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Serum betatrophin levels are increased and associated with insulin resistance in patients with polycystic ovary syndrome

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Abstract

Objective: Betatrophin is a newly identified circulating protein that is significantly associated with type 2 diabetes mellitus (T2DM), adiposity, and metabolic syndrome. The aim of this study was to investigate whether betatrophin levels and polycystic ovary syndrome (PCOS) were associated.
Methods: Circulating betatrophin levels were measured in 162 patients with PCOS and 156 matched control females using specific enzyme-linked immunosorbent assay kits. Correlations between betatrophin levels and PCOS incidence as well as multiple key endocrine PCOS parameters were analyzed using multiple statistical methods.
Results: Betatrophin levels were significantly increased in patients with PCOS (685.3 ± 27.7 *vs.* 772.6 ± 42.5 pg/ml). When sub-grouping all investigated subjects according to the presence of insulin resistance, women with PCOS and insulin resistance exhibited markedly higher betatrophin concentrations. Furthermore, betatrophin levels were significantly correlated with fasting insulin levels and homeostatic model assessment of insulin resistance only in females with PCOS ($r = 0.531$ and $r = 0.628$, respectively).
Conclusion: We provide the first report that betatrophin is strongly associated with PCOS. This study suggests that betatrophin may potentially serve as an independent predictor for the development of PCOS in at-risk women, especially those with insulin resistance.

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Introduction

Polycystic ovary syndrome (PCOS) is the primary cause of anovulatory infertility[1] and affects up to 10% of women of reproductive age.[2] The exact pathophysiology of PCOS is complex and remains largely unclear. However, the aetiology of PCOS is underpinned by both insulin resistance and hyperandrogenism, with insulin resistance exacerbating hyperandrogenism.[3] Insulin resistance occurs in approximately 80% of women with PCOS and occurs independently of obesity.[4] Furthermore, women with PCOS are believed to be at an increased risk of developing type 2 diabetes mellitus (T2DM).[5] A recent meta-analysis of 13 studies reported a 4-fold increased risk of T2DM in women with PCOS.[6] Thus, PCOS is a well-defined clinical model of insulin resistance and the pre-diabetic state.

Betatrophin, also known as angiopoietin-like protein (ANGPTL8), is a newly identified circulating protein predominantly produced in the liver and adipose tissue. Betatrophin is induced as a result of insulin resistance,[7] therefore attracting increasing attention. Betatrophin was reported to promote pancreatic beta cell proliferation and improve metabolic control by increasing the beta cell division rate in insulin resistant mice.[7] However, in humans, the associations of serum betatrophin levels with diabetes, obesity, and lipid profiles remain controversial.[8,9] Some studies have suggested that circulating betatrophin levels are elevated in T2DM as well as type 1 diabetes,[10–17] correlating with lipid profiles,[18] while others reported that betatrophin levels were reduced in subjects with diabetes.[19] Accumulating evidence suggests that betatrophin is significantly associated with adiposity, type 2 diabetes, and the metabolic syndrome.[17,18,20]

To date, there have been no reports on the relationship between betatrophin and PCOS. In fact, most women with PCOS display impaired glucose tolerance and are at higher risk for developing T2DM.[21] Moreover, betatrophin has a close relationship with insulin resistance and T2DM.[8,14,17] These observations raise the question of whether abnormal betatrophin might associate with PCOS. Therefore, the present study aimed to detect circulating betatrophin levels in subjects with PCOS and healthy control female patients. We also evaluated the association between betatrophin levels and clinical, hormonal, and metabolic variables to achieve a better understanding of the relationship between betatrophin and PCOS.

Patients and methods

Study participants

This case-control study was approved by the Institutional Ethical Review Board of Yantai Yuhuangding Hospital (H20130381). Written informed consent was obtained from all patients before the initiation of the study. We included 162 women with a diagnosis of PCOS and 156 non-hirsute ovulatory women (regular cycles and luteal phase progesterone levels higher than 3.8 ng/mL), ranging from 18 to 45 years of age, in the study at our clinic between February 2013 and November 2015. PCOS diagnosis was determined according to the Rotterdam PCOS

Consensus criteria.[22] Each subject underwent a complete medical examination and an endocrine profile and haematological, hepatic, and renal function analysis. Women with body mass index (BMI) ranging from 18.0 to 40.0 kg/m^2 were selected for the study. We made further subgroupings based on the presence of insulin resistance, defined as a homeostatic model assessment (HOMA) index of $\geq$2.4.[23] None of the women from either group had received any drugs known to interfere with hormone levels, blood pressure, or metabolic variables for at least 3 months before the study. Women with diabetes, liver or kidney disease, thyroid dysfunction, or pregnancy were excluded.

Biochemical and hormonal assays

Blood samples were obtained during the mid-follicular phase of the menstrual cycle after at least 12 hours of fasting. Blood samples from all subjects were separated immediately by centrifugation at $4000 \times g$ for 10 min and stored at -80°C until analysis. Automated chemiluminescence immunoassay systems were used for measuring luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone (ADVIA Centaur, Siemens Healthcare Diagnostics, Eschborn, Germany), dehydroepiandrosterone sulfate, and sex hormone–binding globulin (SHBG) (Immulite 2000 XPi, Siemens Healthcare Diagnostics). The free androgen index (FAI) was estimated by dividing total testosterone (nmol/L) by SHBG (nmol/L) $\times$ 100. Low-density lipoprotein cholesterol was estimated indirectly with the Friedewald formula.[24] Total cholesterol, high-density lipoprotein cholesterol, triglyceride, and glucose levels were determined by colorimetric-enzymatic methods (Siemens Advia System, Deerfield, IL, USA). Intra- and inter-assay coefficient of variation values for these parameters were <5% and <8%, respectively.

Glucose tolerance test

In all subjects, a 3-h oral glucose tolerance test was used to evaluate insulin resistance and β-cell function. After a 12-h overnight fast, patients ingested 75 g glucose, and glucose and insulin concentrations were determined at baseline and after 30, 60, 90, 120, and 180 min. For this study, we used only fasting insulin and glucose to determine the HOMA index. Insulin resistance was calculated using the HOMA-insulin resistance (HOMA-IR) formula: glucose (mmol/L) $\times$ fasting insulin (mU/L)/22.5.[25] Fasting insulin was evaluated using a chemiluminescence immunometric assay and commercial kit (Immulite 2000 Analyzer; CPC). Fasting glucose was measured using a glucose oxidase assay (Tosoh Corp., Tosoh, Japan).

Measurement of betatrophin

Fasting serum betatrophin levels were assessed using enzyme-linked immunosorbent assay (ELISA) kits (EIAab Science, Wuhan, China; Catalogue No. E11644h). The procedures were in accordance with the manufacturer's instructions. ELISAs were performed in duplicate, and samples with coefficient of variation values exceeding 5% were excluded.

Statistical analysis

SPSS version 20.0 (SPSS, Chicago, IL) was used for all analyses. Data are presented as mean $\pm$ SD or median [interquartile range]. Differences between groups were evaluated using the unpaired two-tailed Student's t-test for data with Gaussian distributions. The Mann-Whitney U test was used to compare group medians for data with non-Gaussian distributions. Bivariate relations between betatrophin levels and covariates were analysed with Spearman's Rank Correlation Coefficient. A forward stepwise multiple linear regression model was used to test which variables were independent predictors of betatrophin level. We made further subgroupings based on the presence of insulin resistance, defined as a HOMA index $\geq$2.4. Betatrophin concentrations were compared between multi-groups with one-way analysis of variance followed by *LSD-t* tests. Data were considered statistically significant at $P < 0.05$.

Results

Clinical and hormonal features of women in the control and PCOS groups are presented in Table 1. Age, blood pressure, and SHBG, FSH, fasting glucose, total cholesterol, triglyceride, and high-density and low-density lipoprotein cholesterol levels were similar between the groups. As expected, women with PCOS had higher LH, dehydroepiandrosterone sulfate, and total testosterone concentrations and a higher FAI and LH/FSH ratio than those of control women. Patients with PCOS displayed significantly higher mean fasting insulin levels and a higher mean HOMA-IR and were more likely to be insulin resistant ($P = 0.013$).

Table 1. Anthropometric characteristics, hormone concentrations, and metabolic profiles of control patients and patients with PCOS.

Variables	Controls	Patients with PCOS	*P*-value	*P*-value (BMI-adjusted)
Number	156	162		
Age (years)	27.05 ± 5.23	28.78 ± 6.41	0.373	0.367
BMI (kg/m^2)	23.61 ± 3.22	28.71 ± 6.02	**0.013**	
WHR	0.67 ± 0.47	0.85 ± 0.31	**0.034**	**0.047**
Ferriman-Gallwey score	1 (0–2)	4 (2–6)	<**0.001**	<**0.001**
Systolic BP (mmHg)	119.00 ± 4	119.61 ± 16	0.872	0.851
Diastolic BP (mmHg)	76.00 ± 6	76.13 ± 12	0.673	0.483
Total testosterone (ng/ml)	0.52 (0.37–0.62)	1.17 (0.81–1.33)	<**0.001**	<**0.001**
SHBG (nmol/L)	42.5 (34.2–58.1)	33.5 (17.4–48.8)	0.194	0.214
FAI	4.6 (3.7–6.0)	9.3 (6.7–20.4)	**0.001**	**0.002**
FSH (mIU/ml)	6.85 ± 3.37	5.83 ± 1.27	0.087	0.078
LH (mIU/ml)	4.27 ± 2.89	10.43 ± 5.35	**0.001**	<**0.001**
LH/FSH	0.79 ± 0.47	1.86 ± 1.24	<**0.001**	<**0.001**
DHEAS (mg/l)	1.34 ± 1.67	1.94 ± 1.01	**0.001**	**0.003**
Fasting glucose (mg/dL)	84.52 ± 8.45	85.37 ± 7.04	0.439	0.424
Fasting insulin (mIU/L)	7.29 (4.3–11.2)	13.03 (8–19)	**0.004**	**0.010**
HOMA-IR	1.72 (1.4–2.7)	2.93 (1.7–4.1)	**0.013**	**0.015**
Total cholesterol (mmol/L)	5.12 ± 1.36	5.19 ± 1.16	0.872	0.853
Triglycerides (mmol/L)	0.76 ± 0.35	0.83 ± 0.21	0.274	0.304
HDL cholesterol (mmol/L)	1.72 ± 0.92	1.51 ± 0.86	0.085	0.079
LDL cholesterol (mmol/L)	2.65 ± 0.17	3.02 ± 0.19	0.093	0.091
Betatrophin (pg/ml) (pg/ml)	685.3 ± 27.7 (46.6-370.8)	772.6 ± 42.5 (50.00-598.6)	<**0.001**	<**0.001**

Abbreviations: BMI, body mass index; WHR, waist to hip ratio; BP, blood pressure; SHBG, sex hormone–binding globulin; FAI, free androgen index; FSH, follicle stimulating hormone; LH, luteinizing hormone; DHEAS, dehydroepiandrosterone sulfate; HOMA-IR, homeostasis model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Values are expressed as mean ± SD or median (interquartile range). *P*-values were obtained from unpaired two-tailed Student's *t*-test or Mann-Whitney *U*-test. Clinical indexes with significant differences ($P < 0.05$) are in bold.

Notably, betatrophin concentrations were significantly higher in patients with PCOS than those in control patients (Table 1, $P < 0.001$). Further separation of the subjects according to the presence of insulin resistance revealed a significant difference in betatrophin concentrations between the four groups (Figure 1). One-way analysis of variance demonstrated significantly different betatrophin levels between the groups ($F = 21.14$, $P < 0.01$). Further analysis with the *LSD-t* test revealed significantly higher betatrophin levels in patients with PCOS and insulin resistance compared with those in patients with PCOS and control patients without insulin resistance (832.7 ± 98.2 *vs.* 775.5 ± 66.2 pg/ml, $P = 0.013$ and 832.7 ± 98.2 *vs.* 662.9 ± 72.0 pg/ml, $P < 0.001$). However, betatrophin levels did not differ significantly between control patients with insulin resistance and those without ($769.4.7 \pm 43.1$ *vs.* 735.3 ± 72.0 pg/ml).

To study the potential association between PCOS and fasting insulin levels, we further conducted a one-way analysis of covariance using betatrophin levels as the dependent variable, PCOS as the independent variable (two levels), and fasting insulin as the covariate (data not shown). This analysis (between-subjects factor: PCOS, control) indicated that the main effect of PCOS ($F = 0.115$) was statistically significant ($F = 7.03$, $P = 0.013$). Furthermore, the main effect of insulin concentration was also statistically significant ($F = 9.83$, $P = 0.003$), but no statistically significant interaction between the two factors was identified ($F = 2.33$).

Table 2 displays significant positive correlations between betatrophin and fasting insulin levels as well as HOMA-IR ($r = 0.531$, $P < 0.001$ and $r = 0.628$, $P < 0.001$, respectively), which were only identified in patients with PCOS. However, there were no statistically significant

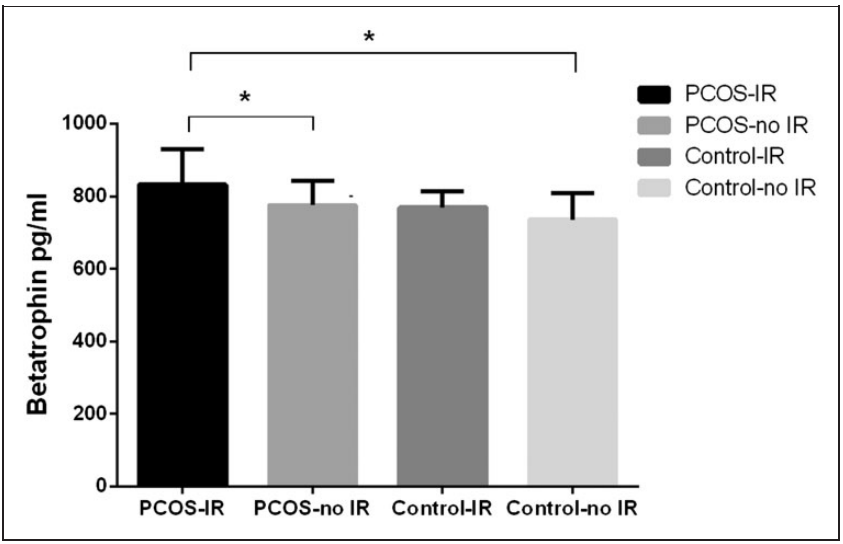

Figure 1. Betatrophin levels in women with PCOS and control women according to insulin resistance status. Data are expressed as means ± SD. *P < 0.05, one-way analysis of variance followed by *LSD-t* tests. Abbreviations: PCOS, polycystic ovary syndrome; IR, insulin resistance.

Table 2. Partial Pearson's or Spearman rank correlation coefficients of betatrophin concentrations and subject characteristics.

	Controls		Patients with PCOS	
	r	*P*-value	*r*	*P*-value
WHR	−0.212	0.462	−0.101	0.751
Ferriman-Gallwey score	−0.373	0.165	−0.076	0.785
Systolic BP (mmHg)	−0.113	0.701	−0.089	0.738
Diastolic BP (mmHg)	−0.041	0.924	−0.119	0.634
Total testosterone (ng/ml)	0.326	0.068	0.334	0.067
SHBG (nmol/L)	−0.070	0.810	−0.058	0.834
FAI	0.338	0.056	0.354	0.055
FSH (mIU/ml)	−0.289	0.091	−0.317	0.144
LH (mIU/ml)	0.306	0.240	−0.085	0.730
LH/FSH	−0.279	0.289	0.071	0.801
DHEAS (mg/l)	0.318	0.228	−0.097	0.718
Fasting glucose (mg/dl)	0.328	0.152	0.272	0.151
Fasting insulin (mIU/ml)	0.258	0.317	0.531	**<0.001**
HOMA-IR	0.363	0.128	0.628	**<0.001**
Cholesterol (mmol/L)	0.180	0.490	0.018	0.933
Triglycerides (mmol/L)	0.102	0.739	0.138	0.551
HDL (mmol/L)	0.103	0.715	0.117	0.618
LDL(mmol/L)	0.053	0.864	0.062	0.810

Abbreviations: WHR, waist to hip ratio; BP, blood pressure; SHBG, sex hormone–binding globulin; FAI, free androgen index; FSH, follicle stimulating hormone; LH, luteinizing hormone; DHEAS, dehydroepiandrosterone sulfate; HOMA-IR, homeostasis model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein. The correlation coefficient (*r*) and *P*-value were adjusted for age and body mass index. Clinical indexes with significant differences ($P < 0.001$) are in bold.

correlations between any variable and betatrophin levels in the control group.

Finally, we used a multivariate linear regression model of betatrophin levels in patients with PCOS, including BMI, fasting insulin levels, fasting glucose levels, HOMA-IR, and FAI as independent variables. Table 3 reveals that only HOMA-IR remained significantly associated with betatrophin levels ($P < 0.001$) and was, thus, concluded to be an independent predictor of betatrophin concentrations.

Discussion

In the present study, our data demonstrated that betatrophin levels were significantly increased in patients with PCOS. When we sub-grouped subjects according to the presence of insulin resistance, women with PCOS and insulin resistance exhibited higher betatrophin concentrations. A one-way analysis of covariance demonstrated that both fasting insulin levels and PCOS diagnosis correlated with betatrophin levels. Furthermore, betatrophin levels were significantly correlated with fasting insulin levels and HOMA-IR only in patients with PCOS.

Betatrophin has recently been introduced as a novel potent stimulator of β-cell replication and improved glucose tolerance by increasing the β-cell division rate in mouse models of insulin resistance.[7] There is evidence suggesting that betatrophin expression can be induced by a high-fat diet and

Table 3. Results of a multivariate linear regression analysis of selected variables performed for betatrophin concentrations in patients with PCOS.

Covariate	Standardized β coefficient	*P*-value
BMI (kg/m^2)	0.46	0.635
Fasting insulin (mIU/ml)	0.83	0.191
Fasting glucose (mg/dl)	0.76	0.225
HOMA-IR	2.91	**<0.001**
FAI	0.62	0.512
Triglycerides (mmol/L)	0.51	0.541

Abbreviations: BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; FAI, free androgen index. Clinical indexes with significant differences ($P < .05$) are in bold.

insulin, resulting in increased serum triglyceride levels and insulin resistance instead of improved glucose metabolism.[13,26] However, several reports have indicated that betatrophin was increased in T2DM and type 1 diabetes mellitus,[10–17] indicating that betatrophin could be a potent diagnostic biomarker for T2DM.[27] Of note, a recent meta-analysis demonstrated that circulating betatrophin levels in patients with T2DM were higher than those of non-diabetic adults in the non-obese, but not in the obese, population.[8] This finding suggests that betatrophin plays a role in the pathogenesis of insulin resistance and T2DM. In addition to T2DM, Ebert et al.[15] determined that women with gestational diabetes mellitus had significantly higher betatrophin levels compared with those of healthy pregnant controls. Furthermore, gestational diabetes mellitus status positively predicted circulating betatrophin levels. Additionally, mounting evidence from recent animal-based studies has suggested that betatrophin associates with lipid metabolism. Mice lacking betatrophin had a 70% reduction in plasma triglyceride levels compared with those of littermate control subjects.[26] However, to date, no studies have examined whether betatrophin is associated with PCOS, though growing evidence has suggested that insulin resistance and dyslipidaemia play critical roles in its pathophysiology.

As indicated in this study, we determined that circulating betatrophin levels were markedly increased in Chinese patients with PCOS compared with those in the control group. Moreover, a Spearman rank analysis demonstrated that serum betatrophin levels were significantly positively associated with indexes of insulin resistance, including fasting insulin levels and HOMA-IR. These findings corroborate those of a previous population-based study that indicated that serum betatrophin levels were elevated in patients with T2DM and associated with insulin resistance.[14] However, it is unclear whether increased betatrophin expression is a compensatory response or only a marker of insulin resistance in PCOS. Notably, increased circulating betatrophin levels were identified in women with PCOS and insulin resistance but not in control women with insulin resistance. Nonetheless, the increased betatrophin levels in subjects with PCOS are interesting and raise the question regarding the actual function of betatrophin, particularly after recent reports confirming that betatrophin does not affect beta cell expansion in mice[28] or humans.[29] Additionally, it is postulated that betatrophin as a novel hormone may be involved in the generation of an atherogenic lipid profile.[18] However, beyond glucose metabolism, we did not find that betatrophin levels significantly associated with the lipid profile. Therefore, it would appear that different mechanisms are involved in the regulation of betatrophin levels in PCOS. However, we cannot exclude the possibility that elevated betatrophin levels may be associated with other etiological factors in PCOS, which may affect insulin resistance. In a recent study, Yi et al.[27] determined that betatrophin could be a potent diagnostic biomarker for T2DM in an indigenous Chinese population, implying that betatrophin might be the driving cause of the disease. More studies are required to determine the mechanisms underlying the role of betatrophin in PCOS.

Similarly, Calan M et al.[30] and Song S et al.[31] revealed that betatrophin levels were higher in patients with PCOS than those in the control group. Moreover, Calan M et al.[30] demonstrated a positive correlation between betatrophin levels and HOMA-IR in patients with PCOS and control subjects, which is consistent with our present result. However, Song S et al.[31] determined that serum betatrophin levels were negatively correlated with HOMA-IR. Conversely, Erbag G et al.[32] in a small sample study (30 patients with PCOS and 27 without PCOS) identified significantly lower betatrophin levels in patients with PCOS. In the same study, betatrophin levels displayed a strong negative correlation with HOMA-IR. The different findings may be related to the use of different ELISA kits, different ethnic groups, or the design and sample size of each study.

To evaluate which parameters were independently associated with betatrophin levels in PCOS, a multiple regression analysis was performed. We identified HOMA-IR as the only parameter that remained statistically significant. We therefore conclude that insulin resistance was the primary contributing factor to elevated betatrophin concentrations in this cohort of patients with PCOS. Thus, betatrophin levels are evidence of a PCOS-associated disorder rather than a PCOS diagnosis, possibly indicating a state of oxidative stress and inflammation, and are strongly associated with insulin resistance in patients with PCOS.

The limitations of the present study included the relatively small sample size, which precluded stratification of groups by BMI for comparison, and the cross sectional nature, which prevented us from establishing causality. Another limitation in our study is the lack of assessment of betatrophin levels on different days of the menstrual cycle in subjects. Therefore, further studies are required to investigate the associations between betatrophin levels and clinical phenotype and pro-inflammatory markers in normal-weight *versus* obese women with PCOS, as well as to better characterize betatrophin secretion throughout the menstrual cycle.

In conclusion, we have provided the first evidence that serum betatrophin concentrations were markedly increased in patients with PCOS compared with those of control subjects. Our findings also suggest a possible association between betatrophin levels and PCOS. However, additional studies are needed to elucidate the role of betatrophin in PCOS development and determine whether targeting betatrophin could hold promise for PCOS treatment.

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Declaration of conflicting interests

The authors declare that there is no conflict of interest.

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References

1. Ehrmann DA. Polycystic ovary syndrome. *N Engl J Med* 2005; 352: 1223–1236.
2. Goodarzi MO, Dumesic DA, Chazenbalk G, et al. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. *Nat Rev Endocrinol* 2011; 7: 219–231.

3. Diamanti-Kandarakis E and Papavassiliou AG. Molecular mechanisms of insulin resistance in polycystic ovary syndrome. *Trends Mol Med* 2006; 12: 324–332.
4. Stepto NK, Cassar S, Joham AE, et al. Women with polycystic ovary syndrome have intrinsic insulin resistance on euglycaemic-hyperinsulaemic clamp. *Hum Reprod* 2013; 28: 777–784.
5. Legro RS, Kunselman AR, Dodson WC, et al. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. *J Clin Endocrinol Metab* 1999; 84: 165–169.
6. Moran LJ, Misso ML, Wild RA, et al. Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reprod Update* 2010; 16: 347–363.
7. Yi P, Park JS and Melton DA. Betatrophin: a hormone that controls pancreatic beta cell proliferation. *Cell* 2013; 153: 747–758.
8. Li S, Liu D, Li L, et al. Circulating betatrophin in patients with type 2 diabetes: a meta-analysis. *J Diabetes Res* 2016; 2016: 6194750.
9. Zhang R and Abou-Samra AB. A dual role of lipasin (betatrophin) in lipid metabolism and glucose homeostasis: consensus and controversy. *Cardiovasc Diabetol* 2014; 13: 133.
10. Espes D, Lau J and Carlsson PO. Increased circulating levels of betatrophin in individuals with long-standing type 1 diabetes. *Diabetologia* 2014; 57: 50–53.
11. Fu Z, Berhane F, Fite A, Seyoum B, et al. Elevated circulating lipasin/betatrophin in human type 2 diabetes and obesity. *Sci rep* 2014; 4: 5013.
12. Hu H, Sun W, Yu S, et al. Increased circulating levels of betatrophin in newly diagnosed type 2 diabetic patients. *Diabetes care* 2014; 37: 2718–2722.
13. Abu-Farha M, Abubaker J, Al-Khairi I, et al. Higher plasma betatrophin/ANGPTL8 level in Type 2 Diabetes subjects does not correlate with blood glucose or insulin resistance. *Sci Rep* 2015; 5: 10949.
14. Chen X, Lu P, He W, et al. Circulating betatrophin levels are increased in patients with type 2 diabetes and associated with insulin resistance. *J Clinl Endocrinol Metab* 2015; 100: E96–E100.
15. Ebert T, Kralisch S, Wurst U, et al. Betatrophin levels are increased in women with gestational diabetes mellitus compared to healthy pregnant controls. *Eur J Endocrinol* 2015; 173: 1–7.
16. Erol O, Ellidag HY, Ayik H, et al. Evaluation of circulating betatrophin levels in gestational diabetes mellitus. *Gynecol Endocrinol* 2015; 31: 652–656.
17. Yamada H, Saito T, Aoki A, et al. Circulating betatrophin is elevated in patients with type 1 and type 2 diabetes. *Endocr J* 2015; 62: 417–421.
18. Ghasemi H, Tavilani H, Khodadadi I, et al. Circulating betatrophin levels are associated with the lipid profile in type 2 diabetes. *Chonnam Med J* 2015; 51: 115–119.
19. Gomez-Ambrosi J, Pascual E, Catalan V, et al. Circulating betatrophin concentrations are decreased in human obesity and type 2 diabetes. *J Clin Endocrinol Metab* 2014; 99: E2004–E2009.
20. Abu-Farha M, Sriraman D, Cherian P, et al. Circulating ANGPTL8/Betatrophin Is Increased in Obesity and Reduced after Exercise Training. *PloS one* 2016; 11: e0147367.
21. Celik C, Tasdemir N, Abali R, et al. Progression to impaired glucose tolerance or type 2 diabetes mellitus in polycystic ovary syndrome: a controlled follow-up study. *Fertil Steril* 2014; 101: 1123–1128.e1.
22. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* 2004; 19: 41–47.
23. Radikova Z, Koska J, Huckova M, et al. Insulin sensitivity indices: a proposal of cut-off points for simple identification of insulin-resistant subjects. *Exp Clin Endocrinol Diabetes* 2006; 114: 249–256.
24. Friedewald WT, Levy RI and Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma,

without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18: 499–502.
25. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28: 412–419.
26. Wang Y, Quagliarini F, Gusarova V, et al. Mice lacking ANGPTL8 (Betatrophin) manifest disrupted triglyceride metabolism without impaired glucose homeostasis. *Proc Natl Acad Sci U S A* 2013; 110: 16109–16114.
27. Yi M, Chen RP, Yang R, et al. Betatrophin Acts as a Diagnostic Biomarker in Type 2 Diabetes Mellitus and Is Negatively Associated with HDL-Cholesterol. *Int j endocrinol* 2015; 2015: 479157.
28. Gusarova V, Alexa CA, Na E, et al. ANGPTL8/betatrophin does not control pancreatic beta cell expansion. *Cell* 2014; 159: 691–696.
29. Jiao Y, Le Lay J, Yu M, et al. Elevated mouse hepatic betatrophin expression does not increase human beta-cell replication in the transplant setting. *Diabetes* 2014; 63: 1283–1288.
30. Calan M, Yilmaz O, Kume T, et al. Elevated circulating levels of betatrophin are associated with polycystic ovary syndrome. *Endocrine* 2016; 53: 271–279.
31. Song S, Wang J, Guo C, et al. [Elevated serum levels of betatrophin in patients with polycystic ovary syndrome and the influential factors]. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 2016; 41: 969–974. [In Chinese, English Abstract].
32. Erbag G, Eroglu M, Turkon H, et al. Relationship between betatrophin levels and metabolic parameters in patients with polycystic ovary syndrome. *Cell Mol Biol (Noisy-le-grand)* 2016; 62: 20–24.