

# Active muscle and whole body lactate kinetics after endurance training in men

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<sup>1</sup>Exercise Physiology Laboratory, Department of Integrative Biology, University of California, Berkeley 94720; <sup>2</sup>University of Colorado Health Sciences Center, Division of Cardiology, Denver, Colorado 80262; <sup>3</sup>Geriatric Research, Education, and Clinical Center, Palo Alto Veterans Affairs Health Care System, Palo Alto, California 95304; and <sup>4</sup>Departments of Pediatrics and Pharmacology, Cardiovascular Research Group, University of Alberta, Edmonton, Alberta, Canada T6G 2S2

**Bergman, Bryan C., Eugene E. Wolfel, Gail E. Butterfield, Gary D. Lopaschuk, Gretchen A. Casazza, Michael A. Horning, and George A. Brooks.** Active muscle and whole body lactate kinetics after endurance training in men. *J. Appl. Physiol.* 87(5): 1684–1696, 1999.—We evaluated the hypotheses that endurance training decreases arterial lactate concentration ( $[\text{lactate}]_a$ ) during continuous exercise by decreasing net lactate release ( $\dot{L}$ ) and appearance rates ( $R_a$ ) and increasing metabolic clearance rate (MCR). Measurements were made at two intensities before [45 and 65% peak  $\text{O}_2$  consumption ( $\dot{V}_{\text{O}_{2\text{peak}}}$ )] and after training [65% pretraining  $\dot{V}_{\text{O}_{2\text{peak}}}$ , same absolute workload (ABT), and 65% posttraining  $\dot{V}_{\text{O}_{2\text{peak}}}$ , same relative intensity (RLT)]. Nine men ( $27.4 \pm 2.0$  yr) trained for 9 wk on a cycle ergometer, 5 times/wk at 75%  $\dot{V}_{\text{O}_{2\text{peak}}}$ . Compared with the 65%  $\dot{V}_{\text{O}_{2\text{peak}}}$  pretraining condition ( $4.75 \pm 0.4$  mM),  $[\text{lactate}]_a$  decreased at ABT (41%) and RLT (21%) ( $P < 0.05$ ).  $\dot{L}$  decreased at ABT but not at RLT. Leg lactate uptake and oxidation were unchanged at ABT but increased at RLT. MCR was unchanged at ABT but increased at RLT. We conclude that 1) active skeletal muscle is not solely responsible for elevated  $[\text{lactate}]_a$ ; and 2) training increases leg lactate clearance, decreases whole body and leg lactate production at a given moderate-intensity power output, and increases both whole body and leg lactate clearance at a high relative power output.

lactate shuttle; exertion; glycogen; glucose; stable isotopes

ARTERIAL LACTATE concentration is decreased at absolute (14, 20, 33) and relative (2, 22, 30) exercise intensities after endurance training. Mechanisms responsible for the attenuated blood lactate response to exercise are equivocal. By using tracers, increased whole body lactate clearance during exercise was first observed in trained rats (14). Subsequently, training was shown to decrease lactate appearance rate ( $R_a$ ) in humans exercising at given relative intensities (30). In contrast, others found unchanged lactate turnover in rats (14) and men (33) exercising at given absolute workloads after endurance training. Thus, although it is possible to conclude that increased lactate clearance contributes to dampened arterial lactate concentration

during exercise after training, the importance of altered lactate appearance is unclear.

Limb lactate balance has also been used to evaluate effects of endurance training on lactate metabolism. After training, decreased limb net lactate release ( $\dot{L}$ ) for the first 10–15 min (19, 22) or throughout 50 min of exercise (39) at a given absolute workload has been reported. At the same relative exercise intensity,  $\dot{L}$  was similar in trained and untrained subjects (45). However, limb lactate uptake and oxidation during  $\dot{L}$  (10–12, 42) confound  $\dot{L}$  as a measure of intramuscular lactate production. Therefore, interpretation of limb lactate balance is tenuous without isotope measurements to quantitate lactate uptake and total  $\dot{L}$  ( $\dot{L}_{\text{tot}}$ ).

The purpose of the present investigation was to quantitate whole body and active-limb lactate metabolism by using both tracer and limb balance techniques at given absolute and relative exercise intensities before and after endurance training. Specifically, we evaluated the hypotheses that training decreases lactate production at absolute workloads and increases lactate clearance at given absolute and relative exercise intensities. Additionally, we evaluated whether maintenance of elevated arterial lactate concentration during exercise is due to sustained active muscle  $\dot{L}$ .

## METHODS

### Subjects

Nine healthy sedentary male subjects aged 19–33 yr were recruited from the University of California, Berkeley, by posted notices. Subjects gave informed consent, were considered untrained if they engaged in no more than 2 h of physical activity per week for the previous year and had a peak oxygen consumption ( $\dot{V}_{\text{O}_{2\text{peak}}}$ ) of  $<45$  ml·kg<sup>-1</sup>·min<sup>-1</sup>. Subjects were included in the study if they had  $<25\%$  percent body fat, were nonsmokers, were diet and weight stable, had a 1-s forced expiratory volume (FEV<sub>1</sub>) of 70% or more of vital capacity, and were injury and/or disease free as determined by physical examination. The study was approved by the Committee for the Protection of Human Subjects at Stanford University and the University of California, Berkeley (CPHS 97–6–34).

### Experimental Design

After interviews and preliminary screening, subjects performed two graded exercise tests to determine  $\dot{V}_{\text{O}_{2\text{peak}}}$  during leg cycle ergometry. Blood lactate threshold was determined during the second screening test. Subjects were then tested in

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a random order at 45 and 65%  $\dot{V}O_{2\text{peak}}$ , with 1 wk between isotope trials (see *Tracer Protocol*). Two days after the second trial, subjects began training on leg cycle ergometers. Post-training isotope trials were also performed in a random order at 65% of pretraining  $\dot{V}O_{2\text{peak}}$  [same absolute workload (ABT)], and 65% of posttraining  $\dot{V}O_{2\text{peak}}$  [same relative intensity (RLT)].

#### *Preliminary Testing*

All exercise tests were performed on an electronically braked cycle ergometer (Monark Ergometric 829E). For determination of  $\dot{V}O_{2\text{peak}}$ , exercise started at a power output of 50 W, which was increased by 25 or 50 W every 3 min until exhaustion. Respiratory gases were analyzed via an indirect open-circuit system and recorded by an on-line, real-time personal computer-based system (2). Body composition was determined via both skinfold measurements (21) and underwater weighing. Three-day diet records were kept to obtain baseline dietary habits and to monitor macronutrient composition and energy intake over the course of study. Dietary analysis was performed by using Nutritionist III software (N-Squared Computing, San Mateo, CA).

#### *Dietary Protocol*

The subjects rested the day before each tracer trial and commenced a standardized dietary protocol that was replicated on each occasion (2, 3).

#### *Catheterizations*

After local lidocaine anesthesia, the femoral artery and vein of the same leg were cannulated by using standard percutaneous techniques as previously described (2, 3). Alternate legs were used for the two trials during both pretraining and posttraining testing. One subject experienced blood leaking from catheter placements during the beginning minutes of exercise at 65% pretraining and did not perform further exercise. Two different subjects did not receive a venous catheter for one of their trials. As a result, a sample size of six to nine subjects was used for calculations and comparisons.

#### *Tracer Protocol*

A venous catheter was placed in an antecubital vein the morning of each trial for infusion of stable isotope solutions during 90 min of rest and 1 h of exercise. Background blood and breath samples were collected after catheterization of the femoral artery and vein. Subjects then received a primed continuous infusion of [6,6- $^2\text{H}$ ]glucose and [3- $^{13}\text{C}$ ]lactate while resting semisupine for 90 min. Glucose kinetics are reported separately (2). The priming bolus was equal to 23 times the resting lactate infusion rate. Tracer lactate was infused via an Intelligent pump 522 (Kendall McGaw, Irvine, CA) at 2.5 mg/min at rest and 7.5 mg/min during exercise at 45% pretraining  $\dot{V}O_{2\text{peak}}$  and 65% old  $\dot{V}O_{2\text{peak}}$  posttraining (ABT), and 10 mg/min at 65% pretraining and 65% posttraining  $\dot{V}O_{2\text{peak}}$  (RLT). Increases in tracer infusion were designed to elicit similar arterial enrichments between exercise intensities during the last 30 min of exercise. Isotopes were obtained from Cambridge Isotope Laboratories (Woburn, MA), diluted in 9% sterile saline, and tested for sterility and pyrogenicity before use (Univ. of California School of Pharmacy, San Francisco, CA).

#### *Muscle Biopsies and Analyses*

Immediately after the isotope infusion was started, one vastus lateralis muscle was prepared for percutaneous needle

biopsy. For each experimental trial, biopsies were taken from two locations separated by 1.5 cm: the distal site for preexercise sampling and the proximal site for immediate postexercise sampling. Right and left vastus lateralis muscles were alternated between trials. Biopsies taken at rest, and within 10 s of exercise cessation, were immediately plunged into liquid nitrogen and subsequently stored under liquid nitrogen and shipped on dry ice. Samples were analyzed for lactate concentration as previously described (17).

#### *Blood Sampling*

Blood temperature was obtained from a thermister at the end of the venous thermodilution catheter immediately before blood sampling. Arterial and venous blood samples were drawn simultaneously and anaerobically over 5 s after 75 and 90 min of rest, and at 5, 15, 30, 45, and 60 min of exercise.  $\text{PO}_2$ ,  $\text{PCO}_2$ , and pH were measured within 30 min of blood sampling (ABL 300, Radiometer, Copenhagen, Denmark). Blood for determination of glucose concentration and lactate enrichment was immediately transferred to tubes containing 8% perchloric acid, shaken, and placed on ice. Blood for determination of arterial and venous lactate concentration was immediately placed on ice. After the final blood sample at the end of exercise, samples were centrifuged at 3,000  $g$  for 10 min, and the supernatant was transferred to storage tubes and frozen at  $-20^\circ\text{C}$  until analysis. Hematocrit measurements were performed for both arterial and venous blood by using the microhematocrit method. Blood hemoglobin concentration was determined in each blood sample by using the cyanomethemoglobin method.

#### *Hemodynamics*

Iliac venous blood flow was determined by thermodilution technique with the use of a cardiac-output computer (model 9520, American Edwards Laboratories) as previously described (3). Measurements were made in triplicate or quadruplicate during rest and exercise immediately after blood sampling.

#### *Metabolite Analyses and Isotope Enrichments*

Glucose concentrations were measured in duplicate by using a hexokinase kit (Sigma Chemical, St. Louis, MO). Plasma lactate concentrations were measured in duplicate by using the method of Gutmann and Wahlefeld (18), which uses lactate dehydrogenase (LDH) corrected to whole blood values by using the method of Pendergrass et al. (32). Samples of arterial and venous blood and breath for measurement of  $^{13}\text{C}$  enrichments were determined by isotope ratio mass spectroscopy (Metabolic Solutions; Merrimack, NH). Lactate isotopic enrichment was measured by using gas chromatography-mass spectrometry (GCMS; GC model 5890 series II and MS model 5989A, Hewlett-Packard) of the *N*-propylamide heptafluorobutyrate derivative. In preparation for GCMS analysis, samples were neutralized with 2 N KOH, transferred to cation (AG 50W-X8, 50- to 100-mesh  $\text{H}^+$  resin)- and anion (AG 1-X8, 100- to 200-mesh formate resin)-exchange columns, and the lactate was eluted with 2 N formic acid. The samples were then lyophilized, transferred to a 2-ml microreaction vial, resuspended in 200  $\mu\text{l}$  of 2,2-dimethoxypropane followed by 20  $\mu\text{l}$  10% HCl in methanol, capped and allowed to sit at room temperature for 60 min. After addition of 50  $\mu\text{l}$  *N*-propylamine, samples were heated for 30 min at  $100^\circ\text{C}$ . Samples were subsequently dried under a stream of  $\text{N}_2$  gas, transferred to GCMS vials via 200  $\mu\text{l}$  ethyl acetate,

and again dried under N<sub>2</sub> gas. Finally, 20 µl heptafluorobutyric anhydride were added to samples and allowed to react for 5 min at room temperature before drying under N<sub>2</sub> gas. The derivatized lactate was then resuspended in ethyl acetate and subsequently analyzed by GCMS.

For GCMS analyses, the injector temperature was set at 200°C; the initial oven temperature was set at 80°C, the transfer line at 250°C, the source temperature at 286°C, and the quadrupole temperature at 126°C. The carrier gas was helium, and splitless injection was used with a 35:1 ml/min ratio. Methane was used for chemical ionization, and selective ion monitoring was used to monitor ion mass-to-charge ratios of 327.25 and 328.25 for [<sup>12</sup>C]- and [<sup>13</sup>C]lactate, respectively.

### Training Protocol

Training was performed on stationary cycle ergometers 5 days/wk, with workloads adjusted to elicit heart rates corresponding to 75% of  $\dot{V}O_{2\text{peak}}$ . Subjects were asked to exercise 1 day/wk on their own in addition to cycle ergometry training so that total training was 6 days/wk. All subjects were exercising at 75% of their  $\dot{V}O_{2\text{peak}}$  for 1 h by the end of the second week of training. After 4 wk of training, subjects performed another maximal exercise test to quantify increases in  $\dot{V}O_{2\text{peak}}$ , and training workloads were adjusted to maintain relative training intensity at 75%  $\dot{V}O_{2\text{peak}}$ . Two weeks preceding posttraining testing, subjects began interval training during the last 10 min of each 1-h workout. Interval training was added to develop recruitment patterns conducive to reaching maximal power outputs during posttraining evaluation. Subjects continued training throughout the 1 wk between posttraining testing with 1 day of rest before an experimental trial and 2 days of rest after an experimental trial to recover from testing procedures. Subjects were weighed daily and asked to increase energy intake to maintain weight during the training program without changing normal macronutrient composition. Three-day diet records were collected after 4 wk of training and at the end of training to ensure maintenance of baseline diet composition.

### Calculations

**Net metabolite exchange.** Net metabolite exchange differences were calculated as the product of leg blood flow and arteriovenous differences, where arterial (a) and venous hematocrit (v) values were used to correct for changes in plasma volume

$$\text{Net lactate release } (\dot{L}) \text{ (mmol/min)} \\ = 2(1 \text{ leg } \dot{Q})([\text{lactate}]_v(\text{Hct}_a/\text{Hct}_v)) - [\text{lactate}]_a$$

$$\text{Total lactate release } (\dot{L}_{\text{tot}}) \text{ (mmol/min)} \\ = \dot{L} + \text{tracer-measured lactate uptake}$$

Tracer-measured leg lactate fractional extraction ( $F_{\text{ex}}$ ) was measured by using two methods

$$F_{\text{ex rest}} (\%) = \frac{([\text{^{13}C}] \text{lactate}_a \text{IE})([\text{lactate}]_a) - ([\text{^{13}C}] \text{lactate}_v \text{IE})([\text{lactate}]_v \times \text{Hct}_a/\text{Hct}_v)}{([\text{^{13}C}] \text{lactate}_a \text{IE})([\text{lactate}]_a)}$$

$$F_{\text{ex exercise}} (\%) = \frac{([\text{^{13}CO}_{2v})(Cv_{\text{CO}_2})] - [(\text{^{13}CO}_{2a})(Ca_{\text{CO}_2})]}{([\text{^{13}C}] \text{lactate}_a \text{IE})([\text{lactate}]_a)}$$

Leg lactate oxidation ( $R_{\text{ox}}$ ) rate was also measured by using two methods

$$R_{\text{ox rest}} \text{ (mmol/min)} = \text{tracer-measured leg fractional extraction } ([\text{lactate}]_a)(2 \times \text{leg } \dot{Q})$$

$$R_{\text{ox exercise}} \text{ (mmol/min)} \\ = \frac{([\text{^{13}CO}_{2v})(Cv_{\text{CO}_2})] - [(\text{^{13}CO}_{2a})(Ca_{\text{CO}_2})]}{\text{lactate}_a \text{IE}}(2 \times \text{leg } \dot{Q})$$

**Blood CO<sub>2</sub> content.** Blood P<sub>CO<sub>2</sub></sub>, P<sub>O<sub>2</sub></sub>, pH, and Hb were measured in both arterial and venous samples and used in the calculations by Douglas et al. (15) and Kelman (27) for determination of blood CO<sub>2</sub> content ( $C_{\text{CO}_2\text{blood}}$ ) (2, 3).

**Lactate kinetics.** Lactate  $R_a$  and rate of disappearance ( $R_d$ ), metabolic clearance rate (MCR), and oxidation were calculated by using equations defined by Steele and modified for use with stable isotopes (47)

$$R_a \text{ (mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) \\ = \frac{F - V[(C_1 + C_2)/2][(IE_2 - IE_1)/(t_2 - t_1)]}{[(IE_2 + IE_1)/2]}$$

$$R_d \text{ (mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = R_a - V[(C_2 - C_1)/(t_2 - t_1)]$$

$$\text{MCR (ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = R_d/[(C_1 + C_2)/2]$$

$$R_{\text{ox}} \text{ (mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = \frac{R_d[(\dot{V}CO_2)(IE_{CO_2})(100)]}{[(F)(k)/89.08]}$$

where  $F$  represents isotope infusion rate,  $IE_1$  and  $IE_2$  are lactate isotopic enrichments at sampling *time points 1* ( $t_1$ ) and *2* ( $t_2$ ), respectively;  $C_1$  and  $C_2$  are lactate concentrations at  $t_1$  and  $t_2$ , respectively;  $V$  is the estimated volume distribution of lactate (180 ml/kg); 89.08 is the molecular weight of lactate; and  $k$  is a correction factor for retention of tracer in CO<sub>2</sub> pools (0.65 during rest and 0.90 during exercise). Isotopic enrichments of lactate were corrected for background enrichments from blood samples taken before isotope infusion. Calculations of steady-state lactate kinetics were performed during the last 15 min of rest (75 and 90 min) and 30 min of exercise (30, 45, and 60 min) when arterial lactate concentration and enrichments were stable, thus minimizing assumptions related to volume and arterial concentration.

### Statistical Analyses

Significance of differences among average arterial glucose and lactate concentrations from the last 30 min of exercise were analyzed by using one-factor ANOVA with repeated measures. Differences between training states for body fat,  $\dot{V}O_{2\text{peak}}$ , and power output at lactate threshold were determined by using a paired Student's  $t$ -test. Differences between groups for venous-arterial limb lactate concentration difference ( $[\text{lactate}]_{v-a}$ ),  $\dot{L}$  and  $\dot{L}_{\text{tot}}$ , lactate  $R_a$ ,  $R_d$ , MCR, oxidation, and arterial enrichment were determined by using a repeated-measures factorial ANOVA. Differences between groups for leg [<sup>13</sup>C]lactate  $F_{\text{ex}}$ , leg lactate uptake, leg lactate oxidation, and muscle lactate concentrations were determined by using a repeated-measures ANOVA. Post hoc comparisons were made by using Fisher's protected least significant difference test. Statistical significance was set at  $\alpha = 0.05$ . All data are presented as means  $\pm$  SE.

## RESULTS

*Subject Characteristics*

Anthropometric data on subjects pre- and posttraining have been reported previously (2) but are repeated in Table 1. Subjects were weight stable throughout the study period.  $\dot{V}O_{2\text{peak}}$  increased significantly by 14.6% as a result of training. Consequently, posttraining trials at  $66 \pm 1.1\%$  of pretraining  $\dot{V}O_{2\text{peak}}$  (the same absolute workload as pretraining, 150 W) were performed at  $54.0 \pm 1.7\%$  of posttraining  $\dot{V}O_{2\text{peak}}$ ; 174 W were required to elicit 65% of  $\dot{V}O_{2\text{peak}}$  posttraining. The power output corresponding to lactate threshold increased 22% ( $P < 0.05$ ) after training. Specific power outputs and rates of  $O_2$  consumption achieved by subjects before and after training have been reported previously (3).

*Muscle Lactate Concentrations*

Resting vastus lateralis lactate concentrations were similar before and after training (Table 2). Additionally, postexercise muscle lactate concentrations and differences between pre- and postexercise muscle lactate concentrations (i.e., delta lactate concentrations) were unaltered by exercise intensity or training status.

*Arterial Lactate and Glucose Concentrations*

Resting arterial lactate concentrations were not altered by endurance training. Arterial lactate concentration increased significantly above rest under all exercise conditions, and lactate concentration was directly related to exercise intensity before and after training (Fig. 1A). Compared with the untrained state, endurance training decreased arterial lactate concentration by 40% at ABT and 20% at RLT ( $P < 0.05$ ). Arterial glucose concentration was stable over time and not significantly different between exercise intensities before and after training (2).

*Leg Lactate Metabolism*

Resting-leg  $[\text{lactate}]_{v-a}$  did not change before or after training (Fig. 1B). Initiation of exercise before and after

Table 1. *Subject characteristics before and after 9 wk of leg cycle endurance training*

Variable	Pretraining	Posttraining	%Difference
Age, yr	27.4 ± 2.0		
Height, in.	70.1 ± 1.0		
Weight, kg	81.8 ± 3.3	81.3 ± 3.2	-0.6
Body fat, %			
Skinfolds	19.7 ± 1.5	17.5 ± 1.6*	-11.0
Underwater weighing	19.5 ± 1.5	17.2 ± 1.4*	-11.6
$\dot{V}O_{2\text{peak}}$			
l/min	3.5 ± 0.10	4.02 ± 0.15*	14.6
ml · kg <sup>-1</sup> · min <sup>-1</sup>	43.5 ± 1.3	50.1 ± 1.6*	15.5
Lactate threshold (% $\dot{V}O_{2\text{peak}}$ )	60.9 ± 2.7	65.4 ± 2.6	7.4
Power output at lactate threshold, W	161.1 ± 4.4	197.2 ± 6.5*	22.4

Values are means ± SE;  $n = 9$  subjects.  $\dot{V}O_{2\text{peak}}$ , peak  $O_2$  consumption. \*Significantly different from pretraining values at  $P < 0.05$ .

Table 2. *Vastus lateralis lactate concentration during rest and immediately after exercise, before and after training*

[Lactate], μmol/g wet wt	45% Pretraining	65% Pretraining	45% ABT Posttraining	65% RLT Posttraining
Rest	5.76 ± 0.69	5.76 ± 0.69	5.43 ± 0.34	5.43 ± 0.34
Postexercise	7.59 ± 0.89	9.25 ± 1.18	10.97 ± 1.11	9.11 ± 0.92
Δ	1.83 ± 1.39	3.49 ± 1.29	5.53 ± 1.24	3.67 ± 0.89

Values are means ± SE;  $n = 9$  subjects. ABT, absolute workload; RLT, relative intensity exercise; Δ, change. Brackets indicate concentration.

training resulted in elevated  $[\text{lactate}]_{v-a}$ , which decreased over time. Before and after training,  $[\text{lactate}]_{v-a}$  was directly related to exercise intensity. Compared with before training,  $[\text{lactate}]_{v-a}$  decreased 60% ( $P < 0.05$ ) at ABT but was unchanged at RLT.

Resting limbs released lactate under all conditions, and limb L was unchanged at rest due to training (Fig. 1C). Under all exercise conditions during commencement of exercise increments in leg blood flow (3) and  $[\text{lactate}]_{v-a}$  caused an increase in L; however, because of changing  $[\text{lactate}]_{v-a}$ , L waned over time despite the constancy of limb blood flow. During exercise, L scaled to exercise intensity, with 210% greater release at 65% compared with 45% pretraining  $\dot{V}O_{2\text{peak}}$  ( $P < 0.05$ ), and 55% greater release at RLT compared with ABT ( $P < 0.05$ ). Compared with before training, L decreased 60% ( $P < 0.05$ ) at ABT but was not significantly different at RLT.

Arterial lactate isotopic enrichment is shown in Fig. 1D. On the basis of prior experience, the tracer infusion rate was adjusted among trials. Consequently, although enrichment fell during exercise, there were no differences in enrichment among trials.

Leg lactate  $F_{\text{ex}}$  approximated 15% (Fig. 2), was unchanged at rest before or after training, and did not change significantly from rest during exercise at any intensity. Before training at 65% compared with 45%  $\dot{V}O_{2\text{peak}}$ , fractional extraction decreased 50% ( $P < 0.05$ ). After training compared with 65% pretraining  $\dot{V}O_{2\text{peak}}$ , it increased 65 and 90% at ABT and RLT, respectively. Fractional extraction was not significantly different between ABT and RLT.

Tracer-measured leg lactate uptake rate (Fig. 3) and oxidation (not shown) were unchanged at rest before and after training and increased from rest to exercise at all intensities. Leg lactate uptake and oxidation scaled to exercise intensity before training, increasing 160% from 45 to 65%  $\dot{V}O_{2\text{peak}}$ , and after training, increasing 70% from ABT to RLT ( $P < 0.05$ ). Compared with 65% pretraining, leg lactate uptake and oxidation after training were unchanged at ABT and were 44% greater ( $P < 0.05$ ) at RLT.

The relationship between leg lactate oxidation and arterial lactate concentration is shown in Fig. 4. Before training, leg lactate oxidation displayed saturation, where leg lactate oxidation plateaued with increased arterial lactate concentration. After training, saturation was not apparent at the concentrations achieved.

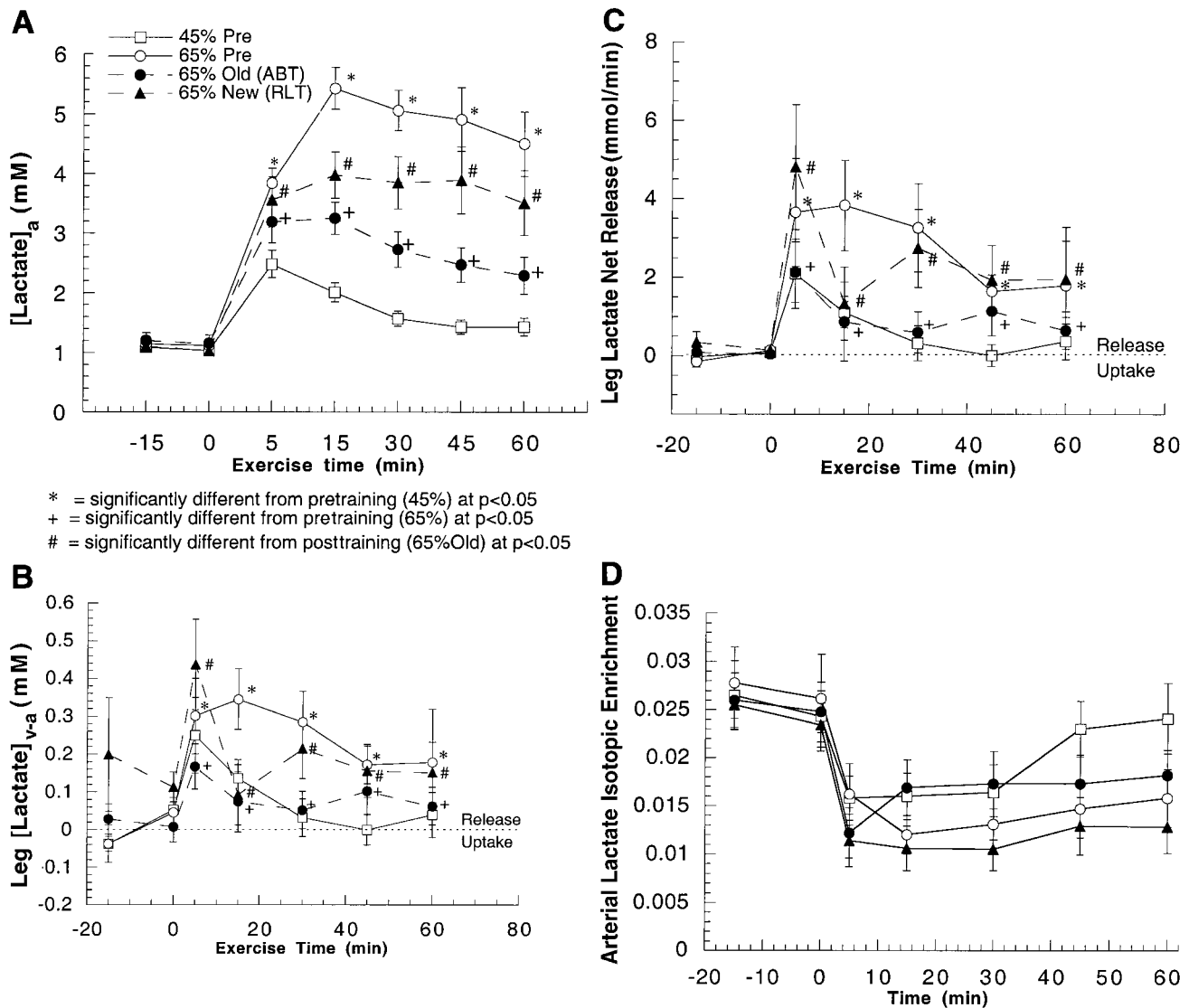


Fig. 1. *A*: arterial lactate concentration during rest and exercise before (Pre) and after endurance training. Values are means  $\pm$  SE for 8–9 subjects. ABT, absolute workload; RLT, relative intensity. *B*: leg venous-arterial (v-a) lactate concentration difference during rest and exercise before and after training. Values are means  $\pm$  SE for 7–8 subjects. *C*: net lactate release during rest and exercise before and after endurance exercise training. Values are means  $\pm$  SE for 7–8 subjects. *D*: arterial lactate isotopic enrichments during rest and exercise before and after endurance exercise training. Values are means  $\pm$  SE for 8–9 subjects.

Table 3 shows the contribution of leg lactate oxidation to leg carbohydrate oxidation determined from leg respiratory quotient (3). Before and after training, resting lactate oxidation accounted for 50% of leg carbohydrate oxidation, which decreased to 15% during exercise at all intensities. There were no differences during exercise before or after training in contribution of leg lactate oxidation to leg carbohydrate oxidation.

#### Whole Body Lactate Kinetics

Lactate  $R_a$  and  $R_d$  were similar at rest before and after training and increased during exercise at all intensities (Fig. 5, *A* and *B*, respectively). Compared with rest, lactate  $R_a$  and  $R_d$  increased by 150% at 45% pretraining  $\dot{V}O_{2\text{peak}}$ , and 500% at 65% pretraining  $\dot{V}O_{2\text{peak}}$  ( $P < 0.05$ ). During exercise after training compared with rest, lactate  $R_a$  and  $R_d$  increased 400% at ABT and

800% at RLT ( $P < 0.05$ ). During exercise before training, lactate  $R_a$  and  $R_d$  increased 140% ( $P < 0.05$ ) at 65% compared with 45%  $\dot{V}O_{2\text{peak}}$ . After training compared with the 65% pretraining  $\dot{V}O_{2\text{peak}}$  condition, lactate  $R_a$  and  $R_d$  decreased 40% ( $P < 0.05$ ) at ABT but were unchanged at RLT. After training, lactate  $R_a$  and  $R_d$  increased 85% ( $P < 0.05$ ) at RLT compared with ABT.

Lactate MCR was similar at rest before and after training (Fig. 5*C*). Lactate MCR increased significantly from rest to exercise by 150% at 45% pretraining  $\dot{V}O_{2\text{peak}}$ , 100% at ABT, and 130% at RLT. Before training, MCR decreased 40% at 65% compared with 45%  $\dot{V}O_{2\text{peak}}$  ( $P < 0.05$ ). During exercise at ABT after training, MCR tended to increase, but the rise in MCR was not significant ( $P = 0.06$ ). During exercise at RLT after training, lactate MCR increased 70% compared with 65% pretraining  $\dot{V}O_{2\text{peak}}$  ( $P < 0.05$ ).

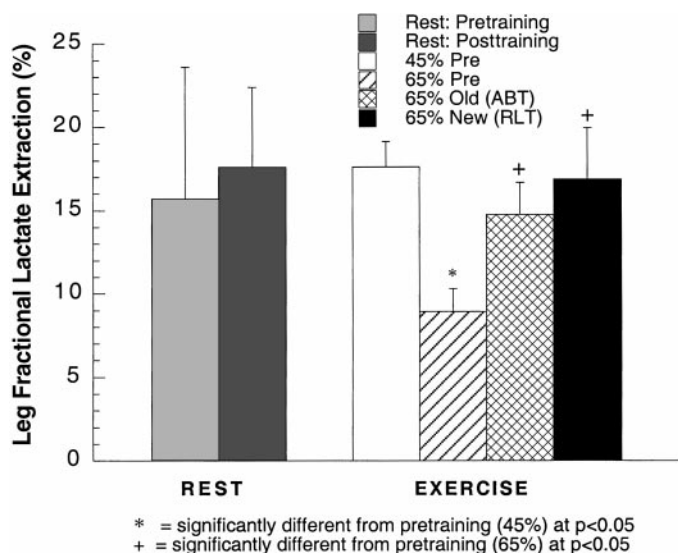


Fig. 2. Tracer-measured leg fractional lactate extraction during rest and exercise before and after training. Values are means  $\pm$  SE for 7–9 subjects.

Whole body lactate oxidation at rest was not significantly different between training states and increased during exercise regardless of intensity (Fig. 5D). Before training, lactate oxidation increased ( $P < 0.05$ ) 700 and 2,000% during exercise compared with rest at 45 and 65%  $\dot{V}O_{2\text{peak}}$ , respectively. After training, lactate oxidation during exercise also increased from rest by 1,300 and 2,500% at ABT and RLT, respectively. Similar to lactate turnover ( $R_a$  and  $R_d$ ), lactate oxidation increased ( $P < 0.05$ ) 160% at 65 vs. 45%  $\dot{V}O_{2\text{peak}}$  before training, and 90% after training at RLT compared with ABT. Compared with 65% pretraining  $\dot{V}O_{2\text{peak}}$ , lactate oxidation after training was 30% lower at ABT ( $P < 0.05$ ), and unchanged at RLT.

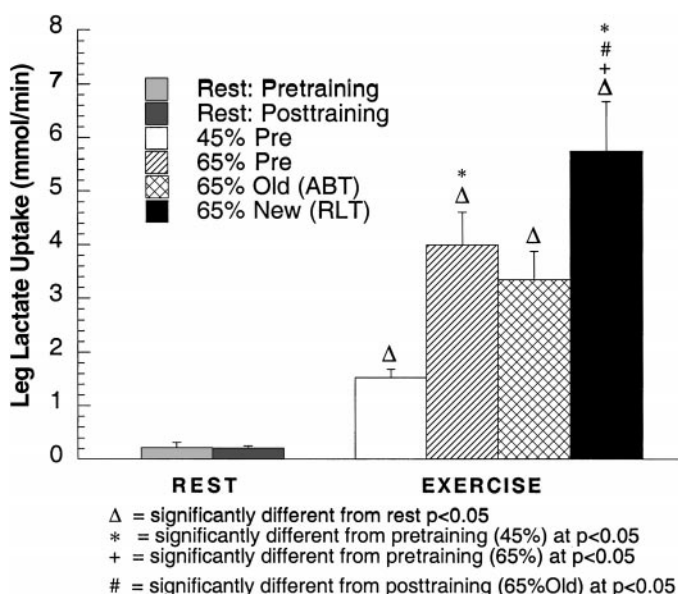


Fig. 3. Tracer-measured leg lactate uptake during rest and exercise before and after training. Values are means  $\pm$  SE for 7–9 subjects.

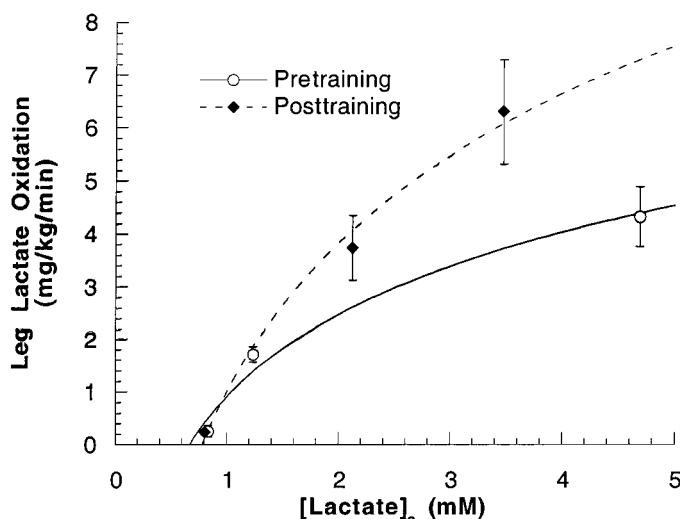


Fig. 4. Relationship between tracer-measured leg lactate oxidation rate and arterial lactate concentration ( $[\text{lactate}]_a$ ) before and after training. Values are means  $\pm$  SE for 7–9 subjects.

Table 3 shows that, during exercise, the percentage of lactate  $R_d$  oxidized increased from rest regardless of training state or exercise intensity. Under all exercise conditions, most (60–80%) lactate was disposed of through oxidation. The percentage of  $R_d$  oxidized increased significantly with increments in exercise intensity, both before and after training, with no differences during ABT or RLT compared with values obtained during the 65% pretraining  $\dot{V}O_{2\text{peak}}$  trial. The contribution of whole body lactate oxidation to whole body carbohydrate oxidation is also shown in Table 3. The percentage of total carbohydrate-derived  $\text{CO}_2$  accounted for by lactate oxidation increased in the transition from rest to exercise when lactate oxidation accounted for 15–25% of total carbohydrate-derived  $\text{CO}_2$ . There were no differences at rest due to training, with increased contributions compared with rest at 65% pretraining  $\dot{V}O_{2\text{peak}}$  and RLT. After training, the percentage of whole body carbohydrate oxidation from whole body lactate oxidation was 47% greater ( $P < 0.05$ ) at RLT compared with ABT.

#### Tracer Lactate Uptake and $\dot{L}_{\text{tot}}$

$\dot{L}$  underestimated  $\dot{L}_{\text{tot}}$  at rest and during every exercise intensity, both before and after training (Table 4).  $\dot{L}_{\text{tot}}$  was dramatically greater than  $\dot{L}$  due to simultaneous limb lactate uptake.  $\dot{L}_{\text{tot}}$  was greater than  $\dot{L}$  by 220% at 45% pretraining  $\dot{V}O_{2\text{peak}}$ , and 180% at 65% pretraining  $\dot{V}O_{2\text{peak}}$ . After training,  $\dot{L}_{\text{tot}}$  was greater than  $\dot{L}$  by 390 and 260% at ABT and RLT, respectively. Thus, regardless of exercise intensity,  $\dot{L}$  underestimated  $\dot{L}_{\text{tot}}$  by  $\sim 200\%$ .

#### Active Limb Contribution to Total Body Lactate Kinetics

At rest, before and after training,  $\dot{L}_{\text{tot}}$  accounted for similar percentages ( $\approx 20\%$ ) of whole body lactate  $R_a$  (Fig. 6A). However, during exercise the working limbs accounted for most (50–80%) of lactate  $R_a$ . Despite the

Table 3. Comparison of parameters of leg and whole body lactate metabolism in men during rest and exercise, before and after training

	Rest Pretraining	Rest Posttraining	45% Pretraining	65% Pretraining	ABT Posttraining	RLT Posttraining
%Lactate $R_d$ oxidized	21.5 ± 1.3	25.4 ± 1.0	61.6 ± 1.5*	69.5 ± 1.8*†	71.8 ± 1.5*†	76.0 ± 2.3*†‡
Contribution of leg CHO oxidation from leg lactate oxidation, %	57.3 ± 20.5	51.1 ± 13.4	13.0 ± 1.3*	17.5 ± 2.8*	14.3 ± 3.1*	16.8 ± 3.2*
Contribution of whole body CHO oxidation from whole body lactate oxidation, %	9.9 ± 2.5	11.7 ± 1.3	15.3 ± 1.4	23.2 ± 3.8*	17.5 ± 2.2	25.7 ± 2.3*§

Values are means ± SE;  $n = 8-9$  subjects. CHO, carbohydrate. \*Significantly different from rest at  $P < 0.05$ . †Significantly different from rest and 45% pretraining at  $P < 0.05$ . ‡Significantly different from 65% pretraining at  $P < 0.05$ . §Significantly different from posttraining (ABT) at  $P < 0.05$ .

apparent trend, a training effect on percentage of lactate  $R_a$  from the legs was not detected. Before training, during exercise compared with rest, the percentage of lactate  $R_a$  from  $\dot{L}_{tot}$  increased 70 and 120% at 45 and 65% pretraining  $\dot{V}O_{2peak}$ , respectively ( $P < 0.05$ ). During exercise after training, the contribution of working-limb  $\dot{L}_{tot}$  to whole body lactate  $R_a$  increased from rest by 210 and 250% at ABT and RLT, respectively ( $P < 0.05$ ). Compared with the 65% pretraining  $\dot{V}O_{2peak}$  trial, there were no differences in the percentage of lactate  $R_a$  from at ABT and RLT. There was a positive linear relationship between the percentage of lactate  $R_a$  from  $\dot{L}_{tot}$  and exercise intensity [ $r = 0.99$ ; pretraining: % $R_a$  from  $\dot{L}_{tot} = 0.59(\% \dot{V}O_{2peak}) + 21.99$ ; posttraining: % $R_a$  from  $\dot{L}_{tot} = 1.05(\% \dot{V}O_{2peak}) + 14.47$ ].

During exercise, approximately one-half of whole body lactate  $R_d$  could be explained by active-limb lactate uptake, which was greater during exercise compared with rest (Fig. 6B). Increasing exercise intensity before and after training did not alter the percentage of lactate  $R_d$  accounted for by limb lactate uptake. After training, at ABT leg lactate uptake accounted for 30% more of lactate  $R_d$  compared with 65% pretraining  $\dot{V}O_{2peak}$ . Even though active legs accounted for a majority of lactate disposal during ABT and RLT after training (Fig. 6B), active legs contributed more to lactate  $R_a$  (Fig. 6A) than to  $R_d$  (Fig. 6B). There was a positive linear relationship between the percentage of lactate  $R_d$  from leg lactate uptake and exercise intensity [ $r = 0.98$ ; pretraining: % $R_d$  from leg lactate uptake =  $0.38(\% \dot{V}O_{2peak}) + 19.58$ ;  $r = 0.96$ ; posttraining: % $R_d$  from leg lactate uptake =  $0.65(\% \dot{V}O_{2peak}) + 13.80$ ].

There was no training effect on the percentage of whole body lactate oxidation attributable to the legs at rest (Fig. 6C). Active limbs accounted for the majority (70%) of whole body lactate oxidation during exercise before and after training (Fig. 6C). The percentage of whole body lactate oxidation from limb lactate oxidation did not increase during exercise compared with rest before or after training. After training at ABT, the percentage of whole body lactate oxidation from active limbs increased 30% compared with 65% pretraining  $\dot{V}O_{2peak}$ , and 20% compared with RLT.

There were strong correlations ( $r = 0.99$ ) between our two measures of lactate production ( $R_a$  and  $\dot{L}_{tot}$ ) during rest and exercise both before and after training (Fig. 5A). Similarly, there were strong correlations ( $r =$

0.97) between whole body lactate  $R_d$  and leg lactate uptake before and after training (Fig. 5B).

## DISCUSSION

This is the first longitudinal investigation of training effects on lactate metabolism by using the combination of tracer technology and limb net exchange measurements. As such, our approach provided two estimates of lactate production during rest and exercise: tracer-derived blood lactate  $R_a$  and  $\dot{L}_{tot}$ . In general, values are highly correlated ( $r = 0.99$ , Fig. 5A) and show active muscle is the predominant, but not exclusive, site of lactate turnover during exercise.

Our data indicate that mechanisms for dampened arterial lactate concentration after endurance training vary depending on exercise intensity. At the same absolute workload, active trained limbs maintained similar lactate  $R_{ox}$  despite attenuated whole body lactate turnover and arterial concentration, due to increased  $F_{ex}$ . Thus, during moderate-intensity exercise after training (i.e., ABT), decreased lactate  $R_a$  and  $\dot{L}_{tot}$  and increased muscle lactate clearance contributed to dampened arterial lactate concentration. However, at a fixed relative exercise intensity (i.e., RLT), lactate turnover was the same after training as before, but leg lactate oxidation and whole body and leg lactate clearance increased.

With regard to circulating lactate concentration during exercise, we found that active-limb lactate release cannot explain the maintained elevation of arterial lactate concentration (Fig. 1A) as limb  $\dot{L}$  fell to close to zero by the end of exercise under all conditions (Fig. 1C). Therefore, other tissues must release lactate on net bases as exercise duration progresses.

Finally, regarding the use of  $\dot{L}$  as a measure of lactate production,  $\dot{L}$  underestimated  $\dot{L}_{tot}$  before and after training. Because limbs simultaneously take up and release lactate,  $\dot{L}$  alone cannot quantitate limb lactate production. Critically, measurements of differences between arterial and venous concentrations miss important parameters of muscle lactate uptake and oxidation.

### Training Adaptations

Our 9-wk training program promoted significant metabolic adaptations (Table 1). Subjects significantly

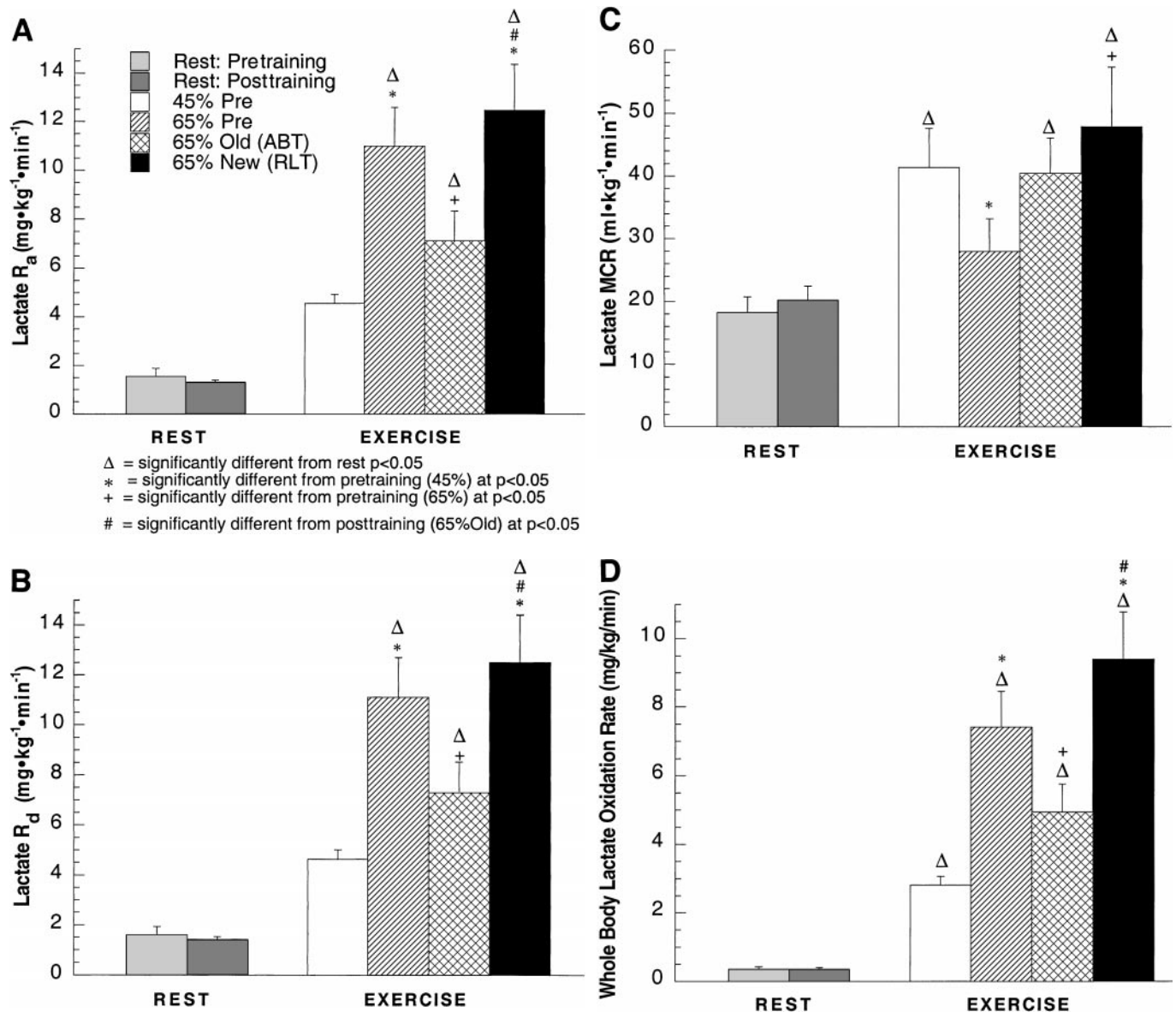


Fig. 5. *A*: effects of exercise intensity and training on lactate rate of appearance ( $R_a$ ). Values are means  $\pm$  SE for 8–9 subjects. There is a positive linear relationship between whole body lactate  $R_a$  and total leg lactate release [ $L_{tot}$ ;  $r = 0.99$ ; pretraining: lactate  $R_a = 1.32(L_{tot}) + 1.65$ , posttraining: lactate  $R_a = 1.35(L_{tot}) + 1.03$ ]. *B*: effects of exercise intensity and training on lactate rate of disappearance ( $R_d$ ). Values are means  $\pm$  SE for 8–9 subjects. There is a positive linear relationship between whole body lactate  $R_d$  and leg lactate uptake [ $r = 0.99$ ; pretraining: lactate  $R_d = 2.18(\text{leg lactate uptake}) + 1.09$ , posttraining: lactate  $R_d = 1.81(\text{leg lactate uptake}) + 0.72$ ]. *C*: effects of exercise intensity and training on lactate metabolic clearance rate (MCR). Values are mean  $\pm$  SE for 8–9 subjects. *D*: effects of exercise intensity and training on whole body lactate oxidation rate. Values are means  $\pm$  SE for 8–9 subjects.

Table 4. *Leg net lactate release, tracer-measured lactate uptake, and total lactate release during rest and the last 30 min of exercise in men before and after training*

	Rest Pretraining	Rest Posttraining	45% Pretraining	65% Pretraining	ABT Posttraining	RLT Posttraining
Net lactate release, mmol/min	0.05 $\pm$ 0.02	0.06 $\pm$ 0.05	0.50 $\pm$ 0.47*	2.23 $\pm$ 0.72*†	0.78 $\pm$ 0.46*‡	2.21 $\pm$ 0.82*†§
Tracer-measured leg lactate uptake, mmol/min	0.21 $\pm$ 0.10	0.21 $\pm$ 0.05	1.53 $\pm$ 0.15*	3.99 $\pm$ 0.61*†	3.35 $\pm$ 0.52*	5.75 $\pm$ 0.92*†‡§
Total lactate release, mmol/min	0.26 $\pm$ 0.10	0.27 $\pm$ 0.05	1.62 $\pm$ 0.33*	6.22 $\pm$ 0.60*†	3.80 $\pm$ 0.66*‡	7.96 $\pm$ 1.25*†§

Values are means  $\pm$  SE;  $n = 8-9$  subjects. \*Significantly different from rest at  $P < 0.05$ . †Significantly different from 45% pretraining at  $P < 0.05$ . ‡Significantly different from 65% pretraining at  $P < 0.05$ . §Significantly different from posttraining (ABT) at  $P < 0.05$ .



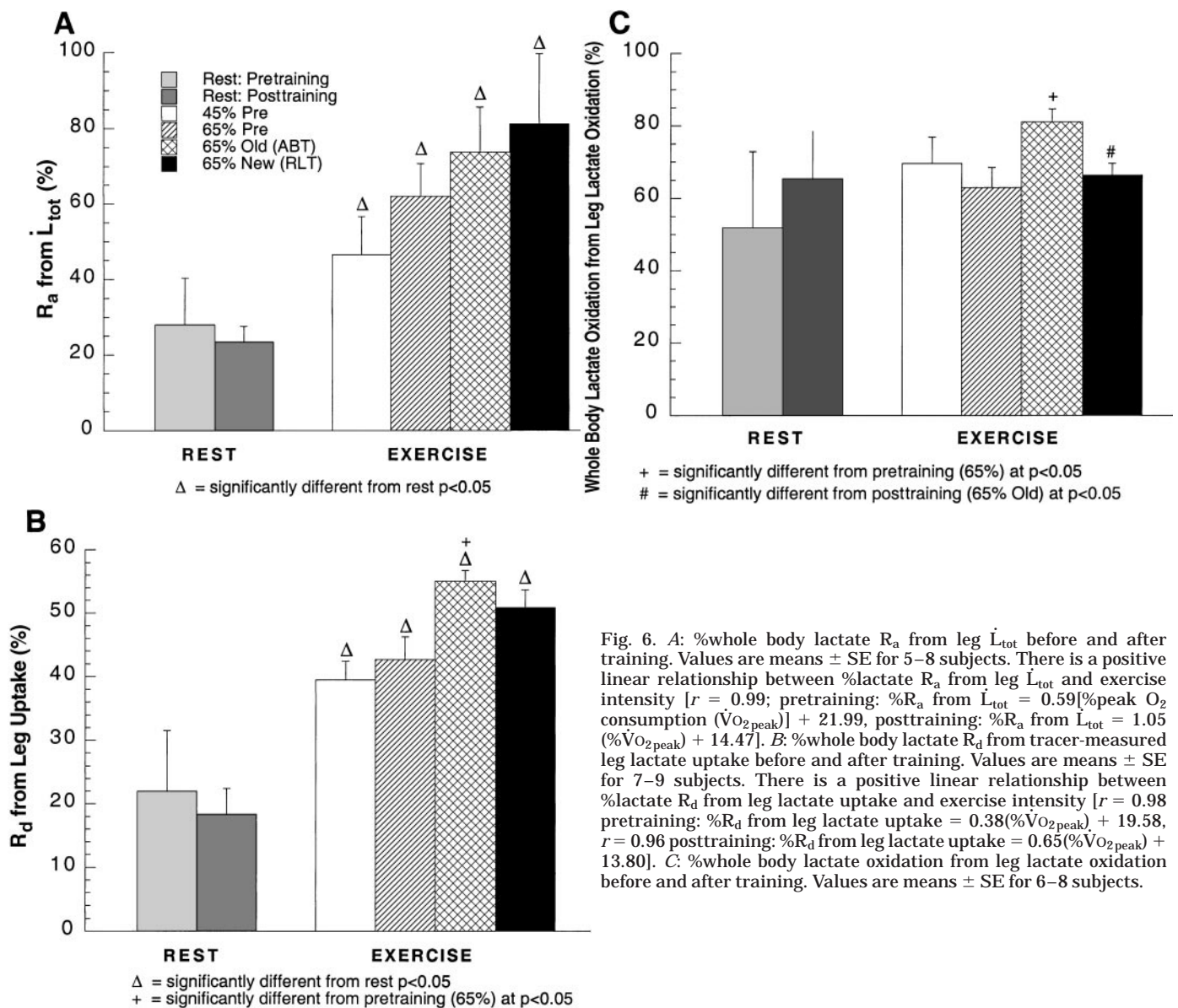


Fig. 6. *A*: %whole body lactate  $R_a$  from leg  $\dot{L}_{tot}$  before and after training. Values are means  $\pm$  SE for 5–8 subjects. There is a positive linear relationship between %lactate  $R_a$  from leg  $\dot{L}_{tot}$  and exercise intensity [ $r = 0.99$ ; pretraining: % $R_a$  from  $\dot{L}_{tot} = 0.59[\%peak\ O_2\ consumption (\dot{V}O_{2peak})] + 21.99$ , posttraining: % $R_a$  from  $\dot{L}_{tot} = 1.05 (\%V_{O_{2peak}}) + 14.47$ ]. *B*: %whole body lactate  $R_d$  from tracer-measured leg lactate uptake before and after training. Values are means  $\pm$  SE for 7–9 subjects. There is a positive linear relationship between %lactate  $R_d$  from leg lactate uptake and exercise intensity [ $r = 0.98$  pretraining: % $R_d$  from leg lactate uptake =  $0.38(\%V_{O_{2peak}}) + 19.58$ ,  $r = 0.96$  posttraining: % $R_d$  from leg lactate uptake =  $0.65(\%V_{O_{2peak}}) + 13.80$ ]. *C*: %whole body lactate oxidation from leg lactate oxidation before and after training. Values are means  $\pm$  SE for 6–8 subjects.

increased  $\dot{V}O_{2peak}$  (15%), decreased the respiratory exchange ratio at a given absolute workload (3.2%), increased the power output eliciting lactate threshold by 22%, decreased arterial lactate concentration at the same relative (26%) and absolute workload (55%), and increased resting muscle glycogen concentration (62%) (2).

#### Muscle Lactate Concentration

Our data for muscle lactate concentration immediately after exercise are similar to those in previous reports (19, 28). Henriksson (19) reported unchanged resting vastus lateralis lactate concentrations after training, as well as nonsignificantly different muscle lactate concentrations after 50 min of exercise at the same relative intensity in men. Kiens et al. (28) also found unchanged vastus lateralis lactate concentrations between trained and untrained subjects at rest and immediately after 2 h of exercise at the same absolute workload. Our data do not agree with those of

others who reported decreased muscle lactate concentrations in trained compared with untrained subjects after exercise at a given absolute (39) or relative (22) intensity. Possibly, this distinction is due to nutritional controls we imposed or the time course of muscle lactate response that first rises, and then falls, as exercise continues (1, 11).

Several investigators have reported significantly decreased muscle lactate concentrations at the beginning of exercise in trained compared with untrained subjects (16, 26). Those data are consistent with our observations of a training effect that dampens most dramatically at the start of exercise (Fig. 1, *B* and *C*). Low muscle lactate concentrations after exercise (Table 2) are attributable to high  $R_{ox}$  (Figs. 4 and 5*D*).

#### Limb Lactate Exchange

Our results show that endurance training decreased  $\dot{L}$  at the same absolute but not the same relative

intensity (Fig. 1C, Table 4). These data are similar to those of others who showed unchanged and low  $\dot{L}$  at rest (19, 22, 28, 39) and dampened  $\dot{L}$  throughout 1 h of exercise at a given absolute workload after training (39). Additionally, our results are similar to those of others who reported unchanged  $\dot{L}$  during exercise in trained compared with untrained subjects at a given relative-intensity task (44).

Muscle  $\dot{L}$  rates computed from arteriovenous difference and blood flow obscure lactate uptake and oxidation during  $\dot{L}$  (11, 12, 42). Stanley et al. (42) reported  $\dot{L}_{\text{tot}}$  ( $\dot{L}$  + tracer-measured lactate uptake) to be roughly twice  $\dot{L}$  by using [ $3\text{-}^{14}\text{C}$ ]lactate infusion during graded-intensity leg cycling in men. Subsequently, using [ $3\text{-}^{13}\text{C}$ ]lactate infusion, Brooks et al. (11) reported  $\dot{L}_{\text{tot}}$  to be 400% greater than  $\dot{L}$  during sea-level leg cycling at 51%  $\text{VO}_{2\text{peak}}$ . We found similar results in the present study, with mean  $\dot{L}_{\text{tot}}$  280% greater than  $\dot{L}$  pretraining and 320% greater posttraining (Table 4). Thus results of our study show that blood concentration and  $\dot{L}$  are inadequate measures of either whole body or tissue lactate metabolism.

Our data show active limb  $\dot{L}$  decreased to close to zero after 45 min of exercise (Fig. 1C), whereas arterial lactate concentration remained elevated (Fig. 1A); similar results have been reported before (1, 11, 19, 45). We interpret these data to suggest that muscle contributes to elevated arterial lactate concentration at the beginning of steady-rate exercise, but, as exercise duration progresses, other tissues become important for maintaining circulating lactate concentration and providing lactate as a substrate for working muscle. Several other tissues, including skin (24), adipose (23), and intestine (40), have been shown to release lactate on net bases. It is possible  $\dot{L}$  increased in these tissues as exercise duration progressed. Thus, our data, as well as those of others (1, 19, 45), suggest that muscle is not responsible for maintaining elevated arterial lactate concentration after as little as 45 min of steady-rate exercise.

#### *Intramuscular Lactate Metabolism*

Studies initially performed independently by Juel (25) and Watt et al. (46) in mouse and rat muscle preparations, respectively, and subsequently confirmed by Roth and Brooks (37, 38) on isolated rat sarcolemmal vesicles, indicated that myocyte lactate exchange is mediated by a lactate transport protein. Western blot analyses indicating increased expression of the putative sarcolemmal lactate transporter protein monocarboxylate transporter 1 (MCT1) after short-term training (5) has been interpreted to mean training decreases muscle net lactate production by facilitating lactate exchange between glycolytic and oxidative fibers according to the cell-cell lactate shuttle hypothesis (7). Western analyses of biopsies obtained from our subjects (H. Dubouchaud, G. E. Butterfield, E. E. Wolfel, B. C. Bergman, and G. A. Brooks, unpublished observations) indicate that 9 wk of training significantly increases muscle MCT1 isoform expression. However, training-induced increases in sarcolemmal lactate transporters cannot explain either the present or previously pub-

lished results. Our data (Figs. 2–4, 5D, and 6; Tables 3 and 4) show that working human muscle takes up and oxidizes lactate and that training increases intramuscular lactate clearance primarily by increasing oxidation. Recently, Brooks et al. (8) demonstrated that muscle, cardiac, and liver mitochondria take up and oxidize lactate directly because of mitochondrial LDH and MCT (lactate-pyruvate) pools. On that basis, and with reference to supporting NMR data showing direct mitochondrial oxidation of lactate by a variety of cells and tissues (4, 6, 43), an “intracellular lactate shuttle” was proposed. Thus our results are consistent with training increasing expression of muscle mitochondrial proteins and constituents, including mitochondrial LDH and MCT, thus facilitating intramuscular lactate oxidation and action of the intracellular lactate shuttle.

#### *Whole Body Lactate Kinetics*

It is well documented that lactate turnover increases as a direct function of exercise intensity (11, 13, 14, 30, 31, 41). Our data are consistent with previous results as lactate  $R_a$  and  $R_d$  increased significantly with increments in exercise intensity both before and after training (Fig. 5, A and B). It has been reported that, at a given absolute workload after endurance training, lactate  $R_a$  was unchanged, whereas MCR significantly increased in both rats (14) and humans (33). Therefore, it was concluded that increased lactate MCR was responsible for decreased circulating lactate concentration after training.

Our results diverge from the literature on effects of endurance training on lactate turnover as we found significantly decreased lactate  $R_a$  and  $R_d$  during ABT after training compared with those parameters determined during the same task before training (Fig. 5, A and B). Thus our data suggest that endurance training promotes decreased arterial lactate concentration at a given absolute workload by decreasing whole body lactate  $R_a$  (Fig. 5A), active-leg total lactate production (Table 4), and  $\dot{L}$  (Fig. 1C, Table 4). We note in this regard that the training-induced increase in whole body lactate MCR at ABT approached, but did not achieve, significance ( $P = 0.06$ ).

There are several potential mechanisms that may have promoted decreased lactate production ( $R_a$  and  $\dot{L}_{\text{tot}}$ ) during exercise at ABT after training. In working muscle, decreased glycogen degradation after training at a given absolute workload (2, 22, 39) should result in less lactate formation because of decreased glycolytic flux. Similarly, although training increases muscle GLUT-4 content, less is translocated to the sarcolemma during exercise at a given power output after training (35). Perhaps more importantly, increased mitochondrial mass enhances intramuscular pyruvate and lactate oxidation and therefore decreases  $\dot{L}$  after training (8). At other tissue sites, glycogenolysis leading to lactate production may have been attenuated by the decline in circulating epinephrine. Consistent with this interpretation,  $\beta$ -adrenergic blockade decreases arterial lactate concentration, whereas muscle  $\dot{L}$  is minimally affected (10).

Donovan and Brooks (14) and Phillips et al. (33) reported unchanged lactate turnover and increased MCR in rats and humans, respectively, exercising at an absolute workload after endurance training. Like the aforementioned researchers, we found that whole body lactate MCR tended to increase after endurance training at ABT ( $P = 0.06$ ) (Fig. 5C); however, we found decreased lactate turnover at ABT. Phillips et al. employed a short, 10-day training program that may explain why they did not find dampened whole body lactate  $R_a$  at ABT. Donovan and Brooks (14) also reported unchanged lactate turnover after endurance training in rats. Possibly, the inability to precisely control exercise intensity of rats during treadmill running may explain the lack of agreement with the present study. Thus the previous data on running rats may be more like the present results obtained during RLT than ABT.

Ours is the first study to report endurance training effects on lactate kinetics at a given relative intensity under steady-state conditions. Our data suggest endurance training does not alter whole body lactate appearance at RLT (Fig. 5A) but increases whole body and leg lactate oxidation and clearance (Figs. 4 and 5, C and D; Table 4). Additionally,  $L$  (Fig. 1C) and  $L_{tot}$  (Table 4) were unchanged at RLT compared with 65% pretraining  $\dot{V}O_{2peak}$ . We attribute the lack of a training effect on whole body lactate flux and increase in leg lactate oxidation at RLT after training to the effect of muscle contraction on glycolysis. Compared with before training, the power output required to elicit 65% of  $\dot{V}O_{2peak}$  increased by 22 W, or 15%, which elicited a similar or greater glycolytic flux. Thus both before and after endurance exercise training, whole body lactate kinetics closely parallel working-muscle lactate production, and relative exercise intensity dictates whole body and active-muscle lactate metabolism.

Donovan and Brooks (14) and Brooks and Gaesser (9) first reported that oxidation was the major fate of lactate during and after exercise, respectively, with 75–80% oxidation of infused tracer. Similarly, using [ $^{13}C$ ]lactate in exercising humans, Mazzeo et al. (31) reported 82% oxidation of lactate  $R_d$  at 50% maximal  $O_2$  consumption and 78% oxidation at 75% maximal  $O_2$  consumption. Our data are consistent with literature showing oxidation as the main fate of lactate  $R_d$  during exercise (Table 3, Fig. 5D). At rest, only 20 and 25% of lactate  $R_d$  was oxidized before and after training, respectively. However, exercise dramatically increased lactate oxidation such that, before training, oxidation accounted for 60 and 70% of lactate  $R_d$  at 45 and 65%  $\dot{V}O_{2peak}$ . After training, 70 and 80% of lactate  $R_d$  was oxidized at ABT and RLT, respectively. Thus oxidation is the major fate of whole body lactate disposal during exercise; lactate oxidation scales to exercise intensity and increases at a given RLT after training.

Leg total lactate production accounted for 54% of whole body lactate  $R_a$  before training and 77% after training (Fig. 6A). Thus our data suggest that most of decreased lactate  $R_a$  after training may be attributable to decreased active muscle lactate release. Active skel-

etal muscle was slightly less influential in determining whole body lactate  $R_d$ , with only 41% of lactate  $R_d$  attributable to active-muscle lactate uptake before training, and 53% after training (Fig. 6B). Other tissues, such as liver and inactive skeletal muscle (34), must have contributed to lactate clearance during exercise before and after training. The majority of whole body lactate oxidation was also due to active muscle lactate oxidation. Leg oxidation accounted for 70% of whole body lactate oxidation before and after training (Fig. 6C). Thus it appears that active muscle is largely responsible for alterations in whole body lactate turnover and oxidation during exercise, both before and after training.

#### *Assumptions and Limitations*

We have made repeated references to active-muscle lactate metabolism throughout this paper with the assumption that the majority of limb lactate metabolism is attributable to alterations in skeletal muscle. However, arteriovenous differences across a limb do not exclusively represent metabolism of skeletal muscle but are influenced by other tissues, including skin, subcutaneous adipose, and adipocytes located among muscle fibers. Because both skin (24) and adipocytes (23) are known to consume glucose and release lactate on net bases, our method of measuring limb lactate exchange may have overestimated skeletal muscle  $L$ .

As with the lack of specificity of venous lactate concentration measurements, a thermodilution technique is unable to determine alterations in blood flow to different muscle fiber types. We assumed that blood flow to individual muscle fibers was unchanged after endurance training, and alterations in leg lactate metabolism were due to changes in skeletal muscle cellular metabolism. It is possible that increased capillary density around type I fibers after training (29) may have decreased transit time specific to type I fibers, resulting in altered muscle fiber perfusion patterns that could alter lactate exchange.

Different equations were used to calculate lactate uptake and oxidation during rest and exercise. Ninety minutes of rest were insufficient to achieve isotopic equilibrium in  $CO_2$  pools as we found  $^{13}CO_2$  consumption across resting limbs. Therefore, to estimate lactate oxidation during rest we determined tracer lactate uptake from isotopic dilution of lactate in femoral venous compared with arterial blood. Thus we may have overestimated resting-limb lactate oxidation. Additionally, tracer fractional extraction was highly variable during exercise, and we estimated muscle lactate uptake from  $^{13}CO_2$  release across limbs. Variable tracer uptake data could be explained in part by [ $^{13}C$ ]lactate release into venous blood from glycogenolysis of  $^{13}C$ -labeled glycogen stored during rest. However, this is unlikely as the period of rest was too short to extensively label blood glucose (2) or muscle glycogen stores with [ $^{13}C$ ]glucose. Alternatively, we considered whether  $^{13}CO_2$  release from active muscle could be due to isotopic equilibration in the TCA cycle during gluconeogenesis. For the present, we are confident  $^{13}CO_2$  release

across working muscle provides an acceptable minimal estimate of lactate uptake as decarboxylation of [ $3\text{-}^{13}\text{C}$ ]lactate tracer in working muscle is due to oxidation in the TCA cycle and not loss of label during gluconeogenesis because key enzymes of gluconeogenesis (e.g., phosphoenolpyruvate carboxykinase) are not expressed in skeletal muscle.

Finally, there has been controversy as to whether lactate tracers measure lactate turnover or pyruvate turnover, and, therefore, total carbohydrate oxidation. Concerns with using tracer lactate to quantitate turnover stem from studies (36) in which tracer lactate or pyruvate infusion resulted in similar lactate and pyruvate enrichments in blood after several minutes. However, the authors failed to appreciate that the lactate-pyruvate equilibrium in blood due to action of LDH in erythrocytes leaves almost all tracer as lactate, because plasma lactate-to-pyruvate ratio rises from 10 to 50 or more during exercise. Hence, infused lactate tracer remains in the blood as lactate.

More importantly, with regard to the use of tracers, our data suggest that lactate turnover does not measure pyruvate turnover because the ratio of whole body and leg lactate oxidation to total body and leg carbohydrate oxidation was always much less than 1 (Table 3). As already discussed, we obtained excellent correlations between lactate  $R_a$  and muscle  $L_{\text{tot}}$  (Fig. 5A). Therefore, our data suggest that carbon-labeled lactate tracers can be used to quantitate blood lactate flux.

### Conclusions

We found that endurance training decreases whole body and working-muscle (leg) lactate production and increases clearance by active muscle at given moderate-intensity workloads. However, at similarly high relative exercise intensities, endurance training increases whole body and active-muscle lactate clearance, but does not influence whole body or muscle production. Thus mechanisms for decreased arterial lactate concentration after endurance training vary depending on exercise intensity. We also found that working skeletal muscle extracts and oxidizes lactate during L, indicating that arteriovenous differences alone underestimate limb lactate production. Additionally, active skeletal muscle likely contributes to elevated arterial lactate concentration during the beginning of steady-rate exercise. However, inactive muscle and other tissues must release lactate during exercise to explain maintenance of elevated arterial lactate concentration, as active muscle consumes and oxidizes blood lactate, whereas  $L$  from active muscle falls to close to zero after 45 min of exercise. Results showing simultaneous lactate production and oxidation in active muscle as well as other tissue beds support functions of cell-cell and intramuscular lactate shuttles in vivo.

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