

# A Trial on The Effects of Magnesium-Zinc-Calcium-Vitamin D Co-Supplementation on Glycemic Control and Markers of Cardio-Metabolic Risk in Women with Polycystic Ovary Syndrome

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## Abstract

**Background:** There is scarce data on the effects of magnesium-zinc-calcium-vitamin D co-supplementation on glycemic control and markers of cardio-metabolic risk among women with polycystic ovary syndrome (PCOS). The objective of this study was to assess the effects of magnesium-zinc-calcium-vitamin D co-supplementation on glycemic control and markers of cardio-metabolic risk in women with PCOS.

**Methods:** Sixty PCOS women were randomized into two groups and treated with 100 mg of magnesium, 4 mg of zinc, 400 mg of calcium plus 200 IU of vitamin D supplements (n = 30) or placebo (n = 30) twice a day for 12 weeks. Glycemic control and markers of cardio-metabolic risk were assessed at baseline and at the end of trial.

**Results:** After the 12-week intervention, compared with the placebo, magnesium-zinc-calcium-vitamin D co-supplementation supplementation resulted in significant reductions in serum insulin levels ( $-1.9 \pm 4.6$  vs.  $+0.4 \pm 2.8$   $\mu$ IU/mL,  $P = 0.01$ ), and homeostatic model of assessment for insulin resistance ( $-0.4 \pm 1.0$  vs.  $+0.1 \pm 0.6$ ,  $P = 0.02$ ), as well as a significant increase in quantitative insulin sensitivity check index ( $+0.01 \pm 0.02$  vs.  $-0.0003 \pm 0.01$ ,  $P = 0.02$ ). In addition, magnesium-zinc-calcium-vitamin D co-supplementation significantly decreased serum triglycerides ( $-26.5 \pm 42.9$  vs.  $+8.9 \pm 17.9$  mg/dL,  $P < 0.001$ ), VLDL-cholesterol concentrations ( $-5.3 \pm 8.6$  vs.  $+1.8 \pm 3.6$  mg/dL,  $P < 0.001$ ), total cholesterol ( $-4.2 \pm 30.7$  vs.  $+11.1 \pm 28.4$  mg/dL,  $P = 0.04$ ) and total-/HDL-cholesterol ratio ( $-0.04 \pm 0.6$  vs.  $+0.3 \pm 0.9$ ,  $P = 0.04$ ) compared with the placebo.

**Conclusions:** Overall, the results of this study demonstrated that magnesium-zinc-calcium-vitamin D co-supplementation for 12 weeks among patients with PCOS had beneficial effects on insulin metabolism and markers of cardio-metabolic risk.

**Keywords:** Cardio-metabolic, glycemic control, polycystic ovary syndrome, supplementation

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## Introduction

Polycystic ovary syndrome (PCOS) is a common endocrinopathy in women of reproductive age, which leads to metabolic disorders, hyperandrogenism, hirsutism and sub-fertility.<sup>1</sup> Its prevalence among premenopausal women is 6–15% based on the diagnostic criteria used.<sup>2</sup> PCOS accompanies significant health conditions, such as type 2 diabetes mellitus (T2DM),<sup>3</sup> various cardiometabolic diseases<sup>4</sup> and metabolic syndrome (MetS).<sup>5</sup> Metabolic diseases in subjects with PCOS are characterized by certain features including insulin resistance, impaired glucose metabolism and dyslipidemia.<sup>6–7</sup> It has been shown that dyslipidemia and hyperinsulinemia are present in PCOS affected women as well as women with MetS.<sup>8</sup>

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The exact cause of PCOS is still unknown. Most women with PCOS, regardless of weight, have insulin resistance.<sup>9</sup> Therefore, lifestyle changes that improve sensitivity to insulin should be the first-line therapy for this syndrome.<sup>10</sup> A few studies have reported that zinc and magnesium levels are significantly lower in patients with PCOS.<sup>11–12</sup> In addition, some studies have reported the beneficial effects of trace elements on metabolic profiles.<sup>13–14</sup> We have previously shown that calcium and vitamin D co-supplementation for 8 weeks among women with PCOS improved markers of insulin metabolism, triglycerides and VLDL-cholesterol levels, but did not affect fasting plasma glucose (FPG) and other lipid profiles.<sup>15</sup> In addition, Guerrero-Romero *et al.*<sup>13</sup> observed that receiving 382 mg/day of magnesium for 4 months had beneficial effects on glycemic status and lipid parameters in non-pregnant women with prediabetes. Joint calcium and vitamin D supplementation could also significantly improve markers of insulin metabolism and lipids fractions in people with T2DM.<sup>16</sup>

Calcium and vitamin D supplementation have been used jointly in other studies. Their co-supplementations may be more functional than single supplementation. There is evidence indicating the importance of magnesium-zinc-calcium-vitamin

D co-supplementation on glycemic control and markers of cardio-metabolic risk in subjects with PCOS. We hypothesized that magnesium-zinc-calcium-vitamin D intake might affect glycemic control and markers of cardio-metabolic risk in subjects with PCOS. The aim of this study is to investigate the effects of magnesium-zinc-calcium-vitamin D intake on glycemic control and markers of cardio-metabolic risk in subjects with PCOS.

## Materials and Methods

### Trial design and subjects

We conducted a randomized, double-blind, placebo-controlled trial, registered in the Iranian registry of clinical trials (<http://www.irct.ir: IRCT201701165623N103>), on 60 patients who were referred to the Research and Clinical Center for Infertility at the Kosar clinic affiliated to Arak University of Medical Sciences (AUMS), Arak, Iran between January 2017 and April 2017. Inclusion criteria were PCOS women, according to the Rotterdam criteria,<sup>17</sup> aged 18–40 years. The study was approved by the research ethics committee of AUMS and written informed consent was taken from all participants prior to the intervention. Intake of magnesium, zinc, calcium and/or vitamin D supplements within the past 3 months, pregnant women, metabolic disorders, including hyperandrogenism, Cushing's syndrome, androgen-secreting tumors, hyperprolactinemia and thyroid dysfunction, and not residing in Arak area were the exclusion criteria.

### Study design

At the onset of the study, subjects were matched for BMI (<25 and  $\geq 25$  kg/m<sup>2</sup>), age (<30 and  $\geq 30$  y), phenotypes A (13 subjects in each group) and D (17 subjects in each group) of PCOS. Then, PCOS women were randomized into two groups to receive either 100 mg magnesium, 4 mg zinc, 400 mg calcium plus 200 IU vitamin D supplements (n = 30) or placebo (n = 30) twice a day for 12 weeks. Shape and size of supplements and placebo tablets were similar and manufactured by Vitane (Wolfratshausen, Germany) and Barij Essence Pharmaceuticals (Kashan, Iran), respectively. All subjects were taking metformin tablet at the initial dose of 500 mg, which was increased in a stepwise manner during the first 3 weeks to a total of 1500 mg/day. Quality control of magnesium-zinc-calcium-vitamin D supplements was done in the laboratory of Food and Drug Administration in Tehran, Iran by high-performance liquid chromatography (for vitamin D) and atomic absorption spectroscopy (for magnesium, zinc and calcium) methods, respectively. Following quality control, we observed that the amounts of magnesium, zinc, calcium and vitamin D in the prescribed supplements were in the range of 95–110 mg, 3.8–4.4 mg, 380–440 mg and 190–210 IU, respectively. All participants were advised to maintain their routine dietary habits, not to change other lifestyle factors, including physical activity during the study. All women completed 3-day food records and three physical activity records as metabolic equivalents (METs) at weeks 0, 3, 6, 9 and 12 of the treatment.

### Treatment adherence

To evaluate compliance, subjects were asked to bring the medication container. To ensure adherence, participants received a daily short message on their cell phones as a reminder for intake of supplements. In addition, compliance to co-supplementation was assessed through quantification of serum magnesium, zinc,

calcium and vitamin D levels.

### Assessment of anthropometric measures

At baseline and end-of-trial, all subjects underwent standard anthropometric measurements: height and weight (Seca, Hamburg, Germany). BMI was calculated as weight in kg divided by height in meters squared.

### Assessment of outcomes

The primary outcomes were markers of glycemic control. The secondary outcomes were markers of cardio-metabolic risk, including lipid profiles, atherogenic index of plasma (AIP), atherogenic coefficient (AC) and cardiac risk ratio (CRR).

### Biochemical assessment

Ten mL fasting blood samples were taken at weeks 0 and 6 of the intervention. To determine FPG, serum magnesium, zinc, calcium, triglycerides, VLDL-, total-, LDL- and HDL-cholesterol concentrations, we used enzymatic kits (Pars Azmun, Tehran, Iran). All inter- and intra-assay coefficient variances (CVs) for FPG, magnesium, zinc, calcium and lipid concentrations were lower than 5%. Serum 25-hydroxyvitamin D concentrations were evaluated using a commercial ELISA kit (IDS, Boldon, UK) with inter- and intra-assay CVs of 4.4 to 6.3%, respectively. Circulating levels of serum insulin were assessed using the ELISA kit (Monobind, California, USA) with the intra- and inter-assay CVs 3.3 and 4.7%, respectively. The homeostatic model of assessment for insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI) were determined according to suggested formulas. AIP, AC and CRR were calculated based on suggested formulas. Measurements of insulin and lipid fractions were conducted in a blinded fashion, in duplicate, in pairs (pre/post-intervention) at the same time, in the same analytical run, and in random order to reduce systematic error and inter-assay variability.

### Randomization

Random assignment was conducted using computer-generated random numbers. Randomization and allocation were concealed from the researchers and participants and were carried out by a trained midwife at the gynecology clinic.

### Statistical methods

To calculate the sample size, we used the standard formula suggested for clinical trials by considering type one error ( $\alpha$ ) of 0.05 and type two error ( $\beta$ ) of 0.20 (power = 80%). Based on a previous study,<sup>15</sup> we used 2.40 as SD and 1.83 as the difference in mean (d) of HOMA-IR as primary variable. The correlation of HOMA-IR between subsequent 12-week periods was  $r = 0.26$ . Based on this, we needed 25 subjects in each group. Considering a drop-out rate of 5 subjects per group, we calculated to have 30 subjects per group.

The Kolmogorov-Smirnov test was applied to control the normal distribution of variables. Independent sample *t*-test was used to establish changes in anthropometric measures and dietary intakes between the two groups. To determine the effects of magnesium-zinc-calcium-vitamin D co-supplementation on glycemic control and markers of cardio-metabolic risk, we used independent samples *t*-test. Adjustment for changes in baseline values of biochemical variables, age and baseline BMI was performed

by analysis of covariance (ANCOVA). The effect size ( $r^2$ ) was calculated as the standardized mean difference between the two groups divided by SD of the population from which the groups were sampled.  $P < 0.05$  was considered statistically significant. All statistical analyses were conducted using the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, Illinois, USA).

## Results

From 65 subjects who were recruited in our study (5 who did not meet the inclusion criteria were excluded from the study), 60 participants in each group completed the trial. On average, more than 90% of supplements were consumed in both groups throughout the study. No side effects were reported following the intake of magnesium-zinc-calcium-vitamin D co-supplements in patients with PCOS throughout the study.

Mean age, height, baseline and end-of-trial of weight and BMI were not significantly different between the two groups (Data not shown).

According to the 3-day dietary records taken throughout the intervention, no statistically significant changes were observed between the two groups in terms of macro- and micro-nutrient intakes (Data not shown).

After the 12-week intervention, compared with the placebo, magnesium-zinc-calcium-vitamin D co-supplementation significantly increased serum magnesium ( $+0.1 \pm 0.1$  vs.  $-0.1 \pm 0.3$  mg/dL,  $P = 0.002$ ), calcium ( $+0.4 \pm 0.3$  vs.  $-0.01 \pm 0.6$  mg/dL,  $P = 0.001$ ) and 25-OH-vitamin D ( $+7.9 \pm 8.4$  vs.  $+0.1 \pm 8.4$  ng/mL,  $P < 0.001$ ). In addition, compared with the placebo, magnesium-zinc-calcium-vitamin D co-supplementation supplementation resulted in significant reductions in serum insulin levels ( $-1.9 \pm 4.6$  vs.  $+0.4 \pm 2.8$   $\mu$ IU/mL,  $P = 0.01$ ), and HOMA-IR ( $-0.4 \pm 1.0$  vs.

$+0.1 \pm 0.6$ ,  $P = 0.02$ ), as well as a significant increase in QUICKI ( $+0.01 \pm 0.02$  vs.  $-0.0003 \pm 0.01$ ,  $P = 0.02$ ) (Table 1). Additionally, magnesium-zinc-calcium-vitamin D co-supplementation significantly decreased serum triglycerides ( $-26.5 \pm 42.9$  vs.  $+8.9 \pm 17.9$  mg/dL,  $P < 0.001$ ), VLDL-cholesterol concentrations ( $-5.3 \pm 8.6$  vs.  $+1.8 \pm 3.6$  mg/dL,  $P < 0.001$ ), total cholesterol ( $-4.2 \pm 30.7$  vs.  $+11.1 \pm 28.4$  mg/dL,  $P = 0.04$ ), total-/HDL-cholesterol ratio ( $-0.04 \pm 0.6$  vs.  $+0.3 \pm 0.9$ ,  $P = 0.04$ ), AIP ( $-0.07 \pm 0.14$  vs.  $+0.04 \pm 0.08$ ,  $P < 0.001$ ), AC ( $-0.04 \pm 0.61$  vs.  $+0.36 \pm 0.89$ ,  $P = 0.04$ ) and CRR ( $-0.04 \pm 0.60$  vs.  $+0.36 \pm 0.89$ ,  $P = 0.04$ ) compared with the placebo. We did not see any significant effect of magnesium-zinc-calcium-vitamin D co-supplementation on LDL- and HDL-cholesterol levels.

There was a significant difference in baseline levels of FPG ( $P = 0.03$ ), magnesium ( $P < 0.001$ ) and calcium ( $P = 0.005$ ) between the two groups. Therefore, we adjusted the analysis for baseline biochemical variables. When we controlled the analysis for baseline values of biochemical variables, the difference in changes in insulin ( $P = 0.06$ ) between the two groups became non-significant, while other findings did not alter (Data not shown). In addition, when we controlled the analysis for baseline values of biochemical variables, age and baseline BMI, the difference in changes in insulin ( $P = 0.10$ ), HOMA-IR ( $P = 0.08$ ), QUICKI ( $P = 0.08$ ), total-/HDL-cholesterol ratio ( $P = 0.06$ ), AC ( $P = 0.06$ ) and CRR ( $P = 0.06$ ) between the two groups became non-significant, while other findings did not alter (Table 2).

## Discussion

We found that magnesium-zinc-calcium-vitamin D co-supplementation for 12 weeks among patients with PCOS had beneficial effects on insulin metabolism and markers of

**Table 1.** Means ( $\pm$  standard deviation) of glycemic control and markers of cardio-metabolic risk at baseline and after the 12-week intervention in patients with polycystic ovary syndrome who received either magnesium-zinc-calcium-vitamin D supplements or placebo.

	Placebo group (n=30)			Magnesium-zinc-calcium-vitamin D group (n = 30)			$P^1$
	Baseline	End-of-trial	Change	Baseline	End-of-trial	Change	
FPG (mg/dL)	90.0 $\pm$ 4.8	91.1 $\pm$ 5.9	1.1 $\pm$ 5.0	86.6 $\pm$ 6.9	86.7 $\pm$ 7.5	0.1 $\pm$ 6.5	0.50
Insulin ( $\mu$ IU/mL)	11.2 $\pm$ 3.9	11.6 $\pm$ 4.5	0.4 $\pm$ 2.8	12.9 $\pm$ 4.4	11.0 $\pm$ 4.6	-1.9 $\pm$ 4.6	0.01
HOMA-IR	2.5 $\pm$ 0.9	2.6 $\pm$ 1.0	0.1 $\pm$ 0.6	2.8 $\pm$ 0.9	2.4 $\pm$ 1.0	-0.4 $\pm$ 1.0	0.02
QUICKI	0.33 $\pm$ 0.01	0.33 $\pm$ 0.02	-0.0003 $\pm$ 0.01	0.33 $\pm$ 0.02	0.34 $\pm$ 0.02	0.01 $\pm$ 0.02	0.02
Triglycerides (mg/dL)	117.7 $\pm$ 50.9	126.6 $\pm$ 55.5	8.9 $\pm$ 17.9	121.6 $\pm$ 67.2	95.1 $\pm$ 38.6	-26.5 $\pm$ 42.9	<0.001
VLDL-cholesterol (mg/dL)	23.5 $\pm$ 10.2	25.3 $\pm$ 11.1	1.8 $\pm$ 3.6	24.3 $\pm$ 13.4	19.0 $\pm$ 7.7	-5.3 $\pm$ 8.6	<0.001
Total cholesterol (mg/dL)	163.3 $\pm$ 28.8	174.4 $\pm$ 34.2	11.1 $\pm$ 28.4	160.2 $\pm$ 37.9	155.9 $\pm$ 28.2	-4.2 $\pm$ 30.7	0.04
LDL-cholesterol (mg/dL)	90.4 $\pm$ 25.7	100.7 $\pm$ 31.1	10.3 $\pm$ 29.3	86.9 $\pm$ 27.8	88.4 $\pm$ 24.4	1.5 $\pm$ 24.5	0.21
HDL-cholesterol (mg/dL)	49.3 $\pm$ 7.6	48.4 $\pm$ 9.3	-0.9 $\pm$ 6.5	48.9 $\pm$ 7.8	48.5 $\pm$ 7.1	-0.4 $\pm$ 7.7	0.77
Total-/HDL-cholesterol ratio	3.4 $\pm$ 0.8	3.7 $\pm$ 1.1	0.3 $\pm$ 0.9	3.3 $\pm$ 0.7	3.25 $\pm$ 0.6	-0.05 $\pm$ 0.6	0.04
AIP	0.34 $\pm$ 0.23	0.38 $\pm$ 0.25	0.04 $\pm$ 0.08	0.34 $\pm$ 0.22	0.26 $\pm$ 0.20	-0.07 $\pm$ 0.14	<0.001
AC	2.39 $\pm$ 0.83	2.75 $\pm$ 1.16	0.36 $\pm$ 0.89	2.29 $\pm$ 0.68	2.25 $\pm$ 0.61	-0.04 $\pm$ 0.60	0.04
CRR	3.39 $\pm$ 0.83	3.75 $\pm$ 1.16	0.36 $\pm$ 0.89	3.29 $\pm$ 0.68	3.25 $\pm$ 0.61	-0.04 $\pm$ 0.61	0.04

All values are means  $\pm$  SDs.  
<sup>1</sup>  $P$  values represent independent sample  $t$ -test.  
 AIP = atherogenic index of plasma; AC = atherogenic coefficient; CRR = cardiac risk ratio; FPG = fasting plasma glucose; HOMA-IR = homeostasis model of assessment-estimated insulin resistance; QUICKI = quantitative insulin sensitivity check index.

**Table 2.** Adjusted changes in metabolic variables in patients with polycystic ovary syndrome who received either magnesium-zinc-calcium-vitamin D supplements or placebo.

	Placebo group (n = 30)	Magnesium-zinc-calcium-vitamin D group (n = 30)	95% CI	Effect size	P <sup>1</sup>
FPG (mg/dL)	1.7±1.0	-0.5±1.0	-0.81, 5.24	0.03	0.14
Insulin (µIU/mL)	0.03±0.7	-1.6±0.7	-0.33, 3.55	0.04	0.10
HOMA-IR	0.02±0.1	-0.3±0.1	-0.04, 0.78	0.05	0.08
QUICKI	0.001±0.003	0.009±0.003	-0.01, 0.001	0.05	0.08
Triglycerides (mg/dL)	7.1±4.8	-24.6±4.8	18.07, 45.28	0.28	<0.001
VLDL-cholesterol (mg/dL)	1.4±1.0	-4.9±1.0	3.61, 9.05	0.28	<0.001
Total cholesterol (mg/dL)	10.0±4.2	-3.1±4.2	1.18, 25.11	0.08	0.03
LDL-cholesterol (mg/dL)	9.3±3.9	2.5±3.9	-4.40, 17.88	0.02	0.23
HDL-cholesterol (mg/dL)	-0.5±1.2	-0.8±1.2	-3.12, 3.71	0.001	0.86
Total-/HDL-cholesterol ratio	0.3±0.1	-0.001±0.1	-0.02, 0.65	0.05	0.06
AIP	0.03±0.02	-0.07±0.02	0.04, 0.16	0.18	0.001
AC	0.31±0.11	-0.001±0.11	-0.02, 0.65	0.05	0.06
CRR	0.31±0.11	-0.001±0.11	-0.02, 0.65	0.05	0.06
All values are means± SEs.					
<sup>1</sup> Obtained from ANCOVA test adjusted for baseline values+ maternal age and baseline BMI.					
AIP = atherogenic index of plasma; AC = atherogenic coefficient; CRR = cardiac risk ratio; FPG = fasting plasma glucose; HOMA-IR = homeostasis model of assessment-estimated insulin resistance; QUICKI = quantitative insulin sensitivity check index.					

cardio-metabolic risk. To our knowledge, this study is the first report of the effects of magnesium-zinc-calcium-vitamin D co-supplementation on glycemic control and markers of cardio-metabolic risk among women with PCOS. In the current study, the effect size of FPG, insulin, HOMA-IR, QUICKI, total-, HDL-cholesterol, total-/HDL-cholesterol ratio, AC and CRR was  $\leq 0.05$ , meaning that the score of the average person in the intervention group was lower than 0.05 standard deviations above the average person in the placebo group, and hence exceeds the scores of 50% of the placebo group. Moreover, the effect size of AIP was nearly 0.2, meaning that the score of the average person in the intervention group was nearly 0.2 standard deviations above the average person in the placebo group, and hence exceeds the scores of 58% of the placebo group. Finally, the effect size of triglycerides and VLDL-cholesterol was nearly 0.3, meaning that the score of the average person in the intervention group was nearly 0.3 standard deviations above the average person in the placebo group, and hence exceeds the scores of 62% of the placebo group. It must be considered that there was a significant difference in baseline levels of FPG, magnesium and calcium between the magnesium-zinc-calcium-vitamin D and the placebo groups at study baseline. This difference might have occurred due to several reasons. The diagnosis of PCOS in our study was conducted based on the Rotterdam criteria. Therefore, different patients might have had different plasma glucose, magnesium and calcium levels, which could in turn have led to a different mean of FPG, magnesium and calcium at study baseline. Furthermore, we did not randomize participants based on their FPG, magnesium and calcium levels because all participants had PCOS. Random assignment to two groups was done after stratification for BMI ( $<25$  and  $\geq 25$  kg/m<sup>2</sup>), age ( $<30$  and  $\geq 30$  y), phenotypes A and D of PCOS, and random assignment was done using computer-generated random numbers. Therefore, the difference in FPG, magnesium and calcium levels between the two groups had occurred randomly. In addition, when we adjusted the analyses for baseline values,<sup>18</sup> no significant changes were observed in our findings except for insulin levels.

Previous studies have reported that PCOS women are

susceptible to metabolic disorders, including insulin resistance and dyslipidemia.<sup>15,19</sup> This is therefore a good group to target with dietary interventions, including magnesium, zinc, calcium and vitamin D supplementation in patients with PCOS. The current study demonstrated that magnesium-zinc-calcium-vitamin D co-supplementation for 12 weeks in PCOS women resulted in a significant reduction in serum insulin concentrations, and HOMA-IR, as well as a significant increase in QUICKI compared with the placebo. When we adjusted the analyses for baseline values of biochemical variables, the change in serum insulin levels was not significantly different between the groups. However, the beneficial effects of single zinc, calcium and vitamin D supplementation on metabolic profiles in patients with PCOS have been previously evaluated and, to our knowledge, this study is the first assessing the favorable effects of magnesium-zinc-calcium-vitamin D co-supplementation on glycemic control and markers of cardio-metabolic risk in patients with PCOS. In a meta-analysis by Morais *et al.*<sup>20</sup>, it was observed that magnesium supplementation decreased insulin resistance in patients with hypomagnesemia presenting insulin resistance. In addition, a 12-week zinc supplementation at a dosage of 20 mg/day as elemental zinc significantly improved metabolic risk parameters among obese subjects.<sup>21</sup> We have previously shown that calcium-vitamin D co-supplementation (1000 mg of calcium/day + 50,000 IU/wk of vitamin D) for 8 weeks among vitamin D deficient subjects with PCOS had beneficial effects on insulin concentrations, HOMA-IR, QUICKI, triglycerides and VLDL-cholesterol levels, but did not influence FPG and other lipid profiles.<sup>15</sup> However, magnesium supplementation at a dosage of 300 mg/day as magnesium oxide for 12 weeks did not affect insulin sensitivity in overweight subjects.<sup>22</sup> Insulin resistance is associated with decreased synthesis and release of nitric oxide (NO), elevated inactivation of NO after its release from endothelial cells, and increased synthesis of vasoconstricting agents, leading to impaired vasodilatory action of insulin in women with PCOS.<sup>23</sup> Regarding magnesium's effects on circulating glucose and insulin, it is emphasized that this mineral participates in phosphorylation reactions of the signaling pathway of this hormone; it is also

part of the  $Mg^{2+}$ -ATP complex.<sup>24-25</sup> In addition, zinc intake may modulate the protein tyrosine phosphatase 1B, a key regulator of the phosphorylation state of the insulin receptor.<sup>26</sup> Zinc element has been documented to inhibit 5  $\alpha$ -reductase, which catalyzes the transformation of testosterone to its non-aromatizable form, di-hydro testosterone (DHT).<sup>27</sup> Thus, elevated levels of zinc may help to reduce PCOS-associated hyperandrogenemia through inhibiting transformation of testosterone to its active form DHT.<sup>11</sup> The effects of vitamin D and calcium intake on glycemic control might be mediated through increased expression of insulin receptors and regulation of serum calcium.<sup>28</sup>

We found that magnesium-zinc-calcium-vitamin D co-supplementation for 12 weeks in PCOS women was associated with a significant reduction in serum triglycerides, VLDL-, total-cholesterol concentrations, total-/HDL-cholesterol ratio, AIP, AC and CRR, but did not influence other lipid profiles. Supporting our study, magnesium supplementation at a dosage of 600 mg/day for 4-8 weeks resulted in a significant decrease in triglycerides levels among patients with T2DM.<sup>29</sup> In addition, zinc supplementation at a dosage of 30 mg/day for 6 weeks among patients with gestational diabetes mellitus had beneficial effects on triglycerides and VLDL-cholesterol levels, but did not affect other lipid profiles.<sup>30</sup> Likewise, in another study by Major *et al.*,<sup>31</sup> it was seen that co-administration of 1,200 mg/day of calcium plus 400 IU/day of vitamin D for 15 weeks significantly improved lipid profiles. However, no significant change was documented in lipid profiles following supplementation with 360 mg/day of elemental magnesium among patients with T2DM for 12 weeks.<sup>32</sup> Moreover, Payahoo *et al.*<sup>33</sup> demonstrated that supplementation with 30 mg/day of zinc gluconate for 12 weeks among healthy obese adults did not affect lipid profiles. Dyslipidemia is one of the most prevalent metabolic aberrations in PCOS, which is most frequently represented by atherogenic dyslipidemia typical of the states of insulin resistance-namely, hypertriglyceridemia, decreased HDL-cholesterol concentrations, and increased LDL-cholesterol levels.<sup>34</sup> Magnesium intake may improve lipid profiles through increased lipoprotein lipase activity.<sup>35</sup> In addition, the activation of peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ ), and the down-regulation of inflammatory markers and endothelial cell adhesion molecules in endothelial cells were documented to be zinc-dependent.<sup>36</sup> The PPAR- $\gamma$  of nuclear receptors, the mediators for lipoprotein metabolism and glucose homeostasis, was shown to play a key protective role in development and progression of atherosclerosis.<sup>37</sup> In addition, calcium and vitamin D co-supplementation may improve lipid profiles through affecting parathyroid hormone concentrations and improving insulin sensitivity.<sup>38</sup>

The current study had a number of limitations. Due to limited funding, we could not evaluate the effects of magnesium-zinc-calcium-vitamin D co-supplementation on gene expression related to insulin resistance and lipid. In addition, further studies are needed with single supplementation of each compared with co-supplementation to evaluate the beneficial effects on glycemic control and markers of cardio-metabolic risk. Considering our main objectives, we did not compare the two groups for ovulation rate. These should be considered when interpreting our findings.

Overall, the results of this study demonstrated that magnesium-zinc-calcium-vitamin D co-supplementation for 12 weeks among patients with PCOS had beneficial effects on insulin metabolism and markers of cardio-metabolic risk.

## Authors' contributions

ZA contributed in conception, design, statistical analysis and drafting of the manuscript. MJ and MM contributed in data collection and manuscript drafting. ZA supervised the study.

**Conflicts of interest:**None.

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