Type 2 Diabetes Impairs Splanchnic Uptake of Glucose but Does Not Alter Intestinal Glucose Absorption During Enteral Glucose Feeding

Additional Evidence for a Defect in Hepatic Glucokinase Activity

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We have previously reported that splanchnic glucose uptake, hepatic glycogen synthesis, and hepatic glucokinase activity are decreased in people with type 2 diabetes during intravenous glucose infusion. To determine whether these defects are also present during more physiological enteral glucose administration, we studied 11 diabetic and 14 nondiabetic volunteers using a combined organ catheterization-tracer infusion technique. Glucose was infused into the duodenum at a rate of 22 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ while supplemental glucose was given intravenously to clamp glucose at ~10 mmol/l in both groups. Endogenous hormone secretion was inhibited with somatostatin, and insulin was infused to maintain plasma concentrations at ~300 pmol/l (i.e., twofold higher than our previous experiments). Total body glucose disappearance, splanchnic, and leg glucose extractions were markedly lower ($P < 0.01$) in the diabetic subjects than in the nondiabetic subjects. UDP-glucose flux, a measure of glycogen synthesis, was ~35% lower ($P < 0.02$) in the diabetic subjects than in the nondiabetic subjects. This was entirely accounted for by a decrease ($P < 0.01$) in the contribution of extracellular glucose because the contribution of the indirect pathway to hepatic glycogen synthesis was similar between groups. Neither endogenous and splanchnic glucose productions nor rates of appearance of the intraduodenally infused glucose in the portal vein differed between groups. In summary, both muscle and splanchnic glucose uptake are impaired in type 2 diabetes during enteral glucose administration. The defect in splanchnic glucose uptake appears to be due to decreased uptake of extracellular glucose, implying decreased glucokinase activity. Thus, abnormal hepatic and muscle (but not gut) glucose metabolism are likely to contribute to postprandial hyperglycemia in people with type 2 diabetes. *Diabetes* 50:1351–1362, 2001

Type 2 diabetes is characterized by both fasting and postprandial hyperglycemia (1–4). In both situations, the degree of hyperglycemia is determined by the difference between the amount of glucose entering and leaving the circulation. After an overnight fast, the liver and kidney are the primary sites of glucose production (5,6). Glucose released from the liver is derived from both glycogenolysis and gluconeogenesis, whereas that released by the kidney is derived exclusively from gluconeogenesis (6,7). The situation becomes more complex after carbohydrate ingestion when glucose enters from the gut (1,3,8,9). However, not all ingested glucose reaches the systemic circulation because a portion of it is metabolized by the gut and/or stored in the liver as glycogen (10–12). In contrast to muscle, where glucose transport appears to be rate-determining, glucose phosphorylation by glucokinase is rate-determining in the liver (13–17). Glucose-6-phosphate, derived from either the direct or indirect (gluconeogenic) pathway, can be converted to glycogen (18). Because hepatic glycogenolysis does not fully suppress after food ingestion, (19,20), the amount of glucose that actually is released into the systemic circulation from splanchnic tissues is determined by an interplay between the rate of glucose absorption, the rate of splanchnic (gut and hepatic) glucose extraction, and the rate of hepatic glucose production.

Numerous studies have shown that insulin-induced stimulation of muscle glucose uptake is impaired in people with type 2 diabetes (9,21–23). We have recently reported that the ability of insulin and glucose to stimulate splanchnic glucose uptake is also impaired (24). We presented evidence that the lower splanchnic glucose uptake was accompanied by a decrease in glycogen synthesis from extracellularly but not intracellularly derived glucose, implying a defect in hepatic glucokinase activity. In those experiments, glucose was clamped at ~9.3 mmol/l and insulin at ~150 pmol/l in an effort to reproduce levels commonly observed between meals and during the night. Under these conditions, the liver accounted for approximately one-third of the defect of total body glucose uptake and muscle accounted for two-thirds. However, in those

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SER, splanchnic glucose extraction ratio.

experiments, all glucose was infused intravenously. Therefore, although these data suggest that impaired hepatic glucose uptake contributes to hyperglycemia in the postabsorptive state (i.e., when no glucose is coming from the gut), their applicability to the fed state remains uncertain for several reasons.

First, studies in both animals and humans have shown that ~15–30% of enterally administered glucose is either not absorbed or metabolized by the gut (10–12). Experimental diabetes in animals has been reported to enhance glucose absorption (25) and increase intestinal glucose metabolism. It is currently not known whether the same phenomenon occurs in diabetic humans. Second, enterally administered glucose has been reported to result in greater hepatic glucose uptake than intravenously infused glucose (8,26,27). This may in part be due to stimulation of glycogen synthesis by intestinal incretins (28) and in part due to the generation of a "portal" signal to the liver (29,30). The latter has been shown to substantially enhance hepatic glucose uptake in dogs (29,30). If this also occurs in diabetic humans, then it may reverse the defect in hepatic glucokinase activity observed during intravenous glucose infusion, thereby normalizing postprandial splanchnic glucose uptake. On the other hand, if incretins and the portal signal increase hepatic glucose uptake in nondiabetic but not diabetic individuals, then the defect in splanchnic glucose uptake may be even more marked when glucose is given orally. Finally, our previous experiments were performed in the presence of relatively low insulin concentrations (~150 pmol/l) because we were trying to mimic postabsorptive conditions. Portal insulin concentrations are likely to be higher in many diabetic patients, particularly those taking insulin secretagogues (31). The higher insulin concentrations may overcome the defect in hepatic glucose uptake, thereby limiting its contribution to postprandial hyperglycemia.

The present experiments therefore were undertaken to determine whether splanchnic glucose uptake is impaired in people with type 2 diabetes during enteral glucose administration. To do so, total body, splanchnic, and leg glucose uptakes were measured in diabetic and nondiabetic subjects during intraduodenal infusion of glucose. To avoid the confounding effects introduced by differences in glucose concentrations, glucose was clamped at ~10 mmol/l by means of a supplemental intravenous glucose infusion while endogenous insulin secretion was inhibited with somatostatin. Exogenous insulin was infused at a rate that would result in insulin concentrations (~300 pmol/l) anticipated to be present in the portal circulation of many diabetic subjects after a meal (3,4,31). The hepatic vein catheterization and dual tracer techniques were combined so that both the rate of appearance of the enterally administered glucose into the portal vein and splanchnic glucose uptake could be measured. Flux through the intrahepatic UDP-glucose pool (the precursor pool of glycogen) was assessed with the acetaminophen glucuronide method (16,32–35).

RESEARCH DESIGN AND METHODS

Subjects. After approval from the Mayo Institutional Review Board, 11 subjects with type 2 diabetes and 14 nondiabetic subjects gave informed written consent to participate in the study. All subjects were Caucasians. Volunteer characteristics are given in Table 1. All subjects were in good health and at a stable weight. None regularly engaged in vigorous physical exercise. None of the first-degree relatives of nondiabetic subjects had a history of diabetes. At the time of screening, three of the diabetic subjects were being treated with diet alone, five with either a sulfonylurea or metformin, and the other three with insulin. Both oral antihyperglycemic drugs were discontinued at least 3 weeks before study. The three subjects who were being treated with insulin were switched to regular insulin 3 days before study. Subjects were on no medications at the time of study other than either thyroxine or estrogen replacement therapy. All subjects were instructed to follow a weight maintenance diet containing 55% carbohydrate, 30% fat, and 15% protein for at least 3 days before the day of study. As anticipated, both fasting glucose and glycosylated hemoglobin concentrations (Bio-Rad, Hercules, CA; normal range 4–7%) were higher in the diabetic subjects than in the nondiabetic subjects.

TABLE 1
Volunteer characteristics

	Nondiabetic (8 M:6 F)	Diabetic (4 M:7 F)
n	14	11
Duration of diabetes (years)		8 ± 1
Age (years)	49 ± 2	58 ± 2
Fat-free mass (kg)	58 ± 4	60 ± 4
BMI (kg/m^2)	29 ± 1	32 ± 2
Body fat (%)	34 ± 2	33 ± 2
HbA_{1c} (%)	5.0 ± 0.2	7.6 ± 0.4
Fasting plasma glucose (mmol/l)	5.3 ± 0.1	9.0 ± 0.5

Data are means ± SE.

Experimental design. Subjects were admitted to the Mayo Clinic General Clinical Research Center at 1700 on the evening before the study. A standard 10 cal/kg meal (55% carbohydrate, 30% fat, and 15% protein) was eaten between 1730 and 1800. After the meal, an 18-gauge catheter was inserted into a forearm vein, and an infusion of insulin was started in the diabetic subjects (100 U regular human insulin in 1 liter of 0.9% saline containing 5 ml of 25% human albumin) and saline in the nondiabetic subjects. The insulin infusion rate was adjusted to maintain glucose concentrations in the diabetic subjects at ~5 mmol/l during the night (36). At ~2100, an 8-French Flexiflo enteral feeding tube (Ross Laboratories, Columbus, OH) was inserted through the nasal route and its position checked radiographically. The tube was flushed every 4 h with 30 ml water throughout the night.

The study design is outlined in Fig. 1. At 0600 on the morning after admission, a urinary catheter was inserted into the bladder in 22 of the 25 subjects. Three subjects elected not to have a bladder catheter placed, and they were able to void on request at the appropriate times. Thereafter, at 0700, the position of the feeding tube was confirmed to be in the distal duodenum by flouroscopy. Subjects were moved to the interventional radiology suite at ~0800. Femoral artery, femoral venous, and hepatic venous catheters were placed as previously described (24,37). In brief, a 5-French Terumo arterial sheath (Cook Company, Bloomington, IN) was inserted under local anesthesia in a retrograde fashion into the right femoral artery just below the inguinal ligament. A 20-cm 4-French catheter with six distally placed holes (special order Cook; Cook Company) was inserted through the sheath and advanced under fluoroscopic guidance to the tip of the common iliac artery. This catheter was used for arterial blood sampling and the sheath for infusion of indocyanine green (Akorn, Buffalo Grove, IL) at 0.25 mg/min. A second sheath (6-French) was placed in the right external iliac vein via the femoral vein through which a 5-French catheter was advanced into the right hepatic vein. This catheter was used for hepatic venous sampling and the sheath for sampling of blood draining from the right leg.

In eight diabetic and nine nondiabetic subjects, a primed continuous infusion of [3-^{3}H]glucose (12 μCi prime, 0.12 μCi/min continuous; New England Nuclear, Boston, MA) was started at 0900. At 0930 (time 0 min), infusions of somatostatin (72 ng · kg^{-1} · min^{-1}), growth hormone (3 ng · kg^{-1} · min^{-1}), and glucagon (0.65 ng · kg^{-1} · min^{-1}) were started and continued until the end of the study. Insulin was infused at a rate of 1.0 mU · kg^{-1} · min^{-1} from 0–240 min. A dextrose infusion also was begun at 0930, and the infusion rate was adjusted to maintain plasma glucose concentrations at ~10 mmol/l over the next 4 h. Glucose, tracers, and hormones all were infused into a forearm vein.

All infused glucose contained [3-^{3}H]glucose to minimize the change in plasma glucose specific activity (24,38,39). In addition, the rate of the basal [3-^{3}H]glucose infusion was reduced to mimic the anticipated changes in endogenous glucose production (40). In the nondiabetic subjects, basal [3-^{3}H]glucose was infused at 100% from −30 to −0 min, 50% from 1–30 min,

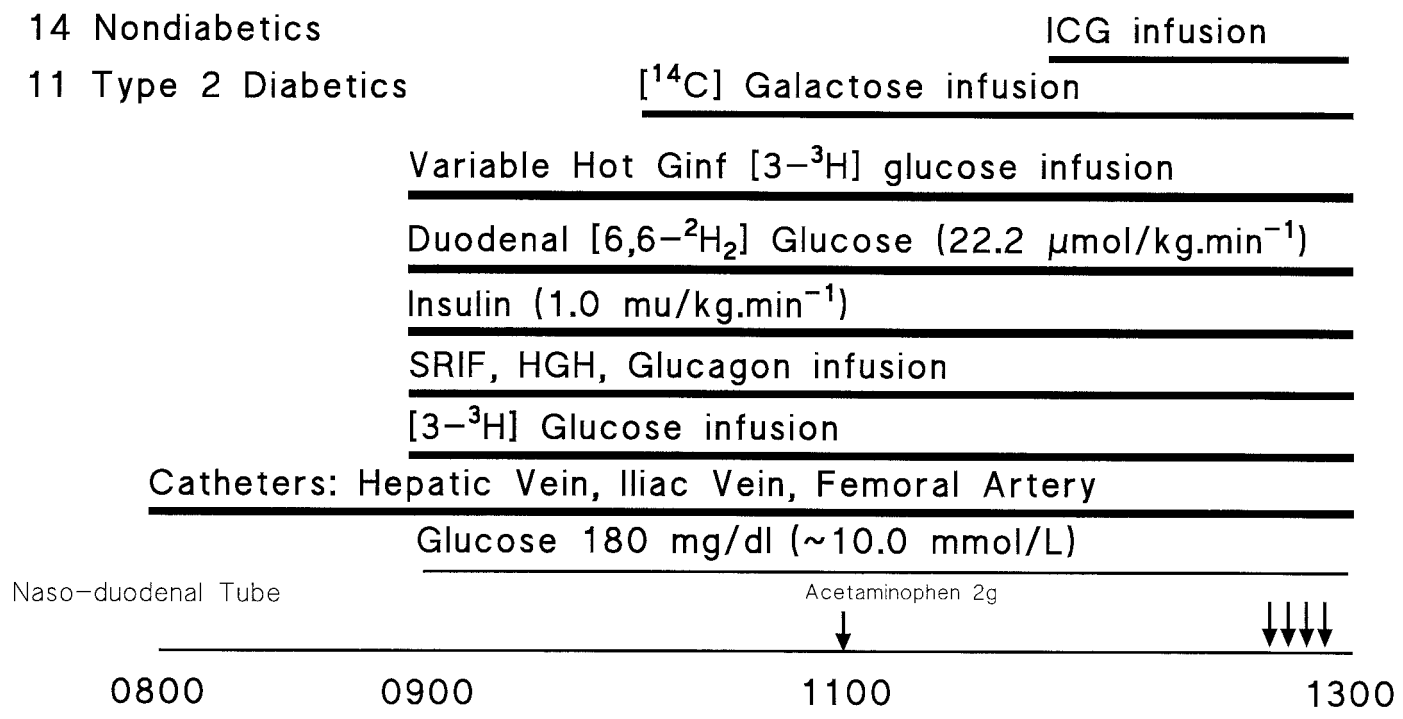

FIG. 1. Experimental design. For additional details regarding this experimental design, please see the text. Ginf, glucose infusion; HGH, human growth hormone; ICG, indocyanine green; SRIF, somatostatin.

43% from 31–60 min, 31% from 61–90 min, 23% from 91–120 min, 19% from 121–150 min, 15% from 151–180 min, 13% from 181–210 min, and 12% from 211–240 min. In the diabetic subjects, the corresponding infusion rates for the same time periods were 100, 50, 45, 39, 34, 30, 27, 25, and 24%. At 0 min, glucose labeled with [6,6-$^2H_2$]glucose at an enrichment of ~6% was infused through the naso-duodenal tube at a rate of 22.2 μmol · kg^{-1} · min^{-1} and continued until the end of the study. To be sure the conclusions were not dependent on the route of tracer infusion, [3-^{3}H]glucose was given through the naso-duodenal tube, and [6,6-$^2H_2$]glucose was infused intravenously in three diabetic subjects and five nondiabetic subjects. In these experiments, all glucose infused intravenously was labeled with [6,6-$^2H_2$] glucose at an enrichment of ~5%. The glucose administered via the enteral route was labeled with 100 μCi [3-^{3}H]glucose. The results were the same regardless of the route of tracer administration; therefore, the data were combined for purpose of analysis.

A primed continuous infusion of [^{14}C]galactose (15 μCi prime, 0.15 μCi/min constant; New England Nuclear) was started at 60 min and continued until the end of the study. Subjects were asked to void at 120 min and then ingest 2 g acetaminophen. Urine was collected from 120–240 min for measurement of urinary acetaminophen glucuronide as previously described (34). All urine passed from 0–240 min also was collected for analysis of urine glucose excretion. Indocyanine green was infused at 0.25 mg/min into the femoral artery sheath from 180–240 min. As part of a separate experiment, an infusion of [9,10-^{3}H]palmitate also was started at 180 min.

Analytical techniques. All samples were placed on ice and centrifuged at 4°C, after which the plasma was removed. Plasma indocyanine green concentration was measured spectrophotometrically at 805 nm on the day of study, as previously described (37). All other samples were stored at −20°C until analysis. Plasma glucose was measured by a glucose oxidase method using a YSI glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was measured using a chemiluminescence method with the Access Ultrasensitive Immunoenzymatic Assay System (Beckman, Chaska, MN). C-peptide and glucagon concentrations were assayed by radioimmunoassay (Linco Research, St. Louis, MO). Growth hormone was measured with the Access hGH two-site immunoenzymatic assay (Beckman). [3-^{3}H]glucose and [^{14}C]glucose specific activities were measured by liquid scintillation counting as previously described (41). Body composition (including fat-free mass and total fat mass) was measured using dual-energy X-ray absorptiometry (DPX-IQ scanner; Hologic, Waltham, MA; SmartScan Version 4.6) (42). Urinary [^{14}C] and [^{3}H]glucuronide specific activities were measured using high-performance liquid chromatography as previously described (34). Plasma [6,6-$^2H_2$]glucose enrichment was measured by gas chromatography mass spectrometry (43).

Calculations. Splanchnic plasma flow was calculated by dividing the indocyanine green infusion rate by the arterial hepatic venous concentration gradient of the dye (44). Likewise, leg plasma flow was calculated by dividing the dye infusion rate by the concentration gradient across the leg (37). The corresponding blood flows were derived by dividing the respective plasma flows by (1 − hematocrit). Blood glucose concentrations were calculated by multiplying the plasma glucose concentrations by 0.85.

When [3-^{3}H]glucose was infused intravenously and [6,6-$^2H_2$]glucose was infused via the naso-duodenal tube, rates of glucose appearance (R_a) and glucose disappearance (R_d) were calculated using the steady-state equations of Steele et al. (45):

$$R_a = R_d = \frac{F_{Glu}}{SA \text{ of } [3 = {}^3H] \text{ glucose}} \quad (1)$$

where F_{Glu} is the infusion rate of [3-^{3}H]glucose and SA of [3-^{3}H] glucose is the plasma specific activity of [3-^{3}H] glucose.

The splanchnic glucose extraction ratio (SER) was calculated as follows:

$$SER = \frac{[A_{Trit} - HV_{Trit}]}{A_{Trit}} \quad (2)$$

where A_{Trit} and HV_{Trit} are the concentrations of [3-^{3}H]glucose in the arterial and hepatic venous plasma, respectively.

Portal appearance (Portal R_a) of duodenally delivered glucose was calculated as follows:

$$\text{Portal } R_a = \frac{\frac{HV_{2H2Glu}}{(1 - SER)} - FA_{2H2Glu}}{Duo_{MPE}} \times Q_{HV} \quad (3)$$

where HV_{2H2Glu} is the concentration of [6,6-$^2H_2$]glucose in the hepatic vein, FA_{2H2Glu} is the femoral artery concentration of [6,6-$^2H_2$]glucose, Q_{HV} is the median of quadruple determinations of splanchnic blood flow, and Duo_{MPE} is the enrichment of [6,6-$^2H_2$]glucose of the intraduodenally infused glucose. This calculation makes the assumption (12) that once absorbed into the splanchnic venous system, the extraction of glucose originating in the duodenum is the same as that originating in the systemic circulation (i.e., equal to SER).

Gut extraction of the duodenally infused glucose was calculated as follows:

$$\text{Gut extraction} = \frac{(G_{Duo} - \text{Portal } R_a)}{G_{Duo}} \times 100 \quad (4)$$

Where G_{duo} is the duodenal glucose infusion rate in micromoles per minute and Portal R_a is derived from Eq. 3.

Splanchnic glucose uptake (SGU) was determined by the sum of the products of arterial glucose concentration, SER, splanchnic blood flow, and Portal R_a and SER:

$$SGU = (FA_{Glu} \times Q_{HV} \times SER) + (Portal\ R_a \times SER) \quad (5)$$

where FA_{Glu} is femoral arterial glucose concentration. Flux through the UDP-glucose pool was calculated as:

$$\text{UDP glucose flux} = \frac{F_{GAL}}{\text{SA of UDP-[}^{14}\text{C]glucose}} \quad (6)$$

where F_{GAL} is the intravenous infusion rate of [^{14}C]galactose and SA of UDP-[^{14}C]glucose is the specific activity of acetaminophen [^{14}C]glucuronide in urine (16,32–35).

The fractional contribution of plasma glucose (i.e., the direct pathway) to UDP-glucose flux was calculated as follows:

$$\text{Direct pathway} = \frac{\text{SA of UDP-[3-}^{3}\text{H]glucose}}{\text{SA of [3-}^{3}\text{H]glucose}} \quad (7)$$

where SA of UDP-[^{3}H]glucose equals the specific activity of acetaminophen [^{3}H]glucuronide in urine, and SA of [3-^{3}H]glucose equals the specific activity of [3-^{3}H]glucose entering the liver.

The SA of [3-^{3}H]glucose entering the liver was calculated by dividing the amount of [3-^{3}H]glucose reaching liver by the amount of unlabeled glucose reaching the liver:

$$\text{SA of [3-}^{3}\text{H]glucose} = \frac{FA_{Trit} \times Q_{HV}}{(FA_{Glu} \times Q_{HV}) + \text{Portal } R_a} \quad (8)$$

where FA_{Trit} is the concentration of [3-^{3}H]glucose in the femoral artery.

The contribution (in $\mu mol \cdot kg^{-1} \cdot min^{-1}$) of the direct pathway to total UDP-glucose flux was calculated by multiplying Eq. 7 by Eq. 6. The contribution of the indirect pathway was calculated as the total UDP flux minus the direct pathway.

Endogenous (total body) glucose production was determined by subtracting the glucose infusion rate and the duodenal glucose rate of appearance in the hepatic vein from the tracer determined rate of glucose appearance.

Splanchnic glucose production (SGP) was calculated as follows:

$$-SGP = [(FA_{Glu} - HV_{Glu}) \times Q_{HV} + \text{Portal } R_a] - SGU \quad (9)$$

While technically a negative number, since SGP represents a rate of release of endogenous glucose from the splanchnic bed, for the sake of clarity, it is presented as a positive number in the figures and text.

Leg glucose uptake (LGU) was calculated as the difference between the femoral arterial (A_{Glu}) and the femoral venous (FV_{Glu}) glucose concentration times the median of quadruple determinations of leg blood flow (Q_L):

$$LGU = [A_{Glu} - FV_{Glu}] \times Q_L \quad (10)$$

The leg glucose extraction ratio (LER) was calculated as follows:

$$LER = \frac{[A_{Trit} - FV_{Trit}]}{A_{Trit}} \quad (11)$$

where A_{Trit} and FV_{Trit} are the concentrations of [3-^{3}H]glucose in the femoral artery and femoral veins, respectively.

The calculations used for the five nondiabetic and three diabetic subjects in whom [6,6-$^2H_2$]glucose was infused intravenously and [3-^{3}H]glucose intraduodenally were the same with the exception that the concentration and enrichment of [6,6-$^2H_2$]glucose were switched with the concentration and specific activity of [3-^{3}H]glucose. In addition, the infusion rate of [6,6-$^2H_2$]glucose also was subtracted from glucose appearance to calculate endogenous glucose production.

Statistical analysis. Data in the text and figures are expressed as means ± SE. Rates are expressed as micromoles per kilogram fat-free mass per minute with the exception of leg glucose uptake, which is expressed as micromoles per kilogram fat-free mass of leg per minute. Responses during the last 30 min of the study were used for statistical analysis. Student's nonpaired one-tailed *t* test was used to test the hypothesis that insulin action is lower in the diabetic subjects than in the nondiabetic subjects. All other tests were two-tailed. $P < 0.05$ was considered statistically significant.

RESULTS

Glucose and insulin concentrations. Despite overnight infusion of insulin, baseline arterial glucose concentrations (Fig. 2*A*) were higher ($P < 0.01$) in the diabetic subjects than in the nondiabetic subjects (8.0 ± 0.6 vs. 5.4 ± 0.1 mmol/l). Arterial glucose concentrations increased in both groups within 60 min of initiation of glucose infusion to ~10 mmol/l. Arterial glucose concentrations were slightly higher ($P < 0.01$) in the diabetic subjects than in the nondiabetic subjects (10.1 ± 0.01 vs. 9.7 ± 0.05 mmol/l) during the final 30 min of the study. Hepatic vein glucose concentrations over the same interval also were higher ($P < 0.01$) in the diabetic subjects than in the nondiabetic subjects (10.8 ± 0.03 vs. 10.2 ± 0.07 mmol/l).

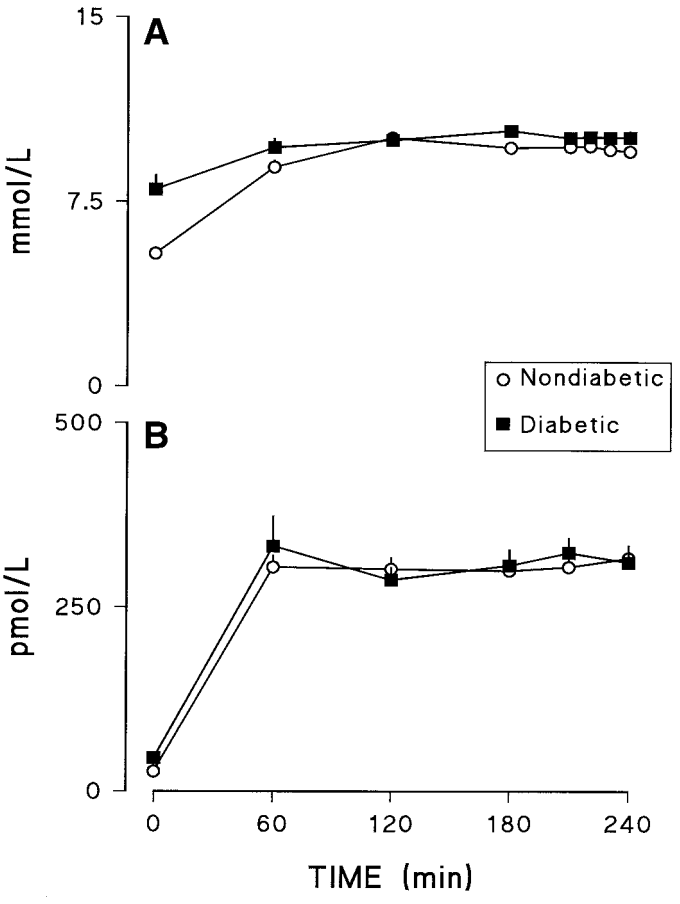

FIG. 2. Glucose (*A*) and insulin (*B*) concentrations observed in the diabetic and nondiabetic subjects during the study. Glucose (intraduodenal and intravenous) and insulin infusions were started at time 0.

Baseline plasma insulin concentrations (Fig. 2*B*) were slightly ($P = 0.06$) higher in the diabetic subjects than in the nondiabetic subjects (45 ± 9 vs. 27 ± 4 pmol/l). The somatostatin plus insulin infusion, begun at time 0, rapidly increased insulin concentrations to ~300 pmol/l in both groups. Insulin concentrations remained constant and equal thereafter. Insulin concentrations during the final 30 min of the study did not differ in the diabetic and nondiabetic subjects (317 ± 22 vs. 311 ± 18 pmol/l).

C-peptide, glucagon, and growth hormone concentrations. C-peptide, glucagon, and growth hormone concentrations did not differ in the diabetic and nondiabetic subjects either before or during the somatostatin infusion (Fig. 3). Somatostatin resulted in prompt and near complete suppression of C-peptide in both groups.

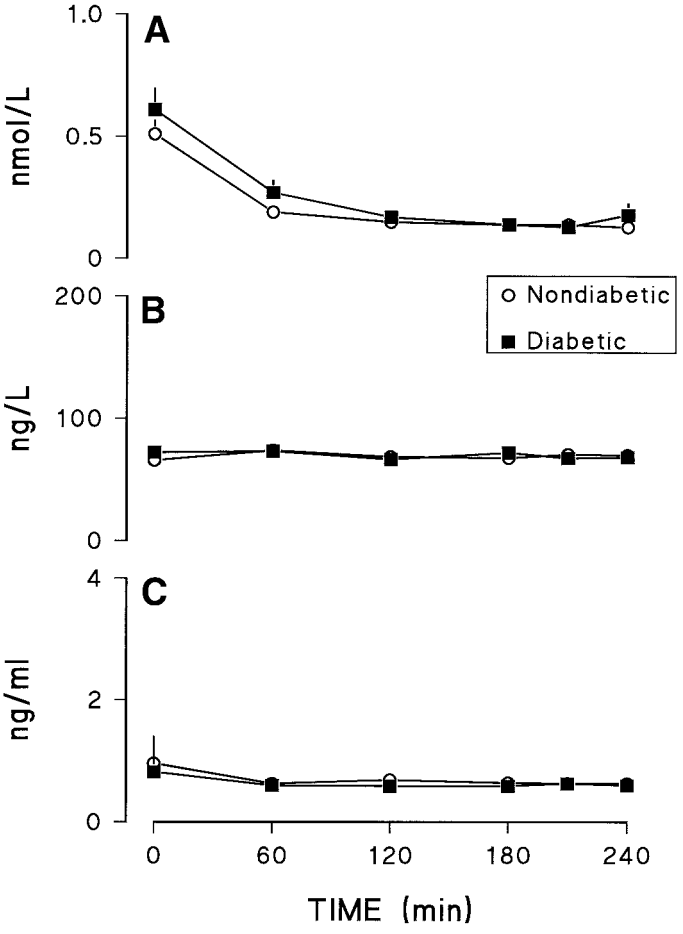

FIG. 3. Plasma C-peptide (*A*), glucagon (*B*), and growth hormone (*C*) concentrations observed in the diabetic and nondiabetic subjects during the study. A somatostatin, glucagon, and growth hormone infusion was started at time 0.

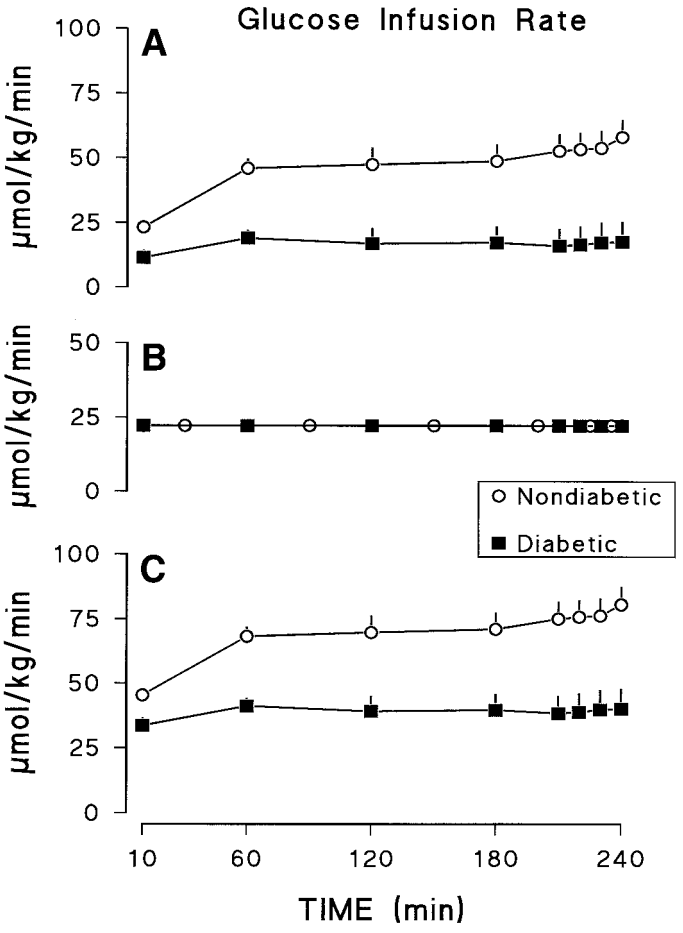

FIG. 4. *A*: The intravenous glucose infusion rate required to maintain glucose concentrations at target levels. *B*: The intraduodenal glucose infusion rate (same rate in all subjects). *C*: The total glucose infusion rate (the sum of the intravenous and duodenal glucose infusion rates).

Intravenous and intraduodenal glucose infusion rates. The intravenous glucose infusion rate necessary to maintain plasma glucose concentrations at target levels was lower ($P < 0.01$) in the diabetic subjects than in the nondiabetic subjects throughout the study (Fig. 4*A*). By design, the intraduodenal infusion rate of glucose was the same in both groups (Fig. 4*B*). Consequently, the total glucose infusion rate (Fig. 4*C*) during the final 30 min of the study also was lower ($P < 0.01$) in the diabetic subjects than in the nondiabetic subjects (40 ± 7 vs. 77 ± 7 µmol · kg^{-1} · min^{-1}).

Glucose specific activities and enrichment. Plasma [3-^{3}H]glucose specific activity plateaued within 60 min of the start of the intravenous tracer infusion and remained constant thereafter (Fig. 5*A*). Because the intraduodenal glucose infusion did not contain [3-^{3}H]glucose and because (due to the lower intravenous glucose infusion rates) the intraduodenal glucose infusion represented a greater proportion of the total glucose infusion in the diabetic subjects, [3-^{3}H]glucose specific activity was consistently lower in the diabetic subjects than in the nondiabetic subjects. The same pattern was observed in the subjects in whom [6,6-$^2H_2$]glucose was infused intravenously and [3-^{3}H]glucose was infused intraduodenally (data not shown).

The [6,6-$^2H_2$]glucose enrichment in plasma gradually increased, approaching a plateau by ~180 min (Fig. 5*B*). Because the intravenous glucose infusion did not contain [6,6-$^2H_2$]glucose and because this intravenous glucose represented a lesser proportion of the total glucose administered in the diabetic subjects, [6,6-$^2H_2$]glucose enrichment was consistently higher in diabetic subjects than in nondiabetic subjects.

After initiation of the [^{14}C]galactose infusion at 60 min, plasma [^{14}C]glucose specific activity increased promptly in both groups, reaching a plateau by 120 min (Fig. 5*C*).

Total body glucose disappearance and endogenous glucose production. Despite similar insulin and slightly higher glucose concentrations, total body glucose disappearance (Fig. 6*A*) was lower ($P < 0.01$) in the diabetic subjects than in the nondiabetic subjects (34.6 ± 9.3 vs. 77.2 ± 7.5 µmol · kg^{-1} · min^{-1}). Although urinary glucose losses were minimal in both groups, they tended to be higher ($P = 0.06$) in the diabetic subjects than in the nondiabetic subjects (2.1 ± 1.0 vs. 0.4 ± 0.1 µmol · kg^{-1} · min^{-1}). Endogenous glucose production (Fig. 6*B*) was equally suppressed in the diabetic and nondiabetic subjects (8.9 ± 1.6 vs. 7.2 ± 1.9 µmol · kg^{-1} · min^{-1}).

Portal appearance of duodenal glucose and duodenal glucose extraction. By design, the duodenal glucose infusion rate was the same (22.2 µmol · kg^{-1} · min^{-1}) in both groups. Of this, 19.4 ± 1.8 and 17.9 ± 1.5 µmol · kg^{-1}

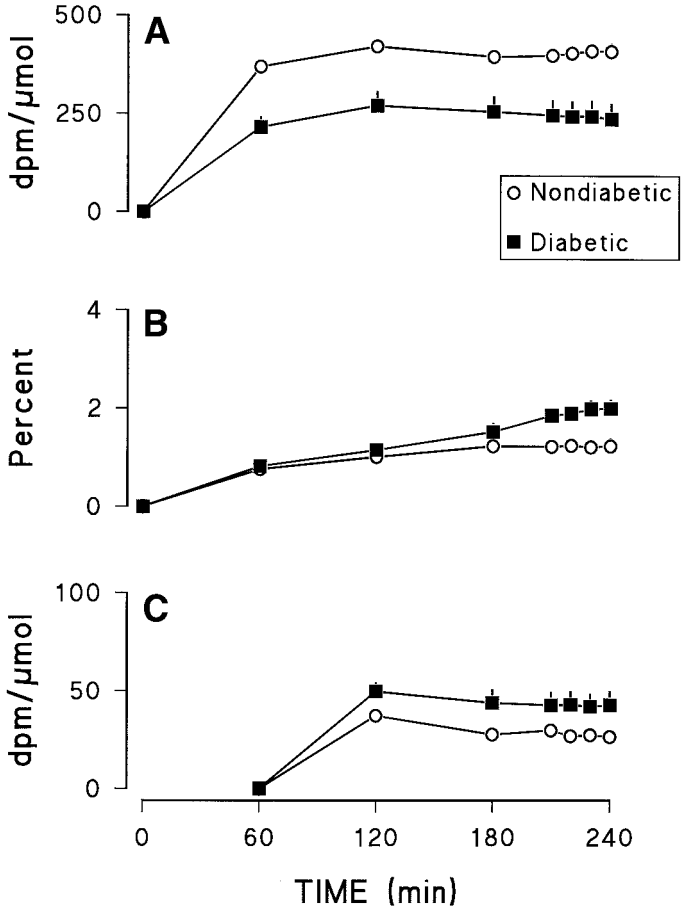

FIG. 5. Plasma [3-^{3}H]glucose specific activity (*A*), [6,6-$^2H_2$]glucose mole percent enrichment (*B*), and [^{14}C]glucose specific activity (*C*) observed in the diabetic and nondiabetic subjects during the study.

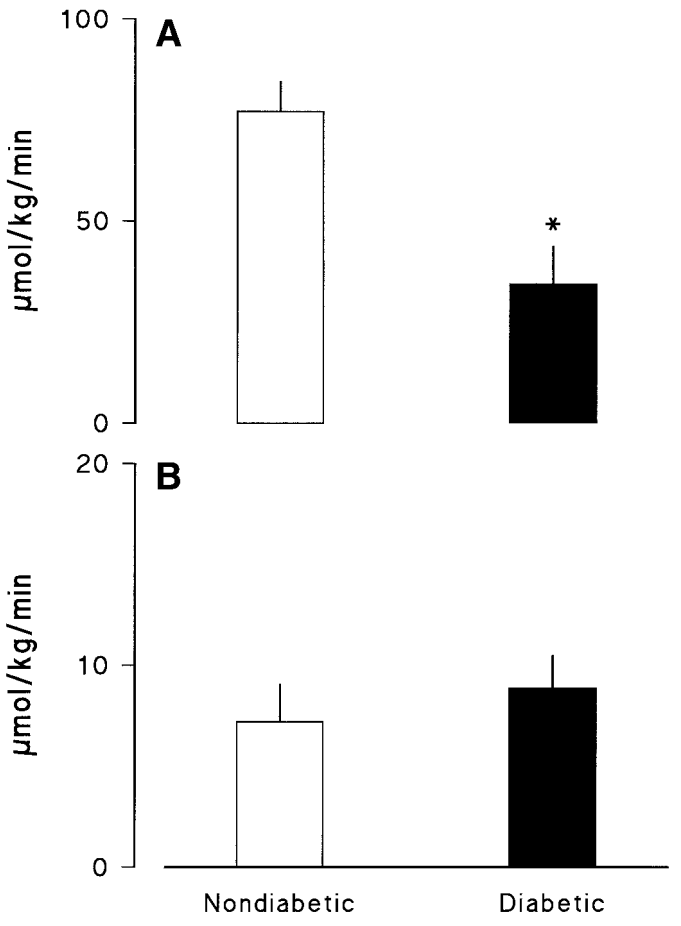

FIG. 6. Rates of glucose disappearance (*A*) and endogenous glucose production (*B*) observed in the diabetic and nondiabetic subjects during the final 30 min of the study.

· min^{-1} appeared in the portal vein in the diabetic and nondiabetic subjects, respectively (Fig. 7*A*). This resulted in a slightly (but not significantly) lower ($P = 0.27$) percentage of glucose extraction across the duodenum in the diabetic subjects than in the nondiabetic subjects (13 ± 8 vs. 19 ± 7%).

Splanchnic extraction of tracer and splanchnic glucose uptake. Splanchnic extraction of tracer (5.0 ± 0.8 vs. 7.7 ± 0.6%, $P < 0.01$) and splanchnic glucose uptake (11.3 ± 1.9 vs. 17.7 ± 2.0 μmol · kg^{-1} · min^{-1}, $P < 0.05$) were lower in the diabetic subjects than in the nondiabetic subjects (Fig. 7*B* and *C*). On the other hand, splanchnic blood flow did not differ between diabetic and nondiabetic subjects (1,391 ± 114 vs. 1,537 ± 238 ml/min).

Flux through UDP pool and the contribution of the direct and indirect pathways to glycogen synthesis. Urinary [^{14}C]-UDP glucuronide specific activity was 931 ± 133 and 758 ± 64 dpm/μmol in the diabetic and nondiabetic subjects, respectively, whereas the corresponding urinary [^{3}H]-UDP glucuronide specific activity was 72 ± 11 in the diabetic subjects and 156 ± 16 dpm/μmol in the nondiabetic subjects. UDP-glucose flux (Fig. 8) was lower ($P < 0.02$) in the diabetic subjects than in the nondiabetic subjects (12.8 ± 1.5 vs. 17.9 ± 1.7 μmol · kg^{-1} · min^{-1}). In addition, both the percentage (29 ± 6 vs. 45 ± 3%, $P < 0.02$) and absolute contribution (3.9 ± 0.9 vs. 8.7 ± 1.3 μmol · kg^{-1} · min^{-1}) of the direct pathway to UDP-glucose flux was lower ($P < 0.01$) in the diabetic subjects than in the nondiabetic subjects. On the other hand, the contribution of the indirect pathway to UDP-glucose flux did not differ between groups (9.0 ± 1.3 vs. 9.2 ± 0.6 μmol · kg^{-1} · min^{-1}).

Splanchnic glucose production. Splanchnic glucose production (5.7 ± 1.4 vs. 9.9 ± 2.8 μmol · kg^{-1} · min^{-1}) did not differ in the diabetic and nondiabetic subjects (Fig. 9).

Leg extraction of tracer and leg glucose uptake. Leg blood flow (547 ± 64 vs. 539 ± 44 ml/min) did not differ between groups. On the other hand, leg tracer extraction (8.5 ± 2.5 vs. 16.0 ± 1.7%) and leg glucose uptake (28.2 ± 8.6 vs. 70.5 ± 9.5 μmol/kg fat-free mass of leg/min) were lower ($P < 0.01$) in the diabetic subjects than in the nondiabetic subjects (Fig. 10).

DISCUSSION

The present studies establish that insulin-induced stimulation of splanchnic and muscle glucose uptake is impaired in people with type 2 diabetes during enteral glucose administration. The decrease in splanchnic glucose uptake was associated with a proportionate decrease in hepatic UDP-glucose flux and presumably, therefore, hepatic glycogen synthesis. This was entirely accounted for by a decrease in the contribution of extracellular glucose to the UDP-glucose pool. On the other hand, the contribution of the indirect gluconeogenic pathway to

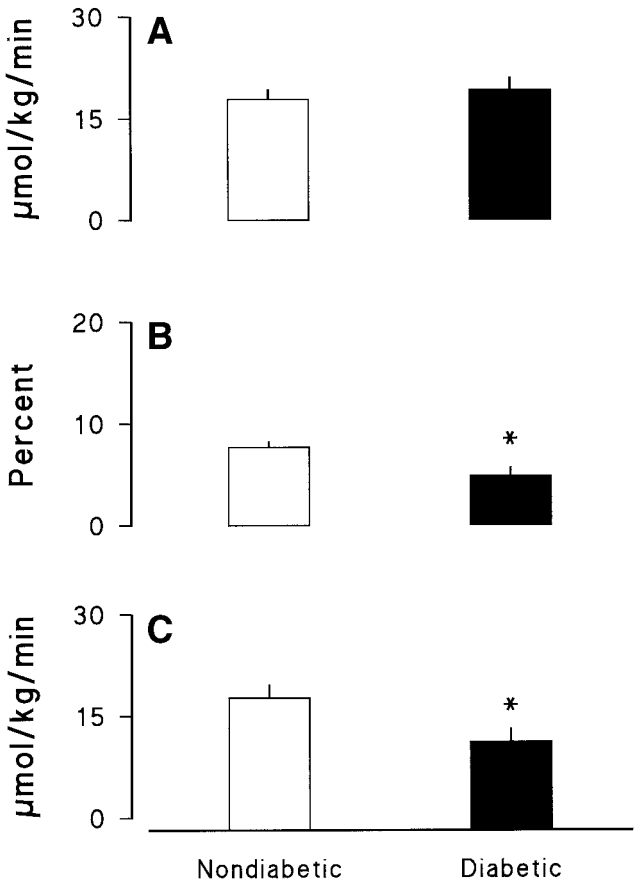

FIG. 7. Portal rate of appearance (*A*), splanchnic extraction of [3-^{3}H]glucose (*B*), and splanchnic glucose uptake (*C*) observed in the diabetic and nondiabetic subjects during the final 30 min of the study.

glycogen synthesis did not differ in the diabetic and nondiabetic subjects. This pattern, which is the same as when glucose was only infused intravenously (24), strongly implies a defect in hepatic glucokinase activity. Taken together with our previous study, these data indicate that alterations in splanchnic and muscle glucose metabolism commonly occur in people with type 2 diabetes and are likely to contribute to both postprandial and postabsorptive hyperglycemia.

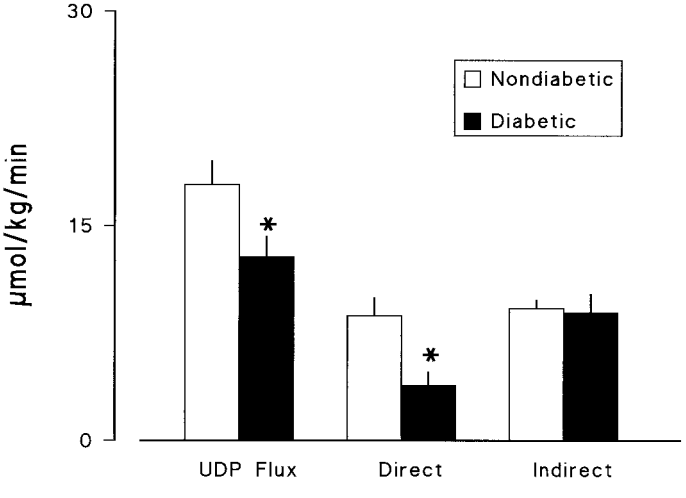

FIG. 8. Flux through the UDP-glucose pool and the contribution of the direct and indirect pathways to glycogen synthesis observed in the diabetic and nondiabetic subjects during the final 30 min of the study. *$P < 0.05$ vs. nondiabetic subjects.

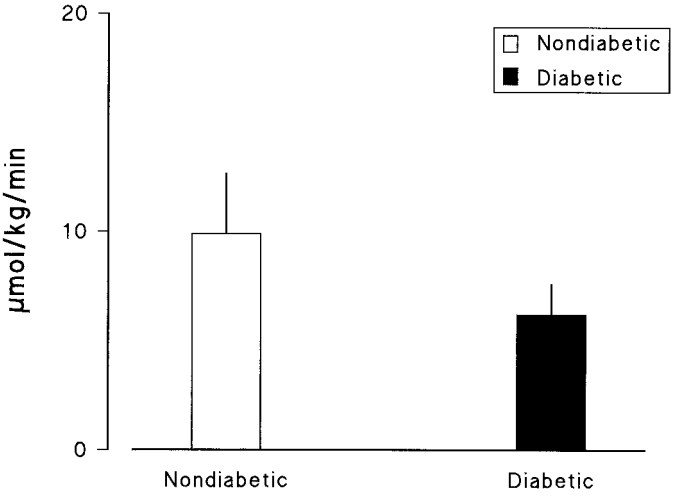

FIG. 9. Rate of splanchnic glucose production observed in the diabetic and nondiabetic subjects during the final 30 min of the study.

Once ingested, glucose must undergo a series of steps before it can reach the systemic circulation. It first must be emptied from the stomach. Several investigators have shown that in the absence of autonomic neuropathy, gastric emptying of solids is normal in people with type 2

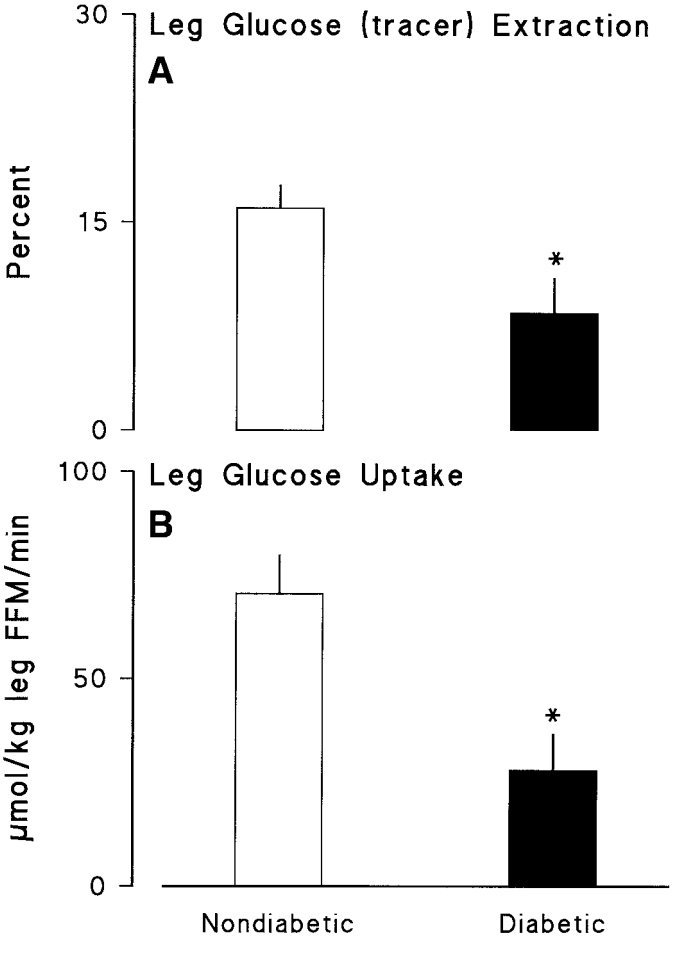

FIG. 10. Leg extraction of [3-^{3}H]glucose (*A*) and leg glucose uptake (*B*) observed in the diabetic and nondiabetic subjects during the final 30 min of the study. FFM, fat-free mass. *$P < 0.05$ vs. nondiabetic subjects.

diabetes, whereas gastric emptying of liquids is (albeit minimally) enhanced (46,47). In the present experiments, glucose was infused directly into the duodenum. This approach avoided potential uncertainty introduced by differences in gastric emptying in the diabetic and nondiabetic subjects. Intraduodenal glucose infusion also was necessitated by the use of somatostatin to inhibit endogenous insulin secretion because somatostatin also is a potent inhibitor of gastric emptying (48). Once in the duodenum, glucose must be absorbed and then transported across the intestinal enterocyte. Although the dual tracer approach used in the present experiments cannot distinguish between these two processes, the portal rate of appearance of enterically delivered glucose was slightly (but not significantly) lower in the diabetic subjects than in the nondiabetic subjects. This contrasts with reports that glucose absorption (25) and intestinal transport (49,50) are enhanced in chronically diabetic animals. Although this could be due to a species difference, it more likely is because the diabetic animals generally were severely hyperglycemic and hyperphagic, which presumably led to intestinal hypertrophy (51). The 15–20% fractional extraction and/or malabsorption of glucose across the gut observed in the present experiments is slightly lower than the 20–30% reported in previous studies using similar methods in nondiabetic animals (10,11) and humans (12). Taken together, these data indicate that accelerated intestinal glucose transport and/or decreased intestinal glucose metabolism are unlikely to contribute to postprandial hyperglycemia in type 2 diabetes.

Having entered the portal vein, enterically administered glucose then passes through the liver. In the present studies, splanchnic glucose uptake was decreased in the diabetic subjects whether measured as splanchnic extraction of tracer or splanchnic glucose uptake. This concordance is reassuring because the calculation of the latter depends on splanchnic blood flow, whereas calculation of the former is not. These results are entirely consistent with our previous observation that splanchnic glucose uptake also was decreased when glucose was only infused intravenously (24). Therefore, the impairment in splanchnic glucose uptake in diabetic subjects is not dependent on the route (enteral vs. parental) of glucose administration. These results are also consistent with the report of Ludvik et al. (52) that net splanchnic balance of glucose was lower during a hyperinsulinemic-euglycemic clamp in type 2 diabetic subjects after glucose ingestion. On the other hand, DeFronzo et al. (9) reported that splanchnic glucose uptake during a hyperinsulinemic-euglycemic clamp did not differ in diabetic and nondiabetic subjects. Because hyperglycemia is the primary stimulus for hepatic glycogen synthesis (8,9,53,54), low rates of hepatic glucose uptake in the presence of euglycemia may have made it difficult to detect a difference in the latter experiment. The similarity of the rates of splanchnic glucose uptake in our previous and current studies is intriguing. Although different subjects were studied, the characteristics of the diabetic and nondiabetic volunteers were quite similar. On the other hand, glucose concentrations were clamped at slightly higher levels in the present study (~10 vs. ~9.3 mmol/l). In addition, the insulin concentrations achieved in the current study (~300 pmol/l) were twofold higher than those present in our previous study (~150 pmol/l). Although the mean splanchnic glucose uptake in the two studies was almost the same in the nondiabetic subjects (17.7 vs. 15.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), it was substantially higher in the diabetic subjects in the present study (11.3 vs. 7.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$).

These data are of interest for several reasons. First, they imply that an increase in insulin above a certain threshold has a minimal effect on splanchnic glucose uptake in nondiabetic individuals, consistent with previous reports that the level of glucose rather than insulin is the primary determinant of the hepatic glucose uptake (8,9,53,54). On the other hand, the higher insulin concentrations in the present study did appear to further enhance splanchnic glucose uptake in the diabetic subjects consistent with a decrease in hepatic insulin sensitivity. It is intriguing to note that whereas splanchnic glucose uptake in the nondiabetic subjects differed minimally between studies, both UDP-glucose flux (17.9 vs. 8.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and flux via the indirect pathway (9.3 vs. 3.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) were substantially higher in the present experiments. These observations confirm in humans the demonstration by Chiasson et al. (55,56) in dogs that physiological increases in insulin do not suppress gluconeogenesis but rather preferentially shunt glucose-6-phosphate derived via the indirect pathway (presumably from carbons originating either within the splanchnic bed or shunted to the liver from peripheral tissues) into glycogen.

Glucose enters the hepatocyte via high K_m GLUT2 transporters and is then phosphorylated to glucose-6-phosphate by glucokinase (13–15,17). Phosphorylation by glucokinase appears to be the rate-determining step for hepatic glucose uptake because GLUT2 transporters have a substantially higher K_m for glucose than glucokinase (13–15,17). People with an autosomal dominant defect in glucokinase activity (commonly referred to as maturity-onset diabetes of the young type 2) have impaired hepatic glucose uptake in response to hyperglycemia and hyperinsulinemia (57). The decrease in UDP-glucose flux in the diabetic subjects observed in the present experiments was entirely accounted for by a decrease in the contribution of extracellular glucose to hepatic UDP flux. Flux via the indirect gluconeogenic pathway was virtually identical in the diabetic and nondiabetic subjects (Fig. 8). This strongly implies that glucokinase activity was lower in the diabetic subjects than in the nondiabetic subjects. The same pattern was observed in our previous experiments at lower insulin concentrations during intravenous glucose infusion (24).

A marked decrease in GLUT2 transporter number or activity also could have led to lower rates of hepatic uptake of extracellular glucose. However, the recent observation that glucokinase overexpression is sufficient to fully restore hepatocyte glucose uptake and glycogen synthesis in Zucker diabetic rats argues against this possibility (58). Increased rates of glucose-6-phosphatase activity could cause a decrease in net hepatic glucose uptake. However, this presumably would result in a decrease in the contribution of both the direct and indirect pathways to hepatic glycogen synthesis rather than a decrease in the direct pathway alone, as observed in the present experiments. In addition, Caro et al. (59) have

shown that glucokinase activity is decreased in liver biopsies obtained from severely obese diabetic patients at the time of bariatric surgery. Thus, decreased glucokinase appears to be common in different forms of diabetes. The present studies show that this defect in not reversed by either high insulin concentrations or enteral delivery of glucose. On the other hand, Nawano et al. (60) have shown that lowering of glucose concentrations in diabetic rats using a phlorizin analog restored glucokinase activity to nondiabetic levels. It remains to be determined whether chronic normalization of plasma glucose concentration also increases hepatic glucokinase activity in diabetic humans.

At first glance, the observation in the present studies that splanchnic extraction of enterally delivered glucose is lower in diabetic subjects than in the nondiabetic subjects may appear to be in conflict with numerous previous reports that the systemic rate of appearance of ingested glucose after either a mixed or carbohydrate-containing meal does not differ in diabetic and nondiabetic subjects (3,4,61–63). However, postprandial glucose concentrations in those studies invariably were far higher in the diabetic subjects than in the nondiabetic subjects. Splanchnic extraction of the ingested glucose clearly was not appropriate for the higher prevailing glucose concentration. On the other hand, those studies indicate that hyperglycemia can compensate for the intrinsically lower rates of hepatic glucose uptake that the current experiments demonstrate to be present under conditions in which glucose and insulin concentrations are matched.

Neither endogenous (total body) nor splanchnic glucose production differed in the diabetic and nondiabetic subjects, indicating that both are equally suppressed at high insulin. Whereas endogenous and splanchnic glucose production in our previous experiments (24) were equal in the nondiabetic subjects, endogenous was higher than splanchnic glucose production in 11 of the 15 diabetic subjects supporting an extrahepatic source of glucose (e.g., the kidney). In the present experiments, endogenous glucose production was not statistically different from splanchnic glucose production in either the diabetic or nondiabetic subjects, implying—but not proving—that the splanchnic bed was the sole source of glucose release.

Leg glucose uptake also was lower in the diabetic subjects than in the nondiabetic subjects. Assuming that appendicular muscle mass (defined as the sum of leg and arm muscle mass measured using DEXA) is equal to 79% of total body muscle mass (64), the percentage reduction in muscle glucose uptake (~56%) closely approximated the percentage reduction in whole-body glucose uptake (~60%). On the other hand, the splanchnic bed accounted for ~15% of the reduction in whole-body glucose uptake seen in the diabetic subjects than in the nondiabetic subjects (Fig. 11). Of interest, nonsplanchnic nonmuscle glucose uptake also was reduced (~29%) in the diabetic subjects. Whereas the higher insulin concentrations in the present study, compared with our previous experiments (24), had a minimal effect on splanchnic glucose uptake, they resulted in a further ~18 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ increase in leg glucose uptake in the nondiabetic subjects and an ~10 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ increase in the diabetic subjects. This suggests that the range of response of insulin-induced stimulation of glucose uptake is greater in muscle than in the liver. These data also indicate that as insulin concentrations increase, muscle makes a proportionately larger contribution to glucose disposal in both diabetic and nondiabetic subjects than the splanchnic bed. Conversely, at lower insulin levels, liver makes a proportionately greater contribution to glucose disposal. A similar pattern has been observed in the presence of euglycemia (9). Therefore, agents that selectively increase insulin action in muscle may have a greater impact than agents that selectively increase insulin action in the liver.

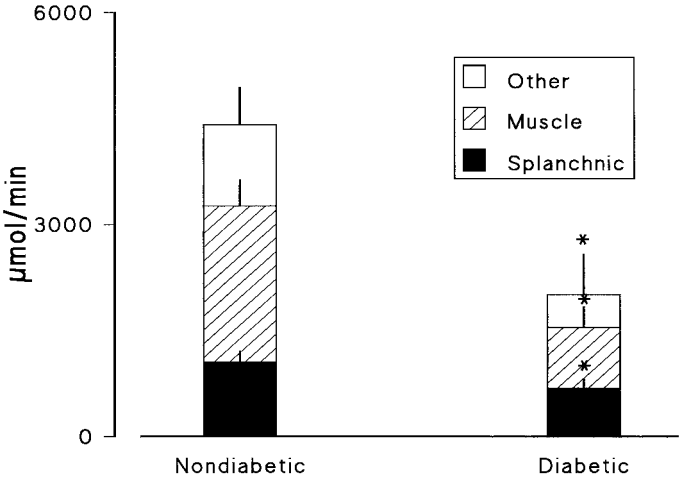

FIG. 11. The relative contributions of splanchnic glucose uptake, muscle glucose uptake, and other to whole-body glucose disappearance (total height of the bars) observed in the diabetic and nondiabetic subjects. The height of all bars in the diabetic subjects are lower (*$P < 0.05$) than those in the nondiabetic subjects.

The present experiments have certain limitations. Glucose was infused into the duodenum at a rate of 22 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (i.e., 4 $mg \cdot kg^{-1} \cdot min^{-1}$), resulting in ~424 mmol (~75 g) being given over the 4 h of the study. Different intraduodenal glucose infusion rates likely would have resulted in different rates of splanchnic glucose uptake. When glucose is given orally, the appearance of ingested glucose generally peaks within 30–60 min at a rate of 25–35 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, presumably resulting in higher portal glucose concentrations than were present during the current experiments (3,4,61–63,65,66). However, the postprandial peak in meal glucose appearance is brief, with rates falling rapidly to values of <22 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ during the next 3–4 h (3,4,61–63,65,66). Insulin concentrations follow a similar pattern, increasing rapidly after carbohydrate ingestion, peaking at 30–60 min, and then falling rapidly back toward basal values (3,4,61–63,65,66). Therefore, the present experimental design, in which the intraduodenal glucose infusion was kept constant while glucose and insulin concentrations were both clamped at elevated levels, likely resulted in rates of splanchnic glucose uptake that were lower than those that occur during peak glucose absorption. On the other hand, this design likely resulted in higher rates of uptake than normally occur from 1 h onward after glucose ingestion. Therefore, although the present study has established that people with type 2 diabetes have impaired splanchnic glucose uptake, it does not provide insight as to the contribution of this defect to postprandial hyperglycemia under conditions of daily living when glucose, insulin, and glucagon concentrations are continuously changing.

Calculation of the portal rate of appearance of enterically infused glucose is based on several assumptions. The systemically infused [3-^{3}H]glucose was used to estimate the splanchnic extraction ratio that in turn was used to calculate the portal rate of appearance of glucose. This ratio measures the percent extraction of systemically infused [3-^{3}H]glucose across splanchnic tissues including the gut, liver, pancreas, and spleen. The calculation assumes that once the enterally infused [6,6-$^2H_2$]glucose leaves the enterocyte and enters the intestinal capillary bed, its metabolism is the same as that of [3-^{3}H]glucose contained in the same capillary bed. This calculation also assumes that the [6,6-$^2H_2$]glucose uniformly mixes with the other glucose that is already present in the splanchnic circulation before reaching the hepatic vein. Both assumptions appear to be reasonable. In addition, errors due to incomplete mixing presumably will be similar in both the diabetic and nondiabetic patients. Nevertheless, for the above reasons, estimates of the portal rate of appearance of glucose in the present experiments should be considered as qualitative rather than quantitative.

The acetaminophen glucuronide method was used to measure UDP-glucose flux (16,32,33,67,68). This method has been extensively validated both in animals and humans (16,33,34). The assumptions of this method are discussed in detail elsewhere (16,32,33,67,68). Steady state is one of the major assumptions. Care was taken in the present experiments to ensure that glucose and insulin concentrations as well as [^{14}C]glucose specific activity all were constant when UDP flux was measured. Somatostatin was infused to inhibit endogenous insulin secretion and thereby ensure that insulin concentrations were equal in the diabetic and nondiabetic subjects. Somatostatin also may have inhibited the secretion of both known and unknown incretin hormones that potentially could influence hepatic glucose uptake (28). However, somatostatin does not appear to abolish the portal signal because it has been extensively used in experiments demonstrating that intraportal glucose infusion enhances hepatic glucose uptake (29,30).

The diabetic subjects were infused with insulin during the night to avoid the confounding effect of marked differences in baseline glucose concentrations between groups. We have previously shown that overnight euglycemia achieved with the same nocturnal insulin infusion improves insulin-induced suppression of glucose production in people with type 2 diabetes but does not alter insulin-induced stimulation of glucose uptake (69). If the overnight infusion of insulin improved insulin-induced stimulation of splanchnic glucose uptake, we may have underestimated the magnitude of the defect that normally is present under conditions of daily living. On the other hand, if the overnight insulin infusion resulted in a substantial increase in hepatic and muscle glycogen, then this in turn may have led to lower subsequent rates of glucose uptake in these tissues. However, we believe the latter to be unlikely because previous studies using nuclear magnetic resonance spectroscopy have demonstrated that hepatic glycogen content is lower in diabetic subjects than in nondiabetic subjects in both the postprandial (i.e., when insulin concentrations are high) and postabsorptive states (70,71). In addition, muscle glycogen content measured with the same technique after an overnight insulin infusion also has been shown to be lower in diabetic subjects than in nondiabetic subjects (72). Finally, glucose is rarely ingested in the absence of other nutrients such as protein and fat. Additional experiments will be required to determine whether splanchnic uptake differed under those conditions.

In summary, the present studies demonstrate that both splanchnic and muscle glucose metabolism are abnormal in people with type 2 diabetes during enteral glucose delivery. Despite equal or higher glucose and insulin concentrations, splanchnic glucose uptake and flux through UDP-glucose during eternal glucose feeding were lower in the diabetic subjects than in the nondiabetic subjects. The defect in hepatic glucose uptake appears to reside at the level of glucokinase because the decrease in glycogen synthesis could be entirely accounted for by a decrease in the rate of entry of extracellular glucose into the UDP-glucose pool. On the other hand, fractional extraction of glucose across the duodenum did not differ in diabetic and nondiabetic subjects. These data indicate that impaired hepatic and muscle but not gut glucose metabolism are likely to contribute to postprandial hyperglycemia in people with type 2 diabetes.

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