

1 **Muscle metabolic and neuromuscular determinants of fatigue**
2 **during cycling in different exercise intensity domains**

3 *Original Investigation*

4
5 **Matthew I. Black^{1,2}, Andrew M. Jones¹, Jamie R. Blackwell¹, Stephen J. Bailey^{1,2}, Lee**
6 **J. Wylie¹, Sinead T.J. McDonagh¹, Christopher Thompson¹, James Kelly¹, Paul**
7 **Summers³, Katya J. Mileva³, Joanna L. Bowtell¹, Anni Vanhatalo¹**

8
9 ¹College of Life and Environmental Sciences, St. Luke's Campus, University of Exeter,
10 Heavitree Road, Exeter, EX1 2LU, United Kingdom. ²School of Sport, Exercise and Health
11 Sciences, Loughborough University, Epinal Way, LE11 3TU, United Kingdom. ³Sport and
12 Exercise Science Research Centre, School of Applied Sciences, London South Bank
13 University, 103 Borough Road, London, SE1 0AA, United Kingdom.

14
15 **Running head:** Metabolic and neuromuscular correlates of fatigue

16
17 Correspondence:

18 Anni Vanhatalo, Ph.D.

19 E-mail: a.vanhatalo@exeter.ac.uk

20 Tel: 01392 722815

21 Fax: 01392 264726

22

23 **ABSTRACT**

24

25 The lactate or gas exchange threshold (GET) and the critical power (CP) are closely
26 associated with human exercise performance. We tested the hypothesis that the limit of
27 tolerance (T_{lim}) during cycle exercise performed within the exercise intensity domains
28 demarcated by GET and CP is linked to discrete muscle metabolic and neuromuscular
29 responses. Eleven males performed a ramp incremental exercise test, 4-5 severe-intensity
30 (SEV; $>CP$) constant-work-rate (CWR) tests until T_{lim} , a heavy-intensity (HVY; $<CP$ but
31 $>GET$) CWR test until T_{lim} , and a moderate-intensity (MOD; $<GET$) CWR test until T_{lim} .
32 Muscle biopsies revealed that a similar ($P>0.05$) muscle metabolic milieu (i.e., low pH and
33 [PCr] and high [lactate]) was attained at T_{lim} (~2-14 min) for all SEV exercise bouts. The
34 muscle metabolic perturbation was greater at T_{lim} following SEV compared to HVY, and also
35 following SEV and HVY compared to MOD (all $P<0.05$). The normalised M-wave
36 amplitude for the m. vastus lateralis (VL) decreased to a similar extent following SEV (-
37 $38\pm 15\%$), HVY ($-68\pm 24\%$), and MOD ($-53\pm 29\%$), ($P>0.05$). Neural drive to the VL
38 increased during SEV ($4\pm 4\%$; $P<0.05$) but did not change during HVY or MOD ($P>0.05$).
39 During SEV and HVY, but not MOD, the rates of change in M-wave amplitude and neural
40 drive were correlated with changes in muscle metabolic ([PCr], [lactate]) and blood
41 ionic/acid-base status ([lactate], $[K^+]$) ($P<0.05$). The results of this study indicate that the
42 metabolic and neuromuscular determinants of fatigue development differ according to the
43 intensity domain in which the exercise is performed.

44

45 **NEW AND NOTEWORTHY**

46

47 The gas exchange threshold and the critical power demarcate discrete exercise intensity
48 domains. For the first time, we show that the limit of tolerance during whole-body exercise
49 within these domains is characterized by distinct metabolic and neuromuscular responses.
50 Fatigue development during exercise >CP is associated with the attainment of consistent
51 'limiting' values of muscle metabolites whereas substrate availability and limitations to
52 muscle activation may constrain performance at lower intensities.

53

54 **KEYWORDS**

55

56 Critical power; gas exchange threshold; neuromuscular fatigue; muscle metabolism; cycling
57 exercise

58

59

60 **INTRODUCTION**

61

62 Intense and/or prolonged excitation of muscle leads to a reversible decline in its force
63 generating capacity and rate of contraction, commonly known as fatigue (21, 22, 23, 55, 27).
64 This temporary reduction in muscle performance may be attributed to central factors that
65 limit the neural drive for muscle contraction, and to peripheral factors, which occur at, or
66 distal to, the neuromuscular junction and that often involve metabolic and ionic perturbations
67 that reduce the muscle's ability to respond to neural stimulation (2, 3, 25, 30, 41).

68

69 The extent of the muscle metabolic and ionic, and blood acid-base and respiratory
70 perturbations experienced during exercise is dependent on the exercise intensity, which can
71 be categorised into three distinct domains demarcated by physiological thresholds (32, 72).
72 The upper limit of the 'moderate' exercise intensity domain is indicated by the lactate
73 threshold (LT; which is often estimated using the gas exchange threshold (GET)), and the
74 boundary between the 'heavy' and 'severe' exercise intensity domains is given by the critical
75 power (CP). Using ^{31}P -magnetic resonance spectroscopy (^{31}P -MRS), it has been
76 demonstrated that severe-intensity, single-leg knee-extension exercise is associated with a
77 progressive loss of muscle homeostasis with time (i.e. progressive reductions in muscle
78 phosphocreatine concentration ([PCr]) and pH and an increase in inorganic phosphate
79 concentration ([P_i])) (9, 31, 33, 69). In contrast, heavy- and moderate-intensity, small muscle
80 mass exercise is associated with much more limited muscle metabolic perturbation with new
81 'steady-state' values of [PCr], pH and [P_i] being achieved within a few minutes of the
82 initiation of exercise (33, 48, 67). These intensity-related differences in muscle metabolic, as
83 well as related blood acid-base and respiratory gas exchange, responses to exercise (33, 51,

84 68, 73) likely underpin the close relationships reported between these threshold phenomena
85 (LT/GET and CP) and human exercise performance (8).

86

87 The role of exercise intensity in defining the extent and dynamics of muscle metabolic
88 perturbation implies that exercise intensity may also influence the nature of neuromuscular
89 fatigue development (3, 22, 24, 39, 41, 52, 53). The peripheral component to fatigue, as
90 estimated non-invasively using surface electromyography (EMG), electrical muscle
91 stimulation and/or transcranial magnetic stimulation, appears to be especially important
92 during high-intensity exercise (45, 64, 66), whereas central fatigue may be more prominent
93 during prolonged, low-intensity exercise (38, 45, 57, 61, 66). The intensity-dependent
94 interaction between peripheral and central components of fatigue is thought to be modulated
95 by changes in afferent feedback arising from the muscle metabolic milieu. Consistent with
96 this, the critical torque (CT; analogous with the CP) for small muscle mass exercise has been
97 shown to represent a threshold in the development of neuromuscular fatigue (10), such that
98 severe-intensity knee-extensor contractions ($>CT$) were associated with elevated motor unit
99 recruitment and a disproportionate increase in the rate of neuromuscular fatigue development
100 relative to heavy-intensity contractions ($<CT$).

101

102 It is presently unclear whether the determinants of neuromuscular fatigue development during
103 whole-body exercise, such as cycling, differ according to the intensity domain in which
104 exercise is performed. Previous studies have assessed neuromuscular fatigue before and after
105 self-paced maximal time trial cycle exercise (66) and during constant-work-rate (CWR)
106 cycling performed ostensibly within the severe-intensity domain (65). These studies
107 suggested that, in contrast to knee extension exercise (10), the level of peripheral fatigue at
108 exhaustion for cycling may also be intensity-dependent above CP (65). Compared to small

109 muscle mass exercise, whole-body exercise is associated with greater rates of pulmonary
110 ventilation and gas exchange (58, 74), differences in cardiac output and muscle perfusion (12,
111 46, 58), and greater activity of type III/IV muscle afferents that may modulate central drive
112 (52, 53). It is possible that these factors impact the relationship between muscle metabolic
113 changes and neuromuscular fatigue development during exercise.

114

115 To date, the physiological and neuromuscular responses to whole-body exercise, and their
116 possible inter-relationship, has not been assessed within distinct exercise intensity domains.
117 The purpose of this study was therefore to evaluate possible differences in the muscle
118 metabolic and systemic responses to different, well-defined, intensities of exercise, with the
119 aim of elucidating whether the exercise intensity domain influences the determinants of
120 neuromuscular fatigue. Based on earlier studies investigating small muscle mass exercise (33,
121 69), we tested the hypotheses that: 1) a consistent muscle metabolic milieu ([ATP], [PCr],
122 [lactate], pH) and neuromuscular responses (muscle excitability and neural drive) will be
123 attained at the limit of tolerance (T_{lim}) during severe-intensity exercise ($>CP$); 2) severe-
124 intensity exercise will be associated with greater muscle metabolic perturbation compared to
125 heavy- and moderate-intensity exercise; and 3) the rate of neuromuscular fatigue
126 development will be greater during severe- compared to heavy- and moderate-intensity
127 exercise due to greater muscle metabolic and ionic perturbations.

128

129 **METHODS**

130

131 *Ethical approval*

132 The protocols were approved by the host institution's Research Ethics Committee and
133 conducted in accordance with the code of the ethical principles of the World Medical

134 Association (Declaration of Helsinki). Subjects gave written informed consent to participate
135 after the experimental procedures, associated risks, and potential benefits of participation had
136 been explained.

137

138 *Subjects*

139 Eleven healthy recreationally active males (mean \pm SD: age 21.8 ± 1.9 years, height $1.79 \pm$
140 0.05 m, body mass 78.2 ± 8.1 kg) volunteered to participate in this study, 8 of whom
141 volunteered to provide muscle tissue samples. One of the subjects who volunteered for the
142 biopsy procedure withdrew from the study having completed only the severe-intensity
143 exercise trials. This subject's data were excluded from statistical difference tests, but included
144 in the correlational analysis. All subjects were in good health and had no known history of
145 neurological or motor disorder. Subjects were instructed to report to all testing sessions in a
146 rested and fully hydrated state, ≥ 3 h post-prandial, and to avoid strenuous exercise and refrain
147 from caffeine and alcohol in the 24 h prior to testing. Each subject started each experimental
148 trial at the same time of day (± 2 h). All trials were performed on the same electronically-
149 braked cycle ergometer (Lode, Excalibur, Groningen, The Netherlands).

150

151 *Experimental design*

152 Each subject visited the laboratory on ~ 7 occasions over a 6-wk period with each visit
153 separated by a minimum of 24 h. A minimum of 7 days recovery was provided following the
154 heavy- and moderate-intensity exercise tests. After the completion of a ramp incremental test
155 (visit 1), subjects performed 4-5 CWR severe-intensity exercise tests to define the power-
156 duration relationship, a heavy-intensity CWR test and a moderate-intensity CWR test,
157 completed in a randomised order (Figure 1) except that the severe-intensity tests always
158 preceded the heavy-intensity test. Pulmonary gas exchange was measured continuously

159 during all tests, with the exception of the moderate-intensity test in which it was measured
160 periodically for 10 min intervals, with the mid-point of collection coinciding with blood
161 sample collection and femoral nerve stimulation (see below). We encouraged the subjects to
162 continue exercising during the moderate-intensity test to enable 10 min of gas exchange data
163 to be collected immediately prior to exercise cessation. EMG data were obtained
164 continuously from m. vastus lateralis (VL) and m. vastus medialis (VM) throughout the
165 exercise period with stimulation of the femoral nerve delivered at regular intervals (Figure 1)
166 to quantify the neuromuscular changes occurring during the exercise protocols. Venous blood
167 samples were obtained before and during exercise for the moderate-, heavy-, and for three of
168 the severe-intensity exercise tests. In addition, muscle tissue was obtained at rest, and
169 immediately following the moderate-, heavy-, and three of the severe-intensity exercise tests
170 (Figure 1). The severe-intensity tests were performed at 3-5 different work-rates (spanning
171 $60\% \Delta$ to $\dot{V}O_{2peak}$; (where Δ refers to the work-rate difference between the GET and the \dot{V}
172 O_{2peak}). Three of these severe-intensity tests (including short $85 \pm 5\% \Delta$, intermediate $75 \pm$
173 $5\% \Delta$, and long $65 \pm 5\% \Delta$) were grouped and compared to test for differences in muscle,
174 neuromuscular, and blood responses within the severe-intensity domain.

175

176 *Incremental test*

177 On the first laboratory visit, subjects completed a ramp incremental test for the determination
178 of the $\dot{V}O_{2peak}$ and gas exchange threshold (GET). The ergometer seat height and handlebars
179 were adjusted for comfort and the same settings were replicated for each subsequent test.
180 Initially, subjects completed 3 min of baseline cycling at 20 W, after which the work-rate was
181 increased by $30 \text{ W} \cdot \text{min}^{-1}$ until volitional exhaustion. The subjects cycled at a constant self-
182 selected pedal rate (80 rpm, $n = 9$, 90 rpm, $n = 2$), which was recorded and reproduced in
183 subsequent tests. The test was terminated when the pedal rate fell more than 10 rpm below

184 the preferred value for more than 5 s despite strong verbal encouragement. Breath-by-breath
185 pulmonary gas exchange data were collected continuously throughout the test and recorded as
186 10-s moving average for data analysis. $\dot{V}O_{2\text{peak}}$ was determined as the highest mean $\dot{V}O_2$
187 during any 30-s period and the GET was determined as previously described (5, 68).

188

189 *CWR tests*

190 All CWR tests started with 3 min of cycling at 20 W, followed by a step increase to the
191 required work-rate. Subjects were instructed to remain seated and to maintain their preferred
192 pedal rate for as long as possible. Strong verbal encouragement was provided, but subjects
193 were not informed of either the work-rate or the elapsed time. The tests were terminated
194 when pedal rate fell more than 10 rpm below the preferred value for more than 5 s. The T_{lim}
195 was recorded to the nearest second.

196

197 The parameters of the power-duration relationship (CP and W') were estimated by
198 completion of 4-5 severe-intensity exercise tests (4 trials, $n = 9$; 5 trials $n = 2$) at different
199 work-rates (approximately 60% Δ , 70% Δ , 80% Δ and 100% $\dot{V}O_{2\text{peak}}$) resulting in T_{lim} ranging
200 between approximately 2 and 14 min. If the standard errors associated with the CP and W'
201 exceeded 5 and 10 %, respectively, after four exercise tests had been completed, a fifth test
202 was performed. Any tests in which the end-exercise $\dot{V}O_2$ was <95% of the individual's ramp
203 test determined $\dot{V}O_{2\text{peak}}$ were excluded from the modelling of the power-duration
204 relationship.

205

206 The CP and W' (the amount of work done above the CP) parameters were estimated using
207 three models: the hyperbolic P- T_{lim} model (Equation 1); the linear work-time (W - T_{lim}) model,

208 where the total work done (W) is plotted against time (Equation 2); and the linear inverse-of-
209 time ($1/T_{lim}$) model, where power output is plotted against the inverse of time (Equation 3):

210

$$211 \quad T_{lim} = W' / (P - CP) \quad [1]$$

$$212 \quad W = CP \cdot T_{lim} + W' \quad [2]$$

$$213 \quad P = W' (1/ T_{lim}) + CP \quad [3]$$

214

215 The standard errors of the estimate associated with the CP and W' were expressed as
216 coefficients of variation ($CV\%$, i.e. relative to the parameter estimate). For each individual,
217 the ‘best fit’ model associated with the lowest $CV\%$ for CP and W' was used for further
218 analyses (7).

219

220 The work-rate for the heavy-intensity CWR trial was equal to the lower bound of the 95%
221 confidence limit in the CP parameter (33). The moderate-intensity CWR trial was performed
222 at a work-rate corresponding to 90% of the GET. Subjects were permitted to ingest water *ad*
223 *libitum* during the heavy- and moderate-intensity tests.

224

225 *Pulmonary gas exchange*

226 Breath-by-breath pulmonary gas exchange and ventilation were measured continuously
227 during all exercise tests, with the exception of the moderate-intensity test, where it was
228 measured at discrete time points (Figure 1). Subjects wore a nose clip and breathed through a
229 mouthpiece and impeller turbine assembly (Jaeger Triple V, Jaeger, Hoechberg, Germany).
230 The inspired and expired gas volume and concentration signals were continuously sampled at
231 100 Hz (Oxycon Pro, Jaeger, Hoechberg, Germany) via a capillary line connected to the
232 mouthpiece. The gas analysers were calibrated before each trial with gases of known

233 concentration and the turbine volume transducer was calibrated using a 3-L syringe (Hans
234 Rudolph, Kansas City, MO). The volume and concentration signals were time aligned by
235 accounting for the delay in capillary gas transit and the analyser rise time relative to the
236 volume signal.

237

238 *Blood analyses*

239 Venous blood samples were drawn into 5-mL heparinised syringes (Terumo Corporation,
240 Leuven, Belgium) from a cannula (Insyte-W™, Becton-Dickinson, Madrid, Spain) inserted
241 into the subject's antecubital vein. The blood was analysed for [lactate] and [glucose] within
242 ~5 min of collection (YSI 2300, Yellow Springs Instruments, Yellow Springs, OH). The
243 remaining whole blood was then centrifuged at 4,000 rpm for 7 min (Hettich EBA 20,
244 Germany) before plasma was extracted and analysed for [K⁺] (9180 Electrolyte Analyser, F.
245 Hoffman-La Roche, Basel, Switzerland).

246

247 *Neuromuscular Function*

248 EMG was used to continuously record the VL and VM activity during exercise, using active
249 bipolar bar electrodes with single differential configuration (DE2.1, DelSys Inc, Boston, MA,
250 USA), positioned over the muscle belly (SENIAM guidelines). The ground electrode was
251 positioned on the patella. Double-sided adhesive interfaces and hypoallergenic medical tape
252 were used to keep the EMG sensors in place and to reduce skin impedance. The leads
253 connected to the electrodes were secured using hypoallergenic medical tape to minimise
254 artefacts due to movement of the leads. The skin area underneath each electrode was shaved,
255 abraded, and cleaned with alcohol swabs prior to electrode placement to minimise skin
256 impedance. The EMG signal was considered of good quality when the average rectified EMG
257 baseline level for each muscle was below 2 μ V (18). The EMG signals were pre-amplified

258 (1,000x), band-pass filtered (20-450 Hz, Bagnoli-8, DelSys Inc, Boston, MA), and digitised
259 at a sampling rate of 2,000 Hz and resolution of 16 bits using a Power 1401 mk-II analog-to-
260 digital converter and Spike 2 data collection software run by custom written sampling
261 configuration (CED, Cambridge Electronic Design, UK).

262

263 The location of the optimal site for transcutaneous femoral nerve stimulation was determined
264 whilst the subject was positioned on the cycle ergometer. Using an adhesive cathode (Boots
265 UK Ltd, Nottingham, England) placed approximately 2 cm medial of the femoral pulse, and
266 an adhesive anode (Boots UK Ltd, Nottingham, England) placed at the anterior aspect of the
267 iliac crest, single electrical pulses generated by a constant current stimulator (DS7 A,
268 Digitimer Ltd, UK) were delivered. The cathode was systematically moved vertically and
269 horizontally and the amplitude of the compound muscle action potential (CMAP, M-wave)
270 was monitored to identify the optimal position of the cathode for attaining maximal peak-to-
271 peak M-wave amplitude during the cycling trials.

272

273 Following the attachment of the EMG and the stimulation electrodes, the crank angle at
274 which stimulation was to be delivered during the trials was determined for each subject. The
275 subject was positioned on the cycle ergometer and cycled at a moderate work-rate (20 W
276 below GET) for 1 min. The EMG activity obtained during this period was rectified and
277 averaged for 20 complete crank revolutions. The duration of each revolution was determined
278 by a custom-made magnetic switch that generated an event marker signal on each occasion
279 that the crank passed top dead centre (i.e. 0°). For each subject, the crank angle at which the
280 rectified VL EMG activity was maximal was determined, and as performed by Sidhu et al
281 (56) stimulations were delivered at the identified crank angle for all subsequent trials for that
282 participant ($65 \pm 5^\circ$ relative to the top dead centre). A custom written sequencer script

283 triggered 3 stimulations, with at least 1 and up to 10 pedal revolutions between stimuli. The
284 intervals were randomly determined using a random number generator incorporated within
285 the sequencer script. This was designed to prevent participants from anticipating the stimulus
286 delivery, which may affect the evoked response.

287

288 A standard M-wave recruitment curve protocol was completed during each laboratory visit.
289 The subject cycled at 20 W below GET throughout the recruitment curve protocol. A single-
290 pulse electrical stimulation (200 μ s) was delivered at the individually identified crank angle
291 as described above. The current was increased in 20 mA increments until the M-wave
292 amplitude plateaued at the maximal M-wave amplitude (M_{\max}). A pulse of 130% M_{\max}
293 current was applied during the exercise tests (mean stimulation intensity: 350 ± 50 mA).

294

295 The EMG signals from the VL and VM were processed using a custom written script to
296 measure peak-to-peak M-wave amplitude and M-wave area. The root-mean-square (RMS) of
297 the EMG signal (an index of the power of the signal) was calculated as the mean over a 25
298 ms pre-stimulation period at each stimulation time point. The EMG RMS amplitudes and the
299 M-wave parameters were normalised to the corresponding values attained after 1 min of
300 exercise during each trial to evaluate temporal changes in the voluntary muscle activation
301 level (i.e. the EMG RMS amplitude) and the peripheral neuromuscular excitability (i.e. the
302 M-wave amplitude and area). In addition, the voluntary EMG RMS amplitude was
303 normalised to the M-wave amplitude recorded at that time point to assess changes in neural
304 drive (RMS/M; 42). The rates of change in M-wave and EMG parameters from baseline
305 cycling to T_{lim} were calculated for each exercise to quantify the rate of neuromuscular fatigue
306 development in each intensity domain.

307

308 *Muscle biopsy*

309 The biopsy site was prepared on the alternate thigh to the EMG and peripheral nerve
310 stimulation setup. Local anaesthesia was applied (2-3 ml of 20 mg.ml⁻¹ lidocaine) and an
311 incision was made in the medial region of the VL. Muscle samples were obtained using
312 needle biopsy with suction (6). Resting muscle samples were obtained prior to any exercise
313 on the first laboratory visit and post-exercise biopsies were taken within ~10 s of the
314 cessation of each exercise test with the subject supported on the ergometer. The muscle tissue
315 was rapidly frozen in liquid nitrogen.

316

317 *Muscle tissue analysis*

318 The frozen muscle samples from each biopsy were weighed before and after freeze-drying to
319 determine water content. After freeze-drying, the muscle samples were dissected free from
320 blood, fat and connective tissue. Prior to muscle metabolite analysis, 200 µl of 3 M perchloric
321 acid was added to approximately 2.5 mg d.w. muscle. The solution was then centrifuged and
322 placed on ice for 30 min. It was subsequently neutralised to pH 7.0 with 255 µl of cooled
323 potassium bicarbonate (KHCO₃) and centrifuged (10,000 g). The supernatant was analysed
324 for PCr, ATP and lactate by fluorometric assays (35). An aliquot containing 1-2 mg d.w.
325 muscle was extracted in 1 M hydrochloric acid (HCl) and hydrolysed at 100°C for 3 h before
326 glycogen content was determined using the hexokinase method (35). Muscle pH was
327 measured using a glass electrode following the homogenisation of 1-2 mg d.w. of muscle in a
328 non-buffering solution containing 145 mM KCl, 10 mM NaCl and 5 mM iodoacetic acid.

329

330 *Statistical analyses*

331 One-way ANOVAs with repeated measures were used to assess differences between severe-
332 intensity exercise tests in $\dot{V}O_{2\text{peak}}$, muscle [ATP], [PCr], [pH], [lactate], and [glycogen], M-

333 wave amplitude, M-wave area, voluntary EMG amplitude and RMS/M, and blood and
334 plasma variables at T_{lim} . The data from the severe-intensity tests were subsequently averaged
335 for each individual for comparison with the heavy- and moderate-intensity tests. Differences
336 in $\dot{V}O_{2peak}$, muscle [ATP], [PCr], [pH], [lactate], and [glycogen] between the severe-, heavy-
337 and moderate-intensity tests were assessed using one-way ANOVAs. Two-way repeated
338 measures ANOVAs (condition x time) were used to analyse differences in M-wave amplitude
339 and area, and voluntary EMG amplitude for the VL and VM, and blood and plasma variables
340 at common time-points (baseline, 1 min, 3 min and T_{lim}) among the severe-, heavy-, and
341 moderate-intensity tests. Significant interaction and main effects were followed up with
342 Bonferroni post-hocs. Relationships between the rates of change of metabolic and
343 neuromuscular variables were assessed using Pearson's product-moment correlation
344 coefficients. Statistical significance was set at $P < 0.05$ and data are presented as mean \pm SD.

345

346 **RESULTS**

347

348 The $\dot{V}O_{2peak}$ measured in the ramp incremental test was $4.32 \pm 0.46 \text{ L}\cdot\text{min}^{-1}$ ($56 \pm 8 \text{ mL}\cdot\text{kg}^{-1}$
349 $\cdot\text{min}^{-1}$) and the peak work-rate was $385 \pm 50 \text{ W}$. The GET occurred at $2.33 \pm 0.34 \text{ L}\cdot\text{min}^{-1}$
350 and $137 \pm 24 \text{ W}$.

351

352 **Physiological responses within the severe-intensity domain**

353 The T_{lim} in the severe-intensity CWR exercise tests ranged from 2.2 to 13.9 min. There were
354 no differences between the three models (Equations 1-3) in the CP or W' estimates ($P > 0.05$;
355 Table 1). The CP from the best fit model corresponded to $64 \pm 7\%$ of ramp test peak work-
356 rate and $45 \pm 11\% \Delta$.

357

358 The $\dot{V}O_{2\text{peak}}$ during the shorter ($\sim 85\% \Delta$: $4.43 \pm 0.50 \text{ L}\cdot\text{min}^{-1}$), intermediate ($\sim 75\% \Delta$: $4.49 \pm$
359 $0.47 \text{ L}\cdot\text{min}^{-1}$) and longer ($\sim 65\% \Delta$: $4.41 \pm 0.47 \text{ L}\cdot\text{min}^{-1}$) severe-intensity tests were not
360 different from the $\dot{V}O_{2\text{peak}}$ achieved during the ramp incremental test (all $P > 0.05$).

361 Moreover, no significant differences were observed at T_{lim} among the three severe-intensity
362 tests for any of the muscle tissue variables or for blood [lactate] (all $P > 0.05$; Figure 2). There
363 were also no differences in plasma $[\text{K}^+]$ at T_{lim} among the shorter ($5.6 \pm 0.6 \text{ mM}$),
364 intermediate ($5.8 \pm 1.1 \text{ mM}$), and longer ($5.7 \pm 0.6 \text{ mM}$) severe-intensity tests ($P > 0.05$).

365

366 **Physiological responses during severe-, heavy- and moderate-intensity exercise**

367 Pulmonary $\dot{V}O_2$, blood [lactate] and plasma $[\text{K}^+]$ during moderate-, heavy- and severe-
368 intensity exercise are illustrated in Figure 3. The T_{lim} for heavy-intensity exercise (231 ± 56
369 W) was $43.5 \pm 16.2 \text{ min}$ (range: 20.5 to 67.4 min) and the $\dot{V}O_2$ at T_{lim} ($3.78 \pm 0.53 \text{ L}\cdot\text{min}^{-1}$;
370 $87 \pm 4\%$ of $\dot{V}O_{2\text{peak}}$) was lower than the ramp test $\dot{V}O_{2\text{peak}}$ ($P < 0.05$). The T_{lim} for the
371 moderate-intensity exercise ($113 \pm 19 \text{ W}$) was $211.1 \pm 57.0 \text{ min}$ (range: 180 to 360 min) and
372 the $\dot{V}O_2$ at T_{lim} ($2.22 \pm 0.38 \text{ L}\cdot\text{min}^{-1}$, $52 \pm 8\%$ of $\dot{V}O_{2\text{peak}}$) was not different from the GET
373 ($P > 0.05$). In 9 out of 11 subjects the $\dot{V}O_2$ remained below the GET throughout the moderate
374 exercise bout.

375

376 During severe-intensity exercise, blood [lactate] increased rapidly until T_{lim} and was
377 significantly greater than baseline after 3 min ($P < 0.05$). During heavy-intensity exercise, the
378 rate of blood [lactate] increase was slower than during severe-intensity exercise such that
379 blood [lactate] did not differ from baseline until after 10 min ($P < 0.05$), and no further
380 increase was observed between 10 min and T_{lim} ($P > 0.05$) (Figure 3B). Plasma $[\text{K}^+]$ was
381 elevated above baseline at all measurement time points during heavy- and severe-intensity
382 exercise (all $P < 0.05$). The $[\text{K}^+]$ continued to rise throughout severe-intensity exercise,

383 whereas it stabilised during heavy-intensity exercise beyond 6 min (Figure 3C). During
384 moderate-intensity exercise, blood [lactate] did not change from baseline ($P>0.05$) while
385 plasma $[K^+]$ was elevated above resting baseline at 1 min ($P<0.05$), with no further increase
386 thereafter (all time points $P>0.05$).

387

388 Muscle metabolic variables at rest and at T_{lim} following moderate-, heavy- and severe-
389 intensity exercise are illustrated in Figure 4. For severe- and heavy-intensity exercise, muscle
390 [ATP], [PCr] and pH were lower and muscle [lactate] was greater at T_{lim} relative to rest (all
391 $P<0.05$). There was no significant muscle [glycogen] depletion during severe-intensity
392 exercise relative to rest ($P>0.05$) but there was a tendency for glycogen depletion during
393 heavy-intensity exercise ($P=0.06$). In contrast, for moderate-intensity exercise, muscle [PCr]
394 at T_{lim} was greater than for severe- and heavy-intensity exercise (all $P<0.05$), and muscle
395 [glycogen] was both lower than at rest and lower than at T_{lim} for heavy- and severe-intensity
396 exercise (all $P<0.05$). Muscle [pH] and [lactate] did not change significantly from rest during
397 moderate-intensity exercise ($P>0.05$).

398

399 **Neuromuscular responses during severe-, heavy- and moderate-intensity exercise**

400 The coefficients of variation (CV%) between trials during unloaded cycling were 25% (VL)
401 and 35% (VM) for the peak-to-peak M-wave amplitude, and 32% (VL) and 32% (VM) for
402 the M-wave total area. The CV% between stimulations during unloaded cycling was 11%
403 (VL) and 9% (VM) for the peak-to-peak M-wave amplitude and 10% (VL) and 9% (VM) for
404 the M-wave total area. The mean M_{max} amplitudes measured during cycling at 20 W below
405 GET (VL 2.77 ± 1.43 and VM 0.99 ± 1.18 mV) were not different between visits (all $P>0.05$).
406 No significant differences were observed between trials in the neural drive to VL and VM
407 during cycling at 20 W below GET.

408

409 *Neuromuscular excitability: M-wave amplitude and M-wave area*

410 The M-wave characteristics at T_{lim} for the three severe-intensity exercise tests, and for
411 moderate-intensity, heavy-intensity and the mean of the severe-intensity exercise tests are
412 shown in Figure 5A-D. Peripheral neuromuscular excitability at T_{lim} , indicated by the M-
413 wave amplitude and M-wave area, did not differ among the severe-intensity tests (all $P<0.05$)
414 (Figure 5A, C). The M-wave amplitude and M-wave area at T_{lim} were greater for severe-
415 intensity exercise compared to both heavy- and moderate-intensity exercise in the VM
416 ($P<0.05$), and the M-wave area at T_{lim} was also greater in severe- than in heavy-intensity
417 exercise in VL ($P<0.05$) (Figure 5B, D). Differences in M-wave characteristics between
418 severe-, heavy- and moderate-intensity exercise at each measurement time point are shown in
419 Figure 6A-D.

420

421 *Voluntary activation and neural drive*

422 Voluntary muscle activation level, measured as EMG RMS amplitude, and neural drive, as
423 indicated by RMS/M-wave amplitude, did not differ at T_{lim} among the severe-intensity
424 exercise tests (all $P<0.05$) (Figure 5E, G). Both EMG RMS and RMS/M were greater at T_{lim}
425 for severe-intensity compared to heavy- and moderate-intensity exercise in the VM ($P<0.05$)
426 (Figure 5F). In the VL, the EMG RMS at T_{lim} was also greater for severe- than for heavy-
427 intensity exercise and the RMS/M was greater for severe- than for moderate-intensity
428 exercise (both $P<0.05$) (Figure 5F, H). The only difference in neuromuscular variables
429 observed at T_{lim} between moderate- and heavy-intensity exercise was a significantly greater
430 EMG RMS in the VL (Figure 5F). Differences in EMG RMS and RMS/M severe-, heavy-
431 and moderate-intensity exercise at each measurement time point are shown in Figure 6E-H.

432

433 **Relationships between physiological and neuromuscular variables**

434 During severe-intensity exercise, the M-wave amplitude decreased in parallel with [PCr]
435 depletion and plasma K^+ accumulation (Table 2). Moreover, increased neural drive (RMS/M)
436 was correlated with high blood [lactate] and plasma [K^+], and to low muscle [PCr], and high
437 muscle [lactate] and [glycogen] (Table 2). During heavy-intensity exercise, the reduction in
438 M-wave amplitude was related to low muscle [PCr] and high plasma [K^+], and increased
439 neural drive was related to high plasma [K^+] and low muscle [PCr], and high muscle [lactate]
440 and [glycogen] (Table 2). During moderate-intensity exercise, the M-wave amplitude was
441 inversely correlated with the reduction in [PCr] (Table 2).

442

443 **DISCUSSION**

444

445 To our knowledge, the present study is the first to combine muscle biopsy, blood analyses
446 and measurements of neuromuscular excitability and neural drive (via electrical stimulation
447 of the femoral nerve during exercise) to assess the muscle metabolic, acid-base and
448 neuromuscular responses to cycling performed within discrete exercise intensity domains
449 (32). The data presented herein provide novel insight into the *in vivo* relationships between
450 exercise intensity, muscle metabolic perturbation and neuromuscular function and support the
451 notion that LT/GET and CP separate exercise intensity domains within which exercise
452 tolerance is limited by discrete fatigue mechanisms. In classical terms, when exercise
453 intensity exceeds CP, the oxidation of fat and carbohydrate cannot keep pace with required
454 ATP turnover and the rate of pyruvate production from glycolysis exceeds the capacity of the
455 Krebs cycle, resulting in progressive increase in intramuscular lactate and H^+ concentrations.
456 We demonstrated that a similar muscle metabolic milieu (i.e., [ATP], [PCr], [lactate] and pH)
457 was attained at T_{lim} irrespective of work-rate within the severe-intensity domain. The muscle

458 metabolic perturbation was greater (i.e., lower [ATP] and pH, and higher [lactate]) at T_{lim}
459 following severe- compared to heavy-intensity exercise, and also following severe- and
460 heavy- compared to moderate-intensity exercise. In contrast, more extensive muscle glycogen
461 depletion occurred during moderate- compared to both severe- and heavy-intensity exercise.

462

463 However, while the results indicate that CP represents a critical threshold for both muscle
464 metabolic control and neuromuscular fatigue development, the importance of the GET in
465 separating exercise intensity domains was less obvious; unlike some muscle metabolic,
466 pulmonary gas exchange and blood [lactate] responses, neuromuscular indices of fatigue
467 development were not strikingly different between moderate-intensity and heavy-intensity
468 exercise.

469

470 **Fatigue during severe-intensity exercise**

471 The T_{lim} during the severe-intensity exercise tests ranged from 2.2 min to 13.9 min and in all
472 cases, subjects achieved $\dot{V}O_{2peak}$. Historically, the amount of work that can be done above
473 CP (i.e., the curvature constant of the power-duration relationship, W'), and therefore the
474 cause(s) of exercise intolerance within the severe-intensity domain, has been linked to the
475 depletion of the high-energy phosphates and a source related to anaerobic glycolysis, along
476 with a finite amount of stored O_2 (43,44). Consistent with this, recent studies have
477 demonstrated that, at least for small muscle mass exercise, the utilisation of this finite energy
478 store (W') coincides with the depletion of muscle PCr and the accumulation of fatigue-related
479 metabolites (i.e. P_i , H^+) until a consistent, presumably 'limiting' value is attained (33, 69).

480 The findings of the current study indicate that, irrespective of work-rate or exercise duration
481 (~2-14 min), T_{lim} during severe-intensity exercise is associated with the attainment of
482 consistently low values of muscle [PCr] (~23% of resting value), [ATP] (~76% of resting

483 value) and pH (~6.56), and consistently high values of muscle [lactate] (~1382% of resting
484 value), as well as blood [lactate] (~838% of resting value). It should be noted that the
485 observed muscle metabolite and substrate changes are reflective of the homogenate muscle
486 sample and therefore reflect the mean values for that particular muscle portion. It is known
487 that the depletion of muscle [PCr] during exercise displays significant regional heterogeneity
488 (13, 54). It is therefore possible that the subjects' eventual failure to maintain the requisite
489 power output was caused by the attainment of sufficiently low values of [PCr] and, perhaps,
490 [ATP], and/or sufficiently high values of muscle metabolites ([P_i], [ADP], [H⁺] and their
491 sequelae) within some of the recruited muscle fibres (51; see also 3,23, 24). Clearly, subjects
492 either could not, or would not, tolerate this 'critical combination' of substrate and metabolite
493 concentrations, but it is not possible to ascertain whether this was related to direct effects of
494 the muscle metabolic milieu on contractile function (17) or to the attainment of some
495 individual sensory 'critical fatigue threshold' which might constrain central motor drive and
496 muscle activation via feedback from type III/IV neural afferents (4). The appreciable
497 metabolic perturbation we observed during severe-intensity exercise was associated with a
498 concomitant decrease in M-wave amplitude in both the VL and VM. A strong inverse
499 correlation was observed between both the voluntary EMG RMS amplitude and neural drive,
500 and the changes in [ATP] and [PCr] (Table 2). This is consistent with there being greater
501 engagement of central neural mechanisms (e.g. muscle fibre recruitment and firing frequency
502 modulation) in order to compensate for peripheral fatigue development.

503

504 We have proposed that the changes in muscle metabolic status that occur concomitantly with
505 the expenditure of the W' are driving the development of the $\dot{V}O_2$ slow component during
506 severe-intensity exercise (8, 33, 70). Thus, exercise intolerance in this intensity domain is
507 associated with the complete utilisation of W' , the attainment of some 'critical' combination

508 of muscle substrate and/or metabolite concentrations, and the achievement of $\dot{V}O_{2\text{peak}}$ (8, 14,
509 47, 70). In the present study, we observed a reduction in muscle excitability in parallel with
510 the increased metabolic stress. The reduction in muscle membrane excitability is likely
511 mediated, at least in part, by changes in plasma $[K^+]$ (Table 2), which may reflect a rise in
512 interstitial $[K^+]$ within the t-tubule weakening propagation of the action potential along the
513 surface membrane. Increased extracellular $[K^+]$ impairs force generation due to
514 depolarisation of the cell membrane, resulting in a reduced amplitude of the action potential
515 (11, 40). This process attenuates Ca^{2+} release from the sarcoplasmic reticulum, reducing
516 cross-bridge formation and the force generating capacity of the myocyte (36). In our study,
517 the increased plasma $[K^+]$ was accompanied by a transient increase in neural drive which was
518 brought about via a preservation of the EMG amplitude with reduced M-wave amplitude. It
519 was notable that the reductions in M-wave amplitude and M-wave area in the VM during
520 exhaustive severe exercise were less pronounced compared to moderate and heavy exercise
521 (Figure 5 B and D), suggesting that the muscle excitability was preserved to a greater extent
522 than at lower exercise intensities. It is important, however, to consider this finding in the
523 context of increasing neural drive during severe exercise (Figure 6 G and H) which implies
524 that exercise cessation was not due to central fatigue. Low muscle pH attained during severe
525 exercise may attenuate the reduction in muscle membrane excitability (3, 24). Furthermore,
526 the muscle glycogen content, a key regulator of sarcoplasmic Ca^{2+} release rate and thus
527 muscle excitability (15, 50), did not fall significantly during severe exercise. Precisely how
528 the utilisation of the W' , the associated alterations in muscle substrate and metabolite
529 concentrations, and ionic changes influence muscle excitability warrants further
530 investigation.

531

532 **Fatigue during heavy-intensity exercise**

533 Heavy-intensity exercise was maintained for an average of 43.5 min (T_{lim} ranged from 20.5 to
534 67.4 min) and, in contrast to severe-intensity exercise, no subject achieved $\dot{V}O_{2peak}$ at T_{lim}
535 ($\sim 87\% \dot{V}O_{2peak}$). Consistent with our second hypothesis, the muscle metabolic perturbation
536 experienced following heavy-intensity exercise was less than that observed following severe-
537 intensity exercise, but was greater than that observed following moderate-intensity exercise.
538 At T_{lim} , significant reductions were observed in muscle [PCr] ($\sim 66\%$), [ATP] ($\sim 12\%$), [pH]
539 ($\sim 97\%$) and [glycogen] ($\sim 59\%$), and there was a significant increase in muscle [lactate]
540 ($\sim 447\%$) relative to resting values. Similarly, blood [lactate] and plasma $[K^+]$ displayed
541 greater perturbation relative to moderate-intensity exercise, but less perturbation relative to
542 severe-intensity exercise (Figure 3). It is of interest that the decrease in muscle excitability
543 from rest to T_{lim} was greater during heavy-intensity than during severe-intensity exercise
544 (Figure 5). Following the onset of exercise, plasma $[K^+]$ increased rapidly to attain a peak
545 value at 10 min which was sustained until T_{lim} ; the reduction in M-wave amplitude followed
546 a similar temporal profile. It is therefore likely that the initial reduction in M-wave amplitude
547 was a result of plasma $[K^+]$ accumulation which reduced the release of Ca^{2+} from the
548 sarcoplasmic reticulum, impairing excitation-contraction coupling (36, 71). As heavy-
549 intensity exercise continued, it is possible that the combined metabolic and ionic perturbation,
550 coupled with the $\sim 60\%$ decrease in muscle [glycogen], may have further impaired Ca^{2+}
551 release and cross-bridge formation (2, 3, 23, 24, 36, 40, 41) and/or the sensitivity of the
552 myofilaments to Ca^{2+} (17). Although more complicated than for severe-intensity exercise,
553 fatigue development during heavy-intensity exercise appears to be related to the combined
554 influence of ionic changes on muscle membrane excitability, muscle metabolite
555 accumulation, and the decrease in energy substrate, which act collectively to impair
556 excitation-contraction coupling.

557

558 **Fatigue during moderate-intensity exercise**

559 Moderate-intensity exercise, performed at a work-rate of 20 W below the GET, was
560 continued for an average of 211 min with subjects working at $\sim 52\%$ $\dot{V}O_{2\text{peak}}$ at T_{lim} . Muscle
561 metabolic perturbation was relatively slight in this domain (Figure 3). For example, at the end
562 of exercise, muscle [PCr] had fallen to $\sim 76\%$ of the baseline value and pH had fallen by 0.1
563 unit from the resting value, while blood [lactate] and plasma $[K^+]$ were also largely
564 unchanged (Figures 3 and 4). There was, however, a large reduction (-83%) in muscle
565 [glycogen] (1, 29, 59, 60). It is therefore likely that the development of peripheral fatigue
566 within the moderate-intensity domain is related to the depletion of muscle glycogen and
567 impairment in neuromuscular excitability and transmission (15, 28, 49, 50, 62). In addition to
568 being an essential substrate for the regeneration of ATP, it has been demonstrated that under
569 conditions where [ATP] is held high, that low muscle [glycogen] can impair muscle function
570 (49, 62). The association between low muscle [glycogen] and impaired muscle function can
571 be attributed to glycogen's modulatory role in the release of Ca^{2+} from the sarcoplasmic
572 reticulum (15, 19, 20, 28, 49, 50). In keeping with glycogen's role in excitation-contraction
573 coupling, individuals deficient in glycogen phosphorylase (McArdle's disease) do not
574 experience a considerable fall in pH but demonstrate an earlier decline in the M-wave
575 amplitude during exercise (16). Furthermore, glucose administration during exercise has been
576 shown to partially restore both the M-wave amplitude and muscle contractility (34, 37, 63)
577 supporting the notion that carbohydrate availability modulates muscle excitability and
578 contractile function. The findings of the present study show that moderate-intensity exercise
579 ($<GET$) can be sustained for a long duration with little change in muscle metabolites and
580 indicate that muscle glycogen depletion is the likely mechanism responsible for the decline in
581 neuromuscular function and exercise intolerance in this domain.

582

583 The majority of research investigating neuromuscular fatigue development during exercise
584 has focused on small muscle groups and has been limited to the assessment of neuromuscular
585 function pre-exercise and as soon as possible (usually within 2-3 minutes) post-exercise.
586 Considering the task-specific nature of neuromuscular fatigue development, and the rapid
587 recovery in muscle function (within 2 min) after high-intensity cycle exercise (26), it is
588 possible that the previously reported changes in neuromuscular function pre- to post-exercise
589 underestimate fatigue development *during* exercise. Recently, Sidhu et al. (56) adopted an
590 approach that uses the motor compound action potential (M-wave) for the assessment of
591 changes in neuromuscular function during cycle exercise. Adopting a similar approach to
592 Sidhu et al. (56), we found large reductions in the M-wave amplitude and M-wave area in
593 both the VL and VM during exercise to T_{lim} in each discrete exercise intensity domain. This
594 suggests that changes in muscle excitability linked to the fatigue process can occur
595 consequent to a wide range of perturbations in muscle and blood chemistry, with limited
596 differentiation between exercise intensity domains. The consistency of indices of
597 neuromuscular fatigue during severe-intensity cycling exercise in our study contrasts with a
598 recent report of Thomas et al. (65) in which peripheral fatigue, assessed post-exercise using
599 electrical stimulation during isometric contractions, was greater at higher work-rates within
600 the severe-intensity domain. It is possible that this reflects differences in the experimental
601 techniques employed, and underlines the importance of accounting for the task-specificity of
602 fatigue and the dynamics of muscle recovery post-exercise (10).

603

604 **Conclusion**

605 This study employed a novel and rather comprehensive combination of invasive and non-
606 invasive techniques that enabled simultaneous assessment of metabolic, ionic, systemic and
607 neuromuscular factors that define muscular performance. Although direct measures of the

608 contribution of central factors to fatigue were not employed, peripheral nerve stimulation permitted
609 elucidation of their relative importance in neuromuscular fatigue development during
610 exhaustive cycle exercise performed within each of the well-defined exercise intensity
611 domains. This study is consistent with the notion that the GET and the CP demarcate exercise
612 intensity domains within which fatigue is mediated by distinct mechanisms. Exercise
613 intolerance within the severe-intensity domain ($>CP$) was associated with the attainment of a
614 consistent critical muscle metabolic milieu (i.e., low [PCr] and pH and high [P_i]). In contrast,
615 moderate-intensity exercise ($<GET$) was associated with more significant depletion of muscle
616 [glycogen]. The cause(s) of fatigue during heavy-intensity exercise ($>GET$, $<CP$) was/were
617 more obscure with intermediate changes in muscle metabolic perturbation and glycogen
618 depletion being apparent. These results are consistent with the notion that both the GET and
619 CP demarcate exercise intensity domains characterised by distinct respiratory and metabolic
620 profiles. Strikingly, CP represents a boundary above which both metabolic and
621 neuromuscular responses conform to a consistent ceiling or nadir irrespective of work-rate
622 and exercise duration.
623

624 **REFERENCES**

625

626 1. Ahlborg B, Bergstrom J, Ekelund LG, Hultman E. Muscle glycogen and muscle
627 electrolytes during prolonged physical exercise. *Acta Physiol Scand* 70: 129-42, 1967.

628

629 2. Allen DG. Fatigue in working muscles. *J Appl Physiol* 106: 358-9, 2009.

630

631 3. Allen DG, Lamb GD, Westerblad H. Skeletal muscle fatigue: Cellular mechanisms.
632 *Physiol Rev* 88: 287-332, 2008.

633

634 4. Amann M, Dempsey JA. Ensemble input of group III/IV muscle afferents to CNS: a
635 limiting factor of central motor drive during endurance exercise from normoxia to
636 moderate hypoxia. *Adv Exp Med Biol* 903: 325-42. 2016.

637

638 5. Beaver WL, Wasserman K, Whipp BJ. A new method for detecting anaerobic
639 threshold by gas exchange. *J Appl Physiol* 60: 2020-7, 1986.

640

641 6. Bergstrom J. Percutaneous needle biopsy of skeletal muscle in physiological and
642 clinical research. *Scand J Clin Lab Invest* 35: 609-16, 1975.

643

644 7. Black MI, Jones AM, Bailey SJ, Vanhatalo A. Self-pacing increases critical power
645 and improves performance during severe-intensity exercise. *Appl Physiol Nutr Metab*
646 40: 662-70, 2015.

647

648 8. Burnley M, Jones AM. Oxygen uptake kinetics as a determinant of sports
649 performance. *Eur J Sport Sci* 7: 63-79, 2007.

650

651 9. Burnley M, Vanhatalo A, Fulford J, Jones AM. Similar metabolic perturbations
652 during all-out and constant force exhaustive exercise in humans: a ^{31}P magnetic
653 resonance spectroscopy study. *Exp Physiol* 95: 798-807, 2010.

654

655 10. Burnley, M. Vanhatalo A, Jones AM. Distinct profiles of neuromuscular fatigue
656 during muscle contractions below and above the critical torque in humans. *J Appl*
657 *Physiol* 113: 215-23, 2012.

658

659 11. Cairns SP, Hing WA, Slack JR, Mills RG, Loiselle DS. Different effects of raised
660 $[\text{K}^+]_o$ on membrane potential and contraction in mouse fast- and slow-twitch muscle.
661 *Am J Physiol* 273: C598-611, 1997.

662

663 12. Calbet JAL, Jensen-Urstad M, van Hall G, Homberg HC, Rosdahl H, Saltin B.
664 Maximal muscular vascular conductances during whole body upright exercise in
665 humans. *J Physiol* 558: 319-31, 2004.

666

667 13. Cannon DT, Howe FA, Whipp BJ, Ward SA, McIntyre DJ, Ladroue C, Griffiths JR,
668 Kemp GJ, Rossiter HB. Muscle metabolism and activation heterogeneity by combined
669 ^{31}P chemical shift and T2 imaging, and pulmonary O_2 uptake during incremental
670 knee-extensor exercise. *J Appl Physiol* 115: 839-49, 2013.

671

672 14. Chidnok W, Fulford J, Bailey SJ, DiMenna FJ, Skiba PF, Vanhatalo A, Jones AM.
673 Muscle metabolic determinants of exercise tolerance following exhaustion:
674 relationship to the “critical power”. *J Appl Physiol* 115: 243-50, 2013.

675

676 15. Chin ER, Allen DG. Effects of reduced muscle glycogen concentration on force,
677 Ca²⁺ release and contractile protein function in intact mouse skeletal muscle. *J*
678 *Physiol* 498: 17-29, 1997.

679

680 16. Cooper RG, Stokes MJ, Edwards RH. Myofibrillar activation failure in McArdle's
681 disease. *J Neurol Sci* 1: 1-10. 1989.

682

683 17. Debold EP, Fitts RH, Sundberg CW, Nosek TM. Muscle fatigue from the perspective
684 of a single cross-bridge. *Med Sci Sports Exerc* In press.
685 DOI:10.1249/MSS.0000000000001047, 2016.

686

687 18. De Luca CJ. The use of surface electromyography in biomechanics. *J Appl Biomech*
688 13: 135-63. 1993.

689

690 19. Duhamel TA, Green HJ, Perco JG, Ouyang J. Effects of prior exercise and a low-
691 carbohydrate diet on muscle sarcoplasmic reticulum function during cycling in
692 women. *J Appl Physiol* 101: 695-706, 2006a.

693

694 20. Duhamel TA, Perco JG, Green HJ. Manipulation of dietary carbohydrates after
695 prolonged effort modifies muscle sarcoplasmic reticulum responses in exercising
696 males. *Am J Physiol Reg Int Comp Physiol* 291: R1100-10, 2006b.

697

698 21. Enoka RM, Duchateau. Muscle fatigue: what, why and how it influences muscle
699 function. *J Physiol* 586: 11-23, 2008.

700

701 22. Enoka RM, Stuart DG. Neurobiology of muscle fatigue. *J Appl Physiol* 72: 1631-48,
702 1992.

703

704 23. Fitts RH. Cellular mechanisms of muscle fatigue. *Physiol Rev* 74: 49-94, 1994.

705

706 24. Fitts RH. The cross-bridge cycle and skeletal muscle fatigue. *J Appl Physiol* 104: 551-
707 8, 2008.

708

709 25. Fowles JR, Green HJ, Tupling R, O'Brien S, Roy BD. Human neuromuscular fatigue
710 is associated with altered Na⁺-K⁺-ATPase activity following isometric exercise. *J*
711 *Appl Physiol* 92: 1585-93, 2002.

712

713 26. Froyd C, Millet GY, Noakes TD. The development of peripheral fatigue and short-
714 term recovery during self-paced high-intensity exercise. *J Physiol* 591: 1339-46.
715 2013.

716

717 27. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev*
718 81: 1725-89, 2001.

719

720 28. Gejl KD, Hvid LG, Frandsen U, Jensen K, Sahlin K, Ortenblad N. Muscle glycogen
721 content modifies SR Ca²⁺ release rate in elite endurance athletes. *Med Sci Sports*
722 *Exerc* 46: 496-505, 2014.

723

- 724 29. Gollnick PD, Piehl K, Saltin B. Selective glycogen depletion pattern in human muscle
725 fibres after exercise of varying intensity and varying pedalling rates. *J Physiol* 241:
726 45-57, 1974.
- 727
- 728 30. Green HJ. Cation pumps in skeletal muscle: potential role in muscle fatigue. *Acta*
729 *Physiol Scand* 162: 201-13, 1998.
- 730
- 731 31. Hogan MC, Richardson RS, Haseler LJ. Human muscle performance and PCr
732 hydrolysis with varied oxygen fractions: a ³¹P-MRS study. *J Appl Physiol* 86: 1367-
733 73, 1999.
- 734
- 735 32. Jones AM, Poole DC. Introduction to oxygen uptake kinetics and historical
736 development of the discipline. In AM Jones and DC Poole (Eds), Oxygen uptake
737 kinetics in sport, exercise and medicine (pp 2-35). London and New York, NY:
738 Routledge, 2005.
- 739
- 740 33. Jones AM, Wilkerson DP, DiMenna F, Fulford J, Poole DC. Muscle metabolic
741 responses to exercise above and below the “critical power” assessed using ³¹P-MRS.
742 *Am J Physiol Regul Integr Comp Physiol* 294: R585-93, 2008.
- 743
- 744 34. Karelis AD, Peronnet F, Gardiner PF. Glucose infusion attenuates muscle fatigue in
745 rat plantaris muscle during prolonged indirect stimulation in situ. *Exp Physiol* 87:
746 585-92, 2002.
- 747

- 748 35. Lowry OH, Passonneau JV. A flexible system of enzymatic analysis. Academic Press,
749 New York, 1972.
- 750
- 751 36. MacIntosh BR, Holash RJ, Renaud JM. Skeletal muscle fatigue – regulation of
752 excitation-contraction coupling to avoid metabolic catastrophe. *J Cell Sci* 125: 2105-
753 14, 2012.
- 754
- 755 37. Marcil M, Karelis AD, Peronnet F, Gardiner PF. Glucose infusion attenuates fatigue
756 without sparing glycogen in rat soleus muscle during prolonged electrical stimulation
757 in situ. *Eur J Appl Physiol* 93: 569-74. 2005.
- 758
- 759 38. Martin V, Kerherve H, Messonnier LA, Banfi JC, Geysant A, Bonnefoy R, Feasson
760 L, Millet GY. Central and peripheral contributions to neuromuscular fatigue induced
761 by a 24-h treadmill run. *J Appl Physiol* 108: 1224-33, 2010.
- 762
- 763 39. Matkowski B, Place N, Martin A, Lepers R. Neuromuscular fatigue differs following
764 unilateral vs bilateral sustained submaximal contractions. *Scand J Med Sci Sports* 21:
765 268-76, 2011.
- 766
- 767 40. McKenna MJ. The roles of ionic processes in muscular fatigue during intense
768 exercise. *Sports Med* 13: 134-45, 1992.
- 769
- 770 41. McKenna MJ, Bangsbo J, Renaud JM. Muscle K^+ , Na^+ and Cl^- disturbances and Na^+ -
771 K^+ pump inactivation: implications for fatigue. *J Appl Physiol* 104: 288-95, 2008.
- 772

- 773 42. Millet GY, Lepers R. Alterations of neuromuscular function after prolonged running,
774 cycling and skiing exercises. *Sports Med* 34: 105-16, 2004.
775
- 776 43. Monod H, Scherrer J. The work capacity of a synergic muscle group. *Ergonomics* 8:
777 329-38, 1965.
778
- 779 44. Moritani T, Nagata A, deVries HA, Muro M. Critical power as a measure of physical
780 work capacity and anaerobic threshold. *Ergonomics* 24: 339-50, 1981.
781
- 782 45. Morris MG, Dawes H, Howells K, Scott OM, Cramp M, Izadi H. Alterations in
783 peripheral muscle contractile characteristics following high and low intensity bouts of
784 exercise. *Eur J Appl Physiol* 112: 337-43, 2012.
785
- 786 46. Mortensen SP, Damsgaard R, Dawson EA, Secher NH, Gonzalez-Alonso J.
787 Restrictions in systemic and locomotor skeletal muscle perfusion, oxygen supply and
788 VO₂ during high-intensity whole-body exercise in humans. *J Physiol* 586: 2621-35,
789 2008.
790
- 791 47. Murgatroyd SR, Ferguson C, Ward SA, Whipp BJ, Rossiter HB. Pulmonary O₂
792 uptake kinetics as a determinant of high-intensity exercise tolerance in humans. *J*
793 *Appl Physiol* (1985) 110:1598-606, 2011.
794
- 795 48. Newham DJ, Cady EB. A ³¹P study of fatigue and metabolism in human skeletal
796 muscle with voluntary, intermittent contractions at different forces. *NMR Biomed* 3:
797 211-19, 1990.

798

799 49. Nielsen J, Schroder HD, Rix CG, Ortenblad N. Distinct effects of subcellular
800 glycogen localization on tetanic relaxation time and endurance in mechanically
801 skinned rat skeletal muscle fibres. *J Physiol* 587: 3679-90, 2009.

802

803 50. Ortenblad N, Nielsen J, Saltin B, Holmberg HC. Role of glycogen availability in
804 sarcoplasmic reticulum Ca²⁺ kinetics in human skeletal muscle. *J Physiol* 589: 711-
805 25, 2011.

806

807 51. Poole DC, Ward SA, Gardner GW, Whipp BJ. Metabolic and respiratory profile of
808 the upper limit for prolonged exercise in man. *Ergonomics* 31: 1265-79, 1988.

809

810 52. Rossman MJ, Garten RS, Venturelli M, Amann M, Richardson RS. The role of active
811 muscle mass in determining the magnitude of peripheral fatigue during dynamic
812 exercise. *Am J Physiol Regul Integr Comp Physiol* 306: R934-40, 2014.

813

814 53. Rossman MJ, Venturelli M, McDaniel J, Amann M, Richardson RS. Muscle mass and
815 peripheral fatigue: a potential role for afferent feedback? *Acta Physiol* 206: 242-50,
816 2012.

817

818 54. Sahlin K, Söderlund K, Tonkonogi M, Hiraoka K. Phosphocreatine content in single
819 fibers of human muscle after sustained submaximal exercise. *Am J Cell Physiol* 273:
820 C172-8, 1997.

821

- 822 55. Sejersted OM, Sjøgaard G. Dynamics and consequences of potassium shifts in
823 skeletal muscle and heart during exercise. *Physiol Rev* 80: 1411-81, 2000.
824
- 825 56. Sidhu SK, Cresswell AG, Carroll TJ. Motor cortex excitability does not increase
826 during sustained cycling exercise to volitional exhaustion. *J Appl Physiol* (1985).
827 113:401-9, 2012.
828
- 829 57. Smith JL, Martin PG, Gandevia SC, Taylor JL. Sustained contraction at very low
830 forces produces prominent supraspinal fatigue in human elbow flexor muscles. *J Appl*
831 *Physiol* 103: 560-8, 2007.
832
- 833 58. Smith JR, Ade CJ, Broxterman RM, Skutnik BC, Barstow TJ. Influence of exercise
834 intensity on respiratory muscle fatigue and brachial artery blood flow during cycling
835 exercise. *Eur J Appl Physiol* 114: 1767-77, 2014.
836
- 837 59. Sjøgaard G. Electrolytes in slow and fast muscle fibers of humans at rest and with
838 dynamic exercise. *Am J Physiol Regul Integr Comp Physiol* 245: R25-31, 1983.
839
- 840 60. Sjøgaard G. Water and electrolyte fluxes during exercise and their relation to muscle
841 fatigue. *Acta Physiol Scand Suppl* 556: 129-36, 1986.
842
- 843 61. Søgaard K, Gandevia SC, Todd G, Petersen NT, Taylor JL. The effect of sustained
844 low-intensity contractions on supraspinal fatigue in human elbow flexor muscles. *J*
845 *Physiol* 573: 511-23, 2006.
846

- 847 62. Stephenson DG, Nguyen LT, Stephenson GM. Glycogen content and excitation-
848 contraction coupling in mechanically skinned muscle fibres of the cane toad. *J*
849 *Physiol* 519: 177-187, 1999.
- 850
- 851 63. Stewart RD, Duhamel TA, Foley KP, Ouyang J, Smith IC, Green HJ. Protection of
852 muscle membrane excitability during prolonged cycle exercise with glucose
853 supplementation. *J Appl Physiol* 103: 331-9, 2007.
- 854
- 855 64. Tomazin K, Morin JB, Strojnik V, Podpecan A, Millet GY. Fatigue after short (100-
856 m), medium (200-m) and long (400-m) treadmill sprints. *Eur J Appl Physiol* 112:
857 1027-36, 2012.
- 858
- 859 65. Thomas K, Elmeua M, Howatson G, Goodall S. Intensity-Dependent Contribution of
860 Neuromuscular Fatigue after Constant-Load Cycling. *Med Sci Sports Exerc* 48:1751-
861 60, 2016.
- 862
- 863 66. Thomas K, Goodall S, Stone M, Howatson G, St Clair Gibson A, Ansley L. Central
864 and peripheral fatigue in male cyclists after 4, 20 and 40 km time trials. *Med Sci Sport*
865 *Exerc* 47: 537-46, 2015.
- 866
- 867 67. Vanderthommen M, Duteil S, Wary C, Raynaud JS, Leroy-Willig A, Crielaard JM,
868 Carlier PG. A comparison of voluntary and electrically induced contractions by
869 interleaved ¹H- and ³¹P-NMRS in humans. *J Appl Physiol* 94: 1012-24, 2003.
- 870

- 871 68. Vanhatalo A, Black MI, DiMenna FJ, Blackwell JR, Schmidt JF, Thompson C, Wylie
872 LJ, Mohr M, Bangsbo J, Krstrup P, Jones AM. The mechanistic bases of the power-
873 time relationship: muscle metabolic responses and relationships to muscle fibre type.
874 *J Physiol* 594: 4407-23, 2016.
- 875
- 876 69. Vanhatalo A, Fulford J, DiMenna F, Jones AM. Influence of hyperoxia on muscle
877 metabolic responses and the power-duration relationship during severe-intensity
878 exercise in humans: a ³¹P magnetic resonance spectroscopy study. *Exp Physiol* 95:
879 528-40, 2010.
- 880
- 881 70. Vanhatalo A, Poole DC, DiMenna FJ, Bailey SJ, Jones AM. Muscle fiber recruitment
882 and the slow component of O₂ uptake: constant work rate vs. all-out sprint exercise.
883 *Am J Physiol Regul Integr Comp Physiol* 300:R700-7, 2011.
- 884
- 885 71. Westerblad H, Allen DG. Cellular mechanisms of skeletal muscle fatigue. *Adv Exp*
886 *Med Biol* 538: 563-70, 2003.
- 887
- 888 72. Whipp BJ, Ward SA. Pulmonary gas exchange dynamics and the tolerance to
889 muscular exercise: effects of fitness and training. *Ann Physiol Anthropol* 11: 207-14,
890 1992.
- 891
- 892 73. Whipp BJ, Wasserman K. Oxygen uptake kinetics for various intensities of constant-
893 load work. *J Appl Physiol* 33: 351-6, 1972.
- 894

895 74. Wuthrich TU, Eberle EC, Spengler CM. Locomotor and diaphragm muscle fatigue in
896 endurance athletes performing time-trials of different durations. *Eur J Appl Physiol*
897 114: 1619-33, 2014.
898

899 **Figure Legends**

900

901 **Table 1** The CP and W' parameter estimates derived from Equations 1-3 and the 'best fit'
902 model.

903

904 **Table 2** The correlation coefficients between the rate of change in blood and muscle tissue
905 variables and the rate of change in neuromuscular variables measured in *m. vastus lateralis*. *
906 $P < 0.05$.

907

908 **Figure 1.** Schematic of the exercise protocol. Group mean work-rates are shown for the
909 severe- (solid line), heavy- (dotted line) and moderate- (dashed line) intensity trials. All trials
910 were started with a 3-min "warm-up" phase at 20 W, followed by an immediate "step"
911 increase to the required work-rate. Subjects were encouraged to continue exercising for as
912 long as possible. The dashed arrows indicate the collection of venous blood, and femoral
913 nerve stimulation. The solid arrows indicate the collection of muscle tissue. N.B., for clarity,
914 the resting muscle sample obtained prior to the first trial is not shown.

915 **Figure 2.** Muscle metabolic responses ([ATP] panel A, [PCr] panel B, pH panel C, [lactate]
916 panel D, [glycogen] panel E) and blood [lactate] (panel F) at T_{lim} were not different following
917 exhaustive exercise at three different severe-intensity work-rates. R = rest; S1 = short trials at
918 $\sim 85\% \Delta$ ($T_{lim} = 224 \pm 41$ s); S2 = intermediate trials at $\sim 75\% \Delta$ ($T_{lim} = 333 \pm 131$ s); and S3 =
919 long trials at $\sim 65\% \Delta$ ($T_{lim} = 475 \pm 145$ s). * Different from S1, S2 and S3 ($P < 0.05$).

920 **Figure 3.** Pulmonary $\dot{V}O_2$ (panel A), blood [lactate], (panel B) and plasma $[K^+]$ (panel C)
921 response to severe- (solid circle), heavy- (clear circle) and moderate- (solid triangle) intensity

922 exercise. To aid clarity error bars have been omitted from all but the final data point. a =
923 different from moderate-intensity $P<0.05$; b = different from heavy-intensity $P<0.05$.

924

925 **Figure 4.** Muscle [ATP] (panel A), [PCr] (panel B), [pH] (panel C), [lactate] (panel D), and
926 [glycogen] (panel E) at rest (white triangle), and following severe- (black circle), heavy-
927 (white circle), and moderate-intensity exercise (black triangle). * = different from rest
928 $P<0.05$; a = different from moderate-intensity $P<0.05$; b = different from heavy-intensity
929 $P<0.05$; c = different from severe-intensity $P<0.05$.

930

931 **Figure 5.** The group mean \pm SD M-wave amplitude and M-wave area (normalised to
932 maximum M-wave during baseline pedalling) indicating peripheral neuromuscular
933 excitability (panels A-D); voluntary EMG RMS amplitude (normalised to M-wave amplitude
934 at 1 min of exercise) indicating muscle activation level (panels E and F); and RMS/M-wave
935 (normalised to corresponding M-wave amplitude at each measurement time point) indicating
936 central fatigue (panels G and H) at the limit of tolerance (T_{lim}) for moderate-, heavy- and
937 severe-intensity exercise (panels B, D, F, H) and for three work-rates (severe 1 $\sim 85\%\Delta$,
938 severe 2 $\sim 75\%\Delta$ and severe 3 $\sim 65\%\Delta$) within the severe-intensity domain (panels A, C, E,
939 G). There were no significant differences among the severe-intensity work-rates in muscle
940 excitability (A, C) or in indices of central fatigue (E, G). VL = *m. vastus lateralis*; VM = *m.*
941 *vastus medialis*; EMG = electromyogram; RMS = root mean square; a = different from
942 moderate-intensity $P<0.05$; b = different from heavy-intensity $P<0.05$; c = different from
943 severe-intensity $P<0.05$.

944

945 **Figure 6.** The normalised M-wave amplitude (panels A and B), M-wave area (panels C and
946 D), voluntary EMG RMS amplitude (panels E and F), and RMS/M-wave amplitude (panels G

947 and H) during severe- (solid circle), heavy- (clear circle), and moderate-intensity (solid
948 triangle) exercise in *m. vastus lateralis* (VL) and *vastus medialis* (VM). M-wave amplitude
949 and area were normalised to maximum M-wave during baseline pedalling, EMG RMS was
950 normalised to M-wave amplitude at 1 min of exercise, and RMS/M-wave was normalised to
951 corresponding M-wave amplitude at each measurement time point. Error bars have been
952 omitted from all but the final data point to aid clarity. ^a Different from rest; ^b different from
953 severe-intensity ($P<0.05$); ^c different from heavy-intensity ($P<0.05$); ^d different from
954 moderate-intensity ($P<0.05$); and ^e trend for difference from heavy-intensity ($P=0.055$).

955

956

957 **Table 1** The parameter estimates derived from Equations 1-3 and the 'optimised fit' model.

958

959

	R²	CP (W)	SEE (W)	CV%	W' (kJ)	SEE (kJ)	CV%
W-Tlim model	0.993 – 1.000	253 ± 54	6 ± 3	2.6 ± 1.4	22.5 ± 5.3	2.3 ± 1.0	11.0 ± 6.2
I/Tlim model	0.939 – 0.999	252 ± 52	7 ± 4	3.0 ± 2.3	20.7 ± 5.2	1.9 ± 1.1	9.5 ± 5.6
P-Tlim model	0.919 – 1.000	248 ± 52	5 ± 3	2.2 ± 1.4	22.4 ± 3.8	2.5 ± 1.8	11.3 ± 9.4
Optimised fit model	0.944 – 1.000	250 ± 53	5 ± 2	2.0 ± 1.2	22.5 ± 6.1	1.8 ± 0.8	8.3 ± 4.5

960

961 **Table 2.** The correlation coefficients between the rate of change in blood and muscle tissue

962 variables and the rate of change in neuromuscular variables measured in *m. vastus lateralis*. *

963 $P < 0.05$.

964

			M-wave Amplitude	Voluntary EMG	Neural Drive
Severe	n = 33	BLa	-0.30	0.57*	0.47*
		Plasma [K ⁺]	-0.39*	0.68*	0.64*
	n = 24	[PCr]	0.59*	-0.80*	-0.80*
		[lactate]	-0.40	0.44*	0.55*
		[glycogen]	-0.22	0.46*	0.56*
		[pH]	-0.13	0.36	0.37
		[ATP]	0.21	-0.60*	-0.59*
Heavy	n = 10	BLa	-0.42	0.13	0.49
		Plasma [K ⁺]	-0.88*	-0.29	0.86*
	n = 7	[PCr]	0.93*	-0.28	-0.72*
		[lactate]	-0.25	0.63	0.66
		[glycogen]	-0.15	0.53	0.77*
		[pH]	0.13	0.78*	0.27
		[ATP]	-0.26	0.32	0.63
Moderate	n = 10	BLa	0.08	0.05	0.10
		Plasma [K ⁺]	0.12	0.18	0.49
	n = 7	[PCr]	-0.67*	-0.36	0.58
		[lactate]	-0.44	-0.34	0.04
		[glycogen]	-0.10	0.43	0.23
		[pH]	0.19	0.06	-0.30
		[ATP]	0.09	0.59	0.24

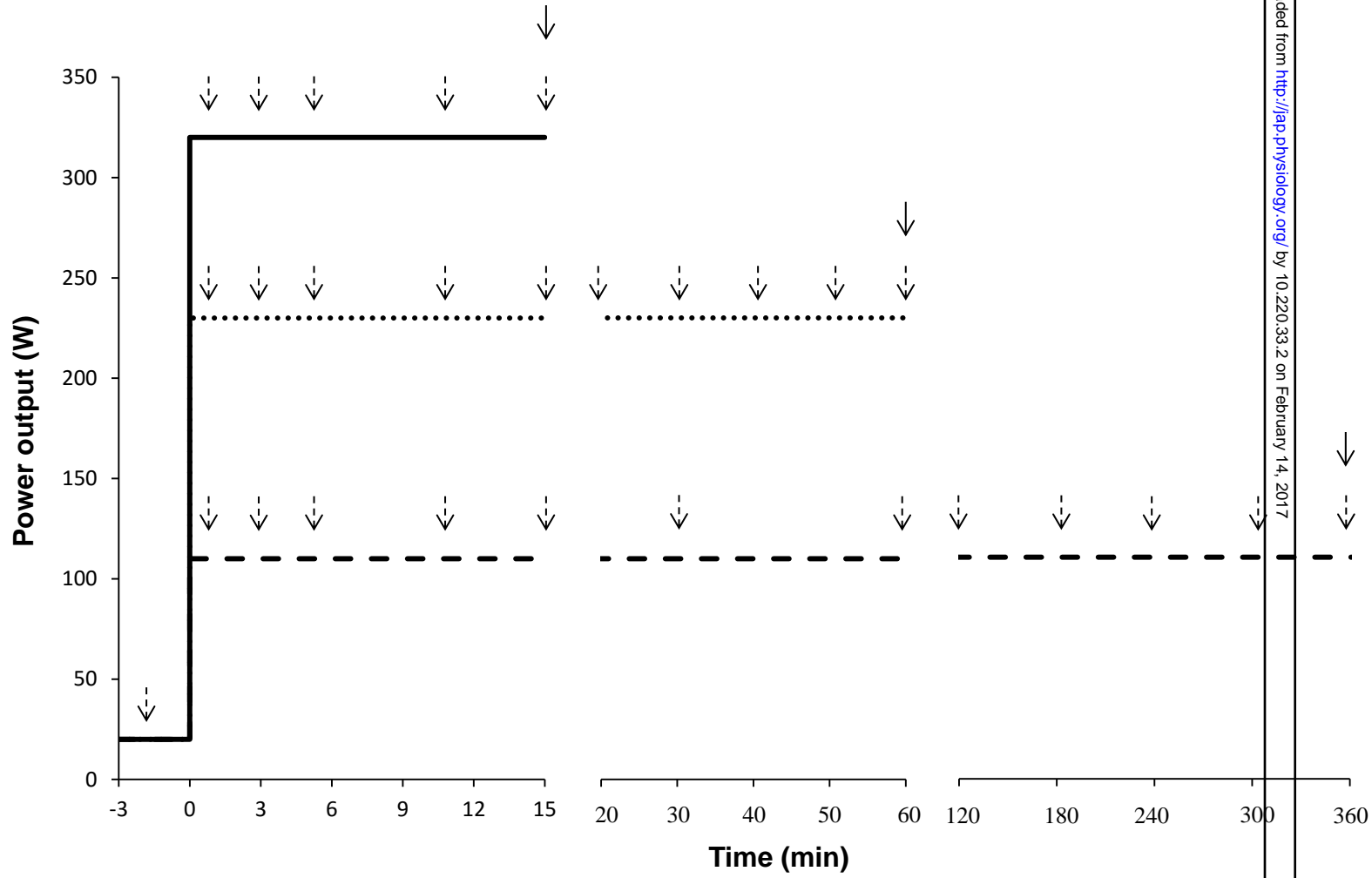


Figure 1

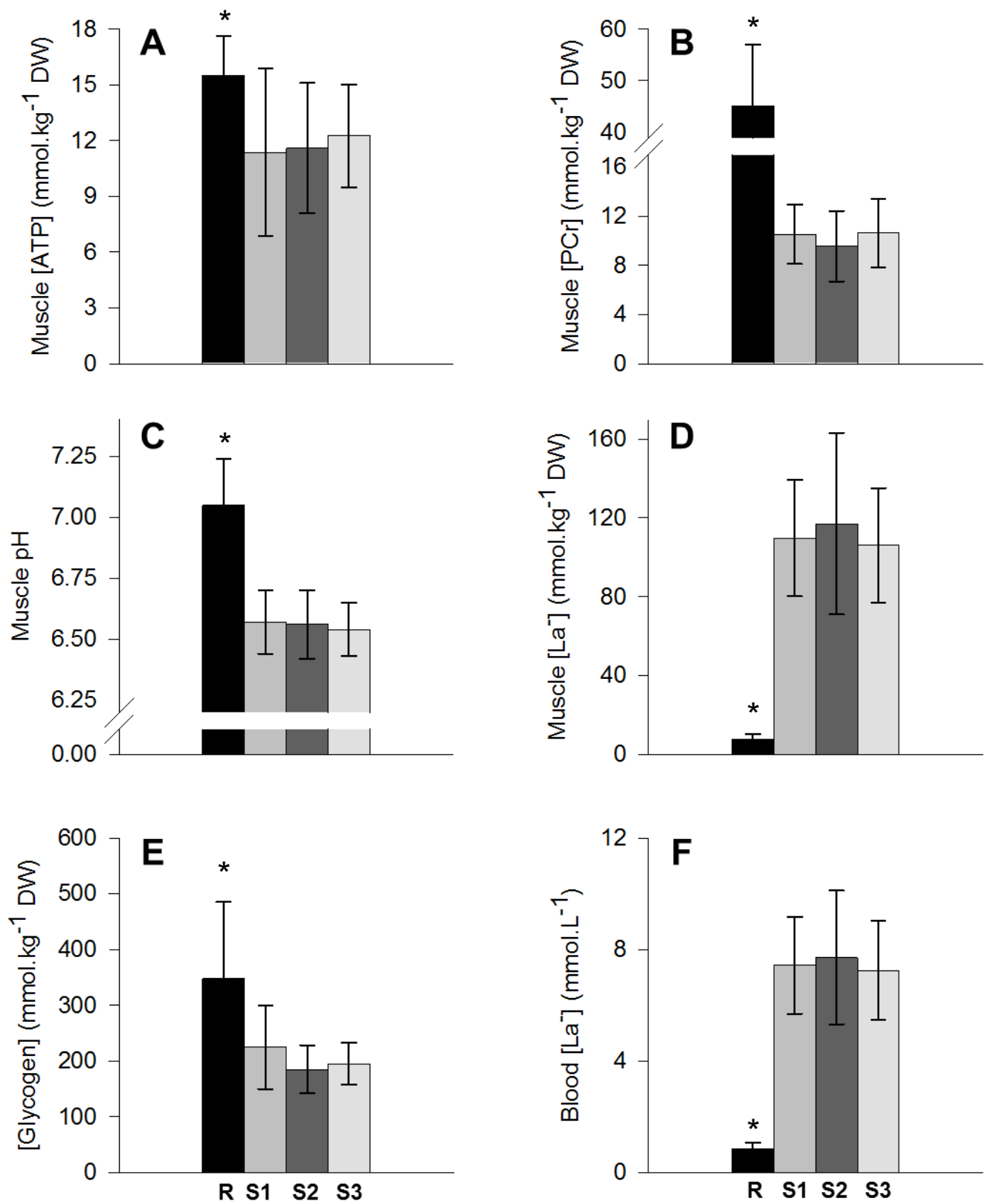


Figure 2

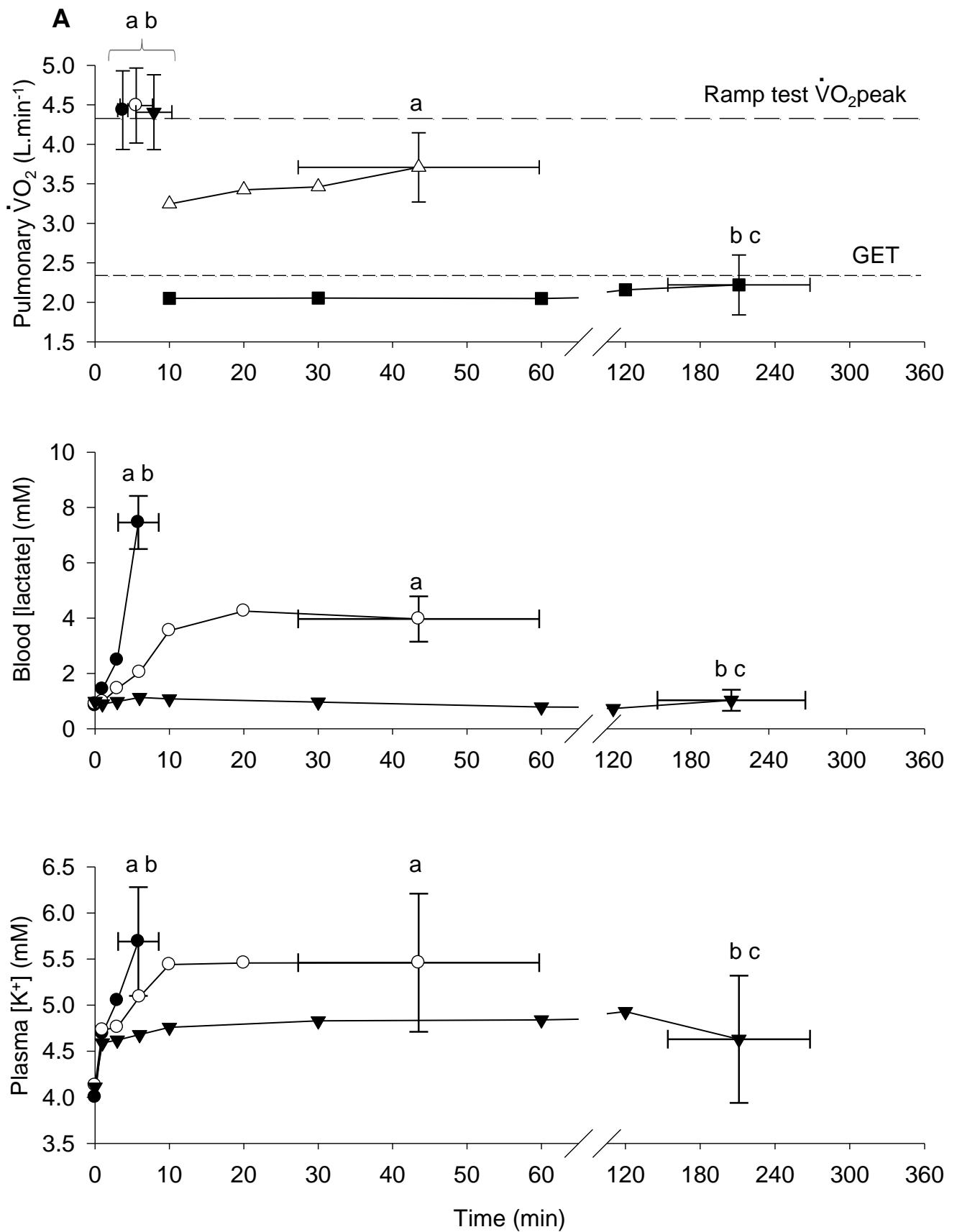


Figure 3

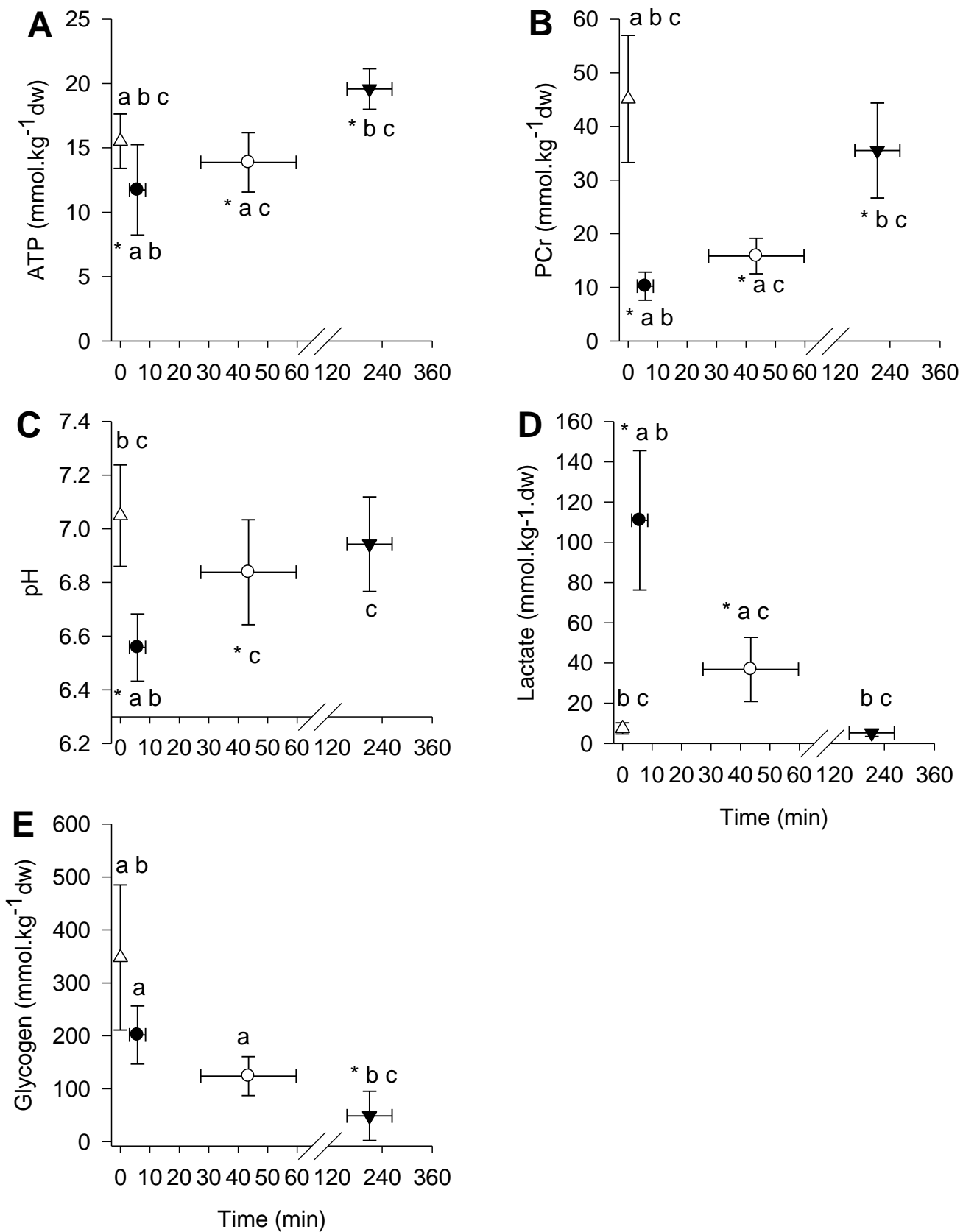
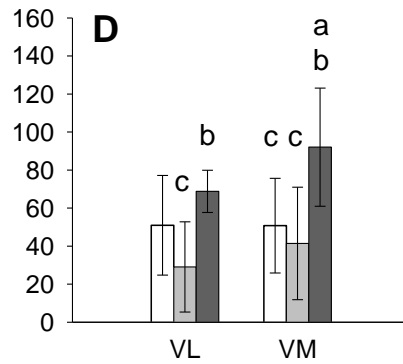
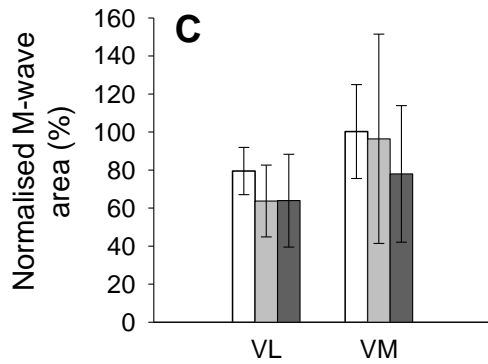
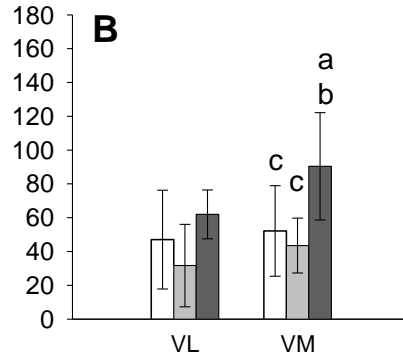
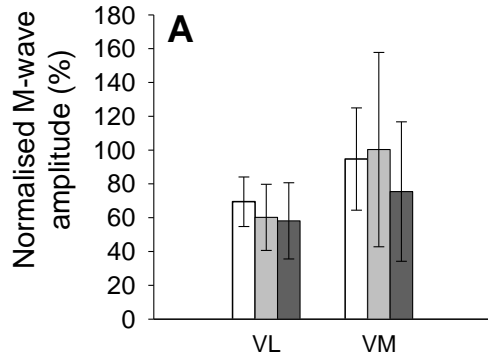


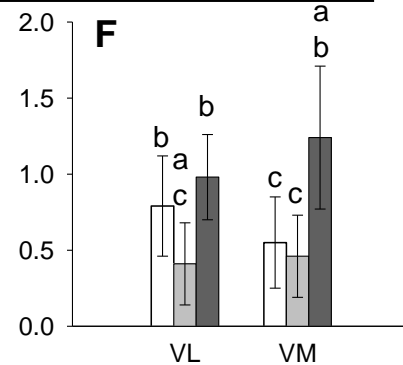
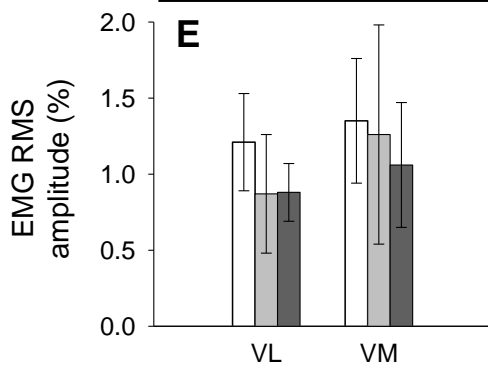
Figure 4



Muscle excitability at T_{lim}



Voluntary activation at T_{lim}



Neural drive at T_{lim}

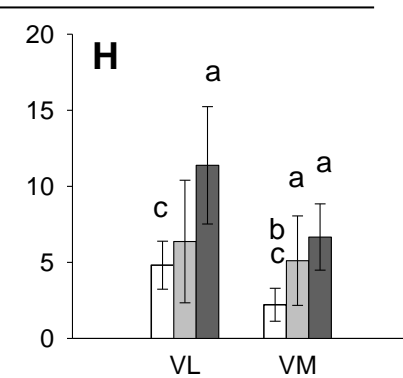
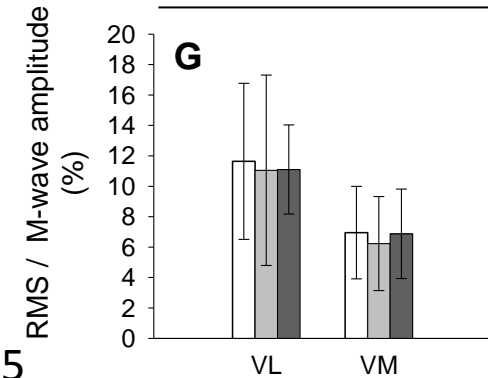


Figure 5

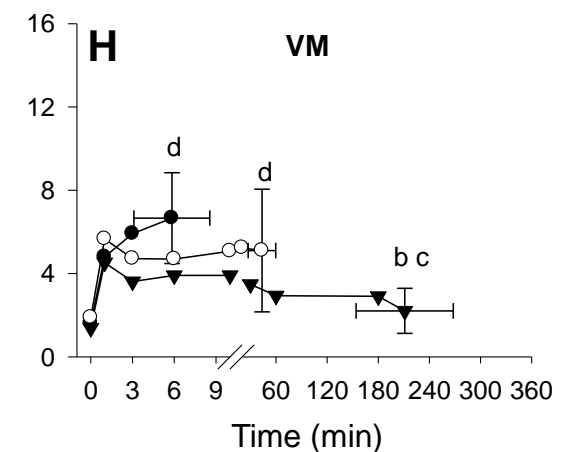
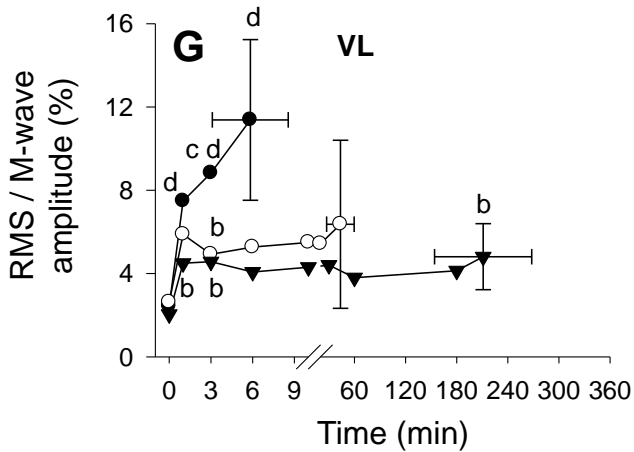
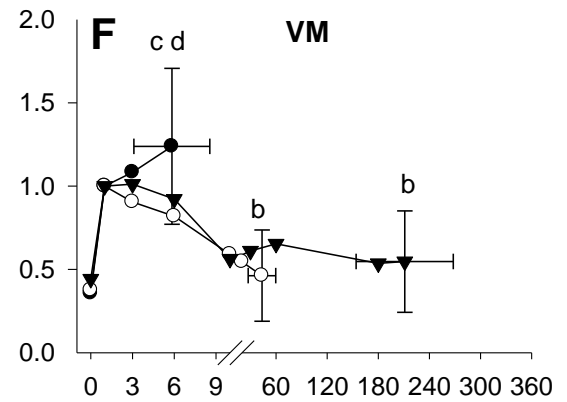
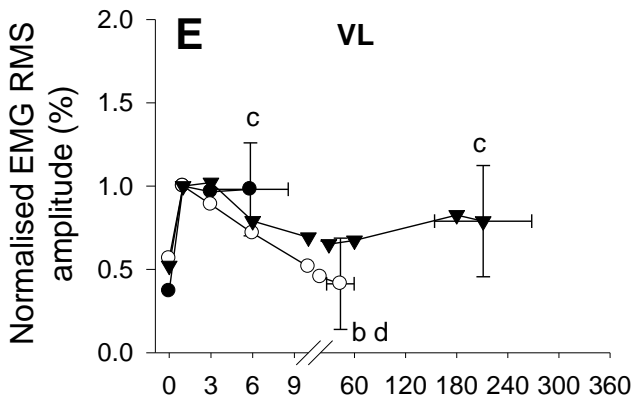
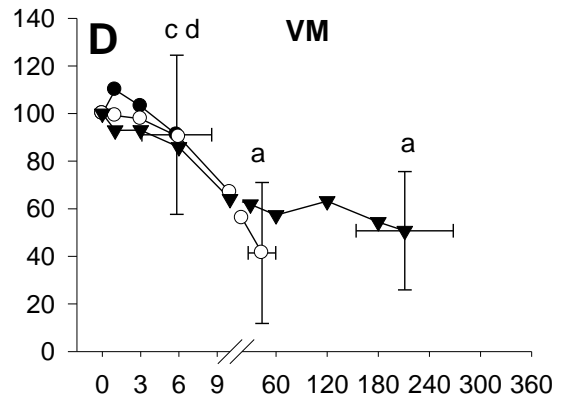
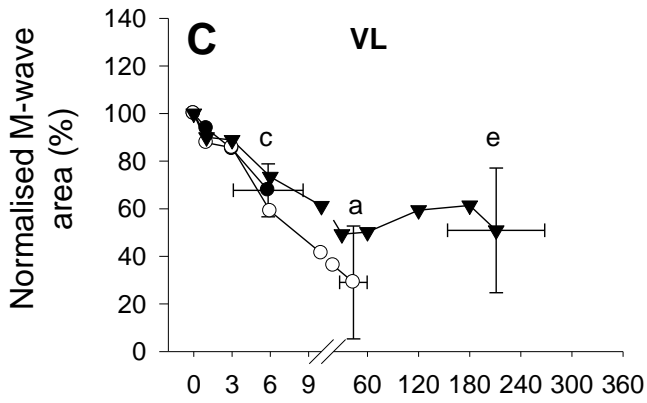
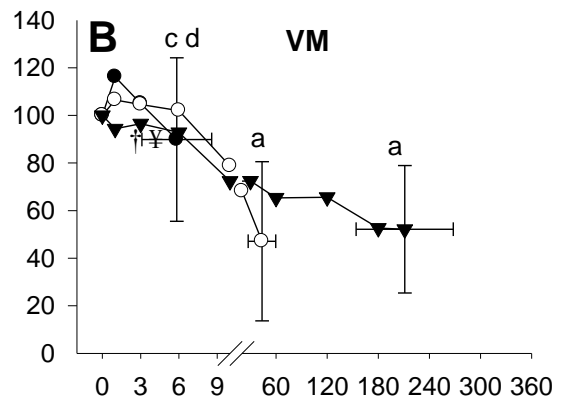
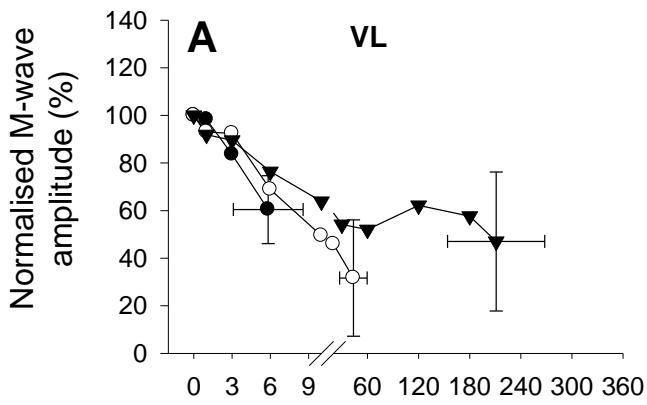


Figure 6