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Original Article

Iron stores and obesity are negatively associated with ovarian volume and anti-Müllerian hormone levels in women with polycystic ovary syndrome

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ABSTRACT

Objective: Obesity and insulin resistance are associated with increased iron stores, but have conflicting effects on ovarian reserve in women with polycystic ovary syndrome (PCOS). Iron-catalyzed oxidative stress might be detrimental to ovarian tissue and granulosa cell function. In this study we determined the association between body iron stores, obesity, and ovarian reserve in women with PCOS.

Materials and Methods: One hundred and fifty-six women diagnosed with PCOS according to Rotterdam criteria and 30 normoweight healthy control women were enrolled in this cross-sectional study. Ovarian volume, total antral follicle count, and the anti-Müllerian hormone (AMH) level were measured as an indicator of ovarian reserve.

Results: Ferritin and transferrin-bound iron levels were significantly higher in women with PCOS than normoweight controls. Obese women with PCOS had higher ferritin levels ($p = 0.006$), but lower AMH levels ($p < 0.0001$) than nonobese women with PCOS. Using univariate analysis, the AMH level and mean ovarian volume were inversely related to the ferritin level, homeostasis model assessment of insulin resistance, and body mass index in women with PCOS. Body mass index and ferritin level remained significantly correlated with a lower AMH level and reduced ovarian volume, respectively, after considering other confounding variables.

Conclusion: An elevated ferritin level and obesity were negatively associated with ovarian volume and the AMH level, respectively, in women with PCOS.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women of reproductive age; PCOS is characterized by chronic anovulation, hyperandrogenism, and polycystic ovaries. Women with PCOS are known to have a higher prevalence of obesity [1,2], metabolism-related disorders [3,4], and insulin resistance, which have been proposed to correlate with oxidative stress caused by excess body iron stores [5,6].

The accumulated iron in mouse ovaries and boar testes has been shown to accelerate gonadal damage during the aging process due to increasing oxidative stress [7,8]. Serum ferritin levels in men and women with transfusion-dependent thalassemia major correlate with the presence of hypogonadism due to injuries, not only involving the hypothalamus and pituitary glands, but also the gonads [9–11]; however, the relationship between iron stores and gonadal function has never been investigated in humans without hemochromatosis.

Anti-Müllerian hormone (AMH) is a sensitive marker of ovarian reserve and granulosa cell function [12,13], and is a predictor of ovarian aging [9,14,15]. Although the reproductive lifespan of women with PCOS has been reported to be no different to [16] or longer than normo-ovulatory women [17], there is a more rapid

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decline in the AMH level in aging women with PCOS than in those without PCOS [17].

Ferritin is the cellular storage protein for iron and has also been the most commonly used indicator of body iron stores in epidemiologic studies [18,19]. Body iron stores and ferritin levels have been reported to be associated with type 2 diabetes, obesity, metabolic syndrome, and nonalcoholic steatohepatitis in the general population [20–23] and in women with PCOS [5,6]—a finding likely related to the tissue injury and organ failure caused by iron-promoted generation of reactive oxygen species.

Factors involved in the process of oxidative stress and the insulin-signaling pathway may be related to granulosa cell dysfunction [24,25]. In addition, obese and diabetic women tend to have an earlier decline of ovarian reserve and an earlier onset of the menopause [26,27]; however, the relationships between ovarian morphology, obesity, and metabolic disturbances in women with PCOS remain controversial [28,29].

We hypothesize that obesity and insulin resistance-related high ferritin levels in women with PCOS might be associated with decreased ovarian reserve due to potential oxidative injuries to the ovary. The aim of this study was to determine the relationships between obesity, ferritin level, and ovarian reserve in women with PCOS. The effects of oligomenorrhea and obesity on ferritin levels were also determined.

Material and Methods

Patients and data collection

One hundred and fifty-six women with PCOS and a chief complaint of irregular menstrual cycles and/or clinical hyperandrogenism, and willing to give informed consent, were consecutively recruited from the Reproductive Endocrinology Clinic (National Taiwan University Hospital, Taipei, Taiwan) at their first visit in this cross-sectional study. Thirty healthy normoweight volunteers with regular ovulatory cycles (mean cycle length < 35 days), < 30 years of age, and without signs of clinical or biochemical hyperandrogenism served as the control group. Control volunteers, but not PCOS patients, were enrolled by the aid of advertisement. None of the volunteers had been prescribed medications before enrollment. This study protocol was approved by the Research Ethics Committee of the National Taiwan University Hospital, Taipei, Taiwan. Written informed consent was obtained from all of the volunteers before participation.

The diagnosis of PCOS was based on the Rotterdam criteria, in which at least two of the following three criteria were met: (1) oligomenorrhea (< 8 spontaneous menstrual cycles per year at least 3 years before enrollment) or amenorrhea; (2) biochemical (serum total testosterone level $\geq$ 0.8 ng/mL) or phenotypic hyperandrogenism, including hirsutism and alopecia (acne was excluded due to an inconsistent correlation with hyperandrogenism in the literature); and (3) polycystic ovaries (> 12 follicles per ovary by transvaginal ultrasonography or an ovarian volume > 10 mL per ovary by transabdominal ultrasonography with a distended bladder for virginal women). The menstrual pattern data was obtained by questionnaire according to the patient's personal annotation or recall. The average of total spontaneous menstrual bleeding events every year for the continuous 3 years before enrollment was counted to define the severity of oligomenorrhea. Women with amenorrhea were defined as having no spontaneous menstrual bleeding without medication and receiving less than one induced menstrual bleeding cycle every year for at least 3 years before enrollment. All of the study volunteers were enrolled after excluding other endocrine, organic, and systemic abnormalities, such as hyperprolactinemia, thyroid dysfunction, Cushing's syndrome, congenital adrenal hyperplasia, adrenal tumors, ovarian tumors, autoimmune diseases, malignancies, central nervous system diseases, current use of oral contraceptives, or the use of medications known to affect the hypothalamic-pituitary-ovarian axis (antiandrogens, ovulation induction agents, antidiabetic medications, antiobesity medications, or glucocorticoids). None of the volunteers received a blood transfusion within 6 months before enrollment.

Overnight fasting blood samples were collected from PCOS patients with amenorrhea for > 3 months before hormone-induced withdrawal bleeding, and in the early follicular phase from those with PCOS who ovulated spontaneously. The blood sample was discarded if the serum progesterone level was > 2 ng/mL, or the serum estradiol level was > 150 pg/mL to exclude the possibility of delayed ovulation. The process for blood sample collection has been described in detail previously [4,30]. Blood was processed within 30 minutes of collection, and the blood glucose and insulin levels were determined on the day of sampling. The remaining serum and plasma were frozen at −70℃ until assayed.

Obesity was defined as a body mass index (BMI) $\geq$ 27 kg/m^2 based on the suggestion for the Asian as Hong Kong Chinese, Singaporean, and Indonesians [2].

Measurement of the total antral follicle count and mean ovarian volume

The mean ovarian volume was measured with transvaginal ultrasound for women with sexual experience and transabdominal ultrasound with a distended bladder for virginal women. The total antral follicle count (AFC) measurement was only performed for those who underwent transvaginal ultrasound examination ($n = 75$ in the PCOS group and $n = 12$ in the control group). Pelvic ultrasound measurements of the total AFC and mean ovarian volume were obtained according to a standard protocol [31] by one physician who was blind to the clinical manifestations of the enrolled volunteers. Briefly, after the longest medial axis of the ovary had been determined, the second dimension was measured, and then the probe was rotated 90° to obtain the third dimension. Ovarian volume was calculated using a suggested simplified formula (0.5 × length × width × thickness) [32]. The mean ovarian volume was defined as the average ovarian volume based on bilateral ovarian measurements. With respect to the AFC measurement, each ovary was scanned in longitudinal and transverse cross-sections from the inner to the outer margins to enumerate the follicles measuring between 2 mm and 9 mm in diameter. The total AFC was defined as the sum of the total number of follicles counted in both ovaries.

Assay methods

The levels of follicle stimulating hormone, luteinizing hormone (LH), estradiol, progesterone, total testosterone, sex hormone binding globulin, insulin, and glucose, and the homeostasis model assessment of insulin resistance (HOMA-IR) were measured and calculated as described previously [3]. The quantitative insulin sensitivity check index (QUICKI) was also applied to evaluate insulin resistance in women with PCOS. The serum levels of ferritin and transferrin-bound iron were measured with a biochemical autoanalyzer (TBA-2000FR; Toshiba Medical Systems Cooperation, Japan). Serum AMH levels were assessed, as in our previous studies [11,31], using a second-generation enzyme immunoassay (Immunotech A Bechman Coulter Company, Marseilles, France), according to the supplier's instructions. The free androgen index was used to estimate the bioavailable testosterone. The intra- and interassay coefficients of variation of all assays were < 10%.

Statistical analysis

Numeric variables are presented as the median with interquartile range in parentheses analyzed with the Wilcoxon rank-sum or Kruskall–Wallis test where appropriate. Categorical variables are presented as the number with the percentage in parentheses, and compared using Chi-square or Fisher's exact test as indicated. The optimal sample size needed for the current study was estimated based on our previous study using the correlation coefficients between AMH and ferritin levels in women with transfusion-dependent beta-thalassemia [11]. Based on the assumption of a correlation coefficient of 0.39, an alpha error of 0.05, and a power of 80%, the estimated total sample size was 46. The sample size of the control group estimation was based on literature studying on the difference of ferritin levels in women with and without PCOS [33]. Based on the assumption of ferritin levels of 56 ng/mL in PCOS and 38 ng/mL in the control group, a standard deviation of 25 ng/mL, an alpha error of 0.05, and a power of 80%, the estimated sample size was 33 in each group. The Shapiro–Wilk test was used to determine whether or not variables were normally distributed. Logarithmic transformation of variables was done before further analyses if variables were shown to deviate from a normal distribution. Spearman rank's correlation coefficients were calculated to determine the correlations between all variables. Multivariate regression models were applied to assess the associations between obesity, ferritin levels, and the surrogate indicators of ovarian reserve as the AMH level, mean ovarian volume, and total AFC after adjustment for the presence of PCOS, the LH and total testosterone levels, and the HOMA-IR. To avoid the effects of collinearity, we used the HOMA-IR instead of fasting glucose and insulin levels, and used the BMI instead of the waist circumference in the final models. Multiple linear regression analyses with and without dummy variables were applied to test the trend and associations of increased ferritin levels and the severity of oligomenorrhea after adjustment for age and BMI. For all analyses, a $p < 0.05$ was considered statistically significant. All of the statistical analyses were performed using the personal computer version of SAS (version 9.1; SAS Institute, Inc., Cary, NC, USA).

Results

Among 156 women with PCOS, 50 (32.1%) were obese with a BMI $\geq$ 27 kg/m^2, according to the definition of obesity for Asian adults, and 31 (19.9%) were amenorrheic for at least 3 years. The BMI, waist circumference, fasting insulin and glucose levels, QUICKI, and HOMA-IR were significantly higher in obese than nonobese women with PCOS. The ferritin levels ($p = 0.006$) were significantly higher, but the AMH levels ($p < 0.0001$) were significantly lower in obese than nonobese women with PCOS (Table 1). Ovarian volume, total AFC, and ferritin, transferrin-bound iron, and AMH levels were significantly higher in nonobese women with PCOS than in control women of similar age (Table 1).

In women with PCOS, the AMH level and mean ovarian volume had positive associations with sex hormone binding globulin, LH, QUICKI, and total testosterone levels, but were negatively correlated with BMI, waist circumference, fasting glucose, fasting insulin, ferritin levels, and the HOMA-IR, as shown in Table 2. The total AFC was positively correlated with the total testosterone level, but negatively correlated with the waist circumference. Ferritin and HOMA-IR were also negatively correlated with the total AFC in women with PCOS; although, the correlation was not statistically significant. The AMH, ovarian volume, and total AFC were still negatively associated with ferritin levels, but did not reach statistical significance, while the fasting glucose levels were negatively correlated with ovarian volume ($r = -0.488$, $p = 0.013$) and the total AFC ($r = -0.630$, $p = 0.028$).

Multivariate linear regression analyses showed that the total testosterone level is the major determinant of the AMH level, mean ovarian volume, and total AFC (Table 3); however, apart from the total testosterone level, the BMI ($\beta = -0.81$, $p = 0.003$) and ferritin level ($\beta = -0.12$, $p = 0.0002$) were negatively correlated with the AMH level and mean ovarian volume, respectively, after adjusting for all the explanatory variables, including the presence of PCOS, BMI, LH, ferritin, total testosterone levels, and the HOMA-IR. The interaction term among all variables was not significant in the linear regression models.

The ferritin level tended to be higher in women with fewer spontaneous menstrual cycles (Table 4). Multiple linear regression analyses revealed that women with fewer menstrual cycles tended to have a higher serum ferritin level than women with more menstrual cycles (p for trend = 0.009), and this effect persisted after further adjustment for age and BMI (p for trend = 0.0087).

Discussion

The current study revealed that obese women with PCOS have higher ferritin levels, but lower AMH levels than nonobese women with PCOS. The ferritin level and obesity were inversely related to the AMH level, mean ovarian volume, and total AFC in women with PCOS. The inverse associations between BMI and AMH level and between ferritin level and mean ovarian volume remained significant after adjustment for all the other explanatory factors, including the presence of PCOS, obesity, insulin resistance, LH level, and hyperandrogenemia. The findings of the present study suggest that increased iron load and the risk of obesity in women with PCOS are not only associated with a higher degree of insulin resistance and metabolic disorders, but also related to diminished ovarian reserve and reduced menstrual period frequency.

Previous studies have demonstrated that an increased ferritin level and marked iron deposition on aging gonads in animal models and human patients with major thalassemia, suggesting an inverse association between iron overload and hypogonadism [7–11,34]. The current study is the first report of an association between the ferritin level and diminished ovarian reserve in women with mildly elevated iron stores, which is related to obesity and insulin resistance. Although the relationship between polycystic ovarian morphology and metabolic disturbances is controversial in women with PCOS [28,29], we found inverse associations between ovarian volume, and the AFC and insulin resistance in women with PCOS when considering the AFC and ovarian volume as continuous variables. These findings suggest that metabolic disturbance and iron loading might affect the degree of polycystic ovarian morphology.

AMH has been reported to be a sensitive marker of diminished ovarian reserve related to aging [13–15]. The AMH level, AFC, and ovarian volume could be considered as surrogate markers to represent ovarian reserve. Although the reproductive lifespan of women with PCOS in comparison with women without PCOS is still in dispute [16,17], there is currently no available evidence to substantiate an earlier onset of menopause in women with PCOS. However, evidence from other populations of women with PCOS showed that obesity, insulin resistance, and diabetes might accelerate ovarian aging, resulting in early ovarian reserve decline and menopause [26,27,35,36]. The inverse association among insulin resistance, obesity, and AMH level has also been previously reported in women without PCOS [36–38]. Dietary restriction restored the ovarian reserve in an animal study [39], and these findings further substantiated the hypothesis that metabolism-related chronic oxidative stress may lead to gonadal damage. The above-mentioned evidence further supports the finding of a lower

Table 1
Comparisons of all demographic variables between nonobese and obese women with polycystic ovary syndrome, and of nonobese women between those with and without polycystic ovary syndrome.

	Nonobese PCOS ($N = 106$)	Obese PCOS ($N = 50$)	p[b]	Nonobese control ($N = 30$)	p[c]
Age (y)	24 (20–28)	25 (20–31)	NS	26 (24–27)	NS
BMI (kg/m^2)	21.63 (19.74–23.44)	31.01 (29.34–35.08)	<0.0001	20.10 (18.62–21.26)	0.006
Waist circumference (cm)	80 (75–84)	100 (95–107)	<0.0001	72 (69–76)	<0.0001
FSH (mIU/mL)	6.57 (5.55–7.55)	5.65 (5.05–6.11)	<0.0001	7.15 (6.10–8.10)	NS
LH (mIU/mL)	12.7 (10.0–16.7)	6.44 (4.91–9.47)	<0.0001	4.85 (3.40–6.30)	<0.0001
E2 (pg/mL)	45.4 (35.0–56.5)	48.5 (39.1–60.0)	NS	34.55 (27.5–29.34)	0.0001
Total testosterone (ng/mL)	0.53 (0.42–0.71)	0.54 (0.41–0.79)	NS	0.47 (0.35–0.64)	0.067
SHBG (nmol/L)	45.5 (28.6–60.2)	20.5 (16.5–25.9)	<0.0001	56.1 (42.7–73.6)	0.013
FAI (%)	4.15 (2.91–7.05)	10.0 (5.79–13.1)	<0.0001	2.74 (2.12–4.02)	0.016
Fasting glucose (mg/dL)	80 (77–84)	84 (80–89)	0.0002	84.5 (82–88)	0.002
Fasting insulin (μU/mL)	2.7 (2.0–5.0)	13.8 (8.3–19.9)	<0.0001	3.9 (2.0–6.3)	NS
HOMA-IR	0.48 (0.38–1.00)	2.89 (1.65–4.16)	<0.0001	0.80 (0.43–1.42)	0.025
QUICKI	0.19 (0.17–0.20)	0.14 (0.13–0.15)	<0.0001	0.17 (0.16–0.19)	0.025
AMH (pM)	64 (43–83)	37 (19–56)	<0.0001	20 (14–26)	<0.0001
Mean ovarian volume (mL)	10.8 (8.7–13.2)	9.9 (7.7–12.8)	NS	4.13 (3.72–5.69)	<0.0001
Total antral follicle count[a]	54 (37–67)	51 (30–60)	NS	10.0 (8–15)	<0.0001
Ferritin (ng/mL)	61.6 (34.1–108.0)	91.3 (61.0–176.3)	0.006	27.7 (16.5–44.2)	<0.0001
Transferrin-bound iron (μg/dL)	96.5 (74–130)	85.5 (70–108)	0.044	74 (58–96)	0.002

Data are presented as median (interquartile range) or n (%).
AMH = anti-Müllerian hormone; BMI = body mass index; E2 = estradiol; FAI = free androgen index; FSH = follicle-stimulating hormone; HOMA-IR = homeostasis model assessment of insulin resistance; LH = luteinizing hormone; NS = nonsignificant; PCOS = polycystic ovary syndrome; QUICKI = quantitative insulin sensitivity check index; SHBG = sex hormone binding globulin.
[a] Total antral follicle counts only available in a total of 12 volunteers without polycystic ovary syndrome (PCOS) and of 75 volunteers with PCOS, among women with PCOS, 21 were obese, and 54 were nonobese.
[b] Comparisons between obese and nonobese women with polycystic ovary syndrome.
[c] Comparisons between nonobese women with and without polycystic ovary syndrome.

ovarian volume and AMH level in obese women compared to nonobese women with PCOS in this study. Our findings are contradictory to previous studies in which polycystic ovary morphology did not predict the metabolic phenotype [28,29] and decreased AMH levels were generally accompanied with weight loss in women with PCOS [40,41]. These discrepant findings might be attributed to ethnic differences because these studies were mostly conducted in Caucasian women who were reported to have more prominent features of hyperandrogenism than East Asian women, and androgen is a well-known determinant of polycystic ovaries in human and animal studies [31,42]. In addition, the decrease in AMH levels after weight loss is always accompanied with a reduction in the androgen level, but independent from the weight change in obese women with PCOS [41]. The finding that the AMH levels did not change in obese women without PCOS and hyperandrogenism after weight loss [40] and the inverse association between ferritin level, obesity, and ovarian reserve after considering the effect of hyperandrogenemia in women with PCOS in this study further substantiate this hypothesis.

The serum ferritin level represents the cellular storage of iron. Unlike transferrin-bound iron that represents the circulating iron level, ferritin is the most commonly used indicator for body iron stores in epidemiologic studies [18,19,21]. Recent studies have reported an association between body iron stores and metabolic

Table 2
Spearman rank correlation analyses of anti-Müllerian hormone levels, mean ovarian volume, and total antral follicle count with each other and with all explanatory variables in women with polycystic ovary syndrome.

	All	AMH		Mean ovarian volume		Total AFC	
	Median (IQR)[a]	r	p	r	p	r	p
Age (y)	24 (20–28.5)	0.048	NS	−0.077	NS	−0.119	NS
BMI (kg/m^2)	23.42 (20.68–29.00)	−0.392	<0.0001	−0.213	0.009	−0.177	NS
Waist circumference (cm)	93.5 (77.0–95.0)	−0.415	<0.0001	−0.220	0.007	−0.247	0.032
FSH (mIU/mL)	6.14 (5.24–7.21)	0.012	NS	−0.055	NS	−0.195	NS
LH (mIU/mL)	11.05 (6.75–15.0)	0.366	<0.0001	0.198	0.016	0.168	NS
E2 (pg/mL)	45.9 (35.6–58.5)	−0.071	NS	−0.050	NS	0.037	NS
Total testosterone (ng/mL)	0.54 (0.42–0.74)	0.372	<0.0001	0.240	0.003	0.350	0.002
SHBG (nmol/L)	31.8 (20.8–52.2)	0.408	<0.0001	0.202	0.014	0.203	NS
FAI (%)	5.79 (3.45–9.94)	−0.112	NS	−0.014	NS	0.039	NS
Fasting glucose (mg/dL)	82 (78–85)	−0.227	0.004	−0.198	0.016	−0.182	NS
Fasting insulin (μU/mL)	4.4 (2.0–9.6)	−0.339	<0.0001	−0.229	0.005	−0.175	NS
HOMA-IR	0.83 (0.40–2.00)	−0.340	<0.0001	−0.220	0.007	−0.193	NS
QUICKI	0.17 (0.15–0.20)	0.340	<0.0001	0.220	0.007	0.193	NS
Ferritin (ng/mL)	77.0 (37.5–125.2)	−0.186	0.020	−0.318	<0.0001	−0.200	NS
Transferrin-bound iron (μg/dL)	92.0 (72.0–119.5)	0.180	0.024	−0.047	NS	−0.043	NS

Data are presented as median with interquartile range.
AFC = antral follicle count; AMH = anti-Müllerian hormone; BMI = body mass index; E2 = estradiol; FAI = free androgen index; FSH = follicle-stimulating hormone; HOMA-IR = homeostasis model assessment of insulin resistance; LH = luteinizing hormone; NS = nonsignificant; QUICKI = quantitative insulin sensitivity check index; r = Spearman rank correlation coefficient; SHBG = sex hormone binding globulin.

Table 3
Multivariate regression models for the association of anti-Müllerian hormone levels, mean ovarian volume, and total antral follicle count with all the explanatory variables in all women.

	logAMH		log(Mean ovarian volume)		log(Total AFC)	
	Model A[a]	Model B[b]	Model A[a]	Model B[b]	Model A[a]	Model B[b]
PCOS	0.89 (<0.0001)	0.84 (<0.0001)	0.97 (<0.0001)	0.97 (<0.0001)	1.62 (<0.0001)	1.66 (<0.0001)
logBMI	−0.81 (0.003)	−0.68 (0.016)	−0.27 (NS)	−0.25 (NS)	−0.42 (NS)	−0.35 (NS)
logLH	0.090 (NS)	0.24 (0.011)	−0.068 (NS)	−0.007 (NS)	−0.068 (NS)	0.014 (NS)
log(total testosterone)	0.60 (<0.0001)	—	0.28 (<0.0001)	—	0.37 (0.005)	—
logHOMA-IR	−0.12 (0.027)	−0.005 (NS)	−0.023 (NS)	0.0004 (NS)	−0.007 (NS)	−0.002 (NS)
logFerritin	−0.024 (NS)	−0.010 (NS)	−0.12 (0.0002)	−0.11 (0.0007)	−0.076 (NS)	−0.075 (NS)
Adjusted R^2 (%)	43.76	34.63	48.29	43.41	62.58	59.09

Data are presented as regression coefficient (β); *p* values are indicated in parenthesis.
AFC = antral follicle count; AMH = anti-Müllerian hormone; BMI = body mass index; HOMA-IR = homeostasis model assessment of insulin resistance; LH = luteinizing hormone; NS = means nonsignificant.
[a] Covariates of all Model A include the presence of polycystic ovary syndrome, body mass index, luteinizing hormone, total testosterone, homeostasis model assessment of insulin resistance, and ferritin levels.
[b] Covariates of all Model B are the same as Model A except total testosterone.

Table 4
Comparisons of age, body mass index, and ferritin levels in women with polycystic ovary syndrome with different severities of oligomenorrhea.

	Mild oligomenorrhea	Modest oligomenorrhea	Moderate oligomenorrhea	Severe oligomenorrhea and/or amenorrhea[a]	*p*	*p* for trend
Average range of interval (d)	35–45	45–90	90–180	>180		
Average spontaneous menstrual cycles/y	> 8	5–8	2–4	≤1		
No. of women with PCOS	25	50	33	48		
Age (y)	21 (19–26)	25.5 (21–28)	25 (23–29)	23 (20–28.5)	NS	NS
BMI (kg/m^2)	22.03 (19.22–27.81)	25.35 (21.27–30.44)	22.65 (21.08–25.69)	23.79 (20.42–28.23)	NS	NS
Ferritin (ng/mL)	40.6 (34.5–84.2)	68.5 (37.9–119.2)	84.5 (30.4–128.0)	95.8 (51.6–167.7)	0.074	0.009

Data are presented as median with interquartile range.
BMI = body mass index; NS = nonsignificant; PCOS = polycystic ovary syndrome.
[a] Among 48 volunteers with severe oligomenorrhea, 35 volunteers were defined as amenorrheic at enrollment.

disturbances, including diabetes, obesity, insulin resistance, hepatic steatosis, and hypertension in the general population and in women with PCOS [5,6,19–23]. Iron depletion therapy, together with phlebotomy to reduce ferritin levels, have been reported to improve insulin resistance [43], decrease blood pressure, treat fatty liver, and improve blood glucose control [44,45]. The association between disordered metabolism and ferritin levels is independent of the secondary role of ferritin as an obesity-related acute phase inflammatory marker [5,19]. Iron accumulation in the ovary/testes [7] accompanied with increased oxidative stress have been reported to accelerate follicle aging [34], ovarian tissue injury [46], reduced sperm count, and testes weight [8]. Taken together, these findings suggest that increased iron stores may be associated with disturbed metabolism and lead to decreased gonadal function in women with PCOS. The causal relationships between obesity, elevated ferritin levels, and diminished ovarian volume in women with PCOS could not be explained by the evidence provided in the current study; indeed, additional studies are needed to clarify the effect of obesity on iron metabolism of the ovary. Furthermore, it is reasonable for us to speculate that obese women with PCOS might be more vulnerable to high ferritin levels with the decline in ovarian reserve and potentially earlier onset of menopause, while further information is needed to determine whether or not this is so.

It is likely that the severity of oligomenorrhea, as it relates to high ferritin level in women with PCOS, is probably due to decreased menstrual blood loss [5] or secondary to other unknown causes. Women with amenorrhea and severe oligomenorrhea are at a higher risk of obesity in comparison with women who are eumenorrheic, whether or not hyperandrogenic [47]. Accordingly, it is known that obesity might confound the association between the ferritin level and severity of oligomenorrhea. We have reported for the first time a significant relationship between ferritin levels and the severity of oligomenorrhea in women with PCOS after considering a common risk factor (obesity).

There were some limitations in this study. Women with PCOS are known to have a higher prevalence of obesity, insulin resistance, and augmented higher AMH levels than women without PCOS [48]. Chinese women with PCOS [49,50] have been reported to have a higher prevalence of polycystic ovary morphology, but less severe hyperandrogenism in comparison with women of other ethnicities [50]. Therefore, the generalizability of the results of the present study to other populations may be limited. In addition, the narrow age range of the enrolled volunteers and lack of obese controls also limit the interpretation of the results in this study.

In the current study, the body iron stores, as represented by the ferritin level, were significantly related to advanced age, obesity, insulin resistance, diminished ovarian reserve, and severity of oligomenorrhea in women with PCOS. The inverse impact of obesity and an elevated ferritin level on the AMH level and mean ovarian volume in women with PCOS persisted after adjustment for the BMI, LH level, insulin resistance index, and hyperandrogenemia. These findings suggest that obesity and elevated iron stores are associated with reduced ovarian reserve in women with PCOS, and might potentially imply a rapid decline in ovarian reserve in obese women with PCOS.

Conflicts of interests

The authors report no conflicts of interest.

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