

Magnesium-Zinc-Calcium-Vitamin D Co-supplementation Improves Hormonal Profiles, Biomarkers of Inflammation and Oxidative Stress in Women with Polycystic Ovary Syndrome: a Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract Data on the effects of magnesium-zinc-calcium-vitamin D co-supplementation on hormonal profiles, biomarkers of inflammation, and oxidative stress among women with polycystic ovary syndrome (PCOS) are scarce. The objective of this study was to assess the effects of magnesium-zinc-calcium-vitamin D co-supplementation on hormonal profiles, biomarkers of inflammation, and oxidative stress in women with PCOS. Sixty PCOS women were randomized into two groups and treated with 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. Hormonal profiles, biomarkers of inflammation, and oxidative stress were assessed at baseline and at end-of-treatment. After the 12-week intervention, compared with the placebo, magnesium-zinc-calcium-vitamin D co-supplementation resulted in significant reductions in hirsutism (-2.4 ± 1.2 vs. -0.1 ± 0.4 , $P < 0.001$), serum high sensitivity C-reactive protein (-0.7 ± 0.8 vs. $+0.2 \pm 1.8$ mg/L, $P < 0.001$), and plasma malondialdehyde (-0.4 ± 0.3 vs. $+0.2 \pm 1.0$ $\mu\text{mol/L}$, $P = 0.03$), and a significant increase in plasma total antioxidant capacity concentrations ($+46.6 \pm 66.5$ vs. -7.7 ± 10.1 $\mu\text{mol/L}$, $P = 0.04$). We failed to find any significant effect of magnesium-zinc-calcium-vitamin D co-supplementation on free androgen index, and other biomarkers of inflammation and oxidative stress. Overall, magnesium-zinc-calcium-vitamin D co-supplementation for 12 weeks among PCOS women had beneficial effects on hormonal profiles, biomarkers of inflammation, and oxidative stress.

Keywords Supplementation · Polycystic ovary syndrome · Endocrine profiles · Inflammation

Introduction

Polycystic ovary syndrome (PCOS) is a frequent gynecological endocrine disorder of women in the childbearing age and is associated with infertility, menstrual irregularities, and biochemical or clinical changes of hyperandrogenism [1]. Approximately, 6–12% of premenopausal women depending on the diagnostic criteria used are affected by this disease [2]. In several previous studies [3, 4], it reported that subjects with PCOS had significantly higher levels of high sensitivity C-reactive protein (hs-CRP). Increased levels of hs-CRP were associated with fat mass, hyperinsulinemia, and insulin resistance in PCOS [5]. Different studies in PCOS women have hinted that insulin resistance plays an important role in the pathophysiology of metabolic syndrome, and that this can induce an increase in biomarkers of oxidative stress and reactive oxygen species (ROS) production by leukocytes [6, 7].

Weight loss and lifestyle change, such as increased physical activity, are the first-line treatment in the management of PCOS [8]. Recently, the role of minerals, including magnesium, zinc and calcium, and vitamin D in the pathogenesis of many metabolic diseases, such as PCOS, has been examined [9, 10]. In addition, few studies have reported that mean zinc concentrations in PCOS women are lower than healthy women [11], while, others demonstrated higher levels of zinc in PCOS [9]. We have previously shown that vitamin D-K-calcium co-supplementation for 8 weeks among subjects with PCOS had beneficial effects on serum dehydroepiandrosterone sulfate (DHEAS), free testosterone, and biomarkers of oxidative stress, but did not affect inflammatory markers [12]. In addition, in a study by Nielsen et al. [13], it was

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observed that taking 320 mg/day of magnesium supplements for 7 weeks improved inflammatory factors in elderly people. Also, calcium and vitamin D co-supplementation in subjects with gestational diabetes had beneficial effects on biomarkers of oxidative stress, but unchanged inflammatory markers [14]. However, among healthy middle-aged overweight subjects, supplementation with 250 mg/day of magnesium as magnesium oxide for 8 weeks did not affect inflammatory factors [15]. In addition, no significant change in inflammatory markers and biomarkers of oxidative stress was observed following 18-month supplementation with fortified milk containing 1000 mg/day of calcium and 800 IU/day of vitamin D among healthy subjects [16].

Calcium and vitamin D co-supplementation have earlier been used by other researchers. Their co-supplementations may be more efficient than single supplementation. In addition, few previous studies have exhibited that joint calcium and vitamin D supplementation might have a strong synergistic effect on metabolic status. For instance, Tabesh et al. [17] observed joint calcium-vitamin D supplementation compared with single supplementation improved systemic inflammation in vitamin D-insufficient individuals with type 2 diabetes mellitus. Furthermore, we have previously shown that combined calcium and vitamin D intake compared with calcium or vitamin D only for 8 weeks among PCOS patients had beneficial effects on metabolic status [18, 19]. Magnesium intake may improve metabolic profiles through effects of its antagonism to calcium and to participate in protein synthesis, and transmembrane ion transport [20]. In addition, calcium and vitamin D intake might affect inflammation and oxidative stress through their effects on activity increase of antioxidant enzymes [21] and inhibiting synthesis of inflammatory cytokines [22]. According to our knowledge, data on the effects of magnesium-zinc-calcium-vitamin D co-supplementation on hormonal profiles, biomarkers of inflammation, and oxidative stress in subjects with PCOS are scarce. Therefore, we hypothesized that magnesium-zinc-calcium-vitamin D intake might affect metabolic status of PCOS population. We investigated this aim by conducting the effects of magnesium-zinc-calcium-vitamin D co-supplementation on hormonal profiles, markers of inflammation, and oxidative stress in women with PCOS.

Subjects and Methods

Trial Design and Participants

This randomized double-blind placebo-controlled clinical trial was performed among 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 subjects with PCOS according to the Rotterdam criteria [23] and age range 18–40 years. All participants attending the center were first examined by the study gynecologist and after diagnosis of PCOS in all subjects; they were instructed to fill hirsutism or modified Ferriman Gallwey (mF-G) scoring form validated for the Iranian population [24]. The research was approved by the research ethics committee of AUMS. Smokers, intake of magnesium, zinc, calcium and/or vitamin D supplements within the last 3 months, pregnant women, other common causes of hyperandrogenism and/or anovulation, including Cushing's syndrome, androgen-secreting tumors, hyperprolactinemia and thyroid dysfunction, and not living in Arak area were excluded in the study.

Study Design

Forty PCOS women were randomized into two groups and treated with 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. Quality control of magnesium-zinc-calcium-vitamin D tablet was conducted 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 amount of magnesium, zinc, calcium and vitamin D in the prescribed tablets was at the range of 95–110 mg, 3.8–4.4 mg, 380–440 mg, and 190–210 IU, respectively. Shape and size of supplements and placebo tablets were similar and manufactured by Vitane (Wolfratshausen, Germany) and Barij Essence Pharmaceuticals (Kashan, Iran), 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 at weeks 0, 3, 6, 9, and 12 of the treatment.

Treatment Adherence

To evaluate the compliance, subjects were asked to bring the medication container. To ensure adherence, participants received a short message on their cell phones to intake the supplements daily.

Anthropometric Measures

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

Assessment of Outcomes

We considered hormonal profiles as primary outcomes, and biomarkers of inflammation and biomarkers of oxidative stress as secondary outcomes. Ten-milliliter fasting blood samples were collected before and after the 12-week treatment at Arak reference laboratory, Arak, Iran. Commercial kits were used to quantify serum magnesium, zinc, and calcium concentrations (Pars Azmun, Tehran, Iran). All inter- and intra-assay coefficient variances (CVs) for magnesium, zinc, and calcium were less than 5%. Serum 25-hydroxyvitamin D levels were determined by a commercial ELISA kit (IDS, Boldon, UK) with inter- and intra-assay CVs of 4.4 to 6.6%, respectively. Serum total testosterone with inter- and intra-assay CVs of 3.9 to 5.7% and sex hormone-binding globulin (SHBG) with inter- and intra-assay CVs of 3.4 to 5.0% were determined by using commercial kits (DiaMetra, Milano, Italy). Free androgen index (FAI) was calculated as the ratio of total testosterone to SHBG. Serum hs-CRP values were quantified using an ELISA kit (LDN, Nordhorn, Germany) with inter- and intra-assay CVs of 4.4 to 6.6%, respectively. The plasma nitric oxide (NO) by the Griess method [25], total antioxidant capacity (TAC) by the use of the ferric reducing antioxidant power method developed by Benzie and Strain [26], total glutathione (GSH) using the method of Beutler et al. [27], and malondialdehyde (MDA) levels by the thiobarbituric acid reactive substance spectrophotometric test [28] were determined. CVs for plasma NO, TAC, GSH, and MDA were 1.0, 1.1, 2.7, and 3.4%, respectively.

Randomization

Randomization assignment was conducted using computer-generated random numbers. Randomization and allocation concealment were done from the researchers and participants and were carried out by a trained staff at the gynecology clinic. Another person, who was not involved in the intervention and not aware of random sequence, assigned patients to the numbered bottles of capsules.

Statistical Methods

To calculate the sample size, we used the standard formula suggested for clinical trials by considering type one error (α) of 0.05 and type two error (β) of 0.20 (power = 80%). Based on a previous study [29], we used 0.11 ng/mL as SD and 0.09 ng/mL as the difference in mean (d) of total testosterone concentrations as primary variable. Based on this, we needed 25 subjects in each group. Considering a dropout of five 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 hormonal profiles, biomarkers of inflammation, and oxidative stress, we used one-way repeated measures analysis of variance. Adjustment for changes in baseline values of biochemical variables, age, and baseline BMI was performed by analysis of covariance. $P < 0.05$ were considered statistically significant. All statistical analyses were conducted using the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, IL, USA).

Results

From 65 subjects who were recruited to our study (5 subjects were excluded from the study because of not meeting inclusion criteria), 60 participants in each group completed the trial (Fig. 1). On average, higher than 90% of supplements were consumed in both groups throughout the study.

Mean age, height, baseline, and end-of-trial of weight and BMI were not significantly different between the two groups (Table 1).

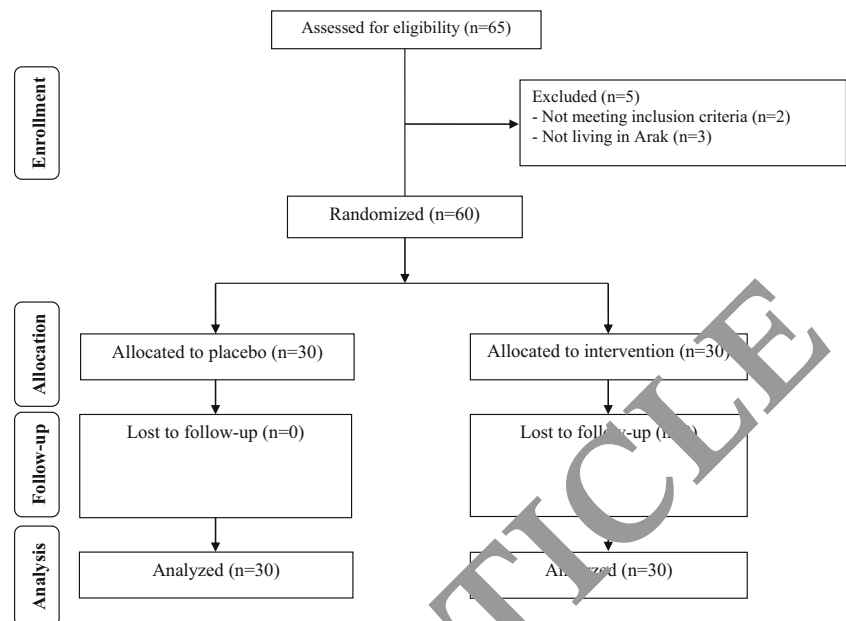
According to the 3-day dietary records taken throughout the intervention, no significant changes was observed between the two groups in terms of macro- and micro-nutrient intakes (data not shown).

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 ± 0.3 mg/dL, $P = 0.002$), calcium ($+0.4 \pm 0.3$ vs. -0.01 ± 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 resulted in significant reductions in hirsutism (-2.4 ± 1.2 vs. -0.1 ± 0.4 , $P < 0.001$), serum hs-CRP (-0.7 ± 0.8 vs. $+0.2 \pm 1.8$ mg/L, $P < 0.001$) and plasma MDA (-0.4 ± 0.3 vs. $+0.2 \pm 1.0$ μ mol/L, $P = 0.01$), and a significant increase in plasma TAC concentrations ($+46.6 \pm 66.5$ vs. -7.7 ± 130.1 mmol/L, $P = 0.04$) (Table 2). We failed to find any significant effect of magnesium-zinc-calcium-vitamin D co-supplementation on FAI, and other biomarkers of inflammation and oxidative stress.

There was a significant difference in baseline levels of TAC ($P = 0.009$) and MDA ($P < 0.001$) between the two groups. Therefore, we adjusted the analysis for baseline of biochemical variables, age, and baseline BMI. When we controlled the analysis for baseline values of biochemical variables, age, and baseline BMI, the difference in changes in TAC ($P = 0.15$) and MDA ($P = 0.95$) between the two groups became non-significant, and serum zinc became significant ($P = 0.01$), while other findings did not alter (Table 3).

Discussion

We found that magnesium-zinc-calcium-vitamin D co-supplementation for 12 weeks among PCOS women had

Fig. 1 Summary of patient flow diagram

beneficial effects on hormonal profiles, biomarkers of inflammation, and oxidative stress. To our knowledge, this study is the first evaluating the effects of magnesium-zinc-calcium-vitamin D co-supplementation on hormonal profiles, biomarkers of inflammation, and oxidative stress among women with PCOS.

Previous studies have supported that PCOS women are susceptible to infertility, hirsutism, increased pro-inflammatory factors, and oxidative stress [30, 31]. Prior studies have documented that vitamin D deficiency is a problem in subjects with PCOS [32, 33]. Many observational studies have reported a relationship between low levels of vitamin D and features of the metabolic syndrome in PCOS women [34, 35]. One hypothesis relies on the regulatory effect of vitamin D on the intracellular and extracellular calcium values that is essential for insulin-mediated intracellular processes and may have influence on insulin resistance [36]. Another theory involves the stimulatory effect of vitamin D on gene expression

related to insulin receptors [37]. This study have indicated that magnesium-zinc-calcium-vitamin D co-supplementation for 12 weeks in 60 PCOS women led to a significant reduction in hirsutism and total testosterone compared with the placebo, but it did not affect SHBG and FAI. Data on the effects of magnesium and zinc supplementation on female fertility and hormonal pictures are scarce. We have previously reported that vitamin D-K-calcium co-supplementation for 8 weeks among women with PCOS resulted in a significant reduction in free testosterone and DHEAS, but it did not influence other hormonal profiles [12]. Furthermore, vitamin D and calcium co-administration in PCOS women for 12 weeks was associated with a significant decrease in total testosterone and androstenedione levels [16]. Firouzabadi et al. [38] demonstrated that taking 100,000 IU vitamin D for 6 months plus 1000 mg/day calcium among PCOS women resulted in improved follicular maturation, regularity of menses, and androgen-related symptoms especially in subjects with

Table 1 General characteristics of study participants

	Placebo group (n = 30)	Magnesium-zinc-calcium-vitamin D group (n = 30)	P ¹
Age (year)	24.8 ± 4.8	23.8 ± 5.7	0.48
Height (cm)	163.5 ± 5.3	164.0 ± 6.6	0.73
Weight at study baseline (kg)	68.3 ± 11.4	65.4 ± 11.8	0.32
Weight at end-of-trial (kg)	67.8 ± 11.8	64.8 ± 11.6	0.31
Weight change (kg)	-0.5 ± 1.1	-0.6 ± 0.5	0.59
BMI at study baseline (kg/m ²)	25.6 ± 4.8	24.2 ± 3.8	0.20
BMI at end-of-trial (kg/m ²)	25.5 ± 4.9	24.0 ± 3.7	0.20
BMI change (kg/m ²)	-0.1 ± 0.4	-0.2 ± 0.2	0.58

Data are means ± SDs

¹ Obtained from independent-samples *t* test

Table 2 Metabolic profiles at baseline and after the 12-week intervention in subjects with polycystic ovary syndrome

	Placebo group (<i>n</i> = 30)			Magnesium-zinc-calcium-vitamin D group (<i>n</i> = 30)			<i>P</i> ¹
	Baseline	End-of-trial	Change	Baseline	End-of-trial	Change	
Magnesium (mg/dL)	1.6 ± 0.3	1.5 ± 0.2	−0.1 ± 0.3	1.5 ± 0.2	1.6 ± 0.2	0.1 ± 0.1	0.002
Zinc (mg/dL)	101.0 ± 14.9	100.1 ± 13.6	−0.9 ± 7.6	106.1 ± 17.9	109.1 ± 18.5	3.0 ± 8.8	0.06
Calcium (mg/dL)	9.3 ± 0.5	9.3 ± 0.8	−0.01 ± 0.6	9.5 ± 0.5	9.9 ± 0.5	0.4 ± 0.3	0.001
25-OH-vitamin D (ng/mL)	10.8 ± 4.6	10.9 ± 4.5	0.1 ± 0.5	10.1 ± 4.9	18.0 ± 10.3	7.9 ± 8.4	<0.001
Total testosterone (ng/mL)	1.4 ± 0.9	1.5 ± 0.9	0.1 ± 0.4	1.6 ± 0.6	1.4 ± 0.5	−0.2 ± 0.5	0.02
SHBG (nmol/L)	41.6 ± 17.3	45.4 ± 16.9	3.8 ± 19.8	43.2 ± 28.2	52.2 ± 34.8	8.9 ± 25.1	0.3
FAI	0.15 ± 0.17	0.12 ± 0.07	−0.03 ± 0.16	0.20 ± 0.15	0.16 ± 0.16	−0.02 ± 0.14	0.86
mF-G scores	12.6 ± 3.9	12.5 ± 3.9	−0.1 ± 0.4	14.3 ± 3.8	11.9 ± 3.1	−2.4 ± 1.2	<0.001
hs-CRP (mg/L)	3.1 ± 2.1	3.3 ± 1.8	0.2 ± 0.8	3.7 ± 1.6	3.0 ± 1.2	−0.7 ± 0.8	<0.001
NO (μmol/L)	47.4 ± 9.9	50.0 ± 10.5	2.5 ± 12.8	46.6 ± 5.8	50.7 ± 6.4	4.1 ± 1.3	0.57
TAC (mmol/L)	881.6 ± 165.0	873.9 ± 189.3	−7.7 ± 130.1	794.9 ± 59.3	841.5 ± 77.2	46.6 ± 66.5	0.04
GSH (μmol/L)	508.7 ± 96.6	562.8 ± 90.1	54.2 ± 128.5	491.2 ± 75.8	555.5 ± 73.0	64.3 ± 104.7	0.74
MDA (μmol/L)	2.2 ± 0.6	2.4 ± 0.9	0.2 ± 1.0	2.8 ± 0.2	2.4 ± 0.2	−0.4 ± 0.3	0.01

All values are means ± SDs

FAI free androgen index, GSH total glutathione, hs-CRP high-sensitivity C-reactive protein, mF-G modified Ferriman Gallwey, MDA malondialdehyde, NO nitric oxide, SHBG sex hormone-binding globulin, TAC total antioxidant capacity

¹ *P* values represent the time × group interaction (computed by analysis of the one-way repeated measures ANOVA)

vitamin D deficiency. Direct/indirect effects of vitamin D and calcium on the steroidogenesis pathway (ovarian and/or adrenal) may be mediated by reducing circulating androgens [16]. In addition, zinc element has been documented to inhibit 5α-reductase, which catalyzes the transformation of testosterone to its non-aromatizable form, di-hydro testosterone (DHT) [39]. Thus, increased circulating levels of zinc may also help to decrease PCOS-associated hyperandrogenemia through inhibiting transformation of testosterone to its active form DHT [9]. The current study indicated that magnesium-zinc-calcium-vitamin D co-supplementation among patients with PCOS for 12 weeks was associated with a significant decrease in serum hs-CRP, but did not affect plasma NO levels. In line with our

Table 3 Adjusted changes in metabolic profile of the patients with polycystic ovary syndrome

	Placebo group (<i>n</i> = 30)	Magnesium-zinc-calcium-vitamin D group (<i>n</i> = 30)	<i>P</i> ¹
Magnesium (mg/dL)	−0.1 ± 0.03	0.1 ± 0.03	0.01
Zinc (mg/dL)	−1.7 ± 1.5	3.7 ± 1.5	0.01
Calcium (mg/dL)	−0.01 ± 0.1	0.4 ± 0.1	0.001
25-OH-vitamin D (ng/mL)	−0.2 ± 1.1	8.1 ± 1.1	<0.001
Total testosterone (ng/mL)	0.03 ± 0.1	−0.2 ± 0.1	0.04
SHBG (nmol/L)	2.8 ± 4.0	9.9 ± 4.0	0.21
FAI	−0.04 ± 0.02	−0.02 ± 0.02	0.40
mF-G scores	−0.2 ± 0.2	−2.3 ± 0.2	<0.001
hs-CRP (mg/L)	0.2 ± 0.1	−0.8 ± 0.1	<0.001
NO (μmol/L)	2.6 ± 1.5	4.0 ± 1.5	0.54
TAC (mmol/L)	−0.6 ± 19.2	39.6 ± 19.2	0.15
GSH (μmol/L)	63.2 ± 15.4	55.3 ± 15.4	0.72
MDA (μmol/L)	−0.1 ± 0.1	−0.1 ± 0.1	0.95

All values are means ± SEs. Values are adjusted for baseline values, age, and BMI at baseline

FAI free androgen index, GSH total glutathione, hs-CRP high-sensitivity C-reactive protein, mF-G modified Ferriman Gallwey, MDA malondialdehyde, NO nitric oxide, SHBG sex hormone-binding globulin, TAC total antioxidant capacity

¹ Obtained from ANCOVA

study, results of a meta-analysis study demonstrated that magnesium supplementation among subjects with inflammation decreased CRP levels [40]. Similarly, supplementation with 30 mg/day of zinc as zinc gluconate for 8 weeks among obese women decreased inflammatory cytokines [41]. We have previously shown that calcium and vitamin D co-supplementation for 8 weeks among overweight and vitamin D-deficient subjects with PCOS had beneficial effects on serum hs-CRP, but did not influence plasma NO levels [18]. However, taking magnesium as magnesium oxide at dosage of 250 mg/day for 8 weeks among overweight women did not significantly attenuate inflammatory markers [15]. The administration of rosuvastatin with or without zinc supplements at dosage of 30 mg/day in subjects with atherosclerosis for 4 months did not influence hs-CRP levels [42]. Previous studies have shown that increased inflammatory cytokines play an important role in insulin resistance and the vascular inflammation process through multiple actions [43, 44]. Anti-inflammatory effects of magnesium may be due to its antagonism to calcium, the ion playing an important role in inflammation [45]. Moreover, zinc intake may be associated with the regulation of nuclear factor- κ B activation via anti-inflammatory proteins A20 and peroxisome proliferator activated receptor- α signaling pathway [46]. Less production of parathyroid hormone following supplementation of calcium and vitamin D supplements may result in decreased production of inflammatory factors [47].

We demonstrated that magnesium-zinc-calcium-vitamin D co-supplementation among women with PCOS for 12 weeks resulted in a significant elevation in plasma TAC and a significant reduction in plasma MDA levels, but unchanged plasma GSH levels. Supporting our study, magnesium supplementation at dosage of 200–270 mg/day as magnesium citrate for 4 weeks in asthmatic children could exert antioxidant activity and affect the glutathione redox system [48]. Furthermore, zinc supplementation (100 mg/day) for 4 weeks increased circulating levels of antioxidant status, such as TAC and GSH, and MDA concentrations decreased in hemodialysis patients [49]. Also, in an experimental study on zinc-deficient rats, zinc administration resulted in lower MDA concentrations, and higher GSH levels [50]. We have previously demonstrated that taking calcium (1000 mg/day) plus vitamin D (50,000 IU weekly) supplements for 8 weeks among PCOS women had beneficial effects on biomarkers of oxidative stress [16]. Oxidative stress may play an important role in the pathophysiology of patients with PCOS and the development of its complications, because free radicals and biomarkers of oxidative stress may modify the structure and function of proteins and lipids due to glycoxidation and peroxidation [51]. Combined magnesium-zinc-calcium-vitamin D might have a better effect on metabolic profiles than magnesium, zinc, calcium, or vitamin D alone. For instance, in a study by Tabesh et al. [17], it was observed that joint

calcium-vitamin D3 supplementation than calcium or vitamin D3 only had more beneficial effects on systemic inflammation through decreasing interleukin 6 and tumor necrosis factor- α concentrations in vitamin D-insufficient subjects with type 2 diabetes mellitus. Magnesium intake might reduce oxidative stress through decreasing ROS production [52] and increasing glutathione-peroxidase activity [53]. Furthermore, zinc intake may cause decreased formation of \cdot OH from hydrogen peroxide through the antagonism of redox-active transition metals, such as iron and copper [54], and increased production of some substances that are the ultimate antioxidant such as metallothioneins [55]. Calcium intake may affect oxidative stress through calcium transport and signaling line [56]. In addition, improvement in the cellular GSH concentrations and a decrease in ROS, and pro-inflammatory factors by vitamin D supplements may explain the beneficial effects on oxidative stress [57].

The current study had some limitations. Due to limited funding, we could not evaluate the effects of magnesium-zinc-calcium-vitamin D co-supplementation on gene expression related to inflammation and oxidative stress. It is also difficult to conclude when four products (magnesium-zinc-calcium-vitamin D) are being used against placebo. Therefore, further studies are necessary with single supplementation for each comparison with co-supplementation to determine the beneficial effects of each on hormonal profiles, biomarkers of inflammation, and oxidative stress.

Overall, magnesium-zinc-calcium-vitamin D co-supplementation for 12 weeks among PCOS women had beneficial effects on hormonal profiles, biomarkers of inflammation, and oxidative stress.

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

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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