Characterization of predictors for GLP-1 secretion showed that postprandial GLP-1 levels positively correlate with fasting FFA and HbA_{1c}, and negatively correlate with DPP-4 activity in Japanese patients with type 2 diabetes (Table 4 and Figure 4). Although fasting GLP-1 also showed strong associations with fasting FFA and DPP-4 (Table 3), incremental AUC-total GLP-1 did not show a significant correlation with fasting FFA ($r = 0.196$ and $P = 0.219$) and DPP-4 activity ($r = -0.100$ and $P = 0.535$), suggesting that fasting FFA and DPP-4 activity could predict basal, but not meal-induced, GLP-1 secretion in Japanese patients with type 2 diabetes. In contrast, incremental AUC-total GLP-1 show a significant correlation with HbA_{1c} ($r = 0.406$ and $P = 0.008$), whereas fasting GLP-1 did not (Table 3), suggesting that HbA_{1c} levels could predict meal-induced GLP-1 secretion in Japanese patients with type 2 diabetes. Further studies are, of course, needed to understand the physiological processes underlying associations of GLP-1 secretion with fasting FFA, HbA_{1c} and DPP-4 activity.

Regarding hormonal regulation of postprandial GLP-1 secretion, it was previously suggested that GLP-1 secretion was enhanced by GIP[27]. However, the present study in Japanese patients with type 2 diabetes does not support the notion that GIP enhances GLP-1 secretion, as no significant association was observed. Glucagon is another potential regulator of postprandial GLP-1 secretion. In Caucasians, not only does administration of exogenous GLP-1 decrease fasting plasma glucagon levels[28], but administration of exendin (9–39)amide, a potent GLP-1 antagonist, elevates fasting plasma glucagon levels[29]. Thus, GLP-1 at physiological levels has glucagonostatic actions, and a reverse relationship between GLP-1 and glucagon might exist. However, it has been recently shown that administration of glucagon does not affect postprandial GLP-1 secretion[30], and, thus, the mechanism underlying the association of fasting glucagon with postprandial GLP-1 secretion observed by Vollmer *et al.* is still unknown. Unlike the Vollmer study, the current study did not show strong associations of fasting and postprandial glucagon with postprandial GLP-1 secretion, suggesting some difference in regulation of GLP-1 secretion between Japanese and Caucasian patients with type 2 diabetes.

Characterization of predictors for GIP secretion showed that postprandial GIP secretion, in drug-naïve patients but not in SU-treated patients, positively correlated with fasting glucose and negatively correlated with fasting insulin, HOMA-β and SUIT (Table 4). Other parameters, including fasting FFA, did not show significant associations with pre- and postprandial GIP secretion, unlike the Vollmer study[15]. The Japanese patients in the present study had much lower BMI, but their age and HbA_{1c} were similar to those of Caucasian patients with type 2 diabetes in the Vollmer study. Further studies are needed to understand the differences in predictors of GLP-1 and GIP secretion between Japanese and Caucasian patients; unequal dependency of postprandial GIP and GLP-1 secretion in the current study confirms that secretion of the two incretin hormones is regulated by separate factors.

In conclusion, the present study has shown the effects of extractions on immunoassays to measure GLP-1 and GIP. Using incretin immunoassays with solid-phase extraction, we have shown that SU-treatment has little effect on pre- and postprandial secretion of GLP-1 and GIP in Japanese patients. Furthermore, we found that secretion of GLP-1 and GIP is predicted by different factors.

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REFERENCES

1. Drucker DJ. The biology of incretin hormones. *Cell Metab* 2006; 3: 153–165.
2. Holst JJ. The physiology of glucagon-like peptide 1. *Physiol Rev* 2007; 87: 1409–1439.
3. Seino Y, Fukushima M, Yabe D. GIP and GLP-1, the two incretin hormones: similarities and differences. *J Diabetes Invest* 2010; 1: 9–23.
4. Yabe D, Kuroe A, Lee S, *et al.* Little enhancement of meal-induced GLP-1 secretion in Japanese: comparison of type 2 diabetes and healthy controls. *J Diabetes Invest* 2010; 1: 56–59.
5. Aaboe K, Knop FK, Vilsboll T, *et al.* Twelve weeks treatment with the DPP-4 inhibitor, sitagliptin, prevents degradation of peptide YY and improves glucose and non-glucose induced insulin secretion in patients with type 2 diabetes mellitus. *Diabetes Obes Metab* 2010; 12: 323–333.
6. Deacon CF, Holst JJ. Immunoassays for the incretin hormones GIP and GLP-1. *Best Pract Res Clin Endocrinol Metab* 2009; 23: 425–432.
7. LaMarca CJ, Perregaux DG, Preston GM, *et al.* Comparison and validation of multiple commercially available methods for active GLP-1 quantification: sample extraction is a ubiquitous requirement. In: ENDD2009. Washington, D.C. 2009.
8. Parker HE, Reimann F, Gribble FM. Molecular mechanisms underlying nutrient-stimulated incretin secretion. *Expert Rev Mol Med* 2010; 12: e1.
9. Parker HE, Habib AM, Rogers GJ, *et al.* Nutrient-dependent secretion of glucose-dependent insulinotropic polypeptide from primary murine K cells. *Diabetologia* 2009; 52: 289–298.
10. Reimann F, Habib AM, Tolhurst G, *et al.* Glucose sensing in L cells: a primary cell study. *Cell Metab* 2008; 8: 532–539.
11. Ross SA, Brown JC, Dupre J. Hypersecretion of gastric inhibitory polypeptide following oral glucose in diabetes mellitus. *Diabetes* 1977; 26: 525–529.

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12. Takemura J, Seino Y, Tsuda K, *et al.* Hypersecretion of gastric inhibitory polypeptide induced by glucose ingestion in diabetes mellitus. *Endocrinol Jpn* 1981; 28: 17–21.
13. Toft-Nielsen MB, Damholt MB, Madsbad S, *et al.* Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. *J Clin Endocrinol Metab* 2001; 86: 3717–3723.
14. Vilsboll T, Krarup T, Deacon CF, *et al.* Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes* 2001; 50: 609–613.
15. Vollmer K, Holst JJ, Baller B, *et al.* Predictors of incretin concentrations in subjects with normal, impaired, and diabetic glucose tolerance. *Diabetes* 2008; 57: 678–687.
16. Nauck MA, Vardarli I, Deacon CF, *et al.* Secretion of glucagon-like peptide-1 (GLP-1) in type 2 diabetes: what is up, what is down? *Diabetologia* 2010; 54: 10–18.
17. Seino Y, Nanjo K, Tajima N, *et al.* Report of the Committee on the Classification and Diagnostic Criteria of Diabetes Mellitus. *J Diabetes Invest* 2010; 1: 212–228.
18. Seino Y, Nanjo K, Tajima N, *et al.* Report of the Committee on the Classification and Diagnostic Criteria of Diabetes Mellitus. *Diabetol Int* 2010; 1: 2–20.
19. Zhu L, Tamvakopoulos C, Xie D, *et al.* The role of dipeptidyl peptidase IV in the cleavage of glucagon family peptides: in vivo metabolism of pituitary adenylate cyclase activating polypeptide-(1-38). *J Biol Chem* 2003; 278: 22418–22423.
20. Matthews DR, Hosker JP, Rudenski AS, *et al.* Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28: 412–419.
21. Yamada Y, Fukuda K, Fujimoto S, *et al.* SUIT, secretory units of islets in transplantation: an index for therapeutic management of islet transplanted patients and its application to type 2 diabetes. *Diabetes Res Clin Pract* 2006; 74: 222–226.
22. Dai H, Gustavson SM, Preston GM, *et al.* Non-linear increase in GLP-1 levels in response to DPP-IV inhibition in healthy adult subjects. *Diabetes Obes Metab* 2008; 10: 506–513.
23. Conlon JM, Bridgeman M, Alberti KG. The nature of big plasma somatostatin: implications for the measurement of somatostatin-like immunoreactivity in human plasma. *Anal Biochem* 1982; 125: 243–252.
24. Tsuda K, Sakurai H, Seino Y, *et al.* Somatostatin-like immunoreactivity in human peripheral plasma measured by radioimmunoassay following affinity chromatography. *Diabetes* 1981; 30: 471–474.
25. Lee S, Yabe D, Nohtomi K, *et al.* Intact glucagon-like peptide-1 levels are not decreased in Japanese patients with type 2 diabetes. *Endocr J* 2010; 57: 119–126.
26. Nielsen LB, Ploug KB, Swift P, *et al.* Co-localisation of the Kir6.2/SUR1 channel complex with glucagon-like peptide-1 and glucose-dependent insulinotrophic polypeptide expression in human ileal cells and implications for glycaemic control in new onset type 1 diabetes. *Eur J Endocrinol* 2007; 156: 663–671.
27. Roberge JN, Brubaker PL. Regulation of intestinal proglucagon-derived peptide secretion by glucose-dependent insulinotropic peptide in a novel enteroendocrine loop. *Endocrinology* 1993; 133: 233–240.
28. Ritzel R, Orskov C, Holst JJ, *et al.* Pharmacokinetic, insulinotropic, and glucagonostatic properties of GLP-1 [7-36 amide] after subcutaneous injection in healthy volunteers. Dose-response-relationships. *Diabetologia* 1995; 38: 720–725.
29. Schirra J, Sturm K, Leicht P, *et al.* Exendin(9-39)amide is an antagonist of glucagon-like peptide-1(7-36)amide in humans. *J Clin Invest* 1998; 101: 1421–1430.
30. Meier JJ, Ueberberg S, Korbas S, *et al.* Diminished glucagon suppression after beta-cell reduction is due to impaired alpha-cell function rather than an expansion of alpha-cell mass. *Am J Physiol Endocrinol Metab* 2011; 300: E717–723.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 | Multiple regression analysis of potential predictors of fasting GLP-1 and GIP levels in Japanese patients with type 2 diabetes

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Effects of glucose and meal ingestion on incretin secretion in Japanese subjects with normal glucose tolerance

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ABSTRACT

Aims/Introduction: Gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are the major incretins; their secretion after various nutrient loads are well-evaluated in Caucasians. However, little is known of the relationship between incretin secretion and differing nutritional loading in Japanese subjects. In the present study, we evaluated GIP and GLP-1 secretion in Japanese subjects with normal glucose tolerance (NGT) after glucose loading (75 g glucose and 17 g glucose) and meal ingestion.

Materials and Methods: A total of 10 Japanese NGT subjects participated in 75 g oral glucose tolerance test (OGTT), 17 g OGTT and meal tolerance test (MTT). Plasma glucose (PG), serum insulin (IRI), serum C-peptide (CPR), plasma total GIP, and plasma total GLP-1 levels during OGTT and MTT were determined.

Results: Area under the curve (AUC)-GIP was increased in proportion to the amount of glucose, and was highest in MTT, showing that GIP secretion is also stimulated by nutrients other than glucose, such as lipid. In contrast, although the larger glucose load tended to induce a larger GLP-1 release, AUC-GLP-1 was not significantly different among the three loading tests (75 g OGTT, 17 g OGTT, MTT) irrespective of the kind or amount of nutrition load.

Conclusions: Our results suggest that nutritional composition might have a greater effect on GIP secretion than that on GLP-1 secretion in Japanese NGT subjects. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2011.00143.x, 2012)

KEY WORDS: Incretin, Meal tolerance test, Oral glucose tolerance test

INTRODUCTION

Oral glucose administration leads to greater insulin release from pancreatic islets than that by intravenous glucose loading yielding equivalent glucose levels. Gut hormonal substances released in response to glucose include the incretins, gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), which are responsible for 50–60% of postprandial insulin secretion[1]. GIP is secreted on meal ingestion from K-cells in the proximal small intestine, whereas GLP-1 is secreted from L-cells in the distal small intestine and colon, and binds to their respective receptors on the surface of pancreatic β-cells to stimulate insulin secretion by increasing the intracellular adenosine 3′,5′-monophosphate concentration[2].

The incretin effect has been shown to be reduced in type 2 diabetic patients compared with that in normal glucose tolerance (NGT) subjects in previous studies[3,4], suggesting that a reduced incretin effect might be associated with hyperglycemia after food intake and glucose loading in type 2 diabetes. Plasma GLP-1 concentrations in type 2 diabetic patients have been reported to be reduced after meal ingestion and glucose loading[4,5]. However, in other studies, it was reported that GLP-1 concentrations did not differ in NGT and type 2 diabetic patients[6–8]. When intravenous infusion of GIP or GLP-1 was carried out in type 2 diabetic patients, GLP-1 potentiated insulin secretion from pancreatic β-cells, but GIP did not, showing that the GIP receptor (GIPR) signal is reduced in β-cells in type 2 diabetes[9]. In contrast, the GIPR signal plays an important role in maintaining blood glucose levels in the non-diabetic obese state[10,11]. Indeed, GIP concentrations are reported to be increased in obese rodent models and obese Caucasian subjects compared with those in lean rodents and lean Caucasian subjects, respectively[12–14]. In addition, we have previously shown hypersensitivity of GIPR to GIP in β-cells of high fat-induced obese mice[11]. In summary, evaluation of incretin secretion and the incretin effect in subjects with various levels of glucose tolerance is important to determine the contribution of incretin deficiency in progression from NGT to type 2 diabetes.

Type 2 diabetes is characterized by both decreased insulin secretion and reduced insulin sensitivity[15–17]. In Caucasians,

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insulin resistance is thought to play a critical role in the pathogenesis of type 2 diabetes. In contrast, insulin sensitivity in Asian subjects has been shown to be higher than that in Mexican Americans and Caucasians in previous reports[18,19], which is partly because of the fact that Asians, including Japanese, are generally less obese. Thus, insulin secretion rather than insulin sensitivity is considered to be the more important factor in progression from NGT to diabetes in Japanese subjects[20]. Indeed, we have reported that early-phase insulin secretion is considerably decreased even in Japanese NGT subjects with 1-h plasma glucose levels higher than 10 mmol/L during an oral glucose tolerance test (OGTT)[21].

A recent study showed that, in both Caucasian NGT subjects and Caucasian type 2 diabetic patients, a meal tolerance test (MTT) elicited a significantly greater response of GIP levels than that elicited by OGTT, whereas GLP-1 levels were not different between OGTT and MTT[6]. In a previous study comparing the incretin secretion measured after different amounts of glucose load in healthy Caucasian subjects and type 2 diabetic Caucasian patients, GLP-1 and GIP were dose-dependently increased[22]. Plasma GLP-1 and GIP levels after glucose load or meal ingestion have been evaluated mainly in Caucasian subjects. In Japanese subjects, there has not been thorough elucidation, and little is known about the relationship between incretin secretion, and the kind and amount of nutrition load.

In the present study, we investigated incretin levels in association with the amount of glucose load and meal ingestion by measuring plasma GLP-1 and GIP levels after administration of 17 or 75 g glucose or mixed meal in Japanese NGT subjects.

MATERIALS AND METHODS

Subjects

A total of 10 healthy Japanese volunteers (eight male and two female) were recruited into the present study. The subjects had no history of hypertension, hyperlipidemia or kidney and liver diseases, and did not take any drugs 2 weeks before the study. The study was designed in compliance with the ethics regulations of the Helsinki Declaration and Kyoto University. Informed consent was obtained from all subjects.

Study Procedure

The subjects' age, height and bodyweight were determined. Blood samples for measurement of liver and kidney function, HbA_{1c} (National Glycohemoglobin Standardization Program), triglycerides (TG), total cholesterol and high-density lipoprotein (HDL)-cholesterol levels were drawn after an overnight fast. All subjects received 75 g OGTT, 17 g (approximately a quarter of 75 g) OGTT and a MTT. The interval between tests was 2–4 weeks. The total caloric content of the test meal was 450 kcal (carbohydrates 57.8 g, protein 17.2 g, fat 16.6 g). After the subjects fasted overnight for 10–16 h, OGTT or MTT was carried out according to the National Diabetes Data Group recommendations[23]. NGT was diagnosed according to World Health Organization (WHO) criteria[24].

Blood samples were collected at 0, 30, 60, 120 and 180 min after glucose loadings or meal ingestion and were centrifuged at 1800 *g* at 4°C for 10 min. After collecting supernatant of the samples, plasma and serum were stocked at −80°C. Blood was distributed into chilled tubes containing ethylenediaminetetraacetic acid and aprotinin (500 kIU/mL blood, Trasylol; SRL Inc., Tokyo, Japan) for analyses of GLP-1 and GIP. Plasma glucose (PG), serum insulin (IRI), serum C-peptide (CPR), plasma total GIP and plasma total GLP-1 were measured at the indicated times. The PG levels were measured by the glucose oxidase method. Serum IRI and CPR levels were measured by enzyme-linked immunosorbent assay. Total GIP and total GLP-1 levels were measured using a human GIP ELISA kit (Linco Research, St Charles, MO, USA) and human GLP-1 ELISA kit (Meso Scale Discovery, Gaithersburg, MD, USA), respectively, as previously described[25].

Calculations and Statistical Analysis

The area under the curve of PG (AUC-PG), IRI (AUC-IRI), CPR (AUC-CPR), total GIP (AUC-GIP) and total GLP-1 (AUC-GLP-1) were calculated by the trapezoidal rule. Statistical analyses were carried out using ANOVA and unpaired Student's *t*-test. *P*-values <0.05 were considered statistically significant. Data are presented as mean ± standard error (SE).

RESULTS

The profiles of the subjects are shown in Table 1. Mean age was 32.2 ± 2.0 years and mean body mass index was 22.4 ± 0.8 kg/m^2. Insulinogenic index, homeostasis model assessment (HOMA)-β and HOMA-insulin resistance were 0.59 ± 0.10, 76.50 ± 12.60, 1.10 ± 0.19, respectively. No subjects had liver or kidney dysfunction. HbA_{1c}, PG, TG, total cholesterol and HDL-cholesterol levels were within normal limits in the fasting state.

The profiles of PG, IRI and CPR in 75 g OGTT, 17 g OGTT and MTT are shown in Figure 1. Judging by the results of 75 g OGTT, all the subjects were diagnosed with NGT according to WHO criteria with fasting plasma glucose and 2 h glucose levels below 6.1 and 7.8 mmol/L, respectively. Fasting concentrations of PG, IRI and CPR were not different among the two OGTT and

Table 1 | Clinical characteristics of the subjects

n (Male/female)	10 (8/2)
Age (years)	32.2 ± 2.0
BMI (kg/m^2)	22.4 ± 0.8
Fasting plasma glucose (mmol/L)	4.9 ± 0.2
HbA_{1c} (%)	5.3 ± 0.1
Triglycerides (mg/dL)	79.4 ± 10.5
Total cholesterol (mg/dL)	169.2 ± 6.1
HDL-cholesterol (mg/dL)	61.5 ± 5.3
LDL-cholesterol (mg/dL)	93.0 ± 9.2

Data represent the mean ± SD. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

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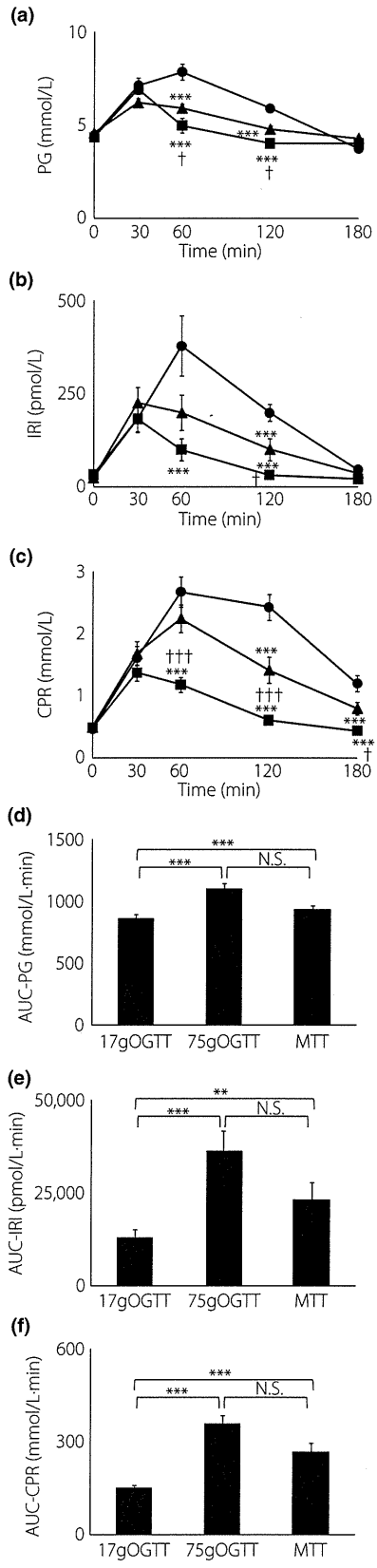

Figure 1 | Concentrations of (a) plasma glucose (PG), (b) serum insulin (IRI) and (c) serum C-peptide (CPR) during the 75 g oral glucose tolerance test (OGTT; closed circle), 17 g OGTT (closed square) and meal tolerance test (MTT; closed triangle) in 10 Japanese subjects. Asterisks indicate significant differences vs 75 g OGTT at individual time-points (*P < 0.05, **P < 0.01, ***P < 0.001); daggers indicate significant differences vs MTT at individual time-points (†P < 0.05, ††P < 0.01, †††P < 0.001). (d) Area under the curve (AUC)-PG, (e) AUC-IRI, (f) AUC-CPR were calculated by the trapezoidal rule. Asterisks indicate significant differences at individual time-points (*P < 0.05, **P < 0.01, ***P < 0.001). Statistical analyses were carried out using ANOVA and unpaired Student's t-test. P-values <0.05 were considered statistically significant. Data are presented as mean ± standard error. N.S., not significant.

MTT. In OGTT studies, AUC-PG, AUC-IRI and AUC-CPR measured by the 75 g OGTT were significantly larger than those measured by the 17 g OGTT (Figure 1d–f). At 30 min after glucose ingestion, the levels of PG, IRI and CPR in the 75 g OGTT and those in the 17 g OGTT were not significantly different. Between MTT and the two OGTT, AUC-PG, AUC-IRI and AUC-CPR in MTT were significantly higher than those in the 17 g OGTT. AUC-PG, AUC-IRI and AUC-CPR in the 75 g OGTT and in MTT were not significantly different.

In the 17 g OGTT, the total GLP-1 level peaked at 30 min and rapidly decreased to the baseline at 60 min after the glucose load. The total GLP-1 level peaked at 30 min after the meal load and was sustained for up to 180 min. In the 75 g OGTT, the GLP-1 level peaked at 60 min and gradually decreased with time, but the level was still higher than baseline even at 180 min. The level of total GLP-1 at 60 min after the 75 g glucose load was significantly higher than that after the 17 g glucose load (Figure 2a). Although a larger glucose load tended to induce a larger GLP-1 release, total AUC-GLP-1 measured by the 75 g OGTT, 17 g OGTT and MTT were not significantly different (Figure 2b).

The baseline levels of GIP were approximately 10 pmol/L. The GIP level rapidly increased, peaked at 30 min after the meal load and gradually decreased with time, but the level was still higher than baseline even at 180 min. In the 75 g OGTT, the GIP level significantly increased at 30 min after the glucose load, peaked at 120 min and were maintained up to 180 min. In the 17 g OGTT, the total GIP level peaked at 30 min after glucose load and gradually decreased to baseline at 180 min. At 30 min after ingestion, total GIP levels in the 75 g OGTT and those in the 17 g OGTT were not significantly different (Figure 3a).

AUC-GIP was significantly higher in the 75 g OGTT than that in the 17 g OGTT. Unlike GLP-1, the peak levels of GIP and the AUC-GIP measured in the MTT were significantly higher than those measured in the 75 g OGTT and 17 g OGTT (Figure 3b).

DISCUSSION

In the present study, incretin levels were estimated after glucose loading or meal ingestion in Japanese NGT subjects.

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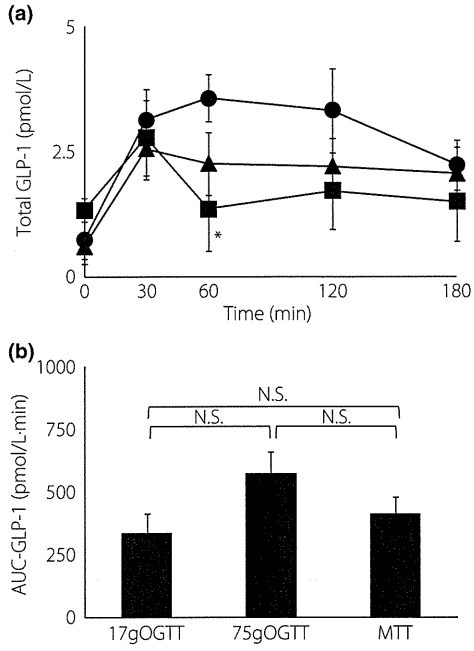

Figure 2 | (a) Concentrations of total glucagon-like peptide-1 (GLP-1) during the 75 g oral glucose tolerance test (OGTT; closed circle), 17 g OGTT(closed square) and meal tolerance test (MTT; closed triangle) in 10 Japanese subjects. Asterisks indicate significant differences vs 75 g OGTT at individual time-points (*P < 0.05, **P < 0.01, ***P < 0.001); daggers indicate significant differences vs MTT at individual time-points (†P < 0.05, ††P < 0.01, †††P < 0.001). (b) Area under the curve (AUC)-GLP-1 was calculated by the trapezoidal rule. Asterisks indicate significant differences at individual time-points (*P < 0.05, **P < 0.01, ***P < 0.001). Statistical analyses were carried out using ANOVA and unpaired Student's t-test. P-values <0.05 were considered statistically significant. Data are presented as mean ± standard error. N.S., not significant.

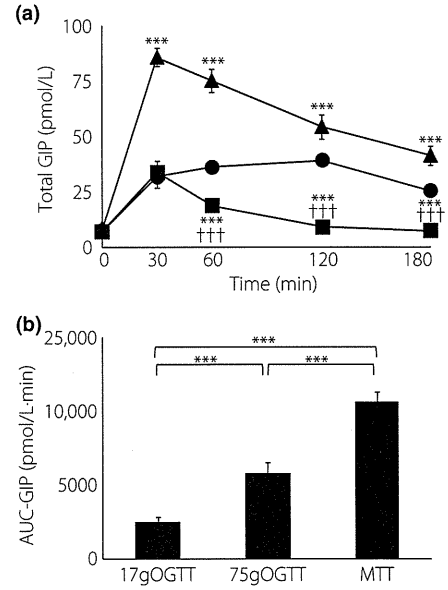

Figure 3 | (a) Concentrations of total gastric inhibitory polypeptide (GIP) during 75 g oral glucose tolerance test (OGTT; closed circle), 17 g OGTT (closed square) and meal tolerance test (MTT; closed triangle) in 10 Japanese subjects. Asterisks indicate significant differences vs 75 g OGTT at individual time-points (*P < 0.05, **P < 0.01, ***P < 0.001); daggers indicate significant differences vs MTT at individual time-points (†P < 0.05, ††P < 0.01, †††P < 0.001). (b) Area under the curve (AUC)-GIP was calculated by the trapezoidal rule. Asterisks indicate significant differences at individual time-points (*P < 0.05, **P < 0.01, ***P < 0.001). Statistical analyses were carried out using ANOVA and unpaired Student's t-test. P-values <0.05 were considered statistically significant. Data are presented as mean ± standard error.

Between the OGTT studies, AUC-PG, AUC-IRI and AUC-CPR in the 75 g OGTT were larger than those in the 17 g OGTT. Regarding incretins, AUC-GIP was significantly larger in the 75 g OGTT than in the 17 g OGTT. In contrast, AUC-GLP-1 was not significantly different between the 75 g OGTT and the 17 g OGTT. Previous studies showed that a larger amount of oral glucose load elicited more GIP and GLP-1 secretion[1,22], whereas a recent study also reported that the secretory response of GIP was more sensitive than that of GLP-1 to changes in intestinal carbohydrate content[26]. The present study also showed that while GLP-1 level was not increased, GIP level was increased dose-dependently in response to glucose load, showing higher sensitivity of GIP to changes of administered nutrient dose.

Between the 75 g OGTT and MTT studies, AUC-PG, AUC-IRI and AUC-CPR were not significantly different. AUC-GIP was significantly larger in MTT than that in the 75 g OGTT. In contrast, there was no significant difference in AUC-GLP-1 among the MTT and the two OGTT. By comparing the results of the three loading tests (75 g OGTT, 17 g OGTT, MTT), we speculate that AUC-GIP is more susceptible to the contents of each loading test than AUC-GLP-1 is. Vollmer *et al.*[6] reported that GIP responses were significantly higher in MTT than in OGTT, whereas GLP-1 levels were similar in both tests in Caucasian NGT, IGT and type 2 diabetic subjects. Because the mixed meal contains not only carbohydrates but also fat, which has been reported to stimulate GIP secretion[27–29], it is likely that the increased GIP concentrations after MTT were largely as a result of the fat content, which might have had no additional impact on GLP-1 secretion.

There are two previous reports that evaluate the incretin levels in both OGTT and MTT in Japanese NGT subjects[8,30]. However, they compared the incretin levels in 75 g glucose or meal load between NGT and type 2 diabetic subjects, but did not compare the incretin levels between 75 g glucose and meal load directly. The present study directly compared the incretin levels in the two OGTT and MTT. Our data clearly show that GIP responses were significantly higher in MTT than those in the two OGTT, whereas GLP-1 levels were not different between the two OGTT and MTT in Japanese NGT subjects.

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According to the study by Yabe *et al.*[8], AUC-GIP is similar between the OGTT and MTT group in Japanese control subjects. It should be noted that the difference between GIP secretion after meal load and that after glucose load was far greater in the present study than that in the study by Yabe *et al.* The total caloric content of the test meal used in their study was 480 kcal (carbohydrates 58.4%, protein 20.8%, fat 20.8%) and that in the present study was 450 kcal (carbohydrates 51.4%, protein 15.3%, fat 33.3%). Therefore, it is possible that the higher amount of contained fat in the test meal used in the present study led to the greater response of GIP secretion in the MTT.

Fasting and peak total GLP-1 concentrations in the present study were approximately 1 pmol/L and 3.5 pmol/L, respectively, and seemed to be lower than those in some published results[8,31]. However, in other reports, total GLP-1 levels after glucose and meal load were not very different from those in the present study. Rijkelijkhuizen *et al.*[32] measured the total GLP-1 concentration with radioimmunoassay, and in their results, the fasting and peak total GLP-1 concentrations in the MTT were approximately 1 pmol/L and 4.5 pmol/L, respectively. In addition, Villareal *et al.*[33] evaluated total GLP-1 concentrations by the same method that we used in the present study, and reported that the fasting and peak total GLP-1 concentrations in OGTT were approximately 1.5 and 6 pmol/L, respectively. Judging by the data in these reports, it is not necessarily the case that total GLP-1 concentrations were extremely low in the present study.

There are some reports showing that GLP-1 secretion is dependent on meal size, especially on carbohydrate and glucose loads. Schirra *et al.*[34] reported that GLP-1 plasma levels rose from basal levels to fourfold after 50 g glucose ingestion and to eightfold after 100 g glucose ingestion. Rijkelijkhuizen *et al.*[32] showed that GLP-1 secretion is increased by the amount of carbohydrate (75 and 109 g) and not by the quantity of the meal. In the present study, however, AUC-GLP-1 was not significantly different among the three loading tests (75 g OGTT, 17 g OGTT, MTT) irrespective of kinds or amounts of nutrition load, although larger glucose load tended to induce a larger GLP-1 release. The most notable difference between the previous studies and the present study was the amount of glucose load. We compared GLP-1 secretion after administration of 17 g glucose, 75 g glucose and 57.8 g of carbohydrate contained in the meal that we used. The amount of glucose and carbohydrate load in the present study were relatively lower than those in the previous studies. It is possible that evaluation of GLP-1 secretion after larger glucose loads could be more appropriate to show the glucose dependency of GLP-1 secretion.

It is also noteworthy that the levels of PG, IRI, CPR, GIP and GLP-1 at 30 min after the 75 g OGTT and 17 g OGTT were similar to each other. In addition, the levels of IRI, CPR and GLP-1 at 30 min after MTT and the two OGTT were not significantly different. By contrast, the GIP level at 30 min after MTT was much higher than those after the 17 g OGTT and 75 g OGTT. Given the similar plasma glucose levels at 30 min after the 17 g OGTT and 75 g OGTT, it is likely that under physiological conditions, the rate at which ingested glucose emptied into the duodenum is regulated finely enough to prevent an abrupt increase in plasma glucose levels irrespective of the amount of ingested glucose. Previous studies have shown that GLP-1 secretion after a test meal or oral glucose load is associated with the rate of gastric emptying, whereas GIP secretion seems to be dependent on nutrient absorption rather than on rate of gastric emptying[34]. Accordingly, a finely regulated rate of gastric emptying might account for the similar levels of GLP-1 at 30 min after MTT, 17 g OGTT and 75 g OGTT. In contrast, the level of total GIP at 30 min after the MTT was much higher than those after the two OGTT, probably because of the presence of fat in the duodenal lumen, as fat is a forcible stimulant of GIP, as discussed earlier.

The present results clearly show that the secretion of GIP and GLP-1 are regulated by different nutrient factors. On the basis of our data, it is also suggested that nutritional composition might have a greater effect on GIP secretion than on GLP-1 secretion in Japanese NGT subjects.

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REFERENCES

1. Nauck MA, Homberger E, Siegel EG, *et al.* Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. *J Clin Endocrinol Metab* 1986; 63: 492–498.
2. Seino Y, Fukushima M, Yabe D. GIP and GLP-1, the two incretin hormone: similarities and difference. *J Diabetes Invest* 2010; 1: 8–23.
3. Nauck M, Stockmann F, Ebert R, *et al.* Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. *Diabetologia* 1986; 29: 46–52.
4. Muscelli E, Mari A, Casolaro A, *et al.* Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and type 2 diabetic patients. *Diabetes* 2008; 57: 1340–1348.
5. Vilsboll T, Krarup T, Deacon CF, *et al.* Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes* 2001; 50: 609–613.
6. Vollmer K, Holst JJ, Baller B, *et al.* Predictors of incretin concentrations in subjects with normal, impaired, and diabetic glucose tolerance. *Diabetes* 2008; 57: 678–687.
7. Faerch K, Vaag A, Holst JJ, *et al.* Impaired fasting glycaemia vs impaired glucose tolerance: similar impairment of pancreatic alpha and beta cell function but differential roles of incretin hormones and insulin action. *Diabetologia* 2008; 51: 853–861.

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8. Yabe D, Kuroe A, Lee S, *et al.* Little enhancement of meal-induced glucagon-like peptide 1 secretion in Japanese: comparison of type 2 diabetes patients and healthy controls. *J Diabetes Invest* 2010; 1: 56–59.
9. Nauck MA, Heimesaat MM, Orskov C, *et al.* Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J Clin Invest* 1993; 91: 301–307.
10. Miyawaki K, Yamada Y, Yano H, *et al.* Glucose intolerance caused by a defect in the entero-insular axis: a study in gastric inhibitory polypeptide receptor knockout mice. *Proc Natl Acad Sci USA* 1999; 96: 14843–14847.
11. Harada N, Yamada Y, Tsukiyama K, *et al.* A novel GIP receptor splice variant influences GIP sensitivity of pancreatic beta-cells in obese mice. *Am J Physiol Endocrinol Metab* 2008; 294: E61–E68.
12. Miyawaki K, Yamada Y, Ban N, *et al.* Inhibition of gastric inhibitory polypeptide signaling prevents obesity. *Nat Med* 2002; 8: 738–742.
13. Flatt PR, Bailey CJ, Kwasowski P, *et al.* Abnormalities of GIP in spontaneous syndromes of obesity and diabetes in mice. *Diabetes* 1983; 32: 433–435.
14. Creutzfeldt W, Ebert R, Willms B, *et al.* Gastric inhibitory polypeptide (GIP) and insulin in obesity: increased response to stimulation and defective feedback control of serum levels. *Diabetologia* 1978; 14: 15–24.
15. Mitrakou A, Kelley D, Mokan M, *et al.* Role of reduced suppression of glucose production and diminished early insulin release in impaired glucose tolerance. *N Engl J Med* 1992; 326: 22–29.
16. Haffner SM, Stern MP, Hazuda HP, *et al.* Increased insulin concentrations in nondiabetic offspring of diabetic parents. *N Engl J Med* 1988; 319: 1297–1301.
17. Saad MF, Knowler WC, Pettitt DJ, *et al.* A two-step model for development of non-insulin-dependent diabetes. *Am J Med* 1991; 90: 229–235.
18. Chiu KC, Chuang LM, Yoon C. Comparison of measured and estimated indices of insulin sensitivity and beta cell function: impact of ethnicity on insulin sensitivity and beta cell function in glucose-tolerant and normotensive subjects. *J Clin Endocrinol Metab* 2001; 86: 1620–1625.
19. Mandavilli A, Cyranoski D. Asia's big problem. *Nat Med* 2004; 10: 325–327.
20. Seino Y, Ikeda M, Yawata M, *et al.* The insulinogenic index in secondary diabetes. *Horn Metab Res* 1975; 7: 323–335.
21. Harada N, Fukushima M, Toyoda K, *et al.* Factors responsible for elevation of 1-h postchallenge plasma glucose levels in Japanese men. *Diabetes Res Clin Pract* 2008; 81: 284–289.
22. Bagger JI, Knop FK, Lund A, *et al.* Impaired regulation of the incretin effect in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2011; 96: 737–745.
23. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. National Diabetes Data Group. *Diabetes* 1979; 28: 1039–1057.
24. Alberi KG, Zimmeret PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998; 54: 539–553.
25. Harada N, Hamasaki A, Yamane S, *et al.* Plasma GIP and GLP-1 levels after glucose loading are associated with different factors in Japanese subjects. *J Diabetes Invest* 2011; 2: 193–199.
26. Yoder SM, Yang Q, Kindel TL, *et al.* Differential responses of the incretin hormones GIP and GLP-1 to increasing doses of dietary carbohydrate but not dietary protein in lean rats. *Am J Physiol Gastrointest Liver Physiol* 2010; 299: G476–G485.
27. Brown JC, Dryburgh JR, Ross SA, *et al.* Identification and actions of gastric inhibitory polypeptide. *Recent Prog Horm Res* 1975; 31: 487–532.
28. Falko JM, Crockett SE, Cataland S, *et al.* Gastric inhibitory polypeptide (GIP) stimulated by fat ingestion in man. *J Clin Endocrinol Metab* 1975; 41: 260–265.
29. Pederson RA, Schubert HE, Brown JC. Gastric inhibitory polypeptide. Its physiologic release and insulinotropic action in the dog. *Diabetes* 1975; 24: 1050–1056.
30. Lee S, Yabe D, Nohtomi K, *et al.* Intact glucagon-like peptide-1 levels are not decreased in Japanese patients with type 2 diabetes. *Endocr J* 2010; 57: 119–126.
31. Kozawa J, Okita K, Imagawa A, *et al.* Similar incretin secretion in obese and non-obese Japanese subjects with type 2 diabetes. *Biochem Biophys Res Commun* 2010; 393: 410–413.
32. Rijkelijkhuizen JM, McQuarrie K, Girman CJ, *et al.* Effects of meal size and composition on incretin, alpha-cell, and beta-cell responses. *Metabolism* 2010; 59: 502–511.
33. Villareal DT, Robertson H, Bell GI, *et al.* TCF7L2 variant rs7903146 affects the risk of type 2 diabetes by modulating incretin action. *Diabetes* 2010; 59: 479–485.
34. Schirra J, Katschinski M, Weidmann C, *et al.* Gastric emptying and release of incretin hormones after glucose ingestion in humans. *J Clin Invest* 1996; 97: 92–103.

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ORIGINAL ARTICLE

Linagliptin monotherapy provides superior glycaemic control versus placebo or voglibose with comparable safety in Japanese patients with type 2 diabetes: a randomized, placebo and active comparator-controlled, double-blind study

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Aims: To evaluate the efficacy and safety of linagliptin 5 and 10 mg vs. placebo and voglibose in Japanese patients with type 2 diabetes mellitus (T2DM).

Methods: This study enrolled patients with inadequately controlled T2DM who were previously treated with one or two oral antidiabetics or were drug naïve. After a 2 to 4-week washout and placebo run-in, 561 patients were randomized (2 : 2 : 2 : 1) to double-blind treatment with linagliptin 5 or 10 mg qd, voglibose 0.2 mg tid or placebo. The primary endpoint was the change from baseline in haemoglobin A1c (HbA1c) with linagliptin vs. placebo after 12 weeks and vs. voglibose after 26 weeks.

Results: Baseline characteristics were well balanced across treatment groups (overall mean HbA1c was 8.01%). The adjusted mean (95% confidence interval) treatment differences at week 12 were −0.87% (−1.04, −0.70; $p < 0.0001$) and −0.88% (−1.05, −0.71; $p < 0.0001$) for linagliptin 5 and 10 mg vs. placebo and at week 26 were −0.32% (−0.49, −0.15; $p = 0.0003$) and −0.39% (−0.56, −0.21; $p < 0.0001$) for linagliptin 5 and 10 mg vs. voglibose. At week 12, mean HbA1c was 7.58, 7.48 and 8.34% in patients receiving linagliptin 5 mg, linagliptin 10 mg and placebo, respectively. At week 26, mean HbA1c was 7.63% with linagliptin 5 mg, 7.50% with linagliptin 10 mg and 7.91% with voglibose. Drug-related adverse event rates were comparable across treatment groups over 12 weeks (9.4% linagliptin 5 mg, 8.8% linagliptin 10 mg and 10.0% placebo) and 26 weeks (11.3% linagliptin 5 mg, 10.6% linagliptin 10 mg and 18.5% voglibose). There were no documented cases of hypoglycaemia.

Conclusions: Linagliptin showed superior glucose-lowering efficacy and comparable safety and tolerability to both placebo and voglibose in Japanese patients with T2DM.

Keywords: α-glucosidase inhibitor, DPP-4 inhibitor, glycaemic control, incretin therapy

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Introduction

An estimated 9 million Japanese individuals suffer from diabetes [1], an increase in prevalence that underlines the need for therapeutic strategies to control this disease in Japan. Deterioration of glycaemic control over time, despite treatment, is characteristic of type 2 diabetes mellitus (T2DM) [2]. In addition to the natural progression of the disease, long-term glycaemic control is often not achieved or not maintained with many oral antidiabetic drugs (OADs) because of limitations to their use, such as treatment-limiting side effects including hypoglycaemia, gastrointestinal (GI) side effects, oedema and weight gain [3], or dose restrictions in patients with declining renal function. Importantly, renal impairment may occur in a large proportion of Japanese T2DM patients, as it has been estimated that ~30% have albuminuria (a condition that often precedes renal impairment and eventually renal failure) [4] and ~44% with early-onset T2DM develop diabetic nephropathy over 30 years [5]. Therefore, new OADs are needed that are effective and safe, can be used in patients who are at risk from or already have renal disease, and show minimal propensity to elicit hypoglycaemia or weight gain.

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Dipeptidyl peptidase (DPP)-4 inhibitors are the most recently developed class of OADs. DPP-4 inhibitors have a unique mechanism of action that increases endogenous glucagon-like peptide-1 levels and consequently promotes glucose-dependent insulin secretion via pancreatic β-cells, reduces glucagon secretion via α-cells, suppresses appetite and delays gastric emptying [6]. These actions reduce hyperglycaemia with minimal risk of hypoglycaemia, no weight gain and may reduce metabolic demands on pancreatic β-cells [7,8].

Linagliptin is a new DPP-4 inhibitor with a xanthine-based structure that is primarily excreted via bile and gut [9], a route of elimination that allows it to be used without dose adjustment in patients with declining renal function [10], unlike other DPP-4 inhibitors. Owing to its favourable pharmacokinetic and pharmacodynamic properties, there are no requirements for adjustment of linagliptin dose in any patient population [11].

Early clinical studies in Caucasian patients demonstrated linagliptin to have suitable pharmacological properties for once-daily oral administration, with similar tolerability to placebo [12–14]. Similar pharmacokinetics, pharmacodynamics and tolerability with all linagliptin doses tested have also been shown in early clinical studies in Japanese individuals [15,16]. Data from Phase II studies on effects on hyperglycaemia and the biomarker of plasma DPP-4 inhibition established linagliptin 5 mg daily as the optimal therapeutic dosage as monotherapy or as add-on to metformin in Caucasian patients [13,14]. Subsequently, several large international Phase III studies, which enrolled many participants from Asian countries including Japan, demonstrated clinically meaningful improvements in glycaemic control, enhanced β-cell function and a good safety profile with linagliptin 5 mg as monotherapy or in combination with other OADs [17–20]. Based on these data, linagliptin was recently licensed for treatment of T2DM in the USA in May 2011, in Japan in July 2011 and in Europe in August 2011.

It is important to validate the therapeutic effects and optimal dose of linagliptin in dedicated studies in Japanese patients with T2DM. Equally important is to compare the efficacy and safety of linagliptin with OADs commonly used in Japanese patients with T2DM to determine if linagliptin represents a therapeutic advance for this patient population.

α-glucosidase inhibitors are OADs with a different mechanism of action to the insulin sensitizers and secretagogues [21]. These compounds inhibit absorption of dietary carbohydrate in the GI tract, which provides postprandial anti-hyperglycaemic effects without substantial hypoglycaemia or weight gain. However, the use of α-glucosidase inhibitors is often limited by GI side effects such as flatulence, abdominal distension and diarrhoea. Voglibose is an α-glucosidase inhibitor that has demonstrated anti-hyperglycaemic effects in Japanese patients when administered three-times daily with food [22,23]. Voglibose is currently widely prescribed in Japan for treatment of T2DM.

This randomized, double-blind, parallel-group Phase IIb/III trial was conducted to examine the efficacy and safety of linagliptin 5 or 10 mg once daily as monotherapy compared with placebo over 12 weeks and with voglibose 0.2 mg three-times daily over 26 weeks in a large number of Japanese patients with T2DM.

Materials and Methods

This study was conducted at 47 centres in Japan between 1 April 2008 and 14 January 2010 (ClinicalTrials.gov, number NCT00654381). The design and methodology have been reported in detail previously [24], and are summarized below. This study was conducted in accordance with the International Conference on Harmonisation Guideline for Good Clinical Practice, the Declaration of Helsinki (October 1996 version) and relevant Japanese laws/regulations. All patients provided written informed consent before participation.

Study Design

Patients were randomized (2 : 2 : 2 : 1) to linagliptin 5 mg, linagliptin 10 mg, voglibose 0.6 mg or placebo following a 2-week placebo run-in (which was preceded by a 2-week washout period in patients who previously received one or two OADs). At week 12, patients receiving placebo were randomized (1 : 1) to linagliptin 5 or 10 mg. At week 26, patients receiving voglibose were randomized (1 : 1) to linagliptin 5 or 10 mg. Patients initially receiving linagliptin continued therapy at weeks 12 and 26. This was followed by an open-label extension period to evaluate the long-term safety/tolerability of linagliptin for up to 52 weeks of treatment, the results of which are to be reported in a second publication to maintain the data integrity in the randomization period.

Study Population

The study enrolled male and non-pregnant female patients with T2DM aged 20–80 years with a body mass index (BMI) $\leq$40 kg/m^{2}and inadequate glycaemic control [haemoglobin A1c (HbA1c) 7.0–10.0% in those previously untreated with OADs; HbA1c 7.0–9.0% at screening and 7.0–10% after washout in those already receiving one or two OADs for $\geq$10 weeks]. Key exclusion criteria were fasting plasma glucose (FPG) >13.3 mmol/l (>240 mg/dl) during washout or placebo run-in; myocardial infarction, stroke or transient ischaemic attack within the previous 6 months; impaired hepatic function (serum alanine aminotransferase, aspartate aminotransferase or alkaline phosphatase levels exceeding three times the upper limit of normal); treatment with a glitazone, insulin or anti-obesity drugs within the previous 3 months or any investigational agent within the previous 2 months; and/or a history of known intolerance, allergy or hypersensitivity to voglibose or any other concomitant drugs.

Study Endpoints

The co-primary endpoints were change from baseline in HbA1c with linagliptin vs. placebo at week 12 and vs. voglibose at week 26. Secondary endpoints included the percentage of patients achieving HbA1c <6.5% or <7.0% at weeks 12 and 26, change from baseline in HbA1c over time, change from baseline in FPG at weeks 12 and 26, and change from

baseline in FPG over time. Exploratory endpoints included the following: plasma proinsulin/insulin ratio; homeostasis model assessment indices for insulin resistance (HOMA-IR) and for insulin secretion (HOMA-IS); disposition index calculated as the product of β-cell function determined from HOMA-IS and HOMA-IR; glycosylated albumin; body weight, BMI and waist circumference; plasma lipid levels; and Diabetes Treatment Satisfaction Questionnaire data. Pharmacodynamic effects were evaluated based on plasma DPP-4 inhibition. Safety and tolerability were assessed by the incidence and intensity of adverse events (AEs) throughout the study period, and clinically relevant changes or findings after physical, vital sign, 12-lead electrocardiogram and laboratory assessments. Hypoglycaemia was defined according to American Diabetes Association guidelines [25]. An independent external clinical event committee conducted blinded adjudication of any suspected cardiovascular or cerebrovascular AEs. All laboratory measurements were performed by a central laboratory (Covance Laboratories, Harrogate, UK).

Statistical Analyses

A sample size of 441 randomized patients was planned (126 patients in each of the linagliptin and voglibose groups, 63 patients in the placebo group), which assumed a 15% drop-out rate. This would provide 90% power to detect a mean difference of 0.5% [standard deviation (s.d.) of 0.9%] in change from baseline in HbA1c between linagliptin and placebo at week 12, and 90% power to detect a mean difference of 0.45% (s.d. of 1.0%) in HbA1c change from baseline at week 26 between linagliptin and voglibose, at a one-sided significance level of 0.025 in both comparisons. To control for type I error, comparisons between linagliptin group and placebo or voglibose were performed using the closed testing procedure.

Efficacy analyses at weeks 12 and 26 were performed using the full analysis set (all randomized patients who had baseline and $\geq$1 post-baseline measurement and received $\geq$1 dose of study medication). Safety analyses at weeks 12 and 26 were performed on the treated set (all randomized patients who received $\geq$1 dose of study medication).

Analysis of covariance (ANCOVA) with baseline value as covariate and treatment and previous OAD therapy as variables was used to evaluate changes in continuous efficacy endpoints. Last observation carried forward was used to impute missing data for HbA1c, FPG, glycosylated albumin, body weight, BMI and waist circumference; observed cases were used for all other efficacy endpoints. Fisher's exact test was used to evaluate changes in categorical efficacy endpoints. Descriptive statistics without imputation of data were used for safety endpoints.

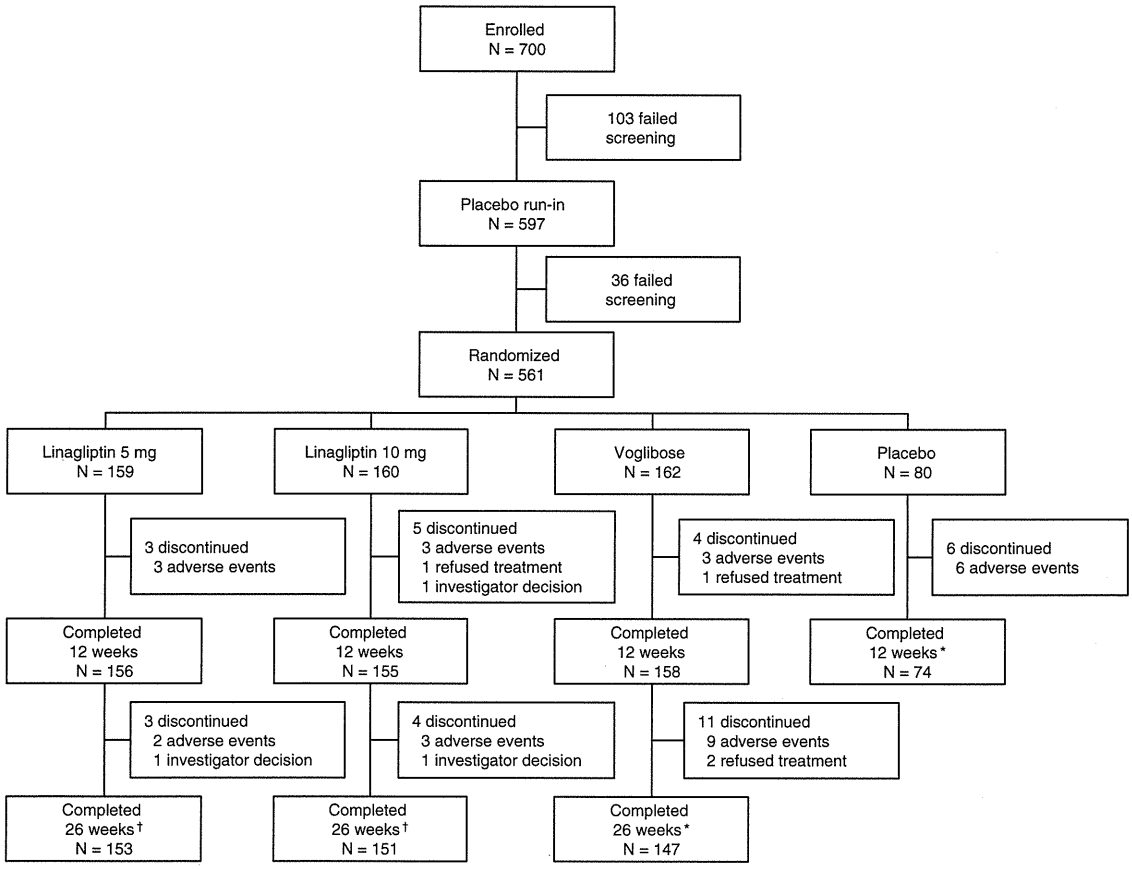

Figure 1. Patient disposition. *Patients randomized to linagliptin 5 or 10 mg for extension study. †Patients continued on linagliptin 5 or 10 mg for extension study.

Table 1. Baseline demographic and clinical characteristics by treatment group (treated set).

	Linagliptin 5 mg	Linagliptin 10 mg	Voglibose	Placebo
N	159	160	162	80
Gender, male [n (%)]	111 (69.8)	112 (70.0)	115 (71.0)	57 (71.3)
Age (years)	60.3 (9.4)	61.3 (10.0)	58.5 (9.9)	59.7 (8.9)
BMI (kg/m^2)	24.6 (4.0)	25.0 (3.8)	25.7 (4.0)	24.3 (3.4)
eGFR, MDRD (ml/min)	113.9 (24.9)	110.6 (26.5)	113.4 (25.8)	111.4 (26.3)
HbA1c (%)	8.07 (0.66)	7.98 (0.68)	8.02 (0.71)	7.95 (0.67)
FPG (mmol/l)	9.1 (1.8)	9.2 (1.9)	9.1 (1.8)	9.0 (1.7)
No. of previous OADs [n (%)]				
0	87 (54.7)	88 (55.0)	90 (55.6)	43 (53.8)
1	58 (36.5)	57 (35.6)	55 (34.0)	29 (36.3)
2	14 (8.8)	15 (9.4)	17 (10.5)	8 (10.0)
Time since diagnosis [n (%)]				
≤1 year	19 (11.9)	19 (11.9)	20 (12.3)	7 (8.8)
>1–5 years	61 (38.4)	59 (36.9)	65 (40.1)	36 (45.0)
>5 years	79 (49.7)	82 (51.3)	77 (47.5)	37 (46.3)
Concomitant disease [n (%)]				
Microvascular disease*	57 (35.8)	54 (33.8)	57 (35.2)	28 (35.0)
Macrovascular disease†	77 (48.4)	91 (56.9)	90 (55.6)	36 (45.0)
Metabolic syndrome	59 (37.1)	75 (46.9)	85 (52.5)	31 (38.8)

Data are mean (s.d.) or number (%) of patients. BMI, body mass index; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HbA1c, haemoglobin A1c; MDRD, modification of diet in renal disease study equation; OADs, oral antidiabetic drugs.
*Including diabetic retinopathy, diabetic nephropathy and diabetic neuropathy.
†Including coronary artery disease, peripheral artery occlusive disease, cerebrovascular disease and hypertension.

Results

Patient Disposition and Characteristics

A total of 700 patients were screened; 597 entered the placebo run-in and 561 were randomized to study treatment (linagliptin 5 mg: 159; linagliptin 10 mg: 160; voglibose: 162; placebo: 80) (figure 1). More patients completed treatment with linagliptin than with placebo or voglibose (week 12: 98.1, 96.9 and 92.5% of those receiving linagliptin 5 mg, linagliptin 10 mg or placebo, respectively; week 26: 96.2, 94.4 and 90.7% of those receiving linagliptin 5 mg, linagliptin 10 mg or voglibose, respectively). The most common cause of discontinuation was AEs both at 12 and 26 weeks.

Baseline demographics and clinical characteristics were comparable across the treatment groups (Table 1). The overall mean (±s.d.) age, BMI and HbA1c were 60.0 (±9.7) years, 25.0 (±3.9) kg/m^2 and HbA1c 8.01% (±0.68%), respectively. In total, 221 (39.4%) patients had T2DM for 1–5 years and 275 (49.0%) for over 5 years, and 99 (17.6%) had some degree of renal impairment (estimated glomerular filtration rate <90 ml/min). Previously drug-naïve patients [n = 308 (54.9%)] were younger, and had slightly lower HbA1c, lower FPG and shorter disease duration than previously treated patients [n = 253 (45.1%)], but the relative proportions of these patients were similar across groups (Table S1).

Efficacy

Both doses of linagliptin elicited reductions in HbA1c that were significantly greater than the changes achieved by either placebo at week 12 or voglibose at week 26 (Tables 2 and 3). Both doses of linagliptin achieved similar treatment differences vs. placebo at week 12, and similar differences vs. voglibose at week 26 (figure 2A).

Significantly greater proportions of patients in both linagliptin groups achieved therapeutic HbA1c targets than those in the placebo or voglibose groups. At week 12, target HbA1c of <7.0% was achieved in 26.4% of patients receiving linagliptin 5 mg ($p = 0.0038$ vs. placebo), 35.7% of those receiving linagliptin 10 mg ($p < 0.0001$ vs. placebo) and 10.0% of the placebo group. At week 26, this target was achieved in 30.2, 34.4 and 22.2% of patients receiving linagliptin 5 mg, linagliptin 10 mg or voglibose, respectively ($p = 0.13$ and $p = 0.02$ vs. voglibose for linagliptin 5 and 10 mg, respectively).

Both doses of linagliptin elicited significantly greater decreases in FPG from baseline than placebo at week 12 and voglibose at week 26 (Tables 2 and 3). In parallel with the significant amelioration of hyperglycaemia, linagliptin also elicited therapeutic changes in indices of β-cell function and insulin sensitivity (Tables 2 and 3). Both the 5 and 10 mg doses produced significant reductions in the proinsulin/insulin ratio compared with placebo at week 12 and voglibose at week 26. Linagliptin 5 mg also showed a tendency to improve fasting insulin, HOMA-IR and disposition index, although these did not reach significance vs. comparators at weeks 12 and 26.

There were very small reductions in body weight, BMI and waist circumference that were similar between linagliptin and placebo, and small but significant reductions in these endpoints with voglibose over linagliptin (Tables S2 and S3). No significant changes were observed in serum levels of triglycerides or total, high-density lipoprotein (HDL) or low-density lipoprotein (LDL) cholesterol with any treatment at weeks 12 and 26, with the exception of a small but significant

Table 2. Measures of glycaemic control in the linagliptin and placebo groups at week 12 (full analysis set).

	N	Week 0 [mean (s.e.)]	Week 12 [mean (s.e.)]	Adjusted mean change from baseline [mean (s.e.)]	Difference in means [linagliptin−placebo (95% CI)]
HbA1c† (%)					
Linagliptin 5 mg	159	8.07 (0.05)	7.58 (0.08)	−0.24 (0.06)	−0.87 (−1.04, −0.70)***
Linagliptin 10 mg	157	7.98 (0.05)	7.48 (0.08)	−0.25 (0.06)	−0.88 (−1.05, −0.71)***
Placebo	80	7.95 (0.07)	8.34 (0.14)	0.63 (0.08)	
FPG† (mmol/l)					
Linagliptin 5 mg	159	9.1 (0.1)	8.2 (0.1)	−0.7 (0.1)	−1.1 (−1.4,−0.8)***
Linagliptin 10 mg	160	9.2 (0.1)	8.3 (0.1)	−0.7 (0.1)	−1.1 (−1.5,−0.8)***
Placebo	80	9.0 (0.2)	9.3 (0.2)	0.4 (0.1)	
Fasting insulin‡ (mU/l)					
Linagliptin 5 mg	28	5.36 (1.06)	4.80 (0.74)	−0.50 (0.61)	−0.27 (−2.20, 1.66)
Linagliptin 10 mg	24	5.61 (0.94)	6.41 (1.39)	0.77 (0.64)	1.00 (−0.98, 2.97)
Placebo	12	3.96 (0.38)	3.88 (0.53)	−0.23 (0.83)	
Proinsulin/insulin ratio‡					
Linagliptin 5 mg	28	0.27 (0.04)	0.24 (0.03)	−0.04 (0.02)	−0.08 (−0.14, −0.02)*
Linagliptin 10 mg	21	0.23 (0.02)	0.18 (0.02)	−0.07 (0.02)	−0.12 (−0.18, −0.06)**
Placebo	12	0.22 (0.03)	0.28 (0.04)	0.05 (0.03)	
HOMA-IR‡ (mU/l × mmol/l)					
Linagliptin 5 mg	28	2.24 (0.43)	1.83 (0.30)	−0.35 (0.23)	−0.35 (−1.07, 0.38)
Linagliptin 10 mg	24	2.34 (0.40)	2.36 (0.48)	0.07 (0.24)	0.08 (−0.66, 0.82)
Placebo	12	1.62 (0.19)	1.71 (0.31)	−0.01 (0.31)	
HOMA-IS‡ [(mU/l)/(mmol/l)]					
Linagliptin 5 mg	28	19.67 (4.76)	21.51 (4.40)	1.65 (3.10)	2.14 (−7.64, 11.92)
Linagliptin 10 mg	24	20.84 (3.67)	29.87 (7.49)	7.91 (3.24)	8.40 (−1.61, 18.42)
Placebo	12	14.75 (1.48)	13.86 (1.96)	−0.49 (4.19)	
Disposition index‡					
Linagliptin 5 mg	37	11.28 (1.11)	13.84 (1.09)	2.07 (1.06)	2.20 (−1.07, 5.47)
Linagliptin 10 mg	36	9.87 (0.92)	12.99 (1.24)	2.44 (1.03)	2.57 (−0.69, 5.82)
Placebo	16	9.43 (0.82)	10.09 (1.66)	−0.13 (1.44)	

CI, confidence interval; FPG, fasting plasma glucose; HbA1c, haemoglobin A1c; HOMA-IR, homeostasis model assessment for insulin resistance; HOMA-IS, homeostasis model assessment indices for insulin secretion; s.e., standard error.
*$p < 0.01$, **$p < 0.001$, ***$p < 0.0001$.
†Full analysis set, last observation carried forward analysis.
‡Full analysis set, observed cases analysis.

increase in LDL cholesterol with voglibose compared with linagliptin 5 and 10 mg at 26 weeks (Tables S2 and S3).

Both doses of linagliptin at weeks 12 and 26 (i.e. at steady state) achieved mean DPP-4 inhibition of ≥80% at trough concentrations (data not shown).

Efficacy Subgroup Analyses

Treatment differences in HbA1c reductions from baseline with linagliptin vs. placebo at week 12 tended to be greater in a pre-specified subgroup with baseline HbA1c ≥8.0% (figure 2B). In other *post hoc* analyses, treatment differences also showed significant HbA1c reductions with linagliptin 5 and 10 mg vs. placebo at week 12 and a trend towards HbA1c reductions vs. voglibose at week 26 across patient subgroups with different disease duration or different renal function (figure 3A and B). In previously drug-naïve patients, treatment differences were slightly smaller than in the overall population, although there were larger HbA1c changes from baseline with each treatment in the drug-naïve subgroup (week 12: −0.75, −0.82 and −0.16% of those receiving linagliptin 5 mg, linagliptin 10 mg or placebo, respectively; week 26: −0.69, −0.83 and −0.51% of those receiving linagliptin 5 mg, linagliptin 10 mg or voglibose, respectively).

A *post hoc* analysis in previously drug-naïve patients with baseline HbA1c <7.0% showed the target HbA1c of <6.5% was achieved at week 12 by three of four patients on linagliptin 5 mg and three of five patients on linagliptin 10 mg, whereas there were no placebo-treated patients in this subgroup; this target was achieved at week 26 in the same subgroup by four of four patients on linagliptin 5 mg, three of five patients on linagliptin 10 mg and only one of four patients on voglibose.

Safety and Tolerability

The rates of overall AEs with linagliptin 5 and 10 mg were comparable with placebo over 12 weeks (linagliptin 5 mg: 56.0%, linagliptin 10 mg: 53.1%, placebo: 56.3%) and voglibose over 26 weeks (linagliptin 5 mg: 72.3%, linagliptin 10 mg: 77.5%, voglibose: 71.6%) (Tables 4 and 5). Similar rates of drug-related AEs were also observed across treatments. No deaths occurred during the study and there were few other serious adverse events (SAEs). None of the SAEs that occurred during the study in any treatment group were deemed by the

Table 3. Measures of glycaemic control in the linagliptin and voglibose groups at week 26 (full analysis set).

	N	Week 0 [mean (s.e.)]	Week 26 [mean (s.e.)]	Adjusted mean change from baseline [mean (s.e.)]	Difference in means [linagliptin–voglibose (95% CI)]
HbA1c† (%)					
Linagliptin 5 mg	159	8.07 (0.05)	7.63 (0.09)	−0.13 (0.07)	−0.32 (−0.49, −0.15)**
Linagliptin 10 mg	157	7.98 (0.05)	7.50 (0.08)	−0.19 (0.07)	−0.39 (−0.56, −0.21)***
Voglibose	162	8.02 (0.06)	7.91 (0.10)	0.19 (0.07)	
FPG† (mmol/l)					
Linagliptin 5 mg	159	9.1 (0.1)	8.6 (0.2)	−0.3 (0.1)	−0.4 (−0.7, 0.0)
Linagliptin 10 mg	160	9.2 (0.1)	8.5 (0.1)	−0.4 (0.1)	−0.5 (−0.9, −0.2)*
Voglibose	162	9.1 (0.1)	8.9 (0.2)	0.1 (0.1)	
Fasting insulin‡ (mU/l)					
Linagliptin 5 mg	27	5.26 (1.07)	5.86 (1.25)	0.53 (0.82)	0.72 (−1.42, 2.86)
Linagliptin 10 mg	26	5.37 (0.87)	5.87 (0.91)	0.42 (0.74)	0.61 (−1.46, 2.69)
Voglibose	20	6.73 (1.41)	6.75 (2.05)	−0.19 (0.80)	
Proinsulin/insulin ratio‡					
Linagliptin 5 mg	27	0.29 (0.04)	0.23 (0.03)	−0.06 (0.02)	−0.09 (−0.14, −0.04)**
Linagliptin 10 mg	23	0.24 (0.02)	0.18 (0.02)	−0.06 (0.02)	−0.09 (−0.14, −0.04)**
Voglibose	19	0.24 (0.03)	0.26 (0.04)	0.03 (0.02)	
HOMA-IR‡ (mU/l × mmol/l)					
Linagliptin 5 mg	27	2.08 (0.41)	2.17 (0.48)	0.09 (0.32)	0.36 (−0.48, 1.19)
Linagliptin 10 mg	26	2.21 (0.36)	2.15 (0.33)	−0.06 (0.29)	0.20 (−0.61, 1.01)
Voglibose	20	2.67 (0.56)	2.36 (0.56)	−0.26 (0.31)	
HOMA-IS‡ [(mU/L)/(mmol/l)]					
Linagliptin 5 mg	27	20.58 (4.92)	25.27 (5.05)	5.58 (4.46)	0.47 (−11.18, 12.12)
Linagliptin 10 mg	26	20.24 (3.47)	27.13 (4.83)	7.86 (4.05)	2.75 (−8.55, 14.05)
Voglibose	20	29.54 (9.29)	39.15 (18.79)	5.11 (4.33)	
Disposition index‡					
Linagliptin 5 mg	34	11.63 (1.18)	13.95 (0.90)	1.39 (1.08)	−0.20 (−3.08, 2.67)
Linagliptin 10 mg	33	9.94 (0.98)	13.42 (1.25)	2.15 (1.02)	0.56 (−2.29, 3.40)
Voglibose	24	10.85 (1.22)	13.28 (1.48)	1.59 (1.14)	

CI, confidence interval; FPG, fasting plasma glucose; HbA1c, haemoglobin A1c; HOMA-IR, homeostasis model assessment for insulin resistance; HOMA-IS, homeostasis model assessment indices for insulin secretion; s.e., standard error.
*$p < 0.01$, **$p < 0.001$, ***$p < 0.0001$.
†Full analysis set, last observation carried forward analysis.
‡Full analysis set, observed cases analysis.

reporting investigators to be related to study drugs. While few patients discontinued because of AEs over 12 and 26 weeks of treatment, the overall drop-out was lower with linagliptin than with placebo or voglibose.

The most common types of AEs were infections/infestations, GI disorders and skin and subcutaneous tissue disorders in all treatment groups. There were no cases of hypoglycaemia with both doses of linagliptin or placebo throughout the first 12 weeks of treatment. There was one case of investigator-defined hypoglycaemia with linagliptin 10 mg and two cases with voglibose—although none of these were documented cases—during the 24-week study period. A *post hoc* analysis showed that the overall incidence of GI disorders in the combined linagliptin 5 or 10 mg group was comparable to that in the voglibose group over 26 weeks. Linagliptin 5 or 10 mg was associated with similar rates of each category of GI event compared with voglibose, except for diarrhoea, the most common GI event, which occurred in fewer linagliptin-treated than voglibose-treated patients (4.4 vs. 9.3%; $p = 0.04$). Three patients were confirmed after blinded adjudication to have experienced a cardiac or cerebrovascular event: one (0.6%) patient receiving linagliptin 5 mg and two (1.2%) receiving voglibose.

Discussion

In this 26-week study in Japanese patients with T2DM and inadequate glycaemic control, linagliptin monotherapy achieved significantly greater mean reductions in HbA1c than voglibose and had an overall safety profile similar to placebo with no documented hypoglycaemia or weight gain. In addition, a higher proportion of linagliptin-treated patients achieved evidence-based targets for glycaemic control and clinically meaningful reductions in HbA1c than those receiving voglibose or placebo.

Greater reductions in HbA1c in patients receiving linagliptin than in those receiving voglibose or placebo were seen regardless of baseline HbA1c level, renal impairment or duration of disease. As expected, reductions in HbA1c were greater in patients with higher baseline HbA1c. Effects on postprandial glucose levels were not determined in this study (because of issues of practicality); however, positive short-term effects

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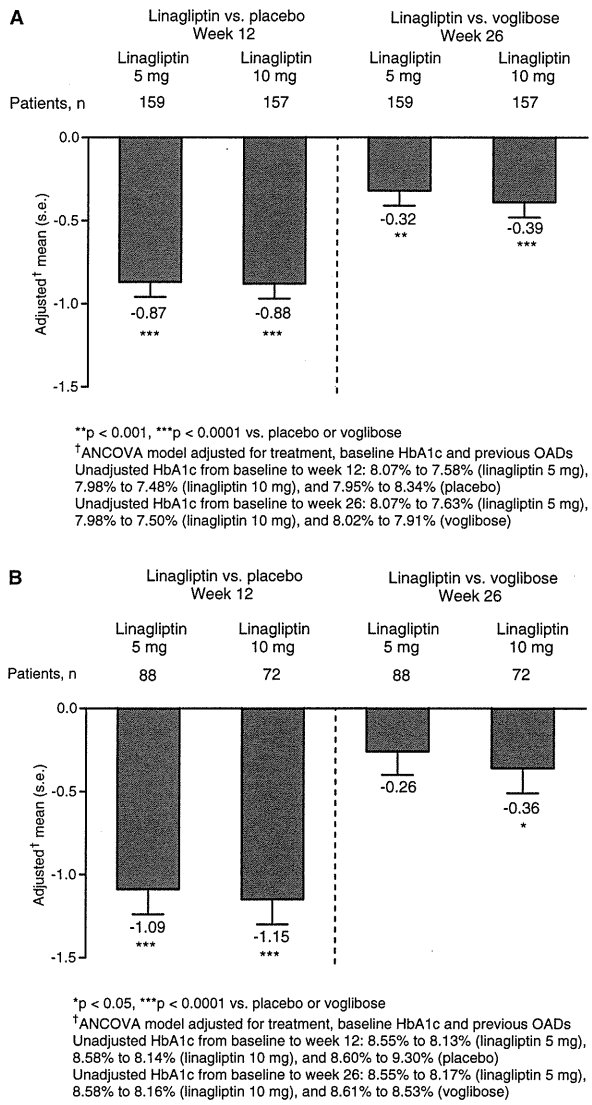

Figure 2. Differences in haemoglobin A1c (HbA1c) changes from baseline between treatments (full analysis set): (A) treatment difference in overall study population and (B) treatment difference in patients with baseline HbA1c ≥8.0%.

of linagliptin on postprandial glucose have been previously observed in an earlier Japanese Phase IIa study [16].

Very minor reductions in body weight, BMI and waist circumference occurred in all treatment groups during the study, but these were of limited clinical relevance for this generally overweight study population.

In this study, the treatment difference between linagliptin and voglibose appeared comparable to previous head-to-head studies of other DPP-4 inhibitors and voglibose in Japanese patients with T2DM [26,27], although direct comparisons between studies must always be interpreted cautiously because of potential confounding factors. In the overall study population, the absolute changes in HbA1c were smaller than those seen in the previous DPP-4 inhibitor studies; however,

Table 4. Clinical AEs in the linagliptin and placebo groups over 12 weeks (treated set).

	Linagliptin 5 mg [n (%)]	Linagliptin 10 mg [n (%)]	Placebo [n (%)]
Total number of patients	159	160	80
Number of patients having ≥1			
AE	89 (56.0)	85 (53.1)	45 (56.3)
Drug-related AE*	15 (9.4)	14 (8.8)	8 (10.0)
SAE	1 (0.6)	4 (2.5)	1 (1.3)
Drug-related SAE*	0 (0.0)	0 (0.0)	0 (0.0)
Number of patients discontinued due to			
AE	3 (1.9)	4 (2.5)	7 (8.8)
Drug-related AE	1 (0.6)	1 (0.6)	2 (2.5)
SAE	1 (0.6)	1 (0.6)	0 (0.0)
AEs of special interest (any cause)			
Hypoglycaemia	0 (0.0)	0 (0.0)	0 (0.0)
Infections and infestations	38 (23.9)	33 (20.6)	12 (15.0)
Metabolism and nutritional disorders	4 (2.5)	4 (2.5)	7 (8.8)
Nervous system disorders	9 (5.7)	10 (6.3)	4 (5.0)
Any GI disorders	29 (18.2)	26 (16.3)	11 (13.8)
Skin and subcutaneous tissue disorders	13 (8.2)	9 (5.6)	2 (2.5)
Musculoskeletal and connective tissue disorders	10 (6.3)	15 (9.4)	8 (10.0)
Any renal or urinary disorder	3 (1.9)	4 (2.5)	2 (2.5)
Hypersensitivity	0 (0.0)	0 (0.0)	0 (0.0)

AE, adverse event; GI, gastrointestinal; SAE, serious adverse event.
*Determined by the investigator to be possibly, probably or definitely drug-related.

this is likely to be a consequence of the strong washout effect in a substantial proportion (~45%) of the current study population who previously received one or two OADs (i.e. glycaemic deterioration during the treatment phase as a result of discontinuation of previous background therapies during the 4-week washout period). This interpretation is supported by the observation that in previously drug-naïve T2DM patients in this study, the absolute HbA1c changes were similar to those previously seen with other DPP-4 inhibitors, and the treatment differences, which account for effects of discontinuation of background therapies across treatment groups, confirmed similar improvements with linagliptin to those seen in the other DPP-4 studies.

Linagliptin was well tolerated in this study with an AE profile similar to placebo, which is consistent with Phase III studies in other populations [17–20]. Both linagliptin and voglibose were associated with very few cases of investigator-defined hypoglycaemia, but none were confirmed or documented by plasma measurements; this is not an unexpected finding given the mechanism of action of these agents and indicates that the anti-hyperglycaemic effects of linagliptin were not associated with any increased risk of hypoglycaemia. Linagliptin also showed GI tolerability that was generally comparable to that of voglibose, although linagliptin was associated with significantly less diarrhoea. The safety/tolerability profile of linagliptin in this Japanese study was consistent with the profiles reported for other DPP-4

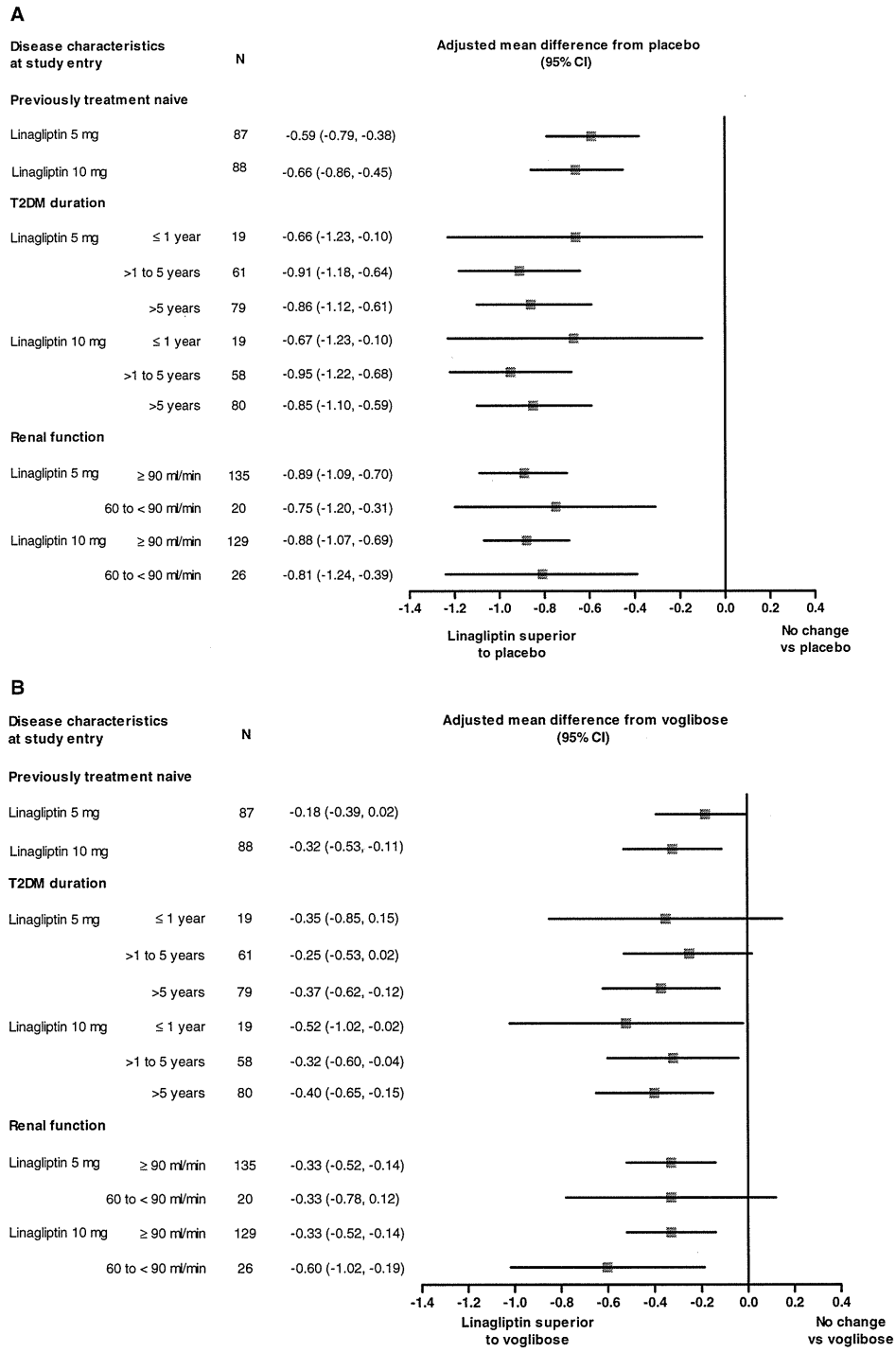

Figure 3. Subgroup analyses of haemoglobin A1c-lowering (full analysis set): (A) linagliptin vs. placebo at week 12 and (B) linagliptin vs. voglibose at week 26 (full analysis set).

inhibitors in Japanese patients, and no new safety concerns were observed [26–28].

There were no apparent differences between the two doses of linagliptin in efficacy and safety/tolerability over 12 to 26 weeks of treatment. This supports the lower 5 mg dose as the optimal therapeutic dose in Japanese patients, as this offers comparable beneficial effects but with lower drug exposure compared to the higher 10 mg dose. This is in agreement with clinical studies in other populations that also demonstrated linagliptin 5 mg was the optimal therapeutic dose [13,14].

Table 5. Clinical AEs in the linagliptin and voglibose groups over 26 weeks (treated set).

	Linagliptin 5 mg [n (%)]	Linagliptin 10 mg [n (%)]	Voglibose [n (%)]
Total number of patients	159	160	162
Number of patients having ≥1			
AE	115 (72.3)	124 (77.5)	116 (71.6)
Drug-related AE*	18 (11.3)	17 (10.6)	30 (18.5)
SAE	5 (3.1)	8 (5.0)	7 (4.3)
Drug-related SAE*	0 (0.0)	0 (0.0)	0 (0.0)
Number of patients discontinued due to			
AE	4 (2.5)	7 (4.4)	12 (7.4)
Drug-related AE	1 (0.6)	1 (0.6)	3 (1.9)
SAE	2 (1.3)	1 (0.6)	2 (1.2)
AEs of special interest (any cause)			
Hypoglycaemia†	0 (0.0)	1 (0.6)	2 (1.2)
Infections and infestations	70 (44.0)	58 (36.3)	56 (34.6)
Metabolism and nutritional disorders	7 (4.4)	12 (7.5)	14 (8.6)
Nervous system disorders	17 (10.7)	15 (9.4)	16 (9.9)
Respiratory, thoracic and mediastinal disorders	9 (5.7)	10 (6.3)	5 (3.1)
Any GI disorders	48 (30.2)	38 (23.8)	49 (30.2)
Skin and subcutaneous tissue disorders	22 (13.8)	13 (8.1)	7 (4.3)
Musculoskeletal and connective tissue disorders	19 (11.9)	24 (15.0)	14 (8.6)
Any renal or urinary disorder	6 (3.8)	7 (4.4)	3 (1.9)
Hypersensitivity	0 (0.0)	1 (0.6)‡	0 (0.0)

AE, adverse event; GI, gastrointestinal; SAE, serious adverse event.
*Determined by the investigator to be possibly, probably or definitely drug-related.
†No documented cases of hypoglycaemia as confirmed by plasma glucose measurements.
‡Hypersensitivity was worsening of pollinosis (allergy), which was reported by the investigator as due to seasonal change.

This study has certain limitations. First, the length of this study does not allow assessment of the long-term durability of glycaemic control with linagliptin. However, its 26-week duration does provide valuable information on the extended effects of DPP-4 inhibition over time compared with voglibose in Japanese patients (randomized double-blind studies comparing sitagliptin and vildagliptin vs. voglibose have reported results over 12 weeks only). To address the need for long-term data in Japanese patients, the open-label extension of this study will determine the long-term safety and durability of linagliptin's therapeutic effects after continued treatment and after switching from voglibose; in addition, another ongoing Japanese study is evaluating linagliptin vs. metformin as add-on to one other OAD (ClinicalTrials.gov, number NCT01204294). Second, as is the case with other DPP-4 inhibitors, large, adequately powered, randomized clinical trials measuring hard clinical endpoints are needed to determine if the efficacy and safety of linagliptin translate into reduced risk of diabetic complications such as vascular events. One such study is ongoing—the CAROLINA study that is taking place in many countries, including Japan, to investigate the effects of linagliptin on cardiovascular outcomes (ClinicalTrials.gov, number NCT01243424).

In conclusion, linagliptin 5 or 10 mg once daily provided superior glycaemic control compared to voglibose with a comparable safety profile in Japanese patients with T2DM. Differences in anti-hyperglycaemic effects and other parameters between the 5 and 10 mg doses of linagliptin were only minor, consistent with previous studies in patients from other racial groups, providing a rationale for a 5 mg once-daily therapeutic dose. The glucose-dependent promotion of insulin secretion by linagliptin, through stimulation and improvement in pancreatic β-cell function, may be particularly helpful for Asian patients with T2DM who are often leaner than their Western counterparts [29] and, consequently, have a pathophysiology in which defective insulin secretion is a stronger contributor to hyperglycaemia than insulin resistance [30]. This study provides support for the use of linagliptin 5 mg once daily as a new oral treatment option in Japanese patients with T2DM that can improve glycaemic control with minimal risk of hypoglycaemia, weight gain or other AEs such as GI symptoms. These effects can be achieved at a single dose strength without regard to declining renal function, which is a frequent limitation of current therapies used in diabetes management.

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Conflict of Interest

R. Kawamori has received consultancy fees from Boehringer Ingelheim and speaker fees from MSD, Novartis, Takeda, Ono, Novo Nordisk and Eli Lilly. N. Inagaki has received consultancy fees from Boehringer Ingelheim, Takeda and Novo Nordisk, speaker fees from MSD, Novartis, Novo Nordisk, Eli Lilly, Takeda and Ono, and research support from Boehringer Ingelheim, MSD, Novartis, Novo Nordisk, Eli Lilly, Takeda and Ono. E. Araki has received consultancy fees from Novo Nordisk, speaker fees from Ono, Novartis, Novo Nordisk, MSD, Eli Lilly and Takeda, and research support from Novartis. H. Watada has received consultancy fees from Boehringer Ingelheim, speaker fees from MSD, Novartis, Takeda, Novo Nordisk and Eli Lilly, and research support from MSD, Novartis, Takeda, Boehringer Ingelheim, Novo Nordisk and Eli Lilly. N. Hayashi, Y. Horie, A. Sarashina, Y. Gong, M. von Eynatten, H. J. Woerle and K. A. Dugi are employees of Boehringer Ingelheim, the study sponsor and manufacturer of linagliptin. All authors meet criteria for authorship as recommended by the International Committee of Medical Journal Editors. All authors contributed to or participated in the design of the study, the analysis of data, the collection of data and the writing or revision of the manuscript. All authors saw and approved the final version of the manuscript.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Baseline demographic and clinical characteristics in previously drug-naïve or treated patients (treated set).

Table S2. Summary of lipids and other exploratory endpoints in the linagliptin and placebo groups at week 12.

Table S3. Summary of lipids and other exploratory endpoints in the linagliptin and voglibose groups at week 26.

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References

1. Japanese Ministry of Health Labour and Welfare. The National Health and Nutrition Survey 2007. Available from URL: http://www.mhlw.go.jp/houdou/2008/12/h1225-5.html. (in Japanese). Accessed 30 November 2011.
2. Turner RC, Cull CA, Frighi V, Holman RR. Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies (UKPDS 49). UK Prospective Diabetes Study (UKPDS) Group. JAMA 1999; **281**: 2005–2012.
3. DeFronzo RA. Current issues in the treatment of type 2 diabetes. Overview of newer agents: where treatment is going. Am J Med 2010; **123**: S38–S48.
4. Yokoyama H, Sone H, Oishi M, Kawai K, Fukumoto Y, Kobayashi M. Prevalence of albuminuria and renal insufficiency and associated clinical factors in type 2 diabetes: the Japan Diabetes Clinical Data Management study (JDDM15). Nephrol Dial Transplant 2009; **24**: 1212–1219.
5. Yokoyama H, Okudaira M, Otani T et al. Higher incidence of diabetic nephropathy in type 2 than in type 1 diabetes in early-onset diabetes. Kidney Int 2000; **58**: 302–311.
6. Habener JF. Insulinotropic glucagon-like peptides. In: LeRoith D, Taylor SI, Olefsky JM eds. Diabetes Mellitus: A Fundamental and Clinical Text, 2nd edn. Philadelphia: Lippincott Williams & Wilkins, 2000.
7. Aaboe K, Krarup T, Madsbad S, Holst JJ. GLP-1: physiological effects and potential therapeutic applications. Diabetes Obes Metab 2008; **10**: 994–1003.
8. Garber AJ. Incretin effects on β-cell function, replication, and mass: the human perspective. Diabetes Care 2011; **34** Suppl 2: S258–S263.
9. Blech S, Ludwig-Schwellinger E, Gräfe-Mody EU, Withopf B, Wagner K. The metabolism and disposition of the oral dipeptidyl peptidase-4 inhibitor, linagliptin, in humans. Drug Metab Dispos 2010; **38**: 667–678.
10. Graefe-Mody U, Friedrich C, Port A et al. Effect of renal impairment on the pharmacokinetics of the dipeptidyl peptidase-4 Inhibitor linagliptin. Diabetes Obes Metab 2011; **13**: 939–946.
11. Boehringer Ingelheim Pharmaceuticals Inc, Ridgefield, CT, USA. Tradjenta (linagliptin) tablets prescribing information 2011.
12. Heise T, Graefe-Mody EU, Hüttner S, Ring A, Trommeshauser D, Dugi KA. Pharmacokinetics, pharmacodynamics and tolerability of multiple oral doses of linagliptin, a dipeptidyl peptidase-4 inhibitor in male type 2 diabetes patients. Diabetes Obes Metab 2009; **11**: 786–794.
13. Forst T, Uhlig-Laske B, Ring A, Ritzhaupt A, Graefe-Mody U, Dugi KA. The oral DPP-4 inhibitor linagliptin significantly lowers HbA1c after 4 weeks of treatment in patients with type 2 diabetes mellitus. Diabetes Obes Metab 2011; **13**: 542–550.
14. Forst T, Uhlig-Laske B, Ring A et al. Linagliptin (BI 1356), a potent and selective DPP-4 inhibitor, is safe and efficacious in combination with metformin in patients with inadequately controlled Type 2 diabetes. Diabet Med 2010; **27**: 1409–1419.
15. Sarashina A, Sesoko S, Nakashima M et al. Linagliptin, a dipeptidyl peptidase-4 inhibitor in development for the treatment of type 2 diabetes mellitus: a Phase I, randomized, double-blind, placebo-controlled trial of single and multiple escalating doses in healthy adult male Japanese subjects. Clin Ther 2010; **32**: 1188–1204.
16. Horie Y, Kanada S, Watada H et al. Pharmacokinetic, pharmacodynamic and tolerability profiles of the dipeptidyl peptidase-4 inhibitor linagliptin: a 4-week, multicenter, randomized, double-blind, placebo-controlled, phase IIa study in Japanese type 2 diabetes patients. Clin Ther 2011; **33**: 973–989.
17. Del Prato S, Barnett AH, Huisman H, Neubacher D, Woerle HJ, Dugi KA. Effect of linagliptin monotherapy on glycaemic control and markers of β-cell function in patients with inadequately controlled type 2 diabetes: a randomized controlled trial. Diabetes Obes Metab 2011; **13**: 258–267.
18. Owens DR, Swallow R, Dugi KA, Woerle HJ. Efficacy and safety of linagliptin in persons with type 2 diabetes inadequately controlled by a combination of metformin and sulphonylurea: a 24-week randomized study. Diabet Med 2011; **28**: 1352–1361.
19. Taskinen MR, Rosenstock J, Tamminen I et al. Safety and efficacy of linagliptin as add-on therapy to metformin in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled study. Diabetes Obes Metab 2011; **13**: 65–74.
20. Gomis R, Espadero RM, Jones R, Woerle HJ, Dugi KA. Efficacy and safety of initial combination therapy with linagliptin and pioglitazone in patients with inadequately controlled type 2 diabetes: a randomized, double-blind, placebo-controlled study. Diabetes Obes Metab 2011; **13**: 653–661.
21. Van de Laar FA, Lucassen PL, Akkermans RP, Van de Lisdonk EH, Rutten GE, Van Weel C. Alpha-glucosidase inhibitors for type 2 diabetes mellitus. Cochrane Database Syst Rev 2005; **2**: CD003639.
22. Matsumoto K, Sera Y, Abe Y, Tominaga T, Ueki Y, Miyake S. Combination therapy of alpha-glucosidase inhibitor and a sulfonylurea compound prolongs the duration of good glycemic control. Metabolism 2002; **51**: 1548–1552.
23. Matsumoto K, Yano M, Miyake S et al. Effects of voglibose on glycemic excursions, insulin secretion, and insulin sensitivity in non-insulin-treated NIDDM patients. Diabetes Care 1998; **21**: 256–260.
24. Horie Y, Hayashi N, Dugi K, Takeuchi M. Design, statistical analysis and sample size calculation of a phase IIb/III study of linagliptin versus voglibose and placebo. Trials 2009; **10**: 82.
25. Workgroup on Hypoglycemia, American Diabetes Association. Defining and reporting hypoglycemia in diabetes: a report from the American Diabetes Association Workgroup on Hypoglycemia. Diabetes Care 2005; **28**: 1245–1249.
26. Iwamoto Y, Tajima N, Kadowaki T et al. Efficacy and safety of sitagliptin monotherapy compared with voglibose in Japanese patients with type 2 diabetes: a randomized, double-blind trial. Diabetes Obes Metab 2010; **12**: 613–622.
27. Iwamoto Y, Kashiwagi A, Yamada N et al. Efficacy and safety of vildagliptin and voglibose in Japanese patients with type 2 diabetes: a 12-week, randomized, double-blind, active-controlled study. Diabetes Obes Metab 2010; **12**: 700–708.
28. Seino Y, Fujita T, Hiroi S, Hirayama M, Kaku K. Efficacy and safety of alogliptin in Japanese patients with type 2 diabetes mellitus: a randomized, double-blind, dose-ranging comparison with placebo, followed by a long-term extension study. Curr Med Res Opin 2011; **27:** 1781–1792.
29. Sone H, Ito H, Ohashi Y, Akanuma Y, Yamada N. Obesity and type 2 diabetes in Japanese patients. Lancet 2003; **361**: 85.
30. Brunetti P. The lean patient with type 2 diabetes: characteristics and therapy challenge. Int J Clin Pract Suppl 2007; **62** Suppl 153: 3–9.

ORIGINAL PAPER

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Sitagliptin add-on to low dosage sulphonylureas: efficacy and safety of combination therapy on glycaemic control and insulin secretion capacity in type 2 diabetes

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SUMMARY

Aims: To assess the efficacy and safety of combination therapy with sitagliptin and low dosage sulphonylureas on glycaemic control and insulin secretion capacity in Japanese type 2 diabetes. Methods: Eighty-two subjects were sequentially recruited for the 52-week, prospective, single arm study. Sitagliptin was added on to sulphonylureas (glimepride or gliclazide) with or without metformin. The primary endpoint was a change in A1C. The secondary endpoints were changes in BMI, insulin secretion capacity, blood pressure and urinary albumin excretion, unresponsive rate, and hypoglycaemia. Insulin secretion capacity was evaluated by glucagon loading test. **Results:** Change in A1C was −0.80% (95% CI −0.90 to −0.68) ($p < 0.001$). Change in BMI, systemic and diastolic blood pressure, and urinary albumin excretion were −0.38 kg/m^2 (95% CI −0.72 to −0.04) ($p < 0.05$), −6.7/−3.6 mmHg (95% CI −10.0 to −3.4/−4.8 to −2.4) ($p < 0.001$), and −43.2 mg/gCr (95% CI −65.7 to −20.8) ($p < 0.001$) respectively. Mild hypoglycaemia was observed in three cases. The unresponsive rate was 6.1%. Glucagon loading test showed that 0-min and 6-min CPR at baseline and 52-week were not significantly changed: 0-min CPR, 1.58 ± 0.58–1.71 ± 0.73 ng/ml; 6-min CPR, 3.48 ± 1.47–3.58 ± 1.21 ng/ml. Insulin secretion capacity, CPI and SUIT index at baseline did not predict the efficacy of the combination therapy. The final dosages of glimepiride and gliclazide were 1.44 ± 0.90 mg and 34.5 ± 15.3 mg respectively. The dosage of sitagliptin was increased from 50 mg to 69.0 ± 24.5 mg in 52-week. **Conclusions:** The combination therapy with sitagliptin and low dosage sulphonylureas was safe and effective for glycaemic control. Glucagon loading test indicated that 1 year administration of sitagliptin and sulphonylureas preserved insulin secretion capacity.

What's known

- Sitagliptin is more effective for glycaemic control in Japanese patients compared with Caucasian patients.
- More than 70 cases of severe hypoglycaemia have been reported in Japanese patients treated with sulphonylureas and sitagliptin.
- A private committee to establish adequate use of incretin-based therapy recommended that the dosage of sulphonylureas should be decreased to less than 1.25 mg/day glibenclamide, 2.0 mg/day glimipiride or 40 mg/day gliclazide at the initiation of incretin-based therapy in Japan.

What's new

- The combination therapy with sitagliptin and low dosage sulphonylureas was safe and effective for glycaemic control.
- Glucagon loading test indicated that 1-year administration of sitagliptin and sulphonylureas preserved insulin secretion capacity.
- The combination therapy is weight neutral, and lowered both the blood pressure level and urinary albumin excretion.
- Because of the decrease in the dosage of sulphonylureas, hypoglycaemia seldom occurs, and there is no severe hypoglycaemia.

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Introduction

Type 2 diabetes is a chronic disease usually requiring multiple antihyperglycaemic agents (AHAs) during the course of the disease. The American Diabetes Association (ADA)/European Association for the Study of Diabetes (EASD) algorithm for treating type 2 diabetes recommends metformin as initial monotherapy (1). However, insulin secretion capacity is genetically lower in Japanese than that it is in Caucasians. Therefore, sulphonylureas (SUs) are frequently used in Japanese type 2 diabetes, and about 60% of the patients are treated with SUs (2). However, SUs increase the likelihood of both hypoglycaemia and weight gain.

Dipeptidyl peptidase-4 (DDP-4) inhibitor is a newly developed AHA that prevents degradation of the incretin hormones, glucagon-like peptide-1 and gastric inhibitory polypeptide (3). The compound promotes glucose-dependent insulin secretion and suppresses glucagon release, and can improve both fasting and postprandial glucose levels. Four different DPP-4 inhibitors are available in Japan: sitagliptin, vildagliptin, alogliptin and linagliptin. Of these, sitagliptin is most widely used because it was the first DPP-4 inhibitor approved in Japan and both its efficacy and safety are accepted in Japanese clinical practice. Sitagliptin is more effective for glycaemic control in Japanese patients compared with Cauca-

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sian patients (4,5,6). Hypoglycaemia does not occur with increased frequency when used as monotherapy or with agents that do not cause hypoglycaemia, such as metformin and thiazolidinediones, but may increase the incidence of hypoglycaemia when used with agents that cause hypoglycaemia (7). A case of severe hypoglycaemia was first reported in an aged Japanese patient when sitagliptin was combined with SUs (8). In this case, 50 mg/day sitagliptin was added to 6 mg/day glimepride. The laboratory data then showed that the plasma glucose level was 1.3 mM, and the immunoreactive insulin (IRI) level was 24.5 μU/ml. Since then, more than 70 cases of severe hypoglycaemia have been reported in patients within a week after the initiation of combination therapy with SUs and sitagliptin (9). In most of these cases, a relatively higher dosage of SUs was administered to elderly patients also having mild renal dysfunction. A private committee of diabetologists to establish adequate use of incretin-based therapy was then established. The committee recommended that the dosage of SUs should be decreased to less than 1.25 mg/day glibenclamide, 2.0 mg/day glimipiride or 40 mg/day gliclazide at the initiation of incretin-based therapy (10). As the recommendation was established in Japan, the number of severe hypoglycaemia cases has decreased remarkably.

However, the add-on effect of sitagliptin to SUs on glycaemic control when the daily dosage of SUs is reduced has not yet been determined. A reduction in the daily dosage of SUs should be helpful for many patients to prevent weight gain as well as hypoglycaemia. Although it also has not been determined whether insulin secretion capacity can be preserved by treatment with sitagliptin, SUs by themselves do not hasten the loss of beta-cell function (11) and improvement of insulin secretion capacity has been reported by vildagliptin, another DPP-4 inhibitor (12). We have conducted a prospective clinical study to determine the efficacy and safety of combination therapy with sitagliptin and lower dosage SUs on glycaemic control and insulin secretion capacity. Here, we show that sitagliptin added to a lower dosage of SUs is effective and well-tolerated for glycaemic control and that beta cell function is preserved at least for 1 year in Japanese type 2 diabetes patients.

Materials and methods

Study design

This study of add-on sitaglipitn to low dosage SUs is a prospective, 52-week, single centre, single arm, intervention study to evaluate the efficacy and safety of sitagliptin on glycaemic control in type 2 diabetes inadequately controlled with SUs. Outpatients of Takashima General Hospital were recruited consecutively for a sample size of 80 subjects who met both inclusion and exclusion criteria. Inclusion criteria were type 2 diabetes treated with SUs (glimepiride or gliclazide) ± metformin ± α-glucosidase inhibitors; aged ≥ 20 years; A1C level ≥ 6.9%; no improvement in A1C ≥ 0.5% within 3 months; and a wish to diet and exercise to improve health. The value for A1C (%) is estimated as an National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by the formula A1C (%) = A1C (JDS) (%) + 0.4%, considering the relational expression of HbA1c (JDS) (%) measured by the previous Japanese standard substance and measurement methods and A1C (NGSP) (13). Exclusion criteria were type 1 diabetes; secondary diabetes; diabetic nephropathy stage 3–5; alcoholism; severe depression or severe psychological condition; malignancy; abnormal haemoglobinaemia; and participation in other clinical trials or studies. The study protocol was approved by the Institutional Review Board of Takashima General Hospital, and registered on the University Hospital Medical Information Network in Japan (UMIN000005498). Written informed consent was obtained from all subjects. The study began on 1 February 2010, and ended on 31 August 2011.

Procedures and intervention

The duration of the study was 52 weeks. Subjects were screened for eligibility and gave informed consent, basic demographic information, medical history and frequency of hypoglycaemia. Within a month before administration of sitagliptin, glucagon loading test was performed without any AHAs for more than 24 h to evaluate insulin secretion capacity. Type 1 diabetes was excluded by examination of autoantibodies to GAD 65 and results of glucagon loading test. At the date sitagliptin treatment was begun the dosage of glimepiride or gliclazide was decreased to equal to or less than 2.0 mg/day or 40 mg/day when the subjects had been treated with more than 2.0 mg/day of glimepiride or 40 mg/day of gliclazide, respectively. If the subjects had been treated with less than 2.0 mg/day of glimepride or 40 mg/day of gliclazide, that dosage of SUs was maintained. Metformin was continued without any changes. α-glucosidase inhibitors were discontinued. During the study, the dosage of SUs was changed depending on the frequency of hypoglycaemic episodes and glycaemic control level. Other AHAs were not added or were discontinued. The dosage of sitagliptin was started at 50 mg/day, the usual initial dosage of sitagliptin in Japan, and it was increased to 100 mg/day if the A1C level did not reach 6.9%, as

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