Glucagon Response to Oral Glucose Challenge in Type 1 Diabetes: Lack of Impact of Euglycemia

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OBJECTIVE

Previous studies have demonstrated aberrant glucagon physiology in the setting of type 1 diabetes (T1D) but have not addressed the potential impact of ambient glycemia on this glucagon response. Thus, our objective was to evaluate the impact of euglycemia versus hyperglycemia on the glucagon response to an oral glucose challenge in T1D.

RESEARCH DESIGN AND METHODS

Ten adults with T1D (mean age 56.6 ± 9.0 years, duration of diabetes 26.4 ± 7.5 years, HbA_{1c} 7.5% ± 0.77, and BMI 24.1 kg/m^2 [22.6–25.4]) underwent 3-h 50-g oral glucose tolerance tests (OGTTs) on two separate days at least 24 h apart in random order under conditions of pretest euglycemia (plasma glucose [PG] between 4 and 6 mmol/L) and hyperglycemia (PG between 9 and 11 mmol/L), respectively.

RESULTS

Glycemic excursion on the OGTT was similar between the euglycemic and hyperglycemic tests (P = 0.72 for interaction between time postchallenge and glycemic setting). Interestingly, glucagon levels increased in response to the OGTT under both glycemic conditions (P < 0.001) and there were no differences in glucagon response between the euglycemic and hyperglycemic days (P = 0.40 for interaction between time postchallenge and glycemic setting). In addition, the incretin responses to the OGTT (glucose-dependent insulinotropic polypeptide, glucagon-like peptide-1, glucagon-like peptide-2) were also not different between the euglycemic and hyperglycemic settings.

CONCLUSIONS

In patients with T1D, there is a paradoxical increase in glucagon in response to oral glucose that is not reversed when euglycemia is achieved prior to the test. This abnormal glucagon response likely contributes to the postprandial hyperglycemia in T1D irrespective of ambient glycemia.

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More than 30 years ago, Unger and Orci first proposed that glucagon, in the setting of absolute or relative insulin deficiency, contributes to hyperglycemia in diabetes: the so-called "bihormonal hypothesis" of diabetes pathogenesis (1). Indeed, impaired suppression of glucagon after oral ingestion of glucose has been demonstrated in both type 1 diabetes (T1D) and type 2 diabetes (T2D) (2–4), suggesting that hyperglucagonemia develops in parallel with hypoinsulinemia. Thus, in addition to insulin deficiency, defects in glucagon physiology may be involved in potentiating fasting and postprandial hyperglycemia in diabetes (5).

The mechanism(s) underlying the aberrant glucagon response of the α-cell in diabetes remains ill-defined. Postprandial hypersecretion of glucagon in diabetes has been attributed to the relative lack of intraislet insulin or the insensitivity of the α-cell to the direct inhibitory effects of glucose (6,7). In addition, recent studies have also implicated certain incretin hormones (i.e., glucose-dependent insulinotropic polypeptide [GIP] or glucagon-like peptide [GLP]-2) as potential glucagonotropic mediators of postprandial hyperglucagonemia in both T1D and T2D (8–10). In this context, understanding the impact of glucagon on the dysregulation of glucose homeostasis in diabetes may be important when devising treatment strategies, particularly in light of newer antidiabetes therapies that can target postprandial hyperglucagonemia (incretin mimetics and dipeptidyl peptidase-4 inhibitors).

Previous studies have demonstrated that, in patients with T1D, a paradoxical increase in glucagon occurs in response to an oral glucose challenge (2,8,11). However, these reports have not accounted for the potential effect of prechallenge ambient glycemia and appropriate insulinemia, which could result in differential glucagon responses. Thus, our primary aim was to determine whether euglycemia versus hyperglycemia prior to glucose ingestion in T1D is an important biologic determinant of postprandial glucagon secretion and glucose excursion.

RESEARCH DESIGN AND METHODS

Study Population

The study population consisted of 10 adult patients with physician-diagnosed T1D on continuous subcutaneous insulin infusion (CSII) therapy. Inclusion criteria included age ≥18 years and hemoglobin A_{1c} (HbA_{1c}) at screening between 6.0 and 9.0% inclusive. Exclusion criteria included uncontrolled hypertension, renal and hepatic dysfunction, malignancy, and chronic infection. The study protocol was approved by the Mount Sinai Hospital Research Ethics Board, and all participants provided written informed consent.

Study Design

Each subject reported to the Test Centre at the Leadership Sinai Centre for Diabetes on two separate days after an overnight fast. In random order, patients underwent a 3-h 50-g oral glucose tolerance test (OGTT) on two separate days at least 24 h apart under conditions of pretest euglycemia (plasma glucose [PG] between 4 and 6 mmol/L) and hyperglycemia (PG between 9 and 11 mmol/L), respectively.

On each experimental day, subjects were placed in a recumbent position and an intravenous catheter was placed in one forearm for administration of an insulin infusion. A standardized insulin dosing protocol was used to target either PG level between 4 and 6 mmol/L on the euglycemic day or PG level between 9 and 11 mmol/L on the hyperglycemic day. Subjects discontinued their CSII prior to administration of intravenous insulin. A second intravenous catheter was inserted in the contralateral arm for blood sampling. Capillary blood glucose levels were measured every 10–15 min for 2 h to ensure that the target PG concentration was maintained. Once the PG range was reached, the insulin infusion was set to the usual basal insulin rate received by the participant. Subjects then ingested a 50-g oral glucose solution (50 g of water-free glucose dissolved in 300 mL water) over 5 min. Blood samples were subsequently collected at 30, 60, 90, 120, 150, and 180 min after the glucose challenge for measurement of PG, C-peptide, glucagon, GIP, GLP-1, GLP-2, growth hormone (GH), and free fatty acids (FFAs). Subjects resumed CSII therapy once the experiment was complete.

Laboratory Measurements

C-peptide was measured using the Roche Modular system and electrochemiluminescence immunoassay kit (Roche Diagnostics). Glucagon was measured using radioimmunoassay with double antibody procedure (Siemens Medical Solutions Diagnostics). GIP and GLP-1 were measured using manual multiplex magnetic beads based on luminex xMAP technology (Millipore). GLP-2 was measured using manual sandwich ELISA assay (Millipore). GH was measured using automated sandwich immunoassay (Beckman Coulter). FFA was measured using manual in vitro enzymatic colorimetric assay (NEFA-HR; Wako). β-Hydroxybutyrate was measured using cyclic enzymatic reaction (Autokit 3-HB; Wako). The total area under the curve (tAUC) and incremental AUC (iAUC) of each PG, C-peptide, glucagon, GIP, GLP-1, GLP-2, GH, and FFA during the OGTT were calculated using the trapezoidal rule.

Statistical Analyses

The sample size of 10 participants provided 80% power to detect a 2.5 pg/mL difference in mean glucagon between the two test conditions, assuming a correlation of $r = 0.7$ between observations at seven time points during the OGTT.

All analyses were conducted using SPSS 18.0 (Chicago, IL). Continuous variables were tested for normality of distribution, and natural log transformations of skewed variables were used where necessary. Variables with normal distribution are presented as mean ± SD, and those with nonnormal distribution are presented as median (25th–75th).

For each analyte (PG, glucagon, GIP, GLP-1, GLP-2, GH, and FFA), generalized estimating equation models were then constructed to assess for *1*) an effect of time postchallenge, *2*) an effect of glycemic setting (euglycemic vs. hyperglycemic), and *3*) a time postchallenge–by–glycemic setting interaction (indicating a significant

difference between the two settings in the rate of change in response over time) (Figs. 1*A* and *B* and 2). In addition, for each analyte, tAUC and iAUC in response to OGTT were compared between the euglycemic and hyperglycemic settings by paired *t* test (Table 2). In order to evaluate the changes in glucagon concentrations per increase of 1 mmol/L glucose in response to the OGTT, we also calculated the change in glucagon concentration divided by the change in glucose as previously described (12) (Fig. 1*C*). $P < 0.05$ was considered statistically significant.

RESULTS

The study population consisted of 10 white participants (4 males) with mean age 56.6 ± 9.0 years, duration of diabetes 26.4 ± 7.5 years, HbA_{1c} 7.5 ± 0.77%, and BMI 24.1 kg/m^2 (22.6–25.4). One participant had residual C-peptide at baseline that increased in response to the OGTT (baseline C-peptide 11 pmol/L and peak postchallenge C-peptide 24 pmol/L), while two participants had residual baseline C-peptide but no response to the OGTT (participant 1, baseline C-peptide 6 pmol/L and peak postchallenge C-peptide 6 pmol/L; participant 2, baseline C-peptide 12 pmol/L and peak postchallenge C-peptide 13 pmol/L) (data shown for normoglycemic day). All participants had normal renal and liver function (Table 1).

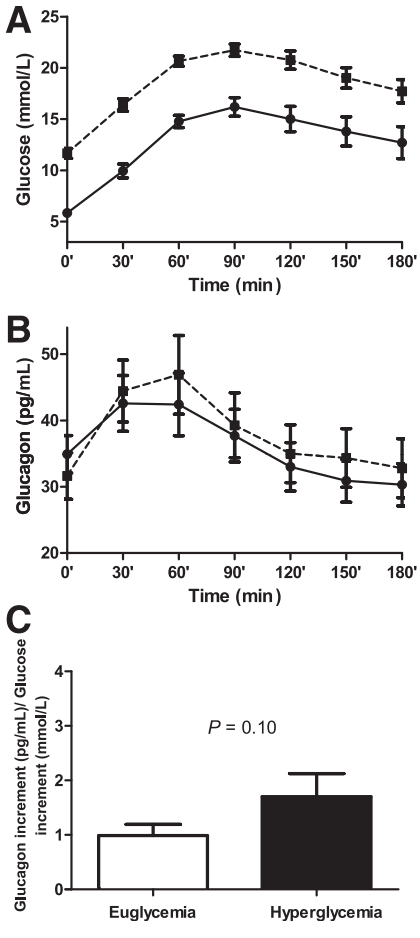

Figure 1—PG (*A*) and glucagon (*B*) levels in response to 50-g OGTT in the setting of euglycemia (solid line) and hyperglycemia (dashed line). *C*: Glucagon increment/ glucose increment in response to the OGTT in the setting of euglycemia and hyperglycemia.

Glucose

Figure 1*A* shows the PG curves in response to the OGTT under both euglycemic and hyperglycemic conditions. As expected, the PG values in response to 50 g glucose were increased at all time points during the hyperglycemic test compared with the euglycemic day ($P < 0.001$); this difference was also evident when tAUCs were compared (Table 2). However, glycemic excursion in response to the OGTT was similar between the euglycemic and hyperglycemic settings as demonstrated by *1*) the absence of significance for the interaction term time postchallenge × glycemic setting ($P = 0.72$) and *2*) the absence of any difference in iAUC glucose between the euglycemic and hyperglycemic settings (43.9 ± 19.4 vs. 43.2 ± 15.0 mmol/L · 180 min, $P = 0.88$) (Table 2).

Glucagon

Figure 1*B* shows the glucagon curves in response to the OGTT under both euglycemic and hyperglycemic conditions. Interestingly, the glucagon levels increased in response to the OGTT in both glycemic settings ($P < 0.001$). However, there were no differences in glucagon levels between the euglycemic and hyperglycemic days ($P = 0.75$) or in tAUC glucagon (euglycemia 219.3 ± 66.7 vs. hyperglycemia 232.1 ± 84.5 pg/mL · 180 min, $P = 0.43$) (Table 2).

The glucagon excursion in response to the OGTT was compared between euglycemic and hyperglycemic days using three approaches. First, we compared the longitudinal changes in glucagon through the interaction term time postchallenge × glycemic setting and found no differences in glucagon response between the euglycemic and hyperglycemic settings ($P = 0.40$). Second, we compared the iAUC glucagon between the euglycemic and hyperglycemic days, which showed a greater increment in glucagon under hyperglycemic conditions compared with the euglycemic setting (39.5 pg/mL · 180 min [−3.6 to 63.2] vs. 4.3 pg/mL · 180 min [−10.6 to 21.7], $P = 0.03$). Finally, in order to determine whether there was differential suppression of glucagon per glucose increment during the OGTT depending on baseline glycemia, we compared the ratio of change of glucagon with change of glucose between the glycemic settings. Figure 1*C* shows that there was a paradoxical increment in glucagon in response to the OGTT under both euglycemic and hyperglycemic conditions and that there was no difference in this increment between the two settings ($P = 0.10$). In sensitivity analyses, we repeated the analyses described above including only the seven participants without residual C-peptide, and the results did not change (data not shown).

Incretins (GIP, GLP-1, and GLP-2)

Figure 2*A*–*C* shows the response curves during the OGTT for GIP, GLP-1, and GLP-2, respectively, under both euglycemic and hyperglycemic conditions. Circulating levels of all of these incretin hormones increased in response to the OGTT in both the euglycemic and hyperglycemic settings (all $P < 0.01$). In addition, there were no differences in GIP, GLP-1, and GLP-2 between the euglycemic and hyperglycemic days (all $P > 0.41$), which was also evident when tAUCs were compared between the euglycemic and hyperglycemic settings: GIP (euglycemia 452.0 ± 156.2 vs. hyperglycemia 497.7 ± 133.11 pg/mL · 180 min, $P = 0.32$), GLP-1 (euglycemia 289.12 ± 62.1 vs. hyperglycemia 279.15 ± 68.33 pg/mL · 180 min, $P = 0.67$), and GLP-2 (euglycemia 8.9 ± 10.4.1 vs. hyperglycemia 9.6 ± 10.6 ng/mL · 180 min, $P = 0.53$) (Table 2).

When incretin excursion in response to the OGTT was compared between the

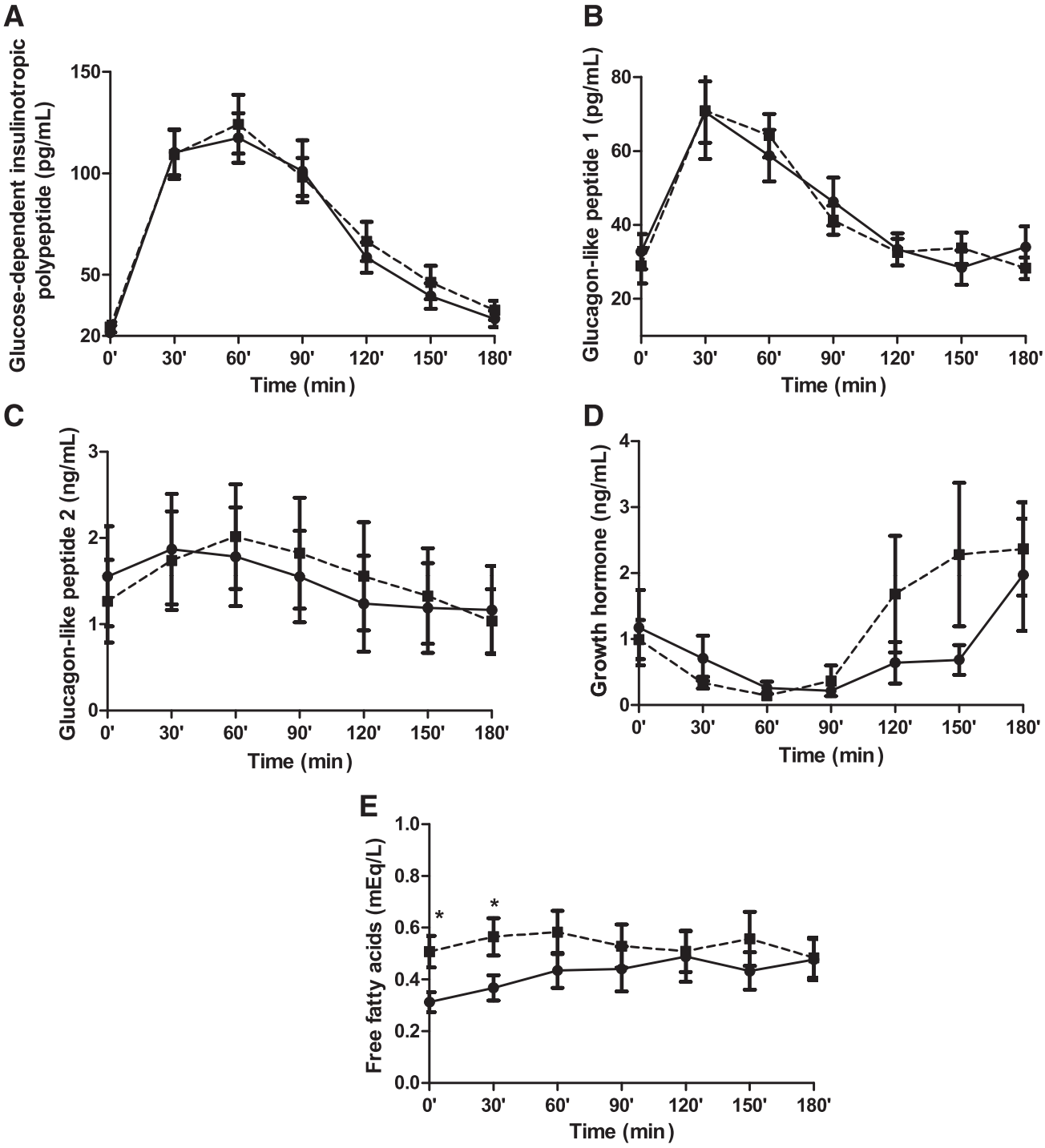

Figure 2—Response to 50-g OGTT in the setting of normoglycemia (solid line) and hyperglycemia (dashed line) for GIP (*A*), GLP-1 (*B*), GLP-2 (*C*), GH (*D*), and FFAs (*E*).

euglycemic and hyperglycemic settings, there were no differences in each response *1*) when the interaction term time postchallenge × glycemic setting was evaluated for each incretin (all $P > 0.27$) and also *2*) when iAUCs were compared between euglycemia and hyperglycemia settings: GIP (euglycemia 322.8 ± 108.3 vs. hyperglycemia 347.0 ± 112.1 pg/mL · 180 min, $P = 0.66$), GLP-1 (euglycemia 79.8 ± 51.8 vs. hyperglycemia 106.4 ± 103.1 pg/mL · 180 min, $P = 0.39$), and GLP-2 (euglycemia: 0 ng/mL · 180 min [−1.0 to 0.52] vs. hyperglycemia 0.38 ng/mL · 180 min [0–5.2], $P = 0.07$) (Table 2).

GH

Figure 2*D* shows the GH curves in response to the OGTT under both euglycemic and hyperglycemic conditions. There were no differences in GH between the euglycemic and hyperglycemic days ($P = 0.41$), which was also evident when tAUCs were

Table 1—Clinical characteristics of included participants (n = 10)

Age (years)	56.6 ± 9.0
Males, *n* (%)	4 (40)
White, *n* (%)	10 (100)
Diabetes duration (years)	26.4 ± 7.5
BMI (kg/m^2)	24.1 (22.6–25.4)
Waist circumference (cm)	89.1 ± 11.8
Systolic blood pressure (mmHg)	116.6 ± 16.1
Diastolic blood pressure (mmHg)	68.5 ± 9.5
Heart rate (bpm)	67 ± 14
Time on insulin pump (years)	10 (6–13.5)
Basal insulin dosage (UI/h)	0.7 ± 0.3
HbA_{1c} (%)	7.5 ± 0.77
HbA_{1c} (mmol/mol)	59 ± 8.4
Creatinine (μmol/L)	69.5 (60.5–71.5)
ALT (IU/L)	18.4 ± 5.6
AST (IU/L)	21.1 ± 4.3
ALP (IU/L)	77.7 ± 20.6
Bilirubin (μmol/L)	8.6 ± 3.8

Data are expressed as mean ± SD or median (25th–75th). ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase.

compared between euglycemia and hyperglycemia settings (4.27 [1.07–6.22] vs. 3.88 [1.46–9.65] ng/mL · 180 min, P = 0.57). In addition, the GH response to the OGTT was similar between glycemic settings as demonstrated by *1*) the nonsignificant interaction term time × glycemic setting (P = 0.33) and *2*) no differences in iAUC (euglycemia −2.9 ± 9.8 vs. hyperglycemia 0.53 ± 8.4 ng/mL · 180 min, P = 0.35).

FFA and β-Hydroxybutyrate

Figure 2*E* shows the FFA curve in response to the OGTT in both euglycemic and hyperglycemic settings. As expected, FFAs were increased at baseline and 30 min postchallenge on the hyperglycemic day compared with euglycemic day (both $P < 0.05$). However, tAUC FFA was similar between euglycemic and hyperglycemic settings (2.55 ± 1.19 vs. 3.23 ± 1.35 mEq/L · 180 min, P = 0.29). The FFA response to the OGTT was not different between the euglycemic and hyperglycemic settings (interaction term time postchallenge × glycemic setting, P = 0.25). This was also evident when iAUC of FFA was compared between euglycemic and hyperglycemic settings (0.68 ± 1.0 vs. 0.19 ± 1.2 mEq/L × 180 min, P = 0.37) (Table 2).

Baseline β-hydroxybutyrate was not different between the euglycemic and hyperglycemic settings (euglycemia 0.26 mmol/L [0.09–0.83] vs. hyper glycemia 0.58 mmol/L [0.09–1.83], P = 0.47).

CONCLUSIONS

In this study, we demonstrate that, in adult patients with long-standing T1D, there is a paradoxical increment in glucagon in response to oral glucose irrespective of ambient glycemia. The achievement of euglycemia, and thus appropriate prechallenge basal insulinemia, prior to the OGTT was not sufficient to reverse the abnormal glucagon response to an oral glucose challenge. Of note, the dynamic changes in incretin hormones (GIP, GLP-1, and GLP-2), GH, and FFA were as expected and also not different between the euglycemic and hyperglycemic conditions.

Previous studies in T1D have demonstrated a dysregulation of glucagon secretion in diverse clinical scenarios. Time course studies in new-onset T1D have shown that declining β-cell function and insulin secretion are associated with increasing postprandial glucagon and glucose excursion in response to a mixed-meal stimulus (13,14). The potential clinically relevant impact of chronic hyperglycemia and hypoinsulinemia on abnormalities of glucagon secretion is also supported by the observation that elevations in glucagon were associated with increased 2-h postchallenge glucose and HbA_{1c} in a study of 60 children with T1D (15). In addition, experimental studies that evaluated glucagon response to OGTT have also demonstrated an abnormal increase in glucagon after carbohydrate ingestion (8,11). Our results reinforce these previous reports by showing a paradoxical rise in glucagon in response to oral glucose ingestion and, most importantly, extend this literature by demonstrating that euglycemia prior to the OGTT does not modify this aberrant glucagon physiology.

Our results provide further insight regarding the mechanism of glucagon hypersecretion in patients with T1D. Although the mechanism through which hyperglucagonemia occurs in diabetes is complex, certain metabolic pathways have been implicated such as the paracrine regulation of glucagon secretion by somatostatin and insulin (16). Indeed, the most widely accepted mechanism is that the inappropriate elevation in glucagon during hyperglycemia is due to the lack of intraislet insulin to restrain the glucose

Table 2—Comparisons of tAUC and iAUC for each analyte under euglycemic and hyperglycemic conditions (n = 10)

	Euglycemia	Hyperglycemia	P
tAUC			
Glucose (mmol/L · 180 min)	78.9 ± 15.6	113.2 ± 10.6	<0.001
Glucagon (pg/mL · 180 min)	219.3 ± 66.7	232.1 ± 84.5	0.43
GLP-1 (pg/mL · 180 min)	289.12 ± 62.1	279.15 ± 68.33	0.67
GLP-2 (ng/mL · 180 min)	8.9 ± 10.4	9.6 ± 10.6	0.53
GIP (pg/mL · 180 min)	452.0 ± 156.2	497.7 ± 133.11	0.32
FFA (mEq/L · 180 min)	2.55 ± 1.19	3.23 ± 1.35	0.29
GH (ng/mL · 180 min)	4.27 (1.07–6.22)	3.88 (1.46–9.65)	0.57
iAUC			
Glucose (mmol/L · 180 min)	43.9 ± 19.4	43.2 ± 15.0	0.88
Glucagon (pg/mL · 180 min)	4.3 (−10.6 to 21.7)	39.5 (−3.6 to 63.2)	0.028
GLP-1 (pg/mL · 180 min)	79.8 ± 51.8	106.4 ± 103.1	0.39
GLP-2 (ng/mL · 180 min)	0 (−1.0 to 0.52)	0.38 (0–5.2)	0.07
GIP (pg/mL · 180 min)	322.8 ± 108.3	347.0 ± 112.1	0.66
FFA (mEq/L · 180 min)	0.68 ± 1.0	0.19 ± 1.2	0.37
GH (ng/mL · 180 min)	−2.9 ± 9.8	0.53 ± 8.4	0.35

Data are means ± SD or median (25th–75th).

effect on α-cells, the so-called "intraislet insulin hypothesis" (7). In other words, in patients with T1D where insulin is deficient, there is a hyperglucagonomia caused by both lack of intraislet insulin and consequent α-cell insensitivity to glucose and insulin (17). However, data from islet transplant recipients have suggested that lack of intraislet insulin is not the sole mechanism of hyperglucagonomia in T1D, since the glucagon response remains abnormal even after the restoration of local insulin secretion (18).

In this context, direct glucagon stimulation by hyperglycemia in T1D was suggested by a study of 257 children with T1D who were evaluated at 1, 6, and 12 months after diagnosis. Using compound symmetrical repeated-measurements models, the authors demonstrated that marked postprandial hyperglycemia was highly associated with increased glucagon levels, suggesting that hyperglycemia directly results in glucagon secretion (13). However, this study did not address the critical question of whether treatment of hyperglycemia and restoration of basal insulin with exogenous insulin therapy result in improvement of the abnormal glucagon secretion in T1D. Our results demonstrate that the euglycemic state achieved through exogenous insulin prior to the oral glucose ingestion was not sufficient to restore a normal glucagon response in patients with long-standing T1D. These results indicate *1*) that glycemic normalization does not reverse the abnormal glucagon secretion in T1D and, possibly, *2*) that current insulin therapies for T1D are not able to impact the glucagon abnormality observed in those individuals. From a clinical perspective, these findings suggest that therapy targeting postprandial hyperglucagonemia (e.g., GLP-1 agonists or dipeptidyl peptidase-4 inhibitors) could play a complementary role in combination with intensive insulin therapy in T1D. Indeed, treatment with either liraglutide (19) or vildagliptin (20) decreases postprandial glucagon levels in these individuals.

The major strength of this study is its novel characterization of the glucagon response to OGTT under distinct glycemic settings: euglycemia and hyperglycemia. As shown in Fig. 1*A*, the protocol was successful in achieving these glycemic conditions before the OGTT. A limitation of our study is that gastric emptying was not assessed, as it is a potential confounder for glucose absorption, gut hormone secretion, and consequent glucagon response. However, all participants denied any relevant gastric symptoms prior to the study, and the crossover design also provided an element of control for this factor. In addition, we should recognize that we evaluated the impact of euglycemia for a short period of time such that the effect of prolonged euglycemia on glucagon response remains uncertain.

In conclusion, our study suggests that an abnormal increment in glucagon secretion in response to OGTT is observed in patients with long-duration T1D irrespective of glycemic setting prior to the test. In addition, incretin, GH, and FFA responses were also similar in both euglycemia and hyperglycemia settings. These results suggest that treating hyperglycemia in T1D with exogenous insulin therapy may not normalize the aberrant glucagon responses to oral glucose, suggesting a potential role for therapies that aim to suppress glucagon in patients with long-standing T1D.

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Author Contributions. C.K.K. performed statistical analyses, wrote the first draft of the manuscript, collected data, contributed to the analysis and interpretation of data, and contributed to the revision of the manuscript for important intellectual content. C.A.B. contributed to the study conception and design, collected data, contributed to the analysis and interpretation of data, and contributed to the revision of the manuscript for important intellectual content. P.V.N. collected data, contributed to the analysis and interpretation of data, and contributed to the revision of the manuscript for important intellectual content. R.R. and B.Z. contributed to the study conception and design, contributed to the analysis and interpretation of data, and contributed to the revision of the manuscript for important intellectual content. B.Z. 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.

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