International Journal of Diabetes in Developing Countries (April–June 2025) 45(2):309–317
https://doi.org/10.1007/s13410-024-01367-9

ORIGINAL ARTICLE

The association of fasting and postprandial GIP and glucagon levels with glycemic variability evaluated by flash glucose monitoring system in type 1 diabetes

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Received: 15 September 2023 / Accepted: 13 June 2024 / Published online: 2 July 2024

Abstract

Objective This study is to explore the relationship between serum fasting and postprandial glucose-dependent insulinotropic polypeptide (GIP) and glucagon levels and glycemic variability in type 1 diabetes (T1D).

Methods Twenty patients with T1D and 20 healthy controls were included in the study. Parameters of glycemic variability were obtained from 14-day sensor data provided by a flash glucose monitoring system. A mixed meal at breakfast was provided for the participants and fasting, and postprandial blood samples were collected to evaluate serum GIP and glucagon levels.

Results There were no significant differences in terms of fasting or postprandial GIP and glucagon levels between the two groups ($p > 0.05$). However, a negative correlation between duration of diabetes and fasting GIP levels ($r = -0.510, p = 0.02$) and a positive correlation between total daily insulin dose and fasting and postprandial GIP levels ($r = 0.48$, $p = 0.03$) were found in patients with T1D. Postprandial glucagon correlated positively with time above range (TAR 180) ($r = 0.56$, $p < 0.001$) and negatively with the number of hypoglycemic events ($r = -0.46, p = 0.03$).

Conclusion Our results indicate that serum GIP was associated with the duration of diabetes and daily insulin dose. Moreover, postprandial glucagon is linked to hyperglycemic and hypoglycemic indices in cases of T1D.

Keywords Type 1 diabetes · Glycemic variability · Flash glucose monitoring systems · GIP · Glucagon

Introduction

Glucagon, which is secreted from the α-cells of the pancreas in the islets of Langerhans, plays a crucial role in maintaining glucose homeostasis. This is achieved by stimulating hepatic glucose production [1]. The balanced secretion of insulin from pancreatic β-cells and glucagon from α-cells is essential for maintaining normal plasma glucose concentrations. The primary regulator of glucagon secretion is circulating glucose. Glucagon release is controlled by both endocrine and paracrine pathways. Hypoglycemia, amino acids, and glucose-dependent insulinotropic polypeptide (GIP) stimulate glucagon release, while hyperglycemia and glucagon-like peptide-1 (GLP-1) inhibit it. Furthermore, factors such as somatostatin, insulin, and amylin paracrinally inhibit the secretion of glucagon [2].

Type 1 diabetes (T1D) is a chronic immune-mediated disease characterized by insulin deficiency due to pancreatic β-cell destruction. However, pancreatic α-cell function

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may also be impaired in such cases, and the aberrant release of glucagon may negatively affect blood glucose regulation [3]. The substantial role of glucagon in the maintenance of euglycemia was demonstrated in previous studies on T1D [4, 5]. In non-diabetic humans, fasting glucagon concentrations increase as β-cell function declines [6]. In the postprandial state, glucagon secretion has been shown to be unaltered or even suppressed after mixed meals in non-diabetic individuals [7]. Nevertheless, in the absence of β-cell insulin secretion, increased α-cell glucagon secretion after carbohydrate-enriched liquid meal has been reported in children with new-onset T1D [8]. In patients with T1D, dysregulation in glucagon secretion is associated with two clinical manifestations: postprandial hyperglucagonemia and impaired glucagon counter regulation to hypoglycemia. Lack of postprandial glucagon suppression contributes to fasting and postprandial hyperglycemia in patients with T1D [9]. However, the normal glucagon response after parenteral administration of glucose in both type 2 diabetes (T2D) and T1D indicates that hormones secreted from the gastrointestinal tract may also have a role in glucagonotropic signaling [10, 11].

Physiologically, hypoglycemia is prevented through various mechanisms. These include the reduction of insulin secretion from β-cells, decreased glucose uptake in peripheral tissues, increased glucagon secretion from α-cells, increased hepatic glucose output, and increased sympathetic neural response and adrenomedullary epinephrine secretion [12]. In cases of both T1D and advanced T2D, hypoglycemia is primarily caused by medical treatment. It occurs due to the interaction between therapeutic hyperinsulinemia and a weakened defense against decreasing blood glucose levels [13]. This situation tends to worsen with the duration of type T1D, likely due to a combination of defective α-cells and reduced α-cell mass [4, 12]. On the other hand, impaired glucagon response during hypoglycemia has been shown even in patients with short durations of T1D [14]. Consequently, inadequate glucagon secretion is considered to be one of the main factors contributing to treatment-related hypoglycemia in T1D [12].

In patients with T2D, the incretin effect is reduced, whereas it is somewhat complicated in T1D. GIP, which is considered to be the most powerful incretin hormone, is secreted at higher levels with oral food intake and the absorption of glucose, protein, and fat. GLP-1 and GIP play important roles in the process of glucoregulation and contribute to 25% to 70% of the postprandial insulin response by enhancing glucose-induced insulin secretion [15, 16]. Acting as an anabolic hormone, GIP, unlike GLP-1, increases glucagon activity in euglycemic or hypoglycemic states [17]. Studies of animal models have shown that GIP is more effective in α-cells in stimulating glucagon secretion when glucose concentrations are lower, whereas in β-cells, GIP is more effective in stimulating insulin secretion in the event of hyperglycemia [18]. GIP has been observed to increase pancreatic glucagon responses during hypoglycemia in patients with T1D [19]. Diminished GIP responses in cases of food consumption have been reported in patients with T1D [20]. Increased GIP levels in patients with long-term T1D compared to healthy individuals were also reported in a previous study [21].

Glycated hemoglobin (HbA1c) only reflects average glucose levels over the past 2–3 months and does not account for fluctuations in glucose levels or glycemic deviations, known as glycemic variability [22]. The association of glycemic variability with the development of acute and chronic complications of diabetes has been shown in various studies [23, 24]. In recent years, research efforts worldwide have focused on screening for intrinsic risk factors such as glucagon and incretin hormones and exogenous risk factors contributing to increased glycemic variability. The goal of such research is to guide the development of appropriate therapeutic regimens that can improve glycemic variability. Continuous glucose monitoring (CGM) systems facilitate an individual approach to patients by providing a more comprehensive glycemic profile [25]. The relationship between glycemic variability and glucagon seems complex and has been evaluated in relatively few studies on T1D. The area under the curve (AUC) of glucagon has been reported to be related to glycemic variability in patients with T1D [26]. Furthermore, arginine-stimulated glucagon secretion has been shown to be associated with glycemic instability in patients with T1D who have no residual β-cell function, suggesting that the effect of glucagon is independent of endogenous insulin [27]. On the other hand, there are limited human studies in the literature addressing the possible effects of GIP on glycemic variability in T1D. A study conducted with men with T1D showed that GIP infusion did not affect most of the parameters of glycemic variability but did reduce time above range (TAR) [28]. Another study showed that exogenous GIP infusions for patients with T1D attenuated postprandial plasma glucose fluctuations without significantly increasing the glucose requirement to prevent hypoglycemia [29]. Although these studies evaluated the effects of supraphysiological GIP infusion, it has not been demonstrated yet how glycemic variability is affected by fluctuations in endogenous GIP levels.

In the present study, we aimed to determine fasting and postprandial GIP and glucagon concentrations as responses to the ingestion of a mixed meal among patients with T1D in comparison to healthy control subjects. Second, the associations of these hormones with glycemic variability as well as clinical parameters were also evaluated after controlling for some clinical and laboratory parameters including age, insulin dose, HbA1c level, and duration of diabetes.

Materials and Methods

Study design

Patients aged 18 years and over who were followed in the endocrinology and metabolism outpatient clinic of our center were included in this study. Patients with previous chronic kidney disease, chronic liver disease, previous history of gastrointestinal surgery, any psychiatric or neurological disease, a diagnosis of malignancy, chronic drug use (especially drugs that affect incretin release), recent use of steroid-containing drugs, chronic alcohol consumption, pregnancy, or breastfeeding were excluded from the study. Patients who were under endocrinology follow-up at our center, diagnosed with T1D for at least 1 year, and not experiencing any acute problems that would affect their glycemic regulation were included in the study.

It has been observed that there may be differences in GIP and glucagon levels measured after the oral glucose tolerance test (OGTT) in patients with prediabetes compared to normoglycemic individuals [30]. Therefore, we excluded individuals with impaired fasting glucose, impaired glucose tolerance, and/or HbA1c levels between 5.7% and 6.4% who were considered prediabetic from our control group, as those variables could have affected our results [31].

This research was designed as a cross-sectional single-center study. Twenty patients with T1D and 20 healthy controls were included. All participants underwent a mixed meal test and were fitted with a CGM device. A mixed meal test and measurements of glucagon and GIP levels were performed on the 5th through 7th days when the sensor was active. The mixed meal test was performed following an overnight fast of at least 9 h. The patients were given a sample breakfast list (200 mL cow's milk + 80 g feta cheese or 60 g cheddar cheese + 10 olives or 2 whole walnuts + 75 g bread + 1 serving of fruit) containing 50% carbohydrates, 36% fat, and 14% protein, totaling 550 kcal. Consumption of tea, coffee, chewing gum, and alcohol was prohibited before the test. Blood samples were drawn before the test and 2 h later. The samples were centrifuged at 3000 rpm for 10 min at -80 °C and preserved for further analyses. Glucagon and GIP levels were studied from the serum samples (Elisa Kit, Raybio, USA). Fasting glucose, HbA1c, lipid levels, and kidney, liver, and thyroid function tests of all participants were evaluated.

A Free Style Libre (Abbot, USA) CGM sensor was attached to the back of the upper arm for continuous glucose measurements for all participants. Participants were instructed to have the sensors read at regular intervals in order to obtain sufficient data. Fourteen days of sensor data were recorded. The CGM data of patients with active sensor time of 70% or more were evaluated. Time in range (TIR) was defined as the period when glucose was between 70 and 180 mg/dL, time above range 180 (TAR 180) when glucose was between 180 and 250 mg/dL, and time above range 250 (TAR 250) when glucose was above 250 mg/dL. Time when glucose was below 70 mg/dL was expressed as time below range 70 (TBR 70) and time when glucose was below 54 mg/dL was expressed as time below range 54 (TBR 54). Data on the coefficients of variation (CVs), mean glucose, estimated HbA1c, glucose monitoring indicator, TIR, TAR 180, TAR 250, TBR 70, TBR 54, and number of hypoglycemic events experienced in 14 days were recorded from CGM results.

Statistical analysis

IBM SPSS Statistics 22.0 program was used for statistical analyses and calculations. The distribution of the data was evaluated with the Kolmogorov–Smirnov and Shapiro–Wilk tests. Categorical variables were expressed as numbers and percentages. For comparisons of the T1D and control groups, median, 25th percentile, and 75th percentile values were used. The chi-square test was used to compare categorical variables. Fisher's exact test was used when the conditions for the chi-square test were not met. Comparisons of the two groups were performed according to the distribution of the data. Differences between the patient and control groups were analyzed using the Mann–Whitney *U* test according to the distribution of the data. The relationships between clinical, laboratory, and CGM data of patients with T1D and GIP and glucagon levels were evaluated using the Spearman correlation coefficient for nonparametrically distributed variables. Partial correlation testing was also used for controlling parameters including age, insulin dose, HbA1c level, and duration of diabetes. The significance level was accepted as $p < 0.05$.

Power analysis was performed using the G*Power 3.1 program, taking into account the effect size suggested by Hare et al. while estimating similar parameters according to the literature [11]. The power of the study was determined based on a total of 38 participants with power of 85% and effect size of $d = 1.00$ for GIP and 24 participants with power of 85% and effect size of $d = 1.33$ for glucagon.

Results

Twenty patients with type T1D and 20 healthy control subjects were included in this study. As shown in Table 1, the median ages were 30.5 (25–40) years in the patient group and 31 (31–39) years in the control group. There

Table 1 Demographic and clinical characteristics of the study participants

	Type 1 diabetes	Control	*p*
Age, years	30.50 (25–40)	31.0 (31–39)	0.94
Body mass index, kg/m^2	24.70 (22.0–29.35)	23.50 (20.90–27.20)	0.38
Male/female ratio, *n* (%)	6/14 (70)	6/14 (70)	1.0
Duration of diabetes, years	13.0 (6–20)	-	-
Insulin pump use, *n* (%)	10 (50.0)	-	-
Total daily insulin dose, units	44.0 (32–64)	-	-
HbA1c, %	7.95 (7.7–8.7)	5.40 (5.2–5.5)	< 0.001
Fasting glucose, mg/dL	152.50 (133.0–185.0)	83.0 (76.0–85.0)	< 0.001
Total cholesterol, mg/dL	184.50 (169.0–206.50)	164.0 (145.0–198.0)	0.15
LDL-cholesterol, mg/dL	115.0 (107.30–122.0)	104.0 (87.0–139.0)	0.07
HDL-cholesterol, mg/dL	52.50 (44.5–71.25)	51.0 (41.0–57.0)	0.13
Triglycerides, mg/dL	89.50 (61.30–108.30)	80.0 (59.0–100.0)	0.39
Fasting GIP, pg/mL	62.76 (52.30–71.06)	66.17 (60.55–80.67)	0.31
Postprandial GIP, pg/mL	61.94 (58.40–70.55)	59.28 (52.80–67.60)	0.16
Fasting glucagon, pg/mL	6.29 (5.94–8.20)	6.95 (5.97–12.30)	0.34
Postprandial glucagon, pg/mL	6.46 (6.22–14.60)	6.71 (6.08–12.47)	0.92

Data are expressed as median and 25th–75th quartiles. *HbA1c* hemoglobin A1c, *GIP* glucose-dependent insulinotropic polypeptide

were 6 men (30%) and 14 women (70%) in the patient group and 6 men (30%) and 14 women (70%) in the control group. There were no significant differences in terms of age or sex between the groups ($p > 0.05$). Median BMI values were 24.70 (22.0–29.35) kg/m^2 for the patient group and 23.50 (20.90–27.20) kg/m^2 for the control group, with no significant difference being observed between the groups ($p = 0.38$). Median diabetes duration was 13 (6–20) years, and 2 (0.4%) patients had diabetic complications. The median HbA1c level of the patients was 7.95% or 63 mmol/mol (7.65–8.77% or 60–72 mmol/mol). The median total daily insulin dose was 44 units/day. Ten (50%) patients were using insulin pump therapy. Fasting GIP ($p = 0.31$), postprandial GIP ($p = 0.16$), fasting glucagon ($p = 0.34$), and postprandial glucagon ($p = 0.92$) levels did not differ between the groups.

Table 2 shows the comparison of the flash glucose monitoring system results of the groups. Both groups had sensor activity indicating adequate data recording (median 97% vs. 96.5%). The median blood glucose value calculated according to CGM values was significantly higher among patients with T1D compared to healthy controls (168 (154–177) mg/dL vs. 92 (87.5–94.5) mg/dL, $p < 0.001$). The estimated HbA1c value of the T1D patients was 7.30%; among healthy controls, it was 5.50%. These results were found to be compatible with the HbA1c values measured from blood samples (Table 1). In the group with T1D, the median average glucose level was 168 mg/dL (154–177 mg/dL), and the median CV value, as a metric of glucose variability, was 38.5% (33.5–44.2%). The median percentage of TIR was 57.5% and that of TBR 70 was 4%. TBR 54 was rare. In healthy controls, TIR was 99%, with no sensor glucose values above

Table 2 Comparison of flash glucose monitoring system results between the groups

	Type 1 diabetes ($n = 20$)	Control ($n = 20$)	*p*
Sensor activity, %	97.0 (95–99)	96.50 (89–98)	0.23
Average glucose, mg/dL	168.0 (154–177)	92.0 (87.5–94.5)	< 0.001
Estimated HbA1c, %	7.30 (7–7.4)	5.50 (5.4–5.6)	< 0.001
CV, %	38.50 (33.5–44.2)	14.40 (12.6–17)	< 0.001
Time in range, 70–180 mg/dL, %	57.50 (50–62.5)	99.0 (97–99)	< 0.001
Time below 70 mg/dL, %	4.0 (1.5–5.5)	1.0 (1–3)	0.02
Time below 54 mg/dL, %	0 (0–1.5)	0 (0–0)	0.01
Time above 180 mg/dL, %	24.50 (20.5–29.5)	0 (0–0)	< 0.001
Time above 250 mg/dL, %	12.0 (6.5–16.5)	0 (0–0)	< 0.001
Low events per 2 weeks, *n*	8.0 (4.5–14)	2.50 (1–4)	< 0.001

Data are expressed as median and 25th–75th quartiles. *HbA1c* hemoglobin A1c, *CV* coefficient of variation

180 mg/dL (equivalent to TAR 180 and TAR 250) or below 54 mg/dL (TBR 54).

As indicated in Table 3, in the T1D group, there was a negative correlation between fasting GIP and duration of diabetes ($r = -0.51, p = 0.02$). Total daily insulin dose correlated positively with fasting and postprandial GIP levels ($r = 0.48$, $p = 0.03$ and $r = 0.44$, $p = 0.05$, respectively). In the T1D and control groups, fasting GIP correlated with postprandial GIP ($r = 0.62$, $p = 0.003$ and $r = 0.79$, $p < 0.001$) and fasting glucagon correlated with postprandial glucagon ($r = 0.88$, $p < 0.001$ and $r = 0.91$, $p < 0.001$), respectively. There was a positive correlation between TAR 180 and postprandial glucagon levels ($r = 0.56$, $p < 0.001$). A negative correlation was also found between postprandial glucagon levels and the number of 14-day hypoglycemic events in the T1D group ($r = -0.46, p = 0.03$). In contrast, a significant positive correlation was found between the number of hypoglycemic events and postprandial glucagon levels among healthy controls ($r = 0.44$, $p = 0.04$). After controlling individually for age, duration of diabetes, insulin dose, and HbA1c, statistically significant positive correlations were found between fasting GIP and fasting glucagon levels in the T1D group (respectively $r = 0.73$, $p < 0.001$; $r = 0.72$, $p < 0.001$; $r = 0.56$, $p = 0.01$; and $r = 0.72$, $p < 0.001$).

As shown in Table 4, when the patients with T1D were evaluated in two groups as < 15 years and ≥ 15 years according to the duration of diabetes, fasting GIP levels were observed to be higher in those with duration of < 15 years (66.40 (62.50–74.40) pg/mL vs. 59.50 (55.90–62.30) pg/mL, $p = 0.03$). There were no significant differences between the groups for postprandial GIP and fasting or postprandial glucagon levels with respect to duration of diabetes ($p = 0.76$, $p = 0.88$, and $p = 0.60$, respectively). The indices of glycemic variability were similar between patients who received a basal bolus and those who received insulin pump therapy ($p > 0.05$).

Discussion

In this study, fasting and postprandial GIP and glucagon levels, which are important glucoregulatory hormones, were investigated for the first time in cases of T1D in relation to glycemic variability based on data from a flash glucose monitoring system. Increased glycemic variability is caused by excessive hyperglycemic peaks and hypoglycemic events. Exaggerated postprandial glucagon secretion, which contributes to postprandial hyperglycemia, has been demonstrated in T1D [32]. The absence of the glucagon response to hypoglycemia has been documented in some cases of T1D with otherwise intact hormonal counterregulatory responses [12]. Furthermore, diminished postprandial GIP secretion

Table 3 Correlation analyses of GIP and glucagon with clinical parameters and index values of glycemic variability in the T1D group

	Type 1 diabetes							
	Fasting GIP		Postprandial GIP		Fasting glucagon		Postprandial glucagon	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Age	-0.27	0.24	-0.05	0.81	-0.10	0.66	-0.19	0.41
Duration of diabetes	**-0.51**	**0.02**	-0.19	0.42	-0.01	0.96	-0.05	0.81
Total daily insulin dose	**0.48**	**0.03**	**0.44**	**0.05**	0.06	0.79	-0.07	0.75
HbA1c	-0.13	0.58	0.01	0.94	0.11	0.63	0.02	0.92
CV	-0.10	0.64	0.03	0.87	-0.13	0.57	-0.36	0.11
TIR	-0.16	0.47	-0.19	0.41	-0.38	0.090	-0.35	0.12
Time below 70 mg/dL	-0.05	0.82	0.09	0.69	-0.19	0.42	-0.40	0.07
Time below 54 mg/dL	-0.04	0.86	0.002	0.99	-0.19	0.40	-0.43	0.05
Time above 180 mg/dL	0.30	0.19	0.02	0.92	0.34	0.13	**0.56**	**0.009**
Time above 250 mg/dL	-0.02	0.90	-0.01	0.94	0.24	0.29	0.19	0.40
Average glucose	0.03	0.87	-0.005	0.98	0.34	0.13	0.40	0.07
GMI	0.16	0.50	0.05	0.81	0.30	0.20	0.39	0.09
Low events per 2 weeks	0.18	0.42	0.19	0.41	-0.27	0.24	**-0.46**	**0.03**
Average duration of low events	-0.21	0.36	0.07	0.76	0.14	0.53	-0.10	0.67
Fasting GIP	-	-	**0.62**	**0.003**	0.28	0.23	0.22	0.36
Postprandial GIP	**0.62**	**0.003**	-	-	0.25	0.29	0.25	0.29
Fasting glucagon	0.28	0.23	0.25	0.29	-	-	**0.88**	**< 0.001**
Postprandial glucagon	0.22	0.36	0.25	0.29	**0.88**	**< 0.001**	-	-

HbA1c hemoglobin A1c, *CV* coefficient of variation, *TIR* time in range, *GMI* glucose monitoring indicator, *GIP* glucose-dependent insulinotropic polypeptide

Table 4 Comparison of GIP and glucagon levels and continuous glucose measurement data of patients with T1D according to duration of diabetes

	Diabetes duration < 15 years ($n=11$)	Diabetes duration ≥ 15 years ($n=9$)	p value
Fasting GIP, pg/mL	66.38 (62.50–74.36)	59.50 (55.85–63.29)	0.03
Postprandial GIP, pg/mL	62.26 (60.44–71.10)	61.76 (57.19–72.07)	0.76
Fasting glucagon, pg/mL	6.22 (5.94–9.11)	6.36 (5.90–7.23)	0.88
Postprandial glucagon, pg/mL	6.36 (6.22–22.88)	6.57 (6.15–12.28)	0.60
Average glucose, mg/dL	169.0 (154.0–181.0)	168.0 (153.0–179.50)	0.76
Estimated HbA1c (GMI), %	7.40 (7.0–7.60)	7.15 (6.92–7.37)	0.39
CV, %	37.40 (34.90–41.80)	39.60 (30.35–50.35)	0.65
Time in range, 70–180 mg/dL, %	58.0 (51.0–62.0)	57.0 (47.0–65.5)	0.65
Time below 70 mg/dL, %	3.0 (1.0–6.0)	4.0 (2.0–4.0)	0.71
Time below 54 mg/dL, %	0 (0–2.0)	0 (0–2.5)	1.00
Time above 180 mg/dL, %	27.0 (21.0–32.0)	24.0 (18.0–27.50)	0.29
Time above 250 mg/dL, %	12.0 (6.0–20.0)	12.0 (6–17.0)	0.94

Data are expressed as median and 25th–75th quartiles. *HbA1c* hemoglobin A1c, *GIP* glucose-dependent insulinotropic polypeptide, *GMI* glucose monitoring indicator, *CV* coefficient of variation

has been reported in patients with T1D [20]. If the dysregulated glucagon and GIP secretion observed in these patients is corrected, glycemic variability, which may contribute to the development of diabetic complications, can be reduced.

With respect to the changes in GIP concentrations in cases of T1D, higher fasting total GIP levels have been reported in patients with T1D compared to control subjects [21]. In contrast, no differences in fasting or post-OGTT GIP levels were reported between T1D and healthy control subjects [11]. Consistently, we found similar fasting and postprandial GIP levels between these groups. In a study conducted by Vilsboll et al., no impairment in fasting or postprandial GIP responses were observed among patients with T1D, which is in line with our findings [33]. The duration of diabetes may be the reason for these contradictory results, as we observed that patients with shorter durations of diabetes had higher fasting GIP levels than those with longer durations of diabetes. Duration of diabetes may also affect glucagon levels in patients with T1D. Li et al. observed high fasting glucagon levels in patients with shorter disease duration compared to patients with longer disease duration, possibly because of gradually decreased counts of β-cells during the course of the disease [34]. On the other hand, no influence of residual β-cell function on peak glucagon response to the mixed meal tolerance test was demonstrated in another previous study [35]. In the present study, we found no effect of disease duration on fasting or postprandial glucagon levels.

There are several potential confounding factors affecting β-cell function, including genetic predisposition, insulin resistance, glucotoxicity, lipotoxicity, and amylin accumulation [36]. Moreover, age and obesity have been suggested to be important factors for β-cell sensitivity to glucose as well as incretin hormones [37, 38]. Regarding the association between β-cell function and glucagon in cases of T1D, a 37% increase in meal-stimulated glucagon secretion was reported 12 months from diagnosis together with a 45% decline in C-peptide secretion [39]. The available data in the literature are limited in terms of postprandial glucagon levels in cases of T1D. Pörksen et al. reported that glucagon levels were increased after carbohydrate enriched liquid meal and associated positively with T1D in the pediatric age group [8]. In that study, postprandial glucagon levels were measured at 90 min after meals. In another previous study on T1D, the time to peak glucagon levels was found to be 45 min in a mixed meal tolerance test [35]. On the other hand, similar glucagon levels between 0 and 120 min with a mixed meal tolerance test in cases of T1D were reported in another study in the literature, although statistical significance was not achieved [32]. In accordance with that study, we found no difference between fasting glucagon values and values obtained 120 min after mixed meals. The inclusion of healthy control subjects is an important feature of our study, and we found similar fasting and post-mixed meal glucagon levels between our diabetic patients and controls.

GIP is a glucagonotropic incretin hormone and its increasing effect on glucagon secretion has been revealed in animal studies [40]. GIP has been demonstrated to stimulate glucagon secretion in a glucose-dependent manner in healthy subjects [41]. Exogenous GIP infusion induces glucagon levels in normoglycemic healthy subjects [42]. Intravenous GIP also increases glucagon secretion during hypoglycemia in patients with T1D [19]. In our study, there was a positive association between fasting GIP and fasting glucagon levels in patients with T1D, which continued after the controlling of clinical parameters including age, duration of diabetes, insulin dose, and HbA1c. Although our findings did not reveal a direct relationship between GIP levels and glycemic variability parameters,

both fasting and postprandial GIP levels showed a positive correlation with total daily insulin dose. This suggests that GIP levels may increase to regulate impaired glucose regulation. In addition, the fact that fasting GIP levels were found to be lower as the duration of diabetes increased may suggest that the effects of enteropancreatic hormones on glycemic regulation decrease over time. These results suggest that the complex effects of various factors such as increasing duration of diabetes, decreasing pancreatic reserve, response of incretin hormones, and appropriate insulin dosage on glycemic regulation.

Glucagon has been suggested to be associated with glycemic variability, but the data on this relationship are limited and contradictory. Bessho et al. reported that arginine-stimulated glucagon did not correlate with high blood glucose index values derived from CGM readings in patients with T1D; however, they found significant correlations of SD and mean amplitude of glycemic excursions with glucagon [27]. In contrast, Takanashi et al. reported no association between SD values obtained from glucagon CGM and AUC data after arginine stimulation testing performed with the ELISA method, as in our study [26]. In the present study, postprandial glucagon levels correlated positively with TAR 180 values derived from a flash glucose monitoring system. These data suggest that measures of glycemic variability increase with increasing postprandial glucagon levels in cases of T1D. On the other hand, Li et al. observed that fasting glucagon levels correlated negatively with low blood glucose index values obtained from CGM applications in cases of T1D without residual β-cell function [34]. Nevertheless, in our T1D group, postprandial glucagon levels after a mixed meal were negatively correlated with the number of 14-day hypoglycemic events. Infusions of amino acids during hypoglycemia, and particularly that of alanine, stimulate glucagon secretion in subjects with T1D despite marked hyperinsulinemia in clamp studies [43]. Therefore, our findings suggest that changes in the glucagon response to meals are associated with daytime hypo- and hyperglycemia in cases of T1D. From these results, one could hypothesize that the relation of postprandial glucagon with hypoglycemic events might be related to the ingestion of certain protein contents in meals. Personalized treatment including a special diet with various nutrient ratios for the regulation of glucagon responses to meals might lead to improved glycemic variability, which would be the result of better glycemic control in cases of T1D. This topic merits further investigation to clarify the complexity of postprandial glucagon regulation in cases of T1D.

Glucose values below 70 mg/dL obtained from CGM data in our healthy control group were accepted as representing hypoglycemia, but none of those patients had measured glucose values below 54 mg/dL. Furthermore, instances of hypoglycemia were generally only observed at night during sleep. Factors such as long-term fasting, skipping meals at night, consuming alcohol before bedtime, intense exercise before bedtime, and acute infections can lower nighttime blood glucose levels below 70 mg/dL. Shah et al. previously showed that sensor glucose values of < 70 mg/dL were obtained for 35% of their participants and hypoglycemic values of < 54 mg/dL were observed in 1% of their healthy participants [44]. In the same study, the CV was found to be 17%, while it was 14% among healthy subjects in our study. In the healthy control group, we found a negative association between postprandial glucagon levels and the number of hypoglycemic events, which was contrary to the findings for the diabetic group. This result suggests that the existence of β-cells has an important role in the relationship between postprandial glucagon and the development of hypoglycemia. On the other hand, a potential explanation that could elucidate this discrepancy might be related to impaired adrenergic mechanisms as a counterregulatory response to hypoglycemia in patients with T1D compared to nondiabetic individuals. Epinephrine has been shown to have modifying effects on glucagon secretion, and there are notable physiological differences in epinephrine action in the liver, muscles, adipose tissues, and pancreas between patients with T1D and healthy subjects [45, 46].

The main limitation of our study is the lack of data on GIP and glucagon levels at 30, 60, and 90 min after the mixed meal test. Multiple measurements at specific time intervals might better represent the dynamics of GIP and glucagon in patients with T1D. Possible correlations between different postprandial time durations and the parameters of glycemic variability would also be meaningful in our patients. Moreover, the response of incretin to a mixed meal measured as an AUC value could have been demonstrated. The sample size of our study was relatively small, but there are very few previous studies in this area. In this respect, our observations of glucoregulatory hormones as well as CGM data in patients with T1D may contribute significantly to the current literature. On the other hand, if more objective methods had been applied for assessing the functionality of intestinal neuroendocrine cells in terms of GIP secretion, different results could have been obtained, which is another limitation of the present study. In future research, larger samples of patients with T1D for whom GIP and glucagon levels are monitored concurrently with CGM data using dynamic measurements will provide a clearer understanding of how intrinsic glucoregulatory factors impact glycemic variability.

Conclusion

In conclusion, our findings showed that GIP was negatively associated with the duration of diabetes and positively associated with total daily insulin dose in our sample of patients,

indicating that GIP may be related to glycemic variability through its effects on clinical parameters. Furthermore, postprandial glucagon is linked to hyperglycemic and hypoglycemic indices derived from flash glucose monitoring systems in cases of T1D. Glycemic variability may be an important target while aiming to achieve glycemic control, particularly for patients with T1D who experience fluctuating blood glucose levels. The intrinsic factors associated with increased glycemic variability are still not fully understood. Therefore, studies examining the factors affecting this variability would be valuable. Our observations in the present study show that future treatments focusing on dysregulated postprandial GIP and glucagon secretion might help in reducing glycemic variability and thus have a positive effect on the development or progression of diabetic complications of T1D to some extent.

Acknowledgments We are grateful to Selma Avci, RN who supports organizing the participants' procedures. We thank all the participants in the study.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical clearance and Consent of patient The study protocol was approved by the local ethics committee (Decision No. 475, Date 13.06.2022) and was designed in line with the principles of the Declaration of Helsinki. All patients were enrolled in the study after providing their written informed consent.

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