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Regulation of Glycemia and Energy Substrate Partitioning in Exercising Humans

By

Chi-An Wang Emhoff

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Integrative Biology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor George A. Brooks, Chair
Professor Steven L. Lehman
Professor Gregory W. Aponte

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Regulation of Glycemia and Energy Substrate Partitioning in Exercising Humans

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Abstract

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Doctor of Philosophy in Integrative Biology

University of California, Berkeley

Professor George A. Brooks, Chair

To gain a more complete understanding of glycemic regulation and energy substrate metabolism during exercise, we examined the effects of endurance training, exercise intensity, and plasma lactate concentration on gluconeogenesis (GNG), hepatic glycogenolysis (GLY), whole body lactate turnover, and direct versus indirect lactate oxidation rates in fasted men exercising at and just below the lactate threshold (LT), where GNG precursor lactate availability is high. The lactate threshold, characterized as the onset of rapid blood lactate accumulation during incremental exercise, marks the transition between steady and non-steady state lactate turnover. We studied six untrained (UT) and six trained (T) subjects during 60-min exercise bouts at power outputs (PO) eliciting the LT. Trained subjects performed two additional exercise bouts at a PO 10% lower (LT-10%), one of which involved a lactate clamp (LC) to match blood lactate concentration ([lactate]$_b$) to that achieved during the LT trial. Flux rates were determined by primed continuous infusion of [6,6-^{2}H$_2$]glucose, [3-^{13}C]lactate, and [^{13}C]bicarbonate tracers during 90 min of rest and 60 min of cycling. Exercise at LT corresponded to 67.6 ± 1.3 and 74.8 ± 1.7 % of peak oxygen consumption (VO$_{2peak}$) in the untrained and trained subjects, respectively ($P < 0.05$). Relative exercise intensity was matched between the untrained group at LT and the trained group at LT-10%, and [lactate]$_b$ during exercise was matched in the LT and LT-10%+LC trials via exogenous lactate infusion. We found that increasing [lactate]$_b$ in the LT-10%+LC trial significantly increased GNG (4.4 ± 0.9 mg·kg^{-1}·min^{-1}) compared to its corresponding LT-10% control (1.7 ± 0.4 mg·kg^{-1}·min^{-1}, $P < 0.05$). Hepatic GLY was higher in T than UT subjects, but not significantly different across conditions. At LT, lactate rate of appearance (R$_a$) was nearly doubled in T than UT subjects (24.1 ± 2.7 vs. 14.6 ± 2.4 mg·kg^{-1}·min^{-1}, respectively, $P < 0.05$), but R$_a$ was not different between UT and T when relative exercise intensities were matched at 67% VO$_{2peak}$. In all trials, [lactate]$_b$ remained constant during exercise, confirming the equivalent rates of lactate appearance and disposal (R$_d$). At LT, metabolic clearance rate (MCR), defined as the ratio of R$_d$/[lactate]$_b$, in T was 34% higher than in UT (62.5 ± 5.0 vs. 46.5 ± 7.0 ml·kg^{-1}·min^{-1}, respectively, $P < 0.05$), and a 10% reduction in PO resulted in a 46% increase in MCR at LT-10% (91.5 ± 14.9 ml·kg^{-1}·min^{-1}, $P < 0.05$), suggesting a lactate clearance limitation at LT. Total lactate oxidation rate (R$_{ox}$) was higher at LT in T (22.7 ± 2.9 mg·kg^{-1}·min^{-1}, 75% VO$_{2peak}$) compared to UT (13.4 ± 2.5 mg·kg^{-1}·min^{-1}, 68%

VO$_{2peak}$, $P < 0.05$). Increasing [lactate]$_b$ significantly increased lactate R$_{ox}$ compared to its corresponding LT-10% control (27.9 ± 3.0 vs. 15.9 ± 2.2 mg·kg^{-1}·min^{-1}, respectively, $P < 0.05$). We partitioned lactate R$_{ox}$ into its direct versus indirect (glucose that is gluconeogenically derived from lactate and subsequently oxidized) components. Direct and indirect lactate oxidation rates increased significantly from rest to exercise and their relative partitioning remained relatively constant in all trials, but differed between T and UT: direct oxidation comprised 75% of total lactate oxidation in UT and 90% in T suggesting the presence of training-induced adaptations. We conclude that *i)* endurance training increases the work capacity at the lactate threshold without a significant decrease in gluconeogenesis, *ii)* gluconeogenesis during exercise can be augmented by increased precursor delivery, *iii)* the lactate threshold represents a limitation in lactate clearance, *iv)* endurance training increases direct oxidation of lactate (90% in trained vs. 75% in untrained), regardless of activity level, suggesting underlying training-induced adaptations independent of exercise parameters, and *v)* exogenous lactate infusion during exercise spares muscle glycogen utilization.

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CHAPTER 1

Introduction

Prolonged exercise at moderate to high intensities imposes a challenge for glycemic homeostasis, as carbohydrate stores are limited yet preferentially utilized over lipids (5). Glycogenolysis in exercising muscle is the primary energy source for fueling contractile activity (14), while hepatic glycogenolysis and gluconeogenesis (GNG) replenish blood glucose levels in the circulation as glucose uptake persists. As the duration and intensity of exercise increase, a key player that emerges within the dynamics of glycemic regulation and energy substrate metabolism is lactate.

An important oxidative fuel and gluconeogenic precursor, lactate and its turnover in the blood have become a topic of interest for exercise physiologists, sports medicine practitioners, coaches and athletes alike. Specifically, the onset of blood lactate accumulation, also termed the lactate threshold (LT), at higher absolute and relative workloads is one of the hallmark adaptations of endurance training (7, 12, 13, 15). Characterized during incremental exercise as the last stage of the slow linear increase in lactate concentration before the rapid acceleration in lactate accumulation, the LT is thought to represent a transition between steady and non-steady state lactate kinetics. Stable isotope tracer studies in humans undergoing an incremental exercise test have shown that at workloads above the LT, lactate rates of appearance (R_a) exceed rates of disposal (R_d) (18). The subsequent accumulation of lactate in the circulation is associated with the predominance, if not to say exclusivity, of carbohydrate fuel sources over lipids for oxidation (5).

Endurance training has been shown to exert a profound effect on the LT through whole body adaptations (2, 3, 11, 16, 19). The LT typically occurs at workloads corresponding to ~60-65% of maximal oxygen consumption (VO_{2max}) in untrained individuals, whereas highly trained endurance athletes may exercise at intensities as high as ~80-85% VO_{2max} before reaching their LT (7, 12, 15). Intramuscular lactate production is determined by glycolytic flux rates, where working muscle accounts for the majority of whole body lactate production (1, 2, 6, 14). The appearance and disposal of lactate in the circulation largely depend on the exercise intensity and oxidative capacity of the individual. Previous studies demonstrating evidence for cell-cell and intracellular lactate shuttles (4) in exercising humans have shown that endurance training triggers changes in lactate flux, most importantly increasing oxidative capacity for lactate clearance (2, 8, 11, 17). These adaptations delay the onset of blood lactate accumulation to higher absolute workloads and relative intensities (10, 12, 13), although the lactate concentration at which the LT occurs changes very little (9, 10).

While glycemic regulation during exercise and the LT are two areas that have been frequently studied, there is surprisingly little data on metabolic flux rates during exercise at the LT. Specifically, lactate-glucose interactions relating to GNG and partitioning of direct and indirect lactate oxidation during exercise in humans at workloads eliciting the LT, to our knowledge, have not been previously studied. In a pilot investigation conducted by our group (Messonnier LA, Emhoff CW, Johnson ML, Carlson TJ, and Brooks GA, unpublished observations), we found that highly trained endurance athletes were able to complete 60 min of constant load exercise at their LT, permitting the use of stable isotope tracers to perform measurements on substrate kinetics. Therefore, the purpose of this dissertation work was to characterize the individual and combined effects of endurance training, exercise intensity, and blood lactate concentration on glucose production, lactate turnover, carbohydrate oxidation, and

energy metabolism in humans. Findings from this study offer a comprehensive understanding in the dynamic relationships between glucose and lactate turnover in the circulation. The use of a ^{13}C tracer allows a direct measurement of gluconeogenesis from lactate, which becomes increasingly relevant during prolonged exercise at intensities that elicit high blood lactate concentrations. Distinct differences in GNG capacity and lactate oxidation between untrained and trained populations further our understanding of the apparent enhancements in exercise performance following endurance training. Additionally, the incorporation of a lactate clamp component in the study design offers unique insight into the specific roles of lactate as an energy substrate and GNG precursor.

In the subsequent chapters, I highlight the role of lactate in gluconeogenesis for glycemic regulation during rest and exercise in the overnight-fasted state (Chapter 2), the effects of endurance training on whole body lactate flux rates during exercise at the lactate threshold (Chapter 3), and the partitioning of lactate oxidation via direct and indirect routes (Chapter 4). Finally, I summarize the results and conclusions from these studies in aggregate and offer suggestions for future research (Chapter 5), including studies that may promote advancements in our understanding of energy substrate metabolism in humans.

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CHAPTER 2

Gluconeogenesis and Hepatic Glycogenolysis during Exercise
at the Lactate Threshold

ABSTRACT

Because the maintenance of glycemia is essential during prolonged exercise, we examined the effects of endurance training, exercise intensity, and blood lactate concentration ([lactate]$_b$) on gluconeogenesis (GNG) and hepatic glycogenolysis (GLY) in fasted men exercising at and just below the lactate threshold (LT), where GNG precursor lactate availability is high. Twelve healthy men (6 untrained, 6 trained) completed 60 min of constant load exercise at power outputs corresponding to their individual LT. Trained subjects completed two additional 60-min sessions of constant load exercise, one at 10% below the LT workload (LT-10%), and the other with a lactate clamp (LT-10%+LC) to match the [lactate]$_b$ of the LT trial. Flux rates were determined by primed continuous infusion of [6,6-^{2}H$_2$]glucose, [3-^{13}C]lactate, and [^{13}C]bicarbonate tracers during 90 min of rest and 60 min of cycling. Exercise at LT corresponded to 67.6 ± 1.3 and 74.8 ± 1.7 %VO$_{2peak}$ in the untrained and trained subjects, respectively ($P < 0.05$). Relative exercise intensity was matched between the untrained group at LT and the trained group at LT-10%, and [lactate]$_b$ during exercise was matched in the LT and LT-10%+LC trials via exogenous lactate infusion. Glucose kinetics (R$_a$, R$_d$, and MCR) were augmented with the lactate clamp. GNG was decreased in the trained subjects exercising at LT and LT-10% compared to the untrained subjects, but increasing [lactate]$_b$ in the LT-10%+LC trial significantly increased GNG (4.4 ± 0.9 mg·kg^{-1}·min^{-1}) compared to its corresponding control (1.7 ± 0.4 mg·kg^{-1}·min^{-1}, $P < 0.05$). Hepatic GLY was higher in the trained than untrained subjects, but not significantly different across conditions. We conclude that GNG plays an essential role in maintaining total glucose production during exercise in fasted men, regardless of training state. However, endurance training increases the ability to achieve a higher relative exercise intensity and absolute power output at the LT without a significant decrease in GNG. Further, raising systemic precursor substrate availability increases gluconeogenesis during exercise but not at rest.

INTRODUCTION

Blood glucose homeostasis is essential during prolonged periods of endurance exercise when large changes occur in tissue oxygen delivery and use, metabolic rate, carbohydrate (CHO) oxidation, blood glucose disposal and hepatic plus renal glucose production. However, compared to the extensive literature on cardiovascular regulation during exercise, the regulation of glucose production for the maintenance of glycemia during exercise in fasted humans has been minimally studied.

Following an overnight fast, gluconeogenesis (GNG) provides 25-50% of total glucose production in resting humans (4, 12, 13, 31, 53), while the remainder is supported by hepatic glycogenolysis (GLY). This relative partitioning of glucose rate of appearance relies more on GNG as the fast progresses and hepatic glycogen stores become depleted (1, 50, 55). Staehr et al. (48) studied glucose metabolism in resting humans after fasting for 40 h, and GNG accounted for nearly 90% of total glucose production. During submaximal exercise, energy demand requires muscle glucose utilization and consequently increases blood glucose disposal (9). Therefore, a consistent glucose delivery into the circulation becomes paramount for preventing hypoglycemia. In contrast to rest, the relative contribution of GNG to total glucose production has been shown to decrease during hard exercise (53); for instance % GNG decreases at power outputs (PO) approaching the lactate threshold (LT). Because hepatic glycogen reserves are partially depleted during the course of an overnight fast (41), the combination of limited hepatic GLY and attenuated GNG has been shown to result in falling blood glucose levels in post-absorptive men after 75-90 min of hard (65% VO_{2max}) exercise (53).

Because lactate is the primary precursor for GNG in the liver and to a lesser extent the renal cortex (2, 16, 38), we hypothesized that increasing lactate availability would increase the rate of GNG, and possibly also the rate of glucose appearance in post-absorptive men during exercise near the LT. For the purpose of testing these hypotheses, we utilized stable isotope tracers and a cross-sectional design to study the maintenance of glycemia in overnight-fasted untrained and trained men at rest and during 60 min of constant load exercise at or just below the PO eliciting the LT. This workload was selected as the target exercise intensity because the LT is widely used in exercise science and sports medicine as a measure of athletic prowess, and, as well, because exercise at the LT results in an elevated lactate (gluconeogenic precursor) supply. Additionally, beyond conducting studies at and just below the LT, to evaluate the effect of precursor concentration on GNG and total glucose production, we also incorporated a lactate clamp component in the experimental design.

METHODS

This manuscript is part of a larger investigation of lactate and glucose metabolism during exercise at the lactate threshold. Some of the data are to be reported elsewhere (37), but for the convenience of the readers, methods are repeated here.

Subjects
Twelve healthy male subjects (6 untrained and 6 trained) aged 19-33 yr were recruited from the University of California Berkeley campus and the surrounding community by posted

notices, word of mouth, and email. The untrained subjects were recreationally active and considered untrained if their peak oxygen consumption (VO_{2peak}) <50 ml·kg^{-1}·min^{-1}. Trained subjects were members of competitive cycling or triathlon teams, currently in the race phase of their training season, and considered well-trained if they had a VO_{2peak} of >55 ml·kg^{-1}·min^{-1}. Subjects were included in the study if they had a body mass index of ≥18 and <26 kg/m^2, were non-smokers, were diet and weight stable, had a 1-s forced expiratory volume of >70% of vital capacity, and were injury/disease free as determined by physical examination by a physician. This study was approved by the University of California Berkeley Committee for the Protection of Human Subjects (CPHS 2010-4-1300) and conformed to the standards set by the Declaration of Helsinki. All subjects gave written informed consent prior to participation in the study.

Preliminary Testing

Exercise tests were performed on an electronically braked leg cycle ergometer (Monark Ergometric 839E, Vansbro, Sweden) and were conducted at least one week apart. Following interviews and screening, subjects performed two graded exercise tests to determine VO_{2peak} and lactate threshold (LT). To determine VO_{2peak}, as per American College of Sports Medicine guidelines (7th ed.), exercise power output (PO) started at 75 or 120 W and was increased by 25 or 30 W for the untrained and trained subjects, respectively, every 3 min until volitional fatigue. Expired respiratory gases were continuously monitored throughout the test via an open-circuit automated indirect calorimetry system (ParvoMedics TrueOne Metabolic System, Salt Lake City, UT) that was calibrated using room air and a certified calibration gas. Finger pricks drawing 10 μl of blood were conducted at the end of each stage to measure lactate concentration via portable lactate analyzers (Nova Lactate Plus, Waltham, MA) and to approximate the PO eliciting the LT.

To determine LT, a second graded exercise test started at 50 W below the approximated LT power output and increased by 10 W every 3 min until volitional exhaustion. At the end of every stage, 1 ml of blood was drawn from an arterialized hand vein for enzymatic analysis of blood lactate concentration ($[lactate]_b$), which increased linearly with exercise work rate until a certain PO. After this point, a rapid acceleration in blood lactate accumulation occurred. The LT was considered to be the last stage of the slow linear increase in $[lactate]_b$ before the rapid acceleration in lactate accumulation. This definition of the LT is termed by some as the second lactate turn point (27), and approximates the maximal lactate steady state (MLSS) (6). For both graded exercise tests, heart rate was monitored continuously using a heart rate monitor (Polar, Gay Mills, WI) and electrocardiography (Quinton 759 ECG, Seattle WA), rating of perceived exertion (RPE) was recorded according to the Borg scale (7), and blood pressure was measured at the middle of every stage by manual auscultation.

Following graded exercise tests, subjects performed 60 min of continuous exercise at the PO corresponding to their LT to ensure stabilization of $[lactate]_b$ over the entire duration of the exercise test. Every 10 min, finger pricks drawing 10 μl of blood were conducted to measure $[lactate]_b$, and heart rate and RPE were monitored.

Experimental Design

The study design consisted of four conditions using stable isotope tracers: one condition within the untrained group, and three conditions within the trained group. Untrained subjects (UT) completed one isotope infusion trial, consisting of a 90-min rest period followed by 60 min of continuous cycling at the LT. Trained subjects completed three isotope infusion trials,

each consisting of a 90-min rest period followed by 60 min of continuous leg ergometer cycling under one of the following conditions: *1)* PO eliciting the lactate threshold (LT), *2)* PO 10% below that eliciting the LT (LT-10%), and *3)* PO 10% below that eliciting the LT, but with [lactate]$_b$ raised to the LT level via exogenous lactate infusion, i.e., clamp (LT-10%+LC). The order of the last two conditions was randomized, and all exercise trials were conducted at least one week apart.

To evaluate the effects of training on metabolic responses, we compared: untrained and trained groups exercising at the same [lactate]$_b$, but different absolute and relative intensities (UT vs. LT); at the same relative intensity, but different [lactate]$_b$ (UT vs. LT-10%); and at the same relative intensity and [lactate]$_b$ (UT vs. LT-10%+LC). Within the trained subjects, we investigated: the effects of exercise intensity given the same [lactate]$_b$ (LT vs. LT-10%+LC); the effects of [lactate]$_b$ given the same exercise intensity (LT-10% vs. LT-10%+LC); and the combined effects of [lactate]$_b$ and exercise intensity (LT vs. LT-10%).

Dietary Controls

Three-day diet records were collected prior to the study to record subjects' caloric intake and macronutrient composition (DietAnalysis Plus, version 6.1 ESHA Research, Salem, OR). Standardized diets (approximately 50% CHO, 30% fat, 20% protein) consisting an average of 2400 and 3200 kcal for the untrained and trained subjects, respectively, were given the day prior to each exercise trial, including an evening snack as the last meal. Subjects came to the laboratory overnight-fasted, and exercise commenced 12 h after consuming the evening snack.

Isotope Tracer Protocol

Subjects reported to the laboratory on the morning of each tracer trial, and expired respiratory gases were sampled for a measurement of background $^{13}CO_2$ enrichment. A catheter was placed into a warmed hand vein for "arterialized" blood sampling, and a background sample was collected. After a second catheter was placed in the antecubital vein of the contralateral arm for infusion of stable isotope tracer solutions, subjects received a primed continuous infusion of $[6,6\text{-}^2H_2]$glucose (i.e., D$_2$-glucose) and $[3\text{-}^{13}C]$lactate (Sigma-Aldrich, St. Louis, MO) while resting semi-supine for 90 min. Isotopes were diluted in 0.9% sterile saline and were tested for pyrogenicity and sterility (School of Pharmacy, University of California, San Francisco, CA) and passed through a 0.2-μm Millipore filter (Nalgene, Rochester, NY) prior to infusion. Priming boluses were the same across trials and contained 250 mg D$_2$-glucose, 57.5 mg $[3\text{-}^{13}C]$lactate, and 136 mg $[^{13}C]$bicarbonate (Isotec, Sigma-Aldrich, St. Louis, MO). Tracers D$_2$-glucose and $[3\text{-}^{13}C]$lactate were then continuously infused for the 90-min rest period via a pump (Baxter Travenol 6300) at 2.0 mg/min for glucose and 2.5 mg/min for lactate in all trials, with the exception of the LT-10%+LC trial that had a resting infusion rate of 7.5 mg/min for lactate. The LT-10%+LC trial also included infusion of a lactic acid-sodium hydroxide mixture (see Lactate Clamp Procedures) to begin raising [lactate]$_b$ during the rest period to the level seen in exercise at the PO eliciting the LT. At the start of all exercise trials, tracer infusion rates were increased to 8 mg/min for glucose and 11.25 mg/min for lactate for the untrained subjects and 10 mg/min for glucose and 15 mg/min for lactate for the trained subjects, and continued for 60 min of exercise. These infusion rates were selected based on prior experience to maintain stable isotopic enrichments (IE) during rest and exercise.

Lactate Clamp Procedure

In the LT-10%+LC trial, a lactate clamp (LC) procedure was performed as previously described (40). An unlabeled "cold lactate" cocktail was prepared by mixing a 30% lactic acid solution (Sigma-Aldrich, St. Louis, MO) in 2N sodium hydroxide to a pH of 4.8, and subsequently tested for pyrogenicity and sterility at the UCSF School of Pharmacy in the same manner as the isotope solutions. During the rest period, cold lactate infusion began at 2.6 mg·kg^{-1}·min^{-1} to raise [lactate]$_b$ to the one obtained individually during the LT trial. Infusion rates were increased or decreased during rest and exercise to maintain the target concentration as determined by a portable lactate analyzer.

Blood and Respiratory Gas Sampling

Arterialized blood was drawn from a warmed hand vein for metabolite, isotopic enrichment (IE), and hormonal analyses at 0 (background), 60, 75, and 90-min of rest and 10, 20, 30, 40, 50, and 60-min of exercise. Hematocrit was also measured at each time point using a circular microcapillary tube reader (International Equipment Company, no. 2201, Needham Heights, MA). Blood for glucose and lactate concentration and IE determinations was immediately deproteinized with 8% perchloric acid, shaken, and placed on ice. Blood for glycerol and non-esterified fatty acid concentration was collected in EDTA tubes, blood for hormones was collected with aprotinin, and blood for catecholamines was collected with glutathione/EGTA. Samples were centrifuged at 3000 g for 18 min, and the supernatant was transferred to storage tubes and frozen at -80°C until analysis.

Respiratory gases were analyzed continuously via indirect calorimetry for 5 min before, and coincident with blood sampling. These measurements were used for calculation of oxygen consumption (VO$_2$), carbon dioxide production (VCO$_2$), respiratory exchange ratio (RER), and minute ventilation (V$_E$). Duplicate samples of expired air were collected in 10-ml evacuated containers for ^{13}CO$_2$ isotopic enrichment determinations. Careful attention was placed on flushing the line and sampling expired CO$_2$ at the same time as blood was drawn for glucose and lactate IE. Heart rate, blood pressure, and RPE measurements were also recorded at the same frequency as blood and breath sampling.

Hormone Analyses

Catecholamines were extracted from the plasma using acid-washed WA-4 Alumina (Sigma-Aldrich, St. Louis, MO) and 1.5 Tris buffer containing 2% EGTA at a pH of 8.6. Perchloric acid (0.1 M) was used to elute the catecholamines. Finally, 100 μl of this eluent were injected in the HPLC system (Electrochemistry Separations Analysis, ESA, model LC/EC, 5200A; Coulochem, Chelmsford, MA). The mobile phase was Cataphase 2 (ESA, Cambridge, MA), and the electrodes were set at +350, +50, and -350 mV. Standard catecholamine solutions were purchased from ESA. Chromatographs were analyzed using an ESA 501 Data Chromatography System. Insulin and glucagon were measured with commercially available radioimmunoassay kits (Coat-A-Count, DPC, Los Angeles, CA).

Metabolite Concentration and Isotopic Enrichment Analyses

Known amounts of uniformly-labeled internal standards [U-^{13}C]glucose and [U-^{13}C]lactate were added to the supernatant samples collected in 8% perchloric acid. Samples were then neutralized with 2N KOH and transferred to ion exchange columns that were previously washed with double deionized water (ddH$_2$O) through a cation resin (Analytical

Grade 50W-X8, 50-100 mesh H$^+$ resin, Bio-Rad Laboratories, Hercules, CA) and with ddH$_2$O followed by 2N formic acid through an anion resin (Analytical Grade 1-X8, 100-200 mesh formate resin). Glucose was eluted first with ddH$_2$O followed by elution of lactate through the anion column with 2N formic acid. Subsequent lactate analyses and kinetics data are reported separately (37).

The glucose effluent was lyophilized and derivatized as previously described (40). Briefly, glucose IE was determined by gas chromatography/mass spectrometry (GC/MS; GC model 6890 series and MS model 5973N, Agilent Technologies) of the pentaacetate derivative, where methane was used for selected ion monitoring of mass-to-charge ratios (m/z) 331 (non-labeled glucose), 332 (M+1 isotopomer, [1-^{13}C]glucose), 333 (M+2 isotopomer, D$_2$-glucose), and 337 (M+6 isotopomer, [U-^{13}C]glucose internal standard). Whole blood glucose concentration was determined by abundance ratios of 331/337. Selected ion abundances were compared against external standard curves for calculation of concentration and IE. Breath samples were analyzed by use of isotope ratio mass spectrometry by Metabolic Solutions (Nashua, NH).

Calculations

All calculations for concentrations and flux rates used the last 30 min of rest (60, 75 and 90 min) and the last 20 min of steady rate exercise (40, 50 and 60 min). Glucose turnover (R_a, rate of appearance and R_d, rate of disposal) and metabolic clearance rate (MCR) were calculated using the non-steady-state equations of Steele modified for use with stable isotopes (57):

$$R_a = \frac{F - V\left(\frac{C_1 + C_2}{2}\right)\left(\frac{IE_2 - IE_1}{t_2 - t_1}\right)}{\left(\frac{IE_1 + IE_2}{2}\right)} \tag{1}$$

$$R_d = R_a - V\left(\frac{C_2 - C_1}{t_2 - t_1}\right) \tag{2}$$

$$MCR = \frac{R_d}{\left(\frac{C_1 + C_2}{2}\right)} \tag{3}$$

where: R_a and R_d are measured in mg·kg^{-1}·min^{-1}; MCR is measured in ml·kg^{-1}·min^{-1}; F is the isotope infusion rate in mg·kg^{-1}·min^{-1}; V is the volume of distribution (180 ml/kg); C_1 and C_2 are concentrations at sampling times t_1 and t_2; and IE_1 and IE_2 are the excess isotopic enrichments at sampling times t_1 and t_2. The percentage of glucose R_a from lactate-derived gluconeogenesis (%GNG) was calculated as previously described (28) in our laboratory. This approach was derived from that of Zilversmit et al. (59):

$$\%GNG \text{ of glucose } R_a = \frac{(\text{glucose M+1 } IE)(H)}{\text{lactate } IE} \times 100 \tag{4}$$

where: glucose M+1 IE is the isotopic enrichment of the M+1 glucose isotopomer; lactate IE is the isotopic enrichment of lactate; and H is the Hetenyi factor to correct for loss of label in the

tricarboxylic acid cycle during GNG (1.45 at rest and 1.0 during exercise). The absolute rate of GNG in mg·kg^{-1}·min^{-1} was calculated as previously (28):

$$GNG = \frac{(\%GNG)(\text{glucose } R_a)}{100} \tag{5}$$

The absolute rate of hepatic glycogenolysis (GLY) was calculated as the difference between total glucose production and gluconeogenesis:

$$GLY = \text{glucose } R_a - GNG \tag{6}$$

Statistical Analyses

Significance of differences in subject characteristics between untrained and trained groups were analyzed using an unpaired Student's t-test. Differences in metabolic parameters between the untrained and the three trained conditions within rest and exercise were analyzed using a one-way analysis of variance. Analyses within the trained conditions were done with repeated measures. Post hoc analyses were made using Fisher's least significant difference multiple comparisons test. Differences from rest to exercise were analyzed using a paired Student's t-test. Statistical significance was set at $\alpha = 0.05$, and values are represented as means ± SE.

RESULTS

Subject Characteristics

Anthropometric data and work capacities of subjects are reported in Table 1. Compared to untrained subjects, trained subjects had a 33% greater peak oxygen consumption ($P < 0.05$), a 44% greater peak power output (PO) ($P < 0.05$), and a 47% greater total daily energy intake ($P < 0.05$). Subjects were weight stable throughout the study, as measured on each day of an exercise trial. Additionally, total energy and macronutrient composition of the subjects' diets did not change throughout the study (approximately 52% CHO, 30% fat, 18% protein).

Work and Cardiovascular Parameters

Exercise trial workloads, exogenous lactate infusion rates, and cardiorespiratory and hemodynamic parameters are reported in Table 2. During exercise, trained subjects reached their LT at an 11% greater VO_{2peak} ($P < 0.05$) and a 62% greater absolute PO ($P < 0.05$). Oxygen consumption (VO_2), carbon dioxide production (VCO_2), minute ventilation (V_E), heart rate (f_H), and hematocrit were increased in all conditions from rest to exercise. A significant increase in mean arterial pressure was observed in the untrained group only.

Lactate Clamp

Exogenous lactate infusion began during the rest period to raise [lactate]$_b$ to approximately 4.3 mmol/l, which was significantly higher than all other resting conditions (Table 3). During exercise, [lactate]$_b$ was significantly elevated in the LT-10%+LC condition compared to its corresponding control (LT-10%) (4.3 ± 0.3 vs. 2.5 ± 0.5 mmol/l, $P < 0.05$), and it was not different from that during exercise in the LT condition (4.3 ± 0.2 mmol/l). Average

lactate infusion rates were 3.91 ± 0.26 and 3.16 ± 0.95 mg·kg^{-1}·min^{-1} for rest and exercise, respectively (Table 2). Exogenous lactate infusion caused mild hemodilution during rest and exercise, but no significant differences were observed in gas exchange variables, ventilation, heart rate, or rating of perceived exertion due to the lactate clamp (LC), with the exception of a decreased respiratory exchange ratio.

Metabolite and Hormone Concentrations

Blood glucose concentrations ([glucose]$_b$) during rest and exercise are shown in Figure 1. Resting [glucose]$_b$ was elevated in untrained subjects (5.4 ± 0.1 mmol/l) compared to trained subjects, particularly during the LT (4.9 ± 0.2 mmol/l, $P < 0.05$) and LT-10%+LC (4.9 ± 0.1 mmol/l, $P < 0.05$) trials. During exercise at LT, [glucose]$_b$ was significantly lower in the untrained subjects (5.1 ± 0.2 vs. 5.9 ± 0.4 mmol/l, $P < 0.05$). In the trained subjects, [glucose]$_b$ was not different between LT-10% and LT-10%+ LC (Figure 1), while an increase in glucagon was observed with the lactate clamp (71 ± 11 vs. 46 ± 8 pg/ml, $P < 0.05$). Mean concentrations for lactate, free fatty acids (FFA), glycerol, and hormones during rest and exercise are reported in Table 3. Blood [lactate] significantly increased from rest to exercise, except in the LT-10%+LC condition where [lactate]$_b$ was clamped. Plasma FFA, glycerol, and catecholamines increased from rest to exercise. The LC procedure suppressed catecholamines and increased glucagon concentration. Insulin decreased during exercise, but was not different between the four trials.

Glucose Kinetics

Arterial enrichments of the glucose M+2 isotopomer (i.e., the D$_2$-glucose tracer) are presented over time in Figure 2A. Glucose rates of appearance (R$_a$) and disposal (R$_d$), calculated from the M+2 isotopomer, were similar during rest across all conditions and proportional to relative workload during exercise, with the exception of the LT-10%+LC condition in trained subjects. Glucose turnover (R$_a$ and R$_d$) was augmented by LC during exercise at 67% VO$_{2peak}$ (Figures 3A and 3B), although this increase did not achieve significance. Metabolic clearance rate (MCR) was also increased during exercise in the LT-10%+LC condition (Figure 3C) compared to its corresponding control (LT-10%). Rates of glucose turnover were higher in the untrained compared to the trained subjects at rest, indicating greater reliance on blood glucose. This elevation in resting glucose turnover achieved significance when compared to the LT-10%+LC condition.

Hepatic Gluconeogenesis (GNG) and Glycogenolysis (GLY)

Arterial enrichments of the glucose M+1 isotopomer from incorporation of ^{13}C from the lactate tracer were stable over time throughout rest and exercise (Figure 2B), but varied due to exercise intensity and lactate tracer infusion rate between subjects. At rest, absolute rates of GNG were 50% higher, although insignificant, in the untrained subjects compared to the trained subjects (Figure 4A). From rest to exercise at LT, GNG rose approximately three-fold for both untrained and trained subjects. From rest to exercise at LT-10%, GNG more than doubled, but then increased nearly seven-fold from rest to exercise in the LT-10%+LC condition.

Absolute rates of hepatic GLY (Figure 4B), determined as the difference between total glucose production (R$_a$) and GNG, were similar across conditions at rest. During exercise, GLY increased by approximately three-fold in all conditions.

Relative partitioning of total glucose production into hepatic GNG and GLY (Figure 4C) showed that approximately 40% of glucose production came from GNG during rest in both untrained and trained groups. During exercise, this percentage dropped to ~25% in the trained subjects exercising at LT and LT-10%. However, these percentages of glucose R_a from GNG were not statistically different from those in the untrained subjects, showing that trained individuals achieved higher exercise workloads without a significant decrease in GNG. The contribution of GNG during exercise persisted at 40% in the untrained subjects and increased to nearly 60% during the LT-10%+LC condition of the trained subjects, with hepatic GLY accounting for the remainder. This increase in GNG as a percentage of total glucose production was significant when compared to the LT and LT-10% trials in the trained group ($P < 0.05$).

DISCUSSION

We examined the individual and combined effects of endurance training, relative exercise intensity, power output and blood lactate concentration ([lactate]$_b$) on glucose kinetics and hepatic gluconeogenesis (GNG) and glycogenolysis (GLY) during exercise at, and just below the lactate threshold. Our main findings were that trained subjects achieved a significantly higher exercise workload at the lactate threshold without experiencing a significant reduction in gluconeogenesis when compared to their untrained counterparts who exercised at a 40% lower power output. Additionally, when [lactate]$_b$ were matched at the same relative exercise intensity using a lactate clamp (LC), a greater gluconeogenic capacity was revealed in the trained compared to untrained subjects. During the LC trial, GNG exceeded GLY in glucose production partitioning, becoming the major contributor (>50%) of glucose production. In other words, providing additional precursor lactate increases gluconeogenesis during exercise.

During fasting or prolonged exercise, GNG plays a crucial role in the maintenance of blood glucose homeostasis. Rates of GNG increase with duration of fast, as shown by studies utilizing dideuterated water and mass isotopomer distribution analysis (MIDA) techniques (12, 23, 31, 53). In our present study, subjects were overnight fasted and likely had reduced capacity for hepatic glucose production from glycogenolysis. Thus, the higher rates of GNG observed, as well as higher relative contribution of GNG to glucose R_a during rest and exercise, were consistent with results of other studies employing a 12-14 h fast (12, 33).

Effects of Exercise Intensity

Rates of GNG depend in part on precursor supply, determined by hepatic blood flow and precursor concentration. Because hepatic blood flow decreases in direct proportion to relative exercise intensity (1, 43), limitations in precursor delivery to gluconeogenic organs may explain results obtained in this and previous studies conducted on subjects exercising at intensities greater than 50% of VO$_{2max}$ in which rates of GNG decrease as workload increases (33). Accordingly, it is reasonable to conclude that differences in GNG observed in the trained subjects between the LT and LT-10%+LC conditions may be partially explained by the reduced hepatic blood flow at the higher exercise power output (PO). However, given the same hepatic blood flow, which can be assumed for the two exercise conditions at LT-10% (i.e., with and without exogenous lactate infusion), increasing precursor [lactate]$_b$ increased GNG. Between the LT and LT-10% conditions, opposing factors of higher hepatic blood flow and lower

[lactate]$_b$ at the LT-10% intensity may have cancelled out and did not significantly alter rates of GNG.

Effects of Precursor Lactate Concentration

Exogenous lactate infusion during the 90 min of rest prior to exercise during the LT-10%+LC condition likely resulted in hepatic glycogen synthesis as a fate of lactate disposal. However, this possibility requires further analysis of lactate flux and oxidation rates to be verified. Despite the presumption that subjects were equipped with greater hepatic glycogen stores for exercise during the LT-10%+LC condition, GNG rates were still significantly higher compared to its corresponding PO-matched control (LT-10%). Accordingly, it is reasonable to conclude that increased GNG during the LT-10%+LC trial was due to the increased blood lactate availability.

Previous studies have reported that exogenous lactate infusion decreased glucose oxidation in humans during rest and moderate-intensity exercise at 55% VO$_{2peak}$ (39), and increased glucose R$_a$ and R$_d$ with no change in oxidation during exercise at 65% VO$_{2peak}$ (40). In the present study, glucose disposal, but not oxidation was quantified. However, based on results of previous determinations on men before and after training (19), either resting (25% glucose R$_d$ via oxidation) or exercising at 65% VO$_{2peak}$ (80% glucose R$_d$ via oxidation), current results can be interpreted to mean that glucose disposal via oxidation was decreased at rest and increased by lactate infusion during exercise at 67% VO$_{2peak}$.

Consistent with results of previous studies (18), we found a reduction in catecholamine concentration during exercise with the lactate clamp. This finding is supported by evidence that high [lactate]$_b$ may be sensed as a sufficiency in fuel supply by the ventromedial hypothalamus (8), suppressing the counter-regulatory release of catecholamines, but not glucagon (35). Indeed, lactate infusion failed to affect pancreatic fuel sensing during hypoglycemic and euglycemic clamps in healthy men (45). As such, in our present study, we observed an increase in glucagon during exercise at LT-10%+LC compared to the LT-10% trial. Alterations in catecholamines and glucagon may affect glucose metabolism by exerting changes in GNG and GLY. Specifically, epinephrine and norepinephrine have been shown to stimulate hepatic GLY directly (15, 47), whereas the augmenting effects of catecholamines on GNG are through increased glycogenolysis in muscle and lipolysis in adipose tissue to release major gluconeogenic precursors, such as lactate, glycerol and alanine (14, 44). Others have also observed an association between plasma glucagon level and GNG (32, 51), and that the role of catecholamines in the stimulation of hepatic glucose production during exercise is probably secondary to the effects of glucagon (11, 26, 30). Therefore, our observations of a suppressed sympathetic response coupled with higher glucagon concentrations in the LT-10%+LC trial may further explain the increase in GNG, and reciprocal decrease in GLY during exogenous lactate infusion.

Effects of Endurance Training

The effect of endurance training on GNG in humans is not well understood, as previous studies have observed decreased, similar, or increased levels of GNG after training (4, 19, 33). Friedlander et al. (19) estimated GNG from three-carbon precursors by determining glucose recycling rate in healthy men before and after 10 weeks of endurance training. Rates of GNG were lower after training, as measured for a given exercise intensity as well as [lactate]$_b$. MacRae et al. (33) reported no change in absolute rates of GNG after 9 weeks of endurance

training for a given metabolic rate during a progressive exercise test-to-exhaustion. Specifically, GNG from lactate decreased after training, while increased oxidation of lactate was simultaneously observed. Conversely, Bergman et al. (4) demonstrated that 9 weeks of endurance training resulted in an increase in GNG at given absolute and relative exercise intensities (65% of pre- and post-training VO_{2peak}). These training effects on GNG were apparent in both absolute GNG rates and relative contributions to total glucose production. Subjects in this latter study were 5-h postprandial, in contrast to the former studies where subjects were 3-h postprandial in the study by Friedlander et al., and 12-h overnight-fasted in the study by MacRae et al. Furthermore, the controls in dietary composition may have varied subtly across studies. Therefore, apparent discrepancies in the effects of endurance training on GNG are judged to be due to factors such as dietary status affecting the independent dynamics of GNG and GLY as they support total glucose production.

Our methodology of controlling dietary status in determining lactate-derived glucose production begs the question of whether carbohydrate-restricted individuals would exhibit gluconeogenic responses different from those observed in this investigation. To our knowledge, neither lactate nor GNG flux rates have been measured in individuals subjected to high-fat diets; such experiments remain to be done. Our calculations of GNG rates were based on measuring incorporation of carbon-3 from infused [3-^{13}C]lactate into the blood glucose pool. This method of determining GNG would also include contributions of metabolites in equilibrium with lactate, such as pyruvate (via lactate dehydrogenase) and alanine (via alanine aminotransferase). However, the contributions of other gluconeogenic precursors such as glycerol would not be included. Hence, in these experiments we provide minimal estimates of GNG. It is reasonable to assume that fat-adapted individuals would be equally or more dependent on GNG to maintain glycemia than in those adapted to a high carbohydrate diet. Compared to those who are carbohydrate-fed, fat-adapted individuals exhibit higher plasma glycerol concentration during prolonged exercise (10). In such a case, increased precursor supply via glycerol might be expected to augment GNG. However, others (29) and us (52) have shown that glycerol is a poor gluconeogenic precursor, suggesting that fat-adapted individuals with low circulating lactate levels would experience compromised capacity for GNG during exercise, unless adaptation to a high-fat diet increases the capacity of GNG from glycerol. Issues surrounding mechanisms of maintaining glycemia in highly trained individuals adapted to a high-fat diet and the role of glycerol warrant further investigation.

Results of the present investigation showed no significant effect of training on partitioning of glucose production at rest, and further, rates of GNG and GLY were not different in resting subjects across conditions following an overnight fast. However, during exercise at LT, trained subjects cycled at a substantially higher relative exercise intensity (75% vs. 67% VO_{2peak}) and absolute PO (259 vs. 161 W) without a significant reduction in GNG compared to the untrained subjects. Still, while not statistically different, the average relative contribution of GNG in glucose production was ~40% lower in trained compared to untrained subjects, while hepatic GLY was ~25% greater during exercise at LT. By way of explanation, we note that trained subjects have larger stores of liver glycogen (21), which can be a determinant of hepatic GLY, particularly in states of low glycogen content (22, 46, 56). However, compared to the LT-10% trial, trained subjects exhibited higher rates of GNG during exercise in the LT-10%+LC trial, despite following a 90-min rest period of exogenous lactate infusion, which presumably preloaded hepatic glycogen reserves prior to exercise. Comparing the trained subjects in the LT-10%+LC trial to the untrained subjects exercising at LT, both groups were cycling at the same

relative exercise intensity (67% VO_{2peak}) and [lactate]$_b$ (~4 mmol/l), thereby characterizing the individual effects of training. We observed higher rates of GNG, although statistically non-significant, in the trained subjects, suggesting that endurance training may increase gluconeogenic capacity during prolonged exercise following an overnight fast.

In terms of energy substrate partitioning, endurance training causes a small, but significant shift in substrate utilization away from carbohydrates and toward fatty acids (9). As well, training reduces blood glucose disposal for a given absolute PO after training (3, 19, 40, 42, 54, 58). In the present study, when untrained and trained subjects exercised at a relative intensity of 67% VO_{2peak} (untrained at LT and trained at LT-10%), we found no significant differences in glucose R_a, R_d, or MCR between groups. Glucose turnover in the trained subjects exercising at LT was also not different from the other conditions, but [lactate]$_b$ was significantly decreased during exercise in the LT-10% trial. This finding is consistent with studies reporting that lactate clearance is increased via oxidation at both given absolute power outputs and relative exercise intensities after training (5, 17, 33, 34, 49). Therefore, endurance trained individuals have increased capacities to use lactate as both an oxidative energy source and a gluconeogenic precursor. When the lactate clamp raised [lactate]$_b$ to match the LT trial, GNG in the trained subjects increased, again showing that providing additional precursor lactate increases GNG during exercise.

Training-induced adaptations in hepatic glucose metabolism are also affected by an attenuated neuroendocrine response during exercise at a given absolute PO (36, 42). In the present study, at a matched relative exercise intensity of 67% VO_{2peak}, we found that the alterations in hormone milieu (i.e., higher insulin, and significantly lower glucagon and epinephrine) persisted in trained compared to untrained subjects (Table 3). Still, untrained subjects were not able to regulate blood glucose as well during exercise, and therefore experienced a greater counter-regulatory response, as seen with higher glucagon concentrations even if matched for relative exercise intensity.

Limitations

Lactate enters the GNG pathway via pyruvate, where tracer enrichment may be reduced when dilution occurs with oxaloacetate from the tricarboxylic acid cycle. Accordingly, we used a correction (Hetenyi, H) factor of 1.45 during rest to account for this reduction in signal (24). Corrections for isotopic dilution may vary according to species, nutritional state, and training state differences (25), yet we assumed the same relative proportion of tracer dilution in both untrained and trained groups. For exercise, we did not apply a correction factor (i.e., H = 1.0) to the data, because hepatic glucose metabolism through oxaloacetate is predominantly anabolic.

While lactate is widely recognized to be the major gluconeogenic precursor, the rates of GNG we calculated for healthy men may have been underestimated because we did not include contributions from glycerol, amino acids, and pyruvate (2). In turn, hepatic GLY may have been overestimated. However, possible underestimation of GNG rates for lack of consideration of the roles of other precursors may have been minimized in the LT-10%+LC condition in which a surfeit of lactate limited the contributions of other gluconeogenic precursors because of the presence of autoregulatory control (29, 51, 52). Further, our findings may have been different in fat-adapted individuals, who have been shown to exhibit altered patterns in substrate utilization during exercise compared to individuals who consume a larger portion of carbohydrates (10).

Throughout the manuscript, we describe glucose production as appearing from the liver, although changes may have occurred in the kidneys as well. Because our measurements reflect

whole body metabolism, we do not exclude the renal cortex as a contributor to GNG and GLY in our results. Importantly, because glycogen storage is low in the kidneys (20), renal glycogenolysis is unlikely to have made a significant contribution to blood glucose appearance.

Summary and Conclusions

We utilized stable isotope tracers to measure glucose metabolism in untrained and trained subjects during steady state exercise at and just below the lactate threshold. We also incorporated a lactate clamp component to study the effect of precursor concentration on gluconeogenesis. From these experiments, it is concluded that lactate-derived gluconeogenesis plays an essential role in hepatic and renal glucose production during exercise in the fasted state regardless of training history. Endurance training increases the work capacity to achieve a higher relative exercise intensity and absolute power output before reaching the lactate threshold and without experiencing a significant decrease in gluconeogenesis. Additionally, gluconeogenesis can be augmented during exercise when blood lactate is increased by endogenous or exogenous supply, suggesting that the contribution of gluconeogenesis to total glucose production may be limited by delivery of gluconeogenic precursors.

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DISCLOSURES

GAB has a financial interest in CytoSport; otherwise, the authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

GAB and LAM were responsible for the conception and design of research; LAM, JAF and CWE performed experiments; TJC was responsible for medical clearance and safe conduct of experimentation; MAH was responsible for gas chromatography and mass spectrometry (GCMS); CWE was responsible for enzymatic, GCMS and statistical analyses; JAF was responsible for enzymatic and hormonal analyses; CWE and GAB analyzed data and drafted the manuscript, and all authors edited and approved the final version of manuscript.

TABLES

Table 1. *Subject characteristics for untrained and trained groups*

Variable	Untrained	Trained
Age (years)	25 ± 1	24 ± 2
Body mass index (kg/m^2)	26.3 ± 0.7	23.9 ± 0.6*
Body fat (%)	13.0 ± 1.0	10.0 ± 1.3
FEV$_1$/FVC (%)	88.7 ± 2.6	79.6 ± 2.9*
Absolute VO$_{2peak}$ (l/min)	3.7 ± 0.1	5.0 ± 0.3*
Relative VO$_{2peak}$ (ml·kg^{-1}·min^{-1})	46.1 ± 1.5	66.6 ± 2.6*
Peak power output (W)	248 ± 7	357 ± 12*
3-Day Diet Records		
Energy (kcal/day)	2363 ± 256	3465 ± 149*
Carbohydrate (%)	53 ± 3	52 ± 4
Fat (%)	28 ± 2	32 ± 4
Protein (%)	19 ± 1	16 ± 1

Values are means ± SE; $n = 6$ for untrained and trained groups; FEV$_1$, forced expiratory volume in 1 s; FVC, forced vital capacity; VO$_{2peak}$, peak oxygen consumption. *Significantly different from untrained group ($P < 0.05$).

Table 2. *Workload, exogenous unlabeled (cold) lactate infusion rate, and cardiorespiratory and hemodynamic parameters during rest and exercise*

	Rest				Exercise			
Variable	Untrained LT	Trained LT	Trained LT-10%	Trained LT-10%+LC	Untrained LT	Trained LT	Trained LT-10%	Trained LT-10%+LC
Power Output (W)	-	-	-	-	161 ± 4	259 ± 10 *	234 ± 9 *£	234 ± 9 *£
$\%VO_{2peak}$ (%)	-	-	-	-	67.6 ± 1.3	74.8 ± 1.7 *	66.6 ± 1.4 £	67.9 ± 1.5 £
Exogenous lactate infusion rate ($mg \cdot kg^{-1} \cdot min^{-1}$)	-	-	-	3.91 ± 0.26	-	-	-	3.16 ± 0.95
VO_2 (l/min)	0.31 ± 0.01	0.31 ± 0.02	0.31 ± 0.02	0.33 ± 0.02	2.52 ± 0.06 ^	3.71 ± 0.19 ^*	3.30 ± 0.16 ^*£	3.36 ± 0.15 ^*£
VCO_2 (l/min)	0.25 ± 0.00	0.28 ± 0.01	0.30 ± 0.03	0.28 ± 0.03	2.33 ± 0.08 ^	3.51 ± 0.18 ^*	3.05 ± 0.15 ^*£	3.05 ± 0.13 ^*£
RER	0.80 ± 0.01	0.85 ± 0.02 *	0.87 ± 0.02 *	0.77 ± 0.01 £†	0.93 ± 0.01 ^	0.95 ± 0.00 ^	0.93 ± 0.01 ^	0.91 ± 0.00 ^£
V_E (l/min)	7.7 ± 0.3	9.1 ± 0.6	10.2 ± 1.6	9.4 ± 1.2	56.2 ± 3.2 ^	84.2 ± 5.8 ^*	68.1 ± 4.2 ^*£	64.5 ± 2.7 ^*£
f_H (beats/min)	62.3 ± 1.8	60.8 ± 2.2	56.8 ± 2.3	58.9 ± 2.3	164.8 ± 5.4 ^	172.4 ± 2.2 ^	158.6 ± 4.5 ^£	155.7 ± 2.6 ^£
MAP (mmHg)	92.0 ± 1.6	91.6 ± 2.6	93.9 ± 2.9	92.2 ± 5.5	101.4 ± 2.9 ^	99.0 ± 3.8	98.4 ± 3.4	92.5 ± 2.4
Hematocrit (%)	43.4 ± 1.1	45.0 ± 0.8	44.3 ± 1.0	39.8 ± 1.1 *£†	46.1 ± 0.8 ^	47.7 ± 0.8 ^	47.2 ± 1.1 ^	42.5 ± 0.9 ^£†
RPE	-	-	-	-	15.9 ± 1.2	14.9 ± 1.4	12.1 ± 0.8 *	13.3 ± 1.2

Values are means $\pm$ SE; $n = 6$ for untrained and trained groups; VO_2, oxygen consumption; VCO_2, carbon dioxide production; RER, respiratory exchange ratio; V_E, minute ventilation; f_H, heart rate; MAP, mean arterial pressure; RPE, rating of perceived exertion. ^ Significantly different from rest within condition ($P < 0.05$); * Significantly different from untrained ($P < 0.05$); £ Significantly different from trained LT ($P < 0.05$); † Significantly different from trained LT-10% ($P < 0.05$).

Table 3. *Metabolite and hormonal concentrations during rest and exercise*

Variable	Rest				Exercise			
	Untrained LT	Trained LT	Trained LT-10%	Trained LT-10%+LC	Untrained LT	Trained LT	Trained LT-10%	Trained LT-10%+LC
Lactate (mmol/l)	0.6 ± 0.1	1.3 ± 0.1 *	1.1 ± 0.1	4.4 ± 0.4 *£†	3.7 ± 0.4 ^	4.3 ± 0.2 ^	2.5 ± 0.5 ^*£	4.3 ± 0.3 †
FFA (μmol/l)	360 ± 50	276 ± 27	200 ± 25 *	259 ± 24 *	491 ± 67 ^	262 ± 47 *	220 ± 21 *	358 ± 37 †
Glycerol (μmol/l)	46 ± 10	28 ± 3	36 ± 4	36 ± 7	218 ± 36 ^	270 ± 39 ^	215 ± 18 ^	248 ± 23 ^
Epinephrine (pg/ml)	39 ± 7	78 ± 17	65 ± 13	44 ± 16	303 ± 16 ^	321 ± 49 ^	216 ± 51 ^*£	144 ± 22 ^*£
Norepinephrine (pg/ml)	214 ± 48	303 ± 83	272 ± 69	254 ± 39	1929 ± 260 ^	4011 ± 750 ^*	2946 ± 594 ^*£	1512 ± 206 ^£†
Insulin (pg/ml)	358 ± 29	303 ± 22	307 ± 17	267 ± 5 *	193 ± 28 ^	187 ± 13 ^	221 ± 21 ^	226 ± 13 ^
Glucagon (pg/ml)	63 ± 8	45 ± 3 *	46 ± 4 *	48 ± 4	85 ± 7 ^	55 ± 8 *	46 ± 8 *	71 ± 11 ^†

Values are means ± SE; $n = 6$ for untrained and trained groups; FFA, free fatty acids. ^ Significantly different from rest within condition ($P < 0.05$); * Significantly different from untrained ($P < 0.05$); £ Significantly different from trained LT ($P < 0.05$); † Significantly different from trained LT-10% ($P < 0.05$).
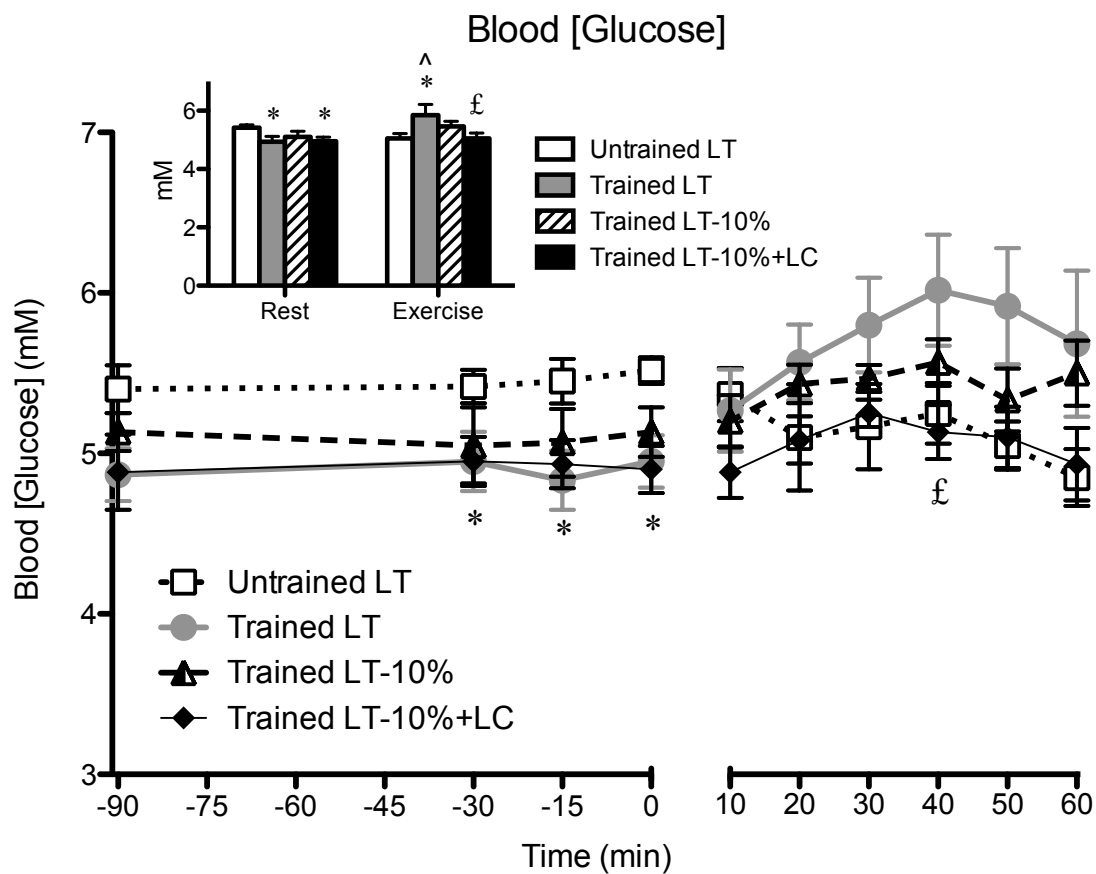
FIGURES

Figure 1. Blood glucose concentration across time. Values are means ± SE; *n* = 6 for untrained and trained groups. Inset shows steady state rest and exercise glucose concentrations for each condition. ^ Significantly different from rest within condition ($P < 0.05$); * Significantly different from untrained ($P < 0.05$); £ Significantly different from trained LT ($P < 0.05$).
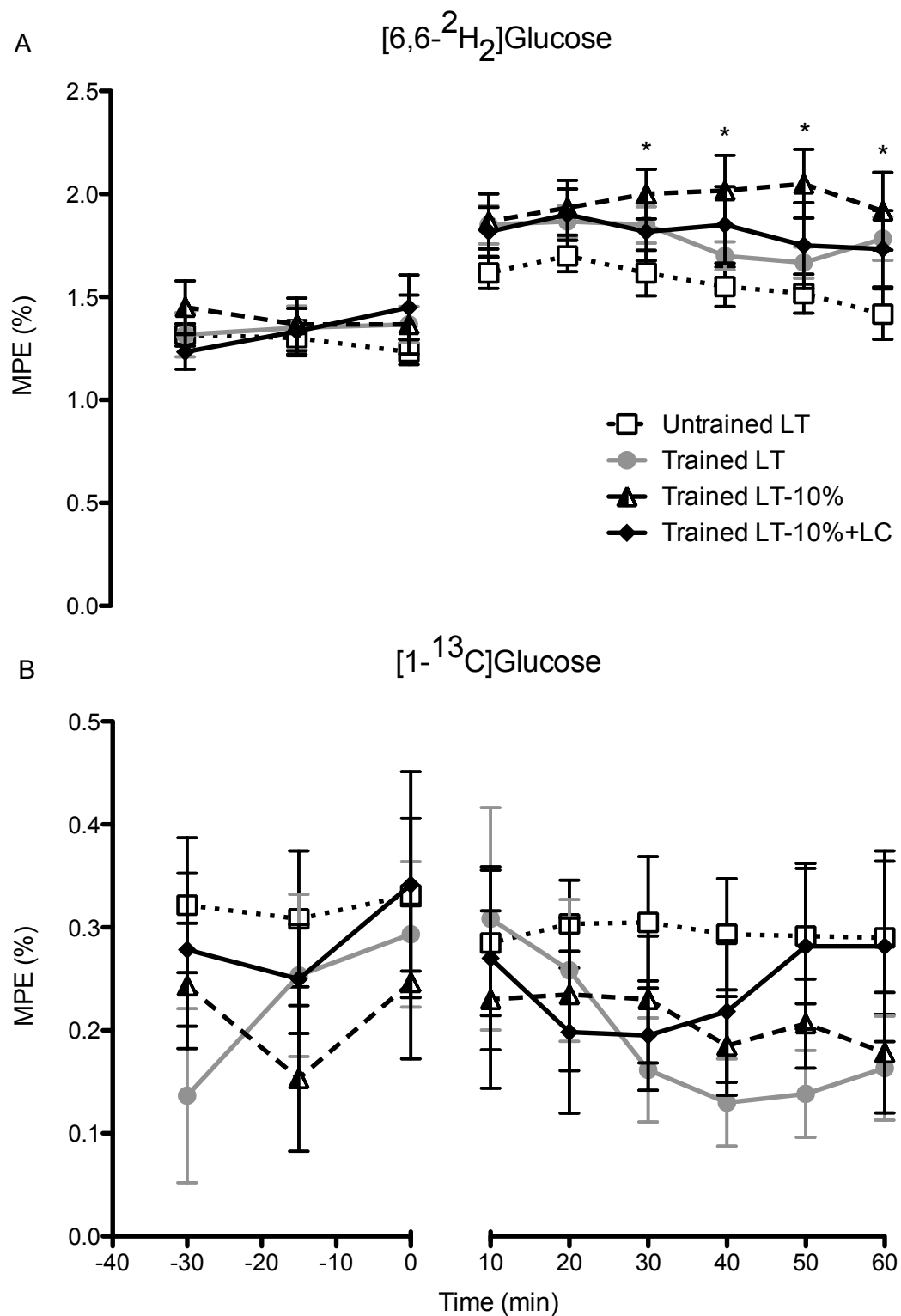
A

$[6,6\text{-}^2H_2]$Glucose

B

$[1\text{-}^{13}C]$Glucose

Figure 2. Mole percent excess of glucose isotopomers M+2 (A) and M+1 (B) across time. Values are means ± SE; $n = 6$ for untrained and trained groups. * Significantly different from untrained ($P < 0.05$).
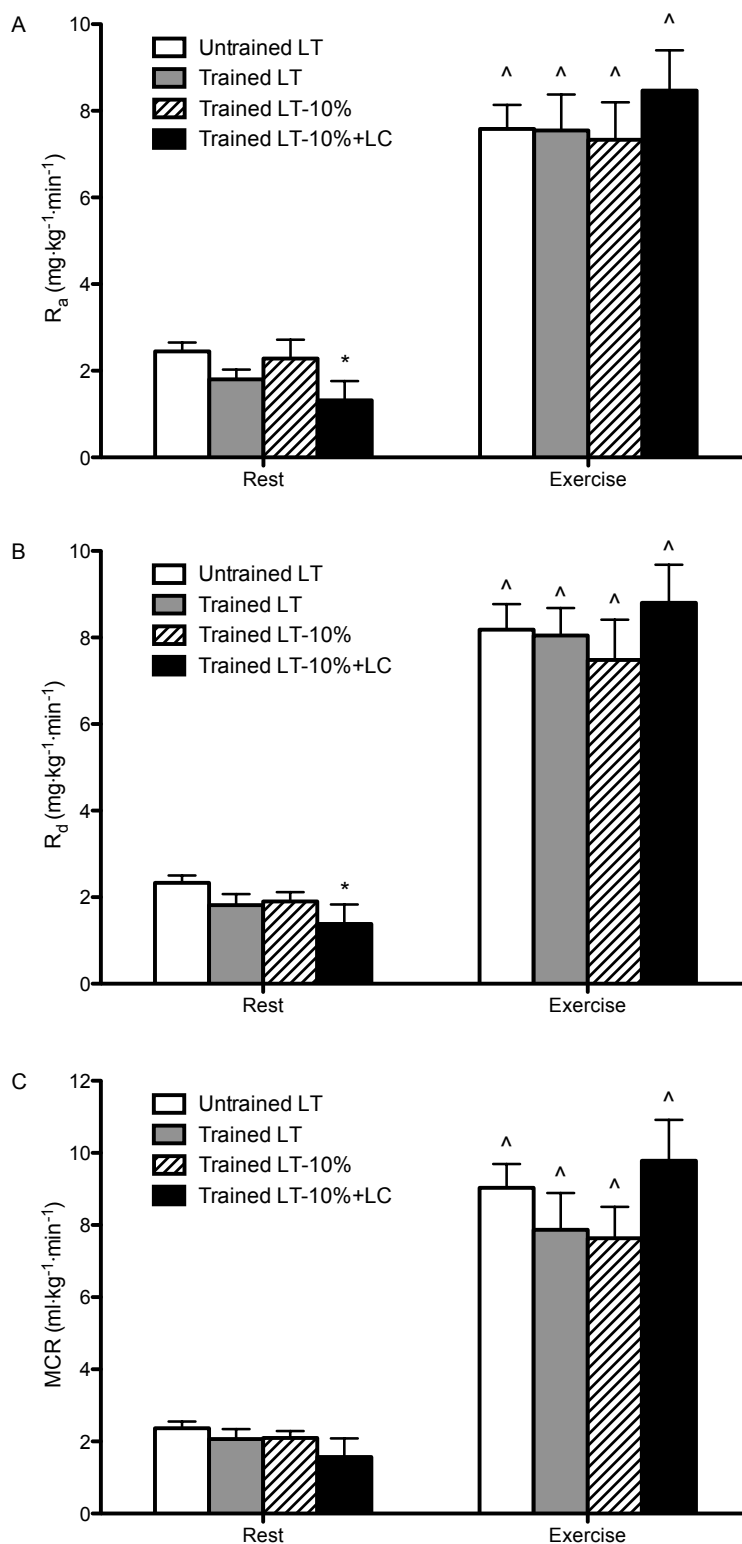
Figure 3. Steady state glucose rates of appearance (R_a; A), disposal (R_d; B), and metabolic clearance (MCR; C) during rest and exercise. Values are means ± SE; $n = 6$ for untrained and trained groups. ^ Significantly different from rest within condition ($P < 0.05$); * Significantly different from untrained ($P < 0.05$).
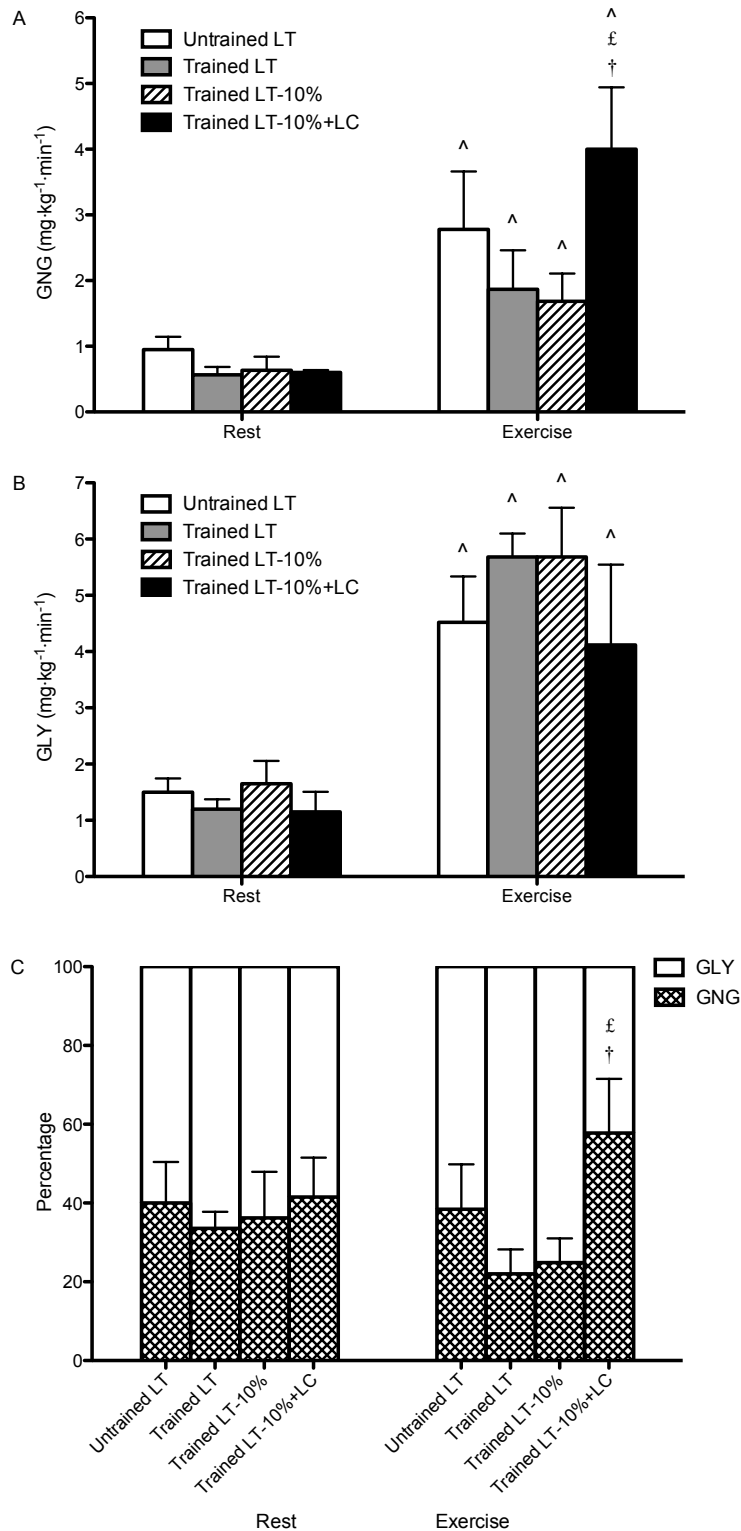
Figure 4. Steady state rates of gluconeogenesis (A) and hepatic glycogenolysis (B), and partitioning of total glucose production in relative terms (C). Values are means ± SE; *n* = 6 for untrained and trained groups. ^ Significantly different from rest within condition ($P < 0.05$); £ Significantly different from trained LT ($P < 0.05$); † Significantly different from trained LT-10% ($P < 0.05$).

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CHAPTER 3

Lactate Kinetics at the Lactate Threshold in Trained and Untrained Men

ABSTRACT

To understand the meaning of the lactate threshold (LT) and to test the hypothesis that endurance training augments lactate kinetics [i.e., rates of appearance and disposal (R_a and R_d, mg·kg^{-1}·min^{-1}) and metabolic clearance rate (MCR, ml·kg^{-1}·min^{-1})], we studied six each untrained (UT) and trained (T) subjects during 60-min exercise bouts at power outputs (PO) eliciting the LT. Trained subjects performed two additional exercise bouts at a PO 10% lower (LT-10%), one of which involved a lactate clamp (LC) to match blood lactate concentration ([lactate]$_b$) to that achieved during the LT trial. At LT, lactate R_a was higher in T (24.1 ± 2.7) than in UT (14.6 ± 2.4, $P < 0.05$), but R_a was not different between UT and T when relative exercise intensities were matched (UT-LT *vs.* T-LT-10%, 67% VO$_{2max}$). At LT, MCR in T (62.5 ± 5.0) was 34% higher than in UT (46.5 ± 7.0, $P < 0.05$), and a reduction in PO resulted in a significant increase in MCR by 46% (LT-10%, 91.5 ± 14.9, $P < 0.05$). At matched relative exercise intensities (67% VO$_{2max}$), MCR in T was 97% higher than in UT ($P < 0.05$). During the LC trial, MCR in T was 64% higher than in UT ($P < 0.05$), where %VO$_{2max}$ and [lactate]$_b$ were similar. We conclude that i) lactate MCR reaches an apex below the LT, ii) LT corresponds to a limitation in MCR, and iii) endurance training augments capacities for lactate production, disposal and clearance.

INTRODUCTION

Blood lactate accumulation increases little with small increments in exercise intensity, but there occurs a power output (PO) above which blood lactate accumulation accelerates in response to further increments in exercise PO. This inflection point has been termed the lactate threshold (LT) (11). The LT has received much attention by exercise physiologists, sports medicine practitioners, coaches and athletes. This interest comes from the fact that the LT is correlated to endurance exercise capability and can be used in the management of endurance training (18, 51, 69, 74). However, the LT remains unclear in its physiological significance, some interpreting the inflection point to indicate the onset of anaerobiosis (77, 78), while others interpret the LT to reflect an imbalance between lactate appearance and removal (12, 72). Moreover, lactate kinetics and its parameters, e.g., rates of appearance (R_a) and disposal (R_d) and metabolic clearance rate (MCR), have never been investigated during sustained exercise at the lactate threshold.

Previous experiments have investigated lactate kinetics as functions of exercise intensity (7, 47, 48, 72). These studies have shown that lactate R_a is matched by R_d during rest, and that both rise with increases in exercise intensity as described by PO, metabolic rate [oxygen consumption (VO_2) or percentage of maximal oxygen consumption ($\%VO_{2max}$)], and blood lactate concentration ([lactate]$_b$). Those investigators also observed that during continual graded exercise, the increase in R_d lags behind the increase of R_a, resulting in rising [lactate]$_b$ (47, 72). As well, previous investigators observed that MCR, a measure of efficiency for lactate disposal (19), increases from rest to moderate intensity exercise, but then decreases from moderate to hard exercise (7, 47, 48, 72, 73). Because none of the earlier investigations studied lactate kinetics at the LT, the question of whether a limitation in lactate clearance occurs at the LT remains. Hence, our first aim of the present study was to interrogate the physiological significance of the LT by determining lactate kinetics in subjects exercising at and just below the LT to test the hypothesis that the LT represents a limitation in lactate clearance. As well, because endurance training has been observed to increase lactate clearance capacity in both laboratory rats (19) and humans (7), our second aim was to test the hypothesis that endurance training augments rates of lactate disposal and clearance at a given relative exercise intensity and blood lactate concentration.

METHODS

Many details of methodology have been presented elsewhere (21), but are repeated here for the convenience of the reader.

Subjects

Six untrained men (UT) and six trained male cyclists (T) took part in the study. Recruitment was done by word of mouth, posting of fliers and e-mail notices within the University of California Berkeley campus and the surrounding community. Untrained subjects were healthy and recreationally active (maximal oxygen consumption, VO_{2max} <50 ml·kg^{-1}·min^{-1}). Trained subjects were members of competitive cycling or triathlon teams, currently in the race phase of their training season (VO_{2max} >55 ml·kg^{-1}·min^{-1}). Subjects were included in the study if they had a body mass index (BMI) of ≥18 and <26 kg/m^2, were non-smokers, were diet and weight stable, had a FEV_1/FVC of >70%, and were injury/disease free as determined by physical examination. This study was approved by the University of California Berkeley Committee for the Protection

of Human Subjects (CPHS 2010-4-1300) and conformed to the standards set by the Declaration of Helsinki. Eligible volunteers were verbally informed of the purposes, procedures and associated risks and gave written informed consent.

Experimental design

The study consisted of a preliminary screening including a medical examination and a maximal exercise stress test to determine VO_{2max}, an incremental exercise test to determine the LT and associated parameters, and one (for UT) or three (for T) isotope tracer trials to measure glucose and lactate kinetics during rest and exercise. Isotope tracer trials took place in the morning after a 12-h fast: study participants rested for 90 min and then exercised for 60 min. During the first tracer trial, untrained and trained subjects exercised at the PO corresponding to their previously determined LT. Trained subjects completed two additional tracer trials at a PO 10% below that eliciting the LT (LT-10%), one of which included exogenous lactate infusion using the "Lactate Clamp" (LC) procedure to match blood lactate concentration ($[lactate]_b$) obtained during the LT trial (LT-10%+LC). During rest and exercise, study participants were infused with stable, non-radioactive tracers of lactate, glucose and bicarbonate. Exercise sessions were separated by at least one week. For the day before each exercise session, study participants were instructed to abstain from structured physical exercise or hard physical activity, but to continue typical activities of daily living.

This experimental design of the tracer trials aimed at assessing the effects of endurance training, $[lactate]_b$, exercise intensity and their combinations on lactate kinetics. Within the trained subjects, we investigated: the effects of exercise intensity given the same $[lactate]_b$ (LT *vs.* LT-10%+LC); the effects of $[lactate]_b$ given the same exercise intensity (LT-10% *vs.* LT-10%+LC); and the combined effects of $[lactate]_b$ and exercise intensity (LT *vs.* LT-10%). To evaluate the effects of training on metabolic responses, we compared UT and T groups exercising: at the LT, i.e., same $[lactate]_b$, but different absolute PO and relative intensities (UT-LT *vs.* T-LT); at the same relative intensity, but different $[lactate]_b$ (UT-LT *vs.* T-LT-10%); and at the same relative intensity and $[lactate]_b$ (UT-LT *vs.* T-LT-10%+LC).

Dietary controls and standardized meals

A three-day diet record was collected prior to the study to verify that the subjects had normal eating patterns. Subjects also provided complete 24-h diet records of the day preceding each exercise session. Diet records were analyzed for macronutrient composition and energy intake by using Diet Analysis Plus, version 6.1 (ESHA Research, Salem, OR). Subjects were instructed to keep their dietary composition and amount constant throughout the duration of the study. The day before each exercise session, subjects reported to the laboratory to pick up their dinner and evening snack. Dinner consisted of approximately 60% carbohydrate (CHO), 25% fat and 15% protein while the evening snack consisted of approximately 55% CHO, 30% fat and 15% protein. The snack was consumed between 9:00 and 9:15 PM. Exercise commenced 12 h following completion of the evening snack, and no food or beverages were allowed except water (*ad libitum*) until the end of the exercise session. Standardized diets consisted on average of 2400 and 3200 kcal/day for the UT and T groups, respectively. Diets were individualized and determined for each participant considering a physical activity level (PAL) of 1.25 for UT and 1.48 for T according to the current dietary reference intake guidelines of the Institute of Medicine for Estimated Energy Requirement.

Preliminary screening, VO$_{2max}$ test and medical examination

Subjects completed a health and exercise history questionnaire developed in cooperation with the Student Health Service. Subsequently, anthropometric data including height, weight and body composition [evaluated by BMI and skinfold measurements at seven sites (abdominal, triceps, chest/pectoral, mid-axillary, subscapular, suprailiac, and thigh)], pulmonary function (FEV$_1$/FVC), and resting blood pressure (determined by auscultation) were measured and recorded. A blood sample was taken for a metabolic panel screening, complete blood count and serological evaluation for HIV infection. On a subsequent day, subjects underwent an exercise stress test in accordance with the American College of Sports Medicine (ACSM) Guidelines for Exercise Testing and Prescription (7th Edition). This test consisted of graded progressive cycle ergometry to volitional exhaustion. Subjects arrived in the laboratory at 8:45 AM in fasted state. After a 10-min rest period during which resting ECG (Quinton Q750 electrocardiograph, Seattle, WA) and pulmonary gas exchange parameters were recorded, exercise started for 3 min at 75 W for UT and at 120, 150 or 180 W for T. Thereafter, resistance increased every 3 min by 25 W for UT and 30 W for T, until volitional exhaustion. ECG, minute ventilation (VE), oxygen consumption (VO$_2$), carbon dioxide production (VCO$_2$) and respiratory exchange ratio (RER = VCO$_2$/VO$_2$) were recorded continuously during exercise. VE, VO$_2$, VCO$_2$ and RER were measured using a Parvo Medics TrueOne$^{®}$ 2400 apparatus (ParvoMedics, Sandy, UT), which was calibrated beforehand using precision-analyzed gas mixtures and a 3-l syringe. Blood pressure was measured in the mid-stage, and rating of perceived exertion (RPE) was recorded during the last 30 s of each stage. Blood [lactate] was determined in 10 μl finger prick samples taken at the end of each stage via a portable lactate analyzer (Nova Lactate Plus, Waltham, MA). This test was performed to assess the maximal heart rate (*f*H$_{max}$) and oxygen consumption (VO$_{2max}$) and VO$_{2max}$-associated power output (PO$_{max}$), as well as to approximate the PO eliciting the LT. Lastly, participants were subjected to a physical examination before final inclusion.

LT determination and confirmation

Subjects arrived in the laboratory at 8:45 AM in fasted state. A catheter was inserted into a warmed dorsal hand vein for "arterialized" blood sampling (38). Following a 10-min rest period, a 1-ml blood sample was collected, and blood pressure was measured. Exercise workload started at 75 W for UT and 120 or 150 W for T, and progressively increased to reach in 3 min ~50% and ~60% of VO$_{2max}$ (i.e., ~40-50 W below the approximated PO at LT) for UT and T, respectively. Thereafter, resistance increased every 3 min by 10 W until volitional exhaustion. Blood pressure was measured in the mid-stage, while RPE was recorded and a 1-ml blood sample was drawn at the end of each exercise stage. ECG and pulmonary gas exchange parameters (VE, VO$_2$, VCO$_2$, RER) were recorded continuously during rest and exercise. This test was performed to determine the LT as defined previously (11) and associated VO$_2$, *f*H, PO, and [lactate]$_b$. On a subsequent day, subjects arrived in the laboratory at 8:45 AM in fasted state. After 10 min of rest, subjects exercised for 60 min at the PO corresponding to their LT to confirm the feasibility of the task and stabilization of [lactate]$_b$ over the 60-min exercise test. Every 10 min, 10 μl finger prick blood samples were assayed for [lactate]$_b$, and heart rate and RPE were monitored. When necessary, PO was adjusted by ~5 W. The mean PO during the last 15 min of exercise was used for the isotope tracer trials.

Isotope Tracer Trials

Subjects performed one (UT) or three (T) isotope tracer trials (see experimental design). Subjects reported to the laboratory at 7:15 AM in fasted state. ECG was monitored continuously during rest and exercise. Resting oxygen consumption was measured, and an aliquot of expired air was taken to determine the $^{13}CO_2$ background enrichment. A catheter was then inserted into a warmed dorsal hand vein. Blood samples were collected for determination of background lactate and glucose isotopic enrichments, and metabolite and hormone concentrations. In the contralateral arm, a second catheter was placed in an antecubital vein for isotope [i.e., [3-^{13}C]lactate, $H^{13}CO_3^-$ and [6,6-$^2H_2$]glucose (D_2-glucose), for all trials] and unlabeled lactate cocktail-saline (i.e., Na-lactate, for LC-10%+LC) infusions. Catheters were kept patent by a saline drip (0.9%, no heparin).

After background sampling, a priming bolus containing 57.5 mg of [3-^{13}C]lactate (i.e., 23 times the resting lactate infusion rate), 250 mg of D_2-glucose (i.e., 125 times the resting lactate infusion rate), and 136 mg of $NaH^{13}CO_3^-$ were injected. The resting infusion rate for [3-^{13}C]lactate was 2.5 mg/min during LT and LT-10%, and 7.5 mg/min during LT-10%+LC. The resting infusion rate of D_2-glucose was 2 mg/min in all trials. Subjects rested semi-supine for 90 min. During the resting period of the LT-10%+LC trial, unlabeled lactate infusion began at 104 ml/h which delivered lactate at ~200 mg/min. Infusion was then adjusted to match the [lactate]$_b$ observed during exercise of the LT trial. Results obtained using D_2-glucose to assess glucose kinetics and gluconeogenesis via secondary labeling of blood glucose with ^{13}C from infused [3-^{13}C]lactate are reported separately (21).

At the onset of exercise, [3-^{13}C]lactate infusion rate was increased to 11.25 mg/min and 15 mg·min^{-1} for UT and T, respectively. During exercise of the LT-10%+LC trial, unlabeled lactate infusion was adjusted to match [lactate]$_b$ observed during the LT trial.

Blood for metabolite concentrations and isotopic enrichments and hormonal analyses was sampled at 0, 60, 75 and 90 min of rest and at 10, 20, 30, 40, 50 and 60 min of exercise. During exercise of the LT-10%+LC trial, [lactate]$_b$ was also immediately analyzed (NOVA Lactate Plus, Nova Biomedical Corp., Waltham, MA) and the information was used to adjust exogenous lactate infusion rate to achieve the desired target level. Pulmonary gas exchange parameters were simultaneously sampled to determine VE, VO_2, VCO_2, and RER. Blood pressure was determined by auscultation 5 min prior to the blood sampling. A sample of expired air was also collected in a 10-ml Vacutainer at each time point of blood sampling to determine $^{13}CO_2$ isotopic enrichment in expired gases. Hematocrit (Hct) was also measured at each time point using the microhematocrit method, and subjects indicated their RPE according to the Borg scale.

Isotope tracer and unlabeled lactate cocktail preparations

Tracers were purchased from Sigma-Aldrich (St. Louis, MO). The individual components were United States Pharmacopeia-National Formulary (USP-NF) certified. Tracer cocktails were prepared in 0.9% sterile saline. For the lactate clamp, unlabeled lactate cocktail was prepared in 0.9% sterile saline by mixing a 88% L(+)-lactic acid solution (Sigma-Aldrich, St. Louis, MO) with 2N NaOH (Spectrum Chemicals, Auburn, WA) to a pH of 4.8 as previously described (53). The tracer and LC infusates were subsequently tested for sterility and pyrogenicity at the University of California, San Francisco, School of Pharmacy, Pharmacy Drug Product Services Division. Infusates passed through a 0.2 mm Millipore filter (Nalgene, Rochester, NY) prior to infusion and were delivered with calibrated pumps (Baxter Colleague 3CX, Deerfield, IL).

Ergometry

All exercises were performed on an electronic braked cycle ergometer (Monark 839E, Vansbro, Sweden). The instantaneous power output and the pedaling frequency were delivered online by a microprocessor. For all exercise tests, the subjects remained in the seated position.

Blood sampling

Arterialized blood samples (~3 ml) for the analyses of lactate and glucose concentrations and isotopic enrichments (IE) were collected in 8% perchloric acid in a 1:2 ratio. Blood samples (~6 ml) for hormone analyses were split in two aliquots of ~3 ml each. Samples were centrifuged for 18 min at 3,000 *g*. Blood (for lactate and glucose IEs and concentrations) and plasma (for catecholamines, insulin and glucagon) were transferred and stored at -80°C until analysis. Results for insulin and glucagon are reported separately (21).

Epinephrine and norepinephrine analyses

Epinephrine and norepinephrine were analyzed as previously described (23). Briefly, catecholamines were extracted from the plasma using acid-washed WA-4 Alumina (Sigma) and 1.5 M Tris buffer containing 2% EGTA at a pH of 8.6. Perchloric acid (0.1 M) was used to elute the catecholamines. Finally, 100 μl of this eluent were injected in the HPLC system (Electrochemistry Separations Analysis, ESA, model LC/EC, 5200A; Coulochem, Chelmsford, MA). The mobile phase was Cataphase 2 (ESA, Cambridge, MA), and the electrodes were set at +350, +50, and -350 mV. Standard catecholamine solutions were purchased from ESA. Chromatographs were analyzed using an ESA 501 Data Chromatography System.

Isotopic enrichments and lactate and glucose concentration analyses

Lactate and glucose were prepared for gas chromatography/mass spectrometry (GC/MS) analysis using the heptafluorobutyric anhydride and pentaacetate derivatives, respectively. Known amounts of uniformly-labeled internal standards [U-^{13}C]lactate and [U-^{13}C]glucose were added to the supernatant samples collected in 8% perchloric acid. Samples were then neutralized with 2N KOH and transferred to ion exchange columns that were previously washed with double deionized water (ddH$_2$O) through a cation resin (Analytical Grade 50W-X8, 50-100 mesh H$^+$ resin, Bio-Rad Laboratories, Hercules, CA) and with ddH$_2$O followed by 2N formic acid through an anion resin (Analytical Grade 1-X8, 100-200 mesh formate resin). Glucose was eluted first with ddH$_2$O followed by elution of lactate through the anion column with 2N formic acid. The samples were then transferred to a 2-ml gas chromatography vial and lyophilized. Glucose analyses and kinetics data are reported separately (21).

For derivatization, lactate samples were resuspended in 200 μl of 2,2-dimethoxypropane and transferred to a vial to which 20 μl 10% HCl in methanol was added. After samples sat at room temperature for 60 min, 50 μl of *N*-propylamine was added. Samples were then heated for 30 min at 100 °C and subsequently dried under a stream of N$_2$ gas, resuspended in 200 μl ethyl acetate, transferred to a GCMS vial and dried again under N$_2$ gas, resuspended in 20 μl of heptafluorobutyric anhydride, left for 5 min at room temperature to react and dried under N$_2$ gas. Finally, the derivatized lactate was resuspended in 50 μl ethyl acetate.

Lactate IEs were determined by GC/MS (GC model 6890 series and MS model 5973N, Agilent Technologies). Methane was used for chemical ionization with selected ion monitoring of mass-to-charge ratios (m/z) 328 (non-labeled lactate), 329 (M+1 isotopomer, [3-^{13}C]lactate),

and 331 (M+3 isotopomer, [U-^{13}C]lactate internal standard). Whole blood lactate concentrations were determined by an abundance ratio of 328/331.

The expired air samples were stored at room temperature until analyzed via isotope ratio mass spectrometry (IRMS) by Metabolic Solutions (Nashua, NH) (data not shown).

Calculations

Calculations of lactate kinetics were performed during the last 30 min of rest and 20 min of exercise. Lactate flux i.e. rate of appearance (R_a, mg·kg^{-1}·min^{-1}), rate of disposal (R_d, mg·kg^{-1}·min^{-1}) and metabolic clearance rate (MCR, ml·kg^{-1}·min^{-1}) were calculated from the equations of Steele modified for use with stable isotopes (80):

$$R_a = \frac{F - V\,[(C_1 + C_2)\,/\,2]\,[(IE_2 - IE_1)\,/\,(t_2 - t_1)]}{(IE_2 + IE_1)\,/\,2} \tag{1}$$

$$R_d = R_a - V\,[(C_2 - C_1)\,/\,(t_2 - t_1)] \tag{2}$$

$$MCR = R_d\,/\,[(C_1 + C_2)\,/\,2] \tag{3}$$

where: *F* represents isotope infusion rate (mg·kg^{-1}·min^{-1}), V is the volume of distribution for lactate (180 ml/kg); C_1 and C_2 are concentrations (mg/l) at sampling times t_1 and t_2, respectively; IE_1 and IE_2 are the excess isotopic enrichments of lactate at these sampling times.

Statistical analyses

Descriptive statistics are expressed as means ± SE. Significance of differences in subject characteristics between UT and T were analyzed using a Mann-Whitney U-test. Differences in responses of parameters in the transition from rest to exercise were analyzed using paired t-tests. Differences in parameters between the UT and the three T conditions within rest and exercise were analyzed using a one-way analysis of variance. Comparisons of results within trained subjects across conditions were done by repeated measures analyses of variance. Post hoc analyses to identify where significant differences occurred across conditions were made by Fisher's least significant difference multiple comparison tests. When called for by stated hypotheses of results of previous investigations (e.g., exercise increases lactate kinetics over rest), one-tailed comparisons were made. Relationships between the different variables were studied by means of linear, polynomial or exponential regression techniques. Statistical significance was set at α = 0.05 i.e., *P* < 0.05.

RESULTS

Anthropometric and dietary data of subjects as well as their cardio-ventilatory responses to graded tests and isotope tracer trials are reported separately (21). Some previously presented results are repeated here for the convenience of the reader or when used for novel calculations.

Physiological responses of subjects to VO$_{2max}$ and LT determination tests

Compared to untrained subjects, cyclists had significantly higher VO$_{2max}$ (*P* < 0.05) and PO$_{max}$ (*P* < 0.05) (Table 1). Figure 1 shows typical blood lactate evolution curves during the

incremental exercise used to determine the LT. LT-associated VO_2 and percentage of VO_{2max} (%VO_{2max}) were significantly higher in trained cyclists compared to untrained subjects ($P < 0.05$).

Exercise power outputs and relative metabolic rates during isotope tracer trials

Absolute POs during the LT trial in untrained subjects (161 ± 4 W) were lower than those during either LT (259 ± 10 W, $P < 0.05$) or LT-10% trials (234 ± 9 W, $P < 0.05$) in cyclists. Relative metabolic rates (%VO_{2max}) were similar in the untrained LT trial compared to cyclists at LT-10% and LT-10%+LC (~67% VO_{2max}). However, relative exercise intensity was significantly higher during the LT trial in the cyclists (75% VO_{2max}, $P < 0.05$).

Lactate, glucose and hormone-concentrations during isotope tracer trials

Before tracer or LC infusions, resting [lactate]$_b$ values were lower ($P < 0.05$) in UT subjects (0.57 ± 0.09 mmol/l) than in T cyclists, no matter the trial (1.08 ± 0.09 mmol/l) (Figure 2). Resting [lactate]$_b$ was similar among the trials in the T group (*NS*). To elevate [lactate]$_b$ to LT levels in T subjects during the LT-10%+LC trial, mean exogenous lactate infusion rates were 3.91 ± 0.26 mg·kg^{-1}·min^{-1} and 3.16 ± 0.95 mg·kg^{-1}·min^{-1} during rest and exercise, respectively. LC increased resting [lactate]$_b$ to 4.30 ± 0.39 mmol/l which was significantly higher than all other conditions ($P < 0.05$). After 10 min of exercise, [lactate]$_b$ rose to reach 4.11 ± 1.01 mmol/l in UT, and 3.71 ± 1.02, 2.57 ± 1.20 and 4.49 ± 1.36 mmol/l in T during LT, LT-10% and LT-10%+LC, respectively. Blood lactate concentrations remained stable during the entire period of exercise in all trials (Figure 2A). During LT trials in UT and T and during LT-10%+LC in T, [lactate]$_b$ was not different (Figure 2B). During LT-10% in T, [lactate]$_b$ was significantly lower than the three other trials ($P < 0.05$).

Resting blood [glucose] was elevated in UT subjects (5.4 ± 0.1 mmol/l) compared with T cyclists (5.0 ± 0.1 mmol/l) (21). During exercise at LT, blood [glucose] was significantly lower in the UT than T subjects (5.1 ± 0.2 *vs.* 5.9 ± 0.4 mmol/l, $P < 0.05$). Blood [glucose] during exercise in LT-10% and LT-10%+LC trials in T were not different from that during LT in UT (21).

At rest, plasma concentrations of epinephrine and norepinephrine ([epinephrine] and [norepinephrine]) were not different between UT and T (21). During exercise, [epinephrine] and [norepinephrine] displayed striking changes, increasing by 3 to 8 and 6 to 13 fold, respectively, compared to their resting values ($P < 0.05$). Epinephrine levels were similar during the LT trials of UT and T (*NS*), which were higher than during the LT-10% and LT-10%+LC trials ($P < 0.05$). Norepinephrine levels were lower during the LT trial in UT than during the LT-10% in T ($P < 0.05$), the latter was itself lower than [norepinephrine] during LT in T ($P < 0.05$). The LC procedure dampened [epinephrine] and [norepinephrine] by 33% (*NS*) and 49% ($P < 0.05$), respectively, as compared to the LT-10% values (21).

Lactate kinetics

Arterialized blood isotopic enrichments achieved steady conditions during each experimental condition (Figure 3), thus justifying use of steady-rate assumptions for computation of metabolic kinetics.

At rest, lactate rate of appearance (R_a, Figure 4A) was significantly increased ($P < 0.05$) by exogenous lactate infusion (i.e., LT-10%+LC trial). Lactate R_a was not different between the three other resting trials. In all treatments, R_a increased from rest to exercise ($P < 0.05$). For the LT-10%+LC trial, R_a increased from rest to exercise by 4.4 fold, while for the three other trials, the increase from rest to exercise ranged between 7.3 to 9.3 fold. Lactate R_a at LT was 65%

higher in T than in UT (24.1 ± 2.7 *vs.* 14.6 ± 2.4 mg·kg^{-1}·min^{-1}, $P < 0.05$). Lactate R_a during LT-10% (18.2 ± 2.6 mg·kg^{-1}·min^{-1}) was not statistically different than R_a during LT in UT (*NS*), but was 24% and 36% lower than R_a during LT ($P = 0.06$) and LT-10%+LC ($P < 0.05$) in T. During the LT-10%+LC trial, R_a (28.4 ± 2.8 mg·kg^{-1}·min^{-1}) was higher than that during exercise at LT in UT ($P < 0.05$) and not significantly different from R_a at LT in T (*NS*).

At rest, lactate rates of disposal (R_d, Figure 4B) were not significantly different among non-LC conditions (ranging from 1.8 ± 0.1 to 2.6 ± 0.6 mg·kg^{-1}·min^{-1}, *NS*). For the LT-10%+LC trial, lactate R_d was significantly higher than during the three other trials (6.6 ± 0.7 mg·kg^{-1}·min^{-1}, $P < 0.05$). R_d increased from rest to exercise in all treatments ($P < 0.05$). R_d during exercise at LT was 61% higher in T than in UT (24.2 ± 2.8 *vs.* 15.0 ± 2.8 mg·kg^{-1}·min^{-1}, $P < 0.05$). R_d during LT-10% (18.1 ± 2.6 mg·kg^{-1}·min^{-1}) was not statistically different from R_d during LT in UT (*NS*), but was 25% and 36% lower than lactate R_d during LT ($P = 0.06$) and LT-10%+LC ($P < 0.05$) in T. During the LT-10%+LC trial, R_d (28.4 ± 2.8 mg·kg^{-1}·min^{-1}) was higher than that during exercise at LT in UT ($P < 0.05$) and not significantly different from R_d at LT in T (*NS*).

At rest, lactate metabolic clearance rates (MCR, Figure 4C) were not different among non-LC conditions, but MCR was decreased in the LT-10%+LC condition as compared to LT in UT ($P < 0.05$). In all T conditions, MCR during exercise was higher than during rest ($P < 0.05$). In contrast, MCR in UT did not change significantly from rest to exercise. During exercise at LT, MCR was 34% greater in T compared to UT (62.5 ± 5.0 *vs.* 46.5 ± 7.0, ml·kg^{-1}·min^{-1}, respectively; $P < 0.05$, one-tailed). In T, MCR during the LT-10% trial (91.5 ± 14.9 ml·kg^{-1}·min^{-1}) was 46% greater than at LT, and 97% greater than in UT who exercised at the same relative intensity ($P < 0.05$). When relative exercise intensity and [lactate]$_b$ were matched in UT and T groups (i.e., comparing UT-LT *vs.* T-LT-10%+LC), MCR was significantly higher by 64% due to endurance training (76.2 ± 5.9 ml·kg^{-1}·min^{-1} in LT-10%+LC trial).

Lactate R_a as functions of exercise intensity and catecholamine concentrations

Lactate R_a rose exponentially as a function of metabolic rate during exercise, whether expressed as VO$_2$ (l/min) (Figure 5A), or as %VO$_{2max}$ (Figure 5B). Similarly, plasma [epinephrine] and [norepinephrine] also rose exponentially as functions of metabolic rate whether expressed on absolute (VO$_2$, l/min), or relative (%VO$_{2max}$) bases (Figure 6). Of note in Figure 6 is that pre-exercise [epinephrine] is elevated in trained athletes, and that the catecholamine response to exercise is greater in athletes than untrained men. Lactate R_a rose linearly as functions of [epinephrine] and [norepinephrine] (Figures 7A and 7B, respectively). Of note in Figure 7A, the slope of the regression between lactate R_a and [epinephrine] is greater in athletes than in untrained men. Also of note is that slopes of regression lines changed in response to the LC procedure because exogenous infusion both raised lactate R_a and suppressed sympathetic nervous system activity.

DISCUSSION

Here we report the first attempt to determine and interpret lactate kinetics in trained and untrained men exercising at the lactate threshold. Major findings are: lactate flux rates at the lactate threshold are much greater in trained cyclists than in untrained subjects, and a decline in MCR occurs as power outputs approach those that elicit the LT. As such, our conclusion is that while endurance training increases the capacities for lactate production, disposal and clearance,

regardless of training state the lactate threshold represents the point at which clearance of lactate becomes limited.

Lactate kinetics at the lactate threshold in trained subjects: a function of exercise intensity

No previous study specifically determined lactate kinetics during exercise at the lactate threshold. In our present study, lactate R_a, R_d and MCR values obtained during exercise performed by trained cyclists at the LT were higher than the values previously reported in the literature during submaximal exercise performed by untrained, active or short-term trained men (7, 13, 46-48, 53, 58, 73), but were very close to those reported in highly-trained cross-country skiers exercising approximately at the same relative exercise intensity, i.e. ~75% of VO_{2max} (75). Because the LT represents very closely the highest absolute and relative workload for which lactate concentrations remain at steady state (35), the training status of the subjects and consequently the elevated absolute and relative (to VO_{2max}) power outputs they achieved while exercising at the LT must be considered when attempting to understand the very high lactate flux values achieved by trained cyclists.

In the aggregate, present and past results (7, 47, 53, 72) indicate a direct, exponential, relationship between lactate R_a and metabolic rate (VO_2) elicited by exercise (Figure 5A). The apparent rightward shift in the lactate R_a *vs*. VO_2 curve (Figure 5A) is due to the greater exercise power outputs sustained by subjects in the present investigation. However, when normalized to relative exercise intensity, it is apparent from results of the several studies depicted that lactate R_a is closely related to %VO_{2max} (Figure 5B).

The relationships between lactate R_a and circulating catecholamines (Figure 7) indicate a role of sympathetic nervous system (SNS) activation in determining lactate kinetics. Increased plasma catecholamine concentrations during exercise result from increased SNS activity and from spillover at terminal SNS nerve endings as well as SNS-stimulated secretions by the adrenal medullas (45, 67). High circulating levels of norepinephrine affect cardio-dynamics as well as regional blood flow distribution, including splanchnic vasoconstriction, whereas elevated circulating epinephrine stimulates muscle glycogenolysis (24, 62, 79) and lactate production. Both vasoactive and metabolic effects of circulating catecholamines have the potential to affect lactate kinetics. The linear relationships between lactate R_a and circulating catecholamines (Figure 7) have been previously observed (13).

Lactate clearance declines as the LT is approached

Initial studies of lactate kinetics using radiotracers in laboratory rats (19) showed that lactate MCR increases from rest to moderate intensity exercise, but then decreases from moderate to hard exercise. Similar results have been subsequently obtained on humans in previous investigations (7, 47, 48, 72, 73). Figure 8 illustrates the relationship between lactate MCR and absolute and relative metabolic rates in the present and former studies involving healthy men of variable fitness levels. The top curve of Figure 8 reflects the very high flux rates obtained in the present investigation on well-trained cyclists (VO_{2max} = 5.0 l/min) as compared to the lower curves obtained on healthy men of variable but lesser fitness levels (VO_{2max} = 2.6, 3.5 and 4.1 l/min). In the present study, when PO was raised from LT-10% to LT workloads, trained cyclists experienced a 60% increase in [lactate]$_b$, but only a 33% increase in R_d, resulting in a 30% decline in MCR. These results suggest that at workloads approaching the LT, lactate MCR declines rapidly.

Oxidation (~70-80%) and gluconeogenesis (~20-30%) account for most whole-body lactate disposal during exercise (6, 7, 48, 54). Lactate uptake and subsequent utilization by consumer tissues (especially active oxidative muscle fibers, liver, kidney, heart, lungs and brain) have been reported to be directly dependent on lactate delivery to these tissues as determined from blood lactate concentration and regional blood flow (1, 2, 5, 7, 15, 29, 40, 46-48, 55, 56, 61, 75, 76). Previous experiments underlined that when PO was increased, hepatic blood flow decreased (55, 56, 65, 66), counterbalancing the associated increase in blood lactate concentrations and curbing lactate uptake by the liver (56). The lack of increase in gluconeogenesis from lactate when exercise intensity increased despite a rise in blood lactate concentrations (21) may come from this leveling-off in hepatic lactate uptake, likely attributable to reductions in splanchnic blood flow, and may contribute to the decline of lactate MCR during elevated intensity exercise.

A limitation in lactate transport into consumer tissues including working muscle may also account for the decline in MCR when exercise intensity increases from moderate to high intensity. Because ~60-80% of lactate R_d is accounted for by active-limb lactate uptake (7), of which ~90-95% is oxidized directly in muscle (15), a limitation of lactate transport into the active muscles during exercise is deleterious for lactate clearance. To be taken up and subsequently utilized by active muscles, lactate needs first to cross cell membranes. This transport occurs by facilitated diffusion via monocarboxylate transporters, i.e. the MCT family, which co-transport lactate and H^+ in a 1:1 ratio (27, 28, 41, 63, 64). The transport of lactate is affected by sarcolemmal transporter content, but is driven by transmembrane lactate anion and H^+ gradients, both of which change with exercise intensity. At low exercise intensities, muscle and blood lactate concentrations and their gradients (16, 44) are favorable for the entry of lactate into myocytes. However, as exercise intensity increases and more muscle and more type II muscle fibers are recruited, muscle and blood lactate concentrations, as well as their gradients, become less favorable for the influx into, but rather facilitate release (efflux) of lactate from active muscle, all effects detrimental for lactate clearance. Conversely, the LC, by increasing [lactate]$_b$, alters the gradients, in effect "pushing" lactate into the tissues that are net lactate consumers, possibly accounting for the observed increase in R_d in the LT-10%+LC trial. Taken together, these results indicate that the transport of lactate across the muscle membrane might constitute a possible limiting factor for subsequent utilization. As well, tissues other than liver, kidneys and active muscles, such as the integument, might be involved in the decline in MCR when exercise intensity transitions from moderate to hard. Further studies would be necessary to specifically investigate the underlying mechanisms limiting MCR during exercises at LT.

Regardless of the mechanisms of lactate transport and uptake, and the limitations to those mechanisms, as shown in Figures 4 and 8, the higher MCR during LT-10% as compared to LT trial strongly suggests that the LT is due to a limitation of MCR. Rephrased, any increase in power output above that which elicits the LT induces an increase in lactate R_a that cannot be accommodated by a corresponding rise in lactate R_d, thus causing a continuous rise in [lactate]$_b$. Hence, results of the present study (Figures 4 and 8) show that the optimal lactate MCR is below the LT. In that sense, it is interesting to note that in most endurance activities (e.g., cross-country skiing and rowing) athletes predominantly train at exercise intensities below the LT (22, 50).

Effects of endurance training on lactate kinetics for exercises performed at the LT and the same relative exercise intensities

Exercise at the LT required higher absolute mechanical and metabolic power outputs to be performed by the trained subjects, as compared to their untrained counterparts. Accordingly,

lactate R_a and R_d were higher by 65% in T than in UT even though exercise at LT resulted in almost similar [lactate]$_b$ in both groups (Figure 2). Consequently, MCR was higher in T than in UT (Figure 4), while T exercised at higher relative workloads than UT (75 vs. 67% of VO_{2max}, respectively).

The comparison of data obtained during the LT trial in UT and the LT-10% trial in T (both at ~67% VO_{2max}) showed the effects of long-term endurance training on lactate kinetics during exercise of similar relative intensity. As already well-described (7, 39, 52), [lactate]$_b$ was lower for a same relative exercise intensity in the trained subjects. Furthermore, R_a and R_d were not different, while MCR was 97% higher in the trained cyclists. Accordingly, our current results of a training-induced increase in lactate MCR agree with results of previous studies describing the effects of endurance training on lactate MCR (7, 47). An important distinction between results of this and previous studies lies in magnitude. Previously, 9 weeks of endurance training increased lactate MCR by ~70-75% (7, 47); in contrast the current results indicate that long-term endurance training can increase lactate MCR by 97%. This latter comparison underlines that long-term training may further improve MCR at a given relative workload.

Our experimental design allowed comparisons of UT and T individuals while exercising at specific blood lactate levels. Elevation of [lactate]$_b$ using the LC procedure allowed comparison of lactate kinetics between untrained and trained subjects at the same relative workload and similar [lactate]$_b$. In other words, comparison of LT in UT and LT-10%+LC in T allowed determination of the effects of endurance training *per se* on lactate kinetics. As suspected, R_d was importantly improved in the trained cyclists subjected to the LC. Consequently, at the same relative workload and similar [lactate]$_b$, R_d of the trained cyclists was 89% higher than that of their untrained counterparts (Figure 4B). As a result, MCR remained significantly higher by 64% ($P < 0.05$) in trained subjects than in untrained for the same relative (i.e., higher absolute) exercise intensity and similar [lactate]$_b$.

In the aggregate, the present results (Figures 4 and 8) extend the current knowledge and support the idea (7, 47) that endurance training improves lactate disposal and clearance, reinforcing the Lactate Shuttle Concept. Because active muscles account for the majority of lactate disposal during exercise (7, 15), improvements in lactate MCR after endurance training are likely due to adaptations involving greater lactate removal and oxidation in active muscles. Endurance training has been shown to alter LDH activity by shifting its distribution towards a higher proportion of its H-LDH isoenzyme (20, 52, 70), which is more favorable for lactate oxidation to pyruvate than the M-LDH isoenzyme. Endurance training has also been shown to increase muscle oxidative capacity and lactate oxidation via enhancements of mitochondrial mass and the expression of mitochondrial constituent proteins, including citrate synthase (CS) and cytochrome oxidase (COx) (4, 20, 30, 36, 37, 52, 57). Importantly, with regard to mitochondrial lactate oxidation, the first step is catalyzed by lactate dehydrogenase (LDH) that has been found to be present in the mitochondria of skeletal muscle (3, 10, 14, 32, 43, 49, 68) and which is necessary for mitochondrial lactate oxidation (10, 14, 43). Furthermore, endurance training has been shown to enhance lactate transport capacity (59, 60) and muscle content of MCT1 (9, 20, 60), the isoform most abundant in the sarcolemmal and mitochondrial membranes of oxidative muscle (20, 32, 34). Of note, LDH, MCT1 and COx constitute a mitochondrial lactate oxidation complex (mLOC) (14, 31, 32, 34). Although the effect of training on the mLOC remains to be investigated, it is interesting to note that lactate, whose turnover is increased during exercise, acts as a hormone (a "lactormone") that activates a cascade which upregulates MCT1 and COX gene and protein expression and mitochondrial biogenesis (31, 33).

Unsolved questions and limitations

In the present study, UT subjects displayed lower resting [lactate]$_b$ than T cyclists (either at LT or LT-10%), for reasons that are not clearly known. Previous studies have not reported any differences in resting [lactate]$_b$ between (short- or long-term) trained and untrained men (7, 8, 17). However, the values we reported for our trained and untrained subjects were within the wide range of the literature for resting [lactate]$_b$ (~ 0.3 to 1.7 mmol·l^{-1}) [e.g., (7, 17, 25, 53)]. One possible explanation would be that the difference in resting [lactate]$_b$ was due to an anticipatory autonomic response in the trained athletes prior to exercise. In a previous report (21) we noted that pre-exercise heart rates were similar in the untrained men and athletes we studied. Knowing the training-induced bradycardia at rest (71), our suspicion of an anticipatory sympathetic response in athletes is justified based on our observations of elevated epinephrine concentrations in trained subjects pre-exercise, and exaggerated epinephrine responses to exercise in trained athletes than in untrained men (Figure 6A). Elevated catecholamine levels in trained men during exercise have been observed by others (42) and us (26).

Based on our experience, we expected that during the LC trial, endogenous lactate production for LT-10% and exogenous infusion would have been additive during exercise (53). Contrary to this expectation, endogenous lactate R$_a$ was substantially augmented during LC (Figure 4A). As well we noted that glucagon concentrations, glucose R$_a$, and RER responded also differently to LC during exercise in the present study on athletes (21) compared to responses seen previously on non-athletes (53). Further studies are required to investigate specifically why LC induces different metabolic responses in trained and untrained subjects.

And finally, because of the cross-sectional design of the present study we cannot exclude the effects of genetic factors to explain the differences in lactate flux rate, exercise power output, and response to the LC procedure of athletes in comparison to results obtained on non-athletes.

Summary and conclusions

We used combinations of exercise intensity and a lactate clamp technique involving exogenous lactate infusion to interrogate the meaning of the lactate threshold in healthy young males and competitive male cyclists. Exercise and exogenous infusion resulted in significant increases in lactate kinetics. In trained cyclists exercising at the lactate threshold, we observed the greatest values of lactate flux ever reported. In both healthy controls and trained cyclists, lactate clearance rose during the transition from rest to moderate intensity exercise, but MCR fell when the exercise task came close to eliciting the LT, suggesting that LT would be determined by a limitation in MCR. In contrast to the comparison in trained men exercising at lactate threshold and a power output lesser by 10%, exogenous lactate infusion resulted in an increase in lactate disposal. That result was interpreted to mean that lactate MCR at the LT was limited by endogenous, intramuscular lactate production. We conclude that while endurance training increases the capacities for lactate production, disposal and clearance for higher absolute as well as relative workload, the lactate threshold represents the point at which clearance of endogenous lactate becomes limited.

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GRANTS

DISCLOSURES

GAB has a financial interest in CytoSport; otherwise, the authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

LAM, JAF and CWE performed experiments; MAH was responsible for gas chromatography and mass spectrometry (GCMS); CWE was responsible for enzymatic and GCMS and statistical analyses; JAF was responsible for enzymatic and hormonal analyses; TJC was responsible for medical clearance and safe conduct of experimentation; TJC, MAH, CWE and GAB edited and revised manuscript; all authors approved final version of manuscript; GAB and LAM were responsible for the conception and design of research; CWE and GAB analyzed data; GAB interpreted results of experiments; GAB and LAM drafted manuscript.

TABLE

Table 1. *Physiological responses to maximal oxygen* consumption *(VO$_{2max}$, upper panel) and lactate threshold (LT, lower panel) determination tests for untrained and trained groups*

Variable	UT	T
fH$_{max}$ (beats/min)	189 ± 5	189 ± 3
VE$_{max}$ (l/min)	115 ± 8	144 ± 13 (*)
VO$_{2max}$ (l/min)	3.7 ± 0.1	5.0 ± 0.3 *
PO$_{max}$ (W)	248 ± 7	357 ± 12 *
fH at LT (beats/min)	158 ± 5	168 ± 3
%fH $_{max}$ at LT (%)	83 ± 3	89 ± 1
VO$_2$ at LT (l/min)	2.57 ± 0.09	3.85 ± 0.21 *
%VO$_{2max}$ (%)	69 ± 3	77 ± 2 *
[lactate]$_b$ at LT (mmol/l)	4.3 ± 0.6	3.6 ± 0.6

Values are means ± SE; n = 6 for untrained (UT) and trained (T) groups; See the text for abbreviations. Differences between groups: $^{(*)}$ = $0.05 < P < 0.10$, * = $P < 0.05$.
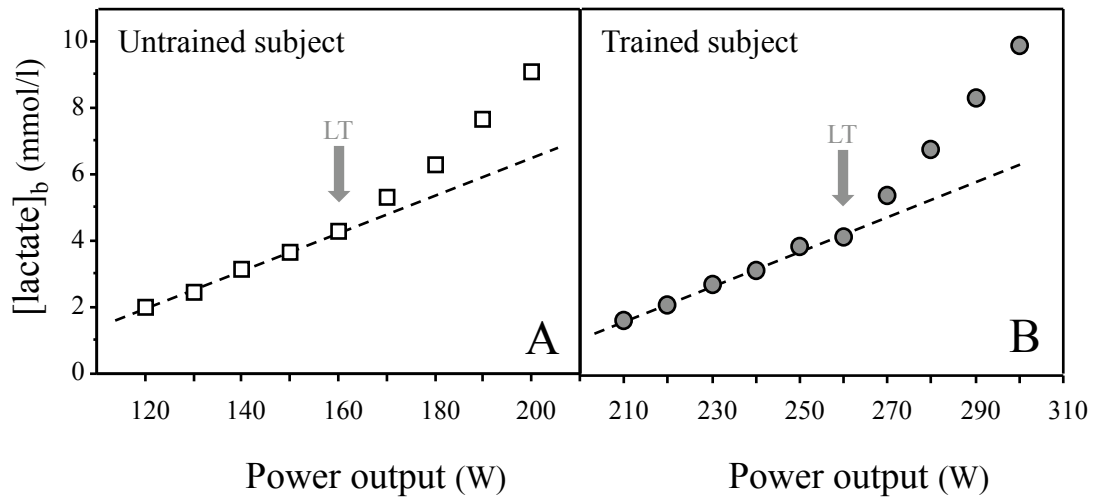
FIGURES

Figure 1: Typical blood lactate evolution curves obtained in untrained (A) and trained (B) subjects during the LT determination test. Deviation from the dashed line is indicative of the acceleration of blood lactate accumulation, i.e. the LT. Of note, the dashed line should not be considered as demonstrating a linear relationship between blood lactate concentrations and power output.
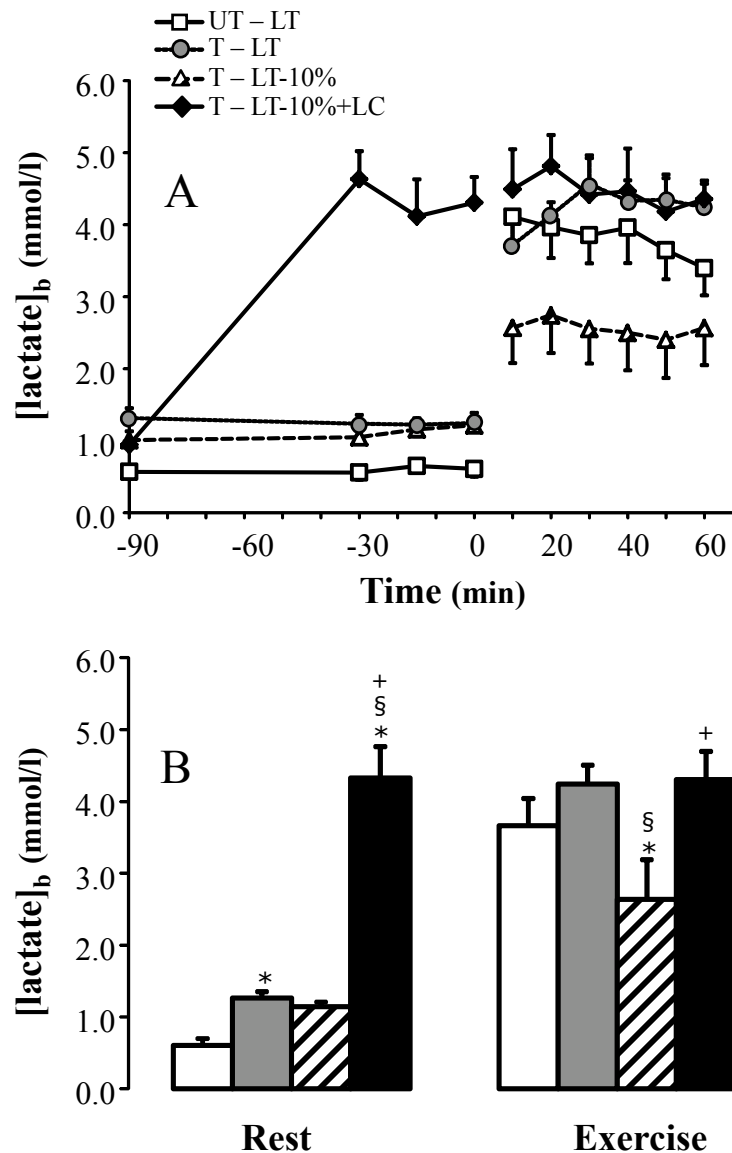
Figure 2: Time course (A) and mean values (B) of blood lactate concentrations at rest and during exercise at LT, LT-10% and LT-10%+LC in untrained (UT) and trained (T) subjects. Values are means ± SE. n = 6 for UT and T groups. Symbols: LT is lactate threshold; LT-10% is 10% below the LT workload; LT-10%+LC is LT-10% with a lactate clamp; White squares are LT trial in UT subjects (UT-LT); dark grey circles, striated triangles and black diamonds are LT, LT-10% and LT-10%+LC trials in T subjects (T-LT, T-LT-10% and T-LT-10%+LC, respectively); * Significantly different from UT-LT ($P < 0.05$); § Significantly different from T-LT ($P < 0.05$); + Significantly different from T-LT-10% ($P < 0.05$). Parentheses mean a trend ($P < 0.10$).
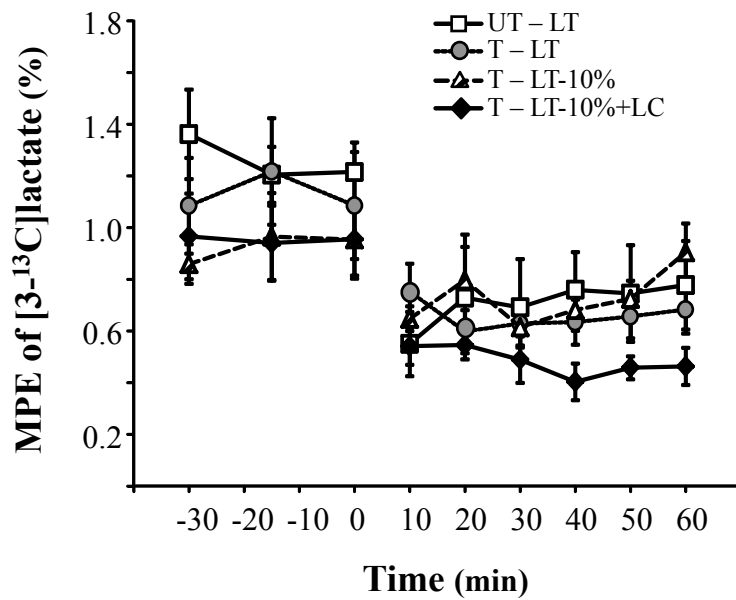
Figure 3: Mole percent excess (MPE, in %) of [3-^{13}C]lactate (M+1 lactate). Values are means ± SE. Symbols are as in Figure 2.
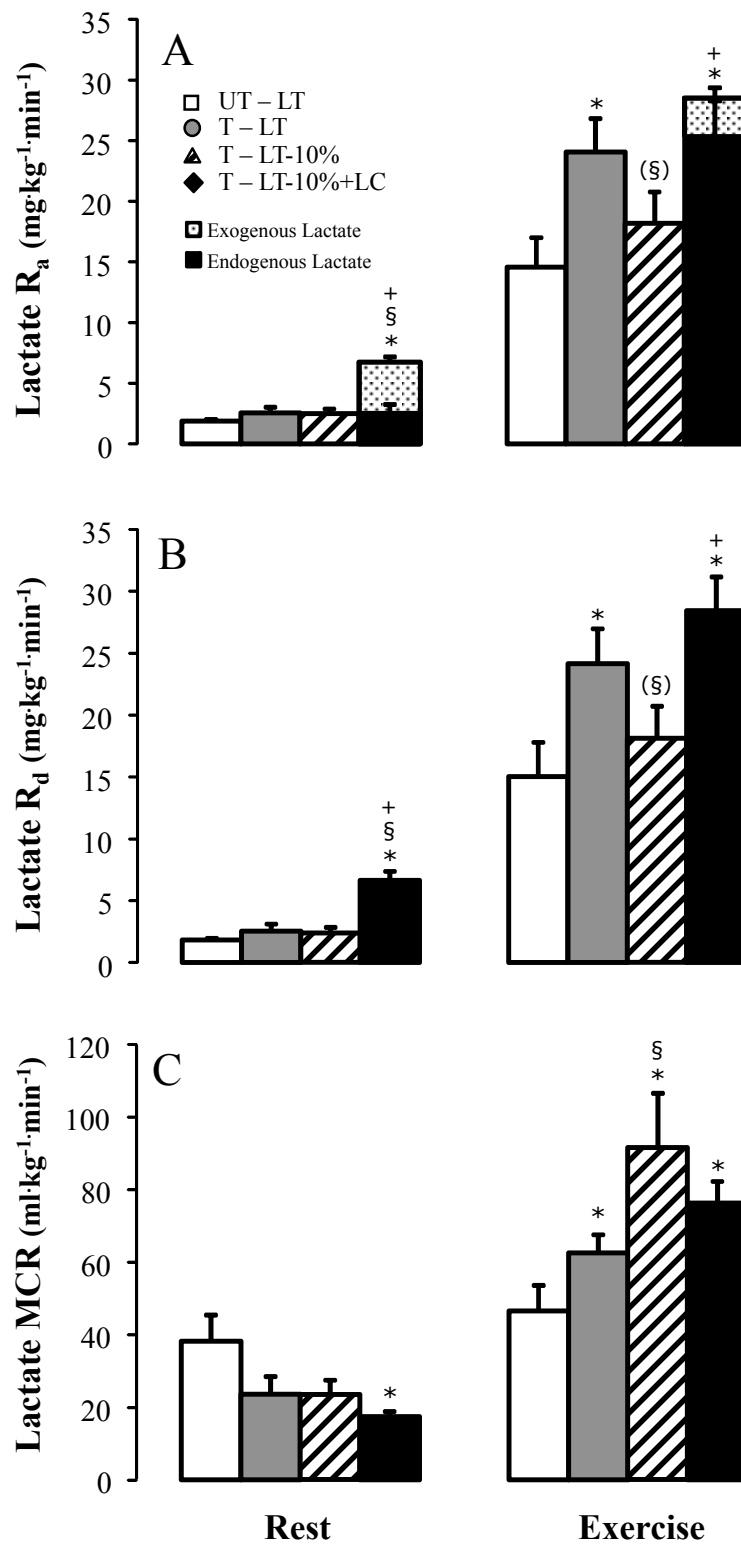
Figure 4: Lactate rates of appearance (Lactate R_a) (A) and disposal (Lactate R_d) (B), and metabolic clearance rate of lactate (Lactate MCR) (C) at rest and during 60 min of exercise at LT, LT-10% and LT-10%+LC in untrained and trained subjects. Values are means ± SE. Symbols are as in Figure 2.

Figure 5: Lactate R_a in function of absolute (VO_2) (A) and relative (to VO_{2max}) (B) metabolic rates elicited at rest and exercise in the present and previous studies involving subjects of different physical fitness statuses. VO_{2max} values (mean ± SE) of UT and T are 3.7 ± 0.1 and 5.0 ± 0.3 l/min.
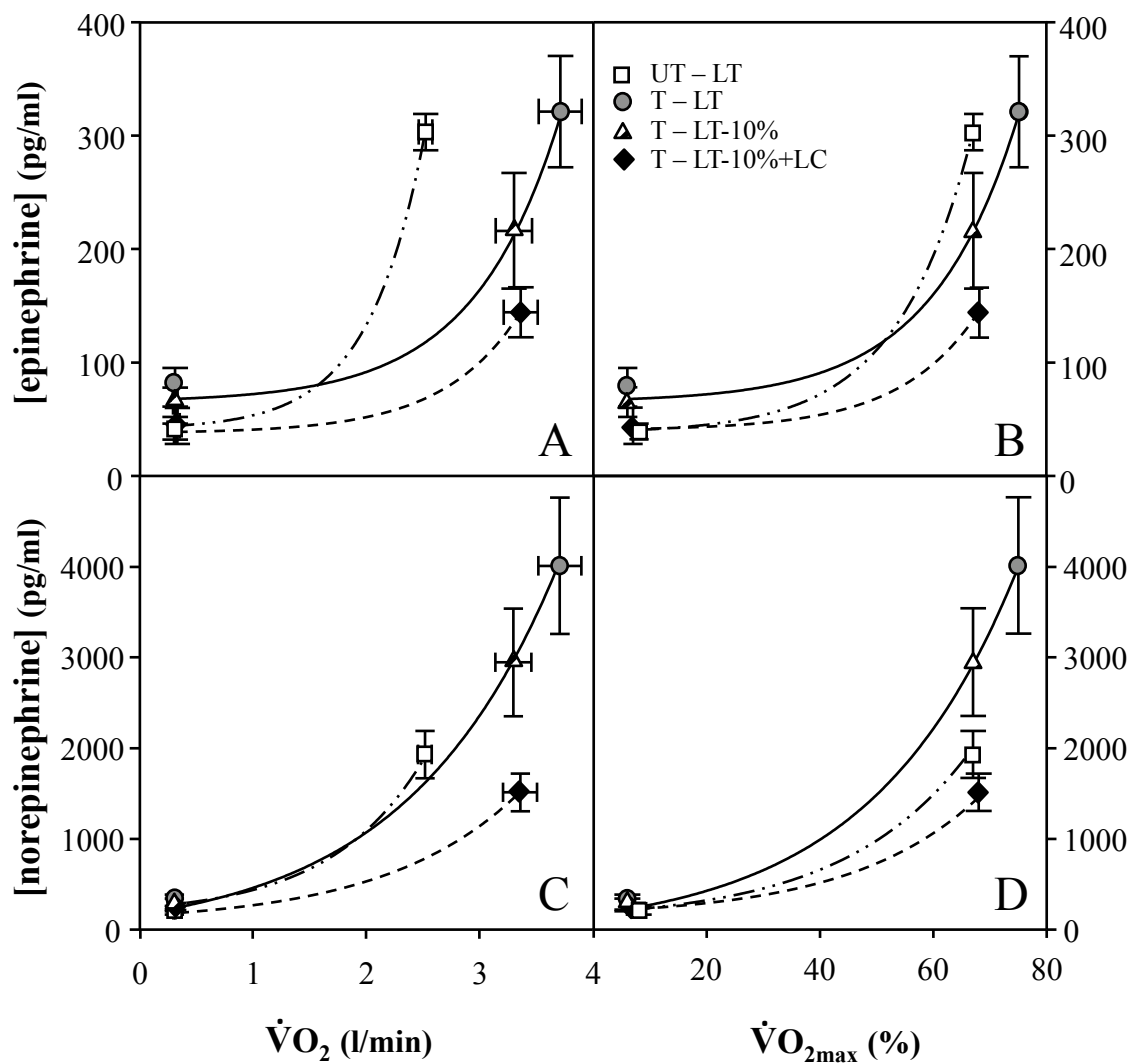
Figure 6: Plasma epinephrine (A and B) and norepinephrine (C and D) concentrations ([epinephrine] and [norepinephrine], respectively) in function of absolute (VO_2) and relative (to VO_{2max}) metabolic rates elicited at rest and exercise. Symbols are as in Figure 2.
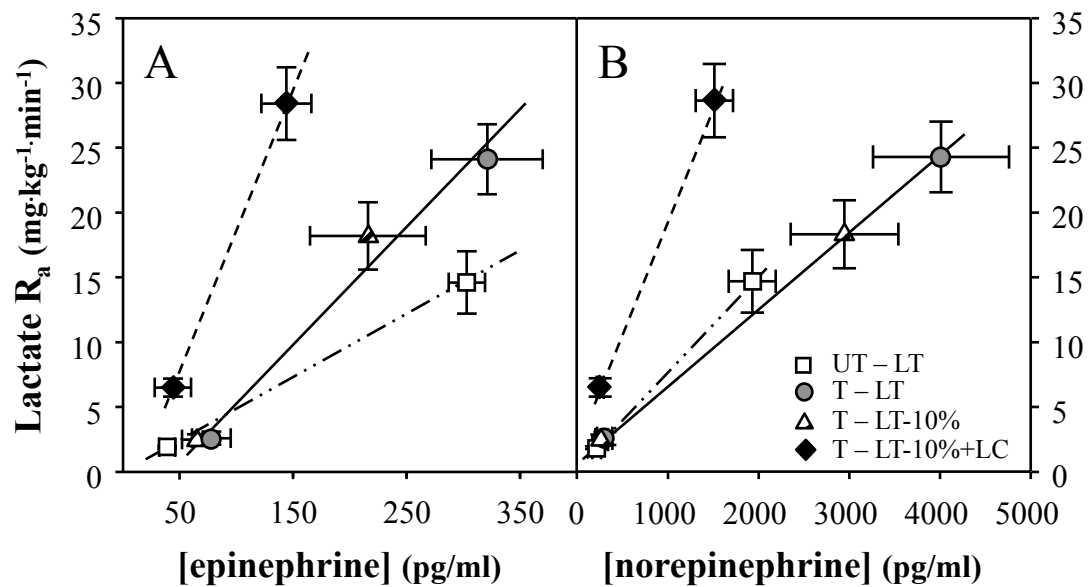
Figure 7: Lactate R_a in function of plasma [epinephrine] (A) and [norepinephrine] (B). Symbols are as in Figure 2.
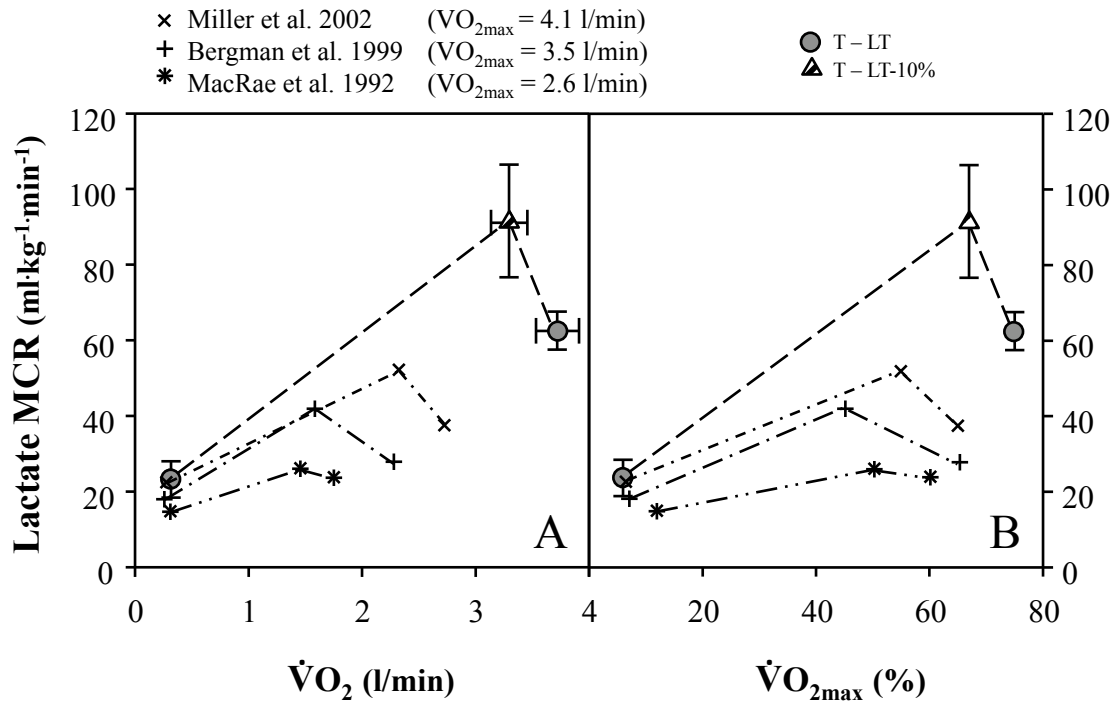
Figure 8: Lactate metabolic clearance rate (MCR) in function of absolute (VO_2) (A) and relative (to VO_{2max}) (B) metabolic rates elicited at rest and exercise in the present and previous studies involving subjects of different physical fitness statuses. Symbols are as in Figure 2. VO_{2max} value (mean ± SE) of T is 5.0 ± 0.3 l/min.

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CHAPTER 4

Direct and Indirect Lactate Oxidation in Trained and Untrained Men

ABSTRACT

Lactate has been shown to be an important oxidative fuel. We aimed to quantify total lactate oxidation rate (R_{ox}) and its direct versus indirect (glucose that is gluconeogenically derived from lactate and subsequently oxidized) components ($mg \cdot kg^{-1} \cdot min^{-1}$) during rest and exercise in humans. We also investigated the effects of endurance training, exercise intensity, and blood lactate concentration ($[lactate]_b$) on direct and indirect lactate oxidation. Six untrained (UT) and six trained (T) men completed 60 min of constant load exercise at power outputs (PO) corresponding to their lactate threshold (LT). Trained subjects completed two additional 60-min sessions of constant load exercise at 10% below the LT workload (LT-10%), one of which included a lactate clamp (LT-10%+LC). R_{ox} was higher at LT in T (22.7 ± 2.9, 75% VO_{2peak}) compared to UT (13.4 ± 2.5, 68% VO_{2peak}, $P < 0.05$). Increasing $[lactate]_b$ (LT-10%+LC, 67% VO_{2peak}) significantly increased lactate R_{ox} (27.9 ± 3.0) compared to its corresponding LT-10% control (15.9 ± 2.2, $P < 0.05$). Direct and indirect lactate oxidation rates increased significantly from rest to exercise and their relative partitioning remained relatively constant in all trials, but differed between T and UT: direct oxidation comprised 75% of total lactate oxidation in UT and 90% in T suggesting the presence of training-induced adaptations. Partitioning of total carbohydrate (CHO) utilization showed that subjects derived one-third of CHO energy from blood lactate, and exogenous lactate infusion significantly increased lactate oxidation, causing a glycogen sparing effect in exercising muscle.

INTRODUCTION

Lactate is now widely recognized as an important oxidative fuel (8, 11, 16, 26, 27, 30, 40, 55, 57) rather than a dead-end waste product of anaerobic metabolism. Under resting conditions, lactate uptake from the circulation occurs in various tissues, including, but not exclusive to, cardiac muscle (21, 39, 54, 74), skeletal muscle (8, 9, 20, 26, 31, 41, 64, 67, 68) and brain (49, 67) for oxidative metabolism, and liver and kidneys for gluconeogenesis (7, 33, 50, 55, 62). Lactate has also been shown to contribute directly to glycogen synthesis in liver and indirectly in muscle (2-4, 13, 58). Previous measurements of lactate disposal and oxidation in humans have used methods including arterio-venous difference measurements (8, 18, 20, 71), infusion of isotope tracers (14, 39, 55-57, 60, 61, 70-72), and muscle biopsies (8, 18, 51), all of which have shown substantial levels of lactate oxidation in active skeletal muscle. However, because lactate is the primary gluconeogenic precursor, a substantial portion of lactate availability in the circulation leads to the production of glucose (7, 30, 33, 50, 55, 62), which may subsequently undergo oxidation.

When circulating lactate enters cells for oxidative disposal, the route is considered to be "direct oxidation" of blood lactate. Conversely, when lactate is converted to glucose through hepatic or renal gluconeogenesis, subsequent oxidation of the lactate-derived glucose monomer can be considered as "indirect oxidation" of the precursor lactate. Most studies utilizing the methodologies described above either do not discriminate between, or lack the means to simultaneously measure these two routes of lactate oxidation. Thus, the present investigation aimed to quantify the rates of direct and indirect lactate oxidation during rest and exercise in humans, and to investigate the individual and combined effects of endurance training, exercise intensity, and blood lactate concentration on their relative partitioning.

We hypothesized that endurance training increases direct lactate oxidation during exercise whether determined at a given relative exercise intensity as assessed by peak oxygen consumption ($\%VO_{2peak}$) or at the lactate threshold. To test this hypothesis, we studied endurance-trained and untrained men exercising at LT, and we used indirect calorimetry and stable isotope tracers to determine glucose and lactate fluxes and lactate oxidation rates. As well, we incorporated a lactate clamp component involving exogenous infusion to test the hypothesis that an increase in blood lactate availability would increase direct lactate oxidation, sparing the utilization of other carbohydrate sources, specifically muscle glycogen and blood glucose.

METHODS

This manuscript presents results from a large investigation of lactate and glucose metabolism during exercise at the lactate threshold. Many details of methodology are reported separately (33, 59), but are repeated here for the convenience of the reader.

Subjects

Twelve healthy male subjects (6 untrained and 6 trained) aged 19-33 yr were recruited from the University of California Berkeley campus and the surrounding community by posted notices, word of mouth, and email. Untrained subjects (UT) were healthy and recreationally active (peak oxygen consumption, VO_{2peak}, <50 ml·kg^{-1}·min^{-1}). Trained subjects (T) were

members of competitive cycling or triathlon teams, currently in the race phase of their training season (VO_{2peak} >55 ml·kg^{-1}·min^{-1}). Subjects were included in the study if they had a body mass index (BMI) ≥18 and <26 kg/m^2, were non-smokers, were diet and weight stable, had a FEV_1/FVC >70%, and were injury/disease free as determined by physical examination. This study was approved by the University of California Berkeley Committee for the Protection of Human Subjects (CPHS 2010-4-1300) and conformed to the standards set by the Declaration of Helsinki. All subjects gave written informed consent prior to participation in the study.

Preliminary testing

Exercise tests were performed on an electronically braked leg cycle ergometer (Monark Ergometric 839E, Vansbro, Sweden) and were conducted at least one week apart. Following interviews and screening, subjects performed two graded exercise tests to determine VO_{2peak} and lactate threshold (LT). To determine VO_{2peak}, as per American College of Sports Medicine guidelines (7th ed.), exercise power output (PO) started at 75 or 120 W and was increased by 25 or 30 W for the UT and T groups, respectively, every 3 min until volitional fatigue. Expired respiratory gases were continuously monitored throughout the test via an open-circuit automated indirect calorimetry system (ParvoMedics TrueOne Metabolic System, Salt Lake City, UT) that was calibrated using room air and a certified calibration gas. Finger pricks drawing 10 μl of blood were conducted at the end of each stage to measure blood lactate concentration ([lactate]$_b$) via portable lactate analyzers (Nova Lactate Plus, Waltham, MA) and to approximate the PO eliciting the LT.

To determine LT, a second graded exercise test started at 50 W below the approximated LT power output and increased by 10 W every 3 min until volitional exhaustion. At the end of every stage, 1 ml of blood was drawn from a warmed "arterialized" hand vein for enzymatic analysis of [lactate]$_b$, which increased slowly with exercise work rate until a certain PO. After this point, a rapid acceleration in blood lactate accumulation occurred. The LT was considered to be the last stage of the slow increase in [lactate]$_b$ before the rapid acceleration in lactate accumulation. This definition of the LT is termed by some as the second lactate turn point (45). For both graded exercise tests, heart rate was monitored continuously using a heart rate monitor (Polar, Gay Mills, WI) and electrocardiography (Quinton 759 ECG, Seattle WA), rating of perceived exertion (RPE) was recorded according to the Borg scale (10), and blood pressure was measured at the middle of every stage by manual auscultation.

On a subsequent day, subjects performed 60 min of continuous exercise at the PO corresponding to their LT to ensure stabilization of [lactate]$_b$ over the entire duration of the exercise test. Every 10 min, finger pricks drawing 10 μl of blood were conducted to measure [lactate]$_b$, and heart rate and RPE were monitored.

Experimental design

The study design consisted of four conditions using stable isotope tracers: one condition within the UT group, and three conditions within the T group. UT subjects completed one isotope infusion trial, consisting of a 90-min rest period followed by 60 min of continuous cycling at the LT. T subjects completed three isotope infusion trials, each consisting of a 90-min rest period followed by 60 min of continuous leg ergometer cycling under one of the following conditions: *1)* PO eliciting the lactate threshold (LT), *2)* PO 10% below that eliciting the LT (LT-10%), and *3)* PO 10% below that eliciting the LT, but with a lactate clamp (exogenous

lactate infusion) to raise [lactate]$_b$ to the LT level (LT-10%+LC). The order of the last two conditions was randomized, and all exercise trials were conducted at least one week apart.

To evaluate the effects of training on metabolic responses, we compared: UT and T groups exercising at the same [lactate]$_b$, but different absolute and relative intensities (UT-LT *vs.* T-LT); at the same relative intensity, but different [lactate]$_b$ (UT-LT *vs.* T-LT-10%); and at the same relative intensity and [lactate]$_b$ (UT-LT *vs.* T-LT-10%+LC). Within the T group, we investigated: the effects of exercise intensity given the same [lactate]$_b$ (LT *vs.* LT-10%+LC); the effects of [lactate]$_b$ given the same exercise intensity (LT-10% *vs.* LT-10%+LC); and the combined effects of [lactate]$_b$ and exercise intensity (LT *vs.* LT-10%).

Dietary controls

Three-day diet records were collected prior to the study to record subjects' caloric intake and macronutrient composition (DietAnalysis Plus, version 6.1, ESHA Research, Salem, OR). Subjects also provided complete 24-h diet records of the day preceding each exercise session. Standardized diets (approximately 50% CHO, 30% fat, 20% protein) were given the day prior to each exercise trial, including dinner and an evening snack as the last meal. Diets, consisting of an average of 2400 and 3200 kcal for the UT and T groups, respectively, were individualized and determined for each participant considering a physical activity level (PAL) of 1.25 for UT and 1.48 for T according to the current dietary reference intake guidelines of the Institute of Medicine for Estimated Energy Requirement. Subjects came to the laboratory overnight-fasted, and exercise commenced 12 h after consuming the evening snack. No food or beverages were allowed except water (*ad libitum*) until the end of the exercise session.

Isotope tracer protocol

Subjects reported to the laboratory on the morning of each isotope tracer trial, and expired respiratory gases were sampled for a measurement of background $^{13}CO_2$ enrichment. A catheter was then placed into a warmed hand vein for arterialized blood sampling, and a background sample was collected. Through a second catheter placed in the antecubital vein of the contralateral arm, subjects received a primed continuous infusion of [6,6-^{2}H$_2$]glucose (D$_2$-glucose) and [3-^{13}C]sodium-lactate (Sigma-Aldrich, St. Louis, MO). Isotopes were diluted in 0.9% sterile saline and were tested for pyrogenicity and sterility (School of Pharmacy, University of California, San Francisco, CA) and passed through a 0.2-μm Millipore filter (Nalgene, Rochester, NY) prior to infusion. Priming boluses contained 250 mg D$_2$-glucose, 57.5 mg [3-^{13}C]sodium-lactate, and 136 mg [^{13}C]sodium-bicarbonate (Isotec, Sigma-Aldrich, St. Louis, MO). Glucose and lactate tracers were then continuously infused for the 90-min rest period via a calibrated pump (Baxter Colleague 3CX, Deerfield, IL) at 2.0 mg/min for glucose and 2.5 mg/min for lactate in all trials, with the exception of the LT-10%+LC trial that had a resting infusion rate of 7.5 mg/min for lactate. The LT-10%+LC trial also included infusion of an unlabeled lactic acid-sodium hydroxide mixture to target a specific [lactate]$_b$ during the rest period (see Lactate Clamp Procedure). At the start of all exercise trials, tracer infusion rates were increased to 8 mg/min for glucose and 11.25 mg/min for lactate for the UT group and 10 mg/min for glucose and 15 mg/min for lactate for the T group, and continued for 60 min of exercise.

Lactate clamp procedure

In the LT-10%+LC trial, a lactate clamp (LC) procedure was performed as previously described (61). An unlabeled lactate cocktail was prepared by mixing a 30% lactic acid solution (Sigma-Aldrich, St. Louis, MO) in 2N sodium hydroxide to a pH of 4.8, and subsequently tested for pyrogenicity and sterility at the UCSF School of Pharmacy. During the rest period, unlabeled lactate infusion began at 4.9 mg·kg^{-1}·min^{-1} to raise [lactate]$_b$ to that obtained individually during the LT trial. Infusion rates were adjusted during rest and exercise to maintain the target [lactate]$_b$ as determined by a portable lactate analyzer.

Blood and respiratory gas sampling

Arterialized blood was drawn from a warmed hand vein for metabolite, isotopic enrichment (IE), and hormonal analyses at 0 (background), 60, 75, and 90-min of rest and 10, 20, 30, 40, 50, and 60-min of exercise. Hematocrit was also measured at each time point using a circular microcapillary tube reader (International Equipment Company, no. 2201, Needham Heights, MA). Blood for glucose and lactate concentration and IE determinations was immediately deproteinized with 8% perchloric acid, shaken, and placed on ice. All samples were centrifuged at 3000 g for 18 min, and the supernatant was transferred to storage tubes and frozen at -80°C until analysis.

Respiratory gases were analyzed continuously via indirect calorimetry for 5 min before, and coincident with blood sampling. These measurements were used for calculation of oxygen consumption (VO$_2$), carbon dioxide production (VCO$_2$), respiratory exchange ratio (RER), and minute ventilation (V$_E$). Duplicate samples of expired air were collected in 10-ml evacuated containers for ^{13}CO$_2$ isotopic enrichment determinations. Careful attention was placed on flushing the line and sampling expired CO$_2$ at the same time as blood was drawn for glucose and lactate IE. Heart rate, blood pressure, and RPE measurements were also recorded at the same frequency as blood and breath sampling.

Metabolite concentration and isotopic enrichment analyses

Methods for the determination of glucose (33) and lactate (59) concentrations and isotopic enrichments have been described previously. Briefly, known amounts of uniformly-labeled internal standards [U-^{13}C]glucose and [U-^{13}C]lactate were added to the supernatant samples collected in 8% perchloric acid. Samples were then neutralized with 2N KOH and transferred to ion exchange columns that were previously washed with double deionized water (ddH$_2$O) through a cation resin (Analytical Grade 50W-X8, 50-100 mesh H$^+$ resin, Bio-Rad Laboratories, Hercules, CA) and with ddH$_2$O followed by 2N formic acid through an anion resin (Analytical Grade 1-X8, 100-200 mesh formate resin). Glucose was eluted first with ddH$_2$O followed by elution of lactate through the anion column with 2N formic acid. Subsequent glucose analyses and kinetics data are reported separately (33).

Lactate IE was determined by gas chromatography/mass spectrometry (GC/MS; GC model 6890 series and MS model 5973N, Agilent Technologies) of the *N*-propylamide heptafluorobutyrate derivative, where methane was used for selected ion monitoring of mass-to-charge ratios (m/z) 328 (unlabeled lactate), 329 (M+1 isotopomer, [3-^{13}C]lactate), and 331 (M+3 isotopomer, [U-^{13}C]lactate internal standard). Whole blood lactate concentration was determined by abundance ratios of 328/331. Selected ion abundances were compared against external standard curves for calculation of concentration and IE.

Breath samples were analyzed via isotope ratio mass spectrometry by Metabolic Solutions (Nashua, NH).

Calculations

For calculations of oxidation rates, data from the last 30 min of rest (i.e., 60, 75 and 90 min) and the last 20 min of steady-rate exercise (i.e., 40, 50 and 60 min) were used. Lactate rates of appearance (R_a), disposal (R_d), and metabolic clearance (MCR) are reported separately (59). Total lactate rate of oxidation (Total lactate R_{ox}, $mg \cdot kg^{-1} \cdot min^{-1}$) was calculated from expired CO_2 and $IECO_2$ (77):

$$\text{\%Lactate } R_d \text{ oxidized} = \frac{(IECO_2 \times VCO_2 \times 90.08) \times 100}{(F \times k \times 22.4)} \quad (1)$$

$$\text{Total lactate } R_{ox} = (\text{Lactate } R_d \times \text{\%Lactate } R_d \text{ oxidized}) / 100 \quad (2)$$

where: $IECO_2$ is the excess isotopic enrichment of expired $^{13}CO_2$; VCO_2 is in l/min; 90.08 is the molecular weight of $[3-^{13}C]$lactate; F is the $[3-^{13}C]$lactate infusion rate in $mg \cdot kg^{-1} \cdot min^{-1}$; k is the correction factor for the retention of CO_2 in body pools as determined previously (44) to be 0.83 at rest and 1.0 during exercise; and 22.4 is the molar volume of CO_2, a non-ideal gas, under STP conditions.

Calculations for the rate of gluconeogenesis from lactate (R_{gng}, $mg \cdot kg^{-1} \cdot min^{-1}$) and glucose rate of disposal (glucose R_d, $mg \cdot kg^{-1} \cdot min^{-1}$) are reported separately (33). Indirect and direct lactate oxidation rates (Indirect lactate R_{ox} and Direct lactate R_{ox}, respectively; both in $mg \cdot kg^{-1} \cdot min^{-1}$) and glucose oxidation rate (Glucose R_{ox}, $mg \cdot kg^{-1} \cdot min^{-1}$) were calculated:

$$\text{Indirect lactate } R_{ox} = R_{gng} \times c \quad (3)$$

$$\text{Direct lactate } R_{ox} = \text{Total lactate } R_{ox} - \text{Indirect lactate } R_{ox} \quad (4)$$

$$\text{Glucose } R_{ox} = \text{Glucose } R_d \times c \quad (5)$$

where: c is relative glucose oxidation as determined previously to be 0.25 at rest, 0.20 at rest with LC (61), 0.80 during exercise with and without LC (36, 37, 60, 61).

Because exogenous lactate infusion was not expected to affect lipid oxidation (22, 34), the overall CHO rate of oxidation (CHO R_{ox}, $mg \cdot kg^{-1} \cdot min^{-1}$) was derived from the equation of Consolazio et al. (25) assuming negligible contribution of protein oxidation:

$$\text{CHO } R_{ox} = \frac{(4.55 \times VCO_2) - (3.21 \times VO_2) \times 1000}{m} \quad (6)$$

where: VCO_2 and VO_2 are in l/min; and m is the subject's body mass in kg. The rate of muscle glycogen oxidation ($mg \cdot kg^{-1} \cdot min^{-1}$) was calculated as the remaining CHO utilization after glucose and lactate:

$$\text{Muscle glycogen } R_{ox} = \text{CHO } R_{ox} - \text{Glucose } R_{ox} - \text{Direct lactate } R_{ox} \quad (7)$$

Because indirect lactate R_{ox} is included in the measurement for glucose R_{ox}, only direct lactate R_{ox} was used for the determination of glycogen R_{ox}. Lipid rate of oxidation (lipid R_{ox}, g/min) was also calculated from the equation of Consolazio et al. (25) assuming negligible contribution of protein oxidation:

$$\text{Lipid } R_{ox} = (1.67 \times VO_2) - (1.67 \times VCO_2) \qquad (8)$$

From oxidation values, we further estimated caloric expenditures (kcal/min) from lactate, glucose, muscle glycogen and lipid using 4.2 and 9.5 for kilocalories provided per gram of oxidized CHO and lipid, respectively.

Statistical analyses

Significance of differences in subject characteristics between untrained and trained groups were analyzed using an unpaired Student's t-test. Differences in metabolic parameters between the untrained and the three trained conditions within rest and exercise were analyzed using a one-way analysis of variance. Analyses within the trained conditions were done with repeated measures. Post hoc analyses were made using Fisher's least significant difference multiple comparisons test. Differences from rest to exercise were analyzed using a paired Student's t-test. Statistical significance was set at $\alpha = 0.05$, and values are represented as means ± SE.

RESULTS

Anthropometric and dietary data of untrained (UT) and trained (T) subjects as well as cardio-ventilatory and hormonal responses to graded tests and isotope tracer trials are reported separately (33, 59). Glucose (33) and lactate (59) kinetics data, including rates of appearance, disposal and metabolic clearance are also reported separately. Some previously presented results are repeated here for the convenience of the reader or when used for novel calculations.

Isotopic enrichment of expired $^{13}CO_2$

Isotopic enrichments of expired $^{13}CO_2$, presented as atom percent excess (APE) over time (Figure 1) was significantly higher in the LT-10%+LC trial compared to the LT and LT-10% trials in the T group at rest ($P < 0.05$). Greater $^{13}CO_2$ during the lactate clamp (LC) protocol was consistent with the increased lactate tracer infusion rates that were three times higher in the LC protocol during the rest period than those during the non-LC trials. During exercise, APE of expired $^{13}CO_2$ in the LT-10%+LC trial was significantly higher compared to the T-LT trial ($P < 0.05$), despite equal [lactate]$_b$ (Table 1) and tracer infusion rates. During the transition from rest to exercise, the increase in tracer infusion rate closely matched the increase in metabolic rate, as a slight, though significant, increase in $^{13}CO_2$ APE was observed in all trials ($P < 0.05$), except for the T-LT trial ($P = 0.07$), corroborating an increased flux and metabolism of endogenous lactate in trained subjects exercising at the LT.

Lactate disposal and its partitioning between oxidation and gluconeogenesis

At rest, lactate rates of disposal (R_d) were not different across non-LC resting conditions in both UT and T groups (Table 1), whereas exogenous lactate infusion raised lactate R_d nearly

threefold (59). In non-LC trials, the percentage of lactate R_d oxidized was ~45% (Table 1). Within the T group, the increase in disposal with LC did not correspond to an increase in oxidation (Figure 2), as the percentage of lactate R_d oxidized in the LT-10%+LC trial was only half the percentage in the non-LC trials (Table 1, $P < 0.05$). During rest, $50.4 \pm 10.6\%$ of lactate R_d in the UT group supported glucose production, whereas in the non-LC trials of the T group approximately 20-23% of lactate R_d appeared as glucose (Table 1, $P < 0.05$). Lactate conversion to glucose was only $6.2 \pm 1.4\%$ of lactate R_d in the LT-10%+LC trial, where [lactate]$_b$ was four times higher than in the non-LC conditions ($P < 0.05$).

During the transition from rest to exercise, lactate R_d and percentage of lactate R_d oxidized significantly increased in all trials (Table 1, $P < 0.05$). During exercise at LT, lactate R_d was 60% greater ($P < 0.05$) in the T group (75% VO_{2peak}) compared to the UT group (68% VO_{2peak}) despite no significant difference in [lactate]$_b$. When trained subjects exercised at LT-10% (67% VO_{2peak}), lactate R_d was not different than UT. However, lactate R_d increased by nearly 60% when exogenous lactate infusion raised [lactate]$_b$ to similar levels as in UT exercising at the same relative work rate ($P < 0.05$). In both UT and T groups exercising at ~67% VO_{2peak}, approximately 89% of lactate R_d was oxidized, which was lower than the LT ($93.5 \pm 1.2\%$) and LT-10%+LC trials in T ($97.6 \pm 2.3\%$), although these differences were significant only when compared to the UT group ($P < 0.05$). Similar to resting conditions, the UT group demonstrated a greater reliance on gluconeogenesis (GNG) for lactate removal during exercise, where $25.7 \pm 6.4\%$ of lactate R_d appeared as glucose. Only ~9% of lactate R_d supported glucose production in the non-LC trials of the T group, and $15.9 \pm 3.7\%$ in the LT-10%+LC trial.

Lactate oxidation and its partitioning between direct and indirect oxidation

During rest, lactate oxidation rates (R_{ox}) were similar across all non-LC trials (Figure 2A). Absolute partitioning of direct and indirect lactate R_{ox} showed that exogenous lactate infusion during the rest period significantly increased direct and decreased indirect lactate R_{ox} compared to those of the UT group ($P < 0.05$).

There was a significant increase in lactate R_{ox} in the transition from rest to exercise in all four trials (Figure 2A). Total lactate R_{ox} was proportional to relative intensity during exercise, with the exception of the LT-10%+LC trial, where total lactate R_{ox} was augmented by 75% during exercise (27.9 ± 3.0 mg·kg^{-1}·min^{-1}) compared to its corresponding LT-10% control (15.9 ± 2.2 mg·kg^{-1}·min^{-1}, $P < 0.05$). At LT, total lactate R_{ox} was 70% higher in the T group (22.7 ± 2.9 mg·kg^{-1}·min^{-1}, 75% VO_{2peak}) compared to the UT group (13.4 ± 2.5 mg·kg^{-1}·min^{-1}, 68% VO_{2peak}, $P < 0.05$). During exercise, absolute direct R_{ox} was significantly higher in the LT and LT-10%+LC trials of the T group compared to the other two trials ($P < 0.05$). Exogenous lactate infusion also significantly increased indirect R_{ox} compared to the non-LC trials of the T group ($P < 0.05$).

Relative partitioning of direct and indirect lactate R_{ox} (Figure 2B) showed a significant training effect that increased direct lactate oxidation from ~75% of total lactate R_{ox} in the UT group to ~90% in the T group non-LC trials ($P < 0.05$) during both rest and exercise. This relative partitioning was consistent regardless of activity level. At rest, LC did not alter direct and indirect lactate oxidation partitioning as compared to the other T trials. During exercise, LC resulted in relative partitioning that was intermediate between the UT and non-LC T trials.

When represented as a percentage of lactate disposal, we estimated that at rest, 33-40% of lactate R_d underwent direct oxidation in UT and non-LC T trials (Table 1). This partition was

significantly decreased by exogenous lactate infusion, where only $22.4 \pm 3.6\%$ of lactate R_d was directly oxidized in the LT-10%+LC trial. During the transition from rest to exercise, percentage of lactate R_d directly oxidized more than doubled, increasing significantly to $68.2 \pm 4.9\%$ in UT and 82-87% in T trials ($P < 0.05$). Differences between groups regarding the indirect lactate oxidation proportion during exercise almost mirrored that of direct oxidation, i.e., $20.5 \pm 5.1\%$ of lactate R_d was indirectly oxidized in the UT group exercising at LT, whereas 7-13% of lactate R_d was indirectly oxidized in the exercise trials of the T group.

Partitioning of carbohydrate oxidation

Determined by pulmonary gas exchange, total carbohydrate (CHO) oxidation rates (R_{ox}) were slightly higher in the T group than that in the UT group during rest (Figure 3A, $P < 0.05$). There was a significant increase in total CHO R_{ox} in the transition from rest to exercise in all four trials ($P < 0.05$). Proportional to absolute workload during exercise, total CHO R_{ox} in the T-LT trial was significantly higher than those in the other trials, and total CHO R_{ox} in the T trials were higher than the UT trials ($P < 0.05$). Absolute partitioning of CHO R_{ox} showed that glucose R_{ox} was not different across trials, with the exception of LT-10%+LC where exogenous lactate infusion slightly increased glucose oxidation compared to its corresponding LT-10% control (7.0 ± 0.7 mg·kg^{-1}·min^{-1} vs. 6.0 ± 0.7 mg·kg^{-1}·min^{-1}, $P < 0.05$). Determined as the remaining CHO utilization next to glucose and direct lactate oxidation, muscle glycogen R_{ox} was significantly higher ($P < 0.05$) during exercise in the T-LT (26.4 ± 3.7 mg·kg^{-1}·min^{-1}) and T-LT-10% (22.9 ± 1.7 mg·kg^{-1}·min^{-1}) trials compared to the UT-LT (14.2 ± 3.2 mg·kg^{-1}·min^{-1}) and T-LT-10%+LC (11.9 ± 2.3 mg·kg^{-1}·min^{-1}) trials.

Relative partitioning of CHO oxidation (Figure 3B) showed that the T group had a lower contribution from blood glucose to CHO metabolism compared to the UT group (~17% vs. 36% at rest, ~13% vs. 22% during exercise, $P < 0.05$). Providing exogenous lactate in the LT-10%+LC trial decreased the reliance on blood glucose at rest ($12.5 \pm 3.3\%$ vs. $18.2 \pm 3.7\%$, $P < 0.05$), but increased glucose utilization during exercise at 67% VO_{2peak} ($16.4 \pm 1.8\%$ vs. $13.9 \pm 1.7\%$, $P < 0.05$). The relative contribution of direct lactate oxidation to total CHO metabolism was significantly augmented due to LC during rest ($43.0 \pm 7.1\%$ vs. $28.6 \pm 5.3\%$, $P < 0.05$) and exercise ($55.8 \pm 4.7\%$ vs. $32.9 \pm 2.9\%$, $P < 0.05$) compared to its corresponding LT-10% control. Due to the combined increases in direct lactate and glucose oxidation in the exercise LT-10%+LC trial, relative muscle glycogen utilization was significantly decreased compared to the non-LC trials in the T group ($P < 0.05$).

Caloric expenditure from carbohydrates and lipids

Resting metabolic rate in both UT and T groups was 1.8 kcal/min (Table 2), where lipid accounted for approximately 47-69% of total caloric expenditure (*NS* across trials). During exercise, total caloric expenditure was proportional to absolute workload, but percentage of lipid contribution was inversely proportional to relative exercise intensity, accounting for ~22% of total caloric expenditure in UT and T groups exercising at 67% VO_{2peak}. In the T-LT trial (75% VO_{2peak}), lipid accounted for only 16% of total caloric expenditure, although these differences did not achieve significance.

DISCUSSION

In this investigation, we examine the individual and combined effects of endurance training, exercise intensity, and blood lactate concentration ($[lactate]_b$) on rates of lactate oxidation during exercise at and just below the lactate threshold (LT). Our main findings support the hypotheses that endurance training increases the capacity for lactate oxidation; in particular, the relative contribution of direct lactate oxidation to total lactate oxidation is increased regardless of whether subjects are resting or exercising. During exercise, an increase in $[lactate]_b$ via exogenous infusion increases lactate oxidation substantially and glucose oxidation slightly, resulting in significant sparing of muscle glycogen during exercise as compared to its corresponding control.

In Figure 4, we summarize our view of the relationship between lactate and glucose turnover in the blood. Lactate appearance is shown to stem primarily from muscle glycogenolysis and to a lesser extent glycolysis of blood glucose. Disposal of lactate includes gluconeogenesis (GNG) and direct oxidation, with muscle glycogen synthesis having a minor role. Lactate that enters GNG can support hepatic glycogen synthesis or glucose production, which is also dependent on hepatic glycogenolysis. Disposal of glucose includes oxidation and muscle or hepatic glycogen synthesis. A small portion of glucose disposal also re-enters the blood lactate pool. In this manuscript, we highlight the distinction between direct and indirect lactate oxidation, where the latter consists of lactate supporting glucose production and then undergoing oxidation as glucose.

Direct and Indirect Lactate Oxidation

Measurements of whole body lactate oxidation have been previously reported in both laboratory mammals (15, 29, 35, 50) and humans (14, 55-57, 60, 61, 70-72). However, to our knowledge, the present investigation is the first to provide estimates of direct and indirect partitioning of lactate oxidation in resting and exercising humans (Figure 2). While absolute direct and indirect oxidation rates increase significantly from rest to exercise, their relative partitioning remains approximately constant regardless of activity level within the untrained (UT) and trained (T) groups. Direct oxidation remains the primary route of removal, comprising 75% of total lactate oxidation in the UT group and increasing up to 90% in the T group. Reciprocally, indirect lactate oxidation was significantly lower in T than in UT.

It is well established that splanchnic blood flow is inversely related to exercise intensity (1, 66), possibly limiting GNG at high exercise intensities (55, 75). In addition to local blood flow, $[lactate]_b$ also determines precursor supply to the liver and therefore GNG. Despite higher PO (LT and LT-10% trials) and/or lower $[lactate]_b$ (LT-10% trial), trained subjects did not exhibit significantly different GNG rates than UT (33). This result indicates that endurance training indeed has enhancing effects on GNG capacity (7, 33). Despite this training-attributed adaptation (i.e., similar GNG in T and UT during exercise at or close to LT), relative contribution of direct and indirect lactate oxidation were respectively higher and lower in T than in UT. These findings suggest that in trained subjects, lactate is a preferred substrate over glucose. Furthermore, the fact that a greater contribution of direct lactate oxidation was observed in the T group under resting conditions with and without LC suggests that blood flow distribution and precursor concentration were unlikely to have been major components in the preferential direct utilization of lactate observed in the present investigation. Rather, there may be underlying training-induced adaptations that allow direct oxidation of lactate to occur, such

as expansion of apparatus described in the intercellular and intracellular lactate shuttles (11, 27). Indeed, endurance training has been shown to increase expression of sarcolemmal and mitochondrial monocarboxylate transporter (MCT) 1 and sarcolemmal MCT 4 (32), facilitating the uptake of circulating lactate and intramuscular lactate exchange and oxidation.

The intracellular lactate shuttle hypothesis (11) suggests that oxidation begins in the mitochondrial intermembrane space where lactate is converted to pyruvate via a posited mitochondrial lactate oxidation complex (mLOC) of MCT1, lactate dehydrogenase (LDH), cytochrome oxidase (19) and CD147 (42, 43). Select colocalization of these putative mLOC components on the inner mitochondrial membrane has been shown using confocal laser-scanning microscopy (CLSM) in skeletal myocytes (42), cardiac myocytes (52), rat neurons (43) and other cells (76). Cellular locations of MCT1 and succinate dehydrogenase (SDH) have also been reported to match (38), further linking MCT1 to the mitochondrial reticulum. Moreover, strong associations found among mitochondrial MCT1, CD147, LDH and COx were not replicated by coprecipitation of complex I NADH dehydrogenase, suggesting a specific coupling with complex IV during mitochondrial electron transport (42). Most recently, a human biopsy study demonstrated that not only are skeletal muscle mitochondria capable of utilizing lactate for mitochondrial respiration, but also the conversion of lactate to pyruvate in the intermembrane space supports the existence of a functional mLOC (51). Therefore, as repeated muscle contraction during long-term training stimulates biogenesis of the mitochondrial reticulum, including a proportional increase in mitochondrial enzymes (5, 46-48, 53), the presence of a mLOC provides a plausible mechanism by which endurance training increases the capacity to clear and oxidize lactate directly regardless of activity level.

Because increasing $[lactate]_b$ via a lactate clamp (LC) provides substrate for both oxidation and GNG, LC did not significantly alter the relative partitioning of direct and indirect lactate oxidation in overnight-fasted subjects under resting conditions. Conversely, during exercise at LT-10% (67% VO_{2peak}), direct and indirect lactate oxidation rates were increased by 70% and 160%, respectively, with LC, suggesting that GNG may exhibit a greater reliance on substrate availability.

Lactate, Glucose, and Muscle Glycogen Oxidation

Total carbohydrate (CHO) oxidation is the summed utilization of blood lactate, blood glucose and muscle glycogen. In our estimates, total CHO oxidation increases proportionally to exercise workload (Figure 3A). Interestingly, while exercise intensity plays little or no significant role in the partitioning of CHO utilization between blood lactate, blood glucose and muscle glycogen, training status and $[lactate]_b$ have much greater impacts on this partitioning.

In the non-LC trials, both UT and T groups obtain approximately 30 to 40% of CHO-derived energy from blood lactate at rest and during exercise (Figure 3B). Since lactate production during exercise is predominantly a result of muscle glycogenolysis (69), the high lactate R_{ox} observed during exercise in this study suggest that lactate is being shuttled from glycolytic cells to oxidative cells, well-described as the cell-cell lactate shuttle (11, 12). Evidently, this exchange of CHO fuel across tissue beds plays a substantial role under resting conditions as well. Blood glucose is a minor source of CHO energy in the T group, comprising less than 20% of CHO oxidation at rest and less than 15% during exercise. Consistent with other training studies investigating glucose metabolism in humans (6, 17, 23, 36, 37, 61, 65, 78), the UT group exhibits significantly greater reliance on blood glucose turnover, comprising over 35% of CHO oxidation at rest and 22% during exercise. Glycogen storage capacity is

known to be greater after training (28, 63, 73), thus providing a plausible explanation for the greater contribution to total CHO oxidation in the T group at rest. However, during exercise, muscle glycogen utilization contributes to $\sim$50-55% of CHO oxidation in both UT and T groups in the non-LC trials. It is important to note that oxidation rates of muscle glycogen in the present study are specific to stores directly oxidized within the same muscle. Whole body muscle glycogen oxidation, including the secondary glycogen utilization via lactate production, circulation and subsequent oxidation may show a significant increase after training as well. However, further studies are required to accurately quantify the shuttling of CHO fuel from one tissue bed to another.

The effect of LC on blood glucose utilization is not well understood. Miller et al. (60) found that glucose R_{ox} was unaffected at rest and significantly depressed due to LC in untrained individuals exercising at 55% VO_{2peak}. However, the present investigation found that estimated glucose R_{ox} was depressed during rest and slightly, yet significantly augmented during exercise at 67% VO_{2peak} in highly trained individuals. It is plausible that the discrepancy in lactate and glucose interactions between studies may be due to differences in training state or exercise intensity, a question that warrants further investigation. Despite the differences in glucose metabolism, it is generally hypothesized that lactate oxidation is augmented by LC, likely by a concentration gradient-driven increase in facilitated diffusion across the plasma membrane. In this investigation, direct lactate oxidation has a tendency to increase at rest ($P = 0.09$) and is significantly increased during exercise ($P < 0.05$) when [lactate]$_b$ is raised due to LC. As a result of the above-mentioned glucose and lactate rates of oxidation, a significant decrease in muscle glycogen utilization is observed during exercise at 67% VO_{2peak} with LC ($P < 0.05$). This sparing of muscle glycogen is primarily due to the near doubling of lactate oxidation when [lactate]$_b$ is increased by a similar proportion, suggesting that lactate may be a preferred fuel in order to delay depletion of glycogen stores within the exercising muscle.

Limitations

In the present study, we infused ^{13}C-lactate tracer to measure lactate oxidation, and, therefore, it was not possible to know glucose oxidation from simultaneous infusion of ^{13}C-glucose. Instead, blood glucose oxidation rates are derived from measured rates of disposal and assumptions based on turnover studies previously conducted in humans using ^{13}C-glucose tracer infusion and subsequent isotopic enrichments of expired [13]CO$_2$. We assume that 25% of glucose R_d is oxidized at rest (20% during LC) (61) and 80% during exercise with or without LC (36, 37, 60, 61). Because the percentages of glucose oxidation have been reproducible within and across studies in our laboratory, and because our tracer-measured flux rates agree with results of others (23, 24), we consider our rates of glucose oxidation to be reasonable estimates within the physiological range. Similarly, muscle glycogen oxidation rates are estimated to be the remaining CHO oxidation next to glucose and lactate, and are therefore subject to similar constraints.

During the LT-10%+LC resting condition, infusion of unlabeled lactate significantly increases lactate R_d, but not lactate-derived glucose production (33), indicating that the increase in lactate disposal may support hepatic glycogen synthesis at rest. Those observations suggest a possibility for preloading of lactate tracer into hepatic glycogen during the 90-min rest period of the LT-10%+LC trial. If the LC procedure resulted in hepatic glycogen preloading, then our measurements of lactate-derived glucose production and as well indirect lactate oxidation rates

are overestimated during exercise. However, that error would further support our conclusion about a training-induced increase in direct versus indirect lactate oxidation partitioning.

The infusion of exogenous lactate also introduces a CHO energy source in overnight-fasted subjects. On average, the LC procedure delivered 1.5 kcal/min during rest and 1.0 kcal/min during exercise. While negligible during exercise, lactate infusion during rest may have altered the fasted state of the subjects. However, aside from the aforementioned effects on lactate and glucose oxidation, the infusion of exogenous lactate did not appear to affect total caloric expenditure or lipid utilization (Table 2).

Summary and Conclusions

We utilized stable isotope tracers and pulmonary gas exchange measurements to determine whole body partitioning of direct and indirect lactate oxidation in untrained and trained men during steady state exercise at and just below the lactate threshold. Additionally, we incorporated a lactate clamp component to examine the effect of exogenous lactate infusion on this partitioning. From these experiments, we conclude that endurance training and blood lactate availability provide physiological conditions for increasing direct lactate oxidation. In trained individuals, the higher direct oxidative disposal of lactate, relative to its indirect oxidation, occurs both at rest and during exercise, suggesting training-induced adaptations on lactate oxidation that are independent of exercise parameters. From the standpoint of substrate utilization, the augmented capacity for direct lactate oxidation allows glycolytic tissues to support oxidative tissues by effectively redistributing carbohydrate fuel, as well as sparing glycogen within exercising muscle.

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DISCLOSURES

GAB has a financial interest in CytoSport; otherwise, the authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

GAB and LAM were responsible for the conception and design of research; LAM, JAF and CWE performed experiments; TJC was responsible for medical clearance and safe conduct of experimentation; MAH was responsible for gas chromatography and mass spectrometry (GCMS); CWE was responsible for enzymatic, GCMS and statistical analyses; JAF was responsible for enzymatic and hormonal analyses; CWE and GAB analyzed data and drafted the manuscript, and all authors edited and approved the final version of manuscript.

TABLES

Table 1. *Workload, lactate concentration, and lactate disposal distribution during rest and exercise*

Variable	Rest				Exercise			
	Untrained LT	Trained LT	Trained LT-10%	Trained LT-10%+LC	Untrained LT	Trained LT	Trained LT-10%	Trained LT-10%+LC
Power Output (W)	-	-	-	-	161 ± 4	259 ± 10 *	234 ± 9 *£	234 ± 9 *£
%VO$_{2peak}$ (%)	-	-	-	-	67.6 ± 1.3	74.8 ± 1.7 *	66.6 ± 1.4 £	67.9 ± 1.5 £
Lactate (mmol/l)	0.6 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	4.4 ± 0.4 *£†	3.7 ± 0.4 ^	4.3 ± 0.2 ^	2.5 ± 0.5 ^*£	4.3 ± 0.3 †
Lactate R$_d$ (mg·kg^{-1}·min^{-1})	1.8 ± 0.1	2.6 ± 0.6	2.4 ± 0.5	6.6 ± 0.7 *£†	15.0 ± 2.8 ^	24.2 ± 2.8 ^*	18.1 ± 2.6 ^	28.4 ± 2.7 ^*†
%Lactate R$_d$ to glucose via GNG	50.4 ± 10.6	20.4 ± 4.7 *	22.6 ± 6.0 *	6.2 ± 1.4 *£†	25.7 ± 6.4 ^	8.5 ± 3.0 ^*	9.0 ± 1.7 ^*	15.9 ± 3.7 ^
%Lactate R$_d$ oxidized (total)	45.9 ± 1.8	45.8 ± 0.7	45.3 ± 2.3	23.7 ± 3.7 *£†	88.7 ± 0.9 ^	93.5 ± 1.2 ^*	88.7 ± 3.9 ^	97.6 ± 2.3 ^*
%Lactate R$_d$ to indirect oxidation	12.6 ± 2.6	5.1 ± 1.2 *	5.0 ± 1.6 *	1.4 ± 0.3 *£†	20.5 ± 5.1 ^	6.8 ± 2.4 *	7.2 ± 1.3 *	12.7 ± 3.0 ^
%Lactate R$_d$ to direct oxidation	33.3 ± 4.1	40.7 ± 1.4 *	40.3 ± 3.1	22.4 ± 3.6 £†	68.2 ± 4.9 ^	86.6 ± 2.1 ^*	81.5 ± 3.7 ^*	84.8 ± 3.7 ^*

Values are means ± SE; $n = 6$ for untrained and trained groups; VO$_{2peak}$, peak oxygen consumption; R$_d$, rate of disposal; GNG, gluconeogenesis. ^ Significantly different from rest within condition ($P < 0.05$); * Significantly different from untrained ($P < 0.05$); £ Significantly different from trained LT ($P < 0.05$); † Significantly different from trained LT-10% ($P < 0.05$). Values for Lactate R$_d$ and %Lactate R$_d$ to glucose via GNG are from Messonnier et al. (59) and Emhoff et al. (33), respectively.

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Table 2. *Partitioning of caloric expenditure during rest and exercise*

Variable	Rest				Exercise			
	Untrained LT	Trained LT	Trained LT-10%	Trained LT-10%+LC	Untrained LT	Trained LT	Trained LT-10%	Trained LT-10%+LC
Total caloric expenditure (kcal/min)	1.80 ± 0.05	1.76 ± 0.09	1.79 ± 0.13	1.79 ± 0.14	13.63 ± 0.40 ^	20.32 ± 1.01 ^*	17.79 ± 0.85 ^*£	17.76 ± 0.91 ^*£
Lactate (kcal/min)	0.21 ± 0.03 (11%)	0.40 ± 0.11 (22%)	0.27 ± 0.06 (16%)	0.43 ± 0.07 (24%) *†	3.54 ± 0.72 (26%) ^	6.79 ± 0.98 (33%) ^*†	4.60 ± 0.57 (26%) ^	7.70 ± 0.74 (44%) ^*†
Glucose (kcal/min)	0.18 ± 0.01 (10%)	0.15 ± 0.1 (9%)	0.18 ± 0.03 (10%)	0.12 ± 0.03 (6%) *†	2.25 ± 0.21 (17%) ^	2.04 ± 0.15 (10%) ^	1.89 ± 0.19 (11%) ^	2.24 ± 0.22 (13%) ^†
Glycogen (kcal/min)	0.16 ± 0.06 (9%)	0.30 ± 0.12 (19%)	0.46 ± 0.07 (27%) *	0.36 ± 0.09 (22%)	4.77 ± 0.99 (34%) ^	8.33 ± 1.07 (41%) ^*	7.32 ± 0.56 (41%) ^*	3.85 ± 0.84 (21%) ^£†
Lipid (kcal/min)	1.25 ± 0.09 (69%)	0.91 ± 0.13 (51%)	0.88 ± 0.17 (47%)	0.88 ± 0.17 (47%)	3.07 ± 0.41 (23%) ^	3.15 ± 0.27 (16%) ^	3.97 ± 0.43 (22%) ^	3.96 ± 0.44 (22%) ^

Values are means ± SE; $n = 6$ for untrained and trained groups; values in parentheses are relative substrate contribution to total caloric expenditure. Statistical symbols are for absolute caloric expenditure values: ^ Significantly different from rest within condition ($P < 0.05$); * Significantly different from untrained ($P < 0.05$); £ Significantly different from trained LT ($P < 0.05$); † Significantly different from trained LT-10% ($P < 0.05$).

FIGURES

Figure 1. Atom percent excess of expired $^{13}CO_2$ during rest and exercise. Values are means ± SE; $n = 6$ for untrained and trained groups. ^ Significantly different from rest within condition ($P < 0.05$); £ Significantly different from trained LT ($P < 0.05$); † Significantly different from trained LT-10% ($P < 0.05$).
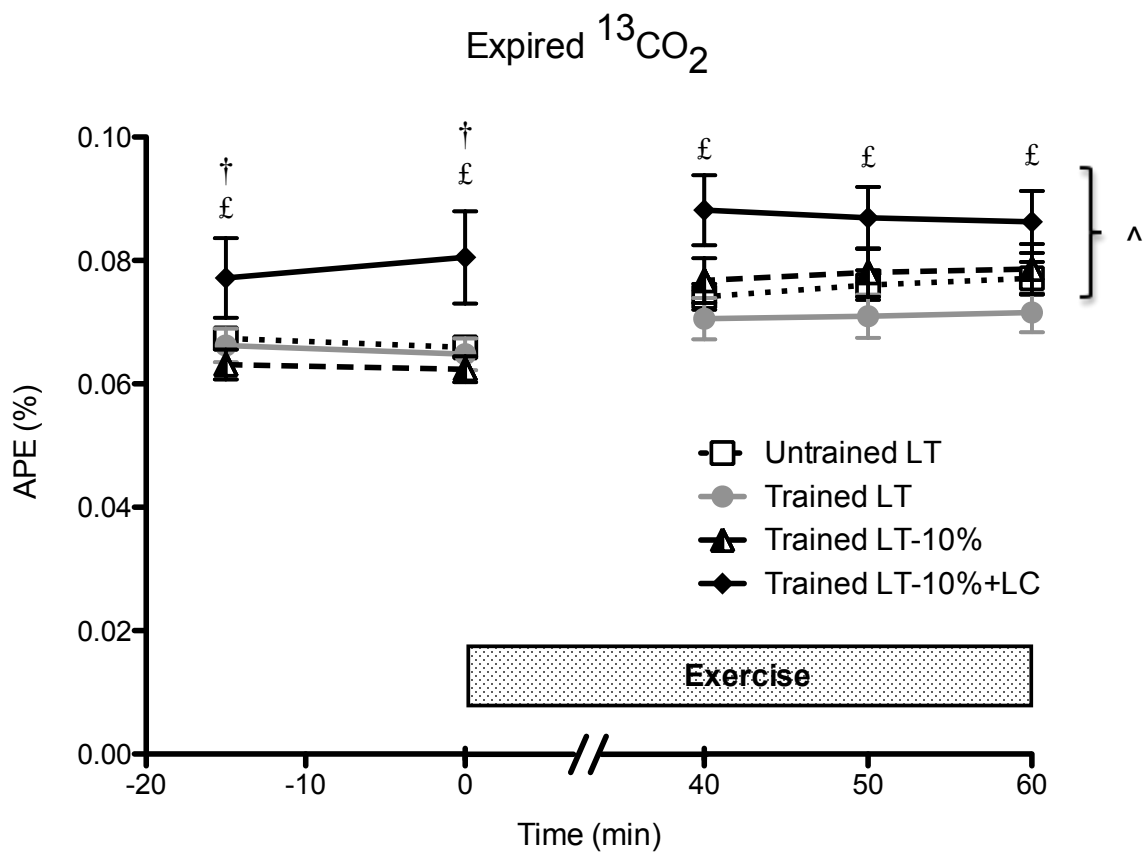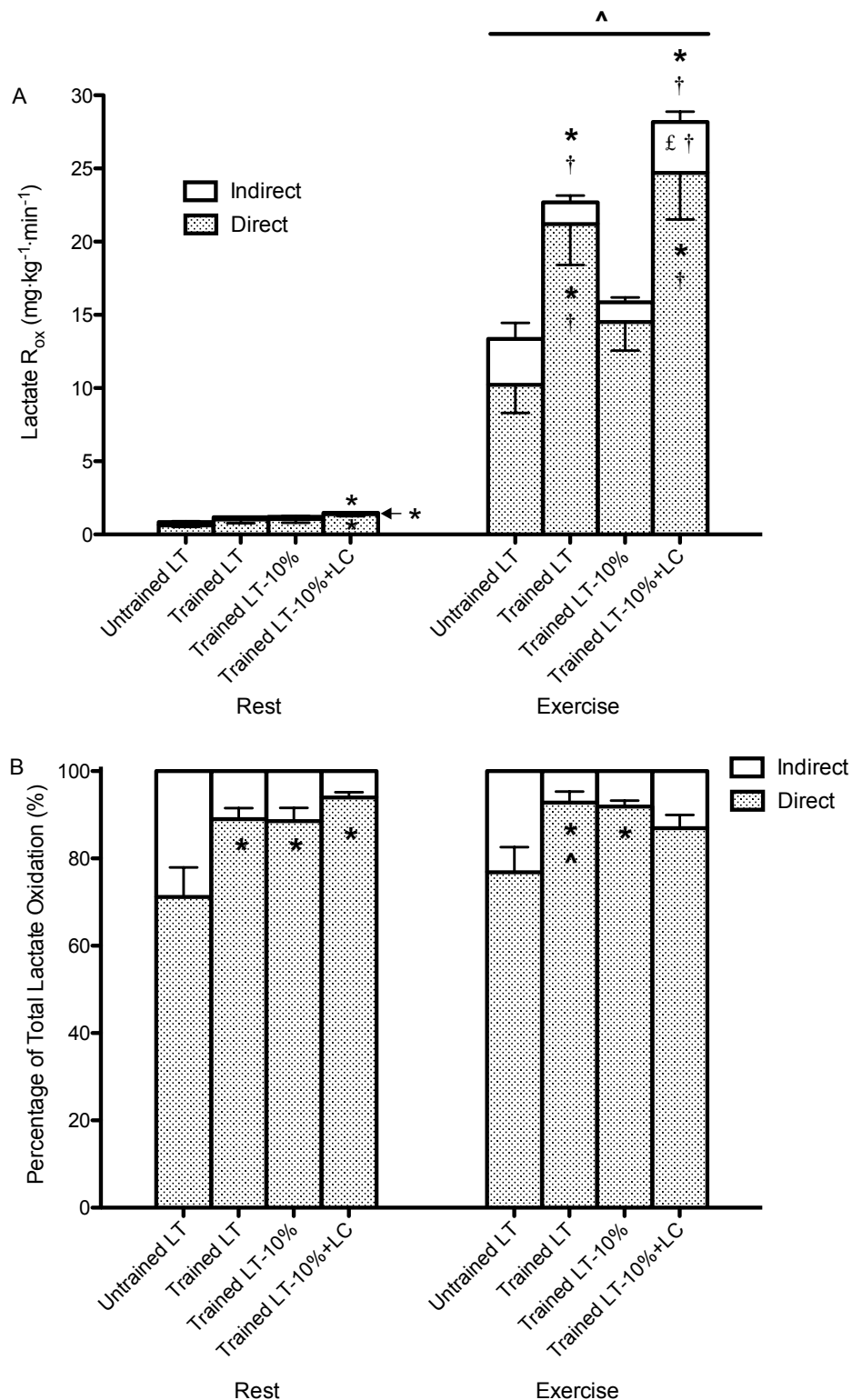
Figure 2. Steady state rates of lactate oxidation (R_{ox}), partitioned into direct and indirect lactate oxidation in absolute (A) and relative (B) terms. Values are means ± SE; $n = 6$ for untrained and trained groups. ^ Significantly different from rest within condition ($P < 0.05$); * Significantly different from untrained ($P < 0.05$); £ Significantly different from trained LT ($P < 0.05$); † Significantly different from trained LT-10% ($P < 0.05$).

Figure 3. Steady state rates of CHO oxidation (R_{ox}), partitioned into lactate, glucose, and muscle glycogen oxidation in absolute (A) and relative (B) terms. Values are means ± SE; $n = 6$ for untrained and trained groups. ^ Significantly different from rest within condition ($P < 0.05$); * Significantly different from untrained ($P < 0.05$); £ Significantly different from trained LT ($P < 0.05$); † Significantly different from trained LT-10% ($P < 0.05$).
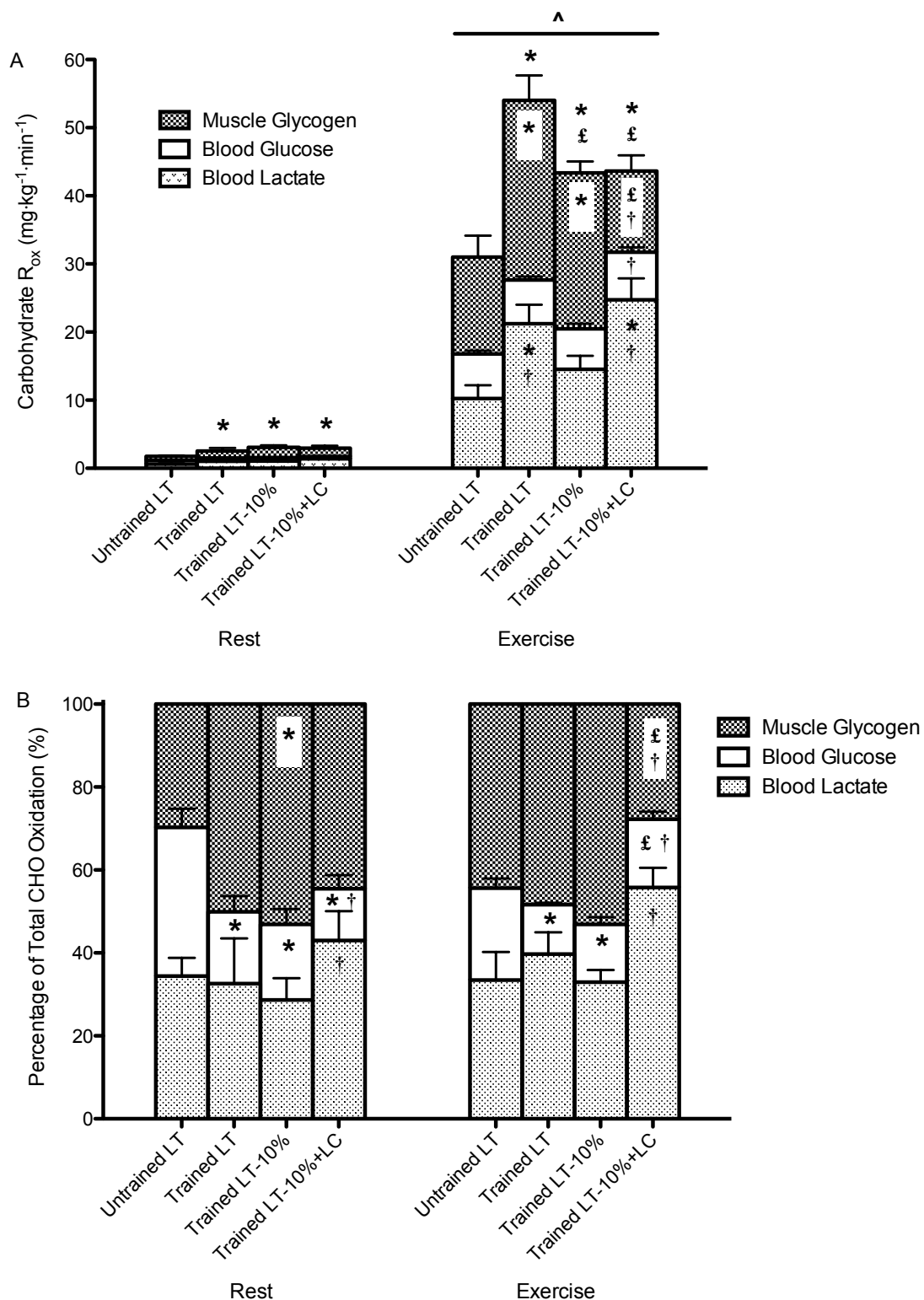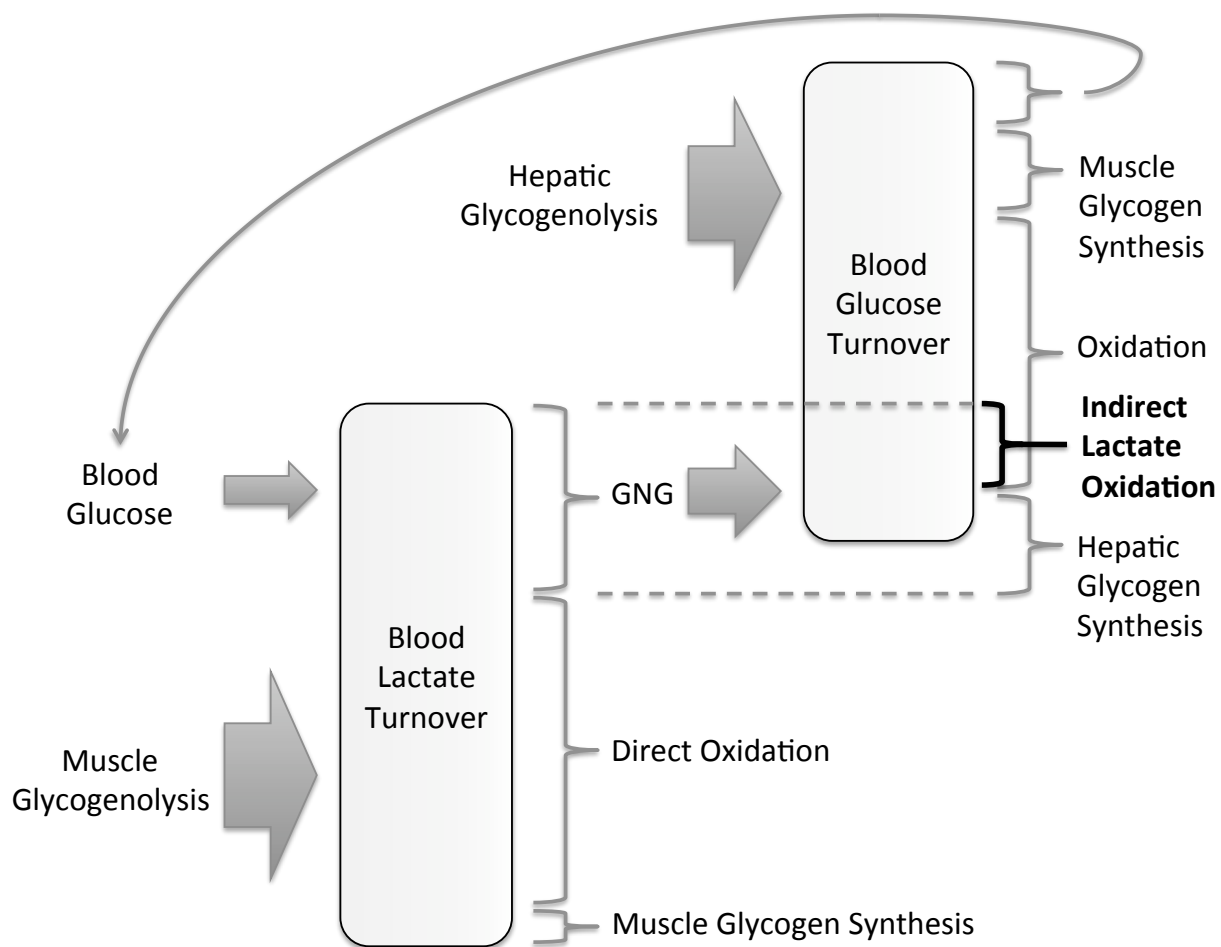
Figure 4. A schematic showing the relationship between lactate and glucose turnover in the blood. Muscle glycogenolysis is the primary source of lactate appearance, followed by glycolysis of blood glucose. Lactate disposal includes direct oxidation, GNG and to a lesser extent glycogen synthesis. Glucose appearance is dependent on hepatic glycogenolysis and GNG, and glucose disposal includes oxidation and glycogen synthesis. A small portion of glucose disposal also re-enters the lactate pool. Indirect lactate oxidation is the portion of lactate that undergoes conversion to glucose via GNG and subsequent oxidation.

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CHAPTER 5

Conclusions and Future Directions

CONCLUSIONS

The results of our present study highlight the individual and combined effects of endurance training, exercise intensity and blood lactate concentration on glucose production, lactate turnover, and carbohydrate oxidation in humans. With the use of stable isotope tracer methodology, we can gain a better understanding of how the body shuttles energy using lactate-glucose interactions during rest and exercise, and how endurance training elicits adaptations to augment lactate utilization through direct oxidative metabolism.

At rest, we measured gluconeogenesis from lactate to comprise approximatly 40% of total glucose production in both untrained and trained subjects. During exercise, endurance training increased the work capacity at the lactate threshold without causing a significant decrease in gluconeogenesis. The resulting glycemic homeostasis was reflected in steady blood glucose concentrations throughout the entire 1-h duration of constand load exercise, whether at a relative intensity of 67% VO_{2peak} or as high as 75% VO_{2peak} in the trained group. Since lactate is the primary gluconeogenic precursor, we found that absolute rates of gluconeogenesis during exercise can be augmented by increasing precursor delivery, accomplished in our study by exogenous lactate infusion.

We reported some of the highest rates of whole body lactate flux in exercising humans, consistent with the highest work rates achieved during steady-rate cycling. The unique experimental design of testing trained subjects at their lactate threshold (LT) as well as at a 10% lower power output (LT-10%) revealed a dramatic shift in lactate clearance. A ratio of lactate rate of disposal to blood lactate concentration, metabolic clearance rate (MCR) was 46% higher at LT-10% compared to LT, suggesting that the lactate threshold indeed represents a limitation in lactate clearance. Interestingly, when relative exercise intensity was held constant between untrained and trained groups, MCR was nearly doubled in the trained group, indicating a massive training effect to increase lactate clearance capacity.

As endurance training requires high levels of sustained oxidative metabolism, we quantified the partitioning of lactate, glucose, and muscle glycogen oxidation in our complete analyses of carbohydrate utilization. Further, we highlighted the distinct routes of lactate utilization between direct oxidation and indirect oxidation (via gluconeogenesis and oxidized as glucose). We found that endurance training increases direct oxidation of lactate (90% in trained vs. 75% in the untrained group), and that this partitioning occurred regardless of activity level. The observation that 90% of lactate is directly oxidized even at rest in endurance-trained individuals suggests that underlying training adaptations must occur in oxidative tissues that are independent of exercise parameters, such as blood flow or blood lactate concentration. Because of the preferential use of lactate, we found that providing exogenous lactate during exercise spared the utilization of muscle glycogen.

In aggregate, our results support the conclusion that lactate is an important oxidative fuel. Endurance training improves an individual's ability to sustain higher work rates by in part expanding the capacity for lactate utilization via gluconeogenesis and direct oxidation. Such studies provide a deeper understanding of how energy homeostasis is maintained during a prolonged bout of moderate to high intensity exercise in healthy humans.

FUTURE DIRECTIONS

Our results demonstrating high rates of lactate oxidation in the LT-10%+LC exercise condition may lead one to speculate where the upper limits of lactate disposal and oxidation occur in highly-trained endurance athletes. We demonstrated a clearance limitation at the LT when blood lactate concentration was clamped at 4 mmol/l, but the question remains how gluconeogenesis and lactate oxidation rates may be affected if blood lactate were to be clamped at a higher concentration, increasing substrate availability. Further, in the context of endurance training adaptations, a full picture of energy substrate metabolism can only be attained when both men and women have been studied. Evidence for highly-trained female athletes exhibiting greater relative changes in oxidative capacity than their male counterparts suggest that women may have altered or augmented training responses than those reported in this study. Therefore, repeating the studies described in this dissertation in women would elucidate some of the sex-related differences in endurance training responses.

Beyond the healthy, young adult populations, understanding lactate metabolism in other populations, such as children and elderly, cancer patients, traumatic brain injury patients, sickle cell trait carriers, and individuals with metabolic disease, would shed significant light on the whole-body strategies for energy partitioning amongst gluconeogenic, glycolytic and oxidative processes, all of which are affected in the aforementioned conditions. For instance, observations of elevated blood lactate concentration in type I and type II diabetes mellitus are not fully explained, despite hypotheses that limitations in lactate clearance may be due to mitochondrial defects. Given a rising prevalence of obesity, insulin resistance, and diabetes, the capability to conduct human studies using stable isotope tracers provides a powerful tool to investigate such questions surrounding the co-morbidities of metabolic syndrome. Similarly, with the increase in human longevity, issues pertaining to the increased risk of cardiovascular disease in post-menopausal women may be linked to simultaneous observations in elevated glucose flux and blood lactate concentrations in this population. Finally, the recent attention on sudden cardiac death in young, otherwise healthy athletes with sickle cell trait has emphasized the importance of understanding the effects of moderate to high intensity exercise on hemoglobinopathies. A candidate in the mechanistic explanation for many of the above conditions, including the results described in this dissertation, is the purported mitochondrial lactate oxidation complex. Further studies combining human tracer studies, exercise protocols, and molecular analyses from muscle biopsies would need to be conducted to investigate mechanisms driving the physiology.

The observations of increased capacities for GNG and direct lactate oxidation in this dissertation can be interpreted to suggest improved glycemic control and mitochondrial function in the trained subjects. One might further propose that these adaptations to exercise training may be directly beneficial for reducing risks associated with cardiovascular, metabolic, and other chronic diseases. Perhaps larger epidemiological studies are needed to solidify the scientific basis for such conclusions. Therefore, the continuation of exercise and metabolism research is paramount in the quest for greater knowledge and appreciation of health benefits associated with exercise training, one of the key components in chronic disease prevention. However, gaps still exist between the body of literature representing current research findings and the

recommendations and treatments that physicians offer their patients. For the ultimate benefit of the general public, and possibly the health care system as a whole, collaboration between scientists and physicians is necessary to integrate exercise physiology into the mainstream medical training curriculum, as well as to create programs that facilitate the incorporation of exercise prescription into regular clinical practice. While efforts do exist, more widespread programs in outreach and community-based research are needed to improve the effectiveness of promoting exercise as preventive medicine and treatment.

The use of tracers is also a unique methodology that crosses the realms of basic research and medicine. For example, current studies employing tracers to measure glucose and lactate metabolism in traumatic brain injury (TBI) patients are revealing a conceivable therapy of supplying lactate to the brain when glucose metabolism is hindered due to the injury. In combination with clinical trials, such studies possess a tremendous potential for advancing the understanding of brain metabolism in TBI. One may speculate that positive outcomes following lactate infusion in TBI may dramatically improve treatment capabilities by first responders and emergency medical physicians.

In summary, the responsibilities of scientists extend far beyond the laboratory. The findings described in this dissertation represent only a small fraction of information pertaining to exercise physiology and energy substrate metabolism that may change the way health care is approached and optimistically promote a more healthful lifestyle in all individuals, given that the information is communicated amongst scientists, educators, physicians, and patients alike.