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Interplay of Insulin Resistance and the Reproductive and Metabolic Changes in Women with Polycystic Ovary Syndrome

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Abstract

Background: Polycystic ovary syndrome (PCOS) is a complex reproductive, endocrine-metabolic disorder with insulin resistance (IR) as a common feature. **Objective:** To clarify whether IR and associated hyperinsulinemia play a key role in the pathophysiological changes in PCOS especially, reproductive and metabolic changes, and the efficacy of metformin therapy in these cases. **Patients and Methods:** Twenty five PCOS patients received metformin 850 mg twice daily for four months and fifteen healthy controls were included in this study. Body mass index (BMI) evaluation followed by ultrasound examination for measurement of antral follicle count (AFC) in both ovaries, oral glucose tolerance test (OGTT), fasting blood glucose, insulin and glucose / insulin ratio (G/I) were measured in addition to serum total testosterone (T), luteinizing hormone (LH) and insulin like growth factor-1 (IGF-1) estimation for all subjects. These parameters were re-evaluated again 4 months after metformin treatment was initiated in PCOS patients.

Results: PCOS patients had significantly increased fasting blood glucose levels ($P < 0.05$), BMI, T, IGF-1 ($P < 0.01$), LH, insulin levels and AFC ($P < 0.001$) but G/I ratio was significantly ($P < 0.001$) lower in comparison to controls. Significant negative correlations between fasting G/I ratio and each of BMI, T, and AFC respectively were evident. Impaired OGTT at baseline was observed in PCOS patients with significant improvement noted after initiation of metformin therapy. Metformin significantly decreased BMI, serum T, LH, IGF-1 levels and AFC and increased the G/I ratio versus pretreatment values. **Conclusion:** IR plays a vital pathophysiological role in PCOS patients as manifested by causal relationship between insulin resistance and the reproductive and metabolic changes of PCOS. Metformin potentially improves these changes.

Key words: Polycystic ovary syndrome, Insulin, Reproductive, Metabolic changes.

Introduction

PCOS is a complex endocrine-metabolic disorder characterized by chronic anovulation and/or clinical/biochemical hyperandrogenism and/or polycystic ovaries at ultrasonography (1). It is an endocrinological disorder affecting 6-10 % of all females of reproductive age (2,3).

IR is a common feature of PCOS and is more prevalent in obese women, suggesting that PCOS and obesity have a synergistic effect on the magnitude of the insulin disorder. It leads to increased insulin secretion by beta-cells and compensatory hyperinsulinemia (4). IR in PCOS was considered a risk factor for gestational diabetes (5). Moreover, if beta-cell compensatory response declines, relative or absolute insulin insufficiency develops which may lead to glucose intolerance and type 2 diabetes (6).

It has been hypothesized that insulin-lowering agents (metformin), by reducing hyperinsulinemia, may improve the metabolic and reproductive abnormalities of PCOS (7,8). Nevertheless, the mechanisms underlying the beneficial effects of metformin in the treatment of PCOS are still in debate (9,10). This study aimed to clarify whether IR and associated hyperinsulinemia play a key role in the pathophysiological changes in PCOS especially, reproductive and metabolic changes, and the efficacy of metformin therapy in these cases.

Patients and Methods

Study population

Twenty-five PCOS patients with age ranging between 22-35 years received metformin 850 mg twice daily for four months according to Trolle et al (11), were included in the study and they were recruited from Gynecology outpatient clinics in cooperation of Gynecology & Obstetrics and Physiology Departments, Faculty of Medicine, Assiut University. They all met the criteria for diagnosis of PCOS according to the Rotterdam Consensus (12). The diagnosis requires 2 of the following 3 criteria: 1) Amenorrhea or chronic oligomenorrhea (≤ 6 menstrual cycle / year) indicating chronic anovulatory disorder 2) Clinical (hirsutism and/or acne) or biochemical hyperandrogenism after exclusion of other causes of hyperandrogenism and 3) Polycystic ovarian morphology by vaginal ultrasound examination.

Fifteen age matched healthy controls with a body mass index (BMI) of less than 25 kg/m², regular menstrual cycles and normal ultrasound examination of the ovaries with no clinical or biochemical signs of hyperandrogenism were recruited from outpatient clinics for routine check-up pur-

poses. The Assiut University Ethics Committee approved the study and every participant signed the informed consent before enrollment. Exclusion criteria included pregnancy, use of hormones or hormonal contraception for at least 3 months prior to the study, renal, hepatic or endocrinal disorders and family history of diabetes.

For both control and patients groups physical examination and BMI [body weight (Kg) divided by height (meters squared)] (13) were done followed by ultrasound examination to determine total antral follicles count (AFC) in both ovaries.

Laboratory assays

Basic oral glucose tolerance test (OGTT) was done at 08.00-10.00 AM after overnight fasting (10-14 h). The fasting glucose sample followed by another 4 samples was taken at 30, 60, 90, and 120 minutes after an oral ingestion of glucose load of 75 grams. The serum was analyzed for glucose and insulin immediately following separation from blood and the remainder of serum was stored at -20°C for later use. Serum glucose was measured by Hexokinase enzymatic method and spectrophotometric quantitation (Glucose kit, Sigma). Fasting insulin was measured by using (UBI- MAGIWEL insulin quantitative kit by ELISA method). Glucose tolerance was assessed by The 1997 American Diabetes Association and 1999 World Health Organization Criteria (14). Fasting blood sample was taken for assaying total testosterone (T) (FERFTIGNX-T-kit), Luteinizing Hormone (LH) (DRG diagnostic kit), and Insulin like Growth Factor -1 (IGF-1) (immunodiagnostic system limited, UK, kit) by ELISA method. According to the basal levels of fasting glucose and insulin, PCOS patients were considered to be IR as fasting Glucose/Insulin (G/I) ratio less than 4.5 and/or fasting insulin level more than 20 uIU/ml (15). All samples were taken in the first week of a spontaneous menstrual cycle (patients and controls) or progesterone withdrawal bleeding (patients). All the above parameters were re-evaluated again 4 months after receiving metformin treatment.

Statistical analysis

Data is expressed as means \pm standard error (SE) for all parameters. The data were analyzed by using GraphPad Prism data analysis program (GraphPad Software, Inc., San Diego, CA, USA). For the comparison of statistical significance between patients and normal subjects, or in patients before and after therapy, Student Newman-Keuls t-test for unpaired and paired data was used. Correlations were as-

sessed using Spearman's non-parametric correlation coefficient according to that described by Knapp and Miller (16). $P \leq 0.05$ was considered statistically significant.

Results

Reproductive and Metabolic characteristics of controls and women with PCOS at baseline are summarized in Table 1. The PCOS patients group had significantly greater BMI ($P < 0.01$), T ($P < 0.01$), LH ($P < 0.001$), IGF-1 ($P < 0.01$), fasting blood glucose ($P < 0.05$) and insulin levels ($P < 0.001$) and AFC ($P < 0.001$) and lower G/I ratio ($P < 0.001$) compared with the control group.

Correlation and linear regression are represented in figure 1A, B and C. At baseline evaluation of PCOS patients, there was significant negative correlation between fasting G/I ratio and each of BMI ($r = -0.72$, $P < 0.001$), T ($r = -0.73$, $P < 0.001$) and AFC ($r = -0.82$, $P < 0.001$) respectively.

The results of OGTT in controls and PCOS patients at baseline and after metformin therapy are depicted in figures 2A and 2B. In OGTT, PCOS patients had impaired fasting glucose (110-126 mg/dl) at baseline ($P < 0.05$ at fasting and at 30 min, $P < 0.01$ at 60 and 90 min, $P < 0.001$ at 120 min) when compared with controls (Fig. 2A). Administration of

Table 1. Reproductive and metabolic characteristics in controls and PCOS women at baseline

Parameter	Controls	PCOS	p-value
Number	15	25	-
Age (year)	28.6 ± 2.6	29.3 ± 4.5	NS
Body Mass Index (kg/m ²)	23.5 ± 2.2	35.9 ± 1.1	<0.01
Serum Testosterone (ng/dl)	42.5 ± 8.7	95.7 ± 13.5	<0.01
Serum LH (IU/L)	5.6 ± 1.2	14.88 ± 2.6	<0.001
Serum IGF-1 (μg/L)	108 ± 29	187.7 ± 34	<0.01
Fasting glucose (mg/dl)	86 ± 11	119 ± 20	<0.05
Fasting insulin (μIU/ml)	12 ± 3	35.5 ± 12	<0.001
Fasting Glucose/Insulin ratio	7.16 ± 3.53	3.35 ± 1.6	<0.001
Antral Follicular Count	8.2 ± 2.4	32.6 ± 5.5	<0.001

LH: Luteinizing Hormone, IGF-1 :Insulin like Growth Factor-1, NS: nonsignificant Data expressed as means ±SE.

Table 2. Comparison of reproductive and metabolic characteristics in PCOS women at baseline and after metformin therapy.

Parameter	Before Metformin	After Metformin	p-Value
Body Mass Index (kg/m ²)	35.9 ± 1.1	28.5 ± 3.7	<0.05
Fasting Glucose/Insulin Ratio	3.35 ± 1.6	5.16 ± 0.6	<0.01
Serum Testosterone (ng/dl)	95.7 ± 13.5	55.3 ± 9.1	<0.05
Serum Luteinizing Hormone (IU/L)	14.88 ± 2.6	9.08 ± 0.37	<0.05
Serum Insulin-like Growth Factor-1 (μg/L)	187.7 ± 34.7	129.5 ± 27.7	<0.05
Antral Follicular Count	32.6 ± 5.5	16.2 ± 3.8	<0.01

P values are for after vs. before. Data is expressed as means ±SE.

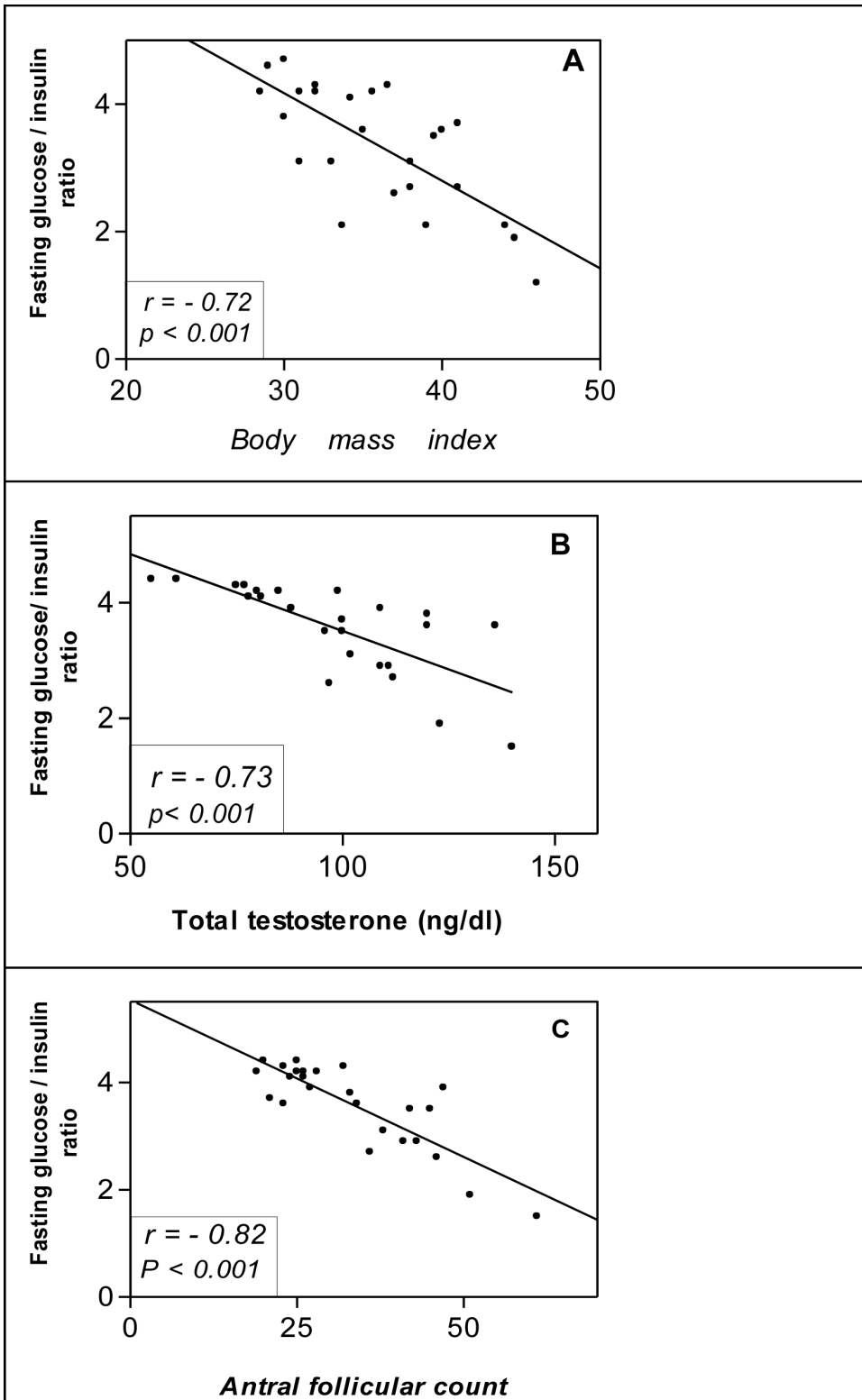


Figure 1. (A, B, and C) (above): Correlation and linear regression between fasting glucose insulin ratio and Body mass index (A), Total testosterone (B) and Antral follicular count (C) in PCOS patients at base line.

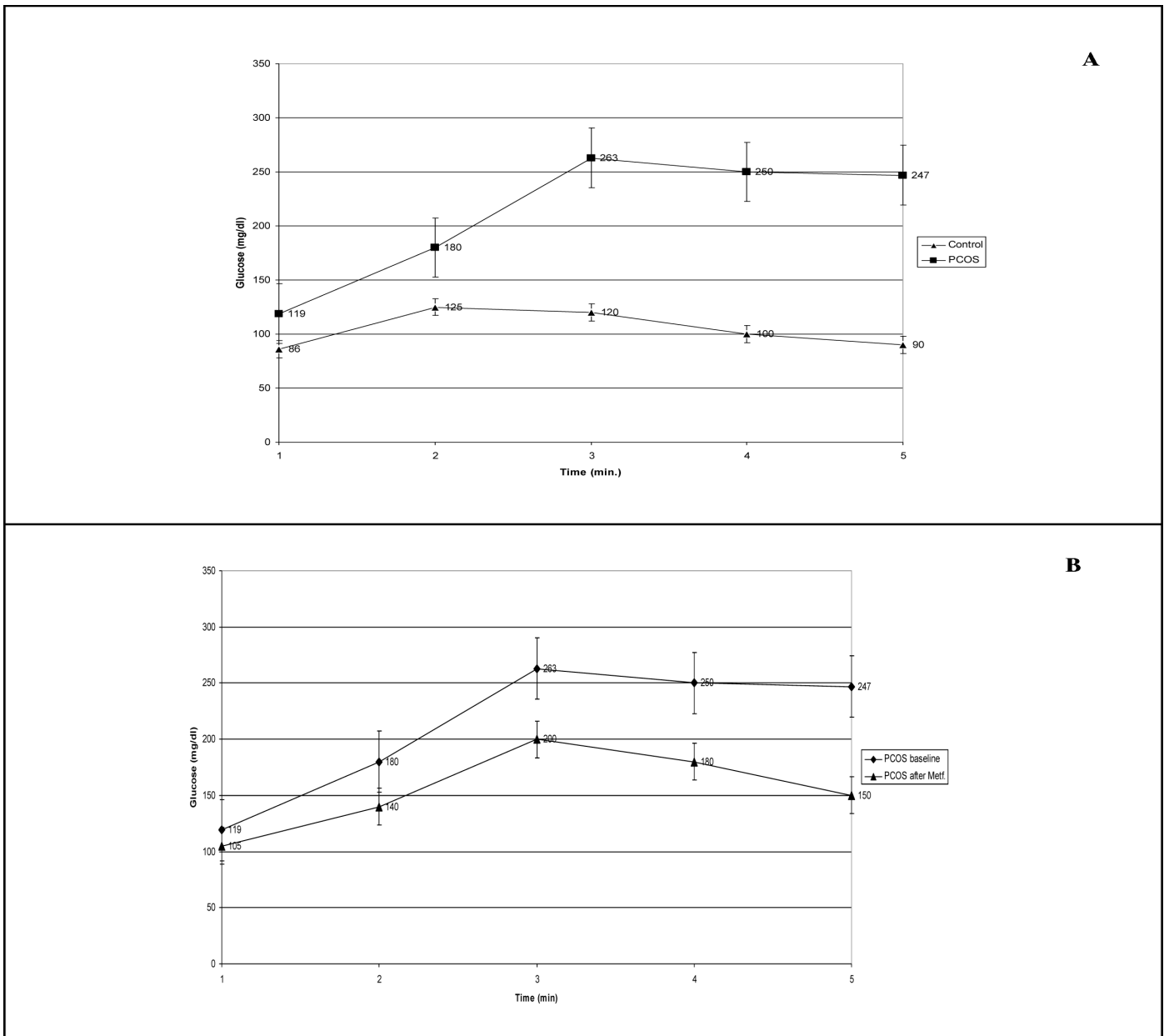


Figure 2 (A and B): Oral glucose tolerance test (OGTT) in control and PCOS patients at baseline and after Metformin treatment.

Figure (2A) : OGTT in controls and PCOS patients at base line, ($P < 0.05$) at fasting and at 30 min, ($P < 0.01$) at 60 and 90 min, ($P < 0.001$) at 120 min as compared to controls. The numbers 1-5 point to time of sampling (at fasting, 30,60,90,120 min respectively).

Fig.(2B): OGTT in PCOS patients at base line and after metformin treatment , (P is non significant) at fasting , ($P < 0.05$) at 30,60 and 90 min and ($P < 0.01$) at 120 min, after treatment vs. at base line. The numbers 1-5 point to time of sampling (at fasting, 30,60,90,120 min respectively).

metformin significantly lowered the blood glucose level (P value was non significant at fasting, $P < 0.05$ at 30, 60 and 90 min and $P < 0.01$ at 120 min) compared with pretreatment values (Fig.2B).

The effects of metformin on reproductive and metabolic parameters in PCOS patients are summarized in Table 2. Administration of metformin significantly decreased BMI, serum Testosterone, LH, IGF-1 levels ($P < 0.05$ for each) and AFC ($P < 0.01$) and increased the G/I ratio ($P < 0.01$) compared with pretreatment values.

Discussion

Insulin resistance is defined as the inability of insulin to exert its physiological effect. It is manifested peripherally (at the tissues) or centrally (at the liver) through reduction in the ability of insulin to lower plasma glucose (17). The pathophysiology of PCOS is still largely unknown and until now, medical care of this patient population has been limited to symptomatic control and infertility (18). While the basic pathophysiological mechanisms seem to be caused by dysfunction of the ovary, the clinical expression and severity of the symptoms are dependent on extra ovarian factors. Our knowledge about the relationship of PCOS to IR is indistinct. In this study, we hypothesized that IR and the associated hyperinsulinemia may play a key role in the pathophysiology of this syndrome.

Clinical evaluation of patients in the present study revealed increased BMI compared with controls that harmonized with previous investigators who reported that obesity could be found in 10 – 50 % of women with PCOS who are have a BMI outside the acceptable range of 19–25 kg/m² (17) and BMI is highly predictive of both insulin and glucose levels in women with PCOS (19). Obesity (the fat mass), body fat location and muscle mass all have important independent effects on insulin sensitivity. Alterations in these parameters could potentially be related to insulin resistance in PCOS in a number of ways. In particular, several metabolites (such as free fatty acid and lactate) as well as tumor necrosis factor-1 and leptin, the production rate of which increases in the obesity state, directly affect the peripheral action of insulin (impair insulin-mediated glucose uptake in skeletal muscle, adipocytes and liver and decrease hepatic insulin sensitivity and increase hepatic glucose output) (20,21).

Significant higher fasting insulin and glucose levels with decreased fasting G/I ratio were observed in PCOS patients of this study when compared with control value. These results are in accordance with previous studies which report-

ed that hyperinsulinemia due to insulin resistance occurs in the majority of women with PCOS (22,23). This is thought to be due to decreased insulin sensitivity with post-receptor defect (reduced autophosphorylation of insulin receptor in the ovary) with decreasing insulin – stimulated glucose uptake (3).

In women with PCOS, basal insulin secretion is increased and hepatic insulin clearance is reduced, resulting in hyperinsulinemia. Insulin-stimulated glucose utilization is decreased by 35-40% in women with PCOS, therefore blood glucose levels become raised, a phenomenon known as hyperglycemia (24,25). Under normal circumstances, insulin secretion increases as insulin sensitivity decreases, in order to maintain glucose homeostasis. This relationship of insulin sensitivity and insulin secretion is known as the deposition index that was described by Muniyapna, (26).

In the OGTT, blood glucose levels were proved to be significantly higher in PCOS patients at baseline in the existing investigation than the control group and when fasting glucose levels were analyzed according to the recommendations of the Expert Committee of the American Diabetes Association (14), we found that most of PCOS patients had impaired fasting glucose (≥ 110 mg/dl and < 126 mg/dl). These results indicated the high prevalence of glucose intolerance in PCOS women, and these patients are at increased risk of developing non insulin dependent diabetes mellitus (NIDDM) that harmonized with other previous studies (27,28).

A significant negative correlation between BMI and fasting G/I ratio was observed in this study. This could support the possibility that obesity in women with PCOS can exacerbate insulin resistance and enhance the risk of NIDDM that is accounted in this study by the high prevalence of impaired oral glucose tolerance test

In the current study, PCOS patients showed significantly higher levels of total testosterone, LH and IGF-1 compared with controls. Also in PCOS women, it was found that total testosterone is negatively correlated with fasting G/I ratio which means that the higher the insulin, the higher the androgen level suggesting a possible causal relationship. The pathophysiological mechanisms of hyperandrogenemia found in this study could be explained by that high circulating insulin levels resulting from insulin resistance stimulate ovarian theca cell androgen production both by a direct action on the ovary (through binding to insulin or IGF-1 receptors) and by stimulating the release of LH. Hyperinsulinemia may also enhance the bioavailability of androgen by decreasing the biosynthesis of IGF- binding protein-1

thus elevating IGF-1 levels which like insulin stimulates ovarian androgen production (29,30). These data support the hypothesis that insulin resistance could play a role in the pathophysiology of reproductive abnormalities characteristic of PCOS.

In the present study, ultrasound examination of the ovaries revealed significantly higher number of antral follicles in the patient group versus controls. These follicles are arrested in development at a size of 2-9 mm, with enlarged stromal volume >10 cc. It is suggested that excess androgens probably play a key role in the etiology of this abnormal ovarian morphology as evident by increasing levels of testosterone in PCOS patients of this study. Androgens encourage the development of primary follicles to the stages of pre-antral and small antral follicles and in the presence of excess androgen; this process is accelerated compared with that of normal ovaries. In addition, several factors that inhibit the endogenous action of Follicle Stimulating hormone (FSH) such as follistatin, epidermal growth factor and a number of anti-apoptotic factors lead to arrest of further follicular growth and slow down the turn over of these arrested follicles. All these factors combine to give the characteristic polycystic ovarian morphology (31). In accordance with Bayrak et al. (15) data of the current study showed a significant negative correlation between G/I ratio and AFC that support that insulin resistance and hyperandrogenism have a significant impact on polycystic ovarian morphology. Further, it was reported that insulin resistance is more severe in women with PCOS who are anovulatory than in those who are equally hyperandrogenic but have normal cycles. This suggests that insulin resistance contributes to anovulation (32,33).

Perhaps, the most convincing evidence linking insulin resistance with reproductive and metabolic abnormalities in women with PCOS is the utilization of insulin-sensitizing drug (metformin) to restore ovulation and metabolic disorders. Therefore, in the present study, metformin therapy improved the measured parameters of IR demonstrated by significant increase G/I ratio and significant decrease total testosterone level, LH, IGF-1, AFC and BMI compared with pretreatment values. These results are in accordance with previous studies (34-36). It was reported that metformin reduces insulin resistance and insulin secretion followed by a reduction of ovarian androgen production. Also, a direct action of metformin on ovarian theca cells to reduce androgen production was also postulated (37,38,9).

In addition, the present study demonstrated that in OGTT, administration of metformin in PCOS patients significantly lowered the blood glucose level compared with pretreatment values. Improved IR by metformin may not only

result from the well-known reduction of hepatic glucose production and increase of peripheral glucose utilization but also from a direct effect on ovarian steroidogenesis, as demonstrated by in vitro studies (39). Moghetti et al. found that metformin effects occur without significant action on beta-cell insulin production but may reduce insulin response to an oral glucose load (40).

The significant decrease in AFC observed in this study following metformin treatment was in agreement with Bayrak et al (15). Therefore, this dose of metformin may be sufficient to achieve improvements in the biochemical markers and polycystic ovarian morphology as evident by our study. On the other hand, other studies that assessed metformin effects in hyperandrogenic subjects did not confirm these findings (18).

In conclusion, insulin resistance plays a vital pathophysiological role in PCOS patients in term of increase, T, IGF-1, LH, AFC and BMI but decrease G/I ratio and impaired OGTT. This suggests a possible causal relationship between insulin resistance and the reproductive and metabolic changes. Metformin potentially improves the reproductive and metabolic features of IR in PCOS women. Further investigations are needed to evaluate the cellular and molecular mechanisms of insulin resistance in PCOS.

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