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ORIGINAL ARTICLE

Elevated circulating levels of betatrophin are associated with polycystic ovary syndrome

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Abstract Betatrophin is a newly identified hormone determined to be a potent inducer of pancreatic beta cell proliferation in response to insulin resistance in mice. Polycystic ovary syndrome (PCOS) is an inflammatory-based metabolic disease associated with insulin resistance. However, no evidence is available indicating whether betatrophin is involved in women with PCOS. The objective of this study was to ascertain whether betatrophin levels are altered in women with PCOS. This study was conducted in secondary referral center. This cross-sectional study included 164 women with PCOS and 164 age- and BMI-matched female controls. Circulating betatrophin levels were measured using ELISA. Metabolic and hormonal parameters were also determined. Circulating betatrophin levels were significantly elevated in women with PCOS compared with controls (367.09 ± 55.78 vs. 295.65 ± 48.97 pg/ml, $P < 0.001$). Betatrophin levels were positively correlated with insulin resistance marker homeostasis model assessment of insulin resistance (HOMA-IR), free-testosterone, high-sensitivity C-reactive protein (hs-CRP), atherogenic lipid profiles, and BMI in PCOS. Multivariate logistic regression analyses revealed that the odds ratio for PCOS was 2.51 for patients in the highest quartile of betatrophin compared with those in the lowest quartile (95 % CI 1.31–4.81, $P = 0.006$). Multivariate regression analyses showed that HOMA-IR, hs-CRP, and free-testosterone were independent factors influencing serum betatrophin levels. Betatrophin levels were increased in women with PCOS and were associated with insulin resistance, hs-CRP, and free-testosterone in these patients. Elevated betatrophin levels were found to increase the odds of having PCOS. Further research is needed to elucidate the physiologic and pathologic significance of our findings.

Keywords Polycystic ovary syndrome · Betatrophin · Insulin resistance · Free-testosterone · Lipids

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Abbreviations

ANGPLT8	Angiopoetin-like 8 protein
BMI	Body mass index
CI	Confidence interval
CV	Coefficient of variability
DHEA-S	Dehydroepiandrosterone sulfate
E_2	Estradiol
FBG	Fasting blood glucose
FG	Ferriman–Gallwey
GDM	Gestational diabetes mellitus
HDL-C	High-density lipoprotein cholesterol
HOMA-IR	Homeostasis model assessment of insulin resistance
hs-CRP	High-sensitivity C-reactive protein

LDL-C	Low-density lipoprotein cholesterol
OR	Odds ratio
PCOS	Polycystic ovary syndrome
RIFL	Re-feeding induced fat and liver protein
TD26	Hepatocellular carcinoma-associated protein
T2DM	Type 2 diabetes mellitus
VIF	Variance inflation factor

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrinopathy affecting 5–10 % of women of reproductive age and is characterized by ovulatory dysfunction, polycystic ovaries, biochemical (elevated androgens), and/or clinical (hirsutism and/or acne) hyperandrogenism. PCOS is associated with low-grade chronic inflammation, insulin resistance, glucose intolerance, metabolic syndrome, obesity, hyperlipidemia, hypertension, and an increased risk of developing type 2 diabetes [1–4]. Although the etiopathogenesis of PCOS is not yet well established, insulin resistance and consequent development of hyperinsulinemia are known to play important roles. Despite extensive research, the mechanisms underlying insulin resistance in PCOS patients are not completely understood. Overall, insulin resistance and compensatory hyperinsulinemia affect up to 70 % of women with PCOS [5–10].

Betatrophin, a newly identified peptide hormone secreted mainly by liver and adipose tissue, is a member of a family of angiopoietin-like proteins and is implicated in the regulation of lipid and glucose metabolism. As the peptide was discovered independently by different groups, it is known by various names that include 're-feeding induced fat and liver (RIFL) protein,' 'lipasin,' 'angiopoetin-like 8 protein' (ANGPLT8), and 'hepatocellular carcinoma-associated protein' (TD26) [11–14]. Originally, betatrophin was determined to function in lipid metabolism by reducing triglyceride clearance through lipoprotein lipase inhibition [12]. It has been implicated that the hormone is a key mediator of the postprandial trafficking of triglyceride-fatty acids to adipose tissue [15]. In addition, betatrophin sequence variations were reported in lipid metabolism [13, 16, 17]. The link between betatrophin and glucose homoeostasis has been recently reported by Yi et al. [14]. The group demonstrated that secretion of betatrophin increases in response to insulin resistance in mice, which stimulates beta cell replication and expansion. Administration of betatrophin was shown to improve glucose homeostasis in these mice [14]. These findings indicate that betatrophin might make a promising target for new therapies in diabetes, although there are also contradictory results which show no such link between the peptide and glucose homeostasis [15, 18]. Furthermore, the results of the studies evaluating the relationship between diabetes and betatrophin are not uniform and any causal relationship between these two remains to be established. Several recent studies have reported betatrophin levels in type 2 diabetes and obesity. Different research groups have shown elevated, reduced, or unchanged betatrophin values depending on the features of the recruited population [19–22]. Controversial results have also been reported regarding the correlation between betatrophin and various metabolic parameters associated with metabolic diseases.

PCOS is a metabolic disease associated with insulin resistance. Therefore, in this study, we aimed to investigate (1) whether betatrophin metabolism is altered in women with PCOS, (2) whether the peptide is associated with the development of PCOS, and (3) whether betatrophin levels are correlated with metabolic disturbances.

Materials and methods

Ethics statement

This study was approved by the local ethics committee of the Dokuz Eylul University Medical School. The subjects gave their oral and written informed consent before their inclusion in the study. The study adhered strictly to the Declaration of Helsinki Principles as revised in 2008.

Subjects and study design

The study was designed as cross-sectional and was conducted between March 2014 and March 2015 in the Department of Obstetrics and Gynecology, Merkezefendi State Hospital, Manisa, Turkey. We consecutively recruited 164 subjects with PCOS and 164 age- and body mass index (BMI)-matched subjects with normal menstrual cycles who either presented to our clinic or volunteered via local advertisements and met the inclusion and exclusion criteria. Clinical and anthropometric variables were evaluated by a single investigator in all subjects. All the recruited women were Caucasian.

PCOS group

PCOS was diagnosed in this study based on the 2003 Rotterdam consensus criteria [23]. To limit metabolic heterogeneity within samples, all three of the following criteria were used for diagnosis (after excluding other causes of hyperandrogenism):

1. Oligo- or anovulatory menstrual dysfunction (manifested as infrequent bleeding at intervals >35 days);
2. Clinical and/or biochemical signs of hyperandrogenism—The Ferriman–Gallwey (FG) method was used

to determine hirsutism [24]. Subjects were deemed hirsute if their FG score was ≥8. Biochemical hyperandrogenism was defined as an increase in the serum concentrations of free-testosterone (normal values: 2.9–31.8 ng/dl) and/or total testosterone (normal values: 0.15–0.7 ng/ml);

3. Typical ultrasonographic finding of polycystic ovaries (with one ovary being sufficient for diagnosis), defined as the presence of ≥12 follicles measuring 2–9 mm in diameter or an ovarian volume >10 ml (without a cyst or dominant follicle in either ovary).

Control group

Control subjects were recruited from healthy women who visited the gynecology clinic for routine annual examination or from hospital workers and university students through local advertisements. All volunteers for the control group had regular menstrual cycles (28–32 days); they had no concomitant health problems or signs of hirsutism, acne, or hyperandrogenism.

Exclusion criteria

The exclusion criteria adopted during subject selection were (1) other causes of irregular menstrual cycles and/or androgen excess (i.e., Cushing's syndrome, hyperprolactinemia, congenital adrenal hyperplasia, or other diseases of the adrenal gland, thyroid disorders, galactorrhea, breastfeeding, and pregnancy); (2) impaired glucose tolerance or type 1/type 2 diabetes; (3) hypertension, hyperlipidemia, active or chronic liver or renal failure, or congestive heart failure; (4) a history of coronary artery disease, gestational diabetes mellitus (GDM), or acute infection (within the preceding 14 days); (5) presence of any chronic inflammatory and autoimmune disease; (6) known malignancy; (7) hormonal contraception and/or anti-androgen therapy (within the preceding 6 months); and (8) BMI ≥35 kg/m^2, age <18 or >35 years. The use of medications for dyslipidemia, hypertension, hyperglycemia, insulin resistance, or obesity was an exclusion criterion for this study. None of the subjects used tobacco or alcohol.

Anthropometric evaluation

A detailed history was obtained from each of the study participants. Following general physical examination of the subjects, anthropometric measurements were performed (age, weight, height, and waist circumference). Height (in centimeters) and weight (in kilograms) were measured with the subject barefoot in light daily clothes. Body mass index (BMI) was calculated using the formula weight (kg)/square meter of height (m^2). Waist circumference (in cm) was measured midway between the lower rib margin and the iliac crest at the end of a gentle expiration. Blood pressure was measured in the sitting position after a rest period of at least 15 min. The average of three measurements was calculated.

Biochemical evaluation

Venous blood samples were collected from the antecubital veins of the subjects during the early follicular phase of menstrual bleeding (3rd to 5th days) in the morning (8:00–9:00 a.m.), after the onset of early spontaneous or progesterone-induced menses, following at least 12 h of fasting. Samples were separated by centrifugation for 15 min at $2000 \times g$ after clotting for 30 min at room temperature. The serum samples were subsequently stored in aliquots at -80 °C prior to the analysis of betatrophin.

Fasting blood glucose (FBG), serum insulin, hs-CRP, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), free-testosterone, total testosterone, dehydroepiandrosterone sulfate (DHEA-S), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E$_2$), and betatrophin levels were measured.

FBG, serum hs-CRP, total cholesterol, total HDL-C, and triglyceride levels were determined using an auto-analyzer (Abbott Architect C 16000, IL, USA) with its dedicated kits (Abbott Diagnostics, Wiesbaden, Germany). LDL-C was calculated using the Friedewald equation: LDL-C = total cholesterol-(HDL-C + Triglyceride/5). Non-HDL-C was calculated as HDL-C subtracted from total cholesterol. Serum insulin levels were measured by an auto-analyzer (Abbott Architect I2000, IL, USA) using chemiluminescent microparticle immunoassay (CMIA) with its dedicated kits (Abbott Diagnostics, Wiesbaden, Germany). Serum FSH, LH, E$_2$, total testosterone, and DHEA-S levels were measured by CMIA (Beckman Coulter Inc., Brea, CA, USA). Serum free-testosterone levels were measured using the radioimmunoassay (RIA) method (DSL 4900 Gamma Counter, Beckman Coulter Inc., Fullerton, CA, USA).

Insulin resistance was calculated for each participant using the homeostasis model assessment of insulin resistance: HOMA-IR = fasting insulin (μU/ml) × fasting glucose (mg/dL)/405 [25].

Betatrophin ELISA

Serum betatrophin levels (Cusabio Biotech, Hubei, China) were measured using commercially available ELISA kits. The intra-assay CV was <6 %, the inter-assay CV was <8 %. All samples were analyzed in duplicate. The assays

have high sensitivity and excellent specificity for the detection of human betatrophin with no significant cross-reactivity or interference.

Statistical analysis

Power analysis

The power analysis was performed using G Power 3.0.10 for Windows. According to our pilot study results for circulating betatrophin levels, the required size of the study population was calculated to be 132 subjects per group ($\alpha = 0.05$ and the study power = 0.90).

All analyses were performed using the Statistical Package for the Social Sciences software version 18.0 (SPSS Inc. Chicago, IL, USA). Distribution of the data was tested using the Kolmogorov–Smirnov test. Triglycerides, DHEA-S, and FSH required logarithmic transformation prior to statistical analysis. Continuous variables were presented as mean ± standard deviation (SD). Demographic and laboratory characteristics of the studied women with and without PCOS were compared using independent samples t test (two-tailed). Subjects in the PCOS and control groups were further divided into two groups according to their BMI levels (BMI <25 kg/m^2 and BMI ≥25 kg/m^2). Betatrophin levels were then compared using independent samples t test (two-tailed). Relationships between betatrophin and other demographic and laboratory characteristics were evaluated using Pearson's correlation analysis. Subjects were divided into four groups according to their betatrophin levels. To assess the association between betatrophin levels (quartiles) and the development of PCOS, odds ratio (OR) was calculated using multivariate logistic regression analysis. The model was adjusted for potential confounders: age, BMI, HOMA-IR, free-testosterone, hs-CRP, and lipids. Model compatibility analysis by Hosmer and Lemeshow test showed that all the models were compatible ($P > 0.05$). Multiple linear regression analyses were performed to adjust the covariates and identify independent relationships between betatrophin levels and age, BMI, HOMA-IR, free-testosterone, lipids, and hs-CRP. All independent variables in the multiple linear regression were tested for multicollinearity. If the variance inflation factor (VIF) exceeded 2.5, the variable was considered to be collinear. Therefore, waist circumference, FBG, insulin, and total cholesterol were not included in the models.

All reported confidence interval (CI) values are calculated at the 95 % level. A two-sided P value <0.05 was considered statistically significant.

Results

Clinical and laboratory characteristics of the study population

The comparative demographic and laboratory parameters of the studied women with and without PCOS are given in Table 1.

As subjects were matched for age and BMI, both parameters were similar in between the two groups ($P > 0.05$). Although mean waist circumference was slightly higher in women with PCOS, it did not reach statistical significance. There were no significant differences between the groups in terms of systolic and diastolic blood pressure.

Circulating betatrophin levels were significantly elevated in women with PCOS compared with controls (367.09 ± 55.78 vs. 295.65 ± 48.97 pg/ml, $P < 0.001$). Serum insulin, FBG, hs-CRP, and HOMA-IR levels were found to be increased in women with PCOS. Furthermore, PCOS patients exhibited more dyslipidemia compared to controls. There was no significant difference in total testosterone and DHEA-S levels between the two groups.

We stratified recruited subjects into two groups according to their BMI levels (<25 kg/m^2 and ≥25 kg/m^2). In the control group, there were 61 subjects with BMI <25 kg/m^2 and 103 subjects with BMI ≥25 kg/m^2; in the PCOS group, there were 49 patients with BMI <25 kg/m^2 and 115 patients with BMI ≥25 kg/m^2. We compared betatrophin levels in each group according to their BMI. There was no significant difference between the number of subjects in the groups ($P = 0.198$) (data not shown). As shown in Fig. 1, overweight/obese women (BMI ≥25 kg/m^2) had significantly higher circulating betatrophin levels than lean subjects both in the control and PCOS groups (BMI <25 kg/m^2).

Correlation of betatrophin with clinical parameters

Correlation coefficients between betatrophin levels and the measured clinical parameters are shown in Table 2.

A strongly positive correlation was found between circulating betatrophin levels and free-testosterone ($r = 0.324$, $P < 0.001$) and hs-CRP ($r = 0.335$, $P < 0.001$) levels in women with PCOS (Fig. 2a, b). There was a positive correlation between HOMA-IR and betatrophin ($r = 0.241$, $P = 0.002$) (Fig. 2c). In addition, we found positive correlations between betatrophin and BMI, waist circumference, serum insulin, FBG, and atherogenic lipids profiles (non-HDL-C, triglycerides, LDL-C, and total cholesterol) in both groups. Betatrophin levels correlated with HOMA-IR and hs-CRP in the control group.

Table 1 Comparison of the demographic and laboratory characteristics of the subjects

Variables	PCOS $n = 164$	Controls $n = 164$	P^a
Age (years)	26.65 ± 3.31	26.82 ± 3.32	0.653
BMI (kg/m^2)	26.66 ± 3.63	26.84 ± 3.77	0.662
Waist circumference (cm)	89.05 ± 9.40	87.35 ± 9.01	0.095
SBP (mmHg)	112.28 ± 10.24	110.15 ± 11.84	0.082
DBP (mmHg)	71.32 ± 6.14	70.27 ± 7.14	0.160
Ferriman–Gallwey score	10.44 ± 1.97	4.37 ± 1.36	<0.001*
FBG (mg/dl)	88.36 ± 5.91	84.98 ± 6.75	<0.001*
Insulin (μIU/ml)	11.91 ± 3.11	8.18 ± 3.27	<0.001*
HOMA-IR	2.61 ± 0.73	1.72 ± 0.76	<0.001*
Total cholesterol (mg/dl)	207.62 ± 41.03	192.07 ± 29.44	<0.001*
LDL-C (mg/dl)	133.64 ± 37.28	122.84 ± 26.53	0.003*
HDL-C (mg/dl)	50.19 ± 12.52	51.75 ± 11.18	0.236
Triglycerides (mg/dl)	135.96 ± 42.79	87.64 ± 37.42	<0.001*
Non-HDL cholesterol (mg/dl)	157.42 ± 40.22	140.31 ± 29.54	<0.001*
hs-CRP (mg/l)	1.17 ± 0.61	0.65 ± 0.34	<0.001*
FSH (mIU/ml)	6.35 ± 0.96	6.17 ± 1.18	0.127
LH (mIU/ml)	9.59 ± 2.43	5.15 ± 1.39	<0.001*
Estradiol (pg/ml)	44.74 ± 9.90	46.63 ± 9.17	0.074
Free-testosterone (ng/dl)	53.33 ± 11.73	14.62 ± 3.30	<0.001*
Total testosterone (ng/ml)	0.42 ± 0.12	0.39 ± 0.11	0.080
DHEA-SO$_4$ (μg/dl)	144.00 ± 37.70	137.04 ± 41.80	0.114
Betatrophin (pg/ml)	367.09 ± 55.78	295.65 ± 48.97	<0.001*

Results are given in mean ± SD

BMI Body mass index, *DBP* diastolic blood pressure, *DHEA-S* dehydroepiandrosterone sulfate, *FBG* fasting blood glucose, *FSH* follicle-stimulating hormone, *HDL-C* high-density lipoprotein cholesterol, *HOMA-IR* homeostasis model assessment of insulin resistance, *hs-CRP* high-sensitivity C-reactive protein, *LDL-C* low-density lipoprotein cholesterol, *LH* luteinizing hormone, *PCOS* polycystic ovary syndrome, *SBP* systolic blood pressure

[a] Independent samples *t* test was used. A *P* value of <0.05 was considered significant (*)
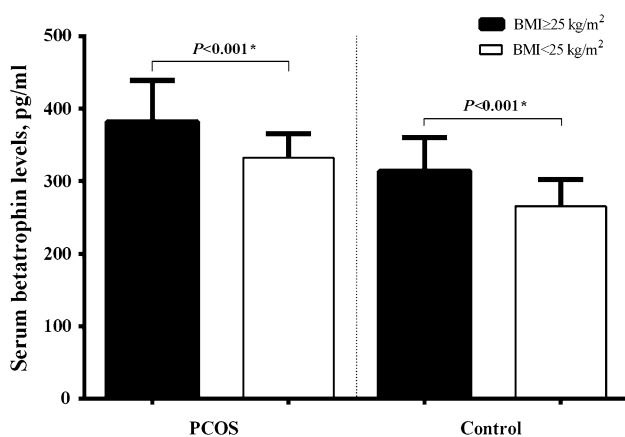

Fig. 1 Circulating betatrophin levels according to BMI (normal weight: BMI <25 kg/m^2; overweight/obese: BMI ≥25 kg/m^2). Overweight/obese women had significantly higher circulating betatrophin levels than lean women, regardless of PCOS

There were no significant correlations between betatrophin levels and age, blood pressure, total testosterone levels and HDL-C.

Multivariate binary logistic regression analysis

The independent association between PCOS and betatrophin levels (in quartiles) was assessed by multivariate binary logistic regression analysis for the potential confounders including age, BMI, HOMA-IR, free-testosterone, hs-CRP and lipids (Fig. 3).

In multivariate logistic regression analysis, subjects in the third (OR 1.37, 95 % CI 1.07–1.76, P = 0.014) and the highest (OR 2.51, 95 % CI 1.31–4.81, P = 0.006) quartiles of betatrophin had significantly higher odds of having PCOS compared with subjects in the lowest quartile. Odds of PCOS were not different between subjects in the second

Table 2 Correlation coefficient between betatrophin levels and clinical parameters

| | Betatrophin | | | |
| | PCOS | | Control | |
	r	P	r	P
Age	0.127	0.168	0.079	0.321
BMI	0.143	0.039*	0.114	0.042*
Waist circumference	0.174	0.027*	0.135	0.038*
Systolic blood pressure	0.142	0.342	0.116	0.267
Diastolic blood pressure	0.138	0.264	0.154	0.325
Insulin	0.216	0.006*	0.145	0.021*
FBG	0.114	0.011*	0.110	0.036*
HOMA-IR	0.241	0.002*	0.128	0.025*
Free-testosterone	0.324	<0.001*	0.124	0.056
Total testosterone	0.237	0.451	0.176	0.298
hs-CRP	0.335	<0.001*	0.113	0.006*
Total cholesterol	0.219	0.029*	0.196	0.035*
LDL-C	0.185	0.037*	0.121	0.041*
HDL-C	0.221	0.431	0.152	0.348
Triglycerides	0.341	0.031*	0.308	0.027*
Non-HDL cholesterol	0.317	0.035*	0.256	0.031*

Pearson's correlation analysis was used. *r*: Pearson's correlation coefficient

BMI Body mass index, *FBG* fasting blood glucose, *HOMA-IR* homeostasis model assessment of insulin resistance, *hs-CRP* high-sensitivity C-reactive protein, *LDL-C* low-density lipoprotein cholesterol

A *P* value of <0.05 was considered significant (*)

and the lowest betatrophin quartiles (OR 1.04, 95 % CI 0.72–1.55, *P* = 0.154). Subjects with betatrophin levels in the highest and third quartiles were approximately 2.5 and 1.4 times more likely to develop PCOS, respectively, compared with those in the lowest quartile after adjustment for potential confounders.

Multivariate regression analysis

To verify independent associations between betatrophin and metabolic profile, multiple linear regression analysis was performed (Table 3). This model explained 57.7 % of the total variability in betatrophin concentration. Multiple linear regression analysis revealed that HOMA-IR, hs-CRP, and free-testosterone independently predicted betatrophin levels.

Discussion

In the present study, we have demonstrated for the first time that circulating betatrophin levels were higher in women with PCOS when compared with control subjects. Betatrophin
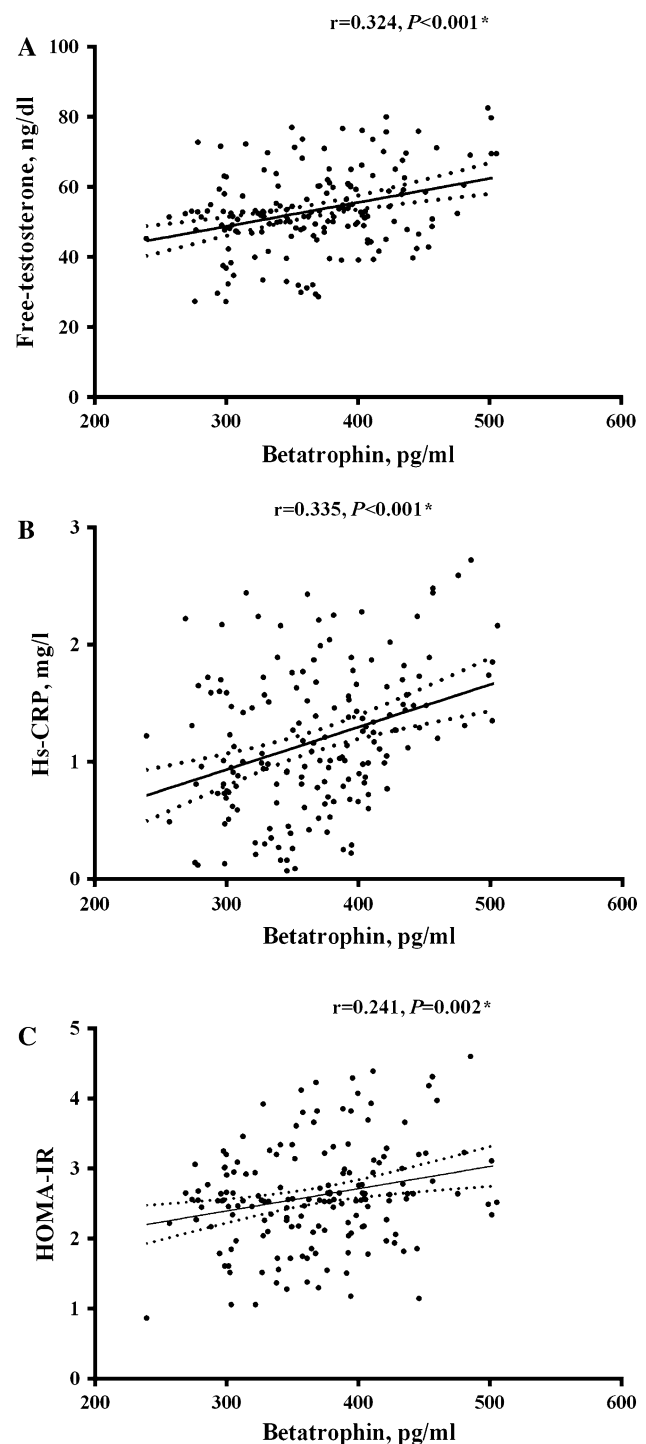

Fig. 2 Scatter plots showing the correlation between circulating betatrophin and free-testosterone levels (**a**), hs-CRP (**b**), and HOMA-IR (**c**) in women with PCOS. There was a positive correlation between betatrophin and free-testosterone, hs-CRP and HOMA-IR

was positively correlated with insulin resistance marker HOMA-IR, inflammatory marker hs-CRP, free-testosterone, atherogenic lipid profiles, and BMI in PCOS. Importantly, overweight/obese women also had significantly higher
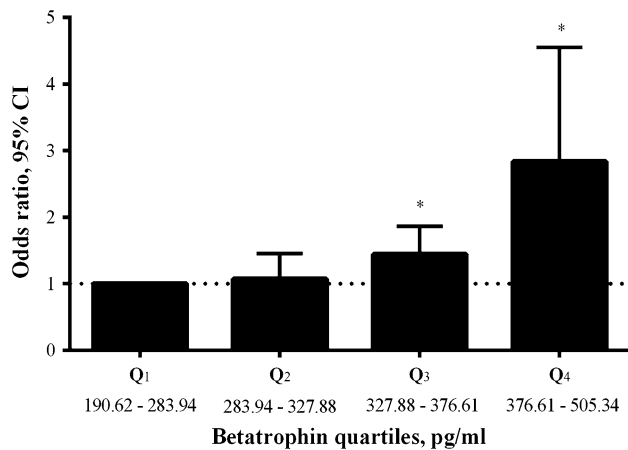
Fig. 3 Association of betatrophin with PCOS in adjusted models. Multivariate adjusted odds ratio (OR) for having PCOS according to the quartiles of betatrophin (reference, the lowest quartile). Model adjusted for age, BMI, HOMA-IR, free-testosterone, hs-CRP and lipids. *OR* Odds Ratio, *CI* Confidence Interval, *Q* Quartile. A *P* value of <0.05 was considered significant (*asterisk*)

Table 3 Evaluation of the effects of age, BMI, HOMA-IR, free-testosterone, LDL-C, triglycerides, and hs-CRP on betatrophin using the multiple linear regression analysis ($R^2 = 0.577$)

Variables	β	95 % CI		P
		Min	Max	
Age	0.189	−0.860	1.237	0.721
BMI	0.155	−0.372	0.683	0.563
HOMA-IR	0.944	0.317	1.572	0.003*
Free-testosterone	1.321	0.097	2.544	0.025*
LDL-C	0.401	−1.350	2.152	0.457
Triglycerides	0.623	−0.584	1.829	0.310
Hs-CRP	0.874	0.366	1.382	0.001*

Multiple linear regression analysis was used. β: Unstandardized regression coefficient; CI: Confidence interval

BMI Body mass index, *HOMA-IR* homeostasis model assessment of insulin resistance, *Hs-CRP* high-sensitivity C-reactive protein, *LDL-C* low-density lipoprotein cholesterol

A *P* value of <0.05 was considered significant (*)

circulating betatrophin levels than lean women, regardless of PCOS. Furthermore, increased circulating betatrophin levels were significantly associated with higher odds of having PCOS after adjusting for potential confounders. Subjects in the highest quartile of betatrophin were approximately 2.5 times more likely to develop PCOS compared with those in the lowest quartile. A similar result was found in subjects in the third quartile of betatrophin, who were nearly 1.4 times more likely to develop PCOS than subjects in the lowest quartile. In addition, multivariate regression analyses showed that HOMA-IR, hs-CRP, and free-testosterone were independent factors influencing serum betatrophin levels. As betatrophin is mainly secreted from the liver, our results suggest that the *hepato-ovarian axis* may play a role in the development of PCOS.

Upon development of insulin resistance in certain metabolic diseases (i.e., diabetes, GDM, obesity and PCOS), beta cells increase proliferation and insulin production to compensate for the increasing insulin demand. Nevertheless, insulin secretion eventually decreases as a result of the exhaustion of beta cell function [26–28]. The mechanisms of increased beta cell proliferation in response to the insulin-resistant state remain unknown. PCOS is associated with insulin resistance. Overall, insulin resistance and compensatory hyperinsulinemia effect up to 70 % of women with PCOS. Moreover, insulin resistance plays a crucial role in the pathogenesis of PCOS. PCOS subjects have a higher prevalence of glucose metabolism disorders. As many as 40 % have impaired glucose tolerance, while 10 % have T2D by their fourth decade [27, 29, 30]. A study published by Yi et al. has reported that secretion of betatrophin increases in response to insulin resistance in mice, which stimulates beta cell replication and mass expansion as well as insulin secretion [14]. Following the publication of this data, a number of studies have been conducted to determine betatrophin levels in metabolic diseases and to determine whether betatrophin levels are correlated with metabolic parameters in these disorders. However, contradictory results have been reported [20, 21, 31, 32]. Hu et al. reported that betatrophin levels were negatively associated with insulin sensitivity in patients with T2DM [31], while Gomez-Ambrossi et al. found a positive association between the two [21]. In a recently published study, betatrophin levels did not correlate with insulin resistance in a large sample size population with T2DM [32]. In addition, several groups have found elevated circulating betatrophin levels in women with GDM [33–35]. Although Ebert et al. did not find a correlation between betatrophin and insulin resistance in GDM, the other two groups reported a positive correlation. Our work here demonstrates a positive correlation between betatrophin and HOMA-IR in PCOS and control subjects. The discrepancies among published data may be related to variations in demographic and ethnic features, as well as the design and sample size of each study. Another possible explanation for the discrepancies in betatrophin levels in these inflammatory-based metabolic diseases may be the degree of inflammation, since we found a strongly positive correlation between betatrophin and the inflammatory marker, hs-CRP. Furthermore, multiple linear regression analyses revealed that hs-CRP independently influenced betatrophin levels. Therefore, the relationship between circulating levels of betatrophin and insulin resistance in humans requires further investigation.

PCOS is a metabolic disease associated with hyperandrogenism, insulin resistance, and low-grade chronic inflammation. Hyperandrogenism, insulin resistance, and inflammation

all contribute to the development of the disorder [10]. In the present study, we found that HOMA-IR, hs-CRP, and free-testosterone levels were independent factors influencing betatrophin levels. Moreover, multivariate logistic regression analyses revealed that increased betatrophin levels were significantly associated with higher odds of having PCOS after adjustment for HOMA-IR, hs-CRP, and other potential confounders. Although the physiological roles of betatrophin in the ovulation system have not been elucidated yet, based on these observations, it is reasonable to speculate that betatrophin may contribute to the development of PCOS independently from associated risk factors including insulin resistance and inflammation.

In addition, betatrophin plays a role in the regulation of lipid metabolism via reduction of triglyceride clearance by lipoprotein lipase inhibition and controls postprandial trafficking of triglyceride-fatty acids to adipose tissue [12, 15]. Moreover, betatrophin sequence variations are known to affect lipid metabolism [13, 16, 17]. Although accumulating evidence indicates a strong link between betatrophin and lipid homeostasis, human studies have been inconclusive. A positive correlation has been reported between betatrophin and both LDL-C and total cholesterol [20]. Gao et al. reported a positive correlation between betatrophin and triglycerides [22]. In another study, on the other hand, circulating betatrophin levels were found to be negatively correlated with triglycerides and positively correlated with HDL-C [21]. Furthermore, Chen et al. reported a negative correlation between betatrophin and total cholesterol, LDL-C and HDL-C [36]. In the present study, PCOS patients exhibited more dyslipidemia compared with controls. Betatrophin levels were found to be positively correlated with atherogenic lipid profiles including triglycerides, LDL-C, total cholesterol, and non-HDL-C, while no significant correlation with HDL-C was detected in PCOS and control groups.

There are some limitations to the current study. Although our sample size is relatively large, the cross-sectional design of the study cannot prove causality. Another limitation of the study is the technique used to evaluate insulin resistance. Due to ethical considerations, we used the less invasive HOMA-IR method, although it is inferior to the clamp technique. Lastly, participants were not screened for betatrophin sequence variations.

In conclusion, our results reveal elevated circulating betatrophin levels in PCOS. However, it is not clear whether an increased betatrophin level is a primary activator or whether it simply results from the disorder. Betatrophin is independently associated with insulin resistance, inflammation, and free-testosterone. More importantly, increased betatrophin levels are independently associated with higher odds of having PCOS, suggesting a potential role of betatrophin in the pathophysiology of PCOS. The physiologic

and pathologic significance of our findings remain to be further elucidated.

Compliance with ethical standards

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