Isotopic Estimation of Plasma Glucose Conversion to Plasma Lactate Using [6-^{3}H] Glucose and [6-^{14}C]Glucose (42953)

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Abstract. **To determine whether [6-^{3}H]glucose could be used to quantitatively estimate the rate of plasma glucose conversion to plasma lactate, we compared the relative transfer of [^{3}H] and [^{14}C]plasma glucose to plasma lactate in nine postabsorptive anesthetized rats infused to isotopic steady state with [6-^{3}H]glucose and [6-^{14}C]glucose. Glucose turnover (μmol/kg/min) measured with [6-^{3}H]glucose (29.4 ± 1.7) and [6-^{14}C] glucose (25.0 ± 1.5), Cori cycle activity (4.35 ± 0.79), and the percentage of plasma lactate derived from plasma glucose calculated from ^{14}C-specific activities (59.8 ± 5.8) were all comparable to previously reported values for the overnight fasted rat. Although the percentage of plasma lactate derived from plasma glucose calculated from ^{3}H-specific activities (30.0 ± 3.3) was only half that calculated from ^{14}C-specific activities ($P < 0.001$), the ^{3}H:^{14}C ratios were constant over a wide range of percentage of lactate derived from glucose ($r = 0.95$, $P < 0.001$). We conclude that ^{3}H-specific activity ratios of plasma lactate to plasma glucose cannot be directly used as a quantitative estimate of the percentage of plasma lactate derived from plasma glucose; however, correction for the apparent constant proportionality of detritiation of lactate relative to ^{14}C loss could permit use of [6-^{3}H]glucose for this purpose.** [P.S.E.B.M. 1989, Vol 192]

Recent studies have demonstrated an important role for gluconeogenesis in hepatic glycogen repletion (1), in recovery from hypoglycemia (2) and in the excessive hepatic glucose output found in diabetes mellitus (3). This has led to renewed interest in the factors controlling gluconeogenesis. One of these factors is substrate availability.

Lactate is considered the predominant gluconeogenic precursor (4) and is also a major product of glucose metabolism (5). In studying glucose-lactate interrelationships, it would obviously be desirable to be able to measure simultaneously the rate of glucose conversion to lactate and the rate of lactate conversion to glucose. Theoretically, this could be done with simultaneous infusion of ^{13}C-labeled glucose and ^{14}C-labeled lactate or vice versa. However, this requires a use of a mass spectrometer, which may not be readily available and stable isotopes are still quite expensive.

An alternative approach would be to infuse [^{14}C] lactate and [6-^{3}H]glucose provided that incorporation of tritium from the labeled glucose into plasma lactate was quantitative. Dunn *et al.* (6) reported similar ^{3}H:^{14}C ratios in plasma lactate and glucose in fed rats simultaneously injected with [6-^{3}H]glucose and [6-^{14}C]glucose, suggesting that such an approach might be feasible. However, Okajima *et al.* (7) found ^{3}H:^{14}C ratios of 0.5–0.7 in 20-hr fasted rats infused simultaneously with [6-^{3}H]glucose and [U-^{14}C]glucose, and Shiota *et al.* (8) found ^{3}H:^{14}C ratios of approximately 0.6 in perfusates of rat hindlimb infused simultaneously with [6-^{3}H] glucose and [U-^{14}C]glucose, observations which cast doubt on whether this approach can be used.

To investigate this issue further, we infused overnight fasted anesthetized rats simultaneously with [6-^{3}H]glucose and [6-^{14}C]glucose to isotopic steady state and determined the percentage of plasma lactate derived from plasma glucose with each isotope, as well as rates of glucose turnover and Cori cycle activity. Although we found that the percentage of [^{3}H]plasma lactate derived from [^{3}H]plasma glucose (~30%) was only half that found for [^{14}C]plasma lactate derived from [^{14}C]plasma glucose, the ^{3}H:^{14}C ratio of plasma

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lactate and glucose was constant over a wide range of rates of glucose conversions to lactate. This finding suggests that combined infusion of [6-^{3}H]glucose and [U-^{14}C]lactate could be used to simultaneously determine rates of plasma glucose conversion to lactate and rates of plasma lactate conversion to glucose provided one can correct for lactate detritiation.

Materials and Methods

Animals. Adult male Sprague-Dawley rats (Hilltop, Scottdale, PA) weighing between 320 and 400 g were adapted to a controlled lighting schedule with the dark period from 7 PM until 7 AM with free access to water and commercial rat chow for at least 1 week. Studies were begun after approval of this protocol was obtained from the University of Pittsburgh's Committee for Care and Use of Laboratory Animals.

Protocol. Food was withdrawn from nine animals at 5 PM the day before experiments. The following morning at approximately 9 AM, animals were anesthetized with ketamine (Ketalar, 100 mg/kg ip). Catheters were inserted in a jugular vein for simultaneous infusion of [6-^{14}C]glucose (0.2–0.4 μCi/min, 20–40 μCi prime; Research Products International, Mt. Prospect, IL) and [6-^{3}H]glucose (0.2–0.4 μCi/min, 20–40 μCi prime; Research Products International) and in a femoral vein for blood sampling. After allowing 2 hr for isotopic equilibration and while the animals were maintained under light anesthesia, three blood samples (1 ml) were collected at 15-min intervals for determination of plasma glucose and lactate concentrations and plasma glucose and lactate radioactivity, from which respective ^{14}C- and ^{3}H-specific activities were calculated.

Analyses. Plasma glucose concentration was measured using a YSI glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma lactate was determined by a microfluorometric enzymatic assay (9). For determination of plasma glucose and lactate ^{3}H and ^{14}C radioactivity, aliquots of plasma were deproteinized with an equal volume of chilled 7% perchloric acid, neutralized with 4 *N* potassium hydroxide and subjected to anion (AGI-formate form) and cation (AG50-hydrogen form) exchange chromatography (10); plasma glucose was eluted off the AG1 and AG50 columns with distilled water. Plasma lactate was eluted off the AGI column with 2 *N* formic acid. Eluates were evaporated under vacuum, were resuspended in 1 ml of distilled water and, after addition of liquid scintillation medium, were counted in a dual channel liquid scintillation spectrometer with corrections for quench and recovery using external standards.

Calculations. Since isotopic steady state was achieved (Table I), steady-state equations were used to calculate glucose turnover and percentage of plasma lactate derived from plasma glucose: glucose turnover = infusion rate of [^{3}H:^{14}C]glucose (dpm/min/kg) ÷ plasma [^{3}H:^{14}C]-specific activity (dpm/μmol) (11); percentage of lactate derived from plasma glucose = [^{3}H:^{14}C]plasma lactate specific activity × 2 ÷ plasma glucose specific activity (11). Glucose carbon recycling (Cori cycle) was estimated as the difference in glucose turnover calculated with [6-^{3}H]glucose and [6-^{14}C]glucose (12).

Statistical Analyses. Data are given as mean ± SE. Paired analyses were used to assess the significance of changes at different time points; paired two-tailed tests were used to evaluate difference obtained with the different tracers. Least squares linear regression was used to assess the relationship between the percentages of plasma derived from plasma glucose obtained with [^{3}H] and [^{14}C]glucose infusions. A probability value less than 0.05 was considered to be statistically significant.

Results

The results of our experiments are summarized in Table I. Plasma glucose and plasma lactate concentrations were constant during the 30-min experimental period averaging 5.89 ± 0.46 and 1.53 ± 0.08 m*M*, respectively. Isotopic steady state was achieved for both plasma [^{14}C:^{3}H]glucose and [^{14}C:^{3}H]lactate since specific activities at 120, 135, and 150 min were not significantly different from one another.

As expected, plasma glucose turnover determined with [6-^{3}H]glucose was significantly greater than that determined with [6-^{14}C]glucose (29.4 ± 1.7 vs 25.0 ± 1.5 μmol/kg/min, $P < 0.001$). Cori cycle activity, calculated as the difference between [6-^{3}H]glucose and [6-^{14}C]glucose turnover, averaged about 15% of glucose turnover (6-^{3}H data) (4.35 ± 0.79 μmol/kg/min).

Using the ratio of [^{14}C]plasma lactate specific activity to that of [^{14}C]plasma glucose, 59.8 ± 5.8% of plasma lactate was calculated to be derived from plasma glucose; using the ratio of [^{3}H]plasma lactate specific activity to that of [^{3}H]plasma glucose, only about half as much (30.3 ± 3.3%) of plasma lactate was calculated to be derived from plasma glucose, $P < 0.001$. However, as shown in Figure 1, the percentage of plasma lactate derived from plasma glucose determined with [6-^{3}H] glucose infusion was highly correlated with that determined with infusion of [6-^{14}C]glucose over a wide range of values ($r = 0.95$, $P < 0.001$).

Discussion

This study was undertaken to determine whether transfer of tritium from [6-^{3}H]glucose into plasma lactate could be used to estimate the conversion of plasma glucose into plasma lactate. For this purpose, we infused overnight fasted rats simultaneously with [6-^{3}H]glucose and [6-^{14}C]glucose and compared the percentages of plasma lactate derived from plasma glucose with each isotope.

The rates of glucose production which we found with [6-^{3}H]glucose (29.4 ± 1.7 μmol/kg/min) and with

Table I. Plasma Glucose and Lactate Concentrations and the [^{14}C]-, ^{3}H-Specific Activities, Rates of Glucose Turnover, Cori Cycle Activity, and Percentage of Plasma Lactate Derived from Plasma Glucose (Mean ± SEM, $n = 9$)

	Time (min)			
	120	135	150	Mean
Plasma glucose (m*M*)	5.77 ± 0.50[a]	5.89 ± 0.45	6.02 ± 0.49	5.89 ± 0.46
Plasma lactate (m*M*)	1.44 ± 0.11	1.50 ± 0.09	1.64 ± 0.08	1.53 ± 0.08
[^{14}C]Plasma glucose spec activity (dpm/μmol)	50,065 ± 7,906	50,162 ± 8,016	49,151 ± 7,450	49,793 ± 7,790
[^{3}H]Plasma glucose spec activity (dpm/μmol)	49,545 ± 9,129	49,777 ± 9,306	48,601 ± 8,597	49,308 ± 8,995
[^{14}C]Plasma lactate spec activity (dpm/μmol)	14,271 ± 1,962	14,164 ± 2,008	13,719 ± 1,700	14,051 ± 1,827
[^{3}H]Plasma lactate spec activity (dpm/μmol)	6,799 ± 974	6,698 ± 918	6,434 ± 898	6,643 ± 900
[^{3}H]Plasma glucose turnover (μmol/kg/min)	29.2 ± 1.5	29.4 ± 2.0	29.5 ± 1.9	29.4 ± 1.7[b]
[^{14}C]Plasma glucose turnover (μmol/kg/min)	24.7 ± 1.2	25.1 ± 1.6	25.2 ± 1.8	25.0 ± 1.5
Cori cycle activity (μmol/kg/min)	4.47 ± 0.80	4.34 ± 0.92	4.24 ± 0.70	4.35 ± 0.79
[^{14}C]Lactate from [^{14}C]glucose (%)	60.2 ± 6.5	60.9 ± 6.8	58.3 ± 5.0	59.8 ± 5.8
[^{3}H]Lactate from [^{3}H]Glucose (%)	30.8 ± 4.1	30.9 ± 3.7	28.4 ± 2.4	30.0 ± 3.3[b]

[a] Mean ± SE ($n = 9$).
[b] $P < 0.001$ vs ^{14}C data.

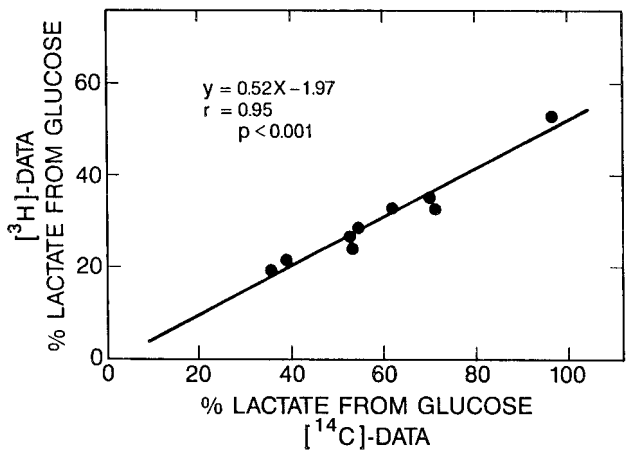

Figure 1. Relationship between percentage of plasma lactate derived from plasma glucose calculated from ratios of [^{3}H] and [^{14}C]plasma lactate/plasma glucose specific activities.

[6-^{14}C]glucose (25.1 ± 1.5 μmol/kg/min) are similar to values previously reported with these isotopes in the postabsorptive rat (13–15). Our estimation of Cori cycle activity (15%) was also comparable to values (10–29%) previously reported for the postabsorptive rat (14–18).

The percentage of plasma lactate derived from plasma glucose calculated using the ratio of the specific activities of plasma [^{14}C]lactate and [^{14}C]glucose was approximately 60% in the present study, a value in general agreement with previously reported values using a carbon-labeled glucose in the rat (17, 19, 20) and other species (20, 21) including the human (22). The percentage of plasma lactate derived from plasma glucose calculated in the present study using the specific activity ratios of plasma [^{3}H]lactate and [^{3}H]glucose was only about 30%. Consequently, the ^{3}H:^{14}C ratios of plasma lactate and plasma glucose in our study was 0.5, a value similar to the 0.6 reported by Shiota *et al.* (8) in the perfused rat hindlimb preparation and the 0.5–0.7 reported by Okajima *et al.* (7) in the 20-hr fasted rat but less than Dunn *et al.* (6) who concluded that ^{3}H:^{14}C ratios of plasma lactate and glucose were similar. However, inspection of their data indicate that the ratio was actually about 0.85. The likely explanation for the higher ratio found by Dunn *et al.* (6) is that their experiments were not performed under isotopic steady-state conditions.

Thus, the present experiments and those of other investigators (6–8) demonstrate that there is less ^{3}H than ^{14}C incorporated into lactate when [^{3}H,^{14}C]glucose is injected or infused. This indicates that the ratio of the specific activity of plasma [^{3}H]lactate to plasma glucose cannot be directly used as a quantitative estimate of the percentage of plasma lactate derived from plasma glucose.

The lower ^{3}H-specific activity of plasma relative to [^{14}C]plasma lactate specific activity in lactate probably results from selective detritiation of lactate as well as loss of ^{3}H from glucose on its way to lactate. According to Katz and Rognstad (12), tritium on position 6 of glucose is retained through its conversion to pyruvate. The tritium in pyruvate could be lost by carboxylation-decarboxylation reactions between pyruvate and dicarboxylic acids in the Krebs cycle and/or by exchange of ^{3}H among pyruvate, lactate, and alanine catalyzed by lactate dehydrogenase and by alanine aminotransferase. The rapid equilibration between lactate, alanine, and pyruvate (23) could lead to substantial loss of ^{3}H but

not that of ^{14}C. These reactions could occur in liver with lactate produced in muscle (12) and in muscle with pyruvate coming directly from plasma glucose (8, 24).

Despite the fact that the ^{3}H:^{14}C ratio of plasma lactate and glucose was less than 1 in the present study, we found that this ratio was constant over a wide range of percentages of plasma lactate derived from plasma glucose. Shiota *et al.* (8) found that ^{3}H:^{14}C ratios were not altered in the perfused rat hindlimb by insulin. It is not yet known whether ^{3}H:^{14}C ratio of 0.5 would remain constant with other hormonal or nutritional perturbations. However, if it did, one could use this constant relationship to correct for excess detritiation of lactate so that [6-^{3}H]glucose might then be used in conjunction with [^{14}C]lactate to estimate simultaneously both sides of the Cori cycle.

The [^{3}H]plasma lactate specific activity/[^{3}H] plasma glucose specific activity ratio obtained during infusion of [6-^{3}H]glucose was less than the [^{14}C]plasma glucose specific activity ratio obtained with infusion of [6-^{14}C]glucose. But the ratio of these ratios was constant over a wide range of percentages of plasma lactate derived from plasma glucose suggesting that, at least under our experimental conditions, the correction for preferential detritiation of lactate might be possible, making the use of [6-^{3}H]glucose feasible for determining the amount of plasma lactate derived from plasma glucose with this tracer.

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