Further evaluation of isotopic equilibration between labeled pyruvate and lactate

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*To evaluate the extent of lactate-pyruvate interconversion, we infused six anesthetized dogs with 1-^{13}C-lactate and U-^{13}C-pyruvate in the basal state (n = 3) or during a hyperinsulinemic euglycemic clamp (n = 3). Blood samples were collected for measurement of both 1-^{13}C- and U-^{13}C-lactate enrichment during and after stopping the isotope infusions. After normalization of infusion rates, there were no significant differences between the enrichments of 1-^{13}C- and U-^{13}C-lactate over time in arterial, venous, or mixed central venous blood in both basal and hyperinsulinemic conditions. We conclude that extensive and very rapid labeling of blood lactate occurs during an infusion of a pyruvate tracer. Given the large mass of the circulating lactate pool relative to the pyruvate pool, it can be concluded that most infused pyruvate tracer circulates in the lactate pool. Because labeled lactate is rapidly converted to pyruvate (*Am. J. Physiol. *254, E532–E535, 1988), these two circulating metabolites can be considered to comprise a single metabolic pool.*

Keywords: stable isotopes; tracer technique; modelling

Introduction

The interpretation of in vivo lactate kinetics as measured by lactate tracers remains unclear, although much knowledge about the problems involved has been obtained in recent years.[1–3] The problems in quantifying whole-body lactate kinetics include selection of the appropriate infusion and sampling sites,[4–7] the sampling of blood that lumps together the blood from various tissues of considerable heterogeneity,[8,9] and validation of the metabolic models.[10–12] Another potentially confounding variable in the quantification of lactate kinetics is the rapid interconversion between lactate and pyruvate. In a previous study, we found that pyruvate enrichment was approximately 92% of the lactate enrichment in anesthetized dogs receiving lactate tracer infusion.[2] This isotopic equilibrium persisted even when the lactate metabolism of the body was perturbed by hypoxia (to stimulate lactate production and hence to increase plasma lactate concentration) or dichloroacetate (to stimulate pyruvate oxidation and hence to decrease plasma lactate concentration).[2] More recently, we have found that complete isotopic equilibration between lactate and pyruvate was achieved within 3 minutes in whole blood in vitro.[13] From these observations, it seems likely that circulating lactate and pyruvate can functionally be represented by a single lactate-pyruvate pool in the blood in vivo. If true, then enrichment of lactate should be similar whether pyruvate or lactate tracer is infused. However, to this point only the response to the infusion of labeled lactate has been tested. Consequently, in this study we simultaneously infused 1-^{13}C-lactate and U-^{13}C-pyruvate into anesthetized dogs and compared 1-^{13}C-lactate and U-^{13}C-lactate enrichments in the basal state and during the hyperinsulinemic euglycemic clamp procedure. The latter situation was used because it is known to affect lactate-pyruvate kinetics.

Materials and methods

The experiments were performed on six anesthetized, overnight-fasted dogs. After a bolus intravenous injection of pentobarbital (30 mg/kg), polyethylene catheters were placed

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in the carotid artery (CA), femoral vein (FV), and left external jugular vein. A Swan-Ganz catheter and a polyethylene catheter were tied together with silk and inserted into a venotomy on the right external jugular vein. Under the guidance of a blood pressure monitor, the distal end of the Swan-Ganz catheter was placed in the pulmonary artery for isotope infusion, and the tip of the polyethylene catheter was adjusted to be immediately proximal to the right atrium for collection of blood from a mixed central vein (MCV). Additional pentobarbital (1 mg/kg·min) and 0.9% saline were given throughout the experiment to maintain a constant level of anesthesia and normal hydration. At the end of the experiment, the animals were given an injection of euthanizing agent T-61. This study was approved by the Animal Welfare Committee of The University of Texas Medical Branch.

Experimental design

After collecting blood samples for the determination of background isotope enrichment, U-^{13}C-pyruvate and 1-^{13}C-lactate (99% enriched; MSD Isotopes, Montreal, Canada) were infused for 2 hours at rates of 1 and 2 μmol/kg·min, respectively. Except for the first dog, priming doses of pyruvate tracer (15 μmol/kg) and lactate tracer (30 μmol/kg) were given before constant infusions. Pyruvate tracer infusate were prepared immediately before the infusion and lactate tracer was prepared as stock solution and diluted to desired concentration with 0.9% saline before infusion. Infusing labeled lactate or pyruvate for 2 hours does not cause appearance of sufficiently enriched glucose for detection of gas chromatography-mass spectometry, meaning that recycling of the label was not a problem. In three of these dogs, insulin was infused via the left jugular vein at a rate of 2.5 U/kg·h (prime, 1 U/kg) along with a variable rate of 50% dextrose infusion to maintain euglycemia. This dose of insulin infusion raises plasma insulin level to approximately 5,000 μU/mL, thereby eliciting the maximal response. After 1 hour infusion of insulin and glucose, background blood samples were collected and the isotope infusion was started. Frequent blood was drawn from CA and FV at 2, 7, 15, 30, 50, 70, 90, 100, 110, and 120 min during tracer infusion, and again at 2, 4, 7, 15, 25, 35, 45, and 60 min after the tracer infusion was stopped, to determine the enrichments of U-^{13}C-lactate (produced from U-^{13}C-pyruvate) and 1-^{13}C-lactate. MCV blood samples were collected at 90, 100, 110, and 120 min during tracer infusion. Plasma glucose concentration was measured every 10 min in the three dogs receiving the hyperinsulinemic clamp, and only before and at the end of the study in the other three dogs not given insulin.

Analysis of samples

One and a half mL of whole blood was added immediately after collection directly to a prechilled tube containing 4 mL of ethyl acetate and 50 μL of 12.5 N HCl. After thorough mixing, the samples were centrifuged at 4° C, and the supernatant was transferred to a tube containing approximately 2 g of sodium sulfate and mixed again. The supernatant was separated by centrifugation and evaporated to dryness under nitrogen, and the trimethylsilyl derivative of lactate prepared using 5:1 mixture of N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) : pyridine. Isotopic enrichment was determined by gas chromatography-mass spectometry using a Hewlett-Packard 5985B (Hewlett Packard, Palo Alto, CA USA) gas chromatography-mass spectrometry system.[14] Methane chemical ionization was used, and ions were selectively monitored at mass-to-charge ratios of 219, 220, and 222. The temperature programming was set at an increment of 30° C per minute from 60 to 250° C with retention time of 0.5 minute. Isotopic enrichment is expressed as tracer:tracee ratio, which includes a correction for the contribution of any tracer of m+1 to the apparent enrichment at m+3.[15]

Arterial plasma glucose concentrations were determined by means of autoanalyzers (Beckmen Instrument Inc., Fullerton, CA USA).

Statistics

The correlation between enrichments of 1-^{13}C-lactate and U-^{13}C-lactate over time was calculated for six dogs using both arterial and venous measurements. The absolute values were compared by means of the paired *t* test.

Results

Before the start of the tracer infusion, plasma glucose concentration in the six dogs was 5.7 ± 0.3 mmol/L. The three basal dogs not given insulin had a stable glucose level throughout the experiment (6.0 ± 0.6 mmol/L before the infusion and 5.9 ± 1.1mmol/L at the end of the infusion). The three hyperinsulinemic dogs received glucose infusion rates of 7.4 ± 0.05, 6.7 ± 0.03, and 12.7 ± 0.4 mg/kg·min (average 8.9 mg/kg·min) to maintain arterial plasma glucose levels at 6.6 ± 0.4, 4.8 ± 0.1, 5.1 ± 0.1 mmol/L, respectively (average 5.5 mmol/L).

The lactate enrichment over time derived from U-^{13}C-pyruvate infusion closely followed the same pattern as that derived from 1-^{13}C-lactate infusion in all experiments. *Figure 1* and *2* show an example in which the U-^{13}C- and 1-^{13}C-lactate enrichments are compared in the carotid artery and femoral vein. The enrichment of 1-^{13}C-lactate was normalized to the U-^{13}C-pyruvate infusion rate by reducing the 1-^{13}C-lactate enrichment to one-half because the infusion rate of 1-^{13}C-lactate was twice that of U-^{13}C-pyruvate. Statistical analysis showed that there were significant correlations be-

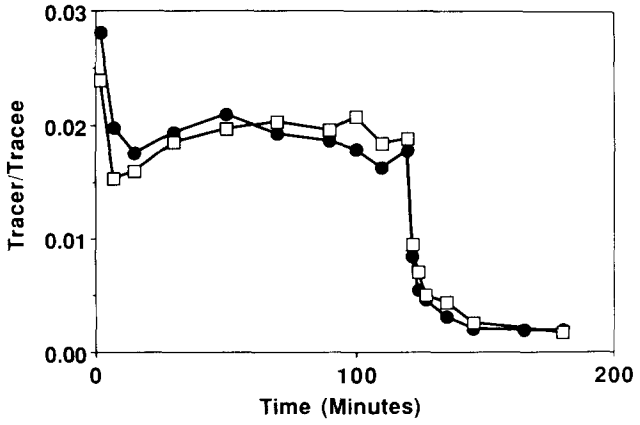

Figure 1 Comparison of [U-^{13}C]lactate and [1-^{13}C]lactate in the arterial blood in a representative dog receiving primed constant infusion of the isotopes at basal state. The enrichment curve of [U-^{13}C]lactate is almost identical to that of [1-^{13}C]lactate. —●— represents [U-^{13}C]lactate enrichment; —□—, [1-^{13}C]lactate enrichment.

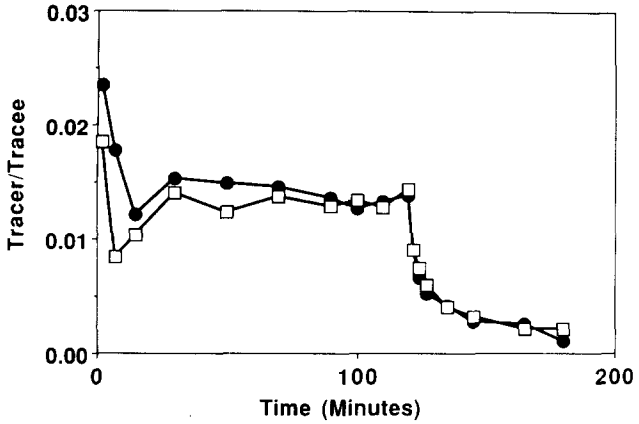

Figure 2 Comparison of [U-^{13}C]lactate and [1-^{13}C]lactate in the femoral vein blood in the same representative dog as *Figure 1*. The enrichment curve of [U-^{13}C]lactate is almost identical to that of [1-^{13}C]lactate. —●— represents [U-^{13}C]lactate; —□—, [1-^{13}C]lactate.

tween 1-^{13}C- and U-^{13}C-lactate. In arterial blood the combined correlation coefficient in six dogs was 0.94 ($n=88$) with a range of 0.90–0.98 for each individual dog ($n=10$–17). In the blood from the femoral vein, the combined coefficient was 0.95 ($n=83$) with a range of 0.88–1.00 ($n=10$–17). From the mixed central vein, blood was collected only between 90–120 min after isotope infusion with a total of 24 samples; the estimated correlation coefficient was 0.94.

Not only was the pattern of enrichment similar over time, but the absolute values of tracer:tracee ratio between 1-^{13}C- and U-^{13}C-lactate were very close (no significant differences). Assuming the mixed central vein is the optimal sampling site to account for the heterogeneity of pyruvate/lactate metabolism in different tissues, the arterial lactate enrichment overestimated the appropriate value by 27–30% and femoral vein lactate enrichment underestimated the appropriate value by 7–10% *(Table 1)*.

To assess the measurement error in measuring the tracer:tracee ratio, we took five aliquots of the same background sample and processed them separately, and analyzed each of them five times on the mass spectrometer. This gives us separate quantification of the error of replicate injections of the same sample into the mass spectrometer, and also the total error of analysis (including errors arising in the derivatization procedure). The total coefficients of variation in the determination of the relative abundances of the M+1, M+2, and M+3 isotopomers of naturally occurring lactate were 0.43%, 1.9%, and 3.0%, respectively. The coefficients of variation for replicate injections of the same samples into the mass spectrometer were 0.52%, 1.7%, and 2.1% for the M+1, M+2, and M+3 isotopomers, respectively; meaning that virtually all error was in the mass spectrometer analysis and not the derivatization procedure. Because the tracer:tracee ratio is the difference between the abundance of each isotopomer after tracer infusion and before infusion, the error in computation of that ratio is dependent on the level of enrichment. For example, the calculation of a tracer:tracee ratio of 0.02 of singly labeled lactate would have a coefficient of variation of between 2.5% and 5%, depending on the extent of correlation of the measured rates of the sample and the background.

Discussion

The quantification of lactate kinetics has been of interest for at least 30 years. Under normal conditions

Table 1 Lactate enrichments derived from 1-^{13}C-lactate or U-^{13}C-pyruvate infusion (lactate tracer infusion rate is normalized to 1 μmol/kg·min)

	Carotid artery		Mixed central vein		Femoral vein	
	1-^{13}C	U-^{13}C	1-^{13}C	U-^{13}C	1-^{13}C	U-^{13}C
A	0.0157 (0.0016)	0.0178 (0.0017)	0.0128 (0.0011)	0.0138 (0.0012)	0.0121 (0.0009)	0.0128 (0.0008)
B	0.0194 (0.0005)	0.0177 (0.0005)	0.0139 (0.0005)	0.0139 (0.0005)	0.0134 (0.0004)	0.0134 (0.0002)
C	0.0235 (0.0004)	0.0242 (0.0003)	0.0195 (0.0002)	0.0194 (0.0002)	0.0183 (0.0005)	0.0186 (0.0005)
D	0.0265 (0.0011)	0.0226 (0.0012)	0.0160 (0.0003)	0.0151 (0.0002)	0.0139 (0.0004)	0.0148 (0.0005)
E	0.0272 (0.0001)	0.0297 (0.0007)	0.0225 (0.0003)	0.0262 (0.0007)	0.0180 (0.0004)	0.0204 (0.0002)
F	0.0137 (0.0004)	0.0173 (0.0012)	0.0123*	0.0134*	0.0111 (0.0007)	0.0121 (0.0008)
mean	0.0210	0.0216	0.0162	0.0170	0.0145	0.0154
SEM	0.0023	0.0020	0.0017	0.0021	0.0012	0.0014

The enrichments are expressed as tracer:tracee ratios; 1-^{13}C represents 1-^{13}C-lactate and U-^{13}C, U-^{13}C-lactate; each value is the mean value of four measurements at 90′, 100′, 110′, 120′, SEM is noted in the parentheses under each mean value.
*Average values of two measurements.

lactate plays an important role as an end product of the peripheral metabolism of glucose and as a precursor for gluconeogenesis. In some situations, such as in intensive exercise or shock, altered lactate kinetics may be central to the physiological response. The ability to quantify pyruvate production would also enable the performance of many important studies of the regulation of glucose metabolism. However, techniques to quantify lactate and pyruvate kinetics are problematical. Net balance studies are unable to provide any information regarding total rates of lactate uptake and release. Consequently, tracers have been commonly used to quantify lactate kinetics and, to a lesser extent, pyruvate kinetics. Unfortunately, application of classical tracer techniques to the study of lactate kinetics has yielded unlikely high values.[16,17] Consequently, we were led to investigate the extent to which infused lactate tracer is directly converted to pyruvate,[2] thereby also reflecting, to at least some extent, pyruvate kinetics. In that paper we found that the conversion was extensively rapid, meaning that lactate kinetics could not be quantified without accounting for the rapid conversion of lactate to pyruvate. To more completely understand the nature of the isotopic exchange between lactate and pyruvate, with the ultimate goal of being able to quantify pyruvate kinetics in addition to lactate kinetics, we performed the current study to determine the rapidity and extent to which labeled pyruvate is converted to lactate.

The results of this study not only indicate that the conversion of labeled pyruvate to lactate is rapid, but suggest that the conversion is so complete that the blood pyruvate and lactate pools can probably be considered to be a single pool. Given that the lactate pool is many times larger than the blood pyruvate pool, one can conclude that when labeled pyruvate is infused, most of the tracer circulates in the form of lactate. This rapid interconversion of pyruvate and lactate makes it impossible to distinguish their individual kinetics by traditional tracer techniques. It is evident that more complex modeling will ultimately be necessary for quantification of lactate and pyruvate kinetics.

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