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Elevated Serum Levels of Interleukin-18 Are Associated with Insulin Resistance in Women with Polycystic Ovary Syndrome

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Overproduction of proinflammatory factors is associated with obesity and diabetes. Interleukin (IL)-18 as a member of IL-1 cytokine family is increased in obese, in diabetic, and even in polycystic ovary syndrome (PCOS) patients. In the present study we evaluated the association of serum IL-18 levels with insulin resistance in PCOS women. Forty-two PCOS women and 38 control subjects were enrolled in this study and matched with respect to age and body mass index (BMI). Serum IL-18 levels and hormones were measured for all subjects. Furthermore, euglycemic hyperinsulinemic clamp test was performed in selected 30 PCOS women and 11 control subjects. Serum IL-18 levels were elevated in PCOS women compared with the control ($p = 0.033$). IL-18 levels were positively correlated with homeostasis model assessment index (HOMA) β index, which assesses β cell function ($p = 0.035$), but were inversely correlated with clamp indices, which best represent insulin resistance status: M, Clamp ISI*100, and MCRg values ($p = 0.006$, 0.010, and 0.009 respectively). No correlation was found between IL-18 and age, BMI, waist-to-hip ratio (WHR), lipid profile, dehydroepiandrosterone-sulfate (DHEAS), sex hormone–binding globulin (SHBG), or fasting insulin levels. In conclusion, in the present study, serum IL-18 levels were significantly increased in PCOS women and firmly associated with insulin resistance displayed by euglycemic hyperinsulinemic clamp test. It indicates that IL-18 may be a contributing factor linking inflammation and insulin resistance in PCOS women.

Key Words: Interleukin-18; polycystic ovary syndrome; insulin resistance; euglycemic hyperinsulinemic clamp test.

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Introduction

Interleukin (IL)-18 was originally identified as a member of the IL-1 family and as an interferon (IFN)-γ-inducing factor (IGIF) *(1–3)*. Proinflammatory cytokines including IL-1β, TNF-α, IL-6, and endotoxin (LPS) induce IL-18 gene expression in Kupffer cells and macrophages *(4,5)*. IL-18 is expressed and secreted quantitatively to the circulation by human adipocytes *(6)*. IL-18 also is expressed in atherosclerotic plaques, and is involved in atherosclerosis and plaque instability *(7,8)*.

Recent studies indicate that inflammatory mediators are overproduced in obesity, in diabetes, in metabolic syndrome, and even in polycystic ovary syndrome (PCOS) *(9–13)*. PCOS is one of the most common endocrine disorders of uncertain etiology, which affects between 6% and 10% of women at the reproductive age *(14)*. It is characterized by menstrual abnormality, hirsutism, acne, anovulatory infertility, and elevated androgens. It has been confirmed that insulin resistance is a common feature in either obese or nonobese women with PCOS *(14–16)*. Escobar-Morreale et al. reported that IL-18 levels were increased in PCOS women *(12)*. Therefore, IL-18, as an inflammatory mediator, is probably associated with insulin resistance in patients with PCOS. In the present study, we investigated the association of elevated IL-18 levels with insulin resistance in PCOS women independent of obesity and type 2 diabetes who demonstrated a high level of insulin resistance assessed by using the method of euglycemic hyperinsulinemic clamp test for the first time.

Results

Univariate Analysis

The clinical and biochemical characteristics for all the study subjects with PCOS and the normal controls are demonstrated in Table 1. Compared with the control, PCOS women had significantly higher serum dehydroepiandrosterone-sulfate (DHEAS) and total testosterone levels. Whereas BMI, waist-to-hip ratio (WHR), blood pressure, the lipids, fasting and postload glucose and insulin levels

Table 1
Physical and Biochemical Characteristics of Study Subjects in Control and PCOS Women

Group	Control subjects	PCOS subjects	*p*
No.	38	42	
Interleukin-18 (pg/mL)	271.7 ± 103.5	326.7 ± 121.4	0.033
Systolic blood pressure (mmHg)	112 ± 11	110 ± 14	0.686
Diastolic blood pressure (mmHg)	73 ± 8	74 ± 10	0.608
Body mass index (kg/m^2)	27.08 ± 5.19	27.47 ± 5.48	0.744
Waist-to-hip ratio	0.84 ± 0.06	0.84 ± 0.08	0.583
DHEAS (μg/dL)	162.8 ± 58.8	208.5 ± 106.3	0.019
SHBG (nmol/L)	64.8 ± 61.1	49.2 ± 43.2	0.195
Follicle stimulating hormone (mIU/mL)	4.83 ± 2.30	6.27 ± 2.86	0.034
Luteinizing hormone (mIU/mL)	9.18 ± 4.89	13.68 ± 18.21	0.252
Total testosterone (ng/mL)	0.53 ± 0.25	0.81 ± 0.29	<0.001
Triglycerides (mmol/L)	1.33 ± 0.62	1.56 ± 0.90	0.459
Total cholesterol (mmol/L)	4.73 ± 0.93	4.61 ± 0.82	0.624
HDL cholesterol (mmol/L)	1.28 ± 0.29	1.29 ± 0.30	0.985
LDL cholesterol (mmol/L)	2.84 ± 0.86	2.61 ± 0.69	0.287
Glucose 0 min (mmol/L)	5.1 ± 0.6	4.9 ± 0.5	0.210
Glucose 120 min (mmol/L)	6.7 ± 1.8	6.8 ± 1.7	0.758
Insulin 0 min (μIU/mL)	16.5 ± 12.6	17.9 ± 11.1	0.596
Insulin 120 min (μIU/mL)	147.7 ± 134.0	168.0 ± 157.1	0.537

*Values are mean ± SD. *p* values between groups result from independent variable *t* test analysis. DHEAS, dehydroepiandrosterone-sulfate; SHBG, sex hormone–binding globulin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

were not different between the two groups. Serum IL-18 levels were significantly increased in PCOS women compared with the control ($p = 0.033$).

The clinical and biochemical characteristics for all the study subjects taking part in the euglycemic hyperinsulinemic clamp test are shown in Table 2. The following indices best represent the insulin resistance status in these women: glucose disposal rate (M), defined as the amount of the glucose supplied to maintain blood glucose levels during the last 30 min of the clamp (M, mg/kg·min); the insulin sensitivity index (ISI), defined as the ratio of M to insulin concentration at the end of the clamp (clamp ISI, mg/kg·min per μIU/mL). Clamp ISI*100 is 100 times of clamp ISI for readability. Metabolic clearance rate of glucose (MCRg), expressed as the ratio of M to blood glucose concentration (MCRg, mL/kg·min). These PCOS subjects were further subdivided into lean and obese groups. The diagnosis of obesity was based on the criteria of Asia–Oceania *(17)*, which was defined by a BMI greater than or equal to 25 kg/m^2. Compared with the lean control subjects, both obese and lean PCOS women had significantly higher homeostasis model assessment index (HOMA) β index (for assessing β cell function), and lower M, clamp ISI*100, and MCRg values.

Among 41 study subjects taking part in the clamp test, univariate correlation analyses showed that IL-18 level was inversely correlated with M ($r = -0.419$, $p = 0.006$), ISI*100 ($r = -0.396$, $p = 0.010$) (Fig. 1A), and MCRg ($r = -0.405$, $p = 0.009$), and positively correlated with HOMA β index ($r = 0.334$, $p = 0.035$) (Fig. 1B). No correlation was observed between IL-18 levels and age, systolic and diastolic blood pressure, BMI, WHR, serum cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, total testosterone, DHEAS, sex hormone–binding globulin (SHBG) and fasting insulin concentrations (data not shown).

Adjusted Analysis

In a linear stepwise regression analysis with a model including age, systolic and diastolic blood pressure, BMI, WHR, total testosterone, HOMA β index, and clamp ISI*100 as independent variables, only clamp ISI*100 showed significant correlation with serum IL-18 levels ($r^2 = 0.134$; $f = 5.123$; $p < 0.05$).

Discussion

The present study demonstrated that serum IL-18 levels were significantly increased in PCOS women who presented with hyperandrogenism and strongly associated with insulin resistance defined by euglycemic hyperinsulinemic clamp test. It indicates that IL-18 may be a contributing factor linking inflammation and insulin resistance in PCOS women.

It is well known that IL-18 acts as an important regulator of both innate and acquired immune responses in many inflammatory diseases *(3,7,18–20)*. A number of studies have recently shown blood IL-18 levels are elevated in patients

Table 2
Physical and Biochemical Characteristics of Study Subjects in Clamp Test

Group	Control subjects	PCOS subjects		*p* ANOVA
	Lean	Lean	Obese	
No.	11	10	20	
Interleukin-18 (pg/mL)	246.6 ± 82.7	354.3 ± 118.0	330.8 ± 145.4	0.115
Systolic blood pressure (mmHg)	104 ± 12	103 ± 12	111 ± 12	0.124
Diastolic blood pressure (mmHg)	69 ± 7	76 ± 10	75 ± 10	0.138
Body mass index (kg/m^2)	19.87 ± 1.62	21.22 ± 2.54	30.11 ± 4.21	<0.001
Waist hip ratio	0.80 ± 0.05	0.79 ± 0.05	0.85 ± 0.07	0.025
DHEAS (μg/dL)	145.3 ± 78.0	192.5 ± 125.3	216.4 ± 105.5	0.205
SHBG (nmol/L)	145.8 ± 54.4	88.7 ± 63.1	36.7 ± 18.7	<0.001
Follicle stimulating hormone (mIU/mL)	4.79 ± 1.94	7.12 ± 4.84	6.28 ± 1.76	0.179
Luteinizing hormone (mIU/mL)	9.51 ± 5.06	23.00 ± 34.77	11.46 ± 5.96	0.170
Total testosterone (ng/mL)	0.48 ± 0.29	0.93 ± 0.23	0.65 ± 0.17	<0.001
Triglycerides (mmol/L)	0.76 ± 0.14	1.01 ± 0.36	1.56 ± 0.65	<0.01
Total cholesterol (mmol/L)	4.07 ± 0.56	4.41 ± 0.77	4.68 ± 0.86	0.249
HDL cholesterol (mmol/L)	1.59 ± 0.19	1.37 ± 0.30	1.29 ± 0.32	0.095
LDL cholesterol (mmol/L)	2.02 ± 0.27	2.39 ± 0.86	2.64 ± 0.64	0.103
Glucose 0 min (mmol/L)	4.9 ± 0.4	4.7 ± 0.4	4.91 ± 0.41	0.461
Glucose 120 min (mmol/L)	4.9 ± 0.9	6.6 ± 2.0	6.9 ± 1.7	0.010
Insulin 0 min (μIU/mL)	5.5 ± 3.9	10.0 ± 4.3	20.5 ± 11.5	<0.001
Insulin 120 min (μIU/mL)	32.0 ± 15.8	138.0 ± 122.5	183.5 ± 183.8	0.027
HOMA β index	80.7 ± 48.7	233.3 ± 245.9	330.2 ± 191.7	<0.01
Values obtained from clamp test				
Glucose disposal rate (mg/kg·min)	8.7 ± 1.9	4.7 ± 1.1	3.8 ± 1.7	<0.001
ISI*100 (mg/kg·min per μIU/mL)	18.42 ± 5.60	9.07 ± 4.77	5.35 ± 2.84	<0.001
MCRg (mL/kg·min)	1.88 ± 0.41	1.04 ± 0.24	0.85 ± 0.41	<0.001

*Values are mean ± SD. *p* values among groups result from one way-ANOVA test analysis. DHEAS, dehydroepiandrosterone-sulfate; SHBG, sex hormone–binding globulin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA β index, homeostasis model assessment index for assessing β cell function; ISI, insulin sensitivity index; MCRg, Metabolic clearance rate of glucose.

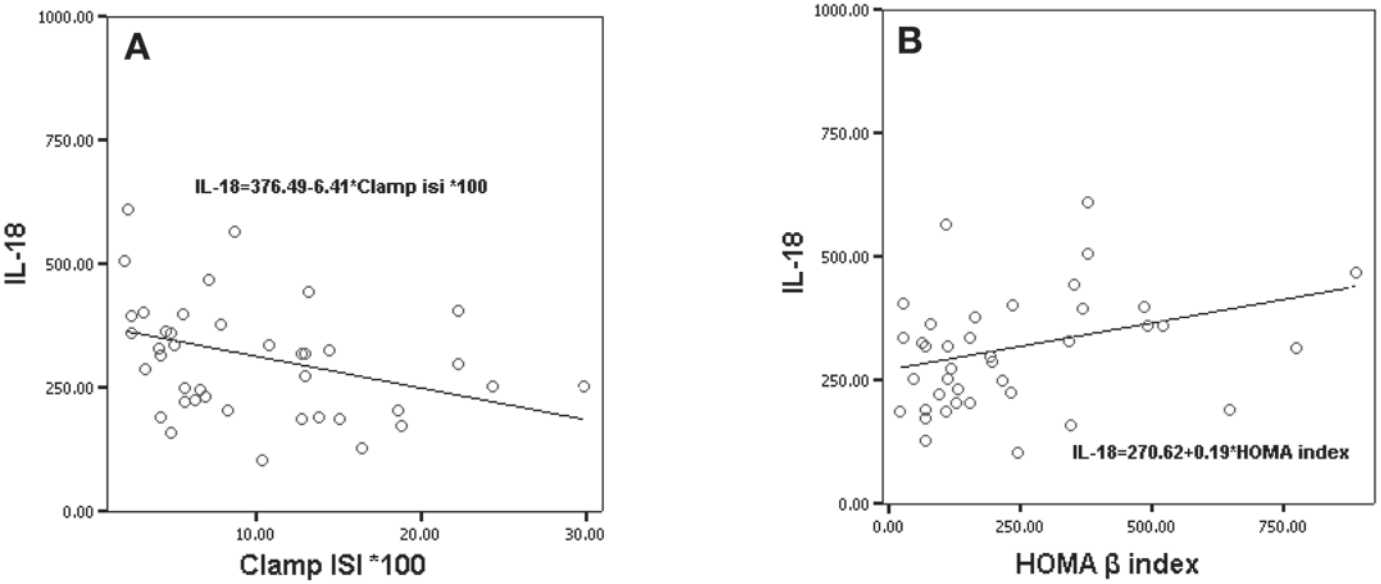

Fig 1. The scatter plot graphs show correlation of serum IL-18 concentrations with Clamp ISI*100 (**A**) and HOMA β index (**B**) among 41 study subjects taking part in the euglycemc hyperinsulinemic clamp test.

with obesity and metabolic syndrome *(6,13)*. In the present study, we observed serum IL-18 levels were significantly increased in PCOS women. The inverse correlations between serum IL-18 levels and insulin sensitivity indices including M, clamp ISI*100, and MCRg values were also demonstrated in PCOS women and the control subjects. Our present results confirmed and expanded preliminary data from Escobar-Morreale et al. *(12)*. Insulin resistance is considered as "common soil" for diabetes and cardiovascular diseases, which also frequently occur in PCOS women *(21,22)*. Moreover, some studies demonstrated an early impairment of endothelial structure and vaso-elastic properties appearing even in young PCOS women with normal weight, normal lipids, and normotension *(22,23)*, which was attributed to insulin resistance and the chronic inflammatory process *(24)*. Chronic inflammation displayed as elevated C-reactive protein, fibrinogen, ferritin, TNF-α, and IL-6 was strongly associated with insulin resistance *(9,10)*. In our present study, the selected PCOS women can be further divided into lean and obese subgroups.

Compared with the lean control women, both lean and obese PCOS women showed significantly lower M, clamp ISI*100, and MCRg values (seen in Table 2). This means the PCOS women considered as a whole in our study showed significantly higher levels of insulin resistance than that of the normal control women. These findings together with the results of univariate correlation analysis and regression analysis indicated that IL-18 might play a role as an proinflammatory mediator linking insulin resistance and inflammation in PCOS women. However, we could not find any correlation between serum IL-18 levels and BMI as it was reported elsewhere *(12,13)* (although serum IL-18 level was slightly increased in the obese subjects). The results favor a hypothesis that inflammatory cytokine IL-18 acts as a contributing factor in the abnormal glucose homeostasis associated with PCOS women even with normal body weight, although until now, a definitive mechanism between the inflammation and insulin resistance is still pending. More research work is needed to further confirm this conclusion.

In the present study, the correlation analysis also showed the positive relationship between β cell secretion (which is calculated by HOMA β index) and serum IL-18 levels in PCOS women. This finding is in accordance with the in vitro study reported by another group *(25)*, which showed that IL-18 has minor stimulatory effects for insulin secretion on β cells. This may indicate that the hypersecretion of β cell in PCOS women with normal glucose status may be associated with the high levels of serum IL-18, which suggests that inflammatory mediator may also be a accelerator of β cell toxication. To confirm and support this hypothesis, more clinical and laboratory studies are required.

In conclusion, serum IL-18 concentrations are significantly increased in PCOS women and are strongly associated with insulin resistance displayed by euglycemic hyperinsulinemic clamp test, which showed the inverse correlation of serum IL-18 with insulin sensitivity index (M, ISI*100, and MCRg). This conclusion indicates that, as a human model of insulin resistance, PCOS women showed high levels of serum IL-18. IL-18, as an inflammatory cytokine, may play a role in mediating insulin resistance and inflammation. All these characteristics may be independent of BMI. But more research works are required to further confirm this hypothesis.

Materials and Methods

Subjects

Forty-two PCOS patients and 38 nonhyperandrogenic women were enrolled in this study. The PCOS and the control group were matched for BMI and fasting plasma glucose levels. The PCOS diagnosis was based on the following criteria *(26)*: (1) chronic anovulation: oligomenorrhea or amenorrhea from menarche; (2) clinical and/or biochemical signs of hyperandrogenism: such as hirsutism, acne, alopecia, and/or increased circulating total or free testosterone, androstenedine; or DHEAS levels; (3) polycystic ovaries. And the congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome, and hyperprolactinemia were ruled out in all the PCOS patients. All subjects were Chinese living in the Shanghai region, and were given informed consent. The Institutional Review Board of the Ruijin Hospital approved the study protocol. None of the study participants was taking any medication or instructed on lifestyle modification. All the subjects in this study were nonsmokers and had no history of atherosclerosis and cardiovascular disease. The plasma glucose concentrations were within normal range (fasting glucose levels <6.1 mmol/L and 2 h post-prandial <7.8 mmol/L) and the blood pressures were less than140/90 mmHg.

Among all the study subjects, 41 subjects were selected to take the euglycemic hyperinsulinemic clamp test. Among them, 11 control subjects were selected from all 11 lean control, 10 lean PCOS subjects were selected from all the lean PCOS women, and the 20 obese PCOS subjects were selected from the 32 obese PCOS women. No subjects were selected from the obese control.

Methods

All the anthropometric measurements were determined by the same physicians from our department. All subjects consumed a diet containing at least 250 g of carbohydrate for at least 3 d before the overnight fasting (12–14 h). Blood samples were taken between d 8 and d 10 of the menstrual cycle or during amenorrhea after excluding pregnancy for measurements of plasma glucose, lipids, serum IL-18, insulin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone, DHEAS, estradiol, and SHBG. A 75 g oral glucose tolerance test (OGTT) was performed and blood samples were obtained for measurement of plasma glucose and serum insulin at 30, 60, 120, and 180 min. The

plasma glucose was measured immediately and the blood samples were frozen at –20°C until assayed.

Euglycemic Hyperinsulinemic Clamp

The euglycemic hyperinsulinemic clamp test was performed 6–8 d after the OGTT as described previously *(27, 28)*. A small polyethylene catheter was inserted into an antecubital vein for blood sampling. A second catheter was inserted into the contralateral forearm vein for infusion of insulin and 20% dextrose solution. After a 30–45 min stabilization period, a 10-min priming insulin infusion was followed by a constant infusion of 40 mU/m^2 surface area per min over 140 min (totally 150 min). The 20% glucose solution was infused 4 min after the initiation of insulin infusion to maintain the blood glucose at baseline level. During the clamp, blood glucose levels were repeatedly determined by glucose analyzer (Biosen 5130, Neckar Healthcare. Co. Ltd. Magdeburg, Germany). The blood samples for insulin determination were collected at the interval of 10 min.

Hormone and Biochemical Assays

Serum LH, FSH, and testosterone concentrations were measured by chemiluminescent assay using an ACCESS autoanalyzer (Beckman Coulter Inc., Fullerton, CA, USA). The concentrations of DHEAS were determined by RIA (DSL Inc., Webster, Texas, USA), SHBG were determined by IRMA (DSL Inc.). Serum IL-18 levels were measured by ELISA (Medical & Biological Laboratories Co., LTD., Nagoya, Japan). The sensitivity of the assay for IL-18 is 12.5 pg/mL and mean intra- and interassay coefficients of variation are 7.3% and 7.5%, respectively. Plasma glucose concentration was determined by a glucose oxidase electrode method (Beckman Coulter Inc.), Plasma insulin concentrations were measured by RIA (DSL Inc.); serum TC and TG were analyzed using the enzymatic kits (Beckman Coulter Inc.). HDL-cholesterol and LDL-cholesterol were determined by an immunoinhibition method (HDL-C, LDL-C Direct, Wake Pure Chemical Industries Ltd. GmbH, Neuss, Germany).

The homeostasis model assessment index (HOMA) for assessing β cell function: HOMA β index was calculated using the following formula: [20× fasting serum insulin (μIU/mL)]/[fasting plasma glucose (mmol/L)–3.5].

Statistical Analysis

Statistical analysis was performed using the SPSS 10.0 system. Measurements with a skewed distribution were normalized by logarithmic transformation. Comparisons of means and proportions were performed with the Student's *t*-test and one-way ANOVA test, respectively. Correlations were tested by Spearsman correlation coefficients. To allow for covariates and confounders, we performed analysis of covariance and multiple linear regression. p value < 0.05 was considered statistically significant. All the data were presented as means ± SD.

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