Hyperglycaemia suppresses the secretion of ghrelin, a novel growth-hormone-releasing peptide: responses to the intravenous and oral administration of glucose

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ABSTRACT

Ghrelin is a novel growth hormone (GH)-releasing peptide, isolated from the stomach, which may also cause a positive energy balance by stimulating food intake and reducing fat utilization. However, whether glucose influences the release of ghrelin remains unknown. Accordingly, we examined circulating levels of ghrelin and GH in response to the intravenous or oral administration of 50 g of glucose in eight healthy humans. After the administration of intravenous glucose (50 g), the plasma ghrelin level decreased significantly from 127 ± 9 to 98 ± 9 fmol/ml (P < 0.01), associated with an increase in plasma glucose from 85 ± 3 to 357 ± 19 mg/dl (P < 0.01). Ingestion of 50 g of glucose decreased the plasma ghrelin level significantly from 134 ± 12 to 97 ± 15 fmol/ml (P < 0.01), associated with an increase in plasma glucose from 93 ± 3 to 166 ± 10 mg/dl (P < 0.01). The decrease in the plasma ghrelin level lasted for more than 30 min after recovery of the plasma glucose level. In conclusion, ghrelin secretion may be suppressed, at least in part, by an increased plasma glucose level in healthy humans.

INTRODUCTION

Ghrelin is a novel growth hormone (GH)-releasing peptide, isolated from the stomach, which has been identified as an endogenous ligand for the GH secretagogue receptor [1]. Interestingly, ghrelin has been shown to cause a positive energy balance by decreasing fat utilization through GH-independent mechanisms [2]. In addition, both intracerebroventricular and peripheral administration of ghrelin have been shown to elicit potent, long-lasting stimulation of food intake via activation of neuropeptide Y neurons in the hypothalamic arcuate nucleus [3–5]. These findings raise the possibility that ghrelin plays an important role in the regulation of metabolic balance. However, whether ghrelin secretion is influenced by circulating levels of glucose, an important energy source, remains unknown.

On the other hand, the serum GH level is known to be suppressed by hyperglycaemia in humans. Since ghrelin strongly stimulates GH release, ghrelin may be involved in hyperglycaemia-induced GH suppression. Accordingly, we sought to investigate whether the intravenous and oral administration of 50 g of glucose influences the plasma ghrelin level in association with a decrease in serum GH levels in healthy humans.

Key words: ghrelin, glucose, growth hormone, hyperglycaemia.

Abbreviations: GH, growth hormone; GHRH, GH-releasing hormone; TFA, trifluoroacetic acid.

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MATERIALS AND METHODS

Subjects
We studied eight healthy men of normal height and body mass index (22 ± 2 kg/m²), aged 27–35 years (mean ± S.D. 31 ± 3 years). None of them had any history of cardiovascular, renal, respiratory, hepatic or metabolic disease. They had not been taking any medications, showed no abnormalities on physical examination or screening biochemical analysis, and had complete blood count findings. Subjects were requested not to consume sodium- or glucose-rich food, caffeine, alcohol, non-steroidal anti-inflammatory agents or nicotine for several hours before the experiments. The study was approved by the ethical committee of the National Cardiovascular Centre, and all subjects signed an informed consent document before participation.

Study protocol
The study was composed of three procedures: intravenous administration of glucose or placebo, and oral administration of glucose. Thus subjects were studied on three separate days, at least 1–2 weeks apart, in a randomized, cross-over fashion. The studies were performed after an overnight fast. The subjects rested in bed quietly early in the morning and were awake during the studies. A 17-gauge catheter was inserted into an ante-cubital vein for blood sampling. Another 17-gauge catheter was inserted for administration of glucose or placebo. After an equilibration period of 30 min, blood sampling was performed for baseline measurements of glucose, GH, serum insulin and ghrelin. Then 50 g of glucose or placebo (0.9 % saline) was administered intravenously over 2 min at a constant rate. Alternatively, 50 g of glucose dissolved in 150 ml of water was administered orally over 2 min. Blood sampling was repeated until 150 min after glucose or placebo loading.

Blood sampling and assay for plasma ghrelin
Blood samples were transferred immediately into chilled glass tubes containing disodium EDTA (1 mg/ml) and aprotinin (500 units/ml) and centrifuged immediately at 4 °C, and the resulting plasma samples were frozen and stored at −20 °C. Plasma samples were extracted before RIA. Briefly, Sep-Pak C18 cartridges (Waters, Milford, MA, U.S.A.) were preconditioned with 5 ml each of chloroform, methanol, 60 % (v/v) acetonitrile containing 0.1 % trifluoroacetic acid (TFA), and saline. Plasma (1 ml) was diluted with 1 ml of saline, and then loaded on to a Sep-Pak C18 cartridge. After the column had been washed with 5 ml each of saline and 5 % acetonitrile containing 0.1 % TFA, the absorbed materials were eluted with 3 ml of 60 % acetonitrile containing 0.1 % TFA. The eluate was then lyophilized.

RIA for plasma ghrelin was performed as described previously [5]. In brief, a polyclonal antibody was raised against the C-terminal fragment (residues 13–28) of rat ghrelin in a rabbit. A maleimide-activated mariculture keyhole-limpet-haemocyanin–[Cys⁸]ghrelin-(13–28) conjugate was used for immunization. Rat [Tyr⁸]ghrelin-(13–28) was radioiodinated by the lactoperoxidase method. A moniodinated ligand was purified by reverse-phase HPLC on a µBondosphere C18 column (3.9 mm × 150 mm; Waters). The tracer was stable for 3 months when stored at −20 °C in 0.1 % BSA. The RIA incubation mixture consisted of 100 µl of standard ghrelin or unknown sample, normal rabbit serum and 200 µl of antiserum at a dilution of 1:10000. After a 12 h incubation at 4 °C, 100 µl of 125I-labelled ligand (15000 c.p.m.) was added to the mixture. After a 36 h incubation at 4 °C, 100 µl of goat anti-(rabbit IgG) serum was added. Free and bound tracers were separated by centrifugation at 12000 × g for 30 min after incubation for 24 h at 4 °C. After aspiration of the supernatant, radioactivity in the pellet was quantified using a γ-radiation counter (ARC-600; Aloka, Tokyo, Japan). The minimum detectable concentration of ghrelin was < 6 fmol/tube. The antisera exhibited 100 % cross-reactivity with rat or human ghrelin-(13–28). No significant cross-reactivity with other peptides was observed. The intraobserver variability for measurement of ghrelin was < 6 %, and the interobserver variability was < 9 %. Day-to-day variation in plasma ghrelin levels in normal subjects was < 9 %.

Other biochemical measurements
The serum GH level (ng/ml) was measured with an immunoradiometric assay kit (Ab Beads HGH Eiken; Eiken Chemical Co., Ltd, Tokyo, Japan). The sensitivity of the assay was 0.1 ng/ml. The inter- and intra-assay coefficients of variation were < 10 % and < 6 % respectively. The plasma glucose level was determined by the glucose oxidase method. Serum insulin was determined by immunoradiometric assay (Insulin-Riabead; Dainabot Co., Ltd, Chiba, Japan).

Statistical analysis
All data were expressed as means ± S.E.M. unless otherwise indicated. Comparisons of the time courses of parameters between the two groups were made by two-way ANOVA for repeated measures, followed by the Newman–Keuls test. A P value of < 0.05 was considered to indicate statistical significance.

RESULTS
The intravenous injection of 50 g of glucose induced marked hyperglycaemia, with a peak plasma glucose level of 357 ± 19 mg/dl at 15 min. The plasma glucose
Decreased plasma ghrelin in hyperglycaemia

Figure 1 Plasma glucose levels following the oral or intravenous administration of 50 g of glucose, or intravenous placebo, in healthy volunteers
Values are means ± S.E.M. Significance of differences: *P < 0.05, †P < 0.01 compared with the placebo group.

Figure 2 Ghrelin responses to a single intravenous dose of 50 g of glucose or placebo in healthy volunteers
Values are means ± S.E.M. Significance of differences: *P < 0.05, †P < 0.01 compared with the placebo group.

level returned gradually to the normal range by 75 min (Figure 1). The injection of glucose significantly decreased the plasma ghrelin level from 127 ± 9 to 98 ± 9 fmol/ml (P < 0.01; Figure 2). The decrease in the plasma ghrelin level was observed from 15 min to 105 min after glucose administration. Thus the decrease lasted for more than 30 min after recovery of the plasma glucose level. Hyperglycaemia significantly decreased the serum GH level (from 0.40 ± 0.12 to 0.10 ± 0.01 ng/ml; P < 0.05) and increased the serum insulin level (4.8 ± 0.8 to 10.3 units/ml; P < 0.01).

The oral administration of 50 g of glucose also induced hyperglycaemia, with a peak plasma glucose level of 166 ± 9 mg/dl at 45 min. The plasma glucose level began to increase at 15 min, and then returned gradually to the normal range by 120 min (Figure 1). Oral glucose loading significantly decreased the plasma ghrelin level from 134 ± 12 to 97 ± 15 fmol/ml (P < 0.01; Figure 3). The decrease in the plasma ghrelin level started at 30 min, when the plasma glucose level was increased slightly (113 ± 13 fmol/ml), and lasted until 150 min after glucose ingestion. Thus the decrease in the plasma ghrelin level lasted for more than 30 min after recovery of the plasma glucose level (Figure 3). Hyperglycaemia significantly decreased the serum GH level (from 0.47 ± 0.20 to 0.15 ± 0.03 ng/ml; P < 0.05).

DISCUSSION
In the present study, we have demonstrated that both the intravenous and the oral administration of glucose decreased the plasma ghrelin level in healthy humans, associated with a decrease in the serum GH level.

Recent studies have shown that food intake decreases plasma ghrelin levels [6,7]. In contrast, the fasting state stimulates ghrelin synthesis and increases plasma ghrelin levels [7,8]. Tschop et al. [2] have demonstrated that oral administration of glucose decreased plasma ghrelin levels in rats. The present study also showed that oral administration of 50 g of glucose markedly decreased the plasma ghrelin level in healthy humans. However, it still remains unknown whether the plasma ghrelin level is influenced by the circulating glucose level. Accordingly, in the present study, 50 g of glucose was administered intravenously to healthy humans. Like the oral ingestion of glucose, the intravenous administration of glucose decreased plasma ghrelin levels in association with an increase in glucose levels. These results suggest that the decrease in the plasma ghrelin level may be attributable, at least in part, to hyperglycaemia. The serum insulin level increased after intravenous glucose administration. This result raises the possibility that plasma ghrelin levels may be decreased by insulin. On the other hand, a recent
experimental study demonstrated that administration of insulin increased ghrelin mRNA levels in the stomach [8]. Further studies are necessary to investigate whether insulin is a regulator of ghrelin.

Peripheral administration of ghrelin has been reported to decrease fat utilization and increase carbohydrate utilization [2]. Ghrelin also elicits a potent, long-lasting stimulation of food intake via activation of neuropeptide Y neurons in the hypothalamic arcuate nucleus [4]. These findings suggest that ghrelin induces a positive energy balance, and raise the possibility that decreased plasma ghrelin levels during hyperglycaemia may represent a compensatory mechanism under conditions of a positive energy balance.

Acute hyperglycaemia is known to suppress both basal GH release [9,10] and GH secretion induced by GH-releasing hormone (GHRH) in healthy humans [11]. It was suggested that suppression of GHRH secretion and/or stimulation of somatostatin secretion are involved in the mechanism by which acute hyperglycaemia suppresses GH secretion. However, because of the difficulty in measuring GHRH and somatostatin levels in portal blood, the precise mechanism behind the hyperglycaemia-induced decrease in GH levels remains unclear. The present study has demonstrated that acute hyperglycaemia significantly decreases plasma ghrelin levels in association with a decrease in serum GH levels. Since ghrelin markedly stimulates GH secretion, comparable with the effect of GHRH, hyperglycaemia-induced suppression of GH may be attributable partly to the decrease in plasma ghrelin.

In conclusion, we have shown that ghrelin secretion may be suppressed, at least in part, by increased plasma glucose levels in healthy humans.

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