Chronic intermittent hypoxia and incremental cycling exercise independently depress muscle in vitro maximal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in well-trained athletes


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Aughey, R. J., C. J. Gore, A. G. Hahn, A. P. Garnham, S. A. Clark, A. C. Petersen, A. D. Roberts, and M. J. McKenna. Chronic intermittent hypoxia and incremental cycling exercise independently depress muscle in vitro maximal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in well-trained athletes. J Appl Physiol 98: 186–192, 2005. First published March 19, 2004; doi:10.1152/japplphysiol.01335.2003.—Athletes commonly attempt to enhance performance by training in normoxia but sleeping in hypoxia (live high and train low [LHTL]). However, chronic hypoxia reduces muscle Na\textsuperscript{+}-K\textsuperscript{+}-ATPase content, whereas fatiguing contractions reduce Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity, which each may impair performance. We examined whether LHTL and intense exercise would decrease muscle Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity and whether these effects would be additive and sufficient to impair performance or plasma K\textsuperscript{+} regulation. Thirteen subjects were randomly assigned to two fitness-matched groups, LHTL (n = 6) or control (Con, n = 7). LHTL slept at simulated moderate altitude (3,000 m, inspired O\textsubscript{2} fraction = 15.48%) for 23 nights and lived and trained by day under normoxic conditions in Canberra (altitude ~600 m). Con lived, trained, and slept in normoxia. A standardized incremental exercise test was conducted before and after LHTL. A vastus lateralis muscle biopsy was taken at rest and after exercise, before and after LHTL or Con, and analyzed for maximal Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity [K\textsuperscript{+}-stimulated 3-O-methylfluorescein phosphatase (3-OMFPase)] and Na\textsuperscript{+}-K\textsuperscript{+}-ATPase content ([	extsuperscript{3}H]ouabain binding sites). 3-OMFPase activity was decreased by ~29 ± 2.6% in LHTL (P < 0.05) and was depressed immediately after exercise (P < 0.05) similarly in Con and LHTL (~13.0 ± 3.2 and ~11.8 ± 1.5%, respectively). Plasma K\textsuperscript{+} concentration during exercise was unchanged by LHTL; [	extsuperscript{3}H]ouabain binding was unchanged with LHTL or exercise. Peak oxygen consumption was reduced in LHTL (P < 0.05) but not in Con, whereas exercise work was unchanged in either group. Thus LHTL had a minor effect on, and incremental exercise reduced, Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity. However, the small LHTL-induced depression of 3-OMFPase activity was insufficient to adversely affect either K\textsuperscript{+} regulation or total work performed.

sodium-potassium pump; potassium; muscle fatigue; altitude

IN SKELETAL MUSCLE, the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase enzyme is mainly located in the sarcolemma and t-tubular system, is critical in maintaining transsarcolemmal Na\textsuperscript{+} concentration ([Na\textsuperscript{+}]) and K\textsuperscript{+} concentration ([K\textsuperscript{+}]) gradients and membrane excitability, and thus has been linked with fatigue (4, 8, 9). Under fatiguing conditions, skeletal muscle intracellular [Na\textsuperscript{+}] (24, 43, 44) and extracellular [K\textsuperscript{+}] (17, 20, 24, 25, 43, 44) can double, causing inactivation of voltage-dependent Na\textsuperscript{+} channels (41) and reducing the muscle membrane potential (43, 44). The importance of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase for muscle contractility is emphasized when Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity is inhibited. Incubation of rat muscle in ouabain, a specific Na\textsuperscript{+}-K\textsuperscript{+}-ATPase inhibitor, or low-[Na\textsuperscript{+}] and high-[K\textsuperscript{+}] buffers increased the Na\textsuperscript{+}/K\textsuperscript{+} leak-to-pump ratio, depressed muscle tetanic force, accelerated muscle fatigue, and reduced recovery rate and M-wave area (4).

In human skeletal muscle, hypoxia induces Na\textsuperscript{+}-K\textsuperscript{+}-ATPase downregulation, with Na\textsuperscript{+}-K\textsuperscript{+}-ATPase content lowered by ~14% in experienced mountain climbers after a 21-day sojourn to high altitude (14). Furthermore, in untrained subjects, exercise training (13) or acute submaximal exercise under hypoxic conditions (42) decreased Na\textsuperscript{+}-K\textsuperscript{+}-ATPase content and activity by ~14 and ~28%, respectively. Given that chronic hypoxia downregulates muscle Na\textsuperscript{+}-K\textsuperscript{+}-ATPase, which could then adversely affect muscle function, it seems somewhat paradoxical that athletes, coaches, and physiologists have used altitude exposure for many years in a bid to improve athletic performance. It is not known if well-trained athletes are as susceptible to the hypoxia-induced depression in Na\textsuperscript{+}-K\textsuperscript{+}-ATPase. More recently, the practice of living high and training low (LHTL), comprising training in normoxia but sleeping in hypoxia, has gained popularity with athletes (18, 19, 28). As training under even moderate hypoxia compromises training intensity (3), LHTL may, therefore, be advantageous by allowing maintenance of training intensity (18). LHTL has been shown to yield small (0.8–1.3%) improvements in the exercise performance of well-trained athletes during a 4-min all-out effort, a 400-m sprint, and 3,000- as well as 5,000-m runs (18). This raises the interesting question about whether the hypoxia-induced depression of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase is less or absent in LHTL (13, 14). Alternately, if LHTL does reduce muscle Na\textsuperscript{+}-K\textsuperscript{+}-ATPase, it will be important to determine the effect on muscular performance. Whether hypoxia imposed by LHTL induces a depression in skeletal muscle Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in well-trained athletes has not yet been investigated.

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Acute single-leg kicking (9) or submaximal cycling exercise in untrained humans (42) and prolonged running in rats (7) each depress maximal Na\(^{+}\)-K\(^{-}\)-ATPase activity in skeletal muscle. Furthermore, repeated isometric muscle contractions for 30 min at 60% of maximal voluntary contraction depressed muscle Na\(^{+}\)-K\(^{-}\)-ATPase activity by as much as 38%, with corresponding reductions in muscle M-wave area and excitability (8). It is unknown whether vigorous daily training, which involves fatiguing exercise bouts, coupled with nightly hypoxic exposure, as occurs in the well-trained athletes utilizing LHTL practice, would result in a synergistic decline in muscle Na\(^{+}\)-K\(^{-}\)-ATPase activity.

This study, therefore, investigated the effects of a LHTL intervention and of intense exercise on skeletal muscle Na\(^{+}\)-K\(^{-}\)-ATPase activity [K\(^{+}\)-stimulated 3-O-methylfluorescein phosphatase (3-O-MFPase) activity], content ([H\(^{+}\)]o/albumin binding sites), K\(^{+}\) regulation, and exercise performance in well-trained humans. We hypothesized that nightly hypoxia imposed by LHTL would not depress muscle maximal Na\(^{+}\)-K\(^{-}\)-ATPase activity and thus allow enhanced muscle performance, whereas fatiguing exercise would acutely depress muscle maximal Na\(^{+}\)-K\(^{-}\)-ATPase activity.

METHODS

Subjects. Thirteen male athletes (9 triathletes, 2 cross-country skiers, and 2 cyclists) gave written, informed consent to participate in this study, which was approved by the Australian Institute of Sport Ethics Committee and Victoria University of Technology Human Research Ethics Committee. Subjects were ranked according to the pre- and postintervention VO\(_{2}\) peak. The sample size of the LHTL group was limited to six. The nine triathletes (4 Con and 5 LHTL) trained together, whereas the remaining athletes completed their own sport-specific training schedules. The physical characteristics of the Con and LHTL groups and their training frequency, intensity, and duration did not differ (Table 1). This study is part of a larger investigation that has previously reported on the effects of LHTL on hematological variables (2), exercise oxygen consumption and performance, skeletal muscle metabolites, pH and buffering capacity, blood gas, and acid base (11).

Experimental design. The study was conducted at the Australian Institute of Sport, Canberra, Australia, at 600 m altitude (barometric pressure, ~948 hPa). LHTL subjects spent ~9.5 h/night for 23 consecutive nights in a room where enriched nitrogen produced hypoxia that simulated 3,000-m altitude [normobaric hypoxia; inspired O\(_{2}\) fraction (FiO\(_{2}\)) 15.48%]. Con subjects slept in their own homes in Canberra under normobaric normoxia for 23 consecutive nights. Training and daytime living for all subjects were at an altitude of ~600 m.

Biopsy trial incremental exercise test. After one habituation trial, subjects completed an incremental test comprising a four-stage submaximal cycle ergometer test, 4-min rest, and then 2 min at 5.6 W/kg, a work rate equivalent to ~105% VO\(_{2}\) peak. This test was undertaken 3 or 4 days before (Pre) and 3 or 4 days after (Post) the LHTL or Con interventions, as earlier detailed (11). The biopsy trial tests took 2 days to complete and were undertaken both Pre and Post intervention after the VO\(_{2}\) peak trial to minimize the possible interference of muscle soreness in the VO\(_{2}\) peak trial. A muscle biopsy was taken before and immediately after exercise, whereas arterialized venous blood samples were taken before, during, and after the test. All tests were completed under normobaric normoxic conditions in Canberra on the same dynamically calibrated ergometer (Excalibur Sport, Lode, Groningen, Holland). The initial work rates and the subjects’ cadences were replicated for subsequent tests.

VO\(_{2}\) peak trial test. The above test was conducted with the addition of 2 min of “all-out” exercise immediately after the 2-min 5.6 W/kg bout 5 days Pre and 2 days Post LHTL or Con interventions, as earlier detailed (11). Total work (kJ) was recorded, and VO\(_{2}\) peak was determined as the highest value averaged over a 60-s interval during the all-out trial; these results have been previously reported (11). The open-circuit indirect calorimetry system for the measurement of respiratory data and its thorough calibration have been described previously (11). Briefly, it comprises chain-compensated gasometers to measure volume and Ametek (Philadelphia, PA) O\(_{2}\) and CO\(_{2}\) analyzers to measure gas fractions. ArterIALIZED venous blood samples were also taken before, during, and after the VO\(_{2}\) peak trial.

Simulated altitude. Ambient air in the Altitude House was diluted with nitrogen gas until the desired percentage of oxygen (FiO\(_{2}\), 15.48%) was obtained, equivalent to an altitude of ~3,000 m above sea level. Throughout each of the 23 nights, FiO\(_{2}\), and inspired CO\(_{2}\) fraction inside the hypoxic room were measured every 30 min (11).

Blood sampling, analyses, and calculations. Before each of the cycle ergometer tests, a catheter (20 gauge, Jelco) was inserted into a superficial dorsal hand vein and covered with an adhesive plastic dressing and waterproof glove. After catheterization, each subject was seated on the cycle ergometer, and the catheterized hand was immersed in a water bath (44.5°C) for 10 min to ensure arterIALIZATION of venous blood. Efficacy of arterIALIZATION was demonstrated because PO\(_{2}\) >70 Torr (data not shown) (6, 35). Two blood samples (1.5 and 2 ml) were taken from a dorsal hand vein during the last 30 s of each work rate and at 1- and 5-min recovery. Blood samples were immediately analyzed for acid-base variables (11), plasma [K\(^{+}\)], and blood hemoglobin concentration, using an automated analyzer (ABL System 625, Radiometer, Copenhagen, Denmark). Hematocrit was determined in quadruplicate via capillary tubes spun in a microcentrifuge (Biofuge, Heraeus Instruments, Osterode, Germany). Plasma acid-base variables have been reported elsewhere (11). The rise in plasma [K\(^{+}\)] above rest (Δ[K\(^{+}\)]) was calculated for each work rate and also normalized for work performed (Δ[K\(^{+}\)]/to-work ratio (Δ[K\(^{+}\)]/work); mmol·l\(^{-1}\)·J\(^{-1}\)) as previously described (9, 34).

Muscle biopsy sampling and analyses. A muscle biopsy was taken at rest and immediately postexercise, both Pre and Post LHTL or Con intervention. The needle biopsy sample was taken from the vastus lateralis muscle under local anesthesia (Xylocaine, 1%), with suction applied to the needle. The postexercise sample was taken immediately after cessation of the 2-min exercise trial at 5.6 ± 0.4 W/kg, with the subject lying supported on the cycle ergometer. Both biopsies in a trial were taken from separate incisions in the same leg, with the exercise sample taken from an incision ~1.5 cm distal to the rest sample. The same experienced medical practitioner took all biopsies at approximately constant depth. Muscle samples (100–120 mg) were removed

Table 1. Subject physical and training characteristics

<table>
<thead>
<tr>
<th></th>
<th>LHTL</th>
<th>Con</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>25.4±3.6</td>
<td>25.1±5.2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>183.5±10.0</td>
<td>181.2±6.3</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>73.0±6.7</td>
<td>73.3±6.1</td>
</tr>
<tr>
<td>Training, sessions/wk</td>
<td>7.1±2.3</td>
<td>6.8±2.3</td>
</tr>
<tr>
<td>Training intensity, Borg units</td>
<td>13.8±1.1</td>
<td>13.6±1.0</td>
</tr>
<tr>
<td>Training, h/wk</td>
<td>13.4±3.8</td>
<td>10.6±5.7</td>
</tr>
</tbody>
</table>

Values are means ± SD. The live high, train low group (LHTL; n = 6) slept at a simulated altitude of 3,000 m (inspired O\(_{2}\) fraction 15.48%) and trained at 600 m (Canberra, Australia), whereas the control group (Con; n = 7) lived and trained at 600 m. No significant differences were found between groups for any variable.

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and blotted on filter paper to remove blood, with obvious fat and connective tissue removed by dissection. The muscle was then rapidly divided into two portions, with one portion immediately frozen and stored for later analysis of \(^{3}H\)ouabain binding sites and the other immediately homogenized for maximal 3-O-MPFase activity.

**Maximal 3-O-MPFase activity.** Thirty to forty milligrams of muscle were quickly blotted on filter paper, weighed, and then homogenized (5% wt/vol) on ice for 2 × 20 s, 20,000 rpm (Omni 1000, Omni International), in a homogenate buffer containing 250 mM sucrose, 2 mM EDTA, and 10 mM Tris (pH 7.40) (9). The homogenate maximal in vitro Na\(^{+}\)-K\(^{+}\)-ATPase activity was determined in triplicate by using the K\(^{+}\)-stimulated 3-O-MPFase activity assay (9, 10). The resting muscle 3-O-MPFase activity was also contrasted between Pre and Post conditions, for both LHTL and Con, by calculating the Pre-Post change in 3-O-MPFase activity and expressed as a percentage of the Pre intervention value (change score). The muscle 3-O-MPFase activity interassay variability was 9.9% (n = 13), and the intra-assay coefficient of variation for 3-O-MPFase was 5.6% (n = 52). The 3-O-MPFase activity was also expressed relative to the muscle homogenate total protein content, which was measured spectrophotometrically (29).

\(^{3}H\)ouabain binding sites. Na\(^{+}\)-K\(^{+}\)-ATPase content was determined in quadruplicate by using \(^{3}H\)ouabain binding site content, as previously described (9, 26, 34, 39). Muscle samples were cut into 2- to 5-mg pieces and washed for 2 × 10 min in 37°C vanadate buffer containing 250 mM sucrose, 10 mM Tris, 3 mM MgSO\(_4\), and 1 mM NaVO\(_4\) (pH 7.2–7.4). This was to thaw the samples and to maintain lower [Na\(^{+}\)] and [K\(^{+}\)] to minimize interference with vanadate-facilitated \(^{3}H\)ouabain binding. Muscle samples were then incubated for 120 min at 37°C in the above buffer with the addition of \(^{3}H\)ouabain (10\(^{-6}\) M, 2.0 μCi/ml). After incubation, muscle samples were washed for 4 × 30 min in ice-cold vanadate buffer to remove any unbound \(^{3}H\)ouabain, blotted on filter paper, and weighed before being soaked overnight in vials containing 0.5 ml of 5% TCA and 0.1 mM ouabain. The following morning, 2.5 ml of scintillation cocktail (Opti-Fluor, Packard) were added before liquid scintillation counting of the \(^{3}H\) activity. The content of \(^{3}H\)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 picomoles per gram wet weight. Due to the limited availability of muscle tissue, muscle Na\(^{+}\)-K\(^{+}\)-ATPase content could not be measured for all subject days and times (Con rest Pre, n = 5; exercise Pre, n = 2; rest Post, n = 3; exercise Post, n = 4; and LHTL rest Pre, n = 6; exercise Pre, n = 2; rest Post, n = 6; and exercise Post, n = 5). Therefore, the resting muscle \(^{3}H\)ouabain binding site content was contrasted between Pre and Post conditions; a complete set of Pre and Post rest samples was available for LHTL (n = 6) and Con (n = 3). The effects of exercise on muscle \(^{3}H\)ouabain binding content were also examined, but the sample size was too small for meaningful comparisons between groups. Data were also pooled from both groups before intervention to examine a possible exercise effect on \(^{3}H\)ouabain binding (n = 11). The resting muscle \(^{3}H\)ouabain binding site content intra-assay coefficient of variation was 14.5% (n = 51). The \(^{3}H\)ouabain binding site content was also expressed relative to muscle protein content.

Statistical analyses. All data are presented as means ± SD. The physical and training characteristics of the two groups were analyzed with an independent t-test. Maximal 3-O-MPFase activity in resting muscle was normalized to preintervention resting levels, and the change score was contrasted between Con and LHTL with an independent t-test, to account for the small sample size and typical intersubject and intra- and interassay variability (22). A three-way ANOVA with independent factor for group (LHTL vs. Con) and with repeated measures for sample time (rest, exercise) and day (Pre, Post) used to test for main and interaction effects for muscle and blood data. Exercise performance data and \(^{3}H\)ouabain binding content were analyzed with a two-way repeated-measures ANOVA for group and day. The Student-Newman-Keuls post hoc test was used to locate differences found in ANOVAs. Statistical significance was accepted at P < 0.05.

**RESULTS**

**Performance.** LHTL reduced \(\dot{V}O_2\) peak by \(-7.2 ± 4.1\% (Pre 5.08 ± 0.34, Post 4.78 ± 0.36 l/min; P < 0.05)\) with no change in Con [Pre 4.95 ± 0.45, Post 4.87 ± 0.44 l/min, not significant (NS)]. Total work did not differ between groups or days (LHTL Pre 50.0 ± 4.2, Post 49.2 ± 4.2; Con Pre 50.5 ± 6.0, Post 50.3 ± 5.8 kJ, NS) (11).

**Muscle 3-O-MPFase activity.** The muscle maximal in vitro 3-O-MPFase activity expressed per gram wet weight (nmol·min\(^{-1}\)·g wet wt\(^{-1}\)) declined by \(-12.4 ± 4.1\%\) immediately after exercise (P < 0.05, Fig. 1). The muscle 3-O-MPFase activity also decreased from Pre to Post (265 ± 27 vs. 262 ± 26 nmol·min\(^{-1}\)·g wt \(^{-1}\), P < 0.05), with no significant difference detected between groups. Maximal 3-O-MPFase activity in resting muscle was normalized to preintervention resting levels, and the change was contrasted between
Con and LHTL to account for the small sample size and typical intersubject and intra- and interassay variability (change score). This indicated that maximal 3-O-MFPase activity was decreased by $-2.9 \pm 2.6\%$ in LHTL ($P < 0.05$) but was unchanged in Con ($-0.4 \pm 1.2\%, \text{NS}$, Fig. 2). When muscle 3-O-MFPase activity was expressed per gram protein (nmol·min$^{-1}$·g protein$^{-1}$), exercise induced a similar depression ($-12.3 \pm 4.4\%$, $P < 0.05$, Fig. 1).

**Muscle $[^{3}H]$ouabain binding content.** Resting muscle $[^{3}H]$ouabain binding content did not differ between group or day when expressed per gram wet weight (Con Pre 307 ± 41, Post 339 ± 54; LHTL Pre 324 ± 52, Post 303 ± 39 pmol/g wet wt) or per gram protein (Con Pre 1,746 ± 144, Post 1,797 ± 252; LHTL Pre 1,843 ± 311, Post 1,751 ± 267 pmol/g protein, NS). There was no effect of acute exercise on $[^{3}H]$ouabain binding content with data pooled for groups before intervention when expressed per gram wet weight (pmol/g wet wt) or per gram protein (rest 1,918 ± 415; end exercise 2,030 ± 611 pmol/g protein, NS).

**Change in plasma $[K^{+}]$.** Plasma $[K^{+}]$ increased above rest with each work rate and fell to below resting levels at 5-min recovery ($P < 0.05$, Fig. 3). A small increase in plasma $[K^{+}]$ was found in the Post intervention test (Pre 4.92 ± 0.68, Post 5.11 ± 0.77 mmol/l, $P < 0.05$, Fig. 3), but plasma $[K^{+}]$ did not differ significantly between groups. The $\Delta[K^{+}]$ increased above 1.5 W/kg during exercise at 2.5, 3.5, and 4.5 W/kg, remaining elevated at the final 5.6 W/kg work bouts ($P < 0.05$, Table 2). The $\Delta[K^{+}]$/work during the first submaximal work rate (1.5 W/kg) was greater ($P < 0.05$, Table 2) than for subsequent work rates (2.5, 3.5, 4.5, and 5.6 W/kg), was higher Pre for LHTL, and also was different from Con Pre ($P < 0.05$, Table 2). Plasma $[K^{+}]$, $\Delta[K^{+}]$, and $\Delta[K^{+}]$/work results were almost identical in the biopsy trial and the performance trial (data not shown).

**DISCUSSION**

*LHTL induces only a small decline in resting Na$^{+}$-K$^{+}$-ATPase activity.* Hypoxic exposure during either an altitude sojourn or during training depressed skeletal muscle Na$^{+}$-K$^{+}$-ATPase total content (13, 14), and the LHTL approach of nightly hypoxic exposure could be anticipated to similarly downregulate Na$^{+}$-K$^{+}$-ATPase in skeletal muscle. Therefore, an important finding of this study was that intermittent hypoxia, imposed through 23 nights at 3,000-m simulated altitude (LHTL), caused only a very small ($-3\%$) depression in muscle maximal Na$^{+}$-K$^{+}$-ATPase activity, as measured by maximal K$^{+}$-stimulated 3-O-MFPase activity (10) in already well-trained individuals. This contrasts with the $-28\%$ reduction in 3-O-MFPase activity recently reported in untrained participants during acute, prolonged submaximal exercise in hypoxia (42). The small reduction in 3-O-MFPase activity that we report was accompanied by no change in plasma K$^{+}$ regulation.
during exercise. If this were also true for muscle intracellular and extracellular [K+] with LHTL, this would suggest no further deterioration in muscle excitability and muscle function with LHTL. Performance, as measured by total work output during high-intensity cycling, was also unchanged by LHTL. This suggests that the duration, magnitude, or intermittent nature of the hypoxic exposure was insufficient to markedly downregulate muscle Na+-K+-ATPase and adversely affect muscle performance or that well-trained athletes may have a differential response to imposed hypoxia. Two further important findings were that intensive cycling exercise depressed muscle 3-O-MFPase activity in well-trained athletes by ~12% and that this depression was not exacerbated by LHTL. The total time spent under hypoxic conditions, or the degree of changes in muscle Na+-K+-ATPase reported here. These findings are difficult to compare, however, as there may be confounding effects of normobaric vs. hypobaric hypoxia, nutritional changes with altitude, training performed under normoxic vs. hypoxic conditions, and possible effects of cold stress.

It is well established that exercise training in normoxia upregulates muscle Na+-K+-ATPase content in humans, with increases of between 13 and 29% reported (5, 12, 16, 30, 34, 36). Participants in this study were highly trained and engaged in strenuous exercise in normoxia for between 10 and 13 h/wk. As little as 6 min of strenuous exercise are enough to increase skeletal muscle Na+-K+-ATPase mRNA (37), suggesting that continual stimuli with repeated training may be important in the elevation in muscle Na+-K+-ATPase with chronic training. This potential stimulatory effect of exercise training in normoxia could counterbalance the potential decrease in Na+-K+-ATPase activity and/or content with hypoxia or training in hypoxia, thereby explaining the minor decrease in activity that we report with LHTL. Compared with the untrained participants in the hypoxic training study (13) or mountain climbers in the altitude sojourn (14), participants in this study had a far more extensive normoxic training history, which may have further protected against the depressive effect of hypoxia. A further possible explanation is that an initial larger depression in muscle 3-O-MFPase activity occurred in the first week of LHTL, or in the first days Post LHTL, but that this was undetected because our biopsies were taken only Pre and Post the 23-night intervention.

### Table 2. Δ[K+] with exercise and Δ[K+] expressed relative to work performed during the biopsy trial (Δ[K+]-to-work ratio) for Con and LHTL

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Day</th>
<th>Exercise Work Rate, W/kg</th>
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</thead>
<tbody>
<tr>
<td>Δ[K+], mmol/l</td>
<td>LHTL</td>
<td>Pre</td>
<td>0.63 ± 0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>0.53 ± 0.15</td>
</tr>
<tr>
<td>Δ[K+]-to-work ratio, μmolH-1J-1</td>
<td>LHTL</td>
<td>Pre</td>
<td>26.7 ± 5.1†§‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>19.0 ± 5.6†</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 7 for Con and n = 6 for LHTL groups. Δ[K+], rise in plasma [K+]. *Different from 1.5 W/kg, †different from all other work rates: P < 0.05 (time main effect). §Greater than Con Pre, ‡LHTL Pre > Post: P < 0.05 (group by time by day interaction effect).
The reduction in muscle maximal 3-O-MFPase activity reported here reflects a reduction in the maximal theoretical 3-O-MFPase activity, as measured under in vitro conditions. Muscle Na\(^{+}\)-K\(^{-}\)-ATPase activity may attain near-maximal theoretical levels in rat muscle, when stimulated at high frequencies (32, 38). However, motor unit discharge rates in contracting human muscles are likely to be at lower frequencies (23, 40), and the Na\(^{+}\)-K\(^{-}\)-ATPase might not attain its maximum theoretical activity in vivo (21, 43). During exercise, Na\(^{+}\)-K\(^{-}\)-ATPase activity achieved in vivo is insufficient to completely counteract the loss of K\(^{+}\) from muscle observed during contraction, because large increases in K\(^{+}\) in circulating plasma and in muscle interstitium occur (17, 20, 25, 33). A 12% decline in the maximal in vitro 3-O-MFPase activity with exercise cannot be directly equated with a reduced in vivo Na\(^{+}\)-K\(^{-}\)-ATPase activity. However, it is likely that, in vivo, maximal Na\(^{+}\)-K\(^{-}\)-ATPase activity would be similarly depressed, which may then exacerbate muscle Na\(^{+}\)-K\(^{-}\) fluxes and thereby contribute to muscle fatigue (4).

Our methodology does not allow us to determine the mechanisms underlying the reduction in maximal muscle Na\(^{+}\)-K\(^{-}\)-ATPase activity with exercise. Given that the 3-O-MFPase activity assay is performed under standardized conditions, the reduction is likely to result from structural changes in the Na\(^{+}\)-K\(^{-}\)-ATPase enzyme or changes in the membrane in which it is embedded. Likely candidates for these changes include increases in muscle accumulation of reactive oxygen species (27) and/or intracellular calcium concentration (45), as previously and fully discussed (9). The lack of a cumulative or synergistic depression in muscle Na\(^{+}\)-K\(^{-}\)-ATPase activity with LHTL and intense exercise might suggest that different mechanisms are responsible for the exercise- and hypoxia-induced depression in muscle maximal Na\(^{+}\)-K\(^{-}\)-ATPase activity.

Depressed Na\(^{+}\)-K\(^{-}\)-ATPase activity yet maintained muscle performance. Intriguing questions addressed here were whether muscle Na\(^{+}\)-K\(^{-}\)-ATPase activity was depressed with LHTL and how this could be compatible with reports of increased muscle performance with LHTL (18). This possible dissociation could then suggest irrelevance of changes in muscle Na\(^{+}\)-K\(^{-}\)-ATPase activity for exercise performance in humans. However, our results were not consistent with this possibility, because only a small decline in muscle maximal Na\(^{+}\)-K\(^{-}\)-ATPase activity was evident with LHTL, whereas muscle performance was maintained Post LHTL, as measured by an unchanged total work performed in the final 2 min of an “all-out” test (11). A small reduction in muscle maximal Na\(^{+}\)-K\(^{-}\)-ATPase activity Post LHTL hypoxic exposure may even be beneficial by reducing muscle ATP utilization, which may make a minor contribution to the small reduction in oxygen consumption and improvement in gross mechanical efficiency noted after both LHTL (11) and an expedition to 6,194 m (15).

In conclusion, LHTL hypoxic exposure induced a small but significant depression (2.9%) in skeletal muscle maximal Na\(^{+}\)-K\(^{-}\)-ATPase activity but with no associated decline in muscle Na\(^{+}\)-K\(^{-}\)-ATPase content, performance, or plasma K\(^{+}\) regulation. These findings are important because they demonstrate that a small reduction in skeletal muscle maximal Na\(^{+}\)-K\(^{-}\)-ATPase activity did not affect muscle performance in exercising humans. It is possible that the intermittent nature of the nightly moderate hypoxic exposure and/or daily training in normoxia prevented a more marked deterioration on muscle maximal Na\(^{+}\)-K\(^{-}\)-ATPase. This may explain why athletes can use LHTL without deterioration in performance. Acute exercise itself induced a much larger depression in skeletal muscle maximal Na\(^{+}\)-K\(^{-}\)-ATPase activity, but there were no cumulative or synergistic effects with LHTL. This depression of maximal Na\(^{+}\)-K\(^{-}\)-ATPase activity with vigorous muscular activity was robust and may be an important component of skeletal muscle fatigue.

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