ORIGINAL ARTICLE

Impact of glucagon response on early postprandial glucose excursions irrespective of residual β-cell function in type 1 diabetes: A cross-sectional study using a mixed meal tolerance test

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ABSTRACT

Aims/Introduction: Controlling postprandial glucose levels in patients with type 1 diabetes is challenging even under the adequate treatment of insulin injection. Recent studies showed that dysregulated glucagon secretion exacerbates hyperglycemia in type 2 diabetes patients, but little is known in type 1 diabetes patients. We investigated whether the glucagon response to a meal ingestion could influence the postprandial glucose excursion in patients with type 1 diabetes.

Materials and Methods: We enrolled 34 patients with type 1 diabetes and 23 patients with type 2 diabetes as controls. All patients underwent a liquid mixed meal tolerance test. We measured levels of plasma glucose, C-peptide and glucagon at fasting (0 min), and 30, 60 and 120 min after meal ingestion. All type 1 diabetes patients received their usual basal insulin and two-thirds of the necessary dose of the premeal bolus insulin.

Results: The levels of plasma glucagon were elevated and peaked 30 min after the mixed meal ingestion in both type 1 diabetes and type 2 diabetes patients. The glucagon increments from fasting to each time point (30, 60 and 120 min) in type 1 diabetes patients were comparable to those in type 2 diabetes patients. Among the type 1 diabetes patients, the glucagon response showed no differences between the subgroups based on diabetes duration (<5 vs ≥5 years) and fasting C-peptide levels (<0.10 vs ≥0.10 nmol/L). The changes in plasma glucose from fasting to 30 min were positively correlated with those in glucagon, but not C-peptide, irrespective of diabetes duration and fasting C-peptide levels in patients with type 1 diabetes.

Conclusions: The dysregulated glucagon likely contributes to postprandial hyperglycemia independent of the residual β-cell functions during the progression of type 1 diabetes.

INTRODUCTION

Type 1 diabetes is a chronic, autoimmune disease characterized by an absolute deficiency of insulin production from β-cells of the Langerhans islets[1]. The residual capacity of β-cells to secrete insulin declines over time in type 1 diabetes patients, leading to fluctuating postprandial glucose levels[2]. A continuous glucose monitoring system showed that not only carbohydrates with a high glycemic index, but also dietary fat and protein can significantly affect postprandial glucose excursions[3]. It has been advocated that diabetes is caused not only by insulin action deficiency, but also by inappropriate glucagon secretion[4,5]. In

healthy individuals, glucagon secretion is suppressed in response to glucose ingestion to lower the plasma blood glucose levels through enhanced glucose uptake in hepatocytes and adipocytes, and this suppresses hepatic gluconeogenesis[6]. In contrast, it was reported that paradoxical hyperglucagonemia after a glucose load exacerbates hyperglycemia in patients with type 2 diabetes[7–10] and those with gestational diabetes[11]. This abnormal glucagon secretion in response to an oral glucose challenge was also observed in patients with type 1 diabetes irrespective of the condition of ambient glycemia[12], suggesting that glucagon plays a pivotal role in exacerbating hyperglycemia in response to an oral glucose challenge in patients with any type of diabetes.

Glucagon plays an essential role in the homeostatic regulation of amino acids[5,13,14], and an intake of amino acids (proteins) resulted in a strong stimulation of pancreatic α-cells to secrete glucagon[15]. As protein-derived calories account for approximately 15% of the total caloric intake in the modern diet[16], a mixed meal tolerance test (MMTT)[17] is suitable rather than an oral glucose tolerance test when evaluating the influence of glucagon abnormality on glycemic control in the daily lives of diabetes patients. The levels of plasma glucagon rose after the ingestion of a mixed meal, even in healthy individuals[18]; however, the glucagon amplitude is very small, because the stimulation of glucagon secretion by amino acids is offset by the intrinsic mechanisms underlying the suppression of glucagon through increased glucose-derived signals[19].

Compared with healthy individuals, patients with type 2 diabetes showed a larger glucagon increase in response to the ingestion of a mixed meal[9,15,20]. Exaggerated postprandial hyperglucagonemia was also observed in patients with type 1 diabetes when a bolus insulin was not administered before the ingestion of a mixed meal[21–24]. The response curve of the effect of the glucose concentration on the glucagon release from α-cells appears to be bell-shaped; that is, low glucose levels stimulate glucagon release, moderately high glucose levels inhibit glucagon secretion and very high glucose concentrations can increase glucagon release from α-cells[19,25]. This indicates that 'very high' concentrations of postprandial plasma glucose, which is observed in some type 1 diabetes patients without administration of premeal insulin, might increase glucagon release. Thus, our primary aim was to determine whether postprandial glucagon dysregulation would be observed even under the condition of "moderately high" glucose levels by the administration of mealtime bolus insulin.

In almost all of the related studies, glucagon values were measured by a widely used conventional radioimmunoassay (RIA), which uses polyclonal antibodies against only the C-terminal region of the glucagon peptide. However, that assay has problems, including the cross-reactivity of antibodies with proglucagon-related peptides[26]. A sandwich enzyme-linked immunosorbent assay (ELISA) was developed in 2014 and has been used to determine the precise glucagon concentrations; this assay's precision is due to the use of monoclonal antibodies against both the C- and N-terminal regions of glucagon[27]. The accuracy of sandwich ELISA for glucagon measurement was confirmed in a comparison with liquid chromatography–mass spectrometry[18].

We carried out the present study using sandwich ELISA to investigate whether the dysregulation of a meal-stimulated glucagon response affects the postprandial glucose excursion in patients with type 1 diabetes, using a premeal bolus insulin in a clinical setting. We also investigated whether the progression of β-cell dysfunction influences the glucagon response to the ingestion of a meal in patients with type 1 diabetes.

MATERIALS AND METHODS

Patients

This was a single-center, prospective cohort study carried out at Nagasaki University Hospital (Nagasaki, Japan) from November 2015 to November 2019. The patients were Japanese individuals with autoimmune type 1 diabetes (i.e., type 1A diabetes) aged >16 years. We also recruited patients with type 2 diabetes as controls. A total of 34 patients with type 1 diabetes and 23 patients with type 2 diabetes were enrolled.

The diagnosis of autoimmune type 1 diabetes was made by diabetologists based on the criteria of type 1 diabetes defined by the Japan Diabetes Society[28]. Patients complicated with other diseases (including cardiovascular disease, liver disease and renal dysfunction undergoing hemodialysis), a history of gastrointestinal surgery or pancreatectomy, alcohol abuse and pregnancy were excluded. Type 2 diabetes patients treated with glucagon-like peptide-1 receptor agonists, dipeptidyl peptidase-4 inhibitors, sodium–glucose cotransporter 2 inhibitors or sulfonylurea were excluded from the study.

Study design

All patients achieved a good level of glycemic control by receiving intensified basal–bolus insulin therapy during hospitalization. The patients underwent an MMTT after an overnight fast. A can of 200-mL (200 kcal) liquid meal composed of 31 g carbohydrate, 7.6 g protein and 4.4 g fat (CalorieMate®; Otsuka Pharmaceutical Co., Tokyo, Japan), which provides a caloric ratio that is similar to the modern Japanese diet[16], was given to each patient to consume for the MMTT. We confirmed that each patient's blood glucose level was within 70–250 mg/dL at the start of the MMTT to avoid the development of extreme hyperglycemia. If a patient's fasting glucose was not within the target level (70–250 mg/dL), the MMTT was canceled or postponed.

Blood specimens were obtained before (0 min), and at 30, 60 and 120 min after the ingestion of the meal for the measurement of plasma glucose, C-peptide and glucagon. The levels of serum C-peptide were measured by an ECLusys kit (Roche, Basel, Switzerland) with a lower detection limit of 0.003 nmol/L (0.01 ng/mL). Blood samples for plasma glucagon were obtained using BD P800 tubes (BD, Franklin Lakes, NJ, USA) and stored at −80°C. The levels of plasma glucagon were

measured by using the sandwich ELISA kit (Mercodia, Uppsala, Sweden)[27].

During the MMTTs, the type 1 diabetes patients treated with an insulin pump were kept on their basal rate of insulin infusion. Patients treated with multiple daily injections received their basal insulin as per usual. The type 1 diabetes patients also received a premeal bolus insulin, which was slightly decreased at two-thirds the necessary dose for a given 31 g of carbohydrates to avoid hypoglycemia during the MMTT. Each patient's necessary dose of bolus insulin was calculated by using his/her insulin-to-carbohydrate ratio, which was determined by each patient's physician. A fast-acting insulin (aspart or lispro) was used as a bolus insulin. The type 2 diabetes patients discontinued all antidiabetic agents, including insulin and oral hypoglycemic drugs, 12 h before the MMTT.

To determine whether the differences in residual β-cell functions of type 1 diabetes affect the patients' glucagon responses, we carried out detailed analyses comparing pairs of groups of the patients with type 1 diabetes. We divided the type 1 diabetes patients into two subgroups based on the duration from the clinical onset of type 1 diabetes (<5 vs ≥5 years) and on the fasting C-peptide levels by the median (<0.10 vs ≥0.10 nmol/L).

Clinical parameters, including physical measurements and biochemical data, were collected from the patients' medical records. The study was approved by the local ethics committee of Nagasaki University Hospital (approval no. 15083102), and was registered with the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (registration no. UMIN000020156). The study was carried out in accord with the principals expressed in the Declaration of Helsinki. Written informed consent for study participation was obtained from each patient.

Statistical analysis

Welch's *t*-test and repeated-measures analysis of variance were used to test differences in the patients' clinical characteristics and the values of glucose, C-peptide and glucagon during the MMTT between the type 1 diabetes and type 2 diabetes groups. The differences between the two pairs of subgroups of the patients with type 1 diabetes (diabetes duration and fasting C-peptide) were assessed by the same analysis method as used to compare the type 1 diabetes versus type 2 diabetes groups.

The changes in the levels of glucagon from baseline (0 min) to each time point (30, 60 and 120 min) during the MMTT are presented as ΔGlucagon 30, 60 and 120 min, as described[11]. The changes in glucose and C-peptide during the MMTT are presented as ΔGlucose 30, 60 and 120 min, and ΔC-peptide 30, 60 and 120 min, respectively. Pearson's correlation coefficient was used to evaluate the correlations between two parameters among ΔGlucagon, ΔGlucose and ΔC-peptide at the same time point during the MMTT. All statistical analyses were carried out using JMP Pro version 14 (SAS Institute, Cary, NC, USA). *P*-values <0.05 were considered significant.

RESULTS

Glucagon responses to mixed meal ingestion in type 1 diabetes patients were comparable to those in type 2 diabetes patients

The characteristics of the 57 patients (type 1 diabetes, *n* = 34; type 2 diabetes, *n* = 23) are summarized in Table 1. Among the 34 patients with type 1 diabetes, nine patients showed undetectable levels of fasting C-peptide (<0.003 nmol/L). Five type 1 diabetes patients were treated with a continuous subcutaneous insulin infusion, and the others were treated with multiple daily injections of insulin. The mean dosage of bolus insulin administered for the meal test in the patients with type 1 diabetes was 2.4 ± 1.4 units, which was calculated using the patients' insulin-to-carbohydrate ratio, as described in the Materials and Methods section. The mean duration of diabetes in the type 1 diabetes group was 8.1 ± 9.1 years. The bodyweight and body mass index were lower in the type 1 diabetes group compared with the type 2 diabetes group. There were no significant between-group differences in the sex ratio, age, height or levels of glycated hemoglobin and creatinine.

Compared with the type 2 diabetes patients, the patients with type 1 diabetes showed higher glucose levels and lower C-peptide levels at all of the time points during the MMTT. As the patients with type 1 diabetes were treated with premeal bolus insulin, the incremental glucose levels from baseline to each time points were comparable between type 1 diabetes and type 2 diabetes. The concentrations of plasma glucagon were elevated, and peaked at 30 min after the ingestion of the mixed-meal in both the type 1 diabetes and type 2 diabetes patients. There were no significant differences in the glucagon levels except at 30 min between the type 1 diabetes and type 2 diabetes patients. To avoid the influence of the volatility in the fasting levels of plasma glucagon, we evaluated the patients' glucagon responses by using the change in the levels from baseline (0 min) to each time point (30, 60 and 120 min) during the MMTT, defined as ΔGlucagon. There were no significant differences in ΔGlucagon 30, 60 or 120 min between the type 1 diabetes and type 2 diabetes patients (Table 1).

Comparison of glucagon responses between type 1 diabetes patients divided by diabetes duration and residual C-peptide levels

We compared the responses to the mixed meal ingestions in the type 1 diabetes patients according to their duration from the clinical onset of type 1 diabetes; <5 (*n* = 16) or ≥5 years (*n* = 18), as shown in the upper panel of Table 2. The group with longer diabetes durations (≥5 years) showed higher body mass index and creatinine levels compared with the group with the shorter duration (<5 years). The patients with longer diabetes durations (≥5 years) required a higher dose of insulin compared with the other group. In the results of the MMTTs, the levels of plasma glucose and ΔGlucose at all of the time points showed no significant differences between these groups

Table 1 | Comparisons of clinical characteristics and results of the mixed meal tolerance test between type 1 diabetes and type 2 diabetes patients

	T1D (n = 34)	T2D (n = 23)	P-value
Male/female (n)	16/18	16/7	0.11
Age (years)	49 ± 16	56 ± 17	0.12
Duration of diabetes (years)	8.1 ± 9.1	NA	NA
Height (cm)	162 ± 3	166 ± 10	0.19
Weight (kg)	59 ± 13	72 ± 16	0.003
Body mass index (kg/m^2)	22.5 ± 4.5	26.2 ± 4.5	0.004
HbA$_{1c}$, NGSP (%)	9.5 ± 2.5	10.4 ± 2.2	0.14
HbA$_{1c}$, IFCC (mmol/mol)	80.2 ± 27.3	90.4 ± 24.2	0.14
Creatinine (μmol/L)	70.9 ± 44.6	57.1 ± 15.7	0.10
Dairy dose of insulin (unit/day)			
Total insulin	34.7 ± 18.5	NA	NA
Basal insulin	10.1 ± 7.4	NA	NA
Bolus insulin	24.7 ± 13.4	NA	NA
Insulin-to-carbohydrate ratio (unit/g)	1.2 ± 0.7	NA	NA
Bolus insulin used in the MMTT (units)	2.4 ± 1.4	Not used	NA
Results of the MMTT			
Glucose (mmol/L)			
0 min	7.9 ± 2.4	6.4 ± 0.9	0.022
30 min	11.8 ± 3.1	10.1 ± 1.9	0.011
60 min	13.8 ± 3.0	11.7 ± 1.8	0.002
120 min	12.3 ± 3.0	10.3 ± 1.9	0.003
C-peptide (nmol/L)			
0 min	0.155 ± 0.182	0.566 ± 0.307	<0.001
30 min	0.232 ± 0.277	1.019 ± 0.524	<0.001
60 min	0.305 ± 0.381	1.245 ± 0.491	<0.001
120 min	0.348 ± 0.425	1.415 ± 0.610	<0.001
Glucagon (ng/L)			
0 min	20.0 ± 13.5	28.8 ± 14.2	0.19
30 min	48.2 ± 31.9	64.6 ± 45.1	0.015
60 min	32.9 ± 22.0	45.0 ± 29.0	0.070
120 min	20.9 ± 12.7	32.3 ± 15.8	0.090
ΔGlucose (mmol/L)			
30 min	3.9 ± 1.5	3.7 ± 1.8	0.72
60 min	5.9 ± 1.6	5.3 ± 1.6	0.17
120 min	4.4 ± 2.2	3.9 ± 1.6	0.36
ΔC-peptide (nmol/L)			
30 min	0.077 ± 0.108	0.453 ± 0.373	<0.001
60 min	0.151 ± 0.214	0.679 ± 0.277	<0.001
120 min	0.194 ± 0.260	0.849 ± 0.423	<0.001
ΔGlucagon (ng/L)			
30 min	28.2 ± 26.5	35.8 ± 41.8	0.44
60 min	12.9 ± 14.3	16.2 ± 27.0	0.59
120 min	0.9 ± 7.6	3.5 ± 11.0	0.34

ΔGlucose, ΔC-peptide and ΔGlucagon 30, 60 and 120 min indicate changes in the levels of plasma glucose, C-peptide and glucagon from fasting (0 min) to 30, 60 and 120 min, respectively. IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; MMTT, mixed-meal tolerance test; NA, not applicable; NGSP, National Glycohemoglobin Standardization Program; T1D, type 1 diabetes; T2D, type 2 diabetes.

(Figure 1a,b). The levels of serum C-peptide at 30, 60 and 120 min, and the values of ΔC-peptide at 60 and 120 min were significantly lower in the longer-duration group than the shorter-duration group (Figure 1c,d). The levels of plasma glucagon and ΔGlucagon were comparable between the groups at all time points (Figure 1e,f).

We also divided the type 1 diabetes patients into two groups based on their fasting C-peptide levels by the median;

Table 2 | Comparisons of clinical characteristics of the patients with type 1 diabetes stratified by diabetes duration or fasting C-peptide levels

Diabetes duration	<5 years (n = 16)	≥5 years (n = 18)	P-value
Male/female (n)	7/9	9/9	0.18
Age (years)	48 ± 16	56 ± 17	0.61
Duration from T1D onset (years)	1.1 ± 1.4	14.2 ± 8.6	<0.001
Height (cm)	164 ± 9	160 ± 11	0.31
Weight (kg)	55 ± 12	63 ± 14	0.10
Body mass index (kg/m^2)	20.5 ± 3.4	24.3 ± 4.6	0.009
HbA1c, NGSP (%)	10.2 ± 2.9	8.8 ± 1.9	0.11
HbA1c, IFCC (mmol/mol)	88.4 ± 32.0	72.9 ± 20.5	0.11
Creatinine (μmol/L)	54.1 ± 14.3	85.8 ± 56.4	0.033
Fasting C-peptide (nmol/L)	0.225 ± 0.183	0.092 ± 0.161	0.21
Dairy dose of insulin (unit/day)			
Total insulin	23.5 ± 14.5	44.7 ± 15.8	<0.001
Basal insulin	6.1 ± 5.5	13.6 ± 7.3	0.002
Bolus insulin	17.4 ± 12.2	31.1 ± 11.1	0.002
Insulin-to-carbohydrate ratio (unit/g)	0.9 ± 0.6	1.5 ± 0.6	0.012
Bolus insulin used in the MMTT (units)	1.6 ± 1.1	3.0 ± 1.2	0.001

Fasting C-peptide level	< 0.10 nmol/L (n = 17)	≥ 0.10 nmol/L (n = 17)	P-value
Male/female (n)	8/9	10/7	0.73
Age (years)	45 ± 17	52 ± 15	0.23
Duration of diabetes (years)	11.1 ± 9.2	5.1 ± 8.2	0.054
Height (cm)	163 ± 9	161 ± 11	0.67
Weight (kg)	60 ± 15	59 ± 12	0.76
Body mass index (kg/m^2)	22.5 ± 4.5	22.6 ± 4.6	0.94
HbA1c, NGSP (%)	9.1 ± 1.8	9.9 ± 3.1	0.32
HbA1c, IFCC (mmol/mol)	75.4 ± 19.3	84.9 ± 33.3	0.32
Creatinine, μmol/L	76.9 ± 51.8	64.8 ± 36.7	0.44
Fasting C-peptide (nmol/L)	0.023 ± 0.036	0.286 ± 0.174	<0.001
Dairy dose of insulin (unit/day)			
Total insulin	43.4 ± 17.9	26.1 ± 14.9	0.004
Basal insulin	13.4 ± 7.9	6.8 ± 5.3	0.008
Bolus insulin	30.0 ± 12.2	19.3 ± 12.7	0.017
Insulin-to-carbohydrate ratio (unit/g)	1.4 ± 0.7	1.0 ± 0.6	0.047
Bolus insulin used in the MMTT (units)	2.9 ± 1.4	1.8 ± 1.2	0.027

IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; MMTT, mixed-meal tolerance test; NGSP, National Glycohemoglobin Standardization Program.

that is, <0.10 nmol/L (n = 17) or ≥0.10 nmol/L (n = 17), as shown in the lower panel of Table 2. The patients with decreased fasting C-peptide levels (<0.10 nmol/L) required a higher dose of insulin than those with preserved C-peptide levels (≥0.10 nmol/L). No other clinical characteristics were significantly different between these groups. The levels of glucose and ΔGlucose at 120 min during the MMTT were significantly higher in the decreased C-peptide group compared with the preserved C-peptide group (Figure 2a,b). There were significant differences in the values of C-peptide and ΔC-peptide at all time points between the groups (Figure 2c,d). The values of both glucagon and ΔGlucagon during the MMTT were comparable between these groups (Figure 2e,f).

Association between the hormonal responses and the glucose increase during the MMTT in type 1 diabetes patients

To assess the effects of glucagon/insulin secretions on the glucose excursions in patients with type 1 diabetes, we determined the correlations of ΔGlucagon (or ΔC-peptide) with ΔGlucose during the MMTT (Figure 3). In the changes at 30 min from baseline, the ΔGlucose 30 min values were positively correlated with those of ΔGlucagon 30 min (r = 0.54, P < 0.001; Figure 3a), but not with those of ΔC-peptide 30 min (Figure 3b). No significant correlation was observed between ΔGlucagon 30 min and ΔC-peptide 30 min (Figure 3c). Additionally, we found no correlation between ΔGlucose 30 min and the dosage of premeal bolus insulin administered in the MMTT (r = − 0.15, P = 0.41). In the changes at 60 and 120 min after the
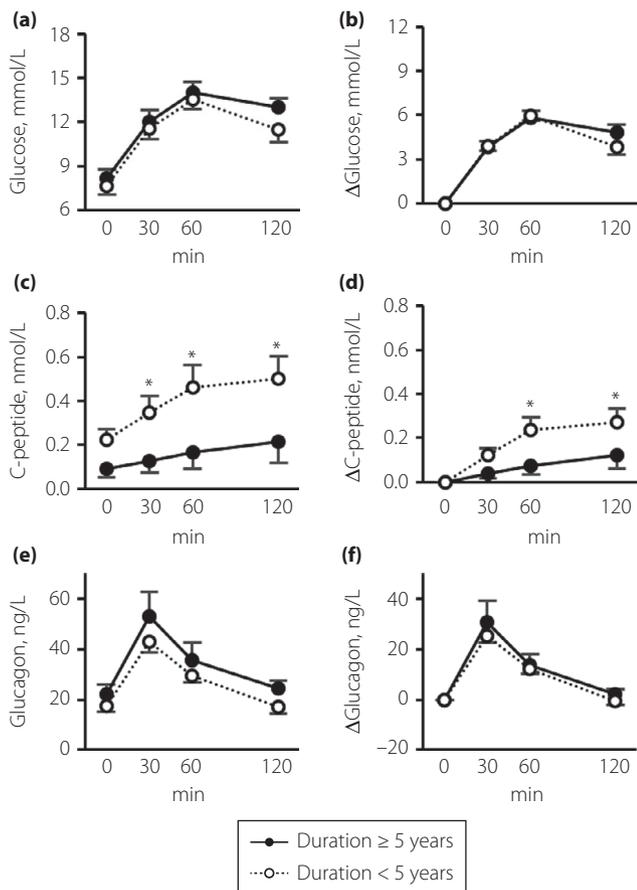
Figure 1 | Results of the mixed meal tolerance test in the patients with type 1 diabetes divided into subgroups according to their diabetes duration: <5 years (open circles with dashed line, *n* = 16) or ≥ 5 years (closed circles with solid line, *n* = 18). (a,c,e) The concentrations of plasma glucose, C-peptide and glucagon before (0 min), and 30, 60 and 120 min after ingestion of the mixed meal, respectively. (b,d,f) The changes in the concentration of plasma glucose, C-peptide and glucagon from baseline (0 min) to 30, 60 and 120 min during the mixed meal tolerance test, which are presented as ΔGlucose, ΔC-peptide and ΔGlucagon, respectively. Bars: standard error. *P < 0.05 versus the ≥5 years group.
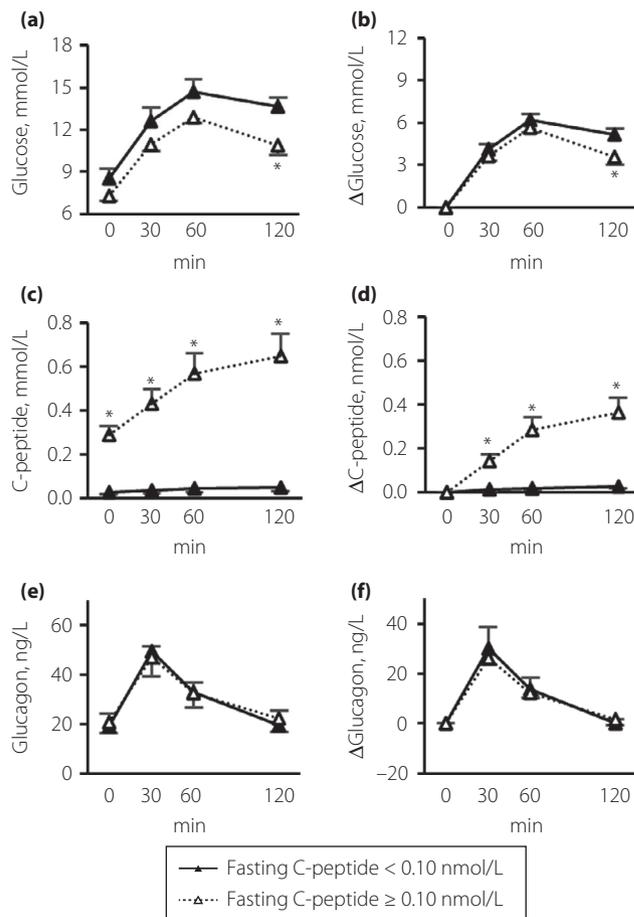

Figure 2 | Results of the mixed meal tolerance test in the patients with type 1 diabetes divided into subgroups according to their fasting C-peptide levels: <0.10 nmol/L (closed triangles with solid line, *n* = 17) or ≥0.1 nmol/L (open triangles with dashed line, *n* = 17). (a,c,e) The concentrations of plasma glucose, C-peptide and glucagon before (0 min), and 30, 60 and 120 min after ingestion of the mixed meal, respectively. (b,d,f) The changes in the concentration of plasma glucose, C-peptide and glucagon from baseline (0 min) to 30, 60 and 120 min during the mixed meal tolerance test, which are presented as ΔGlucose, ΔC-peptide and ΔGlucagon, respectively. Bars, standard error. *P < 0.05 versus the <0.10 nmol/L group.

meal ingestion, no significant correlations were observed between any pair of indexes among ΔGlucose, ΔGlucagon and ΔC-peptide (Figure 3d–i). These results observed in type 1 diabetes were similar to those in type 2 diabetes (Figure S1).

We studied the precise association between ΔGlucose 30 min and ΔGlucagon 30 min in each group of type 1 diabetes patients divided according to diabetes duration or residual C-peptide levels, and significant correlations between ΔGlucose 30 min and ΔGlucagon 30 min were observed independently of the diabetes duration (Figure 4a) and independently of the residual C-peptide levels (Figure 4b). The significant correlation between ΔGlucose 30 min and ΔGlucagon 30 min was preserved in both pairs of groups even though the patients were

divided based on the fasting C-peptide levels by the detection limit of 0.003 nmol/L (data not shown).

DISCUSSION

In the present study using sandwich ELISA for the measurement of plasma glucagon, we evaluated hormonal responses to a mixed meal ingestion in patients with type 1 diabetes using a premeal bolus insulin in a clinical setting. The results of our analyses showed an exaggerated increase in the postprandial glucagon secretion in patients with type 1 diabetes, which was similar to that observed in type 2 diabetes patients. The increase in the glucagon secretion at 30 min after the meal ingestion was positively correlated with the increase of plasma
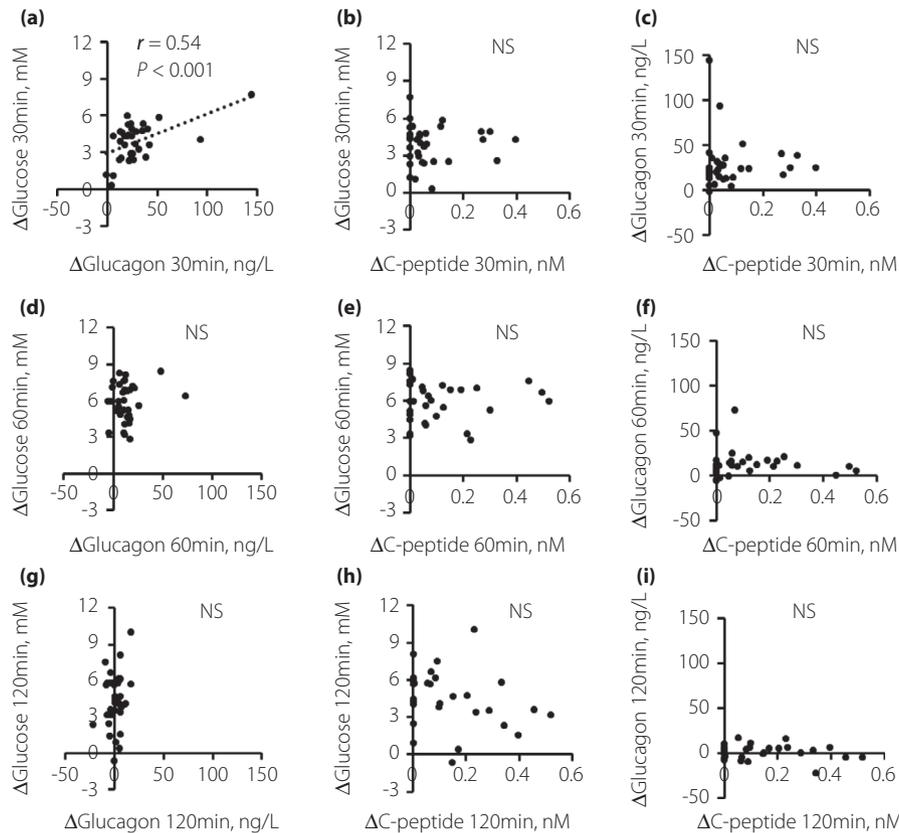
Figure 3 | Correlations between pairs of parameters among ΔGlucose, ΔGlucagon and ΔC-peptide at (a–c) 30, (d–f) 60 and (g–i) 120 min during the mixed meal tolerance test in the patients with type 1 diabetes (*n* = 34). NS, not significant.

glucose levels in the patients with type 1 diabetes, independent of their diabetes duration or residual C-peptide levels. The exaggerations of postprandial hyperglucagonemia thus seem likely to affect the early-postprandial glucose excursions in type 1 diabetes irrespective of the progression of type 1 diabetes, although it should be kept in mind that the present study was cross-sectional.

Previous studies carried out using the RIA, which uses antibodies against only the C-terminal region of glucagon, showed that patients with type 1 diabetes also had a postprandial paradoxical increase of glucagon, although lesser in magnitude to that seen in type 2 diabetes patients[29]. However, Komada et al. recently reported that fasting glucagon levels and glucagon responses to an intravenous arginine challenge test were comparable between type 1 diabetes and type 2 diabetes patients when determined by the RIA[30]. The present study showed that the type 1 diabetes patients developed a magnitude of postprandial hyperglucagonemia that is similar to the magnitude observed in type 2 diabetes patients when the glucagon levels were determined by the recent developed sandwich ELISA (Table 1).

A dysregulated secretion of glucagon was reported to emerge as early as the stage of impaired glucose tolerance (i.e., pre-diabetes), and the dysregulation was established at the stage of overt diabetes in patients with type 2 diabetes[7,9,10]. Several

studies also showed that the magnitudes of glucagon increase among youths with type 1 diabetes in response to the ingestion of a mixed meal were exaggerated during the first year after the onset of type 1 diabetes[31–33]. Although these studies were carried out using the RIA for measurements of glucagon, the finding suggested that postprandial hyperglucagonemia progresses over time in parallel with a decline in the residual capacity of β-cells in both type 1 diabetes and type 2 diabetes due to the lack of suppression by paracrine products including insulin[34,35], gamma-aminobutyric acid[36] and zinc ions[37] from neighboring β-cells in Langerhans islets.

Thivolet et al.[24] first showed the glucagon response to the ingestion of a mixed meal by using the double-antibody sandwich ELISA for glucagon measurements. The glucagon response was not affected by the presence of residual C-peptide levels in both recent-onset and longstanding type 1 diabetes, although they carried out the study without use of meal-time bolus insulin[24]. We observed a dysregulation of glucagon secretion in adults with type 1 diabetes that was not associated with their diabetes durations or residual C-peptide levels, even in a clinical setting using a meal-time bolus insulin (Figures 1f, 2f). The present results reinforce the finding reported by Thivolet et al.[24] that type 1 diabetes patients show postprandial hyperglucagonemia irrespective of their residual β-cell functions.
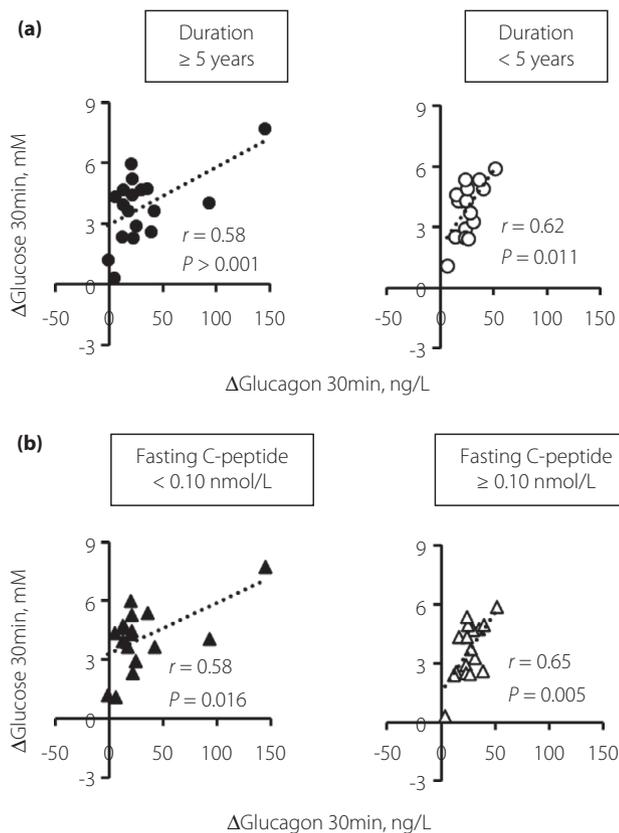
Figure 4 | Correlations between ΔGlucose 30 min and ΔGlucagon 30 min in the respective subgroups of patients with type 1 diabetes. (a) The patients with shorter diabetes durations (<5 years, n = 16; closed circles) and the patients with longer diabetes durations (≥5 years, n = 18; open circles). (b) The patients with decreased fasting C-peptide levels (<0.10 nmol/L, n = 17; closed triangles) and the patients with preserved fasting C-peptide levels (≥0.10 nmol/L, n = 17; open triangles).

Unger *et al.*[4] proposed that a paradoxical increase in glucagon release is the driving factor underlying hyperglycemia in type 1 diabetes and type 2 diabetes patients. Here, we suggest that an increase in glucagon exacerbated the early phase of postprandial hyperglycemia, even in type 1 diabetes patients undergoing intensive insulin therapy (Figure 3a). Interestingly, we observed significant correlations between the changes in the glucagon and glucose levels in the patients with both shorter and longer diabetes durations, and in those with both decreased and preserved C-peptide secretion (Figure 4b). Thus, taken past findings together with these results, the remaining β-cells with minimal endogenous insulin secretion in type 1 diabetes might be insufficient to exert paracrine actions to inhibit glucagon secretion from α-cells. The preserved β-cell function in patients with type 1 diabetes might not have very much impact on the body's inability to prevent increases in postprandial glucose levels, when an adequate dose of premeal insulin is administered.

Maintaining glycemic values as close to the non-diabetic range as possible is effective for preventing or delaying long-term complications in type 1 diabetes[38], but one of the greatest challenges and unmet needs in diabetes management is a limitation in effectively and consistently controlling postprandial hyperglycemia[39]. The uncontrolled early postprandial hyperglycemia (i.e., during the hour after a meal's ingestion) observed in type 1 diabetes has been considered to be mainly a consequence of an inadequate reaction time to a bolus insulin. In fact, the use of rapid-acting insulin analogs results in reduced postprandial hyperglycemia compared with human insulin[40]. Nevertheless, postprandial glucose control is a persistent challenge in both type 1 diabetes and type 2 diabetes[41].

Faster-acting insulin aspart (Fiasp®) and ultra-rapid insulin lispro-aabc (Lyumjev®), which are novel formulations of rapid-acting insulin, have been shown to have more rapid pharmacokinetic and pharmacodynamic profiles. They provided superior control of postprandial glycemia compared with lispro in patients with type 1 diabetes[42,43]. It recently became possible to control glucose levels precisely during the night-time and during fasting in patients with type 1 diabetes with the use of an artificial β-cell system (a so-called closed-loop insulin-delivery system) in continuous subcutaneous insulin infusion[44]. However, both treatments still pose difficulties in controlling postprandial glucose excursions at a level in the non-diabetic range. These difficulties might be caused by the dysregulated glucagon response to meal ingestion, as shown in the present study.

Several therapeutic options, such as glucagon-like peptide-1 receptor agonists[21,45–48] and an analog of amylin[49,50], have been studied to address postprandial glucose control. Both adjunct treatments provided better control for postprandial glucose excursions than insulin monotherapy in patients with type 1 diabetes. However, the clinical use of these treatments is not approved or is limited due to a significant increase in the risk of adverse events. The decreases in postprandial glycemia and the amplitude of glycemic excursions that were provided by an additional treatment of glucagon-like peptide-1 or amylin in type 1 diabetes patients might be due partially to a suppression of postprandial hyperglucagonemia. A new therapeutic strategy to normalize the aberrant glucagon response of the α-cells is thus desired for the optimal regulation of postprandial hyperglycemia.

The present study had some limitations. It was carried out at single center and the sample size was small. Our data of the MMTT included some outliers when type 1 diabetes patients were divided into subgroups, which might affect the statistical analyses. The variability in the patients' fasting glucose levels could potentially affect each glucagon response during the MMTT. It was also unclear whether the premeal bolus with the exogenous insulin injection that we administered to the type 1 diabetes patients affects the glucose excursion and the glucagon response. Recent studies showed that glucagon plays a major role in the regulation of amino acids metabolism, but we did not include measurements of amino acids into the analyses. We evaluated glucagon responses in patients with type 2 diabetes as controls, but did not determine regular glucagon responses in healthy individuals.

In conclusion, in patients with type 1 diabetes, the exaggerated glucagon secretion in response to the ingestion of a mixed meal affected the increase in the patients' glucose levels at the early postprandial phase. The impact of dysregulated glucagon secretion on glucose excursions was observed in the patients irrespective of the residual β-cells. The regulation of postprandial glucagon secretion might be a clue to obtaining further improvements of diurnal glycemic profiles in patients with type 1 diabetes.

DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 | Correlations between pairs of parameters among ΔGlucose, ΔGlucagon and ΔCpeptide at (a–c) 30, (d–f) 60 and (g–1) 120 min (G–I) during the mixed meal tolerance test (MMTT) in the patients with type 2 diabetes ($n = 23$). N.S., not significant.