

# Interspersed normoxia during live high, train low interventions reverses an early reduction in muscle $\text{Na}^+$ , $\text{K}^+$ ATPase activity in well-trained athletes

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**Abstract** Hypoxia and exercise each modulate muscle  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity. We investigated the effects on muscle  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity of only 5 nights of live high, train low hypoxia (LHTL), 20 nights consecutive (LHTLc) versus intermittent LHTL (LHTLi), and acute sprint exercise. Thirty-three athletes were assigned to control (CON,  $n = 11$ ), 20-nights LHTLc ( $n = 12$ ) or 20-nights LHTLi ( $4 \times 5$ -nights LHTL interspersed with 2-nights CON,  $n = 10$ ) groups. LHTLc and LHTLi slept at a simulated altitude of 2,650 m ( $\text{F}_1\text{O}_2$  0.1627) and lived and trained by day under normoxic conditions; CON lived, trained, and slept in normoxia. A quadriceps muscle biopsy was taken at rest and immediately after standardised sprint exercise, before (Pre) and after 5-nights (d5) and 20-nights (Post) LHTL interventions and analysed for

$\text{Na}^+$ ,  $\text{K}^+$ ATPase maximal activity (3-*O*-MFPase) and content ( $[^3\text{H}]$ -ouabain binding). After only 5-nights LHTLc, muscle 3-*O*-MFPase activity declined by 2% ( $P < 0.05$ ). In LHTLc, 3-*O*-MFPase activity remained below Pre after 20 nights. In contrast, in LHTLi, this small initial decrease was reversed after 20 nights, with restoration of 3-*O*-MFPase activity to Pre-intervention levels. Plasma  $[\text{K}^+]$  was unaltered by any LHTL. After acute sprint exercise 3-*O*-MFPase activity was reduced ( $12.9 \pm 4.0\%$ ,  $P < 0.05$ ), but  $[^3\text{H}]$ -ouabain binding was unchanged. In conclusion, maximal  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity declined after only 5-nights LHTL, but the inclusion of additional interspersed normoxic nights reversed this effect, despite athletes receiving the same amount of hypoxic exposure. There were no effects of consecutive or intermittent nightly LHTL on the acute decrease in  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity with sprint exercise effects or on plasma  $[\text{K}^+]$  during exercise.

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## Introduction

In skeletal muscle, the  $\text{Na}^+$ ,  $\text{K}^+$ ATPase enzyme comprises a catalytic  $\alpha$  subunit (100–112 kDa) and a glycosylated  $\beta$  subunit (40–60 kDa) and belongs to a multi-gene family, with different genes encoding four  $\alpha$  ( $\alpha_1$ ,  $\alpha_2$ ,  $\alpha_3$ ,  $\alpha_4$ ) and three  $\beta$  ( $\beta_1$ ,  $\beta_2$ ,  $\beta_3$ ) isoforms (Blanco and Mercer 1998). The  $\text{Na}^+$ ,  $\text{K}^+$ ATPase performs vital functional roles in skeletal muscle, regulating transsarcolemmal  $[\text{Na}^+]$  and  $[\text{K}^+]$  gradients, membrane excitability, and thus contractility (Overgaard et al. 1999; Nielsen and Clausen 2000; Clausen 2003). It is therefore not surprising that the  $\text{Na}^+$ ,  $\text{K}^+$ ATPase is

highly adaptable in skeletal muscle, being increased with physical training (Green et al. 1993; McKenna et al. 1993) and reduced by physical inactivity (Leivseth et al. 1992). A large reduction in the number of functional  $\text{Na}^+$ ,  $\text{K}^+$ ATPase enzymes is deleterious to muscle function. In isolated rat muscle, inhibition of  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity by ouabain increased muscle  $\text{Na}^+$  gain and  $\text{K}^+$  loss during contractions, reduced M-wave area, depressed tetanic force, accelerated fatigue, and also impaired recovery (Clausen and Everts 1989; Clausen et al. 1993; Clausen 2003). Hence, perturbations that reduce muscle  $\text{Na}^+$ ,  $\text{K}^+$ ATPase content and/or activity in human muscle may have similar adverse effects on muscle function.

One such potential intervention is hypoxia, which reduced muscle  $\text{Na}^+$ ,  $\text{K}^+$ ATPase content by ~14% (Green et al. 1999b, 2000) and maximal activity by ~28% (Sandiford et al. 2004). We recently investigated the effects of hypoxia on muscle  $\text{Na}^+$ ,  $\text{K}^+$ ATPase (Aughey et al. 2005), using the live high, train low (LHTL) model commonly employed by elite athletes (Levine and Stray-Gundersen 1997; Hahn and Gore 2001; Hahn et al. 2001). It is now well established that LHTL improves subsequent sea-level performance (Levine and Stray-Gundersen 1992, 1997; Levine et al. 1996; Stray-Gundersen and Levine 1999; Nummela and Rusko 2000; Hahn and Gore 2001; Hahn et al. 2001; Stray-Gundersen et al. 2001; Saunders et al. 2004; Brugniaux et al. 2006). In contrast to previous large reductions with hypoxia, we found surprisingly that  $\text{Na}^+$ ,  $\text{K}^+$ ATPase content was unchanged, with only a small but significant reduction (~3%) in maximal  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity after 23 consecutive nights (n) of LHTL (Aughey et al. 2005). Either methodological factors contributed to our finding of only minor changes in  $\text{Na}^+$ ,  $\text{K}^+$ ATPase with LHTL hypoxia, or elite athletes are resistant to the marked decrease expected in  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity with LHTL (Aughey et al. 2005). It is possible that a large decrease in  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity had occurred early in the 23-n period, but was followed by a compensatory increase. This could in part be consequential to the ongoing, heavy daily training common to well-trained athletes (Hawley et al. 1997), as exercise provides a strong stimulatory effect on muscle  $\text{Na}^+$ ,  $\text{K}^+$ ATPase gene expression (Murphy et al. 2004, 2005; Nordsborg et al. 2005) and training increases  $\text{Na}^+$ ,  $\text{K}^+$ ATPase content (Green et al. 1993, 2004; McKenna et al. 1993; Madsen et al. 1994; Everts et al. 1997; Medbø et al. 2001). An early depressive response would be consistent with the much larger decline in muscle  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity during exercise in acute hypoxia (Sandiford et al. 2004). As the final biopsy was taken 3 days after ces-

sation of LHTL hypoxia in our previous study (Aughey et al. 2005), it is possible that a larger decrease in muscle  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity had already rapidly recovered upon return to normoxia. However, the time course of changes in muscle  $\text{Na}^+$ ,  $\text{K}^+$ ATPase in response to and in recovery from chronic hypoxia is unknown. We therefore measured changes in muscle  $\text{Na}^+$ ,  $\text{K}^+$ ATPase after only 5 nights and within hours after cessation of exposure to 20 nights of LHTL, to test the first hypothesis that LHTL induces a large, early decrease in  $\text{Na}^+$ ,  $\text{K}^+$ ATPase maximal activity that is not sustained with consecutive nightly exposure.

Recent changes to LHTL hypoxic practices for athletes involve interspersing 'blocks' of nightly exposure to hypoxia, with several nights of normoxia (intermittent LHTL, LHTLi), to minimise any adverse psychological impact of LHTL (Saunders et al. 2004). Furthermore, to minimise risks of altitude sickness, athletes now favour slightly lower altitudes up to only ~2,700 m (Saunders et al. 2004). We have also previously reported that 5 n of LHTL was sufficient to improve performance (Roberts et al. 2003), so elected to measure  $\text{Na}^+$ ,  $\text{K}^+$ ATPase function after 5 n. The interspersed nights of normoxia during LHTLi might counter detrimental effects of hypoxic exposure on muscle  $\text{Na}^+$ ,  $\text{K}^+$ ATPase, especially as these athletes continued heavy training. We therefore tested the second hypothesis that LHTLi would attenuate the hypoxia-induced reduction in  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity with LHTLc. Finally we also determined the effects of LHTLi and LHTLc on functional consequences of acute exercise, including the decrease in maximal  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity (Fowles et al. 2002; Fraser et al. 2002; Aughey et al. 2005; Petersen et al. 2005) and the typical rise in plasma  $[\text{K}^+]$ , using sprint exercise matched for power output and duration (Harmer et al. 2000).

## Methods

### Subjects

Thirty-three male endurance-trained athletes (24 cyclists and 9 triathletes) gave written informed consent to participate in the study, which was approved by both the Victoria University Human Research Ethics Committee and the Australian Institute of Sport Ethics Committee and therefore performed in accordance with the 1964 Declaration of Helsinki. Subjects were ranked according to their initial peak  $\text{O}_2$  consumption ( $\dot{V}\text{O}_{2\text{peak}}$ ) and then assigned to one of three groups: control (CON,  $n = 11$ ), live high:train low consecutive

nights (LHTLc,  $n = 12$ ), or live high:train low intermittent nights (LHTLi,  $n = 10$ ). This paper forms part of a larger study also investigating muscle metabolism and respiratory responses to LHTL; hence details of subjects, LHTL, and some test methodologies have already been presented elsewhere (Townsend et al. 2002; Clark et al. 2004). There were no significant differences between groups for age, height, or body mass (Table 1).

### Experimental design

The study was conducted at the Australian Institute of Sport, Canberra, Australia, altitude  $\sim 600$  m ( $P_B \sim 948$  hPa). Due to limited accommodation ( $n = 6$ ) in the altitude house facility used, experimental testing was conducted on four separate occasions over an 11-month period. Each participant was involved in only one of these testing blocks, which lasted  $\sim 7$  weeks. The LHTLc spent 8–10 h night<sup>-1</sup> for 20 consecutive nights in a room enriched with N<sub>2</sub>, at a simulated altitude of 2,650 m ( $F_I O_2$  0.1627; ambient  $P_B \sim 948$  hPa). The LHTLi group also spent a total of 20 nights under the same hypoxic stimulus; however, after every fifth night in hypoxia, subjects underwent 2 n of control. LHTLi subjects spent nights 1–5, 8–12, 15–19, and 22–26 in hypoxia and intervening nights 6, 7, 13, 14, and 20, 21 in normoxia, thus getting the same amount of hypoxic exposure as LHTLc. This method for LHTLi was chosen to reflect current athletic practice (Saunders et al. 2004) and our previous study showing that 5 consecutive nights of LHTL could improve performance (Roberts et al. 2003). CON subjects slept in their own homes in Canberra or in dormitory style accommodation under normobaric normoxia for 20 consecutive nights. Subjects maintained their own training during the study and kept a daily log of duration, mode, and frequency of training beginning

$\geq 1$  week before and continuing throughout the experimental period (Townsend et al. 2002). All training and daytime living and all exercise tests for all subjects were performed under normobaric normoxic conditions, at an altitude of  $\sim 600$  m. All exercise tests were performed on the same electromagnetically braked ergometer (Lode, Groningen, The Netherlands), calibrated using a first-principles calibration rig.

### Peak power output test

All subjects completed an incremental exercise test to voluntary exhaustion to establish their incremental peak power output (PPO) and  $\dot{V}O_{2peak}$  at 18 days prior to altitude exposure (Clark et al. 2004). After a self-paced warm-up, subjects commenced the test at a workrate of 3.3 W kg<sup>-1</sup>, with a 50 W step after 150 s, and then 25 W steps each 150 s until voluntary exhaustion. PPO was determined as the final completed workrate, plus the fraction of the next completed workrate, calculated as  $PPO = W_f + (T_i/150) \times 25$ , where  $W_f$  = final completed workrate;  $T_i$  = time at incomplete workrate; 150 = seconds spent at each workrate; and 25 = the size of the step in workrate. Groups were well matched, with no significant differences between groups for  $\dot{V}O_{2peak}$  or PPO (Table 1).

### Sprint exercise test

After one habituation trial, subjects completed a time to fatigue ( $T_f$ ) trial at a workrate corresponding to 170% PPO, at each of 15 and 8 days before LHTL or CON trials. The protocol for individual subjects was pre-programmed into the ergometer to allow a rapid (1 KW s<sup>-1</sup>) ramp to the desired workrate for the sprint. Prior to the sprint test, subjects underwent a standardised warm-up comprising 10 min at 1.5 W kg<sup>-1</sup> and then 2.5 min at 2 W kg<sup>-1</sup>. During the latter seconds of the warm-up subjects were encouraged to increase cadence to 140 rev min<sup>-1</sup> to ensure they were capable of maintaining pedalling momentum once the desired sprint workrate was reached. The intra-trial (1 and 2) coefficient of variation (CV) for time to fatigue was 13.4% ( $n = 33$ ).

For the experimental sprint tests conducted before and after LHTL intervention or control, sprint exercise power output and duration were carefully matched by limiting the sprint to 90%  $T_f$  for each subject. This allowed comparison of changes in Na<sup>+</sup>, K<sup>+</sup>ATPase activity and plasma [K<sup>+</sup>] during exercise bouts matched for total work, to maximise the likelihood of detecting any intervention-induced changes (Harmer et al. 2000).

**Table 1** Subject physical and peak performance characteristics

	CON	LHTLc	LHTLi
<i>n</i>	11	12	10
Age (year)	26.2 ± 4.5	27.2 ± 5.7	26.2 ± 4.5
Height (cm)	177.6 ± 5.4	181.1 ± 8.0	176.8 ± 11.9
Body mass (kg)	71.3 ± 6.0	73.8 ± 10.7	69.6 ± 8.9
$\dot{V}O_{2peak}$ (l min <sup>-1</sup> )	4.75 ± 0.22	4.66 ± 0.48	4.58 ± 0.50
PPO (W)	362 ± 5	376 ± 14	353 ± 13
Sprint 90% $T_f$ (s)	52 ± 3	46 ± 2	45 ± 5

Data are mean ± SD. No significant differences were found between groups for any variable,  $n = 33$

$\dot{V}O_{2peak}$ , peak oxygen consumption; PPO, peak power output; Sprint 90%  $T_f$ , time to fatigue to 90% of peak sprint power output

Matched sprint tests were conducted 6 days prior to (Pre); after 5-n LHTL (d5); and at 1 day after 20-n LHTL (Post) altitude exposure or control. Mean cadence was calculated as an additional verification of matching of exercise bouts from Pre to Post tests. Prior to intervention, the cadence upon commencement of the sprint did not differ between groups (CON  $142 \pm 6$ ; LHTLc  $140 \pm 4$ ; LHTLi  $140 \pm 5$  rev  $\text{min}^{-1}$ ).

#### Simulated altitude

Details of the operation of the altitude house have previously been reported in detail (Clark et al. 2004). Briefly, ambient air in the 'altitude house' was enriched with nitrogen gas until the desired fraction of oxygen ( $F_{\text{I}}\text{O}_2$  0.1627) was obtained, equivalent to an altitude of approximately 2,650 m above sea level.

#### Blood sampling, analyses, and calculations

Before each of the sprint tests, a catheter (20-G, Jelco) was inserted into a superficial dorsal hand vein, and the site was covered with an adhesive plastic dressing and the hand sheathed in a waterproof glove. After catheterisation, each subject was seated on the cycle ergometer and the catheterised hand was immersed in a water bath (44.5°C) for 10 min to ensure arterialisation of venous blood (McKenna et al. 1997). Efficacy of arterialisation was demonstrated since  $p\text{O}_2 > 70$  mmHg (data not shown) (Forster et al. 1972; McLoughlin et al. 1992). Blood was sampled (2 ml) from the dorsal hand vein beginning at the last 15 s of the sprint workrate to allow time to complete sampling before muscle biopsy sampling and at 1, 3, and 5-min recovery. Blood samples were immediately analysed for plasma potassium concentration  $[\text{K}^+]$  using an automated analyser (Radiometer ABL 700, Copenhagen, Denmark). The rise in plasma  $[\text{K}^+]$  above rest ( $\Delta[\text{K}^+]$ ) was calculated for each test as previously described (McKenna et al. 1993; Fraser et al. 2002).

#### Muscle biopsy sampling and analyses

A muscle biopsy was taken both at rest and immediately after the sprint exercise test, on three separate occasions, before (Pre), after 5-n (d5), and after 20-n (Post) of the LHTL or CON interventions. The needle biopsy sample was taken from the vastus lateralis muscle under local anaesthesia (Xylocaine, 1%), with suction applied to the needle. The post-exercise sample was taken immediately after cessation of the sprint exercise test with the subject lying supported on the cycle ergometer. The two biopsies in each trial were

taken from separate incisions on contra-lateral legs. All biopsies were taken at an approximately constant depth by an experienced medical practitioner. Muscle samples (100–120 mg) were removed, blotted on filter paper to remove blood, with any obvious fat or connective tissue removed by dissection. The muscle was then rapidly divided into two portions by one investigator, with one portion immediately frozen and stored for later analysis of  $[\text{}^3\text{H}]$ -ouabain binding sites and buffering capacity (Clark et al. 2004). Another investigator concurrently rapidly homogenised the second portion for maximal 3-O-MFPase activity (Fraser and McKenna 1998), with this process commencing within 60 s of muscle sampling.

#### Maximal 3-O-MFPase activity

Maximal  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity was measured using the  $\text{K}^+$ -stimulated 3-O-methylfluorescein phosphatase (3-O-MFPase) activity assay (Fraser and McKenna 1998; Fraser et al. 2002). Muscle, 10–15 mg, was quickly blotted on filter paper, weighed, then homogenised (5% wt/vol.) on ice for  $2 \times 20$  s, 20,000 rpm (Omni 1000, Omni International) in an homogenate buffer containing 250 mM sucrose, 2 mM EDTA, and 10 mM Tris (pH 7.40) (Fraser and McKenna 1998). The homogenate maximal in vitro  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity was determined in triplicate. The muscle 3-O-MFPase activity intra-assay variability was 5.6% ( $n = 52$ ). The 3-O-MFPase activity was also expressed relative to the muscle homogenate total protein content, which was measured spectrophotometrically (Lowry et al. 1951).

#### $[\text{}^3\text{H}]$ -ouabain binding sites

$\text{Na}^+$ ,  $\text{K}^+$ ATPase content was determined in quadruplicate using  $[\text{}^3\text{H}]$ -ouabain binding site content (Nørgaard et al. 1984). Muscle samples were cut into 2–5 mg pieces and washed for  $2 \times 10$  min in 37°C vanadate buffer containing 250 mM sucrose, 10 mM Tris, 3 mM  $\text{MgSO}_4$ , and 1 mM  $\text{NaVO}_4$  (pH 7.2–7.4). This was to thaw the samples and to maintain lower  $\text{Na}^+$  and  $\text{K}^+$  concentrations to minimise interference with vanadate-facilitated  $[\text{}^3\text{H}]$ -ouabain binding. Muscle samples were then incubated for 120 min at 37°C in the above buffer with the addition of  $[\text{}^3\text{H}]$ -ouabain ( $10^{-6}$  M,  $2.0 \mu\text{Ci ml}^{-1}$ ). After incubation, muscle samples were washed for  $4 \times 30$  min in ice-cold vanadate buffer to remove any unbound  $[\text{}^3\text{H}]$ -ouabain, blotted on filter paper, and weighed before being soaked overnight in vials containing 0.5 ml of 5% trichloroacetic acid (TCA) and 0.1 mM ouabain. The following morning,



2.5 ml of scintillation cocktail (Opti-Fluor, Packard) was added prior to liquid scintillation counting of the [ $^3\text{H}$ ]-activity. The content of [ $^3\text{H}$ ]-ouabain binding sites was calculated on the basis of the sample wet weight and the specific activity of the incubation medium and samples and expressed as pmol (g wet wt) $^{-1}$ . The effects of exercise on muscle [ $^3\text{H}$ ]-ouabain binding content were also examined, with data pooled from all groups prior to intervention, to examine a possible exercise effect on [ $^3\text{H}$ ]-ouabain binding ( $n = 20$ ). Due to the limited availability of muscle tissue, muscle  $\text{Na}^+$ ,  $\text{K}^+$ ATPase content could not be measured for all subjects, days and times. Therefore, the resting muscle [ $^3\text{H}$ ]-ouabain binding site content was contrasted between Pre, d5 and Post conditions where a complete set of rest samples was available (CON,  $n = 4$ ; LH TLc,  $n = 3$ ; and LH TLi,  $n = 3$ ). For the rest versus exercise comparison, [ $^3\text{H}$ ]-ouabain binding was expressed relative to muscle protein content (pmol (g protein) $^{-1}$ ). The resting muscle [ $^3\text{H}$ ]-ouabain binding site content intra-assay CV was 17.0% ( $n = 92$ ).

#### Statistical analyses

All data are presented as mean  $\pm$  SD. The physical and training characteristics of the three groups were analysed with a one-way ANOVA. The Student–Newman–Kuels post hoc test was used to locate differences found in ANOVAs. Statistical significance was accepted at  $P < 0.05$ . A three-way ANOVA with independent factor for group (CON, LH TLc, and LH TLi) and with repeated measures for sample time (rest, exercise) and day (Pre, d5, and Post) was used to test for main and interaction effects for muscle and blood data. Exercise reproducibility data and [ $^3\text{H}$ ]-ouabain binding content were analysed with a two-way repeated measures ANOVA for group and day. Maximal 3-*O*-MFPase activity in resting muscle was normalised to pre-intervention resting levels at d5 and Post and the change score (Huck and McLean 1975) was contrasted between CON and each LH TL group with a repeated measures (day) ANOVA. The change score is an accepted method to account for the small sample size and typical inter-subject, intra-, and inter-assay variabilities apparent in invasive human trials (Aughey et al. 2005).

## Results

### Sprint exercise

Sprint tests were successfully matched, with no difference between groups or test days for sprint time

(Table 1) or mean cadence (Table 2). Thus, changes with exercise in  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity and plasma [ $\text{K}^+$ ] occurred with an identical exercise stimulus.

### Effects of acute sprint exercise on $\text{Na}^+$ , $\text{K}^+$ ATPase and plasma [ $\text{K}^+$ ]

#### Muscle 3-*O*-MFPase activity

The muscle maximal in vitro 3-*O*-MFPase activity declined by  $12.9 \pm 4.0\%$  immediately after sprint exercise when expressed per gram wet weight (nmol min $^{-1}$  (g $^{-1}$  wet wt) $^{-1}$ ) ( $P < 0.05$ , Fig. 1,  $n = 63$ ) and declined similarly by  $12.0 \pm 1.5\%$  when expressed per gram protein (Rest  $1.545 \pm 115$ ; End-ex  $1.361 \pm 117$  nmol min $^{-1}$  g protein $^{-1}$ ).

#### Muscle [ $^3\text{H}$ ]-ouabain binding

The reduction in 3-*O*-MFPase activity with acute sprint exercise was not due to a decreased number of  $\text{Na}^+$ ,  $\text{K}^+$ ATPase, as [ $^3\text{H}$ ]-ouabain binding content was unchanged. Data pooled for all groups prior to intervention ( $n = 20$ ) did not differ between rest and exercise whether expressed per gram wet weight (Rest  $318 \pm 37$ ; End-Ex  $327 \pm 41$  pmol (g wet wt) $^{-1}$ , NS) or per gram protein (Rest  $1,825 \pm 212$ ; End-Ex  $1,879 \pm 235$  pmol (g protein) $^{-1}$ , NS).

#### Plasma [ $\text{K}^+$ ]

Arterialised venous plasma [ $\text{K}^+$ ] (Fig. 4) increased above rest with sprint exercise and declined by 5-min recovery ( $P < 0.001$ ).

### Effects of LH TL on $\text{Na}^+$ , $\text{K}^+$ ATPase and plasma [ $\text{K}^+$ ]

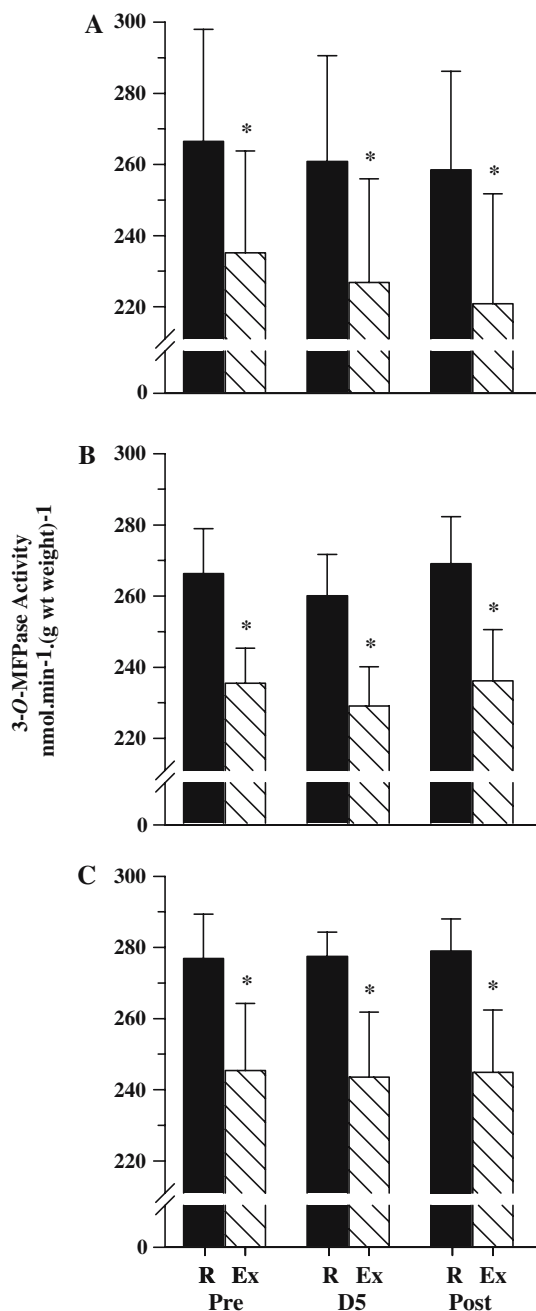
#### Muscle 3-*O*-MFPase activity

Distinctly different patterns of changes in resting muscle maximal 3-*O*-MFPase activity were seen

**Table 2** Matched sprint mean cadence reproducibility data for CON ( $n = 7$ ), LH TLc ( $n = 8$ ), and LH TLi ( $n = 9$ )

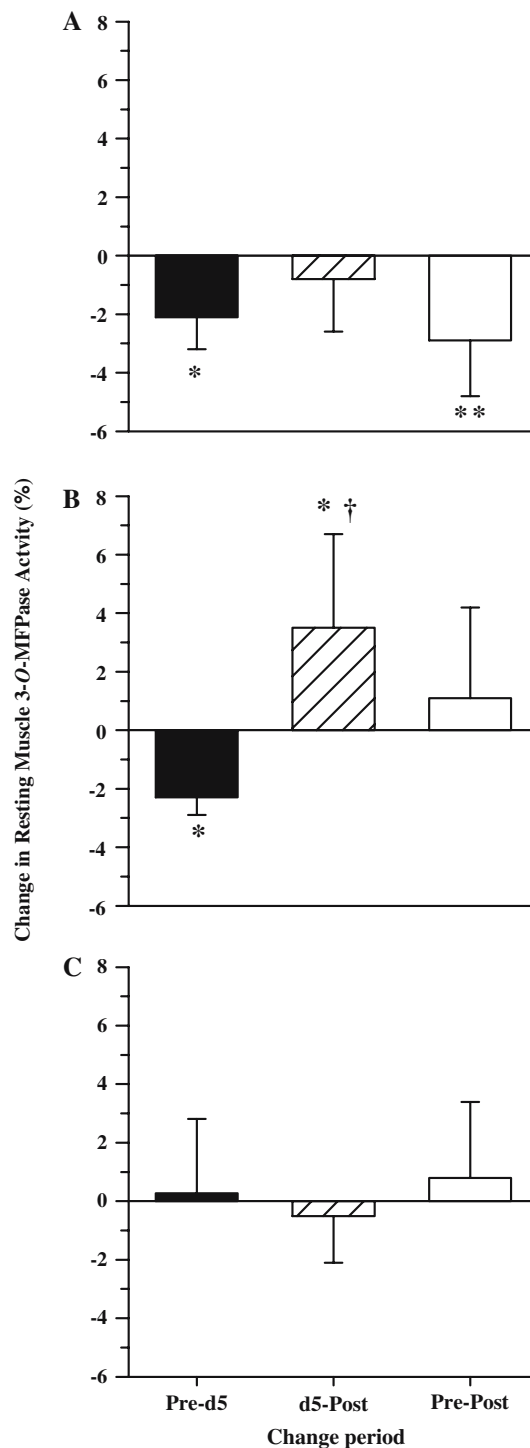
Day	CON	LH TLc	LH TLi
Pre	128 $\pm$ 5	125 $\pm$ 6	122 $\pm$ 7
d5	130 $\pm$ 8	125 $\pm$ 8	126 $\pm$ 6
Post	131 $\pm$ 5	126 $\pm$ 8	124 $\pm$ 5

Data are mean  $\pm$  SD. No significant differences were found between groups



**Fig. 1** Skeletal muscle maximal in vitro K<sup>+</sup>-stimulated 3-O-methylfluorescein phosphatase (3-O-MFPase) activity (Na<sup>+</sup>, K<sup>+</sup>ATPase activity) at rest (R) and end sprint-exercise (Ex) for tests conducted 6 days prior to (Pre); after 5 n LHTL (d5); and at 1 day after 20 n (Post), Live high, train low consecutive (LHTLc) (a) or intermittent (LHTLi) (b) hypoxic or control (CON) (c) interventions. Data are mean ± SD; n = 7 each for CON, LHTLc, and LHTLi. \*End-exercise < Rest (P < 0.05, Exercise main effect)

between the three groups (Fig. 2). Whilst maximal 3-O-MFPase activity was unchanged at any day in CON (Fig. 1), change-score analyses indicated that 3-O-MFPase activity decreased by ~2% in both LHTLc and



**Fig. 2** Changes in resting skeletal muscle maximal 3-O-MFPase activity with LHTL. The resting muscle 3-O-MFPase activity was contrasted between Pre, d5, and Post, for LHTLc (a), LHTLi (b), and CON (c) by calculating the Pre-d5, d5-Post and Pre-Post changes in 3-O-MFPase activity and with changes expressed as a percentage of the Pre-intervention value (change score). Data are mean ± SD; n = 7 for each group; \*different to CON, †different to CON and LHTLc, \*\*different from CON and LHTLi (P < 0.05, group by day interaction effect)

LHTLi at d5 ( $P < 0.05$ , Fig. 2). Maximal 3-*O*-MFPase activity did not change further between d5 and Post in LHTLc and was therefore reduced Pre–Post in LHTLc compared to CON ( $P < 0.05$ , Fig. 2). However, in contrast, a clear divergence was found with LHTLi, with a significant increase in 3-*O*-MFPase activity of ~4% subsequently found between d5 and Post, resulting in no Pre–Post reduction being evident ( $P < 0.05$ , Fig. 2). The individual changes in resting muscle maximal 3-*O*-MFPase activity responses with each intervention are also presented (Fig. 3).

*Muscle [<sup>3</sup>H]-ouabain binding*

Resting muscle [<sup>3</sup>H]-ouabain binding content did not differ between groups or days as a result of LHTL (data not shown).

*Plasma [K<sup>+</sup>]*

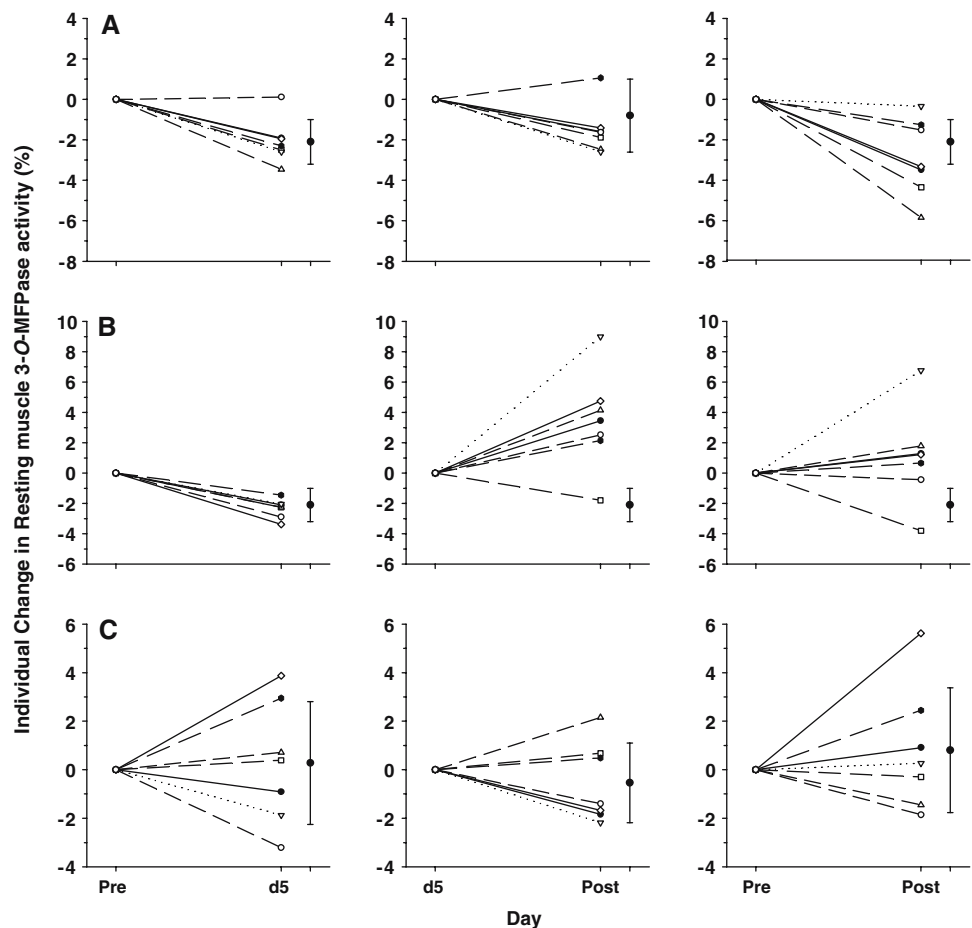
There was no effect of LHTLc or LHTLi on plasma [K<sup>+</sup>] (Fig. 4).

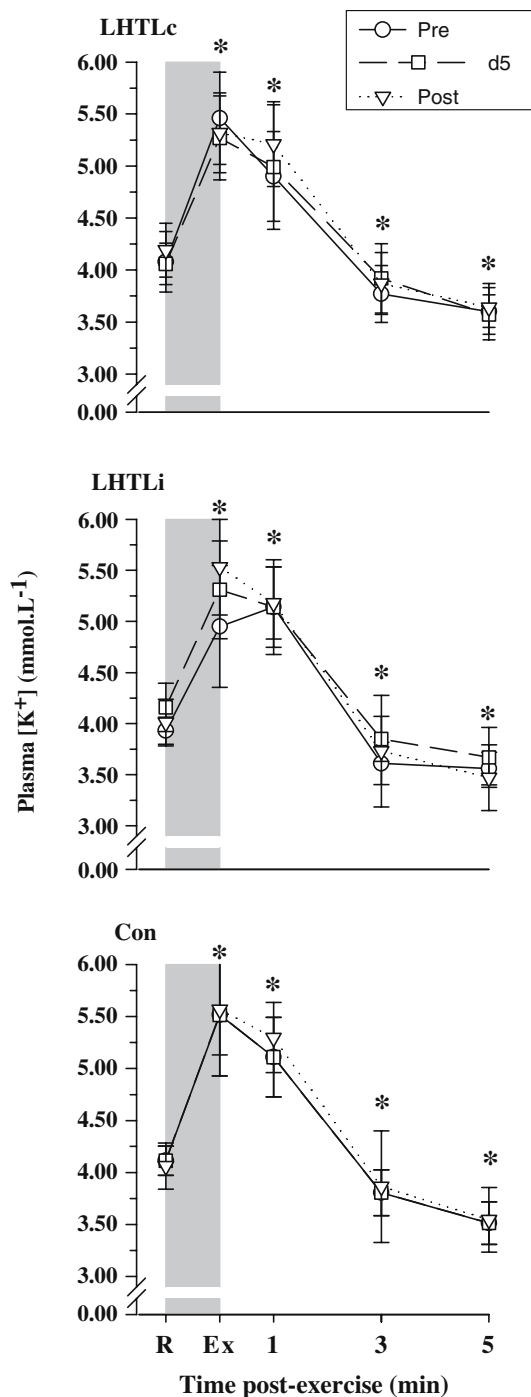
**Discussion**

Early, but small decrease in muscle Na<sup>+</sup>, K<sup>+</sup>ATPase activity with LHTL

A novel finding is that the depressive effects of LHTL on Na<sup>+</sup>, K<sup>+</sup>ATPase activity had already occurred within the first 5 n of modest hypoxic exposure. Thus an early decline in Na<sup>+</sup>, K<sup>+</sup>ATPase activity with LHTLc was evident in these well-trained athletes, likely to be already highly adapted for Na<sup>+</sup>, K<sup>+</sup>ATPase content compared to untrained participants (Klitgaard and Clausen 1989; Fraser et al. 2002). This finding confirms the hypothesised rapid onset of hypoxia-induced decrease in Na<sup>+</sup>, K<sup>+</sup>ATPase maximal activity. However, the magnitude of reduction in maximal Na<sup>+</sup>, K<sup>+</sup>ATPase activity was small and similar to the 3% decline that we previously found after 23 n consecutive LHTL (Aughey et al. 2005). Thus we reject the hypothesis that a large early decline in maximal activity occurred with LHTL. Further, we show that this early decline was still present after continuing

**Fig. 3** Individual changes in resting skeletal muscle maximal 3-*O*-MFPase activity with LHTL. The individual resting muscle 3-*O*-MFPase activity was contrasted between Pre, d5, and Post, for LHTLc (a), LHTLi (b), and CON (c) by calculating the Pre–d5, d5–Post and Pre–Post changes in 3-*O*-MFPase activity and with changes expressed as a percentage of the Pre-intervention value (change score). Data are mean ± SD;  $n = 7$  for each group





**Fig. 4** Arterialised venous plasma  $[K^+]$  for LHTLc (a), LHTLi (b), and CON (c) for blood samples obtained at rest, during sprint exercise, and at 1-, 3- and 5-min recovery prior to (Pre); after 5 days (d5); and at 1 day after 20 nights (Post) interventions;  $n = 11$  for CON,  $n = 12$  for LHTLc, and  $n = 10$  for LHTLi; \*different to preceding sample ( $P < 0.05$ , exercise main effect)

consecutive nightly hypoxic exposure (LHTLc). We therefore also reject the hypothesis of a compensatory upregulation in response to the early decrease in  $Na^+$ ,  $K^+$ ATPase activity in athletes undergoing LHTL.

We also show that the small decrease in maximal activity after LHTL was not due to methodological factors, as was potentially the case previously (Aughey et al. 2005). The Post-LHTL biopsy here occurred within 2–8 h of hypoxic exposure cessation, much earlier than the 3–4 days post-LHTL in our previous study (Aughey et al. 2005). The results of this study and our earlier study (Aughey et al. 2005) therefore suggest that the time required for restoration of  $Na^+$ ,  $K^+$ ATPase activity after hypoxia may be considerably delayed, with reduced  $Na^+$ ,  $K^+$ ATPase activity persisting for at least several days after return to normoxia. This finding may have important implications for muscle recovering from a more pronounced hypoxic insult.

An important question is why these effects with LHTL were so small, given the ~14% reduction in  $Na^+$ ,  $K^+$ ATPase content experienced after chronic hypoxia (Green et al. 1999b, 2000) and the ~29% reduction in  $Na^+$ ,  $K^+$ ATPase activity with exercise under hypoxic conditions (Sandiford et al. 2004). Athletes continue their normal heavy exercise training in normoxia during daytime hours, whilst they are exposed to nightly hypoxia in LHTL. Acute exercise of only a few minutes duration can upregulate  $Na^+$ ,  $K^+$ ATPase mRNA (Murphy et al. 2004, 2005; Nordsborg et al. 2005) whilst just 6 days of training (Green et al. 1993, 2004) and chronic training upregulated  $Na^+$ ,  $K^+$ ATPase content (McKenna et al. 1993; Green et al. 1999a). As athletes in this study maintained ~15 h week<sup>-1</sup> of training, it is therefore possible that a potentially larger hypoxia-induced decrease in  $Na^+$ ,  $K^+$ ATPase activity was attenuated by the upregulatory impact of daily, ongoing, heavy training.

#### Reversal of decrease in $Na^+$ , $K^+$ ATPase activity with interspersed normoxia

An important finding was that 2 days of normoxia interspersed within the LHTL program (LHTLi) reversed the initial depressive effect of 5 days LHTL hypoxia on muscle  $Na^+$ ,  $K^+$ ATPase activity. This clearly contrasts the effect of both the 20-n consecutive LHTL employed here and our previous finding with 23-n consecutive LHTL (Aughey et al. 2005) that each induced a decline in  $Na^+$ ,  $K^+$ ATPase activity. The intermittent LHTL group experienced the same severity and duration of hypoxia as the consecutive LHTL group, yet with different responses in muscle  $Na^+$ ,  $K^+$ ATPase. The time spent living and therefore also training in normoxia was the major difference between consecutive and intermittent LHTL. This gives further support to the notion that the stimulatory



effect of ongoing heavy training under normoxic conditions (McKenna et al. 1993; Green et al. 1999a) undertaken by the LHTLi group was adequate to reverse the small decrease in  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity with consecutive LHTL, itself likely attenuated with daily exercise training. The small magnitude of reduction in  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity with both LHTL programs clearly differs from the 28% reduction during exercise under hypoxic conditions (Sandiford et al. 2004) and the ~14% reductions in  $\text{Na}^+$ ,  $\text{K}^+$ ATPase content after an altitude sojourn (Green et al. 2000) or exercise training under hypoxic conditions (Green et al. 1999b), respectively. None of the total time under hypoxic conditions, the severity of hypoxia, or the training status of participants appear to fully explain the different responses to LHTL and these other studies (Aughey et al. 2005). Furthermore, the small decline in  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity with LHTL occurred without alteration in  $\text{Na}^+$ ,  $\text{K}^+$ ATPase content, suggesting an inactivation of existing  $\text{Na}^+$ ,  $\text{K}^+$ ATPase complexes.

#### Sprint exercise depresses muscle $\text{Na}^+$ , $\text{K}^+$ ATPase activity, independent of LHTL

We and others have found that exercise reduced the maximal  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity in muscle, regardless of whether observed after fatiguing single-leg dynamic kicking exercise (Fraser et al. 2002; Petersen et al. 2005) or intense incremental cycling exercise (Aughey et al. 2005) or isometric contractions (Fowles et al. 2002). We report here that intense sprint cycling exercise also depressed muscle maximal  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity, by ~12%. This decrease occurred despite the subjects being very highly trained athletes performing sport-specific exercise. Depressed maximal  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity during intense exercise could theoretically exacerbate the transmembranous  $\text{Na}^+$  and  $\text{K}^+$  fluxes during muscle contraction, which exceed the rate of  $\text{Na}^+$ ,  $\text{K}^+$ ATPase-mediated transport (Clausen et al. 2004). If so, this could reduce the muscle membrane potential (Sjøgaard et al. 1985; Sejersted and Sjøgaard 2000), contribute to a decline in muscle excitability and M-wave area (Fowles et al. 2002), and therefore accelerate muscle fatigue (Clausen 2003). Given the importance of potassium homeostasis to muscle fatigue (Nordsborg et al. 2003; Mohr et al. 2004; Street et al. 2005) and hence also of maximal  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity to muscle function, it is surprising that there appears to be no obvious protective effect of chronic intensive training on this decrease in these well-trained athletes. This is in agreement with our previous findings of no difference

in the exercise-induced decrease in  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity between untrained, resistance-trained, or endurance-trained participants (Fraser et al. 2002). However, one other study has reported a much larger exercise-induced decrease in  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity in untrained participants (Fowles et al. 2002). Thus, it is unlikely that training protects against the exercise-induced decrease in  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity, but this should not be completely ruled out. Furthermore, this decrease in maximal activity occurred in the absence of any change in skeletal muscle  $\text{Na}^+$ ,  $\text{K}^+$ ATPase content. Consistent with previous findings (Aughey et al. 2005; Petersen et al. 2005) this indicates that sprint exercise results in inactivation, rather than loss of  $\text{Na}^+$ ,  $\text{K}^+$ ATPase units in muscle. Further studies are required to examine the impact of reduced maximal  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity on muscle ion regulation and performance.

#### Functional consequences of LHTL and sprint exercise

In contrast to the greater inhibitory effect of hypoxia on maximal  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity during exercise (Sandiford et al. 2004), we found no effect of LHTL exposure on the reduction of  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity with sprint exercise. Furthermore, the effect of sprint exercise was not different in LHTLc or LHTLi groups, despite a different effect of LHTLc and LHTLi on resting maximal  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity in these groups. Thus there did not appear to be any persistent effects of LHTL on exercise-induced changes in  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity. The small decrease in maximal  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity with LHTL was, however, insufficient to adversely affect plasma  $[\text{K}^+]$  during exercise and therefore presumably  $\text{K}^+$  clearance by both active and inactive muscles. Thus, small reductions in  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity in resting muscle do not appear to affect  $\text{K}^+$  homeostasis during exercise.

Collectively, these observations may explain why elite athletes can use LHTL without hypoxia-induced loss of  $\text{Na}^+$ ,  $\text{K}^+$ ATPase molecules and the expected consequential deterioration in muscular performance. The daily normoxic periods, together with daily training, appear sufficient to counter any nightly hypoxia-induced decline. A further 2-day normoxic break interspersed in the LHTL completely prevented the small decline in maximal  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity.

In conclusion, LHTL caused an early decrease in maximal  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity, but this was much smaller than previously reported with acute or chronic hypoxia exposure in non-athletes. This small decline was reversed with short interspersed periods of norm-

oxia, which may point to important implications in more prolonged hypoxia, such as in patients with chronic respiratory disease. Many years of specific training did not appear to protect against a decline in maximal  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity with sprint exercise, consistent with our previous observations (Fraser et al. 2002) and with our proposals of an important role of depressed  $\text{Na}^+$ ,  $\text{K}^+$ ATPase activity in the development of muscle fatigue. However, even after years of hard training, muscle  $\text{Na}^+$ ,  $\text{K}^+$ ATPase is responsive to this LHTL intervention in well-trained athletes.

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