

METFORMIN IMPROVES MENSTRUAL PATTERNS, ENDOCRINE AND METABOLIC PROFILE IN OBESE HYPERINSULINEMIC WOMEN WITH A POLYCYSTIC OVARY SYNDROME

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Abstract: *Objective:* To evaluate the therapeutic effect of metformin on the clinical features, hormonal and metabolic profile in obese women with a hyperinsulinemic polycystic ovary syndrome (PCOS).

Material and methods: We analyzed 25 women with PCOS, mean age 27.28 ± 7.85 years, BMI 34.42 ± 6.61 kg/m², Ferriman-Gallwey (F/G) score 17.43 ± 5.45 and duration of menstrual cycle 79.2 ± 54.5 days. Basal hormone measurements included: FSH, LH, total testosterone, DHEAS, 17 α OHP and E2. All patients underwent a 75g oral glucose tolerance test (OGTT), during which fasting and stimulated levels of glucose and insulin were measured at 0', 60' and 120'. The lipid profile was also performed. Both basal and stimulated parameters were analyzed before and after treatment with metformin (500 mg orally, three times daily for 9 months).

Results: After metformin treatment the mean frequency of menses became significantly more regular (79.2 ± 54.5 days vs. 31.61 ± 7.7 days, $P < 0.01$) and mean testosterone level had significantly decreased (2.78 ± 1.23 vs. 1.72 ± 0.95 ng/ml, ($P < 0.01$). We also observed a statistically significant decrease in the metabolic parameters, both basal insulinemia (22.18 ± 5.76 vs. 17.19 ± 6.67 μ U/ml, $P < 0.01$), stimulated insulinemia after 60' (179.18 ± 88.96 vs. 136.38 ± 75.43 μ U/ml, $P = 0.04$), stimulated insulinemia after 120' (163.23 ± 89.2 vs. 88.46 ± 61.5 U/ml, $P < 0.01$) and glucose response to OGTT on 120' (7.07 ± 1.82 vs. 6.15 ± 1.52 mmol/L, $P = 0.04$). The levels of HDL cholesterol had increased (0.94 ± 0.16 vs. 1.13 ± 0.19 mmol/L, $P = 0.04$). No changes were noted on the hirsutism score and other steroid levels.

Conclusion: In women with PCOS treatment with metformin is effective in the lowering of hyperinsulinemia and hyperandrogenemia. In most women metformin improves the menstrual pattern, but has no effect on hirsutism.

Key words: PCOS, obesity, insulin resistance, hyperinsulinemia, metformin.

Polycystic ovary syndrome is a disorder characterized by chronic anovulation, hyperandrogenemia, signs of hyperandrogenism, enlarged cystic ovaries and obesity. Most of the patients have metabolic disturbances with life-long consequences on the women's health. There is evidence of decreased insulin sensitivity in both lean and obese patients with PCOS [4], but insulin resistance, accompanied by compensatory hyperinsulinaemia is significant in obese patients with PCOS [8, 10].

A strong positive correlation exists between hyperinsulinaemia and hyperandrogenemia, and there is strong positive correlation between insulin levels and ovary androgen secretion in hyperandrogenic patients with PCOS [2, 3, 8]. According to this, hyperinsulinemia, may cause even more hyperandrogenaemia, but not vice versa [5].

The patients with PCOS are at higher risk of developing type 2 diabetes and dyslipidemia early in their lives (in their thirties and forties). Also, dyslipidemia is associated with hyperinsulinemia to higher degree than with high levels of androgens. Patients with PCOS and hyperinsulinemia often show high levels of low-density lipoproteins (LDL), cholesterol and triglycerides. This lipid pattern is a well-established risk factor for coronary artery disease. Insulin is an atherogenic factor and hyperinsulinemia, independently of other factors, is a risk factor for cardiovascular disease in diabetic and non-diabetic subjects. Furthermore, insulin resistance and hyperinsulinemia are the major risk factors for developing type 2 diabetes in younger patients [9, 11].

Many therapeutic approaches have been used in reducing the clinical features, but many of them have certain limitations. For example, oral contraceptive pills suppress ovarian androgen production but they do not eliminate the basic problem, e.g. insulin resistance. However, metformin reduces the glucose levels through increased intestinal glucose utilization, increased peripheral glucose uptake and inhibition of hepatic glucose production. It also increases insulin sensitivity at the post-receptor levels, but it does not stimulate insulin secretion.

The aim of the study is to evaluate the long-term therapeutic effects of metformin on clinical features, and the hormonal and metabolic profile in obese women with hyperinsulinemic PCOS.

Material and methods

Twenty-five patients with PCOS were analyzed. The diagnosis was established according to the Rotterdam diagnostic criteria (Rotterdam, 2003) (9). Body mass index (BMI calculated as kg/m^2) was used to estimate obesity. The women were considered obese when the BMI was $> 30 \text{ kg}/\text{m}^2$. Blood pressure was measured with a mercury sphygmomanometer after the women had been seated for five minutes. Clinical evaluation of hirsutism was based on the Ferriman-Gallwey score; score ≥ 8 was considered hirsutism. Metformin effects on menstrual abnormality of patients were evaluated by assessing post-treatment changes in the frequency of cycles. Transvaginal sonography was used to evaluate the uterus and ovaries. Basal hormonal measurement included: Follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (T), dehydroepiandrosterone-sulphate (DHEAS), estradiol (E2) and all were measured by electrochemiluminescence (ECLIA-Roche Elecsys 1010). 17α hydroprogesterone (17α OHP) was measured by radioimmunoassay. All hormonal measurements were performed before and after treatment with metformin. The metabolic assessment included an oral glucose tolerance test (OGTT) and lipids. All patients underwent 75 g OGTT, during which fasting and stimulated levels of glucose and insulin were measured at 0', 60', 120'. Insulin levels were measured by radioimmunoassay. Plasma glucose was measured by a glucose oxidase assay on Beckman's analyzer. Glucose tolerance was evaluated using the criteria of the World Health Organization. Levels of serum total cholesterol, triglycerides, high density lipoproteins (HDL) and low density lipoproteins (LDL) were determined by standard methods, (cholesterol-enzymatic colorimetric method; triglyceride-colorimetric enzymatic test using glycerol-3-phosphate oxidase; HDL cholesterol-enzymatic clearance assay; LDL cholesterol is calculated by the Friedwold formula).

The clinical features, hormonal and metabolic parameters were analyzed before and after treatment with metformin (500 mg orally, three times a day for nine months). The first bleeding was considered a response to the therapy. According to the response patients were divided into responders and nonresponders. Descriptive methods were used for statistic analysis. Results are reported as the mean \pm SEM. U- test was used to compare the clinical and hormonal indices between responders and non-responders. *P* value < 0.05 was considered of statistical significance.

Results

We analyzed twenty-five patients who were referred to the Endocrinology Outpatient Department, at the Endocrinology, Diabetes and Metabolic Dis-

orders Clinic. The clinical characteristics of PCOS women were: mean age 27.28 ± 7.85 years, BMI 34.42 ± 6.61 kg/m², Ferriman-Gallwey (F/G) score 17.43 ± 5.45 and duration of menstrual cycle 79.2 ± 54.5 days. All women with PCOS had ovarian ultrasonic findings consistent with the diagnosis (9). Other causes of hyperandrogenisms were excluded. None of the PCOS women had taken any medication for at least 3 months before the study. The patients were treated with metformin and all of them completed the study. Three of them showed minimal side effects on metformin: nausea and diarrhea.

Table 1 – Табела 1

The main clinical and endocrine characteristics of subjects given metformin, at baseline and after treatment

Главни клинички и хормонски карактеристики на пациентките третирани со метформин, базално и по терапија

	Baseline	After treatment	Effect of treatment
Body mass index (kg/m²)	33,57 +/- 6,58	31,61 +/- 5,59	0.0031
Menses (days)	78,26 +/- 56,82	31,61 +/- 7,69	0.0004
F-G score	18,14 +/- 5,11	16,42 +/- 5,22	0.1723
LH/FSH ratio	1,06 +/- 0,64	0,89 +/- 0,54	0.1907
Estradiol (pmol/l)	116,25 +/- 82,58	127,45 +/- 98,18	0.6059
Testosterone (ng/ml)	2,78 +/- 1,23	1,72 +/- 0,95	<0,0001
DHEAS (μmol/L)	7,37 +/- 2,63	7,02 +/- 3,21	0.5208
17 OHP (nmol/L)	4,60 +/- 0,88	6,33 +/- 2,87	0.4204

* $P < 0,05$

We noted the positive effects of metformin on their BMI and menstrual pattern. Thus, there was significant decrease in BMI after treatment ($P < 0,031$). We found very strong and statistically significant effects on the menstrual patterns, so that the mean days of menstrual bleeding before and after treatment were 78.26 ± 56.82 and 31.61 ± 7.69 days ($P < 0.0004$), respectively. There were no differences to mean F/G score, LH/FSH ratio, DHEAS and 17 α OHP before and after treatment. The mean levels of total testosterone were higher (2.78 ± 1.23 ng/ml) in the basal state and decreased (1.72 ± 0.95 ng/ml) significantly after treatment ($P < 0.0001$) (Table 1). The basal mean LH to FSH ratio was within the normal ranges (1.06 ± 0.64), with no significant changes (0.89 ± 0.54) after treatment.

Table 2 – Табела 2

*Main biochemical characteristics of subjects given metformin,
at baseline and after treatment*

*Метаболични карактеристики на пациентките третирани
со метформин, базално и по терапија*

	Baseline	After treatment	Effect of treatment
Basal insulinemia ($\mu\text{U/ml}$)	22,18 +/- 5,77	17,19 +/- 6,67	0,0001
Stimulated insulinemia at 60' ($\mu\text{U/ml}$)	179,18 +/- 88,95	136,38 +/- 75,43	0,0410
Stimulated insulinemia at 120' ($\mu\text{U/ml}$)	163,25 +/- 89,20	88,46 +/- 61,50	<0,0001
Basal glucose (mmol/L)	5,05 +/- 0,66	4,89 +/- 0,67	0,3464
Stimulated glucose at 60' (mmol/L)	8,84 +/- 1,94	8,73 +/- 1,87	0,7817
Stimulated glucose at 120' (mmol/L)	7,07 +/- 1,81	6,15 +/- 1,52	0,0372
Cholesterol (mmol/L)	4,4 +/- 0,64	4,48 +/- 0,83	0,7805
HDL-cholesterol (mmol/L)	0,94 +/- 0,17	1,14 +/- 0,19	0,0237
LDL-cholesterol (mmol/L)	3,04 +/- 0,59	2,82 +/- 0,63	0,3440
Triglycerides (mmol/L)	1,14 +/- 0,45	1,12 +/- 0,48	0,8990

* $P < 0.05$

The major metabolic features are shown in Table 2. We observed a statistically significant decrease in the metabolic parameters, both basal insulinemia (22.18 ± 5.76 vs. 17.19 ± 6.67 $\mu\text{U/ml}$, $P < 0.01$), stimulated insulinemia after 60' (179.18 ± 88.96 vs. 136.38 ± 75.43 $\mu\text{U/ml}$, $P = 0.04$), stimulated insulinemia after 120' (163.23 ± 89.2 vs. 88.46 ± 61.5 U/ml , $P < 0.01$). Glucose response to OGTT on 120' was decreased (7.07 ± 1.82 vs. 6.15 ± 1.52 mmol/L , $P = 0.04$). The mean levels of fasting and stimulated glucose at 60' did not change significantly after treatment. The serum total cholesterol, triglycerides and LDL cholesterol concentrations were within the normal ranges before and after treatment. The low serum levels of HDL-cholesterol had increased significantly at the end of the study ($P < 0.02$).

Table 3 – Табела 3

Mean post-treatment characteristics of women given metformin, divided into responders and non-responders according to the efficacy of treatment on menstrual abnormalities

Терапевскиот ефект на метформинот кај третираните пациентки, поделени во респондери и нереспондери врз основа на ефектот на терапијата на менструалниот циклус

	Responders	Non-responders	p
Body mass index (kg/m ²)	33,82 +/- 6,39	37,22 +/- 7,86	0,7642
Menses (days)	28,55 +/- 2,83	42,6 +/- 9,93	0,0001
LH/FSH ratio	0,45 +/- 0,54	2,36 +/- 0,12	0,0270
Testosterone (ng/ml)	1,58 +/- 0,95	2,16 +/- 0,87	0,1964
Basal insulinemia (μU/ml)	15,55 +/- 5,38	22,40 +/- 8,15	0,0249
Stimulated insulinemia at 120' (μU/ml)	81,66 +/- 46,81	109,98 +/- 97,64	0,3360
Basal glucose (mmol/L)	4,92 +/- 0,66	4,88 +/- 0,73	0,9068
Stimulated glucose at 120' (mmol/L)	6,62 +/- 1,83	8,43 +/- 0,85	0,0308
Cholesterol (mmol/L)	4,8 +/- 0,97	4,9 +/- 0,70	0,8545
HDL-cholesterol (mmol/L)	1,08 +/- 0,97	1,18 +/- 0,70	0,4175
LDL-cholesterol (mmol/L)	3,16 +/- 0,2	3,07 +/- 0,2	0,8429
Triglycerides	1,19 +/- 0,4	1,32 +/- 0,6	0,6642

* $P < 0.05$

In order to avoid bias about the effect of metformin on individual patients we divided the cohort into responders and non-responders. We found a statistically significant difference in the mean length of menstrual cycles, between responders (28.55 ± 2.83 days) and nonresponders (42.6 ± 9.93 days) ($P < 0.0004$). There was no significant difference in BMI between responders and non-responders (N.S.) (Table 3).

As shown in Table 3, the mean basal testosterone levels (1.58 ± 0.95 ng/ml) were lower in responders than in nonresponders (2.16 ± 0.87ng/ml). However, the responders showed a significant decrease in the LH/FSH ratio (0.45 ± 0.54) comparing to nonresponders (2.36 ± 0.12) ($P < 0.027$). Metformin therapy probably improved the menstrual pattern, with the change in the LH/FSH ratio in responders. Moreover, only fasting insulin ($P < 0.025$) and stimulated glucose at 120' ($P < 0.03$) decreased significantly.

Discussion

In the past decades, most uncontrolled short-term studies have shown different results in evaluating the effects of metformin in the treatment of hyperandrogen women with PCOS., Velasquez *et al.* confirmed the clinical usefulness of metformin on the blood pressure and menstrual cycle in seven PCOS women for the first time [1]. Furthermore, Nestle *et al.* [2] reported a decrease in the levels of free testosterone and an increase of serum levels of SHBG, after eight weeks of treatment.

There have been only a small number of studies where metformin has been used for long-term treatment. In our study the outcomes of metformin treatment were examined after nine months, especially the effects on clinical, hormonal and metabolic parameters. The first change was noted in menstrual abnormalities. Moreover, menstrual bleeding appeared during the first month of treatment and became regular in the second and third months. We noted a positive long-term effect on the menstrual pattern with continued therapy with metformin. Actually, we considered the appearance of menstrual bleeding as the first positive sign of treatment. On this ground we divided the patients into responders and non-responders. The meta-analysis shows that metformin is effective in achieving ovulation in women with PCOS [12]. It is recommended to women who desire to become pregnant. However, we did not examine the occurrence frequency of ovulation during the treatment in this study. Spontaneous menses is very important, because it refers to normal ovarian function. In women with PCOS, especially the obese, regular menstrual cycles serve to eliminate the well-known risks of endometrial hyperplasia and carcinoma.

Previous studies have shown that metformin has no effect on body weight [12]. It is not a "weight loss" drug. In our study, non-responders had a higher BMI than responders, but the difference was not statistically significant. This finding proved that metformin itself had no direct effect on body weight, especially in the most obese patients. It is possible that women who do not show the effect on 1.5 g metformin need a higher dose of metformin (e.g. 2.0 g daily) [1, 2, 3]. Furthermore, we recommend metformin to obese PCOS women as an adjuvant therapy to their life-style changes.

In our study, total testosterone levels were elevated at baseline and declined after metformin treatment. It has been suggested that, even without changes in body weight, the insulin-sensitizing agents may reduce increased androgen levels by a reduction of insulin levels [3]. We did not find a reduction in 17α OHP levels after metformin treatment; surprisingly, 17α OHP levels were higher after treatment. This is in contrast with the recent study in which metformin produced an attenuation in serum 17α OHP hyperresponse to GnRH agonist stimulation. This effect was independent of changes in body weight. The investigators postulated that obese patients with PCOS may have an abnormal

intracellular insulin signalling of P450c17 α activity in the ovary, making the enzyme complex more sensitive. Moreover, metformin can reverse enzymatic activities to normal with an improvement in insulin levels [3].

The ratio of LH to FSH was within the normal range at baseline and did not change after treatment (Table 1). However, when we divided the patients into responders and non-responders according to the efficacy of treatment on menstrual abnormalities, the LH/FSH ratio was significantly lower in responders than in non-responders. These data suggest that changes in insulin levels might change ovarian androgen secretion through effects on the pituitary level [10].

PCOS is characterized by insulin resistance. Insulin resistance is a key factor in the metabolic syndrome. If metformin improves some characteristics of the metabolic syndrome, it can reduce the risk of cardiovascular events. After nine months of treatment, metformin significantly decreased the levels of fasting and stimulated insulin during OGTT, as reported previously in some studies [3, 12]. However, we found the greatest decline in fasting insulin and stimulated glucose (120') in responders only. Also, meta-analysis confirms that metformin does significantly reduce fasting insulin levels [12]. So we can recommend metformin as a first line drug. Metformin has little effect on plasma lipids [6, 7]. We found statistically increased levels of HDL cholesterol after metformin treatment and a slight decrease in LDL-C levels. There were no change in triglycerides and total cholesterol. These data are not in agreement with the findings of other investigators [3, 12]. Lord *et al.* reported a significant decrease in LDL-C levels [12]. It is possible that the size of our sample is a reason for this finding.

In summary, this study shows that in women with PCOS metformin treatment is effective in lowering hyperinsulinemia and hyperandrogenism. In most women metformin induces a regulation of menstrual abnormalities and a significant reduction of the LH/FSH ratio in responders only, but has no effect on hirsutism. In future, a large group of patients is needed in order to assess whether some of the factors explored can predict the response to metformin.

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Резиме

МЕТФОРМИНОТ ГИ ПОДОБРУВА МЕНСТРУАЛНИТЕ, ЕНДОКРИНОЛОШКИТЕ И МЕТАБОЛИЧНИТЕ НАРУШУВАЊА КАЈ ОБЕЗНИ ЖЕНИ СО ХИПЕРИНСУЛИНЕМИЧЕН ПОЛИЦИСТИЧЕН ОВАРИЈАЛЕН СИНДРОМ

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Цел: Да се евалуира терапевскиот ефект на метформинот на клиничките карактеристики, хормонските и метаболичните параметри кај обезни пациентки со хиперинсулинемичен ПЦОС.

Материјал и методи: Анализирани се 25 пациентки на возраст од $27,28 \pm 7,85$ години, БМИ од $34,42 \pm 6,61$ kg/m², Ferriman-Gallawey (Ф/Г) скор од $17,43 \pm 5,45$ и должина на менструален циклус од $79,2 \pm 54,5$ дена. Базалните хормонски мерења вклучија: ФСХ, ЛХ, тестостерон, ДХЕАС, 17 ОХП и Е2. Кај сите пациентки беше изведен 75 г орален гликоза толеранс тест (ОГГТ), а базалните и стимулирани вредности на гликемија и инсулинемија беа одредувани на 0, 60 и 120 мин. Липиден профил беше изведуван кај сите пациентки. Базалните и стимулираните параметри беа одредувани пред и по терапијата со метформин (500 мг орално, три пати дневно, за период од 9 месеци).

Резултати: По терапија со метформин постигнато е значајно подобрување во менструалниот циклус (од $79,2 \pm 54,5$ на $31,6 \pm 7,7$ дена, $P < 0,01$), средните вредности на тестостеронот значајно се намалија (од $2,78 \pm 1,23$ на $1,72 \pm 0,95$ ng/ml, $P < 0,01$) а утврдивме значајно намалување во метаболичните параметри, базалната инсулинемија (од $22,18 \pm 5,76$ μ U/ml до $17,19 \pm 6,67$ μ U/ml), стимулираната инсулинемија на 60 мин. (од $179,18 \pm 88,96$ μ U/ml на $136,38 \pm 75,43$ μ U/ml, $P < 0,04$), стимулираната инсулинемија на 120 мин. (од $163,25 \pm 89,2$ μ U/ml до $88,46 \pm 61,5$ μ U/ml, $P < 0,01$) и стимулираната гликемија на 120 мин. од ОГГТ (од $7,07 \pm 1,82$ mmol/l на $6,15 \pm 1,52$ mmol/l, $P = 0,04$). Значајно подобрување беше забележано во нивоата на HDL-C (од $0,94 \pm 0,16$ до $1,13 \pm 0,19$ mmol/l, $P = 0,02$). Не беа забележани промени во хирзутизмот и другите стероидни хормони.

Заклучок: ПЦОС терапијата со метформин е ефикасна за намалување на хиперинсулинемијата и хиперандрогенемията. Метформинот го регулира менструалниот циклус, но нема ефект на хирзутизмот.

Клучни зборови: ПЦОС, дебелина, инсулинска резистенција, хиперинсулинемија, метформин.

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