Effect of feeding on growth hormone response to growth hormone-releasing hormone in polycystic ovarian syndrome: relation with body weight and hyperinsulinism

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The plasma growth hormone (GH) response to direct stimulation with growth hormone-releasing hormone (GHRH) before and after a standard meal was investigated in 14 polycystic ovarian syndrome (PCOS) subjects. Data were compared with those obtained from 14 healthy normovulatory matched patients. All women underwent an oral glucose tolerance test (OGTT) (75 g) and basal plasma hormone concentrations were evaluated. On a different day all subjects had a GHRH test (50 µg GHRH) both before and after lunch randomly. In obese PCOS subjects the GH response to GHRH was blunted after a meal, while in obese control patients there was an enhanced response of GH to GHRH after a meal. Normal control subjects showed an inhibition of the GH response after feeding and lean PCOS subjects showed a trend toward an augmented GHRH related secretion after a meal significantly higher than normal controls (P < 0.05) but not significantly higher than the pre-prandial response. In conclusion, the data indicate in PCOS a derangement of GH secretion related to food ingestion; in particular obese PCOS patients did not exhibit any change of GH response after a meal compared with the paradoxical response observed in obese controls. Several other factors beyond body mass index and hyperinsulinism could be involved in these pathophysiological events.

Key words: body weight/growth hormone/insulin/meal/polycystic ovarian syndrome

Introduction

The debate on the physiological regulation of growth hormone (GH) secretion is still open and several aspects remain to be elucidated. It is well known that GH secretion is blunted in obesity (Williams et al., 1984). Moreover, other metabolic signals may modify the GH response to GH-releasing hormone (GHRH): in normal subjects the plasma GH response to GHRH was impaired after an infusion of glucose (Sharp et al., 1984); similarly lipid–heparin administration, by inducing an increase in circulating free fatty acid (FFA) concentrations, also blunted such response (Imaki et al., 1985). Our previous studies provided information about the meal interference on GH response to GHRH in normal and in obese subjects (De Marinis et al., 1988); particularly, obese patients exhibited a paradoxical increase of GH to GHRH in relation to food ingestion as compared with controls, in whom an inhibitory effect was observed.

Recent studies have well documented an impairment of GH response to several stimuli in women with polycystic ovarian syndrome (PCOS) (Lee et al., 1993; Piaditis et al., 1995). This syndrome is characterized by anovulation, hyperandrogenism and in a considerable percentage of subjects by obesity and hyperinsulinism. The blunted GH response to GHRH in PCOS women could be related only partially to obesity since lean hyperinsulinaemic subjects showed a similar reduction of GH secretion after GHRH administration (Lanzone et al., 1995), thus indicating that several other factors may interfere with GH secretion.

The aim of the present study was to investigate the plasma GH response to direct stimulation with GHRH before and after a standard meal in PCOS subjects in order to study the influence of body weight and insulin on GH secretion.

Materials and methods

A total of 14 women affected by PCOS were studied. Seven of them were obese and seven normal weight. All of the women were in good health and euthyroid, and none had taken any medication known to affect carbohydrate metabolism or gonadal function for at least 3 months before the study. All patients had spontaneous onset of puberty. Adrenal enzymatic defects were excluded by a corticotrophin test according to the criteria of New et al. (New et al., 1983). PCOS was diagnosed by clinical findings (presence of...
amnenorrhoea or oligoamenorrhoea and hirsutism), plasma androgen concentrations at the upper limit or above the normal range (androstenedione 1.98–5.58 nmol/l, testosterone 0.58–2.01 nmol/l), and bilateral normal or enlarged ovaries with the presence of at least 10 microcysts (2–8 mm diameter) (from the inner margin to the outer margin in longitudinal cross-sections) associated with an increase in ovarian stroma (Polson et al., 1988) at the time of ultrasonography. A normal LH to FSH ratio was not considered an exclusion criterion. Data were compared with those obtained from 14 health normovulatory patients, matched for age and body mass index (BMI), seven simply obese patients (i.e. not PCOS) were recruited to our endocrinology department and seven lean controls to our sterility centre. Obesity was defined as a BMI ≥ 27 kg/m² (normal range 19–25 kg/m²). All subjects underwent the same clinical protocol. Informed consent was obtained from each patient. The study was approved by our ethical committee. In the follicular phase, women were hospitalized the day before the beginning of the basal study: this last one was performed after a 3 day standard carbohydrate diet (300 g) and fasting overnight. At 0700 h blood samples were taken for evaluation of LH, FSH, 17β-oestradiol, 17-hydroxyprogesterone (17-OHP), sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulphate (DHEA-S), androstenedione, testosterone, growth hormone (GH), insulin, glucose, and free fatty acid (FFA) plasma concentrations.

Then all women had an oral glucose tolerance test (OGTT). Blood samples were collected every 30 min for 4 h after ingestion of 75 g of glucose; insulin and glucose were assayed in each sample. On different days all subjects underwent GHRH (50 μg GHRH; Geref, Serono, Italy) tests both before and after lunch in randomized order.

**Pre-prandial tests**

After an overnight fast the women had their usual breakfast at 0800 h and then ate nothing thereafter until the last test. At 1245 h, while the women were resting in a quiet room, an indwelling venous cannula was inserted in one arm, through which normal saline (0.9%) was given slowly. At time 0 (1300 h) a 50 μg bolus dose of GHRH was injected i.v. Blood samples were collected 15 min and just before and 30, 60, 90 and 120 min after GHRH administration.

**Post-prandial tests**

Fasting throughout the night the women had their usual breakfast at 0800 h and then ate nothing until the last test. At 1200 h the women ate 800 kcal meal composed of 55% carbohydrate, 30.6% lipid, and 13.6% protein, as previously described (De Marinis et al., 1991). A saline infusion was started at 1245 h and GHRH (50 μg) was administered 15 min later (time 1300 h). Blood samples were collected at –15, 0, 30, 60, 90 and 120 min.

**Assays**

All blood samples were promptly centrifuged (1500 r.p.m.) and stored at –20°C until assayed. LH, FSH, oestradiol, testosterone, 17-OHP, androstenedione, DHEA-S and SHBG were determined in the basal condition. These hormones were measured in duplicate by radioimmunoassay methods using commercial kits (Radim, Pomezia, Italy). The immunoradiometric assay (IRMA) on solid phase (coated tube), based on monoclonal double-antibody technique, was used for LH, FSH and SHBG. Steroids were assayed by a radioimmunoassay direct method in human serum or plasma. Insulin was assayed using radioimmunoassay methods and glucose concentrations were determined by the glucose oxidase technique. Intra-assay and inter-assay coefficients of variation were as follows: LH, 5.6% and 9.1%; FSH, 6.9% and 8.4%; oestradiol, 2.3% and 3.5%; testosterone and androstenedione, 6.1% and 9.3%; insulin, 5.1% and 6.2%; SHBG, 6.9% and 8.5%. GH was determined by the IRMA method using commercial kits from Radim. Intra-assay and inter-assay coefficients of variation were respectively 2.5% and 5.8%. The lowest amount of GH detected was 0.04 μg/l. Free fatty acids were determined by an acyl-coenzyme-A oxidase based colorimetric method (Okabe et al., 1980).

**Data analysis**

An abnormal glycaemic response to the OGTT was defined according to the criteria of National Diabetes Data Group (National Diabetes Group, 1994). No patient showed impaired glucose tolerance. All results were presented as mean ± SD. Insulin and GH plasma concentrations were also expressed as area under the curve (AUC) after OGTT or GHRH test respectively, calculated by the trapezoidal rule and expressed as μIU/ml×240 min for insulin (AUC-I) and μg/l×120 min for GH. Incremental area under curve for GH (AUCi-GH) was calculated by trapezoidal rule after subtracting basal hormone concentration. The difference (AUCd-GH) between the GHRH elicited incremental pre-prandial and post-prandial area (AUCd-GH = AUCi-post-prandial – AUCi-GH pre-prandial) was considered as indicator of GH secretion capacity in relation to a meal. The ratio of testosterone×100/SHBG was used to calculate the free androgen index (FAI). The distribution of the data was tested by Kolmogorov–Smirnov test to verify whether the samples came from a specified distribution and it was found that the data were normally distributed. The significance of differences between the same tests performed before and after a meal was assessed by the non-parametric Wilcoxon rank-sum test. The comparison between different study groups was performed by the non-parametrical Mann–Whitney U-test. Linear regression analysis was used to analyse possible correlation between endocrine findings. The level of statistical significance was set at P < 0.05.

**Results**

Table I lists the endocrine and metabolic parameters for obese and lean groups of PCOS and normal subjects.

Obese PCOS patients showed the lowest SHBG values and their androstenedione and LH plasma concentrations were greater than those found both in lean PCOS and control groups. Lean control subjects showed the lowest FAI index and the lowest FFA concentrations in obese PCOS patients were significantly higher than in the other groups (P < 0.01 and P < 0.05 respectively).

Fasting insulin concentrations were significantly higher in the obese PCOS group and in obese controls with respect to those found in lean controls (P < 0.01). Moreover, AUC-I concentrations in obese PCOS patients were significantly higher than in the other groups (P < 0.001). No differences were found among groups for fasting glycaemia and AUC-glucose values.

Obese patients (both PCOS and controls) had lower baseline GH values versus lean PCOS and control subjects (P < 0.01).

Figure 1 shows plasma GH concentrations after GHRH administration before and after a meal both in lean and obese control and PCOS subjects.

**Pre-prandial test**

In controls the mean peak plasma GH response to GHRH occurred between 30 and 60 min after GHRH injection. In the obese women the mean peak plasma GH concentration was markedly lower than the peak concentration in normal women (5.99 ± 2.71 versus 38.7 ± 13.21 μg/l; P < 0.01).
In lean PCOS subjects GH peak reached 12.3 ± 9.2 µg/l values significantly lower than lean controls (P < 0.05). However, GH concentrations remained significantly higher than those observed in PCOS obese patients at 60, 90 and 120 min (P < 0.05).

**Post-prandial test**

At 1300 h, 1 h after a meal, lean control subjects showed GHRH-induced GH peak concentrations significantly lower (10.7 ± 3.84 µg/l; P < 0.05) than that observed in pre-prandial tests. In obese patients plasma GH response to GHRH injection was significantly increased at 30 min after GHRH injection and the peak GH response was significantly higher in comparison with the one registered without meal administration (14.84 ± 4.95 µg/l; P < 0.05).

The plasma GH response to GHRH in lean PCOS subjects increased in respect to pre-prandial response but not significantly, otherwise the GH post-prandial peak in these subjects was significantly higher than the GH post-prandial peak in normal subjects (23.6 ± 11 µg/l, P < 0.05). In the obese PCOS subjects the GH plasma peak was significantly lower than in lean PCOS women and GH plasma concentrations at 30, 60, 90 and 120 min were not significantly different from those before lunch.

To explore the relative influence of BMI and insulin on GHRH-induced GH secretion the correlation of these parameters with the difference in GH incremental area after and before a meal (AUCd-GH) was examined. A significantly negative linear correlation between AUCd-GH values and BMI (P < 0.001; r = −0.87) as well as AUC-I values (P < 0.001; r = −0.813) was found in the whole PCOS group. However in controls a correlation was found between BMI and AUCd-GH (P < 0.05; r = 0.55) but no correlation between AUC-I and AUCd-GH.

**Discussion**

Body weight and nutritional status are two factors involved in GH secretion and GH response to provocative stimulation (Tanaka et al., 1990; Gianotti et al., 1998). The results presented here concerning obese and lean control subjects are in agreement with those previously described (De Marinis et al., 1988). In fact in obese fasting women GHRH was less effective in causing an increment in plasma GH concentrations than in normal fasting women. Furthermore lean controls showed an inhibition of the GH response after feeding, whereas the obese women had greater plasma GH concentration responses when tested after a meal, compared with those in the fasting state. The data presented here showed that this ‘paradoxical’ response to GHRH after a meal is absent in PCOS population and, on the contrary, obese patients demonstrated a persistent state of GH-releasing inability.

Several authors indicated that obesity is associated with an abnormal elevation in both fasting and post-prandial FFA concentration (Dieguez and Casanueva, 1995; Cordido et al., 1998). Previous studies demonstrated that a glucose infusion or a rise in plasma FFA concentrations impaired GH responsiveness to GHRH (Sharp et al., 1984; Imaki et al., 1985). Such
Growth hormone secretion related to meal in PCOS subjects

Pre-prandial tests:

![Pre-prandial tests graph]

Post-prandial tests:

![Post-prandial tests graph]

**Figure 1.** Growth hormone (GH) plasma concentrations after growth hormone-releasing hormone (GnRH) administration (top) before a meal (pre-prandial tests) and (bottom) after a meal (post-prandial tests) in (right) patients with polycystic ovarian syndrome (PCOS) and (left) controls. Values are expressed as means ± SD. *P < 0.05 lean versus obese controls; †P < 0.05 lean versus obese PCOS. Peak values comparison in pre-prandial and post-prandial tests: ‡P < 0.05 lean PCOS (before meal) versus lean controls (before meal); §§P < 0.05 lean controls (after meal) versus lean controls (before meal); ¶P < 0.05 lean controls (after meal) versus lean controls (before meal).

negative feedback of FFA on GH release may occur both at the pituitary and hypothalamic level (Pontiroli et al., 1986). In the current study, a primary role of FFA seems to be excluded. Obese controls as well as lean PCOS subjects showed an augmented GH secretion after a meal despite higher basal plasma FFA.

Certainly BMI is correlated with GH-stimulated secretion after a meal in PCOS and in the control population. Therefore body weight per se is an important but not the only factor responsible for blunting GHRH-induced release.

In this concern the relation between AUC-I and AUCd-GH is present only in the PCOS population, where the PCOS obese patients showed the highest AUC-I values in respect to lean PCOS patients and also to obese control patients.

The role of insulin is less established and needs to be studied further. Several pieces of experimental data suggest a possible direct effect of insulin on GH axis; for instance, insulin receptors are present in rat hypothalamus and insulin binding sites have been demonstrated in normal rat pituitary adenoma cells as well as in human pituitary adenoma cells (Ceda et al., 1985; Schwartz et al., 1991). In-vitro studies demonstrate that insulin is able to inhibit the peripheral action of GH (Ji et al., 1999). In normal subjects integrated 24 h GH concentration is elevated during fasting (Ho et al., 1988); moreover, during euglycaemic insulin clamp, a reduction of GH response to GHRH has been observed, whereas during hypoglycaemic insulin clamp the GH response to hypoglycaemia is inversely related to the degree of hyperinsulinaemia (Diamond et al., 1991; Press et al., 1992). Recently it has been demonstrated that insulin, independently of FFA, is able to exert an inhibitory effect on GH release (Lanzi et al., 1997).

Thus insulin negative feedback may have physiological relevance, because the insulin concentrations, able to reduce GH response to GHRH, are commonly observed during the post-prandial period. Therefore, elevated insulin concentrations can inhibit pituitary GH release and insulin resistance may be involved in the alteration of GH secretion, particularly in obese PCOS patients.

In the attempt to explain the different behaviour between controls and hyperandrogenized PCOS subjects the influence of hormonal milieu on GH secretion could be considered. Some studies have demonstrated that steroid environment may affect GH dynamics and higher androgen concentrations could modify the sensitivity to regulatory stimuli (De Marinis et al., 1997; Kaltas et al., 1998).

Moreover since previous work indicated that opioids are involved in the post-prandial GH increase in normal subjects (De Marinis et al., 1989) and it was suggested that derangement of opioid tone may have some relation with the blunted response of GH to GHRH in PCOS (Villa et al., 1997), it may...
also be that the difference in opioid tone among controls and PCOS subjects (Lanzoni et al., 1995) may partially explain the results presented here.

In conclusion the data of the present work indicate for the first time a different behaviour of GHRH-induced GH secretion related to food ingestion in PCOS in respect to control subjects. Obese PCOS patients did not exhibit any change of GH response or the paradoxical response observed in matched controls. Several factors apart from BMI may be involved in these pathophysiological events. Further studies are needed in order to elucidate this exciting new field of research.

References


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