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Effects of anagliptin on plasma glucagon levels and gastric emptying in patients with type 2 diabetes: An exploratory randomized controlled trial versus metformin

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ABSTRACT

Aims: Glucagon has an important role in glucose homeostasis. Recently, a new plasma glucagon assay based on liquid chromatography-high resolution mass spectrometry was developed. We evaluated the influence of a dipeptidyl peptidase-4 inhibitor (anagliptin) on plasma glucagon levels in Japanese patients with type 2 diabetes by using this new assay.

Methods: Twenty-four patients with type 2 diabetes were enrolled in a prospective, single-center, randomized, open-label study and were randomly allocated to 4 weeks of treatment with metformin (1000 mg/day) or anagliptin (200 mg/day). A liquid test meal labeled with sodium [^{13}C] acetate was ingested before and after the treatment period. Samples of blood and expired air were collected over 3 h. Plasma levels of glucose, glucagon, C-peptide, glucagon-like peptide-1 (GLP-1), and glucose-dependent insulinotropic polypeptide (GIP) were measured, and gastric emptying was also evaluated.

Results: Twenty-two patients completed the study (metformin group: n = 10; anagliptin group: n = 12). Glycemic control showed similar improvement in both groups. In the anagliptin group, there was a slight decrease of the incremental area under the plasma concentration versus time curve for glucagon after the test meal ($P = 0.048$). In addition, the plasma level of active GLP-1 and GIP was increased, and plasma C-peptide was also increased versus baseline. Neither anagliptin nor metformin delayed gastric emptying.

Conclusions: In patients with type 2 diabetes maintained endogenous insulin secretion, anagliptin increased the plasma level of active GLP-1 and GIP in association with a slight stimulation of insulin secretion and slight inhibition of glucagon secretion, but did not delay gastric emptying.

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1. Introduction

Glucagon is a 29-amino acid peptide hormone secreted by pancreatic α-cells [1] that counteracts the effects of insulin secreted by pancreatic β-cells in a paracrine manner. Glucagon stimulates hepatic glucose production [2], which means that inappropriate or excessive glucagon secretion has a major influence on glucose homeostasis and the pathophysiology of type 2 diabetes [3,4].

However, it has been difficult to measure the plasma glucagon concentration because proglucagon, a precursor of glucagon generated in the intestines and pancreas, is cleaved into glucagon and various other peptides, including glicentin, glicentin-related pancreatic polypeptide, oxyntomodulin, glucagon-like peptide 1 (GLP-1), and GLP-2 [5]. Both radioimmunoassays and enzyme-linked immunosorbent assays (ELISAs), are affected by cross-reaction with these other peptides derived from proglucagon [6,7].

Recently, a new assay was developed for plasma glucagon that employs liquid chromatography-high resolution mass spectrometry (LC-HRMS) to achieve higher accuracy and precision, while also providing a lower limit of quantification [8].

GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) are incretin hormones secreted by intestinal and stimulate insulin secretion and suppresses glucagon secretion in a glucose-dependent manner [9]. Exogenous GLP-1 also contributes to attenuation of postprandial glucose excursions by delaying gastric emptying [10].

Dipeptidyl peptidase-4 (DPP-4) inhibitors are oral hypoglycemic agents that are widely used in Japan [11]. DPP-4 inhibitors block the degradation of incretin hormones, including GLP-1 and GIP, and are expected to suppress glucagon secretion. However, whether DPP-4 inhibitors actually reduce the fasting [12,13] or postprandial [14,15] plasma glucagon level remains controversial.

Therefore, this randomized controlled trial was performed to evaluate the effect of a DPP-4 inhibitor (anagliptin) on plasma glucagon levels in patients with type 2 diabetes by using the new LC-HRMS assay. We selected metformin as an active comparator to assess the influence on the plasma glucose level, since metformin is recommended as a first-line oral hypoglycemic agent in many countries [16]. We also compared the effects of anagliptin and metformin on gastric emptying by performing the [^{13}C]-acetate breath test.

2. Materials and methods

2.1. Study design

This prospective, 4-week, single-center, randomized, open-label study was conducted to compare the effects of metformin and anagliptin on postprandial glucagon secretion.

2.2. Participants

Between August 2017 and October 2018, participants were recruited at the outpatient clinic of St. Marianna University Hospital (Kawasaki, Japan). The inclusion criteria at the time of giving consent were as follows: (1) type 2 diabetes, (2) age between 20 and 75 years, (3) hemoglobin A1c (HbA1c) from 6.5% [47 mmol/mol] to 9.0% [75 mmol/mol] and variation by <0.5% [5 mmol/mol] within 3 months before recruitment, (4) body mass index (BMI) $\leq$ 35 kg/m^2, (5) duration of diabetes $\geq$ 1 years, and (6) current treatment for type 2 diabetes with lifestyle modification or metformin monotherapy ($\leq$1250 mg/day), and (7) the ability to make decisions and provide written consent. The exclusion criteria were as follows: (1) glucagonoma, (2) hospital admission to improve glycemic control within the past 1 year, (3) a history of coronary artery disease, coronary revascularization, stroke, or transient ischemic attacks within the past 1 year, (4) a history of severe infection within the past 1 year, (5) malignancy, (6) severe renal dysfunction (estimated glomerular filtration rate <45 mL/min/1.73 m^2) or nephrotic syndrome, (7) severe liver dysfunction (AST and/or ALT > 3 times the upper limit of the standard range), (8) treatment with steroids, immunosuppressants, azole antifungal agents, or beta-blockers, (9) women who were pregnant, possibly pregnant, planned to become pregnant, or were breastfeeding, (10) a history of gastroduodenal surgery, (11) use of any investigational drug within 12 weeks before recruitment, (12) any change of oral antidiabetic therapy within 12 weeks before recruitment, and (13) patients who were considered to be ineligible for other reasons by the attending doctor. Written informed consent was obtained from all patients. This study was performed in accordance with the Declaration of Helsinki and was approved by the ethics committee of St. Marianna University School of Medicine. This study was registered with the University hospital Medical Information Network Clinical Trials Registry (registration number: UMIN000028293).

2.3. Intervention

Patients were randomized to treatment with metformin (500 mg bid) or anagliptin (100 mg bid) at a 1:1 ratio by the minimization method, taking into account previous treatment (metformin monotherapy or not) and BMI ($\geq$25 or not). Patients using metformin at screening were instructed to take twice a day (before breakfast and dinner). No new lifestyle modifications were introduced. Clinical and laboratory parameters were evaluated at baseline and after 4 weeks of treatment.

2.4. Measurements

2.4.1. Collection of blood samples

Blood samples were collected to evaluate the plasma concentration profiles of glucagon, glucose, C-peptide, active GLP-1, total GLP-1, and active GIP (fasting and 0.5 h, 1.0 h, 2.0 h, and 3.0 h postprandially). The incremental area under the plasma concentration vs. time curve (AUC) for them up to 3.0 h after initiation of the [^{13}C]-acetate breath test was calculated by the trapezoidal rule. HbA1c and other standard laboratory parameters were measured at 0 min after intake of a liquid test meal. Blood samples for determination of active GLP-1, total GLP-1, active GIP, and glucagon were collected into chilled BD™ P800, containing EDTA and a proprietary cocktail of protease, esterase, and DPP-4 inhibitors (Becton,

Dickinson and Company, Franklin Lakes, NJ, USA). Active GLP-1 was determined by ELISA (immuno-Biological Laboratories Co., Ltd.; Code No. 27784 GLP-1, Active form Assay Kit-IBL), total GLP-1 was determined by ELISA (YANAIHARA INSTITUTE INC.; Code No. YK161 total-GLP-1-HS ELISA Kit), active GIP was determined by ELISA (immuno-Biological Laboratories Co., Ltd.; Code No. 27201 Human GIP, Active form Assay Kit-IBL), while other parameters were measured using standard techniques.

2.4.2. *Measurement of plasma glucagon*

Plasma glucagon levels were measured by a recently developed LC-HRMS assay with parallel reaction monitoring without immunoaffinity enrichment [8]. Briefly, an automated nano LC-HRMS system was used that consisted of an Ultimate 3000 Series nano LC system and a Q Exactive Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, GmbH, Bremen, Germany) equipped with a nano-electro spray ionization interface and Black XYZ ion source (AMR, Inc., Tokyo, Japan). For accurate quantification of glucagon, 14-Leu-[$^{13}C_6$]-glucagon was added to every plasma sample as an internal standard. Precipitation of proteins was followed by solid-phase extraction to deplete plasma proteins and unwanted peptides. Parallel reaction monitoring was performed by monitoring the sum of the peak areas of five fragment ions in the + 4 charge state for glucagon (1–29) (*m/z* 871.66), and stable isotope-labeled-glucagon (1–29) (*m/z* 873.17). Using stable isotope-labeled glucagon and 200 μL of plasma, the quantification range of the assay was from 0.5 pmol/L to 100 pmol/L.

2.5. *[^{13}C]-acetate breath test*

At baseline and after 4 weeks of treatment, gastric emptying was measured by the [^{13}C]-acetate breath test, as described previously [17] with slight modifications. Following an overnight fast, expired air was collected in an aluminum bag (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan) at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, and 3 h after ingestion of a liquid test meal (200 mL Racol®NF, Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) labeled with 100 mg of sodium [^{13}C] acetate (acetic-1-^{13}C sodium salt 99%, Sigma-Aldrich Co., St. Louis, MO, U.S.A.). The total calorie content of the test meal was 200 kcal (62% carbohydrate, 20% fat, and 18% protein) and the meal was ingested within 1 min. In each breath sample, the $^{13}CO_2/^{12}CO_2$ ratio was measured by automated stable isotope ratio mass spectrometry (ANCA-GSL®, SerCon Ltd., Cheshire, UK). Then the half gastric emptying time ($T_{1/2}$) and the lag phase (T_{lag}), which corresponds to the time of maximum gastric emptying according to Ghoos et al. [18], were calculated by using special software for gastric emptying analysis (Star Medical Inc., Tokyo, Japan).

2.6. *Assessment of symptoms*

Digestive symptoms were assessed by asking patients to complete a standardized questionnaire, in which they recorded any of the following events on a daily basis during the study period: nausea, epigastric discomfort, abdominal distension, epigastric pain, dyspepsia, loss of appetite, vomiting, diarrhea, and constipation.

2.7. *Outcomes*

The primary outcome of this study was the change from baseline of the incremental plasma glucagon AUC (iAUC) following ingestion of the liquid test meal after the 4-week treatment period. Secondary outcomes included the changes from baseline of the plasma glucose, C-peptide, GLP-1, and GIP iAUC after 4 weeks, as well as the changes of $T_{1/2}$ and T_{lag} of gastric emptying. Furthermore, changes of HbA1c and fasting plasma glucose from baseline after 4 weeks were determined.

2.8. *Statistical analysis*

Because this was a pilot study, the sample size was based on practical considerations rather than a statistical estimate. It was expected that 24 patients could be enrolled during the registration period. With this sample size, the standardized group difference of 1.20 could be detected by two-sided statistical test with alpha and beta error of 0.05 and 0.20, respectively. We evaluated all comparisons in an exploratory manner. All of the randomized patients were analyzed, except those without data at baseline or after initiation of study treatment. Baseline characteristics were summarized as the mean and standard deviation (SD) for continuous variables or as proportions for categorical variables. The change from baseline to the end of treatment in each group, the between-group difference (anagliptin group – metformin group), and the 95% confidence interval (CI) of the difference were calculated. For the primary outcome (plasma glucagon iAUC), a two-sided *t*-test was also conducted for post-hoc analysis. Differences were considered significant if the probability (*P*) value was less than 5%. Because the reported *P* values were exploratory, adjustment for multiplicity was not done. All statistical analyses were performed with R version 3.54 (https://cran.r-project.org).

3. Results

A total of 24 Japanese patients with type 2 diabetes were enrolled in the present study. Two patients did not complete the study because of protocol deviation (n = 1) and scheduling difficulties (n = 1). The remaining 22 patients (9 men and 13 women) completed the study and were analyzed (Fig. 1). At screening, 5 subjects (3 in the anagliptin group and 2 in the metformin group) were on treatment with metformin (500 mg/day). The baseline clinical characteristics of the patients are listed in Table 1. Demographic variables were well balanced between the two groups with regard to age, gender, duration of diabetes, BMI, glycemic control, blood pressure, and lipid profile.

After 4 weeks, the fasting plasma glucose level decreased in the anagliptin group (−11.3 mg/dL; 95%CI −19.4, −3.3) and also in the metformin group (−19.4 mg/dL; 95%CI −27.2, −11.6), the difference between the two groups of 8.1 mg/dL (95% CI −3.9, 20.0). HbA1c also decreased in the anagliptin group (−0.38% [−4.1 mmol/mol]; 95%CI −0.57, −0.20) and the

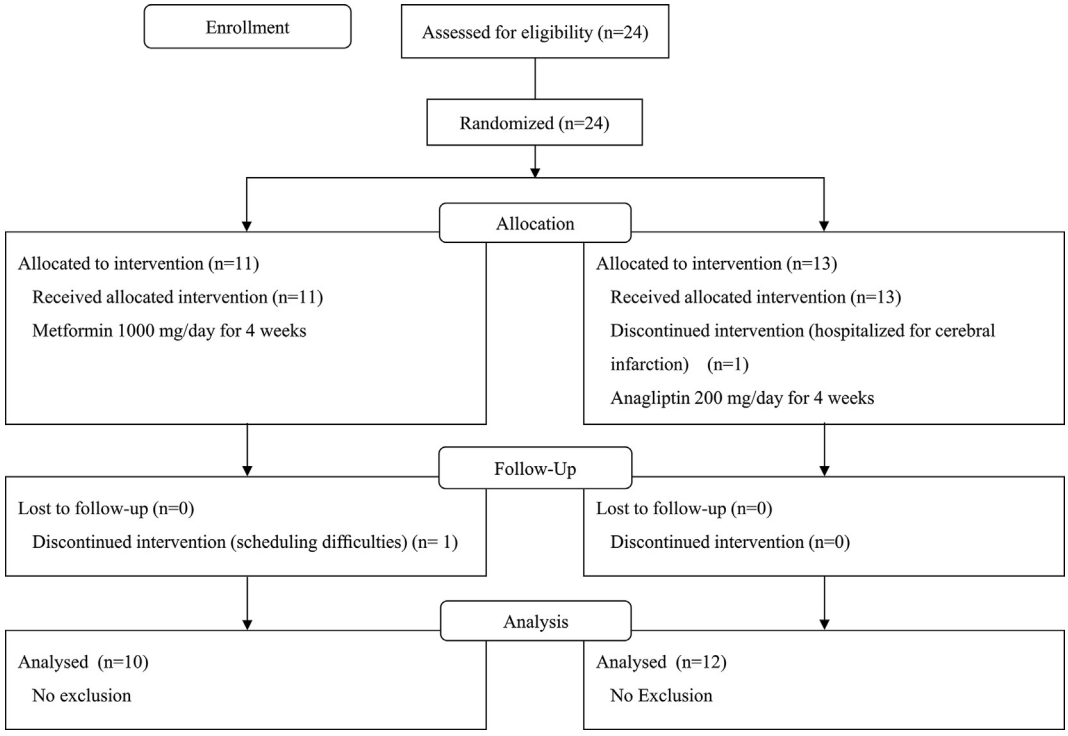

Fig. 1 – Flowchart showing the disposition of the subjects.

metformin group (−0.35% [−3.8 mmol/mol]; 95%CI −0.51, −0.19), with the difference between the two groups of −0.03% [−0.36 mmol/mol] (95% CI −0.29, 0.23). The plasma glucose profile of each group up to 3 h after ingestion of the liquid test meal is displayed in Supplemental Fig. 1A, B. At the end of the treatment period, the mean plasma glucose level was lower at each time point in both groups. The iAUC of plasma glucose was also decreased in both groups, with the difference between them of −14.9 mg·h/dL (95% CI −49.2, 19.4), indicating a similar glucose-lowering effect of both treatments (Table 2).

The plasma glucagon profile and changes of the iAUC for plasma glucagon after ingestion of the liquid test meal are shown in Fig. 2 and Table 2. After 4 weeks of treatment, the

Table 1 – Characteristics of the two groups.

	Metformin group n = 10	Anagliptin group n = 12
Women (*n*)	5	8
Age (years)	63.3 ± 9.8	59.4 ± 8.4
Duration of diabetes (years)	7.5 ± 9.7	4.2 ± 3.0
Body mass index (kg/m^2)	26.0 ± 2.9	26.7 ± 2.8
Fasting plasma glucose (mg/dL)	137.5 ± 15.6	135.8 ± 19.0
Fasting plasma glucagon (pmol/L)	8.2 ± 3.6	8.1 ± 5.0
Fasting plasma C-peptide (ng/ml)	1.9 ± 0.5	1.9 ± 0.6
Fasting active GLP-1 (pmol/L)	5.5 ± 2.6	12.9 ± 18.8
HbA1c (%)	6.9 ± 0.3	7.1 ± 0.7
HbA1c (mmol/mol)	51 ± 3.0	54 ± 7.9
Systolic blood pressure (mmHg)	148.7 ± 12.1	151.9 ± 19.5
Diastolic blood pressure (mmHg)	85.2 ± 8.2	83.4 ± 11.2
Estimated GFR (ml/min/1.73 m^2)	77.0 ± 11.6	76.4 ± 9.1
LDL-C (mg/dL)	111.7 ± 26.9	110.6 ± 19.7
HDL-C (mg/dL)	59.8 ± 15.6	49.1 ± 11.0
Triglycerides (mg/dL)	135.4 ± 74.6	134.8 ± 58.8
Oral hypoglycemic agents at screening (*n*)		
Biguanide	2 (500 mg)	3 (500 mg)

Data are expressed as the mean ± standard deviation for continuous variables or the number for categorical variables. HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Table 2 – $iAUC_{0\text{-}3h}$ after intake of the liquid test meal before and after 4 weeks of treatment, and between-group differences of iAUC changes.

		Metformin group (n = 10)		Anagliptin group (n = 12)		Difference of AUC change between the groups	Confidence Interval	
		Mean (SEM)		Mean (SEM)			Lower	Upper
Glucose (mg·h/dL)	0w	152.9	(12.9)	168.4	(15.1)			
	4w	118.7	(11.6)	119.3	(14.3)			
	Change of AUC	−34.2	(10.8)	−49.1	(12.4)	−14.9	−49.2	19.4
Glucagon (pmol·h/L)	0w	0.3	(2.1)	0.1	(1.6)			
	4w	1.1	(2.2)	−4.1	(1.9)			
	Change of AUC	0.8	(2.1)	−4.2	(0.9)	−5.0	−10.0	−0.1
C-peptide (ng·h/mL)	0w	6.5	(0.7)	6.5	(0.7)			
	4w	6.8	(0.8)	7.2	(0.8)			
	Change of AUC	0.3	(0.4)	0.7	(0.4)	0.4	−0.8	1.5
Total GLP-1 (pmol·h/L)	0w	8.3	(3.7)	12.4	(4.1)			
	4w	15.6	(4.4)	7.0	(3.2)			
	Change of AUC	7.4	(3.2)	−5.4	(2.4)	−12.8	−20.9	−4.7
Active GLP-1 (pmol·h/L)	0w	4.7	(1.9)	11.0	(4.9)			
	4w	11.0	(3.8)	17.5	(8.3)			
	Change of AUC	6.3	(2.9)	6.5	(10.4)	0.2	−23.2	23.6
Active GIP (pmol·h/L)	0w	77.0	(16.4)	58.0	(5.6)			
	4w	58.0	(7.1)	129.6	(14.8)			
	Change of AUC	−19.0	(11.9)	71.6	(17.1)	90.6	45.2	136.0

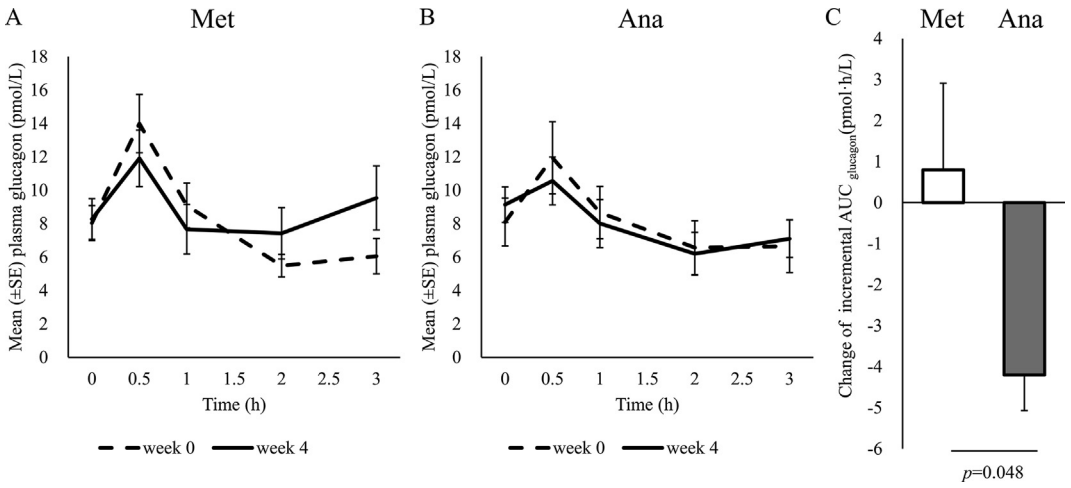

Fig. 2 – Plasma glucagon profile following ingestion of a liquid test meal before (dotted line) and after (solid line) 4 weeks of treatment with (A) metformin (Met, n = 10) or (B) anagliptin (Ana, n = 12). Changes between before and the end of treatment with metformin (open bar: □) or anagliptin (a closed bar: ■) were calculated for incremental AUC of glucagon (pmol·h/L) (C). Data are presented as mean values ± SEM.

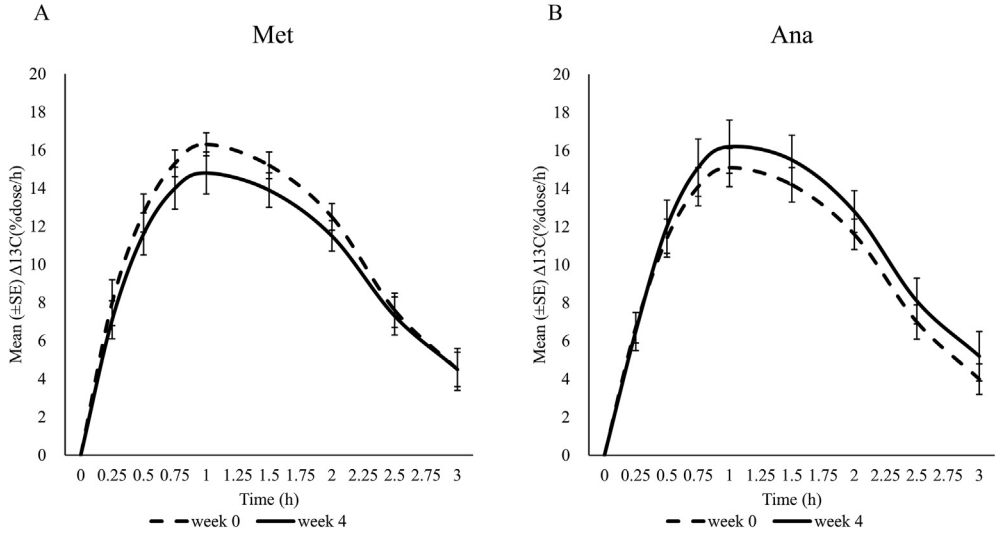

Fig. 3 – Response of ^{13}C to ingestion of a liquid test meal before (dotted line) and after (solid line) 4 weeks of treatment with (A) metformin (Met, n = 10) or (B) anagliptin (Ana, n = 12). Data are presented as mean values ± SEM.

glucagon iAUC was decreased by 4.2 pmol·h/L (95% CI −6.1, −2.3) in the anagliptin group and increased by 0.8 pmol·h/L (95% CI −3.9, 5.6) in the metformin group, with a slight difference between the two groups (−5.0 pmol·h/L; 95%CI −10.0, −0.1, $P = 0.048$).

At baseline, the iAUC of plasma C-peptide was similar in both groups (Table 2). After 4 weeks of treatment, the iAUC showed no change in the metformin group and was increased in the anagliptin group with the difference between the two groups of 0.4 ng·h/mL (95%CI −0.8, 1.5). However, plasma C-peptide levels showed an increase from baseline at 0.5 and 1.0 h in the anagliptin group in Supplemental Fig. 1D.

The iAUC of total GLP-1 decreased after treatment in anagliptin group, while increased in metformin group with the difference of −21.4 pmol·h/L (95%CI −40.3, −2.5). However, the plasma profile of active GLP-1 showed a difference (Supplemental Fig. 1E and F). Its iAUC increased after treatment in both groups, with the difference between them of 0.2 pmol·h/L (95%CI −23.2, 23.6). Active GLP-1 was increased at 0.5 h in the anagliptin group compared with the corresponding time point before treatment in Supplemental Fig. 1F.

The plasma profile of active GIP is also displayed in Table 2 and Supplemental Fig. 1G and H. Its iAUC increased after treatment in anagliptin group, whereas decreased in metformin group with the difference of 90.6 pmol·h/L (95%CI 45.2, 136.0).

The effect of each study treatment on gastric emptying is shown in Fig. 3. At baseline, mean $T_{1/2}$ and T_{lag} were similar in both groups (109.5 min (95%CI 97.5, 121.4) in the metformin group vs. 107.5 min (95%CI 97.5, 117.5) in the anagliptin group and 64.2 min (95%CI 54.1, 75.3) vs. 66.7 min (95%CI 59.5, 73.8), respectively). After 4 weeks of treatment, the mean increment

of $T_{1/2}$ in the metformin group was 4.2 min (95%CI −7.75, 16.1) and that of T_{lag} was 4.6 min (95%CI −3.95, 13.2) (Fig. 3A). Similarly, the increment of $T_{1/2}$ in the anagliptin group was 2.0 min (95%CI −7.9, 11.9) and that of T_{lag} was 2.1 min (95% CI −6.4, 10.5) (Fig. 3B). The differences of $T_{1/2}$ or T_{lag} between the two groups was −2.2 min (95%CI −18.8, 14.4) and −2.6 min (95%CI −15.4, 10.3), respectively.

No severe adverse events occurred in either group during the study period.

4. Discussion

There is increasing evidence that glucagon has a critical role in glucose metabolism [19], but it has been difficult to measure plasma glucagon precisely due to the existence of multiple peptides derived from proglucagon that show cross-reactivity with glucagon.

In the present randomized study, we evaluated the effect of anagliptin on plasma glucagon in patients with type 2 diabetes by using a new LC-HRMS assay and we obtained three main findings. First, after 4 weeks of treatment with metformin (1000 mg/day) or anagliptin (200 mg/day), improvement of glycemic control was comparable, including fasting plasma glucose, HbA1c, and the glucose profile during after ingestion of a 200 kcal liquid test meal. Second, anagliptin slightly decreased the iAUC of plasma glucagon during the meal test, and increased early plasma levels of active GLP-1, active GIP, and C-peptide levels. Third, neither anagliptin nor metformin caused any delay of gastric emptying.

This is the first study employing LC-HRMS for comparison of the plasma glucagon level between metformin and anagliptin. Though anagliptin decreased plasma glucagon, the reduction was small. This result is not surprising because (1) the plasma glucose level improved (with HbA1c being 6.7% [49 mmol/mol]) after 4 weeks of treatment and the glucagon-lowering action of GLP-1 is glucose-dependent [20], and (2) the baseline plasma glucagon level was not elevated in our subjects, while the plasma C-peptide level (representing endogenous insulin secretion) was not low. A recent single-arm study comparing the plasma glucagon level measured by the same LC-HRMS method that we used or by a commercial ELISA kit showed significant reduction of plasma glucagon by the same anagliptin regimen as ours [21]. That study recruited patients on insulin and the baseline plasma C-peptide AUC was lower than in our subjects, while the plasma glucagon AUC was higher. These differences of baseline plasma glucagon and C-peptide levels between the two study populations can be explained because glucagon secretion from pancreatic α-cells is inhibited by endogenous insulin secretion in a paracrine manner [22]. In that study, the baseline plasma glucagon level was higher and the reduction by anagliptin treatment was greater than in the present study. Therefore, it seems difficult to detect a decline of glucagon secretion when baseline secretion is not high in patients with preserved endogenous insulin secretion.

As expected, treatment with anagliptin induced a larger GLP-1 and GIP response to the test meal. In Japanese subjects without DPP-4 inhibitors, total and intact GLP-1 did not show a significant peak, whereas intact GIP reached a peak 30 min after meal ingestion [23]. A single administration of a DPP-4 inhibitor, vildagliptin has been reported to elevate plasma active GLP-1, active GIP, and C-peptide levels within 30 min during the meal tolerance test [24]. In the present study, the anagliptin group showed a similar plasma profile with this study regarding active GLP-1, active GIP, and C-peptide during the meal tolerance test. Our results are consistent with the original action of DPP-4 inhibitors, which increase active GLP-1 and GIP by inhibiting their degradation, thus lowering blood glucose levels by stimulating insulin secretion [25].

Along with the increase of GLP-1, there was also the possibility that anagliptin delayed gastric emptying [26]. However, we found no delay of gastric emptying with either treatment in the present study. It is well recognized that GLP-1 receptor agonists delay gastric emptying by increasing plasma GLP-1 to pharmacological levels (6- to 10-fold increment) [27], but DPP-4 inhibitors only increase plasma GLP-1 levels within the physiological range (2- to 3-fold increment). A previous study reported that sitagliptin did not affect the rate of gastric emptying by determining plasma acetaminophen [28]. Thus, it is reasonable that anagliptin did not delay gastric emptying in the present study.

We found that metformin also did not delay gastric emptying, although there have been recent reports that a single dose of metformin delays gastric emptying in mice [29] and humans [30]. In the present study, the active GLP-1 level was higher at all time points compared with baseline in the metformin group, probably due to increased intestinal secretion of GLP-1, which is one of the gastrointestinal effects of metformin [31–33]. However, four weeks of treatment with metformin could affect gastric emptying via tachyphylaxis, which is often observed during administration of long-acting GLP-1 receptor agonists [34].

The present pilot study had some limitations, including a small sample size and its single-center, open-label design. Because this study were exploratory, multiple comparisons were not appropriate. Also, we measured the active GLP-1 and GIP following the manufacturers' instructions and did not perform extraction treatment. Recent studies have shown that interfering substances in blood could influence the assay for incretin hormones and ethanol or solid-phase extraction treatment is suitable for the more precise assays [35,36]. Therefore, our data include a potential problem overestimating "biologically intact" incretin hormones. Despite these limitations, our data contribute to better understanding of the glucose-lowering mechanisms of DPP-4 inhibitors.

In conclusion, administration of metformin (1000 mg/day) or anagliptin (200 mg/day) for 4 weeks achieved comparable improvement of glycemic control. In patients with type 2 diabetes maintained endogenous insulin secretion, anagliptin increased the active GLP-1 and GIP levels in association with a slight stimulation of insulin secretion and a slight inhibition of glucagon secretion, but did not delay gastric emptying.

Contribution statement

YN designed the study, participated in data collection, and wrote the manuscript. TN participated in data collection, and edited the manuscript. YY participated in data collection.

AM measured glucagon levels using LC-HRMS and edited the manuscript. HH and EM measured glucagon levels using LC-HRMS. MT and EI were involved in statistical analysis and edited the manuscript. YT designed the study and edited the manuscript. All authors have approved the final version to be published.

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Data statement

Will individual participant data be available (including data dictionaries)?

Yes, individual participant data that underlie the results reported in this article, after deidentification (text, tables, figures, and appendices).

Beginning 3 months and ending 36 months following article publication.

What other documents will be available?

Study protocol (in Japanese), Statistical Analysis Plan (in Japanese).

With whom will data be shared, for what types of analyses, and by what mechanism?

Researchers who provide a methodologically sound proposal.

To achieve aims in the approved proposal.

Proposals should be directed to ynagai@marianna-u.ac.jp.

Declaration of Competing Interest

YN has received speaker's fees from Sanwa Kagaku Kenkyusho Co., Ltd. YT has received speaker's fees from Sumitomo Dainippon Pharma Co. Ltd., Sanofi K. K., Astellas Pharma Inc., Kissei Pharmaceutical Co., Ltd., MSD K.K., Novartis Pharma K. K., and Novo Nordisk Pharma Ltd.; research funding from Novartis Pharma K. K., Kowa Pharmaceutical Co. Ltd., Nichirei Company, Sanwa Kagaku Kenkyusho Co., Ltd., and Nippon Boehringer Ingelheim Co., Ltd.; and scholarship donations from Daiichi Sankyo Co. Ltd., Sumitomo Dainippon Pharma Co. Ltd., Shionogi & Co., Ltd., MSD K.K., Astellas Pharma Inc., Sanofi K. K., Ono Pharmaceutical Co., Ltd., Novo Nordisk Pharma Ltd., Tanabe Pharma Corporation, Takeda Pharmaceutical Co. Ltd., Novartis Pharma K. K., Abbott Japan Co., Ltd., Nippon Boehringer Ingelheim Co., Ltd., and Roche Diagnostics K. K.. AM, HH, and EM are employees of Sanwa Kagaku Kenkyusho Co., Ltd. TN, YY, MT, and EI have nothing to declare.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2019.107892.

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