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CLINICAL TRIAL

Comparison of sitagliptin with nateglinide on postprandial glucose and related hormones in drug-naïve Japanese patients with type 2 diabetes mellitus: A pilot study

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Keywords
Dipeptidyl peptidase-4 inhibitor, Glinide, Glucagon

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ABSTRACT

Aims/Introduction: Dipeptidyl peptidase-4 inhibitors and glinides are effective in reducing postprandial hyperglycemia. However, little information is available on the comparative effects of the two drugs on the levels of postprandial glucose. The aim of the present study was to compare the effects of sitagliptin and nateglinide on meal tolerance tests in drug-naïve patients with type 2 diabetes mellitus.

Materials and Methods: The study participants were 19 patients with type 2 diabetes mellitus, which was inadequately controlled by diet and exercise. An open-label, prospective, cross-over trial was carried out to compare the effects of single-dose sitagliptin and nateglinide on the postprandial glucose level and its related hormones during meal tests.

Results: The change in area under the curve (AUC) of glucose from 0 to 180 min ($AUC_{0–180\ min}$) during the meal test by nateglinide was similar to that by sitagliptin. As expected, the change in active glucagon like peptide-1 was significantly higher after a single-dose of sitagliptin than nateglinide. Then, insulin secretion relative to glucose elevation (ISG) ($\Delta ISG_{0–180\ min}$: $\Delta AUC_{0–180\ min}$ insulin/$AUC_{0–180\ min}$ glucose) was significantly enhanced by nateglinide compared with sitagliptin. Conversely, glucagon level ($\Delta AUC_{0–180\ min}$ glucagon) was increased by administration of nateglinide, whereas the glucagon level was reduced by administration of sitagliptin.

Conclusions: The effects of sitagliptin on postprandial glucose levels were similar to those of nateglinide in drug-naïve type 2 diabetes patients. However, the induced changes in insulin, active glucagon-like peptide-1 and glucagon during meal loading suggest that reduction of postprandial hyperglycemia was achieved by the unique effect of each drug.

INTRODUCTION

Postprandial hyperglycemia is widely recognized as a feature of the early stage of type 2 diabetes and impaired glucose tolerance[1]. It is caused at least in part by impairment of early insulin secretion in response to glucose, and correction of this defect is important for long-term glycemic control[2,3].

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Glinides, short-acting insulinotropic agents, improve postprandial hyperglycemia by rapidly increasing insulin secretion with less frequency of hypoglycemia than sulfonylurea, thus they are often used in the early stage of type 2 diabetes[4]. Among the glinides, nateglinide is reported to have some unexpected pharmacological actions. Indeed, experimental data reported that nateglinide inhibited dipeptidyl peptidase-4 (DPP-4) activity[5,6], and enhanced the release of glucagon-like peptide-1 (GLP-1) from L-cells in an adenosine triphosphate-sensitive potassium channel-independent manner[7]. In another study, nateglinide, but not repaglinide, stimulated glucagon secretion from isolated rat islets[8]. These data show that nateglinide might have unique pharmacological actions on other hormones involved in the regulation of glucose metabolism, compared with insulin. However, its importance in the clinical setting has not been elucidated yet.

DPP-4 inhibitors are currently used widely in patients with type 2 diabetes mellitus. Recent studies have reported that these compounds effectively improve postprandial hyperglycemia with the suppression of postprandial glucagon levels[9,10]. In addition, at least in an animal model, DPP-4 inhibitors were reported to have multiple pleiotropic effects that are dependent on[11,12] and independent of incretin action[13,14]. Thus, both DPP-4 inhibitors and nateglinide might be useful for the correction of postprandial hyperglycemia in the early stages of type 2 diabetes, although they could have their own effects on hormones involved in glucose metabolism.

To our knowledge, there are no studies that have compared the effects of glinides and DPP-4 inhibitors on postprandial hyperglycemia in drug-naïve patients with type 2 diabetes mellitus. The present study was a randomized cross-over trial that examined the effects of sitagliptin vs nateglinide on the levels of postprandial glucose, active GLP-1, insulin and glucagon, using a meal test in Japanese type 2 diabetes patients inadequately controlled with exercise and diet. The aim of the study was to elucidate the difference between sitagliptin and nateglinide on postprandial hyperglycemia and related hormones.

METHODS

Participants

We screened type 2 diabetes patients who regularly attended Juntendo University Hospital and Juntendo University Urayasu Hospital between November 2010 and October 2011. Among them, we selected those who met the following criteria: (i) 6.5% ≤glycated hemoglobin (HbA1c) ≤8.0% even after diet and excise therapy for more than 3 months; (ii) fasting glucose ≤140 mg/dL; (iii) age ≥20 years; (iv) absence of concomitant chronic diseases, including anemia (hemoglobin ≤11.0 g/dL), renal dysfunction (plasma creatinine > 1.50 mg/dL) and liver dysfunction (aspartate aminotransferase >80 IU/L or alanine transaminase >80 IU/L), or serious cardiovascular diseases, proliferative diabetic retinopathy and serious diabetic neuropathy.

A total of 19 Japanese subjects matched the aforementioned criteria and were recruited for this study. In the present study, HbA1c levels were expressed as National Glycohemoglobin Standardization Program values (%), calculated by the formula A1c (%) = 1.02 × Japan Diabetes Society (%) + 0.25%, according to the recommendations of the Japanese Diabetes Society[15].

The study protocol was carried out in accordance with the ethical principles stated in the Declaration of Helsinki and approved by the ethics review committee of Juntendo University Hospital. All patients provided written informed consent and confirmed their willingness to attend the study.

Study Design

An open label cross-over design was used. Figure 1 shows the schedule followed in the present study. After inclusion in the study, the 19 participants were randomized into the sitagliptin-

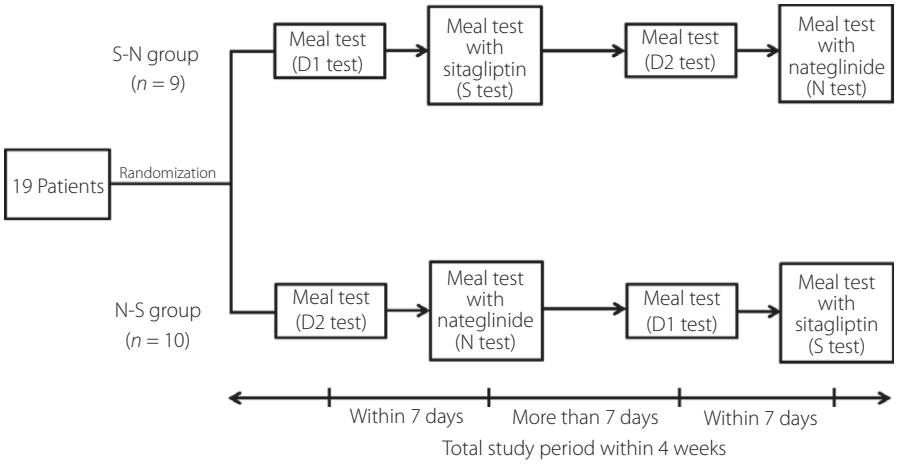

Figure 1 | After screening, patients were randomized into the sitagliptin-nateglinide (S-N) group, who initially received sitagliptin (100 mg), or the nateglinide- sitagliptin (N-S group), who initially received nateglinide (120 mg). The drugs were then switched so that the S-N group received nateglinide and the N-S group received sitagliptin. D1 and D2 test were controls for a single-dose of 100 mg sitagliptin (S test) and a single-dose of 120 mg nateglinide (N test), respectively.

nateglinide group (S-N group, $n = 9$) and the nateglinide-sitagliptin group (N-S group, $n = 10$) based on a computer-generated assignment.

In patients of the S-N group, a meal test was carried out at baseline (without administration of drugs [D1 test]), followed by a meal test with a single dose of 100 mg sitagliptin (S test) within at least 7 days after D1. After an interval of at least 1 week, another meal test was carried out without administration of drugs (D2 test), followed by a meal test with a single dose of 120 mg nateglinide (N test) within at least 7 days after the D2 test.

In patients of the N-S group, a meal test was carried out at baseline (without administration of drugs [D2 test]), followed by a meal test with a single dose of 120 mg nateglinide (N test) within at least 7 days after the D2 test. After an interval of at least 1 week, another meal test was carried out without administration of drugs (D1 test), followed by a meal test with a single dose of 100 mg sitagliptin (S test) within at least 7 days after the D1 test. All tests were carried out within a total of 4 weeks. The effect of the drug was evaluated mainly by the difference in each parameter between the meal test with the drug, and the meal test carried out just before the meal test with the drug. In order to compare the glucose-lowering effect by two drugs more precisely, comparison of the maximum dose of each drug was carried out.

Standard Meal Loading Test

A standard meal was provided, as described by the Japan Diabetes Society[16]. The total energy content of the standard meal was 1,925 kJ (460 kcal), with 56.5 g of carbohydrates, 18.0 g of fat and 18.0 g of protein; with 51.4 energy % (E%) from carbohydrates, 33.3 E% from fat and 15.3 E% from protein. The patients attended the hospital at 09.00 h after a 12-h fast (from 21.00 h on the day before each test). They were instructed to consume the entire meal within 15 min, and to stay at rest and sitting throughout testing. An intravenous line was inserted into one forearm vein before eating the meal, and kept patent using 0.9% NaCl for repeated blood sampling. Blood samples for the meal test were collected at 0 min (immediately before the meal), and 15, 30, 60, 120 and 180 min after the start of the meal. In tests using the specified drugs, sitagliptin was given 2 h before each meal test to achieve enough plasma sitagliptin concentration, whereas nateglinide was given just before each meal test. Plasma glucose, plasma insulin and glucagon were measured at each of the aforementioned time-points, and their areas under the curve (AUC), from the start of the meal tolerance test to 180 min ($AUC_{0–180\ min}$), were calculated using the trapezoidal method[17]. The levels of active GLP-1 were measured at 0, 30 and 120 min after the start of meal, and the $AUC_{0–120\ min}$ from the start of the meal test to 120 min was calculated. HbA1c, plasma glucose and plasma insulin levels were measured using standard methods. The plasma levels of active GLP-1 and glucagon during the meals test were measured by enzyme-linked immunosorbent assay and the Glucagon RIA kit (Cat. no. GL-32K; Millipore Corporation, Billerica, MA, USA), respectively, by a private laboratory (SRL Laboratory, Tokyo, Japan). Regarding the validity of the glucagon assay, the Glucagon RIA kit showed a small cross-reactivity with oxyntomodulin and glicentin, which arose from different processing of the glucagon precursor, proglucagon[18].

The primary outcome of the present study was changes in the $AUC_{0–180\ min}$ during the meal test with either of the two drugs to those during the meal test just before either of the drugs. The secondary outcomes were changes in the AUC of postprandial active GLP-1, insulin and glucagon levels. Furthermore, all adverse events, including a hypoglycemic episode during the meal test, were monitored.

Statistical Analysis

Data are expressed as mean ± standard deviation or median (25–75th percentiles) values. The data in Tables 2 and 3 were presented as medians because they were not normally distributed. The Wilcoxon matched-pair signed-rank test was used for comparisons of variables with skewed distribution. The paired *t*-test was used for comparison of clinical data of the S-N and N-S groups. A *P*-value <0.05 was considered statistically significant. The StatFlex version 6 (Artech Co., Osaka, Japan) software was used for analysis.

RESULTS

A total of 19 patients were enrolled in the study (Table 1). There were no significant differences in age, diabetes duration, body mass index, HbA1c, blood pressure and lipid data between the two drug groups. No adverse events, such as hypoglycemia, were encountered during the study period.

Effects of Sitagliptin and Nateglinide on Plasma Glucose and Insulin

Figure 2a shows the serial changes in plasma glucose levels and a significant difference in plasma glucose at 0 min between the D1 test group and the S test group (8.0 ± 1.2 vs 7.1 ± 1.2 mmol/L, $P < 0.01$) was found. Table 2 shows the $AUC_{0–180\ min}$ glucose during the meal test before and after single-dose sitagliptin. The $AUC_{0–180\ min}$ glucose diminished significantly after a single-dose of sitagliptin (1,788 [1,621 – 2,076] vs 1,535 mmol-min/L [1,318 – 1,796], $P < 0.01$). Figure 2b shows the serial changes in plasma insulin, and Table 2 shows the $AUC_{0–180\ min}$ insulin during the meal test before and after single-dose sitagliptin. Although there was no significant change in the $AUC_{0–180\ min}$ insulin after sitagliptin (4,855 [3,253–7,005] vs 3,952 μIU-min/mL [3,283–5,723]), the $AUC_{0–180\ min}$ insulin during the meal test with sitagliptin tended to be lower than that during the meal test without any drugs. Figure 2c shows the serial changes in plasma glucose, and Table 2 shows the $AUC_{0–180\ min}$ glucose before and after a single-dose of nateglinide. Similar to the meal test with sitagliptin, the $AUC_{0–180\ min}$ glucose diminished significantly after single-dose nateglinide (1,834 [1,558–2,098] vs 1,541 mmol-min/L [1,328–1,725],

Table 1 | Clinical characteristics of the study participants

	S-N group	N-S group	Total	*P*
n	9	10	19	
Sex (male/female)	6/3	8/2	14/5	
Age (years)	57.8 ± 11.6	52.6 ± 14.3	55.1 ± 13.0	NS
Body mass index (kg/m^2)	23.5 ± 2.8	23.7 ± 4.7	23.5 ± 3.8	NS
Diabetes duration (years)	5.0 ± 6.5	5.2 ± 7.6	5.1 ± 6.9	NS
Systolic blood pressure (mmHg)	133.6 ± 20.9	131.5 ± 33.1	132.4 ± 27.6	NS
Diastolic blood pressure (mmHg)	82.5 ± 13.3	79.7 ± 18.6	80.9 ± 16.1	NS
HbA1c (%)	6.8 ± 0.5	6.4 ± 0.8	6.6 ± 0.6	NS
T-CHO (mg/dL)	191.5 ± 36.2	201.6 ± 17.7	196.8 ± 27.7	NS
HDL-C (mg/dL)	56.5 ± 14.4	57.0 ± 14.2	56.7 ± 13.9	NS
TG (mg/dL)	117.3 ± 43.3	130.1 ± 97.1	124.0 ± 74.8	NS

Data are mean ± standard deviation. HDL-C, high-density lipoprotein cholesterol; N-S group, patients who initially received nateglinide then sitagliptin; NS, not significant; S-N group, patients who initially received sitagliptin then nateglinide; T-CHO, total cholesterol; TG, triglyceride.

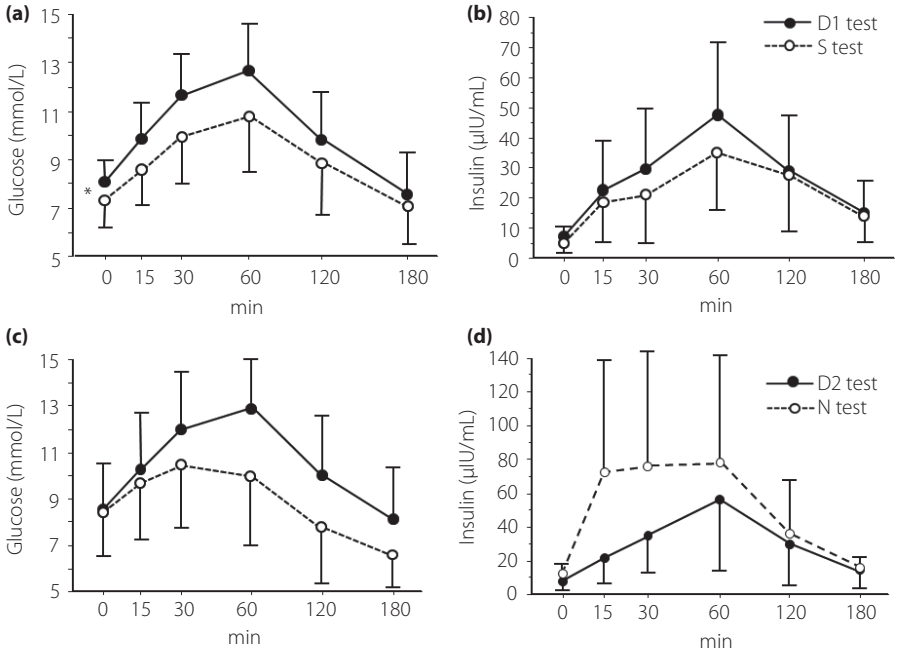

Figure 2 | (a) Plasma glucose and (c) plasma insulin levels before and after sitagliptin administration. (c) Plasma glucose and (d) plasma insulin levels before and after nateglinide administration. *$P < 0.01$, plasma glucose at 0 min D1 vs S test group.

$P < 0.01$). Figure 2d shows the serial changes in plasma insulin, and Table 2 shows $AUC_{0–180\ min}$ insulin before and after a single dose of nateglinide. Different from the meal test with sitagliptin, the $AUC_{0–180\ min}$ insulin increased significantly after single-dose nateglinide (3,728 [3,032–7,914] vs 4,934 μIU-min/mL [3,887–9,421], $P < 0.05$). To assess glucose-induced insulin secretion, we calculated insulin secretion relative to the rise in plasma glucose ($ISG_{0–30\ min}$ and $ISG_{0–180\ min}$). As shown in Table 2, unlike sitagliptin, nateglinide significantly altered $ISG_{0–30\ min}$ and $ISG_{0–180\ min}$.

Effects of Sitagliptin and Nateglinide on Plasma Glucagon and GLP-1 Levels

The serial changes in GLP-1 and glucagon hormones after a meal loading are presented as Figure S1. We compared the effects of single-dose nateglinide and sitagliptin on GLP-1 and glucagon hormone levels at the postprandial state. Sitagliptin, but not nateglinide, significantly increased $AUC_{0–120\ min}$ GLP-1 (S-test). In contrast, nateglinide, but not sitagliptin, significantly increased the $AUC_{0–180\ min}$ glucagon. Table 3 shows changes in the AUC of glucose, insulin, GLP-1, glucagon and ISG induced

Table 2 | Insulin secretion relative to glucose elevation and the area under the curve of insulin, glucagon-like peptide-1 and glucagon during the meal test

	D1 test	S-test	*P*	D2 test	N-test	*P*
$AUC_{0–180\ min}$ glucose (mmol-min/L)	1,788 (1,621–2,076)	1,535 (1,318–1,796)	<0.01	1,834 (1,558–2,098)	1,541 (1,328–1,725)	<0.01
$AUC_{0–180\ min}$ insulin (μIU-min/mL)	4,855 (3,253–7,005)	3,952 (3,283–5,723)	NS	3,728 (3,032–7,914)	4,934 (3,887–9,421)	<0.05
$ISG_{0–30\ min}$ (mIU/mmol)	1.60 (1.30–2.59)	1.78 (1.33–2.50)	NS	1.87 (1.15–2.45)	3.76 (2.56–5.72)	<0.01
$ISG_{0–180\ min}$ (mIU/mmol)	2.68 (1.84–3.49)	2.77 (2.02–3.73)	NS	2.63 (1.84–4.72)	4.48 (2.75–5.74)	<0.01
$AUC_{0–120\ min}$ GLP-1 (pmol-min/L)	332 (228–448)	1,071 (754–1,222)	<0.01	353 (251–501)	372.4 (228–506)	NS
$AUC_{0–180\ min}$ glucagon (pg-min/mL)	11,880 (9,864–13,779)	11,152 (9,422–13,764)	NS	11,779 (10,958–12,975)	14,201 (12,375–15,892)	<0.01

Data are median (interquartile range). S and N-tests are meal tests with sitagliptin and nateglinide, respectively. The meal test carried out at baseline (D1) and again at least 7 days after D1 (D2) are controls for the S and N-tests, respectively. $AUC_{0–120\ min}$ GLP-1, area under the curve of glucagon-like peptide-1 (GLP-1) from 0 to 120 min during the meal test; $AUC_{0–180\ min}$ glucagon, area under the curve of glucagon from 0 to 180 min during the meal test; $ISG_{0–30\ min}$, insulin secretion relative to glucose elevation ($AUC_{0–30\ min}$ insulin / $AUC_{0–30\ min}$ glucose); $ISG_{0–180\ min}$, insulin secretion relative to glucose elevation ($AUC_{0–180\ min}$ insulin/$AUC_{0–180\ min}$ glucose); NS, not significant.

Table 3 | Area under the curve of glucose, insulin, glucagon-like peptide-1, glucagon, and insulin secretion relative to glucose elevation before and after sitagliptin and nateglinide administration

	S-D1 test	N-D2 test	*P*
$\Delta AUC_{0–180\ min}$ glucose (mmol-min/L)	−253 (−310 to 166)	−352 (−445 to 246)	NS
$\Delta AUC_{0–180\ min}$ insulin (μIU-min/mL)	−584 (−1,354 to 534)	1,119 (216 to 1,887)	<0.01
$\Delta ISG_{0–180\ min}$ (mIU/mmol)	0.054 (−0.59 to 0.61)	1.53 (0.70 to 2.29)	<0.01
Δ $ISG_{0–30\ min}$ (mIU/mmol)	0.018 (−0.216 to 0.162)	2.106 (1.01 to 3.87)	<0.01
$\Delta AUC_{0–120\ min}$ GLP-1 (pmol-min/L)	480 (249 to 558)	22.5 (−95 to 195)	<0.01
$\Delta AUC_{0–180\ min}$ glucagon (pmol-min/L)	−1,478 (−2,066 to 351)	1,605 (945 to 4,215)	<0.01

Data are median (interquartile range) values. S-D1 (meal test carried out at baseline – a single-dose of 100 mg sitagliptin) and N-D2 (meal test carried out again at least 7 days after meal test carried out at baseline – a single-dose of 120 mg nateglinide) tests show changes (**Δ**) in glucose, insulin, glucagon-like peptide-1 (GLP-1), and glucagon before and after sitagliptin (S) and nateglinide (N) administration, respectively. Data of each variable were calculated in each patient using the formula: (S test-D1 test) and (N test-D2 test). $AUC_{0–120\ min}$ GLP-1, area under the curve of glucagon-like peptide-1 (GLP-1) from 0 to 120 min during the meal test; $AUC_{0–180\ min}$ glucagon, area under the curve of glucagon from 0 to 180 min during the meal test; $ISG_{0–30\ min}$, insulin secretion relative to glucose elevation ($AUC_{0–30\ min}$ insulin / $AUC_{0–30\ min}$ glucose); $ISG_{0–180\ min}$, insulin secretion relative to glucose elevation ($AUC_{0–180\ min}$ insulin/$AUC_{0–180\ min}$ glucose); NS, not significant.

by single-dose sitagliptin and nateglinide. Sitagliptin and nateglinide induced comparable changes in $AUC_{0–180\ min}$ glucose ($\Delta AUC_{0–180\ min}$ glucose). However, nateglinide induced significantly higher changes in $AUC_{0–180\ min}$ insulin ($\Delta AUC_{0–180\ min}$ insulin), ISG ($\Delta ISG_{0–30\ min}$ and $\Delta ISG_{0–180\ min}$) and $AUC_{0–180\ min}$ glucagon ($\Delta AUC_{0–180\ min}$ glucagon) compared with sitagliptin. In contrast, sitagliptin induced significantly higher changes in $AUC_{0–120\ min}$ GLP-1(Δ $GLP\text{-}1_{0–120\ min}$) than nateglinide.

DISCUSSION

This is the first study to compare the effects of single-dose sitagliptin and nateglinide on glucose, insulin, active GLP-1 and glucagon levels after the meal test in drug-naïve Japanese patients with type 2 diabetes. The results of this cross-over study showed that the effects of the maximum dose of sitagliptin on postprandial glucose levels were similar to those of nateglinide. However, the responses of the hormones involved in the regulation of glucose metabolism, such as active GLP-1 and glucagon, suggest the unique effects of each drug.

Only a few studies have so far compared the effects of glinides and DPP-4 inhibitors. In a previous study, type 2 diabetes patients were randomly assigned to continuous treatment with glinides and switched to sitagliptin. After 12 weeks, the long-term effects of both drugs on postprandial hyperglycemia were investigated using a test meal[19]. Another randomized cross-over trial was carried out in 20 drug-naïve patients with type 2 diabetes who were randomized to receive sitagliptin (50 mg/day) or

mitiglinide + voglibose, and then the treatment was continued for 8 weeks[20]. These comparative studies evaluated the long-term effects of sitagliptin and glinides on glycemic control. However, to our knowledge, there are no studies on the acute effects of a single dose. Therefore, this is the first study to investigate the acute effects by both sitagliptin and nateglinide on postprandial hyperglycemia and its related hormones in drug-naïve patients with type 2 diabetes. For a cross-over study, a washout period of each drug is necessary. In the present study, the washout period was approximately 2 weeks. Previous reports[21,22] showed that the plasma concentration of each drug was below the limit of detection at 60 and 25 h after single administration of sitagliptin 100 mg and nateglinide 120 mg, respectively. Therefore, the washout period of 2 weeks was enough to eliminate a remaining effect of previous medication.

Nateglinide is an insulin secretagogue known to stimulate early-phase insulin secretion from β-cells[2]. In the present study, insulin secretion relative to an increase in blood glucose after a meal load was significantly higher after a single dose of nateglinide. The latter also caused an increase in $ISG_{0–30\ min}$ ($AUC_{0–30\ min}$insulin / $AUC_{0–30\ min}$ glucose), which reflects early-phase glucose-induced insulin secretion. This result is consistent with our previous study that assessed the effect of 3-month treatment with nateglinide[23]. That previous study showed that the combination therapy of vildagliptin and nateglinide improved postprandial hyperglycemia effectively by augmentation of nateglinide-induced early phase insulin secretion. Therefore, the combination thearapy of sitagliptin and nateglinide might also improve postprandial hyperglycemia by the same mechanism. In addition, at least *in vitro*, nateglinide is reported to enhance glucagon secretion[8]. Indeed, nateglinide as well as sulfonylurea closes adenosine triphosphate-sensitive potassium channels by binding to their sulfonylurea receptor subunits in α-cells, and directly stimulates glucagon release[8]. In addition, sulfonylurea was reported to stimulate glucagon secretion also by inhibition of somatostatin released from δ-cells[24], thus nateglinide might have a similar effect. In the present study, nateglinide significantly increased the AUC of glucagon during the meal test compared with sitagliptin. In contrast, regarding the effect on GLP-1 *in vitro*, nateglinide increases the plasma GLP-1 level by inhibition of DPP-4 activity[5,25] and direct stimulation of GLP-1 release from L-cells[7]. However, in the present study, this difference could be related to the study condition. In fact, the use of nateglinide at a dose higher than the clinical dose was reported to have a direct effect on L-cells to enhance GLP-1 release[7]. Taking these previous studies into consideration, the inhibitory effect of DPP-4 activity and the stimulatory effect of GLP-1 after the administration of a clinical dose of nateglinide might be very modest. From these findings, restoration of early insulin secretion by nateglinide seemed to be the main reason for the improvement in postprandial hyperglycemia despite the increase in glucagon by nateglinide.

Finally, our the present had certain limitations. First, as the number of participants was relatively small, this study was a pilot study. However, a cross-over design is statistically efficient and thus requires fewer participants than non-crossover designs. Second, it took 3–4 weeks to complete the present study. Therefore, we cannot exclude the effects of lifestyle changes, such as diet and exercise, on glycemic control. However, the cross-over design of this study mitigated such effects. Third, the difficulty of glucagon assay should be noted. Although the Glucagon RIA kit used in the present study was reported to be the best-performing assay for glucagon among several commercially available assay kits, its accuaracy in the low concentration range was not adequate[18]. Therefore, further improvement for the assay is required.

In conclusion, the present study showed that the effect of single-dose sitagliptin on postprandial glucose level was similar to that of nateglinide in Japanese type 2 diabetes patients inadequately controlled with diet and exercise therapy. However, the study showed that the two drugs have different effects on insulin, glucagon and GLP-1.

DISCLOSURE

AK has received lecture fees from Kissei Pharma, Sanofi and Takeda Pharmaceutical Co. YF has received lecture fees from Novartis Pharmaceuticals and Eli Lilly, and research funds from Novartis Pharmaceuticals, MSD and Takeda Pharmaceutical Co. TH has received lecture fees from MSD, Eli Lilly, Takeda Pharmaceutical Co., Novartis Pharmaceuticals, Dainippon Sumitomo, Novo Nordisk Pharma, Sanofi-Aventis and Daiichi Sankyo, and also research funds from MSD, Eli Lilly, Takeda Pharmaceutical Co., Kowa, Mochida, Sanwakagaku, Novo Nordisk Pharma, Kissei Pharma, Novartis, Boehringer Ingelheim, Tanabe Mitsubishi, Dainippon Sumitomo, Telmo, Sanofi Aventis, Roche, Ono and Daiichi Sankyo. TM has received lecture fees from MSD, Takeda Pharmaceutical Co., and Eli Lilly. HW has received lecture fees from Asteras, Astrazeneca, Boehringer Ingelheim, Daiichi Sankyo Inc., Eli Lilly and Company, Kissei Pharmaceutical Co., Kowa Pharmaceutical CO., Kyowa Hakko Kirin Co., MSD, Novartis Pharmaceuticals, Novo Nordisk Pharma, Ono Pharmaceutical Co., Mitsubishi Tanabe Pharma, Sanofi-Aventis, Sanwakagaku Kenkyusho, and Takeda Pharmaceutical Co., and research funds from Asteras, Astrazeneca, Bristol-Myers Squibb, Boehringer Ingelheim, Daiichi Sankyo Inc., Dainippon Sumitomo Pharma, Eli Lilly, Johnson and Johnson, Kissei Pharmaceutical Co., Kowa Pharmaceutical CO., Kyowa Hakko Kirin Co. MSD, Mitsubishi Tanabe Pharma, Mochida Pharmaceutical Co., Novartis Pharmaceuticals, Novo Nordisk Pharma, Pfizer, Sanwakagaku Kenkyusho, Sanofi, and Takeda Pharmaceutical Co. All the other authors declare no conflict of interest.

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SUPPORTING INFORMATION

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

Figure S1 | Serial changes of plasma glucagon-like peptide-1 (GLP-1) and glucagon.