Glucose-6-Phosphatase Flux In Vitro Is Increased in Type 2 Diabetes

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Despite the effects of hyperinsulinemia and hyperglycemia, 2 factors known to inhibit endogenous glucose production (EGP) in nondiabetic subjects, increased EGP is a consistent feature of type 2 diabetes. Recent studies have suggested that increased glucose-6-phosphatase (G6Pase) and/or decreased glucokinase (GK) may explain the increase in EGP. However, no studies to date have clearly established this relationship in type 2 diabetes. The present studies were designed to determine rates of EGP and the activities of G6Pase and GK in obese patients scheduled for gastric bypass surgery. The study group consisted of 14 obese nondiabetic subjects and 13 patients with type 2 diabetes (BMI 53.7 ± 2.4 vs. 50.1 ± 1.6 kg/m^2). Rates of EGP were determined after an overnight fast with a 4-h infusion of [6,6]-D-glucose, and they were significantly higher in the type 2 diabetic patients (85.9 ± 10.0 vs. 137.8 ± 14.4 mg · m^{-2} · min^{-1}, *P* < 0.001) despite greater plasma glucose (5.1 ± 0.1 vs. 12.0 ± 1.1 mmol/l) and similar insulin concentrations (130.8 ± 19.8 vs. 112.8 ± 16.2 pmol/l, NS). Moreover, resistance to insulin-induced suppression of EGP was observed in the patients with type 2 diabetes when insulin concentrations were increased from ~120 to 180 pmol/l. Hepatic G6Pase activity determined from freshly isolated microsomes was significantly increased in the type 2 diabetic patients compared with the obese control subjects (0.16 ± 0.02 vs. 0.09 ± 0.01 µmol · min^{-1} · mg^{-1} protein, *P* < 0.02), whereas levels of GK were decreased (1.20 ± 0.16 vs. 2.01 ± 0.01 µmol · min^{-1} · mg^{-1} protein, *P* < 0.01). Net flux through G6Pase was significantly increased in type 2 diabetic patients (*P* < 0.01). We conclude that increased EGP is mediated in part by increased G6Pase flux in type 2 diabetes. *Diabetes* 49:969–974, 2000

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EGP, endogenous glucose production; FFA, free fatty acid; FFM, fat-free mass; G6Pase, glucose-6-phosphatase; GC, glucose cycle; GK, glucokinase; HGO, hepatic glucose output; IBW, ideal body weight; R_a, rate of glucose appearance; R_d, rate of glucose disappearance; VCU, Virginia Commonwealth University.

Type 2 diabetes is characterized in part by increased endogenous glucose production (EGP) (1). Although there is increasing appreciation of the potential role of renal gluconeogenesis in EGP (2), much of the increase in EGP is related to increased hepatic glucose output (HGO). Numerous studies using either splanchnic balance techniques (3) or isotopic turnover studies (4–7) have demonstrated increased rates of glucose production in patients with type 2 diabetes. Moreover, the increases in HGO are observed despite elevated levels of plasma insulin and glucose, both of which are known to inhibit the release of glucose in nondiabetic individuals (8). Control of HGO may occur through regulation of gluconeogenesis or glycogenolysis. However, the common final pathway of glucose uptake and release involves the phosphorylation and dephosphorylation of glucose via glucokinase (GK) and glucose-6-phosphatase (G6Pase), respectively. The competing activity between the 2 enzymes has been described as the glucose cycle (GC) and represents an important potential site of regulation (9). Studies demonstrating an increase in the GC have led investigators to suggest that G6Pase activity is increased and that a compensatory increase in GK activity may, at least in part, modulate HGO in patients with type 2 diabetes (9). However, there are few studies that have examined both enzyme activities in liver samples from diabetic and nondiabetic subjects to determine if an increase in 1 activity is associated with a compensatory increase in the other, as might be expected if the GC is increased in patients with type 2 diabetes. The present study was designed to examine this question in morbidly obese patients who were scheduled for gastric bypass surgery. Our studies suggest that G6Pase activity is increased and GK activity is decreased, thereby leading to an increase in net glucose release in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS

The present studies enrolled 14 morbidly obese nondiabetic subjects and 13 patients with type 2 diabetes. All of the patients were scheduled for gastric bypass surgery at the Medical College of Virginia Hospitals for the treatment of severe obesity according to guidelines established by the 1991 National Institutes of Health Consensus Conference on the Surgical Treatment of Obesity (10). The studies were approved by the Committee for the Conduct of Human Research at Virginia Commonwealth University (VCU), and informed consent was obtained from each subject before participation. All of the subjects were admitted to the General Clinical Research Center at VCU 2 days before the planned surgery. The 2 groups were similar in BMI and age (Table 1). Measurement of waist-to-hip ratios was not performed due to the unreliability of the measurement in these patients. The diabetic patients were instructed to discontinue hypoglycemic medication at least 5 days before the study, and blood glucose levels were closely monitored. After an evening meal, subjects were fasted until the studies were completed. An intravenous catheter was

TABLE 1
Demographic information on subjects enrolled in the present studies

	Control subjects	Type 2 diabetic patients
n	14	13
Age (years)	39.8 ± 2.8	44.8 ± 2.4
BMI (kg/m^2)	53.8 ± 2.4	50.1 ± 1.6
Body surface area (m^2)	2.6 ± 0.1	2.4 ± 0.1

Data are *n* or means ± SE.

placed into a forearm vein for the infusion of isotopes and insulin and glucose. Another catheter was retrogradely placed in a hand vein, and the hand was placed in a Plexiglass box heated to 60°C to obtain arterialized venous blood samples for the measurement of isotopic enrichment, intermediary metabolites, and glucoregulatory hormones. Both catheters were kept patent with an infusion of normal saline.

At 4:00 A.M., a primed (26.7 μmol/kg fat-free mass [FFM]) continuous (0.33 μmol · kg^{-1} FFM · min^{-1}) infusion of [6,6]-D-glucose was initiated for determination of EGP and glucose disposal. After a 4-h period of equilibration, baseline blood samples were obtained and a 2-step isoglycemic clamp was performed to determine hepatic insulin sensitivity at 10 and 20 mU · m^{-2} · min^{-1}, as previously described (11). We considered the possibility of performing the insulin clamp studies after an overnight infusion of insulin to normalize plasma glucose levels in the diabetic subjects. However, an isoglycemic clamp was chosen for these studies to avoid the anticipated salutary changes induced by significant increases in plasma insulin levels and lowered glucose levels on the activity of GK and G6Pase (8). Indirect calorimetry was performed at baseline and during the final 30 min of each step in the clamp using a Sensormedics 2000 metabolic cart (Anaheim, CA) equipped with a plastic canopy to measure oxygen consumption (l/min) and carbon dioxide production (l/min).

After completion of the insulin clamps, the subjects consumed a regular midday and evening meal and had no food or liquids by mouth thereafter for the scheduled bariatric surgery the next morning. Rapid-sequence anesthetic induction was achieved with intravenous propofol 2 mg/kg ideal body weight (IBW), succinyl choline 2 mg/kg IBW, lidocaine 60–100 mg, and fentanyl 3–5 mg/kg IBW. After induction, vecuronium was administered for muscle relaxation as needed, and desflurane was administered to achieve a minimal alveolar concentration necessary to keep 50% of the patients from moving with surgical stimulation of 0.5–1 by monitoring the end-tidal levels at 5–8%. The liver tissue was obtained within 1 h of anesthetic induction. Two 2-*O* chromic catgut sutures were placed at a 60° angle to each other and tied. A wedge biopsy of liver tissue was obtained with scalpel dissection. One portion of the sample was immediately frozen in liquid nitrogen and kept at –80°C thereafter for determination of GK activity. The other portion was transported on ice for fresh isolation of liver microsomes to determine G6Pase activity. This procedure was initiated within 20 min of biopsy.

Sample analyses. Arterialized venous blood samples were obtained at selected time intervals and placed immediately into ice-cold fluoridated tubes for determination of glucose enrichment and measurement of plasma hormones and intermediary metabolites. Plasma glucose levels were measured by the glucose oxidase method (Beckman, Fullerton, CA). Plasma insulin (12), C-peptide (13), and glucagon (14) levels were determined with double-antibody radioimmunoassays. Plasma free fatty acid (FFA) levels were determined with enzymatic methods (15). Blood samples for measurement of intermediary metabolites were immediately deproteinized with ice-cold 3M perchloric acid. The supernatant was neutralized with 3M KOH, and the resulting supernatant was assayed for L-lactate, alanine, β-hydroxybutyrate, acetoacetate (16), citrate (17), and glycerol (18) with microfluorometric assays. Urinary nitrogen was determined by the Kjeldahl method. Enrichment of [6,6]-D-glucose was performed as previously described (19). Pentacetate derivates were made from deproteinized blood samples, and aliquots were analyzed with a 5890/5972 gas chromatograph/mass spectrometer (Hewlett Packard, Palo Alto, CA) using selective ion monitoring at *m/z* 333/331 in positive chemical ionization mode.

Hepatic GK activity was measured by a spectrophotometric method as described by Davidson and Arion (20) with modifications described by Barzilai and Rossetti (21). GK activity was assayed at 37°C in a buffer (pH 7.4) containing 50 mmol/l HEPES; 100 mmol/l KCl; 7.5 mmol/l $MgCl_2$; 2.5 mmol/l dithioerythritol; 10 mg/ml albumin; 0.5, 7, 10, 15, 18, or 50 mmol/l glucose; 0.5 mmol/l NAD^+; 4 U glucose-6-phosphate dehydrogenase; and the equivalent of 1 mg liver wet weight. The albumin was added immediately before the assay, and the assay was initiated by the addition of 5 mmol/l ATP. The rate of NAD^+ reduction was measured on a Beckman spectrophotometer at 340 nm after 30 min. Microsomal G6Pase activity was measured by the method of Burchell et al. (22). G6Pase and mannose-6-phosphatase were assayed at 30°C for 10 min in a buffer (pH 6.5) containing 50 mmol/l sodium cacodylate, 2.0 mmol/l EDTA, and the following substrate concentrations: 0, 1.7, 2.5, 3.3, 5, 10, or 15 mmol/l glucose-6-phosphate or 15 mmol/l mannose-6-phosphate in a total volume of 200 μl. The assay was initiated by the addition of 20 μl of the microsomal preparation containing ~10 μg protein. After 10 min, the reaction was stopped by the addition of 0.8 ml of stopping reagent (3.4 mmol/l ammonium molybdate in 0.5 mol/l sulfuric acid, 0.52 mol/l SDS, and 0.6 mol/l ascorbic acid in the proportions of 6:2:1, made fresh daily). The color was developed by incubating at 45°C for 20 min, and absorbance was determined at 820 nm on a Beckman spectrophotometer.

Baseline rates of glucose appearance (R_a) and disappearance (R_d) were determined using steady-state equations. For example: $R_a = I/E - I$, where I equals the rate of infusion of isotope in μmol · m^{-2} · min^{-1}, and E equals the enrichment of [6,6]-D-glucose in atoms percent excess.

During the insulin clamps, R_a and R_d were determined from non–steady-state equations. EGP was determined as the difference between the R_a and the rate of infusion of exogenous glucose. Calculation of carbohydrate and lipid oxidation was performed using the equations of Frayn (23) with the nonprotein respiratory quotient (23). Protein oxidation was estimated as 6.25 × urinary *n* excretion (g/min). Nonoxidative glucose disposal was calculated as the difference between the rates of glucose disposal measured isotopically and of carbohydrate oxidation measured by indirect calorimetry.

Statistical analysis between the groups was performed with analysis of variance. Statistical analysis within the groups was performed using repeated measures of analysis of variance and a multiple comparison test. Statistical significance was assumed at $P < 0.05$. Results are expressed as means ± SE.

RESULTS

Baseline fasting plasma insulin concentrations were similar in the diabetic and nondiabetic subjects (112.8 ± 16.2 vs. 130.8 ± 19.8 pmol/l, NS), despite markedly increased plasma glucose concentrations in the former group (12.0 ± 1.1 vs. 5.1 ± 0.1 mmol/l). As expected, rates of EGP were also significantly increased in the diabetic patients (137.8 ± 14.4 vs. 78.2 ± 5.6 mg · m^{-2} · min^{-1}, $P < 0.001$) (Fig. 1) and were closely correlated with fasting plasma glucose ($r = 0.73$, $P < 0.0001$). As shown in Table 2, plasma ketones were greater in the diabetic patients, and plasma FFA and glycerol levels tended to be greater in diabetic patients compared with obese nondiabetic subjects. However, overall rates of carbohydrate (30.4 ± 0.6 vs. 39.9 ± 0.9 mg · m^{-2} · min^{-1}) and lipid oxidation (54.0 ± 0.4 vs. 48.8 ± 0.5 mg · m^{-2} · min^{-1}) were not significantly different in nondiabetic and diabetic subjects, respectively.

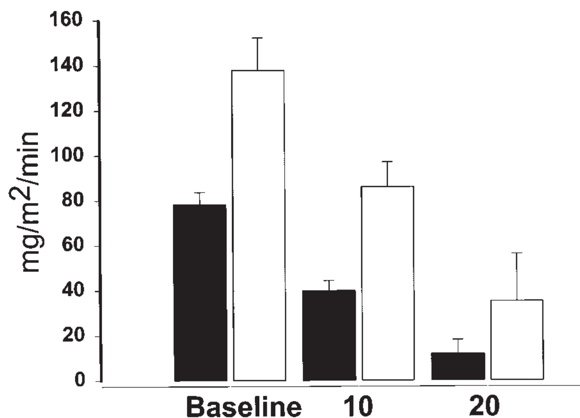

FIG. 1. Rates of EGP at baseline and during the final 30 min of hyperinsulinemic-isoglycemic clamps at 10 and 20 mU · m^{-2} · min^{-1} in nondiabetic (■, *n* = 14) and diabetic subjects (□, *n* = 13). Data are milligrams per meters sqared per minute and are expressed as means ± SE.

TABLE 2
Plasma FFA, glycerol, and β-hydroxybutyrate at baseline and during the last 30 min of a 10 and 20 mU · m^{-2} · min^{-1} isoglycemic clamp in obese type 2 diabetic and nondiabetic control subjects

	Baseline	10 mU	20 mU
FFA (μmol/l)			
Control subjects	512.4 ± 59.1	333.5 ± 60.5*	255.7 ± 44.0*
Type 2 diabetic patients	709.4 ± 83.8	428.4 ± 78.6*	303.9 ± 71.0*
Glycerol (μmol/l)			
Control subjects	180.5 ± 19.5	163.2 ± 24.2*	132.9 ± 15.3*
Type 2 diabetic patients	320.9 ± 32.3	247.1 ± 30.4†	246.3 ± 33.1†
β-Hydroxybutyrate (μmol/l)			
Control subjects	98.5 ± 15.9	55.9 ± 12.1*	29.7 ± 6.2*
Type 2 diabetic patients	195.5 ± 44.1‡	94.2 ± 23.3*	61.4 ± 15.2*

Data are means ± SE. *$P < 0.01$ vs. baseline; †$P < 0.05$ vs. baseline; ‡$P < 0.05$ vs. control.

Thus, rates of nonoxidative glucose disposal were significantly greater in diabetic compared with nondiabetic subjects (98.1 ± 17.4 vs. 44.5 ± 11.7 mg · m^{-2} · min^{-1}, $P < 0.02$). Interestingly, plasma lactate concentrations were not increased in the patients with type 2 diabetes (1.17 ± 0.1 vs. 1.14 ± 0.13 mmol/l, NS).

During the 2-step insulin clamp, mean plasma insulin concentrations increased to 191.4 ± 3.6 and 277.2 ± 5.4 pmol/l at 10 and 20 mU · m^{-2} · min^{-1}, respectively, and did not differ in the diabetic and nondiabetic subjects. A minimal infusion of glucose was required to maintain glucose at baseline levels in each group, but no difference in the glucose infusion rate was observed between the 2 groups (data not shown). Rates of EGP fell similarly in the diabetic (35.7 ± 5.7 and 86.2 ± 12.8%, $P < 0.001$) and nondiabetic subjects (50.4 ± 5.4 and 85.5 ± 10.4%, $P < 0.001$) at 10 and 20 mU · m^{-2} · min^{-1}, respectively. However, absolute rates of EGP were consistently greater in the patients with type 2 diabetes when plasma insulin concentrations were increased from ~120 to 192 pmol/l (86.1 ± 11.0 vs. 39.8 ± 4.5 mg · m^{-2} · min^{-1}, $P < 0.01$) (Fig. 1). The differences in EGP were no longer significant when the insulin concentration was further increased. The insulin infusions also resulted in significant suppression of FFA concentrations (39.7 ± 7.1 vs. 44.6 ± 6.6% and 57.8 ± 4.9 vs. 59.0 ± 5.0%), glycerol concentrations (34.5 ± 3.0 vs. 23.6 ± 7.0% and 43.9 ± 4.4 vs. 32.3 ± 8.7%) and β-hydroxybutyrate concentrations (41.4 ± 8.8 vs. 45.9 ± 7.2% and 65.9 ± 5.3 vs. 62.0 ± 4.9%) (Table 2), but the degree of suppression did not differ significantly between the nondiabetic and diabetic subjects, respectively. Thus, under conditions of identical insulin concentrations and greater plasma glucose concentrations for the diabetic subjects, suppression of peripheral lipolysis and hepatic FFA oxidation was similar in diabetic and nondiabetic subjects.

Enzyme activities for G6Pase and GK in liver samples obtained from the obese subjects at the time of gastric bypass surgery are shown in Fig. 2. Maximal G6Pase activity was significantly increased in the diabetic subjects (0.16 ± 0.02 vs. 0.09 ± 0.01 μmol · min^{-1} · mg^{-1} microsomal protein, $P < 0.05$) (Fig. 2*A*). In contrast, maximal GK activity was significantly decreased in the diabetic compared with the nondiabetic subjects (1.20 ± 0.16 vs. 2.01 ± 0.01 μmol · min^{-1} · mg^{-1} cytosolic protein, $P < 0.001$) (Fig. 2*B*). When the activities of both enzymes were normalized per liver wet weight, rates of G6Pase activity were no longer significantly different (3.97 ± 0.40 and 2.92 ± 0.14 μmol · min^{-1} · g^{-1} liver, NS), whereas GK activity rates were again decreased in the diabetic subjects (1.37 ± 0.16 and 2.07 ± 0.14 μmol · min^{-1} · g^{-1} liver, $P < 0.001$). Perhaps more importantly, however, the ratio of G6Pase to GK per wet weight liver was significantly increased in the patients with type 2 diabetes compared with the control group (3.39 ± 0.47 vs. 1.42 ± 0.24, $P < 0.01$), suggesting an increase in net G6Pase flux in patients with type 2 diabetes. This ratio was also significantly correlated with rates of EGP that were determined before surgery ($r = 0.56$, $P < 0.05$).

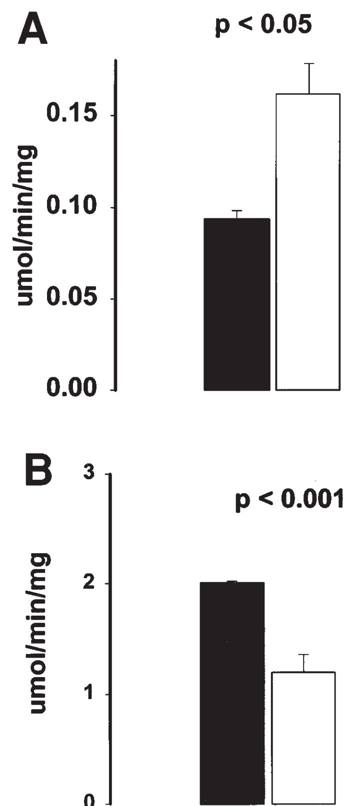

FIG. 2. Mean enzyme activity of G6Pase (*A*) and GK (*B*) determined from liver biopsy samples obtained from morbidly obese nondiabetic (■, *n* = 14) and diabetic (□, *n* = 13) subjects. Data are micromoles per minute per milligram protein and are expressed as means ± SE.

DISCUSSION

Increased EGP, the majority (>75%) of which is thought to be derived from hepatic sources, is a consistent feature of type 2 diabetes. It has been suggested that increased flux through G6Pase is in part responsible for the increased EGP routinely found in patients with type 2 diabetes. However, few studies have been performed in humans with type 2 diabetes because of the obvious difficulty in obtaining adequate liver samples for analysis. The present studies have been performed in patients who underwent gastric bypass surgery after extensive metabolic characterization. By obtaining biopsies at the beginning of the surgical procedure, thereby permitting maximal time to observe the biopsy site, sufficient tissue can be obtained safely to perform numerous measurements on a single biopsy sample. Using this method, we demonstrated an increase in G6Pase activity and a decrease in GK activity in diabetic subjects in whom EGP was also elevated.

In one of the few studies of this topic published (24), no difference in G6Pase activity was observed between diabetic and nondiabetic subjects. However, use of whole liver homogenates rather than microsomal preparations and failure to account for nonspecific phosphatase activity may have masked the differences in these studies. On the other hand, numerous studies performed in animal models of diabetes have demonstrated an increase in G6Pase activity and gene expression (21,25,26). In nearly all of these studies, the increase in G6Pase was associated with a reduction in insulin secretion, and normalization of plasma glucose by insulin treatment also normalized G6Pase activity (21,26). In contrast to these studies, fasting plasma insulin concentrations were not significantly different in the morbidly obese diabetic and nondiabetic subjects studied in the present investigations, which suggests that the increase in G6Pase activity may be mediated by factors other than plasma insulin concentration.

It could be argued that the conditions of anesthesia and surgery alone are responsible for the increase in G6Pase activity observed in diabetic subjects. Inhalation anesthesia has been associated with a reduction in plasma insulin concentrations in nondiabetic individuals (27). Thus, it is possible that greater suppression of insulin secretion in the diabetic subjects during anesthesia and surgery may have increased G6Pase activity. There are no studies, to our knowledge, that have compared the insulin-secretory response to anesthesia in diabetic and nondiabetic subjects. However, changes in G6Pase activity in response to changes in insulin are not generally observed before 3 h (21,28), and the liver samples obtained in the present studies were all obtained within 60 min of anesthesia induction. Finally, incubation of rat liver slices with insulin for up to 90 min in vitro does not alter G6Pase activity (J.N.C., unpublished data). Thus, we believe it is unlikely that the modest changes in insulin secretion, which may have occurred during induction of anesthesia, would have significantly altered enzyme activity in the present studies.

On the other hand, differences in insulin sensitivity may be responsible for the increase in G6Pase that we observed in our diabetic subjects. Results from the 2-step hyperinsulinemic-isoglycemic clamp studies performed before surgery are consistent with this hypothesis. No differences in the percentage of insulin-induced suppression of EGP or peripheral lipolysis were observed when the studies were performed at 5 and 12 mmol/l in the nondiabetic and diabetic subjects, respectively. However, absolute rates of EGP were greater at baseline and during the first step of the insulin clamps in our diabetic subjects compared with the nondiabetic volunteers, despite nearly identical insulin concentrations. Either increased FFA oxidation or hyperglycemia may be responsible for hepatic insulin resistance. Enhanced FFA oxidation has been shown to increase G6Pase activity in rat liver (29), and we have recently shown an inverse relationship between intracellular concentrations of fatty acyl CoA and G6Pase gene expression and enzyme activity (30). In the present studies, total-body lipid oxidation was not increased in the diabetic subjects compared with the nondiabetic control subjects. However, plasma ketone bodies, indirect measures of hepatic FFA oxidation, were significantly increased in the patients with type 2 diabetes, suggesting that increased hepatic FFA oxidation may have altered hepatic insulin sensitivity by increasing G6Pase activity. A direct effect of glucose on G6Pase gene expression and enzyme activity has also been proposed (31). In support of this contention, other studies have demonstrated that overnight infusions of insulin sufficient to normalize plasma glucose concentrations have reversed the defect in hepatic sensitivity to insulin (32,33). However, the ability of glucose to suppress EGP is not normalized by overnight insulin infusion in type 2 diabetic patients (34). Because glucose concentrations were clamped at isoglycemia in the present studies, the observed defect in suppression of EGP may be explained by glucose-mediated induction of G6Pase.

Our studies have also demonstrated a significant decrease in GK activity in type 2 diabetic patients. Studies performed in patients with GK-deficient type 2 maturity-onset diabetes of young (35) and in animal models of GK deficiency (36,37) have suggested that decreased hepatic GK activity plays an important independent role in the regulation of HGO. However, alterations have not been found in the coding sequence for GK in patients with typical type 2 diabetes (38). These findings suggest that decreased GK activity in patients with type 2 diabetes is probably the result of metabolic and/or hormonal factors. Our findings are in apparent conflict with those of Belfiore et al. (24), who demonstrated a nonsignificant reduction in GK in diabetic patients. However, differences in methodology may explain these results. These investigators assayed enzyme activity in a whole liver homogenate in which G6Pase activity may have limited accumulation of G6P, whereas the present studies were performed on postmicrosomal supernatant, the use of which largely eliminates contamination with G6Pase activity (20). On the other hand, the present findings are in agreement with the recent observations of Caro et al. (39) in a similar patient population and the many studies in animal models of diabetes that demonstrated reductions in GK activity (21,36). Most, but not all (21), of the latter studies were performed in insulin-deficient models of diabetes, in which insulin treatment normalized enzyme activity. As previously noted, fasting plasma insulin concentrations were similar in the diabetic and nondiabetic subjects before surgery, suggesting that differences in insulin alone are unlikely to explain the marked reduction in enzyme activity in the diabetic subjects. However, we cannot exclude the

possibility that changes in plasma insulin concentrations during the induction of anesthesia may have led to a greater decrease in GK activity in the diabetic subjects compared with the nondiabetic control subjects. Infusion of anti-insulin serum in nondiabetic animals for 3 h has been shown to reduce GK activity by 33% (28), and marked hyperinsulinemia for 2 h in diabetic animals has been shown to increase GK activity (21). It cannot be determined whether the modest changes in portal insulin secretion, which were expected during the brief period before biopsy, were sufficient to alter GK activity in our subjects. Metabolic alterations in GK activity are also possible. One such effect may be mediated by hyperglycemia per se, as previously indicated, for G6Pase. Hyperglycemia has been reported to induce the accumulation of glucosamine and insulin resistance in numerous cell types. Inhibition of GK activity by glucosamine has been demonstrated in islet cells (40) and in rat liver (41) and could have contributed to our findings. However, marked hyperglycemia (20 mmol/l) is required to acutely increase intracellular glucosamine concentrations sufficient to inhibit GK (41). The plasma glucose levels observed in our diabetic subjects were only slightly more than half of those achieved in these experiments, and none of the patients, based on their HbA_{1c} values, showed evidence of chronic hyperglycemia at this level.

We have also examined the relationship between the activities of G6Pase and GK in our studies. The marked increase in the ratio of G6Pase-to-GK may appear contrary to evidence for an increase in GC (glucose $\leftrightarrow$ glucose-6-phosphate) activity in patients with type 2 diabetes. Noninvasive studies that have examined flux through the GC have supported the contention that net G6Pase flux is increased in type 2 diabetes. By using concomitant infusions of [2-^{3}H]glucose and [6-^{3}H]glucose in the postabsorptive state, an increase in GC activity has been shown in mildly hyperglycemic type 2 diabetic patients compared with normoglycemic nondiabetic subjects (9). In addition, the failure of glucose to inhibit total glucose output in diabetic subjects has suggested a defect in G6Pase, GK, or both (34). The present studies provide important new information on possible explanations for an increase in GC activity measured isotopically in type 2 diabetic patients. First, under fasting conditions in which dephosphorylation of glucose would be expected to exceed phosphorylation, GK activity could be paradoxically increased, thereby leading to a greater loss of label from [2-^{3}H]glucose in the isomerization of glucose-6-phosphate to fructose-6-phosphate (42). The present data do not support such a mechanism. Alternatively, greater production of glucose-6-phosphate through the activity of hexokinase might explain an increase in GC activity. Indeed, a significant increase in hexokinase activity was reported in the studies of Belfiore et al. (24). However, the method used to determine GK activity in the present studies involves the subtraction of hexokinase activity (glucose-6-phosphate generated at 0.5 mmol/l glucose) from total glucose phosphorylation. Levels of hexokinase activity did not differ in the 2 groups of subjects (data not shown). Our data suggest, therefore, that despite decreased GK activity, the increase in GC observed in vivo is the result of mass action and greater flux through G6Pase.

In conclusion, the present studies have demonstrated an increase in G6Pase enzyme activity and a decrease in GK activity in liver samples obtained from morbidly obese patients with type 2 diabetes compared with those obtained from obese nondiabetic subjects. Preoperative studies indicating increased rates of EGP and hepatic insulin resistance in diabetic subjects suggest that the increase in EGP is related to defects in the regulation of G6Pase flux.

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