**special communication**

Limitations in estimating gluconeogenesis and Cori cycling from mass isotopomer distributions using [U-\textsuperscript{13}C\textsubscript{6}]glucose

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Landau, Bernard R., John Wahren, Karin Ekberg, Stephen F. Previs, Dawei Yang, and Henri Brunengraber. Limitations in estimating gluconeogenesis and Cori cycling from mass isotopomer distributions using [U-\textsuperscript{13}C\textsubscript{6}]glucose. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E954–E961, 1998.—Tayek and Katz proposed calculating gluconeogenesis's contributions to glucose production and Cori cycling from mass isotopomer distributions in blood glucose and lactate during [U-\textsuperscript{13}C\textsubscript{6}]glucose infusion [Tayek, J. A., and J. Katz. Am. J. Physiol. 272 (Endocrinol. Metab. 35): E476–E484, 1997]. However, isotopic exchange was not adequately differentiated from dilution, nor was condensation of labeled with unlabeled triose phosphates properly equated. We introduce and apply corrected equations to data from subjects fasted for 12 and 60 h. Impossibly low contributions of gluconeogenesis to glucose production at 60 h are obtained (23–41%). Distributions in overnight-fasted normal subjects calculate to only ~18%. Cori cycling estimates are ~10–15% after overnight fasting and 20% after 60 h of fasting. There are several possible reasons for the underestimates. The contribution of gluconeogenesis is underestimated because glucose production from glycerol and amino acids not metabolized via pyruvate is ascribed to glycogenolysis. Labeled oxaloacetate and α-ketoglutarate can exchange during equilibrium with circulating unlabeled aspartate, glutamate, and glutamine. Also, the assumption that isotopomer distributions in arterial lactate and hepatic pyruvate are the same may not be fulfilled.

lactate; alanine; CO\textsubscript{2} fixation

TAYEK AND KATZ (19) introduced a novel method for estimating the contribution of gluconeogenesis to glucose production from mass isotopomer distribution (MID) values in blood glucose and lactate during [U-\textsuperscript{13}C\textsubscript{6}]glucose administration. However, calculations from observed distributions yielded overestimates (20). Two factors were calculated, one for dilution by unlabeled carbon of [\textsuperscript{13}C\textsubscript{6}]lactate formed from the blood glucose and the other for dilution in the conversion of that lactate to glucose. The product of those factors times an estimated fraction of the blood glucose carbon recycled via lactate to glucose and times glucose production was equated to the rate of gluconeogenesis (19).

Most recently, the same authors presented a new procedure for calculation (20), giving lower estimates of gluconeogenesis. Rates are calculated from the product of glucose production, the factor for the dilution by unlabeled carbon of the labeled lactate formed from the blood glucose, and an estimate of the fraction of glucose molecules recycled, the latter estimate also considered the measure of Cori cycling. However, exchanges of \textsuperscript{13}C with \textsuperscript{12}C in the process of gluconeogenesis are not adequately differentiated from dilution of \textsuperscript{13}C by \textsuperscript{12}C from unlabeled gluconeogenic precursors. Also, the condensation of labeled with unlabeled triose phosphate is not properly equated.

We now present corrected equations for calculating the contributions of gluconeogenesis and Cori cycling from MID. Applying them, we have estimated the contribution of gluconeogenesis in humans after an overnight fast and after fasting for 60 h. If the approach is adequate, the contribution at 60 h should be nearly 100% (12, 18). After an overnight fast, it would be expected to be ~40–50% (6, 11, 17). A critical assumption in the approach is that the MID in arterial blood lactate accurately measures that in pyruvate in liver and in kidney, to the extent kidney contributes to gluconeogenesis. To test that assumption, distributions in arterial blood, lactate, and alanine were compared with distributions in hepatic vein and renal vein lactate and alanine.

**METHODS**

Subjects. Seven healthy normal men, aged 26–31 yr with body mass indexes of 20.5–24.4 kg/m\textsuperscript{2}, were studied. The study was approved by the Human Investigation Committees at the Karolinska Hospital and University Hospitals of Cleveland. Informed consents were obtained.

Materials. [U-\textsuperscript{13}C\textsubscript{6}]glucose, 99% enriched, purchased from Isotec (Miamisburg, OH), was dissolved in isotonic saline and filtered through a sterile Millipore 0.22-µm porosity filter. The solution was shown to be sterile and pyrogen free by the Pharmacy Department of the Karolinska Hospital.

Procedure. At 8 AM, three of the subjects, after 60 h of fasting, were given a priming dose and then an infusion for 5 h of the solution of [U-\textsuperscript{13}C\textsubscript{6}]glucose, 9.8 ml/h, into an antecubital vein of one arm at a rate of ~0.4 µmol of [U-\textsuperscript{13}C\textsubscript{6}]glucose·kg body weight\textsuperscript{−1}·min\textsuperscript{−1}. The priming dose equaled the amount infused in 1 h. Also at 8 AM, a catheter was inserted into the brachial artery of the other arm. At 3 h into the infusion, catheters were inserted under fluoroscopic control into a hepatic vein and a renal vein via a femoral vein. The catheters were kept patent by periodic saline rinses.
Blood was drawn from the artery at 3 h into the infusion and from 4 h to 5 h every 15 min for determination of MID in plasma glucose, lactate, and alanine. Blood was drawn from the hepatic and renal veins every 15 min from 4 h to 5 h for determination of MID in lactate and alanine. Concentrations of blood glucose and β-hydroxybutyrate were determined at 4 and 5 h. Breath was collected at 4 and 5 h for the determination of 13C excess enrichment in breath CO2. Breath was collected before the beginning of [U-13C6]glucose infusion to measure the natural 13C enrichment in breath CO2.

The other four subjects were treated the same as the first three except that the 5 h of infusion of [U-13C6]glucose were begun after 12 rather than 60 h of fasting. Then blood was drawn for the determination of MID in arterial glucose and in arterial, hepatic vein, and renal vein lactate every 15 min from 16 h to 17 h into the fast. Breath was collected at 16 h and 17 h.

Analyses. Blood glucose concentrations were determined using glucose oxidase (YSI, Yellow Springs, OH). β-Hydroxybutyrate concentrations were also determined enzymatically (22). Plasma for MID analyses, collected rapidly after blood drawing, was frozen immediately and shipped to Cleveland. MID values of glucose were determined from the aldonitrile pentaacetate derivative, as described by Tayek and Katz (19), and of lactate from the pentafluorobenzyl derivative as described by Hazey et al. (7). Measured isotopomer distributions were corrected for natural 13C enrichment at all masses (5). To measure 13C enrichment of breath CO2, the CO2 was collected in NaOH, and BaCl2 was added. The rinsed and dried precipitate of BaCO3 was introduced in a vial that was flushed with CO2-free N2 before injection of H2SO4. The evolved CO2 was injected with a gas syringe into a gas chromatograph-mass spectrometer. Linear calibration curves of 13CO2 enrichments (0.1–1.5%) were obtained using NaH13CO3 standards. Measured enrichments of expired CO2 ranged from 0.7 to 1.0%.

Calculations. Rate of appearance of glucose (Ra glucose) in the circulation was calculated using the equation

\[ R_{\text{glucose}} = \frac{R \times (M_6 \text{ of infused [U-}^{13}\text{C}_6\text{]glucose})}{M_6 \text{ of arterial glucose}} \]  

(1)

where R is the rate of infusion of [U-13C6]glucose in mmol·min⁻¹·kg⁻¹, and M6 is the percentage of glucose molecules having six 13C atoms. M6 was 94% of the [U-13C6]glucose infused in the present study. Endogenous glucose production (GP) equals Ra glucose minus R. Because R is small relative to the Ra glucose, GP is a little less than Ra glucose.

D, the dilution by unlabeled lactate of labeled lactate undergoing Cori cycling, was calculated using the equation

\[ D = \frac{0.5 \times (M_1 + M_2 + M_3) + M_6}{m_1 + m_2 + m_3} \]  

(2)

where M1, M2, M3, and M6 are, respectively, the percentages of blood glucose molecules with 1, 2, 3, and 6 13C atoms, i.e., isotopomers M1, M2, M3, and M6. Correspondingly, m1, m2, m3, and m6 are the percentages for blood lactate of isotopomers m1, m2, and m3, respectively.

\[ F, \text{ the fraction of glucose molecules in the blood that recycles, was calculated using the equation} \]

\[ F = \frac{0.5 \times (M_1 + M_2 + M_3)}{0.5 \times (M_1 + M_2 + M_3) + M_6} \]  

(3)

The rate of gluconeogenesis (GNG) equals Ra glucose × D × F.

The percent contribution of GNG (%GNG) to glucose production (GP) equals 100(GNG/GP). The amount of blood glucose undergoing Cori cycling equals (Ra glucose) × F and the percent 100F.

Equations 2 and 3 are applicable only when the rate of glucose infused is small relative to GP. Thus, in the present study, [U-13C6]glucose was infused at only ~4% of the rate of GP. That resulted in relatively low enrichments and with negligible formation of M4 and M5 as well as M6 isotopomers. Small quantities of [U-13C6]glucose must be infused to avoid altering metabolism, even if the cost of [U-13C6]glucose allowed giving quantities to achieve high enrichments (19).

 Correction of M1, to the extent of its formation via fixation by pyruvate of 13CO2 formed from the [U-13C6]glucose, can be made by infusing NaH13CO3 or NaH14CO3 under the same study conditions. Measurements are made of the enrichments or specific activities of blood glucose and breath CO2, combined with measurement of the enrichment of breath CO2 from the [U-13C6]glucose. Thus

\[ [U-13\text{C}_6\text{]}\text{CO}_2 \text{ from [U = 13C]glucose fixed in glucose} = \frac{\text{13CO}_2 \text{ from NaH13CO}_3 \text{ fixed in glucose}}{\text{13CO}_2 \text{ in breath from NaH14CO}_3} \]

The only unknown is then the enrichment of 13CO2 from (U-13C6)glucose fixed in glucose.

Contributions of fixation can also be estimated from just the enrichment of breath 13CO2 labeled from (U-13C6)glucose. For example, assume that 13CO2 in breath from [U-13C6]glucose was 1% enriched in a 64-h-fasted subject in whom GNG is responsible for 100% of GP. Also, assume that this is the enrichment of the CO2 fixed by pyruvate in liver and that there is complete isotopic equilibration with fumarate of the oxaloacetate formed before its conversion to glucose. Then the carboxyls of the oxaloacetate and carbon 3 and carbon 4 of blood glucose would each have 0.5% enrichment, giving a ratio of the enrichment in glucose to that in breath of (2 × 0.5)/1 = 1.0. Therefore, if M1 determined by MID was 2%, the corrected M1 would be 1%.

We infused both NaH13CO3 and NaH14CO3 into normal subjects fasted 60 h (4, 9). The ratio of the enrichments and specific activities of blood glucose to breath CO2 was ~0.65. The ratio being lower than 1.0 is due in large part to incomplete equilibration of the oxaloacetate and the contribution of glycerol to GP. Therefore, the correction to M1 has been estimated in the present study by multiplying the enrichment in the breath CO2 by 0.65. To measure the contribution of 13CO2 fixation to m6, 13CO2 or 14CO2 from carbon-labeled bicarbonate fixed in lactate would have had to be measured.

RESULTS

Arterial glucose concentration of the first three subjects at 65 h into the fast was 3.5 mM in subject 1, 3.4 mM in subject 2, and 2.4 mM in subject 3 and was about the same at 64 h into the fast. Concentrations of β-hydroxybutyrate were, respectively, 2.7, 3.8, and 2.9 mM at 65 h and slightly lower at 64 h into the fast. These concentrations are in accord with the subjects having fasted for 65 h.

The percentage of isotopomer M6 in the MID in glucose did not change between 64 h and 65 h (Table 1). There is a suggestion of a gradual increase in M1, M2, and M3 between 64 h and 65 h. This is particularly
Table 1. Percent mass isotopomer distributions in arterial blood glucose and lactate from normal subjects fasted 65 h and infused with [U-13C6]glucose beginning at 60 h into fast

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Time</th>
<th>Glucose</th>
<th>Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>h</td>
<td>M1</td>
<td>M2</td>
</tr>
<tr>
<td>1</td>
<td>63</td>
<td>0.57</td>
<td>0.41</td>
</tr>
<tr>
<td>2</td>
<td>64</td>
<td>0.66</td>
<td>0.61</td>
</tr>
<tr>
<td>3</td>
<td>64.25</td>
<td>0.77</td>
<td>0.61</td>
</tr>
<tr>
<td>4</td>
<td>64.5</td>
<td>0.72</td>
<td>0.67</td>
</tr>
<tr>
<td>5</td>
<td>64.75</td>
<td>0.84</td>
<td>0.65</td>
</tr>
<tr>
<td>6</td>
<td>65</td>
<td>0.90</td>
<td>0.73</td>
</tr>
</tbody>
</table>

Mean 0.78 0.65 0.73 3.84 0.46 0.33 2.01

Table 2. Rates of GNG, GP, and CC and %contribution of GNG to R₇ of glucose from 4 to 5 h of [U-13C6]glucose infusion

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Ra Glucoseb</th>
<th>Dc (Eq. 2)</th>
<th>Fd (Eq. 3)</th>
<th>GPf (bd)</th>
<th>CCf (bd)</th>
<th>%GNGf (100[e/f])</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.416</td>
<td>9.99</td>
<td>1.76</td>
<td>0.220</td>
<td>3.87</td>
<td>9.58</td>
</tr>
<tr>
<td>2</td>
<td>0.427</td>
<td>10.42</td>
<td>1.38</td>
<td>0.163</td>
<td>2.34</td>
<td>10.00</td>
</tr>
<tr>
<td>3</td>
<td>0.400</td>
<td>9.08</td>
<td>1.73</td>
<td>0.193</td>
<td>3.03</td>
<td>8.68</td>
</tr>
</tbody>
</table>

Rates of gluconeogenesis (GNG), endogenous glucose production (GP), and Cori cycling (CC) are in µmol·min⁻¹·kg⁻¹. Percent contribution of GNG to rate of appearance (R₇) of glucose (%GNG) was calculated from mean mass isotopomer distributions in arterial blood glucose and lactate (see Table 1). R, rate of glucose infusion; D, dilution by unlabeled lactate of labeled lactate undergoing CC; F, fraction of glucose molecules in blood that recycled. Superscript letters refer to calculations that are indicated by letters or eq. nos. in parentheses.

Table 3. % Excess in ¹³C in CO₂ from breath from the subjects of Table 1, multiplied by 0.65

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Time</th>
<th>%Excess ¹³C breath CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>64 h</td>
<td>0.45 (0.66)</td>
</tr>
<tr>
<td>1</td>
<td>65 h</td>
<td>0.58 (0.90)</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.68 (0.49)</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.51 (0.71)</td>
</tr>
</tbody>
</table>

Values within parentheses are M₁ in glucose at 64 h and 65 h from Table 1.

undergoing Cori cycling, D, ~1.5-fold. About 20% of the R₇ of glucose was cycled. The percentage of GP contributed by GNG ranged from 23.4 to 40.4%.

DISCUSSION

Percent GNG, as proposed for calculation most recently by Tayek and Katz (20), derives from two equations, Eqs. 4 and 5

{M₁ + 2M₂ + 3M₃ + 6M₆} / 2(m₁ + 2m₂ + 3m₃)  (4)

Percent contribution of GNG to GP of ~50% (11), again, most of the M₁ appears to have arisen by ¹³CO₂ fixation. The fraction of glucose molecules in the blood that recycled (Eq. 3) was 15.1 ± 1.7%. Enrichments in lactate from arterial, hepatic vein, and renal vein blood were similar (Table 6).
cose has only one $^{13}$C atom. One molecule of glucose was

\[\text{Fraction of recycled glucose molecules} = \frac{M_1 + M_2 + M_3}{M_1 + M_2 + M_3 + M_6} \tag{5}\]

Note that, in Eq. 4, the percentages of mass isotopomers are weighted for the number of their $^{13}$C atoms. The product of those equations times 100 is then equated to percentage Cori cycling (20). Introducing the distributions of isotopomers in Table 1 into Eqs. 4 and 5 yields percent contributions of GNG of 68.8, 43.0, and 62.0%, respectively, rather than 40.4, 23.4, and 34.9% (Table 2). In addition, Cori cycling would range from 28 to 36% rather than from 16 to 22% (Table 2).

Before examination of the reasons why Eqs. 4 and 5 give higher estimates than Eqs. 2 and 3, the difference between isotopic exchange and dilution requires further emphasis (10). Assume that two molecules of lactate, each with three atoms of $^{13}$C, are converted to glucose. Assume that in the conversion each undergoes exchange of $^{13}$C for $^{12}$C in the tricarboxylic acid (TCA) cycle, so that each triose phosphate converted to glucose has only one $^{13}$C atom. One molecule of glucose was formed with one-third of its atoms being $^{13}$C. The same amount of glucose would have been formed if there had been no exchange. Assume instead that the two molecules of $[^{13}$C]lactate are diluted by four unlabeled lactate molecules, and then the six lactates are converted to glucose without exchange. Of the 18 carbons of glucose formed, one-third will again have $^{13}$C, but the net synthesis will be three molecules of glucose. Thus dilution, not exchange, results in a net increase in GNG.

Because Eq. 4 includes isotopomer percentages weighted for $^{13}$C atoms, “dilution” includes both exchange and dilution. Equation 2 removes the contribution of exchange by treating each labeled triose unit in lactate and glucose, no matter how many of its carbons are labeled with $^{13}$C, as having all three carbons labeled. In the calculation of GNG originally proposed by Tayek and Katz (19), the other dilution factor, now Eq. 6 in Ref. 20, was for dilution of pyruvate in the TCA cycle in its conversion to glucose and was set equal to 3 $(M_1 + M_2 + M_3)/(M_1 + M_2 + M_3)$. Because that factor is only more than 1.0 to the extent of exchange (20), its use (19) contributed to the overestimations of GNG.

In Eqs. 2 and 3, the factor 0.5 is required because one-half of the triose units forming the glucose molecules of masses M1, M2, and M3 are unlabeled and are not derived from $[^{13}$C$_6]$glucose. The equations must represent the dilution and fraction only of the $[^{13}$C$_6]$glucose, not the unlabeled glucose formed endogenously.

Multiplying Eq. 2 by Eq. 3, i.e., $D \times F$, yields the contribution of GNG to the $R_g$ glucose.

\[\text{Fractional gluconeogenesis contribution} = \frac{M_1 + M_2 + M_3}{2(m_1 + m_2 + m_3)} \tag{6}\]

Equation 6 provides evidence for the correctness of Eqs. 2 and 3, because their product, if correct, must in theory yield Eq. 6. That is because the estimation of the contribution of GNG by MID is analogous to measuring the ratio of the specific activity or enrichment in arterial blood glucose to that in arterial blood lactate on giving labeled lactate. At steady state, with the assumption that blood lactate specific activity or enrichment is that of intrahepatic pyruvate, if the ratio is 1.0, all blood glucose is formed by GNG. To the extent the ratio is less, the contribution of GNG is less. The need to use isotopomer analysis for quantitation rather than specific activities or enrichments is that the contributions

### Table 4. % Mass isotopomer distributions in artery, hepatic vein, and renal vein lactate from subjects of Table 1

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Compound</th>
<th>Artery</th>
<th>Hepatic Vein</th>
<th>Renal Vein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m$_1$</td>
<td>m$_2$</td>
<td>m$_3$</td>
<td>m$_4$</td>
</tr>
<tr>
<td>1</td>
<td>Lactate</td>
<td>0.46 ± 0.02</td>
<td>0.33 ± 0.01</td>
<td>2.01 ± 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.47 ± 0.01</td>
<td>0.36 ± 0.01</td>
<td>1.98 ± 0.05</td>
</tr>
<tr>
<td>2</td>
<td>Lactate</td>
<td>0.50 ± 0.02</td>
<td>0.39 ± 0.01</td>
<td>2.37 ± 0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.51 ± 0.01</td>
<td>0.46 ± 0.04</td>
<td>2.29 ± 0.07</td>
</tr>
<tr>
<td>3</td>
<td>Lactate</td>
<td>0.54 ± 0.06</td>
<td>0.35 ± 0.04</td>
<td>2.02 ± 0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.57 ± 0.02</td>
<td>0.38 ± 0.01</td>
<td>2.05 ± 0.08</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5 determinations between 16 h and 17 h of fast.

### Table 5. % Mass isotopomer distributions in arterial blood glucose and lactate from normal subjects fasted for 17 h and infused with $[^{13}$C$_6]$glucose beginning at 12 h

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Glucose</th>
<th>Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m$_1$</td>
<td>m$_2$</td>
</tr>
<tr>
<td>1</td>
<td>0.17 ± 0.05</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>2</td>
<td>0.41 ± 0.02</td>
<td>0.30 ± 0.01</td>
</tr>
<tr>
<td>3</td>
<td>0.43 ± 0.02</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>4</td>
<td>0.40 ± 0.03</td>
<td>0.28 ± 0.02</td>
</tr>
</tbody>
</table>
Fig. 1. Flow diagram of distribution of mass isotopomers of glucose and lactate during infusion of [U-\(^{13}\)C\(_6\)]glucose. Gluconeogenesis and glycogenolysis occur simultaneously with 20% Cori cycling and no \(^{13}\)C exchange. No. of molecules is represented for glucose by M and for lactate by m, with no. of their \(^{13}\)C atoms indicated. Percentages of molecules with those numbers of \(^{13}\)C atoms are recorded for blood glucose and lactate. Glucose-6-P, glucose 6-phosphate.

Fig. 2. Conditions of Fig. 1 are modified: no glycogenolysis, 10% Cori cycling, and \(^{13}\)C exchange in the conversion of blood glucose to lactate. Notations as in Fig. 1.
equals 1,122 molecules/unit time, Cori cycling is 10%, and the contribution of GNG to GP is 100%. If conditions are altered, so that the exchange occurred in the conversion of lactate to glucose, i.e., in the TCA cycle rather than in the conversion of glucose to lactate, the percentage of mass isotopomers \( m_1 \) and \( m_3 \) would be different, i.e., \( m_1 = 1.17/2,244 = 0.052% \) and \( m_3 = 21.05/2,244 = 0.938% \). But because \( m_1 \) and \( m_3 \), as well as \( m_1 \) and \( m_3 \), are treated the same in the equations, correct results are still obtained, i.e., \( m_1 + m_3 = 1.82% \) and \( m_1 + m_3 = 0.99% \) in either case. When Eqs. 4 and 5 are applied to the conditions of Fig. 2, GNG is overestimated at 161% and Cori cycling at 18.2%.1

Figure 3 also depicts a prolonged fast when all GP is by GNG, and Cori cycling is 10%. As in Fig. 1, movement is only in three and six \(^{13}\)C units. However, per unit of time, of the 13.34 molecules of lactate from blood glucose mixing with 2,000 molecules of unlabeled lactate, 1,000, rather than being converted to glucose, return to the tissues, producing unlabeled lactate. This is also an exchange (10) that must be considered, because the equilibration between blood lactate and tissue pyruvate is extensive. The result in the example is the net production of 566.67 molecules of glucose from 1,334.34 molecules of lactate per unit time.

From Eqs. 2 and 3, \( D = 15.84/0.99 = 16.0 \), and \( F = 0.84/15.84 = 0.053 \). The rate of GNG is then 666.67 \( \times 16 \times 0.53 = 666.67 \), and therefore the %contribution of GNG to GP still calculates to 100%. However, the introduction of the pyruvate \( \rightarrow \) lactate exchange results in an underestimate of Cori cycling, 5.3% rather than 10%. Therefore, the Cori cycling estimates in Table 2 should be considered underestimates, to the extent labeled lactate formed in the cycling exchanges with unlabeled lactate before conversion to glucose.

Exchanges in the conversion of glucose to lactate and lactate to glucose, resulting in the formation of \( m_0 \) from \( m_3 \), are assumed negligible. This is justified because \( m_3 \) is \( \gg m_1 \), with correction for the contribution of \(^{13}\)CO\(_2\) fixation (Table 3), and \( m_3 \gg m_1 \). The formation of \( m_0 \) from \( m_3 \) should be less than the formation of \( m_1 \) from \( m_3 \). That \( m_1 \) is much less than \( m_2 \) and \( m_3 \) is to be expected, because other than by \(^{13}\)CO\(_2\) fixation, M1 can only be formed via the pentose cycle and by label from \([^{13}\text{C}]\text{lactate}\) after experiencing a turn of the TCA cycle.

In normal subjects fasted overnight and infused with \([^{13}\text{C}]\text{glucose}\) for 3 h (19) and 4 h (20), from the reported data applying Eq. 6, percent contribution of GNG to GP is 18.5 \pm 1.3% (\( n = 14 \)). From the data in Table 5, applying Eq. 4, the percent contribution calculates to 17.3 \pm 2.5% (\( n = 4 \)). That is one-half or less than in other quantifications (6, 11, 17, 21). After 60 h of fasting, GNG calculates to a contribution of 41% or less (Table 2). At 60 h, \(~85%\) would have been expected, allowing for a 10% contribution by glycerol (11, 12) and a 5% contribution by glycogenolysis (20).

There are several reasons why the correct equations give underestimates. Glycerol’s conversion to glucose is included in glycogenolysis rather than GNG. The conversion of amino acids to glucose without pyruvate as an intermediate also results in an underestimation of GNG and an equivalent overestimation of glycogenolysis. Thus the conversion to glucose of aspartate \([\text{via oxaloacetate} \rightarrow \text{phosphoenolpyruvate (PEP)}]\) and of glutamine and glutamate \([\text{via} \alpha\text{-ketoglutarate} \rightarrow \text{oxaloacetate} \rightarrow \text{PEP}\) would calculate as glycogenolysis, except to the extent of PEP cycling, i.e., PEP \( \rightarrow \) pyruvate \( \rightarrow \) oxaloacetate \( \rightarrow \) PEP (16). For PEP cycling into pyruvate to be included in the estimate of the contribution of GNG, the pyruvate would have to equilibrate.

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1 Tayek and Katz, who concluded in calculating gluconeogenesis (19) to provide an adequate approximation when recycling is low (20), doubled the product of the fraction of glucose carbon recycled and the dilution by unlabeled carbon. However, the fraction of glucose recycled times the extent of its dilution equals the fraction of glucose production by gluconeogenesis, not one-half that fraction. When this is combined with the use of the “dilution via TCA cycle” factor, a large overestimation of gluconeogenesis results. The overestimation is reduced by 1) use of an equation for calculating the recycling of glucose carbon, Eq. 3 of Ref. 19 and Eq. 2 of Ref. 20, with weighted isotopomers (so, for example, using that equation, the scheme of Fig. 2 would give a fraction of 0.067 and not 0.100), 2) the extent blood lactate is not the measure of intrahepatic pyruvate, and 3) the extent gluconeogenic substrates are converted to glucose without pyruvate as an intermediate (see DISSCUSSION). Estimates of glucose recycling from differences in \( R_\text{glucose} \) measured with irreversible tracers, e.g., \([3-\text{H}]\text{glucose} \) and \([6-\text{H}]\text{glucose}\), and reversible tracers, e.g., \([^{13}\text{C}]\text{glucose}\) and \([^{13}\text{C}]\text{glucose}\), also expressed in Eq. 3 of Ref. 19 and Eq. 3 of Ref. 20, suffer again from the failure to exclude exchange and to include unlabeled triose phosphate with labeled triose phosphate as having been cycled.
with blood lactate. Labeled oxaloacetate and α-ketoglutarate exchanges during equilibrium with circulating unlabeled aspartate, glutamate, and glutamine also result in underestimations (3, 16). Other non-MID analysis methods for estimating GNG by labeled pyruvate using 13C- or 14C-labeled tracers have the same limitations.

Also, the MID values in blood lactate may not reflect adequately those in intrahepatic and intrarenal pyruvate (to the extent that kidney contributes to GNG). Although there is evidence for an adequate reflection, at least under certain conditions (8, 23), there is also evidence to the contrary (2, 3, 13). Similar enrichments of mass isotopomers when enrichments are low (1, 14), and our evidence (To the extent that kidney contributes to GNG). Although there is evidence for an adequate reflection, at least under certain conditions (8, 23), there is also evidence to the contrary (2, 3, 13). Similar enrichments of mass isotopomers when enrichments are low (1, 14), and our evidence (To the extent that kidney contributes to GNG). Although there is evidence for an adequate reflection, at least under certain conditions (8, 23), there is also evidence to the contrary (2, 3, 13). Similar enrichments of mass isotopomers when enrichments are low (1, 14), and our evidence (To the extent that kidney contributes to GNG). Although there is evidence for an adequate reflection, at least under certain conditions (8, 23), there is also evidence to the contrary (2, 3, 13). Similar enrichments of mass isotopomers when enrichments are low (1, 14), and our evidence (To the extent that kidney contributes to GNG). Although there is evidence for an adequate reflection, at least under certain conditions (8, 23), there is also evidence to the contrary (2, 3, 13). Similar enrichments of mass isotopomers when enrichments are low (1, 14), and our evidence (To the extent that kidney contributes to GNG). Although there is evidence for an adequate reflection, at least under certain conditions (8, 23), there is also evidence to the contrary (2, 3, 13). Similar enrichments of mass isotopomers when enrichments are low (1, 14), and our evidence (To the extent that kidney contributes to GNG). Although there is evidence for an adequate reflection, at least under certain conditions (8, 23), there is also evidence to the contrary (2, 3, 13). Similar enrichments of mass isotopomers when enrichments are low (1, 14), and our evidence (To the extent that kidney contributes to GNG). Although there is evidence for an adequate reflection, at least under certain conditions (8, 23), there is also evidence to the contrary (2, 3, 13). Similar enrichments of mass isotopomers when enrichments are low (1, 14), and our evidence (To the extent that kidney contributes to GNG). Although there is evidence for an adequate reflection, at least under certain conditions (8, 23), there is also evidence to the contrary (2, 3, 13).

If the correct equation is used, estimates might be more reasonable were it not for systematic bias in mass spectral analysis. Thus, when methods for analyzing spectral data are used, there can be errors in estimates of mass isotopomers when enrichments are low (1, 14), as encountered here in M3, M2, m2, and m3. The optimal approach to eliminating systematic bias is yet to be achieved (1). Despite giving prime and injecting [U-13C6]glucose doses for 5 h, labeling from lactate, as reflected in isotopomer M3, still had not reached steady state. That is so even when we consider that the contribution of GNG increases with the duration of fasting (11). The failure to achieve steady state presumably reflects the time needed for [13C]lactate to equilibrate with unlabeled lactate/pyruvate and recycle into glucose (15).

In conclusion, when correctly calculated, estimates of the contributions of GNG to GP and Cori cycling from isotopomer distributions in blood glucose and lactate on [U-13C6]glucose administration are underestimated. The reasons for that are several.

**NOTE ADDED IN PROOF**

Data were recently reported from experiments in which [U-13C6]glucose was infused into fasted pigs (Wykes, L. J., P. J. Reeds. Gluconeogenesis measured with [U-13C6]glucose and mass isotopomer analysis of apoB-100 amino acids in pigs. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E365–E376, 1998). The authors calculated by several methods the contribution of gluconeogenesis to glucose production 20–22 h into the fast. Values calculated by using the equation of Tayek and Katz (20) were consistently >100%. With the use of the data of Wykes et al. (Table 1 in Wykes et al.) and our Eq. 6, the contribution calculates to 59%.

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