

1 **Title:** Locomotor muscle fatigue is not critically regulated after prior upper body exercise

2

3 **Authors:** Johnson MA<sup>1</sup>, Sharpe GR<sup>1</sup>, Williams NC<sup>1</sup>, Hannah R<sup>2</sup>

4

5 **Affiliations:** <sup>1</sup>Sport, Health and Performance Enhancement (SHAPE) Research Group,  
6 Department of Sports Science, School of Science and Technology, Nottingham Trent  
7 University, Nottingham, UK; <sup>2</sup>Sobell Department of Motor Neuroscience and Movement  
8 Disorders, Institute of Neurology, University College London, London, UK.

9

10 **Author Contributions:** M.A.J., N.C.W., G.R.S., and R.H., conception and design of  
11 research; M.A.J., N.C.W., and R.H. performed experiments; M.A.J. and R.H. analyzed data;  
12 M.A.J., G.R.S., and R.H. interpreted results of experiments; M.A.J. prepared figures; M.A.J.  
13 drafted manuscript; M.A.J., G.R.S., and R.H. edited and revised manuscript; M.A.J., N.C.W.,  
14 G.R.S., and R.H. approved final version of manuscript.

15

16 **Running head:** Prior upper body exercise and locomotor muscle fatigue

17

18 **Corresponding Author:**

19 Dr Michael A Johnson, School of Science and Technology, Nottingham Trent University,  
20 Nottingham, NG11 8NS, UK

21 Telephone: +44 (0)115 8483362

22 Fax: +44 (0)115 8486680

23 E-mail: Michael.johnson@ntu.ac.uk

24 **ABSTRACT**

25 This study examined the effects of prior upper body exercise on subsequent high-intensity  
26 cycling exercise tolerance and associated changes in neuromuscular function and perceptual  
27 responses. Eight males performed 3 fixed work-rate (85% peak power) cycling tests: (1) to  
28 the limit of tolerance (CYC); (2) to the limit of tolerance after prior high-intensity arm-  
29 cranking exercise (ARM-CYC); (3) without prior exercise and for an equal duration as ARM-  
30 CYC (ISOTIME). Peripheral fatigue was assessed via changes in potentiated quadriceps  
31 twitch force during supramaximal electrical femoral nerve stimulation. Voluntary activation  
32 was assessed using twitch interpolation during maximal voluntary contractions. Cycling time  
33 during ARM-CYC and ISOTIME ( $4.33 \pm 1.10$  min) was 38% shorter than CYC ( $7.46 \pm 2.79$   
34 min) ( $P < 0.001$ ). Twitch force decreased more after CYC ( $-38 \pm 13\%$ ) than ARM-CYC ( $-26$   
35  $\pm 10\%$ ) ( $P = 0.004$ ) and ISOTIME ( $-24 \pm 10\%$ ) ( $P = 0.003$ ). Voluntary activation was  $94 \pm 5\%$   
36 at rest and decreased after CYC ( $89 \pm 9\%$ ,  $P = 0.012$ ) and ARM-CYC ( $91 \pm 8\%$ ,  $P = 0.047$ ).  
37 Rating of perceived exertion for limb discomfort increased more quickly during cycling in  
38 ARM-CYC ( $1.83 \pm 0.46$  AU $\cdot$ min $^{-1}$ ) than CYC ( $1.10 \pm 0.38$  AU $\cdot$ min $^{-1}$ ,  $P = 0.003$ ) and  
39 ISOTIME ( $1.05 \pm 0.43$  AU $\cdot$ min $^{-1}$ ,  $P = 0.002$ ), and this was correlated with the reduced  
40 cycling time in ARM-CYC ( $r = -0.72$ ,  $P = 0.045$ ). In conclusion, cycling exercise tolerance  
41 after prior upper body exercise is potentially mediated by central fatigue and intolerable  
42 levels of sensory perception rather than a critical peripheral fatigue limit.

43

44

45

46

## 47 INTRODUCTION

48 A consistent reduction (~35%) in the potentiated quadriceps twitch force is observed  
49 after high-intensity cycling (4-6, 66, 73, 79). It is proposed that this reduction represents an  
50 “individual critical threshold” of peripheral locomotor muscle fatigue beyond which the  
51 degree of associated sensory perception would not be tolerable (3). The observation of similar  
52 intramuscular metabolic perturbation at the end of exhaustive exercise irrespective of the  
53 work-rate (20, 77) supports the notion that it is probably not peripheral fatigue *per se* that is  
54 monitored / regulated but the associated fatigue-inducing biochemical changes within the  
55 muscle (3). The critical limit of peripheral fatigue observed under “normal” conditions is also  
56 unchanged when exercise tolerance is reduced (i.e. the critical limit is reached more quickly)  
57 due to moderate hypoxia ( $F_{iO_2}$  0.13-0.15) (7, 66), superimposed inspiratory muscle loading  
58 (67), volitionally-induced inspiratory or expiratory muscle fatigue (73, 80), prior high-  
59 intensity cycling exercise (5), and prior electrically-induced quadriceps muscle fatigue (34).  
60 Conversely, the degree of peripheral fatigue observed after cycling exercise in severe hypoxia  
61 ( $F_{iO_2}$  0.10) is about two-thirds of that observed in normoxia, suggesting that the major  
62 determinant of exercise tolerance switches from a peripheral to central origin, possibly due to  
63 brain hypoxia (8). Individual critical limits to peripheral fatigue are thought to be mediated  
64 by thin fiber group III/IV muscle afferents (3, 31), which may influence central motor drive,  
65 and thereby exercise tolerance, by providing inhibitory feedback to the central nervous  
66 system in response to intramuscular metabolic perturbation (1, 10, 25, 51). However, despite  
67 growing support for an important role of peripheral fatigue in determining exercise tolerance,  
68 this notion has been challenged (53, 54). Marcora (53) has proposed a psychobiological  
69 model of endurance exercise tolerance, which primarily attributes exercise intolerance to a  
70 conscious decision to stop exercise due to perception of effort, mediated exclusively by feed-  
71 forward mechanisms (i.e. corollary discharge), reaching a level that the individual is

72 unwilling to tolerate. A pivotal role for the rating of perceived exertion (RPE) in limiting  
73 exercise tolerance is also depicted in the ‘flush model’ proposed by Millet (57). However,  
74 this model differs from the psychobiological model because it attributes RPE to both feed-  
75 forward *and* feedback (i.e. peripheral) mechanisms, thereby also emphasizing the importance  
76 of intramuscular metabolic perturbation and peripheral fatigue. The importance of sensory  
77 perception in influencing exercise tolerance is also evident in the striking ability of the RPE  
78 to predict the tolerable duration of exercise after prior fatiguing exercise (32), and at various  
79 exercise intensities (64), muscle glycogen concentrations (59) and ambient temperatures (24).  
80 Thus the rate of increase in RPE ( $\Delta\text{RPE}/\Delta\text{time}$ ), and possibly dyspnea ( $\Delta\text{dyspnea}/\Delta\text{time}$ ),  
81 may be considered major contributors to the attainment of a “critical sensory tolerance limit”  
82 (35, 57) and subsequent cessation of exercise.

83         Several studies have also shed light on the determinants of exercise tolerance by  
84 showing reduced lower body exercise tolerance after prior high-intensity upper body exercise  
85 (12, 17, 36, 44, 46, 47, 61). This has been attributed to an accelerated development of  
86 peripheral locomotor muscle fatigue secondary to faster intramuscular metabolite (i.e.  $\text{K}^+$ ,  $\text{H}^+$ ,  
87 and  $\text{La}^-$ ) accumulation resulting from the prior upper body exercise. However, this  
88 explanation remains conjecture because peripheral fatigue was not evaluated in these studies.  
89 An alternative explanation is that rather than accelerating the development of peripheral  
90 fatigue, prior upper body exercise might reduce lower body exercise tolerance by accelerating  
91 the attainment of an intolerable level of sensory perception that is mediated, in part, by the  
92 ensemble input of group III/IV muscle afferents. Specifically, since group III/IV muscle  
93 afferent input may remain elevated for up to 15 min after high-intensity upper body exercise  
94 (25, 45), the ensemble group III/IV muscle afferent input would be elevated during  
95 subsequent high-intensity lower body exercise. Subsequently, increases in  $\Delta\text{RPE}/\Delta\text{time}$   
96 and/or  $\Delta\text{dyspnea}/\Delta\text{time}$  may reduce exercise tolerance with *less* lower body peripheral

97 fatigue incurred. This notion is supported by the observation of increased RPE and reduced  
98 exercise tolerance with less peripheral fatigue incurred during single-leg knee extensor  
99 exercise preceded by fatiguing knee extensor exercise using the contralateral leg (10).

100 Therefore, the present study aimed to elucidate the mechanism(s) by which prior  
101 high-intensity upper body exercise reduces subsequent leg cycling exercise tolerance.  
102 Specifically, we tested the hypothesis that prior upper body exercise reduces subsequent leg  
103 cycling exercise tolerance and that this is associated with less peripheral fatigue, but an  
104 accelerated rise in  $\Delta\text{RPE}/\Delta\text{time}$  and  $\Delta\text{dyspnea}/\Delta\text{time}$  .

## 105 **METHODS**

### 106 **Participants**

107 Eight healthy, non-smoking, moderately trained males (age:  $26 \pm 4$  years; height:  $182$   
108  $\pm 4$  cm; body mass:  $83 \pm 4$  kg; peak oxygen uptake:  $50 \pm 10$  mL·kg<sup>-1</sup>·min<sup>-1</sup>) provided written  
109 informed consent to participate in the study. Five of the participants had previously taken part  
110 in investigations that included assessment of neuromuscular function using the methods  
111 described in the present study (38-41). Participants refrained from strenuous exercise and  
112 alcohol the day preceding and the day of an exercise test, abstained from caffeine on test  
113 days, and reported to the laboratory at least 2 h post-prandial. The study was approved by the  
114 Nottingham Trent University Human Ethics Committee, and all procedures were conducted  
115 in accordance with the Declaration of Helsinki.

### 116 **Experimental design**

117 Participants attended the laboratory on five separate occasions, at a similar time of  
118 day, separated by at least 48 h. The initial visit comprised a maximal incremental cycling test  
119 for the determination of peak oxygen uptake and peak cycling power ( $\dot{W}_{\text{peak}}$ ). The second  
120 visit comprised familiarization with the knee extensor neuromuscular function assessments

121 and arm-cranking protocol. The subsequent three visits comprised the experimental trials.  
122 The first two experimental trials were performed in a randomized order and comprised a  
123 fixed work-rate cycling test at 85%  $\dot{W}_{\text{peak}}$ , and exercise was performed to the limit of  
124 tolerance. These two cycling tests were performed without (hereafter termed CYC) and with  
125 (hereafter termed ARM-CYC) prior high-intensity arm-cranking exercise. For the third  
126 experimental trial, the CYC protocol was repeated except that the cycling test was terminated  
127 after an identical duration to that achieved during ARM-CYC (hereafter termed ISOTIME).  
128 Knee extensor force and surface electromyographic (EMG) signals were recorded during a  
129 series of electrically-evoked and voluntary isometric contractions of the dominant leg to  
130 quantify the presence and magnitude of central and peripheral locomotor muscle fatigue. For  
131 an illustration of the protocol for the experimental trials please refer to Figure 1.

### 132 **Neuromuscular Function**

133 *Dynamometer* Participants were seated in a rigid, custom built dynamometer adapted from  
134 Hannah et al. (40), with hip and knee joint angles of 100° and 95° (180° = full extension)  
135 respectively. Adjustable strapping across the pelvis and shoulders prevented extraneous  
136 movement during muscle activation. A non-compliant strap was attached to the dominant leg  
137 of the participant ~2 cm proximal to the medial malleolus and was in series with a linear  
138 strain gauge (615, Tedeo-Huntleigh, Herzliya, Israel) oriented perpendicular to the tibia. The  
139 dynamometer configuration was established during the familiarization session and replicated  
140 thereafter. The force signal was amplified ( $\times 1000$ ) in the frequency range 0-500 Hz, and  
141 sampled at 2000 Hz using an external A/D converter (1401; CED, Cambridge, UK)  
142 interfaced with a personal computer using Spike 2 software (CED). Force data were low-pass  
143 filtered in both directions at 450 Hz using a fourth-order zero-lag Butterworth filter prior to  
144 analysis. Baseline resting force was subtracted from all force recordings to correct for the  
145 effect of gravity.

146 **Electromyography** EMG signals were recorded from the superficial quadriceps (rectus  
147 femoris, vastus medialis, and vastus lateralis) and hamstring (biceps femoris) muscles, as  
148 described previously (40). After preparation of the skin by shaving, light abrasion, and  
149 cleaning with alcohol, bipolar surface electrodes (2.5 cm inter-electrode distance; silver/silver  
150 chloride, 95 mm<sup>2</sup> area, Ambu Blue Sensor; Ambu, Ballerup, Denmark) were attached over  
151 each muscle at standardized percentages of thigh length measured from the knee joint space  
152 to the greater trochanter (rectus femoris, 55%; vastus medialis, 25%; vastus lateralis and  
153 biceps femoris, 45%). These sites were selected to avoid the innervation zones of each  
154 muscle (65). Electrodes were positioned parallel to the presumed orientation of the muscle  
155 fibers. EMG signals were pre-amplified by active EMG leads (input impedance 100 MΩ,  
156 common mode rejection ratio > 100 dB, base gain 500, 1st order high pass filter set to 10 Hz;  
157 Noraxon, Scottsdale, USA) connected in series to a custom-built junction box and  
158 subsequently to the same A/D converter and computer software that enabled synchronization  
159 with the force data. The signals were sampled at 2000 Hz. Prior to analysis EMG data were  
160 band-pass filtered in both directions between 20 and 450 Hz using a fourth-order zero-lag  
161 Butterworth filter (26, 27, 55).

162 **Electrical stimulation** Equipment and procedures for electrical stimulation have been  
163 described previously (41). A constant current variable voltage stimulator (DS7AH; Digitimer  
164 Ltd, Welwyn Garden City, UK) was used to assess knee extensor contractile properties whilst  
165 the participant was voluntarily passive. Square-wave pulses (0.2 ms duration) were delivered  
166 via supramaximal femoral nerve stimulation to evoke maximal potentiated twitch and triplet  
167 (3 pulses at 300 Hz) contractions (24, 41). Stimulation of the femoral nerve was achieved via  
168 a 1 cm diameter cathode stimulation probe (Electro Medical Supplies, Wantage, UK) pressed  
169 into the femoral triangle. The surface of the anode, a 4 × 7 cm carbon rubber electrode  
170 (Electro Medical Supplies), was coated in electrode gel and located over the greater

171 trochanter. The precise location of the cathode was determined as the position that evoked the  
172 greatest twitch response for a particular submaximal electrical current (typically 30–50 mA),  
173 and was marked on the skin using indelible ink to ensure accurate repositioning within each  
174 trial.

175 **Procedure** Initially discrete electrical stimuli were delivered via percutaneous stimulation of  
176 the femoral nerve in the femoral triangle to elicit twitch contractions of the quadriceps.  
177 Stepwise increments in the current were delivered, separated by 10 s to allow for  
178 neuromuscular recovery, until plateaus were reached in the amplitude of twitch force and  
179 compound muscle action potentials (M-waves). The stimulus intensity was then increased by  
180 25% above the value required to elicit a plateau to ensure supramaximal stimulation.  
181 Participants subsequently performed sub-maximal warm-up contractions of the knee  
182 extensors, lasting ~3 s and interspersed by ~30 s rest, at ~50, 75 and 90% of their perceived  
183 maximal force. Thereafter, and following baseline measurements for heart rate and  $[La^-]_B$ ,  
184 participants performed four maximum voluntary contractions (MVCs) lasting 3-4 s and  
185 interspersed by ~30 s rest. Participants were instructed to extend the knee “as hard and as fast  
186 as possible”. During and after each contraction participants received strong verbal  
187 encouragement. Online feedback of the force signal was provided and a marker showing  
188 maximum force during that session was displayed onscreen in order to assist participants in  
189 attempting to maintain a high and stable force level. Each MVC was followed within 1-2 s by  
190 two supramaximal electrical stimuli, separated by 1 s, delivered to the femoral nerve to elicit  
191 maximal potentiated twitches (49). Single electrically-evoked triplet contractions (3  
192 supramaximal stimuli delivered at 300 Hz) were superimposed on the 3<sup>rd</sup> and 4<sup>th</sup> MVC, and at  
193 rest ~1-2 s after the two potentiated twitch contractions (29, 48). Triplets were used in the  
194 calculation of voluntary activation (see below), because the detection of single superimposed  
195 twitches becomes increasingly difficult at high forces as a result of the decreasing signal-to-



196 noise ratio. This may lead to the erroneous conclusion that voluntary activation is maximal  
197 (i.e. 100%). Consequently, some studies have suggested the use of multiple stimuli (48, 63,  
198 72). Furthermore, triplets may offer advantages over potentiated twitches as an indicator of  
199 peripheral fatigue since pilot data from our lab and that of de Haan (28, 29) has found the  
200 force evoked by 300 Hz bursts (3-8 pulses) to be insensitive to potentiation, and because they  
201 evoke much greater force than potentiated twitches they may better reflect the functional  
202 changes observed during maximal voluntary contractions.

203         The maximum voluntary force (MVF) of the quadriceps was defined as the greatest  
204 instantaneous force produced during the relevant series of MVCs. The root mean square  
205 (RMS) amplitude of the EMG signal for each agonist muscle (vastus medialis, vastus lateralis  
206 and rectus femoris) was calculated over a 500 ms epoch surrounding MVF (250 ms either  
207 side) (19). Agonist EMG RMS values were averaged to calculate a mean quadriceps  
208 ( $QEMG_{max}$ ) value and normalized to the peak-to-peak amplitude of the M-wave (see below)  
209 to provide a measure of neuromuscular activation. Potentiated twitches were measured for  
210 peak force and the amplitude of M-wave response for the three quadriceps electrodes, which  
211 were then averaged across the three sites to provide a mean quadriceps value. Mean  
212 quadriceps M-wave amplitude and potentiated peak twitch force were averaged across the  
213 latter four twitch contractions (i.e. after the 3<sup>rd</sup> and 4<sup>th</sup> MVC) within each time period because  
214 it typically takes three MVC's to fully potentiate twitches (49). The mean quadriceps M-wave  
215 amplitude across the four potentiated twitches was defined as the maximal M-wave amplitude  
216 ( $M_{max}$ ) and was used for normalization of voluntary quadriceps EMG RMS (19). Measures of  
217 triplet peak force were averaged across the two contractions within each time period. To  
218 evaluate the presence and magnitude of central fatigue voluntary activation was evaluated for  
219 the 3<sup>rd</sup> and 4<sup>th</sup> MVC using the formula for the twitch interpolation technique (56) as described  
220 previously (29, 48):

221            Voluntary activation (%) = 100 – [(triplet force increment / resting triplet force) × 100]

222    where the triplet force increment refers to that produced by superimposed triplet stimulation.

223    The highest voluntary activation of the two MVCs was retained for analysis. Assessment of

224    neuromuscular function (from the first MVC to the last triplet) took ~2 min.

### 225    **Maximal incremental cycling test**

226            Participants initially performed a maximal incremental cycling test using an

227    electromagnetically-braked cycle ergometer (Excalibur Sport; Lode, Groningen, The

228    Netherlands). Tests began at 0 W and power was increased by discrete 20 W increments

229    every 60 s and exercise was performed to the limit of volitional tolerance or task failure (i.e.

230    cycling cadence below 60 rpm) (46). Participants wore a facemask (model 7940; Hans

231    Rudolph, Missouri, USA) connected to a flow sensor (ZAN variable orifice pneumotach;

232    Nspire Health, Oberthulba, Germany) that was calibrated using a 3 L syringe. Gas

233    concentrations were measured using fast responding laser diode absorption spectroscopy

234    sensors, which were calibrated using gases of known concentration (5% CO<sub>2</sub>, 15% O<sub>2</sub>,

235    balance N<sub>2</sub>; BOC, Guilford, UK), and ventilatory and pulmonary gas exchange variables were

236    measured breath-by-breath (ZAN 600USB; Nspire Health) as described previously (46). Peak

237    oxygen uptake was defined as the highest recorded value over any 30 s period, and  $\dot{W}_{\text{peak}}$  was

238    calculated as the sum of the power output in the last completed stage plus the product of ramp

239    increment (20 W) and the fraction of the final stage actually completed.

### 240    **Experimental trials**

241            During the experimental trials (CYC, ARM-CYC, and ISOTIME) heart rate was

242    measured using short-range telemetry (Polar S610; Polar, Kempele, Finland) and fingertip

243 capillary blood samples were taken and analyzed for blood lactate concentration ( $[La^-]_B$ )  
244 using an automated analyzer (Biosen C\_line Sport; EKF Diagnostics, Barleben, Germany).

245 An illustration of the timing of measurements taken during the experimental trials is  
246 shown in Figure 1. Each experimental trial comprised a fixed work-rate cycling test at 85%  
247  $\dot{W}_{peak}$  preceded by a standardized 23.5 min period. During the first 6 min of this period  
248 baseline measures of heart rate,  $[La^-]_B$ , and neuromuscular function were taken.

249 After baseline measurements, participants remained seated in the dynamometer and  
250 then either rested (CYC and ISOTIME) or performed intense intermittent arm-cranking  
251 exercise (ARM-CYC) using an electromagnetically-braked arm-cranking ergometer (Angio;  
252 Lode). The arm-cranking protocol comprised  $8 \times 1$  min arm-cranking bouts, interspersed with  
253 30 s rest, at a fixed work-rate of 1.0-1.5  $W \cdot kg^{-1}$  body mass (mean:  $1.2 \pm 0.2 W \cdot kg^{-1}$  body  
254 mass,  $100 \pm 15 W$ ) (46). As in our previous study (46), the arm-cranking work-rate for each  
255 individual was selected based on their habitual upper body exercise regimen. This work-rate  
256 was trialed during the familiarization session and, based on successful completion by all  
257 participants, was deemed suitable for subsequent testing. Cadence was maintained between  
258 90-110 rpm. Heart rate was measured at the end of each arm-cranking bout during ARM-  
259 CYC, and  $[La^-]_B$  was also measured after the final arm-cranking bout. These measurements  
260 were taken at equivalent time points whilst participants rested during CYC and ISOTIME.  
261 Quadriceps and hamstring muscle EMG was recorded throughout the arm-cranking protocol  
262 and displayed online with a high gain to aid visual detection of EMG activity. Participants  
263 received verbal feedback regarding EMG activity in order to ensure minimal activation of the  
264 leg muscles.

265 Arm-cranking (ARM-CYC) or seated rest (CYC and ISOTIME) was followed by  
266 another 6 min period before the start of the fixed work-rate cycling test. During this period

267 measures of neuromuscular function were taken and participants then transferred to the cycle  
268 ergometer (positioned ~2 m from the dynamometer). Immediately before the start of the  
269 cycling test heart rate and  $[La^-]_B$  were measured along with dyspnea (defined as breathing  
270 “effort”) and RPE for leg discomfort using Borg’s modified CR10 scale (18).

271 Participants adopted a self-selected cadence between 80-100 rpm during the first  
272 cycling test and this was replicated during subsequent tests. Quadriceps and hamstring  
273 muscle EMG was synchronized with the cycle ergometer crank position via a reed switch  
274 attached to the crank and ergometer. The RMS amplitude of the EMG signal of the  
275 quadriceps muscle was measured at the start and end of each minute during each arm-  
276 cranking exercise bout and normalized to  $QEMG_{max}$  to quantify quadriceps neuromuscular  
277 activation. EMG RMS amplitude of the quadriceps and hamstring muscles was also measured  
278 over 10 consecutive pedal revolutions at the end of the first, third and final minute of cycling  
279 exercise and normalized to  $M_{max}$  (quadriceps only) to quantify changes in neuromuscular  
280 activation during cycling. Onsets and offsets of EMG bursts were determined visually by the  
281 same investigator according to a previously published method (22, 23). Threshold methods  
282 for determining EMG onsets and offsets are sensitive to changes in background EMG (42)  
283 and are unsuitable for this type of analysis because bursts of EMG activity occur with  
284 background activity already present in the muscles and the amplitude of background activity  
285 varies between muscles (22, 23). Heart rate, RPE and dyspnea were measured after 3 min of  
286 cycling. During CYC and ARM-CYC, cycling exercise was performed to the limit of  
287 volitional tolerance. An additional criterion for terminating a cycling test was a fall in  
288 cadence below 60 rpm. During ISOTIME, cycling exercise was terminated by the  
289 investigators after an identical duration to that achieved during ARM-CYC.

290           Upon cessation of cycling exercise heart rate,  $[La^-]_B$ , RPE and dyspnea were  
291 measured immediately and participants were assisted to the dynamometer for neuromuscular  
292 function evaluation with the first MVC initiated after 2 min ( $\pm 9$  s).

### 293 **Statistical analyses**

294           Data were analyzed using SPSS for Windows (IBM, Chicago, IL). Trial-to-trial  
295 variation in baseline neuromuscular function was calculated as the within-participant  
296 coefficient of variation (CV). Measurement error and reproducibility of baseline  
297 neuromuscular function were calculated, and the smallest meaningful change was  
298 subsequently determined (16, 43). A one-way repeated measures ANOVA followed by  
299 Tukey's post-hoc test was used to analyze differences between trials for cycling exercise  
300 duration and rates of change in perceptual responses expressed relative to absolute exercise  
301 time ( $\Delta RPE/\Delta time$  and  $\Delta dyspnea/\Delta time$ ) and when normalized to total cycling exercise  
302 duration ( $\Delta RPE/\%time$  and  $\Delta dyspnea/\%time$ ). All other data were analyzed using a two-way  
303 (trial  $\times$  time) repeated measures ANOVA. Significant interactions were further explored by  
304 performing one-way repeated measures ANOVA: (i) within each trial, and (ii) across trials at  
305 individual time-points, followed by Tukey's post-hoc test. When differences were observed  
306 within or between trials, 95% confidence intervals (CI) for the difference were calculated (2).  
307 Pearson's correlation coefficient was used to determine the relationship between selected  
308 variables. Statistical significance was set at  $P < 0.05$ . Results are presented as mean  $\pm$  SD.

## 309 **RESULTS**

### 310 **Cycling exercise tolerance**

311           There was an effect of trial on cycling exercise duration at 85%  $\dot{W}_{peak}$  ( $273 \pm 26$  W)  
312 [ $F(2,14) = 16.8$ ,  $P < 0.001$ ], which was, as expected, identical ( $4.33 \pm 1.10$  min) during

313 ARM-CYC and ISOTIME and  $38 \pm 17\%$  shorter than CYC ( $7.46 \pm 2.79$  min) (mean  
314 difference =  $3.13 \pm 2.15$  min, 95% CI = 1.50 to 4.75 min,  $P < 0.001$ ). Cycling cadence at the  
315 termination of cycling exercise in CYC ( $68 \pm 6$  rpm, range: 61-78 rpm), ARM-CYC ( $66 \pm 5$   
316 rpm, range: 60-74 rpm) and ISOTIME ( $92 \pm 10$  rpm, range: 85-104 rpm) was always  $\geq 60$   
317 rpm. Therefore, cycling exercise during CYC and ARM-CYC was always performed to the  
318 limit of volitional tolerance rather than being terminated by the investigators.

### 319 **Neuromuscular function**

320 Baseline measures of neuromuscular function are shown in Table 1 and these were  
321 highly reproducible between trials. Raw traces of force from a representative participant at  
322 baseline performing a MVC with superimposed triplet, followed by twitch and triplet  
323 contractions, are shown in Figure 2. In all trials measures of neuromuscular function were  
324 unchanged from baseline to pre-cycling (data not shown). Thus arm-cranking *per se* did not  
325 result in central or peripheral locomotor muscle fatigue.

326 For MVF, there was a trial  $\times$  time interaction [ $F(4,28) = 6.2$ ,  $P < 0.001$ ] and an effect  
327 of time in CYC [ $F(2,14) = 14.3$ ,  $P < 0.001$ ], ARM-CYC [ $F(2,14) = 11.5$ ,  $P = 0.001$ ] and  
328 ISOTIME [ $F(2,14) = 8.5$ ,  $P = 0.003$ ]. MVF decreased from baseline to post-cycling in CYC  
329 (mean difference =  $95 \pm 70$  N, 95% CI = 45 to 145 N,  $P < 0.001$ ), ARM-CYC (mean  
330 difference =  $56 \pm 39$  N, 95% CI = 22 to 89 N,  $P = 0.002$ ) and ISOTIME (mean difference =  
331  $49 \pm 48$  N, 95% CI = 13 to 85 N,  $P = 0.008$ ). Furthermore, there was an effect of trial on the  
332 decrease in MVF [ $F(2,14) = 8.3$ ,  $P = 0.004$ ], which was greater in CYC than ARM-CYC  
333 (mean difference =  $39 \pm 38$  N, 95% CI = 7 to 71 N,  $P = 0.02$ ) and ISOTIME (mean difference  
334 =  $46 \pm 43$  N, 95% CI = 14 to 78 N,  $P = 0.005$ ) (Fig. 3A).

335 For voluntary activation, there was a trial  $\times$  time interaction [ $F(4,28) = 3.8, P = 0.013$ ]  
336 and an effect of time in CYC [ $F(2,14) = 8.0, P = 0.005$ ] and ARM-CYC [ $F(2,14) = 4.7, P =$   
337  $0.027$ ], but not ISOTIME [ $F(2,14) = 0.8, P = 0.46$ ]. Voluntary activation decreased from  
338 baseline (see Table 1) to post-cycling in CYC ( $89 \pm 9\%$ , mean difference =  $5.0 \pm 4.8\%$ , 95%  
339 CI = 1.4 to 8.7%,  $P = 0.012$ ) and ARM-CYC ( $91 \pm 8\%$ , mean difference =  $3.8 \pm 4.7\%$ , 95%  
340 CI = 0.1 to 7.4%,  $P = 0.047$ ). Furthermore, there was an effect of trial on the decrease in  
341 voluntary activation [ $F(2,14) = 5.2, P = 0.021$ ], which was greater in CYC than ISOTIME  
342 (mean difference =  $4.4 \pm 4.9\%$ , 95% CI = 0.7 to 8.0%,  $P = 0.019$ ) (Fig. 3B).

343 For potentiated twitch force, there was a trial  $\times$  time interaction [ $F(4,28) = 8.8, P <$   
344  $0.001$ ] and an effect of time in CYC [ $F(2,14) = 49.4, P < 0.001$ ], ARM-CYC [ $F(2,14) = 48.3,$   
345  $P < 0.001$ ], and ISOTIME [ $F(2,14) = 22.7, P < 0.001$ ]. Potentiated twitch force decreased  
346 from baseline to post-cycling in CYC (mean difference =  $77 \pm 30$  N, 95% CI = 55 to 98 N,  $P$   
347  $< 0.001$ ), ARM-CYC (mean difference =  $52 \pm 21$  N, 95% CI = 38 to 66 N,  $P < 0.001$ ) and  
348 ISOTIME (mean difference =  $50 \pm 24$  N, 95% CI = 30 to 70 N,  $P < 0.001$ ). Furthermore,  
349 there was an effect of trial on the decrease in potentiated twitch force [ $F(2,14) = 10.9, P =$   
350  $0.001$ ], which was greater in CYC than ARM-CYC (mean difference =  $25 \pm 17$  N, 95% CI =  
351 8 to 41 N,  $P = 0.004$ ) and ISOTIME (mean difference =  $27 \pm 22$  N, 95% CI = 10 to 43 N,  $P =$   
352  $0.003$ ) (Fig. 3C).

353 For potentiated triplet force, there was a trial  $\times$  time interaction [ $F(4,28) = 9.1, P <$   
354  $0.001$ ] and an effect of time in CYC [ $F(2,14) = 11.1, P = 0.001$ ], ARM-CYC [ $F(2,14) = 5.4,$   
355  $P = 0.018$ ], and ISOTIME [ $F(2,14) = 7.2, P = 0.007$ ]. Potentiated triplet force decreased from  
356 baseline to post-cycling in CYC (mean difference =  $63 \pm 50$  N, 95% CI = 26 to 100 N,  $P =$   
357  $0.001$ ), ARM-CYC (mean difference =  $37 \pm 40$  N, 95% CI = 7 to 66 N,  $P = 0.014$ ) and  
358 ISOTIME (mean difference =  $31 \pm 26$  N, 95% CI = 9 to 52 N,  $P < 0.001$ ). Furthermore, there

359 was an effect of trial on the decrease in potentiated triplet force [ $F(2,14) = 9.0, P = 0.003$ ],  
360 which was greater in CYC than ARM-CYC (mean difference =  $27 \pm 18$  N, 95% CI = 5 to 48  
361 N,  $P = 0.015$ ) and ISOTIME (mean difference =  $33 \pm 28$  N, 95% CI = 11 to 54 N,  $P = 0.004$ ).  
362 (Fig. 3D).

363 Quadriceps  $M_{\max}$  and neuromuscular activation (i.e. RMS EMG normalized to  $M_{\max}$ )  
364 at MVF remained unchanged in all trials.

### 365 **Leg muscle EMG during cycling**

366 Quadriceps EMG RMS during arm-cranking was  $\leq 3\%$  of the  $QEMG_{\max}$  during an  
367 MVC (data not shown), thus demonstrating minimal leg activation. For quadriceps  
368 neuromuscular activation (EMG RMS normalized to  $M_{\max}$ ) during cycling, there was a trial  $\times$   
369 time interaction [ $F(4,28) = 6.1, P = 0.001$ ] and an effect of time in CYC [ $F(2,14) = 36.3, P <$   
370  $0.001$ ], ARM-CYC [ $F(2,14) = 11.6, P = 0.001$ ] and ISOTIME [ $F(2,14) = 36.3, P = 0.012$ ].  
371 There was also an effect of trial on neuromuscular activation in the final minute of cycling  
372 [ $F(2,14) = 6.2, P = 0.012$ ], which was greater in CYC than ARM-CYC (mean difference =  
373  $0.76 \pm 0.84 \% M_{\max}$ , 95% CI = 0.03 to  $1.5 \% M_{\max}$ ,  $P = 0.040$ ) and ISOTIME (mean difference  
374 =  $0.91 \pm 0.87 \% M_{\max}$ , 95% CI = 0.18 to  $1.64 \% M_{\max}$ ,  $P = 0.014$ ). (Fig. 4). The absolute  
375 hamstrings EMG RMS remained constant during cycling and was not different between trials  
376 (pooled data:  $0.08 \pm 0.05$  mV).

### 377 **Heart rate and blood lactate concentration**

378 For heart rate, there was a trial  $\times$  time interaction [ $F(24,168) = 81.7, P < 0.001$ ] and  
379 an effect of trial on the mean of the eight heart rate measurements taken during the 11.5 min  
380 period of arm-cranking in ARM-CYC or seated rest in CYC and ISOTIME (see Fig. 1)  
381 [ $F(2,24) = 144.3, P < 0.001$ ]. The mean heart rate during this period was higher in ARM-



382 CYC ( $153 \pm 19$  bpm) than CYC ( $72 \pm 14$  bpm) (mean difference =  $81 \pm 20$  bpm, 95% CI =  
383 67 to 96 bpm,  $P < 0.001$ ) and ISOTIME ( $73 \pm 11$  bpm) (mean difference =  $80 \pm 16$  bpm, 95%  
384 CI = 66 to 95 bpm,  $P < 0.001$ ). There was also an effect of trial on heart rate measured pre-  
385 cycling [ $F(2,14) = 21.7$ ,  $P < 0.001$ ], after 3 min of cycling [ $F(2,14) = 17.8$ ,  $P < 0.001$ ], and  
386 post-cycling [ $F(2,14) = 12.3$ ,  $P < 0.001$ ]. Pre-cycling, heart rate was higher in ARM-CYC  
387 than CYC (mean difference =  $34 \pm 21$  bpm, 95% CI = 18 to 49 bpm,  $P < 0.001$ ) and  
388 ISOTIME (mean difference =  $34 \pm 15$  bpm, 95% CI = 18 to 49 bpm,  $P < 0.001$ ). After 3 min  
389 of cycling, heart rate was higher in ARM-CYC than CYC and ISOTIME (mean difference  
390 from both trials =  $10 \pm 6$  bpm, 95% CI = 5 to 15 bpm,  $P < 0.001$ ). Post-cycling, heart rate  
391 was lower in ISOTIME than CYC (mean difference =  $10 \pm 6$  bpm, 95% CI = 3 to 16 bpm,  $P$   
392 = 0.005) and ARM-CYC (mean difference =  $12 \pm 8$  bpm, 95% CI = 5 to 18 bpm,  $P = 0.001$ )  
393 (Fig. 5A).

394 For  $[La^-]_B$ , there was a trial  $\times$  time interaction [ $F(6,42) = 79.7$ ,  $P < 0.001$ ] and an  
395 effect of trial on  $[La^-]_B$  measured immediately after the period of arm-cranking in ARM-CYC  
396 or seated rest in CYC and ISOTIME [ $F(2,14) = 167.2$ ,  $P < 0.001$ ]. Immediately after this  
397 period,  $[La^-]_B$  was higher in ARM-CYC than CYC and ISOTIME (mean difference from both  
398 trials =  $10.3 \pm 2.2$  mmol $\cdot$ L $^{-1}$ , 95% CI = 8.6 to 12.0 mmol $\cdot$ L $^{-1}$ ,  $P < 0.001$ ). There was also an  
399 effect of trial on  $[La^-]_B$  measured pre-cycling [ $F(2,14) = 158.2$ ,  $P < 0.001$ ], which was higher  
400 in ARM-CYC than CYC (mean difference =  $8.5 \pm 2.0$  mmol $\cdot$ L $^{-1}$ , 95% CI = 7.0 to 10.0  
401 mmol $\cdot$ L $^{-1}$ ,  $P < 0.001$ ) and ISOTIME (mean difference =  $8.6 \pm 1.8$  mmol $\cdot$ L $^{-1}$ , 95% CI = 7.2 to  
402 10.1 mmol $\cdot$ L $^{-1}$ ,  $P < 0.001$ ). Furthermore, there was an effect of trial on  $[La^-]_B$  measured post-  
403 cycling [ $F(2,14) = 31.9$ ,  $P < 0.001$ ], which was higher in ARM-CYC than CYC (mean  
404 difference =  $2.3 \pm 1.0$  mmol $\cdot$ L $^{-1}$ , 95% CI = 0.8 to 3.9 mmol $\cdot$ L $^{-1}$ ,  $P = 0.003$ ) and ISOTIME  
405 (mean difference =  $4.6 \pm 1.8$  mmol $\cdot$ L $^{-1}$ , 95% CI = 3.1 to 6.1 mmol $\cdot$ L $^{-1}$ ,  $P < 0.001$ ). Post-

406 cycling,  $[La^-]_B$  was also higher in CYC than ISOTIME (mean difference =  $2.3 \pm 2.0$  mmol·L<sup>-1</sup>,  
407 95% CI = 0.8 to 3.8 mmol·L<sup>-1</sup>,  $P = 0.004$ ) (Fig. 5B).

#### 408 **Rating of perceived exertion and dyspnea**

409 There was a trial × time interaction for RPE [ $F(4,28) = 14.7$ ,  $P < 0.001$ ] and an effect  
410 of trial on RPE measured after 3 min of cycling [ $F(2,14) = 11.7$ ,  $P = 0.001$ ], which was  
411 higher in ARM-CYC than CYC (mean difference =  $2.4 \pm 1.7$  AU, 95% CI = 1.0 to 3.9 AU,  $P$   
412 = 0.002) and ISOTIME (mean difference =  $2.3 \pm 1.9$  AU, 95% CI = 0.8 to 3.8 AU,  $P =$   
413 0.003). There was also an effect of trial on RPE measured post-cycling [ $F(2,14) = 18.4$ ,  $P <$   
414 0.001], which was lower in ISOTIME than CYC (mean difference =  $3.3 \pm 1.9$  AU, 95% CI =  
415 1.4 to 5.2 AU,  $P = 0.001$ ) and ARM-CYC (mean difference =  $4.1 \pm 2.6$  AU, 95% CI = 2.2 to  
416 5.9 AU,  $P < 0.001$ ) (Fig. 6A).

417 There was a trial × time interaction for dyspnea [ $F(4,28) = 5.8$ ,  $P < 0.001$ ] and an  
418 effect of trial on dyspnea measured after 3 min of cycling [ $F(2,14) = 16.3$ ,  $P < 0.001$ ], which  
419 was higher in ARM-CYC than CYC (mean difference =  $1.9 \pm 1.4$  AU, 95% CI = 0.9 to 3.0  
420 AU,  $P < 0.001$ ) and ISOTIME (mean difference =  $2.1 \pm 1.1$  AU, 95% CI = 1.0 to 3.2 AU,  $P <$   
421 0.001). There was also a main effect of trial on dyspnea measured post-cycling [ $F(2,14) =$   
422 11.8,  $P = 0.001$ ], which was lower in ISOTIME than CYC (mean difference =  $2.8 \pm 2.4$  AU,  
423 95% CI = 0.9 to 4.6 AU,  $P = 0.004$ ) and ARM-CYC (mean difference =  $3.1 \pm 2.6$  AU, 95%  
424 CI = 1.3 to 5.0 AU,  $P = 0.002$ ) (Fig. 6B).

425 There was an effect of trial on  $\Delta RPE/\Delta time$  [ $F(2,14) = 11.7$ ,  $P = 0.001$ ], which was  
426 higher in ARM-CYC than CYC (mean difference =  $0.72 \pm 0.63$  AU·min<sup>-1</sup>, 95% CI = 0.25 to  
427 1.21 AU·min<sup>-1</sup>,  $P = 0.003$ ) and ISOTIME (mean difference =  $0.79 \pm 0.55$  AU·min<sup>-1</sup>, 95% CI  
428 = 0.31 to 1.27 AU·min<sup>-1</sup>,  $P = 0.002$ ) (Table 2). There was also an effect of trial on

429  $\Delta$ dyspnea/ $\Delta$ time [ $F(2,14) = 4.5, P = 0.031$ ], which was higher in ARM-CYC than ISOTIME  
430 (mean difference =  $0.46 \pm 0.58 \text{ AU}\cdot\text{min}^{-1}$ , 95% CI = 0.02 to  $0.90 \text{ AU}\cdot\text{min}^{-1}$ ,  $P = 0.038$ ) (Table  
431 2).

432 There was an effect of trial on  $\Delta$ RPE/ $\Delta$ %time [ $F(2,14) = 19.1, P < 0.001$ ] (Fig. 6C  
433 and Table 2), which was lower in ISOTIME than CYC (mean difference =  $0.03 \pm 0.01$   
434  $\text{AU}\cdot\%\text{time}^{-1}$ , 95% CI = 0.01 to  $0.05 \text{ AU}\cdot\%\text{time}^{-1}$ ,  $P < 0.001$ ) and ARM-CYC (mean  
435 difference =  $0.04 \pm 0.02 \text{ AU}\cdot\%\text{time}^{-1}$ , 95% CI = 0.02 to  $0.05 \text{ AU}\cdot\%\text{time}^{-1}$ ,  $P < 0.001$ ). There  
436 was also an effect of trial on  $\Delta$ dyspnea/ $\Delta$ %time [ $F(2,14) = 7.5, P = 0.006$ ] (Fig. 6D and Table  
437 2), which was lower in ISOTIME than CYC (mean difference =  $0.03 \pm 0.01 \text{ AU}\cdot\%\text{time}^{-1}$ , 95%  
438 CI = 0.01 to  $0.05 \text{ AU}\cdot\%\text{time}^{-1}$ ,  $P = 0.006$ ) and ARM-CYC (mean difference =  $0.02 \pm 0.01$   
439  $\text{AU}\cdot\%\text{time}^{-1}$ , 95% CI = 0.001 to  $0.04 \text{ AU}\cdot\%\text{time}^{-1}$ ,  $P = 0.036$ ).

440 When data from CYC and ARM-CYC were pooled,  $\Delta$ RPE/ $\Delta$ time was negatively  
441 correlated with the time to the limit of cycling exercise tolerance ( $r = -0.74, P = 0.001$ ).  
442 Furthermore, the reduction in cycling exercise tolerance during ARM-CYC compared with  
443 CYC was negatively correlated with the increases in  $\Delta$ RPE/ $\Delta$ time ( $r = -0.72, P = 0.045$ ) and  
444  $\Delta$ dyspnea/ $\Delta$ time ( $r = -0.80, P = 0.018$ ).

## 445 DISCUSSION

446 The present study examined the effects of prior high-intensity upper body exercise on  
447 subsequent high-intensity leg cycling exercise tolerance and associated changes in  
448 neuromuscular function and perceptual responses. Our main findings were threefold: (I) prior  
449 upper body exercise in ARM-CYC reduced subsequent cycling exercise tolerance by 38%; (II)  
450 the reduced cycling exercise tolerance in ARM-CYC was associated with less peripheral  
451 muscle fatigue incurred but a similar reduction in voluntary activation compared with CYC;

452 and (III) the reduced cycling exercise tolerance in ARM-CYC was related to increases in  
453  $\Delta RPE/\Delta time$  and  $\Delta dyspnea/\Delta time$ . These findings suggest that exercise tolerance is not  
454 regulated by a critical level of peripheral fatigue. Instead, central fatigue and an exacerbation  
455 of perceptual responses are the potential mechanisms underlying the reduced cycling exercise  
456 tolerance after prior upper body exercise.

457 We recently showed that high-intensity cycling exercise tolerance was reduced by a  
458 strikingly similar extent after an identical upper body exercise protocol (46). Several authors  
459 suggest that reduced lower limb exercise tolerance after prior upper body exercise occurs  
460 because of accelerated development of peripheral fatigue caused by greater intramuscular  
461 metabolic perturbation (12, 17, 36, 44, 46, 61). This notion is supported, indirectly, by the  
462 observation that prior high-intensity upper body exercise elevated leg muscle  $[La^-]$  and  $[H^+]$   
463 at the onset of isolated knee extensor exercise (11, 12), accelerated the exercise-induced  
464 increase in interstitial  $[K^+]$  (61), and reduced exercise tolerance (12, 61). However, although  
465 such metabolite accumulation has been implicated in the etiology of peripheral fatigue (21,  
466 33), previous prior upper body exercise studies did not measure peripheral fatigue or  
467 neuromuscular activation. Comparisons of our work with isolated knee extensor exercise  
468 studies are also complicated by the task-specificity of fatigue etiology (13, 74). Two  
469 observations from the present study suggest that peripheral fatigue during cycling exercise  
470 was not accelerated by prior upper body exercise. Firstly, the extent of peripheral fatigue in  
471 ARM-CYC and ISOTIME was the same even though there was considerable systemic  
472 metabolic perturbation in ARM-CYC. Indeed, in our previous study the same upper body  
473 exercise protocol reduced the strong ion difference by 15%, increased plasma  $[H^+]$  by 33%,  
474 reduced  $[HCO_3^-]$  by 29%, and accelerated the increase in plasma  $[K^+]$  during subsequent  
475 cycling exercise by 56% (46). Secondly, if peripheral fatigue during cycling exercise was  
476 accelerated this would be expected to result in greater neuromuscular activation (i.e.

477 increased motor unit recruitment and/or firing frequency) to compensate for the reduced force  
478 generating capacity (14, 30); however, this was not observed. Collectively, these observations  
479 therefore suggest that systemic metabolite perturbation plays a minor role in peripheral  
480 fatigue generation.

481         Our findings contrast previous cycling exercise studies in which moderate hypoxia (7,  
482 66), superimposed inspiratory muscle loading (67), volitionally-induced inspiratory or  
483 expiratory muscle fatigue (73, 80), prior high-intensity cycling exercise (5), and prior  
484 electrically-induced quadriceps muscle fatigue (34), reduced exercise tolerance but resulted  
485 in the same degree of peripheral fatigue incurred compared with control conditions. These  
486 observations are taken as evidence for inhibitory group III/IV muscle afferent feedback to the  
487 central nervous system regulating central motor drive to confine the development of  
488 peripheral fatigue to a critical threshold (3, 31). However, although the 38% reduction in  
489 twitch force after CYC is comparable to the proposed critical threshold of peripheral fatigue  
490 previously reported after high-intensity fixed work-rate cycling exercise (4, 5, 66, 73, 80),  
491 this degree of peripheral fatigue was not reached during ARM-CYC (26% reduction in twitch  
492 force). This finding is similar to the observation of less peripheral fatigue incurred after high-  
493 intensity cycling exercise in severe hypoxia ( $F_{I}O_2$  0.10) compared with normoxia (8). The  
494 notion that peripheral fatigue is not critically regulated is also supported by two recent  
495 isolated muscle studies: Rossman et al. (68) observed greater quadriceps muscle fatigue  
496 during single-leg compared with double-leg knee extensor exercise, whereas Amann et al. (10)  
497 observed less quadriceps muscle fatigue during single-leg knee extensor exercise after  
498 fatiguing knee extensor exercise with the contralateral leg. The present study thus extends  
499 these observations to whole-body exercise by providing novel evidence that peripheral  
500 fatigue is not independently regulated during high-intensity fixed work-rate cycling exercise  
501 to volitional tolerance.

502 Whether peripheral fatigue plays an important role in governing exercise tolerance  
503 remains controversial (9, 53, 54, 60). Consistent with previous observations (5, 7, 54, 71, 75),  
504 submaximal quadriceps muscle recruitment was observed at the limit of exercise tolerance in  
505 CYC and ARM-CYC (~55% and 50%, respectively, of the  $QEMG_{max}$ ) and Noakes (58)  
506 argues that this negates peripheral fatigue as the single limiting factor to exercise tolerance.  
507 Furthermore, Decorte et al. (30) have shown that peripheral fatigue during cycling exercise at  
508 80%  $W_{peak}$  develops mostly during the first half of the test, such that the limit of tolerance  
509 approaches without further peripheral fatigue, but with a significant reduction in voluntary  
510 activation. The similar reduction in voluntary activation after CYC and ARM-CYC indicates  
511 that central fatigue developed more quickly in ARM-CYC, possibly due to a ‘spill-over’ of  
512 central fatigue from the exercised upper body muscles to the leg locomotor muscles. In  
513 support, it was recently demonstrated that fatiguing leg cycling exercise resulted in a ‘spill-  
514 over’ of central fatigue (i.e. reduced voluntary activation) to the remote unexercised elbow  
515 flexors (69). This effect was attributed to inhibitory group III/IV muscle afferent feedback  
516 originating in fatigued leg muscle since attenuating this feedback using intrathecal fentanyl  
517 abolished the decline in voluntary activation of the elbow flexors. Whether a fall in voluntary  
518 activation limits cycling exercise that is characterized by submaximal muscle contractions  
519 remains uncertain (74). However, it is also recognized that a limiting influence of central  
520 fatigue on exercise tolerance may be manifest by changes in sensory perception (57, 74).

521 The conscious perception of fatigue is thought to reflect the complex integration and  
522 interpretation of central motor drive and an associated corollary discharge, somatosensory  
523 feedback (4, 50, 70, 78), and cognitive functions such as motivation and emotional state (70).  
524 After 3 min of cycling, RPE for leg discomfort was greater in ARM-CYC compared with  
525 CYC and ISOTIME despite similar levels of quadriceps neuromuscular activation, which  
526 supports observations made during single-leg knee extensor exercise after fatiguing knee

527 extensor exercise with the contralateral leg (10). Furthermore, at the end of cycling, RPE was  
528 greater in ARM-CYC compared with ISOTIME despite similar levels of peripheral fatigue  
529 incurred, whereas RPE was similar at the end of cycling in CYC and ARM-CYC despite less  
530 peripheral fatigue incurred during ARM-CYC. Collectively, our findings suggest that the  
531 perception of leg discomfort during cycling exercise does not exclusively reflect the extent of  
532 quadriceps neuromuscular activation or degree of peripheral fatigue incurred. Similar  
533 observations have been made in COPD patients who sometimes stop exercise because of leg  
534 discomfort and in the absence of quadriceps muscle fatigue (52). These observations suggest  
535 that the conscious perception of leg discomfort likely reflects a complex interplay between  
536 multiple factors other than peripheral fatigue and neuromuscular activation (70). Minute  
537 ventilation was not measured in the present study and thus it cannot be ruled out that the  
538 greater  $\Delta$ dyspnea/ $\Delta$ time during ARM-CYC resulted, in part, from a greater ventilatory  
539 response (10). However, afferents involved in the perception of dyspnea and limb discomfort  
540 project to the same sensorimotor brain areas (62) and, therefore, a heightened level of one  
541 perception may potentiate the other. In support, quadriceps fatigue induced by sustained  
542 contractions increased dyspnea during a subsequent inspiratory loaded breathing challenge  
543 without affecting breathing pattern or pleural pressure swings (37). Thus although we could  
544 not elucidate the precise causative mechanism(s), we propose that the greater  $\Delta$ RPE/ $\Delta$ time  
545 and  $\Delta$ dyspnea/ $\Delta$ time during ARM-CYC reflects, in part, greater ensemble group III/IV  
546 afferent projections to integrated sensorimotor brain structures due to cycling commencing  
547 with pre-existing afferent input originating from the previously exercised respiratory (50) and  
548 upper body musculature (10, 25, 45), lungs (50), and heart (78). During cycling exercise the  
549 pre-existing afferent input would have been added to the prevailing inputs related to central  
550 motor drive, and locomotor muscle (10) and cardiorespiratory (50, 78) activity, thereby  
551 accelerating the increase in perceptual responses and reducing exercise tolerance. The

552 correlation between increased  $\Delta\text{RPE}/\Delta\text{time}$  and  $\Delta\text{dyspnea}/\Delta\text{time}$  and reduced cycling  
553 exercise tolerance in ARM-CYC also supports sensory perception as an important mediator  
554 of exercise tolerance. Our findings are therefore consistent with the ‘flush model’ proposed  
555 by Millet (57), which suggests that exercise tolerance is mediated primarily by  $\Delta\text{RPE}/\Delta\text{time}$   
556 which, in turn, depends mainly on feedback (i.e. peripheral) and feed-forward (i.e. central)  
557 mechanisms.

558         The greater  $\Delta\text{RPE}/\Delta\text{time}$  and  $\Delta\text{dyspnea}/\Delta\text{time}$  during ARM-CYC compared with  
559 CYC, but similar  $\Delta\text{RPE}/\Delta\%time$  and  $\Delta\text{dyspnea}/\Delta\%time$ , suggests that the pre-existing  
560 afferent input at the onset of cycling in ARM-CYC affected perceptual responses by  
561 increasing their gain. Similar effects on the absolute and normalized RPE are observed when  
562 exercise tolerance is reduced by muscle glycogen depletion (59), warm and cold ambient  
563 temperatures (24), and prior fatiguing activity using the same muscle groups (32). These  
564 observations underpin the notion that perceptual responses are set in anticipation, otherwise  
565 known as teleoanticipation (76), so that exercise terminates at a critical sensory tolerance  
566 limit (32, 58, 60, 75). By limiting exercise tolerance the sensory tolerance limit will, therefore,  
567 also mediate the degree of peripheral fatigue incurred, which is consistent with the findings  
568 of recent studies using the isolated knee extensor exercise model (10, 68). We note, however,  
569 that the limit of cycling exercise tolerance during CYC and ARM-CYC was sometimes  
570 associated with submaximal RPE and dyspnea, suggesting that additional influences, such as  
571 psychological factors (15, 54), were also mediating the limit of exercise tolerance.

572         In conclusion, reductions in cycling exercise tolerance due to prior upper body  
573 exercise are associated with an acceleration of central fatigue and greater perceptual  
574 responses rather than an accelerated development of peripheral fatigue. These findings  
575 suggest that peripheral fatigue is not independently regulated during high-intensity fixed  
576 work-rate cycling exercise to volitional tolerance, and that exercise tolerance, and thus the



577 degree of peripheral fatigue incurred, is potentially determined by intolerable levels of  
578 sensory perception.

579

580 **ACKNOWLEDGEMENTS**

581 None.

582 **GRANTS**

583 No funding was received for this research.

584 **DISCLOSURES**

585 The authors report no conflicts of interest.

586

587

588

589

590

591

592

593

594

595

- 597 1. **Adreani CM, Hill JM and Kaufman MP.** Responses of group III and IV muscle  
598 afferents to dynamic exercise. *J Appl Physiol* 82: 1811-1817, 1997.
- 599 2. **Altman DG and Gardner MJ.** Means and their differences. In: *Statistics with Confidence*,  
600 edited by Altman DG, Machin D, Bryant TN and Gardner MJ. London: BMJ Books, 2000, p.  
601 28-35.
- 602 3. **Amann M.** Central and peripheral fatigue: interaction during cycling exercise in humans.  
603 *Med Sci Sports Exerc* 43: 2039-2045, 2011.
- 604 4. **Amann M, Blain GM, Proctor LT, Sebranek JJ, Pegelow DF and Dempsey JA.**  
605 Implications of group III and IV muscle afferents for high-intensity endurance exercise  
606 performance in humans. *J Physiol* 589: 5299-5309, 2011.
- 607 5. **Amann M and Dempsey JA.** Locomotor muscle fatigue modifies central motor drive in  
608 healthy humans and imposes a limitation to exercise performance. *J Physiol* 586: 161-173,  
609 2008.
- 610 6. **Amann M, Proctor LT, Sebranek JJ, Pegelow DF and Dempsey JA.** Opioid-mediated  
611 muscle afferents inhibit central motor drive and limit peripheral muscle fatigue development  
612 in humans. *J Physiol* 587: 271-283, 2009.
- 613 7. **Amann M, Romer LM, Pegelow DF, Jacques AJ, Hess CJ and Dempsey JA.** Effects of  
614 arterial oxygen content on peripheral locomotor muscle fatigue. *J Appl Physiol* 101: 119-127,  
615 2006.
- 616 8. **Amann M, Romer LM, Subudhi AW, Pegelow DF and Dempsey JA.** Severity of  
617 arterial hypoxaemia affects the relative contributions of peripheral muscle fatigue to exercise  
618 performance in healthy humans. *J Physiol* 581: 389-403, 2007.
- 619 9. **Amann M and Secher NH.** Point: Afferent feedback from fatigued locomotor muscles is  
620 an important determinant of endurance exercise performance. *J Appl Physiol* 108: 452-454,  
621 2010.
- 622 10. **Amann M, Venturelli M, Ives SJ, McDaniel J, Layec G, Rossman MJ and**  
623 **Richardson RS.** Peripheral fatigue limits endurance exercise via a sensory feedback-  
624 mediated reduction in spinal motoneuronal output. *J Appl Physiol* 115: 355-364, 2013.
- 625 11. **Bangsbo J, Aagaard T, Olsen M, Kiens B, Turcotte LP and Richter EA.** Lactate and  
626 H<sup>+</sup> uptake in inactive muscles during intense exercise in man. *J Physiol* 488: 219-229, 1995.
- 627 12. **Bangsbo J, Madsen K, Kiens B and Richter EA.** Effect of muscle acidity on muscle  
628 metabolism and fatigue during intense exercise in man. *J Physiol* 495: 587-596, 1996.
- 629 13. **Barry BK and Enoka RM.** The neurobiology of muscle fatigue: 15 years later. *Integr*  
630 *Comp Biol* 47: 465-473, 2007.
- 631 14. **Bigland-Ritchie BR, Dawson NJ, Johansson RS and Lippold OC.** Reflex origin for  
632 the slowing of motoneurone firing rates in fatigue of human voluntary contractions. *J Physiol*  
633 379: 451-459, 1986.
- 634 15. **Blanchfield AW, Hardy J, De Morree HM, Staiano W and Marcora SM.** Talking  
635 yourself out of exhaustion: the effects of self-talk on endurance performance. *Med Sci Sports*  
636 *Exerc* 46: 998-1007, 2014.
- 637 16. **Bland JM and Altman DG.** Measurement error. *BMJ* 313: 744, 1996.
- 638 17. **Bogdanis GC, Nevill ME and Lakomy HK.** Effects of previous dynamic arm exercise  
639 on power output during repeated maximal sprint cycling. *J Sports Sci* 12: 363-370, 1994.
- 640 18. **Borg G.** *Borg's Perceived Exertion and Pain Scales*. IL: Human Kinetics, 1998.
- 641 19. **Buckthorpe MW, Hannah R, Pain TG and Folland JP.** Reliability of neuromuscular  
642 measurements during explosive isometric contractions, with special reference to  
643 electromyography normalization techniques. *Muscle Nerve* 46: 566-576, 2012.

- 644 20. **Burnley M, Vanhatalo A, Fulford J and Jones AM.** Similar metabolic perturbations  
645 during all-out and constant force exhaustive exercise in humans: a <sup>31</sup>P magnetic resonance  
646 spectroscopy study. *Exp Physiol* 95: 798-807, 2010.
- 647 21. **Cairns SP and Lindinger MI.** Do multiple ionic interactions contribute to skeletal  
648 muscle fatigue? *J Physiol* 586: 4039-4054, 2008.
- 649 22. **Chapman AR, Vicenzino B, Blanch P and Hodges PW.** Patterns of leg muscle  
650 recruitment vary between novice and highly trained cyclists. *J Electromyogr Kinesiol* 18:  
651 359-371, 2008.
- 652 23. **Chapman AR, Vicenzino B, Blanch P, Knox JJ and Hodges PW.** Leg muscle  
653 recruitment in highly trained cyclists. *J Sports Sci* 24: 115-124, 2006.
- 654 24. **Crewe H, Tucker R and Noakes TD.** The rate of increase in rating of perceived exertion  
655 predicts the duration of exercise to fatigue at a fixed power output in different environmental  
656 conditions. *Eur J Appl Physiol* 103: 569-577, 2008.
- 657 25. **Darques JL, Decherchi P and Jammes Y.** Mechanisms of fatigue-induced activation of  
658 group IV muscle afferents: the roles played by lactic acid and inflammatory mediators.  
659 *Neurosci Lett* 257: 109-112, 1998.
- 660 26. **De Luca CJ.** The use of electromyography in biomechanics. *J Appl Biomech* 13: 135-163,  
661 1997.
- 662 27. **De Luca CJ, Gilmore LD, Kuznetsov M and Roy SH.** Filtering the surface EMG signal:  
663 Movement artifact and baseline noise contamination. *J Biomech* 43: 1573-1579, 2010.
- 664 28. **de Ruiter CJ, Hoddenbach JG, Huurnink A and de Haan A.** Relative torque  
665 contribution of vastus medialis muscle at different knee angles. *Acta Physiol* 194: 223-237,  
666 2008.
- 667 29. **de Ruiter CJ, Kooistra RD, Paalman MI and de Haan A.** Initial phase of maximal  
668 voluntary and electrically stimulated knee extension torque development at different knee  
669 angles. *J Appl Physiol* 97: 1693-1701, 2004.
- 670 30. **Decorte N, Lafaix PA, Millet GY, Wuyam B and Verges S.** Central and peripheral  
671 fatigue kinetics during exhaustive constant-load cycling. *Scand J Med Sci Sports* 22: 381-391,  
672 2012.
- 673 31. **Dempsey JA.** New perspectives concerning feedback influences on cardiorespiratory  
674 control during rhythmic exercise and on exercise performance. *J Physiol* 590: 4129-4144,  
675 2012.
- 676 32. **Eston R, Faulkner J, St Clair Gibson A, Noakes T and Parfitt G.** The effect of  
677 antecedent fatiguing activity on the relationship between perceived exertion and  
678 physiological activity during a constant load exercise task. *Psychophysiology* 44: 779-786,  
679 2007.
- 680 33. **Fitts RH.** The cross-bridge cycle and skeletal muscle fatigue. *J Appl Physiol* 104: 551-  
681 558, 2008.
- 682 34. **Gagnon P, Saey D, Vivodtzev I, Laviolette L, Mainguy V, Milot J, Provencher S and**  
683 **Maltais F.** Impact of preinduced quadriceps fatigue on exercise response in chronic  
684 obstructive pulmonary disease and healthy subjects. *J Appl Physiol* 107: 832-840, 2009.
- 685 35. **Gandevia SC.** Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* 81:  
686 1725-1789, 2001.
- 687 36. **Grant MC, Robergs R, Baird MF and Baker JS.** The effect of prior upper body  
688 exercise on subsequent wingate performance. *Biomed Res Int* 2014: 329328, 2014.
- 689 37. **Grippo A, Carrai R, Chiti L, Bruni GI, Scano G and Duranti R.** Effect of limb  
690 muscle fatigue on perception of respiratory effort in healthy subjects. *J Appl Physiol* 109:  
691 367-376, 2010.
- 692 38. **Hannah R, Minshull C, Buckthorpe MW and Folland JP.** Explosive neuromuscular  
693 performance of males versus females. *Exp Physiol* 97: 618-629, 2012.

- 694 39. **Hannah R, Minshull C and Folland JP.** Whole-body vibration does not influence knee  
695 joint neuromuscular function or proprioception. *Scand J Med Sci Sports* 23: 96-104, 2013.
- 696 40. **Hannah R, Minshull C, Smith SL and Folland JP.** Longer electromechanical delay  
697 impairs hamstrings explosive force versus quadriceps. *Med Sci Sports Exerc* 46: 963-972,  
698 2014.
- 699 41. **Hannah R, Stannard RL, Minshull C, Artioli GG, Harris RC and Sale C.** beta-  
700 Alanine supplementation enhances human skeletal muscle relaxation speed but not force  
701 production capacity. *J Appl Physiol* 118: 604-612, 2015.
- 702 42. **Hodges PW and Bui BH.** A comparison of computer-based methods for the  
703 determination of onset of muscle contraction using electromyography. *Electroencephalogr  
704 Clin Neurophysiol* 101: 511-519, 1996.
- 705 43. **Hopkins WG.** Measures of reliability in sports medicine and science. *Sports Med* 30: 1-  
706 15, 2000.
- 707 44. **Jacobs I, Hermiston AJ and Symons JD.** Effects of prior exercise or ammonium  
708 chloride ingestion on muscular strength and endurance. *Med Sci Sports Exerc* 25: 809-814,  
709 1993.
- 710 45. **Jammes Y and Balzamo E.** Changes in afferent and efferent phrenic activities with  
711 electrically induced diaphragmatic fatigue. *J Appl Physiol* 73: 894-902, 1992.
- 712 46. **Johnson MA, Mills DE, Brown PI and Sharpe GR.** Prior upper body exercise reduces  
713 cycling work capacity but not critical power. *Med Sci Sports Exerc* 46: 802-808, 2014.
- 714 47. **Karlsson J, Bonde-Petersen F, Henriksson J and Knuttgen HG.** Effects of previous  
715 exercise with arms or legs on metabolism and performance in exhaustive exercise. *J Appl  
716 Physiol* 38: 763-767, 1975.
- 717 48. **Kooistra RD, de Ruiter CJ and de Haan A.** Conventionally assessed voluntary  
718 activation does not represent relative voluntary torque production. *Eur J Appl Physiol* 100:  
719 309-320, 2007.
- 720 49. **Kufel TJ, Pineda LA and Mador MJ.** Comparison of potentiated and unpotentiated  
721 twitches as an index of muscle fatigue. *Muscle Nerve* 25: 438-444, 2002.
- 722 50. **Laviolette L, Laveneziana P and ERS Research Seminar Faculty.** Dyspnoea: a  
723 multidimensional and multidisciplinary approach. *Eur Respir J* 43: 1750-1762, 2014.
- 724 51. **Light AR, Hughen RW, Zhang J, Rainier J, Liu Z and Lee J.** Dorsal root ganglion  
725 neurons innervating skeletal muscle respond to physiological combinations of protons, ATP,  
726 and lactate mediated by ASIC, P2X, and TRPV1. *J Neurophysiol* 100: 1184-1201, 2008.
- 727 52. **Mador MJ, Kufel TJ and Pineda L.** Quadriceps fatigue after cycle exercise in patients  
728 with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 161: 447-453, 2000.
- 729 53. **Marcora S.** Counterpoint: Afferent feedback from fatigued locomotor muscles is not an  
730 important determinant of endurance exercise performance. *J Appl Physiol* 108: 454-456,  
731 2010.
- 732 54. **Marcora SM and Staiano W.** The limit to exercise tolerance in humans: mind over  
733 muscle? *Eur J Appl Physiol* 109: 763-770, 2010.
- 734 55. **Merletti R and di Torino P.** Standards for reporting EMG data. *J Electromyogr Kinesiol*  
735 9: III-IV, 1999.
- 736 56. **Merton PA.** Voluntary strength and fatigue. *J Physiol* 123: 553-564, 1954.
- 737 57. **Millet GY.** Can neuromuscular fatigue explain running strategies and performance in  
738 ultra-marathons?: the flush model. *Sports Med* 41: 489-506, 2011.
- 739 58. **Noakes TD.** Fatigue is a Brain-Derived Emotion that Regulates the Exercise Behavior to  
740 Ensure the Protection of Whole Body Homeostasis. *Front Physiol* 3: 82, 2012.
- 741 59. **Noakes TD.** Linear relationship between the perception of effort and the duration of  
742 constant load exercise that remains. *J Appl Physiol* 96: 1571-1572, 2004.

- 743 60. **Noakes TD and Marino FE.** Arterial oxygenation, central motor output and exercise  
744 performance in humans. *J Physiol* 585: 919-921, 2007.
- 745 61. **Nordsborg N, Mohr M, Pedersen LD, Nielsen JJ, Langberg H and Bangsbo J.**  
746 Muscle interstitial potassium kinetics during intense exhaustive exercise: effect of previous  
747 arm exercise. *Am J Physiol Regul Integr Comp Physiol* 285: R143-R148, 2003.
- 748 62. **O'Donnell DE, Banzett RB, Carrieri-Kohlman V, Casaburi R, Davenport PW,**  
749 **Gandevia SC, Gelb AF, Mahler DA and Webb KA.** Pathophysiology of dyspnea in  
750 chronic obstructive pulmonary disease: a roundtable. *Proc Am Thorac Soc* 4: 145-168, 2007.
- 751 63. **Oskouei MA, Van Mazijk BC, Schuiling MH and Herzog W.** Variability in the  
752 interpolated twitch torque for maximal and submaximal voluntary contractions. *J Appl*  
753 *Physiol* 95: 1648-1655, 2003.
- 754 64. **Pires FO, Noakes TD, Lima-Silva AE, Bertuzzi R, Ugrinowitsch C, Lira FS and Kiss**  
755 **MA.** Cardiopulmonary, blood metabolite and rating of perceived exertion responses to  
756 constant exercises performed at different intensities until exhaustion. *Br J Sports Med* 45:  
757 1119-1125, 2011.
- 758 65. **Rainoldi A, Melchiorri G and Caruso I.** A method for positioning electrodes during  
759 surface EMG recordings in lower limb muscles. *J Neurosci Methods* 134: 37-43, 2004.
- 760 66. **Romer LM, Haverkamp HC, Amann M, Lovering AT, Pegelow DF and Dempsey JA.**  
761 Effect of acute severe hypoxia on peripheral fatigue and endurance capacity in healthy  
762 humans. *Am J Physiol Regul Integr Comp Physiol* 292: R598-R606, 2007.
- 763 67. **Romer LM, Lovering AT, Haverkamp HC, Pegelow DF and Dempsey JA.** Effect of  
764 inspiratory muscle work on peripheral fatigue of locomotor muscles in healthy humans. *J*  
765 *Physiol* 571: 425-439, 2006.
- 766 68. **Rossmann MJ, Garten RS, Venturelli M, Amann M and Richardson RS.** The role of  
767 active muscle mass in determining the magnitude of peripheral fatigue during dynamic  
768 exercise. *Am J Physiol Regul Integr Comp Physiol* 306: R934-R940, 2014.
- 769 69. **Sidhu SK, Weavil JC, Venturelli M, Garten RS, Rossmann MJ, Richardson RS,**  
770 **Gmelch BS, Morgan DE and Amann M.** Spinal mu-opioid receptor-sensitive lower limb  
771 muscle afferents determine corticospinal responsiveness and promote central fatigue in upper  
772 limb muscle. *J Physiol* 592: 5011-5024, 2014.
- 773 70. **St Clair Gibson A, Baden DA, Lambert MI, Lambert EV, Harley YX, Hampson D,**  
774 **Russell VA and Noakes TD.** The conscious perception of the sensation of fatigue. *Sports*  
775 *Med* 33: 167-176, 2003.
- 776 71. **St Clair Gibson A, Schabert EJ and Noakes TD.** Reduced neuromuscular activity and  
777 force generation during prolonged cycling. *Am J Physiol Regul Integr Comp Physiol* 281:  
778 R187-R196, 2001.
- 779 72. **Suter E and Herzog W.** Effect of number of stimuli and timing of twitch application on  
780 variability in interpolated twitch torque. *J Appl Physiol* 90: 1036-1040, 2001.
- 781 73. **Taylor BJ and Romer LM.** Effect of expiratory muscle fatigue on exercise tolerance and  
782 locomotor muscle fatigue in healthy humans. *J Appl Physiol* 104: 1442-1451, 2008.
- 783 74. **Taylor JL and Gandevia SC.** A comparison of central aspects of fatigue in submaximal  
784 and maximal voluntary contractions. *J Appl Physiol* 104: 542-550, 2008.
- 785 75. **Tucker R, Marle T, Lambert EV and Noakes TD.** The rate of heat storage mediates an  
786 anticipatory reduction in exercise intensity during cycling at a fixed rating of perceived  
787 exertion. *J Physiol* 574: 905-915, 2006.
- 788 76. **Ulmer HV.** Concept of an extracellular regulation of muscular metabolic rate during  
789 heavy exercise in humans by psychophysiological feedback. *Experientia* 52: 416-420, 1996.
- 790 77. **Vanhatalo A, Fulford J, DiMenna FJ and Jones AM.** Influence of hyperoxia on  
791 muscle metabolic responses and the power-duration relationship during severe-intensity

792 exercise in humans: a 31P magnetic resonance spectroscopy study. *Exp Physiol* 95: 528-540,  
793 2010.  
794 78. **Williamson JW.** The relevance of central command for the neural cardiovascular control  
795 of exercise. *Exp Physiol* 95: 1043-1048, 2010.  
796 79. **Wuthrich TU, Eberle EC and Spengler CM.** Locomotor and diaphragm muscle fatigue  
797 in endurance athletes performing time-trials of different durations. *Eur J Appl Physiol* 114:  
798 1619-1633, 2014.  
799 80. **Wuthrich TU, Notter DA and Spengler CM.** Effect of inspiratory muscle fatigue on  
800 exercise performance taking into account the fatigue-induced excess respiratory drive. *Exp*  
801 *Physiol* 98: 1705-1717, 2013.  
802  
803

804 Table 1. Baseline neuromuscular function and between-trial reproducibility. Measured  
805 variables are shown as mean  $\pm$  SD. CV, coefficient of variation; SMC, smallest meaningful  
806 change.

	CYC	ARM-CYC	ISOTIME	Within-participant CV (%)	Measurement error	Reproducibility	SMC
MVF (N)	616 $\pm$ 75	602 $\pm$ 84	616 $\pm$ 73	4	19	53	27
Potentiated twitch force (N)	201 $\pm$ 33	200 $\pm$ 20	203 $\pm$ 22	3	11	29	15
Potentiated triplet force (N)	339 $\pm$ 35	328 $\pm$ 37	337 $\pm$ 33	3	10	28	14
Voluntary activation (%)	94 $\pm$ 5	95 $\pm$ 6	94 $\pm$ 6	2	1.8	5.1	2.6
Quadriceps M-wave amplitude (mV)	6.3 $\pm$ 2.1	6.1 $\pm$ 1.9	5.8 $\pm$ 2.5	10	0.6	1.7	0.8
Quadriceps EMG RMS at MVF (%M <sub>max</sub> amplitude)	8.8 $\pm$ 2.9	8.5 $\pm$ 2.9	8.9 $\pm$ 2.5	11	1.0	2.7	1.3

807 MVF, maximal voluntary force.

808

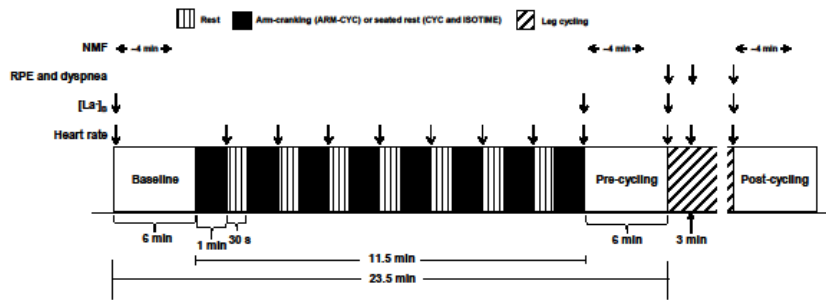
809 Table 2. Rates of change in the rating of perceived exertion (RPE) and dyspnea expressed  
810 relative to absolute exercise time and when normalized to total cycling exercise duration.  
811 Data are mean  $\pm$  SD.

	CYC	ARM-CYC	ISOTIME
$\Delta$ RPE/ $\Delta$ time (AU $\cdot$ min <sup>-1</sup> )	1.10 $\pm$ 0.38	1.83 $\pm$ 0.46**	1.05 $\pm$ 0.43
$\Delta$ RPE/ $\Delta$ %time (AU $\cdot$ %time <sup>-1</sup> )	0.07 $\pm$ 0.02	0.08 $\pm$ 0.02	0.04 $\pm$ 0.02**
$\Delta$ dyspnea/ $\Delta$ time (AU $\cdot$ min <sup>-1</sup> )	0.93 $\pm$ 0.39	1.33 $\pm$ 0.55*	0.87 $\pm$ 0.03
$\Delta$ dyspnea/ $\Delta$ %time (AU $\cdot$ %time <sup>-1</sup> )	0.07 $\pm$ 0.03	0.06 $\pm$ 0.02	0.04 $\pm$ 0.02 <sup>#†</sup>

812 \*\* $P$  < 0.01 vs. other two trials; \* $P$  < 0.05 vs. ISOTIME; # $P$  < 0.01 vs. CYC; † $P$  < 0.05 vs.  
813 ARM-CYC.

814

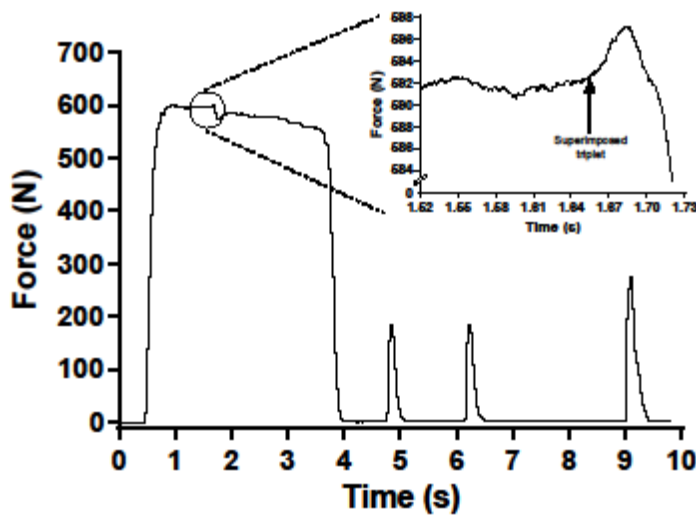
815 **Figures**



816

817 Fig. 1. Experimental protocol. Arrows denote timing of measurement. Note that  $[La]_B$ , heart  
 818 rate, rating of perceived exertion (RPE) and dyspnea were measured immediately before the  
 819 start of leg cycling exercise.

820



821

822 Fig. 2. Raw traces of force at baseline from a representative participant. Force was measured  
 823 during a maximal voluntary contraction with superimposed triplet, and subsequently during  
 824 two potentiated twitch contractions and one triplet contraction. Inset figure provides a close  
 825 up view of changes in force with the superimposed triplet.

826

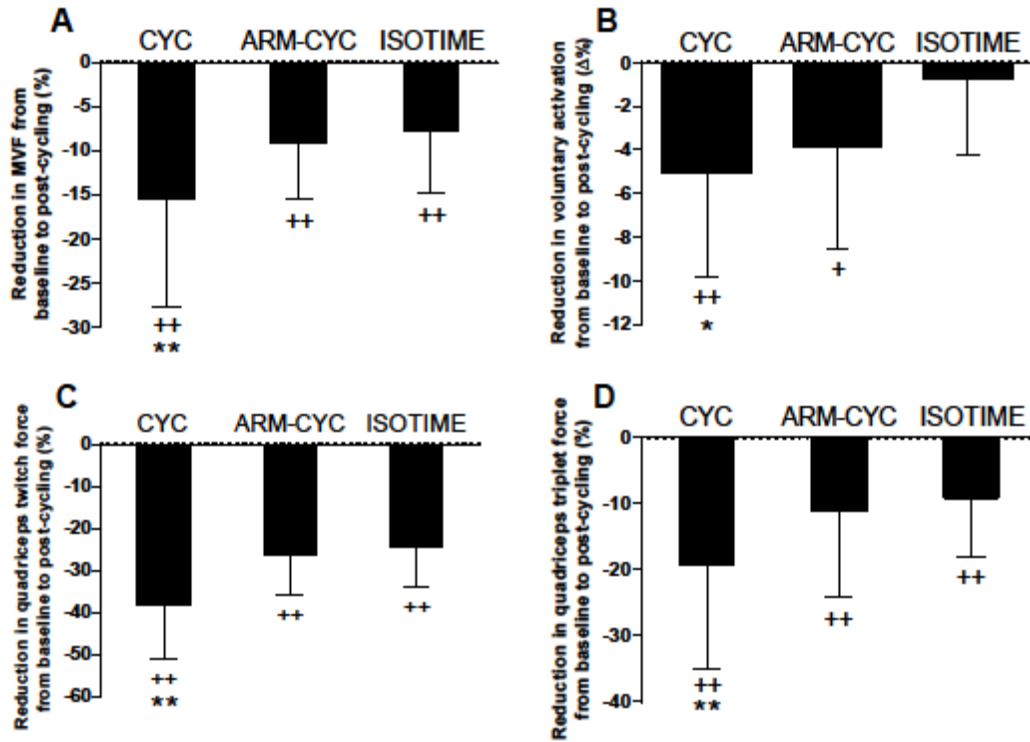
827

828

829

830

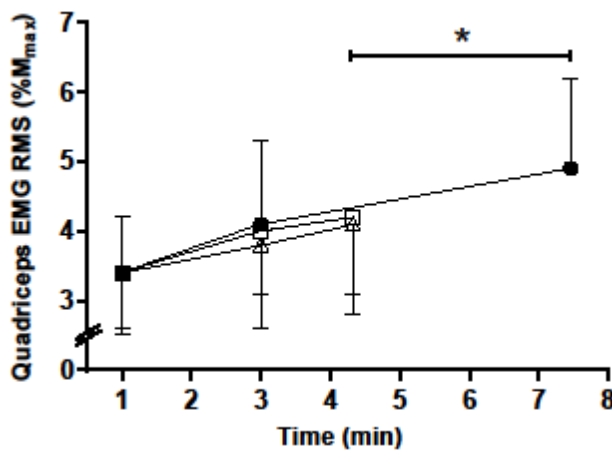
831



832

833 Fig. 3. Reductions in maximal voluntary force (MVF) (A), voluntary activation (B), and  
 834 electrically-evoked potentiated twitch (C) and triplet (D) force after cycling exercise. Data  
 835 are mean  $\pm$  SD. Reduction from baseline ( $^+P < 0.05$ ,  $^{++}P < 0.01$ ). \*Greater reduction  
 836 compared with ISOTIME ( $P < 0.05$ ). \*\*Greater reduction compared with ARM-CYC and  
 837 ISOTIME ( $P < 0.01$ ).

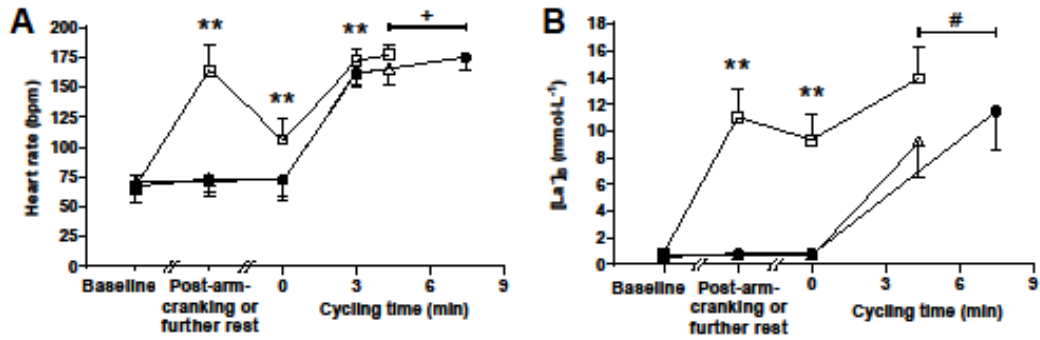
838



839

840 Fig. 4. Quadriceps neuromuscular activation measured as EMG RMS normalized to  $M_{max}$   
 841 during cycling in CYC (●), ARM-CYC (□) and ISOTIME (Δ). Data are mean  $\pm$  SD with x-  
 842 error bars omitted at the end of cycling exercise to improve clarity. \*Significant difference:  
 843 CYC vs. ARM-CYC and ISOTIME at the end of cycling ( $P < 0.05$ ).

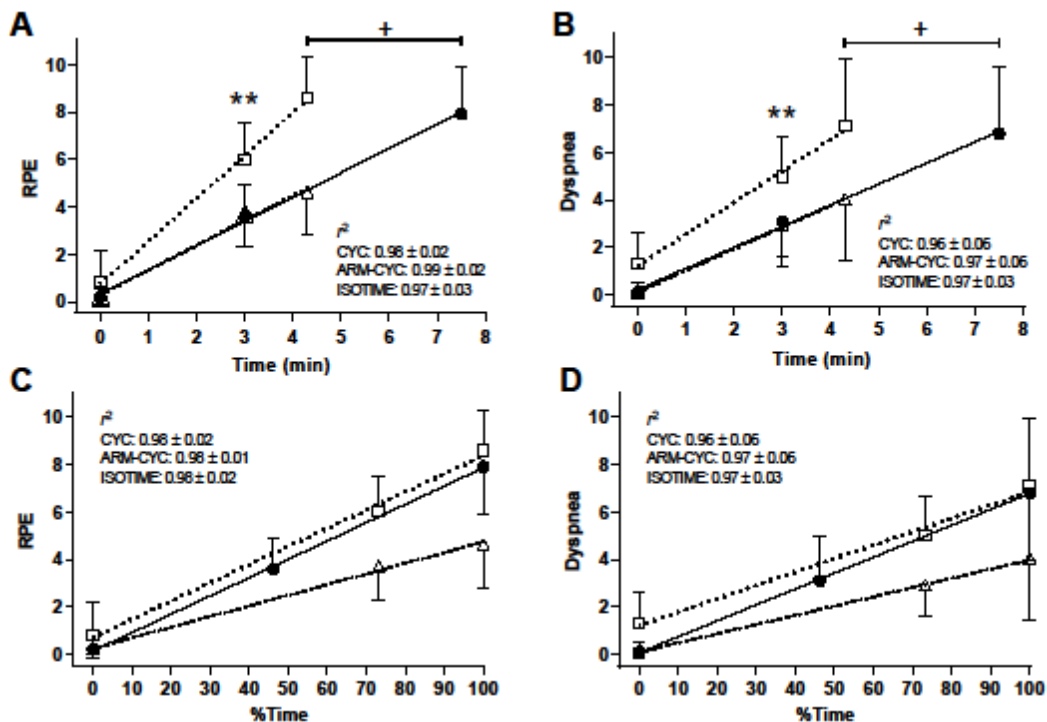




844

845 Fig. 5. Heart rate (A) and blood lactate concentration ( $[La^-]_B$ ) (B) during CYC (●), ARM-  
 846 CYC (□) and ISOTIME (Δ). Data are mean  $\pm$  SD and x-error bars are omitted at the end of  
 847 cycling exercise to improve clarity. Measurements at 0 min were taken immediately before  
 848 the start of cycling exercise. Significant difference between trials ( $P < 0.01$ ): \*\*ARM-CYC  
 849 vs. CYC and ISOTIME; +CYC and ARM-CYC vs. ISOTIME at the end of cycling; #all trials  
 850 at the end of cycling.

851



852

853 Fig. 6. Rating of perceived exertion (RPE) and dyspnea during cycling exercise in CYC (●),  
 854 ARM-CYC (□) and ISOTIME (Δ). Data are mean  $\pm$  SD and expressed relative to absolute  
 855 exercise time (A and B) and when normalized to total cycling exercise duration (C and D).  
 856 Measurements at 0 min and 0 %time were taken immediately before the start of cycling  
 857 exercise. X-error bars in A and B are omitted at the end of cycling exercise to improve clarity.  
 858 Significant difference ( $P < 0.01$ ): \*\*ARM-CYC vs. CYC and ISOTIME; +CYC and ARM-  
 859 CYC vs. ISOTIME at the end of cycling.