

Quantitation of Gluconeogenesis by ^2H Nuclear Magnetic Resonance Analysis of Plasma Glucose Following Ingestion of $^2\text{H}_2\text{O}$

John G. Jones,* Rui A. Carvalho,† A. Dean Sherry,*‡ and Craig R. Malloy*§

*Mary Nell and Ralph B. Rogers Magnetic Resonance Center, Department of Radiology, University of Texas Southwestern Medical Center, Dallas, Texas 75235-9085; †Department of Biochemistry, University of Coimbra, Coimbra, Portugal;

‡Department of Chemistry, University of Dallas, Richardson, Texas; and §Department of Veterans Affairs Medical Center, Dallas, Texas

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We present a simple ^2H NMR assay of the fractional contribution of gluconeogenesis to hepatic glucose output following ingestion of $^2\text{H}_2\text{O}$. The assay is based on the measurement of relative deuterium enrichment in hydrogens 2 and 3 of plasma glucose. Plasma glucose was enzymatically converted to gluconate, which displays fully resolved deuterium 2 and 3 resonances in its ^2H NMR spectrum at 14.1 T. The signal intensity of deuterium 3 relative to deuterium 2 in the gluconate derivative as quantitated by ^2H NMR was shown to provide a precise and accurate measurement of glucose enrichment in hydrogen 3 relative to hydrogen 2. This measurement was used to estimate the fractional contribution of gluconeogenesis to hepatic glucose output for two groups of rats; one group was fasted for 7 h and the other was fasted for 29 h. Rats were administered $^2\text{H}_2\text{O}$ to enrich total body water to 5% over the last 4–5 h of each fasting period. For the 7-h fasted group, the hydrogen 3/hydrogen 2 enrichment ratio of plasma glucose was 0.32 ± 0.09 ($n = 7$). This indicates that gluconeogenesis contributed $32 \pm 9\%$ of total hepatic glucose output with glycogenolysis contributing the remainder. For the 29-h fasted group, the hydrogen 3/hydrogen 2 enrichment ratio of plasma glucose was 0.81 ± 0.10 ($n = 6$), indicating that gluconeogenesis supplied the bulk of hepatic glucose output ($81 \pm 10\%$).

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When functioning hepatocytes are presented with $^2\text{H}_2\text{O}$, they generate glucose enriched with deuterium at predictable positions. Enrichment of hydrogen 2 (H2) from $^2\text{H}_2\text{O}$ occurs at the level of glucose 6-phosphate

(G6P)¹ and thereby reflects the sum of glycogenolytic and gluconeogenic fluxes. Enrichment of H3–H6 occurs at the level of triose phosphates or below, representing the contribution of gluconeogenic precursors to the total glucose output (1–3). Enrichment of H5 is considered the most representative of total gluconeogenesis since it reflects the contributions of phosphoenolpyruvate (PEP) plus glycerol to hepatic glucose output (2). Enrichment of H3 is another potential marker of total gluconeogenesis since this position is enriched by $^2\text{H}_2\text{O}$ at the level of triose phosphate isomerase. In an early study of $^3\text{H}_2\text{O}$ metabolism by hepatocytes, it was concluded that glucose H3 and H5 are labeled to the same extent following gluconeogenic activity and that either site is a good marker of gluconeogenesis (3).

Because $^2\text{H}_2\text{O}$ is relatively safe, inexpensive, and easily administered to humans, measurement of the plasma glucose H5/H2 ratio has been applied in the clinical setting to determine the fractional contribution of gluconeogenesis to hepatic glucose output (1, 2, 4–7). However, the GC/MS derivatization procedure, while elegant, is technically very demanding. 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. Therefore, we used ^2H NMR as an alternative method of measuring positional deuterium enrichment in glucose. The effective sensitivity of ^2H is comparable to that of ^{13}C (8) while its low natural abundance (0.015%) gives an acceptably low back-

¹ Abbreviations used: G6P, glucose 6-phosphate; PEP, phosphoenolpyruvate; G3P, glyceraldehyde 3-phosphate; DHAP, dihydroxyacetone phosphate.

ground signal. This includes human measurements where glucose ^2H enrichments range from about 0.15 to 0.50%² (1, 2). Most significantly, ^2H NMR is insensitive to tracer levels of ^{13}C enrichment, providing the option of measuring ^2H enrichment in the presence of labeling from ^{13}C tracers.³ However, these advantages are offset by the poor chemical shift dispersion of the ^2H NMR spectrum even at high fields. This has largely restricted the application of ^2H NMR to metabolic studies of specific ^2H -enriched precursors such as [$1\text{-}^2\text{H}$]glucose (8) or pharmacological agents (9, 10). Typically, these precursors generate a small number of ^2H -enriched end products with well-resolved ^2H resonances. In comparison, $^2\text{H}_2\text{O}$ metabolism generates a diversity of deuterated products due to the ubiquity of biochemical exchange reactions between metabolite and water hydrogens. Consequently, ^2H NMR spectra of plasma and tissue extracts following $^2\text{H}_2\text{O}$ administration feature numerous, but poorly resolved, deuterium signals (11, 12). In the case of plasma glucose, all seven aliphatic hydrogens are labeled to some extent under physiological conditions (3). As a result, the ^2H NMR spectrum of glucose obtained from these studies is highly congested with only the H1 α and H1 β signals adequately resolved (11).

Conversion of glucose to gluconate (13), while removing hydrogen 1, substantially simplifies the NMR spectrum of the remaining six hydrogens. We show here at 14.1 T that the signals of $^2\text{H}_2$ and $^2\text{H}_3$ are fully resolved by ^2H NMR, providing a simple and direct measurement of the $^2\text{H}_3/{}^2\text{H}_2$ enrichment ratio and an estimate of the fractional contribution of gluconeogenesis to hepatic glucose output.

MATERIALS AND METHODS

Rat experiments. All animal studies were given prior approval by the Institutional Animal Care and Research Advisory Committee of the University of Texas Southwestern Medical Center. Conscious, male Sprague–Dawley rats (250–300 g) were given two oral priming doses of 3.5 ml 99% $^2\text{H}_2\text{O}$, 60 min apart. After the first dose, rats were given access *ad libitum* to a maintenance dose of 5% $^2\text{H}_2\text{O}$ over the next 4 or 5 h. Recent human studies have shown that deuterium enrichment of body water and the deuterium enrichment ratios of plasma glucose reach steady-state levels within 3 h using a similar procedure (4). At the end of this time the rats were terminally anesthetized with sodium pentobarbital and 4–6 ml of blood was har-

vested from the aorta. Rats were divided into two groups. The first group was given the first $^2\text{H}_2\text{O}$ priming dose after 3 h of fasting and blood was collected after 7 h of fasting. The second group had the first priming dose of $^2\text{H}_2\text{O}$ after 24 h of fasting and blood was collected after 29 h of fasting.

Sample processing. Blood was immediately centrifuged and the plasma supernatant was deproteinized with perchloric acid, neutralized with KOH, and lyophilized. In early experiments, the extract was resuspended in 10 ml potassium acetate buffer, pH 6.5, containing 10 units each of glucose oxidase and lactate oxidase and a few grains of sodium azide. The solution was aerated overnight at room temperature, deproteinized with perchloric acid, neutralized with KOH, and lyophilized. In subsequent experiments, lactate oxidase was omitted and the plasma extract was instead purified by anion exchange chromatography prior to glucose oxidation and cation exchange chromatography following oxidation. A typical extract was dissolved in 2 ml water, the pH was adjusted to 8.0, and the solution stirred with 2 g of Dowex-1X8–acetate for 1 h. Subsequently, the resin was removed by filtration, the pH was adjusted to 6.0 by using dilute acetic acid, and glucose was oxidized as previously described (13). The sample was deproteinized with perchloric acid and then stirred with 2 g of cation-exchange resin (Dowex-50H⁺) for 1 h. After removal of the resin, the pH was adjusted to 7.0 with KOH, and the sample was lyophilized. The lyophilized samples were resuspended in 0.6 ml deuterium-depleted water and the pH was adjusted to either 7.0 or 2.8. For the low-pH conditions, the pH was adjusted to 2.8–3.0 with 70% perchloric acid and spectra were obtained within 2–3 h of pH adjustment, during which less than 5% of the gluconic acid had undergone lactonization. [$2\text{-}^2\text{H}$]Glucose and [$3\text{-}^2\text{H}$]glucose standards were dissolved in 10 ml of 10 mM sodium acetate buffer, pH 6.0, oxidized to gluconate, and analyzed by ^2H NMR. [$2\text{-}^2\text{H}$]Glucose, $^2\text{H}_2\text{O}$ and deuterium-depleted water were obtained from Cambridge Isotopes (Cambridge, MA) and [$3\text{-}^2\text{H}$]glucose was obtained from Isotec (Miamisburg, OH).

NMR methods. All spectra were obtained using a Varian Inova 14.1 T spectrometer equipped with a standard 5-mm broadband probe with the observe coil tuned to ^2H (92.1 MHz). Shimming was performed on selected gluconate resonances in the water-suppressed ^1H NMR spectrum acquired by the decoupling coil. Proton decoupling for ^2H spectroscopy was performed using a standard WALTZ-16 pulse sequence. Proton-decoupled ^2H NMR spectra were acquired using a 90° pulse and a sweep width of 920 Hz digitized into 1984 points, giving an acquisition time of 1 s. No additional interpulse delays were used in this pulse sequence. Spectra were collected with 512–5000 acquisitions and

² For example, measured enrichments of 0.15% in H5 and 0.50% in H2 give a H5/H2 enrichment ratio of 0.30. After subtracting the natural abundance contribution, the true excess enrichment ratio is 0.28 (0.135/0.485).

³ The ^{13}C NMR spectrum is likewise insensitive to tracer levels of ^2H enrichment.

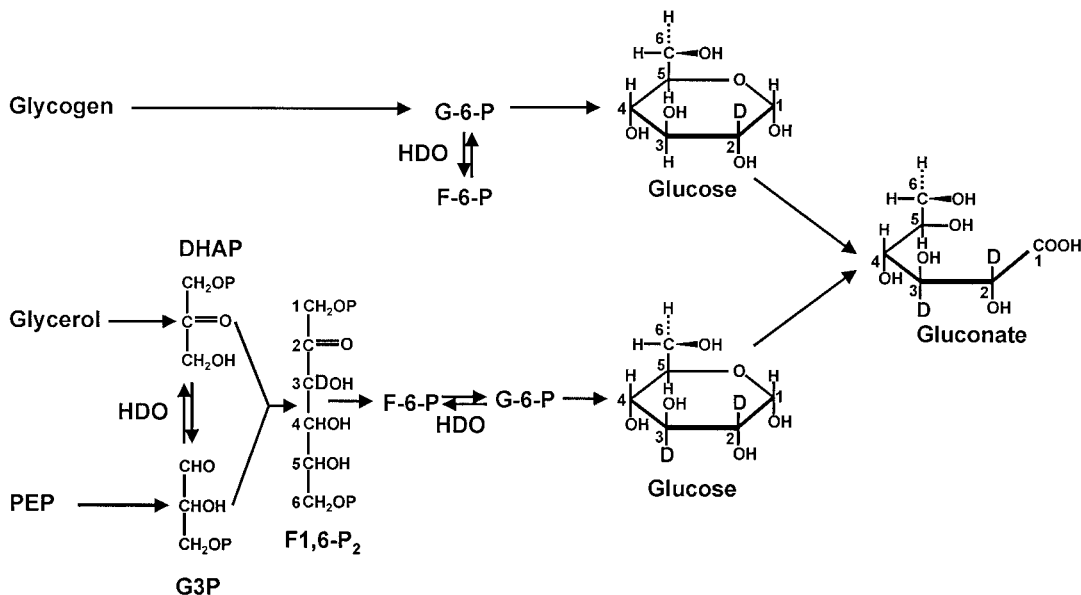


FIG. 1. Metabolic incorporation of deuterium from $^2\text{H}_2\text{O}$ into positions 2 and 3 of hepatic glucose under postabsorptive conditions. Hydrogen 2 of glucose is labeled by G6P-isomerase activity and hydrogen 3 of glucose is labeled via triose phosphate isomerase activity. Enrichment at these two sites is conserved following oxidation of glucose to gluconate.

were routinely processed by zero-filling the free-induction decay to 4K and applying 0.5–1.0 Hz of exponential multiplication. Peak areas were analyzed using the curve-fitting routine supplied with the NUTS PC-based NMR spectral analysis program (Acorn NMR Inc., Fremont, CA). ^2H relaxation times were calculated from inversion recovery experiments using the Varian exponential fitting program.

^1H NMR spectra were acquired using a 45° pulse angle, 6 kHz sweep width and 32 K points. The water peak was attenuated by using a standard presaturation pulse sequence. For each spectrum, 16 free-induction decays were collected. Gluconate ^1H chemical shift assignments were determined by using homonuclear (^1H – ^1H) and heteronuclear (^1H – ^{13}C) COSY experiments.

Theory. Figure 1 shows the incorporation of solvent protons into H2 and H3 of the hexose skeleton. The underlying assumptions include: (1) the exchange reactions shown are complete compared to the subsequent committing step, i.e., G6P–F6P exchange is complete before G6P conversion to glucose and triose phosphate exchange is complete before F1,6- P_2 synthesis; (2) all hexose units formed by glycogenolysis proceed directly to G6P and glucose, bypassing the triose phosphate pools; and (3) there is no cycling between glycogen and G6P carbons. Given these assumptions, G6P isomerase incorporates deuterium at H2 for all G6P molecules, while only glucose molecules derived from triose phosphates will also have deuterium incorporated at H3. Therefore, the H3/H2 ratio is equivalent to the H5/H2 ratio used by Landau and co-workers (2,

5) to estimate the fractional contribution of gluconeogenesis to hepatic glucose output.

RESULTS

Oxidation of glucose to gluconate. It is mandatory that the deuterium labels at H2 and H3 do not exchange with solvent protons during enzymatic oxidation of glucose to gluconate. This was tested using commercially available 99% [2 - ^2H]glucose and 98% [3 - ^2H]glucose (see Fig. 2). A ^1H NMR spectrum of gluconate generated from 99% [2 - ^2H]glucose had no proton signal for H2, indicating that no exchange had

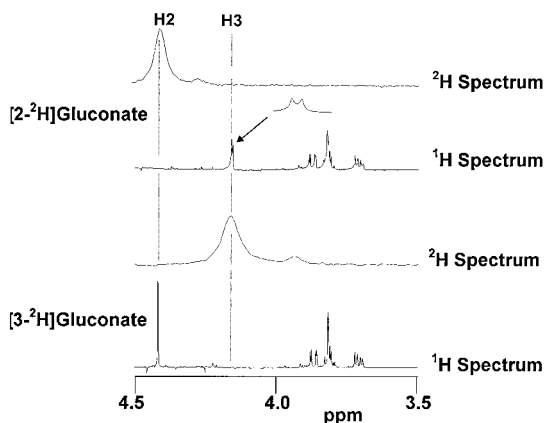


FIG. 2. ^1H and ^2H NMR spectra of gluconate derived from the oxidation of 99% [2 - ^2H]glucose (top pair) and 98% [3 - ^2H]glucose (bottom pair). Also shown is an expanded plot of the hydrogen 3 proton signal from [2 - ^2H]glucose.

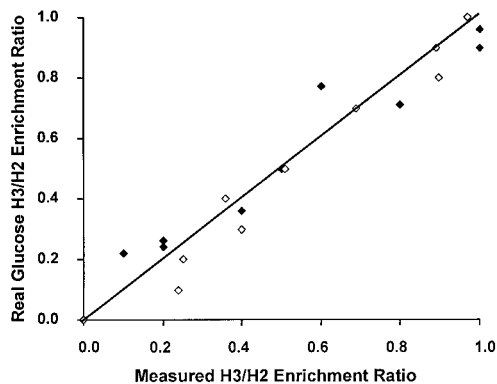


FIG. 3. Comparison of the glucose H3/H2 enrichment ratio as measured by ^2H NMR of gluconate product vs the real composition of the sample. Samples consisted of gluconate derived from 20 mg of glucose with 5% enrichment of hydrogen 2 and 0–5% enrichment of hydrogen 3. The gluconate products were analyzed at pH 2.8 (\diamond) and 7.0 (\blacklozenge) using 512 acquisitions. The collection time per spectrum was 17 min. The line of identity is also shown.

occurred between the solvent protons and deuterium label. Further confirmation was provided by the H3 proton resonance, which was split into a doublet by a single neighboring H4 proton (see inset). Likewise, a ^1H NMR spectrum of the gluconate product of 98% [$3\text{-}^2\text{H}$]glucose demonstrates that deuterium enrichment at H3 is also fully preserved during glucose oxidation. No proton signals were detected for H3, and the singlet character of the H2 resonance confirmed the absence of neighboring H3 protons.

The chemical shift difference between the H2 and H3 resonances of gluconate was found to be pH dependent, with the maximal separation of 0.27 ppm (25 Hz for ^2H at 14.1 T) occurring at pH 2.5–3.0. At pH values above 4.0, the chemical shift difference between the two resonances was substantially smaller (0.12 ppm, or 11 Hz). Our experimental deuterium linewidths were 5–7 Hz at a sample temperature of 30°C, providing baseline resolution of the resonances at pH 2.8 and adequate resolution for deconvolution of the resonances at pH 7.0. While heating the sample to 50°C significantly improved the gluconate linewidths, it also reduced the dispersion between the water and gluconate resonances because of an upfield shift in the water signal.

Quantitation of the glucose H2/H3 ratio by ^2H NMR analysis of gluconate. Standards consisting of glucose enriched with known levels of [$2\text{-}^2\text{H}$]glucose and [$3\text{-}^2\text{H}$]glucose were oxidized to gluconate and the H3/H2 ratio was determined by ^2H NMR. The NMR measurement had a precise and accurate correlation with the composition of the parent glucose samples when analyzed at both pH 2.8 and pH 7.0 (Fig. 3). For the pH 7.0 data set, the slope, intercept, and correlation coefficient were 0.964, 0.033, and 0.985, respectively, and for the pH 2.8 data set, values of 1.043,

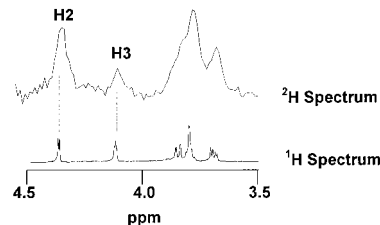


FIG. 4. ^1H and ^2H NMR spectra of gluconate derived from plasma glucose of a rat given $^2\text{H}_2\text{O}$ at 3–7 h of fasting. Spectra were obtained at a pH of 2.8 and a temperature of 25°C. The ^2H NMR spectrum represents the sum of 3000 acquisitions and took 59 min to collect.

–0.053, and 0.970 were obtained. The spin-lattice relaxation times of [$2\text{-}^2\text{H}$]gluconate and [$3\text{-}^2\text{H}$]gluconate were found to be identical to each other at both 30°C (64 ± 3 and 64 ± 6 ms, respectively) and 50°C (116 ± 8 and 115 ± 9 ms, respectively). Therefore, the 1-s acquisition time allowed full relaxation of the deuterium nuclei in between the 90° excitation pulses.

Plasma glucose analysis from rats given $^2\text{H}_2\text{O}$. Figure 4 shows representative ^1H and ^2H NMR spectra of gluconate obtained from the plasma glucose of a 7-h fasted rat. The ^1H spectrum has a crucial dual role in providing an assessment of sample purity and chemical shift registration for the ^2H signals. At low pH, the H2 and H3 deuterium resonances are fully resolved and their relative intensities provide a direct measure of the glucose H3/H2 enrichment ratio. For this group of animals, the glucose H3/H2 ratio was 0.32 ± 0.09 ($n = 7$, \pm standard deviation). This enrichment ratio indicates that gluconeogenesis supplied $32 \pm 9\%$ of total hepatic glucose output under this experimental condition. In comparison, perfused livers from overnight-fasted rats generated glucose with a H6/H2 ratio of 0.35 when presented with 5% $^2\text{H}_2\text{O}$ (14). Other studies of intact rats indicate that gluconeogenesis contributes 50% or less of the total hepatic glucose output over the initial hours of fasting (15, 16).

Figure 5 shows the ^2H and ^1H NMR spectra of glu-

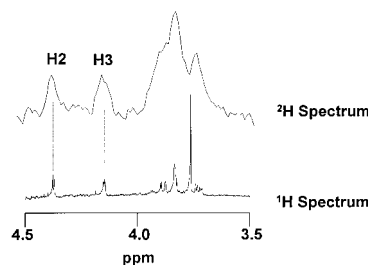


FIG. 5. ^1H and ^2H NMR spectra of gluconate derived from plasma glucose of a rat given $^2\text{H}_2\text{O}$ at 24–29 h of fasting. Spectra were obtained at a pH of 2.8 and a temperature of 25°C. The ^2H NMR spectrum represents the sum of 5000 acquisitions and took 92 min to collect.

conate obtained from a rat after 29 h of fasting. In contrast to the ^2H spectrum from the 7-h fasted rat, the H3 and H2 deuterium signals are nearly equal in this spectrum. For this group of animals, the H3/H2 ratio was 0.81 ± 0.10 ($n = 6$, \pm standard deviation), indicating that gluconeogenesis accounted for the bulk of hepatic glucose production ($81 \pm 10\%$). These findings are consistent with other reports of substantially depleted hepatic glycogen levels (17) and a high gluconeogenic contribution to hepatic glucose output (15) in rats fasted for 24 h.

DISCUSSION

Measurement of the fractional gluconeogenic and glycogenolytic contribution to the total hepatic glucose output using $^2\text{H}_2\text{O}$ is being increasingly applied to study human hepatic physiology and pathophysiology (5–7, 18, 19). In addition to its low cost and the convenience of oral administration, $^2\text{H}_2\text{O}$ rapidly distributes into body water and metabolite hydrogens that are in fast exchange with body water, such as those of G6P (1, 2, 4). Therefore, enrichment of $^2\text{H}_2\text{O}$ across the liver is uniform and the gluconeogenic flux estimate is representative of the whole liver. In comparison, carbon tracers of gluconeogenesis are not always evenly distributed across the liver and therefore may not provide representative estimates of hepatic gluconeogenesis (20, 21). However, carbon tracers are essential for measuring metabolic fluxes at the Krebs cycle level (22–25), the principal source of both carbons and energy for gluconeogenesis. The method reported here will allow the integration of $^2\text{H}_2\text{O}$ and ^{13}C tracer measurements of gluconeogenesis and hepatic Krebs cycle fluxes.

The stoichiometry between enrichment at H3 of glucose and gluconeogenesis is contingent on complete randomization of glyceraldehyde 3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP) carbon skeletons by triose phosphate isomerase. Studies of both [^{13}C]glycerol and [^{13}C]lactate have shown that the distribution of ^{13}C is randomized in the triose phosphate pools to an extent of 85–90% (15). The effects of incomplete randomization can be understood given the fact that 1*R*-[1- ^2H]DHAP, the precursor of [3- ^2H]glucose, is formed during the conversion of G3P to DHAP. If PEP is the sole source of triose phosphate carbons, enrichment of glucose H3 via triose phosphate isomerase is obligatory since all DHAP molecules must have been derived from G3P. Therefore under these conditions, the glucose H3/H2 ratio is exactly equal to the fraction of glucose molecules derived from gluconeogenesis. However, if DHAP is derived from an alternative source such as glycerol, enrichment of glucose H3 by $^2\text{H}_2\text{O}$ is dependent on a complete triose phosphate isomerase cycle, i.e., glycerol \rightarrow DHAP \rightarrow G3P \rightarrow 1(*R*)-[1- ^2H]DHAP \rightarrow [3- ^2H]glucose. To the extent

that exchange between triose phosphates is incomplete, the glucose H3/H2 ratio will underestimate gluconeogenesis under these conditions. Since glycerol normally contributes less than 15% of the total gluconeogenic carbons (26, 27), underrepresentation of total gluconeogenesis should be small given the extent of G3P–DHAP randomization. Cycling between glycerol and DHAP (20) provides an additional opportunity for enriching glucose H3 from glycerol. In comparison, the stoichiometry between glucose H5 enrichment and gluconeogenesis is preserved regardless of the source of gluconeogenic carbons and the extent of triose phosphate randomization (2). Hence the H5/H2 ratio of glucose is probably a more reliable measurement of gluconeogenesis especially when glycerol is a significant contributor, i.e., during prolonged fasting (2).

^2H enrichment of metabolites can also be measured indirectly by both ^{13}C and ^1H NMR spectroscopy (8, 28–30). Substitution of ^1H by ^2H perturbs the both the spin–spin coupling and chemical shifts of neighboring ^1H and ^{13}C nuclei providing a rich source of information about positional ^2H enrichment and isotopomer distributions. However, neither approach is optimal for measuring tracer levels of ^2H enrichment because the signals of interest are difficult to quantitate against a very large background signal from nondeuterated sites.

In this report, we have demonstrated that ^2H NMR can measure the H3/H2 ratio of plasma glucose enriched at 2–5% and harvested from ~ 5 ml of whole rat blood. In comparison, human plasma glucose is enriched to ~ 0.2 – 0.5% following the $^2\text{H}_2\text{O}$ ingestion protocol of Landau and co-workers, about 10% of the enrichment levels used here. Therefore, in the setting of Landau's experiment, ~ 50 ml of blood would need to be sampled to preserve the sensitivity of the ^2H NMR measurement, assuming similar blood glucose levels for rats and humans. While this sample size is large compared to the 3–5 ml used in GC/MS measurements of glucose ^2H enrichment (1, 2, 4), it is comparable to the 20–100 ml of blood harvested for human carbon tracer studies (22, 23, 25). To this end, we have recently demonstrated the measurement of glucose H3/H2 ratios using ^2H NMR from 30 ml of human blood in the setting of Landau's $^2\text{H}_2\text{O}$ ingestion protocol and in the presence of ^{13}C tracers (11).

In conclusion, we have shown that ^2H NMR spectroscopy of gluconate can be used to resolve and quantitate positional deuterium enrichment in H2 and H3 of plasma glucose following ingestion of $^2\text{H}_2\text{O}$. The method is sufficiently sensitive to be of practical use for both small animal and human studies of glucose enrichment from $^2\text{H}_2\text{O}$. Finally, the measurement is insensitive to tracer levels of ^{13}C enrichment in glucose, allowing the integration of ^2H and ^{13}C tracers for metabolic studies.

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REFERENCES

- Landau, B. R., Wahren, J., Chandramouli, V., Schumann, W. C., Ekberg, K., and Kalhan, S. C. (1995) *J. Clin. Invest.* **95**, 172–178.
- Landau, B. R., Wahren, J., Chandramouli, V., Schumann, W. C., Ekberg, K., and Kalhan, S. C. (1996) *J. Clin. Invest.* **98**, 378–385.
- Rognstad, R., Clark, G., and Katz, J. (1974) *Eur. J. Biochem.* **47**, 383–388.
- Chandramouli, V., Ekberg, K., Schumann, W. C., Kalhan, S. C., Wahren, J., and Landau, B. R. (1997) *Am. J. Physiol.* **273**, E1209–E1215.
- Dekker, E., Romijn, J. A., Ekberg, K., Wahren, J., Van, T. H., Ackermans, M. T., Thuy, L. T., Chandramouli, V., Kager, P. A., Landau, B. R., and Sauerwein, H. P. (1997) *Am. J. Physiol.* **272**, E1059–E1064.
- Petersen, K. F., Krssak, M., Navarro, V., Chandramouli, V., Hundal, R., Schumann, W. C., Landau, B. R., and Shulman, G. I. (1999) *Am. J. Physiol.* **276**, E529–E535.
- Chen, X., Iqbal, N., and Boden, G. (1999) *J. Clin. Invest.* **103**, 365–372.
- Aguayo, J. B., Gamcsik, M. P., and Dick, J. D. (1988) *J. Biol. Chem.* **263**, 19552–19557.
- Holmes, E., Caddick, S., Lindon, J. C., Wilson, I. D., Kryvavych, S., and Nicholson, J. K. (1995) *Biochem. Pharmacol.* **49**, 1349–1359.
- Farrant, R. D., Cupid, B. C., Nicholson, J. K., and Lindon, J. C. (1997) *J. Pharm. Biomed. Anal.* **16**, 1–5.
- Jones, J. G., Solomon, M. A., Carvalho, R. A., Sherry, A. D., and Malloy, C. R. (1999) ENC 40th Meeting, Abstract 149.
- Vianello, F., Scarpa, M., Viglino, P., and Rigo, A. (1997) *Biochem. Biophys. Res. Commun.* **237**, 650–652.
- Jones, J. G., Cottam, G. L., Miller, B. C., Sherry, A. D., and Malloy, C. R. (1994) *Anal. Biochem.* **217**, 148–152.
- Hazey, J. W., Yang, D., Powers, L., Previs, S. F., David, F., Beaulieu, A. D., Puchowicz, M. A., Potter, J. L., Palmquist, D. L., and Brunengraber, H. (1997) *Anal. Biochem.* **248**, 158–167.
- Neese, R. A., Schwarz, J. M., Faix, D., Turner, S., Letscher, A., Vu, D., and Hellerstein, M. K. (1995) *J. Biol. Chem.* **270**, 14452–14466.
- Giacconi, A., Morviducci, L., Pastore, C., and Zoretta, D. (1998) *Diabetologica* **41**, 307–314.
- Katz, J., Wals, P. A., and Lee, W. N. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 2103–2107.
- Bugianesi, E., Kalhan, S., Burkett, E., Marchesini, G., and McCullough, A. (1998) *Gastroenterology* **115**, 1530–1540.
- Kalhan, S., Rossi, K., Gruca, L., Burkett, E., and O'Brien, A. (1997) *J. Clin. Invest.* **100**, 1775–1781.
- Previs, S. F., Fernandez, C. A., Yang, D., Soloviev, M. V., David, F., and Brunengraber, H. (1995) *J. Biol. Chem.* **270**, 19806–19815.
- Landau, B. R., Fernandez, C. A., Previs, S. F., Ekberg, K., Chandramouli, V., Wahren, J., Kalhan, S. C., and Brunengraber, H. (1995) *Am. J. Physiol.* **269**, E18–E26.
- Magnusson, I., Schumann, W. C., Bartsch, G. E., Chandramouli, V., Kumaran, K., Wahren, J., and Landau, B. R. (1991) *J. Biol. Chem.* **266**, 6975–6984.
- Beylot, M., Soloviev, M. V., David, F., Landau, B. R., and Brunengraber, H. (1995) *J. Biol. Chem.* **270**, 1509–1514.
- Large, V., Brunengraber, H., Odeon, M., and Beylot, M. (1997) *Am. J. Physiol.* **272**, E51–E58.
- Jones, J. G., Solomon, M. A., Sherry, A. D., Jeffrey, F. M., and Malloy, C. R. (1998) *Am. J. Physiol.* **275**, E843–E852.
- Remesy, C., and Demigne, C. (1983) *Ann. Nutr. Metab.* **27**, 57–70.
- Peroni, O., Large, V., and Beylot, M. (1995) *Am. J. Physiol.* **269**, E516–E523.
- Moldes, M., Cerdan, S., Erhard, P., and Seelig, J. (1994) *NMR Biomed.* **7**, 249–262.
- Nicholson, J. K., Timbrell, J. A., Bales, J. R., and Sadler, P. J. (1985) *Mol. Pharmacol.* **27**, 634–643.
- Willem, R., Biesemans, M., Kayser, F., and Malaisse, W. J. (1994) *Magn. Reson. Med.* **31**, 259–267.