consume alcohol and caffeine and not to perform vigorous exercise in the 24h prior to testing.

Training protocol

The SIT intervention consisted of three weekly supervised sessions of 4-6 bouts of 30 second all-out sprints, as previously utilized (Burgomaster et al. 2008) and one non-supervised weekly ET session consisting of 25% of the subject's self-reported weekly distance prior to intervention. Thus, 75% of the subject's usual weekly ET was replaced by the SIT, and the 25% ET was included to mimic a more wholesome approach to periodized utilization of SIT. The intensity for these ET runs was instructed to reflect the subject's usual intensity when doing distance training. For the intervention group, all training sessions were conducted on a 400m outdoor track. Prior to each training session, subjects performed a warm-up consisting of two different dynamic stretching exercises, each done for 10 repetitions; a 400m jog at self-selected pace; 10 dynamic standing leg swings for each leg; six 100m runs of increasing speed, with the last 20m of the final

run being at maximal possible speed. A two minute rest was maintained between this and the start of the first sprint bout. Subjects were lined at the starting line, and a three second countdown initiated the sprint. The subjects were encouraged to go as hard as possible, right out of the blocks and attempt to maintain this for the entire 30 seconds, and repeat this for each bout without consideration for economization for the remaining number of bouts. During the entire 30 seconds bout, strong verbal encouragement was given as well as time cues with 15, 10 and 5 seconds remaining. A four minute active rest was made up of the subjects walking back to the starting line, with a particular encouragement to remain mobile for the first 30 seconds of the pause to avoid venous pooling. A maximum of six subjects trained at the same session. Each session was separated from the previous session by at least 46 hours.

The distance covered by each subject for each bout was estimated to the nearest meter using fixed 10m markers on the track. After each bout subjects rated the bout on an exertion scale (RPE) ranging from 0-10 (Appendix 1). After the test, subjects were asked to perform a short cool down.

The control group was instructed to maintain their regular training (volume and intensity) and both groups kept a training diary which was handed in by the end of the intervention period. To control for compliance and compare training volume between groups, these diaries were analyzed to produce and compare a mean two-week volume (km) for each group.

3000 meter performance test

The outdoor performance test was conducted on a 400m rubberized artificial running surface. The supervised warm-up consisted of two different dynamic stretching exercises, each done for 10 repetitions; a 400m jog at self-selected pace; 10 dynamic standing leg swings for each leg; a 100m run of increasing speed (nearing 70% maximal velocity) and finally another 400m run at a pace approximating expected 3000m pace. Two minutes after the warm-up was completed, the 3000m test was initiated. The instructions for the subjects were to cover the distance in the minimal amount of time in their own pace, and to the best of their ability not let their pacing be influenced by other subjects. The time it took the subjects to cover the 3000m was measured using a stopwatch. The cool down from the SIT sessions was repeated after the performance test.

NIRS

The NIRS measurements were performed in a dedicated laboratory. Subjects were seated in a KIN COM (Chattanooga Group, Inc. 1997, software v. 5.28, Chattanooga, TN, USA) dynamometer during all measurements. The NIRS protocol is illustrated in Figure 5.

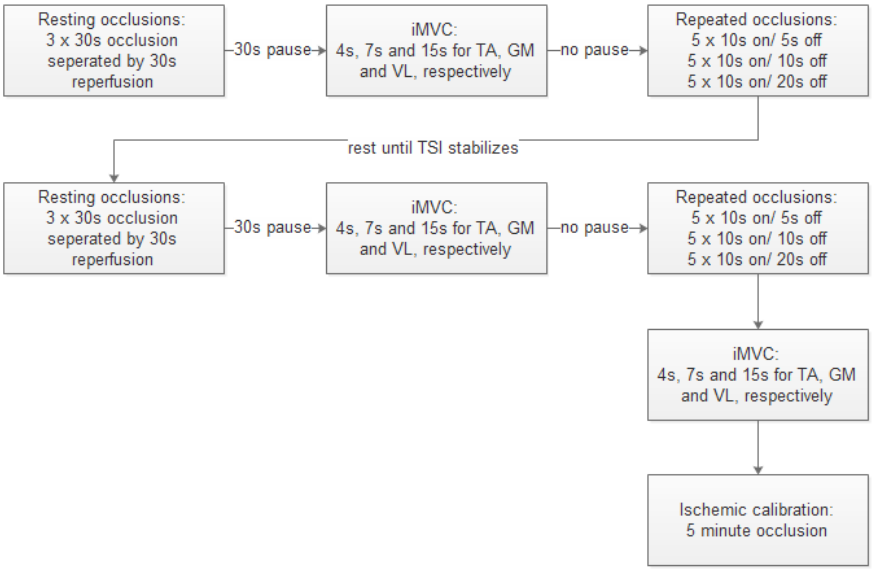


Figure 5. Illustration of the NIRS protocol for measurements on VL, GM and TA.

The NIRS probe (Oxymon Mk III, Artinis Medical Systems b.v., Zetten, The Netherlands) was placed directly on the skin over the muscles of interest. The placement of the probe for the VL muscle was 2/3 on the line from the anterior spina iliaca superior to the lateral side of the patella. For the GM and the TA muscles, the probe was placed on the most prominent bulge of the muscles. The cuff (140mm Velcro-closed, custom build compressor system) for arterial occlusion was placed on the thigh, proximal to the NIRS probe. The cuff was set to 300mmHg pressure for all arterial occlusions, and time to inflation and deflation was set to 0.02 seconds.

During the resting occlusions, the subject was instructed to remain motionless. For the measurements of repeated occlusions and ischemic calibration, the subject performed an isometric maximal voluntary contraction (iMVC) immediately prior to the first engagement of the cuff, in order to stimulate muscle oxidative metabolism, and then remained motionless. Based on pilot studies the duration of the iMVC was set to 4s for the TA, 7s for GM and 15s for VL, in order to avoid a TSI below 30%, since low oxygen tensions may affect NIRS measurements (Ryan, Brizendine & McCully 2013). At the end of each iMVC repeated arterial occlusions were applied (see below). For the respective muscles, the KIN KOM settings were changed according to the manufacturer's instructions. Joint angles were 120° plantar flexion of the ankle joint for measurements of TA (Maganaris 2001) and GM (Maganaris 2003) and 70° knee flexion for measurement of VL (Ichinose et al. 1997) as these angles have been reported to be optimal for force development of the respective muscles. For each person, the same order of tests between muscles was used at pre- and post-test. For an illustration of the experimental setup, see Figure 6.

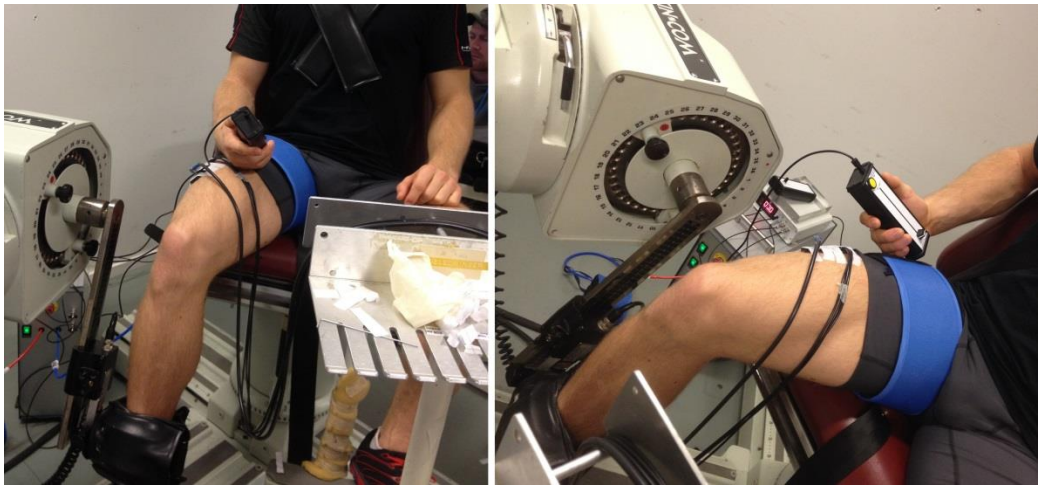


Figure 6. Experimental setup of NIRS measurement of the vastus lateralis muscle.

NIRS data were recorded with Oxysoft DAQ Version 2.1.6(Artinis Medical Systems b.v., Zetten, The Netherlands). Data was recorded at 10Hz, differential path length factor was set to 4 and transmitter-receiver distance was 40mm.

Treadmill running test

A treadmill running test was adopted from Skovgaard et al. (2014) in which subjects performed a series of walking and running bouts on an electrically braked treadmill (Woodway Pro XL, Waukesha, WI, USA). Measurements were obtained via breath-by-breath analysis using a Jaeger Oxycon Pro (Cardinal Health GMBH, Hoechberg, Germany) and the appertaining software (LABManager v. 5.3.0.4, Cardinal Health Germany, Hoechberg, Germany). The test began with the subject doing a 5 $km*h^{-1}$ warm-up walk for two min, followed by six min of running at steady state, a velocity corresponding to 80% of the subject's average velocity during the 3000m field test performed pre and post intervention. This procedure was repeated three times, each separated by 20 min of rest. To determine the VO_{2max} of the subject, at the end of the third 6 min run, the speed of the treadmill was increased by 1 $km*h^{-1}$ at the minute mark until exhaustion. Strong verbal encouragement was given during the final minutes of testing.

Data analysis

Training volume

To control for the training volume of the SIT and CON group, respectively, mean values of distances covered during the two weeks of intervention were calculated. For the SIT group these values consisted of distances accumulated during all six SIT bouts plus the two ET maintenance runs. For the CON group training diaries were analyzed to give a mean distance for the group. All recorded SIT bout distances and RPEs were averaged to present descriptive data of the training intervention.

VO_2 kinetics

To determine VO_{2p} kinetics, breath-by-breath values were converted by LABManager to give bin-averaged 5s values. All data files were blinded and the six minutes of running were manually selected to start at the beginning of the steep rise in VO_2 from the transition from walking to running. The data points from the six minutes of running were then fitted to a monoexponential curve using the following equation:

$$y = y_0 + a\left(1 - e^{-bx}\right)$$

y represents the oxygen uptake in milliliters to a given time point and y_0 represents the VO_2 value in the transition from walking to running. *a* is the amplitude of the curve; *b* is the time constant, representing the rate of the rise in VO_2 to achieve 63% of the amplitude. This was done for each six minute running bout for a total of three bouts to strengthen the reliability of the τ value. Data for each six minute running bout was manually analyzed while blinded, and data points considered outliers were removed. For further analysis, the time constants for the three bouts were averaged for each subject.

Running economy

For analysis of running economy (RE) bin averaged 15s values were exported from LABManager, and RE was determined by averaging the last two minutes of VO_2 data at steady state for the three running bouts and inserting it in the following equation:

$$RE\ (ml * kg^{-1} * km^{-1}) = \frac{VO_2(ml * min^{-1}) * 60\ (min/h)}{BM(kg) * v(km * h^{-1})}$$

RE represents the running economy in milliliters per kilogram body mass per kilometers; VO_2 is the average oxygen uptake at steady state; BM is the body mass and *v* is the running velocity at steady state. For further analysis, the RE for the three bouts were averaged for each subject.

VO_{2max}

For analysis of VO_{2max}, bin averaged 15s values were exported from LABManager. During the final portion of the treadmill running test, VO_{2max} was determined by the greatest 15s average VO_2 value attained. Respiratory exchange ratio (RER) and heart rate (HR) was measured at the end of the test.

NIRS

Raw data correction

Analysis of the NIRS data was done using custom-written scripts in Matlab (v. 8.5.0.197613, The Mathworks, Natick, MA). To determine each subject's full range of O_2Hb tissue saturation the lowest point

from the ischemic calibration and the highest point from the hyperemic response were used. All data was expressed as a percentage change per second of total tissue saturation of O_2Hb relative to each person's full range.

A blood volume correction factor (β) was calculated based on the assumption that the microvascular (i.e. arterioles, venules and capillaries) environment under the NIRS probe is a closed system, when arterial occlusion is applied, changes in O_2Hb and HHb happens with a 1:1 ratio, which represents the mitochondrial oxygen consumption. However, since the change in the NIRS signal also has shown to be caused by a blood volume flux (*Δblood volume*) from redistribution of heme between high-pressure arteries/arterioles and low-pressure veins/venules this influence must be corrected for, before a valid measure of mVO_2 is possible:

$$mVO_2 = \Delta NIRS - \Delta blood\ volume$$

To correct for the changes in blood volume, the following equation was applied:

$$\beta(t) = \frac{|O_2Hb(t)|}{(|O_2Hb(t)| + |HHb(t)|)}$$

For the equation above, β(t) is the blood volume correction factor to a given time point, which represents the proportionality of the blood volume change with values ranging between 0 and 1. This β was calculated for each NIRS data point, after which each data point was corrected using its consequent β. The corrected mVO_2 signal was calculated with the following equations:

$$O_2Hb_{corrected} = O_2Hb - [tHb * (1 - \beta)]$$

$$HHb_{corrected} = HHb - (tHb * \beta)$$

For the above equations $O_2Hb_{corrected}$ and $HHb_{corrected}$ are the corrected oxygenated and deoxygenated NIRS signals; O_2Hb, HHb and tHb are the uncorrected NIRS signals of oxygenated, deoxygenated and total hemoglobin, respectively. In both equations the NIRS signal is corrected by subtracting the proportion of the blood volume change attributed to either O_2Hb or HHb.

Muscle oxygen consumption

mVO_2 was defined using linear regression as the rate of decrease in O_2Hb as a percentage of each subjects full range during each occlusion (Figure 7). For resting occlusions the change in O_2Hb was calculated during the first 10 seconds of data. For repeated occlusions the first three seconds were used to calculate the rate of change in O_2Hb during each occlusion. All descending curves (reflecting start of arterial occlusion) were marked by event keys during the NIRS testing, and later during data analysis the exact location of event

keys was corrected manually for all data. The rate of change in $m\text{VO}_2$ during all 15 (repeated) occlusions was fitted to a monoexponential curve as follows:

$$y = y_0 + a\left(1 - e^{-bx}\right)$$

For the equation above, *y* represents the relative mVO_2 during occlusion. y_0 is the mVO_2 right after the iMVC, *a* is the amplitude in mVO_2 from end exercise to rest, and *b* is the fitting time constant, which represents mitochondrial function.

Muscle reoxygenation

As an indicator of microvascular function, a rate constant was calculated for the recovery of the rise in O_2Hb during free flow period. The free flow periods are the ascending slopes between the red lines on Figure 7. For these ascending curves, which are the relative increase in O_2Hb during free flow periods, the first two seconds of data was used. The beginning of all ascending curves was marked manually, as the trough appearing ~10s after an event key marking the beginning of arterial occlusion. The change in slope of ascending curves from occlusion 1 to 15 was fitted to a monoexponential curve as with mVO_2.

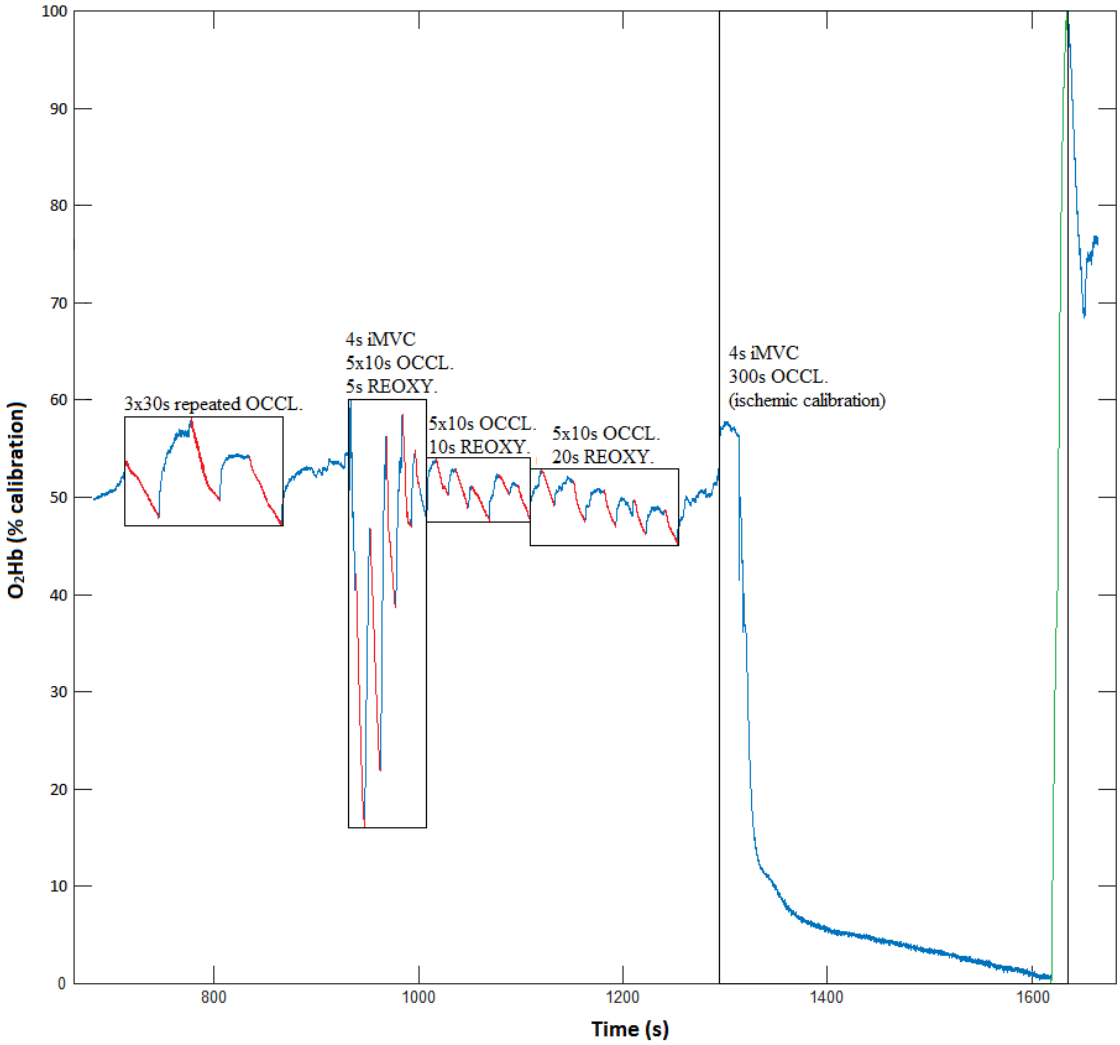


Figure 7. Example of data collection from NIRS testing on the TA, as well as the final ischemic calibration. The line represents O_2Hb as a percentage of the individuals' full range of O_2Hb saturation over time. Red parts of the line indicate descending curves for data collection of mVO_2. Ascending curves immediately following a red line during the 15 repeated occlusions is where data was collected for muscle reoxygenation. The green line represents the hyperemic response following the ischemic calibration. OCCL = occlusion; REOXY = ascending curve during free flow period; iMVC = isometric maximal voluntary contraction.

Hyperemic response

The hyperemic response following the ischemic calibration was fitted to a monoexponential curve as with mVO_2. An example of the hyperemic response is represented by the green line on figure 7 at around 1600s on the x-axis. Based on pilot studies, 60 seconds of data was used for the GM and TA, and 150s of data was used for the VL. The amount of data was chosen in order to ensure that the data only contained the hyperemic response and no data for the decay towards resting values.

Statistical analysis

All statistical analysis was conducted using SPSS (IBM SPSS Statistics, IBM Corp. 2013).

Baseline values correlation

At baseline, absolute values for mitochondrial function and microvascular function were compared using a one-factor ANOVA (factor: muscle). Further paired t-tests were performed for values with a significant difference in the ANOVA, in order to investigate the origin of the differences. For the paired t-tests, Bonferroni corrections were applied. To investigate for correlations between baseline measurements of all variables, including mitochondrial function and microvascular function of individual muscles, a Pearson's Correlation Analysis was used. For VO_{2p} kinetics and RE, an average value of all three bouts was used. For NIRS measurements of resting mVO_2 a mean of six measurements at baseline was used. For mVO_2 during repeated occlusions, as well as muscle reoxygenation, the mean value obtained from two measurements during baseline testing was used.

Delta values

For all variables measured from performance, treadmill and NIRS tests differences between pre and post values were calculated for each individual. An independent samples t-test was conducted to detect differences in delta values between groups (SIT and CON). For each variable, a two factor ANOVA (factors: time, group) was also conducted (Appendix 2).

Compliance and training volume

Training volume for the SIT group was calculated as the mean distance for the two weekly distance runs plus the mean distance covered in all SIT bouts. For the CON group training volume was calculated as the mean volume for two weeks reported in training diaries. Difference in two-week values between groups was tested using an independent samples t-test. A paired t-test was also used to compare CON group's reported weekly distance prior to engaging in the study with their mean weekly distance during intervention/control-period as reported by training diaries.

Results

In this chapter the results from the baseline values and correlations between NIRS data from three different muscles, treadmill running test data and 3000m performance will be presented first. Thereafter subject characteristics for the subjects included in the post-test will be presented, as well as results for the training intervention.

Baseline correlations

At baseline there were significant differences in curves of mVO_2, reoxygenation and the hyperemic response between muscles. The GM was the muscle with the highest oxidative capacity (mVO_2) and microvascular function (reoxygenation and hyperemic response), followed by the TA and VL in that order. There were no significant differences in resting oxygen consumption between muscles (table 2).

Table 2. ***ANOVA for baseline values for NIRS measurements.***

Muscle	Mvo2 (1/τ)	Reoxy (1/τ)	Hyperemic (1/τ)	Resting (%*s^{-1})
VL	82.7 ± 73.3	67. 7 ± 37.6	14.1 ± 6.3	-2.6 ± 1.2
GM	34.7 ± 19.7	39.1 ± 26.8	7.0 ± 3.0	-3.1 ± 1.4
TA	47.1 ± 20.9	40.6 ± 18.8	8.3 ± 2.4	-2.7 ± 0.7
P value	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P = 0.36$

Values are expressed as means ± standard deviations for the time constants for curves of muscle oxygen consumption (mVO2), reoxygenation (REOXY) and hyperemic response for each muscle at baseline, as well as resting oxygen consumption at baseline. VL is vastus lateralis; GM is medial gastrocnemius and TA is tibialis anterior. *P* values are for differences between muscles.

Table 3 shows paired samples t-tests for differences between muscles. With a Bonferroni correction, significance was considered at $P < 0.017$. Significant differences in mVO_2 were only present between the VL and GM. Reoxygenation curves did not differ between any muscles. The hyperemic response differed significantly between the VL and TA and the VL and GM.

Table 3. ***Paired samples t-tests for differences between muscles at baseline.***

	Pair of muscles	Mean	SD	*P*-value
Pair 1	VLmVO_2 - TA mVO_2	35.8	82.7	.09
Pair 2	VL mVO_2 - GM mVO_2	58.2	64.6	.01*
Pair 3	GM mVO_2 - TA mVO_2	-11.2	27.5	.11
Pair 4	VLREOXY - TAREOXY	28.0	44.6	.02
Pair 5	VLREOXY - GMREOXY	25.6	47.2	.04
Pair 6	GMREOXY - TAREOXY	-2.6	32.8	.74
Pair 7	VLhyperemic - TAhyperemic	5.7	7.4	<.01*
Pair 8	VLhyperemic - GMhyperemic	7.1	7.4	<.01*
Pair 9	GMhyperemic - TAhyperemic	-1.3	3.2	.08

The table shows paired t-tests for differences in baseline values of muscle oxygen consumption (mVO2), reoxygenation (REOXY) and hyperemic response (hyperemic) between muscles. TA is tibialis anterior, VL is vastus lateralis, GM is medial gastrocnemius. * = significant at the 0.017 level (Bonferroni correction).

There was a significant inverse correlation between VO_{2max} and 3000m performance (r = -0.721; $P < 0.001$) (Figure 8). There was no correlation between VO_{2max} and VO_{2p} kinetics, or 3000m performance and VO_{2p} kinetics. RE and 3000m performance showed a weak, non-significant correlation (r = 0.419; $P = 0.059$); RE and oxygen uptake kinetics also showed a weak, non-significant correlation (r = 0.417; $P = 0.060$). There was no correlation between RE and VO_{2max}. The mVO_2 for the VL correlated with the mVO_2 for the GM (r = 0.698, $P = 0.005$). The reoxygenation curves for the TA correlated with the hyperemic response for the TA (r = 0.527, $P = 0.017$). For a table containing all correlations, including non-significant values, between all measured variables, see appendix 3.

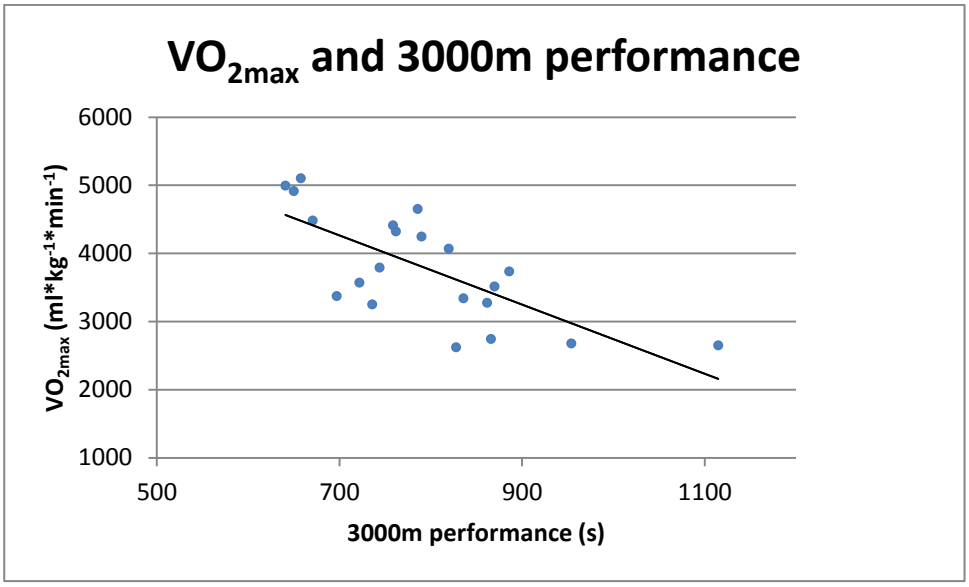


Figure 8. Relationship between baseline values of maximal oxygen uptake and 3000m performance. A Pearson's correlation test showed a significant negative correlation (r= -0.721; p<0.001).

Subject characteristics

Due to the high dropout rate (see Figure 9) from both the SIT and CON groups, a table with updated baseline characteristics of subjects included in the data analysis of delta values, is shown below (Table 4).

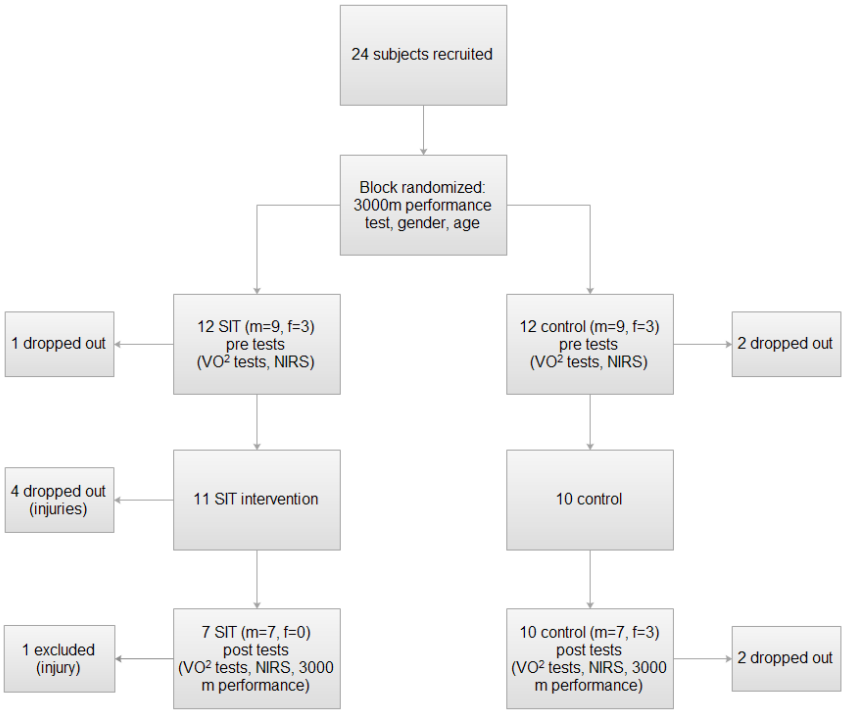


Figure 9. Illustration of the recruitment and dropouts of subjects throughout the intervention and pre- and post-testing periods.

The SIT group had only six subjects due to dropouts and one exclusion due to injury while there were ten subjects in the CON group. Also, SIT group ended up consisting of only males, while CON group consisted of seven males and three females. There were no significant differences between groups for age, weekly running distance before intervention, VO_{2max}, and 3000m performance.

Table 4. ***Subject characteristics at baseline.***

Chacteristic	Group	n	Minimum	Maximum	Mean	SD	*P*-value
Age (y)	SIT	6	37.0	49.0	42.8	5.0	0.81
	CON	10	21.0	67.0	44.2	17.2	
Weekly running distance pre INT (km)	SIT	6	15.0	45.0	29.2	9.8	0.30
	CON	10	10.0	50.0	36.0	12.7	
VO_{2max} ($ml*kg^{-1}*min^{-1}$)	SIT	6	48.4	61.4	55.8	4.9	0.18
	CON	8	40.1	59.7	50.7	6.3	
3000m performance (s)	SIT	6	650.0	886.0	740.0	90.4	0.25
	CON	10	641.0	1115.0	817.3	139.8	

Data for baseline characteristics of subjects included in the post-test and thus data analysis. An independent samples t-test showed no differences between groups. SD is standard deviation. Due to dropouts, only eight subjects from CON group completed VO_{2max} testing.

SIT intervention

Results for the 3000m performance tests and treadmill running test are shown in table 5 below. Data is shown for an independent t-test on delta values (post-pre). SIT group decreased 3000m time by 2.8% (740 ± 90.4s to 719.2 ± 82.8s) and CON group decreased 3000m time by 0.5% (817.3 ± 139.8s to 813.1 ± 147.7s) (between group difference *P* = 0.10). A power test revealed that one more subject would have resulted in a statistically significant improvement in the SIT group for 3000m performance. SIT group improved running economy by 1.97% (217.5 ± 9.1 $ml*kg^{-1}*km^{-1}$ to 213.2 ± 10.3 $ml*kg^{-1}*km^{-1}$) and CON group improved running economy by 0.01% (219.5 ± 15.7 $ml*kg^{-1}*km^{-1}$ to 219.5 ± 24.9 $ml*kg^{-1}*km^{-1}$) (between group difference *P* = 0.38). Pulmonary oxygen uptake kinetics time constants decreased by 7.2% (32.6 ± 3.6s to 30.2 ± 2.4s) in SIT group and 5.6% (36.2 ± 12.8s to 34.2 ± 11.0s) in CON group (between group difference *P* = 0.91). VO_{2max} decreased by 0.5% (4293.0 ± 666.0 ml O_2*min^{-1} to 4272.5 ± 695.0 ml O_2*min^{-1}) in SIT group and increased by 0.5% (3714.9 ± 668.1 ml O_2*min^{-1} to 3732.9 ± 589.9 ml O_2*min^{-1}) in CON group (between group difference *P* = 0.70). These results were also analyzed with a two-factor ANOVA (Appendix 2).

Table 5 *Results for independent t-test on 3000m performance, RE, VO_{2p} kinetics and VO_{2max}.*

Test	Group	n	Δ Mean	Δ SD	*P*-value	CV (%)
3000m (s)	SIT	6	-20.83	9.24	0.10	
	CON	10	-4.20	21.79		
RE ($ml*kg^{-1}*km^{-1}$)	SIT	6	-4.29	4.81	0.38	1.7
	CON	8	-0.03	11.76		
VO_{2p} kinetics (s)	SIT	6	-2.35	2.54	0.91	15.8
	CON	8	-2.03	7.43		
VO_{2max} ($ml\ O_2*min^{-1}$)	SIT	6	-20.50	224.99	0.70	
	CON	8	18.00	141.91		

Between group differences were tested using an independent samples t-test. RE is running economy; VO_{2p} is pulmonary oxygen uptake; VO_{2max} is maximal oxygen uptake; SD is standard deviation; CV is the coefficient of variation between three repeated measures of RE and VO_{2p} kinetics.

Results for the different variables measured by NIRS are shown in Table 6 below. Data is shown for an independent t-test on delta values (post-pre). Values are expressed as time constants (1/τ) for the respective monoexponential curves. There were no changes in time constants of mVO_2, reoxygenation or hyperemic response for any muscles between groups.

Table 6. *Results for variables measured by NIRS.*

Test	Group	n	Mean Δ	SD Δ	P-value
GM mVO_2 (s)	SIT	6	30.91	72.76	0.40
	CON	6	3.05	39.49	
GM Reoxy (s)	SIT	5	-1.84	17.10	0.17
	CON	6	-23.42	27.97	
GM Hyperemic (s)	SIT	6	-0.01	2.61	0.59
	CON	6	-1.13	4.19	
GM Resting (%*s^{-1})	SIT	7	-1.21	58.00	0.88
	CON	8	-4.30	11.73	
TA mVO_2 (s)	SIT	6	18.31	40.64	0.21
	CON	6	-7.22	22.38	
TA Reoxy (s)	SIT	5	-3.04	7.18	0.55
	CON	6	0.28	10.00	
TA Hyperemic (s)	SIT	6	-1.19	1.32	0.23
	CON	6	0.22	2.37	
TA Resting (%*s^{-1})	SIT	7	6.14	11.80	0.14
	CON	8	-22.87	48.63	
VL mVO_2 (s)	SIT	6	33.61	51.64	0.26
	CON	6	-33.61	129.07	
VL Reoxy (s)	SIT	5	69.93	143.33	0.23
	CON	6	-6.26	30.66	
VL Hyperemic (s)	SIT	6	-0.43	2.94	0.24
	CON	7	-3.31	5.02	
VL Resting (%*s^{-1})	SIT	7	-6.26	46.63	0.38
	CON	8	13.21	35.73	

Between group differences were tested using an independent samples t-test for delta values (post-pre). GM is medial gastrocnemius; TA is tibialis anterior; VL is vastus lateralis; mVO_2 is muscle oxygen consumption; SD is standard deviation. Some measurements were excluded due to bad quality of data, which explains the variation in n between measurements.

Coefficients of variation (CV) for included subjects during NIRS testing are shown in Table 7 below. CV was calculated for the repeated measures of mVO2 performed for each muscle during the same session, pre and post intervention. Furthermore, averaged r-values for the fit to the monoexponential curve for all mVO_2 and reoxygenation curves for each muscle during the same session, pre and post intervention are also illustrated in Table 7.

Table 7. Coefficients of variations and r-values for all NIRS measurements of each muscle

Muscle	mVO_2 (%)	Reoxygenation (%)	Resting occlusion (%)	r-value mVO_2	r-value Reoxy
VL	41	38	18	0.89	0.84
GM	36	34	25	0.88	0.84
TA	28	29	22	0.92	0.88

Coefficients of variations were calculated using Pearson's Correlation Analysis. r-values of fit to the monoexponential curves are expressed as means for each muscle . GM is medial gastrocnemius; TA is tibialis anterior; VL is vastus lateralis; mVO_2 is rate of muscle oxygen consumption; Reoxy is rate of reoxygenation between repeated occlusions.

Sprint distances and rated perceived exertions

Table 8 shows distances covered during SIT bouts for each session. The minimum distance covered during a bout was 170m and the maximal distance was 221m. Mean values during all sessions were above 190m.

Table 8. *Distances covered during SIT sessions*.

	Number of bouts (subjects * bouts)	Minimum distance (m)	Maximum distance (m)	Mean distance (m)	Std. Deviation (m)
Session1	12	181	214	198.4	10.6
Session2	24	179	221	194.8	11.5
Session3	30	175	212	190.8	10.0
Session4	30	170	210	190.8	11.2
Session5	36	180	212	194.4	9.4
Session6	34	170	210	194.7	10.8

For each session the total number of bouts performed by the total body of subjects is listed. For session 1 data for three subjects were not recorded and therefore not included. For sessions 6 one subject performed only four bouts instead of six but still achieved the 90% training compliance.

Table 9 lists the rating of perceived effort for each SIT session. The minimum value rated during a bout was 8, on a scale of 0-10, and the maximal value was 10. The mean value rated during any session was always above 9.

Table 9. ***Rated perceived exertion of subjects during SIT sessions.***

	Number of bouts (subjects* bouts)	Minimum	Maximum	Mean	Std. Deviation
Session1	20	8	10	9.1	0.8
Session2	24	8	10	9.3	0.7
Session3	30	8	10	9.4	0.6
Session4	30	8	10	9.4	0.7
Session5	36	8	10	9.3	0.7
Session6	34	8	10	9.3	0.7

For each session a rated perceived exertion was recorded for each subject. The second column denotes the total number of recorded RPEs. For session 1 data for three subjects were not recorded and therefore not included. For session 6 one subject performed only four bouts instead of six but still achieved the 90% training compliance.

Training volume

Table 10 below shows average running distance covered during the two weeks of intervention. SIT group ran 15.2 ± 5.9 km and CON group ran 38.8 ± 19.1 km (between group differences $p<0.001$)

Table 10. ***Training volume during the two week intervention for SIT and CON groups.***

Group	**n**	**Mean (km)**	**SD (km)**	***P*-value**
SIT	5	15.2	5.9	<0.01
CON	7	38.8	19.1	

Training volume expressed in kilometers for SIT and CON group during the two week intervention period. From the SIT group, one subject failed to hand in training diary, while three subjects from the CON group failed to do this. Between group differences were tested using an independent samples t-test. SD is standard deviation.

Discussion

The main finding of this study was that no effect on mitochondrial function and microvascular function was observed following two weeks of SIT in trained runners. In addition, no effect was observed on VO_{2max}, VO_{2p} kinetics and RE, despite a non-significant improvement in 3000m performance for the SIT group. Possibly, 3000m performance was improved by other mechanisms than those measured in this study.

Secondly, at baseline the GM had a significantly higher mitochondrial function than the VL in trained runners. Furthermore, the TA and GM had faster hyperemic responses following arterial occlusion than the VL, which indicates better microvascular function in these muscles. A significant inverse correlation was found between VO_{2max} and 3000m running performance. Also weak, non-significant, correlations was found between RE and 3000m running performance and RE and VO_{2p} kinetics, respectively. Finally a significant correlation between the reoxygenation curves for TA and the hyperemic response for the TA was found.

The discussion will be chronologically presented, thus beginning with the comparisons of NIRS data collected at baseline for the three different muscles, followed by correlations between all variables measured at baseline. Secondly, the results from the two week SIT intervention will be discussed, and finally methodological considerations will be presented.

Baseline data and correlations

At baseline, the GM had significantly higher mitochondrial function than the VL. This is in agreement with a previous study (Larsen et al. 2009, Layec et al. 2013), that reported the GM to have a higher oxidative capacity compared to the VL in recreationally trained subjects. This difference in muscle oxidative capacity may largely be a result of habitual usage patterns in this group of trained runners, since previous studies have shown that such patterns may be the primary determinant of muscle oxidative capacity (Larsen et al. 2009, Larsen et al. 2012). The activation of the VL compared to the GM has been shown to increase with running velocity (Cappellini et al. 2006), and our findings of a higher oxidative capacity in the GM may be a reflection of a slow habitual running velocity of our subjects.

The hyperemic response was significantly higher in the GM and TA compared to the VL, indicating better microvascular function in these lower leg muscles in comparison to the VL. Again, this may be reflective of usage patterns in the runners' habitual activity when comparing the GM to the VL. Further, the TA is primarily a slow oxidative muscle with 73-75% type I fibers (Gregory, Vandenborne & Dudley 2001), while the VL has a mixed fiber type composition (Staron et al. 2000). This difference in fiber type composition may explain the differences in the hyperemic response, since type I fibers have a larger capillary to fiber area (Ingjer 1979). Furthermore, fast twitch fibers need a higher exercise intensity in order to be recruited

and thus adapt to a training stimulus (Dudley, Abraham & Terjung 1982). Since the subjects in this study ran mostly longer distances and were not habitually performing sprints, it is plausible that the type II fibers in the VL had not regularly been recruited.

Previous studies have found the VL to have a higher oxidative capacity than the TA in younger men (Larsen et al. 2009, Larsen et al. 2012), but the same authors also found the reverse in older men regardless of activity level (Larsen et al. 2012). In this study, we observed no significant differences in oxidative capacity between the TA and the VL and thus our results conflict with those of previous studies. The reason for this is unknown, but the high CV between measurements may have masked any differences, since the *P* value was approaching significance (*P* = 0.09). Forbes et al. (2009) observed a significantly greater oxidative potential in the GM compared to the TA. This was not the case in our study, and may be explained by differences between subjects. The subjects in the study by Forbes et al. (2009) were recreationally active, in contrast to our subjects who were trained runners. As shown by Cappellini et al. (2006), the activity of the TA is higher during running than during walking, and this increased activity may cause an adaptation in oxidative capacity in the TA. The activity of the GM also increases when transitioning from walking to running (Cappellini et al. 2006), but the GM is however loaded with a high volume during everyday activities. A proportionally greater adaptation in the TA than in the GM from habitual running in our subjects may thus explain why no differences were observed between these muscles in our study.

Taken together, the patterns in adaptations related to oxidative metabolism (mitochondrial and microvascular function) observed in this study may reflect the usage patterns of these muscles during running, and also the habitual run intensity of the subjects(Cappellini et al. 2006).

There was a strong, significant inverse correlation between VO_{2max} and 3000m performance, which has been observed previously (Bassett, Howley 2000). This is to be expected since a higher aerobic capacity allows for greater O_2 delivery to support ATP production during aerobic activities. This does not imply, however, that an improved VO_{2max} will always result in improved performance, as performance is also impacted upon by other variables, one of which is RE. In this study a moderate correlation approaching significance was observed between RE and 3000m performance (*P* = 0.059, r = 0.419). This is in agreement with previous literature (Bassett, Howley 2000), indicating that RE may be an important determinant of endurance performance. One of the reasons for the stronger correlation between VO_{2max} and performance may be, that this variable can be improved upon much more than RE. For example, there may be only a 10% difference in RE between elite runners and untrained persons, whereas there may be a 100% difference in VO_{2max}.

Interestingly, we observed no correlation between VO_{2p} kinetics and 3000m performance. This may be due to relatively similar time constants for VO_{2p} kinetics between subjects at baseline, since other studies point towards that VO_{2p} kinetics is an important factor in aerobic performance (Burnley, Jones 2007, Demarle et al. 2001). Thus, if our subjects had included untrained persons and/or elite athletes, it is possible that a correlation would have been present.

The oxidative capacity in the VL and GM showed a moderate correlation (r = 0.698, *P* = 0.005). This may indicate that these two muscles adapt in a similar fashion to oxidative phosphorylation demands, although at different absolute levels (Table 2). One possible explanation for the correlation is that both muscles perform large amounts of work during running (Nicola, Jewison 2012). The oxidative capacity for the TA did not correlate with that of the GM or VL, possibly due to a different activation pattern in running (Nicola, Jewison 2012).

There was a significant correlation between reoxygenation curves and the hyperemic response for the TA. Since the hyperemic response has been used as a measure of microvascular function (Bopp, Townsend & Barstow 2011), this correlation supports the use of reoxygenation curves between repeated occlusions as a measure of microvascular function. Using NIRS to measure vascular function allows for quantification also of microvascular characteristics, compared to methods such as laser Doppler flowmetry which measures more at a macro level (i.e. large arteries) (Bopp et al. 2014). In addition, when correcting for blood volume tissue oxygenation is expressed as a percentage of maximal tissue oxygenation, which may give a more functional measure of O_2Hb saturation as a relative value of physiological maximum. In this study no correlation was found between the hyperemic response and reoxygenation curves for the GM or VL. This may be due to the higher CV observed for measurements in those muscles. Further research is needed in order to test for correlation between reoxygenation curves and the hyperemic response measured using NIRS.

We observed no correlations between oxidative capacity in any of the three muscles and VO_{2max}. This is not in agreement with other literature, which has shown a linear relationship between mitochondrial mass and VO_{2max} (Hoppeler 1990). Possibly, our subjects were within too narrow a range of mitochondrial function and VO_{2max} to detect a correlation. The high CV of the NIRS measurements may also play a part in this. It should also be mentioned that we only measured mitochondrial function in three muscles of the leg, while the VO_{2max} is a product of oxygen consumption from muscles all over the body. Furthermore, it is also possible that VO_{2max} is mainly limited by central factors in our subjects, and that this explains the lack of correlation between oxidative capacity in the VL, GM and TA and VO_{2max}.

3000m performance

In other studies improvements of 3.8 – 10.1% are seen on aerobic time trial performances following 2-8 weeks of SIT (Macpherson et al. 2011, Burgomaster, Heigenhauser & Gibala 2006, Gibala et al. 2006, Skovgaard et al. 2014). Generally, the improvements appear to be smaller in trained compared with untrained subjects (Weston et al. 2014). For example, trained runners showed a 3.8% improvement in 10km running performance after four weeks of concurrent SIT and strength training (Skovgaard et al. 2014), whereas subjects unaccustomed to cycling showed a 10.1% improvement in 30km cycling time trial after only two weeks of SIT (Gibala et al. 2006). In this study we observed a non-significant improvement of 20.8 ± 9.24s in 3000m performance in the SIT group, following two weeks of SIT. This is equal to a performance improvement of 2.8%, which is in in agreement with abovementioned literature, when the duration of intervention and fitness level of subjects is taken into account. .

It is possible that a larger sample-size in the SIT group, would have resulted in a statistically significant improvement, since a power analysis using the mean and SD of improvements in SIT group showed that one additional subject would have resulted in statistical significance. Notably, a higher sample size would have been attained if the dropout rate, due to injuries, had not been so unexpectedly high. Many potential mechanisms, both central and peripheral, can contribute to increases in performance after short term SIT, however none of the variables measured in this study could explain changes in performance.

Maximal oxygen consumption

SIT interventions have generally been reported to elicit improvements in VO_{2max} in the range of 4-13.5% (Sloth et al. 2013), however, not all studies have reported improvements in VO_{2max} (Burgomaster et al. 2005, Burgomaster, Heigenhauser & Gibala 2006). There seems to be a fitness dependent component to these improvements, as SIT studies using subjects of higher fitness status found no significant changes in VO_{2max} (Macpherson, Weston 2015, Skovgaard et al. 2014). This is in agreement with our findings, as we observed no change in VO_{2max} following two weeks of SIT. Macpherson & Weston (2015) trained subjects with a baseline VO_{2max} of 52.7 ± 4.7 $ml*kg^{-1}*min^{-1}$, and Skovgaard et al. (2014) had subjects who tested a VO_{2max} baseline of 60.7 ± 1.2 $ml*kg^{-1}*min^{-1}$. These values are comparable to the 55.8 ± 4.9 $ml*kg^{-1}*min^{-1}$ in our subjects. That improvements in VO_{2max} following HIT become progressively smaller as fitness level increases, is in agreement with (Weston et al. 2014), and may explain the absence of changes in VO_{2max} in our SIT subjects. It is plausible, that longer interventions are needed to elicit improvements in VO_{2max} in subjects of a higher fitness status, although the Skovgaard et al. study, who used eight weeks SIT two $times*week^{-1}$, along with concurrent aerobic training and strength training, found no improvements.

Nonetheless, further research is warranted to clarify the effects of intervention length of SIT and fitness status on VO_{2max} improvements.

Macpherson et al. (2011) concluded that SIT primarily increases VO_{2max} by means of peripheral adaptations in oxidative capacity, whereas ET increases VO_{2max} by increasing stroke volume and thus cardiac output. Our data is thus in agreement with the literature, since no changes were seen in neither VO_{2max} nor oxidative capacity in the VL, GM and TA.

Running economy

The literature examining the effect of HIT/SIT on RE is equivocal, with a recent meta-analysis showing that RE is improved by 1-7% following some HIT interventions, whereas other interventions show no improvement (Barnes, Kilding 2015). Barnes et al. goes on to suggest that training volume is an important factor in improving RE, and that sprint-interval type training may not include sufficient volume to improve RE, and furthermore suggests that running at too high velocities may disrupt biomechanics of running at lower velocities, thus decreasing RE. Also, a study by Macpherson et al. (2011) reported no change in RE following six weeks of running HIT. These reports are in agreement with our findings of no change in RE following two weeks of run SIT. In contrast, Iaia et al. (2009) observed a 5.7-7.6% improvement in RE at velocities varying from 11-16 $km*h^{-1}$ following four weeks of run SIT. This improvement could not be explained by changes in ventilation, UCP3 or changes in substrate utilization. Iaia and colleagues speculated, among other things, that the degree of proton leak through the mitochondrial membrane could be altered following the SIT in a way that was not detectable to the authors. Skovgaard et al. (2014) observed a 3.1% improvement in RE following 8 weeks of concurrent SIT and strength training. It is possible that the improvement in RE seen by Skovgaard et al. (2014) was induced by strength training, since strength training has been shown to increase RE (Barnes, Kilding 2015). In conclusion, no effect of SIT on RE was observed in the present study, and more research is needed to clarify the role of SIT on running economy.

Pulmonary oxygen uptake kinetics

SIT and HIT has been shown to improve VO_{2p} kinetics in several studies lasting only a few weeks (Da boit, Mckay, Bailey, Williams). McKay et al. (2009) observed a 20% improvement in VO_{2p} kinetics following only two sessions of HIT, showing that these adaptations occur rapidly. This is in contrast to our findings, as we observed no changes in VO_{2p} kinetics following two weeks of SIT. The main difference between our study and cited studies is the fitness level of subjects. Our subjects had a mean VO_{2max} of 55.8 ± 4.9 $ml*kg^{-1}*min^{-1}$, which is about 10 $ml*kg^{-1}*min^{-1}$ higher than abovementioned studies. Another study using subjects at a

fitness level similar to our subjects was done by Skovgaard et al. (2014), who also observed no change in VO_{2p} kinetics following eight weeks of SIT. Thus, it seems that rapid adaptations in factors speeding VO_{2p} kinetics may not occur in individuals with a relatively high fitness level.

Peripheral adaptations

The main adaptation leading to faster VO_{2p} kinetics in the early stages of training has been suggested to be improved microvascular function (McKay, Paterson & Kowalchuk 2009, Williams, Paterson & Kowalchuk 2013, Bailey et al. 2009). However, the cited studies used changes in HHb, measured by NIRS, as a measure of muscle oxygen consumption. With this method, it cannot be concluded whether any increases in oxygen consumption are caused by improved mitochondrial function or better oxygen delivery to mitochondria (microvascular function). In our study, arterial occlusion was applied in order to isolate oxygen consumption during occlusion, and look at reoxygenation between occlusions as a measure of microvascular function. Furthermore we corrected for blood volume and standardized measurements to the hyperemic response following ischemic calibration, which makes the O_2Hb signal between occlusions an indicator of oxygenation as a percentage of the maximal possible oxygenation.

In contrast to previous studies (McKay, Paterson & Kowalchuk 2009, Williams, Paterson & Kowalchuk 2013, Bailey et al. 2009, Da Boit et al. 2014), we did not observe any changes in VO_{2p} kinetics following two weeks of running SIT. In line with this result, we did not observe any changes in measures of mitochondrial or microvascular function in any of the three investigated muscles. Other studies have shown increases in markers of muscle oxidative capacity following 2-6 weeks of SIT (Burgomaster et al. 2005, Larsen, Befroy & Kent-Braun 2013, Burgomaster et al. 2008, Burgomaster, Heigenhauser & Gibala 2006), however this is also in contrast to our findings. Both the absence of changes in muscle oxidative capacity and microvascular function in this study may be due to the already high training status of the subjects. The main purpose of our study was to compare adaptations in VO_{2p} kinetics with peripheral adaptations in mitochondrial and microvascular function in three different muscles. As mentioned, we did not see any changes in VO_{2p} kinetics following two weeks of SIT, which is consistent with our findings regarding peripheral adaptations, where no changes were apparent.

Methodological Considerations

Recruitment and dropouts

The main consideration for this study was the high dropout rate in the SIT group. Specifically, 5 out of 12 subjects experienced an injury that forced them to drop out. Injuries consisted mainly of hamstring and quadriceps strains and were severe enough to cause an inability to complete the SIT protocol. Also in the

control group the dropout rate was high, although in this case it was due to compliance, causing a drop out of another five subjects. The high injury rate could be related to the high intensity nature of the training intervention and possibly also to the characteristics of the subjects recruited. The training proved to be very demanding for the subjects as evidenced by high RPE scores obtained after each SIT bout. In addition to this, all subjects reported cases of delayed onset muscle soreness (DOMS) when reporting for each session following the first session. It should be noted that the said high injury rate occurred in spite of thorough supervised warm up prior to each SIT session. This injury prevalence is not in agreement with the current literature of SIT running studies (Macpherson, Weston 2015, Macpherson et al. 2011, Iaia et al. 2009, Skovgaard et al. 2014, Sandvei et al. 2012, Rowan, Kueffner & Stavrianeas 2012), but provides new and important insights to practical, and possibly ethical, considerations when implementing SIT in a training regimen. Thus, the practical knowledge brought forth by this intervention has a high value for application of SIT in training programs. Considerations should be given to the possibility of utilizing SIT over longer periods of time, possibly allowing for a gradual ramping up of intensity as well as a more conservative increase in volume than done in this study.

Attention should, however, be given to the characteristics (habitual training and age) of the recruited subjects in this study as it may pertain to the injury prevalence. The runners recruited were all long distance runners (i.e. competing in marathons), and as such not accustomed to running intervals at high velocities. Therefore, the population represented by the recruited subjects may have been more prone to injuries incurred by the SIT protocol, mainly due to a lack of specific conditioning. In this study subjects unaccustomed to SIT were recruited, since rapid adaptations were expected to occur in this population. In future studies, a balance between the characteristic of subjects versus the propensity for injuries should be thoroughly considered. If choosing an unaccustomed population, a longer intervention period is warranted to allow for a more conservative progression.

To round up recruitment considerations, a brief note should be given to the inclusion criteria of volume. Subjects reported their habitual training volume from the previous six months by questionnaire. A strength of this method is, that it takes into account the training volume over a long period of time. However, recall questionnaires are known to be inaccurate, and including a detailed training log during a shorter period could possibly have provided more reliable information about training level.

Testing protocols

This study tried to take into account any familiarization of lab tests by including a control group and comparing measurements between groups, thus negating any learning effects. However, some aspects of the individual tests will be evaluated in the following paragraphs.

Treadmill running test

Regarding laboratory testing, several aspects of the treadmill running test should be evaluated. First, the subjects ran at 80% of their respective pre or post-test 3000m performance velocity. This approach allowed us to test RE and Vo2p kinetics at a velocity that corresponded to the same relative intensity for all subjects. The velocity of the treadmill was adjusted from pre to post based on the 3000 m test performed pre and post, so the 80% would correspond to any changes in capacity taking place during the course of the intervention period.

NIRS testing

Previous studies have used PCr recovery kinetics or biopsies measuring enzyme activities to infer information about mitochondrial function (Burgomaster et al. 2005, Larsen et al. 2009). However, PCr recovery is under some conditions dependent on O_2 availability, and as such improvements on PCr recovery may reflect improvements in both microvascular and/or mitochondrial function. Measuring enzyme content is, on the other hand, a robust method to estimate mitochondrial function, but still does not take into account any change in microvascular function. A strength of this study was that NIRS testing allowed us to investigate changes in oxygen delivery and consumption independently. However, there are both pros and cons regarding the use of an in-vivo measurement such as NIRS. Using a non-invasive method such as NIRS provides a functional measure of mitochondrial function and microvascular function, but does not give further information on the mechanism behind the end result.

A few points could be improved upon during NIRS testing. Firstly, anatomical landmarks were used to standardize placements of the NIRS probe at both pre- and post-test. This has some limitations when it comes to locating the exact same spot at both pre- and post-test. A more exact method would be to tattoo or have the subjects mark up a spot daily, such that the exact same site could be measured pre and post intervention.

With regards to usage of the arterial occlusions, the cuff did inflate and deflate rapidly but not instantaneously. However, this was amended in this study by manually analyzing all NIRS data (blinded), and choosing peaks and troughs from where to measure mVO2 and influx of O2Hb respectively. Furthermore, since a delayed occlusion may result in only a few seconds of total arterial occlusion, data was only analyzed for three seconds of the descending curve and two seconds for ascending curves. Notably, manual inspection of the curves confirmed linear slopes of these curves, suggesting that this approach did not affect data analysis.

The NIRS ascending curves had a repeated contamination of the first data point. It is possible that a slowed reoxygenation occurs following the iMVC plus occlusion which can explain the low value of this data point.

It was assumed that the oxygenation and de-oxygenation of hemoglobin during the repeated occlusions followed a monoexponential function (Ryan et al. 2013). In a few cases, greatly outlying data points resulted in a poor fit. Therefore, to correct for these erroneous artifacts, a manual inspection of the data was done to remove the first point on the ascending curve (i.e., reoxygenation) when the data point was an outlier. An example of this correction in the fit of reoxygenation data is illustrated in Figure 10. Following correction, average r-values for reoxygenation ranged between 0.84-0.88 Table 7.

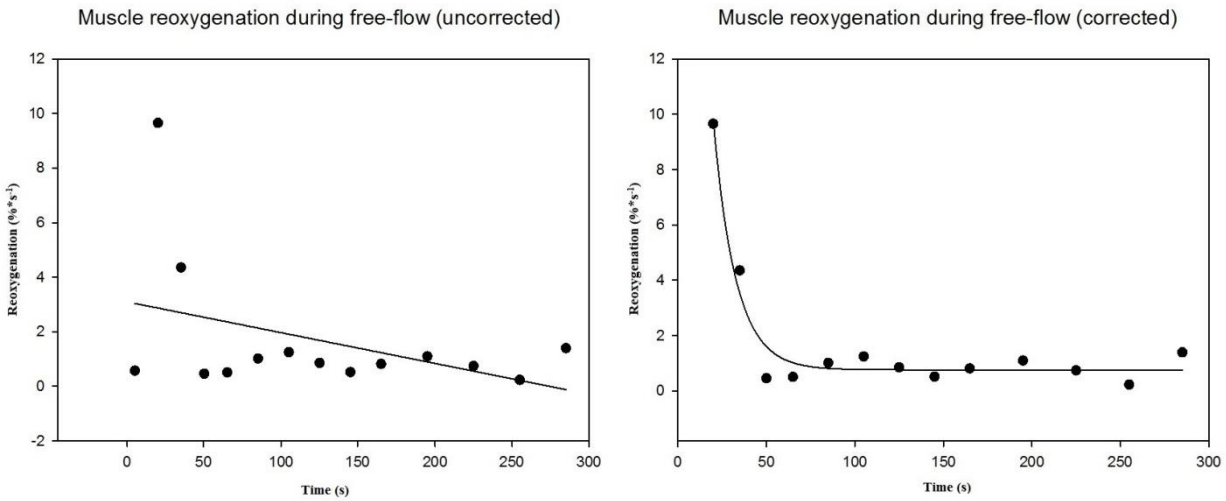


Figure 10. Data correction for muscle reoxygenation. Illustration of the effect of removing the first erroneous data point on monoexponential fit of curves for muscle reoxygenation. The graph on the left is the original data. The graph on the right shows the corrected data. Y-axes on both graphs shows change in muscle reoxygenation per second as a percentage of the ischemic calibration, and x-axes show time in seconds.

Analysis of the descending curves (i.e., de-oxygenation) showed a pattern with the second data point (i.e., slope of second curve) being lower than the first point (i.e., higher mVO2). It is possible that the MVC followed by cuff occlusions result in a lag in muscle oxygen consumption rate due to O2 limitations within the active muscle fibers. The de-oxygenation curves showed good fits (r = 0.89-0.92). Other studies using the same approach to estimate mitochondrial function have not reported any manual correction of the descending curves (Ryan et al. 2014, Ryan et al. 2013, Ryan et al. 2012, Ryan, Brizendine & McCully 2013), so the fits were done using all data points. An example of the fit of these functions can be seen in Figure 11.

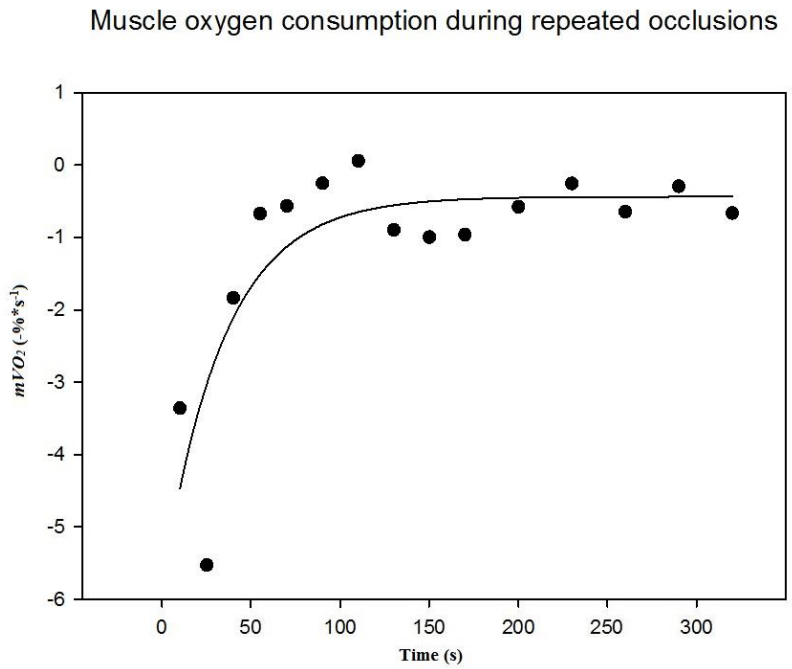


Figure 11. Example of representative data for muscle oxygen consumption during 15 repeated occlusions. The y-axis shows muscle oxygen consumption per second as a percentage of the ischemic calibration, and the x-axis shows time.

Some outlying mVO_2 data showed a poor fit to the monoexponential function, but were still included uncorrected. Manual correction of this data would result in better fits, and time constants that were more in line with those of other subjects. Since it is known that resynthesis of ATP, of which the mVO_2 is an indicator, follows a monoexponential function (Lanza et al. 2011), it could be argued that some outlying data points are not representative of a physiological response. In the future when analyzing NIRS data, correcting such values manually should be considered. An example of the effect of data correction on some outlying data for mVO_2 can be seen in figure 12. In this example, the fit changes notably from a linear function to a monoexponential function, and the rate constant becomes within those values observed in other subjects.

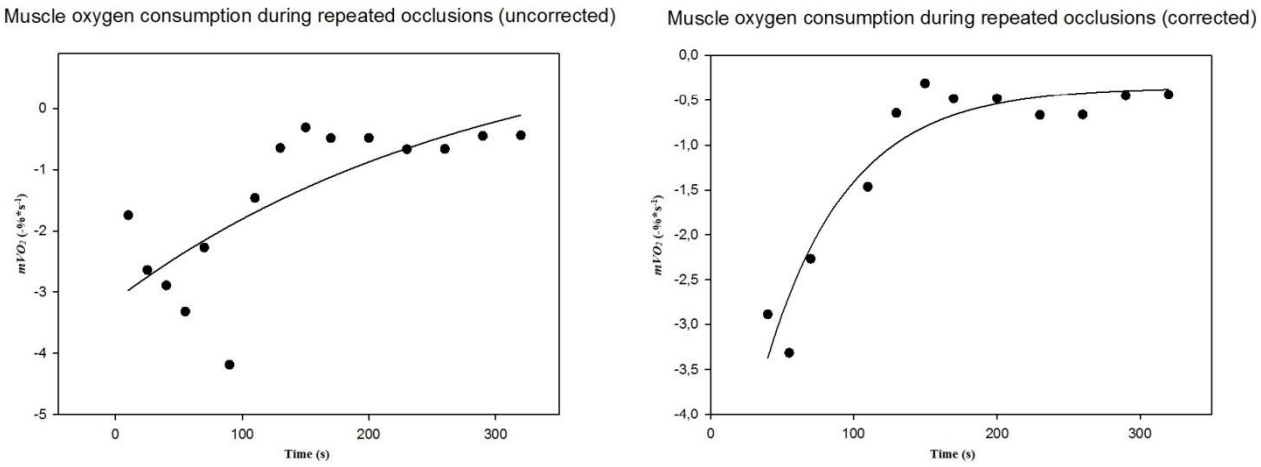


Figure 12. Effect of manual data correction on outlying time constants. An example of outlying uncorrected data for muscle oxygen consumption (mVO_2) is presented on the left, and an example of corrected data is shown on the right. In data analysis, no correction on mVO_2 data was made. Y-axes on both graphs shows change mVO_2 per second as a percentage of the ischemic calibration, and x-axes show time in seconds.

While analyzing NIRS data, an error encountered was seemingly a displacement of the absolute value of O2Hb signal. In other words, the slope of the relative increase or decrease in O2Hb seemed unaffected, but a jump in the data appeared. It is possible these jumps occur due to a displacement of the probe relative to the tissue, either by touch to the probe or movement of the tissue below the probe. Furthermore, spikes sometimes appeared in the data. Spikes could possibly be caused by muscle twitches (voluntary or involuntary) or short duration movement of the tissue beneath the probe. In this study these errors were accounted for by making sure no jumps or spikes in data were present during measurements of mVO2, reoxygenation or full physiological calibration. An example of both a spike and a jump in the curve after ischemic calibration is shown in Figure 13. In such a case, the jump was excluded from the script defining the minimum and maximal value of the full range.

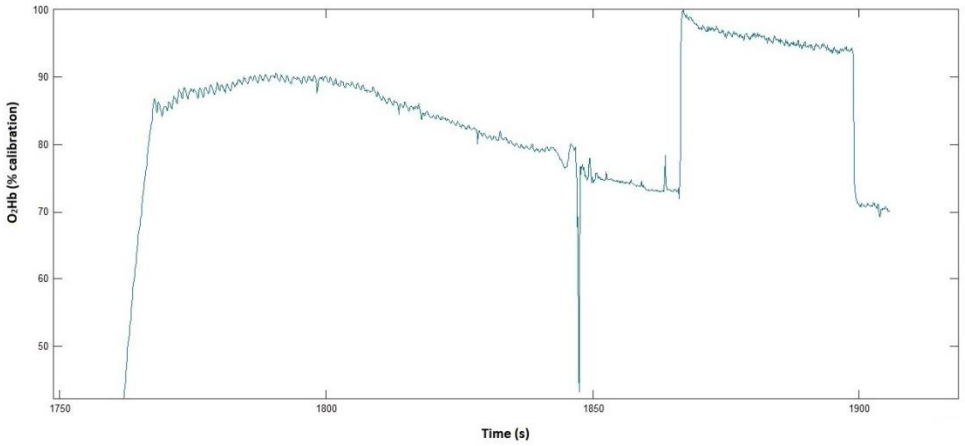


Figure 13 illustrates sampling of NIRS data following the ischemic calibration. At 1845 seconds on the x-axis a spike in the data is shown. Around 1870-1900 on the x-axis a jump in the data is shown, where the absolute value of data displaces.

This study also tested intra-session validity of NIRS measurements. Overall high coefficients of variation were observed for both measurements of mVO_2 (CV = 28-41%) and reoxygenation (CV = 29-38 %), which makes it harder to detect small changes in mitochondrial function or microvascular function as could be expected following a two-week intervention. This high coefficient of variation is not in agreement with previous studies using NIRS or PCr recovery to measure mitochondrial function (Larsen, Befroy & Kent-Braun 2013, Ryan et al. 2013, Ryan et al. 2012, Brizendine et al. 2013, Ryan, Brizendine & McCully 2013). One study using PCr recovery did, however, show a high CV similar to our study, with 42% CV for the quadriceps, and 44% CV for the plantarflexors (Layec et al. 2013). The main difference between our study and abovementioned studies by Ryan et al. is the method of stimulation. In our study, subjects performed an iMVC before measurements of mVO_2, whereas previous studies have used either electrical stimulation or submaximal contractions (Ryan et al. 2014, Ryan et al. 2013, Ryan et al. 2012, Brizendine et al. 2013, Ryan, Brizendine & McCully 2013). It is possible that the iMVC resulted in movement of the NIRS probe and thus played a part in the high CV observed in this study. An iMVC was used in order to ensure activation of as many fibers as possible in the given muscles, and to standardize the contraction from pre to post-test. Furthermore, despite basing the contraction times on pilot testing in order to not desaturate the muscle below 30% O_2Hb, manual data analysis revealed that some subjects desaturated the muscle completely during the iMVC. This desaturation may change the pH level in the muscle, which can affect the monoexponential fit of the mVO_2 curve (Yoshida, Watari 1993, Walter et al. 1997). Also, during measurements of the VL, it was observed that inflation of the cuff caused the skin underneath the NIRS probe to move, and it is possible that this in turn affected the recordings of the VL. This would also explain why the CV was larger for the VL than the TA or GM, since the cuff was placed further away from the probe when recording from the latter two muscles. In addition to this, it was observed by the authors, that subjects for whom data was excluded due to bad quality generally were subjects with a thicker layer of subcutaneous adipose tissue. Although Ryan et al. (2012) claims that calibrating the O2hb signal to each individuals' full range removes the influence of adipose tissue, this may not be the case when the adipose tissue layer exceeds a certain thickness. This is the case, since the NIRS device may not penetrate through the adipose layer, and thus measurements will be of adipose tissue oxygenation instead of muscle oxygenation.

3000m performance test

The 3000m performance test was chosen based on the aerobic nature of this event. Not all subjects were familiar with 3000m running, and as such a learning effect could take place from pre- to post-test. This was countered by having a control group, but could be further improved upon by choosing a distance with which the subjects were more familiar. Another possibility would be to include a familiarization run before

baseline testing, or use this familiarization run as a reliability test. Furthermore, subjects may have been subjected to a pacing effect as test groups were not standardized pre to post, and thus some subjects ran with different fellow subjects, pre to post. In order to counter this, subjects should have been running in pre-organized groups, or individually.

Sprint interval training

The high intensity nature of the SIT intervention was not only physically demanding but also mentally taxing. Therefore, and consistent with studies using similar interventions (Macpherson et al. 2011, Burgomaster et al. 2005, Burgomaster, Heigenhauser & Gibala 2006, Iaia et al. 2009, Gibala et al. 2006), all SIT sessions were supervised. Recording the individual distances covered during all SIT bouts as well as individual RPEs for each bout for the intervention group allowed for a number of descriptive variables. A quantification of the SIT distances covered enables a portraying of the subject's ability to sprint for 30 seconds, and thereby of their anaerobic conditioning. This is a valuable measure of the training status of the recruited runners, as it arguably indicates a greater specific measure of these subjects ability to perform, and in turn improve brief, all out intervals. To accompany the recorded distances, the RPEs make possible for a control of the intra-subject effort and compliance with the instruction for pacing, i.e. go as fast as possible from start to finish, as changes in distances from bout to bout and a change in RPE could indicate discrepancies. With the purpose of integrating the SIT intervention to a more ecological context, a weekly distance run of 25% of reported individual weekly volume was implemented. This would to a greater extend lend itself to situations were SIT is implemented in a periodized programming in which SIT is not entirely replacing all aerobic distance training for a longer period of time. This may diminish the comparability of the study to others using the same SIT protocol for running, but on the other hands offers a perspective of the utilization of SIT in a more real life context.

When greatly reducing volume and increasing intensity, as occurred during this intervention (Table 10) a tapering effect may occur, which could increase performance (Mujika 2010). However, since training intensity was so greatly increased, resulting in subjective reports of severe DOMS in the subjects, it is the authors' belief that a tapering effect did not affect the results.

Conclusion

At baseline, the runners in this study had better mitochondrial function in the GM compared to the VL. The hyperemic response was faster in the GM and TA than in the VL, indicating better microvascular function in these muscles compared with the VL. As expected, there was a significant correlation between VO_{2max} and 3000m performance.

Two weeks of running SIT led to a non-significant improvement in 3000m performance of 2.8% (740.0s and 719.2s pre and post, respectively). This could not be explained by any changes in VO_{2max}, RE, VO_{2p} kinetics, mitochondrial or microvascular function, and it is possible that performance was improved by mechanisms not measured in this study. This two week SIT intervention did not improve RE, and thus helps to clarify the time course of adaptations in RE. The results also highlight that once a certain fitness level is reached, short term SIT may not lead to rapid adaptations in VO_{2p} kinetics as seen in previous studies using subjects with a smaller VO_{2max}. We were unable to test our hypothesis regarding the effects of changes in microvascular or mitochondrial function in three different muscles on changes in VO_{2p} kinetics, since no adaptations were observed in either variable. Future studies should examine the relationship between changes in VO_{2p} kinetics and peripheral adaptations using subjects with a smaller fitness level.

From a practical standpoint, this study showed the potential drawback to running SIT as five of twelve subjects in the SIT group were injured. In future studies, a more conservative running SIT intervention should be considered, when subjects are unaccustomed to this type of training.

Bibliography

Bailey, S.J., Wilkerson, D.P., Dimenna, F.J. & Jones, A.M. 2009, "Influence of repeated sprint training on pulmonary O2 uptake and muscle deoxygenation kinetics in humans", *Journal of applied physiology (Bethesda, Md.: 1985),* vol. 106, no. 6, pp. 1875-1887.

Barnes, K.R. & Kilding, A.E. 2015, "Strategies to Improve Running Economy", *Sports Medicine,* vol. 45, no. 1, pp. 37-56.

Barnett, C., Carey, M., Proietto, J., Cerin, E., Febbraio, M. & Jenkins, D. 2004, "Muscle metabolism during sprint exercise in man: influence of sprint training", *Journal of Science and Medicine in Sport,* vol. 7, no. 3, pp. 314-322.

Bassett, D.R.,Jr & Howley, E.T. 2000, "Limiting factors for maximum oxygen uptake and determinants of endurance performance", *Medicine and science in sports and exercise,* vol. 32, no. 1, pp. 70-84.

Bopp, C.M., Townsend, D.K. & Barstow, T.J. 2011, "Characterizing near-infrared spectroscopy responses to forearm post-occlusive reactive hyperemia in healthy subjects", *European journal of applied physiology,* vol. 111, no. 11, pp. 2753-2761.

Bopp, C.M., Townsend, D.K., Warren, S. & Barstow, T.J. 2014, "Relationship between brachial artery blood flow and total [hemoglobin myoglobin] during post-occlusive reactive hyperemia", *Microvascular research,* vol. 91, pp. 37-43.

Brizendine, J.T., Ryan, T.E., Larson, R.D. & Mccully, K.K. 2013, "Skeletal muscle metabolism in endurance athletes with near-infrared spectroscopy", *Med Sci Sports Exerc,* vol. 45, no. 5, pp. 869-875.

Burgomaster, K.A., Howarth, K.R., Phillips, S.M., Rakobowchuk, M., MacDonald, M.J., McGee, S.L. & Gibala, M.J. 2008, "Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans", *The Journal of physiology,* vol. 586, no. 1, pp. 151-160.

Burgomaster, K.A., Heigenhauser, G.J. & Gibala, M.J. 2006, "Effect of short-term sprint interval training on human skeletal muscle carbohydrate metabolism during exercise and time-trial performance", *Journal of applied physiology (Bethesda, Md.: 1985),* vol. 100, no. 6, pp. 2041-2047.

Burgomaster, K.A., Hughes, S.C., Heigenhauser, G.J., Bradwell, S.N. & Gibala, M.J. 2005, "Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans", *Journal of applied physiology (Bethesda, Md.: 1985),* vol. 98, no. 6, pp. 1985-1990.

Burnley, M. & Jones, A.M. 2007, "Oxygen uptake kinetics as a determinant of sports performance", *European Journal of Sport Science,* vol. 7, no. 2, pp. 63-79.

Cappellini, G., Ivanenko, Y.P., Poppele, R.E. & Lacquaniti, F. 2006, "Motor patterns in human walking and running", *Journal of neurophysiology,* vol. 95, no. 6, pp. 3426-3437.

Da Boit, M., Bailey, S.J., Callow, S., Dimenna, F.J. & Jones, A.M. 2014, "Effects of interval and continuous training on O2 uptake kinetics during severe-intensity exercise initiated from an elevated metabolic baseline", *Journal of applied physiology (Bethesda, Md.: 1985),* vol. 116, no. 8, pp. 1068-1077.

Demarle, A.P., Slawinski, J.J., Laffite, L.P., Bocquet, V.G., Koralsztein, J.P. & Billat, V.L. 2001, "Decrease of O(2) deficit is a potential factor in increased time to exhaustion after specific endurance training", *Journal of applied physiology (Bethesda, Md.: 1985),* vol. 90, no. 3, pp. 947-953.

Dudley, G.A., Abraham, W.M. & Terjung, R.L. 1982, "Influence of exercise intensity and duration on biochemical adaptations in skeletal muscle", *Journal of applied physiology: respiratory, environmental and exercise physiology,* vol. 53, no. 4, pp. 844-850.

Forbes, S.C., Slade, J.M., Francis, R.M. & Meyer, R.A. 2009, "Comparison of oxidative capacity among leg muscles in humans using gated 31P 2-D chemical shift imaging", *NMR in biomedicine,* vol. 22, no. 10, pp. 1063-1071.

Gibala, M.J., Little, J.P., Van Essen, M., Wilkin, G.P., Burgomaster, K.A., Safdar, A., Raha, S. & Tarnopolsky, M.A. 2006, "Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance", *The Journal of physiology,* vol. 575, no. 3, pp. 901-911.

Gist, N.H., Fedewa, M.V., Dishman, R.K. & Cureton, K.J. 2014, "Sprint interval training effects on aerobic capacity: a systematic review and meta-analysis", *Sports Medicine,* vol. 44, no. 2, pp. 269-279.

Gollnick, P.D., Armstrong, R.B., Saltin, B., Saubert, C.W.,4th, Sembrowich, W.L. & Shepherd, R.E. 1973, "Effect of training on enzyme activity and fiber composition of human skeletal muscle", *Journal of applied physiology,* vol. 34, no. 1, pp. 107-111.

Gregory, C.M., Vandenborne, K. & Dudley, G.A. 2001, "Metabolic enzymes and phenotypic expression among human locomotor muscles", *Muscle & nerve,* vol. 24, no. 3, pp. 387-393.

Hamaoka, T., McCully, K.K., Niwayama, M. & Chance, B. 2011, "The use of muscle near-infrared spectroscopy in sport, health and medical sciences: recent developments", *Philosophical transactions.Series A, Mathematical, physical, and engineering sciences,* vol. 369, no. 1955, pp. 4591-4604.

Hoppeler, H. 1990, "The different relationship of V̇O2 to muscle mitochondria in humans and quadrupedal animals", *Respiration physiology,* vol. 80, no. 2, pp. 137-137-145.

Iaia, F.M., Hellsten, Y., Nielsen, J.J., Fernstrom, M., Sahlin, K. & Bangsbo, J. 2009, "Four weeks of speed endurance training reduces energy expenditure during exercise and maintains muscle oxidative capacity despite a reduction in training volume", *Journal of applied physiology (Bethesda, Md.: 1985),* vol. 106, no. 1, pp. 73-80.

Ichinose, Y., Kawakami, Y., Ito, M. & Fukunaga, T. 1997, "Estimation of active force-length characteristics of human vastus lateralis muscle", *Cells Tissues Organs,* vol. 159, no. 2-3, pp. 78-83.

Ingjer, F. 1979, "Capillary supply and mitochondrial content of different skeletal muscle fiber types in untrained and endurance-trained men. A histochemical and ultrastructural study", *European journal of applied physiology and occupational physiology,* vol. 40, no. 3, pp. 197-209.

Jones, A.M. & Carter, H. 2000, "The effect of endurance training on parameters of aerobic fitness", *Sports medicine,* vol. 29, no. 6, pp. 373-386.

Kime, R., Hamaoka, T., Sako, T., Murakami, M., Homma, T., Katsumura, T. & Chance, B. 2003, "Delayed reoxygenation after maximal isometric handgrip exercise in high oxidative capacity muscle", *European journal of applied physiology,* vol. 89, no. 1, pp. 34-41.

Lanza, I.R., Bhagra, S., Nair, K.S. & Port, J.D. 2011, "Measurement of human skeletal muscle oxidative capacity by 31P-MR spectroscopy: A cross-validation with in vitro measurements", *Journal of Magnetic Resonance Imaging,* vol. 34, no. 5, pp. 1143-1150.

Larsen, R.G., Callahan, D.M., Foulis, S.A. & Kent-Braun, J.A. 2012, "Age-related changes in oxidative capacity differ between locomotory muscles and are associated with physical activity behavior", *Applied Physiology, Nutrition, and Metabolism,* vol. 37, no. 1, pp. 88-99.

Larsen, R.G., Befroy, D.E. & Kent-Braun, J.A. 2013, "High-intensity interval training increases in vivo oxidative capacity with no effect on P(i)-->ATP rate in resting human muscle", *American journal of physiology.Regulatory, integrative and comparative physiology,* vol. 304, no. 5, pp. R333-42.

Larsen, R.G., Callahan, D.M., Foulis, S.A. & Kent-Braun, J.A. 2009, "In vivo oxidative capacity varies with muscle and training status in young adults", *Journal of applied physiology (Bethesda, Md.: 1985),* vol. 107, no. 3, pp. 873-879.

Laursen, P.B. & Jenkins, D.G. 2002, "The scientific basis for high-intensity interval training", *Sports Medicine,* vol. 32, no. 1, pp. 53-73.

Layec, G., Malucelli, E., Le Fur, Y., Manners, D., Yashiro, K., Testa, C., Cozzone, P.J., Iotti, S. & Bendahan, D. 2013, "Effects of exercise-induced intracellular acidosis on the phosphocreatine recovery kinetics: a 31P MRS study in three muscle groups in humans", *NMR in biomedicine,* vol. 26, no. 11, pp. 1403-1411.

Liljedahl, M.E., Holm, I., Sylvén, C. & Jansson, E. 1996, "Different responses of skeletal muscle following sprint training in men and women", *European journal of applied physiology and occupational physiology,* vol. 74, no. 4, pp. 375-383.

MacDougall, J.D., Hicks, A.L., MacDonald, J.R., McKelvie, R.S., Green, H.J. & Smith, K.M. 1998, "Muscle performance and enzymatic adaptations to sprint interval training", *Journal of applied physiology (Bethesda, Md.: 1985),* vol. 84, no. 6, pp. 2138-2142.

Macpherson, R.E., Hazell, T.J., Olver, T.D., Paterson, D.H. & Lemon, P.W. 2011, "Run sprint interval training improves aerobic performance but not maximal cardiac output", *Medicine and science in sports and exercise,* vol. 43, no. 1, pp. 115-122.

Macpherson, T.W. & Weston, M. 2015, "The effect of low-volume sprint interval training on the development and subsequent maintenance of aerobic fitness in soccer players", *International journal of sports physiology and performance,* vol. 10, no. 3, pp. 332-338.

Maganaris, C.N. 2003, "Force-length characteristics of the in vivo human gastrocnemius muscle", *Clinical Anatomy,* vol. 16, no. 3, pp. 215-223.

Maganaris, C.N. 2001, "Force–length characteristics of in vivo human skeletal muscle", *Acta Physiologica Scandinavica,* vol. 172, no. 4, pp. 279-285.

McKay, B.R., Paterson, D.H. & Kowalchuk, J.M. 2009, "Effect of short-term high-intensity interval training vs. continuous training on O2 uptake kinetics, muscle deoxygenation, and exercise performance", *Journal of applied physiology (Bethesda, Md.: 1985),* vol. 107, no. 1, pp. 128-138.

Menard, M.R., Penn, A.M., Lee, J.W., Dusik, L.A. & Hall, L.D. 1991, "Relative metabolic efficiency of concentric and eccentric exercise determined by 31P magnetic resonance spectroscopy", *Archives of Physical Medicine and Rehabilitation,* vol. 72, no. 12, pp. 976.

Mujika, I. 2010, "Intense training: the key to optimal performance before and during the taper", *Scandinavian Journal of Medicine & Science in Sports,* vol. 20, no. s2, pp. 24-31.

Nicola, T.L. & Jewison, D.J. 2012, "The anatomy and biomechanics of running", *Clinics in sports medicine,* vol. 31, no. 2, pp. 187-201.

Poole, D.C. & Jones, A.M. 2012, "Oxygen uptake kinetics", *Comprehensive Physiology,* .

Racinais, S., Connes, P., Bishop, D., Blonc, S. & Hue, O. 2005, "Morning versus evening power output and repeated-sprint ability", *Chronobiology international,* vol. 22, no. 6, pp. 1029-1039.

Rowan, A.E., Kueffner, T.E. & Stavrianeas, S. 2012, "Short duration high-intensity interval training improves aerobic conditioning of female college soccer players", *International Journal of Exercise Science,* vol. 5, no. 3, pp. 6.

Ryan, T.E., Brophy, P., Lin, C., Hickner, R.C. & Neufer, P.D. 2014, "Assessment of in vivo skeletal muscle mitochondrial respiratory capacity in humans by near-infrared spectroscopy: a comparison with in situ measurements", *The Journal of physiology,* vol. 592, no. 15, pp. 3231-3241.

Ryan, T.E., Brizendine, J.T. & McCully, K.K. 2013, "A comparison of exercise type and intensity on the noninvasive assessment of skeletal muscle mitochondrial function using near-infrared spectroscopy", *Journal of applied physiology (Bethesda, Md.: 1985),* vol. 114, no. 2, pp. 230-237.

Ryan, T.E., Erickson, M.L., Brizendine, J.T., Young, H.J. & McCully, K.K. 2012, "Noninvasive evaluation of skeletal muscle mitochondrial capacity with near-infrared spectroscopy: correcting for blood volume changes", *Journal of applied physiology (Bethesda, Md.: 1985),* vol. 113, no. 2, pp. 175-183.

Ryan, T.E., Southern, W.M., Reynolds, M.A. & McCully, K.K. 2013, "A cross-validation of near-infrared spectroscopy measurements of skeletal muscle oxidative capacity with phosphorus magnetic resonance spectroscopy", *Journal of applied physiology (Bethesda, Md.: 1985),* vol. 115, no. 12, pp. 1757-1766.

Sandvei, M., Jeppesen, P.B., Støen, L., Litleskare, S., Johansen, E., Stensrud, T., Enoksen, E., Hautala, A., Martinmäki, K. & Kinnunen, H. 2012, "Sprint interval running increases insulin sensitivity in young healthy subjects", *Archives of Physiology and Biochemistry,* vol. 118, no. 3, pp. 139-147.

Skovgaard, C., Christensen, P.M., Larsen, S., Andersen, T.R., Thomassen, M. & Bangsbo, J. 2014, "Concurrent speed endurance and resistance training improves performance, running economy, and muscle NHE1 in moderately trained runners", *Journal of applied physiology (Bethesda, Md.: 1985),* vol. 117, no. 10, pp. 1097-1109.

Sloth, M., Sloth, D., Overgaard, K. & Dalgas, U. 2013, "Effects of sprint interval training on VO2max and aerobic exercise performance: A systematic review and meta-analysis", *Scandinavian Journal of Medicine & Science in Sports,* vol. 23, no. 6, pp. e341-e352.

Staron, R.S., Hagerman, F.C., Hikida, R.S., Murray, T.F., Hostler, D.P., Crill, M.T., Ragg, K.E. & Toma, K. 2000, "Fiber type composition of the vastus lateralis muscle of young men and women", *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society,* vol. 48, no. 5, pp. 623-629.

Walter, G., Vandenborne, K., McCully, K.K. & Leigh, J.S. 1997, "Noninvasive measurement of phosphocreatine recovery kinetics in single human muscles", *The American Journal of Physiology,* vol. 272, no. 2 Pt 1, pp. C525-34.

Weston, M., Taylor, K.L., Batterham, A.M. & Hopkins, W.G. 2014, "Effects of Low-Volume High-Intensity Interval Training (HIT) on Fitness in Adults: A Meta-Analysis of Controlled and Non-Controlled Trials", *Sports Medicine,* vol. 44, no. 7, pp. 1005-1017.

Williams, A.M., Paterson, D.H. & Kowalchuk, J.M. 2013, "High-intensity interval training speeds the adjustment of pulmonary O2 uptake, but not muscle deoxygenation, during moderate-intensity exercise transitions initiated from low and elevated baseline metabolic rates", *Journal of applied physiology (Bethesda, Md.: 1985),* vol. 114, no. 11, pp. 1550-1562.

Yoshida, T. & Watari, H. 1993, "31P-nuclear magnetic resonance spectroscopy study of the time course of energy metabolism during exercise and recovery", *European journal of applied physiology and occupational physiology,* vol. 66, no. 6, pp. 494-499.

Appendix

Appendix 1 – RPE scale

”Hvor anstrengende var bout’en for dig?”

Sæt kryds på tallet der bedst repræsenterer din oplevelse

Table 11. Rating of perceived exertion (RPE) scale. Subjects marked their RPE on this scale following each bout.

0	1	2	3	4	5	6	7	8	9	10
Ingen anstrengelse										Uudholdeligt, må stoppe øjeblikkeligt!

Appendix 2 – Results

Table 12. ANOVA for between groups differences.

	Value	F	Hypothesis df	Error df	Sig.
3000m performance					
Time	0,667	7,002[b]	1	14	0,019*
Time*group	0,819	3,091[b]	1	14	0,101
VO_{2p} kinetics					
Time	,864	1,881[b]	1,000	12,000	,195
Time*group	,999	,010[b]	1,000	12,000	,921
RE					
Time	0,901	1,315[b]	1	12	0,274
Time*group	1	,000[b]	1	12	0,987
VO_{2max}					
Time	1	,001[b]	1	12	0,98
Time*group	0,987	,155[b]	1	12	0,701

Table 12 shows results for a two-factor ANOVA testing for an interaction between effects of group (SIT/CON) or time. 3000m is 3000m performance time, VO_{2p} kinetics is pulmonary oxygen uptake kinetics, RE is running economy and VO_{2max} is maximal oxygen consumption.

Table 13. ANOVA for between groups differences for NIRS measurements.

	Value	F	Hypothesis df	Error df	Sig.
GM_mVO_2					
Time	0,902	1,199[b]	1	11	0,297
Time*group	0,938	,726[b]	1	11	0,412
GM_REOXY					
Time	0,72	3,893[b]	1	10	0,077
Time*group	0,762	3,124[b]	1	10	0,108
GM hyperemic					
Time	0,919	,972[b]	1	11	0,345
Time*group	0,92	,952[b]	1	11	0,35
GM resting					
Time	0,998	,022[b]	1	11	0,884
Time*group	0,828	2,291[b]	1	11	0,158
TA mVO_2					
Time	0,931	,811[b]	1	11	0,387
Time*group	0,902	1,188[b]	1	11	0,299
TA_REOXY					
Time	0,956	,458[b]	1	10	0,514
Time*group	0,971	,297[b]	1	10	0,598
TA_hyperemic					
Time	0,833	2,201[b]	1	11	0,166
Time*group	0,853	1,889[b]	1	11	0,197
TA_resting					
Time	0,957	,497[b]	1	11	0,495
Time*group	0,88	1,507[b]	1	11	0,245
VL mVO_2					
Time	0,982	,207[b]	1	11	0,658
Time*group	0,961	,443[b]	1	11	0,519
VL_REOXY					
Time	0,887	1,145[b]	1	9	0,312
Time*group	0,846	1,640[b]	1	9	0,232
VL_hyperemic					
Time	0,765	3,686[b]	1	12	0,079
Time*group	0,833	2,402[b]	1	12	0,147
VL_resting					
Time	0,973	,329[b]	1	12	0,577
Time*group	0,877	1,685[b]	1	12	0,219

Table 13 shows results for a two-factor ANOVA testing for an interaction between effects of group (SIT/CON) or time. GM is medial gastrocnemius, TA is tibialis anterior, VL is vastus lateralis. mVO_2 is muscle oxygen consumption, REOXY is reoxygenation curves, hyperemic is the hyperemic response following 5 minutes occlusion and resting is the resting oxygen consumption.

Appendix 3 – Baseline correlations

Correlations

		3000m time	Vo2max	Vo2p kinetics	RE	VL MVO2	VL REOXY	VL Ischemic	VL Resting	GM MVO2	GM REOXY	GM Ischemic	GM Resting	TA MVO2	TA REOXY	TA Ischemic
3000m time	r	1														
	p															
	N	22														
Vo2max	r	-,721**														
	p	,000														
	N	21														
Vo2p kinetics	r	,313	-,276													
	p	,167	,226													
	N	21	21													
RE	r	,419	-,351	,417												
	p	,059	,119	,060												
	N	21	21	21												
VL MVO2	r	-,196	-,203	-,137	,139											
	p	,452	,434	,601	,594											
	N	17	17	17	17											
VLREOXY	r	,092	-,145	,086	-,218	,491										
	p	,716	,567	,733	,384	,053										
	N	18	18	18	18	16										
VL Ischemic	r	,227	-,205	,129	,155	,445	,383									
	p	,335	,385	,589	,515	,073	,116									
	N	20	20	20	20	17	18									
VL Resting	r	-,003	-,189	-,272	,056	-,019	-,225	,072								
	p	,991	,425	,246	,814	,944	,369	,764								
	N	20	20	20	20	17	18	20								
GM MVO2	r	-,218	-,230	-,207	,037	,698**	,274	,480	,251							
	p	,400	,374	,426	,887	,005	,304	,051	,331							
	N	17	17	17	17	14	16	17	17							
GM REOXY	r	-,241	,242	,067	,128	,023	-,016	,184	,090	,538*						
	p	,319	,319	,786	,602	,931	,951	,451	,713	,026						
	N	19	19	19	19	16	17	19	19	17						
GM Ischemic	r	,159	,049	,061	,183	-,228	-,554*	-,147	-,081	-,099	,192					
	p	,492	,836	,799	,439	,378	,017	,535	,735	,705	,432					
	N	21	20	20	20	17	18	20	20	17	19					
GM Resting	r	,005	,131	,125	,448*	-,095	-,297	,013	-,297	,111	,297	,458*				
	p	,984	,583	,598	,048	,716	,231	,957	,203	,672	,216	,037				
	N	21	20	20	20	17	18	20	20	17	19	21				
TA MVO2	r	,052	-,067	-,009	-,318	-,308	-,019	-,240	,391	-,017	,193	-,216	-,306			
	p	,823	,778	,969	,171	,229	,940	,309	,088	,948	,429	,348	,178			
	N	21	20	20	20	17	18	20	20	17	19	21	21			
TA REOXY	r	-,092	-,113	,380	-,002	-,176	-,115	-,222	,177	-,221	,038	,196	-,185	,442		
	p	,699	,645	,108	,992	,515	,660	,360	,469	,411	,881	,408	,435	,051		
	N	20	19	19	19	16	17	19	19	16	18	20	20	20		
TA Ischemic	r	,156	,025	,045	-,069	-,335	-,001	-,278	-,093	-,599*	-,148	,337	-,267	,222	,527*	
	p	,501	,918	,849	,773	,188	,996	,235	,697	,011	,544	,136	,243	,334	,017	
	N	21	20	20	20	17	18	20	20	17	19	21	21	21	20	
TA Resting	r	,180	-,084	-,351	-,191	-,344	-,227	-,504*	-,077	-,243	-,221	,086	,315	,102	-,126	-,076
	p	,434	,725	,129	,419	,177	,366	,023	,748	,348	,362	,710	,164	,660	,596	,743
	N	21	20	20	20	17	18	20	20	17	19	21	21	21	20	21

Figure 14. Pearson's correlations between variables measured at baseline. 3000m time is 3000m performance time, VO_{2max} is maximal oxygen consumption, VO_{2p} kinetics is pulmonary oxygen uptake kinetics, RE is running economy, GM is medial gastrocnemius, TA is tibialis anterior, VL is vastus lateralis, mVO_2 is muscle oxygen consumption, REOXY is reoxygenation curves, ischemic is the hyperemic response following 5 minutes occlusion and resting is the resting oxygen consumption.