

Serum zonulin is elevated in women with polycystic ovary syndrome and correlates with insulin resistance and severity of anovulation

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Abstract

Objective: Evidence suggests that increased gut permeability may be associated with polycystic ovary syndrome (PCOS). Human zonulin is currently the only physiological mediator known to reversibly regulate gut permeability by disassembling intestinal tight junctions. So far, no data on serum zonulin levels in patients with PCOS are available. This study aimed to determine circulating serum zonulin levels in women with PCOS and discuss the relationship between zonulin, insulin resistance, and menstrual disorders in this group.

Design: A case–control study.

Methods: The study includes 78 women recently diagnosed with PCOS and 63 age-matched healthy controls recruited. Serum zonulin levels were determined by ELISA. Insulin resistance was assessed by homeostasis model assessment of insulin resistance (HOMA-IR) and Matsuda and DeFronzo's insulin sensitivity index (ISI).

Results: PCOS women had higher serum zonulin levels ($P=0.022$). After adjustment for age and BMI, zonulin levels significantly correlated with HOMA-IR and ISI. Furthermore, PCOS women with more severe menstrual disorders had significantly higher zonulin levels and displayed an inverse correlation between zonulin and the number of menstrual cycles per year ($r=-0.398$, $P<0.001$).

Conclusions: Serum zonulin, a biomarker for gut permeability, is increased in PCOS women and correlates with insulin resistance and severity of menstrual disorders. It suggests that alterations in gut permeability may play a role in the pathophysiology of PCOS, and serum zonulin might be used as a biomarker for both risk stratification and therapeutic outcomes in PCOS women.

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women of reproductive age and is associated with several clinical consequences, including an increased risk for infertility, obesity, insulin resistance, and metabolic alterations. Moreover, these subjects have an increased risk of endometrial carcinoma and cardiovascular disease (1, 2). Unfortunately, the exact underlying etiology is presently unknown.

Evidence suggests that increased gut permeability may play a role in the pathogenesis of PCOS (3). Obesity is the most common co-morbidity in PCOS. Both animal models (4, 5) and human studies (6) have established that obesity is characterized by increased gut permeability. In addition, irritable bowel syndrome and chronic fatigue syndrome, two conditions linked with increased gut permeability (7, 8), are more common in PCOS populations (9, 10).

Furthermore, insulin resistance affects ~70% of women with PCOS (11), and is associated with increased gut permeability (12). Taken collectively, it is probable that increased gut permeability may contribute to the development of PCOS.

Human zonulin, the eukaryotic counterpart of *Vibrio cholerae* zonula occludens toxin, is the only physiological mediator currently known to reversibly regulate gut permeability by disassembling intestinal tight junctions (13, 14). It increases intestinal permeability in the small intestines and participates in intestinal innate immunity. Zonulin serum concentration is strongly correlated with lactulose: mannitol urine ratio, a widely used clinical indicator of gut permeability (15), and is considered as a non-invasive biomarker for gut permeability (14, 16). Previous studies have revealed a key role of zonulin-dependent gut barrier dysfunction in the pathogenesis of autoimmune diseases (e.g. type 1 diabetes, celiac disease) (15, 17). However, the association between zonulin and metabolic diseases has also been recently recognized. Clinical studies have demonstrated that zonulin levels are significantly increased in obese subjects and type 2 diabetes patients, and positively correlates with insulin resistance and inflammatory markers (e.g. tumor necrosis factor α , interleukin 6) (18, 19). However, the association between zonulin and patients with PCOS remains unknown. This study aimed to determine serum zonulin levels in PCOS and to investigate the potential links between zonulin levels and clinical and biochemical measures of glucose, lipid metabolism, insulin resistance, and severity of menstrual disorders in PCOS subjects.

Subjects and methods

Seventy-eight newly diagnosed PCOS women were recruited from the endocrine outpatient clinic in Xiangya Hospital of Central South University. The diagnosis of PCOS was established according to the 2003 Rotterdam Consensus Criteria, which require the presence of at least two of the following: oligoovulation and/or anovulation, biochemical and/or clinical signs of hyperandrogenism, polycystic ovaries (by gynecologic ultrasound), after exclusion of other known causes of hyperandrogenemia and ovulatory dysfunction, such as intense physical activity, hypothyroidism, hyperprolactinemia, Cushing's syndrome, congenital adrenal hyperplasia, androgen-secreting tumors (20), and those with other concurrent medical illness (kidney, liver disease) also were excluded from the study. PCOS was newly diagnosed in patients who had not previously received any treatment.

The control group were recruited from amongst our hospital staff, and from female patients who were overweight/obese or normal weight with a family history of diabetes, attending our clinic. All of them underwent a 75 g oral glucose tolerance test (OGTT), and had been tested for luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, prolactin, and total testosterone. It consisted of 63 age-matched healthy women with regular menses (28 ± 2 days) and normal ovaries as assessed by ultrasonography. The participants were excluded if they had thyroid, hepatic, or renal diseases, or took medication known to affect serum sex steroids and blood glucose level for at least 6 months before the start of the study.

A written informed consent for this study was obtained from all the participants, and was approved by the medical Ethics Committee of Central South University Xiangya Hospital.

Anthropometry and laboratory measurements

Anthropometric data were collected at the time of recruitment. BMI (kg/m^2) was calculated based on the height and body weight. According to the Asia-Pacific perspective (21), the degree of obesity was classified as nonobese ($\text{BMI} < 25.0 \text{ kg}/\text{m}^2$) or obese ($\text{BMI} \geq 25.0 \text{ kg}/\text{m}^2$). Waist circumferences (WC) and hip circumferences were measured, starting at the midpoint of the inferior border of the lowest rib and following the iliac crest on the mid-axillary line by around the abdomen, and the waist to hip ratio (WHR) was calculated.

At the time of recruitment, a questionnaire was used to determine the family history and history of diabetes, age of menarche, menstrual cycles per year, and use of oral contraceptives.

A 75-g OGTT was conducted after an 8–12 h overnight fast. The venous blood samples were obtained at 0, 30, 60, and 120 min after the glucose load to measure glucose and insulin. The serum samples were frozen and stored at -70°C for subsequent zonulin assays.

Plasma glucose, total cholesterol, triglyceride (TG), HDL-C, LDL-C, and uric acid were measured enzymatically on an automatic analyzer (Hitachi) with reagents from Wako Pure Chemical Industries (Osaka, Japan). HbA1c was determined using a high-performance liquid chromatography method on the Bio-Rad D10 Analyzer using reagents from Roche Diagnostics. LH, FSH, estradiol, prolactin, total testosterone, and insulin were measured by commercially available immunochemiluminescent assay (Roche Diagnostic).

Serum zonulin concentrations were measured in duplicate by using the ELISA Kit (Immundiagnostik AG, Bensheim, Germany). The sensitivity of the assay was <0.01 ng/ml. Intra- and interassay coefficients of variation for these determinations were between 2.8 and 8.1% and between 4.8 and 11.6% respectively. The ELISA Kit used for zonulin measurement only detects the active (uncleaved) form of zonulin.

Visceral adipose index (VAI) was calculated using the proposed formula: $VAI = WC / (36.58 + (1.89 \times BMI)) \times (TG / 0.81) \times (1.52 / HDL-C)$ (22). The indexes of homeostasis model assessment of insulin resistance (HOMA-IR) and β -cell insulin secretion (HOMA- β) were calculated based on the plasma glucose and insulin concentrations as described previously (23). Insulin sensitivity index (ISI) derived from the OGTT was assessed via Matsuda and DeFronzo's ISI (24). ($ISI = 10\,000 / \sqrt{(\text{fasting plasma glucose} \times \text{fasting plasma insulin}) \times (\text{mean glucose} \times \text{mean insulin})}$)).

Statistical analysis

Statistical analyses were performed using SPSS 19.0 (SPSS). The one-sample Kolmogorov–Smirnov test was used to determine data normality. Normally distributed data were

shown as mean \pm s.d., and skewed data were expressed as medians (interquartile range). Differences in clinical characteristics between two groups were analyzed using two-tailed Student's *t*-tests or Mann–Whitney *U* test where appropriate. Differences among the three groups of patients with PCOS were analyzed using one-way ANOVA or Kruskal–Wallis test. Multiple *post hoc* comparisons were performed using Tukey's correction. Correlation coefficients between serum zonulin with anthropometric parameters and biochemical indexes were determined by simple and partial correlation analyses where appropriate. The Spearman's correlation analysis or partial correlation analysis was applied to assess the correlation of number of menstrual cycles per year along with VAI, HOMA-IR, zonulin and ISI. $P < 0.05$ was considered statistically significant.

Results

Clinical characteristics of study participants

Clinical characteristics are given in Table 1. Compared with controls, PCOS women had higher systolic blood pressure, WC, WHR, BMI, TGs, VAI, OGTT plasma glucose at 0 min (Glu₀) and 120 min (Glu₁₂₀), OGTT insulin at

Table 1 Clinical and biochemical data of the women with PCOS and control women.

Variables	PCOS	Controls	P
<i>n</i>	78	63	–
Age (years)	29 \pm 5	30 \pm 5	0.250
Systolic blood pressure (mmHg)	121 \pm 14	110 \pm 12	0.021
Diastolic blood pressure (mmHg)	82 \pm 12	77 \pm 7	0.205
Waist circumference (cm)	91.8 \pm 8.2	82.3 \pm 8.2	0.003
WHR	0.93 \pm 0.05	0.86 \pm 0.06	0.001
BMI (kg/m ²)	27.6 \pm 4.0	25.3 \pm 3.0	0.001
Triglycerides (mmol/l)	2.61 \pm 1.70	1.44 \pm 1.15	0.023
Total cholesterol (mmol/l)	4.77 \pm 0.86	4.90 \pm 0.95	0.468
HDL-C (mmol/l)	1.33 \pm 0.31	1.96 \pm 0.38	0.012
LDL-C (mmol/l)	2.88 \pm 0.87	2.42 \pm 0.99	0.065
Glu ₀ (mmol/l)	5.79 \pm 1.82	5.05 \pm 0.52	0.041
Glu ₁₂₀ (mmol/l)	7.63 \pm 3.38	6.07 \pm 0.99	0.044
Ins ₀ (mU/l)	15.1 (11.2–22.9)	8.0 (7.5–11.1)	0.004
Ins ₁₂₀ (mU/l)	70.1 (43.1–134.9)	46.6 (25.0–60.6)	0.008
VAI	3.98 \pm 2.24	1.61 \pm 1.03	0.015
Total testosterone (ng/ml)	0.64 \pm 0.29	0.43 \pm 0.12	0.001
LH (mIU/ml)	9.05 \pm 5.93	5.43 \pm 3.12	0.016
FSH (mIU/ml)	5.86 \pm 1.52	5.92 \pm 1.56	0.651
LH/FSH	1.57 \pm 0.94	0.93 \pm 0.68	0.001
HOMA-IR	3.91 (2.80–7.41)	1.88 (1.46–2.51)	0.002
HOMA- β	223.8 (104.0–250.2)	142.7 (93.8–169.6)	0.081
ISI	2.53 (1.73–3.29)	5.59 (2.64–7.87)	0.001
Zonulin (pg/ml)	7.78 \pm 5.26	4.57 \pm 4.05	0.022

Statistically significant *P* values are in bold. WHR, waist-to-hip ratio; Glu₀, OGTT plasma glucose 0 min; Glu₁₂₀, OGTT plasma glucose at 120 min; Ins₀, OGTT insulin at 0 min; Ins₁₂₀, OGTT insulin at 120 min; VAI, visceral adiposity index; LH, luteinizing hormone; FSH, follicle stimulating hormone; HOMA-IR, HOMA of insulin resistance index; HOMA- β , HOMA of β -cell secretion index; ISI, insulin sensitivity index derived from OGTT.

Table 2 Comparison of clinical parameters between women with PCOS and BMI-matched controls.

	PCOS (n=78)		Controls (n=63)		P	
	Non-obese	Obese	Non-obese	Obese	P1	P2
n	21	57	39	24	–	–
Age	28±6	29±5	29±8	30±5	0.456	0.383
BMI (kg/m ²)	22.5±2.2	29.3±3.1	22.9±1.7	28.8±1.6	0.934	0.106
Waist circumference (cm)	81.5±4.8	95.0±6.1	77.6±5.8	90.7±3.5	0.004	0.003
WHR	0.90±0.05	0.94±0.05	0.83±0.06	0.91±0.06	0.001	0.012
VAI	2.08±1.14	4.81±3.25	1.32±1.02	2.79±1.36	0.086	0.002
Total testosterone (ng/ml)	0.32±0.12	0.78±0.45	0.31±0.10	0.63±0.14	0.856	0.183
HOMA-IR	2.80 (1.40–3.88)	4.60 (3.0–7.6)	1.80 (1.19–2.51)	2.02 (1.78–3.02)	0.034	0.021
HOMA-β	128.3 (92–224.4)	246.5 (113.8–366.7)	123.8 (73.4–146.3)	127.1 (96.0–205.5)	0.221	0.042
ISI	3.30 (2.21–6.02)	2.42 (1.44–2.88)	5.62 (4.64–6.87)	5.33 (3.94–7.04)	0.032	0.001
Menstrual cycles/year	10 (9–12)	7 (4–10)	12	12	0.001	0.001
Zonulin (pg/ml)	4.86±2.26	8.93±5.56	3.23±2.18	6.77±3.53	0.043	0.030

Statistically significant *P* values are in bold. WHR, waist-to-hip ratio; VAI, visceral adiposity index; HOMA-IR, HOMA of insulin resistance index; HOMA-β, HOMA of β-cell secretion index; ISI, insulin sensitivity index derived from OGTT; P1, non-obese women with PCOS vs non-obese controls; P2, obese women with PCOS vs obese controls.

0 min (Ins₀) and 120 min (Ins₁₂₀), total testosterone, LH and LH/FSH, and a lower HDL-C level (*P*<0.05 for all).

Serum zonulin levels and insulin resistance in PCOS patients and controls

Serum zonulin levels in PCOS women were significantly higher than those in controls (*P*=0.022). Compared with controls, the PCOS group also had increased HOMA-IR and decreased ISI, suggesting insulin resistance in PCOS women (Table 1).

After adjusting for BMI, serum zonulin levels, HOMA-IR, and ISI in PCOS subjects were still statistically different from those in BMI-matched controls (Table 2).

Relationships between serum zonulin and metabolic parameters

As given in Table 3, serum zonulin levels correlated significantly with BMI, WHR, TGs, HDL-C, VAI, Glu₀, Glu₁₂₀, Ins₀, Ins₁₂₀, HOMA-IR, and ISI (*P*<0.05 for all). After adjusting for age and BMI, the correlation between serum zonulin levels and HOMA-IR (*r*=0.315, *P*=0.044) and ISI (*r*=−0.262, *P*=0.019) remained significant.

Relationship between serum zonulin and severity of anovulation

PCOS patients were classified into three groups according to the severity of menstrual disorders: Group A (*n*=23) with severe menstrual disorders (≤6 cycles/year), Group B (*n*=42) with mild menstrual disorders (7–10 cycles/year),

and Group C (*n*=13) without menstrual disorders (11–12 cycles/year) (Table 4). Group A subjects had higher blood pressure, WC, BMI, TGs, VAI, glucose levels, insulin, HOMA-IR, and lower HDL-C and ISI, than either groups B

Table 3 Correlation of serum zonulin with anthropometric parameters and biochemical indexes.

Variables	Zonulin		Zonulin (adjust for age and BMI)	
	R	P	R	P
Age	−0.019	0.702	–	–
BMI	0.535	0.000	–	–
Systolic blood pressure	0.259	0.133	0.289	0.103
Diastolic blood pressure	0.260	0.131	0.279	0.116
WHR	0.401	0.015	−0.015	0.935
Triglycerides	0.422	0.031	−0.091	0.673
Total cholesterol	0.038	0.858	−0.248	0.254
HDL-C	−0.412	0.031	−0.239	0.272
LDL-C	0.136	0.512	−0.196	0.371
VAI	0.432	0.011	−0.092	0.678
Glu ₀	0.335	0.049	0.351	0.045
Glu ₁₂₀	0.445	0.007	0.347	0.045
Ins ₀	0.605	0.000	0.258	0.141
Ins ₁₂₀	0.527	0.001	0.246	0.161
Total testosterone	0.411	0.051	0.01	0.972
LH	−0.313	0.106	−0.174	0.519
FSH	0.093	0.714	−0.034	0.901
LH/FSH	−0.291	0.241	−0.181	0.503
HOMA-IR	0.645	0.000	0.315	0.044
HOMA-β	−0.082	0.349	−0.074	0.681
ISI	−0.542	0.000	−0.262	0.019

Statistically significant *P* values are in bold. BMI; WHR, waist-to-hip ratio; HDL-C; LDL-C; VAI, visceral adiposity index; Glu₀, OGTT plasma glucose at 0 min; Glu₁₂₀, OGTT plasma glucose at 120 min; Ins₀, OGTT insulin at 0 min; Ins₁₂₀, OGTT insulin at 120 min; LH, luteinizing hormone; FSH, follicle-stimulating hormone; HOMA-IR, HOMA of insulin resistance index; HOMA-β, HOMA of β-cell secretion index; ISI, insulin sensitivity index derived from OGTT.

Table 4 Characteristics of PCOS patients in groups classified by the severity of menstrual disorders.

Variables	Group A	Group B	Group C
<i>n</i>	23	42	13
Menstrual cycles/year	4 (3–6) ^{†§}	9 (8–10) [†]	11 (11–12)
Age (years)	30±6	28±5	30±4
Systolic blood pressure (mmHg)	133±15 ^{*†}	117±12	119±12
Diastolic blood pressure (mmHg)	91±10 ^{*†}	80±12	79±13
Waist circumference (cm)	95.5±9.3 ^{*†}	90.4±7.9	90.1±7.8
WHR	0.93±0.05	0.94±0.04	0.92±0.05
BMI (kg/m ²)	30.4±4.0 ^{*†}	26.6±3.5	26.4±4.0
Triglycerides (mmol/l)	3.56±2.17 ^{*†}	2.28±1.55	2.16±1.50
Total cholesterol (mmol/l)	5.09±0.93	4.81±1.01	4.15±1.18
HDL-C (mmol/l)	1.15±0.33 ^{*†}	1.38±0.34	1.43±0.24
LDL-C (mmol/l)	3.10±0.76	2.83±0.92	2.58±0.60
VAI	5.09±2.04 ^{††}	3.75±1.79	2.99±1.96
Glu ₀ (mmol/l)	7.30±2.87 ^{*†}	5.22±0.61	5.07±0.34
Glu ₁₂₀ (mmol/l)	8.49±3.51 [*]	7.61±2.03	6.22±1.04
Ins ₀ (mU/l)	29.3(14.1–35.2) ^{*†}	14.2 (10.6–18.9)	15.4 (6.9–28.8)
Ins ₁₂₀ (mU/l)	149.2(40.5–223.8) ^{*†}	78.3(46.6–109.9) [*]	57.0 (13.6–90.4)
Total testosterone (ng/dl)	0.60±0.19	0.68±0.32	0.56±0.29
LH (mIU/ml)	8.63±4.41	9.16±3.34	9.44±4.78
FSH (mIU/ml)	5.69±1.02	5.88±1.65	6.07±1.74
LH/FSH	1.50±0.96	1.58±0.66	1.57±0.65
HOMA-IR	7.54 (4.16–8.29) ^{††}	3.55 (2.44–4.69)	2.77 (1.54–3.87)
HOMA-β	272.2 (47.9–405.7)	219.5 (100.7–259.8)	192.8 (91.6–421.4)
ISI	2.29 (1.49–3.46) [†]	3.25 (2.26–4.80) [*]	4.43 (1.36–7.22)
Zonulin (pg/ml)	10.39±6.01 ^{††}	7.33±4.27 [*]	4.99±2.56

Compared with group C, ^{*}*P*<0.05, [†]*P*<0.01; compared with group B, [‡]*P*<0.05, [§]*P*<0.01; WHR, waist-to-hip ratio; BMI; HDL-C; LDL-C; VAI, visceral adiposity index; Glu₀, OGTT plasma glucose at 0 min; Glu₁₂₀, OGTT plasma glucose at 120 min; Ins₀, OGTT insulin at 0 min; Ins₁₂₀, OGTT insulin at 120 min; LH, luteinizing hormone; FSH, follicle stimulating hormone; HOMA-IR, HOMA of insulin resistance index; HOMA-β, HOMA of β-cell secretion index; ISI, insulin sensitivity index derived from OGTT.

or C, suggesting more severe metabolic dysfunction and insulin resistance in women with more severe menstrual disorders. Of particular interest, serum zonulin level was statistically different between each group (Table 4). In addition, an inverse correlation between zonulin and the number of menstrual cycles per year was observed ($r = -0.398$, $P < 0.001$; Fig. 1). After adjustment for BMI, this correlation remained significant ($r = -0.401$, $P < 0.001$). However, this inverse correlation was no longer significant after adjustment for HOMA-IR ($r = -0.128$, $P = 0.135$).

Discussion

To date, there have been no investigations on the role of zonulin in PCOS. In this study, we showed that serum zonulin levels were significantly elevated in women with PCOS. In addition, we demonstrated a strong relationship between serum zonulin level and obesity, insulin resistance, dyslipidemia, and severity of menstrual disorders.

To our knowledge, this is the first study to illustrate that gut permeability, determined by serum zonulin, is significantly increased in PCOS women. This might be explained by higher prevalence of obesity in the PCOS

group, as obesity is characterized by an increased gut permeability (4, 5, 6), and zonulin levels have been reported to be higher in obese subjects (19). Nevertheless, this study showed a difference in zonulin levels between PCOS women and controls, even after controlling for BMI. Our results suggest that serum zonulin levels are not independently determined by the degree of adiposity in PCOS women. Rather, other contributory factors are likely to play a role. One such factor might be insulin resistance, which was observed in both lean and obese women with PCOS (25), and was confirmed in our study. Our data show PCOS subjects had higher HOMA-IR and lower ISI as compared with BMI-matched controls. In addition, in accordance with Moreno-Navarrete *et al.* (19), an association between zonulin and insulin resistance was shown in our subjects. Therefore, we speculate that insulin resistance might be a link between elevated zonulin level (increased gut permeability) and development of PCOS. Alterations in gut permeability can compromise the intestinal barrier, allowing access of infectious agents and dietary antigens to facilitate mucosal immune elements, leading to production of inflammatory cytokines. It is well known that inflammatory cytokines, such as interleukin 6

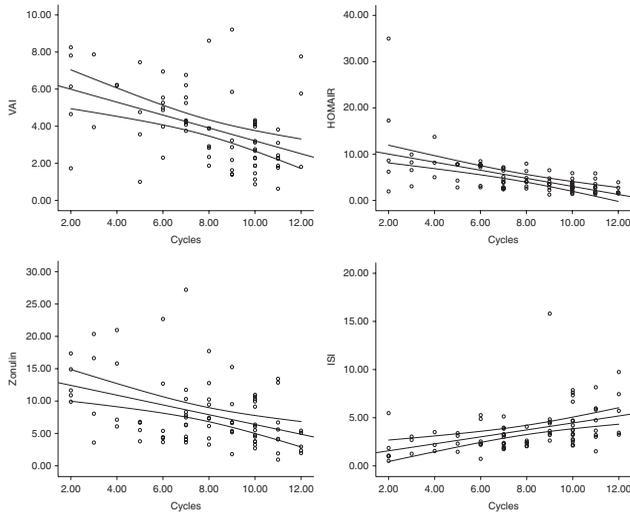


Figure 1

Correlation of number of menstrual cycles per year with VAI, HOMA-IR, zonulin, and ISI in PCOS women studied. (VAI, visceral adiposity index; HOMA-IR, HOMA of insulin resistance index; ISI, insulin sensitivity index derived from OGTT. With VAI, $r = -0.478$, $P < 0.001$; with HOMA-IR, $r = -0.605$, $P < 0.001$; with ISI, $r = 0.552$, $P < 0.001$; with zonulin, $r = -0.398$, $P < 0.001$).

(IL6) and $\text{TNF}\alpha$, can lead to insulin resistance (26). In addition, cytokines control zonulin production, as the *HP2* gene (which encodes zonulin production) promoter is under IL6 control through STAT3 activation and miR-18a induction (27). Thus, increased gut permeability leads to insulin resistance through a continuous inflammatory state.

In this study, we found PCOS women with more severe menstrual disorders had significantly higher zonulin levels, as well as an inverse correlation between zonulin and the number of menstrual cycles per year indicates an association between zonulin levels and severity of menstrual disorders. However, the correlation between serum zonulin and the number of menstrual cycles per year was no longer significant after adjusting for HOMA-IR. This suggests that insulin resistance is an essential link between zonulin and severity of menstrual disorders. In support of this, our data also show an inverse correlation between the number of menstrual cycles per year and insulin resistance, as estimated by HOMA-IR and ISI (Fig. 1).

Visceral adiposity index (VAI), a surrogate marker of adipose tissue function and distribution, is strongly associated with visceral adipose (22). VAI could be a useful tool for the assessment of cardiometabolic risk associated

with PCOS (28) in both clinical settings and in population studies. This study showed a strong relationship between serum zonulin level and VAI, suggesting serum zonulin may be valuable to detect patients at high risk to develop cardiometabolic disease.

Several animal studies have shown that prebiotic and probiotic supplements can help to improve intestinal barrier function and prevent impaired insulin resistance (29, 30). Therefore, we may expect that prebiotic and probiotic supplements would be an effective treatment for PCOS.

Hyperandrogenemia is the biochemical hallmark of PCOS (31). It was proposed that hyperandrogenemism can induce insulin resistance in PCOS, which in turn also plays a role in the development of hyperandrogenemism (32, 33). Hyperandrogenemia and ovulatory disturbances are commonly encountered in the syndromes of extreme insulin resistance (34). We failed to identify any association between total testosterone and zonulin level in our study. This may be a result of poor sensitivity of the methods used for total testosterone assays. The free androgen index may be more precise for hyperandrogenemia (35, 36).

Our study is not without limitations. The total number of participants was limited. Also, it would be appropriate to match groups for age and BMI (and not just for age), although we tried to overcome this limitation by using multivariate correction in the analysis of the correlation between zonulin with anthropometric and biochemical parameters. Also, given the cross-sectional nature, our study does not prove a mechanistic link between zonulin (increased gut permeability) and the development of PCOS. In addition, as increased gut permeability leads to insulin resistance through a continuous inflammatory state, it would be noteworthy to measure related cytokines, such as interleukin 1 β , tumor necrosis factor α , interleukin 6, in order to further explore the potential mechanisms between zonulin and PCOS.

In conclusion, our results show that serum levels of zonulin, a biomarker for gut permeability, were increased in PCOS women, and correlated with insulin resistance and severity of menstrual disorders. It suggested that alterations in gut permeability may play a role in the pathophysiology of PCOS, and serum zonulin might be used as a marker for both risk stratification and therapeutic outcomes in PCOS women.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

All authors listed in this manuscript contributed significantly to the development of the research and writing of the manuscript. Dr D Zhang designed the study. Drs L Zhang, F Yue, and Y Zheng contributed to sample collecting, data collection, and testing. Dr R Russell contributed to data analyses. Drs D Zhang and R Russell prepared the manuscript for submission.

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