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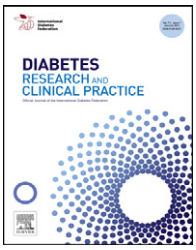

Effect of sitagliptin monotherapy on serum total ghrelin levels in people with type 2 diabetes

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ABSTRACT

Aim: Sitagliptin is not associated with weight gain and has neutral effects on body weight. It is unclear whether sitagliptin treatment alters serum ghrelin levels in people with type 2 diabetes.

Methods: Forty-four subjects with type 2 diabetes were randomly assigned to receive sitagliptin or medical nutrition therapy (MNT) for 12 weeks. Changes in anthropometric variables, glycemic control, insulin resistance, lipid parameters, and total ghrelin levels were evaluated at baseline and following 12 weeks of treatment.

Results: Significant decreases in body weight and body mass index were observed over the entire study period in both treatment groups. Glycosylated hemoglobin and postprandial plasma glucose levels were statistically significant decreased in the groups receiving sitagliptin compared with baseline values ($p = 0.021$ and $p = 0.021$, respectively), while they were unchanged in the groups receiving MNT. There was a significant decrease in total ghrelin in the groups receiving sitagliptin ($p = 0.04$) compared with baseline values but not in the groups receiving MNT ($p = 0.46$) at the end of the 12 weeks.

Conclusions: In this study of patients with type 2 diabetes, treatment with sitagliptin was associated with a significant decrease in serum ghrelin levels. These results suggest that the neutral effect of sitagliptin on weight might be associated with the suppression of fasting serum ghrelin levels.

1. Introduction

Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are incretin hormones that are released from the gastrointestinal tract after food intake. The incretin hormone GLP-1 retards gastric emptying and decreases caloric intake. Today, therapies based on incretin are being used for type 2 diabetes with an increasing frequency. Sitagliptin administered once daily is a potent and tolerable dipeptidyl peptidase-4 (DPP-4) inhibitor [1]. DPP-4 inhibitors are effective in glucose metabolism by preventing DPP-4 from deactivating GLP-1 and GIP quickly. Weight gain is also another important problem in the treatment of type 2 diabetes mellitus as well as glycemic control. Nevertheless, the effect of sitagliptin on weight is neutral, unlike that of sulfonylurea, thiazolidinedione and insulin, which are used in diabetes therapy.

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Ghrelin is an orexigenic peptide produced mainly in the stomach [2]. Plasma ghrelin concentrations increase with fasting and decrease after feeding, suggesting a physiological role for ghrelin in meal initiation in humans [3,4]. Plasma ghrelin concentration also shows a diurnal rhythm, and is inversely proportional to food intake (acute effect) and obesity (chronic effect) [5,6]. Serum ghrelin levels are positively correlated with insulin sensitivity whereas they are negatively correlated with serum insulin and glucose levels [3,7–10].

Bearing in mind the effects of ghrelin on food intake and weight gain, the neutral effect of sitagliptin on body weight might be associated with serum ghrelin levels. There is only one study in the literature evaluating the effect of sitagliptin monotherapy on serum ghrelin levels, and it is unclear whether sitagliptin treatment alters serum ghrelin levels in people with type 2 diabetes. In this study, we aimed to evaluate the effects of sitagliptin monotherapy on serum ghrelin levels in patients with type 2 diabetes.

2. Subjects, materials and methods

2.1. Patients

The study protocol was approved by our Institutional Review Board and was conducted in accordance with the Declaration of Helsinki. Forty-four newly diagnosed (duration <6 months) patients with type 2 diabetes [according to the American Diabetes Association (ADA) criteria] were enrolled [11]. Patients were excluded if they had a history of impaired hepatic function (defined as plasma aminotransferase and/or gamma-glutamyltransferase level higher than the upper limit of normal [ULN] for age and sex) or impaired renal function (defined as serum creatinine level higher than the ULN for age and sex). Patients with serious cardiovascular disease (e.g., New York Heart Association class I–IV congestive heart failure or a history of myocardial infarction or stroke) or cerebrovascular conditions were also excluded. Women who were pregnant or breastfeeding were excluded. Patients younger than 18 years were not included our study because sitagliptin is not approved for childhood period. We also excluded patients older than 65 years since they usually have co-morbid diseases and take multiple medications, which may influence with our results and impair homogeneity of the study groups.

Patients were randomly assigned to receive 100 mg/day sitagliptin plus medical nutrition therapy (MNT) ($n = 28$) or MNT alone ($n = 16$) for 12 weeks. MNT was based on ADA recommendations and contained 50% of calories from carbohydrates, 30% from fat (6% saturated) and 20% from proteins, with a maximum cholesterol content of 300 mg/day, and 35 g/day of fiber. All patients provided written informed consent to participate.

2.2. Study design

Before starting the study, all patients underwent an initial screening assessment that included a medical history, physical examination, calculation of body mass index (BMI), assessment of glycemic control (glycosylated hemoglobin A1c (HbA1c), fasting plasma glucose (FPG), postprandial glucose (PPG), and insulin levels (FPI)), homeostasis model assessment-insulin resistance (HOMA-IR) index and serum ghrelin levels. The estimate of insulin resistance was calculated using the HOMA-IR, with the following formula: insulin resistance = FPI (μU/mL) $\times$ FPG (mmol/L)/22.5 (normal if <2.5, presence of insulin-resistance if $\geq$2.5). BMI was calculated by the investigators as weight in kilograms divided by the square of height in meters. All measurements were repeated after 12 weeks.

2.3. Biochemical analysis

Venous blood samples were drawn in all patients between 8:00 AM and 9:00 AM. Blood was collected into tubes with EDTA as the anticoagulating substance for plasma preparation and serum separator tubes with clot activator. Samples were centrifuged at 2500 $\times$ g for 15 min to separate plasma and serum. Blood samples were frozen and stored at −80 °C until analysis. Serum ghrelin levels were measured with commercially available radioimmunoassay (RIA) kits (Linco Research, St. Charles, MO, USA). The limit of sensitivity of total ghrelin was 93 pg/mL. Serum total ghrelin was measured in duplicate and the difference between duplicate results of samples was <10% coefficient variation. HbA1c level was measured using high-performance liquid chromatography (BIO-RAD Diagnostic Group, CA, USA), with intra- and inter-assay coefficients of variation (CsV) of <2%. Plasma glucose was assayed using a glucose-oxidase method (GOD/PAP, Roche Diagnostics, Mannheim, Germany) with intra- and inter-assay CsV <2%. Serum insulin was assayed with Phadiaseph Insulin Radioimmunoassay (Pharmacia, Uppsala, Sweden) using a second antibody to separate the free and antibody-bound 125 I-insulin (intra- and inter-assay CsV, 4.6% and 7.3%, respectively). Laboratory parameters including total cholesterol (T chol), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) were measured using autoanalyzer (Aeroset System Abbott, Abbott Laboratories, Diagnostic Division, IL, USA).

2.4. Data analysis

χ^2-test was used for clinical data variables. Mann–Whitney U test analysis was used to asses the differences between the two study groups. Wilcoxon test was used for within-group comparisons. Analysis of covariance (ANCOVA) was also performed to determine any difference between two groups after controlling for the effects of covariates. Statistical analysis of data was performed using the Statistical Package for Social Sciences software version 17.0 (IBM Acquires SPSS Inc., Somers, NY, USA), which is used in the Uludag University network. Data are presented as mean $\pm$ standard deviation (SD). In all statistical analyses, $p < 0.05$ was considered statistically significant.

3. Results

A total of 44 patients (13 males, 31 females; mean age: 56.5 $\pm$ 8.8 years) were enrolled in the study. The characteristics of the

Table 1 – Baseline characteristics and parameter changes at 12 weeks in the two study groups.

	MNT (n = 16)			Sitagliptin (n = 28)		
	Baseline	12 weeks	p	Baseline	12 weeks	p
Body weight (kg)	77.1 ± 11.2	74.9 ± 10.7	0.003	84.1 ± 17.0	82.1 ± 16.4	0.003
BMI (kg/m^2)	29.8 ± 4.5	29.0 ± 4.5	0.003	31.6 ± 5.8	29.9 ± 5.1	0.002
FPG (mg/dL)	130.4 ± 24.8	120.7 ± 21.8	NS	135.4 ± 20.2	129.7 ± 38.1	NS
PPG (mg/dL)	178.6 ± 63.4	155.8 ± 45.8	NS	196.4 ± 52.9	165.2 ± 45.1	0.021
HbA1c (%)	6.5 ± 0.8	6.4 ± 0.7	NS	6.9 ± 0.7	6.6 ± 0.8	0.021
TC (mg/dL)	195.4 ± 34.8	195.0 ± 28.6	NS	191.3 ± 31.2	201.6 ± 40.9	NS
HDL-C (mg/dL)	49.1 ± 12.1	47.6 ± 12.0	NS	47.4 ± 9.3	47.0 ± 9.1	NS
LDL-C (mg/dL)	114.1 ± 32.5	120.1 ± 26.2	NS	111.1 ± 28.2	125.8 ± 45.0	NS
TG (mg/dL)	161.6 ± 74.4	136.6 ± 58.6	NS	162.2 ± 72.9	168.8 ± 93.2	NS
HOMA-IR	3.1 ± 1.5	2.5 ± 1.6	NS	4.9 ± 3.4	4.8 ± 2.9	NS
Total ghrelin	883.2 ± 361.2	992.7 ± 397.0	NS	649.2 ± 221.5	568.7 ± 159.0	0.040

MNT: medical nutrition therapy, BMI: body mass index, FPG: fasting plasma glucose, PPG: postprandial plasma glucose, HbA1c: hemoglobin A1c, TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, TG: triglyceride, HOMA-IR: homeostasis model assessment-insulin resistance, and NS: not significant.

patient population in the initial period of the study were similar in both groups with the exception of HOMA-IR (p = 0.039). Baseline body weight, BMI, FPG, PPG, HbA1c, lipid variables, and serum ghrelin levels did not differ significantly between the study groups. Sitagliptin was well tolerated and used regularly by the patients. No serious adverse effect was seen during the study period.

3.1. *Body mass index*

There was a significant reduction in BMI of subjects in the groups receiving MNT (−2.7 ± 3.1%, p = 0.003) and sitagliptin (−3.5 ± 3.7%, p = 0.002) compared with baseline values (Table 1). When the two groups were compared, no significant difference was found between the groups (Table 2).

3.2. *Glycemic control*

HbA1c and PPG levels were statistically significant decreased in the groups receiving sitagliptin compared with baseline values (p = 0.021 and p = 0.021, respectively), while they were unchanged in the groups receiving MNT (Table 1). However, percentage changes in HbA1c, FPG, PPG, and HOMA-IR were not different in inter-group comparisons (p = 0.303, p = 0.687, p = 0.393, p = 0.191, respectively). Percent decreases in HOMA-IR at the 12th week from baseline levels also demonstrated no statistically significant difference in either group.

3.3. *Lipid variables*

No significant changes in T chol, HDL-C and LDL-C were observed in the two study groups at the 12-week assessment when compared with the baseline values.

3.4. *Measurements of serum ghrelin*

After 12 weeks of treatment, serum total ghrelin level was decreased −9.3 ± 21.8% in the groups receiving sitagliptin, and the difference was statistically significant compared with baseline values (p = 0.04) (Fig. 1). However, percentage changes in ghrelin level were not different between the two groups at the end of the study period (p = 0.082) (Table 2). ANCOVA was also performed to determine any differences

Table 2 – Percentage changes in clinical and biochemical parameters after 12 weeks of intervention.

% change	MNT	Sitagliptin	p
Body weight (kg)	−2.7 ± 3.1	−2.3 ± 3.7	0.751
BMI (kg/m^2)	−2.7 ± 3.1	−3.5 ± 3.7	0.352
FPG (mg/dL)	−6.0 ± 15.2	−2.8 ± 29.4	0.687
PPG (mg/dL)	−5.6 ± 31.6	−11.2 ± 27.0	0.393
HbA1c (%)	−1.4 ± 5.9	−4,1 ± 7.7	0.303
TC (mg/dL)	2.0 ± 17.9	7.3 ± 23.5	0.903
HDL-C (mg/dL)	−1.7 ± 18.2	0.6 ± 18.3	0.893
LDL-C (mg/dL)	14.4 ± 41.8	13.0 ± 39.1	0.880
TG (mg/dL)	−5.1 ± 47.8	9.5 ± 48.8	0.150
HOMA-IR	−13.3 ± 45.8	25.1 ± 77.8	0.191
Total ghrelin	21.4 ± 53.3	−9.3 ± 21.8	0.082

MNT: medical nutrition therapy, BMI: body mass index, FPG: fasting plasma glucose, PPG: postprandial plasma glucose, HbA1c: hemoglobin A1c, TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, TG: triglyceride, and HOMA-IR: homeostasis model assessment-insulin resistance.

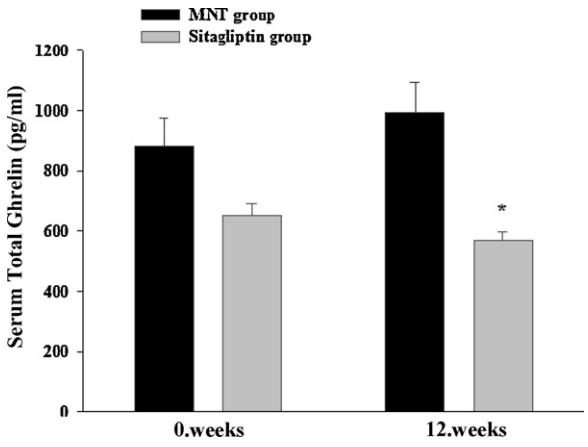

Fig. 1 – Serum ghrelin levels after 12 weeks in MNT and sitagliptin groups. * p = 0.04 according to the baseline values.

between the two groups after controlling for the effects of age, serum glucose and insulin covariates. When the effects of age, glucose and insulin were removed, the effect of the group variable on serum ghrelin levels was found statistically significant ($p = 0.038$). Estimated marginal means (EMM) adjusted for the effects of covariates were 0.209 ± 0.107 for the groups receiving MNT and -0.09 ± 0.8 for the groups receiving sitagliptin. Covariates serum glucose, insulin and age were not significantly related to serum ghrelin levels ($p = 0.642$, $p = 0.962$, $p = 0.923$, respectively).

4. Discussion

In this study, we found that fasting serum ghrelin levels were significantly decreased following 12 weeks of sitagliptin monotherapy compared with baseline values in patients with type 2 diabetes.

Ghrelin is an important orexigenic peptide secreted mainly by the stomach. Endogenous ghrelin levels change with acute and chronic nutritional status. Fasting causes elevation in ghrelin levels, which reduce 60–120 min after food intake [3,12,13]. Some other studies have also shown that chronic administration of ghrelin causes weight gain by not only increasing food intake but also decreasing energy consumption and fat catabolism [14,15].

A considerable number of patients with type 2 diabetes are overweight or obese, and weight gain is an important problem in the treatment of type 2 diabetes mellitus [16]. Excessive energy intake is an important cause of the observed weight gain in type 2 diabetes [17,18]. As a result, physicians should take into consideration the effect of oral anti-diabetic drugs on weight gain when treating type 2 diabetic patients. However, metformin is the only treatment providing weight loss [19], and weight gain is exacerbated by treatment with thiazolidinediones, insulin or insulinotropic agents such as sulfonylureas. Currently, therapies based on incretin are being used for type 2 diabetes with an increasing frequency. Interestingly, it was reported that sitagliptin and other DPP-4 inhibitors had neutral effects on weight despite the insulin secretory effects of these drugs [20,21]. In present study, there was a statistically significant decrease in BMI of patients (31.6 ± 5.8 vs 29.9 ± 5.1, $p = 0.002$) treated with sitagliptin monotherapy, in concordance with previous studies. BMI of patients was also decreased in the groups receiving MNT (29.8 ± 4.5 vs 29.0 ± 4.5, $p = 0.003$), and inter-group analysis revealed no significant difference in percentage change of BMIs.

The exact mechanism of this observed weight-protecting effect of DPP-4 inhibitors is unclear. Taking the effects of ghrelin on food intake and body weight into consideration, ghrelin might be one of the contributors and/or might play a role in the actions of sitagliptin. Previously, we observed that serum total and acylated ghrelin levels were similar in patients with type 2 diabetes treated with metformin, pioglitazone or diet therapy alone after a mixed meal test [22]. Postprandial ghrelin suppression was also found to be longer in metformin-treated subjects compared with the diet group. However, it is unclear whether sitagliptin treatment alters serum ghrelin levels in people with type 2 diabetes. Huang et al. [23] reported that an oral dose of sitagliptin did not affect preprandial circulating ghrelin, insulin or glucose concentrations in normal subjects. They found that only after meal loading was there a progressive and persistent decline in ghrelin levels until the end of the study period, while a rapid and temporary rise in gastrin, insulin and glucose levels at 0.5 h was observed. However, Huang et al.'s study was a cross-sectional study, and the study population consisted of normal subjects, in contrast with the present study. In our study, sitagliptin were administered to patients with type 2 diabetes for 12 weeks and only fasting ghrelin levels were evaluated.

There was also an approximately 0.3% decrease in HbA1c levels of our patients in the sitagliptin group compared with baseline values (from $6.9 \pm 0.7\%$ to $6.6 \pm 0.8\%$, $p = 0.021$), and no significant change was observed in serum lipid levels of patients (Table 1). These results are concordant with previous studies reporting that sitagliptin therapy causes a decrease in HbA1c by 0.5–0.8% and has no effect on serum lipid levels [20,21,24,25].

This study has several limitations. The present investigation was designed as an exploratory pilot project. The primary outcome measures in this study were body weight and fasting serum ghrelin levels. We did not measure post-prandial serum ghrelin levels. Another potential limitation of the present study is that the interval of 12 weeks may have been too short to observe the full effects of sitagliptin on body weight and serum ghrelin levels. Greater number of patients and longer treatment durations are required to fully evaluate this possibility. However, our findings provide a rationale for future studies.

In conclusion, these results suggest that the weight-neutral effect of sitagliptin might be associated with the suppression of fasting serum ghrelin levels.

Conflict of interest

The authors declare that they have no conflict of interest.

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