CLINICAL STUDY

Acute plasma glucose increase, but not early insulin response, regulates plasma ghrelin

Lucia Briatore, Gabriella Andraghetti and Renzo Cordera
Department of Endocrinology and Metabolism, University of Genoa, Viale Benedetto XV 6, 16132 Genoa, Italy
(Correspondence should be addressed to R Cordera; Email: record@unige.it)

Abstract
Objective: The independent role of glucose and insulin in ghrelin regulation is still controversial; this is also because in healthy subjects it is difficult to isolate the increase of glucose from that of insulin. The aim of this study was to discriminate the effect of glucose increase alone and early insulin response on plasma ghrelin, comparing ghrelin variation after i.v. glucose between healthy subjects and type 2 diabetic (T2DM) subjects, in whom the early insulin response to i.v. glucose is abolished.

Methods: Plasma glucose, insulin and ghrelin levels were measured 0, 3, 5, 10, 30, 45 and 60 min after a 5 g glucose i.v. bolus in seven healthy control subjects and eight T2DM subjects.

Results: There were no significant differences in body mass index, basal insulin and basal ghrelin between T2DM and healthy subjects. Basal glucose levels were higher in T2DM subjects than in controls. After i.v. glucose administration, plasma glucose increased significantly in both groups and the glucose peak was higher in T2DM subjects than in controls (9.67 ± 1.25 (S.D.) vs 6.88 ± 1.00 mmol/l, P < 0.01). Insulin increased rapidly in controls, while in T2DM subjects, plasma insulin did not rise in the first 10 min. After the glucose bolus, plasma ghrelin showed a significant reduction both in controls and in T2DM subjects after 5 min.

Conclusion: These findings indicate that a low-dose i.v. glucose bolus reduces ghrelin both in controls and in T2DM subjects and therefore that early insulin response does not affect plasma ghrelin.

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Introduction
Ghrelin is a 28 amino acid peptide identified as the endogenous ligand for the growth hormone (GH) secretagogue receptor (1). Produced predominantly by the stomach, ghrelin acts as a potent stimulator of GH secretion (2), but it may also play a role in different aspects of food intake and energy balance control.

In humans and rats, plasma ghrelin increases during fasting and falls after meals (3–5), showing therefore a nutritional modulation opposite to that of insulin. Post-prandial ghrelin variations seem independent of gastric distension (3, 6) and they are probably regulated by metabolic and endocrine responses to meals. In healthy subjects it has been suggested that both oral and i.v. glucose decrease ghrelin rapidly, within 30 and 10–15 min respectively (6–8). It has also been suggested that exogenous insulin administration reduces plasma ghrelin independently of plasma glucose concentrations (9–12), but some studies did not confirm the influence of insulin on ghrelin (13, 14). The independent role of glucose and insulin on ghrelin secretion in humans is still controversial. Since in healthy subjects it is difficult to isolate the increase of glucose from the increase of insulin, we thought to compare plasma ghrelin concentrations after a small i.v. glucose bolus between a group of healthy control volunteers and a group of subjects with type 2 diabetes mellitus (T2DM) and mild fasting hyperglycaemia, in whom the early insulin response to i.v. glucose is abolished or greatly blunted (15). In this condition it should be possible to discriminate the effect of glucose increase alone and early insulin response on ghrelin secretion. Here we show that i.v. glucose reduces ghrelin also in the absence of early insulin secretion.

Subjects and methods
The study included seven healthy subjects (four men and three women; mean age ± s.d. 48.7 ± 11.3 years; mean body mass index (BMI) ± s.d. 23.0 ± 1.9 kg/m²) and eight patients with T2DM (five men and three women; age 52.2 ± 9.9 years; BMI 24.3 ± 3.2 kg/m²). Diabetes mellitus was defined according to American Diabetes Association criteria as fasting plasma glucose
> 7 mmol/l or an oral glucose tolerance test with 2 h post-load plasma glucose > 11.1 mmol/l. The healthy subjects had normal physical examinations, routine blood examinations, thyroid function tests and a stable body weight for at least 3 months prior to the study. Among the T2DM patients, five were treated with diet and exercise, one with metformin and one with netaglinide; all of them had good glycaemic control (mean glycosylated haemoglobin 5.4%) and they did not present any microvascular and macrovascular diabetic complications or evidence of gastrointestinal disease, cancer, thyroid disorders or infections. The study was approved by the local Ethics Committee and all subjects gave informed consent.

Subjects were admitted to the Clinical Research Centre in the morning at 0830–0900 h after an overnight fast. An i.v. catheter was inserted into an antecubital vein and the i.v. line was kept open by slow infusion of isotonic saline. This line was used for blood sampling. Subjects received 5 g/25 ml glucose i.v. in 60 s. Blood samples were collected at 0, 3, 5, 10, 30, 45 and 60 min after the glucose injection to assay ghrelin, insulin and glucose concentrations.

Plasma immunoreactive ghrelin levels were measured using a commercial RIA that uses 125I-labelled bioactive ghrelin as a tracer and a rabbit polyclonal antibody raised against full-length octanoylated human ghrelin (Phoenix Pharmaceuticals, Belmont, CA, USA) that recognised both acylated and desacylated ghrelin (intra-assay coefficient of variation 8.7%). Plasma insulin was determined using a commercial IRMA (Tecnogenetics, Milan, Italy). Glucose levels were determined by the glucose oxidase method (Sigma Diagnostics). All samples from a single subject were run in duplicate in the same assay.

Data are presented as means ± S.D. The area under the curve (AUC) values were calculated using the trapezoidal rule. As the data were not normally distributed, statistical analysis was performed with a Mann–Whitney U test or Friedman test followed by Wilcoxon test. Values of P < 0.05 were considered statistically significant.

**Results**

There was no significant difference between healthy controls and patients with T2DM in BMI (controls 23.0 ± 1.9 vs T2DM subjects 24.3 ± 3.2 kg/m², P = NS), basal plasma insulin (50.9 ± 12.1 vs 67.8 ± 38.9 pmol/l, P = NS) and basal plasma ghrelin (189.8 ± 78.0 vs 133.6 ± 93.5 fmol/ml, P = NS). Basal plasma glucose in T2DM subjects was significantly higher than in controls (6.83 ± 0.95 vs 4.05 ± 0.31 mmol/l, P < 0.01) (Table 1). After i.v. glucose administration, in T2DM subjects plasma glucose reached a higher peak than in controls (9.67 ± 1.25 vs 6.88 ± 1.00 mmol/l, P < 0.01) (Fig. 1A) and the 10 min glucose AUC was greater (AUC_{0–10 min} 87.13 ± 13.46 vs 61.72 ± 6.28 mmol/l per 10 min, P < 0.01). The glucose increment, calculated as Δ glucose 0–3 min, was not different between T2DM subjects and controls (2.86 ± 0.59 vs 2.92 ± 1.02 mmol/l, NS).

**Table 1** Characteristics of healthy control subjects and T2DM patients. Data are means ± S.D.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>T2DM</th>
<th>P</th>
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<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>23.0 ± 1.9</td>
<td>24.3 ± 3.2</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.2 ± 10.0</td>
<td>52.0 ± 8.0</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>4.05 ± 0.31</td>
<td>6.83 ± 0.95</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>50.9 ± 12.1</td>
<td>67.8 ± 38.1</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting ghrelin (fmol/ml)</td>
<td>189.8 ± 78.0</td>
<td>133.6 ± 93.5</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose increment 0–3 min (mmol/l)</td>
<td>2.92 ± 1.02</td>
<td>2.86 ± 0.59</td>
<td>NS</td>
</tr>
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</table>
Glucose disappearance was faster in controls than in T2DM subjects (t1/2 glucose 40 vs 65 min). In healthy subjects, glucose induced a rapid increase of plasma insulin concentration from 50.9±12.1 to 225.7±65.0 pmol/l (P < 0.01), while in T2DM subjects, as expected, plasma insulin did not rise significantly during the 60 min (Fig. 1B). The 10 min insulin AUC was significantly greater in control subjects than in T2DM subjects (AUC0–10 min 1588.6±621.1 vs 620.3±238.7 pmol/l per 10 min, P < 0.01). After i.v. glucose administration, plasma ghrelin significantly decreased in controls and T2DM subjects at 5 min, reaching a nadir of −14.1% and −17.5% respectively (P < 0.05), and then it returned slowly to basal levels (Fig. 1C).

**Discussion**

The present data show that 5 g glucose i.v. is able to reduce ghrelin concentrations both in patients with T2DM and in control subjects. Since i.v. glucose in healthy subjects induces a rapid and transient insulin increase that is absent in T2DM, we concluded that insulin does not acutely regulate plasma ghrelin.

In our study, as expected, i.v. glucose induced in control subjects a peak in insulin concentrations during the first 10 min (early insulin response). Intravenous glucose decreased plasma ghrelin in controls and T2DM subjects, in whom the early insulin response was absent. Therefore, this increment in plasma insulin did not affect ghrelin concentrations. Our data are in accordance with those of Caixas et al. (14), who reported that s.c. administration of pulse of a short-acting insulin analogue did not affect plasma ghrelin. Moreover, Schaller et al. (13) did not find a role of insulin at physiological concentrations in the regulation of plasma ghrelin, although they did not study periods shorter than 30 min. In contrast, other studies using hyperinsulinaemic clamp techniques reported that insulin infusion reduces ghrelin concentration (9–12) and these inhibitory effects seem independent of glucose concentrations. In these studies, insulin was infused i.v. for a period between 10 and 210 min and insulinopenia was maintained at concentrations like postmeal ones or greater. Instead, we induced an endogenous insulin secretion that caused lower plasma insulin concentrations and had a rapid decrease. It is possible that a more sustained or protracted hyperinsulinaemia, rather than a short-lived burst like the early insulin response, is required for the inhibition of plasma ghrelin.

It has been suggested that glucose has an inhibitory effect on ghrelin concentration, because ghrelin decreased after oral and i.v. glucose (6–8). However, glucose administration increases insulin, which could mediate or contribute to the inhibitory effect of glucose on plasma ghrelin. In our study it has been possible to observe the effect of hyperglycaemia alone on plasma ghrelin. In fact, in diabetic subjects, i.v. glucose induced hyperglycaemia but it did not increase plasma insulin. In these conditions we observed in T2DM subjects a significant reduction in plasma ghrelin after i.v. glucose bolus, suggesting that hyperglycaemia per se could play a role in ghrelin regulation.

We infused a small i.v. glucose bolus that induced a similar increase in plasma glucose both in controls and in T2DM subjects independently of different basal glycaemia. This little glucose increase induced a small but significant decrease in plasma ghrelin in both groups, suggesting that glucose increment reduces ghrelin independently of basal glucose concentration.

In conclusion, the present study demonstrates that in healthy subjects and in subjects with T2DM a low-dose i.v. glucose bolus reduces plasma ghrelin concentrations. These data exclude a role of the early insulin response in postprandial ghrelin reduction.

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