Brief Twice-Weekly Episodes of Hypoglycemia Reduce Detection of Clinical Hypoglycemia in Type 1 Diabetes Mellitus

Fernando Ovalle, Carmine G. Fanelli, Deanna S. Paramore, Tamara Hershey, Suzanne Craft, and Philip E. Cryer

We tested the hypothesis that as few as two weekly brief episodes of superimposed hypoglycemia (i.e., doubling the average frequency of symptomatic hypoglycemia) would reduce physiological and behavioral defenses against developing hypoglycemia and reduce detection of clinical hypoglycemia in patients with type 1 diabetes mellitus (T1DM). Compared with nondiabetic controls, six patients with well-controlled T1DM (HbA_{1c}, 7.5 ± 0.7% [mean ± SD]) exhibited absent glucagon responses and reduced epinephrine (P = 0.0027), norepinephrine (P = 0.0007), pancreatic polypeptide (P = 0.0030), and neurogenic symptom (P = 0.0451) responses to hypoglycemia as expected. In these patients, 2 h of induced hypoglycemia (50 mg/dl, 2.8 mmol/l) twice weekly for 1 month, compared in a random-sequence crossover design with an otherwise identical 2 h of induced hyperglycemia (150 mg/dl, 8.3 mmol/l) twice weekly for 1 month, further reduced the epinephrine (P = 0.0001) and pancreatic polypeptide (P = 0.0030) responses, tended to further reduce the norepinephrine and neurogenic symptom responses to hypoglycemia, and reduced cognitive dysfunction during hypoglycemia (P = 0.0271), all assessed in the investigational setting. In the clinical setting, induced hypoglycemia did not alter overall glycemic control, but did reduce the total number of symptomatic hypoglycemic episodes detected by the patients from 49 to 30 per month and lowered the mean ± SE self-monitored blood glucose level during symptomatic hypoglycemia from 51 ± 2 mg/dl (2.8 ± 0.1 mmol/l) to 46 ± 3 mg/dl (2.6 ± 0.2 mmol/l) (P < 0.01). It also reduced the proportion of low regularly scheduled self-monitored values that were symptomatic by ~33%. Thus as little as doubling the frequency of symptomatic hypoglycemia further reduced both the key epinephrine response and clinical awareness of developing hypoglycemia, changes reasonably expected to increase the risk of severe iatrogenic hypoglycemia in T1DM. *Diabetes* 47:1472–1479, 1998

From the Division of Endocrinology, Diabetes, and Metabolism, and the General Clinical Research Center and Diabetes Research and Training Center, Washington University School of Medicine, St. Louis, Missouri.

Address correspondence and reprint requests to Dr. Philip E. Cryer, Division of Endocrinology, Diabetes and Metabolism, Washington University School of Medicine (Campus Box 8127), 660 South Euclid Ave., St. Louis, MO 63110. E-mail: pcryer@imgate.wustl.edu.

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T1DM, type 1 diabetes mellitus.

Iatrogenic hypoglycemia is the limiting factor, both conceptually and in practice, in the management of diabetes (1). Although glycemic control prevents or delays several of the long-term complications of diabetes (2), attempts to achieve near-normal glycemia increase the risk of hypoglycemia (1,2). People with type 1 diabetes mellitus (T1DM) experience an average of two episodes of symptomatic hypoglycemia per week during intensive therapy (1).

Iatrogenic hypoglycemia is the result of the interplay of relative or absolute insulin excess, which must occur from time to time because of the imperfections of current insulin replacement regimens, and compromised glucose counterregulation, rather than insulin excess alone in T1DM (1). The concept of hypoglycemia-associated autonomic failure in T1DM (1,3) posits that periods of relative or absolute therapeutic insulin excess in the absence of glucagon secretory responses to falling plasma glucose levels lead to episodes of iatrogenic hypoglycemia; that these episodes, in turn, cause reduced autonomic (adrenomedullary and sympathetic as well as parasympathetic) responses to falling glucose levels on subsequent occasions; and that these reduced autonomic responses result in reductions in the symptoms of and resultant behavioral defense against (e.g., food ingestion) developing hypoglycemia (i.e., the clinical syndrome of hypoglycemia unawareness) and, because epinephrine responses are reduced when glucagon responses are absent, lead to impaired physiological defense against developing hypoglycemia (i.e., the clinical syndrome of defective glucose counterregulation). Thus a vicious cycle of recurrent hypoglycemia is created and perpetuated. There is considerable support for this concept (3–8). Perhaps the most compelling is that hypoglycemia unawareness and, in part, defective glucose counterregulation are generally reversible by scrupulous avoidance of iatrogenic hypoglycemia (5–8).

To assess the impact of hypoglycemia-associated autonomic failure on the daily lives of people with T1DM, we tested the hypothesis that as few as two weekly brief episodes of superimposed hypoglycemia (i.e., doubling the average frequency of symptomatic hypoglycemia) reduces physiological and behavioral defenses against developing hypoglycemia and reduces detection of hypoglycemia in the clinical setting. To do so we used a random-sequence crossover design to contrast the effects of twice-weekly 2-h episodes of insulin-induced hypoglycemia for 1 month with the effects of otherwise identical twice-weekly episodes of hyperglycemia for 1 month in patients with well-controlled T1DM.

TABLE 1
Characteristics of the patients with T1DM

	Age (years)	Sex	BMI (kg/m^2)	Duration (years)	Insulin regimen	Insulin dose (U/day)	HbA_{1c} (%)
Patient							
1	37	F	19.5	21	CSII	34	6.9
2	34	F	37.4	22	CSII	70	7.6
3	33	F	29.7	9	CSII	35	7.0
4	33	M	23.3	8	MDI	65	7.7
5	44	M	26.3	17	CSII	35	7.0
6	39	F	22.8	33	CSII	32	8.6
Mean ± SD (SE)	37 ± 4 (2)	—	26.5 ± 6.3 (2.6)	18 ± 9 (4)	—	45 ± 17 (7)	7.5 ± 0.7 (0.3)

CSII, continuous subcutaneous insulin infusion; MDI, multiple daily injection.

RESEARCH DESIGN AND METHODS

Subjects. Study subjects included six patients with T1DM and 12 nondiabetic control subjects, each of whom gave their written informed consent to participate in this study, which was approved by the Washington University Human Studies Committee and conducted at the Washington University General Clinical Research Center. The characteristics of the patients are listed in Table 1. The 12 nondiabetic subjects (7 women, 5 men) had a mean age (± SD) of 27 ± 4 years and a mean BMI of 24.8 ± 3.6 kg/m^2.

Protocol. The patients with T1DM followed their established prestudy management regimens (Table 1) throughout the study. They were assigned randomly to either twice-weekly clamped hypoglycemia for 1 month or twice-weekly clamped hyperglycemia for 1 month and then, after a 1-month interval, crossed over to twice-weekly hyperglycemia or hypoglycemia for 1 month. These episodes involved intravenous infusion of regular human insulin (Novolin R; Novo Nordisk, Bagsvaerd, Denmark) in a dosage of 2.0 mU · kg^{-1} · min^{-1} (12.0 pmol · kg^{-1} · min^{-1}) and variable infusion of 20% glucose to hold plasma glucose concentrations at 50 mg/dl (2.8 nmol/l) or 150 mg/dl (8.3 mmol/l) for 2 h. The time of day of these clamps was individualized to fit each patient's schedule, but was the same in a given patient throughout the study.

Patients performed self-monitoring of blood glucose with One Touch Profile devices with memory capability (Lifescan, Milpitas, CA) at a minimum before meals and at bedtime throughout the study. They were instructed to record all episodes of symptomatic hypoglycemia; the timing of these was ascertained by the investigator during their frequent visits for hypoglycemic or hyperglycemic clamps.

Hyperinsulinemic stepped hypoglycemic clamps (9) were performed before the study (baseline) and at the end of the twice-weekly hypoglycemia and hyperglycemia arms, ~48 h after the last episode of induced hypoglycemia or hyperglycemia. The patients were admitted to the research center for overnight intravenous insulin infusions in dosages that held plasma glucose levels at ~100 mg/dl (5.6 mmol/l). The following morning, after an overnight fast, hyperinsulinemic (2.0 mU · kg^{-1} · min^{-1}, 12.0 pmol · kg^{-1} · min^{-1}) stepped hypoglycemic clamps with hourly steps targeted at plasma glucose concentrations of 85, 75, 65, 55, and 45 mg/dl (4.7, 4.2, 3.6, 3.0, and 2.5 mmol/l) were performed. Arterialized venous blood samples were serially obtained through an indwelling needle in a hand vein, with the

TABLE 2
Plasma insulin, glucagon, and pancreatic polypeptide concentrations during hyperinsulinemic stepped hypoglycemic clamps in nondiabetic subjects and patients with T1DM at baseline, after twice-weekly hypoglycemia for 1 month, and after twice-weekly hyperglycemia for 1 month

	Nominal glucose (mg/dl)	Nondiabetic subjects (n = 12)	T1DM patients (n = 6)			P value (analysis of variance)
			Baseline	After hypoglycemia	After hyperglycemia	
Insulin (μU/ml)	~100	10 ± 1	29 ± 4	23 ± 4	25 ± 3	NS (nondiabetic vs.
	85	127 ± 6	119 ± 13	108 ± 16	115 ± 9	T1DM baseline)
	75	129 ± 4	124 ± 14	113 ± 20	126 ± 14	NS (T1DM baseline vs.
	65	132 ± 6	124 ± 11	114 ± 20	125 ± 9	after hypoglycemia)
	55	126 ± 5	128 ± 11	109 ± 18	121 ± 13	NS (T1DM baseline vs.
	45	134 ± 5	130 ± 12	114 ± 24	116 ± 16	after hyperglycemia)
Glucagon (pg/ml)	~100	112 ± 9	47 ± 5	48 ± 4	51 ± 6	0.0561 (nondiabetic vs.
	85	101 ± 9	46 ± 4	44 ± 4	48 ± 5	T1DM baseline)
	75	92 ± 7	44 ± 4	43 ± 4	45 ± 4	NS (T1DM baseline vs.
	65	99 ± 9	43 ± 4	43 ± 4	46 ± 5	after hypoglycemia)
	55	115 ± 10	42 ± 4	42 ± 4	44 ± 5	NS (T1DM baseline vs.
	45	121 ± 18	42 ± 6	43 ± 5	48 ± 6	after hyperglycemia)
Pancreatic polypeptide (pg/ml)	~100	66 ± 5	73 ± 21	70 ± 12	71 ± 17	0.0030 (nondiabetic vs.
	85	54 ± 5	50 ± 11	53 ± 9	43 ± 10	T1DM baseline)
	75	49 ± 6	42 ± 6	51 ± 6	52 ± 9	0.0430 (T1DM baseline vs.
	65	152 ± 38	60 ± 10	46 ± 9	42 ± 10	after hypoglycemia)
	55	415 ± 52	57 ± 9	43 ± 7	59 ± 13	NS (T1DM baseline vs.
	45	567 ± 40	408 ± 78	225 ± 79	294 ± 76	after hyperglycemia)

Data are means ± SE. To convert glucose to millimoles per liter, multiply by 0.05551; insulin to picomoles per liter, multiply by 6.0; glucagon to picomoles per liter, multiply by 0.2871; and pancreatic polypeptide to picomoles per liter, multiply by 0.239.

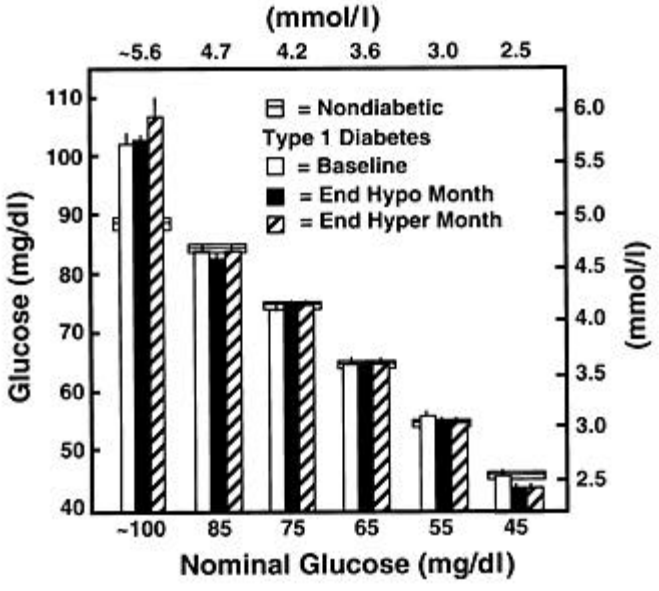

FIG. 1. Means ± SE of plasma glucose concentrations during hyperinsulinemic (2.0 mU · kg^{-1} · min^{-1}, 12.0 pmol · kg^{-1} · min^{-1}) stepped hypoglycemic clamps in nondiabetic control subjects (⊟) and patients with T1DM studied at baseline (□), after twice-weekly 2-h episodes of induced hypoglycemia (50 mg/dl, 2.8 mmol/l) for 1 month (■), and after twice-weekly 2-h episodes of induced hyperglycemia (150 mg/dl, 8.3 mmol/l) for 1 month (▨).

hand kept in a box at ~65°C. Heart rate and blood pressure were also determined serially, and the electrocardiogram was monitored throughout. The nondiabetic subjects underwent identical hyperinsulinemic stepped hypoglycemic clamps on one occasion after an overnight fast as outpatients.

Analytical methods. Plasma glucose concentrations were determined with a glucose oxidase method (Beckman Glucose Analyzer II; Beckman Instruments, Fullerton, CA). Plasma epinephrine and norepinephrine were measured with a single isotope–derivative (radioenzymatic) method (10), and plasma pancreatic polypeptide (11), free insulin (12), C-peptide (12), glucagon (13), growth hormone (14), and cortisol (15) levels were measured with radioimmunoassays. Serum nonesterified fatty acids were measured with an enzymatic colorimetric method (16), and blood lactate (17), β-hydroxybutyrate (18), and alanine (19) were measured with enzymatic techniques. HbA_{1c} was measured with an ion-exchange high-performance liquid chromatography method (20); the nondiabetic range is 4.0–6.0%.

Symptoms were quantitated by asking the subject to score (from 0 for "none" to 6 for "severe") each of 12 symptoms: 6 neurogenic (3 adrenergic—pounding heart, shaky/tremulous, nervous/anxious, and 3 cholinergic—sweaty, hungry, tingling) and 6 neuroglycopenic (having difficulty thinking/confused, tired/drowsy, weak, warm, faint, dizzy) symptoms (21).

Our battery of cognitive function tests has been described in detail elsewhere (22). They include measures of attention (Stroop arrow-word test), information processing (paced serial addition test), pattern recognition and memory (delayed nonmatch to sample test), declarative memory (delayed paragraph recall test), and memory retrieval (memory scanning test).

Statistical methods. Data are expressed as means ± SE, except where SD is specified. Data were analyzed by repeated-measures analysis of variance. In addition, a standard score, the unitless z score (23), was computed for each cognitive function data set and is presented as the sum of z scores for the four tests that were sensitive to hypoglycemia. Self-monitored glucose levels during symptomatic hypoglycemia were compared with a paired t test. $P < 0.05$ was considered significant.

RESULTS

Hyperinsulinemic stepped hypoglycemic clamps. During hyperinsulinemic stepped hypoglycemic clamps, target plasma glucose concentrations were achieved (Fig. 1); plasma free insulin and glucose concentrations were similar in nondiabetic control subjects and in patients with T1DM at baseline, after twice-weekly hypoglycemia for 1 month, and after twice-weekly hyperglycemia for 1 month (Table 2).

Compared with the responses of nondiabetic subjects, plasma glucagon responses to hypoglycemia were absent (Table 2) and plasma epinephrine ($P = 0.0027$) (Fig. 2), pancreatic polypeptide ($P = 0.0030$), norepinephrine ($P = 0.0007$), neurogenic symptom ($P = 0.0451$) (Fig. 3), and serum nonesterified fatty acid ($P = 0.0009$) responses to hypoglycemia were reduced in patients with T1DM (Tables 2–4). Plasma cortisol and blood β-hydroxybutyrate and lactate responses tended to be reduced, but none of these was reduced significantly (Table 4). Plasma growth hormone responses also tended to be increased (Table 4). Performance on all cognitive function tests except that on the memory scanning task deteriorated during hypoglycemia (Table 5). Thus the latter measure of memory retrieval was again not sensitive to hypoglycemia (22). Patients with T1DM performed less well than nondiabetic subjects on that test ($P = 0.0136$) (Table 5) and also on the four tests that were sensitive to hypoglycemia (sum of z scores, $P = 0.0001$) (Fig. 4). Based on the z scores for each individual test (data not shown), the patients with T1DM also performed less well on the delayed nonmatch to sample task ($P = 0.0002$), the delayed paragraph recall task ($P = 0.0047$), the paced serial addition task ($P = 0.0006$), and the Stroop arrow-word task ($P = 0.0001$).

Compared with responses during the baseline stepped hypoglycemic clamps, after twice-weekly hypoglycemia for 1 month, but not after twice-weekly hyperglycemia for 1 month, plasma epinephrine ($P = 0.0001$), pancreatic polypeptide ($P = 0.0430$), and blood lactate ($P = 0.0422$) responses to hypoglycemia were reduced further, and the plasma growth hormone ($P = 0.0405$) response was reduced in patients with T1DM (Tables 2–4, Fig. 2). Plasma norepinephrine and symptom responses tended to be reduced further and plasma cortisol responses tended to be reduced, but none of these was reduced significantly (Tables 3 and 4). As assessed by the sum of z scores, overall cognitive function in patients with T1DM deteriorated less during hypoglycemia after twice-weekly hypoglycemia, but not after twice-weekly hyperglycemia ($P = 0.0271$) (Fig. 4). After twice-weekly hypoglycemia, performance on the delayed nonmatch to sample task (overall $P = 0.0461$, increment $P = 0.0154$), the delayed paragraph recall task (overall $P = 0.0288$, decrement $P = 0.0343$), and the Stroop arrow-word task (increment $P = 0.0061$) was preserved significantly, and performance on the paced serial addition task tended to be preserved (Table 5).

Clinical hypoglycemia. Patients with T1DM self-monitored their blood glucose concentrations just as frequently and overall glycemic control was the same in the two arms of the study. They performed 99 ± 5 determinations with a mean glucose value of 136 ± 6 mg/dl (7.5 ± 0.3 mmol/l) during the twice-weekly hyperglycemia month and 106 ± 5 determinations with a mean glucose value of 146 ± 8 mg/dl (8.1 ± 0.4 mmol/l) during the twice-weekly hypoglycemia month.

The total number of symptomatic hypoglycemic episodes detected by the patients was reduced from 49 in the twice-weekly hyperglycemia month to 30 in the twice-weekly hypoglycemia month, and the mean self-monitored glucose level during symptomatic hypoglycemia was reduced from 51 ± 2 mg/dl (2.8 ± 0.1 mmol/l) to 46 ± 3 mg/dl (2.6 ± 0.2 mmol/l) ($P < 0.01$) in the hyperglycemia and hypoglycemia months, respectively (Fig. 5). The number of symptomatic episodes of hypoglycemia with self-monitored glucose levels <70 mg/dl (3.9 mmol/l) were 49 and 30, those with glucose levels <60 mg/dl (3.3 mmol/l) were 45 and 27, and those with glucose levels <50 mg/dl (2.8 mmol/l) were 29 and 18 during the hyper-

TABLE 3
Plasma norepinephrine concentrations, neuroglycopenic symptom scores, and plasma growth hormone and cortisol concentrations during hyperinsulinemic stepped hypoglycemic clamps in nondiabetic subjects and patients with T1DM at baseline, after twice-weekly hypoglycemia for 1 month, and after twice-weekly hyperglycemia for 1 month

	Nominal glucose (mg/dl)	Nondiabetic subjects (n = 12)	T1DM patients (n = 6)			P value (analysis of variance)
			Baseline	After hypoglycemia	After hyperglycemia	
Norepinephrine (pg/ml)	~100	197 ± 15	220 ± 16	174 ± 19	207 ± 28	0.0007 (nondiabetic vs.
	85	198 ± 11	223 ± 16	200 ± 23	236 ± 33	T1DM baseline)
	75	200 ± 11	238 ± 16	207 ± 26	230 ± 30	NS (T1DM baseline vs.
	65	246 ± 14	224 ± 14	210 ± 24	255 ± 40	after hypoglycemia)
	55	296 ± 14	249 ± 23	224 ± 26	260 ± 36	NS (T1DM baseline vs.
	45	413 ± 20	276 ± 28	230 ± 31	296 ± 26	after hyperglycemia)
Neuroglycopenic symptom score	~100	1.5 ± 0.2	2.8 ± 1.4	2.1 ± 1.0	1.4 ± 0.6	NS (nondiabetic vs.
	85	1.5 ± 0.2	1.9 ± 0.6	3.4 ± 1.0	1.4 ± 0.4	T1DM baseline)
	75	2.0 ± 0.2	2.2 ± 0.6	4.3 ± 1.1	1.9 ± 0.9	NS (T1DM baseline vs.
	65	3.6 ± 0.5	2.3 ± 0.4	4.4 ± 0.9	3.2 ± 1.2	after hypoglycemia)
	55	4.9 ± 0.5	5.9 ± 3.4	4.3 ± 1.0	5.6 ± 1.8	NS (T1DM baseline vs.
	45	10.5 ± 1.3	10.2 ± 5.0	7.3 ± 2.6	9.3 ± 4.5	after hyperglycemia)
Growth hormone (ng/ml)	~100	2.8 ± 0.6	6.7 ± 2.7	5.8 ± 2.5	2.9 ± 0.8	NS (nondiabetic vs.
	85	3.6 ± 0.4	4.1 ± 1.9	3.5 ± 2.3	1.2 ± 0.1	T1DM baseline)
	75	1.2 ± 0.1	4.5 ± 2.6	1.4 ± 0.5	0.9 ± 0.0	0.0405 (T1DM baseline vs.
	65	8.9 ± 1.6	5.1 ± 2.6	3.3 ± 1.3	4.1 ± 2.1	after hypoglycemia)
	55	13.6 ± 2.5	12.3 ± 5.3	8.7 ± 4.0	6.4 ± 2.1	NS (T1DM baseline vs.
	45	15.8 ± 2.2	40.6 ± 8.8	23.2 ± 5.6	29.6 ± 6.4	after hyperglycemia)
Cortisol (μg/dl)	~100	16.9 ± 1.6	13.6 ± 2.3	12.2 ± 1.0	11.5 ± 1.5	NS (nondiabetic vs.
	85	15.6 ± 1.2	10.7 ± 1.6	8.7 ± 0.8	8.3 ± 0.6	T1DM baseline)
	75	13.5 ± 0.9	10.2 ± 2.1	7.6 ± 1.2	7.6 ± 0.7	NS (T1DM baseline vs.
	65	16.4 ± 1.2	9.9 ± 1.9	7.8 ± 1.0	10.1 ± 1.7	after hypoglycemia)
	55	22.8 ± 2.2	9.9 ± 1.1	9.8 ± 1.4	9.8 ± 0.8	NS (T1DM baseline vs.
	45	33.0 ± 2.2	19.3 ± 1.5	13.9 ± 1.9	15.9 ± 2.2	after hyperglycemia)

Data are means ± SE. To convert norepinephrine to nanomoles per liter, multiply by 0.005911; growth hormone to picomoles per liter, multiply by 44.15; and cortisol to millimoles per liter, multiply by 27.59.

glycemia and hypoglycemia months, respectively (Fig. 5). Among all of the regularly scheduled self-monitored glucose levels <70 mg/dl (3.9 mmol/l), 36% were associated with symptoms during the hyperglycemia month and 26% were symptomatic during the hypoglycemia month. The corresponding percentages at glucose values <60 mg/dl (3.3 mmol/l) were 52 and 38%, respectively, and at glucose values <50 mg/dl (2.8 mmol/l) were 70 and 41%, respectively.

There were only two episodes of severe hypoglycemia (i.e., an episode requiring the assistance of another person) during the study, one in each of the two study arms.

DISCUSSION

These data document that as few as two episodes of brief hypoglycemia each week, superimposed on a background of episodes of treatment-associated hypoglycemia, reduce both physiological and potential behavioral defenses against developing hypoglycemia assessed in the investigational setting and patient detection of hypoglycemia in the clinical setting in patients with T1DM.

Compared with nondiabetic controls, patients with well-controlled T1DM exhibited absent glucagon responses to hypoglycemia, reduced autonomic—adrenomedullary (epinephrine), sympathetic neural (norepinephrine), and parasympathetic neural (pancreatic polypeptide)—responses to developing hypoglycemia, and, correspondingly, reduced neurogenic (i.e., autonomic) symptoms of hypoglycemia, as expected (1). Given these features of compromised glucose counterregulation central to the concept of hypoglycemia-associated autonomic failure (1,3), and the patients' near-normal glycemic control, they would be expected to be at high risk of iatrogenic hypoglycemia (24–26). Indeed, during the control month of the study (twice-weekly hyperglycemia), they experienced an average of two episodes of symptomatic hypoglycemia per week. Interestingly, this figure is identical to the average frequency of symptomatic hypoglycemia during intensive therapy in larger groups of patients with T1DM (1).

In these patients with T1DM, 2 h of superimposed induced hypoglycemia (50 mg/dl, 2.8 mmol/l) twice-weekly for 1 month, compared in a crossover design with an otherwise identical 2 h of induced hyperglycemia twice-weekly for 1 month, further reduced the epinephrine and pancreatic polypeptide responses and tended to further reduce the norepinephrine and neurogenic symptom responses to a given level of hypoglycemia. Blood lactate responses were also reduced, perhaps a reflection of the reduced epinephrine responses; serum nonesterified fatty acid and blood β-hydroxybutyrate levels were suppressed comparably. The

TABLE 4
Nonesterified fatty acid, blood β-hydroxybutyrate, and lactate concentrations during hyperinsulinemic stepped hypoglycemic clamps in nondiabetic subjects and patients with T1DM at baseline, after twice-weekly hypoglycemia for 1 month, and after twice-weekly hyperglycemia for 1 month

	Nominal glucose (mg/dl)	Nondiabetic subjects (n = 12)	T1DM patients (n = 6)			P value (analysis of variance)
			Baseline	After hypoglycemia	After hyperglycemia	
Nonesterified fatty acids (μmol/l)	~100	512 ± 34	160 ± 45	136 ± 39	134 ± 28	0.0009 (nondiabetic vs.
	85	134 ± 20	69 ± 19	53 ± 6	55 ± 9	T1DM baseline)
	75	56 ± 7	51 ± 10	66 ± 13	45 ± 8	NS (T1DM baseline vs.
	65	64 ± 1	51 ± 12	52 ± 7	46 ± 8	after hypoglycemia)
	55	74 ± 15	53 ± 10	57 ± 14	42 ± 9	NS (T1DM baseline vs.
	45	137 ± 26	77 ± 10	80 ± 24	53 ± 8	after hyperglycemia)
β-Hydroxybutyrate (μmol/l)	~100	152 ± 12	78 ± 16	99 ± 27	114 ± 19	NS (nondiabetic vs.
	85	100 ± 6	83 ± 20	74 ± 19	86 ± 20	T1DM baseline)
	75	116 ± 11	87 ± 25	79 ± 28	90 ± 13	NS (T1DM baseline vs.
	65	112 ± 12	69 ± 16	70 ± 15	83 ± 13	after hypoglycemia)
	55	102 ± 8	108 ± 27	66 ± 13	119 ± 26	NS (T1DM baseline vs.
	45	107 ± 6	106 ± 22	70 ± 12	161 ± 78	after hyperglycemia)
Lactate (μmol/l)	~100	1,060 ± 70	780 ± 110	860 ± 80	710 ± 40	NS (nondiabetic vs.
	85	1,270 ± 70	1,100 ± 170	1,020 ± 60	940 ± 80	T1DM baseline)
	75	1,210 ± 40	1,010 ± 90	1,010 ± 50	1,060 ± 80	0.0422 (T1DM baseline vs.
	65	1,080 ± 40	950 ± 120	930 ± 100	1,050 ± 80	after hypoglycemia)
	55	1,230 ± 40	980 ± 130	860 ± 40	910 ± 80	NS (T1DM baseline vs.
	45	1,520 ± 50	1,160 ± 120	880 ± 40	1,120 ± 90	after hyperglycemia)

Data are means ± SE. To convert norepinephrine to nanomoles per liter, multiply by 0.005911; growth hormone to picomoles per liter, multiply by 44.15; and cortisol to millimoles per liter, multiply by 27.59.

extent to which these reduced responses to hypoglycemia were the result of a cumulative effect of twice-weekly induced hypoglycemia over the month or of the most proximate episode of induced hypoglycemia 2 days earlier cannot be determined from these data. In either case, however, since hypoglycemia was induced twice weekly, it is reasonable to conclude that these responses to hypoglycemia were reduced most of the time in this arm of the study. A different experimental design would be required to determine if there is an additive effect of recurrent antecedent hypoglycemia on reduced responses to subsequent hypoglycemia.

Although reduced autonomic and symptomatic responses to hypoglycemia are expected results of antecedent hypoglycemia (1), the impact of antecedent hypoglycemia on hypoglycemic cognitive dysfunction is less well established (1,22,27). Veneman et al. (28) found less deterioration on a battery of cognitive function tests during hypoglycemia after nocturnal hypoglycemia in nondiabetic subjects, but an overall effect following afternoon hypoglycemia in patients with T1DM (3) or nondiabetic subjects (22) was not apparent in our earlier studies. However, the hypoglycemic clamps were carried to plasma glucose levels of only 50 mg/dl (2.8 mmol/l) in the former study; the data from the latter indicated that pattern recognition and memory and information processing were preserved to a greater extent during hypoglycemia after afternoon hypoglycemia. More recent studies have also demonstrated relative preservation of cognitive functions after antecedent hypoglycemia (29,30). Nonetheless, based on other evidence, one current view is that the glycemic threshold for hypoglycemic cognitive dysfunction is not altered by antecedent hypoglycemia (31–33). The present data show less deterioration of cognitive function overall during hypoglycemia after twice-weekly hypoglycemia, but not after twice-weekly hyperglycemia, for 1 month in patients with T1DM. During hypoglycemia, pattern recognition and memory (as assessed by the delayed nonmatch-to-sample task), declarative memory (as assessed by the delayed paragraph recall task), and attention (as assessed by the Stroop arrow-word task) were preserved, and information processing (as assessed by the paced serial addition task) tended to be preserved to a greater extent after twice-weekly hypoglycemia. Thus the present data provide additional evidence that glycemic thresholds for hypoglycemic cognitive dysfunction, like those for autonomic and symptomatic responses to hypoglycemia, shift to lower plasma glucose concentrations after antecedent hypoglycemia (22,28–30).

These effects of twice-weekly induced hypoglycemia to reduce autonomic responses, symptomatic, and cognitive dysfunction during subsequent hypoglycemia documented in the investigational setting (hypoglycemic clamps) were reflected in the clinical setting. Superimposed induced hypoglycemia reduced the total number of symptomatic hypoglycemic episodes detected by these patients with T1DM by nearly 40%. It also lowered the mean self-monitored blood glucose level during symptomatic episodes by 10%. Finally, it reduced the proportion of regularly scheduled self-monitored glucose values <70, 60, or 50 mg/dl (3.9, 3.3, or 2.8 mmol/l) that were symptomatic by ~33%. Thus doubling the frequency of symptomatic hypoglycemia reduced awareness of clinical hypoglycemia. Although these effects might be extrapolated from the findings in the investigative setting, they

TABLE 5
Results of cognitive function tests during hyperinsulinemic stepped hypoglycemic clamps in nondiabetic subjects and patients with T1DM at baseline, after twice-weekly hypoglycemia for 1 month, and after twice-weekly hyperglycemia for 1 month

	Nominal glucose (mg/dl)	Nondiabetic subjects (n = 12)	T1DM patients (n = 6)			P value (analysis of variance)
			Baseline	After hypoglycemia	After hyperglycemia	
Delayed nonmatch to sample (reaction time, ms)	~100	—	—	—	—	0.0546 (nondiabetic vs. T1DM baseline)
	85	1,425 ± 156	1,810 ± 91	2,216 ± 77	2,242 ± 86	
	75	1,354 ± 120	1,915 ± 137	2,118 ± 107	2,240 ± 100	0.0461* (T1DM baseline vs. after hypoglycemia)
	65	1,522 ± 188	1,940 ± 89	2,755 ± 451	2,515 ± 258	
	55	1,793 ± 249	2,254 ± 149	2,480 ± 226	2,658 ± 399	NS (T1DM baseline vs. after hyperglycemia)
	45	1,988 ± 243	2,774 ± 217	2,537 ± 286	2,787 ± 454	
Delayed paragraph recall (bits recalled)	~100	—	—	—	—	NS (nondiabetic vs. T1DM baseline)
	85	27.5 ± 1.4	25.5 ± 1.4	15.3 ± 2.5	19.0 ± 4.0	
	75	25.1 ± 1.7	19.8 ± 2.1	19.9 ± 3.8	22.5 ± 1.4	0.0288† (T1DM baseline vs. after hypoglycemia)
	65	21.2 ± 2.9	19.0 ± 2.2	17.2 ± 1.8	19.4 ± 2.6	
	55	17.6 ± 2.2	12.5 ± 2.4	19.3 ± 4.0	18.0 ± 3.8	NS (T1DM baseline vs. after hyperglycemia)
	45	20.8 ± 2.2	13.4 ± 3.3	15.0 ± 1.9	12.2 ± 3.4	
Paced serial addition (errors)	~100	—	—	—	—	NS (nondiabetic vs. T1DM baseline)
	85	2.1 ± 0.7	3.5 ± 1.1	3.3 ± 1.1	3.7 ± 1.1	
	75	2.8 ± 0.7	7.5 ± 1.2	2.7 ± 1.1	2.5 ± 0.8	NS (T1DM baseline vs. after hypoglycemia)
	65	2.3 ± 0.7	3.0 ± 1.2	4.0 ± 1.1	4.5 ± 1.1	
	55	2.8 ± 1.0	4.8 ± 1.4	4.5 ± 1.5	4.8 ± 1.4	NS (T1DM baseline vs. after hyperglycemia)
	45	5.1 ± 1.0	9.0 ± 2.7	6.7 ± 2.0	9.7 ± 3.0	
Stroop arrow-word (reaction time, ms)	~100	—	—	—	—	0.0007 (nondiabetic vs. T1DM baseline)
	85	563 ± 35	608 ± 21	639 ± 27	647 ± 47	
	75	520 ± 24	626 ± 29	606 ± 26	611 ± 43	NS‡ (T1DM baseline vs. after hypoglycemia)
	65	545 ± 21	532 ± 29	587 ± 40	607 ± 40	
	55	547 ± 25	741 ± 59	661 ± 50	664 ± 50	NS (T1DM baseline vs. after hyperglycemia)
	45	614 ± 35	904 ± 95	700 ± 48	788 ± 83	
Memory scanning (reaction time, ms)	~100	—	—	—	—	0.0136 (nondiabetic vs. T1DM baseline)
	85	1,026 ± 95	1,264 ± 79	1,135 ± 64	1,357 ± 179	
	75	802 ± 70	1,122 ± 56	1,140 ± 147	1,214 ± 99	NS (T1DM baseline vs. after hypoglycemia)
	65	773 ± 61	1,119 ± 101	1,088 ± 108	1,035 ± 62	
	55	799 ± 63	1,131 ± 90	1,121 ± 129	1,109 ± 79	NS (T1DM baseline vs. after hyperglycemia)
	45	825 ± 74	1,133 ± 34	1,108 ± 86	1,137 ± 96	

Data are means ± SE. *Increment P = 0.0154; †decrement P = 0.0343; ‡increment P = 0.0061.

have not been previously documented in the clinical setting, the daily lives of people with T1DM.

It is notable that all of these findings were derived from a study with a small number of patients with T1DM. The extent to which a larger sample size might have disclosed additional effects of an increased frequency of hypoglycemia is unknown.

In summary, in patients with well-controlled T1DM, as little as doubling the frequency of symptomatic hypoglycemia reduced physiological defenses against developing hypoglycemia and patient detection of clinical hypoglycemia, with the resultant reduction in behavioral defenses against severe hypoglycemia. This study was not designed to assess the impact of these changes on the frequency of severe iatrogenic hypoglycemia. Because patients practicing intensive therapy have been reported to experience an average of roughly one episode of severe hypoglycemia each year (2), a substantially larger sample size and duration of observation would be required to assess that end point. However, the present data demonstrate that doubling the frequency of symptomatic hypoglycemia further impairs a central feature (the reduced epinephrine response) of the syndrome of defective glucose counterregulation and the central feature (the reduced perception of hypoglycemia) of the syndrome of hypoglycemia unawareness. These two syndromes are associated with a substantially increased risk of severe iatrogenic hypoglycemia in T1DM (1,24–26).

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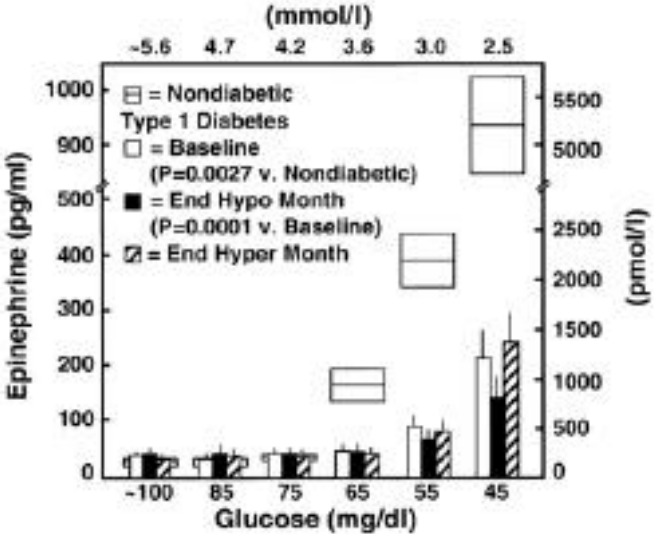

FIG. 2. Means ± SE of plasma epinephrine concentrations during hyperinsulinemic (2.0 mU · kg^{-1} · min^{-1}, 12.0 pmol · kg^{-1} · min^{-1}) stepped hypoglycemic clamps in nondiabetic control subjects (⊟) and patients with T1DM studied at baseline (□), after twice-weekly 2-h episodes of induced hypoglycemia (50 mg/dl, 2.8 mmol/l) for 1 month (■), and after twice-weekly 2-h episodes of induced hyperglycemia (150 mg/dl, 8.3 mmol/l) for 1 month (▨).

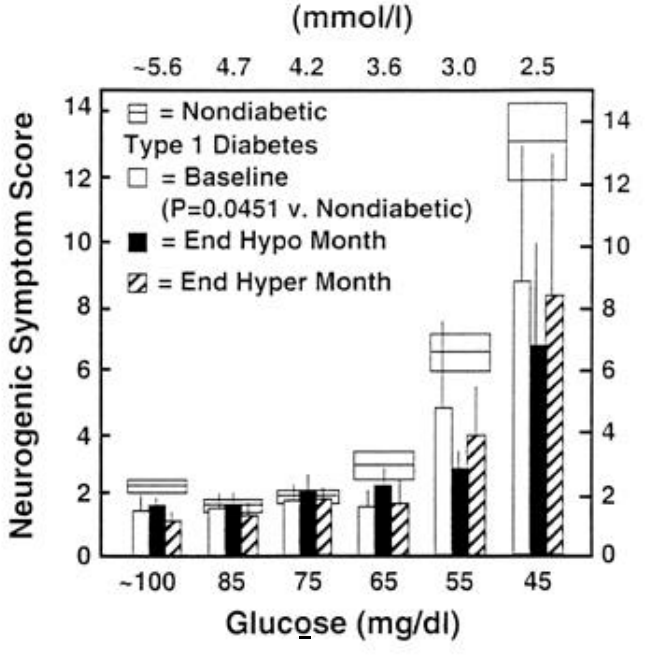

FIG. 3. Means ± SE neurogenic (autonomic) symptom scores during hyperinsulinemic (2.0 mU · kg^{-1} · min^{-1}, 12.0 pmol · kg^{-1} · min^{-1}) stepped hypoglycemic clamps nondiabetic control subjects (⊟) and patients with T1DM studied at baseline (□), after twice-weekly 2-h episodes of induced hypoglycemia (50 mg/dl, 2.8 mmol/l) for 1 month (■), and after twice-weekly 2-h episodes of induced hyperglycemia (150 mg/dl, 8.3 mmol/l) for 1 month (▨).

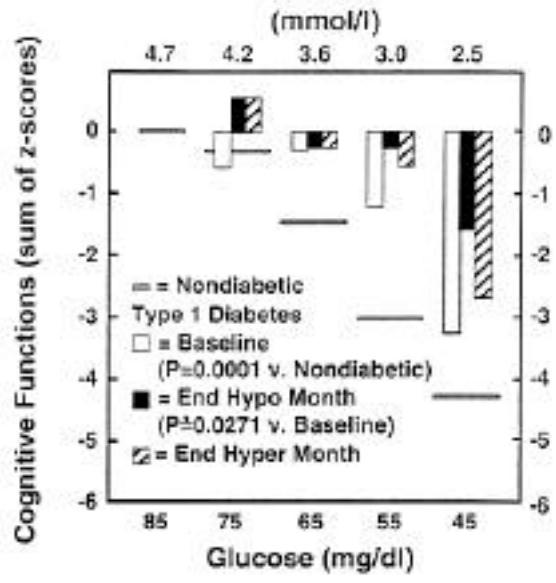

FIG. 4.Sum of *z* scores for cognitive function tests (delayed nonmatch to sample, delayed paragraph recall, paced serial addition, and Stroop arrow-word tasks) during hyperinsulinemic (2.0 mu · kg^{-1} · min^{-1}, 12.0 pmol · kg^{-1} · min^{-1}) stepped hypoglycemic clamps in nondiabetic control subjects (═) and patients with T1DM studied at baseline (□), after twice-weekly 2-h episodes of induced hypoglycemia (50 mg/dl, 2.8 mmol/l) for 1 month (■), and after twice-weekly 2-h episodes of induced hyperglycemia (150 mg/dl, 8.3 mmol/l) for 1 month (▨).

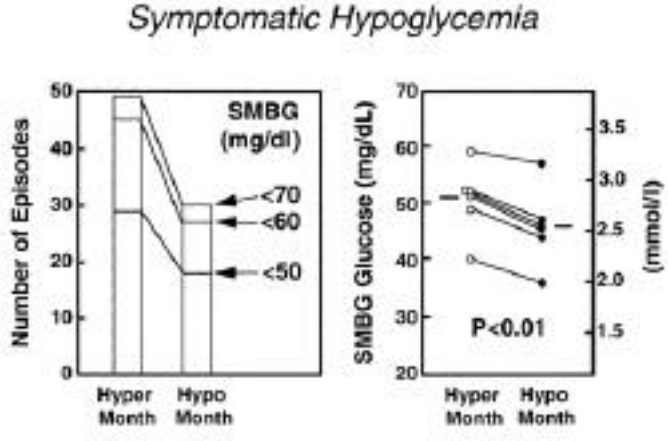

FIG. 5. Number of episodes of symptomatic hypoglycemia with self-monitored blood glucose levels of <70, 60, or 50 mg/dl (3.9, 3.3, or 2.8 mmol/l) (left) and mean self-monitored blood glucose (SMBG) levels (right) during symptomatic hypoglycemia in six patients with T1DM during twice-weekly 2-h episodes of induced hyperglycemia (150 mg/dl, 8.3 mmol/l) for 1 month (hyper month) and during twice-weekly 2-h episodes of induced hypoglycemia (50 mg/dl, 2.8 mmol/l) for 1 month (hypo month).

REFERENCES

1. Cryer PE: Hypoglycemia in diabetes mellitus. In *Hypoglycemia: Pathophys - iology, Diagnosis and Treatment.* New York/Oxford, Oxford University Press, 1997, p. 91–125
2. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
3. Dagogo-Jack SE, Craft S, Cryer PE: Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus. *J Clin Invest* 91:819–828, 1993
4. Rattarasarn C, Dagogo-Jack SE, Zachwieja JJ, Cryer PE: Hypoglycemia-induced autonomic failure in T1DM is specific for the stimulus of hypoglycemia

and is not attributable to prior autonomic activation per se. *Diabetes* 43:809–818, 1994
5. Fanelli CG, Epifano L, Rambotti AM, Pampanelli S, Di Vincenzo A, Modarelli F, Lepore M, Annibale B, Ciofetta M, Bottini P, Porcellati F, Santeusanio F, Brunetti P, Bolli GB: Meticulous prevention of hypoglycemia normalizes the glycemic thresholds and magnitude of most neuroendocrine responses to, symptoms of, and cognitive function during hypoglycemia in intensively treated patients with short-term IDDM. *Diabetes* 42:1683–1689, 1993
6. Cranston I, Lomas J, Maran A, Macdonald I, Amiel SA: Restoration of hypoglycemia unawareness in patients with long duration insulin dependent diabetes mellitus. *Lancet* 344:283–287, 1994
7. Dagogo-Jack SE, Rattarasarn C, Cryer PE: Reversal of hypoglycemia unawareness, but not defective glucose counterregulation, in IDDM. *Diabetes* 43:1426–1434, 1994
8. Fanelli C, Pampanelli S, Epifano L, Rambotti AM, Di Vincenzo A, Modarelli F, Ciofetta M, Lepore M, Annibale B, Torlone E, Perriello G, De Feo P, Santeusanio F, Brunetti P, Bolli GB: Long-term recovery from unawareness, deficient counterregulation and lack of cognitive dysfunction during hypoglycemia following institution of rational intensive therapy in IDDM. *Diabetologia* 37:1265–1276, 1994
9. Schwartz NS, Clutter WE, Shah SD, Cryer PE: Glycemic thresholds for activation of glucose counterregulatory systems are higher than the threshold for symptoms. *J Clin Invest* 79:777–781, 1987
10. Shah S, Clutter W, Cryer PE: External and internal standards in the single isotope derivative (radioenzymatic) assay of plasma norepinephrine and epinephrine in normal humans and patients with diabetes mellitus and chronic renal failure. *J Lab Clin Med* 106:624–629, 1985
11. Gingerich R, Lacy P, Chance R, Johnson M: Regional pancreatic concentration and in vivo secretion of canine pancreatic polypeptide, insulin and glucagon. *Diabetes* 27:96–101, 1978
12. Kuzuya H, Blix P, Horwitz D, Steiner D, Rubenstein A: Determination of free and total insulin and C-peptide in insulin treated diabetics. *Diabetes* 26:22–29, 1977
13. Ensinck J: Immunoassays for glucagon. In *Glucagon: Handbook of Experimental Pharmacology*. P Lefebrve, Ed. New York, Springer-Verlag, 1983, p. 203–221
14. Schalch D, Parker M: A sensitive double antibody radioimmunoassay for growth hormone in plasma. *Nature* 703:1141–1142, 1964
15. Farmer R, Pierce C: Plasma cortisol determination: radioimmunoassay and competitive binding compared. *Clin Chem* 20:411–414, 1974
16. Hosaka K, Kikuchi T, Mitsuhida N, Kawaguchi N: A new colorimetric method for the determination of free fatty acids with acyl-CoA synthetase and acyl-CoA oxidase. *J Biochem* 89:1799–1803, 1981
17. Lowry O, Passoneau J, Hasselberger F, Schultz D: Effect of ischemia on known substrates and co-factors of the glycolytic pathway of the brain. *J Biol Chem* 239:18–30, 1964
18. Pinter J, Hayaski J, Watson J: Enzymatic assay of glycerol, dihydroxyacetone and glyceraldehyde. *Arch Biochem Biophys* 121:404–414, 1967
19. Cahill G, Herrera M, Morgan A, Soeldner J, Steinke J, Levy P, Reichard G, Kipnis D: Hormone-fuel interrelationships during fasting. *J Clin Invest* 45:1751–1769, 1966
20. Halwachs-Baumann G, Katzensteiner S, Schnedl W, Purstner P, Pieber T, Wilders-Truschnig M: Comparative evaluation of three assay systems for automated determination of hemoglobin A_{1c}. *Clin Chem* 43:511–517, 1997
21. Towler DA, Havlin CE, Craft S, Cryer PE: Mechanisms of awareness of hypoglycemia: perception of neurogenic (predominantly cholinergic) rather than neuroglycopenic symptoms. *Diabetes* 42:1791–1798, 1993
22. Hvidberg A, Fanelli CG, Hershey T, Terkamp C, Craft S, Cryer PE: Impact of recent antecedent hypoglycemia on hypoglycemic cognitive dysfunction in nondiabetic humans. *Diabetes* 45:1030–1036, 1996
23. Lezak MD: *Neuropsychological Assessment*. New York/Oxford, Oxford University Press, 1995, p. 154–156
24. White NH, Skor DA, Cryer PE, Bier DM, Levandoski L, Santiago JV: Identification of type 1 diabetic patients at increased risk for hypoglycemia during intensive therapy. *N Engl J Med* 308:485–491, 1983
25. Bolli GB, De Feo P, De Cosmo S, Perriello G, Ventura MM, Massi-Benedetti M, Santeusanio F, Gerich JE, Brunetti P: A reliable and reproducible test for adequate glucose counterregulation in type I diabetes mellitus. *Diabetes* 33:732–737, 1984
26. Gold AE, MacLeod KM, Frier BM: Frequency of severe hypoglycemia in patients with type I diabetes with impaired awareness of hypoglycemia. *Diabetes Care* 17:697–703, 1994
27. Fanelli CG, Pampanelli S, Porcellati F, Bolli GB: Shift of glycaemic thresholds for cognitive function in hypoglycaemia unawareness in humans. *Diabetologia*. In press
28. Veneman T, Mitrakou A, Mokan M, Cryer P, Gerich J: Induction of hypoglycemia unawareness by asymptomatic nocturnal hypoglycemia. *Diabetes* 42:1233–1237, 1993
29. Fanelli C, Paramore D, Hershey T, Terkamp C, Craft S, Cryer P: Impact of nocturnal hypoglycemia on hypoglycemic cognitive dysfunction in type 1 diabetes mellitus (Abstract). *Diabetes* (Suppl.1) 47: A108, 1998
30. Fanelli C, Pampanelli S, Ciofetta M, Lalli C, Del Sindaco P, Lepore M, Brunetti P, Bolli G: Effect of duration of recent, antecedent hypoglycemia in humans (Abstract). *Diabetes* 46:41A, 1997
31. Blackman JD, Towle VL, Sturis J, Lewis GF, Spire J-P, Polonsky KS: Hypoglycemic thresholds for cognitive dysfunction in IDDM. *Diabetes* 41:392–399, 1992
32. Widom B, Simonson DC : Glycemic control and neuropsychologic function during hypoglycemia in patients with insulin-dependent diabetes mellitus. *Ann Intern Med* 112:904–912, 1990
33. Maran A, Lomas J, Macdonald IA, Amiel SA: Lack of protection of higher brain function during hypoglycaemia in patients with intensively treated IDDM. *Diabetologia* 38:1412–1418, 1995