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

Sally A. Clark, 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

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 2141, Australia

Submitted 30 July 2003; accepted in final form 23 September 2003

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 Rm were measured by lactate tracer; monocarboxylate transporters; and pH regulation were unchanged by the LHTL intervention. 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. Rd during the last 60 min of exercise was not different between groups. During the last 25 min of exercise at 85% VO2 peak, Rd was higher compared with exercise at 65% of VO2 peak and was decreased in LHTLc (P < 0.05) compared with the other groups. R4 followed a similar pattern to Rd. Although Rox was significantly increased during exercise at 85% compared with 65% of VO2 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 Rm during intense exercise in well-trained athletes. However, muscle markers of lactate metabolism and pH regulation were unchanged by the LHTL intervention.

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.

Address for reprint requests and other correspondence: J. A. Hawley, School of Medical Sciences, RMIT Univ., PO Box 71, Bundoora, Victoria 3083, Australia (E-mail: john.hawley@rmit.edu.au).

http://www.jap.org 8750-7587/04 $5.00 Copyright © 2004 the American Physiological Society

517
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<sub>a</sub>) and increase lactate oxidation (R<sub>ox</sub>), 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); HLTLC, 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<sub>2</sub> that simulated an altitude of 2,650 m (normobaric hypoxia; F<sub>I</sub>O<sub>2</sub> = 16.2%); 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 Residency Halls under normobaric normoxic conditions. During night time, the O<sub>2</sub> and CO<sub>2</sub> concentrations inside the altitude house were measured every 30 min with O<sub>2</sub> and CO<sub>2</sub> 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<sub>2</sub> (BOC Gases Australia). When subjects slept under hypoxic conditions, heart rate (HR) and blood O<sub>2</sub> saturation were determined every 30 min by pulse oximetry (model 505-US, Criticare, Waukesha, WI). Training and daytime living for all subjects were at an altitude of 600 m.

**Preliminary Testing**

V<sub>O2</sub><sub>peak</sub>. 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 a 200-liter aluminumized foil bags (Scholle Industries, Elizabeth, South Australia). Gas fractions were measured with O<sub>2</sub> and CO<sub>2</sub> 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<sub>O2</sub> and CO<sub>2</sub> production (V<sub>CO2</sub>), minute ventilation (V<sub>E</sub>), 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<sub>2</sub> and CO<sub>2</sub> content that spanned the physiological range. The analyzers were checked for drift after each test, and this never exceeded ±0.03%. V<sub>O2</sub><sub>peak</sub> was defined as the highest V<sub>O2</sub> 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<sub>O2</sub><sub>peak</sub> of ≥60 ml/kg·min<sup>-1</sup>). 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<sup>+</sup>3 analyzer (Bayer Diagnostics, Tarrytown, NY). Four milliliters of blood were collected into a tube prepared with K<sub>3</sub>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 Tinaquant 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 ng/ml was prescribed an oral iron supplement, Ferrogred 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<sub>O2</sub> 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<sub>O2</sub><sub>peak</sub> 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 V<sub>O2</sub><sub>peak</sub>. 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></th>
<th>Age, yr</th>
<th>Mass, kg</th>
<th>V&lt;sub&gt;O2&lt;/sub&gt;&lt;sub&gt;peak&lt;/sub&gt;, 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>
<td>5.2 ± 0.5</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>
<td>5.0 ± 0.2</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>
<td>5.1 ± 0.4</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. V<sub>O2</sub><sub>peak</sub>, peak O<sub>2</sub> 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 rest 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 V̇O₂peak after 0, 10, 15, 40, 54, 60, 70, and 85 min for the determination of V̇O₂peak. 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 V̇O₂ and V̇CO₂. In addition, the content of 14C 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 CO₂ had been trapped. Ten milliliters of scintillation fluid (Ultima gold XR, Packard Bioscience, Groningen, The Netherlands) were then added, and the content of 14C 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 [14C]lactate tracer recovered as expired 14CO₂ was calculated as:

%Recovery of 14C = (V14CO₂/F) × 100

where V14CO₂ is the rate of expired 14CO₂ [V̇(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.

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 LHTLc) 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 1-[14C]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 V̇O₂peak. For the final 30 min the workload was decreased to a power output that elicited ~85% of V̇O₂peak. 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 V̇O₂ and V̇CO₂. In addition, the content of 14CO₂ in the expired air was determined immediately after each respiratory measurement by

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

where \( V_{14\text{C}} \) is the rate of expired \(^{14}\text{C}O_2\) \([\text{V}\dot{\text{O}}_2/\text{STPD}] \times \) \(^{14}\text{C}O_2\) concentration (dpm/l)), and \( F \) is the \(^{14}\text{C}\) 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) and 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 (Accucare 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 \(^{14}\text{C}\) bicarbonate as \(^{14}\text{CO}_2\), 3 ml of distilled H₂O₂ (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 \(^{14}\text{C}\) lactate from any \(^{14}\text{C}\) 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 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

J Appl Physiol • VOL 96 • FEBRUARY 2004 • www.jap.org
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):

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

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

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), $\Delta S A/\Delta t$ 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 $\Delta Lac/\Delta t$ is the change in lactate concentration ([Lac]) (in μmol·mL$^{-1}$·min$^{-1}$).

$$R_{ox} = \frac{SA_{CO_2}}{SA} \times V_{CO_2}$$

where $SA_{CO_2}$ is the specific radioactivity of expired $^{14}$CO$_2$ (dpm/μmol), SA is the lactate specific activity (dpm/μmol), and $V_{CO_2}$ is the rate of CO$_2$ 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 ($SA_{mv}$) from our arterialized venous blood lactate concentrations (in μmol/L). The remaining blood was added to an aliquot of preser -

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%). The power output at 4 mmol/L lactate was attained and the $VO_2$ peak were increased in all groups from pre- to postintervention. Despite iron supplementation, serum ferritin concentration was significantly lower postintervention for all groups (LHTLc, 891 ± 189 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, $VO_2$ peak, and peak power output. Both the work rate at which 4 mmol/L of lactate was attained and the $VO_2$ peak were increased in all groups from pre- to postintervention. Despite iron supplementation, serum ferritin concentration was significantly lower postintervention for all groups (LHTLc, 891 ± 189 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; $P < 0.05$).

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

<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, $P < 0.05$. 

J Appl Physiol • VOL 96 • FEBRUARY 2004 • www.jap.org
postintervention ($P < 0.05$; Table 3). There was no difference between groups.

**Experimental Trial**

$V_{O_{2}}$, $V_{CO_{2}}$, $V_{E}$, and $RER$. $V_{O_{2}}$ (l/min), $V_{CO_{2}}$ (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 ($\sim 0.90$–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% $V_{O_{2}}$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 $S_{A_{hi}}$ values of the estimates of the lactate $R_{a}$ and $R_{b}$ were not significantly different from $S_{A_{art}}$ (Fig. 1). Accordingly, all subsequent data are presented for the venous infusion and arterialized venous sampling mode.

**Lactate $S_{A}$ and blood lactate concentration.** Changes in blood $S_{A}$ 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% $V_{O_{2}}$peak and from 65 to 90 min undertaken at 85% $V_{O_{2}}$peak. Lactate $S_{A}$ 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% $V_{O_{2}}$peak. During the final 30 min of exercise at 85% $V_{O_{2}}$peak there was a gradual increase in $[Lac]$ from $\sim 1.8$ 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.

$R_{pa}$, $R_{pa}$, and MCR. Fig. 3 shows $R_{pa}$ (A), $R_{pa}$ (B), $R_{pa}$ (C), and MCR (D) determined during 90 min of exercise. During the first 60 min of exercise at 65% of $V_{O_{2}}$peak, $R_{pa}$ 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 $R_{pa}$ averaged during the last 25 min of exercise at 85% $V_{O_{2}}$peak was $\sim 230$ $\mu$mol·kg$^{-1}$·min$^{-1}$ for all three groups and was significantly higher compared with exercise undertaken at 65% $V_{O_{2}}$peak ($P < 0.05$). Lactate $R_{pa}$ was significantly decreased at 75 and 90 min in the LHTLi group ($P < 0.05$) after intervention. There was no difference in lactate $R_{pa}$ for the LHTLi and Con groups. $R_{pa}$ (Fig. 4B) ranged between 65 and 90 $\mu$mol·kg$^{-1}$·min$^{-1}$ at 65% of $V_{O_{2}}$peak, 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>$V_{O_{2}}$peak, mg/kg·min$^{-1}$</th>
<th>PPO, W/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>LHTLi</td>
<td>28.7±43</td>
<td>301.4±4*</td>
</tr>
<tr>
<td>LHTLi</td>
<td>277.49</td>
<td>283.45*</td>
</tr>
<tr>
<td>Con</td>
<td>281.32</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, $P < 0.05$.}

---

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

**Prehypoxic Exposure**

<table>
<thead>
<tr>
<th>$V_{O_{2}}, \text{l/min}$</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>LHTLi</td>
<td>3.02±0.31</td>
<td>3.12±0.31</td>
<td>3.15±0.31</td>
<td>3.88±0.36*</td>
<td>4.08±0.43*</td>
<td>4.13±0.43*</td>
</tr>
<tr>
<td>LHTLi</td>
<td>2.91±0.32</td>
<td>2.96±0.30</td>
<td>3.00±0.28</td>
<td>3.72±0.38*</td>
<td>3.87±0.37*</td>
<td>4.00±0.33*</td>
</tr>
<tr>
<td>Con</td>
<td>2.94±0.12</td>
<td>3.04±0.11</td>
<td>3.08±0.11</td>
<td>3.70±0.38*</td>
<td>3.99±0.12*</td>
<td>4.11±0.16*</td>
</tr>
<tr>
<td>$V_{CO_{2}}, \text{l/min}$</td>
<td>2.80±0.30</td>
<td>2.85±0.31</td>
<td>2.84±0.32</td>
<td>3.68±0.41*</td>
<td>3.80±0.47*</td>
<td>3.81±0.46*</td>
</tr>
<tr>
<td>LHTLi</td>
<td>2.67±0.31</td>
<td>2.71±0.27</td>
<td>2.66±0.24</td>
<td>3.45±0.33*</td>
<td>3.52±0.32*</td>
<td>3.62±0.30*</td>
</tr>
<tr>
<td>LHTLi</td>
<td>2.72±0.31</td>
<td>2.80±0.14</td>
<td>2.77±0.09</td>
<td>3.59±0.10</td>
<td>3.70±0.32*</td>
<td>3.75±0.17*</td>
</tr>
</tbody>
</table>

---

### Table 5. Respiratory values during 90 min of submaximal cycling before and after hypoxic exposure in the 3 experimental groups

**Posthypoxic Exposure**

<table>
<thead>
<tr>
<th>$V_{O_{2}}, \text{l/min}$</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>LHTLi</td>
<td>2.98±0.34</td>
<td>3.08±0.37</td>
<td>3.13±0.38</td>
<td>3.91±0.48*</td>
<td>4.07±0.53*</td>
<td>4.14±0.50*</td>
</tr>
<tr>
<td>LHTLi</td>
<td>2.93±0.32</td>
<td>2.98±0.30</td>
<td>3.01±0.29</td>
<td>3.68±0.41*</td>
<td>3.82±0.44*</td>
<td>4.02±0.39*</td>
</tr>
<tr>
<td>Con</td>
<td>2.95±0.14</td>
<td>3.05±0.13</td>
<td>3.08±0.10</td>
<td>3.77±0.14*</td>
<td>3.94±0.15*</td>
<td>4.08±0.20*</td>
</tr>
<tr>
<td>$V_{CO_{2}}, \text{l/min}$</td>
<td>2.74±0.34</td>
<td>2.83±0.34</td>
<td>2.80±0.36</td>
<td>3.67±0.46*</td>
<td>3.75±0.49*</td>
<td>3.80±0.47*</td>
</tr>
<tr>
<td>LHTLi</td>
<td>2.61±0.31</td>
<td>2.69±0.29</td>
<td>2.62±0.29</td>
<td>3.38±0.40*</td>
<td>3.46±0.42*</td>
<td>3.63±0.38*</td>
</tr>
<tr>
<td>LHTLi</td>
<td>2.75±0.17</td>
<td>2.80±0.12</td>
<td>2.77±0.08</td>
<td>3.57±0.12*</td>
<td>3.63±0.16*</td>
<td>3.74±0.15*</td>
</tr>
<tr>
<td>$V_{E}, \text{l/min}$</td>
<td>60.9±8.6</td>
<td>62.5±8.3</td>
<td>63.9±8.9</td>
<td>86.5±12.7*</td>
<td>97.7±18.7*</td>
<td>105.2±21.2*</td>
</tr>
<tr>
<td>LHTLi</td>
<td>54.1±4.9</td>
<td>56.8±4.6</td>
<td>56.6±4.7</td>
<td>75.6±6.2*</td>
<td>82.1±5.6*</td>
<td>87.8±9.1*</td>
</tr>
<tr>
<td>LHTLi</td>
<td>57.5±5.6</td>
<td>59.4±5.3</td>
<td>59.6±5.1</td>
<td>78.4±7.7*</td>
<td>85.2±8.1*</td>
<td>91.0±15.2*</td>
</tr>
<tr>
<td>LHTLi</td>
<td>57.0±3.7</td>
<td>59.5±3.3</td>
<td>59.8±4.7</td>
<td>77.7±5.8*</td>
<td>85.3±7.6*</td>
<td>93.2±9.6*</td>
</tr>
</tbody>
</table>

Values are means ± SD. LHTLi, n = 9; LHTLi, n = 10; Con, n = 10. $V_{O_{2}}$, $O_{2}$ uptake; $V_{CO_{2}}$, $CO_{2}$ 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 $V\dot{O}_2$ peak was $230 \mu$mol$\cdot$kg$^{-1}$$\cdot$min$^{-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, $145.3 \pm 18.5$ vs. $148.1 \pm 18.4$; LHTLi, $141.0 \pm 14.6$ vs. $145.9 \pm 15.9$; Con, $149.5 \pm$

---

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.
20.9 ± 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 \( \dot{V}O_2 \text{max} \) (24) result in improved \( \dot{O}_2 \) 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 \( \dot{O}_2 \) delivery (1, 2, 3, 16, 32). This is the first study to systematically investigate the effects of LHTL hypoxic exposure on a multitude of independent measures related to whole body and muscle lactate metabolism and \( \dot{O}_2 \) delivery 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% \( \dot{V}O_2 \text{max} \)) 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 \( \dot{R}_a \) was significantly lower during intense cycling after LHTLC. Accordingly, lactate \( \dot{R}_d \) 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 \( \dot{R}_a \) 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 \( \dot{V}O_2 \text{max} \) 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 \( \dot{R}_a \) and \( \dot{R}_d \). 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 \( \dot{R}_a \) during exercise performed at the same absolute workload, which corresponded to 65% of pretraining \( \dot{V}O_2 \text{max} \). However, in that study (6), lactate \( \dot{R}_a \) was similar before and after training during more intense exercise. In contrast, we observed a decrease in lactate \( \dot{R}_a \) during intense (85% \( \dot{V}O_2 \text{peak} \)) but not moderate (65% \( \dot{V}O_2 \text{peak} \)) 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 ~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 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 ~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 sarclemma (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 $R_m$ would be increased. Although lactate $R_m$ 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 $R_m$. 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 $R_m$ 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.

ACKNOWLEDGMENTS

The authors acknowledge the technical support of staff in the Department of Physiology at the Australian Institute of Sport, Dale Bickham from The School of Health Sciences, Deakin University for assistance with the MCT analysis, and Dr. Nigel K. Stepto from Monash University.

GRANTS

This study was funded by Australian Research Council Grant C0002552.

REFERENCES


