

Impact of Nocturnal Hypoglycemia on Hypoglycemic Cognitive Dysfunction in Type 1 Diabetes

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To test the hypothesis that glycemic thresholds for cognitive dysfunction during hypoglycemia, like those for autonomic and symptomatic responses, shift to lower plasma glucose concentrations after recent antecedent hypoglycemia in patients with type 1 diabetes mellitus (T1DM), 15 patients were studied on two occasions. Cognitive functions were assessed during morning hyperinsulinemic stepped hypoglycemic clamps (85, 75, 65, 55, and 45 mg/dl steps) after, in random sequence, nocturnal (2330–0300) hypoglycemia (48 ± 2 mg/dl) on one occasion and nocturnal euglycemia (109 ± 1 mg/dl) on the other. Compared with nondiabetic control subjects ($n = 12$), patients with T1DM had absent glucagon ($P = 0.0009$) and reduced epinephrine ($P = 0.0010$), norepinephrine ($P = 0.0001$), and neurogenic symptom ($P = 0.0480$) responses to hypoglycemia; the epinephrine ($P = 0.0460$) and neurogenic symptom ($P = 0.0480$) responses were reduced further after nocturnal hypoglycemia. After nocturnal hypoglycemia, in contrast to nocturnal euglycemia, there was less deterioration of cognitive function overall ($P = 0.0065$) during hypoglycemia based on analysis of the sum of standardized scores (z-scores). There was relative preservation of measures of pattern recognition and memory (the delayed non-match to sample task, $P = 0.0371$) and of attention (the Stroop arrow-word task, $P = 0.0395$), but not of measures of information processing (the paced serial addition task) or declarative memory (the delayed paragraph recall task), after nocturnal hypoglycemia. Thus, glycemic thresholds for hypoglycemic cognitive dysfunction, like those for autonomic and symptomatic responses to hypoglycemia, shift to lower plasma glucose concentrations after recent antecedent hypoglycemia in patients with T1DM. *Diabetes* 47:1920–1927, 1998

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ANOVA, analysis of variance; GCRC, General Clinical Research Center; T1DM, type 1 diabetes mellitus.

Glycemic control prevents or delays several of the long-term complications of type 1 diabetes mellitus (T1DM) (1,2). However, euglycemia, or even near-normal plasma glucose concentrations, cannot be achieved safely in the vast majority of people with T1DM because of the barrier of iatrogenic hypoglycemia (1–3). Hypoglycemia is the limiting factor in the management of T1DM (3).

Iatrogenic hypoglycemia is the result of the interplay of relative or absolute therapeutic 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 (3). The concept of hypoglycemia-associated autonomic failure in T1DM (3–7) posits that periods of therapeutic insulin excess, in the setting of absent 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 both reduced symptoms of, and the resultant behavioral defense (e.g., food ingestion) against, developing hypoglycemia (i.e., the clinical syndrome of hypoglycemia unawareness) and—because epinephrine responses are reduced in the setting of absent glucagon responses—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.

A central pathophysiological feature of the concept of hypoglycemia-associated autonomic failure in T1DM is the fact that glycemic thresholds for autonomic (including adrenomedullary epinephrine) and symptomatic responses to falling glucose levels are shifted to lower plasma glucose concentrations after recent antecedent hypoglycemia (3,5,6,8–12). Recent iatrogenic hypoglycemia undoubtedly largely underlies this pattern of reduced responses to a given level of hypoglycemia in people with effectively intensively treated T1DM (13–15), although a component of classical diabetic autonomic neuropathy may also contribute (16).

In contrast to its impact on neuroendocrine and symptomatic responses, the impact, or lack of impact, of recent antecedent hypoglycemia on glycemic thresholds for hypoglycemic cognitive dysfunction is controversial (5,17–24). Veneman et al. (17) found less overall deterioration on a battery of cognitive function tests during hypoglycemia after

nocturnal hypoglycemia in nondiabetic subjects, but an overall effect after afternoon hypoglycemia in patients with T1DM (5) or in nondiabetic subjects (18) was not apparent in our earlier studies. However, the stepped hypoglycemic clamps were carried to plasma glucose levels of only 50 mg/dl (2.8 mmol/l) in the former study (5), and the latter data indicated that measures of pattern recognition and memory and of information processing were preserved to a greater extent during hypoglycemia after afternoon hypoglycemia (18). More recent studies have also demonstrated overall relative preservation of cognitive function after antecedent hypoglycemia (19,23). Indeed, we recently found less overall deterioration of cognitive function during hypoglycemia after brief twice weekly episodes of induced hypoglycemia for 1 month, but not after otherwise identical brief twice weekly episodes of induced hyperglycemia for 1 month, in patients with T1DM (23). However, performance on only three of four tests—measures of pattern recognition and memory, of declarative memory, and of attention—were preserved significantly, although that on the other test—a measure of information processing—tended to be preserved. In contrast to the studies just summarized, which were designed to assess the impact of recent antecedent hypoglycemia directly, other studies have used intensively treated T1DM, a history of hypoglycemia unawareness, or both as surrogates for recent antecedent hypoglycemia and concluded that glycemic thresholds for hypoglycemic cognitive dysfunction are not altered by recent antecedent hypoglycemia (20–22,24).

Resolution of this controversial issue is fundamentally important from both clinical and pathophysiological perspectives. Clinically, if glycemic thresholds for activation of physiological and behavioral defenses against falling plasma glucose levels are shifted to lower plasma glucose concentrations, but those for hypoglycemic cognitive dysfunction are not, during intensive therapy of T1DM, cognitive failure might precede activation of those defenses as plasma glucose levels fall, resulting in frequent severe clinical hypoglycemia. Pathophysiologically, dissociation of the glycemic thresholds for neuroendocrine and symptomatic responses to hypoglycemia from those for hypoglycemic cognitive dysfunction would be difficult to rationalize with a simple unifying mechanism for the shifts in glycemic thresholds, such as a generalized increase in blood-to-brain glucose transport (25,26), after recent antecedent hypoglycemia. Accordingly, we tested the hypothesis that nocturnal hypoglycemia reduces hypoglycemic cognitive dysfunction the next morning in patients with T1DM.

RESEARCH DESIGN AND METHODS

Subjects. Twelve healthy nondiabetic subjects (7 women, 5 men), with a mean (\pm SD) age of 27 ± 4 years, a mean body wt of 71.6 ± 16.2 kg, and a mean BMI of 24.8 ± 3.6 kg/m², and 15 patients with T1DM gave 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 (GCRC). Characteristics of the patients with T1DM (7 women, 8 men) included a mean (\pm SD) age of 27 ± 6 years, a mean body wt of 72.4 ± 8.3 kg, a mean BMI of 24.9 ± 1.8 kg/m², a mean duration of T1DM of 12.5 ± 7.2 years, a mean HbA_{1c} level of $8.7 \pm 1.4\%$, and a mean insulin dose of 45 ± 8 U/day. None had a history of recurrent severe hypoglycemia, untreated proliferative retinopathy (fundoscopic examination), autonomic neuropathy (cardiovascular reflex tests), nephropathy (serum creatinine, urine albumin), clinically overt atherosclerotic disease (history, physical examination, electrocardiogram), hypertension, or a history of seizures or of any other central nervous system disease; these were exclusion criteria for this study. The comparison data from the nondiabetic subjects used here were also used in another report from our laboratory (23).

Protocol. Patients with T1DM were admitted to the GCRC at 1700 on two occasions separated by at least 4 weeks. They were instructed to carefully avoid hypoglycemia for 3 days before study, and they used regular insulin only to control their diabetes on the day of admission (i.e., intermediate or long-acting insulin was omitted that day). They took their usual dose of regular insulin at 1730 and were given a meal at 1800. (They then fasted until completion of the study at 1300 the next day when they were given subcutaneous regular insulin and lunch before discharge.) Intravenous lines—one in an antecubital vein for infusions and one in a hand vein, with that hand kept in an -55°C Plexiglas box, for arterialized venous sampling—were inserted at 1900. Plasma glucose levels, measured every 15 min, were adjusted to and held at ~ 110 mg/dl (6.1 mmol/l) with variable intravenous infusion doses of regular insulin (Novolin R; Novo Nordisk, Bagsvaerd, Denmark) until 2300. The insulin infusion rate was increased to $2.0 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ($12.0 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) from 2300 to 0300, plasma glucose was measured every 5 min, and 20% glucose was infused to clamp plasma glucose levels, in random sequence, at ~ 110 mg/dl (6.1 mmol/l) on one admission (nocturnal euglycemia) and at ~ 50 mg/dl (2.8 mmol/l) on the other admission (nocturnal hypoglycemia). The insulin infusion rate was then decreased to $0.5 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ($3.0 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) from 0300 to 0800. After baseline observations (see below) at ambient plasma glucose concentrations, hyperinsulinemic stepped hypoglycemic clamps (27) were performed. From 0800 to 1300, insulin was infused in a dose of $2.0 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ($12.0 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), and 20% glucose was infused in doses that clamped plasma glucose levels at 85, 75, 65, 55, and 45 mg/dl (4.7, 4.2, 3.6, 3.0, and 2.5 mmol/l) in sequential hourly steps. Some patients required higher insulin infusion rates to achieve the final (45 mg/dl) glucose step. In those individuals, the same higher insulin dose was given during the final step on both study occasions. Arterialized venous blood samples were drawn at 5-min intervals for glucose measurements and at 15-min intervals for hormone and metabolic substrate/intermediate measurements (see below). Heart rates and blood pressures were recorded, and symptom scores (see below) were determined at 15-min intervals; the electrocardiogram was monitored throughout. Cognitive function tests (see below) were performed during the final 30 min of each glycemic step. All of the data from these morning hyperinsulinemic stepped hypoglycemic clamps reported here are from the final 30 min of each step (i.e., after steady-state glycaemia was achieved).

Nondiabetic subjects presented to the GCRC at 0700 after an overnight fast and underwent identical hyperinsulinemic stepped hypoglycemic clamps on one occasion.

Analytical methods. Plasma glucose concentrations were determined with a glucose oxidase method (Glucose Analyzer 2; Beckman Instruments, Fullerton, CA). Plasma epinephrine and norepinephrine were measured with a single isotope derivative (radioenzymatic) method (28), while plasma free insulin (29), C-peptide (29), glucagon (30), pancreatic polypeptide (31), growth hormone (32), and cortisol (33) were measured with radioimmunoassays. Serum nonesterified fatty acids were measured with an enzymatic colorimetric method (34), while blood lactate (35), β -hydroxybutyrate (36), and alanine (37) were measured with enzymatic techniques.

Symptom scores. Symptoms were quantitated by asking the subject to score (from 0 [none] to 6 [severe]) each of 12 symptoms: six neurogenic (adrenergic: heart pounding, shaky/tremulous, and nervous/anxious; cholinergic: sweaty, hungry, and tingling) and six neuroglycopenic (difficulty thinking/confused, tired/drowsy, weak, warm, faint, and dizzy) symptoms (38).

Cognitive function tests. Our battery of cognitive function tests has been described in detail elsewhere (18). These tests include measures of 1) memory retrieval, the memory scanning task; 2) pattern recognition and memory, the delayed non-match to sample task; 3) attention, the Stroop arrow-word task; 4) information processing, the paced serial addition task; and 5) declarative memory, the delayed paragraph recall task. Each subject and patient practiced these tasks on each study occasion, i.e., before each morning hyperinsulinemic stepped hypoglycemic clamp. Their performance during those practice sessions is not reported here. Thus, while baseline (ambient glycemia) data are presented for all other measurements, cognitive function data are presented only from the final 30 min of each glycemic step during the clamps. The sequence of testing, after presentation of the paragraph, was delayed non-match to sample, Stroop arrow-word, memory scanning, paced serial addition, and delayed paragraph recall.

Statistical methods. Data are expressed as the mean \pm SE except where SD is specified. In addition, a standard score, the unitless z-score (39), 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. Data from the stepped hypoglycemic clamps were analyzed by repeated measures analysis of variance (ANOVA). The *P* values provided refer to group (or group \times time interaction) contrasts, not time contrasts. *P* values <0.05 were considered to indicate significant differences.

RESULTS

Plasma glucose concentrations. Target plasma glucose concentrations were achieved during the morning hyperinsulinemic stepped hypoglycemic clamps in all three groups (Table 1) and, in the patients with T1DM, during nocturnal euglycemia and hypoglycemia (Fig. 1).

Nondiabetic subjects versus patients with T1DM. Compared with nondiabetic subjects, patients with T1DM had absent glucagon ($P = 0.0009$, Table 1) and reduced epinephrine ($P = 0.0010$, Table 2), norepinephrine ($P = 0.0001$, Table 2), neurogenic symptom score ($P = 0.0480$, Table 2), and pancreatic polypeptide ($P = 0.0070$, Table 1) responses to hypoglycemia. Neuroglycopenic symptom score responses (Table 2) were not reduced significantly. Cortisol ($P = 0.0040$) and diastolic blood pressure ($P = 0.0100$), but not growth hormone, responses were also reduced (data not shown). Patients also had slightly higher insulin levels ($P = 0.0330$, Table 1) despite similar insulin infusion rates. Nonesterified fatty acid levels were lower at baseline in the patients ($P = 0.0100$); β -hydroxybutyrate, lactate, and alanine levels did not differ significantly, nor did heart rates or systolic blood pressures (data not shown).

Performance on our measure of memory retrieval (the memory scanning task) did not deteriorate during hypoglycemia in the nondiabetic subjects or in the patients with T1DM (Fig. 2). Performance on the other four cognitive func-

tion tests deteriorated during hypoglycemia and, overall, deteriorated to a greater extent in the patients with T1DM (after nocturnal euglycemia) ($P = 0.0425$, Fig. 3).

Patients with T1DM performed less well on four of the five cognitive function tests. These included measures of memory retrieval (the memory scanning task, $P = 0.0007$, Fig. 2), pattern recognition and memory (the delayed non-match to sample task, $P = 0.0097$, Fig. 4), attention (the Stroop arrow-word task, $P = 0.0037$, Fig. 5), and information processing (the paced serial addition task, $P = 0.0232$, Fig. 6).

Patients with T1DM after nocturnal euglycemia versus after nocturnal hypoglycemia. Compared with testing after nocturnal euglycemia, after nocturnal hypoglycemia, epinephrine ($P = 0.0460$, Table 2), neurogenic symptom score ($P = 0.0486$, Table 2), and pancreatic polypeptide ($P = 0.0165$, Table 1) responses to morning hypoglycemia were reduced further in the patients with T1DM. Absent glucagon (Table 1) and reduced norepinephrine (Table 2) and cortisol (data not shown) responses were not altered significantly.

Overall, based on the sum of z-scores, performance on the four cognitive function tests that were sensitive to hypoglycemia deteriorated to a lesser extent ($P = 0.0060$) after nocturnal hypoglycemia than it did after nocturnal euglycemia in the patients with T1DM (Fig. 3). Indeed, while hypoglycemic cognitive dysfunction was greater in the

TABLE 1

Mean plasma glucose, insulin, glucagon, and pancreatic polypeptide concentrations during morning (0800–1300) hyperinsulinemic stepped hypoglycemic clamps in nondiabetic subjects and in patients with T1DM

	Nominal glucose (mg/dl)	Nondiabetic subjects (n = 12)	T1DM patients (n = 15)		ANOVA P
			After Eu	After Hypo	
Glucose (mg/dl)	Base	89 ± 1	113 ± 1	111 ± 2	Nondiabetic vs. T1DM: NS
	85	85 ± 1	88 ± 1	89 ± 1	
	75	75 ± 0	75 ± 1	75 ± 1	
	65	65 ± 0	66 ± 1	65 ± 1	T1DM after Eu vs. after Hypo: NS
	55	55 ± 0	55 ± 1	54 ± 0	
	45	46 ± 0	46 ± 0	46 ± 0	
Insulin (μ U/ml)	Base	10 ± 1	33 ± 2	75 ± 44	Nondiabetic vs. T1DM: 0.0330
	85	127 ± 6	120 ± 7	153 ± 11	
	75	129 ± 4	132 ± 8	148 ± 8	
	65	132 ± 6	140 ± 8	140 ± 8	T1DM after Eu vs. after Hypo: NS
	55	126 ± 5	143 ± 10	165 ± 15	
	45	134 ± 5	168 ± 11	176 ± 12	
Glucagon (pg/ml)	Base	112 ± 9	72 ± 4	63 ± 3	Nondiabetic vs. T1DM: 0.0009
	85	101 ± 9	70 ± 4	60 ± 3	
	75	92 ± 7	67 ± 4	58 ± 3	
	65	99 ± 9	71 ± 4	60 ± 3	T1DM after Eu vs. after Hypo: NS
	55	115 ± 10	71 ± 4	60 ± 3	
	45	121 ± 18	74 ± 4	64 ± 4	
Pancreatic polypeptide (pg/ml)	Base	66 ± 5	57 ± 7	57 ± 5	Nondiabetic vs. T1DM: 0.0070
	85	54 ± 5	63 ± 17	49 ± 4	
	75	49 ± 6	49 ± 8	46 ± 3	
	65	152 ± 38	58 ± 6	46 ± 4	T1DM after Eu vs. after Hypo: 0.0165
	55	415 ± 52	177 ± 33	84 ± 13	
	45	567 ± 40	305 ± 45	218 ± 40	

Data are means \pm SE. T1DM patients were studied after nocturnal (2330–0300) euglycemia (Eu, 109 \pm 1 mg/dl, 6.0 \pm 0.1 mmol/l) and after nocturnal hypoglycemia (Hypo, 48 \pm 2 mg/dl, 2.7 \pm 0.1 mmol/l). 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.

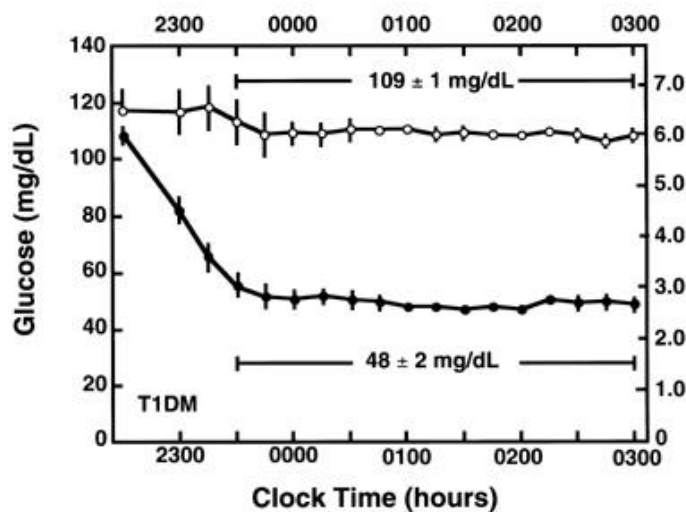


FIG. 1. Mean \pm SE plasma glucose concentrations during intravenous insulin infusions ($2.0 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ from 2330 to 0300) in 15 patients with T1DM on the nocturnal euglycemia night (\circ) and on the nocturnal hypoglycemia night (\bullet).

patients with T1DM than in the nondiabetic subjects after nocturnal euglycemia, it was comparable in both groups after nocturnal hypoglycemia (Fig. 3). Among the individual cognitive function tests there was less deterioration on the measures of pattern recognition and memory (the delayed non-match to sample task, $P = 0.0371$, Fig. 4) and of attention (the Stroop arrow-word task, $P = 0.0395$, Fig. 5) after nocturnal hypoglycemia. Performance on the measures of information processing (the paced serial addition task, Fig. 6) and declarative memory (the delayed paragraph recall task, Fig. 7) was not preserved significantly after nocturnal hypoglycemia.

DISCUSSION

These data indicate that cognitive functions deteriorate to a lesser extent during hypoglycemia after nocturnal hypoglycemia than they do after nocturnal euglycemia in patients with T1DM. Because cognitive functions deteriorated progressively in a hypoglycemia dose-related fashion during hypoglycemia under both conditions, but to a lesser extent after nocturnal hypoglycemia, it can be concluded that glycemic thresholds for hypoglycemic cognitive dysfunction, like those for autonomic and symptomatic responses to hypoglycemia (3,5,6,8–12), shift to lower plasma glucose concentrations after recent antecedent hypoglycemia. This generic conclusion is based on analysis of a standardized score (z-score) that demonstrated statistically significant relative preservation of performance on four cognitive function tests, which have been shown in our studies to be sensitive to hypoglycemia, after nocturnal hypoglycemia. Significant effects were found on two of these tests analyzed individually.

There are several reports that glycemic thresholds for hypoglycemic cognitive dysfunction, assessed with end points ranging from a battery of cognitive function tests to a single test, are unaltered in patients with T1DM (20–22,24). A premise of the interpretation of such data is that well-controlled (i.e., frequently hypoglycemic) T1DM is a surrogate for recent antecedent hypoglycemia. This is a plausible premise,

since reduced autonomic and symptomatic responses to a given level of hypoglycemia are the rule in such patients (3) and reduced symptomatic (20–22,24) and epinephrine (21,22), among other neuroendocrine, responses to hypoglycemia in the patients were documented in the studies reporting no alteration in hypoglycemic cognitive dysfunction. Blackman et al. (20) found prolongation of the P300 wave latency and of reaction time to a visual stimulus at a plasma glucose level of 45 mg/dl (2.5 mmol/l), but not at a glucose level of 63 mg/dl (3.5 mmol/l), in both patients with T1DM (who were not selected for tight glycemic control) and nondiabetic subjects. They concluded that hypoglycemic thresholds for cognitive dysfunction in poorly controlled T1DM are similar to those in control subjects. Widom and Simonson (21), using a battery of five cognitive function tests, found no differences in the glycemic thresholds for deterioration of performance on these tests in patients with well-controlled T1DM compared with patients with poorly controlled T1DM and nondiabetic control subjects. Notably, however, the glycemic thresholds were defined as the plasma glucose level at which the test score had a persistent decrement of at least 2 SDs from the mean score of the previous determinations. With that definition, the ranges of calculated thresholds extended to values lower than the lowest measured plasma glucose level (40 mg/dl, 2.2 mmol/l) on all five tests in the nondiabetic subjects and the patients with well-controlled T1DM and on three of the five tests in the patients with poorly controlled T1DM. Thus, this approach might have been too insensitive to demonstrate glycemic thresholds for hypoglycemic cognitive dysfunction at significantly lower plasma glucose concentrations in the patients with well-controlled T1DM even if that were the case. Maran et al. (22) found no difference in the estimated glycemic thresholds for deterioration of performance on a single cognitive function test, i.e., four-choice reaction time, in patients with well-controlled T1DM compared with those with poorly controlled T1DM. Significant deterioration in reaction time was considered to have occurred when there were two or more consecutive increments of 5% or more, and the glycemic threshold was defined as the blood glucose concentration at which these increments occurred. If a significant change, so defined, did not occur during hypoglycemia, the glucose nadir (36 mg/dl, 2.0 mmol/l) was entered as the threshold for statistical analysis. The number of instances in which the latter was necessary was not reported. Thus, as in the earlier report (21), only derived data—the arbitrarily defined glycemic thresholds—were reported. Recently, Hopkins et al. (24), from the same investigative group, reported no alteration of similarly calculated glycemic thresholds for performance on four-choice reaction time and on four other cognitive function tests, but relative preservation of performance on the Stroop reading task, during hypoglycemia in patients with T1DM and a history of hypoglycemia unawareness compared with patients who were aware of their hypoglycemia. In contrast to these generally negative reports (20–22,24), there are two reports that lower plasma glucose concentrations are required to alter auditory P300 event-related potentials in well-controlled T1DM (40,41).

The impact of recent antecedent hypoglycemia on hypoglycemic cognitive dysfunction has been assessed directly in five previous studies, each using a battery of cognitive function tests. Based on analysis of standardized scores (the sum of z-scores [39]), overall relative preservation of cognitive

TABLE 2

Mean plasma epinephrine and norepinephrine concentrations and neurogenic and neuroglycopenic symptoms scores during morning (0800–1300) hyperinsulinemic stepped hypoglycemic clamps in nondiabetic subjects and in patients with T1DM

	Nominal glucose (mg/dl)	Nondiabetic subjects (n = 12)	T1DM patients (n = 15)		ANOVA P
			After Eu	After Hypo	
Epinephrine (pg/ml)	Base	26 ± 3	41 ± 4	39 ± 3	Nondiabetic vs. T1DM: 0.0010
	85	26 ± 3	46 ± 5	46 ± 4	
	75	39 ± 5	57 ± 6	53 ± 5	T1DM after Eu vs. after Hypo: 0.0460
	65	168 ± 31	89 ± 10	77 ± 7	
	55	389 ± 48	194 ± 24	146 ± 15	
45	934 ± 39	387 ± 45	304 ± 28		
Norepinephrine (pg/ml)	Base	197 ± 15	205 ± 13	180 ± 10	Nondiabetic vs. T1DM: 0.0001
	85	198 ± 11	206 ± 14	180 ± 9	
	75	200 ± 11	201 ± 13	188 ± 10	T1DM after Eu vs. after Hypo: NS
	65	246 ± 14	206 ± 13	199 ± 9	
	55	296 ± 14	229 ± 15	221 ± 11	
45	413 ± 20	277 ± 18	271 ± 12		
Neurogenic symptom score (unitless)	Base	2.2 ± 0.2	1.3 ± 0.3	1.6 ± 0.2	Nondiabetic vs. T1DM: 0.001
	85	1.7 ± 0.2	1.5 ± 0.2	2.0 ± 0.3	
	75	1.9 ± 0.2	2.0 ± 0.2	2.9 ± 0.3	T1DM after Eu vs. after Hypo: 0.0480
	65	3.0 ± 0.4	2.8 ± 0.3	3.6 ± 0.3	
	55	6.6 ± 0.6	5.4 ± 0.4	4.9 ± 0.4	
45	12.4 ± 1.2	9.0 ± 0.9	8.5 ± 0.8		
Neuroglycopenic symptom score (unitless)	Base	1.5 ± 0.2	1.4 ± 0.2	1.2 ± 0.2	Nondiabetic vs. T1DM: NS
	85	1.5 ± 0.2	1.9 ± 0.3	2.0 ± 0.2	
	75	2.0 ± 0.2	3.0 ± 0.4	3.2 ± 0.4	T1DM after Eu vs. after Hypo: NS
	65	3.6 ± 0.5	4.5 ± 0.6	3.6 ± 0.4	
	55	4.9 ± 0.4	5.8 ± 0.4	4.8 ± 0.4	
45	10.5 ± 1.3	8.6 ± 0.8	7.4 ± 0.8		

Data are means ± SE. T1DM patients were studied after nocturnal (2330–0300) euglycemia (Eu, 109 ± 1 mg/dl, 6.0 ± 0.1 mmol/l) and after nocturnal hypoglycemia (Hypo, 48 ± 2 mg/dl, 2.7 ± 0.1 mmol/l). To convert epinephrine to picomoles per liter, multiply by 5.458, and norepinephrine to nanomoles per liter, multiply by 0.005911.

functions during hypoglycemia after recent antecedent hypoglycemia was found in three of these studies, two of which were performed in nondiabetic subjects (17,19) and one in patients with T1DM (23). Analyses of individual cognitive function tests were not reported in the former studies (17,19). However, in the third study, performance on only three of four cognitive function tests that were sensitive to hypoglycemia—measures of pattern recognition and memory, declarative memory, and attention—was preserved after antecedent hypoglycemia (23). Overall relative preservation of cognitive functions during hypoglycemia after recent antecedent hypoglycemia was not found in the other two studies, one of which was performed in patients with T1DM (5) and the other in nondiabetic subjects (18). However, standardized scores were not reported in either of these studies, and relative preservation of cognitive function followed antecedent hypoglycemia on two of four cognitive function tests—measures of pattern recognition and memory and of information processing—in the latter study (18).

Five cognitive function tests were used in the present study. One of these, a measure of memory retrieval (the memory scanning task), was not sensitive to hypoglycemia. Performance on this task did not deteriorate during hypoglycemia in the nondiabetic subjects or in the patients with

T1DM. This has been a consistent finding (18,23). On the other hand, performance on the other four tasks did deteriorate during hypoglycemia. Analysis of the sum of z-scores on these four cognitive function tests disclosed significant overall preservation of cognitive performance during hypoglycemia after nocturnal hypoglycemia relative to that after nocturnal euglycemia in these patients with T1DM. However, performance on only two of the four individual tasks—measures of pattern recognition and memory (the delayed non-match to sample task) and of attention (the Stroop arrow-word task)—was preserved significantly after nocturnal hypoglycemia. Performance on measures of information processing (the paced serial addition task) and of declarative memory (the delayed paragraph recall task) was not preserved. Thus, among the cognitive function tests we have used, the delayed non-match to sample task, a measure of pattern recognition and memory that is known to be sensitive to hippocampal and medial temporal disruption (42–44), is the most robust. It has been consistently sensitive to hypoglycemia and preserved after recent antecedent hypoglycemia (18,23, this study). Had we relied on this single test, our conclusion that glycemic thresholds for hypoglycemic cognitive dysfunction shift to lower plasma glucose concentrations after recent antecedent hypoglycemia would have been the

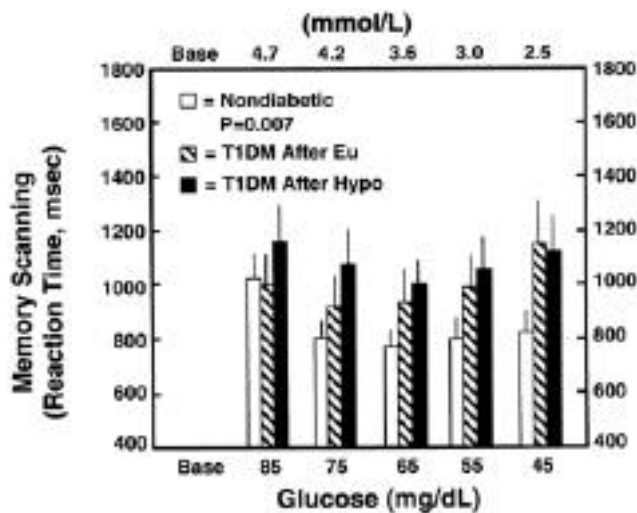


FIG. 2. Mean \pm SE reaction times on the memory scanning task during morning (0800–1300) hyperinsulinemic stepped hypoglycemic clamps in 12 nondiabetic subjects and in 15 patients with T1DM, the latter studied after nocturnal (2330–0300) euglycemia (Eu, 109 ± 1 mg/dl, 6.0 ± 0.1 mmol/l) and after nocturnal hypoglycemia (Hypo, 48 ± 2 mg/dl, 2.7 ± 0.1 mmol/l). Repeated measures ANOVA P values <0.05 are shown between the groups to which they apply. Those >0.05 are not shown.

same. However, had we relied on any one of the other four tests, our conclusions would have been discordant.

In contrast to that on the delayed non-match to sample task, performance on the delayed paragraph recall task, also a test of hippocampal and medial temporal functions, was not preserved significantly after hypoglycemia in the present study. However, performance on the latter task is consistently more variable; indeed, it was preserved significantly after

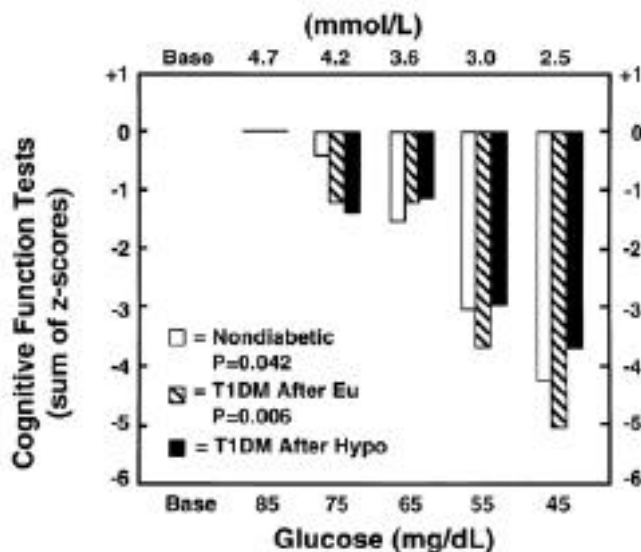


FIG. 3. Sum of z-scores for performance on the four cognitive function tests that were sensitive to hypoglycemia (the delayed non-match to sample, Stroop arrow-word, paced serial addition, and delayed paragraph recall tasks) during morning (0800–1300) hyperinsulinemic stepped hypoglycemic clamps in 12 nondiabetic subjects and in 15 patients with T1DM, the latter studied after nocturnal (2330–0300) euglycemia (Eu, 109 ± 1 mg/dl, 6.0 ± 0.1 mmol/l) and after nocturnal hypoglycemia (Hypo, 48 ± 2 mg/dl, 2.7 ± 0.1 mmol/l). Repeated measures ANOVA P values <0.05 are shown between the groups to which they apply.

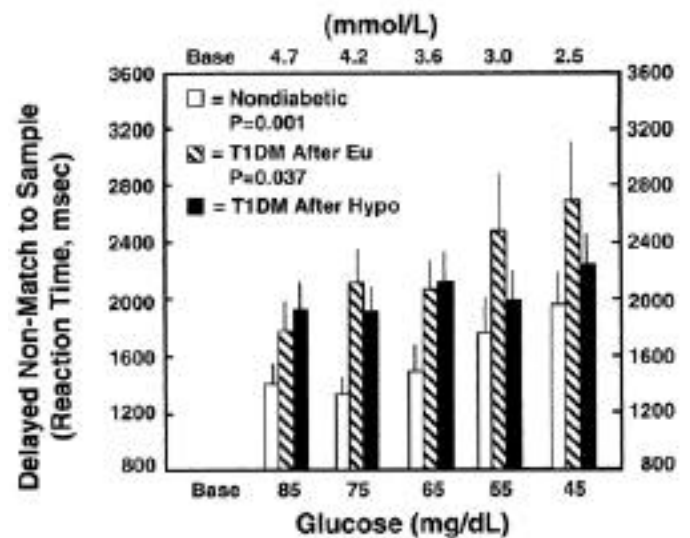


FIG. 4. Mean \pm SE reaction times on the delayed non-match to sample task during morning (0800–1300) hyperinsulinemic stepped hypoglycemic clamps in 12 nondiabetic subjects and in 15 patients with type 1, the latter studied after nocturnal (2330–0300) euglycemia (Eu, 109 ± 1 mg/dl, 6.0 ± 0.1 mmol/l) and after nocturnal hypoglycemia (Hypo, 48 ± 2 mg/dl, 2.7 ± 0.1 mmol/l). Repeated measures ANOVA P values <0.05 are shown between the groups to which they apply.

hypoglycemia in an earlier study (23). Nonetheless, it is conceivable that there is some special vulnerability of spatial memory (as assessed by the delayed non-match to sample task) as opposed to verbal memory (as assessed by the delayed paragraph recall task) (45). Fundamentally, we do not know the extent to which the apparent discrepancies reported here and in the literature are the result of effects of

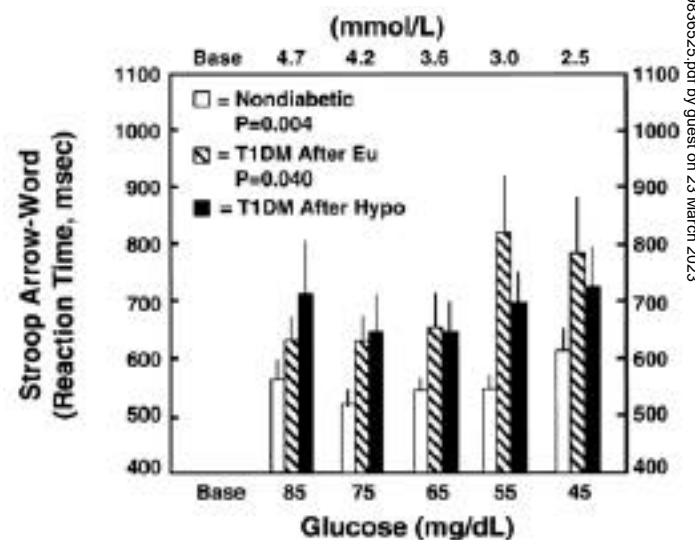


FIG. 5. Mean \pm SE reaction times on the Stroop arrow-word task during morning (0800–1300) hyperinsulinemic stepped hypoglycemic clamps in 12 nondiabetic subjects and in 15 patients with T1DM, the latter studied after nocturnal (2330–0300) euglycemia (Eu, 109 ± 1 mg/dl, 6.0 ± 0.1 mmol/l) and after nocturnal hypoglycemia (Hypo, 48 ± 2 mg/dl, 2.7 ± 0.1 mmol/l). Repeated measures ANOVA P values <0.05 are shown between the groups to which they apply.

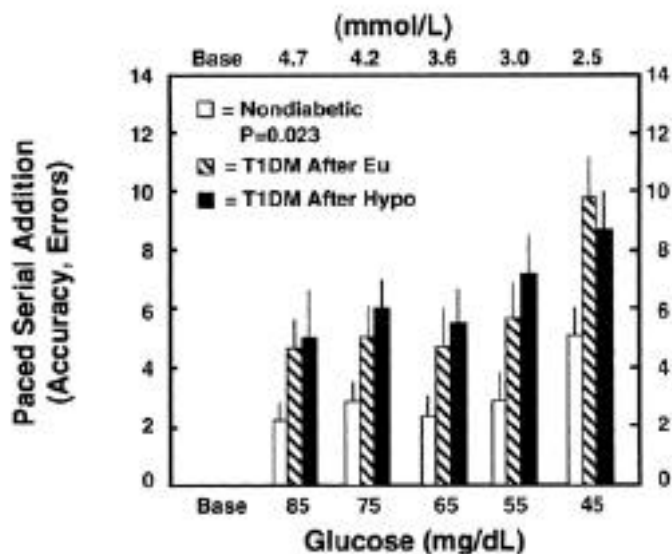


FIG. 6. Mean \pm SE number of errors on the paced serial addition task during morning (0800–1300) hyperinsulinemic stepped hypoglycemic clamps in 12 nondiabetic subjects and in 15 patients with T1DM, the latter studied after nocturnal (2330–0300) euglycemia (Eu, 109 ± 1 mg/dl, 6.0 ± 0.1 mmol/l) and after nocturnal hypoglycemia (Hypo, 48 ± 2 mg/dl, 2.7 ± 0.1 mmol/l). Repeated measures ANOVA *P* values <0.05 are shown between the groups to which they apply. Those >0.05 are not shown.

antecedent hypoglycemia on some cognitive functions but not others or of the instruments used to assess different cognitive functions.

Because we did not perform paired hyperinsulinemic-euglycemic clamps in these subjects, we could not calculate quantitative glycemic thresholds for the various responses to hypoglycemia (27,46,47). Nonetheless, because there was progressive hypoglycemia dose-related deterioration of cognitive functions during hypoglycemia on all four tests that have been consistently shown to be sensitive to hypoglycemia (18,23, this study), the finding of significant relative preservation of performance, by repeated measures ANOVA using the primary data, on the delayed non-match to sample and Stroop arrow-word tasks indicates that the glycemic thresholds for dysfunction of the corresponding cognitive functions shifted to lower plasma glucose concentrations after nocturnal hypoglycemia. The same reasoning applies to overall hypoglycemic cognitive dysfunction based on analysis of the sum of z-scores.

The additional finding that the patients with T1DM performed significantly less well than the nondiabetic subjects on four of the five cognitive function tests (the memory scanning, delayed non-match to sample, Stroop arrow-word, and paced serial addition tasks) is largely consistent with known neuropsychological deficits associated with T1DM (48,49). Impaired motor speed, memory, and visuospatial functioning have been associated with aspects of T1DM, such as age at onset and a history of severe hypoglycemia (49–51). However, we interpret this finding with caution because this study was not designed specifically to assess this relationship. For instance, the nondiabetic subjects were medical center personnel who were not matched to the patients with respect to intelligence or educational level.

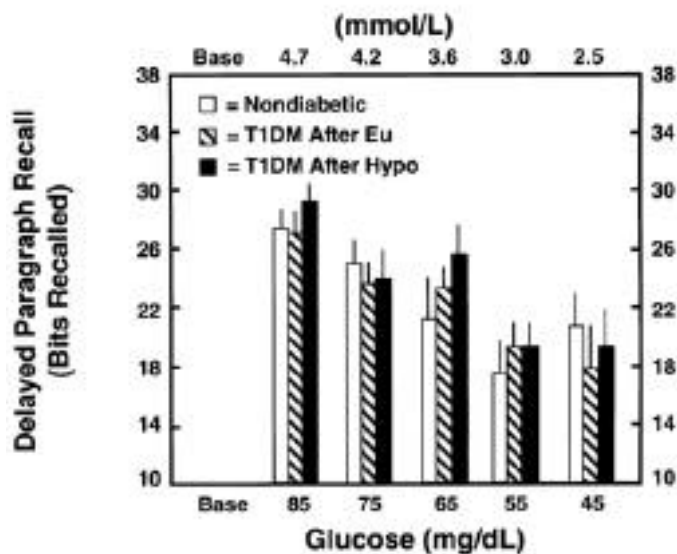


FIG. 7. Mean \pm SE number of bits recalled on the delayed paragraph recall task during morning (0800–1300) hyperinsulinemic stepped hypoglycemic clamps in 12 nondiabetic subjects and in 15 patients with T1DM, the latter studied after nocturnal (2330–0300) euglycemia (Eu, 109 ± 1 mg/dl, 6.0 ± 0.1 mmol/l) and after nocturnal hypoglycemia (Hypo, 48 ± 2 mg/dl, 2.7 ± 0.1 mmol/l). Repeated measures ANOVA disclosed no significant differences.

As expected (3), the patients with T1DM had absent glucagon and reduced autonomic—adrenomedullary (epinephrine), sympathetic neural (norepinephrine), and parasympathetic neural (pancreatic polypeptide)—and neurogenic symptom responses to hypoglycemia. The adrenomedullary and neurogenic symptom responses to hypoglycemia were reduced further after nocturnal hypoglycemia. Notably, the neuroglycopenic symptom score response was not reduced further, although the trend was in that direction.

In summary, the data indicate that glycemic thresholds for hypoglycemic cognitive dysfunction, like those for autonomic and symptomatic responses to hypoglycemia, shift to lower plasma glucose concentrations after recent antecedent hypoglycemia in patients with T1DM.

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REFERENCES

1. Reichard P, Berglund B, Britz A, Cars I, Nilsson B-Y, Rosenqvist U: Intensified conventional insulin treatment retards the microvascular complications of insulin-dependent diabetes mellitus (IDDM): the Stockholm Diabetes Intervention Study (SDIS) after 5 years. *J Intern Med* 230:101–108, 1991
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. Cryer PE: Hypoglycemia in diabetes mellitus. In *Hypoglycemia: Pathophysiology, Diagnosis, and Treatment*. Cryer PE, Ed. New York/Oxford, Oxford University Press, 1997, p. 91-125
 4. Cryer PE: Iatrogenic hypoglycemia as a cause of hypoglycemia-associated autonomic failure in IDDM: a vicious cycle. *Diabetes* 41:255-260, 1992
 5. Dagogo-Jack SE, Craft S, Cryer PE: Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus. *J Clin Invest* 91:819-828, 1993
 6. Rattarasarn C, Dagogo-Jack SE, Zachwieja JJ, Cryer PE: Hypoglycemia-induced autonomic failure in IDDM is specific for the stimulus of hypoglycemia and is not attributable to prior autonomic activation per se. *Diabetes* 43:809-818, 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. Heller SR, Cryer PE: Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia after one episode of hypoglycemia in nondiabetic humans. *Diabetes* 40:223-226, 1991
 9. Davis M, Shamon H: Counterregulatory adaptation to recurrent hypoglycemia in normal humans. *J Clin Endocrinol Metab* 73:995-1001, 1991
 10. Widom B, Simonson DC: Intermittent hypoglycemia impairs glucose counterregulation. *Diabetes* 41:1597-1602, 1992
 11. Davis MR, Mellman M, Shamon H: Further defects in counterregulatory responses induced by recent hypoglycemia in type I diabetes. *Diabetes* 41:1335-1340, 1992
 12. Lingenfelter T, Renn W, Sommerwerck U, Jung MF, Buettner UW, Zaiser-Kaschel H, Kaschel R, Eggstein M, Jakob B: Compromised hormonal counterregulation, symptom awareness, and neurophysiological function after recurrent short-term episodes of insulin-induced hypoglycemia in IDDM patients. *Diabetes* 42:610-618, 1993
 13. Simonson DC, Tamborlane WV, DeFonzo RA, Sherwin RS: Intensive insulin therapy reduces counterregulatory responses to hypoglycemia in patients with type I diabetes. *Ann Intern Med* 103:184-190, 1985
 14. Amiel SA, Tamborlane WV, Simonson DC, Sherwin RS: Defective glucose counterregulation after strict glycaemic control of insulin-dependent diabetes mellitus. *N Engl J Med* 316:1376-1383, 1987
 15. Amiel SA, Sherwin RS, Simonson DC, Tamborlane WV: Effect of intensive insulin therapy on glycaemic thresholds for counterregulatory hormone release. *Diabetes* 37:901-907, 1988
 16. Bottini P, Boschetti E, Pampanelli S, Ciofetta M, Del Sindaco P, Scionti L, Brunetti P, Bolli GB: Contribution of autonomic neuropathy to reduced plasma adrenaline responses to hypoglycemia in IDDM. *Diabetes* 46:814-823, 1997
 17. Veneman T, Mitrakou A, Mokan M, Cryer P, Gerich J: Induction of hypoglycemia unawareness by asymptomatic nocturnal hypoglycemia. *Diabetes* 42:1233-1237, 1993
 18. 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
 19. 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
 20. 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
 21. 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
 22. 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
 23. Ovalle F, Fanelli CG, Paramore DS, Craft S, Cryer PE: Brief twice-weekly episodes of hypoglycemia reduce detection of clinical hypoglycemia in type 1 diabetes. *Diabetes* 47:1472-1479, 1998
 24. Hopkins D, Evans M, Lomas J, Pernet A, Amiel S: Variable effect of previous glycaemic experience on cognitive function and symptoms of hypoglycemia (Abstract). *Diabetes* 47:A110, 1998
 25. Boyle PJ, Nagy R, O'Connor AM, Kempers SF, Yeo RA, Qualls C: Adaptation in brain glucose uptake following recurrent hypoglycemia. *Proc Natl Acad Sci USA* 91:9352-9356, 1994
 26. Boyle PJ, Kempers SF, O'Connor AM, Nagy RJ: Brain glucose uptake and unawareness of hypoglycemia in patients with insulin-dependent diabetes mellitus. *N Engl J Med* 333:1726-1731, 1995
 27. 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
 28. 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
 29. 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
 30. Ensink J: Immunoassays for glucagon. In *Glucagon: Handbook of Experimental Pharmacology*. Lefebvre P, Ed. New York, Springer-Verlag, 1983, p. 203-221
 31. 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
 32. Schalch D, Parker M: 1964: a sensitive double antibody radioimmunoassay for growth hormone in plasma. *Nature* 703:1141-1142, 1974
 33. Farmer R, Pierce C: 1974: plasma cortisol determination: radioimmunoassay and competitive binding compared. *Clin Chem* 20:411-414, 1974
 34. Hosaka K, Kikuchi T, Mitsuhide 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
 35. 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
 36. Pinter J, Hayaski J, Watson J: Enzymatic assay of glycerol, dihydroxyacetone, and glyceraldehyde. *Arch Biochem Biophys* 121:404-414, 1967
 37. 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
 38. 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
 39. Lezak MD: *Neuropsychological Assessment*. New York/Oxford, Oxford University Press, 1995, p. 154-156
 40. Ziegler D, Høbinger A, Muhlen H, Gries FA: Effects of previous glycaemic control on the onset and magnitude of cognitive dysfunction during hypoglycaemia in type 1 (insulin-dependent) diabetic patients. *Diabetologia* 35:828-834, 1992
 41. Jones TW, Borg WP, Borg MA, Boulware SD, McCarthy G, Silver D, Tamborlane WV, Sherwin RS: Resistance to neuroglycopenia: an adaptive response during intensive insulin treatment of diabetes. *J Clin Endocrinol Metab* 82:1713-1718, 1997
 42. Sidman M, Stoddard LT, Mohr JP: Some additional quantitative observations of immediate memory in a patient with bilateral hippocampal lesions. *Neuropsychologia* 6:245-254, 1968
 43. Aggleton JP, Shaw C, Gaffan EA: The performance of postencephalitic amnesic subjects on two behavioral tests of memory: concurrent discrimination learning and delayed matching to sample. *Cortex* 28:359-372, 1994
 44. Mishkin M, Murray EA: Stimulus recognition. *Curr Opin Neurobiol* 4:200-206, 1994
 45. Hershey T, Bhargava N, White N, Craft S: Standard vs. intensive insulin treatment in children with insulin-dependent diabetes mellitus: effects on memory and reaction time (Abstract). *J Intern Neuropsych Soc* 4:2, 1998
 46. Mitrakou A, Ryan C, Veneman T, Mokan M, Jenssen T, Kiss I, Durrant J, Cryer P, Gerich J: Hierarchy of glycaemic thresholds for counterregulatory hormone secretion, symptoms, and cerebral dysfunction. *Am J Physiol* 260:E67-E74, 1991
 47. Fanelli C, Pampanelli S, Epifano L, Rambotti AM, Ciofetta M, Modarelli F, Di Vincenzo A, Annibale B, Lepore M, Lalli C, Del Sindaco P, Brunetti P, Bolli GB: Relative roles of insulin and hypoglycaemia on induction of neuroendocrine responses to, symptoms of, and deterioration of cognitive function in hypoglycaemia in male and female humans. *Diabetologia* 37:797-807, 1994
 48. Holmes CS: Neuropsychological sequelae of acute and chronic blood glucose disruption in adults with insulin-dependent diabetes. In *Neuropsychological and Behavioral Aspects of Diabetes*. Holmes CS, Ed. New York, Springer-Verlag, 1990, p. 123-153
 49. Ryan CM: Neuropsychological consequences and correlates of diabetes in childhood. In *Neuropsychological and Behavioral Aspects of Diabetes*. Holmes CS, Ed. New York, Springer-Verlag, 1990, p. 58-84
 50. Ryan CM, Vega A, Drash A: Cognitive deficits in adolescents who developed diabetes early in life. *Pediatrics* 75:921-927, 1985
 51. Hershey T, Craft S, Bhargava N, White NH: Memory and insulin-dependent diabetes (IDDM): effects of childhood onset and severe hypoglycemia. *J Intern Neuropsych Soc* 3:509-520, 1997

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