Transpulmonary pyruvate kinetics

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Transpulmonary pyruvate kinetics. Am J Physiol Regul Integr Comp Physiol 301: R769–R774, 2011. First published June 15, 2011; doi:10.1152/ajpregu.00206.2011.—Shuttling of intermediary metabolites, such as pyruvate, contributes to the dynamic energy and biosynthetic 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. However, we understood that during each circulatory passage, the entire cardiac output transits the pulmonary circulation. Therefore, we examined the transpulmonary pyruvate kinetics in an anesthetized rat model during an unstimulated (Con), lactate clamp (LC), and epinephrine infusion (Epi) conditions using a primed-continuous infusion of [U-13C]pyruvate. Compared with Con and Epi stimulation, LC significantly increased mixed central venous ([V]l) and arterial ([a]l) pyruvate concentrations (P < 0.05). 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. Transpulmonary net pyruvate balances were positive during all three conditions, indicating net pyruvate uptake. Net balance was significantly greater during epinephrine stimulation compared with the unstimulated 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 LC 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 data 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 a major site of circulating pyruvate, or epinephrine stimulation, pyruvate extraction is increased.

lactate; metabolism; exercise; isotope tracers

PYRUVATE IS A MAJOR METABOLITE at the junction of glycolytic and oxidative pathways. In 1973, Scholz et al. (33) showed that rat lung slices were capable of oxidizing [14C]pyruvate. That study established a role for exogenous pyruvate in lung tissue metabolism that subsequent investigators were able to elaborate upon (2–7, 12, 29). Among those findings was that environmental insults (e.g., low or high PO2, ozone) altered lactate to pyruvate ratios (L/P) and that exogenous pyruvate infusions increased lactate concentration in the effluent of perfused lung preparations (29). 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, 21, 24, 28, 30).

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 in vivo (11). The anatomical positioning of the lungs receives the entire cardiac output, resulting in high tissue-specific blood flow rates in relation to their metabolic requirements. High relative blood flow rates through the pulmonary circulation cause mixed central venous (v) to arterial (a) difference measurements for substrates to be small and difficult to reliably assay.

Monocarboxylate transporters (MCTs) are symporters that facilitate transmembrane exchange of protons and lactate and pyruvate anions. The presence of MCT isoforms in skeletal muscle, liver, and heart tissue is well documented (31, 32). To date, investigators have documented MCT-1 and -4 protein expression in cancerous lung cells (21). However, little is known about MCT expression in noncancerous lung cells. Specifically, MCT2, the main pyruvate transporter, has not been found in lung tissue (16).

Given the dearth of information on lung pyruvate metabolism and its transport, we sought to develop a rat model that would allow transpulmonary metabolic measurements during times of stress in vivo. In this respect, 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 are involved in changes in lactate and pyruvate concentration ([L]/[P]) and isotope enrichment values because they receive the total cardiac output during each circulatory passage. Therefore, we used nonradioactive 13C-labeled isotope tracers of pyruvate to assess transpulmonary pyruvate kinetics in anesthetized rats during an unstimulated condition that acted as the control (Con), compared with two interventions both designed to increase pyruvate turnover, but through different mechanisms. The first, a lactate clamp (LC), is named after the commonly performed glucose clamp. Physiologically the lactate clamp 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 (Epi), and thus mimic pyruvate production during exercise. On the basis of previous work on resting and exercising muscle tissue, along with the work done by previous investigators on rat lung preparations ex vivo, we hypothesized that the lungs, specifically the pulmonary capillary beds, are sites of simultaneous production and removal of pyruvate and contribute significantly to whole body carbohydrate intermediary metabolism (18, 33).
METHODS

Animals and experimental preparations. Twenty-one female Wistar rats (n = 7 per condition, body mass range, 240–290 g) 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 2 per cage on a 12:12-h light-dark cycle (light from 7:00 AM to 7:00 PM), in a 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 (S\text{O}2, by pulse oximetry positioned at the base of the tail). S\text{O}2 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 for blood sampling were positioned in the left common carotid artery and the right atrium (Intramedic PE-50, BD), as previously described (10). A third catheter was placed in the right iliac vein for infusion purposes. The surgical preparation of animals took ~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 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) with a tracer prime corresponding to 15 times the minute infusion rate. Lactate tracer was infused at rates corresponding to the tracer prime, and the infusion rate was determined from results obtained during preliminary studies and was continuously adjusted during experimental trials to consistently elevate lactate concentration levels to those seen during strenuous exercise (i.e., 4–5 mM). During each LC trial, blood lactate concentration was determined at time points 40, 50, and 60 min (Nova Biomedical, Lactate Plus analyzer), and the exogenous lactate infusion rate was adjusted to produce an arterial [lactate] in the target range. For Epi trials, the hormone cocktail was prepared immediately after infusion commenced. ANOVA indicated that 13C-isotopic enrichment was different among conditions and, therefore, values were pooled to obtain best estimates of background isotopic enrichments. Single one-way ANOVA was used to test for treatment effects. Because concentrations did not always plateau during trials, a two-way ANOVA was used to test for treatment effects.
Table 1. Pyruvate concentration, net balance, and total release

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pyruvate Concentration (µM)</th>
<th>Net Balance (µg/min)</th>
<th>Total Release (µg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed central venous</td>
<td>Con: 44.7 ± 9.1</td>
<td>LC: 139.6 ± 8.5</td>
<td>Epi: 88.9 ± 18.5</td>
</tr>
<tr>
<td>Arterial</td>
<td>Con: 32.4 ± 7.3</td>
<td>LC: 81.2 ± 3.0</td>
<td>Epi: 37.2 ± 8.3</td>
</tr>
</tbody>
</table>

Net pyruvate balance was positive during all three conditions (Table 1). One-way ANOVA revealed a treatment effect on net pyruvate balance ($P = 0.001$), and multiple comparisons with Tukey’s HSD showed that pyruvate net balance (i.e., uptake) was greatest during Epi ($P = 0.001$).

Transpulmonary concentration measured pyruvate fractional extraction averaged $33.1 ± 5.0\%$ for the three conditions, and one-way ANOVA revealed a significant treatment effect ($P = 0.001$). Multiple comparisons with Tukey’s HSD showed that the epinephrine stimulation ($47.2 ± 7.0\%$) significantly increased the concentration-measured transpulmonary pyruvate fractional extraction above that during the unstimulated condition ($18.2 ± 10.0\%, P = 0.042$) (Fig. 2).

Tracer measured transpulmonary pyruvate fractional extraction averaged $42.8 ± 5.8\%$ across all three conditions. Although epinephrine stimulation produced the highest FEXT ($60.9 ± 7.1\%$), a one-way ANOVA did not reveal a treatment effect.

Transpulmonary tracer measured pyruvate uptake was positive for all three conditions (Con, 75.1 ± 13.6 µg/min; Epi, 389.6 ± 75.2 µg/min; LC, 147.1 ± 25.9 µg/min; Fig. 3), and a one-way ANOVA revealed a treatment effect ($P = 0.001$). Multiple comparisons with Tukey’s HSD showed that epinephrine stimulation produced significantly higher pyruvate uptake than both the unstimulated ($P = 0.001$) and LC conditions ($P = 0.034$).

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 unstimulated and LC conditions (Table 1) One-way ANOVA revealed a treatment effect on total pyruvate release ($P = 0.043$), and multiple comparisons with Tukey’s HSD showed that epinephrine stimulation significantly increased total release above a lactate load ($P = 0.041$).

Lung MCT expression. MCT1, MCT2, and MCT4 were detected with immunoblotting in lung tissue homogenates (Fig. 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.

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**RESULTS**

**Mixed central venous and arterial pyruvate concentrations.**

Pyruvate concentrations in the mixed central venous blood were highest during the lactate load (LC). One-way ANOVA revealed an effect of treatment on [V] pyruvate ($P = 0.001$), and multiple comparisons with Tukey’s HSD showed that the lactate load significantly increased pyruvate concentration over the control ($212.3 ± 8.1\% \Delta, P = 0.001$) and epinephrine-stimulated condition ($98.9 ± 10.1\% \Delta, P = 0.03$, Table 1). Levels of arterial pyruvate were highest during the lactate load, and one-way ANOVA revealed an effect of treatment on arterial pyruvate ($P = 0.001$). Multiple comparisons with Tukey’s HSD showed that the lactate load significantly increased arterial pyruvate concentration over both the unstimulated ($P = 0.001, 250\%\Delta$) and epinephrine-stimulated condition ($P = 0.001, 21.8\%\Delta$, Table 1).

**Transpulmonary pyruvate metabolism.**

The pyruvate concentration gradient ([V] – [a]) was positive across the tissue bed, indicating net uptake during all three conditions (Fig. 1). One-way ANOVA revealed a treatment effect on the pyruvate concentration gradient ($P = 0.001$), and multiple comparisons with Tukey’s HSD showed that both lactate load ($P = 0.001$) and epinephrine stimulation ($P = 0.032$) significantly increased pyruvate uptake compared with the unstimulated Con condition.

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**Fig. 1.** Mixed central venous-arterial concentration difference (µM) across the rat lung during control (Con), lactate clamp (LC), and epinephrine infusion (Epi) conditions. Values are expressed as means ± SE. *Significantly different from Con, $P < 0.05$.

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**Fig. 2.** Pyruvate fractional extraction (%) for Con, LC, and Epi conditions. Calculated as tracer measured extraction and concentration measured fractional extraction. Values are expressed as means ± SE. *Significantly different from Con, $P < 0.05$. 

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**Table 1.** Pyruvate concentration, net balance, and total release

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<td>Epi</td>
<td>7.3 ± 8.3</td>
<td>34.9 ± 59.9</td>
<td>40.8 ± 29.6</td>
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Values are expressed as means ± SE. Pyruvate concentration in the mixed central venous [V] and arterial [a] blood; transpulmonary pyruvate balance (uptake), and pyruvate total release during Con, lactate clamp (LC), and epinephrine infusion (Epi) conditions, respectively. *Significantly different from Con, $P < 0.05$. §Significantly different than LC, $P < 0.05$. 

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**Note:** The image contains figures and tables that are not transcribed here due to the nature of the task. The content is focused on the text that is readable from the page.
The large transpulmonary tracer and concentration measured fractional extractions of pyruvate are our most reliable and physiologically significant measurements. This is because our estimates of cardiac output come from published values obtained on rats studied under similar conditions. In future trials we will need to determine cardiac outputs in control and stimulated conditions.

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. However, there remains an absence of data concerning the cellular and subcellular distributions of MCT proteins in lung tissues. To date, investigators have focused on interpreting the overexpression of MCT1 and MCT4 in lung cancer and have considered targeting MCT proteins as a means to kill tumors (21). Although effective lung cancer therapies are needed, an increased knowledge of MCTs and other associated proteins (e.g., CD147, cytochrome oxidase, lactate dehydrogenase, and other components, including plasma membrane and mitochondrial MCT protein complexes) is critical to advancing our understanding of roles of MCTs in lung normophysiology and pathophysiology (19).

During moderate-intensity exercise, higher venous pyruvate (200 μM) compared with arterial concentration (20 μM) is documented in humans (1, 18). The venous-arterial difference tends to scale with exercise intensity, as a percentage of maximal oxygen consumption (18, 27). For example, in resting men, Henderson et al. (18) found venous and arterial pyruvate to approximate 50–60 μM, with no significant difference between the two blood compartments. However, in men during exercise at 65% of maximal oxygen consumption, Henderson et al. found the venous and arterial difference to approach 180 μM (18). Those observations fit with our current results because both concentration difference and total release approximated zero during the unstimulated condition when tracer...
measured uptake and net balance for pyruvate were similar, resulting in a small transpulmonary \( \dot{v} - a \). The \( \dot{v} - a \) 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 provides 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 \(^{13}\)CO2 enrichments in mixed venous and arterial blood or expired CO2. A second alternative explanation is that \( \beta \)-adrenergic stimulation causes conversion of pyruvate to lactate in lung parenchyma with release of lactate into the pulmonary vein and subsequently, arterial systemic circulation. The latter is a viable possibility. Hence, the 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, 35), and now rats (22) 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 (IEL/IEP) can change by nearly 70% (Johnson ML, Horning MA, and Brooks GA, unpublished results). However, the reason for the conversion is not clear. For example, \( \beta \)-adrenergic stimulation would seemingly favor the conversion of pyruvate to lactate by the enzyme lactate dehydrogenase 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 regarding the compartment and cells to which pyruvate is extracted from the circulation is premature. Further, other factors, such as blood flow heterogeneity during Con and Epi conditions or rest and exercise, are considerations still to be tested (25). For now, it can be said that during times of epinephrine stimulation, metabolically active cells in 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 (23, 30, 33). However, the source of the lactate released is not known. Longmore and Mourning (26) showed net lactate release by the lungs from a nonglucose precursor during exposure to hypoxia, but they were unable to identify the precursor ex vivo. Because the arterial oxygen saturation was maintained between 94 and 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 and Mourning, as well as reconcile them with the data of Bassett and Bowen-Kelly (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 in vivo. 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, 23, 30, 34). And finally, the results that we report here on lung parenchymal pyruvate uptake are consistent with the possibility of pyruvate to lactate conversion, as blood courses through the lung. In this way, our present results help reconcile previous results from our laboratory (18) and others (35) regarding pyruvate uptake and lactate release by the lungs at all times and especially in the lungs under stress.

**Perspectives and Significance**

That the lungs are a metabolic organ and possess roles beyond those of gas exchange and trapping emboli is perhaps new to researchers of metabolism. The lungs anatomical location positions itself to receive the entire blood flow during rest, exercise, health, and disease. No other organ except the heart shares this privilege, and in the heart, blood flows through chambers as opposed to a capillary bed that precludes metabolite exchange. Still, by analogy with the heart, in which atrial distension affects the release of atrial natriuretic peptide, it may also turn out that beyond metabolite overload and adrenergic stimulation, physical distension of the lungs causes the release of endocrine and paracrine factors that have downstream effects.

The data presented here not only expand our understanding of pyruvate kinetics in the lungs, but also allow us to better understand the integrative and evolutionary nature of metabolism. Imagine for a moment that pyruvate turnover in the lungs is a major signal for increasing MCT protein and allowing for yet greater pyruvate turnover, and hence conversion to lactate during times of stress. Perhaps this trait developed through natural selection over a million of years ago, as it allowed for greater arterial delivery of potential energy to working muscle beds during times of physical activity. Using the pyruvate-to-lactate conversion data from Henderson et al. (18) during exercise, the NADH generated during this conversion accounts for roughly 2% of total energy expenditure for the exercising muscle. This is a small amount for sure, but significant to an Olympian today, and very possibly significant to the survival of our ancestors.

**ACKNOWLEDGMENT**

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**GRANTS**

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**DISCLOSURES**

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

**REFERENCES**
