Additional Evidence That Transaldolase Exchange, Isotope Discrimination During the Triose-Isomerase Reaction, or Both Occur in Humans

Effects of Type 2 Diabetes

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OBJECTIVE—To determine whether deuterium enrichment on carbons 5 and 3 (C5/C3) in plasma glucose is influenced by processes other than gluconeogenesis and, if so, whether these processes are altered by type 2 diabetes.

RESEARCH DESIGN AND METHODS—In this study, 10 obese diabetic and 10 obese nondiabetic subjects were infused intravenously with [3,5-$^2H_2$] galactose enriched at a C5-to-C3 ratio of 1.0 as well as the enrichment of deuterium on C5 and C3 of plasma glucose, measured with nuclear magnetic resonance using the acetaminophen glucuronide method.

RESULTS—The ratio of deuterium enrichment on C5 and C3 of glucose was <1 ($P < 0.001$) in all of the diabetic and nondiabetic subjects, resulting in a means ± SE C5-to-C3 ratio that did not differ between groups (0.81 ± 0.01 vs. 0.79 ± 0.01, respectively).

CONCLUSIONS—That the C5-to-C3 glucose ratio is <1 indicates that transaldolase exchange, selective retention of deuterium at the level of the triose-isomerase reaction, or both occur in humans. This also indicates that the net effect of these processes on the C5-to-C3 ratio is the same in people with and without type 2 diabetes. The possible effects of transaldolase exchange or selective retention of deuterium (or tritium) at the level of the triose-isomerase reaction on tracee labeling and tracer metabolism should be considered when the deuterated water method is used to measure gluconeogenesis or [3-^{3}H] glucose is used to measure glucose turnover in humans. ***Diabetes* 57:1539–1543, 2009**

The deuterated water method is extensively used to measure gluconeogenesis in humans (1–7). One premise of this method is that there is negligible exchange of the lower three carbons of fructose or sedoheptulose via the transaldolase exchange reaction (8). If such exchange does occur, then glucose can be labeled on the fifth carbon (C5) by simple exchange with a labeled carbon three (C3) precursor without net hexose synthesis (9–11). Whereas it is established that transaldolase exchange can occur in vitro (9,12), until recently it was not known whether exchange also occurs in vivo. To address this question, we infused [3,5-$^2H_2$] glucose enriched at a C5-to-C3 ratio of 1.07 intravenously in nondiabetic subjects (13). We observed that the C5-to-C3 deuterium enrichment in uridine-diphosphoglucose glucose measured using the acetaminophen glucuronide method was <1 in all six subjects studied, averaging 0.75 before and 0.67 during a 4-h hyperinsulinemic-euglycemic clamp. Jones et al. (14) have observed similar effects of transaldolase activity on overestimation of the indirect pathway of glycogen synthesis in five healthy humans.

These observations are both surprising and disconcerting because they indicate that deuterium on C5 of fructose-1,6-phosphate was lost during exchange with unlabeled C3 precursors, presumably via transaldolase exchange; that deuterium was selectively retained on C3, presumably due to a kinetic isotope effect at the level of the triose-isomerase reaction; or that both occurred (Fig. 1). If substantial transaldolase exchange does occur in humans, then the extent of labeling of C5 glucose with deuterium following administration of deuterated water will be determined by both the rate of transaldolase exchange and the rate of gluconeogenesis (8). Therefore, the plasma C5 glucose–to–C2 glucose ratio (which is labeled by both glycogenolysis and gluconeogenesis) would overestimate the percent of glucose derived from gluconeogenesis. This also would preclude accurate measurement of gluconeogenesis with any other tracer method because all assume negligible transaldolase exchange (15,16).

Alternatively, selective retention of deuterium on C3 also would reduce the C5-to-C3 ratio. This would be consistent with in vitro studies that have shown slower removal of deuterium during the triose-isomerase reaction due to a kinetic isotope effect (9–11,17). If this were to also occur in humans, then it would call into question the ability of [3-^{3}H] glucose to accurately measure glucose turnover because retention of tritium due to a kinetic isotope effect could result in an underestimation of glucose turnover if it caused the hepatic glucose-6-phosphate pool to be enriched with tracer. If the rate of transaldolase exchange or the degree of retention of tritium during the triose-isomerase reaction differs in diabetic and nondiabetic humans, this would be particularly problematic because it would confound comparison of gluconeogene-

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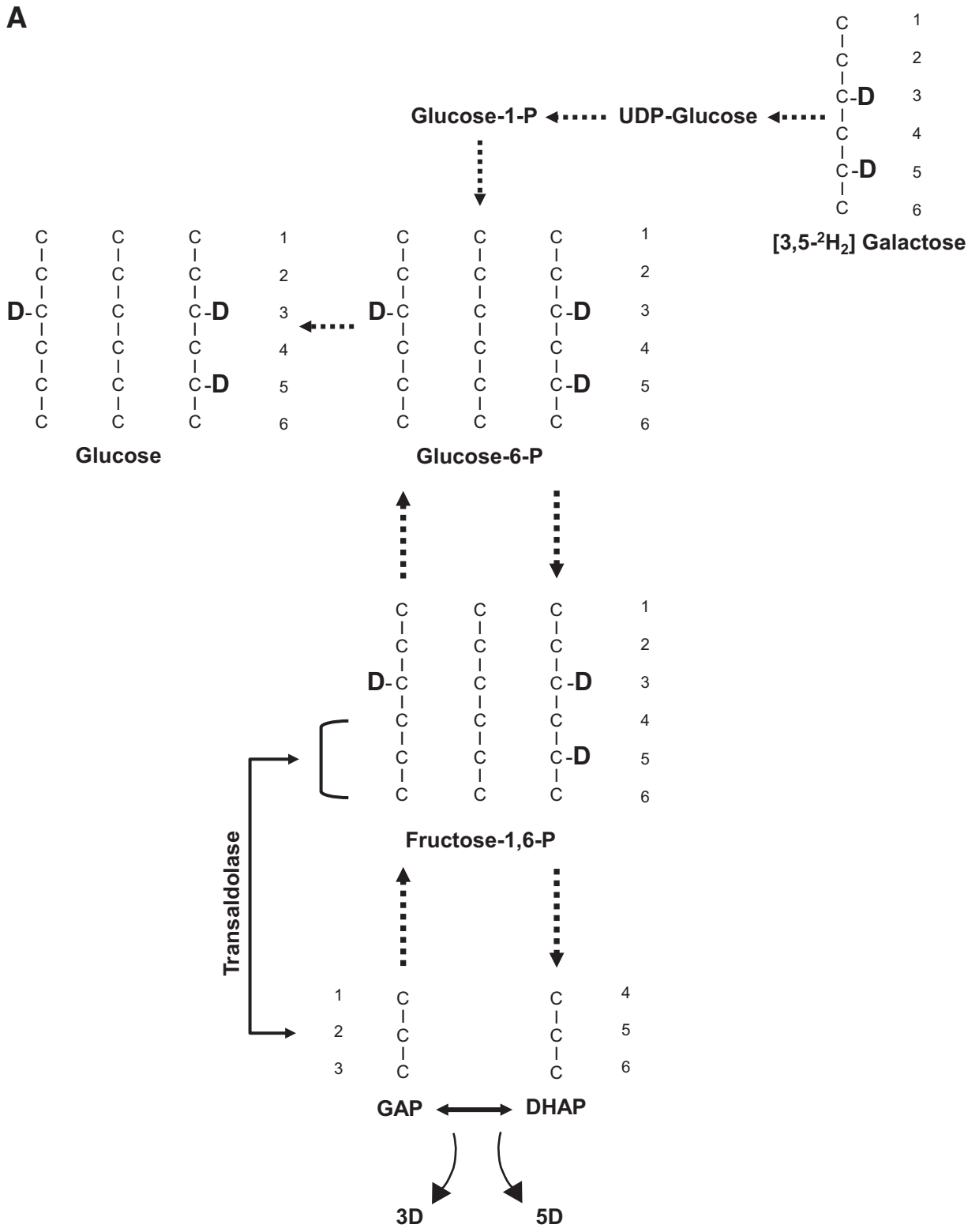

FIG. 1. The C5-to-C3 ratio of plasma glucose can decrease if unlabeled glyceraldhyde-3-phosphate exchanges with the bottom three carbons of C5– and C3–labeled fructose-1,6-phosphate via the transaldolase reaction (*A*), if the deuterium on dihydroxyacetone originating from the third carbon of frucose-1,6-posphate is retained relative to that originating from the fifth carbon of fructose-1,6-phosphate due to kinetic isotope effect (i.e., a slower removal) during the triose isomerase reaction (*B*), or if a combination of both occurs. Hydrogens and oxygens have been omitted for the sake of clarity. C, carbon; D, deuterium; DHAP, dihydroxyacetone phosphate; fructose-1,6-P, fructose-1,6-phosphate; GAP, glyceraldehyde-3-phosphate; glucose-1-P, glucose-1-phosphate; glucose-6-P, glucose-6-phosphate; UDP, uridine-diphosphoglucose.

sis measured with the deuterated water method and glucose turnover measured with [3-^{3}H] glucose between groups.

The present study was undertaken to confirm or refute, in a larger number of subjects, our previous observation that the C5-to-C3 ratio measured in the plasma glucose

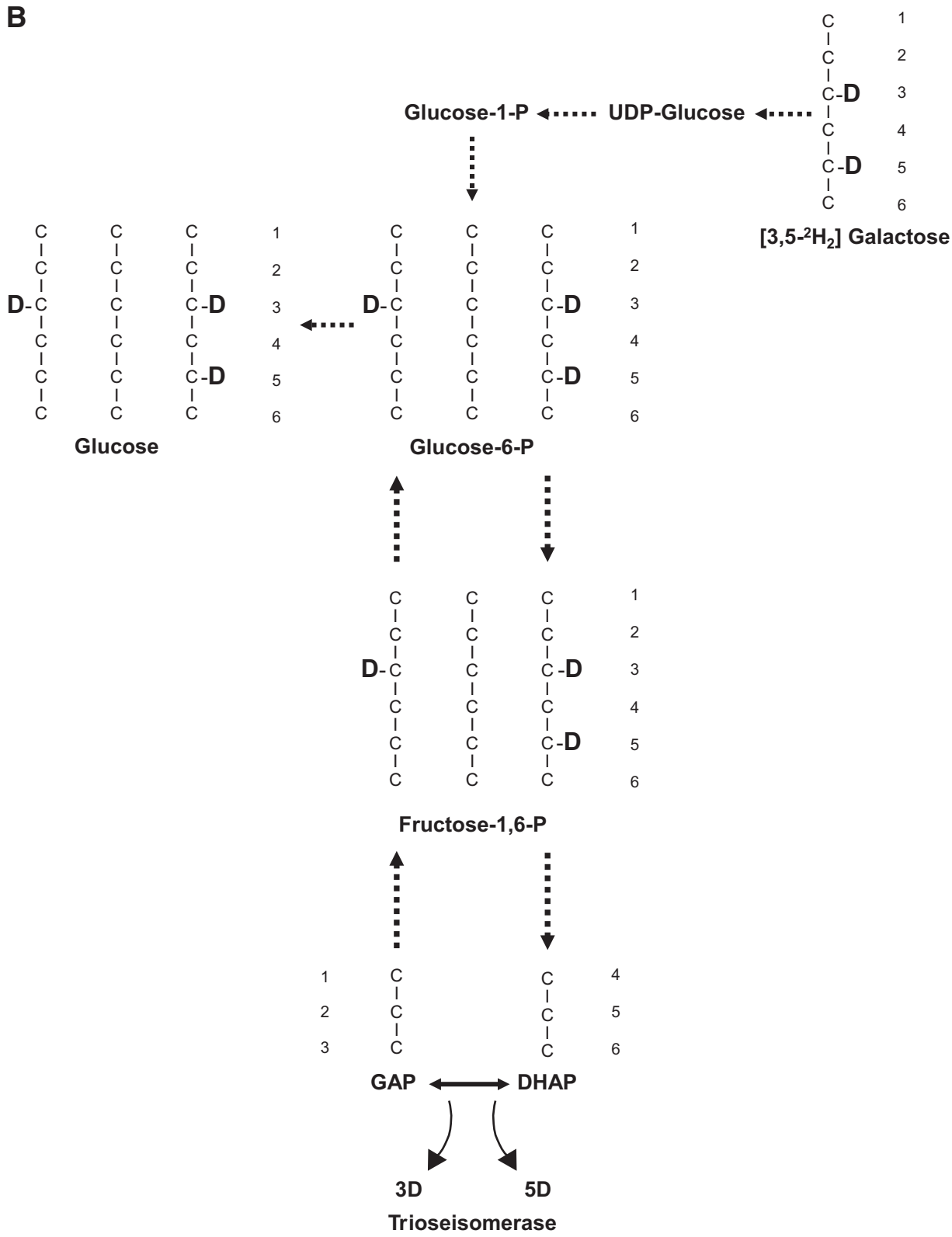

FIG. 1. Continued.

pool is lower than that of the intravenously infused tracer (13). We also sought to determine whether the degree of reduction of the C5-to-C3 plasma glucose ratio differs in diabetic and nondiabetic humans. We addressed these questions by infusing [3,5-$^2H_2$] galactose to directly label the plasma-glucose pool in obese nondiabetic and obese diabetic subjects after an 18-h fast in order to reduce hepatic glycogen, thereby maximizing plasma glucose enrichment by minimizing the rate of entry of unlabeled plasma glucose into the pool. We report that the C5-to-C3 plasma glucose ratio was <1 in all subjects. We also report that the degree of reduction in the C5-to-C3 ratio did not

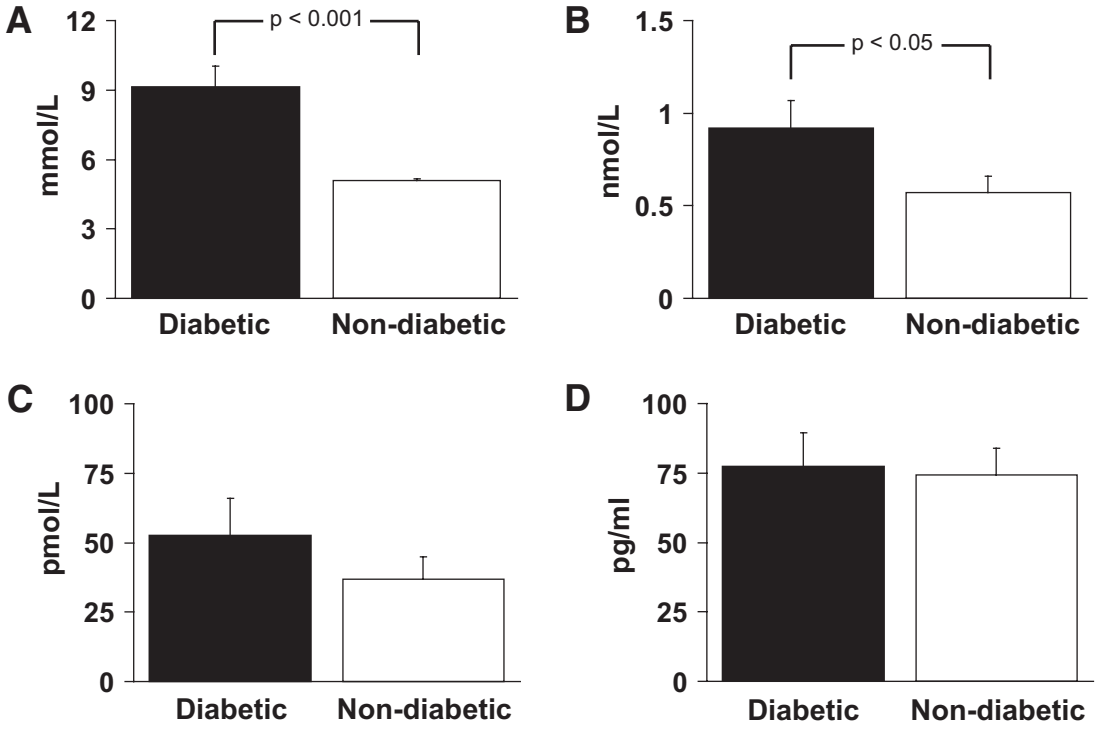

FIG. 2. Glucose (*A*), C-peptide (*B*), insulin (*C*), and glucagon (*D*) concentrations observed in the diabetic and nondiabetic subjects.

differ for diabetic and nondiabetic subjects, indicating that the net effect of transaldolase exchange or retention of deuterium on C3 was comparable in the two groups.

RESEARCH DESIGN AND METHODS

After approval of the Mayo Institutional Review Board, 10 diabetic and 10 nondiabetic subjects, matched for age (mean ± SE 62 ± 4 vs. 53 ± 5 years, respectively), BMI (31.2 ± 1.2 vs. 30.3 ± 1.7 kg/m^2), lean body mass (47.4 ± 4.5 vs. 43.6 ± 2.8 kg), and body fat (44.7 ± 2.5 vs. 46.5 ± 3.3%) provided written informed consent to participate in the study. Subjects were in good health, at a stable weight, and did not engage in regular vigorous exercise. The nondiabetic subjects did not have a history of diabetes in first-degree relatives. Diabetic subjects discontinued oral hypoglycemic medications at least 10 days before the study. As expected, fasting plasma glucose (152 ± 15 vs. 90 ± 2 mg/dl) and A1C (7.1 ± 0.3 vs. 5.4 ± 0.1%) at the time of study were higher for diabetic than nondiabetic subjects.

Subjects were instructed to follow a weight maintenance diet consisting of 55% carbohydrates, 30% fat, and 15% protein for a least one week prior to the study. Subjects were admitted to the Mayo Clinical Research Unit and Center for Clinical and Translation Science Activities 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 into a forearm vein for tracer infusions. A second 18-gauge cannula was inserted in a retrograde fashion into a dorsal hand vein of the opposite arm, and the hand was placed in a heated box (~55°C) to enable sampling of arterialized venous blood. A primed continuous infusion of [6,6-$^2H_2$] glucose (88 mg prime; 50 mg/h infusion) was started at 0800 h as well as an infusion of [3,5-$^2H_2$] galactose (Omicron, Biochemicals, IN; C5-to-C3 ratio 1.0) at 200 mg/h until the study end at 1300 h. Arterialized venous blood samples were collected at 1230, 1240, 1250, and 1300 h for measurements of glucose, insulin, and C-peptide as well as glucagon concentrations and tracer enrichment.

Analytical techniques. Plasma samples were placed on ice, centrifuged at 4°C, separated, and stored at 20°C until assay. Plasma glucose concentrations were measured using a glucose oxidase method (Yellow Springs Instrument, Yellow Springs, OH). Plasma insulin, C-peptide, and glucagon concentrations were measured using a chemiluminescence assay (Access Assay, Beckman, Chaska, MN). Body composition was measured using dual-energy X-ray absorptiometry (Lunar, Madison, WI). Plasma [6,6-$^2H_2$] glucose enrichment was measured using mass spectrometry (8), and analysis of C3 and C5 deuterium enrichment on plasma glucose was measured using 2H nuclear magnetic resonance spectroscopy as previously described (18).

Statistical analysis. Data are expressed as means ± SE. Values from 1230, 1240, 1250, and 1300 h were averaged for statistical analysis. A paired Student's t test was used to determine if the C5-to-C3 ratio was <1, and an unpaired Student's t test was used to determine if the C5-to-C3 ratio differed between the diabetic and nondiabetic subjects. A $P < 0.05$ was considered statistically significant.

RESULTS

Plasma glucose concentrations during the final hour of the study were higher ($P < 0.001$) for diabetic than nondiabetic subjects (9.13 ± 0.9 vs. 5.1 ± 0.1 mmol/l, respectively). Insulin concentrations were slightly higher (52.5 ± 10.6 vs. 36.9 ± 5.1 pmol/l; $P = 0.06$) and C-peptide concentrations significantly higher (0.92 ± 0.12 vs. 0.57 ± 0.01 nmol/l); $P < 0.05$) for diabetic than nondiabetic subjects. However, glucagon concentrations did not differ between groups (77.5 ± 6.4 vs. 74.3 ± 7.8 pg/ml) (Fig. 2).

Endogenous glucose production was slightly but not significantly higher for diabetic than nondiabetic subjects (11.9 ± 0.4 vs. 10.6 ± 0.5 μmol · kg^{-1} · min^{-1}, respectively). The C5-to-C3 ratio in plasma glucose was <1 ($P <$ 0.001) for all of the diabetic and nondiabetic subjects, resulting in a mean that did not differ between groups (0.81 ± 0.01 vs. 0.79 ± 0.01) (Fig. 3).

DISCUSSION

The present study indicates that despite infusing [3,5-$^2H_2$] galactose enriched at a C5-to-C3 ratio of 1.0, the C5-to-C3 ratio in plasma glucose was <1 in all subjects, with the extent of the reduction being comparable in those with and those without type 2 diabetes. To our knowledge, there are only two possible ways this ratio can be reduced. As discussed in detail elsewhere (13) and as shown in Fig. 1*A*, C5 deuterium can be selectively lost during exchange of the lower three carbons of fructose-1,6-phosphate (or sedoheptulose) with unlabeled glyceraldhyde-3-phos-

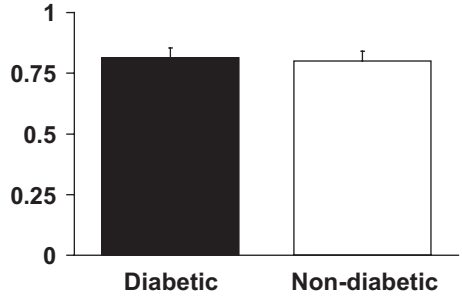

FIG. 3. The C5-to-C3 ratio of plasma glucose observed in diabetic and nondiabetic subjects following a 5-h intravenous infusion of [3,5-$^2H_2$] galactose enriched with deuterium at a C5-to-C3 ratio of 1.0.

phate. In contrast, as shown in Fig. 1*B*, C3 deuterium can be selectively retained due to a kinetic isotope effect (i.e., dihydroxyacetone phosphate labeled with deuterium is converted to glyceraldhyde-3-phosphate more slowly than unlabeled dihydroxyacetone phosphate) at the level of the triose-isomerase reaction.

Because we only have measured the C5-to-C3 ratio in plasma glucose, we cannot distinguish between these two possibilities. However, the fact that the C5-to-C3 plasma glucose ratio does not differ in obese diabetic (~0.81) and obese nondiabetic (~0.79) subjects fasted for ~18 h, is comparable to that previously observed in nondiabetic subjects (~0.75) fasted for ~12 h (13), and does not appear to change during infusion of insulin (13) is reassuring. This implies that error introduced by labeling of plasma C5 with deuterium via transaldolase exchange following ingestion of ^2H_2O or error introduced by use of [3-^{2}H] glucose (and presumably [3-^{3}H] glucose) to measure glucose turnover is also likely to be the same under these conditions. However, it remains possible that the rate of loss of C5 due to an increase or decrease in the rate of transaldolase exchange in one group could be offset by a proportionate decrease or increase in retention of C3 at the level of the triose-isomerase exchange reaction, resulting in comparable C5-to-C3 glucose ratios in both groups, albeit via different mechanisms.

Whereas the present study indicates that the C5-to-C3 ratio is comparably decreased in nondiabetic and well-controlled type 2 diabetic subjects, we hesitate to recommend applying a correction factor for the calculation of gluconeogenesis using the deuterated water method because we do not know the extent to which the decrease in the C5-to-C3 ratio is due to transaldolase exchange (which would increase C5 deuterium labeling) or to retention of deuterium on C3 (which would not influence C5 deuterium labeling). In addition, because glucose turnover differed only minimally in the diabetic and nondiabetic subjects in the current experiments, we also do not know whether a correction factor (if necessary) would be the same in the presence of marked differences in glucose production (e.g., poorly controlled diabetes). Future studies will need to address these questions. In the interim, the possible effects of transaldolase exchange and selective retention of deuterium (or tritium) at the level of the triose-isomerase reaction should be considered when the deuterated water method is used to measure gluconeogenesis and when [3-^{3}H] glucose is used to measure glucose turnover in humans.

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