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Live high:train low increases muscle buffer capacity and submaximal cycling efficiency

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ABSTRACT

This study investigated whether hypoxic exposure increased muscle buffer capacity (βm) and mechanical efficiency during exercise in male athletes. A control (CON, $n = 7$) and a live high:train low group (LHTL, $n = 6$) trained at near sea level (600 m), with the LHTL group sleeping for 23 nights in simulated moderate altitude (3000 m). Whole body oxygen consumption ($\dot{V}O_2$) was measured under normoxia before, during and after 23 nights of sleeping in hypoxia, during cycle ergometry comprising 4 × 4-min submaximal stages, 2-min at 5.6 ± 0.4 W kg^{-1}, and 2-min 'all-out' to determine total work and $\dot{V}O_{2peak}$. A vastus lateralis muscle biopsy was taken at rest and after a standardized 2-min 5.6 ± 0.4 W kg^{-1} bout, before and after LHTL, and analysed for βm and metabolites. After LHTL, βm was increased (18%, $P < 0.05$). Although work was maintained, $\dot{V}O_{2peak}$ fell after LHTL (7%, $P < 0.05$). Submaximal $\dot{V}O_2$ was reduced (4.4%, $P < 0.05$) and efficiency improved (0.8%, $P < 0.05$) after LHTL probably because of a shift in fuel utilization. This is the first study to show that hypoxic exposure, per se, increases muscle buffer capacity. Further, reduced $\dot{V}O_2$ during normoxic exercise after LHTL suggests that improved exercise efficiency is a fundamental adaptation to LHTL.

Keywords altitude training, cycling efficiency, hypoxia, muscle buffering.

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Altitude training for improved performance at sea level remains highly contentious (Rusko 1996, Saltin 1996, Wolski *et al.* 1996). In part, this may be a consequence of any performance change being small and variable between individuals (Rusko 1996). Recently an alternative approach to enhance athletic performance has been mooted, where athletes live at moderate altitude and train near sea level. This method of using hypobaric hypoxia improved the sea-level 5000 or 3000 m run time in both college (Levine & Stray-Gundersen 1997) and elite level runners (Stray-Gundersen *et al.* 2001), but enhanced performance is a relatively rare outcome among those studies of altitude training that have used a control (CON) group. Because many countries lack suitable geography, the so-called 'live high:train low' (LHTL) approach (Levine & Stray-Gundersen 1997) has been further refined to include living at simulated altitude under normobaric conditions (Rusko 1996).

Regardless of whether LHTL or natural altitude sojourns are used by athletes there is some evidence to challenge the traditional paradigm that the key adaptation for any performance benefit is increased red cell mass (Mairbäurl 1994) and the concomitant increase in maximal aerobic power ($\dot{V}O_{2max}$) that has otherwise been associated with polycythaemia (Buick *et al.* 1980). Two studies have reported that training at altitude (~2000–2700 m) induced a 5–6% increase in skeletal muscle *in-vitro* buffer capacity (βm) (Mizuno *et al.* 1990, Saltin *et al.* 1995a). Furthermore, a carefully conducted study has recently reported a significant (5%) improvement in the net mechanical efficiency of submaximal cycling subsequent to a 21-day mountain ascent (6194 m) (Green *et al.* 2000b). The mechanism

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of increased βm and mechanical efficiency is unclear, but in both cases hypoxia is a likely candidate.

Given the potential importance of anaerobic metabolism (Bulbulian *et al.* 1986) and efficiency (Snell & Mitchell 1984) to performance, even in highly trained endurance athletes, further investigation of possible anaerobic adaptations to hypoxia is clearly warranted. Based on the reported effect of 2 weeks living and training at natural altitude (Saltin *et al.* 1995a), we hypothesized that merely sleeping in moderate hypoxia (LHTL) for sufficient duration would improve βm. Secondly, based on the observation of Green *et al.* (2000b), we hypothesized that LHTL of sufficient duration would improve gross mechanical efficiency during submaximal cycle ergometry conducted in normobaric normoxia.

MATERIALS AND METHODS

Subjects

Thirteen male athletes (nine triathletes, two cross-country skiers and two cyclists) gave written consent to participate in this study, which was approved by the Australian Institute of Sport Ethics Committee. Subjects were ranked according to the power output achieved during the last 2 min of an incremental cycle ergometer test, that also established their peak oxygen consumption ($\dot{V}O_{2peak}$). The ranking was used to assign subjects to two fitness-matched groups: the CON group ($n = 7$) and LHTL group ($n = 6$). The physical characteristics of the CON and LHTL groups and their training frequency, intensity and duration did not differ (Table 1). The nine triathletes (four CON and five LHTL) trained together and the remaining athletes completed their own sport-specific training schedules.

Table 1 Physical and training characteristics. The live high:train low group (LHTL, $n = 6$) lived at 3000 m simulated altitude and trained at 600 m (Canberra, Australia), while the control group (CON, $n = 7$) lived and trained in Canberra. The data for peak oxygen consumption ($\dot{V}O_{2peak}$) and 'all-out' 2-min power output are those achieved during habituation (see Fig. 1). Data are mean and (SD). No significant differences were found between groups for any variable

Variable	LHTL	CON
Age (year)	25.4 (3.6)	25.1 (5.2)
Height (cm)	183.5 (10.0)	181.2 (6.3)
Body mass (kg)	73.0 (6.7)	73.3 (6.1)
$\dot{V}O_{2peak}$ (L min^{-1})	5.08 (0.34)	4.95 (0.45)
All-out 2-min power output (W kg^{-1})	5.74 (0.46)	5.72 (0.31)
Training (sessions week^{-1})	7.1 (2.3)	6.8 (2.3)
Training intensity (Borg units)	13.8 (1.1)	13.6 (1.0)
Training (h week^{-1})	13.4 (3.8)	10.6 (5.7)

Experimental design

The study was conducted in Canberra, Australia at 600 m altitude, $P_B \sim 711$ mmHg. The LHTL subjects spent 9.5 h night^{-1} for 23 consecutive nights in a room where enriched nitrogen produced hypoxia that simulated 3000 m altitude (normobaric hypoxia; $O_2 = 15.48\%$). The CON subjects slept in their own homes under normobaric normoxia. Training and daytime living for all subjects was at an altitude of 600 m.

Submaximal workloads. After one habituation trial, subjects completed five, four-stage submaximal cycle ergometer tests before, during and after the LHTL group slept at simulated altitude. The timing of these submaximal ergometer tests was 4 and 5 days before (PRE), 2 and 3 days after (POST), as well as after 11 of the 23 nights of simulated altitude (MID) (Fig. 1). All tests were completed under normobaric normoxic conditions in Canberra on one ergometer (Excalibur Sport model, Lode, Groningen, Holland) that was dynamically calibrated with a torquemeter. Based on the habituation trial, workloads for each subject were programmed using the Lode 'hyperbolic' mode at 1.5, 2.5, 3.5 and 4.5 W kg^{-1}; and for the baseline test (day 5-PRE) these corresponded to an overall group mean of 36, 52, 68, and 84% $\dot{V}O_{2peak}$. The workloads programmed on day 5-PRE were replicated for each subject's subsequent tests and their cadence on day 5-PRE was recorded each minute and then matched for the four subsequent tests. Controlling cadence was a necessary precaution when using the 'hyperbolic' mode of the Lode ergometer because in this condition power output is constant and cadence independent, and yet cadence can markedly alter the $\dot{V}O_2$ of cycling (Woolford *et al.* 1999).

All-out trials. On days 5-PRE, 11-MID and 2-POST an 'all-out' trial was conducted in which the submaximal ergometer test was followed by 4 min of rest and then by a 4-min maximal effort. The first 2 min was set at an individual load (mean ± SD = 5.6 ± 0.4 W kg^{-1}) equivalent to 105% of the workload achieved at $\dot{V}O_{2peak}$ in the habituation incremental test, and the last 2 min was an 'all-out' effort. The 2 min workload at 5.6 ± 0.4 W kg^{-1} was programmed using the 'hyperbolic' mode of the Lode ergometer, after which the 2 min all-out workload reverted immediately to the 'linear' mode of the ergometer with the linear factor (gearing) programmed according to individual requirements. In the 'linear' mode, power output on the Lode is cadence dependent and appropriate gearing is important for optimal performance. For each subject, the hyperbolic and linear factors used during the

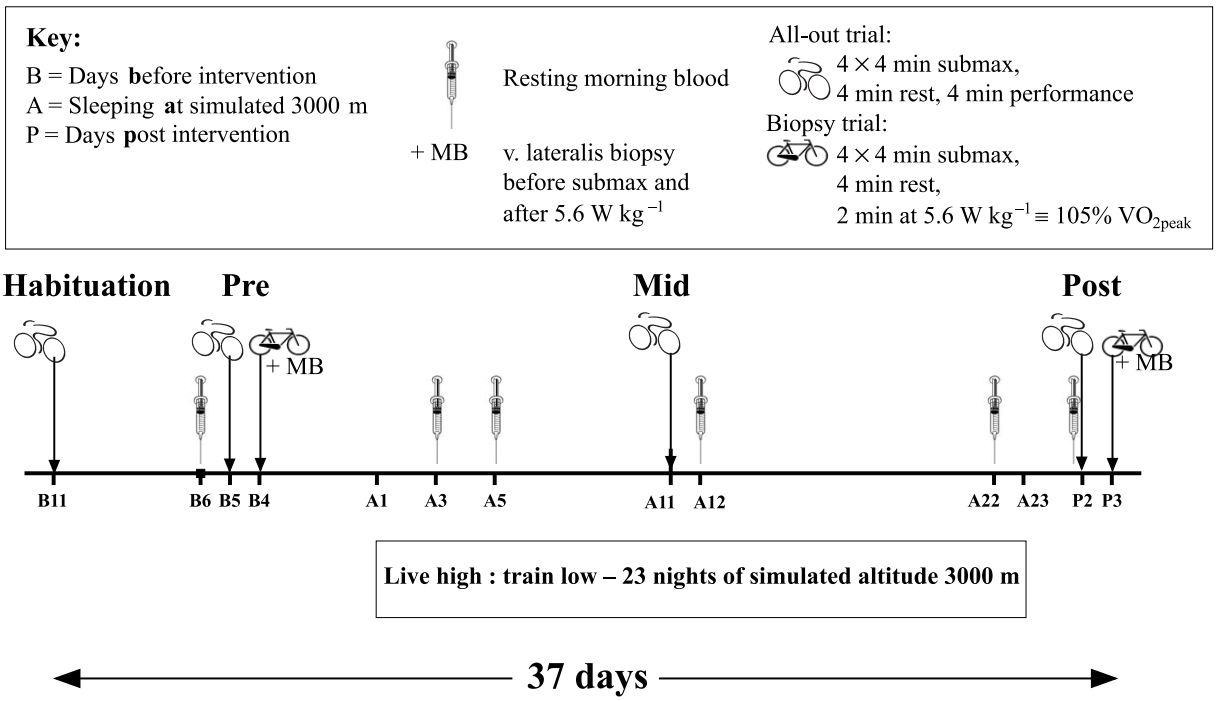

Figure 1 Testing schedule and simulated altitude exposure of control (CON, $n = 7$) and live high:train low (LHTL, $n = 6$) groups. Both the CON and LHTL trained in normobaric normoxia in Canberra (600 m altitude), Australia, while LHTL spent 23 nights in normobaric hypoxia.

habitation trial were replicated for the three subsequent all-out tests. During the all-out effort, total work, $\dot{V}O_2$ and $\dot{V}O_{2peak}$ were recorded.

Biopsy trials. On days 4-PRE and 3-POST a 'biopsy trial' was conducted in which each subject had two muscle biopsies (vastus lateralis), one at rest ~30 min before the four-stage submaximal ergometer test and a second biopsy taken immediately (<15 s) after completing 2 min at 5.6 ± 0.4 W kg^{-1} (Fig. 1).

Subject preparation and analyser calibration

Simulated altitude. Throughout each of the 23 nights, $\%O_2$ and $\%CO_2$ inside the hypoxic room were measured every 30 min with Ametek (Pittsburgh, PA, USA) O_2 and CO_2 gas analysers (model S-3A and CD-3A, respectively) calibrated every 2 h at two points; with air from outside the laboratory and with one precision grade gas (BOC Gases Australia, Sydney, Australia). The LHTL subjects had their resting heart rate (HR) and blood oxyhaemoglobin saturation (S_pO_2) estimated with finger-tip pulse oximetry (model 505-US, Criticare, Waukesha, WI, USA) every 30 min.

Morning resting blood acid-base status. Resting venous blood was collected under normoxic conditions within 30 min of waking for both LHTL and CON. Samples were taken on the sixth day before entering the altitude house (6-PRE); after 3, 5, 12 and 22 nights at simulated altitude (A3, A5, A12 and A22, respectively), as well as after one night of sleeping in normoxia (2-POST) for determination of acid–base variables (Fig. 1). With each subject supine, blood was sampled from a superficial forearm vein via a winged infusion set into a heparinized 2 mL blood gas syringe. Resting samples were also analysed for red blood cell parameters, with data reported elsewhere (Ashenden *et al.* 1999).

Exercise blood sampling. Before each of the five cycle ergometer tests, a catheter was inserted into a superficial dorsal hand vein and covered with an adhesive plastic dressing and latex glove. After catheterization, each subject was seated on the cycle ergometer and the catheterized hand was immersed in a water bath (44.5 °C) to ensure arterialization of venous blood. After 10 min in this posture, a 1.5-mL pre-exercise blood sample was acquired via a heparinized 2 mL blood gas syringe. Blood samples (1.5 mL) were taken from a dorsal hand vein during the last 30 s of each of the four submaximal workloads and at 5.6 ± 0.4 W kg^{-1} during the biopsy trials, and on the days of the all-out trial during the last 15 s of the final 2-min effort.

Blood analyses. Blood samples were stored on ice (<1 h) until analysis in triplicate for plasma pH and bicarbonate concentration [HCO^{3-}], lactate concentration [La^-]$_p$, and carbon dioxide partial pressure (P_{CO_2}) using an automated analyser (ABL System 625,

Radiometer, Copenhagen, Denmark), which was calibrated daily in accordance with the manufacturer's specifications.

Muscle biopsies and analyses

A needle biopsy sample was taken at rest from the vastus lateralis muscle via one of the two incisions made ipsilaterally under local anaesthesia (Xylocaine, 1%; Astra Pharmaceuticals, Sydney, Australia), with suction applied to the needle. 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. All biopsies were taken at constant depth by the same, experienced medical practitioner. The second sample was taken immediately after cessation of the 2 min exercise trial at 5.6 ± 0.4 W kg^{-1}, with the subject lying supported on the cycle ergometer. The samples for metabolite and βm analyses were rapidly frozen in liquid nitrogen.

Muscle pH, buffer capacity and total protein content. Before analysis in duplicate, the samples were freeze-dried (Modulo, Edwards, Crawley, UK) and dissected free of connective tissue, blood and fat. The sample was diluted 1:200 in 5 mM NaIAA, 145 mM KCl, and 10 mM NaCl, pH 7.0 and then homogenized (Omni 1000, Omni International, Warrenton, VA, USA) on ice for 60 s. Muscle homogenate pH (expressed as $[H^+]$) was measured at 37 °C under magnetic stirring with a glass microelectrode (MI-145, Microelectrodes, Bedford, TX, USA). The *in-vitro* buffer capacity (βm) was then measured by titration of the homogenate from pH 7.1 to 6.1 and expressed relative to muscle dry mass (μmol H^+ g muscle dm^{-1} pH^{-1}). Total protein content was determined spectrophotometrically (Lowry *et al.* 1951). The reliability of the duplicate measures was calculated as the within subject standard deviation or typical error of measurement (TEM) (Hopkins 2000). The TEM for βm was 3.6 μmol H^+ g muscle dm^{-1} pH^{-1} or 1.9% of the mean, and the corresponding values for total protein were 0.016 mg (mg muscle)$^{-1}$ equivalent to 1.2% of the mean.

Muscle metabolites. The muscle lactate (La^-_m), adenosine triphosphate (ATP), PCr, creatine (Cr) and glycogen contents were measured in triplicate on freeze-dried muscle using standard fluorometric techniques (Lowry & Passoneau 1972). Muscle ATP, PCr and Cr contents were corrected to the total Cr content. Muscle anaerobic ATP production was estimated from the rest to end-exercise changes (Δ) in ATP, PCr and La^-_m, and calculated as $\Delta ATP + \Delta PCr + 1.5\Delta La^-_m$. Because of technical difficulties, the sample size for these measures was $n = 4$ and $n = 6$ for the LHTL and CON, respectively. The respective TEMs for La^-_m, ATP, PCr and glycogen were 1.5, 0.6, 1.5 and 14 mmol kg dm^{-1} equivalent to 2.0, 2.5, 5.8 and 2.5% of the mean values.

Oxygen consumption and mechanical efficiency

During each cycle ergometer test $\dot{V}_{O_2}$, carbon dioxide output ($\dot{V}_{CO_2}$), minute ventilation ($\dot{V}_E$) and respiratory exchange ratio (RER) were measured continuously and results were displayed every 30 s. Data from the last 60 s of each of the four 4-min submaximal workloads were used to indicate the 'steady-state' level, and $\dot{V}_{O_{2peak}}$ was determined as the highest value recorded in any 60-s interval during the last 4 min of the all-out trial. The open-circuit indirect calorimetry system comprised Ametek O_2 and CO_2 gas analysers as well as two chain-compensated gasometers and has been described previously (Pierce *et al.* 1999). The analysers were calibrated before, and checked for drift after, each test using three α grade gases (BOC Gases Australia). The average TEM for $\dot{V}_{O_2}$ was 0.12 and 0.09 L min^{-1}, respectively, for the duplicated PRE (5- and 4-PRE) and POST (2- and 3-POST) four stages of submaximal ergometry. At any submaximal workload the mean difference between either of the two repeat tests was $<\pm 52$ mL min^{-1} for both CON and LHTL groups. The corresponding PRE and POST TEMs for $\dot{V}_E$ during submaximal ergometry were 5.0 and 4.7 L min^{-1}, equivalent to 6.1 and 4.9% of the respective mean values.

Gross mechanical efficiency (%) was determined from the ratio of power output (kJ min^{-1}) to energy expended (kJ min^{-1}), as calculated from $\dot{V}_{O_2}$ and RER (Elia & Livesey 1992).

Heart rate

Overnight resting heart rate (HR) each night was calculated for the LHTL group as the grand mean from 11:00 PM to 05:00 AM. The HR during cycle ergometry was assessed every 5 s by telemetry (Polar Vantage, Polar Electro OY, Kempele, Finland). The TEMs for HR during the four-stage submaximal ergometer tests at PRE and POST were 3 and 4 beats min^{-1}, equivalent to 2.6 and 3.5% of the respective mean values.

Statistical analysis

All values are reported as mean ± SD. The physical and training characteristics of the two groups were assessed with independent *t*-tests. Three-way analysis of variance (ANOVA) with repeated measures was used to test for interaction and main effects for most of the dependent variables measured during exercise. The three factors were group (CON and LHTL), day (PRE, MID and POST simulated altitude), and stage of

exercise (rest, end of exercise and where relevant the four submaximal workloads). When the three-way interaction was not significant, the data of LHTL and CON groups were analysed with separate two-way repeated measures ANOVA for day and stage of exercise. Peak exercise data were analysed with two-way repeated measures ANOVA for group by day. When interactions or main effects achieved statistical significance, Tukey post hoc tests were used to identify differences between cell means. Statistical significance was tested at the $P < 0.05$ level using Statistica software (StatSoft, Tulsa, OK, USA). In addition, and as a method to partially circumvent the likelihood of a type II error as a consequence of our small sample size, the effect size [ES = ($mean_1$ – $mean_2$)/SD] was calculated for selected results that did not achieve significance and the pooled SD was calculated when the SDs were unequal (Cohen 1988). Cohen's (Cohen 1988) conventions for effect size were adopted for interpretation, where ES = 0.2, 0.5 and 0.8 are considered as small, medium and large, respectively.

RESULTS

All-out trials

Performance and $\dot{V}_{O_2}$. The $\dot{V}_{O_{2peak}}$ of LHTL fell significantly by –3.8 ± 1.9% at MID and –7.2 ± 4.1% at POST, whilst CON $\dot{V}_{O_{2peak}}$ was unchanged (Table 2). Total V_{O_2} in the 2 min all-out effort was also significantly depressed in LHTL at POST compared with PRE, although, the corresponding work output was not changed in either group (Table 2). Total V_{O_2} during 2 min at 5.6 ± 0.4 W kg^{-1} was not different between groups ($P > 0.2$) but tended to be less after 23 nights of sleeping in hypoxia in LHTL (Δ PRE vs. POST = –4.0%) than in CON (Δ = 1.1%).

Cadence at PRE ranged from 90 ± 7 to 102 ± 2 rev min^{-1} for 1.5 W kg^{-1} and all-out workloads, respectively, for LHTL, and 93 ± 15 to 102 ± 3 rev min^{-1} for CON. No significant differences were found between groups or between different days of exercise.

Submaximal $\dot{V}_{O_2}$ *and mechanical efficiency*. During the first four stages of the all-out trial, LHTL had a significantly lower submaximal $\dot{V}_{O_2}$ at both MID (−3.1 ± 2.9%) and POST (–4.4 ± 3.3%) compared with PRE (Fig. 2). Submaximal $\dot{V}_E$ was significantly increased after 23 nights of sleeping in moderate hypoxia (Fig. 2). Although RER of LHTL was not significantly different between days, the effect sizes tended to be large (at 1.5 W kg^{-1} PRE vs. MID, ES = 2.27; PRE vs. POST, ES = 1.67). Overall, RER for LHTL was 0.88 ± 0.07 PRE and 0.91 ± 0.07 POST. The CON showed no change in submaximal $\dot{V}_{O_2}$, $\dot{V}_E$ and RER for MID and POST vs. PRE (Fig. 2).

Submaximal efficiency of LHTL was significantly different between days and stage of exercise ($P = 0.02$). Each POST value (16.6 ± 1.5, 19.6 ± 0.8, 20.9 ± 0.7 and 21.5 ± 0.7%) was higher than the corresponding PRE value (15.8 ± 1.4, 18.7 ± 0.9, 20.2 ± 1.0 and 21.0 ± 0.7%) at 1.5, 2.5, 3.5 and 4.5 W kg^{-1}, respectively. Overall, submaximal efficiency of the LHTL group was improved 0.8% from PRE (18.9 ± 2.7%) to POST (19.7 ± 2.4%) ($P < 0.01$).

Heart rate. Submaximal HR was significantly different between groups when comparing the three test days and four submaximal stages of exercise [$F_{(6,66)} = 2.43$, $P = 0.03$). The PRE HR was not different between

Table 2 All-out trials. Peak and total $\dot{V}_{O_2}$, work, peak HR, and end exercise $[La^-]_p$ and pH for 2-min all-out cycle ergometry. The groups and the intervention are described in Table 1 and the timing of tests is illustrated in Fig. 1. Data are mean and (SD)

		Day of measurement		
Variable	Group	Day 5-PRE	Day 11-MID	Day 2-POST
$\dot{V}_{O_{2peak}}$ (L min^{-1})	LHTL	5.08 (0.34)	4.90 (0.33)*	4.78 (0.36)*
	CON	4.95 (0.45)	4.92 (0.47)	4.87 (0.44)
$V_{O_{2total}}$ in 2 min (L)	LHTL	9.99 (0.72)	9.63 (0.71)	9.24 (0.66)*
	CON	9.60 (1.09)	9.77 (0.93)	9.64 (0.92)
Work in 2 min (kJ)	LHTL	50.0 (4.2)	51.0 (3.9)	49.2 (4.2)
	CON	50.5 (6.0)	51.5 (6.5)	50.3 (5.8)
Heart $rate_{peak}$ (beats min^{-1})	LHTL	183 (9)	185 (6)	183 (6)
	CON	189 (8)	189 (9)	190 (9)
$[La^-]_p$ (mmol L^{-1})	LHTL	15.4 (3.3)	16.7 (2.5)†	17.3 (2.6)†
	CON	17.4 (1.2)	21.1 (3.1)*	22.4 (1.7)*
pH	LHTL	7.26 (0.03)	7.25 (0.03)	7.26 (0.03)
	CON	7.24 (0.03)	7.23 (0.03)	7.24 (0.02)

*Significantly different from PRE.
†Significantly different between groups on the same day.

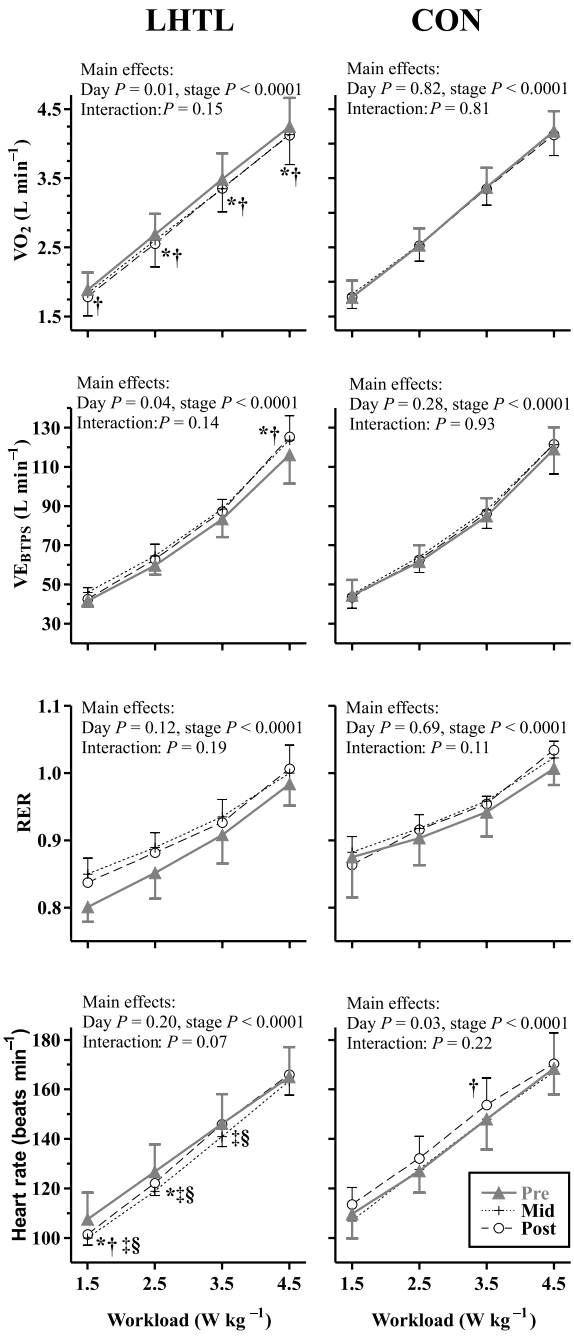

Figure 2 Oxygen consumption ($\dot{V}_{O_2}$), ventilation ($\dot{V}_E$), respiratory exchange ratio (RER) and heart rate (HR) for Live High:Train Low (LHTL, $n = 6$, *left panels*) and Control (CON, $n = 7$, *right panels*) groups during submaximal cycle ergometry before (PRE), after 11 nights (MID), and 2 days after (POST) 23 nights of simulated altitude. Values are mean and SD. Significant differences within groups; *MID vs. PRE, †POST vs. PRE; significant differences between groups at matched time, §MID vs. MID, ‡POST vs. POST. Main effects for Day (PRE, MID, POST), exercise stage (1.5–4.5 W kg^{-1}), as well as the day by stage interaction are indicated in each subpanel.

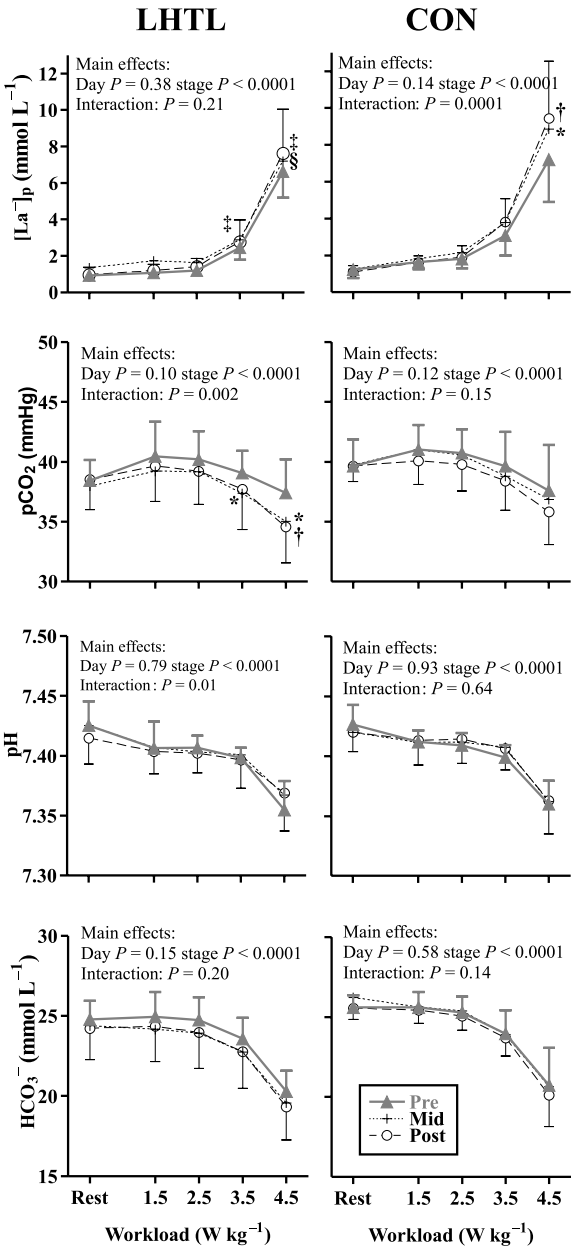

Figure 3 Arterialized venous plasma lactate concentration $[La^-]_p$, CO_2 tension (P_{CO_2}), pH and bicarbonate ion concentration $[HCO_3^-]$ for the LHTL (*left panel*) and CON (*right panel*) groups as described in Fig. 2. Values are mean and SD. Significant differences within group; *MID vs. PRE, †POST vs. PRE; significant differences between groups at matched time, §MID vs. MID, ‡POST vs. POST.

groups at any workload, however, HR was significantly lower for LHTL than CON at the first three submaximal workloads at both MID and POST (Fig. 2). In addition, HR of LHTL during the first two stages of the MID test and the first stage of the POST test were significantly lower (6–8 beats min^{-1}) than at PRE. The HR_{peak} was not different within or between groups for PRE vs. POST (Table 2).

Blood biochemistry. The three-way interaction between groups, test days and the five stages of rest or submaximal exercise was significant for $[La^-]_p$

($F_{(8,88)} = 2.22$, $P = 0.03$). During submaximal exercise, $[La^-]_p$ for LHTL was not different between test days, but within CON $[La^-]_p$ at 4.5 W kg^{-1} was significantly higher than PRE at both MID and POST (Fig. 3). Furthermore, $[La^-]_p$ for LHTL was significantly lower than that of CON at MID at 3.5 W kg^{-1}, and at both MID and POST at 4.5 W kg^{-1}. At the end of the 2 min all-out effort, LHTL $[La^-]_p$ was not different between PRE, MID and POST tests, although CON $[La^-]_p$ was significantly higher at both MID and POST than PRE (Table 2).

The LHTL P_{CO_2} during MID was lower than at PRE at both 3.5 and 4.5 W kg^{-1}, and at POST was lower than PRE at 4.5 W kg^{-1} (Fig. 3). Although Tukey post hoc tests did not identify differences between cell means, pH at 4.5 W kg^{-1} tended to be higher compared with PRE at both MID (ES = 0.50) and POST (ES = 0.49). While not significantly different between days, LHTL $[HCO_3^-]$ at rest and during submaximal exercise tended to be lower at both MID and POST compared with PRE (ES ~ 0.5). At the end of all-out exercise, pH was stable in each group for the three tests (Table 2).

Biopsy trials

Performance and $\dot{V}O_2$. The LODE ergometer was programmed to ensure that the amount of work completed during 2 min at 5.6 ± 0.4 W kg^{-1} was the same on both days (48.2 ± 5.1 kJ). There was a non-significant ($P > 0.20$) trend for total $\dot{V}O_2$ during the 2 min to be lower in LHTL ($\Delta = -3.5\%$) but not CON ($\Delta = 0.2\%$).

Muscle buffer capacity and metabolites. Resting βm increased significantly in LHTL (17.7 ± 4.9%) but was unchanged in CON (0.5 ± 5.8%, Fig. 4). Analysis of βm in post-exercise samples confirmed this finding of an elevation in the LHTL group only (data not shown). The increased βm was not the result of an increased total muscle protein content, as the latter did not differ between or within groups (Table 3). Muscle $[H^+]$ was not significantly different between the groups. The pooled data of both groups indicated no difference in resting muscle $[H^+]$ PRE vs. POST (70.0 ± 4.1 vs. 66.9 ± 4.9 nmol kg dm^{-1}, respectively), although at the end of exercise it tended to be lower POST than PRE [140.2 ± 19.6 vs. 159.7 ± 21.6 nmol kg dm^{-1}; day by exercise interaction ($P = 0.06$)]. The $[H^+]$ accumulation and calculated *in vivo* βm after exercise at 5.6 ± 0.4 W kg^{-1} ($\Delta[H^+]$) was unchanged in either LHTL or CON (Table 3). Muscle ATP, PCr and glycogen decreased with exercise whereas Cr increased, but these were not different between groups nor affected by 23 nights sleeping in hypoxia (Table 3). The La^-_m accumulation (ΔLa^-_m) and estimated anaerobic energy production after exercise at 5.6 ± 0.4 W kg^{-1} was unchanged from PRE to POST in either group (Table 3).

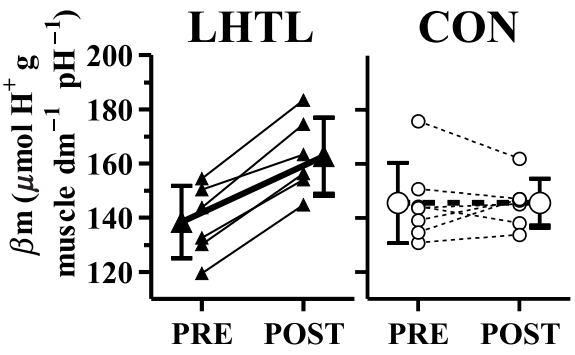

Figure 4 Change in resting *in-vitro* muscle buffering capacity (βm) PRE and POST 23 nights of simulated altitude. *Left panel* shows individual data points of LHTL group ($n = 6$) that lived high and trained low with mean ± SD data indicated with large symbols. The *right panel* is for the control (CON, $n = 7$) group.

Heart rate. No differences between or within groups were found for PRE vs. POST HR at 5.6 ± 0.4 W kg^{-1} (Table 4).

Blood biochemistry. At 5.6 ± 0.4 W kg^{-1} $[La^-]_p$, P_{CO_2}, pH, and $[HCO_3^-]$ were not different between groups or from PRE to POST (Table 4).

Morning blood biochemistry

Morning resting plasma pH was not different between groups at baseline or after one night of sleeping in hypoxia, but was significantly higher at day A5 in LHTL than in CON (Fig. 5). Within LHTL, pH at A5 tended to be higher than at baseline ($P = 0.07$, ES = 2.29). Morning resting $[HCO_3^-]$ was not different between groups on any day, although it tended to be lower (at least 1.2 mmol L^{-1}) in LHTL during and 2 days after simulated altitude (Fig. 5). The between group effect sizes at days A3 and A22 were 0.51 and 1.04, respectively.

Overnight heart rate and blood saturation

Overnight resting HR of the LHTL group was unchanged across the 23 nights, with a grand mean of 57 ± 11 beats min^{-1}, and the S_pO_2 was 91 ± 3% for the 219 h spent in normobaric hypoxia.

DISCUSSION

Our major findings challenge conventional concepts of adaptation to chronic hypoxic exposure. We show

Table 3 Biopsy trials. Muscle protein content, H^+ concentration and metabolites at rest and immediately after 2 min of cycle ergometry at 5.6 ± 0.4 W kg^{-1}. The groups and the intervention are described in Table 1 and the timing of tests is illustrated in Fig. 1. The sample size for ATP, PCr, glycogen, Cr and La^-_m are $n = 4$ and $n = 6$ for the LHTL and CON groups, respectively. Data are mean and (SD). Differences within and between groups are not significant

		LHTL		CON	
Variable	Condition	Day 4-PRE	Day 3-POST	Day 4-PRE	Day 3-POST
Protein (mg (mg muscle^{-1}))	Rest	0.177 (0.010)	0.174 (0.013)	0.168 (0.014)	0.166 (0.015)
	Exercise	0.175 (0.013)	0.174 (0.012)	0.168 (0.012)	0.166 (0.014)
	Δ (Ex – Rest)	38.6 (6.6)	34.0 (12.7)	42.6 (2.4)	43.4 (3.4)
$[H^+]$ (nmol L^{-1})	Rest	71.1 (3.8)	67.3 (5.5)	69.0 (4.4)	66.4 (4.8)
	Exercise	156.6 (22.8)	139.9 (20.4)	162.3 (21.9)	140.5 (20.5)
	Δ (Ex – Rest)	85.5 (21.7)	72.6 (19.2)	93.3 (18.3)	74.1 (22.5)
$\beta_{in\text{-}vivo} = (\Delta[H^+]/\Delta[La^-]_m)$		105.6 (30.0)	104.7 (40.1)	115.4 (17.8)	136.5 (30.0)
ATP	Rest	28.6 (0.8)	28.6 (0.6)	28.7 (1.2)	28.7 (1.0)
(mmol kg dm^{-1})	Exercise	18.0 (0.4)	18.1 (0.5)	18.0 (0.3)	17.9 (0.4)
PCr	Rest	87.9 (0.7)	87.9 (0.5)	87.9 (3.0)	89.0 (1.9)
(mmol kg dm^{-1})	Exercise	62.5 (0.9)	62.3 (0.7)	62.9 (0.7)	63.0 (1.1)
Glycogen	Rest	576 (98)	571 (79)	611 (68)	599 (56)
(mmol glucosyl units kg dm^{-1})	Exercise	245 (15)	247 (10)	235 (19)	227 (27)
Cr	Rest	47.5 (1.4)	47.6 (1.2)	47.7 (1.3)	47.7 (1.3)
(mmol kg dm^{-1})	Exercise	72.9 (1.0)	73.1 (1.1)	73.8 (0.7)	73.7 (0.9)
$[La^-]_m$	Rest	5.9 (2.9)	6.0 (2.5)	4.9 (0.6)	4.9 (0.5)
(mmol kg dm^{-1})	Exercise	44.5 (7.1)	40.1 (12.1)	47.5 (2.5)	48.2 (3.4)
Anaerobic ATP production (mmol kg dm^{-1})	Δ (Rest to end Ex)	93.9 (9.7)	87.1 (18.9)	100.7 (3.7)	101.9 (5.8)

for the first time that muscle *in-vitro* buffer capacity was increased after sleeping in hypoxia, and thus can be attributed to chronic hypoxic exposure alone. However, after LHTL this did not coincide with enhanced muscle H^+ regulation, evidenced by an unchanged post-exercise muscle $[H^+]$, or by a general up-regulation of anaerobic metabolism during intense exercise. The second major finding was that whole body $\dot{V}O_2$ during submaximal cycle ergometry under normobaric, normoxic conditions was significantly lower after 23 nights of sleeping at 3000 m simulated altitude. The finding of reduced $\dot{V}O_2$ at a constant exercise workload, without a corresponding elevation in anaerobic metabolism suggests that 23 nights exposure to moderate hypoxia enhances mechanical efficiency during exercise.

Table 4 Biopsy trials. Plasma metabolites and acid–base status, as well as HR at the immediate end of 2 min of cycle ergometry at 5.6 ± 0.4 W kg^{-1}. The groups and the intervention are described in Table 1 and the timing of tests is illustrated in Fig. 1. Data are mean and (SD). Differences within and between groups are not significant

Variable	Group	Day 4-PRE	Day 3-POST
$[La^-]_p$ (mmol L^{-1})	LHTL	9.3 (2.1)	11.3 (3.2)
	CON	10.1 (2.3)	14.3 (4.0)
P_{CO_2} (mmHg)	LHTL	31.6 (1.9)	34.3 (2.5)
	CON	32.5 (2.1)	33.8 (2.4)
pH	LHTL	7.34 (0.03)	7.35 (0.04)
	CON	7.34 (0.02)	7.34 (0.03)
HCO_3^- (mmol L^{-1})	LHTL	14.5 (1.8)	14.2 (1.7)
	CON	15.1 (1.5)	14.4 (1.5)
Heart rate (beats min^{-1})	LHTL	166 (9)	168 (6)
	CON	169 (9)	172 (9)

Muscle buffer capacity, anaerobic metabolism and acid–base regulation

This is the first study to report that merely sleeping, rather than living and training, in hypoxia elevates βm and thus strongly suggests that hypoxia is the key factor in improving βm. The increase in βm was not the result of increased muscle protein content but apparently reflected a qualitative change in the buffer capacity of the dipeptides or protein expressed. This may be a consequence of a higher intramuscular carnosine concentration as suggested by others (Saltin *et al.* 1995a), but the mechanism remains unknown. Elevated βm after LHTL is consistent with the 5–6% increase reported after training and living at ~2000–2700 m (Mizuno *et al.* 1990, Saltin *et al.* 1995a). In contrast, a recent report indicated an unspecified decrease in βm after living at 2500 m and training at 2200–3000 m (Stray-Gundersen *et al.* 1999).

This is also the first report of the effects of LHTL on skeletal muscle H^+ regulation during exercise. Surprisingly, there did not appear to be a positive modulation of intramuscular H^+ regulation, with an unchanged post-exercise muscle $[H^+]$ during intense exercise after LHTL, in comparison with the CON group. Such an effect with LHTL would be expected to be evident from

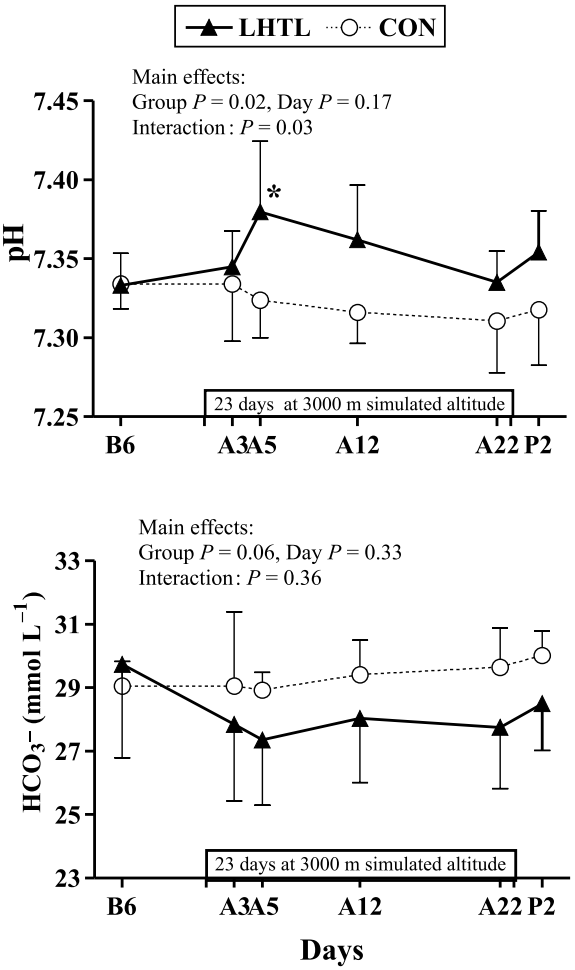

Figure 5 Morning resting plasma pH (*top panel*) and bicarbonate concentration (*bottom panel*) of live high:train low (LHTL, $n = 6$) and control (CON, $n = 7$) groups before, during and after LHTL spent 23 nights sleeping in hypoxia. Values are mean and SD. *Significant difference between groups.

the matched work bout used in this study because identical, rather than exhausting, work bouts are a salient method to compare markers of muscle metabolism and ion regulation (Harmer *et al.* 2000). Further, the calculated *in-vivo* βm was not enhanced after LHTL, although considerable variability was found in the data. This suggests that intramuscular H^+ regulation was not improved after LHTL. As we measured $[H^+]$ in dried muscle and CO_2 is lost during the freeze-drying process, our $[H^+]$ values are slightly lower than expected in wet muscle. Nonetheless, both F_ECO_2 (data not shown) and arterialized venous P_{CO_2} were lower during exercise after LHTL, suggesting that intramuscular CO_2 would also tend be less in the LHTL group. Hence, the CO_2-dependent H^+ accumulation would be lower in LHTL, consistent with our conclusions that an increased βm was not associated with improved muscle H^+ regulation. Thus, our results are incompatible with the concept that the primary importance of increased βm is to confer benefits for muscle H^+ regulation. In addition to βm, muscle H^+ regulation during exercise will be affected by the sarcolemmal lactate$^-$/H^+ and Na^+/H^+ exchange mechanisms, capillarization and muscle blood flow (Juel 1998) and by changes in the intracellular strong ion difference (Kowalchuk *et al.* 1988). The effects of LHTL on each of these remain unknown. An interesting finding was that the increased βm in the LHTL group occurred without any corresponding elevation in other markers of anaerobic metabolism, in contrast with the suggestion of others who used natural altitude exposure (Mizuno *et al.* 1990, Saltin *et al.* 1995a); although with a small sample size our analyses are prone to type II errors. The degradation of muscle ATP, PCr and glycogen during intense exercise were unchanged, as were the intramuscular and blood accumulation of La^- and H^+ ions. Each of these changes was highly reproducible with low TEM, and was identical in the PRE and POST trials in both the CON and LHTL groups. The decline in ATP was most likely because of the 2-min exercise bout at 105% $\dot{V}_{O_{2peak}}$. The work completed in this trial (~48 kJ) was similar to that in the last 2 min of the all-out trial (~50 kJ), when subjects were asked to produce as much work as possible. Thus, reductions in ATP are not unexpected with this heavy exercise. The decline in PCr and rise in Cr was surprisingly small relative to the rise in La^-. This may reflect the usual slight delay in biopsy sampling and a likely rapid PCr resynthesis in these endurance-trained athletes. The anaerobic ATP production may consequently be slightly underestimated, but importantly, this was clearly not enhanced after LHTL.

The typical lactate response to exercise during chronic altitude exposure is an initial elevation in lactate accumulation in arterial and venous blood as well as in muscle, together with elevated muscle lactate release, each of these subsequently decline with acclimatization (Hochachka 1988, Brooks *et al.* 1992, 1998, Reeves *et al.* 1992). Our data clearly demonstrate that La^- accumulation was not elevated during intense exercise after LHTL. The muscle and blood lactate data are inconsistent with the premise that lactate production was greater after sleeping in hypoxia, consistent with our conclusion that anaerobic metabolism is not enhanced after LHTL. The $[La^-]_p$ also was not increased within the LHTL group after 23 nights spent in hypoxia. The $[La^-]_p$ during the latter stages of exercise was lower in LHTL than in CON subsequent to simulated altitude but this was because of an unexpected increase in the CON group. Thus, our data suggest it is unlikely that the typical lactate response to natural altitude occurred after LHTL, possibly because of both the simulated altitude and duration of exposure being insufficient to elicit such a response.

Reduced submaximal oxygen consumption and enhanced efficiency

A clear finding in the current study was that under normoxic conditions $\dot{V}O_2$ of the LHTL group was depressed and efficiency was increased at each of the four, 4-min submaximal workloads after both 11 and 23 nights of sleeping in hypoxia (Fig. 3). These results challenge the conventional concept that, at sea level, $\dot{V}O_2$ at any given submaximal power output remains unchanged after returning from an altitude or simulated altitude sojourn (Levine & Stray-Gundersen 1997, Piehl Aulin *et al.* 1998). Most other studies have reported no change in submaximal $\dot{V}O_2$ at sea level (Wolfel *et al.* 1991, Grassi *et al.* 1996, Levine & Stray-Gundersen 1997, Piehl Aulin *et al.* 1998). However, our data are consistent with a recent report that $\dot{V}O_2$ was significantly lower (8–10%) during prolonged submaximal cycle ergometry subsequent to a 21-day climb (2160–6194 m) (Green *et al.* 2000b). Collectively, our data and those of subjects living and climbing at natural altitude (Green *et al.* 2000b) suggest that one can attribute the increase in mechanical efficiency to hypoxia per se rather than hypobaria, cold or the effects of heavy athletic training. Interestingly, our results are consistent with those of several cross-sectional studies that have reported higher exercise efficiency in altitude natives compared with lowlanders (Hochachka *et al.* 1991, Saltin *et al.* 1995b). We cannot be completely sure why our finding differs from those of most others. However, our indirect calorimetry system has good precision and we were very careful to maintain identical pedalling cadences for a subject across all exercise trials. Failure to control cadence may have confounded the work of others (Sutton *et al.* 1988, Wolfel *et al.* 1991) as for example, a cadence of 90 vs. 120 rev min^{-1} lowers submaximal $\dot{V}O_2$ by an average of 0.47 L min^{-1} (Woolford *et al.* 1999).

The reduction in whole body $\dot{V}O_2$ during exercise after LHTL can likely be explained by a shift from fat to carbohydrate oxidation, rather than a shift from oxidative to anaerobic metabolism. In our study, submaximal RER was marginally higher (0.03) POST than PRE, which is sufficient to entirely explain the 0.8% improvement in efficiency of exercise after LHTL. The higher RER is consistent with the suggestion that at altitude increased carbohydrate and lactate fluxes reflect an overall shift towards carbohydrate utilization which optimizes the available energy for a given oxygen consumption (Brooks *et al.* 1998). Preferential use of carbohydrate fuels rather than fats at 4300 m altitude has been shown at rest and during submaximal exercise (Brooks *et al.* 1992, Roberts *et al.* 1996a,b). Our findings of a lower $\dot{V}O_2$ at the same absolute workload are consistent with the postulate that altitude acclimatization improves coupling of ATP demand and supply (Hochachka 1988).

Other mechanisms which might contribute to increased exercise efficiency after LHTL include a reduction of ATP consuming processes within skeletal muscle as shown recently by down-regulation of Na^+, K^+-ATPase after an altitude sojourn (Green *et al.* 2000a), and a reduction in $\dot{V}O_2$ of the respiratory musculature. The latter is unlikely because exercise ventilation was increased after LHTL and during heavy cycle exercise the respiratory muscles consume a significant fraction of the pulmonary $\dot{V}O_2$ (Harms *et al.* 1997). Lastly, type I fibres are energetically more efficient when cycling (Coyle *et al.* 1992), and both fibre recruitment and cycling $\dot{V}O_2$ are cadence-dependent (Barstow *et al.* 1996, Woolford *et al.* 1999). However, it seems doubtful that the LHTL group may have increased their type I fibre recruitment because all fibres would be expected to be recruited during the maximal work bouts, when $\dot{V}O_{2peak}$ was also subnormal. Reduced submaximal cycling cadence also cannot explain the subnormal $\dot{V}O_2$ as this was maintained constant for each subject in all tests.

$\dot{V}O_2$ peak and performance after simulated altitude. A novel finding in this study was that after LHTL $\dot{V}O_{2peak}$ was depressed by 7%, although total work was unchanged, during 2 min of all-out cycling. It is implausible that this decrease in $\dot{V}O_{2peak}$ could be explained by detraining of the LHTL group. Even studies of well-trained athletes who completely cease training for 2–3 weeks have reported a decrease in $\dot{V}O_{2max}$ of only 2–7% (Houston *et al.* 1979, Coyle *et al.* 1984, Laforgia *et al.* 1999). Before and during the period of LHTL, most of the CON group trained with the LHTL group and the former maintained a stable $\dot{V}O_{2peak}$ throughout this study. It therefore seems unlikely that the LHTL group which spent 13 h $week^{-1}$ in athletic preparation at a mean intensity of 13.8 Borg units would have detrained. Finally, our $\dot{V}O_2$ system has high precision, which suggests that measurement error was not the cause for the observed reduction in $\dot{V}O_2$. In this context it is notable that tests on LHTL and CON subjects were interspersed. Although there is a widespread paradigm that acclimatization to hypoxia increases red cell mass and consequently $\dot{V}O_{2max}$ (Cerretelli & Hoppeler 1996, Rusko 1996, Levine & Stray-Gundersen 1997, Rodríguez *et al.* 1999, Fulco *et al.* 2000), we (Hahn & Gore 2001) and others (Sawka *et al.* 2000) oppose this view. Our LHTL subjects exhibited no change in haemoglobin mass or reticulocyte indices of accelerated erythropoiesis (Ashenden *et al.* 1999) but their $\dot{V}O_{2peak}$ was depressed. The potential mechanisms underlying decreased $\dot{V}O_{2peak}$ require further investigation.

Total work as an indicator of performance was unchanged in the LHTL group but this may be a problem of insufficient statistical power. There is mounting evidence from three independent groups that LHTL may yield small improvements (0.8–1.3%) in events lasting from ~50 s to 17 min; 400 m sprint (Nummela & Rusko 2000), 4-min all-out effort (Hahn *et al.* 2001), as well as 3000 m (Stray-Gundersen *et al.* 2001) and 5000 m (Levine & Stray-Gundersen 1997) run times.

CONCLUSIONS

Chronic nightly hypoxic exposure using the LHTL model for 23 days increased βm by ~18%, but this occurred in the absence of enhanced muscle H^+ regulation during intense exercise. Living high:training low also significantly reduced whole body oxygen utilization during exercise in normoxia, including during standardized submaximal exercise workloads, and by 7% at $\dot{V}_{O_2peak}$. Thus, submaximal cycling efficiency was increased by 0.8%, which could be attributed to increased carbohydrate oxidation. Our results suggest that increased βm may be merely an indicator of adaptation and that greater efficiency may be of more practical importance.

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