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Definition of insulin resistance using the homeostasis model assessment (HOMA-IR) in IVF patients diagnosed with polycystic ovary syndrome (PCOS) according to the Rotterdam criteria

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ABSTRACT

Introduction: Polycystic ovary syndrome (PCOS) women are more insulin resistant than general population. Prevalence data on insulin resistance (IR) in PCOS varies depending on population characteristics and methodology used. The objectives of this study were to investigate whether IR in PCOS is exclusively associated with body mass and to assess the prevalence of IR in lean and overweight/obese PCOS.

Methods: Study included 250 consecutive women who attended a Department of Human Reproduction diagnosed as having PCOS according to the Rotterdam criteria. Control group comprised 500 healthy women referred for male factor infertility evaluation during the same period as the PCOS women.

Results: PCOS women (n=250) were more insulin resistant than controls (n=500) even after adjustment for age and BMI ($P=0.03$). Using logistic regression analysis BMI ≥ 25 kg/m² (OR 6.0; 95%CI 3.3-11.0), PCOS (OR 2.2; 95%CI 1.4-3.5) and waist circumference (WC) ≥ 80 cm (OR 2.0; 95%CI 1.1-3.8) were identified as independent determinants of IR ($P<0.001$). IR was more prevalent in overweight/obese controls (n=100) than in lean PCOS women (n=150), 31% vs 9.3%, but less prevalent than in overweight/obese PCOS (n=100), 31% vs 57%. The prevalence of IR between lean controls (5%) and lean PCOS (9.3%) did not significantly differ.

Conclusions: Both PCOS-specific and obesity-related IR independently contribute to IR in PCOS. Using HOMA-IR cut-off value of 3.15 specific for Croatian women in our clinical setting, the assessed prevalence of IR in lean and overweight/obese PCOS women was 9.3% and 57%, respectively.

Keywords

insulin resistance, polycystic ovary syndrome, body mass index

INTRODUCTION

The polycystic ovary syndrome (PCOS) is the most common endocrine disorder in reproductive age women with prevalence estimates ranging from 6% to 20% depending on the diagnostic criteria applied and the characteristics of population studied [1-4]. It is a very heterogeneous syndrome, with ethnicity, race, geographic region, and environmental factors contributing to both different clinical manifestation of PCOS and PCOS-associated long-term health risk [5].

Insulin resistance (IR) is typically defined as decreased sensitivity or responsiveness to the metabolic action of insulin [6]. IR is a prominent feature of PCOS, but it is not diagnostic criterion for PCOS. In addition, although insulin resistant women with PCOS are at an increased metabolic and cardiovascular risk, there is no general consensus on screening for IR in all PCOS women [5,7]. Direct, dynamic methods for measuring IR are accurate but inconvenient for clinical practice and epidemiological studies [6]. Among static IR indices, homeostasis model assessment for IR index (HOMA-IR) is the most widely used as a surrogate measure of IR in large population studies. However, the reports of studies investigating prevalence of IR in PCOS are highly inconsistent mainly due to differences in methods used and cut-offs selected for defining IR [8-10]. Moreover, there is an ongoing debate whether IR in PCOS is related to obesity alone or obesity aggravates IR intrinsic to PCOS [11,12].

Since IR in PCOS contributes to both reproductive and metabolic disturbances [6,7], it is clinically important to identify prevalence and degree of IR in PCOS population by using appropriate HOMA-IR cut-off value for identifying IR. Therefore, the objectives of this study were to investigate the relationship between IR and overweight/obesity in IVF patients diagnosed with PCOS according to the Rotterdam criteria and to derive HOMA-IR cut-off

value for identifying IR in our clinical setting in order to assess the prevalence and degree of IR in lean and overweight/obese PCOS.

METHODS

This cross sectional study included 250 consecutive women who attended a Department of Human Reproduction for infertility treatment between October 2010 and December 2012 and were diagnosed as having PCOS according to the Rotterdam criteria [13]. Oligomenorrhoea was defined as the mean menstrual cycle length >35 days in the preceding year. Hyperandrogenism (HA) was defined as serum testosterone concentration >2.8 nmol/L and/or clinically by hirsutism defined as a modified Ferriman-Gallwey (mFG) score >7 [14]. Polycystic ovarian morphology (POM) were defined as the presence of >11 follicles measuring 2-9 mm in diameter in at least one ovary [15].

The routine laboratory tests were performed to exclude other endocrine and metabolic disorders. The women using medications known to have an influence that might affect glucose metabolism and insulin sensitivity were excluded from the study.

Control group comprised 500 women referred to the Department of Human Reproduction for infertility evaluation during the same period as the PCOS women. They were randomly selected from the department database if met the following inclusion criteria: 1) infertility attributable only to male factor; 2) age ≤ 40 years; 3) no family history of diabetes or hypertension; 4) regular menstrual intervals (21-35 days) in the preceding year; 5) no clinical or biochemical signs of hyperandrogenism; 6) normal ovarian morphology; 7) serum glucose concentration ≤ 6.0 mmol/L; 8) no previous or current, treated or not-treated, conditions that could interfere with study results; 9) no medications used in the past year.

Physical examination, blood sample collection and ultrasound assessment of ovarian morphology were performed on day 3-5 of a menstrual cycle or a withdrawal bleeding induced by gestagens [16].

Blood samples for the determination of glucose and hormone concentrations were taken between 8:00 and 9:30 hours after an overnight fast. Testosterone, follitropin (FSH) and lutropin (LH) concentrations were determined by chemiluminescent immunoassays on Access[®] 2 analyser (Beckman Coulter, Inc., Brea, USA). Insulin concentration was measured using the ADVIA Centaur[®] XP immunoassay system (Siemens Healthcare Diagnostics, Inc., Tarrytown, USA). Glucose concentration was measured using a hexokinase method (Beckman Coulter, Inc., Brea, USA).

HOMA-IR was calculated using the formula: $\text{HOMA-IR} = [\text{fasting insulin } (\mu\text{IU/mL}) \times \text{fasting glucose (mmol/L)}] / 22.5$ [17].

AFC was assessed using a two-dimensional transvaginal probe 5-7 MHz (Toshiba, Nemio, Japan).

Anthropometric measurements (height, weight, waist circumference (WC)) were performed on the same day as the transvaginal ultrasound scan and body mass index (BMI) was calculated. PCOS patients and controls were divided according to BMI in lean subgroups ($\text{BMI} < 25 \text{ kg/m}^2$; $n=150$ and $n=400$, respectively) and overweight/obese subgroups ($\text{BMI} \geq 25 \text{ kg/m}^2$; $n=100$ and $n=100$, respectively).

For the purpose of this study the 95th percentile of distribution HOMA-IR values in lean, non-hyperandrogenic Caucasian women with regular menstrual cycles, normal (non-polycystic) ovarian morphology, $\text{WC} \leq 80 \text{ cm}$, and no family history of diabetes was used as cut-off value for identifying IR. These women ($n=382$) were selected from the control group.

As the present study is retrospective and included only analysis of data obtained from routine clinical and laboratory measurements, the Institutional Review Board approval was not required.

Statistical analysis

Data analysis was performed with MedCalc[®] statistical software, version 12.6.1 (MedCalc Software, Ostend, Belgium). The Man-Whitney test was used to test for difference between PCOS and control group. Analysis of covariance (ANCOVA) was used to compare HOMA-IR values in PCOS and controls after controlling for age and BMI. The Kruskal-Wallis test with post-hoc pairwise comparison was used to compare the HOMA-IR in the lean and overweight/obese PCOS women with that of corresponding control subgroups. The chi-square test was used to compare the prevalence of IR in PCOS and controls before and after stratification in lean and overweight subgroups. The logistic regression analysis was applied to check for confounding effects of overweight/obesity ($\text{BMI} \geq 25 \text{ kg/m}^2$) and PCOS as dichotomous variables on IR. *P* value < 0.05 was considered as statistically significant.

RESULTS

PCOS women were younger and had significantly higher insulin concentrations, HOMA-IR, WC and BMI compared with controls. Insulin and HOMA-IR remained higher in PCOS women than in controls even after controlling for age and BMI (Table 1). The between-group difference in glucose concentration was not found. As expected, obesity was more prevalent in PCOS than in controls (15.6% vs 4.2%; $P < 0.001$).

Comparison of HOMA-IR between the lean subgroups and the overweight/obese subgroups of controls and PCOS women after being matched for BMI is shown in Figure 1. PCOS women were more insulin resistant i.e. they had higher HOMA-IR than controls when the

lean PCOS subgroup was compared with the lean control subgroup, and when the overweight/obese PCOS subgroup was compared with the overweight/obese control subgroup, respectively ($P<0.05$). However, HOMA-IR was found to be significantly lower in lean PCOS women than in overweight/obese controls ($P<0.05$).

The HOMA-IR values obtained from 382 lean ($\text{BMI}<25\text{kg/m}^2$, $\text{WC}\leq 80$ cm) women, who were selected from the control group, were used for determination of cut-off value for IR. Their baseline characteristics are summarized in Table 2. The cut-off value for IR was defined as 95th percentile of the distribution of HOMA-IR observed in these lean, non-hyperandrogenic, eumenorrhoeic women of reproductive age with normal ovarian morphology and no family history of diabetes i.e. IR was defined if $\text{HOMA-IR} > 3.15$ (95%CI 2.9-3.4).

The HOMA-IR cut-off value of 3.15 was then used to assess the prevalence of IR in IVF patients with PCOS. In the overall study population, there were 16.5% women with $\text{HOMA-IR} > 3.15$. As expected, the prevalence of IR in PCOS women was significantly higher ($P<0.001$) than in controls, 28.4% (71/250) vs 10.6% (53/500), respectively.

After stratification of PCOS women and controls into subgroups according to BMI, the prevalence of IR was 5.0%, 9.3%, 31.0% and 57.0% in lean controls ($n=400$), lean PCOS women ($n=150$), overweight/obese controls ($n=100$) and overweight/obese PCOS women ($n=100$), respectively. The prevalence of IR was higher in overweight/obese controls than in lean PCOS women (31.0% vs 9.3%; $P<0.001$) but lower compared to overweight/obese PCOS women (31.0% vs 57.0%; $P<0.001$). The difference in prevalence of IR between lean controls (5.0%) and lean PCOS (9.3%) did not reach the level of statistical significance.

Using logistic regression analysis, $\text{BMI}\geq 25$ kg/m^2 (OR 6.0; 95%CI 3.3-11.0), PCOS (OR 2.2; 95%CI 1.4-3.5) and $\text{WC}\geq 80$ cm (OR 2.0; 95%CI 1.1-3.8) were identified as independent determinants of IR ($P<0.001$).

DISCUSSION

In this study, PCOS women had higher HOMA-IR than non-PCOS women (controls) even after controlling for BMI and age which is indicative of decreased insulin sensitivity in PCOS and, thus, a higher risk for developing IR-associated metabolic disorders such as impaired glucose tolerance, type 2 diabetes, the metabolic syndrome and potentially cardiovascular disease (Table 1) [18-20]. However, no difference was found in concentration of fasting glucose between PCOS and controls supporting previous findings that fasting glucose could not serve as a sensitive indicator of IR in PCOS women. BMI and WC were also higher in PCOS women which corroborate previously demonstrated positive association of obesity/visceral adiposity with the prevalence and degree of IR [21,22].

Lean PCOS were more insulin resistant than BMI-matched controls but less insulin resistant than overweight/obese PCOS (Figure 1). These results were similar to those recently obtained using the clamp technique for measurement of IR [23] and support the hypothesis on the intrinsic, PCOS-specific IR which could be augmented by obesity-related IR [11,12]. The difference in IR between lean PCOS and overweight/obese controls could be explained by pronounced effect of body mass on IR [5].

Although, the prevalence of obesity ($\geq 30 \text{ kg/m}^2$) in Croatian woman was estimated at 20.6% [24], obesity is not prominent clinical feature of women admitted to our department for infertility treatment. Only 8.0% women in this study cohort were obese. The prevalence of obesity in PCOS group was also very low (15.6%) compared with reported prevalence of obese women in studies conducted in the U.S. and Australia. In these countries, 61% and 76% PCOS women were considered obese [5]. The observed between-studies differences in prevalence of overweight/obese women are reflection of geographic location, ethnicity, environmental factors (lifestyle, diet) and criteria used for diagnosing PCOS.

It is clinically important to identify insulin resistant women in infertile IVF population in order to reduce their long-term metabolic risk and/or improve reproductive outcomes through lifestyle changes and pharmacological interventions [5,25]. Insulin promotes primordial to primary follicle transition [26]. In addition, FSH-responsiveness of granulosa cells of gonadotropin-dependent stages of folliculogenesis are enhanced by insulin growth factors [27]. Therefore, in the IVF setting, the multifolliculogenesis as an response to exogenous gonadotropin stimulation is more frequent in insulin resistant patients who are, thus, more prone to develop ovarian hyperstimulation syndrome [28,29]. The identification of insulin resistant patients prior to the IVF procedure could help clinicians to decrease the risk of IVF complications by choosing appropriate ovarian stimulation protocol and optimal gonadotropin dose and/or to assess potential benefit from insulin-sensitizing therapy.

In this study, the 95th percentile of HOMA-IR in healthy, lean women was selected as the optimal cut-off for distinguishing insulin resistant from insulin sensitive individuals. Recent study using pre-selected HOMA-IR cut-off ≥ 2.5 identified 22.6% non-obese PCOS women as being insulin resistant while the overall prevalence of IR was estimated at 31.6% in the similar sample of women with PCOS [17,30]. If the same cut-off has been selected as criterion in this study, more than 10% of healthy lean controls with WC < 80 cm would be identified as being IR. Therefore, the use of pre-selected HOMA-IR cut-off for identifying those with IR should be discouraged since, even in the ethnically homogeneous population, substantial differences in HOMA-IR cut-off value used could have an influence on identification of insulin resistant women and therefore, their healthcare management [9,31].

Thus, IR was defined as HOMA-IR > 3.15. The observed prevalence of IR in PCOS was 28.4% and significantly higher compared with controls (10.6%), but lower compared with other reports (44-70%) [6,32]. The prevalence of IR was similar in lean controls and lean PCOS but higher in overweight PCOS compared with overweight controls. Nevertheless, an

independent association of overweight/obesity ($\text{BMI} \geq 25 \text{ kg/m}^2$), PCOS (the Rotterdam criteria) and abdominal obesity ($\text{WC} \geq 80 \text{ cm}$) with IR demonstrated in this study corroborates the role of intrinsic, PCOS-specific IR [6,11,12].

The limitation of this study is concern about sensitivity and accuracy of HOMA-IR to assess IR compared with the gold standard technique for measuring IR. However, due to convenience and cost-saving, HOMA-IR is considered appropriate for large scale and epidemiological studies with cross-sectional design [33]. The fact that control group included women undergoing IVF could be recognized as one of the study limitation. However, all women from the control group underwent IVF treatment due to the male factor infertility and not any other infertility issue, whatsoever. Controls could therefore be considered as representatives of the general population or a true control group although included patients referred to the Department of Human Reproduction for infertility treatment. Moreover, all women from the control group underwent transvaginal ultrasound examination and those with polycystic ovaries were not included in the control group of the study.

The main advantages of this study were homogeneity of study population with respect to racial, ethnic, and geographic origin and absence of selection bias other than being evaluated for infertility treatment. Furthermore, the ultrasound ovarian examination and the assessment of hirsutism were performed by the same physician, thus eliminating interobserver bias.

In conclusion, the prevalence of IR, as defined by $\text{HOMA-IR} > 3.15$, was 28.4% in infertile Croatian women with PCOS. The prevalence of IR in lean and overweight/obese PCOS was 9.3% and 57%, respectively. Both PCOS-specific and obesity-related IR independently contribute to IR in PCOS. These study results support the necessity of determination of the clinical setting-specific HOMA-IR cut-off value for identifying IR for routine clinical practice and in studies aimed to investigate IR prevalence. Accordingly, the HOMA-IR cut-off value used in our clinical setting could not be advised to be applied universally.

Declaration of interest

The authors have no conflict of interest to disclose.

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Table 1. Baseline characteristics of study population

Variable	Controls (n=500)	PCOS women (n=250)	<i>P</i>	<i>P</i> *
Age (years)	33.1 (30.4 - 36.0)	29.9 (27.4 - 32.5)	<0.001	NA
BMI (kg/m ²)	23 (21 - 25)	24 (21 - 29)	<0.001	NA
Waist circumference (cm)	70 (66 - 78)	76 (68 - 87)	<0.001	0,103
Testosterone (nmol/L)	1.4 (1.0 - 1.8)	2.0 (1.5 - 2.7)	<0.001	<0.001
mFG score	2 (1 - 2)	6 (3 - 10)	<0.001	<0.001
FSH (IU/L)	7.4 (6.2 - 9.1)	6.0 (4.9 - 7.4)	<0.001	<0.001
LH (IU/L)	4.5 (3.5 - 5.9)	6.0 (4.1 - 8.6)	<0.001	<0.001
Glucose (mmol/L)	5.2 (5.0 - 5.5)	5.2 (4.9 - 5.5)	0.241	0,112
Insulin (mIU/L)	6.9 (5.3 - 9.0)	9.7 (6.5 - 14.6)	<0.001	0,011
HOMA-IR	1.6 (1.1 - 2.1)	2.3 (1.5 - 3.4)	<0.001	0,023

Values are median (interquartile range). NA = not applicable; mFG score = modified Ferriman Gallwey score.

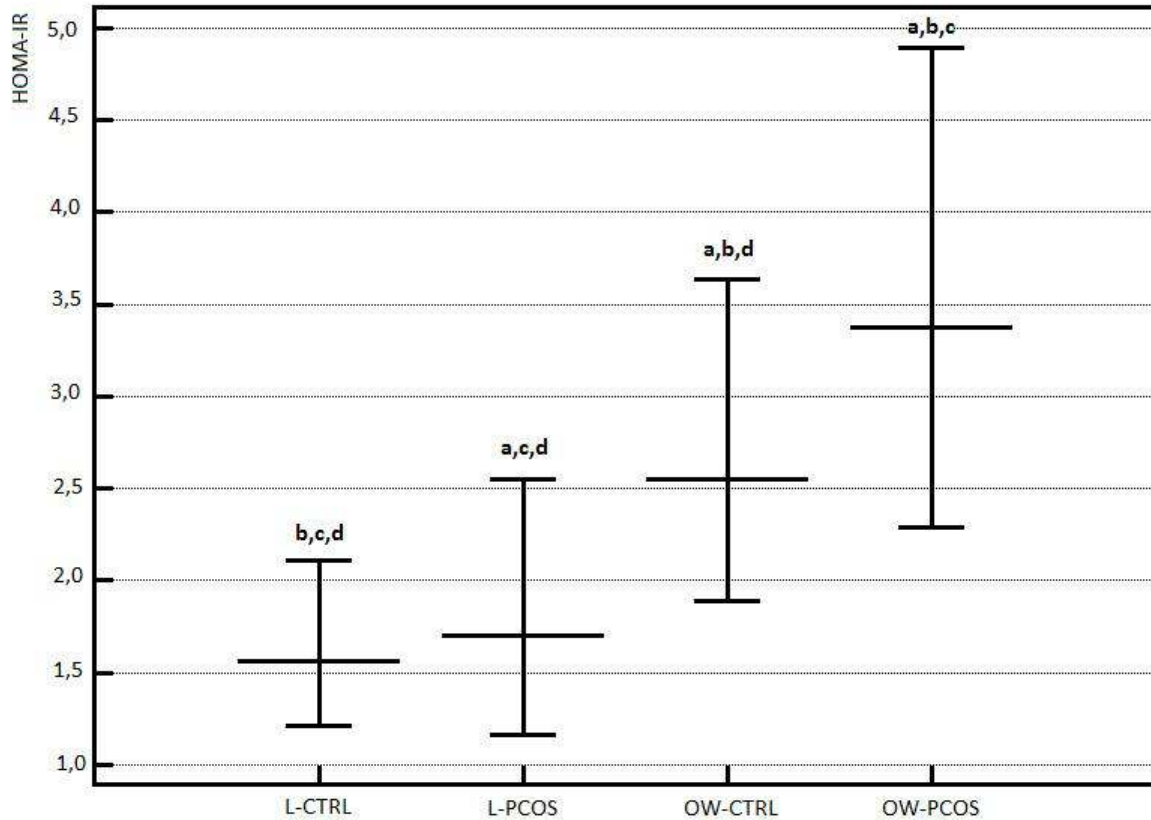
P - value was assessed using Mann-Whitney test; *P* - value* was assessed using ANCOVA after controlling for age and BMI; *P* < 0.05 was considered statistically significant.

Table 2. Baseline characteristics of lean controls (BMI < 25 kg/m², waist circumference < 80 cm) selected to determine the clinical setting-specific HOMA-IR cut-off value for identification of insulin resistance

Variable	Lean controls (n=382)
Age (years)	33.3 (30.3 - 36.0)
BMI (kg/m ²)	22 (20 - 24)
Waist circumference (cm)	69 (65 - 73)
Menstrual cycle length (days)	29 (28 - 30)
Modified Ferriman-Gallwey score	2 (1 - 2)
Antral follicle count	12 (8 - 15)
Testosterone (nmol/L)	1.4 (1.0 - 1.7)
FSH (IU/L)	7.6 (6.2 - 9.4)
LH (IU/L)	4.6 (3.6 - 6.1)
Insulin (mIU/L)	6.9 (5.3 - 9.0)
Glucose (mmol/L)	5.2 (5.0 - 5.5)
HOMA-IR	1.5 (1.2 - 2.1)

Values are median (interquartile range).

Figure 1: Comparison of HOMA-IR in BMI-matched PCOS women and controls



L-CTRL - lean ($\text{BMI} < 25 \text{ kg/m}^2$) controls ($n=400$); L-PCOS - lean PCOS ($n=150$); OW-CTRL - overweight/obese ($\text{BMI} \geq 25 \text{ kg/m}^2$) controls ($n=100$); OW-PCOS - overweight/obese PCOS ($n=100$). Middle horizontal lines represent the medians. Horizontal small bars indicate 27-75th percentile range. P - value was calculated by Kruskal-Wallis test with pairwise between-group comparison according to Conover. The P - value < 0.05 was considered as statistically significant (a - denotes the significant difference compared with L-CTRL, b - denotes the significant difference compared with L-PCOS, c - denotes the significant difference compared with OW-CTRL and d - denotes the significant difference compared with OW-PCOS)