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Blunted Counter-regulatory Hormone Responses to Hypoglycemia in Young Children and Adolescents with Well-controlled Type 1 Diabetes

Diabetes Research in Children Network (DirecNet) Study Group*

*The DirecNet Study Group is listed in the online appendix at http://care.diabetesjournals.org, the writing group is listed in the Acknowledgements at the end of the text.

Short Running Title: *Counter-regulatory hormones in hypoglycemia*

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Objectives: Hypoglycemia in young children with T1DM 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 sympatho-adrenal responses has not been established in youth with T1DM due to difficulties in testing procedures.

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 counter-regulatory hormone concentrations were measured in 14 young children (3 to <8 years, HbA1c 7.7 ± 0.6%) vs. 14 adolescents (12 to <18 years, HbA1c 7.6 ± 0.8%).

Results: Plasma glucose decreased to similar nadir concentrations in the two groups. Four young children and 4 adolescents never had an epinephrine response. In the 4 young children and 5 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 to our data, epinephrine response to hypoglycemia in 14 non-diabetic adolescents studied at the Children's Hospital of Pittsburgh was higher.

Conclusions: These data suggest that even young children and adolescents with T1DM 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.

Severe hypoglycemia is a life-threatening complication of intensive therapy of type 1 diabetes (T1DM), 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, since 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 non-diabetic children hypoglycemia triggers counter-regulatory responses that include increases in plasma glucagon and epinephrine concentrations. In non-diabetic adolescents and in conventionally treated adolescents with poorly controlled T1DM, 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 non-diabetic adults (3). Since glucagon responses to hypoglycemia are lost early in the disease (4), an intact plasma epinephrine response is critical in patients with T1DM.

In adults with T1DM, the episodes of mild hypoglycemia that accompany intensive treatment induce a defect in sympatho-adrenal responses that has been termed hypoglycemia-associated autonomic failure (5-8). Whether intensive treatment causes similar defects in youth with T1DM has not been well studied, due in part to 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 well-controlled, insulin pump treated youth with T1DM. Counter-regulatory hormone concentrations were measured sequentially in order 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 non-diabetic adolescents to provide a frame of reference to judge responses to hypoglycemia in the T1DM subjects.

METHODS

Subjects with T1DM: Subjects with T1DM were studied at the 5 DirecNet clinical centers. A Data and Safety Monitoring Board (DSMB) and Institutional Review Boards at each center approved the study protocol, consent and assent form. A parent or guardian and each subject >7 years of age gave written consent and assent, respectively.

Eligibility Criteria: Eligibility criteria for T1DM subjects were: 1) age 3 to <8 years or 12 to <18 years, 2) duration of T1DM of ≥1 year, 3) using an insulin pump and 4) hemoglobin A1c ≤10.0% (DCA2000+ analyzer, Siemens Healthcare Diagnostics, Indianapolis, IN). Exclusion criteria included 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 T1DM were admitted to the research center on the evening prior to 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, 12a.m., 3a.m., 6a.m., and 7a.m. Oral carbohydrates were given to prevent low glucose levels with the goal of having the glucose values ≥110mg/dl at 8a.m. If glucose levels were high and it was predicted that it would take a long time for the glucose level to drop to 110mg/dl, small insulin correction doses were given.

At the start of the test, a bolus dose of insulin equal to approximately one 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 minutes until the glucose value was $\leq$100mg/dL and at 5-10 minute intervals thereafter, until the end of the study. Blood samples were obtained for determination of glucose, epinephrine, norepinephrine, cortisol, glucagon and growth hormone (GH) at baseline, when the meter glucose was between 95 and 110mg/dL (in duplicate) and then <90, <80, <70, and <60mg/dL. Once the glucose level fell to <60mg/dL, intravenous glucose was given, the basal rate was returned to normal, and breakfast eaten. An additional blood sample was collected for glucose and hormone concentrations 15 minutes following treatment with glucose.

At each blood sampling time, parents were asked whether their child "looked low" and adolescents 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 'Some' and 3 'Very much'.

Laboratory Procedures: Blood samples were frozen prior to shipping. Glucagon, cortisol, GH, 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 from goat. The lower limit of detection was 20pg/mL (6pmol/L). Coefficients of variation (CVs) were 6.5–8.8% on 3 controls. 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. Lower limit of detection was 0.5µg/dL (14nmol/L). CVs were 11 – 12% on 2 controls. GH 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. Lower limit of detection was 0.1ng/mL (4pmol/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) HPLC column to separate norepinephrine and epinephrine, which were detected coulometrically, using an ESA Coulochem II instrument. Lower limit of detection is 10 pg/mL (0.06nmol/L and 55pmol/L, respectively). CVs were 7-11% and 6-7% on 2 controls. Catecholamine samples were collected in EDTA tubes and immediately frozen. This method was determined to not have any difference in values vs. when collected in EDTA-Na bisulfite tubes.

Non-Diabetic Subjects: Studies in 14 non-diabetic adolescents between 12-17 years of age (14.8$\pm$2.1 years, mean$\pm$SD) were performed at the Children's Hospital of Pittsburgh (Pittsburgh) under a protocol approved by its IRB between 1999 and 2002. Subjects were studied in the morning following an overnight fast. Two intravenous catheters, one for blood sampling and one for glucose and insulin infusion, were inserted in the non-dominant arm. After obtaining baseline blood samples for glucose and catecholamines, intravenous insulin infusion was infused at a rate of 0.1 unit $\cdot$ kg^{-1} $\cdot$ h^{-1} throughout the study. Glucose levels were maintained at 103$\pm$6mg/dL (mean $\pm$ SD) for

60 minutes by a variable rate infusion of 20% dextrose. Subsequently, glucose levels were allowed to fall to 64±4mg/dL by decreasing the intravenous glucose infusion over a 20 to 25-minute period. The hypoglycemic nadir was maintained for 60 minutes. Blood was withdrawn every 15 minutes for measurement of plasma epinephrine concentrations.

Epinephrine concentrations on 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^{o}C. A subset (N=18) of these samples was re-assayed 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 186pg/mL. In the 9 samples obtained during the one hour hypoglycemic phase of the Pittsburgh study, the original median peak plasma epinephrine concentration was 312pg/ml (range 126-848) versus 186pg/ml (range 10-758) on re-analysis in the DirecNet laboratory.

Statistical Methods: Based on the study by Jones et al (11), 50 subjects (25 in each of the two age groups with T1DM) 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 300pg/mL with a standard deviation of 306pg/mL. The study was discontinued following 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 2-sample t-test. Analyses involving plasma glucose concentrations utilized laboratory rather than meter glucose values. Time intervals between the <90mg/dL and post glucose treatment blood samplings were compared using paired t-test. The plasma glucose threshold that stimulated a counter-regulatory 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 26pg/mL for epinephrine, 44pg/mL for norepinephrine, 0.83ug/dL for cortisol, 1.2ng/mL for GH and 16pg/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 analysis of covariance (ANCOVA) model based on van der Waerden normal scores and adjusted for the corresponding baseline value.

The study was completed by 30 subjects with T1DM. One in each age group was not included in the analysis because of blood sampling problems.

Since the Pittsburgh study used a one-step hypoglycemic clamp, lowering glucose in one step from ~100 to 60mg/dL, plasma epinephrine responses in this study were used only to describe the magnitude of the plasma epinephrine responses in non-diabetic subjects.

RESULTS

Primary analysis included 14 young children (4 to 7 years old; duration of diabetes 3.3±1.1 years) and 14 adolescents (12 to 17 years old; duration of diabetes 6.6±3.4 years, p=0.003) with T1DM. Twenty-one percent were female and 86% were Caucasian in young children versus 43% and 100% correspondingly in adolescents. Mean HbA1c was 7.7 ± 0.6% in young children and 7.6±0.8% in adolescents. The 14 non-diabetic adolescents studied in Pittsburgh were 29%

female and 93% Caucasian. HbA1c for all these subjects was less than 6.1%.

Plasma Glucose during Hypoglycemia Testing in T1DM Subjects: Individual baseline and nadir plasma glucose concentrations in the patients with T1DM are shown in the Figure. Plasma glucose was reduced gradually to a mean nadir concentration of 61mg/dL in the young children and 59mg/dL in adolescents. Nadir glucose concentration was <60mg/dL in 8 of 14 subjects in each group and between 60 and <70mg/dL in 5 subjects in each group. In only one child and one adolescent was the nadir greater than 70mg/dL (i.e., 73 and 74mg/dL). The mean time interval between <90mg/dL and post glucose treatment blood sampling tended to be longer in adolescents than in younger children (71 versus 53 minutes, P=0.06).

Counter-regulatory Hormone Responses inT1DM Subjects: Individual baseline and peak plasma epinephrine concentrations inT1DM subjects are also shown in the Figure. 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 56-67mg/dL in the young children and 58-74mg/dL in the adolescents. Four young children and 5 adolescents did not manifest an epinephrine response until plasma glucose fell to <60mg/dL. Only 2 children and no adolescents had 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, GH and glucagon were not different in young children and adolescents (Table 2). In addition, the change in hormone concentrations and the number of subjects that had a response in each group was not different. In contrast, the median plasma epinephrine concentrations in non-diabetic subjects rose from 77pg/mL to 582pg/mL. in response to hypoglycemia..

Symptom Scores: Table 3 shows the responses to the question "I feel like my glucose is low" by the adolescents and by parents of the young children to the question "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 percent of adolescents who reported more than minimal symptoms of hypoglycemia increased when glucose levels fell below 70mg/dl.

DISCUSSION

The principal aim of this study was to compare the epinephrine responses to hypoglycemia in young children and adolescents with T1DM. 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 counter-regulatory hormone responses between the two groups. The nadir glucose tended to be achieved faster in young children but the rate of glucose fall has not been shown to affect counter-regulatory 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 <60mg/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 T1DM would be similar to the response that had been reported in non-diabetic subjects and reduced by up to 50% in young children with T1DM. Previous studies of plasma epinephrine responses to hypoglycemia carried out more than 15-20 years ago (11,12) demonstrated that in non-diabetic children 8-18 years of age, plasma epinephrine concentrations rose to more than 600pg/mL, when plasma glucose was lowered below 60mg/dL. Youth with poorly-controlled T1DM (mean HbA1c 15.1%) had an even higher threshold for release of epinephrine but the peak epinephrine response was similar to that in non-diabetic subjects (11). These poorly-controlled adolescents did not have hypoglycemia unawareness as confirmed by their symptom scores, which were similar to the non-diabetic controls. In contrast, 29% of our adolescents reported little or no symptoms of hypoglycemia with plasma glucose <60mg/dL.

Since the epinephrine responses observed in our adolescents with T1DM were so dramatically different from those previously reported in diabetic and non-diabetic 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 non-diabetic adolescents, which showed epinephrine responses that were similar to the studies (11,12) were available for re-analysis. 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 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 T1DM have demonstrated that recent antecedent hypoglycemia impairs the counter-regulatory 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 counter-regulatory 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 T1DM (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 counter-regulatory defense mechanisms are impaired in children and adolescent with T1DM 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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REFERENCES

1. The Diabetes Control and Complications Trial Research Group: Hypoglycemia in the Diabetes Control and Complications Trial. *Diabetes* 46:271-286, 1997

2. Jones TW, Davis EA: Hypoglycemia in children with type 1 diabetes: current issues and controversies. *Pediatr Diabetes* 4:143-150, 2003

3. Davis EA, Jones TW: Hypoglycemia in children with diabetes: incidence, counterregulation and cognitive dysfunction. *J Pediatr Endocrinol Metab* 11:177-182, 1998

4. Bolli GB, De Feo P, De Cosmo S, Perriello G, Ventura MM, Massi Benedetti M, Santeusanio F, Gerich J, Brunetti P: A reliable and reproducible test for adequate glucose counterregulation in type 1 diabetes mellitus. *Diabetes* 33:732-737, 1984

5. Dagogo-Jack SE, Craft S, Cryer PE: Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus. *J Clin Invest* 91:819-828, 1993

6. Davis MR, Mellman M, Shamoon H: Further defects in counterregulatory responses induced by recurrent hypoglycemia in IDDM. *Diabetes* 41:1335-1340, 1992

7. Davis SN, Galassetti P, Wasserman DH, Tate D: Effects of antecedent hypoglycemia on subsequent counterregulatory responses to exercise. *Diabetes* 49:73-81, 2000

8. Sherwin RS: Bringing light to the dark side of insulin: a journey across the blood-brain barrier. *Diabetes* 57:2259-2268, 2008

9. Neese JW, Duncan P, Bayse D, Robinson M, Cooper T, Stewart C: Development and evaluation of a hexokinase/glucose-6-phosphate dehydrogenase procedure for use as a national glucose reference method. Atlanta, Centers for Disease Control, 1976

10. Passey RB, Gillum RL, Fuller JB, Urry FM, Giles ML: Evaluation and comparison of 10 glucose methods and the reference method recommended in the proposed product class standard (1974). *Clin Chem* 23:131-139, 1977

11. Jones TW, Boulware SD, Kraemer DT, Caprio S, Sherwin RS, Tamborlane WV: Independent effects of youth and poor diabetes control on responses to hypoglycemia in children. *Diabetes* 40:358-563, 1991

12. Amiel SA SD, Tamborlane WV, DeFronzo RA, Sherwin RS.: The rate of glucose fall does not affect the counterregulatory hormone responses to hypoglycemia in normal and diabetic man. *Diabetes* 36:518-522, 1987

13. Fanelli C, Epifano L, Rambotti AM, Pampanelli S, Di Vincenzo A, Modarelli F, Lepore M, Annibale B, Ciofetta M, Bottini P, Porcellati F, Scionti L, Santeusanio F, Brunetti P, Bolli GB: Meticulous prevention of hypoglycemia (near-)normalizes magnitude and glycemic thresholds of neuroendocrine responses to, symptoms of, and cognitive function during hypoglycemia in intensively treated patients with IDDM of short duration. *Diabetes* 42:1683-1689, 1993

14. Diabetes Research in Children Network (DirecNet) Study Group: The effects of aerobic exercise on glucose and counter-regulatory hormone concentrations in children with type 1 diabetes. *Diabetes Care* 29:20-25, 2006

15. Diabetes Research in Children Network (DirecNet) Study Group: Prevention of Hypoglycemia during exercise in children with type 1 diabetes by suspending basal insulin. *Diabetes Care* 29:2200-2204, 2006

16. Diabetes Research in Children Network Study Group: Continuous glucose monitoring in children with type 1 diabetes. J Pediatr 151:388-393, 2007

Table 1. Plasma Glucose concentrations at Epinephrine and Norepinephrine Response

	Epinephrine Response*		Norepinephrine Response*	
	Young children N=14	**Adolescents N=14**	**Young children N=14**	**Adolescents N=14**
Plasma Glucose at time of first response *				
≥90 mg/dL †	2	0	0	2
80-<90 mg/dL	0	0	0	2
70-<80 mg/dL	1	1	1	0
60-<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 Standard Deviations above baseline.
† The maximum lab glucose concentration triggering epinephrine response was 96 mg/dL (from a young child with a baseline value of 125 mg/dL) and 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.

Table 2. Counter-regulatory Hormone Concentrations by Age Group (N=28)

	Young children	**Adolescents**
	median (25th, 75th percentile)	*median (25th, 75th percentile)*
Epinephrine	**N=14**	**N=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)
N (%) increased ≥ 3SDs†	10 (71%)	10 (71%)
Norepinephrine	**N=14**	**N=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)
N(%) increased ≥3SDs†	5 (36%)	6 (43%)
Cortisol	**N=14**	**N=14**
Baseline (ug/dL)	9.4 (8.2, 10.9)	12.7 (10.1, 14.1)
Peak (ug/dL)	10.1(8.5, 11.7)	12.0 (9.8, 14.4)
Change* (ug/dL)	-0.5 (-1.4, +1.9)	+0.1 (-0.6, +0.8)
N(%) increased ≥3SDs†	5 (36%)	3 (21%)
Growth Hormone	**N=14**	**N=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)
N(%) increased ≥3SDs†	9 (64%)	9 (64%)
Glucagon	**N=13‡**	**N=14**
Baseline (pg/mL)	42 (33, 50)	48 (32, 57)
End (pg/mL)	42 (36, 43)	50 (31,57)
Change* (pg/mL)	-3 (-4, +2)	+1 (-2, +9)
N(%) increased ≥3SDs†	1 (8%)	1 (7%)

* defined as peak minus baseline.
† 3 Standard Deviations described in Methods.
‡ samples for glucagon were only drawn at baseline and at Meter glucose value <60mg/dL. One subject was missing the end draw.

Table 3. Scores on Question: "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 (N=13)				Adolescents Subject Response (N=14)			
	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)
Plasma glucose								
Baseline	11	100%	0%	0%	11	82%	18%	0%
>90 mg/dL	5	100%	0%	0%	8*	63%	13%	25%
80-<90 mg/dL	7	100%	0%	0%	12*	75%	8%	17%
70-<80 mg/dL	13*	85%	15%	0%	13*	54%	15%	31%
60-<70 mg/dL	11*	100%	0%	0%	13*	46%	31%	23%
<60 mg/dL	7	71%	29%	0%	7	29%	43%	29%

* include multiple responses from same subject.

Figure. Plasma Glucose Concentrations from Baseline to Nadir and Epinephrine Concentrations from Baseline to Peak
Each line and symbol combination represents a unique subject in glucose and epinephrine plots.