

# Insulin Dose-Response Curves for Stimulation of Splanchnic Glucose Uptake and Suppression of Endogenous Glucose Production Differ in Nondiabetic Humans and Are Abnormal in People With Type 2 Diabetes

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To determine whether the insulin dose-response curves for suppression of endogenous glucose production (EGP) and stimulation of splanchnic glucose uptake (SGU) differ in nondiabetic humans and are abnormal in type 2 diabetes, 14 nondiabetic and 12 diabetic subjects were studied. Glucose was clamped at ~9.5 mmol/l and endogenous hormone secretion inhibited by somatostatin, while glucagon and growth hormone were replaced by an exogenous infusion. Insulin was progressively increased from ~150 to ~350 and ~700 pmol/l by means of an exogenous insulin infusion, while EGP, SGU, and leg glucose uptake (LGU) were measured using the splanchnic and leg catheterization methods, combined with a [<sup>3</sup>-<sup>3</sup>H]glucose infusion. In nondiabetic subjects, an increase in insulin from ~150 to ~350 pmol/l resulted in maximal suppression of EGP, whereas SGU continued to increase ( $P < 0.001$ ) when insulin was increased to ~700 pmol/l. In contrast, EGP progressively decreased ( $P < 0.001$ ) and SGU progressively increased ( $P < 0.001$ ) in the diabetic subjects as insulin increased from ~150 to ~700 pmol/l. Although EGP was higher ( $P < 0.01$ ) in the diabetic than nondiabetic subjects only at the lowest insulin concentration, SGU was lower ( $P < 0.01$ ) in the diabetic subjects at all insulin concentrations tested. On the other hand, in contrast to LGU and overall glucose disposal, the increment in SGU in response to both increments in insulin did not differ in the diabetic and nondiabetic subjects, implying a right shifted but parallel dose-response curve. These data indicate that the dose-response curves for suppression of glucose production and stimulation of glucose uptake differ in nondiabetic subjects and are abnormal in people with type 2 diabetes. Taken together, these data also suggest that agents that enhance SGU in diabetic patients (e.g. glucokinase activators) are likely to improve glucose tolerance. *Diabetes* 53:2042–2050, 2004

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EGP, endogenous glucose production; LGU, leg glucose uptake; SGU, splanchnic glucose uptake.

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**T**ype 2 diabetes is associated with hepatic and extrahepatic insulin resistance (1–5). Hepatic insulin resistance is characterized by both excessive hepatic glucose production and decreased hepatic glucose uptake (2,3,6–15). Impaired suppression of glucose production is most evident at low insulin concentrations (less than ~300 pmol/l), with normal suppression eventually being observed at insulin concentrations (~600 pmol/l) that are commonly observed in the portal vein of nondiabetic individuals (1–3,6,13–15). However, the insulin dose-response curve for stimulation of hepatic glucose uptake has not been as extensively studied. It also is not known whether defects in hepatic glucose uptake evident in people with type 2 diabetes at low insulin concentrations (6,7) resolve at high insulin concentrations. If so, this would imply that the origin of the defect (e.g. decreased glucokinase activity) is relative rather than absolute.

In one of the earliest studies examining the effects of insulin on splanchnic glucose uptake, Ferrannini et al. (8) reported that neither splanchnic glucose uptake (SGU) nor splanchnic (tracer) glucose extraction increased in nondiabetic volunteers when plasma insulin concentrations were increased from ~330 to ~1,200 pmol/l. However, the number of subjects studied at high insulin concentrations was small ( $n = 2$ ). In subsequent experiments, DeFronzo et al. (16) reported that SGU in nondiabetic subjects remained unchanged when insulin was increased from ~220 to ~7,200 pmol/l. The effects of insulin also may not have been detected in those experiments (16) because glucose was clamped at euglycemic levels, thereby limiting hepatic glucose uptake and, with the exception of the ~600 pmol/l group ( $n = 6$ ), the number of subjects studied at the other insulin concentration ( $n = 2-4$ ) was also small. In contrast, Meyers et al. (17) reported that both hepatic glucose uptake and fractional hepatic glucose extraction increased in nondiabetic dogs as insulin increased from ~200 to ~1,200 pmol/l. In addition, Petersen et al. (18) demonstrated that in the presence of hyperglycemia and glucagon deficiency, glycogen synthesis from extracellular glucose (and therefore presumably hepatic glucose uptake) increased in nondiabetic subjects when

insulin concentrations were increased from ~40 to 400 pmol/l, implying a concurrent increase in hepatic glucose uptake. More recently, we observed (6) that SGU, while lower in type 2 diabetic than nondiabetic subjects, increased in both groups when insulin was increased from ~70 to ~140 pmol/l. On the other hand, in a separate series of experiments (7) performed at higher insulin concentrations (~300 pmol/l), the difference in SGU between the diabetic and nondiabetic subjects appeared to narrow, suggesting that the dose-response relationship differed in the two groups. However, comparison of rates of SGU in those studies is difficult because the route of glucose delivery (intravenous versus intraduodenal) and the insulin concentrations differed.

There are theoretical reasons why the shape of the dose-response curves for insulin-induced suppression of glucose production and stimulation of SGU may not be the same and may differ in diabetic and nondiabetic individuals. Insulin decreases glucose production by both direct and indirect mechanisms (19,20). Low insulin concentrations inhibit glycogenolysis, whereas higher insulin concentrations are required to decrease gluconeogenesis (11,21–23). The resultant decrease in intrahepatic glucose-6-phosphate concentrations and possibly glucose-6-phosphatase activity results in a decrease in hepatic glucose release (18,24). In contrast, in the absence of hyperglycemia, insulin has only a minimal effect on hepatic glucose uptake (18). However, in the presence of hyperglycemia, insulin can increase hepatic glucose uptake, at least in part by increasing glucokinase activity, intrahepatic glucose-6-phosphate concentrations, and hepatic glycogen synthetase activity (18,24,25). Diabetes has been shown to be associated with defects in essentially all of the processes that regulate hepatic glucose uptake and hepatic glucose production (10,25–29). It therefore would not be surprising if the insulin dose-response curves for the suppression of glucose production and stimulation of SGU differed in nondiabetic humans and were abnormal in people with type 2 diabetes.

The present experiments sought to address these questions by using the hepatic catheterization technique in combination with the tracer dilution method to concurrently measure glucose production and SGU in both diabetic and nondiabetic subjects. Glucose concentrations were clamped at ~9.5 mmol/l in both diabetic and nondiabetic subjects, while insulin concentrations were increased from ~150 to ~700 pmol/l to concurrently stimulate hepatic and muscle glucose uptake. Endogenous hormone secretion was inhibited with somatostatin, and glucagon was replaced to ensure that portal insulin and glucagon concentrations were the same in both groups. We report that although the shapes of the dose-response curves differ, insulin concentrations spanning the physiologic range inhibit endogenous glucose production (EGP) and stimulate SGU in both diabetic and nondiabetic humans. However, although increases in insulin concentrations resulted in a progressive increase in SGU in both groups, rates remained lower in the diabetic than nondiabetic subjects at all insulin concentrations tested. These data indicate that defects in the ability of insulin to stimulate hepatic glucose uptake likely contribute to hyperglycemia in people with type 2 diabetes. They also add

further support to the concept that the mechanisms by which insulin regulates hepatic glucose uptake and production differ in diabetic and nondiabetic humans.

## RESEARCH DESIGN AND METHODS

After approval from the Mayo Institutional Review Board, 14 nondiabetic subjects and 12 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 parents, siblings, and children (first-degree relatives) of the nondiabetic subjects had a history of diabetes. At the time of screening, two of the diabetic subjects were being treated with lifestyle modifications alone and seven with either a sulfonylurea or metformin. One subject was on insulin alone, and two were on both insulin and metformin. Oral hypoglycemic agents were discontinued 3 weeks prior to study and long-acting insulin switched to regular insulin with meals, beginning with the midday meal 2 days prior to study. Subjects were on no medications at the time of the study other than either thyroxine or hormone 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 prior to the day of study. Diabetic and nondiabetic subjects, respectively, were matched for age ( $65 \pm 1$  vs.  $63 \pm 2$  years), sex (6/6 vs. 7/7 men/women), total body weight ( $89 \pm 4.0$  vs.  $86 \pm 3.3$  kg), BMI ( $32.1 \pm 1.2$  vs.  $30.1 \pm 1.3$  kg/m<sup>2</sup>), and body fat ( $39.1 \pm 3.0$  vs.  $34.2 \pm 2.5\%$ ). Fasting plasma glucose ( $8.0 \pm 0.3$  vs.  $5.3 \pm 0.1$  mmol/l) and HbA<sub>1c</sub> ( $9.1 \pm 1.0$  vs.  $5.2 \pm 1.0\%$ ), measured at the time of screening, were significantly ( $P < 0.001$ ) higher in diabetic versus nondiabetic subjects.

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 (100 units regular human insulin in 1 l of 0.9% saline containing 5 ml of 25% human albumin) was started in the diabetic subjects or 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 (30). In addition, 0.9% saline along with 20 mEq of KCl per l was infused at 60 ml/min throughout the night in the diabetic subjects and during the insulin clamp in both the nondiabetic and diabetic subjects.

At 0600 on the morning after admission, a urinary bladder catheter was placed, after which the subjects were taken to an interventional radiology suite where femoral artery, femoral venous, and hepatic venous catheters were placed as previously described (6,7). The arterial catheter was used for blood sampling, and the arterial sheath was used for indocyanine green infusion (Akorn, Buffalo Grove, IL). The venous catheters were used for blood sampling. Although, the experimental design is similar to that previously reported by us (6,7), none of the subjects in the present experiments participated in those studies.

Subjects were then returned to the General Clinical Research Center, where at ~0900, a primed-continuous infusion of [<sup>3</sup>H] glucose (12 μCi primed, 0.12 μCi/min continuous; New England Nuclear, Boston, MA) into a forearm vein was started. Infusions of somatostatin (60 ng · kg<sup>-1</sup> · min<sup>-1</sup>), growth hormone (3 ng · kg<sup>-1</sup> · min<sup>-1</sup>), and glucagon (0.65 ng · kg<sup>-1</sup> · min<sup>-1</sup>) were also started ( $t = 0$  min) and continued until the end of the study. Insulin was infused at a rate of 0.78 mU · kg lean body wt<sup>-1</sup> · min<sup>-1</sup> (~0.5 mU · kg total body wt<sup>-1</sup> · min<sup>-1</sup>) from 0 to 180 min, 1.56 (~1.0) from 181 to 300 min, and 3.1 (~2.0) from 301 to 420 min. A dextrose infusion also was begun and the rate adjusted so as to maintain plasma glucose concentrations at ~9.3 mmol/l (~165 mg/dl) over the next 7 h of study. All infused glucose contained [<sup>3</sup>H] glucose to minimize the change in plasma glucose specific activity (31,32). In addition, the rate of the "basal" [<sup>3</sup>H] glucose infusion also was reduced to mimic the anticipated changes in EGP (33).

**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 (34). 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 (RIA; Linco Research, St. Louis, MO). Growth hormone was measured with the Access hGH two-site immunoassay (Beckman). Body composition, including percentage of fat and lean body mass, were measured using dual-energy X-ray absorptiometry (DPX-IQ scanner, SmartScan version 4.6; Hologic, Waltham, MA).

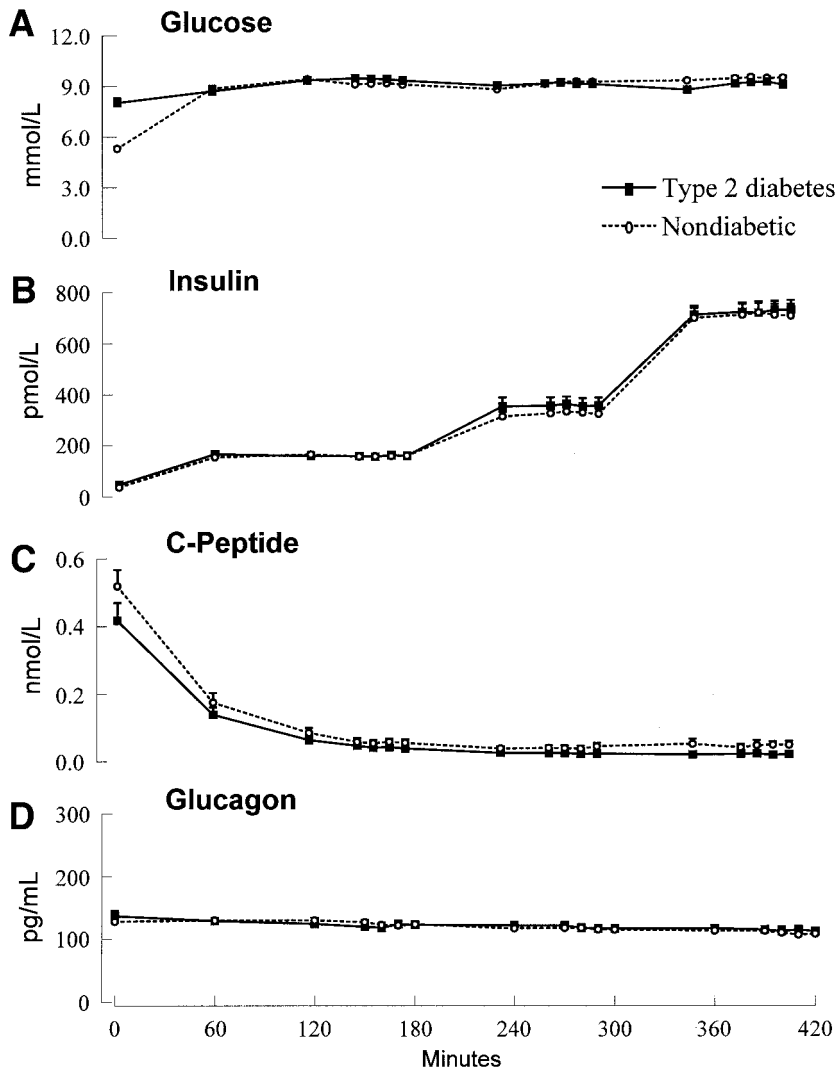


FIG. 1. Plasma glucose (A), insulin (B), C-peptide (C), and glucagon (D) concentrations observed in the diabetic and nondiabetic subjects prior to and during a hyperglycemic clamp. The insulin, somatostatin, glucagon, and growth hormone infusions started at time 0.

**Calculations.** Splanchnic plasma flow was calculated by dividing the indocyanine green infusion rate by the arterial-hepatic venous concentration gradient of the dye, and leg plasma flow was calculated by dividing the dye infusion rate by the concentration gradient across the leg (34,35). The corresponding blood flows were calculated by dividing the respective plasma flow by  $(1 - \text{hematocrit})$ . Blood glucose concentrations were calculated by multiplying the plasma glucose concentrations by 0.85. Net splanchnic glucose balance was calculated as the product of the arterial-hepatic vein glucose difference and the median of quadruple determinations of splanchnic blood flow. The splanchnic-to-glucose extraction ratio was calculated as the difference of arterial and hepatic venous  $[3\text{-}^3\text{H}]$  glucose concentrations divided by the arterial  $[3\text{-}^3\text{H}]$  glucose concentration. SGU was determined by multiplying the arterial glucose concentration by splanchnic-to-glucose extraction ratio and splanchnic blood flow. Leg glucose uptake (LGU) was calculated as the difference between the femoral arterial and the femoral venous glucose concentration times the median of quadruple determinations of leg blood flow. The leg-to-glucose extraction ratio was calculated as the difference in arterial and femoral venous  $[3\text{-}^3\text{H}]$  glucose concentration divided by the arterial  $[3\text{-}^3\text{H}]$  glucose concentration. Rates of glucose appearance ( $R_a$ ) and disappearance ( $R_d$ ) were calculated using the steady-state equations of Steele et al. (36). EGP was determined by subtracting the glucose infusion rate from the tracer-determined rate of glucose appearance.

**Statistical analysis.** Data in the text and figures are expressed as mean  $\pm$  SE. All rates (including SGU and LGU) are expressed as micromoles per kilogram of lean body mass per minute. Responses during the three dose insulin infusions were determined by meaning the results present, respectively, from 150 to 180 min, 270 to 300 min, and 390 to 420 min. Student's unpaired *t* test was used to compare results between groups (e.g. diabetic versus nondiabetic subjects), and ANOVA was used to compare results within a group (e.g. low- versus mid- versus high-dose insulin infusion), followed by

Student's paired *t* test wherever appropriate.  $P < 0.05$  was considered statistically significant.

## RESULTS

**Plasma glucose, insulin, C-peptide, and glucagon concentrations.** Plasma glucose concentrations were higher ( $P < 0.001$ ) in the diabetic than in the nondiabetic subjects (Fig. 1A) prior to the clamp ( $8.0 \pm 0.3$  vs.  $5.5 \pm 0.1$  mmol/l), but did not differ during the clamp ( $9.3 \pm 0.1$  vs.  $9.3 \pm 0.1$  mmol/l). Plasma insulin concentrations were slightly but not significantly higher in the diabetic than in the nondiabetic subjects prior to the clamp ( $48 \pm 6$  vs.  $39 \pm 5$  pmol/l) and did not differ during either the low ( $161 \pm 8$  vs.  $158 \pm 10$  pmol/l)-, middle ( $358 \pm 31$  vs.  $326 \pm 20$ )-, or high ( $727 \pm 37$  vs.  $711 \pm 46$ )-dose insulin infusions (Fig. 1B). Plasma C-peptide concentrations were slightly but not significantly lower in the diabetic than in the nondiabetic subjects prior to the clamp ( $0.42 \pm 0.05$  vs.  $0.52 \pm 0.04$ ) and were comparably suppressed to almost undetectable levels in both groups (Fig. 1C). Plasma glucagon concentrations (Fig. 1D) did not differ in the diabetic subjects compared with the nondiabetic subjects either prior to ( $137 \pm 9$  vs.  $128 \pm 5$  pg/ml) or during the clamp ( $118 \pm 5$  vs.  $117 \pm 3$ ). Plasma growth hormone concentrations also

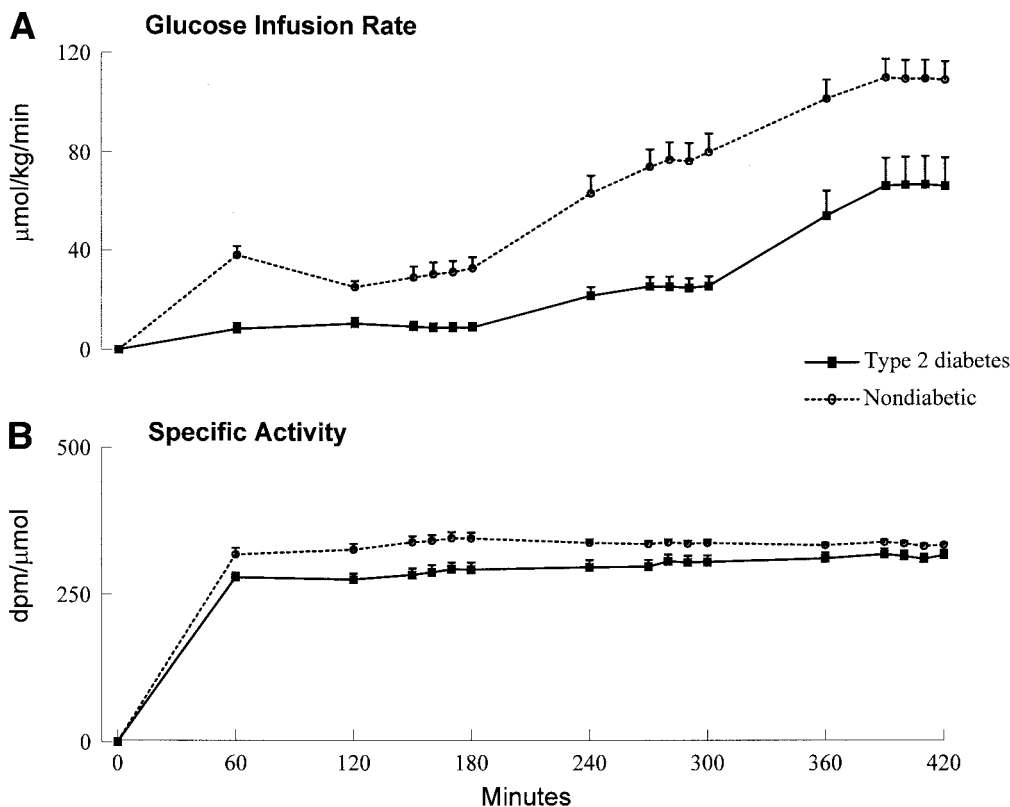


FIG. 2. Glucose infusion rates (A) required to maintain target glucose concentrations and plasma [ $3\text{-}^3\text{H}$ ]glucose specific activity (B) observed in the diabetic and nondiabetic subjects prior to and during a hyperglycemic clamp. The insulin, somatostatin, glucagon, and growth hormone infusions started at time 0.

did not differ in the diabetic and nondiabetic groups either prior to or during the clamps (data not shown).

**Glucose infusion rates required to maintain target glucose concentrations and [ $3\text{-}^3\text{H}$ ] glucose specific activity.** The glucose infusion rate required to maintain plasma glucose at target concentrations was lower ( $P < 0.002$ ) in the diabetic than nondiabetic subjects during the low ( $9 \pm 2$  vs.  $31 \pm 5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), middle ( $25 \pm 4$  vs.  $77 \pm 7$ ), and high ( $66 \pm 11$  vs.  $110 \pm 7$ )-dose insulin infusions, documenting the presence of insulin resistance (Fig. 2A). All exogenous glucose contained [ $3\text{-}^3\text{H}$ ] glucose in an effort to minimize change in glucose specific activity during the clamp. This caused plasma glucose specific activity to reach a plateau within 60 min, enabling accurate measurement of glucose production and disappearance thereafter (Fig. 2B).

**Net splanchnic glucose balance.** Splanchnic blood flow did not differ in the diabetic and nondiabetic subjects during the low ( $1,157 \pm 79$  vs.  $1,524 \pm 288 \text{ ml/min}$ ), middle ( $1,205 \pm 79$  vs.  $1,553 \pm 271$ ), or high ( $1,235 \pm 65$  vs.  $1,687 \pm 337$ )-dose insulin infusions. Despite hyperglycemia, net splanchnic glucose balance was negative (net release) in the diabetic subjects during the lowest-dose insulin infusion, becoming positive (net uptake) during the middle-dose insulin infusion (Fig. 3). On the other hand, net splanchnic glucose balance was positive in the nondiabetic subjects at all three insulin infusion rates. Net splanchnic glucose balance increased ( $P < 0.001$ , ANOVA) with increasing insulin concentrations in both groups. However, net splanchnic glucose balance remained lower ( $P < 0.05$ ) in the diabetic than in the nondiabetic subjects during the low ( $-3.5 \pm 1.4$  vs.  $2.6 \pm 1.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), middle ( $2.6 \pm 1.0$  vs.  $7.8 \pm 1.2$ ), and high ( $7.1 \pm 1.2$  vs.  $11.6 \pm 1.5$ )-dose insulin infusions.

**Splanchnic glucose (tracer) extraction and uptake.** Splanchnic (tracer) glucose extraction increased ( $P < 0.001$ , ANOVA) with increasing insulin concentrations in both groups. However, splanchnic glucose extraction remained lower ( $P < 0.02$ ) in the diabetic than in the nondiabetic subjects during the low ( $2.0 \pm 0.5$  vs.  $4.1 \pm 0.5\%$ ), middle ( $3.2 \pm 0.4$  vs.  $5.2 \pm 0.4$ ), and high ( $4.3 \pm 0.5$  vs.  $7.2 \pm 0.5$ )-dose insulin infusions.

The pattern of change in SGU paralleled that of splanchnic glucose extraction. SGU (Fig. 4A) increased ( $P < 0.001$ , ANOVA) with increasing insulin concentrations in both the diabetic and nondiabetic subjects. On the other

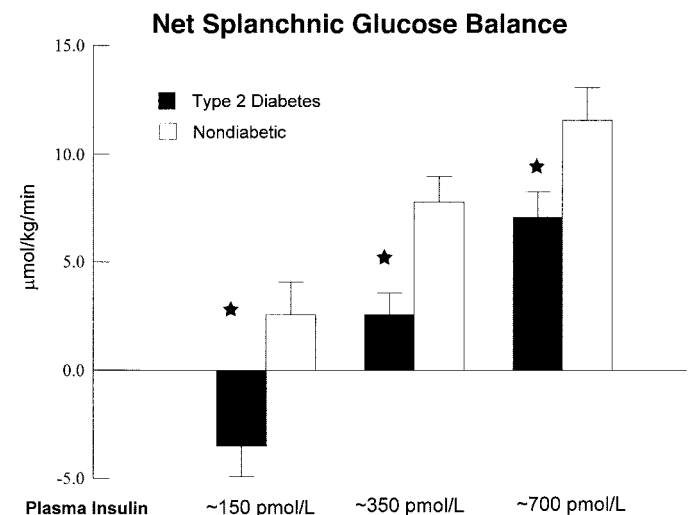


FIG. 3. Net splanchnic glucose balance observed in the diabetic and nondiabetic subjects during the final 30 min of the low ( $\sim 150 \text{ pmol/l}$ ), middle ( $\sim 350 \text{ pmol/l}$ ), and high ( $\sim 700 \text{ pmol/l}$ )-dose insulin infusions. \* $P < 0.05$  versus nondiabetic values.

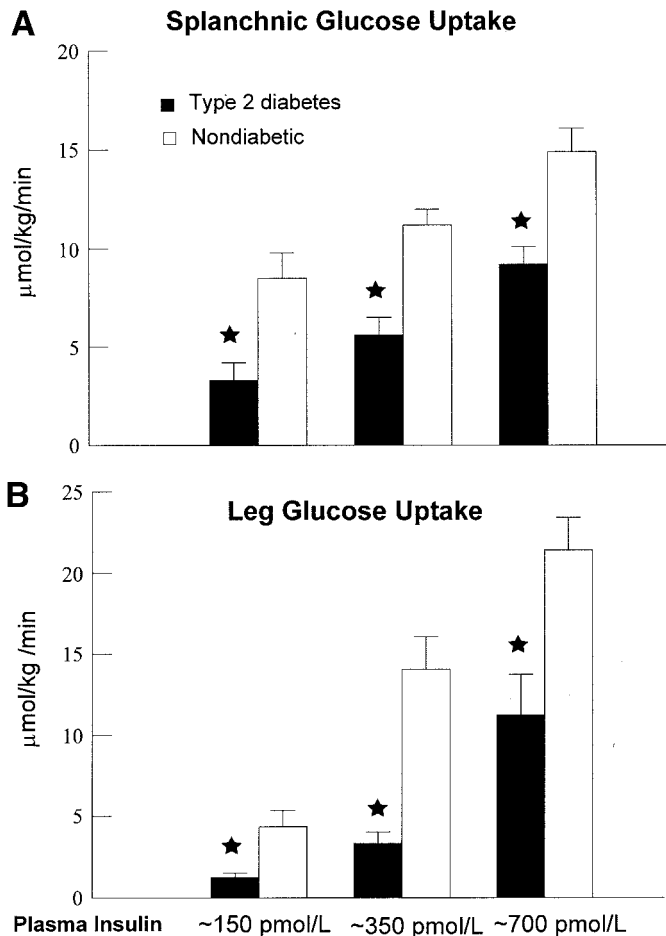


FIG. 4. SGU (A) and LGU (B) observed in the diabetic and nondiabetic subjects during the final 30 min of the low (~150 pmol/L)-, middle (~350 pmol/L)-, and high (~700 pmol/L)-dose insulin infusions. \* $P < 0.01$  versus nondiabetic values.

hand, SGU was lower ( $P < 0.01$ ) in the diabetic than nondiabetic subjects during the low ( $3.3 \pm 0.9$  vs.  $8.5 \pm 1.3$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), middle ( $5.6 \pm 0.9$  vs.  $11.2 \pm 0.8$ ), and high ( $9.2 \pm 0.9$  vs.  $14.9 \pm 1.2$ )-dose insulin infusions. Of note, the increment in SGU observed when the insulin was increased from the low- to middle-dose ( $2.3 \pm 1.2$  vs.  $2.6 \pm 1.2$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) insulin infusions, and the middle- to high-dose ( $3.6 \pm 1.1$  vs.  $4.2 \pm 1.1$ ) insulin infusions did not differ in the diabetic and nondiabetic subjects.

**Leg glucose (tracer) extraction and uptake.** Leg blood flow did not differ in the diabetic and nondiabetic subjects during the low ( $514 \pm 40$  vs.  $483 \pm 53$  ml/min)-, middle ( $457 \pm 30$  vs.  $511 \pm 45$ ), or high ( $551 \pm 49$  vs.  $630 \pm 60$ )-dose insulin infusions. Leg (tracer) glucose extraction increased ( $P < 0.001$ , ANOVA) with increasing insulin concentrations in both groups. However, leg glucose extraction remained lower ( $P < 0.001$ ) in the diabetic than in the nondiabetic subjects during the low ( $1.7 \pm 0.0$  vs.  $7.1 \pm 2.0\%$ ), middle ( $6.0 \pm 1.5$  vs.  $17.1 \pm 2.4$ ), and high ( $15.0 \pm 2.0$  vs.  $20.6 \pm 2.0$ )-dose insulin infusions.

The pattern of change in LGU also paralleled that of leg glucose extraction. LGU (Fig. 4B) increased ( $P < 0.001$ , ANOVA) with increasing insulin concentrations in both the diabetic and nondiabetic subjects. On the other hand, LGU was lower ( $P < 0.001$ ) in the diabetic than in the nondia-

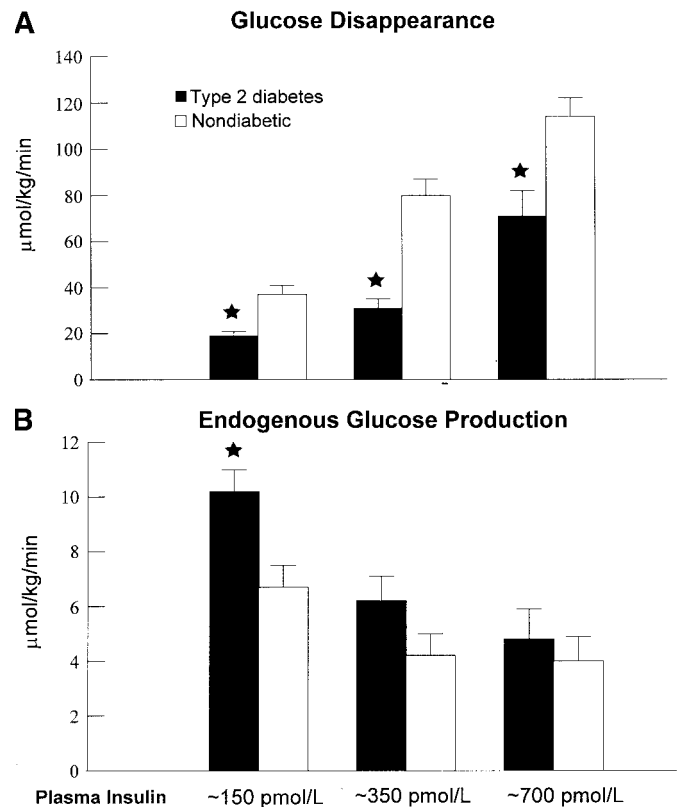


FIG. 5. Rates of glucose disappearance (A) and EGP observed in the diabetic and nondiabetic subjects during the final 30 min of the low (~150 pmol/L)-, middle (~350 pmol/L)-, and high (~700 pmol/L)-dose insulin infusions. \* $P < 0.01$  versus nondiabetic values.

betic subjects during the low ( $1.2 \pm 0.3$  vs.  $4.4 \pm 1.0$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), middle ( $3.3 \pm 0.7$  vs.  $14.1 \pm 2.0$ ), and high ( $11.2 \pm 2.5$  vs.  $21.4 \pm 2.0$ )-dose infusions. Although the increment in LGU observed when insulin was increased from the low to middle infusion rate ( $2.1 \pm 0.6$  vs.  $9.7 \pm 1.5$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) was lower ( $P < 0.001$ ) in the diabetic than in the nondiabetic subjects, it did not differ when insulin was increased from the middle to high infusion rate ( $7.9 \pm 1.9$  vs.  $7.3 \pm 0.7$ ).

**Glucose disappearance and EGP.** Total glucose disappearance increased ( $P < 0.001$ , ANOVA) with increasing insulin concentrations in both the diabetic and nondiabetic subjects (Fig. 5A). Glucose disappearance was lower ( $P < 0.01$ ) in the diabetic than nondiabetic subjects during the low ( $19 \pm 2$  vs.  $37 \pm 4$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), middle ( $31 \pm 4$  vs.  $80 \pm 7$ ), and high ( $71 \pm 11$  vs.  $114 \pm 8$ )-dose insulin infusions. As with LGU, the increment in total body glucose disappearance observed when the insulin was increased from the low to middle infusion rate was lower ( $P < 0.001$ ) in the diabetic than nondiabetic subjects ( $11.9 \pm 3.1$  vs.  $43.1 \pm 5.1$ ), but did not differ when insulin was increased from the middle to high infusion rate ( $40.3 \pm 8.0$  vs.  $33.3 \pm 4.2$ ).

EGP in the diabetic subjects progressively decreased ( $P < 0.001$ , ANOVA) with increasing insulin concentrations (Fig. 5B). On the other hand, EGP in the nondiabetic subjects decreased ( $P < 0.02$ ) when insulin was increased from the low to middle insulin infusion rates but did not decrease thereafter. This resulted in EGP rates that were higher ( $P < 0.01$ ) in the diabetic than nondiabetic subjects

TABLE 1  
7-h low-dose insulin infusion in nondiabetic subjects

	0 min	150–180 min	270–300 min	390–420 min
Glucose (mmol/l)	5.7 ± 0.3	9.1 ± 0.1	9.5 ± 0.1	9.5 ± 0.1
Insulin (pmol/l)	37.0 ± 6.0	194.0 ± 24.0	199.0 ± 20.0	196.0 ± 26.0
C-peptide (nmol/l)	0.55 ± 0.09	0.06 ± 0.03	0.04 ± 0.01	0.04 ± 0.01
Glucagon (pg/ml)	121.0 ± 15.0	122.0 ± 9.0	114.0 ± 10.0	104.0 ± 8.0
Glucose infusion rate ( $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ )	—	52 ± 4	60 ± 5	57 ± 2
[3- <sup>3</sup> H]glucose specific activity (dpm/ $\mu\text{mol}$ )	—	373 ± 28	390 ± 33	395 ± 30

Data are means ± SE.

during the low but not the middle or high insulin infusion rates.

**Effects of duration of hyperinsulinemia on splanchnic glucose (tracer) extraction and uptake.** In order to determine whether SGU increased with increasing duration of hyperinsulinemia, insulin was infused at a rate of  $0.78 \text{ mU} \cdot \text{kg lean body wt}^{-1} \cdot \text{min}^{-1}$  in four nondiabetic subjects while glucose was clamped at  $\sim 9.5 \text{ mmol/l}$  for 7 h. As shown in Table 1, glucose, insulin, C-peptide, and glucagon concentrations reached a plateau within 1 h and did not change thereafter. The glucose infusion rate increased gradually for the first 5 h, then decreased slightly during the succeeding 2 h. Plasma [3-<sup>3</sup>H] glucose specific activity achieved a steady state within 1 h.

Splanchnic blood flow did not differ when measured from 150 to 180 min ( $1,066 \pm 96 \text{ ml/min}$ ), 270 to 300 min ( $1,034 \pm 95$ ), or 390 to 420 min ( $1,197 \pm 126$ ). Splanchnic (tracer) glucose extraction ( $7.9 \pm 0.4$  vs.  $7.6 \pm 0.8$  vs.  $6.9 \pm 0.1\%$ , respectively) and SGU ( $12.3 \pm 2.0$  vs.  $12.9 \pm 2.1$  vs.  $12.6 \pm 3.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , respectively) measured over the same intervals also did not differ (Fig. 6).

## DISCUSSION

The present studies indicate that in the presence of hyperglycemia, an increase in insulin from  $\sim 150$  to  $\sim 700 \mu\text{mol/l}$  resulted in a progressive increase in SGU in both diabetic and nondiabetic humans. On the other hand, EGP

was maximally suppressed in the nondiabetic subjects at insulin concentrations of  $\sim 350 \text{ pmol/l}$ , but was further suppressed in the diabetic subjects at insulin concentrations up to  $\sim 700 \mu\text{U/ml}$ . SGU was lower in the diabetic than nondiabetic subjects at all insulin concentrations tested, whereas glucose production only was higher in the diabetic than in the nondiabetic subjects during the lowest-dose insulin infusion. These data indicate that the insulin dose-response curves for stimulation of SGU and suppression of glucose production differ in nondiabetic humans and are abnormal in people with type 2 diabetes. **Effects of insulin on SGU and splanchnic glucose production in nondiabetic humans.** In the presence of hyperglycemia, SGU increased with increasing insulin concentrations in the nondiabetic subjects. This observation is consistent with that previously reported by Myers et al. (17) in nondiabetic dogs and by Petersen et al. (18) and ourselves (6,7) in humans. At the lowest insulin concentrations tested ( $\sim 150 \text{ pmol/l}$ ), SGU averaged  $\sim 8.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , accounting for 23% of total body uptake. However, this comparison likely underestimates the potential contribution of hepatic glucose uptake to overall carbohydrate tolerance in the postprandial setting because portal insulin concentrations are several fold higher than peripheral insulin concentrations following food ingestion (i.e., when both glucose and insulin concentrations are normally increased). Assuming a portal venous-to-peripheral venous insulin gradient of  $\sim 2.3$  to 1 (13,15), the  $37 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  of total glucose uptake observed in the presence of peripheral insulin concentrations of  $150 \text{ pmol/l}$  would be accompanied by portal venous insulin concentrations of  $\sim 350 \text{ pmol/l}$  and SGU of  $11.2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ . Under these circumstances, the splanchnic bed would account for  $\sim 30\%$  of insulin-stimulated glucose uptake. These numbers are estimates of the potential rather than the actual effects of insulin observed after eating a carbohydrate-containing meal, because in that situation glucose and insulin concentrations are not maintained at a constant level, as was done in the current experiments, but rather are continuously changing. Therefore, the prolonged insulin infusions may have resulted in higher rates of SGU and extrasplanchnic glucose uptake than would occur following a briefer increase in insulin concentrations. On the other hand, glucose concentrations following food ingestion are higher in the portal than hepatic vein, likely leading to greater hepatic glucose uptake than occurs when all glucose is given intravenously, as was done in the present experiments (17).

In the presence of hyperglycemia and hyperinsulinemia, the majority of nonhepatic glucose uptake is believed to occur in muscle. In the present experiments, leg glucose

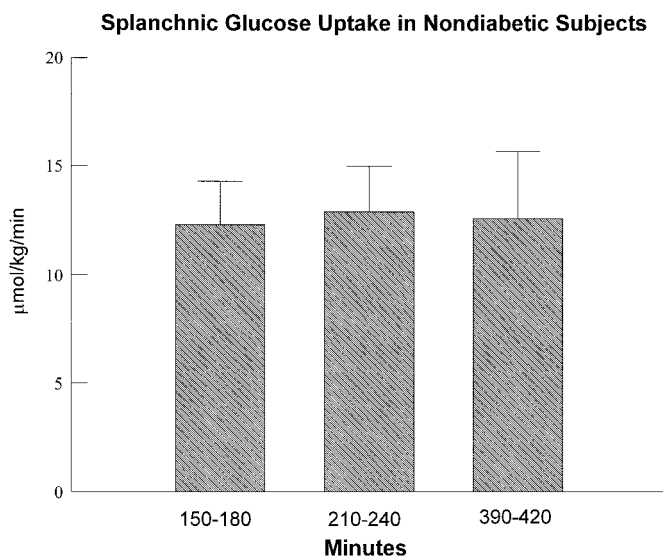


FIG. 6. SGU observed in nondiabetic subjects from 150 to 180 min, 210 to 240 min, and 390 to 420 min during a 7-h low-dose ( $\sim 150 \text{ pmol/l}$ ) insulin infusion. The insulin, somatostatin, glucagon, and growth hormone infusions started at time 0.

extraction and uptake increased 3- and 4.8-fold, respectively, as insulin was increased from  $\sim 150$  to  $\sim 700$  pmol/l. The proportionately greater increase in LGU than leg glucose extraction resulted from a slight but nonsignificant increase in leg blood flow with increasing insulin concentrations. On the other hand, splanchnic glucose extraction and SGU only increased 1.75-fold, indicating a greater range of response to insulin in the leg than in the liver. The true dose-response curves for these tissues are not known because the present experiments began with insulin concentrations in the midphysiologic range and there was no evidence of a maximal response in either tissue at the highest insulin concentration tested. Nevertheless, the wider range of response to insulin in the leg than in the splanchnic bed indicates the relative contribution of each to glucose disposal; therefore, carbohydrate tolerance will change as insulin concentrations change.

Consistent with previous experiments (1,3,6,13,14), maximal suppression of glucose production in the nondiabetic subjects occurred at insulin concentrations between  $\sim 150$  and  $\sim 350$  pmol/l. In contrast, as noted above, SGU continued to increase at insulin concentrations up to  $\sim 700$  pmol/l. Therefore, the mechanism by which insulin regulates these processes must differ. The amount of glucose released by the liver is determined by the balance between the rate that intrahepatic free glucose is phosphorylated to glucose-6-phosphate by glucokinase and the rate at which glucose-6-phosphate is dephosphorylated to glucose by glucose-6-phosphatase. Insulin regulates flux through the glucose-6-phosphatase pathway both directly by altering the expression of the enzyme and indirectly by lowering other regulators such as free fatty acid concentrations (18,28,37–40). On the other hand, insulin increases hepatic glucose uptake by increasing the activity of glucokinase and glycogen synthetase (18,40,41). In the presence of hyperglycemia, insulin can also stimulate glycogen synthetase by increasing intrahepatic glucose-6-phosphate concentrations (24). Since glucokinase is believed (41–43) to be rate limiting for hepatic glucose uptake, the present experiments imply that insulin concentrations sufficient to maximally suppress flux through glucose-6-phosphatase are not sufficient to maximally stimulate flux through glucokinase. Therefore the signals regulating these pathways must differ.

Glucagon concentrations were maintained constant at all insulin concentrations. In addition, while we did not measure free fatty acid concentrations, previous experiments (14) have shown that they are maximally suppressed in nondiabetic subjects at insulin concentrations  $< 150$  pmol/l. Therefore neither likely played a role in the modulation of glucose production and uptake under the present experimental conditions. Insulin can increase both the amount and activity of hepatic glucokinase, leaving open the possibility that the 7-h insulin infusion stimulated glucokinase synthesis (28). However, the effects of insulin on SGU did not appear to change with time because the rates did not differ from 180 min onward during the 7-h 0.5-mU/kg insulin infusion. This observation is consistent with previous reports (16,17) wherein the effects of insulin on splanchnic glucose balance reach a plateau within 90 min.

Taken together, the present data indicate that the shape

of the insulin dose-response curves for the regulation of hepatic glucose release and uptake differ, with the former being very sensitive to small changes in insulin and the latter continuing to increase at insulin concentrations spanning the physiologic range. They also indicate that in the presence of physiologic insulin concentrations, the splanchnic bed can account for  $\sim 25$ – $30\%$  of total body glucose uptake.

#### **The effects of type 2 diabetes on the ability of insulin to regulate SGU and splanchnic glucose production.**

The present data once again demonstrate that type 2 diabetes impairs insulin-induced suppression of glucose production and stimulation of SGU and LGU (1–3,6,7,13). However, although glucose production was eventually suppressed to nondiabetic rates, both SGU and LGU remained lower than nondiabetic rates at insulin concentrations spanning the physiologic range. As with the nondiabetic subjects, the relative contribution of the splanchnic bed and muscle (as reflected by LGU) to total body uptake in the diabetic subjects differed depending on the prevailing insulin concentration. In the nondiabetic subjects, leg glucose extraction was two- to threefold greater than splanchnic glucose extraction at all insulin concentrations examined. In contrast, splanchnic and leg fractional glucose extraction (both 2%) were virtually identical in the diabetic subjects at insulin concentrations of  $\sim 150$  pmol/l (Figs. 4 and 5). In addition, the absolute amount of glucose taken up in the diabetic subjects by the splanchnic bed at those insulin concentrations ( $\sim 3.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) exceeded that taken up by both legs ( $\sim 2.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , assuming equivalent uptake in each leg). This emphasizes the marked degree of muscle insulin resistance in people with type 2 diabetes as well as the importance of hepatic glucose uptake in the regulation of glucose tolerance in the presence of low physiologic insulin concentrations. On the other hand, the relative contribution of muscle and SGU changed as insulin concentrations increased. Leg fractional glucose extraction in the diabetic subjects was twofold greater than splanchnic fractional glucose extraction when insulin was increased to  $\sim 350$  pmol/l (6 vs. 3%) and almost four times that of splanchnic glucose extraction at insulin concentrations of  $\sim 700$  pmol/l (15 vs. 4%). This resulted in rates of SGU that were slightly lower than those of both legs at insulin concentrations of  $\sim 350$  pmol/l ( $\sim 5.6$  vs.  $\sim 6.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) and substantially lower than those of both legs at insulin concentrations of  $\sim 700$  pmol/l ( $\sim 9.2$  vs.  $22.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). Thus, although the ability of insulin to stimulate muscle glucose uptake is severely impaired in diabetic subjects, as with nondiabetic subjects, the range of response to insulin substantially exceeds that of the liver. Nevertheless, in the presence of insulin concentrations likely to be present in diabetic individuals under the conditions of daily living, SGU is likely to make a substantial contribution to total glucose uptake in people with type 2 diabetes.

The highest insulin concentration examined in the present experiments was  $\sim 700$  pmol/l. We therefore do not know whether SGU and LGU would have eventually reached the same maximal rates if the insulin concentrations were increased to sufficiently high concentrations. Previous studies (1,3,13,14) have shown that maximal

rates of total body glucose disposal are lower in obese diabetic than nondiabetic subjects, similar to those who participated in the current experiments. Interestingly, the increment in both LGU and total body glucose disposal when insulin was increased from ~150 to ~350 pmol/l was lower in the diabetic than in the nondiabetic subjects, but did not differ when insulin was further increased to ~700 pmol/l. This indicates that the insulin dose-response curve for both LGU and total body disposal was steeper in the nondiabetic than diabetic subjects at lower insulin concentrations but appeared to become parallel at higher concentrations. This pattern could have occurred if the step that was both defective and rate limiting for muscle glucose uptake at low insulin concentrations (e.g. glucose transport and/or phosphorylation) in the diabetic subjects was no longer rate limiting at higher insulin concentrations. In contrast, while the absolute rate of SGU was lower in the diabetic than nondiabetic subjects, the increment in uptake with each increment in insulin did not differ between groups. Glucokinase is believed to be rate limiting for hepatic glucose uptake, and the amount and activity of hepatic glucokinase has been reported (42–45) to be lower in animal models of diabetes and in people with diabetes. Therefore, the right-shifted (i.e. lower but parallel) insulin dose curve for stimulation of SGU could be explained if the amount of active glucokinase was reduced in the diabetic subjects in the basal state with preserved translocation and activation of glucokinase in response to an increment in insulin. It would be of interest in this regard if the insulin dose-response curve for the stimulation of hepatic glucose uptake also is shifted to the right in individuals known to have genetic defects (i.e., MODY2) in the activation of hepatic glucokinase (43).

In summary, the present experiments indicate that shape of the insulin dose-response curves for suppression of glucose production and stimulation of SGU differ in nondiabetic subjects. Whereas a relatively small increase in insulin results in maximal suppression of glucose production, SGU continues to increase at insulin concentrations spanning the physiologic range. Type 2 diabetes impairs both processes, with the defect in insulin-induced suppression of glucose production only being evident at lower insulin concentrations. In the presence of hyperglycemia, insulin-induced stimulation of SGU and muscle glucose uptake both contribute to overall glucose disposal in both diabetic and nondiabetic subjects. However, due to the presence of marked insulin resistance in muscle, the relative contribution of SGU to overall glucose disposal is greater in diabetic subjects, particularly at insulin concentrations likely to be present under conditions of daily living. Finally, the right shifted but parallel insulin dose-response curve for the stimulation of SGU observed in the diabetic subjects suggests a defect that lowers basal rates of uptake but does not alter the subsequent response to insulin. However, because the subjects who participated in the present studies were on average either overweight or obese and were predominantly 50–70 years of age, additional studies will be required to determine whether these conclusions also apply to people with type 2 diabetes whose age and degree of adiposity differs. Nevertheless, taken together, these data emphasize the importance of SGU as a contributor to overall glucose disposal. They also

suggest that agents that enhance SGU in diabetic patients (e.g. glucokinase activators) are likely to have favorable effects on glucose tolerance.

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