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Metformin doses and body mass index: clinical outcomes in insulin resistant polycystic ovary syndrome women

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Abstract. – OBJECTIVE: PCOS is the most common endocrinopathy among reproductive age women. Approximately 60% of PCOS women have insulin resistance. While the efficacy of metformin in reducing insulin resistance and decreasing androgen level has been widely validated, there is no agreement on the dose of metformin to be used.

PATIENTS AND METHODS: Prospective non-randomized cohort study of 108 insulin resistant, overweight and obese PCOS women, aged between 22 and 35 years. All patients received 1500 mg of metformin (500 mg x 3 times/day) for the first 6 months. At the end of this period, the patients' HOMA index was evaluated. In subjects, who did not demonstrate normalization of the HOMA index, the dose was increased to 2500 mg/day (500 mg at breakfast and 1000 mg at lunch and dinner) for additional 6 months. The hormonal blood profile, fasting insulin and fasting glucose levels, HOMA index, anthropometric assessment, pelvic ultrasound, FAI index and cholesterol were evaluated.

RESULTS: Overall results showed a good response to metformin therapy in insulin-resistant PCOS patients with BMI >25, while in patients with higher BMI (31.15 ± 0.40), no normalization of HOMA was found. At the higher dose of metformin, obese patients achieved a good response to therapy, with improvement in BMI, menstrual pattern, cholesterol levels and hyperandrogenism.

CONCLUSIONS: Our results demonstrate a correlation between the required dose of metformin, BMI and hyperandrogenism. The dose of metformin should be adjusted to patients' BMI in order to obtain significant results in terms of clinical, metabolic and hormonal responses.

Key Words:
PCOS, Insulin resistance, Metformin, HOMA index, Hyperandrogenism.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy among women of reproductive age[1,2]. These patients present with a combination of signs and symptoms, varying from amenorrhea to sonographic picture of polycystic ovaries. Other signs include subtle phenotypic abnormalities, hyperandrogenism and advanced PCOS syndrome with its associated long-term sequelae[3]. Moreover, most women with PCOS also exhibit features of metabolic disorders including insulin resistance, obesity and dyslipidemia[4]. Insulin resistance in women with PCOS is present both in obese and non-obese patients[5].

Insulin resistance and compensatory hyperinsulinemia are thought to contribute to hyperandrogenism by multiple mechanisms. Hyperinsulinemia stimulates ovarian production of androgens directly by acting on thecal cells and indirectly by inhibiting FSH action on granulosa cells. Insulin stimulates thecal cell proliferation, as demonstrated *in vitro*, induces secretion of androgens mediated by LH and increases cytochrome P450 expression of LH and IGF-1 receptors[6]. Furthermore, insulin receptors also present in the hypothalamus and pituitary stimulate the release of FSH and LH under basal conditions and after GnRH stimulation[7].

Moreover, insulin influences hyperandrogenism through the inhibition of SHBG synthesis, leading to an increase in testosterone availability[8]. IGF-1/IGFBP-1 ratio increases significantly in women with PCOS[9], making IGF-1 easily available to thecal cells, that in turn can promote production of androgens. Additionally, IGF-1, stimulates oestrogen production by granulosa cells, and can synergistically act with FSH and

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LH in modulating the expression of aromatase in these cells[10]. Insulin induces ACTH-mediated androgen production in the adrenal glands, thus acting positively on LH-stimulated ovarian steroidogenesis[11].

Improving insulin sensitivity through both lifestyle and pharmacological intervention has been suggested to ameliorate the hyperandrogenism, to restore ovulation and enhance pregnancy rate in women with PCOS. Treatment with metformin, the most widely studied insulin-sensitizing drug in PCOS patients, is often associated with a significant reduction in plasma concentrations of various androgens[12].

Moreover, metformin treatment has been associated with weight reduction in overweight and obese PCOS patients, both insulin sensitive and insulin resistant[13]. It has been further demonstrated[14,15] that metformin therapy can significantly reduce visceral and subcutaneous adipose tissue.

Currently, many studies have confirmed the efficacy of metformin in reducing insulin resistance and its beneficial effects in PCOS subjects, but there is no consensus regarding the optimal metformin dose to be used in these patients. The rationale of our study is based on the correlation between metformin dose and BMI. In our study we aimed to evaluate the effect of different doses of metformin in PCOS subjects with different BMI on their Homeostasis Model Assessment Insulin Resistance (HOMA) index and their hormonal and lipid levels.

Patients and Methods

Our study consisted of 108 PCOS women aged 22 to 35 attending the Infertility and IVF Unit of Department of Molecular and Development Medicine of the University of Siena, Italy, during a two-years period (from 2016 to 2018).

Inclusion criteria were: PCOS according to the Rotterdam criteria, HOMA [fasting plasma glucose (mg/dl) x fasting insulin (mIU/l)/405]>2.5 and BMI >25.

All patients enrolled had no co-morbidities and had not taken, for the previous 6 months, any medications interfering with the levels of sex hormones, blood glucose, and insulin.

Recruited patients underwent anthropometric assessment (waist circumference and BMI), evaluation of their menstrual cycle and transvaginal pelvic ultrasound. Hormonal blood profile, fasting insulin, fasting glucose, and cholesterol were measured during the follicular phase (3rd to 5th day of the menstrual cycle). In patients with amenorrhea, menstrual flow was induced by progestin (medroxyprogesterone acetate -10 mg for 8 days).

In order to reduce the side effects of metformin, such as abdominal discomfort and diarrhea, its dose was gradually increased: one third of the required dose was initially administered, after 7 days it was increased to two-thirds and after 14 days the full dose was administrated.

During the study, all participants were advised to undergo lifestyle modification, including dietary adjustments and moderate-strength physical exercises three times a week for 30-40 minutes per session. They were asked to record their daily dietary and exercise activities, which were monitored through hospital visits.

All patients received 1500 mg of metformin (500 mg x 3 times/day) for 6 months. At the end of this period, the patients' HOMA index was evaluated, and the patients were further divided into two groups: Group 1 was those who responded with a normalization of the HOMA index and Group 2 were subjects who did not demonstrate normalization of the HOMA index (non responders).

In women of Group 2, the dose was increased to 2500 mg/day (500 mg at breakfast and 1000 mg at lunch and dinner) for 6 additional months.

Plasma concentration of Testosterone (T), FSH and LH were measured using Access Immunoassay System (Beckman Coulter, Milan, Italy), for Andostenedione (A), SHBG, estradiol were used Immunolite 2000 system Kits (Siemens, Los Angeles, CA, USA).

The samples were analysed twice with two dilutions. For each test, controls at low, medium and high concentration were included. The dosing limits were 277 pmol/L to T, 104 pmol/L for A, 0.02 nmol/L for SHBG, 15 pg/ml for estradiol, 0.2 mUI/ml for LH and FSH. Variations between samples were 3.4% and 4.6% for T, 5.6% and 6.4% for A, 6.9% and 13% for SHBG. Plasma glucose was measured with a glucose oxidation method (Beckman Instruments, Fullerton, CA USA) and cholesterol was determined by an enzymatic assay (Bristol, Paris, France). The methods used are highly specific for each hormone and have low cross-reactivity (b.0.5%) with other hormones or drugs present in the samples. FAI Index was calculated: the total testosterone level was divided by the sex hormone binding globulin (SHBG) level, and then, multiplied by 100.

Statistical analysis was performed with the software GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA). The standardized values of skewness and kurtosis will be used to verify the normal distribution of data. Parametric (*t*-test) or non-parametric (Mann Whitney and Wilcoxon signed-rank test) tests were used when appropriate. Statistical significance was set at a value of $p<0.05$.

Ethical Approval

This study was approved by our Local Ethics Committee (Review Board of Department of Obstetrics and Gynaecology, University of Siena). All patients provided written informed consent. Local Ethical Committee Approval protocol number: 325/05 A on 23rd June 2015.

Results

Of the 108 PCOS women initially treated, eight dropped out during the first part of the study: two patients conceived, one suffered from thyroid disease and five patients withdrew due to the side effects caused by metformin (nausea and occasional diarrhoea attacks). Patients were divided into two groups based on normalization of the HOMA index after first treatment period with metformin 1500 mg and their baseline characteristics, both anthropometric and hormonal features, are presented in Table I. Between the two groups (responders *vs.* non-responders) significant differences emerged: HOMA index is higher in non-responders (3.09 ± 0.09 *vs.* 3.44 ± 0.09; *p*-value 0.010), as well as BMI (28.46 ± 0.39 *vs.* 31.15 ± 0.40; *p*-value < 0,0001), waist circumference (83.85 ± 1.02 *vs.* 89.34 ± 0.83; *p*-value < 0,0001) and total cholesterol (202.7 ± 4.29 *vs.* 218.9 ± 4.61; *p*-value 0.012). The number of menstrual cycles during the first 6 months was greater in group 1 (7.02 ± 0.37 *vs.* 5.50 ± 0.28; *p*-value 0,0014). LH (6.87 ± 0.16 *vs.* 7.39 ± 0.15; *p*-value 0,0186) and estradiol levels (31.55 ± 1.004 *vs.* 35.74 ± 1.10; p 0,0065) were higher in non-responders, with no in-between group differences in FSH, Testosterone, SHBG, FAI and Androstenedione.

At the end of the first 6 months of therapy, general improvements in HOMA index, SHBG and FAI were recorded in both groups (Table II). Reduction in BMI was also observed, although the differences were not statistically significant. Testosterone levels dropped significantly in group 1 but not in group 2 (Table II).

The subjects of group 2, with an average of basal BMI of 31.15 ± 0.40, showed a significant reduction in the HOMA index after 6 months of 1500 mg per day metformin, but the HOMA index levels did not normalized (HOMA index <2.5). In these patients, the daily metformin dose was increased to 2500 mg for additional 6 months.

At the end of the second treatment period these patients showed a statistically significant improvement with normalization of the HOMA index, together with a significant amelioration of the fasting glucose, insulin, and hyperandro-

Table I. Basal characteristics of study population. Data are presented as Mean(M) ± Standard Deviation (SD).

	Group 1 "Responders" No. 47	Group 2 "Non- responders" No. 53	*p*-value
Age	24.91 ± 0.51	25.30 ± 0.40	0.5494
HOMA	3.09 ± 0.09	3.44 ± 0.09	0.0109
N. of menstrual cycles in 6 months	7.021 ± 0.37	5.51 ± 0.28	0.0014
BMI	28.46 ± 0.39	31.15 ± 0.40	< 0.0001
Fasting glucose mg/dl	89.03 ± 1.31	94.12 ± 1.18	0.0047
Fasting insulin mIU/mL	13.98 ± 0.22	14.67 ± 0.247	0.0433
Total cholesterol mg/dl	202.7 ± 4.29	218.9 ± 4.61	0.0124
Waits circumference cm	83.85 ± 1.02	89.34 ± 0.83	< 0.0001
LH mUI/ml	6.87 ± 0.16	7.39 ± 0.15	0.0186
FSH mUI/ml	6.07 ± 0.11	5.98 ± 0.11	0.5597
Estradiol pg/ml	31.55 ± 1.004	35.74 ± 1.10	0.0065
Testosterone pmol/l	0.6132 ± 0.01	0.611 ± 0.004	0.7808
SHBG nmol/l	31.89 ± 0.13	31.79 ± 0.12	0.5844
FAI	1.92 ± 0.019	1.92 ± 0.012	0.9804
Androstenedione pmol/l	2.72 ± 0.071	2.77 ± 0.075	0.6302

Table II. HOMA, BMI, basal glucose and insulin and androgens before and after 6 months treatment with Mefotrmin 1500 mg (500 mg three times a day): Data are presented as Mean(M) ± Standard Deviation (SD).

Parameters	Responders (No. 47)		Non-responders		
	At baseline	After 6 months	At baseline	After 6 months	*p*-value
HOMA index	3.09 ± 0.09	2.25 ± 0.03	3.44 ± 0.09	3.06 ± 0.06	< 0.0001[a] 0.0109[b] 0.0012[c]
BMI	28.46 ± 0.39	27.73 ± 0.36	31.15 ± 0.40	30.69 ± 0.37	0.1728[a] < 0.0001[b] 0.4050[c]
Fasting glucose mg/dl	89.03 ± 1.31	81.34 ± 0.62	94.12 ± 1.18	89.68 ± 0.83	< 0.0001[a] 0.0047[b] 0.0027[c]
Fasting insulin mIU/mL	13.98 ± 0.22 7	11.23 ± 0.11	14.67 ± 0.25	13.78 ± 0.18	< 0.0001[a] 0.0433[b] 0.0044[c]
Testosterone pmol/l	0.61 ± 0.01 N = 47	0.58 ± 0.005	0.61 ± 0.004	0.60 ± 0.004	0.0009[a] 0.7808[b] 0.2417[c]
SHBG pmol/l	31.89 ± 0.13 47	34.36 ± 0.22	31.79 ± 0,12	32.79 ± 0.18	< 0.0001[a] 0.5844[b] < 0.0001[b]
FAI	1.92 ± 0.02 = 47	1.70 ± 0.017	1.92 ± 0.01	1.84 ± 0.013	< 0.0001[a] 0.9804[b] < 0.0001[c]

[a]between responders. [b]between responders and non-responders at baseline.

genism (Table III, Figure 1). Testosterone levels (0.61 ± 0.004 pmol/l *vs.* 0.58 ± 0.005 pmol/l; p<0.0001) and BMI (31.15 ± 0.40 *vs.* 30.08 ± 0.37; p<0.05) decreased statistically significantly only after the treatment with metformin 2500 mg in non-responders subjects (0.61 ± 0.004 *vs.* 0.58 ± 0.005; p<0.0001). Total cholesterol, waist circumference and the menstrual pattern were also improved with the higher dose of metformin (Table III). No side effects are reported in this group and metabolic acidosis did not occur in any patient. The parameters recorded in the group 2 after metformin treatment with 2500 mg, were similar to those obtained in the group of responders after metformin therapy with 1500 mg.

Table III. Subjects without normalization of HOMA index after 6 months treatment with 1500 mg metformin (Group 2), treated with 2500 mg/day (500 mg at breakfast and 1000 mg at lunch and dinner) for additional 6 months.

Parameters	Group 2 "Non-responders"		
	At baseline	After 6 months of Metformin 2500 gr	*p*-value
HOMA index	3.44 ± 0.09	2.49 ± 0.04	< 0.0001
BMI	31.15 ± 0.40	30.08 ± 0.37	< 0.05
Fasting glucose mg/dl	94.12 ± 1.18	82.53 ± 0.69	< 0.0001
Fasting insulin mIU/mL	14.67 ± 0.2469	12.20 ± 0.14	< 0.0001
Testosterone pmol/l	0.61 ± 0.004	0.58 ± 0.005	< 0.0001
SHBG nmol/l	31.79 ± 0.12	36.02 ± 0.25	< 0.0001
FAI	1.92 ± 0.01	1.62 ± 0.02	< 0.0001
Total cholesterol mg/dl	218.9 ± 4.61	203.48 ± 5.21	< 0.0001
Waist circumference cm	89.34 ± 0.83	86.21 ± 4.10	< 0.05
No. of menstrual cycles in 6 months	5.51 ± 0.28	6.97 ± 0.31	< 0.001

Data are presented as Mean (M) ± Standard Deviation (SD).

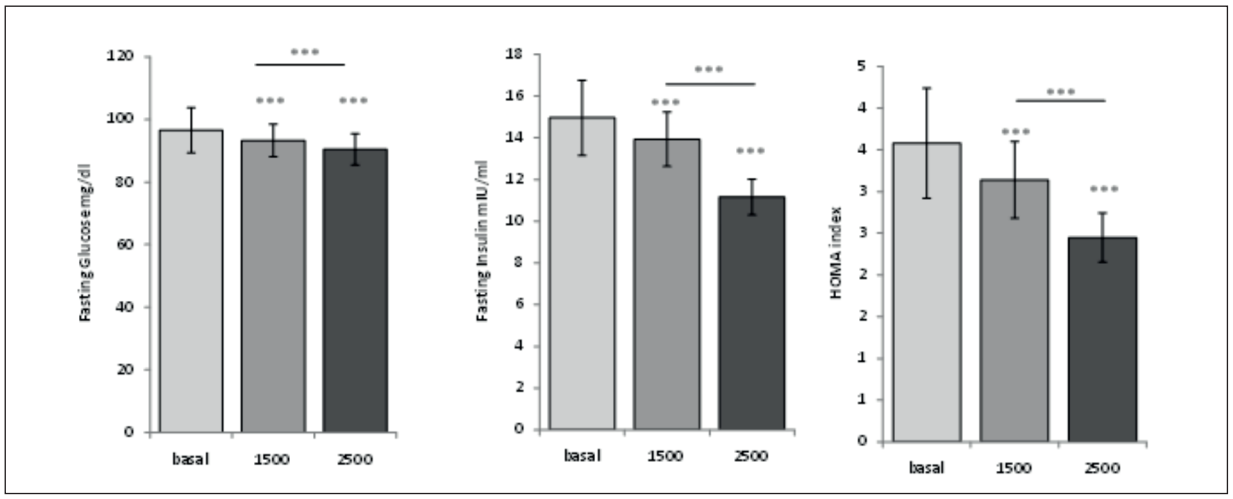

Figure 1. Effect of metformin on fasting glucose, fasting insulin and HOMA index after 1500 mg daily and after 2500 mg daily.

Overall, these results show a good response to metformin therapy for insulin resistant PCOS patients with BMI >25. In those patients with higher BMI, who had not normalized their HOMA index, the increase in metformin dose induced a significant amelioration in all the tested variables.

Discussion

The use of insulin sensitizers, particularly metformin, is well accepted for the treatment of insulin resistance PCOS patients. However, there is still no consensus in choosing the optimal dose and there is no agreement on the minimum effective dose.

Our data clearly show that the dose of metformin should be adjusted to patients' BMI in order to obtain significant results in terms of clinical, metabolic and hormonal responses. A higher BMI generally requires higher doses of metformin. Moreover, dose escalation does not affect the safety of the drug, since metformin does not cause hypoglycaemia[16]. On the other hand, one of its most worrying side effects is metabolic acidosis, but it is very rare in patients with normal kidney function[17]. The minor side effects of metformin, such as nausea and diarrhea[18], can be limited by a gradual weekly increase in the starting dose.

It has been long known that adipose tissue plays a role in maintaining metabolic homeostasis and that adipose tissue expansion can enhance insulin resistance and induce a state of inflammation. These conditions are associated with a chronic inflammatory response characterized by abnormal cytokine production and activation of inflammatory signalling pathways[19,20].

The effectiveness of metformin is not only demonstrated by the reduction of insulin resistance and the consequent beneficial effects on reproductive function, but it also has long-term beneficial effects on health[21,22]. Many studies have been more successful in reducing body weight, using higher dosage of metformin[23]. Metformin leads to a reduction in body weight and glucose production by the liver, due to its anoreptic effect and its interaction with AMP-activated protein kinase (AMPK)[15,24,25]. AMPK regulates several intracellular systems, including the glucose uptake, the b-oxidation of fatty acids, and the biogenesis of glucose transporter 4 and mitochondria. Since it causes a switch from synthesis of cellular nutrient stores to their breakdown, AMPK might be involved in metformin action[26].

Body weight reduction was observed when the relationship between metformin dose and effects on body weight was assessed in a group of PCOS women stratified by weight. In the obese group, weight loss following metformin treatment appeared to be dose dependent. In fact, at end of the treatment, the body weight reduction was higher in the group treated with 2550 mg compared to the group taking 1500 mg of metformin[27].

There is no general agreement on the use of metformin as a dose dependent treatment. Indeed, patients with PCOS have been reported

to benefit from metformin therapy regardless of dosage[28]. However, in our study, we reported that PCOS women with higher BMI showed a significant amelioration of the fasting glucose, insulin, and hyperandrogenism only if a higher dosage of metformin was used. It has also been shown that treatment with 1500 mg of metformin was more effective in improving baseline values of insulin, total and free testosterone and androstenedione in non-obese patients compared to obese patients, in whom this treatment induced only a decrease in free testosterone values[29]. The androgenic parameters ameliorate not only by the effect of metformin-mediated insulin resistance reduction. In theca cells, metformin demonstrates a direct dose-dependent inhibitory effect on androgen production by targeting 3β-hydroxysteroididosidrogenasetype 2 (HSD3B2) and 17α-hydroxylase/17.20 lyase (CYP17A1)[30].

Beneficial effects of other insulin-sensitizers are also studied in polycystic ovary syndrome. Thiazolidinediones (TZDs) can reduce insulin resistance in PCOS women mainly acting on the adipocytes and the muscle cells. In addition to improving insulin resistance and compensatory hyperinsulinemia, administration of TZDs may increase the ovulation rate and pregnancy and cause a substantial decrease in circulating androgen concentrations. Overall, in some conditions these drugs may be considered as protective agents even if, due to their side effects, their use should come as a secondary option in the treatment of the metabolic problems linked to PCOS[30]. Inositols have also been investigated for the treatment of PCOS: they can regulate menstrual cycles and improved metabolic parameters. However, Inositol (in any form) should currently be considered an experimental therapy in PCOS, with emerging evidence on efficacy highlighting the need for further research[31].

When surrogate indexes, like HOMA, were used for the identification of insulin resistant PCOS women, Tosi et al[32] found a high PPV (90-96%), but a low NPV (36-45%) if compared to euglycemic-hyperinsulinemic clamp values. We support Tosi conclusions indicating that surrogate indexes work better in women with obesity and/or metabolic syndrome; in fact, our patient sample consisted entirely of overweight or obese women. The evaluation of IR with HOMA index is not the gold standard for determining IR, but this method can easily be repeated without causing discomfort to patients, and moreover it is reliable in identifying IR.

Conclusions

Our results demonstrate a correlation between metformin dose, BMI and hyperandrogenism in overweight PCOS women, indicating that the higher is BMI index, the higher should be the dose of metformin needed to achieve an effective reduction in insulin resistance in these patients.

Further studies are needed to better evaluate metformin doses and BMI in PCOS patients.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

All authors have made a substantial contribution to research design, to the acquisition, analysis and interpretation of data. All authors reviewed/edited the manuscript and given approval of the submitted and final versions.

References

1) Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. J Clin Endocrinol Metab 2004; 89: 2745-2749.
2) Escobar-Morreale HF. Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. Nat Rev Endocrinol 2018;14: 270-284.
3) Stein IF, Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. Am J Obstet Gynecol 1935; 29: 181-191.
4) Zhao X, Zhong J, Mo Y, Chen X, Chen Y, Yang D. Association of biochemical hyperandrogenism with type 2 diabetes and obesity in Chinese women with polycystic ovary syndrome. Int J Gynaecol Obstet. 2010; 108: 148-51.
5) Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocr Rev 1997; 18: 774-800.

6) Bremer AA, Miller WL. The serine phosphorylation hypothesis of polycystic ovary syndrome: a unifying mechanism of hyperandrogenemia and insulin resistance. Fertil Steril 2008; 89: 1039-1048.

7) Unger JW, Livingston JN, Moss AM. Insulin receptors in the central nervous system: localization, signalling mechanisms and functional aspects. Prog Neurobiol 1991; 36: 343-362.

8) Plymate SR, Matej LA, Jones RE, Friedl KE. Inhibition of sex hormone-binding globulin production in the human hepatoma (Hep G2) cell line by insulin and prolactin. J Clin Endocr Metab 1988; 67: 460-464.

9) Poretsky L, Chandrasekher YA, Bai C, Liu HC, Rosenwaks Z, Giudice L. Insulin receptor mediates inhibitory effect of insulin, but not of insulin-like growth factor (IGF)-I, on IGF binding protein 1 (IGFBP-1) production in human granulosa cells. J Clin Endocrinol Metab 1996; 81: 493-496.

10) Yong EL, Baird DT, Yates R, Reichert LE Jr, Hillier SG. Hormonal regulation of the growth and steroidogenic function of human granulosa cells. J Clin Endocrinol Metab 1992; 74: 842-849.

11) Baptiste CG, Battista MC, Trottier A, Baillargeon JP. Insulin and hyperandrogenism in women with polycystic ovary syndrome. J Steroid Biochem Mol Biol 2010; 122: 42-52.

12) De Leo V, La Marca A, Petraglia F. Insulin-lowering agents in the management of polycystic ovary syndrome. Endocr Rev 2003; 24: 633-667.

13) Pasquali R, Gambineri A, Biscotti D, Vicennati V, Gagliardi L, Colitta D, Fiorini S, Cognigni GE, Filicori M, Morselli-Labate AM. Effect of long-term treatment with metformin added to hypocaloric diet on body composition, fat distribution, and androgen and insulin levels in abdominally obese women with and without the polycystic ovary syndrome. J Clin Endocrinol Metab 2000;85: 2767-2774.

14) Aghili R, Malek M, Valojerdi AE, Banazadeh Z, Najafi L, Khamseh ME. Body composition in adults with newly diagnosed type 2 diabetes: effects of metformin. J Diabetes Metab Disord 2014; 13: 88.

15) Zhou J, Massey S, Story D, Li L. Metformin: an old drug with new applications. Int J Mol Sci. 2018; 19: 2863.

16) Dumitrescu R, Mehedintu C, Briceag I, PurcĐrea VL, Hudita D. Metformin-clinical pharmacology in PCOS. J Med Life 2015; 8: 187-192.

17) Bailey CJ,Turner RC. Metformin. N Engl J Med 1996; 334: 574-579.

18) Mathur R, Alexander CJ,Yano J, Trivax B, Azziz R. Use of metformin in polycystic ovary syndrome. Am J Obstet Gynecol 2008; 199: 596-609.

19) Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. Gastroenterology 2007; 132: 2169-2180.

20) Patel S. Polycystic ovary syndrome (PCOS), an inflammatory, systemic, lifestyle endocrinopathy. J Steroid Biochem Mol Biol 2018; 182: 27-36.

21) Morin-Papunen L, Rautio K, Ruokonen A, Hedberg P, Puukka M, Tapanainen JS. Metformin reduces serum C-reactive proteinlevels in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2003; 88: 4649-4654.

22) Wang YW, He SJ, Feng X, Cheng J, Luo YT, Tian L, Huang Q. Metformin: a review of its potential indications. Drug Des Devel Ther. 2017; 11: 2421-2429.

23) Nieuwenhuis-Ruifrok AE, Kuchenbecker WK, Hoek A, Middleton P, Norman RJ. Insulin sensitizing drugs for weight loss in women of reproductive age who are overweight or obese: systematic review and meta-analysis. Hum Reprod Update 2009; 15: 57-68.

24) Chau-Van C,Gamba M, Salvi R, Gaillard RC, Pralong FP. Metformin inhibits adenosine 5'-monophosphate-activated kinase activation and prevents increases in neuropeptide Y expression in cultured hypothalamic neurons. Endocrinology 2007; 148: 507-511.

25) Tsilchorozidou T, Batterham RL, Conway GS. Metformin increases fasting plasma peptide tyrosine tyrosine (PYY) in women with polycystic ovarian syndrome (PCOS). Clin Endocrinol (Oxf) 2008; 69: 936-942.

26) Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia 2017; 60: 1577-1585.

27) Harborne LR, Sattar N, Norman JE, Fleming R. Metformin and weight loss in obese women with polycystic ovary syndrome: comparison of doses. J Clin Endocrinol Metab 2005; 90: 4593-4598.

28) Fulghesu AM, Romualdi D, Di Florio C, Sanna S, Tagliaferri V, Gambineri A, Tomassoni F, Minerba L, Pasquali R, Lanzone A. Is there a dose-response relationship of metformin treatment in patients with polycystic ovari syndrome? Result from a multicentric study. Hum Reprod 2012; 27: 3057-3066.

29) Maciel GAR, Junior JMS, Alves da Motta EL, Abi Haidar M, de Lima GR, Baracat EC. Nonobese women with polycystic ovary syndrome respond better than obese women to treatment with metformin. Fertil Steril 2004; 81: 355-360.

30) Banaszewska B, Pawelczyk L, Spaczynski R. Current and future aspects of several adjunctive treatment strategies in polycystic ovary syndrome. Reprod Biol 2019; 19: 309-315.

31) Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, Piltonen T, Norman RJ7; International PCOS Network. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Fertil Steril 2018; 110: 364-379.

32) Tosi F, Bonora E, Moghetti P. Insulin resistance in a large cohort of women with polycystic ovary syndrome: a comparison between euglycaemic-hyperinsulinaemic clamp and surrogate indexes. Hum Reprod 2017; 32: 2515-2521.