Adropin: a key component and potential gatekeeper of metabolic disturbances in policystic ovarian syndrome

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Summary

Purpose: The aim of the current study was to evaluate potential relationships between serum adropin levels and metabolic parameters in polycystic ovary syndrome (PCOS). *Materials and Methods*: Twenty women with PCOS and 20 healthy, age and body mass index (BMI) matched controls were included in the study. All subjects underwent venous blood drawing on the early follicular phase after an overnight fasting. Serum adropin levels were measured with enzyme immunosorbent assay (EIA). The relationships between serum adropin levels and metabolic parameters were also assessed. *Results:* Serum adropin levels were found to be significantly lower in women with PCOS when compared to control group ($p < 0.001$). Serum adropin level was correlated negatively with fasting serum insulin levels, homeostasis model of assessment - insulin resistance (HOMA-IR) and serum lipid markers including cholesterol, very low-density lipoprotein, and triglycerides (TG) in PCOS patients ($p < 0.05$). *Conclusion:* The findings of current study suggest that women with PCOS have low serum adropin levels that may contribute to the underlying pathogenesis of metabolic disturbances in PCOS.

Key words: Adropin; PCOS; Insulin resistance; HOMA-IR.

Introduction

Adropin is a recently identified protein encoded by the energy homeostasis associated gene (Enho) that is expressed in the brain and the liver [1,2]. It has been demonstrated that the expression of adropin is regulated by energy status and dietary nutrient content, and altered with body fat composition [1,2]. In diet-induced obese (DIO) mice, systemic administration of adropin markedly attenuated insulin resistance and glucose intolerance [1-4]. Furthermore, the experimental studies have reported important findings that DIO mice are associated with diminished expression of adropin transcript in liver and low circulating adropin concentrations [1, 2]. Evidence from previous animal study showed that adropin knockout mice exhibit hepatic steatosis, insulin resistance, increased fasting triglycerides (TG), and propensity for impaired glucose tolerance [2].

PCOS affecting up to 20% of women of reproductive age is characterized by oligomenorrhea, hyperandrogenism, and a characteristic ovarian morphology on ultrasonographic examination [5]. The etiology of PCOS is complex and not fully known. Nevertheless, a significant proportion of women with PCOS suffer from insulin resistance (IR) and IR appears to be a central pathophysiological feature of PCOS. Despite its frequent association with obesity, the relation of IR in women with PCOS cannot be precisely explained solely due to obesity because data showing existence of higher IR in lean PCOS women than normal healthy controls is present [6].

Based on aforementioned observations, the authors hypothesized that adropin, an energy regulatory peptide, may be a factor in development of IR and dyslipidemia in women with PCOS. To date, adropin and its role in relation to IR, and lipid metabolism has not been studied in patients with PCOS. The aim of the current study was therefore to evaluate potential relationships between adropin levels and metabolic parameters, in women at reproductive age with PCOS.

Materials and Methods

This study was carried out in collaboration with Departments of Obstetrics and Gynecology in Pamukkale University in Denizli, in Inonu University Turgut Ozal Medical Centre, and Department of Medical Biochemistry, Firat University, Elazig in Turkey. The study protocol was approved by Instutional Ethics Committee. All subjects participating in the study were fully informed of the aim of the study, and informed consents were obtained.

This study enrolled 20 women with PCOS and 20 age- and body mass index (BMI)-matched healthy women as the control subjects. The subjects with PCOS were selected from a group of PCOS patients who were seeking treatment for menstrual irregularity, acne, hirsutism or infertility. The control group consisted of 20 age- and BMI-matched healthy volunteers who were menstruating regularly, normo-ovulatory, non-hirsute, and had normal biochemical and hormonal profiles, thereby excluding the diagnosis of PCOS in this group.

PCOS was defined when at least two of the following three features were present after the exclusion of other etiologies (Rotterdam criteria): (I) oligo/amenorrhea (fewer than six menstrual periods in the preceding year), (II) clinical and/or biochemical signs of hyperandrogenism, and (III) ultrasonographic finding [7]. The ultrasound criteria used for diagnosis of PCOS were: pres-

Revised manuscript accepted for publication March 28, 2013

ence of 12 or more follicles in each ovary measuring two to nine mm in diameter, and/or increased ovarian volume (> ten ml). Clinical hyperandrogenism was quantified by the Ferriman-Gallwey scoring system [8] and the diagnosis was established when it was greater than 8. Patients taking antiandrogen drugs, antidiabetics, lipid lowering medication, glucocorticoids or other hormonal drugs were excluded. Patients with anemia pregnancy, and adrenal disorders including congenital adrenal hyperplasia, diabetes mellitus, hypertension, myocardial infarction, stroke, and peripheral vascular disease were also eliminated.

For each subject, height, weight and BMI were evaluated by standard methods. BMI was measured as the ratio of the weight to the square of the height. All subjects underwent venous blood drawing for complete hormonal assays, lipids, adropin, fasting glucose, and insulin analysis. The blood of subjects was sampled in the morning following an overnight fast during early follicular phase (day 2–5) of spontaneous or progesterone induced withdrawal bleeding.

Biochemical analysis

The blood samples were centrifuged and then the plasma aliquots were frozen at -80°C until assayed. Serum adropin concentration was analyzed using an enzyme immunosorbent assay (EIA) kit with a minimum detectable level less than 0.3 ng/ml. The intra- and interassay coefficient of variance ranged from 7.8 to 8.1 and from 9.2 to 11.3, respectively. Samples were processed by technicians that were blinded to the identity of samples.

Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, sex hormone-binding globulin (SHBG), insulin, and dehydroepiandrosterone sulfate (DHEAS) levels were measured by competitive chemiluminescent enzyme immunoassay method using the same trademark kits. Fasting glucose, TG, total cholesterol, and high-density lipoprotein cholesterol (HDL-C) concentrations were measured by enzymatic colorimetric assay methods using an autoanalyzer and commercially available kits. Low-density lipoprotein cholesterol (LDL-C) concentrations were calculated using Friedewald formula [8]: LDL-C = TC - HDL-C – TG/5. Fasting insulin levels were measured in both PCOS and control subjects to estimate the insulin sensitivity. IR was calculated using the homeostasis model assessment IR index (HOMA-IR), according to the formula; HOMA-IR = fasting serum insulin (mU/ml) x fasting plasma glucose (mg/dl) /405[9].

Statistical analysis

The normality of distribution of variables was tested by using the Kolmogorov-Smirnov test. Categorical variables, presented as numbers and percentages, were compared using Chi square test between the outcome groups. Variables with a skewed distribution (serum adropin level, LH, and LH/FSH levels) were log-transformed. Continuous variables are presented as mean and standard deviation (SD). Comparison of continuous variables between the groups was assessed using Mann-Whithney U test. The correlation of variables was assessed by Pearson correlation test. For all comparisons, statistical significance was defined by $p < 0.05$. The data were assessed using the Statistical Package for Social Sciences software 19.0 for Windows package software.

Table 1. — *Comparison of demographic features, hormone, and metabolic profiles between the patients with PCOS and control groups.*

Baseline variables	PCOS group (n = 20)	Control group (n = 20)	*p* value
Age	27.85 ± 8.36	23.39 ± 5.90	0.14
BMI (kg/m²)	24.98 ± 5.14	23.66 ± 3.96	0.58
FG score (median, IQR)	13 (12-16)	6 (5-8)	<0.001*
FSH (mIU/ml)	5.14 ± 2.06	6.38 ± 3.02	0.25
LH (mIU/ml)	8.13 ± 4.73	3.50 ± 1.58	< 0.001*
FSH/LH	0.67 ± 0.17	2.16 ± 1.42	< 0.001*
Estradiol (mIU/ml)	68.84 ± 58.74	57.07 ± 42.89	0.60
Total testosterone (ng/dl)	45.02 ± 24.43	21.60 ± 9.57	0.004*
Free testosterone (ng/dl)	5.31 ± 4.49	2.23 ± 1.74	0.01*
FAI	5.71 ± 3.22	1.29 ± 0.81	< 0.001*
DHEAS (µg/dl)	278.28 ± 141.43	139.85 ± 55.38	0.001*
TSH (mIU/ml)	1.37 ± 0.77	1.39 ± 0.70	0.80
Fasting insulin (mU/ml)	14.81 ± 9.95	7.66 ± 6.86	0.03*
Fasting glucose (mg/dl)	94.90 ± 9.61	91.22 ± 9.60	0.45
HOMA-IR	3.56 ± 2.38	1.72 ± 1.52	0.02*
Cholesterol (mg/ml)	170.15 ± 35.34	148.39 ± 30.01	0.04*
Triglycerides (mg/ml)	122.10 ± 95.22	76.11 ± 32.84	0.04*
HDL (mg/ml)	48.60 ± 10.66	52.67 ± 10.30	0.16
LDL (mg/ml)	102.91 ± 30.59	87.68 ± 24.86	0.08
VLDL (mg/ml)	24.09 ± 19.30	15.50 ± 6.75	0.08

Values are given in mean ± standard deviation unless otherwise stated; *Statistically significant (*p* value < 0.05).

Table 2. — *Pearson correlations (r values) of demographic features, hormone, and metabolic profiles with serum adropin levels in patients with PCOS.*

Baseline demographic features	Serum adropin concentration (ng/ml) Correlations	
	r	*p*
Age (year)	0.31	0.06
BMI (kg/m²)	-0.09	0.60
FSH (mIU/ml)	-0.35	0.03*
LH (mIU/ml)	-0.06	0.70
LH/FSH	-0.20	0.23
Estradiol (mIU/ml)	-0.11	0.52
Total testosterone (ng/dl)	-0.007	0.97
Free testosterone (ng/dl)	-0.21	0.26
SHBG (nmol/ml)	0.29	0.09
FAI	-0.04	0.84
DHEAS (µg/dl)	0.13	0.45
TSH (mIU/ml)	-0.02	0.92
Fasting Insulin (mU/ml)	-0.47	0.006*
Fasting glucose (mg/dl)	0.18	0.29
HOMA-IR	-0.49	0.003*
Cholesterol (mg/ml)	-0.37	0.02*
Triglycerides (mg/ml)	-0.33	0.04*
HDL-C (mg/ml)	-0.16	0.34
LDL-C (mg/ml)	-0.30	0.06
VLDL-C (mg/ml)	-0.33	0.05*

*Statistically significant (*p* value < 0.05).

Results

The demographic characteristics of study and control groups are compared in Table 1. Mean age and BMI were not different in the PCOS group compared to the controls ($p = 0.14$ and $p = 0.58$, respectively). All patients and subjects had normal renal, hepatic, and thyroid functions.

The mean LH, ratio of FSH to LH, HOMA-IR, fasting insulin, serum cholesterol, and TG levels were higher in PCOS group when compared those of the controls ($p < 0.001$, $p < 0.001$, $p = 0.02$, $p = 0.03$, $p = 0.04$, and $p = 0.04$, respectively); while there were no significant differences between the groups regarding mean serum estradiol, FSH, and thy-

roid stimulating hormone (TSH) levels ($p = 0.60$, $p = 0.24$ and $p = 0.80$). The mean SHBG, free androgen index (FAI), and free and total testosterone levels increased in the PCOS group when compared to the controls ($p = 0.006$, $p < 0.001$, $p = 0.004$ and $p = 0.01$, respectively).

The mean and SD of maternal serum adropin levels in the PCOS group were significantly lower than those of the controls (2.56 ± 1.63 vs 7.47 ± 1.87, respectively, $p < 0.001$). The serum adropin level negatively correlated with FSH, fasting insulin, HOMA-IR, and serum lipid markers including cholesterol, very-low-density lipoprotein (VLDL-C) and TG (Table 2).

Discussion

This study is the first, to the authors' knowledge, study demonstrating the decrease of serum adropin levels in women with PCOS cases in whom each one of those patients had increased fasting serum insulin levels and HOMA-IR. Moreover, the current study has demonstrated significant negative correlation between serum adropin concentrations and fasting insulin, HOMA-IR, and lipid concentrations. These data has indicated an association between low serum adropin levels with IR and dyslipidemia in women with PCOS. In accordance with these findings, a previous study on humans has demonstrated that the low plasma adropin level is associated with an increased risk in metabolic factors including IR and dyslipidemia [10].

The mechanisms regulating the synthesis and secretion of adropin in PCOS women are not well known. However being a novel metabolic marker, its levels can be altered by any metabolic factor associated with PCOS. A recent study has demonstrated that overexpression of adropin in obese mice resulted in marked improvement in insulin sensitivity, a reduction in diabetes, and weight loss [1, 2]. A significant proportion of women with PCOS suffer from IR that appears to play a role in the pathophysiology of PCOS [11, 12]. However, there is no data whether the decrease in adropin levels seen in PCOS is mediated through IR or is a consequence of other metabolic factors.

Another possible explanation for the observed lower adropin levels in women with PCOS might be dyslipidemia. In the current study, the authors found a significant negative correlation between serum adropin and total cholesterol, TG, and VLDL-C levels. In addition, PCOS subjects with low serum adropin levels exhibited higher TG, HOMA-IR, fasting insulin, and VLDL-C levels compared to controls. Because most women with PCOS have IR [6, 11, 13, 14], we do not know whether decreased adropin level is secondary to IR or dyslipidemia contributes to IR and decreased adropin levels per se.

The current study has some disadvantages as involving a single measurement and a small population of women with PCOS. Second, the authors did not compare adropin levels in lean and obese PCOS patients. In spite of these limitations, this study was able to detect a difference between the serum adropin levels of the women with PCOS and those of controls.

Conclusion

In conclusion, the present results provide further indication of the significance of serum adropin in maintaining glucose homeostasis, lipid metabolism, and IR in PCOS subjects. Adropin deficiency associated with PCOS may attenuate the development of IR and dyslipidemia and might play a role in the pathophysiology of PCOS.

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