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THE EFFECT OF MYOINOSITOL AND METFORMIN ON CARDIOVASCULAR RISK FACTORS IN WOMEN WITH POLYCYSTIC OVARY SYNDROME: A RANDOMIZED CONTROLLED TRIAL

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Abstract

Context. Cardiovascular risk is increased in women with polycystic ovary syndrome (PCOS). Do insulin sensitizing agents such as metformin (MET) and myoinositol (MI) ameliorate biomarkers of cardiovascular risk?

Objective. To compare the effects of MET and MI on blood pressure, lipid profile and high sensitive C-reactive protein (hs-CRP) in women with PCOS in respect to their body mass index (BMI).

Design. Open label, parallel randomized, single center study.

Subjects and Methods. Sixty six women with PCOS (33 normal-weight and 33 overweight/obese) were randomized to either MI (4 g/day) or MET (1500 mg/day) for a period of 6 months. Serum concentration of hormones, lipid profile, oxidized LDL (ox-LDL), hs-CRP, blood pressure measurement and clinical assessment of BMI, waist circumference (WC) and Ferriman Gallwey score (FG score) were performed before and after treatment.

Results. Thirty patients in each group completed the trial. Compared with MET, MI significantly decreased diastolic blood pressure (DBP) (p=0.036) and significantly increased serum hs-CRP (p=0.043). No differences between groups in total cholesterol (TC), HDL-cholesterol, LDL-cholesterol, ox-LDL and triglycerides were reported after 6 months. Treatment with MI reduced BMI (p=0.037), WC (p=0.005), DBP (p=0.021) and TC (p=0.008). During MET treatment a significant decrease in BMI (p=0.005), WC (p=0.004), FG score (p=0.001), testosterone (p=0.013) and free androgen index (FAI) (p=0.006) was observed.

Conclusions. Our study showed an advantage of MI in reduction of DBP and TC thus predicting favorable metabolic and cardiovascular outcomes in PCOS women. MET more effectively decrease indices of hyperandrogenism.

Keywords: polycystic ovary syndrome, metformin, myoinositol, cardiovascular risk.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common form of metabolic diseases affecting 6–10% of women of reproductive age (1). The pathogenesis of PCOS includes insulin resistance (IR) and low grade inflammation (2). IR places these women at an increased risk to develop hypertension, dyslipidemia, type 2 diabetes mellitus (T2DM), and cardiovascular disease (CVD) (3).

Current epidemiological data suggest increased prevalence of classic and non-classic cardiovascular (CV) risk factors in women with different phenotypes of PCOS (4).

Several risk factors modify overall metabolic outcomes in PCOS. Obesity acts synergistically with PCOS to impair insulin sensitivity. Moreover, emerging data suggest that adipose tissue dysfunction induces chronic low-grade inflammation that may be involved in the development of metabolic and reproductive dysfunctions of PCOS (5).

In addition to obesity, dyslipidemia and hypertension are more prevalent compared to the general population. The prevalence of at least one feature of metabolic syndrome (MS) has been found to be present in more than 50% of adult PCOS women, commonly constituted by lipid alterations, such as HDL-cholesterol (HDL) decrease and LDL-cholesterol (LDL) and total cholesterol (TC) increase (6). Arterial

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hypertension represents an uncommon and inconsistent finding in young women with PCOS, but its prevalence increases to 40% in the perimenopausal period (7). While some authors showed that a higher prevalence of hypertension was related to obesity and not to PCOS per se (7), others demonstrated that a high value of the FAI even in young women with PCOS was associated with hypertension independent of insulin resistance, body mass index (BMI) and dyslipidemia (8). Therefore, PCOS women are at risk for severe metabolic and CVD.

Due to all the possible implications that PCOS can have on medium and long term, great attention has been focused on the management of PCOS women as patients at risk of severe cardiovascular and metabolic diseases.

Metformin (MET) is known to decrease IR and increase insulin-mediated glucose disposal in PCOS women (9). MET treatment in a long-term could delay or prevent deterioration of glucose tolerance including type 2 diabetes in PCOS women (10). Other beneficial effects of metformin therapy in PCOS include reduction of C-reactive protein (CRP) (11), intima-mediated thickness (IMT), an enhancement of flow mediated dilation (FMD) (12).

Inositol phosphoglycans (IPGs) are potentially important putative intracellular mediators of insulin action (13). Insulin-like actions of both biologically derived and chemically synthesized myoinositol (MI) and D-chiroinositol (DCI) containing IPGs have been clearly documented (14).

A significant improvement in metabolic parameters of insulin sensitivity and to a lesser extent of testosterone levels was seen after MI supplementation (15). It was recently shown that MI therapy significantly improved insulin sensitivity and reduced serum levels of TC, LDL, and homocysteine in patients over 30 years of age, suggesting consequent decrease in CVD risk (16).

In the present study, we aim to evaluate whether therapy with insulin sensitizers MI and MET is able to improve the lipid profile, blood pressure and high sensitive C-reactive protein (hsCRP) in PCOS subjects and consequently to reduce CV risk.

MATERIAL AND METHODS

Study participants

A 6-month, randomized, open-label study was performed. The study protocol was consistent with the principles of the Declaration of Helsinki and has been approved by Ethics Committee of University Clinical Center of the Republic of Srpska (01-9-742.2/16). All participants gave written informed consent. Eighty seven eligible PCOS women (18-40 years of age) were screened, and 66 randomized [33 normal weight and 33 overweight/obese] for the study. Subjects were recruited from the outpatient endocrinology clinic, because of irregular menstrual cycles, infertility problem, hirsutism, or acne. PCOS was diagnosed according to the revised Rotterdam Consensus Conference criteria (17). Hirsutism was defined as modified Ferriman-Gallwey (F-G) score ≥8 (18). Biochemical hyperandrogenemia was defined as total testosterone >2.0 nmol/L and/or FAI ≥6 (19).

Exclusion criteria were clinical or laboratory evidence of confounding systemic diseases (chronic renal or hepatic failure and chronic inflammatory diseases), diabetes and cardiovascular disorders. Women having thyroid dysfunction, hyperprolactinemia, Cushing syndrome, nonclassical congenital adrenal hyperplasia (NCAH), and androgen-secreting tumors were excluded by appropriate tests. Women having history of alcohol or drug abuse, and medical history of breast and uterine cancer were excluded from the study. None of the subjects received oral contraceptives, glucocorticoids, antiandrogens, and other hormonal agents within the three months prior to the initiation of the study.

Study design

All PCOS women entered into the prospective, open label, randomized, comparative single center study. In order to evaluate the influence of BMI, participants were randomized to treatment groups in a 1:1 ratio using stratification by BMI (≤25 kg/m^2 or >25 kg/m^2). Each study group had 33 patients to receive either of the following two treatments: myoinositol (MI) 2g plus 200 mcg folic acid twice daily and metformin (MET) 500 mg thrice daily for 6 months. At the end of a six-month investigation period, we repeated all the clinical and biochemical evaluations. No changes of life style or diet were required.

Anthropometric and biochemical characteristics of the subjects

The study protocol included two visits (baseline and 6 months). BMI and waist circumference (WC) were determined at the first visit and at the 6-month endpoint. BMI (kg/m^2) was calculated as the ratio of body weight (kg) and body height (m) squared. WC was measured at the midpoint between the lower

border of the rib cage and the iliac crest by using a flexible centimeter tape. Upon enrollment, all patients also underwent transvaginal ultrasonography and the grade of hirsutism was assessed using the F-G score. Systolic blood pressure (SBP) (mmHg) and diastolic blood pressure (DBP) (mmHg) was measured using a mercury sphygmomanometer on the left arm in a sitting position by the same examiner. The average of two measurements was calculated. According to the criteria used in all definitions for metabolic syndrome (20), hypertension was defined as SBP ≥130mmHg and/or DBP ≥85mmHg.

Blood samples were collected after 12 hours of fasting in the follicular phase of the cycle (between the 3rd and the 7th days) or randomly in a case of amenorrhea. The following analyses were determined: TC, HDL, LDL, triglycerides (TG), ox-LDL, hsCRP, glucose, insulin, total testosterone, sex hormone-binding globulin (SHBG), and dehydroepiandrosterone sulfate (DHEAS). The samples for hormone analysis were stored at -80˚C until measurement. For estimation of IR the following indexes were used: HOMA-IR (homeostasis model assessment) index calculated using formula: [fasting insulin (mU/L) × fasting glucose (mmol/L)]/22.5 (21). All the biochemical analyses and calculations were performed at baseline and after 24 weeks of treatment.

Biochemical and hormonal assays

The samples for hormone analysis were stored on -80˚C until measurement. Insulin, testosterone, SHBG, DHEA-S and AMH were measured by an electrochemiluminescence immunoassay method (ECLIA, Roche Diagnostics, Mannheim, Germany; inter- and intra-assay coefficients of variability (CVs) were 0.9% and 3.7%, 2.1% and 2.5%, 1.3% and 2.1%, 2.6% and 2.7%, 1.6% and 1.8%, respectively). CRP concentrations were determined by an ultrasensitive immunoturbidimetric assay (CRP Latex HS; Roche Diagnostics, Mannheim, Germany) with a sensitivity of 0.03 mg/liter and intra- and interassay coefficients of variation of 1.3 and 5.7%, respectively. Plasma glucose, TC, LDL , HDL and TG were measured immediately after sampling with enzymatic calorimetric tests (Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instructions. Ox-LDL levels were determined by enzyme-linked immunosorbent assay (ELISA) (Immundiagnostik AG, Bensheim, Germany). The intra- and interassay coefficients of variability were 5.7% and 11.8%, respectively.

Outcome measures

Primary outcome was change from baseline of androgen levels and at least one of the metabolic parameters: blood pressure, TC, LDL, HDL, ox-LDL, TG and hs-CRP. Secondary outcome was the improvement of BMI and WC.

Statistical analysis

To estimate the required sample size, we used a randomized clinical trial sample size formula where type one (α) and type two errors (β) were 0.05 and 0.20 (power=80%), respectively. The sample size was calculated according to suspected change of serum testosterone as the key variable. Based on a previous study, we used a standard deviation (SD) of 1.0 nmol/L and a difference in mean (d) of 0.8 nmol/L (22). The calculation indicated that 25 subjects were needed in each group. Assuming a dropout of 10-20% subjects in each group, the final sample size was determined to be 33 subjects in each group.

Statistical analysis was performed using the Statistical Package for Social Science (version 21.0, SPSS Inc., Chicago, IL). T-tests and analysis of variance were used for comparison between groups. Two-way analyses of variance (ANOVA) with repeated measures were performed to assess the effects of treatment and/or time. Paired t-test was used to compare parameters before and after treatment in the same patients, and nonparametric Wilcoxson and McNemar test, as appropriate. Results are presented as mean ± standard deviation (SD). $p<0.05$ was considered to be statistically significant.

RESULTS

Effects of MET and MI on clinical and biochemical variables in women with PCOS

At the end of the 6 months treatment period, 3 participants were excluded from MET group due to intolerable gastrointestinal side effects, and 3 from MI group (2 for personal reasons, 1 for pregnancy). Finally, 30 participants from each group completed the trial (Fig. 1).

The reduction in the mean BMI and WC was statistically significant in both groups, but on comparing the two groups, the difference was not significant (Table 1). Significant improvement in FG score was found in metformin treated group. On comparing the two groups, no treatment difference in FG score was found (Table 1). Both treatments led to reduction of testosterone level and FAI, but significant only in MET

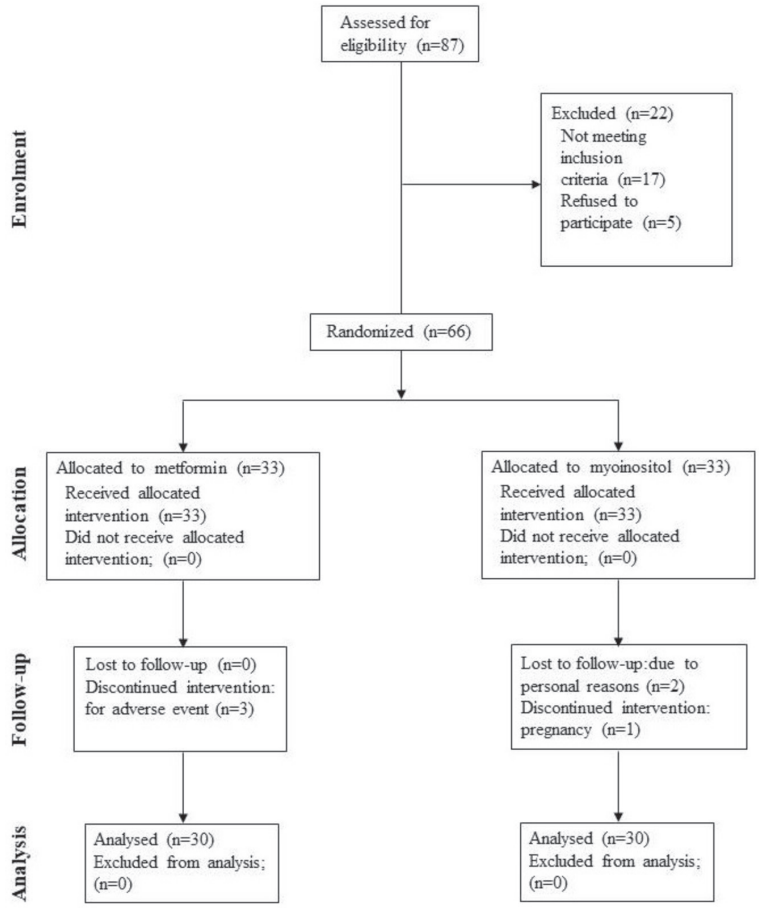

Figure 1. Recruitment, follow-up and drop outs over the course of the study.

group. Compared with MI, MET significantly decreased testosterone, with a tendency to FAI reduction (Table 1).

After 6-months of treatment with metformin no significant change was observed in the lipid parameters. TC was significantly reduced in MI treatment group. No difference in lipid levels (TC, HDL, LDL, TG, ox-LDL) between groups was reported at 6 months (Table 1).

There was a statistically significant two-way interaction between treatment and time, for DBP, F(1, 29)=4.841, p=0.036; Systolic blood pressure (F=3.487, p=0.072) had tendency for significance (Table 1).

Diastolic blood pressure was 4.5 mmHg higher (95% CI, 1.382 to 7.618) at the beginning in the MI arm as opposed to the MET arm, a difference that was statistically significant, F(1, 29)=8.175, p=0.006. DBP was 2.467 mmHg higher after 6 months (95% CI, -0.409 to 5.342) in the MI arm as opposed to the MET arm, a difference that was not statistically significant, F(1, 29)=3.079, p=0.090. DBP was significantly different over time in the MI arm, F(1, 29)=4.241, p=0.049. In the MET arm a statistical significance was not observed, F(1, 29)=0.450, p=0.508. SBP was not significantly modified by the 2 treatments.

There was a statistically significant two-way interaction between treatment and time, for hsCRP, F(1, 29)=4.466, p=0.043. Mean CRP concentration was statistically significantly different over time in the MI arm, F(1, 29)=5.609, p=0.025. In the MET arm a statistical significance was not observed, F(1, 29)=0.596, p=0.446.

DISCUSSION

Few studies have directly compared the outcome of therapy with MET and MI in PCOS women, and in particular the efficacy on the cardiovascular disease. The present study shows that supplementation with MI and folic acid positively affects metabolic parameters and the indices of cardiovascular disease in women with PCOS.

Inositol supplementation resulted in reduction of diastolic blood pressure, similar to other studies (23). In animals model MI exerts vascular actions by generating neurogenic and endothelium dependent relaxation, preserves NO signaling, prevents and reverses endothelial dysfunction (24). In addition, MI is

Table 1. Clinical and hormonal features at baseline and after 6 months of myoinositol and metformin treatment

	MET (n=30)			MI (n=30)			MET *vs*. MI Baseline	MET vs MI After 6 months	MET *vs*. MI#
	Baseline	After 6 months	p	Baseline	After 6 months	p	p	p	p
WC (cm)	82.27 (±13.35)	80.07 (±13.07)	**0.004**	84.37 (±14.84)	81.87 (±13.56)	**0.005**	0.567	0.603	0.917
BMI	25.76 (±5.64)	24.70 (±5.31)	**0.005**	26.49 (±5.36)	25.69 (±5.15)	**0.037**	0.608	0.612	0.777
FG score	14.53 (±5.31)	13.47 (±4.81)	**0.001**	16.70 (±6.27)	16.23 (±6.59)	0.288	0.154	0.069	0.262
hsCRP (mg/L)	2.41 (±2.35)	2.12 (±1.96)	0.446	1.93 (±1.98)	2.56 (±2.35)	**0.025**	0.399	0.434	**0.043**
Ox-LDL (ng/mL)	110.50 (±54.97)	105.73 (51.65)	0.181	102.27 (±44.39)	100.13 (±41.71)	0.670	0.526	0.646	0.661
SDP (mmHg)	118.93 (±9.75)	119.00 (±9.21)	0.943	122.53 (±12.08)	120.63 (±11.72)	0.326	0.209	0.551	0.072
DBP (mmHg)	78.20 (±6.71)	78.63 (±5.45)	0.508	82.70 (±10.13)	81.10 (±8.28)	**0.021**	**0.047**	0.178	**0.036**
TC (mmol/L)	4.56 (± 0.89)	4.36 (±0.69)	0.116	4.88 (±0.94)	4.59 (±0.68)	**0.008**	0.184	0.201	0.633
LDL (mmol/L)	2.76 (±0.77)	2.66 (±0.74)	0.309	3.08 (±0.82)	2.99 (±0.75)	0.247	0.123	0.092	0.948
HDL (mmol/L)	1.46 (±0.35)	1.48 (±0.34)	0.581	1.40 (±0.31)	1.38 (±0.31)	0.579	0.500	0.221	0.446
TG (mmol/L)	1.21 (±0.73)	1.09 (±0.54)	0.280	1.18 (±0.53)	1.08 (±0.57)	0.157	0.835	0.928	0.866
HOMA-IR	2.52 (±0.90)	2.43 (±1.26)	0.632	2.82 (±1.26)	2.50 (±1.45)	0.127	0.293	0.858	0.709
Testosterone (ng/mL)	0.53 (±0.17)	0.45 (±0.13)	**0.013**	0.69 (±0.23)	0.62 (±0.27)	0.115	**0.004**	**0.004**	**0.043**
SHBG (nmol/L)	45.07 (±27.64)	48.42 (±24.79)	0.108	44.97 (±22.70)	49.82 (±29.21)	0.067	0.987	0.842	0.663
FAI	5.78 (±4.54)	4.13 (±2.78)	**0.006**	6.69 (±4.96)	6.50 (±7.48)	0.786	0.463	0.113	0.053
DHEAS (mcg/dL)	369.85 (±126.21)	375.11 (±111.24)	0.644	350.96 (±141.62)	374.57 (±157.39)	0.110	0.588	0.988	0.900

ALL values are means± SDs.

#P values represent the time × group interaction (computed by analysis of the two-way repeated measures ANOVA).

BMI: body mass index; WC: waist circumference; F-G score: Ferriman-Gallwey score; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; HDL-C:high-density lipoprotein; LDL-C: low-density lipoprotein; TG: triglycerides; hsCRP: high sensitive C reactive protein; Ox-LDL:oxidised LDL; HOMA-IR: homeostasis model assessment–insulin resistance index; SHBG: sex hormone-binding globulin; FAI: free androgen index; DHEAS: dehydroepiandrosterone sulfate;

a substrate for the biosynthesis of phosphatidylinositol, a membrane molecule that is the precursor of phosphatidylinositol- 4,5-bisphosphate (PIP2), a key modulator of many ion channels (25). Consequently, MI transporters may have a profound effect on ion channels function in the vasculature, in particular K+ channels, which is a key mechanism modulating arterial contractility (26). According to national register-based study that investigated the risk of developing CVD in women with PCOS, high baseline BP was an important predictor of CVD development (2). The positive effect of MI therapy on DBP in our study suggests that the metabolic risk may be modified by MI treatment.

Cardiovascular risk in PCOS was closely associated with BMI (2). Both drugs showed a similar positive effect on BMI and WC, a marker of visceral adiposity. This is in line with previous studies that compared the effect of both drugs on BMI and body composition (27).

Data on the comparison of MI and MET on inflammatory cytokines in PCOS women are limited. MET inhibits nuclear factor kappa B (NF-κB) and thus reduces the inflammation. MI targets at the disease per se and not on the inflammation produced by the disease. We obtained a marginally statistical treatment difference in mean hsCRP. The mean hsCRP level after MI treatment increased, which can be atributed to a higher proportion of smokers in MI group (28). MET treatment did not change hsCRP levels in population of normal-weight and overweight/obese PCOS women. This finding is in agreement with the results of another 6-months MET treatment study (29). The lack of treatment effect on inflammatory marker hsCRP could be due to a lower average BMI in our study group, and unchanged HOMA-IR during treatment since Mohlig *et al.* showed a positive relationship between the obesity, insulin resistance and inflammatory markers (30). We believe that the obtained difference in mean hsCRP is not clinicaly relevant due to a relatively small group of investigated subjects. Our observation on the effect of insulin sensitizing agents on hsCRP need to be proved in the following studies on a larger group of PCOS women.

In women with PCOS, MI therapy is able to influence the metabolism leading to an improved lipid profile. In our study, MI had a significant beneficial effect on total cholesterol, as was shown before (31). No significant difference was found in the LDL, HDL and TG levels in MI group, and some studies demonstrated that the combination of MI and DCI in their physiological plasma ratio 40:1 is more effective than MI alone also in reducing LDL, TG and HOMA-

index (32). Nevertheless, no significant differences were found between the MET and the MI groups. MET treatment showed a decrease in BMI values, whereas no changes in TG, TC, LDL and HDL levels were detected. These results are consistent with some other studies (33) and opposite to others (34). The existing inconsistencies could be due to the difference in selecting women with PCOS. In our study, patients were selected based on the Rotterdam criteria, which means the inclusion of patients without metabolic disorders or with minor abnormalities. The fact that MET had no effect on lipids in our study can be related to the lower average BMI and lower fasting insulin levels in our participants, since studies have shown that obese patients and patients with hyperinsulinemia respond better to metformin (35).

PCOS is associated with oxidative stress (19). Oxidized LDL is a marker of oxidative stress-related inflammation and the risk of development of CVD. Women with PCOS have elevated ox-LDL (19, 36). The similar level of ox-LDL between PCOS and controls since obesity is directly associated with oxidative stress and contributes to the increased oxidative stress in PCOS (37).

The novelty of our study is the assessment of serum ox-LDL in patients with PCOS treated with MET *vs*. MI. The treatment with insulin sensitizers did not reduce ox-LDL levels in both study groups. Previously, beneficial role of MET in reducing serum ox-LDL levels was shown in patients with diabetes explained with its antioxidative properties and effect on the immune system (38). In another study MET failed to inhibit the oxidation of LDL, similarly to our study (39).

The treatment with MI is effective in reducing oxidative abnormalities in PCOS patients by improving IR (13). Serum ox-LDL was unaffected by 6-month MI treatment in our study. We believe this is the first study addressing the effect of inositol administration on serum ox-LDL in women with PCOS.

Our study has a few limitations. Firstly, we did not examine the compliance to myoinositol and metformin intake by biochemical variables. The other limitation of this RCT is that the treatment could not be blinded, due to the different administration of the drugs.

In conclusion, our study showed that MET and MI are effective in improving metabolic and anthropometric parameters such as BMI and WC in PCOS women. This study confirms the advantage of MET treatment on biochemical hyperandrogenism, that was probably not sufficient to make difference in clinical signs of hyperandrogenism as well. Overall, MI compared to MET treatment had favorable effects on DBP in PCOS patients. The effect on blood pressure reduction, observed in our study, suggested the potential effect of MI treatment in reduction of future CVD in PCOS. Further randomized and properly sized studies are needed in order to confirm our data.

Conflict of interest

The authors declare that they have no conflict of interest.

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