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Prolonged constant load cycling exercise is associated with reduced gross efficiency and increased muscle oxygen uptake

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Running Head: Oxygen consumption during cycling

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ABSTRACT

This study investigated the effects of prolonged constant load cycling exercise on cycling efficiency and local muscle oxygen uptake responses. Fourteen well trained cyclists each completed a 2h steady state cycling bout at 60% of their maximal minute power output to assess changes in gross cycling efficiency (GE) and muscle oxygen uptake ($m\dot{V}O_2$) at time points 5, 30, 60, 90 and 120 min. Near-infrared spatially resolved spectroscopy (NIRS) was used to continually monitor tissue oxygenation of the Vastus Lateralis muscle, with arterial occlusions (OCC) applied to assess $m\dot{V}O_2$. The half-recovery time of oxygenated hemoglobin (HbO_2) was also assessed pre and post the 2h cycling exercise by measuring the hyperaemic response following a 5 min OCC. GE significantly declined during the 2h cycling bout (18.4±1.6 to 17.4±1.4%; $P < 0.01$). Conversely, $m\dot{V}O_2$ increased, being significantly higher after 90 and 120 min than at min 5 (+0.04 $mlO_2.min^{-1}.100g^{-1}$; $P = 0.03$). The half-recovery time for HbO_2 was increased comparing pre- and post- the 2h cycling exercise (+7.1 ±± 19s), albeit not significantly (*d*:0.48; $P = 0.27$). This study demonstrates that GE decreases during prolonged constant load cycling exercise and provides evidence of an increased $m\dot{V}O_2$, suggestive of progressive mitochondrial or contractile inefficiency.

Keywords: Cycling efficiency, lactate threshold, maximal oxygen uptake, endurance performance, muscle efficiency

INTRODUCTION

Cycling gross efficiency (GE) has been demonstrated to be a key determinant of cycling performance (Hopker et al., 2013). GE is defined as the ratio of power output to power input from measures of oxygen uptake ($\dot{V}O_2$) and carbon dioxide ($\dot{V}CO_2$) during steady state cycling (Hopker et al., 2012). Sustained moderate intensity exercise has been shown to reduce GE, via an unexplained increase in $\dot{V}O_2$ measured at the mouth (Hagan et al., 1992; Hagberg et al., 1978; Passfield & Doust, 2000), and subsequently reduce high intensity cycling performance (Passfield & Doust, 2000). However, both the rate of decline and the underlying mechanisms are yet to be fully established.

Oxidative phosphorylation is the main process by which ATP is produced under aerobic conditions. Mitochondrial efficiency has been shown to be an important component in exercise efficiency (Fernstrom et al., 2007), and so changes in the efficiency of oxidative phosphorylation will therefore affect cycling efficiency. Key questions remain unanswered regarding the efficiency of energy transfer within the mitochondria and the possible role of the uncoupling of oxidative phosphorylation. Uncoupling accounts for around 50% of resting oxygen consumption in rodent muscle (Tonkonogi et al., 1998), and has been seen to increase by 18% after prolonged exhaustive exercise in human skinned muscle fibers (Whipp & Wasserman, 1969). Further in a recent study, muscle uncoupling protein content (UCP3) has been negatively correlated with work efficiency in a cohort of mixed-ability cyclists (Mogensen et al., 2006). Another potential mechanism responsible for an apparent additional $\dot{V}O_2$ slow component (Poole et al., 1994) during prolonged constant intensity exercise might be related to muscle contractile inefficiency. Specifically,

during prolonged exercise above the lactate threshold it has been suggested that the $\dot{V}O_2$ slow component might be the product of an increased phosphate cost of power production (Rossiter et al., 2002; Cannon et al., 2014). Indeed, previous research has demonstrated a close relationship between muscle and whole body $\dot{V}O_2$ measured via pulmonary oxygen consumption (Poole et al., 1992). Therefore, an increase in mitochondrial uncoupling and muscle $\dot{V}O_2$ ($m\dot{V}O_2$) during prolonged cycling exercise has the potential to increase the $\dot{V}O_2$ from constant load exercise, and consequently reduce cycling efficiency.

Near-infrared spectroscopy (NIRS) can provide information about the changes in tissue oxygenation at rest and during exercise (Ferrari et al., 2011) from the oxygen dependent absorption characteristics of infrared light. This non-invasive technique therefore allows continuous monitoring of the dynamics of tissue oxygenation. Indeed, NIRS has been used to provide information about relative changes in oxygenated haemoglobin/myoglobin (HbO_2), deoxygenated haemoglobin/myoglobin (HHb), and total haemoglobin or blood volume (tHb). Resultantly, NIRS has been used to measure skeletal muscle oxygenation (Chuang et al., 2002) and provide an estimate of blood flow (De Blasi et al., 1997; Nioka et al., 2006). Moreover, repeated arterial occlusions have been used both during and after exercise to assess muscle oxygen consumption as an index of mitochondrial function (Van Beekvelt et al., 2001). During arterial occlusion an absolute value for muscle O_2 consumption can be calculated, under the assumption that tHb remains constant (due to the occlusion), from the decreasing slope of HbO_2. The disassociation of oxygen molecules from oxyhaemoglobin/myoglobin reflects the requirement of oxidative phosphorylation,

and therefore will be indicative of the tightness of mitochondrial coupling and changes in the rate ATP consumption per unit of power production.

The aim of this study was to determine the relationship between whole body measures of GE calculated from pulmonary $\dot{V}O_2$, and $m\dot{V}O_2$ (measured via NIRS) during 2 h constant load cycling exercise at 60 % of maximal minute power output. We simultaneously measured whole body oxygen uptake via pulmonary gas exchange and local muscle oxygen consumption via NIRS in order to identify how each measure changed during the prolonged bout of cycling. To control for the confounding effects of changes in blood flow, $m\dot{V}O_2$ was measured during arterial occlusion. We hypothesized that GE would decrease, and $m\dot{V}O_2$ would increase during 2h constant load cycling exercise.

MATERIALS AND METHODS

Fourteen well-trained male cyclists (mean ± SD: age 30 ± 14 years, mass 66 ± 11 kg, $\dot{V}O_{2max}$ 73 ± 2 $mL.kg^{-1}.min^{-1}$, maximum minute power output [MMP] 319 ± 15W) volunteered to participate in the study. All participants had a minimum of two years training and racing experience, and were in preparation for a full competitive season. The study was completed with full ethical approval from the local institutional ethics committee according to the Declaration of Helsinki standards. All cyclists provided written informed consent prior to participating.

Study Design and Experimental Procedures

Participants visited the exercise testing laboratory on two separate occasions in a euhydrated state. During visit 1 participants undertook an incremental exercise test (see *Maximal incremental test* for more details) for the identification of $\dot{V}O_{2max}$ and MMP. The protocol used at Visit 2 is shown in Figure 1, and consisted of participants undertaking 2 h of constant load cycling at 60% maximal minute power output (see *2-hours cycling test* for more details) to assess changes in GE and $\dot{V}O_2$. Prior to, and immediately following the 2 h cycling bout, participants completed a 6 s maximal isokinetic sprint test at set cadences of 60, 90 and 120 rev.min^{-1} (see *Sprint tests* for more details). All tests were completed on an electromagnetically braked cycle ergometer (Schoberer Rad Messtechnik GmbH, Jülich, Germany). An electric cooling fan was used to cool participants for the whole duration of the 2 h constant load cycling exercise. Participants were also provided with water to drink adlibitum during both visits. Visits were conducted on non-concurrent days, with participants instructed to refrain from any exercise in the day prior to testing and intense exercise in the two days prior, not to consume caffeine 3 h before each visit.

INSERT FIGURE 1 HERE

Maximal incremental test

The maximal incremental test started with a 10 min warm-up at 100 W, after which required cycling power output increased by 5 W every 15 s until the participant reached volitional exhaustion (operationally defined as a cadence of <60 revolutions/min for >5 s, despite strong verbal encouragement). Respiratory gas exchange data were assessed using an online gas analyzer (Metalyzer 3B, CORTEX Biophysik GmbH, Leipzig, Germany) throughout the test, by use of a facemask

covering the nose and mouth. Participant's $\dot{V}O_{2max}$ was assessed as the highest $\dot{V}O_2$ that was attained during a 60 s period in the test. MMP was assessed as being the highest average 60 s power output during the test. Following the maximal incremental exercise test, participants were familiarized with the testing procedures used during visit 2. This consisted of familiarization with muscle occlusions, practice 6 s sprint trial, and a 10 min bout of cycling at the target 2 h power output in order to determine preferred cadence.

2-hour cycling test

Following a 10 min warm-up at 100 W and the sprint tests, participants cycled at 60 % MMP continuously for 120 min. During this time, expired air was collected for one minute using non-diffusible gas bags (Hans - Rudolph, USA), at time points 5, 30, 60, 90 and 120 min, and were analyzed immediately following collection using a Servomex 5200 gas analyzer (Servomex, Crowborough, East Sussex) by the procedures outlined by Hopker et al. (2012). During the final 20 s of gas collection, and whilst the cyclist continued to pedal at the required $rev.min^{-1}$, an arterial occlusion (see NIRS below) was applied to the right leg to determine local muscle oxygen uptake ($m\dot{V}O_2$). This procedure allows controlling for the confounding effects of changes in blood flow on $m\dot{V}O_2$. Throughout the 120 min of cycling, subjects were required to maintain a constant self-selected cadence (range: 80-88 $rev.min^{-1}$), which was determined during Visit 1. Heart rate was recorded continuously throughout the exercise test (S810i, Polar Electro Oy, Finland).

Blood lactate concentration was measured using a fingertip capillary blood sample and was taken during exercise at the same time points as the expired gases and NIRS measurements. Blood samples were analyzed using a Biosen C-Line (EKF

Diagnostic, London, UK). RPE measurements were also taken at the same time points using the Borg 6-20 scale (Borg, 1998).

Calculation of cycling efficiency

Cycling efficiency was calculated as the ratio of work done to energy expended during the sampling minute in the form:

Gross Efficiency % = (Work accomplished/Energy Expenditure) ×100

In order to establish the 'Work accomplished', the mean power recorded during the same period as the respiratory collection was determined and converted into kcal.min^{-1} via the following equation:

'Work accomplished' (kcal.min^{-1}) = Power (W) × 0.01433

Energy expenditure was calculated from the 1 min respiratory collection to ascertain $\dot{V}O_2$ and Respiratory Exchange Ratio (RER). The calorific equivalent of O_2 was then determined from the corresponding RER according the data of Peronnet and Massicotte (1991):

'Energy expenditure' (kcal.min^{-1}) = $\dot{V}O_2$ (L.min^{-1}) × kcal.L^{-1} of O_2

Near-infrared Spectroscopy

Muscle oxygenation and consumption in the right *Vastus Lateralis (VL)* was continuously monitored using wireless spatially resolved dual-wavelength spectrometers (Portamon, Artinis Medical Systems, BV, the Netherlands). The small unit measures 83 x 52 x 20 mm and weighs 84g, including the battery. The device has three pairs of diodes emitting light of wavelengths 760 and 850nm. Resultantly it is

possible to detect combined concentration changes in the chromophores haemoglobin (Hb) and myoglobin (Mb). The distance between light source and detector was 40mm. The inability to measure absolute chromophore concentrations can be accommodated for by using Spatially Resolved Spectroscopy (SRS). The Portamon device utilizes three light sources in a spatially resolved configuration, distanced 30, 35, and 40mm from the one light receiver. A differential path-length factor of 4.0 was assumed during all tests. The gradient of light attenuation allows a deeper more muscle-biased measurement with less interference from superficial skin and fat layers. SRS is also insensitive to light scattering, allowing the diffusion equation for light transport to be used to yield an absolute measure of tissue oxygen saturation (TSI%). Using these methods, changes are reported from an arbitrary baseline value taken prior to the start of exercise.

The NIRS optode was situated on the cyclist's right leg, over the belly of the VL muscle and 10 cm proximal to the knee joint on a line between the greater trochanter of the femur and the lateral epicondyle. Skinfold thickness was measured at the location of the probes using a skinfold caliper. The skinfold thickness was 11.1 + 2.8 mm. To ensure the optodes and detector did not move relative to the subject's skin, the device was fixed into position using surgical tape, and then secured with a bandage.

A pressure cuff (Hokanson SC12D; Bellevue, WA, USA) was secured around the thigh and proximal to the NIRS device. During occlusions, the pressure cuff was rapidly (< 0.3 s) inflated to 300 mmHg for 5 min using a semi-automated inflation system (Hokanson E20; Bellevue, WA, USA). This was used as a measure of baseline

$m\dot{V}O_2$ following the warm-up, but prior to the 2 h constant load cycling exercise. Following release of the cuff, the hyperaemic response was used to assess the half-recovery time and re-oxygenation rate at baseline. Finally, 5 min after release of the occlusion and resolution of the hyperaemic response, baseline NIRS measurements were taken in the two minutes immediately prior to the start of exercise. The NIRS data were collected wirelessly at 10 Hz, then for the purposes of further analysis, a 10-point moving average was applied.

Calculation of changes in NIRS parameters

All TSI and chromophore concentration changes were collected from a 30 s period concomitant with expired gas sampling, and are presented relative to a baseline value taken immediately prior to the start of the 2 h period of cycling. $m\dot{V}O_2$ was derived from NIRS using arterial occlusion by evaluating the rate of decline in Hb_{diff} (Hb_{diff} = HbO_2-HHb) with the assumption that tHb is constant (De Blasi et al., 1997). A typical recording of an occlusion is shown in figure 2. The use of 20 s arterial occlusions enabled the measurement of $m\dot{V}O_2$ whilst controlling for potential blood volume changes (Van Beekvelt et al., 2001). A 3 s period of data was selected and used for the calculation of $m\dot{V}O_2$, and R^2 values were used to check the linearity of the regressions during the determination of $m\dot{V}O_2$ with a mean value of 0.99 (range 0.97-1.00). Concentration changes of HHb and Hb_{diff} were expressed in micromoles per second and converted to milliliters O_2 per minute per 100 grams tissue ($mlO_2.min^{-1}.100g^{-1}$). A value of 1.04 $kg.l^{-1}$ was used for muscle density (Van Beekvelt et al., 2001). The recovery of HbO_2 after exercise or ischemia represents the time needed for resaturation of deoxygenated haemoglobin and myoglobin and is thought to reflect both the influx of oxygenated arterial blood and the continued O_2 consumption during recovery (Chance et al., 1992). The half time recovery of HbO_2 (s) was calculated

from maximum deoxygenation at the end of the 5 min occlusions (pre- and post- 2 h exercise) to 50% of the maximum re-oxygenation during hyperaemia (Chance et al, 1992). The reoxygenation rate (ΔHbO_2 in $\mu M.s^{-1}$) was calculated as the rate of increase in HbO_2 during the initial 3 s after cessation of the occlusion both pre- and post- 2 h constant load cycling exercise. This variable reflects the initial inflow of HbO_2 over a fixed time period following the release of the occlusion and is therefore not influence by the magnitude of the hyperaemic response. Thus, the half time recovery of HbO_2 and re-oxygenation rates were used to provide an indication of the recovery of vascular components and the continued oxygen consumption following exercise cessation. We speculated that in the presence of increased mitochondrial uncoupling and reduced cycling efficiency, there is likely to be a slowed half time recovery of HbO_2 and re-oxygenation rate.

*** INSERT FIGURE 2 HERE***

Sprint Tests

To assess muscle fatigue via maximal voluntary cycling power output, prior to, and immediately following the 2 h constant load cycling exercise, participants were asked to perform three maximal sprints, each of 6 s followed by 60 s active recovery (with no resistance). Sprints were performed at three fixed cadences (60, 90 and 120 $rev.min^{-1}$) using the isokinetic mode of the electromagnetically braked cycle ergometer. Peak 1 s power output was obtained from each sprint in order to assess the maximal voluntary power producing capability of the exercising muscles and consequently highlights the presence of exercise-induced muscle fatigue (i.e. decrease in the ability to produce maximal power).

Statistical Analysis

Prior to all data analysis, data was checked for normality of distribution. Repeated measures analysis of variance (ANOVA) with least significant difference unadjusted post hoc analysis used to analyze data from the 2h constant load cycling exercise. Differences in sprint power output at the cadences of 60, 90 and 120 rev.min^{-1} were assessed using two-way repeated measures ANOVA. Effect sizes were calculated using partial eta squared (η_p^2) and were defined as small, moderate or large based upon .02, .13 and .26, respectively (Cohen et al., 1998). The difference in half-recovery time and reoxygenation rate of HbO_2 pre- to post- 2 h constant load cycling exercise were assessed using a paired t-test, with Cohen's *d* effect sizes being defined as 0.2, 0.5 and 0.8 for small, medium and large effects respectively (Cohen et al., 1998). Statistical analyses were conducted using IBM SPSS Statistics 22 (IBM®, Armonk, NY), and a $P < 0.05$ was used as the criteria for detection of significance in all cases. Data are reported as mean and standard deviation (mean ± SD) unless specified otherwise.

RESULTS

Cardiorespiratory measurements

Mean cycling power output was 192 ± 9 W with mean cadence being 84 ± 1 rev.min^{-1} during the 2h constant load cycling exercise. Both submaximal $\dot{V}O_2$ and $\dot{V}CO_2$ increased significantly over the period of cycling ($\dot{V}O_2 = \eta_p^2$: 0.40; $P = <0.01$; Figure 3c; $\dot{V}CO_2 = \eta_p^2$: 0.34; $P = 0.01$; Figure 3d). GE significantly declined during the 2 h constant load cycling exercise (η_p^2: 0.38; $P < 0.01$; Figure 3a) from an initial value of 18.4 ± 1.6 % at min 6 to 17.4 ± 1.4 % at minute 120. RER significantly declined between 90 and 120 min of constant load cycling exercise (η_p^2: 0.28; $P = 0.01$; Figure 3b). $\dot{V}_E$ significantly increased during the 2 h cycling period with time points 90 and

120 min being greater than time points 5 and 30 min (η_p^2: 0.49; $P < 0.01$; Figure 3e). Heart rate significantly increased over the 2 h constant load cycling exercise, being higher at 90 and 120 min than minute 5 (η_p^2: 0.42; $P < 0.05$; Figure 3f).

INSERT FIGURE 3 HERE

Blood lactate and perceived exertion

Blood lactate concentration rose significantly from baseline after 5 min of cycling (η_p^2: 0.37; $P = 0.01$, Figure 3g), stayed unchanged between 5 and 30 min and then reduced significantly between 60 ($P = 0.03$) and 90 min ($P = 0.04$). At the end of the cycling exercise, blood lactate was significantly higher than baseline ($P < 0.01$), but not different from any of the other time points. Even though the required work rate was held constant at 60 % MMP, perceived exertion rose continuously throughout the 2 h constant load cycling exercise. RPE at all measured time points was significantly higher than the previous (η_p^2: 0.73; $P < 0.05$, Figure 3h).

NIRS measurements

The responses of NIRS parameters during the 2 h constant load cycling exercise are shown in Figure 4. There was no significant change in HbO_2 (η_p^2: 0.20; $P > 0.05$; Figure 4a), however HHb increased significantly (η_p^2: 0.25; $P = 0.02$), with values at 90 and 120 min being statistically higher than at min 5 (Figure 4b). tHb increased steadily over time after 30 min of constant load cycling with time points of 60, 90 and 120 min being significantly greater than 5 and 30 min (η_p^2: 0.30; $P < 0.04$; Figure 4c). There was a trend for a reduction in TSI% levels over the 2 h constant load cycling exercise (η_p^2: 0.15; $P = 0.20$, Figure 4d). As can be seen in the typical m$\dot{V}O_2$ trace,

figure 2, during the occlusion O_2Hb decreased, HHb increased and tHb remained stationary, indicating that the blood flow was occluded. Resting m$\dot{V}O_2$ was 0.04±0.02 $mlO_2.min^{-1}.100g^{-1}$ and demonstrated a 7.5±3.8 fold increase after 6 min cycling at 60% MMP. m$\dot{V}O_2$ increased further during the 2 h constant load cycling exercise, being significantly higher after 90 (10.0±5.5 fold increase) and 120 min (10.3±6.2 fold increase) than at min 5 (η_p^2: 0.29; P = 0.03, Figure 5a). There was a trend for both the half-recovery time (d: 0.48; P = 0.27; Figure 5b), and reoxygenation rate (d: 0.60; P = 0.11; Figure 5c) of HbO_2 to be slower following occlusion after the 2 h constant load cycling exercise.

INSERT FIGURE 4 & 5 HERE

Sprint tests

There was no interaction effect between 6-s sprint time point (i.e. pre vs. post 2 h of cycling) and sprint cadence (η_p^2: 0.09; P = 0.29). Regardless of cadence, sprint power output was significantly lower at each cadence following the 2 h constant load cycling exercise (η_p^2: 0.51; P = 0.04; Figure 6). However, the reduction in GE was not related to the decline in 6-s sprint power output at any cadence (P > 0.05).

INSERT FIGURE 6 HERE

DISCUSSION

This study used NIRS to investigate the relationship between local muscle and whole body physiological responses to prolonged constant load cycling exercise. The main findings of this study was that GE declined significantly during 2 h constant load cycling exercise in accordance with the findings of previous studies (Hagan et al., 1992; Hagberg et al., 1978; Passfield & Doust, 2000), despite maintenance of constant power output and cadence. The physiological data recorded during the constant load exercise trial may provide some answers to the origins of the reduction in efficiency recorded. GE is the ratio of work accomplished to energy expenditure and expressed as a percentage (Hopker et al., 2012), where work accomplished is determined by the mean cycling power output of the corresponding data-sampling period. Energy expenditure is determined by the oxygen cost of the exercise multiplied by the caloric equivalent per liter of oxygen determined from the corresponding RER. The reduction of GE seen in the current study was associated with a significant increase in the oxygen cost of the exercise, i.e. the emergence of a $\dot{V}O_2$ slow component (see Figure 3c). Previous research has demonstrated that increases in fat metabolism, ventilation, lactate metabolism, and body temperature cannot account for the increased oxygen cost of work after sustained moderate-intensity cycling exercise (Hagan et al., 1992, Hagberg et al., 1978). The present data support this conclusion. RER decreased by 0.02 units (0.96-0.94) across the 2 h period constant load cycling exercise and so there were minimal changes in substrate metabolism. There was a significant increase in pulmonary ventilation (mean 16 $L.min^{-1}$) during the 2 h constant load cycling exercise but this was estimated to only increase $\dot{V}O_2$ by a negligible 29 $mL.min^{-1}$ O_2 (Aaron et al., 1992). Blood lactate was significantly higher during the cycling bout than at baseline, but once elevated to ~3 $mmol.L^{-1}$ at min 5, there were no further

increases even though GE continued to decline. Unfortunately no measures of core temperature were taken during the current study, although Passfield and Doust (2000) demonstrate that following an initial rise, core body temperature reaches a plateau during constant load cycling at 60% $\dot{V}O_{2peak}$. Therefore, we are confident that changes in core temperature significantly affected gross efficiency in the current study.

It is possible that the cause of this reduction in efficiency is related to changes at the local muscle level (Gonzalez-Alonso et al., 1998). Interestingly the reduction in efficiency does not seem to be related to the loss in maximal muscle function assessed by the 6 s sprints before and after the cycling bout. However, in support of previous findings by Passfield and Doust (2000), there were reductions in gross efficiency and maximal cycling power output of a similar magnitude (~10%). Therefore further studies should clarify the hypothetical relationship between changes in GE and muscle fatigue induced by prolonged constant load cycling exercise.

NIRS provides the ability to investigate the balance between O_2 supply and utilization within the exercising muscle (Hamaoka et al., 1996). As shown in Figure 4, there was a relative increase in HHB and tHb during the exercise test, indicating that there was an increase in blood volume coupled with increased local muscle deoxygenation during the constant load cycling exercise. HbO_2 remained statistically unchanged throughout the cycling bout. The general trend for progress local Vastus Lateralis muscle desaturation (as shown by the HHb and TSI%) to occur as the trial progressed suggests a greater metabolic demand rather than O_2 supply to exercising muscle. Therefore, mitochondrial oxygen consumption could be assumed to have progressively increased during the 2 h constant load cycling exercise. It is important

to note that muscle oxygenation (TSI%) does not directly reflect $m\dot{V}O_2$, but reflects the balance between oxygen supply and consumption (Hamaoka et al., 1996). A more robust measure of $m\dot{V}O_2$ is performed using arterial occlusions to control inflow and outflow of blood to the limb, i.e. to limit changes in blood volume (Van Beekvelt et al., 2001). Most previous studies have used occlusions of the upper limb (e.g. Van Beekvelt et al., 2001), with few using arterial occlusions on large muscle groups i.e. the legs (Brizendine et al., 2013; Nagasawa, 2008; Nioka et al., 2006). To the author's knowledge, this is the first study to use occlusions of the quadriceps muscle during whole-body dynamic exercise to evaluate $m\dot{V}O_2$.

$m\dot{V}O_2$ increased steadily over the course of the 2 h constant load cycling exercise, even though work rate remained unchanged, being 10.0±5.6 fold higher at min 90 and 10.3±6.2 fold higher at min 120. There is a paucity of research on $m\dot{V}O_2$ during cycling exercise. To our knowledge, the only previous research using NIRS to determine $m\dot{V}O_2$ via arterial occlusions of the quadriceps was performed after, rather than during exercise (Brizendine et al., 2013; Nagasawa et al., 2008), making direct comparisons difficult. We are aware of only one previous study to use arterial occlusions during exercise to determine $m\dot{V}O_2$. Van Beekvelt et al. (2001) demonstrated a ~6 fold increase in $m\dot{V}O_2$ during a 10% isometric MVC of the forearm. Submaximal cycling at 70% $\dot{V}O_{2max}$ has been shown to require ~20% MVC (Lollgen et al., 1980) and so our ~10 fold magnitude of increase in $m\dot{V}O_2$ ($mlO_2.min^{-1}.100g^{-1}$) is, unsurprisingly, higher than the forearm data.

The reasons for the progressive increase in $m\dot{V}O_2$ despite no change in exercise intensity are unclear. One possibility is an alteration of the ratio between

mitochondrial ADP phosphorylation and oxygen consumption (P/O ratio), which reflects the efficiency of oxidative phosphorylation. Specifically, back leak of protons across the inner membrane without driving ATP-synthase would reduce the P/O ratio, and thus increase uncoupling. Increased content or activation of uncoupling protein-3 (UCP3) appear to be important in mediating this process (Mogensen et al., 2006). Alternatively, the rise in m$\dot{V}O_2$ could be caused by some mitochondrial ATP generation being used to reduce ROS generation within the cell (Brand, 2000). A high proton motive force that drives efficient ATP synthesis is associated with an additional ROS production. Proton leak across the mitochondrial membrane without driving ATP production may therefore assist in limiting the oxidative damage associated with high levels of ROS generated during the prolonged cycling exercise (Sahlin et al., 2010).

While it is possible that the energetic cost of exercise might increase if the O_2 cost of ATP production increases with progressive mitochondrial uncoupling, an alternative possibility is that the ATP cost of contraction changes during prolonged exercise. In support of this proposition Cannon et al. (2014), have demonstrated that there is an increased phosphate cost of power production during constant load moderate intensity bilateral knee extensor exercise. Cannon et al. (2014) suggest that an increase in ATP turnover rate and $\dot{V}O_2$ during constant load exercise is consequent to a rise in contractile inefficiency due to muscle fatigue (Rossiter et al., 2002). Indeed the reduction of maximal voluntary cycling power at 60, 90 and 120 rev.min^{-1} shown in the current study after 2 h constant load cycling indicates the presence of muscle fatigue. As prolonged cycling exercise is known to induce both peripheral and central components of muscle fatigue (Lepers et al., 2000; Lepers et al., 2002), we are

confident that at least part of the decrease in maximal voluntary cycling power is due to presence of peripheral fatigue, i.e. fatigue produced by changes at or distal to the neuromuscular junction (Gandevia et al., 2001). Therefore it is possible to speculate that due to progressive peripheral fatigue encountered during the 2 h constant intensity cycling, there was an increase in the ATP cost of muscle contraction, which in turn might have contributed to the increased $m\dot{V}O_2$. Furthermore, as perception of effort is i) known to be influenced by both mental and muscle fatigue (Pageaux 2014, Pageaux et al., 2015) and ii) a main feature of fatigue (Enoka and Stuart, 1992), the progressive increase in perception of effort during the 2 h constant load cycling exercise strongly suggests a progressive development of muscle fatigue through the exercise.

It should be noted that there are some methodological limitations that have to be considered when interpreting the findings of the current study. Firstly, NIRS measurements were made at only one site of the Vastus Lateralis and whether the results hold true for other sites (Koga et al., 2007), or other muscles (Kalliokoski et al., 2006) involved in the cycling action remains to be determined. Secondly the study used continuous-wave NIRS to measure HbO_2 and HHb signals meaning that there are potential confounding factors including an unknown optical path length, absorption and scattering coefficients (Hamaoka et al., 2011). This study used an assumed differential path-length factor to estimate absolute changes in chromophore oxygenation. However, Ferreira et al. (2007) have previously demonstrated that the scattering coefficient can change during exercise, and assuming a constant coefficient can lead to an overestimation of the changes in NIRS variables during exercise. However, it is important to note that the study of Ferreira et al. (2007) investigated

incremental exercise, rather than constant intensity as used in this study. Adipose tissue thickness is a potential major confounder for the NIRS measurements used in the current study (Ferrari et al., 2011). However, there were no repeated measurements used in the current study, and participants were used as their own control. In addition, all participants were lean and had an adipose tissue thickness of less than 12mm, therefore, the impact of adipose tissue thickness on NIRS measurements are likely to be minimal. Moreover, the use of spatially resolved spectroscopy within the TSI% measurement is able to account for some of these limitations (Ferrari et al., 2004).

The use of arterial occlusions allowed the quantitative measurement of muscle oxygen consumption independently of blood flow and oxygen delivery. The NIRS data suggest an increase in muscle blood flow and oxygen consumption over the 2 h cycling period. However, it is possible that heterogeneity in the NIRS response (Koga et al., 2007) could have influenced our data and conclusions. The increased blood flow over the 2 h cycling could have been accessing regions of the muscle that are not directly contributing to, or are less efficient in force production. To address this possibility, topographical MRI or fNIRS would be required.

In conclusion, the present study demonstrates that during constant load cycling exercise at 60% MMP a $\dot{V}O_2$ slow component is evident, leading to a resultant reduction in cycling gross efficiency. *In vivo* Vastus Lateralis mitochondrial oxygen consumption measured via NIRS during arterial occlusions demonstrates concomitant increases in $m\dot{V}O_2$ over time. The increased $m\dot{V}O_2$ during the 2 h constant intensity cycling exercise is likely indicative of progressive mitochondrial / contractile

inefficiency, or the use of the mitochondrial proton motive force for tasks other than ATP production. To further test the relationships between whole-body GE, NIRS derived $m\dot{V}O_2$, and mitochondrial/contractile efficiency, future studies intervention studies might be considered.

Perspectives

Cycling efficiency has been demonstrated to be an important determinant of endurance cycling performance (Coyle et al., 1992; Horowitz et al., 1994; Hopker et al., 2013), which can be improved by endurance training (Hopker et al., 2010). However, to date the underpinning physiological determinants of exercise efficiency are yet to be fully elucidated. Prolonged endurance exercise has been shown to result in reductions in cycling efficiency (Passfield and Doust, 2000), and so therefore provides a method that can be used to investigate its physiological determinants. Over the 2 h period of constant intensity cycling exercise, the emergence of a $\dot{V}O_2$ slow component is seen to reduce whole body exercise efficiency. With negligible changes in fat metabolism, ventilation, and lactate metabolism it is likely that the main determinant of the pulmonary slow component is the exercising skeletal muscle. Indeed, the increases in the NIRS derived $m\dot{V}O_2$ signal suggest the greater O_2 consumption may arise from a combination of both an increased O_2 cost of ATP resynthesis, and an increased ATP cost of power production.

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FIGURE LEGENDS

Figure 1. Overview of the protocol and timing of measurements used during visit 2.

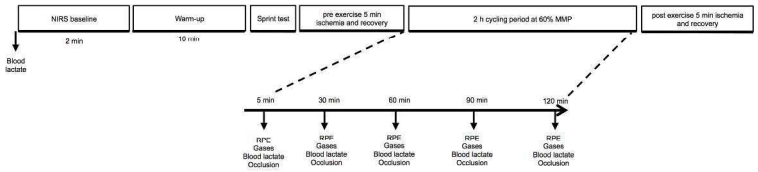
Figure 2. A typical NIRS trace showing HbO_2, HHb and tHb signals during a 20 s occlusion of the Vastus Lateralis muscle during cycling. Trace data has been filtered using a 10-point average. Shaded area identifies the 3 s period of data selected for the calculation of $m\dot{V}O_2$.

Figure 3. Changes in a.) Gross Efficiency, b.) RER, c.) $\dot{V}O_2$, d.) $\dot{V}CO_2$, e.) Ventilation, f.) Heart rate, g.) Blood lactate, h.) Rating of perceived exertion during 2 h constant load cycling exercise. Values are means ± SEM for figures a-f. * = significantly different from min 6. # = significantly different from min 30. ^ = significantly different from min 60. $ significantly different from min 90.

Figure 4. Changes from baseline in a) ΔHbO_2, b) ΔHHb, c) ΔtHb and d) ΔTSI% during 2 h constant load cycling exercise. Values are means ± SEM. * = significantly higher than 5 min. ^ = significantly higher than 5 and 30 min.

Figure 5. $m\dot{V}O_2$ response from 2 h cycling constant load cycling exercise. a) Time course of $m\dot{V}O_2$ response during 2 h constant load cycling exercise, b) half time of oxygenation recovery and c) reoxygenation rate following release of 5 min occlusion pre and post exercise. Values are means ± SEM.

Figure 6. Sprint power output at cadences of 60, 90 and 120 $rev.min^{-1}$ pre- and post-2 h constant load cycling exercise. Values are means ± SEM. * significant main effect of time.



Overview of the protocol and timing of measurements used during visit 2.
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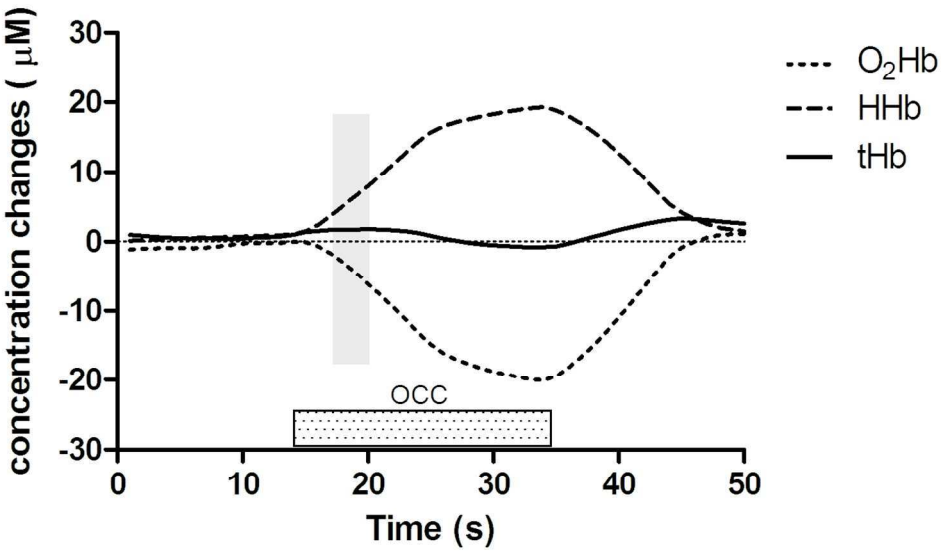

A typical NIRS trace showing HbO_2, HHb and tHb signals during an occlusion to the Vastus Lateralis muscle during cycling. Trace data has been filtered using a 10-point average. Shaded area identifies the period of data selected for the calculation of mVO_2.
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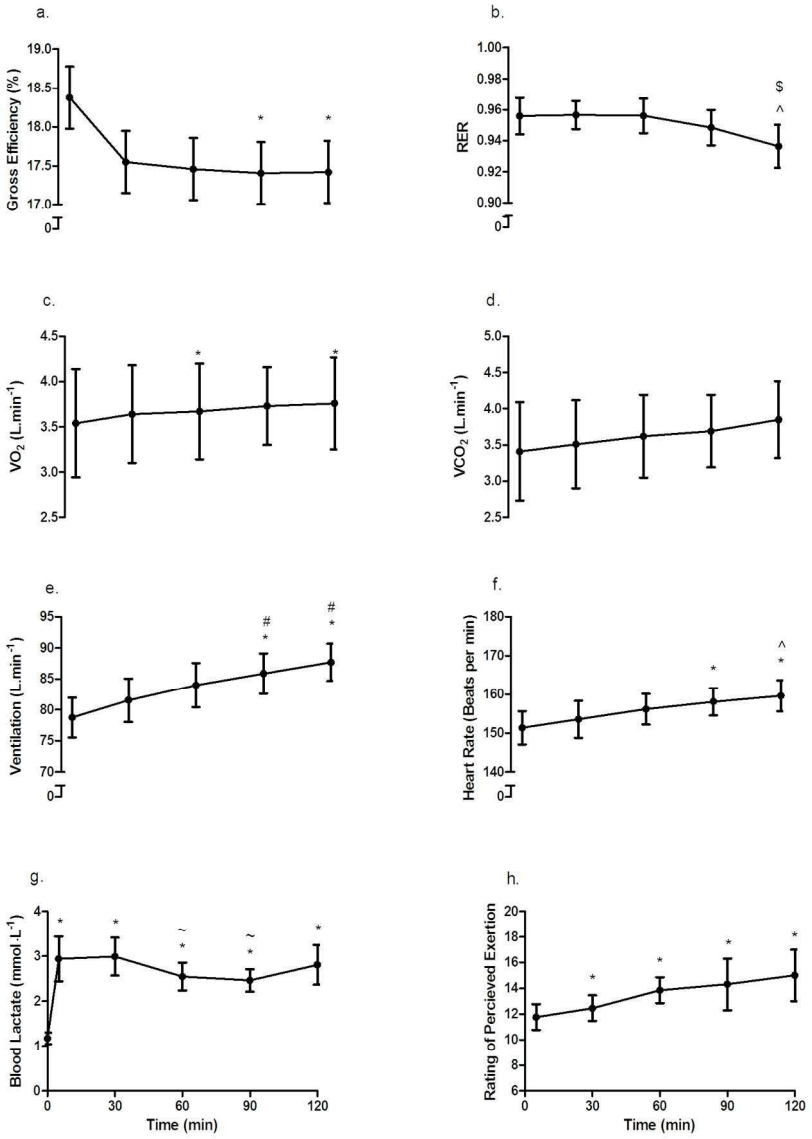
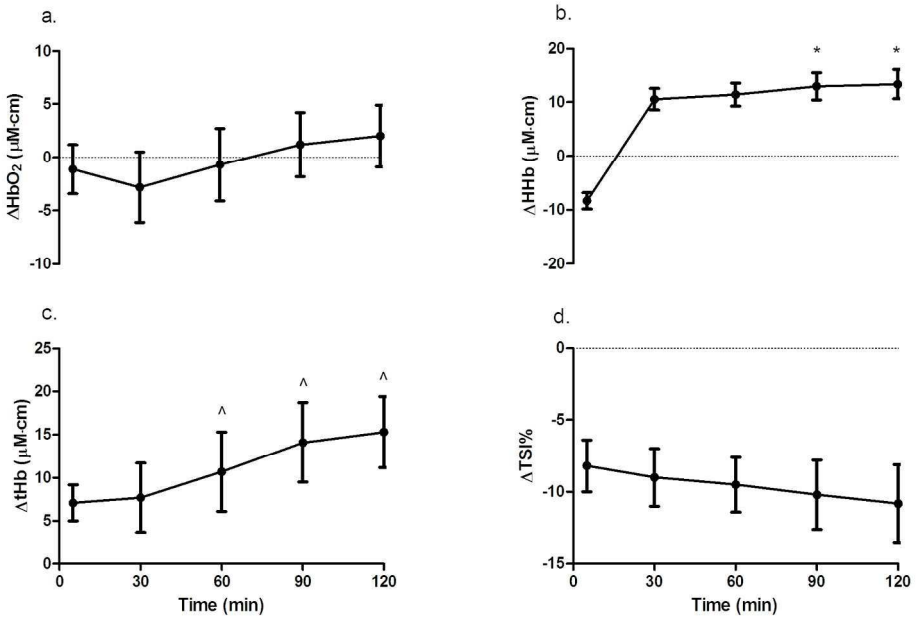
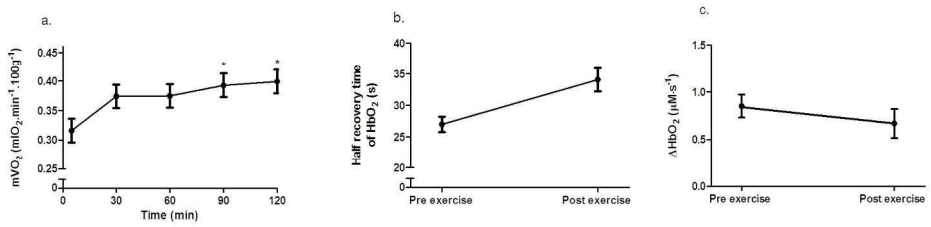


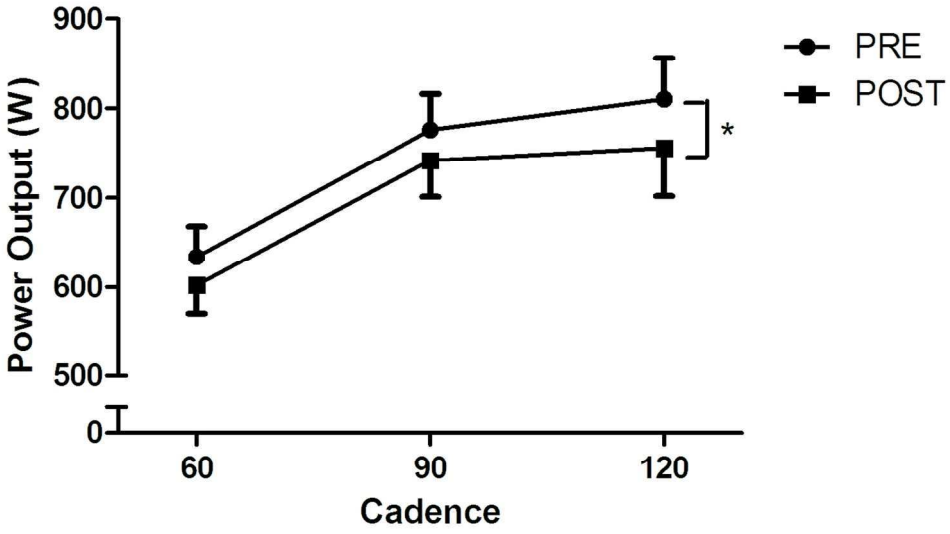
Changes in a.) Gross Efficiency, b.) RER, c.) VO_2, d.) VCO_2, e.) Ventilation, f.) Heart rate, g.) Blood lactate, h.) Rating of perceived exertion during 2 h constant load cycling exercise. Values are means ± SD for figures a-f. * = significantly different from min 6. # = significantly different from min 30. ^ = significantly different from min 60. $ significantly different from min 90.
148x201mm (300 x 300 DPI)



Mean values for a) ΔHbO_2, b) ΔHHb, c) ΔtHb and d) ΔTSI% during 2 h constant load cycling exercise. Values are means ± SEM. * = significantly higher than 5 min. ^ = significantly higher than 5 and 30 min.
181x124mm (300 x 300 DPI)



mVO2 response from 2 h cycling constant load cycling exercise. a) Time course of mVO2 response during 2 h constant load cycling exercise, b) half time of oxygenation recovery and c) reoxygenation rate following release of 5 min occlusion pre and post exercise. Values are means ± SEM.
178x50mm (300 x 300 DPI)



Sprint power output at cadences of 60, 90 and 120 rev.min^{-1} pre- and post- 2 h constant load cycling exercise. Values are means ± SEM. * significant main effect of time.
122x73mm (300 x 300 DPI)