Lactate kinetics at the lactate threshold in trained and untrained men

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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 (Ra and Rd, 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 Ra was higher in T (24.1 ± 2.7) than in UT (14.6 ± 2.4, P < 0.05), but Rd was not different between UT and T when relative exercise intensities were matched (UT-LT vs. T-LT-10%, 67% VO₂max). 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% VO₂max), 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 %VO₂max 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.

Key words: exercise, maximal lactate steady state (MLSS), exertion, endurance training, lactate oxidation, gluconeogenesis from lactate, intermediary metabolism
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 (Rₐ) and disposal (Rₜ) 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 Rₐ is matched by Rₜ during rest, and that both rise with increases in exercise intensity as described by PO, metabolic rate [oxygen consumption (VO₂) or percentage of maximal oxygen consumption (%VO₂max)], and blood lactate concentration ([lactate]ₜ). Those investigators also observed that during continual graded exercise, the increase in Rₜ lags behind the increase of Rₐ, resulting in rising [lactate]ₜ (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.
Materials and 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, $\text{VO}_{2\text{max}} < 50 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Trained subjects were members of competitive cycling or triathlon teams, currently in the race phase of their training season ($\text{VO}_{2\text{max}} > 55 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Subjects were included in the study if they had a body mass index (BMI) of $\geq 18$ and $< 26 \text{ kg} \cdot \text{m}^{-2}$, 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 $\text{VO}_{2\text{max}}$, 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 per day for the UT and T groups, respectively. Diets were individualized and determined for each participant considering a Physical Activity Level 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, suprailliac, and thigh)], pulmonary function (FEV1/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 \([\text{lactate}]\) was determined in 10 \(\mu\)l 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 \(f_{\text{Hmax}}\) and oxygen consumption \(\text{VO}_{2\text{max}}\) and \(\text{VO}_{2\text{max}}\)-associated power output \(\text{PO}_{\text{max}}\), 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 \(\text{VO}_{2\text{max}}\) (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 \(\text{VE, VO}_2, \text{VCO}_2, \text{RER}\) were recorded continuously during rest and exercise. This test was performed to determine the LT as defined previously (11) and associated \(\text{VO}_2, f_{\text{H}}, \text{PO}, \text{ and } [\text{lactate}]_b\). 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 \([\text{lactate}]_b\) over the 60-min exercise test. Every 10 min, 10 \(\mu\)l finger prick blood samples were assayed for \([\text{lactate}]_b\), 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-$^2$H$_2$]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$^{-1}$ during LT and LT-10%, and 7.5 mg·min$^{-1}$ during LT-10%+LC. The resting infusion rate of D$_2$-glucose was 2 mg·min$^{-1}$ 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$^{-1}$ which delivered lactate at ~200 mg·min$^{-1}$. 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\(^{-1}\) 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 \(V_E\), \(V_O2\), \(V_CO2\), 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).
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 (ddH₂O) through a cation resin (Analytical Grade 50W-X8, 50-100 mesh H⁺
resin, Bio-Rad Laboratories, Hercules, CA) and with ddH₂O followed by 2N formic acid through
an anion resin (Analytical Grade 1-X8, 100-200 mesh formate resin). Glucose was eluted first
with ddH₂O 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 N₂ gas, resuspended in 200 µl ethyl
acetate, transferred to a GCMS vial and dried again under N₂ gas, resuspended in 20 µl of
heptafluorobutyric anhydride, left for 5 min at room temperature to react and dried under N₂ 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, [3-13C]lactate),
and 331 (M+3 isotopomer, [U-13C]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):

$$Ra = F - V \left[ \frac{(C_1 + C_2)}{2} \right] \frac{(IE_2 - IE_1)}{(t_2 - t_1)}$$

$$Rd = Ra - V \left[ \frac{(C_2 - C_1)}{(t_2 - t_1)} \right]$$

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

where: $F$ represents isotope infusion rate (mg·kg$^{-1}$·min$^{-1}$), $V$ is the volume of distribution for lactate (180 ml·kg$^{-1}$); $C_1$ and $C_2$ are concentrations (mg·l$^{-1}$) 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 $\alpha = 0.05$ 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\textsubscript{2max} and LT determination tests

Compared to untrained subjects, cyclists had significantly higher VO\textsubscript{2max} (\(P < 0.05\)) and PO\textsubscript{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\textsubscript{2} and percentage of VO\textsubscript{2max} (%VO\textsubscript{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\textsubscript{2max}) were similar in the untrained LT trial compared to cyclists at LT-10% and LT-10%+LC (~67% VO\textsubscript{2max}). However, relative exercise intensity was significantly higher during the LT trial in the cyclists (75% VO\textsubscript{2max}, \(P < 0.05\)).

Lactate, glucose and hormone concentrations during isotope tracer trials

Before tracer or LC infusions, resting \([\text{lactate}]_b\) values were lower (\(P < 0.05\)) in UT subjects (0.57 ± 0.09 mmol·l\(^{-1}\)) than in T cyclists, no matter the trial (1.08 ± 0.09 mmol·l\(^{-1}\)) (Figure 2). Resting \([\text{lactate}]_b\) was similar among the trials in the T group (NS). To elevate \([\text{lactate}]_b\) 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 \([\text{lactate}]_b\) to 4.30 ± 0.39 mmol·l\(^{-1}\) which was significantly higher than all other conditions (\(P < 0.05\)). After 10 min of exercise, \([\text{lactate}]_b\) rose to reach 4.11 ± 1.01 mmol·l\(^{-1}\) in UT, and 3.71 ± 1.02, 2.57 ± 1.20 and 4.49 ± 1.36 mmol·l\(^{-1}\) in T during LT, LT-10% and LT-10%+LC trials.
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⁻¹) compared with T cyclists (5.0 ± 0.1 mmol·l⁻¹) (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⁻¹, 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 (Rₐ, Figure 4A) was significantly increased (P < 0.05) by exogenous lactate infusion (i.e., LT-10%+LC trial). Lactate Rₐ 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$^{-1}$) (Figure 5A), or as %VO$_{2\text{max}}$ (Figure 5B). Similarly, plasma [epinephrine] and [norepinephrine] also rose exponentially as functions of metabolic rate whether expressed on absolute (VO$_2$, l·min$^{-1}$), or relative (%VO$_{2\text{max}}$) 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 V$O_2$max (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 V$O_2$max) 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 (V$O_2$) elicited by exercise (Figure 5A). The apparent rightward shift in the lactate $R_a$ vs. V$O_2$ 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 $\%VO_{2\text{max}}$ (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 ($VO_{2\text{max}} = 5.0 \, \text{l} \cdot \text{min}^{-1}$) as compared to the lower curves obtained on healthy men of variable but lesser fitness levels ($VO_{2\text{max}} = 2.6, 3.5$ and $4.1 \, \text{l} \cdot \text{min}^{-1}$). In the present study, when PO was raised from LT-10% to LT workloads, trained cyclists experienced a 60% increase in $[\text{lactate}]_b$, 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 as the LT is approached.

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 Rd 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 sarcomemmal 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]₀, alters the gradients, in effect “pushing” lactate into the tissues that are net lactate consumers, possibly accounting for the observed increase in Rₐ 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 Rₐ that cannot be accommodated by a corresponding rise in lactate Rₐ, thus causing a continuous rise in [lactate]₀. 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 Rₐ and Rₐ were higher by 65% in T than in UT even though exercise at LT resulted in
Messonnier et al. Lactate Kinetics at the LT

almost similar \([\text{lactate}]_b\) 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\(_{2\text{max}}\), respectively).

The comparison of data obtained during the LT trial in UT and the LT-10% trial in T (both at \(-67\%\) VO\(_{2\text{max}}\)) showed the effects of long-term endurance training on lactate kinetics during exercise of similar relative intensity. As already well-described (7, 39, 52), \([\text{lactate}]_b\) 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 \([\text{lactate}]_b\) using the LC procedure allowed comparison of lactate kinetics between untrained and trained subjects at the same relative workload and similar \([\text{lactate}]_b\). In other words, comparison of LT in UT and LT-10%+LC in T allowed determination of the effects of endurance training \emph{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 \([\text{lactate}]_b\), \(R_d\) 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 \([\text{lactate}]_b\).
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 \([\text{lactate}]_b\) 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 \([\text{lactate}]_b\) (~0.3 to 1.7 mmol·L\(^{-1}\)) [e.g., (7, 17, 25, 53)]. One possible explanation would be that the difference in resting \([\text{lactate}]_b\) 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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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 1. *Physiological responses to maximal oxygen consumption (VO_{2\text{max}}, upper panel) and lactate threshold (LT, lower panel) determination tests for untrained and trained groups*

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.
Figure captions

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 [3-13C]lactate (M+1 lactate). Values are means ± SE. Symbols are as in Figure 2.

Figure 4: Lactate rates of appearance (Lactate Ra) (A) and disposal (Lactate Rd) (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 Ra in function of absolute (VO$_2$) (A) and relative (to VO$_{2\text{max}}$) (B) metabolic rates elicited at rest and exercise in the present and previous studies involving subjects of different physical fitness statuses. VO$_{2\text{max}}$ values (mean ± SE) of UT and T are 3.7 ± 0.1 and 5.0 ± 0.3 l·min$^{-1}$.

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$_{2\text{max}}$) metabolic rates elicited at rest and exercise. Symbols are as in Figure 2.

Figure 7: Lactate Ra 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$_{2\text{max}}$) (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$_{2\text{max}}$ values (mean ± SE) of T is 3.7 ± 0.1 and 5.0 ± 0.3 l·min$^{-1}$.
References


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

<table>
<thead>
<tr>
<th>Variable</th>
<th>UT</th>
<th>T</th>
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<tr>
<td>( f_{H_{\text{max}}} ) (\text{min}^{-1})</td>
<td>189 ± 5</td>
<td>189 ± 3</td>
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<tr>
<td>( V_{E_{\text{max}}} ) (l·min(^{-1}))</td>
<td>115 ± 8</td>
<td>144 ± 13 (*)</td>
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<tr>
<td>( V_{O_{2\text{max}}} ) (l·min(^{-1}))</td>
<td>3.7 ± 0.1</td>
<td>5.0 ± 0.3 *</td>
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<tr>
<td>( P_{O_{\text{max}}} ) (W)</td>
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<td>357 ± 12 *</td>
</tr>
<tr>
<td>( f_{H} ) at LT (\text{min}^{-1})</td>
<td>158 ± 5</td>
<td>168 ± 3</td>
</tr>
<tr>
<td>%( f_{H_{\text{max}}} ) at LT (%)</td>
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<td>89 ± 1</td>
</tr>
<tr>
<td>( V_{O_{2}} ) at LT (l·min(^{-1}))</td>
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<td>3.85 ± 0.21 *</td>
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<tr>
<td>%( V_{O_{2\text{max}}} ) (%)</td>
<td>69 ± 3</td>
<td>77 ± 2 *</td>
</tr>
<tr>
<td>( [\text{lactate}]_{b} ) at LT (mmol·l(^{-1}))</td>
<td>4.3 ± 0.6</td>
<td>3.6 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 6 \) for untrained (UT) and trained (T) groups; See the text for abbreviations. Differences between groups: (*) = 0.05 < \( P < 0.1 \), * = \( P < 0.05 \).
Figure 1

[Graph showing the relationship between lactate concentration ([lactate]₀) and power output (W) for untrained and trained subjects. The graph is divided into two sections labeled A and B. The untrained subject section (A) shows a linear increase in lactate concentration with power output, marked with squares and an arrow indicating LT. The trained subject section (B) shows a similar linear increase, marked with circles and an arrow also indicating LT.]
Figure 2

(A) Time course of lactate concentration ([lactate]_b) in blood during exercise at different intensities. UT = Ultraressing, LT = Low-intensity training, T = Training, T - LT - 10% = Training at 90% of LT, T - LT - 10% + LC = Training at 90% of LT with low-carbohydrate diet. Error bars represent ± SEM.

(B) Bar graph showing lactate concentration ([lactate]_b) in blood at rest and during exercise. * indicates significant difference from rest, § indicates significant difference from exercise at different intensities. Error bars represent ± SEM.
Figure 3

[Graph showing the MPE of [3-13C]lactate (%) over time (min) for different conditions: UT - LT, T - LT, T - LT-10%, T - LT-10%+LC.]
Figure 4

A

Lactate $R_a$ (mg kg$^{-1}$ min$^{-1}$)

- UT – LT
- T – LT
- T – LT-10%
- T – LT-10%+LC

Exogenous Lactate
Endogenous Lactate

B

Lactate $R_d$ (mg kg$^{-1}$ min$^{-1}$)

C

Lactate MCR (ml kg$^{-1}$ min$^{-1}$)

Rest
Exercise

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Figure 5

- Miller et al. 2002 ($\dot{V}O_{2\text{max}} = 4.1 \text{l.min}^{-1}$)
- Bergman et al. 1999 ($\dot{V}O_{2\text{max}} = 3.5 \text{l.min}^{-1}$)
- MacRae et al. 1992 ($\dot{V}O_{2\text{max}} = 2.6 \text{l.min}^{-1}$)

The graph shows the relationship between Lactate ($R_a$, mg kg$^{-1}$ min$^{-1}$) and $\dot{V}O_2$ (l.min$^{-1}$) and $\dot{V}O_{2\text{max}}$ (%). The data points represent different studies with various $\dot{V}O_{2\text{max}}$ values.
Figure 7

Graph A and B show the relationship between Lactate \( R_a \) (mg kg\(^{-1}\) min\(^{-1}\)) and epinephrine and norepinephrine concentrations (pg ml\(^{-1}\)). The graphs are labeled A and B, with different conditions such as UT - LT, T - LT, T - LT-10%, and T - LT-10%+LC represented by various symbols and lines.
Figure 8

- Miller et al. 2002 (VO_{2max} = 4.1 \text{l.min}^{-1})
- Bergman et al. 1999 (VO_{2max} = 3.5 \text{l.min}^{-1})
- MacRae et al. 1992 (VO_{2max} = 2.6 \text{l.min}^{-1})
- T – LT
- T – LT-10%