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Comment

'A quantitative analysis of the metabolic pathways of hepatic glucose synthesis in vivo with ^{13}C-labeled substrates' by B. Kalderon, A. Gopher and A. Lapidot [(1987) FEBS Lett. 213, 209–214]

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Kalderon et al. [1] infused intragastrically [2-^{13}C]acetate into starved rats and isolated glucose, lactate, alanine and glutamate from the liver. The enrichment of the individual carbons of glucose and glutamate was determined by NMR and GC-MS analysis. They claim that their results show that there is negligible dilution of the carbons of pyruvate in their conversion to phospho*enol*-pyruvate (PEP) and glucose by the exchange with carbons of acetyl-CoA. The dilution is said to be 7% (abstract) or as little as 1.2% (table 2).

This conclusion goes counter to a large body of studies in vivo and in vitro. Moreover, it is a priori most implausible. As is well known, O_2 uptake by liver is 2–4-times higher than normal rates of gluconeogenesis, and most of the O_2 uptake is via oxidation in the Krebs cycle. Thus, according to universally accepted metabolic schemes, there should be a large flux of acetyl-CoA into citrate and oxaloacetate and extensive mixing of carbons from pyruvate and acetate in oxaloacetate and subsequently PEP and glucose.

The authors' calculation is based on the findings that the ^{13}C enrichment in carbons of glucose and lactate-alanine is much the same, as well as the ^{13}C-enrichment pattern in glutamate. I am unable to follow the rationale of their calculations. Lactate-alanine molecules were formed extrahepatically and their enrichments are not related to the Krebs cycle. Actually, it is clear from their data that there is extensive flux in the Krebs cycle and the dilution of pyruvate carbon of oxaloacetate by that of acetyl-CoA is extensively, completely opposite to the authors' conclusions.

In 1952, Strisower et al. [2] (see also the appendix in review [3]) showed for administration of [2-^{14}C]acetate how the relative rates of gluconeogenesis and Krebs cycle oxidation can be calculated from the ratios of specific activities of carbons 2 and 3 of oxaloacetate to the carboxyl carbons. This ratio corresponds to the ratio of specific activity of carbons 1, 2, 5 and 6 (which are equal) to carbons 3 and 4 of glucose reviews [4,5].

Strisower et al. [2] have shown that the tracer patterns in oxaloacetate or glucose are a function of the relative rates of the flux to PEP (or pyruvate carboxylation) to that of citrate synthesis (oxidative flux). If the ratio of the specific activity (or enrichment) of the inner carbons to the carboxyl carbons of oxaloacetate, y, or the corresponding

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ratios in glucose are designated as R, then

$$y = \frac{\text{pyruvate carboxylation}}{\text{citrate synthesis}} = \frac{R-2}{2}$$

It follows that the limiting value of R, when there is virtually no PEP synthesis, but only oxidation, is 2. The ratio increases with the relative increase in pyruvate carboxylation and gluconeogenesis.

The authors report (table 1) for glucose near equal enrichment of carbons 1, 2, 5 and 6 of 2.85% and that of carbons 3 and 4 of 1.15%. The ratio (1.62) is even below the rate-limiting value of 2. The deviation is most likely due to recycling of carbon from glucose via labelled lactate formed in extrahepatic cells and entering the Krebs cycle in liver. This introduces tracer into the carboxyl groups of oxaloacetate. In any case, the ratio suggests extensive oxidation via the Krebs cycle.

An alternative value for the ratio R is obtained from glutamate, since C-2 and C-3 correspond to carbons 2 and 3 of oxaloacetate and C-1 of glutamate to its carboxyl carbons. From table 1 the enrichments are 5.6 and 3.1, or a ratio of 2.8. Calculation leads to a value of $y = 0.4$, or that the rate of Krebs cycle flux is some 2.5-times that of pyruvate carboxylation. This is the range of values observed in many in vitro studies, for example [6], and in vivo studies (e.g. the large series of experiments in rats and dogs by Hetenyi and Ferraroto [7]). From this value it can be calculated, according to Strisower or Katz, that 72% of the carbons of PEP exchanged with those of acetyl-CoA.

In view of the extensive recycling via lactate (formed from labelled glucose in non-hepatic tissues) and the unknown extent of pyruvate decarboxylation, no exact calculation is possible for in vivo experiments, but the results indicate that at least half of the carbons of oxaloacetate and PEP arise from exchange with acetyl-CoA, a far cry from the 7% claimed by the authors.

The authors also miscalculate the enrichment in PEP. At steady state this equals that in C-1, C-2 and C-3 of glutamate and is 3.1, 8.6 and 8.6, respectively, rather than the values 4.3, 13.4 and 13.4 calculated by the authors.

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