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ORIGINAL ARTICLE

The effect of metformin and myoinositol on metabolic outcomes in women with polycystic ovary syndrome: role of body mass and adiponectin in a randomized controlled trial

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Abstract

Purpose To compare the effects of insulin sensitizers metformin (MET) and myo-inositol (MI) on adiponectin levels and metabolic characteristics in women with polycystic ovary syndrome (PCOS) with respect to their body mass index (BMI).

Methods In this open label, parallel randomized clinical trial, 66 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 adiponectin, hormonal and metabolic laboratory outcomes and clinical assessment of BMI, body composition and Ferriman–Gallwey score (FG score) were evaluated before and after treatment.

Results After the 6-month intervention, comparison between MET and MI in time to treatment analysis showed no significant differences between the two treatments for all analyzed parameters. Only borderline significantly lower AUC glucose was found in the MET group in comparison to the MI group ($p=0.071$). The main effect of treatment was shown for glucose concentration at 120 min OGTT ($p=0.032$) and testosterone ($p=0.002$). The main effect of time was shown for body mass ($p=0.004$), waist circumference ($p<0.001$), BMI ($p=0.003$), body fat mass ($p=0.001$), adiponectin ($p=0.020$), fasting glucose ($p=0.001$), testosterone ($p=0.015$), SHBG ($p=0.013$), 17OH progesterone ($p=0.008$), LH ($p=0.004$) and estradiol ($p=0.014$).

Conclusion Our study showed similar effects of MET and MI on BMI, body composition, hormonal profile, metabolism of glucose and insulin, and adiponectin level. The two insulin sensitizers, MET and MI, were useful in reducing BMI and improving body composition without significant differences between the two treatments in PCOS women.

Trial registration ISRCTN13199265. Trial registration date: 14.04.2021. (ISRCTN Registry), retrospectively registered.

Keywords Polycystic ovary syndrome · Body mass · Metformin · Myoinositol · Adiponectin

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Introduction

Polycystic ovary syndrome (PCOS) is the most prevalent endocrine disease affecting 6–20% of women during the reproductive period [1]. Obesity is present among half of PCOS women and is characterized by central fat distribution, which is a clinical predictor of metabolic abnormalities detected from the early stages of the syndrome [1]. Emerging evidence suggests that obesity may induce adipocytes expansion, as observed in women with PCOS [2]. Also, higher percentage of body fat mass and androgen excess have been associated with IR [3]. Recent studies investigating phenotypes of PCOS confirmed that even milder forms of the syndrome are characterized by IR [4].

Adiponectin is an adipose tissue-specific protein, which decreases with obesity [5]. Decreased plasma adiponectin and upregulation of adiponectin receptors in adipose tissue occur with the development of IR in PCOS [6]. The specific role of adiponectin in PCOS is not clear: it may have an insulin-sensitizing activity, or it could be regulated by insulin and serve as a marker for IR [7]. 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 [8].

Insulin sensitizers are commonly used in the management of IR in women with PCOS [1]. Metformin (MET) has been extensively investigated and showed its efficacy in reducing insulin levels, improving insulin sensitivity, menstrual irregularities and hyperandrogenemia in PCOS women [9, 10]. Controversial results have been reported on the ability of MET to influence adiponectin synthesis and secretion in adipose tissue [11]. Recent reports indicated that MET treatment was associated with a significant increase in adiponectin level [12]. This effect of metformin on adipose tissue represents an additional mechanism through which this compound may improve the metabolic and hormonal disturbances in patients with PCOS. AMPK activation is the central mechanism whereby adiponectin regulates insulin sensitivity, glucose uptake and inflammation [11]. Hypoadiponectinemia reflects a higher risk for metabolic dysfunction in the phenotypic groups classified as classic PCOS, as a consequence of higher insulin resistance, and can serve as a surrogate marker for the assessment of insulin resistance [13, 14].

Recent studies have reported that alteration in the insulin pathway could be due to defects in insulin signaling, namely inositol phosphoglycans (IPGs) pathway [15]. It was shown that women with PCOS are characterized by IPGs deficiency or their altered metabolism could enhance insulin resistance state [16]. Myoinositol (MI) is an insulin-sensitizing agent. However, the 6-month MI treatment was not associated with an increase in plasma adiponectin level in postmenopausal women with metabolic syndrome [17]. The role of MI in the modulation of adiponectin expression and secretion in women with PCOS has not been established so far. In relation to these findings, we aimed at compare the effects of MET to those of MI therapy, and establish whether administration of insulin sensitizer agents such as MET and MI can upregulate serum adiponectin levels in PCOS with respect to their body mass index (BMI).

Materials and methods

Study participants

The study was performed in the University Clinical Centre Banja Luka between November 2017 and May 2019. Subjects were recruited from the outpatient endocrinology clinic which they visited due to irregular menstrual cycles, infertility problem, hirsutism or acne. Eighty-seven eligible PCOS women between 18 and 40 years of age were screened, and 66 randomized [33 normal weight and 33 overweight/obese] were included in the study. PCOS was diagnosed according to the revised Rotterdam Consensus Conference criteria [18].

Hirsutism was defined as Ferriman-Gallwey score (FG score) score ≥ 8 [19]. Biochemical hyperandrogenemia was defined as total testosterone > 2.0 nmol/L and/or free androgen index (FAI) ≥ 6 [20].

Women suffering from thyroid dysfunction, hyperprolactinemia, Cushing syndrome, non-classical congenital adrenal hyperplasia (NCAH), and androgen-secreting tumors were excluded. Women suffering from diabetes, hepatic, renal and cardiovascular disorders, or having a history of alcohol or drug abuse or medical history of breast or uterine cancer were excluded from the study. None of the subjects had received oral contraceptives, glucocorticoids, antiandrogens, and other hormonal agents within the 3 months prior to the initiation of the study.

Study design

To evaluate the influence of BMI, PCOS women had been recruited to form two subgroups: non-obese and overweight/obese. All subjects were classified into one of the two groups:

- Group 1—non-obese PCOS (BMI ≤ 25 kg/m^2).
- Group 2—obese or overweight PCOS (BMI > 25 kg/m^2).

All PCOS women entered into this prospective, open label, randomized, comparative single center study. Participants were randomly allocated into treatment groups in a 1:1 ratio using stratification by BMI (≤ 25 kg/m^2 or > 25 kg/m^2).

Allocation was achieved using random permuted blocks of size three. Allocation of the subjects was performed in the endocrinology clinic by a pharmacist, who was blinded to the randomization schedule. Since commercially available MI and MET were used, there was no blinding after randomization; consequently, investigators and subjects could identify the actual treatment. Patients allocated to MI (17 normal weight and 16 overweight/obese) received 2 g MI plus 200 mcg folic acid twice daily and patients allocated to MET (16 normal weight and 17 overweight/obese) received 500 mg thrice daily for 6 months. No changes in lifestyle or diet were recommended.

At enrollment, a complete history was obtained and physical examination and biochemical evaluation were performed. At the end of a 6-month investigation period, clinical and biochemical evaluation was repeated.

In all subjects, BMI and waist circumference (WC) were determined at the first visit and at the 6-month end point. 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. Body composition was estimated by using bioelectrical impedance analysis (InBody 370, Biospace Co. Ltd., Seoul, South Korea). Upon enrollment, all patients also underwent transvaginal ultrasonography and hirsutism was evaluated according to the FG score.

Baseline blood samples were collected after 12 h of fasting during the early follicular phase (between the 3rd and the 7th day) of the regular menstrual cycle, or at any time in case of severe oligo- or amenorrhea. Biochemical measurements included determination of glucose (mmol/L), insulin (mIU/L), follicle-stimulating hormone (FSH, IU/L), luteinizing hormone (LH, IU/L), estradiol (E2, pg/mL), prolactin (PRL, mIU/L), total testosterone (ng/mL), sex hormone-binding globulin (SHBG, nmol/L), dehydroepiandrosterone sulfate (DHEAS, μg/dL), 17-hydroxyprogesterone (17-OHP, ng/mL), anti-Müllerian hormone (AMH, ng/mL) and adiponectin (μg/mL). FAI was calculated using the formula 100xT (ng/dL)/28.84xSHBG (nmol/L) [21].

Two-hour oral glucose tolerance test (OGTT) was performed and serum glucose and insulin were determined from blood samples collected at the baseline (0 min) and 30, 60, 90, and 120 min after the oral assumption of 75 g of glucose. For estimation of IR the following indexes were used: HOMA-IR (homeostasis model assessment) index calculated using the formula: [fasting insulin (mU/l) × fasting glucose (mmol/l)]/22.5; and quantitative insulin sensitivity check index QUICKI [I/(log[fasting insulin (mU/l)] × log[fasting glucose (mg/dl)])] [22]. The area under the curve in OGTT (AUC) was analyzed using the trapezoid formula for the assessment of glucose and insulin response to oral glucose load. All biochemical analyses and calculations were performed at baseline and after 6 months of treatment.

Biochemical and hormonal assays

Samples for hormone analysis were stored at − 80 °C until measurement. Insulin, testosterone, SHBG, DHEAS 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). FSH, LH and E2 were measured by a chemiluminescent immunometric assay (CLIA, Immulite 2000, Siemens Healthcare Diagnostics, Erlangen, Germany; inter- and intra-assay CVs were 2.9% and 3.9%, 2.9% and 3.8%, 5.6% and 7.1%, respectively). Serum 17-OHP was measured by RIA (CISBio International, Gif-sur-Yvette, France; inter- and intra-assay CVs 2.7 and 2.9%). Adiponectin was determined by immune-enzymatic assays (ELISA, DRG Diagnostics, Marburg, Germany; inter- and intra-assay CV was 5.9% and 6.3%., respectively). Plasma glucose was measured immediately after sampling with enzymatic calorimetric test (Roche Diagnostics, Mannheim, Germany).

Outcome measures

The primary outcome measure was to compare the effects of MET and MI on BMI, adiponectin levels, body composition, hormonal profile and metabolism of glucose and insulin at baseline and after 6 months of respective therapy. Secondary outcome measures included; (i) verifying the correlations of adiponectin with clinical and biochemical parameters in the PCOS group; (ii) to ascertain the differences in clinical and biochemical parameters in groups of obese and non-obese PCOS women in MET and MI arm.

Statistical analysis

The sample size was calculated according to the suspected change of serum adiponectin after MET and MI therapy. Assuming that MI does not change adiponectin level [17] and that 25% difference in adiponectin level before and after MET treatment [12] would be of significant clinical and biochemical value, we needed 60 patients in the PCOS group to demonstrate this difference at a power of 80% and an α error of 0.05. We expected that some of the patients might have become pregnant or dropped out during follow-up, so an extra 10% of the required sample size was enrolled. Statistical analysis was performed using the Statistical Package for Social Science version 21 (IBM Corp, Released 2012, IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp). The two-way repeated measures ANOVA (TW

RMA) was used to determine if there is a statistically significant interaction effect between MET and MI treatments, on a continuous dependent variable. Before analysis was performed, normality of a continuous dependent variable was determined. The two within-subject factors were time and treatment, and the dependent variable was a continuous variable. Because this was 2 × 2 interaction (treatment × time), sphericity was always assumed. If a statistically significant two-way interaction was seen, we performed tests to determine whether there were any statistically significant simple main effects. If there was not a significant two-way interaction, we looked for any statistically significant main effects. Results were shown with 95% confidence intervals (95% CI), with Bonferroni adjustment. Differences between the same tests performed before and after treatment were assessed using the paired *t* test and nonparametric Wilcoxon rank-sum test, as appropriate. The correlations between adiponectin and other indicators were analyzed using Spearman correlation analysis. Results are presented as mean ± standard deviation (SD). $p < 0.05$ was considered to be statistically significant.

Results

The number of patients screened and recruited as well as dropouts over the course of the study is shown in Fig. 1 CONSORT flowchart. As demonstrated in the study flow diagram, during the intervention phase of the study, three

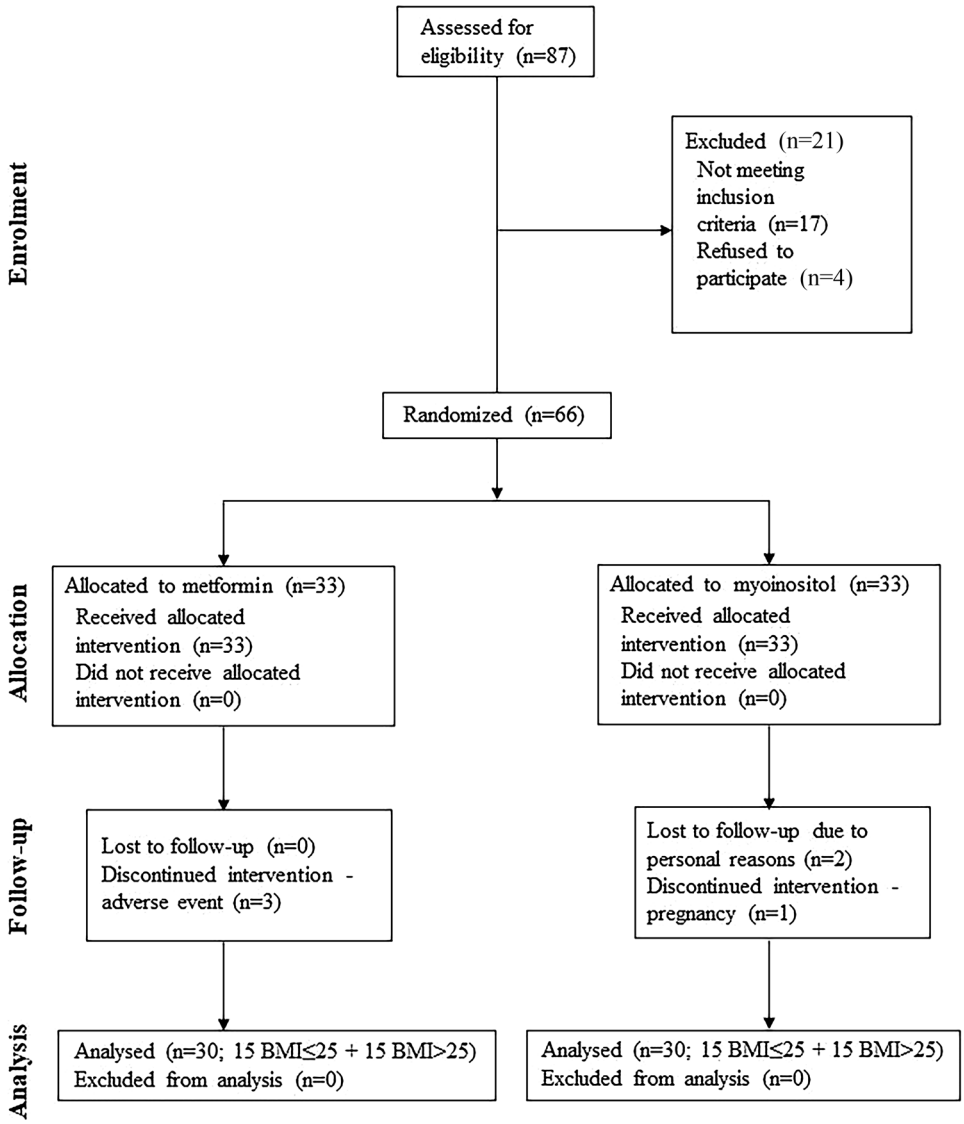

Fig. 1 CONSORT flowchart

participants were excluded from MET group due to intolerable gastrointestinal side effects, and three from MI group (two for personal reasons, one for pregnancy). Finally, 30 participants [15 normal weight and 15 overweight/obese) from each group completed the trial.

Myoinositol and metformin effect on clinical and biochemical parameters

Detailed results are shown in Table 1, Fig. 2 and 3. The decision was to keep the outliers, except in the case of FAI (basic and post-therapy value had outlier > 3 in the same person).

Two-way interaction (time × treatment)

Comparison between MET and MI in time to treatment analysis showed no significant differences between two treatments for all analyzed parameters. Only borderline significantly lower AUC glucose was found in the MET group in comparison to the MI group ($p = 0.071$).

Main effect of treatment

In the results that did not have a statistically significant interaction, the main effect of treatment was calculated. The main effect of treatment showed a statistically significant difference in glucose concentration at 120 min OGTT, $F(1, 29) = 5.072$, $p = 0.032$, and MET arm had median concentration of glucose of 0.72 mmol/L lower than the MI arm. Testosterone was 0.16 ng/mL higher in the MI arm compared to the MET arm, with significance of $F(1, 29) = 11.506$, $p = 0.002$. The two groups deteriorate at about the same rate, hence the difference between groups is about the same across occasions, thus suggesting that there is no group-by-time interaction effect. Tendency for significance was observed in body mass (F 3.392, $p = 0.076$).

Main effect of time

The main effect of time was also calculated for findings that did not have a statistically significant reaction. Variables

Table 1 Comparison of both treatments on clinical, metabolic and hormonal characteristics of women with PCOS

			Treatment × time interaction			Treatment main effect			Time main effect	
	Outliers	Shapiro–Wilk	*F*	*p*	η^2	*F*	*p*	Difference	*F*	*p*
Body weight (kg)		Normal	0.125	0.727	0.004	3.392	0.076	4.758	9.991	**0.004**
WC (cm)		Normal	0.082	0.777	0.003	0.708	0.407	1.950	17.790	**< 0.001**
BMI (kg/m^2)		Normal	0.011	0.917	< 0.001	0.962	0.335	0.712	10.951	**0.003**
Body fat mass (kg)		Mixed	0.437	0.514	0.015	0.674	0.419	1.140	13.529	**0.001**
Adiponectin (µg/ml)		Mixed	0.231	0.634	0.008	0.901	0.350	-0.883	6.060	**0.020**
Fasting glucose (mmol/l)		Mixed	0.005	0.945	< 0.001	0.559	0.461	-0.082	13.750	**0.001**
Glucose 120' (mmol/l)	1	Mixed	0.464	0.501	0.016	5.072	**0.032**	-0.720	0.742	0.396
AUC glucose		Mixed	3.519	0.071	0.108	2.739	0.109	-0.985	2.175	0.151
Fasting insulin (µIU/ml)		Mixed	0.667	0.421	0.022	0.621	0.437	0.792	0.859	0.362
Insulin120' (µIU/ml)		Mixed	0.924	0.344	0.031	0.001	0.973	0.393	0.539	0.449
AUC insulin	1	Mixed	1.408	0.245	0.046	0.737	0.398	16.756	0.646	0.428
HOMA-IR		Mixed	0.709	0.407	0.024	0.595	0.447	0.235	2.268	0.143
QUICKI	1	Mixed	0.011	0.918	< 0.001	0.066	0.800	-0.006	3.592	**0.068**
Testosterone (ng/ml)	2	Mixed	0.043	0.837	0.001	11.506	**0.002**	0.166	6.719	**0.015**
SHBG (nmol/l)	2	Non-normal	0.194	0.663	0.007	0.011	0.917	-4.102	7.085	**0.013**
FAI	3	Non-normal	2.754	0.108	0.087	1.665	0.207	1.636	4.084	**0.053**
DHEAS (µg/dl)		Normal	0.900	0.350	0.030	0.079	0.781	-9.712	2.877	1.01
17-OHP (ng/ml)	1	Mixed	0.375	0.545	0.013	0.236	0.631	0.067	8.166	**0.008**
LH (mIU/ml)		Mixed	1.033	0.318	0.034	1.071	0.309	0.886	9.630	**0.004**
FSH (mIU/ml)	1	Mixed	0.031	0.861	0.001	0.119	0.732	-0.150	0.007	0.935
Estradiol (pg/ml)	3	Mixed	0.894	0.352	0.030	0.389	0.537	16.526	6.890	**0.014**
AMH (ng/ml)	2	Mixed	2.118	0.156	0.068	0.251	0.620	0.521	1.194	0.284

$p < 0.05$ was considered to be statistically significant. All statistically significant values are given in bold

BMI body mass index, *WC* waist circumference, *AUCglucose* area under the glucose curve, *AUCinsulin* area under the insulin curve, *HOMA–IR* homeostasis model assessment–insulin resistance index, *QUICKI* quantitative insulin sensitivity check index, *SHBG* sex hormone-binding globulin, *FAI* free androgen index, *DHEAS* dehydroepiandrosterone sulfate, *17-OHP* 17-hydroxy progesterone, *LH* luteinizing hormone, *FSH* follicle-stimulating hormone, *AMH* anti-Müllerian hormone

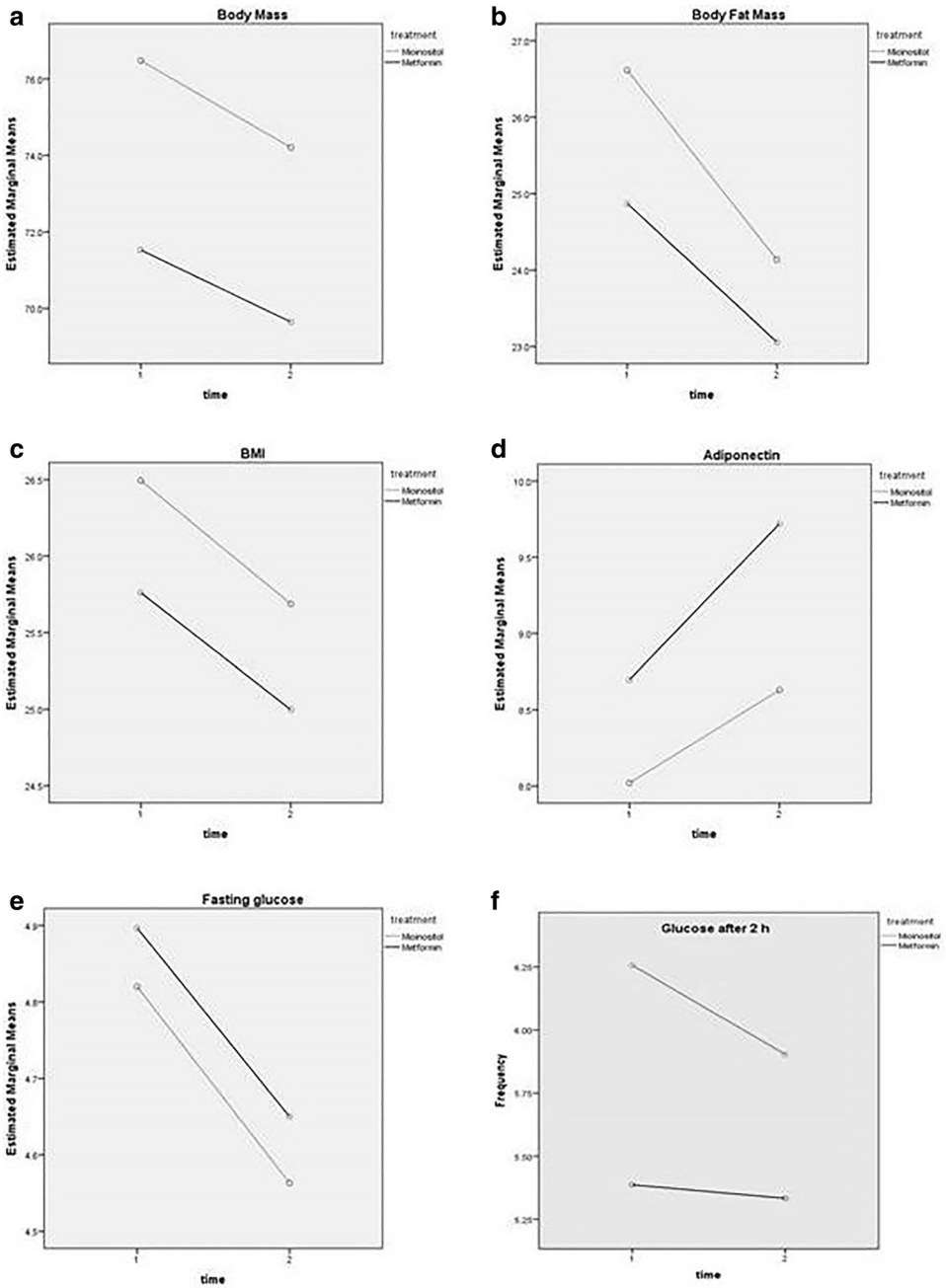

Fig. 2 BMI, body weight, body fat mass, FPG, 2 h OGTT glucose, and adiponectin levels for both metformin and myoinositol treatment

that had a statistically significant difference were: body mass (F 9.991, $p=0.004$), waist circumference (F 17.790, $p<0.001$), BMI (F 10.951, $p=0.003$), body fat mass (F 13.529, $p=0.001$), adiponectin (F 6.060, $p=0.020$), fasting glucose (F 13.750, $p=0.001$), testosterone (F 6.719, $p=0.015$), SHBG (F 7.085, $p=0.013$), 17OH progesterone (F 8.166, $p=0.008$), LH (F 9.630, $p=0.004$) and estradiol (F 6.890, $p=0.014$). Tendency for significance was observed in QUICKI and FAI.

Secondary outcomes

Correlations of adiponectin with clinical and biochemical parameters in the PCOS group

We found significant inverse correlation of adiponectin with BMI ($r=-0.503$; $p<0.001$), body weight ($r=-0.434$; $p=0.001$), WC ($r=-0.478$; $p<0.001$), body fat mass (%) ($r=-0.405$; $p=0.001$), HOMA-IR ($r=-0.547$;

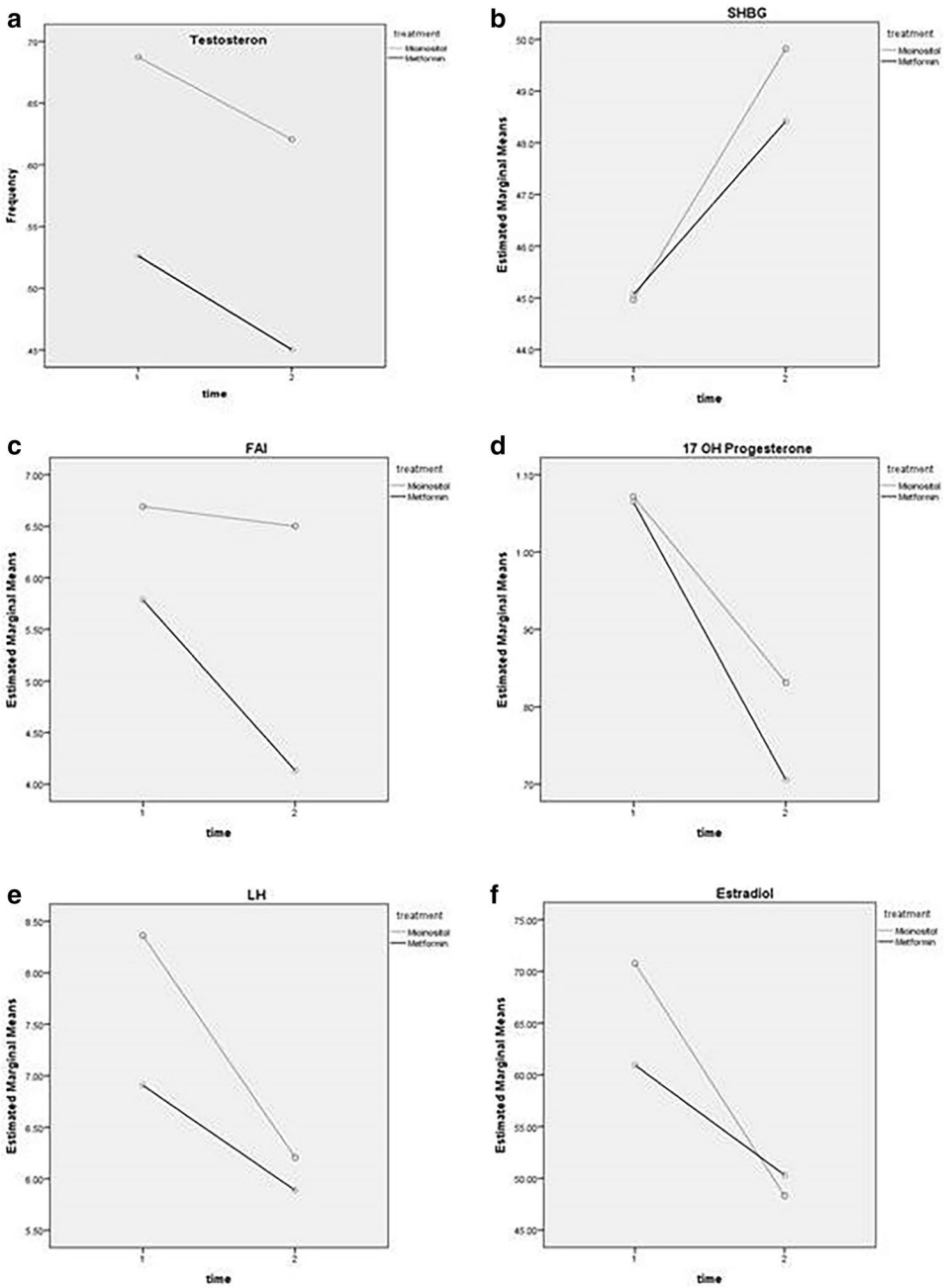

Fig. 3 Testosterone, SHBG, FAI, 17-OH progesterone, LH, estradiol levels for both metformin and myoinositol treatment

$p < 0.001$), $AUC_{glucose}$ ($r = -0.345$; $p = 0.007$) and $AUC_{insulin}$ ($r = -0.393$; $p = 0.002$) and positive correlation of adiponectin with QUICKI ($r = 0.496$; $p < 0.001$). Adiponectin had significant negative correlation with FAI ($r = -0.425$; $p = 0.001$) and positive correlation with SHBG ($r = 0.532$; $p < 0.001$).

Differences between obese and non-obese women in the MET and MI arm

The intra-group analysis of clinical, anthropometric and biochemical characteristics of PCOS women before and after 6 months of treatment on two regimens is presented in Table 2.

Table 2 Clinical indices at baseline and after 6 months of metformin (MET) and myoinositol (MI) treatment in women with PCOS

	MET ($n=30$)						MI ($n=30$)					
	BMI ≤ 25 kg/m^2 ($n=15$)			BMI > 25 kg/m^2 ($n=15$)			BMI ≤ 25 kg/m^2 ($n=15$)			BMI > 25 kg/m^2 ($n=15$)		
	Baseline	After 6 mts	*p*	Baseline	After 6 mts	*p*	Baseline	After 6 mts	*p*	Baseline	After 6 mts	*p*
Body weight (kg)	57.99 (±5.67)	57.07 (±5.08)	0.108	85.06 (±12.09)	82.22 (±11.89)	**0.029**	62.46 (±7.02)	61.60 (±7.06)	0.225	90.50 (±10.58)	86.81 (±12.78)	0.072
WC (cm)	72.60 (±7.82)	70.47 (±7.34)	**0.022**	91.93 (±10.40)	89.67 (±10.13)	0.067	71.93 (±7.77)	70.87 (±7.77)	0.154	96.820 (±8.03)	92.87 (±7.83)	**0.016**
BMI (kg/m^2)	20.96 (±2.04)	20.61 (±1.79)	0.093	30.50 (±3.52)	29.38 (±3.76)	**0.019**	21.96 (±2.34)	21.67 (±2.46)	0.249	31.03 (±3.16)	29.72 (±3.80)	0.073
Body fat mass (kg)	15.44 (±5.51)	14.99 (±4.66)	0.410*	34.30 (±8.20)	31.13 (±7.92)	**0.008**	15.73 (±5.24)	14.74 (±4.71)	0.151	37.50 (±7.17)	33.53 (±9.70)	**0.026**
F–G score	13.93 (±4.99)	12.93 (±4.60)	0.069	15.13 (±5.72)	14.00 (±5.11)	**0.009***	15.93 (±5.91)	15.00 (±5.84)	0.340*	17.47 (±6.73)	17.47 (±7.26)	1.00
Fasting glucose (mmol/l)	4.67 (±0.43)	4.45 (±0.59)	0.419*	5.13 (±0.53)	4.85 (±0.35)	0.081	4.64 (±0.47)	4.31 (±0.35)	**0.006**	5.00 (±0.47)	4.82 (±0.68)	0.180
Glucose 120' (mmol/l)	6.01 (±1.79)	5.34 (±1.95)	0.262*	6.50 (±2.02)	6.47 (±1.66)	0.953	4.93 (±1.11)	5.12 (±1.55)	0.659	5.84 (±1.67)	5.55 (±1.54)	0.460
AUC glucose	13.67 (±2.86)	12.06 (±2.88)	**0.017**	15.37 (±3.17)	14.98 (±2.92)	0.540	11.86 (±1.54)	12.21 (±2.82)	0.591	14.22 (±2.48)	13.85 (±2.70)	0.730*
Fasting insulin (µIU/ml)	9.80 (±3.73)	8.69 (±2.61)	0.334	13.51 (±3.76)	14.42 (±6.22)	0.427	9.90 (±3.00)	9.21 (±3.40)	0.440	15.94 (±5.42)	14.54 (±5.83)	0.396
Insulin120' (µIU/ml)	66.08 (±52.72)	57.13 (±36.21)	0.650*	78.96 (±66.57)	84.69 (±52.57)	0.745	55.54 (±40.13)	79.24 (±61.66)	**0.015***	77.79 (±41.89)	75.86 (±41.98)	0.869
AUC insulin	158.43 (±116.07)	115.60 (±35.75)	0.427*	181.50 (±72.11)	176.79 (±80.87)	0.846	141.86 (±72.90)	163.12 (±105.85)	0.159	204.87 (±115.20)	189.49 (±65.79)	0.543
HOMA-IR	2.03 (±0.76)	1.76 (±0.60)	0.274	3.02 (±0.76)	3.11(±1.41)	0.770	2.08 (±0.71)	1.79 (±0.77)	0.119	3.57 (±1.26)	3.20 (±1.65)	0.320*
QUICKI	0.35 (±0.018)	0.36 (±0.032)	0.280	0.33 (±0.012)	0.33 (±0.022)	0.779	0.35 (±0.022)	0.355 (±0.023)	0.322	0.32 (±0.017)	0.33 (±0.021)	0.251
Adiponectin (µg/ml)	10.61 (±4.45)	10.46 (±3.48)	0.513*	6.79 (±3.83)	8.98 (±4.63)	**0.030***	8.60 (±3.72)	9.49 (±4.38)	0.362	7.44 (±1.82)	7.77 (±2.31)	0.478*
FSH (mIU/ml)	6.80 (±1.80)	6.77 (±1.23)	0.960	6.46 (±1.95)	6.34 (±2.71)	0.840	6.00 (±1.31)	6.13 (±2.06)	0.910*	6.85 (±2.69)	6.78 (±2.13)	0.901
LH (mIU/ml)	7.99 (±3.37)	7.58 (±3.49)	0.688	5.83 (±3.31)	4.19 (±2.37)	**0.047**	9.64 (±6.43)	6.71 (±3.78)	0.088*	7.08 (±3.62)	5.71 (±3.46)	0.061*
Estradiol (pg/ml)	49.97 (±16.12)	49.46 (±18.86)	0.922	71.91 (±36.45)	51.19 (±27.67)	**0.047***	80.62 (±63.85)	45.39 (±14.89)	**0.047***	60.92 (±57.23)	51.26 (±15.47)	0.532*
17-OHP (ng/ml)	0.93 (±0.51)	0.69 (±0.38)	0.178	1.19 (±1.03)	0.72 (±0.25)	**0.041***	1.13 (±0.83)	0.80 (±0.58)	0.163	1.01 (±0.45)	0.86 (±0.53)	0.206
Testosterone (ng/ml)	0.54 (±0.14)	0.49 (±0.14)	0.268	0.51 (±0.20)	0.41 (±0.11)	**0.019**	0.67 (±0.24)	0.59 (±0.22)	0.105*	0.71 (±0.23)	0.65 (±0.32)	0.551*
SHBG (nmol/l)	59.60 (±31.66)	63.41 (±24.96)	0.315	30.54 (±11.27)	33.43 (±12.99)	0.147	54.30 (±26.27)	60.55 (±35.67)	0.427*	35.63 (±13.82)	39.09 (±15.76)	0.103
FAI	4.41 (±3.94)	2.99 (±1.12)	0.159	7.16 (±4.81)	5.28 (±3.46)	**0.008**	4.84 (±2.06)	4.545 (±3.97)	0.124*	8.55 (±6.27)	8.45 (±9.59)	0.730*

Table 2 (continued)

	MET ($n=30$)						MI ($n=30$)					
	BMI ≤ 25 kg/m^2 ($n=15$)			BMI > 25 kg/m^2 ($n=15$)			BMI ≤ 25 kg/m^2 ($n=15$)			BMI > 25 kg/m^2 ($n=15$)		
	Baseline	After 6 mts	p	Baseline	After 6 mts	p	Baseline	After 6 mts	p	Baseline	After 6 mts	p
DHEAS (µg/dl)	362.18 (± 132.80)	357.07 (± 100.19)	0.758	377.51 (± 123.42)	393.15 (± 122.06)	0.335	366.02 (± 139.56)	371.75 (± 138.49)	0.746	335.91 (± 146.91)	377.39 (± 179.21)	0.085
AMH (ng/ml)	9.60 (± 7.25)	8.57 (± 5.71)	**0.017***	5.81 (± 2.67)	5.20 (± 2.36)	0.184	7.95 (± 3.54)	8.51 (± 5.19)	0.527	7.49 (± 3.65)	7.32 (± 3.80)	0.752

Data is shown as mean ± SD

WC waist circumference, *BMI* body mass index, *AUCglucose* area under the glucose curve, *AUCinsulin* area under the insulin curve, *HOMA–IR* homeostasis model assessment–insulin resistance index, *QUICKI* quantitative insulin sensitivity check index, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *17-OHP* 17-hydroxyprogesterone, *SHBG* sex hormone-binding globulin, *FAI* free androgen index, *DHEAS* dehydroepiandrosterone sulfate, *AMH* anti-Müllerian hormone

*Obtained by Wilcoxon signed rank test

Drug tolerability and compliance

MI was better tolerated than MET. In the MI arm, no patients experienced any significant side effects. There were two dropouts due to personal reasons, and one patient became pregnant. In the metformin arm, three patients dropped out due to intolerable gastrointestinal disturbances that were not corrected by temporary reduction in the dose.

Discussion

In the present study, we analyzed the relevance of treatment with two insulin-sensitizing agents on clinical and biochemical indices in women with PCOS. Both MET and MI had similar effect on BMI, adiponectin levels, body composition, hormonal profile and metabolism of glucose and insulin in PCOS women.

During both treatments, BMI, WC and body fat mass decreased with time. A beneficial effect of MET on BMI and abdominal obesity was observed when added to lifestyle modification [23]. Very few studies have examined the impact of MET on visceral and subcutaneous fat depots. The addition of MET to a low-calorie diet resulted in modest impact on body weight and abdominal fat distribution in PCOS [24]. We showed that our obese PCOS women treated with MI significantly reduced predominantly abdominal fat, followed by a trend toward reduction in BMI. Other authors suggested that MI can positively influence body composition in obese PCOS patients [25]. The decrease in white adipose tissue deposition was also observed in experimental studies, but the exact mechanism was not elucidated [26]. The reduction in fat mass can precede the long-term insulin-sensitizing effect of MI treatment [27]. In relation to BMI, we showed differences in the effect of the examined compounds on body composition. As expected, the effect of MET was more pronounced in overweight/obese PCOS women. Moreover, these effects are direct effects of the compounds used, as we did not recommend any specific or strict dietary recommendation.

Another important consequence of IR in PCOS patients is decrease of adiponectin [13, 28]. Our study did not confirm the advantage of one drug in comparison to another in the effect on adiponectin concentration. Negative correlation of adiponectin with indices of body composition, insulin sensitivity and hyperandrogenemia was shown. However, others did not confirm hypoadiponectinemia in PCOS women, but suggested the potential role of obesity in determining adiponectin level [29]. Adiposity, and not IR, could be the main determinant factor for adiponectin, further supported with inverse association of BMI and WC with adiponectin levels in our PCOS women. Furthermore, BMI was a significant

predictor of changes in adiponectin level with calculated decrease of 0.328 μg/mL for the 1 kg/m^2 increment of BMI.

Experimental data demonstrated that MET up-regulates adiponectin gene expression in the subcutaneous white adipose tissue, both in vivo and in vitro, and stimulates adiponectin protein secretion in vitro [30]. Serum adiponectin increased in time after MET and MI treatment in our PCOS women. The exact mechanism for improved body composition needs to be clarified. One of the possibilities could be the effect of MET on appetite regulation through the inhibition of secretion of other cytokines, like ghrelin [31]. Although fat mass reduction was found in overweight/obese PCOS women in both treatment arms, adiponectin level only increased in overweight/obese MET treated PCOS (12). We did not observe differences between treatments used, probably due to short duration and limited number of subjects as well lack of phenotype classification of our PCOS women which will be included in our future studies. To our knowledge, this is the first study in PCOS women that evaluated change in adiponectin level during a 6-month MI treatment period.

Testosterone concentrations decreased at about the same rate in both treatments groups, without difference related to treatment. Our results, in agreement with other studies, indicated the possibility for MET to reduce plasma androgen concentrations in overweight/obese PCOS women [32–34]. MET decreases testosterone level through reduction of fasting and glucose-stimulated insulin level and concomitant decrease in ovarian cytochrome P450c17 activity [35]. However, reduction of testosterone level in our study could not be explained by reduction of insulin, since insulin sensitivity indices were unaffected by MET treatment irrespectively of BMI. Direct inhibitory effect of MET on ovarian steroidogenesis targeting HSD3B2 and CYP17-lyase through inhibition of mitochondrial Complex I was suggested [36]. Similarly, a suppression of the adrenal steroidogenesis by MET caused by significant reduction in the ACTH-stimulated steroid levels and consequent decrease in C17 hydroxylase activity were suggested [37]. The inhibitory effects of MET on ACTH secretion resulted from the diminished POMC expression following MET-induced AMPK and LXRα phosphorylation in the pituitary [38]. However, we did not observe any substantial effect of MET treatment on DHEAS levels during the 6-month treatment period. Our results are in line with the others, which showed a reduction in various steroid enzymatic activities in both ovaries and adrenals during a long-term MET treatment without apparent changes in basal steroid levels and in insulin sensitivity [39].

Comparison between MET and MI showed no significant differences between two treatments in the indices of glucose and insulin metabolism. Significantly lower AUC glucose was found in the MET group in comparison to the MI group. During therapy with MI, we failed to find a significant decrease in insulin resistance indices Previous reports documented an improvement in the hormonal and metabolic milieu after MI treatment in PCOS patients [40]. However, Artini et al. observed a significant reduction of insulin levels without changes in circulating androgen levels after a 12-week treatment with MI [41]. Several hypotheses were formulated explain these inconsistencies. Combined MI and D-chiro-inositol (DCI) treatment compared to the treatment with MI alone has shown more efficacy on the metabolic profile [42]. Myoinositol affects insulin metabolism mainly at the ovary level, while DCI rather reduces peripheral hyperinsulinemia and hyperandrogenemia [43]. The lack of improvement in both fasting insulin concentrations and insulin response to glucose load in our patients treated with MI suggests that compensatory hyperinsulinemia in these patients is triggered only in part by a change in DCI–IPG synthesis/release.

We did not demonstrate reduction in fasting glucose and insulin levels along with the change in insulin sensitivity indexes in our PCOS patients treated with MET. Increase in insulin sensitivity after MET treatment was not accompanied by a significant decrease in fasting insulin even when euglycemic clamp was performed [9]. Other studies with PCOS women found an invariable decrease in fasting insulin levels [44, 45]. Treatment effect on 2 h glucose during OGTT and the reduction of AUC glucose during OGTT in MET-treated subjects indicate that the primary glucose-lowering effect of insulin sensitizers could be achieved by improving glucose effectiveness mediated by mechanisms such as inhibition of mitochondrial complex I, which is an AMPK-dependent effect [46, 47].

The influence of MET on serum AMH concentrations has been debated. Several studies have suggested that the reduction in AMH levels after MET treatment is primarily observed in highly insulin-resistant patients with PCOS [48, 49]. The present study demonstrated no difference in treatment effect of both insulin-sensitizing compounds on serum AMH. However, serum AMH only decreased in normal-weight PCOS subjects during MET treatment. Similar results in normal-weight PCOS women were hypothesized by a peripheral effect of prolonged MET treatment. This effect is independent of its insulin-sensitizing properties, which lead to reduction of ovarian volume and stromal compartment in normal-weight PCOS, and may result from a direct action of MET on granulosa cell viability and phosphorylated AKT and MAPK protein expression in human granulosa cells [50, 51].

Besides anthropometric, metabolic and hormonal effects, the novelty of the study is the effect of MET on AMH in

normal-weight patients with PCOS. To the best of our knowledge, this is a first study that directly compared the effects of both insulin-sensitizing compounds in normal-weight and overweight/obese PCOS women over a longer treatment period.

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

In conclusion, our study showed similar effects of MET and MI on BMI, body composition, hormonal profile, glucose and insulin metabolism, and adiponectin level in PCOS patients. However, this is the first study analyzing the prolonged effects of MI on adiponectin levels in PCOS. Our study indicates that weight loss is crucial and may precede the change in insulin sensitivity remaining the first-line therapeutic option in women with the syndrome. The effects of inositols on body composition and cardiovascular risk in PCOS need to be further elucidated during long-term clinical studies.

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Author contributions VSS: involved in study design, data collection and article preparation. SPP: involved in study design implementation and supervision. DM: involved in study design, implementation and supervision, expert knowledge and article preparation. SS was the trial coordinator and performed the statistical analyses. AP, GM, JBM, SL, SO, GM and DM: participated in the analysis, article revision and expert knowledge. All authors provided critical discussion and contributed to the preparation of the manuscript.

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Data availability The data underlying this article cannot be shared publicly due to the privacy of individuals that participated in the study. The data will be shared on reasonable request to the corresponding author.

Code availability Not applicable.

Declarations

Conflict of interest The authors have no conflict of interest to declare.

Ethical approval Ethics approval was obtained from the Ethics Committee of University Clinical Center of the Republic of Srpska (01-9-742.2/16).

Research involving human participants and/or animals All procedures performed in human participants were in accordance with the ethical standards of the institutional research committees and with the 1964 Helsinki Declaration and its later amendments of ethical standards.

Informed consent Written informed consent was obtained from all individual human participants included in the study.

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