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

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3 Prolonged constant load cycling exercise is associated with reduced gross
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5 efficiency and increased muscle oxygen uptake
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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 $17.4\pm 1.4\%$; $P < 0.01$). Conversely, $m\dot{V}O_2$ increased, being significantly higher after 90 and 120 min than at min 5 ($+0.04 \text{ mlO}_2 \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$; $P = 0.03$). The half-recovery time for HbO_2 was increased comparing pre- and post- the 2h cycling exercise ($+7.1 \pm 19\text{s}$), 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

1 INTRODUCTION

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

11
12 Oxidative phosphorylation is the main process by which ATP is produced under
13 aerobic conditions. Mitochondrial efficiency has been shown to be an important
14 component in exercise efficiency ([Fernstrom et al., 2007](#)), and so changes in the
15 efficiency of oxidative phosphorylation will therefore affect cycling efficiency. Key
16 questions remain unanswered regarding the efficiency of energy transfer within the
17 mitochondria and the possible role of the uncoupling of oxidative phosphorylation.
18 Uncoupling accounts for around 50% of resting oxygen consumption in rodent muscle
19 (Tonkonogi et al., 1998), and has been seen to increase by 18% after prolonged
20 exhaustive exercise in human skinned muscle fibers (Whipp & Wasserman, 1969).
21 Further in a recent study, muscle uncoupling protein content (UCP3) has been
22 negatively correlated with work efficiency in a cohort of mixed-ability cyclists
23 (Mogensen et al., 2006). Another potential mechanism responsible for an apparent
24 additional $\dot{V}O_2$ slow component (Poole et al., 1994) during prolonged constant
25 intensity exercise might be related to muscle contractile inefficiency. Specifically,

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2
3 26 during prolonged exercise above the lactate threshold it has been suggested that the
4
5 27 $\dot{V}O_2$ slow component might be the product of an increased phosphate cost of power
6
7 28 production (Rossiter et al., 2002; Cannon et al., 2014). Indeed, previous research has
8
9 29 demonstrated a close relationship between muscle and whole body $\dot{V}O_2$ measured via
10
11 30 pulmonary oxygen consumption (Poole et al., 1992). Therefore, an increase in
12
13 31 mitochondrial uncoupling and muscle $\dot{V}O_2$ ($m\dot{V}O_2$) during prolonged cycling exercise
14
15 32 has the potential to increase the $\dot{V}O_2$ from constant load exercise, and consequently
16
17 33 reduce cycling efficiency.
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22
23 35 Near-infrared spectroscopy (NIRS) can provide information about the changes in
24
25 36 tissue oxygenation at rest and during exercise (Ferrari et al., 2011) from the oxygen
26
27 37 dependent absorption characteristics of infrared light. This non-invasive technique
28
29 38 therefore allows continuous monitoring of the dynamics of tissue oxygenation.
30
31 39 Indeed, NIRS has been used to provide information about relative changes in
32
33 40 oxygenated haemoglobin/myoglobin (HbO_2), deoxygenated haemoglobin/myoglobin
34
35 41 (HHb), and total haemoglobin or blood volume (tHb). Resultantly, NIRS has been
36
37 42 used to measure skeletal muscle oxygenation (Chuang et al., 2002) and provide an
38
39 43 estimate of blood flow (De Blasi et al., 1997; Nioka et al., 2006). Moreover, repeated
40
41 44 arterial occlusions have been used both during and after exercise to assess muscle
42
43 45 oxygen consumption as an index of mitochondrial function (Van Beekvelt et al.,
44
45 46 2001). During arterial occlusion an absolute value for muscle O_2 consumption can be
46
47 47 calculated, under the assumption that tHb remains constant (due to the occlusion),
48
49 48 from the decreasing slope of HbO_2 . The disassociation of oxygen molecules from
50
51 49 oxyhaemoglobin/myoglobin reflects the requirement of oxidative phosphorylation,
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3 50 and therefore will be indicative of the tightness of mitochondrial coupling and
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5 51 changes in the rate ATP consumption per unit of power production.
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10 53 The aim of this study was to determine the relationship between whole body measures
11
12 54 of GE calculated from pulmonary $\dot{V}O_2$, and $m\dot{V}O_2$ (measured via NIRS) during 2 h
13
14 55 constant load cycling exercise at 60 % of maximal minute power output. We
15
16 56 simultaneously measured whole body oxygen uptake via pulmonary gas exchange and
17
18 57 local muscle oxygen consumption via NIRS in order to identify how each measure
19
20 58 changed during the prolonged bout of cycling. To control for the confounding effects
21
22 59 of changes in blood flow, $m\dot{V}O_2$ was measured during arterial occlusion. We
23
24 60 hypothesized that GE would decrease, and $m\dot{V}O_2$ would increase during 2h constant
25
26 61 load cycling exercise.
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30 62

31 63 **MATERIALS AND METHODS**

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34 64
35 65 Fourteen well-trained male cyclists (mean \pm SD: age 30 ± 14 years, mass 66 ± 11 kg,
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37 66 $\dot{V}O_{2max}$ 73 ± 2 mL.kg⁻¹.min⁻¹, maximum minute power output [MMP] 319 ± 15 W)
38
39 67 volunteered to participate in the study. All participants had a minimum of two years
40
41 68 training and racing experience, and were in preparation for a full competitive season.
42
43 69 The study was completed with full ethical approval from the local institutional ethics
44
45 70 committee according to the Declaration of Helsinki standards. All cyclists provided
46
47 71 written informed consent prior to participating.
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76 Study Design and Experimental Procedures

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

92 ***INSERT FIGURE 1 HERE***

93 *Maximal incremental test*

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

1
2
3 100 covering the nose and mouth. Participant's $\dot{V}O_{2\max}$ was assessed as the highest $\dot{V}O_2$
4
5 101 that was attained during a 60 s period in the test. MMP was assessed as being the
6
7 102 highest average 60 s power output during the test. Following the maximal incremental
8
9
10 103 exercise test, participants were familiarized with the testing procedures used during
11
12 104 visit 2. This consisted of familiarization with muscle occlusions, practice 6 s sprint
13
14 105 trial, and a 10 min bout of cycling at the target 2 h power output in order to determine
15
16 106 preferred cadence.

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18
19 107 *2-hour cycling test*
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21

22 108 Following a 10 min warm-up at 100 W and the sprint tests, participants cycled at 60
23
24 109 % MMP continuously for 120 min. During this time, expired air was collected for one
25
26 110 minute using non-diffusible gas bags (Hans - Rudolph, USA), at time points 5, 30, 60,
27
28 111 90 and 120 min, and were analyzed immediately following collection using a
29
30 112 Servomex 5200 gas analyzer (Servomex, Crowborough, East Sussex) by the
31
32 113 procedures outlined by Hopker et al. (2012). During the final 20 s of gas collection,
33
34 114 and whilst the cyclist continued to pedal at the required rev.min⁻¹, an arterial
35
36 115 occlusion (see NIRS below) was applied to the right leg to determine local muscle
37
38 116 oxygen uptake ($m\dot{V}O_2$). This procedure allows controlling for the confounding effects
39
40 117 of changes in blood flow on $m\dot{V}O_2$. Throughout the 120 min of cycling, subjects were
41
42 118 required to maintain a constant self-selected cadence (range: 80-88 rev.min⁻¹), which
43
44 119 was determined during Visit 1. Heart rate was recorded continuously throughout the
45
46 120 exercise test (S810i, Polar Electro Oy, Finland).

51
52 121 Blood lactate concentration was measured using a fingertip capillary blood sample
53
54 122 and was taken during exercise at the same time points as the expired gases and NIRS
55
56 123 measurements. Blood samples were analyzed using a Biosen C-Line (EKF
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3 124 Diagnostic, London, UK). RPE measurements were also taken at the same time points
4
5 125 using the Borg 6-20 scale (Borg, 1998).
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8 126 *Calculation of cycling efficiency*
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11 127 Cycling efficiency was calculated as the ratio of work done to energy expended
12
13 128 during the sampling minute in the form:
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15
16
17 129 $\text{Gross Efficiency \%} = (\text{Work accomplished}/\text{Energy Expenditure}) \times 100$
18

19
20 130 In order to establish the 'Work accomplished', the mean power recorded during the
21
22 131 same period as the respiratory collection was determined and converted into
23
24 132 $\text{kcal}\cdot\text{min}^{-1}$ via the following equation:
25

26
27 133 'Work accomplished' ($\text{kcal}\cdot\text{min}^{-1}$) = Power (W) \times 0.01433
28

29
30 134 Energy expenditure was calculated from the 1 min respiratory collection to ascertain
31
32 135 $\dot{V}\text{O}_2$ and Respiratory Exchange Ratio (RER). The calorific equivalent of O_2 was then
33
34 136 determined from the corresponding RER according the data of Peronnet and
35
36 137 Massicotte (1991):
37

38
39
40 138 'Energy expenditure' ($\text{kcal}\cdot\text{min}^{-1}$) = $\dot{V}\text{O}_2$ ($\text{L}\cdot\text{min}^{-1}$) \times $\text{kcal}\cdot\text{L}^{-1}$ of O_2
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43 139
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45
46 140 *Near-infrared Spectroscopy*
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48
49 141 Muscle oxygenation and consumption in the right *Vastus Lateralis* (VL) was
50
51 142 continuously monitored using wireless spatially resolved dual-wavelength
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53 143 spectrometers (Portamon, Artinis Medical Systems, BV, the Netherlands). The small
54
55 144 unit measures 83 x 52 x 20 mm and weighs 84g, including the battery. The device has
56
57 145 three pairs of diodes emitting light of wavelengths 760 and 850nm. Resultantly it is
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1
2
3 146 possible to detect combined concentration changes in the chromophores haemoglobin
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5 147 (Hb) and myoglobin (Mb). The distance between light source and detector was 40mm.
6
7 148 The inability to measure absolute chromophore concentrations can be accommodated
8
9
10 149 for by using Spatially Resolved Spectroscopy (SRS). The Portamon device utilizes
11
12 150 three light sources in a spatially resolved configuration, distanced 30, 35, and 40mm
13
14 151 from the one light receiver. A differential path-length factor of 4.0 was assumed
15
16 152 during all tests. The gradient of light attenuation allows a deeper more muscle-biased
17
18 153 measurement with less interference from superficial skin and fat layers. SRS is also
19
20 154 insensitive to light scattering, allowing the diffusion equation for light transport to be
21
22 155 used to yield an absolute measure of tissue oxygen saturation (TSI%). Using these
23
24 156 methods, changes are reported from an arbitrary baseline value taken prior to the start
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26
27 157 of exercise.
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30 158
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32
33 159 The NIRS optode was situated on the cyclist's right leg, over the belly of the VL
34
35 160 muscle and 10 cm proximal to the knee joint on a line between the greater trochanter
36
37 161 of the femur and the lateral epicondyle. Skinfold thickness was measured at the
38
39 162 location of the probes using a skinfold caliper. The skinfold thickness was 11.1 ± 2.8
40
41 163 mm. To ensure the optodes and detector did not move relative to the subject's skin,
42
43 164 the device was fixed into position using surgical tape, and then secured with a
44
45 165 bandage.
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49 166 A pressure cuff (Hokanson SC12D; Bellevue, WA, USA) was secured around the
50
51 167 thigh and proximal to the NIRS device. During occlusions, the pressure cuff was
52
53 168 rapidly (< 0.3 s) inflated to 300 mmHg for 5 min using a semi-automated inflation
54
55 169 system (Hokanson E20; Bellevue, WA, USA). This was used as a measure of baseline
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3 170 $m\dot{V}O_2$ following the warm-up, but prior to the 2 h constant load cycling exercise.

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5 171 Following release of the cuff, the hyperaemic response was used to assess the half-
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7 172 recovery time and re-oxygenation rate at baseline. Finally, 5 min after release of the
8
9 173 occlusion and resolution of the hyperaemic response, baseline NIRS measurements
10
11 174 were taken in the two minutes immediately prior to the start of exercise. The NIRS
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13 175 data were collected wirelessly at 10 Hz, then for the purposes of further analysis, a
14
15 176 10-point moving average was applied.

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19 177 *Calculation of changes in NIRS parameters*

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22 178 All TSI and chromophore concentration changes were collected from a 30 s period
23
24 179 concomitant with expired gas sampling, and are presented relative to a baseline value
25
26 180 taken immediately prior to the start of the 2 h period of cycling. $m\dot{V}O_2$ was derived
27
28 181 from NIRS using arterial occlusion by evaluating the rate of decline in Hb_{diff} ($Hb_{diff} =$
29
30 182 $HbO_2 - HHb$) with the assumption that tHb is constant (De Blasi et al., 1997). A typical
31
32 183 recording of an occlusion is shown in figure 2. The use of 20 s arterial occlusions
33
34 184 enabled the measurement of $m\dot{V}O_2$ whilst controlling for potential blood volume
35
36 185 changes (Van Beekvelt et al., 2001). A 3 s period of data was selected and used for
37
38 186 the calculation of $m\dot{V}O_2$, and R^2 values were used to check the linearity of the
39
40 187 regressions during the determination of $m\dot{V}O_2$ with a mean value of 0.99 (range 0.97-
41
42 188 1.00). Concentration changes of HHb and Hb_{diff} were expressed in micromoles per
43
44 189 second and converted to milliliters O_2 per minute per 100 grams tissue ($mlO_2 \cdot min^{-1}$
45
46 190 $\cdot 100g^{-1}$). A value of $1.04 kg \cdot l^{-1}$ was used for muscle density (Van Beekvelt et al.,
47
48 191 2001). The recovery of HbO_2 after exercise or ischemia represents the time needed for
49
50 192 resaturation of deoxygenated haemoglobin and myoglobin and is thought to reflect
51
52 193 both the influx of oxygenated arterial blood and the continued O_2 consumption during
53
54 194 recovery (Chance et al., 1992). The half time recovery of HbO_2 (s) was calculated

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3 195 from maximum deoxygenation at the end of the 5 min occlusions (pre- and post- 2 h
4
5 196 exercise) to 50% of the maximum re-oxygenation during hyperaemia (Chance et al,
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7 197 1992). The reoxygenation rate (ΔHbO_2 in $\mu\text{M}\cdot\text{s}^{-1}$) was calculated as the rate of
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9
10 198 increase in HbO_2 during the initial 3 s after cessation of the occlusion both pre- and
11
12 199 post- 2 h constant load cycling exercise. This variable reflects the initial inflow of
13
14 200 HbO_2 over a fixed time period following the release of the occlusion and is therefore
15
16 201 not influence by the magnitude of the hyperaemic response. Thus, the half time
17
18 202 recovery of HbO_2 and re-oxygenation rates were used to provide an indication of the
19
20 203 recovery of vascular components and the continued oxygen consumption following
21
22 204 exercise cessation. We speculated that in the presence of increased mitochondrial
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24 205 uncoupling and reduced cycling efficiency, there is likely to be a slowed half time
25
26 206 recovery of HbO_2 and re-oxygenation rate.

27
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30 207 *** INSERT FIGURE 2 HERE***

31 32 33 208 *Sprint Tests*

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35
36 209 To assess muscle fatigue via maximal voluntary cycling power output, prior to, and
37
38 210 immediately following the 2 h constant load cycling exercise, participants were asked
39
40 211 to perform three maximal sprints, each of 6 s followed by 60 s active recovery (with
41
42 212 no resistance). Sprints were performed at three fixed cadences (60, 90 and 120
43
44 213 $\text{rev}\cdot\text{min}^{-1}$) using the isokinetic mode of the electromagnetically braked cycle
45
46 214 ergometer. Peak 1 s power output was obtained from each sprint in order to assess the
47
48 215 maximal voluntary power producing capability of the exercising muscles and
49
50 216 consequently highlights the presence of exercise-induced muscle fatigue (i.e. decrease
51
52 217 in the ability to produce maximal power).

53 54 55 56 57 218 *Statistical Analysis*

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3 219 Prior to all data analysis, data was checked for normality of distribution. Repeated
4
5 220 measures analysis of variance (ANOVA) with least significant difference unadjusted
6
7 221 post hoc analysis used to analyze data from the 2h constant load cycling exercise.
8
9 222 Differences in sprint power output at the cadences of 60, 90 and 120 rev.min⁻¹ were
10
11 223 assessed using two-way repeated measures ANOVA. Effect sizes were calculated
12
13 224 using partial eta squared (η_p^2) and were defined as small, moderate or large based
14
15 225 upon .02, .13 and .26, respectively (Cohen et al., 1998). The difference in half-
16
17 226 recovery time and reoxygenation rate of HbO₂ pre- to post- 2 h constant load cycling
18
19 227 exercise were assessed using a paired t-test, with Cohen's *d* effect sizes being defined
20
21 228 as 0.2, 0.5 and 0.8 for small, medium and large effects respectively (Cohen et al.,
22
23 229 1998). Statistical analyses were conducted using IBM SPSS Statistics 22 (IBM®,
24
25 230 Armonk, NY), and a $P < 0.05$ was used as the criteria for detection of significance in
26
27 231 all cases. Data are reported as mean and standard deviation (mean \pm SD) unless
28
29 232 specified otherwise.
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233

234 **RESULTS**

235

236 *Cardiorespiratory measurements*

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40
41 237 Mean cycling power output was 192 ± 9 W with mean cadence being 84 ± 1 rev.min⁻¹
42
43 238 during the 2h constant load cycling exercise. Both submaximal $\dot{V}O_2$ and $\dot{V}CO_2$
44
45 239 increased significantly over the period of cycling ($\dot{V}O_2 = \eta_p^2: 0.40; P = <0.01$; Figure
46
47 240 3c; $\dot{V}CO_2 = \eta_p^2: 0.34; P = 0.01$; Figure 3d). GE significantly declined during the 2 h
48
49 241 constant load cycling exercise ($\eta_p^2: 0.38; P < 0.01$; Figure 3a) from an initial value of
50
51 242 18.4 ± 1.6 % at min 6 to 17.4 ± 1.4 % at minute 120. RER significantly declined
52
53 243 between 90 and 120 min of constant load cycling exercise ($\eta_p^2: 0.28; P = 0.01$; Figure
54
55 244 3b). \dot{V}_E significantly increased during the 2 h cycling period with time points 90 and
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2
3 245 120 min being greater than time points 5 and 30 min (η_p^2 : 0.49; $P < 0.01$; Figure 3e).

4
5 246 Heart rate significantly increased over the 2 h constant load cycling exercise, being

6
7 247 higher at 90 and 120 min than minute 5 (η_p^2 : 0.42; $P < 0.05$; Figure 3f).

8
9
10 248 ***INSERT FIGURE 3 HERE***

11
12
13 249 *Blood lactate and perceived exertion*

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15
16 250 Blood lactate concentration rose significantly from baseline after 5 min of cycling

17
18 251 (η_p^2 : 0.37; $P = 0.01$, Figure 3g), stayed unchanged between 5 and 30 min and then

19
20 252 reduced significantly between 60 ($P = 0.03$) and 90 min ($P = 0.04$). At the end of the

21
22 253 cycling exercise, blood lactate was significantly higher than baseline ($P < 0.01$), but

23
24 254 not different from any of the other time points. Even though the required work rate

25
26 255 was held constant at 60 % MMP, perceived exertion rose continuously throughout the

27
28 256 2 h constant load cycling exercise. RPE at all measured time points was significantly

29
30 257 higher than the previous (η_p^2 : 0.73; $P < 0.05$, Figure 3h).

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38 259 *NIRS measurements*

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41 260 The responses of NIRS parameters during the 2 h constant load cycling exercise are

42
43 261 shown in Figure 4. There was no significant change in HbO₂ (η_p^2 : 0.20; $P > 0.05$;

44
45 262 Figure 4a), however HHb increased significantly (η_p^2 : 0.25; $P = 0.02$), with values at

46
47 263 90 and 120 min being statistically higher than at min 5 (Figure 4b). tHb increased

48
49 264 steadily over time after 30 min of constant load cycling with time points of 60, 90 and

50
51 265 120 min being significantly greater than 5 and 30 min (η_p^2 : 0.30; $P < 0.04$; Figure 4c).

52
53 266 There was a trend for a reduction in TSI% levels over the 2 h constant load cycling

54
55 267 exercise (η_p^2 : 0.15; $P = 0.20$, Figure 4d). As can be seen in the typical m $\dot{V}O_2$ trace,

1
2
3 268 figure 2, during the occlusion O_2Hb decreased, HHb increased and tHb remained
4
5 269 stationary, indicating that the blood flow was occluded. Resting $m\dot{V}O_2$ was 0.04 ± 0.02
6
7 270 $mlO_2\cdot min^{-1}\cdot 100g^{-1}$ and demonstrated a 7.5 ± 3.8 fold increase after 6 min cycling at
8
9 271 60% MMP. $m\dot{V}O_2$ increased further during the 2 h constant load cycling exercise,
10
11 272 being significantly higher after 90 (10.0 ± 5.5 fold increase) and 120 min (10.3 ± 6.2
12
13 273 fold increase) than at min 5 (η_p^2 : 0.29; $P = 0.03$, Figure 5a). There was a trend for
14
15 274 both the half-recovery time (d : 0.48; $P = 0.27$; Figure 5b), and reoxygenation rate (d :
16
17 275 0.60; $P = 0.11$; Figure 5c) of HbO_2 to be slower following occlusion after the 2 h
18
19 276 constant load cycling exercise.

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24 277 ***INSERT FIGURE 4 & 5 HERE***

25 26 27 278 *Sprint tests*

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29
30 279 There was no interaction effect between 6-s sprint time point (i.e. pre vs. post 2 h of
31
32 280 cycling) and sprint cadence (η_p^2 : 0.09; $P = 0.29$). Regardless of cadence, sprint power
33
34 281 output was significantly lower at each cadence following the 2 h constant load cycling
35
36 282 exercise (η_p^2 : 0.51; $P = 0.04$; Figure 6). However, the reduction in GE was not related
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38 283 to the decline in 6-s sprint power output at any cadence ($P > 0.05$).

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41 284 ***INSERT FIGURE 6 HERE***
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285 **DISCUSSION**

286 This study used NIRS to investigate the relationship between local muscle and whole
287 body physiological responses to prolonged constant load cycling exercise. The main
288 findings of this study was that GE declined significantly during 2 h constant load
289 cycling exercise in accordance with the findings of previous studies (Hagan et al.,
290 1992; Hagberg et al., 1978; Passfield & Doust, 2000), despite maintenance of constant
291 power output and cadence. The physiological data recorded during the constant load
292 exercise trial may provide some answers to the origins of the reduction in efficiency
293 recorded. GE is the ratio of work accomplished to energy expenditure and expressed
294 as a percentage (Hopker et al., 2012), where work accomplished is determined by the
295 mean cycling power output of the corresponding data-sampling period. Energy
296 expenditure is determined by the oxygen cost of the exercise multiplied by the caloric
297 equivalent per liter of oxygen determined from the corresponding RER. The reduction
298 of GE seen in the current study was associated with a significant increase in the
299 oxygen cost of the exercise, i.e. the emergence of a $\dot{V}O_2$ slow component (see Figure
300 3c). Previous research has demonstrated that increases in fat metabolism, ventilation,
301 lactate metabolism, and body temperature cannot account for the increased oxygen
302 cost of work after sustained moderate-intensity cycling exercise (Hagan et al., 1992,
303 Hagberg et al., 1978). The present data support this conclusion. RER decreased by
304 0.02 units (0.96-0.94) across the 2 h period constant load cycling exercise and so there
305 were minimal changes in substrate metabolism. There was a significant increase in
306 pulmonary ventilation (mean 16 L.min⁻¹) during the 2 h constant load cycling
307 exercise but this was estimated to only increase $\dot{V}O_2$ by a negligible 29 mL.min⁻¹ O₂
308 (Aaron et al., 1992). Blood lactate was significantly higher during the cycling bout
309 than at baseline, but once elevated to ~3 mmol.L⁻¹ at min 5, there were no further

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3 310 increases even though GE continued to decline. Unfortunately no measures of core
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5 311 temperature were taken during the current study, although Passfield and Doust (2000)
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7 312 demonstrate that following an initial rise, core body temperature reaches a plateau
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10 313 during constant load cycling at 60% $\dot{V}O_{2peak}$. Therefore, we are confident that changes
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12 314 in core temperature significantly affected gross efficiency in the current study.

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16 316 It is possible that the cause of this reduction in efficiency is related to changes at the
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18 317 local muscle level (Gonzalez-Alonso et al., 1998). Interestingly the reduction in
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21 318 efficiency does not seem to be related to the loss in maximal muscle function assessed
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23 319 by the 6 s sprints before and after the cycling bout. However, in support of previous
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25 320 findings by Passfield and Doust (2000), there were reductions in gross efficiency and
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27 321 maximal cycling power output of a similar magnitude (~10%). Therefore further
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29 322 studies should clarify the hypothetical relationship between changes in GE and
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31 323 muscle fatigue induced by prolonged constant load cycling exercise.

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36 325 NIRS provides the ability to investigate the balance between O_2 supply and utilization
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38 326 within the exercising muscle (Hamaoka et al., 1996). As shown in Figure 4, there was
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40 327 a relative increase in HbB and tHb during the exercise test, indicating that there was
41
42 328 an increase in blood volume coupled with increased local muscle deoxygenation
43
44 329 during the constant load cycling exercise. HbO₂ remained statistically unchanged
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46 330 throughout the cycling bout. The general trend for progress local Vastus Lateralis
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48 331 muscle desaturation (as shown by the HHb and TSI%) to occur as the trial progressed
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50 332 suggests a greater metabolic demand rather than O_2 supply to exercising muscle.
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52 333 Therefore, mitochondrial oxygen consumption could be assumed to have
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54 334 progressively increased during the 2 h constant load cycling exercise. It is important
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3 335 to note that muscle oxygenation (TSI%) does not directly reflect $m\dot{V}O_2$, but reflects
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5 336 the balance between oxygen supply and consumption (Hamaoka et al., 1996). A more
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7 337 robust measure of $m\dot{V}O_2$ is performed using arterial occlusions to control inflow and
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10 338 outflow of blood to the limb, i.e. to limit changes in blood volume (Van Beekvelt et
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12 339 al., 2001). Most previous studies have used occlusions of the upper limb (e.g. Van
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14 340 Beekvelt et al., 2001), with few using arterial occlusions on large muscle groups i.e.
15
16 341 the legs (Brizendine et al., 2013; Nagasawa, 2008; Nioka et al., 2006). To the author's
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18 342 knowledge, this is the first study to use occlusions of the quadriceps muscle during
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20 343 whole-body dynamic exercise to evaluate $m\dot{V}O_2$.

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24 345 $m\dot{V}O_2$ increased steadily over the course of the 2 h constant load cycling exercise,
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26 346 even though work rate remained unchanged, being 10.0±5.6 fold higher at min 90 and
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28 347 10.3±6.2 fold higher at min 120. There is a paucity of research on $m\dot{V}O_2$ during
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30 348 cycling exercise. To our knowledge, the only previous research using NIRS to
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32 349 determine $m\dot{V}O_2$ via arterial occlusions of the quadriceps was performed after, rather
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34 350 than during exercise (Brizendine et al., 2013; Nagasawa et al., 2008), making direct
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36 351 comparisons difficult. We are aware of only one previous study to use arterial
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38 352 occlusions during exercise to determine $m\dot{V}O_2$. Van Beekvelt et al. (2001)
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40 353 demonstrated a ~6 fold increase in $m\dot{V}O_2$ during a 10% isometric MVC of the
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42 354 forearm. Submaximal cycling at 70% $\dot{V}O_{2max}$ has been shown to require ~20% MVC
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44 355 (Lollgen et al., 1980) and so our ~10 fold magnitude of increase in $m\dot{V}O_2$ ($mlO_2 \cdot min^{-1}$
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46 356 $\cdot 100g^{-1}$) is, unsurprisingly, higher than the forearm data.

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50 358 The reasons for the progressive increase in $m\dot{V}O_2$ despite no change in exercise
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52 359 intensity are unclear. One possibility is an alteration of the ratio between

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3 360 mitochondrial ADP phosphorylation and oxygen consumption (P/O ratio), which
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5 361 reflects the efficiency of oxidative phosphorylation. Specifically, back leak of protons
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7 362 across the inner membrane without driving ATP-synthase would reduce the P/O ratio,
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9 363 and thus increase uncoupling. Increased content or activation of uncoupling protein-3
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11 364 (UCP3) appear to be important in mediating this process (Mogensen et al., 2006).

14 365 Alternatively, the rise in $\dot{m}\dot{V}O_2$ could be caused by some mitochondrial ATP
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16 366 generation being used to reduce ROS generation within the cell (Brand, 2000). A high
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18 367 proton motive force that drives efficient ATP synthesis is associated with an
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20 368 additional ROS production. Proton leak across the mitochondrial membrane without
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22 369 driving ATP production may therefore assist in limiting the oxidative damage
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24 370 associated with high levels of ROS generated during the prolonged cycling exercise
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26 371 (Sahlin et al., 2010).

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31 373 While it is possible that the energetic cost of exercise might increase if the O_2 cost of
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33 374 ATP production increases with progressive mitochondrial uncoupling, an alternative
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35 375 possibility is that the ATP cost of contraction changes during prolonged exercise. In
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37 376 support of this proposition Cannon et al. (2014), have demonstrated that there is an
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39 377 increased phosphate cost of power production during constant load moderate intensity
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41 378 bilateral knee extensor exercise. Cannon et al. (2014) suggest that an increase in ATP
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43 379 turnover rate and $\dot{V}O_2$ during constant load exercise is consequent to a rise in
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45 380 contractile inefficiency due to muscle fatigue (Rossiter et al., 2002). Indeed the
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47 381 reduction of maximal voluntary cycling power at 60, 90 and 120 rev.min⁻¹ shown in
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49 382 the current study after 2 h constant load cycling indicates the presence of muscle
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51 383 fatigue. As prolonged cycling exercise is known to induce both peripheral and central
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53 384 components of muscle fatigue (Lepers et al., 2000; Lepers et al., 2002), we are
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3 385 confident that at least part of the decrease in maximal voluntary cycling power is due
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5 386 to presence of peripheral fatigue, i.e. fatigue produced by changes at or distal to the
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7 387 neuromuscular junction (Gandevia et al., 2001). Therefore it is possible to speculate
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9 388 that due to progressive peripheral fatigue encountered during the 2 h constant
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11 389 intensity cycling, there was an increase in the ATP cost of muscle contraction, which
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13 390 in turn might have contributed to the increased $m\dot{V}O_2$. Furthermore, as perception of
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15 391 effort is i) known to be influenced by both mental and muscle fatigue (Pageaux 2014,
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17 392 Pageaux et al., 2015) and ii) a main feature of fatigue (Enoka and Stuart, 1992), the
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19 393 progressive increase in perception of effort during the 2 h constant load cycling
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21 394 exercise strongly suggests a progressive development of muscle fatigue through the
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23 395 exercise.
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30 397 It should be noted that there are some methodological limitations that have to be
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32 398 considered when interpreting the findings of the current study. Firstly, NIRS
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34 399 measurements were made at only one site of the Vastus Lateralis and whether the
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36 400 results hold true for other sites (Koga et al., 2007), or other muscles (Kalliokoski et
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38 401 al., 2006) involved in the cycling action remains to be determined. Secondly the study
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40 402 used continuous-wave NIRS to measure HbO₂ and HHb signals meaning that there
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42 403 are potential confounding factors including an unknown optical path length,
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44 404 absorption and scattering coefficients (Hamaoka et al., 2011). This study used an
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46 405 assumed differential path-length factor to estimate absolute changes in chromophore
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48 406 oxygenation. However, Ferreira et al. (2007) have previously demonstrated that the
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50 407 scattering coefficient can change during exercise, and assuming a constant coefficient
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52 408 can lead to an overestimation of the changes in NIRS variables during exercise.
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55 409 However, it is important to note that the study of Ferreira et al. (2007) investigated
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3 410 incremental exercise, rather than constant intensity as used in this study. Adipose
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5 411 tissue thickness is a potential major confounder for the NIRS measurements used in
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7 412 the current study (Ferrari et al., 2011). However, there were no repeated
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9 413 measurements used in the current study, and participants were used as their own
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11 414 control. In addition, all participants were lean and had an adipose tissue thickness of
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13 415 less than 12mm, therefore, the impact of adipose tissue thickness on NIRS
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15 416 measurements are likely to be minimal. Moreover, the use of spatially resolved
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17 417 spectroscopy within the TSI% measurement is able to account for some of these
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21 418 limitations (Ferrari et al., 2004).

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25 420 The use of arterial occlusions allowed the quantitative measurement of muscle oxygen
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27 421 consumption independently of blood flow and oxygen delivery. The NIRS data
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29 422 suggest an increase in muscle blood flow and oxygen consumption over the 2 h
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31 423 cycling period. However, it is possible that heterogeneity in the NIRS response (Koga
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33 424 et al., 2007) could have influenced our data and conclusions. The increased blood
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35 425 flow over the 2 h cycling could have been accessing regions of the muscle that are not
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37 426 directly contributing to, or are less efficient in force production. To address this
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39 427 possibility, topographical MRI or fNIRS would be required.

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45 429 In conclusion, the present study demonstrates that during constant load cycling
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47 430 exercise at 60% MMP a $\dot{V}O_2$ slow component is evident, leading to a resultant
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49 431 reduction in cycling gross efficiency. *In vivo* Vastus Lateralis mitochondrial oxygen
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51 432 consumption measured via NIRS during arterial occlusions demonstrates concomitant
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53 433 increases in $m\dot{V}O_2$ over time. The increased $m\dot{V}O_2$ during the 2 h constant intensity
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55 434 cycling exercise is likely indicative of progressive mitochondrial / contractile
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3 435 | inefficiency, or the use of the mitochondrial proton motive force for tasks other than
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5 436 | ATP production. To further test the relationships between whole-body GE, NIRS
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7 437 | derived $\dot{m}\dot{V}O_2$, and mitochondrial/contractile efficiency, future studies intervention
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9 438 | studies might be considered.
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14 440 **Perspectives**

16 441 | Cycling efficiency has been demonstrated to be an important determinant of
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18 442 | endurance cycling performance (Coyle et al., 1992; Horowitz et al., 1994; Hopker et
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20 443 | al., 2013), which can be improved by endurance training (Hopker et al., 2010).
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23 444 | However, to date the underpinning physiological determinants of exercise efficiency
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25 445 | are yet to be fully elucidated. Prolonged endurance exercise has been shown to result
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27 446 | in reductions in cycling efficiency (Passfield and Doust, 2000), and so therefore
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29 447 | provides a method that can be used to investigate its physiological determinants. Over
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31 448 | the 2 h period of constant intensity cycling exercise, the emergence of a $\dot{V}O_2$ slow
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33 449 | component is seen to reduce whole body exercise efficiency. With negligible changes
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35 450 | in fat metabolism, ventilation, and lactate metabolism it is likely that the main
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37 451 | determinant of the pulmonary slow component is the exercising skeletal muscle.
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39 452 | Indeed, the increases in the NIRS derived $\dot{m}\dot{V}O_2$ signal suggest the greater O_2
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41 453 | consumption may arise from a combination of both an increased O_2 cost of ATP
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43 454 | resynthesis, and an increased ATP cost of power production.
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50 456 **Acknowledgements**

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52 457 | None.
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PROOF

FIGURE LEGENDS

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

Figure 2. A typical NIRS trace showing HbO₂, HHb and tHb signals during a 20 s occlusion of f 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 $\dot{V}O_2$.

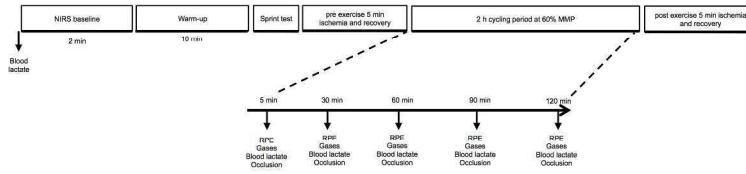
Figure 3. Changes in a.) Gross Efficiency, b.) RER, c.) $\dot{V}O_2$, d.) $\dot{V}CO_2$, e.) Ventilation, f.) Heart rate, g.) Blood lactate, h.) Rating of perceived exertion during 2 h constant load cycling exercise. Values are means \pm SEM for figures a-f. * = significantly different from min 6. # = 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) $\Delta TSI\%$ during 2 h constant load cycling exercise. Values are means \pm SEM. * = significantly higher than 5 min. ^ = significantly higher than 5 and 30 min.

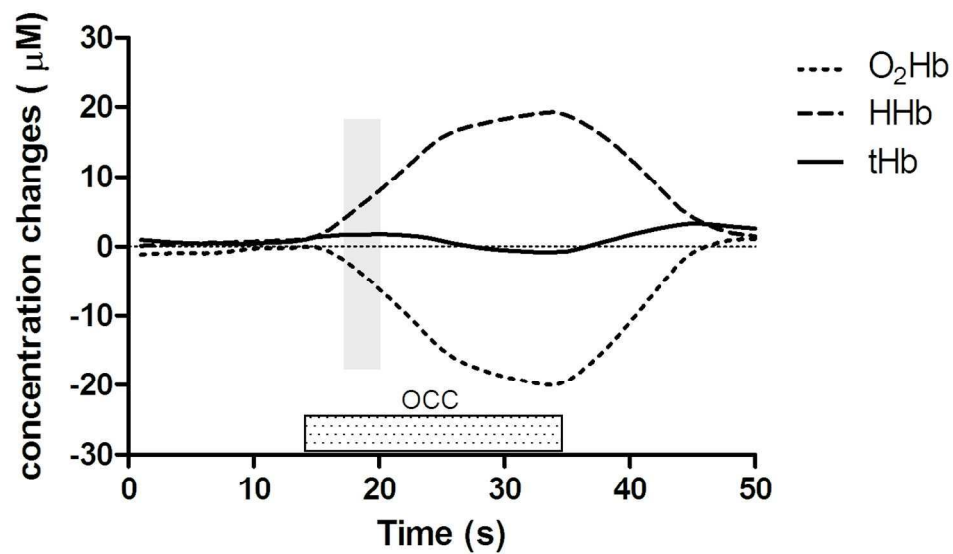
Figure 5. m $\dot{V}O_2$ response from 2 h cycling constant load cycling exercise. a) Time course of m $\dot{V}O_2$ response during 2 h constant load cycling exercise, b) half time of oxygenation recovery and c) reoxygenation rate following release of 5 min occlusion pre and post exercise. Values are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

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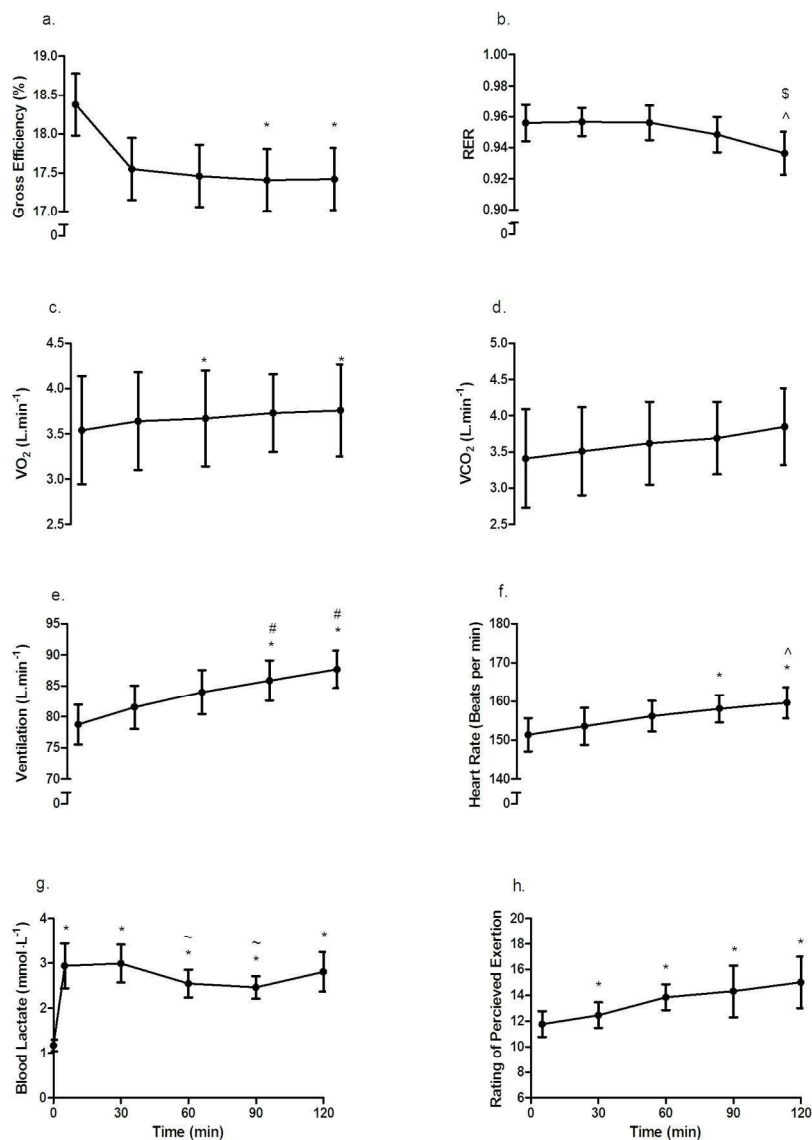


Overview of the protocol and timing of measurements used during visit 2.
297x209mm (300 x 300 DPI)



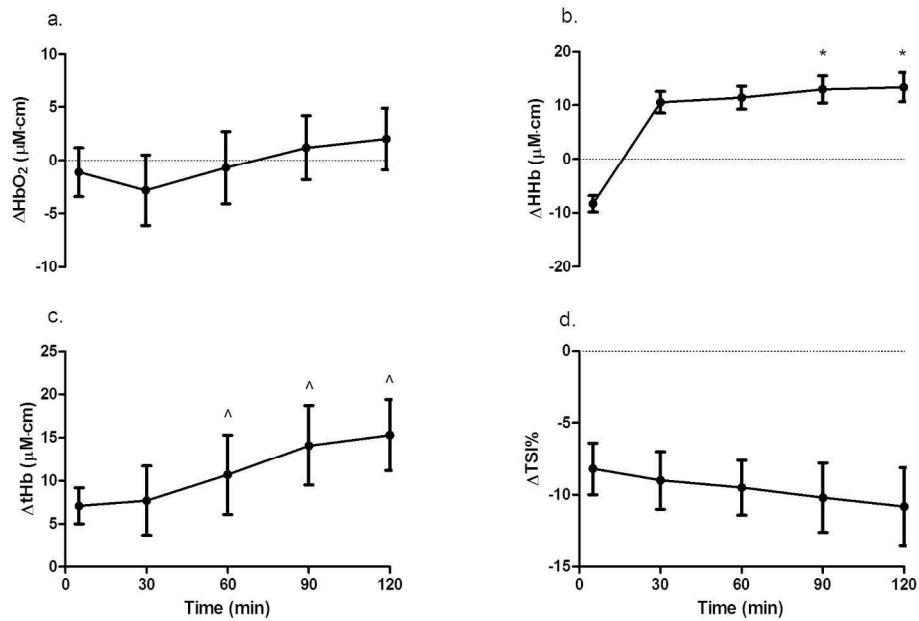
A typical NIRS trace showing HbO_2 , HHb and tHb signals during an occlusion to the Vastus Lateralis muscle during cycling. Trace data has been filtered using a 10-point average. Shaded area identifies the period of data selected for the calculation of mVO_2 .

121x73mm (300 x 300 DPI)

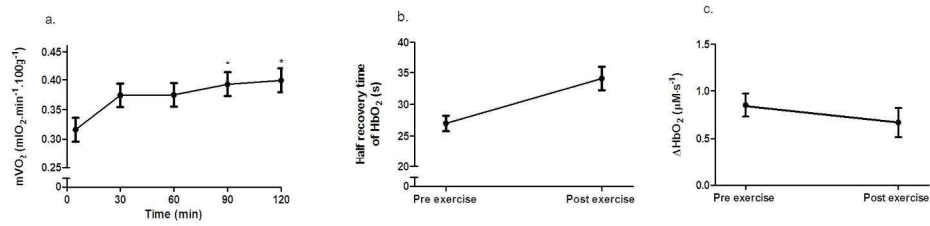


Changes in a.) Gross Efficiency, b.) RER, c.) VO_2 , d.) VCO_2 , e.) Ventilation, f.) Heart rate, g.) Blood lactate, h.) Rating of perceived exertion during 2 h constant load cycling exercise. Values are means \pm SD for figures a-f. * = significantly different from min 6. # = significantly different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

148x201mm (300 x 300 DPI)

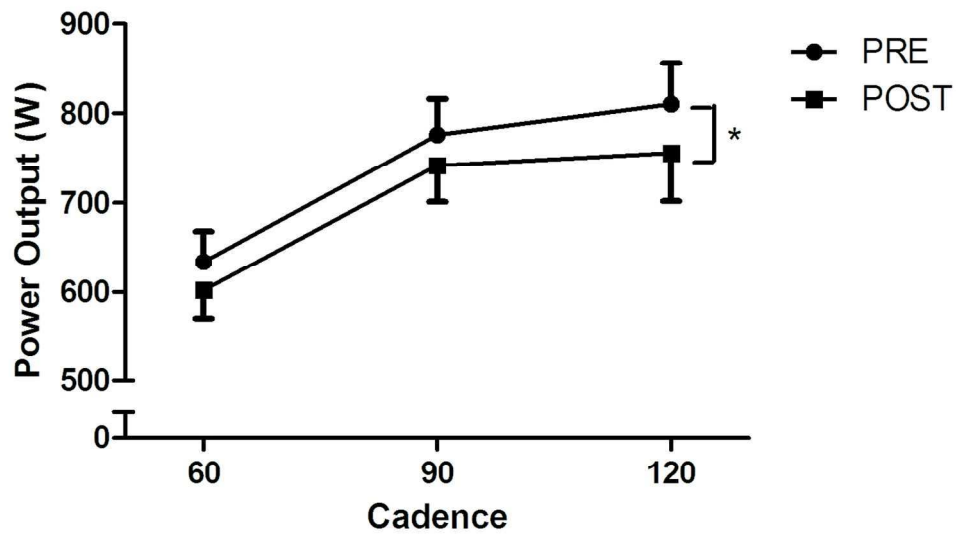


Mean values for a) ΔHbO_2 , b) ΔHHb , c) ΔtHb and d) $\Delta\text{TSI}\%$ during 2 h constant load cycling exercise. Values are means \pm SEM. * = significantly higher than 5 min. [^] = significantly higher than 5 and 30 min.
181x124mm (300 x 300 DPI)



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 \pm SEM.
178x50mm (300 x 300 DPI)

PROOF



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 \pm SEM. * significant main effect of time.
122x73mm (300 x 300 DPI)