Transpulmonary Lactate and Pyruvate Kinetics

By

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Shuttling of intermediary metabolites such as lactate and pyruvate contribute to the dynamic energy needs of tissues. Tracer kinetic studies offer a powerful method to measure the metabolism of substrates like lactate and pyruvate that are simultaneously taken up from and released into the circulation by organs. We examined the transpulmonary lactate and pyruvate kinetics in an anesthetized rat model during a control (Con), lactate load (LL) and epinephrine infusion (Epi) using a primed-continuous infusion of [U-13C]lactate and pyruvate. Compared to Con and Epi stimulation, LL significantly increased mixed central venous ([ v ]) and arterial ([a]) pyruvate and lactate concentrations (P < 0.05). Transpulmonary net lactate and pyruvate balances were positive during all three conditions indicating net metabolite uptake. Net balance was significantly greater during epinephrine stimulation compared to the control for both lactate and pyruvate (P < 0.05). Tracer measured lactate fractional extractions were similar for control and lactate load conditions, but negative during Epi stimulation when a transpulmonary production of lactate from venous pyruvate occurred. Tracer measured pyruvate fractional extraction averaged 42.8 ± 5.8% for all three conditions, and was significantly higher during epinephrine stimulation (P < 0.05) than during either Con or LL conditions, which did not significantly differ from each other. Lactate total release (= tracer measured uptake - net release) increased due to a lactate load (i.e., LL) and decreased (i.e., negative) from epinephrine stimulation because of the transpulmonary pyruvate \(\rightarrow\) lactate conversion. Pyruvate total release was significantly higher during epinephrine stimulation (400 ± 100 \(\mu\)g/min) vs. Con (30 ± 20 \(\mu\)g/min) (P < 0.05). For lactate, we conclude that transpulmonary concentration difference measurements across the lungs provide an incomplete – and perhaps misleading – picture of parenchymal lactate metabolism, especially during epinephrine stimulation. Regarding pyruvate, significant extraction occurs during circulatory transport across the lungs. The extent of pulmonary parenchymal pyruvate extraction predicts high expression of monocarboxylate (lactate/pyruvate) transporters (MCTs) in the tissue and western blot analysis of whole lung homogenates detected three isoforms: MCT1, MCT2, and MCT4. We conclude that a critical site of circulating pyruvate extraction resides in the lungs. Furthermore, during times of elevated circulating pyruvate or epinephrine stimulation, pyruvate extraction increases.
DEDICATION

This work is dedicated to my companion and best friend, Haley. I would also like to dedicate this work to my family for their enthusiastic support of my endeavors.
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INTRODUCTION

An integrated view of metabolic regulation requires an understanding of the metabolism of carbon-derived fuel sources both within and between tissues, at rest, and throughout the metabolic scope of the organism. Several notable concepts provide a structure for understanding and integrating metabolic regulation at the level of macronutrients and the body’s organ systems. For example, the Randle Cycle describes the relationship between lipid and carbohydrate metabolism at rest (20), while the Crossover Concept describes exercise (9). The Cori Cycle integrates glucose metabolism with that of its primary glycolytic intermediates, lactate and pyruvate, together with the liver in the maintenance of glycaemia (18). Finally, the Lactate Shuttle, first described in exercising muscle (7), defines the role of lactate in the distribution of carbohydrate potential energy during exercise. Combining scientific observations to form useful concepts provides the basis for future hypothesis, research, and, in the case of the Lactate Shuttle, therapies with clinical application.

Originally described between muscle fiber types of the same muscle bed and between anatomically distinct muscles such as an exercising limb and the heart (7), the Lactate Shuttle concept repurposed a “dead-end” metabolite in biology. Shortly after the Lactate Shuttle was proposed, the monocarboxylate transporters (MCT) were discovered. Four MCT proteins transport lactate and pyruvate: isoforms 1, 2, 3, and 4 (13), and knowledge of their distribution at the cellular and subcellular level expanded the original Lactate Shuttle concept to within cells (8).

Today the Lactate Shuttle is recognized in multiple fields of biology. The Astrocyte-Neuron Lactate Shuttle (19) describes the role of lactate in brain metabolism and provides a basis for lactate as a signaling molecule from astrocytes to neurons in the field of neurobiology. In cancer research, inhibiting the Lactate Shuttle is an effective method of reducing tumor growth (15, 23). The utilization of MCT expression in the gastrointestinal wall now provides some of the world’s elite athletes with an alternative fuel (8) and may be part of an alternative fuel support protocol for victims of traumatic brain injury in the intensive care unit in the future.

Expanding the Lactate Shuttle to include pyruvate (14), we recently observed that on a net basis working human skeletal muscle releases similar quantities of lactate and pyruvate. Moreover, based on $^{13}$C appearances onto pyruvate across the exercising muscle bed during a $^{13}$C lactate infusion study, the largest source of pyruvate released by working muscle into the venous circulation was attributed to intramuscular conversion of arterial lactate. Pyruvate concentration and $^{13}$C enrichment in arterial blood were reduced to the level of instrumental noise. These data indicated that the pyruvate released by working muscle was almost completely cleared in one circulatory pass during exercise. We hypothesized that a significant fraction of working muscle-derived pyruvate was metabolized in the lungs (14). Because the lungs are the only anatomically positioned organ to receive the entire cardiac output during rest and exercise our hypothesis expanded the lungs’ central role in blood gas exchange to encompass an active role in whole body lactate metabolism.
Early researchers demonstrated the ability of the lung to produce lactate from glucose under fully aerobic conditions (11). Subsequent work identified the lungs as a complex metabolic organ with respect to lactate and pyruvate metabolism. For example, at times the lungs consumed lactate, while at other times, most notably after lung injury, the lungs released lactate on a net basis in vivo (3, 4, 6, 10, 16). Of importance, Longmore and colleagues (17) demonstrated that the majority of lactate released during aerobic conditions is derived from glucose ex vivo. However, lactate production doubles upon exposure to a stress (hypoxia) even though only 60% of the lactate comes from glucose. The work of Longmore et al. highlights the diverse metabolic properties of the lung under conditions of stress, and that lactate release may come from a variety of precursors given different metabolic states (17).

While the literature on pyruvate metabolism is scarce in vivo, in 1973 Scholz and colleagues showed that rat lung slices were capable of oxidizing [14C]pyruvate (22). Future work by Scholz and others in the field found that environmental insults (e.g., low or high PO2, ozone) alter lactate to pyruvate ratios (L/P) across the lungs, and most notably, that exogenous infusion of pyruvate increases lactate concentration in the effluent of perfused lung preparations (1-3, 5, 12, 17). Together with our work on pyruvate shuttling at rest and during exercise, the literature on pyruvate metabolism suggests a role by the lungs in the exchange of pyruvate and lactate (21).

There are few studies of lung carbohydrate metabolism in vivo. To date, the results are ambiguous, regarding the lungs’ role in lactate and pyruvate metabolism and none of the previous studies account for the fact that lactate is simultaneously taken up and produced within most tissue beds. Therefore, it is unclear whether the small venous to arterial (substrate supply and removal) concentration measurements indicated a large turnover of lactate or very little. Additionally, while pyruvate concentration data are scarce in the literature, the high venous pyruvate and low arterial pyruvate ratio is documented in humans and the difference increases with exercise intensity. Therefore, we developed an anesthetized rat model to address these questions using isotopically labeled lactate and pyruvate.

In the present study we utilized a continuous infusion of uniformly labeled carbon-13 lactate or pyruvate ([U-13] lactate or pyruvate) via a femoral vein catheter in anesthetized female Wistar rats. Samples were taken simultaneously from the right atrium (v) and carotid artery (a) to determine lactate and pyruvate exchange across the lung tissue. By simultaneously measuring the isotopic enrichment of lactate and pyruvate across the lung tissue together with concentration measurements and blood flow estimates, we can measure the substrate kinetics for uptake, release, and the transpulmonary conversion of pyruvate to lactate (P → L).

A control condition was compared to two interventions designed to increase monocarboxylate turnover through different mechanisms. The first intervention, a lactate infusion, raised blood lactate levels into the range of moderate exercise without stimulating glycolysis. Physiologically, the lactate infusion increases the lactate load on the tissue without increasing energy requirements. The second intervention was designed to stimulate glycolysis through β-adrenergic stimulation via epinephrine infusion and thus mimic lactate and pyruvate production during exercise. During all conditions we infused [U-13C] lactate or pyruvate stable isotopes for the duration of the experiments and collected tissue samples for analysis of MCT protein in whole lung homogenate.
Using our novel rat model and stable isotope tracer technology we set out to test the hypothesis that mixed central venous pyruvate concentration would be elevated while pyruvate concentration in the blood leaving the lungs (arterial) would be paradoxically suppressed during the lactate load and epinephrine infusion, thereby indicating substantial net pyruvate extraction across the lungs. Further, utilizing mass spectrometry to measure the blood $^{13}$C isotopic enrichments of pyruvate and lactate, we tested the hypothesis that the lungs would actively extract and release both lactate and pyruvate from the circulation. Our rat model also allowed us to hypothesize that both lung lactate and pyruvate turnover would be elevated during the two interventions in comparison to the control condition.
REFERENCES


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CHAPTER 1

Transpulmonary Lactate Shuttle
Title: *Transpulmonary lactate shuttle*

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ABSTRACT

The shuttling of intermediary metabolites such as lactate contributes to the dynamic energy needs of tissues. Tracer kinetic studies offer a powerful tool to measure the metabolism of substrates like lactate that are simultaneously taken up from and released into the circulation by organs. To determine if transpulmonary lactate shuttling affects whole-body lactate kinetics in vivo, we examined the effects of a lactate load (LL) procedure and epinephrine (Epi) stimulation on transpulmonary lactate kinetics in an anesthetized rat model using a primed-continuous infusion of [U-13C]lactate. Under all conditions studied, control (Con), LL and Epi, net transpulmonary lactate uptake occurred. In comparison to Con, a lactate load (via LL) significantly increased mixed central venous ([v]) and arterial ([a]) lactate concentrations (P < 0.05). Transpulmonary lactate gradient ([v] – [a]) was highest under the lactate load condition and lowest under Epi stimulation (P < 0.05). Tracer measured lactate fractional extractions were similar under Con and LL conditions, but negative during Epi stimulation, when there was a transpulmonary production of lactate from venous pyruvate. Lactate total release (= tracer measured uptake - net release) was increased by a lactate load (i.e., under LL) and decreased (i.e., release was negative) under Epi because of the transpulmonary pyruvate to lactate conversion. We conclude that pulmonary arterial-vein concentration difference measurements across the lungs provide an incomplete, and perhaps misleading picture of pulmonary lactate metabolism, especially during epinephrine stimulation.
INTRODUCTION

Early researchers demonstrated the ability of the lungs to produce lactate from glucose under fully aerobic conditions (9). Subsequent work identified the lungs as a complex metabolic organ with respect to carbohydrate intermediary metabolism; at times the lungs were net consumers of lactate, while at other times, such as in patients with acute respiratory distress syndrome, the lungs released lactate on a net basis (1-3, 7, 18). Many studies were unable to show transpulmonary net uptake or release due to the high blood flow of the lung in relationship to its metabolic requirements (11, 21).

Using [U-\textsuperscript{14}C]glucose and an isolated perfused rat lung preparation, Longmore and colleagues (20) demonstrated that the majority of lactate released under 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 the hypoxic lung was re-exposed to aerobic conditions following hypoxia, lactate release reverted back to production from glucose. The work of Longmore et al illustrates the diverse metabolic properties of lungs under conditions of stress. Further, the work of Longmore et al demonstrates how lactate release may come from a variety of precursors under differing metabolic states, such as hypoxia.

During recent work on lactate metabolism in our laboratory we observed that working human skeletal muscle releases similar amounts of lactate and pyruvate (12). Moreover, the ratio of isotopically labeled lactate to pyruvate (IE\textsubscript{L}/IE\textsubscript{P}) decreased in blood as it transited through working muscle. Paradoxically, the arterial IE\textsubscript{L}/IE\textsubscript{P} remained significantly higher than in the venous efflux during muscle exercise (12). Because the lungs were 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 in pulmonary arterial (mixed, central venous) blood reaching the pulmonary capillary bed. We further posited that pulmonary lactate metabolism affected circulating monocarboxylate (lactate and pyruvate) levels during conditions of high peripheral glycolytic flux, such as during exercise and ß-adrenergic stimulation.

In an attempt to better understand the diverse metabolic properties of lactate shuttling in the lungs under conditions of physiological stress we investigated transpulmonary lactate metabolism using an anesthetized rat model utilizing continuous infusions of [U-\textsuperscript{13}C]lactate during a control (Con) condition and two interventions. The first, a lactate load (LL), increased the lactate load on the tissue without increasing metabolic rate; the second intervention, epinephrine stimulation (Epi), increased lactate levels by increasing glycolytic flux.
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 # R017-1007). Prior to experimentation rats were housed two per cage in a light (light from 7:00 am to 7:00 pm), temperature and humidity controlled environment with unrestricted access to food and water.

Preparation for each experiment began with the induction of surgical anesthesia (isofluorane inhalation; 4% in 100% O₂ for induction, 2% for maintenance) followed by loose securing of the animal in dorsal recumbence and connection to a system for continuous monitoring of arterial O₂ saturation (SO₂, by pulse oximetry positioned at the base of the tail). Arterial SO₂ remained between 94-99% for all experiments and core temperature was maintained at ~37 ºC 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 (8). A third catheter was placed in the right iliac vein for infusion purposes. The surgical preparation of animals took approximately 20 min.

Experimental Protocol. Immediately following surgical preparation a blood sample 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/min with a tracer prime corresponding to 15 times the minute infusion rate. Lactate tracer infusion rates corresponded to 288 and 577 µg/kg/min during Epi and LL 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 of sodium pentobarbital (150-200 mg/kg). Accurate catheter placement was confirmed by visual inspection during necropsy.

Processing and Analysis of Blood. Upon collection, blood samples were 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 3000 rpm, 4 °C) and the supernatants 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 (GCMS: GC, model 6890 series; MS, model 5973N, Agilent Technologies) of the N-propylamide heptafluorobutyrate derivative (22). Briefly, neutralized perchloric extracts were lyophilized re-suspended 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 re-suspended 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 IE were determined using gas chromatography-mass spectrometry GCMS of the trimethylsilyl-quinoxalinol derivative, with α-ketovalerate as the internal standard for concentration measurements (12). Briefly, 150 µl of perchloric extract was spiked with α-ketovalerate, mixed (1:1) with an ortho-phenylenediamine 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).

Calculations. The transpulmonary concentration gradient for lactate was calculated from concentrations in the right atrium (mixed central venous sample, \( \bar{v} \)) and carotid artery (a):

\[
\text{Transpulmonary concentration gradient (mmol/l) = } [\bar{v}] - [a]
\]

\[
\text{Net (lactate) balance (mmol/l) = } ([\bar{v}] - [a]) \times Q
\]

where \([\bar{v}]\) and \([a]\) are the concentrations of lactate collected from the mixed central venous and arterial samples sites respectively, and \(Q\) is blood flow as determined previously by Lin et al (19). 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 was calculated as:

\[
\text{F}_{\text{EXC}} = ([\bar{v}] - [a]) / [\bar{v}] \times 100\%
\]

Tracer measured transpulmonary fractional extraction (\(\text{F}_{\text{EXTM}}\)), tracer measured uptake, and total lactate release were calculated as:
\[ F_{\text{EXTM}} = \frac{([^{13}\text{C}]{\text{lactate}} \rightarrow \text{IE}) ([\text{lactate}] \rightarrow)}{([^{13}\text{C}]{\text{lactate}} \text{IE}) ([\text{lactate}] \rightarrow)} \times 100\% \]

Tracer Measured Uptake (mg/min) = \( Q \times F_{\text{EXTM}} \times [\text{lactate}] \rightarrow \)

Total Lactate Release (mg/min) = Tracer Measured Uptake - Net Balance

Net rate of conversion of mixed central venous to arterial lactate (P \rightarrow L) was estimated as:

\[ \text{P} \rightarrow \text{L}, \text{umol/min} = ((\text{IEL}_a/\text{IEp} \rightarrow) \times [\text{lactate}] \rightarrow) - ((\text{IEL}_v/\text{IEp} \rightarrow) \times [\text{lactate}] \rightarrow) \times Q \]

where IEl_a is IE of lactate in arterial blood, IEp \rightarrow is IE of pyruvate in the mixed central venous blood, and IEl \rightarrow is IE of lactate in the mixed central blood.

Here we assume that in measuring transpulmonary monocarboxylate exchange the \([U-^{13}\text{C}]\) tracer came only from labeled mixed central venous pyruvate (first term) or labeled mixed central venous lactate (second term) and that flux of tracee is proportional to flux of tracer. Because of tracer atom scrambling in gluconeogenesis, we assumed that the \([U-^{13}\text{C}]\) label in arterial or central venous lactate or pyruvate did not come from glucose. The percent isotopic equilibration in the blood was calculated as \(\frac{\text{IEL}_a}{\text{IEp}}\) for arterial or mixed central venous blood.

**Statistics.** Data are presented as mean ± standard error (SE). 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 that \(^{13}\text{C}\)-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 analyses of variance (ANOVA) 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 treatment on lactate concentration. Following ANOVA, post-hoc testing to identify significant treatment effects was done using Tukey’s honest significance difference (HSD) tests; an \(\alpha\) of 0.05 was used throughout. To evaluate effects of the lungs on the isotopic enrichments of lactate and pyruvate in blood transiting through the lungs an analysis of covariance (ANCOVA) were performed. To evaluate the effect of the lungs on the lactate to pyruvate ratios, Student’s paired t-tests with sequential Bonferroni correction factors were performed. Statistical tests were performed using SPSS Graduate Pack 11.0 software.
RESULTS

**Mixed Central Venous and Arterial Lactate Concentrations.** Lactate in the mixed central venous blood was lowest under the Con condition (Figure 1A). Both the lactate load and epinephrine stimulation increased lactate concentration and a two-way ANOVA revealed an effect of treatment on [\(\bar{v}\)] lactate (P < 0.05). Multiple comparisons with Tukey’s HSD showed that the lactate load (LL) significantly increased [\(\bar{v}\)] lactate concentration (P < 0.05). Levels of [a] lactate were lowest under the control condition (Figure 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 (under LL) significantly increased [a] lactate values (P < 0.05) and while epinephrine stimulation increased lactate levels it was not significantly elevated.

**Transpulmonary Lactate Metabolism.** Transpulmonary lactate gradients ([\(\bar{v}\] – [a]) were positive under 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 ± 0.2 for Con, 1.9 ± 0.5 for LL, 1.9 ± 0.8 for Epi mg/dl) (Table 1). One-way ANOVA did not reveal a treatment effect on net balance (P < 0.05).

Concentration-measured fractional extraction of lactate calculated as a percent of net uptake averaged 13.1 ± 2.1%. One-way ANOVA did not reveal a treatment effect on F\(_{\text{EXC}}\) (P < 0.05).

Tracer-measured fractional extraction averaged 30.4 ± 16.6% under the Con condition and 8.2 ± 3.4% for the lactate load (Table 1). Epinephrine stimulation resulted in a negative F\(_{\text{EXTM}}\) (-25.3 ± 9.7%), which is production of lactate from mixed central venous pyruvate (12.8 ± 5.7 µmol/min). One-way ANOVA revealed a treatment effect on F\(_{\text{EXTM}}\) (P < 0.05) (P < 0.05), and multiple comparisons with Tukey’s HSD showed epinephrine stimulation significantly decreased F\(_{\text{EXTM}}\).

Under both Con and LL there appeared to be an association between F\(_{\text{EXTM}}\) and net balance. However, when epinephrine stimulation was considered, any possibility of a correlation across conditions disappeared, because F\(_{\text{EXTM}}\) from net balance was positive (8.2 ± 4.7%) whereas F\(_{\text{EXTM}}\) was negative (-25.3 ± 9.7%).

Tracer measured uptake was positive under Con and lactate load (LL) conditions, whereas epinephrine stimulation resulted in a negative tracer measured lactate uptake (Figure 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 epinephrine stimulation significantly decreased tracer measured lactate uptake.

Lactate to pyruvate ratios were significantly lower in the mixed central venous compared to 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 ± 4.7 to 77 ± 8.5 in the mixed central venous and arterial blood respectively (Figure 3).
Total lactate release was not significantly different from zero under the Con (-0.3 ± 0.3 mg/min) and LL (-0.9 ± 0.7 mg/min) conditions. However, total lactate release was negative during epinephrine stimulation (-6.9 ± 2.2 mg/min, P < 0.05) indicating pulmonary lactate production.

The mixed central venous isotopic equilibration ratio (i.e., IE_L/IE_P) averaged 2.6 ± 0.5, 3.6 ± 0.7, and 2.2 ± 0.4 under the Con, LL, 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 ± 0.3) and epinephrine stimulation (3.2 ± 0.3) in comparison to the control (Con) (1.3 ± 0.7). One-way ANOVA revealed a treatment effect and multiple comparisons and Tukey’s HSD analysis showed that the lactate load significantly increased the arterial isotopic equilibration compared to the Con condition (P < 0.05). The relationships among the isotopic enrichments of central venous and arterial lactate and pyruvate are shown for each condition in Figures 4A 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). Also, 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 molar percent excesses (MPEs) are shown in Figure 4C to highlight the effect of epinephrine on increasing lactate MPE as blood transits through the lungs.
DISCUSSION

Main findings are that: net lactate uptake by the lungs occurs under both stimulated and control conditions; however net lactate uptake by the lungs does not provide an accurate picture of simultaneous lactate extraction (tracer measured uptake) and production (total release), and epinephrine stimulates transpulmonary pyruvate to lactate conversion. Furthermore, isotopic equilibration between lactate and pyruvate did occur following tracer lactate infusion, but depending on compartment (arterial or venous) and physiological stimulus, the \( \text{IE}_L/\text{IE}_P \) ratio ranged from 1.3 to 3.6, and the \( [L]/[P] \) concentration ratio ranged from 38 to 77. These results mean that several hundred times more tracer existed in arterial blood as lactate than as pyruvate, regardless of whether lactate or pyruvate (16) was infused. These effects are largely due to monocarboxylate metabolism in the lungs. Hence, assertions that the IE’s of lactate and pyruvate are equivalent in mammalian blood following tracer infusion (6, 23) are simply incorrect.

Early studies on rat lung preparations (6, 9, 17, 21) 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 rate of uptake (9, 11, 21). Indeed our data support that view because it took exogenous lactate infusion to produce a large \([v]\) difference in anesthetized, but living rats. Of importance is that concentration measurements alone underrepresent 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 (Figure 2).

Seemingly, some of the variability in the literature on transpulmonary lactate exchange is due to monocarboxylate turnover and inter-conversion in the lungs (10, 14, 18). Here we now show significant lactate turnover under control conditions and pyruvate-to-lactate conversion during epinephrine stimulation. In the control and lactate load conditions the tracer measured fractional extractions for lactate are (16.6% ± 5.2) and (8.2% ± 3.4) respectively. The \( \text{Con} \) and \( L \) \( \text{FEXTM} \) values obtained on anesthetized rats are reminiscent of femoral arterial-venous difference measurements made on young healthy men during rest and exercise following primed-continuous infusion of \([3-\text{\textsuperscript{13}C}]\)lactate (4). During leg ergometer cycling working muscle released \([3-\text{\textsuperscript{13}C}]\)pyruvate into the venous effluent causing a decrease in the \( \text{IE}_L/\text{IE}_P \) ratio when compared to that in the arterial blood. However, because the arterial \( \text{IE}_L/\text{IE}_P \) ratio rose significantly in one circulatory passage, the researchers hypothesized (12) that the conversion took place in the pulmonary circulation. Our results support the earlier hypothesis of pulmonary monocarboxylate turnover and transpulmonary \( P \rightarrow L \) conversion during times of physiologic stress (e.g., \( \beta \)-adrenergic stimulation (this study) or human exercise (12)).

The effects of epinephrine in producing arterial lactate from central venous pyruvate provide an explanation for the negative tracer measured uptake value during the
epinephrine stimulation despite the net lactate uptake. Lactate production from pyruvate is supported by the work of Longmore and colleagues where “other” lactate production was observed during hypoxic stress (20).

While an increase in $[\text{v}]$ 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 $\text{P} \rightarrow \text{L}$ conversion, because LL produced higher venous pyruvate concentration than did Epi (16), but Epi caused a greater $\text{P} \rightarrow \text{L}$ conversion. Both the present results on anesthetized rats and our earlier results on exercising humans show that the $\text{L}/\text{P}$ 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, concerning monocarboxylate metabolism. The metabolically active cells that are participating in monocarboxylate exchange become reduced during times of physiological stress, resulting in transpulmonary production of lactate from pyruvate, therefore contributing to the whole body lactate response to exercise. Our results point to the lungs as a metabolic organ, which by their anatomical location, cause them to function as a switch of whole-body intermediary metabolism; meaning that at rest the lungs do not alter monocarboxylate concentrations and isotopic enrichments significantly. However under conditions of metabolic stress the lungs switch to releasing arterial lactate from venous pyruvate.

In this investigation uniformly labeled $^{13}$C tracers were used, and so the question may arise: ‘Did $^{13}$C-glucose from infused tracer lactate contribute to the $^{13}$C lactate and pyruvate isotopomers measured?’ The answer is clearly “no” because in our studies we were careful to compare the exchanges of [U-$^{13}$C]-lactate and -pyruvate across the lung. In terms of mass spectrometry, we relied on the m+3 signals. We have no doubt that over time hepatic and renal lactate and pyruvate to glucose conversions occurred. However, because of atom rearrangement in gluconeogenesis, the labeled precursors were unlikely to produce either of the m+3 isotopomers we infused and traced.

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, 23). Some have asserted that, because equivalent isotope enrichments were measured, the lactate tracer was a surrogate for pyruvate flux, thus negating use of lactate tracer to measure lactate flux. In contrast, we show different lactate and pyruvate IEs in arterial and mixed central venous pools and different responses to each. The present results show that regardless of the sampling site, the majority of tracer and IE are in the form of lactate, and further, the results show how the lungs affect the lactate/pyruvate concentration ratios, especially under the influence of epinephrine stimulation.

From the current results and those of Henderson et al (12), it is apparent 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 the blood sample was taken. In Henderson et al (12) working muscle represented an oxidized environment and hence $^{13}$C-lactate to pyruvate occurred. In contrast, in the current experiments as well as in Henderson et al in terms of redox status, 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 (15) 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.

In conclusion, the lungs simultaneously extract and release lactate into the circulation \textit{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, and epinephrine stimulates the conversion of pyruvate to lactate.
ACKNOWLEDGMENT
We thank A. Zhou, D. Kluck, A. Afifi, E. Thomas, and L. Arena for their help in performing the surgeries and isotopic enrichment analyses of lactate.

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).
REFERENCES


Table
Table 1.

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<th>Con</th>
<th>LL</th>
<th>Epi</th>
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<td>0.6 ± 0.2</td>
<td>0.2 ± 0.1§</td>
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<td>Net Lactate Balance, mg/min</td>
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<td>1.9 ± 0.8</td>
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<td>Fex, % (Tracers)</td>
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<td>( P \Rightarrow L ), ( \mu )mol/min</td>
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<td>Arterial IE( L )/ IE( P )</td>
<td>1.3 ± 0.7</td>
<td>2.9 ± 0.3*</td>
<td>3.2 ± 0.3</td>
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Table 1. Net lactate balance, fractional extraction of lactate, production of lactate from pyruvate, lactate to pyruvate ratio and mixed central venous to arterial lactate to pyruvate ratio by concentration and isotopic enrichment during Con, LL, and Epi conditions respectively. Values are means ± SE; *Significantly different that Con, \( P < 0.05 \). § Significantly different than LL, \( P < 0.05 \).
Figures

Figure 1: A. Mixed central venous lactate concentration under Con, LL, and Epi conditions respectively. Values are means ± SE; *Significantly different that Con, P < 0.05. B. Arterial lactate concentration under Con, LL, and Epi conditions respectively. Values are means ± SE; *Significantly different that Con, P < 0.05.

Figure 2: Transpulmonary lactate total release and its components (tracer measured uptake – net balance) under Con, LL, and Epi conditions respectively. Values are means ± SE. *Significantly different that Con, P < 0.05. § Significantly different than LL, P < 0.05 for tracer measured uptake.

Figure 3: Lactate to pyruvate ratio in central mixed venous blood and arterial blood respectively by trial. Values are means ± SE, ^Significantly different than the corresponding trial, P < 0.05

Figure 4: A. Relationship between mixed central venous lactate and pyruvate MPE in rat blood under Con, LL, and Epi conditions respectively. B. Relationship between arterial lactate and pyruvate MPE in rat blood under Con, LL, and Epi conditions respectively. C. Isotopic enrichment of [U-13C]lactate in mixed central venous and arterial blood of control, lactate load and epinephrine stimulated rats. Inset figure, shows isotopic enrichment of [U-13C]lactate in mixed central venous and arterial blood of epinephrine stimulated rats by time. Values are means ± SE.
Fig. 1A. Mixed central venous lactate concentration under Con, LL, and Epi conditions respectively. Values are means ± SE; *Significantly different that Con, P < 0.05
Fig. 1B. Arterial lactate concentration during Con, LL, and Epi conditions respectively. Values are means ± SE; *Significantly different that Con, P < 0.05.
Fig. 2. Transpulmonary lactate total release and its components (tracer measured uptake – net balance) under Con, LL, and Epi conditions respectively. Values are means ± SE. *Significantly different that Con, P < 0.05. § Significantly different than LL, P < 0.05 for tracer measured uptake.
Fig. 3. Lactate to pyruvate ratio in central mixed venous blood and arterial blood respectively by trial. Values are means ± SE, ^Significantly different than the corresponding trial v, P < 0.05
Fig 4A – Relationship between mixed central venous lactate and pyruvate MPE in rat blood under Con, LL, and Epi conditions respectively.
Fig 4B - Relationship between arterial lactate and pyruvate MPE in rat blood under Con, LL, and Epi conditions respectively.

\[ y = 0.3203x + 0.3985 \]

\[ R^2 = 0.44807 \]
Fig 4C – Isotopic enrichment of [U-^{13}C]lactate in mixed central venous and arterial blood of control, lactate load and epinephrine stimulated rats. Inset figure, shows isotopic enrichment of [U-^{13}C]lactate in mixed central venous and arterial blood of epinephrine stimulated rats by time. Values are means ± SE.
CHAPTER 2

Transpulmonary Pyruvate Kinetics
Title: *Transpulmonary pyruvate kinetics*

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Running Title: The lung extracts pyruvate

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Shuttling of intermediary metabolites such as pyruvate contribute to the dynamic energy needs of tissues. Tracer kinetic studies offer a powerful tool to measure the metabolism of substrates like pyruvate that are simultaneously taken up from and released into the circulation by organs. We examined the transpulmonary pyruvate kinetics in an anesthetized rat model during a control (Con), lactate load (LL) and epinephrine infusion (Epi) conditions using a primed-continuous infusion of [U-\textsuperscript{13}C]pyruvate. Compared to Con and Epi stimulation, LL significantly increased mixed central venous ([\textsubscript{v}]) and arterial ([\textsubscript{a}]) pyruvate concentrations (P < 0.05). Transpulmonary net pyruvate balances were positive during all three conditions indicating net pyruvate uptake. Net balance was significantly greater during epinephrine stimulation compared to the control (P < 0.05). Tracer-measured pyruvate fractional extraction averaged 42.8 ± 5.8% for all three conditions, and was significantly higher during epinephrine stimulation (P < 0.05) than during either Con or LL conditions, that did not differ from each other. Pyruvate total release (tracer measured uptake - net balance) was significantly higher during epinephrine stimulation (400 ± 100 μg/min) vs. Con (30 ± 20 μg/min) (P < 0.05). These results are interpreted to mean that significant pyruvate extraction occurs during circulatory transport across lung parenchyma. The extent of pulmonary parenchymal pyruvate extraction predicts high expression of monocarboxylate (lactate/pyruvate) transporters (MCTs) in the tissue. Western blot analysis of whole lung homogenates detected three isoforms, MCT1, MCT2, and MCT4. We conclude that the lungs constitute a major site of circulating pyruvate extraction, and that under conditions of elevated pyruvate circulation or epinephrine stimulation, pyruvate extraction is increased.
INTRODUCTION

In 1973, Scholz et al (31) showed that rat lung slices were capable of oxidizing \(^{14}\text{C}\)pyruvate. That study established a role for exogenous pyruvate in lung tissue metabolism that subsequent investigators were able to elaborate upon (2-7, 12, 27). Among those findings were that environmental insults (e.g., low or high PO\(_2\), ozone) altered lactate to pyruvate ratios (L/P), and that exogenous pyruvate infusion increased lactate concentration in the effluent of perfused lung preparations (27). Together with elevated lactate to pyruvate ratios during lung injury, those data suggest a metabolic role for the lungs in the metabolism of circulating pyruvate (17, 19, 21, 22, 26, 28).

Despite a significant amount of research characterizing the metabolic properties of lungs from studies on isolated perfused lung preparations, the ability to make meaningful transpulmonary measurements is difficult \textit{in vivo} (11). Anatomical positioning of the lungs and resulting high blood flow rates (= cardiac output) in relation to the metabolic requirements make mixed central venous (v) - arterial (a) differences small and difficult to measure reliably.

Given the dearth of information on pyruvate metabolism we sought to develop a rat model that would allow transpulmonary metabolic measurements during times of stress \textit{in vivo}. Our interest was heightened because we observed significant differences in pyruvate concentrations and isotopic enrichments between the venous effluent of working muscles and arterial blood of healthy young men at rest and during leg ergometer cycling exercise (18). As a result, we reasoned that the lungs must be involved in changes in lactate and pyruvate concentration ([L]/[P]) and isotope enrichment values, because it receives the total cardiac output.

Therefore, we used non-radioactive \(^{13}\text{C}\)-labeled isotope tracers of pyruvate to assess transpulmonary pyruvate kinetics in anesthetized rats during a control condition that acted as the control (Con), in comparison to two interventions both designed to increase pyruvate turnover, but through different mechanisms. The first, a lactate load (LL), is named after the commonly performed glucose clamp. Physiologically the lactate load increases the lactate load on the tissue without increasing energy requirements. The second intervention was designed to stimulate glycolysis through \(\beta\)-adrenergic stimulation via epinephrine infusion (Epi), and thus mimic pyruvate production during exercise. Based upon previous work on resting and exercising muscle tissue, along with the work done by previous investigators in rat lung preparations \textit{ex vivo}, we hypothesized that the lungs, specifically the pulmonary capillary beds, are sites of simultaneous production and removal of pyruvate and contributes significantly to whole body carbohydrate intermediary metabolism (18, 31).
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 # R017-1007). Prior to experimentation rats were housed two per cage in a light (light from 7:00 am to 7:00 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 dorsal recumbence and connection to a system for continuous monitoring of arterial O₂ saturation (SO₂, by pulse oximetry positioned at the base of the tail). SO₂ remained between 94-99% 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 for blood sampling were positioned in the left common carotid artery and the right atrium (Intramedic PE-50, BD) as previously (10). A third catheter was placed in the right iliac vein for infusion purposes. The surgical preparation of animals took about 20 min.

Experimental Protocol. Immediately following surgical preparation a blood sample was collected from the carotid artery for measurement of background isotope enrichments of pyruvate and lactate. Next, a primed-continuous infusion of [U-¹³C]pyruvate, 99% enriched, Cambridge Isotope Laboratories (Andover, MA) were administered for 60 min via the iliac vein catheter using a syringe pump (Harvard Apparatus, South Natick, MA). Tracers were dissolved in 0.9% saline. For Con, pyruvate tracer was infused at 90 µg/kg/min with a tracer prime corresponded to 15 times the minute infusion rate. Lactate tracer was infused at rates corresponding to 288 and 577 µg/kg/min during Epi, and LL 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 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, the animal was euthanized with an intravenous injection of sodium pentobarbital (150-200 mg/kg). Accurate placement of the catheters was confirmed by visual inspection during necropsy.

Sample preparation and Western blotting. Tissues from female Wistar rat (lungs, liver, heart, plantaris skeletal muscle, brain) and L6 myocytes were homogenized and fractionated as previously described (8). Total protein concentrations were determined using BCA protein assay kits (Pierce Biotechnology, Radford, IL). The same amount of
total protein (~ 30 µg) was loaded into electrophoresis wells for separation by SDS-PAGE, and Western blotting was performed as previously described (8). Primary antibodies used were rabbit anti-MCT1 (Brooks custom antibody), rabbit anti-MCT4 (Brooks custom antibody), and rabbit anti-MCT2 (Chemicon, Temecula, CA).

Processing and Analysis of Blood. Upon collection, all blood samples were 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 3000 g, 4 ºC) and the supernatant transferred to separate tubes for storage at -20 ºC until further analysis.

Blood [pyruvate] concentrations and isotope enrichments (IE) were determined using gas chromatography-mass spectrometry (GCMS: GC, model 6890 series; MS, model 5973N, Agilent Technologies) of the trimethylsilyl-quinoxalinol derivative, with α-ketovalerate as the internal standard for concentration measurements (18). Briefly, 150 µl of perchloric extract was spiked with α-ketovalerate, mixed (1:1) with an orthophenylendiamine solution (5 mg/ml in 3 M HCl) and heated for 60 min at 90 ºC. 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)trifluoroacetamide 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).

Calculations. The transpulmonary concentration gradient for pyruvate was calculated from concentrations in the right atrium (mixed central venous sample, \( \bar{v} \)) and carotid artery (a):

Transpulmonary concentration gradient (µmol/l) = \( [\bar{v}] - [a] \)

Transpulmonary pyruvate net balance was determined from the product of the concentration gradient and cardiac output (Q):

Net Balance (µg/min) = \{([\bar{v}] - [a]) \times Q \}

where \([\bar{v}]\) and \([a]\) are the concentrations of pyruvate collected from the mixed central venous and arterial samples sites respectively, and Q is blood flow as determined previously by Lin et al (23). 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 was calculated as:

\[ F_{EXC} = \frac{([\bar{v}] - [a])}{[\bar{v}]} \times 100\% \]

Tracer-measured transpulmonary fractional extraction, tracer-measured uptake, and total pyruvate release was calculated as:

\[ F_{EXTM} = \frac{([^{13}C]pyruvate\bar{v} \text{IE})([pyruvate] \bar{v})}{([^{13}C]pyruvate_{v} \text{IE})([pyruvate]_{v})} - \frac{([^{13}C]pyruvate_{a} \text{IE})([pyruvate]_{a})}{([^{13}C]pyruvate_{v} \text{IE})([pyruvate]_{v})} \]
Tracer Measured Uptake = Q \times \text{Fex} \times \text{[pyruvate]}$
overline{v}$

Total Pyruvate Release (µg/min) = Tracer-Measured Uptake - Net Balance

Statistical analyses. Data are presented as mean ± standard error (SE). For statistical analysis, representative values of blood pyruvate concentrations and isotopic enrichments were obtained by averaging data taken during the last 20 min of each trial (i.e., at 40, 50, and 60 min) after infusion commenced. ANOVA indicated that $^{13}$C-isotopic enrichments of pyruvate 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 analyses of variance (ANOVA) 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 treatment on pyruvate concentration. Following ANOVA, post-hoc testing to identify significant treatment effects was done using Tukey’s honest significance difference (HSD) tests, and $\alpha$ of 0.05 was used throughout. Statistical tests were performed using SPSS Graduate Pack 11.0 software.
RESULTS

Mixed Central Venous and Arterial Pyruvate Concentrations. Pyruvate concentrations in the mixed central venous blood were highest during the lactate load (LL). One-way ANOVA revealed an effect of treatment on \([ v ]\) pyruvate \((P < 0.05)\) and multiple comparisons with Tukey’s HSD showed that the lactate load significantly increased pyruvate concentration over both the epinephrine stimulated \((68.0 \pm 8.8\%)\) and control condition \((36.4 \pm 13.8\%)\), Table 1. Levels of arterial pyruvate were highest during the lactate load and one-way ANOVA revealed an effect of treatment on arterial pyruvate \((P < 0.05)\). Multiple comparisons with Tukey’s HSD showed that the lactate load significantly increased arterial pyruvate concentration over both the control \((60.1 \pm 5.2\%)\) and epinephrine stimulated \((54.2 \pm 7.8\%)\) condition (Table 1).

Transpulmonary Pyruvate Metabolism. The pyruvate concentration gradient \(( [ v ] – [a] )\) was positive across the tissue bed indicating net uptake during all three conditions (Figure 1). One-way ANOVA revealed a treatment effect on the pyruvate concentration gradient \((P < 0.05)\), and multiple comparisons with Tukey’s HSD showed that both lactate load and epinephrine stimulation significantly increased pyruvate uptake compared to during the control Con condition \((P < 0.05)\).

Net pyruvate balance was positive during all three conditions (Table 1). One-way ANOVA revealed a treatment effect on net pyruvate balance \((P < 0.05)\), and multiple comparisons with Tukey’s HSD showed that pyruvate net balance (i.e., uptake) was greatest during \((P < 0.05)\).

Transpulmonary concentration measured pyruvate fractional extraction averaged \(33.1 \pm 5.0\%\) for the three conditions and one-way ANOVA revealed a significant treatment effect \((P < 0.05)\). Multiple comparisons with Tukey’s HSD showed that the epinephrine stimulation \((47.2 \pm 7.0\%)\) significantly increased concentration measured transpulmonary pyruvate fractional extraction above that during the control condition \((18.2 \pm 10.0\%)\) (Figure 3).

Tracer measured uptake was positive under all three conditions and greatest for epinephrine stimulation (Figure 2). One-way ANOVA revealed a treatment effect on tracer measured pyruvate uptake, and multiple comparisons by Tukey’s HSD showed that epinephrine stimulation significantly increased tracer measured pyruvate uptake \((P < 0.05)\).

Tracer measured transpulmonary pyruvate fractional extraction averaged \(42.8 \pm 5.8\%\) across all three conditions. Although epinephrine stimulation produced the highest \(F_{EXTM}\) at \(60.9 \pm 7.1\%\), a one-way ANOVA did not reveal a treatment effect \((P < 0.05)\).

Transpulmonary tracer measured pyruvate net balance was positive for all three conditions and a one-way ANOVA revealed a treatment effect \((P < 0.05)\). Multiple comparisons with Tukey’s HSD showed that epinephrine stimulation was significantly higher than both the control and lactate load conditions \((P < 0.05)\).

Total transpulmonary pyruvate release (= tracer measured pyruvate uptake – net balance) provides an estimate of pyruvate flux in the tissue bed studied. Transpulmonary total pyruvate release was highest during epinephrine stimulation and near zero or
slightly negative for the control and lactate load conditions. One-way ANOVA revealed a treatment effect on total pyruvate release (P < 0.05), and multiple comparisons with Tukey’s HSD showed that epinephrine stimulation significantly increased total release above a lactate load (P < 0.05).

*Lung MCT expression.* MCT1, MCT2, and MCT4 were detected by immunoblotting in lung tissue homogenates (Figure 4). Lung MCT1 expression was reported in lung tissue by others (12, 13). Our data show for the first time that MCT2 and MCT4 are also expressed in rat lung tissue.
DISCUSSION

As evidenced by the large net balance and tracer-measured uptake rates under all conditions studied, it appears that the lungs extract a significant fraction of circulating pyruvate in vivo. During the control, the rate of total pyruvate release from the lungs is approximately zero. However increasing pyruvate turnover by lactate loading or epinephrine stimulation increases net uptake of pyruvate. Most impressive was that epinephrine stimulation significantly increased tracer-measured pyruvate uptake over that during lactate loading. The epinephrine-stimulated tracer measured uptake was similar to that observed in our earlier work in exercising humans (18). However, the present results provide more direct evidence that the lungs are responsible for significant changes in venous – arterial pyruvate concentration and isotopic enrichment differences (1, 18). The presence of three MCT isoforms in lungs, and position of the lungs in the pulmonary circulatory bed in relation to the systemic circulation offers an explanation of how the lungs affect whole-body monocarboxylate metabolism.

The large transpulmonary tracer- and concentration-measured fractional extractions of pyruvate are perhaps our most striking observations. The \( F_{\text{EXTM}} \) averaged 43% (Figure 3) and is three times higher than that of lactate across the lungs under the same conditions (20). Both lactate and pyruvate are exchanged across the lung parenchyma by proton-linked transport (29, 30) involving the MCT proteins (15). The most relevant MCT for pyruvate exchange is MCT2 because of its high affinity for substrates, Km’s for pyruvate and lactate are 0.1 mM and 0.7 mM respectively (16). Gene transcripts for MCT2 were previously found in rodent lung homogenates (14), and we now show for the first time that MCT2 and MCT4 (Km of 150 mM for pyruvate, and Km of 28 mM for lactate) protein are expressed in rat lungs (Fig. 4). MCT1 (Km of 0.7 mM for pyruvate, and Km of 3-5 mM for lactate) was previously found in the lungs (14, 15), and our data confirmed this finding. Again, the presence of MCT isoforms in the lungs helps provide a mechanism for the high fractional extraction of pyruvate in blood traversing the pulmonary capillary bed. It is likely that the high Km of MCT2 for pyruvate is causing the lung to extract pyruvate when delivery is increased, such as under a lactate load, or epinephrine stimulation.

During exercise, higher venous pyruvate compared with arterial concentration is documented in humans (1, 18). The venous - arterial difference tends to scale with exercise intensity (18, 25). Those observations fit with our current results because total release approximated zero during the control condition when tracer measured uptake and net balance for pyruvate were similar, resulting in a small transpulmonary \([v-a]\). The \([v-a]\) for pyruvate increased during lactate loading and epinephrine stimulation, when circulating pyruvate concentrations increased significantly. These data show that net balance for pyruvate is in part concentration driven and provide evidence that the lungs are responsible for the extraction of venous pyruvate during times of increased venous concentration such as during exercise (1, 18).

There are at least two possible explanations for the effect of epinephrine on increasing lung pyruvate uptake. One possibility is increased pyruvate oxidation and
excretion as CO₂. Unfortunately, we lacked means to determine ¹³CO₂ enrichments in mixed venous and arterial blood or expired CO₂. An alternative explanation is that β-adrenergic stimulation cause conversion of pyruvate to lactate in lung parenchyma with release of lactate into the pulmonary vein, and subsequently in arterial systemic circulation. Results in our companion report (20) support the latter possibility. Hence an effect of epinephrine on pyruvate metabolism across the lung is to increase pyruvate extraction (Fig. 3) and convert a significant portion of the extracted pyruvate to lactate.

Studies on humans (18), dogs (9, 33), and now our data on rats (20) provide additional evidence for transpulmonary conversion of pyruvate to lactate during exercise or epinephrine stimulation. In fact, the isotopic enrichment ratio between lactate and pyruvate ([IEₖ/IEₚ]) can change by nearly 70% (20). However the reason for the conversion is less clear. For example β-adrenergic stimulation would seemingly favor the conversion of pyruvate to lactate by the enzyme lactate dehydrogenase (LDH) to regenerate NAD⁺ during times of high glycolytic flux. However the subcellular distribution of the MCT proteins in the lungs is not known at this time. Therefore speculation as to the compartment pyruvate is extracted into from the circulation is premature. Further, other factors such as blood flow heterogeneity under Con and Epi conditions or rest and exercise are considerations still to be tested (23). For now it can be said that during times of epinephrine stimulation the lungs are responsible for converting a significant portion of venous pyruvate into arterial lactate and that this is likely occurring during exercise as well.

The lungs are known to release lactate on a net basis during times of “stress” such as exercise, low partial pressure of oxygen, or lung injury (21, 28, 31). However the source of the lactate released is not known. Longmore and colleagues (24) showed net lactate release by the lungs from a non-glucose precursor during exposure to hypoxia, but they were unable to identify the precursor ex vivo. Because the arterial oxygen saturation was maintained between 94-99% for all experiments, high lung lactate production from cellular hypoxia is unlikely during our conditions (13). However, conversion of circulating pyruvate to lactate as blood transits through the pulmonary capillary bed provides a means to explain the results of Longmore as well as reconcile them with the data of Bassett et al. (2) who found transpulmonary production of lactate during a pyruvate infusion in lung preparations studied ex vivo.

In conclusion, our data on transpulmonary pyruvate kinetics show that the lungs actively extract pyruvate from pulmonary arterial (mixed venous) blood. The robust expression of MCT isoforms in lung parenchyma may provide a mechanism to explain pulmonary pyruvate uptake. While reports of venous and arterial pyruvate concentrations are scarce in the literature, overall our data are consistent with those reports. Moreover, the present observations of high venous and low arterial pyruvate concentrations are consistent with measurements in exercising humans (18, 21, 28, 32). Finally, the results we report here on lung parenchymal pyruvate uptake are consistent with the possibility of pyruvate to lactate conversion as blood courses through the lung parenchyma. In this way our present results help reconcile previous results of others (33) and us (18)
regarding pyruvate uptake and lactate release by the lungs always and especially in the lungs under stress.
ACKNOWLEDGMENT
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).
REFERENCES

Table 1. Pyruvate Concentration, Net Balance, and Total Release

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<th>Con</th>
<th>LL</th>
<th>EPI</th>
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<tr>
<td><strong>Mixed Central Venous, µmol</strong></td>
<td>44.7 ± 9.1§</td>
<td>139.6 ± 8.5</td>
<td>88.9 ± 18.5§</td>
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<td><strong>Arterial, µmol</strong></td>
<td>32.4 ± 7.3§</td>
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<td><strong>Net Balance (µg/min)</strong></td>
<td>30.0 ± 20.0</td>
<td>200.0 ± 40.0</td>
<td>400.0 ± 100.0*</td>
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<td><strong>Total Release (µg/min)</strong></td>
<td>-13.1 ± 15.1</td>
<td>-40.5 ± 31.9</td>
<td>40.8 ± 29.6§</td>
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Table 1. Pyruvate concentration in the mixed central venous [v] and arterial [a] blood; transpulmonary pyruvate balance (uptake), and pyruvate total release during Con, LL, and Epi conditions respectively. Values and are means ± SE; *Significantly different that Con, P < 0.05. §Significantly different from LL, P < 0.05.
Figures

**Figure 1:** Mixed central venous – arterial concentration difference (µM) across the rat lung during Con, LL, and Epi conditions respectively. Values are means ± SE; *Significantly different that Con, P < 0.05

**Figure 2:** Tracer measured pyruvate uptake (mg/min) during Con, LL, and Epi conditions respectively. Values are means ± SE; *Significantly different that Con, P < 0.05. §Significantly different than LL, P < 0.05.

**Figure 3:** Pyruvate fractional extraction (%) for Con, LL, and Epi conditions respectively. Calculated as tracer-measured extraction (left) and concentration-measured fractional extraction (right). Values are means ± SE; *Significantly different that Con, P < 0.05.

**Figure 4:** Representative immunoblots showing the expression of (A) MCT1, (B) MCT2, and (C) MCT4 in rat tissue homogenates from liver (LI), heart (H), lung (Lu), brain (B), skeletal muscle (SM), and rat liver mitochondrial fraction (LM), and sarcolemmal fraction of L6 cells (SL). Data show MCT2 and MCT4 proteins are expressed in the rodent lung as well as MCT1.
Fig. 1. [v - a] difference across the rat lung during Con, LL, and Epi conditions respectively. Values are means ± SE; *Significantly different that Con, P < 0.05
Fig. 2. Tracer measured pyruvate uptake during Con, LL, and Epi conditions respectively. Values are means ± SE; *Significantly different that Con, P < 0.05. §Significantly different than LL, P < 0.05.
Fig. 3. Pyruvate fractional extraction for Con, LL, and Epi conditions respectively. Calculated as tracer-measured extraction (left) and concentration-measured fractional extraction (right). Values are means ± SE; *Significantly different from Con, P < 0.05.
<table>
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<tr>
<td>MCT1 40 KDa</td>
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<td>B</td>
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<td>MCT4 40 KDa</td>
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Fig. 4. Representative immunoblots showing the expression of (A) MCT1, (B) MCT2, and (C) MCT4 in rat tissue homogenates from liver (Li), heart (H), lung (Lu), brain (B), skeletal muscle (SM), and rat liver mitochondrial fraction (LM), and sarcolemmal fraction of L6 cells (SL). Data show MCT2 and MCT4 proteins are expressed in the rodent lung as well as MCT1.
CHAPTER 3

Conclusions And Future Directions
The present studies expand the lungs’ metabolic role beyond gas exchange and position it as a switch of intermediary carbohydrate metabolism. Our results describe the kinetics of lactate, pyruvate, and the interplay between the two metabolites across the lung parenchyma *in vivo*. The simultaneous uptake and release of lactate across the lungs during the control and lactate load conditions are redolent of the femoral arterial-venous difference measurements made on men during rest and exercise across contracting skeletal muscle. Interestingly, epinephrine stimulation produced similar pyruvate venous to arterial difference tracer kinetic values as exercising muscle. Taken as a whole these results confirm that the lungs participate in the Lactate Shuttles, and secondarily provide insight into the lungs metabolic response to exercise.

The use of a lactate tracer is an area of debate in the field of exercise physiology. Our results now provide evidence that the lungs are responsible for the arterial blood isotopic equilibration of lactate and pyruvate. Furthermore, the lungs under epinephrine stimulation produced similar pyruvate net uptake values as exercising humans. It seems the lungs’ metabolic participation in exercise extends well beyond gas exchange. Future work should use a larger animal model to allow for cardiac and pulmonary vein catheterization during exercise. Transpulmonary tracer kinetic studies during exercise will expand our understanding of the lungs role in monocarboxylate metabolism during periods of increased metabolic demand.

Our pyruvate results lead one to speculate upon precisely what compartment monocarboxylates are taken into from the circulation. It is possible that lactate and pyruvate are extracted from the circulation by MCTs directly into the mitochondria. This mechanism would explain why in Henderson *et al.* during exercise arterial lactate was oxidized across a working muscle bed, while in the lungs, a less metabolically active tissue would reduce pyruvate during times of increased substrate delivery. Finally it would reconcile the results of others, such as Chatham *et al.* who found that lactate delivered to the heart by the cardiac circulation was preferentially oxidized to carbon dioxide, while blood glucose was converted and released to lactate by the heart.

Our characterization of MCT1, 2, and 4 in whole lung homogenate suggest a mechanism; further work should focus on the distribution of MCTs within the intracellular domains of the lung tissue. Knowledge of the subcellular compartment(s) the extracted pyruvate enters will hopefully explain the observed conversion of venous pyruvate to arterial lactate by the pulmonary tissue.

The large extraction of pyruvate across the lungs is truly remarkable and reconciles venous to arterial concentration differences in the literature. The distinctive metabolic response to a lactate load in comparison to epinephrine stimulation beckons future investigation. The large net uptake and $^{13}$C tracer data provide compelling evidence that the lungs extract a significant portion of circulating pyruvate under high concentrations, yet during times of stress convert extracted pyruvate to lactate. Using a larger animal model where exercise and beta-blockade is possible will provide further insight into epinephrine’s metabolic affects on the lungs.

The results of the present investigation describe the lungs effects on intermediary carbohydrate metabolism. It is apparent that the lungs actively participate in the whole body metabolism of lactate and pyruvate. We have reported on three different...
physiologic conditions and the effects the lungs exert on lactate and pyruvate metabolism under those conditions. Our work expands the Lactate Shuttle to the lungs, and opens the door to future work on tracer kinetics across metabolically active tissue beds. Understanding how the lung parenchyma alters substrate kinetics during normal and stress conditions will provide valuable information to pulmonary researchers along with those in other fields for basic science and clinical settings.