

## INSULIN RESISTANCE DECREASED DURING TREATMENT IN POLYCYSTIC OVARY SYNDROME (PCOS) WOMEN

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### ABSTRACT

Polycystic ovary syndrome (PCOS) is a highly prevalent endocrine-metabolic disorder that implies various severe consequences to female health, including alarming rates of infertility. Although its exact etiology remains elusive, it is known to feature several hormonal disturbances, including hyperandrogenemia, insulin resistance (IR), and hyperinsulinemia. Insulin appears to disrupt all components of the hypothalamus-hypophysis-ovary axis, and ovarian tissue insulin resistance results in impaired metabolic signaling but intact mitogenic and steroidogenic activity, favoring hyperandrogenemia, which appears to be the main culprit of the clinical picture in PCOS. material and method The study group which complete study period included

(95) PCOS women(**group A**) include (35) women will treated with **Metformine** for six months and (**group B**) include (32) women will treated with **Choline & Inositol** and **Metformine** for six months and (**group C**) include (28) women will changed lifestyle and followed diet for six months we measured INS, IR and FBG ,before and after treatm. **Result:** highly significant decrease( $p<0.01$ ) in INS level in group (A,B), AND significant decrease( $p<0.05$ ) in IR in all group (A,B,C),while non significant change in FBG. **Conclusions** used cholin & inositol plus metformin could be as a first- line treatment in patients with PCOS.

**KEYWORDS:** hormonal disturbances, including hyperandrogenemia, insulin resistance (IR), and hyperinsulinemia.

## INTRODECTION

Polycystic ovary syndrome (PCOS) is an endocrine-metabolic disorder characterized by multiple hormonal imbalances, reflecting on a clinical presentation dominated by manifestations of hyperandrogenism, which generate short and long term consequences on female health.<sup>[1]</sup> Among these, infertility is one of the most alarming associated morbidities, as it currently affects approximately 48.5 million women aged 20–44 years<sup>[2]</sup>, with PCOS accounting for 6–15% of these cases<sup>[3]</sup>, although up to 70% of women with PCOS may be undiagnosed.<sup>[4]</sup> Indeed, its optimal diagnosis is often hindered due to its apparent similarities with several other pathologies remarkably, obesity as well as Cushing's syndrome, ovarian and adrenal neoplasms, and congenital adrenal hyperplasia.<sup>[5]</sup>

IR, defined as a metabolic state characterized by a decrease in cellular ability to respond to insulin signaling, appears to be an essential pathophysiologic mechanism in the development of all metabolic complications of PCOS.<sup>[6]</sup> As a consequence, outstanding proportions of women with PCOS are also diagnosed with DM2 or MS, as well as isolated criteria from the latter.<sup>[7]</sup> Compensatory hyperinsulinemia appears to mediate many of these deleterious effects. This phenomenon stems as a response of pancreatic  $\beta$  cells in order to preserve lipid and carbohydrate homeostasis in face of diminished insulin sensitivity.<sup>[8]</sup> This compensation leads to  $\beta$  cell exhaustion and the genesis of not only DM2, but also a series of collateral effects originated by hyperinsulinemia, including the aforementioned frequent comorbidities of PCOS.<sup>[9]</sup>

Notwithstanding the importance of IR in the development of PCOS, both obese and nonobese patients have specific mechanisms leading to ovarian dysfunction independent of IR, reflecting the complexity of this syndrome.<sup>[10]</sup> Therefore, considering the severe consequences PCOS exerts on the health and lifestyle of the affected women, it is of utmost importance to unravel the intricate pathophysiologic cross-talk among PCOS, IR, and obesity.

## Insulin

Insulin is a pancreatic peptide hormone produced by  $\beta$ -cells of islets of Langerhans.<sup>[11]</sup> The classical target organ for its action are muscles, adipose tissue and liver.<sup>[12]</sup>

Insulin plays a major role in regulation of carbohydrate, fat and protein metabolism, it suppresses hepatic glucose output, inhibits glycogenolysis and gluconeogenesis, and promotes glycogen synthesis. It stimulates peripheral glucose uptake in muscle and fat

tissues, induces protein synthesis, cell growth and differentiation, and inhibits lipolysis.<sup>[12]</sup> Insulin plays a role as a co-gonadotropin in regulation of ovarian function.<sup>[13]</sup> Co-gonadotropin is a term applied to any extra ovarian hormone, that exhibits potentiating or synergistic effect through a specific receptors for gonadotropin mediating growth of follicles and steroidogenesis.<sup>[14]</sup>

Insulin plays both direct and indirect roles in the pathogenesis of androgen excess in PCOS. Although women with PCOS have peripheral insulin resistance, ovarian steroidogenesis appears to be hypersensitive to insulin.<sup>[15]</sup> Insulin acts synergistically with LH to enhance theca cell androgen production in women with PCOS by activating a specific signaling pathway via its own receptor.<sup>[16]</sup> In addition, insulin can stimulate human theca cell proliferation, and can also enhance ovarian growth and follicular cyst formation in rats.<sup>[17]</sup>

Hyperinsulinemia may also have adverse effects in women with PCOS through its action at non-ovarian sites including the liver, adrenal glands and pituitary.<sup>[18]</sup>

Insulin also potentiates ACTH-mediated adrenal androgen production. The concept that hyperinsulinemia affects GnRH pulse frequency and inappropriate gonadotropin secretion in PCOS by acting at pituitary level is mainly based on in vitro studies in which insulin has been shown to increase LH secretion from cultured rat pituitary cells. This suggests that insulin has an important pathophysiological role in PCOS, although its role in neuroendocrine dysfunction remains unclear.<sup>[19]</sup>

## TREATMENT OF PCOS

The course of treatment for women with PCOS largely depends on the severity of an individual's symptoms. Well-defined published data indicate a high risk for development of T2DM and CVD in women with PCOS.<sup>[20,21]</sup> Metformin is now thought to be of therapeutic value directly and/or indirectly in the management of PCOS.<sup>[22]</sup>

In addition to the expected improvements in insulin sensitivity and glucose metabolism, metformin therapy also ameliorates hyperandrogenism and menstrual irregularity, the favorable effect of metformin on hyperandrogenism in PCOS.

Treatment with Choline and Inositol "Inositol" is a term used to refer to a group of naturally occurring carbohydrate compounds that exist in nine possible chemical orientations called stereoisomers. The most common being myo-inositol, which is often sold as a dietary

supplement labeled simply as inositol. Inositol, particularly myo-inositol and another less common stereoisomer called D-chiro-inositol, plays a critical, but underappreciated, role in insulin signaling. Conditions such as hyperglycemia and diabetes are associated with disrupted inositol signaling, leading many researchers to suggest that this may be a key pathologic feature of insulin resistance.<sup>[23]</sup>

**Natural Treatments for PCOS** Over the past few years, research into the naturopathic and nutritional approach to PCOS has revolutionized the condition's treatment. It is important to treat the factors that lead to PCOS. Following *The Natural PCOS Diet*, making lifestyle changes and taking supplements can influence a healthy outcome. One of the most important things is to address insulin resistance, if this is an apparent problem. This can be done by incorporating dietary changes and taking suitable nutritional supplements. What you eat can have a direct influence on how balanced (or unbalanced) your hormones are. This is why it's important to have a healthy diet.<sup>[24]</sup>

## MATERIAL AND METHODS

In this study, 25 healthy women (without PCOS) and 150 women with PCOS were included, they were divided according to type of treatment.<sup>[55]</sup> PCOS women were removed from the result because they did not continue in the study period, and this group included<sup>[25]</sup> PCOS women which were treated with Choline & Inositol alone and 30 PCOS women of all groups which were treated but did not complete the study period so that the result was removed.

The study group which completed the study period included ninety five<sup>[95]</sup> PCOS women, (group A) included<sup>[35]</sup> women which were treated with Metoformine for six months and (group B) included<sup>[32]</sup> women which were treated with Choline & Inositol and Metoformine for six months and (group C) included<sup>[28]</sup> women which changed lifestyle and followed diet for six months.

This study was carried out in Kamal AL-Samarrae Hospital- Baghdad during the period from July 2013 to February 2014. It included<sup>[95]</sup> Iraqi women with (PCOS); their age range was<sup>[18-39]</sup> years.

### *Determination of Serum Insulin Hormone*

Serum insulin hormone was measured by ELISA using a kit supplied by Diagnostic automation Company-USA

**A. Assay Procedure**

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-25°C).

1. Pipette 50µl of the appropriate calibrators, controls and samples into the assigned wells.
2. Add 100µl of the biotinylated/enzyme labeled antibodies to each well. It is important to dispense all reagents close to the bottom of the micro well.
3. Swirl the micro plate gently for 20-30 seconds to mix cover with a plastic wrap.
4. Incubate for 120 minutes at room temperature (22-25°C).
5. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
6. Add 300µL of wash buffer, decant (tap and blot) or aspirate. Repeat two additional times for a total of three washes. An automatic or manual plate washer can be used. If a squeeze bottle is used, fill each well to the top by squeezing the container. Avoiding air bubbles. Decant the wash and repeat two additional times.
7. One hundred micro liters was added of working substrate solution to all wells. Always add reagents in the same order to minimize reaction time differences between wells.
8. Incubate at room temperature for fifteen minutes.
9. Add 50µl of stop solution to each well and mix gently for 15-20 seconds; always add reagents in the order to minimize reaction time difference between wells.
10. Read the absorbance in each well at 450nm in a micro plate reader. The results should be read within thirty minutes of adding the stop solution.

**B. Calculation**

1. Record the absorbance obtained from the printout of micro plate reader.
2. Plot the absorbance versus the corresponding insulin concentration in µU/ml on linear graph paper.
3. Draw a dose response curve using the best fit through the plotted points.

Interpolate the concentration of the controls and the unknowns from the dose response curve.

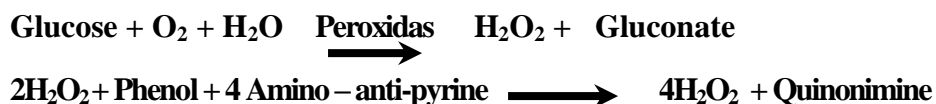
**Determination of Fasting Serum Glucose (F.S.G.)**

Diagnostic by BioMeriux – France.

### A. Principle

By using an enzymatic colorimetric method with a commercially available kit, the fasting plasma glucose (FPG) was determined. It is based on the principle that glucose is oxidized by glucose-oxidase (GOD) to gluconate and hydrogen peroxide, according to the following equation.

### GOD



### B. Assay procedure

The content of the vial marked R2 was dissolved in the buffer of R1, and such solution was considered the working reagent. To carry out the assay, three tubes were marked as blank, standard and sample, and into each tubes the materials were added as the following.

Materials	Tubes		
	Blank	Standard	Sample
Standard	-	10 µl	-
Sample	-	-	10 µl
Working Reagent	1.0 ml	1.0 ml	1.0 ml

The tubes were mixed gently and incubated for 10 minutes at 37°C, and then the optical density was recorded for each tube at a wavelength 505 nm.

### C. Calculations

The fasting plasma glucose was measured using the following equation.

$$\text{Fasting Plasma Glucose (mg/dL)} = \left( \frac{\text{Optical Density of Sample}}{\text{Optical Density of Standard}} \right) \times \text{Concentration of Standard}$$

### Homeostasis Model Assessment (HOMA)

Insulin resistance (IR) was determined by a number of different methods including fasting insulin, glucose for calculation (insulin measured µU/ml, glucose measured in mg/dl), the homeostasis model assessment (HOMA). The estimation of insulin resistance by HOMA score was calculated with the formula (Mathews DR, et al., 1985).

$$\text{HOMA} = [\text{Fasting serum insulin (µU/ml)} \times \text{Fasting blood glucose (mmol/L)}] / 22.5$$

This index is useful for measuring insulin sensitivity, which is the inverse of IR. It has the advantage that it can be obtained from a fasting blood sample, and is the preferred method for certain types of clinical research.

### Statistical Analysis

The Statistical Analysis System- SAS (2012) was used to effect of different factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study. Estimate of correlation coefficient between difference variables.

### RESULT

The result of Insulin hormone, Fasting blood glucose and Insulin resistance showed in table (a), Group (A) which the PCOS women treated by metformine (850 mg tow time /day) we found the significant decrease in Insulin hormone level, non significant decrease in fasting blood glucose and significant decrease in insulin resistance after treatment with metformine for sixth month.

The table (a). Showed group( B) which treated with cholin and inositole(500/500 mg tow time /day) plus metformin(850 mg tow time /day) we found insulin level before treatment ( $34.11 \pm 1.99$  a) became( $20.15 \pm 1.8$  c) after treatment , higher significant decrease during (0and3)month and significant decrease during (3 and 6)month in insulin level, non significant decrease in fasting blood glucose level and IR before treatment( $7.68 \pm 0.53$  a)became ( $4.90 \pm 0.34$  b) after treatment ,the significant decrease in insulin resistance during (3 and 6)month while non significant during (0 and3)month.

The same table (a) found group (C ) which treated by changing lifestyle and followed up diet the result was no significant decrease of insulin hormone level and blood glucose level while significant decreased in insulin resistance.



Table (a) showed insulin hormone level, fasting blood glucose and insulin resistance for study group which treated with different treatment during (0,3 and 6)month.

Group	Duration of treatment	Mean $\pm$ SE		
		INS	FBS	IR
A	0	33.10 $\pm$ 1.98 a	5.50 $\pm$ 0.18 a	8.14 $\pm$ 0.62 a
	3	26.89 $\pm$ 1.46 b	5.24 $\pm$ 0.12 a	6.29 $\pm$ 0.41 b
	6	23.25 $\pm$ 1.35 b	5.02 $\pm$ 0.09 a	5.16 $\pm$ 0.30 c
LSD value		5.734 **	0.562 NS	1.746 *
B	0	34.11 $\pm$ 1.99 a	5.14 $\pm$ 0.16 a	7.68 $\pm$ 0.53 a
	3	27.42 $\pm$ 1.78 b	5.11 $\pm$ 0.15 a	6.36 $\pm$ 0.37 a
	6	20.15 $\pm$ 1.8 c	5.04 $\pm$ 0.14 a	4.90 $\pm$ 0.34 b
LSD value		6.102 **	0.364 NS	1.602 *
C	0	40.02 $\pm$ 2.36 a	5.29 $\pm$ 0.12 a	9.63 $\pm$ 0.68 a
	3	36.92 $\pm$ 2.41 a	5.03 $\pm$ 0.09 a	8.38 $\pm$ 0.64 ab
	6	34.41 $\pm$ 2.13 a	4.93 $\pm$ 0.08 a	7.65 $\pm$ 0.56 b
LSD value		7.315 NS	0.492 NS	1.412 *
* (P $\leq$ 0.05), ** (P $\leq$ 0.01).				

group( A) which treated with metformine(850mg tow time /day )

group (B) which treated with Choline&Inositol (500\500 tow time /day) and Metoformin (850 mg tow time /day ),group (C) change life style and followed diet.

## DISCUSSION

This result which was in agreement with many studies Zelija – velija<sup>[24]</sup> reported the metformine therapy significantly improved insulin resistance, imbalance of endocrine hormones in women with PCOS.

Another studies showed no significant decreasing in fasting blood glucose. Plasma insulin level showed a significant improvement after 3and 6month of therapy.<sup>[25]</sup>

Longer term metformine therapy in young obese pcos women reduced fasting insulin and produced borderline reduction in HOMA – IR (P=0.05).<sup>[26]</sup>

The present study disagree with anthere studies, Agarwal N., *et al* showed metformin did not reduce HOMA – IR<sup>[27]</sup>, anther studies showed glucose, insulin level and insulin resistance not improved by metformin.<sup>[28]</sup>

Metformin has been the main stay of treatment for IR in PCOS women , metformin is abiguanide that acts principally on the liver to inhibit hepatic gluconeogenesis it also inhibits acetyl co A carboxylase activity and suppresses fatty acid production ,and acts on skeletal



muscle to inhibit lipid production and acts peripherally on adipose tissue to stimulate glucose transport and uptake, metformin reduces insulin level and promote improved insulin activity.<sup>[29]</sup>

The results of group (B) was in agreement with many studies ,researchers looking at the effects of metformin in PCOS women concluded that the drug's benefits could be related to its ability to improve the function of D-chiro- inositol (DCI) phosphoglycans in the body. Thus it appears that DCI may be highly effective when used in combination with metformin for PCOS.<sup>[30]</sup>

Another studies which reported that the Inositol was significant decrease insulin level and insulin resistance.<sup>[31,32]</sup>

The result in present study of blood glucose level disagree with anther studies, Minozzi M., *et al* rported that the myo– inositol significantly decreased hyperinsulinamia by positively effecting the fasting insulin and glucose level.<sup>[33]</sup>

Nestler JE.,*et al*<sup>[34]</sup> suggests that some actions of insulin are mediated by putative inositolphosphoglycan (IPG) mediators, also known as second messengers. We review studies indicating that the IPG signaling system transduces insulin's stimulation of human thecal androgen biosynthesis, thus offering a mechanism by which insulin can stimulate ovarian androgen production even in women with PCOS whose tissues are resistant to insulin's stimulation of glucose metabolism. Furthermore, a deficiency in a specific D-chiro-inositol-containing IPG may contribute to insulin resistance in women with PCOS. In support of this idea, administration of D-chiro-inositol has been demonstrated to improve glucose tolerance, decrease serum androgens and improve ovulation in PCOS. The hypothesis is advanced that PCOS may be characterized by a defect in the conversion of myo-inositol to D-chiro-inositol, and that such a defect would contribute to both insulin resistance and hyperandrogenism in the syndrome.

In fact, some researchers have suggested that improving insulin-mediated release of inositolphosphoglycan mediators is part of how metformin improves insulin resistance in women with PCOS.<sup>[48]</sup>

The result of group (C) was in agreement with many studies which reported no differences were observed between the diet groups with the respect to changes in insulin, and decreased significantly in insulin resistance.<sup>[35,36]</sup>

Another studies showed that the reducing insulin resistance by lifestyle modification such as diet and exercise improve endocrine and menstrual function on PCOS. These lifestyle modification or the boost initial means of improving insulin resistance.<sup>[37]</sup>

Palomba S., *et al* showed the different changed observed in insulin sensitivity indices after exercise and diet interventions, we hypothesized that in both cases, insulin sensitivity improvement itself is the pivotal factor involved in the restoration of ovarian function. The exercise program potentially improved insulin sensitivity in two way. First of all exercise results in large reduction of waist circumference and, thus, of visceral adipose tissue, even if related to a modest weight loss and BMI change.<sup>[38]</sup>

The visceral adipose tissue is more metabolically active than subcutaneous fat, and the central fat distribution is closely related to insulin resistance, they showed a smaller reduction in body weight.<sup>[39]</sup>

Huber-Buchholz *et al*<sup>[40]</sup> found that improving insulin sensitivity restored normal menstrual function and fertility in obese women with PCOS. by using lifestyle program that sets realistic weight loss .subjects were able to sustain improvement carbohydrate metabolism over a 6- month period , although weight loss as percentage of body weight was small.

The present study disagree with Mariana K., *et al*<sup>[41]</sup> study which reported the central obesity and insulin resistance were not change with macronutrient composition or energy intake in women with PCOS.

Another studies showed no significant differences were observed in diet on fasting insulin levels, or insulin sensitivity as assessed by homeostatic model assessment (HOMA).<sup>[42]</sup>

In the present study we used different type of management used mix between drugs, used drug alone and used physicals management by change lifestyle and followed up diet. After complete the period of study take the results and compare between groups in the study (every group treated with different treatment)and healthy women (without PCOS) table (B)in all parameter.

Group (A) which include women with PCOS treated with metformine (850 mg two time /day) for six months. The result in this group will good and that metformin therapy restores normal levels of insulin and testosterone, AMH and another hormone in study this result was similar to results pawekczyk *et al*<sup>[43]</sup> report that metformin therapy not only restores normal

levels of insulin and testosterone but also decreases the lipid profile . the role of metformin in improving the frequency Ovulation and balance the another hormone will be observed by other authors.<sup>[44]</sup>

The present study showed group A results lower effective than group B, may need long term period of treatment.

**Table (B) hormonal profile and biochemical parameter in healthy women (without PCOS)**

Parameter	Mean $\pm$ SE
INS	16.75 $\pm$ 0.68
FBS	4.84 $\pm$ 0.08
IR	3.91 $\pm$ 0.24

The present study showed group B which treatment with Cholin & inositol 500 / 500mg(two times /day) and metformin (850mg two times /day ) this type of treatment used first time in Iraq and now used as a first line of treatment PCOS patient in Kamal AL-Samarrae Hospital – Baghdad. Showed the best result because all parameter which was massaged in the study returned to the normal level or near to it when compared with healthy women in table (B)(women without PCOS), the women with PCOS can become pregnant in shorter time and the rate of pregnancy higher than group A which treatment with metformin alone. Cholin & inositol and metformin will give the best result D-chiro inositol (DCI) administration ameliorates insulin resistance, hyperandrogenemia and improves ovulation in this disorder, while metformin does not increase the amount of DCI available, it may act to release the DCI – IPG mediator that to the cell to use and / or store glucose, Deficiency of DCI in women with PCOS may be why metformin is effective in some but not all PCOS patients<sup>[31]</sup>, so that we give two drugs together.

The present study is the first to treat patient with PCOS by giving two insulin agents(inositol and metformin together)because not found data published similar to the present study.

The group( C) which change life style and followed diet did not give the best result because used diet alone without any chemical treatment did not have role to manage the PCOS because big number of patient in this group will follow diet by meal replacement and this type of lifestyle modification (diet alone)an effective in weight loss and hormonal balance in PCOS patient, this result was similar to the result Moran LJ *et al*<sup>[45]</sup> which reported that the

use of twice - daily meal replacements was an effective for achieving weight loss and gaining associated hormonal and clinical benefits in women with PCOS and that long -term weight loss may not be needed for fertility improvement in all women with PCOS.

Furthermore, Lifestyle modifications in the treatment of PCOS do not escape criticism and controversy despite being widely accepted recommendations. Physical activity (PA) has been reported to ameliorate anovulation, IR, blood pressure, and lipid profiles in women with PCOS, sometimes independently of weight loss<sup>[46]</sup> yet PA alone does not seem to be able to equal these parameters to non-PCOS subjects.<sup>[47]</sup> Therefore, it should be accompanied by a complementary diet plan in order to fully potentiate the effects of a lifestyle-modification therapeutic program. In this study, combination between choline & inositol and metformin offered a significant advantage over another type of management. In addition, patients on this management reported no side effects during the course of treatment. Choline & inositol and metformin could be as a first – line treatment in patients with PCOS.

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