Glucagon responses to glucose challenge in patients with idiopathic postprandial syndrome

Running title: Glucagon in postprandial syndrome

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Abstract

Background: Postprandial syndrome is characterized by hunger, weakness and anxiety neurosis occurring after meals. Although abnormal glucagon response has been suggested, inaccuracies of the conventional glucagon measurement method have prevented from precise analysis. Recently, a more reliable dual-antibody sandwich enzyme-linked immunosorbent assay for glucagon has been developed.

Methods: We conducted a 75 g oral glucose tolerance test (OGTT) extending to 4 hours in 14 patients with idiopathic postprandial syndrome. In addition to blood glucose and insulin, we have measured glucagon concentrations using the novel method and analyzed retrospectively.

Results: Median (lower quartile, upper quartile) of age and BMI were 40 years old (30, 49) and 24.9 (23.1, 26.2), respectively. The OGTT revealed that one patient had a diabetic pattern, and two were glucose intolerant. Fasting insulin was 7.6 $\mu\text{U/mL}$ (6.8, 8.8) and reached 73.7 (54.3, 82.6) at 30 min. Insulin remained elevated until 180 min. The fasting glucagon was 21.1 pg/mL (16.1, 33.8), falling at 60 min to a nadir of 6.9 (3.5, 10.3), one-third of the baseline, then remaining suppressed until 180 min. Furthermore, we have found that two types of glucagon dynamics: one is lower fasting glucagon with further suppression and the other is normal or higher fasting glucagon with subsequent big drop.

Conclusions: These data suggest that glucagon suppression is stronger in patients with idiopathic postprandial syndrome than in normal subjects previously reported. The present data will contribute to further understanding and future research of this syndrome.

Key words: Glucagon, Postprandial syndrome, Hypoglycemia

Introduction

Postprandial syndrome refers to a condition characterized by hunger, weakness, palpitations and anxiety neurosis usually occurring 2 to 4 hours after a meal^{1,2}. Although hypoglycemia has been suggested to cause such symptoms, studies have demonstrated that hypoglycemia below 55 mg/dL (defined as hypoglycemia in persons without diabetes)³ does not always co-exist with these features of postprandial syndrome^{4,5}. Nonetheless, patients exhibiting postprandial syndrome with relatively low blood glucose levels are occasionally referred to endocrine specialists for evaluation of suspected hypoglycemic disorders including insulinoma.

For patients with symptoms of postprandial syndrome, we conducted a 75 g oral glucose tolerance test (OGTT) extending to 4 hours to identify abnormalities in glucose homeostasis. For analysis of OGTT samples, we have measured blood glucose and immunoreactive insulin (IRI) as well as glucagon concentrations. Until recently, glucagon assessment was unreliable due to the inaccuracies of commercially available radioimmunoassays. A dual-antibody sandwich enzyme-linked immunosorbent assay (ELISA) has resolved these issues⁶. Herein, we retrospectively analyzed plasma glucagon levels measured using the new ELISA during prolonged OGTT in patients exhibiting postprandial syndrome.

Method

This study was designed in accordance with the principles of the Declaration of Helsinki, and all study procedures were approved by the ethics committee of the Nihon University Itabashi Hospital (RK-200609-7). The protocol of this study has been released on the web page of the Ethics Committee, and we have provided the opportunity to opt out for study

subjects.

We have included 17 patients who visited at Nihon University Itabashi Hospital, because of symptoms of so-called postprandial syndrome containing symptoms occurred by postprandial hypoglycemia between October 2017 and October 2020. Sex, age, body-mass index (BMI), smoking habit, systolic and diastolic blood pressures, diabetes, anti-hypertension drug use, statin use, and biochemical variables including lipid metabolism, uric acid and HbA1c were evaluated in all subjects. Biochemical variables were measured after an overnight fast. Total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, uric acid, creatinine and HbA1c were measured with an automatic analyzer. Oral glucose tolerance tests were performed for all patients. Blood were drawn at 0, 30, 60, 90, 120, 180, and 240 min. Glucagon was measured using a sandwich ELISA (Mercodia AB, Uppsala, Sweden). Measurements below the lower limit of detection (3.5 pg/mL) were treated as 3.5 pg/mL. Data are presented as median (lower quartile (Q1) and upper quartile (Q3) values). Differences were assessed by using Welch's *t*-test.

Results

The postprandial symptoms experienced by our 17 patients with this syndrome included hunger, sweating, palpitations and weakness. Some but not all patients had ambient blood glucose levels of 45 - 70 mg/dL. One patient had already been diagnosed with polycystic ovary syndrome (PCOS). In another female patient, an insulinoma was subsequently detected. In addition, one female patient lacked glucagon measurements. Thus, data from 14 patients with idiopathic postprandial syndrome were analyzed. Patient characteristics are shown in Table 1. Only three patients were male. Three of the women suffered from

neurosis or depression and had been prescribed anti-depressants. Laboratory examinations yielded essentially normal results.

Homeostatic model assessment of insulin resistance (HOMA-IR) and of β -cell function (HOMA-beta) were calculated with data at fasting during OGTT. HOMA-IR 1.78 (1.58, 1.93) and HOMA-beta was 88.5 (78.1, 103.1), indicating the subjects to be slightly insulin resistant while having normal or exaggerated insulin secretory capacities.

Glucose, IRI and plasma glucagon dynamics during OGTT are presented in Figure 1. One patient showed a diabetic pattern, and two were glucose intolerant. Three patients had glucose levels below 70 mg/dL during the tests. The median (Q1, Q3) of fasting IRI was 7.6 μ U/mL (6.8, 8.8) and reached 73.7 μ U/mL (54.3, 82.6) at 30 min. Insulin remained elevated until 180 min. The fasting glucagon peaked at 21.1 pg/mL (16.1, 33.8), immediately falling thereafter and reaching at 60 min a nadir of 6.9 pg/mL (3.5, 10.3), one-third of the baseline. The glucagon levels remained suppressed until 180 min and returned to the baseline at 240 min.

We then divided our subjects into two groups. One group consisted of patients whose blood glucose dropped below their fasting levels during OGTT (group D (dropped), n = 9). Patients in the other group maintained blood glucose levels above the fasting values (group ND (not dropped), n = 5). No significant differences of clinical characteristics between group D and ND was indicated, excluding serum total cholesterol level (the median of group D and ND were 174 and 226 mg/dL, $P = 0.04$). As expected from the grouping criteria, blood glucose levels tended to be lower in the D than in the ND group from 60 to 180 min and were significantly lower at 240 min (Figure 2). Although insulin levels were similar, glucagon levels clearly differed between the two groups. The fasting plasma glucagon concentration in group D was twice that in group ND (Figure 2).

Glucagon was suppressed more than three-fold in both groups, but recovered only in the D group at 240 min.

For reference, OGTT results are presented of patients with insulinoma (Figure 3A-C) and with PCOS (Figure 3D-F). An insulinoma patient was female of 40s and BMI of 24.7 and HbA1c of 4.7. Other laboratory tests were normal. During OGTT, insulin levels does not fully decline after 120 min and blood glucose dropped to 60 and 45 mg/dL at 180 and 240 min, respectively. Glucagon levels appear to be properly suppressed but did not recover at 120 min and delayed to 180 min. In PCOS patient (late 30s and BMI of 33.4 and HbA1c of 5.8) with severe insulin resistance (HOMA-IR of 6.96), both fasting insulin and glucagon levels were very high. After glucose challenge, insulin secretion was delayed with the peak being at 90 min. Glucagon secretion was greatly dropped by 60 pg/mL. Blood glucose dropped to 58 mg/dL at 240 min. Patient felt dizziness at this point.

Discussion

In this study, we demonstrated, for the first time, plasma glucagon levels measured using the recently developed ELISA in idiopathic postprandial syndrome. Minimization of cross-reactivity to other proglucagon products allows us to evaluate dynamic changes in glucagon responses after glucose challenge. We found that glucagon levels were promptly suppressed, decreasing to one third of the fasting levels, and that this drop persisted until 180 min in patients with postprandial syndrome.

Since this study was retrospective and we analyzed data obtained in routine medical practice, we do not have corresponding data from normal subjects. Therefore, we have used as a reference data for Japanese subjects from recent studies⁷⁻⁹. Glucagon responses were measured using the same ELISA in two of these prior studies^{7,8}. Glucose excursions

in patients with postprandial syndrome were essentially normal. Although insulin secretion was rapid, it appeared to be higher than in normal subjects and did not diminish even at 180 min, resembling the pattern seen in prediabetic patients⁹. Indeed, HOMA-IR analysis showed mild insulin resistance. Glucagon secretion was rapidly suppressed, as in normal subjects⁸. However, while glucagon secretion reportedly recovers at 180 min in normal subjects, suppression lasted until 180 min in our patients with postprandial syndrome. In addition, plasma glucagon was suppressed by nearly 70% in our patients, while in normal subjects, glucagon was reportedly suppressed by approximately 50%⁸. These data indicated that patients with idiopathic postprandial syndrome have some abnormalities in insulin and glucagon secretion.

An intriguing observation was made when patients were divided into two groups based on whether or not glucose had dropped below fasting levels during prolonged OGTT. Thus, we found two types of glucagon dynamics. In patients who did not show a glycemic drop, fasting glucagon levels were slightly lower and strongly suppressed to below the lower limit. In other patients with a glycemic drop, fasting glucagon was normal or higher, reaching the levels seen in patients with type 2 diabetes⁸. Glucose challenge greatly reduced glucagon concentrations to below one third of fasting values. We can reasonably speculate that in these patients with relative fasting hyperglucagonemia, the contribution of hepatic glucose output to maintaining fasting glycemic levels might be high, and that once glucagon secretion is suppressed, patients may be prone to hypoglycemia. However, it remains unclear the effect of exaggerated glucagon secretion of pancreatic α -cell against insulin secretion of β -cell and blood glucose level in our study subjects. We speculate that patients in group D include subjects who might develop type 2 diabetes and indicate excess of glucagon secretion due to pancreatic α -cell insulin resistance (A. Tsuchiyama,

et al. *Diabetes Care* 2007; 30: 2583-2587.). Further, sandwich ELISA in glucagon measurement of our study might show cross-reaction with gastrointestinal glucagon-related peptides (B. Kobayashi, et al. *J Diabetes Investg* 2020 Jun 16. doi: 10.1111/jdi.13325.).

Present data suggests that glucagon suppression is stronger in patients with idiopathic postprandial syndrome than in normal subjects previously reported. The mechanism underlying this abnormality is not clear. Insulin resistance and hyperinsulinemia might be causes, since insulin could be an intra-islet regulator of glucagon secretion¹⁰. In this regard, OGTT results of a PCOS patient are informative. Higher insulin secretion in the PCOS might cause a great suppression of glucagon secretion. Although symptoms of postprandial syndrome resemble those of hypoglycemia, the syndrome is not always associated with hypoglycemia. Dynamic changes in blood glucagon may cause these symptoms directly, since glucagon acts on diverse physiological function including gastrointestinal tract motility and satiety regulation¹¹. It is our hope that the present data will contribute to further understanding of this syndrome and glucagon pathophysiology.

Since islet hormone secretion in patients with idiopathic postprandial syndrome is found abnormal, though not severe, timely follow-up might be recommended. In addition, although symptoms of postprandial syndrome are often considered psychological, we should bear in mind that they could be presented as early manifestation of insulinoma as in our case.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Figure Legends

Figure 1.

Blood glucose (A), insulin (B) and glucagon (C) levels during OGTT in patients with postprandial syndrome. Data are median with error bars representing quartile ranges.

Figure 2.

Blood glucose (A), insulin (B) and glucagon (C) levels during OGTT in patients with postprandial syndrome whose glucose dropped below their fasting levels (filled squares) or whose glucose maintained above the fasting values (open circles) during OGTT. Data are median with error bars representing quartile ranges. * $p < 0.05$, ** $p < 0.01$

Figure 3.

Blood glucose (A), insulin (B) and glucagon (C) levels during OGTT in a patient with insulinoma. Blood glucose (D), insulin (E) and glucagon (F) levels in a PCOS patient.

Characteristics		Characteristics	
Male : Female	3 : 11	HDL chol (mg/dL)	65.0 (56.5, 70.5)
Age	40 (30, 49)	LDL chol (mg/dL)	113.0 (85.0, 145.0)
BMI	24.9 (23.1, 26.2)	ALT (IU/L)	19.0 (15.3, 20.8)
HbA1c (%)	5.3 (5.2, 5.5)	AST (IU/L)	18.0 (16.0, 22.3)
Systolic BP (mmHg)	122 (114, 126)	γ GTP (IU/L)	19.0 (17.0, 27.0)
Diastolic BP (mmHg)	76 (69, 85)	Cr (mg/dL)	0.68 (0.62, 0.79)
TSH (μ IU/mL)	1.99 (1.70, 2.58)	eGFR (ml/min)	82.1 (71.1, 90.3)
Free T4 (ng/dL)	1.34 (1.20, 1.50)	Na (mEq/L)	141.0 (140.0, 141.8)
Triglyceride (mg/dL)	105.0 (63.5, 149.8)	K (mEq/L)	4.1 (4.0, 4.4)

Data represent as number or median (lower quartile, upper quartile).