Counterregulation During Spontaneous Nocturnal Hypoglycemia in Prepubertal Children With Type 1 Diabetes

KRYSTYNA A. MATYKA, MRCP
ELIZABETH C. CROWNE, MD
PETER J. HAVEL, PHD
IAN A. MACDONALD, PHD
DAVID MATTHEWS, FRCP
DAVID B. DUNGER, FRCP

OBJECTIVE — To examine counterregulatory responses during spontaneous nocturnal hypoglycemia in prepubertal children with type 1 diabetes.

RESEARCH DESIGN AND METHODS — A total of 29 prepubertal patients with type 1 diabetes underwent two overnight profiles. Data were analyzed from 16 children (median [range] 8.7 [5.9–12.9] years of age) with a night of hypoglycemia and a nonhypoglycemic night. Children hypoglycemic (<3.5 mmol/l) on night 1 were given 25% extra carbohydrate as uncooked cornstarch with their usual evening snack on night 2 to avoid hypoglycemia. Glucose, growth hormone, and cortisol were measured every 15 min, catecholamines every 30 min, and glucagon, pancreatic polypeptide, insulin, and ketones every 60 min. A group of 15 healthy control subjects, aged 9.5 (5.6–12.1) years, underwent one overnight profile.

RESULTS — Median duration of hypoglycemia was 225 (30–630) min, and glucose nadir was 2.0 (1.2–3.3) mmol/l. Insulin levels were not different on the two nights ($P = 0.9$, analysis of variance), but children with diabetes had higher insulin levels than normal control subjects between 2300 and 0300, maximal at 0200 (mean ± SEM 57.4 ± 5.7 vs. 31.6 ± 5.0 pmol/l, $P = 0.002$). Peak epinephrine was higher on the night of hypoglycemia (0.98 [0.52–2.09] nmol/l) versus nonhypoglycemia (0.32 [0.21–0.62] nmol/l), $P = 0.001$, but norepinephrine (1.29 [1.07–2.64] vs. 1.26 [1.04–1.88] nmol/l, $P = 0.5$), glucagon (93 [64.2–125.6] vs. 100.5 [54.6–158] ng/l, $P = 0.6$), pancreatic polypeptide (410.2 [191–643.2] vs. 270.8 [158.2–777.8] ng/l, $P = 0.5$), and cortisol (513 [300–679] vs. 475 [235–739] nmol/l, $P = 0.6$) were not different. Glucose threshold for epinephrine release was very low, 1.9 ± 0.2 mmol/l. There was a short-lived rise in growth hormone from 75–105 min after onset of hypoglycemia, maximal at 90 min (7.8 ± 1.2 vs. 3.5 ± 0.9 ng/ml, $P = 0.02$).

CONCLUSIONS — The prolonged nature of nocturnal hypoglycemic episodes may be explained in part by defective counterregulation. The risk of nocturnal hypoglycemia needs to be reduced before intensification of insulin therapy can be contemplated in this age-group.

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Hypoglycemia continues to be a serious hazard for all patients with type 1 diabetes and is the major obstacle in the achievement of tight glycemic control (1). The main defense against a severe episode of hypoglycemia, resulting in coma or convulsion, is the adequate recognition of falling glucose levels (2). This recognition relies on an intact counterregulatory defense mechanism, usually generating an autonomic symptom complex that alerts the individual to the falling glucose.

Children appear to be at greater risk of severe hypoglycemia (3,4). The precise reason for this is unclear, although it may in part represent the inability of young children to express autonomic symptoms to the same degree (5). There have been few studies of glucose counterregulation in childhood, and most of these have been in the adolescent population. The results have shown both exaggerated hormone release (6,7) and blunted responses (8,9), differences which may be explained by different methods of inducing hypoglycemia (insulin infusion or hyperinsulinemic clamp).

Nocturnal hypoglycemia is a special problem. It is common in children and adults with type 1 diabetes (10–13), yet, despite many episodes being profound and prolonged, the majority are asymptomatic, even in individuals with good awareness of hypoglycemia during waking hours (14). The possibility of modified counterregulatory responses during sleep, resulting in defective nighttime awareness, has been little studied, and the results so far are conflicting. Nocturnal hypoglycemia in adults provoked greater catecholamine release than daytime hypoglycemia (14), whereas adolescents studied during slow wave sleep were found to have significantly blunted catecholamine responses (15).

Nocturnal hypoglycemia is more common in young prepubertal children (12,16) than in adolescents, and younger children may also be more vulnerable to any long-term adverse effects of hypoglycemia (17). The possibility that impaired counterregulatory responses contribute to this serious problem has been little studied. We report a study performed in a group of prepubertal children with type 1 diabetes to examine the counterregulatory hormone responses to spontaneous overnight hypoglycemia.

RESEARCH DESIGN AND METHODS

— Studies were approved by the Central Oxford Research Ethics Committee, and consent was obtained from the parents, once assent had been obtained from the children.

From the Department of Paediatrics (K.A.M., E.C.C., D.B.D.) and the Diabetes Research Laboratories (D.M.), John Radcliffe Hospital, Oxford; the School of Biomedical Sciences (I.A.M.), Nottingham, U.K.; and the Department of Nutrition (P.J.H.), University of California–Davis, Davis, California.

Address correspondence and reprint requests to Dr. D. Dunger, Department of Paediatrics, John Radcliffe Hospital, Headington, Oxford OX3 9DU, U.K. E-mail: david.dunger@paediatrics.ox.ac.uk.

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Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; GH, growth hormone; HPLC, high-performance liquid chromatography; PP, pancreatic polypeptide.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Demographic details of diabetic and nondiabetic participants*

	Type 1 diabetic subjects	Control subjects
n	16	15
Age (years)	8.7 (5.9 to 12.9)	9.5 (5.6 to 12.1)
HbA_{1c} (%)	8.7 (6.9 to 10.3)	5.3 (5.1 to 5.7)
Duration (years)	3.1 (1.1 to 10.2)	—
Insulin dose ($U \cdot kg^{-1} \cdot day^{-1}$)	0.8 (0.5 to 1.1)	—
BMI SD	0.34 (−1.14 to 1.89)	−0.48(−1.48 to 2.00)
Positive C-peptide status	4	—

Data are *n* or medians (range).

Children were recruited from the pediatric diabetic clinic of the John Radcliffe Hospital, Oxford, U.K., on the basis of their willingness to participate. Prepubertal children over the age of 5 years, with a diabetes duration of >12 months and with no other significant medical condition, were eligible to participate. Of a total of 52 eligible children, 29 were studied. All children were on conventional insulin regimens, receiving twice-daily mixtures of soluble and isophane insulin.

A group of 15 healthy children, siblings or friends of children attending the diabetic clinic, were also studied to provide normal overnight metabolic data (Table 1).

Studies on the children with type 1 diabetes took place in their home following a normal day's routine as regards diet, activity, and dose of insulin. Two overnight metabolic profiles were performed 1–2 weeks apart. At 1930, an intravenous cannula was sited on the dorsum of the hand, and patency was maintained with heparinized normal saline. Samples for HbA_{1c} were taken at 2000 and for C-peptide at 0700. Venous samples were taken in the home for glucose every 15 min between 2000–0800, but analyzed the following day. Hypoglycemia was defined as a blood glucose <3.5 mmol/l on two successive 15-min measurements.

Of the 29 children, 16 had a night of hypoglycemia and a night of nonhypoglycemia for comparison. Characteristics of these children are summarized in Table 1. Ten were hypoglycemic on study night 1 and were given 25% extra carbohydrate in the form of uncooked cornstarch with their usual evening snack on night 2.

Blood was taken for growth hormone (GH) and cortisol every 15 min, and for free insulin and ketones every 60 min. Eight of these children had samples analyzed for glucagon and pancreatic polypeptide (PP); selection was purely on the basis of sample availability.

The final eight children to take part in the study also had blood taken for catecholamines, and comparison between hypoglycemic ($n = 7$) and nonhypoglycemic nights ($n = 9$) were not in the same children.

In the 15 children without diabetes, samples were taken for GH and glucose every 15 min and insulin and ketones every 60 min. Seven of these children also had blood taken for catecholamines every 30 min, for glucagon every 60 min, and for cortisol every 120 min.

Biochemical analysis

Samples for measurement of blood glucose were collected into fluoride tubes and stored at room temperature over-night. Whole blood glucose was measured using a glucose oxidase method (YSI analyzer; Clandon Scientific, Farnborough, Hampshire, U.K.). HbA_{1c} was measured by high-performance liquid chromatography (HPLC) (Diamat, Bio-Rad, Hemel Hempstead, U.K.), normal range 4.3–6.1%. The intra-assay coefficients of variation were 1.9 and 2.2% at HbA_{1c} levels of 6.9 and 11.5%, respectively. The interassay coefficients of variation were 2.7 and 2.3% at HbA_{1c} levels of 7.0 and 11.6%, respectively.

Samples for GH analysis were initially kept at room temperature until completion of each study, before being spun and separated for storage at −20°C. Plasma GH was then measured by immunoradiometric assay (NETRIA, St. Bartholomew's Hospital, London, U.K.) using an international reference standard 80/505. All samples from each individual were analyzed in the same batch. The intra-assay coefficients of variation at GH concentrations of 1.1, 5.6, and 27.1 ng/ml were 8.0, 2.0, and 3.4%, respectively, while interassay coefficients of variation at GH concentrations of 1.4, 5.9, and 30.2 ng/ml were 9.4, 7.7, and 10.5%, respectively.

Plasma glucagon was measured by radioimmunoassay using guinea pig anti-human glucagon antibodies specific for pancreatic glucagon, ^{125}I-labeled glucagon as tracer, and glucagon standards, with reagents supplied by Linco Research, St. Charles, MO. Inter- and intra-assay coefficients of variation were 5–10%.

PP was assayed by double antibody radioimmunoassay using rabbit anti-human PP antibodies, ^{125}I-labeled human PP, and human PP standard with reagents supplied by Linco Research.

For catecholamine analysis, samples were added to EGTA/glutathione preservative, spun for 10 min, and then separated immediately before being stored at −80°C. Epinephrine and norepinephrine were extracted from 50-µl samples of plasma with an organic solvent method and separated and quantified by HPLC with electrochemical detection (18). Intra-assay coefficients of variation were 4% for norepinephrine and 6% for epinephrine; interassay values were 6 and 8%, respectively.

Free insulin was measured by first mixing 1.0 ml of whole blood into a 25% solution of polyethylene glycol 6000 (Merck, Dorset, U.K.) to remove insulin antibodies (19). This was then centrifuged and the supernatant separated and stored at −20°C until assayed with a double antibody radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX). The intra-assay coefficients of variation were 8.2, 4.8, and 6.3% at 29, 106, and 328 pmol/l, respectively. The interassay coefficients of variation were 11.2, 9.2, and 4.7% at 30, 97, and 318 pmol/l, respectively. Polyethylene glycol was not added to samples from nondiabetic children.

Cortisol was measured using a coated tube radioimmunoassay (DPC, Llanberis, U.K.). The intra-assay coefficient of variation at levels of 86, 552, and 938 nmol/l was 4.8, 3.0, and 4.4%, respectively. The interassay coefficient of variation at levels of 91, 579, and 993 nmol/l was 5.2, 4.0, and 6.4%, respectively.

Ketones (acetoacetate and β-hydroxybutyrate) were assayed using a standard technique (20,21) after the addition of ice-cold 10% perchloric acid and separation of samples.

Data analysis

Overnight comparisons were made using analysis of variance (ANOVA) for repeated measures using log-transformed data. Peak data were defined as the maximal level

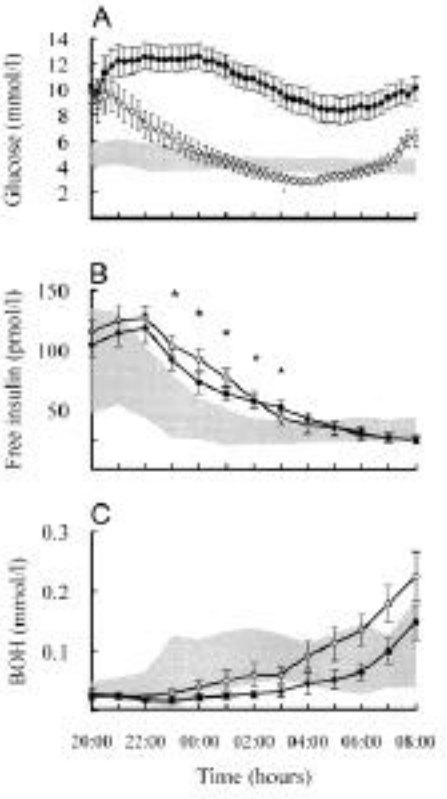

Figure 1—*A: Glucose profiles on the night of hypoglycemia (○) and the night without (●). The gray shaded area represents the range of glucose values from the overnight profiles of children without diabetes. B and C: Insulin and β-hydroxybutyrate (BOH) profiles on the night of hypoglycemia (○) and the night without (●). The gray shaded area represents the 95% CIs of the values from the children without diabetes. *Significant difference (P < 0.05) at these time points between the values in children with and those without diabetes.*

achieved overnight and area under the curve (AUC) data were calculated using the trapezoidal method. Comparisons between the night of hypoglycemia and the nonhypoglycemic night were made using nonparametric, paired analyses except in the case of catecholamines, where unpaired analyses were used. Data were also compared with data obtained from the nondiabetic children.

Serial array averaging was used to examine the effect of the onset of hypoglycemia on the release of cortisol and GH for the subsequent 2 h, in 16 paired profiles. The point of hypoglycemia was identified as the time at which the glucose level first fell below 3.5 mmol/l, and a comparison was made of successive 15-min values during hypoglycemia, and the corresponding time points from the night without hypoglycemia, using *t* tests. A glucose threshold for epinephrine release was also calculated, this being defined as the glucose level at which epinephrine rose by >2 SD above basal levels.

Data are shown as medians (range) or means ± SEM, unless otherwise stated. A *P* value <0.05 was taken to be significant. The computer program SPSS 7.5 for Windows 95 was used for statistical analyses.

RESULTS

Glucose

Mean blood glucose levels were 5.2 ± 0.5 mmol/l during the night of hypoglycemia and 10.3 ± 0.8 mmol/l during the nonhypoglycemic night in 16 children where paired comparisons were possible (Fig. 1*A*). The median glucose nadir was 2.0 (range 1.2–3.3) mmol/l on the night of hypoglycemia. The median duration of these paired episodes of hypoglycemia was 225 (30–630) min. The median time of glucose nadir was 0345 (2145–0645), and the median time of onset of hypoglycemia was 0115 (2100–0515).

Despite a slight fall in glucose levels during the early part of the night, none of the nondiabetic children had a blood glucose <3.5 mmol/l overnight (mean ± SEM, 4.3 ± 0.1 mmol/l).

Free insulin

Free insulin levels were not significantly different between the night of hypoglycemia and the night without using either ANOVA (*P* = 0.9) or AUC (*P* = 0.4). Using unpaired comparisons of insulin data at matched time points, insulin levels were significantly higher in children with diabetes on both nights when compared with normal control subjects between 2300–0300 (Fig. 1*B*).

Median levels of insulin at the time of onset of hypoglycemia were not different from those obtained at the same time point on the night without hypoglycemia (95.4 [range 17.9–211.7] vs. 68.2 [17.9–137.0] pmol/l, *P* = 0.06). During six episodes of hypoglycemia, low glucose levels persisted for 245 ± 41 min despite insulin levels at the lower limit of detection of the assay (17.9 pmol/l).

Ketones

Only β-hydroxybutyrate will be presented because acetoacetate results were similar. Ketone levels were suppressed at the beginning of the night but increased as insulin levels waned (Fig. 1*C*). There were no overnight differences in β-hydroxybutyrate between the night of hypoglycemia and the night without, as defined by ANOVA, *P* = 0.08. However, peak values were significantly higher on the night of hypoglycemia, *P* = 0.01 (Table 2), and the AUC data were also significantly greater, *P* = 0.01 (Table 3).

Comparing data from the night of hypoglycemia with data obtained from the nondiabetic control subjects, there were no significant differences in either peak levels or AUC.

GH

Mean overnight levels of GH were similar on the night of hypoglycemia and the night

Table 2—*Peak responses*

	n	Hypoglycemia	No hypoglycemia	Control subjects
Epinephrine (nmol/l)	8	0.98 (0.52–2.09)*	0.32 (0.21–0.62)	0.52 (0.13–1.91)
Norepinephrine (nmol/l)	8	1.29 (1.07–2.64)	1.26 (1.04–1.88)	1.59 (0.71–2.58)
Glucagon (ng/l)	8	93 (64.2–125.6)	100.5 (54.6–158)	76.5 (60.3–81.3)
PP (ng/l)	8	443.6 (191–643.2)	257.6 (158.2–777.8)	—
Cortisol (nmol/l)	16	513 (300–679)	475 (235–739)	477 (384–552)
β-hydroxybutyrate (mmol/l)	16	0.29 (0.04–0.53)†	0.12 (0.02–0.44)	0.13 (0.03–0.51)
Acetoacetate (mmol/l)	16	0.14 (0.02–0.28)‡	0.09 (0.03–0.21)	0.10 (0.03–0.27)

Data are *n* or medians (range). *n* = number of children with type 1 diabetes in whom sample analysis was possible. **P* = 0.001, †*P* = 0.01, ‡*P* = 0.02 for hypoglycemia vs. no hypoglycemia.

Table 3—*AUC data*

	n	Hypoglycemia	No hypoglycemia	Control subjects
Epinephrine (nmol · l^{-1} · 12 h)	8	277 (121–357)*	132 (89–245)	150 (84–244)
Norepinephrine (nmol · l^{-1} · 12 h)	8	747 (662–874)*	628 (533–746)	772 (354–993)
Glucagon (ng · l^{-1} · 12 h)	8	44,385 (36,579–66,474)	50,118 (28,632–81,063)	42,980 (38,676–47,790)
PP (ng · l^{-1} · 8 h)	8	87,828 (57,516–108,042)	64,074 (50,004–172,176)	—
Cortisol (nmol · l^{-1} · 12 h)	16	144,330 (83,040–274,920)	148,140 (86,940–211,440)	126,540 (101,700–163,980)
β-hydroxybutyrate (mmol · l^{-1} · 12 h)	16	50.1 (12.0–132.6)*	26.2 (7.1–80.5)	42.5 (3.8–155.6)
Acetoacetate (mmol · l^{-1} · 12 h)	16	52.1 (9.3–98.9)*	39.6 (15.8–61.2)	47.4 (11.8–114.4)

Data are *n* or medians (range). *n* = number of children with type 1 diabetes in whom sample analysis was possible. *$P < 0.01$ for hypoglycemia vs. no hypoglycemia.

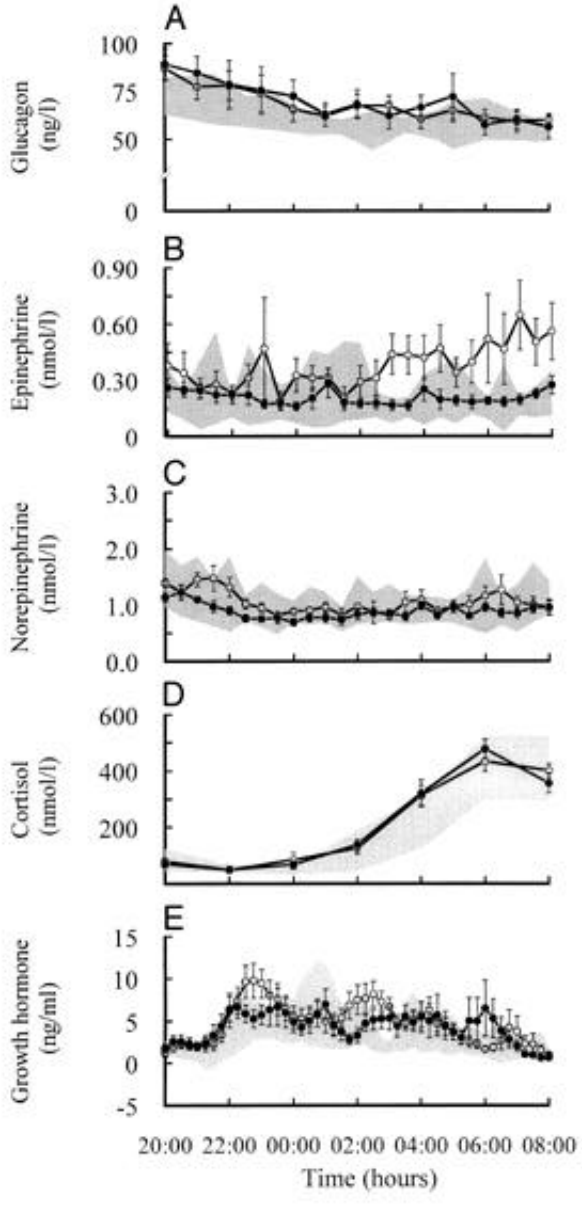

Figure 2—*Counterregulatory hormone profiles for glucagon* (A)*, epinephrine* (B)*, norepinephrine* (C)*, cortisol* (D)*, and GH* (E) *on the night of hypoglycemia* (○) *and the night without* (●)*. The gray shaded areas represent the 95% CIs of the values from the children without diabetes.*

without hypoglycemia (4.8 ± 0.5 vs. 4.3 ± 0.5 ng/ml, $P = 0.3$) (Fig. 2*E*). Furthermore, mean levels from the night without hypoglycemia and mean levels from the nondiabetic control subjects were not significantly different (4.3 ± 0.5 vs. 3.5 ± 0.3 ng/ml, $P = 0.2$).

When analyzed by serial array averaging, a small rise in GH levels was detected 75–105 min following the onset of hypoglycemia (difference maximal at 90 min, 7.8 ± 1.2 vs. 3.5 ± 0.9 ng/ml, $P = 0.02$), and levels remained higher until 105 min (Fig. 3*A*). After this time, values were similar to that on the night without hypoglycemia.

Cortisol

There were no differences in cortisol levels between the night of hypoglycemia and the night without hypoglycemia (Fig. 2*D*) when analyzed by ANOVA, AUC, or peak levels (Tables 2 and 3). When comparing data from the night without hypoglycemia with that obtained from the nondiabetic control subjects, there were no significant differences in either peak levels or AUC ($P = 0.5$ and 0.3, respectively). No change in cortisol levels after hypoglycemia was detected by serial array averaging (Fig. 3*B*).

Glucagon

There were no significant increases in glucagon during hypoglycemia (Fig. 2*A*) detected either by ANOVA ($P = 0.7$), peak levels achieved ($P = 0.9$) (Table 2), or AUC values ($P = 0.4$) (Table 3). Generally, absolute levels were not different when compared with nondiabetic children.

PP

Fasting PP levels were analyzed (fasting values taken from the midnight sample). There were no significant differences between the night of hypoglycemia and the nonhypoglycemic night, by ANOVA ($P = 1.0$), by peak levels ($P = 0.5$), or by AUC ($P = 1.0$) (Tables 2 and 3).

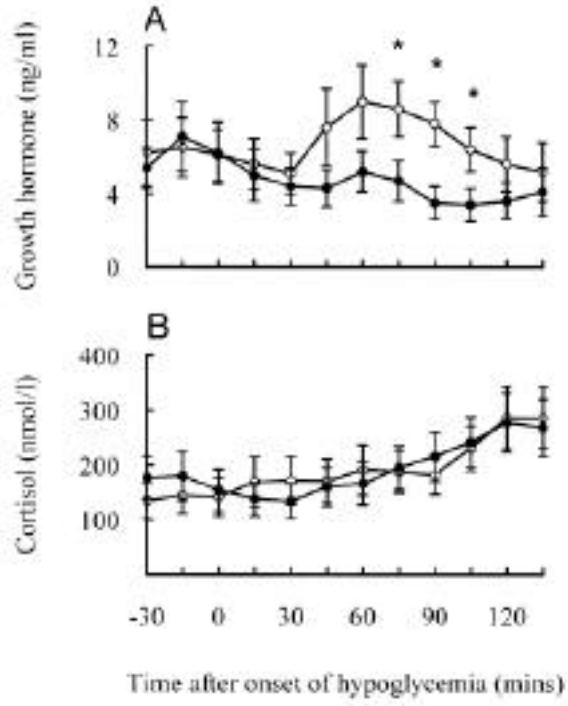

Figure 3—*Serial array averaging for consecutive 15-min values for GH (A) and cortisol (B) after onset of hypoglycemia (0 min). ○, night of hypoglycemia; ●, night without. *Significant difference (P < 0.05) at these time points between hypoglycemia and no hypoglycemia.*

Catecholamines

Peak epinephrine levels (Fig. 2*B*) were significantly higher on the night of hypoglycemia (0.98 [0.52–2.09] nmol/l) than on the nonhypoglycemic night (0.32 [0.21–0.62] nmol/l) (P = 0.001), with a mean increment from baseline of 0.9 ± 0.3 nmol/l (Table 2). Although peak levels after hypoglycemia were higher than those observed in the nondiabetic control subjects, this difference did not achieve statistical significance (0.98 [0.52–2.09] vs. 0.52 [0.13–1.91] nmol/l, P = 0.09).

The mean glucose threshold for significant epinephrine release was 1.9 ± 0.2 mmol/l. The time between the glucose falling below 3.5 mmol/l and a significant increment in epinephrine (>2 SD above baseline) was 170 ± 24 min. No correlation was observed between changes in epinephrine and subsequent change in blood glucose.

Although no significant differences in norepinephrine profiles were observed by ANOVA and peak levels were no different (Table 2) (Fig. 2*C*), the AUC was significantly higher on the night of hypoglycemia when compared to the night without (Table 3).

CONCLUSIONS — In this study, we found a high prevalence of severe hypoglycemia of long duration in young children on conventional insulin regimens. Insulin levels were higher in the middle of the night than those seen in nondiabetic control subjects, reflecting the kinetics of intermediate-acting insulins given in the evening (22). Hypoglycemia also persisted even when insulin levels waned, suggesting defective counterregulation. Ketone levels were suppressed in the early part of the night, when insulin levels were high, and then rose gradually in the early hours of the morning as insulin levels waned. Although peak ketone levels were higher following a night of hypoglycemia, they were not significantly different from those observed in control subjects. This lack of a significant rise also suggests defective counterregulation.

We examined counterregulatory responses during these episodes of spontaneous nocturnal hypoglycemia. Blood was taken for catecholamimes only on the last eight children to take part in the study, so paired comparison of these data was not possible. Although we found a significant rise in epinephrine in response to hypoglycemia, the mean peak response of 0.98 nmol/l was small when compared to previous studies (6,7). In one of those studies, in which a hyperinsulinemic clamp was used to induce hypoglycemia, peak epinephrine levels were threefold greater than those found in our study (6). In that study, both pubertal and prepubertal children were studied, and epinephrine was still higher when their data were analyzed separately. In contrast, a more recent study of adolescents, again using a hyperinsulinemic clamp, found that increases in epinephrine were significantly lower when compared with the nondiabetic control subjects, although the increment of 1.9 nmol/l was still greater than that found in our subjects (9). These discrepancies could be explained by the younger age of our study group, because peak epinephrine responses have been found to increase with the age of the child (8). In the only study of pre–school-age children experiencing recurrent severe hypoglycemia, epinephrine responses were again higher than those found in our study, with an incremental value of 2 nmol/l (23). In addition to the low peak levels, the glucose threshold for epinephrine release was 1.9 mmol/l, which is much lower than the previously reported threshold of 3.6 mmol/l found in healthy children and the threshold of 3.0 mmol/l for adults from the same study (24). It is possible that the differences seen in our study, compared with the previous studies quoted, may have been the spontaneous nature of the episodes we studied. We also found a marked time delay between the glucose falling below 3.5 mmol/l and the rise in epinephrine, 170 min, in contrast to the normal rapid response in stepped hyperinsulinemic-hypoglycemic clamp studies. The markedly blunted and delayed epinephrine responses to hypoglycemia in our prepubertal subjects suggests that counterregulatory failure may be responsible for the lack of glucose recovery overnight.

Glucagon responses to hypoglycemia have been found to be defective in adults within 2–5 years of the development of type 1 diabetes, irrespective of the presence of other counterregulatory or autonomic abnormalities; the precise reason for this is unclear (25,26). We also found no significant change in glucagon even following profound nocturnal hypoglycemia, and this is in agreement with other studies in children. Blunted glucagon responses have been consistently reported (6,7) even in the youngest children with the shortest duration of diabetes (23). There is evidence that autonomic activation contributes to the glucagon response to hypoglycemia in the absence of diabetes (27,28). Because our children have impaired autonomic responses, as evi-

denced by the reduced epinephrine (sympathetic) and blunted PP (parasympathetic) responses, it is possible that this is one reason why glucagon levels do not increase, despite profound hypoglycemia (27). The autonomic impairment is likely to be a result of previous hypoglycemia (29) rather than autonomic neuropathy, in view of the short duration of diabetes.

Glucagon and epinephrine are the first-line hormones in the acute recovery from hypoglycemia, whereas GH and cortisol are considered to be of lesser importance in the hierarchy of counterregulatory hormones unless hypoglycemia is prolonged (2). In our study we found no significant changes in cortisol despite prolonged hypoglycemia. Previous studies have indicated variable cortisol responses, with reports of similar increases in children with diabetes and control subjects (6,7), smaller increases (9), and no increases in either children with diabetes or control subjects (23). Given the lack of increase in cortisol levels and the presence of other evidence suggestive of defective counterregulation, it was surprising to find a small rise in GH in response to hypoglycemia. However, the magnitude and short duration of the response indicate that it is unlikely to be effective in restoring glucose levels. In previous studies of counterregulation, the GH response to hypoglycemia was preserved in subjects with diabetes (6,7,9,23). In one study of 24-h profiles in children with diabetes, GH responses were in fact greater when hypoglycemia occurred during the night than during the day, although some of this difference may relate to the fact that daytime episodes were symptomatic and therefore treated (30).

There may be a number of reasons to explain the differences we have found in our study group compared with that found in previous studies. We considered the possibility that the 16 children who had one night of hypoglycemia were not representative of the group as a whole; thus we compared mean hormone responses on the night without hypoglycemia in these children with responses in the 9 children who did not become hypoglycemic on either night (the remaining children had two nights of hypoglycemia). We found no significant differences in mean ketone, insulin, cortisol, or GH levels between the two groups or the nondiabetic control subjects (data not shown). A potential weakness of our study is the lack of control data for normal counterregulatory responses to hypoglycemia in these young children. Ethical considerations would prevent the study of induced hypoglycemia in these young children, and so we have relied on data comparisons from other studies (6–9,23,24). In this respect it is important, when comparing our results with previous studies of hypoglycemia in children, to consider that all episodes occurred at night while the children were asleep. Bendtson et al. (14) examined the possibility that hormonal responses to hypoglycemia are altered by sleep, which could provide an explanation for the lack of symptoms of hypoglycemia occurring during sleep. They found that hormonal responses to induced hypoglycemia were either similar or greater at night than during the day, although they did not control for the stage of sleep at the time of hypoglycemia. However, Jones et al. (15) found significantly diminished epinephrine responses during hypoglycemia induced during stage 3/4 sleep when compared with hypoglycemia either during the day or at night when the patient was awake. Interestingly, they also found preserved GH responses to hypoglycemia, even during sleep, despite blunting of cortisol and glucagon. In our study, the wide variability in the time of onset of hypoglycemia and the long duration of the episodes make it difficult to interpret hormonal responses in relation to sleep staging. Nevertheless, the possibility that counterregulation may be entrained to sleep stage, reflecting altered cerebral metabolism at different times of the night (31), remains an interesting question that deserves further investigation.

The possible defective counterregulation we have observed in these young children may have its origins in prior episodes of hypoglycemia, occurring either during the day or at night, that had gone undetected. Studies in nondiabetic adults have found that even one episode of nocturnal hypoglycemia can lead to subsequent deficiencies in glucoregulation (29), yet this finding has not been confirmed in diabetic adults (32). It is possible that in young children, who may have a poor understanding of the practical issues surrounding their illness together with an inability to articulate their symptoms, repeated episodes of hypoglycemia that go undetected could lead to impaired counterregulation to subsequent episodes of hypoglycemia. Further study is required to study this hypothesis.

In summary, we have shown a high frequency of profound asymptomatic nocturnal hypoglycemia of long duration in young children on standard insulin therapy. Although these episodes may be precipitated by overinsulinization during the early part of the night, their prolonged nature may result from defective counterregulation. These data provide strong evidence that intensification of insulin therapy may be especially hazardous in this age-group.

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