Plasma ghrelin concentrations in patients with polycystic ovary syndrome before and after 6-months therapy: Correlation with androgen levels

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Abstract

OBJECTIVE: Ghrelin is a natural ligand of the growth hormone secretagogue receptor and has been shown to be a potent stimulant of GH secretion. It has also orexigenic effects and regulates energy homeostasis. Recent studies claim that ghrelin influences the androgen level and probably takes part in PCOS pathomechanism. The aim of the study was an assessment of ghrelin level in plasma in women with PCOS before and after the treatment and ghrelin's influence on androgen level change.
MATERIAL AND METHODS: The study included 25 women with the diagnosed PCOS (mean age 25.3±4.05 yr). The tests were done twice: before the treatment and after 6-month therapy with Diane 35 (cyproterone acetate 2 mg with ethinylestradiol 35 μg). Following hormones were measured: ghrelin, free testosterone, androstendione, dehydroepiandrosterone sulfate, 17-OH-progesterone and estradiol.
RESULTS: The received results in both groups were compared with the control group (11 healthy women, mean age 26.0±2.6 yr). No statistically significant differences in ghrelin levels before (187.8±8.1 fmol/ml) and after the therapy (185.6±9.5 fmol/ml) were found. Similar results were received when two groups of women compared with the control (186.5±8.7 fmol/ml). No correlations between ghrelin and androgen levels were confirmed.
CONCLUSIONS: Final conclusion is that there is no direct impact of ghrelin level on PCOS pathogenesis, however, its role in development of obesity, hyperinsulinemia and insulin resistance co-occurring with metabolic disorders syndrome cannot be excluded.

ORIGINAL ARTICLE

Introduction

Polycystic Ovarian Syndrome (PCOS) is one of the most often diagnosed endocrynologic disordes in women at the reproductive age and refers to about 5–10% of this population [24,25]. Diagnostic criteria of PCOS, based on the agreements of National Institutes of Health from 1990 [29], include menstruation disorders caused by oligoovulation or an absence of ovulation and clinical or biochemical features of androgenisation. Clinical symptoms are often accompanied by characteristic pathomorphologic picture of ovaries with a presence of 12 or more follicles in each ovary measuring 2–9 mm in diameter, and/or increased ovarian volume(>10 ml) [1].

The women with PCOS are exposed to a danger of metabolic disordes. The symptoms in those patients include obesity, lowered cell insulin sensitivity and hyperinsulinemia increasing a risk of type 2 diabetes mellitus [25,6]. The observed dislipidemia, arterial hypertension and coagulation disorders are vital risk factor of cardiac and vascular disorders [25].

Etiopathogenesis of PCOS is complicated and has not been clearly explained yet. The basis of PCOS development is a subject of many studies defining the factors influencing the development of clinical symptoms [25,28,5]. Recent studies claim that ghrelin influences the androgen level and probably takes part in PCOS pathomechanism [16,9,7].

Ghrelin is the particle with a function of natural ligand of GHS-R (growth hormone secretagogue receptor). It stimulates the pituitary to release GH (growth hormone) and takes part in the regulation of energy homeostasis causing effects opposite to leptin. Ghrelin affects the centers of appetite and satiety in brain. It can stimulate the appetite through neuropeptide Y and AGRP (agouti–related protein) activation in the neurons of the arcuate hypothalamus nucleus [4,18,26]. The level of ghrelin is lowered in obese persons and in hyperglycemia, however it is raised in persons suffering from anorexia or in starvation cachexia [13,19].

The aim of the study was an assessment of plasma ghrelin level in women with PCOS before and after the treatment and ghrelin's influence on androgen level change.

Materials and methods

The study included 25 women with the diagnosed PCOS (mean age 25.3±4.05 yr). The diagnosis was based on the criteria presented at the conference of National Institutes of Health in 1990 [29]. Increased levels of androstendione in serum and menstruation disorders of oligomenorrhoea type were found in all patients. Other diseases causing anovulation and androgenisation features, hyperprolactinemia, Cushing's syndrome, tumors secreting androgens, classical and non-classical form of innate adrenomegaly, abnormal thyroid function were excluded. The women did not take any medicines containing estrogens or progestogens or other medicines influencing the level of androgens. The tests were done twice: before the treatment and after 6-month therapy with Diane 35 (cyproterone acetate 2 mg with ethinylestradiol 35 µg). The received results in both groups were compared with the control group which consisted of 11 healthy women (mean age 26.0±2.6 yr).

Blood was sampled from ulnar vein after overnight fast, between 8.00 and 9.00 o'clock, at the early follicular phase. After centrifugation the serum was stored in temperature –70 °C for further analysis. To obtain the plasma needed for ghrelin marking, blood samples were placed in test tubes containing EDTA. The plasma was extracted in order to remove multimolecular proteins and the collected eluate was left to evaporate. The extract was stored in temperature –70 °C for further analysis.

Serum free testosterone (fT) was determined by radioimmunoassay (RIA) (DSL, USA) with a lower sensitivity of 0.18 pg/ml and intra- and interassay coefficients of variation (CV) of 5.03% and 8.3%, respectively. Androstendione was measured by enzyme immunoassay (ELISA) (IBL, Hamburg) with lower detectable concentrations of 0.04 ng/ml and intra- and interassay CV of 4.8% and 6.86%, respectively. Dehydroepiandrosterone sulfate (DHEAS) was assayed by a chemiluminescence immunoassay (DPC, USA). Minimal detectable concentrations was 3 µg/dl (0.08 µmol/l) and intra- and interassay CV of 8.24% and 12.03%, respectively. Serum 17-OH-progesterone was determined by RIA (BIOSOURCE, Belgia) with a lower level of sensitivity 0.02 ng/ml and intra- and interassay CV of 5.63% and 7.2%, respectively. Estradiol (E_2) was measured by chemiluminescence immunoassay (Roche, USA) with lower detectable concentrations of 5 pg/ml and intra- and interassay CV of 2.74% and 3.42%, respectively. Serum ghrelin was determined by ELISA (Phoenix Pharmaceuticals, Inc., Kalifornia, USA) with minimal detectable concentrations of 0.9 ng/ml and intra- and interassay CV of less then 5% and 14%, respectively.

The study protocol was approved by the Ethics Committee of the Silesian Medical University.

Statistic analysis was made with STATISTICA program. Arithmetic means and standard deviations were calculated and correlation analysis was performed. To define significance of the difference among means, t-Student test with the statistic confidence level $p<0,05$ was applied.

Results

The mean arithmetic values and standard deviations for the examined parameters in the group of women with PCOS before and after treatment and in control group are presented in Table 1. The values of arithmetic means and standard deviations of ghrelin for each group are presented in Figure 1.

No statistically significant differences in ghrelin levels before (187.8±8.1 fmol/ml) and after the therapy (185.6±9.5 fmol/ml) were found. Similar results were received when two groups of women compared with the

Table 1. Comparison of anthropometric data and hormonal parameters between PCOS women before and after Diane 35 therapy and control group.

Variables	PCOS before therapy (n=25) mean (SEM)	PCOS after therapy (n=25) mean (SEM)	Control group (n=11) mean (SEM)
age (yr)	25.3 (4.0)	25.3 (4.0)	26.0 (2.6)
height (cm)	162 (6.5)	162 (6.5)	164 (2.2)
weight (kg)	68.9 (21.4)	68.0 (21.8)	63.2 (14.1)
BMI (kg/m^2)	26.2 (7.6)	25.9 (7.9)	23.8 (5.2)
fT (pg/ml)	2.7 (2.8)	1.2 (1.4)	1.4 (0.9)
androstendione (ng/ml)	5.3 (1.6)**	2.8 (1.6)	2.5 (1.1)
DHEAS (µg/dl)	289.3 (127.7)*	188.2 (123.4)	208.7 (41.7)
ghrelin (fmol/ml)	187.8 (8.1)	185.6 (9.5)	186.5 (8.7)
17OH-P (ng/ml)	2.2 (2.0)	1.5 (0.9)	2.0 (0.6)
E_2 (pg/ml)	47.0 (23.7)	35.9 (22.6)	49.3 (21.9)

BMI – body mass indexes; fT – free testosterone; DHEAS – dehydroepiandrosterone sulfate; 17OH-P – 17-OH-progesterone; E_2 – estradiol.
**– statistically significant differences ($p < 0,05$) between PCOS women before and after therapy and between PCOS women before therapy and control group.
* - statistically significant differences ($p<0,05$) between PCOS women before and after therapy.

control (186.5±8.7 fmol/ml). No correlation between the ghrelin level and other parameters in all the three groups were found. While observing the patients before and after treatment, statistically significant differences in androstendione ($p<0.0001$) and DHEAS ($p<0.02$) levels were found. No statistically significant differences among other parameters were fund for each group of women.

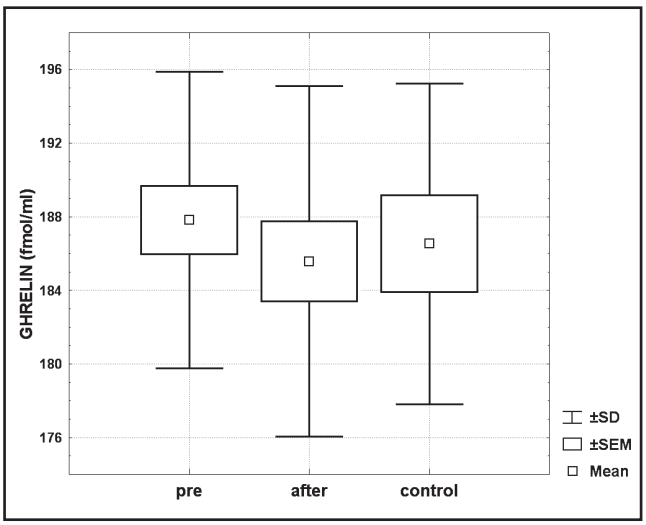

Figure 1. The values of arithmetic means and standard deviations of ghrelin.

Discussion

Pathogenesis of PCOS is not fully recognized. Special controversies are evoked by the origin of surplus of testosterone and androstendione [28,2,12]. Many studies concentrate on the problem whether the increased androgen level is the result of inner disorders within ovary hilus cells or is a response to disorders in other organs. Recently, reports indicating the role of ghrelin in steroidogenesis regulation and gonad function[16,9]. A presence of ghrelin and its GHS-R receptors was revealed in human ovaries and other tissues producing testosterone, such as placenta and testicles[9,8,17,3]. Some authors report on inhibiting influence of ghrelin on secretion of lutenizing hormone [9,8]. It is suggested that ghrelin can take part in regulation of androgen secretion by ovary hilus cells and therefore, influence the development of hyperandrogenization symptoms in women with PCOS [16,9,7].

Pogotto et al. [16] observed a presence of negative correlation between the level of ghrelin and the level of androstendione in a group of obese women with PCOS. However Gambinerii et al. [7] examined a similar group of women and found out the increased ghrelin level after anti-androgen treatment. In our study, no correlation between ghrelin and androgen levels in serum of women with PCOS was found both before and after the treatment. As opposed to the mentioned studies, our study group did not consist of obese women (mean BMI 26.2±7.6). It is known that ghrelin influence energy homeostasis and its level changes are observed mainly in persons with upset energy balance, i.e. obese and hunger devastated organisms [13,23]. Many studies have shown a decreased level of ghrelin in people with high values of BMI, returning to their norm after body mass loss [21,27]. According to those facts it can be expected that obese patients with PCOS will have decreased levels of ghrelin. Pogotto et al. [16] admit that the correlation between ghrelin and androstendione levels was not

limited only to women with PCOS but also occurred in obese patients without PCOS. The differences observed in Pogotto's [16] and Gambinerii's [7] studies may relate to the bigger body mass in the examined women. This conclusion seems to be confirmed by the fact that the studies including women with PCOS with normal BMI, as well as our study, did not reveal any relations between the ghrelin level and changes in androgen level [15,22].

The ghrelin levels in blood serum of the women with PCOS in both our groups, before and after the treatment, was mean 187.82 fmol/l and are comparable to the results of other authors [16,15,22]. No changes in ghrelin level were found both in the group before and after the treatment and in the control. The similar results were found by Orio et al. [15], whereas Schofl et al. [22] report lower ghrelin levels in serum of women with PCOS as compared with the control group. The changes only referred to the patients with diagnosed insulin resistance, which suggest that the decreased ghrelin level was a consequence of insulin resistance common for all the patients. There are many reports showing the ghrelin impact on glucose and insulin metabolism. Many authors report about decreased ghrelin levels in people with lowered insulin sensitiveness or suffering from type 2 diabetes mellitus [11,20,14]. That information may explain that fact that Schofl et al. [22] results were not confirmed in case of women with PCOS who did not hag insulin resistance. The level of ghrelin in that group, similarly to our results, was comparable with the control [22].

No correlation between ghrelin level and other hormones, such as DHEAS, 17-OHP and E_2 was found in our study. These results confirm the findings of the authors who did not show a correlation between ghrelin and the parameters such as DHEAS, 17-OHP, E_2, prolactin, lutenizing hormone, follicle-stimulating hormone, progesterone and sex hormone binding globulin [16,15,22]. However ghrelin levels in the recent Glintborget et al. [10] study were decreased in hirsute PCOS patients and showed a significant, negative correlation with testosterone independent of body composition.

Summing up the above presented considerations, our study did not show differences in ghrelin level plasma of women with PCOS in comparison with the control. No changes in ghrelin level after the half-year treatment were found out, additionally no correlations between ghrelin and androgen levels were confirmed. Therefore our final conclusion is that there is no direct impact of ghrelin level on PCOS pathogenesis, however, its role in development of obesity, hyperinsulinemia and insulin resistance co-occurring with metabolic disorders syndrome cannot be excluded.

REFERENCES

1 Balen A. Ovulation induction for polycystic ovary syndrome. Hum Fertil. 2003; **3**: 106–11.
2 Calvo R, Asuncion M, Telleria D, Sancho J, San Millan S, Escobar-Morreale H. Screening for mutations in the steroidogenic acute regulatory protein and steroidogenic factor-1 genes, and in CYP11A and dosage-sensitive sex reversal-adrenal hypoplasia gene on the X chromosome, gene-1 (DAX-1), in hyprandrogenic hirsute women. J Clin Endocrinol Metab. 2001; **86**: 1746–9.
3 Caminos J, Tena-Sempere M, Gaytăn F, Sanchez–Criado J, Barreiro M, Nogueiras R, et al.. Expression of ghrelin in the cyclic and pregnant rat ovary. Endocrinology. 2003; **144**: 1594–1602.
4 Chen H, Trumbauer M, Chen A, Weingarth D, Adams J, Frazier E, et al. Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. Endocrinology. 2004; **145**: 2607–12.
5 Dunaif A. Genes and environment in the pathogenesis of PCOS. Gynecol Endocrinol. 2004; **18**: 123.
6 Fica S, Gheorghisan A, Virtej I, Vladareanu R, Bunghez R.. Prevalence of glucose metabolism abnormalities in polycystic ovary syndrome PCOS - insulin resistance and associated risks. Gynecol Endocrinol. 2004; **18**: 308
7 Gambineri A, Pagotto U, Tschop M, Vicennati V, Manicardi E, Carcello A, et al. Anti-androgen treatment increases circulating ghrelin levels in obese women with polycystic ovary syndrome. J Endocrinol Invest. 2003; **26**: 629–34.
8 Gaytan F, Barreiro M, Caminos J, Chopin L, Herington A, Morales C, et al. Expression of ghrelin and its functional receptor, the type 1a growth hormone secretagogue receptor, in normal human testis and testicular tumors. J Clin Endocrinol Metab. 2004; **89**: 404–9
9 Gaytan F, Barreiro M, Chopin L, Herington A, Morales C, Pinilla L, et al. Immunolocalization of ghrelin and its functional receptor, the type 1a growth hormone secretagogue receptor, in the cyclic human ovary. J Clin Endocrinol Metab. 2003; **88**: 879–87
10 Glintborg D, Andersen M, Hagen C, Frystyk J, Hulstrom V, Flyvbjerg A, et al. Ev aluation of metabolic risk markers in polycystic ovary syndrome (PCOS). Adiponectin, ghrelin, leptin and body composition in hirsute PCOS patients and controls. Eur J Endocrinol. 2006; **155**(2): 337–45.
11 Katsuki A, Urakawa H, Gabazza EC, Murashima S, Nakatani K, Togashi K, et al. Circulating levels of active ghrelin is associated with abdominal adiposity, hyperinsulinemia and insulin resistance in patients with type 2 diabetes mellitus. Eur J Endocrinol. 2004; **151**: 573–7.
12 Legro R, Driscoll D, Strauss J, Fox J, Dunaif A.. Evidence for a genetic basis for hyperandrogenemia in polycystic ovary syndrome. Proc. Natl. Acad. Sci. U S A. 1998; **95**: 14956–0.
13 Marzullo P, Verti B, Savia G, Walker G, Guzzaloni G, Tagliaferri M, et al. The relationship between active ghrelin levels and human obesity involves alterations in resting energy expenditure. J Clin Endocrinol Metab. 2004; **89**: 936–9.
14 McLaughlin T, Abbasi F, Lamendola C, Frayo S, Cummings D. Plasma ghrelin concentrations are decreased in insulin-resistant obese adults relative to equally obese insulin-sensitive controls. J Clin Endocrinol Metab. 2004; **89**: 1630–5.
15 Orio F, Lucidi P, Palomba S, Tauchmanova L, Cascella T, Russo T, et al. Circulating ghrelin concentrations in the polycystic ovary syndrome. J Clin Endocrinol Metab. 2003; **88**: 942–5.
16 Pagotto U, Gambineri A, Vicennati V, Heiman M, Tschop M, Pasquali R. Plasma ghrelin, obesity, and the polycystic ovary syndrome: correlation with insulin resistance and androgen levels. J Clin Endocrinol Metab. 2002; **87**: 5625–9.
17 Papotti M, Ghe C, Cassoni P, Catapano F, Deghenghi R, Ghigo E, et al. Growth hormone secretagogue binding sites in peripheral human tissues. J Clin Endocrinol Metab. 2000; **85**: 3803–7.
18 Popovic V, Miljic D, Micic D, Damjanovic, Arvat E, Ghigo E, et al. Ghrelin main action on the regulation of growth hormone release is exerted at hypothalamic level. J Clin Endocrinol Metab. 2003; **88**: 3450–3.

19 Poykko S, Kellokoski E, Horkko S, Kauma H, Kesaniemi A, Ukkola O. Low plasma ghrelin is associated with insulin resistance, hypertension, and prevalence of type 2 diabetes. Diabetes. 2003; **52**: 2546–53.
20 Purnell J, Weigle D, Breen P, Cummings D. Ghrelin levels correlate with insulin levels, insulin resistance, and high-density lipoprotein cholesterol, but not with gender, menopausal status, or cortisol levels in humans. J Clin Endocrinol. Metab. 2003; **88**: 5747–52.
21 Rosickă M, Kršek M, Matoulek M, Jarkovskă Z, Marek J, Justovă V, et al. Serum ghrelin levels in obese patients: the relationship to serum leptin levels and soluble leptin receptors levels. Physiol Res. 2004; **52**: 61–6.
22 Schofl C, Horn R, Schill T, Schlosser H, Muller M, Brabant G. Circulating ghrelin levels in patients with polycystic ovary syndrome. J Clin Endocrinol Metab. 2002; **87**: 4607–10.
23 Shiiya T, Nakazato M, Mizuta M, Date Y, Mondal M, Tanaka M, et al. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. J Clin Endocrinol Metab. 2002: **87**: 240–4.
24 Silfen M, Denburg M, Manibo A, Lobo R, Jaffe R, Ferin M, et al. Oberfield S. Early endocrine, metabolic, and sonographic characteristics of polycystic ovary syndrome (PCOS): comparison between nonobese and obese adolescents. J Clin Endocrinol Metab. 2003; **88**: 4682–8.
25 Staszewicz P, Kos–Kudła B, Strzelczyk J, Nasiek M. Metabolic consequences of policystic ovary syndrome. Diabetol Pol. 2003; 10: 346–52 (in polish).
26 Toshinai K, Date Y, Murakami N, Shimada M, Mondal M, Shimbara T, et al. Ghrelin – induced food intake is mediated via the orexin pathway. Endocrinology. 2003; **144**: 1506–12.
27 Vendrell J, Broch M, Vilarrasa N, Molina A, Gómez J, Gutiérrez C, et al. Resistin, adiponectin, ghrelin, leptin, and proinflammatory cytokines: relationships in obesity. Obes Res. 2004;**12**: 962–71.
28 Wood J, Nelson V, Ho C, Jansen E, Wang C, Urbanek M, et al. The molecular phenotype of polycystic ovary syndrome (PCOS) theca cells and new candidate PCOS genes defined by microarray analysis. J Biol Chem. 200; **278**: 26380–90.
29 Zawadzki JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome: Towards a rational approach. In: , Dunaif A, Givens JR, Haseltine FP, Merriam GR (Eds), (Series Ed: Hershman, SM), Current Issues in Endocrinology and Metabolism, 4 , Blackwell Scientific Publications, Boston.1992, chap.32.