

## Research Article

# Age, Body Mass Index, and Waist-to-Hip Ratio Related Changes in Insulin Secretion and Insulin Sensitivity in Women with Polycystic Ovary Syndrome: Minimal Model Analyses

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Insulin resistance is believed to be an integral component of the polycystic ovary syndrome (PCOS). Beta ( $\beta$ ) cell dysfunction is also found in PCOS. In the study, we determined the influence of age, body mass index (BMI), and waist-to-hip ratio (WHR) on insulin response to oral glucose load (OGTT) and on insulin sensitivity ( $S_i$ ) and  $\beta$ -cell function in young women with PCOS. One hundred fourteen patients with PCOS and 41 controls with normal basal plasma glucose were studied. A 75-g OGTT was performed to determine glucose tolerance and insulin response. Insulin sensitivity and  $\beta$ -cell function were studied using a modified frequently sampled IV glucose tolerance test (FISGTT) to determine the acute insulin response ( $AIR_G$ ), as well as  $S_i$  by minimal model analysis.  $S_i$  was decreased in PCOS women ( $2.49 \pm 0.18$  vs.  $3.41 \pm 0.36$ ,  $p < 0.05$ ), but no difference in  $AIR_G$  existed between the PCOS and control group ( $75.1 \pm 4.6$  vs.  $63.4 \pm 4.6$ ,  $p < 0.05$ ). BMI and WHR correlated negatively with  $S_i$  ( $r = -0.43$ ;  $r = -0.289$ ,  $p < 0.001$ , respectively), but not with  $AIR_G$  ( $r = 0.116$ ;  $r = -0.02$ ,  $p > 0.05$ , respectively). Increasing age correlated negatively with  $AIR_G$  ( $r = -0.285$ ,  $p < 0.001$ ). There was a significant interaction between disease (PCOS), BMI, and WHR on  $S_i$  as well as between age and PCOS on  $AIR_G$ . Thus, patients below the age of 25 with PCOS showed enhanced  $AIR_G$  ( $89.5 \pm 7.1$  vs.  $65.1 \pm 6.7$ ,  $p < 0.05$ ) and decreased  $S_i$  ( $2.43 \pm 0.25$  vs.  $4.52 \pm 0.62$ ,  $p < 0.05$ ) compared to age-matched controls. In conclusion, these data suggest that not all patients with PCOS have basal and stimulated hyperinsulinemia, insulin resistance, and impaired glucose tolerance. Based on these data in young PCOS subjects, the development of insulin resistance and T2DM may be prevented with appropriate treatment strategies.

## 1. Introduction

Polycystic ovary syndrome (PCOS) is one of the most frequent endocrinopathies in women of reproductive age [1]. The diagnosis of PCOS is suggested by the findings of hyperandrogenism and infertility [2]. Insulin resistance is a coexisting characteristic of this disorder in many mature patients with established PCOS, but its role in the pathogenesis of PCOS is unclear [3–5]. While insulin resistance may be a factor in the development of PCOS, the associated

failure of pancreatic  $\beta$ -cell function may also be an important determinant of impaired glucose tolerance or type 2 diabetes (T2DM). Knockout experiments confirm that type 2 diabetes is a “2-hit” disease, in which insulin resistance is necessarily accompanied by a  $\beta$ -defect, preventing the compensatory up-regulation of insulin secretion [6]. The prevalence of impaired glucose tolerance (IGT) and T2DM is increased in PCOS [6]. The clinical characteristics of PCOS, including insulin resistance, have been studied in adolescent persons with this disorder. It is postulated that

the disorder begins at menarche, and some characteristics change with age [7]. A previous study [8] suggested that adolescents with PCOS are severely insulin resistant, compared with a control group matched for body composition and abdominal obesity. Middle-aged PCOS women have been observed to have an increased prevalence of T2DM when compared to an age-matched control population [9].

Thus, the aim of the present study was to investigate the oral glucose tolerance test (OGTT), insulin sensitivity ( $S_i$ ), and acute insulin response ( $AIR_G$ ) during a frequently sampled glucose tolerance test (FSIGTT) as well as the interactions of age, body mass index (BMI), and waist-to-hip ratio (WHR) with the PCOS disorder in a relatively young group of women with PCOS.

## 2. Materials and Methods

**2.1. Subjects.** 114 women with PCOS and 41 years of age and BMI-matched healthy women were referred consecutively to the outpatient clinic of the Clinic for Endocrinology, Diabetes, and Diseases of Metabolism for clinical hyperandrogenism and/or menstrual dysfunction. PCOS was diagnosed according to the Rotterdam workshop criteria, i.e., the presence of at least two among the three following features: clinical and/or biochemical hyperandrogenism, chronic oligoanovulation, and polycystic ovary morphology (PCOM), after exclusion of secondary causes [2]. Appropriate tests were used to confirm the absence of specific diseases of the adrenal, ovary or pituitary, such as nonclassic 21-hydroxylase deficiency, hyperprolactinemia, or androgen-secreting neoplasms [1]. No women were taking medications which could potentially interfere with the evaluations carried out in the study. Moreover, patients had not received oral contraceptives, insulin-sensitizing agents, antiandrogens, or glucocorticoids in the six months prior to the investigation. BMI was calculated as body weight/height ( $\text{kg}/\text{m}^2$ ), and WHR was determined by measuring the waist in centimeters at its smallest circumference and the hip in centimeters at the largest circumference. A BMI of  $25 \text{ kg}/\text{m}^2$  was determined as the borderline between overweight and nonoverweight subjects. All the investigated subjects had normal fasting plasma glucose ( $\leq 5.6 \text{ mmol}/\text{l}$ ) except one PCOS patient who had fasting plasma glucose of  $5.8 \text{ mmol}/\text{L}$ . All controls had normal glucose tolerance based on 2-hr plasma glucose levels during OGTT [10]. Women with PCOS were studied in their follicular phase of the menstrual cycle or were amenorrhoeic for more than three months, while control women were tested during their follicular phase. The local human investigation committee approved the study protocol, and all participants gave informed consent.

**2.2. Protocol.** Oral glucose tolerance test (OGTT) and intravenous glucose tolerance test (IVGTT) with frequent blood sampling (FSIGTT) were conducted on separate occasions in all subjects. Tests were performed after 3 days on a 300 g per day carbohydrate diet and after an overweight fast of 10 hr. Blood samples for plasma glucose and plasma

TABLE 1: Characteristics of studied PCOS women and controls (mean  $\pm$  SEM).

Characteristics	PCOS	Controls
	( $n = 114$ ) (Mean $\pm$ SE)	( $n = 41$ ) (Mean $\pm$ SE)
AGE (years)	$24.88 \pm 0.59$	$26.60 \pm 0.89$
BMI ( $\text{kg}/\text{m}^2$ )	$28.80 \pm 0.67$	$28.55 \pm 1.35$
WHR	$0.81 \pm 0.006$	$0.82 \pm 0.015$
Fasting plasma glucose (mmol/L)	$4.42 \pm 0.06$	$4.37 \pm 0.09$
Total testosterone (nmol/L)*	$3.73 \pm 0.16$	$1.89 \pm 0.10$
Systolic BP (mmHg)	$125.12 \pm 1.13$	$126.39 \pm 2.30$
Diastolic BP (mmHg)	$80.61 \pm 0.93$	$82.92 \pm 1.85$

\*  $p < 0.05$ .

insulin were drawn at baseline and every 30 min for 2 hr, after a 75-g glucose load. The modified IVGTT (FISGTT) was also performed after overnight fasting, according to the previously published procedures [11, 12]. After an overnight fast, catheters were placed in a forearm vein and a hand vein of the contralateral arm. Basal samples were collected for glucose and insulin at  $-15$ ,  $-10$ ,  $-5$ , and  $-1$  min. Glucose ( $300 \text{ mg}/\text{kg}$ ) was injected as a bolus at time 0 over 1 min and flushed with saline to ensure complete delivery. After 20 min,  $0.05 \text{ IU}/\text{kg}$  of short-acting insulin (Actrapid HM, NovoNordisk) was injected. Blood samples were drawn at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 90, 100, 120, 140, 160, and 180 min for measuring plasma glucose and insulin levels. Glucose was measured in a Beckman glucose analyser, using the glucose oxidase method. Insulin ( $\text{mU}/\text{L}$ ) and testosterone ( $\text{nmol}/\text{L}$ ) (basal) were measured by radioimmunoassay (RiA INEP, Zemun).

**2.3. Data Analysis.** The area under the glucose (AUCG) and insulin (AUCI) response curves during OGTT was calculated by the standard trapezoidal rule. The insulin sensitivity index ( $S_i$ ) was calculated by a minimal model analysis using the MINMOD computer program [13]. An acute insulin response ( $AIR_G$ ) was calculated as the mean increase in insulin levels calculated from 2 to 10 min of IVGTT. The disposition index was calculated according to the following formula:  $DI = S_i \times AIR$ . The glucose tolerance to IV glucose load (Kg) was calculated according to the standard procedures [12]. Data were compared using  $T$ -test, and age and BMI multivariate probability distribution was compared using the peacock test. Multivariate probability distributions were not different between the groups ( $p = 0.305$ ).

Best subset regression was done using the leaps package in order to estimate the best predictor of BMI as well as WHR [14].

Data are presented as the mean SEM. Comparisons between groups were performed using the general factorial analysis of the covariance model, controlling for the effect of BMI and WHR (ANCOVA). All analysis was done for the whole group, but some data are presented for obese and nonobese separately. All analysis was performed using SPSS and the R software package.

TABLE 2: Investigated indices in PCOS and controls.

Parameter	PCOS	Controls	P value
Fasting glucose (mmol/L)	4.42 ± 0.06	4.37 ± 0.089	<i>p</i> > 0.05
Plasma glucose at 120 min of OGTT (mmol/L)	5.29 ± 0.10	4.72 ± 0.17	<i>p</i> < 0.05
Fasting insulin (mU/L)	16.35 ± 0.99	12.34 ± 1.47	<i>p</i> < 0.05
Plasma insulin at 120 min of OGTT (mU/L)	78.52 ± 5.93	52.07 ± 8.69	<i>p</i> < 0.05
Area under glucose curve (OGTT)	729.47 ± 13.43	699.40 ± 24.81	<i>p</i> > 0.05
Area under insulin curve (OGTT)	9931.62 ± 594.49	7816.08 ± 892.38	<i>p</i> < 0.05
Si (insulin sensitivity, minimal model analysis)	2.49 ± 0.18	3.41 ± 0.36	<i>p</i> < 0.05
AIR (acute insulin response, minimal model analysis)	76.29 ± 4.56	65.69 ± 3.28	<i>p</i> > 0.05
Di (disposition index, minimal model analysis)	171.07 ± 13.07	220.28 ± 28.12	<i>p</i> > 0.05

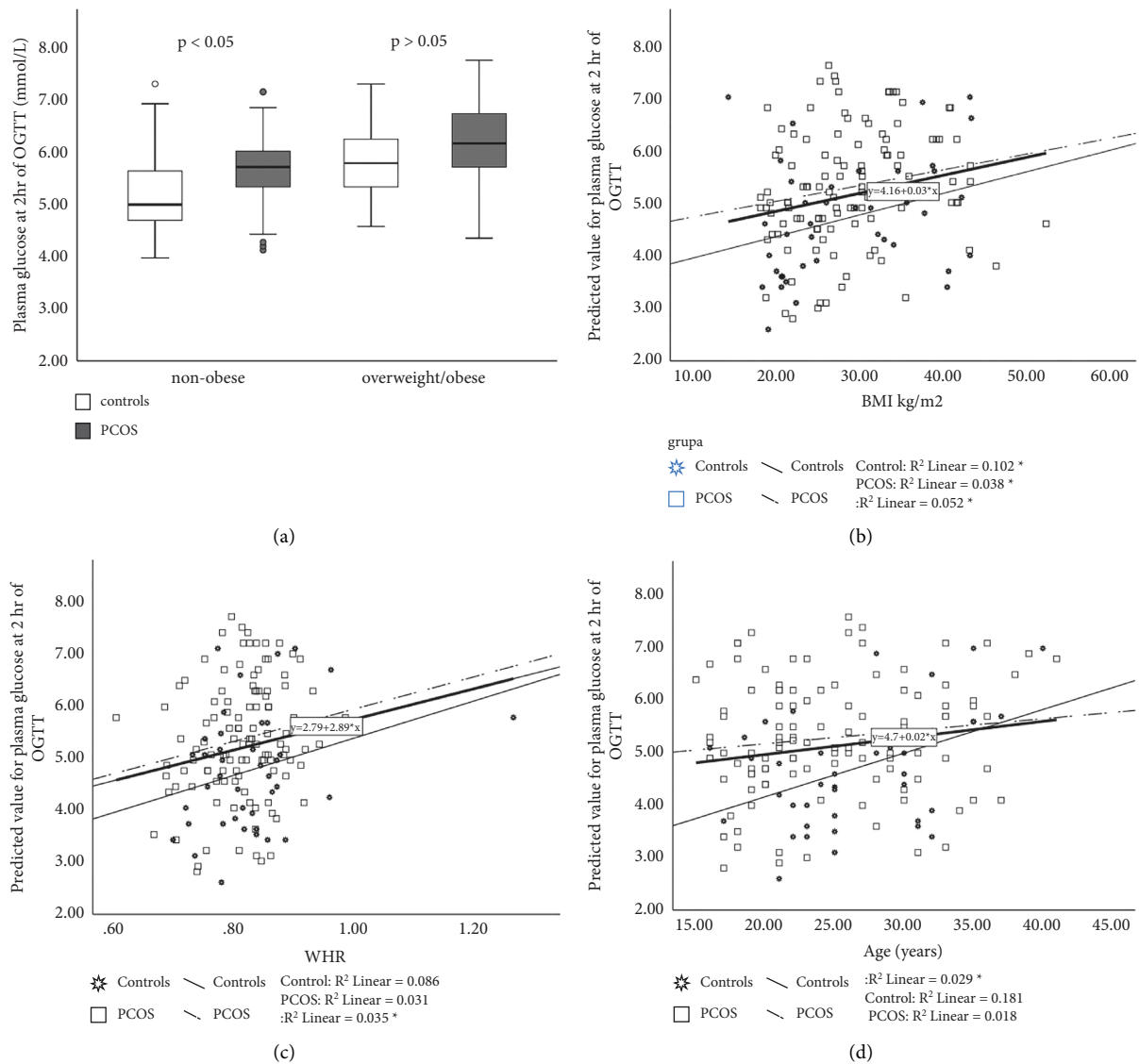


FIGURE 1: Plasma glucose at 2 hr of OGTT in PCOS patients and controls. PCOS vs. controls, *p* < 0.05. Data are presented as the mean ± SEM, separately for nonobese subjects (BMI < 25 kg/m<sup>2</sup>) and overweight/obese (BMI > 25 kg/m<sup>2</sup>) (a). Relationship between BMI and plasma glucose at 2 hr of OGTT (b). Relationship between WHR and plasma glucose at 2 hr of OGTT (c). Relationship between age and plasma glucose at 2 hr of OGTT (d). \* *p* < 0.05.

TABLE 3: Investigated indices in PCOS and controls (nonobese vs. overweight/obese).

Parameter	PCOS vs. controls (nonobese)	P value	PCOS vs. controls (overweight/obese)	P value
Fasting glucose (mmol/L)	4.34 ± 0.11 vs. 4.14 ± 0.13	<i>p</i> > 0.05	4.46 ± 0.08 vs. 4.58 ± 0.10	<i>p</i> > 0.05
Plasma glucose at 120 min of OGTT (mmol/L)	4.87 ± 0.16 vs. 4.26 ± 0.23	<i>p</i> < 0.05	5.50 ± 0.13 vs. 5.10 ± 0.21	<i>p</i> > 0.05
Fasting insulin (mU/L)	12.33 ± 1.35 vs. 8.93 ± 1.09	<i>p</i> > 0.05	18.351.28 vs. 15.58±±2.51	<i>p</i> > 0.05
Plasma insulin at 120 min of OGTT (mU/L)	48.19 ± 5.13 vs. 31.50 ± 4.29	<i>p</i> < 0.05	93.68 ± 7.99 vs. 71.65 ± 15.45	<i>p</i> > 0.05
Area under glucose curve (OGTT)	669.28 ± 21.58 vs. 651.53 ± 37.89	<i>p</i> > 0.05	759.57 ± 16.01 vs. 744.99 ± 29.86	<i>p</i> > 0.05
Area under insulin curve (OGTT)	7163.16 ± 852.17 vs. 5681.55 ± 460.25	<i>p</i> > 0.05	11315.86 ± 736.86 vs. 9848.96 ± 1579.33	<i>p</i> > 0.05
Si (insulin sensitivity, minimal model analysis)	3.39 ± 0.38 vs. 4.48 ± 0.52	<i>p</i> > 0.05	2.03 ± 0.39 vs. 2.40 ± 0.39	<i>p</i> < 0.05
AIR (acute insulin response, minimal model analysis)	61.78 ± 6.12 vs. 69.37 ± 4.47	<i>p</i> > 0.05	83.56 ± 5.96 vs. 62.21 ± 4.76	<i>p</i> > 0.05
Di (disposition index, minimal model analysis)	190.29 ± 24.16 vs. 298.05 ± 45.58	<i>p</i> < 0.05	161.45 ± 15.44 vs. 146.22 ± 25.40	<i>p</i> > 0.05

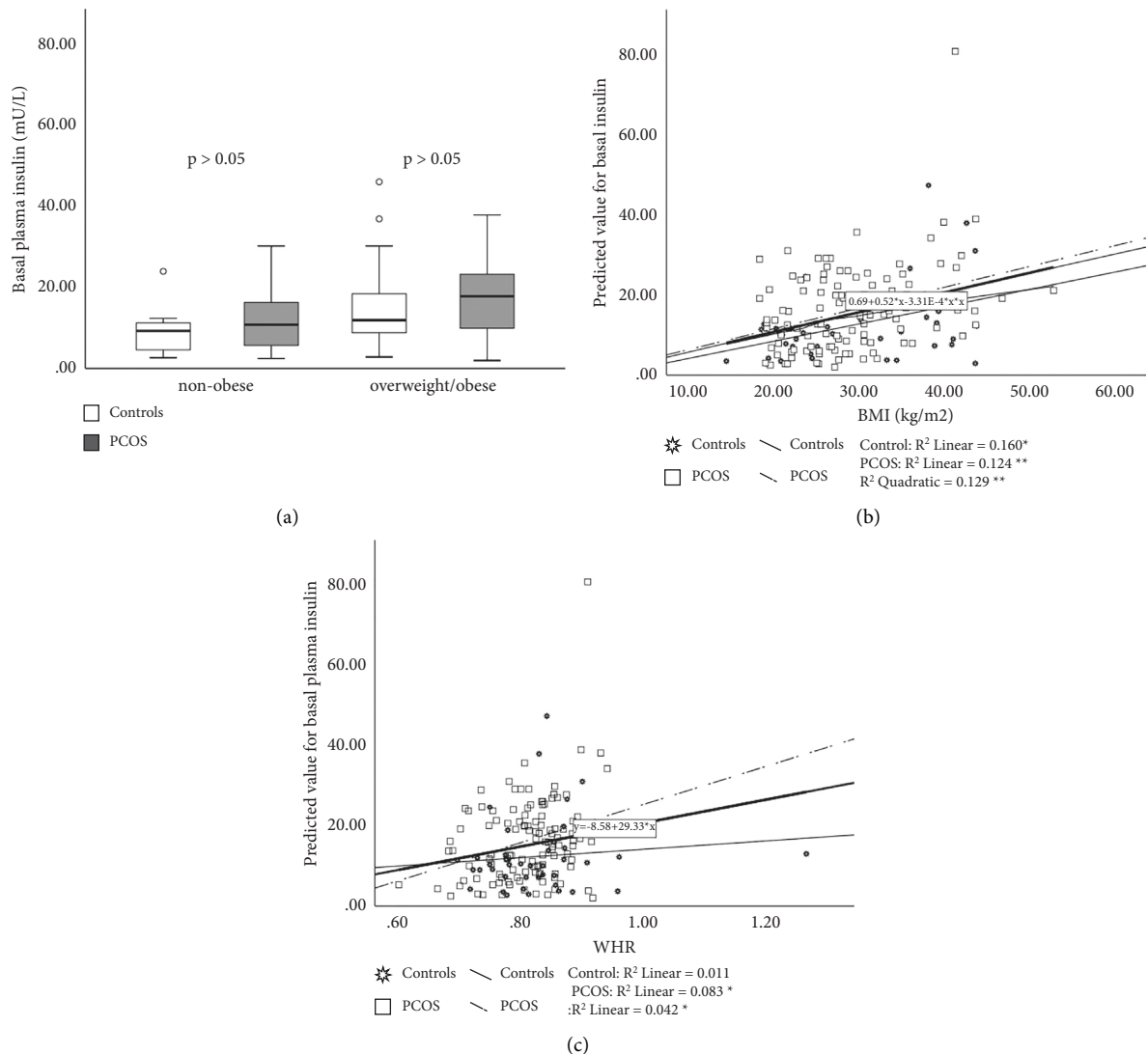


FIGURE 2: Basal plasma insulin in PCOS patients and controls. PCOS vs. controls, *p* < 0.05. Data are presented as mean ± SEM, separately for nonobese subjects (BMI < 25 kg/m<sup>2</sup>) and overweight/obese (BMI > 25 kg/m<sup>2</sup>) (a). Relationship between BMI and PCOS on basal plasma insulin (*p* < 0.001) (b). Relationship between WHR and PCOS on basal plasma insulin (*p* < 0.05) (c). \* *p* < 0.05, \*\* *p* < 0.001.

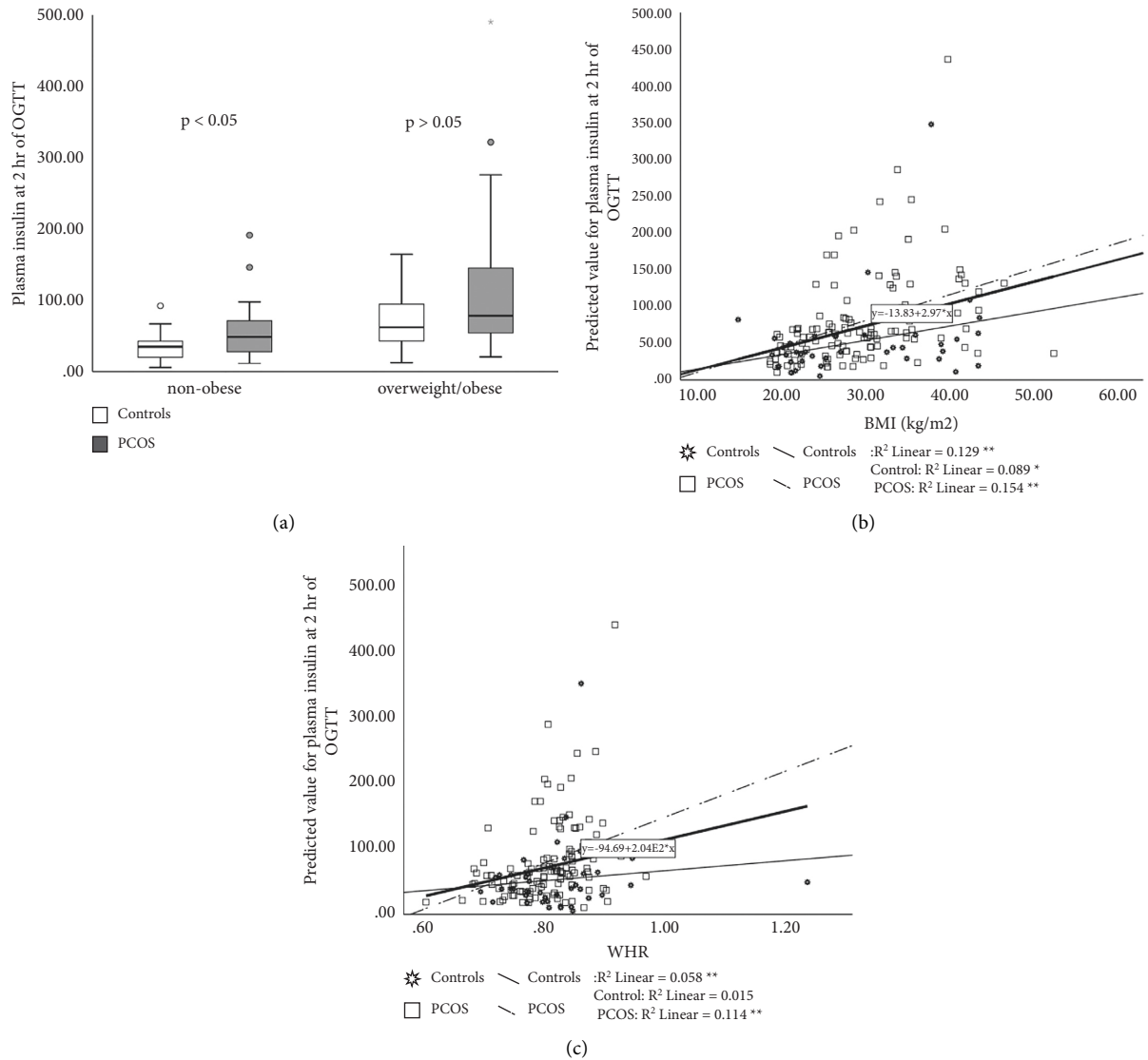


FIGURE 3: Plasma insulin at 2 hr of OGTT in PCOS patients and controls. PCOS vs. controls,  $p < 0.05$ . Data are presented as mean  $\pm$  SEM, separately for nonobese subjects (BMI <math>< 25 \text{ kg/m}^2</math>) and overweight/obese (BMI >math>> 25 \text{ kg/m}^2</math>) (a). Relationship between BMI and PCOS on plasma insulin at 2 hr of OGTT ( $p < 0.001$ ) (b). Relationship between WHR and PCOS on plasma insulin at 2 hr of OGTT ( $p < 0.001$ ) (c).  $^{**} p < 0.001$ .

### 3. Results

**3.1. Clinical Characteristics.** The clinical characteristics of PCOS women and controls are presented in Table 1. There is no difference in age, BMI, WHR, fasting glucose, or blood pressure between PCOS and controls. In the PCOS group, 67.53% patients were overweight/obese. In control group 51.22% women were overweight/obese. Total testosterone levels were substantially higher ( $p < 0.001$ ) in PCOS than in control women ( $3.73 \pm 0.16$  vs.  $1.89 \pm 0.10$  nmol/L). As regards PCOS clinical phenotypes, 109 (95.62%) women had hyperandrogenism and polycystic ovary morphology (PCOM) and 4.38% (5 women) had hyperandrogenism and oligoanovulation.

**3.2. Fasting Plasma Glucose, Insulin, and OGTT.** After adjusting for BMI and using an analysis of covariance, it was

observed that there was no difference in fasting plasma glucose between PCOS and controls ( $4.42 \pm 0.06$  vs.  $4.37 \pm 0.09$  mmol/L,  $p > 0.05$ ) (Table 2). There was a positive interaction between BMI and PCOS ( $p < 0.05$ ). Plasma glucose at the 2 hr point of OGTT was higher in the PCOS group than in controls ( $p < 0.05$ ) (Figure 1(a), Table 2) and in nonobese subgroup of PCOS in comparison with nonobese control (Table 3). BMI (Figure 1(b)) and WHR (Figure 1(c)) were positively correlated with plasma glucose at 2 hr of OGTT while positively correlated with this parameter only in the control group (Figure 1(d)). AUCG was not significantly higher in PCOS group vs. controls ( $729.47 \pm 13.43$  vs.  $699.40 \pm 24.81$  mmol/L/120 min,  $p > 0.05$  (Table 2)).

Fasting plasma insulin and plasma insulin at 2 hr of OGTT were significantly higher in PCOS patients than in controls ( $p < 0.05$ ) (Figures 2(a) and 3(a)) while AUCI was not

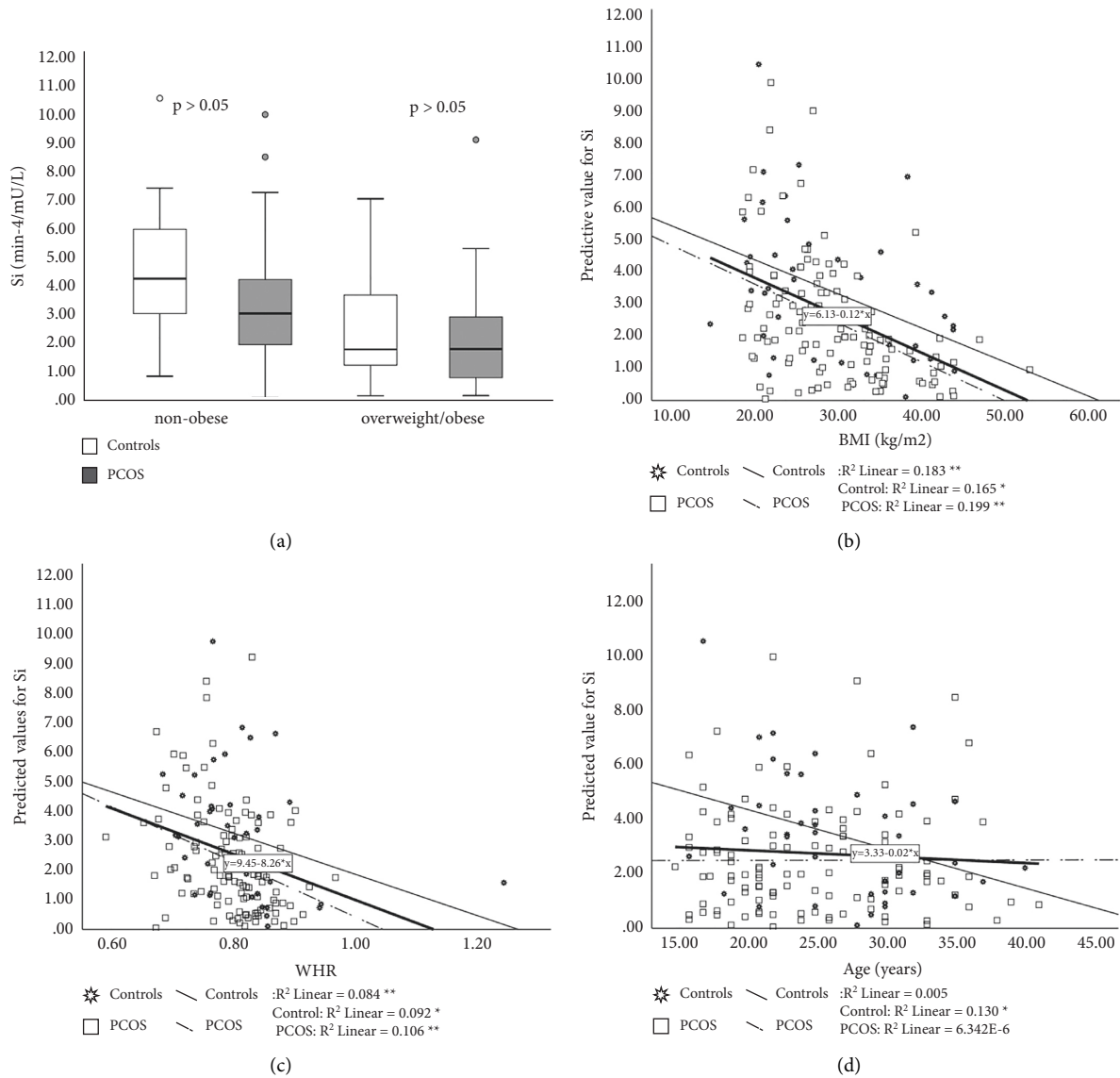


FIGURE 4: Insulin sensitivity ( $S_i$ ) in PCOS patients and controls. PCOS vs. controls,  $p < 0.05$ . Data are presented as mean  $\pm$  SEM, separately for nonobese subjects (BMI  $< 25$  kg/m<sup>2</sup>) and overweight/obese (BMI  $\geq 25$  kg/m<sup>2</sup>) (a). Relationship between BMI and PCOS on  $S_i$  ( $p < 0.001$ ) (b). Relationship between WHR and PCOS on  $S_i$  ( $p < 0.001$ ) (c). Relationship between age and controls on  $S_i$  ( $p < 0.05$ ) (d). \*  $p < 0.05$ , \*\*  $p < 0.01$ .

significantly higher in PCOS group vs. controls ( $9931.62 \pm 594.49$  vs.  $7816.08 \pm 892.38$  mU/L/120 min,  $p > 0.05$ ) (Tables 2 and 3). BMI and WHR correlated positively with these parameters ( $p < 0.05$ ) (Figures 2(b) and 3(b)). Significant interactions were found between PCOS and BMI on basal and stimulated plasma insulin ( $p < 0.001$ ) (Figures 2(b) and 3(b)), as well as between WHR and PCOS on basal/stimulated insulin ( $p < 0.001$ , respectively) (Figures 2(c) and 3(c)).

**3.3. Minimal Model Assessment of  $S_i$  and  $AIR_G$ .**  $S_i$  was decreased in patients with PCOS compared to controls ( $p < 0.05$ ) (Figure 4(a)). There was a significant interaction between PCOS and BMI as well as between WHR and PCOS in relation to  $S_i$  ( $p < 0.001$ ) (Figures 4(b) and 4(c)). Results obtained by measuring  $AIR_G$ , show that  $AIR_G$  was not

different between the PCOS and the control group ( $p > 0.05$ ) (Figure 5(a)). With increasing age,  $AIR_G$  decreased in both groups. Interaction between age and PCOS on this parameter shows that  $AIR_G$  declines more with aging in the PCOS group ( $p < 0.001$ ) (Figure 5(b)). Disposition index ( $S_i \times AIR$ ) was decreased in the PCOS group but not significantly compared to controls ( $p > 0.05$ ) (Figure 6). When PCOS patients and controls were collated into two subgroups based on age (subgroup A  $< 25$  years, subgroup B  $\geq 25$  years)  $AIR_G$  was higher in the PCOS subgroup A than in age-matched controls ( $p < 0.05$ ) (Figure 7(a)).  $S_i$  was decreased in the PCOS group ( $p < 0.05$ ) and DI was not significantly decreased in the PCOS subgroup A compared to the control subgroup A ( $p = 0.061$ ) (Figures 7(b) and 7(c)) (Table 4). The entire group of women with PCOS had a

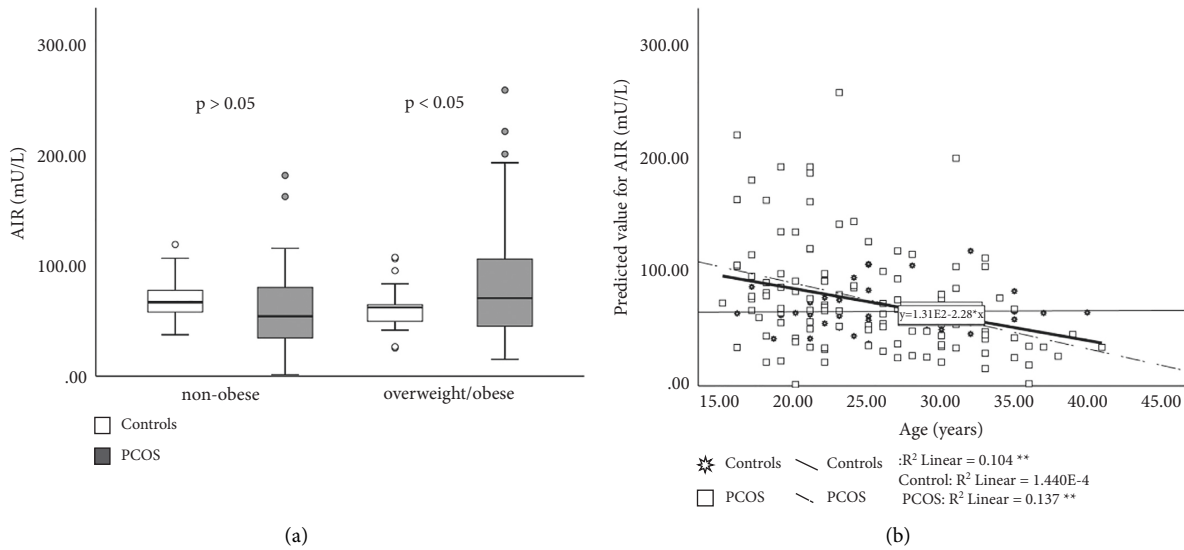


FIGURE 5: Acute insulin response during IVGTT (AIR<sub>G</sub>) in PCOS patients and controls. PCOS vs. controls,  $p > 0.05$ . Data are presented as the mean  $\pm$  SEM, separately for subjects overweight/obese (BMI  $\geq 25$  kg/m<sup>2</sup>) and nonobese (BMI < 25 kg/m<sup>2</sup>) (a). Relationship between age and PCOS on AIR<sub>G</sub> ( $p < 0.001$ ) (b). \*\*  $p < 0.01$ .

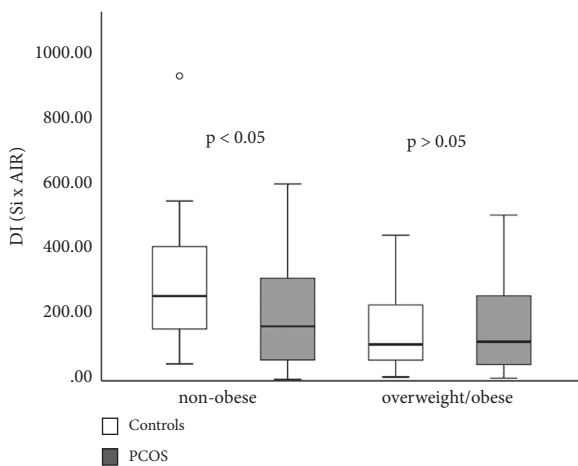


FIGURE 6: Disposition index (S<sub>i</sub> × AIR<sub>G</sub>) in PCOS patients and controls. PCOS vs. controls,  $p > 0.05$ . Data are presented as the mean  $\pm$  SEM, separately for nonobese subjects (BMI < 25 kg/m<sup>2</sup>) and overweight/obese (BMI  $\geq 25$  kg/m<sup>2</sup>).

normal glucose tolerance during IVGTT (K<sub>g</sub>), not different from controls ( $2.01 \pm 0.11$  vs.  $1.88 \pm 0.14 \times 10^2$ ,  $p > 0.05$ ). A positive relationship between BMI and WHR with PCOS was also confirmed ( $p < 0.05$ ). The best predictors of BMI as well as WHR were presented in Table 5.

#### 4. Discussion

The prevalence of impaired glucose tolerance (IGT) and diabetes increased in PCOS [14, 15]. Our data confirmed that plasma glucose at the 2 hr point of OGTT was higher in the PCOS group than in controls although all investigated subjects had normal fasting glucose and normal glucose tolerance. The PCOS subjects in this investigation had higher basal plasma insulin and higher plasma insulin levels at 2 hr

of OGTT compared to controls. Our results agree with the notion that high BMI and central obesity cause exaggerated insulin responses in PCOS women [16, 17]. Insulin resistance occurs in 40%–70% of women with PCOS [18]. Obesity may increase PCOS prevalence and exacerbate IR in women with PCOS [19], while insulin resistance in lean women with PCOS is not consistently demonstrated [20]. The euglycemic-hyperinsulinemic clamp is the gold standard to directly measure insulin sensitivity. In some previous studies [21, 22] it was showed that women with PCOS have intrinsic reduction in insulin sensitivity on euglycemic-hyperinsulinemic clamp and almost all obese women with PCOS have more serious IR than lean women with PCOS. A systematic review and meta-analysis of euglycemic-hyperinsulinemic clamp studies by Cassar et al. showed a reduction in insulin sensitivity of 27% and obesity exacerbates the reduction in insulin sensitivity by 15% in women with PCOS [23]. Our results confirmed the existence of insulin resistance in young PCOS patients. After adjusting for BMI, S<sub>i</sub> remained lower in PCOS women than in controls. Overweight contributed to the impairment of insulin sensitivity in PCOS as well as controls [24]. Furthermore, our results showed that the interaction between disease (PCOS) and being overweight exists. These data suggest that increased BMI may have a more deleterious effect on insulin sensitivity in PCOS than in controls. This is important because overweight or obesity is detected in about 30–50% women with PCOS [9]. Furthermore, our data indicate that individual lean patients with PCOS often have no hyperinsulinemia and insulin resistance as it was shown in previous study [25].

Android obesity, clinically confirmed as increased WHR, is an especially strong risk for insulin resistance and other factors that predispose to premature cardiovascular disease [20]. Although we were unable to demonstrate a difference in WHR between PCOS and healthy women, our results

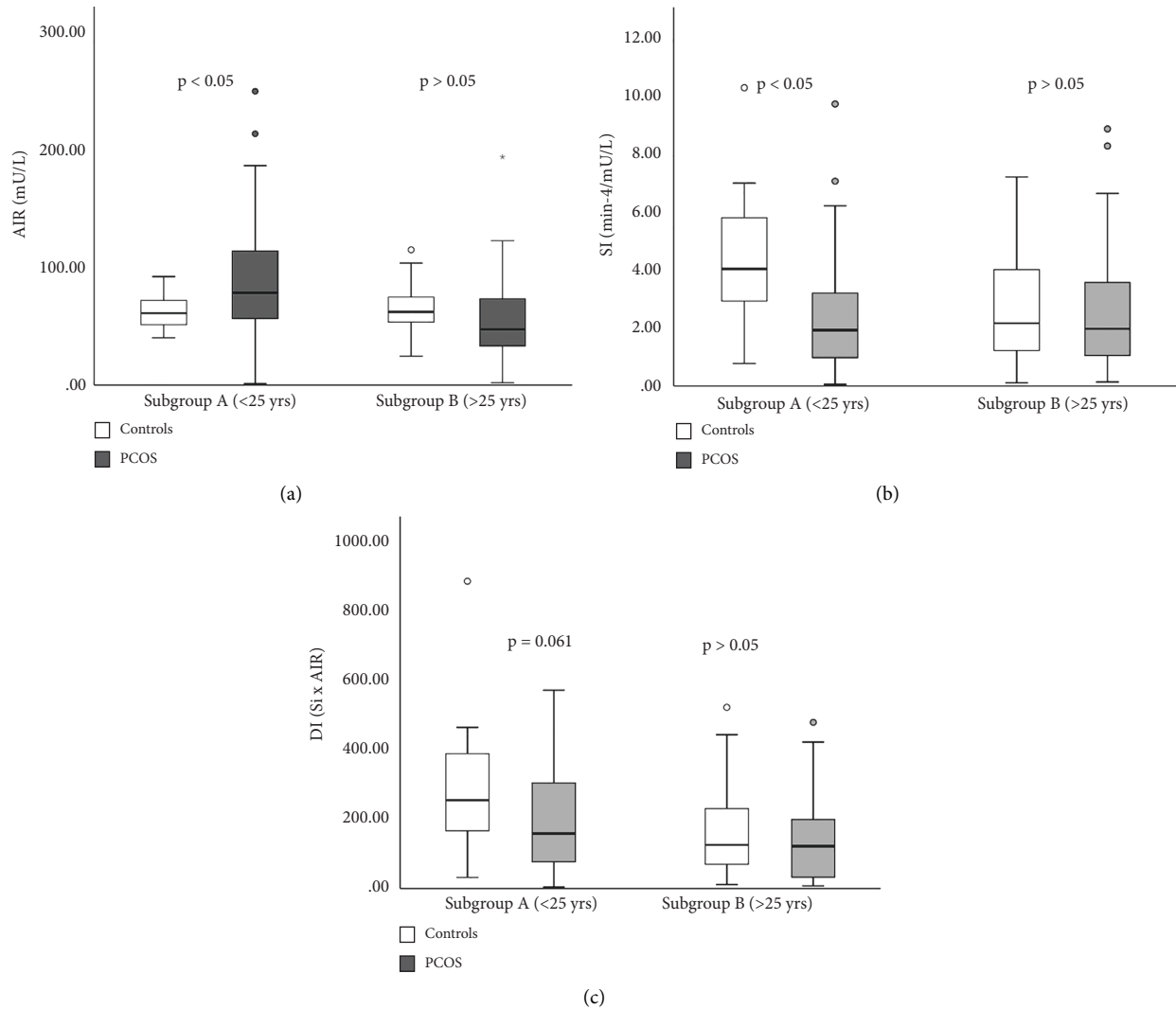


FIGURE 7: AIR<sub>G</sub> in age subgroups of PCOS patients and controls. Subgroup A: <25 years old. Subgroup B: ≥25 years old. Data are presented as the mean ± SEM (a). S<sub>i</sub> in age subgroups of PCOS patients and controls. Data presented as the mean ± SEM (b). DI in age subgroups of PCOS patients and controls. Data presented as the mean ± SEM (c).

TABLE 4: Investigated indices in PCOS and controls (<25 years old vs. ≥25 years old).

Parameter	PCOS vs. controls (<25 years old)	P value	PCOS vs. controls (≥25 years old)	P value
Fasting glucose (mmol/L)	4.39 ± 0.09 vs. 4.33 ± 0.18	$p > 0.05$	4.45 ± 0.09 vs. 4.39 ± 0.09	$p > 0.05$
Plasma glucose at 120 min of OGTT (mmol/L)	5.16 ± 0.14 vs. 4.36 ± 0.23	$p < 0.05$	5.44 ± 0.15 vs. 4.94 ± 0.24	$p > 0.05$
Fasting insulin (mU/L)	16.09 ± 1.18 vs. 14.02 ± 3.20	$p > 0.05$	16.63 ± 1.66 vs. 11.26 ± 1.29	$p < 0.05$
Plasma insulin at 120 min of OGTT (mU/L)	86.30 ± 9.82 vs. 42.42 ± 6.49	$p < 0.05$	69.87 ± 6.04 vs. 58.24 ± 13.61	$p > 0.05$
Area under glucose curve (OGTT)	704.59 ± 18.19 vs. 665.72 ± 32.78	$p > 0.05$	757.12 ± 19.36 vs. 720.96 ± 34.69	$p > 0.05$
Area under insulin curve (OGTT)	10508.03 ± 934.95 vs. 7000.97 ± 834.59	$p = 0.061$	9291.17 ± 703.45 vs. 8337.75 ± 1367.11	$p > 0.05$
Si (insulin sensitivity, minimal model analysis)	2.43 ± 0.25 vs. 4.52 ± 0.62	$p < 0.05$	2.54 ± 0.27 vs. 2.71 ± 0.38	$p > 0.05$
AIR (acute insulin response, minimal model analysis)	92.57 ± 6.88 vs. 64.14 ± 3.88	$p < 0.05$	58.22 ± 4.82 vs. 66.69 ± 4.82	$p > 0.05$
Di (disposition index, minimal model analysis)	195.77 ± 18.89 vs. 308.23 ± 53.15	$p > 0.05$	143.61 ± 17.34 vs. 163.99 ± 26.33	$p > 0.05$



TABLE 5: Regression analysis: best predictors of BMI and WHR.

	Estimate	Std. error	Coefficient	<i>p</i> value
<b>BMI</b>				
Intercept	24.12239	2.48639		
Age	0.20362	0.08492		
Basal insulin	0.19604	0.05077		
Si	-1.29620	0.25430		
			$r = 0.521$	$< 0.001$
<b>WHR</b>				
Intercept	0.67519190	0.03245457	20.804	
Age	0.00352968	0.00088745	3.977	
AUCG	0.00009734	0.00003786	2.571	
Si	-0.00778025	0.00255817	-3.041	
			$r = 0.474$	$< 0.001$

confirmed a significant influence of android obesity towards insulin resistance in both PCOS and controls. Thus, our data also indicated that PCOS women are more susceptible to increasing WHR regarding the development of insulin resistance.

The product of insulin sensitivity ( $S_i$ ) and insulin response ( $AIR_G$ ) in healthy subjects is a constant value that has been termed the disposition index (DI) [12]. It is expected that  $AIR_G$  will be higher as a compensatory mechanism in the state of insulin resistance [13]. On the other hand, the DI is low in subjects with IGT or T2DM [25]. In our study, we demonstrated decreased DI in nonobese PCOS compared to controls. A possible explanation for this finding is the lack of a compensatory insulin response in nonobese PCOS women as would be expected in an insulin resistant state.

Interestingly, the data show that there is no difference in  $AIR_G$  between PCOS and controls. Results from this study clearly show a significant negative correlation between  $AIR_G$  and increasing age. With an increase in age,  $AIR_G$  falls faster in patients with PCOS. Interestingly, our data did not confirm the significant influence of obesity on this parameter [24].

While insulin resistance is a factor in the development of PCOS, the associated failure of pancreatic  $\beta$ -cell function could be an important determinant of the development of T2DM in many of these women [26–28]. Although most of the observed hyperinsulinemia in PCOS is probably secondary to insulin resistance, there seems to be an important component of abnormal insulin secretion, which is independent of insulin resistance, body weight, and body fat distribution [3, 8, 29, 30]. Thus, besides insulin resistance,  $\beta$ -cell dysfunction seems to be an integral characteristic of this syndrome [27]. A recent study showed that in women with PCOS, metabolic clearance of insulin is reduced, contributing to developing hyperinsulinemia, as well as that serum androgens are independent predictors of this phenomenon [28]. But it was demonstrated that a higher prevalence of impaired insulin secretion than impaired insulin action exists in first-degree relatives of patients with PCOS [27, 31].

According to a previously published report [26],  $\beta$ -cell secretory defects may contribute to increased carbohydrate intake and subsequently obesity and insulin resistance.

Current data support the notion that glucose intolerance and frank T2DM are found in combination with the sign of  $\beta$ -cell exhaustion in a significant portion of young women with PCOS. These subjects with PCOS may manifest later stage carbohydrate intolerance and T2DM after long-standing insulin resistance in susceptible women [26]. In this regard, our data could suggest that an intrinsic defect in insulin secretion exists in PCOS patients.

In the youngest PCOS women (subgroup A), we demonstrated exaggerated  $AIR_G$  in comparison with age-matched controls as well as compared to older PCOS women (subgroup B). The exaggerated  $AIR_G$  in our young patients with PCOS could be a compensatory response to underlying insulin resistance. Despite the compensatory response, this group failed to achieve a DI observed in controls.

Current data are in concert with prior reports that significant abnormalities in insulin secretion are already present in patients with PCOS <25 years [31, 32]. Although, in our younger PCOS women,  $AIR_G$  was enhanced, the DI was still decreased compared to age-matched controls due to significantly impaired  $S_i$ , placing these patients at heightened risk for T2DM. Studies on young adolescent girls may provide clues about the pathophysiology of PCOS since this is an age when early clinical signs are manifested. Thus, this may be the time to initiate hygienic and medical interventions to retard the development of impaired glucose intolerance and T2DM [27, 29–31, 33]. This study suggests that young PCOS women can indeed be identified and placed on therapy to reduce the cardiovascular risk factors and development of T2DM [10, 34–49].

## 5. Conclusion

The major strength of this study is the large number of PCOS women investigated using minimal model analyses to evaluate acute insulin response and assess insulin secretion as well as insulin sensitivity. However, there are also limitations in the study. This is an observational study, and cause-effect relationships cannot be firmly established. In addition, as most women in this cohort had a hyperandrogenic PCOS phenotype, the results of the study may be more applicable to these subjects.

In conclusion, current observations underlie the importance of interactions between PCOS, BMI, age, and WHR. This investigation has also shown that not all patients with PCOS demonstrate basal and stimulated hyperinsulinemia, insulin resistance, and impaired glucose tolerance, particularly early in the evolution of PCOS as a clinical entity. Our data concerning subjects younger than 25 years underscores the importance of establishing the diagnosis of PCOS in adolescence, and the institution of appropriate therapy targeting insulin resistance and  $\beta$ -cell secretion before T2DM develops.

## Data Availability

Data can be made available on reasonable request (msumaracdumanovic@gmail.com).

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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