

Effects of Type 2 Diabetes on the Ability of Insulin and Glucose to Regulate Splanchnic and Muscle Glucose Metabolism

Evidence for a Defect in Hepatic Glucokinase Activity

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Insulin-induced stimulation of muscle glucose uptake (MGU) is impaired in people with type 2 diabetes. To determine whether insulin-induced stimulation of splanchnic glucose uptake (SGU) is also impaired, we simultaneously measured leg glucose uptake (LGU) and SGU in 14 nondiabetic subjects and 16 subjects with type 2 diabetes using a combined organ catheterization-tracer infusion technique. Glucose was clamped at ~ 9.3 mmol/l, while insulin concentrations were maintained at ~ 72 pmol/l (low) and ~ 150 pmol/l (high) for 3 h each. Endogenous hormone secretion was inhibited with somatostatin. Total body glucose disappearance was lower ($P < 0.01$) and glucose production higher ($P < 0.01$) during both insulin infusions in the diabetic compared with the nondiabetic subjects, indicating insulin resistance. Splanchnic glucose production was higher ($P < 0.05$) in the diabetic subjects during the low but not the high insulin infusion. SGU was slightly lower in the diabetic than in the nondiabetic subjects during the low insulin infusion and 50–60% lower ($P < 0.05$) during the high insulin infusion. LGU ($P < 0.001$), but not SGU, was inversely correlated with the degree of visceral adiposity. The contribution of the indirect pathway to hepatic glycogen synthesis did not differ in the diabetic and nondiabetic subjects. In contrast, both flux through the UDP-glucose pool ($P < 0.05$) and the contribution of the direct pathway to glycogen synthesis ($P < 0.01$) were lower in the diabetic than in the nondiabetic subjects, indicating decreased uptake and/or phosphorylation of extracellular glucose. On the other hand, glycogenolysis was equally suppressed in both groups. In summary, type 2 diabetes impairs the ability of insulin to stimulate both MGU and SGU. The defect appears to reside at a proximal (e.g., glucokinase)

metabolic step and is not related to the degree of visceral adiposity. These data suggest that impaired hepatic glucose uptake as well as MGU contribute to hyperglycemia in people with type 2 diabetes. *Diabetes* 49:272–283, 2000

Type 2 diabetes is associated with a decrease in insulin-induced stimulation of glucose uptake (1–9). The ability of glucose to enhance its own uptake (commonly referred to as glucose effectiveness) is also abnormal (10–14). The sites of these defects remain an area of active investigation. Although a large number of studies have shown that the response of muscle to glucose and insulin is impaired in people with type 2 diabetes (1–9), the effects of diabetes on splanchnic glucose metabolism are less clear (2,15,16). This distinction is important because while insulin and glucose stimulate glucose uptake in both tissues, the mechanism by which they do so differs.

In nondiabetic humans, glucose transport appears to be rate limiting in muscle, whereas phosphorylation appears to be rate limiting in the liver (6,17–21). Insulin increases glycogen synthase activity in both muscle and liver but inhibits glycogen phosphorylase activity only in liver (5,22–26). Perhaps more importantly, in the presence of euglycemia, insulin markedly stimulates glucose uptake in muscle but not liver (2,22,23,27–29). In contrast, hyperglycemia combined with hyperinsulinemia substantially increases glucose uptake in both tissues (27–30). Thus, therapies that enhance muscle glucose uptake (MGU) in insulin-resistant people may not also improve hepatic glucose uptake (HGU), and conversely, therapies that enhance HGU may not necessarily increase MGU.

Only a few studies have examined the effects of type 2 diabetes on splanchnic glucose metabolism. Felig et al. (16), using the splanchnic catheterization technique, were the first to report that net splanchnic release of glucose is greater in diabetic than in nondiabetic subjects after glucose ingestion. Firth et al. (31), as well as other investigators (32–34), subsequently used the dual isotope technique to demonstrate that the greater net splanchnic glucose release was due to excessive endogenous glucose production (EGP) rather than lower initial splanchnic extraction of the ingested glucose. However, the insulin and glucose concentrations differed in the diabetic and nondiabetic subjects in all of those studies,

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DXA, dual-energy X-ray absorptiometry; EGP, endogenous glucose production; FFA, free fatty acid; FFM, fat-free mass; G-6-P, glucose-6-phosphate; HGU, hepatic glucose uptake; LER, leg glucose extraction ratio; LGU, leg glucose uptake; MGU, muscle glucose uptake; NSGB, net splanchnic glucose balance; R_a , rate of glucose appearance; R_d , rate of glucose disappearance; SER, splanchnic glucose extraction ratio; SGP, splanchnic glucose production; SGU, splanchnic glucose uptake.

precluding direct comparison of the efficiency of splanchnic glucose uptake (SGU). DeFronzo et al. (2) and Ludvik et al. (15) attempted to circumvent this problem by measuring SGU during a euglycemic-hyperinsulinemic clamp, when glucose was either infused intravenously or both ingested and infused intravenously. The results of these studies were not concordant, with Ludvik et al. (15) reporting decreased SGU in diabetic subjects and De Fronzo et al. (2) reporting no difference in SGU between diabetic and nondiabetic subjects. However, as discussed above, since hyperinsulinemia in the absence of hyperglycemia only minimally stimulates SGU, the significance of these findings remains uncertain. Furthermore, neither fractional splanchnic glucose extraction nor completeness of absorption of glucose from the intestine was directly assessed in the studies of Ludvik et al. (15), further confounding interpretation of the data.

The present studies therefore were undertaken to determine whether in the presence of hyperglycemia and modest elevation of insulin (i.e., conditions resembling those commonly observed in diabetic individuals between meals and during the night), splanchnic as well as muscle glucose metabolism is impaired in people with type 2 diabetes. To do so, we used the splanchnic and leg catheterization methods in combination with the isotope technique to concurrently measure leg glucose uptake (LGU), SGU, splanchnic glucose production (SGP), and total body glucose production and disposal in diabetic and nondiabetic subjects. To avoid the confounding effects introduced by differences in glucose and insulin concentrations, glucose was clamped at 9.3 mmol/l (~165 mg/dl), endogenous insulin secretion was inhibited with somatostatin, and exogenous insulin was infused in both groups. To gain insight into the effects of type 2 diabetes on intrahepatic glucose metabolism, flux through the UDP-glucose pool (the precursor pool of glycogen) was assessed with the acetaminophen glucuronide method (35–42). In addition, the contribution of glycogenolysis to systemic glucose appearance was estimated by measuring release of [¹⁴C]glucose from glycogen previously labeled by an intravenous infusion of [¹⁴C]galactose (35,37–40,42).

RESEARCH DESIGN AND METHODS

Subjects. After approval from the Mayo Institutional Review Board, 14 nondiabetic subjects and 16 subjects with type 2 diabetes gave informed written consent to participate in the study. 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 the nondiabetic subjects had a history of diabetes. Five of the diabetic subjects were being treated with diet alone, and the other 11 with either a sulfonylurea or metformin. Both drugs were discontinued at least 3 weeks before the study. Subjects were on no medications at the time of the 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. Characteristics of the subjects are listed in Table 1. The two groups did not differ in age, fat-free mass (FFM), BMI, percent body fat, or total abdominal fat. Visceral fat content, however, was higher ($P < 0.01$) in the diabetic than in the nondiabetic subjects. As anticipated, both fasting glucose and glycosylated hemoglobin concentrations (Bio-Rad, Hercules, CA; normal range 4–7%) were higher in the diabetic 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 (43).

TABLE 1
Volunteer characteristics

	Nondiabetic	Diabetic
n	14	16
Duration of diabetes (years)	—	6 ± 2
Age (years)	50 ± 3	55 ± 2
FFM (kg)	46 ± 3	51 ± 3
BMI (kg/m ²)	28 ± 1	30 ± 1
Body fat (%)	34 ± 2	33 ± 2
Total abdominal fat (cm ²)	1,018 ± 57	1,151 ± 32
Visceral fat (cm ²)	127 ± 21	268 ± 21*
GHb (%)	4.9 ± 0.1	8.2 ± 0.5*
Fasting plasma glucose (mmol/l)	5.0 ± 0.1	9.4 ± 0.9*

Data are means ± SE. * $P < 0.01$ diabetic vs. nondiabetic volunteers.

The study design is outlined in Fig. 1. At 0600 on the morning after admission, a urinary bladder catheter was inserted in 28 of the 30 subjects. Two subjects elected not to have a bladder catheter placed; they were able to void upon request at the appropriate times. Subjects were moved to the interventional radiology suite at ~0800. Femoral artery, femoral vein, and hepatic venous catheters were placed as previously described (44,45). 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.

At 0900, a primed-continuous infusion of [³-³H]glucose (12 μCi prime, 0.12 μCi/min continuous; Du Pont–NEN, Boston, MA) was started into a forearm vein. At 0930 (time 0 min), infusions of somatostatin (60 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 0.25 mU · kg⁻¹ · min⁻¹ from 0 to 180 min and 0.5 mU · kg⁻¹ · min⁻¹ from 181 to 360 min. [³-³H]palmitate (Du Pont–NEN) was infused at 0.3 μCi/min from 120 to 180 min and 300 to 360 min as part of a separate experiment. A dextrose infusion was also begun at 0930, and the infusion rate was adjusted to maintain plasma glucose concentrations at ~9.3 mmol/l (~165 mg/dl) over the next 6 h. All infused glucose contained [³-³H]glucose to minimize the change in plasma glucose specific activity (46–48). In addition, the rate of basal [³-³H]glucose infusion was varied to mimic the anticipated changes

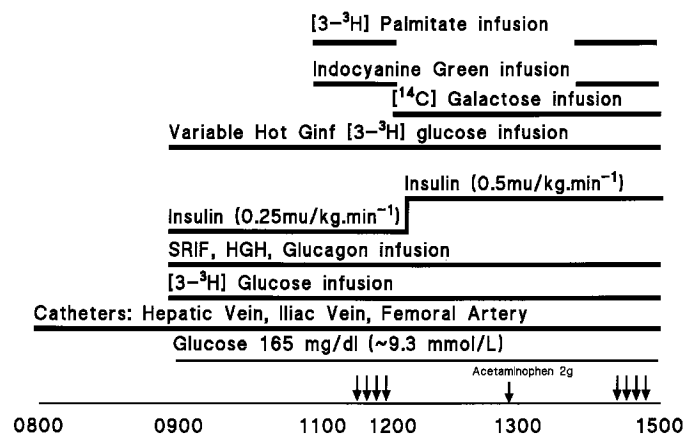


FIG. 1. Schematic outline of the experimental design (14 nondiabetic subjects and 16 subjects with type 2 diabetes). HGH, human growth hormone; SRIF, somatostatin; Variable Hot Ginf, the infusion of glucose containing [³-³H]glucose.

in EGP (10,14). In the nondiabetic subjects, the basal [^3H]glucose was infused at 100% from -30 to 0 min, 25% from 1 to 180 min, 21.5% from 181 to 210 min, 15.5% from 211 to 240 min, 11.5% from 241 to 270 min, 9.5% from 271 to 300 min, 7.5% from 301 to 330 min, and 6% from 331 to 360 min. In the diabetic subjects, the corresponding infusion rates for the same time periods were 100%, 25%, 23%, 19.5%, 17%, 15%, 13.5%, and 12%.

A primed-constant infusion of [^{14}C]galactose (15 μCi prime; 0.15 $\mu\text{Ci}/\text{min}$ constant; Du Pont-NEN) was started at 180 min and continued until the end of the study. Subjects were asked to void at 240 min and then ingest 2 g of acetaminophen. Urine was collected from 240 to 360 min for measurement of urinary acetaminophen glucuronide, as previously described (42). All urine passed from 0 to 240 min was also collected for analysis of urine glucose excretion.

Analytical techniques. All samples were placed in ice, centrifuged at 4°C , and separated. Plasma indocyanine green concentration was measured spectrophotometrically at 805 nm on the day of study, as previously described (44). 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, OH). Plasma insulin was measured using a chemiluminescence method with the Access Ultrasensitive Immunoassay 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 immunoassay (Beckman). [^3H]glucose and [^{14}C]glucose specific activities were measured by liquid scintillation counting, as previously described (49). Body composition (including FFM, total fat mass, visceral fat mass, and appendicular muscle mass) was measured using dual-energy X-ray absorptiometry (DXA) (DPX-IO scanner, SmartScan Version 4.6; Hologic, Waltham, MA) combined with a single-slice computerized tomograph scan at the level of L2/L3, as previously described (50). Urinary [^{14}C]glucuronide and [^3H]glucuronide specific activities were measured using high-performance liquid chromatography, as previously described (42).

Calculations. Splanchnic plasma flow was calculated by dividing the indocyanine green infusion rate by the arterial-hepatic venous concentration gradient of the dye (51). Likewise, leg plasma flow was calculated by dividing the dye infusion rate by the concentration gradient across the leg (44,45). 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. Rates of glucose appearance (R_a) and disappearance (R_d) were calculated using the steady-state equations of Steele et al. (52):

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

where F_{Glu} is the infusion rate of [^3H]glucose and SA of [^3H]glucose is the plasma specific activity of [^3H]glucose. EGP was determined by subtracting the glucose infusion rate from tracer-determined R_a .

Net splanchnic glucose balance (NSGB) was calculated as the product of the arterial-hepatic vein glucose difference times the splanchnic blood flow:

$$\text{NSGB} = [A_{\text{Glu}} - \text{HV}_{\text{Glu}}] \times Q_{\text{HV}} \quad (2)$$

where A_{Glu} is the concentration of glucose in the femoral artery, HV_{Glu} is the concentration of glucose in the hepatic vein, and Q_{HV} is the median of quadruple determinations of splanchnic blood flow.

The splanchnic glucose extraction ratio (SER) was calculated as

$$\text{SER} = \frac{[A_{\text{Trit}} - \text{HV}_{\text{Trit}}]}{A_{\text{Trit}}} \quad (3)$$

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

SGU was determined by multiplying arterial glucose concentration by SER and splanchnic blood flow:

$$\text{SGU} = A_{\text{Glu}} \times Q_{\text{HV}} \times \text{SER} \quad (4)$$

SGP was calculated by subtracting SGU from NSGB:

$$\text{SGP} = \text{NSGB} - \text{SGU} \quad (5)$$

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

$$\text{LGU} = [A_{\text{Glu}} - \text{FV}_{\text{Glu}}] \times Q_L \quad (6)$$

Leg glucose extraction ratio (LER) was calculated as

$$\text{LER} = \frac{[A_{\text{Trit}} - \text{FV}_{\text{Trit}}]}{A_{\text{Trit}}} \quad (7)$$

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

Flux through the UDP-glucose pool was calculated as

$$\text{UDP glucose flux} = \frac{F_{\text{GAL}}}{\text{SA of UDP-}[^{14}\text{C}]\text{glucose}} \quad (8)$$

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 (37-40,42).

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

$$\text{Direct pathway} = \frac{\text{SA of UDP-}[^3\text{H}]\text{glucose}}{\text{SA of } [^3\text{H}]\text{glucose}} \quad (9)$$

where SA of UDP- ^3H]glucose equals the specific activity of acetaminophen [^3H]glucuronide in urine and SA of [^3H]glucose equals the specific activity of [^3H]glucose in plasma. The contribution (in micromoles per kilogram per minute) of the direct pathway to total UDP-glucose flux was calculated by multiplying equation 8 by equation 9 (36,37,40-42). The contribution of the indirect pathway equals total UDP-glucose flux minus direct pathway.

The percentage of SGP derived from glycogen (40) was determined by dividing the hepatic venous specific activity of [^{14}C]glucose (product) by the specific activity of UDP- ^{14}C]glucose (precursor):

$$\text{Percent glycogenolysis} = \frac{\text{SA of } [^{14}\text{C}]\text{glucose}}{\text{SA of UDP-}[^{14}\text{C}]\text{glucose}} \quad (10)$$

We have previously shown that the liver quantitatively extracts [^{14}C]galactose (53). Preliminary studies conducted in two nondiabetic and two diabetic subjects used high-pressure liquid chromatography to once again confirm that all [^{14}C] counts in hepatic venous blood were contained in glucose (i.e., no [^{14}C]galactose was present).

The net rate of glycogenolysis was calculated by multiplying percent glycogenolysis by SGP:

$$\text{Net glycogenolysis} = \text{percent glycogenolysis} \times \text{SGP} \quad (11)$$

This calculation is based on the assumption that the specific activity of UDP-glucose pool approximates that of glycogen (29,35-42,54,55).

Statistical analysis. Data in the text and figures are expressed as means \pm SE. Rates are expressed as micromoles per kilogram FFM per minute. Responses during the low- and high-dose insulin infusions were determined by using the mean of the results present, respectively, from 150 to 180 min and from 330 to 360 min. Nonpaired Student's *t* test was used to compare results between groups (e.g., diabetic vs. nondiabetic subjects), and paired Student's *t* test was used to compare results within a group (e.g., low- vs. high-dose insulin infusion). One-tailed tests were used to test the hypotheses that insulin increases LGU and SGU and that type 2 diabetes impairs insulin action. All other tests were two-tailed. $P < 0.05$ was considered statistically significant.

RESULTS

Arterial, hepatic, venous, and femoral venous glucose concentrations. Before initiation of the somatostatin, insulin, and glucose infusions at time 0 min, arterial glucose concentrations were slightly higher ($P < 0.01$) in the diabetic than in the nondiabetic subjects (7.1 ± 0.6 vs. 5.1 ± 0.1 mmol/l). The glucose infusion promptly increased arterial glucose concentration to ~ 9.3 mmol/l by 60 min in both groups (Fig. 2). Arterial glucose concentrations then remained relatively constant from 120 to 180 min, dipped slightly when the insulin infusion rate was increased at 180 min, then remained constant from 300 to 360 min. Arterial glucose concentrations were slightly higher ($P < 0.001$) in the diabetic than in the nondiabetic subjects during the final 30 min of the low-dose insulin infusion

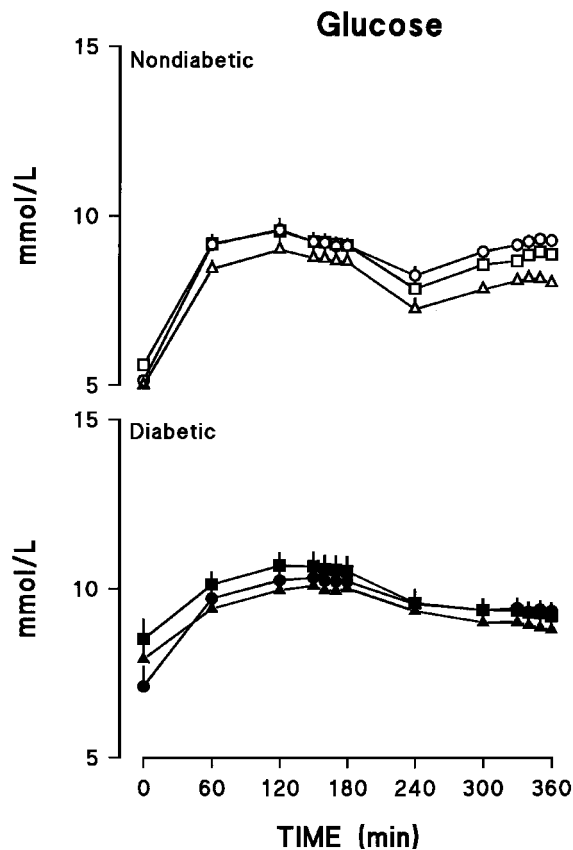


FIG. 2. Femoral arterial (\circ , \bullet), hepatic venous (\square , \blacksquare), and femoral venous (Δ , \blacktriangle) glucose concentrations observed in the diabetic (\bullet , \blacksquare , \blacktriangle) and nondiabetic (\circ , \square , Δ) subjects during the low-dose (from 0 to 180 min) and high-dose (from 181 to 360 min) insulin infusions.

(10.3 ± 0.03 vs. 9.2 ± 0.03 mmol/l), but they did not differ during the final 30 min of the high-dose insulin infusion (9.4 ± 0.02 vs. 9.3 ± 0.04 mmol/l).

Hepatic venous glucose concentrations were lower ($P < 0.001$) than arterial glucose concentrations in the nondiabetic subjects during the high-dose insulin infusion (8.8 ± 0.06 vs. 9.3 ± 0.04 mmol/l) but not during the low-dose insulin infusion (9.2 ± 0.02 vs. 9.2 ± 0.03 mmol/l). In contrast, hepatic venous glucose concentrations were higher ($P < 0.001$) than arterial glucose concentrations in the diabetic subjects during the low-dose insulin infusion (10.6 ± 0.03 vs. 10.3 ± 0.03 mmol/l) but not during the high-dose insulin infusion (9.3 ± 0.03 vs. 9.4 ± 0.02 mmol/l).

Femoral venous glucose concentrations were lower ($P < 0.01$) than arterial glucose concentrations in the nondiabetic subjects during the low-dose insulin infusion (8.7 ± 0.03 vs. 9.2 ± 0.03 mmol/l), with the difference becoming even greater ($P < 0.001$) during the high-dose insulin infusion (8.1 ± 0.03 vs. 9.3 ± 0.04 mmol/l). Femoral venous glucose concentrations were also lower ($P < 0.01$) than arterial concentrations in the diabetic subjects during both the low-dose (10.0 ± 0.03 vs. 10.3 ± 0.03 mmol/l) and high-dose (8.9 ± 0.05 vs. 9.4 ± 0.02 mmol/l) insulin infusions; however, the differences were less marked than those observed in the nondiabetic subjects. Insulin, C-peptide, glucagon, and growth hormone concentrations. Before initiation of the somatostatin, insulin, and glucose infusions at time 0 min, plasma insulin

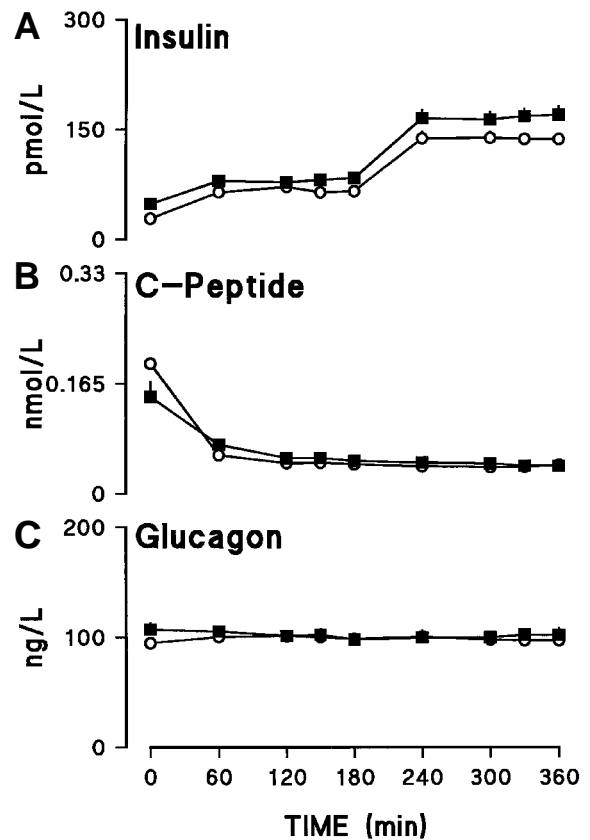


FIG. 3. Plasma insulin (A), C-peptide (B), and glucagon (C) concentrations observed in the diabetic (\blacksquare) and nondiabetic (\circ) subjects during the low-dose (from 0 to 180 min) and high-dose (from 181 to 360 min) insulin infusions.

concentrations (48 ± 9 vs. 28 ± 3.6 pmol/l) were greater ($P = 0.05$) in the diabetic compared with the nondiabetic subjects, whereas C-peptide concentrations did not differ (0.19 ± 0.02 vs. 0.15 ± 0.01 nmol/l) between the diabetic and nondiabetic subjects. After initiation of the hormone infusions, insulin concentrations rose to levels that were slightly higher ($P < 0.01$) in the diabetic than in the nondiabetic groups during both the low-dose (80 ± 1 vs. 67 ± 2 pmol/l) and high-dose (167 ± 2 vs. 138 ± 1 pmol/l) insulin infusions (Fig. 3A). The somatostatin infusion resulted in comparable and near total suppression of insulin secretion in both groups (Fig. 3B). Plasma glucagon concentrations did not differ in the diabetic and nondiabetic subjects either before or during somatostatin infusion (Fig. 3C). Growth hormone concentrations remained constant and equal in both the diabetic and nondiabetic subjects (0.82 ± 0.01 vs. 0.83 ± 0.05 $\mu\text{g/l}$) throughout the study (data not shown).

Glucose infusion rates and glucose specific activities. The glucose infusion rates required to maintain arterial glucose concentrations at ~ 9.3 mmol/l were lower ($P < 0.0001$) in the diabetic than in the nondiabetic subjects during both the low-dose (6.6 ± 0.2 vs. 24.1 ± 0.6 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and the high-dose (22.7 ± 0.3 vs. 62.1 ± 0.8 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) insulin infusions (Fig. 4A). Plasma [^3H]glucose specific activity (Fig. 4B) plateaued within 60 min of initiation of the [^3H]glucose infusion, then remained constant throughout the remainder of the experiment. Plasma [^{14}C]glucose specific activity

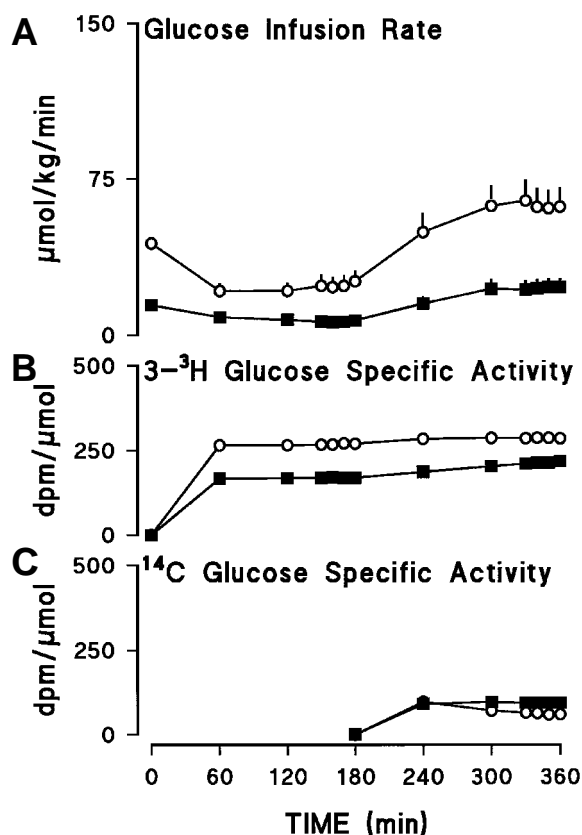


FIG. 4. The glucose infusion rate required to maintain glucose concentrations at target levels (A), plasma [$^3\text{-}^3\text{H}$]glucose specific activity (B), and plasma [^{14}C]glucose specific activity (C) observed in the diabetic (■) and nondiabetic (○) subjects during the low-dose (from 0 to 180 min) and high-dose (from 181 to 360 min) insulin infusions.

increased promptly after initiation of the [^{14}C]galactose infusion at 180 min, reaching a plateau by 300 min (Fig. 4C). Rate of EGP and R_{d} . Despite slightly higher insulin concentrations, EGP was greater ($P < 0.01$) in the diabetic than in the nondiabetic subjects during both the low-dose (12.2 ± 1.0 vs. $9.1 \pm 0.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and the high-dose (10.8 ± 1.3 vs. $5.9 \pm 0.9 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) insulin infusions (Fig. 5A). Conversely, R_{d} was lower ($P < 0.01$) in the diabetic than in the nondiabetic subjects during both the low-dose (18.9 ± 1.8 vs. $33.2 \pm 5.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and the high-dose (33.5 ± 4.0 vs. $68.0 \pm 9.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) insulin infusions (Fig. 5B). Urinary glucose losses were minimal in both the diabetic and nondiabetic subjects and did not differ between groups (0.26 ± 0.11 vs. $0.05 \pm 0.02 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$).

The increase from the low to the high insulin infusion rate was associated with a decrease ($P < 0.001$) in EGP in the nondiabetic but not the diabetic subjects. It also caused an increase ($P < 0.001$) in R_{d} in both the diabetic and nondiabetic subjects. However, the insulin-induced increment in R_{d} was smaller ($P < 0.01$) in the diabetic than in the nondiabetic subjects (14.6 ± 3.2 vs. $34.8 \pm 6.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). NSGB. NSGB is the algebraic sum of SGU and SGP. Splanchnic blood flow was slightly but not significantly higher in the diabetic than in the nondiabetic subjects during both the low-dose ($1,522 \pm 114$ vs. $1,121 \pm 99 \text{ ml}/\text{min}$) and the high-dose ($1,503 \pm 119$ vs. $1,234 \pm 108 \text{ ml}/\text{min}$) insulin infusions. Splanchnic blood flow during the low-dose insulin infusion did

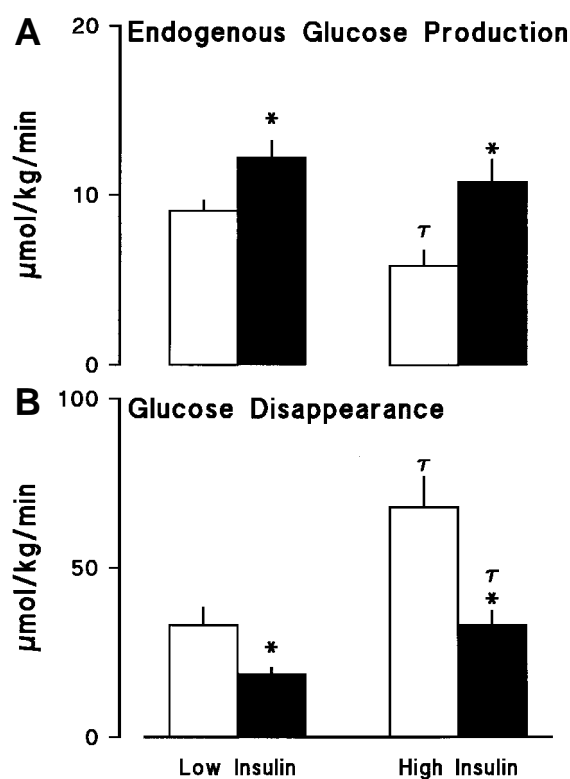


FIG. 5. Rate of EGP (A) and R_{d} (B) observed in the diabetic (■) and nondiabetic (□) subjects during the low- and high-dose insulin infusions. * $P < 0.05$ between groups, i.e., diabetic vs. nondiabetic subjects; $\tau P < 0.05$ within groups, i.e., high- vs. low-dose insulin infusion.

not differ from that during the high-dose insulin infusion in either the diabetic or the nondiabetic subjects. NSGB during the low-dose insulin infusion was negative (i.e., net production) in the diabetic subjects but did not differ from zero in the nondiabetic subjects (Fig. 6A). This resulted in lower ($P < 0.01$) NSGB in the diabetic than in the nondiabetic subjects (-7.9 ± 1.8 vs. $-0.5 \pm 1.0 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). NSGB became positive (i.e., net uptake) during the high-dose insulin infusion in both groups. However, NSGB remained lower ($P < 0.01$) in the diabetic than in the nondiabetic subjects (2.1 ± 1.2 vs. $10.3 \pm 2.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) during the high-dose insulin infusion.

SGU. SGU (Fig. 6B) was slightly lower in the diabetic than in the nondiabetic subjects during the low-dose insulin infusion (5.1 ± 1.9 vs. $7.6 \pm 1.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and significantly lower ($P < 0.05$) during the high-dose insulin infusion (7.5 ± 2.3 vs. $15.4 \pm 2.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). A similar pattern was observed for splanchnic (tracer) extraction, which was slightly lower (2.1 ± 0.9 vs. $3.7 \pm 0.6\%$) in the diabetic than in the nondiabetic subjects during the low-dose insulin infusion and significantly lower ($P < 0.05$) during the high-dose insulin infusion (4.1 ± 1 vs. $6.9 \pm 1\%$). The increase from the low- to the high-dose insulin infusion rate resulted in an increase ($P < 0.02$) in splanchnic extraction of tracer in both the diabetic and nondiabetic subjects and an increase ($P < 0.01$) in SGU in the nondiabetic subjects.

SGP. SGP (Fig. 6C) was greater ($P < 0.05$) in the diabetic than in the nondiabetic subjects during the low-dose insulin infusion (13.0 ± 1.9 vs. $8.1 \pm 1.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), but it did not

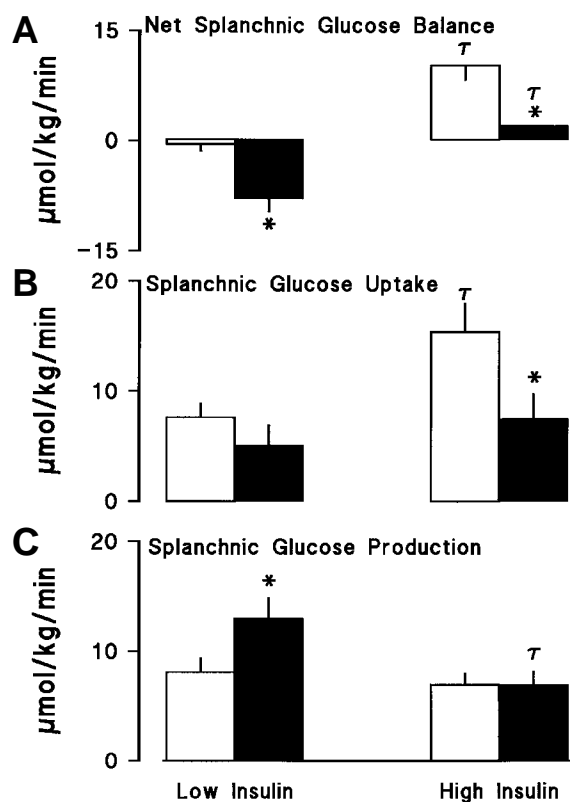


FIG. 6. NSGB (A), SGU (B), and SGP (C) observed in the diabetic (■) and nondiabetic (□) subjects during the low- and high-dose insulin infusions. Negative splanchnic balance represents net production, and positive balance represents net uptake. * $P < 0.05$ between groups, i.e., diabetic vs. nondiabetic subjects; $\tau P < 0.05$ within groups, i.e., high- vs. low-dose insulin infusion.

differ between groups during the high-dose insulin infusion (7.0 ± 1.3 vs. $6.9 \pm 1.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). The increase from the low- to the high-dose insulin infusion rate resulted in further suppression ($P < 0.01$) of glucose production in the diabetic but not in the nondiabetic subjects.

The percent contribution of glycogen to SGP was calculated by dividing the specific activity of plasma [^{14}C]glucose (i.e., the specific activity of the product) by urinary [^{14}C]acetaminophen glucuronide specific activity (i.e., the specific activity of the precursor UDP-glucose pool). The actual rate of glycogenolysis was then calculated by multiplying the percent contribution by the rate of SGP. During the high-dose insulin infusion, the plasma [^{14}C]glucose specific activity averaged 95 ± 8 and 70 ± 15 dpm/ μmol , and the urinary [^{14}C]acetaminophen glucuronide specific activity averaged $1,055 \pm 151$ and $1,056 \pm 93$ dpm/ μmol in the diabetic and nondiabetic subjects, respectively. Glycogenolysis was comparable in the diabetic and nondiabetic subjects whether calculated as the percent contribution (9.5 ± 1.6 vs. $7.7 \pm 1.7\%$) to SGP or the actual rate of glycogenolysis (0.6 ± 0.2 vs. $0.6 \pm 0.2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$).

Flux through UDP pool and contribution of direct and indirect pathways to glycogen synthesis. Flux through the UDP-glucose pool (Fig. 7) was estimated during the high-dose insulin infusion by dividing the infusion rate of [^{14}C]galactose by the [^{14}C]glucuronide specific activity in urine. UDP-glucose flux was lower ($P < 0.05$) in the diabetic

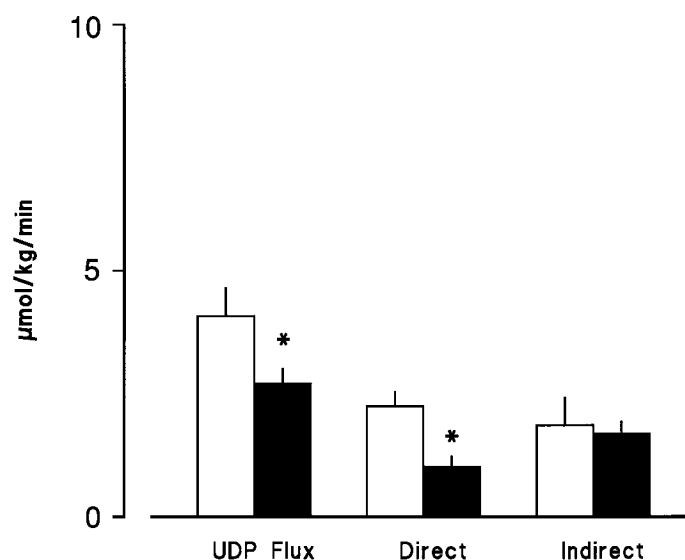


FIG. 7. Flux through the UDP-glucose pool and the contributions of the direct and indirect pathways to glycogen synthesis observed in the diabetic (■) and nondiabetic (□) subjects during the high-dose insulin infusion. * $P < 0.05$ between groups, i.e., diabetic vs. nondiabetic subjects.

than in the nondiabetic subjects (5.4 ± 0.6 vs. $8.1 \pm 1.2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). In addition, the ratio of the urinary [^3H]glucuronide specific activity to plasma [^3H]glucose specific activity was also lower ($P < 0.05$) in the diabetic than in the nondiabetic subjects (0.37 ± 0.06 vs. 0.60 ± 0.09), indicating a lower percent contribution of the direct pathway to UDP-glucose flux. This resulted in a lower ($P < 0.01$) absolute contribution of the direct pathway (2.0 ± 0.4 vs. $4.5 \pm 0.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) to UDP-glucose flux in the diabetic than in the nondiabetic subjects (Fig. 7). On the other hand, the contribution of the indirect pathway to glycogen synthesis (3.4 ± 0.5 vs. $3.7 \pm 1.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) did not differ in the diabetic and nondiabetic subjects (Fig. 7).

LGU. LGU was measured using both the tracer and arterial-venous difference techniques. Leg glucose (tracer) extraction (Fig. 8A) was lower ($P < 0.05$) in the diabetic than in the nondiabetic subjects during both the low-dose (4 ± 1 vs. $6 \pm 1\%$) and the high-dose (7 ± 1 vs. $12 \pm 2\%$) insulin infusions. Leg glucose extraction increased ($P < 0.05$) in both groups when the insulin infusion rate was increased.

Leg blood flow tended ($P = 0.2$) to be higher in the diabetic than in the nondiabetic subjects during both the low-dose (673 ± 120 vs. 454 ± 47 ml/min) and the high-dose (600 ± 128 vs. 425 ± 35 ml/min) insulin infusions. Leg blood flow during the low-dose insulin infusion did not differ from that during the high-dose insulin infusion in either the diabetic or the nondiabetic subjects. LGU (Fig. 8B) was slightly lower during the low-dose insulin infusion (14.0 ± 2.7 vs. $23.1 \pm 7.1 \mu\text{mol} \cdot \text{kg}^{-1}$ FFM leg $\cdot \text{min}^{-1}$) and significantly lower ($P < 0.01$) during the high-dose insulin infusion (18.9 ± 5.2 vs. $51.8 \pm \mu\text{mol} \cdot \text{kg}^{-1}$ FFM leg $\cdot \text{min}^{-1}$) in the diabetic than in the nondiabetic subjects. The increase from the low- to the high-dose insulin infusion rate resulted in an increase ($P < 0.01$) in LGU in the nondiabetic but not in the diabetic subjects. These results were the same when LGU was calculated as a function of the whole leg weight (obtained from DXA analysis).

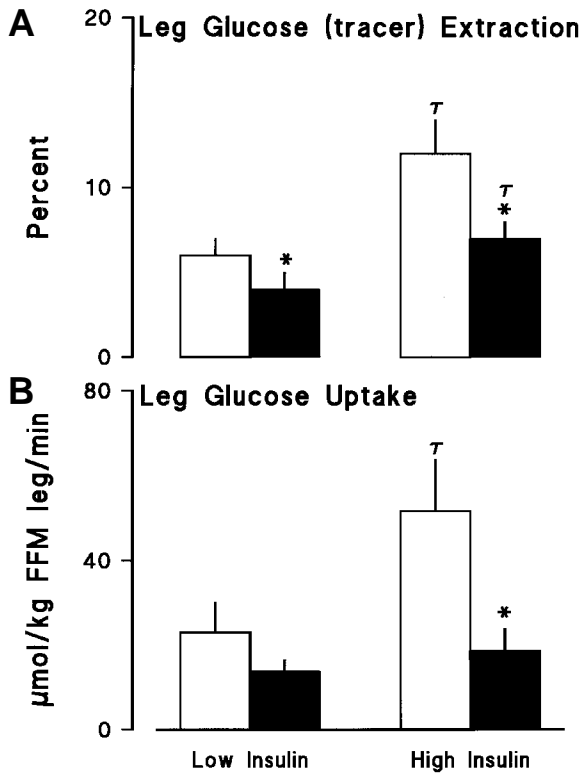


FIG. 8. Leg glucose (tracer) extraction (A) and LGU (B) observed in the diabetic (■) and nondiabetic (□) subjects during the low- and high-dose insulin infusions. * $P < 0.05$ between groups, i.e., diabetic vs. nondiabetic subjects; ** $P < 0.05$ within groups, i.e., high- vs. low-dose insulin infusion.

Correlation between visceral adiposity and glucose uptake or production. Degree of adiposity, particularly in the visceral region, has been reported to be inversely correlated with insulin action (56–60). To determine whether this relationship differs in splanchnic and extrasplanchnic tissues, we examined the extent to which glucose disappearance, LGU, SGU, and SGP correlated with visceral adiposity during the high-dose insulin infusion. Both total body glucose disappearance ($P < 0.001$) and LGU ($P < 0.001$) were inversely correlated with visceral adiposity (Fig. 9A and C). On the other hand, SGU, SGP (Fig. 9B and D), and rates of glycogenolysis (data not shown) were not correlated with visceral adiposity. Total body glucose disappearance ($r = -0.5$; $P < 0.01$), but not LGU ($r = -0.3$; $P = 0.15$), was also inversely correlated with visceral adiposity during the low-dose insulin infusion. Of interest, there was a strong correlation between whole body glucose disappearance and LGU during both the low-dose ($r = 0.8$; $P < 0.0001$) and high-dose ($r = 0.95$; $P < 0.0001$) insulin infusions. In contrast, there was no correlation between whole body glucose disappearance and SGU during either the low-dose ($r = -0.02$; $P = 0.7$) or the high-dose ($r = 0.3$; $P = 0.2$) insulin infusions.

Palmitate concentrations. The plasma palmitate concentration was higher ($P < 0.02$) in the diabetic than in the nondiabetic subjects at baseline (174.8 ± 14.9 vs. $131.4 \pm 8.4 \mu\text{mol/l}$) as well as during the low-dose (78.4 ± 16.1 vs. $35.7 \pm 8.6 \mu\text{mol/l}$) and high-dose (37.7 ± 11.5 vs. $13.1 \pm 1.9 \mu\text{mol/l}$) insulin infusions. The percent decline from baseline in the palmitate concentrations did not differ during the low-dose insulin infusion rate (61.8 ± 5.1 vs. $73.5 \pm 5.5\%$) but was less in the diabetic than in the nondiabetic

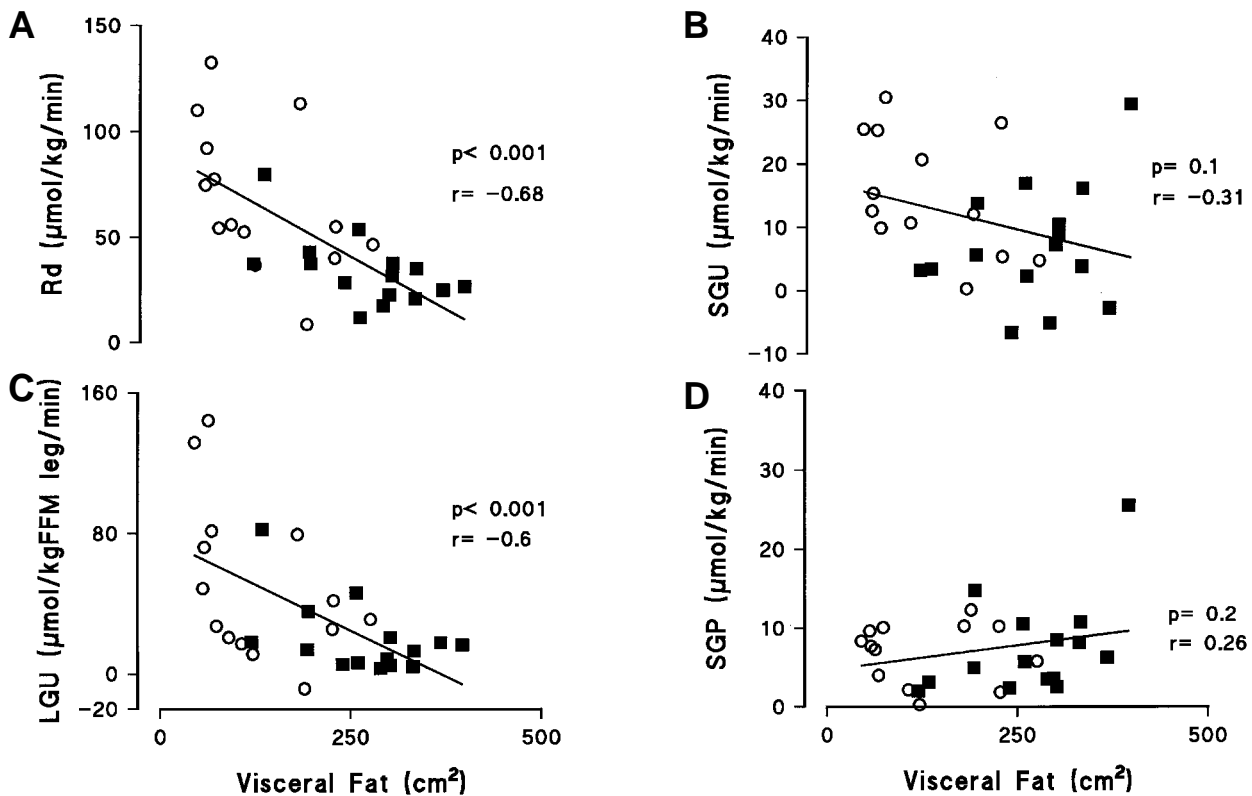


FIG. 9. Correlation between the amount of visceral fat and R_d (A), SGU (B), LGU (C), and SGP (D) observed in the diabetic (■) and nondiabetic (○) subjects during the high-dose insulin infusion.

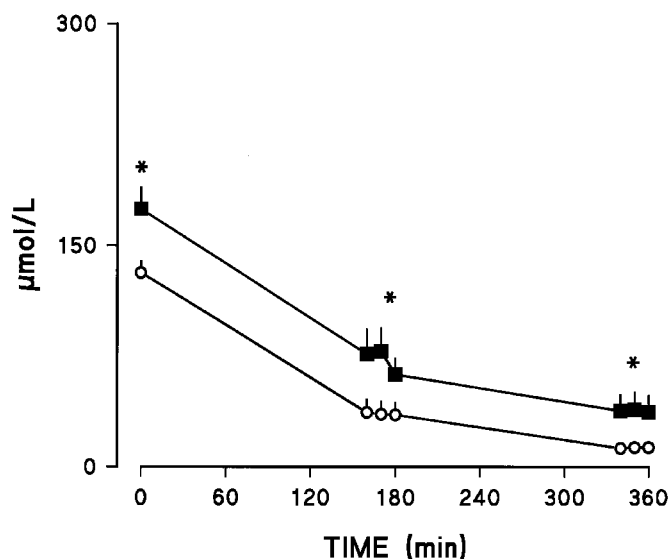


FIG. 10. Palmitate concentrations observed in the diabetic (■) and nondiabetic (○) subjects at baseline and during the low- and high-dose insulin infusions. * $P < 0.05$ between groups, i.e., diabetic vs. nondiabetic subjects.

subjects (80.5 ± 4.0 vs. $90.3 \pm 1.1\%$; $P < 0.05$) during the high-dose insulin infusion (Fig. 10)

DISCUSSION

It is well established that type 2 diabetes impairs insulin-induced stimulation of MGU (1–9). In contrast, the effect of type 2 diabetes on HGU has been controversial (2,15,16). The present studies resolve this controversy by measuring LGU and SGU in diabetic and nondiabetic subjects in the presence of comparable but elevated glucose and insulin concentrations. These conditions were chosen because they are known to result in concurrent stimulation of MGU and liver glucose uptake in nondiabetic humans (27,28). In addition, they mimic those commonly observed in diabetic individuals between meals and during the night (61–64). Under these conditions, whole body glucose disposal was lower and EGP was higher in the diabetic than in the nondiabetic subjects. The lower rates of disposal were due to a 50–60% decrease in both SGU and LGU. The degree of visceral adiposity was inversely correlated with LGU but not SGU, implying a differing effect of obesity on muscle and liver glucose metabolism. Both the flux through the UDP-glucose pool and the contribution of the direct pathway to glycogen synthesis were also decreased in the diabetic subjects, indicating a decrease in HGU and/or phosphorylation of extracellular glucose. On the other hand, hyperglycemia and modest hyperinsulinemia resulted in comparable suppression of SGP and net glycogenolysis in the diabetic and nondiabetic subjects. Taken together, these data indicate that type 2 diabetes impairs the ability of insulin and glucose to stimulate both MGU and SGU. They also strongly suggest that defects in HGU are likely to contribute to hyperglycemia in people with type 2 diabetes.

Effects of type 2 diabetes on SGU and SGP. In nondiabetic humans, an increase in glucose concentration is invariably accompanied by an increase in insulin concentration, cre-

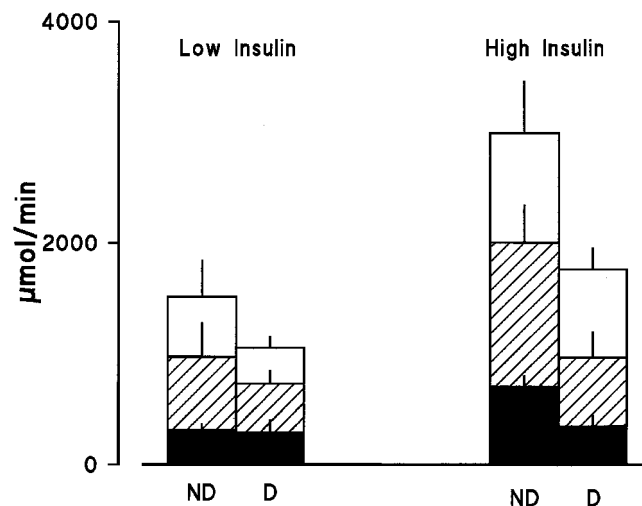


FIG. 11. The relative contributions of MGU (▨), SGU (■), and other (□) to whole body glucose disappearance (total height of the bars) observed in the diabetic (D) and nondiabetic (ND) subjects during the low- and high-dose insulin infusions.

ating a metabolic milieu that stimulates HGU and suppresses hepatic glucose production (1,2,27–29,49). In the present studies, glucose concentrations were clamped at ~ 9.3 mmol (~ 165 mg/dl), since previous studies have demonstrated that insulin-induced stimulation of whole body glucose uptake is impaired at this level in people with type 2 diabetes (11,14). Hyperglycemia of this degree in the presence of low insulin concentrations (~ 70 pmol/l) was sufficient to create an NSGB of zero in the nondiabetic subjects, indicating that net SGU and release were equal. In contrast, the diabetic subjects continued to have a negative NSGB, indicating that the liver was releasing more glucose than it was taking up. These differences are particularly noteworthy in view of the fact that both glucose and insulin were higher in the diabetic than in the nondiabetic subjects during the low-dose insulin infusion. Because the same amounts of insulin were infused in both groups, the higher insulin levels in the diabetic subjects likely reflect a decrease in insulin clearance. The higher glucose concentrations in the diabetic subjects resulted from the fact that once glucose was acutely increased to ~ 165 mg/dl, very little additional glucose was required to maintain this level. It was therefore difficult to lower the glucose concentration if it drifted above target. Presumably, the difference in splanchnic glucose balance between the diabetic and nondiabetic subjects would have been even greater if the glucose and insulin concentrations had been better matched.

The increase in insulin concentration that occurred when the insulin infusion was increased from the lower to the higher rate (i.e., ~ 70 to ~ 140 pmol/l) doubled SGU in the nondiabetic subjects, thereby creating a net positive splanchnic glucose balance. This synergistic effect of glucose and insulin on SGU is consistent with previous reports in both animals and humans (27,28,30). In contrast, the increase in insulin failed to increase SGU in the diabetic subjects and minimally increased splanchnic glucose (tracer) extraction. A statistically significant increase in splanchnic glucose extraction but not uptake presumably was due to the variability intro-

duced into the latter by measurement of splanchnic blood flow. The lower rate of SGU observed in the present experiments in diabetic humans is consistent with reports of decreased rates of glycogen synthesis in animal models of diabetes (65–67). Because those animals did not have a genetic form of diabetes, and since their defects in glycogen synthesis could be reversed by treatment with insulin (65–67), it is likely that the decreased HGU observed in those and in the present experiments is due to the metabolic milieu associated with diabetes (e.g., chronic hyperglycemia). In light of this, it should be noted that the diabetic subjects were infused with insulin during the night to avoid uncertainty that could potentially be introduced by marked differences in glucose concentrations on the morning of study. We have previously shown that overnight infusion with insulin improves insulin-induced suppression of EGP in people with type 2 diabetes (68). If it also improves HGU, then the present experimental design likely underestimated the severity of the defect that would be present in diabetic subjects whose glucose concentrations are not as well controlled during the night.

Of interest, the increase in the insulin infusion from low to high resulted in further suppression of SGP in the diabetic subjects but no further suppression of EGP. Because EGP measures all glucose entering the systemic circulation, whereas SGP measures only that released by the liver, this discrepancy implies a difference in the source of glucose production between the two groups. There is currently considerable debate regarding the relative contributions of the kidney and the liver to EGP (69–71). Meyer et al. (70) have recently reported that renal glucose production after an overnight fast is increased in people with type 2 diabetes. In the present experiments, EGP did not exceed SGP in either the diabetic (12.2 ± 1.0 vs. $13.0 \pm 1.9 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) or the nondiabetic (9.1 ± 0.6 vs. $8.1 \pm 1.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) subjects during the low-dose insulin infusion. Although EGP in the nondiabetic subjects fell slightly more during the high-dose insulin infusion than did SGP (accounting for further suppression of the former but not the latter), there continued to be no statistically significant difference between the two rates (5.9 ± 0.9 vs. $6.9 \pm 1.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively). On the other hand, EGP was higher than SGP in 11, equal in 1, and lower in 4 of the diabetic subjects during the high-dose insulin infusion (10.8 ± 1.3 vs. $7.0 \pm 1.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). This finding implies (but does not prove) that under the present experimental conditions, type 2 diabetes altered the relative contribution of the liver and kidney to EGP. Future studies in which glucose production is simultaneously measured across the renal and splanchnic beds will be required to specifically answer this question. Furthermore, it should be borne in mind that glucose entered the splanchnic bed in the present experiments solely via the arterial circulation, mimicking the situation that occurs in diabetic individuals between meals and during the night. Additional studies will be required to determine whether comparable defects in SGU are also present when glucose enters the splanchnic bed via the enteral route, as normally occurs after food ingestion. Effects of type 2 diabetes on intrahepatic glucose metabolism. Glycogen synthesis can occur via direct or indirect pathways (72). The direct pathway refers to synthesis of glycogen from glucose-6-phosphate (G-6-P) that is derived from extracellular glucose, whereas the indirect pathway refers to synthesis of glycogen from G-6-P that is gen-

erated by the gluconeogenic pathway. In both instances, G-6-P can successively be converted to glucose-1-phosphate, then UDP-glucose, then glycogen. Because glucose derived from plasma must first be phosphorylated, a decrease in hepatic glucokinase activity will decrease flux through the direct pathway but will not alter flux through the indirect pathway. On the other hand, a decrease in conversion of UDP-glucose to glycogen because of impaired glycogen synthase activity would proportionately decrease flux through both the direct and indirect pathways.

The present experiments used an infusion of [^{14}C]galactose in combination with the acetaminophen glucuronide method to distinguish between these two possibilities. As discussed in detail elsewhere (36–42,73), glucuronidation appears to occur primarily within the liver. Therefore, measurement of acetaminophen glucuronide specific activity provides a non-invasive assessment of the intrahepatic UDP-glucose pool, whereas infusion of [^{14}C]galactose provides a means of labeling the UDP-glucose pool. UDP-glucose flux was only measured during the steady-state portion (i.e., the final 2 h) of the high-dose insulin infusion. Because the half-time of excretion of urinary acetaminophen glucuronide is only 20 min (39), we also could have measured UDP-glucose flux during the low-dose insulin infusion. However, we elected not to in order to avoid potential confounding if a portion of glucuronide formed during the low-dose infusion were secreted during the high-dose insulin infusion. UDP-glucose flux and percent contribution of the direct pathway during the high-dose insulin infusion averaged $8.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and 60%, respectively. These values are virtually identical to those observed under similar experimental conditions by Hellerstein et al. (37) in nondiabetic humans and by Rother et al. (42) in nondiabetic dogs. In contrast, both UDP-glucose flux ($5.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and percent contribution of the direct pathway (37%) were decreased in the diabetic patients. On the other hand, although the percent contribution of the indirect pathway to glycogen synthesis was greater in the diabetic than in the nondiabetic subjects (63 vs. 40%), the absolute contribution of the indirect pathway to UDP-glucose flux was the same (3.4 vs. $3.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). The proportionate decrease (~35%) in both UDP-glucose flux and percent contribution of extracellular glucose to glycogen synthesis combined with no change in the contribution of the indirect (intracellular) pathway strongly implies a defect in glucose transport and/or phosphorylation.

Several lines of evidence suggest that this defect likely resides at the level of glucokinase. In an elegant series of investigations, Velho et al. (74) demonstrated a similar pattern of change in hepatic glucose metabolism in people who have an autosomal dominant defect in glucokinase activity (commonly referred to as maturity-onset diabetes of the young type 2). Furthermore, hyperglycemia in animals has been shown to decrease glucokinase activity while increasing GLUT2 transporter number; both abnormalities can be reversed by treatment with insulin (75,76). Perhaps most intriguing, Caro et al. (77) reported that hepatic glucokinase activity is decreased in liver biopsies obtained from severely obese diabetic patients at the time of bariatric surgery. Finally, glucokinase activity rather than glucose transport appears to be rate limiting for liver glucose uptake (20,21,40,78).

Net glycogen accumulation is determined by the balance between glycogen synthesis and glycogen breakdown. The

present data add to the growing body of evidence showing that glycogen synthesis and breakdown occur concurrently (27,35,54,79,80). Both the diabetic and nondiabetic subjects continued to release [^{14}C]glucose into the hepatic vein during the high-dose insulin infusion. However, the percent contribution of glycogenolysis to SGP (~9%) was quite small and did not differ between groups. That SGP was the same in both groups implies that gluconeogenesis was also equal in both groups. These results are consistent with those previously reported by Rossetti et al. (55) in diabetic rats. The divergence in the diabetic subjects between normal suppression of SGP and glycogenolysis versus impaired stimulation of SGU and glycogen synthesis (as reflected by decreased flux through the UDP-glucose pool) is intriguing in that it implies differential regulation of these processes. An impaired hepatic response to insulin but not to glucose could readily explain these data and would be consistent with previous observations showing that while hyperglycemia is the primary regulator of glycogenolysis, insulin (in the presence of hyperglycemia) is the primary regulator of both glucokinase and hepatic glycogen synthase activity (23,26,27,55,81). If so, then agents that improve hepatic insulin action have the potential to fully normalize splanchnic glucose metabolism in people with type 2 diabetes.

Effects of type 2 diabetes on the relative contribution of liver and muscle to whole body glucose uptake. LGU during the high-dose insulin infusion was reduced by ~60% and SGU by ~50% in the diabetic subjects relative to the nondiabetic subjects. Both LGU and SGU were also lower in the diabetic than in the nondiabetic subjects during the low-dose insulin infusion; however, the differences did not reach statistical significance. In an effort to gain insight into how changes in LGU could affect total body glucose uptake, both leg muscle mass and appendicular muscle mass (defined as the sum of leg and arm muscle mass) were measured in each subject using DXA. Because appendicular muscle mass has previously been shown to equal ~79% of total body muscle mass (82), total MGU was calculated by dividing appendicular glucose uptake by 0.79. Total body glucose disappearance (tracer-determined) during the high-dose insulin infusion was ~1,240 $\mu\text{mol}/\text{min}$ lower in the diabetic than in the nondiabetic subjects (Fig. 11). Assuming that LGU during the high-dose insulin infusion primarily reflects uptake by skeletal muscle, MGU averaged ~600 and ~1,300 $\mu\text{mol}/\text{min}$ in the diabetic and nondiabetic subjects, respectively. On the other hand, SGU averaged ~350 and ~700 $\mu\text{mol}/\text{min}$, respectively. This means that MGU was ~700 $\mu\text{mol}/\text{min}$ lower in the diabetic than in the nondiabetic subjects, accounting for ~55% of the 1,240 $\mu\text{mol}/\text{min}$ decrease in total body glucose disappearance. In contrast, SGU was ~350 $\mu\text{mol}/\text{min}$ lower in the diabetic than in the nondiabetic subjects, accounting for ~30% of the decrease in total body glucose disappearance. Thus, impaired glucose uptake in muscle and liver accounted almost entirely (~85%) for the decrease in total body glucose disappearance, with a defect in the former making an approximately twofold greater contribution than the latter. Note, however, that these relative contributions of muscle and liver to total body glucose uptake likely are dependent on the prevailing insulin level. Because the insulin dose-response curve for HGU in diabetic and nondiabetic humans is not known, future studies will be required to

determine whether the same relative impairment in MGU and SGU persists at higher insulin concentrations.

There is increasing evidence that the degree of visceral obesity is an important determinant of insulin action (56–60). Previous studies have shown that whole body glucose disposal in the presence of euglycemia is inversely correlated with visceral fat mass in both diabetic and nondiabetic subjects, implying an effect on MGU (56–60). The present study establishes that this relationship also holds in the presence of hyperglycemia. It also directly implicates muscle, since LGU was inversely correlated ($P < 0.001$) with visceral adiposity. However, neither SGU nor SGP correlated with the degree of visceral adiposity. This is somewhat surprising, since release of free fatty acids (FFAs) by visceral adipose depots into the portal venous system presumably would result in exposure to higher FFA concentrations in the liver than in muscle. There are several possible explanations for this apparent paradox. The first may relate to the conditions of the experiment. The elevated insulin and glucose concentrations may have resulted in near-complete suppression of visceral lipolysis but may not have altered lipolysis of intramuscular fat (56,83). Although we cannot rule this possibility out, the observation by Meek et al. (45) that visceral lipolysis in nondiabetic humans is more resistant to insulin than leg lipolysis speaks against this possibility. Alternatively, FFAs may exert different effects on MGU and liver glucose uptake. Several investigators (84–86) have shown that experimentally induced increases in FFA concentrations impair muscle glucose transport and/or phosphorylation. Because uptake and phosphorylation occur via GLUT4 transporters and hexokinase, respectively, in muscle and via GLUT2 transporters and glucokinase in liver (76,78,81,87,88), a different effect of FFAs on glucose metabolism would not be surprising. Finally, SGU and SGP in 1 diabetic subject appeared to differ markedly from that observed in the other 15 subjects (Fig. 9B and D). If data from this subject are excluded, the correlation between SGU and visceral fat becomes significant ($r = -0.5$; $P = 0.008$), whereas the relationship to SGP does not change ($r = 0.01$; $P = 0.9$). In either case, the present data emphasize the heterogeneity of response between splanchnic and extrasplanchnic tissues, adding further caution to the extrapolation of the response in one tissue to the other.

Summary and conclusions. The present studies demonstrate that in the presence of comparable but elevated glucose concentrations (~9.3 mmol/l) and modestly elevated insulin concentrations (~150 pmol/l), people with type 2 diabetes have lower rates of both SGU and LGU than do nondiabetic subjects. Rates of glucose uptake in the leg but not the splanchnic bed were inversely correlated with the degree of visceral adiposity. Flux through the UDP-glucose pool and the contribution of extracellular glucose to glycogen synthesis both were lower in the diabetic than in the nondiabetic subjects, implying a defect in hepatic glucokinase activity. On the other hand, the contribution of the indirect pathway to glycogen synthesis was equal in the two groups. Although EGP was higher in the diabetic than in the nondiabetic subjects, SGP and glycogenolysis did not differ between groups. Taken together, these data strongly suggest that defects in the ability of insulin to regulate both MGU and liver glucose uptake contribute to hyperglycemia in people with type 2 diabetes. Presumably, therapies that correct both abnormalities will be more effective than those that correct only one.

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