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Original Article

Decreased circulating levels of betatrophin in Chinese women with polycystic ovary syndrome

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Abstract: The release and regulation of betatrophin in women with polycystic ovary syndrome (PCOS) haven't been well known. The study was to investigate circulating betatrophin levels in women with PCOS and the correlations with insulin resistance and biochemical parameters in two cohorts. In cohort 1, 90 women with PCOS and 85 age-matched healthy women were recruited in a cross-sectional study. Serum concentrations of betatrophin were measured. Serum betatrophin levels were significantly lower in PCOS patients than that in healthy control subjects (0.43 ± 0.15 ng/mL vs 1.19 ± 0.57 ng/mL, P<0.001). Serum betatrophin negatively correlated with fasting plasma glucose (FPG), 2 h postprandial plasma glucose (2hPG), fasting insulin (FINS), triglycerides (TG), homeostasis model assessment of insulin resistance (HOMA-IR), total testosterone (T), and high-sensitivity C-reaction protein (hs-CRP), even after controlling for age and body mass index (BMI) in PCOS participants (all P<0.05). Moreover, stepwise multiple regression analysis showed that HOMA-IR was independently related to betatrophin in PCOS subjects (P<0.05). In cohort 2, 72 PCOS patients were observed longitudinally after 3 months of rosiglitazone + metformin treatment. After treatment, PCOS patients showed higher betatrophin concentrations than the baseline (0.85 ± 0.51 ng/ml vs 0.43 ± 0.18 ng/ml, P<0.001). Circulating betatrophin levels were decreased in patients with PCOS, and associated with indexes of insulin resistance and/or other metabolic abnormalities of PCOS (e.g., hyperandrogenemia).

Keywords: Betatrophin, polycystic ovary syndrome, insulin resistance

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine-metabolic disease, affecting 5-10% of women in the reproductive age [1]. PCOS is characterized by chronic anovulation, androgen excess and insulin resistance (IR) [2]. Insulin resistance plays a critical role in the pathophysiology of PCOS, and as a clinical symptom as well [3, 4]. Given the well documented association between PCOS and IR, insulin-sensitizing agents have been considered an important part of therapy in PCOS patients.

Betatrophin is a newly identified hormone produced in liver and adipose tissue that has been shown to be associated with insulin resistance and regulate glucose and lipid metabolism. Yi et al [5] reported that its expression was increased in mice with insulin resistance (IR), such as S961 administration mice, ob/ob mice, db/db mice and pregnant mice. Increasing evidence then revealed an association between betatrophin levels and IR; however, the results of varying studies on IR models of obesity, diabetes, etc, proved inconsistent. Studies in diabetes revealed inconsistent results with regard to betatrophin. Some studies found that betatrophin levels were higher than that in normal glucose tolerance (NGT) [6-8], while others found the opposite [9] or no difference [10] in T2D subjects. However, reports that investigated betatrophin in gestational diabetes mellitus (GDM) agreed that betatrophin levels were increased, despite the inconsistency with the association of betatrophin with IR [11-15]. Hence, the relationship of betatrophin and IR remains unclear. Rencently, Calan et al [16] conducted a cross-sectional study in a Turkish Caucasian population and found that the betatrophin levels were higher in PCOS patients. Since the different race and living habits may deeply influence the study results, it is neces-

Table 1. Characteristics in patients and controls (Mean ± SD)

Group	CON	PCOS	*p*-value
n	85	90	
Age (year)	25.50 ± 3.70	26.40 ± 4.86	0.417
Weight (Kg)	57.23 ± 8.63	64.23 ± 13.47	0.010
BMI (Kg/m^2)	22.38 ± 2.74	25.27 ± 4.83	0.002
WHR	0.81 ± 0.04	0.83 ± 0.07	0.075
SBP (mm Hg)	115.73 ± 11.25	113.07 ± 11.73	0.353
DBP (mm Hg)	77.54 ± 9.34	78.44 ± 7.09	0.646
FPG (mmol/L)	5.13 ± 0.38	5.42 ± 0.51	0.012
2hPG (mmol/L)	4.78 ± 0.63	8.34 ± 1.57	<0.001
FINS (uU/mL)	5.96 ± 4.84	13.10 ± 9.84	<0.001
INS_{120} (mU/L)	5.85 ± 5.83	133.50 ± 95.48	<0.001
HbA1c (%)	5.44 ± 0.25	5.51 ± 0.29	0.315
HOMA-IR	1.37 ± 1.17	3.22 ± 2.52	<0.001
HOMA-β	75.36 ± 56.64	137.32 ± 105.19	0.001
TG (mmol/L)	1.04 ± 0.40	1.38 ± 0.72	0.014
TC (mmol/L)	4.88 ± 0.98	4.64 ± 0.87	0.276
HDL-c (mmol/L)	1.62 ± 0.44	1.45 ± 0.52	0.154
LDL-c (mmol/L)	2.84 ± 0.93	2.75 ± 0.76	0.642
hs-CRP (mg/L)	0.89 ± 0.65	1.99 ± 2.12	0.047
DHEAS (ug/dL)	195.21 ± 53.62	271.71 ± 99.69	<0.001
T (ng/mL)	0.28 ± 0.13	0.50 ± 0.21	<0.001
SHBG (nmol/L)	46.89 ± 22.98	32.86 ± 15.96	0.003
FAI (%)	2.64 ± 1.97	7.12 ± 4.82	<0.001
LH (mIU/mL)	4.10 ± 1.65	13.11 ± 7.17	<0.001
FSH (mIU/mL)	5.77 ± 1.58	5.87 ± 2.14	0.839
LH/FSH ratio	0.72 ± 0.24	2.29 ± 1.05	<0.001
Betatrophin (ng/mL)	1.19 ± 0.57	0.43 ± 0.15	<0.001

Abbreviations: BMI, body mass index; WHR, waist hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; 2hPG, 2 h postprandial plasma glucose; FINS, fasting insulin; INS_{120}, 2 h postprandial serum insulin; HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, homeostasis model assessment of β cell function; hs-CRP, high-sensitivity C-reaction protein; TC, total cholesterol; TG, triglycerides; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; FSH, follicular-stimulating hormone; LH, luteinizing hormone; T, total testosterone; PRL, prolactin; DHEAS, Dehydroepiandrosterone sulfate; SHBG, sex hormone-binding globulin; FAI, free androgen index.

sary to detect this indicator in a Chinese PCOS population. Furthermore, there is no longitudinal study to find the changes of betatrophin levels during the improvement of IR in PCOS patients. Therefore, our study is aimed to investigate betatrophin levels and its association with insulin resistance as well as other biochemical parameters in women with PCOS, and to re-confirm the relationship between betatrophin and IR in a longitudinal study.

Methods

Study design and setting

In cohort 1, a cross-sectional comparison of PCOS patients and control subjects was performed. In cohort 2, another 72 PCOS patients were recruited in a longitudinal, prospective study, and were followed-up for 3-month, all these patients were prescribed rosiglitazone and metformin. This study was conducted in the First Affiliated Hospital, Chongqing Medical University, Chongqing, China, during June 2015 and November 2015. The study was approved by the Ethical Committee of Chongqing Medical University. Signed informed consents were obtained from all participants in this study.

Study population

90 women with polycystic ovarian syndrome were recruited from our outpatient endocrine clinic. The diagnosis of PCOS was based on European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine guidelines (Rotterdam criteria, 2003), as including at least two of the following three criteria: 1) chronic anovulation; 2) clinical or biochemical signs of hyperandrogenism; and 3) polycystic ovary morphology shown on ultrasound scan, defined as the presence of R12 follicles (with one ovary being sufficient for diagnosis) measuring 2-9 mm in diameter. As the exclusion criteria: other known causes of hyperandrogenemia and ovulatory dysfunction including 21-hydroxylase deficiency, congenital adrenal hyperplasia, Cushing's syndrome, androgen secreting tumors, thyroid disease, and hyperprolactinemia.

85 age-matched healthy women were recruited among the relatives who were accompanying for health medical visits. The inclusion criteria for controls were: over 18 years of age, had a normal menstrual cycle, and had no clinical and/or biochemical hyperandrogenism. The

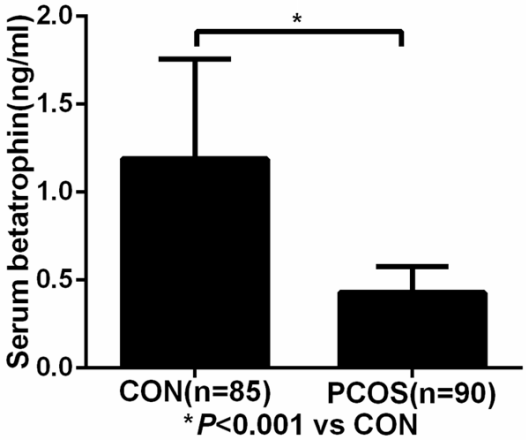

Figure 1. Serum betatrophin levels in the control and PCOS groups.

control group did not include relatives of the PCOS patients.

Exclusion criteria for both groups included concurrent hormone therapy within the previous 6 weeks, any chronic disease, and renal or liver disease

Measures

Baseline anthropometric measurements

Weight (without shoes and in light outdoor clothing) and height were measured, and body mass index (BMI) was calculated using the standard BMI formula: body weight (in kilograms) divided by height (in meters squared). Waist and hip circumferences were measured to the nearest cm with a soft tape at the narrowest part of the torso and at the widest part of the gluteal region, and the waist-to-hip ratio (WHR) was calculated. Seated blood pressure was taken by a trained nurse after the subjects had rested for 10 min.

Baseline biochemistry measurements

Blood samples were obtained after fasting overnight for at least 10 hours when a standard 75-g oral glucose tolerance test (OGTT) was performed. After clotting, blood specimens were separated by centrifugation for 15 min at 1,000 g. Then serum was extracted and stored at -80°C for analysis of betatrophin.

Plasma glucose levels were determined using the glucose oxidase method; insulin levels were measured using chemiluminescence method; Serum triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c) were measured using enzymatic methods; and the serum levels of high-sensitivity C-reactive protein (hs-CRP) were measured by latex particle-enhanced immunoturbidimetric assay; glycated hemoglobin (HbA_{1c}) was measured using isoelectric focusing method; Liver and kidney function were determined using automatic biochemistry analyzer analysis; The levels of hormones containing follicular-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (T), prolactin (PRL), and Dehydroepiandrosterone sulfate (DHEAS) were measured by chemiluminescence immunoassays. Serum sex hormone-binding globulin (SHBG) was measured by electrochemiluminescence. The free androgen index (FAI) was calculated according to the equation: FAI (%) = T (ng/mL) × 3.47 × 100/SHBG (nmol/L). The homeostasis model assessment (HOMA) of IR was computed as: HOMA - IR = FINS (mU/L) × FPG (mmol/L)/22.5, homeostasis model assessment (HOMA) of β was calculated as: HOMA-β = 20 × FINS (mU/L)/(FPG (mmol/L) - 3.5).

Measurement of serum betatrophin

Serum betatrophin levels in the fasting serum were assessed using a validated ELISA kit (Human ANGPTL8 ELISA kit, SEW803Hu, Uscn, Wuhan, China) with intra- and interassay coefficients of variation being <10% and <12%, respectively. The procedures were in accordance with the manufacturer's instructions. All samples were analyzed in duplicate.

Reassessment after rosiglitazone + metformin treatment

A subset of 72 patients from the PCOS group received rosiglitazone (4 mg/d) + metformin in a weight-adapted dose for 3 months (body weight <60 kg: 750 mg/d, 60-100 kg: 1000 mg/d, and >100 kg or BMI >30 kg/m^2: 1500 mg/d). The patients were assessed after 3 months using methods described above.

Statistical analysis

All statistical analyses were performed using SPSS version 19.0. Descriptive and demographic data are presented as mean ± SEM.

Table 2. Correlations of betatrophin levels with clinical and biochemical parameters in women diagnosed with PCOS

	Betatrophin		Betatrophin (age and BMI adjusted)	
	r	P	r	P
BMI	-0.390	0.008	-	-
WHR	-0.302	0.044	-0.167	0.285
FPG	-0.391	0.008	-0.355	0.019
2hPG	-0.395	0.007	-0.363	0.017
FINS	-0.482	0.001	-0.341	0.025
INS_{120}	-0.232	0.125	-0.145	0.353
HbA_{1C}	-0.373	0.012	-0.285	0.064
TG	-0.356	0.017	-0.341	0.025
TC	-0.225	0.137	-0.176	0.259
HDL-c	0.393	0.008	0.273	0.077
LDL-c	-0.378	0.010	-0.256	0.095
HOMA-β	-0.384	0.009	-0.221	0.154
HOMA-IR	-0.502	<0.001	-0.369	0.01
T	-0.302	0.044	-0.299	0.050
FAI	-0.389	0.008	-0.340	0.026
hs-CRP	-0.476	0.001	-0.345	0.023
FSH	0.267	0.076	0.191	0.219

Abbreviations: BMI, body mass index; WHR, waist hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; 2hPG, 2 h postprandial plasma glucose; FINS, fasting insulin; INS_{120}, 2 h postprandial serum insulin; HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β: homeostasis model assessment of β cell function; TC, total cholesterol; TG, triglycerides; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; T, total testosterone; FAI, free androgen index; hs-CRP, high-sensitivity C-reaction protein; FSH, follicular-stimulating hormone.

Normal distribution of the data was tested using Kolmogorox-Smirnov test. Variables not normally distributed were natural-logarithmically transformed. Independent Student t test was used to compare differences between the PCOS and control subjects. For comparison between the pre-treatment and post-treatment in PCOS group, a two-tailed t-test was used for normally distributed variables. The correlations between variables were assessed using a Pearson correlation analysis and partial correlation analysis. Stepwise multiple regression analysis was conducted for betatrophin as a dependent variable, including all variables of interest at the same time as independent variables to demonstrate the relative contribution of each of these variables to the outcome ones. A two-sided value of $P<0.05$ was considered statistically significant.

Results

Cross sectional study

*The clinical characteristics were stated in **Table 1:*** We enrolled 90 PCOS participants and 85 healthy controls. The average age of participants was 25.50 ± 3.70 for the control subjects and 26.40 ± 4.86 for subjects with PCOS. There were no statistically significant differences between PCOS patients and control subjects with respect to age, WHR, SBP, DBP, HbA_{1c}, TC, HDL-c, LDL-c and FSH. Subjects with PCOS had a significantly higher weight, BMI, FPG, 2hPG, FINS, INS120, HOMA-IR, HOMA-β, TG, hs-CRP, DHEAS, T, FAI, LH and LH/FSH ratio while had lower SHBG (all *P* values <0.05).

*Betatrophin levels in the control and PCOS groups were presented in **Figure 1:*** Serum betatrophin levels were significantly lower in subjects with PCOS compared with control subjects (0.43 ± 0.15 ng/mL vs 1.19 ± 0.57 ng/mL, P<0.001).

*Relationships between betatrophin levels and insulin resistance as well as biochemical parameters were described in **Table 2:*** We investigated the relationships of circulating betatrophin levels with various clinical and biochemical parameters. In women with PCOS, we observed that betatrophin levels negatively correlated with FPG, 2hPG, FINS, TG, HOMA-IR, T, and hs-CRP (all *P* values <0.05). All these correlations remained statistically significant after adjustments for age and BMI. However, in the control subjects, betatrophin only showed significant correlation with T (r = -0.431, P = 0.028). Multivariate regression analyses showed that HOMA-IR (β = -0.214; P<0.001) was an independently related factor influencing serum betatrophin levels in PCOS.

Longitudinal cohort study

In our study, a total of 72 subjects were followed-up longitudinally during the course of treatment. Among 18 patients who did not complete this study, five subjects were lost to follow up, three subjects were excluded from the study for pregnancy, six subjects left the study due to personal reasons, and four

Table 3. The Comparison between before and after treatment in PCOS women

Group	Pre-treatment	Post-treatment	*p*-value
n	72	72	
BMI (Kg/m^2)	25.75 ± 4.84	25.24 ± 4.50	<0.001
WHR	0.85 ± 0.08	0.84 ± 0.07	0.356
FPG (mmol/L)	5.38 ± 0.57	5.25 ± 0.56	0.277
2hPG (mmol/L)	8.30 ± 1.66	6.91 ± 0.84	<0.001
FINS (uU/mL)	14.21 ± 11.48	8.44 ± 4.62	<0.001
INS_{120} (mU/L)	141.40 ± 98.90	75.96 ± 36.36	<0.001
HOMA-IR	3.51 ± 2.95	2.03 ± 1.22	<0.001
TG (mmol/L)	1.56 ± 0.76	1.36 ± 0.36	0.326
HDL-c (mmol/L)	1.41 ± 0.52	1.51 ± 0.41	0.032
hs-CRP (mg/L)	1.94 ± 1.91	1.08 ± 1.06	<0.001
T	0.53 ± 0.22	0.48 ± 0.17	0.009
FAI	7.54 ± 5.26	5.67 ± 3.24	0.366
Betatrophin	0.43 ± 0.18	0.85 ± 0.51	<0.001

Abbreviations: BMI, body mass index; WHR, waist hip ratio; FPG, fasting plasma glucose; 2hPG, 2 h postprandial plasma glucose; FINS, fasting insulin; INS_{120}, 2 h postprandial serum insulin; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reaction protein; TG, triglycerides; HDL-c, high-density lipoprotein cholesterol; T, total testosterone; FAI, free androgen index.

patients left the study for gastrointestinal side-effects of metformin. After 3 months of metformin + rosiglitazone treatment, BMI, 2hPG, FINS, INS120, HOMA-IR, hs-CRP, and T significantly decreased (**Table 3**). Interestingly, circulating betatrophin levels significantly increased from 0.43 ± 0.18 ng/ml to 0.85 ± 0.51 ng/ml (P<0.001, **Figure 2**).

Discussion

The circulating concentrations of betatrophin in other IR related diseases, such as T2DM and obesity, were controvasal [6-15], and the results were extremely limited in PCOS patients. In the present study, we first reported that the serum betatrophin concentrations were significantly decreased in women with polycystic ovary syndrome as compared with the healthy subjects in a Chinese population. However, in a recently published study, Calan et al [16] found betatrophin levels were increased in Turkey Caucasian women with PCOS. The disparities may be due to the difference in race/ethnicity, living habits, sample sizes and/or metabolic abnormalities, including the degree of IR.

The mechanisms underlying decreased betatrophin levels in PCOS patients remain unknown. In women with PCOS, it has been established that IR and hyperinsulinemia may represent two distinct features of the insulin disorders. We speculated that the decreased levels of betatrophin in PCOS might be due to hyperinsulinemia and insulin resistance. Because, in this study, betatrophin concentrations were strongly and negatively correlated with FINS and HOMA-IR. Supporting this notion, our longitudinal study found betatrophin concentrations were increased after FINS and HOMA-IR decreased through the 3 months of therapy. Indeed, recently Guo et al [17] demonstrated betatrophin alleviates insulin resistance via the Akt-GSK3β or Akt-FoxO1 pathway in HepG2 Cells, and the effect of betatrophin in ameliorating insulin resistance is dose dependent. Therefore, we speculate that betatrophin may be a defensive hormone in women with polycystic ovary syndrome, and involve in the pathogenesis of the disease via influencing insulin resistance. However, the lower betatrophin levels may be partly associated with another factor in patients with PCOS. Javier et al [9] reported that the dramatic gender dimorphism observed significantly lower circulating betatrophin concentrations in men than in women. Hyperandrogenemia is considered to be part of the metabolic abnormalities of PCOS [2, 18]. And previous studies in humans indicated that androgens decreased insulin sensitivity [19]. In present study, we found that betatrophin inversely correlated with T in PCOS and CON groups. Therefore, it is possible that the decreased betatrophin observed in women with PCOS might be related directly or indirectly to changes in the levels of androgens. Further studies are needed to delineate the mechanisms of decreased betatrophin in PCOS.

The involvement of betatrophin in lipid regulation had been confirmed by previous labs [22-25]. Two mice studies have manifested that overexpression of betatrophin increased triacylglycerol concentrations [22, 24]. Moreover betatrophin deficiency reduced triacylglycerol levels [19, 25]. Chen et al [21] and Espes et al [6] found betatrophin levels were positively associated with TG, but negatively associated with HDL-cholesterol in T2DM. Recently, betatrophin increased TG accumulation via decreasing the expression of ATGL (adipose tri-

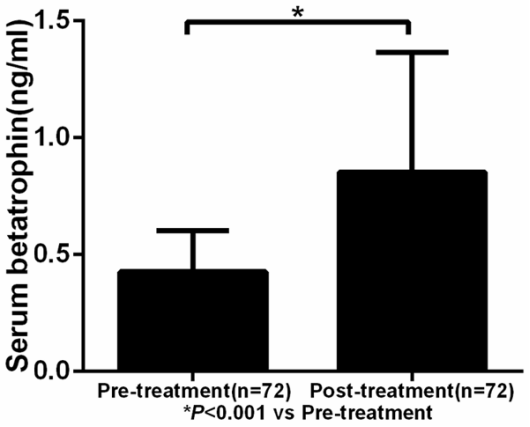

Figure 2. Serum betatrophin levels in the pre-treatment and post-treatment groups.

glyceride lipase) in mammalian cells [26]. Clinical data revealed no correlation of betatrophin and triglyceride levels in T2DM [20]. In addition, a research suggested that betatrophin did not correlate with lipid metabolism in gestational diabetes mellitus [15]. Nevertheless betatrophin concentrations in obese humans were strongly and correlated with triglyceride levels (negatively) and with HDL-c (positively) [9]. In this current study, we found that betatrophin levels were negatively associated with TG in PCOS group, but had no correlation with TC, LDL-c, and HDL-c. The disparity is unclear. It may potentially be due to sample size, the complex metabolic abnormalities of PCOS and diverse factors affecting betatrophin levels. Future studies are needed to elucidate this point.

However, this study is limited by its relatively small sample size. Thus, researches including a large number of subjects are needed. In addition, we investigated betatrophin levels only in Chinese subjects, and the results only could be applied in this population.

In conclusion, we demonstrated that betatrophin concentrations were decreased in PCOS Patients, and were associated with indexes of insulin resistance. Moreover we re-confirm this relationship through a longitudinal study. Our findings indicated that the betatrophin levels might be an effective indicator in the diagnosis and treatment evaluation of PCOS in the future.

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Disclosure of conflict of interest

None.

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