Total ghrelin levels during acute insulin infusion in patients with polycystic ovary syndrome

D. Micic[1], M. Sumarac-Dumanovic[1], A. Kendereski[1], G. Cvijovic[1], S. Zoric[1], D. Pejkovic[1], J. Micic[2], N. Milic[3], C. Dieguez[4], and F.F. Casanueva[4]

[1]Center for Metabolic Disorders in Endocrinology, Institute of Endocrinology, Diabetes and Diseases of Metabolism; [2]Institute of Gynecology, and Obstetrics, Clinical Center of Serbia; [3]Institute of Medical Statistics, School of Medicine, University of Belgrade, Belgrade, Serbia; [4]Department of Endocrinology, University of Santiago de Compostela, Santiago de Compostela, Spain

ABSTRACT. Controversial data were reported concerning fasting ghrelin (decreased, normal or elevated) in polycystic ovary syndrome (PCOS). The aim of our study was to clarify ghrelin levels in non-obese, overweight, and obese PCOS patients; to investigate the effect of acute insulin infusion on ghrelin in PCOS as a chronic insulin-resistant state, with and without the impact of obesity, and to examine ghrelin-androgen interaction. In that order, we evaluated 1) ghrelin levels among 8 non-obese patients with PCOS [body mass index (BMI): 20.52±1.31 kg/m^2], 8 overweight and obese patients with PCOS (BMI: 34.36±6.53 kg/m^2) and their respective controls, 2) ghrelin suppression during euglycemic hyperinsulinemic clamp, and 3) ghrelin-androgen interrelationship. After overnight fast, 2-h euglycemic hyperinsulinemic clamp, was performed in all investigated women. Fasting ghrelin was significantly lower in non-obese PCOS than in controls (64.74±25.69 *vs* 108.36±52.60; *p*<0.05) as well as in overweight and obese PCOS in comparison with controls (38.71±14.18 *vs* 98.77±40.49; *p*<0.05). Insulin infusion significantly suppressed ghrelin in all subgroups of investigated women. Analysis of variance for repeatable measures confirmed that there was no significant difference in pattern of response between PCOS and controls. In conclusion, women with PCOS had lower fasting ghrelin and decreased insulin sensitivity independently of their BMI, compared to the controls. In addition, there were no differences between fasting ghrelin levels among non-obese, overweight, and obese women with PCOS. During euglycemic hyperinsulinemic clamp, ghrelin decreased in all studied groups to a similar extent, implying that, compared to chronic hyperinsulinemia, acute hyperinsulinemia reduces ghrelin levels independently of the degree of insulin resistance.
(J. Endocrinol. Invest. 30: 820-827, 2007)

INTRODUCTION

Ghrelin is a gastric peptide, which stimulates GH and has orexigenic and adipogenic properties (1-3). Plasma ghrelin levels are influenced by nutritional status and it is postulated that they play a role in regulating food intake and body weight. Inverse correlation between ghrelin and adiposity has been demonstrated, with higher ghrelin levels among lean subjects in comparison with obese subjects (4, 5).

Controversial data were reported concerning fasting ghrelin levels in patients with polycystic ovary syndrome (PCOS). Ghrelin levels were reported to be decreased (6-9), normal (10) or elevated (11). Inverse relationship between circulating ghrelin and androgens was demonstrated in patients with PCOS and it was suggested that ghrelin might be involved in steroidal synthesis and/or action in PCOS (9), although it was not confirmed in other studies (6).

PCOS is associated with insulin resistance and obesity and it is known that associated obesity may worsen conditions of insulin resistance (12), so that it represents a natural human model in which ghrelin levels could be evaluated in the light of different possible influences of insulin levels, insulin resistance, obesity, and androgen levels.

Controversial data were reported concerning effects of insulin infusion on the ghrelin levels: from the sup-

Key-words: Ghrelin, PCOS, euglycemic clamp, insulin, SHBG.

Correspondence: D. Micic, MD, PhD, Center for Metabolic Disorders in Endocrinology, Institute of Endocrinology, Diabetes and Diseases of Metabolism, Clinical Center of Serbia, Dr Subotica 13, 11000 Belgrade, Serbia.

E-mail: micicd@eunet.yu

Accepted March 2, 2007.

pression (10) to the lack of suppression (13). However, hyperinsulinemia during a euglycemic clamp is able to suppress plasma ghrelin concentrations in uncomplicated obesity, and this effect appears to be positively related to insulin sensitivity (14). The aim of our study was first, to further clarify controversial data reported for ghrelin levels in non-obese, overweight, and obese PCOS patients (and its possible impact on PCOS phenotype), second, to investigate the effect of acute insulin infusion on ghrelin levels in a chronic insulin-resistant state such as PCOS with and without the impact of obesity, and third, to examine possible interaction between ghrelin and androgen levels. In order to do that, we evaluated 1) ghrelin levels among non-obese, overweight, and obese patients with PCOS and their respective, age- and body mass index (BMI)-matched controls, 2) relation of ghrelin suppression during euglycemic hyperinsulinemc clamp with basal insulin levels and indices of insulin sensitivity, and 3) interrelationship between ghrelin and androgen levels.

MATERIALS AND METHODS

Sixteen women with PCOS and 16 age- and weight-matched healthy women were included in the study. Anthropometric characteristics of the investigated groups are presented on Table 1. The diagnosis of PCOS was based on the presence of oligo/amenorrhea, hyperandrogenism, and polycystic ovaries as confirmed by ultrasound imaging (15). Patients having thyroid, renal, liver, or cardiovascular dysfunctions as well as previous gastrointestinal surgery and Type 2 diabetes based on an oral glucose tolerance test (OGTT) (16) were excluded. The presence of Cushing's syndrome and congenital adrenal hyperplasia, conditions both associated with hyperandrogenism, were excluded by appropriate testing. All controls had regular menstrual cycles and did not show any signs or symptoms of hyperandrogenism. All subjects were without medication for at least 3 months before the study; none of the studied individuals were undergoing caloric restrictions at the time of the study or taking regular physical exercise. All 32 investigated women gave informed consent for participating in the study and the local Ethics Committee approved the study. PCOS patients and control women were divided in 4 subgroups according to BMI (greater or less than 25 kg/m^2) in group A (non-obese PCOS), group B (non-obese control), group C (overweight and obese PCOS) and group D (overweight and obese control). Basal hormone levels (means±SE) in PCOS women and control women are presented on Table 1.

After an overnight fast, standard 2-h euglycemic hyperinsulinemic clamp was performed in all investigated women. An iv catheter was placed in an antecubital vein for insulin and glucose infusions. Another catheter was placed retrograde in a dorsal vein of the contralateral hand for blood sampling. The hand was placed in a heating pad to arterialize the blood. Regular human insulin (NovoNordisk) 0.1 IU/kg body weight (BW)/h was infused for 2 h and a variable infusion of 20% glucose was given to maintain plasma glucose at the fasting level. Subsequently, the insulin infusion was discontinued and the 20% glucose infusion was tapered gradually to maintain plasma glucose at the same level for another 2 h. Arterialized plasma glucose was measured every 10 min during the procedure to guide the glucose infusion. Blood samples were collected at 0, 30, 45, 60, 90, 120, and 240 min for ghrelin determination and at 0, 15, 30, 45, 60, 90, 100, 110, 110, 120, 150, 180, 210, and 240 min in the period 0-240 min for determination of plasma insulin. The computation of M index was done according to previously reported equation (17). The total M was calculated from the means of the five 20-min periods from 20 to 120 min of the study and is expressed in the dimensions of mg/(kgxmin). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as follows: plasma insulin x fasting plasma glucose/22.5 using SI U (18). Plasma glucose (glucose-oxydase method, Randox, UK; mmol/l), insulin [RIA, INEP, Zemun; mU/l, Intra-assay coefficients of variation (CV) were 20.4, 7.6, and 6.1% for

Table 1 - *Baseline anthropometric and hormonal characteristics of polycystic ovary syndrome (PCOS) patients and controls (*p<0.05 between PCOS and comparative controls, †p<0.05 between group A and group C).*

Group	Group A non-obese PCOS	Group B non-obese controls	Group C overweight and obese PCOS	Group D overweight and obese controls
No.	8	8	8	8
Age	22.12±2.85	24.87±6.91	25.06±6.37	28.50±4.98
BMI	20.52±1.31	20.19±1.16	34.36±6.53	31.00±3.66
Insulin (mU/l)	16.26±8.25	13.74±4.88	27.83±13.59	14.52±6.91
HOMA-IR	3.32±1.84	2.63±1.13	5.91±3.48*†	2.80±1.65
M index	4.44±2.17*	7.80±3.66	1.82±0.64	3.92±2.87
Testosterone (nmol/l)	3.60±2.36	1.94±0.69	4.89±3.05*	2.11±0.54
SHBG (nmol/l)	26.16±8.67*	54.66±17.29	15.83±7.08*	43.20±14.36
FAI (%)	18.44±22.91	3.59±1.52	38.73±31.46*†	5.00±0.94
Ghrelin (pg/ml)	64.74±25.69*	108.36±52.60	38.71±14.18*	98.77±40.49

BMI: body mass index; HOMA-IR: homeostasis model assessment of insulin resistance; FAI: free androgen index.

concentrations 9.4, 23.7, and 181.6 mU/l, while inter-assay CV were 22.1, 10.6, and 16.8% for concentrations 8.6, 33.8, and 153.9 mU/l], ghrelin (RIA, Phoenix Pharmaceuticals, USA; pg/ml; CV for intra-assay 5.5%, CV for inter-assay <10%); SHBG (RIA, CIS bio International, France; nmol/l, intra-assay CV were 2.5, 3.6, 4.6, 3.9, and 6.1% for concentrations 17.1, 24.1, 35.0, and 71.9 nmol/l, while inter-assay CV were 4.1, 5.5, 4.6, and 4.7% for concentrations 15.8, 24.0, 34.5 and 69.6 nmol/l) and testosterone (RIA, CIS bio International, France; nmol/l, intra-assay CV were 4.5, 3.8, and 5.5% for concentrations 4.8, 11.4, and 26.5 nmol/l, while inter-assay CV were 5.1, 4.8, and 4.8% for concentrations 3.5, 9.1, and 23.3 nmol/l) levels were measured in respective time intervals. All samples from individual subjects were analyzed in duplicate for each hormone. All samples were immediately chilled on ice, centrifuged, and serum or plasma aliquots frozen at –80 C until assayed. Free androgen index (FAI) was calculated as a ratio of total testosterone to SHBG (19).

Statistical analysis

Results are reported as the mean values±SEM. Normal distribution and homoscedasticity of continuous variables were tested by means of the Kolmogorov-Smirnov test. Relationships between variables were assessed by the Pearson's and Spearman's correlation test, as appropriate. To avoid multiple comparisons, the data at the different times of the study were evaluated by means of two-way analysis of variance (ANOVA), applying a within-treatment and group design. The within-subject ANOVA, with the same design, was used to compare the modifications observed during the course of the study. Univariate and Multivariate Linear Regression Analysis were also performed. Statistical evaluations were performed by running the Statistical Package for the Social Sciences (SPSS)/PC+software package (SPSS, Inc., Chicago, IL) on a personal computer; *p*-values of less than 0.05 were regarded as statistically significant.

RESULTS

Baseline characteristics of PCOS and control women are presented in Table 1. There were no differences among the ages between the investigated groups ($p>0.05$). There were no significant differences in BMI between PCOS subgroups and respective controls ($p>0.05$). There were significant differences between BMI among group A and B compared to group C and D ($p<0.05$). There was no difference in basal insulin levels among the investigated groups ($p>0.05$). HOMA-IR index was significantly higher in group C compared to respective control (5.91±3.48 *vs* 2.80±1.65, $p<0.05$). HOMA-IR index was also significantly higher in group C compared to group A (5.91±3.48 *vs* 3.32±1.84; $p<0.05$). M index in group A was significantly lower compared to group B (4.44±2.17 *vs* 7.80±3.66, $p<0.05$). There were no differences between M index among group C and D ($p>0.05$). There were no significant difference between M index among group A and group C ($p=0.053$). There were no differences among testosterone levels between group A and group B ($p>0.05$). Testosterone in group C was significantly higher compared to testosterone in group D (4.89±3.05 *vs* 2.11±0.54, $p<0.05$). SHBG in group A was significantly lower compared to SHBG in group B (26.16±8.67 *vs* 54.66±17.29, $p<0.05$). SHBG was significantly lower in group C compared to group D (15.83±7.08 *vs* 43.20±14.36, $p<0.05$). There were no difference in FAI levels between group A and B (18.44±22.91 *vs* 3.59±1.52, $p>0.05$). FAI was significantly higher in group C compared to group D (38.73±1.46 *vs* 5.00±0.94, $p<0.05$). There was significant difference in FAI level between group A and C (18.44±22.91 *vs* 38.73±31.46, $p<0.05$). Fasting ghrelin level was significantly lower in group A compared to group B (64.74±25.69 *vs* 108.36±52.60; $p<0.05$) (Fig. 1). Fasting ghrelin level was also significantly lower in group C compared to group D (38.71±14.18 *vs* 98.77±40.49; $p<0.05$). There were no significant differences between fasting ghrelin levels among groups A and C (64.74±25.69 *vs* 38.71±14.18, $p>0.05$).

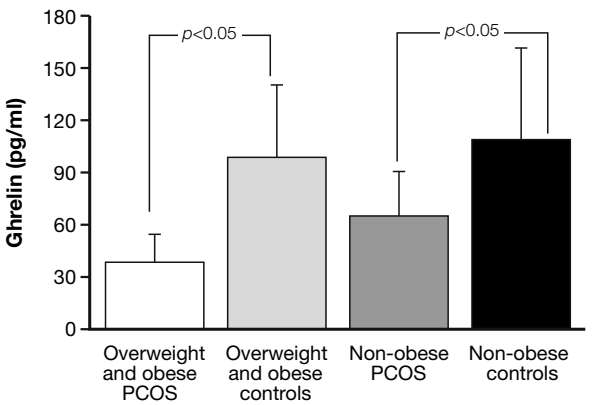

Fig. 1 - *Basal ghrelin levels in polycystic ovary syndrome (PCOS) patients and controls.*

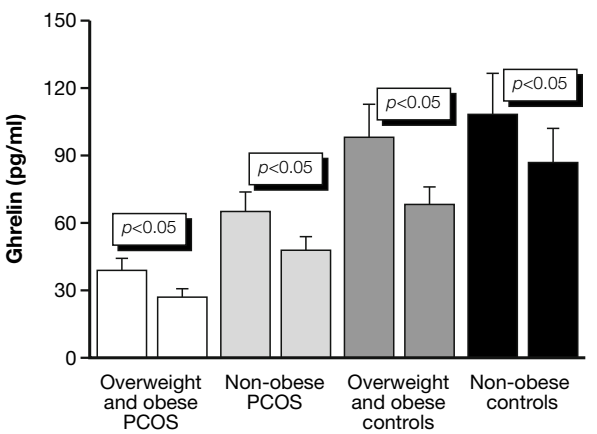

Fig. 2 - *Basal ghrelin levels and nadir of ghrelin during euglycemic clamp in polycystic ovary syndrome (PCOS) patients and controls.*

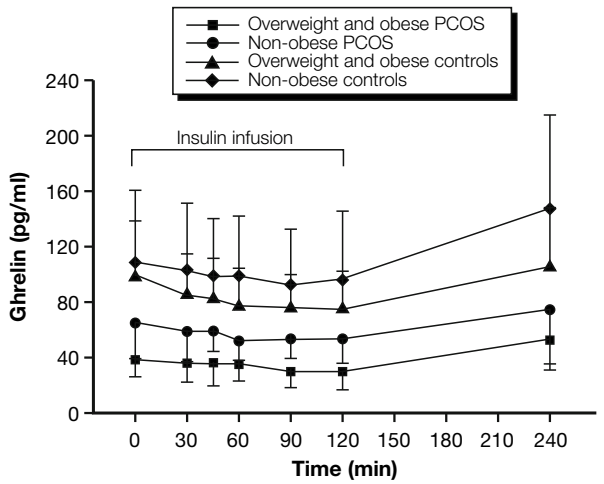

Fig. 3 - *Ghrelin levels during euglycemic clamp in polycystic ovary syndrome (PCOS) patients and controls.*

There were also no differences between group B and D, concerning ghrelin level (108.36±52.60 *vs* 98.77±40.49, $p>0.05$). There were no difference in insulin plateau levels during the euglycemic clamp (30-120 min of clamp study) between PCOS and controls (101.94±15.24 *vs* 98.48±13.94 mU/l, $p>0.05$). There was significant suppression of ghrelin levels during insulin infusion in all subgroups of investigated women (Fig. 2). There were no significant differences between percentage of ghrelin nadir among group A (–21.63±6.49), group B (–18.77±5.08), group C (–29.47±6.40), and group D (–25.44±7.68) ($p>0.005$) (Fig. 2). ANOVA for repeatable measures confirmed that there was no significant difference in pattern of response between PCOS and control women (Fig. 3). Ghrelin returned to basal level at 120 min after discontinuation of insulin infusion in all groups (Fig. 3). There was no significant correlation between ghrelin and BMI (r=–0.278, $p=0.123$), taking into the consideration the whole group of investigated women. However, when only patients with PCOS (non-obese, overweight, and obese) were taken into account, a correlation coefficient between ghrelin and BMI (r=–0.474, $p=0.064$) was at the border of statistical significance (Fig. 4). Significant correlation was obtained between basal ghrelin level and SHBG (r=0.688, $p<0.0001$) (Fig. 5); testosterone (r=–0.460, $p=0.008$) and FAI (r=–0.499, $p=0.004$) (Fig. 6); basal insulin (r=–0.547, $p=0.001$); M index (r=0.429, $p=0.014$) and HOMA-IR (r=–0.432, $p=0.014$) (Fig. 7). Multiple linear regressions were performed to ascertain the predictive value of BMI, testosterone, SHBG, FAI, basal insulin, M index, and HOMA on fasting ghrelin. The best predictive value of SHBG on fasting ghrelin was confirmed in all investigated women (r=0.688, $p<0.0001$) (Table 2, Fig. 5).

DISCUSSION

Controversial data were reported concerning fasting ghrelin levels in patients with PCOS: from normal (10) to decreased (6-20) or elevated levels (11). We demonstrated in our PCOS patients reduced level of total ghrelin, compared to their respective con-

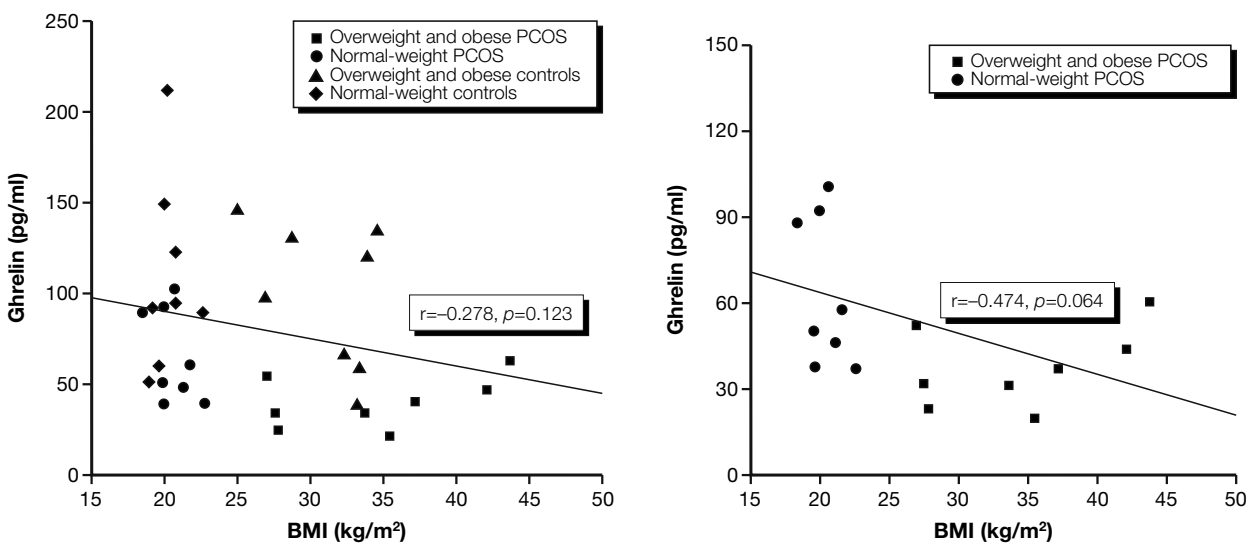

Fig. 4 - *Correlations between ghrelin levels and body mass index (BMI) in whole group and in patients with polycystic ovary syndrome (PCOS).*

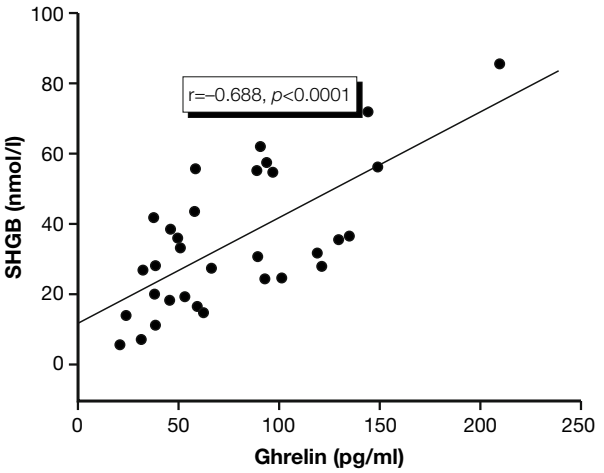

Fig. 5 - *Correlation between basal ghrelin levels and SHBG.*

trols, no matter whether they were non-obese or overweight and obese. Looking for interrelation between insulin and ghrelin, suppression of ghrelin was obtained during insulin infusion under euglycemic clamp condition in some studies (21, 22) but not in all (13). To our knowledge, our study is the first to use euglycemic hyperinsulinemic clamp to investigate ghrelin levels during insulin infusion in patients with PCOS. Suppression of ghrelin during insulin infusion was confirmed in our PCOS women as well as in controls. There was no difference in pattern of ghrelin response during euglycemic clamp between PCOS and healthy women. Ghrelin levels returned to near basal values after insulin infusion was stopped, as it was previously reported in other studies (21, 22). Our data indicated that the insulin-induced suppression of ghrelin levels is preserved in insulin-resistant states, such as PCOS and obesity. It was previously demonstrated that acute hyperinsulinemic condition (as it is found in post-prandial state and during euglycemic hyperinsulinemic clamp) and chronic hyperinsulinemic condition (as insulin resistance) are associated with decreased circulating total ghrelin levels in humans (23-25). Furthermore, insulin-sensitive individuals display higher fasting total ghrelin concentrations than insulin-resistant obese subjects (26). Our study demonstrated a positive correlation between total ghrelin level and M index as a measure of insulin sensitivity and in addition a negative correlation between total ghrelin level and HOMA-IR (Fig. 7). It was recently proposed that acylated ghrelin could possibly convey detrimental effects on insulin sensitivity (27). Quite recently, total and non-acylated ghrelin levels were found to decrease throughout the euglycemic hyperinsulinemic clamp in insulin-sensitive and insulin-resistant obese subjects (28), as we found in our study. In contrast to total ghrelin levels, acylated ghrelin concentrations were only significantly reduced in insulin sensitive individuals, whereas, these levels increased slightly in insulin-resistant obese subjects under hyperinsulinemia (28). It would be of course necessary to perform a similar study in patients with PCOS and to relate ratio of acylated to non-acylated ghrelin under hyperinsulinemic clamp conditions in different subsets of patients with PCOS (29).

Lack of significant differences in fasting insulin among the investigated groups, that was expected accord-

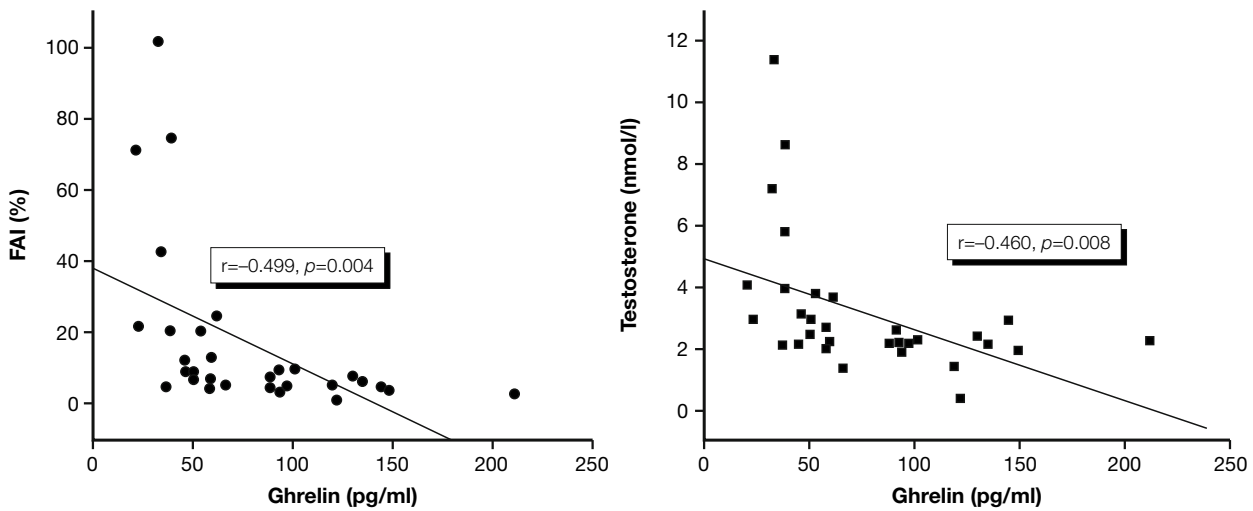

Fig. 6 - *Correlations between basal ghrelin levels and androgens level [testosterone and free androgen index (FAI)].*

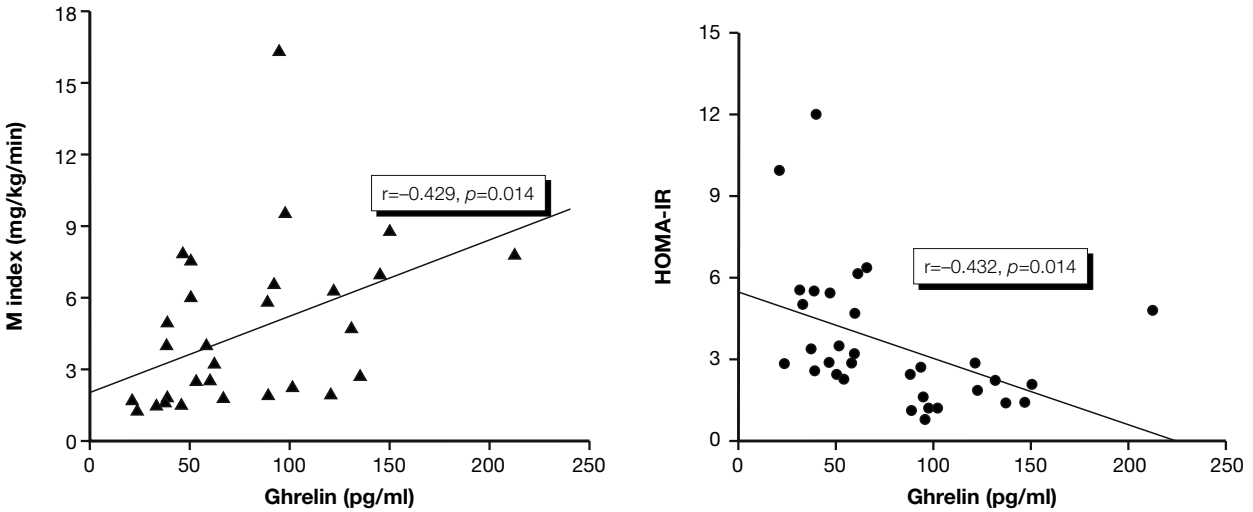

Fig. 7 - *Correlations between ghrelin levels and indices of insulin sensitivity [(M index and homeostasis model assessment of insulin resistance (HOMA-IR)].*

ing the differences in BMI, may be due to relatively small number of the investigated subjects and to relatively high SD among the groups. Insulin may influence ghrelin levels through its central direct and/or indirect peripheral effects. Recently, it was postulated that insulin might play a role in regulating body weight throughout direct central effect, *via* its own receptors in central nervous system (30-32). Intracerebroventricular administration of insulin in the rats lowered circulating ghrelin concentrations and it was demonstrated that phosphoinositide-3 kinase [PI(3)K] is a specific mediator of this action (33). Another possibility is that insulin performs its action through indirect peripheral effects (34, 35). Increase of insulin level with weight gain could also underlie the decrease in plasma ghrelin (36, 37). In addition to regulating plasma ghrelin, insulin in physiological concentrations modulates adipocyte leptin production. While ghrelin stimulates appetite and promotes weight gain (2, 38), leptin inhibits food intake and further weight loss (39, 40). Thus, a rise in plasma insulin due to increased caloric intake could increase leptin and decrease ghrelin levels resulting in a reduced food intake and *vice versa*. In our study, fasting ghrelin was negatively correlated with fasting insulin, as it was demonstrated in other studies (41). Negative interrelation between ghrelin and BMI was found in whole group of investigated women, although not significant, similar to those already reported (10, 11, 26). The same interrelation in PCOS patients was at borderline of statistical significance. The lack of significance in these relations may be due to a relatively limited number of patients in each subgroup and to relatively high SD among the groups. The fact, however, that ghrelin significantly correlated to the degree of insulin sensitivity but not to BMI in our subjects may indicate that factors other than simple adiposity could determine ghrelin levels. In some studies, inverse relationship between circulating ghrelin and androgens was found (9), while others (6) did not establish such relations. Different clinical and biochemical phenotypes of the syndrome may be associated with different ghrelin concentrations (7). Anti-androgen treatment with flutamide in PCOS patients leads to an increase in ghrelin levels, demonstrating that plasma ghrelin changes were mainly

Table 2 - *Univariate and multivariate linear regression analysis (dependent variable: basal ghrelin; /= ns).*

Variable	Linear regression analysis			
	Univariate		Multivariate	
	r	*p*	r	*p*
BMI	0.278	0.123	/	/
Testosterone	0.460	0.008	/	/
SHBG	0.688	<0.0001	0.688	<0.0001
FAI	0.499	0.004	/	/
Basal insulin	0.547	0.001	/	/
M index	0.429	0.014	/	/
HOMA	0.432	0.014	/	/

BMI: body mass index; HOMA: homeostasis model assessment; FAI: free androgen index.

due to changes of androgen levels rather than to improved insulin sensitivity (42). In obese PCOS patients, marked negative correlation between ghrelin and androstendion levels suggested possible interaction between ghrelin and steroid effects (7, 42, 43). Androgens were reported as independent modulators of circulating ghrelin concentration (42). Our data demonstrated significant correlation between fasting ghrelin and SHBG in PCOS women as well as in obese insulin-resistant control women, contrary to the observations where such relationship was not established (10). The best predictive value of SHBG on fasting plasma ghrelin in our study could suggest significance of insulin resistance as well as possible influence of some other factor including androgen levels on fasting ghrelin levels in PCOS patients and should be confirmed in studies with larger number of investigated subjects. However, a previous study, which takes into consideration SHBG and some parameters of insulin status, demonstrated that this relationship seems to be weak in patients with PCOS and SHBG cannot be used as a predictor of insulin resistance (44). In conclusion, women with PCOS had lower fasting ghrelin levels and decreased insulin sensitivity independently of their BMI, compared to controls. In addition, there were no differences among fasting ghrelin levels between non-obese, overweight, and obese women with PCOS. During euglycemic hyperinsulinemic clamp, ghrelin levels were decreased in all studied groups to a similar extent, implying that, compared to chronic hyperinsulinemia, acute hyperinsulinemia reduces ghrelin levels independently of the degree of insulin resistance.

ACKNOWLEDGMENTS

This study was supported by the project 145067, Ministry of Science and Environmental Protection, Republic of Serbia.

REFERENCES

1. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999, 402: 656-60.
2. Wren AM, Seal LJ, Cohen MA, et al. Ghrelin enhances appetite and increase food intake in humans. J Clin Endocrinol Metab 2001, 86: 5992-5.
3. Horvath TL, Diano S, Sotonyi P, Heiman M, Tschöp M. Minireview: ghrelin and the regulation of energy balance - A hypothalamic perspective. Endocrinology 2001, 142: 163-9.
4. Tschöp M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature 2000, 407: 908-13.
5. Shiiya T, Nakazato M, Mizuta M, et al. Plasma ghrelin levels in lean and obese humans and the effects of glucose on ghrelin secretion. J Clin Endocrinol Metab 2002, 87: 240-4.
6. Schöfl C, Horn R, Schill T, Schlösser HW, Müller MJ, Brabant G. Circulating ghrelin levels in patients with polycystic ovary syndrome. J Clin Endocrinol Metab 2002, 87: 4607-10.
7. Pagotto U, Gambineri A, Vicennati V, Heiman ML, Tschöp 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.
8. Moran LJ, Noakes M, Clifton PM, et al. Ghrelin and measures of satiety are altered in polycystic ovary syndrome but not differentially affected by diet composition. J Clin Endocrinol Metab 2004, 89: 3337-44.
9. Panidis D, Farmakiotis D, Koliakos G, et al. Comparative study of plasma ghrelin levels in women with polycystic ovary syndrome, in hyperandrogenic women and in normal controls. Human Reprod 2005, 20: 2127-32.
10. Orio F Jr, Lucidi P, Palomba S, et al. Circulating ghrelin concentrations in the polycystic ovary syndrome. J Clin Endocrinol Metab 2003, 88: 942-5.
11. Wasko R, Komarowska H, Warenik-Szymankiewicz A, Sowinski J. Elevated ghrelin plasma levels in patients with polycystic ovary syndrome. Horm Metab Res 2004, 36: 170-3.
12. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implication for pathogenesis. Endocrine Rev 1997, 18: 774-800.
13. Schaller G, Schmidt A, Pleiner J, Woloszczuk W, Wolzt M, Luger A. Plasma ghrelin concentrations are not regulated by glucose or insulin: a double blind, placebo-controlled crossover clamp study. Diabetes 2003, 52: 16-20.
14. Leonetti F, Iacobellis G, Riabaudo MC, et al. Acute insulin infusion decreases plasma ghrelin levels in uncomplicated obesity. Reg Pept 2004, 122: 179-83.
15. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004, 81: 19-25.
16. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2000, 23, (Suppl 1): S4-19.
17. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979, 237: E214-23.
18. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function frm fasting glucose and insulin concentration in man. Diabetologia 1985, 28: 412-9.
19. Stanczyk FZ. Diagnosis of hyperandrogenism: biochemical criteria. Best Pract Res Clin Endocrinol Metab 2006, 20: 177-91.
20. Glintborg D, Andersen H, Hagen C, et al. Evaluation 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: 337-45.
21. Flanagan DE, Evans ML, Monsod TP, et al. The influence of insulin on circulating ghrelin. Am J Physiol Endocrinol Metab 2003, 284: E313-6.

22. Saad MF, Bernaba B, Hwu CM, et al. Insulin regulates plasma ghrelin concentration. J Clin Endocrinol Metab 2002, 87: 3997-4000.
23. Ikezaki A, Hosoda H, Ito K, et al. Fasting plasma ghrelin levels are negatively correlated with insulin resistance and PAI-1, but not with leptin, in obese children and adolescents. Diabetes 2002, 51: 3408-11.
24. le Roux CW, Patterson M, Vincent RP, Hunt C, Ghatei MA, Bloom SR. Postprandial plasma ghrelin is suppressed proportional to meal calorie content in normal weight but not obese subjects. J Clin Endocrinol Metab 2005, 90: 1068-71.
25. Mohlig M, Spranger J, Otto B, Ristow M, Tschöp M, Pfeiffer AF. Euglycemic hyperinsulinemia, but not lipid infusion, decreases circulating ghrelin levels in humans. J Endocrinol Invest 2002, 25: RC36-8.
26. McLaughlin T, Abbasi F, Lamendola C, Frayo RS, Cummings DE. Plasma ghrelin concentrations are decreased in insulin-resistant obese adults relative to equally obese inulin-sensitive controls. J Clin Endocrinol Metab 2004, 89: 1630-5.
27. van der Lely AJ, Tschöp M, Heiman ML, Ghigo E. Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. Endocr Rev 2004, 25: 426-57.
28. St-Pierre DH, Karelis AD, Coderre L, et al. Association of acylated and nonacylated Ghrelin with insulin sensitivity in overweight and obese postmenopausal women. J Clin Endocrinol Metab 2007, 92: 264-9.
29. Welt CK, Gudmundsson JA, Arason G, et al. Characterizing discrete subsets of polycystic ovary syndrome as defined by the Rotterdam criteria: the impact of weight on phenotype and metabolic features. J Clin Endocrinol Metab 2006, 91: 4842-8.
30. Porte D Jr, Baskin DG, Schwartz MW. Insulin signaling in the central nervous system: a critical role in metabolic homeostasis and disease from C. elegans to humans. Diabetes 2005, 54: 1264-76.
31. Plum L, Schubert M, Brüning JC. The role of insulin receptor signaling in the brain. Trends Endocrinol Metab 2005, 16: 59-65.
32. Pliquett RU, Führer D, Falk S, Zysset S, von Cramon DY, Stumvoll M. The effects of insulin on the central nervous system-focus on appetite regulation. Horm Metab Res 2006, 38: 442-6.
33. Ueno M, Carvalheira JB, Oliveira RL, Velloso LA, Saad MJ. Circulating ghrelin concentrations are lowered by intracerebroventricular insulin. Diabetologia 2006, 49: 2449-52.
34. Wierup N, Svensson H, Mulder H, Sundler F. The ghrelin cell: a novel developmentally regulated islet cell in the human pancreas. Regul Pept 2002, 107: 63-9.
35. Caixas A, Bashore C, Nash W, PI-Sunyer FX, Laferrere B. Insulin, unlike food intake, does not suppress ghrelin in human subjects. J Clin Endocrinol Metab 2002, 87: 1902-6.
36. Ott V, Fasshauer M, Dalski A, et al. Direct peripheral effects of ghrelin includes suppression of adiponectin expression. Horm Metab Res 2002, 34: 640-5.
37. Ravussin E, Tschöp M, Morales S, Bouchard C, Heiman ML. Plasma ghrelin concentration and energy balance: overfeeding and negative energy balance studies in twins. J Clin Endocrinol Metab 2001, 86: 4547-51.
38. Kalra SP, Ueno N, Kalra PS. Stimulation of appetite by ghrelin is regulated by leptin restraint: peripheral and central sites of action. J Nutr 2005, 135: 1331-5.
39. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998, 395: 763-70.
40. Bagnasco M, Kalra PS, Kalra SP. Ghrelin and leptin pulse discharge in fed and fasted rats. Endocrinology 2002, 143: 726-9.
41. Tschöp M, Weyer C, Tataranni A, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. Diabetes 2001, 50: 707-9.
42. Gambineri A, Pagotto U, Tschöp M, et al. Anti-androgen treatment increases circulating ghrelin levels in obese women with polycystic ovary syndrome. J Endocrinol Invest 2003, 26: 629-34.
43. Tena-Sempere M, Barreiro MI, Gonzáles IC, et al. Novel expression and functional role of ghrelin in rat testis. Endocrinology 2002, 143: 717-25.
44. Dahan MH, Goldstein J. Serum sex-hormone binding globulin levels show too much variability to be used effectively as a screening marker for insulin resistance in women with polycystic ovary syndrome. Fertil Steril 2006, 86: 934-41.