Four weeks of normobaric “live high-train low” do not alter muscular or systemic capacity for maintaining pH and K⁺ homeostasis during intense exercise


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“ALTITUDE TRAINING” is usually performed to increase endurance exercise performance at sea level, but hypoxic exposure may also lead to increased brief intense exercise capacity, as illustrated by the finding that world class track cyclists achieved a 4% performance improvement in an ~5-min all-out test following 31 days of training and living at ~2,700 m (17). Also, 10 days of “live high-train low” (LHTL), where elite runners resided at an altitude equivalent to 2,200 m in normobaric hypoxia while training at sea level, resulted in improved 400-m running performance (34). In contrast, LHTL for 23 days at an inspired O₂ fraction corresponding to 3,000 m with training conducted at 600 m did not alter 2-min all-out cycling performance in endurance athletes (18), and living and training at 1,850 m did not alter 5 × 200 m swimming performance (39). Thus, at present, it is not clear if LHTL increases brief, intense exercise performance or not. An important aspect in answering this question could be the use of a double-blind, placebo-controlled design, because the placebo effect to LHTL may be significant (5, 44).

Enhancement of intense exercise performance following hypoxic exposure may occur through several mechanisms: increased maximal muscular aerobic energy production, improved muscular efficiency, and increased capacity to maintain the homeostasis of fatigue-inducing agents, such as H⁺, K⁺, and P. Controversy exists regarding the potential mechanisms by which LHTL may improve endurance exercise performance, as illustrated by 39 responses to a point-counterpoint discussion in this journal (19, 25). In a recently published part of the present double-blind and placebo-controlled study, LHTL was demonstrated not to affect either hemoglobin mass (Hbmass), maximal oxygen uptake (V̇O₂max), or 26-km time-trial performance (44). It may be noted, however, that 5 of 10 participants did experience an increase in Hbmass, leaving the possibility open that erythropoesis occurred in some individuals. This variation, however, was of no greater magnitude than in the control group, and, since the variations are within the range of biological variation for this measure (36), the observed changes are very likely to be related to biological variation. However, other nonplacebo-controlled LHTL studies have reported increases in Hbmass and V̇O₂max (15, 24, 38) or only Hbmass (10). Moreover, in a LHTL study where a placebo-controlled design was attempted, but unfortunately failed, Hbmass was increased by the intervention (38). Even if the capacity for aerobic energy production and efficiency is unaltered (44), intense exercise performance may still be increased if fatigue tolerance is improved. The existence of such a mechanism was put forward as an explanation for the improved ~5-min all-out exercise capacity (17) and improved maximal 4-min cycling performance (15) previously reported after LHTL and is partly supported by the observation of increased maximal O₂ deficit and improved short-term running performance after 2 wk of living and training at 2,000 m (31), as well as increased maximal O₂ deficit in well-trained cyclist after 5, 10, and 15 days of LHTL at 2,650 m (37). However, following LHTL, the maximal accumulated oxygen deficit may also be unchanged (24), and it remains unclear if LHTL can induce physiological adaptations beneficial for supramaximal exercise performance.

Exercise-induced muscular acidosis has been linked with the development of muscle fatigue for more than a century (13), and muscle function is likely to be impaired if intramuscular pH reaches ~6.7 pH units (14). However, low muscle pH has also been challenged as a fatigue-inducing agent (48). If muscular metabolic acidosis is of importance for muscle func-
tion, then altitude-induced adaptations leading to an augmented muscle buffer capacity could also improve performance. Living at ~2,000 m and training at 2,000–2,700 m for 2 wk has been demonstrated to increase muscle buffer capacity by ~5% (31, 40). In accordance, 75 days of exposure to >5,250 m increases muscle buffer capacity in both leg and arm muscles by ~5–10% (32). LHTL for 21 days caused an even greater (~18%) increase of muscle buffer capacity (18). However, this did not change intramuscular H+ regulation or 2-min all-out work capacity (18). In addition to the possible effect of altitude acclimatization on muscle buffer capacity, living for 37 days above 2,800 m results in an ~40% increase in exercise-induced change (Δ) in lactate concentration ([La]/Δ[H+], termed “extracellular pH defense” (6), defined as −Δ[La]×Δ[PH]−1. The apparent increase in extracellular buffer capacity may be partly related to the improved muscle buffer capacity, but could also be caused by increased capacity for muscular H+ transmembrane transport. However, sarcolemmal expression of Na+/H+ exchanger 1 (NHE1) and La−H+ cotransporters [monocarboxylate transporter (MCT) 1, MCT4] are unchanged by 8-wk exposure to 4,100 m in habitually active subjects (22), and MCT1, as well as MCT4, was also found to be unchanged in well-trained subjects following LHTL (9). Nevertheless, it remains unknown if NHE1 muscle membrane H+ transport capacity is altered in athletes following LHTL. If extracellular pH defense and/or muscle membrane H+ transport capacity is increased with LHTL, the muscle metabolic acidosis could be reduced without changes in muscle buffer capacity per se. Taken together, it appears plausible that hypoxic exposure may alter pH regulation during exercise, with potential implications for muscle fatigue development. However, it also remains unresolved if expression of specific subunits (α1, α2, α3, B1) or the activity-regulating protein FXYD1 (4) is affected differently by LHTL. This seems likely, since the Na+/K+ pump expression in vitro activity has been observed to decrease after LHTL, despite unchanged total Na+/K+ content (1).

In the present study, a 30-s all-out cycling test (“Wingate test”) is applied to investigate if the high-intensity exercise fatigue profile of elite endurance athletes is altered following LHTL. During a 30-s all-out test, the fractional utilization of the maximal accumulated oxygen deficit (30) is 60–95% (7), which demonstrates the high anaerobic energy demand during the test. For elite athletes, the relative anaerobic energy contribution during the test may be as high as 70–80% (8). These observations are in accordance with the observed reduction in muscle pH from 7.17 to 6.68 during a Wingate test in untrained

<table>
<thead>
<tr>
<th>Table 1. Detailed training registrations</th>
<th>Lead-In</th>
<th>LHTL</th>
<th>Post</th>
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<tbody>
<tr>
<td><strong>Distance, km</strong></td>
<td><strong>Week 0</strong></td>
<td><strong>Week 1</strong></td>
<td><strong>Week 2</strong></td>
</tr>
<tr>
<td></td>
<td>111.6±26.0</td>
<td>115.2±29.6</td>
<td>122.8±26.0</td>
</tr>
<tr>
<td><strong>Time spent in intensity zone: 60%</strong></td>
<td><strong>Week 0</strong></td>
<td><strong>Week 1</strong></td>
<td><strong>Week 2</strong></td>
</tr>
<tr>
<td></td>
<td>111.6±26.0</td>
<td>115.2±29.6</td>
<td>122.8±26.0</td>
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| **O2 uptake (V˙O2max)** power output, time, and distance were recorded using either SRM or PowerTap power meters. Power output was normalized to mean of maximum
subjects (29). After 10 days of training in a hypoxic environment, Wingate mean power increase 3.0% in athletes (21). Thus the test is both eliciting a high anaerobic energy production, muscular acidosis, and is sensitive to changes in high-intensity performance induced by environmental factors. In relation to disturbance of ion-homeostasis, venous plasma K⁺ increases to at least 6.5 mM in the femoral vein within the first 30 s of high-intensity exercise (47). Thus very high and potentially fatigue-inducing levels of K⁺ must be expected in the muscular t-tubular system (11). Taken together, the 30-s all-out sprint test is appropriate for evaluating high-intensity exercise performance and fatigue development.

The aim of the present study was to investigate if athletes subjected to 4 wk of normobaric LHTL would experience an altered capacity for maintaining muscle pH. It was hypothesized that LHTL would increase muscle buffer capacity, as well as muscle membrane H⁺ transport capacity. Accordingly, it was hypothesized that the change in blood lactate normalized to the change in blood pH would be reduced after LHTL. It was also the aim to evaluate if LHTL would affect either 30-s all-out exercise capacity or the amount of work completed above the VO2max eliciting intensity during an incremental cycling test. Lastly, the effect of LHTL on ion-homeostasis was evaluated, and it was hypothesized that adaptation to LHTL is associated with reduced K⁺ levels at a given absolute exercise intensity whereas Na⁺-K⁺ pump isoform-specific expression was hypothesized to be unaffected.

METHODS

Subjects. Seventeen highly trained endurance athletes (16 men, 1 woman, age 29 ± 6 yr, height 179 ± 8 cm, body weight 69 ± 9 kg) from various countries in North America and Europe attended as subjects in the present study. All of them regularly participated in endurance competitions on at least national level in disciplines related to cycling, i.e., road cycling, triathlon, cycle cross, and/or mountain bike. To prevent bias from previous altitude acclimatization, we excluded subjects who traveled to altitudes higher than 2,500 m within the last month before the study. All subjects gave written consent to participation, and the study was approved by the ethical boards of Zurich and Waadt (Switzerland).

During the course of the experiment, one subject decided to withdraw participation for personal reasons, and hence his data were not included in the analysis. The remaining 16 subjects all completed the study. Other parts of the study have been published previously (44).

Study design. The study was conducted in Prémonton (France, 1,135 m) over a period of 8 wk. The participants were housed in a facility where the oxygen fraction could be individually adjusted for each room. All experimental procedures were performed at hospital La Vallée (Le Sentier, Switzerland), located at an altitude of 1,020 m.

Table 2. Wingate pedaling rates

<table>
<thead>
<tr>
<th></th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
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<tbody>
<tr>
<td><strong>LHTL</strong></td>
<td></td>
<td></td>
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<tr>
<td>RPMmean</td>
<td>100 ± 3</td>
<td>114 ± 4</td>
<td>111 ± 3</td>
<td>110 ± 3</td>
<td>111 ± 3</td>
</tr>
<tr>
<td>RPMmax</td>
<td>134 ± 4</td>
<td>162 ± 7</td>
<td>150 ± 7</td>
<td>172 ± 4</td>
<td>168 ± 4</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>RPMmean</td>
<td>100 ± 4</td>
<td>110 ± 6</td>
<td>114 ± 3</td>
<td>113 ± 3</td>
<td>113 ± 3</td>
</tr>
<tr>
<td>RPMmax</td>
<td>138 ± 5</td>
<td>147 ± 9</td>
<td>169 ± 8</td>
<td>173 ± 5</td>
<td>164 ± 5</td>
</tr>
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</table>

Values are means ± SD in min⁻¹. RPMmean and RPMmax, mean and maximum revolutions/min, respectively. Significantly different from week −2: *P < 0.05; †P < 0.01; ‡P < 0.001.

Fig. 1. Thirty-second all-out cycling test performed before and after a 4-wk “live high-train low” (LHTL) double-blind, placebo-controlled protocol with an intervention (n = 10) and placebo group (n = 6). The recorded mean power (A), peak power in any 5-s period (B), and fatigue index calculated as the lowest power recorded in any 5-s period relative to the peak power (C) are shown. Values are means ± SD. *Significant (P < 0.05) difference from the value at −1 wk.

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normoxia (placebo group, *n* = 6). Because blinding toward the intervention previously has proved difficult (41), subjects were relocated every week and assigned new roommates. All rooms were controlled and calibrated with precision gases by the company that built the facilities (Fieldbrook, London, UK) immediately before the study. Participants and investigators were blinded with respect to group assignments. Only the principal investigator (who did not perform any measurements) was aware of group allocation. A successful blinding was indicated by questionnaires, as described in Siebenmann et al. (44). In brief, 50% or less of the participants guessed right when asked if they believed themselves to be in the LHTL group. All rooms were similar, and air pumps were constantly turned on, irrespective of participant group assignment. The *O*₂ fraction in each room was continuously monitored from two independent *O*₂ probes (T7OX-V, Oxygen CiticeL, City Technology, Portsmouth, UK) and controlled by the main investigator 5 days/wk or by a qualified technician from the used facility who was not involved in any measurement. In addition, *O*₂ fraction in each room was controlled twice daily via a portable *O*₂ sensor (VNO₂, Cambridge Sensotec, Cambridge, UK) by either the main investigator or the facility technician.

The LHTL group was exposed to normobaric hypoxia equivalent of 2,500 m for 2 days, and subsequently the *O*₂ fraction was decreased to the equivalent of 3,000 m. As a result, morning arterial *O*₂ saturation, estimated by pulse oximetry (NPB-290, Nellcor Puritan Bennett, Pleasanton, CA) was 92 ± 2% in the LHTL group (at 3,000 m) and 97 ± 1% in the placebo group (*P* < 0.05). Participants were asked to stay in their rooms from 2000 to 0700, from 0800 to 1000, and again from 1600 to 1900 during the 4-wk intervention period. Adherence was rigorously supervised by the main investigator 5 days/wk and by other investigators the remaining 2 days. During the last 2 wk of the study, the postintervention period, participants were relieved of the room confinement and hypoxic exposure. The purpose of this period was to investigate the temporal response of potential LHTL effects subsequent to hypoxic exposure. Thus blinding was maintained for participants and investigators until the end of the 8-wk study period.

Six testing sessions were distributed over the 8-wk duration of the study. Two testing sessions were completed during the first 2 wk. Subsequently, testing was performed after 3 and 4 wk in the intervention period, as well as 1 and 2 wk after the intervention period. Specific testing time points are evident from the graphs in the RESULTS section.

**Measurements.** Brief high-intensity exercise performance was evaluated using a 30-s all-out exercise test on a Monark ergometer (Monark, Varberg, Sweden), modified to allow instant application of braking resistance. Participants performed a 10-min warm-up consisting of 5 min at 150 W and 5 min at 200 W. After a brief (<2 min) resting period, participants were instructed to reach >100 rpm unloaded pedaling within 5 s. At 5 s, a resistance of 0.085 × body weight to the nearest 100 g was applied instantly, and participants were verbally encouraged to pedal as fast as possible at any time point during the following 30 s. The braking resistance was chosen based on previous studies of competitive cyclist (23), as well as a mixed athletic population (50), as well as the ranges suggested for optimal mean power determination (3, 46). Flywheel revolutions were recorded with a resolution of 16 points per revolution using a custom-build infrared light-sensitive reflector system. The recordings were used to calculate the average power (mean power): the highest average power in any 5-s period (peak power), and the lowest average power in any 5-s period (minimum power). No correction for flywheel inertia or inherent ergometer braking resistance was performed.

To estimate the exercise capacity when oxygen demand exceeds the supply and when systemic ion-homeostasis and acid/base balance is severely challenged, high-intensity exercise performance was determined as the amount of work completed above the workload calculated to elicit VO₂max, as determined from an exhaustive VO₂max test. The VO₂max test protocol was completed using an electronically braked bicycle ergometer (Monark, Varberg, Sweden), as reported elsewhere (44). Briefly, a 5-min warm-up period at 150 W was followed by 5 min at 200 W, except for the female athlete for whom workloads were 100 and 150 W. Thereafter, the workload was increased by 25 W each minute until exhaustion. Pulmonary *O*₂ and *CO*₂ concentration in expired gas were continuously measured.
All respiratory variables were averaged in blocks of 30 s. The highest recorded average oxygen uptake (\(V_O_2\)) during the test was defined as \(V_O_2_{max}\). Peak workload (\(W_{max}\)) was calculated as \(W_{max} = W_{compl} + 25 \times (t/60)\), with \(W_{compl}\) being the last completed workload, and \(t\) the number of seconds in the not completed workload. The \(V_O_2_{max}\) eliciting workload, if applied for several minutes, was estimated by performing linear regression on \(V_O_2\) recorded at 150, 200, 225, and 250 W. Based on this regression and the recorded \(V_O_2_{max}\), the workload that can be expected to elicit \(V_O_2_{max}\) was calculated. The difference between \(W_{max}\) and the workload estimated to elicit \(V_O_2_{max}\) was termed “high-intensity exercise performance” and used as an index of the capacity to perform work at intensities above \(V_O_2_{max}\).

On the second test before the intervention period and 3 wk into the intervention period, an arterial catheter was inserted into a. radialis before testing. After local anesthesia with 2% lidocaine, a 20-gauge catheter (model 80115.09R, Vygon Laboratories, Ecouen, France) was inserted percutaneously using the Seldinger technique. Arterial blood was sampled anaerobically in heparinized syringes and immediately analyzed for plasma \(K^+\), \(L^+\), and \(pH\) using an ABL 800 blood analyzer (Radiometer, Copenhagen, Denmark). Arterial samples were collected at 200 W and at exhaustion. The index \(-\Delta[L^+]\times\Delta pH^{-1}\) was quantified and interpreted as an index of the in vivo buffer capacity using delta values calculated from the sample obtained at 200 W and at exhaustion. The index has previously been termed “extracellular pH defense” (6). Arterial bicarbonate (\(HCO_3^-\)) concentration was calcu-

![Fig. 5. Plasma pH (A and B), plasma bicarbonate concentration (\(\text{cHCO}_3^-\); C and D), standardized base excess (SBE; E and F), and Pco2 (G and H) in a. radialis before and after a 4-wk LHTL double-blind, placebo-controlled protocol with an intervention (LHTL) and placebo group, determined at a submaximal 200-W workload (A, C, E, and G) and at exhaustion after an incremental test (B, D, F, and H) in normoxia (n = 6 in LHTL and n = 4 in placebo). Values are means ± SD.](http://jap.physiology.org/)

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lated according to the Henderson-Hasselbalch equation solved for $\text{HCO}_3^-$: $[\text{HCO}_3^-] = 0.03 \times \text{PCO}_2 \times 10^{(\text{pH}_a-6.1)}$, where $\text{pH}_a$ is arterial pH. The standardized arterial base excess (SBE$_a$) was calculated (45) as SBE$_a$ = $(1 - [\text{Hb}] \times 0.023) \times ([\text{HCO}_3^-] - 24.4) + (2.3 \times [\text{Hb}] + 7.7) \times ([\text{Hb}] - 7.4)$, where [HCO$_3^-$] is arterial HCO$_3^-$ concentration, and [Hb]$_a$ is arterial Hb concentration. In some cases, catheterization was not possible, and for some samples the automatic analysis resulted in errors. Thus the number of included participants for each analysis and time point is reported in the RESULTS section or in a figure. During the second of the lead-in weeks before the intervention period and after the 4-wk intervention period, a muscle biopsy was obtained from *m. vastus lateralis*. Local anesthesia (Xylocaine, 20 mg/ml) was applied, and a small incision was made through the skin and subcutaneous tissue. The biopsy was obtained using a Bergstrom needle with suction. The muscle tissue was rapidly dissected free of visible fat, blood, and connective tissue, and a part was rapidly frozen (within 120 s) in liquid $N_2$ for later analysis of protein expression using Western blotting. Before analysis of protein expression, muscle tissue was freeze dried and dissected free from blood, fat, and connective tissue. Subsequently, samples were homogenized (Qiagen Tissuelyser II, Retisch, Haan, Germany) in a fresh batch of buffer containing the following (in mM): 10% glycerol, 20 sodium-pyrophosphate, 150 NaCl, 50 HEPES (pH 7.5), 1% NP-40, 20 β-glycerophosphate, 2 Na$_3$VO$_4$, 10 NaF, 2 PMSF, 1 EDTA (pH 8), 1 EGTA (pH 8), 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 3 benzamidine. Afterwards the samples were rotated end over end for 1 h at 4°C and centrifuged at 16,500 g for 30 min at 4°C, and the supernatant (lysate) was used for further analysis. Total protein concentration in each sample was determined by a bovine serum albumin standard kit (Pierce, Rockford, IL), and samples were mixed with 6 × modified Laemmli buffer (7 ml 0.5 M Tris base, 3 ml glycerol, 0.93 g DTT, 1 g SDS, and 1.2 mg bromophenol blue). Samples were loaded together with protein markers (all blue and dual color, Bio-Rad Laboratories) on precasted gels (Bio-Rad Laboratories). Proteins were separated by SDS page gel electrophoresis and semidyed transferred to a polyvinylidene fluoride membrane (Millipore). The membranes were blocked in either 2% skimmed milk or 3% BSA in Tris-buffered saline, including 0.1% Tween 20 (TBST) before an overnight incubation in primary antibody. To determine changes in total protein expression, the following antibodies were used with the localization of the quantified signal noted: Na$^+$–K$^+$ pump α$_1$-subunit: 100 kDa, C464.6 (Millipore); Na$^+$–K$^+$ pump α$_2$-isoform: 100 kDa, 07–647 (Upstate/Millipore); Na$^+$–K$^+$ pump β$_1$-isoform: 50 kDa, MA3–930 (Affinity Bioreagents); unspecific FXYD1 phosphorylation: 12 kDa, AB_FXYD1 (kindly donated by Dr. J. Randall Moorman, University of Virginia); caveolin 3 (CAV3): 21 kDa, ab2912 (Abcam, Cambridge, UK); total actin: 42 kDa, A2066 (Sigma); NHE1: 110 kDa, MAB3140 (Chemicon/Millipore); sarco(endo)plasmic reticulum Ca$^{2+}$ ATPase isofrom 1 (SERCA1): 100 kDa, MA3–912 (Thermo Scientific); MCT1: 43 kDa, AB3538P (Millipore/Chemicon); MCT4: 50 kDa, AB3316P (Millipore/Chemicon); myosin heavy chain I (MHC I): ~200 kDa, A4.840 (Developmental Studies Hybridoma Bank), and MHC II: ~200 kDa, A4.74 (Developmental Studies Hybridoma Bank). The secondary antibodies used were horseradish peroxidase-conjugated goat anti-mouse and goat anti-rabbit (P-0447, DAKO). All samples were analyzed in duplicates on two separate occasions. Samples were excluded if the sample intensity normalized to the total sample intensity was more than 2 SDs from the mean of all analyzed samples. Subsequently, samples were excluded if the post-to-pre ratio was more than 2 SDs from the mean of all analyzed ratios. The actual number of samples included for each analysis was as follows (LHTL and placebo, respectively): Na$^+$–K$^+$ pump α$_1$-subunit (6, 5); Na$^+$–K$^+$ pump α$_2$-subunit (8, 6); Na$^+$–K$^+$ pump β$_1$-subunit (8, 6); FXYD1 (7, 6); MCT1 (8, 6); MCT4 (7, 6); NHE1 (8, 6); SERCA1 (7, 5); actin (7, 6); GAPDH (7, 6); CAV3 (8, 6); MHC I (7, 6); MHC II (6, 6).

Muscle pH was measured by a small glass electrode (Micro pH-elektrode N6000 BNC, SCHOTT Instruments, Mainz, Germany) after homogenization of 1 mg freeze-dried samples in a nonbuffered solution containing 145 mM KCl, 10 mM NaCl, and 5 mM sodium fluoride (28). After having adjusted pH of the sample to 7.1 with 0.01 M NaOH, the sample was titrated to pH 6.5 by serial additions of 0.01 M HCl. The pH was measured after each addition. The non-HCO$_3^-$ physiochemical buffer capacity was determined from the number of moles of H$^+$ required to change pH from 7.1 to 6.5 and was expressed as millimoles H$^+$ per kilogram dry weight per pH (28).

**Training and nutrition.** Subjects were instructed to follow their usual training habits and to keep training intensity and volume as constant as possible throughout the 8 wk. Training was supervised using heart rate monitors (Polar, Suunto or Garmin). To prevent iron deficiency, all subjects were supplemented with daily oral intake of 256 mg dried ferrous sulfate (Tadyferon 80 mg, Pierre Fabre). Details on the performed training are reported elsewhere (44). Not all riders had access to power meters. However, example data are provided for four riders in Table 1. Two riders achieved special advice from the investigators to reduce their training volume and intensity for a period of 4 days because they showed signs of overtraining (tiredness; poor high-intensity performance).

**Statistics.** A mixed model analysis (12) with “time” (for example, −2 wk, −1 wk, 0 wk, 3 wk, 4 wk, 5 wk, 6 wk) and “group” (LHTL and placebo) as fixed factors and with specification of repeated measures for subject was performed in IBM SPSS version 19 statistical software package. If a significant main effect was observed for either “time”, “group”, or an interaction between the two, a post hoc analysis was performed using the Holm-Sidak adjustment for multiple comparisons. Results are means ± SD. When $n$ is different from 10 in the LHTL group and 6 in the placebo group, the actual number is reported. Detection limits were calculated from observed standard deviations of measured parameters using SigmaStat 11.0 power calculation.
Fig. 7. Muscle (m. vastus lateralis) protein expression determined before and after a 4-wk LHTL double-blind, placebo-controlled protocol with an intervention (LHTL) and placebo group. MCT1, monocarboxylate transporter 1; MCT4, monocarboxylate transporter 4; NHE1, Na⁺/H⁺ exchanger 1; SERCA1, sarcoplasmic reticulum Ca²⁺/ATPase isoform 1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; CAV3, caveolin 3; MHC I, myosin heavy chain I; MHC II, myosin heavy chain II. Example Western blots for one duplicate analysis for one subject are shown, together with molecular weight controls (arrows), on top of each panel. Values are means ± SD.
RESULTS

Thirtieth-second all-out cycling test. Mean power during the 30-s all-out cycling test (Fig. 1A) was similar in the LHTL and placebo group at all test occasions. Likewise, the 5-s peak power recorded during the test (Fig. 1B) did not differ significantly between the LHTL and placebo group at any time point. The 30-s all-out fatigue index (Fig. 1C) was unaltered in both groups during the entire study period. In addition, pedaling rates can be seen in Table 2.

High-intensity exercise performance. No significant changes occurred in high-intensity exercise performance, as illustrated in Fig. 2. Before the intervention, the W_max was 399 ± 59 and 407 ± 27 W in LHTL and placebo, respectively. The V̇O₂max workload was 360 ± 47 and 363 ± 42 W in LHTL and placebo, respectively.

Arterial plasma K⁺, La⁻, HCO₃⁻ concentration, SBE, Pco₂, and pH. Arterial plasma K⁺ determined during submaximal exercise and maximal exercise was not affected by the intervention in either group (Fig. 3). Likewise, arterial La⁻ did not differ between the groups, neither before nor after the intervention (Fig. 4). Arterial calculated HCO₃⁻, SBE, Pco₂, and pH at submaximal workload and W_max was also unaltered by the intervention in both groups (Fig. 5).

\[-\Delta[La] \times \Delta pH^{-1}\]. Calculation of the total in vivo buffer capacity revealed no difference between the two groups, neither before nor after the intervention (Fig. 6).

Muscle buffer capacity. The 4-wk intervention period did not cause significant changes in muscle buffer capacity between groups or with time. Muscle buffer capacity before the intervention was 140 ± 12 mmol H⁻ kg dry wt⁻¹ pH⁻¹ in the LHTL (n = 8) and 145 ± 5 mmol H⁺ kg dry wt⁻¹ pH⁻¹ in the placebo (n = 6) group. After the intervention, the muscle buffer capacity was 140 ± 16 mmol H⁺ kg dry wt⁻¹ pH⁻¹ (n = 8) in the LHTL and 140 ± 8 mmol H⁺ kg dry wt⁻¹ pH⁻¹ (n = 6) in the placebo group.

M. vastus lateralis protein expression. For the investigated proteins, no significant interaction between group and time effects was apparent, demonstrating no significant change as a result of the intervention (Fig. 7).

DISCUSSION

Major finding. The major findings in the present study were that 4 wk of placebo-controlled normobaric LHTL did not alter resting muscle buffer capacity, the in vivo buffer capacity index \(-\Delta[La] / \Delta pH\), muscle membrane ion transport capacity, or plasma K⁺ levels during exercise. In accordance with these findings, both 30-s all-out and high-intensity exercise performance remained unchanged.

Intense exercise performance. The present study is the first to investigate 30-s all-out exercise performance after LHTL. The finding of unaltered mean power, peak power, and fatigue index all demonstrate that LHTL does not improve very brief high-intensity exercise performance. This is in contrast to the findings that 10 days of LHTL increase performance in 400-m elite runners (34) and that 5 days of LHTL increase mean power in a 4-min maximal cycling test. However, in the study of 400-m runners, the performance effect was only ~1%. It has previously been discussed that a significant placebo effect may exist in studies of LHTL (5). Furthermore, it should be noted that the maximal speed attained in an anaerobic interval run-

ning test was increased to a similar extent in the control and intervention group (34), thereby demonstrating that LHTL is not more efficient than normal training for this type of exercise. The present observation of unchanged supramaximal exercise capacity did not verify the study hypothesis, but is in line with the observation of unchanged maximal O₂ deficit after 4 wk of LHTL (24) and unchanged ability to perform 2-min all-out exercise performance after 23 days of LHTL (18).

It may be noted that the present mean power results from the Wingate test (~9.6 W/kg) correspond with the reported elite level (>9.8 W/kg) of a large athletic population (n = 1,374 men) (50). However, the observed Wingate mean power of trained endurance cyclists in the present study is lower than what has previously been reported for elite endurance cyclists (12.4 W/kg) (8) and elite BMX riders (10.4 W/kg) (49). Possible reasons for the apparent discrepancy may be that Calbet et al. (8) used a higher braking resistance (0.11/body weight) than in the present study (0.085/body weight) and that BMX riders are specifically sprint trained athletes (49). Moreover, Calbet et al. (8) corrected for flywheel inertia and inherent unloaded ergometer friction, which was not done in the present study. In the present study, a rather pronounced increase in Wingate mean power from the first to the second test was apparent (Fig. 1). This is likely to reflect that the participating endurance athletes were unaccustomed to the test before entering the study and demonstrates the importance of the applied lead-in period.

pH homeostasis. In the present study, neither plasma pH, plasma lactate, SBE, arterial HCO₃⁻, arterial Pco₂, or the calculated arterial \(-[La] / [H⁺] (i.e., in vivo buffer capacity) was altered by the LHTL intervention. This is in agreement with the observation of unchanged muscle buffer capacity and unaltered expression of the muscle membrane H⁺ transport proteins, MCT1, MCT4, and NHE1. These observations also contrast the study hypothesis of improved muscle H⁺ handling capacity. The hypothesis was based on previous reports of increased muscle buffer capacity with continuous altitude acclimatization (18, 31, 40) and increased extracellular pH defense (6). A notable difference between the present study and previous studies is that the athletes in one study both lived and trained at >2,000 m (31, 40), which may have caused more severe muscular pH perturbations. It is, however, possible that prolonged and more severe hypoxic exposure can increase muscle buffer capacity, as demonstrated after 75 days of exposure to >5,250 m (32). In most apparent conflict with the present results are the observation that LHTL for 21 days caused an ~18% increase of muscle buffer capacity (18). However, in that study, both muscle in vivo buffer capacity \((-[H⁺] / [La]⁻) and postexercise muscle [H⁺⁻] were unaltered. Also, the current finding of unchanged expression of sarcolemmal NHE1, MCT1, and MCT4 is in accordance with

<table>
<thead>
<tr>
<th>Value</th>
<th>Within LHTL</th>
<th>Within placebo</th>
<th>Between LHTL and placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁺, mM</td>
<td>0.29</td>
<td>0.25</td>
<td>0.42</td>
</tr>
<tr>
<td>pH</td>
<td>0.115</td>
<td>0.109</td>
<td>0.145</td>
</tr>
<tr>
<td>La⁻, mM</td>
<td>0.94</td>
<td>0.74</td>
<td>1.12</td>
</tr>
<tr>
<td>(-[La] / [H⁺]⁻)</td>
<td>27.5</td>
<td>25.3</td>
<td>44.1</td>
</tr>
</tbody>
</table>

Values are changes in arterial values that are likely to have been detected with the current number of subjects and observed SD for each analysis. Power = 0.8 and α = 0.05.
the observation of unchanged expression of these proteins after acclimatization to 4,100 m for 8 wk (22).

$K^+$ homeostasis. Four weeks of LHTL did not alter the arterial $K^+$ levels at submaximal or maximal exercise, indicating an unaltered capacity for maintenance of ion-homestasis. This is in line with the finding of unaltered Na$^+$-$K^+$ pump $\alpha_1$-, $\alpha_2$-, and $\beta_1$-subunit expression, as well as unaltered expression of the Na$^+$-$K^+$ pump activity regulating protein FXYD1. Our laboratory has previously demonstrated that 8 wk of acclimatization to 4,100 m causes a reduced net $K^+$ release from the exercising muscles during ergometer cycling and reduced plasma $K^+$ levels at a given workload in hypoxia (33). Furthermore, 21 days of gradual ascent to 6,194 m and subsequent decent have been found to reduce muscle Na$^+$-$K^+$ pump expression by $\sim$14% (20). However, 23 days of LHTL do not affect Na$^+$-$K^+$ pump protein expression or plasma $K^+$ during exercise (1, 35), which is in agreement with the present observations. Thus it appears that $K^+$ homeostasis and Na$^+$-$K^+$ pump expression may be affected by prolonged severe hypoxic exposure, but not by 4 wk of LHTL at $\sim$3,000 m.

Muscle proteins. In the present study, no normalization of the expression of the investigated proteins to the so-called “housekeeping” proteins ($\beta$-actin, MHC I, MHC II, GAPDH, CAV3) was performed. Instead, normalization was performed to total protein content as described. The reason for this approach is that biological and analytic variation exists for both target and housekeeping proteins. Thus the variability of the target protein-to-housekeeping protein ratio must be expected to be higher than for the target protein alone. The similar expression of muscle actin, MHC I, MHC II, and GAPDH expression before and after the intervention indicates that no change in cytoskeletal properties or non-key regulatory enzymes occurred. Furthermore, the similar SERCA1 expression before and after the intervention indicates that sarcosomal Ca$^{2+}$ reuptake capacity was also unaltered. This agrees with previous findings with prolonged and continuous altitude exposure in regards to cytoskeletal structure (26, 27, 32). Also, the expression of CAV3, which forms caveolae localized on the sarcolemmal membrane (16), was unchanged. Thus no change in membrane to total protein expression appeared to occur with LHTL. It may be noted that the reported results for the investigated target proteins were similar when normalized to housekeeping protein expression (data not shown). It may be speculated that the total muscle mass changed during the intervention period and thus an undetected global change of protein expression pattern may have existed. However, the athletes were weight stable, and no significant gain or loss of muscle mass would be expected with the applied training procedures.

Limitations. The recruitment of elite-level athletes and the study period of 2 mo resulted in limitations primarily related to the statistical power. This is especially true for the arterial measurements, where $n = 6$ in the LHTL group and $n = 4$ in the placebo group. Therefore, detection limits for the primary arterial values is shown in Table 3. With regard to changes in muscle characteristics, the calculated detection level of muscle buffer capacity was 12% between groups and 8% within the LHTL group. For muscle protein expression, detection limits varied from $\sim$15 to 40%, dependent on the investigated protein. Based on the observed standard deviation of the group mean difference in Wingate mean power in successive tests, a change of $\sim$20 W in group mean difference would have been detected.

Conclusion. In conclusion, 4 wk of placebo-controlled normobaric LHTL do not alter muscular or systemic capacity for maintaining pH and $K^+$ balance during exercise. In accordance, the capacity to perform work at intensities just above the $V_{O_2\text{max}}$ and a 30-s all-out sprint is also unaltered.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


