

Evidence That Processes Other Than Gluconeogenesis May Influence the Ratio of Deuterium on the Fifth and Third Carbons of Glucose

Implications for the Use of $^2\text{H}_2\text{O}$ to Measure Gluconeogenesis in Humans

Gerlies Bock,¹ William C. Schumann,² Rita Basu,¹ Shawn C. Burgess,³ Zheng Yan,³ Visvanathan Chandramouli,² Robert A. Rizza,¹ and Bernard R. Landau^{2†}

OBJECTIVE—The deuterated water method uses the ratio of deuterium on carbons 5 and 2 (C5/C2) or 3 and 2 (C3/C2) to estimate the fraction of glucose derived from gluconeogenesis. The current studies determined whether C3 and C5 glucose enrichment is influenced by processes other than gluconeogenesis.

RESEARCH DESIGN AND METHODS—Six nondiabetic subjects were infused with [3,5- $^2\text{H}_2$]glucose and insulin while glucose was clamped at ~ 5 mmol/l; the C5-to-C3 ratio was measured in the in UDP-glucose pool using nuclear magnetic resonance and the acetaminophen glucuronide method.

RESULTS—Whereas the C5-to-C3 ratio of the infusate was 1.07, the ratio in UDP-glucose was <1.0 in all subjects both before (0.75 ± 0.07) and during (0.67 ± 0.05) the insulin infusion.

CONCLUSIONS—These data indicate that the deuterium on C5 of glucose is lost more rapidly relative to the deuterium on C3. The decrease in the C5-to-C3 ratio could result from exchange of the lower three carbons of fructose-6-phosphate with unlabeled three-carbon precursors via the transaldolase reaction and/or selective retention of the C3 deuterium at the level of triosephosphate isomerase due to a kinetic isotope effect. After ingestion of $^2\text{H}_2\text{O}$, these processes would increase the enrichment of C5 and decrease the enrichment of C3, respectively, with the former causing an overestimation of gluconeogenesis using the C2-to-C5 ratio and the latter an underestimation using the C3-to-C2 ratio. Future studies will be required to determine whether the impact of these processes on the measurement of gluconeogenesis differs among the disease states being evaluated (e.g., diabetes or obesity). *Diabetes* 57:50–55, 2008

From the ¹Division of Endocrinology, Diabetes, Metabolism, and Nutrition, Department of Medicine, Mayo Clinic College of Medicine, Rochester, Minnesota; ²The Advanced Imaging Research Center, Case Western Reserve University School of Medicine, Cleveland, Ohio; and ³the University of Texas Southwestern Medical Center, Dallas, Texas.

Address correspondence and reprint requests to Robert A. Rizza, MD, Mayo Clinic, 200 1st St. SW, Rm. 5-194 Joseph, Rochester, MN 55905. E-mail: rizza.robert@mayo.edu.

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DHAP, dihydroxyacetone phosphate; G3P, glyceraldehyde-3 phosphate; MAG, monoacetoneglucose; NMR, nuclear magnetic resonance; THF, tetrahydrofuran.

†B.R.L. is deceased.

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Plasma glucose concentration is determined by the balance between the rate of glucose production and glucose uptake. Glucose released from the liver is derived from either the degradation of glycogen, referred to as glycogenolysis, or the synthesis of new glucose from three-carbon precursors, referred to as gluconeogenesis. Multiple methods have been used to estimate rates of glycogenolysis and gluconeogenesis in humans with the deuterated water method perhaps being the most widely accepted (1–7). This method is based on the assumption that after ingestion of $^2\text{H}_2\text{O}$, the fifth carbon of glucose (C5) is labeled with deuterium at the level of the triosephosphate isomerase reaction during gluconeogenesis, whereas the second carbon of glucose (C2) is labeled with deuterium during both glycogenolysis and gluconeogenesis (1). Therefore the plasma C5-to-C2 glucose ratio is used to estimate the fraction of glucose derived from gluconeogenesis. Because the third carbon of glucose (C3) and C5 are concurrently labeled during the triose isomerase reaction, the C3-to-C2 ratio also has been used to assess gluconeogenesis (8). The absolute rate of gluconeogenesis is calculated by multiplying the fraction of glucose derived from gluconeogenesis times the tracer-determined rate of endogenous glucose production (9–11). The rate of glycogenolysis is then determined by subtracting the rate of gluconeogenesis from glucose production.

In general, estimates derived with the deuterated water method are concordant with those derived using other methods of estimating gluconeogenesis (2,3). In addition, gluconeogenesis measured with the deuterated water method changes in a biologically plausible manner during perturbations such as fasting or diabetes (1,11–14). However, unfortunately there is no "gold standard" against which results derived with this method can be compared. An alternative approach is to test the assumptions of the method. As noted above, a key assumption is that labeling of the fifth carbon of glucose with deuterium after ingestion of $^2\text{H}_2\text{O}$ only occurs during gluconeogenesis (1). The specific biochemical step in which the C5 hydrogen is exchanged for body water is during the equilibration between glyceraldehyde-3 phosphate (G3P) and dihydroxyacetone phosphate (DHAP) at the triosephosphate isomerase reaction (1). The C3 hydrogen also is lost during this exchange. Therefore, whenever a triose molecule is con-

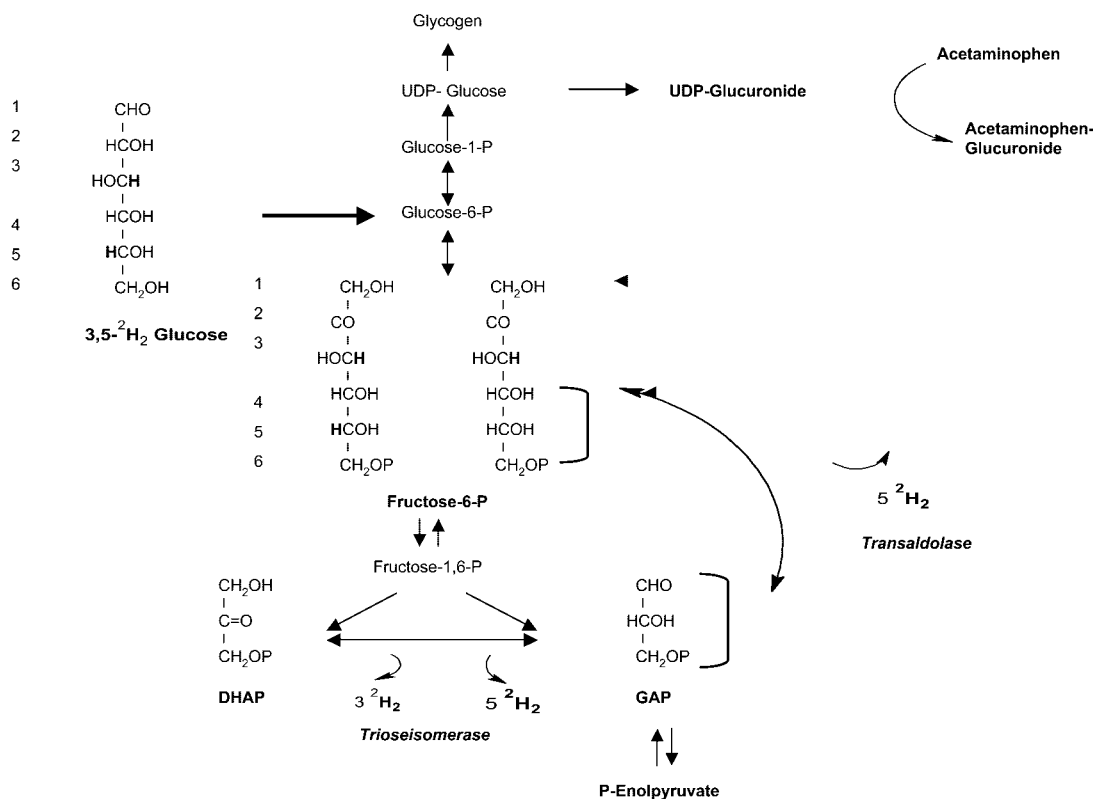


FIG. 1. Reactions involved in the synthesis of glucose, glucose-6-phosphate, and UDP-glucose.

verted to glucose, enrichment at either C5 or C3 can, in principle, be used to index gluconeogenesis. However, processes other than gluconeogenesis can influence enrichment of these carbons. For instance, C5 of glucose can become labeled when the lower three carbons of fructose-6-phosphate and/or sedoheptulose exchange with glyceraldehyde-3-phosphate by the transaldolase reaction (Fig. 1). Because this represents a simple exchange of the bottom three carbons of fructose-6-phosphate (C4–C6) with glyceraldehyde-3-phosphate rather than new hexose synthesis, generation of C5-enriched glucose via transaldolase will result in an overestimate of gluconeogenesis (15). Conversely, exchange of the C3 hydrogen with deuterium during the triosephosphate isomerase reaction may not be complete because of a primary kinetic isotope effect (16,17). If so, this would result in a lower than expected enrichment in C3 and therefore an underestimate of gluconeogenesis if the C3-to-C2 ratio is used. However, the extent (if at all) to which these processes occur in humans, is not known.

The present experiments addressed these questions by infusing [3,5-²H₂]glucose labeled in a 1:1 ratio (i.e., C5:C3 glucose ratio equaled 1.0) with deuterium on both the fifth and third carbons in nondiabetic humans before and during a hyperinsulinemic-euglycemic clamp. Enrichment in the UDP-glucose pool was measured with the acetaminophen glucuronide method. Because at steady state, UDP-glucose is in equilibrium with the glucose-6-phosphate pool and glucose-6-phosphate is derived from both plasma glucose and the fructose-6-phosphate pools, a C5-to-C3 ratio in the UDP-glucose pool that differs from that of the infusate indicates that processes in addition to gluconeogenesis influence this ratio.

RESEARCH DESIGN AND METHODS

After approval of the Mayo Institutional Review Board, six subjects (four women and two men, age 53 ± 5 , BMI 33.7 ± 1.6 , lean body mass 41.7 ± 3.0) gave written informed consent to participate in the study. Subjects were in good health, stable at weight, did not engage in vigorous exercise, were on no medication at the time of the study, and did not have a history of diabetes in first-degree relatives. All subjects were interviewed by a dietitian and instructed to follow a weight-maintaining diet consistent of 55% carbohydrate, 30% fat, and 15% protein for a least 1 week before the study. Subjects were admitted to the Mayo General Clinical Research Center the evening before the study, ingested a standard 10 kcal/kg dinner at 1800 h and then remained fasting thereafter. At 0600 h the following morning, an 18-gauge cannula was inserted in a forearm vein for tracer and hormone infusions. A second 18-gauge cannula was inserted in a retrograde fashion in a dorsal hand vein of the opposite arm, and the hand was placed in a heated box ($\sim 55^\circ\text{C}$) to enable sampling of arterialized venous blood. A urinary catheter was placed for urine collection. A primed (fasting glucose [in millimoles per liter] divided by 5.5 mmol/l times 12 μCi) continuous (0.12 $\mu\text{Ci}/\text{min}$) infusion of [3-³H]glucose (New England Nuclear, Boston, MA) was started at 0700 h and continued until the end of the study. In addition, 2 g liquid sugar-free acetaminophen was given by mouth in split doses at 0800 and 0930 h to enable measurement of urinary acetaminophen glucuronide specific activity. At 0900 h, a primed (133 mg in the first four subjects; 62.5 mg in the final two subjects), continuous (80 mg/h in the first four subjects; 40 mg/h in the final two subjects) infusion of [3,5-²H]glucose (Omicron Biochemicals, South Bend, IN) was started to enable the measurement of contribution of the transaldolase reaction to endogenous glucose production. A constant infusion containing somatostatin ($60 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), glucagon ($0.65 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), growth hormone ($3 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), and insulin ($0.50 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was started at 1000 h (i.e., time 0) and continued for 4 h. Dextrose (D₅₀W) containing [3-³H]glucose was infused as necessary to maintain plasma glucose concentrations at ~ 5.0 mmol/l as previously described (18). Arterialized venous blood samples were collected for measurements of glucose, hormones, and tracer concentrations. Urine was collected from 0900 to 1000, 1000 to 1200, and 1200 to 1400 h for analysis of urine acetaminophen glucuronide.

Analytical techniques. Plasma samples were placed on ice, centrifuged at 4°C , separated, and stored at -20°C until assay. Plasma glucose concentration was measured using a glucose oxidase method (Yellow Springs Instrument, Yellow Springs, OH). Plasma insulin concentrations were measured using a

chemiluminescence assay with reagents (Access Assay) obtained from Beckman (Chaska, MN). Body composition was measured using dual-energy X-ray absorptiometry (DPX scanner; Lunar, Madison, WI). Plasma [$3\text{-}^3\text{H}$]glucose specific activity was measured using liquid scintillation counting as previously described (19).

Acetaminophen glucuronide was isolated as previously described (20). Briefly urine was defrosted and concentrated to a small volume. The residue was dissolved in methanol, and the methanol solution was filtered and evaporated to dryness. The residue was dissolved in water, the pH was raised to 9.0 with 10N NaOH, and the residue was applied to an ion exchange column (spectragel 1×8 75–150 μm Spectrum) in its acetate form. The column was washed with water until urea test of fractions was negative and acetaminophen glucuronide was eluted with 5 mol/l acetic acid. The acetic acid fractions were evaporated off, and the residue was dissolved in 0.1 mol/l acetate buffer, pH 5.0. The acetaminophen glucuronide was converted to monoacetoneglucose (MAG) by a slight modification of the method of Jones et al. (21). Acetaminophen glucuronide in acetate buffer was added with glucuronidase (Helix Pomatia) and incubated for 48 h at 37°C. At the end of the incubation $\text{Ba}(\text{OH})_2$ and ZnSO_4 were added to the reaction mixture and the supernatant was passed down a column (Dowex 50W) in H^+ form. The eluent was evaporated to dryness and then stored in a desiccator overnight to give glucuronolactone. The lactone was stirred overnight with anhydrous acetone containing 2% H_2SO_4 . Water was added to quench the reaction, and pH was raised to 5.0 with 1 mol/l sodium carbonate. This solution was evaporated off to dryness and extracted three times with tetrahydrofuran (THF) 10 ml each. The combined THF solution was reduced with 1 mol/l lithium borohydride in THF. The solution was quenched with water, made acidic with acetic acid, and evaporated to dryness. The residue was dissolved in 10 ml methanol and evaporated. This process was repeated twice more. The MAG was dissolved in water, put through a 3-ml Sep-Pak C-18 cartridge (Waters), and washed with water, and MAG was eluted in 5.0% acetonitrile in water. This fraction evaporated off and dissolved in acetonitrile for nuclear magnetic resonance (NMR).

Analysis of C3 and C5 deuterium enrichments in MAG was performed by ^2H NMR as previously described (22). The MAG samples were dissolved in 160 μl HPLC grade acetonitrile with 5–10 μl water and transferred to a 3-mm NMR tube. Deuterium NMR spectra of MAG were collected using a 14.1 T Varian INOVA spectrometer and 3-mm broadband probe, tuned to 92 MHz as previously described. A 90-degree pulse was applied, and the signal was acquired over 1 s (sweep width = 1,000 Hz) with no further delay. Deuterium spectra were collected in blocks of 256 acquisitions without lock followed by a single pulse on the proton channel. The proton position of the solvent peak was ascertained, and Z_0 was automatically adjusted as a correction to any field drift since the collection of the last block. The end of acquisition all blocks were summed for the final spectrum. ^2H NMR spectra were typically signal-averaged for 1–4 h at 50°C. NMR peak areas were fitted using the PC-based NMR analysis software ACD (Advanced Chemistry Development, Toronto, Canada). The H3 and H5 represent the ^2H peak area at the 3 and 5 positions of MAG, respectively. Baseline was chosen as the center of the noise across the span of the spectrum and corrected by the NMR software. Operator error was minimized by allowing the ADC software to line fit the NMR peaks automatically. Repeated measurements of NMR data by a single operator typically yield <5% SD but are dependent on the signal/noise quality of the spectrum.

Statistical analysis. Data in the text and figures are expressed as mean \pm SE. Rates are expressed as per kilogram of fat free mass. Values from –30 to 0 min were averaged and considered as basal, and values from 210 to 240 min were averaged and considered as clamp. Student's paired *t* test was used to determine whether the C5-to-C3 ratio was <1. A *P* < 0.05 was considered as statistically significant. Values are means \pm SE.

RESULTS

Plasma glucose, insulin, and C-peptide concentrations (Fig. 2). Plasma glucose concentrations did not differ before and during the clamp (5.1 ± 0.1 vs. 5.1 ± 0.1 mmol/l). Plasma insulin concentrations increased from 35 ± 5 pmol/l before the clamp to 130 ± 13 pmol/l during the clamp. Somatostatin resulted in essentially complete suppression of C-peptide.

Endogenous glucose production and glucose disappearance (Fig. 3). Endogenous glucose production averaged 17.3 ± 1.0 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ before the clamp and decreased to 1.5 ± 1.4 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ by the end of the clamp. On the other hand, glucose disappearance

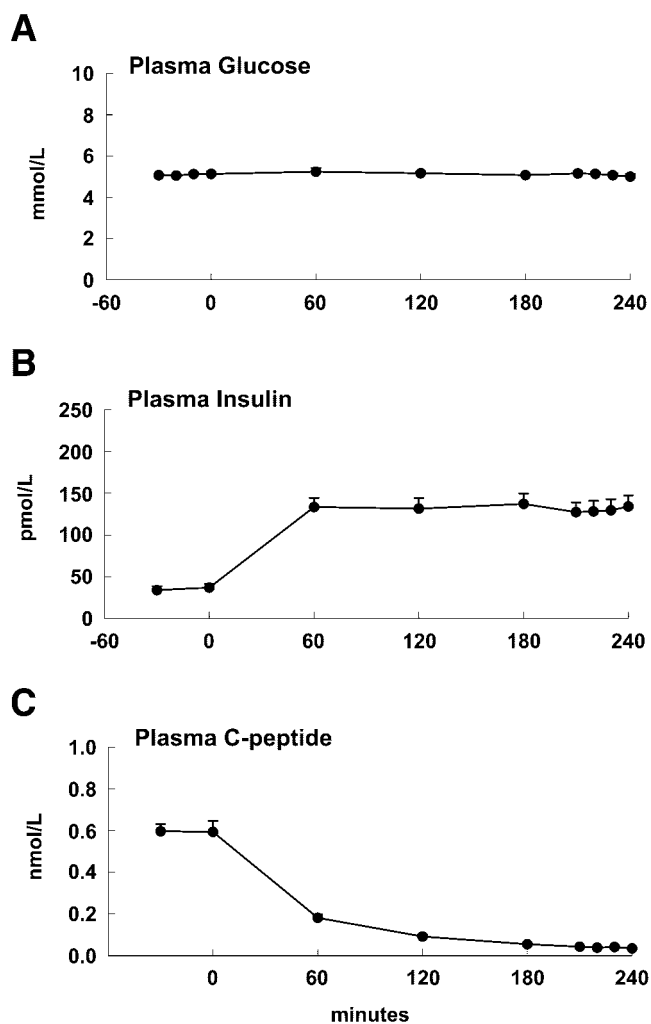


FIG. 2. Plasma glucose (A), insulin (B), and C-peptide (C) concentrations observed before and during a hyperinsulinemic-euglycemic clamp. An infusion of [$3,5\text{-}^2\text{H}_2$]glucose was started at time –60 min and insulin at 0 min.

averaged 16.9 ± 0.8 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ before the clamp and increased to 22.4 ± 2.3 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ by the end of the clamp.

C5-to-C3 glucose ratio (Figs. 4 and 5). A typical spectra of MAG derived from UDP-glucose is shown in Fig. 4. Peak areas correspond to the relative deuterium enrichments of the two hydrogen positions of glucose. The C5-to-C3 glucose ratio in the infusate was 1.07, indicating that fifth and third carbons of glucose were equally labeled with deuterium. The C5-to-C3 ratio in the UDP-glucose pool (Fig. 5) was <1.0 before the insulin infusion in all four subjects in whom it was measured (0.75 ± 0.07) and during the insulin infusion in all six subjects (0.67 ± 0.05), indicating either selective loss of deuterium from the fifth carbon of glucose and/or selective retention of deuterium on the third carbon of glucose.

DISCUSSION

The deuterated water method for the measurement of gluconeogenesis relies on the assumption that there is extensive equilibration between the precursor hydrogens and body water (1,2,3). Therefore, enrichment of plasma with $^2\text{H}_2\text{O}$ results in the labeling of newly synthesized glucose with deuterium. The ratio of the deuterium on C5

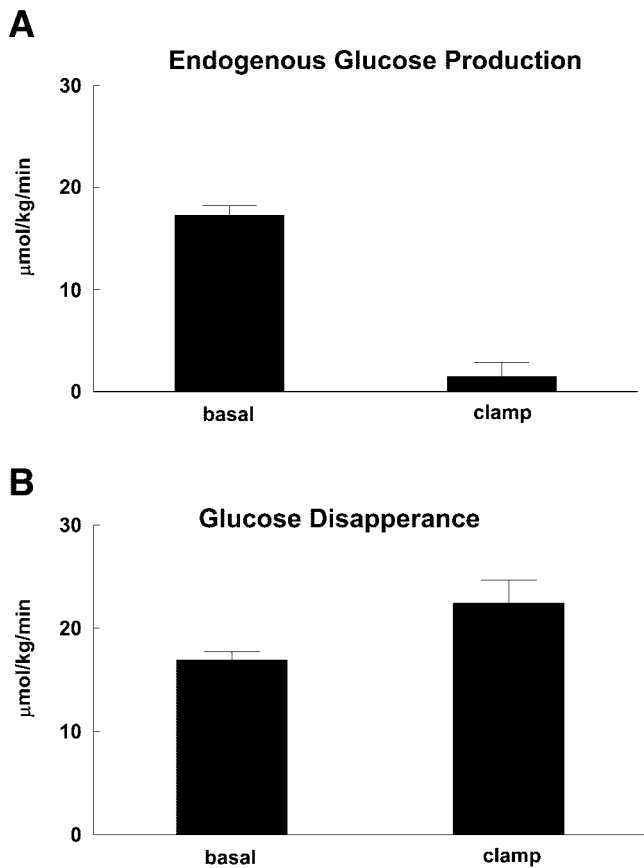


FIG. 3. Rates of endogenous glucose production (A) and glucose disappearance (B) observed before and during a hyperinsulinemic-euglycemic clamp. An infusion of $[3,5\text{-}^2\text{H}_2]\text{glucose}$ was started at time -60 min and insulin at 0 min.

(labeled during gluconeogenesis) to that on C2 (labeled during both gluconeogenesis and glycogenolysis due to equilibration between glucose-6-phosphate and fructose-6-

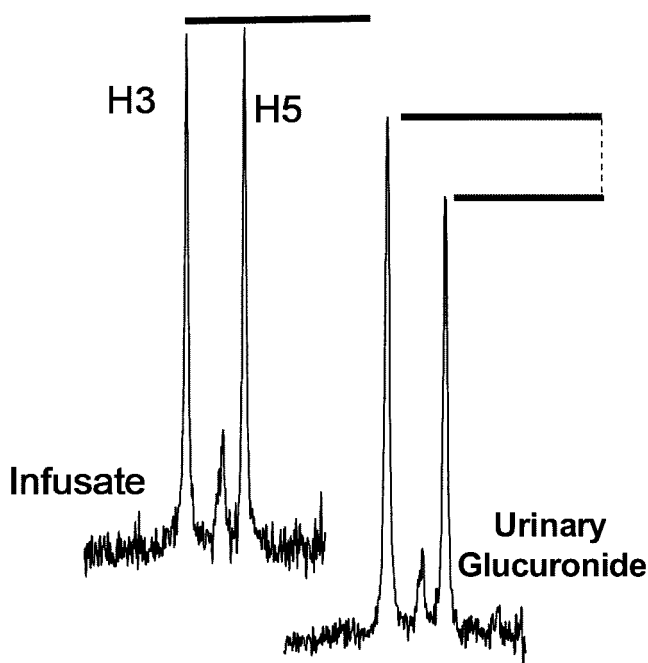


FIG. 4. Sample ^2H NMR spectra of MAG derived from UDP-glucose.

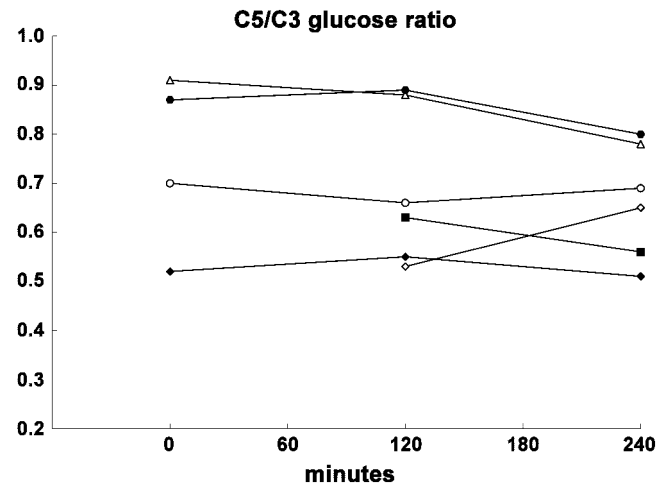


FIG. 5. The individual ratios of the deuterium enrichment on third and fifth carbons of UDP-glucose (C5/C3) measured in four subjects before and in six subjects during a hyperinsulinemic-euglycemic clamp. An infusion of $[3,5\text{-}^2\text{H}_2]\text{glucose}$ was started at time -60 min and insulin at 0 min.

phosphate pools) of glucose provides an estimate of the fraction of plasma glucose derived from gluconeogenesis (1). Because in the absence of an isotope effect, C5 and C3 are equally labeled during the triosephosphate isomerase reaction, in theory, the C3-to-C2 ratio also can be used to assess the fractional contribution of gluconeogenesis (8). The present study provides evidence that factors other than gluconeogenesis influence enrichment of C5 and/or C3 because the C5-to-C3 ratio of UDP-glucose was <1.0 both before and during infusion of insulin despite the fact the enrichment of the infused C5/C3 glucose equaled 1.0. This means that during equilibration of the fructose-6-phosphate, glucose-6-phosphate, and UDP-glucose pools, the deuterium on the fifth carbon of glucose was selectively lost and/or the deuterium on the third carbon of glucose was selectively retained. Both scenarios have implications when the deuterated water method is used to measure gluconeogenesis.

Transaldolase exchange. The transaldolase reaction can exchange the bottom three carbons of C5-labeled fructose-6-phosphate with glyceraldehyde-3-phosphate. Selective loss of C5 deuterium occurs when the lower three carbons of fructose-6-phosphate exchange with unlabeled glyceraldehyde-3-phosphate via the transaldolase reaction (15,24). It is unlikely that the pentosephosphate pathway contributed significantly to our observations because deuterium on C3, but not on C5 is lost when glucose-6-phosphate traverses the pentose phosphate pathway, which would increase rather than decrease the C5-to-C3 ratio (15). On the other hand, in the absence of an isotope effect (see below), the deuteriums on C3 and C5 are equally lost during equilibration of glyceraldehyde-3-phosphate and dihydroxyacetone at the level of the triose isomerase reaction (15). It is conceivable that glyceraldehyde-3-phosphate can become labeled with deuterium during its equilibration with dihydroxyacetone at the level of the triose isomerase reaction. However, the probability would be very low under the present experimental conditions because $^2\text{H}_2\text{O}$ only was generated during metabolism of C5/C3 glucose which was infused in trace amounts.

We are unaware of prior studies in humans that have measured the effect of the transaldolase reaction on labeling of the fifth carbon of glucose. On the other hand,

this reaction is well documented *in vitro* (15,24). Of note, in a parallel study using a similar approach, Jones et al. (personal communication) observed a C5-to-C3 ratio of ~ 0.77 in six healthy subjects after an overnight fast and ingestion of $[U-^2H_7]$ glucose. This ratio is entirely consistent with the ratios observed in the present experiment of 0.75 in the state and 0.67 during infusion of insulin. Although exchange of the lower three carbons of fructose-6-phosphate with unlabeled glyceraldehyde-3-phosphate would result in a decrease in C5 enrichment in the present study, the opposite would have occurred if plasma had been enriched with 2H_2O . Under those conditions, glyceraldehyde-3-phosphate would be extensively labeled with deuterium during its equilibration with dihydroxyacetone, and carbon exchange via the transaldolase reaction would increase C5 glucose enrichment in the absence of an increase in gluconeogenesis.

Triosephosphate isomerase kinetic isotope effect. Selective retention of deuterium on C3 relative to C5 due to a kinetic isotope effect at the level of the triosephosphate isomerase reaction also would reduce the C5-to-C3 ratio. This effect is well known *in vitro* (16,17,25–27) but has not yet been described *in vivo*. The origin of the kinetic isotope effect lies in the fact that 2H and 3H isotopes have one and two extra neutrons, respectively, which doubles and triples their mass compared with the naturally abundant 1H . If the location of the isotope label is in a position that participates in the transition state of a biochemical reaction, then the heavier mass isotope increases the activation energy for this step and a slower reaction rate is observed for isotopically labeled substrates compared with unlabeled substrates. This effect is particularly important in hydrogen isotope-labeled substrates because they have highest degree of mass difference compared with their natural abundant isotope and because their bonds are the most likely to be broken and formed in the transition state of biochemical reactions. This appears to be the case in the triosephosphate isomerase reaction. When $[3,5-^2H_2]$ glucose is metabolized in the liver to the level of triosephosphates, $[1-^2H]$ -labeled DHAP and $[3-^2H]$ -labeled G3P are initially formed. In principle, the interconversion of these two metabolites by the triosephosphate isomerase reaction leads to an equivalent loss of 2H label in both positions. In practice, Knowles and colleagues (26) showed that 77% of label in $[3-^3H]$ -labeled G3P is exchanged with water, but only 13% of label in $[1-^3H]$ -labeled DHAP is exchanged with water during the triosephosphate isomerase reaction (16). For deuterium, the kinetic isotope effect at the C1 position of DHAP is threefold but essentially negligible for the C2 position of G3P (25). This represents a substantial primary kinetic isotope effect during the exchange of the C1 hydrogen of DHAP (destined to become glucose C3 hydrogen) but not in the C2 hydrogen of G3P (destined to become glucose C5 hydrogen). Under the present experimental conditions, selective retention of deuterium enrichment in the three position compared with the five position also would result in a reduction of the C5-to-C3 ratio.

Implications for the use of 2H_2O to measure gluconeogenesis *in vivo*. Deuterium enrichment of plasma glucose and urinary glucuronide has been measured after administration of deuterated water under a variety of conditions in both human (8,28,29) and animal studies by NMR (14,22,30). Whereas the C3 enrichment typically has not been reported in these studies, it is easily visualized in the NMR spectra shown in the above references. We are

unaware of any instance in which glucose C3 and C5 deuterium enrichment become equal to one another after administration of 2H_2O . In addition, measurement of gluconeogenesis in overnight-fasted humans using the C3 enrichment yields a lower-estimate of gluconeogenesis than does measurement of C5 enrichment (8). Although this observation is consistent with the reduced C5-to-C3 ratio observed in the present studies, it provides no insight into the origin of the discrepancy. However, studies in long term-fasted rodents (24 h) are helpful, because under these conditions, liver glycogen is depleted, which means that essentially all of the glucose production originates from gluconeogenesis (14,22). Under these conditions, measurement of C5 enrichment indicates that $>95\%$ of glucose production is derived from gluconeogenesis. In contrast, measurement of C3 enrichment yielded a less likely value of $\sim 75\%$ gluconeogenesis (14,22). Thus, whereas the C5-to-C2 ratio approached 1.0 after an extended fast, the C3-to-C2 ratio did not, which is consistent with a primary kinetic isotope effect. It should be noted that the fact that measurement of C5 enrichment during a prolonged fast yields plausible estimates of gluconeogenesis does not rule out ongoing transaldolase exchange, because under these conditions, C5 enrichment will approach plasma 2H_2O enrichment and therefore cannot be further increased by exchange by labeled three-carbon precursors. This situation differs from that which occurs under conditions of daily living where gluconeogenesis does not account for 100% of glucose production. In this circumstance, C5 enrichment is substantially less than plasma 2H_2O and therefore can be increased by exchange with labeled three-carbon precursors.

Limitations. The present study is subject to limitations. Because enrichment in plasma was low, enrichment of C5/C3 in the UDP-glucose pool was measured by collecting urine at 2-h intervals and extracting acetaminophen glucuronide. Because there presumably is a delay between the time glucuronide is formed within the liver and the time it is excreted in the urine, this method looks back at what occurred during the interval before sampling of the urine. Therefore the enrichment of glucuronide in the liver and urine at any given time may differ. However, the C5-to-C3 ratio remained essentially constant over the 5 h of study. This suggests that equilibrium between hepatic and urine glucuronide was reached during the first 2 h of the study and remained unchanged throughout the remainder of the study. Perhaps more importantly from the perspective of the present experiments, our conclusions are based on the C5-to-C3 ratio rather than the degree to which steady state was achieved. This is of interest because endogenous glucose production gradually decreased over the same period of time to values that no longer differed from zero by the end of the study. Consistent with previous studies in animals (31), the progressive fall in endogenous glucose production in the absence of a change in the C5-to-C3 ratio suggests that insulin inhibited hepatic glucose release by suppressing glucose-6-phosphatase activity over time without altering the equilibration among the fructose-6-phosphate, glucose-6-phosphate, and UDP-glucose pools.

The C5-to-C3 ratio only was measured in the fasting state and during a euglycemic-hyperinsulinemic clamp. We therefore do not know whether the increase in hepatic glucose uptake that occurs in the presence of combined hyperglycemia and hyperinsulinemia would alter the rate of the transaldolase reaction and/or the rate of the isomer-

ase reaction. Only six subjects were studied. Because the C5-to-C3 ratio in those subjects ranged from ~0.5 to ~0.9, we do not know whether the same degree of variation would be observed in a larger population. However the ratio was <1 in all subjects, strongly supporting our conclusion that processes other than gluconeogenesis influence this ratio. We only studied obese nondiabetic subjects. We therefore do not know whether other metabolic conditions (e.g., obesity or diabetes) influence the C5-to-C3 ratio. On the other hand, if the C5-to-C3 ratio remains constant under the conditions in which such comparisons are made, then while enrichment of C5 glucose via the transaldolase reaction would make determination of the absolute rates of gluconeogenesis problematic, it would not preclude comparisons among groups.

CONCLUSIONS

In summary, the present data indicate that processes other than the rate of gluconeogenesis influence the C5-to-C3 deuterium ratio of urinary glucuronide after infusion of [3,5-²H]glucose in humans. The 25–30% reduction of the C5-to-C3 ratio in UDP-glucose relative to the ratio of 1.0 in the infused C5/C3 glucose both in the fasting state and during a hyperinsulinemic-euglycemic clamp is consistent with transaldolase exchange, a kinetic isotope effect at the level of triosephosphate isomerase or a combination of both. In any case, our results do not obviate the utility of the deuterated water method to estimate gluconeogenesis. While both the ²H₂O and isotopomer methods are affected by transaldolase exchange and/or an isotope effects at the level of the triosephosphate isomerase step, we believe that they remain the best available methods for assessing gluconeogenesis in vivo. However, it will be important in future studies to determine whether the processes other than gluconeogenesis that increase labeling C5 or reduce labeling of C3 after ingestion of deuterated water differ among the disease states being evaluated (e.g., diabetes or obesity).

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