Testosterone Replacement Therapy Restores Normal Ghrelin in Hypogonadal Men

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We recently described a connection between androgens and ghrelin in women affected by the polycystic ovary syndrome. To further investigate the interaction between sex steroids and ghrelin, we investigated circulating ghrelin levels in a group of hypogonadal men before and after therapeutic intervention aiming at normalization low testosterone (T) concentrations. Seven hypogonadal men were compared with nine overweight/moderately obese men matched for body mass index and body fat distribution parameters, as well as with 10 normal weight controls. Total and free T and plasma ghrelin levels were significantly lower in the hypogonadal men than in the control groups. Hypogonadal men also had a significantly higher insulin resistance state. Ghrelin levels were positively correlated with both total and free T concentrations. A significant correlation was also found between ghrelin and the anthropometric parameters and the insulin resistance indexes. However, in a multiple regression analysis in which a correction for all covariants was performed, only the relationship with total and free T persisted. After the 6-month replacement T therapy, ghrelin levels of hypogonadal patients increased and did not differ significantly in comparison with both control groups. The positive correlation between ghrelin and androgens still persisted after T replacement therapy, after adjusting for confounding variables. These data further indicate that sex hormones modulate circulating ghrelin concentrations in humans. This may be consistent with the concept that ghrelin may exert a relevant role in the endocrine network connecting the control of the reproductive system with the regulation of energy balance. (J Clin Endocrinol Metab 88: 4139–4143, 2003)

THREE YEARS AFTER its identification, ghrelin has turned out to be the only circulating factor to promote appetite, food intake, and positive energy balance (1–3). Ghrelin is predominantly produced and secreted by the stomach and the gut (4). Its production site, in combination with its main biological effects, already indicated that this novel peptide hormone might represent a mediator transducing quantitative and qualitative changes in nutrient intake into central circuits regulating metabolic balance (1). Although ghrelin has a potent orexigenic activity and causes weight gain (1, 4), a widespread peripheral distribution of ghrelin receptor suggests a pleiotropic and multifunctional role of this gastric hormone (5). In this context, gonadal tissues have been proposed as being a target for ghrelin based on a high number of binding sites in both ovary and testis (5). In addition to this first descriptive evidence that ghrelin might play a role in the regulation of reproductive physiology, a recent study has shown that ghrelin affects the chorionic gonadotropin- and cAMP-induced testosterone (T) secretion in rat testis by inhibiting key enzymes of steroidogenic pathways (6). Moreover, it has been recently demonstrated that gonads are not only target tissues for ghrelin, but also relevant sites of ghrelin production. Notably, in both testis and ovary, ghrelin is coexpressed with sex hormones in androgen-producing cells like Leydig and hilar interstitial cells (7, 8). These in vitro findings suggested a potential interaction between ghrelin and androgens and prompted us to additionally investigate the existence of mutual influences between ghrelin and sex steroids in vivo. Recently, we found that obese women with the polycystic ovary syndrome (PCOS), a condition characterized by hyperandrogenism, have even lower ghrelin levels than expected on the basis of their obese phenotype (9). We also described a highly significant negative correlation between ghrelin and circulating androgen parameters, particularly androstenedione, and we suggested that suppressed ghrelin concentrations observed in PCOS women may be caused by high androgen levels. The results of a controlled follow-up study in a separate group of obese PCOS women demonstrated that long-term treatment with the antiandrogen agent flutamide significantly increased circulating ghrelin levels, regardless of changes in body weight, fat topography, and insulin sensitivity (10). To provide direct proof that androgens regulate ghrelin secretion, we have now investigated basal ghrelin levels along with insulin sensitivity and body composition in overweight/moderately obese hypogonadal men before and after T replacement therapy in comparison with age- and weight-matched eugonadal men as well as with normal weight age-matched healthy individuals.

Subjects and Methods

The study population consisted of three groups. The first group included seven hypogonadal men (four with Klinefelter’s syndrome, one with primary testicular dysfunction due to long-term complicated
Hormone assays and data analysis

After sampling, blood was immediately chilled on ice and centrifuged, and serum as well as plasma aliquots were frozen at −80 °C until assayed. All samples from individual subjects were analyzed in duplicate for each endocrine and biochemical parameter. Plasma glucose levels were determined by the glucose-oxidase method. Insulin, T, and SHBG were analyzed as previously described (13). Free T values were obtained by calculation from T and SHBG values in agreement with the method proposed by Vermeulen et al. (14). The intraassay coefficient of variation in our laboratory was 7.0% for total T, 6.5% for SHBG, and 3.0% for insulin. The Quantitative Insulin-Sensitivity Check Index (QUICKI) was calculated according to the formula proposed by Mather et al. (15). The Homeostasis Model Assessment (HOMA) insulin resistance index was calculated as proposed by Matthews et al. (16). Plasma samples were assayed in duplicate for immunoreactive ghrelin concentration by a commercially available RIA (Phoenix Pharmaceuticals, Mountain View, CA) using 125I-labeled bioactive ghrelin as a tracer and a rabbit polyclonal antibody raised against the c-terminal end of human ghrelin. This assay recognizes both acylated and deacylated ghrelin (17). The antisemum does not cross-react with any relevant peptide as previously shown (17, 18). Intra- and interassay coefficients of variation were less than 5.3 and 13.6%, respectively.

Statistical analysis

Results are reported as the mean values ± sd, unless stated otherwise. Statistical analysis between groups was performed by using ANOVA. Within-subject ANOVA was used to compare the modifications observed in the group of hypogonadal men during the T replacement therapy. All statistical analyses including correlation and multiple regression analysis were performed using StatView software (Abacus Concepts Inc., Berkeley, CA). P values of less than 0.05 were regarded as statistically significant.

Results

Baseline hormonal and metabolic parameters (Table 1)

Compared with the two control groups, hypogonadal men had lower total and free T, not significantly different SHBG concentrations, higher fasting glucose and insulin levels, lower QUICKI, and higher HOMA values. However, within the two control groups, fasting insulin and the HOMA index were significantly higher in the obese compared with the normal weight subjects, without any difference in the other parameters. Ghrelin levels were significantly lower in hypogonadal men when compared with both control groups.

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Interestingly, men with primary hypogonadism had ghrelin values (92.1 ± 66.6 fmol/ml) comparable to those with secondary hypogonadism (108.1 ± 57.7 fmol/ml). Blood concentrations of ghrelin were positively correlated with both total (r = 0.749; P < 0.0001) and free (r = 0.693; P < 0.0001) T concentrations in all groups (Fig. 1). Moreover, they were negatively correlated with BMI (r = −0.510; P < 0.01), WHR (r = −0.463; P < 0.05), fasting glucose (r = −0.530; P < 0.01), fasting plasma insulin levels (r = −0.493; P < 0.01), and HOMA values (r = −0.567; P < 0.01), whereas ghrelin plasma levels were positively correlated with the QUICKI values (r = 0.576; P < 0.01). However, in a multiple regression model, only the relationship between ghrelin and total (t = 3.014; P < 0.01) as well as free (t = 2.351; P < 0.05) T remained significant.

Effect of T replacement in hypogonadal men (Table 2)

Six months of T replacement therapy caused a significant increase in total and free T, a decrease in SHBG and fasting insulin levels and an improvement in insulin resistance in the hypogonadal men, whereas no significant changes occurred in BMI, WHR, and the fasting glucose levels. Moreover, plasma ghrelin significantly increased to values similar to those of both normal weight and BMI-matched overweight controls (Table 2 and Fig. 2). When data derived from pre- and postandrogen replacement therapy were analyzed together, a positive correlation between plasma ghrelin and both total (r = 0.563; P < 0.05) and free (r = 0.629; P < 0.01) T concentrations was still observed.

**Discussion**

This is the first report showing that circulating ghrelin levels in hypogonadal men are lower when compared with weight-matched eugonadal men or normal weight controls. In agreement with other studies, normal weight subjects tended to present with higher circulating ghrelin levels in comparison with overweight/obese eugonadal males (17, 19, 20). In the past, the correlation between decreased ghrelin concentration and increased body weight has been attributed to the presence of an insulin resistance state (20, 21). On the other hand, controversy still remains on this issue, as documented by the findings in patients with type 2 diabetes in whom ghrelin concentrations remained within the normal range in lean subjects and only decreased in obese patients (19). To clarify this assumption, we investigated the relationship between plasma ghrelin levels and indexes of insulin resistance, proving the existence of a negative corre-
loration between these parameters. This generally agrees with similar findings observed in subjects with states of insulin resistance, such as obesity, type 2 diabetes, and PCOS (9, 10, 17, 19, 21). However, after statistically adjusting for androgen levels, the correlation between ghrelin and insulin resistance was abrogated at least in the cohort of PCOS (9, 10). On the other hand, a positive correlation between plasma ghrelin and circulating T levels was found throughout the examined population enrolled in this study, regardless of the androgen status. In fact, the relationship between ghrelin and T levels was found in both hypogonadal and eugonadal subjects. We therefore hypothesize that the correlation between ghrelin and androgens that we have described here and in previous studies (9, 10) is a phenomenon that is independent of body fat, body composition, and the presence of insulin resistance. The demonstration of a marked increase of plasma ghrelin in the hypogonadal men after T replacement therapy, which occurred regardless of changes in body weight and insulin sensitivity, rather provides further support to the notion of a close relationship between androgens and ghrelin. The fact that posttreatment plasma levels of ghrelin in hypogonadal patients became similar to values observed in the BMI-matched controls even suggests that changes in androgen levels rather than variations in body composition are responsible for this normalization. Although our results were derived from one single measurement of ghrelin that was also not able to discriminate between the active and the inactive ghrelin, these data confirm and extend our recent findings in a population of obese women with PCOS, a condition featured by a hyperandrogenic state, in whom we found a significant correlation between ghrelin and androgens independent of body weight, fat distribution, and insulin resistance (9). Furthermore, when the PCOS patients underwent a long-term antiandrogen therapy, a significant increase in plasma ghrelin levels was observed, again regardless of changes in body weight and insulin sensitivity (10). In summary, our findings support the concept that androgens play an important regulatory role regarding ghrelin secretion or catabolism. However, the relationship between androgens and ghrelin is negative in female, but positive in male individuals. Further studies have to show why there is a gender-specific regulatory influence of androgens on ghrelin. We however propose that the normalization of the androgen status, rather than an unnatural increase or decrease, may recover suppressed ghrelin secretion and therewith may possibly reestablish a balanced energy homeostasis. Until now, no significant gender difference regarding circulating ghrelin levels has been found (22). Data derived from experimental studies, all performed in animal models, are also contradictory. In fact, Gualillo et al. (23) reported that ghrelin expression in rat stomach was similar in male and female rats, whereas Liu et al. (24) found a relevant sexual dimorphism in aged mice, showing higher stomach ghrelin mRNA expression in females when compared with males. We speculate that a serious imbalance of endocrine factors regulating reproduction may suppress ghrelin levels, whereas physiological sexual hormone levels in both genders do not affect them.

Mechanisms by which abnormal androgen conditions may alter ghrelin concentrations are at present unknown. Theoretically, androgens may act directly on both peptide expression and synthesis as well as on its metabolic pathways. This mode of action, however, has not yet been investigated. Alternatively, androgens may modulate ghrelin in an indirect way, through other regulatory factors. These players may putatively be represented by leptin or free fatty acids (FFA). T substitution is known to normalize serum leptin secretion in hypogonadal patients; we can therefore hypothesize that leptin normalization may be involved in the elevation of ghrelin levels observed in our patients (25). Moreover, it is well known that the adipose visceral depots are important sites of production of FFA and that androgens influence visceral fat mass in a gender-specific manner. In particular, in males, T stimulates lipolysis inducing an increase of FFA release from the visceral fat depots, whereas in females, androgen administration increases lipogenesis in the visceral depots (26). This is clearly mirrored by the fact that low T levels in men as well as hyperandrogenism in women are usually associated with increased abdominal fatness (27).

A link between FFA and ghrelin has been shown very recently by Broglio et al., who demonstrated that FFA infusion reduces the ability of ghrelin to induce GH secretion from the pituitary (28). Additionally, it has been shown that a fat-rich diet, known to increase circulating FFA, was able to decrease circulating ghrelin levels in experimental rats (29). On the other hand, there are also studies in humans showing no changes in ghrelin levels caused by lipid infusion, a stimulus known to increase circulating FFA levels (30). Therefore, the putative role of FFA on ghrelin regulation still remains to be defined. Gonadotropins may also be involved in the modulation of ghrelin expression or secretion. In fact, Dieguez and colleagues (31) have shown that cyclic ovarian mRNA ghrelin expression was disrupted by blockade of preovulatory gonadotropin surge as obtained by the administration of a potent GnRH antagonist. Moreover, the same group found that the Leydig cell-specific expression of ghrelin in rat testis is under the regulation of LH (7). In our patients, no correlation was found between gonadotropins and ghrelin (data not shown), and no differences were found in circulating ghrelin between patients with primary hypogonadism and those with secondary hypogonadism. Therefore, the possibility that gonadotropins play a role in the regulation of ghrelin blood concentrations cannot be confirmed by us and needs further investigation.

A further improvement will be also given by the possibility to understand by more specific RIAs whether the changes in total ghrelin observed will also be followed by a similar change in active ghrelin in the same experimental setting.

In conclusion, this work provides further evidence that androgens influence blood ghrelin concentrations. Considering the role of ghrelin in the regulation of metabolic processes, our present data seem to further support the notion that ghrelin may constitute an important link between the regulation of reproduction and the control of metabolic homeostasis. Further highlights on this issue will be provided by studies in which the interaction of androgens and ghrelin will be monitored in physiological conditions as the prepuberal and postpuberal phases or in situations in which abnormal and acute changes of androgens will be induced by pharmacological or surgical treatments.
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