Independent Effects of Youth and Poor Diabetes Control on Responses to Hypoglycemia in Children

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To evaluate the effects of childhood and poorly controlled insulin-dependent diabetes mellitus (IDDM) on counterregulatory hormone and symptomatic responses to hypoglycemia, we studied 16 nondiabetic children (13 ± 2 yr), 19 nondiabetic adults (26 ± 3 yr), and 13 children with IDDM (14 ± 2 yr, HbA, 15.1 ± 3.3%) during a gradual reduction in plasma glucose with the glucose-clamp technique. Plasma glucose was reduced from ~5 to ~2.8 mM over 240 min with serial assessment of counterregulatory hormone levels and symptom awareness. The plasma glucose level that triggered a sustained rise in plasma epinephrine was consistently higher in nondiabetic children than in adults (3.9 ± 0.06 vs. 3.2 ± 0.06 mM, $P < 0.001$). Poorly controlled IDDM further elevated the glucose threshold for epinephrine release to normoglycemic levels (4.9 ± 0.2 mM, $P < 0.001$ vs. both control groups). Age and IDDM also produced an upward shift in the glucose level at which growth hormone release and symptom awareness were initiated. In contrast to the effect on glucose thresholds, maximal epinephrine responses and symptom scores were increased only by age and not IDDM (2-fold higher in children). We conclude that childhood and poor diabetes control independently contribute to an upward shift in glucose thresholds for counterregulatory hormone release and symptom awareness during mild hypoglycemia. Normoglycemic counterregulation may interfere with efforts to control diabetes in young patients. *Diabetes* 40:358–63, 1991

Glycemic lability is a frequent problem in the management of insulin-dependent diabetes mellitus (IDDM) in childhood. Although it has long been recognized that developmental and behavioral features peculiar to childhood are important determinants of this metabolic instability, other physiological factors have been identified that may also contribute to management difficulties in IDDM children. Children with and without IDDM become insulin resistant during puberty (1). In addition, both prepubertal and pubertal children show exaggerated epinephrine responses to moderate hypoglycemia compared with adults (2). Whether this phenomenon reflects an alteration in the magnitude of the adrenomedullary response or involves an upward shift of the glycemic level initiating epinephrine release is uncertain. Regardless, age-related changes in epinephrine responsiveness in IDDM children might make these patients particularly vulnerable to rebound hyperglycemia after hypoglycemia, especially in the setting of insulin resistance.

Poor metabolic control of diabetes might itself influence the hormonal response to hypoglycemia in diabetic children by raising the plasma glucose level at which counterregulatory responses occur. This possibility is in keeping with animal studies indicating that brain glucose transport is less efficient in chronically hyperglycemic rats (3,4). Nevertheless, an upward shift in the glucose threshold for counterregulation in poorly controlled IDDM has only rarely been reported (5), with normal or suppressed responses being more commonly observed (6,7). For example, Boyle et al. (7), in a study designed specifically to address this issue, found no alteration in glycemic thresholds for hormonal responses to hypoglycemia in adult IDDM patients in poor metabolic control, although symptoms did appear at a higher glucose level than in nondiabetic control subjects. This question, however, remains of particular relevance for young IDDM patients, because they are more likely to have poor glycemic control (8) and less likely than adults to have blunted epinephrine responses as a result of autonomic neuropathy or increased disease duration (9–11).

This study was undertaken to examine the effects of age and diabetes on responses to hypoglycemia by applying a

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modification of the glucose-clamp technique designed to produce a standardized reduction in plasma glucose. With this approach, we were able to compare the onset and magnitude of counterregulatory hormone responses and the evolution of symptoms during a gradual glucose fall in nondiabetic children and adults and children with IDDM. Our findings suggest that normoglycemic counterregulation in children with IDDM may be an important physiological and developmental adaptation contributing to the difficulty in achieving glycemic control in this age-group.

RESEARCH DESIGN AND METHODS

Thirty-five healthy nonobese nondiabetic subjects (19 adults, 16 children) were studied. They had normal fasting glucose (4.8 ± 0.1 mM), insulin (65 ± 14 pM), and HbA_1 levels (Table 1). In addition, 13 children with IDDM who attend the Yale Children's Diabetes Center were studied. They were eligible for study if their disease duration was >1 yr, their HBA_1 level was >10% (adult nondiabetic reference range 4–8%), and they were taking no more than two daily insulin injections. All were healthy except for diabetes and were taking no medication other than insulin. Clinical characteristics of the three subject groups are shown in Table 1. The protocol was approved by the Human Investigation Committee of the Yale University School of Medicine, and informed written consent was obtained from all subjects and their parents (for the children).

Patients with diabetes were admitted to the Yale Clinical Research Center (children's unit) 24 h before the study. Plasma glucose was measured every 60 min, and during the night, regular insulin was given to ensure near-normal fasting glucose concentrations the next morning. Nondiabetic children were admitted on the evening before the study so that they could become adapted to the hospital setting. All studies were performed after a 10- to 12-h overnight fast.

A modification of the glucose-clamp technique was used to produce a gradual and standardized reduction in plasma glucose. The methods used for this procedure (hypoglycemic clamp) have been described previously (12). Two catheters were inserted in all subjects: one into an antecubital vein for infusion of glucose and insulin, and the second into a dorsal hand vein in the contralateral arm for blood sampling. The latter hand was placed in a heated box (~65°C) to arterialize venous blood (13).

After a 30-min rest period, three blood samples were obtained over a 40-min interval for measurement of baseline glucose and hormone levels. A primed continuous infusion of rapid-acting human insulin (Novolin, Squibb-Novo, Princeton, NJ) was then begun: the continuous rate was 80 mU · m^{-2} · min^{-1}, and the priming rate was 240 mU · m^{-2} · min^{-1} for 5 min and 160 mU · m^{-2} · min^{-1} for 5 min. After the insulin infusion was begun, the desired plasma glucose level was achieved by varying the rate of infusion of 20% glucose based on measurements of plasma glucose at 5-min intervals. Blood samples for measurement of insulin and counterregulatory hormones (epinephrine, norepinephrine, growth hormone, cortisol, and glucagon) were taken at 10- to 20-min intervals throughout the study.

In nondiabetic subjects, plasma glucose was initially maintained at fasting euglycemic levels for 60 min and then reduced in hourly ~0.5-mM steps to a nadir of 2.8 mM (Fig. 1). For the diabetic patients, plasma glucose was maintained at ~5.5 mM for 60 min before commencement of the procedure and then gradually reduced to a nadir value of ~2.8 mM over an additional 240 min. All subjects were masked to the plasma glucose levels during the study.

TABLE 1
Clinical characteristics

	Nondiabetic adults	Nondiabetic children	IDDM children
Age (yr)	25.6 ± 3.1	13.0 ± 2.4	14.1 ± 2.3
Tanner stage	NA		
I		4	3
II–IV		9	7
V		3	3
n (F/M)	7/12	6/10	7/6
Duration IDDM (yr)			7.5 ± 3.2
HbA_1 (%)	5.4 ± 0.6	4.8 ± 0.5	15.1 ± 3.3

Data are expressed as means ± SD. IDDM, insulin-dependent diabetes mellitus; NA, not applicable.

Subjects were instructed to immediately report the onset of any symptoms that developed during the procedure. The presence of symptoms was also systematically determined by questionnaire in 10 of 19 adults and in all of the children. This was completed in all subjects before the procedure and in the final 20 min of each hour (in IDDM children, an additional report was obtained 20 min after the commencement of the procedure). Subjects rated symptoms (presented and recorded on a laptop computer) on a scale of 0 (not at all) to 6 (extremely). Symptoms presented included pounding heart, feeling shaky, weakness, headache, having difficulty concentrating or thinking, slowed thinking, and feeling sweaty. The sum of these symptoms at each observation point constituted the total symptom score at that time. In addition, four filler items were included (earache, pain in joints, watery eyes, ringing in ears) to control for nonspecific effects not referable to hypoglycemia; the sum of these four items at each observation point constituted the control symptom score.

Plasma glucose was measured in duplicate at the bedside with a glucose analyzer (Beckman, Fullerton, CA). Glucose was measured in common units (mg/dl) and converted to Système International units after analysis (conversion factor 0.056). Catecholamines were measured with a radioenzymatic assay (Amersham, Arlington Heights, IL), and growth

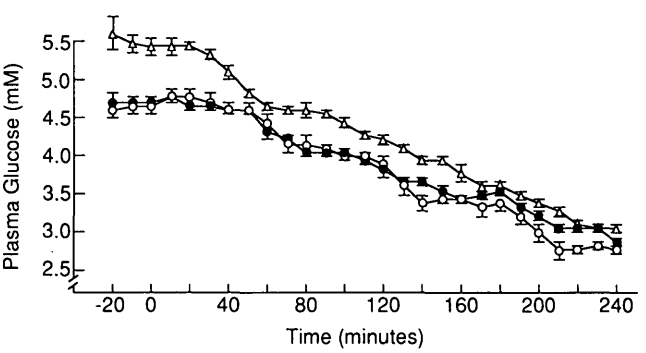

FIG. 1. Plasma glucose levels during hypoglycemic clamp procedure in nondiabetic adults (○), nondiabetic children (●), and insulin-dependent diabetic children (△). Insulin infusion was 80 mU · m^{-2} · min^{-1}. Values are means ± SE.

hormone (Karllsted, Austin, TX), glucagon (ICN Biomedicals, Carson, CA), and cortisol (Clinical Assays, Cambridge, MA) were determined by radioimmunoassay. The intra-assay coefficients of variation for these assays were 20.2% at 175 pM and 6.7% at 1168 pM for epinephrine, 6.2% at 3.5 μg/L for growth hormone, 9% at 122 ng/L for glucagon, and <2% for cortisol. Plasma insulin was measured by double-antibody radioimmunoassay (Ventrex, ME). Plasma samples from patients with IDDM were treated with polyethylene glycol immediately after separation to precipitate antibody-bound insulin, and the supernatant was later measured for free insulin (14). HbA_1 was measured chromatographically (Glyc-Affin, Isolab, Akron, OH).

Statistical methods and analyses. Demographic data are expressed as means ± SD and all other data as means ± SE. Comparisons of hormone responses and symptom reports between groups were made with analysis of variance with repeated measures design. Within groups, the onset of hormone responses above basal values was tested by analysis of variance. For this purpose, basal values included all values obtained before plasma glucose was reduced. Because growth hormone levels commonly fell during the initial phase of the insulin infusion, the four nadir values were used to define basal values for this hormone.

In addition to group analysis of hormone increments in response to hypoglycemia, the plasma glucose threshold for hormone release was calculated for individual subjects. This was defined as the glucose level at which an unequivocal sustained increment in circulating hormone concentration above basal was first observed. To avoid errors caused by spontaneous fluctuations in hormone levels, an unequivocal increment was determined in each subject in three ways: *1*) a rise >2SD over basal values, *2*) a rise >3 times the intra-assay coefficient of variation of the assay at that level, and *3*) for epinephrine, a rise >410 pM above basal values (this value was selected as one of probable physiological significance; 15). These thresholds were compared between groups with analysis of variance and unpaired Student's *t* test. $P < 0.05$ was significant.

RESULTS

The glucose profiles achieved during the glucose-clamp procedure in all the groups are shown in Fig. 1. In nondiabetic children and adults, plasma glucose was gradually reduced from similar fasting levels (4.7 ± 0.1 vs. 4.7 ± 0.1 mM, children vs. adults) in a nearly identical manner over 240 min; the nadir glucose level was slightly higher in the children (2.9 ± 0.06 vs. 2.7 ± 0.06 mM, NS). During this time in the diabetic children, plasma glucose fell from higher values of 5.5 ± 0.1 mM to a nadir glucose level of 3.1 ± 0.1 mM. As a result, for the initial 180 min of the study, glucose levels were higher in diabetic children compared with both groups of nondiabetic subjects ($P < 0.05$). Minimal variability in plasma glucose levels was achieved in all three groups of subjects (coefficient of variation <3% at each hypoglycemic level). The steady-state insulin levels achieved during the insulin infusion were similar in all three groups (847 ± 36 pM in nondiabetic adults, 898 ± 43 pM in nondiabetic children, 919 ± 72 pM in diabetic children, NS).

In Fig. 2, mean plasma epinephrine concentrations are plotted against the mean glucose level at each of the sampling times in the three groups. There were no differences between the groups in basal plasma epinephrine levels at the start of the study. In nondiabetic adults, plasma epinephrine values did not rise significantly until plasma glucose levels fell below 3.4 mM. In contrast, plasma epinephrine levels rose much earlier in nondiabetic children than adults ($P < 0.05$). At plasma glucose levels of ≤3.9 mM, plasma epinephrine was significantly higher in nondiabetic children compared with nondiabetic adults, reaching nearly twofold higher values in the nondiabetic children at the end of the study (4150 ± 524 vs. 2168 ± 251 pM, $P < 0.01$). In IDDM children, the plasma glucose level stimulating release of epinephrine was increased further to 4.7 ± 0.1 mM ($P <$ 0.05 vs. other 2 groups; Fig. 2). Nevertheless, the mean epinephrine level at the end of the study in this group (4089 ± 464 pM) was not increased compared with nondiabetic children, although it was substantially greater than values in nondiabetic adults ($P < 0.01$). Individually calculated plasma glucose thresholds for epinephrine release are shown in Table 2. Significant differences were noted among all three groups regardless of the method used to calculate

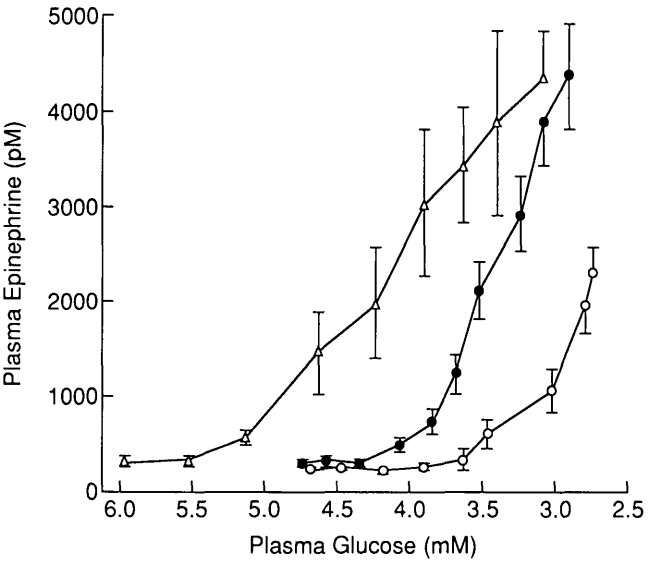

FIG. 2. Mean plasma epinephrine concentrations plotted against corresponding mean plasma glucose at sampling time for nondiabetic adults (○), nondiabetic children (●), and insulin-dependent diabetic children (△).

TABLE 2
Glycemic thresholds (mM) for epinephrine release and symptom onset

	Nondiabetic		
	Adults	Children	IDDM children
Epinephrine*			
>2SD over basal values	3.3 ± 0.06	3.9 ± 0.06†	5.1 ± 0.2‡
>3 × intra-assay variation	3.2 ± 0.06	3.9 ± 0.06†	4.9 ± 0.2‡
>410 pM	3.1 ± 0.06	3.8 ± 0.06†	4.7 ± 0.2‡
Symptom onset	3.1 ± 0.06	3.8 ± 0.06†	4.9 ± 0.2‡

IDDM, insulin-dependent diabetes mellitus.
*Increment above basal values (see text).
†$P < 0.001$ vs. adults.
‡$P < 0.001$ vs. nondiabetic children and adults.

the threshold (Table 2). Thus, nondiabetic children released epinephrine at a higher glucose level than nondiabetic adults, and in diabetic children, this threshold was further elevated.

Differences between the three groups in the glucose threshold triggering release of growth hormone mirrored that of epinephrine (i.e., IDDM children > nondiabetic children > nondiabetic adults, $P < 0.05$; Table 3). However, in contrast to epinephrine, the peak growth hormone values achieved at the end of the study in all three groups were not significantly different. Plasma cortisol levels did not rise significantly in any of the groups until the end of the study, and there were no differences in the peak values observed (Table 3). The time of onset and the magnitude of the glucagon response to hypoglycemia did not differ between nondiabetic adults and children, whereas no significant rise in plasma glucagon was detected in the IDDM children (basal, 120 ± 24 vs. 117 ± 30 ng/L at 240 min; Table 3).

The plasma glucose level at which the subjects spontaneously reported the onset of symptoms was significantly higher in diabetic than in nondiabetic children, which was higher than glucose levels in adults (Table 2). These differences were also observed on a systematic symptom questionnaire; Fig. 3 shows the total symptom score at each glucose level in all three groups of subjects. The symptom score was similar in all groups before commencement of the procedure. However, the symptom score increased significantly at a higher glucose level in IDDM children (4.2 ± 0.06 mM, $P < 0.05$) compared with either control group. In nondiabetic children, the symptom score increased after reduction of the plasma glucose level to 3.5 ± 0.06 mM, but by the end of the study, there was no difference in the reported magnitude of symptomatic awareness between both groups of children (25 ± 4 vs. 24 ± 5, IDDM vs. nondiabetic children). In contast, symptom scores were significantly lower in nondiabetic adults at this glucose level (12 ± 2, $P < 0.05$ vs. nondiabetic and IDDM children). The difference in total symptom scores at the final glucose level between nondiabetic adults and both groups of children was mainly due to increased reporting of feeling shaky, weak, sweaty, and having a pounding heart. There were no significant changes in the control filler symptom score for any group over the course of the study (nondiabetic adults, baseline 2.0 ± 0.8 to 2.0 ± 0.7 at the final glucose level; nondiabetic children, 2.0 ± 0.5 to 3 ± 1; IDDM children, 1.0 ± 0.5 to 2.0 ± 0.7).

TABLE 3
Growth hormone, cortisol, and glucagon responses to hypoglycemia

	Nondiabetic		IDDM
	Adults	Children	children
Growth hormone			
Glucose threshold*	3.00 ± 0.06	3.70 ± 0.06†	4.2 ± 0.1‡
Peak level (μg/L)	14 ± 1	14 ± 2	21 ± 3
Cortisol			
Glucose threshold*	3.10 ± 0.06	3.1 ± 0.1	3.4 ± 0.1
Peak level (nM)	20 ± 1	20 ± 1	22 ± 2
Glucagon			
Glucose threshold*	3.00 ± 0.06	2.90 ± 0.06	
Peak level (ng/L)	139 ± 30	150 ± 29	

IDDM, insulin-dependent diabetes mellitus.
*Plasma glucose level (mM) associated with sustained increment of hormone of >2SD over basal values.
†$P < 0.001$ vs. adults.
‡$P < 0.001$ vs. nondiabetic children and adults.

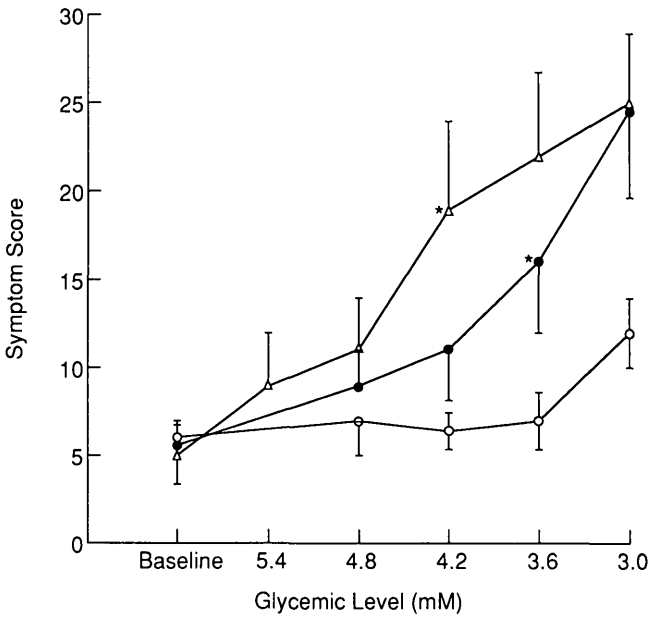

FIG. 3. Total symptom scores at each observation point in nondiabetic adults (○), nondiabetic children (●), and insulin-dependent diabetic children (△). *Symptom score at which change from baseline values ($P < 0.05$) was first noted.

DISCUSSION

This study demonstrates an upward shift in the epinephrine response curve during a gradual reduction in plasma glucose in nondiabetic children compared with nondiabetic adults. As a result, epinephrine was released at a higher plasma glucose level, and the magnitude of the response was markedly increased in children. This age-related difference in responsiveness appears to be unique to childhood, because previous studies have failed to observe impaired epinephrine release in healthy elderly versus young adult subjects (16). Furthermore, differences in hormonal responses to mild hypoglycemia in nondiabetic children were not limited to epinephrine because growth hormone release was also stimulated at a higher glucose level. Thus, these data indicate that glucose counterregulation in childhood is initiated by decrements in plasma glucose only slightly below usual overnight fasting levels.

To evaluate further whether the chronic hyperglycemia that is characteristic of poorly controlled IDDM during childhood might independently affect the timing and degree of epinephrine responses, young diabetic patients with mean HbA_1 levels more than twofold higher than values in nondiabetic children were studied. The experimental protocol for these children differed from that in the nondiabetic control subjects in that plasma glucose was reduced from slightly higher values so that we might be able to detect an elevation in glucose thresholds for hormone release. When IDDM children were compared with nondiabetic children, epinephrine release was initiated earlier at a substantially higher glucose level. In contrast, the magnitude of the peak epinephrine

response was not significantly affected by IDDM. The similarity in peak responses between nondiabetic and diabetic children was consistent with our previous report, in which plasma glucose was lowered from ~5 to ~2.8 mM over 10–15 min (2). In that study, the peak epinephrine responses in both groups of children were nearly identical to that observed herein (~4000 pM). Thus, it is unlikely that the slightly faster rate of glucose fall in diabetic patients compared with both groups of nondiabetic subjects influenced our results.

Of particular importance, the children with poorly controlled diabetes demonstrated increases in plasma epinephrine and growth hormone and symptom scores at plasma glucose levels that were well into the normoglycemic range. Thus, chronic hyperglycemia in these subjects seems to have caused a further upward shift in the glucose level at which counterregulation and hypoglycemic symptoms developed. These results differ from those of Boyle et al. (7), who used techniques similar to those of this study to compare responses to hypoglycemia between nondiabetic adults and adult IDDM patients. Although in that study symptoms developed at a significantly higher glucose level in IDDM, a similar shift in glucose thresholds for hormone release was not observed. This apparent discrepancy may be explained by the fact that we studied younger diabetic patients, and our patients had HbA_1 levels that were more than twofold higher than the upper limit of values in age-matched control subjects, and their adult subjects had HbA_1 levels ~40% above the upper limit of normal. Moreover, our patients had a shorter duration of diabetes than those studied by Boyle et al. As a result, they were less likely to have had the blunted adrenergic responses that are often seen in adult IDDM patients with prolonged disease (11).

It has been reported that counterregulatory responses may be precipitated by a fall in glucose levels in the supranormal range (from 11 to 6 mM) in both nondiabetic (6) and diabetic (5) adult subjects. The hormonal responses described in those studies, although small and transient, imply that the counterregulatory response may be modified by antecedent glycemia. Studies describing suppressed glucose thresholds for counterregulation in intensively treated IDDM are consistent with this view (17). This finding provides further support for this concept by demonstrating that sustained and significant hormonal responses may be initiated in chronically hyperglycemic individuals at glucose levels above those seen in nondiabetic subjects.

The differences in the glucose thresholds for the release of epinephrine and growth hormone between children and adults and children with IDDM were paralleled by differences in glucose levels at which symptomatic awareness of hypoglycemia developed. Symptoms not only followed the onset of hormonal responses, as described previously by Schwartz et al. (18), but they developed after a rise in circulating epinephrine levels of ~410 pM, an increment that has been suggested to be of physiological significance (15). Furthermore, in keeping with this, the symptoms that were most prominent in children compared with adults were adrenergic in nature. The finding that healthy nondiabetic children developed epinephrine responses and hypoglycemic symptoms after reductions in plasma glucose to only slightly below fasting values is of potential importance. Similar modest decrements in plasma glucose are commonly observed in the postprandial period after ingestion of simple sugars. In preliminary studies, we observed that glucose ingestion provokes greater elevations of epinephrine and more symptoms in otherwise healthy children compared with adults (19). The clinical importance of these findings may be more relevant for the young patient with poorly controlled IDDM. Although the importance of the Somogyi phenomenon continues to be debated (20–25), exaggerated epinephrine responses generated at normoglycemia (and the early release of growth hormone) may contribute to further hyperglycemia. In addition to these hormonal effects, hyperglycemia is likely to be compounded by food intake after the development of hypoglycemic symptoms at inappropriate glucose levels. Thus, the combined effects of age and poor glycemic control on responses to reductions in plasma glucose may confound symptom assessment and aggravate lability in children with IDDM.

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