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 \(22 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\) while supplemental glucose was given intravenously to clamp glucose at \(\approx 10 \text{ mmol/l}\) in both groups. Endogenous hormone secretion was inhibited with somatostatin, and insulin was infused to maintain plasma concentrations at \(\approx 300 \text{ pmol/l}\) (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 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 \(\approx 9.3 \text{ mmol/l}\) and insulin at \(\approx 150 \text{ pmol/l}\) 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
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 thiazide 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.

**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. The subjects elected not to have a bladder catheter placed, and they were able to void on request at the appropriate times. Therefore, at 0700, the position of the feeding tube was confirmed to be in the distal duodenum by fluoroscopy. 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 (8-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-H]glucose (12 μCi prime, 0.12 μCi/min continuous; New England Nuclear, Boston, MA) was started at 0000. At 0030 (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 0030, and the infusion rate was adjusted to maintain plasma glucagon concentrations at ~10 pmol/l over the next 4 h. Glucose, tracers, and hormones all were infused into a forearm vein.

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

### Table 1: Volunteer characteristics

<table>
<thead>
<tr>
<th></th>
<th>Nondiabetic (8 M:6 F)</th>
<th>Diabetic (4 M:7 F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>8 ± 1</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Age (years)</td>
<td>49 ± 2</td>
<td>58 ± 2</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>58 ± 4</td>
<td>60 ± 4</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>29 ± 1</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>34 ± 2</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>5.0 ± 0.2</td>
<td>7.6 ± 0.4</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>5.3 ± 0.1</td>
<td>9.0 ± 0.5</td>
</tr>
</tbody>
</table>

Data are means ± SE.
Nondiabetics

Type 2 Diabetics

ICG infusion

 Variable Hot Ginf [3-3H] glucose infusion

 Duodenal [6,6-2H2] Glucose (22.2 μmol/kg.min⁻¹)

 Insulin (1.0 μu/kg.min⁻¹)

 SRIF, HGH, Glucagon infusion

 [3-3H] Glucose infusion

 Catheters: Hepatic Vein, Iliac Vein, Femoral Artery

Glucose 180 mg/dl (~10.0 mmol/L)

Naso-duodenal Tube

Acetaminophen 2g

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.

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 − hemotocrit). Blood glucose concentrations were calculated by multiplying the plasma glucose concentrations by 0.85.

When [3-3H]glucose was infused intravenously and [6,6-2H2]glucose was infused via the naso-duodenal tube, rates of glucose appearance (RA) and glucose disappearance (RD) were calculated using the steady-state equations of Steele et al. (45):

\[
R_a = \frac{F_{\text{gin}}}{SA_{\text{of [3-3H]glucose}}} \tag{1}
\]

where \( F_{\text{gin}} \) is the infusion rate of [3-3H]glucose and \( SA_{\text{of [3-3H]glucose}} \) is the plasma specific activity of [3-3H]glucose.

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

\[
\text{SER} = \frac{A_{\text{trm}} - H\text{V}_{\text{trm}}}{A_{\text{trm}}} \tag{2}
\]

where \( A_{\text{trm}} \) and \( H\text{V}_{\text{trm}} \) are the concentrations of [3-3H]glucose in the arterial and hepatic venous plasma, respectively.

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

\[
\text{Porta}l \ R_p = \frac{\text{HV}_{\text{Gin}}}{1 - \text{SER}} - \frac{\text{FA}_{\text{Gin}}}{\text{Duo}_{\text{MPE}}} \times Q_{\text{HV}} \tag{3}
\]

where \( \text{HV}_{\text{Gin}} \) is the concentration of [6,6-2H2]glucose in the hepatic vein, \( \text{FA}_{\text{Gin}} \) is the femoral arterial concentration of [6,6-2H2]glucose, \( Q_{\text{HV}} \) is the median of quadruple determinations of splanchnic blood flow, and \( \text{Duo}_{\text{MPE}} \) is the enrichment of [6,6-2H2]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:

\[
\text{Gut extraction} = \left( \frac{G_{\text{Gin}} - \text{Portal } R_p}{G_{\text{Gin}}} \right) \times 100 \tag{4}
\]
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Where G\textsubscript{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:

\[
SGU = (FA\textsubscript{Ain} \times Q\textsubscript{inv} \times SER) + (Portal \ R_a \times SER)
\]  

(5)

where \( FA\textsubscript{Ain} \) is femoral arterial glucose concentration. Flux through the UDP-glucose pool was calculated as:

\[
UDP \text{ glucose flux} = \frac{F\textsubscript{GAL}}{SA \text{ of UDP-}[^{14}\text{C}] \text{glucose}}
\]

(6)

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

The specific activity of \([^{3}\text{H}]\text{glucuronide}\) in urine, and \( SA \text{ of } [^{3}\text{H}] \text{glucose} \) equals the specific activity of \([^{3}\text{H}]\text{glucose}\) entering the liver was calculated by dividing the amount of \([^{3}\text{H}]\text{glucose}\) reaching liver by the amount of unlabeled glucose reaching the liver:

\[
SA \text{ of } [^{3}\text{H}] \text{glucose} = \frac{FA\textsubscript{Ain} \times Q\textsubscript{inv}}{(FA\textsubscript{Ain} \times Q\text{inv}) + Portal \ R_a}
\]

(8)

where \( FA\textsubscript{Ain} \) is the concentration of \([^{3}\text{H}]\text{glucose}\) in the femoral artery.

The contribution (in \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \)) 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:

\[
SGP = [FA\textsubscript{Ain} - HV\textsubscript{a} \times Q\textsubscript{inv} \times Portal \ R_a] - SGU
\]

(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 (\( A\textsubscript{Glu} \)) and the femoral venous (\( F\textsubscript{Glu} \)) glucose concentration times the median of quadruple determinations of leg blood flow (\( Q\text{L} \)):

\[
LGU = (A\textsubscript{Glu} - F\textsubscript{Glu}) \times Q\text{L}
\]

(10)

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

\[
LER = \frac{[A\textsubscript{Glu} - F\textsubscript{Glu}]}{A\textsubscript{Glu}}
\]

(11)

where \( A\textsubscript{Glu} \) and \( F\textsubscript{Glu} \) are the concentrations of \([^{3}\text{H}]\text{glucose}\) in the femoral artery and femoral veins, respectively.

The calculations used for the five nondiabetic and three diabetic subjects in whom \([6,6\text{-}_2\text{H}_2]\text{glucose}\) was infused intravenously and \([^{3}\text{H}]\text{glucose}\) intraduodenally were the same with the exception that the concentration and enrichment of \([6,6\text{-}_2\text{H}_2]\text{glucose}\) were switched with the concentration and specific activity of \([^{3}\text{H}]\text{glucose}\). In addition, the infusion rate of \([6,6\text{-}_2\text{H}_2]\text{glucose}\) 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 concentra-

tions (Fig. 2A) were higher \( (P < 0.01) \) in the diabetic subjects than in the nondiabetic subjects \( (8.0 ± 0.6 \text{ vs. } 5.4 ± 0.1 \text{ mmol/l}) \). Arterial glucose concentrations increased in both groups within 60 min of initiation of glucose infusion to \( \approx 10 \text{ mmol/l} \). Arterial glucose concentrations were slightly higher \( (P < 0.01) \) in the diabetic subjects than in the nondiabetic subjects \( (10.1 ± 0.01 \text{ vs. } 9.7 ± 0.05 \text{ mmol/l}) \) 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 \( (10.8 ± 0.03 \text{ vs. } 10.2 ± 0.07 \text{ mmol/l}) \).

Baseline plasma insulin concentrations (Fig. 2B) were slightly \( (P = 0.06) \) higher in the diabetic subjects than in the nondiabetic subjects \( (45 ± 9 \text{ vs. } 27 ± 4 \text{ pmol/l}) \). The somatostatin plus insulin infusion, begun at time 0, rapidly increased insulin concentrations to \( \approx 300 \text{ pmol/l} \) 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 \( (317 ± 22 \text{ vs. } 311 ± 18 \text{ pmol/l}) \).

**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.

\[\text{FIG. 2. Glucose (A) and insulin (B) concentrations observed in the diabetic and nondiabetic subjects during the study. Glucose (intra-}\]

\[\text{duodenal and intravenous) and insulin infusions were started at time 0.}\]
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. 4A). By design, the intraduodenal infusion rate of glucose was the same in both groups (Fig. 4B). Consequently, the total glucose infusion rate (Fig. 4C) during the final 30 min of the study also was lower \( (P < 0.01) \) in the diabetic subjects than in the nondiabetic subjects \( (40 \pm 7 \text{ vs. } 77 \pm 7 \text{ } \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) \).

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. 5A). 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 \( (40 \pm 7 \text{ vs. } 77 \pm 7 \text{ } \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) \).

The \([6,6-2^H_2]\)glucose enrichment in plasma gradually increased, approaching a plateau by \(~180\text{ min} \) (Fig. 5B). Because the intravenous glucose infusion did not contain \([6,6-2^H_2]\)glucose and because this intravenous glucose represented a lesser proportion of the total glucose administered in the diabetic subjects, \([6,6-2^H_2]\)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. 5C).

Total body glucose disappearance and endogenous glucose production. Despite similar insulin and slightly higher glucose concentrations, total body glucose disappearance (Fig. 6A) was lower \( (P < 0.01) \) in the diabetic subjects than in the nondiabetic subjects \( (34.6 \pm 9.3 \text{ vs. } 77.2 \pm 7.5 \text{ } \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) \). 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 \( (2.1 \pm 1.0 \text{ vs. } 4.0 \pm 0.1 \text{ } \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) \). Endogenous glucose production (Fig. 6B) was equally suppressed in the diabetic and nondiabetic subjects \( (8.9 \pm 1.6 \text{ vs. } 7.2 \pm 1.9 \text{ } \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) \).

Portal appearance of duodenal glucose and duodenal glucose extraction. By design, the duodenal glucose infusion rate was the same \( (22.2 \text{ } \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) \) in both groups. Of this, \( 19.4 \pm 1.8 \text{ and } 17.9 \pm 1.5 \text{ } \mu \text{mol} \cdot \text{kg}^{-1} \)
appeared in the portal vein in the diabetic and nondiabetic subjects, respectively (Fig. 7A). 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 \((13.6 \pm 8.0\% \text{ vs. } 19.5 \pm 6.7\%)\).

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

**Flux through UDP pool and the contribution of the direct and indirect pathways to glycogen synthesis.** Urinary \[^{14}\text{C}]\text{-UDP glucuronide specific activity was } 931 \pm 133 \text{ and } 758 \pm 64 \text{ dpm/μmol in the diabetic and nondiabetic subjects, respectively, whereas the corresponding urinary }[^{3}\text{H}]\text{-UDP glucuronide specific activity was } 72 \pm 11 \text{ in the diabetic subjects and } 156 \pm 16 \text{ dpm/μmol in the nondiabetic subjects. UDP-glucose flux (Fig. 8) was lower } (P < 0.02) \text{ in the diabetic subjects than in the nondiabetic subjects } (12.8 \pm 1.5 \text{ vs. } 17.9 \pm 1.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) \text{. In addition, both the percentage } (29 \pm 6 \text{ vs. } 45 \pm 3\%, P < 0.02) \text{ and absolute contribution } (3.9 \pm 0.9 \text{ vs. } 8.7 \pm 1.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) \text{ of the direct pathway to UDP-glucose flux was lower } (P < 0.01) \text{ 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 \pm 1.3 \text{ vs. } 9.2 \pm 0.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) \text{.}

**Splanchnic glucose production.** Splanchnic glucose production \((5.7 \pm 1.4\% \text{ vs. } 9.9 \pm 2.8 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})\) did not differ in the diabetic and nondiabetic subjects (Fig. 9).

**Leg extraction of tracer and leg glucose uptake.** Leg blood flow \((547 \pm 64 \text{ vs. } 539 \pm 44 \text{ ml/min})\) did not differ between groups. On the other hand, leg tracer extraction \((8.5 \pm 2.5 \text{ vs. } 16.0 \pm 1.7%)\) and leg glucose uptake \((28.2 \pm 8.6 \text{ vs. } 70.5 \pm 9.5 \mu\text{mol/kg fat-free mass of leg/min})\) 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
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.

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 diabetes.

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

**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.

**FIG. 10.** Leg extraction of [3-3H]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.
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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 μmol · kg⁻¹ · min⁻¹), it was substantially higher in the diabetic subjects in the present study (11.3 vs. 7.5 μmol · kg⁻¹ · 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 μmol · kg⁻¹ · min⁻¹) and flux via the indirect pathway (9.3 vs. 3.7 μmol · kg⁻¹ · 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 Kₘ 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 Kₘ 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 (~50%) 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 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) increase in leg glucose uptake in the nondiabetic subjects and an ~10 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) 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 present experiments have certain limitations. Glucose was infused into the duodenum at a rate of 22 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) (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 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \), 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 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) 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 enterally infused glucose is based on several assumptions. The systemically infused [3-3H]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 [3-3H]glucose across splanchnic tissues including the gut, liver, pancreas, and spleen. The calculation assumes that once the enterally infused [6,6-2H2]glucose leaves the enterocyte and enters the intestinal capillary bed, its metabolism is the same as that of [3-3H]glucose contained in the same capillary bed. This calculation also assumes that the [6,6-2H2]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 [14C]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 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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