Limitations in the use of [U-\textsuperscript{13}C\textsubscript{6}]glucose to estimate gluconeogenesis

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The efforts of Drs. J. Kelleher, W.-N. P. Lee, and J. Radziuk to resolve the differences between our formulas and those of Drs. J. A. Tayek and J. Katz are greatly appreciated. However, with deep regret and due respect for Drs. Radziuk and Lee, they (26) misinterpret the contributions of my colleagues and myself (21). We have never proposed a method for measuring rates of gluconeogenesis using [U-\textsuperscript{13}C\textsubscript{6}]glucose. Tayek and Katz proposed such a method that, applied under fasting conditions, resulted in physiologically reasonable estimates (13, 30). However, their estimates were based on a theoretically unsound equation, which we corrected. Then we showed using our equation that their method gave “impossibly low” estimates. It is incorrect to interpret that to mean we proposed a method that was the same as that of Tayek and Katz, except for use of an equation that we knew gave impossibly low estimates.

There is only one method using [U-\textsuperscript{13}C\textsubscript{6}]glucose under discussion, that of Tayek and Katz. Conclusions given by Radziuk and Lee in their abstract (26) are what we concluded with regard to the proposed method of Tayek and Katz, but those conclusions should not be extended to a method we never proposed. Because Radziuk and Lee recognize the need to correct the equations of Tayek and Katz, as we did, they conclude, as we did, that the method gives estimates lower than physiologically expected. The only difference between their conclusions and ours is that they conclude that assumptions made by Tayek and Katz may not hold, and we conclude that they do not hold. We hope no one uses our equation to avoid confusion, because later we will consider another factor of 2.

As to the need for the 0.5 factor, we believe it is not as evident as it should be from Refs. 15 and 26. Consider the situation when glucose is formed only by gluconeogenesis from lactate. Mathematically, if there are 200 molecules of triose-P and 4 are labeled from [U-\textsuperscript{13}C\textsubscript{6}]glucose via lactate, the fraction labeled is 0.02, i.e., 4/200. Those 200 triose-P molecules will yield 100 glucose molecules, and 4 will be labeled. The fraction labeled is 0.04, i.e., 4/100. The fractional contribution of gluconeogenesis to glucose formation is then 0.5/M = 0.5(0.04)/0.02 = 1.0, i.e., 100% gluconeogenesis. Thus the 0.5 is needed because two molecules of lactate form one molecule of glucose, and in the equations the M and m values are the fractions or percentages of glucose and lactate molecules that are labeled. It must be emphasized that our equation holds when [U-\textsuperscript{13}C\textsubscript{6}]glucose is given in a tracer dose, i.e., the amount is so small that the frequency of two labeled molecules of triose-P condensing can be neglected (21).

Conceptually, the 0.5 is needed because of every labeled glucose molecule formed by gluconeogenesis, the source of one-half of its carbons is the tracer, i.e., a triose-P molecule whose carbons were never labeled. The equation to calculate the fractional contribution must express the fate of the tracer. Therefore, M\textsubscript{1}, M\textsubscript{2}, and M\textsubscript{3} must be multiplied by 0.5. If the [U-\textsuperscript{13}C\textsubscript{6}]glucose is a true tracer, it will then reflect the fate of the tracer (21). This is an important principle in the use of tracers.

As to the need for the 0.5 factor, historically, Radziuk and Lee (26) indicate that the development of the Tayek-Katz method stems from a previous analysis by Katz et al. (12) to assess the indirect pathway of glycogen synthesis. Indeed, the same considerations apply in developing equations for estimating the indirect pathway, i.e., lactate → glycogen, and for gluconeogenesis, i.e., lactate → glucose. However, the roots, as well as the stem, of that development are important for the present. Katz et al. (10) first used [U-\textsuperscript{13}C\textsubscript{6}]glucose to...
assess the indirect pathways on the basis of equations proposed by Kalderon et al. (7). The required 0.5 factor was lacking in their equations. Des Rosiers et al. (4) recognized that omission and its consequence. Katz and associates (11, 12) then accepted the need for the 0.5. In a note added in proof at that time (12), Katz et al. concluded that, although it was needed, by including the 0.5 impossibly low estimates of the indirect pathway were obtained unless other factors were taken into account. Their conclusion, at that time, is then in complete agreement with the conclusion of Landau et al. (21) that including the 0.5 in estimates of gluconeogenesis results in impossibly low estimates unless other factors are taken into account.

Before those other factors are considered, differences between the formulas need further clarification. In Table 2 of Ref. 26, the equations of Tayek and Katz (29) based upon recycled carbon are recorded. Their equation for the fractional contribution of gluconeogenesis equals the product of fractional recycled label, dilution of lactate label, and tricarboxylic acid (TCA) cycle dilution, but with the omission of a 2 in the denominator. That equation is recorded in Table 1 of Ref. 15 as "Tayek and Katz 1996." The product of fractional recycled label times dilution of lactate label with the inclusion of 2 in the denominator is used by Radziuk and Lee (26), their Eq. 5', to calculate fractional gluconeogenesis from the data of Tayek and Katz and from our data (Table 1 of Ref. 26). Because Radziuk and Lee include the 2, the contributions, as expected, are impossibly low, in agreement with Landau et al. (21) by use of Eq. 1 (Eq. 3' of Ref. 26).

Although we recognize that those 1996 equations, in terms of carbon atoms, can be compared with the equations relating to the fate of 13C from [U-13C6]glucose and Dr. Lee's collaboration in the 1996 study, we believe there should have been attention in Ref. 26 to the 1997 equation of Tayek and Katz for estimating the contribution of gluconeogenesis (30). Although the 1996 equation is only 3 years old, we have considered it of historical interest in the development by Tayek and Katz of their approach. Referring to their 1996 equation, although not recognizing the need for the 0.5 factor, Tayek and Katz stated in 1997 (30), "our calculation was an approximation adequate when recycling is low. It overestimated gluconeogenesis when recycling is high. Here we present a revised equation." That revised equation, labeled "Tayek and Katz 1997," is found in Table 1 of Ref. 15. It still lacks the 0.5 factor and uses molar enrichments in calculating dilution of lactate label. The presence of a 2 in the denominator of the 1997 equation, noted in Ref. 15, does not negate the need for that 0.5 factor. Omitting the 0.5 factor results in estimates about twice those using Eq. 1. The 1996 equation gives estimates higher than the 1997 equation, at least in part because of inclusion in the 1996 equation of a correction for TCA cycle exchange (15). It is the 1997 equation that is now being applied by investigators to estimate gluconeogenesis (13, 28, 31) and to which attention must therefore be directed.

The revision by Tayek and Katz in 1997 (30) was to replace their 1996 equation for recycling of glucose carbon by an equation for recycling of glucose molecules (M1 + M2 + M3)/(M2 + M3 + M4 + M6). That equation was introduced by Kalderon et al. (8). It is incorrect because of the omission of the 0.5 factor, as shown by Des Rosiers et al. (4). The correct equation for glucose recycling, i.e., Cori cycling, is (21)

\[ \frac{0.5(M1 + M2 + M3)}{0.5(M1 + M2 + M3) + M6} \]  

Again the 0.5 factor is required, because one-half of the carbons of M1, M2, and M3 have the tracer as their source. Again, including that 0.5 factor in the 1997 equation for gluconeogenesis of Tayek and Katz gives impossibly low estimates. By using the equation of Kalderon et al., if the 0.5 factor had been added, Tayek and Katz would have had a beginning toward use of a molecular approach, with its correctness as emphasized by Kelleher (15), and with its mathematical and conceptual simplicity, as concluded by Radziuk and Lee (26). The strength of the approach is illustrated by its use to remove the effect of TCA cycle exchange.

Isotopomers carry a record of their past, allowing estimates of the metabolic pathways traversed in their formation. However, the assumption that all information necessary to obtain a valid estimate of rates of gluconeogenesis is contained in the isotopomers of lactate and glucose is not correct. Tayek and Katz, having obtained reasonable estimates, assumed it was

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1. Heading to the first column of Table 2 of Ref. 26 would better be "calculation" than "method." Again, there is only one method using [U-13C6]glucose, with calculations shown either based on recycled carbon or recycled molecules. Also, the last column should more precisely be labeled "TCA cycle exchange." In the TCA cycle 13C is exchanged for 13C with no increase in the amount of carbon. That is also so in the exchange of 13C with 12C-labeled molecules in the equilibration of labeled 1-ketoglutarate with unlabeled glutamate and of labeled oxaloacetate with unlabeled aspartate. In the dilution of labeled carbon by the conversion of unlabeled substrate (e.g., glycerol) to glucose, there is a net increase in the amount of carbon (21).

2. Because Radziuk and Lee corrected the 1996 equation of Tayek and Katz and then obtained impossibly low estimates does not mean they proposed a method to estimate rates of gluconeogenesis using their corrected equation, making the same assumptions as Tayek and Katz.

3. Tayek and Katz's (29, 30) equation for dilution of lactate label is (M1 + 2M2 + 3M3 + 6M4)/(M2 + 2M3 + 3M4). Because experimentally M1, M2, and M3 are less than M4 and M6, less than M6, as a first approximation the equation reduces to 6M4/2(3M4) = M4/M6. When the M4/M6 is multiplied (Kalderon's equation, i.e., our Eq. 2 without the 0.5) again as a first approximation, because M1 and M2 are less than M3 and those less than M6, fractional gluconeogenesis equals (M3/M6) (M6/M3) (M4/M6) = (M3/M6). That expression would then be correct, except for the omission of the 0.5, if there were no TCA cycle exchange (and 13CO2 fixation) to form M3 and M6 and hence M1 and M2. When that approximation is extended, the 1997 equation for fractional gluconeogenesis of Tayek and Katz (30) becomes (M1 + M2 + M3)/(M1 + M2 + M3). That is the expression Katz and Tayek in correspondence have concluded their 1997 equation can be reduced to in molecular terms. It is then equivalent to our Eq. 1, except that again the required 0.5 is lacking.
correct. We (21), having obtained impossibly low estimates by use of Eq. 1, concluded it was not correct, although Radziuk and Lee (26) claim we assumed it was correct for a method that we did not propose. Using Eq. 1, with the data of Tayek and Katz, we calculated after an overnight fast a contribution of -20%, less than by any other method. We gave [U-13C6]glucose in long-term fasting, because then a valid estimate would be near 100%. Having calculated -40% from the data we obtained, we gave [U-13C6]glucose in overnight fasting in case a question arose as to the reliability of our mass isotopomer analyses. The data we then obtained calculated to -20%, in accord with the estimates made by use of the data of Tayek and Katz.

Those impossibly low estimates were predictable because of those other factors (21), if only from the experience of Katz et al. (12) 10 years before. Calculation using Eq. 1, or the equations of Tayek and Katz corrected by addition of the required 0.5 factor, only gives estimates of the fraction contributed to gluconeogenesis by conversion to glucose of hepatic pyruvate and its precursors (e.g., lactate, alanine) traced with [U-13C6]glucose. If the enrichments in the isotopomers of hepatic pyruvate were less than in arterial lactate, the contribution of gluconeogenesis will be underestimated.4 Any process, beyond pyruvate's entrance, by which carbon from an unlabeled glucogenic substrate is converted to glucose or unlabeled carbon is exchanged with labeled carbon, other than in the TCA cycle, will also result in underestimation. Thus there are 13 glucogenic amino acids released by proteolysis, at least in small quantity, during fasting and therefore unlabeled, that are converted to glucose via α-ketoglutarate, fumarate, succinate, or oxaloacetate.5 There are the exchanges, that appear to be considerable (14, 24), in the equilibration of aspartate and glutamate with their ketoacids. There will be equilibration between those unlabeled carbons and those of labeled hepatic pyruvate via pyruvate cycling, as previously considered (21). However, that equilibration is far from complete (19, 23), which will be evident when we discuss the "F factor."

Furthermore, glycerol from the circulation, converted to glucose, will dilute the labeled carbons in their conversion to glucose. The glycerol will be unlabeled because its source in fasting is mainly lipolysis of triglyceride stores.6 Katz and Tayek (13) raised the possibility that glycogen cycling could result in underestimation. Evidence is that glycogen cycling in fasting is not significant (1, 17). For example, Petersen et al. (25) estimated glycogen synthesis in overnight-fasted subjects at a rate <2% the rate of glycogenolysis. The assumptions of Hellerstein et al. (6), on the basis of which they concluded cycling in the fasting state, do not appear tenable (1, 17). The title of the paper by Magnusson et al. [Liver glycogen turnover in fed and fasted humans (22)] can be misleading. Measurements were made not in the fasting state but in subjects that had fasted and were then infused with glucose to a concentration of 9.5 mM, and that concentration was maintained for several hours. Other factors could contribute to the underestimates, including compartmentation, which was not discussed, and the pentose pathway, which was only briefly considered (21).

Radziuk and Lee (26) indicate that Tayek and Katz (29), as well as my colleagues and I (21), discussed the reason why gluconeogenesis in the 60-h-fasted state is only estimated at 40%, with equations containing the 0.5 factor. We did discuss the reasons (those other factors), but Tayek and Katz did not. Katz and Tayek, in a note added in proof to their recent paper (13), concluded that we claimed as valid our estimates with the corrected equation that are about one-half of expected estimates. They made this claim, even though we had stated that our estimates were impossibly low.

If Eq. 1 is used, correction for those other factors that result in underestimates have to be taken into account (21). Designating "F" for that correction (17), the fractional contribution of gluconeogenesis equals

\[
\frac{0.5 \cdot F \cdot (M_1 + M_2 + M_3)}{m_1 + m_2 + m_3}
\]

That may be considered the equation to estimate "total" fractional contribution, i.e., from all glucogenic substrates. Not only could F be significant, as Radziuk and Lee surmise (26), but it is significant, and the 1.4 value they refer to is not a measure of its size. That 1.4 is the factor calculated by Katz et al. (12) to correct for exchange in the TCA cycle. Our equation removes that need. The title of Ref. 12 (Determination of pathways of glycogen synthesis and the dilution of the three-carbon pool with [U-13C6]glucose) clearly indicates attention...
given to “dilution of the three-carbon pool.” Thus Katz et al. (12) estimated an F of ~2.5 for the dilution of the triose-P pool by nonglucose carbon. Katz et al. in their

The reader can now understand why Tayek and Katz obtained reasonable estimates using their equation. If the 0.5 is omitted, because 0.5 \times 2 = 1, that omission is the equivalent of not omitting the 0.5 and fixing F = 2. If they, using their incorrect equation, had taken those factors into account, they would have had remarkable overestimates, e.g., ~200% in long-term fasting (21).

The above considerations provide an important message for investigators contemplating the use of [U-13C6]glucose to estimate gluconeogenesis from isotope-pomer distributions in arterial lactate and glucose. That message goes beyond statements (26) that it is left to the investigators to assess their precise experimental situation and all methods are based on assumptions that lead to certain approximations. An investigator should have a measure of the precision to be expected using the Tayek-Katz method. There is no way to know what the value of F will be under a given experimental condition. Results thus far appear to be in a range from perhaps 1.5 to 2.5. Thus Wykes et al. (31), using the equation of Tayek and Katz (30), estimated the contribution of gluconeogenesis in fasting piglets at over 100%. Probably F, being fixed at 2, was responsible for the overestimate, F actually then being less than 2. If an investigator calculates with Eq. 1 a contribution of gluconeogenesis of 30%, and, if F were 1.5, the actual contribution would be 45%, and if 2.5, it would be 75%. It is that uncertainty that resulted in our not proposing a method to quantitate gluconeogenesis from isotope-pomer distributions obtained using [U-13C6]glucose.

The use of Eq. 2 to estimate glucose recycling, i.e., Cori cycling, also gives underestimates and requires correction with F along with its uncertainty. Thus correction must be made for the exchange of 13C by 12C other than in the TCA cycle, and dilution by unlabeled substrates in the conversion of labeled lactate formed from the [U-13C6]glucose in its conversion to glucose. Tayek and Katz (23, 30) claim that Eq. 2 without the 0.5 is correct, because it then gives the same estimates as obtained from the difference in glucose production measured with radioactive glucose tracers that are irreversible [e.g., [3-H]glucose, in which no label is cycled] and reversible [e.g., [1-14C]glucose, in which label is cycled]. However, that same 0.5 factor must be used in calculating glucose production with the reversible tracer (21). Thus, when 14C-labeled triose-P is formed from [1-14C]glucose and condenses with unlabeled triose-P, 0.5 of the carbons of the glucose formed has the tracee as its source.

F is so large that the errors in the estimates of gluconeogenesis from factors detailed by Radziuk, Lee, and Kelleher become less significant. Furthermore, those factors that produce underestimates are included with the other factors in F, because F is an overall correction factor. The reason for including m1 in the equation, as concluded by Radziuk and Lee as well as by Kelleher, is well taken. The principle is important, as is the reminder that m1 can be significantly less than 1 in some situations (15). However, as they note, with the low dose of [U-13C6]glucose that must be given to avoid significant condensing of two labeled triose-P molecules, the correction is small. Thus, if 0.97 of the molecules of lactate are from the tracer and 0.03 from the other factors, the correction is only 1.0/0.97 = 1.03. Radziuk and Lee (26) also indicate that theoretical accuracy increases when our equation is used, as either m1 becomes greater than m1 + m2 or the TCA cycle is negligible. A negligible TCA cycle is probably incompatible with life. As we noted, experimentally, m1 has been much more than m1 + m2, particularly when m2 is corrected for 13CO2 fixation (21). 13CO2 fixation may also affect to some extent the amount of m2 relative to m2.

The reader should also have a measure of the y, the gluconeogenesis-to-TCA cycle flux ratio, because it determines the extent to which a labeled molecule in the TCA cycle will have all its 13C exchanged for 12C. In fasting and in diabetes in humans, we estimated y to be 2 or more (19, 23), resulting in a correction of 45% or less, according to Radziuk and Lee (26). In fasting, a y of ~0.5 has been estimated (3), but that estimate was an artifact of a method (16, 27) that also gave reasonable estimates of gluconeogenesis but lacked validity (2, 16, 27). There two wrongs seemed to make a right, as here in the Tayek-Katz proposal, by not taking into account the 0.5 and those other factors. There both y and the 14C specific activity of acetyl-CoA, required in the calculation, were underestimated. The assumption that pyruvate dehydrogenase activity is negligible compared with pyruvate carboxylase activity appears reasonable. At least in long-term fasting, we estimated the conversion of pyruvate to acetyl-CoA to be one-thirtieth or less than the rate of pyruvate’s conversion to oxaloacetate (19, 23). The advantages of using 13C over 14C are noted by Kelleher (15). I believe derivations using 13C can be made as easily without analogy to derivations made using 14C. In any event, with restrictions in the use of 14C, justified or not, at least for humans (20), equations using [1-13C]glucose are probably already obsolete.

Equation 1 will be valid as long as two molecules of lactate yield one molecule of glucose and the principles in the use of tracers hold. The equation can be easily derived without a glucose cycling model (17), as Kelleher concludes (15). Furthermore, I am pleased to have the validity of our equation, our molecular approach, and our conclusions regarding the Tayek and Katz equations tested by application of the binomial probabil-
In preparing this commentary I have had three major objectives. First, I want to put an end to the false claim (13, 26, 28) that my colleagues and I proposed a method for calculating rates of gluconeogenesis that gives impossibly low estimates. Second, in the light of Refs. 15 and 26, I wish to make clear the limitations in use of the method of Tayek and Katz, that the overwhelming difference in the formulas is that the formula of Tayek and Katz lacks a factor of 0.5, and that estimates by the method are uncertain to a large extent. Third, in no way do I wish to detract from the many contributions Dr. Katz has made to tracer methodology and calculations of pathway contributions over almost half a century, for which he has my continued admiration.

I remain amazed that a mathematically correct method for calculation, conceptually easy to understand, coupled to an 0.5 factor whose need was established 10 years ago, could evoke so much effort and as a result generate so much heat. I had hoped understanding would come more easily. I am sorry my attempts failed to make Drs. Tayek and Katz and others understand.

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REFERENCES


