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

Title

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Running head

Rest interval duration and muscle pH regulation

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Abstract

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

Keywords

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

Introduction

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

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

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

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

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

Materials & Methods

Participants

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

Experimental Design

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

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

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

Insert Figure 1 here

Sprint-Interval Training

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

Insert Table 1 here

Experimental Trials

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

First SIT Session (SIT1)

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

Needle Muscle Biopsies

Muscle biopsies were taken from the belly of the vastus lateralis, approximately halfway between the knee and hip, using the needle biopsy technique modified with suction (25). Subsequent samples were taken approximately 1 cm from a previous biopsy site. An incision was made under local anesthesia (1% Xylocaine) and a muscle sample taken using a Bergström needle (9). Samples were blotted on filter paper to remove blood, before being immediately snap-frozen in liquid nitrogen and then stored at −80°C until subsequent analyses. Muscle samples were taken from the dominant leg before (Pre) and immediately after (Post) the first training session, 3 d after the twelfth training session (+4 wk), and 3 d after the seventeenth training session (+10 wk).

Repeated-Sprint Ability Test

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

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

where total work = actual work performed in all 5 sprints, and ideal work = work performed in sprint number 1 × 5.

Graded-Exercise Test

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

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

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

Peak Oxygen Uptake Test

Immediately after the graded-exercise test, participants cycled for 5 min at 20 W, followed by a square-wave $\dot{V}O_{2peak}$ test at 105% of W_{peak} achieved during the graded-exercise test. Participants were instructed to accelerate to 90–100 rpm at the commencement of a 5-s countdown, and to maintain a high, but not fixed cadence until volitional fatigue. Consistent verbal encouragement was provided throughout. Expired gases were analyzed every 15 s using a custom-made metabolic cart. A two-point calibration of the gas analyzers (S-3lA/II and CD-3A analyzers, Ametek, Berwyn, PA, USA) was performed before each test using one certified gravimetric gas (16.1% O_2, 4.17% CO_2; BOC Gases, Chatswood, NSW, Australia) and ambient air. Ventilation was recorded every 15 s. The ventilometer (KL Engineering, Sunnyvale, CA, USA) was calibrated at the start of each day using a 3-L syringe (MedGraphics, St. Paul, MN). $\dot{V}O_{2peak}$ was calculated as the mean of the two highest consecutive 15-s values. Exercise duration was 145 (30) s [mean (SD)].

Muscle Buffer Capacity

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

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

Muscle Lactate Assay

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

Quantitative Western Blotting

Muscle homogenate preparation

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

Protein assay

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

Immunoblotting

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

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

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

Insert Table 2 here

Statistical Analyses

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

Results

Training Data

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

Physiological Responses to SIT1

Short-term physiological responses to SIT1 are shown in Figure 2 and Figure 3. Following SIT1, muscle [La^{-}] increased to 84.2 (20.3) mmol•kg dm^{-1} and 61.8 (31.9) mmol•kg dm^{-1} for Rest-1 and Rest-5, respectively (time main effect: $F_{1,15}$ = 209.26, P < 0.001, pooled ES: 5.49, 90% CL ×/÷0.72), but there was no clear difference between groups (group×time interaction: $F_{1,15}$ = 0.86, P = 0.37, ES: 0.70, ×/÷1.42). Assuming a muscle water content of 3.3 L•kg dm^{-1} (48), this equates to 25.5 (6.2) mM 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 (0.14), than Rest-5, 6.89 (0.13) to 6.63 (0.18) (group×time interaction: $F_{1,15}$ = 3.50, P = 0.08, ES: 1.07; ±1.11).

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

Insert Figure 2 here

Venous blood pH decreased from 7.36 (0.03) Pre to 7.21 (0.07) at +2 min following SIT1 (time main effect: $F_{5,68.4}$ = 34.1, P < 0.001). There were no clear differences in blood pH between the two groups (group×time interaction: $F_{5,68.4} = 0.48$, $P = 0.79$) due to the high within-group variability (between-group +2 min ES: 0.97; ±2.13). Mean venous blood [La^-] peaked at +7 min (6.60 mM and 5.50 mM for Rest-1 and Rest-5), with a greater increase apparent for Rest-1 at +5 min and +7 min (group×time interaction: $F_{5,64.7} = 2.77$, $P = 0.025$, +5 min ES: 1.08, ×/÷1.12, +7 min ES: 1.24, ×/÷1.16).

Insert Figure 3 here

Protein Abundance

MCT1

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

MCT4

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

Basigin

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

NHE1

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

Insert Figure 4 here

NBCe1

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

Insert Figure 5 here

CAII

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

CAIII

The CAIII antibody recognized a single band just above 25 kDa (Fig. 6B). There was no overall change in CAIII content for the two groups (time main effect: $F_{2,26.9} = 0.32$, $P = 0.73$, pooled +4 wk ES: 0.19, ×/÷0.42). There was no difference between the two groups after four weeks training (group×time interaction: $F_{2,26.9} = 1.74$, $P = 0.20$, ES: 0.26, ×/÷0.91). At +10 wk the difference between groups was unclear (ES: 1.04, ×/÷1.42).

CAIV

The CAIV antibody had poor signal-to-noise ratio but recognized a single or double band near the predicted molecular mass of 35 kDa (Fig. 6C) in the muscle homogenate and positive control (293T Cell Transient Overexpression Lysate, Abnova, Taipei, Taiwan). The CAIV response to training did not differ between the two groups (group×time interaction: $F_{2,18.6} = 0.26$, $P = 0.77$, +4 wk ES: 0.01, ×/÷1.06). On pooling the data, CAIV content progressively decreased following training, 0.87-fold ×/÷1.40 and 0.79-fold ×/÷1.36 at +4 wk and +10 wk, respectively (time main effect: $F_{2,18.6} = 3.50$, $P = 0.051$, pooled +4 wk ES: −0.39, ×/÷0.47, +10 wk ES −0.72, ×/÷0.46).

CAXIV

The CAXIV antibody recognized a single band below 50 kDa in the muscle homogenate and the positive control (Fig. 6D). There was no difference between groups in the CAXIV response (group×time interaction: $F_{2,26.0} = 0.40$, $P = 0.68$, +4 wk ES: 0.08, ×/÷0.96). Overall CAXIV abundance increased in response to training 1.63-fold ×/÷1.66 (time main effect: $F_{2,26.0} = 5.77$, $P = 0.008$, pooled +4 wk ES: 1.00, ×/÷0.49). After SIT volume was reduced to 1 d per week, mean CAXIV abundance decreased to baseline values (pooled +10 wk ES: 0.20, ×/÷0.62).

Insert Figure 6 here

Muscle Buffer Capacity

$\beta m_{in\ vitro}$ did not change in response to training for either group (Fig. 7) (time main effect: $F_{2,32.0} = 0.11$, $P = 0.89$, pooled +4 wk ES: 0.11; ±0.39). As noted above, $\beta m_{in\ vitro}$ was lower throughout for Rest-5 (group main effect: $F_{1,15} = 14.4$, $P = 0.002$, Pre ES: −1.06; ±0.87).

Insert Figure 7 here

Repeated-Sprint Ability

Total work performed during the RSA test improved only in the Rest-5 group (Fig. 8A) (group×time interaction: $F_{2,27.0} = 4.60$, $P = 0.02$, +4 wk ES: 0.51; ±0.37). On reducing SIT volume to 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-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 difference between groups at +10 wk (ES: 0.42; ±0.50). Comparing the change in work performed during each individual sprint (Fig. 8C), Rest-5 had better improvements for the last three sprints at +4 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). For both groups work decrement during the RSA test improved following four weeks of training (Fig. 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 differences between the two groups (group×time interaction: $F_{2,27.4} = 1.84$, $P = 0.18$, +4 wk ES: 0.67; ±1.05, +10 wk ES: 0.04; ±1.08). There was no difference between the pooled +4 wk and +10 wk data, indicating that better work decrement was maintained with 1 d per week training (pooled ES: −0.20; ±0.32).

Insert Figure 8 here

Aerobic Capacity

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

Insert Figure 9 here

Discussion

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

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

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

Second to the MCTs, NHE1 provides the next most important contribution to H^+ efflux during high-intensity exercise (4, 5). This study found small increases in NHE1 abundance after four weeks of SIT for Rest-1 (1.12-fold ×/÷1.09) and Rest-5 (1.13-fold ×/÷1.22). All of the research to date on the NHE1 protein response to training has emanated from Copenhagen (3, 32, 33, 49, 54, 66, 78, 81), with mean fold-change following SIT/HIIT interventions ranging from 0.95-fold (81) to 1.35-fold (78). The one study to include females, in a group consisting of both sexes, found a 1.14-fold increase with training (32). Thus, it seems while NHE1 abundance may increase following HIIT/SIT in different populations, the magnitude of any increase is typically small. An unexpected finding in the current study was a further increase in NHE1 content for Rest-5 of 1.25-fold ×/÷1.23, compared to +4 wk, after SIT volume was reduced to 1 d per week. In contrast, NHE1 abundance returned to baseline for Rest-1, indicating the stress imposed by the two training regimens differentially induced NHE1, but only when training load was reduced. One study has shown NHE1 abundance to increase 1.35-fold in elite footballers who stopped training for 2 weeks at the end of their season, in contrast to a group who performed 2 weeks of mixed HIIT/SIT (81). It appears that following a period of intensive training, a reduced training stimulus is favorable for NHE1 upregulation. This may relate to the long fractional degradation rate for NHE1 mRNA, whereby steady-state mRNA content is not apparent until 24–48 h post-HIIT (62). If there is a subsequent delay in protein synthesis, then upregulation of NHE1 may benefit from a longer duration between exercise sessions. Further evidence is required to confirm this.

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

There were contrasting changes in each of the CA isozymes after four weeks of training. Of the cytosolic CAs, CAII content decreased slightly for both groups, whereas there was no change in CAIII content. Of the sarcolemmal CAs, there were large increases in CAXIV abundance for both Rest-1 and Rest-5, but a small decrease in CAIV content for both groups at +4 wk. There are no published data on the CA response to SIT, although we reported increases in CAII and CAXIV content, but decreases or no change in CAIII or CAIV, following four weeks of 2-min HIIT in men (63). Given there were no differences between groups in the present study, despite differing metabolic stresses imposed by 1-min versus 5-min rest intervals, nor after differing intensities of HIIT (63), there is little insight as to the potential mechanisms for regulation of CA content.

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

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

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

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

Perspectives and Significance

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

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Figure Captions

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

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

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

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

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

Figure 6 Representative immunoblots and protein abundance of (A) CAII, (B) CAIII, (C) CAIV, and (D) CAXIV before (Pre) and after 4 weeks (+4 wk) and 10 weeks (+10 wk) of sprint interval training (SIT) for Rest-1 and Rest-5. Protein abundance is relative to a calibration curve for a mixed-homogenate internal standard run on every gel. Individual data points and geometric means (horizontal bars) are plotted. Positive controls (+ve) were loaded for CAIV and CAXIV. Non-adjacent lanes from the same blots are indicated by vertical lines (A and D). CV of duplicate or triplicate gels: CAII (15%), CAIII (single gels), CAIV (23%), and CAXIV (29%).

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

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

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

Tables

Table 1 Title

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

Table 1 Legend

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

Table 2 Title

Details of primary and secondary antibodies used for western blotting