Testosterone Is a Strong Correlate of Ghrelin Levels in Men and Postmenopausal Women

Yona Greenman Vanessa Rouach Rona Limor Susan Gilad Naftali Stern
Institute of Endocrinology, Metabolism and Hypertension, Tel Aviv Sourasky Medical Center and Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

Abstract

Background/Aims: The secretion and regulation of several hormones such as leptin and growth hormone (GH) is sexually dimorphic. Gender effects on ghrelin, a hormone involved in the regulation of GH secretion and appetite control, are controversial. Our aim was to study the relationship between plasma ghrelin and serum sex steroid hormone concentrations. Methods: Forty-five subjects (19 men, 12 premenopausal and 14 postmenopausal women) were evaluated at the Institute of Endocrinology and Metabolism, Tel Aviv Sourasky Medical Center, Israel. After an overnight fast, blood samples were collected for measurements of ghrelin, testosterone, bioavailable testosterone (BT) and estradiol. Statistical analysis was performed with adjustments for age and body mass index. Results are given as mean ± standard deviation. Results: Ghrelin levels were significantly higher in women (510 ± 489 pg/ml) than in men (319 ± 237 pg/ml; p = 0.02). There was a positive correlation between ghrelin and both total testosterone (r = 0.5, p = 0.039) and BT (r = 0.719, p = 0.0011) in male subjects. In premenopausal women, no significant correlations were found between ghrelin and testosterone or BT (r = –0.39, p = 0.2). In contrast, ghrelin strongly and positively correlated with total testosterone (r = 0.7, p = 0.01) and BT (r = 0.821, p = 0.001) in postmenopausal women. Estradiol and ghrelin were positively correlated in the group as a whole (r = 0.356, p = 0.019), but not significantly when analyzed separately by gender. Conclusions: Circulating ghrelin in humans is sexually dimorphic. Testosterone correlates positively with ghrelin levels in men and postmenopausal women.

Introduction

The secretion and regulation of several hormones is sexually dimorphic in humans. The growth hormone (GH) axis has been extensively studied in this regard, pointing to complex interactions between sex steroids and the neuroendocrine regulation of GH secretion [1]. GH is secreted in large nocturnal and smaller diurnal pulses in men, whereas women have more frequent and uniform GH output throughout the day, with higher inter-pulse valley levels [2]. In men, testosterone augmentation of spontaneous and stimulated GH secretion depends on its aromatization and subsequent GH releasing hormone stimulation by estrogen [3, 4] and is accompanied by a parallel increase in insulin growth factor 1 (IGF-1) levels. In contrast, estrogen stimulation of GH secretion in women is probably indirect and involves de-
creased IGF-1 generation in the liver with consequent attenuation of IGF-1 feedback inhibition [5].

Sexual dimorphism has also been reported in systems involved in the regulation of energy homeostasis. Leptin levels are significantly higher in women at any level of body adiposity [6]. Furthermore, leptin and testosterone levels are inversely correlated [7], and cross-sex hormone administration in transsexuals reverses the sex difference in leptin levels [8].

Ghrelin is a hormone involved both in the regulation of GH secretion and in appetite control, and as such, could potentially be affected by sex steroids as well. Nevertheless, reports on variations in ghrelin levels according to gender have not been consistent. No gender differences were found in ghrelin mRNA levels in the stomach of young and adult rats [9] and mice [10], but higher levels were detected in female aged mice [10]. Ovariectomy has been reported to increase the number of gastric ghrelin cells and plasma ghrelin levels in young rats [11], but other studies found no changes in gastric mRNA or plasma ghrelin levels after gonadectomy of male and female adult rats [9, 12]. Notwithstanding, estrogen produced locally in the stomach induced gastric ghrelin gene expression and production in rats of both genders [12]. In humans, hourly ghrelin levels measured during 24 h were clearly higher in young lean women studied in the late follicular stage of their cycle than in young male subjects [13]. We and others have reported higher fasting total ghrelin levels in women [14, 15]. Fasting plasma levels of acylated ghrelin, but not of desacyl ghrelin, were found to be higher in female compared with male subjects [16]. In contrast, other investigators did not find variations in ghrelin levels [17, 18] or in the ghrelin-stimulated GH, prolactin and adrenocorticotropic response according to gender [19].

To further investigate this issue, we studied plasma ghrelin concentration in subjects within a broad range of age and body mass index (BMI), including women in pre- and postmenopausal status, in relation to testosterone, bioavailable testosterone (BT) and estradiol levels.

Subjects and Methods

Subjects consisted of volunteers recruited from the Endocrine Clinic and among medical personnel at the Tel Aviv Sourasky Medical Center. Women with a clinical history of menstrual irregularities or hirsutism, as well as hypogonadal men, were excluded from this study. Twenty subjects had hypertension, 13 had hyperlipidemia, 6 had hypothyroidism and 2 suffered from osteoporosis. All subjects were well controlled with appropriate medical therapy. Two premenopausal women received oral contraceptives but no postmenopausal women were on hormonal replacement therapy. Subjects were studied after an overnight fast. Weight, height, BMI and blood pressure were recorded upon arrival and blood was collected for hormonal measurements. Partial data from 24 subjects have been previously reported [9]. The study was conducted in accordance with the principles of the Declaration of Helsinki and informed consent was obtained from all participants.

Laboratory Measurements

Blood samples were collected in prechilled tubes containing EDTA (1 mg/ml) and aprotinin (500 U/ml; Phoenix Pharmaceuticals, Belmont, Calif., USA) and immediately centrifuged. Plasma was stored at –80°C until assayed. Human total plasma ghrelin was measured with a commercial radioimmunoassay (Phoenix Pharmaceuticals) with an interassay coefficient of variation (CV) of <12% and an intra-assay CV of <7%. Blood was also collected for serum measurements of total testosterone (electrochemiluminescence immunoassay on Roche Elecsys 1010 analyzer, intra-assay CV 1.4% and interassay CV 2.2%), estradiol 17β (chemiluminescence immunoassay on Immulite 2000 analyzer, intra-assay CV 7.8% and interassay CV 11%) and BT. BT was measured by a competitive binding assay first reported by Tremblay and Dube [20] with some modifications, as previously described by us [21] (intra- and interassay CV 11.5%).

Statistical Analysis

Results are given as mean ± SD. Comparisons between groups were performed by unpaired Student’s t test, Mann-Whitney test for nonparametric data and by analysis of variance with adjustments for BMI and age. Associations between continuous parameters were evaluated by Pearson correlation analysis with adjustments for BMI and age. Stepwise multivariate logistic regression analysis was applied to the data to assess the relationship between ghrelin concentrations (dependent variable) and explanatory variables (gender, age, BMI and testosterone levels). A p level <0.05 was considered statistically significant. Statistical analyses were carried out using the SAS for Windows version 9.1.

Results

There were 19 male and 26 female subjects, 14 of whom were postmenopausal. There were no gender-related differences in age (52 ± 14.4 years, range 29–74, for male, and 46 ± 16 years, range 23–72, for female subjects) and BMI (29.9 ± 6.8, range 19–45, in male, and 30.4 ± 8.5, range 16–47, in female subjects). Both systolic (129 ± 19.4 vs. 117 ± 14 mm Hg; p = 0.02) and diastolic (81 ± 8.8 vs. 72 ± 9.7 mm Hg; p = 0.001) blood pressure levels were higher in men than in women in this cohort. Ghrelin levels (mean ± SD) were significantly higher in women (510 ± 489 pg/ml, range 122–2,120) than in men (319 ± 237 pg/ml, range 97–1,054), after adjustments for age and BMI (p = 0.02).
There was a significant negative correlation between ghrelin levels and BMI ($r = –0.4265$, $p = 0.0035$, $n = 45$), that was more evident in female ($r = –0.478$, $p = 0.01$, $n = 26$) than in male ($r = –0.327$, $p = 0.17$) subjects. Furthermore, after adjustments for BMI, there was a positive but weak correlation between age and ghrelin levels ($r = 0.298$, $p = 0.049$, $n = 45$), that was significant in female ($r = 0.45$, $p = 0.02$) but not in male ($r = 0.22$, $p = 0.36$) subjects.

There was a positive correlation between ghrelin levels and both total testosterone ($r = 0.507$, $p = 0.02$) and BT ($r = 0.493$, $p = 0.03$) in male subjects. As both testosterone and ghrelin are modulated by BMI and age, statistic procedures were repeated with adjustments for these 2 parameters. The correlation between ghrelin and total testosterone remained significant ($r = 0.5$, $p = 0.039$; fig. 1a), whereas the correlation between ghrelin and BT became stronger ($r = 0.719$, $p = 0.0011$; fig. 1b) after adjustments. In premenopausal women, no significant correlations were found between ghrelin and testosterone ($r = 0.08$, $p = 0.8$) or BT ($r = –0.39$, $p = 0.2$). In contrast, ghrelin strongly and positively correlated with total testosterone ($r = 0.7$, $p = 0.01$; fig. 2a) and BT ($r = 0.821$, $p = 0.001$; fig. 2b) in postmenopausal women. There was a positive adjusted correlation between estradiol and ghrelin levels in the population as a whole ($r = 0.356$, $p = 0.019$) that lost its significance when analyzed separately by gender ($r = 0.29$, $p = 0.16$ and $r = 0.34$, $p = 0.17$ for female and male subjects, respectively). Ghrelin levels were higher in postmenopausal (612 ± 630 pg/ml) than in premenopausal (390 ± 214 pg/ml) women, but the difference did not attain statistical significance ($p = 0.7$).

To evaluate potential explanatory variables for the variation in ghrelin plasma levels, stepwise multivariate logistic regression analyses were performed. BMI, age and gender accounted for 17.3, 7.3 and 9% of the variation in ghrelin levels in the study population. In female subjects, 51.8% of the variation in ghrelin levels was related to BMI (22.9%), age (15.8%) and total testosterone levels (13%). In male subjects, total testosterone levels alone explained 25.7% of the variation in circulating ghrelin, whereas BMI was not a significant contributor to the variation in circulating ghrelin concentration. Details of the regression analyses are summarized in table 1.

No correlation was found between ghrelin levels and systolic or diastolic blood pressure. Systolic blood pressure was negatively correlated with total testosterone ($r = –0.62$, $p = 0.0076$) and BT ($r = –0.46$, $p = 0.058$) in male but not in female subjects.
Ghrelin levels were higher in female subjects in our study, thus supporting the assertion that ghrelin levels are sexually dimorphic. Nevertheless, gender accounted for only 9% of ghrelin variance in our cohort, thus possibly explaining why this effect went undetected in some studies. A strong correlation between ghrelin and testosterone levels was evident in men and in postmenopausal women, suggesting that testosterone is an important factor affecting ghrelin levels. In fact, among male subjects, testosterone was the major correlate of ghrelin levels, accounting for over 25% of its variability. The inter-relationship between sex steroids and ghrelin has not been thoroughly explored and few reports on this subject can be found in the literature. Our findings are in agreement with a previous report in which low ghrelin levels found in hypogonadal men were restored to the eugonadal range with testosterone replacement therapy [22]. In that study, a positive correlation between testosterone and ghrelin levels was evident in both the hypogonadal and eugonadal states. The relationship between ghrelin and androgens has been studied in women diagnosed with the polycystic ovary syndrome, but to the best of our knowledge, this is the first report on the subject in normo-androgenemic women in general, and in the postmenopausal period in

**Table 1.** Summary of stepwise multivariate logistic regression analysis for ghrelin as the dependent variable

<table>
<thead>
<tr>
<th>Explanatory variables</th>
<th>All (n = 45)</th>
<th>Females (n = 26)</th>
<th>Males (n = 19)</th>
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<tr>
<td></td>
<td>partial $R^2$</td>
<td>p value</td>
<td>partial $R^2$</td>
</tr>
<tr>
<td>BMI</td>
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<td>0.004</td>
<td>0.2290</td>
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<td>0.04</td>
<td>0.1584</td>
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<td>0.26</td>
<td>0.1306</td>
</tr>
<tr>
<td>Gender</td>
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<td>0.02</td>
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<tr>
<td>Model $R^2$</td>
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</table>

**Discussion**

Ghrelin levels were higher in female subjects in our study, thus supporting the assertion that ghrelin levels are sexually dimorphic. Nevertheless, gender accounted for only 9% of ghrelin variance in our cohort, thus possibly explaining why this effect went undetected in some studies. A strong correlation between ghrelin and testosterone levels was evident in men and in postmenopausal women, suggesting that testosterone is an important factor affecting ghrelin levels. In fact, among male subjects, testosterone was the major correlate of ghrelin levels, accounting for over 25% of its variability. The inter-relationship between sex steroids and ghrelin has not been thoroughly explored and few reports on this subject can be found in the literature. Our findings are in agreement with a previous report in which low ghrelin levels found in hypogonadal men were restored to the eugonadal range with testosterone replacement therapy [22]. In that study, a positive correlation between testosterone and ghrelin levels was evident in both the hypogonadal and eugonadal states. The relationship between ghrelin and androgens has been studied in women diagnosed with the polycystic ovary syndrome, but to the best of our knowledge, this is the first report on the subject in normo-androgenemic women in general, and in the postmenopausal period in
particular. Ghrelin plasma levels are reported to be decreased in polycystic ovary syndrome [23], a condition characterized by hyperandrogenism, obesity and insulin resistance. The latter features are well-established negative modulators of ghrelin, but reports on the relationship between androgen and ghrelin levels in this population have been inconsistent: Although ghrelin was reported to inversely correlate with androstenedione [24] and testosterone [25] levels, these findings have not been confirmed by other studies [19, 26]. In our group of premenopausal women, no significant correlations were found between testosterone and ghrelin levels. In contrast, in postmenopausal women, there was a strong positive correlation between testosterone and ghrelin levels. In male subjects.

Interactions between ghrelin and the reproductive axis may exist at several levels. Several studies suggest that ghrelin has a central (hypothalamic) inhibitory effect on gonadotropin secretion: luteinizing hormone (LH) pulsatility is suppressed by central administration of ghrelin and estradiol concentrations [27]. Here, we report a weak positive correlation between estrogen and ghrelin levels in our study population. We are unable to further characterize this relationship as premenopausal women were not studied uniformly throughout the menstrual cycle. Furthermore, it is possible that the estrogen/ghrelin association, secondary to testosterone aromatization to estradiol in male and postmenopausal women. Interactions between ghrelin and the reproductive axis may exist at several levels. Several studies suggest that ghrelin has a central (hypothalamic) inhibitory effect on gonadotropin secretion: luteinizing hormone (LH) pulsatility is suppressed by central administration of ghrelin to rats, monkeys and sheep [28], and ex vivo gonadotropin-releasing hormone (GnRH) secretion by hypothalamic explants from female rats is also inhibited by ghrelin treatment [29]. On the other hand, although ghrelin inhibits the GnRH-induced LH release by pituitary explants, it also has the opposite effect of directly stimulating both LH and follicle-stimulating hormone secretion in vitro [30]. Another level of complexity is added by the differential effects of hormones when given in a fixed or pulsatile manner. Hence, a single injection of ghrelin had no effect on LH secretion, but when given in a pulsatile manner, ghrelin significantly suppressed LH secretion in humans [31]. Ghrelin levels are markedly elevated in patients with anorexia nervosa, characterized by extremely low body weight and amenorrhea [32]. This ghrelin rise may be one of the factors signaling and connecting energy-deficient states with the inhibition of the hypothalamo-pituitary-gonadal axis. Ghrelin levels are also elevated in amenorrheic exercising women of normal weight, thus reflecting more subtle states of chronic energy deficiency [33]. Interestingly, it has recently been shown in the rhesus monkey that activation of the hypothalamo-pituitary-adrenal axis may mediate the ghrelin inhibition of LH pulsatility. Pretreatment with a corticotropin-releasing hormone receptor antagonist prevented ghrelin-induced reduction in LH pulsatility [34].

At the peripheral level, it has been shown that both ghrelin and ghrelin receptors are expressed in the testis and ovary of several species [35] including the human gonads [36, 37], suggesting that circulating or locally produced ghrelin may have direct effects on gonadal function. Alternatively, it is possible that sex steroids may directly regulate ghrelin levels, thus creating a feedback loop regulatory mechanism. Cyproterone acetate given to healthy men as short-term contraceptive treatment, suppressed LH, follicle-stimulating hormone, estradiol and testosterone levels, with a parallel increase in ghrelin levels. During cotreatment with cyproterone acetate and testosterone enanthate, gonadotropin levels remained low, as expected, but the high testosterone levels were associated with decreased ghrelin levels [38]. These results and those pointing to inhibition of gonadotropins by ghrelin seem to be in contradiction to ours and those of Pagotto et al. [22], where testosterone and ghrelin levels were positively correlated. Nevertheless, these inhibitory effects were found as a result of acute pharmacological manipulations, which cannot be readily compared with hormone measurements in the steady state, as performed by us and Pagotto et al. [22].

Our study is limited in that we measured total ghrelin levels, but not the individual acylated and nonacylated forms. Nevertheless, most information gathered to date on ghrelin effects and regulation is based on the measurements of total ghrelin in the plasma, as well as on the study of effects of total ghrelin infusion in animals and humans. Furthermore, it has recently been shown that both acylated and unacylated ghrelin is similarly inhibit LH secretion in rats [39]. Nonetheless, we cannot exclude the possibility that sex steroids could differentially affect acylated and nonacylated ghrelin receptors. Another possible limitation of our study is that a single point measurement may not accurately reflect the overall secretory status of hormones that are pulsatile in nature. Standardization of sampling conditions (e.g., fasting state, time of the day) seems to overcome, at least in part, this drawback. For example, well validated data on negative correlations be-
between insulin and ghrelin levels [13, 14] have been obtained in a similar fashion.

Finally, although a negative correlation between blood pressure and ghrelin levels has been found in previous studies [40, 41], we were not able to confirm this finding. A possible explanation may be an inadequate statistical power to demonstrate such a correlation in a small cohort.

In conclusion, the strong associations between sex steroids and ghrelin levels reported in our study, support the notion that ghrelin, like leptin, may play a role in the integration of energy balance and reproduction. The mechanisms of these interactions need to be further explored and characterized.

References


34. Vulliemoz NR, Xiao E, Xia-Zhang L, Rivier J, Ferin M: A nonselective corticotrophin-releasing hormone receptor antagonist, prevents the inhibitory effect of ghrelin on luteinizing hormone pulse frequency in the ovariecetomized rhesus monkey. Endocrinology 2008;149:869–874.


