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Effects of Oral Magnesium Supplementation on DNA Damage, Obesity, Insulin Resistance, and Intracellular Magnesium in Women With Polycystic Ovary Syndrome

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Abstract

Background: Disturbances in magnesium (Mg) homeostasis may contribute to the metabolic abnormalities associated with polycystic ovary syndrome (PCOS). Evidence on the relation between Mg status, circulating glucose transporter type 4 (GLUT-4), and selected adipokines remains limited. This study evaluated the effects of oral Mg supplementation on metabolic, hormonal, and selected molecular biomarkers in women with PCOS.

Methods: This prospective, nonrandomized, open-label controlled intervention study enrolled 60 women with PCOS diagnosed according to the Rotterdam criteria. Participants were allocated according to baseline serum Mg concentration to a low-Mg intervention group (n=30; 1.2–1.7 mg/dL), which received oral Mg (365 mg/day) for 12 weeks, or a normomagnesemic comparison group (n=30; 1.8–2.4 mg/dL), which received no supplementation. Anthropometric measures, lipid variables, and serum concentrations of GLUT-4, chemerin, betatrophin, and oxidative-stress markers (8-OHdG, TAS, and TOS) were assessed at baseline and at weeks 6 and 12.

Results: Serum Mg increased significantly in the intervention group (p=0.013). Total cholesterol and LDL cholesterol were higher in the low-Mg group at baseline; both declined numerically during follow-up, and between-group differences were no longer statistically significant at week 12. Circulating GLUT-4, chemerin, and betatrophin concentrations were higher in the normomagnesemic comparison group than in the low-Mg group at the assessed time points. Mg supplementation was not associated with statistically significant changes in HOMA-IR, HbA1c, or BMI over 12 weeks.

Conclusion: Oral Mg supplementation corrected low serum Mg concentrations but did not produce statistically significant improvements in glycaemic indices or BMI over 12 weeks. The nonrandomized allocation according to baseline Mg status precludes separating treatment effects from pre-existing group differences. Larger randomized trials are needed to determine whether Mg supplementation improves lipid, hormonal, or molecular outcomes in PCOS.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common, heterogeneous endocrine disorder affecting women of reproductive age. Although its aetiology is incompletely understood, PCOS is thought to result from interactions between genetic susceptibility and environmental factors. Its broad phenotypic spectrum is associated with metabolic comorbidities, including obesity, insulin resistance, dyslipidaemia, and metabolic syndrome, from adolescence through adulthood. Obesity commonly exacerbates the PCOS phenotype and clinical course [1]. Its pathophysiological effects include worsening insulin resistance, increased androgen and luteinizing hormone (LH) secretion, and reduced sex hormone-binding globulin (SHBG) concentrations. Adipokine dysregulation and chronic low-grade inflammation may further compound these abnormalities. Obesity has also been associated with higher hirsutism scores, reduced response to ovulation induction, chronic anovulation, menstrual irregularity, adverse pregnancy

outcomes, and miscarriage. The coexistence of obesity and PCOS may partly reflect shared genetic susceptibility [2].

The long-term consequences of PCOS extend beyond metabolic disease and include an increased risk of endometrial hyperplasia and cancer in premenopausal women. First-degree male relatives of women with PCOS have also been reported to have higher frequencies of metabolic syndrome, type 2 diabetes, and hypertension [3]. PCOS-associated genetic variants may influence gonadotrophin secretion, androgen biosynthesis, glucose homeostasis, folliculogenesis, and age at menopause [4]. Interest has therefore grown in the role of micronutrients in the metabolic and hormonal management of PCOS. Magnesium (Mg) is involved in insulin signalling and glucose homeostasis; however, direct clinical effects of Mg supplementation in PCOS remain uncertain. Women with PCOS have been reported to have lower serum Mg concentrations than healthy controls, and Mg supplementation has been proposed as an adjunctive strategy for improving metabolic outcomes [5]. A recent randomized clinical trial reported improvements in insulin resistance and selected metabolic variables after Mg supplementation in women with PCOS [6]. Nevertheless, the effects of Mg supplementation on circulating GLUT-4 and selected adipokines, including chemerin and betatrophin, remain insufficiently characterized.

We hypothesised that Mg replacement in women with PCOS and low serum Mg concentrations would restore Mg status and may be associated with changes in metabolic, hormonal, and molecular biomarkers. This prospective study therefore examined the effects of oral Mg supplementation on these outcomes, including circulating GLUT-4, chemerin, and betatrophin.

MATERIALS AND METHODS

The study protocol, including participant selection, intervention, and analyses, is described below. The protocol was not prospectively registered in a public clinical-trial registry.

Study design and participants: This was a prospective, nonrandomized, open-label controlled intervention study conducted in accordance with the Declaration of Helsinki and approved by the Clinical Research Ethics Committee of Van Yüzüncü Yıl University (10 February 2021; Decision No. 17; Appendices 1–2).

Sample-size calculation: The primary outcome was change in serum Mg concentration. Using G*Power, a minimum of 23 participants per group was estimated to be required to detect the prespecified minimum between-group difference with 80% power at a two-sided α level of 0.05. Sixty women aged 18–40 years who met the Rotterdam diagnostic criteria for PCOS and attended the Adult Endocrinology Outpatient Clinic between March and June 2021 were enrolled. Written informed consent was obtained from all participants (Appendix 3).

Study groups and intervention: Allocation was nonrandomized and based solely on baseline serum Mg concentration. Participants were assigned to one of the following groups:

1. Low-Mg intervention group: Thirty women with PCOS and serum Mg concentrations of 1.2–1.7 mg/dL received one oral Mg tablet daily (365 mg/day; $MgCl_2$, MgO, or $Mg(OH)_2$; 40–60 mEq) for 12 weeks, in accordance with Turkish Endocrinology and Metabolism Association guidance [7].

2. Normomagnesemic comparison group: Thirty women with PCOS and serum Mg concentrations of 1.8–2.4 mg/dL, with similar age and BMI characteristics, received no supplement and underwent routine follow-up.

The groups were comparable with respect to use of spironolactone, metformin, and antiandrogenic oral contraceptives, as well as reported diet and exercise habits. Exclusion criteria were heart block, renal failure (estimated glomerular filtration rate <60 mL/min), anuria, myasthenia gravis, Mg allergy, severe respiratory depression, or clinically significant electrolyte abnormalities. During the 12-week follow-up, 14 participants (7 per group) withdrew because of pregnancy or inability to attend the study centre during the COVID-19 pandemic. The final analysis therefore included 46 participants.

Data collection and sampling procedures

Sample-size calculation: Using G*Power (RRID:SCR_013726), a minimum of 23 participants per group was estimated to provide 80% power at a two-sided α level of 0.05 for the primary outcome of change in serum Mg concentration.

Clinical and laboratory evaluations were performed at baseline (week 0) and at weeks 6 and 12. Fasting venous blood samples were collected from the antecubital vein after at least 8 hours of overnight fasting and, where applicable, during the follicular phase of the menstrual cycle (days 3–5). Specimens were allocated as follows:

Whole blood (EDTA tube): HbA1c and intracellular Mg analyses.

Serum (gel tube): Routine biochemistry, hormone profile, and ELISA-based measurement of GLUT-4, 8-OHdG, TAS, TOS, leptin, chemerin, and betatrophin.

Biochemical and hormonal analyses: Although clinical follow-up was open label, laboratory personnel who performed the biochemical and molecular analyses were blinded to group allocation and clinical information. Serum was separated by centrifugation at 4000 rpm for 10 minutes and stored at −80 °C until analysis. Routine variables (glucose, lipid profile, and electrolytes) were measured photometrically using an Abbott ARCHITECT c16000 analyser. Hormones (FSH, LH, total testosterone, DHEA-S, SHBG, insulin, C-peptide, E2, TSH, fT3, fT4, prolactin, and PTH) were measured on an Abbott ARCHITECT i1000SR system (RRID:SCR_019328) using chemiluminescent microparticle immunoassays. All measurements were performed according to the manufacturers' instructions [8,9].

ELISA-based biomarker analyses: Serum concentrations of GLUT-4, 8-OHdG, TAS, TOS, leptin, chemerin, and betatrophin were measured using commercially available ELISA kits (Sunred Biological Technology,

Shanghai, China). The manufacturer-reported intra-assay and inter-assay coefficients of variation were <10%. Optical density was read at 450 nm using a BioTek ELx800 microplate reader (USA).

Intracellular Mg measurement: For erythrocyte intracellular Mg measurement, plasma and the buffy coat were removed from EDTA-anticoagulated blood. Erythrocytes were washed three times with 0.9% NaCl and haemolysed with distilled water. Haemolysates were analysed by atomic absorption spectrophotometry (Thermo Scientific ICE-3000, USA) using a modified method described by Polat et al. (2010) (285.2 nm; 0.2% HNO_3 dilution) [10,11].

Anthropometric measurements and insulin resistance: Height, body weight, waist circumference, and hip circumference were measured using standard protocols. BMI and waist-to-hip ratio (WHR) were calculated [12,13]. Insulin resistance was estimated using the homeostatic model assessment of insulin resistance (HOMA-IR): HOMA-IR = [fasting insulin (μU/mL) × fasting plasma glucose (mg/dL)] / 405. A HOMA-IR value >2.5 was considered indicative of insulin resistance [14].

Statistical analysis: Analyses were performed using IBM SPSS Statistics version 26.0 (RRID:SCR_002865; IBM Corp., Armonk, NY, USA). Normality was assessed using the Kolmogorov–Smirnov test. Continuous variables are presented as mean ± standard deviation (SD), and categorical variables as frequency (n) and percentage (%). Between-group comparisons at each time point were conducted using two-tailed independent-samples t tests when normality and homogeneity-of-variance assumptions (Levene's test) were satisfied. Within-group baseline-to-week-12 comparisons used two-tailed paired-samples t tests. Changes over time were assessed using repeated-measures analysis of variance; where applicable, post-hoc comparisons used Duncan's test. Categorical variables were compared using Pearson's chi-square test or Fisher's exact test when expected cell counts were insufficient. Two-sided p values <0.05 were considered statistically significant. No custom code or algorithms were used.

RESULTS

Study population and anthropometric characteristics

Of the 60 participants enrolled, 46 (23 per group) completed follow-up and were included in the analyses. Smoking status ($\chi^2[1, N=46]=0.534$, $p=0.465$), alcohol consumption ($\chi^2[1, N=46]=0.001$, $p>0.999$), and use of spironolactone, metformin, or oral contraceptives ($\chi^2[1, N=46]=1.073$, $p=0.300$) did not differ significantly between groups.

Table 1 presents the anthropometric characteristics. Body weight, BMI, and WHR did not differ significantly between groups (all $p>0.05$). BMI declined numerically in both groups by week 12; the patterns of change were similar between groups.

Metabolic profile, lipids, and electrolytes

Table 2 summarises metabolic and biochemical variables. At baseline, serum Mg (p=0.001) and intracellular Mg (p=0.009) were lower in the low-Mg intervention group than in the normomagnesemic comparison group. Serum Mg increased in the intervention group at week 6; however, it remained lower than in the comparison group at week 12 (p=0.013).

Total cholesterol and LDL cholesterol were higher in the low-Mg intervention group at baseline. Both values declined numerically by week 12, and the between-group differences were no longer statistically significant. The within-group reductions did not reach statistical significance.

Glycaemic variables (glucose, insulin, C-peptide, HbA1c, and HOMA-IR) did not differ significantly between groups over follow-up. Among electrolytes, calcium concentrations were higher in the normomagnesemic comparison group than in the low-Mg intervention group at baseline and week 12 (both $p<0.05$).

Hormonal profile

Table 3 presents thyroid, parathyroid, and sex-hormone variables. fT4 concentrations were higher in the comparison group at baseline. fT3 and TSH varied over time, without evidence of clinically overt thyroid dysfunction.

PTH concentrations were higher in the low-Mg intervention group than in the comparison group at baseline and at weeks 6 and 12 (all $p<0.05$). Estradiol concentrations were higher in the low-Mg intervention group at week 6 (p=0.034). Total testosterone and DHEA-S concentrations were numerically higher in that group but did not differ significantly between groups.

Adipokines and oxidative-stress markers

Table 4 presents circulating adipokines and oxidative-stress markers. Circulating GLUT-4 concentrations were lower in the low-Mg intervention group than in the comparison group at all measurement time points (all $p<0.05$).

Chemerin concentrations were higher in the comparison group at all time points, and betatrophin concentrations were higher in the comparison group at weeks 6 and 12 (all $p<0.05$). 8-OHdG showed a similar pattern in the two groups, whereas total antioxidant status (TAS) was higher in the comparison group at week 6.

Table 1. Comparison of anthropometric measurements

Parameter	*Week*	*Control (Mean ± SD)*	*Case (Mean ± SD)*	*t-value*	*p value**
Weight (kg)	0.	65.83 ± 13.78	70.30 ± 14.71	-1.214	0.230
	6.	65.38 ± 13.07	70.59 ± 14.46	-1.465	0.148
	12.	63.70 ± 12.21	67.74 ± 11.78	-1.143	0.259
BMI (kg/m²)	0.	25.50 ± 5.12	27.11 ± 5.45	-1.170	0.247
	6.	25.30 ± 4.98	27.16 ± 5.60	-1.403	0.166
	12.	24.71 ± 4.80	26.63 ± 5.10	-1.329	0.191
Waist-to-Hip Ratio	0.	0.85 ± 0.13	0.81 ± 0.06	1.436	0.156
	6.	0.82 ± 0.10	0.81 ± 0.06	0.214	0.832
	12.	0.84 ± 0.14	0.82 ± 0.07	0.595	0.555

SD, standard deviation; NS, not significant. A two-sided p value <0.05 was considered statistically significant.

Table 2. Comparison of metabolic, lipid, and electrolyte profiles

Parameter	*Week*	*Control (Mean ± SD)*	*Case (Mean ± SD)*	*t-value*	*p value**
Serum Mg (mg/dL)	0.	2.03 ± 0.12	1.66 ± 0.05	15.550	0.001
	6.	2.01 ± 0.08	1.90 ± 0.13	3.992	0.001
	12.	2.03 ± 0.24	1.89 ± 0.12	2.595	0.013
Intracellular Mg (mg/dL)	0.	76.30 ± 15.10	66.87 ± 11.81	2.695	0.009
	6.	62.55 ± 12.37	68.78 ± 8.96	-2.233	0.029
	12.	76.28 ± 12.11	67.31 ± 9.04	2.807	0.007
Glucose (mg/dL)	0.	91.90 ± 12.48	95.77 ± 36.08	-0.555	0.581
	12.	96.48 ± 58.94	86.35 ± 5.80	0.820	0.416
Insulin (µU/mL)	0.	14.76 ± 10.20	11.04 ± 7.77	1.587	0.118
	12.	11.50 ± 11.38	10.19 ± 6.20	0.486	0.629
HOMA-IR	0.	3.44 ± 2.56	2.67 ± 2.18	1.255	0.215
	12.	2.34 ± 2.14	2.17 ± 1.23	0.329	0.744
HbA1c (%)	0.	5.40 ± 0.65	6.95 ± 0.72	-0.975	0.334
	12.	5.47 ± 0.78	5.40 ± 0.60	0.319	0.751
C-Peptide (ng/mL)	0.	2.20 ± 1.14	2.10 ± 0.65	0.416	0.679
	12.	2.19 ± 0.94	1.88 ± 0.65	1.305	0.199
T. Cholesterol (mg/dL)	0.	154.90 ± 26.48	170.40 ± 30.30	-2.110	0.039
	12.	156.00 ± 25.64	163.17 ± 25.04	-0.960	0.342
LDL-C (mg/dL)	0.	83.43 ± 24.80	98.76 ± 28.66	-2.216	0.031
	12.	82.67 ± 25.13	90.51 ± 23.29	-1.098	0.278
HDL-C (mg/dL)	0.	51.52 ± 10.80	53.05 ± 9.38	-0.586	0.560
	12.	52.76 ± 12.23	52.85 ± 13.74	-0.025	0.980
Triglyceride (mg/dL)	0.	99.73 ± 49.71	93.17 ± 31.52	0.611	0.544
	12.	102.87 ± 55.18	99.04 ± 30.53	0.291	0.772
VLDL-C (mg/dL)	0.	19.94 ± 9.95	18.63 ± 6.30	0.608	0.546
	12.	20.57 ± 11.04	19.81 ± 6.11	0.291	0.772
Calcium (Ca) (mg/dL)	0.	9.64 ± 0.45	9.31 ± 0.36	3.130	0.003
	12.	9.54 ± 0.39	9.24 ± 0.45	2.450	0.018

Phosphorus (P) (mg/dL)	0.	3.65 ± 0.51	3.46 ± 0.54	1.400	0.167
	12.	3.67 ± 0.45	3.39 ± 0.35	2.359	0.023
Sodium (Na) (mmol/L)	0.	140.07 ± 1.28	139.13 ± 1.57	2.520	0.015
	12.	138.70 ± 2.14	139.96 ± 1.22	-2.452	0.018
Potassium (K) (mmol/L)	0.	4.46 ± 0.37	4.38 ± 0.28	0.866	0.390
	12.	4.50 ± 0.45	4.31 ± 0.28	1.691	0.098
Albumin (g/dL)	0.	47.33 ± 2.82	46.67 ± 2.12	1.034	0.305
	12.	47.43 ± 3.01	45.09 ± 3.81	2.317	0.025

SD, standard deviation. A two-sided p value <0.05 was considered statistically significant.

Table 3. Hormonal profile (thyroid, parathyroid, and sex hormones)

Parameter	*Week*	*Control (Mean ± SD)*	*Case (Mean ± SD)*	*t-value*	*p value**
TSH (µIU/mL)	0.	1.57 ± 1.14	1.94 ± 0.74	-1.525	0.133
	12.	1.53 ± 0.81	1.92 ± 0.75	-1.656	0.105
fT3 (pg/mL)	0.	3.50 ± 0.32	3.21 ± 0.28	3.685	0.001
	12.	3.12 ± 0.33	3.40 ± 0.51	-2.266	0.028
fT4 (ng/mL)	0.	1.03 ± 0.11	0.94 ± 0.09	3.187	0.002
	12.	1.07 ± 0.19	1.02 ± 0.08	1.105	0.275
PTH (pg/mL)	0.	65.35 ± 31.41	87.47 ± 26.25	-2.960	0.004
	6.	63.57 ± 23.87	84.67 ± 25.27	-3.324	0.002
	12.	57.75 ± 20.90	73.33 ± 26.03	-2.240	0.030
FSH (mIU/mL)	0.	4.73 ± 2.16	5.66 ± 2.50	-1.532	0.131
	12.	5.03 ± 2.16	4.92 ± 2.38	0.166	0.869
LH (mIU/mL)	0.	8.99 ± 9.17	11.52 ± 12.17	-0.907	0.368
	12.	5.31 ± 4.12	8.15 ± 6.77	-1.716	0.093
LH/FSH Ratio	0.	1.79 ± 1.33	1.92 ± 1.23	-0.406	0.686
	12.	1.41 ± 0.90	1.63 ± 0.78	-2.776	0.008
T. Testosterone (ng/mL)	0.	1.47 ± 0.45	1.50 ± 0.50	-0.196	0.846
	12.	1.32 ± 0.50	1.57 ± 0.58	-1.551	0.128
DHEA-S (µg/dL)	0.	300.02 ± 135.88	322.96 ± 121.46	-0.690	0.493
	12.	293.31 ± 104.51	317.22 ± 150.87	-0.625	0.535
SHBG (nmol/L)	0.	59.34 ± 55.61	54.51 ± 38.22	0.392	0.696
	12.	76.97 ± 65.21	85.08 ± 67.84	-0.414	0.681
Estradiol (pg/mL)	0.	68.93 ± 54.56	101.87 ± 96.32	-1.630	0.109
	6.	49.61 ± 30.51	68.90 ± 37.83	-2.174	0.034
Prolactin (ng/mL)	0.	17.25 ± 11.44	16.49 ± 4.94	0.335	0.739
	12.	18.26 ± 9.06	16.83 ± 6.95	0.601	0.551

SD, standard deviation. A two-sided p value <0.05 was considered statistically significant.

Table 4. Circulating adipokines and oxidative-stress markers

Parameter	*Week*	*Control (Mean ± SD)*	*Case (Mean ± SD)*	*t-value*	*p value**
GLUT-4 (ng/mL)	0.	33.04 ± 22.54	20.63 ± 15.84	2.468	0.017
	6.	31.80 ± 22.83	19.56 ± 12.92	2.554	0.013
	12.	26.86 ± 11.70	15.79 ± 7.73	3.788	0.001
Chemerin (ng/mL)	0.	461.25 ± 268.61	306.50 ± 243.42	2.338	0.023
	6.	452.23 ± 253.50	264.22 ± 216.19	3.091	0.003
	12.	371.06 ± 199.11	238.04 ± 197.45	2.269	0.028
Betatrophin (ng/L)	0.	1302.43 ± 1487.50	833.54 ± 1163.69	1.360	0.179
	6.	1226.67 ± 1495.41	483.61 ± 429.95	2.616	0.011
	12.	1042.11 ± 905.29	362.79 ± 227.90	3.490	0.001
Leptin (ng/mL)	0.	35.27 ± 29.49	29.57 ± 32.16	0.716	0.477
	6.	35.44 ± 33.01	20.40 ± 26.53	1.946	0.057
	12.	21.52 ± 14.17	17.94 ± 23.55	0.624	0.536
TAS (U/mL)	0.	33.30 ± 21.86	25.70 ± 18.40	1.457	0.150
	6.	39.36 ± 22.60	18.36 ± 10.04	4.657	0.001
	12.	36.42 ± 16.47	29.26 ± 15.25	1.531	0.133
TOS (pg/mL)	0.	23.73 ± 13.22	19.40 ± 9.74	1.446	0.153
	6.	26.88 ± 14.48	23.15 ± 14.02	1.014	0.315
	12.	27.13 ± 9.91	19.98 ± 12.42	2.157	0.036
8-OHdG (ng/mL)	0.	42.34 ± 30.84	30.84 ± 20.73	1.695	0.095
	6.	39.67 ± 28.84	35.92 ± 24.03	0.547	0.586
	12.	49.08 ± 23.50	35.97 ± 21.67	1.987	0.056

SD, standard deviation. A two-sided p value <0.05 was considered statistically significant.

DISCUSSION

Magnesium homeostasis may be relevant to the metabolic phenotype of PCOS, but the present nonrandomized design does not permit causal attribution of between-group differences to Mg supplementation. The groups were broadly comparable in age, height, and waist-to-hip ratio at baseline. Prior studies have reported mixed findings on anthropometric outcomes with Mg supplementation in PCOS. Farsinejad-Marj et al. (2020) and studies in obesity reported reductions in BMI or waist

circumference [15–17], whereas Muneyyirci-Delale et al. (2013) observed no significant change over 12 weeks. In the present study, BMI declined numerically in the low-Mg group but not significantly. This finding should be interpreted as hypothesis-generating rather than evidence of a weight-management effect [18].

Magnesium is involved in insulin signalling and glucose homeostasis, including pathways related to tyrosine kinase activity [19]. Reports in metabolic disease and PCOS populations have linked Mg supplementation or Mg status with glycaemic and hormonal outcomes, although the clinical findings are heterogeneous [20,21]. In this cohort, both serum and intracellular Mg concentrations were lower in the low-Mg intervention group at baseline and serum Mg increased after treatment. These findings document correction of serum Mg status in the treated group. Higher PTH and lower calcium concentrations in the low-Mg group may reflect altered mineral homeostasis; however, this observational between-group pattern cannot establish a compensatory parathyroid response [22].

Magnesium deficiency has been associated with an atherogenic lipid profile [23]. In this study, total cholesterol and LDL cholesterol were higher at baseline in the low-Mg group and declined numerically after supplementation. Because the within-group changes were not statistically significant and allocation was based on baseline Mg concentration, these data do not establish a lipid-lowering effect of Mg. The proposed role of lipoprotein lipase modulation remains mechanistic speculation [17].

Insulin resistance and hyperinsulinaemia can increase ovarian androgen synthesis in PCOS [24,25]. SHBG increased numerically in the intervention group, but the change was not statistically significant; no inference about clinically meaningful androgen neutralisation is therefore warranted. Likewise, the decline in estradiol after week 6 should be interpreted cautiously because estradiol varies across the menstrual cycle and the study was not designed to test effects on aromatase activity or oestrogen metabolism.

The molecular findings concern circulating GLUT-4 concentrations measured by ELISA, rather than tissue GLUT-4 expression or translocation. Adipocyte GLUT-4 expression has been reported to be reduced in PCOS and may contribute to impaired glucose uptake [26,27]. In the present study, circulating GLUT-4 was lower in the low-Mg group at each time point. Any numerical increase after treatment should not be interpreted as evidence that Mg supplementation increased cellular GLUT-4 expression or glucose transport without tissue-based mechanistic measurements.

Chemerin and betatrophin concentrations were higher in the normomagnesemic comparison group than in the low-Mg group at several time points. This direction differs from reports linking chemerin to insulin resistance [28]. The study cannot determine whether this pattern reflects Mg status, adipose-tissue function, assay characteristics, medication exposure, or other unmeasured differences. Explanations based on adipocyte “exhaustion” or impaired adipokine synthesis are speculative and require targeted mechanistic studies.

8-OHdG is commonly used as a marker of oxidative DNA damage. An increase after supplementation cannot be assumed to represent enhanced DNA repair or clearance of oxidised bases without direct measures of DNA-repair activity or urinary excretion. The observed pattern should therefore be reported descriptively and interpreted cautiously [29].

Although glucose, HbA1c, and HOMA-IR changed numerically after Mg supplementation, no statistically significant changes were observed. This may reflect the short follow-up, sample size, baseline metabolic status, or a lack of treatment effect; the study cannot distinguish among these explanations [30].

In this prospective, nonrandomized study, oral Mg supplementation increased serum Mg concentrations in women with PCOS and low baseline Mg. No statistically significant improvement was observed in glycaemic outcomes, BMI, or within-group lipid changes over 12 weeks. Baseline Mg-based allocation and the absence of a placebo-treated, Mg-matched control group limit causal interpretation. Randomized, adequately powered trials with prespecified clinical and mechanistic outcomes are needed before Mg supplementation can be recommended as an adjunctive treatment for PCOS.

Declarations

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Funding: This study was supported by the Scientific Research Projects Coordination Unit of Van Yüzüncü Yıl University under Project No. FDK-2022-9838.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Van Yüzüncü Yıl University Faculty of Medicine (10 February 2021; Decision No. 17; Appendices 1–2).

Trial Registration: The study protocol was not prospectively registered in a public clinical-trial registry.

Informed Consent Statement: Written informed consent was obtained from all adult participants before enrolment.

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to applicable ethical and institutional requirements.

Conflicts of Interest: The authors declare no conflicts of interest.

Author Contributions: Conceptualization, A.K.Z.; methodology, A.K.Z., I.Ç., and M.A.; validation, A.K.Z., M.A., and I.Ç.; formal analysis, A.K.Z., M.A., and I.Ç.; investigation, A.K.Z., M.A., and I.Ç.; resources, A.K.Z., M.A., H.I.A., D.D., S.Y., and I.Ç.; data curation, A.K.Z.; writing—original draft preparation, A.K.Z., M.A., I.Ç., and H.I.A.; writing—review and editing, A.K.Z., M.A., and I.Ç.; visualization, A.K.Z.; supervision, M.A. and

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