

## DECREASED EPINEPHRINE RESPONSES TO HYPOGLYCEMIA DURING SLEEP

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## ABSTRACT

**Background** In patients with type I diabetes mellitus, hypoglycemia occurs commonly during sleep and is frequently asymptomatic. This raises the question of whether sleep is associated with reduced counterregulatory-hormone responses to hypoglycemia.

**Methods** We studied the counterregulatory-hormone responses to insulin-induced hypoglycemia in eight adolescent patients with type I diabetes and six age-matched normal subjects when they were awake during the day, asleep at night, and awake at night. In each study, the plasma glucose concentration was stabilized for 60 minutes at approximately 100 mg per deciliter (5.6 mmol per liter) and then reduced to 50 mg per deciliter (2.8 mmol per liter) and maintained at that concentration for 40 minutes. Plasma free insulin, epinephrine, norepinephrine, cortisol, and growth hormone were measured frequently during each study. Sleep was monitored by polysomnography.

**Results** The plasma glucose and free insulin concentrations were similar in both groups during all studies. During the studies when the subjects were asleep, no one was awakened during the hypoglycemic phase, but during the final 30 minutes of the studies when the subjects were awake both the patients with diabetes and the normal subjects had symptoms of hypoglycemia. In the patients with diabetes, plasma epinephrine responses to hypoglycemia were blunted when they were asleep (mean  $\pm$  SE peak plasma epinephrine concentration,  $70 \pm 14$  pg per milliliter [ $382 \pm 76$  pmol per liter];  $P=0.3$  for the comparison with base line), as compared with when they were awake during the day or night ( $238 \pm 39$  pg per milliliter [ $1299 \pm 213$  pmol per liter],  $P=0.004$  for the comparison with base line, and  $296 \pm 60$  pg per milliliter [ $1616 \pm 327$  pmol per liter],  $P=0.004$ , respectively). The patients' plasma norepinephrine responses were also reduced during sleep, whereas their plasma cortisol concentrations did not increase and their plasma growth hormone concentrations increased slightly. The patterns of counterregulatory-hormone responses in the normal subjects were similar.

**Conclusions** Sleep impairs counterregulatory-hormone responses to hypoglycemia in patients with diabetes and normal subjects. (N Engl J Med 1998;338:1657-62.)

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INTENSIVE therapy aimed at achieving plasma glucose concentrations and glycosylated hemoglobin values as close to normal as safely possible has been recommended for most patients with type I diabetes mellitus, because such treatment lowers the risks of the complications of diabetes.<sup>1</sup> However, intensive therapy also increases the risk of severe hypoglycemia,<sup>2</sup> and hypoglycemia is the main obstacle to the success of intensive therapy in such patients.

Patients with type I diabetes are susceptible to hypoglycemia for many reasons, including the non-physiologic nature of insulin treatment, inconsistencies in food intake and exercise, and most important, defective counterregulatory responses to hypoglycemia.<sup>3-7</sup> Because glucagon responses to hypoglycemia are lost early in the course of the disease,<sup>6</sup> patients with diabetes are dependent on sympathoadrenal responses. Furthermore, mild hypoglycemia itself reduces plasma epinephrine responses and symptomatic awareness of subsequent episodes of hypoglycemia.<sup>8</sup>

Most severe episodes of hypoglycemia occur at night,<sup>2,9,10</sup> and in studies in which plasma glucose was measured at night in patients with diabetes, asymptomatic hypoglycemia was common and its duration often prolonged for more than four hours.<sup>11,12</sup> The propensity to nocturnal hypoglycemia has been assumed to be due to a mismatch between the action of intermediate- or long-acting insulin given before supper or bedtime and the increased hepatic sensitivity to insulin that occurs in the middle of the night. However, the effect of sleep on counterregulatory-hormone responses to hypoglycemia has not been systematically evaluated. This study was undertaken to examine the hypothesis that deep sleep makes patients with diabetes more susceptible to hypoglycemia by impairing plasma epinephrine responses to it.

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## METHODS

### Study Subjects

We studied eight adolescents with type I diabetes and six normal adolescents. Among the patients with diabetes, four were male and four were female, with a mean ( $\pm$ SD) age of  $15 \pm 1$  years. The duration of diabetes was  $4 \pm 1$  years. All the patients were taking insulin twice daily (mean [ $\pm$ SD] total dose,  $1.2 \pm 0.5$  U per kilogram daily) and had moderate glycemic control, with a mean glycosylated hemoglobin value of  $8.1 \pm 0.5$  percent (normal value,  $<6.2$ ). None had clinical evidence of autonomic neuropathy, severe hypoglycemia within the preceding three months, symptoms of hypoglycemia, or a blood glucose concentration (ascertained by self-monitoring) of  $<72$  mg per deciliter ( $4.0$  mmol per liter) during the 24 hours before any study; none were receiving any medication other than insulin or had an acute illness. Among the normal subjects, four were male and two were female, with a mean age of  $14 \pm 1$  years. None had a history of illnesses that might affect sleep, glucose regulation, or hormonal responses to hypoglycemia. All 14 subjects were of normal weight. The study was approved by the Princess Margaret Hospital Ethics Committee and the Yale Human Investigation Committee, and all the subjects and their parents gave written informed consent.

### Protocol

The patients with diabetes were studied while awake during the day, asleep at night, and awake at night, and the normal subjects were studied while awake during the day and asleep at night. The daytime studies were performed between 10 a.m. and noon after a 10-to-12-hour overnight fast, and the nighttime studies were performed between 11 p.m. and 1 a.m. after an early dinner (at approximately 4 p.m.). On the evenings of the nighttime studies, the patients with diabetes received subcutaneous injections of regular insulin before dinner but no intermediate- or long-acting insulin and no food after dinner. Similarly, in the morning no intermediate- or long-acting insulin was given before the study. The order of the studies for each subject was random, and each study was separated from the others by an interval of at least 10 weeks.

### Procedures

To ensure a standardized hypoglycemic stimulus, we performed a one-step hypoglycemic-clamp procedure, as described by Simonson et al.,<sup>13</sup> in all the subjects. Two cannulas were inserted, one in an antecubital vein for infusion of insulin and glucose and the other in a dorsal hand vein for blood sampling. The hand was placed in a heated ( $65^{\circ}\text{C}$ ) box to "arterialize" the venous blood. The subjects were given continuous intravenous infusions of insulin ( $80$  mU per square meter of body-surface area per minute), and target plasma glucose values were achieved by varying the rate of an infusion of 20 percent glucose in water. Plasma glucose was measured at the bedside at five-minute intervals (Beckman glucose analyzer, Beckman, Fullerton, Calif., or YSI analyzer, YSI, Yellow Springs, Ohio). In the patients with diabetes, the insulin infusion was commenced at the time of the usual insulin injection (i.e., at least three to four hours before the hypoglycemic phase). In all the subjects, plasma glucose concentrations were stabilized between  $90$  and  $108$  mg per deciliter ( $5.0$  and  $6.0$  mmol per liter) before the induction of hypoglycemia. The length of this euglycemic phase varied but was at least 60 minutes in all subjects. When the subjects were asleep and had entered stage 3 or 4 non-rapid-eye-movement (REM) sleep, the plasma glucose concentration was reduced to  $50$  mg per deciliter ( $2.8$  mmol per liter) over a period of approximately 20 minutes by reducing the rate of glucose infusion. Plasma glucose concentrations were then maintained at that level for a further 40 minutes (hypoglycemic phase, 0 to 60 minutes). During all sleeping studies, sleep states were monitored continuously by polysomnography, and all studies were performed

in sleep laboratories to ensure that the subjects were not awakened during the hypoglycemic phase of the procedures.

### Measurements

Blood samples were taken at 10-to-20-minute intervals (from 60 minutes before the hypoglycemic period to 60 minutes after) for measurements of plasma free insulin, epinephrine, norepinephrine, cortisol, and growth hormone. Because plasma glucagon responses to hypoglycemia are negligible in patients with type I diabetes, plasma glucagon was measured only in the normal subjects.

Sleep was monitored by polysomnography with the use of standard electroencephalographic, electrooculographic, electromyographic, electrocardiographic, oximetric, and respiratory channels. It was classified according to the standard criteria as wakefulness, sleep stages 1 to 4, or REM sleep.

In subjects who were awake, symptoms of hypoglycemia were assessed at 15-minute intervals with a questionnaire in which the subjects were asked to rate a set of symptoms on a scale of 1 (non-existent) to 7 (extreme). The symptoms were pounding heart, shakiness, sweating, headache, difficulty thinking, and slowed thinking. The scores for the symptoms were added to give a total hypoglycemic score (possible range, 6 to 42). Heart rates were measured continuously and averaged over 30-second intervals.

### Statistical Analysis

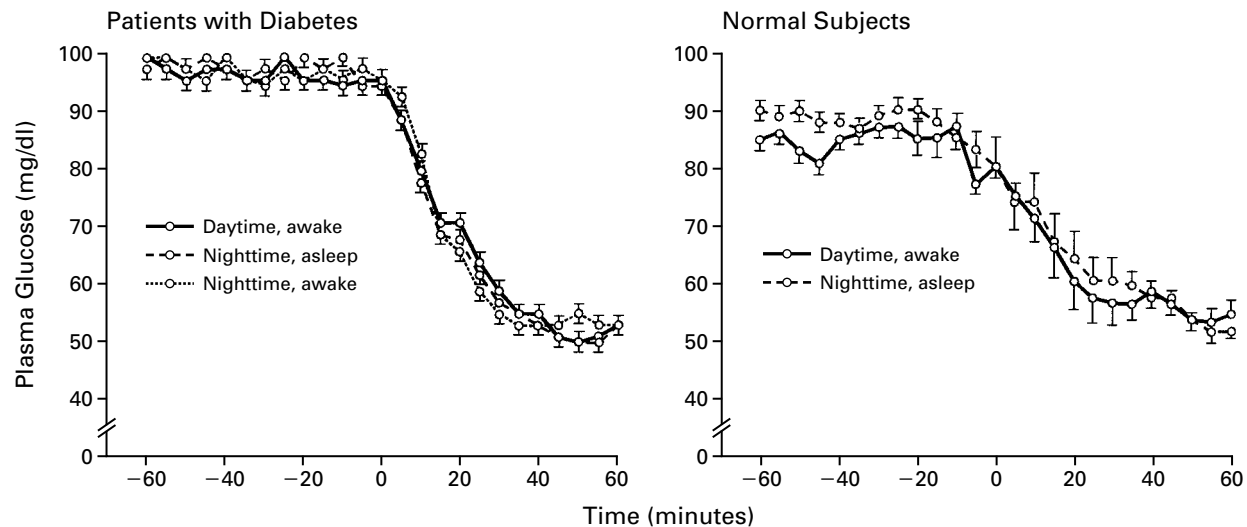
Plasma catecholamines were measured by radioenzymatic assay (Pharmacia & Upjohn, Bridgewater, N.J.) and plasma free insulin (measured after precipitation with polyethylene glycol), growth hormone, cortisol, and glucagon were measured by double-antibody radioimmunoassays. All the samples from each subject were analyzed in a single assay. The intraassay variations for the growth hormone, glucagon, and cortisol assays were  $<10$  percent, and for the epinephrine, norepinephrine, and free insulin assays they were 19, 11, and 19 percent, respectively.

The plasma glucose concentrations, hormone responses, and symptom responses in the three studies were compared by analysis of variance, with repeated-measures design when appropriate. Base-line plasma concentrations of each hormone were defined as the means of the values at  $-20$  and  $0$  minutes of the euglycemic-hyperinsulinemic phase. Post hoc analyses included one-way analysis of variance with Tukey's procedure for multiple comparisons and Student's *t*-test with Bonferroni corrections to localize effects at single points in time. All statistical tests were two-sided.

## RESULTS

### Plasma Glucose and Insulin Concentrations

The mean plasma glucose concentrations during the hypoglycemic-clamp procedures were similar in the patients with diabetes and the normal subjects (Fig. 1), and during each study the target plasma glucose concentration of  $50$  mg per deciliter was reached. In the patients with diabetes, the mean ( $\pm$ SE) plasma free insulin concentrations during the hypoglycemic phases of the daytime study with the patients awake, the nighttime study with the patients asleep, and the nighttime study with the patients awake were similar ( $52 \pm 6$ ,  $48 \pm 5$ , and  $58 \pm 9$   $\mu\text{U}$  per milliliter [ $312 \pm 36$ ,  $288 \pm 30$ , and  $348 \pm 54$  pmol per liter], respectively). The plasma free insulin concentrations in the normal subjects during the day while they were awake and during the night while they were asleep were similar ( $65 \pm 6$  and  $71 \pm 8$   $\mu\text{U}$  per milliliter [ $390 \pm 36$  and  $426 \pm 48$  pmol per liter], respectively).



**Figure 1.** Mean ( $\pm$ SE) Plasma Glucose Concentrations in Eight Patients with Type I Diabetes and Six Normal Subjects during Periods of Hypoglycemia When They Were Awake during the Day, Awake at Night, and Asleep at Night.

To convert plasma glucose values to millimoles per liter, multiply by 0.056. The zero on the x axis indicates the beginning of the hypoglycemic period.

#### Plasma Catecholamine Responses to Hypoglycemia

The plasma epinephrine concentrations did not change during euglycemic hyperinsulinemia in either group during any of the studies (Fig. 2). In the patients with diabetes, the plasma epinephrine concentrations increased during the periods of hypoglycemia during the day (from  $64 \pm 4$  to  $238 \pm 39$  pg per milliliter [ $349 \pm 22$  to  $1299 \pm 213$  pmol per liter],  $P=0.004$  for the comparison with base line) or during the night when they were awake (from  $45 \pm 6$  to  $296 \pm 60$  pg per milliliter [ $246 \pm 33$  to  $1616 \pm 327$  pmol per liter] at 60 minutes,  $P=0.004$ ). In contrast, the plasma epinephrine response to hypoglycemia was markedly blunted in the patients with diabetes when they were studied at night during deep sleep (from  $52 \pm 3$  to only  $70 \pm 14$  pg per milliliter [ $284 \pm 16$  to  $382 \pm 76$  pmol per liter] at 60 minutes,  $P=0.30$  for the comparison with base line). Similarly, the mean plasma epinephrine concentration increased to  $449 \pm 52$  pg per milliliter ( $2451 \pm 284$  pmol per liter,  $P=0.005$  for the comparison with base line) in the normal subjects during the periods of hypoglycemia when they were awake but to only  $86 \pm 49$  pg per milliliter ( $469 \pm 267$  pmol per liter) when they were asleep ( $P=0.30$ ).

In the patients with diabetes, the mean plasma norepinephrine concentrations increased slightly during both studies with the patients awake but decreased slightly when hypoglycemia was induced during sleep at night (Fig. 3). The plasma norepinephrine concentrations did not change during either study in the normal subjects.

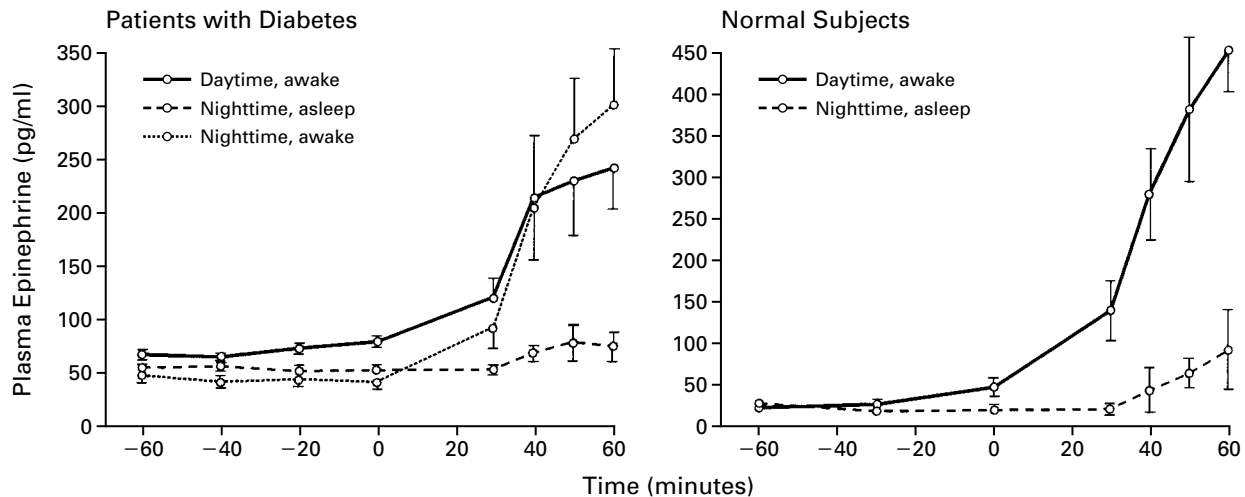
#### Plasma Cortisol, Growth Hormone, and Glucagon Responses to Hypoglycemia

In the patients with diabetes, the plasma cortisol concentrations were higher during the daytime study than during the two nighttime studies — a finding consistent with the diurnal variation in cortisol secretion (Fig. 3). Plasma cortisol concentrations did not increase during hypoglycemia when the patients were asleep at night (Fig. 3) ( $P=0.60$ ) but did increase during the periods of hypoglycemia when they were awake during the day or night ( $P=0.02$ ). The results were similar in the normal subjects, with the plasma cortisol concentrations increasing during hypoglycemia when the subjects were awake during the day ( $P=0.02$ ) but not when they were asleep at night ( $P=0.60$ ). In contrast, plasma growth hormone concentrations increased when hypoglycemia was induced during both sleep and wakefulness in both the patients with diabetes and the normal subjects (Fig. 3).

In the normal subjects, plasma glucagon responses increased during the periods of hypoglycemia, both during the day and during the night (from  $44 \pm 8$  to  $102 \pm 15$  pg per milliliter and from  $46 \pm 7$  to  $82 \pm 8$  pg per milliliter;  $P=0.001$  and  $P=0.007$ , respectively, for the comparisons with base line).

#### Symptoms

Both the patients with diabetes and the normal subjects had increases in hypoglycemic scores during the final 30 minutes of the studies with the patients awake ( $P=0.04$  for the comparison with base line,



**Figure 2.** Mean ( $\pm$ SE) Plasma Epinephrine Concentrations in Eight Patients with Type I Diabetes and Six Normal Subjects during Periods of Hypoglycemia When They Were Awake during the Day, Awake at Night, and Asleep at Night.

To convert plasma epinephrine values to picomoles per liter, multiply by 5.458. The zero on the x axis indicates the beginning of the hypoglycemic period.

data not shown). For the nighttime studies during sleep, polysomnography confirmed that all the subjects had achieved stage 3 or 4 sleep at the time of the onset of hypoglycemia, and no subjects awakened during the procedure. The mean heart rates did not change significantly during the periods of hypoglycemia when the subjects were asleep (at base line,  $84 \pm 6$  beats per minute; during the final 10 minutes of hypoglycemia during sleep,  $81 \pm 7$  beats per minute), or awake (at base line,  $86 \pm 5$  beats per minute; during the final 10 minutes of hypoglycemia,  $92 \pm 6$  beats per minute).

### DISCUSSION

We found that patients with diabetes have impaired plasma epinephrine responses to hypoglycemia when they are asleep. This impairment would be expected to make them more susceptible to hypoglycemia during that time, because a rise in plasma epinephrine is their main hormonal defense against hypoglycemia. To limit potential confounding factors, we studied only young patients who had had diabetes for a few years and who had no autonomic neuropathy, recent severe hypoglycemia, or even mild hypoglycemia on the day before each study. Moreover, we used the same hypoglycemic stimulus during all studies. Sleep had the same adverse effect on the plasma epinephrine responses of the normal subjects, indicating that the defect in adrenomedullary responsiveness was due to the sleep state itself.

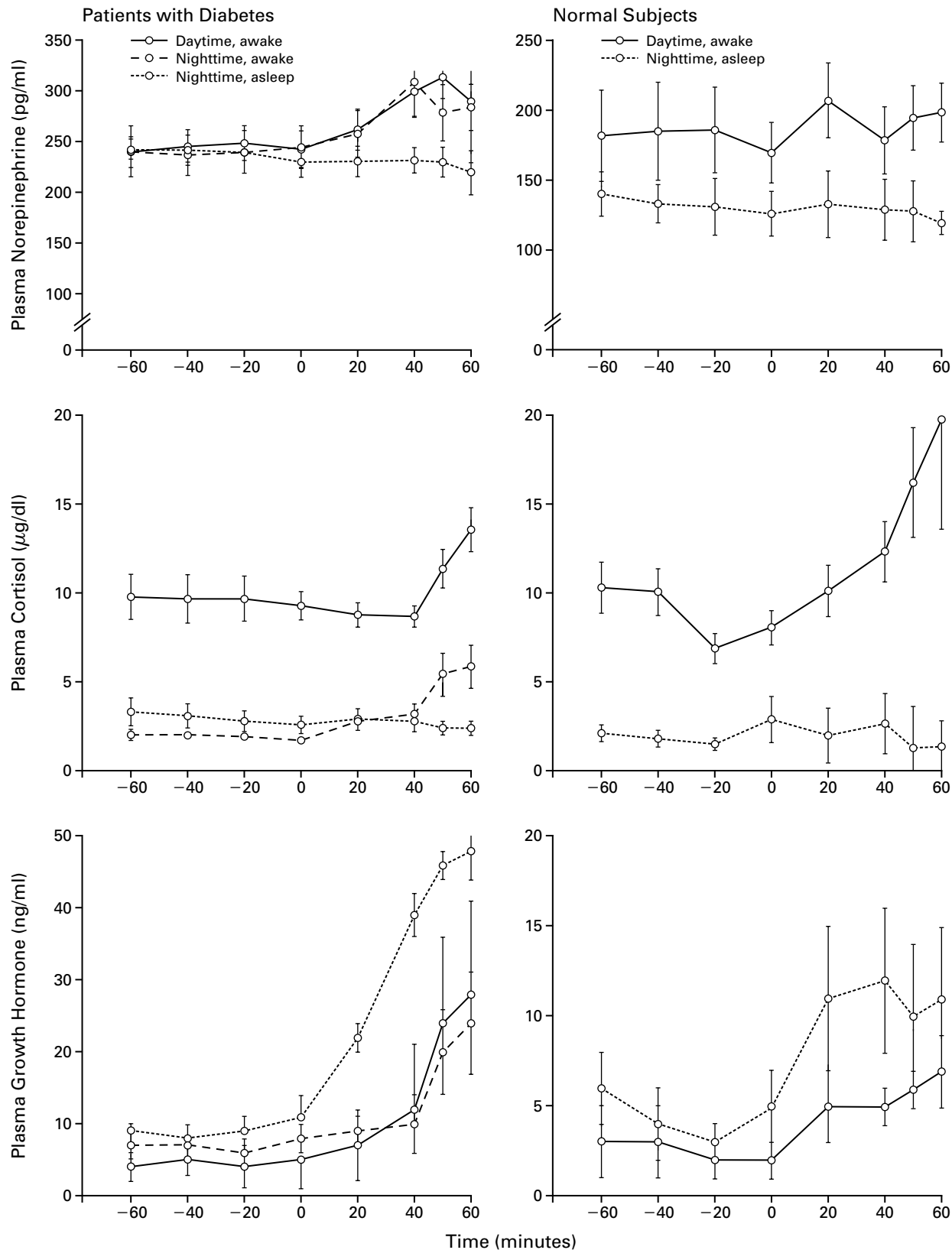
The increase in plasma norepinephrine concentrations (an index of central sympathetic activation) during hypoglycemia was less during sleep in the pa-

tients with diabetes, but the magnitude of the differences in these concentrations was much less than that of the differences in plasma epinephrine concentrations. This is not surprising, because changes in plasma norepinephrine concentrations during hypoglycemia do not fully reflect the amount of norepinephrine released at peripheral neuroeffector junctions after central sympathetic stimulation.<sup>14</sup> Other indexes of central sympathetic activity, such as heart rate, systemic blood pressure, and peripheral vascular resistance, are also reduced during the deeper stages of non-REM sleep.<sup>15</sup> Thus, the impairment of catecholamine responses to hypoglycemia may be part of a general reduction in sympathetic activity that occurs in stages 3 and 4 of non-REM sleep.<sup>16,17</sup> This sleep stage predominates during the first third of the nighttime-sleep cycle, the time when patients with diabetes are most prone to severe hypoglycemia.

Few studies have addressed the issue of diurnal variations in catecholamine release in patients with diabetes. In one study of 10 patients, the plasma catecholamine responses to hypoglycemia were similar when the patients were awake during the day and

**Figure 3.** Mean ( $\pm$ SE) Plasma Norepinephrine, Cortisol, and Growth Hormone Concentrations in Eight Patients with Type I Diabetes and Six Normal Subjects during Periods of Hypoglycemia When They Were Awake during the Day, Awake at Night, and Asleep at Night.

To convert plasma norepinephrine values to picomoles per liter, multiply by 5.911, and to convert plasma cortisol values to nanomoles per liter, multiply by 27.59. The zero on the x axis indicates the beginning of the hypoglycemic period.



asleep at night; however, the hypoglycemia was severe (glucose concentration, 27 mg per deciliter [1.5 mmol per liter]).<sup>18</sup> In another study of 22 patients, plasma catecholamine concentrations increased during hypoglycemia at night but were measured only at two-hour intervals, and the sleep stage was not reported. In addition, no daytime studies were done.<sup>19</sup> In both these reports the hypoglycemic stimulus varied, making the results difficult to compare with those of our study, in which a uniform degree of hypoglycemia was produced by using the glucose-clamp technique.

Nocturnal hypoglycemia has serious implications for patients with diabetes. Hypoglycemia is more likely to cause seizures during the night than during the day.<sup>2,9</sup> Perhaps as important, asymptomatic nocturnal hypoglycemia may itself result in further deficits in counterregulatory-hormone responses.<sup>20</sup> Thus, impaired defenses against hypoglycemia during deep sleep may contribute to the vicious cycle of hypoglycemia, impaired counterregulatory-hormone responses, and unawareness of hypoglycemia while patients are awake or asleep.

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