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Clinical and Metabolic Responses to Magnesium Supplementation in Women with Polycystic Ovary Syndrome

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Abstract

We hypothesized that magnesium supplementation might help improve metabolic profiles and clinical symptoms of polycystic ovary syndrome (PCOS) through its role in insulin action. The present study aimed to investigate the effect of magnesium supplementation on metabolic profiles and levels of sex hormones in women with PCOS. In this parallel randomized, double-blind, placebo-controlled clinical trial, 60 women with PCOS aged 20–45 years were recruited. After stratification for body mass index (BMI), age, and types of medications, participants were randomly assigned to consume magnesium supplements (containing 250 mg magnesium oxide) or placebo for 8 weeks. To assess biochemical indicators, a venous blood sample was taken after an overnight fasting. The mean age of study participants was 26.4 years. We found that magnesium supplementation for 8 weeks among women with PCOS had favorable effects on BMI compared with the placebo group (changes from baseline in intervention group: -0.31 ± 0.07 vs. 0.07 ± 0.09 kg/m^2 in control group). In addition, the supplementation lead to preventing the increase in waist circumference in intervention group compared with the control group (0.02 vs. 1.15 cm). No significant effects on glycemic variables and lipid profile were seen following the magnesium supplementation. A significant increase in serum LH levels in intervention group and a decrease in placebo group were observed ($P = 0.01$). Although we found a significant decrease in serum testosterone levels in intervention and placebo groups, comparing the changes between the two groups, a marginally significant difference in serum testosterone levels was found (51.65 vs. 47.80 in intervention, 43.41 vs. 39.46 in placebo, $P = 0.08$). A significant increase in serum dehydroepiandrogens (DHEA) (136.32 vs. 172.37 intervention, 102.74 vs. 120.15 placebo, $P = 0.01$) was seen in two groups. Magnesium supplementation had no significant effects on FSH, 17OH-progesteron, sex hormone–binding globulin (SHBG), and free androgen index (FAI) levels. We found evidence indicating that magnesium supplementation did not influence serum lipid profiles and glycemic indicators among women with PCOS. Magnesium supplementation resulted in reduced BMI and testosterone levels as well as increased DHEA concentrations in women with PCOS. Also, magnesium supplementation may increase serum LH levels. ClinicalTrials.gov IRCT registration no. NCT02178150

Keywords Magnesium · Supplementation · PCOS · Sex hormones · Metabolic profiles · Insulin

Abbreviations

PCOS	Polycystic ovary syndrome
FPG	Fasting plasma glucose
BMI	Body mass index
DHEA	Dehydroepiandrogens
Mg	Magnesium

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SHBG	Sex hormone–binding globulin
FAI	Free androgen index
HOMA-IR	Homeostatic model assessment of insulin resistance
HOMA-B	Homeostatic model assessment of β-cell function
QUICKI	Quantitative insulin sensitivity check index
CV	Coefficients of variability
SD	Standard deviation
BP	Blood pressure

Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder affecting 6–10% of reproductive-aged women [1, 2]. Although the pathogenesis remains unclear, earlier evidence indicated the pivotal role of insulin resistance, hyperandrogenism, and obesity in its etiology. Individuals with PCOS are at increased risk of metabolic syndrome, infertility, diabetes, and cardiovascular diseases [3]. This condition imposes a considerable burden on the health care system. In the USA, the total cost of evaluating and providing care to reproductive-aged PCOS women in 2004 was $4.36 billion [4].

Management of PCOS typically includes insulin-lowering drugs, anti-androgen therapy, oral contraceptives pills, and lifestyle modification, including weight loss and dietary intervention with energy restriction and/or altered diet composition [2, 4]. Among others, dietary intake of minerals, including magnesium, might also play a key role in the pathogenesis of PCOS due to its contribution to insulin sensitivity [5]. Inadequate intake of magnesium has been shown to be linked with insulin resistance through its influence on tyrosine-kinase activity, enhancing oxidative stress and inflammation [6–8]. Besides observational studies, some studies have demonstrated that oral magnesium supplementation improved insulin sensitivity not only in mild uncomplicated hypertensive patients [9] but also in normo-magnesemic, overweight, non-diabetic subjects [10]. However, some studies have failed to find any significant association between serum Mg levels and insulin resistance [11, 12]. A 12-week magnesium and vitamin E co-supplementation had beneficial effects on parameters of insulin metabolism and serum triglycerides, VLDL, and total cholesterol in PCOS women [13]. Another trial indicated that magnesium-zinc-calcium-vitamin D co-supplementation for 12 weeks in PCOS women led to a significant reduction in total testosterone compared with the placebo, but it did not affect SHBG and FAI [14]. Despite the role of magnesium in insulin sensitivity and potential role of insulin resistance in the pathogenesis of PCOS, few studies have applied mono-nutrient therapy with magnesium supplementation in the management of PCOS. We are aware of just one report examining the effect of magnesium supplementation in PCOS, in which supplementation with 400 mg of magnesium oxide for 12 weeks revealed no significant effect on serum free fatty acid levels and insulin resistance. The mentioned study did not assess the effect of supplementation on sex hormones, blood lipids, and inflammation [15]. We hypothesized that magnesium supplementation might help improve metabolic profiles and clinical symptoms of PCOS through its role in insulin action. The present study, therefore, aimed to investigate the effect of magnesium supplementation on metabolic profiles and levels of sex hormones in women with PCOS.

Subjects and Methods

Participants This randomized, double-blind, placebo-controlled clinical trial was conducted in Isfahan, Iran, during 2015. On the basis of the sample size formula suggested for randomized clinical trials, considering a type I error of 5% ($\alpha = 0.05$) and type II error of 10% (study power = 90%) and serum insulin concentration as a key variable [16], we determined a sample size of 25 persons for each group. Considering a drop-out rate of 5 subjects per group, we calculated to have 30 subjects per group.

Women with PCOS aged 20–45 years were recruited into this study. Diagnosis of PCOS was the Rotterdam criteria, based on having at least two of the following characteristics: clinical or laboratory indices of increased androgen levels, anovulatory menstrual dysfunction, and polycystic ovaries on ultrasonography [4]. We did not include those who were lactating, individuals who were on a specific diet during the last 3 months, smokers, those with diabetes, cardiovascular, liver, kidney, and thyroid diseases, and persons who were using dietary supplements. Those with hormone therapy, with severe weight loss (more than 5% of body weight) during the study, and who went on a specific disease during the study were excluded. Participants were selected from among those referring to Isfahan Shahid Beheshti Hospital, one of the central hospitals in Isfahan. After making phone calls to those who met our inclusion criteria ($n = 76$), 60 patients agreed to participate in the study. After stratification for body mass index (BMI), age, and types of medications they were taking, they were randomly assigned to consume magnesium supplements or placebo for 8 weeks. Computer-generated random numbers were used to perform random assignment by a person who was not involved in the trial. Both researchers and participants were blinded from randomization and allocation until the final analyses were completed. Another person, who was not aware of random sequences, assigned the subjects to the numbered bottles of tables. The ethics committee of the Isfahan University of Medical Sciences approved the study,

and an informed written consent was obtained from all participants. The trial was registered at the Iranian website (www.irct.ir) for the registration of clinical trials (NCT02178150).

Study Design At study baseline and after stratification for BMI, age and types of medications, subjects were randomly assigned to receive either magnesium supplements ($n = 30$) or placebo ($n = 30$) for 8 weeks. Individuals in the magnesium group received one tablet every day, containing 250 mg magnesium oxide and 47 mg calcium carbonate, and those in the placebo group received one tablet containing lactose, starch, calcium stearate, and calcium phosphate. The appearance of the placebo tablets, such as color, shape, size, and packaging, was identical to the magnesium tablets. Participants were asked not to alter their routine physical activity or usual dietary intakes throughout the study and not to consume any supplements other than the one they were requested to by the investigators. Dietary intakes of study participants were examined throughout the study by means of four non-consecutive dietary records, one in every 2 weeks. Participants were also requested to record their physical activities at the same day they were recording their dietary intakes; therefore, data on physical activity were available for 4 days throughout the study.

Assessment of Variables Required information on other variables such as age, education, socioeconomic status, smoking, medical history, diseases, and medications were collected through the use of a pre-tested questionnaire. Body weight was measured to the nearest 0.1 kg after overnight fasting, without shoes and wearing minimal clothing, by the use of a digital scale. Height was measured to the nearest 0.1 cm by using a non-stretched tape measure. BMI was calculated as weight in kilograms divided by height in meters squared. Waist circumference was examined at its narrowest part near the last rib at the navel and at the end of a normal expiration, with an accuracy of 0.1 cm using a non-stretched tape measure. Blood pressure was assessed after 15 min of rest in the sitting position using a mercury sphygmomanometer. Serum insulin was considered as the primary outcome and other metabolic profiles were defined as the secondary outcomes. In order to quantify biochemical indicators, a venous blood sample of 10 mL was taken after a 12-h overnight fasting at the Aseman laboratory at study baseline and week 8 of intervention. Participants did not avoid from taking medications until the night before blood sample collection. Fasting plasma glucose (FPG) levels were measured on the day of blood sampling. To separate the serum, blood samples were centrifuged immediately (Hettich D-78532, Tuttlingen, Germany) at 1465*g* for 10 min. Serum lipid profiles were measured based on standard protocols on the same day and then serums were frozen in −70 °C until further measurements. Commercial available kits were used to measure FPG, serum magnesium, total cholesterol, triacylglycerol, and HDL cholesterol (Pars Azmun, Tehran, Iran). The intra- and inter-assay CVs for FPG were 1.5 and 2.7%, respectively. All inter- and intra-assay CVs were less than 5% for lipid profile measurements. ELISA kits were used to assay serum 17α-OH progesterone (DRG Progesterone ELISA, EIA1561, Germany), dehydroepiandrogens (DHEA) (DiaSorin, Saluggia, Italy), testosterone (Testosterone II Cobas, Roche), FSH (DiaSorin, Italy), LH (DiaSorin, Italy), and sex hormone–binding globulin (SHBG) levels (DiaSorin, Italy). The intra- and inter-assay CVs for all these variables were less than 10%. Serum insulin levels were measured by ELISA (Cobas Integra 800 Autoanalyzer, Roche Diagnostics, Germany). The intra- and inter-assay CVs for serum insulin were 1.9% and 2.6%, respectively. HOMA-IR and beta-cell function (HOMA-B), quantitative insulin sensitivity check index (QUICKI), and free androgen index (FAI) were calculated on the basis of suggested formulas [17, 18].

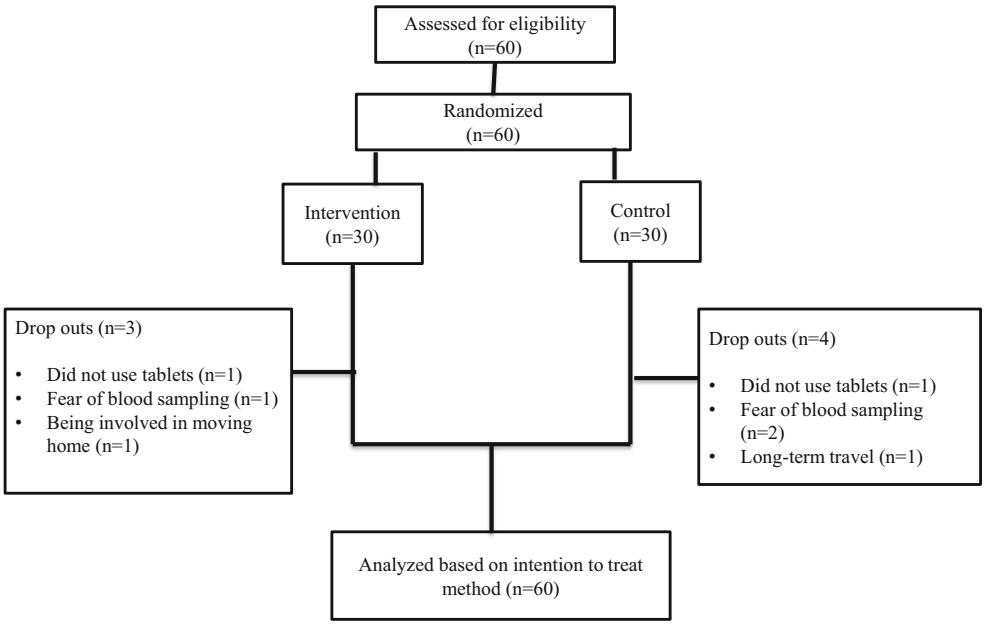
Statistical Analysis We used the Kolmogrov-Smirnov test to examine the normal distribution of variables. Log transformation was conducted for non-normally distributed variables. The analyses were performed on the basis of an intention-to-treat approach. Missing values were treated according to the last-observation-carried-forward method. The independent-samples Student's *t* test was used to detect differences in general characteristics and dietary intakes between the two groups. To determine the effects of magnesium supplementation on metabolic profiles and sex hormone levels, we used repeated measures ANOVA. To assess if the magnitude of the change in outcome variables was dependent on the baseline value, we conditioned all analyses on baseline values to avoid the potential bias that might have resulted. $P < 0.05$ was considered significant. All statistical analyses were conducted by using the SPSS, version 17 (SPSS Inc).

Results

Mean age of study participants was 26.4 years. Three women in the magnesium group and four women in the placebo group were excluded due to not using tablets ($n = 2$) and moving from the area ($n = 2$) and lack of willingness to continue the study ($n = 3$). A total of 53 participants (intervention ($n = 27$) and placebo ($n = 26$)) completed the trial (Fig. 1). However, the statistical analyses were performed on the original 60 participants on the basis of the intention-to-treat approach, and missing values for these 7 excluded participants were determined on the basis of the last-observation carried-forward method.

Based on physical activity records, we found that the activity levels of study participants were not significantly different between intervention and control groups (Fig. 2). The

Fig. 1 Study flow diagram



effects of magnesium supplementation on serum magnesium levels are given in Fig. 3. Baseline and end-of-trial means of serum magnesium concentrations were not significantly different between the two groups. No significant differences were found between the two groups in terms of mean age, weight, body mass index (BMI), and other demographic characteristics (Table 1). On the basis of dietary records obtained throughout the intervention, no significant differences were seen between the two groups in terms of dietary intakes of macronutrients and micronutrients (Table 2).

The effects of magnesium supplementation on anthropometric measures and blood pressure (BP) are summarized in Table 3. Magnesium supplementation for 8 weeks resulted in a significant reduction in BMI (changes from baseline in intervention group: -0.31 ± 0.07 vs. 0.07 ± 0.09 kg/m^2 in control group). In addition, this supplementation lead to preventing the increase in waist circumference in intervention group compared with the control group (0.02 vs. 1.15 cm). No significant reduction in systolic or diastolic blood pressure was seen after supplementation.

The effects of magnesium supplementation on glycemic indicators are provided in Table 4. Plasma concentrations of fasting blood glucose and insulin as well as scores of QUICKI, HOMA-IR, and HOMA-B were significantly changed during 8 weeks in either group. However, when we compared changes from baseline between the two groups, we failed to find a significant difference.

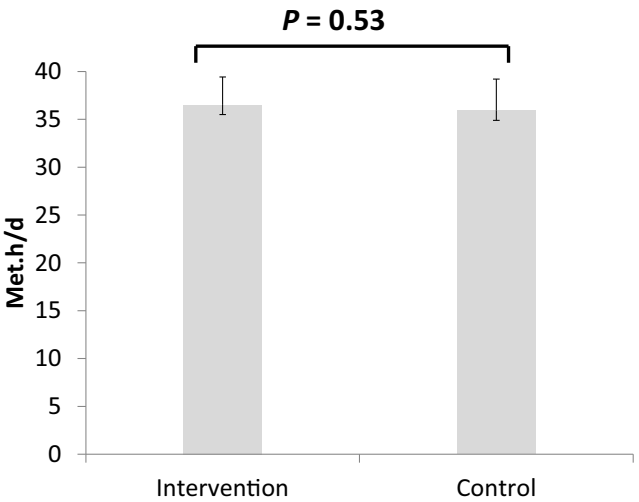


Fig. 2 Comparison of physical activity between the two groups during the trial. Individuals in the magnesium group ($n = 30$) received one tablet of magnesium supplements containing 250 mg magnesium oxide every day and those in the placebo group ($n = 30$) received one tablet containing lactose, starch, calcium stearate, and calcium phosphate

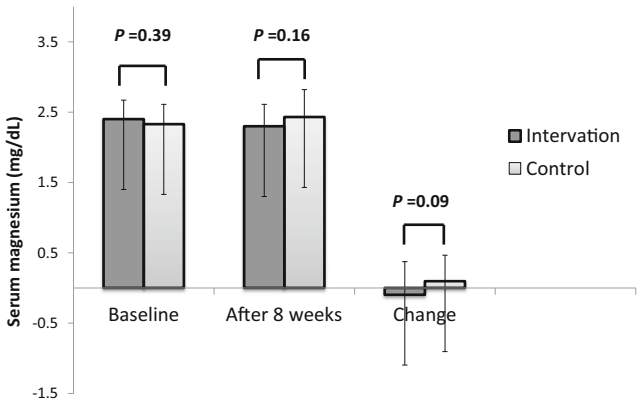


Fig. 3 Changes in serum magnesium levels during the trial. Individuals in the magnesium group ($n = 30$) received one tablet of magnesium supplements containing 250 mg magnesium oxide every day and those in the placebo group ($n = 30$) received one tablet containing lactose, starch, calcium stearate, and calcium phosphate

Table 1 General characteristics of study participants at baseline of the trial[1]

	Intervention	Control	P^2
Age (year)	26.32 ± 3.92	26 ± 5.06	0.78
Weight (kg)	72.09 ± 12.71	72.24 ± 13.31	0.96
Height (cm)	161.10 ± 4.70	161.70 ± 6.46	0.68
Body mass index (kg/m^2)	27.80 ± 5.07	27.72 ± 5.40	0.95
Married	74.20	69	0.43
Education (≥ diploma)	77.40	89.70	0.18
Socioeconomic status			
Family number (≥ 4)	29	41.40	0.23
House owners	54.80	72.40	0.12
Weight changes during 3 months before the study (yes)	48.40	58.60	0.29
Metformin usage (yes)	9.7	17.2	0.32

[1] Data are means ± SD or percent

[2] Obtained from independent-sample *t* test for continuous variable and chi-square for categorical variables

The effects of magnesium supplementation on lipid profiles are presented in Table 5. We did not observe any significant effect of the supplementation on levels of serum triglyceride, total cholesterol, LDL, and HDL cholesterol in either the crude or adjusted model. Although magnesium supplements resulted in a decrease in serum triglyceride levels and total and LDL-cholesterol concentrations, comparing the two groups, this finding was not statistically significant.

The effects of magnesium supplementation on sex hormone levels are given in Table 6. A significant increase in serum LH levels in intervention group and a decrease in placebo group was observed ($P = 0.01$). Although we found a significant decrease in serum testosterone levels in intervention and placebo groups, comparing the changes between the two groups, we found a marginally significant difference in serum testosterone levels (47.80 vs. 51.65 intervention, 39.46 vs. 43.41 placebo, $P = 0.08$). A significant increase in serum dehydroepiandrogens (DHEA) (136.32 vs. 172.37 intervention, 102.74 vs. 120.15 placebo, $P = 0.01$) was seen in two groups. Magnesium supplementation had no significant effects on FSH, 17OH-progesteron, sex hormone–binding globulin (SHBG), and free androgen index (FAI) levels.

Discussion

We found that magnesium supplementation for 8 weeks among women with PCOS had favorable effects on BMI compared with the placebo group; however, no significant effect on glycemic variables and lipid profile was seen following the magnesium supplementation. Also, we found a significant decrease in serum concentrations of testosterone and a significant increase in serum DHEA concentrations by magnesium supplementation, which were statistically significant although might not be clinically significant. To our knowledge, this is the first study that examined the effects of the magnesium supplementation on sex hormone levels in women with PCOS.

The origin and pathophysiology of PCOS is multifaceted, complex, and incompletely understood. A combination of genetic, epigenetic, and environmental mechanisms appears to account for the varied phenotypic manifestations of this syndrome. Magnesium intake may improve metabolic profiles through effects of its antagonism to calcium and to participate in protein synthesis and transmembrane ion transport [19], and also may decrease the circulating levels of triglycerides and VLDL-cholesterol through increased excretion of fecal fat [20] and increased lipoprotein lipase activity [21]. Increased activity of the acetyl-CoA carboxylase enzyme [22] and inhibiting the voltage-dependent calcium channel by magnesium may improve insulin sensitivity [23]. Intracellular magnesium plays a pivotal role in both insulin and glucose metabolism and is as a cofactor in a number of other metabolic and physiologic processes including blood pressure regulation. Therefore, it would be logical to pursue dietary studies and more interventional trials with magnesium supplements [1].

In the analysis on women with PCOS, carefully defined by the Rotterdam criteria, no significant effect of magnesium supplementation on glycemic and lipid profile was found in the present study. Although opposite associations between an individual's magnesium intake and risk of type 2 diabetes have been reported, most of earlier studies were carried out in Western countries [24–26], and their results may not be directly applied to Iranian and other Asian people. Some epidemiologic studies have shown an inverse association between dietary magnesium intake and insulin concentrations or the incidence of type 2 diabetes [27–29]; however, such associations may reflect other beneficial dietary components such as fibers in foods that are high in magnesium. Opposite to our findings, results from a clinical trial revealed a beneficial effect of magnesium supplementation on plasma glucose levels and insulin sensitivity in type 2 diabetics with low total

Table 2 Dietary intake of participants during the study[1]

	Intervention	Control	P^2
Energy intake (kcal)	1808.10 ± 88.42	1891 ± 90.02	0.51
Carbohydrates (% energy)	55.05 ± 1.57	53.90 ± 1.40	0.60
Protein (% energy)	14.26 ± 0.42	14.47 ± 0.43	0.73
Fat (% energy)	32.43 ± 1.44	33.22 ± 1.33	0.69
Saturated fatty acid (g/day)	18.50 ± 1.19	18.55 ± 1.37	0.98
Cholesterol (mg/day)	200.17 ± 19.74	250.50 ± 18.84	0.07
Magnesium (mg/day)	236.25 ± 15.16	218.0 ± 19.88	0.46
Calcium (mg/day)	650.46 ± 39.20	650.08 ± 47.07	0.99
Sodium (mg/day)	1035 ± 79.58	1195.90 ± 142.99	0.31
Potassium (mg/day)	2323 ± 100.77	2305 ± 173.86	0.92
Dietary fiber (g/day)	14.50 ± 0.98	13.67 ± 1.23	0.61
Folate (μg/day)	241.97 ± 13.16	232.40 ± 21.33	0.69

[1] Data are means ± SE

[2] Obtained from independent-sample *t* test

serum magnesium levels [30]. There is also evidence that magnesium supplementation has a small beneficial effect on insulin sensitivity in non-diabetics with insulin resistance and hypomagnesemia [31]. However, we observed no significant effect of magnesium supplementation on insulin sensitivity in the present study. Some of these discrepancies may result from differences in the duration of intervention or design of the study, type and dose of magnesium, or ethnic background of the subjects [24, 28–30].

A cross-sectional study suggested that exceeding the recommended dietary intake of magnesium might not provide additional benefit with respect to insulin sensitivity [32]. A potent inverse correlation has been shown between magnesium intake and risk of type 2 diabetes in subjects with low magnesium intake [25]. Hence, magnesium supplementation may mostly useful for individuals with magnesium deficiency; the benefits of supplementation on insulin sensitivity may vary among different ethnic groups. The dietary magnesium intake of Taiwanese adults was found to be relatively lower than that of adults in Western countries; however, no association between diabetes and low dietary magnesium was found [33].

Hypertension is another pathological condition resulting from altered cellular magnesium metabolism. Studies of extracellular ion levels have shown that the intracellular free magnesium level is strongly related to hypertension, proposing a pathophysiological relationship between magnesium depletion and hypertension [34]. However, the therapeutic role of magnesium in hypertension remains unclear, although it is currently used in critical situations as malignant hypertension and preeclampsia [35]. Thus, considering the available data, a role for decreased magnesium levels in the pathophysiology of hypertension appears likely, despite no confirmation of a consistent, reproducible effect of magnesium supplementation on BP [36]. Furthermore, there are few studies showing that magnesium supplementation reduces BP in normal subjects [37]. Our results are not consistent with those of the previous study; however, the subjects in that study were hypertensive; they reported that magnesium

Table 3 The effect of magnesium supplementation on anthropometric features of the participants in the study[1]

	Intervention				Control				P^2 between groups
	Baseline	8th week	Change	P^2	Baseline	8th week	Change	P^2	
Weight (kg)	72.09 ± 2.30	71.54 ± 2.25	−0.54 ± 0.38	0.16	72.24 ± 2.47	72.30 ± 2.50	0.06 ± 0.20	0.76	0.17
BMI (kg/m^2)	27.80 ± 0.91	27.48 ± 0.92	−0.31 ± 0.07	< 0.001	27.72 ± 1.01	27.79 ± 1.03	0.07 ± 0.09	0.41	0.002
Waist circumference (cm)	94.58 ± 2.36	94.85 ± 2.41	0.27 ± 0.51	0.60	94.24 ± 2.51	95.39 ± 2.52	1.15 ± 0.48	0.02	0.21
Systolic blood pressure (mmHg)	10.16 ± 0.19	9.93 ± 0.14	−0.22 ± 0.13	0.10	9.61 ± 0.21	9.30 ± 0.19	−0.31 ± 0.14	0.04	0.66
Diastolic blood pressure (mmHg)	7.28 ± 0.15	7.30 ± 0.15	0.02 ± 0.15	0.88	6.70 ± 0.20	6.40 ± 0.18	−0.30 ± 0.17	0.09	0.16

[1] Data are means ± SE

[2] Obtained from independent-sample *t* test

Table 4 The effect of magnesium supplementation on features of glycemic variables in participants[1]

	Intervention		Control		*P*		
	Baseline	8th week	Baseline	8th week	Time	Group	Time × group
Fasting blood glucose (mg/dL)							
Crude	92.32 ± 2.85	89.74 ± 2.9	94.69 ± 2.75	92.93 ± 2.20	0.16	0.43	0.79
Model I[2]	93.12 ± 3.06	90.17 ± 2.9	94.98 ± 3.24	93.04 ± 3.06	0.98	0.55	0.77
Insulin (uIU/mL)							
Crude	7.82 ± 0.88	13.83 ± 2.15	10.91 ± 1.51	12.38 ± 1.93	0.004	0.70	0.07
Model I	8.15 ± 1.3	14.8 ± 2.23	11.71 ± 1.37	13.42 ± 2.36	0.075	0.63	0.08
Quicki							
Crude	0.35 ± 0.005	0.33 ± 0.007	0.34 ± 0.007	0.34 ± 0.007	0.008	0.74	0.04
Model I	0.35 ± 0.006	0.33 ± 0.008	0.34 ± 0.007	0.33 ± 0.008	0.14	0.75	0.04
HOMA-IR							
Crude	1.80 ± 0.22	3.30 ± 0.63	2.62 ± 0.38	2.93 ± 0.49	0.01	0.67	0.09
Model I	1.89 ± 0.32	3.50 ± 0.63	2.82 ± 0.34	3.19 ± 0.66	0.13	0.63	0.11
HOMA-B							
Crude	106.51 ± 13.57	191.25 ± 20.99	134.66 ± 19.40	159.11 ± 23.12	< 0.001	0.93	0.019
Model I	109.57 ± 17.74	203.44 ± 23.49	143.68 ± 18.78	171.98 ± 24.89	0.02	0.96	0.02

[1] Data are means ± SE. *P* obtained from repeated measure ANOVA

[2] Adjustment was done for menstrual cycle phase

Table 5 The effect of magnesium supplementation on features of lipid profile in participants[1]

	Intervention		Control		*P*		
	Baseline	8th week	Baseline	8th week	Time	Group	Time × group
Serum Triglyceride (mg/dL)[2]							
Crude	139.13 ± 16.31	133.97 ± 18.12	135.07 ± 19.03	128.80 ± 17.88	0.46	0.84	0.94
Model I	142.67 ± 15.7	137.38 ± 16.21	123.08 ± 16.62	115.32 ± 17.15	0.85	0.33	0.89
Total cholesterol (mg/dL)							
Crude	208.55 ± 7.04	201.42 ± 6.33	198.03 ± 9.32	190.07 ± 7.34	0.16	0.24	0.93
Model I	209.08 ± 9.04	201.35 ± 7.44	199.82 ± 9.57	190.39 ± 7.88	0.60	0.33	0.89
HDL-C (mg/dL)							
Crude	47.03 ± 1.11	44.64 ± 1.32	46.17 ± 1.79	45.48 ± 1.41	0.07	0.99	0.31
Model I	46.56 ± 1.47	43.95 ± 1.32	46.45 ± 1.56	45.61 ± 1.4	0.21	0.67	0.35
LDL-C (mg/dL)							
Crude	133.61 ± 6.85	129.97 ± 4.84	130.21 ± 7.83	118.80 ± 7.40	0.07	0.40	0.35
Model I	134.02 ± 7.71	129.93 ± 6.4	134.89 ± 8.16	121.7 ± 6.78	0.86	0.69	0.34
Total cholesterol/HDL							
Crude	4.53 ± 0.20	4.68 ± 0.25	4.56 ± 0.31	4.32 ± 0.24	0.76	0.63	0.21
Model I	4.59 ± 0.27	4.76 ± 0.25	4.54 ± 0.29	4.26 ± 0.26	0.31	0.43	0.21
Serum triglyceride/HDL							
Crude	3.19 ± 0.46	3.40 ± 0.62	3.47 ± 0.75	3.25 ± 0.72	0.98	0.94	0.38
Model I	3.31 ± 0.45	3.55 ± 0.53	2.90 ± 0.48	2.64 ± 0.57	0.99	0.33	0.37

[1] Data are means ± SE. *P* Obtained from repeated measure ANOVA

[2] Adjustment was done for menstrual cycle phase

Table 6 The effect of magnesium supplementation on features of sex hormone[1]

	Intervention		Control		*P*		
	Baseline	8th week	Baseline	8th week	Time	Group	Time × group
FSH (mIU/mL)							
Crude	7.37 ± 0.50	6.78 ± 0.36	7.72 ± 0.55	7.33 ± 0.40	0.14	0.43	0.76
Model I[2]	7.19 ± 0.56	6.56 ± 0.38	8.02 ± 0.59	7.53 ± 0.40	0.83	0.13	0.85
LH (mIU/mL)							
Crude	11.50 ± 0.94	13.01 ± 0.98	9.30 ± 1.13	8.72 ± 0.96	0.46	0.01	0.10
Model I	11.21 ± 0.97	12.88 ± 0.80	9.01 ± 1.03	8.33 ± 0.85	0.34	0.003	0.10
Testosterone (ng/dL)							
Crude	51.65 ± 4.70	47.80 ± 3.65	43.41 ± 3.65	39.46 ± 2.91	0.15	0.08	0.98
Model I	52.08 ± 4.28	47.91 ± 3.20	44.23 ± 4.53	39.54 ± 3.40	0.89	0.08	0.93
Dehydroepiandrogens (DHEA) (μg/dL)							
Crude	136.32 ± 14.91	172.37 ± 15.07	102.74 ± 8.47	120.15 ± 9.99	< 0.001	0.01	0.16
Model I	135.80 ± 13.58	175.49 ± 14.40	101.19 ± 14.37	121.64 ± 15.24	0.10	0.02	0.20
17OH-progesteron (ng/mL)							
Crude	1.54 ± 0.14	1.80 ± 0.23	1.32 ± 0.14	1.41 ± 0.17	0.12	0.17	0.46
Model I	1.56 ± 0.14	1.85 ± 0.21	1.31 ± 0.14	1.41 ± 0.23	0.30	0.14	0.49
Sex hormone–binding globulin (SHBG) (nmol/L)							
Crude	39.34 ± 3.21	43.57 ± 5.23	42.21 ± 5.01	45.41 ± 6.07	0.27	0.70	0.87
Model I	39.24 ± 3.68	43.55 ± 5.53	39.57 ± 3.90	43.71 ± 5.85	0.64	0.96	0.98
Free androgen index (FAI)							
Crude	5.86 ± 0.79	5.26 ± 0.74	4.88 ± 0.62	4.53 ± 0.64	0.24	0.36	0.75
Model I	6.01 ± 0.72	5.37 ± 0.70	5.06 ± 0.77	4.62 ± 0.74	0.67	0.36	0.82

[1] Data are means ± SE. *P* Obtained from repeated measure ANOVA

[2] Adjustment was done for menstrual cycle phase

supplementation lowered blood pressure in hypertensive subjects and that the effect was greater in subjects with higher initial BP [38].

We found no correlation between Mg supplementation and indexes of insulin secretion or IR in our study. This result has been supported by Kaufmann et al. [39]. They reported that magnesium levels do not correspond with insulin sensitivity, glycemic levels, and lipid levels in reproductive-age women with PCOS. While, another study showed a significant negative correlation between magnesium and lipid profiles [40].

Despite the possible role of magnesium in insulin sensitivity and the potential role of insulin resistance in the pathogenesis of PCOS, few studies have examined the effect of magnesium supplements in patients with PCOS. In the only study in this field, supplementation with 400 mg of magnesium oxide for 12 weeks in women with PCOS had no effect on serum FFA level and is not a result of insulin resistance. The mentioned study had no data on the levels of blood lipids and inflammation, and did not provide the level of sex hormones [15]. In the present study, we investigate the effect of magnesium supplementation on sex hormone levels, and magnesium was associated with a significant decrease in testosterone and a significant rise in DHEA levels, respectively. LH levels were decreased in the placebo and increased in the intervention group. Since PCOS patients might show normal or elevated LH concentration [41], our finding in the case of LH might be not related to the effect of magnesium supplementation, and this finding might not be clinically significant. A randomized, placebo-controlled trial indicated that magnesium-zinc-calcium-vitamin D co-supplementation for 12 weeks in PCOS women led to a significant reduction in total testosterone, but it did not affect SHBG and FAI levels [14]. Also, in another trial, magnesium and vitamin E co-supplementation for 12 weeks among PCOS women had beneficial effects on parameters of insulin metabolism and serum triglycerides, VLDL, and total cholesterol [13]. These investigations showed the favorable effects of combined magnesium-zinc-calcium-vitamin D–vitamin E on metabolic profiles and suggested new studies to investigate the effects of mono-nutrient therapy. The present study showed that magnesium supplementation in women with PCOS might reduce testosterone levels and increase DHEA concentrations. The decrease in serum testosterone and increase in serum DHEA appeared in both intervention and placebo

groups and might not be the effect of magnesium. No other study is available investigating the effect of magnesium supplementation on sex hormone levels.

Our study has some limitations, including the relatively short duration of the study and the fact that no attempt was made to measure ionized and urinary magnesium. Applied dose of elemental magnesium in the present study was rather modest, and it may not be high enough to achieve the desired outcomes of our research. Furthermore, since our results were obtained in subjects with normal serum magnesium levels, the present findings cannot be generalized to conditions associated to magnesium depletion.

In conclusion, the present study provides the evidence showing that magnesium supplementation did not influence serum lipid profiles and glycemic indicators among women with PCOS. Magnesium supplementation resulted in reduced BMI and testosterone levels as well as increased DHEA concentrations in women with PCOS. Also, magnesium supplementation might increase serum LH levels.

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Authors' Contribution MF, LA, FM, PS, and AE contributed in conception, design, statistical analyses, data interpretation, and manuscript drafting. All authors approved the final manuscript for submission.

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Compliance with Ethical Standards

The study was approved by the ethics committee of Isfahan University of Medical Sciences.

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

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