Analysis of ^2H Enrichment in all Positions of Plasma Glucose by ^2H NMR Spectroscopy Following Infusion of ^2H_2O

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INTRODUCTION: When functioning hepatocytes are presented with deuterated water, they generate glucose enriched with deuterium. The glucose ^2H-enrichment pattern reflects the metabolic origins of hepatic glucose-6-phosphate hence its measurement provides a valuable insight into hepatic glucose metabolism. This includes quantitation of the contribution of glycogen, glycerol and phosphoenolpyruvate (PEP) to the total hepatic glucose output (see Figure 1) based on the relative enrichment of ^2H in positions 2, 5 and 6 of glucose[1]. Current GC-MS methods for measuring ^2H-

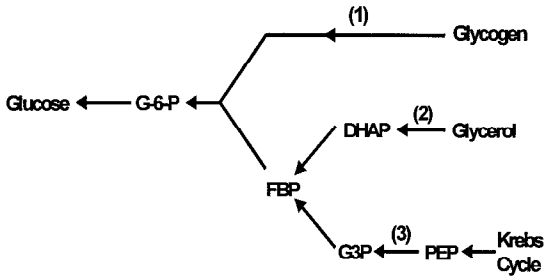

Figure 1: Sources of plasma glucose during fasting: (1), glycogenolysis; (2) gluconeogenesis from glycerol and (3) gluconeogenesis from PEP and the Krebs cycle.

enrichment in glucose have the necessary sensitivity but involve highly complicated derivatization methods. Furthermore, the analysis cannot be performed on glucose containing tracer levels of excess ^{13}C-enrichment since it contaminates the mass isotopomer signal assigned for ^2H-enrichment. ^2H NMR analysis of glucose has sufficient sensitivity but the ^2H-resonances are poorly resolved. We therefore applied a new method based on ^2H NMR spectroscopy of a monoacetone derivative of glucose[2] (Fig 2).

The ^2H NMR spectrum of monoacetone glucose at

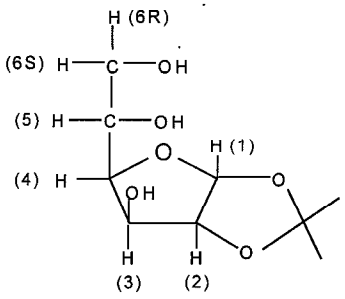

Figure 2: Chemical structure of the monoacetone glucose derivative. Parentheses refer to the hydrogen positions of the parent glucose molecule.

14.1T has fully resolved resonances allowing deuterium enrichment in all positions of glucose to be measured. The analysis is insensitive to the presence of low levels of ^{13}C-enrichment from ^{13}C-tracers of hepatic glucose output and gluconeogenesis. Conversion of plasma glucose to the monoacetone derivative is simple and quantitative.

METHODS: Rats weighing 180-220g, after fasting for 4 hours, were cannulated via the jugular vein under ketamine/xylazine anesthesia. They were infused with 99% ^2H_2O at a rate of 1 ml/hour over 2.5 hours. Body water ^2H-enrichment at the end of infusion was estimated to be 2.7± 0.1%. At 2.5 hours, 4 ml of blood was drawn from the carotid artery and the animal was sacrificed. Blood was immediately centrifuged and the plasma was deproteinized with perchloric acid and the protein precipitated by centrifugation. The supernatant was neutralized with KOH, centrifuged to remove $KClO_4$, and lyophilized to complete dryness. The extract was dissolved in 5 ml methanol, heated to 50°C, stirred for 15 minutes and centrifuged. The methanolic supernatant was lyophilized and glucose was converted to monoacetone glucose using the method of Landau *et al*[1]. Monoacetone glucose extracts were dissolved in a 90% acetonitrile-10% water containing a few grains of sodium bicarbonate. Fully-relaxed ^2H NMR spectra were gathered at 50°C using a 90° pulse and a 1-second acquisition time[2]. Spectra were obtained with a 5-mm broadband probe in a Varian Inova 14.1T spectrometer. Peak areas were measured using the NUTS curve-fitting program. The contribution of glycogen, glycerol and PEP to total glucose output was calculated from the relative enrichments in H2, H5 and H6[1]. Experimental data are reported as the mean ± standard deviation.

RESULTS: Figure 3 shows a representative ^2H NMR

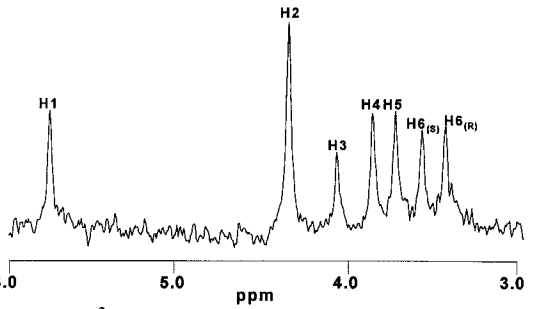

Figure 3: ^2H NMR Spectrum of monoacetone glucose obtained from rat plasma glucose. Spectrum represents the sum of 2,000 acquisitions (33 minute collection time).

spectrum of monoacetone glucose obtained from a single rat blood sample. All deuterium resonances, including the prochiral H6 signals, are fully resolved. Relative enrichments (with H2 set at 100%) were as follows: H1=56 ± 2; H3=40 ± 3; H4=50 ± 3; H5=52 ± 5; $H6_{(R)}$= 44 ± 2 and $H6_{(S)}$=42 ± 4.

The relative contributions to hepatic glucose output were as follows: glycogenolysis = 48 ± 5%, glycerol = 8 ± 7% and PEP = 43 ± 4%. The relative contributions of glycogenolysis and total gluconeogenesis (PEP + glycerol) are in good agreement with recent GC/MS measurements in postabsorptive rats[3]. In conclusion, ^2H NMR spectroscopy of monoacetone glucose provides a simple, sensitive and complete analysis of ^2H-enrichment patterns in plasma glucose following administration of tracer levels of ^2H_2O.

REFERENCES:

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3. Peroni *et al.* (1995) *Am. J. Physiol.* **269**, E516-E523.