Suppression of Gluconeogenesis After a 3-Day Fast Does Not Deplete Liver Glycogen in Patients With NIDDM

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To determine the effect of inhibition of gluconeogenesis on liver glycogen stores in patients with non-insulin-dependent diabetes mellitus (NIDDM) after a 3-day fast, 10% ethanol (EtOH) was administered intravenously to nine obese patients with NIDDM and six obese nondiabetic subjects. Rates of glucose appearance (3-[^{3}H]glucose) and [U-^{14}C]alanine incorporation into glucose (alanine gluconeogenesis [Ala-GNG]) were determined before and during EtOH administration, and residual glycogen stores were assessed by the incremental glucose response to glucagon ($glucose_{AUC}$). Hepatic glucose output (HGO) was closely correlated with plasma glucose levels ($r = 0.71$, $P < 0.001$) after the 3-day fast and was significantly greater in the diabetic compared with the nondiabetic subjects (13.8 ± 1.4 vs. 7.6 ± 0.6 $\mu mol \cdot kg^{-1}$ FFM $\cdot$ min^{-1}, $P < 0.01$). During the 120-min EtOH infusion, Ala-GNG fell by more than 50% in both groups and did not increase after intravenous glucagon administration. HGO fell modestly in both the diabetic and nondiabetic subjects during the first 30 min of EtOH infusion and stabilized thereafter. In contrast to Ala-GNG, HGO increased significantly after intravenous glucagon administration in both the diabetic and nondiabetic subjects, but the increase was significantly greater in the patients with NIDDM ($P < 0.01$). The glucose area under the curve in response to glucagon ($glucose_{AUC}$) was lower in the presence of EtOH than in its absence (14.9 ± 7 vs. 68 ± 15.6 mM/min, $P < 0.01$) in the obese nondiabetic subjects, which suggests a decrease in liver glycogen stores. In contrast, EtOH infusion did not alter the $glucose_{AUC}$ in obese diabetic subjects compared with saline control studies (134.7 ± 17.8 vs. 102.6 ± 20 mM/min, NS). The persistent increase in $glucose_{AUC}$ suggests that glycogen stores are not depleted by EtOH-induced suppression of gluconeogenesis in patients with NIDDM after a 3-day fast. *Diabetes* 43:256–62, 1994

Non-insulin-dependent diabetes mellitus (NIDDM) is characterized by increased hepatic glucose output (HGO) (1), which may be derived from either glycogenolysis or gluconeogenesis, or both. In nondiabetic subjects, postabsorptive rates of gluconeogenesis have been variously reported to account for 30 (2) to 70% (3) of HGO, increasing to more than 90% of HGO as the fast is extended to 64 h (2,3). At that time, liver glycogen stores are depleted as indicated by the appearance of hypoglycemia when gluconeogenesis is inhibited by ethanol (EtOH) (4). The resistance to EtOH-induced hypoglycemia after a 3-day fast observed in obesity has been attributed to the persistence of liver glycogen sustaining HGO (4). Thus, maintenance of HGO during EtOH-inhibited gluconeogenesis must reflect glycogen status.

Decreased glycogen synthesis and increased glycogenolysis are expected consequences of the diabetic state and would predict low hepatic glycogen concentrations. However, actual glycogen determinations have produced conflicting data (5). Recently, the elegant studies of Shulman et al. (6) using ^{13}C NMR have reported lower hepatic glycogen stores in patients with NIDDM compared with nondiabetic control subjects after an overnight fast and have suggested that gluconeogenesis accounts for nearly 90% of HGO in NIDDM after a 23-h fast (6). However, we have previously presented evidence indicating that after a 3-day fast liver glycogen stores are increased in patients with NIDDM compared

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Received for publication 5 May 1993 and accepted in revised form 7 October 1993.

NIDDM, non-insulin-dependent diabetes mellitus; EtOH, ethanol; $glucose_{AUC}$, glucose area under the curve in response to glucagon; HGO, hepatic glucose output; G-6-P, glucose-6-phosphate; FFA, free fatty acid; R_a, rate of glucose appearance; R_d, rate of glucose disappearance; ANOVA, analysis of variance; β-OHB, β-hydroxybutyrate; AcAc, acetoacetate; Ala-GNG, alanine gluconeogenesis.

Table 1
Baseline demographic data for obese NIDDM and nondiabetic subjects

	Nondiabetic subjects	NIDDM subjects
n	14	11
Age (years)	32 ± 3	40 ± 2
Height (m)	1.67 ± 0.01	1.63 ± 0.01
Weight (kg)	101.6 ± 8.2	108.1 ± 8.1
Body mass index (kg/m^2)	38.2 ± 2.7	38.4 ± 2.4
Body fat (%)	37.7 ± 1.3	40.1 ± 1.6

Data are means ± SE. Body fat was based on skin-fold determinations (42).

with control subjects fasted similarly (7). For liver glycogen to be preserved or perhaps even increased during fasting, gluconeogenesis must equal or exceed HGO with some shunting of glucose-6-phosphate (G-6-P) formed from gluconeogenesis into glycogen. Plasma concentrations of gluconeogenic promoters and substrates in NIDDM during a fast are more than adequate to sustain the increased rates of gluconeogenesis (8). Thus, the fall in HGO during a 3-day fast in patients with NIDDM may alter carbohydrate economy in the form of an increase in liver glycogen (7). The present studies were designed to determine the effect of EtOH-induced inhibition of gluconeogenesis on HGO after a 3-day fast in NIDDM patients. Under these conditions, glycogenolysis would be required to sustain HGO. The studies have demonstrated that the level of glycogen stores in patients with NIDDM after a 3-day fast is sufficient to prevent a fall in HGO during EtOH-induced suppression of gluconeogenesis, and to elicit a normal response to a subsequent injection of intravenous glucagon. The liver glycogen that accumulates during a 3-day fast appears to be derived from gluconeogenesis.

RESEARCH DESIGN AND METHODS

A total of 14 obese patients with recent-onset NIDDM and 11 age- and weight-matched obese nondiabetic subjects (Table 1) were admitted to the Clinical Research Center at the Medical College of Virginia and underwent a 3-day fast, during which time the subjects received only water and ice-chips. On the third day of the fast, a primed, continuous infusion of [3-^{3}H]glucose and [U-^{14}C]-alanine was administered intravenously, and baseline blood samples were obtained after steady state had been achieved (3 h for the patients with NIDDM and 2 h for the nondiabetic control subjects). In some of the subjects (5 diabetic and 5 nondiabetic subjects), an intravenous injection of glucagon (1.0 mg) was administered and sequential blood samples were obtained every 15 min for the next hour as described previously (7). The remaining subjects (9 diabetic and 6 nondiabetic subjects) received an intravenous infusion of 10% EtOH (200 ml/h for 3 h). Blood samples were obtained every 30 min for the first 2 h of EtOH administration. To assess residual glycogen stores, glucagon was administered as above and blood samples were obtained every 15 min for the final hour of study.

Analyses. Arterialized venous blood samples were obtained before and after glucagon administration at the times listed above for the determination of insulin (9), C-peptide (10), glucagon (11), and cortisol (γ-Coat, Clinical Assays, Cambridge, MA). Plasma glucose was measured immediately by the glucose oxidase method (Beckman, Fullerton, CA). Plasma free fatty acid (FFA) was determined by enzymatic methods (12). Blood samples for measurement of intermediary metabolites were immediately deproteinized with ice-cold 3 M perchloric acid. The supernatant was neutralized with 3 M KOH and the resulting supernatant assayed for L-lactate (13), alanine (14), β-hydroxybutyrate (β-OHB) (14), acetoacetate (AcAc) (14), and glycerol (15).

To assess plasma radioactivity, additional blood samples were immediately deproteinized with BaOH and $ZnSO_4$ (16). The supernatant was then chromatographed over sequential anion (Dowex 1X8, Cl^- form) and cation (Dowex 50X8, H^+ form) exchange resins and the neutral fraction was air-dried and counted in a Beckman LS-1700 liquid scintillation counter (Glenroth, Scotland). Labeled alanine and lactate were eluted separately from the exchange resins (17) and counted. After isotopic equilibration (3 h), rates of glucose appearance (R_a) and disappearance (R_d) were calculated from the equations of Steele as modified by DeBodo (18) using a glucose pool fraction of 0.65 (19). Because no glucose was infused during the isotopic studies, HGO is equal to R_a. For the determination of gluconeogenesis, the dual isotope technique of Chiasson et al. (20) was used. Because the infusion of EtOH during concomitant [^{14}C]alanine infusion is associated with an increase in [^{14}C]lactate radioactivity, as well as an increase in alanine specific activity, the addition of lactate specific activity to that of alanine might logically be used to calculate gluconeogenesis. However, the inclusion of lactate radioactivity in these calculations is controversial. We have included only the alanine specific activity for our calculations, with the understanding that the anticipated fall in gluconeogenesis during EtOH administration will be underestimated by this omission. Although the measurement of Ala-GNG represents a fraction of total gluconeogenesis, we (21) and others (17,22) have shown that the use of labeled alanine reflects overall gluconeogenic trends in the absence and presence of EtOH. The incremental glucose response to glucagon was calculated as the area under the curve (AUC) using the trapezoidal rule (× minute − value at baseline), where baseline is defined as the mean of the −15- and 0-min sample before glucagon administration (23).

Compilation and analysis of data was performed using CLINFO (BBN), a database management package provided by the General Clinical Research Centers, National Institutes of Health. Statistical analysis between groups was performed using repeated measures analysis of variance (ANOVA) or analysis of covariance and within groups using repeated measures ANOVA and Dunnett's multiple comparison test (SAS, Cary, NC). Where appropriate, Student's *t* test was performed. Data are expressed as means ± SE. Statistical significance was assumed when $P < 0.05$. The investigations were ap-

Table 2
Baseline plasma insulin, C-peptide, glucagon, and cortisol levels after an overnight (12- to 14-h) and a 3-day (64-h) fast in obese NIDDM and nondiabetic subjects

	Nondiabetic subjects		NIDDM subjects	
	14 h	64 h	14 h	64 h
Glucose (mM)	5.5 ± 0.1	3.9 ± 0.1*	14.9 ± 1.1†	9.9 ± 0.8*†
Insulin (pM)	53.4 ± 6.6	10.8 ± 1.8*	133.2 ± 30.6	36.0 ± 7.8*
C-peptide (nM)	0.83 ± 0.09	0.26 ± 0.03*	0.76 ± 0.09	0.48 ± 0.06†
Glucagon (pg/ml)	94.5 ± 4.9	114.3 ± 9.7*	151.4 ± 13.7‡	143.1 ± 12.4
Cortisol (nM)	496.8 ± 45.8	331.2 ± 58.2	434.7 ± 47.5	404.1 ± 50.5

Data are means ± SE.
*$P < 0.01$ compared with 14-h value.
†$P < 0.01$ compared with control subjects.
‡$P < 0.05$ compared with control subjects.

proved by the Medical College of Virginia Committee for the Conduct of Human Research, and informed consent was obtained from each subject before the study.

RESULTS

Effect of fasting on baseline glucoregulatory hormones and intermediary metabolites. Fasting plasma glucose levels fell significantly during the fast in both the diabetic (14.9 ± 1.1 to 9.9 ± 0.8 mM, $P < 0.0001$) and nondiabetic subjects (5.4 ± 0.1 to 3.9 ± 0.1 mM, $P < 0.01$). As shown in Table 2, plasma insulin and C-peptide levels also fell during the fast and were consistently greater in the patients with NIDDM compared with the nondiabetic subjects. Plasma glucagon levels were also greater in patients with NIDDM compared with obese control subjects after an overnight fast. However, glucagon levels did not change during the 3-day fast in the diabetic subjects, but rose significantly in the obese nondiabetic subjects. Plasma cortisol levels were not different in the two groups on either day of study. Levels of intermediary metabolites (Table 3) in patients with NIDDM after a 14-h fast reflect an increase in gluconeogenic substrates (lactate) as well as increased FFA and concomitant oxidation (as assessed by ketone body concentrations) compared with obese nondiabetic subjects. In contrast, plasma FFA and gluconeogenic substrate levels were comparable in the two groups after the fast had been extended for an additional 48 h. In five of the patients with NIDDM, plasma glucose fell below 7.8 mM after the 3-day fast. Plasma glucose levels after 14 h of fasting were also significantly lower in these five subjects compared with those who failed to respond to fasting (17.4 ± 1.2 vs. 10.6 ± 0.5 mM, $P < 0.01$). However, no other differences in glucoregulatory hormones or intermediary metabolite levels could be detected that distinguished these subjects from the remaining nine patients. For this reason, they are included in the mean data for the NIDDM group.

Effect of EtOH administration on HGO and Ala-GNG. After 64 h of fasting, HGO was significantly correlated with plasma glucose (Fig. 1, $r = 0.71$, $P < 0.001$) and was greater in the patients with NIDDM compared with the control group (13.8 ± 1.4 vs. 7.6 ± 0.6 $\mu mol \cdot kg^{-1}$ $FFM \cdot min^{-1}$, $P < 0.01$). Baseline rates of Ala-GNG were similar in the diabetic (1.6 ± 0.2 $\mu mol \cdot kg^{-1}$ $FFM \cdot min^{-1}$) and nondiabetic subjects (2.4 ± 0.3 $\mu mol \cdot kg^{-1}$ $FFM \cdot min^{-1}$). After the initiation of EtOH administration, plasma EtOH levels rose progressively in both subject groups to a maximum of 14 mM at 180 min (Fig. 2*A*). Evidence of prompt intrahepatic EtOH metabolism is provided by a marked increase in the β-OHB/AcAc ratio during the first 30 min of EtOH infusion in both the obese nondiabetic subjects (3.7 ± 0.5 to 5.2 ± 0.6, $P < 0.01$) and in the patients with NIDDM (2.3 ± 0.2 to 8.1 ± 2.4,

Table 3
Baseline plasma alanine, lactate, FFAS, glycerol, β-OHB, and AcAc after 14 and 64 h of fasting in obese NIDDM and nondiabetic subjects.

	Nondiabetic subjects		NIDDM subjects	
	14 h	64 h	14 h	64 h
Alanine (mM)	0.41 ± 0.02	0.28 ± 0.02*	0.39 ± 0.02	0.25 ± 0.01*
Lactate (mM)	1.27 ± 0.10	0.99 ± 0.07	1.96 ± 0.23†	1.11 ± 0.10*
FFA (mM)	0.52 ± 0.08	0.91 ± 0.10‡	0.98 ± 0.09§	0.87 ± 0.09
Glycerol (mM)	0.14 ± 0.02	0.15 ± 0.02	0.11 ± 0.01	0.08 ± 0.01*
β-OHB (mM)	0.10 ± 0.02	1.59 ± 0.25‡	0.33 ± 0.09	1.25 ± 0.23‡
AcAc (mM)	0.05 ± 0.01	0.44 ± 0.06*	0.17 ± 0.06	0.45 ± 0.08*

Data are means ± SE.
*$P < 0.001$ compared with 14-h value.
†$P < 0.05$ compared with control.
‡$P < 0.01$ compared with 14-h value.
§$P < 0.01$ compared with control subjects.

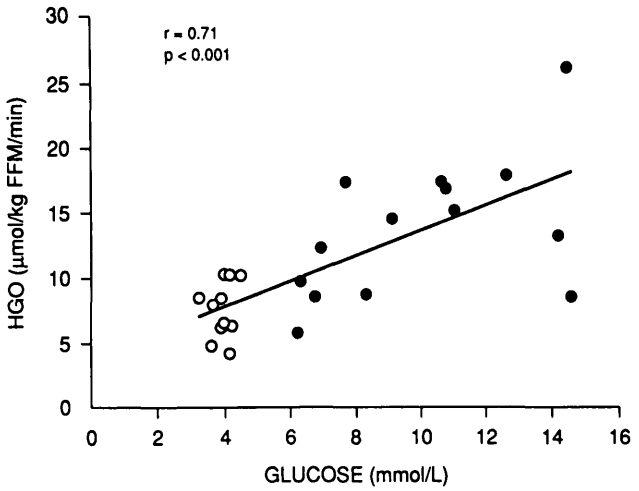

FIG. 1. Correlation between basal plasma glucose levels (mM) and HGO (μmol · kg^{-1}FFM · min^{-1}) after a 3-day fast in obese diabetic (n = 14, ●) and nondiabetic (n = 11, ○) subjects.

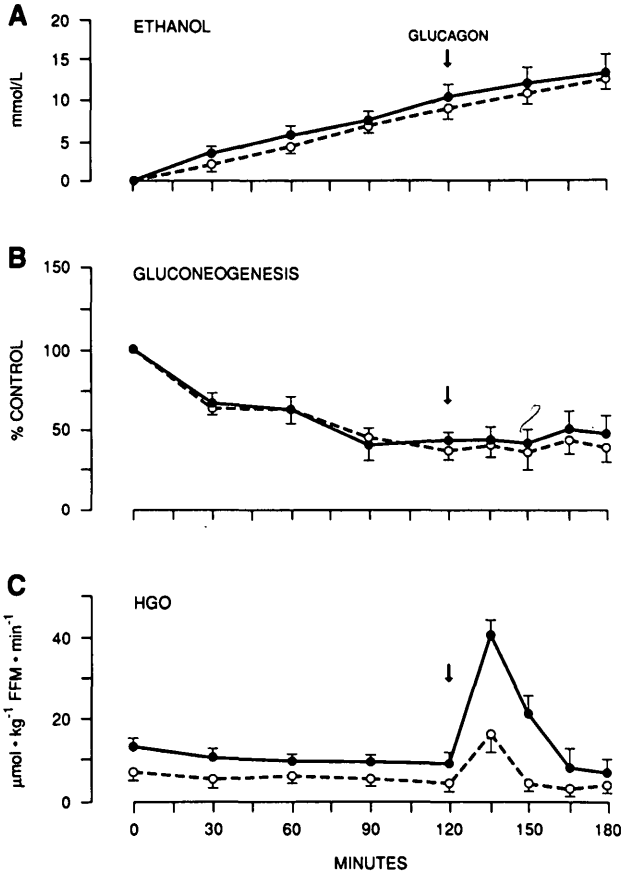

FIG. 2. Effect of 10% EtOH infusion on plasma EtOH levels (*A*), [U-^{14}C]alanine incorporation into glucose (gluconeogenesis, expressed as percentage of control, *B*), and overall HGO (expressed as μmol · kg−1FFM · min−1, *C*) in obese diabetic (n = 9, ●) and nondiabetic (n = 6, ○) subjects after a 3-day fast before and after 1.0 mg intravenous glucagon administration. Data are expressed as means ± SE.

$P < 0.01$), which did not change significantly thereafter. Plasma lactate levels rose (0.92 ± 0.09 to 2.00 ± 0.16 mM, $P < 0.01$ and 1.04 ± 0.14 to 2.52 ± 0.33 mM, $P < 0.01$) and plasma alanine levels fell (0.30 ± 0.03 to 0.22 ± 0.01 mM, $P < 0.01$ and 0.25 ± 0.01 to 0.21 ± 0.01 mM, $P < 0.01$) during 120 min of EtOH infusion in the nondiabetic and diabetic subjects, respectively. In addition, [^{14}C]lactate radioactivity increased significantly during 120 min of EtOH administration in the obese nondiabetic (849 ± 186%) and diabetic (530 ± 151%) individuals, presumably as a result of transamination of [^{14}C]alanine to lactate (22). Even when this loss of [^{14}C]alanine radioactivity is not accounted for in the calculations (see METHODS), Ala-GNG was suppressed by >50% in both groups during EtOH infusion (Fig. 2*B*). Examination of [^{14}C]glucose radioactivity profiles (DPM/ml) before and during EtOH infusion in the patients with NIDDM revealed a delay in the fall in plasma radioactivity compared with the nondiabetic subjects, which may represent the release of labeled glucose from glycogen. Plasma [^{14}C]glucose radioactivity fell 10% in the first 60 min of EtOH administration in the nondiabetic subjects, and rose 4% in the patients with NIDDM ($P < 0.05$ compared with nondiabetic subjects). Thereafter, [^{14}C]glucose radioactivity fell in the patients with NIDDM to 93.5 ± 4.5% of baseline after 120 min of EtOH infusion. In contrast, a progressive fall in [^{14}C]glucose radioactivity was observed in the nondiabetic subjects to 80.4 ± 3.6% of baseline at 120 min.

HGO fell modestly in both groups during the first 30 min of infusion but did not change further as the infusion was continued for an additional 90 min (Fig. 2*C*). Glucose utilization (R_d) also fell during the EtOH infusion in the nondiabetic (9.3 ± 0.4 to 6.8 ± 0.8 μmol · kg^{-1} FFM · min^{-1}, $P < 0.01$) and diabetic subjects (13.8 ± 1.2 to 11.8 ± 1.5 μmol · kg^{-1} FFM · min^{-1}, $P < 0.01$). However, R_d consistently exceeded HGO during the EtOH infusion. As a result of the greater rates of peripheral glucose utilization compared with HGO, plasma glucose levels fell during the 120-min EtOH infusion in the nondiabetic (3.9 ± 0.1 to 3.1 ± 0.3 mM, $P < 0.01$) and diabetic (9.9 ± 0.9 to 8.2 ± 0.7 mM, $P < 0.01$) subjects. Plasma insulin, C-peptide, and glucagon levels did not change from baseline during EtOH infusion (data not shown).

After the intravenous administration of glucagon, a prompt and significant rise in HGO was observed in all of the diabetic and nondiabetic subjects (Fig. 2*C*). However, the response was significantly greater in the patients with NIDDM ($P < 0.01$). No change in Ala-GNG was observed in response to glucagon administration, which suggests that the increase in HGO observed after glucagon administration reflects an increase in glycogenolysis.

To compare the effect of EtOH-diminished gluconeogenesis with saline control on glycogen stores in each study group, the glucose response to glucagon in the absence and presence of EtOH administration was examined (Fig. 3). After a 3-day fast in obese nondiabetic subjects (nonpaired data), the glucose$_{AUC}$ was significantly diminished by 2 h of EtOH infusion (68 ± 15.6 vs. 14.9 ± 7 mM · min, $P < 0.01$). Surprisingly, the 2-h EtOH infusion did not reduce the glucose$_{AUC}$ in the patients with NIDDM (102.6 ± 20 vs. 134.7 ± 17.8 mM/min, NS,

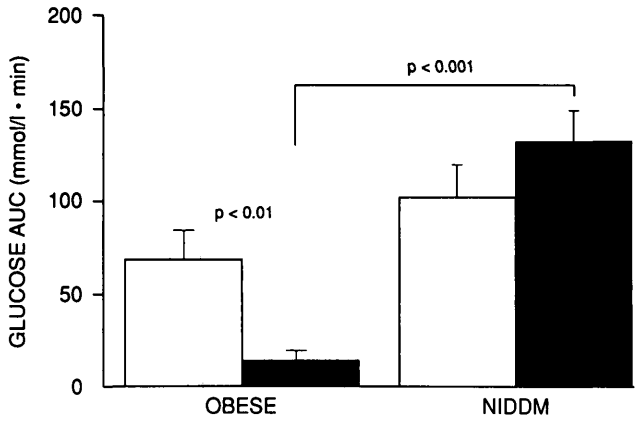

FIG. 3. Glucose response to glucagon ($glucose_{AUC}$) in obese diabetic and nondiabetic subjects after a 3-day fast in the absence (□) and presence (■) of intravenous infusion of 10% EtOH for 120 min. Data are expressed as means ± SE.

nonpaired data). Thus, the $glucose_{AUC}$ in the NIDDM patients was significantly greater than in the nondiabetic subjects after EtOH infusion ($P < 0.001$).

DISCUSSION

The present studies have indicated that liver glycogen stores are increased in patients with NIDDM after a 3-day fast, and that inhibition of gluconeogenesis for 2 h is insufficient to deplete those stores, in contrast to age- and weight-matched control subjects. We have previously used the glucose response to glucagon to show that liver glycogen stores are increased in patients with NIDDM (7). This method was first validated by Landau et al. (24). In this study, which incorporated measures of HGO, it can be seen that the $glucose_{AUC}$ nicely reflects the increase in HGO in response to glucagon. The increase in HGO is probably attributable solely to increased glycogenolysis because gluconeogenesis was inhibited by EtOH. The failure of glucagon to acutely increase alanine incorporation into glucose after fasting also indicates that glucagon's ability to increase HGO is mainly attributable to glycogenolysis. Under these conditions (prolonged fast and/or EtOH infusion), we believe the $glucose_{AUC}$ and HGO response to glucagon reflects glycogen stores. Thus, the greater response of patients with NIDDM compared with the nondiabetic control subjects after EtOH administration supports our initial observations that glycogen stores are increased in patients with NIDDM after a 3-day fast (7).

Although we have demonstrated similar EtOH-induced suppression of Ala-GNG in the diabetic and nondiabetic subjects, it is possible that differences in the percentage suppression of overall gluconeogenesis by EtOH could have contributed to our findings. To our knowledge, no studies have directly compared the effect of EtOH on gluconeogenesis and glycogenolysis in diabetic and nondiabetic animals in vivo. However, data obtained from studies in humans using the techniques employed in the present studies suggest that EtOH-induced suppression of gluconeogenesis is equivalent in diabetic and nondiabetic persons. Incorporation of labeled lactate and glycerol into glucose is decreased by ~70% in nonobese patients with NIDDM during EtOH administration (25). Similar studies in nonobese nondiabetic subjects using labeled alanine or lactate have shown nearly identical suppression of gluconeogenesis (17,26). Given greater HGO in the diabetic subjects after fasting and assuming overall rates of gluconeogenesis that account for more than 70% of HGO, similar suppression of gluconeogenesis during EtOH infusion in the diabetic subjects would require greater release of glycogen to sustain HGO in the diabetic subjects. On the other hand, if, after fasting, *1*) glycogenolysis contributes >30% to HGO in the patients with NIDDM and *2*) the EtOH-induced suppression of gluconeogenesis is only 50% of that of the nondiabetic subjects, then the greater glucose response to glucagon in the diabetic subjects would suggest similar but not necessarily greater glycogen stores before EtOH infusion. In either case, levels of liver glycogen after a 3-day fast in patients with NIDDM are sufficient to prevent a fall in HGO during EtOH infusion.

Attempts to quantitate precisely hepatic glycogen stores in patients with NIDDM have been hindered by methodological concerns (5) and have yielded conflicting results. Recent studies using ^{13}C-NMR spectroscopy to assess [1-^{13}C]glycosyl residues in vivo have suggested that glycogen stores are diminished in patients with NIDDM after an overnight fast (14–16 h) compared with nondiabetic control subjects, and fall further as the fast is extended to 23 h (6). However, the levels of liver glycogen reported in patients with NIDDM after a 23-h fast (6) are similar to those reported after a 64-h fast in nondiabetic subjects (50 mM, 3). Thus, it is surprising that infusions of EtOH do not suppress HGO in patients with NIDDM after an overnight fast (25) but do in fasted nondiabetic subjects with similar hepatic glycogen content (4). Moreover, acute suppression of FFA, known promoters of gluconeogenesis (27), does not alter the increased HGO of NIDDM (28–30), whereas chronic (>10–12 h) suppression of lipolysis (31) or FFA oxidation (32) reduces HGO, presumably after liver glycogen stores have fallen below a threshold value (50 mM?). In this study, EtOH was used to suppress gluconeogenesis and yet the expected fall in HGO was prevented, consonant with the above observations.

Although it is not possible to quantitate liver glycogen with the methods used in this study, calculations based on these as well as other published data may provide some insight into the regulation of hepatic glycogen metabolism in NIDDM. Glucose appearance in patients with NIDDM after an overnight fast has been reported to exceed that of nondiabetic subjects by ~2–3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (1). During a fast, HGO falls from 12 to 7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in nondiabetic subjects and from 14 to 10 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in NIDDM. If rates of gluconeogenesis, which account for nearly all of HGO, do not change during a 3-day fast in patients with NIDDM, but HGO falls, it is reasonable to assume that some of the newly synthesized G-6-P could be stored as liver glycogen. During a 3-day fast, the diversion of as little as 1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ of G-6-P would increase liver glycogen by more than 90 mM of liver in 24 h and 180 mM in

2 days. Unfortunately, we do not have an accurate estimation of the amount of G-6-P shunted into the glycogen pathway. However, we can make a reasonable estimate of the least amount of glycogen present based on two assumptions. The first assumption is that liver glycogen concentrations of 50 mM are not sufficient to sustain HGO during an EtOH infusion. The second assumption is that EtOH inhibits gluconeogenesis by 60%, based on figures from the literature. Indeed, rates of alanine incorporation into glucose fell by more than 50% during EtOH administration in this study. However, this figure is likely to be an underestimation of the inhibition of ALA-GNG that occurred during EtOH administration because of the rapid conversion of [^{14}C]alanine to [^{14}C]lactate (22). Previous investigators have demonstrated that an increase in reducing equivalents (NADH/NAD) correlates well with the reduction in gluconeogenesis observed with EtOH administration (22). We have chosen the β-OHB/AcAc ratio as a reflection of the redox state of the liver because ketone production is limited to the liver and is therefore a specific marker for hepatic EtOH metabolism (33). In addition to changes in the latter ratio, plasma EtOH levels increased progressively in both groups to levels (10 mM) at 120 min of EtOH infusion that have been shown previously to suppress gluconeogenesis by 65% in vitro (22) and in vivo (17,25). Thus, if one assumes that EtOH infusion inhibits gluconeogenesis by 60% for 120 min, liver glycogen would fall by ~45 mM ([6 $\mu mol \cdot kg^{-1} \cdot min^{-1}$] × 90 kg × 120 min]/1.5 L liver), an amount that would not lower liver glycogen below 50 mM. Thus, our data are consistent with an increase in liver glycogen to levels exceeding 95 mM in patients with NIDDM after a 3-day fast.

This study design is similar to that of Kreisberg et al. (26) in that we have administered tracer before as well as during EtOH infusion to test the hypothesis that glycogen repletion in patients with NIDDM during a fast was the result of diversion of G-6-P derived from gluconeogenesis. If so, a fraction of the labeled G-6-P derived from [U-^{14}C]alanine would be incorporated into liver glycogen during the 3 h of infusion before EtOH administration. The isotopic data appear to support this contention. After a period of isotopic equilibration, EtOH infusion resulted in a prompt fall in [^{14}C]glucose radioactivity in nondiabetic subjects, which was delayed in patients with NIDDM. Based on the work of Radziuk et al. (34), which showed that glycogen is formed and released in layers, with the most recently formed glycogen released first, it seems likely that the delayed fall in [^{14}C]glucose radioactivity during EtOH administration in the patients with NIDDM is explained by depletion of labeled glycogen.

Restoration of liver glycogen stores as an adaptation to fasting (35,36) or to diabetes (37) has been described previously. However, a mechanism for this increase has not been elucidated. Decreases in the glucagon:insulin ratio that would explain an increase in glycogen storage in NIDDM have not been observed in this or previous studies with fasting (7). In fact, the glucagon:insulin ratio increased significantly in both groups during the 3-day fast (Table 2). Plasma cortisol levels were also assessed because of the known stimulatory effect of glucocorticoids on glycogen storage. However, no change in cortisol was observed in either group that might explain the increase in liver glycogen. Changes in plasma concentrations of intermediary metabolites are also potential explanations for our findings. Zhang and Radziuk (38) have recently demonstrated an augmentation of liver glycogen storage in the presence of increased lactate availability. Although plasma lactate levels were clearly increased in the patients with NIDDM after an overnight fast compared with obese nondiabetic subjects (Table 3), this difference was not apparent after the fast had been extended. It is also possible that the increase in liver glycogen is the result of direct incorporation of glucose in our hyperglycemic patients. Previous investigators have failed to demonstrate direct incorporation of labeled glucose into glycogen in the postabsorptive state in patients with NIDDM (39). Similarly, [3-^{3}H]glucose radioactivity did not increase during either saline (7) or EtOH administration (present study) in our diabetic subjects after glucagon administration (data not shown). Rather, the increase in liver glycogen after fasting in patients with NIDDM may be the result of enhanced hepatic insulin sensitivity, as proposed previously (40,41). The decrease in plasma glycerol observed in our patients with NIDDM after fasting despite changes in glucagon and insulin, which would be expected to increase lipolysis, appears to support this hypothesis. Whether this increased sensitivity is at the level of glycogen synthase, glycogen phosphorylase, or glucose-6-phosphatase is unknown.

In conclusion, this study suggests that liver glycogen stores are not depleted by a 3-day fast in patients with NIDDM. The greater glycogen stores in patients with NIDDM compared with nondiabetic subjects after fasting is supported by the failure of EtOH-induced suppression of gluconeogenesis to decrease the glucose response to glucagon in patients with NIDDM after a 3-day fast, in contrast to obese nondiabetic subjects.

ACKNOWLEDGMENTS

These studies were supported in part by National Institutes of Health Grants R29-DK43013 and RR-00065.

We would like to thank the nursing staff of the Clinical Research Center, Shirley Helm, and Livia Griffith, Systems Manager for the General Clinical Research Center, for valuable assistance in the performance of these studies.

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