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Effects of live high, train low hypoxic exposure on lactate metabolism in trained humans

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Clark, Sally A., Robert J. Aughey, Christopher J. Gore, Allan G. Hahn, Nathan E. Townsend, Tahnee A. Kinsman, Chin-Moi Chow, Michael J. McKenna, and John A. Hawley. Effects of live high, train low hypoxic exposure on lactate metabolism in trained humans. *J Appl Physiol* 96: 517–525, 2004. First published September 26, 2003; 10.1152/japplphysiol.00799.2003.—We determined the effect of 20 nights of live high, train low (LHTL) hypoxic exposure on lactate kinetics, monocarboxylate lactate transporter proteins (MCT1 and MCT4), and muscle in vitro buffering capacity (βm) in 29 well-trained cyclists and triathletes. Subjects were divided into one of three groups: 20 consecutive nights of hypoxic exposure (LHTLc), 20 nights of intermittent hypoxic exposure [four 5-night blocks of hypoxia, each interspersed with 2 nights of normoxia (LHTLi)], or control (Con). Rates of lactate appearance (R_a), disappearance (R_d), and oxidation (R_{ox}) were determined from a primed, continuous infusion of L-[U-^{14}C]lactic acid tracer during 90 min of steady-state exercise [60 min at 65% peak O_2 uptake ($\dot{V}O_{2\,peak}$) followed by 30 min at 85% $\dot{V}O_{2\,peak}$]. A resting muscle biopsy was taken before and after 20 nights of LHTL for the determination of βm and MCT1 and MCT4 protein abundance. R_a during the first 60 min of exercise was not different between groups. During the last 25 min of exercise at 85% $\dot{V}O_{2\,peak}$, R_a was higher compared with exercise at 65% of $\dot{V}O_{2\,peak}$ and was decreased in LHTLc ($P < 0.05$) compared with the other groups. R_d followed a similar pattern to R_a. Although R_{ox} was significantly increased during exercise at 85% compared with 65% of $\dot{V}O_{2\,peak}$, there were no differences between the three groups or across trials. There was no effect of hypoxic exposure on βm or MCT1 and MCT4 protein abundance. We conclude that 20 consecutive nights of hypoxia exposure decreased whole body R_a during intense exercise in well-trained athletes. However, muscle markers of lactate metabolism and pH regulation were unchanged by the LHTL intervention.

lactate tracer; monocarboxylate transporters; muscle buffering

TRAINING AT MODERATE ALTITUDE (~2,500 m) is common practice among athletes. However, the effect of such a regimen on subsequent sea-level endurance performance is equivocal (4, 13–15). Potential mechanisms underlying any observed performance enhancements include changes in a multitude of central and peripheral responses that, collectively, result in improved O_2 delivery and utilization during exercise (5, 10, 30, 31, 40, 43). However, altitude-induced hypoxia has also been shown to reduce the intensity at which elite athletes can train (11, 23, 24), resulting in a relative loss of adaptation. Accordingly, it has been recommended that acclimatization to moderate altitude be combined with training at low (~1,000 m) altitude ("living high, training low") to confer the optimal adaptations for improving sea-level performance (17, 24, 37).

The mechanisms proposed to underlie the improved endurance capacity after live high, train low hypoxic (LHTL) exposure include an increase in red blood cell volume, an increase in maximal O_2 uptake ($\dot{V}O_{2\,max}$) (24, 33), and the maintenance of sea-level O_2 flux during low-altitude training that preserves skeletal muscle function (35). On the other hand, the results of several studies suggest that the key adaptations underlying any performance enhancement may be due to improvements in local skeletal muscle metabolism rather than systemic changes in hematological and O_2 transport capabilities. Several lines of evidence support this latter paradigm. First, three separate studies involving highly trained athletes (with matched controls) found no change in reticulocytes or hemoglobin after LHTL (1–3). Second, we have recently reported a reduction (4.4%) in submaximal O_2 consumption ($\dot{V}O_2$) over a range of power outputs despite a significant depression (7%) in peak O_2 uptake ($\dot{V}O_{2\,peak}$) (13). Finally, we observed a significant increase (~18%) in in vitro skeletal muscle buffer capacity (βm) after sleeping (rather than training) in hypoxic conditions (16). The improvement in βm occurred despite no changes in muscle or blood lactate concentrations after a bout of standardized incremental cycling (16). However, without the use of tracer techniques in that study (16), we were unable to determine whether hypoxic exposure was associated with changes in lactate production and/or removal.

An increase in lactic acid production in response to exercise is associated with an increase in hydrogen ions (H^+) that may disturb the pH of the cell. In general, skeletal muscle pH homeostasis is a balance between H^+ accumulation and H^+ removal via diffusion of undissociated lactic acid or via facilitated transport in the sarcolemma (19). The transport of lactate in skeletal muscle is facilitated by two known monocarboxylate transporters, MCT1 and MCT4 (19). The transport of lactate is coupled to the transport of H^+ in a 1:1 ratio (20), and therefore an increase in MCT1 and MCT4 protein expression could minimize perturbations in intracellular pH. In this regard, Bonen et al. (7) have previously reported that short-term endurance training increases MCT1 transporters. Such a finding has been proposed to reflect a training-induced decrease in muscle lactate production by facilitating lactate exchange be-

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tween glycolytic and oxidative fibers according to the cell-cell lactate shuttle hypothesis (9). Accordingly, we hypothesized that sleeping under hypoxic conditions would decrease lactate production (R_a) and increase lactate oxidation (R_{ox}), while concomitantly increasing the lactate transporters, MCT1 and MCT4.

METHODS

Subjects

Thirty-three well-trained male cyclists or triathletes volunteered to participate in this study, which was approved by the Human Research Ethics Committee of Royal Melbourne Institute of Technology, the Human Research Ethics Committee of Victoria University, and the Ethics Committee of the Australian Institute of Sport. Subjects were fully informed of all testing procedures and the associated potential risks involved before providing their written consent.

Overview of Study Design

Details of the operation of the altitude house have been described in detail previously (3, 41). Because of limited accommodation in the altitude house facility used for this study, experimental testing was conducted on four separate occasions over an 11-mo period. During this time, subjects were assigned to one of three groups: LHTL, consecutive exposure (LHTLc); LHTL, intermittent exposure (LHTLi); and a control group (Con). The LHTLc group spent 9–10 h/night for 20 consecutive nights in an altitude house enriched with N_2 that simulated an altitude of 2,650 m (normobaric hypoxia; F_IO_2 = 16.27%). The LHTLi group spent 9–10 h/night for 20 nights exposed to the same level of hypoxia, comprised of four cycles of five consecutive nights at simulated altitude followed by two nights sleeping under normobaric normoxic conditions (Canberra, Australia; 600 m altitude, ambient barometric pressure ~711 mmHg). Con slept in either their own homes or the Australian Institute of Sport Residence Halls under normobaric normoxic conditions. During night time, the O_2 and CO_2 concentrations inside the altitude house were measured every 30 min with O_2 and CO_2 gas analyzers (Ametek model S3A and CD-3A, respectively; Pittsburgh, PA) that were calibrated every 2 h at two points, with air from outside the laboratory and with precision grade gas containing 16.51% O_2 (BOC Gases Australia). When subjects slept under hypoxic conditions, heart rate (HR) and blood O_2 saturation were determined every 30 min via fingertip pulse oximetry (model 505-US, Criticare, Waukesha, WI). Training and daytime living for all subjects were at an altitude of 600 m.

Preliminary Testing

$\dot{V}O_{2\,peak}$. On their first visit to the laboratory, all subjects performed a maximal, incremental cycle test to volitional exhaustion (18) on an electromagnetically braked ergometer (Lode, Groningen, The Netherlands) calibrated by using a first-principles calibration rig. Throughout the maximal test and the subsequently described experimental trials, subjects inspired air through a two-way valve (model R2700, Hans Rudolph, Kansas City, MO), with expirate directed to a custom-built, automated, indirect calorimetry system. Expirate was directed into 200-liter aluminized foil bags (Scholle Industries, Elizabeth, South Australia). Gas fractions were measured with O_2 and CO_2 analyzers (Ametek model S3A and CD-3A, respectively), and a precision-bore piston (Tufnol, Birmingham, UK), instrumented for real-time measurement of displacement, pressure, and temperature, was used to determine volume. The rates of $\dot{V}O_2$ and CO_2 production ($\dot{V}CO_2$), minute ventilation ($\dot{V}E$; BTPS), and the respiratory exchange ratio (RER) were calculated every 30 s from conventional equations. Before each maximal test and all subsequent experimental trials, the analyzers were calibrated with three commercially available α-grade gases of known O_2 and CO_2 content that spanned the physiological range. The analyzers were checked for drift after each test, and this never exceeded ±0.03%. $\dot{V}O_{2\,peak}$ was defined as the highest $\dot{V}O_2$ a subject attained during two consecutive 30-s sampling periods. Peak power output was defined as the last completed work rate (in W) plus the fraction of time spent in the final uncompleted work rate multiplied by 25 W (18). The purpose of this preliminary test was to ensure that subjects met the inclusion criteria for the investigation (i.e., a $\dot{V}O_{2\,peak}$ of ≥60 ml·kg^{-1}·min^{-1}). Four subjects did not meet the inclusion criteria, and accordingly 29 subjects participated in the study. The physical characteristics of the three experimental groups are presented in Table 1.

Morning Blood Status

Hemoglobin, hematocrit, and ferritin. Before any experimental intervention, each subject reported to the laboratory a second time in an overnight fasted state, and a resting venous blood sample was collected while the subjects were supine. Hemoglobin concentration ([Hb]) and hematocrit (Hct) were determined by using the Technicon H*3 analyzer (Bayer Diagnostics, Tarrytown, NY). Four milliliters of blood were collected into a tube prepared with K_3EDTA (Greiner Labortecnik, Kremsmunster, Germany) for the determination of serum ferritin concentration by use of an immunoturbidimetric assay on a Boehringer Mannheim/Hitachi 911 analyzer (Boehringer Mannheim). The analyzer was calibrated regularly with the use of Tinaquant Ferritin (Boehringer Mannheim) and checked daily with Lyphochek (Bio-Rad Laboratories, Anaheim, CA) level 1, level 2, and anemia controls. Any subject with a serum ferritin concentration <100 ng/ml was prescribed an oral iron supplement, Ferrograd C (325 mg dried ferrous sulfate, 562.4 mg sodium ascorbate) to be taken daily for the duration of the study.

Training

Subjects were instructed to maintain their normal training program throughout the study and kept a detailed training log that included the exercise mode and duration of each workout. Total training time was calculated from the information recorded in each subject's training log.

LT/Peak $\dot{V}O_2$ Test

In the week before allocation to an experimental condition, and then after 18 or 19 nights of simulated altitude exposure (*day 19* or *20* for LHTLc and Con and *day 26* for LHTLi), all subjects performed a lactate "threshold" (LT) and $\dot{V}O_{2\,peak}$ test. Thirty-six hours before a test, the training and nutritional status of each subject were controlled in an attempt to standardize muscle and liver glycogen stores. Two days before the test, all subjects reported to the laboratory between 1700 and 1900 and completed a 60-min ride at ~75% of $\dot{V}O_{2\,peak}$. They were then provided with a standard diet consisting of 55 kcal/kg body mass, composed of 57% carbohydrate (8 g/kg body mass), 29% fat, and 14% protein, to be consumed over the subsequent 36 h. During this time, subjects refrained from training.

Table 1. *Physical characteristics of subjects*

	Age, yr	Mass, kg	$\dot{V}O_{2\,peak}$, l/min	PPO, W	PPO, W/kg
LHTLc	27.2±5.7	72.6±9.7	4.9±0.6	374±47	5.2±0.5
LHTLi	26.5±4.7	69.9±8.9	4.6±0.6	352±43	5.0±0.2
Con	26.2±4.5	70.8±5.1	4.8±0.3	362±16	5.1±0.4

Values are means ± SD. LHTLc (n = 9), live high, train low continuous; LHTLi (n = 10), live high, train low intermittent; Con (n = 10), control. $\dot{V}O_{2\,peak}$, peak O_2 uptake; PPO, peak sustained power output determined during the maximal test.

On the morning of a LT test, subjects reported to the laboratory between 0700 and 0800, 12–14 h after an overnight fast. A Teflon cannula was inserted into an antecubital vein and attached to a three-way sterile stopcock to allow for blood sampling. The cannula was regularly flushed with 1–2 ml of heparinized 0.9% sterile saline to keep the vein patent. Subjects then consumed a standard breakfast, providing 2 g/kg of carbohydrate. This meal was consumed within 15 min, after which subjects rested for 2 h. At this time, subjects voided and then mounted the ergometer and commenced a discontinuous cycling protocol starting at an initial workload of 100 W. Each workload was maintained for 6 min with a 1-min rest period. Subjects remained seated on the ergometer between work bouts. The workload was increased by 50 W until a power output of 200 W was attained. Thereafter power output increments were 15 W. During the 1-min rest period, 1.5 ml of blood were collected into a heparinized 2-ml blood-gas syringe (QS90, Radiometer Medical, Copenhagen, Denmark). Samples were immediately analyzed in duplicate for whole blood lactate concentration ($[Lac]_b$) by use of a blood-gas analyzer (ABL 700 series, Radiometer Medical). The LT test was terminated at a power output that elicited a $[Lac]_b$ of $\geq$4 mmol/l. Such a lactate concentration is purely arbitrary but is commonly employed in testing of elite athletes in our laboratory. After completion of the LT test, subjects rested for 5 min before commencing an incremental maximal test for the determination of $\dot{V}O_{2\,peak}$. The starting power output for the maximal test was that at which each subject reached a $[Lac]_b$ of ~4 mmol/l. Thereafter the power output was increased by 25 W every 150 s until exhaustion. A blood sample was collected immediately on completion of the maximal test for the determination of $[Lac]_b$. The power output (W/kg) at a $[Lac]_b$ of 4 mmol/l was determined for each subject from the individual's $[Lac]_b$-vs.-power curve by linear interpolation from the two consecutive $[Lac]_b$ values that were above and below this value.

Experimental Trial

Lactate turnover. In the week before the altitude exposure and 3 days postexposure (*day 23* or *24* for both LHTLc and Con and *day 30* for LHTLi) subjects performed a prolonged, submaximal cycling test for the determination of lactate turnover. Thirty-six hours before a test, the training and nutritional status of each subject was controlled (described previously). On the morning of each experimental ride, subjects reported to the laboratory between 0600 and 0800 h, after a 10–12 h overnight fast. A cannula was inserted into a superficial dorsal hand vein to which a minimum volume extension tube was attached. The cannula and extension tube was kept patent with heparinized saline. The site was covered with an adhesive plastic dressing and plastic wrap. During the exercise test (described subsequently), the hand was immersed in a water bath (44.5°C) for 10 min before each blood sample to arterialize venous blood (28). A second cannula was inserted in an antecubital vein on the opposite arm for the infusion of the lactate tracer. After a baseline blood sample was taken (10 ml), subjects consumed a standard breakfast (as described), that was consumed within 15 min. Then a primed-continuous infusion of L-[U-^{14}C]lactic acid (Amersham Pharmacia Biotech, Essex, UK) was commenced for 180 min by using an automatic syringe pump (Terufusion TE-312, Terumo, Tokyo, Japan). A bolus dose of 20 ml was injected over a 1-min period. Thereafter, a constant-rate infusion (~10 μCi/h) was maintained. Blood samples were drawn after 15, 30, 60, and 90 min of rest to confirm a plateau in blood lactate specific radioactivity. Ninety minutes after beginning the tracer infusion, subjects mounted the ergometer and commenced 90 min of cycling exercise. During the first 60 min, a power output was chosen to elicit ~65% of each subject's $\dot{V}O_{2\,peak}$. For the final 30 min the workload was increased to a power output that elicited ~85% of $\dot{V}O_{2\,peak}$. Throughout exercise, respiratory gas was collected for 5-min periods after 0, 10, 15, 40, 54, 60, 70, and 85 min for the determination of $\dot{V}O_2$, and $\dot{V}CO_2$. In addition, the content of $^{14}CO_2$ in the expired air was determined immediately after each respiratory measurement by having subjects exhale into a Hans-Rudolph two-way valve attached to a 2-liter aluminized bag. The trapped air was then passed through a solution that contained 1 ml of 1 N hyamine hydroxide (ICN Biomedicals, Aurora, OH), 1 ml of 96% ethanol, and 1–2 drops of phenolphthalein (ICN Biomedicals) until the phenolphthalein indicator changed color, indicating that exactly 1 ml of CO_2 had been trapped. Ten milliliters of scintillation fluid (Ultima gold XR, Packard Bioscience, Groningen, The Netherlands) were then added, and the content of ^{14}C was subsequently determined by a liquid scintillation counter (Tri-Carb 1500, Packard Instruments). Bicarbonate or acetate correction factors were not used, but all counts were corrected for differences in quench and background. Specific activity was expressed as disintegrations per minute (dpm), and the percentage of infused [^{14}C]lactate tracer recovered as expired $^{14}CO_2$ was calculated as

$$\%\text{Recovery of } ^{14}C = (V^{14}CO_2/F) \times 100$$

where $V^{14}CO_2$ is the rate of expired $^{14}CO_2$ [$\dot{V}_E$ (STPD) (l/min) × $^{14}CO_2$ concentration (dpm/l)], and F is the [^{14}C]lactate infusion rate (dpm).

A limitation to this method is that some of the isotopic carbon atoms will recycle back into the tracer pool. Lactate is in equilibrium with pyruvate, which undergoes gluconeogenesis in the liver and kidneys. Approximately 25% of whole-body lactate tracer disappearance recycles back to blood glucose, and up to 20% of the glucose disappearance can enter the lactate pool (36). This reentry of lactate into the tracer pool results in a true rate of lactate tracer entry that is ~5% greater than the measured infusion rate. Because these errors can only be estimated and are relatively small, the data have not been corrected for recycling errors. More to the point, such an error is systematic and likely to be the same between trials.

At the same time that expired gas was collected, blood samples (10 ml) were taken for the subsequent analyses of a variety of metabolites and hormones (described below). In addition, 1.5 ml of blood were collected into a heparinized 2-ml blood-gas syringe, and the samples were immediately analyzed in duplicate for blood gases by use of the ABL 700 series blood-gas analyzer.

Muscle biopsies. In the 72 h before a subject completed an experimental trial (pre- and postexperimental intervention), a resting muscle sample was obtained from the vastus lateralis muscle via an incision made under local anesthesia (Xylocaine, 1%, Astra Pharmaceuticals, Sydney, Australia), with suction applied to the needle. The sample was quickly frozen in liquid N_2.

For all testing, laboratory conditions were maintained between 20 and 22ºC and between 45 and 50% relative humidity. Subjects were cooled with a fan (wind speed 7 m/s) and provided with water ad libitum throughout exercise. HR was monitored via telemetry (Accuex Plus; Polar Electro Oy, Kempele, Finland), and ratings of perceived exertion (RPE) using the 6–20 point Borg scale (8) were recorded at regular intervals.

Sample Analyses

Plasma lactate specific activity. One milliliter of plasma was used for this assay. To deproteinize each sample and to drive off any [^{14}C]bicarbonate as $^{14}CO_2$, 3 ml of distilled H_2O, (pH 7–8) were added to the plasma, mixed, and then heated for 5 min at 100°C. The samples were then cooled on ice for 10 min. Samples were then centrifuged at 4,000 *g* for 10 min at 4°C, and the protein-free supernatant was removed and refrigerated. Separation of [^{14}C]lactate from any [^{14}C]glucose that may have been formed via gluconeogenesis was achieved by passing the supernatant through a 1 × 4-cm column of Dowex (AG 1-X Cl mesh size 100–200, Bio-Rad) anion-exchange resin. Glucose was eluted with distilled H_2O (5 ml), and lactate was eluted with 0.2 M $CaCl_2$ (5 ml). Samples were evaporated in an oven at 35°C for ~20 h to reduce the volume of sample to <1 ml. After cooling, liquid scintillation cocktail (10 ml) was added to

each sample and counted. Recovery of ^{14}C was assessed by spiking a nonradioactive blood sample with [^{14}C]lactate and then processing the sample with those collected during the [^{14}C]lactate infusion. Lactate specific activity (SA) was expressed as dpm per micromole. The R_a and R_d of lactate were calculated by using the non-steady-state equations of Steele (39)

$$R_a = [F - (V \times Lac \times \Delta SA/\Delta t)/SA]$$

$$R_d = R_a - (V \times \Delta Lac/\Delta t)$$

where F is the infusion rate (dpm/kg, determined for each subject); V is the predicted non-steady-state distribution volume (100 ml/kg); Lac is the mean lactate concentration in consecutive samples (μmol), $\Delta SA/\Delta t$ is the change in lactate specific radioactivity ($dpm \cdot \mu mol^{-1} \cdot min^{-1}$); SA is the mean lactate specific activity in successive samples (dpm/μmol), and $\Delta[Lac]/\Delta t$ is the change in lactate concentration ([Lac]) (in $\mu mol \cdot ml^{-1} \cdot min^{-1}$).

R_{ox} (in $\mu mol \cdot ml^{-1} \cdot kg$ body $mass^{-1}$) was estimated as follows

$$Lac\ R_{ox} = (SA_{CO_2}/SA) \times \dot{V}_{CO_2}$$

where SA_{CO_2} is the specific radioactivity of expired $^{14}CO_2$ (dpm/μmol), SA is the lactate specific activity (dpm/μmol), and $\dot{V}_{CO_2}$ is the rate of CO_2 production (in $\mu mol \cdot kg^{-1} \cdot min^{-1}$).

Metabolic clearance rate (MCR) (expressed as $ml \cdot kg^{-1} \cdot min^{-1}$) was calculated by dividing R_d by the corresponding [Lac] values ($\mu mol \cdot ml^{-1} \cdot min^{-1}$). It has been reported that mixed venous, rather than arterial or venous, blood represents a more accurate blood sample in lactate tracer studies (22). Accordingly we performed calculations of mixed venous lactate SA (SA_{mv}) from our arterialized venous lactate SA

$$SA_{mv} = SA_{art} - (I/\dot{Q}[Lac])$$

where SA_{mv} is mixed venous SA (in dpm/μmol), SA_{art} is arterialized SA (in dpm/μmol); I is the infusion rate (in dpm/min); $\dot{Q}$ is the estimated cardiac output (in ml/min) (34), and [Lac] is the arterialized venous blood lactate concentration (in μmol/ml).

Plasma glucose, lactate, and FFA concentrations. Ten milliliters of blood were collected at each sampling point, of which 5 ml were placed in a tube containing fluoride and spun at 4,000 *g* for 8 min. Plasma was taken off, separated into two aliquots, and stored at −80°C. One of the duplicate supernatants was analyzed for lactate and glucose concentration by use of an automated analyzer (Yellow Springs Instruments 2300 Stat plus glucose and L-lactate analyzer; Yellow Springs, OH). The other sample was used to determine [^{14}C]lactate. The remaining blood was added to an aliquot of preservative consisting of EGTA and reduced glutathione in normal saline, mixed gently, and spun in a centrifuge. The plasma was later analyzed for free fatty acid (FFA) concentration by using an enzymatic colorimetric method (Wako, NEFAC test kit Tokyo, Japan).

Muscle buffering capacity. βm was measured in duplicate on freeze-dried muscle (~2 mg) by using a pH microelectrode (MI-145, Microelectrodes, Bedford, TX) by titration as previously described (16).

MCT1 and MCT4 protein. Muscle samples (~20 mg) obtained from a subset of subjects ($n = 16$) were homogenized in ice-cold buffer (210 mM sucrose, 2 mM EGTA, 40 mM NaCl, 30 mM HEPES, pH 7.4, and freshly added protease inhibitor cocktail) for ~30 s by using a Polytron PT1200 (Kinematica, Luzern, Switzerland). Homogenates were vortexed and then spun at 600 *g* for 10 min at 4°C. A portion of the supernatant (60 μl) was stored for MCT4 analysis. The remaining supernatant was then spun in an ultracentrifuge at 60,000 *g* for 15 min at 4°C. The pellet was resuspended in 60 μl of the homogenizing buffer and stored for MCT1 analysis. An aliquot of each sample was set aside for subsequent total protein analysis (Micro BCA protein assay reagent kit; Pierce, Rockford, IL) with bovine serum albumin as the standard, whereas the remainder was stored at −80°C until further analysis. SDS-PAGE was performed by using a Multiphor II (Pharmacia Biotech, Uppsala, Sweden) system. Aliquots of muscle homogenates containing 30 or 60 μg protein (MCT1 and MCT4, respectively) were separated by SDS-PAGE (10% resolving gel), transferred to nitrocellulose membrane (Nitrobind 0.45μm, Geneworks), and blocked for 2 h [1 × Tris-buffered saline + Tween 20 (TBST) in 5% non-fat milk]. Membranes were then incubated in the appropriate primary antibody (MCT1 1:500 and MCT4 1:250 dilution) (Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. Membranes were washed in blocking buffer (1 × TBST in 5% non-fat milk) and then incubated with the secondary antibody [anti-goat conjugated to horseradish peroxidase (1:5,000)] dilution for 60 min. After five washes in TBST, the membrane was placed in a chemiluminescent substrate (Pierce Supersignal Chemiluminescent) for 60 s and then visualized by use of a Kodak Image Station (440 CF; Perkin-Elmer, Life Sciences). Band density was analyzed with the use of Kodak 1D software (Kodak 1D 3.5).

Statistical Analysis

A two-way ANOVA for repeated measures was used to test for interaction and main effects for the dependent variables measured during exercise. With the use of Statistica software (version 5, Statsoft, Tulsa, OK), the two factors were Group (3 levels: Control, LHTLc, and LHTLi) and Time (2 levels: Pre and Post). Statistical significance was established at the $P < 0.05$ level. All values are reported as means ± SD. When main effects or interactions reached significance, the Newman-Keuls post hoc statistic was used to identify significant differences between means.

RESULTS

Morning Blood Status

[Hb], Hct, and ferritin. [Hb] (LHTLc, 14.6 ± 0.5 vs. 14.9 ± 0.9; LHTLi, 14.6 ± 0.7 vs. 15.1 ± 1.0; Con, 15.4 ± 1.1 vs. 15.4 ± 0.8 g/dl) and Hct (LHTLc, 0.42 ± 0.01 vs. 0.43 ± 0.02; LHTLi, 0.42 ± 0.02 vs. 0.43 ± 0.03; Con, 0.44 ± 0.04 vs. 0.44 ± 0.02%) were not different between groups pre- or postintervention. Despite iron supplementation, serum ferritin concentration was significantly lower postintervention for all groups (LHTLc, 94.6 ± 44.3 vs. 67.9 ± 31.6; LHTLi, 122.0 ± 44.7 vs. 85.6 ± 36.6; Con, 96.1 ± 64.1 vs. 66.6 ± 46.0 ng/ml; $P < 0.05$), although there were no differences between groups.

Training

The weekly training duration (min) for the three groups is presented in Table 2. There was no difference in training duration between LHTLi and Con. However, the duration was greater in the LHTLc group compared with Con ($P < 0.05$).

LT/Maximal Tests

Power output at 4 mmol/l, $\dot{V}_{O_2\ peak}$, and peak power output. Both the work rate at which 4 mmol/l of lactate was attained and the $\dot{V}_{O_2\ peak}$ were increased in all groups from pre- to

Table 2. *Total time engaged in exercise training during each week of the study for the 3 experimental groups*

	Week 1	*Week 2*	*Week 3*	*Week 4*	Total
LHTLc	873±343	930±326	1,087±283	758±189	3,647±874*
LHTLi	708±298	901±280	993±261	578±233	3,180±891
Con	672±179	630±240	847±299	520±223	2,670±762

Values are means ± SD in minutes. LHTLc, $n = 9$; LHTLi, $n = 10$; Con, $n = 10$. *Significantly different from Con, $P < 0.05$.

Table 3. *Work rate at which 4 mmol/l of lactate was attained*

	Power (W) at 4 mmol/l		$\dot{V}O_{2\,peak}$, ml·kg^{-1}·min^{-1}		PPO, W/kg	
	Pre	Post	Pre	Post	Pre	Post
LHTLc	287±43	301±40*	64.5±7.5	66.4±6.8*	5.06±0.6	5.26±0.6*
LHTLi	277±49	283±45*	65.4±4.4	67.4±5.1*	5.09±0.2	5.29±0.3*
Con	251±32	271±24*	67.3±4.4	69.7±4.9*	4.99±0.4	5.20±0.4*

Values are means ± SD. LHTLc, $n = 9$; LHTLi, $n = 10$; Con, $n = 10$. Pre, before hypoxic intervention; Post, after intervention. *Significantly greater than pretest, $P < 0.05$.

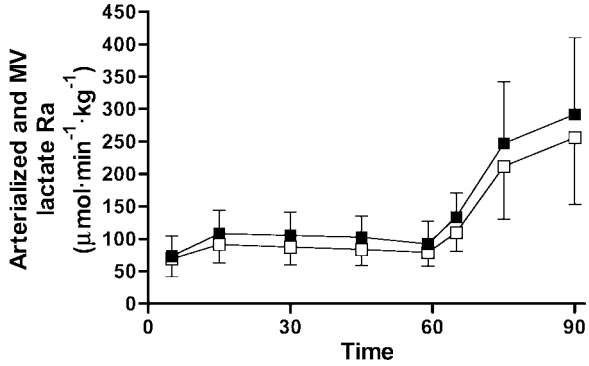

Fig. 1. Comparison between arterialized venous lactate rate of appearance (R_a; ■) and estimated mixed venous lactate R_a (□). Values are not significantly different.

postintervention ($P < 0.05$; Table 3). There was no difference between groups.

Experimental Trial

$\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$, and RER. $\dot{V}O_2$ (l/min), $\dot{V}CO_2$ (l/min), and $\dot{V}E$ (l/min) were stable throughout the first 60 min and increased during the last 30 min of exercise as a direct result of the increase in intensity (Table 4). RER remained unchanged (~0.90–0.93) during the 90 min of exercise. There was no difference in any of these parameters after 20 nights of LHTLc or LHTLi compared with Con, or between groups.

HR and RPE. There was a significant increase in HR and RPE when the exercise intensity was increased from 65% to 85% $\dot{V}O_{2\,peak}$. There was a decrease in HR at 59 and 65 min in the posttrial compared with the pretrial ($P < 0.05$), although there was no difference between the three groups (data not shown).

Blood lactate kinetics. The SA_{mv} values of the estimates of the lactate R_a and R_d were not significantly different from SA_{art} (Fig. 1). Accordingly, all subsequent data are presented for the venous infusion and arterialized venous sampling mode.

Lactate SA and blood lactate concentration. Changes in blood SA during 90 min of exercise are shown in Fig. 2*A*. Steady-state conditions were attained for both the first 59 min of exercise at 65% $\dot{V}O_{2\,peak}$ and from 65 to 90 min undertaken at 85% of $\dot{V}O_{2\,peak}$. Lactate SA decreased with an increase in exercise intensity ($P < 0.05$). There was no difference between trials or groups. [Lac] during 90 min of exercise is shown in Fig. 2*B*. Steady-state [Lac] was attained throughout the first 60 min of cycling at 65% $\dot{V}O_{2\,peak}$. During the final 30 min of exercise at 85% $\dot{V}O_{2\,peak}$ there was a gradual increase in [Lac] from ~1.8 to 4.9 mmol/l ($P < 0.05$), which was similar for all groups. There was a decrease in [Lac] at 65, 75, and 90 min in the LHTLc group ($P < 0.05$), and at 75 and 90 min in the Con group ($P < 0.05$) after intervention. There was no difference in [Lac] in the LHTLi group pre- and postintervention.

R_a, R_d, R_{ox}, and MCR. Fig. 3 shows R_a (*A*), R_d (*B*), R_{ox} (*C*), and MCR (*D*) determined during 90 min of exercise. During the first 60 min of exercise at 65% of $\dot{V}O_{2\,peak}$, R_a ranged between 65 and 90 μmol·kg^{-1}·min^{-1} and was not different between either the two treatment groups or Con. Lactate R_a averaged during the last 25 min of exercise at 85% $\dot{V}O_{2\,peak}$ was ~230 μmol·kg^{-1}·min^{-1} for all three groups and was significantly higher compared with exercise undertaken at 65% of $\dot{V}O_{2\,peak}$ ($P < 0.05$). Lactate R_a was significantly decreased at 75 and 90 min in the LHTLc group ($P < 0.05$) after intervention. There was no difference in lactate R_a for the LHTLi and Con groups. R_d (Fig. 4*B*) ranged between 65 and 90 μmol·kg^{-1}·min^{-1} at 65% of $\dot{V}O_{2\,peak}$, and there were no

Table 4. *Respiratory values during 90 min of submaximal cycling before and after hypoxic exposure in the 3 experimental groups*

	Prehypoxic Exposure						Posthypoxic Exposure					
	5 min	30 min	59 min	65 min	75 min	90 min	5 min	30 min	59 min	65 min	75 min	90 min
$\dot{V}O_2$, l/min												
LHTLc	3.02±0.31	3.12±0.31	3.15±0.31	3.88±0.36*	4.08±0.43*	4.13±0.43*	2.98±0.34	3.08±0.37	3.13±0.38	3.91±0.48*	4.07±0.53*	4.14±0.50*
LHTLi	2.91±0.32	2.96±0.30	3.00±0.28	3.72±0.38*	3.87±0.37*	4.00±0.33*	2.93±0.32	2.98±0.30	3.01±0.29	3.68±0.41*	3.82±0.44*	4.02±0.39*
Con	2.94±0.12	3.04±0.11	3.08±0.11	3.70±0.38*	3.99±0.12*	4.11±0.16*	2.95±0.14	3.05±0.13	3.08±0.10	3.77±0.14*	3.94±0.15*	4.08±0.20*
$\dot{V}CO_2$, l/min												
LHTLc	2.80±0.30	2.85±0.31	2.84±0.32	3.68±0.41*	3.80±0.47*	3.81±0.46*	2.74±0.34	2.83±0.34	2.80±0.36	3.67±0.46*	3.75±0.49*	3.80±0.47*
LHTLi	2.67±0.31	2.71±0.27	2.66±0.24	3.45±0.33*	3.52±0.32*	3.62±0.30*	2.61±0.31	2.69±0.29	2.63±0.29	3.38±0.40*	3.46±0.42*	3.63±0.38*
Con	2.72±0.11	2.80±0.14	2.77±0.09	3.59±0.10*	3.70±0.32*	3.75±0.17*	2.75±0.17	2.80±0.12	2.77±0.08	3.57±0.12*	3.63±0.16*	3.74±0.15*
$\dot{V}E$, l/min												
LHTLc	60.9±8.6	62.5±8.3	63.9±8.9	86.5±12.7*	97.7±18.7*	105.2±21.2*	60.9±9.3	62.0±8.7	62.5±9.0	84.4±11.9*	93.4±14.2*	100.0±17.1*
LHTLi	54.1±4.9	56.8±4.6	56.6±4.7	75.5±6.2*	82.1±5.6*	87.8±9.1*	57.5±5.6	59.4±5.3	59.6±5.1	78.4±7.7*	85.2±8.1*	91.0±15.2*
Con	54.8±3.7	57.4±3.6	58.5±3.2	77.7±4.3*	87.9±6.9*	96.1±7.3*	57.0±3.7	59.5±3.3	59.8±4.7	77.7±5.8*	85.3±7.6*	93.2±9.6*
RER												
LHTLc	0.93±0.04	0.91±0.02	0.90±0.02	0.95±0.03	0.93±0.02	0.92±0.03	0.92±0.04	0.92±0.02	0.89±0.02	0.94±0.03	0.92±0.03	0.91±0.03
LHTLi	0.92±0.04	0.91±0.02	0.90±0.02	0.93±0.01	0.91±0.02	0.91±0.03	0.89±0.04	0.90±0.02	0.87±0.02	0.92±0.02	0.90±0.02	0.90±0.03
Con	0.92±0.02	0.92±0.02	0.90±0.01	0.95±0.02	0.93±0.01	0.91±0.01	0.93±0.02	0.92±0.01	0.90±0.02	0.95±0.03	0.92±0.03	0.92±0.03

Values are means ± SD. LHTLc $n = 9$; LHTLi, $n = 10$; Con, $n = 10$. $\dot{V}O_2$, O_2 uptake; $\dot{V}CO_2$, CO_2 production; $\dot{V}E$, minute ventilation; RER, respiratory exchange ratio.

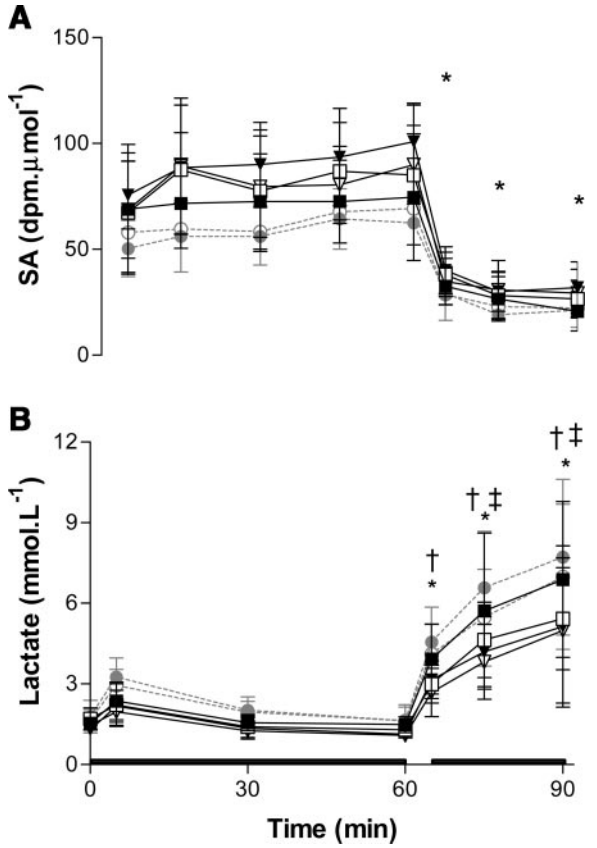

Fig. 2. Lactate specific activity (SA; *A*) and plasma lactate concentrations (*B*) during 90 min of submaximal cycling before and after hypoxic exposure. ■, Before (Pre) live high-train low continuous exposure (LHTLc); □, after (Post) LHTLc; ▼, live high-train low intermittent (LHTLi) Pre; ▽, LHTLi Post; ●, control (Con) Pre; ○, Con Post. Values are means ± SD. *Significantly greater than exercise at 65% peak O_2 uptake ($\dot{V}O_{2\,peak}$) for all groups; †significantly lower after intervention in the LHTLc group; ‡significantly different after intervention in the Con group.

differences either between the three groups or after intervention. Blood lactate R_d averaged during the last 25 min of exercise at 85% of $\dot{V}O_{2\,peak}$ was ~230 μmol·kg^{-1}·min^{-1}, which was higher than at 65% of $\dot{V}O_{2\,peak}$ ($P < 0.05$). Lactate R_d was significantly decreased at 75 and 90 min in the LHTLc group ($P < 0.05$) postintervention. There was no difference for lactate R_d in the LHTLi and Con groups. $\dot{R}_{ox}$ progressively decreased during 60 min of exercise at 65% $\dot{V}O_{2\,peak}$ (Fig. 4*C*), although this change was not statistically significant. There was a significant increase in R_{ox} when the exercise intensity was increased to 85% $\dot{V}O_{2\,peak}$ ($P < 0.05$). However, there was no difference in R_{ox} between the three groups or across the two trials. MCR (Fig. 4*D*) decreased when the exercise intensity increased from 65 to 85% of $\dot{V}O_{2\,peak}$ ($P < 0.05$), with the magnitude of change in MCR similar in all three groups and in all trials.

Plasma glucose and plasma FFA. There was no change in either plasma glucose (range 4.5–5.5 mmol/l) or plasma FFA concentration (range 0.50- 0.77 mmol/l) throughout the 90 min of exercise for all groups and across all trials.

Muscle buffering capacity. There was no effect of LHTL hypoxic exposure on βm (LHTLc, 145.3 ± 18.5 vs. 148.1 ± 18.4; LHTLi, 141.0 ± 14.6 vs. 145.9 ± 15.9; Con, 149.5 ±

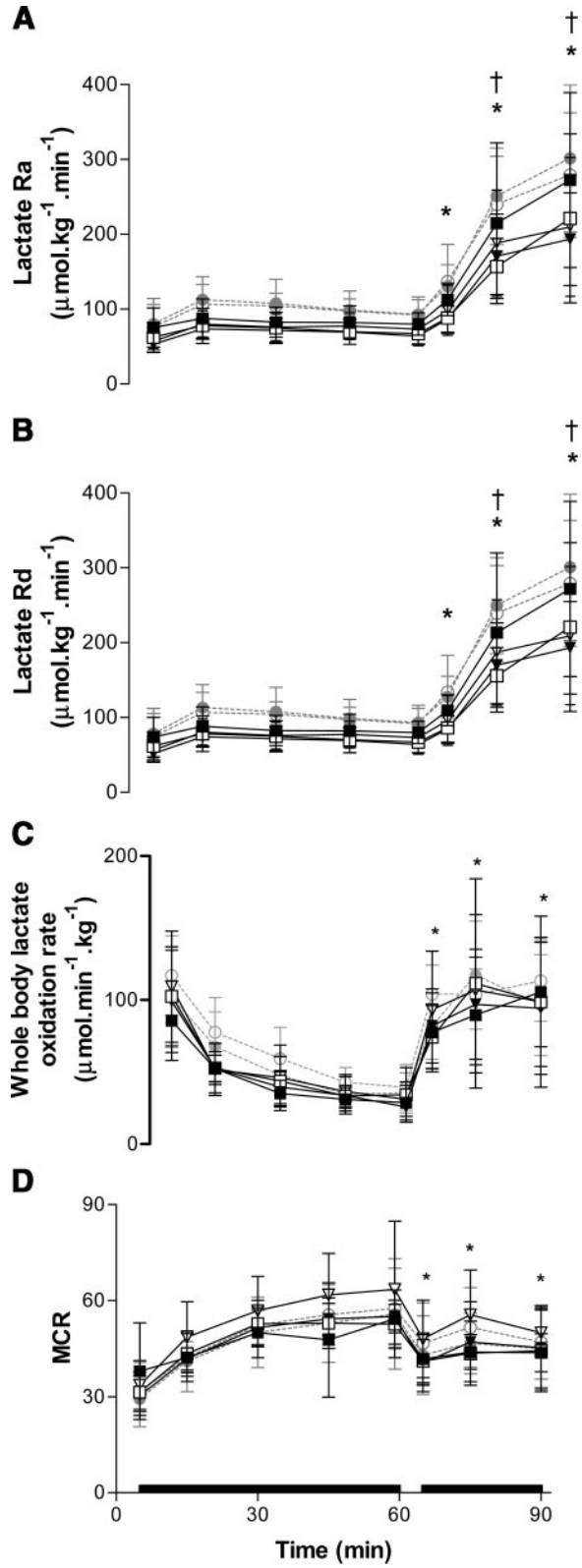

Fig. 3. Lactate R_a (*A*), rate of disappearance (R_d; *B*), rate of oxidation (*C*), and metabolic clearance rate (MCR, *D*) determined during 90 min of submaximal cycling before and after hypoxic exposure. ■, LHTLc Pre; □, LHTLc Post; ▼, LHTLi Pre; ▽, LHTLi Post; ●, Con Pre; ○, Con Post. Values are means ± SD. *Significantly different than exercise at 65% $\dot{V}O_{2\,peak}$ for all groups; †significantly lower after intervention in the LHTLc group.

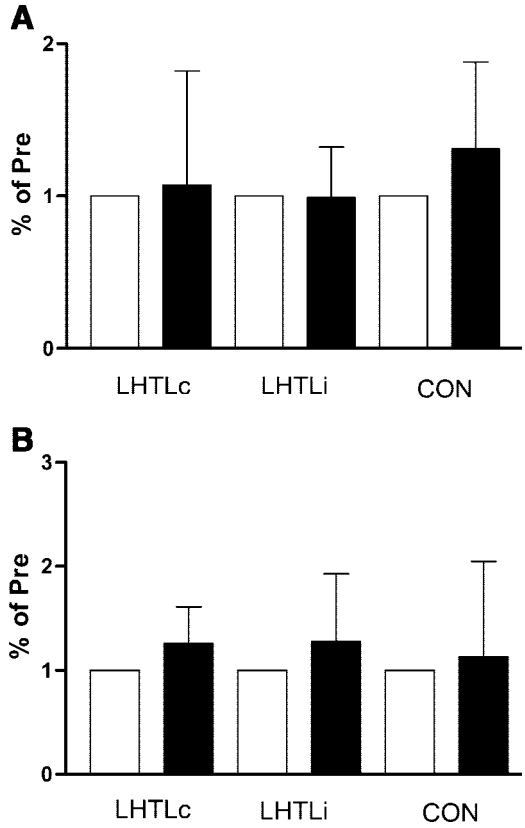

Fig. 4. Muscle membrane transport protein MCT1 (*A*) and MCT4 (*B*) abundance before (open bar) and after (solid bar) 20 nights of hypoxic exposure. LHTLc, $n = 6$; LHTLi, $n = 5$; Con, $n = 5$. Values are means ± SD.

20.9 vs. 151.0 ± 22.2, μmol H^+·g muscle dry wt^{-1}·pH^{-1}, values are pre- and postintervention, respectively).

MCT1 and MCT4 analysis. There was no difference in the abundance of MCT1 or MCT4 between groups before or after the intervention (Fig. 4).

DISCUSSION

The mechanisms underlying the improved endurance capacity reported after LHTL hypoxic exposure has received much scientific enquiry. The results of several investigations suggest that a hypoxia-induced increase in red blood cell volume and an associated increase in $\dot{V}O_{2\,max}$ (24) result in improved O_2 delivery and utilization during exercise that leads to enhancement of subsequent sea-level performance (12, 37). In contrast, it has been proposed that the key adaptations underpinning any hypoxia-induced performance improvement may be due to upregulation of skeletal muscle metabolism rather than changes in hematological variables and O_2 delivery (1, 2, 3, 16, 32). This is the first study to systematically investigate the effects of LHTL hypoxic exposure on a multitude of interdependent measures related to whole body and muscle lactate metabolism and pH regulation in well-trained athletes.

Our first finding was that, in association with a small but significant decrease in plasma lactate concentration during intense exercise (85% $\dot{V}O_{2\,max}$) and the marked increase in the workload at 4 mmol/l, whole body lactate kinetics were altered by LHTL hypoxic exposure (Fig. 2). Specifically, lactate R_a was significantly lower during intense cycling after LHTLc. Accordingly, lactate R_d was decreased in this group after hypoxic exposure. This finding suggests that sleeping under hypoxic conditions and training close to sea level may confer an advantage to whole body lactate metabolism (and thus performance) compared with training at sea level alone and argues for an adaptation in skeletal muscle that allows it to adopt a more oxidative mode of energy provision. The lack of change in R_a in the LHTLi group suggests that this intermittent hypoxic exposure does not result in the same physiological response. This finding is in contrast to the results of our previous study (32) that showed similar physiological responses to either 5, 10, or 15 nights of hypoxic exposure. It is unclear in the present study why the LHTLi group responded differently than the LHTLc group, considering that the hypoxic exposure was of similar magnitude. Despite our best efforts, the LHTLc group reported a greater training volume throughout the study compared with Con but not, it should be noted, LHTLi (Table 2). Unfortunately, it is difficult to interpret the likely effect of any training-induced response on the basis of the total training time reported by our subjects. The global measure of training time does not take into account the likely differences in training duration and intensity between triathletes and cyclists. More to the point, we chose a priori to match subjects to the different groups on the basis of similar $\dot{V}O_{2\,max}$ values rather than sporting discipline. Although it is possible that changes in whole body lactate kinetics may, partially, reflect a training rather than a hypoxic-induced response, training per se cannot fully explain our findings because there was no difference in training volume between the LHTLc and the LHTLi groups despite observed differences in lactate R_a and R_d. In this regard, Bergman et al. (6) have previously reported that 9 wk of endurance training in previously healthy but sedentary men significantly decreased lactate R_a during exercise performed at the same absolute workload, which corresponded to 65% of pretraining $\dot{V}O_{2\,max}$. However, in that study (6), lactate R_a was similar before and after training during more intense exercise. In contrast, we observed a decrease in lactate R_a during intense (85% $\dot{V}O_{2\,peak}$) but not moderate (65% $\dot{V}O_{2\,peak}$) exercise. Difference between the training status of the subjects and the interventions employed to investigate lactate kinetics makes direct comparisons between the two studies difficult.

In contrast to our previous study that 23 nights of LHTL increased βm by ~18% (13), a second finding of the present investigation was that 20 nights of LHTL hypoxic exposure had no effect on βm. Differences in results from the present investigation may be due to the ~13% higher simulated altitude in our previous study (16). Although it seems unlikely that this difference would result in such a change to βm, the possibility of a "dose-response" effect cannot be completely ruled out. Differences in results between investigations are, however, unlikely to be explained by the variability of measurement techniques, which are highly reproducible in our hands (16). Although previous studies have reported small (5–6%) increases (29, 35) or, alternatively, unspecified decreases (38) in βm after a period of training at terrestrial altitudes ranging from ~2,000 to 2,700 m, we are not aware of any other measures of βm after LHTL hypoxic exposure. Although there is always likely to be large individual variation in response to hypoxic exposure (12), we suggest that future studies of the LHTL paradigm attempt to randomize subjects to treatment groups on

the basis of either strict performance criteria or some direct measure of training status (i.e., oxidative enzyme capacity).

Our third finding was a failure to observe an increase in the abundance of lactate transport proteins after LHTL hypoxic exposure. To the best of our knowledge this is the first study to examine the effects of LHTL hypoxic exposure on MCT1 and MCT4 in skeletal muscle in humans. MCT1 transporters facilitate lactate influx into muscle and its subsequent oxidation (20, 27) and may also enhance lactate removal from the cell depending on the lactate concentration gradient across the sarcolemma (7). The MCT4 transporters are found predominantly in glycolytic fibers and are thought to facilitate lactic acid removal (20, 42). We originally speculated that LHTL hypoxic exposure would upregulate MCT1 and MCT4 lactate transporters and that whole body lactate production would be decreased whereas R_{ox} would be increased. Although lactate R_a was indeed lower after hypoxic exposure, at least in those subjects who were exposed to the stimulus of "continuous" hypoxia, we were unable to detect any effect of LHTL on lactate R_{ox}. The lactate-H^+ transport system contributes to skeletal muscle and blood acid-base control and has been proposed to be more active during exercise compared with rest (21). Accordingly, the lack of increase in lactate transport proteins in the present investigation maybe a consequence of already well-trained athletes sleeping (and not training) under hypoxic conditions. To the best of our knowledge, only two previous studies have measured lactate transporters after hypoxic exposure, and the results are equivocal. McClelland and Brooks (26) measured MCT1, MCT4, and lactate dehydrogenase isoforms in whole muscle and mitochondrial enriched fractions after 8 wk of hypobaric hypoxia (~4,300 m) in rats. Acclimation resulted in a 34% increase in MCT4 in heart and a decrease in MCT1 (−47%) and MCT4 (−47%) in plantaris whole muscle (26). The authors were unable to fully explain the tissue specific response to chronic hypoxia. Juel et al. (21) found no change in MCT1 and MCT4 lactate transporters after 8 wk acclimation to high altitude (4,100 m) in untrained humans. It should be noted that the models used by McClelland and Brooks (26) and Juel et al. (21) to investigate the effects of hypoxic exposure on MCT transporters are different from the LHTL model employed in the present investigation, making direct comparisons difficult.

In conclusion, and in accordance with one of our original hypotheses, 20 nights of LHTL hypoxic exposure decreased lactate production during intense exercise in well-trained athletes. However, the lower lactate R_a after LHTL exposure was limited to those subjects who underwent a continuous rather than intermittent mode of exposure. Finally, muscle markers of lactate metabolism and pH regulation were unchanged by either of the hypoxic interventions.

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GRANTS

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