Enrichment of hepatic glycogen and plasma glucose from $H_2{}^{18}O$ informs gluconeogenic and indirect pathway fluxes in naturally feeding mice

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Deuterated water (^2H_2O) is a widely used tracer of carbohydrate biosynthesis in both basic science and clinical research, but the significant kinetic isotope effects (KIE) of ^2H can distort metabolic information and mediate toxicity. *^{18}O-water ($H_2{}^{18}O$) has no significant kinetic isotope effects and is incorporated into specific carbohydrate oxygens via well-defined mechanisms, but to date, it has not been evaluated in any animal model.*

Here, mice were given $H_2{}^{18}O$ during overnight feeding, and ^{18}O-enrichments of liver glycogen, triglyceride glycerol (TG), and blood glucose were quantified using ^{13}C NMR spectroscopy. The ^{18}O isotope causes a small shift in the ^{13}C signal, compared to ^{16}O, allowing fractional enrichments to be measured at levels of 1-2%. A very high signal to noise ratio is required to detect the small amount of shifted ^{13}C signals and requires the use of a highly sensitive cryoprobe at 800 MHz to be feasible. Enrichment of oxygens 5 and 6 relative to body water informed indirect pathway contributions from the Krebs cycle and triose phosphate sources (shown in the figure to the right). Compared with mice fed normal chow, mice whose diet was supplemented with a fructose/glucose mix had significantly higher indirect pathway contributions from triose phosphate sources, consistent with fructose glycogenesis. Blood glucose and liver triglyceride glycerol ^{18}O-enrichments were quantified by mass spectrometry. Blood glucose ^{18}O-enrichment was significantly higher for high sugar versus control mice and was consistent with gluconeogenic fructose metabolism. Triglyceride glycerol ^{18}O-enrichment was extensive for both Control and High sugar mice, indicating a high turnover of liver triglyceride, independent of diet. *Thus, $H_2{}^{18}O$ informs hepatic carbohydrate biosynthesis in similar detail to ^2H_2O but without kinetic isotope-associated risks that can skew data.* This basic research may lead to more accurate analyses of metabolic pathways in the future.

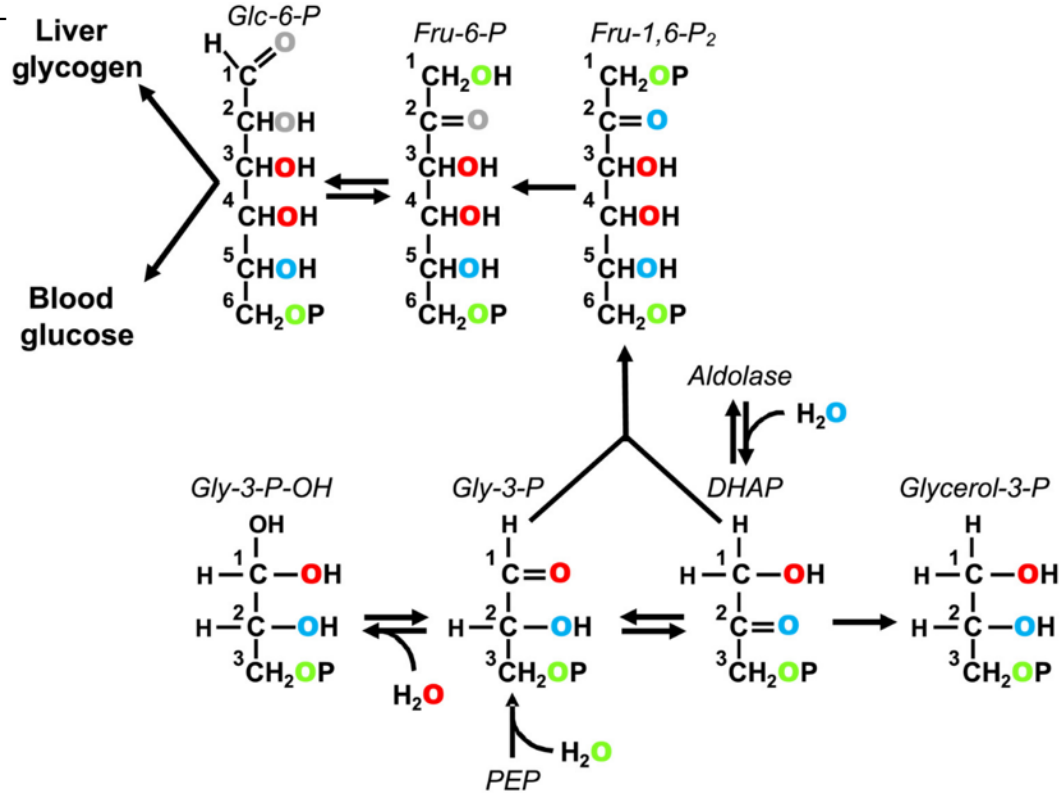

Figure 1. Incorporation of ^{18}O from water into carbohydrate precursor metabolites at the level of phosphoenolpyruvate (PEP) and triose phosphates. Exchange of dihydroxyacetone phosphate (DHAP) with aldolase results in the incorporation of ^{18}O into position 2 (**blue**). The reversible hydration of Gly-3-P to form an acetal (Gly-3-P-OH) results in the incorporation of ^{18}O into position 1 of Gly-3-P **(red)**. Addition of water to PEP via enolase incorporates ^{18}O into position 3 of Gly-3-P **(green)**. Triose phosphate isomerase exchange and aldolase generate Fru-1,6-P_2 enriched in positions 1 and 6. The position 2 oxygen of fructose-6-phosphate (Fru-6-P) can undergo additional exchange with water via anomerization (shown in gray). Likewise, the position 1 oxygen of both glucose-6-phosphate (Glc-6-P) and glucose can be exchanged with water via anomerization (also shown in gray)

Facilities and instrumentation used: AMRIS, Bruker Avance III 800MHz.

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