Sources of hepatic glycogen synthesis during an oral glucose tolerance test: effect of transaldolase exchange on flux estimates

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Introduction: Following ingestion of a meal and carbohydrate absorption, plasma glucose levels increase and promote the secretion of insulin by pancreatic β-cells. Insulin enhances glycogen synthesis from hepatic glucose-6-phosphate (G6P) primarily by activating glycogen synthase and inhibiting glycogen phosphorylase. The G6P used for hepatic glycogen synthesis is derived from two distinct pathways: direct conversion from glucose *via* glucokinase and indirect synthesis involving 3-carbon precursors (1, 2). The 3-carbon sources for the indirect pathway may originate from hepatic glycolysis of glucose as well as from non-glucose (gluconeogenic) precursors such as lactate, amino acids and glycerol. Direct and indirect pathway contributions to hepatic glycogen synthesis can be examined following ingestion of a tracer and analysis of plasma glucose and hepatic uridine diphosphate-glucose (UDP-glucose) enrichments. Previously, indirect pathway was found to contribute for 30-50 % to hepatic glycogen synthesis in healthy subjects following a mixed meal (3-5) or a glucose load (6, 7).

Methods: Sources of hepatic glycogen synthesis during an oral glucose tolerance test (OGTT) were evaluated in six healthy subjects by enrichment of a 75 gram glucose load with 6.67% [U-^{13}C]glucose and 3.33% [U-$^{2}H_7$]glucose and analysis of plasma glucose and hepatic UDP-glucose enrichments (sampled as urinary menthol glucuronide) by ^{2}H and ^{13}C NMR spectroscopy.

Results: The direct pathway contribution, as estimated from the dilution of [U-^{13}C]glucose between plasma glucose and hepatic UDP-glucose, was unexpectedly low (36 ± 5 %). With [U-$^{2}H_7$]glucose, direct pathway estimates based on the dilution of position 3 ^{2}H-enrichment between plasma glucose and glucuronide were significantly higher (50 ± 6 %, p = 0.05). These differences reflect the exchange of the carbon 456 moiety of fructose-6-phosphate and glyceraldehyde-3-phosphate catalyzed by transaldolase. As further evidence of this exchange, ^{2}H-enrichments in glucuronide positions 4 and 5 were less than that of position 3. From the difference in glucuronide positions 5 and 3 enrichments, the fraction of direct pathway carbons that experienced transaldolase TA) exchange was estimated at 21±4%.

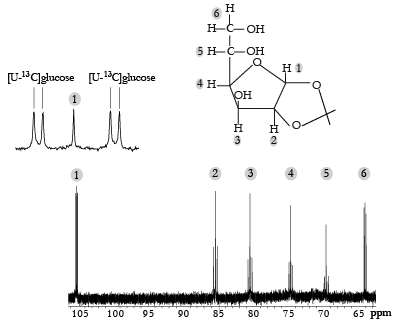

Figure 1. ^{13}C NMR spectrum of monoacetone glucose (MAG) derived from plasma glucose collected 3 h post-load. ^{13}C NMR signals from carbons 1-6 of MAG are indicated. In the inset, C1 signals are expanded and the singlet corresponding to the natural abundance and the isotopomers resultant of [U-^{13}C]glucose are observed.

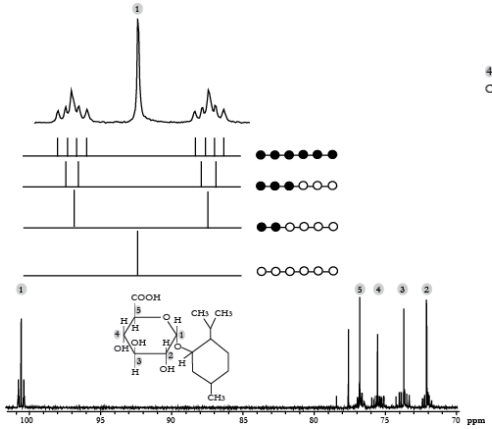

Figure 2. ^{13}C NMR spectrum of urinary menthol glucuronide collected 2-4 h post-load. ^{13}C NMR signals from carbons 1-5 of menthol glucuronide are indicated. In the inset, C1 signals are expanded and the isotopomers resultant of hepatic glucose metabolism assigned.

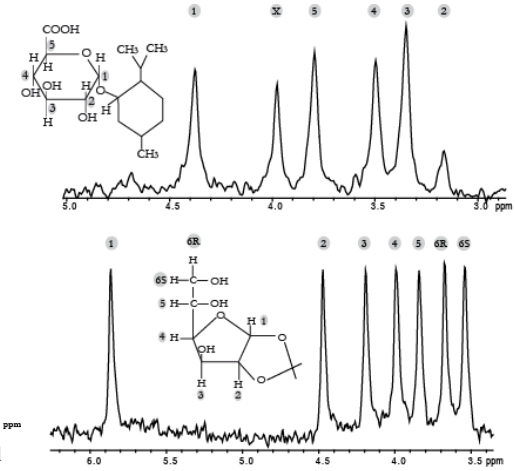

Figure 3. ^{2}H NMR spectra of monoacetone glucose (MAG) derived from plasma glucose collected post-load (bottom) and from menthol glucuronide collected 2-4 h post-load (upper). ^{2}H NMR signals from protons 1-6 of MAG and 1 to 5 of menthol glucuronide are indicated.

Conclusions: Novel ^{13}C and ^{2}H glucose tracers were used to determine direct and indirect pathway contributions to hepatic glycogen synthesis during OGTT by ^{13}C and ^{2}H NMR analysis of plasma glucose and urinary glucuronide enrichments. Exchanges of both carbon and hydrogen moieties during the direct pathway metabolism of glucose were further revealed and quantified. Enrichment of glucuronide from any glucose tracer that is enriched in carbons 4, 5 and 6 (including [U-^{13}C]glucose) will be modified by TA exchange in addition to direct and indirect pathway fluxes. During OGTT, ~21% of direct pathway flux was involved in this exchange hence the values derived from the [U-^{13}C]glucose tracer resulted in underestimates of the direct pathway contribution. For glucose enriched with ^{2}H, the position 3 label is unaffected by hexose phosphate exchanges hence direct pathway estimates based on [3-^{2}H]glucose/glucuronide enrichment ratios are insensitive to TA exchange and therefore reflect true direct and indirect pathway fluxes. In conclusion, the results presented in here demonstrate that during an OGTT in healthy humans, half of hepatic glycogen synthesis is derived from 3-carbon precursors rather than directly from the glucose load.

References: 1. Newgard CB *et al.* (1983) J Biol Chem;258(13):8046-8052; 2. Newgard CB *et al. (*1984) J Biol Chem;259(11):6958-6963; 3. Roy T *et al.* (1996) J Clin Invest 1996;97:126-132; 4. Bischof MG *et al.* (2002) Diabetes;51(1):49-54; 5. Jones JG *et al.* (2006) Diabetes;55(8):2294-2300; 5. Napoli R *et al*. (1992) J Clin Physiol;12(6):641-652; 6. Petersen KF *et al.* (2001) Metabolism 2001;50(5):598-601.