



# Evolution of Abnormal Plasma Glucagon Responses to Mixed-Meal Feedings in Youth With Type 1 Diabetes During the First 2 Years After Diagnosis

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## OBJECTIVE

To examine the evolution of the dysregulated glucagon responses to mixed-meal tolerance tests (MMTTs) in youth with recent-onset type 1 diabetes (T1D).

## RESEARCH DESIGN AND METHODS

MMTTs were performed in 25 youth (9–18 years of age) with 1.5–12 months disease duration (year 1); 22 subjects were restudied 1 year later (year 2). Twenty nondiabetic (ND) control children were also studied.

## RESULTS

In T1D children, MMTT-stimulated increases in glucagon were significantly greater than that in ND children (median increments: year 1, 21 pg/mL [16–30]; year 2, 25 pg/mL [16–30]; ND, 9 pg/mL [5–16];  $P = 0.001$  and  $P < 0.001$ , respectively).

## CONCLUSIONS

In comparison with ND control children, exaggerated plasma glucagon responses to mixed-meal feedings are observed in youth with T1D within the first 2 years of diagnosis. Further studies to determine whether suppression of these abnormal responses may help to improve glycemic control are warranted.

Many patients with type 1 diabetes (T1D) are vulnerable to severe hypoglycemic events because they fail to stimulate glucagon responses to falling blood glucose levels (1,2). Conversely,  $\alpha$ -cell response to amino acid stimulation is exaggerated in T1D, which may contribute to postprandial hyperglycemia (3,4). However, the evolution of this aspect of  $\alpha$ -cell dysfunction beyond the 1st year of T1D in children and adolescents has not been determined or compared with that in nondiabetic (ND) children. This study was undertaken to examine this question and to compare differences in the magnitude of glucagon responses to a standard mixed-meal feeding in T1D and ND children.

## RESEARCH DESIGN AND METHODS

### Subjects

Twenty-five youth with T1D were enrolled at the five centers in the Diabetes Research in Children Network (DirecNet), as part of a study examining the loss of glucagon responses to hypoglycemia (5). They were between 9 and 18 years of age ( $13.4 \pm 2.7$  years) and had a duration of T1D between 1.5 and 12 months.

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\*A complete list of the members of the Diabetes Research in Children Network can be found in the APPENDIX.

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Subjects were recruited into four disease duration bins: 5 subjects with duration of diabetes between 6 and 13 weeks, 7 between 14 and 26 weeks, 7 between 27 and 39 weeks, and 6 between 40 and 52 weeks; 22 of the 25 subjects ( $13.2 \pm 2.7$  years of age) were restudied  $\sim 12$  months later. Twenty healthy, age- and sex-matched ND children ( $13.5 \pm 3.1$  years of age) were also studied. Supplementary Table 1 summarizes the demographic data of the ND children to the T1D subjects on study entry.

Each center received approval of the study from their institutional review board. In subjects  $<18$  years of age, informed written consent was obtained from the parents/guardians and written assent from the subjects. Subjects  $\geq 18$  years of age provided their own written consent.

#### Procedures

Mixed-meal tolerance tests (MMTTs) were performed after an overnight fast, with subjects drinking Boost High Protein (24% protein, 55% carbohydrate, and 21% fat) at a dose of 6 mL/kg (maximum dose 360 mL), as previously described (5). Blood was obtained for measurement of plasma glucose, C-peptide, and glucagon levels at  $-10$ ,  $0$ ,  $15$ ,  $30$ ,  $60$ ,  $90$ ,  $120$ ,  $150$ ,  $180$ ,  $210$ , and  $240$  min.

#### Laboratory Methods

C-peptide concentrations were measured at Northwest Lipid Metabolism

and Diabetes Research Laboratories (Seattle, WA) using Tosoh AIA 1800. The lower limit of detection was  $0.05$  ng/mL ( $0.0167$  nmol/L), and the median coefficient of variation was 6.5%. Plasma glucagon concentrations were measured at the University of Minnesota (Minneapolis, MN) 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).

#### Analytical Methods

Standard deviation of baseline glucagon levels (based on the coefficient of variation of split duplicate plasma glucagon measurements) was  $4$  pg/mL. A clinically relevant increase in plasma glucagon levels during the MMTT was defined as a rise in plasma glucagon concentrations  $\geq 12$  pg/mL or greater than three times the standard deviations above baseline concentrations, as previously described (5). Data are presented as median (25–75%). Wilcoxon rank sum tests were performed to compare the plasma glucagon and C-peptide responses in diabetic and ND subjects. Paired Student *t* tests or signed rank tests were used to assess changes in responses in the T1D subjects from year 1 to year 2. Fisher exact test was used to assess the presence of a glucagon response between cohorts of subjects. Calculations were performed using SAS 9.3.

#### RESULTS

Mean plasma glucose, C-peptide, and glucagon levels during the MMTT are shown in Supplementary Fig. 1. Fasting and peak stimulated plasma C-peptide levels during the 1st year ( $0.29$  nmol/L [ $0.25$ – $0.36$ ] and  $0.87$  nmol/L [ $0.57$ – $1.12$ ], respectively) were lower than corresponding values in ND subjects ( $0.43$  nmol/L [ $0.37$ – $0.57$ ] and  $1.92$  nmol/L [ $1.70$ – $2.43$ ];  $P < 0.001$  for both). Nevertheless, only one T1D subject had a peak stimulated C-peptide value  $<0.2$  mmol/L during year 1. By year 2, baseline and peak stimulated C-peptide values fell to  $0.10$  nmol/L ( $0.04$ – $0.18$ ) and  $0.28$  nmol/L ( $0.19$ – $0.54$ ), and seven subjects (32%) had peak stimulated values  $<0.2$  nmol/L.

During years 1 and 2, fasting plasma glucagon levels in T1D subjects were similar to each other ( $44$  pg/mL [ $34$ – $53$ ] and  $46$  pg/mL [ $41$ – $54$ ]) and to baseline concentrations in ND children ( $50$  pg/mL [ $46$ – $57$ ]). As shown in Fig. 1, in years 1 and 2, the peak increments in plasma glucagon levels during the MMTT ( $21$  pg/mL [ $16$ – $30$ ] and  $25$  pg/mL [ $16$ – $30$ ], respectively) were significantly greater than median increments in ND children ( $9$  pg/mL [ $5$ – $16$ ];  $P = 0.001$  and  $P < 0.001$ , respectively). Moreover, all but two subjects with T1D in year 2 had clinically relevant increases in plasma glucagon (i.e.,  $\geq 12$  pg/mL) versus only 40% of control subjects ( $P < 0.001$ ).

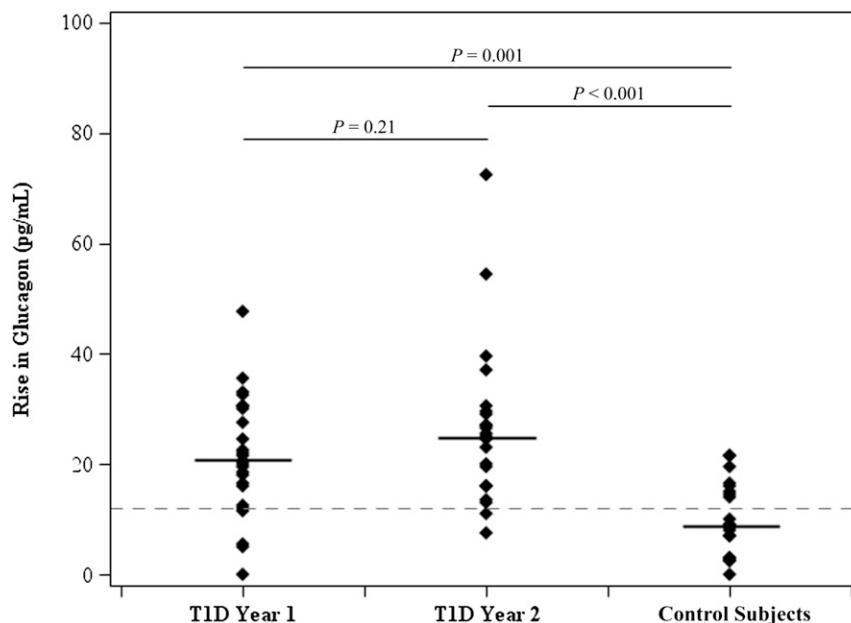


Figure 1—Glucagon response to MMTT.

A progressive rise in glucagon AUC was noted with increasing disease duration of T1D ( $P = 0.009$ ) reaching values that were significantly greater than in ND control subjects ( $P < 0.03$  and  $P < 0.002$  at years 1 and 2, respectively).

## CONCLUSIONS

More than 40 years ago, Unger et al. (6,7) suggested that diabetes was a bi-hormonal disease characterized by too little insulin and too much glucagon. Interest in this question faded during the early intensive treatment era when attention turned to impaired glucagon responses to hypoglycemia (8). The introduction of pharmacological agents that suppress plasma glucagon and difficulties in controlling postprandial hyperglycemia with external closed-loop systems that use the subcutaneous route of insulin delivery (9) have served to refocus attention on the role of  $\alpha$ -cell dysregulation on postprandial hyperglycemia. Although concomitant use of insulin and glucagon during closed-loop control has been explored (10–12), fewer studies have investigated the benefits of glucagon suppression during closed-loop therapy (13).

The most important finding in this study was that exaggerated increases in plasma glucagon levels in response to mixed-meal feedings appear to be fully established during the 1st year of T1D, at a time when almost all of our subjects retained substantial residual C-peptide responses, corroborating the results of Brown et al. (4). Moreover, peak increments in plasma glucagon during the first 2 years of diabetes differed markedly from ND children, in whom plasma glucagon concentrations remained within error of the assay in many subjects. Additionally, the glucagon responses to mixed-meal feedings in our youngsters with T1D approximated the increase in plasma glucagon that we previously reported in ND young adults in response to hypoglycemia (median increment 38 pg/mL) (14). These observations provide a compelling rationale for further studies on the benefits of agents like pramlintide and GLP1 agonists that can suppress abnormal glucagon responses to feeding (15–18) in the treatment of youth with T1D.

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**Author Contributions.** J.S. and V.V.T. researched data, contributed to discussion, wrote the manuscript, and reviewed and edited the manuscript. E.T., L.F., B.B., S.W., N.H.W., A.M.A., C.K., K.J.R., P.C., and R.W.B. researched data, contributed to discussion, and reviewed and edited the manuscript. R.W.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

## Appendix

### Clinical Centers

(Listed in alphabetical order. Personnel are listed as PI for principal investigator, I for co-investigator, and C for coordinator.) Stead Family Department of Pediatrics, University of Iowa Carver College of Medicine: Eva Tsalikian, MD (PI), Michael J. Tansey, MD (I), Julie Coffey, MSN (C), Joanne Cabbage (C), and Sara Salamati (C); Nemours Children's Clinic: Nelly Mauras, MD (PI), Larry Fox, MD (I), Kim Englert, RN (C), Joe Permuy, ARNP (C), and Kaitlin Sikes (C); Division of Pediatric Endocrinology and Diabetes, Stanford University: Bruce Buckingham, MD (PI), Darrell M. Wilson, MD (I), Paula Clinton, RD, CDE (C), and Kimberly Caswell, APRN (C); Department of Pediatrics, Yale University School of Medicine: Stuart Weinzimer, MD (PI), William V. Tamborlane, MD (I), Jennifer Sherr, MD (I), Amy Steffen, BS (C), Kate Weyman, MSN (C), Melinda Zgorski, BSN (C), and Eileen Tichy, MMS (C);

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### Data and Safety Monitoring Board

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