Meticulous Prevention of Hypoglycemia Normalizes the Glycemic Thresholds and Magnitude of Most of Neuroendocrine Responses to, Symptoms of, and Cognitive Function During Hypoglycemia in Intensively Treated Patients With Short-Term IDDM

CARMINE G. FANELLI, LUCILLA EPIFANO, ANNA MARIA RAMBOTTI, SIMONE PAMPANELLI, ANTONIO DI VINCENZO, FRANCESCO MODARELLI, MAURO LEPORE, BRUNO ANNIBALE, MARCO CIOFETTA, PAOLO BOTTINI, FRANCESCA PORCELLATI, LUCIANO SCIONTI, FAUSTO SANTEUSANIO, PAOLO BRUNETTI, AND GEREMIA B. BOLLI

To test the hypothesis that hypoglycemia unawareness is largely secondary to recurrent therapeutic hypoglycemia in IDDM, we assessed neuroendocrine and symptom responses and cognitive function in 8 patients with short-term IDDM (7 yr) and hypoglycemia unawareness. Patients were assessed during a stepped hypoglycemic clamp, before and after 2 wk and 3 mo of meticulous prevention of hypoglycemia, which resulted in a decreased frequency of hypoglycemia (0.49 ± 0.05 to 0.045 ± 0.03 episodes/patient-day) and an increase in HbA_{1c} (5.8 ± 0.3 to 6.9 ± 0.2%) ($P < 0.05$). We also studied 12 nondiabetic volunteer subjects. At baseline, lower than normal symptom and neuroendocrine responses occurred at lower than normal plasma glucose, and cognitive function deteriorated only marginally during hypoglycemia. After 2 wk of hypoglycemia prevention, the magnitude of symptom and neuroendocrine responses (with the exception of glucagon and norepinephrine) nearly normalized, and cognitive function deteriorated at the same glycemic threshold and to the same extent as in nondiabetic volunteer subjects. At 3 mo, the glycemic thresholds of symptom and neuroendocrine responses normalized, and surprisingly, some of the responses of glucagon recovered. We concluded that hypoglycemia unawareness in IDDM is largely reversible and that intensive insulin therapy and a program of intensive education may substantially prevent hypoglycemia and at the same time maintain the glycemic targets of intensive insulin therapy, at least in patients with IDDM of short duration. *Diabetes* 42:1683–89, 1993

From the Department of Internal Medicine and Endocrine and Metabolic Sciences, University of Perugia, and Chair of Gastroenterology, University La Sapienza, Rome, Italy.

Address correspondence and reprint requests to Dr. Geremia B. Bolli, Dipartimento di Medicina Interna e Scienze Endocrine e Metaboliche, Via E. Dal Pozzo, 06126 Perugia, Italy.

Received for publication 29 June 1993 and accepted in revised form 26 July 1993.

IDDM, insulin-dependent diabetes mellitus; RIA, radioimmunoassay; HPLC, high-performance liquid chromatography; CI, confidence interval; ANOVA, analysis of variance.

Hypoglycemia unawareness, i.e., the loss of autonomic warning symptoms to impending hypoglycemia (1), is a major risk factor for severe hypoglycemia in IDDM (1,2). Autonomic neuropathy (3), long duration of diabetes (4), and strict glycemic control (5), have all been associated with hypoglycemia unawareness. However, its pathogenesis remains unknown (1).

Recently, it has been shown that antecedent hypoglycemia reduces the counterregulatory and symptom responses to hypoglycemia (6–8) and protects cognitive function during subsequent hypoglycemia in nondiabetic subjects (9), patients with IDDM (10,11), and patients with insulinoma (12,13). In the latter patients, curing hypoglycemia by surgically removing the pancreatic adenoma normalizes hormone and symptom responses (12,13) and cognitive function during hypoglycemia (13). This observation not only indicates that antecedent, recurrent hypoglycemia may induce hypoglycemia unawareness, but also that normal or near-normal counterregulatory hormone and symptom responses to hypoglycemia may be regenerated as long as hypoglycemia is prevented, as recently postulated (14,15). However, this hypothesis (14) has never been tested on clinical grounds in IDDM patients with hypoglycemia unawareness.

These studies were undertaken to test the hypothesis (14) that the syndrome of hypoglycemia unawareness in IDDM is largely iatrogenic, i.e., secondary to recurrent therapeutic hypoglycemia (14). Whether successful prevention of hypoglycemia in the everyday life of IDDM patients reverses hypoglycemia unawareness was assessed. Therefore, symptom and counterregulatory responses to hypoglycemia and cognitive function during hypoglycemia were assessed before and after meticulous prevention of hypoglycemia in a group of patients

with short-term IDDM (7 yr) and hypoglycemia unawareness.

RESEARCH DESIGN AND METHODS

Institutional Review Board approval was obtained for these studies. Informed consent was given by 12 age-, sex-, and BMI-matched nondiabetic volunteer subjects and 8 IDDM patients: 4 men and 4 women; 26 ± 2 yr of age (22–36 yr); BMI of 22 ± 0.7 kg/m^2; diabetes duration for 5.0 ± 0.6 yr (4–7 yr); HbA_{1c} of 5.8 ± 0.3% (5.1–7.3%); and estimated duration of hypoglycemia unawareness of 1.2 ± 0.3 yr (0.5–2 yr). The studies in IDDM patients and nondiabetic volunteer subjects were concurrent. The diabetic patients were recruited from those referred to the inpatient and/or outpatient units of the Department of Internal Medicine and Endocrine and Metabolic Sciences, University of Perugia, based on the following criteria: *1*) short duration of IDDM (≤7 yr); *2*) treatment with intensive insulin therapy and a consistent history of frequent hypoglycemia, i.e., capillary blood glucose concentrations <3 mM measured by reflectometer-read chemistrips in the absence of warning symptoms (autonomic, described below) for at least 6 mo before the study; and *3*) absence of clinically overt autonomic neuropathy, as judged by a standard battery of cardiovascular tests (16). All patients were on 3 or 4 daily injections of insulin strictly aimed at normoglycemia. Two patients had suffered severe hypoglycemia (coma or need for external assistance) at least once during the year before the study. At the time of the study, all diabetic patients were free of any detectable diabetic complications. They had no other disease apart from diabetes and were not taking any drugs other than insulin.

After a 2-wk run-in period during which the diabetic patients kept constant the model of insulin treatment, glycemic targets, diet, and lifestyle, and continued home blood glucose monitoring (before each main meal and at bedtime), the neuroendocrine and symptom responses and cognitive function were examined during a stepped hypoglycemic clamp (baseline examination). Afterward, in the patients who initially were on 3 daily insulin injections, the model of insulin treatment was changed into regular insulin at breakfast, lunch, and supper and intermediate-acting insulin (NPH) at 2300–2330. The diet was also changed to 3 daily main meals with no snacks. The decided doses of insulin were based on the capillary blood glucose concentration read by a reflectometer, always determined before each insulin injection. The patients received daily counseling by telephone with two of us (C.G.F. and G.B.B.). To prevent hypoglycemia, the doses of insulin were aimed at fasting, preprandial, and bedtime blood glucose measurements of ~7.2–8.3 mM. The patients were restudied after 2 wk and 3 mo of such a therapeutic strategy. All patients were asked to measure and record capillary blood glucose concentrations (reflectometer) at least 4 times/day, i.e., before each insulin injection.

Stepped hyperinsulinemic-hypoglycemic clamp. To quantify thresholds and magnitude of responses of counterregulatory hormones, and symptoms and deterioration of cognitive function, the stepped hyperinsulinemic-hypoglycemic clamp was used as described previously (17), with minor modifications. In brief, the diabetic patients were admitted to the Clinical Research Center of the Department of Internal Medicine and Endocrine and Metabolic Sciences, University of Perugia the night before the study between 2100 and 2130. The diabetic patients had been withdrawn from their long-acting insulin for 48 h and given injections of regular insulin at each meal, at bedtime, and at 0300. On the night before the study, normoglycemia was maintained in the diabetic patients by an intravenous infusion of insulin into a superficial vein of the left arm above the elbow as described previously (18). Nondiabetic volunteer subjects were admitted on the morning of the studies. All subjects were in the fasting state (12 h). Between 0700 and 0730, a hand vein of the left arm was cannulated retrogradely and maintained in a plexiglass thermoregulated box (65°C) for sampling of arterialized venous blood. After a 60-min equilibration period, infusion of insulin was started (1 mU · kg^{-1} · min^{-1} for 270 min followed by 2 mU · kg^{-1} · min^{-1} for an additional 90 min). Plasma glucose was clamped by variable glucose infusion at sequential target glucose concentrations of 4.3, 3.6, 3.0, and 2.3 mM, based on plasma glucose concentration measured every 5 min. Each of these steps lasted 90 min. The first 45 min were used to reach the desired plateau plasma glucose concentration and the last 45 min to maintain it in steady state. Arterialized venous blood samples were drawn every 15–30 min for determination of plasma free insulin, growth hormone, glucagon, cortisol, adrenaline, noradrenaline, and pancreatic polypeptide.

A semiquantitative symptom questionnaire was compiled every 15 min. Subjects scored from 0 (none) to 5 (severe) for each of the following symptoms: dizziness, tingling, blurred vision, difficulty in thinking, faintness, anxiety, palpitations, hunger, sweating, irritability, or tremor. In accordance with previous classifications (17), the first 5 symptoms were considered neuroglycopenic and the last 6 autonomic. The sum of each of these constituted the symptom score. In addition, at baseline and during each plateau the following standard cognitive tests were compiled: trail-making part B, verbal fluency, interference subtest from the Stroop test, simple and choice visual reaction time, word and color subtest from the Stroop test, digit vigilance test, trail-making part A, verbal memory test, and forward and backward digit span, as described previously (17). The evening before the study, subjects extensively practiced each test. For the actual study, six alternate forms were prepared. Diabetic patients, nondiabetic volunteer subjects, and the person who administered the symptom and cognitive tests were all blinded to the experimental conditions.

Analytical methods. Plasma glucose was measured using a Beckman Glucose Analyzer (Palo Alto, CA). Plasma insulin, growth hormone, cortisol, adrenaline, noradrenaline (19), and pancreatic polypeptide (20) were measured by assays described previously. To remove antibody-bound insulin in IDDM patients, plasma was mixed with an equal volume of 30% polyethylene

glycol immediately after blood collection (18). Plasma glucagon was measured by RIA using a commercially available kit (ICN Biomedical, Costa Mesa, CA). HbA_{1c} was determined by an HPLC method (range in nondiabetic subjects of 3.8–5.5%).

Statistical methods. Glycemic thresholds for a given parameter were defined as the plasma glucose concentration at which the parameter first exceeded the 95% CI observed for changes in that parameter at the corresponding time point in normoglycemic control experiments as reported previously (4,13). Euglycemic control experiments were performed in all 12 volunteer subjects. Because in 5 IDDM patients the euglycemic control experiments did not yield results different from those of nondiabetic volunteer subjects, the data collected in the latter were used to calculate glycemic thresholds in IDDM patients (C.G.F., unpublished observations). Data are given as means ± SE. Because of the differences in units of treatment, results of cognitive tests were transformed to Z scores (individual values minus means divided by SD) (21) to permit their summation to obtain one unitless value for evaluation (17). A commercially available software package (CSS, Stasoft, Tulsa, OK) was used for statistical analysis. The differences among the groups were analyzed using either ANOVA followed by the least significant different test (22) or the χ^2 test (21).

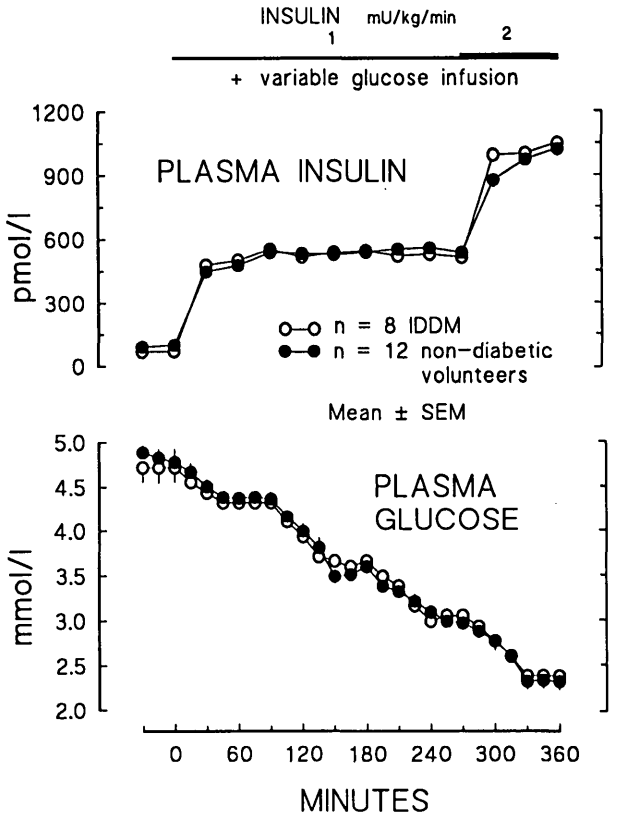

FIG. 1. Plasma insulin (free insulin in IDDM patients) and plasma glucose concentrations during the stepped hypoglycemic clamp of the baseline study.

RESULTS

Frequency of hypoglycemia and percentage HbA_{1c}. After initiation of the therapeutic program, the frequency of hypoglycemia decreased from 0.49 ± 0.05 episodes/patient-day at baseline to 0.05 ± 0.004 episodes/patient-day at 2 wk, and to 0.04 ± 0.03 episodes/patient-day at 3 mo ($P < 0.05$). Severe hypoglycemia (coma, seizures, or need for external assistance) never occurred over the 3-mo period of meticulous prevention of hypoglycemia. HbA_{1c} increased from 5.8 ± 0.3% at baseline to 6.9 ± 0.2% at 3 mo ($P < 0.05$).

Stepped hyperinsulinemic-hypoglycemic clamp. After insulin infusion, plasma insulin concentration increased to similar values in IDDM patients and nondiabetic volunteer subjects. In IDDM patients, plasma glucose concentration was 4.78 ± 0.15 mM at baseline, 4.38 ± 0.07 mM between 45 and 90 min, 3.58 ± 0.05 mM between 135 and 180 min, 3 ± 0.06 mM between 225 and 270 min, and 2.33 ± 0.1 mM between 315 and 360 min. Plasma glucose concentrations of nondiabetic volunteer subjects were superimposable to those of IDDM patients (Fig. 1). Plasma insulin and glucose concentrations in the stepped hypoglycemic clamps repeated after 2 wk and 3 mo did not differ from those of the clamp at baseline.

At baseline, the neuroendocrine and symptom responses in IDDM patients were all reduced compared with nondiabetic volunteer subjects (Figs. 2 and 3), both in terms of glycemic thresholds and magnitude of responses (Table 1). However, after 2 wk of meticulous prevention of hypoglycemia, the magnitude of responses of symptoms increased (autonomic symptoms by ~100%, neuroglycopenic symptoms by ~40%). At 3 mo, not only the magnitude of responses, but also glycemic thresholds were no longer different from those of nondiabetic volunteer subjects (Table 1).

The response of plasma adrenaline improved at 2 wk (increase in magnitude of ~50%). At 3 mo, the magnitude of response increased further, although it was still lower than in nondiabetic volunteer subjects, whereas the glycemic thresholds normalized. The glycemic threshold of responses of plasma noradrenaline was blunted in IDDM patients studied at baseline (2.34 ± 0.1 mM vs. 3.06 ± 0.05 nM in IDDM patients and nondiabetic volunteer subjects, respectively, $P < 0.05$) and did not improve after 2 wk or after 3 mo. However, the magnitude of noradrenaline responses in IDDM patients at baseline (1.65 ± 0.17 nM) were no different compared with nondiabetic volunteer subjects (1.85 ± 0.15 nM) (NS). Baseline plasma glucagon was lower in IDDM patients compared with nondiabetic volunteer subjects and did not respond to hypoglycemia (Fig. 2). Glucagon did not improve after 2 wk of meticulous prevention of hypoglycemia. However, after 3 mo, an increase in glucagon response to hypoglycemia occurred (Fig. 2), although the threshold was higher than normal and the magnitude was considerably lower than normal (Table 1). The responses of pancreatic polypeptide improved after 2 wk of meticulous prevention of hypoglycemia, both in terms of threshold and magnitude, and were normal at 3 mo (Table 2). The magnitude of responses of growth hor-

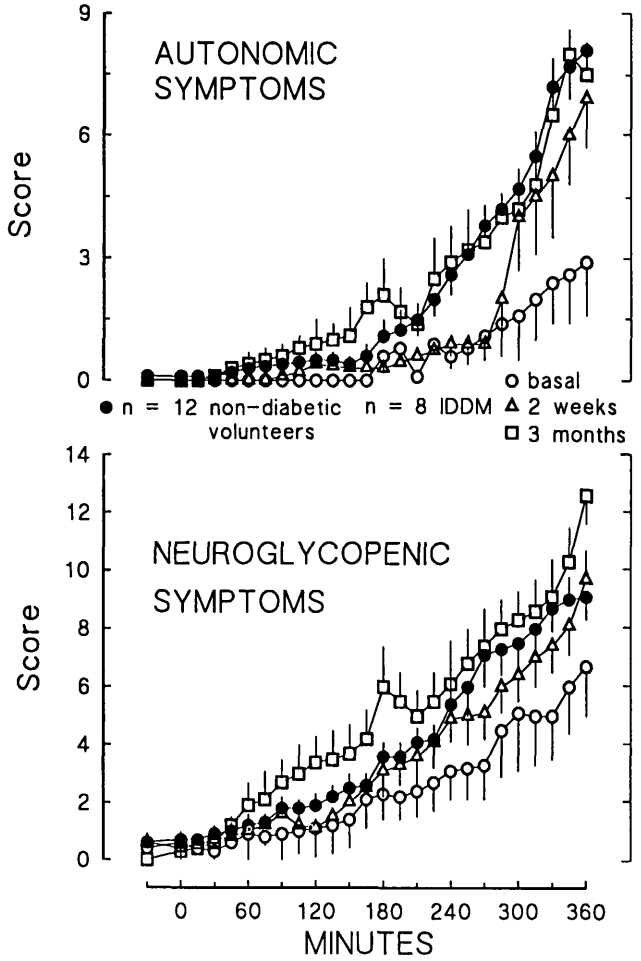

FIG. 2. Score of autonomic and neuroglycopenic symptom responses to the stepped hypoglycemia in nondiabetic volunteer subjects and IDDM patients studied at baseline and after 2 wk and after 3 mo of meticulous prevention of hypoglycemia.

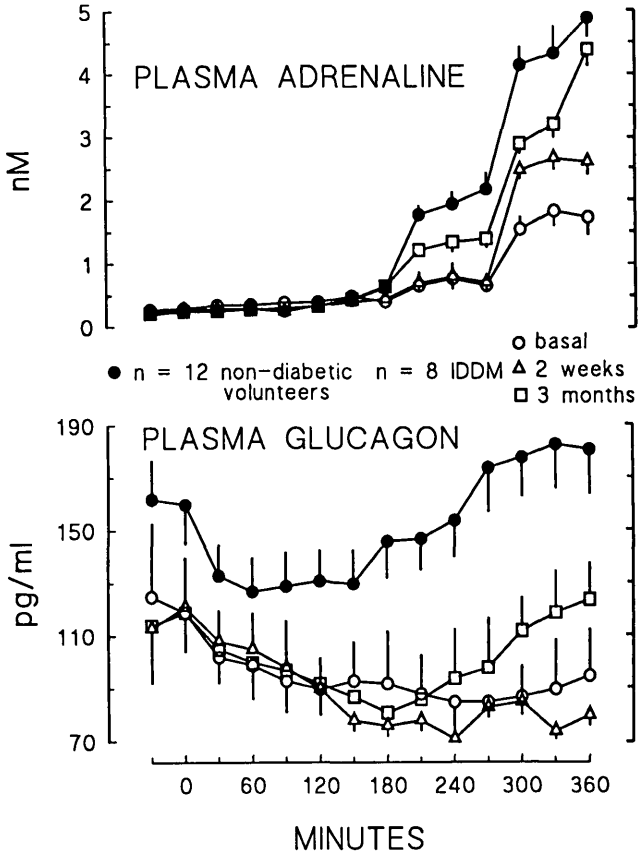

FIG. 3. Plasma adrenaline and plasma glucagon responses to the stepped hypoglycemia in nondiabetic volunteer subjects and IDDM patients studied at baseline and after 2 wk and after 3 mo of meticulous prevention of hypoglycemia.

mone and cortisol normalized after 2 wk of meticulous prevention of hypoglycemia. After 3 mo, the glycemic thresholds of responses of both growth hormone and cortisol also were normal (Table 2).

Cognitive function (Table 2) in IDDM at baseline deteriorated at a lower plasma glucose concentration (2.39 ± 0.06 mM) than in nondiabetic volunteers (2.78 ± 0.06 mM, $P < 0.05$). Also, the magnitude of deterioration during the last hypoglycemic step of the clamp was lower at baseline in IDDM than in nondiabetic volunteers. However, after 2 wk of meticulous prevention of hypoglycemia, cognitive function in response to hypoglycemia deteriorated in IDDM patients at the same glycemic threshold and to the same extent as nondiabetic volunteers. This result was maintained at 3 mo.

The mean rate of glucose infusion needed to maintain plasma glucose concentrations at the plateaus of ~3.0 and ~2.3 mM during the last two steps of the clamp was greater in IDDM patients at baseline (30.1 ± 2.88 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) compared with nondiabetic volunteers (21.4 ± 2.22 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) ($P < 0.05$). However, the rate of glucose infusion decreased to 28 ± 1.91 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ after 2 wk and 25.8 ± 2.71 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ after 3 mo of meticulous prevention of hypoglycemia ($P < 0.05$ vs. baseline), although it was still greater than in the nondiabetic volunteers ($P < 0.05$).

DISCUSSION

This study confirms that recurrent hypoglycemia, a drawback of intensive insulin therapy (22), is associated with high glycemic thresholds of neuroendocrine and symptom responses to hypoglycemia and protection of cognitive function during hypoglycemia in IDDM, as reported previously (4,5). However, these studies demonstrate that a therapeutic program directed primarily at meticulous prevention of hypoglycemia in the everyday life of patients with IDDM nearly normalized the magnitude of neuroendocrine and symptom responses and induced cognitive dysfunction during hypoglycemia within 2 wk, with the exception of the responses of glucagon and noradrenaline, which remained suppressed. Note also that, at 3 mo, glycemic thresholds of neuroendocrine and symptom responses normalized and the response of glucagon improved, but not noradrenaline. Although more than one mechanism probably is involved in the pathogenesis of hypoglycemia unawareness (1), the results of these studies in patients with IDDM of relatively short duration (7 yr), free of classical autonomic neuropathy, strongly support the hypothesis that hypoglycemia unawareness is largely secondary to recurrent therapeu-

TABLE 1
Glycemic thresholds and magnitude of symptom and neuroendocrine responses to the stepped hypoglycemic clamp in nondiabetic volunteer subjects and IDDM patients examined at baseline and after 2 wk and 3 mo of meticulous prevention of hypoglycemia

	Glycemic threshold (mM plasma glucose)	Magnitude of response
Autonomic symptoms (score)		
IDDM patients		
At baseline	2.34 ± 0.07*	2.15 ± 0.9*
After 2 wk	2.58 ± 0.06*	4.7 ± 1.7*†
After 3 mo	3.1 ± 0.05†	5.8 ± 0.6†
Nondiabetic volunteer subjects	3.04 ± 0.06	6.2 ± 0.8
Neuroglycopenic symptoms (score)		
IDDM patients		
At baseline	2.78 ± 0.11*	5.4 ± 1.5*
After 2 wk	3.00 ± 0.07*	7.4 ± 1.7*†
After 3 mo	3.18 ± 0.08†	9.4 ± 1.1†
Nondiabetic volunteer subjects	3.11 ± 0.06	8.3 ± 0.8
Adrenaline (mM)		
IDDM patients		
After baseline	2.80 ± 0.11*	1.71 ± 0.3*
After 2 wk	2.95 ± 0.08*	2.59 ± 0.25*†
After 3 mo	3.45 ± 0.07†	3.50 ± 0.22*†
Nondiabetic volunteer subjects	3.54 ± 0.06	4.5 ± 0.37
Glucagon (pg/ml)		
IDDM patients		
At baseline	none	91 ± 11*
After 2 wk	none	80 ± 12*
After 3 mo	2.63 ± 0.08*†	118 ± 13*†
Nondiabetic volunteer subjects	3.61 ± 0.06	181 ± 17
Pancreatic polypeptide (pM)		
IDDM patients		
At baseline	2.81 ± 0.06*	45 ± 6*
After 2 wk	3.19 ± 0.05*†	108 ± 11*†
After 3 mo	3.58 ± 0.08†	169 ± 20†
Nondiabetic volunteer subjects	3.83 ± 0.06	178 ± 24
Growth hormone (pM)		
IDDM patients		
At baseline	3.00 ± 0.05*	0.54 ± 0.17*
After 2 wk	3.10 ± 0.06*	1.71 ± 0.39†
After 3 mo	3.58 ± 0.05†	1.44 ± 0.37†
Nondiabetic volunteer subjects	3.66 ± 0.06	1.44 ± 0.21
Cortisol (nM)		
IDDM patients		
At baseline	2.89 ± 0.06*	202 ± 37*
After 2 wk	3.05 ± 0.06*	653 ± 42†
After 3 mo	3.56 ± 0.05†	697 ± 47†
Nondiabetic volunteer subjects	3.61 ± 0.06	653 ± 55

Data are means ± SE; n = 8 IDDM patients and 12 nondiabetic volunteer subjetcs.
*P < 0.05 IDDM patients vs. nondiabetic volunteer subjects.
†P < 0.05 IDDM vs. baseline study.

tic hypoglycemia (14). In addition, the results are consistent with the observation in insulinoma patients (12,13) that cure of hypoglycemia normalizes neuroendocrine and symptom responses as well as cognitive dysfunction during hypoglycemia. In these studies, the fact that meticulous prevention of hypoglycemia largely, if not fully, recovered neuroendocrine and symptom responses within a few days and cognitive dysfunction during hypoglycemia, militates in favor of an effect on brain glucose transport by antecedent blood glucose concentration, as demonstrated in rats (23) and more recently in humans (24).

A surprising finding in this study was the improved response of glucagon after 3 mo of meticulous prevention of hypoglycemia. To the best of our knowledge, this is the first observation of a recovery, albeit small, of responses of glucagon to hypoglycemia in IDDM. Because the improved glucagon response occurred under conditions of supraphysiological hyperinsulinemia (~1000 pM), which suppresses the glucagon response to hypoglycemia (25,26), it is likely that greater glucagon responses might have been observed if the lower plasma insulin concentrations of the everyday life of IDDM patients (~70–300 pM) were used in these studies to induce hypoglycemia. Although the overall glucagon increase was quantitatively small, the fact that, in these studies, prevention of hypoglycemia was able to regenerate some of the glucagon response to hypoglycemia is conceptually important. It indicates that a functional mechanism, either neural and sensitive to changes in the brain glucose transport or sensitive to changes in the pancreatic A-cell itself, modulates the responses of glu-

TABLE 2
Cognitive function (sum of *Z* scores) during stepped hypoglycemic clamp in nondiabetic volunteer subjects and IDDM patients studied at baseline and after 2 wk and 3 mo of meticulous prevention of hypoglycemia

		Time of stepped hypoglycemic clamp (min)				
	n	0	90	180	270	360
IDDM patients	8					
At baseline		−0.0 ± 3.12	−0.05 ± 2.7	−9 ± 2.4	−1.1 ± 3.7*	−7.34 ± 2.01*†
After 2 wk		−0.0 ± 3.02	−0.9 ± 2.9	−1.5 ± 3.1	−2.88 ± 3.67	−12.3 ± 3.93‡
After 3 mo		0.0 ± 2.89	1.1 ± 2.5	−1.7 ± 2.9	−2.07 ± 3.61	−11.6 ± 2.86‡
Nondiabetic volunteer subjects	12	0.0 ± 2.5	−1.4 ± 1.9	−2.9 ± 1.5	−4.5 ± 2.3	−12.1 ± 2.9†

Data are means ± SE.
*$P < 0.05$ IDDM patients vs. nondiabetic volunteer subjects.
†$P < 0.05$ vs. time 0.
‡$P < 0.05$ IDDM vs. baseline study.

cagon to hypoglycemia in IDDM, as it does in nondiabetic subjects (7,11). However, the fact that in these studies, the responses of pancreatic polypeptide were normalized and those of adrenaline near normalized, whereas those of glucagon were still largely abnormal after meticulous prevention of hypoglycemia, is consistent with the concept of an intrinsic A-cell defect in the pancreatic islets of IDDM (27). The A-cell defect develops early in the course of diabetes (28), whereas the secretion of the other counterregulatory hormones appears to be nearly intact, at least in IDDM of short duration.

In these studies, no specific test (11) was conducted to demonstrate improved defenses against hypoglycemia by the recovered counterregulatory hormone responses. However, the fact that the amount of exogenous glucose infused during the last two steps of the hypoglycemic clamp was lower after 2 wk and 3 mo compared with baseline indicates that the defenses against hypoglycemia by the improved counterregulatory hormone response were more efficient after meticulous prevention of hypoglycemia.

This study also indicates that an intensive therapeutic program involving primarily education of the IDDM patients and frequent contact in person or by telephone successfully reduces the frequency of hypoglycemia to a minimum (~0.04 episodes/patient-day), without drifting blood glucose control into the opposite condition of sustained hyperglycemia. This is indicated in our study by a moderate increase in HbA_{1c} from 5.8 ± 0.3 to 6.9 ± 0.2% in which an HPLC assay was used with an upper limit of percentage HbA_{1c} in nondiabetic subjects of 5.5. Note that the mean percentage HbA_{1c} reported for the experimental group on intensive insulin therapy in the DCCT is ~7.0%, with an HPLC assay with an upper limit of 6.1% in nondiabetic subjects (ADA Annual Meeting Las Vegas, June 1993). Thus, the fact that in our study the percentage HbA_{1c} after prevention of hypoglycemia is only slightly greater than that of the DCCT suggests that in our studies the strategy of prevention of hypoglycemia resulted in a mean blood glucose hopefully protecting against the onset and/or progression of microvascular complications.

Several questions remain open. Will meticulous prevention of hypoglycemia in patients with long-term IDDM, free of classical autonomic neuropathy, be as successful as in patients with relatively short duration of IDDM? Will patients with classical autonomic neuropathy benefit? Finally, what risk is there that HbA_{1c} will increase excessively over a long term when using meticulous prevention of hypoglycemia as the primary goal rather than using a target of blood glucose near normoglycemia? Clearly, additional studies are needed to answer these questions.

ACKNOWLEDGMENTS

This work was supported by grant 91-00325-PF40 for C.N.R.'s finalized project on Aging and by the Juvenile Diabetes Foundation Grant 193108.

The dedicated editorial help of Patricia Boyce, the superb technical assistance of Giampiero Cipiciani, and the friendly support from the Desert Inn, Las Vegas (FP3312GT), are gratefully acknowledged.

Presented in part at the 52nd Annual Meeting of the American Diabetes Association, San Antonio, Texas, June 1992.

REFERENCES

1. Gerich J, Mokan M, Veneman T, Korytkowski M, Mitrakou A: Hypoglycemia unawareness. *Endocrinol Rev* 12:356–71, 1991
2. MacLeod KM, Gold AE, Frier BM: Frequency of severe hypoglycemia in insulin-dependent diabetic patients with altered awareness of hypoglycemia. *Diabetes* 42 (Suppl. 1):26A, 1993
3. Heller S, Herbert M, MacDonald I, Tattersall R: Influence of sympathetic nervous system on hypoglycemic warning symptoms. *Lancet* II:359–63, 1987
4. Mokan M, Mitrakou A, Raptis G, Platanisiotis D, Grannakopoulos F, Korytkowski M, Cryer P, Gerich J: Differential effects of duration of diabetes and glycemic control on thresholds and magnitude of responses to hypoglycemia. *Diabetes* 40 (Suppl. 1):556A, 1991
5. Amiel S, Pottinger R, Cunnah DC, Chusney G, Archibald H, Prior PF, Gale EAM: Effect of antecedent glucose control on cerebral function during hypoglycemia. *Diabetes Care* 14:109–18, 1991
6. Davis M, Shamoon H: Counterregulatory adaptation to recurrent hypoglycemia in normal humans. *J Clin Endocrinol Metab* 73:995–1001, 1991
7. Heller S, Cryer P: Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia after one episode of hypoglycemia in nondiabetic humans. *Diabetes* 40:223–26, 1991
8. Widom B, Simonson DC: Intermittent hypoglycemia impairs glucose counterregulation. *Diabetes* 41:1597–1602, 1992
9. Veneman T, Mitrakou A, Mokan M, Cryer P, Gerich JE: Induction of hypoglycemia unawareness by asymptomatic nocturnal hypoglycemia. *Diabetes* 42:1233–37, 1993
10. Lingenfelser T, Renn W, Sommerwerck U, Jung MF, Buettner UW, Zaiser-Kaschel H, Kaschel R, Eggstein M, Jacober B: Compromised hormonal counterregulation, symptom awareness, and neurophysiological function after recurrent short-term episodes of insulin-induced hypoglycemia in IDDM patients. *Diabetes* 42:610–18, 1993

11. Dagogo-Jack SE, Cryer PE: Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus: recent antecedent hypoglycemia reduces autonomic responses to, symptoms of, and defense against subsequent hypoglycemia. *J Clin Invest* 91:819–28, 1993
12. Maran A, Taylor J, MacDonald IA, Amiel S: Evidence for reversibility of defective counterregulation in a patient with insulinoma. *Diabetic Med* 9:765–68, 1992
13. Mitrakou A, Fanelli C, Veneman T, Perriello G, Platanisiotis, Rambotti A, Raptis S, Brunetti P, Cryer P, Gerich J, Bolli G: Reversibility of the hypoglycemia unawareness phenomenon: studies in insulinoma patients before and after surgery. *N Engl J Med.* In press
14. Cryer PE: Iatrogenic hypoglycemia as a cause of hypoglycemia-associated autonomic failure in IDDM: A vicious circle. *Diabetes* 41:255–60, 1992
15. Lingefelser T, Renn W, Eggstein M, Reinauer M: Restoration of impaired counterregulatory hormone response, symptom perception and cerebral function by short-term euglycemia in IDDM. *Diabetes* 42 (Suppl 1): 243A, 1993
16. Ewing DJ, Clarke BF. Diagnosis and management of diabetic autonomic neuropathy. *Br Med J* 285:916–18, 1982
17. Mitrakou A, Ryan C, Veneman T, Mokan M, Jenssen T, Kiss I, Durrant J, Cryer P, Gerich J: Hierarchy of glycemic thresholds for counterregulatory hormone secretion, symptoms, and cerebral dysfunction. *Am J Physiol* 260:E67–74, 1991
18. De Feo P, Perriello G, Ventura MM, Calcinaro F, Basta G, Lolli C, Cruciani C, Dell'Olio A, Santeusanio F, Brunetti P, Bolli GB: Studies on overnight insulin requirements and metabolic clearance rate of insulin in normal and diabetic man: relevance to the pathogenesis of the dawn phenomenon. *Diabetologia* 29:475–80, 1986
19. Fanelli C, De Feo P, Porcellati F, Perriello G, Torlone E, Santeusanio F, Brunetti P, Bolli GB: Adrenergic mechanisms contribute to the late phase of hypoglycemic glucose counterregulation in humans by stimulating lipolysis. *J Clin Invest* 89:2005–13, 1992
20. Kohn A, Annibale B, Suriano G, Severi C, Spinella S, Delle Fave G: Gastric acid and pancreatic polypeptide responses to modified sham feeding: indication of an increased basal vagal tone in a subgroup of duodenal ulcer patients. *Gut* 26:776–82, 1985
21. Zar J: *Biostatistical analysis.* Englewood Cliffs, Prentice Hall, 1984
22. The DCCT Research Group: Epidemiology of severe hypoglycemia in the diabetes control and complications trial. *Am J Med* 90:450–59, 1991
23. McCall A, Fixman L, Fleming N, Tornheim K, Chick W, Ruderman N: Chronic hypoglycemia increases brain glucose transport. *Am J Physiol* 251:E442–47
24. Nagy RJ, O'Connor A, Robinson B, Boyle PJ: Hypoglycemia unawareness results from adaptation of brain glucose metabolism. *Diabetes* 42 (Suppl. 1):133A, 1993
25. Diamond MP, Hallarman L, Starick-Zych, Jones TW, Connolly-Howard M, Tamborlane WV, Sherwin RS: Suppression of counterregulatory hormone response to hypoglycemia by insulin per se. *J Clin Endocrinol Metab* 72:1388–90, 1991
26. Liu D, Moberg E, Kollind M, Lins P-E, Adamson U: A high concentration of circulating insulin suppresses the glucagon response to hypoglycemia in normal man. *J Clin Endocrinol Metab* 73:1123–28, 1991
27. Gerich J, Langlois M, Noacco C, Karam J, Forsham P: Lack of glucagon response to hypoglycemia in diabetes: evidence for an intrinsic pancreatic alpha cell defect. *Science* 182:171–73, 1973
28. Bolli GB, De Feo P, Compagnucci P, Cartechini MG, Angeletti G, Santeusanio F, Brunetti P, Gerich JE: Abnormal glucose counterregulation in insulin-dependent diabetes mellitus: interaction of anti-insulin antibodies and impaired glucagon and epinephrine secretion. *Diabetes* 32:134–41, 1983