The Endocrine Response to Ghrelin as a Function of Gender in Humans in Young and Elderly Subjects

FABIO BROGLIO, ANDREA BENSO, CARLOTTA CASTIGLIONI, CRISTINA GOTTERO,
FLAVIA PRODAM, SILVIA DESTEFANIS, CARLOTTA GAUNA, AART JAN van der LELY,
ROMANO DEGHENGHI, MARIO BO, EMANUELA ARVAT, and EZIO GHIGO

Division of Endocrinology and Metabolism, Department of Internal Medicine (F.B., A.B., C.Go., F.P., S.D., C.Ga., R.D., E.A.,
E.G.), and Division of Geriatrics, Department of Medical and Surgical Disclipines (C.C., M.B.), University of Turin, 10126
Turin, Italy; and Division of Endocrinology and Metabolism, Department of Internal Medicine (A.J.v.d.L.), Erasmus
University of Rotterdam, 3015 Rotterdam, The Netherlands

Ghrelin modulates somatotroph, lactotroph, corticotroph, and insulin secretion and glucose metabolism. To clarify the influence of gender and age on the endocrine actions of ghrelin in humans, we studied the effects of ghrelin (1.0 μg/kg iv) or placebo on GH, prolactin (PRL), ACTH, cortisol, insulin, glucagon, and glucose levels in 18 young subjects (YS) and 16 elderly subjects (ES) of both genders. The GH response to GHRH (1.0 μg/kg iv) was also studied. The GH response to ghrelin in YS was higher (P < 0.01) than in ES and both higher (P < 0.01) than to GHRH, without gender-related differences. In YS ghrelin also induced: 1) gender-independent increase in (P < 0.01) in PRL, ACTH, and cortisol levels; 2) gender-independent increase in glucose levels (P < 0.01); 3) decrease (P < 0.01) in insulin levels in male YS; and 4) no change in glucagon. In ES, ghrelin induced gender-independent PRL, ACTH, and cortisol responses (P < 0.01). In ES ghrelin elicited gender-independent transient decrease in insulin (P < 0.01) coupled with increase in glucose levels (P < 0.05). In conclusion, the GH-releasing effect of ghrelin is independent of gender but undergoes age-related decrease. The effect of ghrelin on lactotroph and corticotroph secretion is age and gender independent. In both ES and YS, ghrelin influences insulin secretion and glucose metabolism. (J Clin Endocrinol Metab 88: 1537–1542, 2003)

I T IS WELL KNOWN that GH secretion varies as a function of age and gender in humans (1, 2). The mechanisms underlying the age-related changes in GH secretion certainly include the influence of gonadal steroids and adiposity (1, 2). However, there is evidence that the influence of aging on the GH/IGF-I axis mainly reflects age-related changes in the neuroendocrine control of GH secretion (1, 2). In fact, GH secretion is primarily controlled by the central nervous system, and it is mainly regulated by the tight interplay between the hypothalamic neurohormones GHRH and somatostatin with the cooperation of neurotransmitters, peripheral hormones, and metabolic factors (1, 2).

The existence of another major unknown factor involved in the control of somatotroph function had been hypothesized on the basis of evidence that synthetic, nonnatural, peptidyl, and nonpeptidyl molecules named GH secretagogues (GHS) possess a strong GH-releasing effect acting on the pituitary and mainly on the hypothalamus, in which specific receptors (GHS-Rs) are present (3, 4). An endogenous ligand for the GHS-R, named ghrelin, has recently been purified from both rat and human stomach (5). Ghrelin is a 28-amino acid peptide and shows a unique structure with an n-octanoyl ester at its third serine residue that is essential for its potent stimulatory activity on somatotroph secretion. Another ligand showing the same activity has been purified from rat stomach; it is a 27-amino acid peptide named des-Gln14-ghrelin, the sequence of which is identical to ghrelin

except for one glutamine (6). Brain ghrelin immunoreactive neurons have also been localized in the hypothalamic arcuate nucleus (3, 5–7).

Ghrelin specifically stimulates GH secretion in rats from both pituitary cells in culture and in vivo (5, 6). Ghrelin releases more GH than GHRH and even more than the synthetic GHS hexarelin in humans; moreover, it synergizes with GHRH but not with hexarelin (8–11). This evidence agrees with the assumption that it exerts its effects via the activation of the GHS-R at the pituitary and mainly at the hypothalamic level, likely enhancing the activity of GHRH-secreting neurons and also acting as functional somatostatin antagonist (3, 4).

The GHS-R and its subtypes are not restricted to the hypothalamus-pituitary unit but are also present in other central and peripheral tissues (12, 13), and the activity of ghrelin as well as of synthetic GHS is not fully specific for GH. In fact, ghrelin stimulates lactotroph and corticotroph secretion, has orexigenic activity and modulates energy balance also via influence on glucose metabolism and insulin secretion, exerts cardiovascular actions, modulates cell proliferation, and regulates gastric motility and acid secretion through vagal mediation (3, 9, 11).

On the basis of studies with synthetic GHS, it had been hypothesized that the hypoactivity of the natural ligand of GHS-R could play a major role in the age-related changes of somatotroph function (3, 14). In fact, synthetic GHS have been shown able to induce considerable GH discharge in elderly subjects, but their response is reduced with regard to that in young adults (3, 15, 16). The age-related decrease in

the GH response to GHS had been surprisingly found uncoupled to the reduction in both PRL- and ACTH-releasing activity (17).

Because the endocrine activities of ghrelin as function of age and gender have never been studied so far, in the present study, we measured the GH, PRL, ACTH, cortisol, insulin, glucagon, and glucose responses to acute iv ghrelin or placebo administration in normal young adults and elderly subjects of both genders. The GH response to ghrelin was compared with that of GHRH.

Subjects and Methods

Eighteen healthy young volunteers (YS) [nine males and nine females; age (mean ± SD), 28.5 ± 8.4 yr; body mass index, 22.7 ± 3.0 kg/m^2] and 16 normal elderly subjects (ES) (eight males and eight females; age, 71.4 ± 8.1 yr; body mass index, 22.6 ± 3.9 kg/m^2) were studied. All female YS were free of contraceptive therapy and were studied in their early follicular phase; none of the female ES was on hormone replacement therapy. All subjects gave their written informed consent to participate in the study, which had previously been approved by the independent Ethical Committee of the University of Turin.

All subjects underwent the following three testing sessions in random order and at least 3 d apart: 1) placebo (2 ml isotonic saline iv at 0 min); 2) ghrelin (1.0 μg/kg iv at 0 min); and 3) GHRH-29 (1.0 μg/kg iv at 0 min).

After overnight fasting, the tests were begun in the morning at 0830–0900 h, 30 min after an indwelling catheter had been inserted in an anterocubital vein of the forearm kept patent by slow infusion of isotonic saline.

Blood samples were taken every 15 min from −15 up to +90 min. GH levels were assayed at each time point in all sessions; prolactin (PRL), ACTH, cortisol, insulin, glucagon, and glucose levels were also assayed after placebo or ghrelin administration.

Serum GH levels (micrograms per liter; 1 μg/liter = 45.4 pmol/liter) were measured in duplicate by immunoradiometric assay (IRMA) (hGH-CTK, IRMA, SORIN Biomedica, Saluggia, Italy). The sensitivity of the assay was 0.15 μg/liter. The inter- and intra-assay variation coefficients were 2.9–4.5% and 2.4–4.0%, respectively.

Serum PRL levels (micrograms per liter; 1 μg/liter = 43.5 pmol/liter) were measured in duplicate by IRMA (PRL-CTK, IRMA, SORIN Biomedica). The sensitivity of the assay was 0.15 μg/liter. The inter- and intra-assay variation coefficients ranged between 3.9% and 6.8% and between 3.3% and 7.5%, respectively.

Plasma ACTH levels (picograms per milliliter; 1 pg/ml = 0.2202 pmol/liter) were measured in duplicate by IRMA (Allegro HS-ACTH, Nichols Institute Diagnostic, San Juan Capistrano, CA). The sensitivity of the assay was 1 pg/ml. The inter- and intra-assay variation coefficients ranged between 2.4% and 8.9% and between 3.9% and 9.9%, respectively.

Serum cortisol levels (micrograms per liter; 1 μg/liter = 2.759 nmol/liter) were measured in duplicate by RIA (CORT-CTK 125, IRMA, SORIN Biomedica). The sensitivity of the assay was 4.0 μg/liter. The inter- and intra-assay variation coefficients ranged between 6.6% and 7.5% and between 3.8% and 6.6%, respectively.

Serum insulin levels (milliunits per liter; 1 mU/liter = 7.175 pmol/liter) were measured in duplicate by IRMA (INSIK-5, SORIN Biomedica). The sensitivity of the assay was 2.5 ± 0.3 mU/liter. The inter- and intra-assay coefficients of variation were 6.2–10.8% and 5.5–10.6%, respectively.

Plasma glucagon levels (micrograms per liter; 1 μg/liter = 3.57142 pmol/liter) were measured by IRMA (GLUCAGON, Biochem Immunosystems, Guidonia Montecelio, Italy). The sensitivity of the assay was 14.5 μg/liter. The inter- and intra-assay coefficients of variation were 8.2–9.0% and 8.0–9.5%, respectively.

Plasma glucose levels (milligrams per deciliter; 1 mg/dl = 0.05551 mmol/liter) were measured by glucooxidase colorimetric method (GLUCOFIX, Menarini Diagnostici, Florence, Italy).

All samples from an individual subject were analyzed together. The hormonal responses are expressed as mean ± SD Δ changes *vs.* baseline and Δ areas under curves calculated by trapezoidal integration. The

TABLE 1. Mean (±SD) GH, PRL, ACTH, cortisol, insulin, glucagon, and glucose changes after acute ghrelin (1.0 μg/kg iv) administration in young or elderly male and female normal subjects

	Young male subjects		Young female subjects		Elderly male subjects		Elderly female subjects	
	Mean Δ changes *vs.* baseline	Δ AUC	Mean Δ changes *vs.* baseline	Δ AUC	Mean Δ changes *vs.* baseline	Δ AUC	Mean Δ changes *vs.* baseline	Δ AUC
GH (μg/liter)	43.9 ± 27.3	4432.9 ± 1166.8	40.2 ± 28.6	4103.3 ± 1446.3	24.5 ± 16.9	2498.7 ± 2009.2	21.4 ± 16.3	2198.4 ± 962.9
PRL (μg/liter)	4.7 ± 3.4	480.1 ± 361.4	5.1 ± 5.0	545.7 ± 569.7	5.1 ± 2.5	503.9 ± 69.1	8.2 ± 5.7	835.3 ± 230.0
ACTH (pg/ml)	13.71 ± 23.8	1506.5 ± 2150.2	9.7 ± 15.4	1049.0 ± 1550.7	6.8 ± 10.3	761.8 ± 859.9	11.9 ± 12.3	1244.4 ± 1704.6
Cortisol (μg/liter)	15.05 ± 16.4	1607.5 ± 2394.3	4.1 ± 14.5	539.6 ± 2064.1	9.4 ± 10.9	1013.5 ± 3357.8	45.5 ± 30.5	4658.1 ± 4476.5
Insulin (mU/liter)	-2.3 ± 1.5	-222.9 ± 163.0	-0.4 ± 0.5	-42.1 ± 154.9	-2.5 ± 2.0	-254.3 ± 402.9	-3.2 ± 1.6	-313.8 ± 316.3
Glucagon (μg/liter)	-13.7 ± 8.2	-1255.6 ± 1599.5	2.3 ± 6.7	280.4 ± 1457.5	0.02 ± 3.8	24.8 ± 1901.7	-1.3 ± 5.7	-142.6 ± 895.3
Glucose (mg/dl)	6.2 ± 3.8	594.2 ± 1065.3	4.27 ± 2.9	424.7 ± 569.8	4.1 ± 2.0	380.2 ± 599.8	4.2 ± 2.3	399.4 ± 434.7

statistical analysis was carried out using nonparametric ANOVA (Friedman test) and then Mann-Whitney U test as appropriate.

Results

No significant changes in GH, PRL, insulin, and glucose levels were observed after placebo administration both in YS and ES of both genders. On the other hand, a significant ($P <$ 0.05) trend toward decrease of spontaneous ACTH, cortisol, and glucagon levels was observed.

In YS ghrelin induced strong GH increase, which was similar in both genders (Table 1 and Fig. 1).

The GH response to ghrelin was markedly higher ($P <$ 0.01) than that induced by GHRH in YS in both males (mean Δ change *vs.* baseline, 13.7 $\pm$ 8.7 μg/liter; Δ areas under curves, 1383.9 $\pm$ 1204.2 μg·min/liter) and females (11.8 $\pm$ 8.4 μg/liter; 1212.1 $\pm$ 502.1 μg·min/liter) (Fig. 1).

The GH responses to ghrelin in male and female ES were similar and both lower ($P <$ 0.05) than those in male and female YS (Table 1 and Fig. 1).

The GH responses to GHRH in male (4.0 $\pm$ 2.2 μg/liter; 397.5 $\pm$ 271.6 μg·min/liter) and female (2.3 $\pm$ 1.5 μg/liter; 230.5 $\pm$ 227.6 μg·min/liter) ES subjects were lower ($P <$ 0.01) than those in YS as well as those induced by ghrelin in ES ($P <$ 0.01) (Fig. 1).

In YS ghrelin also induced: 1) a significant ($P <$ 0.01) increase in PRL, ACTH, and cortisol levels, which was similar in both genders (Table 1 and Fig. 2); 2) a gender-independent increase ($P <$ 0.05) in glucose levels (Table 1 and Fig. 3); 3) a significant decrease ($P <$ 0.01) in insulin levels in young men only (Table 1 and Fig. 3); and 4) no change in glucagon levels (Table 1).

In ES the ghrelin-induced increase ($P <$ 0.01) in PRL levels was similar to that in YS, without significant differences between males and females (Table 1 and Fig. 2).

Similarly, the ACTH and cortisol responses ($P <$ 0.01) to ghrelin in ES were similar to those in YS and independent of gender (Table 1 and Fig. 2).

Moreover, in ES ghrelin administration elicited gender-independent transient decrease in insulin levels ($P <$ 0.01), which was coupled with significant increase in glucose levels ($P <$ 0.05) (Table 1 and Fig. 3).

Side effects

A transient facial flushing was observed after administration of GHRH in six subjects. Twelve subjects sensed a peculiar sudden increase in appetite directly following ghrelin administration.

Discussion

The results of the present study demonstrate that: 1) the GH-releasing effect of ghrelin is independent of gender but undergoes an age-related decrease; 2) the stimulatory effect of ghrelin on lactotroph and corticotroph secretion is independent of either gender or age; and 3) acute ghrelin administration increases glucose levels coupled with inhibitory influence on insulin secretion independently of age.

That acute administration of acylated ghrelin induces strong increase in GH secretion coupled with significant increase in lactotroph and corticotroph secretion (9, 11) as well as hyperglycemic effect coupled with transient insulin decrease (18) had already been shown in normal adult volunteers. This is the first study describing the endocrine response to ghrelin in humans in both genders in YS and ES.

The lack of gender-related difference in the GH response to ghrelin in YS and ES agrees with similar findings obtained studying the effects of synthetic GHS throughout life (3, 19). At puberty only, the GH response to GHS has been reported higher in females than in males, and an enhancing effect of pharmacological doses of estrogens on the GH response to GHS in postmenopausal women has been reported by some but not by other authors (3, 20–22). In fact, hypothalamic GHS receptor expression is modulated by estradiol (23). Our present findings showing that the GH response to ghrelin is independent of gender in YS and ES suggest that changes in estrogen milieu in adulthood do not affect the somatotroph response to this gastric hormone. The possibility that exposure to pharmacological levels of estrogens might affect the somatotroph response to ghrelin remains to be verified.

Our present data also fully agree with previous findings from studies with synthetic GHS showing that even the stimulator effect of ghrelin on either lactotroph or corticotroph
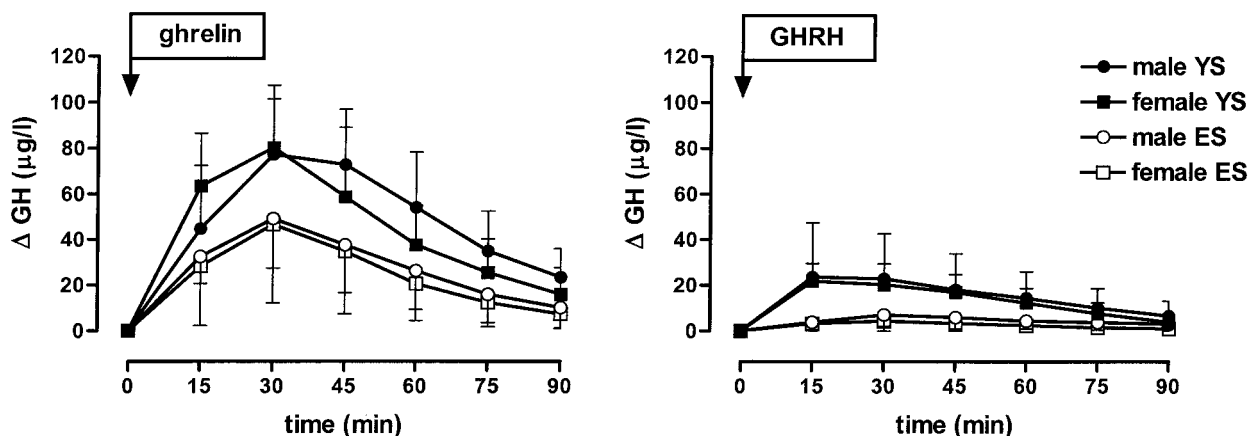

FIG. 1. Mean ($\pm$SD) Δ GH responses to acute ghrelin (1.0 μg/kg iv at 0 min) or GHRH (1.0 μg/kg iv at 0 min) administration in young or elderly male and female normal subjects.

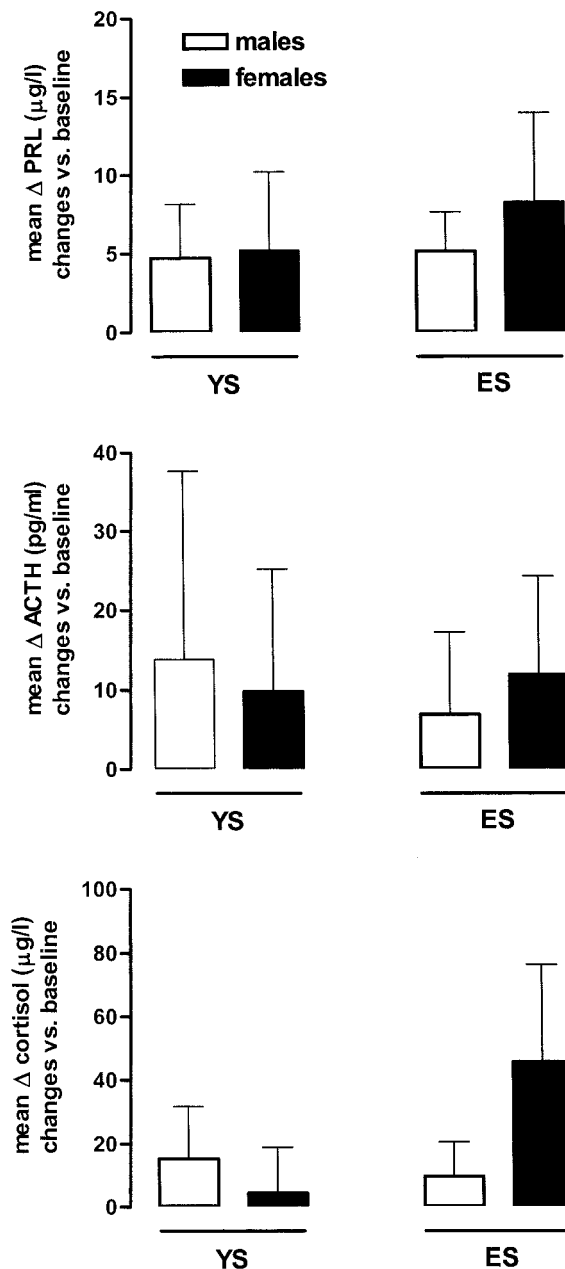FIG. 2. Mean (±SD) Δ PRL, ACTH, and cortisol changes *vs.* baseline after acute ghrelin (1.0 μg/kg iv) administration in young or elderly male and female normal subjects.

FIG. 3. Mean (±SD) Δ insulin and glucose changes *vs.* baseline after acute ghrelin (1.0 μg/kg iv) administration in young or elderly male and female normal subjects.

secretion is gender independent and therefore unlikely dependent on the estrogenic milieu (3).

As opposed to no dependence on gender, the GH response to ghrelin shows clear age-related decrease in both genders. This evidence agrees with previous findings showing that the GH response to either peptidyl or nonpeptidyl synthetic GHS in ES of both genders is lower than in adult subjects (3, 15, 16). Anyway, it has also to be considered that, theoretically, although a more frequent sampling would have provided more details in terms of qualitative description of the secretory events, no substantial different information in terms of integrated quantitative amount of hormone secreted would have been obtained by simply increasing sampling frequency.
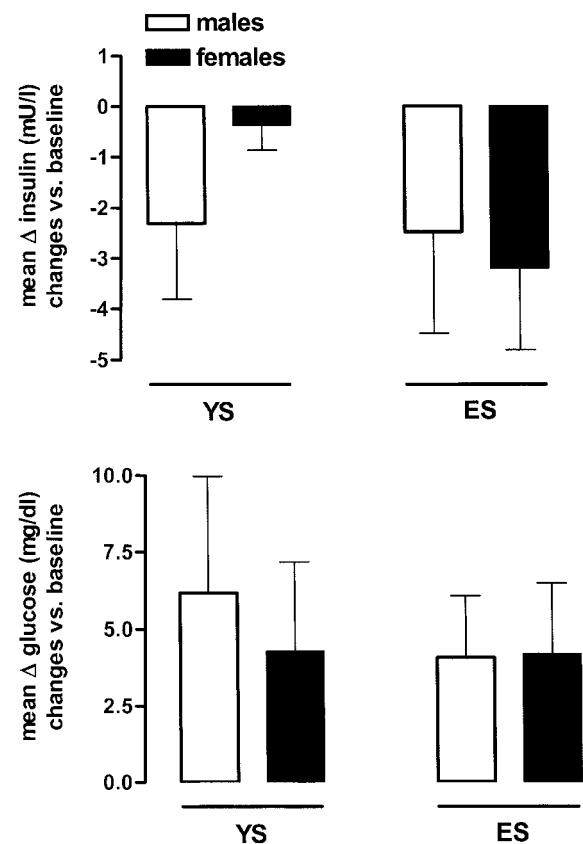
Actually, the absolute mean GH response to ghrelin (in the present study) as well as to synthetic GHS in ES was remarkable in agreement with the availability of almost preserved GH-releasable pool in the aged pituitary (3). This evidence was also the rational basis to hypothesize that chronic treatment with orally active GHS in aging would rejuvenate the function of the GH/IGF-I axis (15, 24). The age-related decrease of the GH response to ghrelin and GHS agrees with the well known *in vitro* and *in vivo* hyporesponsiveness of the aged somatotroph cells to the majority of provocative stimuli, including GHRH, despite the availability of remarkable GH-releasable pool (1–3).

It has been clearly shown that the age-related reduction of both spontaneous and stimulated GH secretion reflects age-related changes in the neural control of somatotroph function (1, 2). These changes include concomitant reduction in GHRH and enhancement in somatostatin activity (1, 2) that would *per se* explain the reduced response to ghrelin and GHS in aging. In fact, the GH-releasing activity of natural and synthetic GHS depends on the functional integrity of the hypothalamus-pituitary unit and in particular GHRH-secreting neurons (3).

However, somatotroph insufficiency in aging would also reflect some impairment in the ghrelin/GHS system. In fact, GHS-R expression has been found reduced in the aged human hypothalamus in both genders (3). This would further explain the reduced GH response to ghrelin in ES of both genders.

At variance with the influence of age on the stimulatory effect of ghrelin on somatotroph secretion, we found that its stimulatory effect on lactotroph and corticotroph secretion is independent of age. The lack of any influence of aging on the PRL- and ACTH-releasing activity of ghrelin replicates what had been found studying the effects of synthetic GHS (17) and is intriguing. In fact, the PRL-releasing activity of GHS more likely depends on direct pituitary action, but the ACTH-releasing activity is totally dependent on actions at the central nervous system level only, partially involving CRH- and/or arginine vasopressin-mediated mechanisms (3).

Evidence that the ghrelin-induced PRL release does not vary with age indicates that the PRL-releasing activity is not mediated by estrogens and dopaminergic tone that play a major role in age-dependent changes of lactotroph function (25).

On the other hand, the age independence of the stimulatory effect of ghrelin on corticotroph secretion agrees with evidence that the ACTH-releasing activity of ghrelin as well as synthetic GHS is not mediated by CRH-mediated mechanisms (3). In fact, both spontaneous and CRH-stimulated ACTH secretion are reported increased in aging, probably as a consequence of altered sensitivity to glucocorticoid feedback at the hippocampal level (26).

The age-related dissociation between the stimulatory effect of ghrelin on somatotroph cells on one hand and on lactotroph and corticotroph cells on the other hand suggests that ghrelin acts at different levels and/or on different receptor subtypes to modulate these pituitary hormonal secretions; moreover, this evidence makes unlikely the possibility that the age-related differences in the GH-responses to ghrelin might be due to different ghrelin half-lives during the lifespan, although further studies to describe ghrelin kinetic are needed to definitely rule out this possibility.

With regard to the impact of ghrelin on glucose and insulin levels in humans, it had already been shown that ghrelin, but not hexarelin, a synthetic peptidyl GHS, has hyperglycemic effect coupled with transient inhibition of insulin secretion in normal male adults (18). Our present findings confirm these effects of ghrelin in humans and show that they are basically independent of both gender and age, although in the present study, young women did not show significant change in insulin levels following ghrelin administration.

The functional relationship between ghrelin and insulin is still unclear, although ghrelin and GHS-R expression have been demonstrated in the endocrine pancreas either in animals and humans (3, 13, 27, 28). There is clear negative association between insulin and ghrelin secretion (29), and insulin has been reported able to decrease ghrelin secretion in humans (30). Actually, ghrelin has been found able to inhibit or stimulate insulin secretion, depending on different experimental conditions in animals (28, 31–33). The hypothetical existence of a functional feedback mechanism linking ghrelin and insulin secretion has, therefore, to be taken into account.

The transient inhibitory effect of ghrelin on insulin levels is coupled with significant increase in glucose levels that is apparent independent of either age or gender. This rise in glucose levels would be unrelated to insulin decrease because often this follows the hyperglycemic effect, at least when evaluated in the general circulation. We show here that rise in plasma glucose levels is not reflecting increase in glucagon secretion that, in fact, was unchanged after ghrelin administration in YS as well as ES. Thus, to explain the hyperglycemic effect of ghrelin, a direct glycogenolitic effect at the hepatic level would be hypothesized, and it would likely occur via a GHS-R non-type 1a receptor because synthetic, peptidyl GHS do not affect glucose levels (18).

In conclusion, this study shows that the GH-releasing effect of ghrelin is independent of gender but undergoes age-related decrease. On the other hand, the stimulatory effect of ghrelin on lactotroph and corticotroph secretion is independent of either age or gender. Ghrelin shows influence on insulin secretion and glucose metabolism in ES as well as YS. These findings further indicate that ghrelin is not simply a natural GHS but has a wide spectrum of endocrine actions that are variably dependent on age and gender.

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