Assessment of Insulin Action in Insulin-dependent Diabetes Mellitus Using [6^{14}C]Glucose, [3^{3}H]Glucose, and [2^{3}H]Glucose

Differences in the Apparent Pattern of Insulin Resistance Depending on the Isotope Used

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Abstract

To determine whether [2^{3}H], [3^{3}H], and [6^{14}C]glucose provide an equivalent assessment of glucose turnover in insulin-dependent diabetes mellitus (IDDM) and nondiabetic man, glucose utilization rates were measured using a simultaneous infusion of these isotopes before and during hyperinsulinemic euglycemic clamps. In the nondiabetic subjects, glucose turnover rates determined with [6^{14}C]glucose during insulin infusion were lower ($P < 0.02$) than those determined with [2^{3}H]glucose and higher ($P < 0.01$) than those determined with [3^{3}H]glucose. In IDDM, glucose turnover rates measured with [6^{14}C]glucose during insulin infusion were lower ($P < 0.05$) than those determined with [2^{3}H]glucose, but were not different from those determined with [3^{3}H]glucose. All three isotopes indicated the presence of insulin resistance. However, using [3^{3}H]glucose led to the erroneous conclusion that glucose utilization was not significantly decreased at high insulin concentrations in the diabetic patients. [6^{14}C] and [3^{3}H]glucose but not [2^{3}H]glucose indicated impairment in insulin-induced suppression of glucose production. These results indicate that tritiated isotopes do not necessarily equally reflect the pattern of glucose metabolism in diabetic and nondiabetic man.

Introduction

Isotope dilution techniques are used extensively in clinical research to measure glucose turnover in diabetic and nondiabetic man. The ideal tracer for these measurements should both accurately reflect the pattern of nonradioactive glucose metabolism and not require complex processing. Although early studies in animals generally used [^{14}C]-labeled glucose as a tracer (1, 2), in recent years, glucose labeled with tritium has become the isotope most commonly used in clinical research. Use of tritiated glucose does not require correction for reincorporation of radioactivity back into the glucose molecule due to Cori cycle activity, whereas [^{14}C]glucose does (3, 4). Glucose labeled with tritium in the second or third position has the additional advantage that samples do not have to be processed to remove radioactive three-carbon fragments as does glucose labeled with tritium in the sixth position (3, 5). [3^{3}H]Glucose has gained wide acceptance in human studies after the demonstration in animals that results obtained with this isotope are comparable to those obtained with [^{14}C]glucose corrected for Cori cycle activity (3, 5). Whether this relationship applies to postabsorptive

humans and whether it holds under conditions of insulin-induced changes in glucose utilization rates is unknown.

Use of [3^{3}H]glucose has several theoretical disadvantages. First, there is considerable evidence from in vitro studies that [3^{3}H]glucose can be detritiated as a result of futile cycling between fructose-6-phosphate and fructose diphosphate (6, 7) and during recycling through the pentose phosphate pathway (8). Second, [3^{3}H]glucose may be incorporated into and released from glycogen without losing its tritium (9). Therefore, under conditions of rapid glycogen synthesis concurrent with or followed by glycogen breakdown (e.g., transition from the fed to the postabsorptive state), radioactive glucose will be released from glycogen along with nonradioactive glucose resulting in an underestimate of hepatic glucose production and total glucose utilization. This error will be increased if, as has been reported, most recently formed glycogen is the first to be released during glycogen breakdown (10).

By contrast, [2^{3}H]glucose should lose its label during passage into and out of glycogen (11, 12). However in mammals, [2^{3}H]glucose also loses its label as part of the glucose/glucose-6-phosphate futile cycle (3, 5, 8, 13). This results in an overestimate of glucose turnover rates in the postabsorptive state. However, in dogs the percent overestimate observed with the use of [2^{3}H]glucose is reduced when glucose flux increases during the hyperglycemia and hyperinsulinemia produced by infusion of glucose (14, 15).

The extent of futile cycling and cycling through glycogen is dependent on the relative flux through the glycogenic, glycogenolytic, and Embden-Meyerhof pathways (16). Because the activities of the enzymes in these pathways may be markedly different in diabetic and nondiabetic man (17, 18), the present studies were undertaken to determine: (a) whether estimates of glucose utilization and production measured with [2^{3}H] and [3^{3}H]glucose are equivalent to those obtained with [6^{14}C]glucose in the postabsorptive state and during insulin-induced stimulation of glucose turnover in diabetic and nondiabetic man; (b) whether the relationships between turnover rates measured with these three isotopes are the same in both groups; and (c) whether each isotope provides an equal assessment of hepatic and extrahepatic insulin resistance.

To examine these questions, we measured glucose turnover in diabetic and nondiabetic subjects before and during infusion of insulin, using simultaneous infusions of [6^{14}C]glucose, [3^{3}H]glucose, and [2^{3}H]glucose. Results with [6^{14}C]glucose were corrected for resynthesis of glucose from glycolytic metabolites (Cori cycle activity). The simultaneous use of [2^{3}H]glucose and [3^{3}H]glucose was made possible by modification of an enzymatic method for selective detritiation of [2^{3}H]glucose without detritiation of [3^{3}H]glucose. We report that whereas [6^{14}C] and [3^{3}H]glucose accurately reflect glucose turnover in diabetic patients, [3^{3}H]glucose underestimates glucose turnover in nondiabetic man. [2^{3}H]Glucose slightly overestimates glucose turnover

in both diabetic and nondiabetic man, which indicates the presence of glucose/glucose-6-phosphate cycling. This overestimate does not change with changes in the rate of glucose flux. All three isotopes provide different assessments of the degree of insulin resistance in diabetic and nondiabetic subjects. Using [6^{14}C]glucose as the standard, the current studies (a) indicate that patients with insulin-dependent diabetes mellitus (IDDM)[1] have both hepatic and extrahepatic insulin resistance and (b) emphasize that while use of tritiated isotopes of glucose may be convenient, the isotopes do not equally reflect the pattern of metabolism in diabetic and nondiabetic man.

Methods

Subjects. After approval from the Mayo Clinic Institutional Review Board, eight normal weight subjects with IDDM (two male, six female; body mass index 23.0±0.8 kg/m^2; age 35.6±3.3 yr) and nine matched healthy nondiabetic patients (five male, four female; body mass index 23.1±1.0 kg/m^2; age 34.2±5.5 yr) with no family history of diabetes mellitus gave informed written consent to participate. All diabetic patients had a history of ketosis and an absent or minimal plasma C-peptide response to glucagon stimulation. Mean duration of diabetes was 9.6±2.9 yr and mean glycosylated hemoglobin at the time of study was 8.6±0.7% (normal range 4–7%). None of the diabetic patients had clinically significant retinopathy or nephropathy.

Insulin dose-response studies. Subjects were admitted at 7:00 a.m. to the outpatient facility of the Clinical Research Center after an overnight (12 h) fast. On the day before the study, the insulin dose of the diabetic patients was reduced by 20%, with no long or intermediate acting insulin given that evening. Subjects were placed at bed rest and remained supine throughout the study. An antecubital vein was cannulated with an 18-gauge Cathlon (Critikon, Tampa, FL) which was used for infusion of glucose, insulin, and radioactive glucose each by separate Harvard pumps (Harvard Instrument Co., Ayer, MA). A contralateral wrist vein was cannulated with an 18-gauge Cathlon for blood withdrawal by a Biostator glucose monitor (Life Science Instruments, Miles Laboratories Inc., Elkhart, IN). An additional contralateral hand vein was cannulated retrogradely with a 19-gauge butterfly (Terumo, Tokyo, Japan) and the hand was placed in a thermo-regulated plexiglass box maintained at 55°C to allow intermittent sampling of arterialized blood.

A primed continuous infusion (all isotopes, New England Nuclear, Boston, MA) of [6^{14}C]glucose (0.13 μCi/min, specific activity 49.1 mCi/mmol), [3^{3}H]glucose (0.26 μCi/min, specific activity 10.1 Ci/mmol), and [2^{3}H]glucose (0.26 μCi/min, specific activity 24.0 Ci/mmol) mixed in 0.9% NaCl, was started at 7:00 a.m. All isotopes were contained in a single syringe. All isotopes were >98% pure, as demonstrated by paper chromatography and paper electrophoresis. Incubation of [2^{3}H]glucose with phosphoglucose isomerase (see below) demonstrated that >96% of tritium was on the second carbon. Incubation of [3^{3}H]glucose with whole blood resulted in <1% of radioactivity of label being recovered in lactate consistent with tritium being present on the third carbon. Decarboxylation of [6^{14}C]glucose (see below) revealed essentially no radioactivity in the C^1 carbon. A 2-h equilibration period was allowed before the beginning of the experiments.

Insulin dose-response studies were performed as described previously (19). Insulin was infused sequentially at rates of 0.4 (180 min), 1.0 (120 min), and 2.0 (120 min) mU-kg^{-1}-min^{-1}. Data were not available in one nondiabetic subject during the 1.0 mU-kg^{-1}-min^{-1} infusion due to technical difficulties. The insulin (Actrapid Human, E. R. Squibb & Sons, Inc., Princeton, NJ) was dissolved in 0.9% NaCl containing 1% human serum albumin (Baxter Travenol Laboratories, Deerfield, IL) and made up to a final concentration of 53 mU-kg^{-1}-ml^{-1}. Both K$^+$ (0.13 meq-

ml^{-1}) and PO$_4$$^{2-}$ (0.09 meq-ml^{-1}) were infused during the dose-response studies to prevent hypokalemia and hypophosphatemia. Glucose (~45 g/100 ml) was infused via a separate Harvard pump so as to clamp the arterialized venous plasma at 95 mg-dl^{-1}. The actual glucose concentration of the glucose solution was measured on each occasion.

Analytical techniques. Arterialized venous samples were used for all analyses. Samples for free insulin (20) were collected in EDTA-containing tubes (Becton-Dickinson & Co., Rutherford, NJ) and centrifuged at the end of each experiment). The resultant plasma was stored at −20°C until radioimmunoassay. Samples for glucose and glucose specific activity were collected in NaF–oxalate tubes (Becton-Dickinson & Co.) and centrifuged immediately. An aliquot was used for determining in duplicate of glucose concentration using a glucose oxidase method (Yellow Springs Instruments Co., Yellow Springs, OH). The remainder was stored at −20°C before determination of glucose specific activities.

[2^{3}H]Glucose, [3^{3}H]glucose, and [6^{14}C]glucose specific activity. Plasma for glucose specific activity was deproteinized using Ba(OH)$_2$ and ZnSO$_4$ by the method of Somogyi (21). After centrifugation, the supernatant was passed sequentially through anion (AG1-X8, BioRad Laboratories, Richmond, CA) and cation (AG 50W X8, BioRad Laboratories) exchange columns to remove charged [^{14}C] intermediates. The eluate was evaporated under N$_2$ and reconstituted to the original volume of plasma using 133 mM phosphate buffer (pH 7.4). This reconstituted material containing [2^{3}H], [3^{3}H], and [6^{14}C]glucose was divided into three aliquots. The above and all subsequent procedures were performed in glass containers.

One aliquot was used for determination of total [^{14}C] and total [^{3}H] counts. After addition of 0.5 ml 133 mM phosphate buffer and 10 ml Safety-Solve (Research Products International Corp., Mount Prospect, IL) this 0.25-ml aliquot was counted in a dual channel refrigerated liquid scintillation spectrometer. The external ratios method was used to correct for quenching. Radioactivity in this aliquot represented total [^{14}C] and total [^{3}H] (both [2^{3}H] and [3^{3}H] radioactivity).

The second 0.25-ml aliquot was used for determining [3^{3}H]glucose radioactivity. This was accomplished by selective enzymatic detritiation of [2^{3}H]glucose during which [3^{3}H]glucose was left intact. Subtraction of the [3^{3}H]glucose radioactivity from total tritiated radioactivity yielded [2^{3}H]glucose radioactivity. Selective detritiation of [2^{3}H]glucose was performed using a modification of the method of Issekutz (22). 0.5 ml of a mixture containing 1.2 U hexokinase (Sigma Chemical Co., St. Louis, MO), 5 U phosphoglucose isomerase (Sigma Chemical Co.), MgCl$_2$ (final concentration in reaction mixture 6 mM), and ATP (Sigma Chemical Co., final concentration 5.6 mM) was added to a 0.25 ml aliquot of reconstituted plasma. The mixture was incubated at 37°C for 2 h. The samples were then dried under a stream of air, reconstituted in water, and counted as above. External standards of [3^{3}H]glucose and [2^{3}H]glucose from each infusate were added to patient plasma and processed in parallel with every patient assay. The results were used to calculate the degree of detritiation of each isotope during each patient assay. Overall completion of detritiation of [2^{3}H]glucose was 96.5±0.3 (interassay coefficient of variation 1.63%, intraassay coefficient of variation 0.9%) while 98.7±0.6 (interassay coefficient of variation 2.6%, intraassay coefficient of variation 2.3%) of [3^{3}H]glucose remained intact. Essentially all of the residual radioactivity after the detritiation reaction and evaporation could be bound by the ion exchange columns, ruling out the presence of ionic nonglucose radioactive contaminants in the infusate.

The third aliquot was used to correct [^{14}C]glucose specific activity for Cori cycle activity. To do so, [1^{14}C]glucose present in the aliquots was enzymatically decarboxylated to estimate the amount of radioactivity that had been randomized from the C$_6$ position of infused glucose to the C$_1$ position. Assuming carbon from C$_6$ is equally randomized to C$_1$, C$_2$, C$_5$, and C$_6$, four times this amount was subtracted from total [^{14}C]glucose radioactivity to correct for Cori cycle activity according to the method of Reichard et al. (4). The modification of the enzymatic decarboxylation of Kalhan et al. (23) has been described fully elsewhere (24). In brief, an 0.7-ml aliquot of patient sample was placed in a glass tube to which 0.31% bovine serum albumin, 6 mM MgCl$_2$, 6.8 mM ATP, 6.8 mM NADP, 6 U hexokinase and 6 U glucose-6-phosphate

1. *Abbreviation used in this paper:* IDDM, insulin-dependent diabetes mellitus.

dehydrogenase were added (all reagents, Sigma Chemical Co.; concentrations given were those of final reaction mixture). The tube was capped with a tightly fitting rubber stopper (Bittner Industries, Inc., Mobile, AL) and the reaction was started by the injection through the stopper of 15 U glutamate dehydrogenase (Calbiochem, LaJolla, CA) 9 mM alpha-ketoglutarate (Sigma Chemical Co.) and 6 U 6-phosphogluconate dehydrogenase (Sigma Chemical Co.). The mixture was incubated for 24 h at 37°C. Completion of the reaction was assessed using external standards of [1^{14}C] processed in parallel with the patient samples. Overall decarboxylation was 92.7±1.4% (interassay coefficient of variation 4.4%, intraassay coefficient of variation 2.7%).

Carbon dioxide produced by the decarboxylation was trapped in 0.1 ml hyamine (1 mM in methanol, J. T. Baker Chemical Co., Philipsburg, NJ) contained in a plastic well (Kontes Co., Vineland, NJ) suspended from the rubber stopper. After the 24-h incubation period, the well containing hyamine was placed in a scintillation vial along with 10 ml Safety-Solve and 1 ml water, and [^{14}C] radioactivity was counted as above. The efficiency of hyamine trapping was assessed by the external standards. Overall trapping was 62.1±1.1% (interassay coefficient of variation 9.5%, intraassay coefficient of variation 10.5%), which corrected for quench averaged 96.4±0.4%.

Calculation of glucose utilization. Glucose utilization rates were determined for each isotope before the first insulin infusion and during the last 40 min of each subsequent infusion as described previously (19). Glucose utilization rates were calculated by employing the non-steady-state equations of Steele (2) as modified by DeBodo (1). The pool fraction in these equations was assumed to be 0.5.

Statistics. Data in the text and figures are given as mean±SEM. Statistical analyses were performed using the paired and nonpaired two-tailed t test for normally distributed data, and the Wilcoxon paired-sample and Mann-Whitney tests for nonnormally distributed data. Overall differences between turnover rates during insulin infusions both between isotopes and between diabetic and nondiabetic subjects were tested using the sum of all observations for each parameter.

Results

Plasma glucose and insulin concentrations and glucose infusion rates (Fig. 1). Plasma glucose concentrations were greater in the diabetic patients than nondiabetic subjects (180±29 vs. 94±1 mg-dl^{-1}), $P < 0.02$). Plasma glucose concentrations were allowed to decrease during the first insulin infusion in the diabetic subjects

until euglycemia was achieved. Thereafter, plasma glucose concentrations were maintained at 95±1 mg-dl^{-1} (mean coefficient of variation, 4.4±0.4%), which did not differ from the plasma glucose concentrations in the nondiabetic subjects (95±1 mg-dl^{-1} mean coefficient of variation 5.0±0.4%) during the insulin infusions.

Plasma-free insulin concentrations were significantly greater in the diabetic than nondiabetic subjects both before (18±7 vs. 3±1 μU-ml^{-1}, $P = 0.01$) and during the 0.4 mU-kg^{-1}-min^{-1} insulin infusion (31±7 vs. 20±1 μU-ml^{-1}, $P = 0.005$), and slightly but not significantly greater during the 1.0 and 2.0 mU-kg^{-1}-min^{-1} insulin infusions (72±12 vs. 60±4, 147±16 vs. 127±10 μU-ml^{-1}, respectively). The glucose infusion rates required to maintain euglycemia were significantly lower in the diabetic patients than the nondiabetic subjects at all insulin infusion rates (1.7±0.5 vs. 4.9±0.5 mg-kg^{-1}-min^{-1}, $P = 0.001$, 5.6±0.8 vs. 9.0±0.5 mg-kg^{-1}-min^{-1}, $P = 0.004$, 8.0±0.7 vs. 10.2±0.4 mg-kg^{-1}-min^{-1}, $P = 0.01$ mg-kg^{-1}-min^{-1} during the 0.4, 1.0, and 2.0 mU-kg^{-1}-min^{-1} insulin infusions, respectively).

Glucose turnover in the postabsorptive state. Before infusion of insulin, glucose turnover (glucose utilization and production) rates in the diabetic patients determined with [6^{14}C]glucose (3.1±0.3 mg-kg^{-1}-min^{-1}) were significantly greater than those determined with [3^{3}H]glucose (2.6±0.3 mg-kg^{-1}-min^{-1}, $P = 0.007$) and slightly but not significantly lower than those with [2^{3}H]glucose (3.4±0.4 mg-kg^{-1}-min^{-1}, $P = 0.16$). In the nondiabetic subjects, glucose turnover rates determined with [6^{14}C]glucose (2.3±0.1 mg-kg^{-1}-min^{-1}) were slightly but not significantly greater than those determined with [3^{3}H]glucose (2.1±0.1 mg-kg^{-1}-min^{-1}, $P = 0.10$) and less than those determined with [2^{3}H]glucose (2.7±0.2 mg-kg^{-1}-min^{-1}, $P = 0.008$).

Glucose turnover rates determined with [6^{14}C]glucose were significantly greater ($P = 0.02$) in the diabetic than nondiabetic subjects. Glucose turnover rates were also greater in the diabetic than nondiabetic subjects when determined with both [2^{3}H] and [3^{3}H]glucose; however, these differences did not reach statistical significance ($P = 0.13$ and 0.16, respectively).

Glucose turnover during insulin infusion (Fig. 2). During insulin infusion, glucose turnover rates in the diabetic patients
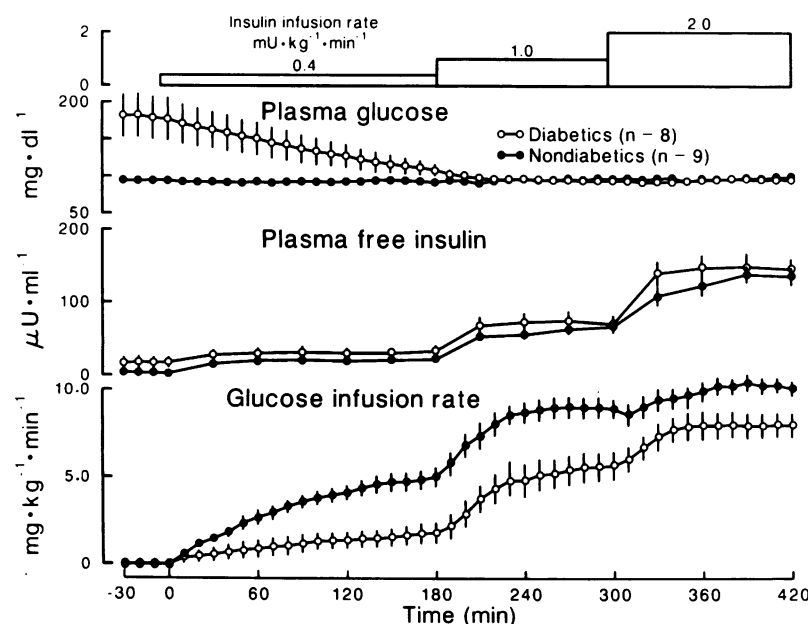

Figure 1. Plasma glucose and insulin concentrations and glucose infusion rates required to maintain euglycemia during infusion of insulin.
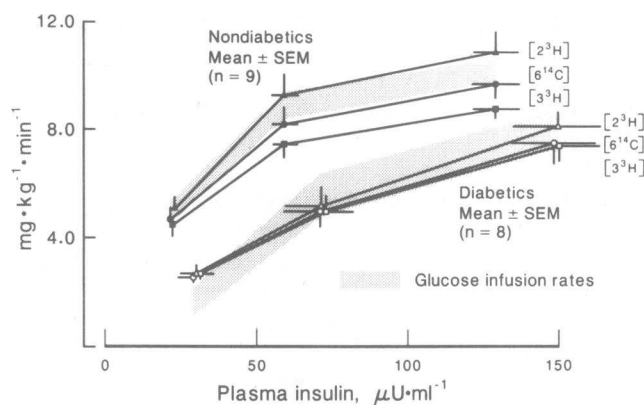
Figure 2. Isotopically determined glucose utilization rates are plotted versus the prevailing plasma insulin concentrations. Data from diabetic patients, *open circles.* Data from nondiabetic subjects, *solid circles.* The shaded area represents the mean±SEM of the glucose infusion rate required to maintain euglycemia.

determined with [6^{14}C] (2.6±0.2, 5.0±0.6, and 7.5±0.8 mg·kg^{-1}-min^{-1}) and [3^{3}H]glucose (2.7±0.2, 5.0±0.6, and 7.5±0.7 mg-kg^{-1}-min^{-1}) were equivalent. On the other hand, glucose turnover rates determined with [2^{3}H]glucose (2.7±0.3, 5.2±0.7, and 8.1±0.9 mg-kg^{-1}-min^{-1}) were slightly greater than those determined with [6^{14}C]glucose (P = 0.04 overall). Glucose turnover rates determined with all three isotopes during the highest insulin infusion (when glucose production was presumably completely suppressed, and, therefore, the glucose infusion rate should equal glucose turnover) did not differ from the actual exogenous glucose infusion rate required to maintain euglycemia.

In contrast, in nondiabetic subjects, glucose turnover rates determined with [3^{3}H]glucose (4.5±0.4, 7.5±0.5, and 8.8±0.4 mg-kg^{-1}-min^{-1}) were lower than those determined with [6^{14}C]glucose (4.7±0.4, 8.2±0.6, and 9.7±0.5 mg-kg^{-1}-min^{-1}, P = 0.01 overall). As in the diabetic patients, glucose turnover rates in the nondiabetic subjects determined with [2^{3}H]glucose (5.1±0.5, 9.3±0.7, and 10.9±0.7 mg-kg^{-1}-min^{-1}) were greater than those determined with [6^{14}C]glucose (P = 0.02). The glucose turnover rates in the nondiabetic subjects determined with either [6^{14}C] or [2^{3}H]glucose during the highest insulin infusion did not differ significantly from the actual amount of glucose infused. On the other hand, glucose turnover rates determined with [3^{3}H]glucose were significantly lower than the exogenous glucose infusion rates (P = 0.006) during the 2.0 mU-kg^{-1}-min^{-1} insulin infusion.

Comparison of insulin-induced glucose utilization in diabetic and nondiabetic subjects (Fig. 3). To determine whether use of different isotopes would influence conclusions regarding the degree of insulin resistance in diabetes mellitus, glucose utilization rates measured with each isotope were compared in the diabetic and nondiabetic subjects. Diabetic patients would be judged to have decreased glucose utilization no matter which isotope was used (overall P = 0.004, 0.008, and 0.002 for [6^{14}C], [3^{3}H], and [2^{3}H]glucose, respectively). To determine where differences existed at physiologic and supraphysiologic insulin concentrations, differences were sought at the individual insulin infusion rates. This analysis indicates that [6^{14}C] and [2^{3}H]glucose would lead to the conclusion that glucose utilization was decreased at all insulin concentrations (P = 0.03–0.001). On the other hand, use of [3^{3}H]glucose would lead to the conclusion that glucose utilization in the diabetic subjects was significantly lower than that in the nondiabetic subjects during the 0.4 (P = 0.002) and 1.0 (P = 0.005) mU-kg^{-1}-min^{-1} insulin infusions but not during the highest insulin infusion (P = 0.15).

Comparison of insulin-induced suppression of endogenous glucose production in diabetic and nondiabetic subjects (Fig. 4). Because glucose production rates are calculated by subtracting the actual amount of glucose being infused from the isotopically determined glucose turnover rates, differences between turnover rates determined with the three isotopes resulted in different relative patterns of suppression of glucose production in the diabetic and nondiabetic subjects. While endogenous glucose release during the 0.4 mU-kg^{-1}-min^{-1} insulin infusion was greater in the diabetic than nondiabetic subjects with [6^{14}C]glucose (0.6±0.3 vs. −0.3±0.2 mg-kg^{-1}-min^{-1}, P = 0.02), [3^{3}H]glucose (0.7±0.3 vs. −0.5±0.1 mg-kg^{-1}-min^{-1}, P = 0.002), and [2^{3}H]glucose (0.7±0.3 vs. 0.1±0.2 mg-kg^{-1}-min^{-1}, P = 0.17), [6^{14}C] and [3^{3}H]glucose but not [2^{3}H]glucose would lead to the conclusion that there was a "statistically" significant difference in suppression of glucose production.

To determine whether differences existed between diabetic and nondiabetic subjects once maximal suppression had occurred, glucose production rates were compared during the 1.0 and 2.0 mU-kg^{-1}-min^{-1} insulin infusions. Suppression determined with [6^{14}C]glucose in the nondiabetic subjects (−0.8±0.3 and −0.5±0.4 mg-kg^{-1}-min^{-1}) did not differ from that observed in diabetic patients (−0.7±0.3 and −0.5±0.3 mg-kg^{-1}-min^{-1}, P = 0.93). On the other hand, suppression determined with [3^{3}H]glucose was less in the diabetic patients (−0.7±0.3 and −0.5±0.3 mg-kg^{-1}-min^{-1}) than in the nondiabetic subjects (−1.5±0.2 and −1.4±0.4 mg-kg^{-1}-min^{-1}, P = 0.04). Suppression determined with [2^{3}H]glucose was greater in the diabetic patient
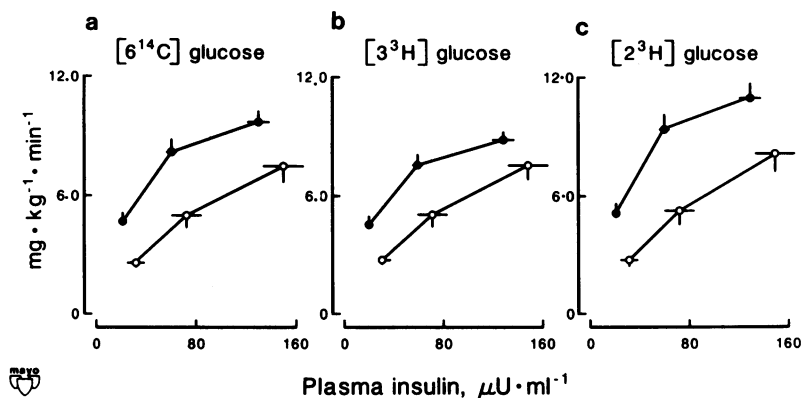
Figure 3. Isotopically determined glucose utilization rates determined with [6^{14}C]glucose (*a*), [3^{3}H]glucose (*b*), and [2^{3}H]glucose (*c*) are plotted versus the prevailing insulin concentrations in the diabetic (*open circles*) and nondiabetic (*solid circles*) subjects.

Figure 4. Isotopically determined glucose production rates determined with [6^{14}C]glucose (a), [3^{3}H]glucose (b), and [2^{3}H]glucose (c) are plotted versus the prevailing insulin concentrations in the diabetic (open circles) and nondiabetic (solid circles) subjects.
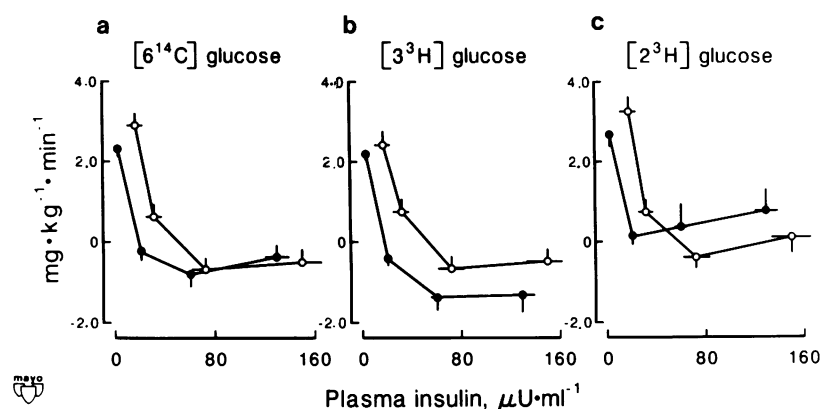
(−0.4±0.3 and 0.1±0.4 mg-kg^{-1}-min^{-1}) than nondiabetic subjects (0.4±0.6 and 0.7±0.6 mg-kg^{-1}-min^{-1}), but this difference did not reach statistical significance ($P = 0.21$).

Cori cycle activity. Cori cycle activity calculated as four times the [^{14}C] radioactivity percent in the C_1 position during the infusion of [6-^{14}C]glucose averaged 0.15±0.02, 0.13±0.03, 0.19±0.09, and 0.18±0.07 mg-kg^{-1}-min^{-1} in the nondiabetic and 0.36±0.04, 0.15±0.03, 0.13±0.06, and 0.23±0.09 mg-kg^{-1}-min^{-1} in the diabetic subjects at baseline and during the 0.4, 1.0, and 2.0 mU-kg^{-1}-min^{-1} insulin infusions, respectively.

Discussion

Tritiated isotopes of glucose have been used extensively to measure rates of glucose production and utilization in diabetic and nondiabetic man. Use of these isotopes assumes that their metabolism reflects the metabolism of the carbon skeleton of glucose. This assumption is based primarily on studies in postabsorptive nondiabetic animals and man (3, 5, 6, 12–14, 25). The current studies indicate that in the postabsorptive state, glucose turnover rates in hyperglycemic patients with IDDM determined with [2^{3}H]glucose are slightly higher, and rates determined with [3^{3}H]glucose slightly lower than those determined with [6^{14}C]glucose. These relationships were qualitatively similar to those observed in nondiabetic individuals. On the other hand, the relationship between isotopes differed in diabetic and nondiabetic subjects during insulin infusion. Whereas turnover rates determined with [2^{3}H]glucose were significantly greater than rates determined with [6^{14}C]glucose in both groups, rates determined with [3^{3}H]glucose were equivalent to those determined with [6^{14}C]glucose in the diabetic patients, but were lower in the nondiabetic subjects.

Both futile cycling and incorporation of radioactivity into and release from glycogen can alter isotopically determined glucose turnover rates (16). The slightly higher glucose utilization rates observed with [2^{3}H]glucose than those with [6^{14}C]glucose in the current experiments after an overnight fast are in agreement with studies in mammals (3, 5, 11–13) and recent studies in nondiabetic man (12, 26, 27). This overestimation of glucose utilization has been shown in animals to result from detritiation of [2^{3}H]glucose during passage from glucose-6 to fructose-6 to glucose-6-phosphate (8). Because the glucose/glucose-6-phosphate cycle results in hydrolysis of ATP without full metabolism of glucose, this process has been referred to as futile cycling (28).

The absolute rate of this cycle tended to increase with insulin-induced glucose utilization, reaching a maximum during the

highest insulin infusion; however, the percentage of glucose detritiated by this process did not increase. These results are consisent with those reported by Issekutz et al. and Radziuk during glucose infusion in dogs (13, 15). In contrast to a recent report by Effendic et al. that the glucose/glucose-6-phosphate cycle is increased in patients with impaired glucose tolerance (29) (measured as the difference between turnover rates determined with [2^{3}H] and [3^{3}H]glucose), futile cycling (measured as the difference between rates determined with [2^{3}H] and [6^{14}C]glucose) was not increased in patients with IDDM, either before or during insulin infusion. In fact, if the differences between rates determined with [2^{3}H] and [3^{3}H]glucose were used as an indicator in the current study, futile cycling at the glucose/glucose-6-phosphate level was less ($P = 0.002$) in the diabetic patients during infusion of insulin. Of note, glucose utilization rates determined with both [6^{14}C] and [2^{3}H]glucose more closely reflected the exogenous glucose infusion rates during the highest two insulin infusions (i.e., when glucose production was completely suppressed) in both the diabetic and nondiabetic subjects than did [3^{3}H]glucose.

The current experiments, however, have not excluded the possibility that glucose turnover rates determined with [6^{14}C]glucose were artifactually low due to uptake and release of glucose from glycogen without loss of radioactive label. This could be a particular problem if the outer layers of glycogen are selectively labeled (10). We believe this explanation to be unlikely because the difference between turnover rates determined with [2^{3}H] and [6^{14}C]glucose, if anything, increased with increasing insulin concentrations at a time when hepatic glucose release (and therefore potential release of radioactive label from glycogen) was decreasing. Overall, our results strongly support the presence of a glucose/glucose-6-phosphate cycle in both diabetic and nondiabetic man.

A variety of in vitro studies have demonstrated that futile cycling can also occur at the fructose-6-phosphate/fructose diphosphate level (6, 7). In vivo, this would result in an overestimate rather than an underestimate of glucose utilization measured using [3^{3}H]glucose. The lack of significant difference between glucose utilization rates in the nondiabetic subjects calculated with [3^{3}H]glucose and those calculated with [6^{14}C]glucose in the postabsorptive state are consistent with the previous report by Streja et al. (25), and recent reports by Shulman et al. (26) and Argound et al. (30). Taken together, these findings suggest that futile cycling in the postabsorptive man at the fructose-6-phosphate/fructose diphosphate level is insignificant. However, this conclusion rests on the assumption that

there is no isotope effect hindering the metabolism of [3^3H]glucose (see below). If an isotope effect did occur, it may have obscured the presence of a fructose-6-phosphate/fructose diphosphate cycle.

During infusion of insulin, glucose utilization rates determined with [3^3H] and [6^{14}C]glucose in the diabetic subjects were virtually identical and accurately reflected the exogenous glucose infusion rate in the diabetic patients. These data suggest that [3^3H]glucose may be a useful tracer in patients with IDDM. Unfortunately, because [3^3H]glucose consistently underestimated both the glucose turnover rates determined with [6^{14}C]glucose and the exogenous glucose infusion rate in the nondiabetic subjects, using [3^3H]glucose could potentially lead to erroneous conclusions when diabetic and nondiabetic subjects are compared. Preliminary data reported by Argound et al. (30) reveal a similar underestimate during a hyperinsulinemic clamp in nondiabetic subjects when flux rates determined with [3^3H] are compared with those determined with 6,6 didueterated glucose, an isotope whose metabolism is believed to be similar to [6^{14}C]glucose.

The etiology of the underestimate of [6^{14}C]glucose utilization rates and the glucose infusion rates by [3^3H]glucose in the nondiabetic subject is not known. This observation may provide a partial explanation why, under conditions of high glucose utilization such as are present during hyperinsulinemic euglycemic clamps, glucose flux rates determined with [3^3H]glucose not uncommonly are lower than the glucose infusion rate in both man (31–35) and dog (36). This results in the disconcerting conclusion that glucose production rates are negative (31–36). A similar relationship was evident in the nondiabetic subjects in the current studies with glucose utilization rates determined with [3^3H]glucose being slightly (~ 1 mg-kg^{-1}-min^{-1}) but consistently lower than the actual glucose infusion rates. The difference between glucose utilization rates determined with [3^3H]glucose during the highest insulin infusion from those determined with [6^{14}C]glucose remained significant ($P = 0.007$) even if the [^{14}C]glucose specific activity was not corrected for Cori cycle activity. The difference between glucose utilization rates determined with [6^{14}C] and [3^3H]glucose becomes even larger if Cori cycle activity is calculated by multiplying the radioactivity in the C_1 position of glucose by 5 (25) rather than 4, as was done in the present study. The true rate of the Cori cycle in man is unknown, because dilution of the intracellular precursor pool under non–steady-state conditions has not been determined. The difference between the glucose utilization rate determined with [3^3H]glucose and the glucose infusion rate also cannot be attributed solely to use of an inappropriate pool correction factor (13, 37), because the differences in glucose utilization rates determined with [3^3H]glucose and the glucose infusion rates persisted both when the pool correction factor was considered to be 1 (i.e., no slowly equilibrating compartment) or when the steady-state form of Steele's equation (2) was used in the calculation (data not shown).

The underestimate of glucose flux in the nondiabetic subjects is unlikely to be due to the presence of three-carbon tritiated intermediates because: (a) they are removed during passage over the anion and cation exchange resins, (b) they presumably would be accompanied by a proportionate number of [^{14}C]-labeled compounds, and (c) they were not evident in the diabetic patients.

Although glycerol would not be extracted by the exchange resins, it is difficult to envision how tritium relative to ^{14}C could be selectively incorporated into this compound. In addition, tritiated glycerol presumably would be present in both diabetic and nondiabetic subjects whereas the underestimate was only observed in the latter. Because large amounts of tritiated water and presumably tritiated NADPH are generated during insulin-stimulated glucose metabolism, it is possible that lipogenesis resulted in inclusion of radioactivity into plasma lipids or glucose. Theoretically, radioactivity from [2^3H]glucose could have been randomized from [2^3H]glucose to the third position of [3^3H]glucose if detritiation at the level of phosphoglucose isomerase was incomplete. This possibility appears unlikely however, because tritium is rapidly and quantitatively recovered in body water after injection of [2^3H]glucose (14). The difference between [3^3H] and [6^{14}C]glucose cannot be attributed to selective release of [3^3H]glucose from glycogen because both isotopes retain their radioactive label when glucose is directly incorporated into glycogen, and [3^3H]glucose, if anything, will lose its label if it is incorporated into glycogen via the indirect pathway.

Differences in metabolism between tritiated and nontritiated glucose could account for the present observations. Isotope effects have been well documented both in vitro and in vivo (38–40). Deuterated dihydroxyacetone-phosphate is metabolized by the triosephosphate isomerase reaction (i.e., the site of detritiation of [3^3H]glucose) at approximately one-half the rate of the nondeuterated substrate (39, 40). This isotope effect has been demonstrated in both muscle (39) and liver (40). Because the mass of tritium is greater than that of deuterium, it can be calculated that tritiated dihydroxyacetone-phosphate would be metabolized at only one-third the rate of ordinary substrate (39). If a similar isotope effect occurs in man, the ratio of [3^3H]glucose to ordinary glucose metabolized could be altered, leading to an apparent underestimate of glucose turnover. Whether normalization of glycemia in IDDM restores [3^3H]glucose metabolism toward that seen in nondiabetic individuals, thereby also resulting in an underestimate of glucose turnover, is unknown.

Many (41–44), but not all (45), investigators have reported that conventionally treated patients with IDDM are resistant to insulin. Most of these studies have used the infusion rate of exogenous glucose required to maintain euglycemia as an indicator of insulin action. Using this criterion, the current studies demonstrate that significantly lower glucose infusion rates were required to maintain euglycemia in the diabetic patients at all insulin concentrations. However, because the glucose infusion rate required to maintain euglycemia equals the algebraic sum of the insulin-induced decrease in glucose production and the insulin-induced increase in glucose uptake, it cannot be equated with glucose utilization unless glucose production is totally suppressed.

If either [2^3H] or [6^{14}C]glucose were chosen as the tracer for determination of glucose utilization, the same conclusions would have been reached, namely that diabetic patients had decreased glucose utilization at all insulin concentrations examined. On the other hand, [3^3H]glucose indicated that the diabetic patients had lower glucose utilization rates than nondiabetic subjects at insulin concentrations of <100 μU/ml but did not differ significantly from normal at insulin concentrations >100 μU/ml. Because response to insulin at supraphysiologic concentrations has been equated with postbinding events (46), the [3^3H]glucose data could be interpreted as indicating normal postreceptor function in the diabetic patients. Obviously, this latter conclusion is erroneous and stems from the fact that [3^3H]glucose underestimated glucose utilization in the nondiabetic subjects most markedly at the highest insulin concentrations. Such as under-

estimate may be only a minor irritant when there are large differences in insulin action between diabetic and nondiabetic subjects, but may be a major problem when relatively small differences are present.

Hepatic response to insulin has been reported to be normal in IDDM (41, 42, 44). However, hepatic insulin resistance may have been overlooked since all these studies used insulin concentrations well above those required to produce maximal suppression of glucose production in normal man. The current studies demonstrate impaired suppression of glucose production in the IDDM patients at submaximal insulin concentrations when measured by $[6^{14}C]$ or $[3^{3}H]$glucose. $[2^{3}H]$Glucose also yielded results consistent with impaired glucose production at low insulin concentrations in the diabetic patients, but these differences did not reach statistical significance. $[3^{3}H]$Glucose but not $[6^{14}C]$ or $[2^{3}H]$glucose indicated impaired suppression of hepatic glucose production in IDDM at high insulin concentrations only if the absolute rates were considered (i.e., negative glucose production rates retained). If negative glucose production were considered as zero, then $[3^{3}H]$glucose also would lead to the conclusion that suppression of glucose production was equivalent at high insulin concentrations in the diabetic and nondiabetic subjects.

It should be noted that the differences in the pattern of suppression of glucose production at submaximal and maximal insulin concentrations using the three different isotopes were due to differences in the perceived suppression in the nondiabetic rather than the diabetic patients. This stemmed from the fact that $[3^{3}H]$glucose underestimated actual turnover in the nondiabetic patients. Using $[6^{14}C]$glucose as the standard, the current studies indicate that diabetic patients have hepatic as well as extrahepatic insulin resistance. Even this conclusion must be qualified by the possibility that there was greater incorporation into and release of $[6^{14}C]$glucose from glycogen in the nondiabetic patients than in the diabetic patients. Because this label does not lose its label during passage through glycogen, incorporation into and release from glycogen would lead to an underestimate of glucose production with apparent greater suppression in the nondiabetic subjects. This possibility seems unlikely, however, because it would imply greater activity of glycogen phosphorylase in the nondiabetic subjects than in the diabetic patients, the opposite of what has been demonstrated in vitro (17).

In summary, the current studies indicate that similar estimates of glucose turnover were obtained using $[6^{14}C]$, $[3^{3}H]$, and $[2^{3}H]$glucose in patients with IDDM both in the postabsorptive state and during insulin-induced stimulation of glucose utilization. The slightly greater turnover rates determined with $[2^{3}H]$ than $[6^{14}C]$glucose support the presence of futile cycling at the level of glucose/glucose-6-phosphate in both diabetic and nondiabetic subjects. Use of any of these isotopes or simply the glucose infusion rates required to maintain euglycemia leads to the conclusion that the diabetic patients are insulin resistant. However, due to a systematic underestimate of glucose turnover by $[3^{3}H]$glucose in the nondiabetic subjects, using this isotope leads to the incorrect conclusion that glucose utilization does not differ significantly between groups at the highest insulin infusion rates. On the other hand, using $[6^{14}C]$ and $[3^{3}H]$glucose, but not $[2^{3}H]$glucose, leads to the conclusion that diabetic patients have impaired suppression of hepatic glucose production.

Overall, using turnover rates determined by $[6^{14}C]$glucose as the standard, the current studies indicate that patients with

IDDM have both hepatic and extrahepatic insulin resistance. They also emphasize that while tritiated isotopes of glucose may be more convenient than $[^{14}C]$glucose, they do not necessarily equally reflect the pattern of glucose metabolism in diabetic and nondiabetic man. Depending on the accuracy required, tritiated isotopes may still be useful in clinical research. However, caution clearly must be exercised in interpreting the data thus derived. Whether equivalent relationships pertain in other disease states or during hyperglycemic induced changes in glucose flux remains to be determined.

Acknowledgments

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