Metabolic and cardiorespiratory responses to “the lactate clamp”

BENJAMIN F. MILLER, JILL A. FATTOR, KEVIN A. JACOBS, MICHAEL A. HORNING, SANG-HOON SUH, FRANCO NAVAZIO, AND GEORGE A. BROOKS

Department of Integrative Biology, University of California, Berkeley, California 94720

Received 17 June 2002; accepted in final form 11 July 2002

Miller, Benjamin F., Jill A. Fattor, Kevin A. Jacobs, Michael A. Horning, Sang-Hoon Suh, Franco Navazio, and George A. Brooks. Metabolic and cardiorespiratory responses to “the lactate clamp.” Am J Physiol Endocrinol Metab 283: E889–E898, 2002. First published July 17, 2002; 10.1152/ajpendo.00266.2002.—To evaluate the hypothesis that precursor supply limits gluconeogenesis (GNG) during exercise, we examined training-induced changes in glucose kinetics [rates of appearance (Ra) and disappearance (Rd)], oxidation (Rox), and recycling (Rr) with an exogenous lactate infusion to 3.5–4.0 mM during rest and to pretraining 65% peak O2 consumption (Vo2peak) levels during exercise. Control and clamped trials (LC) were performed at rest pre- (PRR, PRR-LC) and posttraining (POR, POR-LC) and during exercise pre- (PEREX) and posttraining at absolute (POAB, P0AB-LC) and relative (P0RLC, P0RLC-LC) intensities. Glucose R0 was not different in any rest or exercise condition. Glucose Rox did not differ as a result of LC. Glucose Rox was significantly decreased with LC at P0R (0.38 ± 0.03 vs. 0.56 ± 0.04 mg·kg−1·min−1) and P0AB (3.82 ± 0.51 vs. 5.0 ± 0.62 mg·kg−1·min−1). Percent glucose Rox oxidized decreased with all LC except P0RLC-LC (P0R, 32%; P0R-LC, 22%; P0R, 27%; P0R-LC, 20%; P0AB, 95%; P0AB-LC, 77%), which resulted in a significant increase in oxidation from alternative carbohydrate (CHO) sources at rest and P0AB. We conclude that 1) increased arterial [lactate] did not increase glucose R0 measured during rest or exercise after training, 2) glucose disposal or production did not change with increased precursor supply, and 3) infusion of exogenous CHO in the form of lactate resulted in the decrease of glucose Rox.

Lactate; glucose kinetics; glucose recycling; training

MAINTENANCE of blood [glucose] homeostasis requires coordination of delivery and utilization, or else hypo- or hyperglycemia results. Prolonged exercise and disease states, such as type 2 diabetes, represent situations in which glucose homeostasis is challenged. During postabsorptive rest (40) and exercise (3, 40), hepatic and renal gluconeogenesis (GNG) can increase to maintain glucose production (GP) and spare finite hepatic glycogen stores. Regular exercise training is accompanied, in part, by beneficial adaptations pertaining to glucose homeostasis (17).

Address for reprint requests and other correspondence: G. A. Brooks, Exercise Physiology Laboratory, Dept. of Integrative Biology, 5101 Valley Life Science Bldg., Univ. of California, Berkeley 94720-3140 (E-mail: gbrooks@socrates.berkeley.edu).

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.
Neither of those studies matched arterial [lactate] to a pretraining concentration or examined the effect of lactate infusion on glucose turnover, GNG capacity, or substrate selection.

To examine training-induced changes in GNG capacity, we employed an exogenous lactate infusion to “clamp” blood [lactate] [lactate clamp (LC)] during periods of rest and exercise, and we measured glucose recycling rate (Rg). LC values at rest were ~3.5–4.0 mM, whereas during exercise they were targeted to pretraining 65% peak O2 consumption (VO2peak) levels (PBE), again ~3.5–4.0 mM. Posttraining trials were performed with and without LC at both absolute (POAB, POAB-LC) and relative (PORL, PORL-LC) exercise intensities compared with pretraining (PBE). Our hypotheses were that 1) GNG was substrate limited during exercise and that endurance training would increase glucose Rg during exercise when blood lactate level was restored to pretraining levels by exogenous infusion, 2) glucose kinetics and concentration would not change with exogenous lactate infusion due to autoregulation of GP, and 3) whole body substrate oxidation patterns would change during exercise due to infusion of lactate, an additional CHO source.

METHODS

Subjects. Nine healthy sedentary male subjects aged 18–35 yr were recruited from the University of California, Berkeley campus (UC-Berkeley), by posted notices. Subjects were considered for study if they were nonsmoking, not taking medications, healthy by physical examination, training regularly <2 h/wk, weight stable for the last 6 mo, under 25% body fat, and had normal lung function as determined by a 1-s forced expiratory volume (FEV1) 75% of vital capacity. Subjects gave informed consent, and the study protocol was approved by the UC-Berkeley Committee for the Protection of Human Subjects (CPSH 2000-6-98).

Preliminary testing. After preliminary screening, subjects performed two progressive exercise tests a minimum of 1 wk apart. All VO2peak, lactate threshold (LT), and exercise trials were performed on an electrically braked cycle ergometer (Monarch Ergometric 839E). To determine VO2peak and LT, subjects pedaled at a self-selected cadence at 100 W. Every 3 min, the workload was increased by 25 or 50 W until voluntary cessation. During the LT test, blood was collected from an antecubital vein during the last 30 s of each 3-min stage. Respiratory gases were collected and analyzed continuously by a real-time, online PC-based system (Ametek S-3A1 Ox and Ametek CD-3A CO2 analyzers). Finally, body composition was assessed by skin-fold measurements, as previously reported (17).

Study design. Subjects completed a series of trials pre- (PBE) and posttraining (PBE). Trials had rest (R) and exercise (E) components pretraining, as well as rest (R), exercise at the same absolute intensity (AB), and exercise at the same relative intensity (RB) posttraining. Additionally, during rest pre- and posttraining and exercise posttraining at absolute and relative intensities, an LC involving infusion of exogenous lactate was performed. In detail, after screening, subjects performed two isotope trials. In random order, subjects completed a rest-only trial with LC (PBE-LC) or PBE at 65% VO2peak preceded by a rest period with no LC (PBE). During PBE-LC, subjects were infused with a sodium lactate-lactic acid mixture (Lactate clamp) to a target of 4 mM. After the second tracer trial, subjects began training on a cycle ergometer 5 days/wk for 12 wk. At week 5, subjects performed another progressive exercise test to determine training-induced changes in VO2peak and LT. Although a 6-wk period of endurance training is sufficient to achieve a steady-state mitochondria content (24), posttraining trials were randomized to eliminate the possibility of training-induced changes after the final 4 wk. Posttraining, subjects performed one exercise trial at PBE and one trial at PBE. In addition, POAB and POAB trials were performed with blood [lactate] clamped at PBE level (PBE-LC and PORL-LC, respectively). The clamped exercise trials were preceded by a rest period with blood [lactate] clamped at PBE-LC concentrations.

Training protocol. Subjects were required to report to the laboratory 5 days/wk for 1 h of monitored cycle ergometer exercise training. Additionally, subjects were asked to train 1 day on the weekend with the activity of their choice. All training done in the laboratory was monitored by undergraduates currently or previously enrolled in the Department of Integrative Biology’s exercise physiology course. Subjects were weighed each day and were advised to adjust diet accordingly to minimize changes in weight. Training began at ~60% VO2peak as estimated by heart rate (fH) and the preliminary VO2peak test. Subjects gradually increased exercise intensity over the course of 2 wk until they were exercising at 75% VO2peak. Thereafter, subjects exercised at the fH corresponding to 75% VO2peak, as determined by a portable heart rate monitor (Polar USA, Woodbury, NY). At week 6, subjects performed another VO2peak test to monitor improvements in aerobic fitness. At week 6, interval training was added 2 days/wk to achieve maximal increases in VO2peak. Subjects continued to train through the final 4 wk, although each exercise trial was preceded by 1 day of rest.

Dietary controls. Three-day diet records were collected before, midway, and at the completion of the study to ensure consistent dietary habits throughout the experiment. Diet analysis was performed with Nutritionist III software (N-Squared Computing, Salem, OR). In addition to a pretraining, midpoint, and posttraining diet analysis, subjects’ diets were standardized the day before an isotope trial. Lunch and dinner were prepared by the laboratory staff and together consisted of 2,309 kcal (64% CHO, 23% fat, and 14% protein). At 2145 on the day before isotope trials, subjects consumed a standardized snack of 584 kcal (54% CHO, 30% fat, and 16% protein). The exact time the subjects finished the evening snack was recorded, and exercise commenced 12 h after the evening snack.

Isotope trials. Subjects reported to the laboratory at 0730 on the day of a trial. After a breath sample for determination of background CO2 enrichment, a catheter was inserted into a dorsal hand vein that was warmed for collection of an “arterialized” sample, as previously described (17). In the contralateral arm, a second catheter was placed in a forearm vein for isotope and unlabeled lactate/saline infusion. After a background blood sample was obtained, a primed continuous infusion of [6,6-2H2]glucose (D-glucose) and [1,13C]glucose (Cambridge Isotope Laboratories, Woburn, MA) began and represented time 0. Isotopes were diluted in 0.9% sterile saline and were pyrogenicity and sterility tested (University of California, San Francisco School of Pharmacy), and passed through a 0.2-μm Millipore filter (Nalgene, Rochester, NY) before infusion. The priming bolus contained 250 mg of each glucose isotope; continuous infusion, delivered via a peristaltic pump (Baxter Travolen 6300 infusion pump), was 2.0 mg/min during rest and 8.0 mg/min during exercise. For LC trials, an additional lactate/saline infusion was begun (see...
**Lactate clamp.** Subjects rested for a total of 90 min in a supine or semi-supine position. At the completion of rest, subjects were transferred to the cycle ergometer, where they exercised for 90 min. Exercise trials began exactly 12 h after the last meal.

**Lactate clamp.** Infusion cocktail was prepared by mixing a 30% L(+)-lactic acid solution (Sigma) in 2 N NaOH to a pH of 4.8. The lactate solutions were tested for pyrogenicity and sterility in the same manner as the isotope solutions. During the PrR-LC, LC began at 104 ml/h, which delivered lactate at 202.8 mg/min or roughly 2.6 mg·kg⁻¹·min⁻¹. Lactate infusion rates were increased or decreased to maintain target [lactate] as determined by a portable lactate monitor (Accusport, Hawthorne, NY). The infusion procedure was repeated during the resting period of PAA-LC and PCrR-LC. During exercise, lactate infusion was adjusted to match each individual subject's [lactate] encountered during the PrR trial. To match PAA [lactate], lactate infusion rates had to be decreased or stopped in order to account for the additive effect of endogenous lactate production at the initiation of exercise and [lactate] already elevated during resting LC.

**Sampling and analysis.** Blood for metabolite, isotope enrichment, and hormonal analyses was sampled at 0, 60, 75, and 90 min of rest and at 30, 45, 60, 75, and 90 min of exercise. Additional small aliquots of blood were sampled at 10, 20, 30, and 45 min of rest and at 10 and 20 min of exercise for blood [lactate] and hematocrit (Hct). Blood samples for metabolites, tracer enrichment, and hormones were immediately chilled on ice and centrifuged at 3,000 g for 18 min, and the supernatant was collected and frozen at −80°C until analysis. Blood for glucose isotopic enrichment (IE) and glucose and lactate concentrations was collected in 8% perchloric acid. Samples for free fatty acid (FFA) and glycerol concentrations were collected in a vacutainer containing EDTA. Insulin, glucagon, and cortisol were collected in a vacutainer containing EDTA and aprotinin. Hct was monitored at each time point and was used to correct for changes in plasma volume. Respiratory gases were analyzed for the 5 min before blood sampling. These gases were used for calculation of the volume of O₂ inspired per minute (VeO₂), the volume of CO₂ expired per minute (VeCO₂), exercise output (Ve), and respiratory exchange ratio (RER). During respiratory sampling, subjects indicated their rating of perceived exertion (RPE) by pointing to a Borg Scale.

Glucose concentration was measured in duplicate and triplicate using a hexokinase kit (Sigma Chemical, St. Louis, MO). Lactate concentrations were measured enzymatically in duplicate or triplicate (21). FFA and glycerol were measured enzymatically with commercially available kits (NEFA-C, Wako, Richmond, VA, and GPO-Trinder, Sigma). Insulin, glucagon, and cortisol were measured with commercially available radioimmunoassays ( Coat-A-Count, DPC, Los Angeles, CA).

**IE analysis.** For glucose isotopic analysis, the pentacacetate derivative was prepared. Briefly, glucose samples were neutralized with KOH and transferred to cation (AG 1-X8, 50 to 100 mesh H+ resin) and anion (AG1-X8, 100 to 200 mesh formate resin) exchange columns and eluted with doubly-deionized water. Samples were then lyophilized and resuspended in methanol, from which 200 μl were transferred to a 2-ml microreaction vial and blown off under a stream of nitrogen gas. One hundred microliters of a 2:1 acetic anhydride-pyridine solution were added to each vial and heated at 60°C for 10 min. Samples were again dried under nitrogen, resuspended in 200 μl of ethyl acetate, and transferred to microwavels for analysis.

Glucose enrichments were measured with a gas chromatography-mass spectrometer (GC-MS; GC model 5890, series II, and MS model 5890A, Hewlett-Packard, Palo Alto, CA) by use of positive chemical ionization. Injector temperature was set at 210°C and increased 5°C/min until 225°C was reached. Analysis was performed with a 35-to-1 ml/min splitless injection ratio with helium as the carrier gas. Transfer line temperature was set to 200°C, and source and quadrupole temperatures were 200°C and 115°C, respectively. Using selected ion monitoring, or SIM, ions 331, 332, and 333 were monitored representing M+0, M+1, and M+2, respectively.

Duplicate samples of expired air were collected in a 10-ml evacuated container for 13CO₂ IE at sampling points. Breath samples were analyzed by use of isotope ratio mass spectrometry, or IRMS, by Metabolic Solutions (Acton, Nashua, NH).

**Calculations.** Glucose Rₐ, Rₐ⁴, metabolic clearance rate (MCR), and Rₑ were calculated from the equations of Steele, modified for use with stable isotopes (41)

\[
Rₐ (mg \cdot kg⁻¹ \cdot min⁻¹) = (F - V[(C₁ + C₂)/2]) \times [(IE₂ - IE₁)(t₂ - t₁)]/[((IE₂ + IE₁)/2)]
\]

(1)

\[
Rₐ⁴ (mg \cdot kg⁻¹ \cdot min⁻¹) = Rₑ - [(V(C₁ - C₂)(t₂ - t₁)]
\]

(2)

\[
MCR (mg \cdot kg⁻¹ \cdot min⁻¹) = Rₑ/[(C₁ + C₂)/2]
\]

(3)

\[
Rₑ (mg \cdot kg⁻¹ \cdot min⁻¹) = Rₑ/[(C₁ + C₂)/2]
\]

(4)

where F represents the isotopic infusion rate (mg·kg⁻¹·min⁻¹); volume distribution (V) of glucose is equal to plasma volume (180 ml/kg); C₁ and C₂ are concentrations at sampling times t₁ and t₂; IE₁ and IE₂ are the excess isotopic enrichments of glucose; and Rₑ,H and Rₑ,C are glucose rates of appearance measured using D₃-glucose and [1-13]C-glucose, respectively. Measured IE values were corrected for background of blood samples taken before isotope infusion.

Glucose rate of oxidation (Rₒx) was calculated from expired CO₂

relative glucose oxidation (%) = [(IECO₂)(VCO₂)(100)]/(F × k)

(5)

where IEKO₂ is the isotopic enrichment of expired ¹³CO₂; VCO₂ is the volume of CO₂ expired per minute; F is the [1-¹³]C-glucose infusion rate; and k is the correction factor for the retention of CO₂ in body pools. Values for k were selected to be 0.7 for PrR and PAA, 0.67 for PrR-LC and PAA-LC, 0.78 for POAB-LC, 0.83 for POAB-LC, and 1.0 for POA1 and POA2. These values were chosen on the basis of another study in our laboratory (32) that examined the recovery of H²¹C₃O₂ during control and LC conditions.

Because of changes in plasma volume during LC, all metabolite concentrations were corrected for percent change in plasma volume (%ΔPV) according to the formula of Harrison (23). However, %ΔPV was small and did not affect calculations of glucose kinetics.

**Data analysis and statistics.** Data are presented as means ± SE. POₐR and POₐR-LC represent combined average resting values from POA1 and POA2, and from POAB-LC and POAB-LC, respectively. Metabolite, cardiovascular, and respiratory values are presented as the averages of the last 30 min of rest and exercise. Glucose kinetic values are presented as the averages of the last 15 min of rest and the last 30 min of exercise. We wished to explore the independent effects of training, intensity, and LC. All comparisons were made with
Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Pr</th>
<th>Po</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>27.8 ± 0.98</td>
<td>77.5 ± 2.4*</td>
<td>-1.4</td>
</tr>
<tr>
<td>Height, cm</td>
<td>70.9 ± 0.44</td>
<td>15.34 ± 1.6*</td>
<td>-7.9</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>78.6 ± 2.3</td>
<td>80.20 ± 2.2*</td>
<td>15.7</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>16.66 ± 1.7</td>
<td>300.00 ± 25*</td>
<td>20.0</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>0.80 ± 0.01</td>
<td>51.24 ± 2.2*</td>
<td>17.7</td>
</tr>
<tr>
<td>VO2peak, mg·kg⁻¹·min⁻¹</td>
<td>43.55 ± 1.9</td>
<td>51.24 ± 2.2*</td>
<td>17.7</td>
</tr>
<tr>
<td>VO2peak, l/min</td>
<td>3.45 ± 0.22</td>
<td>3.99 ± 0.20*</td>
<td>15.7</td>
</tr>
<tr>
<td>Watt max</td>
<td>250 ± 25</td>
<td>300 ± 25*</td>
<td>20.0</td>
</tr>
<tr>
<td>Lactate threshold</td>
<td>66 ± 1.7</td>
<td>74 ± 1.6*</td>
<td>12.1</td>
</tr>
<tr>
<td>%VO2</td>
<td>150 ± 0</td>
<td>200 ± 0*</td>
<td>33.3</td>
</tr>
<tr>
<td>Diet, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHO</td>
<td>53</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>Fat</td>
<td>30</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>Protein</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 9. Pr, pretraining; Po, posttraining; FEV, forced expiratory volume; FVC, forced vital capacity; VO2peak, peak oxygen consumption; CHO, carbohydrate. *Significantly different from pretraining values at P < 0.05.

RESULTS

Subject characteristics and dietary control. Subjects lost 1 kg and 1% body fat over 12 wk of endurance training (Table 1). VO2peak, maximum wattage, VO2 at LT, and power output at LT increased by 17.7, 20.0, 12.1, and 33.3%, respectively. Because of the training-induced increase in VO2peak, workloads at POAB corresponded to 55% of posttraining VO2peak. Additionally, according to 3-day diet records, percentages of calories from CHO, fat, and protein were unchanged after 12 wk of training (Table 1).

Lactate clamp. During PR-LC and PO-LC, blood lactate was significantly elevated above that of corresponding control trials (Fig. 1). Additionally, the concentration to which lactate was clamped during rest was not different between PR-LC and PO-LC. PRex, lactate increased to ~4.5 mM by 30 min and gradually decreased over time to ~3.5 mM. Additionally, both POAB-LC and POAB-LC were not significantly different from PRex at any time point (although at the 150-min time point, P = 0.053 for POAB-LC compared with PRex). At all time points POAB-LC and POAB-LC were significantly increased over POAB and POAB-LC, respectively. Finally, blood lactate during POAB and POAB-LC were significantly decreased compared with PRex at all time points except 150 and 165 min, when POAB-LC was not significantly decreased.

Work, ventilatory, and cardiovascular parameters. Subjects worked at an average of 141 W during PRex, POAB, and POAB-LC trials (Table 2). During POAB and POAB-LC trials, subjects worked at a significantly greater workload, 165 W. Lactate infusion was not significantly different between PR-LC and PO-LC. There was a significantly larger lactate infusion rate during the POAB-LC compared with the POAB-LC trial (Table 2).

Minute ventilation (VE) increased from rest to exercise (Table 2). POAB-LC was significantly decreased compared with PRex, whereas POAB was not. Additionally, POAB and POAB-LC were significantly increased compared with POAB and POAB-LC, respectively. There was an intensity effect on VO2peak and VO2 but no difference as a result of LC. RER decreased significantly during PR-LC and PO-LC compared with PR and PO, respectively. Slight changes in VE caused significant differences in VE/VO2 and VE/VCO2. Both ratios were significantly decreased during exercise with LC.

Training decreased resting fH (P ≤ 0.05) (Table 2). Additionally, fH was increased during POAB-LC compared with PR. During exercise, fHt was lower at POAB compared with POAB with and without LC. Additionally, at POAB, fHt was significantly decreased compared with POAB-LC. A fH difference was not noted at POAB.
Changes in $\dot{f}_1$ associated with LC had little effect on mean arterial pressure (MAP). All LC trials caused a significant drop in Hct compared with their appropriate control. Consequently, all metabolite concentrations were corrected for changes in PV. There was an intensity effect with RPE, but no difference due to LC procedure at any exercise intensity.

**Metabolite and hormone concentrations.** No differences were seen in free fatty acid concentrations ([FFA]) during rest (Table 3). After 90 min of exercise, [FFA] were significantly increased compared with rest at all intensities except POAB. Glycerol decreased significantly during rest after training (Table 3). From rest to exercise, [glycerol] increased in all trials. There was no effect of LC on [glycerol].

LC did not affect insulin during resting trials (Table 3). However, insulin levels during POAB-LC and PORI-LC were increased compared with P3EX, whereas POAB and PORI were not. Glucagon was only significantly decreased during POAB-LC compared with P3EX (Table 3). These changes resulted in significantly increased insulin-to-glucagon ratios (I/G) at POAB-LC and PORI-LC compared with P3EX (Table 3). Cortisol was significantly decreased during exercise after training at POAB-LC, POAB-PO, and PORI-LC intensities (Table 3).

**Blood glucose concentration and kinetics.** Glucose IE was steady over time for both glucose tracers (Fig. 2, A and B). As neither rest nor exercise had any significant changes in [glucose] (Fig. 3A), changes in PV did not affect calculation of glucose Ra.

Glucose Ra was not different in any condition during rest (Fig. 3B). Glucose Ra increased significantly from rest to exercise in all conditions. Additionally, POAB and POAB-LC were decreased compared with P3EX, whereas PORI and PORI-LC were not. Additionally, POAB-LC was significantly decreased compared with PORI-LC. Glucose Ra followed the same pattern (Fig. 3C). Glucose MCR was significantly increased from rest to exercise in all conditions (Fig. 3D). Again, POAB and POAB-LC were decreased compared with P3EX, and POAB-LC was decreased compared with PORI-LC.

### Table 2. Workload, lactate infusion rate, and cardiorespiratory parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rest</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PnR</td>
<td>PnR-LC</td>
</tr>
<tr>
<td>Workload, W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate infusion rate, mg kg(^{-1}) min(^{-1})</td>
<td>7.23 ± 0.38</td>
<td>7.11 ± 0.26</td>
</tr>
<tr>
<td>$V_\text{o}_2$, l min(^{-1})</td>
<td>0.27 ± 0.007</td>
<td>0.27 ± 0.007</td>
</tr>
<tr>
<td>$V_\text{CO}_2$, ml kg(^{-1}) min(^{-1})</td>
<td>3.39 ± 0.08</td>
<td>3.57 ± 0.11</td>
</tr>
<tr>
<td>FFA mmol l(^{-1})</td>
<td>0.3 ± 0.05</td>
<td>0.22 ± 0.007</td>
</tr>
<tr>
<td>LC did not affect insulin during resting trials</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Values are means ± SE; $n = 9$. PnR, pretraining rest; PoR, posttraining rest; P3EX, pretraining exercise; POAB, absolute workload; PORI, relative workload; LC, lactate clamp; Ve, minute ventilation; $V_\text{CO}<em>2$, volume expired carbon dioxide per minute; $V</em>\text{o}_2$, volume expired oxygen per minute; RER, respiratory exchange ratio; $f_1$, heart rate; MAP, mean arterial pressure; RPE, rating of perceived exertion; Hct, hematocrit. ‡ Significantly different from rest at $P &lt; 0.05$; ¤ significantly different from $P_3$ at $P &lt; 0.05$; § significantly different from corresponding control at $P &lt; 0.05$; † significantly different from corresponding POAB at $P &lt; 0.05$.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
During exercise, the percent glucose oxidized was decreased with low carbohydrate (LC) intensities. During rest, both before and after training, there were no significant differences in %oxidation of glucose between the groups. However, these differences were not noted at relative %oxidation of glucose (Fig. 4B). During exercise, 101 ± 3% of R4 was oxidized during PREX. After training, %R4 oxidized at POAB and POAB-LC was 95 ± 6 and 77 ± 5%, respectively. POAB-LC was significantly decreased compared with POAB. At POAB and POAB-LC, %R4 oxidized was 85 ± 6 and 82 ± 4%, and these were not significantly different from each other. Glucose Rr was not significantly different at any point during rest or exercise (Fig. 4C).

**Energy expenditure.** Because O2 consumption was not different between a control trial and its corresponding low carbohydrate (LC) trial, %energy, energy from CHO and lipid, and total energy were calculated using an LC trial's corresponding control (Table 4). However, energy from blood glucose was calculated from glucose Rox, whereas "other CHO" was calculated by means of energy from CHO minus glucose energy equivalents. A training effect was noted in %energy partitioning at POAB, but not POAB-LC, intensities. Energy from CHO was significantly lower at POAB intensities compared with PREX. Additionally, POAB intensities were greater than POAB-LC but not different from PREX. Energy from blood glucose was significantly decreased at POAB-LC compared with POAB. Energy from blood glucose was also significantly decreased between POAB and POAB-LC. Decreased energy from blood glucose was the result of an increased "other CHO" energy source to CHO energy supply.

**DISCUSSION**

We report first efforts to “clamp” blood [lactate] during rest and exercise in humans. Key findings are that 1) increased arterial [lactate] did not increase tracer-measured glucose Rr during rest or during exercise after training, 2) GP did not change with increased precursor supply during rest or exercise, and 3) infusion of exogenous CHO in the form of lactate resulted in the decrease of glucose Rox. Also, lactate infusion during exercise did not affect measures of physiological or perceived stress.

**Training adaptations.** Observed training effects were as expected on the basis of previous findings (3, 17). After training, resting fH decreased significantly (Table 2). V02peak, maximum wattage, V02 at LT, and watts at LT increased by 17.7, 20, 12.1, and 33.3%, respectively (Table 1). Additionally, workload at 65% V02peak increased by 18% over PREX workload (Table 1). Finally, blood [lactate] decreased at both POAB (55% of Preme) and POAB-LC (24% of Preme) (Fig. 2). Taken together, these data indicate that our subjects received a significant training stimulus.

**Dietary controls.** Because we were interested in examining the effect of training on GNG, we chose to maximize GNG conditions and study subjects in a 12-h postabsorptive condition. Because the time since last feeding affects contribution to GNG (38), it is important to note that all subjects started their rest protocol at 10.5 h postfeeding and started exercise exactly 12 h postfeeding. Thus we were able to eliminate time since feeding as a variable in our measures of glucose Rr. Our subjects consumed a mixed diet, which did not change macronutrient composition over the course of 12 wk (Table 1).

**Lactate clamp.** We were successful at varying and holding [lactate] during periods of rest and exercise (Fig. 1). Our variable lactate infusion method resembled a glucose clamp, whereby lactate infusion rates were adjusted on the basis of measured concentration. Furthermore, the procedure did not require infusion of insulin, somatostatin, or other counterregulatory hormones. Our procedure differs from that of other investigators who have raised [lactate] during rest with a primed continuous infusion (1, 5) or a prolonged continuous infusion (9, 22, 26, 27, 34, 36). We are the first to attempt clamping [lactate] to a predetermined blood lactate curve during exercise. As previously demonstrated (4, 35), at the onset of exercise there is a net...
release of lactate at the muscle, and as exercise continues, net release decreases until it is not different from zero. The rate of decrease of the net release is intensity dependent. Thus, at the onset of exercise, exogenous lactate infusion was low, or in some cases at PORL-LC, ceased. As exercise continued, lactate infusion increased to maintain blood [lactate]. Thus, at POR intensity, the average exogenous lactate infusion was decreased compared with POAB intensity (1.74 compared with 4.08 mg·kg$^{-1}$·min$^{-1}$; Table 2).

Cardiovascular and respiratory parameters. Lactate clamping caused a slight hypoventilation, as indicated by decreases in $\dot{V}_{E)/\dot{V}_{O_2}}$ and $\dot{V}_{E)/\dot{V}_{CO_2}}$ (Table 2). Associated with hypoventilation was a decreased RER during resting LC that in some subjects decreased below 0.7. The finding of a decreased RER due to lactate infusion has been previously reported (9, 16, 34, 36). We believe that these changes are associated with a changing acid-base status as a result of lactate infusion (BF Miller, MI Lindinger, JA Fattor, KA Jacobs, F Navazio, and GA Brooks, unpublished observations). Hct decreased with LC, which indicates an increased PV (Table 2). Additionally, $f_H$ was increased during POAB-LC and POAB-LC compared with their appropriate controls, but not at POR-LC (Table 2). Despite these differences, MAP was maintained in all trials, indicating an unchanged pressor response. Finally, RPE was not different between LC and non-LC trials (Table 2). Together, the MAP and RPE data indicate that the physiological or perceived stress was not different with LC.

Glucose kinetics. An increase in GNG precursors in the form of lactate (22, 26, 27), alanine (42), or glycerol (37) does not change GP during resting conditions. Additionally, it has been demonstrated that increased levels of glycerol during exercise do not increase GP (37). We separately studied the effects of LC during rest and exercise after training. Blood [glucose] was maintained in all conditions before and after training (Fig. 3A). Additionally, glucose $R_a$ and $R_d$ were decreased after training at POAB but not at POR (Fig. 3, B and C). Results are in agreement with previous studies from our laboratory, which indicate that glucose kinetics scale to relative exercise intensity (3, 17). LC did not affect glucose $R_a$, $R_d$, or MCR at any intensity compared with its appropriate control (Fig. 3, A, B, and C). Thus these data extend the current knowledge of the effect of precursor supply on GP (1, 25–27, 37, 42). Specifically, an infusion of exogenous lactate equivalent to $P_E{\text{[lactate]}}$ during exercise at the same POAB and POR intensities does not change GP.
Energy lipid, kcal/min 0.51
Other CHO, kcal/min 0.62

Glucose recycling. After training men and studying them at the same exercise power output as before training, Coggan et al. (10) and MacRae et al. (31) found decreased and unchanged GNG rates, respectively. However, by the incorporation of [13C]lactate into glucose, Bergman et al. (3) found an increase in GNG rate at both POAB and PORL exercise intensities. We attempted to measure training-induced increases in GNG capacity by use of the glucose Rr technique.

To measure glucose Rr, the Rr of [13C]glucose is subtracted from the Rr of D2-glucose. The theory is that, after passing through glycolysis, the heavy hydrogen atoms of D2-glucose are lost irreversibly to the body’s water pool, whereas the [13C]glucose can incorporate into lactate and recycle to glucose. Thus the difference between the Rr values of the two tracers is an indirect measure of GNG (41). Our data on glucose Rr are in the same range as previously reported values in humans (17, 28, 37). However, values were unchanged from rest to exercise and unchanged during exercise conditions (Fig. 4C). Although GNG, measured as glucose Rr, did not increase with increased precursor supply, there may be confounding factors. Recently Trimmer et al. (37) demonstrated that the

Table 4. Percent energy expenditure

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P0R</td>
<td>P0R-LC</td>
</tr>
<tr>
<td>% Energy CHO</td>
<td>60 ± 5.1</td>
<td>60 ± 4.9</td>
</tr>
<tr>
<td>% Energy lipid</td>
<td>40 ± 5.1</td>
<td>40 ± 4.9</td>
</tr>
<tr>
<td>Energy CHO, kcal/min</td>
<td>0.81 ± 0.06</td>
<td>0.81 ± 0.06</td>
</tr>
<tr>
<td>Energy from blood glucose, kcal/min</td>
<td>0.18 ± 0.02</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td>Other CHO, kcal/min</td>
<td>0.62 ± 0.06</td>
<td>0.67 ± 0.06</td>
</tr>
<tr>
<td>Energy lipid, kcal/min</td>
<td>0.51 ± 0.07</td>
<td>0.51 ± 0.07</td>
</tr>
<tr>
<td>Total energy, kcal/min</td>
<td>1.32 ± 0.03</td>
<td>1.32 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>P0EX</td>
<td>P0AβI</td>
</tr>
<tr>
<td>% Energy CHO</td>
<td>72 ± 3.3</td>
<td>62 ± 2.6*</td>
</tr>
<tr>
<td>% Energy lipid</td>
<td>28 ± 3.3</td>
<td>38 ± 2.6*</td>
</tr>
<tr>
<td>Energy CHO, kcal/min</td>
<td>8.50 ± 0.63</td>
<td>6.77 ± 0.5*</td>
</tr>
<tr>
<td>Energy from blood glucose, kcal/min</td>
<td>2.26 ± 0.16</td>
<td>1.63 ± 0.21*</td>
</tr>
<tr>
<td>Other CHO, kcal/min</td>
<td>6.24 ± 0.5*</td>
<td>5.13 ± 0.41</td>
</tr>
<tr>
<td>Energy lipid, kcal/min</td>
<td>3.15 ± 0.44</td>
<td>3.87 ± 0.41</td>
</tr>
<tr>
<td>Total energy, kcal/min</td>
<td>11.64 ± 0.71</td>
<td>10.64 ± 0.8*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 9. ‡significantly different from rest at P < 0.05; *significantly different from P0R at P < 0.05; §significantly different from corresponding control at P < 0.05; †significantly different from corresponding P0Aβ at P < 0.05.

AJP-Endocrinol Metab • VOL 283 • NOVEMBER 2002 • www.ajpendo.org
infusion of a supraphysiological concentration of glycerol completely inhibits glucose $R_g$. Those results were consistent with the idea that the increase of one precursor (glycerol) decreases the contribution of other precursors (e.g., alanine and lactate) to GNG (25, 42).

An important consideration, however, is the entry of substrate into the GNG pathway. Glycerol enters GNG at the level of the triose phosphate pool; in contrast, lactate enters the pathway as phosphoenolpyruvate after oxidation to pyruvate. Thus, by mass action, glycerol can functionally inhibit upstream reactions, whereas lactate cannot.

Furthermore, the dilution of precursor $^{13}$C enrichment during GNG must be considered. In our LC trials, we created a situation in which the size of the lactate pool increased (Fig. 1). Thus, when $P_{\text{OAB}}$ was compared with $P_{\text{OAB-LC}}$, or $P_{\text{R}}$, with $P_{\text{R-LC}}$, it is possible that the $^{13}$C label from glucose was diluted in a larger lactate pool, resulting in decreased $R_g$ of the $[^{13}$C$]glucose. This possibility is supported by a separate study on different subjects, in which we (BF Miller, JA Fattor, KA Jacobs, F Navazio, and GA Brooks, unpublished observations) infused $[3-{^{13}$C}$]lactate and observed increased $^{13}$C incorporation into glucose during LC trials compared with control.

**Glucose and alternative substrate oxidation rates.** Our data provide insight into the importance of lactate in CHO metabolism during rest and exercise. During exercise, lactate oxidation is proportional to [lactate] and can account for as much as 17% of muscle CHO oxidation and 26% of whole body CHO (4). Again, LC resulted in changes in respiratory parameters; however, if we assume that the overall CHO oxidation does not change during LC, we can calculate CHO partitioning (Table 4). We observed a significant decrease in glucose $R_{\text{ox}}$ at $P_{\text{R-LC}}$ and $P_{\text{OAB-LC}}$ compared with their non-LC controls (Fig. 4A and Table 4). Additionally, $P_{\text{R-PC}}$, $P_{\text{R-LC}}$, and $P_{\text{OAB-LC}}$ had significant decreases in the percent glucose $R_g$ oxidized compared with their non-LC controls (Fig. 4B). Thus raising blood [lactate] by exogenous infusion increased lactate and suppressed glucose oxidation by mass action (7). These results are consistent with those of Lombardi et al. (30), who have demonstrated in rats that chronic lactate infusion can reduce glucose uptake.

There are two possible fates for glucose in this scenario. The first is storage as glycogen. It is thought that muscle glycogen cycles during rest and exercise (2); thus there could be an increased uptake of the blood glucose into muscle glycogen (33) with lactate infusion. Second, glucose could be left in the circulation. It is likely that glucose sensors in the body monitor glucose concentration (13). However, the actual content of the blood glucose during LC is significantly higher than during control because of plasma volume expansion with LC (Fig. 3A). Thus, although the kinetics were not different, the amount of glucose in the blood had increased significantly.

Finally, it is interesting to note that the effect of LC on glucose $R_{\text{ox}}$ is exercise intensity dependent. During $P_{\text{R-LC}}$, $P_{\text{R-PC}}$, and $P_{\text{OAB-LC}}$, we observed a decreased $R_g$ oxidized but not at $P_{\text{R-LC}}$ (Fig. 4B).

Decreased glucose oxidation in men during LC exercise at low intensity is consistent with results on isolated rat muscle, in which infusion of lactate decreased glycolytic flux and glucose $R_{\text{ox}}$ and increased glycogen synthesis (33). However, with both humans and isolated rat muscle, increased exercise intensity and, consequently, increased contraction-stimulated glucose uptake, there is increased flux of glucose units through glycolysis independent of substrate availability.

**Lipid mobilization and hormonal concentration.** Hard exercise that produces a rise in blood [lactate] is accompanied by a suppression of the rise in FFAs, but not necessarily glycerol (18). Hence, it has been suggested that acidosis, which corresponds to increased glycolysis and lactate accumulation, either suppresses lipolysis or promotes reesteryification. In our study, FFA and glycerol concentrations did not change significantly as a result of LC during rest, $P_{\text{OAB}}$, or $P_{\text{R}}$ (Table 3). Thus we conclude that any effect of lactate or acidosis on lipolysis or reesterification is attributable to protons, and not lactate anions.

In the present study, there was no significant difference in [glucagon] with LC (Table 3), which is in agreement with previous studies during rest (16, 26, 27, 36) and exercise (5). Differences in [insulin] and I/G between control and LC approached, but did not reach, significance. However, $P_{\text{OAB-LC}}$ and $P_{\text{R-LC}}$ were significantly greater than $P_{\text{R-EX}}$, whereas $P_{\text{OAB}}$ and $P_{\text{R}}$ were not. Thus there was a differential response of insulin secretion and, consequently, I/G, in response to LC. These results are interpreted to mean that lactate or some parameter of acid-base status resulted in increased pancreatic $\beta$-cell secretion despite constant [glucose].

**Summary and conclusions.** Ours is the first study to employ a lactate clamp on men before and after training during rest and exercise. With infusion of a sodium lactate-lactic acid cocktail sufficient to raise and hold blood lactate concentration, cardiovascular and respiratory measures changed slightly due to an acid-base disturbance; however, the physiological and perceived stress did not change. Additionally, glucose recycling and glucose kinetics did not change, confirming that gluconeogenic substrate load does not affect glucose production. Finally, at the site of carbohydrate disposal, another carbohydrate source, which was likely lactate, was preferentially oxidized at rest and at lower exercise intensities after training.

First, we thank the subjects for their time and effort during all aspects of the study. We also thank the student trainers for their work during the training period, and Dr. Cindy Chang for assistance during the pilot trials. Finally, we are especially thankful to Peter Lee, Jean Lin, Jenny Rodriquez, and Nellie Taleux for their laboratory assistance.

This work was supported by National Institute of Arthritis and Musculoskeletal and Skin Diseases Grant AR-42906.

**REFERENCES**


