Measurement of gluconeogenesis and mass isotopomer analysis based on \([U-^{13}C]\)glucose

Jerry Radziuk and W.-N. Paul Lee

The Ottawa Hospital and the University of Ottawa, Ottawa, Canada K1Y 4E9; and Department of Pediatrics, Harbor-University of California Los Angeles Medical Center, Torrance, California 90502

Radziuk, Jerry, and W.-N. Paul Lee. Measurement of gluconeogenesis and mass isotopomer analysis based on \([U-^{13}C]\)glucose. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E199–E207, 1999.—Two methods of measuring rates of gluconeogenesis based on label redistribution after the introduction of \([U-^{13}C]\)glucose into the whole body are examined. These methods are compared with methods previously derived for carbon-14 tracers. It is shown that the three approaches (stoichiometric, dilution, and combinatorial) are equivalent, provided the same set of assumptions are used. Barring a factor of two [see Am. J. Physiol. 270 (Endocrinol. Metab. 33): E709–E717, 1996], the differences (10–15\%) in the carbon-based dilutional and the molecule-based estimates of the rate of gluconeogenesis from published isotopomer data likely arise from small differences in the assumptions that concern the relative rate of label loss from the different isotopomers. The production of unlabeled substrate for glucose synthesis (phosphoenolpyruvate) from the different isotopomers of lactate is shown to be a potential source of error in these methods. This error is estimated using models of the interaction of the gluconeogenetic pathway and the tricarboxylic acid (TCA) cycle and is shown to vary from negligible to 30\% depending on the relative flux of the two pathways through the oxaloacetate pool. Because the estimates obtained by both methods considered are lower than is physiologically expected, some of the assumptions made may not hold. Future work will exploit the rich information content of isotopomer data to yield improved estimates.

isotopomers; tracers; turnover; tricarboxylic acid cycle; mathematical models

The measurement of the gluconeogenic component of glucose production has been the goal of much developmental effort. Briefly, gluconeogenesis is the conversion of nonglucose substrate to glucose. A maximal rate of net gluconeogenesis can therefore be estimated from the uptake of all possible substrates by the liver (let us consider only this organ for the sake of simplicity) (1, 20, 28). An alternative, direct measurement is the simultaneous determination of glycogenolysis [by biopsy or NMR measurements (19, 25)] and the rate of total glucose production, gluconeogenesis being the difference. Clearly, these more direct methods cannot be frequently implemented in humans because of the degree of invasiveness or expense involved. Indirect measures of gluconeogenesis have therefore been sought. These have almost always involved the use of tracers, or isotopically labeled substrate and glucose. The basis of those estimates is the dilution principle in the measurement of total hepatic glucose production (HGP) and the transfer of label from substrate to the product, glucose. The appearance of label in glucose, corrected for the substrate specific activity or enrichment, yields an index of gluconeogenic activity. For such an index to be an exact measure, the substrate in question would have to be the only substrate or be in complete equilibrium with all substrates so that its specific activity or enrichment would be representative of that of all the substrates. In addition, there should be no loss of label either by dilution or exchange along the conversion pathway.

Clearly, these assumptions do not hold in general. Lactate, perhaps the predominant substrate, is not in complete equilibrium even with alanine (3), for example. Moreover, it was recognized (12) that carbons involved in the gluconeogenic flux can exchange with those originating in acetyl-CoA without any net synthesis from fatty acids. Early estimates of the degree of incorporation of fatty acid carbon into glucose (29) were
initially applied as a correction for the loss of labeled carbon in the exchange with tricarboxylic acid (TCA) cycle carbon (6).

Such exchanges with labeled CO₂ (22), acetate (2), and water (4, 13, 21), furthermore, have been used as markers or probes of the gluconeogenic process at various points along its pathway. Again, assumptions must be made about the completeness of the equilibration, i.e., the rate of the exchange process relative to the flux to glucose and whether any spurious labeling can take place, such as labeling with no net flux to glucose. Methods in the context of incomplete equilibration have also been developed (18). This very brief overview has been provided to emphasize the importance of the assumptions that underlie any of the methods proposed.

These assumptions are also involved in specific ways in the development of the isotopomer-based methods discussed here. Isotopomers can be defined as compounds that are chemically identical but that differ in the degree of isotopic substitution. Isotopomers may be characterized by their mass (M₁, M₂, ... or M₀, M₁, M₂,...) or by the position at which different isotopes (¹³C, ²H) are substituted (positional isotopomers). The distribution of glucose mass isotopomers after the introduction of [U-¹³C]glucose in vivo has been proposed as the basis of methods to estimate fractional gluconeogenesis (or the rate of gluconeogenesis if the total rate of glucose production is known) (8, 14, 16, 26, 27, 30). This is based on the premise that this distribution, along with that of lactate, contains the necessary information both on the dilution of substrate molecules by unlabeled substrate and on the dilution of labeled carbons by ¹²C. Depending on the approach taken, this information is used to estimate these dilution factors (26) or to obviate the necessity of this calculation (14).

APPROACHES AND ASSUMPTIONS

The two recently published methods under consideration here (14, 26) are based on the same measurements and therefore depend on the same assumptions. It is useful to list these assumptions explicitly:

1) The ¹³C distribution in lactate is representative of that in intrahepatic pyruvate, which is the direct substrate for the gluconeogenic process. This implies either that lactate is the principal substrate, and therefore its labeling pattern essentially determines that of the pyruvate, or that hepatic pyruvate and circulating lactate are in rapid equilibrium so that their labeling patterns are identical.

2) Flux through pyruvate dehydrogenase is negligible so that the TCA cycle dilution of labeled carbons is exclusively with ¹³C arising from acetyl-CoA.

3) Pyruvate kinase flux can also be neglected.

4) No other exchanges of ¹³C occur to a measurable degree. The possible impact of ¹³C incorporation from circulating ¹³CO₂/¹³H is, however, discussed in Ref. 14.

5) The tracer concentration is low enough that the probability of combinations of two labeled molecules is negligibly low.

The contributions of glycerol are neglected for the purposes of this calculation. It is also implicitly assumed that all of the corrections for background glucose (and derivative) enrichments and for tracer contamination with nonuniformly labeled glucose molecules have been made.

When it is the carbon atoms that are labeled in the glucose molecule, glycolysis and subsequent resynthesis of lactate or pyruvate to glucose induce a redistribution or randomization of the labeled carbon (¹⁴C or ¹³C). It is therefore distinguishable from the original label. For [¹-¹³C]glucose, for example, the recycled ¹³C is found in all the other positions, although primarily in positions 1, 2, 5, and 6. The amount of recycled label can thus be quantitated (24), and its rate of appearance in the glucose molecule can be used as an index of gluconeogenesis (23). Identical considerations hold for [¹⁴C]glucose. The recycled molecule will be labeled as M₁, M₂, and M₃, a measure that is somewhat more quantitative than the radioactive label because with small amounts of tracer no M₀ glucose will be reformed. The rate of appearance of these isotopomers can then also be used as an indicator of gluconeogenesis. It may be instructive to first derive an estimate of the gluconeogenic rate based on the ¹⁴C label.

Let us define the following quantities:

- Rₐ: Rate of appearance of glucose
- Rₜ*: Rate of infusion of tracer ([¹-¹³C]glucose)
- Rₜ⁺: Rate of appearance of recycled [¹⁴C]glucose
- C*: Plasma concentration of [¹-¹³C]glucose
- C: Plasma concentration of [¹⁴C]glucose
- Cᵃ: Specific activity of infused label in plasma, C/Cᵣ:

\[ Rₐ = Rₜ⁺/Cᵣ \]
\[ Rₜ⁺ = Rₜ⁺C/C⁺ \]

The reason that the rate of appearance of glucose corresponds to Eq. 1 and that of recycled label to Eq. 2 is that Rₜ⁺/Cᵣ defines a steady-state measure of the metabolic clearance of glucose (MCR, ml/min or ml·kg⁻¹·min⁻¹), which then clearly applies to both glucose and labeled glucose. This can be formalized as

\[ Rₐ = MCR·C \] and

\[ Rₜ⁺ = MCR·C⁺ \]

The applicability of MCR to either glucose or tracer is inherent in the linearity property of tracers. Any tag that is attached to glucose will be cleared in the same way as glucose so long as the tag itself does not affect the clearance mechanisms. Thus, barring isotope effects at the level of enzymes, [¹⁴C]- or [¹³C]glucose will be removed (proportionally) at the same rate as [¹²C]glucose, but 3-O-methylglucose might not. It does not take any extrapolation to conclude that each molecule of glucose, as well as each carbon within the glucose molecule, will be cleared at the same MCR.
[1-14C]glucose is a good example of a tracer where the measurement of the label concentration, C^+ (dpm/ml), yields what could either be considered a carbon or a molecular approach. In the terminology of mass spectrometry, each labeled glucose molecule has one carbon labeled originally at the first position, and therefore corresponds to an M + 2 (because 14C has two additional mass units). Clearly, with the very low enrichments in 14C, any metabolic process that recycles the label back to glucose will also yield an M + 2 molecule. If the label is lost (e.g., in exchanges), only an M + 0 molecule will remain. Therefore, we have a situation in which only M + 2 and M + 0 molecules will exist. If one considers carbons, C^-/C = (1)M_2, where C^-/C is the specific activity, and M_2 is the enrichment in M + 2 or 14C-labeled molecules, and there is only one 14C per molecule. If one considers molecules, then C^-/C = M_2, which is identical to the above. CM_2 can therefore be substituted for C^- in the equations given above. For 14C, M_2 is extremely low, not easily detectable by use of mass spectrometry, but C^- or CM_2 can be measured using liquid scintillation counting.

To convert R_a into a rate of gluconeogenesis, the immediate precursor of the glucose molecule needs to be considered. If, as assumed here, lactate is well equilibrated with hepatic pyruvate, it is the nearest measured representative of such a precursor, and dilution of label at this level (e.g., peripheral glycogenolysis) can be taken into account. Each labeled molecule incorporated into glucose (recycled molecule) is now representative of a certain number of unlabeled pyruvate (lactate) molecules. By the same token, each labeled carbon is representative of a certain number of unlabeled carbons. Therefore, under steady-state conditions, dividing the rate of incorporation of labeled carbons into glucose (R_a) by this ratio of labeled to unlabeled molecules (or carbons) would yield the mass rate of appearance of new glucose. The (net) stoichiometry of lactate and lactate tracer conversion to glucose is as follows:

\[
2 \cdot \text{lactate} \rightarrow 1 \cdot \text{glucose}
\]

If this reaction is considered from the point of view of the simplest chemical kinetics, then the equilibrium constant, K, for the reaction is defined by

\[
\text{glucose} = K \cdot \text{lactate}^2
\]  

(i)

Now consider a perturbation of the system in which a (very) small amount of tracer lactate is added. This will have the effect of producing a small amount of additional glucose, which will also be labeled. By the definition of a tracer, it is distinguishable from the perspective of measurement but indistinguishable in terms of its physical, chemical, or metabolic behavior. Equation i can therefore be rewritten for the new concentrations of glucose and lactate in the system as

\[
[\text{glucose} + \text{glucose}^*] = K \cdot [\text{lactate} + \text{lactate}^*]^2
\]  

(ii)

Because the volume of distribution of the glucose and tracer is the same, we can also write

\[
[\text{glucose} + \text{glucose}^*] = [\text{glucose}] + [\text{glucose}^*]
\]

and

\[
[\text{lactate} + \text{lactate}^*] = [\text{lactate}] + [\text{lactate}^*]
\]

It follows then, from the chemical and physical equivalence of metabolite and tracer, that Eq. ii can be rewritten as

\[
[\text{glucose}] + [\text{glucose}^*] = K \cdot ([\text{lactate}] + [\text{lactate}^*])^2
\]  

(iii)

where [glucose^*] and [lactate^*] are the concentrations of label. Expanding, subtracting Eq. i and dropping the term in [lactate]^2, because it is small, will yield the following

\[
[\text{glucose}^*] = 2K[\text{lactate}][\text{lactate}^*]
\]  

(iv)

Dividing Eq. iv by Eq. i then gives

\[
[\text{glucose}^*] = 2 \frac{[\text{lactate}^*]}{[\text{glucose}]}
\]

This implies that a = 2a_l (where a_l is the measured molar specific activity of lactate), if no dilution takes place. This is equivalent to the probabilistic expression of this issue: because we have a mixture of m_0 and m_2, we must consider the number of possible ways in which these two molecules can combine. The probability of each combination can be determined from the binomial expansion (5, 11)

\[
(m_0 + m_2)^2 = m_0^2 + 2m_0m_2 + m_2^2
\]

where

\[
M_0 = m_0^2
\]

\[
M_2 = 2m_0m_2
\]

\[
M_4 = m_2^2
\]

Because m_0 is essentially 1, M_2 is simply equal to 2m_2. Thus, because two molecules of lactate condense to form one molecule of glucose, but only one lactate label has a detectable probability of entering the glucose molecule, we finally have

\[
R_a(gng) = \frac{R_a^+C^+}{C^+ \cdot 2a_l}
\]

(3)

where R_a(gng) is the rate of gluconeogenesis. Again, the factor two is included in the denominator because the combination of two lactate molecules leads to one glucose molecule, and therefore the molar specific activity of glucose (with respect to 14C) will be double that of lactate.

Finally, to account for TCA cycle dilution, an appropriate correction factor, F, should be used (6, 7, 10, 17). Often this is extrapolated from another experiment.
The final formula is therefore

$$R_a(gng) = FR_a^o C^+/(2C^+ a_l) \quad (4)$$

An analysis very similar to this one was used to estimate glycogen synthesis by the gluconeogenic pathway in humans (23).

**[U-13C]GLUCOSE: TRACKING CARBONS**

The development proposed by Tayek and Katz (26) also stems from a previous analysis to assess this “indirect” pathway of glycogen synthesis (9). In these studies (9, 26), M6 glucose was infused. Converting the molar infusion rate to labeled carbons, we have

$$R_a = \frac{R \cdot 6}{M_6 \cdot 6} \quad (1')$$

where R is the rate of infusion of [U-13C]glucose in millimoles per minute, and M6 is the measured enrichment of plasma glucose in this isotopomer. Equation 1’ is thus (for small enrichments) exactly equivalent to Eq. 1, with 6 × M6 equivalent to a. It should be noted that because the infusions of tracer are not massless, the calculated Ra should be corrected for this rate of infusion

$$R_a \to R_a (1/M_6 - 1)$$

The next step involves estimating the fraction of newly produced glucose that is recycled. This can be viewed from the perspective of either molecules or carbons. Since, because of its extremely low specific activity, the recycled 14C-labeled glucose considered in the last section is labeled at one 14C atom per molecule, considerations of numbers of atoms and molecules are essentially the same. Equation 3 can therefore be rewritten as

$$R_a(gng) = \frac{R^o C^+ \cdot (C^+ / C) \cdot (C^+ + C^+ / C) \cdot 2(C^+ / C)}{C^+ \cdot (C^+ + C^+ / C) \cdot 2(C^+ / C)} \quad (5)$$

where C+ and C− are the labeled and unlabeled lactate concentrations, and a = C+/C−. The second factor in Eq. 5, (C+ / C)/(C^+ + C^−) represents the fraction of 14C glucose label that is recycled, and [(C^+ + C^−) / (C^+ + C^−)] is the dilution of labeled glucose carbon by unlabeled lactate. Hence the fraction in Eq. 5, C^-/ (C^+ + C^-), applies to both cases. With the use of [U-13C]glucose, the issue is less transparent. Tayek and Katz in their first paper (26) chose the alternative of considering the problem entirely in terms of carbon fluxes and treating it analogously to that based on a [14C]glucose tracer. Thus the fraction of labeled carbon that is recycled and that would correspond to C^-/ (C^+ + C^-) or (C^- / C)/(C^+ + C^-) is

$$\sum_{i=1}^{3} iM_i / \sum_{i=1}^{6} iM_i \quad (6)$$

Whether this fraction is representative of the fraction of recycled glucose carbon can be questioned. In this context it should be noted that when [1-14C]glucose is the starting label, it is clear (because of the very low final specific activity) that as 14C is distributed among the different positions of the glucose molecule, there will not be more than one 14C per molecule. Each measured 14C will therefore represent both a single labeled carbon and a single labeled molecule. When [U-13C]glucose is the starting label, all of the carbons of one molecule are substituted with 13C. Whether the fractions of Eq. 3 and Eq. 6 will lead to the same estimates may therefore be open to discussion. [14C]glucose can be considered as six positional isotopomers of the M6, mass isotopomer. For [13C]glucose, we could consider M1, M2, and M3 to be in some sense equivalent, so that it may not be relevant whether the 13C is on the same molecule or on different molecules. Certainly without carbon dilution by label exchange in oxaloacetate (OAA), these labels would recycle onto themselves in a similar manner as the 14C label. Only with the M6 isotopomer may other considerations hold. Clearly each M6-labeled glucose (with no TCA cycle exchange) will yield two M3 glucose molecules. The number (or mass) of recycled carbons, however, remains the same.

When the strategy of dealing with 13C in the same way as with 14C is used, the rate of appearance of labeled glucose carbons can then be calculated analogously to Eq. 2

$$R_a(gng) = \frac{R \cdot 6}{CM_6 \cdot 6} \cdot \frac{1}{2} \sum_{i=1}^{3} iM_i \quad (2')$$

Note that to illustrate the analogy with [14C]glucose, we consider C (M6 × 6) and C · 2 · iM1, which correspond to the mass of labeled glucose carbons divided by volume of plasma.

The remaining problem is twofold: accounting for the dilution of pyruvate carbon by unlabeled substrate (e.g., amino acids or lactate from muscle glycogen) and for the loss of labeled carbon in label exchange with the TCA cycle.

Approaching the first problem analogously with [14C]glucose, the equation equivalent to Eq. 3 is

$$R_a(gng) = \frac{R \cdot 6}{M_6 \cdot 6} \cdot \frac{1}{2} \sum_{i=1}^{3} iM_i \quad (3')$$

where mi is the molar enrichment of lactate mass isotopomers for i = 1 to 3. The factor 2 is included in the denominator for the same reasons as before. Again, Eq. 3’ can be expressed as

$$R_a(gng) = \frac{R \cdot 6}{M_6 \cdot 6} \cdot \frac{1}{2} \sum_{i=1}^{3} iM_i \quad (5')$$
The second factor in the equation represents the fraction of recycled labeled carbon, and the third, the dilution of the labeled carbon in the lactate (or, by assumption, liver pyruvate) pool. This demonstrates the equivalence of the development here, for both the $^{14}$C- and $^{13}$C-labeled glucose, with that of Ref. 26, where fractional gluconeogenesis was estimated from a product of labeled carbon dilution factors.

Finally, there is the issue of loss of labeled carbon atoms by exchange with unlabeled TCA cycle carbon. Tayek and Katz (26) used a simple method based on the enrichments of the different mass isotopomers, information which is not available with radioactive labels. Because there would be no loss of label without this exchange, all recycled glucose would be $M_3$, and the fraction of labeled carbons would be $3 \Sigma_3 M_i$. The appearance of $M_2$ and $M_3$ isotopomers quantifies the loss of $^{13}$C in the OAA pool. The dilution factor is therefore simply

$$\frac{3}{1} M_3 / \frac{3}{1} iM_i$$

and the final equation for the rate of gluconeogenesis

$$R_a(gng) = \frac{M_6 \cdot 6}{R} \cdot \sum \frac{3}{1} iM_i \cdot \sum \frac{3}{1} M_i$$

or

$$R_a(gng) = \frac{R}{M_6} \cdot \frac{3}{2} \sum \frac{3}{1} M_i \cdot \sum \frac{3}{1} iM_i$$

which (except for a factor of 2) corresponds to the final equation proposed in Ref. 26.

One major assumption in the exchange dilution factor, and therefore in Eq. 4', is that there is no loss of labeled glucose molecules, i.e., that

$$\sum \frac{3}{1} M_i \equiv \sum \frac{3}{1} M_i + M_6$$

where, however, $M_6$ refers only to the unlabeled molecules arising from the previously labeled molecules. To the extent that $M_6$ is generated, an error in the dilution factor will occur; this will be approximated by the ratio of the two terms in the above equation.

Second, the dilution correction is essentially applied to all of the terms in the second factor of Eq. 6'. As discussed in Ref. 26, it only applies exactly to the $M + 3$ situation, because it accounts (with the exception of the production of $M_6$) for the loss of $^{13}$C in the TCA cycle. Clearly this loss of labeled carbons is not proportional for those molecules starting as $M_2$ or $M_1$. The correction should be smaller, and the overall correction made will overestimate the rate of gluconeogenesis. As pointed out in Ref. 26, the proportion of $M_2$ and $M_1$ is small relative to $M_3 + 2M_6$, so the error will not be large.

[U-13C]GLUCOSE: THE MOLECULAR APPROACH

An alternative approach to estimating the rate of gluconeogenesis ($R_a(gng)$) using [U-13C]glucose and mass isotopomer analysis was provided by Landau et al. (14). The same equation (Eq. 1') clearly holds for total glucose production. The development of the remaining equations remains completely analogous to that for [14C]glucose. When $^{13}$C is used as the label, each molecule is either labeled or unlabeled. In an exactly similar fashion, when $^{13}$C is used as the label, each molecule is also to be labeled ($M_1$, $M_2$, or $M_3$) or unlabeled. The appearance of labeled (and recycled) glucose molecules is therefore simply

$$R_a = \frac{R}{M_6} \cdot \sum \frac{3}{1} M_i$$

As before, because the immediate substrate is hepatic pyruvate (represented by circulating lactate), the appearance of labeled molecules can be converted to the rate of gluconeogenesis by the enrichment of lactate in labeled molecules. This corresponds to $\Sigma_3 m_i$ since different isotopomers are not distinguished. Therefore, Eq. 3 becomes

$$R_a(gng) = \frac{R}{M_6} \cdot \sum \frac{3}{1} M_i$$

with the factor of 2 again present, because two molecules of lactate yield one of glucose, so that twice the fraction of glucose molecules will be labeled relative to lactate molecules.

Note that, again, Eq. 3' can be written (14) as

$$R_a(gng) = \frac{R}{M_6} \cdot \sum \frac{3}{1} M_i \cdot \sum \frac{3}{1} M_i + 2M_6$$

where the second factor is the recycled fraction of glucose molecules, and the third factor is the dilution of labeled lactate molecules arising from glucose by unlabeled lactate. $M_6$ is multiplied by 2 because each molecule of glucose labeled in all 6 carbons yields 2 molecules of labeled lactate and, therefore, 2 molecules of recycled glucose.

The innovative feature of Eq. 3' is that, so long as one assumes (as before) that there is no loss of labeled molecules (i.e., no complete loss of carbon from any labeled molecule) in the TCA cycle, then the number of labeled (in any way) molecules remains the same between the lactate/pyruvate pool and the phosphoenolpyruvate (PEP) pool, and hence the recycled glucose. No additional correction for loss of labeled molecules, therefore, needs to be made.
Based on the consideration of the existence of only two types of molecules, labeled (in any way) and unlabeled, an alternative approach based on the probabilities of different molecular combinations can be used to calculate the isotopomer distribution in glucose synthesized from the set of labeled lactate molecules $(m_0,m_1,m_2,m_3)$. The probabilities can be obtained from the expansion of

$$(m_0 + \sum_{i=1}^{3} m_i)^2 = m_0^2 + 2 \cdot m_0 \cdot \sum_{i=1}^{3} m_i + \left(\sum_{i=1}^{3} m_i\right)^2$$

The newly synthesized glucose will have the following distribution

$$M_0 = m_0^2$$

$$\sum_{i=1}^{3} M_i = 2 \cdot m_0 \cdot \sum_{i=1}^{3} m_i$$

with the remainder of the terms neglected, because the probability of the combination of two labeled molecules is very low. When this model is used, the fractional gluconeogenesis is expressed as

$$\frac{\sum_{i=1}^{3} M_i}{2 \cdot m_0 \cdot \sum_{i=1}^{3} m_i}$$

Because $m_0$ in studies such as these is $\sim 95–97\%$, the fractional rate of gluconeogenesis will be corrected slightly, by $\sim 3\%$.

Finally, as before, $M_0$ isotopomers will be formed in the process of recycling, and exactly the same correction as before will hold

$$\left(\frac{\sum_{i=1}^{3} M_i + M_0}{\sum_{i=1}^{3} M_i}\right)^3$$

where $M_0$ again refers only to unlabeled molecules that were previously labeled. A relatively subtle correction needs to be considered when a factor such as the above is applied. The lactate labeled as $m_0$, $m_1$, and $m_2$ will not become $M_0$(PEP) at the same rate. Therefore, a labeled lactate that starts as $m_3$ will have a much smaller chance of losing all its label than, for example, $m_0$, on a single pass through the TCA cycle or even the OAA pool. Stated differently, in the presence of any equilibration with the TCA cycle, Eq. 3 used with pure $m_1$ lactate tracer and pure $m_2$ lactate tracer will yield very different answers. A single correction factor is thus unlikely to apply to all three terms in the numerator of Eq. 3. A factor such as Eq. 7 needs to be generated for each set of $(m_0,m_1,m_2)$ and will not simply be dependent on properties of the enzymes in the TCA cycle. Thus the nonequivalence of the three isotopomers (the factor will be larger for $m_1$ and $m_2$ than for $m_3$) will yield an underestimation of the gluconeogenic rate if a correction factor dependent only on the TCA cycle and derived on the basis of the conversion of $m_3$ to $M_0$(PEP) is used.

### Table 1. Estimates of fractional gluconeogenesis from published data using Eqs. 4' and 3''

<table>
<thead>
<tr>
<th>Subject Category</th>
<th>Subject No.</th>
<th>$\sum_i M_i$</th>
<th>$\sum_i m_i$</th>
<th>$\sum_i m_i$</th>
<th>Fractional Gluconeogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\sum_i M_i$</td>
<td>$\sum_i m_i$</td>
<td>$\sum_i m_i$</td>
<td></td>
</tr>
<tr>
<td>12-h-fasted</td>
<td>N1</td>
<td>1.87</td>
<td>4.9</td>
<td>12.7</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>N2</td>
<td>0.91</td>
<td>1.1</td>
<td>2.86</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>N3</td>
<td>0.71</td>
<td>1.4</td>
<td>3.48</td>
<td>0.31</td>
</tr>
<tr>
<td>(Ref. 14)</td>
<td>N4</td>
<td>1.33</td>
<td>3.8</td>
<td>9.98</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>N5</td>
<td>1.05</td>
<td>2.3</td>
<td>5.91</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>N6</td>
<td>1.12</td>
<td>2.0</td>
<td>5.46</td>
<td>0.31</td>
</tr>
<tr>
<td>12-h-fasted</td>
<td>D1</td>
<td>0.56</td>
<td>1.3</td>
<td>3.34</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>D2</td>
<td>0.96</td>
<td>1.7</td>
<td>4.28</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>D3</td>
<td>1.38</td>
<td>2.1</td>
<td>4.96</td>
<td>0.41</td>
</tr>
<tr>
<td>(Ref. 18)</td>
<td>D4</td>
<td>0.71</td>
<td>2.5</td>
<td>6.72</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>D5</td>
<td>0.84</td>
<td>1.9</td>
<td>4.84</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>D6</td>
<td>1.48</td>
<td>3.1</td>
<td>7.48</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>D7</td>
<td>0.73</td>
<td>2.6</td>
<td>6.03</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>D8</td>
<td>0.90</td>
<td>1.7</td>
<td>4.59</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>D9</td>
<td>0.39</td>
<td>1.3</td>
<td>3.35</td>
<td>0.17</td>
</tr>
<tr>
<td>Means ± SE</td>
<td></td>
<td>0.25 ± 0.02</td>
<td>0.21 ± 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60-h-fasted</td>
<td>1</td>
<td>2.16</td>
<td>2.80</td>
<td>7.15</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.87</td>
<td>3.26</td>
<td>8.39</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.93</td>
<td>2.91</td>
<td>7.30</td>
<td>0.40</td>
</tr>
<tr>
<td>Means ± SE</td>
<td></td>
<td>0.37 ± 0.06</td>
<td>0.32 ± 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This relative underestimation will again be small, because the $m_3$ value is higher than $m_1+m_2$.

The two formulas attributed to Tayek and Katz (26) (without including the factor of 2, Eq. 5') and Landau et al. (Ref. 14, Eq. 3'') are similar. Both formulas will be exact (given the assumptions) and therefore identical, if there is no $^{13}$C exchange and if only $m_3$ is present. This is illustrated by the calculated fractional gluconeogenetic rate of 56.3% using both formulas, in Fig. 1 of Ref. 14. On the other hand, Table 1 shows a comparison of the estimates of fractional gluconeogenesis made from data in Refs. 14 and 26. It can be seen that the estimates using both formulas are similar, although those arising from Eq. 4' are consistently somewhat higher, by $\sim 15–20\%$. Because of the nonequivalence of the three isotopomers of lactate, the (corrected) formula of Tayek and Katz (26) should slightly overestimate, and that of Landau et al. (14) slightly underestimate the rate of gluconeogenesis, relative to the theoretical case in which we have only $m_3$. These approximations

![Fig. 1. Interactions between the gluconeogenetic pathway and the tricarboxylic acid (TCA) cycle: equilibration of oxaloacetate (OAA) with fumarate and exchange with carbons arising from acetyl-CoA. The flux to citrate has a nominal rate, 1, and the gluconeogenic flux, a relative rate, $y$.](http://ajpendo.physiology.org/)}
This can be done by considering the positional isotopomers of OAA that compose the three mass isotopomers and that arise from the equivalent isotopomers of lactate. We define these isotopomeric states in Fig. 2.

The transition matrix, T, an extension of that defined in Ref. 15, and the isotopomer vector C are

$$\begin{align*}
T &= 0.5 \cdot \\
\tilde{C} &= C_0 \\
C_{31} &= dm_3 \\
C_{32} &= (1 - d)m_3 \\
C_{33} &= \frac{1}{1 + y} (\tilde{C} + C_0) T A \tilde{C} \\
\end{align*}$$

where $C_0$ is the $M_0$ isotopomer of OAA. The initial distribution, $C_{i0}$, can be defined, for example, by starting with $m_0$ lactate, so that

$$C_{i0} = dm_3$$

with the remaining $C_{ij} = 0$. Each multiplication by $T$ describes a turn of the TCA cycle. The final distribution of the $C_{ij}$ can then be defined either by

$$\tilde{C} = \frac{1}{1 + y} (\tilde{C} + C_0) T A \tilde{C}$$

By use of approaches based on Eq. 10, corrections ranging from 0.24% for $y = 10$ to 4.5% for $y = 2$ and 28%

---

**Table 2. Summary of calculations of gluconeogenesis**

<table>
<thead>
<tr>
<th>Method</th>
<th>Hepatic Glucose Production: $R_a$</th>
<th>Fractional Recycled Label</th>
<th>Dilution of Lactate Label</th>
<th>TCA Cycle Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recycled [1-14C]glucose</td>
<td>$(C^+/C)$</td>
<td>$(C^+ + C^*)/C$</td>
<td>$2 \cdot a_i$</td>
<td>F, based on other data</td>
</tr>
<tr>
<td>Recycled carbon from [U-13C]glucose</td>
<td>$R \times M_6$</td>
<td>$\sum \frac{1}{1} i M_i$</td>
<td>$\sum \frac{1}{1} i M_i$</td>
<td>$3 \cdot \sum \frac{1}{1} M_i$</td>
</tr>
<tr>
<td>Recycled molecules labeled with 13C</td>
<td>$R \times M_6$</td>
<td>$\sum \frac{1}{1} i M_i$</td>
<td>$\sum \frac{1}{1} i M_i$</td>
<td>$3 \cdot \sum \frac{1}{1} i M_i$</td>
</tr>
</tbody>
</table>

See text for explanation of abbreviations.
for $y = 0.5$ were obtained. Equation 9 was also used to estimate $m_0(PEP)$ for individual experiments, with account taken of the existence of $m_1$ and $m_2$. Iterating and regrouping the $C_m$ in terms of the isotopomers of PEP, values were obtained for the $m_0(PEP)$. The correction factors obtained in this way were near 33% for $y = 0.5$, or only somewhat higher than the previous estimate, but they illustrate the fact that a significant correction may arise under physiological circumstances. It may also be interesting to note that pyruvate kinase (PK) flux (which is assumed to be zero in the current but does contain assumptions that increase its theoretical accuracy only as an indication that some of the assumptions implicit in various derivations are not identical, leading to variances in the proposed formulas. A summary of the formulas based on the different approaches is presented in Table 2. It is left to the investigator to assess their precise experimental situation and what approach might be most suitable to build on. It should be restated that the principal difference between the formula proposed by Ref. 26 and that shown above is a factor of 2, which is not present in the presentation above. The development made by Landau and colleagues (14) is conceptually and mathematically simpler but does contain assumptions that increase its theoretical accuracy only as $m_3$ becomes much greater than $m_1 + m_2$, or the TCA cycle interaction becomes negligible.

Gluconeogenesis is a complex metabolic process that is likely not amenable to exact measurement. Advances in methodology have continuously yielded improvements in the approximations which must perforce be made. The richness of information present in the mass spectrometric determinations of mass isotopomers of precursors and products has provided a major step forward in allowing consideration of precursor-to-glucose flux and TCA cycle label exchange in the same experiment. In addition to the potential in providing better estimates of gluconeogenesis, much information can simultaneously be obtained about interacting metabolic processes, such as the TCA cycle. Counting labeled molecules is a major advantage in simplifying approaches. The underestimation of gluconeogenesis that results in its use is an indication that some of the assumptions do not hold. As illustrated above in estimating the label distribution in PEP, one returns at that point to the consideration of positional isotopomers and, therefore, by definition, carbons. Clearly, given the present state of the approximations, further work is needed to exploit the wealth of information present in the isotopomer data.

The summary presented here was solicited by the Journal to resolve and understand the differences in formulas developed in Refs. 14 and 26. The help of Dr. Joanne Kelleher (Dept. of Physiology, Georgetown University School of Medicine) in the combinatorial approaches to determining product isotopomer enrichments is gratefully acknowledged.

This work was supported by Medical Research Council (Canada) Grant 7334 and National Institutes of Health grants to the General Clinical Research Center (M01-RR-00425) and to the Clinical Nutrition Research Unit (P01-CA-42710).

Address for correspondence and reprint requests: J. Radziuk, Ottawa Hospital (Civic Site), 1053 Carling Ave, Ottawa, Ontario, Canada K1Y 4E9 (E-mail: jradziuk@ottomanhospital.on.ca).

REFERENCES


