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ORIGINAL ARTICLE

Magnitude of slowing gastric emptying by glucagon-like peptide-1 receptor agonists determines the amelioration of postprandial glucose excursion in Japanese patients with type 2 diabetes

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Keywords
Continuous ^{13}C breath test, Gastric emptying, Glucagon-like peptide-1 receptor agonists

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ABSTRACT

Aims/Introduction: Pharmacological levels of glucagon-like peptide-1 (GLP-1) can decelerate gastric emptying (GE) and reduce postprandial glucose levels. Most previous studies have used liquid meals to evaluate GE. We evaluated the effects of GLP-1 receptor agonists (GLP-1 RAs) on GE and postprandial glucose excursion in Japanese type 2 diabetes mellitus patients using a combination of solid and liquid meals.

Materials and Methods: In this single-center, prospective, open-label study, nine healthy individuals and 17 patients with type 2 diabetes mellitus consumed a 460-kcal combination of a solid and liquid meal labeled with ^{13}C-acetic acid. GE was measured from t = 0 to 150 min in a continuous ^{13}C breath test. Eight participants with type 2 diabetes mellitus were administered GLP-1 RAs, and we examined the relationship between GE and blood glucose excursion.

Results: There were no differences in the average GE coefficient (GEC) and lag time between the healthy and type 2 diabetes mellitus groups. However, the type 2 diabetes mellitus group showed larger GEC variations ($P < 0.05$). The coefficient of variation of R-R intervals was a significant predictor of GEC in type 2 diabetes mellitus patients ($P < 0.01$). The short-acting GLP-1 RA reduced the GEC at 1 month ($P = 0.012$), whereas the long-acting GLP-1 RA did not significantly change the GEC after treatment. A positive relationship was observed between postprandial glucose excursion from $T_{0\ min}$ to $T_{60\ min}$ and the GEC ($r^2 = 0.75$; $P < 0.01$).

Conclusions: The reduction in GE rate by the administration of GLP-1 RAs can predict the improvement in postprandial glucose excursion in type 2 diabetes mellitus patients.

INTRODUCTION

Type 2 diabetes mellitus is characterized by progressive hyperglycemia, and a high risk of microvascular and macrovascular complications. The maintenance of strict glycemic control is required for the prevention of complications. Postprandial hyperglycemia, particularly, is an independent cardiovascular risk factor[1,2].

Postprandial glucose is controlled by numerous factors, including gastric emptying (GE), intestinal carbohydrate digestion and absorption, and postprandial insulin secretion. GE is a major factor in the determination of postprandial glucose levels in healthy people, as well as those with diabetes, and accounts for ~35% of the variance in the initial glycemic response to oral carbohydrates[3,4]. After bariatric surgery, meal ingestion results in earlier and higher glucose peaks. This pattern is attributed to the more rapid transit of nutrients into the small intestine from the restricted gastric compartments inherent to such types of surgery[5].

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GE can be measured by a number of methods, including direct techniques, such as scintigraphy, and indirect techniques such as the quantification of ^{13}C and plasma acetaminophen[6]. Indirect methods depend on the speed of delivery of the substrate into the duodenum as a rate-limiting step for absorption and metabolism. ^{13}C acetic or octanoic acid breath tests are well correlated to scintigraphy – the gold standard for GE measurement[7] and are non-invasive, quantitative and less expensive than scintigraphy[6]. They are reproducible and have been validated in a cohort of diabetes patients, with a reported sensitivity and specificity of 75–86% and 80–86%, respectively, in the detection of delayed GE[8,9]. However, most previous studies reporting on GE used nutrient-containing liquid meals or low-caloric solid foods (200–255 kcal)[10–12]. The liquid or solid meal portions were small, compared with daily meals. Additionally, there is little evidence on the relationship between solid and liquid meals in terms of GE in type 2 diabetes mellitus patients[3,10,13]. The intragastric distribution of solid and liquid meal components is different, with increased retention of solid and liquid in the proximal stomach, and increased retention of solid, but not liquid, in the distal stomach. In solid meals, GE involves the storage of food, mixing with gastric secretions, grinding of solid food into particles sized 1–2 mm in diameter, and the subsequent delivery into the small intestine at a rate designed to optimize digestion and absorption[14].

Glucagon-like peptide-1 (GLP-1) is an incretin hormone released predominantly from the small intestine in response to food intake, which reduces postprandial glucose levels by enhancing the rate of glucose-dependent insulin secretion. Additionally, the administration of pharmacological levels of GLP-1 decelerates GE, which is associated with the relaxation of the proximal stomach, inhibition of antral and duodenal motility, and stimulation of pyloric pressure[15]. Furthermore, postprandial glucose level reductions, induced by intravenous GLP-1 administration, are closely related to the magnitude of GE reductions in healthy people[4,16,17]. GLP-1 receptor agonists (GLP-1 RAs), classified as "short"- and "long"-acting by the duration of action, are important in the deceleration of GE, besides stimulatory insulin secretion[18]. In previous reports, short-acting GLP-1 RAs slowed GE and reduced postprandial glucose excursions (PPGE) in a ^{13}C octanoic acid breath test[19,20]. Whether there is a difference in GE and glucose excursion between short-acting and long-acting GLP-1 RAs is unclear.

GE is important for determining postprandial glucose levels. However, limited studies have been carried out to evaluate GE after GLP-1 RA treatment using solid meals. It remains unclear how GLP-1 RA can affect the interaction between GE and blood glucose excursion in Japanese patients with type 2 diabetes mellitus. The present study aimed to evaluate GE rates by combined meals of solid and liquid, and compare the effects of GLP-1 RAs on GE and PPGEs in Japanese patients with type 2 diabetes mellitus.

METHODS

Study participants

Healthy controls and participants with type 2 diabetes mellitus, aged ≥20 years, were recruited for the investigation of GE, between April 2014 and January 2017. This was a prospective, open-label study carried out in Akita University Hospital, Akita, Japan. At screening, participants with type 1 diabetes, gastrointestinal tract diseases including gastroparesis, history of gastrointestinal surgery, cardiac disease, pulmonary disease, pancreatic disease, liver disease, renal disease, alcohol or drug abuse, malignancy and pregnancy were excluded. Additionally, participants taking medications known to affect gastrointestinal motility or appetite, or who smoked >10 cigarettes per day were excluded. Type 2 diabetes mellitus was diagnosed according to the Japanese Diabetes Society criteria[21]. First, we examined GE between healthy controls and type 2 diabetes mellitus patients. Second, we evaluated the relationship between GE and PPGE. GLP-1 RAs were administered to eight type 2 diabetes mellitus patients. Their glycated hemoglobin (HbA1c) values were >7%. We selected individuals with stable retinopathy and until the appearance of macroalbuminuria, and obtained informed consent to administer GLP-1 RAs. Drug-naive patients were excluded. Lixisenatide, exenatide and liraglutide were approved for use in Japan at the time of this study. We administered lixisenatide at 20 μg, liraglutide at 0.9 mg once daily and exenatide at 10 μg twice daily.

Meal test

Participants underwent a morning meal test after an overnight fast. The test meal comprised creamed chicken, crackers and pudding (total energy 460 kcal, carbohydrate 56.5 g, protein 18.0 g, fat 18.0 g). The creamed chicken contained 100 mg of ^{13}C-acetic acid (Cambridge Isotope Laboratories, Inc, Tewksbury, MA, USA). Participants took turns to ingest the creamed chicken and crackers first, and then the pudding. Then, they drank 100 mL of water. Time 0 was considered to be the beginning of the breakfast, which was consumed within 15 min. To analyze the GE rate, a standardized ^{13}C acetic acid breath test was carried out. Breath samples were continuously collected through a nasal tube, and the $^{13}CO_2/^{12}CO_2$ ratio was measured using the BreathID system (Exalenz Bioscience Ltd, Modiin, Israel) up to 150 min after the ingestion of the test meal. The rationale underlying the breath test methodology was that, as a result of GE, as ^{13}C acetic acid is rapidly absorbed and transported to the liver where it is oxidized, the $^{13}CO_2/^{12}CO_2$ ratio over time provides a measure of GE. GE was evaluated at baseline, and after 1 week and 1 month of the administration of GLP-1 RAs. To measure the levels of plasma glucose and serum C-peptide, blood samples were collected at T = 0, 30, 60, 120 and 180 min after test meal ingestion.

Data analysis of the ^{13}C-acetic acid breath test

The $^{3}CO_2/^{12}CO_2$ ratio values were expressed as percentage doses per hour of ^{13}C recovered (PDR) over time for each time

interval, and as cumulative values over 150 min (CPDR). Based on the method reported by Ghoos *et al.*[7], the PDR was fitted to the formula: $y\ (t) = at^b e^{-ct}$ by non-linear regression analysis, where '*y*' stands for the percentage of ^{13}C excretion in the breath per hour, '*t*' is time in hours, and 'a', 'b' and 'c' are constants. The GE coefficient (GEC) was calculated by ln (a). These parameters were analyzed using Oridion Research Software, β version (Oridion Medical Ltd., Modiin, Israel). GEC indicates the inclination of pulmonary $^{13}CO_2$ excretion in the early phase. A faster (slower) emptying corresponds to a larger (smaller) GEC. The time to linearly increased ^{13}C excretion commencement was estimated as the lag time from the CPDR. For solid meals, digestion has been shown to have a lag phase representative of the milling function, followed by linear emptying of gastric contents. This period of minimal or absent emptying was defined as the lag time[22].

Safety evaluation

Safety and tolerability assessments were carried out based on adverse events, including symptomatic hypoglycemia (defined as an event with clinical symptoms that were considered to result from hypoglycemia), abdominal discomfort, laboratory data (standard hematology, clinical chemistry and urinalysis parameters) and local tolerability at the site of injection.

Statistical analysis

Continuous variables are expressed as the mean ± standard deviation or median (range), as appropriate. The primary outcome was the GE rate with administration of GLP-1 RAs. The significance of the differences in the values was investigated by a Mann-Whitney *U*-test. Stepwise multiple linear regression was used to determine the predictors of the GEC. A Pearson's correlation test was used to determine the relationships between the changes in the blood glucose levels from $T_{0\ min}$ to $T_{60\ min}$ ($\Delta BG_{60-0\ min}$) and several parameters. Glycemic excursion and GE after the administration of GLP-1 RAs were analyzed using one-way analysis of variance (ANOVA) for repeated measures (the Bonferroni correction method was used for the post-hoc analysis). In the present study, for a 90% chance of a reduction in GEC of approximately 1 at a level of 0.025, the power calculations indicated that each of the two groups required at least four patients.

All statistical analyses of recorded data were carried out using the Excel statistical software package (BellCurve for Excel; Social Survey Research Information Co., Ltd., Tokyo, Japan). A *P*-value <0.05 was considered to show significant differences.

Ethics

The protocol for this research project was approved by a suitably constituted ethics committee of the institution, and it conforms to the provisions of the Declaration of Helsinki. Committee of Akita University Graduate School of Medicine, Approval No. 1160. Written informed consent was obtained from all study participants.

RESULTS

GE in type 2 diabetes mellitus

GE was evaluated in a total of nine healthy controls and 17 type 2 diabetes mellitus patients (Table 1). Figure 1a and b shows the CPDR and PDR during 150 min in both the healthy

Table 1 | Characteristics of the participants with type 2 diabetes and healthy controls

	Healthy control participants	Type 2 diabetes patients
n (male/female)	9 (7/2)	17 (9/8)
Age (years)	36.5 ± 7.4	63.1 ± 15.2
BMI (kg/m^2)	22.6 ± 2.8	26.9 ± 5.59
Duration of diabetes (years)	–	16.9 ± 12.9
Fasting plasma glucose (mg/dL)	88 ± 8.8	134.9 ± 29.5
HbA1c (%)	5.2 ± 0.42	8.96 ± 1.24
HOMA-R/HOMA-β	1.0 ± 0.35/63.5 ± 16.1	–
CPI/SUIT	–	1.65 ± 0.89/39.0 ± 24.1
Hypertension (*n*)/dyslipidemia (*n*)	0/0	12/12
Retinopathy: none	–	7
Simple		6
Preproliferative		1 (Stable)
Proliferative		3
Nephropathy: none	–	10
Microalbuminuria		5
Macroalbuminuria		2
Neuropathy: peripheral	–	14
Autonomic		3

Data are presented the mean ± standard deviation. BMI, body mass index; SUIT, secretory units of islets in transplantation; CPI, C-peptide immunoreactivity index; HbA1c, glycated hemoglobin; HOMA-β, homeostasis model assessment of β-cell function; HOMA-R, homeostasis model assessment of insulin resistance.

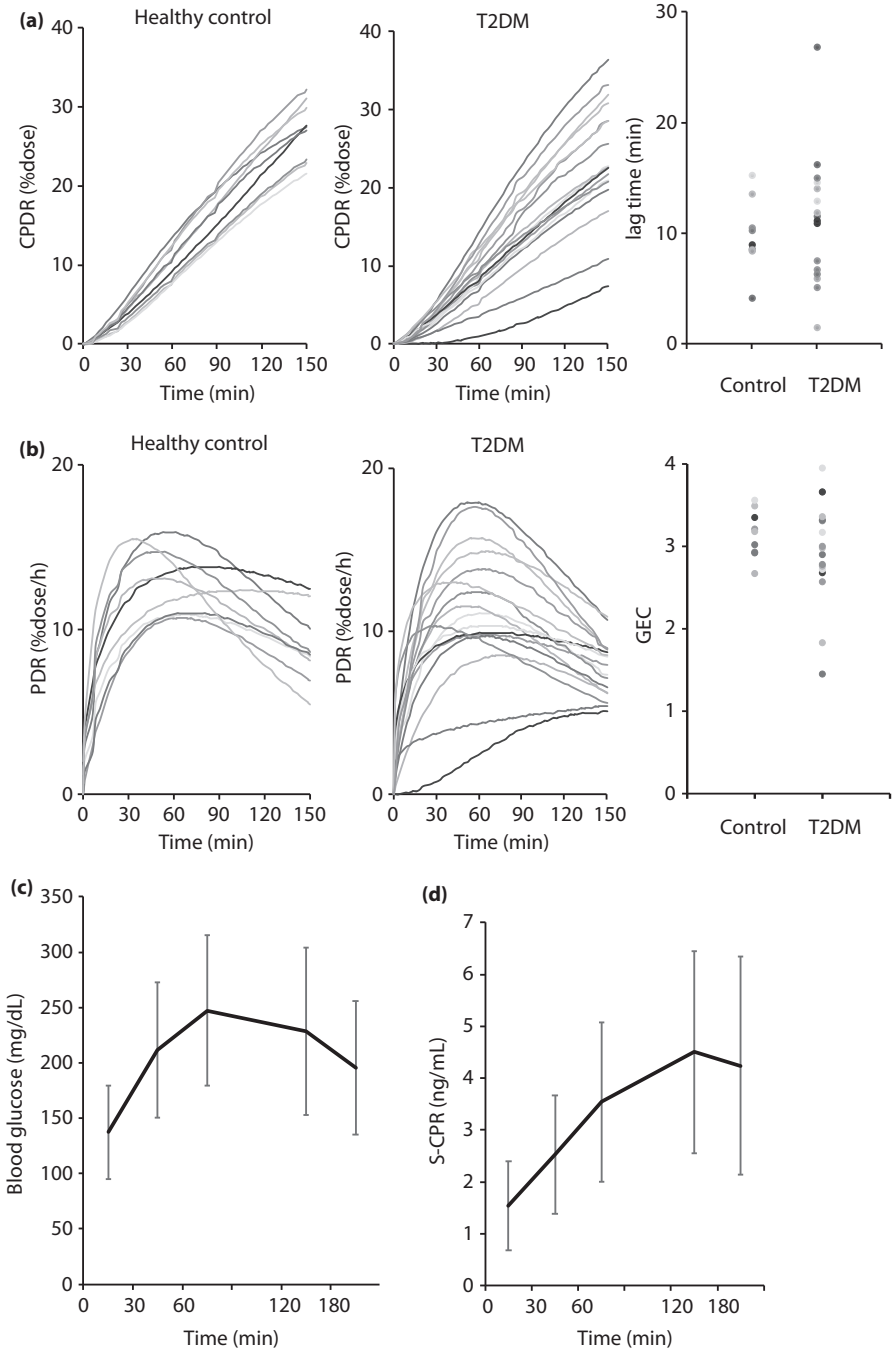

Figure 1 | $^{13}CO_2$ excretion curves after the administration of a meal labeled with ^{13}C acetic acid, lag time and the gastric emptying coefficient (GEC) of the gastric emptying values in healthy control participants and type 2 diabetes mellitus patients. (a) Cumulative percentage dose (CPDR) and lag time. (b) Percentage dose per hour of ^{13}C recovered (PDR) and the GEC. (c,d) Blood glucose profile. S-CPR, serum C-peptide. [Correction added on 6 August, after first online publication: Figure 1b has been amended.]

controls and type 2 diabetes mellitus patients after consumption of a meal with ^{13}C-labeled acetic acid. The average lag time was not different between the two groups (healthy controls: 9.82 ± 3.21 min, type 2 diabetes mellitus: 10.9 ± 5.76 min, $P = 0.73$). Variations in lag time tended to be larger in the type 2 diabetes mellitus group ($P = 0.09$). Peak $^{13}CO_2$ excretion occurred at 63.8 ± 22.7 min in healthy controls, and 71.9 ± 36.4 min in the type 2 diabetes mellitus group ($P = 0.98$). The GEC, the breath test parameter, was 3.15 ± 0.29 in healthy controls and 2.95 ± 0.63 in the type 2

Table 2 | Multiple linear regression equation

Variable	Coefficient (β)	SE	95% CI	*P*-value
Intercept	2.2706	0.2600	1.77–2.77	<0.001
CVR-R	0.1833	0.0549	0.07–0.30	0.0049

CI, confidence interval; CVR-R, coefficient of variation of R-R intervals; SE, standard error.

diabetes mellitus group. There were no significant differences in the average GEC between the two groups ($P = 0.40$), whereas variations in the GEC were significantly larger in the type 2 diabetes mellitus group ($P = 0.033$).

Stepwise multiple linear regression was used to predict the GEC based on age, duration, body mass index, fasting plasma glucose, urinary C-peptide, C-peptide index, secretory units of islets in transplantation (SUIT), HbA1c and the electrocardiogram R-R interval variation coefficient (CVR-R)[23]. Only CVR-R was a significant predictor of the GEC ($P < 0.01$). A significant regression equation was observed ($F(1,15) = 11.1454$, $P = 0.0049$), with an r^2 of 0.4432 (Table 2). The type 2 diabetes mellitus patients' predicted GEC was equal to 2.2706 + 0.1833 × CVR-R. However, there was no predictor observed that defined the lag time.

Relationships between GE and postprandial glucose excursion by GLP-1 RA administration

The baseline characteristics of the diabetes patients are listed in Table 3. None of these showed positive results in the Schellong test or a decreased CVR-R, suggesting the absence of diabetic autonomic neuropathy. After GLP-1 RA administration, eight participants with type 2 diabetes mellitus completed the study with no adverse events, including gastrointestinal symptoms. Their postprandial glucose levels at 120–180 min after 1 month were significantly decreased ($T = 120$ min, $P = 0.017$; $T = 180$ min, $P = 0.043$; Figure 2a).

Figure 2b shows the relationship between GE and PPGEs. The increase in the blood glucose level from $T_{0\ min}$ to $T_{60\ min}$ ($\Delta BG_{60\text{-}0\ min}$) was significantly related to the GEC in the overall population ($r^2 = 0.7815$, $P < 0.001$). In contrast, there was no correlation between the magnitude of the postprandial rise in blood glucose levels and the lag time, insulin secretion. The relationships between the rate of change in various factors and the rate of change in GEC by the administration of GLP-1 RAs are provided in Figure 3. The rate of change was calculated as follows: (1 − [the value at 1 month / before value]) × 100 (%). A positive correlation between the rates of change for GEC and $\Delta BG_{60\text{-}0\ min}$ was observed ($r^2 = 0.8170$, $P = 0.0021$). However, no significant relationships were observed between the rates of change for GEC and the rates of change for HbA1c level, bodyweight and insulin secretion rate at 0 min by SUIT (SUIT0).

Comparison of the effects of short- and long-acting GLP-1 RAs on GE and postprandial glucose excursion

Principal component analysis was carried out to determine the effects of GLP-1 RAs, and we found that, using the GEC as the first main component (91.0%) and the stimulated insulin secretion (stimulated SUIT60) as the second component (8.98%), GLP-1 RAs can be categorized into two groups (Figure 3E). One corresponds to short-acting GLP-1 RAs and the other to long-acting GLP-1 RAs.

Table 3 | Characteristics of the participants with type 2 diabetes by glucagon-like peptide-1 receptor agonist administration

	Group 1	Group 2
n (male/female)	4 (2/2)	4 (3/1)
Age (years)	55.5 ± 22.1	55.8 ± 14.9
BMI (kg/m^2)	32.0 ± 4.1	26.9 ± 4.4
Duration of diabetes (years)	18.0 ± 20.8	13.3 ± 7.70
Fasting plasma glucose (mg/dL)	162 ± 57.2	152.5 ± 37.6
HbA1c (%)	9.8 ± 2.3	8.50 ± 0.60
CPI/SUIT	1.01 ± 0.76/26.8 ± 23.4	1.61 ± 0.60/45.0 ± 22.3
Retinopathy: none	3	1
Simple	0	2
Preproliferative	1 (Stable)	1 (Stable)
Proliferative	0	0
Nephropathy: none	2	3
Microalbuminuria	2	0
Macroalbuminuria	0	1
Peripheral neuropathy (*n*)	3	4
GLP-1 RA		
Short-acting	4 (Exenatide: 1, Lixisenatide: 3)	0
Long-acting	0	4 (Liraglutide: 4)

Data are presented the mean ± standard deviation. BMI, body mass index; CPI, C-peptide immunoreactivity index; GLP-1 RA, glucagon-like peptide-1 receptor agonist; HbA1c, glycated hemoglobin; SUIT, secretory units of islets in transplantation.

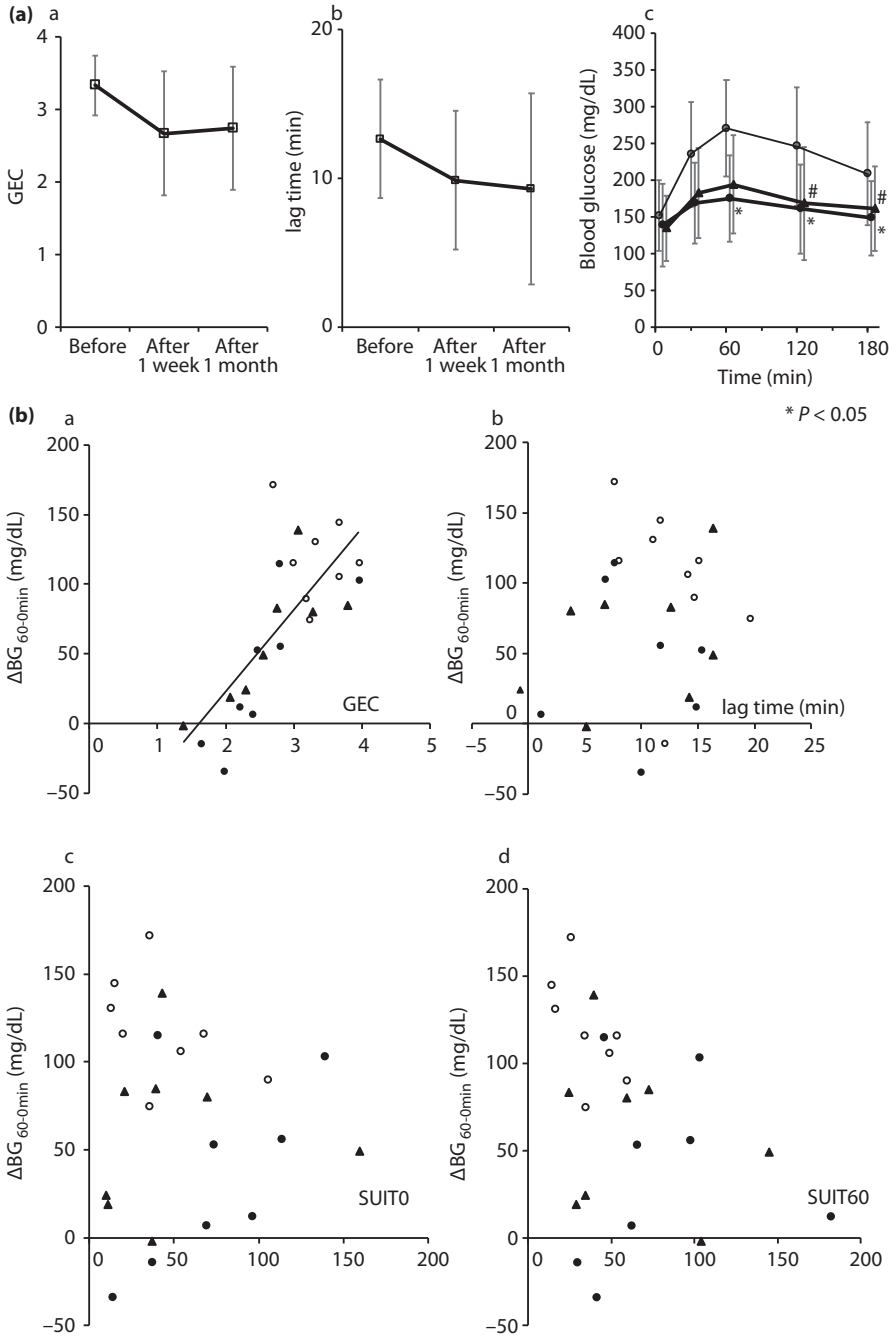

Figure 2 | (a) Changes in the gastric emptying values and glucose excursion in type 2 diabetes mellitus patients by the administration of glucagon-like peptide-1 receptor agonists during three different stages. Gastric emptying coefficient (GEC), lag time and blood glucose profile (before, white circles; after 1 week, black circles; after 1 month; black triangles). (b) Plots of the relationships among the changes in the gastric values, insulin secretion and changes in the blood glucose levels from $T_{0\ min}$ to $T_{60\ min}$ during three different stages. GEC, lag time, insulin secretion rate at 0 min by secretory units of islets in transplantation (SUIT0) and insulin secretion rate at 60 min by secretory units of islets in transplantation (SUIT60). The Pearson correction coefficient (r^2) and *P*-values were calculated as follows: GEC $r^2 = 0.7815$, $P < 0.001$; lag time $r^2 = 0.0025$, $P = 0.4846$; SUIT0 $r^2 = 0.005$, $P = 0.74$; and SUIT60 $r^2 = 0.120$, $P = 0.09$. $\Delta BG_{60\text{-}0\ min}$, change in the blood glucose level from $T_{0\ min}$ to $T_{60\ min}$.

Assessments of glucose excursion and insulin secretion, in addition to PDR and CPDR, were carried out during the meal test in each category (Figure 4). The GEC was significantly declined after 1 week and 1 month, compared with before treatment, indicating that short-acting GLP-1 RA use led to delayed GE; the GEC values were 3.49 (range 2.68–3.66), 2.09

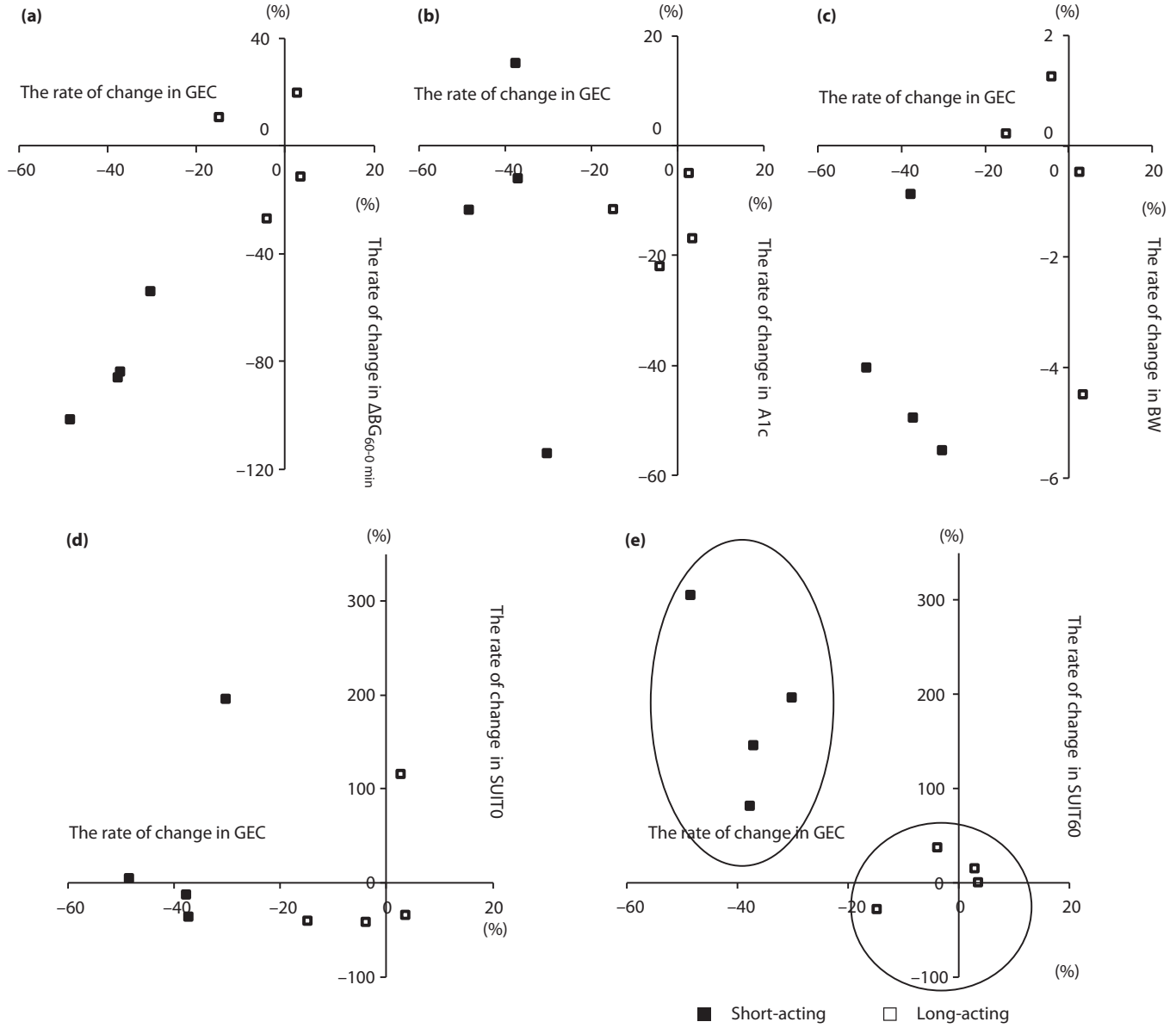

Figure 3 | Plots of the relationships among the rate of change in the blood glucose level from $T_{0\ min}$ to $T_{60\ min}$, glycated hemoglobin, bodyweight, insulin secretion and the rate of change in the gastric emptying coefficient (GEC) by the administration of glucagon-like peptide-1 receptor agonists. (a) Change in the blood glucose level from $T_{0\ min}$ to $T_{60\ min}$ ($\Delta BG_{60-0\ min}$), (b) glycated hemoglobin, (c) bodyweight, (d) insulin secretion rate at 0 min by secretory units of islets in transplantation (SUIT0) and (e) insulin secretion rate at 60 min by secretory units of islets in transplantation (SUIT60). Short-acting, black square; long-acting, white square. BW, bodyweight.

(range 1.64–2.39) and 2.20 (range 1.38–2.55) before, 1 week after and 1 month after treatment, respectively (before vs 1 week after treatment, $P = 0.0112$; before vs 1 month after treatment, $P = 0.0122$), whereas there was no change in the lag time with short-acting GLP-1 RA treatment (Figure 4C). The rate of change in the stimulated SUIT60 was increased. Therefore, glucose elevations from $T_{0\ min}$ to $T_{60\ min}$ were suppressed after 1 month (before vs 1 week after, $P = 0.0131$; before vs 1 month after, $P = 0.0111$, respectively; Figure 4d) and there was a positive relationship between $\Delta BG_{60-0\ min}$ and the GEC in the short-acting GLP-1 RA group ($r^2 = 0.6620$, $P = 0.0013$; Figure 5a).

In contrast, there was no significant difference in the GEC before and after treatment with long-acting GLP-1 RAs; the GEC values were 3.20 (range 2.98–3.90), 2.79 (range 2.46–3.95) and 3.17 (range 2.75–3.79) before, 1 week after and 1 month after treatment, respectively (before vs 1 week after, $P = 0.22$; before vs 1 month after, $P = 1.00$; Figure 4C). The change in

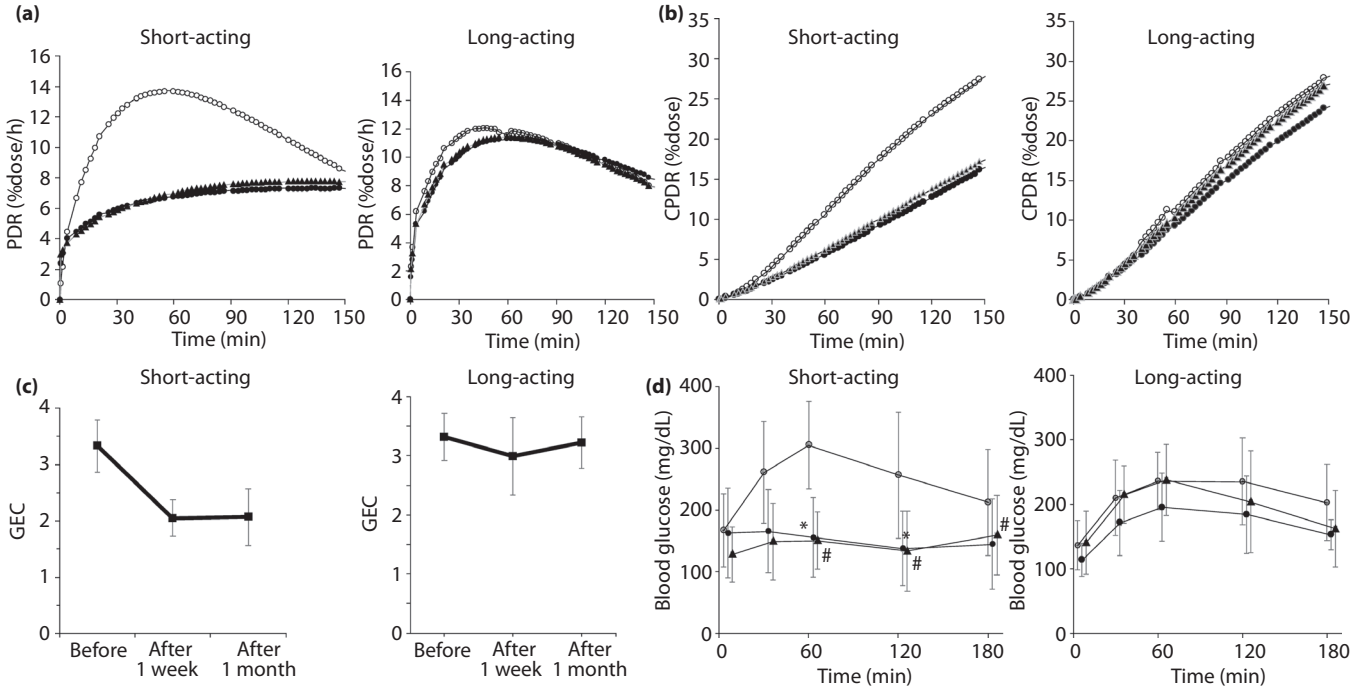

Figure 4 | Time-course curves for gastric emptying and glucose excursion in short-acting glucagon-like peptide-1 receptor agonists and long-acting glucagon-like peptide-1 receptor agonists during the three stages of examination (before, white circles; after 1 week, black circles; after 1 month; black triangles). (a) Percentage dose per hour of ^{13}C recovered (PDR), (b) cumulative percentage dose (CPDR), (c) gastric emptying coefficient (GEC) and (d) blood glucose. Data were analyzed by one-way analysis of variance for repeated measures; * and # indicate $P < 0.05$ (before vs after 1 week, and before vs after 1 month, respectively).

the stimulated SUIT30-180 was significantly increased only after 1 week ($P < 0.05$); however, the glucose levels did not change for 1 month (Figure 4d). The rate of change in the stimulated SUIT60 was not increased, and there was no significant decrease in $\Delta BG_{60-0\ min}$ for 1 month. Therefore, there was no relationship between the magnitude of the postprandial rise in blood glucose levels and the GEC in the long-acting GLP-1 RA group (Figure 5b).

DISCUSSION

In the present study, we evaluated the GE rates in healthy control participants and type 2 diabetes mellitus patients using $^{13}CO_2$ excretion after the consumption of a combined solid and liquid meal. We showed an association between GE and PPGEs in type 2 diabetes mellitus patients after the administration of GLP-1 RAs. The BreathID system allows continuous evaluation of GE and can serve as real-time breath analysis for patients. To our knowledge, we are the first to show that GLP-1 RAs reduce the GE rate in the early phase, using solid and liquid meals, and control PPGE improvements in type 2 diabetes mellitus patients, using a continuous real-time breath test.

The poor correlation between intragastric distribution and solid and liquid components has been reported in diabetes[10]. The emptying of digestible solids is characterized by an initial lag phase, after which, an emptying phase approximates a linear pattern. In contrast, non-nutrient liquids empty from the stomach in an overall mono-exponential pattern; that is, the GE of non-nutrient liquids usually lacks an initial lag phase[14], and the initial emptying rate of liquids might be more rapid in diabetes patients than those without the disease[3], indicating that the measurement of the GE of solids is more sensitive than that of liquids in the diagnosis of gastroparesis[24]. In the present study, the GEC, but not the lag time, could be predicted from the CVR-R value, suggesting that the administration of a combination of solid and liquid meals and performance of continuous breath tests can be useful in testing the GE ability in type 2 diabetes mellitus patients. Exogenous GLP-1 attenuates antral motility, increases pyloric tone, stimulates duodenal motility and relaxes the proximal stomach[25]. With the administration of GLP-1 RAs, while the GEC was decreased, the lag time was not affected. Therefore, the GE observed through the administration of GLP-1 RA in the case of combined meals predominantly points to increased proximal and distal stomach retention[17].

GE is mainly controlled by the vagal nervous systems for the regulation of the contraction and relaxation of gastric muscles[15,26]. Vagal damage is an important cause of delayed GE; delayed GE has been shown after vagotomy[27]. Intragastric meal distribution might also be associated with diabetic autonomic neuropathy. Measurement of the CVR-R provides objective

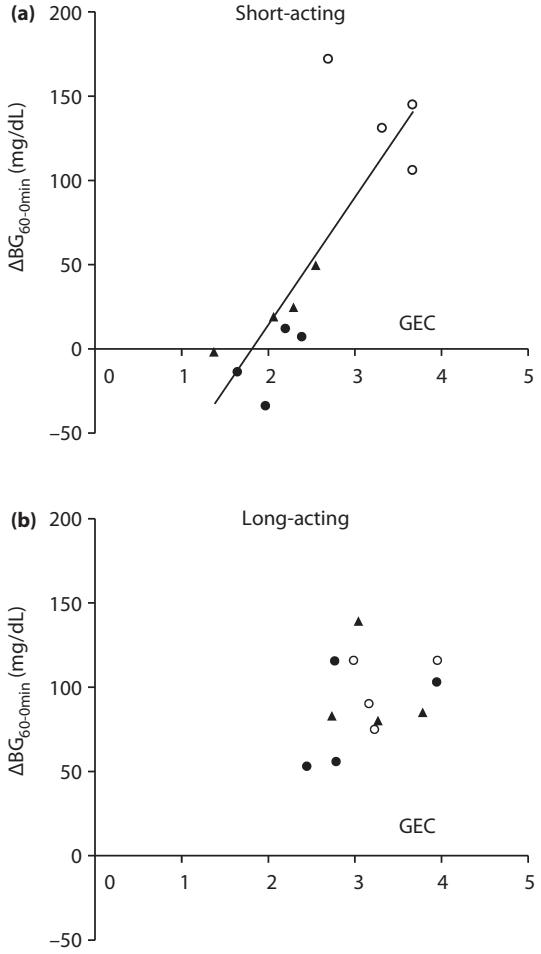

Figure 5 | Plots of the relationships between the change in blood glucose levels from $T_{0\ min}$ to $T_{60\ min}$ ($\Delta BG_{60-0\ min}$) and the gastric emptying coefficient (GEC; before, white circles; after 1 week, black circles; after 1 month; black triangles) in the (a) short-acting cases and (b) long-acting cases. The Pearson correction coefficient (r^2) and *P*-values were calculated as follows: (a) $r^2 = 0.6620$, $P = 0.0013$; and (b) $r^2 = 0.1131$, $P = 0.2851$.

evidence for the presence of autonomic neuropathy[28]. Although we excluded patients with orthostatic hypotension, we observed a significant relationship between autonomic nerve function and GE, suggesting that autonomic nerve function plays an important role in the pathogenesis of GE. Although the GEC is a good indicator of GE, it is difficult to evaluate the GEC of the GE index through daily medical examinations. The CVR-R might be a reasonable surrogate marker for GE.

The reduction in the magnitude of PPGEs by GLP-1 RA injection was significantly related to the magnitude of GE deceleration. The deceleration of GE had a stronger effect on the reductions in the magnitude of PPGEs than stimulated postprandial insulin secretion. Exogenous GLP-1 has a direct relationship with postprandial glycemic response with the deceleration of GE by GLP-1 in healthy participants[15]. Our observation that GE can be controlled by the administration of GLP-1 RA for type 2 diabetes mellitus patients, and that it is a major determinant of postprandial glucose levels, is of interest. The magnitude of the deceleration of GE by the administration of GLP-1 RA can predict postprandial glucose elevations. Although several drugs, in addition to GLP-1 RA, might modulate GE, whether these drugs affect postprandial glucose levels should be further examined[29,30].

Previous studies have shown that the glucose-lowering effects of GLP-1 RA rely on the remaining β-cell function and that it improves HbA1c[31,32]. As the present study was short term, the effect of GLP-1 RA on blood glucose excursion was examined by improvement of postprandial glucose rather than HbA1c. We examined the relationship between $\Delta BG_{60-0\ min}$ and SUIT0 before and after administering GLP-1 RAs, and found that there was no significant relationship between PPGE and insulin secretion (short-acting: $r^2 = 0.0123$, $P = 0.7314$; long-acting: $r^2 = 0.0723$, $P = 0.3980$). We suggested that GE rather than insulin secretion of β-cells was more effective in the improvement in postprandial blood glucose excursion after the administration of GLP-1 RAs.

The present findings show that short-acting GLP-1 RA administration led to the maintenance of decelerated GE for 1 month, and the reduction in the postprandial glucose level was marked. Short-acting GLP-1 RA use can lead to continued receptor stimulation without attenuation, and ensure the sustained deceleration of GE in the early phase. Therefore, short-acting GLP-1 RA administration might have a powerful impact on the deceleration of GE. However, long-acting GLP-1 RA use did not lead to the deceleration of GE, and did not reduce postprandial glucose levels after 1 month of treatment, consistent with the observation of tachyphylaxis after continuous infusion of GLP-1[33]. In the present study, GLP-1 RAs were categorized into two groups of the GEC and stimulated SUIT index. The effects on the GEC and stimulated SUIT index might lead to differences in the magnitude of postprandial excursions between the short- and long-acting groups.

The present study had several limitations. First, the number of patients was limited. When we administered GLP-1 RAs, we examined only the mild complications associated with diabetes. None of the patients had diabetic autonomic neuropathy. Second, it is unclear whether GLP-1 RA use can affect the GEC and lag time of GE and PPGEs in patients with gastroparesis. Third, in the present study, the patients did not have gastrointestinal symptoms as a result of GLP-1 RA administration (nausea, vomiting, diarrhea). The present findings did not show if gastrointestinal symptoms were related to deceleration in GE.

In conclusion, we showed that there was no significant difference in the GE rates with the intake of a combined meal between type 2 diabetes mellitus patients and healthy control participants. However, the variations in the GE rates were larger in type 2 diabetes mellitus patients, and we found that autonomic nerve function plays an important role in the pathogenesis of GE. The difference in the effects on the deceleration

of GE and stimulation of postprandial insulin secretion leads to variations in postprandial glycemic excursion reductions between short- and long-acting GLP-1 RAs. Through the evaluation of GE, GLP-1 RAs can be selected appropriately, and the effect of reduced PPGE in type 2 diabetes mellitus patients can be predicted.

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