Mechanisms for abnormal postprandial glucose metabolism in type 2 diabetes


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Woerle, Hans J., Ervin Szoke, Christian Meyer, Jean M. Dostou, Steven D. Wittlin, Niyaz R. Gosmanov, Stephen L. Welle, and John E. Gerich. Mechanisms for abnormal postprandial glucose metabolism in type 2 diabetes. Am J Physiol Endocrinol Metab 290: E67–E77, 2006. First published August 16, 2005; doi:10.1152/ajpendo.00529.2004.—To assess mechanisms for postprandial hyperglycemia, we used a triple-isotope technique ([3-3H]glucose and [14C]bicarbonate and oral [6,6-dideutero]glucose iv) and indirect calorimetry to compare components of glucose release and pathways for glucose disposal in 26 subjects with type 2 diabetes and 15 age-, weight-, and sex-matched normal volunteers after a standard meal. The results were as follows: 1) diabetic subjects had greater postprandial glucose release (P < 0.001) because of both increased endogenous and meal-glucose release; 2) the greater endogenous glucose release (P < 0.001) was due to increased gluconeogenesis (P < 0.001) and glycogenolysis (P = 0.01); 3) overall tissue glucose uptake, glycolysis, and storage were comparable in both groups (P > 0.3); 4) glucose clearance (P < 0.001) and oxidation (P = 0.004) were reduced, whereas nonoxidative glycolysis was increased (P = 0.04); and 5) net splanchnic glucose storage was reduced by ~45% (P = 0.008) because of increased glycogen cycling (P = 0.03). Thus in type 2 diabetes, postprandial hyperglycemia is primarily due to increased glucose release; hyperglycemia overcomes the effects of impaired insulin secretion and sensitivity on glucose transport, but intracellular defects persist so that pathways of glucose metabolism are abnormal and glucose is shunted away from normal sites of storage (e.g., liver and muscle) into other tissues.

Gluconeogenesis; insulin; glycolysis; glycogenolysis; oxidation

CONSIDERABLE ATTENTION has recently been focused on the importance of postprandial hyperglycemia as a risk factor for cardiovascular disease (12, 14, 32) and as a rate-limiting factor for achieving optimal glycemic control in people with type 2 diabetes (T2DM) (68). Nevertheless, the mechanisms responsible for postprandial hyperglycemia are still poorly understood.

Although excessive postprandial release of glucose into the circulation has been uniformly found in patients with impaired glucose tolerance and T2DM (2, 22, 23, 27, 37, 45, 49), it is still unclear to what extent this depends on impaired splanchnic sequestration of the ingested carbohydrate and impaired suppression of endogenous glucose release (EGR) (9, 17, 23, 24, 37, 40, 47, 49, 51, 61). Moreover, although there is evidence for increased postprandial hepatic glycogen cycling and gluconeogenesis in T2DM (9, 29, 42, 45, 47), the individual contributions of these processes have not been examined.

With respect to routes and sites of postprandial glucose disposal, although overall tissue glucose uptake is generally “normal” in a quantitative sense in T2DM (2, 27, 37, 45, 49), overall glucose clearance is obviously abnormal because postprandial plasma glucose levels are higher in people with T2DM. Moreover, the finding of reduced postprandial hepatic and muscle glycogen accumulation in the face of “normal” overall tissue glucose uptake (10, 42) suggests that glucose might be taken up excessively by tissues that are not normally the major sites of postprandial glucose disposal (46) and that fluxes of glucose through specific pathways might be altered.

In humans, glucose taken up by tissues is, for the most part, either directly stored as glycogen or glycolyzed (67). The glucose undergoing glycolysis is then either oxidized or converted to gluconeogenic intermediates (67). In T2DM, postprandial glucose oxidation has been reported to be reduced (21, 37, 61), but postprandial glycolysis has not been assessed. Therefore, it remains unclear whether direct storage and non-oxidative glycolysis are also abnormal in T2DM.

We have recently applied a triple-isotope approach to characterize pathways for glucose disposal after meal ingestion in individuals with normal glucose tolerance (67). In this report, we used a similar approach to assess the same pathways in additional volunteers with normal glucose tolerance and in a larger number of subjects with T2DM than have been typically included in previous studies, thus providing greater statistical power to identify potential differences.

METHODS

Subjects. After the protocol had been approved by the University of Rochester Institutional Review Board, informed written consent was obtained from 15 healthy volunteers (7 men and 8 women, 49 ± 3 yr of age, 89 ± 4 kg body wt, BMI 30 ± 1 kg/m²; 36 ± 3 %body fat) with no family history of diabetes mellitus and normal glucose tolerance (according to World Health Organization criteria) (70), and 26 subjects with T2DM (16 men and 10 women, 53 ± 2 yr of age, 93 ± 4 kg body wt, BMI 30 ± 1 kg/m²; 34 ± 3 %body fat). The diabetic subjects had a mean Hb A1c of 8.6 ± 0.3% and had been treated with diet and exercise or oral hypoglycemic agents (metformin and or sulfonylureas), which had been withdrawn 4 days before the experiment. All subjects had normal physical examinations and routine laboratory tests, had been on a weight-maintaining diet containing >200 g of carbohydrate for 3 days before the study, and had abstained from alcohol and exercise.

Protocol. Subjects were admitted to the University of Rochester General Clinical Research Center between 6 and 7 PM the evening before the experiments, consumed a standard meal between 6:30 and 8 PM, and fasted thereafter, except for drinking water ad libitum. A 12-h overnight urine collection was started at 8 PM for measurement of urea nitrogen and glucose excretion. Urine was also collected for these determinations during the 6-h postprandial period. At 4 AM, primed (~20 μCi) continuous (~0.20 μCi/min) infusions of [3-3H]glucose and [14C]NaHCO₃ (100 μCi, 1.1 μCi/min) were started in a forearm vein. The normal prime of [3-3H]glucose, which...
was given in healthy volunteers, was adjusted for the prevailing plasma glucose concentration in the diabetic subjects (normal prime × millimolar plasma glucose/5 mM). Five hours were allowed for isotope equilibration. In eight of the healthy volunteers, at ~8 AM an infusion of p-aminohippuric acid (12 mg/min) was started for determination of renal blood flow (8) for calculation of renal glucose uptake and release, which have been separately reported. In these eight subjects, a renal vein was catheterized between 8 and 9 AM (67).

At ~9 AM, a dorsal hand vein was cannulated and kept in a thermostatically controlled Plexiglas box at 65°C for sampling arterialized venous blood. About 1 h later, blood sampling was started from the dorsal hand vein at 30-min intervals for the next 7 h. Whole body CO₂ production and O₂ consumption were determined over 20-min intervals three times during the baseline period and hourly thereafter during the remainder of the experiment, using a canopy indirect calorimetry system (DeltaTrac, Anaheim, CA) (36). To allow for equilibration within the canopy, only data during the last 10 min were used for analysis. Throughout the experiment, arterialized samples were collected at 30-min intervals for measurements of glucose, free fatty acids (FFA), insulin, glucagon, lactate, glycerol, and alanine levels, glucose and water specific activities, and glucose enrichments. Breath samples for ¹⁴CO₂ specific activities were collected at 30-min intervals beginning at ~60 min.

At 10 AM, subjects ingested a meal for 5 min. The size and composition of this meal were designed to simulate a normal breakfast. The size of the meal was 6 kcal/kg, with a composition of 50% carbohydrate, 30% fat, and 20% protein. It consisted of solid (cheese omelet) and liquid (dextrose, Dextol; Baxter Healthcare, McGaw Park, IL) components. On average, the meal contained 84 g of glucose, 10 g of fat, and 26 g of protein, including 3 g of [6,6-d²]glucose to permit determination of the system appearance of ingested glucose. Subjects remained supine throughout the study, except when they were ingesting the meal.

Analytical procedures. Blood samples were collected for glucose, lactate, alanine, and glycerol concentrations, [¹³C]H₂O, and [¹⁴C]glucose specific activities, [6,6-d²]glucose enrichments, and tritiated water in oxalate-fluoride tubes for FFAs concentrations in EDTA tubes and for insulin and glucagon concentrations in EDTA tubes containing a protease inhibitor. Samples were immediately placed in a 4°C ice bath, and plasma was separated within 30 min by centrifugation at 4°C. Plasma glucose was determined in triplicate with a glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH).

Plasma glucose [¹³C] and [³H] specific activities and breath [¹⁴CO₂] specific activities and plasma deuterated glucose enrichments were measured by previously described methods (13, 62, 67). Plasma tritiated water specific activity (67) was measured as follows: protein-free filtrates of plasma were passed over ion exchange columns, and the resultant eluate ([³H₂O + [³H]glucose] was divided; one aliquot was evaporated to remove [³H₂O and represented disintegrations per minute (dpm) only in glucose; this was resuspended and counted, as well as the other aliquot, representing [³H₂O + [³H]glucose (60). The difference between the two represents the counts in [³H₂O. Plasma lactate, alanine, glycerol, and FFA concentrations were determined by standard microfluorometric assays (39, 48, 66). Plasma insulin and glucagon were determined by standard radioimmunoassays as previously described (64).

Calculations. Homeostasis model assessment of insulin resistance (HOMA-IR), an index of insulin resistance, was calculated using the equation of Matthews et al. (43). Total glucose release into plasma and removal of glucose from peripheral plasma were determined with steady-state equations before meal ingestion (69) and subsequently after meal ingestion with non-steady-state equations of DeBodo et al. (15), using a pool fraction of 0.65 and a volume of distribution of 200 ml/kg. Release of glucose contained in the meal into the systemic circulation was calculated with the equation of Chiaisson et al. (11), using the plasma and meal [6,6-dideuterogluucose enrichments. In contrast to our previous studies, in which postprandial endogenous glucose production was calculated as the difference between total glucose release and meal glucose release, EGR was calculated independently as follows: from the ratio of [6,6-dideuterogluucose enrichments in plasma and in the meal, the proportions of plasma glucose attributable to exogenous and endogenous glucose were determined (61). The plasma endogenous glucose concentration was used to determine the specific activity of endogenous glucose. This was used in the nonsteady-state equation of Grill et al. (33).

Glucose entering plasma via gluconeogenesis was calculated from the incorporation of [¹³C] into plasma glucose from [¹⁴C]HCO₃ by using modifications of the above-mentioned equations for the postabsorptive and postprandial states (20, 44). In the postabsorptive steady state, it was calculated as Rₕ × 1.38 × Sₘₕ/Sₘₕ, where Rₕ is the overall rate of plasma glucose appearance, Sₘₕ is the [¹⁴C]plasma glucose specific activity (dpm/µmol), and Sₘₕ is the [¹³C] specific activity of CO₂ in exhaled breath (dpm/µmol). The constant 1.38 represents a correction factor for TCA cycle carbon exchange. Consoli et al. (13) and Dairaison et al. (19) determined correction factors for TCA cycle carbon exchange to be 1.39 and 1.34, respectively, in nondiabetic adults and 1.42 and 1.39, respectively, in T2DM subjects, none of which were significantly different from another. We therefore used the mean value from these studies. Although we did not determine correction factors in our subjects, use of the reported mean value of 1.38 seems reasonable because, in the studies to be presented, during maximum suppression of postprandial EGR all of the EGR would have been accounted for by gluconeogenesis if a correction factor of 1.5 had been used.

After meal ingestion, the nonsteady-state equation was used:

\[
(1.38)(Rₕ + Rₖ)(0.5)(Sₘₕ + Sₖ)(0.5) + \left( (T₂ - T₁)⁻¹(Sₘₕ × C₢₂) - (Sₖ × C₢₂) ⁄ 0.5(Sₘₕ × C₢₂) \right)
\]

where Rₕ, Sₘₕ, C₢₂, and CO₂ are rates of plasma glucose disposal, [¹⁴C]plasma glucose specific activities, plasma glucose concentrations, and breath [¹⁴CO₂] specific activities at times T₁ and T₂. Although two molecules of pyruvate are required to synthesize one molecule of glucose, there is no factor for this in the equation because, for each two molecules of [¹⁴CO₂ incorporated into oxaloacetate, one is lost during randomization of oxaloacetate with fumarate (7, 20).

Glycogenolysis was calculated by subtracting gluconeogenesis from EGR (1).

Overall splanchnic glucose uptake was calculated as the sum of first-pass extraction from the portal vein and uptake of glucose from the arterial circulation. First-pass glucose extraction of the ingested glucose was calculated as the difference between the amount of glucose ingested and the total appearance of the ingested glucose in the systemic circulation during the 6-h postprandial period (67), assuming absorption of the ingested glucose had been completed, as has been reported by Radziuk et al. (57). Splanchnic uptake of glucose from the arterial circulation was calculated as the product of arterial glucose concentration, splanchnic blood flow, and splanchnic glucose fraction extraction. We used values for splanchnic blood flow of 1.320 ml/min and values for splanchnic fraction extraction (3.3% diabetic subjects and 4.1% normal subjects), as reported by DeFronzo et al. (16). Peripheral glucose uptake was calculated by subtracting splanchnic uptake of glucose from the arterial circulation from total glucose uptake, determined isotopically as described above. Whole body glucose tissue uptake was calculated as the sum of peripheral and splanchnic glucose uptake minus glucose excreted in urine (25).

Rates of whole body glucose oxidation were calculated using indirect calorimetry, with protein oxidation estimated from measurement of urine urea nitrogen (30, 36, 63).

Glucose oxidation in g/min = 4.55 VCO₂ - 3.21 VO₂ - 2.87 N, where VCO₂ is CO₂ production, VO₂ is O₂ consumption, and N is urinary nitrogen excretion in liters per minute, liters per minute, and grams per minute, respectively.
Basal and postprandial glycolysis were calculated from the production of tritiated water from [3-3H]glucose as previously described (67). The validation and limitations of this technique have been discussed elsewhere in detail (26, 59). For graphical purposes, glycolysis of peripheral glucose was calculated as described by Féry et al. (26) and Rossetti et al. (60) as the increment per 30 min in plasma 3H2O calculated × body water mass ÷ the plasma glucose 3-3H specific activity. Plasma water was assumed to be 93% of total plasma volume, and total body water was measured using bioelectric impedance (41).

Whole body glycolysis was calculated as the sum of plasma and splanchnic glycolysis. To estimate glycolysis occurring individually in splanchnic and peripheral tissues, we used the method of Féry et al. (25). This involves calculation of a glycolytic fraction (total tritiated water production during the 6-h postprandial period divided by the total amount of tritiated glucose infused over the 6-h period). This glycolytic fraction was then multiplied by splanchnic and peripheral tissue glucose uptake to estimate glycolysis in splanchnic and peripheral tissues. This assumes that the glycolytic fractions for splanchnic and peripheral tissues are comparable. That this is a reasonable assumption is supported by the observations of Lukaski (41) that virtually identical proportions of intravenously and intraduodenally infused tritiated glucose undergo glycolysis in humans. Furthermore, even if glycolysis in tissues supplied by portal venous and arterial blood differed somewhat, a substantial proportion (~25%) of splanchnic glycolysis occurs from the arterial circulation (67). Whole body nonoxidative glycolysis was calculated as the difference between whole body glycolysis and whole body glucose oxidation as assessed by indirect calorimetry.

Because in normal humans virtually all glucose initially taken up by tissues can be considered either to be directly incorporated into glycogen or to undergo glycolysis (18, 67), whole body, splanchnic, and peripheral tissue direct pathway glucose storage was taken as the difference between their respective glucose uptake and their glycolysis (67). Because three carbon intermediates generated from nonoxidative glycolysis are almost exclusively consumed in the gluconeogenesis pathway (67), whole body indirect glucose storage was calculated as the difference between whole body nonoxidative glycolysis and the amount of glucose entering the plasma via gluconeogenesis (67). This was assumed to occur in splanchnic tissues. Therefore, total splanchnic glucose storage was taken as the sum of splanchnic glucose-direct and -indirect storage.

Statistical analysis. Unless stated otherwise, data are expressed as means ± SE. Two-tailed, nonpaired Student’s t-tests were used to compare data of diabetic and nondiabetic subjects. A P value of <0.05 was considered statistically significant. As a caveat to the reader, we wish to point out that measurement errors might be compounded when a value is derived as a function of several variables. Therefore, the accuracy of absolute values for various processes/pathways should be evaluated in view of their standard errors, and the differences found between diabetic and nondiabetic subjects should be considered qualitative rather than quantitative (i.e., the difference in direction, up or down, is more reliable than the absolute difference).

RESULTS

Arterial concentrations of plasma glucose, insulin, and glucagon. Diabetic subjects started with greater preprandial plasma glucose concentrations (11.7 ± 0.6 vs. 4.7 ± 0.1 mM, \( P < 0.001 \)) and had more prolonged and greater increases after meal ingestion Fig. 1. Consequently, their mean plasma glucose concentration during the 6-h postprandial period was almost three times as great as that of the nondiabetic volunteers (15.5 ± 0.7 vs. 6.0 ± 0.2 mM, \( P < 0.001 \)).

Fasting plasma insulin concentrations were greater in the diabetic subjects (77 ± 6 vs. 52 ± 5 pM, \( P = 0.015 \)). HOMA-IR, an index of insulin resistance, was greater in the diabetic subjects (6.82 ± 0.71 vs. 1.86 ± 0.19 mM/pM in nondiabetic volunteers, \( P < 0.001 \)). During the initial 90 min after meal ingestion, plasma insulin increased less in the diabetic subjects, averaging 179 ± 19 vs. 290 ± 29 pM in the nondiabetic volunteers (\( P = 0.016 \)).

Fasting plasma glucagon concentrations were comparable in diabetic and nondiabetic subjects (74 ± 3 vs. 69 ± 5 pM/ml, respectively, \( P = 0.41 \)). After meal ingestion, plasma glucagon increased in the diabetic subjects and decreased in the nondiabetic volunteers so that, during the initial 90 min, plasma glucagon in the diabetic subjects averaged 84 ± 4 vs. 53 ± 3 pg/ml in the nondiabetic volunteers (\( P < 0.001 \)). During the entire 6-h postprandial period, plasma glucagon levels in the
diabetic subjects were greater than in the normal volunteers (79 ± 3 vs. 64 ± 4 pg/ml, P = 0.02).

As a result of the above differences in plasma insulin and glucagon responses, the plasma insulin-to-glucagon molar ratio during the initial 90 min after meal ingestion was reduced by >50% in the diabetic subjects (8.3 ± 1.4 vs. 19.6 ± 2.3 in nondiabetic volunteers, P < 0.001). Over the entire 6-h postprandial period, the plasma insulin-to-glucagon molar ratio was also reduced in the diabetic subjects (7.6 ± 1.0 vs. 11.7 ± 1.3 in normal volunteers, P = 0.02).

**Arterial substrate concentrations.** Fasting plasma lactate concentrations were greater in the diabetic subjects (1.15 ± 0.20 vs. 0.85 ± 0.05 mM in normal volunteers, P = 0.035), whereas alanine (0.30 ± 0.02 vs. 0.29 ± 0.02 mM, P = 0.95), glycerol (0.08 ± 0.01 vs. 0.09 ± 0.01 mM, P = 0.96), and FFA concentrations (620 ± 25 vs. 624 ± 51 μM, P = 0.72) were not significantly different (Fig. 2).

During the postprandial period, plasma lactate and alanine concentrations increased in both groups; plasma lactate concentrations were greater in the diabetic subjects (1.29 ± 0.11 vs. 0.90 ± 0.04 mM in normal volunteers, P = 0.01), whereas plasma alanine concentrations were not significantly different (0.36 ± 0.02 vs. 0.32 ± 0.01 mM, P = 0.25). Over the 6-h postprandial period, plasma FFA and glycerol concentrations decreased comparably in both groups and were not significantly different in the diabetic subjects vs. the nondiabetic volunteers (364 ± 27 and 0.06 ± 0.01 vs. 398 ± 42 and 0.06 ± 0.01 μM, both P > 0.40). During the initial 90 min, however, decrements in FFA and glycerol concentrations were less in the diabetic subjects (204 ± 21 and 0.036 ± 0.003 μmol/l, respectively) than in the normal subjects (319 ± 47 and 0.047 ± 0.009 μmol/l, respectively, P = 0.01 and 0.04).

**Total, meal, and endogenous glucose release into the systemic circulation and rates of gluconeogenesis.** The time course of changes in plasma glucose, water, and breath CO₂ specific activities and/or enrichments are given in Fig. 3.

Postabsorptive EGR was significantly greater in the diabetic subjects (10.8 ± 0.4 vs. 8.3 ± 0.5 μmol·kg⁻¹·min⁻¹ in nondiabetic subjects, P = 0.002; Fig. 4, Table 1). This was attributable to increases in both glyceroneogenesis (7.0 ± 0.4 vs. 5.5 ± 0.6 μmol·kg⁻¹·min⁻¹, P = 0.051) and gluconeogenesis (3.8 ± 0.3 vs. 2.6 ± 0.2 μmol·kg⁻¹·min⁻¹, P = 0.009). The relative contribution of gluconeogenesis to EGR was comparable in both groups (32.5 ± 2.4 vs. 33.5 ± 3.4% in diabetic and nondiabetic subjects, respectively, P = 0.65).

After meal ingestion, total and meal glucose release into the systemic circulation followed similar patterns in both groups, increasing to a maximum at 30–60 min and subsequently decreasing toward basal rates. EGR decreased in both groups over the initial 90 min by comparable amounts (3.3 ± 0.7 and 4.6 ± 0.5 μmol·kg⁻¹·min⁻¹ in diabetic and nondiabetic subjects, respectively, P = 0.36). But because the diabetic subjects had greater preprandial values, their EGR during this period (7.4 ± 0.7 μmol·kg⁻¹·min⁻¹) was almost twice as great as that of the nondiabetic volunteers (3.7 ± 0.5 μmol·kg⁻¹·min⁻¹, P = 0.002).

During this period, gluconeogenesis did not change in either group. Although glycogenolysis decreased promptly and substantially in both groups, it was greater in the diabetic subjects than in the nondiabetic volunteers (3.8 ± 0.7 vs. 1.3 ± 0.6 μmol·kg⁻¹·min⁻¹, P = 0.022), representing suppression of ~45 and 75%, respectively.

During the 6 h after meal ingestion, total glucose release into the systemic circulation (97.8 ± 3.2 vs. 78.9 ± 4.2 g, P < 0.001) was significantly greater in the diabetic subjects. However, of the diabetic subjects’ ~20 g of overall greater glucose release, ~90% (~18 g) occurred during the initial 90 min. This was predominantly due to the ~12 g of greater meal glucose release into the systemic circulation (30.7 ± 1.7 vs. 18.9 ± 2.5 g in nondiabetic subjects, P < 0.001) because EGR was only ~6 g greater in the diabetic subjects during this period (11.2 ± 1.1 vs. 5.5 ± 0.9 g, P = 0.003).

During this period, meal glucose release was inversely correlated with the insulin-to-glucagon molar ratio (r = -0.41, P = 0.01) and directly correlated with decrements in plasma glucagon (r = 0.51, P < 0.001) and increments in plasma insulin adjusted for insulin resistance, i.e., HOMA-IR (r = -0.38, P = 0.02) but not plasma FFA decrements (r = 0.07, P = 0.68). EGR was also significantly correlated with the insulin-to-glucagon molar ratio (r = -0.48, P = 0.002) and plasma insulin increments (~0.46, P = 0.003) and plasma glucagon decrements (r = -0.54, P < 0.001), as well as plasma FFA decrements (r = 0.45, P = 0.003).

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**Fig. 2.** Arterial substrate concentrations. Values are means ± SE. FFA, free fatty acids.
Of the remaining overall greater glucose release into the systemic circulation in diabetic subjects during the last 4.5 h, all was attributable to greater EGR (26.3 ± 2.1 vs. 12.4 ± 2.1 g in diabetic and nondiabetic subjects, respectively, \( P < 0.001 \)), because meal glucose release was not significantly different in diabetic and nondiabetic subjects (31.6 ± 2.1 vs. 37.8 ± 4.9 g, respectively, \( P = 0.18 \)). EGR during this period was significantly correlated to the changes in plasma insulin (\( r = 0.48, P = 0.002 \)) and glucagon (\( r = 0.53, P < 0.001 \)) during the initial 90 min but not to either contemporaneous plasma insulin (\( r = -0.23, P = 0.16 \)) or glucagon (\( r = 0.17, P = 0.30 \)) responses.

Increased gluconeogenesis and glycogenolysis both contributed to the greater EGR in diabetic subjects. During the 6-h postprandial period, gluconeogenesis was almost twofold greater in the diabetic subjects (26.9 ± 2.2 vs. 15.3 ± 1.2 g in nondiabetic volunteers, \( P < 0.001 \)). Glycogenolysis was increased slightly more than twofold in the diabetic subjects (10.1 ± 1.2 vs. 4.3 ± 1.7, \( P = 0.007 \)) but accounted for roughly the same proportion of EGR as in the nondiabetic subjects (~22 vs. ~27%).

Peripheral glucose uptake, glycolysis, and direct storage. The patterns of postprandial changes in diabetic and nondiabetic subjects were similar. Moreover, during the 6-h postprandial period, peripheral tissue uptake of glucose, glycolysis, and direct storage were not significantly different in diabetic and nondiabetic subjects (72.9 ± 4.5 vs. 71.3 ± 5.4 g, \( P = 0.82 \); 42.7 ± 4.1 vs. 46.0 ± 3.8 g, \( P = 0.60 \); 30.2 ± 2.8 vs. 25.2 ± 3.4 g, respectively, \( P = 0.29 \); Fig. 5 and Table 2). The proportion of glucose taken up by peripheral tissues that underwent glycolysis tended to be lower in diabetic subjects (57 ± 3 vs. 68 ± 5% in nondiabetic volunteers, \( P = 0.061 \)).

Splanchnic glucose uptake, glycolysis, and direct storage. During the 6-h postprandial period, total splanchnic glucose uptake was comparable in diabetic and nondiabetic subjects (33.1 ± 1.4 vs. 29.9 ± 1.6 g, \( P = 0.17 \); Table 2). However, diabetic subjects had greater glucose uptake from the arterial circulation (10.3 ± 0.7 vs. 5.2 ± 0.3 g in nondiabetic volunteers, \( P < 0.001 \)), whereas their first-pass splanchnic uptake of ingested glucose was slightly reduced (22.7 ± 1.1 vs. 25.0 ± 1.7 g in nondiabetic volunteers, \( P = 0.35 \)). Splanchnic glycolysis was comparable in diabetic (18.7 ± 1.3 g) and nondiabetic subjects (21.0 ± 2.1 g, \( P = 0.34 \)). Direct splanchnic storage was greater in diabetic subjects (14.3 ± 0.9 vs. 8.9 ± 1.0 g in nondiabetic volunteers, \( P = 0.004 \)). By contrast, indirect storage was markedly reduced in the diabetic subjects (1.8 ± 1.2 vs. 6.5 ± 1.5 g in nondiabetic subjects, \( P = 0.03 \)). Nevertheless, as a result of these reciprocal changes in direct and indirect storage, total splanchnic glucose storage in the diabetic subjects (16.1 ± 1.2 g) was not significantly different from that of the nondiabetic subjects (15.3 ± 1.5 g, \( P = 0.73 \)).

Renal glucose excretion, total body glucose uptake, glycolysis, oxidation, and storage. During the 6-h postprandial period, diabetic subjects excreted 17.4 ± 2.7 g of glucose in their...
urine, whereas glycosuria was negligible in the nondiabetic volunteers ($0.7 \pm 0.4$ g, $P < 0.001$). Total body tissue glucose uptake, glycolysis, and direct storage (the sum of plasma and splanchnic values) were comparable in the diabetic and nondiabetic subjects ($106 \pm 4.6$ vs. $101 \pm 4.6$ g, $P = 0.50$; $61.5 \pm 4.9$ vs. $67.1 \pm 4.8$ g, $P = 0.47$; $44.5 \pm 3.7$ vs. $34.1 \pm 5.7$ g, $P = 0.12$, respectively; Table 2). However, direct storage accounted for a greater proportion of total storage in the diabetic subjects ($96 \pm 3.0$ vs. $84 \pm 4\%$ in nondiabetic subjects, $P = 0.03$). Postprandial whole body carbohydrate oxidation was reduced in the diabetic subjects ($32.8 \pm 2.8$ vs. $45.6 \pm 2.6$ g in nondiabetic volunteers, $P = 0.004$), whereas nonoxidative glycolysis ($28.7 \pm 2.2$ vs. $21.5 \pm 2.2$ g in nondiabetic volunteers, $P = 0.04$) and lipid oxidation ($12.4 \pm 0.8$ vs. $9.6 \pm 0.8$ g in nondiabetic volunteers, $P < 0.03$) were both increased in the diabetic subjects.

Postprandial glucose clearance (total body tissue uptake divided by mean arterial glucose concentration) was markedly reduced in the diabetic subjects ($1.2 \pm 0.2$ vs. $3.0 \pm 0.2$ ml·kg$^{-1}$·min$^{-1}$, $P < 0.001$). Values for clearance were significantly correlated with those of HOMA-IR ($r = -0.80$, $P < 0.001$).

**DISCUSSION**

The present study provides new information on several aspects of the pathophysiology of postprandial hyperglycemia.

**Table 1. Total, meal, and endogenous postprandial glucose release in nondiabetic and diabetic subjects**

<table>
<thead>
<tr>
<th>Process</th>
<th>Nondiabetic</th>
<th>Diabetic</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>78.9±4.2</td>
<td>97.8±3.2</td>
<td>&lt;0.001</td>
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<tr>
<td>Meal</td>
<td>58.9±2.8</td>
<td>60.9±1.5</td>
<td>0.53</td>
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<tr>
<td>Endogenous</td>
<td>19.9±2.1</td>
<td>37.0±3.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gluconeogenesis</td>
<td>15.3±1.2</td>
<td>26.9±2.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glycogenolysis</td>
<td>4.3±1.7</td>
<td>10.1±1.2</td>
<td>0.007</td>
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</table>

Values are means ± SE in g over 6 h.

in T2DM that have not been previously assessed, namely 1) the time-dependent relative contributions of excessive entry of ingested and endogenously produced glucose; 2) the involvement of increased glycogenolysis and gluconeogenesis; and 3) alterations in specific pathways of glucose disposal, especially with regard to glycolysis and splanchnic glycogen metabolism. It should be pointed out that many of the processes/pathways estimated in these studies are interdependent and subject to compounding of measurement errors (e.g., subtracting considerable urinary glucose losses from total glucose disposal to calculate tissue glucose uptake). Therefore, the accuracy of absolute values for various processes/pathways should be evaluated on the basis of their standard errors, and the differences found between diabetic and nondiabetic subjects should be considered qualitative rather than quantitative (i.e., the difference in direction, up or down, is more reliable than the absolute difference).

**Total postprandial glucose release into the systemic circulation.** Consistent with all previous studies (2, 22, 23, 27, 37, 45, 49), we found that total (endogenous plus ingested) postprandial release of glucose into the systemic circulation was increased in diabetic subjects ($98 \pm 3$ vs. $79 \pm 4$ g in nondiabetic volunteers, $P < 0.001$). In contrast, total postprandial uptake of glucose by tissues in the diabetic subjects was normal ($106 \pm 5$ vs. $101 \pm 5$ g in nondiabetic volunteers, $P = 0.61$), as has been found in several previous studies (2, 27, 37, 45, 49). It thus appears that hyperglycemia, by its mass action effects, was able to compensate for insulin resistance and impaired insulin secretion insofar as tissue glucose uptake was concerned but not with respect to glucose release.

**Postprandial release of ingested glucose into the systemic circulation.** Postprandial release of glucose into plasma represents the sum of the ingested glucose reaching the systemic circulation and the continued release of endogenously produced glucose. The amount of ingested glucose reaching the systemic circulation depends on the amount of glucose escap-
During the 90-min after-meal ingestion, release of ingested glucose was ~12 g greater in diabetic subjects (P = 0.001). During the same interval, release of endogenously produced glucose was ~5 g greater in diabetic subjects (P = 0.004). The excessive release of ingested glucose during the initial 90 min after meal ingestion accounted for ~90% of the increased glucose entry into the circulation in diabetic subjects over the entire 6-h postprandial period. These results contrast with the general impression that increased postprandial glucose release into the systemic circulation is mainly due to increased EGR (31).

The relatively greater importance of ingested, compared with endogenous, glucose entering the circulation during this early postprandial period has not generally been appreciated. However, Butler and Rizza (9) found increased hepatic glucose cycling in T2DM during the initial 120 min after meal ingestion. Our observation that glycogenolysis was twofold greater in diabetic subjects during this period supports this view.

In examining potential factors that are responsible for the diabetic subjects’ excessive release of ingested glucose into the circulation (plasma insulin, glucagon, and FFA concentrations, as well as underlying insulin resistance as assessed by HOMA), only changes in plasma glucagon (r = 0.51, P < 0.001) and insulin (r = -0.38, P = 0.02) were significantly correlated with the release of ingested glucose. It is of note that Butler and Rizza found a correlation between hepatic cycling and plasma glucagon. A role for glucagon in the increased release of ingested glucose vis-a-vis glycogen cycling, as suggested by our correlation data and the diabetic subjects’ increased postprandial plasma glucagon concentrations, is consistent with the fact that infusion of glucagon increases hepatic cycling (50) and suppression of glucagon secretion reduces glycogen turnover (58).

**Postprandial EGR: role of gluconeogenesis and glycogenolysis.** Postprandial EGR has been uniformly found to be increased in T2DM (2, 17, 37, 45, 47, 49, 61). This was also the case in the present study, where postprandial EGR was almost twofold greater in diabetic subjects (37 ± 3 vs. 20 ±
2 g in nondiabetic volunteers, \( P < 0.001 \)). The amount of endogenously produced glucose entering the circulation depends on changes in glycogen breakdown, glycogen formation, and production of glucose 6-phosphate via gluconeogenesis, which gets released as free glucose into circulation.

Gluconeogenesis in the diabetic subjects started out higher and did not decrease after meal ingestion. As a consequence, over the entire 6-h postprandial period, gluconeogenesis was almost twice as great in diabetic subjects (27 ± 2 vs. 15 ± 1 g in nondiabetic volunteers, \( P < 0.001 \)). Previous studies have also found evidence for increased postprandial gluconeogenesis in T2DM (29, 45, 47).

Postprandial glycogenolysis in T2DM has not been previously examined. In the present study, glycogenolysis started out higher in the diabetic subjects, and although it decreased substantially and by a comparable absolute amount in diabetic and nondiabetic subjects, it still remained greater postprandially in diabetic subjects. Thus, during the 6-h postprandial period, glycogenolysis was more than twofold greater in diabetic subjects (10 ± 1 vs. 4 ± 2 g in nondiabetic volunteers, \( P = 0.007 \)). Although both increased gluconeogenesis and glycogenolysis contributed to increased postprandial EGR in diabetic subjects, quantitatively, gluconeogenesis appeared to be more important, because it was ~12 g greater in diabetic subjects whereas glycogenolysis was only ~6 g greater.

The observations above illustrate the influence of initial preprandial gluconeogenesis and glycogenolysis on the subjects’ subsequent postprandial rates and thus on postprandial hyperglycemia. It is of note that changes in plasma glucagon and insulin during the initial 90 min after meal ingestion (when 90% of the differences in glucose release occurred between diabetic and nondiabetic subjects) were correlated not only with EGR during this period but also with EGR occurring during the last 4.5 h of the study. By contrast, no correlation was found between EGR and contemporaneous plasma insulin and glucagon levels during the last 4.5 h. These observations provide additional support for the concept that early changes in insulin and glucagon secretion are important determinants of overall postprandial glucose tolerance (49, 56).

It is of note that, in both the diabetic and nondiabetic subjects, glucose entering plasma via gluconeogenesis increased ~4 h after meal ingestion. In both the nondiabetic subjects and the diabetic subjects, rates increased to values significantly above those observed preprandially (both \( P < 0.001 \)). In both groups of subjects, EGR was increasing from maximally suppressed rates during this period. Thus this phenomenon could be explained by 1) derepression of the flux through the gluconeogenic pathway; 2) reduced suppression of glucose-6-phosphatase (i.e., more gluconeogenic carbon entering plasma than glycogen); and 3) desuppression of glycogenolysis, resulting in release of \(^{14}C\)glycogen that had been formed via the indirect pathway. We cannot, on the basis of our data, quantify the relative contributions of these possible mechanisms. However, on the basis of the time course of net hepatic glycogen accumulation reported by Taylor et al. (65) and the observations of Firth et al. (28) that meal-derived 3-carbon intermediates appear in plasma glucose at greater rates 4–6 h after meal ingestion than earlier, we favor a combination of the latter two processes.

Pathways for postprandial glucose disposal. Use of the tritiated water technique in conjunction with indirect calorimetric measurements in the present study permitted estimation of routes of postprandial glucose disposal not previously examined in T2DM. Because essentially the only immediate fates of glucose taken up by tissues in humans are glycolysis and storage (18, 67), use of the tritiated water technique allowed us to partition glucose taken up by tissues into those undergoing initial glycolysis or direct storage, and use of indirect calorimetry allowed us to partition glycolysis in oxidative and non-oxidative pathways.

Total postprandial tissue glucose uptake (peripheral plus splanchnic) was comparable in diabetic and nondiabetic subjects, as has been previously reported (2, 27, 37, 45, 47, 49). We presume that this was because of the ability of hyperglycemia to compensate for decreased insulin release and insulin sensitivity, as has been demonstrated in glucose clamp experiments (35).

Despite total tissue glucose uptake being normal, several routes of glucose disposal were abnormal in diabetic subjects. As has been previously found (5, 21, 37, 61), postprandial glucose oxidation was significantly reduced in diabetic subjects. This intracellular defect, which persisted despite the apparent ability of hyperglycemia to overcome defects in glucose transport, is consistent with and provides additional support for the concept that impaired mitochondrial activity might represent a fundamental defect in T2DM (52, 53).

Because overall glycolysis was comparable in both groups whereas oxidative glycolysis was reduced in diabetic subjects, nonoxidative glycolysis was found to be increased in diabetic subjects. Use of glucose-derived carbons undergoing nonoxidative glycolysis is essentially limited to gluconeogenesis, and these carbons are either incorporated into glycogen via the indirect pathway or released into plasma as free glucose (18, 67). Consequently, subtracting the amount of plasma glucose formed via gluconeogenesis from nonoxidative glycolysis provides an estimate of the remaining gluconeogenic carbon available for indirect pathway glycogen storage. We found that diabetic subjects had significantly reduced indirect pathway storage. However, because their direct storage, presumably due to the mass action effects of hyperglycemia, was greater than that of nondiabetic subjects, neither total postprandial glucose storage nor their total splanchnic glucose storage was significantly different from that of nondiabetic subjects.

At face value, these observations appear to be at variance with previous reports indicating that in T2DM there is reduced postprandial splanchnic and skeletal muscle glucose uptake (9, 24, 38, 40, 45, 47, 49), reduced postabsorptive hepatic and skeletal muscle glycogen stores (10, 42), and reduced postprandial glycogen accumulation in skeletal muscle (10). However, possible explanations for the apparent inconsistencies can be offered.

It is important to note that direct glucose storage was calculated as the difference between tissue glucose uptake and glycolysis because other pathways for glucose disposal are normally trivial. It is possible that, under conditions in which there is insulin deficiency, insulin resistance, and hyperglycemia, these normally trivial pathways (e.g., pentose phosphate shunt, sorbitol pathway) might be increased. If this were the case, our calculation of direct tissue glucose storage would be overestimated.

Finally, although we found that direct glucose storage in peripheral tissues was not reduced in diabetic subjects, this
does not necessarily indicate that postprandial glycogen accumulation in muscle was normal. Recently, Meyer et al. (47) reported that postprandial renal glucose uptake is increased in subjects with T2DM, whereas their skeletal muscle glucose uptake is reduced. In addition to the kidney (3), diabetic neural tissue (55) and skin (54) have been shown to accumulate excessive glycogen. Thus, to some extent, glucose storage in tissues other than skeletal muscle, as well as glycosylation of various proteins, might be increased in T2DM.

**Glycogen cycling.** Regarding the liver, previous studies reporting splanchnic glucose uptake considered only first-pass extraction (9, 24, 34, 49). In the present study we included an estimate of splanchnic uptake of glucose from the peripheral circulation. This was found to be increased in diabetic subjects, presumably because of the mass action effect of hyperglycemia, and compensated for their reduced first-pass splanchnic extraction so that their total splanchnic uptake was not significantly different from that of nondiabetic subjects. It is important to note that our method of calculating splanchnic glucose storage does not represent net storage but rather initial sequestration. Thus, if glycogen breakdown had been increased, the diabetic subjects could have ended up with less net storage.

As illustrated in Table 3, this appeared to be the case. Although total splanchnic glucose storage was comparable in diabetic and nondiabetic subjects, glycogen breakdown was increased more than twofold in diabetic subjects ($P = 0.008$). Consequently, net storage (storage minus breakdown) was reduced by $\sim 50\%$ in diabetic subjects ($P = 0.008$). Of interest, Magnusson et al. (42) found that, 4 h after meal ingestion, subjects with T2DM had a 55% reduction in hepatic glycogen content compared with normal volunteers, results quite similar to those in the present 6-h postprandial study. Our finding that T2DM subjects have increased postprandial glycogen breakdown is consistent with increased hepatic phosphorylase activity, which has been reported in diabetic animals (6) and could be explained, at least in part, by the reduced postprandial plasma insulin-to-glucagon molar ratios found in our diabetic subjects.

**Relationship between glucose oxidation and gluconeogenesis.** Despite overall tissue glucose uptake being comparable in diabetic and nondiabetic subjects, postprandial glucose oxidation was reduced in the diabetic subjects, consistent with previous reports (21, 37, 61). This reduced postprandial glucose oxidation, in the face of presumably hyperglycemia-driven normal glycogenesis (18), resulted in increased nonoxidative glycolysis, which would have provided increased availability of substrates for gluconeogenesis. That this was a factor contributing to EGR is suggested by a significant correlation found between nonoxidative glycolysis and glucose entering plasma via gluconeogenesis in our T2DM subjects ($r = 0.45$, $P = 0.03$). However, it is of note that whereas $\sim 60\%$ of nonoxidative glycolysis was used for plasma glucose from gluconeogenesis in the nondiabetic subjects, $\sim 90\%$ was used in the diabetic subjects ($P = 0.036$). This suggests that factors other than mere substrate availability were involved.

The diabetic subjects in the present study on average were not under good glycemic control, as evidenced by their mean Hb A1c of 8.6% (range 6.6–11.8%), and they had been withdrawn from their medications for 4 days before the experiments. There is no doubt that these factors contributed to the differences between the diabetic and nondiabetic subjects. Indeed, as is shown in Table 4, significant correlations were found among the diabetic subjects between their Hb A1c levels and their postprandial EGR, nonoxidative glycolysis, glyco-genolysis, glycogen cycling, glucose oxidation, glucose clearance, and their preprandial glucose release, HOMA insulin resistance, and HOMA insulin secretion. Curiously, there was no significant correlation between Hb A1c and either postprandial gluconeogenesis or total postprandial glucose release.

It is of note that the plasma FFA levels before and during most of the postprandial period were virtually identical in our diabetic and nondiabetic subjects. One would have expected increased plasma FFA levels in the diabetic subjects, because most studies have shown fasting plasma FFA levels to be increased in T2DM (4) and not to decrease to quite the same extent postprandially as in nondiabetic subjects (5). However, postprandial FFA levels were positively correlated with Hb A1c values in the diabetic subjects (Table 4). Although our data do not allow us to provide an explanation for this apparent discrepancy, several factors may have been involved. First, our diabetic subjects had increased fasting levels of insulin, which should have suppressed their FFA levels. Thus their “normal” levels were inappropriate. The apparently “normal” FFA levels observed postprandially might have resulted partly from their hyperglycemia suppressing lipolysis and partly from the increased postprandial lipid oxidation, which we and others (5) have found. Clearly, further studies are needed regarding postprandial FFA turnovers, oxidation, incorporation in lipids, and use by specific tissues (e.g., splanchnic vs. muscle) in T2DM.

Table 3. **Splanchnic glycogen formation, glycogenolysis, and cycling in diabetic and nondiabetic subjects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subjects</th>
<th>Nondiabetic</th>
<th>Diabetic</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total splanchnic storage*</td>
<td></td>
<td>15.3±1.5</td>
<td>16.1±1.2</td>
<td>0.73</td>
</tr>
<tr>
<td>Glycogenolysis</td>
<td></td>
<td>4.3±1.7</td>
<td>10.1±1.2</td>
<td>0.007</td>
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<tr>
<td>Net storage</td>
<td></td>
<td>11.0±1.9</td>
<td>6.0±0.8</td>
<td>0.008</td>
</tr>
<tr>
<td>Glycogen cycling†</td>
<td></td>
<td>28±3</td>
<td>63±8</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Sum of direct and indirect storage; †glycogenolysis × 100 ÷ total storage.

Table 4. **Correlations between Hb A1c and various metabolic parameters in diabetic subjects**

<table>
<thead>
<tr>
<th>Correlation</th>
<th>$r$ Value</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postprandial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endogenous glucose release</td>
<td>0.51</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>Total glucose release</td>
<td>0.17</td>
<td>NS</td>
</tr>
<tr>
<td>Glucoseogenesis</td>
<td>0.09</td>
<td>NS</td>
</tr>
<tr>
<td>Nonoxidative glycolysis</td>
<td>0.39</td>
<td>$&lt;0.05$</td>
</tr>
<tr>
<td>Metabolic clearance</td>
<td>0.38</td>
<td>$&lt;0.05$</td>
</tr>
<tr>
<td>Glycogenolysis</td>
<td>0.66</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Glycogen cycling</td>
<td>0.41</td>
<td>$&lt;0.04$</td>
</tr>
<tr>
<td>Glucose oxidation</td>
<td>$-0.53$</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>Plasma FFA</td>
<td>0.55</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>Preprandial glucose release</td>
<td>0.36</td>
<td>$&lt;0.04$</td>
</tr>
<tr>
<td>HOMA-insulin resistance</td>
<td>0.47</td>
<td>$&lt;0.02$</td>
</tr>
<tr>
<td>HOMA-insulin secretion</td>
<td>$-0.47$</td>
<td>$&lt;0.02$</td>
</tr>
</tbody>
</table>

Values are means ± SE. FFA, free fatty acids; HOMA, homeostasis model assessment.
The present studies indicate that, in T2DM, 1) release of ingested and endogenously produced glucose into the systemic circulation are both increased postprandially; 2) the postprandial increased EGR involves both increased gluconeogenesis and glycogenolysis; 3) increased hepatic glycogen cycling probably plays a role in diminishing direct and indirect hepatic glucose storage; 4) preprandial rates of gluconeogenesis and glycogenolysis, as well as early changes in insulin and glucagon secretion, substantially influence postprandial rates of glycogenolysis and gluconeogenesis; 5) postprandial glucose oxidation is reduced, resulting in increased nonoxidative glycolysis, which supplied additional substrates to support increased gluconeogenesis, thus providing a link between abnormal peripheral glucose metabolism and abnormal EGR; and 6) although whole body glucose storage is normal in a quantitative sense, hyperglycemia, in the face of insulin resistance and abnormal insulin and glucagon secretion, may alter glucose fluxes through specific pathways and cause glucose carbon to be shunted away from normal sites of storage (e.g., liver and muscle) into other tissues.

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