Integrated NMR Measurements of Hepatic Glucose Production and Gluconeogenic Fluxes in Humans

John G. Jones, Michael A. Solomon, A. Dean Sherry, F. Mark H. Jeffrey and Craig R. Malloy
The Mary Nell and Ralph Rogers Magnetic Resonance Center, U.T. Southwestern Medical Center, Dallas TX.

INTRODUCTION The hepatic Krebs cycle is the principal conduit of carbons and energy for glucose synthesis and recycling thereby playing a major role in glucose homeostasis. While stable-isotope tracer measurements of human hepatic Krebs cycle activity are currently well developed in terms of metabolic models and flux predictions, they remain poorly developed as practical clinical tools.

Recently, we demonstrated that relative measurements of Krebs cycle fluxes, pyruvate recycling and gluconeogenic outflow could be simply obtained by ^{13}C-NMR analysis of plasma glucose following ingestion of [U-^{13}C]propionate[1]. These can be converted to absolute values if the absolute flux through one of the component pathways can be measured. In postabsorptive humans, hepatic glucose output (as measured by glucose turnover) is the only accessible measurement of absolute hepatic carbon flux. Following a prolonged fast, hepatic glucose output provides a good estimate of absolute gluconeogenic outflow since there is little contribution of glucose carbons from other sources[2]. With shorter fasts however, a significant fraction of hepatic glucose is derived from glycogen[2]. If this is not accounted for, absolute gluconeogenic outflow and related Krebs cycle fluxes will be significantly and systematically overestimated.

As a first step towards obtaining absolute gluconeogenic and Krebs cycle flux measurements from plasma glucose, we demonstrate that hepatic glucose output and relative fluxes at the Krebs cycle level can be simultaneously measured from glucose isotopomer analysis. This is achieved by infusion of [1,6-$^{13}C_2$] glucose, a novel tracer of hepatic glucose output[3] and concurrent ingestion of [U-^{13}C]propionate. Furthermore, we demonstrate that our measurements can be performed in the setting of Landau's D_2O-ingestion protocol for estimating the fractional contribution of gluconeogenesis to the total glucose output[2]. Thus, integration of both methods could provide absolute gluconeogenic outflow and Krebs cycle flux measurements within the practical constraints of clinical applications.

METHODS All protocols were approved by the institutional review board for human studies at U.T. Southwestern. Subjects (3F, 2M, 70 ± 14 kg) began fasting at 6:00 pm the previous day. At 11:00 pm and 3:00 am, subjects ingested 99% D_2O (2.5g/kg body water) and for the remainder of the study they ingested 1% D_2O *ad libitum*. From 7:00-8:00 am, subjects ingested phenyl-acetate (20 mg/kg) and Tylenol (1000 mg). At 8:00 am, a 3-hour primed infusion of [1,6-$^{13}C_2$]glucose (133 mg, 1.33 mg/min) was initiated for each subject. In addition, the subjects ingested [U-^{13}C]propionate (10 mg/kg, packaged into 3 gelcaps) from 8:00-9:00 am. Beginning at 8:00 am, 10 mls of blood were drawn every 20 minutes for 2 hours with additional draws at 2.5 and 3 hours. The first blood sample was drawn immediately before administration of the ^{13}C tracers. Urine was also collected every hour up to 6 hours. Blood samples were deproteinized, lyophilized and resuspended in 0.6 ml D_2O. ^{13}C NMR spectra were obtained using a 5 mm broadband probe on a 14.1T Varian INOVA spectrometer operating at 150.9 MHz. ^{1}H Spectra were obtained with the same spectrometer using a 5-mm indirect probe. For ^{13}C NMR spectra, 18,000 acquisitions were taken over 14 hours and for ^{1}H NMR spectra, 256-512 acquisitions were taken over 1-2 hours. ^{13}C and ^{1}H NMR spectra were analyzed using NUTS (Acorn NMR, Fremont, CA). Hepatic glucose output and relative Krebs cycle fluxes were calculated from ^{13}C and ^{1}H NMR spectra of plasma glucose as described previously.[3] All data are reported as mean ± standard deviation.

RESULTS AND DISCUSSION ^{13}C NMR spectra of blood samples taken before ^{13}C-tracer administration showed natural-abundance singlets and no multiplets. The glucose C1 enrichment in these samples as measured by ^{1}H NMR[3] was 1.00 ± 0.06%. ^{13}C NMR spectra of subsequent blood draws featured a doublet contribution from [1,6-$^{13}C_2$]glucose and multiplets arising from the conversion of [U-^{13}C]propionate to glucose isotopomers. The C1 enrichment at 40 minutes was 2.43 ± 0.27%, cresting at 120 minutes (3.07 ± 0.57%). Since metabolism of [1,6-$^{13}C_2$]glucose does not enrich the C2 position to a significant extent[3], C2 enrichments were systematically less than those of C1, ranging from 1.4 ± 0.3% at 40 minutes to 2.0 ± 0.4% at 120 minutes. Figure 1 shows the ^{13}C spectrum and isotopomer assignments for a 120-minute blood glucose sample. The C1β resonance features a well-resolved contribution from [1,6-$^{13}C_2$]glucose (D16) while D12 and Q represent contributions from 1,2 and 1,2,3-labeled isotopomers generated from gluconeogenic metabolism of [U-^{13}C]propionate. The C2β multiplet also has components from 1,2 and 1,2,3-glucose isotopomers (D12+Q) plus an additional contribution from the 23-isotopomer (D23).

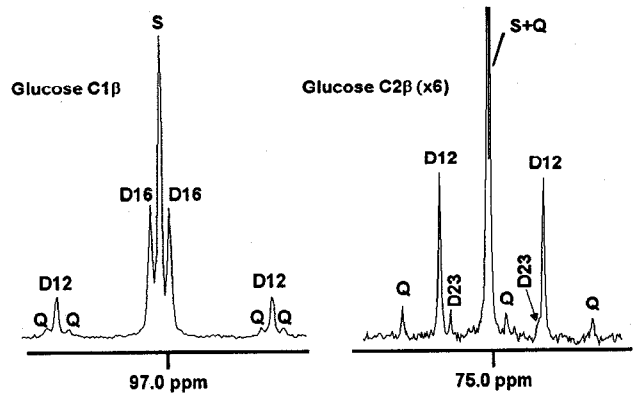

Figure 1: Glucose C1β and C2β resonances from a ^{13}C NMR spectrum of blood drawn at 120 minutes.

From the [1,6-$^{13}C_2$]glucose isotope dilution measurements, hepatic glucose output was 1.90 ± 0.21 mg/kg/min. These estimates are in good agreement with recent measurements of glucose output in overnight-fasted subjects using other tracers and analytical methods[2,4]. From the glucose C2β isotopomer analysis, the following fluxes (relative to a citrate synthase flux of 1.0) were estimated[3]. Total anaplerotic influx into the Krebs cycle was estimated to be 6.64 ± 0.66; total pyruvate recycling flux (pyruvate kinase + malic enzyme + Cori cycle) was estimated to be 4.79 ± 0.54 and net gluconeogenic outflow (expressed as PEP units) was found to be 1.85 ± 0.35. As was found in our previous study of 24-hour fasted subjects[1], a substantial fraction of anaplerotic carbons are recycled via pyruvate and the net gluconeogenic flux is about twice that of citrate synthase.

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