

Obesity and Type 2 Diabetes Impair Insulin-Induced Suppression of Glycogenolysis as Well as Gluconeogenesis

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To determine whether the hepatic insulin resistance of obesity and type 2 diabetes is due to impaired insulin-induced suppression of glycogenolysis as well as gluconeogenesis, 10 lean nondiabetic, 10 obese nondiabetic, and 11 obese type 2 diabetic subjects were studied after an overnight fast and during a hyperinsulinemic-euglycemic clamp. Gluconeogenesis and glycogenolysis were measured using the deuterated water method. Before the clamp, when glucose and insulin concentrations differed among the three groups, gluconeogenesis was higher in the diabetic than in the obese nondiabetic subjects ($P < 0.05$) and glycogenolysis was higher in the diabetic than in the lean nondiabetic subjects ($P < 0.05$). During the clamp, when glucose and insulin concentrations were matched and glucagon concentrations were suppressed, both glycogenolysis and gluconeogenesis were higher ($P < 0.01$) in the diabetic versus the obese and lean nondiabetic subjects. Furthermore, glycogenolysis and gluconeogenesis were higher ($P < 0.01$) in the obese than in the lean nondiabetic subjects. Plasma free fatty acid concentrations correlated ($P < 0.001$) with glucose production and gluconeogenesis both before and during the clamp and with glycogenolysis during the clamp ($P < 0.01$). We concluded that defects in the regulation of glycogenolysis as well as gluconeogenesis cause hepatic insulin resistance in obese nondiabetic and type 2 diabetic humans. *Diabetes* 54:1942–1948, 2005

Type 2 diabetes is characterized by both fasting and postprandial hyperglycemia. Numerous studies have established that glucose production in people with type 2 diabetes is either elevated or not appropriate for the prevailing glucose and insulin concentrations (1–8). The cause(s) of these inappropriately elevated rates of glucose production remains an area of active investigation. A series of studies have shown that gluconeogenesis, whether measured with magnetic reso-

nance spectroscopy (7) or the deuterated water method, is increased in people with type 2 diabetes (2,9–13). On the other hand, rates of glycogenolysis have been reported to not differ in diabetic and nondiabetic individuals (2,9,10,12). However, because both hyperglycemia and hyperinsulinemia are potent inhibitors of glycogenolysis (14–17), equal rates of glycogenolysis, despite higher glucose and insulin concentrations in diabetic subjects, imply abnormal regulation of the glycogenolytic as well as the gluconeogenic pathway.

We recently confirmed this supposition (11) by demonstrating that the contribution of glycogenolysis to endogenous glucose production (henceforth referred to as glycogenolysis) was fully suppressed in nondiabetic subjects when insulin concentrations were clamped at levels slightly above basal and glucose concentrations were raised to levels typically observed in people with type 2 diabetes (i.e., ~ 11 mmol/l). In contrast, glycogenolysis persisted in people with mild (e.g., fasting glucose ~ 8 mmol/l) or severe (e.g., fasting glucose ~ 12 mmol/l) diabetes who were studied under the same conditions. Of interest, the contribution of gluconeogenesis to endogenous glucose production (henceforth referred to as gluconeogenesis) was elevated in the subjects with severe diabetes before but not during the clamp when glucagon as well as glucose and insulin concentrations were matched.

Although the results of our previous study (11) indicated that glycogenolysis is inappropriately elevated in people with type 2 diabetes, we could not determine whether this was due to impaired insulin-induced suppression, glucose-induced suppression, or a combination of both as glucose and insulin concentrations were clamped at equal but elevated levels in all groups. Furthermore, because glucagon concentrations were maintained at basal levels via a concurrent glucagon infusion, we could not exclude the possibility that an unrecognized increase in the portal venous glucagon-to-insulin ratio in the diabetic subjects led to what appeared to be excessive rates of glycogenolysis. Finally, because both the diabetic and nondiabetic subjects in that study were obese, we could not determine if obesity per se impaired insulin-induced suppression of glycogenolysis as well as gluconeogenesis.

The present study was undertaken to address these questions raised by our previous study. Endogenous glucose production, gluconeogenesis, and glycogenolysis were measured in age- and sex-matched lean nondiabetic, obese nondiabetic, and obese diabetic subjects after an

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FFA, free fatty acid; G6P, glucose-6-phosphate.

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TABLE 1
Characteristics of study subjects

	Lean nondiabetic	Obese nondiabetic	Type 2 diabetic
<i>n</i>	10	10	11
Age (years)	55 ± 4	54 ± 4	59 ± 3
Sex (M/F)	4/5	4/6	5/6
BMI (kg/m ²)	23.0 ± 0.9	33.9 ± 1.6*	30.0 ± 1.7*
Body fat (%)	27.9 ± 3.1	43.0 ± 3.4*	35.4 ± 3.6*
Fat free mass (kg)	44.6 ± 3.3	50.7 ± 3.4*	51.3 ± 2.3*
Visceral fat (cm ²)	88.8 ± 15.8	219.6 ± 36.0*	187.5 ± 17.5*
Total abdominal fat (cm ²)	196.7 ± 23.2	486.2 ± 44.2*	430.8 ± 43.7*
A1C (%)	5.5 ± 0.1	5.6 ± 0.1†	8.2 ± 0.9‡

Data are means ± SE. **P* < 0.01 vs. lean nondiabetic; †*n* = 7 subjects; ‡*P* < 0.01 vs. lean and obese nondiabetic subjects.

overnight fast and during a hyperinsulinemic-euglycemic clamp. Somatostatin was infused to inhibit endogenous insulin secretion and thereby ensure that portal insulin concentrations were constant and equal in all groups. Glucagon concentrations were permitted to fall to determine if the increased rates of glycogenolysis observed in the diabetic subjects in our previous study (11) persisted when glucagon is suppressed. Gluconeogenesis was measured using the deuterated water method (18,19).

In our results, we report that obesity as well as type 2 diabetes impairs insulin-induced suppression of glycogenolysis as well as gluconeogenesis and that the degree of impairment correlates with plasma free fatty acid (FFA) concentrations.

RESEARCH DESIGN AND METHODS

Characteristics of the 11 obese diabetic, 10 obese nondiabetic, and 10 lean age- and sex-matched nondiabetic subjects studied are given in Table 1. All subjects were in good health and at a stable weight. None regularly engaged in vigorous physical exercise. None of the parents, siblings, or children (i.e., first-degree relatives) of the nondiabetic subjects had a history of diabetes. At the time of the screening, three of the diabetic subjects were being treated with lifestyle modifications alone, one with a sulfonylurea alone, two with metformin alone, two with a combination of a sulfonylurea and metformin, two with insulin alone, and one with both insulin and metformin. Oral hypoglycemic agents were discontinued 2 weeks before the study, and long-acting insulin was switched to regular insulin with meals beginning with the mid-day meal 2 days before the study. Subjects were on no medications at the time of study other than thyroxine. All subjects were instructed to follow a weight-maintenance diet (55% carbohydrate, 30% fat, and 15% protein) for at least 3 days before the day of the study.

Subjects were admitted to the Mayo Clinic General Clinical Research Center at 1700 on the evening before the study. A standard 10 kcal/kg meal (55% carbohydrate, 30% fat, and 15% protein) was eaten between 1730 and 1800. After blood was sampled for baseline enrichment, 1.67 g ²H₂O per kg weight of body water was given in three divided doses at 1800, 2000, and 2200. Small sips of water (containing ²H₂O) were permitted upon request. At 0500 on the morning of the study, an intravenous catheter was placed in a forearm vein in the left arm for the infusion of saline, isotopes, and hormone solutions. At ~0600, an infusion of 9,11,12,12-[²H₄]cortisol was started. Femoral arterial and hepatic venous catheters were placed as part of a separate protocol, the results of which have been reported in part elsewhere (20). A primed (fasting glucose in millimoles per liter divided by 5.5 mmol/l × 12 μCi), continuous (0.12 μCi/min) infusion of [³-³H]glucose (New England Nuclear, Boston, MA) was started at 0700 and continued until the end of the study. An infusion containing somatostatin (60 ng · kg⁻¹ · min⁻¹) and growth hormone (3 ng · kg⁻¹ · min⁻¹) was started at ~1000 (*t* = 0 min) and continued until the end of the study. An infusion of insulin was also started at *t* = 0 at a rate of 0.45 mU · kg lean body wt⁻¹ · min⁻¹ and continued until 240 min. Dextrose containing [³-³H]glucose was infused as necessary to maintain plasma glucose concentrations at ~ 5.0 mmol/l over the 4 h of the study. The rate of the concurrent

infusion of [³-³H]glucose was also decreased at *t* = 0, as previously described (21), to minimize the change in plasma glucose-specific activity.

Analytic techniques. Plasma samples were placed on ice, centrifuged at 4°C, separated, and stored at -20°C until being assayed. Glucose concentrations were measured using a glucose oxidase method (Yellow Springs Instrument, Yellow Springs, OH). Plasma insulin and growth hormone concentrations were measured using a chemiluminescence assay with reagents obtained from Beckman (Access Assay; Beckman, Chaska, MN). Plasma glucagon and C-peptide concentrations were measured by radioimmunoassay using reagents supplied by Linco Research (St. Louis, MO). FFA concentrations were measured using a calorimetric assay (COBAS; Roche Diagnostics, Indianapolis, IN). HbA_{1c} (A1C) was measured by affinity chromatography (Gly-Affin, Akron, OH; normal range 4–6.3%). (A1C was inadvertently not measured in three obese nondiabetic subjects due to a protocol error.) Body composition was measured using dual-energy X-ray absorptiometry (DPX scanner; Hologic, Waltham, MA) combined with a computerized tomographic scan at T11–12 and L3–4. Plasma [³-³H]glucose-specific activity and enrichments of deuterium on the 2nd and 5th carbon of plasma glucose were measured as previously described (18,22–25).

Calculations. The mean concentrations and rates from -30 to 0 (basal) and 210 to 240 (clamp) min were used for analysis. All rates are expressed per kilogram of lean body mass. Glucose appearance and disappearance were calculated using the steady-state equations of Steele et al. (26). Endogenous glucose production during the clamp was calculated by subtracting the exogenous glucose infusion rate from the total glucose appearance rate. The rate of gluconeogenesis was calculated by multiplying the plasma ratio of C5 and C2 glucose enrichments times endogenous glucose production (18,22). Glycogenolysis was then calculated by subtracting the rate of gluconeogenesis from endogenous glucose production (2,5,16).

Statistical analysis. Data in the text and figures are expressed as means ± SE. Rates are expressed as micromoles per kilogram of lean body mass per minute. Responses before and during the insulin infusion were assessed by taking the mean of the results present from -30 to 0 min and 210 to 240 min, respectively. ANOVA was used to compare results among groups, followed by Student's paired *t* test to determine whether rates of endogenous glucose production, gluconeogenesis, and glycogenolysis were higher in the diabetic versus the nondiabetic subjects and in the obese versus the lean subjects. Paired *t* tests were performed to determine if rates of glucose production, gluconeogenesis, and glycogenolysis at the end of the insulin infusion differed from zero. *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 obese and lean nondiabetic subjects before the insulin infusion (10.2 ± 0.8 vs. 5.4 ± 0.1 vs. 5.2 ± 0.1 mmol/l) but did not differ during the insulin infusion (5.5 ± 0.3 vs. 5.1 ± 0.1 vs. 5.0 ± 0.1 mmol/l) (Fig. 1). Fasting plasma insulin concentrations did not differ in the diabetic and obese nondiabetic subjects (52 ± 9 vs. 47 ± 9 pmol/l) but were higher (*P* < 0.01) in both groups than concentrations in the lean nondiabetic subjects (19 ± 2 pmol/l). Plasma insulin concentrations increased during the clamp to levels that did not differ among the three groups (144 ± 4 [diabetic] vs. 139 ± 10 [obese nondiabetic] vs. 138 ± 8 [lean nondiabetic] pmol/l). Fasting plasma C-peptide concentrations were higher (*P* < 0.01) in the obese diabetic and obese nondiabetic subjects than in the lean nondiabetic subjects before the clamp (0.65 ± 0.08 vs. 0.55 ± 0.07 vs. 0.33 ± 0.03 nmol/l). On the other hand, C-peptide concentrations were comparably suppressed during the clamp in all three groups (0.05 ± 0.01 vs. 0.03 ± 0.00 vs. 0.02 ± 0.00 nmol/l).

Plasma glucagon concentrations were higher (*P* < 0.05) in the diabetic and obese nondiabetic subjects than in the lean nondiabetic subjects both before (132 ± 5 vs. 145 ± 8 vs. 111 ± 6 pg/ml) and during (94 ± 5 vs. 102 ± 7 vs. 83 ± 3 pg/ml) the clamp. In contrast, glucagon concentrations did not differ in the obese diabetic and obese nondiabetic subjects either before or during the clamp.

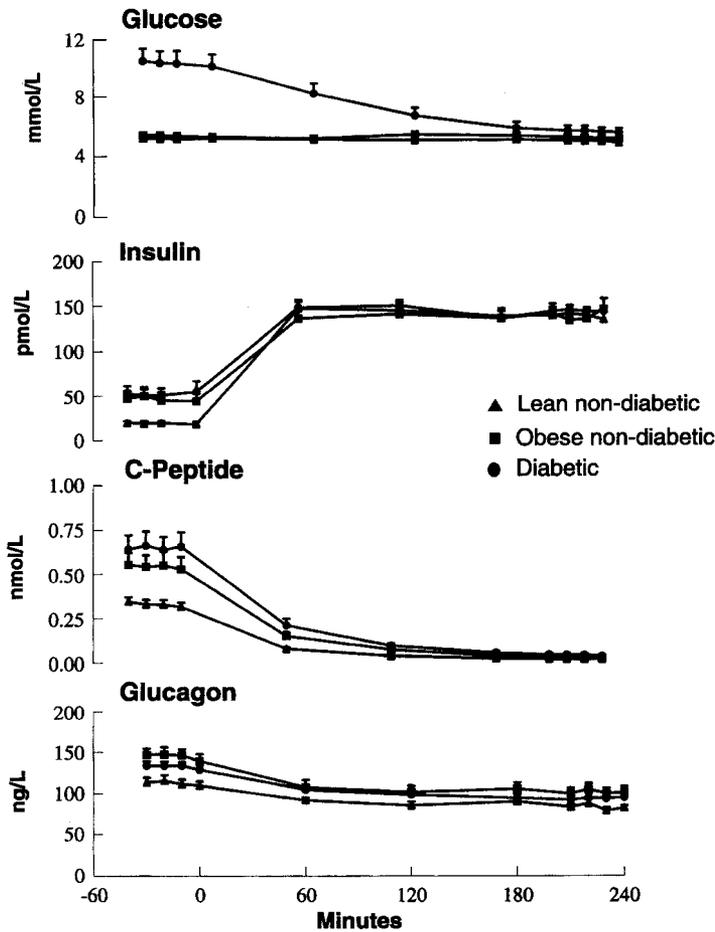


FIG. 1. Arterial plasma glucose, insulin, C-peptide, and glucagon concentrations before and during infusion of insulin in lean nondiabetic, obese nondiabetic, and diabetic subjects. The insulin infusion was started at $t = 0$.

Plasma FFA and growth hormone concentrations.

Plasma FFA levels did not differ among the diabetic, obese nondiabetic, and lean nondiabetic subjects (0.41 ± 0.03 vs. 0.35 ± 0.02 vs. 0.39 ± 0.03 mmol/l) before the clamp. During the clamp, plasma FFA concentrations did not differ between the diabetic and the obese nondiabetic subjects (0.09 ± 0.02 vs. 0.05 ± 0.01 mmol/l, $P = 0.08$) but were higher ($P < 0.05$) than those in the lean nondiabetic subjects (0.03 ± 0.00 mmol/l).

Growth hormone concentrations did not differ among the diabetic, obese nondiabetic, or lean nondiabetic groups either before (0.3 ± 0.2 vs. 0.7 ± 0.5 vs. 1.2 ± 0.5 ng/ml) or during (0.8 ± 0.0 vs. 0.7 ± 0.0 vs. 0.9 ± 0.1 ng/ml) the clamp.

Plasma C5 glucose and C2 glucose enrichments.

Plasma C5 glucose enrichment before the clamp did not differ among the groups (Table 2). On the other hand, plasma C5 glucose enrichment was higher ($P < 0.05$) in the diabetic than in the obese and lean nondiabetic subjects during the clamp. Plasma C5 glucose enrichment was also higher ($P < 0.001$) in the obese nondiabetic than in the lean nondiabetic subjects during the clamp. Plasma C5 glucose enrichment was lower ($P < 0.01$) during than before the clamp in all groups.

Plasma C2 glucose enrichment was higher ($P < 0.05$) in the diabetic than in the obese or lean nondiabetic subjects before and during the clamp. Plasma C2 glucose enrichment was lower ($P < 0.01$) during than before the insulin infusion in all groups. This resulted in plasma C5 glucose-to-C2 glucose ratios that did not differ among

the diabetic, obese nondiabetic, and lean nondiabetic subjects either before or during the clamp and were not altered during the clamp by insulin infusion.

Total glucose appearance and glucose clearance. Total glucose appearance (i.e., exogenously infused and endogenously produced glucose) was higher ($P < 0.05$) in the lean nondiabetic than in the obese nondiabetic or diabetic subjects (26.1 ± 2.2 vs. 19.2 ± 0.6 vs. 17.6 ± 2.5 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Glucose clearance was also higher ($P < 0.01$) in the lean nondiabetic than in the obese nondiabetic and diabetic subjects (5.3 ± 0.4 vs. 3.8 ± 0.4 vs. 3.3 ± 0.5 $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$).

Endogenous glucose production, gluconeogenesis, and glycogenolysis. Endogenous glucose production was

TABLE 2
Plasma C5 glucose and C2 glucose enrichment

	C5 glucose	C2 glucose	C5-to-C2 glucose ratio
Basal			
Lean nondiabetic	0.28 ± 0.02	0.47 ± 0.03	0.61 ± 0.05
Obese nondiabetic	0.33 ± 0.02	0.60 ± 0.02	0.55 ± 0.02
Diabetic	0.30 ± 0.01	$0.53 \pm 0.01^*$	0.58 ± 0.03
Clamp			
Lean nondiabetic	0.06 ± 0.01	0.12 ± 0.01	0.51 ± 0.04
Obese nondiabetic	$0.13 \pm 0.01^\dagger$	0.23 ± 0.02	0.56 ± 0.03
Diabetic	$0.22 \pm 0.03^*$	$0.39 \pm 0.04^\dagger$	0.56 ± 0.04

Data are means \pm SE. * $P < 0.05$ vs. lean and obese nondiabetic; $^\dagger P < 0.001$ vs. lean nondiabetic.

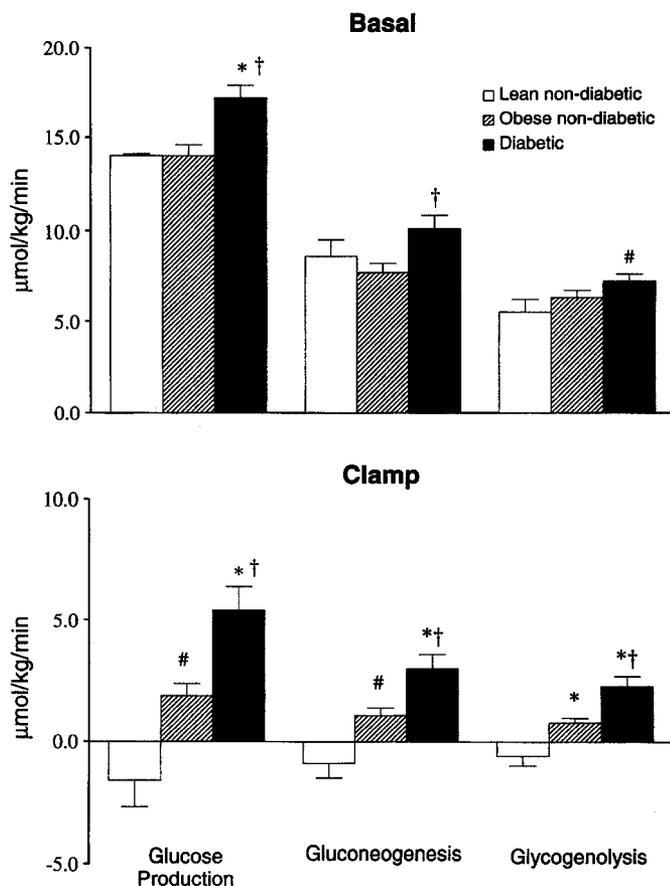


FIG. 2. Glucose production, gluconeogenesis, and glycogenolysis observed in lean nondiabetic, obese nondiabetic, and diabetic subjects after an overnight fast (basal) and during the final 40 min of a 4-h insulin infusion (clamp). * $P < 0.005$ vs. lean nondiabetic; # $P < 0.05$ vs. lean nondiabetic; † $P < 0.01$ vs. obese nondiabetic

higher ($P < 0.005$) in the diabetic subjects than in the obese or lean nondiabetic subjects before (17.2 ± 0.7 vs. 14.0 ± 0.6 vs. $14.0 \pm 0.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and during (5.4 ± 1.0 vs. 1.9 ± 0.5 vs. $-1.6 \pm 1.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) the insulin infusion (Fig. 2). Despite twofold higher insulin concentrations, endogenous glucose production in the obese nondiabetic subjects before the clamp did not differ from that in the lean nondiabetic subjects, thereby implying the presence of insulin resistance. This was confirmed during the insulin infusion when endogenous glucose production measured in the presence of matched insulin levels was higher ($P < 0.05$) in the obese than in the lean nondiabetic subjects.

Gluconeogenesis was higher ($P < 0.01$) before the insulin infusion in the diabetic than in the obese nondiabetic subjects (10.1 ± 0.7 vs. $7.7 \pm 0.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) but did not differ from that in the lean nondiabetic subjects ($8.6 \pm 0.9 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Gluconeogenesis in the diabetic subjects also was higher during the clamp than in either the obese ($P < 0.01$) or lean ($P < 0.001$) nondiabetic subjects (3.0 ± 0.6 vs. 1.1 ± 0.3 vs. $-0.9 \pm 0.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). In addition, gluconeogenesis during the clamp was higher ($P < 0.05$) in the obese than in the lean nondiabetic subjects. Of note, although gluconeogenesis was not fully suppressed (i.e., was greater than zero) in the diabetic ($P < 0.01$) and obese nondiabetic ($P < 0.01$)

subjects, it was fully suppressed (i.e., did not differ from zero) in the lean nondiabetic subjects.

Despite the higher glucose concentrations that were present before the clamp, the rates of glycogenolysis in the diabetic subjects were higher ($P < 0.05$) than in the lean nondiabetic subjects (7.2 ± 0.4 vs. $5.5 \pm 0.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and tended ($P < 0.08$) to be higher than in the obese nondiabetic subjects ($6.3 \pm 0.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), thereby implying abnormal regulation of glycogenolysis. This was confirmed during the clamp when glucose and insulin concentrations were matched. Glycogenolysis during the clamp was greater ($P < 0.005$) in the diabetic than in the obese and lean nondiabetic subjects (2.3 ± 0.4 vs. 0.8 ± 0.2 vs. $-0.6 \pm 0.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Glycogenolysis during the clamp was also greater ($P < 0.005$) in the obese nondiabetic subjects than in the lean nondiabetic subjects. Of interest, glycogenolysis was completely suppressed (i.e., did not differ from zero) in the lean nondiabetic subjects but was incompletely suppressed (i.e., was greater than zero) in both the diabetic ($P < 0.001$) and obese nondiabetic ($P < 0.005$) subjects.

Correlations. Plasma FFAs correlated with endogenous glucose production both before ($r = 0.57$; $P < 0.001$) and during ($r = 0.61$; $P < 0.001$) the clamp. Plasma FFAs also correlated with gluconeogenesis both before ($r = 0.61$; $P < 0.001$) and during the clamp ($r = 0.60$; $P < 0.001$). In contrast, although plasma FFAs did not correlate with glycogenolysis before the clamp ($r = 0.15$; $P = 0.5$), they correlated with glycogenolysis during the clamp ($r = 0.60$; $P < 0.005$). Visceral fat did not correlate with endogenous glucose production, gluconeogenesis, or glycogenolysis either before ($r = 0.17$; $P = 0.4$) or during ($r = 0.35$; $P = 0.06$) the clamp.

DISCUSSION

The present results indicate that insulin-induced suppression of glycogenolysis as well as gluconeogenesis is impaired by both obesity and type 2 diabetes. Because hyperglycemia and hyperinsulinemia are both potent inhibitors of glycogenolysis (14), the defect in its suppression was evident only when glucose and insulin concentrations were matched. This defect persisted when glucagon secretion was inhibited by a somatostatin infusion, indicating that factors other than glucagon excess impaired the ability of insulin to suppress gluconeogenesis and glycogenolysis in our type 2 diabetic subjects. Plasma FFAs correlated with rates of gluconeogenesis and glycogenolysis, suggesting that they influenced the regulation of both processes.

Previous studies using magnetic resonance spectroscopy or the deuterated water method have established that gluconeogenesis is increased in type 2 diabetic subjects after an overnight fast (2,7,9,9–13), with the magnitude of the increase correlating with the severity of diabetes and the degree of obesity (2,5,10,27). As in the present study, glycogenolysis in the basal state in those studies did not differ when rates in the diabetic subjects were compared with those in obese nondiabetic subjects (9). This is in contrast to our earlier study where rates of glycogenolysis were higher in the diabetic than in the nondiabetic subjects, presumably because the diabetic subjects in those studies were more severely hyperglycemic than those in

the present studies. In contrast, rates of glycogenolysis in the obese diabetic subjects in the basal state were elevated when compared with those in lean nondiabetic subjects, likely because of the combined effects of obesity and diabetes. However, because small increases in insulin and glucose alone or in combination result in marked suppression of glycogenolysis (14–17), a reexamination of the basal data in relation to the higher fasting glucose concentrations from present as well as previous studies indicates that the “normal” rates of glycogenolysis in the diabetic subjects were, in fact, excessive.

To our knowledge, only the study of Gastaldelli et al. (9) has attempted to circumvent the problem introduced by differences in glucose and insulin concentrations by measuring glycogenolysis and gluconeogenesis in diabetic and nondiabetic subjects during a hyperinsulinemic glucose clamp. That study, consistent with the present study, demonstrated that insulin-induced suppression of gluconeogenesis was impaired in obese diabetic compared with in obese nondiabetic subjects. However, in contrast to the present study, Gastaldelli et al. (9) reported glycogenolysis to be completely suppressed in both groups. There are several reasons why Gastaldelli et al. may have failed to detect an abnormality in insulin-induced suppression of glycogenolysis in their diabetic subjects. First, the insulin concentrations used in that study were approximately fivefold higher than those used in our study. Because glycogenolysis is sensitive to small changes in insulin, the very high insulin concentrations resulted in maximal suppression in both groups (15,16). Second, glucose concentrations in the Gastaldelli et al. (9) study were clamped at ~ 8.1 mmol/l in the diabetic subjects vs. ~ 5.0 mmol/l in the nondiabetic subjects. The higher glucose concentrations in the diabetic subjects presumably enhanced suppression of glycogenolysis and, in so doing, obscured differences between groups (14,17). Third, rates of gluconeogenesis were determined using the C5-to- $^2\text{H}_2\text{O}$ method. If steady state was not achieved in that study, then the plasma C5 glucose concentrations during the clamp could have been higher in the more insulin-resistant diabetic subjects due to a slower clearance of the C5 glucose present before the clamp rather than higher rates of gluconeogenesis during the clamp. If so, this would lead to falsely low rates of glycogenolysis, because glycogenolysis is calculated by subtracting gluconeogenesis from endogenous glucose production. Using the plasma C5-to-C2 glucose ratio rather than the plasma C5-to- $^2\text{H}_2\text{O}$ ratio minimizes this problem, as the ratio of plasma C5 to C2 glucose is not influenced by differences in glucose clearance. Of note, the observation in the present study that insulin-induced suppression of both glycogenolysis and gluconeogenesis is impaired in type 2 diabetes is consistent with previous studies in which these processes were measured under optimized conditions in diabetic rats (14).

Plasma FFA concentrations correlated with endogenous glucose production and gluconeogenesis both before and during the clamp. This finding is in agreement with the report of Gastaldelli et al. (9) and the well-established stimulatory effect of FFAs on gluconeogenesis (9,28,29). Boden et al. (30) have shown that an increase in plasma FFAs produced by means of an intralipid heparin infusion impairs insulin-induced suppression of glycogenolysis in

nondiabetic subjects. In contrast, Chu et al. (28) have reported that elevated FFAs in the presence of hyperglycemia enhance, rather than impair, suppression of glycogenolysis. The observation that plasma FFAs were positively correlated with glycogenolysis during the clamp in the current study suggests that endogenous FFAs also modulate the ability of insulin to suppress glycogenolysis in obese nondiabetic and diabetic humans. Although correlation does not prove causality, the fact that the contribution of both glycogenolysis and gluconeogenesis to endogenous glucose production during the clamp increased with increasing FFA concentrations suggests that FFAs influence a pathway common to both processes (e.g., glucose-6-phosphatase flux). The lack of correlation between FFA concentrations and glycogenolysis before the clamp in our study was likely due to the confounding effects of differences in glucose, insulin, and glucagon concentrations among the three groups. However, these differences did not obscure the relation between fatty acid concentrations and gluconeogenesis that was equally evident before and during the clamp, perhaps reflecting a more marked and/or additional effect of FFAs or a closely associated substrate (e.g., glycerol) on gluconeogenesis.

Glucagon stimulates both glycogenolysis and gluconeogenesis (31). Glucagon concentrations were higher in the diabetic subjects than in the lean nondiabetic subjects but did not differ from those present in the obese nondiabetic subjects. In addition, glucagon concentrations fell in all three groups during the clamp. Therefore, although elevated fasting glucagon concentrations could exacerbate an underlying defect in the regulation of the gluconeogenic and glycogenolytic pathways, hyperglucagonemia is unlikely to be the sole cause of the increased rates of glycogenolysis and gluconeogenesis observed in the diabetic subjects. Regardless of the etiology of these abnormalities, the observations that insulin-induced suppression of glycogenolysis and gluconeogenesis was impaired in the obese nondiabetic subjects relative to in the lean nondiabetic subjects and that obesity commonly precedes diabetes add further support to the premise that hepatic insulin resistance occurs early in the evolution of type 2 diabetes.

Although the deuterated water method is generally accepted as the most accurate method for measuring gluconeogenesis, it too has limitations. The infusion of exogenous glucose does not alter the plasma C5-to-C2 glucose ratio, as it comparably lowers the concentration of both C5 glucose and C2 glucose. Therefore, differences in the infusion rate of exogenous glucose cannot account for the differences in the glycogenolysis and gluconeogenesis rates among groups. This method measures only the contribution of gluconeogenesis to glucose production rather than the contribution of gluconeogenesis to glycogen formation (18,23). Glucose cycling in which extracellular glucose is taken up by the liver, phosphorylated to glucose-6-phosphate (G6P), then dephosphorylated back to glucose can result in the labeling of the 2nd carbon of glucose with deuterium during equilibration of G6P and fructose-6-phosphate within the hepatocyte (18). This could result in an overestimate of glycogenolysis. Efendic et al. (32) have reported that glucose cycling is increased in patients with type 2 diabetes in the presence of hyper-

glycemia. However, we have observed no difference in glucose cycling in diabetic and nondiabetic subjects in the presence of euglycemia and insulin concentrations equivalent to those used in the present study, presumably because there is limited hepatic glucose uptake under these conditions (33). On the other hand, the transaldolase reaction can lead to an overestimation of gluconeogenesis by causing C5 labeling of glycogen-derived G6P, which is then released as glucose (23). In addition, C5 G6P synthesized via the gluconeogenic pathway can be incorporated into glycogen then subsequently released from glycogen back to C5 G6P. Diabetes may increase "glycogen cycling" (14,18,23); if so, the contribution of gluconeogenesis may have been overestimated and the contribution of glycogenolysis to glucose production underestimated in the diabetic subjects. In addition, incorporation of the systematically infused [$^3\text{-}^3\text{H}$]glucose into glycogen followed by subsequent release back into plasma can cause an underestimation of endogenous glucose production. Therefore, rates of endogenous glucose production may also have been underestimated in the diabetic subjects. Finally, measurement of gluconeogenesis and glycogenolysis in vivo in humans is complex and lacks a "gold standard." For this reason, rates reported in this and other studies in humans should be considered as qualitative rather than quantitative.

In summary, the results of the present study indicate that the hepatic insulin resistance associated with obesity and type 2 diabetes results in impaired insulin-induced suppression of glycogenolysis as well as gluconeogenesis. These defects persist when glucagon secretion is inhibited, indicating that factors other than hyperglucagonemia cause these abnormalities. The correlation between plasma FFAs and rates of glycogenolysis and gluconeogenesis suggest that FFAs may be one such factor. The observation that glycogenolysis contributes to excessive glucose production in type 2 diabetic patients provides insight as to why prolonged fasting and its associated decrease in hepatic glycogen content results in a marked decrease in glucose concentration and restoration of glucose production to rates observed in nondiabetic subjects (34,35). Furthermore, these data add to the growing body of evidence indicating that obesity and diabetes both cause hepatic insulin resistance (6,36–40). They also suggest that complete normalization of hepatic glucose metabolism will require correction of the pathogenic processes that alter regulation of glycogenolysis as well as gluconeogenesis in people with type 2 diabetes.

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