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Rest interval duration does not influence adaptations in acid/base transport proteins following 10 wk of sprint-interval training in active women

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1 **Title**

2 Rest interval duration does not influence adaptations in acid/base transport proteins following 10 weeks
3 of sprint-interval training in active women

4 **Running head**

5 Rest interval duration and muscle pH regulation

6 **Authors**

7 Cian McGinley^{1,3}, David J Bishop^{1,2}

8 ¹College of Sport and Exercise Science, ²Institute of Sport, Exercise and Active Living (ISEAL),
9 Victoria University, PO Box 14428, Melbourne, VIC 8001, Australia

10 ³Sportscotland Institute of Sport, Stirling, Scotland

11

12 **Corresponding author**

13 Cian McGinley

14 Sportscotland Institute of Sport

15 Airthrey Road

16 Stirling

17 FK9 5PH

18 Scotland

19

20 E: cian.mcginley@live.vu.edu.au

21 T: +44 (0) 1786 460100

22 F: +44 (0) 1786 460101

23 **Abstract**

24 The removal of protons (H^+) produced during intense exercise is important for skeletal muscle
25 function, yet it remains unclear how best to structure exercise training to improve muscle pH regulation.
26 We investigated whether 4 weeks of work-matched, sprint-interval training (SIT), performed 3 days per
27 week, with either 1 min (Rest-1; $n = 7$) or 5 min (Rest-5; $n = 7$) of rest between sprints, influenced
28 adaptations in acid/base transport protein content, non-bicarbonate muscle buffer capacity ($\beta_{m\text{in vitro}}$),
29 and exercise capacity in active women. Following one week of post-testing, comprising a biopsy, a
30 repeated-sprint ability (RSA) test, and a graded-exercise test, maintenance of adaptations was then
31 studied by reducing SIT volume to one day per week for a further 5 weeks. After 4 weeks of SIT, there
32 was increased protein abundance of monocarboxylate transporter (MCT)1, sodium/hydrogen exchanger
33 (NHE)1, and carbonic anhydrase (CA)XIV for both groups, but rest interval duration did not influence
34 the adaptive response. In contrast, greater improvements in total work performed during the RSA test
35 after 4 weeks of SIT was evident for Rest-5 compared to Rest-1 [effect size (ES): 0.51; 90% confidence
36 limits ± 0.37], whereas both groups had similarly modest improvements in $\dot{V}O_{2\text{peak}}$. When training
37 volume was reduced to one day per week, enhanced acid/base transport protein abundance was
38 maintained, although NHE1 content increased further for Rest-5 only. Finally, our data support
39 intracellular lactate as a signaling molecule for inducing MCT1 expression, but neither lactate nor H^+
40 accumulation appear to be important signaling factors in MCT4 regulation.

41

42 **Keywords**

43 pH regulation, lactate transport, intracellular buffering, repeated-sprint ability, detraining

44

45 **Introduction**

46 High-intensity exercise demands a large non-mitochondrial ATP turnover and results in
47 accumulation of protons (H^+) in skeletal muscle, which have been implicated in excitation–contraction
48 coupling inhibition (69), impairment of cross-bridge cycling (27), and inactivation of key glycolytic
49 enzymes (74). Mitigation of the exercise-induced reduction in intracellular pH (pH_i) entails uptake of
50 H^+ by physicochemical buffers, such as histidyl-imidazole residues and inorganic phosphate (P_i), and
51 buffering of H^+ by metabolic reactions in parallel with energy production (48). Acid/base transport
52 across the sarcolemma by specific proteins provides a means to remove H^+ from the myofiber, or to
53 introduce bicarbonate (HCO_3^-) or equivalents (CO_3^{2-} , $NaCO_3^-$) (50, 77). Given the importance of pH_i
54 regulation for muscle function and exercise capacity, much research has focused on the effects of
55 exercise training on acid/base transport proteins and muscle buffer capacity (β_m). Most of these studies
56 have employed different modalities of high-intensity interval training [HIIT; repeated exercise bouts at
57 intensities $\rightarrow 100\%$ maximal oxygen uptake ($\dot{V}O_{2max}$)], or sprint interval training [SIT; repeated exercise
58 bouts at intensities $\geq 100\% \dot{V}O_{2max}$] (84). However, equivocal findings to date indicate the stimuli
59 required for upregulation of the individual components of pH_i regulation remain to be determined.

60 The acid/base transport proteins can be categorized as either lactate (La^-)-coupled or
61 nonlactate-coupled. The former comprises the monocarboxylate transport proteins (MCT1 and MCT4)
62 and their chaperone protein basigin. The latter consists of the sodium hydrogen exchanger (NHE)
63 proteins, the skeletal muscle isoform being NHE1. H^+ -equivalent transport is provided by the sodium-
64 coupled bicarbonate transporter (NCBT) family of proteins, of which, isoform-specific protein evidence
65 has been shown for only the electrogenic sodium/bicarbonate cotransporter (NBCe)1 (63). In addition,
66 a functional, and perhaps physical interaction, has been demonstrated in vitro between each of these
67 acid/base transport proteins and the skeletal muscle carbonic anhydrase (CA) isozymes (6). While there
68 has been much investigation of the response to training of the MCTs and NHE1, though little of these
69 other proteins, the signaling networks responsible for regulation of any of the acid/base transport
70 proteins remain poorly understood compared to, for example, the well-described Akt/mTOR and
71 AMPK/PGC1- α pathways for resistance and endurance exercise, respectively (e.g. 29).

72 Notwithstanding this limited picture of transcriptional and translational events for the acid/base
73 transporters, there must be an initial signal(s) activated by exercise that initiates a phenotypic change
74 (65). One such signal previously proposed for different acid/base transporters is the accumulation, or
75 sustained production, of intracellular La^-/H^+ (38, 51, 53, 80). Through manipulating the rest period
76 between high-intensity work intervals, it should be possible to design contrasting exercise protocols
77 that result in either high absolute La^-/H^+ levels, or sustained production of La^-/H^+ . For example, muscle
78 pH has been shown to decrease even further in the first 90 s after a single 30-s sprint (17); therefore,
79 exercise comprising high-intensity work intervals, interspersed with rest periods < 90 s, should result
80 in large peaks in La^-/H^+ . However, given insufficient recovery of pH_i between intervals may result in
81 reduced glycogen phosphorylase (Phos) activity during subsequent intervals (74), sustained production
82 of La^-/H^+ may require longer rest between work intervals. With muscle pH recovering by ~50% within
83 4–6 min after single or multiple sprints (8, 17, 64), similar duration rest periods may maximize
84 anaerobic glycolysis for sequential intervals (74). Ultimately, by manipulating rest interval duration,
85 while matching intensity and total work, it may be possible to test whether different exercise-induced
86 changes in intracellular La^-/H^+ subsequently influence upregulation of specific acid/base transport
87 proteins.

88 In addition to the uncertainty as how best to elicit improvements in pH_i -regulatory components,
89 it is also not clear how detraining might be mitigated. Adaptations of acid/base transport proteins to
90 training have been shown to be transient following cessation of HIIT. In a previous study (63), we
91 found almost total reversal of adaptations in all of the acid/base transporters 6 weeks after completing
92 4 weeks of HIIT. Reductions in MCT1 and MCT4 abundance have similarly been reported 6 weeks
93 after stopping SIT (18). It is known that maintenance of some training-induced adaptations can be
94 achieved, or dysregulation mitigated, by reducing the volume of training while maintaining the intensity
95 (41, 42, 46, 47, 67). Although, it is possible that fractional degradation of some or all the acid/base
96 transport proteins can be mitigated by a reduced volume of high-intensity training, this hypothesis has
97 not been tested.

98 The present study therefore compared two different recovery durations between 30-s sprints
99 during work-matched SIT. We hypothesized that through altering the duration of the rest interval,
100 subsequent variations in intracellular La^-/H^+ would influence the adaptive responses of the acid/base
101 transport proteins. In addition, we postulated that improvements in βm and exercise performance would
102 reflect potential differences in substrate usage. Finally, we proposed that performing SIT one day per
103 week for 5 weeks, following a 4-week period of training 3 days per week, would be sufficient to
104 maintain any potential adaptations in acid/base transport proteins, βm , and repeated-sprint ability.

105

106 **Materials & Methods**

107 *Participants*

108 Twenty-one recreationally-active women gave written informed consent to participate in this
109 study. Six participants withdrew during the familiarization period because of either unrelated
110 illness/injury, or a lack of time, and one participant withdrew after the first SIT session (SIT1) because
111 of an unwillingness to undergo further biopsies. Fourteen participants completed the entire study [age:
112 24 (6) y; height: 164.6 (3.8) cm; mass: 62.9 (7.8) kg; $\dot{V}\text{O}_{2\text{peak}}$: 39.3 (5.3) $\text{mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$; mean (SD)].
113 Before the training intervention began, weekly training load was 1,005 (593) arbitrary units [mean
114 (SD)], calculated from session rating of perceived exertion and training duration (28). All procedures
115 were approved by the Victoria University Human Research Ethics Committee.

116 *Experimental Design*

117 The study employed a two-group parallel design (Fig. 1). There were two work-matched SIT
118 groups, differing only in the duration of the rest component of the duty cycle – either 1-min rest (Rest-
119 1: $n = 7$) or 5-min rest (Rest-5: $n = 7$). Following familiarization trials and pre-training tests, participants
120 were ranked on total work performed during a repeated-sprint ability (RSA) test, and coded
121 consecutively with either an A or a B in ABBA format. Using random generation of binary numbers,
122 type of training was then randomly allocated to group A (Rest-5) and group B (Rest-1).

123 The study consisted of two training phases, participants trained three days per week for the first
124 four weeks and, following one week of post-testing, they then trained one day per week for five weeks.
125 Before (Pre) and after four weeks (+4 wk) and ten weeks (+10 wk) of training, the following
126 experimental trials were conducted at least two days apart: 1) an RSA test, 2) a graded-exercise test,
127 followed 5 min later by a square wave peak oxygen uptake ($\dot{V}O_{2\text{peak}}$) test (Pre and +4 wk only), and 3)
128 a resting muscle biopsy. The resting muscle biopsy Pre was conducted before the first training session,
129 with a second biopsy (Post) taken immediately following exercise [detailed in First SIT Session (SIT1)
130 subsection].

131 Participants were familiarized with all exercise tests before undertaking experimental trials.
132 They performed two familiarization trials of the RSA test on separate days, and on another day they
133 performed familiarization trials of the graded-exercise test and peak oxygen uptake ($\dot{V}O_{2\text{peak}}$) test. In
134 the week preceding the +10-wk trial, participants performed a re-familiarization of the RSA test.

135 Insert Figure 1 here

136 *Sprint-Interval Training*

137 The training intervention consisted of supervised, work-matched SIT performed on an
138 electromagnetically-braked cycle ergometer (Velotron, Racer-Mate, Seattle, WA). Participants
139 performed a standardized 5-min steady state warm-up at 60 W, followed by 4 × 30-s sprints in the first
140 session, increasing to a maximum of 10 × 30-s sprints in the tenth session (see Table 1 for training
141 program). All sprints were interspersed with either 1 min (Rest-1) or 5 min (Rest-5) of passive recovery.
142 Training intensity was initially set at 200% of the power at the pre-training lactate threshold (LT),
143 increasing to 305% of the pre-training LT by week 4, which was equivalent to between 121 (14)% and
144 186 (17)% of baseline peak aerobic power (W_{peak}) during the first 4 weeks. The 13th session training
145 intensity was adjusted to 300% of the +4 wk LT, or where necessary to avoid a reduction in intensity,
146 adjusted to up to 350% of the LT. This was equivalent to between 190 (13)% and 204 (14)% of the +4
147 wk W_{peak} during weeks 6 to 10.

148 Insert Table 1 here

149 *Experimental Trials*

150 All exercise and/or biopsy trials were performed in the morning (06.00–11.00) following an
151 overnight fast, with participants recording and subsequently replicating a food diary for the 24 h prior
152 to each exercise trial. They were also instructed not to drink caffeine on any trial day, and to abstain
153 from drinking alcohol or exercising in the preceding 24 h, with compliance verified by pre-trial
154 questionnaires. Participants were asked to maintain their normal level of activity throughout the study
155 and completed a bespoke web-based daily training diary to monitor compliance.

156 *First SIT Session (SIT1)*

157 To characterize both training protocols, changes in blood and muscle pH and $[La^-]$, and βm ,
158 were measured in response to the first SIT session. The exercise bout consisted of 4 × 30-s sprints at
159 200% LT, with either 1 min or 5 min of rest between sprints, as per group allocation. Muscle biopsies
160 were taken at rest before exercise (Pre) and immediately following the final sprint (Post). Venous blood
161 samples were taken at rest (before the muscle biopsy), and 2, 3, 5, 7, and 10 min after the final sprint.
162 A 22 g IV cannula was inserted into an antecubital vein. Before each sample the cannula was flushed
163 with a small volume of sterile saline and then ~1 mL of blood was drawn into a dry syringe and
164 discarded. Samples were taken with the participant recumbent on a plinth. Approximately 3 mL of
165 blood was drawn into a heparinized syringe (Rapidlyte, Siemens Healthcare, Melbourne, VIC,
166 Australia). Air was immediately removed from the sample by ejecting blood into the cap and carefully
167 tapping the syringe. The syringe was then gently rolled to mix the sample thoroughly. Unless the
168 sample was ready to be analyzed without delay, the syringe was placed in an ice slurry to minimize
169 changes in pH (23), and subsequently analyzed for blood pH (Rapidpoint 405, Siemens Healthcare,
170 Melbourne, VIC, Australia). Prior to this, about 0.5 mL of blood was dispensed into a microtube and
171 immediately analyzed for lactate (2300 STAT Plus, YSI Inc., Yellow Springs, OH), or kept in an ice
172 slurry for imminent analysis.

173 *Needle Muscle Biopsies*

174 Muscle biopsies were taken from the belly of the vastus lateralis, approximately halfway
175 between the knee and hip, using the needle biopsy technique modified with suction (25). Subsequent

176 samples were taken approximately 1 cm from a previous biopsy site. An incision was made under local
177 anesthesia (1% Xylocaine) and a muscle sample taken using a Bergström needle (9). Samples were
178 blotted on filter paper to remove blood, before being immediately snap-frozen in liquid nitrogen and
179 then stored at -80°C until subsequent analyses. Muscle samples were taken from the dominant leg
180 before (Pre) and immediately after (Post) the first training session, 3 d after the twelfth training session
181 (+4 wk), and 3 d after the seventeenth training session (+10 wk).

182 *Repeated-Sprint Ability Test*

183 An isokinetic RSA test was conducted on a separate day before training commenced (Pre), and
184 after 4 weeks (+4 wk) and 10 weeks of SIT (+10 wk). The test was performed at 110 rpm while seated
185 on an electromagnetically-braked cycle ergometer (Excalibur Sport, Lode, Groningen, The
186 Netherlands). It followed the same protocol to that previously reported (63), comprising 5×6 -s
187 maximal sprints, separated by 24 s of rest. During the recovery period participants remained stationary.
188 Five seconds before the start of each sprint participants assumed the ready position – crank of the
189 dominant leg at an angle of 45° . Initially, participants performed a 5-min steady-state warm-up at 60
190 W, followed by 2×3 -s practice sprints at 90% of perceived maximal effort, separated by 24 s of rest.
191 A criterion 6-s maximal sprint was then performed 90 s later, followed by 5 min of rest prior to the RSA
192 test. To minimize pacing, participants were required to achieve $\geq 90\%$ of their criterion score in the
193 first sprint of the 5×6 -s test. If this criterion was not achieved, participants were required to rest for a
194 further five minutes before restarting the test (one Rest-1 participant's +10 data were later excluded
195 from the analyses for failing to meet this criterion). Before the first practice sprint, the single 6-s sprint,
196 and the first sprint of the 5×6 -s test, participants were required to accelerate to 110 rpm and stop
197 pedaling instantly, followed 24 s later by the requisite sprint. This ensured similar velocity of the
198 flywheel before each sprint. Consistent verbal encouragement was given throughout. Total work and
199 work decrement were calculated from the raw data as follows (14):

$$200 \text{ work decrement (\%)} = \left(\frac{\text{total work} - \text{ideal work}}{\text{ideal work}} \times 100 \right)$$

201 where total work = actual work performed in all 5 sprints,

202 and ideal work = work performed in sprint number 1 × 5.

203 *Graded-Exercise Test*

204 Graded-exercise tests were performed pre-training and at +4 wk to determine the LT from
205 venous blood samples (taken from an antecubital vein, as described above), and W_{peak} was also
206 recorded. Blood samples were taken at rest and at the end of every stage during the graded-exercise
207 test. Samples were drawn into a 3-mL dry syringe and aliquoted into a microtube for instant analysis
208 of lactate (2300 STAT Plus, YSI Inc., Yellow Springs, OH). The tests were performed seated on the
209 same ergometer used for the RSA test, employing an intermittent protocol, with 4-min exercise stages
210 and 30-s rest stages. Beginning at 50 W, the power was subsequently increased by 25 W every 4.5 min
211 and consistent verbal encouragement was provided for the latter stages. Participants were required to
212 maintain a set cadence that was self-selected during the familiarization trial and repeated for each of
213 their subsequent graded-exercise tests. The test was terminated either volitionally by the participant, or
214 by the assessors when the participant could no longer maintain the required cadence (± 10 rpm), despite
215 strong verbal encouragement. The LT was identified as the power at which venous blood lactate
216 increased by 1 mM above baseline, and was calculated using Lactate-E version 2.0 software (72). W_{peak}
217 was calculated as previously reported (57):

$$218 \quad W_{\text{peak}} = W_{\text{final}} + \left(\frac{t}{240} \times 25 \right)$$

219 where W_{final} was the power output of the last completed stage and t was the time in seconds of any final
220 incomplete stage.

221 *Peak Oxygen Uptake Test*

222 Immediately after the graded-exercise test, participants cycled for 5 min at 20 W, followed by
223 a square-wave $\dot{V}O_{2\text{peak}}$ test at 105% of W_{peak} achieved during the graded-exercise test. Participants were
224 instructed to accelerate to 90–100 rpm at the commencement of a 5-s countdown, and to maintain a
225 high, but not fixed cadence until volitional fatigue. Consistent verbal encouragement was provided
226 throughout. Expired gases were analyzed every 15 s using a custom-made metabolic cart. A two-point
227 calibration of the gas analyzers (S-31A/II and CD-3A analyzers, Ametek, Berwyn, PA, USA) was

228 performed before each test using one certified gravimetric gas (16.1% O₂, 4.17% CO₂; BOC Gases,
229 Chatswood, NSW, Australia) and ambient air. Ventilation was recorded every 15 s. The ventilometer
230 (KL Engineering, Sunnyvale, CA, USA) was calibrated at the start of each day using a 3-L syringe
231 (MedGraphics, St. Paul, MN). $\dot{V}O_{2\text{peak}}$ was calculated as the mean of the two highest consecutive 15-s
232 values. Exercise duration was 145 (30) s [mean (SD)].

233 *Muscle Buffer Capacity*

234 Non-bicarbonate muscle buffer capacity ($\beta_{\text{in vitro}}$) was measured in duplicate on 2–3 mg dry
235 mass (dm) of freeze-dried muscle samples using the titration technique (60). Samples were dissected
236 free of visible blood and connective tissue, then homogenized on ice for 3 × 30 s in a 10 mM solution
237 of the glycolytic inhibitor NaF (0.1 mL of NaF per 3 mg dm). pH measurements were performed at
238 37°C with a glass microelectrode (MI-410, Microelectrodes, Bedford, NH) connected to a pH meter
239 (Lab 850, Schott Instruments GmbH, Mainz, Germany). After the initial pH measurement,
240 homogenates were adjusted, if necessary, to pH 7.1–7.2 with 0.02 M NaOH, and then titrated to pH
241 6.1–6.2 with the serial addition of 2 μL of 0.01 M HCl. From linear regression, the number of moles
242 of H⁺ required to change pH from 7.1–6.5 was interpolated, then expressed per whole pH unit and
243 relative to sample mass. The typical error (within-sample standard deviation) for repeat titrations was
244 10.3 mmol H⁺•kg dm⁻¹•pH⁻¹, equivalent to a CV of 7.7% (16).

245 To determine the potential short-term effects of the different SIT protocols on muscle buffer
246 capacity, non-protein $\beta_{\text{in vitro}}$ was also assayed on the pre- and post-SIT1 samples using the titration
247 technique (11). Briefly, the homogenate was deproteinized with the addition of 0.03% 5-sulfosalicylic
248 acid hydrate solution (100% w/v in 10 mM NaF), and centrifuged for 10 min at 1,000g. Following a
249 similar procedure to above, the pH of the supernatant was adjusted to 7.1–7.2 with the minimum volume
250 of strong concentration NaOH, and then titrated to pH 6.1–6.2 with the serial addition of 2 μL aliquots
251 of 5 mM HCl. Non-protein $\beta_{\text{in vitro}}$ was calculated as above, with the protein $\beta_{\text{in vitro}}$ estimated from
252 the difference between whole muscle and non-protein $\beta_{\text{in vitro}}$. The typical error for repeat titrations
253 was 6.7 mmol H⁺•kg dm⁻¹•pH⁻¹, equivalent to a CV of 10.4% (16).

254 *Muscle Lactate Assay*

255 Approximately 4 mg of freeze-dried pre- and post-SIT1 muscle samples were assayed
256 enzymatically for lactate by spectrophotometric analysis (xMark microplate absorbance
257 spectrophotometer, Bio-Rad Laboratories, Hercules, CA) using a modification of a previously described
258 assay (75). Samples were dissected free of visible blood and connective tissue, then homogenized in
259 0.6 M perchloric acid using a TissueLyser II (Qiagen, Valencia, CA). Homogenates were then
260 centrifuged at 10,000g for 10 min at 4°C and the deproteinized supernatant removed. 1.2 M KOH and
261 0.125 M HEPES were added and the supernatant adjusted to pH 9.0, with 3.0 M KCl then added.
262 Following further centrifugation at 10,000g for 10 min at 4°C, the perchlorate salts were discarded and
263 the supernatant stored at -80°C until analysis. Lactate content was measured in triplicate against 9
264 dilutions of a sodium lactate standard (L7022, Sigma-Aldrich, St. Louis, MI). The typical error was 1.5
265 mmol H⁺•kg dm⁻¹, equivalent to a CV of 2.4% (15).

266 *Quantitative Western Blotting*

267 *Muscle homogenate preparation*

268 Approximately 30 mg of frozen muscle tissue were homogenized (Kontes Pellet Pestle, Kimble
269 Chase, NJ, USA) in a 1:20 dilution of ice-cold RIPA buffer (pH 7.4) containing: 0.15 M NaCl, 1%
270 Triton-X100, 0.5% sodium deoxycholate, 0.05 M Tris, 0.1% SDS, 1 mM EDTA, 1X
271 protease/phosphatase inhibitor cocktail (5872, Cell Signaling Technology, Danvers, MA, USA), and 1
272 mM PMSF. NBCe1 was assayed in muscle tissue homogenized in a HEPES-sucrose buffer (pH 7.4)
273 containing: 210 mM sucrose, 30 mM HEPES, 40 mM NaCl, 2 mM EGTA, 5 mM EDTA, 1% protease
274 inhibitor cocktail (P8340, Sigma-Aldrich), 1% phosphatase inhibitor cocktail (P5726, Sigma-Aldrich),
275 and 1 mM PMSF. Homogenates were rotated end-over-end for 60 min at 4°C and centrifuged twice at
276 15,000g for 10 min at 4°C. The supernatants were collected and the pellets discarded.

277 *Protein assay*

278 Protein content of muscle homogenate was measured in triplicate using a Bradford assay (Bio-
279 Rad protein assay dye reagent concentrate) against serial dilutions of bovine serum albumin standards
280 (A9647, Sigma-Aldrich).

281 *Immunoblotting*

282 Immunoblotting for each antibody has been previously described in detail, including validation
283 steps taken for specific antibodies (63). Briefly, homogenate was diluted in Laemmli buffer and equal
284 amounts of total protein (1–25 µg) were loaded in different wells on 10% or 12% Tris-Glycine-HCl
285 SDS-PAGE gels, or in Criterion 4–12% Bis-Tris SDS-PAGE gels (Bio-Rad) for NBCe1 and CAIV.
286 All samples for a participant were loaded on the same gel. Five or six different dilutions of a mixed-
287 homogenate internal standard were also loaded on every gel and a calibration curve plotted of density
288 against protein amount. From the subsequent linear regression equation, protein abundance was
289 calculated from the measured band intensity for each sample on the gel (68). Coomassie blue (Phastgel
290 Blue R-350, GE Healthcare, Rydalmere, NSW, Australia) staining of total protein was used as a loading
291 control.

292 Gel electrophoresis ran for 60–80 min at 100 V or 140 V, and proteins were then wet-transferred
293 at 100 V to a 0.2 µm polyvinylidene fluoride membrane. Membranes were blocked for 60 min at room
294 temperature in 5% non-fat dry milk diluted in Tris-buffered saline with 0.1% Tween-20 (TBST).
295 Following TBST wash steps, membranes were incubated either overnight or for 2 h with the appropriate
296 primary antibody (see Table 2 for conditions). After further TBST washes, the membranes were
297 incubated for 90 min at room temperature in the appropriate secondary antibody. After further washes,
298 membranes were incubated in chemiluminescent solution for 2 min and images were taken with a
299 VersaDoc Imaging System (Bio-Rad, Hercules, CA) fitted with a CCD camera (Bio-Rad).
300 Densitometry was performed with Image Lab 5.0 software (Bio-Rad) using the volume calculation and
301 background correction was applied individually to each lane using a rolling-ball algorithm. Images of
302 example blots are displayed in their entirety, except where membranes were cut as described. CVs of
303 replicate gels for individual proteins are presented in their respective figure captions.

304 A Pre sample for one Rest-5 participant was blood contaminated, necessitating exclusion of her
305 western blot data for MCT1, NHE1, NBCe1, and CAII. In addition, there was insufficient muscle
306 biopsied to allow analyses of all proteins for another Rest-5 participant's +10 wk sample.
307 Consequently, there are no +10 wk data for CAIV, CAXIV, and NBCe1 for this participant. Finally,
308 in analyzing NBCe1 at +10 wk for one Rest-1 participant, the signal for this band was below the
309 threshold of detection.

310 Insert Table 2 here

311 *Statistical Analyses*

312 Measures of centrality and dispersion are mean (SD) unless otherwise stated. To reduce bias
313 from non-uniformity of error, data were logarithmically-transformed where heteroscedasticity was
314 present (70), such as for western blot data. For these data, geometric mean and standard deviation are
315 reported (geometric mean \times/\div SD). Data were analyzed using linear mixed models with, for example,
316 'time' (repeated-measure), 'group', and 'group \times time' as fixed factors; and 'subject' and 'intercept' as
317 random factors. First-order autoregressive covariance structures were used for all models and model
318 fit was assessed by -2 log-likelihood (26). Effect sizes (ES) were assessed using Cohen's d , with ES
319 thresholds defined as trivial < 0.2 , small < 0.6 , moderate < 1.2 , large < 2.0 , very large < 4.0 , and
320 extremely large ≥ 4.0 (45). Uncertainty of effects is expressed as 90% confidence limits (90% CL) and
321 P values, with the latter presented as precise values unless $P < 0.001$ (19). Effects were not considered
322 meaningful if there was $< 75\%$ probability of being substantially positive relative to the smallest
323 worthwhile change (ES = 0.2), and were deemed unclear if there was a greater than 5% probability of
324 also being substantially negative (44, 45). If no between-group differences were found, within-group
325 effects are reported for pooled group data. Linear mixed models were analyzed using IBM SPSS
326 Statistics V21 (IBM Corporation, Somers, NY, USA), and effect sizes and confidence limits were
327 calculated using custom Excel spreadsheets (44). Total work performed by the two groups during the
328 training intervention was compared by an independent sample, unequal variance t test, and by effect
329 size and confidence limits calculated from a spreadsheet for comparison of two independent groups
330 (43).

331 **Results**

332 *Training Data*

333 Both training groups performed similar volumes of total work throughout the training
334 intervention, 1.19 (0.15) MJ and 1.15 (0.23) MJ for Rest-1 and Rest-5, respectively ($t_{12} = 0.37$, $P =$
335 0.72 , ES: 0.18; 90% CL ± 0.90). There was excellent training compliance, with only a single training
336 session being missed by one participant.

337 *Physiological Responses to SIT1*

338 Short-term physiological responses to SIT1 are shown in Figure 2 and Figure 3. Following
339 SIT1, muscle $[La^-]$ increased to 84.2 (20.3) $mmol \cdot kg \text{ dm}^{-1}$ and 61.8 (31.9) $mmol \cdot kg \text{ dm}^{-1}$ for Rest-1
340 and Rest-5, respectively (time main effect: $F_{1,15} = 209.26$, $P < 0.001$, pooled ES: 5.49, 90% CL $\times/\div 0.72$),
341 but there was no clear difference between groups (group \times time interaction: $F_{1,15} = 0.86$, $P = 0.37$, ES:
342 0.70 , $\times/\div 1.42$). Assuming a muscle water content of $3.3 \text{ L} \cdot kg \text{ dm}^{-1}$ (48), this equates to 25.5 (6.2) mM
343 and 18.7 (9.7) mM for Rest-1 and Rest-5. Muscle pH decreased more for Rest-1, 6.92 (0.11) to 6.53
344 (0.14), than Rest-5, 6.89 (0.13) to 6.63 (0.18) (group \times time interaction: $F_{1,15} = 3.50$, $P = 0.08$, ES: 1.07;
345 ± 1.11).

346 Mean $\beta_{m_{in \text{ vitro}}}$ did not change for either group following SIT1 (time main effect: $F_{1,15} = 0.008$,
347 $P = 0.93$, pooled ES: 0.03; ± 0.65). $\beta_{m_{in \text{ vitro}}}$ was lower Pre for Rest-5 than Rest-1, 146.7 (11.4) and
348 134.8 (9.5) $mmol \text{ H}^+ \cdot kg \text{ dm}^{-1} \cdot pH^{-1}$, respectively (group main effect: $F_{1,15} = 14.4$, $P = 0.002$, Pre ES:
349 1.06 ; ± 1.37). There was also no change in mean non-protein $\beta_{m_{in \text{ vitro}}}$ after SIT1 (time main effect: $F_{1,15}$
350 $= 0.66$, $P = 0.43$, pooled ES: 0.29; ± 0.63); nor was there a meaningful difference between groups (group
351 main effect: $F_{1,15} = 1.92$, $P = 0.19$, ES: 0.48; ± 1.36). The measured non-protein $\beta_{m_{in \text{ vitro}}}$ at rest was 62.6
352 (8.0) $mmol \text{ H}^+ \cdot kg \text{ dm}^{-1} \cdot pH^{-1}$, which was 44 (6)% of total $\beta_{m_{in \text{ vitro}}}$ (range: 34% to 59%). Therefore, the
353 estimated protein $\beta_{m_{in \text{ vitro}}}$ was 76.5 (9.1) $mmol \text{ H}^+ \cdot kg \text{ dm}^{-1} \cdot pH^{-1}$.

354 Insert Figure 2 here

355 Venous blood pH decreased from 7.36 (0.03) Pre to 7.21 (0.07) at +2 min following SIT1 (time
356 main effect: $F_{5,68.4} = 34.1$, $P < 0.001$). There were no clear differences in blood pH between the two

357 groups (group×time interaction: $F_{5,68.4} = 0.48$, $P = 0.79$) due to the high within-group variability
358 (between-group +2 min ES: 0.97; ± 2.13). Mean venous blood $[La^-]$ peaked at +7 min (6.60 mM and
359 5.50 mM for Rest-1 and Rest-5), with a greater increase apparent for Rest-1 at +5 min and +7 min
360 (group×time interaction: $F_{5,64.7} = 2.77$, $P = 0.025$, +5 min ES: 1.08, $\times/\div 1.12$, +7 min ES: 1.24, $\times/\div 1.16$).

361 Insert Figure 3 here

362 *Protein Abundance*

363 *MCT1*

364 The MCT1 antibody recognized a single band at approximately 50 kDa (Fig 4A). MCT1
365 protein content did not differ between the two groups (group×time interaction: $F_{2,25.2} = 0.17$, $P = 0.84$,
366 +4 wk ES: 0.04, $\times/\div 0.57$). On pooling the data there was an increase at +4 wk of 1.18-fold $\times/\div 1.23$
367 (time main effect: $F_{2,25.2} = 3.18$ $P = 0.06$, ES: 0.44, $\times/\div 0.27$). Comparing the pooled difference scores
368 from +4 wk to +10 wk, i.e. when training volume reduced from 3 d to 1 d per week, there was no change
369 in mean MCT1 abundance (ES: -0.13 , $\times/\div 0.37$).

370 *MCT4*

371 The MCT4 antibody recognized a strong band at ~50 kDa and a weaker band at ~75 kDa in
372 some samples (Fig 4B); only the 50 kDa band was quantified. There were no differences between the
373 two groups (group×time interaction: $F_{2,28.1} = 0.30$, $P = 0.74$, +4 wk ES: 0.21, $\times/\div 0.82$). Overall, mean
374 MCT4 abundance did not change in response to training (time main effect: $F_{2,28.1} = 0.46$, $P = 0.64$,
375 pooled +4 wk ES: 0.09, $\times/\div 0.39$).

376 *Basigin*

377 The basigin antibody detected a single band just above 37 kDa (Fig 4C), likely the canonical
378 basigin-2 isoform (63). Basigin content did not differ between the two groups (group×time interaction:
379 $F_{2,28.3} = 0.20$, $P = 0.82$, +4 wk ES: 0.06, $\times/\div 0.61$). On pooling the data there were no meaningful changes
380 (time main effect: $F_{2,28.3} = 3.04$, $P = 0.06$, +4 wk ES: 0.30, $\times/\div 0.29$, +10 wk ES: 0.02, $\times/\div 0.31$).

381 *NHE1*

382 The NHE1 antibody recognized a single or a double band just below 100 kDa (Fig. 4D). The
383 predicted molecular mass of NHE1 is 91 kDa, therefore it is probable that the identified band(s)
384 represent non- or partially-glycosylated NHE1 protein (39). As per previous reports, both bands were
385 quantified if present (55). Overall there was an increase in NHE1 abundance following training (time
386 main effect: $F_{2,25.3} = 4.00$, $P = 0.03$, pooled +4 wk ES: 0.57, $\times/\div 0.33$). The two groups responded
387 differently to training (group \times time interaction: $F_{2,25.3} = 5.23$, $P = 0.013$). There were similar increases
388 at +4 wk of 1.11-fold $\times/\div 1.09$ and 1.15-fold $\times/\div 1.22$, for Rest-1 and Rest-5 respectively (ES: 0.16,
389 $\times/\div 0.80$), but when training volume was reduced to 1 d per week NHE1 decreased to baseline for Rest-
390 1, while for Rest-5 NHE1 increased 1.44-fold $\times/\div 1.18$ compared to baseline (+10 wk ES: 1.68, $\times/\div 0.97$).

391 Insert Figure 4 here

392 *NBCe1*

393 The NBCe1 antibody recognized a band close to the predicted molecular mass of 121 kDa for
394 the canonical NBCe1-B splice variant (Fig. 5). Stronger signal bands of unknown origin were also
395 detected at about 50 kDa and 75 kDa; only the ~120 kDa band was quantified (63). Overall there was
396 no meaningful change in NBCe1 abundance following training (time main effect: $F_{2,17.8} = 0.23$, $P =$
397 0.80, pooled +4 wk ES: 0.15, $\times/\div 0.38$). Mean NBCe1 content increased for Rest-1 (1.30-fold $\times/\div 1.55$)
398 and decreased for Rest-5 (0.89-fold $\times/\div 1.47$) after 4 weeks of SIT due to high individual
399 variability/outliers (group \times time interaction: $F_{2,17.8} = 1.14$, $P = 0.34$, +4 wk ES: 0.64, $\times/\div 0.72$). There
400 was no meaningful difference between groups at +10 wk (ES: 0.42, $\times/\div 0.94$).

401 Insert Figure 5 here

402 *CAII*

403 The CAII antibody recognized a single band just above 25 kDa (Fig. 6A). There was no
404 difference between groups in CAII content over time (group \times time interaction: $F_{2,24.9} = 0.08$, $P = 0.92$,
405 +4 wk ES: 0.09, $\times/\div 0.64$). On pooling the data CAII content decreased 0.92-fold $\times/\div 1.11$ following
406 training (time main effect: $F_{2,24.9} = 3.79$, $P = 0.04$, +4 wk ES: -0.47, $\times/\div 0.31$).

433

Insert Figure 7 here

434 *Repeated-Sprint Ability*

435 Total work performed during the RSA test improved only in the Rest-5 group (Fig. 8A)
436 (group×time interaction: $F_{2,27.0} = 4.60$, $P = 0.02$, +4 wk ES: 0.51; ± 0.37). On reducing SIT volume to
437 1 d per week, total work for Rest-5 decreased slightly from 13.9 (2.2) kJ to 13.5 (2.6) kJ, while for Rest-
438 1 it did not change from 14.0 (2.1) kJ at +4 wk to 14.0 (2.5) kJ at +10 wk. Hence, there was still a clear
439 difference between groups at +10 wk (ES: 0.42; ± 0.50). Comparing the change in work performed
440 during each individual sprint (Fig. 8C), Rest-5 had better improvements for the last three sprints at +4
441 wk compared to Rest-1 (Sprint 3 ES: 0.57; ± 0.49 , Sprint 4 ES: 0.78; ± 0.50 , Sprint 5 ES: 0.51; ± 0.46).
442 For both groups work decrement during the RSA test improved following four weeks of training (Fig.
443 8B) (time main effect: $F_{2,27.4} = 9.57$, $P = 0.001$, pooled +4 wk ES: 0.95; ± 0.52). There were no clear
444 differences between the two groups (group×time interaction: $F_{2,27.4} = 1.84$, $P = 0.18$, +4 wk ES: 0.67;
445 ± 1.05 , +10 wk ES: 0.04; ± 1.08). There was no difference between the pooled +4 wk and +10 wk data,
446 indicating that better work decrement was maintained with 1 d per week training (pooled ES: -0.20 ;
447 ± 0.32).

448

Insert Figure 8 here

449 *Aerobic Capacity*

450 Power at the LT changed little in response to training for either group (Fig. 9A) (time main
451 effect: $F_{1,14} = 2.47$, $P = 0.14$, pooled ES: 0.24; ± 0.59). There was also no clear difference between the
452 two groups (group×time interaction: $F_{1,14} = 1.79$, $P = 0.20$, ES: 0.41; ± 0.59). As with the LT, mean
453 W_{peak} did not change with training (Fig. 9B) (time main effect: $F_{1,14} = 2.84$, $P = 0.11$, pooled ES: 0.19;
454 ± 0.21), nor was there a difference between the two groups (group×time interaction: $F_{1,14} = 0.23$, $P =$
455 0.64, ES: 0.10; ± 0.42). $\dot{V}O_{2\text{peak}}$ did not change differently between the two groups (Fig. 9C)
456 (group×time interaction: $F_{1,14} = 0.01$, $P = 0.91$, ES: 0.03; ± 0.44). On pooling the data there was a small
457 increase in $\dot{V}O_{2\text{peak}}$ of 2.2 mL·min⁻¹·kg⁻¹ (90% CL ± 1.1 mL·min⁻¹·kg⁻¹) (time main effect: $F_{1,14} = 12.00$,
458 $P = 0.004$, pooled ES: 0.38; ± 0.20).

459

Insert Figure 9 here

460 **Discussion**

461 This study provides a comprehensive analysis of the acid/base transport protein response to SIT
462 in active women. Our main finding was that selective upregulation of these proteins was evident
463 following SIT, but manipulation of rest interval duration (1 or 5 min) did not influence the adaptive
464 response. Increased abundance of MCT1, NHE1, and CAXIV was seen after 4 weeks, with either no
465 change or a decrease evident for MCT4, NBCe1, basigin, CAII, CAIII, and CAIV. There was also no
466 change in $\beta m_{in\ vitro}$. Greater improvements in RSA were seen with Rest-5 compared to Rest-1, while
467 both groups had similar improvements in aerobic capacity. A second major finding was that enhanced
468 protein abundance and RSA were maintained when training volume was reduced to 1 d per week,
469 although disparate responses between groups were seen for NHE1. Overall, these data demonstrate that
470 short-term, supramaximal-intensity exercise has modest effects on pH-regulatory components
471 compared to, for example, the rapid mitochondrial remodeling reported following SIT (30).

472 MCT1 protein content increased for both Rest-1 and Rest-5, but in common with most studies
473 there were no changes in MCT4 protein content following 4 weeks of SIT (3, 10, 32, 49, 66, 76).
474 Although the signaling factors responsible for regulation of the MCTs in vivo remain to be determined,
475 the current study supports the existing in vitro evidence that they respond to distinct stimuli (34, 38,
476 79). For example, increased content of MCT1, but not MCT4, has been shown in L6 cells 1 h after
477 incubation with either 10 mM or 20 mM La^- (38). In our study, immediately after SIT1 muscle $[La^-]$
478 increased to 25.5 (6.2) mM and 18.7 (9.7) mM for Rest-1 and Rest-5, respectively. Therefore, consistent
479 with in vitro data (38), intracellular La^- accumulation may be a stimulus for MCT1, but not MCT4
480 upregulation, in human skeletal muscle. However, given the similar increase in MCT1 abundance for
481 both groups, and that exercise-induced decreases in pH_i differed by only ~0.1 pH unit following SIT1,
482 our data prove inconclusive as to whether H^+ accumulation is an important signal for inducing MCT1
483 expression. The absence of any change in MCT4 abundance indicates that neither La^- nor H^+
484 accumulation are likely to be important signaling factors in MCT4 regulation.

485 The factors affecting regulation of the MCT chaperone protein basigin in human skeletal
486 muscle have not been described. The present study provides the first investigation of the response of
487 basigin to a SIT intervention, finding no meaningful changes in protein abundance after 4 or 10 weeks
488 of training for either group. Given the substantial increase in muscle $[La^-]$ following both Rest-1 and
489 Rest-5 noted above, these results contrast with previous data from L6 cells (38), whereby basigin protein
490 content was increased by 1.85-fold to 2.78-fold after 1 h of La^- incubation (10 or 20 mM), but decreased
491 after 6–48 h of incubation. However, an difference between the cell model and exercising human
492 muscle is that prolonged $[La^-]$ of 10 mM or 20 mM are not physiological in human muscle in vivo.
493 While accumulation of similar levels of intracellular La^- was found in the present study, and is typical
494 of high-intensity exercise, activity of the MCTs causes La^- efflux from the cytosol, resulting in
495 oxidation of La^- to pyruvate or entry of La^- into the Cori cycle for gluconeogenesis (24). Thus, while
496 prolonged high $[La^-]$ may stimulate an increase in basigin content in L6 cells, the present data suggest
497 the high $[La^-]$ typical of SIT does not provoke increases in basigin content in exercising human muscle.

498 Second to the MCTs, NHE1 provides the next most important contribution to H^+ efflux during
499 high-intensity exercise (4, 5). This study found small increases in NHE1 abundance after four weeks
500 of SIT for Rest-1 (1.12-fold $\times/\div 1.09$) and Rest-5 (1.13-fold $\times/\div 1.22$). All of the research to date on the
501 NHE1 protein response to training has emanated from Copenhagen (3, 32, 33, 49, 54, 66, 78, 81), with
502 mean fold-change following SIT/HIIT interventions ranging from 0.95-fold (81) to 1.35-fold (78). The
503 one study to include females, in a group consisting of both sexes, found a 1.14-fold increase with
504 training (32). Thus, it seems while NHE1 abundance may increase following HIIT/SIT in different
505 populations, the magnitude of any increase is typically small. An unexpected finding in the current
506 study was a further increase in NHE1 content for Rest-5 of 1.25-fold $\times/\div 1.23$, compared to +4 wk, after
507 SIT volume was reduced to 1 d per week. In contrast, NHE1 abundance returned to baseline for Rest-
508 1, indicating the stress imposed by the two training regimens differentially induced NHE1, but only
509 when training load was reduced. One study has shown NHE1 abundance to increase 1.35-fold in elite
510 footballers who stopped training for 2 weeks at the end of their season, in contrast to a group who
511 performed 2 weeks of mixed HIIT/SIT (81). It appears that following a period of intensive training, a

512 reduced training stimulus is favorable for NHE1 upregulation. This may relate to the long fractional
513 degradation rate for NHE1 mRNA, whereby steady-state mRNA content is not apparent until 24–48 h
514 post-HIIT (62). If there is a subsequent delay in protein synthesis, then upregulation of NHE1 may
515 benefit from a longer duration between exercise sessions. Further evidence is required to confirm this.

516 The NBCe1 protein response to a SIT intervention has been reported here for the first time.
517 Although no changes in mean NBCe1 content were seen for either group, there were highly variable
518 responses. We previously found NBCe1 abundance to increase in men undergoing 4 weeks of HIIT
519 performed at different intensities between the LT and W_{peak} (63). Using a non-isoform specific
520 antibody, others have reported NBC content to increase in rat soleus following 5 weeks of HIIT,
521 regardless of whether rats received sodium bicarbonate or a placebo prior to each training session (80).
522 By buffering extracellular H^+ , sodium bicarbonate enhances H^+ efflux by creating a greater pH gradient
523 relative to the intracellular space (58). In the current study, 5 min of rest would also have enabled
524 greater H^+ efflux compared to 1 min of rest. Therefore, from these limited data it seems that H^+ efflux
525 does not influence changes in NBC(e1) protein content. While there may be sex-specific responses on
526 comparing our two human studies, it seems that longer duration intervals of high-intensity training may
527 be required to provoke adaptations in NBC(e1). The signaling factors required for regulation of NBCe1
528 in human muscle in vivo are unknown, but in vitro research has shown that IRBIT (inositol 1,4,5
529 trisphosphate receptor binding protein released with inositol 1,4,5 trisphosphate) activates most of the
530 NCBT proteins, except for NBCe2 (73). Whether IRBIT is important in vivo remains to be shown, but
531 it is a potential target for future study.

532 There were contrasting changes in each of the CA isozymes after four weeks of training. Of
533 the cytosolic CAs, CAII content decreased slightly for both groups, whereas there was no change in
534 CAIII content. Of the sarcolemmal CAs, there were large increases in CAXIV abundance for both
535 Rest-1 and Rest-5, but a small decrease in CAIV content for both groups at +4 wk. There are no
536 published data on the CA response to SIT, although we reported increases in CAII and CAXIV content,
537 but decreases or no change in CAIII or CAIV, following four weeks of 2-min HIIT in men (63). Given
538 there were no differences between groups in the present study, despite differing metabolic stresses

539 imposed by 1-min versus 5-min rest intervals, nor after differing intensities of HIIT (63), there is little
540 insight as to the potential mechanisms for regulation of CA content.

541 There were no changes in $\beta_{m_{in\ vitro}}$ for either group following 4 or 10 weeks of SIT. Previous
542 research postulated that H^+ accumulation might be a stimulus for upregulation of $\beta_{m_{in\ vitro}}$ (21, 83). If
543 so, then $\beta_{m_{in\ vitro}}$ might have been expected to improve more for Rest-1 because the shorter rest intervals
544 would prevent recovery of pH_i between sprints. As expected, muscle pH was lower for Rest-1 than
545 Rest-5, but given there were no improvements in $\beta_{m_{in\ vitro}}$ for either group, the low muscle pH associated
546 with both types of training was not a factor in improving $\beta_{m_{in\ vitro}}$. These data add to the existing
547 evidence showing improvements in $\beta_{m_{in\ vitro}}$ are typically not found with short-duration work intervals
548 (2, 20, 37, 49, 71).

549 To profile the metabolic stress imposed by the two exercise bouts, we also assayed total and
550 non-protein $\beta_{m_{in\ vitro}}$ before and after the first bout of SIT. While a small reduction in $\beta_{m_{in\ vitro}}$ might
551 theoretically be expected post-exercise due to the greater content of organic phosphates and lower free
552 P_i content, mean non-protein $\beta_{m_{in\ vitro}}$ changed little. The randomness of individual responses suggests
553 methodological rather than physiological variation. Our group has previously reported lower $\beta_{m_{in\ vitro}}$
554 immediately after various bouts of high-intensity exercise (11, 13). In one study, because non-protein
555 $\beta_{m_{in\ vitro}}$ was unchanged, the lower total $\beta_{m_{in\ vitro}}$ was inferred to be due to reduced protein buffering
556 (11). However, in the current study, with no change in either total $\beta_{m_{in\ vitro}}$ or non-protein $\beta_{m_{in\ vitro}}$, there
557 was consequently no change in estimated protein $\beta_{m_{in\ vitro}}$. Therefore, the present data cast doubt on
558 whether a short-term reduction in protein buffering is likely immediately following high-intensity
559 exercise. This adds to our data questioning the sensitivity and ecological validity of the titration assay
560 (62). Notwithstanding these concerns, our estimated protein $\beta_{m_{in\ vitro}}$ of 76.5 (9.1) $mmol\ H^+ \cdot kg$
561 $dm^{-1} \cdot pH^{-1}$, or 23.2 (2.8) $mmol \cdot L^{-1} \cdot pH^{-1}$, is similar to the 26 $mmol \cdot L^{-1} \cdot pH^{-1}$ predicted from the
562 Henderson–Hasselbalch equation for protein-bound histidine (48).

563 We anticipated that performance improvements would be specific to training that more closely
564 matched the substrate usage of the exercise test; however, not all the performance improvements were
565 as expected. RSA did improve more for Rest-5, with greater total work performed and better fatigue

566 resistance. This was not due to improved single (i.e. first) sprint performance, but to progressively
567 greater total work performed in sequential sprints at +4 wk compared to Pre. Greater total work
568 probably relates to an enhanced anaerobic capacity providing greater relative substrate level
569 phosphorylation for sequential sprints, in part because of improved PCr resynthesis (12). Rest-1 had
570 no improvement in total work, but did have improved fatigue resistance because of a lower reduction
571 in work for the later sprints. Better fatigue resistance more likely reflects that the ability to sustain
572 power for later sprints is dependent on oxidative rather than glycolytic capacity (61). Accordingly, both
573 groups displayed similarly modest improvements in $\dot{V}O_{2\text{peak}}$ of ~6%, or $2.2 \text{ mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ (90% CL
574 $\pm 2.4 \text{ mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$), and $2.1 \pm 1.0 \text{ mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$, for Rest-1 and Rest-5, respectively. These data are
575 less than the aggregate reported in a meta-analysis of SIT studies of 8%, or $3.6 \text{ mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ (31), but
576 greater than the 3.6% improvement in a subgroup analysis of non-athletic active females, reported in a
577 separate meta-analysis (85). It appears that short-duration SIT interventions produce only moderate
578 improvements in $\dot{V}O_{2\text{peak}}$ in active women. Finally, there were no group mean improvements in the LT
579 or W_{peak} for Rest-1 or Rest-5, but there was notable individual variability within both groups. Those
580 factors determining the individual training response require further research (59), with particular
581 emphasis on study design to enable making robust inferences (1, 40).

582 We have shown that adaptations in some acid/base transport proteins can be maintained by a
583 reduction in training volume for a subsequent 5-week period of similar intensity SIT. One unexpected
584 finding was that NHE1 protein content increased further for Rest-5 after reducing SIT volume to one
585 day per week. Why this occurred is uncertain, but it may be that with comparatively long mRNA and
586 protein half-lives (62), NHE1 protein synthesis peaks with longer recovery between training sessions.
587 Finally, improved performance was evident during the RSA test for both groups, but to different
588 degrees. On comparing total work and work decrement at +10 wk and +4 wk there were no differences,
589 demonstrating that where RSA was enhanced after 4 weeks of training, it was maintained with just one
590 day per week of training at a similar intensity. This confirms that once performance adaptations are
591 achieved with a short period of intensified training, they can be readily maintained with a lower volume
592 of high-intensity training (41, 42, 46, 47, 67).

593

594 **Perspectives and Significance**

595 Selective upregulation of acid/base transport proteins was found in active women after 4 weeks
596 of SIT. The similar adaptations of two work-matched training groups, differing only in rest intervals
597 of 1 or 5 min, indicates duration of recovery does not influence short-term adaptations in these proteins,
598 at least not in an active female population. We also report evidence supporting lactate as a signaling
599 molecule for inducing MCT1, but not MCT4 expression. Furthermore, four weeks of SIT produced
600 only modest improvements in $\dot{V}O_{2\text{peak}}$, indicating there was insufficient training volume to produce the
601 primarily central adaptations that determine $\dot{V}O_{2\text{peak}}$. Overall, there was little change in mean LT or
602 W_{peak} , but possible evidence of high- and low-responders in both groups merits further exploration.
603 Meanwhile there were better improvements in RSA for Rest-5, likely reflecting the greater anaerobic
604 training stimulus provided by longer rest intervals. Finally, maintaining the physiological stimulus of
605 training intensity, but reducing the volume of training, was sufficient to mitigate reversal of adaptations
606 for acid/base transport proteins and RSA. The implication from this research is that rest intervals of 5
607 minutes during SIT lead to better improvements in RSA than 1-minute rest intervals, and these
608 improvements can be maintained despite a reduction in the volume of SIT performed.

609

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615 **References**

- 616 1. **Atkinson G and Batterham AM.** True and false interindividual differences in the
617 physiological response to an intervention. *Exp Physiol* 100: 577-588, 2015.
- 618 2. **Baguet A, Everaert I, De Naeyer H, Reyngoudt H, Stegen S, Beeckman S, Achten E,**
619 **Vanhee L, Volkaert A, Petrovic M, Taes Y, and Derave W.** Effects of sprint training combined with
620 vegetarian or mixed diet on muscle carnosine content and buffering capacity. *Eur J Appl Physiol* 111:
621 2571-2580, 2011.
- 622 3. **Bangsbo J, Gunnarsson TP, Wendell J, Nybo L, and Thomassen M.** Reduced volume and
623 increased training intensity elevate muscle Na⁺-K⁺ pump α_2 -subunit expression as well as short- and
624 long-term work capacity in humans. *J Appl Physiol* 107: 1771-1780, 2009.
- 625 4. **Bangsbo J, Johansen L, Graham T, and Saltin B.** Lactate and H⁺ effluxes from human
626 skeletal muscles during intense, dynamic exercise. *J Physiol* 462: 115-133, 1993.
- 627 5. **Bangsbo J, Juel C, Hellsten Y, and Saltin B.** Dissociation between lactate and proton
628 exchange in muscle during intense exercise in man. *J Physiol* 504: 489-499, 1997.
- 629 6. **Becker HM, Klier M, and Deitmer JW.** Carbonic Anhydrases and Their Interplay with
630 Acid/Base-Coupled Membrane Transporters. In: *Carbonic Anhydrase: Mechanism, Regulation, Links*
631 *to Disease, and Industrial Applications*, edited by Frost SC and McKenna R. Netherlands: Springer,
632 2014, p. 105-134.
- 633 7. **Becker HM, Klier M, Schüler C, McKenna R, and Deitmer JW.** Intramolecular proton
634 shuttle supports not only catalytic but also noncatalytic function of carbonic anhydrase II. *Proc Natl*
635 *Acad Sci U S A* 108: 3071-3076, 2011.
- 636 8. **Bell GJ and Wenger HA.** The effect of one-legged sprint training on intramuscular pH and
637 nonbicarbonate buffering capacity. *Eur J Appl Physiol Occup Physiol* 58: 158-164, 1988.

- 638 9. **Bergström J.** Muscle electrolytes in man determined by neutron activation analysis on needle
639 biopsy specimens. *Scand J Clin Lab Invest* 14: 1-110, 1962.
- 640 10. **Bickham DC, Bentley DJ, Le Rossignol PF, and Cameron-Smith D.** The effects of short-
641 term sprint training on MCT expression in moderately endurance-trained runners. *Eur J Appl Physiol*
642 96: 636-643, 2006.
- 643 11. **Bishop D, Edge J, Mendez-Villanueva A, Thomas C, and Schneiker K.** High-intensity
644 exercise decreases muscle buffer capacity via a decrease in protein buffering in human skeletal muscle.
645 *Pflügers Arch* 458: 929-936, 2009.
- 646 12. **Bishop D, Edge J, Thomas C, and Mercier J.** Effects of high-intensity training on muscle
647 lactate transporters and postexercise recovery of muscle lactate and hydrogen ions in women. *Am J*
648 *Physiol Regul Integr Comp Physiol* 295: R1991-1998, 2008.
- 649 13. **Bishop D, Edge J, Thomas C, and Mercier J.** High-intensity exercise acutely decreases the
650 membrane content of MCT1 and MCT4 and buffer capacity in human skeletal muscle. *J Appl Physiol*
651 102: 616-621, 2007.
- 652 14. **Bishop D, Spencer M, Duffield R, and Lawrence S.** The validity of a repeated sprint ability
653 test. *J Sci Med Sport* 4: 19-29, 2001.
- 654 15. **Bland JM and Altman DG.** Measurement error. *BMJ* 313: 744, 1996.
- 655 16. **Bland JM and Altman DG.** Measurement error proportional to the mean. *BMJ* 313: 106, 1996.
- 656 17. **Bogdanis GC, Nevill ME, Boobis LH, Lakomy HK, and Nevill AM.** Recovery of power
657 output and muscle metabolites following 30 s of maximal sprint cycling in man. *J Physiol* 482 (Pt 2):
658 467-480, 1995.

- 659 18. **Burgomaster KA, Cermak NM, Phillips SM, Benton CR, Bonen A, and Gibala MJ.**
660 Divergent response of metabolite transport proteins in human skeletal muscle after sprint interval
661 training and detraining. *Am J Physiol Regul Integr Comp Physiol* 292: R1970-1976, 2007.
- 662 19. **Curran-Everett D and Benos DJ.** Guidelines for reporting statistics in journals published by
663 the American Physiological Society: the sequel. *Adv Physiol Educ* 31: 295-298, 2007.
- 664 20. **De Smet S, Van Thienen R, Deldicque L, James R, Sale C, Bishop DJ, and Hespel P.** Nitrate
665 Intake Promotes Shift in Muscle Fiber Type Composition during Sprint Interval Training in Hypoxia.
666 *Front Physiol* 7: 233, 2016.
- 667 21. **Edge J, Bishop D, and Goodman C.** Effects of chronic NaHCO₃ ingestion during interval
668 training on changes to muscle buffer capacity, metabolism, and short-term endurance performance. *J*
669 *Appl Physiol* 101: 918-925, 2006.
- 670 22. **Edge J, Bishop D, and Goodman C.** The effects of training intensity on muscle buffer capacity
671 in females. *Eur J Appl Physiol* 96: 97-105, 2006.
- 672 23. **Eichhorn JH.** Inaccuracy in Blood Gas pH Measurements Caused by the Blood Sample. *J Med*
673 *Technol* 2: 23-26, 1985.
- 674 24. **Emhoff CA, Messonnier LA, Horning MA, Fattor JA, Carlson TJ, and Brooks GA.** Direct
675 and indirect lactate oxidation in trained and untrained men. *J Appl Physiol* 115: 829-838, 2013.
- 676 25. **Evans WJ, Phinney SD, and Young VR.** Suction applied to a muscle biopsy maximizes
677 sample size. *Med Sci Sports Exerc* 14: 101-102, 1982.
- 678 26. **Field AP.** Discovering statistics using IBM SPSS statistics : and sex and drugs and rock 'n' roll.
679 (4th ed.). Los Angeles, CA: Sage, 2013, p. 825-826.
- 680 27. **Fitts RH.** The cross-bridge cycle and skeletal muscle fatigue. *J Appl Physiol* 104: 551-558,
681 2008.

- 682 28. **Foster C.** Monitoring training in athletes with reference to overtraining syndrome. *Med Sci*
683 *Sport Exer* 30: 1164-1168, 1998.
- 684 29. **Fyfe JJ, Bishop DJ, and Stepto NK.** Interference between concurrent resistance and
685 endurance exercise: molecular bases and the role of individual training variables. *Sports Med* 44: 743-
686 762, 2014.
- 687 30. **Gibala MJ, Little JP, van Essen M, Wilkin GP, Burgomaster KA, Safdar A, Raha S, and**
688 **Tarnopolsky MA.** Short-term sprint interval versus traditional endurance training: similar initial
689 adaptations in human skeletal muscle and exercise performance. *J Physiol* 575: 901-911, 2006.
- 690 31. **Gist NH, Fedewa MV, Dishman RK, and Cureton KJ.** Sprint interval training effects on
691 aerobic capacity: a systematic review and meta-analysis. *Sports Med* 44: 269-279, 2014.
- 692 32. **Gunnarsson TP and Bangsbo J.** The 10-20-30 training concept improves performance and
693 health profile in moderately trained runners. *J Appl Physiol* 113: 16-24, 2012.
- 694 33. **Gunnarsson TP, Christensen PM, Thomassen M, Nielsen LR, and Bangsbo J.** Effect of
695 intensified training on muscle ion kinetics, fatigue development, and repeated short-term performance
696 in endurance-trained cyclists. *Am J Physiol Regul Integr Comp Physiol* 305: R811-821, 2013.
- 697 34. **Halestrap AP.** The monocarboxylate transporter family--Structure and functional
698 characterization. *IUBMB life* 64: 1-9, 2012.
- 699 35. **Halestrap AP.** Monocarboxylic acid transport. *Compr Physiol* 3: 1611-1643, 2013.
- 700 36. **Hallerdei J, Scheibe RJ, Parkkila S, Waheed A, Sly WS, Gros G, Wetzel P, and Endeward**
701 **V.** T tubules and surface membranes provide equally effective pathways of carbonic anhydrase-
702 facilitated lactic acid transport in skeletal muscle. *PloS ONE* 5: e15137.
703 15110.11371/journal.pone.0015137, 2010.

- 704 37. **Harmer AR, McKenna MJ, Sutton JR, Snow RJ, Ruell PA, Booth J, Thompson MW,**
705 **Mackay NA, Stathis CG, Crameri RM, Carey MF, and Eager DM.** Skeletal muscle metabolic and
706 ionic adaptations during intense exercise following sprint training in humans. *J Appl Physiol* 89: 1793-
707 1803, 2000.
- 708 38. **Hashimoto T, Hussien R, Oommen S, Gohil K, and Brooks GA.** Lactate sensitive
709 transcription factor network in L6 cells: activation of MCT1 and mitochondrial biogenesis. *FASEB J*
710 21: 2602-2612, 2007.
- 711 39. **Haworth RS, Fröhlich O, and Fliegel L.** Multiple carbohydrate moieties on the Na⁺/H⁺
712 exchanger. *Biochem J* 289 (Pt 3): 637-640, 1993.
- 713 40. **Hecksteden A, Kraushaar J, Scharhag-Rosenberger F, Theisen D, Senn S, and Meyer T.**
714 Individual response to exercise training - a statistical perspective. *J Appl Physiol* 118: 1450-1459, 2015.
- 715 41. **Hickson RC, Foster C, Pollock ML, Galassi TM, and Rich S.** Reduced training intensities
716 and loss of aerobic power, endurance, and cardiac growth. *J Appl Physiol* 58: 492-499, 1985.
- 717 42. **Hickson RC and Rosenkoetter MA.** Reduced training frequencies and maintenance of
718 increased aerobic power. *Med Sci Sports Exerc* 13: 13-16, 1981.
- 719 43. **Hopkins WG.** A spreadsheet to compare means of two groups. *Sportscience* 11: 22-23, 2007.
- 720 44. **Hopkins WG.** Spreadsheets for analysis of controlled trials with adjustment for a predictor.
721 *Sportscience* 10: 46-50, 2006.
- 722 45. **Hopkins WG, Marshall SW, Batterham AM, and Hanin J.** Progressive statistics for studies
723 in sports medicine and exercise science. *Med Sci Sports Exerc* 41: 3-13, 2009.
- 724 46. **Houmard JA, Costill DL, Mitchell JB, Park SH, Hickner RC, and Roemmich JN.** Reduced
725 training maintains performance in distance runners. *Int J Sports Med* 11: 46-52, 1990.

- 726 47. **Houmard JA, Tyndall GL, Midyette JB, Hickey MS, Dolan PL, Gavigan KE, Weidner**
727 **ML, and Dohm GL.** Effect of reduced training and training cessation on insulin action and muscle
728 GLUT-4. *J Appl Physiol* 81: 1162-1168, 1996.
- 729 48. **Hultman E and Sahlin K.** Acid-base balance during exercise. *Exerc Sport Sci Rev* 8: 41-128,
730 1980.
- 731 49. **Iaia FM, Thomassen M, Kolding H, Gunnarsson T, Wendell J, Rostgaard T, Nordsborg**
732 **N, Krstrup P, Nybo L, Hellsten Y, and Bangsbo J.** Reduced volume but increased training intensity
733 elevates muscle Na⁺-K⁺ pump α_1 -subunit and NHE1 expression as well as short-term work capacity in
734 humans. *Am J Physiol Regul Integr Comp Physiol* 294: R966-974, 2008.
- 735 50. **Juel C.** Regulation of pH in human skeletal muscle: adaptations to physical activity. *Acta*
736 *Physiol (Oxf)* 193: 17-24, 2008.
- 737 51. **Juel C.** Skeletal muscle Na⁺/H⁺ exchange in rats: pH dependency and the effect of training.
738 *Acta Physiol Scand* 164: 135-140, 1998.
- 739 52. **Juel C and Halestrap AP.** Lactate transport in skeletal muscle - role and regulation of the
740 monocarboxylate transporter. *J Physiol* 517: 633-642, 1999.
- 741 53. **Juel C, Holten MK, and Dela F.** Effects of strength training on muscle lactate release and
742 MCT1 and MCT4 content in healthy and type 2 diabetic humans. *J Physiol* 556: 297-304, 2004.
- 743 54. **Juel C, Klarskov C, Nielsen JJ, Krstrup P, Mohr M, and Bangsbo J.** Effect of high-
744 intensity intermittent training on lactate and H⁺ release from human skeletal muscle. *Am J Physiol*
745 *Endocrinol Metab* 286: E245-251, 2004.
- 746 55. **Juel C, Lundby C, Sander M, Calbet JAL, and van Hall G.** Human skeletal muscle and
747 erythrocyte proteins involved in acid-base homeostasis: adaptations to chronic hypoxia. *J Physiol* 548:
748 639-648, 2003.

- 749 56. **Kirk P, Wilson MC, Heddle C, Brown MH, Barclay AN, and Halestrap AP.** CD147 is
750 tightly associated with lactate transporters MCT1 and MCT4 and facilitates their cell surface
751 expression. *EMBO J* 19: 3896-3904, 2000.
- 752 57. **Kuipers H, Verstappen FT, Keizer HA, Geurten P, and van Kranenburg G.** Variability of
753 aerobic performance in the laboratory and its physiologic correlates. *Int J Sports Med* 6: 197-201, 1985.
- 754 58. **Mainwood GW and Worsley-Brown P.** The effects of extracellular pH and buffer
755 concentration on the efflux of lactate from frog sartorius muscle. *J Physiol* 250: 1-22, 1975.
- 756 59. **Mann TN, Lamberts RP, and Lambert MI.** High responders and low responders: factors
757 associated with individual variation in response to standardized training. *Sports Med* 44: 1113-1124,
758 2014.
- 759 60. **Mannion AF, Jakeman PM, and Willan PL.** Determination of human skeletal muscle buffer
760 value by homogenate technique: methods of measurement. *J Appl Physiol* 75: 1412-1418, 1993.
- 761 61. **McGawley K and Bishop DJ.** Oxygen uptake during repeated-sprint exercise. *J Sci Med Sport*
762 18: 214-218, 2015.
- 763 62. **McGinley C and Bishop DJ.** Distinct protein and mRNA kinetics of skeletal muscle proton
764 transporters following exercise can influence interpretation of adaptations to training. *Exp Physiol* 101:
765 1565-1580, 2016.
- 766 63. **McGinley C and Bishop DJ.** Influence of training intensity on adaptations in acid/base
767 transport proteins, muscle buffer capacity, and repeated-sprint ability in active men. *J Appl Physiol* 121:
768 1290-1305, 2016.
- 769 64. **Mendez-Villanueva A, Edge J, Suriano R, Hamer P, and Bishop D.** The Recovery of
770 Repeated-Sprint Exercise Is Associated with PCr Resynthesis, while Muscle pH and EMG Amplitude
771 Remain Depressed. *PLoS ONE* 7: e51977. 51910.51371/journal.pone.0051977, 2012.

- 772 65. **Miller BF, Konopka AR, and Hamilton KL.** The rigorous study of exercise adaptations: why
773 mRNA might not be enough. *J Appl Physiol*: jap 00137 02016, 2016.
- 774 66. **Mohr M, Krstrup P, Nielsen JJ, Nybo L, Rasmussen MK, Juel C, and Bangsbo J.** Effect
775 of two different intense training regimens on skeletal muscle ion transport proteins and fatigue
776 development. *Am J Physiol Regul Integr Comp Physiol* 292: R1594-1602, 2007.
- 777 67. **Mujika I and Padilla S.** Detraining: loss of training-induced physiological and performance
778 adaptations. Part II: Long term insufficient training stimulus. *Sports Med* 30: 145-154, 2000.
- 779 68. **Murphy RM and Lamb GD.** Important considerations for protein analyses using antibody
780 based techniques: down-sizing Western blotting up-sizes outcomes. *J Physiol* 591: 5823-5831, 2013.
- 781 69. **Nelson CR and Fitts RH.** Effects of low cell pH and elevated inorganic phosphate on the pCa-
782 force relationship in single muscle fibers at near-physiological temperatures. *Am J Physiol Cell Physiol*
783 306: C670-678, 2014.
- 784 70. **Nevill A.** Why the analysis of performance variables recorded on a ratio scale will invariably
785 benefit from a log transformation. *J Sport Sci* 15: 457-458, 1997.
- 786 71. **Nevill ME, Boobis LH, Brooks S, and Williams C.** Effect of training on muscle metabolism
787 during treadmill sprinting. *J Appl Physiol* 67: 2376-2382, 1989.
- 788 72. **Newell J, Higgins D, Madden N, Cruickshank J, Einbeck J, McMillan K, and McDonald**
789 **R.** Software for calculating blood lactate endurance markers. *J Sport Sci* 25: 1403-1409, 2007.
- 790 73. **Parker MD and Boron WF.** The divergence, actions, roles, and relatives of sodium-coupled
791 bicarbonate transporters. *Physiol Rev* 93: 803-959, 2013.
- 792 74. **Parolin ML, Chesley A, Matsos MP, Spriet LL, Jones NL, and Heigenhauser GJ.**
793 Regulation of skeletal muscle glycogen phosphorylase and PDH during maximal intermittent exercise.
794 *Am J Physiol* 277: E890-900, 1999.

- 795 75. **Passonneau JV and Lowry OH.** *Enzymatic analysis : a practical guide.* Totowa, NJ: Humana
796 Press, 1993.
- 797 76. **Puype J, Van Proeyen K, Raymackers JM, Deldicque L, and Hespel P.** Sprint interval
798 training in hypoxia stimulates glycolytic enzyme activity. *Med Sci Sports Exerc* 45: 2166-2174, 2013.
- 799 77. **Romero MF, Chen AP, Parker MD, and Boron WF.** The SLC4 family of bicarbonate
800 (HCO_3^-) transporters. *Mol Aspects Med* 34: 159-182, 2013.
- 801 78. **Skovgaard C, Christensen PM, Larsen S, Andersen TR, Thomassen M, and Bangsbo J.**
802 Concurrent speed endurance and resistance training improves performance, running economy, and
803 muscle NHE1 in moderately trained runners. *J Appl Physiol* 117: 1097-1109, 2014.
- 804 79. **Summermatter S, Santos G, Pérez-Schindler J, and Handschin C.** Skeletal muscle PGC-
805 1α controls whole-body lactate homeostasis through estrogen-related receptor α -dependent activation
806 of LDH B and repression of LDH A. *Proc Natl Acad Sci U S A* 110: 8738-8743, 2013.
- 807 80. **Thomas C, Bishop D, Moore-Morris T, and Mercier J.** Effects of high-intensity training on
808 MCT1, MCT4, and NBC expressions in rat skeletal muscles: influence of chronic metabolic alkalosis.
809 *Am J Physiol Endocrinol Metab* 293: E916-922, 2007.
- 810 81. **Thomassen M, Christensen PM, Gunnarsson TP, Nybo L, and Bangsbo J.** Effect of 2-wk
811 intensified training and inactivity on muscle $\text{Na}^+\text{-K}^+$ pump expression, phospholemman (FXD1)
812 phosphorylation, and performance in soccer players. *J Appl Physiol* 108: 898-905, 2010.
- 813 82. **Weston AR, Myburgh KH, Lindsay FH, Dennis SC, Noakes TD, and Hawley JA.** Skeletal
814 muscle buffering capacity and endurance performance after high-intensity interval training by well-
815 trained cyclists. *Eur J Appl Physiol Occup Physiol* 75: 7-13, 1997.
- 816 83. **Weston AR, Wilson GR, Noakes TD, and Myburgh KH.** Skeletal muscle buffering capacity
817 is higher in the superficial vastus than in the soleus of spontaneously running rats. *Acta Physiol Scand*
818 157: 211-216, 1996.

- 819 84. **Weston KS, Wisløff U, and Coombes JS.** High-intensity interval training in patients with
820 lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. *Br J Sports Med* 48:
821 1227-1234, 2014.
- 822 85. **Weston M, Taylor KL, Batterham AM, and Hopkins WG.** Effects of low-volume high-
823 intensity interval training (HIT) on fitness in adults: a meta-analysis of controlled and non-controlled
824 trials. *Sports Med* 44: 1005-1017, 2014.
- 825 86. **Wetzel P, Hasse A, Papadopoulos S, Voipio J, Kaila K, and Gros G.** Extracellular carbonic
826 anhydrase activity facilitates lactic acid transport in rat skeletal muscle fibres. *J Physiol* 531: 743-756,
827 2001.
- 828 87. **Wilson MC, Meredith D, Fox JEM, Manoharan C, Davies AJ, and Halestrap AP.** Basigin
829 (CD147) is the target for organomercurial inhibition of monocarboxylate transporter isoforms 1 and 4:
830 The ancillary protein for the insensitive MCT2 is embigin (gp70). *J Biol Chem* 280: 27213-27221, 2005.
831

832 **Figure Captions**

833 Figure 1 Experimental design. Abbreviations: GXT (graded-exercise test); RSA (repeated-sprint ability
834 test); SIT1 (sprint-interval training session 1).

835 Figure 2 (A) Muscle pH, (B) muscle lactate, (C) total $\beta_{\text{in vitro}}$ (non-bicarbonate muscle buffer capacity),
836 and (D) non-protein $\beta_{\text{in vitro}}$ before (Pre) and immediately after (Post) a single bout of sprint-interval
837 training (SIT1) for Rest-1 (triangles, $n = 7$) and Rest-5 (circles, $n = 8$; including one participant who
838 dropped out after SIT1). Individual data points are plotted. CV of duplicate samples: pH (0.6%), lactate
839 (3.8%), total $\beta_{\text{in vitro}}$ (6%), and non-protein $\beta_{\text{in vitro}}$ (8%).

840 Figure 3 (A) Venous blood pH, and (B) venous lactate before (Pre) and 2, 3, 5, 7, and 10 min after a
841 single bout of sprint-interval training (SIT1) for Rest-1 (triangles, $n = 7$) and Rest-5 (circles, $n = 8$;
842 including one participant who dropped out after SIT1). Data are mean (SD). CV of duplicate samples:
843 pH (0.05%), lactate (5.3%).

844 Figure 4 Representative immunoblots and protein abundance of (A) MCT1, (B) MCT4, (C) basigin,
845 and (D) NHE1 before (Pre) and after 4 weeks (+4 wk) and 10 weeks (+10 wk) of sprint interval training
846 (SIT) for Rest-1 and Rest-5. Protein abundance is relative to a calibration curve for a mixed-
847 homogenate internal standard run on every gel. Individual data points and geometric means (horizontal
848 bars) are plotted. Non-adjacent lanes from the same blots are indicated by vertical lines (A, B, and D).
849 CV of duplicate or triplicate gels: MCT1 (16%), MCT4 (16%), basigin (11%), and NHE1 (15%).

850 Figure 5 (A) Representative immunoblot and (B) NBCe1 protein abundance before (Pre) and after 4
851 weeks (+4 wk) and 10 weeks (+10 wk) of sprint interval training (SIT) for Rest-1 and Rest-5. Protein
852 abundance is relative to a calibration curve for a mixed-homogenate internal standard run on every gel.
853 Individual data points and geometric means (horizontal bars) are plotted. CV of duplicate gels was
854 26%.

855 Figure 6 Representative immunoblots and protein abundance of (A) CAII, (B) CAIII, (C) CAIV, and
856 (D) CAXIV before (Pre) and after 4 weeks (+4 wk) and 10 weeks (+10 wk) of sprint interval training
857 (SIT) for Rest-1 and Rest-5. Protein abundance is relative to a calibration curve for a mixed-

858 homogenate internal standard run on every gel. Individual data points and geometric means (horizontal
859 bars) are plotted. Positive controls (+ve) were loaded for CAIV and CAXIV. Non-adjacent lanes from
860 the same blots are indicated by vertical lines (A and D). CV of duplicate or triplicate gels: CAII (15%),
861 CAIII (single gels), CAIV (23%), and CAXIV (29%).

862 Figure 7 Non-bicarbonate muscle buffer capacity ($\beta_{m_{in\ vitro}}$) before (Pre) and after 4 weeks (+4 wk) and
863 10 weeks (+10 wk) of sprint interval training(SIT) for Rest-1 and Rest-5. Individual data points are
864 plotted. CV of duplicate titrations = 7.7%.

865 Figure 8 (A) Total work and (B) work decrement during a repeated-sprint ability test (5×6 s) before
866 (Pre) and after 4 weeks (+4 wk) and 10 weeks (+10 wk) of sprint interval training (SIT) for Rest-1 and
867 Rest-5 (individual data points), and (C) work performed per individual sprint for Rest-1 (triangles) and
868 Rest-5 (circles) at Pre and +4 wk [mean (SD)].

869 Figure 9 (A) Lactate threshold, (B) peak aerobic power (W_{peak}), and (C) peak oxygen uptake ($\dot{V}O_{2peak}$)
870 before (Pre) and after 4 weeks (+4 wk) of sprint interval training (SIT) for Rest-1 and Rest-5. Individual
871 data points are plotted.

872

873 **Tables**

874 Table 1 Title

875 Sprint-interval training programs performed by the 1-min rest (Rest-1) and 5-min rest (Rest-5)
876 training groups

877 Table 1 Legend

878 Relative intensity for weeks 1 to 4 was calculated as a percentage of the lactate threshold (LT)
879 determined during the pre-training graded-exercise test. Relative intensity for weeks 6 to 10 was
880 calculated as a percentage of the LT determined during the +4 wk graded-exercise test. Data are mean
881 (SD).

882

883 Table 2 Title

884 Details of primary and secondary antibodies used for western blotting