The reciprocal pool model for the measurement of gluconeogenesis by use of [U-\(^{13}\)C]glucose

MOREY W. HAYMOND AND AGNETA L. SUNEHAG

Baylor College of Medicine, Children’s Nutrition Research Center, United States
Department of Agriculture, Agricultural Research Service, Houston, Texas 77030

Haymond, Morey W., and Agneta L. Sunehag. The reciprocal pool model for the measurement of gluconeogenesis by use of [U-\(^{13}\)C]glucose. Am. J. Physiol. Endocrinol. Metab. 278: E140–E145, 2000.—To improve upon the [U-\(^{13}\)C]glucose method to estimate “gluconeogenesis” as described by J. Katz and J. A. Tayek (Am. J. Physiol. Endocrinol. Metab. 272: E476–E484, 1997, and 275: E537–E542, 1998), we describe the reciprocal pool model by using only the isotopomer data of plasma glucose during infusion of [U-\(^{13}\)C]glucose. The glucose pool serves as both precursor and product for the calculation of the fraction of molecules generated by gluconeogenesis and to correct for exchange and loss of labeled carbon at the level of the tricarboxylic acid cycle. We have applied this model to both our own data and those of other investigators using [U-\(^{13}\)C]glucose and have demonstrated excellent agreement between the Katz and Tayek model and our reciprocal pool model. When we compare the results of the reciprocal pool model with those of Hellerstein ([2-\(^{13}\)C]glycerol) and Landau ([\(^2\)H\(_2\)O-glucose-C-5]), the results are similar in short- and long-term fasted adult humans. Finally, when we apply the reciprocal pool model to our data from premature infants, it is clear that we account for the inflow of unlabeled glycerol and presumably amino acids. This is not surprising, because the vast majority of gluconeogenesis is the result of recycling of glucose and pyruvate carbon.

THE SYNTHESIS OF GLUCOSE via gluconeogenesis is complicated by our inability to sample the true precursor pool(s) (i.e., isotopic enrichment and the distribution of label in the three carbon units at the level of the triose phosphates) and to know the degree of isotopic exchange that occurs as substrate is processed through glycolysis, the tricarboxylic acid (TCA) cycle, and gluconeogenesis. Over the past several years, three new and independent methods have been developed to estimate the fraction of glucose production derived from gluconeogenesis in vivo (5–8, 10, 11, 14, 23). Each has its own unique strengths (and weaknesses), but each also relies on plausible assumptions that are not directly verifiable in vivo.

J. Katz and J. A. Tayek (8, 23) have proposed a method employing the infusion of [U-\(^{13}\)C]glucose and the measurement of the isotopomer distribution of \(^{13}\)C in glucose. Gluconeogenesis is calculated as the product of the fraction of glucose molecules generated via gluconeogenesis and a correction factor. The latter is the ratio of all \(^{13}\)C atoms in glucose to all \(^{13}\)C atoms in plasma lactate (8, 23). Much controversy has surrounded the application of this method to in vivo studies (8, 12, 21).

We present an alternative model for the analysis of the glucose isotopomer information obtained during infusion of [U-\(^{13}\)C]glucose. This model builds on that proposed by Katz and Tayek (8, 23). However, we have derived the equations employed and use the isotopomer information in the plasma glucose pool to calculate the molecules generated by gluconeogenesis and to correct for loss and exchange of labeled carbon at the level of the TCA cycle. Thus the analytical problems and assumptions associated with the use of the lactate measurements are avoided. The assumptions of our approach are straightforward, and the resultant calculations are simplified, as is the analytical work required. We have utilized our own data, as well as those from the literature, to explore the validity of this new approach.

THE RECIPROCAL POOL MODEL

Assumptions of the Model

1) Labeled and unlabeled glucose are metabolized identically, i.e., there is no isotope discrimination.
2) During infusion of [U-\(^{13}\)C]glucose at a constant rate, substrate and isotopomer steady state is approximated.
3) Entry and exit of labeled glucose from hepatic glycogen are equal and constant.
4) The only source of \(^{13}\)C\(_6\)glucose is from the exogenous infusion of tracer, i.e., the probability of a \(^{13}\)C\(_6\)glucose being formed from two \(^{13}\)C\(_3\) molecules at the triose phosphate level of gluconeogenesis is negligible.
5) Accurate measurement of all isotopomers can be made and appropriately corrected for natural abundance and the isotopomer distribution of the infused tracer.
6) \(^{13}\)CO\(_2\) fixation is assumed to be negligible unless \(M_1\) exceeds \(M_2\) or \(M_3\). (See definitions that follow.)
Definitions

1) Unlabeled glucose molecules (12C6) are denoted M0, glucose molecules with one 13C and five 12C in any position (13C12C5) are denoted M1, glucose molecules with two 13C and four 12C in any position (13C212C4), M2, and so on. Glucose molecules uniformly labeled with 13C (13C6) are denoted M6.

2) The enrichment (Ei) of any glucose species (Mi) is the mole ratio (R) for each species corrected for the isotopic contribution of natural abundance and the distribution of the isotopomers in the tracer, divided by the sum of all other glucose species (including the fraction of M6 denoted Fm6), that is

\[ Fm0 = Rm0(Rm0 + Rm1 + Rm2 + Rm3 + Rm4 + Rm5 + Rm6) \]
\[ E1 = Rm1(Rm0 + Rm1 + Rm2 + Rm3 + Rm4 + Rm5 + Rm6) \]
\[ E2 = Rm2(Rm0 + Rm1 + Rm2 + Rm3 + Rm4 + Rm5 + Rm6) \]
\[ E6 = etc. \]
\[ \sum Ei = E0 + E1 + E2 + E3 + E4 + E5 = 1.0 \]

where i is the number of 13C in each species of glucose.

PRODUCT/PRECURSOR RELATIONSHIP

The primary precursor for all labeled species of glucose is infused [U-13C]glucose. During the course of a constant infusion of this tracer, labeled species of glucose appear in the plasma glucose pool as M1...M5 isotopomers. These species can only be derived as the result of return of tracer 13C atoms via the gluconeogenic process. However, once entering the glucose pool, each of these labeled species of glucose, i.e., M1...M5, will serve as a source of 13C from which new glucose can be labeled. Thus the M1...M5 species are labeled products of gluconeogenesis, and the M1...M6 species represent the labeled precursors.

RATE OF ENTRY OF UNLABELED AND LABELED SPECIES OF Glucose

The entry of each species of glucose (rate of appearance, Ra) can be calculated using the following standard formula

\[ R_{a, total} = \frac{[U-13C]glucose infusion rate}{E_{M6}} \]
\[ = \frac{[U-13C]glucose infusion rate, \mu mol \cdot kg^{-1} \cdot min^{-1}}{E_{M6} \sum E_{Mi}} \]

that is, Ra,total is glucose produced endogenously via glycolysis and gluconeogenesis + infused [U-13C]glucose, and Mi is the molecular species of glucose with i 13C atoms.

Similar calculations can be made for the entry of individual or groups of species of glucose. The entry rate (Ra) of glucose species labeled as a result of the process of gluconeogenesis is the sum of the appearance rates of the M1...M5 glucose species, or

\[ R_{a, M1-M5} = \frac{[U-13C]glucose infusion rate}{E_{M6} \sum \frac{E_{Mi}}{5}} \]

and the entry of all 13C-labeled species of glucose is

\[ R_{a, M1-M6} = \frac{[U-13C]glucose infusion rate}{E_{M6} \sum \frac{E_{Mi}}{6}} \]

Therefore, the fraction of all of the labeled species of glucose derived from gluconeogenesis is

\[ \frac{[U-13C]glucose infusion rate}{E_{M6} \sum \frac{E_{Mi}}{5}} \]

To save space, in the examples and tables this equation is written: \( \sum_{M1-M5} \frac{E_{Mi}}{5} \).

CORRECTION FACTOR FOR LOSS OF LABEL

The fraction of labeled species derived from gluconeogenesis shown above, however, does not take into account the loss and exchange of labeled carbon for unlabeled carbon at the level of oxaloacetate. Each labeled glucose molecule (M1...M6) is the result of the condensation of two three-carbon molecules (triose phosphates), of which at least one contains 13C. Therefore, the distribution of labeled and unlabeled carbon in the M1...M5 glucose species reflects the isotopomer distribution of the labeled and unlabeled carbons in the glyceroldehyde-3-phosphate and the dihydroxyacetone phosphate pools. Were there no exchange as a result of the enzymatic events that occur in the TCA cycle, only M3 species of glucose would be derived via gluconeogenesis from the [U-13C]glucose tracer, and the number of 13C and 12C atoms in labeled glucose molecules derived from gluconeogenesis would be equal, i.e., 13C/12C = 1.0. This does not occur. Some of the 13C from labeled pyruvate (generated from M1...M6 species of glucose) will be lost as a result of carbon exchange and oxidation in the TCA cycle. This will result in a total loss of 13C in some phosphoenolpyruvate (PEP) molecules generated from labeled pyruvate. Conversely, 12C will be gained as a result of the entry of unlabeled substrates (lactate, alanine, glycerol, and glutamine) with their subsequent randomization and exchange in the TCA cycle. Collectively, these processes will result in an underestimation of the fraction of glucose derived from gluconeogenesis, a process early appreciated by Dr. Krebs (9) as the essential issue in quantifying gluconeogenesis by use of a tracer.
Thus, during infusion of [U-13C]glucose tracer, all labeled carbon in glucose comes directly or indirectly from this tracer, but those glucose species containing fewer than three labeled carbons reflect replacement of a labeled carbon by an unlabeled carbon due to loss and exchange. For this reason, the ratio of 12C- to 13C-labeled M1-M5 glucose molecules is an index of this loss of labeled carbon. The loss of label is reflected in the sums of the entry rates of the 12C and 13Ci n 5 species of glucose can be calculated in a fashion similar to that just described for labeled glucose species with the following formulas:

\[
R_{a}^{13C_{Mi}} = \frac{[U-13C]glucose\text{ infusion rate}}{E_{M6}/[E_{Mi} \times (i)]}
\]

and

\[
R_{a}^{12C_{Mi}} = \frac{[U-13C]glucose\text{ infusion rate}}{E_{M6}/[E_{Mi} \times (6-i)]}
\]

where i is 1–5 and represents the number of 12C in each species of glucose.

Thus the ratio of entry rates of the 12C and 13C carbons in the glucose species labeled as a result of the process of gluconeogenesis (M1 – M5) is the ratio of the sums of the entry rates of the 12C and 13C in M1, . . . M5 species, respectively, or

\[
\frac{[U-13C]glucose\text{ infusion rate}}{E_{M6}/5} = \frac{E_{M6}/5 \sum_{i} E_{Mi} \cdot (6-i)}{E_{M6}/5 \sum_{i} E_{Mi} \cdot (i)}
\]

\[\Sigma_{M1-M5}(12C)/\Sigma_{M1-M5}(13C)\]

Consequently, the fraction of circulating glucose derived from gluconeogenesis, including the correction for loss of label, is the product of the fraction of labeled molecules derived via gluconeogenesis and the ratio of 12C to 13C in these molecules.

\[
\text{Fractional gluconeogenesis} = \frac{\sum_{i} E_{Mi} \times \sum_{i} 12C_{Mi}}{\sum_{i} E_{Mi} \times \sum_{i} 13C_{Mi}}
\]

\[\Sigma_{M1-M5}(12C)/\Sigma_{M1-M5}(13C)\]

**APPLICATION OF THE RECIPROCAL POOL MODEL TO PUBLISHED DATA**

In the examples below, we have used our own data (21), as well as data published by other investigators (12), to explore in detail each step in the proposed model. The calculations are based on the average isotopomer values from these publications.

Example 1

In this first example, premature infants (n = 8) received [U-13C]glucose (93.5% 13C6) over 10 h at a rate of 16.7 µmol·kg\(^{-1}\)·min\(^{-1}\) as part of total parenteral nutrition that also includes lipids and amino acids (21). The [U-13C]glucose represented the total amount of exogenous glucose delivered and is an extreme of isotope administration.

<table>
<thead>
<tr>
<th>Glucose Species</th>
<th>E(_{M6}) (µmol·kg(^{-1}))</th>
<th>12C/100 Glucose Molecules</th>
<th>13C/100 Glucose Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>M(_6) 13C(_5)12C(_6)</td>
<td>29.80</td>
<td>21.2</td>
<td>4.24</td>
</tr>
<tr>
<td>M(_5) 13C(_4)12C(_5)</td>
<td>4.24</td>
<td>13.08</td>
<td>6.54</td>
</tr>
<tr>
<td>M(_4) 13C(_3)12C(_4)</td>
<td>3.27</td>
<td>10.71</td>
<td>10.71</td>
</tr>
<tr>
<td>M(_3) 13C(_2)12C(_3)</td>
<td>3.57</td>
<td>1.58</td>
<td>3.16</td>
</tr>
<tr>
<td>M(_2) 13C(_1)12C(_2)</td>
<td>0.79</td>
<td>0.47</td>
<td>2.35</td>
</tr>
<tr>
<td>M(_1) 13C(_0)12C(_1)</td>
<td>0.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M(_0) 13C(_0)12C(_0)</td>
<td>57.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (\Sigma_{M1-M5})</td>
<td>12.34</td>
<td>47.04</td>
<td>27.0</td>
</tr>
<tr>
<td>Total (\Sigma_{M1-M6})</td>
<td>70.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>((\Sigma_{M1-M5}/\Sigma_{M1-M6}))</td>
<td>0.176</td>
<td>(\Sigma_{13C}/\Sigma_{12C})</td>
<td>1.74</td>
</tr>
</tbody>
</table>

*"As Published" values represent calculations performed according to Katz and Tayek (8, 23), i.e., the correction for loss of labeled carbon was based on lactate data (8, 23). In this example, about one-half of the glucose Ra was provided exogenously as [U-13C]glucose, and gluconeogenesis as a fraction of GPR was calculated by dividing the gluconeogenic rate by the GPR.

Example 2

In this study, healthy adults (n = 3) were studied after a 65-h fast. [U-13C]glucose was infused at a low rate of 0.4 µmol·kg\(^{-1}\)·min\(^{-1}\) over 5–6 h (12).
The described correction factor compensates for this loss. The published data are based on the "corrected" equation as described by Landau et al. (12), which does not include any correction factor for loss of labeled carbon, resulting in a value for gluconeogenesis that is about one-half that obtained by both the Tayek and Katz method (8, 23) and the proposed reciprocal pool model.

**DISCUSSION**

The proposed model significantly simplifies that of Katz and Tayek (8, 23) in that only the isotopomer analysis of glucose needs to be made; e.g., analysis of plasma lactate is not necessary. Thus the entire calculation of gluconeogenesis is made on the basis of the ratio of 12C/13C in the substrate amount of tracer (i.e., up to 10–15% enrichment in the 12C-to-13C ratio in the M1–M5, not from circulating lactate (8, 23) or from an indirect estimate of the enrichment at the level of the triose phosphates (6).

The concept that M1 through M5 glucose molecules can only be generated via gluconeogenesis is clear. However, the correction for exchange and loss of labeled carbon is more difficult. It is impossible to know the precise locations of each of these labels in the M1–M5 species of glucose, and tracing the precursors through the myriad of potential metabolic pathways (e.g., carboxylations, decarboxylations, and rearrangements) becomes futile when one understands that the precursor product is product and the product is precursor. However, exchange and loss of labeled carbon do occur and result in an increase in the 12C-to-13C ratio in the M1–M5 glucose and an underestimation of gluconeogenesis. The described correction factor compensates for this loss.

### Table 1. Correction for loss of 13C by use of the reciprocal pool model and lactate measurements as described in Refs. 8 and 23

<table>
<thead>
<tr>
<th>Glucose Species</th>
<th>E&lt;sub&gt;mono&lt;/sub&gt; (%)</th>
<th>12C/100 Glucose Molecules</th>
<th>13C/100 Glucose Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0 12C&lt;sub&gt;0&lt;/sub&gt;12C&lt;sub&gt;6&lt;/sub&gt;</td>
<td>94.26</td>
<td>3.05</td>
<td>0.61</td>
</tr>
<tr>
<td>M1 12C&lt;sub&gt;1&lt;/sub&gt;12C&lt;sub&gt;5&lt;/sub&gt;</td>
<td>0.61</td>
<td>2.52</td>
<td>1.26</td>
</tr>
<tr>
<td>M2 12C&lt;sub&gt;2&lt;/sub&gt;12C&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.63</td>
<td>1.83</td>
<td>1.83</td>
</tr>
<tr>
<td>M3 12C&lt;sub&gt;3&lt;/sub&gt;12C&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M4 12C&lt;sub&gt;4&lt;/sub&gt;12C&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M5 12C&lt;sub&gt;5&lt;/sub&gt;12C&lt;sub&gt;1&lt;/sub&gt;</td>
<td>3.89</td>
<td>7.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Total ΣM1-M5</td>
<td>1.85</td>
<td>7.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Total ΣM1-M6 (ΣM1-M5/ΣM1-M6)</td>
<td>0.3222</td>
<td>Σ1-5(12C)/Σ1-5(13C)</td>
<td>2.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Glucose Ra, µmol·kg&lt;sup&gt;-1&lt;/sup&gt;·min&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>Reciprocal Pool Model</th>
<th>As Published (13)</th>
<th>Method of Tayek and Katz (8, 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Ra, µmol·kg&lt;sup&gt;-1&lt;/sup&gt;·min&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>9.6</td>
<td>9.6</td>
<td>9.6</td>
</tr>
<tr>
<td>GPR, % of glucose Ra</td>
<td>9.2</td>
<td>9.2</td>
<td>9.2</td>
</tr>
<tr>
<td>GNG, % of glucose Ra</td>
<td>64.4</td>
<td>33</td>
<td>58.0</td>
</tr>
<tr>
<td>GNG, % of GPR</td>
<td>6.5</td>
<td>3.2</td>
<td>5.6</td>
</tr>
<tr>
<td>Glycogenolysis, %</td>
<td>32.8</td>
<td>65.2</td>
<td>39.1</td>
</tr>
</tbody>
</table>

It is of interest to note that the correction factor calculated using the reciprocal pool model is very similar to that derived from the isotopomer distribution of plasma lactate and glucose as described by Katz and Tayek (8, 23) (Table 1). Some of the differences observed in the correction factors (Table 1) could, in part, be due to the low infusion rates of [U-13C]glucose employed in these studies, resulting in low enrichments of M1 through M5 in plasma glucose and of M1 through M1 in lactate (12, 23). In our opinion, the measurement of the enrichments of molecular species below the level of 0.7–0.8% is associated with potential inaccuracies. Fortunately, the price of [U-13C]glucose has decreased dramatically over the past 2 yr, making such studies in adult humans only expensive, not financially impossible. Therefore, the investigator must weigh the potential confounding variables of an infusion of a near-substrate amount of tracer (i.e., up to 10–15% enrichment in the M6, which will result in more accurate and reproducible measurement of all glucose isotopomers, vs. potential errors associated with low enrichments of all glucose isotopomers due to a lower rate of tracer infusion.

Table 2 depicts the estimates of gluconeogenesis by use of the Katz and Tayek model (8, 23) and the reciprocal pool model, with isotopomer information derived from published studies, employing the infusion of [U-13C]glucose to estimate gluconeogenesis. The results obtained by the reciprocal pool model were close to those calculated using the Katz and Tayek model. In

<table>
<thead>
<tr>
<th>Fractional gluconeogenesis (% of glucose Ra)</th>
<th>Reciprocal Pool Model</th>
<th>Katz and Tayek's Model (8, 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ref. 8</td>
<td>28</td>
<td>41</td>
</tr>
<tr>
<td>Ref. 8</td>
<td>70</td>
<td>71</td>
</tr>
<tr>
<td>Ref. 8</td>
<td>79</td>
<td>92</td>
</tr>
<tr>
<td>Ref. 12</td>
<td>64</td>
<td>58</td>
</tr>
<tr>
<td>Ref. 12</td>
<td>51</td>
<td>36</td>
</tr>
<tr>
<td>Ref. 21</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>Ref. 22</td>
<td>37</td>
<td>49</td>
</tr>
<tr>
<td>Ref. 22</td>
<td>41</td>
<td>45</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>50 ± 7</td>
<td>53 ± 7</td>
</tr>
</tbody>
</table>

Ra, rate of appearance.
Table 3. Rates of gluconeogenesis calculated using the reciprocal pool model compared with models of Hellerstein et al. (6) and Landau et al. (11) after different periods of fasting in normal volunteers

<table>
<thead>
<tr>
<th>Hours Fasted</th>
<th>Reciprocal Pool Model</th>
<th>[2-13C]glycerol (Ref. 6)</th>
<th>C-5 Glucose Deuterium (Ref. 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12–14</td>
<td>30*</td>
<td>36</td>
<td>67</td>
</tr>
<tr>
<td>20–22</td>
<td>70†</td>
<td>67</td>
<td>93</td>
</tr>
<tr>
<td>40–60</td>
<td>79†</td>
<td>78</td>
<td>93</td>
</tr>
</tbody>
</table>

*Calculations were based on data from Refs. 8 and 22. †Calculations were based on data from Ref. 8.

addition, these estimates of gluconeogenesis are similar to those obtained after comparable periods of fasting, in which the methods of Hellerstein et al. (6) and Landau et al. (11) were used (Table 3). This is remarkable, considering that these results are derived from different laboratories and follow different periods of isotope infusion at different enrichments of tracer.

Whether our model, or any of the other proposed models, measures absolute rates of gluconeogenesis, pure Cori cycle activity, or some value in between, remains to be determined, because no "gold standard" exists to which the results of these models can be compared. The events regulating the delivery of metabolic precursors and the myriad of potential pathways and futile cycles through which substrate and label might pass make the modeling of this process intellectually intriguing but not necessarily enlightening. The major concern of the opponents of the Katz and Tayek method, and most likely our approach as well, is whether this method measures the influx of unlabeled gluconeogenic precursors derived from proteolysis and lipolysis, i.e., amino acids and glycerol.

Of the potential gluconeogenic amino acids, it is generally accepted that alanine and glutamine are the most important and account for ~10% of total glucose production after an overnight fast (2, 3, 17). About 65–80% of alanine and 80% of glutamine turnover are derived via de novo synthesis (1, 4, 17, 19). Isotope labeling studies have demonstrated that 65% of alanine is derived from pyruvate (1) and that infusion of glucose at 4 mg·kg\(^{-1}\)·min\(^{-1}\) is associated with a 40% increase in de novo synthesis of alanine (19). In addition, glucose infusion at a similar rate doubled the contribution of glucose carbon to glutamine (R. G. Hankard, M. W. Haymond, and D. Darmoun, unpublished data). Thus, a significant portion of alanine and glutamine carbon is derived from glucose via pyruvate and will result in a progressive labeling of these precursor pools for gluconeogenesis.

Glycerol derived from the hydrolysis of triglycerides contributes ~3–5% of glucose production in the postabsorptive state and 10–15% in the prolonged fasting state (13, 18, 24). Landau et al. (13) recently reported data from 60-h-fasted humans demonstrating that a maximum of 38% of [U-\(^{13}\)C\(_{3}\)]glycerol is converted directly to glucose (M + 3), whereas 35% of glycerol label entered glucose (\(^{13}\)C-1, \(^{14}\)C-4, and \(^{14}\)C-6) via pyruvate (13). We presume that the remaining 27% is lost via oxidation. Therefore, some 62% (35% + 27%) of glycerol derived from lipolysis (together with alanine and glutamine) passes through the pyruvate pool and may be labeled as a result of isotopic exchange during infusion of [U-\(^{13}\)C\(_{3}\)]glucose. However, unlabeled lactate, alanine, glycerol, and glutamine provide \(^{13}\)C atoms that enter the TCA cycle and are involved in the randomization and exchange of carbons, possibly resulting in an underestimate of gluconeogenesis.

We recently demonstrated that the [2-\(^{13}\)C\(_{2}\)]glycerol and [U-\(^{13}\)C\(_{3}\)]glucose methods provided similar estimates of gluconeogenesis in premature infants studied under nearly identical conditions (21). The C-6 deuterium method, which does not include the contribution of glycerol, provided an estimate that was <30% of estimates obtained using the other two methods. However, when the contribution of glycerol to gluconeogenesis measured by the [2-\(^{13}\)C\(_{2}\)]glycerol method is added to that of the C-6 deuterium method, the resultant value is similar to values obtained with the [2-\(^{13}\)C\(_{2}\)]glycerol and the [U-\(^{13}\)C\(_{3}\)]glucose methods (21). Applying our reciprocal pool model to these data leads to similar results (Fig. 1). Were our method and that of Katz and Tayek unable to measure the contribution of glycerol to gluconeogenesis, we would have observed a result similar to that of the C-6 deuterium method alone, but this was not the case. Therefore, we conclude that our model, as well as that of Katz and Tayek, accounts for at least the majority of the contribution of glycerol to gluconeogenesis. This is not at all surprising in that, as discussed above, the vast majority of glucose production derived from gluconeogenesis is the result of recycling of pyruvate (i.e., Cori cycle activity). The objective facts speak for themselves.

The reciprocal pool model is uniquely internal to itself and makes no assumptions or extrapolations as to the "true" precursor pool at the level of the triose phosphates and is analytically much simpler than the
$^2$H$_2$O method, with measurement of the deuterium enrichment at the C-2 and C-5 positions of glucose. It is, however, important to recognize that each of the fundamental methods to estimate "gluconeogenesis" has its unique strengths and weaknesses, and only through continued exploration of the models and the substrate and hormone factors that regulate gluconeogenesis will we gain further insights into these and perhaps other models to measure gluconeogenesis. We should not permit the present controversy regarding methodology to inhibit the progress in exploring the factors that regulate gluconeogenesis in humans.

We thank Drs. David Cohen and Dennis Bier, Children's Nutrition Research Center, Houston, TX, for their invaluable help during the discussions of the model and in the preparation of this manuscript.

This work is a publication of the United States Department of Agriculture (USDA)/Agricultural Research Service Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX. The project was supported by grants from USDA Cooperative Agreement no. 58–6250–6-001 and National Institute of Diabetes and Digestive and Kidney Diseases Grant 1RO1-DK 55478–01. The contents of this publication do not necessarily reflect the views or policies of the USDA, nor does mention of trade names, commercial products, or organizations imply endorsement from the US Government.

Address for reprint requests and other correspondence: M. W. Haymond, Children's Nutrition Research Center, 1100 Bates St., Houston, TX 77030–2600 (E-mail: mhaymond@bcm.tmc.edu).

Received 14 May 1999; accepted in final form 2 September 1999.

REFERENCES


