

BRAIN GLUCOSE UPTAKE AND UNAWARENESS OF HYPOGLYCEMIA IN PATIENTS WITH INSULIN-DEPENDENT DIABETES MELLITUS

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Abstract Background. In patients with insulin-dependent diabetes mellitus (IDDM) whose treatment results in nearly normal mean plasma glucose concentrations, an unawareness of hypoglycemia can develop, and such patients are at increased risk for seizures and coma. We tested the hypothesis that during hypoglycemia, these patients would have normal glucose uptake in the brain and that consequently no sympathoadrenal activation would begin, resulting in an unawareness of hypoglycemia.

Methods. We measured glucose uptake in the brain at plasma glucose concentrations of 105 and 54 mg per deciliter (5.8 and 3.0 mmol per liter) in 24 patients with IDDM, stratified into three groups according to their glycosylated hemoglobin values (mean [\pm SD] values, 7.2 ± 0.5 , 8.5 ± 0.4 , and 10.2 ± 1.3 percent) and compared the values for brain glucose uptake with those measured in 15 normal subjects at plasma glucose concentrations of 85 and 55 mg per deciliter (4.2 and 3.1 mmol per liter). We also recorded the subjects' hypoglycemic-symptom scores and measured their plasma concentrations of counterregulatory hormones.

Results. There was no significant change in the up-

take of glucose in the brain (calculated as the uptake during hypoglycemia minus the uptake during normoglycemia) among the patients with IDDM who had the lowest glycosylated hemoglobin values ($+0.6\pm 2.0$ mg [3.3 ± 11.1 μ mol] per 100 g of brain tissue per minute, $P=0.39$). Conversely, glucose uptake in the brain fell in both the group with intermediate values (a decrease of 1.3 ± 1.0 mg [7.2 ± 5.6 μ mol] per 100 g per minute, $P=0.009$) and the group with the highest values (a decrease of 1.8 ± 1.6 mg [10.0 ± 9.0 μ mol] per 100 g per minute, $P=0.01$), as it did in the normal subjects (a decrease of 1.6 ± 1.8 mg [9.0 ± 10.1 μ mol] per 100 g per minute, $P=0.003$). The responses of plasma epinephrine and pancreatic polypeptide and the frequency of symptoms of hypoglycemia were lowest in the group with the lowest glycosylated hemoglobin values.

Conclusions. During hypoglycemia, patients with IDDM who have nearly normal glycosylated hemoglobin values have normal glucose uptake in the brain, which preserves cerebral metabolism, reduces the responses of counterregulatory hormones, and causes an unawareness of hypoglycemia. (N Engl J Med 1995;333:1726-31.)

PATIENTS with insulin-dependent diabetes mellitus (IDDM) who have nearly normal mean plasma glucose concentrations with treatment, as in the intensive-treatment group of the Diabetes Control and Complications Trial, more often have seizures or coma or require another person's assistance to recover from hypoglycemia than do patients treated less intensively, and the hypoglycemia may occur without warning symptoms.^{1,2} The term "unawareness of hypoglycemia" has been used to describe the state in which autonomic warning symptoms do not occur or are not recognized before neuroglycopenia develops.³ Given the clear benefit of improved glycemic control in reducing the microvascular complications of diabetes,¹ it is essential to understand the origin of unawareness of hypoglycemia in order to reduce the risk associated with intensified treatment of the disease.

Fundamental to the patient's recognition of a falling plasma glucose concentration is an increase in autonomic tone, which is predominantly reflected in increased sympathoadrenal activity, resulting in the tachycardia, nervousness, and tremor that characterize hypoglycemia.⁴ Specific glucose-sensing centers in the ventromedian hypothalamic nuclei⁵ of the brain mediate the sympathoadrenal response,⁶ which begins at an arterial

plasma glucose concentration of approximately 75 mg per deciliter (4.2 mmol per liter) in normal subjects.^{7,8} Intensive treatment of diabetes with either multiple daily injections of insulin or insulin-pump therapy is known to reduce the secretion of epinephrine, so that the response begins at lower plasma glucose concentrations.⁹

The central nervous system depends on a continuous supply of glucose for fuel, because it cannot synthesize or store more than a few minutes' worth of glucose for use during hypoglycemia.¹⁰ In animals, however, the rise in plasma concentrations of epinephrine and glucagon is attenuated if the brain is infused with glucose while systemic hypoglycemia is induced.¹¹ Glucose reaches the central nervous system by crossing the blood-brain barrier by means of the specific glucose transport protein, GLUT 1.¹² When endothelial cells in capillaries in the brain are deprived of glucose in vitro, there is an associated increase in the transcription¹³ and translation¹⁴ of this protein. In rodents, prolonged hypoglycemia increased the number of glucose transporters in the brain¹⁵ and the extraction of glucose¹⁶ and normalized brain concentrations of ATP.¹⁷ If such an adaptation in the capacity to transport glucose were operative in humans, the normal energy metabolism in the brain could potentially be maintained in the presence of low systemic plasma glucose concentrations.¹⁸

We tested the hypothesis that patients with IDDM who have nearly normal plasma glucose concentrations because of treatment (and consequently have an increased frequency of hypoglycemia) have an increased rate of whole-brain uptake of glucose during hypogly-

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emia. If the glucose metabolism in the brain is maintained, sympathoadrenal activation will not occur, and therefore patients will be unaware of subnormal plasma glucose concentrations.

METHODS

Subjects

We studied 24 patients with IDDM (plasma concentration of C peptide, <0.2 ng per milliliter [0.07 nmol per liter]) who were recruited from the diabetes clinic and 15 normal subjects. The patients were being treated with a variety of insulin-replacement schemes, including multiple daily injections with regular insulin and either isophane insulin suspension (NPH) or extended insulin zinc suspension (ultralente). One patient was using a subcutaneous insulin-infusion pump. Six patients were Hispanic and 18 were non-Hispanic whites; among the normal subjects, 4 were Hispanic, 1 Native American, and 10 white. The patients were stratified into three groups, each containing eight patients, according to whether their glycosylated hemoglobin values were low (glycosylated hemoglobin, 6.4 to 7.8 percent), intermediate (7.9 to 8.9 percent), or high (9.0 to 12.7 percent) at the time of the study (Table 1).

The results of home monitoring of blood glucose values before meals and at bedtime during the seven days before the study began showed that glucose values below 70 mg per deciliter (3.9 mmol per liter) were more frequent in the group with the lowest glycosylated hemoglobin concentrations than in the other two groups ($P = 0.002$). Two patients in each group had blood glucose concentrations below 55 mg per deciliter (3.1 mmol per liter) during the 24 hours before administration. All the study subjects were within 10 percent of their ideal body weights, and no patient with diabetes had complications of the disease on physical examination or laboratory testing. The results in the 15 normal subjects have been previously reported and are included here for comparison with the results in the patients with IDDM.⁷ The studies were approved by the Human Research Review Committee at the University of New Mexico, and all subjects gave informed consent.

Protocol

The study subjects were admitted to the General Clinical Research Center the afternoon before the study began. During the 24 hours before admission, the patients measured their blood glucose concentrations before and 2 hours after each meal. The night before the study, regular human insulin was administered subcutaneously before dinner, followed by a variable intravenous infusion of insulin, beginning at 11 p.m., to maintain the patients' venous blood glucose concentrations at levels similar to the mean values measured before and after meals on the preceding day. The initial rate of the infusion was 0.2 mU per kilogram of body weight per minute, and it was adjusted by 5 to 10 percent per hour as needed in order to achieve the target blood glucose concentration. Between 3 a.m. and 7 a.m., the patients' blood glucose concentrations were reduced to 105 mg per deciliter (5.8 mmol per liter). At 7 a.m., an 18-gauge, flexible, 20-cm (8-in.) cannula was introduced into the right internal jugular vein and advanced retrogradely until it met with resistance at the level of the internal jugular bulb, a point at which all the subjects experienced ear discomfort. We¹⁹ and others²⁰ have previously used roentgenography to demonstrate correct placement of the cannula at the jugular bulb by this technique. Left radial arterial and right antecubital intravenous cannulas were introduced for the collection of blood and for infusion, respectively.

A continuous intravenous infusion of regular human insulin (1.5 mU per kilogram per minute) was begun, and 20 percent dextrose

Table 1. Characteristics of the Patients with IDDM, Grouped According to Their Glycosylated Hemoglobin Values at the Time of the Study, and the Normal Subjects.*

CHARACTERISTIC	PATIENTS WITH IDDM			NORMAL SUBJECTS (N = 15)	P VALUE†
	LOWEST GLYCOSYLATED HEMOGLOBIN, 6.4–7.8% (N = 8)	INTERMEDIATE GLYCOSYLATED HEMOGLOBIN, 7.9–8.9% (N = 8)	HIGHEST GLYCOSYLATED HEMOGLOBIN, 9.0–12.7% (N = 8)		
Sex (M/F)	3/5	5/3	6/2	8/7	—
Age (yr)	32±9	30±8	22±4	30±9	0.10
Age (yr) at onset of diabetes	18±8	22±8	12±8	—	0.06
Duration of diabetes (yr)	14±5	8±4	10±6	—	0.09
Glycosylated hemoglobin (%)	7.2±0.5	8.5±0.4	10.2±1.3	4.4 to 6.8	—
No. of blood glucose measurements ≤70 mg/dl (3.9 mmol/liter) in preceding week	6±3	2±2‡	3±2§	—	0.001

*Plus-minus values are means ±SD.

†By analysis of variance among the groups of patients with IDDM.

‡ $P = 0.002$ by a post hoc Newman-Keuls test for the comparison with the group with the lowest glycosylated hemoglobin values.

§ $P = 0.01$ by a post hoc Newman-Keuls test for the comparison with the other two groups.

was infused to produce a stable arterial plasma glucose concentration of 105 mg per deciliter. The arterial plasma glucose concentration was measured every five to seven minutes, and the dextrose infusion was adjusted manually to meet the target concentration.⁷ After a period of 30 minutes in which the glucose concentration in the brain equilibrated with the systemic plasma glucose concentration, the glucose uptake in the brain (calculated as the arteriovenous difference in the glucose concentration across the brain multiplied by the cerebral blood flow) was determined. The subjects inhaled 9 percent nitrous oxide as an inert tracer gas, and the nitrous oxide content of jugular venous and arterial blood was measured frequently over a 20-minute period for the calculation of cerebral blood flow by the Fick method.^{7,19,21} Arterial and jugular venous plasma glucose concentrations were determined in quintuplicate at the beginning and end of each determination of blood flow (the means of the values recorded at these times are reported here). At the end of the 20-minute period, the subjects scored themselves with respect to 12 symptoms of hypoglycemia, from 0 (for none at all) to 7 (for most intense).^{7,22} The symptoms were classified as either autonomic (if the subject was nervous, tingly, shaky, sweating, hungry, or had pounding of the heart) or neuroglycopenic (if the subject was tired, dizzy, faint, had blurred vision, or was unable to think easily).⁴ The total score for symptoms included these symptom scores and one subjective symptom score, for "feeling different in any way." The plasma glucose concentration was then slowly decreased over a period of 60 to 80 minutes to 54 mg per deciliter (3.0 mmol per liter). The sequence of equilibration and determinations of the rate of glucose uptake in the brain and other tests was then repeated. Figure 1 shows a summary of the study protocol.

Samples of arterial plasma were collected for the measurement of counterregulatory hormones at the beginning and end of each blood-flow measurement. The results included here for the normal subjects were obtained in our earlier study,⁷ in which the plasma glucose concentration was decreased from 85 to 45 mg per deciliter (4.7 to 2.5 mmol per liter) in steps of 10 mg per deciliter (approximately 0.6 mmol per liter); the values reported here are those obtained in that study at plasma glucose concentrations of 85 and 55 mg per deciliter.

Analytical Methods

Glycosylated hemoglobin was measured by ion-exchange chromatography (Abbott Laboratories, Abbott Park, Ill.; normal range, 4.4 to 6.8 percent). Plasma glucose was measured by the glucose oxidase technique (Beckman Instruments, Fullerton, Calif.). Plasma concentrations of free insulin,²³ C peptide,²³ glucagon,²⁴ and pancreatic polypeptide²⁵ were measured by radioimmunoassay, those of epinephrine by a single-isotope radioenzymatic technique,²⁶ and those of ni-

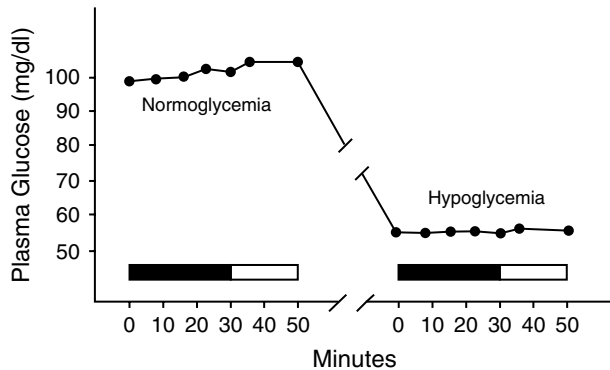


Figure 1. Experimental Method and Mean Arterial Plasma Glucose Concentrations in Patients with IDDM during Normoglycemia and Hypoglycemia.

Each 50-minute period of the test included a 30-minute period of equilibration between the glucose concentration in the brain and that in plasma (shaded bars), followed by a 20-minute period in which glucose uptake in the brain was measured (open bars). The break in the time axis represents a variable period during which the plasma glucose concentration was adjusted to 54 mg per deciliter (3.0 mmol per liter). To convert values for glucose to millimoles per liter, multiply by 0.05551.

trous oxide with an infrared spectrophotometer designed to detect trace amounts of nitrous oxide (Traverse Medical Monitors, Saline, Mich.).

Statistical Analysis

Dividing the nitrous oxide concentration at equilibrium by the integrated area between the time-concentration curve for the arterial

nitrous oxide concentration and that for the venous concentration yields the cerebral blood flow.²¹ The differences between groups were initially assessed by an analysis of variance with covariates as needed. Once statistical significance was established, a post hoc Newman-Keuls multiple-comparison test was performed. Paired, two-tailed Student's t-tests were used to compare the measurements obtained during periods of normoglycemia and hypoglycemia within groups, and unpaired, two-tailed Student's t-tests were used to compare measurements between groups. When data were non-normally distributed, a Wilcoxon signed-rank test was used to assess significance. The results are presented as means \pm SD unless otherwise noted.

RESULTS

Mean Arterial Plasma Glucose Concentrations in Patients with IDDM

The mean plasma glucose concentrations in the three groups of patients were similar at the two levels of glycemia studied, and therefore the mean plasma glucose concentrations in all 24 patients are shown (Fig. 1). The time required to lower the patients' plasma glucose concentration to 54 mg per deciliter varied; therefore, no intermediate data are presented. The mean initial plasma concentrations of free insulin in the three groups were similar, ranging from 80 to 87 μ U per milliliter (480 to 522 pmol per liter).

Normoglycemic and Hypoglycemic Values

The rates of cerebral blood flow during normoglycemia were similar in the three groups and did not change during hypoglycemia (Table 2). The differences between the arterial and the venous plasma glucose concentrations in the three groups were also similar

Table 2. Glucose Uptake in the Brain and Plasma Glucagon Concentrations during Normoglycemia and Hypoglycemia in Patients with IDDM, Grouped According to Their Glycosylated Hemoglobin Values at the Time of the Study, and Normal Subjects.*

VARIABLE	PATIENTS WITH IDDM			NORMAL SUBJECTS (N = 15)	P VALUE†
	LOWEST GLYCOSYLATED HEMOGLOBIN, 6.4–7.8% (N = 8)	INTERMEDIATE GLYCOSYLATED HEMOGLOBIN, 7.9–8.9% (N = 8)	HIGHEST GLYCOSYLATED HEMOGLOBIN, 9.0–12.7% (N = 8)		
Normoglycemia					
Cerebral blood flow (ml/100 g of tissue/min)	64.2 \pm 10.0	59.1 \pm 18.1	71.0 \pm 16.0	65.4 \pm 11.3	0.30
Plasma glucose, arteriovenous difference (mg/dl)	9.4 \pm 2.0	10.4 \pm 1.4	11.1 \pm 1.7	9.9 \pm 2.0	0.14
Glucose uptake in the brain (mg/100 g of tissue/min)	6.0 \pm 1.8	6.0 \pm 1.5	7.8 \pm 1.7	6.6 \pm 1.9	0.07
Plasma glucagon (pg/ml)	66 \pm 10	60 \pm 13	66 \pm 8	84 \pm 23	0.47
Hypoglycemia					
Cerebral blood flow (ml/100 g of tissue/min)	73.7 \pm 14.1	63.4 \pm 25.6	71.3 \pm 15.8	65.5 \pm 12.2	0.54
Plasma glucose, arteriovenous difference (mg/dl)	9.0 \pm 1.1	8.0 \pm 1.7‡	8.6 \pm 1.5‡	7.6 \pm 0.9‡	0.34
Glucose uptake in the brain (mg/100 g of tissue/min)	6.7 \pm 1.5	4.7 \pm 1.1§	6.0 \pm 1.0¶	5.0 \pm 1.1	0.02
Plasma glucagon (pg/ml)	74 \pm 13	87 \pm 36**	77 \pm 16	104 \pm 32††	0.56

*Plus-minus values are means \pm SD. To convert values for glucose to millimoles per liter, multiply by 0.05551; to convert values for glucose uptake in the brain to micromoles per 100 g per minute, multiply by 5.6; and to convert values for glucagon to picomoles per liter, multiply by 0.29.

†By analysis of variance among the groups of patients with IDDM.

‡P=0.001 by the t-test for the comparison with the value obtained during normoglycemia.

§P=0.009 by the t-test for the comparison with the value obtained during normoglycemia, and P=0.01 for the comparison during hypoglycemia with the group with the lowest glycosylated hemoglobin values.

¶P=0.01 by the t-test for the comparison with the value obtained during normoglycemia.

||P=0.003 by the t-test for the comparison with the value obtained during normoglycemia.

**P=0.03 by the t-test for the comparison with the value obtained during normoglycemia.

††P<0.01 by the Wilcoxon signed-rank test for the comparison with the value obtained during normoglycemia.

during both normoglycemia and hypoglycemia, and these values were lower during hypoglycemia than during normoglycemia in the groups with the intermediate and the highest glycosylated hemoglobin values. The absolute values for glucose uptake in the brain were similar during normoglycemia; during hypoglycemia, the patients in the group with the lowest glycosylated hemoglobin values had no significant change in glucose uptake in the brain, but this value decreased significantly in the other groups ($P=0.004$).

The base-line plasma glucagon concentrations were similar in the three groups but were slightly higher in the normal subjects. This value increased only in the group with intermediate glycosylated hemoglobin values and in the normal subjects.

Changes in Glucose Uptake in the Brain, Symptom Scores, and Plasma Responses of Epinephrine and Pancreatic Polypeptide

The normal subjects had a 24 percent reduction in glucose uptake in the brain during hypoglycemia (Fig. 2), and the decrease in the patients in the groups with intermediate and highest glycosylated hemoglobin values was similar, whereas the patients in the group with the lowest glycosylated hemoglobin values had no significant change ($P=0.01$). The overall hypoglycemic-symptom score increased in all groups except the group with the lowest glycosylated hemoglobin values,

so that the patients in the other two groups had higher symptom scores during hypoglycemia than the group with the lowest values ($P=0.003$). The changes were similar when the symptom scores were subdivided into the score for autonomic symptoms ($P=0.02$) and that for neuroglycopenic symptoms ($P=0.002$).⁴ Plasma epinephrine concentrations increased significantly in all groups ($P<0.001$), whereas plasma concentrations of pancreatic polypeptide increased in all the groups except the one with the lowest glycosylated hemoglobin values ($P=0.01$). The increase in the plasma epinephrine concentration was significantly smaller in the group with the lowest glycosylated hemoglobin values ($P=0.02$).

DISCUSSION

Glucose uptake in the brain did not decrease during moderate hypoglycemia in patients with IDDM who had nearly normal mean plasma glucose concentrations with treatment, as determined on the basis of glycosylated hemoglobin values. As a consequence, no counter-regulation of glucose began in these patients, so that they had very little increase in plasma concentrations of epinephrine and pancreatic polypeptide and few symptoms of hypoglycemia. In contrast, in the patients with less well controlled diabetes and the normal subjects, glucose uptake in the brain decreased at similar levels of hypoglycemia.⁷ We conclude that increased uptake

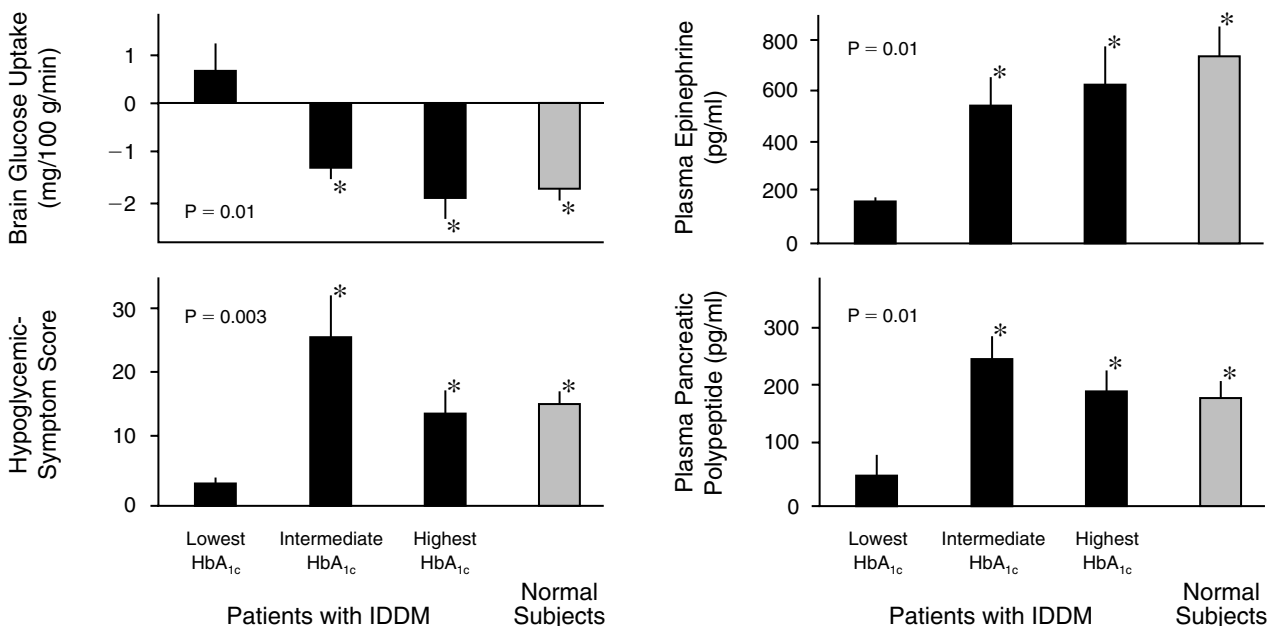


Figure 2. Mean (\pm SD) Changes from Base Line in Glucose Uptake in the Brain, Hypoglycemic-Symptom Scores, and Plasma Concentrations of Epinephrine and Pancreatic Polypeptide during Hypoglycemia.

Black bars indicate patients with IDDM, and gray bars normal subjects. HbA_{1c} denotes glycosylated hemoglobin. The ranges of values for glycosylated hemoglobin in the three groups of patients are the same as in Table 1. P values shown in the figure reflect differences only among the groups of patients with IDDM, by analysis of variance. Asterisks indicate $P\leq 0.03$ by t-test for the comparison with the group with the lowest glycosylated hemoglobin values. To convert values for glucose uptake in the brain to micro-moles per 100 g per minute, multiply by 5.6; to convert values for plasma epinephrine and pancreatic polypeptide to picomoles per liter, multiply by 5.46 and 0.24, respectively.

of glucose in the brain during hypoglycemia is a major mechanism for inducing the unawareness of hypoglycemia in patients with IDDM.

Previous studies using positron-emission tomography in patients with well-controlled IDDM demonstrated that the rate of glucose metabolism in the brain during hypoglycemia is similar to that of normal subjects,²⁷ but the accuracy of these studies has been questioned because of quantitative inconsistencies in the basal rates of glucose metabolism as compared with established norms. The validity of the methods we used relies on the knowledge that blood collected from the internal jugular vein represents actual cerebral venous drainage, with less than 1 percent being derived from extracranial sources. We have previously discussed in detail the limitations of our procedure and those of others.^{7,19}

Recurrent hypoglycemia is probably the mechanism that leads to increased glucose uptake in the brain. Glycosylated hemoglobin is a surrogate marker of recent hypoglycemia, but its disadvantage is that it reflects the overall level of glycemia during the preceding three months.²⁸ The frequency and duration of hypoglycemia required to induce the augmented uptake are not certain. In normal subjects, adaptation in glucose uptake in the brain and tolerance to a plasma glucose concentration of 45 mg per deciliter (2.5 mmol per liter) are complete when recurrent hypoglycemia is present for 56 hours.⁷ In the current study, the patients with normal glucose uptake in the brain during hypoglycemia had slightly low blood glucose values on home monitoring an average of six times during the week before the study. Defective glucose counterregulation can occur after a single recent episode of hypoglycemia.^{29,30} Even patients with poorly controlled diabetes, as assessed by their glycosylated hemoglobin values, can have hypoglycemia without being aware of it if they have hypoglycemia shortly before they are studied.³¹ Indeed, three patients in our group with intermediate glycosylated hemoglobin values had hypoglycemia more often than the other members of that group in the days immediately before the study. These three patients had higher rates of glucose uptake in the brain during hypoglycemia than did the other patients in the group.

In summary, patients with IDDM who have nearly normal plasma glucose concentrations because of treatment have what could be considered a physiologically useful adaptation in glucose uptake in the brain that preserves brain function in the presence of episodic hypoglycemia. However, the induced defect in glucose counterregulation may contribute to a vicious circle of asymptomatic hypoglycemia that further increases the uptake of glucose in the brain, causing an unawareness of even lower plasma glucose concentrations.³² Ultimately, there is a threshold at which the provision of glucose from the circulation will be insufficient to support neural activity, even with increased uptake. The plasma glucose concentration at which the metabolism of the brain is finally impaired may be so close to the

concentration that causes serious neuroglycopenia that patients have limited opportunity to correct the situation. Therefore, increased glucose uptake in the brain may be considered maladaptive. Defects in the secretion of epinephrine and in the ability to perceive symptoms in patients with IDDM may be reversible with strict avoidance of hypoglycemia.^{31,33,34} Such reversibility implies that glucose uptake in the brain is dynamic and changes with the antecedent level of glycemia.¹⁸ These results should challenge patients with IDDM and their physicians to achieve the tightest level of glycemic control in order to minimize microvascular complications, while at the same time avoiding even a slight degree of hypoglycemia, because hypoglycemia is likely to lead to a reversible, maladaptive central nervous system tolerance to subnormal plasma glucose concentrations.

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