Transpulmonary lactate shuttle

Matthew L. Johnson, Chi-An W. Emhoff, Michael A. Horning, and George A. Brooks
Exercise Physiology Laboratory, Department of Integrative Biology, University of California, Berkeley California

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Using [U-14C]glucose and an isolated perfused rat lung preparation Longmore and Mourning (22) demonstrated that the majority of lactate released during aerobic conditions was derived from glucose. However, upon exposure to hypoxia, lactate production nearly doubled in the preparation, but with only 60% of the lactate produced coming from glucose. When previously hypoxic lungs were reexposed to aerobic conditions, lactate release reverted to production exclusively from glucose. The work of Longmore and Mourning illustrates the diverse metabolic properties of lungs under conditions of stress. Further, the work of Longmore and Mourning demonstrates how lactate release may come from a variety of precursors under differing metabolic states, such as hypoxia.

During recent work, we observed that working human skeletal muscle releases similar amounts of lactate and pyruvate (12). Moreover, the ratio of isotopic enrichment (IE)-labeled lactate to pyruvate (IEL/IEP) decreased in blood as it transited through working muscle, indicating that arterial lactate was a substantial substrate for the pyruvate released into the venous circulation. Paradoxically, the arterial IEL/IEP remained significantly higher than in the venous effluent during muscle exercise (12). Because the pulmonary parenchyma was the only metabolically active tissue bed receiving all the cardiac output in one circulatory pass, we posited that the lungs metabolized a significant fraction of pyruvate delivered by pulmonary arterial blood. We further posited that pulmonary lactate metabolism affected circulating lactate levels during conditions of high peripheral glycolytic flux, such as during exercise that elevates circulating lactate concentrations and results in β-adrenergic stimulation.

In an attempt to better understand the diverse metabolic properties of lactate shuttling in the lungs under conditions of physiological stress and the meaning of IEL/IEP, we investigated transpulmonary lactate metabolism using an anesthetized rat model utilizing continuous infusions of [U-13C]lactate during a control (Con) condition and two interventions. The first, a lactate clamp (LC) increases the lactate load on the tissue significantly higher than in the venous effluent during muscle exercise (12). Because the pulmonary parenchyma was the only metabolically active tissue bed receiving all the cardiac output in one circulatory pass, we posited that the lungs metabolized a significant fraction of pyruvate delivered by pulmonary arterial blood. We further posited that pulmonary lactate metabolism affected circulating lactate levels during conditions of high peripheral glycolytic flux, such as during exercise that elevates circulating lactate concentrations and results in β-adrenergic stimulation.

MATERIALS AND METHODS

Animals and experimental preparations. Twenty-one female Wistar rats (n = 7 per condition, body mass range, 240–290 g; Charles River Laboratories, Wilmington, MA) were used in these experiments, which were approved by the Animal Care and Use Committee at the University of California, Berkeley (AUP no. R017–1007). Prior to experimentation, rats were housed two per cage in a light- (light from 0700 AM to 0700 PM), temperature-, and humidity-controlled environment with unrestricted access to food and water.

Preparation for each experiment began with the induction of surgical anesthesia (isoflurane inhalation; 4% in 100% O2 for induction, 2% for maintenance) followed by loose securing of the animal in supine position and connection to a system for continuous monitoring.

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

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of arterial O2 saturation (SaO2) by tail pulse oximetry. Arterial SO2 remained between 94 and 99% for all experiments, and core temperature was maintained at ~37°C by use of a thermostatically controlled heating pad. Two small skin incisions (~5 mm) were made over the ventral thorax, and catheters inserted for blood sampling were positioned in the left common carotid artery and the right atrium (Intramedic PE-50; BD), as previously described (8). A third catheter was placed in the right iliac vein for infusion purposes. Surgical preparation of animals took ~20 min.

Experimental protocol. Immediately following surgical preparation, a blood sample of ~200 μl was collected from the carotid artery for measurement of background isotope enrichments of lactate and pyruvate. Next, a primed continuous infusion of [U-13C]lactate, 99% enriched (Cambridge Isotope Laboratories, Andover, MA) was administered for 60 min via the iliac vein catheter using a syringe pump (Harvard Apparatus, South Natick, MA). Tracer was dissolved in 0.9% saline. For Con, lactate tracer was infused at 90 μg·kg−1·min−1 with a tracer prime corresponding to 15 times the minute infusion rate, an infusion rate and prime that we found to give steady-state values of ~2% molar percent excess (MPE). Lactate tracer infusion rates corresponded to 288 and 577 μg·kg−1·min−1 during Epi and LC conditions, respectively, again with the tracer prime corresponding to 15 times the minute tracer infusion rate. For Epi trials, the hormone cocktail was prepared immediately prior to infusion by dissolving stock epinephrine in 0.9% saline with ~4 μg/ml sodium bisulfite to give a final epinephrine concentration of 4.35 μg/ml. Epinephrine was infused at 0.2 μg/kg/min. The total volumes of solutions administered (prime + constant infusion) were similar in all experiments (~4 ml/kg).

Blood samples (150–200 μl) were collected simultaneously from arterial and right atrium sample sites (mixed central venous, v) after 40, 50, and 60 min of infusion. Cannulas were flushed with an equivalent amount of 0.9% saline after each collection. Animals remained anesthetized for the entire experiment, and after the last blood collection, animals were euthanized with an intravenous injection ofpentobarbital sodium (150–200 mg/kg). Accurate catheter placement was confirmed by visual inspection during necropsy.

Processing and analysis of blood. Blood lactate, pyruvate, and glucose concentrations were assayed enzymatically, as previously described (12). Briefly, sampled blood was immediately transferred to ice-chilled tubes containing 0.6 M of perchloric acid, shaken, and stored on ice until the end of the experiment. Within 1 h of collection, perchloric acid extracts were centrifuged [10 min at 3,000 rpm (g = 2,000), at 4°C], and the supernatants were transferred to separate tubes for storage at −20°C until further analysis.

Blood [lactate] was measured in neutralized perchloric extracts using an enzymatic method, as previously described (4). Blood lactate IE was determined using GCMS of the n-propylamide heptafluorobutyrate derivative (25). Briefly, neutralized perchloric extracts were lyophilized, resuspended in 200 μl of 2,2-dimethoxypropane and 20 μl 10% HCl in methanol, capped, and incubated at room temperature for 60 min. Following the addition of 50 μl of n-propylamine, the samples were heated at 100°C for 30 min, dried under a stream of N2 and transferred to GCMS vials using ethyl acetate. Thereafter, the samples were dried under N2, derivatized by adding 20 μl of heptafluorobutyric anhydride (5 min at room temperature), dried again under N2, and resuspended in ethyl acetate for GCMS analysis. Methane was used for chemical ionization with selected ion monitoring for mass to charge ratios 328 (unlabeled lactate) and 331 (labeled lactate), respectively.

Blood [pyruvate] concentrations and IEs were determined using gas chromatography-mass spectrometry (GCMS; GC, model 6890 series; MS, model 5973N; Agilent Technologies, Santa Clara, CA) of the trimethylsilyl-quinoxalinol derivative, with α-ketovalerate as the internal standard for concentration measurements (12, 17). Briefly, 150 μl of perchloric extract was spiked with α-ketovalerate, mixed (1:1) with an orthophenylenediamine solution (5 mg/ml in 3 M HCl) and heated for 60 min at 90°C. Pyruvate was subsequently extracted with methylene chloride, the aqueous layer discarded, and the remaining solution evaporated under a stream of N2. The samples were subsequently derivatized with 50 μl of a pyridine-bis(trimethylsilyl)-trifluoracetamide mixture (1:1). Chemical ionization (methane gas) was used with selected ion monitoring for mass to charge ratios 233 (unlabeled pyruvate), 236 (labeled pyruvate), and 261 (α-ketovalerate).

Blood [glucose] was measured in neutralized perchloric extracts using an enzymatic hexokinase set (Pointe Scientific, Canton, MI).

Calculations. The transpulmonary concentration gradient for lactate was calculated from concentrations in the right atrium (mixed central venous sample, v) and carotid artery [a]: transpulmonary concentration gradient (mmol/l) = [v] − [a] and net (lactate) balance (mmol/l) = ([v] − [a]) × Q, where [v] and [a] are the concentrations of lactate collected from the mixed central venous and arterial samples respectively, and Q is blood flow, as determined previously by Lin et al. (21). The value of Q used for the Con and LC conditions was 37 ml/min, and for the Epi condition, the estimated Q was doubled to 74 ml/min. Both positive transpulmonary concentration gradients and net balances reflect net pulmonary metabolite uptake, while a negative value reflects net metabolite release.

Concentration derived transpulmonary fractional extraction (FEXM) was calculated as FEXM = [v] − [a] / [v] × 100%

Tracer measured transpulmonary fractional extraction (FETXM), tracer measured uptake, and total lactate release was calculated as FETXM = [(13C]lactatev IE[(lactatev) − [(13C]lactatev IE[(lactatev)])/(13C]lactatev IE[(lactatev)] × 100%, tracer measured uptake (mg/min) = Q × FETXM × [lactatev], and total lactate release (mg/min) = tracer measured uptake − net balance. Net rate of conversion of mixed central venous pyruvate to arterial lactate (P → L) was estimated as P → L, μmol/min = [(IELv/IEPv) × [lactatev] − (IELv/IEPv) × [lactatev]] × Q, where IELv is IE of lactate in arterial blood, IEPv is IE of pyruvate in the mixed central venous blood, and IELv is IE of lactate in the mixed central blood.

While body lactate rate of appearance (Ra), rate of disappearance (Rd), and metabolic clearance rate (MCR) were calculated using the three blood sampling time points (40, 50, and 60 min into infusion) during each trial and the equations of Steele (25), as modified for use with stable isotopes.

\[
Ra = \frac{(C_1 + C_v)/2}{(IE_2 - IE_1)/(t_2 - t_1)} \\
Rd = \frac{MCR}{(C_1 + C_v/2)} \\
MCR = \frac{RD}{(C_1 + C_v/2)}
\]

where C1 is the arterial lactate concentration at time 1 (t1), and C2 is the lactate concentration at time 2 (t2). The percent that the lungs accounted for whole body lactate turnover (Ra) was calculated as (Raendogenous − Raartrial)Raartrial×100% where Raendogenous used the enrichment values from the mixed central venous blood and Raartrial used the enrichment values from the arterial blood. The percent isotopic enrichment equilibration in the blood was calculated as IEL/IEP in simultaneously sampled arterial and mixed central venous blood samples, respectively.

Statistics. Data are presented as means with deviation (SD). For statistical analysis, representative values of blood lactate concentrations and isotopic enrichments were obtained by averaging data taken during the last 20 min of each trial (i.e., from blood sampled at 40, 50, and 60 min after infusion commenced. ANOVA indicated 13C-isotopic enrichments of lactate in blood sampled prior to commencement of trials were not different among conditions and, therefore, values were pooled to obtain best estimates of background isotopic enrichments. Single one-way ANOVAs were used to test for treatment effects. Because concentrations did not always plateau during trials, a two-way ANOVA was used to determine the effect of...
Mixed central venous and arterial lactate concentrations. Preperfusion blood lactate [1.1 mM (SD 0.3)] and pyruvate [43.7 μM (SD 17.8)] concentrations were in the expected ranges (11, 12, 17, 19). Lactate concentration in the mixed central venous blood was lowest during the Con condition (Fig. 1A). Two-way ANOVA revealed an effect of treatment on lactate (P < 0.05; open triangles in Fig. 1) and multiple comparisons with Tukey’s HSD showed that the LC significantly increased [v] lactate concentration (P < 0.05). Similarly, levels of [a] lactate were lowest during the control condition (Fig. 1B). Two-way ANOVA revealed an effect of treatment on arterial lactate concentration (P < 0.05), and multiple comparisons with Tukey’s HSD showed that the lactate load significantly increased [a] lactate values (P < 0.05).

Transpulmonary lactate metabolism. Transpulmonary lactate gradients ([v] − [a]) were positive during all three conditions (Table 1). One-way ANOVA revealed a treatment effect on the transpulmonary lactate gradient (P < 0.05), and multiple comparisons with Tukey’s HSD showed that lactate loading significantly increased the transpulmonary lactate gradient above that during epinephrine stimulation (P < 0.05).

Net lactate balance was positive during all three conditions, indicating net uptake [1.2 mg/min (SD 0.7) for Con, 1.9 for LC, and 1.9 (SD 3.6) for Epi] (Table 1). One-way ANOVA did not reveal a treatment effect on net transpulmonary lactate balance (P > 0.05).

Concentration-measured fractional extraction of lactate calculated as a percent of net uptake averaged 13.1% (SD 16.6). One-way ANOVA did not reveal a treatment effect on FEXC (P > 0.05).

Tracer-measured fractional extraction averaged 16.6% (SD 23.8) for the Con and 8.2% (SD 15.3) for LC (Table 1). Epinephrine stimulation resulted in a negative FEXC, −25.3% (SD 44.5), which indicated transpulmonary lactate production from mixed central venous pyruvate [12.8 μmol/min (SD 24.8)]. One-way ANOVA revealed a treatment effect on FEXC (P < 0.05).

Table 1. Parameters of lactate kinetics and isotopic enrichment ratios during control, exogenous lactate load, and epinephrine stimulation conditions

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>LC</th>
<th>Epi</th>
</tr>
</thead>
<tbody>
<tr>
<td>α difference mM</td>
<td>0.4 (0.2)</td>
<td>0.6 (0.7)</td>
<td>0.2 (0.5)</td>
</tr>
<tr>
<td>Net lactate balance, mg/min</td>
<td>12.0 (2.7)</td>
<td>19.2 (3.5)</td>
<td>19.2 (3.5)</td>
</tr>
<tr>
<td>FEXC, % (net balance)</td>
<td>19.4 (11.1)</td>
<td>11.8 (13.9)</td>
<td>8.2 (21.6)</td>
</tr>
<tr>
<td>FEXTM, % (tracers)</td>
<td>16.0 (15.3)</td>
<td>8.2 (15.3)</td>
<td>−25.3 (45.5)</td>
</tr>
<tr>
<td>P → L, μmol/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate total release, mg/min</td>
<td>−0.3 (1.6)</td>
<td>−0.9 (3.2)*</td>
<td>−6.9 (10.2)*</td>
</tr>
<tr>
<td>Lactate Ra, mg/min</td>
<td>1.2 (0.3)</td>
<td>8.4 (1.9)*</td>
<td>3.3 (1.6)*</td>
</tr>
<tr>
<td>Lactate Rd, mg/min</td>
<td>1.2 (0.3)</td>
<td>8.4 (1.9)*</td>
<td>3.3 (1.6)*</td>
</tr>
<tr>
<td>Lactate MCR, ml/min</td>
<td>8.6 (2.8)</td>
<td>21.4 (2.7)*</td>
<td>12.6 (4.1)*</td>
</tr>
<tr>
<td>% Lung lactate turnover of whole body</td>
<td>5.1%</td>
<td>12.9%</td>
<td>43.0%*</td>
</tr>
<tr>
<td>L/P concentration ratio Δ (a−v)</td>
<td>28.4 (0.6)</td>
<td>16.2 (0.1)</td>
<td>39.6 (2.6)</td>
</tr>
<tr>
<td>Mixed central venous IEL/IEP</td>
<td>2.6 (1.3)</td>
<td>3.6 (1.8)</td>
<td>2.2 (1.2)</td>
</tr>
<tr>
<td>Arterial IEL/IEP</td>
<td>1.3 (1.8)</td>
<td>2.9 (0.9)*</td>
<td>3.2 (0.8)</td>
</tr>
</tbody>
</table>

Values are expressed as means and SD (SD). Con, control; LC, lactate load; Epi, epinephrine stimulation; FEXC, fractional extraction from concentration; FEXTM, tracer-measured fractional extraction; Ra, rate of appearance; Rd, rate of disappearance; L/P, lactate to pyruvate; IEL, isotopically enriched lactate; and IEP, isotopically enriched pyruvate. *Significantly different than Con, P < 0.05. §Significantly different than LC, P < 0.05.

RESULTS
on FEXTM ($P < 0.05$), and multiple comparisons with Tukey’s HSD showed epinephrine stimulation significantly decreased FEXTM.

For both control and lactate load conditions, there appeared to be an association between FEXTM and net balance. However, when epinephrine stimulation was considered, any possibility of a correlation across conditions disappeared, because FEXC from net balance was positive [8.2% (SD 15.3%)] whereas FEXTM was negative [−25.3% (SD 44.5%)].

Tracer measured uptake was positive for Con and LC conditions, whereas epinephrine stimulation resulted in a negative tracer measured lactate uptake (Fig. 2). One-way ANOVA revealed a treatment effect on tracer measured lactate uptake ($P < 0.05$), and multiple comparisons by Tukey’s HSD showed that Epi significantly decreased tracer measured lactate uptake.

Lactate-to-pyruvate ratios were significantly lower in the mixed central venous compared with arterial blood under all three conditions ($P < 0.05$). The transpulmonary change in $[L]/[P]$ ratio provides further evidence for lactate formation in the pulmonary bed and averaged 39 (SD 19.7) to 77 (SD 36.9) in mixed central venous and arterial blood pools, respectively (Fig. 3).

The rate at which lactate appeared and disappeared from the blood (Ra and Rd) increased significantly during epinephrine stimulation and lactate load conditions (Table 1, $P < 0.05$). Lactate Ra during the Con condition [1.2 mg/min (SD 0.3)] was lower than that for pyruvate [2.0 ± 0.8 mg/min (SD 0.8)] under similar conditions (16). The rate at which lactate was cleared from the blood, i.e., metabolic clearance rate, or MCR, showed very different patterns during the lactate load and epinephrine stimulation (Table 1). The Con condition produced the lowest MCR at 8.6 ml/min (SD 2.7), while Epi stimulation increased MCR slightly [12.9 ml/min (SD 4.1)], and the lactate load significantly increased lactate clearance to 21.4 ± 2.7 ml/min (SD 2.7) ($P < 0.05$). The lungs accounted for 5.1% of whole body lactate turnover during Con, and significantly more during LC and Epi stimulation conditions, 12.9% and 43%, respectively (Table 1, $P < 0.05$).

Total transpulmonary lactate release was not significantly different from zero for Con [−0.3 mg/min (SD 1.6)] and lactate load [−0.9 mg/min (SD 3.2)] conditions. However, total lactate release was negative during epinephrine stimulation [−6.9 mg/min (SD 10.2), $P < 0.05$], indicating pulmonary lactate production.

Mixed central venous isotopic equilibration ratios (i.e., IEL/IEP) averaged 2.6 (SD 1.9), 3.6 (SD 2.5), and 2.2 (SD 0.9) during Con, LC, and Epi conditions, respectively. One-way ANOVA did not reveal a treatment effect on mixed central venous isotopic equilibration. The arterial isotopic equilibration, calculated from arterial lactate IE and arterial pyruvate IE following the infusion of tracer lactate was higher during the lactate load [2.9 (SD 1.1)] and epinephrine stimulation [3.2 (SD 1.6)] compared with Con [1.3 (SD 0.9)]. One-way ANOVA revealed a treatment effect and multiple comparisons and Tukey’s HSD analysis showed that the lactate load significantly increased the arterial IEL/IEP compared with Con ($P < 0.05$). The relationships among the isotopic enrichments of central venous and arterial lactate and pyruvate are shown for each condition in Fig. 4, A and B. There was a higher correlation between lactate and pyruvate IE in central venous ($R^2 = 0.7$) than arterial blood ($R^2 = 0.4$). As well, slopes and $y$-intercepts of the relationships were significantly different ($P < 0.05$). Clearly, epinephrine had profound effects on metabolism in the pulmonary parenchyma. Arterial blood MPEs are shown in Fig. 4C to highlight the effect of epinephrine on increasing lactate MPE as blood transits through the lungs.

**Mixed central venous and arterial glucose concentrations.** Glucose concentration in the mixed central venous blood was lowest during the control condition (Table 2). One-way ANOVA revealed an effect of treatment on mixed venous glucose concentration ($\bar{v}$) ($P < 0.05$), and multiple comparisons with Tukey’s HSD showed that Epi significantly increased [$\bar{v}$] glucose concentration ($P < 0.05$). Mean levels of arterial glucose concentration [$a$] were lowest during the control condition, but there was no evidence for an effect of treatment. Transpulmonary glucose concentration differences ([$\bar{v}$] − [$a$]) were positive during all three conditions (Table 2). One-way ANOVA revealed a treatment effect on the transpulmonary glucose gradient ($P < 0.05$), and multiple comparisons with Tukey’s HSD showed that Epi significantly increased the
Glucose uptake was highest in the Epi condition due to the estimated elevation in cardiac output.

DISCUSSION

The main findings are that net lung lactate uptake occurs under both stimulated and control conditions; net lactate uptake by the lungs belies simultaneous lactate extraction (tracer measured uptake) and production (total release), and epinephrine stimulates transpulmonary pyruvate to lactate conversion. Further, isotopic equilibration between lactate and pyruvate occurs following tracer lactate infusion, but depending on compartment (arterial or venous) and physiological stimulus, IEL/IEP and [L]/[P] concentration ratios ranged widely. Those results mean that several hundred times more tracer existed in arterial blood as lactate than pyruvate regardless of whether lactate or pyruvate (17) was infused. These effects are largely due to monocarboxylate metabolism in the lungs. Hence, assertions that the IEs of lactate and pyruvate are equivalent in mammalian blood following tracer infusion (27) are simply incorrect. In brief, the present results show that the lungs metabolize substrates delivered in the pulmonary artery and, further, that lung metabolism affects metabolite concentrations and isotopic enrichments when tracers are infused into the systemic circulation.

The literature contains few reports of mixed venous and arterial values of lactate, and still fewer values of pyruvate. However, the results obtained in the control (Con) condition were comparable to published values for [L] and [P], with the caveat that anesthesia tends to raise arterial [L].

Early studies on rat lung preparations (9, 18, 23) showed that the lungs were capable of taking up glucose and producing lactate. Subsequent measurements in healthy humans, though limited, could not detect a significant difference in transpulmonary lactate concentration. Accordingly, researchers concluded that the rate of pulmonary lactate production was equal to its uptake (9, 11, 23). Indeed, our data support that view because it took exogenous lactate infusion in the form of a lactate clamp to produce a large [v]–[a] difference in anesthetized, but living rats. Furthermore, the percent contribution of lactate turnover by the lungs compared with the whole body is small during the Con condition (Table 1). Of importance is that concentration measurements alone under-represent total lactate metabolism because tissue beds, including the lungs, simultaneously extract and produce lactate. Results of the current study show that total lactate metabolism in lungs is underestimated by an average of 45% without the addition of tracer-derived data (Fig. 2) and that different physiological conditions (e.g., LC and Epi) result in significantly different contributions.
to whole body lactate turnover (Table 1). Those results show that the lungs can contribute, sometimes significantly, to whole body lactate metabolism.

Seemingly, some of the variability in the literature on transpulmonary lactate exchange is due to monocarboxylate (lactate and pyruvate) turnover and interconversion in the lungs (10, 15, 21). We now show significant lactate turnover during Con conditions and pyruvate-to-lactate conversion during epinephrine stimulation. In control and lactate load conditions, the tracer measured fractional extractions for lactate were 16.6% (SD23.8) and 8.2% (SD15.3), respectively. The control and lactate clamp FEXTM values obtained on anesthetized rats are similar to measurements made on young healthy men during rest and exercise following primed-continuous infusion of [3-13C]lactate (4). During leg ergometer cycling, working muscle released [3,13C]pyruvate into the venous effluent, causing a decrease in the IEL/IEP ratio compared with that in the arterial blood. However, because the arterial IEL/IEP ratio rose significantly in one circulatory passage, it was posited (12) that the conversion took place in the pulmonary circulation. The results now support the earlier hypothesis of pulmonary monocarboxylate turnover and transpulmonary P→L conversion during times of physiological stress [e.g., β-adrenergic stimulation (this study) or human exercise (12)].

The effects of epinephrine on producing arterial lactate from central venous pyruvate provide an explanation for the negative tracer measured uptake value during the epinephrine stimulation despite net lactate uptake. Lactate production from pyruvate is supported by the work of Longmore and Mourning (22), in which “other” lactate production was observed during hypoxic stress. It is important to note that because circulating lactate levels are at a minimum 10 times higher than circulating pyruvate levels, any P→L conversion and subsequent release of lactate would not (and, indeed, it does not) affect lactate [V-a] difference across the tissue to a measureable extent.

It is important to highlight that in the current investigation, we did not have the capabilities to measure rat Q (cardiac output), but instead, we relied on estimates from Lin et al. (21) who measured Q in rats of similar average body weights. Further, as noted in MATERIALS AND METHODS, we assumed that Epi stimulation increased rat Q in proportion to increases in lactate Ra and heart rate. While estimating such an important variable is never optimal, we have been careful to ensure that the conclusions drawn from the data are not influenced by the values chosen for Q. Specifically, net lactate uptake was determined from carefully measured transpulmonary concentration gradient values. Importantly also, we obtained repeated measures of IEL, IEP, and the IEL/IEP, which were independent of Q, measured or predicted.

At this time, we are able to make several comments about the cause of mixed central venous pyruvate conversion to arterial lactate during pulmonary perfusion. First, while an increase in [V] pyruvate may be partially responsible for the increase in conversion to lactate across the lungs (1), our results show that increased delivery is not the primary cause of P→L conversion because the LC produced higher central venous pyruvate concentrations than did epinephrine stimulation (17), but Epi caused a greater P→L conversion. Second, both the present results on anesthetized rats and our earlier results on exercising humans show the ratio is in favor of lactate in the transpulmonary arterial efflux during times of physiological stress (1, 7, 11). Compartmentalization of lactate uptake from the blood is documented in situations as disparate as perfused rat heart preparations (5) and exercising human muscle (12). Our new data suggest that the pulmonary capillary bed represents a unique metabolic compartment, especially for monocarboxylate metabolism. Redox status in pulmonary parenchyma becomes reduced during times of physiological stress, such as exercise or β-adrenergic stimulation, resulting in transpulmonary production of lactate from mixed central venous pyruvate. At this time, we can only speculate as to the specific location of monocarboxylate turnover; however, recent findings have linked type II pneumocystis with glucose metabolism for the synthesis of surfactant phospholipid (14). Consistent with early rat lung preparations, we also observed glucose uptake across the lung in all three conditions, particularly during epinephrine stimulation. Therefore, our results point to the lungs as a metabolic organ, which by their location, cause them to function as a fulcrum of whole-body intermediary metabolism.

In this investigation, uniformly labeled 13C tracers were used, and so the question may arise did 13C-glucose from infused tracer lactate contribute to the 13C lactate and pyruvate isotopomers measured? The answer to such a concern is clearly “no,” because in our studies we were careful to compare the exchanges of [U-13C]lactate and pyruvate across the lung. In terms of mass spectrometry, we relied on the m+3 signals. Additionally, we measured the incorporation of the carbon-13 label into either α or β glucose isotope m+3 peaks and did not find a significant increase over background during the course of our study. We have no doubt that over time, hepatic and renal lactate and pyruvate-to-glucose conversions occurred. However, because we infused tracer for only 1 h, the enrichment of conflicting isotopomers was minimized.

The meaning of the percent isotopic equilibration in blood between lactate and pyruvate following infusion of either tracer has been an area of controversy (6, 12, 13, 27). Some have asserted (27) that because equivalent isotope enrichments were measured, the lactate tracer was a surrogate for pyruvate flux, thus negating the use of lactate tracer to measure lactate flux. However, that same group then reported that any exchange would only affect lactate flux calculations to a small extent, especially during exercise, and noted that the IEL/IEP may change on the basis of the physiological state being studied (24). In furthering our understanding of the shuttling of carbohydrate intermediates, we now show different lactate and pyruvate IEs in arterial and mixed central venous pools and different responses in each compartment in response to control and two different physiological interventions (Fig. 4, A and B). The present and recent results (17), the calculations of Chinkes et al. (6), and our data on humans at rest and during exercise (12), show that isotopic equilibration between lactate and pyruvate in the blood is most heavily influenced by monocarboxylate metabolism in the most recent upstream tissue bed from which a blood sample is taken. Furthermore, changes in the IEL/IEP across a tissue bed likely represent the tissue’s metabolic state. In support of our conclusion that the changes in lactate and pyruvate concentrations and IEs occurred in the pulmonary parenchyma and not in blood transiting the pulmonary circulation, we note that Romijn et al. (24) showed that the changes in IEL/IEP, which occurred in a suspension of erythrocytes, took several minutes to occur ex vivo. Therefore,
in a resting human, a lactate or pyruvate molecule would circulate approximately three times before an intravascular effect. In the rats that we studied, blood would circulate many more times before an intravascular effect on IEL/IEP occurred. Therefore, we believe that highly perfused and metabolically active tissue beds, including the lungs, and not LDH in erythrocytes are the primary determinants of blood IEL/IEP ratios in vivo.

Henderson et al. (12) found that working muscle represented an oxidized environment (16), and, hence, 13C-lactate-to-pyruvate conversion occurred. In contrast, in terms of redox status, the current experiments, as well as those of Henderson et al. (12), show that the pulmonary capillary bed created a reducing environment in which pyruvate-to-lactate conversion occurred. In support of this assertion, we cite the work of Jöbsis and Stainsby (16), who showed that mitochondria NAD+ levels increased in canine muscle contracting in situ compared with rest. While similar measurements have not been made on lung tissue, our current results suggest that epinephrine stimulation would create a reducing environment in the lung parenchyma. Hence, L/P isotopic equilibration in arterial blood reflects the redox state of the lungs, whereas the L/P isotopic equilibration in central venous blood reflects the redox state in diverse upstream tissues comprised mostly of skeletal muscle during exercise.

In conclusion, the lungs simultaneously extract and release lactate into the circulation in vivo; during times of stress, the lungs actively contribute to the whole body lactate and pyruvate responses observed. Lactate uptake is, in part, concentration dependent, while epinephrine stimulates the conversion of pyruvate to lactate and lactate release into the systemic circulation. Further, assertions that the IEs of lactate and pyruvate are equivalent in mammalian blood following tracer infusion are incorrect.

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DISCLOSURES

G. A. Brooks has a financial interest in CytoSport, Inc. Otherwise, no conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES