Blunted Counterregulatory Hormone Responses to Hypoglycemia in Young Children and Adolescents With Well-Controlled Type 1 Diabetes

DIABETES RESEARCH IN CHILDREN NETWORK (DIRECNET) STUDY GROUP*

OBJECTIVE — Hypoglycemia in young children with type 1 diabetes is an acute complication of intensive insulin therapy and is commonly observed in the absence of signs or symptoms. The effect of intensive treatment and patient age on sympathoadrenal responses has not been established in youth with type 1 diabetes because of difficulties in testing procedures.

RESEARCH DESIGN AND METHODS — We developed a standardized inpatient continuous subcutaneous insulin infusion protocol to produce a progressive fall in plasma glucose concentrations in insulin pump–treated patients. Plasma glucose and counterregulatory hormone concentrations were measured in 14 young children (3 to <8 years, A1C 7.7 ± 0.6%) vs. 14 adolescents (12 to <18 years, A1C 7.6 ± 0.8%).

RESULTS — Plasma glucose decreased to similar nadir concentrations in the two groups. Four young children and four adolescents never had an epinephrine response. In the four young children and five adolescents who had a modest epinephrine response, this only occurred when plasma glucose fell to <60 mg/dl. In evaluating symptom scores, 29% of parents of young children felt that their child looked hypoglycemic, even at the lowest plasma glucose concentrations. Adolescents were better able to detect symptoms of hypoglycemia. In comparison with our data, epinephrine response to hypoglycemia in 14 nondiabetic adolescents studied at the Children's Hospital of Pittsburgh was higher.

CONCLUSIONS — These data suggest that even young children and adolescents with type 1 diabetes are prone to develop hypoglycemia-associated autonomic failure regardless of duration. Whether these abnormalities can be reversed using continuous glucose monitoring and closed-loop insulin delivery systems awaits further study.

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Severe hypoglycemia is a life-threatening complication of intensive therapy of type 1 diabetes, especially in youth. In the Diabetes Control and Complications Trial, adolescents had a higher rate of severe hypoglycemia than adults (1). Young children are at even greater risk (2) and pose a particular therapeutic dilemma, because recurrent episodes of hypoglycemia may have adverse effects on brain development, and anecdotal reports from parents indicate that hypoglycemic events are commonly observed in this age-group in the absence of any signs or symptoms. In nondiabetic children hypoglycemia triggers counterregulatory responses that include increases in plasma glucagon and epinephrine concentrations. In nondiabetic adolescents and in conventionally treated adolescents with poorly controlled type 1 diabetes, the plasma glucose threshold that stimulates an epinephrine response has been reported to be higher and the rise in epinephrine levels is greater than in nondiabetic adults (3). Because glucagon responses to hypoglycemia are lost early in the disease (4), an intact plasma epinephrine response is critical in patients with type 1 diabetes.

In adults with type 1 diabetes, the episodes of mild hypoglycemia that accompany intensive treatment induce a defect in sympathoadrenal responses that has been termed hypoglycemia-associated autonomic failure (5–8). Whether intensive treatment causes similar defects in youth with type 1 diabetes has not been well studied, in part because of difficulties in performing controlled hypoglycemia clamps in children.

We developed a continuous subcutaneous insulin infusion protocol to produce a progressive fall in plasma glucose in insulin pump–treated youth with well-controlled type 1 diabetes. Counterregulatory hormone concentrations were measured sequentially to compare the plasma glucose threshold for and magnitude of these hormone responses in young children versus adolescents. Although not strictly comparable, we also report the epinephrine responses to a similar degree of hypoglycemia in nondiabetic adolescents to provide a frame of reference to judge responses to hypoglycemia in the type 1 diabetic subjects.

RESEARCH DESIGN AND METHODS

— Subjects with type 1 diabetes were studied at the five DirecNet clinical centers. A data and safety monitoring board and institutional review boards at each center approved the study protocol and consent and assent forms. A parent or guardian and each subject aged >7 years gave written consent and assent, respectively.

Eligibility criteria

Eligibility criteria for type 1 diabetic subjects were *1*) age 3 to <8 years or 12 to <18 years, *2*) duration of type 1 diabetes of ≥1 year, *3*) use of an insulin pump, and *4*) A1C ≤10.0% (DCA2000+ analyzer;

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Siemens Healthcare Diagnostics, Indianapolis, IN). Exclusion criteria included a severe hypoglycemic event resulting in seizure or loss of consciousness in the last month, use of systemic or inhaled corticosteroids in the last month, or cystic fibrosis.

Study procedures

Subjects with type 1 diabetes were admitted to the research center on the evening before the study. An intravenous catheter was inserted in an arm vein for blood sampling. Glucose measurements were made using the One Touch Ultra meter at bedtime, 12:00 A.M., 3:00 A.M., 6:00 A.M., and 7:00 A.M. Oral carbohydrates were given to prevent low glucose levels with the goal of having the glucose values ≥110 mg/dl at 8:00 A.M. If glucose levels were high, and it was predicted that it would take a long time for the glucose level to drop to 110 mg/dl, small insulin correction doses were given.

At the start of the test, a bolus dose of insulin equal to ~1 hour of the subject's basal rate at that time was given, and the basal insulin infusion rate was increased by 25–50%. The basal insulin rate was increased further, and additional insulin doses were given, if needed, to achieve a gradual decline in plasma glucose.

Blood samples for meter glucose measurements were obtained every 15 min until the glucose value was ≤100 mg/dl and at 5- to 10-min intervals thereafter, until the end of the study. Blood samples were obtained for determination of glucose, epinephrine, norepinephrine, cortisol, glucagon, and growth hormone at baseline, when the meter glucose was between 95 and 110 mg/dl (in duplicate) and then <90, <80, <70, and <60 mg/dl. Once the glucose level fell to <60 mg/dl, intravenous glucose was given, the basal rate was returned to normal, and breakfast was eaten. An additional blood sample was collected for glucose and hormone concentrations 15 min after treatment with glucose.

At each blood sampling time, parents were asked whether their child "looked low," and adolescents were asked whether they "felt low." Parents and adolescents were masked to the meter glucose, and they ranked their response on a 4-point scale where 0/1 denoted "Not at all"/"Very little," 2 denoted "Some," and 3 denoted "Very much."

Laboratory procedures

Blood samples were frozen before shipping. Glucagon, cortisol, growth hormone, and glucose concentrations were measured at the DirecNet Central Laboratory (University of Minnesota). Glucagon was measured by a radioimmunoassay (Linco Research, St. Charles, MO) with the primary antibody from guinea pig and the secondary antibody from goat. The lower limit of detection was 20 pg/ml (6 pmol/l). Coefficients of variation (CVs) were 6.5–8.8% on three control samples. Cortisol was assayed with a competitive chemiluminescence assay (Bayer Advia Centaur; Bayer HealthCare, Diagnostics Division, Tarrytown, NY), using a polyclonal rabbit antibody and a mouse monoclonal antibody coupled with paramagnetic particles. The lower limit of detection was 0.5 μg/dl (14 nmol/l). CVs were 11–12% on two control samples. Growth hormone was measured by a sandwich chemiluminescence assay (DPC Immulite; Diagnostic Products Corporation, Los Angeles, CA). Monoclonal mouse antibody was coated on the bead with a rabbit polyclonal antibody in the reagent. The lower limit of detection was 0.1 ng/ml (4 pmol/l). CVs were 5.9–9.1%. Glucose determinations were made using a hexokinase enzymatic method in the laboratory (9,10) and measured by the meter at the bedside (5).

Epinephrine and norepinephrine concentrations were measured at the Mayo Clinic Laboratory (Rochester, MN) using a reverse-phase (C18) high-pressure liquid chromatography column to separate norepinephrine and epinephrine, which were detected coulometrically, using an ESA Coulochem II instrument. The lower limit of detection was 10 pg/ml (0.06 nmol/l and 55 pmol/l, respectively). CVs were 7–11% and 6–7% on two control samples. Catecholamine samples were collected in EDTA tubes and frozen immediately. This method was determined to not cause any difference in values versus when samples were collected in EDTA-sodium bisulfite tubes.

Nondiabetic subjects

Studies in 14 nondiabetic adolescents between 12 and 17 years of age (14.8 ± 2.1 years, mean ± SD) were performed at the Children's Hospital of Pittsburgh (Pittsburgh, PA) under a protocol approved by its institutional review board between 1999 and 2002. Subjects were studied in the morning after an overnight fast. Two intravenous catheters, one for blood sampling and one for glucose and insulin infusion, were inserted in the nondominant arm. After baseline blood samples for glucose and catecholamines were obtained, an intravenous insulin infusion was begun at a rate of 0.1 unit · kg^{-1} · h^{-1} throughout the study. Glucose levels were maintained at 103 ± 6 mg/dl for 60 min by a variable rate infusion of 20% dextrose. Subsequently, glucose levels were allowed to fall to 64 ± 4 mg/dl by decreasing the intravenous glucose infusion over a 20- to 25-min period. The hypoglycemic nadir was maintained for 60 min. Blood was withdrawn every 15 min for measurement of plasma epinephrine concentrations.

Epinephrine concentrations in these samples were originally measured using a high-pressure liquid chromatographic method on either the LCEC capsule N-46 Bioanalytical System (Lafayette, IN) or the ESA system (ESA, Chelmsford, MA). Extra plasma was frozen and stored at −70°C. A subset (n = 18) of these samples was reassayed in the DirecNet Central Laboratory. The 25th, 50th, and 75th quartile levels for the original analysis were 96, 174, and 312 pg/ml and the corresponding values from the DirecNet laboratory were 10, 73, and 186 pg/ml. In the nine samples obtained during the 1-h hypoglycemic phase of the Pittsburgh study, the original median peak plasma epinephrine concentration was 312 pg/ml (range 126–848) vs. 186 pg/ml (range 10–758) on reanalysis in the DirecNet Central Laboratory.

Statistical methods

Based on the study by Jones et al. (11), 50 subjects (25 in each of the two age-groups with type 1 diabetes) were estimated to be needed to detect, with 90% power and a type 1 error rate of 5%, a difference between the two groups in the peak epinephrine response, assuming a true mean difference of 300 pg/ml with an SD of 306 pg/ml. The study was discontinued after a preplanned interim analysis, when it was determined that a significant mean difference would be unachievable, even with a much larger sample size.

The comparison of the diabetes duration in the two age-groups was performed using a two-sample t test. Analyses involving plasma glucose concentrations used laboratory rather than meter glucose values. Time intervals between the <90 mg/dl and postglucose treatment blood samplings were compared using a paired t

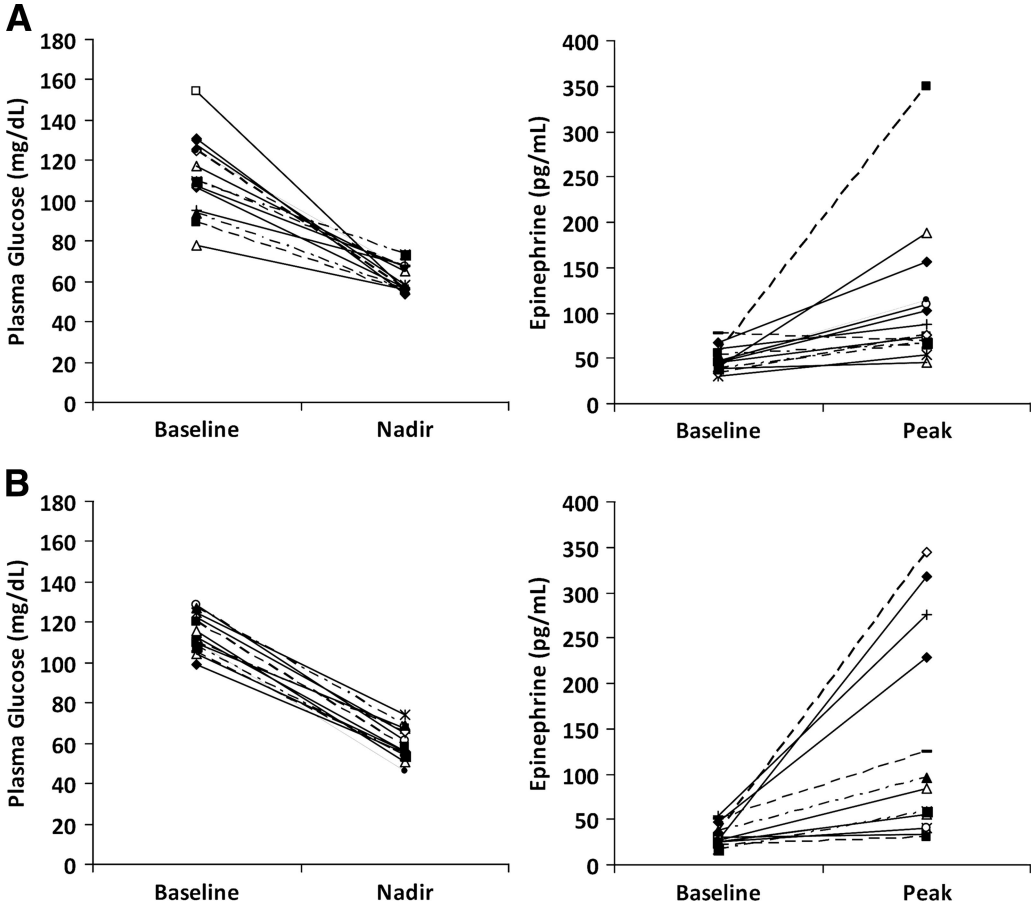

Figure 1—*Plasma glucose concentrations from baseline to nadir and epinephrine concentrations from baseline to peak in young children* (A) *and adolescents* (B)*. Each line and symbol combination represents a unique subject in the glucose and epinephrine plots.*

test. The plasma glucose threshold that stimulated a counterregulatory hormone response was defined as the point at which the hormone concentration was ≥3 SD above baseline (with the SD based on the duplicate blood samples at baseline). The 3-SD limits were 26 pg/ml for epinephrine, 44 pg/ml for norepinephrine, 0.83 μg/dl for cortisol, 1.2 ng/ml for growth hormone, and 16 pg/ml for glucagon. The proportions of subjects who had hormone responses in each age-group were compared using Fisher's exact test. Group comparisons of the change in hormone concentration from baseline to peak were performed using an ANCOVA model based on van der Waerden normal scores and adjusted for the corresponding baseline value.

The study was completed by 30 subjects with type 1 diabetes. One subject in each age-group was not included in the analysis because of blood sampling problems. Because the Pittsburgh study used a one-step hypoglycemic clamp, lowering glucose in one step from ~100 to 60 mg/dl, plasma epinephrine responses in this study were used only to describe the magnitude of the plasma epinephrine responses in nondiabetic subjects.

RESULTS — Primary analysis included 14 young children (4–7 years old; duration of diabetes 3.3 ± 1.1 years) and 14 adolescents (12–17 years old; duration of diabetes 6.6 ± 3.4 years, $P = 0.003$) with type 1 diabetes. Of the young children, 21% were female and 86% were Caucasian vs. 43 and 100%, respectively, for adolescents. Mean A1C was 7.7 ± 0.6% in young children and 7.6 ± 0.8% in adolescents. The 14 nondiabetic adolescents studied in Pittsburgh were 29% female and 93% Caucasian. A1C for all these subjects was <6.1%.

Plasma glucose during hypoglycemia testing in type 1 diabetic subjects

Individual baseline and nadir plasma glucose concentrations in the patients with type 1 diabetes are shown in Fig. 1. Plasma glucose was reduced gradually to a mean nadir concentration of 61 mg/dl in the young children and 59 mg/dl in adolescents. Nadir glucose concentration was <60 mg/dl in 8 of 14 subjects in each group and between 60 and <70 mg/dl in 5 subjects in each group. In only one child and one adolescent was the nadir >70 mg/dl (i.e., 73 and 74 mg/dl). The mean time interval between <90 mg/dl and postglucose treatment blood sampling tended to be longer in adolescents than in younger children (71 vs. 53 min, $P = 0.06$).

Counterregulatory hormone responses in type 1 diabetic subjects

Individual baseline and peak plasma epinephrine concentrations in type 1 diabetic subjects are also shown in Fig. 1. As summarized in Table 1, 4 of 14 subjects in each age-group never had an epinephrine response, despite nadir plasma glucose concentrations that ranged between 56 and 67 mg/dl in the young children and 58 and 74 mg/dl in the adolescents. Four young children and five adolescents did not manifest an epinephrine response until plasma glucose fell to <60 mg/dl. Only two children and no adolescents had an

Table 1—*Plasma glucose concentrations at epinephrine and norepinephrine response*

Plasma glucose at time of first response*	Epinephrine response*		Norepinephrine response*	
	Young children	Adolescents	Young children	Adolescents
n	14	14	14	14
≥90 mg/dl†	2	0	0	2
80 to <90 mg/dl	0	0	0	2
70 to <80 mg/dl	1	1	1	0
60 to <70 mg/dl	3	4	2	1
<60 mg/dl	4	5	2	1
Never responded‡	4‡	4‡	9§	8§

*The hormone response was defined as 3 SDs above baseline. †The maximum laboratory glucose concentration triggering epinephrine response was 96 mg/dl (from a young child with a baseline value of 125 mg/dl) and the triggering norepinephrine response was 116 mg/dl (from an adolescent with a baseline value of 125 mg/dl). ‡The nadir glucose values ranged from 56 to 67 mg/dl in young children and 58 to 74 in adolescents. §The nadir glucose values ranged from 54 to 68 mg/dl in young children and 46 to 69 in adolescents.

abnormally high glucose threshold for epinephrine release. Nine of 14 young children and 8 of 14 adolescents never had a norepinephrine response to hypoglycemia (Table 1). Two adolescents had a high glucose threshold for norepinephrine response.

Median, 25th, and 75th percentile of baseline and peak plasma concentrations of epinephrine, norepinephrine, cortisol, growth hormone, and glucagon were not different in young children and adolescents (Table 2). In addition, the change in hormone concentrations and the number of subjects who had a response in each group were not different. In contrast, the median plasma epinephrine concentrations in nondiabetic subjects rose from 77 to 582 pg/ml in response to hypoglycemia.

Symptom scores

Table 3 shows the responses to the statement "I feel like my glucose is low" by the adolescents and by parents of the young children to the statement "My child looks low." Only a small fraction of parents felt that their child looked hypoglycemic even at the lowest plasma glucose concentrations. In contrast, the percentage of adolescents who reported more than minimal symptoms of hypoglycemia increased when glucose levels fell to <70 mg/dl.

CONCLUSIONS

— The principal aim of this study was to compare the epinephrine responses to hypoglycemia in young children and adolescents with type 1 diabetes. We hypothesized that, despite a shorter duration of diabetes, the glucose threshold and magnitude of the epinephrine response would be lower in the young children. The study protocol allowed us to lower plasma glucose concentrations to the same nadir and to estimate the glucose concentrations that stimulated epinephrine and other counterregulatory hormone responses between the two groups. The nadir glucose tended to be achieved faster in young children, but the rate of the fall in glucose has not been shown to affect counterregulatory hormone responses to hypoglycemia (12).

Our most concerning finding was that 29% of subjects in both age-groups failed to stimulate any epinephrine response, despite in most cases, reaching a blood glucose level <60 mg/dl, and plasma epinephrine concentrations only rose modestly in those who did. An even greater percentage of subjects in both groups failed to mount a plasma norepinephrine or cortisol response and the glucagon response to hypoglycemia was virtually absent.

We had postulated that the plasma epinephrine response in adolescents with type 1 diabetes would be similar to the response that had been reported in nondiabetic subjects and reduced by up to 50% in young children with type 1 diabetes. Previous studies of plasma epi-

Table 2—*Counterregulatory hormone concentrations by age-group*

	Young children	Adolescents
Epinephrine		
n	14	14
Baseline (pg/ml)	47 (39, 56)	27 (25, 37)
Peak (pg/ml)	82 (68, 115)	71 (41, 229)
Change (pg/ml)*	36 (23, 68)	50 (16, 182)
Increased ≥3 SDs†	10 (71)	10 (71)
Norepinephrine		
n	14	14
Baseline (pg/ml)	120 (107, 128)	153 (108, 226)
Peak (pg/ml)	138 (122, 186)	219 (169, 271)
Change (pg/ml)*	31 (9, 64)	40 (26, 76)
Increased ≥3 SDs†	5 (36)	6 (43)
Cortisol		
n	14	14
Baseline (μg/ml)	9.4 (8.2, 10.9)	12.7 (10.1, 14.1)
Peak (μg/ml)	10.1 (8.5, 11.7)	12.0 (9.8, 14.4)
Change (μg/ml)*	−0.5 (−1.4, +1.9)	+0.1 (−0.6, +0.8)
Increased ≥3 SDs†	5 (36)	3 (21)
Growth hormone		
n	14	14
Baseline (ng/ml)	1.4 (0.5, 4.2)	2.0 (0.3, 9.7)
Peak (ng/ml)	5.2 (2.2, 13.9)	6.7 (2.6, 18.8)
Change (ng/ml)*	1.6 (−0.3, 9.4)	2.5 (−0.4, 17.3)
Increased ≥3 SDs†	9 (64)	9 (64)
Glucagon		
n	13‡	14
Baseline (pg/ml)	42 (33, 50)	48 (32, 57)
Peak (pg/ml)	42 (36, 43)	50 (31, 57)
Change (pg/ml)*	−3 (−4, +2)	+1 (−2, +9)
Increased ≥3 SDs†	1 (8)	1 (7)

Data are median (25th, 75th percentile) or *n* (%). Total *n* = 28. *Defined as peak minus baseline. †3 SDs described in RESEARCH DESIGN AND METHODS. ‡Samples for glucagon were drawn only at baseline and at meter glucose value <60 mg/dl. One subject was missing the end draw.

Table 3—*Scores on the statement: "My child looks low" (parent response) or "I feel like my glucose is low" (subject response) at different plasma glucose concentrations during insulin-induced hypoglycemia*

	Young children parent response				Adolescent subject response			
Plasma glucose	*n*	Not at all/very little (score 0 or 1)	Some (score 2)	Very much (score 3)	*n*	Not at all/very little (score 0 or 1)	Some (score 2)	Very much (score 3)
n			13				14	
Baseline	11	100	0	0	11	82	18	0
>90 mg/dl	5	100	0	0	8*	63	13	25
80 to <90 mg/dl	7	100	0	0	12*	75	8	17
70 to <80 mg/dl	13*	85	15	0	13*	54	15	31
60 to <70 mg/dl	11*	100	0	0	13*	46	31	23
<60 mg/dl	7	71	29	0	7	29	43	29

Data are %. *Includes multiple responses from same subject.

nephrine responses to hypoglycemia performed >15–20 years ago (11,12) demonstrated that in nondiabetic children aged 8–18 years, plasma epinephrine concentrations rose to >600 pg/ml, when plasma glucose was lowered to <60 mg/dl. Youth with poorly controlled type 1 diabetes (mean A1C 15.1%) had an even higher threshold for release of epinephrine, but the peak epinephrine response was similar to that in nondiabetic subjects (11). These adolescents with poorly controlled diabetes did not have hypoglycemia unawareness as confirmed by their symptom scores, which were similar to those of the nondiabetic control subjects. In contrast, 29% of our adolescents reported few or no symptoms of hypoglycemia with plasma glucose <60 mg/dl.

Because the epinephrine responses observed in our adolescents with type 1 diabetes were so dramatically different from those reported previously in diabetic and nondiabetic subjects (11,12), we were concerned that changes in assay methods over the years were responsible for the marked discrepancy. Fortunately, frozen samples from hypoglycemia studies performed in Pittsburgh in nondiabetic adolescents, who showed epinephrine responses that were similar to those in the previous studies (11,12), were available for reanalysis. Even though the samples from Pittsburgh showed evidence of loss of epinephrine concentrations after 6–9 years of storage, the assay results were similar enough to the assay at the DirecNet laboratory to indicate that the marked blunting of the epinephrine responses in our subjects could not simply be due to differences in assay methods. However, the difference in the method of developing hypoglycemia in our study versus that in the older studies (slower versus rapid fall in plasma glucose) adds another note of caution in interpreting these results.

A number of elegant studies in adults with and without type 1 diabetes have demonstrated that recent antecedent hypoglycemia impairs the counterregulatory hormone responses to subsequent hypoglycemia and increases the risk for severe hypoglycemic events (6–8). Meticulous prevention of hypoglycemia is able to at least partially reverse impaired counterregulatory hormone responses to hypoglycemia (13). Clinical research center–based exercise studies and outpatient continuous glucose monitoring studies conducted by DirecNet have shown that asymptomatic biochemical hypoglycemia is very common in youth with type 1 diabetes (14–16). Unfortunately, it appears from the results of this study that children are also prone to develop hypoglycemia-associated autonomic failure, as observed in adults (5). In addition, as illustrated by the symptom assessment carried out in this study, the risk of severe hypoglycemia may be increased in young children because their parents are unable to recognize glucose levels falling into a dangerous range in the absence of a meter glucose measurement. Clearly, further studies are needed to clarify how often and to what extent counterregulatory defense mechanisms are impaired in children and adolescents with type 1 diabetes and whether these abnormalities can be reversed with intensive diabetes management using continuous glucose monitoring and closed-loop insulin delivery systems.

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APPENDIX— The writing committee of Diabetes Research in Children Network (DirecNet) Study Group consists of the following: Eva Tsalikian, MD; William Tamborlane, MD; Dongyuan Xing, MPH; Dorothy M. Becker, MBBCh; Nelly Mauras, MD; Rosanna Fiallo-Scharer, MD; Bruce Buckingham, MD; Stuart Weinzimer, MD; Michael Steffes, MD, PhD; Ravinder Singh, PhD; Roy Beck, MD, PhD; Katrina Ruedy, MSPH; and Craig Kollman, PhD.

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