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Answer

Can two intramitochondrial oxaloacetate pools unravel the dispute?

A reply to Katz

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In response to Katz' comment, we shall focus on the central issue: a discussion of the validity of the calculation, introduced by Strisower et al. [1] in the early 1950's, for estimating the rates of gluconeogenesis and Krebs cycle oxidation for the in vivo experiments presented in our recent publication [2]. Their method was designed to measure the relative contribution of acetyl-CoA carbon atoms to hepatic oxaloacetate, using the ratio of individual glucose carbons: (SA) [1-^{14}C]glucose/(SA) [3-^{14}C]glucose in their calculations.

A different approach was presented by Hetenyi and Ferrarotto [3], which is not based on the distribution of carbon atoms in the oxaloacetate or other Krebs cycle intermediates. The measurements consist of an i.v. infusion of [2-^{14}C]acetate and determination of the steady-state specific activity of plasma glucose. The apparent synthesis of glucose from acetyl-CoA is calculated from the ratio of (SA) glucose to (SA) acetoacetate in blood. This study and others [4] have been reviewed critically by Katz [5].

Our new approach [2] consists of intraintestinal administration of [2-^{13}C]acetate to starved rats and measurement of the individual ^{13}C enrichment of hepatic glucose and glutamate carbons by ^{13}C NMR and GC-MS techniques. This method avoids the laborious process of molecular degradation and isolation of specific carbons. Glutamate ^{13}C enrichment was used as a probe of Krebs cycle flux, as it represents the ^{13}C enrichment of oxoglutarate. The individual ^{13}C enrichments were compared to hepatic glucose carbon enrichments and the ratio of the ^{13}C enrichment of each carbon atom in glucose (C-1, C-2, C-5, C-6/C-3, C-4) could be tested if they really represent the ratio of the individual ^{13}C enrichments of oxaloacetate C-2, C-3/C-1, C-2. The calculated PEP enrichment used in our study [2] reflects oxaloacetate (OAA) enrichment before being diluted with the OAA pool reaching the tricarboxylic acid cycle from pyruvate carboxylated by pyruvate carboxylase. We thank Dr Katz for drawing our attention to using the correct PEP fragment derived from glutamate. Glutamate carbons 1, 2 and 3 with an average enrichment of 6.8% correspond to PEP enrichment in the steady state; the corresponding OAA enrichment is 5.9% derived fromt the equation: [2(C-1) + C-2 + C-3]/4.

According to conventional metabolic schemes all non-labeled and labeled OAA precursors are channelled towards one mitochondrial OAA pool. Hence, the parameter y, 0.4, and the derived value for the exchange of PEP carbons with those of acetyl-CoA is 72% as calculated by Katz. This cal-

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culation neglects the contribution of other sources of labeling. We have found that both lactate and alanine become labeled [2] and should be included in the calculation of the parameter y. The ^{13}C enrichment of pyruvate (2.2%) may be the result of recycling of the label from PEP to pyruvate by pyruvate kinase in the liver, and recycling of labeled glucose through extrahepatic tissue. Another source of labeled pyruvate could be the trioses synthesized in the muscle from [^{13}C]-acetyl-CoA as a result of Krebs cycle flux and malic enzyme activity, since muscle glutamate has been found to be ^{13}C-labeled (unpublished). Dilution of muscle labeled pyruvate may occur by muscle glycogenolysis.

The ^{a}OAA enrichment, 5.9% (derived from glutamate carbons 1, 2 and 3), is the result of two [^{13}C]OAA sources - from pyruvate carboxylated by pyruvate carboxylase the enrichment is $2.2 \times 0.75 = 1.65\%$ (due to introducing one non-labeled carbon) (pathway a in scheme 1), and from oxoglutarate carbons (C-2, C-3, C-4, C-5) the average enrichment is 8.8% (pathway g, scheme 1) - as was calculated in our study [2]. The ratio of the two fluxes is calculated as follows: $5.9 = 8.8Z + 1.65(1 - Z)$; $Z = 0.59$ corresponding to the rate of OAA formed in the tricarboxylic acid flux and $1 - Z = 0.41$ corresponding to the rate of OAA formed from pyruvate carboxylation by pyruvate carboxylase. The parameter y can be calculated from the new values of Z and $1 - Z$:

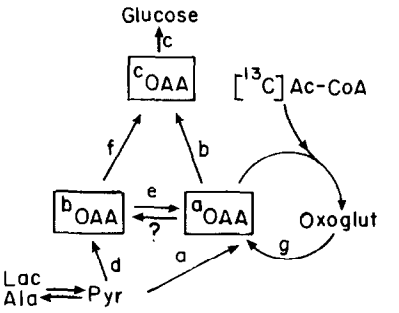
$$y = \frac{\text{pyruvate carboxylase (a)}}{\text{tricarboxylic acid flux (g)}} = 0.69$$

From the new value 0.69, it can be calculated that only 59%, instead of 72% of the carbons of PEP exchanged with those of acetyl-CoA.

To compare both methods of calculation, that presented by Strisower et al. [1] and ours, the enrichment of OAA derived from pyruvate was considered to be zero and the relative fluxes were calculated as above:

$$y = \frac{\text{pyruvate carboxylase (a)}}{\text{tricarboxylic acid flux (g)}} = 0.49$$

From $y = 0.49$, the extent of PEP carbons exchanging with those of acetyl-CoA was calculated to be 67%. The small difference obtained between the two methods of calculation indicates the validity of our approach.



Scheme 1. A model for incorporation of ^{13}C into liver metabolites from [2-^{13}C]acetate.

The above calculations for the magnitude of 'metabolic exchange' are based on the conventional concept of a common OAA pool for the tricarboxylic acid cycle and gluconeogenesis. Hence, the ^{13}C enrichment and pattern of isotope distribution of PEP corresponding to C-1, C-2 and C-3 of glutamate should be equal to that of glucose. Instead, the ^{13}C enrichment of glucose (2.3%) is markedly lower than the average ^{13}C enrichment of C-1, C-2 and C-3 of glutamate (6.8%). The significant difference is surprising. This may arise from incomplete mixing of OAA and/or dilution by a large pool of non-labeled precursors entering the system not via pyruvate. However, the estimated dilution is no more than 25% (10–25%) [6,7], and therefore cannot account for the 3-fold dilution of [^{13}C]glucose in comparison to its precursor PEP. The possibility that glycogen-glucose is a source for such dilution is excluded, since liver glycogen is depleted after a 24 h fast.

Recently, Heath and Rose [7] in their in vivo study, using bicarbonate fixation into glucose and other metabolites in the liver of starved rats, have suggested metabolic channelling of oxaloacetate formed from pyruvate towards gluconeogenesis as a result of incomplete mixing of mitochondrial oxaloacetate. The significant difference in ^{13}C enrichment of glucose and PEP (derived from glutamate) found in our study [2] could not be explained only by one mixed pool of oxaloacetate in the mitochondria. To explain the incorporation pattern of ^{13}C into hepatic metabolites from [2-^{13}C]acetate, a similar scheme to that of Heath and Rose is proposed. The proposed pathway ^{b}OAA enabled us to evaluate the contribution of two metabolic pathways for hepatic glucose synthesis: the conven-

tional pathways denoted by a, b and c and the proposed pathways via d, e and b or via d and f, for metabolic channelling of OAA from pyruvate toward gluconeogenesis.

In view of this proposal, the metabolic parameter y, should be applied only to the tricarboxylic acid cycle ^{a}OAA, therefore even the corrected value 59% for PEP carbons exchanging with acetyl-CoA via the Krebs cycle is overestimated.

If we assume that the experimental result for glucose enrichment (2.3%) is an underestimate, due to ~20% dilution by unlabeled non-pyruvate precursors, then the corrected value will be 23/0.8 = 2.9%. When this value is used to calculate the contribution of ^{a}OAA to the newly synthesized glucose, the ratio of glucose enrichment (2.9%) to ^{a}PEP (6.8%) is 43%. Hence, 57% of the newly synthesized glucose is not formed via ^{a}OAA, instead channelling of pyruvate via ^{b}OAA is suggested. Therefore, the parameter y should be corrected and applied only to 43% of the newly synthesized glucose; the resulting metabolic exchange should be $43 \times 0.59 = 25\%$. In this calculation the contribution of labeled pyruvate via ^{b}OAA to cytosolic ^{c}OAA is not accounted for. When the enrichment of pyruvate is included in the calculation, the ratio of two OAA pools can be estimated as follows: $^{c}\text{PEP} = {}^{a}\text{PEP} \cdot X + \text{Pyr}(1 - X)$, which corresponds to $2.9 = 6.8 \cdot X + 2.2(1 - X)$; $X = 15\%$. The ^{a}OAA pool is only 15% of the ^{c}OAA pool and the corresponding metabolic exchange value is $15 \times 0.59 = 9\%$, indicating that only 9% of the newly synthesized glucose carbons are derived by exchanging with acetyl-CoA via the tricarboxylic acid cycle.

This value might be underestimated, due to the unknown contribution of glucose recycling to pyruvate. Nevertheless, our results suggest that the metabolic exchange values are in the range of 9–25%.

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