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## Recycling of deuterium from dideuterated glucose during moderate exercise

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A doubly labelled tracer molecule of glucose, [6,6-<sup>2</sup>H]glucose, has been used to measure the rate of glucose appearance in blood due to endogenous glucose production (by the liver and kidneys).<sup>1,2</sup> Considered 'non-recirculating', this tracer generally provides a realistic estimate of glucose kinetics.<sup>2,3</sup> Used as a dual tracer in conjunction with [1-<sup>13</sup>C]glucose, the extent of glucose recycling can be quantitated,<sup>2,4</sup> but the extent of possible recirculation of a single deuterium atom on newly formed glucose must then be considered.

The purpose of this study was to examine whether recirculation of a singly-deuterated molecule ([6-<sup>2</sup>H]glucose) does occur to a significant extent during a constant infusion of dideuterated tracer ([6,6-<sup>2</sup>H]glucose) and to quantify the extent to which it may cause overestimation of the glucose (M+1) signal and, therefore, glucose recycling determined with dual glucose tracers. Twelve men (age 28.0 ± 1.4 years) were studied in the post-absorptive state. Using a forearm vein catheter, a primed, continuous infusion of [6,6-<sup>2</sup>H]glucose was administered at a rate of 2 mg/min during 90 min of rest and 6 mg/min during 60 min of cycle exercise at 50% of the pre-determined oxygen consumption (VO<sub>2</sub>) peak in six subjects, while the other six completed the same protocol without infusion of any tracer to determine the contribution to the (M+1) signal from natural isotopic abundance (both [<sup>13</sup>C] and [<sup>2</sup>H] sources).

Arterialized blood samples were obtained from a heated hand vein in the contra-lateral arm from tracer infusion. These were analysed for glucose isotopic enrichment (measuring atoms per cent excess; APE) by gas chromatograph mass spectroscopy (GC/MS; Hewlett

Packard GC Model 5890, Series II, and an MS Model 5989A; Hewlett Packard, Palo Alto CA, USA) in the chemical ionization (CI) mode. A penta-acetate derivative of glucose allowed ions to be selectively monitored at *m/z* 331.2, 332.2, and 333.2.<sup>1</sup>

Steady-state conditions for enrichment of the (M+2) tracer were achieved during the final 30 min of rest and exercise (1.795 ± 0.181 and 2.429 ± 0.198, respectively). The increase in enrichment of the (M+1) signal was 0.107 ± 0.053% above baseline at rest and 0.203 ± 0.043% during moderate exercise during infusion of the glucose tracer (see Fig. 1). In control subjects, the (M+1) signal at each time point was unchanged from baseline values.

Data from the current study were used to recalculate glucose recycling rates from our previous work.<sup>4</sup> The corrected values were 6.88 ± 3.28% during rest instead of 8.60 ± 3.85, and 9.93 ± 5.21% during exercise instead of 11.90 ± 5.22, representing a 20.0 and 17.0% previous overestimation of recycling during rest and exercise conditions, respectively.

In this study, infusion of a dideuterated glucose isotopic tracer resulted in a measurable amount of tracer recycling into newly formed deuterated glucose, thus increasing the (M+1) signal. This recycling caused underestimation of total glucose production calculated previously with a [1-<sup>13</sup>C]glucose tracer as well as overestimation of total glucose recycling calculated from the simultaneous use of [1-<sup>13</sup>C]glucose and [6,6-<sup>2</sup>H]glucose under this protocol.

In many studies, both stable isotope carbon and hydrogen glucose tracers are infused simultaneously in order to assess glycolytic carbon recycling through gluconeogenesis in the liver.<sup>2</sup> In the gluconeogenic process, labelled lactate is reconverted to glucose, thereby raising the measured enrichment of the carbon tracer by the amount of recycled [<sup>13</sup>C] label. Deuterium on

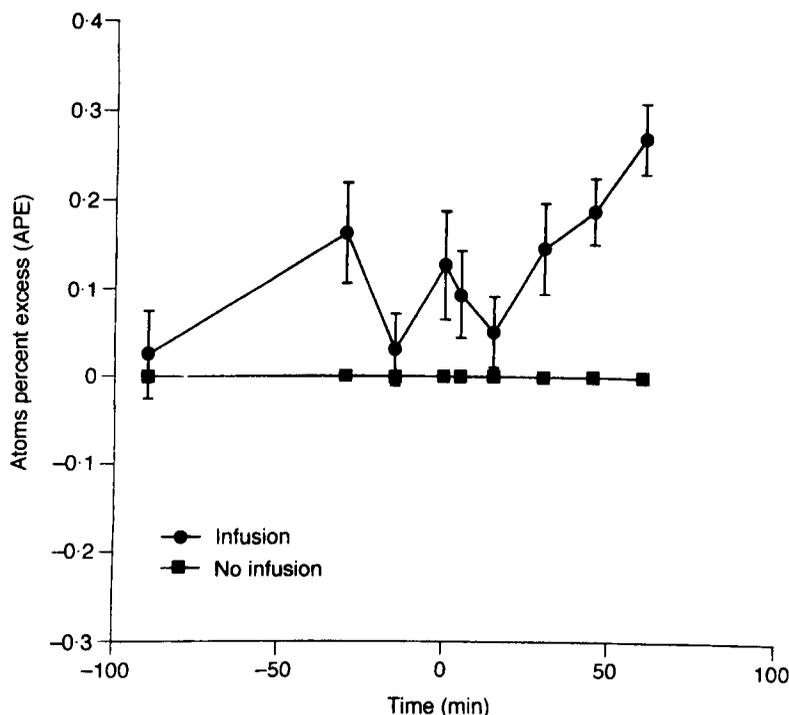


FIGURE 1. Increase in  $(M+1)$  enrichment (APE) with  $[6,6-^2\text{H}]$ glucose infusion during rest ( $-90$  to  $0$  min) and exercise ( $0$  to  $60$  min). Data are given as mean  $\pm$  standard error of the mean for six subjects at each time point.

the sixth carbon is not lost during glycolysis *per se*, but rather at two possible sites during gluconeogenesis. One deuterium is lost in the pyruvate carboxylase reaction converting pyruvate to oxaloacetate.<sup>5</sup> A deuterium may also be lost from the fifth carbon in the equilibration with the mitochondrial hydrogen pool during equilibration between oxaloacetate, malate and fumarate in the Krebs cycle. If only one deuterium is lost from the dideuterated tracer instead of both, then newly formed glucose will actually be  $[6-^2\text{H}]$ glucose instead of unlabelled glucose, thus increasing the  $(M+1)$  signal as our data indicate. In accordance with our results, Kalhan<sup>5</sup> presented data from one subject showing an increase in the  $(M+1)$  enrichment in blood during a constant infusion of  $[6,6-^2\text{H}]$ glucose at rest, indicating recycling of a single deuterium atom.

In summary, our data demonstrate that recirculation of a singly-deuterated glucose during a constant infusion of  $[6,6-^2\text{H}]$ glucose will cause an overestimation of the glucose  $(M+1)$  signal. While all calculated recycling values from our previous study<sup>4</sup> were in range comparable to published research using similar

tracers at rest,<sup>3</sup> we have now demonstrated that glucose recycling in our study was actually overestimated with the simultaneous use of these two tracers. These results are applicable to all studies incorporating dual isotopic tracers where one of the tracers is  $[6,6-^2\text{H}]$ glucose and the other tracer increases the  $(M+1)$  signal. Our new data allow for the actual quantification and correction of recycling under these specific conditions and rates of tracer infusion. However, another solution to this problem may be to use an alternate methodology for analysis that may be able to effectively account for the recycling of deuterium.

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