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Comparison of myo-inositol and metformin on glycemic control, lipid profiles, and gene expression related to insulin and lipid metabolism in women with polycystic ovary syndrome: a randomized controlled clinical trial

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ABSTRACT

This investigation was conducted to evaluate comparison of myo-inositol and metformin on glycemic control, lipid profiles, and gene expression related to insulin and lipid metabolism in women with polycystic ovary syndrome (PCOS). This randomized controlled trial was conducted on 53 women with PCOS, aged 18–40 years old. Subjects were randomly allocated into two groups to take either myo-inositol ($n = 26$) or metformin ($n = 27$) for 12 weeks. Myo-inositol supplementation, compared with metformin, significantly reduced fasting plasma glucose (FPG) (β −5.12 mg/dL; 95% CI, −8.09, −2.16; p=.001), serum insulin levels (β −1.49 μIU/mL; 95% CI, −2.28, −0.70; p<.001), homeostasis model of assessment-insulin resistance (β −0.36; 95% CI, −0.55, −0.17; p<.001), serum triglycerides (β 12.42 mg/dL; 95% CI, −20.47, −4.37; p=.003) and VLDL-cholesterol levels (β −2.48 mg/dL; 95% CI, −4.09, −0.87; p=.003), and significantly increased the quantitative insulin sensitivity check index (β 0.006; 95% CI, 0.002, 0.01; p=.006) compared with metformin. Moreover, myo-inositol supplementation upregulated gene expression of peroxisome proliferator-activated receptor gamma (PPAR-γ) (p=.002) compared with metformin. Overall, taking myo-inositol, compared with metformin, for 12 weeks by women with PCOS had beneficial effects on glycemic control, triglycerides and VLDL-cholesterol levels, and gene expression of PPAR-γ.

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Myo-inositol; metformin; glycemic control; lipid profiles; polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting 6–15% of reproductive-aged women among different geographic regions based on the Rotterdam criteria [1]. This disorder is characterized by androgen excess including hyperandrogenemia and/or clinical evidence of hyperandrogenism, and oligo-ovulation [2]. In addition, insulin resistance, glucose intolerance, and dyslipidemia may play a crucial role in the development of PCOS, cardiovascular disorder (CVD), type 2 diabetes mellitus (T2DM), various cancers such as ovarian cancer, and hypertension [3–5].

Recently, new insulin sensitizers containing inositol have been suggested in the treatment of patients with PCOS. In addition, inositol(s) supplementation could fruitfully influence different pathophysiological aspects of disorders pertaining Obstetrics and Gynecology [6]. Few studies have reported the beneficial effects of myo-inositol on insulin sensitivity, metabolic status and gene expression related to insulin and lipid metabolism [7, 8]. In addition, myo-inositol intake improved reproductive axis functioning in patients with PCOS [9]. In contrast to metformin, no side effects have been reported following treatment with myo-inositol [10]. On the other hand, the peroxisome proliferator-activated receptor gamma (PPAR-γ) is involved in several metabolic pathways such as insulin and lipid metabolism, and cellular differentiation [11]. Furthermore, PPAR-γ may regulate the steroidogenesis, which in turn contributes to the regulation of ovarian function [12]. Also, low-density lipoprotein receptor (LDLR) is a cell surface glycoprotein that plays a pivotal role in the homeostatic control of blood cholesterol by mediating the removal of cholesterol-containing lipoprotein particles from the circulation [13].

Myo-inositol produces a second messenger of the inositol triphosphate that regulates some hormones reviled thyroid-stimulating hormone and follicle-stimulating hormone [14], and is responsible for the glucose uptakes, which in turn increase insulin sensitivity [15]. According to our knowledge, data on comparison of myo-inositol and metformin on glycemic control, lipid profiles, and gene expression related to insulin and lipid metabolism in patients with PCOS are limited [16]. Therefore, the aim of this study was to compare myo-inositol and metformin on glycemic control, lipid profiles, and gene expression related to insulin and lipid metabolism in patients with PCOS.

Subjects and methods

This randomized controlled clinical trial, registered in the Iranian website for registration of clinical trials as http://www.irct.ir: IRCT2017082733941N10, was conducted among 53 subjects, aged 18–40 years old with PCOS diagnosed based on according to the Rotterdam criteria [17] which were referred to the Kosar Clinic in

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Table 1. Specific primers used for real-time quantitative PCR.

Gene	Primer	Product size (bp)	Annealing temperature (C)
GAPDH	F: AAGCTCATTTCCTGGTATGACAACG R: TCTTCCTCTTGTGCTCTTGCTGG	126	61.3
PPAR-γ	F: ATGACAGACCTCAGACAGATTG R: AATGTTGGCAGTGGCTCAG	210	54
GLUT-1	F: TATCTGAGCATCGTGGCCAT R: AAGACGTAGGGACCACACAG	238	62.1
LDLR	F: ACTTACGGACAGACAGACAG R: GGCCACACATCCCATGATTC	223	57

GAPDH: glyceraldehyde-3-Phosphate dehydrogenase; GLUT-1: glucose transporter 1; LDLR: low-density lipoprotein receptor; PPAR-γ: peroxisome proliferator-activated receptor gamma.

Arak, Iran, from September 2017 to December 2017. The study protocol was approved by the Ethics Committee of Arak University of Medical Sciences (AUMS). Written informed consent was obtained from all participants prior to the intervention. Exclusion criteria were as follows: pregnancy, adrenal hyperplasia, androgen-secreting tumors, hyperprolactinemia, thyroid dysfunction, and diabetes at enrollment.

Intervention

At baseline, participants were matched according to BMI (<25 and ≥25 kg/m^2), age (<30 and ≥30 y), phenotypes A (13 subjects in each group) and D (14 subjects in metformin group and 13 subjects in myo-inositol group) of PCOS. Then, participants were randomly allocated into two groups to receive either 500 mg of metformin three times a day ($n = 27$) or myo-inositol containing 2 g of myo-inositol plus 200 µg of folic acid two times a day ($n = 26$) for 12 weeks. Myo-inositol and metformin were provided by LO.LI. Pharma (Rome, Italy) and Tehran Darou Pharma (Tehran, Iran), respectively. Randomization assignment was performed using computer-generated random numbers. At the time of randomization, sequentially numbered, sealed envelopes were opened. Allocation of the subjects was performed by a trained midwife member at the gynecology clinic who was blinded to the randomization schedule. All participants provided 3-d dietary records and three physical activity records to verify that they maintained their usual diet and physical activity during the intervention. Both dietary records and physical activity were taken at weeks 0, 3, 6, 9, and 12 during the intervention. To obtain information on participant nutrient intake based on these 3-d food diaries, we used Nutritionist IV software (First Databank, San Bruno, CA) modified for Iranian foods. Physical activity was described as metabolic equivalents (METs) in hours per day by standard tables.

Anthropometric measures

A trained midwife took anthropometric measurements in the clinic at baseline and the end of the intervention. Height and weight (Seca, Hamburg, Germany) were measured light clothing with shoes removed. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared.

Assessment of outcomes

In this study, glycemic control was considered as the primary outcome. Lipid profiles, gene expression of PPAR-γ, glucose transporter 1 (GLUT-1) and LDLR were recognized as the secondary outcomes.

Biochemical assessment

Fasting blood samples were collected from participants (15 mL) at weeks 0 and 12 of the intervention. To determine fasting plasma glucose (FPG) and lipid profiles, enzymatic kits (Pars Azmun, Tehran, Iran) with inter- and intra-assay coefficient variances (CVs) lower than 5% were used. Serum insulin values were assessed using an ELISA kit (Monobind Inc., Lake Forest, CA) with the intra- and inter-assay CVs lower than 6%. The homeostatic model assessment for insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI) was determined according to the suggested formulas [18].

Isolation of lymphocyte cells

Lymphocyte cells were extracted from blood samples of women with PCOS with a 50% percoll (Sigma-Aldrich, Dorset, UK). Samples were taken for cell count and viability testing using trypan blue, RNA, and DNA extraction [19].

RNA extraction and real-time PCR

RNA extraction was performed with use of the RNX-plus kit (Cinnacolon, Tehran, Iran). Following extraction of the total RNAs from each sample, the RNA quantification was evaluated by the UV spectrophotometer. Samples OD 260/280 ratio was standardized between 1.7 and 2.1 showing no contamination for both protein and DNA [19]. The isolated RNA was reverse transcribed to cDNA library using the moloney murine leukemia virus reverse transcriptase (M-MLV RT). Gene expressions of PPAR-γ, GLUT-1, and LDLR were evaluated by quantitative RT-PCR, using the LightCycler technology (Roche Diagnostics, Rotkreuz, Switzerland) with SYBR green detection and Amplicon Kit (Table 1). Glyceraldehyde-3-phosphate dehydrogenase primers were used as housekeeping gene. To design primers, Primer Express Software (Applied Biosystems, Foster City, CA) and Beacon designer software (Takaposizt, Tehran, Iran) were utilized. Relative transcription levels were calculated using the methods of Pffafi or $2^{-\Delta\Delta CT}$.

Statistical analyses

The Kolmogorov–Smirnov test was done to determine the normality of data. Differences in anthropometric measures, dietary intakes, and gene expression between treatment groups were detected with independent-sample t-tests. Multiple linear regression models helped to assess the treatment effects on study outcomes after adjusting for confounding parameters including age and BMI. Significance of the treatment effects was presented as the mean differences with 95% confidence interval. Bootstrapping was also used as a sensitivity analysis of confidence interval. p values <.05 were considered statistically significant. All statistical analyses were done using the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, IL).

Results

As demonstrated in the study flow diagram (Figure 1), during the enrollment phase of the study, there were 67 women with PCOS; however, 7 participants did not meet the inclusion criteria and thus were excluded. During the follow-up, 7 participants dropped out of the study due to personal reasons (3 participants

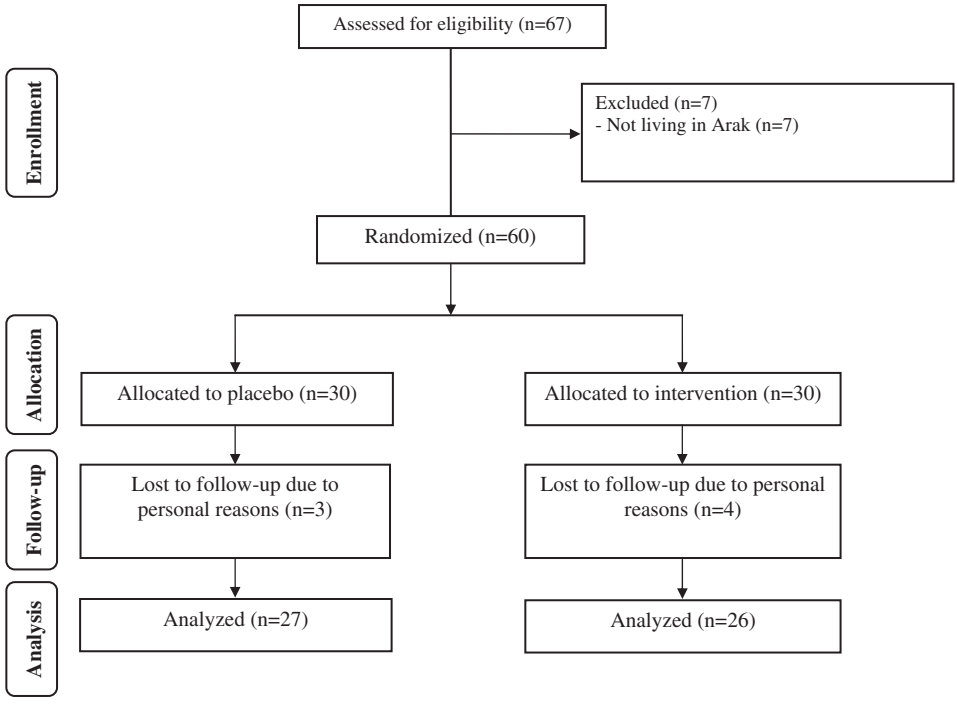

Figure 1. Summary of patient flow diagram.

Table 2. General characteristics of study participants.

	Metformin group ($n=27$)	Myo-inositol group ($n=26$)	*p*a
Age (years)	27.7 ± 3.2	28.3 ± 4.9	.54
Height (cm)	161.9 ± 4.8	161.4 ± 3.3	.66
Weight at study baseline (kg)	71.5 ± 8.7	73.2 ± 8.9	.49
Weight at end-of-trial (kg)	71.0 ± 8.5	72.5 ± 8.6	.53
Weight change (kg)	−0.5 ± 1.0	−0.6 ± 1.2	.54
BMI at study baseline (kg/m^2)	27.3 ± 3.3	28.1 ± 3.1	.38
BMI at end-of-trial (kg/m^2)	27.1 ± 3.3	27.8 ± 3.0	.41
BMI change (kg/m^2)	−0.2 ± 0.4	−0.3 ± 0.5	.60

Data are means ± SD.
[a]Obtained from independent *t*-test.

in metformin group and 4 participants in myo-inositol group). Finally, 53 participants [metformin ($n=27$) and myo-inositol ($n=26$)] completed the trial.

Participants' mean age, height, and weight and BMI at baseline and end-of-trial were not statistically different between the two groups (Table 2).

Considering the 3-d dietary records obtained during the intervention, there was no statistically significant difference in terms of dietary macro- and micro-nutrient intakes between myo-inositol and metformin groups (Data not shown).

Myo-inositol supplementation, compared with metformin, significantly reduced FPG (β −5.12 mg/dL; 95% CI, −8.09, −2.16; p=.001), serum insulin levels (β −1.49 μIU/mL; 95% CI, −2.28, −0.70; p<.001), HOMA-IR (β −0.36; 95% CI, −0.55, −0.17; p<.001), serum triglycerides (β 12.42 mg/dL; 95% CI, −20.47, −4.37; p=.003) and VLDL-cholesterol (β −2.48 mg/dL; 95% CI, −4.09, −0.87; p=.003), and significantly increased QUICKI (β 0.006; 95% CI, 0.002, 0.01; p=.006) compared with metformin (Table 3). Myo-inositol supplementation did not affect other lipid profiles.

Myo-inositol supplementation upregulated gene expression of PPAR-γ (p=.002) compared with metformin (Figure 2). Carnitine and chromium co-supplementation did not affect gene expression of GLUT-1 and LDLR (Figures 2 and 3).

Discussion

In this study, we compared the effects of myo-inositol and metformin administration for 12 weeks on glycemic control, lipid concentrations, and gene expression related to insulin and lipid metabolism among women with PCOS. We found that taking myo-inositol for 12 weeks by women with PCOS had beneficial effects on glycemic control, triglycerides and VLDL-cholesterol levels, and gene expression of PPAR-γ, but did not affect other lipid profiles and gene expression of GLUT-1 and LDLR.

Effects on glycemic control

Subjects with PCOS are susceptible to several metabolic disorders such as insulin resistance, reduced insulin sensitivity, CVD, and T2DM [20, 21]. We found that myo-inositol administration for 12 weeks to women with PCOS had beneficial effects on FPG, insulin levels, HOMA-IR, and QUICKI. In addition, myo-inositol administration upregulated gene expression of PPAR-γ, but did not affect gene expression of GLUT-1. However, data on the comparison of myo-inositol and metformin on glycemic control and gene expression related to insulin metabolism among women with PCOS are limited; few studies have assessed the effects of myo-inositol administration on and metabolic parameters among patients with PCOS. In a study by Costantino et al. [22], it was observed that mayo-inositol had beneficial effects on QUICKI among patients with PCOS. Also, Dona et al. [23] demonstrated that treatment with myo-inositol for 12 weeks to women with PCOS was effective in improving metabolic status and insulin resistance. In addition, treatment with myo-inositol and contraceptive pill had beneficial effects on insulin sensitivity and insulin resistance in patients with PCOS than oral contraceptive solely [24]. In another study, combined treatment with myo-inositol and D-chiro-inositol increased insulin sensitivity in obese PCOS patients [25]. Also, Rago et al. [26] found combined treatment with myo-inositol and α-lipoic acid in women with PCOS undergoing an IVF cycle could help to improve their

Table 3. Metabolic profiles at baseline and after the 12-week intervention in women with polycystic ovary syndrome that received either myo-inositol supplements or metformina.

	Metformin group ($n = 27$)		Myo-inositol group ($n = 26$)		Difference in outcome measures between myo-inositol and placebo groups[a]	
Variables	Baseline	Week 12	Baseline	Week 12	β (95% CI)	p^b
FPG (mg/dL)	97.0 ± 7.6	94.8 ± 9.7	97.5 ± 6.6	89.8 ± 8.5	−5.12 (−8.09, −2.16)	.001
Insulin (μIU/mL)	12.6 ± 2.2	11.9 ± 2.4	13.0 ± 3.4	10.8 ± 3.0	−1.49 (−2.28, −0.70)	<.001
HOMA-IR	3.0 ± 0.7	2.8 ± 0.7	3.1 ± 0.9	2.6 ± 0.8	−0.36 (−0.55, −0.17)	<.001
QUICKI	0.32 ± 0.008	0.32 ± 0.01	0.32 ± 0.01	0.33 ± 0.01	0.006 (0.002, 0.01)	.006
Triglycerides (mg/dL)	111.1 ± 46.2	110.1 ± 47.9	109.7 ± 36.8	97.4 ± 36.2	−12.42 (−20.47, −4.37)	.003
VLDL-cholesterol (mg/dL)	22.2 ± 9.2	22.0 ± 9.6	21.9 ± 7.4	19.5 ± 7.2	−2.48 (−4.09, −0.87)	.003
Total cholesterol (mg/dL)	165.5 ± 27.0	168.2 ± 30.0	166.1 ± 35.3	164.9 ± 37.9	−3.58 (−12.53, 5.36)	.42
LDL-cholesterol (mg/dL)	95.4 ± 23.8	98.5 ± 26.6	100.6 ± 31.4	101.1 ± 30.9	−1.97 (−10.37, 6.43)	.63
HDL-cholesterol (mg/dL)	47.9 ± 5.2	47.7 ± 7.1	43.5 ± 11.3	44.3 ± 12.3	1.20 (−1.54, 3.94)	.38
Total-/HDL-cholesterol ratio	3.5 ± 0.7	3.6 ± 1.0	4.0 ± 1.3	3.9 ± 1.1	−0.20 (−0.49, 0.08)	.16

Data are mean ± SD.
[a]'Outcome measures' refers to the change in values of measures of interest between baseline and week 12. β (difference in the mean outcomes measures between treatment groups [myo-inositol group =1 and metformin group =0]).
[b]Obtained from multiple regression model (adjusted for baseline values of each biochemical variables, age, and baseline BMI).
FPG: fasting plasma glucose; HOMA-IR: homeostasis model of assessment-insulin resistance; HDL-cholesterol: high-density lipoprotein-cholesterol; LDL-cholesterol: low-density lipoprotein-cholesterol; QUICKI: quantitative insulin sensitivity check index; VLDL-cholesterol: very low-density lipoprotein-cholesterol.

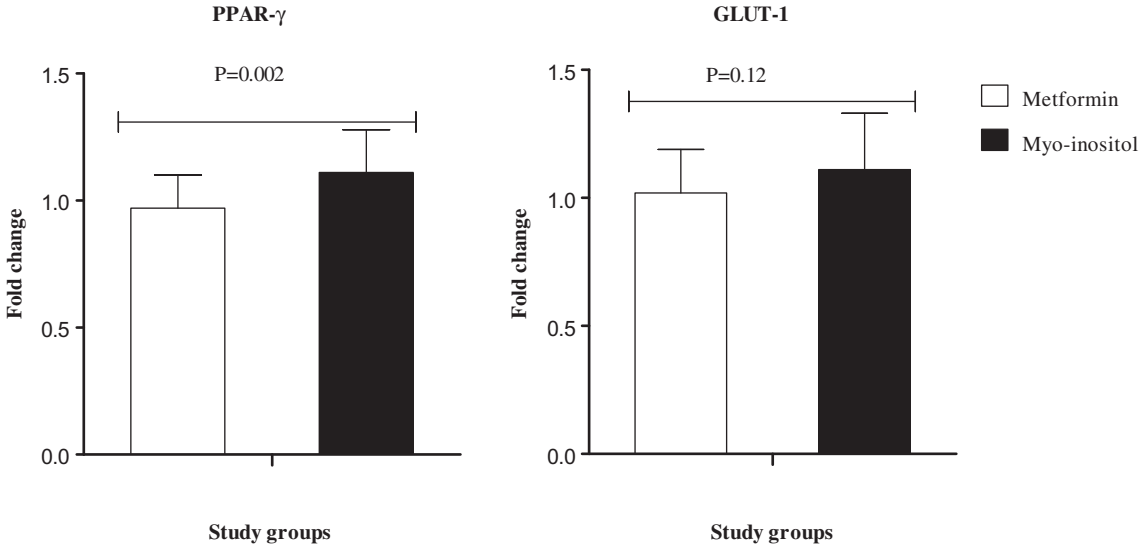

Figure 2. Effect of the 12-week supplementation with myo-inositol or metformin on expression ratio of PPAR-γ and GLUT-1 gene in PBMCs of PCOS women.

reproductive outcome and also their metabolic parameters. Artini et al. [27] observed a significant amelioration of both menstrual regularity and insulin metabolism. Moreover, it has been shown that follicles containing good quality oocytes have higher values of myo-inositol in follicular fluid [28]. Decreased PPAR-γ expression was also reported in PCOS rats compared with the control group [29]. Several studies have demonstrated that the mechanism of insulin deficiency results from in the inositolphosphoglycan (IPG) mediator and that a deficiency of inositol in the IPG is responsible for insulin resistance. It has been shown that the use of D-chiro-inositol reduces insulin resistance [30, 31]. Several studies have suggested that a defect of the insulin pathway can be due to impairment in the IPG second messenger [32, 33]. IPG play a role in activating enzymes that control glucose metabolism [30, 34]. In PCOS patients, impairment in tissue availability or altered metabolism of inositol or IPG mediators may contribute to insulin resistance [35]. Earlier, it has been suggested that myo-inositol can restore spontaneous ovarian activity in women with PCOS and consequently fertility in many of these patients [36].

Effects on lipid parameters

Triglycerides, LDL-, and VLDL-cholesterol levels are higher in PCOS subjects as compared with non-PCOS women [37]. We found that myo-inositol administration for 12 weeks to women with PCOS had beneficial effects on triglycerides and VLDL-cholesterol, but did not affect other lipid profiles and gene expression of LDLR. In accordance to these results, combined treatment with myo-inositol and D-chiro-inositol improved lipid profiles in obese PCOS woman [9]. In addition, combined therapy of myo-inositol and monacolin k for 6 months to women with PCOS reduced triglycerides, total-, and LDL-cholesterol levels [38]. Also, decreasing levels of total cholesterol and triglycerides were observed following the administration of myo-inositol among subjects with PCOS [39]. However, data on comparison of myo-inositol and metformin on lipid profiles among PCOS patients are limited, several studies have evaluated the impact of folate supplementation on lipid profiles among patients with/or without PCOS. We have previously shown that folate supplementation (5 mg/d) to subjects with PCOS decreased total-, LDL-, and total-/HDL-cholesterol ratio [40]. Also,

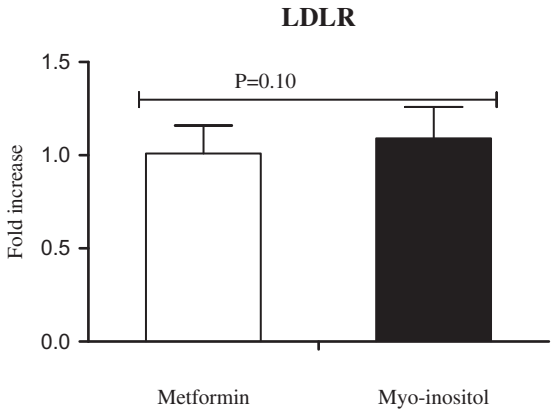

Figure 3. Effect of the 12-week supplementation with myo-inositol or metformin on expression ratio of LDLR gene in PBMCs of PCOS women. GLUT-1: glucose transporter 1; LDLR: low-density lipoprotein receptor; PPAR-γ: peroxisome proliferator-activated receptor gamma; PCOS: polycystic ovary syndrome; PBMCs: peripheral blood mononuclear cells.

folate administration (5 mg/d) among subjects with end-stage renal disease for 6 months reduced total cholesterol [41]. In contrast to our findings, in a meta-analysis by Carvalho et al. [42], myo-inositol administration did not effect on cholesterol and triglycerides levels. In addition, it has been demonstrated folate supplementation (5 mg/d) for 4 weeks to familial hypercholesterolemia did not influence lipid profiles [43]. Also, these data support the role of inositol as a modulator of insulin-mediated metabolic parameters and fit with studies previous evidence emphasizing the association between insulin resistance and inositol-phosphoglycan intracellular balance [30].

This study had a few limitations. First, in the present study, the evaluation of insulin resistance was only based on HOMA-IR. We did assess no direct dynamic test, including glucose tolerance test or hyperinsulinemic euglycemic clamp. Therefore, this should be taken into account in the interpretation of our findings. In addition, the sample size and duration of the intervention were low. Future studies with longer duration and bigger sample size are needed to confirm our findings.

Overall, taking myo-inositol for 12 weeks by women with PCOS had beneficial effects on glycemic control, triglycerides, and VLDL-cholesterol levels, and gene expression of PPAR-γ, but did not affect other lipid profiles and gene expression of GLUT-1 and LDLR.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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