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CITATION:

ISSUE DATE:
2011-10-21

URL:
http://hdl.handle.net/2433/197320

RIGHT:
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Plasma gastric inhibitory polypeptide and glucagon-like peptide-1 levels after glucose loading are associated with different factors in Japanese subjects

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ABSTRACT

Aims/Introduction: Gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are major incretins that potentiate insulin secretion from pancreatic β-cells. The factors responsible for incretin secretion have been reported in Caucasian subjects, but have not been thoroughly evaluated in Japanese subjects. We evaluated the factors associated with incretin secretion during oral glucose tolerance test (OGTT) in Japanese subjects with normal glucose tolerance (NGT).

Materials and Methods: We measured plasma GIP and GLP-1 levels during OGTT in 17 Japanese NGT subjects and evaluated the factors associated with GIP and GLP-1 secretion using simple and multiple regression analyses.

Results: GIP secretion (AUC-GIP) was positively associated with body mass index ($P < 0.05$), and area under the curve (AUC) of C-peptide ($P < 0.05$) and glucagon ($P < 0.01$), whereas GLP-1 secretion (AUC-GLP-1) was negatively associated with AUC of plasma glucose ($P < 0.05$). The insulinogenic index was most strongly associated with GIP secretion ($P < 0.05$); homeostasis model assessment β-cell was the most the strongly associated factor in GLP-1 secretion ($P < 0.05$) among the four indices of insulin secretion and insulin sensitivity.

Conclusions: Several distinct factors might be associated with GIP and GLP-1 secretion during OGTT in Japanese subjects.

KEY WORDS: Gastric inhibitory polypeptide, Glucagon-like peptide-1, Incretin

INTRODUCTION

Oral glucose administration leads to greater insulin release from pancreatic islets than intravenous glucose loading that yields 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. 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 (GIP receptor [GIPR] and GLP-1 receptor) on the surface of pancreatic β-cells to stimulate insulin secretion by increasing the intracellular adenosine 3',5'-monophosphate (cAMP) concentration.

Type 2 diabetes is characterized by both decreased insulin secretion and reduced insulin sensitivity. The incretin effect has been shown to be reduced in type 2 diabetic subjects compared with those with normal glucose tolerance (NGT) in previous studies, suggesting that a reduced incretin effect might be associated with hyperglycemia after food intake and glucose loading in type 2 diabetes. When intravenous infusion of GIP or GLP-1 was carried out in type 2 diabetic subjects, GLP-1 potentiated insulin secretion from pancreatic β-cells, but GIP did not, showing that the GIPR signal is downregulated in β-cells in type 2 diabetes. In studies using rodent models, it was reported that GIPR mRNA and protein expression levels in islets are decreased in the diabetic state. In contrast, in the non-diabetic obese state, GIP plays an important role in maintaining blood glucose levels. The GIP signal might be enhanced as a result of increased GIPR sensitivity of β-cells to GIP or increased GIP secretion from K-cells in the non-diabetic obese state. Indeed, GIP concentrations are reported to be increased in obese rodent models and human subjects compared with those in lean rodents and human subjects, respectively. Furthermore, we have previously shown the hypersensitivity of GIPR to GIP in β-cells of high fat-induced obese mice. Plasma GLP-1 concentrations in type 2 diabetic patients are reported to be reduced after meal ingestion and glucose loading. However, in other studies it was reported that GLP-1 concentrations did not differ...
in NGT and type 2 diabetic subjects\textsuperscript{18–20}. Thus, the measurement of GIP and GLP-1 concentrations in various metabolic states is important to evaluate the effects of incretin on insulin secretion.

Insulin sensitivity in Asian subjects has been shown to be higher than in Mexican Americans and Caucasians in previous reports\textsuperscript{21,22}, which is partly as a result of the fact that Asians, including Japanese, are generally less obese. Furthermore, insulin secretion rather than insulin sensitivity is the more important factor in progression from NGT to diabetes in Japanese subjects\textsuperscript{23}. We have reported that early-phase insulin secretion is considerably decreased, even in Japanese NGT subjects with 1-h plasma glucose (PG) levels during oral glucose tolerance test (OGTT) of more than 180 mg/dL\textsuperscript{24}. Thus, it is especially important to evaluate incretin secretion and determine the factors associated with incretin secretion in Japanese NGT subjects, because GIP and incretin is responsible for more than 50% of postprandial insulin secretion after glucose ingestion. The factors responsible for incretin secretion have been reported in Caucasian subjects, but have not been thoroughly elucidated in Japanese subjects.

In the present study, we evaluated GIP and GLP-1 levels during OGTT and determined the factors involved in GIP and GLP-1 secretion (area under the curve [AUC] of GIP and GLP-1 during OGTT) in Japanese NGT subjects.

**MATERIALS AND METHODS**

**Subjects**
We recruited 17 Japanese healthy volunteers. 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 the measurement of liver and kidney function, HbA1c, serum triglyceride (TG), total cholesterol and high-density lipoprotein (HDL)-cholesterol levels were drawn after an overnight fast. All subjects received OGTT. After the subjects fasted overnight for 10–16 h, standard OGTT with 75 g glucose was given according to the National Diabetes Data Group recommendations\textsuperscript{25}. NGT was diagnosed according to World Health Organization (WHO) criteria\textsuperscript{26}.

Blood samples were collected at −15, 0, 10, 20, 30, 60, 90, 120, 150 and 180 min after glucose loading and were centrifuged at 1800 g at 4°C for 10 min. After collecting supernatant of the samples, plasma and serum were stored at −80°C. Plasma GIP, GLP-1 levels and the various parameters (PG, serum immunoreactive insulin [IRI], serum C-peptide reactivity [CPR], TG, serum free fatty acid [FFA] and plasma glucagon) were measured at the indicated times (plasma GIP and GLP-1 levels were measured at −15, 0, 10, 30, 60, 90, 120 and 180 min after glucose loading, and plasma glucagon levels were measured at −15, 0, 30, 60, 90, 120 and 180 min after glucose loading). The PG levels were measured by glucose oxidase method. Serum IRI levels were measured by two-site radioimmunoassay. Total GIP and total GLP-1 levels were measured using human GIP ELISA kit (Linco Research, St Charles, MO, USA; range of detection from 8.2 pg/mL to 2000 pg/dL) and human GLP-1 ELISA kit (Meso Scale Discovery, Gaithersburg, MD, USA; range of detection from 2.4 pg/mL to 1,000,000 pg/dL), respectively, as previously described\textsuperscript{27,28}. The AUC of PG, IRI, CPR, TG, FFA, glucagon, total GIP (AUC-GIP) and total GLP-1 (AUC-GLP-1) were calculated. We then analyzed the relationship between the AUC of GIP (GIP secretion) and GLP-1 (GLP-1 secretion) and age, body mass index (BMI) and the parameters during OGTT.

**Statistical Analysis**
Basal insulin secretion and sensitivity were evaluated by homeostasis model assessment (HOMA) \(\beta\)-cell function and homeostasis model assessment of insulin resistance (HOMO-IR)\textsuperscript{29,30}, respectively. Early-phase insulin secretion and systemic insulin sensitivity during OGTT were evaluated by insulinogenic index\textsuperscript{31} and insulin sensitivity index (ISI) composite\textsuperscript{32}. The calculations of the four indices were as follows:

\[
\text{HOMO } \beta\text{-cell} = 20 \times \text{fasting IRI level (FIRI)} (\text{pmol/L}) / (\text{fasting PG level [FPG]} (\text{mmol/L}) - 3.5)
\]

\[
\text{HOMO-IR} = \text{FIRI} (\text{pmol/L}) \times \text{FPG} (\text{mmol/L}) / 22.5
\]

\[
\text{Insulinogenic index} = (30 \text{ min IRI} - \text{FIRI} (\text{pmol/L})) / (30 \text{ min PG} - \text{FPG} (\text{mmol/L}))
\]

\[
\text{IRI composite} = 10,000 / [\text{FPG} (\text{mg/dL}) \times \text{FIRI} (\mu\text{U/mL})] \times \text{mean OGTT PG [mg/dL]}
\]

\[
\times \text{mean OGTT IRI} [\mu\text{U/mL}]^{0.5}
\]

All analyses were carried out using statistical analysis software (SPSS version 17.0, IBM, Somers, NY, USA) system. Statistical analysis was carried out by \textit{ANOVA} with Fisher’s PLSD test for changing levels of GIP, GLP-1, and the parameters during OGTT and differences between the two groups were assessed by unpaired \(t\)-test. We used simple regression analysis to determine the relationship between AUC-GIP or AUC-GLP-1 and the age, BMI and the parameters during OGTT, and we carried out multiple regression analysis to determine the factors most strongly associated with AUC-GIP and AUC-GLP-1, and the indices of insulin secretion and sensitivity. Probability (\(P\)) values <0.05 were considered statistically significant. Data are presented as mean ± standard error (SE).

**RESULTS**
Table 1 shows clinical characteristics of the subjects. Mean age was 31.7 ± 1.3 years and mean BMI was 23.1 ± 0.9 kg/m\(^2\). No subjects had liver or kidney dysfunction. HbA1c, FPG, TG, total
The levels of GIP, GLP-1, PG, IRI, CPR, TG, FFA and glucagon after glucose loading were measured (Figure 1). The subjects were diagnosed NGT according to WHO criteria with fasting plasma glucose and 2-h glucose levels below 6.1 and 7.8 mmol/L, respectively. Levels of PG, IRI and CPR were significantly increased from 10 min after glucose loading compared with fasting level (Figure 1a–c). FFA levels were significantly decreased from 30 min after glucose loading (Figure 1d). TG levels were not significantly changed during OGTT (Figure 1e). Glucagon levels were significantly decreased from 30 min after glucose loading (Figure 1d). TG levels were significantly increased from 10 min during OGTT (Figure 1f). Total GIP levels were significantly increased from 10 min during OGTT (Figure 1g). Total GLP-1 levels were significantly increased from 10 min during OGTT with peaks at 30 and 120 min (Figure 1h).

We analyzed the relationship between AUC-GIP or AUC-GLP-1 and age, BMI and the several parameters (AUC of PG, IRI, CPR, TG, FFA and glucagon). AUC-GIP were positively related to BMI and AUC of CPR, IRI and glucagon, but AUC-GLP-1 was not related to these factors (Figure 2a–c; AUC data of IRI during OGTT are not shown; P < 0.05). In contrast, AUC-GLP-1 was inversely related to AUC of PG (Figure 2d), but AUC-GIP was not.

We then analyzed the relationship between AUC-GIP or AUC-GLP-1 and indices of insulin secretion and insulin sensitivity. AUC-GIP was positively related to insulinogenic index and HOMA-IR, whereas AUC-GLP-1 was positively related to HOMA-β cell function (Figure 3a–c). ISI composite was not related to either AUC-GIP or AUC-GLP-1 (Figure 3d). In addition, multiple regression analysis was carried out to determine the factors strongly associated with AUC-GIP and AUC-GLP. The insulinogenic index was the most strongly associated factor in AUC-GIP (correlation coefficients 0.56, standardized β 0.56, P < 0.05) of the four indices; HOMA-β cell function was the strongest factor in AUC-GLP-1 (HOMA-β cell function: correlation coefficients 0.524, standardized β 0.870, P < 0.01, ISI composite; correlation coefficients 0.063, standardized β 0.581, P < 0.05).

**DISCUSSION**

In the present study, we estimated the incretin level after glucose loading in Japanese NGT subjects and found that plasma GIP and GLP-1 levels during OGTT are related to different factors. Incretin action of GIP is reduced in the diabetic state as a result of decreased GIP receptor expression on pancreatic β-cells, whereas GIP signaling is enhanced and maintains...
is a positive relationship between AUC-GIP and AUC of FFA during OGTT. In the present study, AUC-GIP after glucose loading was not associated with AUC of FFA, but was positively associated with BMI, HOMA-IR, and AUC of IRI and CPR after glucose loading. In fact, obese subjects are known to have hyperinsulinemia and insulin resistance, and BMI was strongly associated with AUC of IRI and CPR. Thus, GIP secretion from K-cells may well be associated with insulin resistance to maintain postprandial hyperinsulinemia in Japanese NGT subjects. It is unknown why there was no correlation between AUC-GIP and AUC-glucose. It might be explained by the fact that GIP secretion is associated with the amount of glucose loading, whereas blood glucose levels are maintained within normal levels by GIP-induced compensatory insulin secretion in NGT subjects.

GLP-1 secretions of type 2 diabetes subjects after glucose or meal ingestion are diverse in human studies. Some studies report that GLP-1 secretion is decreased in Caucasian type 2 diabetes. Recently, it is reported that GLP-1 levels after ingestion of glucose and mix meal in Japanese type 2 diabetic subjects were not decreased compared with those in NGT subjects, suggesting that GLP-1 secretion is not decreased in Japanese type 2 diabetes. Two studies of Caucasian subjects found that AUC-GLP-1 during OGTT is positively associated with age and AUC of glucagon, whereas AUC of GLP-1 is negatively associated with BMI or bodyweight and AUC of FFA.
present study, AUC-GLP-1 was negatively related to AUC of PG during OGTT, showing that the increase in GLP-1 secretion after glucose loading is associated with a decrease in postprandial glucose levels in Japanese NGT subjects. It has been reported that GLP-1 levels after glucose loading are positively related to gastric emptying in Caucasian subjects. Although we did not measure gastric emptying of the subjects in the present study, increasing GLP-1 secretion after glucose loading might decrease postprandial glucose levels through gastric emptying. In the present study, BMI and AUC of FFA were not associated with AUC-GLP-1 during OGTT. Obese subjects have higher FFA levels than lean subjects. However, because Japanese subjects are less obese than Caucasian subjects, the difference observed in the relationship between AUC-GIP and GLP-1, and AUC of FFA might reflect this ethnic difference in Caucasians and Japanese.

Insulin secretion, rather than insulin sensitivity, is the more important factor in the progression from NGT to type 2 diabetes in Japanese patients. Because incretin is an intestinal hormone that induces postprandial insulin secretion, we hypothesize that GIP and GLP-1 secretion is more crucial in Japanese subjects than in Caucasian subjects. Indeed, GLP-1 mimetics and DPP-4 inhibitors improve glycemic control better in Japanese type 2 diabetic patients than in Caucasian type 2 diabetic patients in clinical trials.

We therefore evaluated the correlation between GIP secretion (AUC-GIP) and GLP-1 secretion (AUC-GLP-1), and the indices of insulin secretion and insulin sensitivity in Japanese NGT subjects during OGTT. The values of HOMA \( \beta \)-cell, insulogenic index, HOMA-IR and ISI composite were similar to those in previous studies of Japanese subjects. AUC-GIP was positively associated with the insulogenic index and HOMA-IR, and the insulogenic index was strongly associated with AUC-GIP, whereas AUC-GLP-1 was associated only with HOMA \( \beta \)-cell among the four indices. It has been reported that early-phase insulin secretion is an important factor in the progression from NGT to type 2 diabetes in Japanese patients. Thus, enhancing the GIP and GLP-1 signals might be particularly useful in inhibiting the progression of type 2 diabetes in Japanese patients. Recently, variants at the GIP receptor gene locus associated with 2-h glucose levels during OGTT were identified by meta-analysis of genome-wide association studies. In subjects who carry this GIP receptor risk allele, early-phase insulin secretion is decreased. These data seem to support our results that GIP secretion is associated with insulogenic index in Japanese NGT subjects.

In conclusion, we evaluated plasma GIP and GLP-1 levels during OGTT in Japanese NGT subjects. GLP-1 secretion was associated with PG during OGTT, and basal insulin secretion (HOMA \( \beta \)-cell) and GIP secretion was associated with BMI and early-phase insulin secretion (insulogenic index). Thus, there might be different factors associated with GIP and GLP-1 secretion during OGTT in Japanese subjects.

ACKNOWLEDGEMENTS

We thank Dr Yutaka Seino (Kansai Electric Power Hospital) for his helpful suggestions. This study was supported by Scientific Research Grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and by Kyoto University Global COE Program ‘Center for Frontier Medicine’, and also by Novo Nordisk Pharma Ltd.

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