Pyruvate shuttling during rest and exercise before and after endurance training in men

Gregory C. Henderson, Michael A. Horning, Steven L. Lehman, Eugene E. Wolfe, Bryan C. Bergman, and George A. Brooks. Pyruvate shuttling during rest and exercise before and after endurance training in men. J Appl Physiol 97: 317–325, 2004. First published February 27, 2004; 10.1152/japplphysiol.01367.2003.—We describe the isotopic exchange of lactate and pyruvate after arm vein infusion of [3-13C]lactate in men during rest and exercise. We tested the hypothesis that working muscle (limb net lactate and pyruvate exchange) is the source of the elevated systemic lactate-to-pyruvate concentration ratio (L/P) during exercise. We also hypothesized that the isotopic equilibration between lactate and pyruvate would decrease in arterial blood as glycolytic flux, as determined by relative exercise intensity, increased. Nine men were studied at rest and during exercise before and after 9 wk of endurance training. Although during exercise arterial pyruvate concentration decreased to below rest values (P < 0.05), pyruvate net release from working muscle was as large as lactate net release under all exercise conditions. Exogenous (arterial) lactate was the predominant origin of pyruvate released from working muscle. With no significant effect of exercise intensity or training, arterial isotopic equilibration or elevated arterial L/P during exercise. We also hypothesized that the isotopic equilibration between lactate and pyruvate in arterial blood decreases significantly during exercise; 2) working muscle is not solely responsible for the decreased arterial isotopic equilibration or elevated arterial L/P occurring during exercise; 3) working muscle releases similar amounts of lactate and pyruvate, the predominant source of the latter being arterial lactate; 4) pyruvate clearance from blood occurs extensively outside of working muscle; and 5) working muscle also releases alanine, but alanine release is an order of magnitude smaller than lactate or pyruvate release. These results portray the complexity of metabolic integration among diverse tissue beds in vivo.

LACTATE KINETICS HAVE BEEN measured in previous attempts to model whole body glycolytic flux, but the relationship between pyruvate and lactate metabolism at the whole body level has not been as extensively studied. Some have attempted to measure the production of pyruvate by working muscle, but this involved estimations based on rates of glucose uptake and changes in intramuscular contents of glycogen, glucose, and intermediates of carbohydrate metabolism (33). Others have used isotope tracers but only studied anesthetized dog prepa-
rations (18, 47), thus employing models quite distinct from studies examining kinetics in humans in vivo.

It has been proposed that there is rapid interconversion between lactate and pyruvate in mammalian blood (36). After infusion of labeled lactate in anesthetized dogs, it was reported that blood pyruvate became nearly as enriched with carbon tracer as blood lactate (47). Another investigation by the same group also reported that during infusion of pyruvate or lactate tracer in anesthetized dogs, the isotopic enrichments (IEs) of pyruvate or lactate in blood were essentially equal (18). However, at physiological lactate concentrations, lactate transport activity in dog erythrocytes is about six times greater than in human erythrocytes (37, 38), presumably increasing isotopic equilibration of lactate and pyruvate in dog blood via greater access to erythrocyte lactate dehydrogenase (LDH).

Sumegi et al. (41) lamented conversion of pyruvate tracer to lactate in rat blood in vivo, and Wolfe and associates (18, 47) concluded that isotopic lactate and pyruvate equilibration in blood of anesthetized dogs meant that infused lactate tracer was of little use for estimation of lactate flux in vivo. However, others have obtained different results and reached contrary conclusions. In a study on anesthetized rats, Large et al. (27) found only ~70% isotopic equilibration of lactate to pyruvate in arterial blood. Similarly, Avogaro et al. (4) reported isotopic equilibration of lactate to pyruvate approximating 60% in arterial blood of resting postabsorptive humans. Considering the large equilibrium constant of LDH (~105), and as it is commonly reported that the lactate-to-pyruvate concentration ratio (L/P) in arterial blood is at least 10 in resting humans, it is clear that the balance between lactate and pyruvate interconversion strongly favors lactate in vivo. Furthermore, data obtained on intact rats (27, 41) and humans (4), as well as isolated working rat heart preparations (17), indicate that tissue pools are also not in complete isotopic equilibration. Regrettably, to our knowledge, there have yet to be any studies investigating the extent of isotopic equilibration between lactate and pyruvate in blood of humans during exercise. Similarly, we judged the literature deficient with respect to describing the roles of pyruvate in monokarboxylate exchange within and among tissue beds of resting and exercising humans.

To date it is well established that arterial lactate can provide energy substrate for the working heart (20). It is also known that during exercise lactate is the major gluconeogenic precur-

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it is known that working muscle is not the sole source of blood lactate in exercising humans (13, 15), and, moreover, that nonworking muscle can be a net consumer of lactate whereas working human muscle simultaneously produces and consumes lactate (10, 13, 15, 39). Given the demonstrated importance of lactate shuttling within and between tissues (13, 15, 39), it was appropriate to suspect that pyruvate shuttling could be involved in the distribution of carbohydrate potential energy. Hence, we sought to test hypotheses regarding the relationships among the lactate and pyruvate pools during rest and exercise. We hypothesized (erroneously) that the working muscle is the source of the elevated arterial L/P during exercise and would therefore release lactate at a substantially greater rate than pyruvate both before and after endurance training. We also hypothesized (correctly) that increased glycolytic flux rates, as seen at higher relative exercise intensities, would affect the isotopic equilibration of lactate and pyruvate in arterial blood.

Evaluation of these hypotheses revealed unexpected roles of working muscles and other tissues in the integration of carbohydrate metabolism during physical activity.

METHODS

Details of experimental protocols in terms of subject selection, evaluation, treatment, and endurance training were reported previously (10). In the present report, we focus on measures of pyruvate concentration and IE but reiterate salient aspects of methodology.

Subjects

Nine healthy sedentary male subjects age 19–33 yr were recruited from the University of California, Berkeley, campus 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 (\( \dot{V}O_2 \) peak) of < 45 ml/kg \(^{-1}\) min \(^{-1}\). Subjects were included in the study if they had < 25% body fat, were nonsmokers, were diet and weight stable, had a 1- s forced inspiratory volume of 70% or more of vital capacity, and were injury and disease free as determined by physical examination. This study was approved by the Committee for the Protection of Human Subjects at Stanford University, the University of Colorado, Denver, and the University of California, Berkeley (CPHS 97-6-34).

Experimental Design

After interviews and preliminary screening were completed, subjects performed two graded exercise tests to determine \( \dot{V}O_2 \) peak during leg cycle ergometry. During the second assessment of \( \dot{V}O_2 \) peak, forearm vein blood was taken for assessment of lactate threshold. Subjects were then tested in a random order at 45 and 65% \( \dot{V}O_2 \) peak within 1 wk between isotope trials (see Tracer Protocol). Two days after the second trial, subjects began training on leg cycle ergometers. After 9 wk of training, posttraining isotope trials were performed in a random order at 65% of pretraining \( \dot{V}O_2 \) peak [same absolute workload (ABT)], and 65% of posttraining \( \dot{V}O_2 \) peak [same relative intensity (RLT)].

Preliminary Testing

All exercise tests were performed on an electronically braked cycle ergometer (Monark Ergometric 829E). For determination of \( \dot{V}O_2 \) 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 online, real-time personal computer-based system (8). Body composition was determined via both skinfold measurements (23) 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 the study. Dietary analysis was performed using the Nutritionist III software (N-Squared Computing, San Mateo, CA).

Dietary Protocol

Subjects rested the day before each tracer trial and commenced a standardized dietary protocol that was replicated on each occasion (7, 8). Our procedures involved collection of dietary records, weighing participants daily to maintain stable body weights, and the participants consuming standardized meals before tracer trials.

Catheterizations

After local lidocaine anesthesia, the femoral artery and vein of the same leg were cannulated via standard percutaneous techniques as previously described (7, 8). 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 was used for calculations and comparisons.

Tracer Protocol

An antecubital vein catheter was placed 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-\(^{2}\)H]glucose and [3,3-\(^{14}\)C]lactate while resting semisupine for 90 min. Glucose kinetics (8) and lactate kinetics (10) are reported separately. 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 \( \dot{V}O_2 \) peak (45% Pre) and 65% old \( \dot{V}O_2 \) peak postraining (ABT), and 10 mg/min at 65% pretraining (65% Pre) and 65% postraining \( \dot{V}O_2 \) 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 (University of California School of Pharmacy, San Francisco, CA).

Blood Sampling

Blood temperature was obtained from a thermistor 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. Blood for determination of glucose concentration, alanine concentration, and lactate IE was immediately transferred to tubes containing 8% perchloric acid, shaken, and placed on ice. As pyruvate has been shown to be stabilized by storage in an acidic medium (44), blood for determination of pyruvate concentration and IE was collected in 8% perchloric acid in the same manner. Blood for determination of 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°C until analysis. Hematocrit measurements were performed on both arterial and venous blood using the microhematocrit method. Blood hemoglobin concentration was determined on each blood sample by the cyanomethemoglobin method.

Hemodynamics

Iliac venous blood flow was determined by thermodilution technique using a cardiac output computer (model 9520, American Ed-
Training Protocol

Training was performed on stationary leg cycle ergometers 5 days/wk for 9 wk with workloads adjusted to elicit heart rates corresponding to 75% of \( V_{\text{O2peak}} \). 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 \( V_{\text{O2peak}} \) 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 \( V_{\text{O2peak}} \), and training workloads were adjusted to maintain relative training intensity at 75% \( V_{\text{O2peak}} \). 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.

Calculations

Values were averaged from samples at 45 and 60 min of exercise, because steady state for arterial pyruvate concentration and arterial pyruvate IE had not yet been reached at 30 min of exercise.

\[
\text{Net rate of pyruvate release from the legs was calculated as:}\]

\[
([\text{pyruvate}]_r - [\text{pyruvate}]_v)/(2 \dot{Q}_{\text{bo}})
\]

The first term is the difference between blood pyruvate concentrations in the femoral vein and femoral artery and the second is blood flow in the two legs.

\[
\text{Net rate of conversion of arterial lactate to femoral venous pyruvate (L} \rightarrow \text{P)} \text{ was estimated as:}
\]

\[
L \rightarrow P = ([\text{IEp}/\text{IEl}]_v[\text{pyruvate}]_v - ([\text{IEp}/\text{IEl}]_a[\text{pyruvate}]_a)/(2 \dot{Q}_{\text{bo}})
\]

where IEp is IE of pyruvate in femoral venous blood, IEp is IE of pyruvate in arterial blood, and IEl is IE of lactate in arterial blood.

Because we report novel patterns for [pyruvate] (brackets denote concentration) change during exercise, we sought to confirm that a traditional [pyruvate] assay would also have been able to uncover these patterns. Therefore, for a subset of isotope trials, arterial and femoral venous samples from steady-state time points of rest and exercise were also analyzed by an enzyme-linked spectrophotometer assay (42), and the results were not significantly different from those obtained by GC-MS analysis.

Metabolite Analyses and IEs

Alanine. Blood alanine concentrations were measured in perchloric acid extracts by the method of Pfleiderer (31) using glutamate-pyruvate transaminase and LDH. These assays were conducted last in the series, and insufficient material remained to evaluate alanine IEs in blood.

Pyruvate. Pyruvate IE and concentration were measured via gas chromatography-mass spectrometry (GC-MS; GC model 6890 series and MS model 5973N, Agilent) of the trimethylsilyl-quinoxalinol derivative, using \( \alpha \)-ketovalerate as the internal standard for concentration measurement. The method of Rocchiccioli et al. (34) was modified for use with perchloric acid extracts. After spiking with \( \alpha \)-ketovalerate, 500 \( \mu \)l of perchloric acid extracts were mixed 1:1 with an ortho-phenylenediamine solution (5 mg/ml in 3 N HCl). The solution was heated for 60 min at 90 \(^\circ\)C and subsequently extracted with 4 ml of methylene chloride. The aqueous layer was discarded, and the remaining solution was dried under \( N_2 \), 50 ml of pyridine and 50 \( \mu \)l of bis(trimethylsilyl)trifluoroacetamide were added and the solution was vortexed and transferred to autosampler vials for GC-MS analysis.

For GC-MS analyses, an HP-1701 column was used, the inlet temperature was set at 250 \(^\circ\)C, the initial oven temperature was set at 70 \(^\circ\)C, the source temperature at 250 \(^\circ\)C, and the quadrupole temperature at 106 \(^\circ\)C. Splitless injection was used, and the carrier gas was helium with a constant flow of 50 ml/min. Methane was used for chemical ionization, and selective ion monitoring was used to monitor ions \( m/z \) of 261 for \[^{12}C\]- and \[^{13}C\]-pyruvate, respectively.

Because we report novel patterns for [pyruvate] (brackets denote concentration) change during exercise, we sought to confirm that a traditional [pyruvate] assay would also have been able to uncover these patterns. Therefore, for a subset of isotope trials, arterial and femoral venous samples from steady-state time points of rest and exercise were also analyzed by an enzyme-linked spectrophotometer assay (42), and the results were not significantly different from those obtained by GC-MS analysis.

Lactate. Lactate IEs and concentrations were measured and reported previously (10).

Statistical Analyses

Data are presented as means ± SE. Statistical analyses were performed by use of SPSS Graduate Pack 10.0 (SPSS, Chicago, IL). Comparisons were made between averaged values from the last 15 min of rest and from the last 15 min of exercise by paired-sample t-tests with the Bonferroni adjustment for repeated measures. Effects of time were determined by ANOVA with repeated measures with the least significant difference test. Significance was set at \( P < 0.05 \).

RESULTS

Subject Characteristics

Anthropometric data on subjects pre- and posttraining have been reported previously (8) but are repeated in Table 1 for

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pretraining</th>
<th>Posttraining</th>
<th>%Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>27.4 ± 2.0</td>
<td>27.9 ± 1.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Height, in.</td>
<td>70.1 ± 1.0</td>
<td>70.5 ± 0.9</td>
<td>0.6</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>81.8 ± 3.3</td>
<td>81.3 ± 3.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>19.7 ± 1.5</td>
<td>17.5 ± 1.6</td>
<td>-11.0</td>
</tr>
<tr>
<td>Skin folds</td>
<td>19.5 ± 1.5</td>
<td>17.2 ± 1.4</td>
<td>-11.6</td>
</tr>
<tr>
<td>Underwater weighing</td>
<td>3.5 ± 0.10</td>
<td>4.02 ± 0.15</td>
<td>14.6</td>
</tr>
<tr>
<td>( V_{\text{O2peak}} ) l/min</td>
<td>43.5 ± 1.3</td>
<td>50.1 ± 1.6</td>
<td>15.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 9 \). \( V_{\text{O2peak}} \) peak \( O_2 \) consumption. *Significantly different from pretraining values, \( P < 0.05 \).
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convenience. Subjects were weight stable throughout the study period. \( V_{\text{O2, peak}} \) increased significantly by 14.6% as a result of training. Consequently, postraining trials at 66 ± 1.1% of pretraining \( V_{\text{O2, peak}} \) (the same absolute workload as pretraining, 149 ± 7 W) were performed at 54.0 ± 1.7% of postraining \( V_{\text{O2, peak}} \), and 174 ± 7 W were required to elicit 65% of \( V_{\text{O2, peak}} \) postraining. Specific power outputs and rates of \( O_2 \) consumption achieved by subjects before and after training have been reported previously (8).

Pyruvate and Lactate Concentrations During Assessment of \( V_{\text{O2, peak}} \)

Forearm vein [lactate] and [pyruvate] tended to rise during progressive exercise to \( V_{\text{O2, peak}} \), but the trend was greater for [lactate], causing L/P to reach 86 ± 43 at the highest consistently reached workload of 225 W, whereas L/P at rest was 10 ± 1.4. The [lactate] became significantly greater than rest concentrations at a lower workload than was required for a significant rise in [pyruvate]. At a given workload, after training, subjects showed a trend for decreased [lactate] and [pyruvate] as well as decreased L/P, and this trend achieved significance for [lactate] at 200 W. During \( V_{\text{O2, peak}} \) assessment, the workloads corresponding to those studied in the isotope trials were insufficient to cause a significant rise in forearm venous [pyruvate].

Arterial Pyruvate Concentrations During Continuous Exercise

Arterial and femoral venous lactate concentration and IE values have been reported previously (10) and are not repeated here because of space considerations. Before training (45% Pre and 65% Pre), there was a trend for arterial pyruvate concentration ([pyruvate]a) to rise at exercise onset, but after training (ABT and RLT) this phenomenon was no longer present (Fig. 1). During each of the four trials, [pyruvate]a gradually declined to values significantly \((P < 0.05)\) below rest values. There was no statistically significant difference between rest or between exercise values in comparisons of any of the groups to one another, and when data from the four trials were combined, the trend was maintained and was even more significant \((P < 0.05)\) for a given absolute exercise power output.

Femoral Venous Pyruvate Concentrations

All exercise intensities showed that femoral venous pyruvate concentrations ([pyruvate]fv) were significantly \((P < 0.05)\) elevated above rest values (Fig. 2). Before training, [pyruvate]fv was significantly \((P < 0.05)\) higher at 65% \( V_{\text{O2, peak}} \) than at 45% \( V_{\text{O2, peak}} \). However, training decreased [pyruvate]fv \((P < 0.05)\) for a given absolute exercise power output.

Arterial L/P Concentration

During tracer trials, the arterial L/P (Fig. 3) in resting men approximated 10 both before and after endurance training. During exercise, the arterial L/P scaled with relative exercise intensity (i.e., rest < 45% < 65%). However, training significantly decreased arterial L/P \((P < 0.05)\) for a given absolute power output (i.e., ABT < 65% Pre). All exercise intensities show arterial L/P significantly \((P < 0.05)\) elevated above rest values.
Femoral Venous L/P Concentration

Training had no significant effect on rest values, and the femoral venous L/P was not significantly different from rest for any of the exercise intensities (Fig. 4). Despite this, there was a significant training effect \( P < 0.05 \) on values during exercise at the same absolute power output (i.e., ABT < 65% Pre). Also, before training, there was a significant association \( P < 0.05 \) between exercise intensity and femoral venous L/P (i.e., 65% Pre > 45% pre).

Arterial Isotopic Equilibration

The arterial isotopic equilibration, calculated from arterial pyruvate IE (Fig. 5) and arterial lactate IE (10), did not change significantly at rest as a result of training and averaged 60 ± 3.1% (Fig. 6). Arterial isotopic equilibration fell significantly \( P < 0.05 \) to 12 ± 2.7% during exercise with no significant exercise intensity or training effect.

Femoral Venous Isotopic Equilibration

Femoral venous isotopic equilibration was not significantly affected by exercise or training. The combined average femoral venous isotopic equilibration from all conditions was 60 ± 3.3%, which was not different from the rest value for arterial isotopic equilibration.

Pyruvate Net Release

Rather than showing an early spike in net release as generally seen for lactate, pyruvate release rose and approached plateau values over the course of the exercise session (Fig. 7). At rest, there was no training effect and the leg was a net pyruvate consumer \( (0.014 ± 0.008 \text{ mmol/min}) \) [and net lactate releaser (10)], but during all exercise intensities working muscle became a significant pyruvate net producer. At all exercise time points pyruvate net release at 65% Pre and ABT was greater \( P < 0.05 \) than at 45% Pre (Fig. 7), and net release at RLT was greater \( P < 0.05 \) than at 45% Pre. Pyruvate net release was as great as lactate net release at all exercise intensities, but alanine net release was \( \sim 1/15 \) of pyruvate net release. Net \( L\rightarrow P \) was undetectable at rest and thereby not included in Table 2, because there was net pyruvate uptake yet dilution of pyruvate IE across the resting leg (between the femoral artery and femoral vein). Figure 8 shows previously published lactate net release (10) and glucose net uptake (8) data along with pyruvate and alanine net release data to provide a context for the magnitude of pyruvate and lactate shunting during exercise.
alnine release. The sum of net releases of lactate, pyruvate, and alanine (with unsubstantial contribution from alanine) was comparable to net glucose uptake by working skeletal muscle. However, as has been shown previously, muscle glycogen would provide additional carbohydrate substrate for the sustained substantial lactate and pyruvate net releases during exercise (8).

**DISCUSSION**

We provide data on systemic lactate and pyruvate concentrations and IEs after infusion of carbon-labeled isotopic tracer in resting and exercising men, before and after endurance training. Furthermore, we provide similar arterial-venous IE concentration, and net release data obtained on resting and working muscle. The results lead to general conclusions regarding 1) net pyruvate release from resting and working human skeletal muscle, 2) compartmentation of pyruvate pools within working human skeletal muscle, 3) effect of exercise on the isotopic equilibration of lactate and pyruvate in human arterial blood, and 4) the significant roles of tissues outside working skeletal muscle for pyruvate clearance. These main conclusions are discussed in order, followed by discussion of pyruvate concentration data and methodological considerations.

**Net Pyruvate Release**

In agreement with results of previous investigations studying dogs (45) and humans (1), we found that the muscle bed of interest (i.e., the leg) was a slight net consumer of pyruvate at rest and that during exercise, regardless of exercise intensity or training state, working muscle switched to significant net pyruvate release (Fig. 7). Most of the increase in the pyruvate arterial-venous difference that accompanied exercise was due to the increased [pyruvate]v, although [pyruvate], also decreased during exercise (Figs.1 and 2). The rate of pyruvate net release was similar to the rate of lactate net release, and the sum of [pyruvate] and lactate net releases was comparable to net glucose uptake (Fig. 8). Alanine release was relatively small (Fig. 8). Endurance training significantly lowered net lactate release at a given (absolute) power output (10), but the same was not true for net pyruvate release.

**Compartmentation of Pyruvate Pools Within Working Skeletal Muscle**

During exercise, accompanying the large pyruvate net release was a significant gain in pyruvate IE in blood passing from the femoral artery sampling site to the femoral vein sampling site. Thus exogenous lactate was a substantial substrate for pyruvate production; ~65% of net pyruvate release was from exogenous lactate (L→P in Table 2). During exercise, both before and after endurance training, this mode of lactate removal by the working limb was quantitatively important. Because net alanine release during exercise (Fig. 8) was relatively small (~1/15 of net pyruvate release and ~1/10 of L→P), total working muscle lactate clearance was approximately the sum of L→P plus lactate oxidation. Comparing L→P from Table 2 with our previously reported lactate ox-

![Fig. 8. Net release of pyruvate, lactate, and alanine and net uptake of glucose by the legs during rest and exercise. Values are means ± SE; n = 7–9.](image)

**Table 2. Working muscle net pyruvate release and L→P**

<table>
<thead>
<tr>
<th></th>
<th>45% Pre</th>
<th>65% Pre</th>
<th>ABT</th>
<th>RLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net pyruvate release, mmol/min</td>
<td>0.70±0.07</td>
<td>2.17±0.30*</td>
<td>1.66±0.25*</td>
<td>2.90±0.47*</td>
</tr>
<tr>
<td>L → P, mmol/min</td>
<td>0.53±0.16</td>
<td>1.50±0.60</td>
<td>1.04±0.37</td>
<td>1.70±0.35</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7–9. 45% Pre, 45% pretraining VO2peak; 65% Pre, 65% pretraining VO2peak; ABT, 65% old VO2peak postraining; RLT, 65% postraining VO2peak; L → P, conversion of arterial lactate to venous pyruvate. *Significantly different from 45% Pre, P < 0.05.
Pyruvate and lactate shuttling

Dilation rates (10), ~25% of leg lactate uptake was cleared as pyruvate release, whereas ~75% was cleared via oxidation.

Net pyruvate release from exogenous lactate by the working limb indicates that the environment of contracting muscle makes conversion of lactate to pyruvate favorable. In the working muscle, the majority of L→P cannot have occurred in the same compartment in which the bulk of glycolytic flux occurs. If it had, then increasing the glycolytic flux by a factor of at least 8 from rest to exercise should have diluted the venous pyruvate IE by about the same factor. Instead, despite large glycolytic flux, L→P accounts for most of the pyruvate in the femoral vein during exercise. It is known that oxidative decarboxylation is the main mode of lactate disposal during exercise (10, 29, 30, 40, 43), and the first step in the utilization of lactate is oxidation to pyruvate (14). Hence, pyruvate formation from lactate requires LDH to function as an oxidase and proceed in the normally energetically unfavorable direction. During exercise, both working muscle and whole body lactate oxidation rates increase to a greater degree than either intramuscular or plasma lactate concentration (10), so a mass action effect alone cannot explain the increase in lactate oxidation accompanying exercise. In the cytosol, glycolysis increases [NADH] and ATP hydrolysis increases [H⁺], potentially driving the LDH reaction toward lactate, not toward pyruvate. In the subjects we studied, working limb muscles were taking up glucose (8) and releasing lactate (10). Thus glycolysis, as classically defined, was occurring in working muscle beds, a situation unfavorable for lactate oxidation to pyruvate in the cytosol. Conversion of lactate to pyruvate may be energetically favorable inside the mitochondrial matrix, where [H⁺] must be lower than in the cytosol and free [NADH] may be lower because NADH may be bound to the electron transport chain and where high [NADH] would inhibit pyruvate dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase, slowing the tricarboxylic acid cycle and the malate shuttle. Although there are insufficient data to establish [NAD⁺]/[NADH] in the mitochondrial matrix, our interpretation is consistent with the data of Józsás and Stainsby (24) showing that the mitochondrial [NAD⁺]/[NADH] of canine muscle contracting in situ became more oxidized compared with rest, and in agreement with Chatham et al. (17), who posited separate compartments of glycolytic flux and lactate oxidation in working rat heart preparations on the basis of their results using NMR spectroscopy. In other reports, our laboratory demonstrated that isolated mitochondria are capable of oxidizing lactate directly and provided evidence of how mitochondrial lactate oxidation might occur (12, 14). Therefore, for working skeletal muscle, we propose that lactate-to-pyruvate conversion may have occurred in a portion of the mitochondrial reticulum where the lactate and pyruvate, although derived from cytosolic pools, are in an environment more favorable for lactate oxidation, because of segregation of the cytosolic and mitochondrial NADH and NAD⁺ pools.

Isotopic Equilibration in Arterial Blood

Others (18, 47) have shown isotopic equilibration of lactate and pyruvate in blood of anesthetized dog preparations. We note no significant effect of exercise intensity or endurance training and show that this isotopic equilibration decreases from ~60% at rest to ~12% during exercise (Fig. 6). With benefit of the present results, it now seems that discussion of “isotopic equilibration” is of little physiological relevance to humans in vivo. Accepting the validity of data from studies on anesthetized mammalian preparations, from a physiological perspective interpretation of those data was faulty. In humans, the arterial L/P ranges from 12 at rest to over 300 during continuous sustained physical activity (Fig. 3). Therefore, even if for some reason infused lactate or pyruvate tracer were to reach isotopic equilibration in arterial blood in vivo, most of the tracer would be in the form of lactate, not pyruvate. Hence, irrespective of the data shown here demonstrating isotopic equilibration between lactate and pyruvate to approximate 60% at rest and fall to ~12% during exercise (Fig. 6), the reasonable and appropriate conclusion when both infused pyruvate and lactate tracers circulate predominantly as lactate should have been that such tracers cannot be used to provide an estimate of the pyruvate flux. From a physiological perspective, to our knowledge in no report in which lactate flux was measured in humans with tracers did lactate rate of disappearance approximate carbohydrate oxidation rate from indirect calorimetry. Lactate oxidation estimated from tracers is always much less than pyruvate oxidation (10, 29, 30, 40, 43). Hence, from neither tracer nor physiological data is it appropriate to conclude that lactate tracer provides a measure of pyruvate flux.

Pyruvate Clearance Outside of Working Muscle

During rest, arterial pyruvate IE (1.4 ± 0.1 mole percent excess) was greater than femoral venous pyruvate IE (1.0 ± 0.1 mole percent excess). During exercise, the arterial pyruvate IE was profoundly smaller than the femoral venous IE. Modest tracer dilution occurring during exercise between the femoral vein and artery would not be perplexing because there exist numerous potential sites of pyruvate turnover outside of the working muscle. For example, pyruvate-to-lactate conversion may occur in nonworking tissues such as muscle, adipose (19), and skin (25), and the increasing cardiac workload may facilitate greater exogenous pyruvate utilization by the myocardium. Total active limb blood flow accounted for ~50% of cardiac output during exercise (8), but under all exercise conditions pyruvate concentration and IE decreased by >80% in transit between the femoral vein and femoral artery, and arterial pyruvate IE (Fig. 5) declined to the measurement noise level during the most strenuous exercise. Therefore, dilution due to mixing with venous blood from inactive tissue is an insufficient explanation for the dilution of pyruvate concentration and IE occurring in transit between the femoral vein and femoral artery during exercise.

To explain how pyruvate label might disappear in transit between the femoral vein and artery, we posit three possibilities. The first possibility is that labeled pyruvate could be entirely converted to labeled lactate by red blood cells in circulation. Although red blood cells remove pyruvate from blood (16), and this conversion can be very fast (41), complete conversion should then also happen at rest when circulation time is about four times longer than during exercise. The second possibility is that labeled pyruvate could be so completely diluted by unlabeled pyruvate from other tissues not drained by the femoral vein that the IE reaches noise level. However, such dilution did not occur at rest, and during exercise, as already stated above, the active limbs received too...
large a portion of cardiac output for such a mixing effect to fully explain the results. So dilution of tracer due to mixing in the right heart could only partially explain the pyruvate IE data. The remaining possibility we see is that a significant fraction of labeled pyruvate was utilized by the lungs. The lungs are in fact capable of oxidizing exogenous pyruvate (5, 6), and, alternatively, pyruvate could have been converted to lactate in the lungs and subsequently released into the pulmonary vein.

Arterial Pyruvate Concentration and L/P Concentration

Our results from the $\dot{V}O_2$-peak assessment using an incremental exercise protocol agree with those of Wasserman et al. (46) in that we also saw a trend for increased blood [pyruvate] with progressively increasing workloads. However, during sustained submaximal exercise, we did not observe elevated [pyruvate] in arterial blood. Before training, at either workload, there was a trend for transient rise in [pyruvate]a, but during continuous exercise [pyruvate]a fell to values significantly below those at rest (Fig. 1). A tendency for [pyruvate]a to increase during sustained submaximal exercise of at least 30-min duration, compared with rest, has been previously reported in the arterial blood of humans (1, 35). This tendency has also been shown in normoxic dogs (48). However, under similar exercise conditions, the opposite trend, decreasing [pyruvate]a, has been shown for hypoxic dogs (48). It has also been shown, again in dogs, that moderate intensity exercise has no effect on [pyruvate]a (45). The majority of studies on exercising humans reporting [pyruvate]a (2, 3, 22, 28, 32) did not utilize an exercise duration corresponding to the timeframe we studied in which the decrease of [pyruvate]a became apparent. Regrettably, there are insufficient published data from exercising humans to make ample comparisons for prolonged exercise conditions.

Arterial L/P increased with higher exercise workload and decreased after training for a given (absolute) exercise intensity. However, working muscle did not cause the femoral venous L/P to increase above rest values, whereas L/P increased substantially in arterial blood during exercise (Fig. 3). Hence, a tissue or tissues other than the contracting muscle must drive the increase in arterial L/P that occurs during exercise. As stated above, the lungs may be involved, and also perhaps the heart and sympathetic effects on inactive tissues (e.g., noncontracting muscle, adipose, and liver) may account for some increase in arterial L/P.

Methodological Considerations

As discussed previously (10), calculation of metabolite net release from working tissue beds depends on components of the Fick relationship (Eq. 1); of these, placement of the femoral venous catheter is critical to obtain representative blood flow and venous content measurements. As there is no objective means to know whether the venous flow and content measurements are representative of working muscle, instead we base validity of our measurements on basic principles of muscle energetics. In our studies that involved repeated measurements of pulmonary oxygen consumption rate ($\dot{V}O_2$) at four discrete exercise power outputs (i.e., 45 and 65% $\dot{V}O_2$-peak, Pre, ABT, and RLT), the $\dot{V}O_2$-exercise muscle power output relationship (in liters O2/min and W, respectively) was $y = 0.012x + 0.55$, $r^2 = 0.99$. For the two legs, the simultaneously determined relationship between oxygen consumption and muscle power output was $y = 0.012x + 0.04$, $r^2 = 0.95$. Hence, as increments in working leg and pulmonary $\dot{V}O_2$ paralleled, both providing $\Delta$ efficiency estimates of 24%, we have reason to believe that our flow and venous content measurements did not suffer unduly from admixture of blood from nonworking muscle and other tissue beds and that the data obtained reliably portray the parameters of interest.

Summary and Conclusions

The data on IE of pyruvate present three paradoxes. First, despite a large increase in flux through pyruvate during exercise, pyruvate IE in the femoral vein greatly exceeds IE in the femoral artery; labeled pyruvate is not diluted but rather increased. Second, at the higher exercise intensities, labeled pyruvate almost entirely disappears somewhere between the femoral vein and femoral artery (i.e., in tissues beyond the main exercising muscle). Third, although the femoral artery sample contains little or no labeled pyruvate during exercise, it still contains unlabeled pyruvate, even at the highest exercise intensity. Each paradox produces a strong constraint on the mechanisms that might feasibly produce the data. The first implies a compartmentalized lactate-to-pyruvate shuttle in exercising muscle. The second and third imply pyruvate metabolism by the lungs (some clearance and a small amount of pyruvate production).

In our studies we have demonstrated that conversion of lactate to pyruvate occurs substantially in working human skeletal muscle and that on net bases pyruvate and lactate are released at similar rates from working muscle. Working muscle also releases alanine but at a rate an order of magnitude smaller than that of pyruvate or lactate. As such, we have discovered a new and previously unrecognized aspect of the lactate shuttle, our model for describing the integration of glycolytic and oxidative metabolic processes. Working muscle releases pyruvate, but [pyruvate]a decreases during sustained submaximal exercise. Arterial lactate is a major source of pyruvate released from working muscle, but the majority of venous pyruvate is cleared before entry into arterial circulation. It is apparent that working skeletal muscle is not solely responsible for the rise in the arterial lactate-to-pyruvate concentration ratio or the decrease in arterial isotopic equilibration accompanying exercise. With regard to carbohydrate metabolism in resting and exercising individuals both before and after endurance training, our data highlight the importance of separate metabolic compartments within working muscle and elsewhere.

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