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

Sally A. Clark,1 Robert J. Aughey,2 Christopher J. Gore,3 Allan G. Hahn,3 Nathan E. Townsend,4 Tahnee A. Kinsman,4 Chin-Moi Chow,4 Michael J. McKenna,2 and John A. Hawley1

1Exercise Metabolism Group, School of Medical Sciences, RMIT University, Victoria 3083; 2Muscle Ions and Exercise Group, School of Human Movement, Recreation and Performance, Centre for Rehabilitation, Exercise and Sports Science, Victoria University of Technology, Victoria 3011; 3Australian Institute of Sport, ACT 2616; 4School of Exercise and Sports Science, The University of Sydney, Sydney, NSW 2114, Australia

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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), and the lactate tracer; monocarboxylate transporters; muscle buffering were determined from a primed, continuous infusion of \( [\text{U-}^{13}\text{C}] \) lactate tracer during 60 min at 65% peak \( \dot{\text{V}} \dot{\text{O}}_2 \) uptake (\( \dot{\text{V}} \dot{\text{O}}_2 \)peak) followed by 30 min at 85% \( \dot{\text{V}} \dot{\text{O}}_2 \)peak. A resting muscle biopsy was taken before and after 20 nights of LHTL for the determination of \( \beta m \) and MCT1 and MCT4 protein abundance. \( R_d \) during the first 60 min of exercise was not different between groups. During the last 25 min of exercise at 85% \( \dot{\text{V}} \dot{\text{O}}_2 \)peak, \( R_a \) was higher compared with exercise at 65% of \( \dot{\text{V}} \dot{\text{O}}_2 \)peak and was decreased in LHTLi (\( P < 0.05 \)) 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 \( \dot{\text{V}} \dot{\text{O}}_2 \)peak, there were no differences between the three groups or across trials. There was no effect of hypoxic exposure on \( \beta m \) or MCT1 and MCT4 protein abundance. We conclude that 20 consecutive nights of hypoxic exposure decreased whole body \( R_a \) during intense exercise in well-trained athletes. However, muscle markers of lactate metabolism and \( \dot{\text{V}} \dot{\text{O}}_2 \) regulation were unchanged by the LHTL intervention.

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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 (Ra) and increase lactate oxidation (Ro3), 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 N2 that simulated an altitude of 2,650 m (normobaric hypoxia; F IO2 = 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 O2 and CO2 concentrations inside the altitude house were measured every 30 min with O2 and CO2 gas analyzers (Ametek model S3A and CD-3A, respectively; Pittsburgh, PA) that were calibrated every 2 h at all points, with air from outside the laboratory and with precision grade gas containing 16.5% O2 (BOC Gases Australia). When subjects slept under hypoxic conditions, heart rate (HR) and blood O2 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 O2 and CO2 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 CO2 production (\( \dot{V}CO_2 \)), minute ventilation (Ve; ml/min), 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 O2 and CO2 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 K3EDTA (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 Tina-quant 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**

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Mass, kg</th>
<th>( \dot{V}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 continuous; LHTLi (n = 10), live high, train low intermittent; Con (n = 10), control. \( \dot{V}O_2\)peak, peak \( \dot{V}O_2 \) 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, 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 (Q590, 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 VO2peak. 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 LHTLi 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 testing trial (pre- and postexperimental intervention), a resting muscle sample was obtained from the vastus lateralis muscle via an experimental incision made under local anesthesia (Xylocaine, 1%, Astra Pharma- maceuticals, Sydney, Australia), with suction applied to the needle. The sample 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 (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 [14C]carbonate 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 X 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

% Recovery of [14C]lactate tracer recovered as expired 14CO2 was calculated as

\[
\text{% Recovery} = \left( \frac{V_{14CO2}/F}{V_{14CO2}} \right) \times 100
\]

where \(V_{14CO2}\) is the rate of expired 14CO2 [VE (STPD) (l/min) \times 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 sample was quickly frozen in liquid N2.

Plasma lactate specific activity. One milliliter of plasma was used for this assay. To deproteinize each sample and to drive off any [14C]carbonate 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 X 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
each sample and counted. Recovery of $^{14}$C was assessed by spiking a non-radioactive 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)

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

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

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); ΔSA/Δt 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 Δ[Lac]/Δt is the change in lactate concentration ([Lac]) in (μmol·ml⁻¹·min⁻¹).

$$R_{ox} = (\text{SA}_{CO_2}/\text{SA}) \times \text{VCO}_2$$

where $\text{SA}_{CO_2}$ is the specific radioactivity of expired $^{14}$CO₂ (dpm/μmol), SA is the lactate specific activity (dpm/μmol), and $\text{VCO}_2$ is the rate of CO₂ production (in μmol·kg⁻¹·min⁻¹).

Metabolic clearance rate (MCR) (expressed as ml·kg⁻¹·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 represents a more accurate blood sample in lactate tracer studies (22). Accordingly we performed calculations of mixed venous lactate SA (SAmv) from our arterialized venous blood samples with those collected during the $[^{14}$C]lactate infusion. Lactate was calculated by dividing $R_d$ by the corresponding [Lac] values (μmol/L). $R_{ox} (\text{mol·lmin}^-1·\text{kg body mass}^-1)$ was estimated as follows

$$\text{Lac}_{ox} = \text{SA}_{CO_2}/\text{SA} \times \text{VCO}_2$$

$\text{SA}_{CO_2}$ is the specific radioactivity of expired $^{14}$CO₂ (dpm/μmol), SA is the lactate specific activity (dpm/μmol), and $\text{VCO}_2$ is the rate of CO₂ production (in μmol·kg⁻¹·min⁻¹).

Muscle buffering capacity. ββ 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 100,000 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 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 0.45 μm, Generooks) and blocked for 2 h with 1 Trucks-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).

<table>
<thead>
<tr>
<th>LT/Maximal Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>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</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Week</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.
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>VO₂peak, mg/kg·h⁻¹·l⁻¹</th>
<th>PP0, W/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>LHTLi</td>
<td>287±43</td>
<td>301±40*</td>
</tr>
<tr>
<td>LHTLi</td>
<td>277±49</td>
<td>283±45*</td>
</tr>
<tr>
<td>Con</td>
<td>251±32</td>
<td>271±24*</td>
</tr>
</tbody>
</table>

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

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

Experimental Trial

VO₂, VCO₂, Ve, and RER. VO₂ (l/min), VCO₂ (l/min), and Ve (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 LHTLi 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% VO₂peak. 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 SAvo values of the estimates of the lactate Ra and Rb were not significantly different from SAart (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 [Lac] was attained throughout the first 60 min of cycling at 65% VO₂peak. During the final 30 min of exercise at 85% VO₂peak 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 LHTLi 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.

 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% VO₂peak. During the final 30 min of exercise at 85% VO₂peak 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 LHTLi 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.

Rm, Rb, Rsv, and MCR. Fig. 3 shows Rm (A), Rb (B), Rsv (C), and MCR (D) determined during 90 min of exercise. During the first 60 min of exercise at 65% of VO₂peak, Ra ranged between 65 and 90 µmol·kg⁻¹·min⁻¹ and was not different between either the two treatment groups or Con. Lactate Ra averaged during the last 25 min of exercise at 85% VO₂peak was ~230 µmol·kg⁻¹·min⁻¹ for all three groups and was significantly higher compared with exercise undertaken at 65% of VO₂peak (P < 0.05). Lactate Ra was significantly decreased at 75 and 90 min in the LHTLi group (P < 0.05) after intervention. There was no difference in lactate Ra for the LHTLi and Con groups. Rb (Fig. 4B) ranged between 65 and 90 µmol·kg⁻¹·min⁻¹ at 65% of VO₂peak, and there were no

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

<table>
<thead>
<tr>
<th>Prehypoxic Exposure</th>
<th>Posthypoxic Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 min</td>
<td>30 min</td>
</tr>
<tr>
<td>VO₂, l/min</td>
<td>LHTLi</td>
</tr>
<tr>
<td>LHTLi</td>
<td>2.91±0.32</td>
</tr>
<tr>
<td>Con</td>
<td>2.94±0.12</td>
</tr>
<tr>
<td>VCO₂, l/min</td>
<td>LHTLi</td>
</tr>
<tr>
<td>LHTLi</td>
<td>2.67±0.31</td>
</tr>
<tr>
<td>Con</td>
<td>2.72±0.11</td>
</tr>
<tr>
<td>Ve, l/min</td>
<td>LHTLi</td>
</tr>
<tr>
<td>LHTLi</td>
<td>54.1±4.9</td>
</tr>
<tr>
<td>Con</td>
<td>54.8±3.7</td>
</tr>
</tbody>
</table>

Values are means ± SD. LHTLi, n = 9; LHTLi, n = 10; Con, n = 10. VO₂, O₂ uptake; VCO₂, CO₂ production; Ve, minute ventilation; RER, respiratory exchange ratio.

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differences either between the three groups or after intervention. Blood lactate $R_d$ averaged during the last 25 min of exercise at 85% of $V\dot{O}_2$ peak was 230 μmol·kg$^{-1}$·min$^{-1}$, which was higher than at 65% of $V\dot{O}_2$ 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. $R_{ox}$ progressively decreased during 60 min of exercise at 65% $V\dot{O}_2$ peak (Fig. 4C), although this change was not statistically significant. There was a significant increase in $R_{ox}$ when the exercise intensity was increased to 85% $V\dot{O}_2$ peak ($P < 0.05$). However, there was no difference in $R_{ox}$ between the three groups or across the two trials. MCR (Fig. 4D) decreased when the exercise intensity increased from 65 to 85% of $V\dot{O}_2$ 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 $\beta$m (LHTLc, 145.3 ± 18.5 vs. 148.1 ± 18.4; LHTLi, 141.0 ± 14.6 vs. 145.9 ± 15.9; Con, 149.5 ±

**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 ($V\dot{O}_2$peak) 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% $V\dot{O}_2$peak for all groups; †significantly lower after intervention in the LHTLc group.
workload at 4 mmol/l, whole body lactate kinetics were altered
an associated increase in $V_o^\text{max}$ that a hypoxia-induced increase in red blood cell volume and
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cult. measures related to whole body and muscle lactate
effects of LHTL hypoxic exposure on a multitude of interde-
changes in hematological variables and $O_2$ delivery (1, 2, 3, 16,
upregulation of skeletal muscle metabolism rather than
hypoxia-induced performance improvement may be due to
the intervention (Fig. 4).

**DISCUSSION**

The mechanisms underlying the improved endurance capacity
reported after LHTL hypoxic exposure has received much
scientific question. The results of several investigations suggest
that a hypoxia-induced increase in red blood cell volume and
an associated increase in $V_o^\text{max}$ (24) result in improved $O_2$
delivery and utilization during exercise that leads to enhance-
cement 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 interde-
pendent 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% $V_o^\text{max}$) 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 $R_e$ 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 through-
out 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 triath-
letes and cyclists. More to the point, we chose a priori to match
subjects to the different groups on the basis of similar $V_o^\text{max}$
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 $V_o^\text{max}$. 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% $V_o^\text{peak}$) but not
moderate (65% $V_o^\text{peak}$) exercise. Difference between the
training status of the subjects and the interventions employed
to investigate lactate kinetics makes direct comparisons be-
tween the two studies difficult.

In contrast to our previous study that 23 nights of LHTL
increased $\beta_m$ by ~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 ~13% higher simulated alti-
tude 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 measure-
ment techniques, which are highly reproducible in our
hands (16). Although previous studies have reported small
(5–6%) increases (29, 35) or, alternatively, unspecified de-
creases (38) in $\beta_m$ 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 $\beta_m$ 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 \( R_a \) 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 \( R_a \). 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 isofoms in whole muscle and mitochondrial enriched fractions after 8 wk of hypobaric hypoxia (\( \sim 4,300 \) m) in rats. Acclimation resulted in a 34% increase in MCT4 heart and its decrease in MCT1 (\( \sim 47\% \)) and MCT4 (\( \sim 47\% \)) 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.

REFERENCES


HYPOXIC EXPOSURE AND LACTATE METABOLISM


