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 (Ra), disappearance (Rd), and oxidation (Rox) were determined from a primed, continuous infusion of L-[U-14C]lactic acid tracer during 90 min of steady-state exercise [60 min at 65% peak O2 uptake (V O2 peak) followed by 30 min at 85% V O2 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. Ra during the first 60 min of exercise was not different between groups. During the last 25 min of exercise at 85% V O2 peak, Ra was higher compared with exercise at 65% of V O2 peak and was decreased in LHTLC (P < 0.05) compared with the other groups. Rox followed a similar pattern to Ra. Although Rox was significantly increased during exercise at 85% compared with 65% of V O2 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 Ro 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 O2 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 O2 uptake (V O2 max) (24, 33), and the maintenance of sea-level O2 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 O2 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 O2 consumption (V O2) over a range of power outputs despite a significant depression (7%) in peak O2 uptake (V O2 peak) (13). Finally, we observed a significant increase (~18%) in intravascular muscle buffer capacity (βm) after sleeping (rather than training) in hypoxic conditions (16). The improvement in βm occurred with 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 lactate 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 between intracellular and extracellular pools of lactate (25, 26). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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between 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 ($R_{ao}$), 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; FIO$_2$ 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 finger opti turbidimetric assay (model 505-US, Cricare, Waukesha, WI). Training and daytime living for all subjects were at an altitude of 600 m.

Preliminary Testing

$V\dot{O}_2$ 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 aluminumized 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 $V\dot{O}_2$ and CO$_2$ production ($V\dot{O}_2$, minute ventilation ($V_{E}$), 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 $\alpha$-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 $\pm 0.03\%$. $V\dot{O}_2$ peak was defined as the highest $V\dot{O}_2$ 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 $V\dot{O}_2$ peak of $\geq 60$ 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$^+$ analyzer (Bayer Diagnostics, Tarrytown, NY). Four milliliters of blood were collected into a tube prepared with K$_2$EDTA (Greiner Labortecnic, 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 Tinsquant Ferritin (Boehringer Mannheim) and checked daily with Lyphocheck (Bio-Rad Laboratories, Anaheim, CA) level 1, level 2, and anemia controls. Any subject with a serum ferritin concentration < 100 mg/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 $V\dot{O}_2$ 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 $V\dot{O}_2$ 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 $\sim 75\%$ of $V\dot{O}_2$ 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

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Mass, kg</th>
<th>$V\dot{O}_2$ peak, l/min</th>
<th>PPO, W</th>
<th>PPO, W/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>LHTLc</td>
<td>27.2±5.7</td>
<td>72.6±9.7</td>
<td>4.9±0.6</td>
<td>374±47</td>
</tr>
<tr>
<td>LHTLi</td>
<td>26.5±4.7</td>
<td>69.9±8.9</td>
<td>4.6±0.6</td>
<td>352±43</td>
</tr>
<tr>
<td>Con</td>
<td>26.2±4.5</td>
<td>70.8±5.1</td>
<td>4.8±0.3</td>
<td>362±16</td>
</tr>
</tbody>
</table>

Values are means ± SD. LHTLc ($n = 9$), live high, train low intermittent; LHTLi ($n = 10$), live high, train low continuous; Con ($n = 10$), control. $V\dot{O}_2$ peak, peak $O_2$ uptake; PPO, sustained power output determined during the maximal test.
On the morning of a LT test, subjects reported to the laboratory between 0700 and 0800. 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 ≈ 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 VO2max. 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 15 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 12–1 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 film a plateau in blood lactate specific activity. One milliliter of plasma was used for this assay. To deproteinize each sample and to drive off any [14C]bicarbonate as 14 CO2, 3 ml of distilled H2O, (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 [14C]lactate from any [14C]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 H2O (5 ml), and lactate was eluted with 0.2 M CaCl2 (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

where V14CO2 is the rate of expired 14CO2 [VE (STPD) (l/min) × 14CO2 concentration (dpm/l)], and F is the [14C]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 needle was quickly frozen in liquid N2.

For all testing, laboratory conditions were maintained between 20 and 22°C 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 (Accux 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 [14C]bicarbonate as 14CO2, 3 ml of distilled H2O, (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 [14C]lactate from any [14C]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 H2O (5 ml), and lactate was eluted with 0.2 M CaCl2 (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
HYPOXIC EXPOSURE AND LACTATE METABOLISM

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)

$$ R_a = \frac{\text{F} - (V \times \text{Lac} \times \Delta \text{SA}/\Delta t)}{\text{SA}} $$

$$ R_d = \frac{R_a - (V \times \Delta \text{Lac}/\Delta t)}{\text{SA}} $$

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), $\Delta \text{SA}/\Delta t$ is the change in lactate specific radioactivity (dpm·μmol$^{-1}$·min$^{-1}$); SA is the mean lactate specific activity in consecutive samples (dpm/μmol), and $\Delta \text{Lac}/\Delta t$ is the change in lactate concentration (Lac) (in μmol·ml$^{-1}$·min$^{-1}$).

$R_{ox}$ (in μmol·mol$^{-1}$·kg body mass$^{-1}$) was estimated as follows

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

where $\text{SA}_{CO_2}$ is the specific radioactivity of expired $^{14}$CO$_2$ (dpm/μmol), SA is the lactate specific activity (dpm/μmol), and $\text{V}_{CO_2}$ is the rate of $CO_2$ production (in μmol·kg$^{-1}$·min$^{-1}$).

Metabolic clearance rate (MCR) (expressed as ml/kg·min$^{-1}$) was calculated by dividing $R_d$ by the corresponding [Lac] values (μmol·ml$^{-1}$·min$^{-1}$). It has been reported that mixed venous, rather than arterial or venous, blood presents 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

$$ SA_{mv} = SA_{ar} - (I/Q[Lac]) $$

where $SA_{mv}$ is mixed venous SA (in dpm/μmol), $SA_{ar}$ is arterialized SA (in dpm/μmol); $I$ is the infusion rate (in dpm/min); $Q$ 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. Plasma glucose, lactate, and FFA concentrations were measured by use of an automated analyzer (Yellow Springs Instruments 2300 Stat plus glucose and L-lactate analyzer; Sprinfs Instruments) and the $V_{CO_2}$ peak was attained by 10.22±0.33. Similarly, the $V_{O_2 \text{peak}}$ was attained by 10.22±0.33. The $V_{O_2 \text{peak}}$ was attained by 10.22±0.33. Both the work rate at which 4 mmol/l of lactate was attained and the $V_{O_2 \text{peak}}$ were increased in all groups from pre- to postintervention. Despite iron supplementation, serum ferritin concentration was significantly lower postintervention for all groups (LHTLc, 94.6±4.3 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 ($P<0.05$).

<table>
<thead>
<tr>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>LHTLc</td>
<td>873±343</td>
<td>930±326</td>
<td>1,087±283</td>
<td>758±189</td>
</tr>
<tr>
<td>LHTLi</td>
<td>708±298</td>
<td>901±280</td>
<td>993±261</td>
<td>578±233</td>
</tr>
<tr>
<td>Con</td>
<td>672±179</td>
<td>630±240</td>
<td>847±299</td>
<td>520±223</td>
</tr>
</tbody>
</table>

Values are means ± SD in minutes. LHTLc, $n=9$; LHTLi, $n=10$; Con, $n=10$. *Significantly different from Con, $P<0.05$.
postintervention ($P < 0.05$; Table 3). There was no difference between groups.

**Experimental Trial**

$VO_2$, $VCO_2$, $V_{E}$, and $RER$. $VO_2$ (l/min), $VCO_2$ (l/min), and $V_{E}$ (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% $VO_{2peak}$. 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 $SA_{max}$ 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. 2A. Steady-state conditions were attained for both the first 59 min of exercise at 65% $VO_{2peak}$ and from 65 to 90 min undertaken at 85% of $VO_{2peak}$. 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. 2B. Steady-state [Lac] was attained throughout the first 60 min of cycling at 65% $VO_{2peak}$. During the final 30 min of exercise at 85% $VO_{2peak}$ 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.

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

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

<table>
<thead>
<tr>
<th>Power (W) at 4 mmol/l</th>
<th>$VO_{2peak}$, mg/kg·h$^{-1}$·min$^{-1}$</th>
<th>PPO, W/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>LHTLc</td>
<td>287±33 301±40* 64.5±7.5 66.4±6.8* 5.06±0.6 5.26±0.6*</td>
<td></td>
</tr>
<tr>
<td>LHTLi</td>
<td>277±39 283±45* 65.4±4.4 67.4±5.1* 5.09±0.2 5.29±0.3*</td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>251±32 271±24* 67.3±4.4 69.7±4.9* 4.99±0.4 5.20±0.4*</td>
<td></td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Prehypoxia Exposure</th>
<th>5 min</th>
<th>30 min</th>
<th>59 min</th>
<th>65 min</th>
<th>75 min</th>
<th>90 min</th>
<th>5 min</th>
<th>30 min</th>
<th>59 min</th>
<th>65 min</th>
<th>75 min</th>
<th>90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>$VO_2$, l/min</td>
<td>LHTLc</td>
<td>3.02±0.31 3.12±0.31 3.15±0.31 3.88±0.36 4.08±0.43 4.13±0.43*</td>
<td>2.98±0.34 3.08±0.37 3.13±0.38 3.91±0.48 4.07±0.53 4.14±0.50*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LHTLi</td>
<td>2.91±0.32 2.96±0.30 3.00±0.28 3.72±0.38 3.87±0.37 4.00±0.33*</td>
<td>2.93±0.32 2.98±0.30 3.01±0.29 3.68±0.41 3.82±0.44* 4.02±0.39*</td>
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<td>Con</td>
<td>2.94±0.12 3.04±0.11 3.08±0.11 3.70±0.38 3.99±0.12* 4.11±0.16*</td>
<td>2.95±0.14 3.05±0.13 3.08±0.10 3.77±0.14 3.94±0.15* 4.08±0.20*</td>
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<td>$VCO_2$, l/min</td>
<td>LHTLc</td>
<td>2.80±0.30 2.85±0.31 2.84±0.32 3.68±0.41 3.80±0.47 3.81±0.46*</td>
<td>2.74±0.34 2.83±0.34 2.80±0.36 3.67±0.46 3.75±0.49* 3.80±0.47*</td>
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<td>LHTLi</td>
<td>2.67±0.31 2.71±0.27 2.66±0.24 3.45±0.33 3.52±0.32* 3.62±0.30*</td>
<td>2.61±0.31 2.69±0.29 2.62±0.29 3.38±0.40 3.46±0.42* 3.63±0.38*</td>
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<tr>
<td>Con</td>
<td>2.72±0.11 2.80±0.14 2.77±0.09 3.59±0.10 3.70±0.32* 3.75±0.17*</td>
<td>2.75±0.17 2.80±0.12 2.77±0.08 3.57±0.12 3.63±0.16 3.74±0.15*</td>
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<td>$V_{E}$, l/min</td>
<td>LHTLc</td>
<td>60.9±8.6 62.5±8.3 63.9±8.9 86.5±12.7 97.7±18.7 105.2±21.2*</td>
<td>60.9±9.3 62.0±8.7 62.5±9.0 84.4±11.9 93.4±14.2 100.0±17.1*</td>
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<tr>
<td>LHTLi</td>
<td>54.1±4.9 56.8±4.6 56.6±4.7 75.5±6.2* 82.1±5.6* 87.8±9.1*</td>
<td>57.5±5.6 59.4±5.3 59.6±5.1 78.4±7.7* 85.2±8.1* 91.0±15.2*</td>
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<tr>
<td>Con</td>
<td>54.8±3.7 57.4±3.6 58.5±3.2 77.7±4.3* 87.9±6.9* 96.1±7.3*</td>
<td>57.0±3.7 59.5±3.3 59.8±4.7 77.7±5.8* 85.3±7.6* 93.2±9.6*</td>
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Values are means ± SD. LHTLc, n = 9; LHTLi, n = 10; Con, n = 10. $VO_3$, $O_2$ uptake; $VCO_2$, CO$_2$ production; $V_{E}$, minute ventilation; RER, respiratory exchange ratio.
differences either between the three groups or after intervention. Blood lactate $R_d$ averaged during the last 25 min of exercise at 85% of $V_\text{O}_2\text{peak}$ was 230 mmol/kg·min, which was higher than at 65% of $V_\text{O}_2\text{peak}$ ($P < 0.05$). 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. $R_\text{ox}$ progressively decreased during 60 min of exercise at 65% $V_\text{O}_2\text{peak}$ (Fig. 4C), although this change was not statistically significant. There was a significant increase in $R_\text{ox}$ when the exercise intensity was increased to 85% $V_\text{O}_2\text{peak}$ ($P < 0.05$). However, there was no difference in $R_\text{ox}$ between the three groups or across the two trials. MCR (Fig. 4D) decreased when the exercise intensity increased from 65 to 85% of $V_\text{O}_2\text{peak}$ ($P < 0.05$), 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 $\beta$m (LHTLc, 145.3 ± 18.5 vs. 148.1 ± 18.4; LHTLi, 141.0 ± 14.6 vs. 145.9 ± 15.9; Con, 149.5 ±

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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 $V_\text{O}_2$ for all groups; †significantly lower after intervention in the LHTLc group; ‡significantly different after intervention in the Con group.

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% $V_\text{O}_2\text{peak}$ for all groups; †significantly lower after intervention in the LHTLc group.
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 $V_{O2_{peak}}$ 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 $V_{O2_{max}}$. 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% $V_{O2_{peak}}$) but not moderate (65% $V_{O2_{peak}}$) exercise. Differences 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 $\beta m$ by $\sim 18\%$ (13), a second finding of the present investigation was that 20 nights of LHTL hypoxic exposure had no effect on $\beta m$. Differences in results from the present investigation may be due to the $\sim 13\%$ higher simulated altitude in our previous study (16). Although it seems unlikely that this difference would result in such a change to $\beta m$, 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 $\beta m$ after a period of training at terrestrial altitudes ranging from $\sim 2,000$ to $2,700$ m, we are not aware of any other measures of $\beta m$ 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 lactate 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 Rₐ would be increased. Although lactate Rₐ 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 Rₐ. 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 isoenzymes 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 Rₐ 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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