

# Role of Hepatic Glycogen Breakdown in Defective Counterregulation of Hypoglycemia in Intensively Treated Type 1 Diabetes

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Impairment of hypoglycemic counterregulation in intensively treated type 1 diabetes has been attributed to deficits in counterregulatory hormone secretion. However, because the liver plays a critical part in recovery of plasma glucose, abnormalities in hepatic glycogen metabolism per se could also play an important role. We quantified the contribution of net hepatic glycogenolysis during insulin-induced hypoglycemia in 10 nondiabetic subjects and 7 type 1 diabetic subjects (HbA<sub>1c</sub> 6.5 ± 0.2%) using <sup>13</sup>C nuclear magnetic resonance spectroscopy, during 2 h of either hyperinsulinemic euglycemia (plasma glucose 92 ± 4 mg/dl) or hypoglycemia (plasma glucose 58 ± 3 mg/dl). In nondiabetic subjects, hypoglycemia was associated with a brisk counterregulatory hormone response (plasma epinephrine 246 ± 38 vs. 2,785 ± 601 pmol/l during hypoglycemia, plasma norepinephrine 1.9 ± 0.2 vs. 2.5 ± 0.3 nmol/l, and glucagon 38 ± 7 vs. 92 ± 17 pg/ml, respectively, *P* < 0.001 in all), and a relative increase in endogenous glucose production (EGP 0.83 ± 0.14 mg · kg<sup>-1</sup> · min<sup>-1</sup> during euglycemia yet ~50% higher with hypoglycemia [1.30 ± 0.20 mg · kg<sup>-1</sup> · min<sup>-1</sup>], *P* < 0.001). Net hepatic glycogen content declined progressively during hypoglycemia to 22 ± 3% below baseline (*P* < 0.024). By the final 30 min of hypoglycemia, hepatic glycogen fell from 301 ± 14 to 234 ± 10 mmol/l (*P* < 0.001) and accounted for ~100% of EGP. In marked contrast, after an overnight fast, hepatic glycogen concentration in type 1 diabetic subjects (215 ± 23 mmol/l) was significantly lower than in nondiabetic subjects (316 ± 19 mmol/l, *P* < 0.001). Furthermore, the counterregulatory response to hypoglycemia was significantly reduced with small increments in plasma epinephrine and norepinephrine (126 ± 22 vs. 448 ± 16 pmol/l in hypoglycemia and 0.9 ± 0.3 vs. 1.6 ± 0.3 nmol/l, respectively, *P* < 0.05 for both) and no increase in plasma glucagon. EGP decreased during hypoglycemia with no recovery (1.3 ± 0.5 vs. 1.2 ± 0.3 mg · kg<sup>-1</sup> · min<sup>-1</sup> compared with euglycemia, *P* = NS),

and hepatic glycogen concentration did not change significantly with hypoglycemia. We conclude that glycogenolysis accounts for the majority of EGP during the first 90 min of hypoglycemia in nondiabetic subjects. In intensively treated type 1 diabetes, despite some activation of counterregulation, hypoglycemia failed to stimulate hepatic glycogen breakdown or activation of EGP, factors that may contribute to the defective counterregulation seen in such patients. *Diabetes* 55:659–666, 2006

An appropriate increase in endogenous glucose production (EGP) is critical for the recovery from hypoglycemia (1–3). Glycogen breakdown and gluconeogenesis represent the two major sources of glucose-6-phosphate destined for dephosphorylation and glucose release. Glycogenesis and glycogenolysis take place simultaneously, with the preponderance favoring glycogenolysis when an increase in EGP is required, whereas gluconeogenesis is favored in the fasting, postabsorptive state (4,5). Gluconeogenesis contributes to glycogenesis and also by providing glucose-6-phosphate for dephosphorylation into glucose, hence, directly contributing to EGP (5,6).

Recent in vivo studies in dogs and humans suggest that glycogen breakdown may be the primary mechanism responsible for the increase in EGP during the initial recovery from insulin-induced hypoglycemia, followed by an increase in gluconeogenesis (4,7). These reports suggest that glycogenolysis is activated rapidly but quickly wanes (within the first 1–3 h of hypoglycemia [8,9]). Gluconeogenesis, in contrast, is activated more slowly but becomes the predominant process that sustains EGP during prolonged hypoglycemia (4,8).

Delayed and deficient recovery from hypoglycemia in patients with type 1 diabetes and the concern about severe hypoglycemia represent major clinical impediments to intensive insulin therapy (10,11). Patients with type 1 diabetes suffer from an impaired counterregulatory hormonal response to hypoglycemia characterized by severe blunting or absence of the glucagon response and both a delayed threshold for and a reduced magnitude of epinephrine secretion that are exacerbated by intensive therapy (1,12). However, type 1 diabetes may also be associated with alterations in hepatic glycogen metabolism. Hwang et al. (13) have previously demonstrated impaired net hepatic glycogen synthesis in poorly controlled type 1 diabetic patients during mixed meals. Bishof et al. (14) have demonstrated that poorly controlled type 1

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EGP, endogenous glucose production; GCRC, General Clinical Research Center; NMR, nuclear magnetic resonance.

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diabetic patients display a marked reduction in both hepatic glycogen synthesis and breakdown, which only partially improve with short-term glycemic control, suggesting that antecedent glycemic control and/or insulinization may not entirely account for defects in hepatic glycogen handling. Furthermore, a recent report by Petersen et al. (15) shows that moderately controlled type 1 diabetic patients exhibit increased rates of EGP both at rest and during exercise, which can be entirely accounted for by increased gluconeogenesis. Taken together, these studies suggest that type 1 diabetes may be associated with alterations in hepatic glycogen metabolism that could play a role in the EGP response to various stimuli and possibly also in the EGP response to hypoglycemic counterregulation.

Previous studies in humans used indirect methods to measure fluctuations in hepatic glycogen during hypoglycemia (gluconeogenesis estimated from the appearance of plasma glucose from labeled lactate and glycogen breakdown estimated by subtracting gluconeogenesis from EGP) were not strictly quantitative (7). Furthermore, no studies have been performed to directly examine glycogenolysis during hypoglycemia in type 1 diabetic humans, in part due to technical difficulties in quantifying hepatic glycogen *in vivo* and in real time.

Because the main pathway contributing to EGP recovery during early hypoglycemia is glycogenolysis, we hypothesized that intensively treated type 1 diabetic patients (who suffer from alterations in glycogen metabolism) will demonstrate a decrease in glycogenolysis during hypoglycemia with a consequent deficit in recovery in EGP. Thus, we used  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectroscopy to directly measure rates of net hepatic glycogenolysis, combined with  $[6,6\text{-}^2\text{H}_2]\text{glucose}$  administration, to calculate the rates of EGP during clamped hypoglycemia in nondiabetic subjects and in subjects with type 1 diabetes.

## RESEARCH DESIGN AND METHODS

We studied 10 lean nondiabetic volunteers (5 men and 5 women, aged  $28 \pm 2$  years, BMI  $22 \pm 1$  kg/m $^2$ ) and 7 type 1 diabetic patients (4 men and 3 women, aged  $31 \pm 3$  years, BMI  $23 \pm 1$  kg/m $^2$ ). All type 1 diabetic subjects were treated intensively with insulin (HbA $_{1c}$  [A1C]  $6.5 \pm 0.2\%$  [normal range 4.7–6.4%], four subjects using insulin pump therapy and three using multiple insulin injections). Except for diabetes, all type 1 diabetic subjects were in general good health. Eligibility for the study was determined by history, physical examination, and hematological and biochemical tests. Individuals with clinical neuropathy, anemia, bleeding disorders, recent weight changes, or type 1 diabetes with unstable metabolic control were excluded. Type 1 diabetic subjects were instructed to avoid any episodes of hypoglycemia in the 2-week period before the studies. Informed written consent was obtained in accordance with the policy of the Committee on Clinical Investigations of the Albert Einstein College of Medicine.

Before any infusion study, subjects were placed in the MR scanner to acclimate them to the enclosed environment and for visualization of the liver and acquisition of hepatic glycogen signal. Each subject participated in two clamp studies separated by an interval of 6 weeks. In random order, the studies consisted of either euglycemic or hypoglycemic clamps.

**Type 1 diabetic subjects.** Long-acting insulin was withheld the morning of admission. Subjects were admitted to the General Clinical Research Center (GCRC) the evening before the study for low-dose overnight insulin infusion to establish euglycemia by the morning of study. Beginning at 2200 on the night before the study, subjects were asked to fast, and a variable intravenous infusion of insulin (Humulin Regular; Eli Lilly, Indianapolis, IN) was started. The overnight insulin infusion rate was adjusted according to an algorithm based on hourly blood glucose measurements, assuring a gradual normalization of plasma glucose levels. The experimental protocol was initiated the following morning. Nondiabetic subjects were admitted to the GCRC for each experiment. Studies were performed after a 14-h overnight fast.

At 0700 on the day of study, two indwelling cannulas were inserted, one in an antecubital vein for infusions and the second in the contralateral forearm for blood sampling. To obtain arterialized venous blood samples, this hand

was maintained at 55°C with a nonmagnetic heating pad. At  $t = -120$  min, a primed continuous infusion of  $\text{D-}[6,6\text{-}^2\text{H}_2]\text{glucose}$  (Isotec, Miamisburg, OH) was begun at  $0.38$  mg  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$  for 10 min and continued at  $0.038$  mg  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$  for the entire period of study. In the nondiabetic subjects, at  $t = 0$  min, a primed continuous infusion of insulin (Humulin Regular; Eli Lilly, Indianapolis, IN) at a rate of  $0.8$  mU  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$  was initiated for 10 min followed by  $0.4$  mU  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$ , and a variable infusion of 20% dextrose was begun. In the type 1 diabetic subjects, at  $t = 0$  min, the insulin infusion was increased from baseline to a rate of  $0.4$  mU  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$ , and a variable infusion of 20% dextrose was initiated.

In the euglycemic experiment, the plasma glucose concentration was held at  $\sim 90$  mg/dl for the entire study. In the hypoglycemic experiment, the plasma glucose concentration was allowed to decrease to  $\sim 60$  mg/dl and then maintained by dextrose infusion. The plasma glucose was clamped at the desired range (either 90 or 60 mg/dl) by varying the dextrose infusion according to plasma glucose measured at 5-min intervals. Blood samples were obtained at 10-min intervals for the determinations of plasma insulin, C-peptide, glucagon, epinephrine, and norepinephrine, as well as for glucose kinetics. At the end of the clamp ( $t = 120$  min), all of the infusions were discontinued, and the subject was given a meal and discharged from the GCRC.

To achieve steady-state conditions for tracer analysis, we started an intravenous infusion of  $\text{D-}[6,6\text{-}^2\text{H}_2]\text{glucose}$  2 h ( $t = -120$  min) before the initiation of the clamp. The initial enrichment and calculations were performed at  $t = 0$  min for the basal EGP. Because plasma glucose decreased by  $\sim 30$  mg/dl (from baseline) during the hypoglycemic clamp, we used the final two measurements of  $\text{D-}[6,6\text{-}^2\text{H}_2]\text{glucose}$  for calculating rate of glucose appearance ( $R_a$ )/rate of glucose disappearance ( $R_d$ ). Thus, an additional 80 min was allotted for re-equilibration at this glycemic level (because hypoglycemia was sustained from  $t = \sim 40$  min until  $t = 120$  min). The enrichment of infused dextrose was kept equivalent to plasma glucose enrichment by addition of  $\text{D-}[6,6\text{-}^2\text{H}_2]\text{glucose}$  to the infusate (16).

**Analytical methods.** Plasma glucose was measured with a Beckman glucose analyzer (Fullerton, CA) using the glucose oxidase method. Plasma  $\text{D-}[6,6\text{-}^2\text{H}_2]\text{glucose}$  enrichment was measured using gas chromatography-mass spectrometry as previously described (17). Calibration standards for  $\text{D-}[6,6\text{-}^2\text{H}_2]\text{glucose}$  were made up from 0.251–2.01% APE in unlabeled glucose. All analyses were carried out with a 1- $\mu$ l injection from an Agilent 7683 autoinjector into a 6890 gas chromatograph interfaced to a 5973 mass spectrometer using an Equity 5 gas chromatography column (Supelco, Bellefonte, PA). The carrier gas was helium at 0.6 ml/min constant flow, with a split ratio of 40:1. The mass spectrometer was operated in electron ionization mode at 70 eV, retention time of 4 min;  $m/z$  of the  $m + 0$ ,  $m + 1$ , and  $m + 2$  fragments were monitored by selective ion monitoring at 217.1, 218.1, and 219.1 with a 20-ms dwell time and triplicate injection. Sample peaks were integrated using the Chemstation integrator. APE over background enrichment obtained from a preinfusion plasma blank was calculated with a standard curve taking into account  $\text{D-}[6,6\text{-}^2\text{H}_2]\text{glucose}$  tracer as a percentage of total glucose:  $m + 2 / [(m + 0) + (m + 1) + (m + 2)] \times 100$ .

The methods for measurement of plasma insulin, glucagon, epinephrine, norepinephrine, cortisol, and growth hormone and their intra- and interassay variations have been previously reported (18).

**NMR spectroscopy.** *In vivo*  $^{13}\text{C}$  NMR proton-coupled spectroscopy of the liver was performed on a 4T Varian/Magnex human MR system using a  $^{13}\text{C}$  11-cm circular surface coil with a 13.5-cm coplanar butterfly  $^1\text{H}$  coil. The RF coil was placed over the lateral aspect in the supine subject. Initial coil placement was determined by percussing the borders of the liver, and the final position was confirmed by the image. T1 weighted gradient echo images (TR/TE = 250/8 ms) were acquired through the  $^1\text{H}$  coil for localization. To remove the signals from subcutaneous fat and muscle above the liver, a one-dimensional spectroscopic imaging method was used. The spectroscopic imaging acquisition parameters were as follows: a repetition time of 0.3 s; 333- $\mu$ s nonselective excitation pulse, 15,000-Hz sweep width, and 2,048 complex points. RF power was calibrated using a microsphere filled with 99% enriched [ $^{13}\text{C}$ ]formate at the center of the coil. The tip angle of 270° at the coil center was used for excitation.

The field of view of 60.0 cm with 32 phase-encoding steps resulted in a one-dimensional nominal voxel resolution of 1.875 cm (equivalent to slice thickness), orientation of  $\sim 30\text{--}40^\circ$ , perpendicular to the  $^{13}\text{C}$  coil surface. Because the liver is located in an oblique fashion in the abdomen, the oblique localization was achieved by the simultaneous use of vertical and horizontal gradients. A Gaussian-weighted phase-encoding scheme was used to improve the signal-to-noise ratios without sacrificing the efficiency of localization (19). A total of 505 phase-encoding steps over 32 k-space values was acquired per cycle of Gaussian-weighted sampling. One data point of hepatic glycogen was acquired every 15 min.

**Spectral analysis and quantification.** All spectroscopic imaging files were transferred from the Varian MR scanner to a personal computer. Each

spectroscopic image was processed using customized programs in MATLAB (MathWorks, Natick, MA). Extracted spectroscopic imaging spectra from the liver were properly phased, and C-1 glycogen doublets were fitted to assess integrations of the glycogen peaks at 100.1 ppm. Quantification of the glycogen resonance was performed using a matching glycogen phantom composed of 300 mmol/l glucosyl units of oyster glycogen (Sigma, St. Louis, MO) and 60 mmol/l KCl. A [ $^{13}\text{C}$ ]formate sphere placed at the center of the  $^{13}\text{C}$  coil served to calibrate the pulse angle and coil loading. The integrated area of the glycogen (100.1 ppm) resonance was then referenced to an equivalently acquired phantom sample, corrected for receiver gain and reception sensitivity. Finally, individual variations in liver volume and shape were also corrected by image corrections. The liver outline of each subject determined from in vivo magnetic resonance imaging was superimposed on the pulse sequence image, and the relative ratio of those integrals was used as the correction factor to obtain the final concentrations of hepatic glycogen. The intrasubject variability of the  $^{13}\text{C}$  MR method was evaluated in control subjects using repeated hepatic glycogen measurements. Studies on the same and separate days were performed after an overnight fast. The coefficients of variation for this method were  $4.8 \pm 1.4\%$  ( $n = 13$ ) for same day-repeated studies and  $6.5 \pm 3.0\%$  ( $n = 4$ ) when studies were performed on separate days.

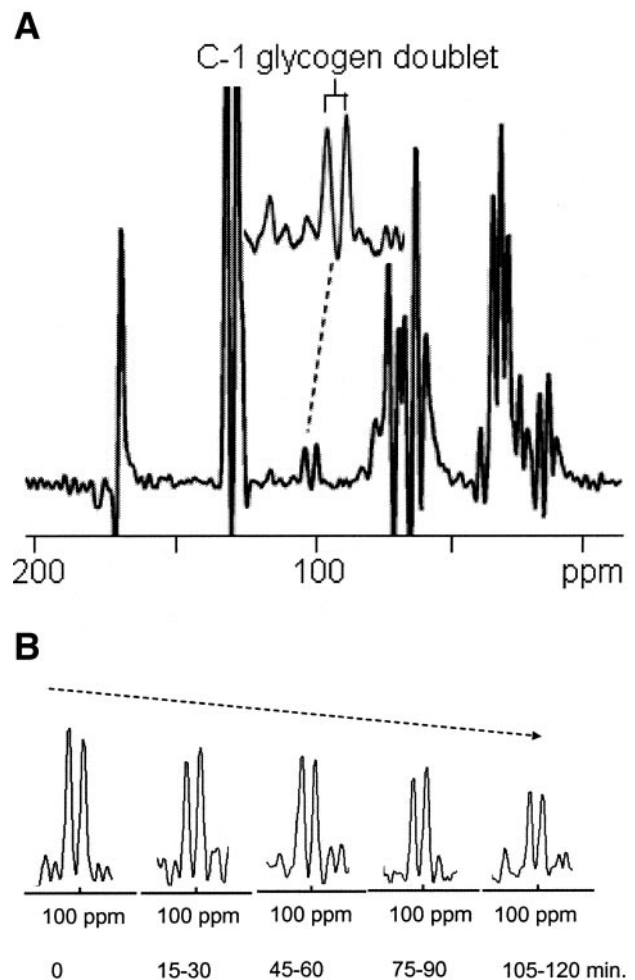
Glycogen measurements were acquired every 15 min during the euglycemic and hypoglycemic clamp.  $^{13}\text{C}$  NMR spectroscopy measures net changes in liver glycogen concentration; thus, independent rates of glycogen synthesis and rates of glycogenolysis were not assessed. Individual rates of net glycogenolysis were calculated by linear regression of the net glycogen concentration-time curves during each clamp (14,20). The slope of change in liver glycogen concentration (millimoles/liter) from the glycogen content at the beginning of the study to the end of the clamp was multiplied by the liver volume (liters) to calculate the change in net hepatic glycogen content (millimoles; Fig. 1).

Liver volume was quantified using liver volume MR imaging performed in a 1.5T scanner before and immediately after the clamp studies. Images were acquired with a phased array body coil in a Philips 1.5T Gyroscan NT imager with Intera upgrade, at 3D T1 gradient. Three 10-mm-thick images were obtained through the liver in a single breath hold, above the dome of diaphragm. The data were analyzed at a Philips EasyVision workstation for volumetric calculation using Philips software. The capsular margins of liver were hand drawn with the workstation mouse, and the regions were evaluated with semiautomated volume calculation software. The liver volumes were averaged from three acquisitions for each subject.

**Data analysis.** The data in the text, figures, and tables are presented as means  $\pm$  SE. Values for hormones, glucose infusion rate, EGP, and glucose uptake, obtained at 10-min intervals, were also averaged over the final 30 min of each study. Statistical analyses were performed using repeated measures ANOVA for multiple comparisons and paired Student's *t* test for comparing means. A value of  $P < 0.05$  was considered significant.  $R_a$  and  $R_d$  (glucose uptake) were calculated using Steele's steady-state equation (21). Rates of EGP were determined by subtracting rates of glucose infusion from the tracer-determined  $R_a$ .

## RESULTS

**Plasma glucose and glucose kinetics.** Figure 2 depicts the concentrations of plasma glucose in nondiabetic subjects and in the subjects with type 1 diabetes. The estimates of EGP and glucose uptake ( $R_d$ ) derived from isotopic analysis are depicted in Fig. 3. On the hypoglycemic study day, plasma glucose was maintained at  $\sim 60$  mg/dl, whereas during the euglycemic study, the plasma glucose target was 90 mg/dl. Given the rates of insulin infusion used, hypoglycemia reached a stable plateau by 40 min in both groups. An insulin infusion rate of  $0.4 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  was used to induce mild hypoglycemia without completely suppressing EGP. Thus, with the initiation/increase of insulin infusion there was a 36% decrease in EGP in the nondiabetic subjects and a 32% decrement in the type 1 diabetic subjects. However, in nondiabetic control subjects, hypoglycemic counterregulation resulted in a proportional increase or "recovery" in EGP ( $1.5 \pm 0.6$  vs.  $0.8 \pm 0.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in type 1 diabetic subjects,  $P < 0.001$ , during the last 30 min of the hypoglycemic clamp) and a corresponding decrease in glucose uptake ( $R_d$ ) ( $3.5 \pm 0.2$  vs.  $4.3 \pm 0.4 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P < 0.001$ ,



**FIG. 1.** Proton-coupled  $^{13}\text{C}$  NMR spectra from the liver. **A:** C-1 glycogen coupled with H-1 glycogen to form doublet at 100.1 ppm clearly exhibited together with the carbons of hepatic lipids. The inlayed trace is the zoomed C-1 glycogen doublet. **B:** The time course spectra of glycogen at 100.1 ppm during hypoglycemic clamp on a control subject.

respectively). In marked contrast, in the type 1 diabetic subjects, there was no significant difference in EGP and  $R_d$  related to hypoglycemic counterregulation between the euglycemic and hypoglycemic studies ( $1.3 \pm 0.5$  vs.  $1.2 \pm 0.3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  and  $2.4 \pm 0.5$  vs.  $2.6 \pm 0.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , NS).

**Glucoregulatory hormone concentrations.** In the nondiabetic subjects, plasma insulin concentrations under baseline fasting conditions were comparable in both sets of studies (Table 1). With insulin infusion, plasma insulin was raised by 9- to 10-fold and maintained at those levels. Although insulin was infused at identical rates in the two sets of studies, plasma insulin concentrations tended to be higher in the hypoglycemic studies (the difference, however, was not significant). The greater suppression of endogenous insulin secretion during hypoglycemia resulted in plasma C-peptide values 40–50% lower during hypoglycemia ( $0.51 \pm 0.09$  vs.  $0.24 \pm 0.04 \text{ nmol/l}$ ,  $P < 0.01$ ). In the type 1 diabetic subjects, basal plasma insulin levels were higher (approximately sevenfold) due to the overnight insulin infusion intended to maintain basal insulin requirements. However, during the final 30 min of the hypoglycemic clamps, plasma insulin was comparable within and between studies (Table 1).

During euglycemia, the concentrations of all three coun-

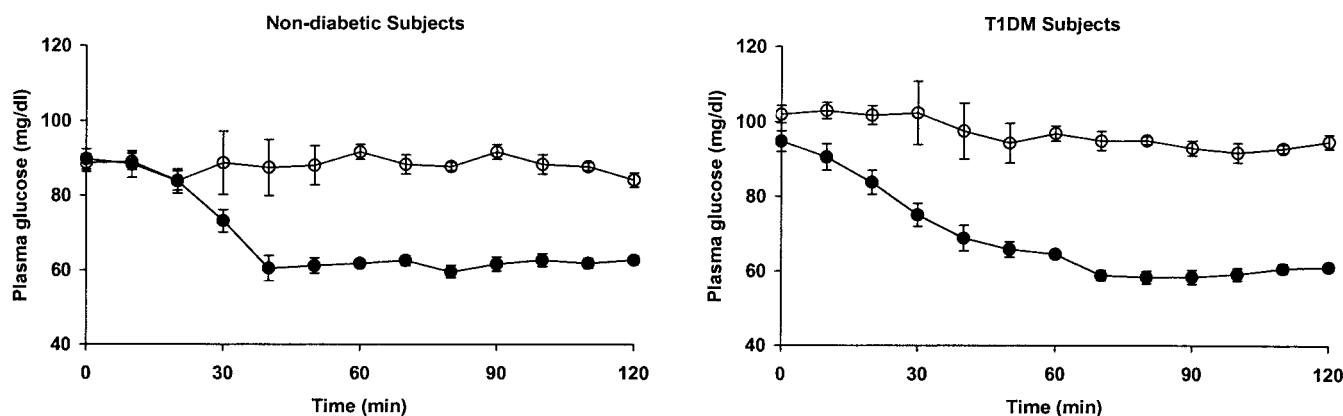


FIG. 2. Plasma glucose concentrations during clamped hypoglycemic (●) and euglycemic (○) studies. T1DM, type 1 diabetic subjects.

terregulatory hormones remained stable at baseline values in both groups (Table 1). The counterregulatory hormonal response to hypoglycemia was predictable; in nondiabetic subjects, plasma glucagon, epinephrine, and norepinephrine rose significantly (197, 1,113, and 133%, respectively,  $P < 0.001$  in all). In the type 1 diabetic subjects, plasma epinephrine and norepinephrine rose significantly during hypoglycemia (356 and 160%, respectively,  $P < 0.05$  for both) but were markedly reduced compared with nondiabetic subjects ( $P < 0.01$  for all). As expected, the glucagon response to hypoglycemia was absent, and the epineph-

rine response was markedly blunted in the type 1 diabetic subjects (Table 1).

**Glycogen quantitation in response to hypoglycemia.** In all groups, liver volumes averaged  $1,411 \pm 60$  ml before the initiation of the hypoglycemic studies and did not change significantly with hypoglycemia ( $1,528 \pm 82$  ml, NS). Figure 4 depicts the changes in hepatic glycogen concentrations estimated from NMR spectroscopy. In the nondiabetic subjects, basal hepatic glycogen concentrations averaged  $316 \pm 19$  mmol/l for the euglycemic studies and  $301 \pm 14$  mmol/l for hypoglycemic

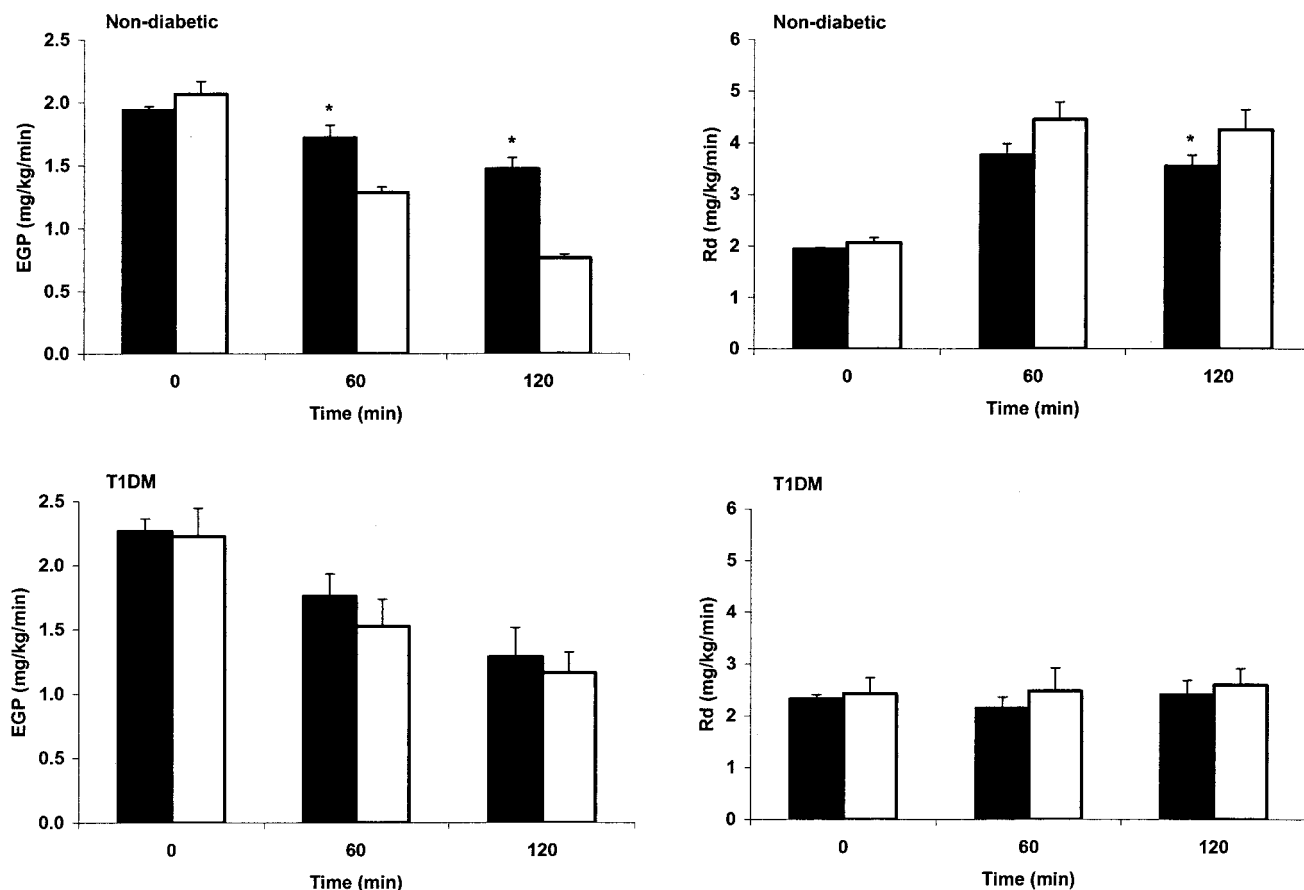


FIG. 3. EGP and rates of glucose uptake ( $R_d$ ) in nondiabetic subjects and type 1 diabetic subjects (T1DM). Data during hypoglycemia (■) and euglycemia (□) are shown. \* $P < 0.001$  vs. euglycemia.

TABLE 1  
Plasma insulin, C-peptide, epinephrine, norepinephrine, and glucagon concentrations in nondiabetic and type 1 diabetic subjects

	Subjects	Hypoglycemia			Euglycemia		
		0 min	60 min	120 min	0 min	60 min	120 min
Insulin (pmol/l)	Nondiabetic	11 ± 4	184 ± 25	185 ± 30	27 ± 9	155 ± 11	149 ± 11
	Type 1 diabetic	89 ± 24	253 ± 60	150 ± 24	105 ± 21	205 ± 61	168 ± 40
C-peptide (nmol/l)	Nondiabetic	0.85 ± 0.12	0.40 ± 0.07	0.24 ± 0.04*	0.71 ± 0.10	0.59 ± 0.06	0.51 ± 0.09
	Type 1 diabetic	0.19 ± 0.04	0.16 ± 0.01	0.15 ± 0.02	0.22 ± 0.06	0.18 ± 0.04	0.16 ± 0.04
Epinephrine (pmol/l)	Nondiabetic	253 ± 66	1491 ± 464	2,796 ± 601*	175 ± 31	169 ± 31	246 ± 38
	Type 1 diabetic	124 ± 23	265 ± 54	449 ± 193*	111 ± 46	97 ± 56	122 ± 70
Norepinephrine (nmol/l)	Nondiabetic	1.9 ± 0.2	2.4 ± 0.2	2.5 ± 0.3*	1.4 ± 0.2	1.5 ± 0.3	1.6 ± 0.3
	Type 1 diabetic	1.0 ± 0.3	1.4 ± 0.4	1.6 ± 0.3*	0.6 ± 0.2	1.0 ± 0.3	0.8 ± 0.3
Glucagon (ng/l)	Nondiabetic	47 ± 11	61 ± 13	92 ± 17*	48 ± 5	39 ± 6	38 ± 7
	Type 1 diabetic	37 ± 5	36 ± 4	39 ± 6	43 ± 6	35 ± 5	30 ± 4

Data are means ± SE. \* $P < 0.001$  vs. corresponding time point during euglycemia.

studies (NS). In these subjects, we observed a progressive decrease in glycogen concentrations during hypoglycemia beginning at the 60- to 75-min acquisition period, declining to a nadir of  $234 \pm 10$  mmol/l by the final acquisition period at 120 min ( $P < 0.001$ ). This decline represented a  $22 \pm 3\%$  decrease from fasting glycogen concentrations and contrasted to an average hepatic glycogen increment of  $6 \pm 7\%$  (NS) with hyperinsulinemic euglycemia.

In the type 1 diabetic subjects, baseline hepatic glycogen was  $\sim 100$  mmol/l lower compared with the nondiabetic subjects,  $P < 0.001$  (Fig. 4). This value was sig-

nificantly lower than that in overnight-fasted control subjects, despite identical periods of fasting. In addition, there were no fluctuations in hepatic glycogen during the experimental protocols: baseline hepatic glycogen averaged  $215 \pm 23$  and  $207 \pm 16$  mmol/l in the euglycemic and hypoglycemic studies (NS) and reached  $219 \pm 226$  and  $214 \pm 22$  mmol/l, respectively, during the final 15 min of the studies (NS). Taken together, the overall percent change in hepatic glycogen during hypoglycemia corrected for the effect of insulin infusion was estimated at  $\sim 28\%$  in nondiabetic subjects versus essentially no change in the type 1 diabetic subjects (Fig. 5).

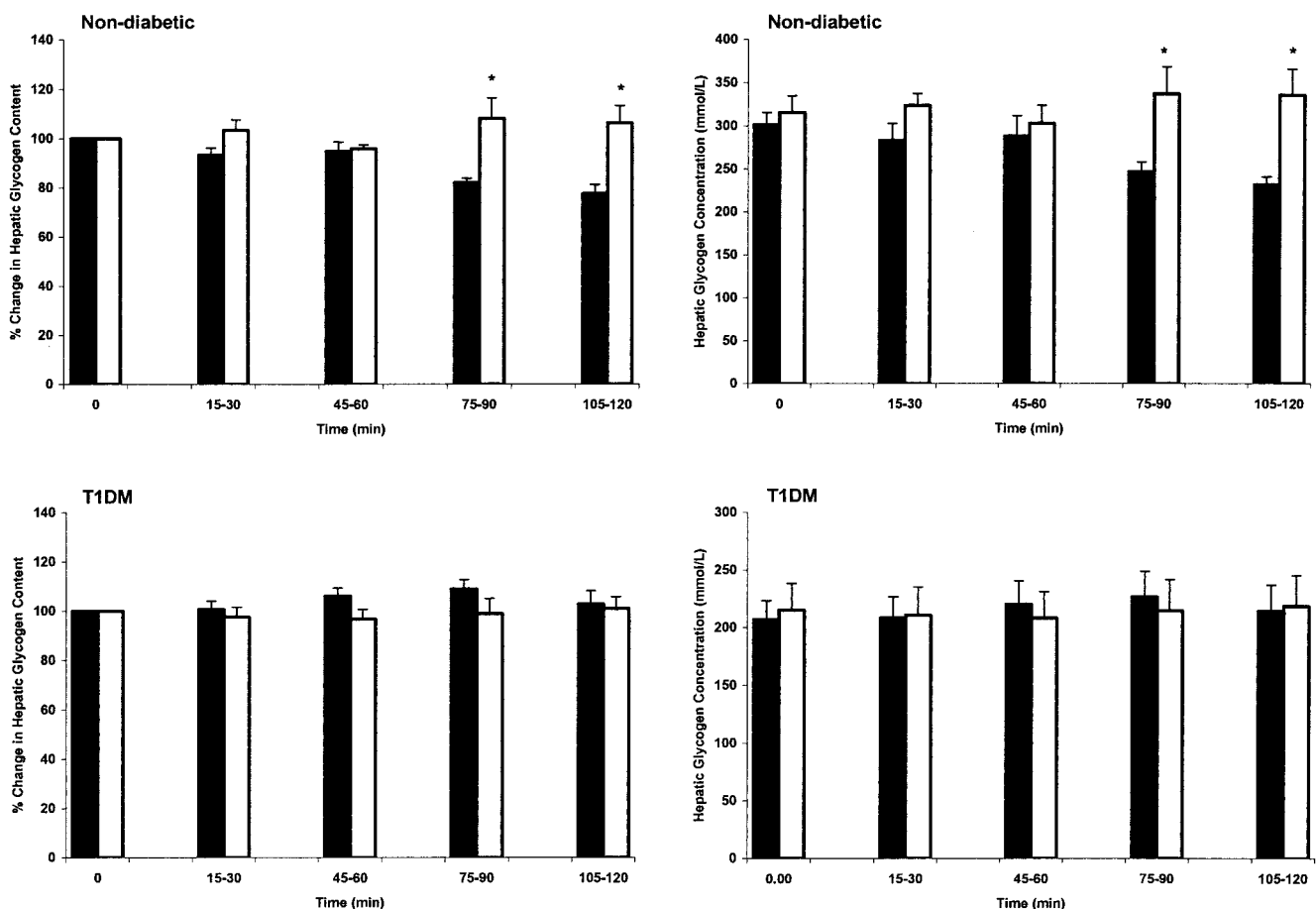


FIG. 4. Percent change in hepatic glycogen and total hepatic glycogen concentration (millimoles/liter) in nondiabetic subjects and type 1 diabetic subjects (T1DM). ■, hypoglycemia; □, euglycemia. \* $P < 0.001$  vs. hypoglycemia.

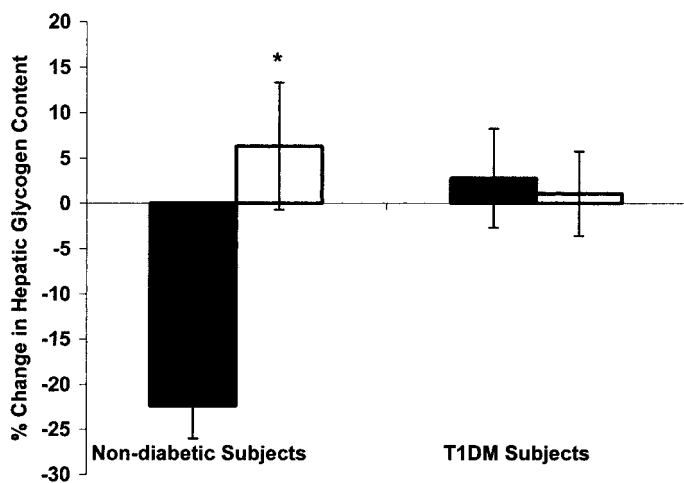


FIG. 5. Percent change in hepatic glycogen averaged for the final 45 min of each clamp study in nondiabetic subjects and type 1 diabetic subjects. ■, hypoglycemia; □, euglycemia. \* $P < 0.001$  vs. hypoglycemia.

## DISCUSSION

We examined the relative contribution of net hepatic glycogen content to the counterregulatory rise in EGP in nondiabetic subjects and in type 1 diabetic subjects during insulin-induced hypoglycemia. We compared these parameters during euglycemia using the same insulin infusion rate and maintaining plasma glucose by a variable dextrose infusion. In nondiabetic subjects, euglycemia (together with hyperinsulinemia) resulted in a relative increase in hepatic glycogen (6% from baseline), probably due to the stimulatory effect of insulin on hepatic glycogenesis. However, during mild hypoglycemia induced by a similar insulin infusion, hepatic glycogen decreased significantly (22% from baseline). Taking these data together to eliminate the effect of insulin on net glycogen turnover, the estimated decrease in hepatic glycogen during hypoglycemia would thus be  $22 + 6$  or  $\sim 28\%$ . In concert with these data, the rates of EGP decreased proportionally during the euglycemic studies because of the suppressive effect of hyperinsulinemia on EGP, but activation of the counterregulatory hormones during hypoglycemia (glucagon and epinephrine in particular) contributed to the relative increase in EGP in the face of hyperinsulinemia. Thus, the fluctuations in hepatic glycogen paralleled the rates of EGP both in the euglycemic and in the hypoglycemic studies. Coinciding with the achievement of hypoglycemia at 45 min, mean glycogen concentrations decreased linearly ( $R^2 = 0.81$ ) at a rate of  $\sim 720 \mu\text{mol} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$  (Fig. 1). Because the influx of new glucose into the plasma glucose pool is assumed to be in a 1:1 ratio with hepatic C1 glucosyl units, we used the decline in hepatic glycogen (in millimoles per liter) and the corresponding total liver volume to estimate EGP derived from glycogen over time. The calculated rate of glycogen breakdown accounted for  $\sim 100\%$  of EGP during the final 30 min of hypoglycemia.

The effects of hypoglycemia in humans on the pathways of hepatic glycogen synthesis/breakdown have not been extensively studied, in part because of the difficulty of obtaining data on liver glycogen in vivo in physiological models of hypoglycemia. Studies in animals demonstrated that epinephrine, a major component of the counterregulatory hormone response to hypoglycemia, induces a shift in glycogen metabolism toward glycogenolysis and, indirectly, toward an increase in gluconeogenesis by activat-

ing the peripheral delivery of gluconeogenic precursors to the liver (22). Furthermore, glucagon released in response to hypoglycemia activates both glycogenolysis and gluconeogenesis (23–25); however, its effects on gluconeogenesis appear to be more persistent (9). Taken together, these interactions that occur at the level of glycogen metabolism and gluconeogenesis act in concert to produce rapid recovery from insulin-induced hypoglycemia.

Lecavalier et al. (7) examined the contribution of gluconeogenesis to hypoglycemia using two methods: an isotopic approach based on appearance of plasma glucose from lactate and experiments in which ethanol infusion was used to block gluconeogenesis. Their findings also suggested that glycogenolysis represents the primary mechanism responsible for the increased EGP during hypoglycemia, followed by a sustained activation of gluconeogenesis. However, the methods used in that study were not strictly quantitative, because estimation of gluconeogenesis from the rate of appearance of glucose from lactate underestimates the overall rate of gluconeogenesis because of carbon exchange in the Krebs cycle and because it measures gluconeogenesis from only one precursor (lactate). Additionally, ethanol infused in their experiments may have affected counterregulatory hormone responses. In the rat, acute alcohol administration reduces the glucagon and catecholamine responses to stress (26). Ethanol administration may also increase circulating acetate levels, which by itself may modulate the counterregulatory response to hypoglycemia (27). Nevertheless, their estimation of the contribution of glycogen breakdown to the early phase of glucose counterregulation in normal subjects is borne out by our direct observations of the decrement in net hepatic glycogen.

In the type 1 diabetes studies, baseline glycogen concentration was 33% lower compared with the nondiabetic subjects. This difference in hepatic glycogen could not be accounted for by differences in duration of fasting, because both the nondiabetic and the type 1 diabetic subjects were fasted overnight for the same duration before the study. Additionally, the continuous insulin infusion administered to the type 1 diabetic subjects overnight would be expected to have increased baseline hepatic glycogen. These findings are in agreement with previous studies in less well-controlled type 1 diabetic subjects that demonstrated lower concentrations of hepatic glycogen in the postabsorptive state (13,28). However, Bischof et al. (28) reported no difference in hepatic glycogen between healthy volunteers and type 1 diabetic subjects who were well controlled on multiple-injection therapy. In their study, the glycogen concentration in type 1 diabetic subjects after  $\sim 14$  h of fasting was essentially similar to our type 1 diabetic subjects ( $\sim 200$  mmol/l), whereas in their healthy control subjects, glycogen content was somewhat lower than in our subjects. The reported range of mean hepatic glycogen content from  $\sim 250$  to  $\sim 340$  mmol/l in healthy subjects has been obtained under differing study conditions (29,30). Thus, our finding of reduction of fasting hepatic glycogen content in intensively treated type 1 diabetic subjects will require further study.

In addition to the apparent difference in fasting hepatic glycogen, we also observed dramatic differences in the time course of changes in hepatic glycogen during the clamp studies. In type 1 diabetic subjects, there was no significant change in hepatic glycogen during either euglycemic or hypoglycemic studies. These findings were in contrast to those observed in the nondiabetic subjects in

whom hepatic glycogen fell during hypoglycemia. In concert with these data, EGP in type 1 diabetic subjects decreased proportionally during the euglycemic and hypoglycemic studies without any recovery even by the end of the hypoglycemic clamps.

The primary mechanism responsible for the lack of glycogen breakdown during hypoglycemia in type 1 diabetic subjects is likely due, at least in part, to the blunted hormonal counterregulatory response (i.e., no increase in plasma glucagon concentrations and modest increments in plasma epinephrine). However, the significantly lower concentration of basal, fasting hepatic glycogen content in type 1 diabetic subjects even after overnight insulinization could also play a role in their defective glucose counterregulation. Previous studies in type 1 diabetic subjects demonstrated defects in regulation of glycogenolysis and gluconeogenesis, although these experiments used different physiological paradigms (8,14,28,31). Interestingly, Petersen et al. (15) have recently reported that the increase in EGP seen with exercise in nondiabetic subjects is fully accounted for by increased hepatic glycogenolysis, whereas in "moderately controlled" type 1 diabetic subjects (A1C averaging 7.9%) increased rates of EGP with exercise were not associated with hepatic glycogen decrements; thus, they conclude that the increase in EGP was due to enhanced gluconeogenesis. Consistent with these studies, the lack of change in hepatic glycogen that we observed in the type 1 diabetic subjects during hypoglycemia suggests that gluconeogenesis played the preponderant role in maintaining EGP during hypoglycemic counterregulation.

Although evidence of hepatic resistance to the effects of counterregulatory hormones is not strong, a recent report suggests attenuation of the effects of infused epinephrine on EGP in poorly controlled type 1 diabetes (32). In contrast to these findings, Berk et al. (33) demonstrated enhanced glyemic responsiveness to epinephrine in type 1 diabetic subjects compared with nondiabetic subjects. Alternatively, it is conceivable that the underlying mechanism that activates glycogen breakdown may be a combination of hormonal changes operative in the nondiabetic subjects but not present in type 1 diabetic subjects, i.e., a drop in portal insulin (as reflected by the decrease in plasma C-peptide concentrations in nondiabetic subjects) may be an additional signal that is required for activation of glycogenolysis (34). It is also possible that had we induced a more severe degree or a more prolonged period of hypoglycemia in the type 1 diabetic subjects, we would have observed evidence of greater activation of counterregulation and/or hepatic glycogenolysis. Finally, a potential role for the reduction in basal hepatic glycogen content cannot be excluded.

In summary, these are the first direct measurements of hepatic glycogen content in nondiabetic subjects and in type 1 diabetic patients during insulin-induced hypoglycemia. Our data clearly demonstrate that in nondiabetic subjects, hepatic glycogen depletion (~22% of basal) is associated with the early response to insulin-induced hypoglycemia. This decrease in liver glycogen suggests that glycogen breakdown is the major pathway contributing to the increased EGP for recovery of plasma glucose. In contrast, intensively treated patients with type 1 diabetes and defective hormonal counterregulation demonstrated no quantitative change in their liver glycogen content during hypoglycemia. Our observations suggest that in type 1 diabetes, impaired counterregulatory hor-

mone response, defects in baseline hepatic glycogen content, and the preponderant contribution of gluconeogenesis to EGP could all have an impact on the recovery of plasma glucose from insulin-induced hypoglycemia. These defects may contribute to the recurrent episodes of severe hypoglycemia and possibly to the hypoglycemia-associated autonomic failure seen in intensively treated patients with type 1 diabetes.

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