

## RAPID COMMUNICATION

## Ghrelin-induced growth hormone secretion in humans

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### Abstract

Ghrelin is a novel growth hormone (GH) releaser acylated peptide that has recently been purified from stomach, and which potently binds to the GH secretagogue receptor. Ghrelin releases GH *in vitro* and *in vivo* in animal models, however its actions, potency and specificity in humans are unknown. In the present study, 12 healthy subjects were studied: 6 underwent four tests with ghrelin administered i.v. at the dose of 0 (placebo), 0.25, 0.5 and 1 µg/kg which corresponds to 0, 18, 37 and 75 µg total dose. A further 6 volunteers underwent two tests on different days with ghrelin at the dose of 3.3 or 6.6 µg/kg which corresponds to 250 µg and 500 µg total dose.

Ghrelin-mediated GH secretion showed a dose–response curve, in which 1 µg/kg was the minimally effective dose in some individuals, but not as a group. On the contrary, the total doses of 250 µg and 500 µg elicited a powerful GH secretion, with a mean peak of  $69.8 \pm 9.2$  µg/l and  $90.9 \pm 16.9$  µg/l respectively, and areas under the curve of  $4435 \pm 608$  and  $6125 \pm 1008$  µg/l per 120 min respectively. All of them statistically significant vs placebo and vs the 1 µg/kg dose.

Ghrelin administration also elicited a relevant dose–response mediated prolactin secretion suggesting no specificity of its actions. No relevant side effects were observed with ghrelin apart from a hyperhydrosis episode in two individuals tested with the higher ghrelin doses.

In conclusion, ghrelin is a potent releaser of GH in normal individuals, with a dose–response pattern of operation. No saturating dose was observed.

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### Introduction

The widely held view that growth hormone (GH) secretion is regulated by the interplay of GHRH and somatostatin has been placed in question by the insurgence of growth hormone secretagogues (GHS) into the field of endocrinology (1). GHS, of which the most emblematic compound is the growth hormone releasing hexapeptide, GHRP-6, are artificial compounds invented by Bowers' group, long before the discovery of GHRH, in order to have useful GH releasers as clinical and experimental tools (2). After thorough testing, it became clear that these compounds were a new physiological system involved in GH regulation, and that they were independent from GHRH or somatostatin (1). This view was confirmed after the cloning of the receptor for GHS (3). Interestingly, the site of action of GHRP-6 and similar compounds are both the hypothalamus and the pituitary (4), and their role in regulating GH secretion is far from understood. In any case, the complexity of GH regulation which clearly exceeds that of any other pituitary hormone (5),

has long ago suggested that new unknown regulatory factors are implicated in this function. The availability of biological functions exerted by GHS and an orphan receptor able to bind these artificial compounds made it clear that an endogenous factor able to activate the GHS-receptor should exist (6, 7).

The cloning and partial characterization of an 3300 molecular weight endogenous ligand for the GHS-receptor, called ghrelin, has been recently reported (8). Surprisingly, this new peptide was isolated not from the hypothalamus, but from the stomach; secondly it was an acylated peptide representing a new type of molecular structures (9). Ghrelin stimulates rat GH secretion *in vitro* and *in vivo* in anaesthetised animals (8), which suggests that ghrelin may well be the expected third factor involved in GH regulation. As this new peptide circulates in normal subjects at considerable plasma concentrations, it has been postulated that ghrelin is secreted by the stomach, and that it circulates in plasma to stimulate pituitary and hypothalamic structures involved in the somatotrope cell function. In any

case, the physiology and physiopathology of this compound have yet to be precisely assessed.

The present work studies the capability of ghrelin for releasing GH in humans, as well as its specificity.

## Subjects and methods

Twelve normal, healthy male volunteers, aged  $26.2 \pm 1.1$  years (range 21–35 years) took part in this study after providing informed consent. All of them had normal lifestyles, were taking no medication, and were within 10% of their ideal body weight (BMI  $24.0 \pm 0.4$ ). The study was approved by an independent ethical committee. Six subjects were tested with the administration of ghrelin (National Cardiovascular Center Research Institute, Osaka), prepared as described (8) at four different doses, 0 (placebo), 0.25, 0.5 and  $1.0 \mu\text{g}/\text{kg}$  on four different days. Six different subjects were tested with ghrelin at either  $250 \mu\text{g}$  or  $500 \mu\text{g}$  total dose, equivalent to  $3.3 \mu\text{g}/\text{kg}$  and  $6.6 \mu\text{g}/\text{kg}$  respectively, on two different occasions.

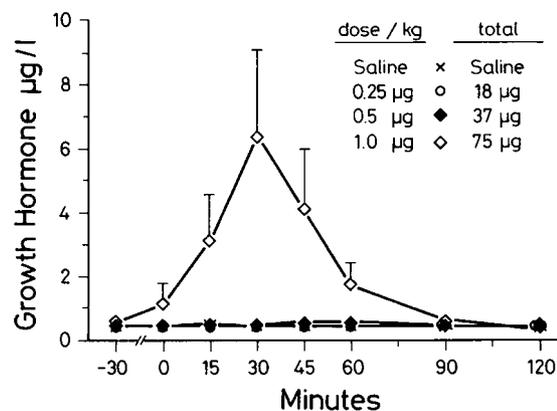
Tests were performed at 0800 h with the subjects recumbent after an overnight fast, an indwelling catheter was placed in a forearm vein and kept permeable with a slow infusion of  $150 \text{ mmol}/\text{l}$  of NaCl. Two basal blood samples were obtained at  $-30$  and  $0$  min and ghrelin as GH stimulus was administered at  $0$  min, the additional blood samples were obtained at 15, 30, 45, 60, 90 and 120 min. After centrifugation, plasma was kept at  $-20^\circ\text{C}$  until assay.

Serum GH concentrations were determined by immunoenzymometric assay (IEMA, RADIM SpA, Italy) with a sensitivity of  $0.1 \mu\text{g}/\text{l}$  and coefficients of variation of 5.6% ( $3.2 \mu\text{g}/\text{l}$ ), 5.6% ( $8.9 \mu\text{g}/\text{l}$ ) and 4.7% ( $19.2 \mu\text{g}/\text{l}$ ). Serum prolactin (PRL) was measured by Automated Chemiluminescence System (Chiron Diagnostics Corporation, East Walpole, MA, USA) with a sensitivity of  $0.3 \mu\text{g}/\text{l}$ , and coefficients of variation of 2.5% ( $2.7 \mu\text{g}/\text{l}$ ), 2.8% ( $34.0 \mu\text{g}/\text{l}$ ) and 3.8% ( $121 \mu\text{g}/\text{l}$ ).

Results are presented as absolute values (means  $\pm$  s.e.) and the areas under the secretory curve (AUC) were calculated by a trapezoidal method. Groups were compared by the Mann–Whitney non-parametric test and ANOVA. The statistical level of significance was set at  $P < 0.05$ .

## Results

In the dose–response study performed, ghrelin at the dose of 0.25, 0.5 and  $1.0 \mu\text{g}/\text{kg}$  (18, 37 and  $75 \mu\text{g}$  total dose) elicited a peak GH secretion of  $0.5 \pm 0.007$ ,  $0.6 \pm 0.09$  and  $6.5 \pm 2.6 \mu\text{g}/\text{l}$  respectively that was not significantly different than that observed in the placebo group ( $0.6 \pm 0.08 \mu\text{g}/\text{kg}$ ) (Fig. 1). Examined on an individual basis, the dose of  $1 \mu\text{g}/\text{kg}$  stimulated GH release in some subjects but was inconsistent and some subjects did not respond at all. In order to find a more robust response, control subjects were tested with

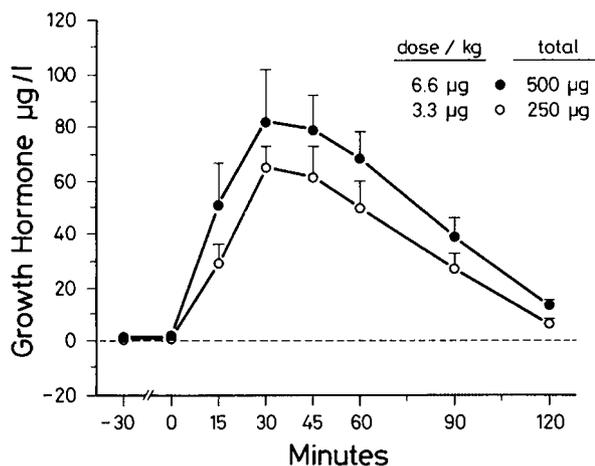


**Figure 1** Mean  $\pm$  s.e. of GH secretion after the administration of different doses of ghrelin at time 0 min.

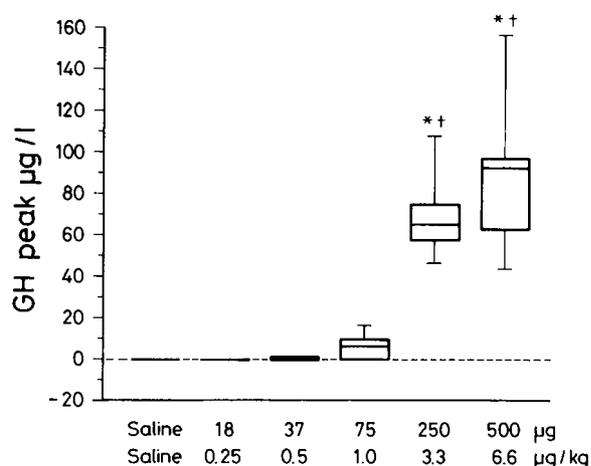
ghrelin at the dose of 250 and  $500 \mu\text{g}$  total dose ( $3.3$  and  $6.6 \mu\text{g}/\text{kg}$  respectively). As Fig. 2 shows, a clear-cut GH response was obtained with GH mean peaks of  $69.8 \pm 9.2 \mu\text{g}/\text{l}$  and of  $90.9 \pm 16.9 \mu\text{g}/\text{l}$  respectively. These higher ghrelin doses elicited a robust GH release significantly higher than the placebo ( $P < 0.005$ ) and than the  $1 \mu\text{g}/\text{kg}$  dose ( $P < 0.005$ ), considering either the mean peak (Fig. 3), and the AUC (Fig. 4), due to a prolonged secretion across time.

Ghrelin also elicited a clear-cut dose-dependent increase in PRL (Fig. 5), with a peak of  $8.3 \pm 1.1 \mu\text{g}/\text{kg}$  after placebo and  $14.3 \pm 6.6$ ,  $24.8 \pm 8.5$  and  $35.0 \pm 1.1 \mu\text{g}/\text{kg}$  after the administration of a total dose of 75, 250 and  $500 \mu\text{g}$  of ghrelin. The two higher doses elicited a significant ( $P < 0.05$ ) secretion of PRL compared with saline.

No relevant side effects were observed with ghrelin, and pulse rate and blood pressure were not altered under any dose. Two subjects showed a marked episode of perspiration in the upper part of the trunk after the



**Figure 2** Mean  $\pm$  s.e. of GH secretion after the administration of ghrelin at the total dose of either  $250 \mu\text{g}$  or  $500 \mu\text{g}$ .

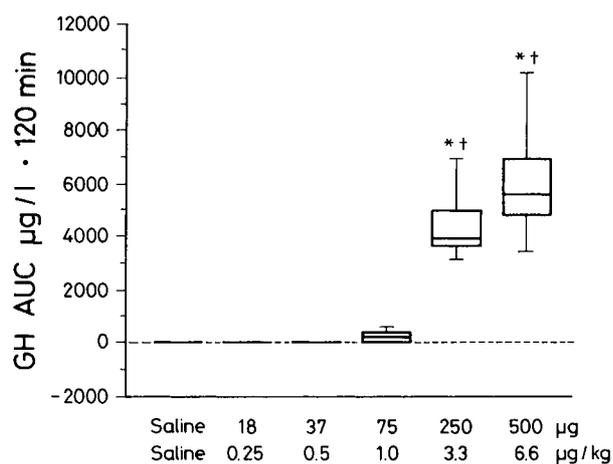


**Figure 3** Box plot of the mean GH peak elicited by different doses of ghrelin compared with placebo (saline). The error bars represent the 10–90% range, the box represents the median. \* $P < 0.005$  vs saline; † $P < 0.005$  vs the 1 µg/kg dose.

250 and 500 dose that lasted nearly 60 min. No increase in appetite was observed after ghrelin administration.

## Discussion

In collaboration with GHRH and somatostatin, ghrelin may well be the third peptidergic factor involved in GH regulation. Although its receptor is well characterized and several of its putative functions have already been described using its artificial surrogate GHRP-6, the physiology of this new regulatory system can only be addressed now after its isolation. In any case, certain facts concerning ghrelin are relevant: a) it was isolated using the GHS receptor as bioassay; b) it releases GH in



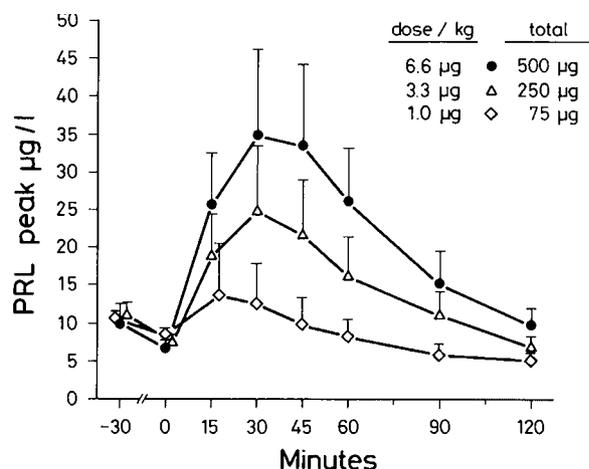
**Figure 4** Box plot of the GH area under the curve (AUC) elicited by different doses of ghrelin compared with placebo (saline). The error bars represent the 10–90% range, the box represents the median. \* $P < 0.005$  vs saline; † $P < 0.005$  vs the 1 µg/kg dose.

rat dispersed anterior pituitary cells and in anaesthetised rats, c) it was isolated from stomach, a fact that need further clarification and d) it possesses a peculiar structure because of its acylated chain. Whether ghrelin is the ligand or just one of the ligands of the GHS receptor will require further studies. By the same token, if the GHS-receptor used for its isolation is the only one or if it represents one of a kind will need to be clarified in the future (9).

A step toward the understanding of its physiological role has been the report that ghrelin is able to release GH also in freely moving rats with a greater potency than GHRH (10), a fact that provides further evidence that it may play an important role in the physiological control of GH secretion. Recently, we have provided preliminary evidence that ghrelin was able to release GH in humans (11), however a thorough evaluation of the potency, selectivity and interactions of this compound is still required.

In the present work it was shown that ghrelin is a potent releaser of GH in normal subjects with a dose-dependent pattern. Since the 1 µg/kg dose produced a very variable secretion, with several subjects not responding at all and others providing a mild response, we studied two larger doses of 250 and 500 µg total dose, equivalent to 3.3 and 6.6 µg/kg respectively. These doses elicited a powerful release of GH in all the individuals tested, and their potency was evident not only in the GH peak but also in the area under the curve. In fact the GH release was highly sustained over time. These results unambiguously demonstrate that ghrelin stimulates GH secretion in a dose-dependent manner, the most effective dose being 250 µg and that saturation was not observed, keeping open the possibility that larger doses may be used clinically, providing adequate tolerance. This was a relevant difference as for GHRH 1 µg/kg is the maximal saturating dose and further increments are not effective (12), while a saturating dose or plateau was not observed for either GHRP-6 or ghrelin. It is evident that ghrelin appears to be highly potent, probably being the strongest GH secretagogue up to date when administered alone. Whether this stronger potency is due to a higher affinity for the GHS-receptor or to a prolonged plasma half-life remains to be established.

It has been reported that GHS are not selective for GH and that they release both PRL and ACTH/cortisol in significant amounts (13, 14). In the present work, ghrelin was shown to release PRL in a dose-related manner, where the release is significantly higher than that after placebo at 250 and 500 µg total dose. This partial lack of specificity of ghrelin comes as no surprise if one considers that similar observations have been reported with GHRP-6, hexarelin and with non-peptidyl compounds. Whether these lateral actions are maintained after repetitive administration of ghrelin and their relevance to a future clinical application of this compound will need further clarification.



**Figure 5** Mean  $\pm$  S.E. of prolactin secretion after the administration of different doses of ghrelin at time 0 min.

The saga of GH secretagogues is coming to a conclusion. Bowers and co-workers invented a peptide, (it was not discovered as it does not exist in nature), and later on they postulated that GHRP-6 and similar compounds constituted a new physiological system involved in GH regulation, a bold hypothesis that was received with resounding scepticism by the experts in the field. However, the cloning of the GHS-receptor and recently the isolation and characterization of ghrelin would seem to lend a great deal of credence to Bowers hypothesis, and it seems probable that GH secretion is regulated not by two, but at least by three peptidergic hormones (15). The detailed interplay among them will require extensive work in the coming years but, in any case, the presence of the GHS receptor in a fossil like the puffer fish (16) and the high presence of ghrelin in stomach (1) convincingly suggest that ghrelin will provide us not only with physiological, but also with data on the evolution of the somatotroph system. The presence of ghrelin in placental tissue and its relevant changes through pregnancy in both humans and rat models (17) are contributing data to the overall evaluation of a peptide that may soon be considered a new hormone.

In conclusion, ghrelin is a powerful non-selective GH releaser in humans and at doses over 3  $\mu$ g/kg appears as the most potent GH secretagogue to date. Its role in the physiology of GH regulation and its clinical implication await further assessment.

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