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The Effect of Magnesium Supplementation on DNA Damage, Obesity, Insulin Resistance and Intracellular Magnesium Levels in Patients With Polycystic Ovary Syndrome

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Abstract

Background

Altered magnesium (Mg) homeostasis is increasingly implicated in the metabolic dysregulation of Polycystic Ovary Syndrome (PCOS). However, the interplay between Mg status, glucose transporter-4 (GLUT-4) expression, and novel adipokines remains underexplored. This study aimed to evaluate the impact of oral magnesium supplementation on metabolic profiles, hormonal balance, and specific molecular markers in women with PCOS.

Methods

This case-control study enrolled 60 women diagnosed with PCOS according to Rotterdam criteria. Participants were stratified based on baseline serum Mg levels: a hypomagnesemic intervention group (n = 30; Mg: 1.2–1.7 mg/dL) receiving 365 mg/day oral magnesium for 12 weeks, and a normomagnesemic control group (n = 30; Mg: 1.8–2.4 mg/dL) followed without supplementation. Anthropometric indices, lipid profiles, and serum levels of GLUT-4, chemerin, betatrofin, and oxidative stress markers (8-OHdG, TAS, TOS) were assessed at baseline, week 6, and week 12.

Results

Magnesium treatment significantly restored serum Mg concentrations in the intervention group ($p = 0.013$). While baseline Total Cholesterol and LDL-cholesterol were significantly higher in the hypomagnesemic group, supplementation led to a numerical reduction, eliminating the statistical disparity with the control group by week 12. Surprisingly, contrary to typical insulin resistance patterns, serum levels of GLUT-4, chemerin, and betatrofin were found to be significantly higher in the healthy normomagnesemic controls compared to the intervention group throughout the study. Magnesium intake did not yield statistically significant changes in glucose homeostasis parameters (HOMA-IR, HbA1c) or BMI within the 12-week period.

Conclusion

This study posits that magnesium deficiency is not merely a secondary consequence in PCOS pathogenesis, but potentially a precipitating factor in the dysregulated 'oxidative stress–insulin resistance–adipokine' axis. Given its potential to ameliorate LDL-cholesterol levels, modulate GLUT-4 expression, and enhance SHBG concentrations, magnesium supplementation emerges as a promising adjuvant therapeutic candidate in the clinical management of PCOS.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common complex endocrine disorder in women of reproductive age, although its etiology has not been fully elucidated, it involves the interaction of genetic predisposition with various environmental factors. This syndrome, characterized by a broad phenotypic spectrum and significant clinical heterogeneity, predisposes patients to metabolic comorbidities such as obesity, insulin resistance, dyslipidemia, and metabolic syndrome from adolescence to adulthood. Obesity is a common finding that significantly aggravates the PCOS phenotype and clinical course [1]. The pathophysiological effects of obesity emerge along the axis of intensified insulin resistance, increased testosterone and luteinizing hormone (LH) secretion, and decreased sex hormone-binding globulin (SHBG) levels. This picture is further complicated by adipokine imbalances and chronic low-grade inflammation. Additionally, obesity is known to elevate hirsutism scores, reduce response to ovulation induction, exacerbate chronic anovulation and menstrual irregularities, and increase pregnancy complications and miscarriage risk. Current literature indicates that the coexistence of obesity and PCOS may be associated with common genetic predispositions [2].

The long-term effects of PCOS are not only metabolic but also include oncological risks; premenopausal patients have an increased risk of endometrial hyperplasia and cancer. Additionally, as an indicator of genetic transmissibility, first-degree male relatives (fathers and brothers) of women with PCOS have been reported to have increased frequency of metabolic syndrome, type 2 diabetes, and hypertension [3] Genetic variants associated with the syndrome have been shown to modulate critical physiological processes such as gonadotropin secretion, androgen biosynthesis, glucose homeostasis, folliculogenesis, and age of menopause [4]. The role of micronutrients in the metabolic and hormonal management of PCOS is gaining increasing attention. In this context, Magnesium (Mg) stands out with its key role in androgen-estrogen conversion, insulin sensitivity, and glucose homeostasis. Additionally, it carries therapeutic value in conditions such as endometriosis due to its potential to suppress vascular endothelial growth factor (VEGF) and its effects on menstruation. However, PCOS patients have been observed to have lower serum Mg levels compared to healthy controls. Therefore, Mg supplementation is considered as a potential adjuvant treatment strategy to improve insulin metabolism, reduce the risk of metabolic comorbidities, and contribute to menstrual regulation [5]. Indeed, studies show that Mg supplementation provides significant improvements in insulin resistance, total cholesterol, LDL, and fasting blood glucose levels in patients with PCOS [6]. Although current literature focuses on the metabolic effects of magnesium, its effects on GLUT-4 translocation and next-generation adipokines (Chemerin, Betatrophin), which are critically important in PCOS pathogenesis, have not yet been elucidated.

The main hypothesis of this study is that magnesium replacement in PCOS patients will not only correct serum levels but also provide metabolic improvement by increasing GLUT-4 expression and regulating the adipokine profile. In this regard, the study aims to be one of the first researches examining the effects of magnesium supplementation on metabolic, hormonal, and molecular markers in patients with PCOS through a holistic and prospective design. Although current literature has focused on the metabolic effects of magnesium, its effects on GLUT-4 translocation and next-generation adipokines (Chemerin, Betatrophin), which are critical in PCOS pathogenesis, have not yet been elucidated.

Materials and Methods

The detailed study protocol regarding participant selection, intervention, and analysis is fully described in the 'Materials and Methods' section of this article; the protocol was not deposited in a public repository

Study Design and Participants: This research is a prospective, case-controlled, and open-label clinical intervention study conducted in accordance with the principles of the Helsinki Declaration, with the approval of Van Yüzüncü Yıl University Clinical Research Ethics Committee (Date: 10.02.2021, Decision No: 17; Appendix 1-2).

Power Analysis: The primary endpoint of the study was determined as the change in serum magnesium level. Using G*Power software, it was calculated that at least 23 participants in each group were required to detect the minimum significant difference between groups with 80% power and 5% Type-1 error margin (α=0.05). In accordance with the pathophysiology of the disease, all participants enrolled in this study were biological females. A total of 60 patients aged 18-40 years who were diagnosed with PCOS according to Rotterdam criteria and who presented to the Adult Endocrinology Outpatient Clinic between March 2021 and June 2021 were included in the study. Written informed consent was obtained from all participants (Appendix-3).

Study Groups and Protocol: Group allocation was non-randomized; participants were assigned to the study groups strictly based on their baseline serum magnesium concentrations. Participants were divided into two groups based on their baseline serum magnesium (Mg) levels:

1. Case Group (Intervention Group): 30 PCOS patients with slightly low serum Mg levels (1.2-1.7 mg/dL). This group was prescribed 365 mg oral magnesium tablets once daily ($MgCl_2$, MgO, or $Mg(OH)_2$; 40-60 mEq) in accordance with the Turkish Endocrinology and Metabolism Association (TEMD) guidelines [7].

2. Control Group: 30 PCOS patients with normal serum Mg levels (1.8-2.4 mg/dL) with similar age and BMI characteristics. No supplements were administered to this group; they underwent routine follow-ups.

Patients in both groups were ensured to have similar characteristics in terms of spironolactone, metformin, or anti-androgenic oral contraceptive use, as well as diet and exercise habits. Patients with heart block, renal failure (GFR <60 mL/min), anuria, myasthenia gravis, magnesium allergy, severe respiratory depression, or serious electrolyte imbalance (hypotension/hypokalemia) were excluded from the study. During the 12-week follow-up period, an attrition rate of 23.3% was observed; a total of 14 participants (7 from the intervention group and 7 from the control group) were withdrawn from the study due to the occurrence of pregnancy or difficulties in accessing the healthcare facility amidst the COVID-19 pandemic. Consequently, the final statistical analysis was performed on the remaining 46 participants.

Data Collection and Sampling Procedure

Power Analysis: The primary endpoint of the study was determined as the change in serum magnesium levels. Using G*Power software (Using G*Power software (RRID:SCR_013726), it was calculated that at least 23 participants in each group were required to detect the minimum significant difference between groups with 80% power and 5% Type-1 error margin (α=0.05).), it was calculated that at least 23 participants in each group would be required to detect a minimum significant difference between groups with 80% power and 5% Type-1 error margin (α=0.05).

Clinical and laboratory evaluations were repeated at three time points: baseline (week 0), week 6, and week 12. Blood samples were collected from the antecubital vein during the follicular phase of the menstrual cycle (days 3-5) and following at least 8 hours of overnight fasting. The sample distribution is as follows:

Whole Blood (EDTA tube): For HbA1c and intracellular Mg analysis.

Serum (Gel tube): For routine biochemistry, hormone profile, and ELISA method markers (GLUT-4, 8-OHdG, TAS, TOS, leptin, chemerin, betatrophin).

Biochemical and Hormonal Analyses: Although the clinical follow-up was open-label, to minimize detection bias, the laboratory personnel performing the biochemical and molecular analyses were blinded to the participants' group allocations and clinical details. Serum samples were separated by centrifugation at 4000 rpm for 10 minutes and stored at -80°C until the day of analysis. Routine parameters (Glucose, lipid profile, electrolytes) were analyzed using the photometric method on the Abbott Architect c16000 instrument; the hormone panel (FSH, LH, Testosterone, DHEA-S, SHBG, Insulin, C-peptide, E2, TSH, fT3, fT4, PRL, PTH) levels were analyzed using the Abbott ARCHITECT i1000sr System (RRID:SCR_019328) based on the chemiluminescent microparticle immunoassay (CMIA) method. All measurements were performed according to manufacturer protocols [8,9].

Biomarker Analysis with ELISA Method: Serum levels of GLUT-4, 8-OHdG, TAS, TOS, leptin, chemerin, and betatrophin were analyzed using the ELISA method with commercially available kits (Sunred Biological Technology, Shanghai, China). The intra-assay and inter-assay coefficient of variation (CV) of the kits were reported to be <10%. Optical density (OD) readings were performed at a wavelength of 450 nm using a microplate reader (Biotek ELx800, USA).

Intracellular Magnesium Measurement: For erythrocyte intracellular Mg level, plasma and leukocyte layer were removed from EDTA-treated blood. The pure erythrocytes obtained were washed three times with 0.9% NaCl and then hemolyzed with distilled water. The hemolysates were analyzed using atomic absorption spectrophotometry (Thermo Scientific ICE-3000, USA) by the 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 and hip circumference measurements were performed according to standard protocols; Body Mass Index (BMI) and Waist/Hip Ratio (WHR) were calculated [12,13]. The HOMA-IR index was used for insulin resistance

assessment and was based on the following formula: HOMA-IR = [Fasting Insulin (μU/mL) × Fasting Plasma Glucose (mg/dL)] / 405. In light of literature data, a HOMA-IR value > 2.5 was accepted as the presence of insulin resistance [14].

Statistical Analysis: Data were analyzed using the IBM SPSS Statistics 26.0 (RRID:SCR_002865) package (IBM Corp., Armonk, NY, USA). As a result of the power analysis, the test power was predicted as 80% with a Type-I error of 5%. The normality of the data distribution was assessed using the Kolmogorov-Smirnov test. As the data exhibited a normal distribution, parametric statistical methods were employed for the analysis. Descriptive statistics are presented as mean ± standard deviation (SD), frequency (n), and percentage (%). Repeated Measures ANOVA was used for the analysis of changes over time within groups, while Continuous variables were compared between the two independent groups (Case vs. Control) using the Independent Samples T-test (two-tailed), provided that the assumptions of normality and homogeneity of variances (Levene's test) were met. For intra-group comparisons (Baseline vs. 12th Week), the Paired Samples T-test (two-tailed) was employed. The assumption of independence of observations was maintained for the unpaired tests. Effect sizes were calculated where significant differences were observed. All analyses were conducted using IBM SPSS Statistics 26.0 (RRID:SCR_002865). or One-Way ANOVA was used for comparisons between groups. Post-hoc comparisons were performed with Duncan's test; Categorical data (e.g., presence of obesity or other binary outcomes) were analyzed using the Pearson Chi-square test for independence. The assumptions of mutually exclusive categories and independence of observations were met for these analyses. In cases where the assumption of a minimum expected cell frequency of 5 was not met (i.e., when more than 20% of cells had expected frequencies < 5), Fisher’s Exact Test was employed. All analyses were performed using IBM SPSS Statistics 26.0 (RRID:SCR_002865), and a p-value < 0.05 was considered statistically significant.. The level of statistical significance was accepted as $p<0.05$. No custom code or software algorithms were developed for this study; all statistical analyses were performed using the commercially available software packages mentioned above.

RESULTS

Study Group and Anthropometric Data:

Out of 60 participants initially included in the study, analyses were completed with a total of 46 patients (23 Cases, 23 Controls) following dropouts during the follow-up period. There were no significant differences between the groups regarding smoking habits (X^2 (1, N=46) = 0.534, p = 0.465) or alcohol consumption (X^2 (1, N=46) = 0.001, p > 0.999). Furthermore, the use of medications such as spironolactone, metformin, or oral contraceptives was similar across both groups (X^2 (1, N=46) = 1.073, p = 0.300).

The anthropometric characteristics of the groups are detailed in Table 1. No statistically significant difference was detected between case and control groups in terms of body weight, Body Mass Index

(BMI), and Waist/Hip ratio ($p>0.05$). Although a numerical decreasing trend in BMI values was observed towards the 12th week in both groups, the change between groups followed a similar course.

Metabolic Profile, Lipids, and Electrolytes

The complete breakdown of metabolic and biochemical parameters is presented in Table 2. Serum Mg ($p=0.001$) and Intracellular Mg ($p=0.009$) levels, which were the main parameters of the study, were found to be significantly lower in the case group compared to the control group at baseline. Although serum Mg levels in the case group increased at week 6 with Mg supplementation, they still remained below the control group at the end of week 12 ($p=0.013$).

When examining the lipid profile; Total Cholesterol and LDL-Cholesterol levels, which were statistically higher in the case group at baseline, showed a numerical decreasing trend with Mg treatment at week 12, and the statistical difference between the case group and the control group disappeared ($p>0.05$). However, this decrease did not reach the level of statistical significance ($p<0.05$) in within-group analyses.

No statistical difference was observed between groups in glycemic parameters (Glucose, Insulin, C-peptide, HbA1c, and HOMA-IR), and glucose metabolism followed a similar course. In the electrolyte panel, the most notable finding was in Calcium (Ca) metabolism; the Ca levels of the control group were found to be significantly higher than the case group both at baseline and at the end ($p<0.05$).

Hormonal Profile

A broad panel including thyroid, parathyroid, and sex hormones is presented in Table 3. In thyroid function tests, while fT4 levels were initially higher in the control group, fluctuations were observed in fT3 and TSH levels over time, but no clinical pathology was detected.

The most striking hormonal finding is in PTH levels; in the case group, PTH remained significantly higher compared to the control group at all time points of the study (0, 6, and 12 weeks) ($p<0.05$). In the androgen profile, it was determined that Estradiol (E2) levels in the case group were significantly higher than the control group, especially at week 6 ($p=0.034$), while Total Testosterone and DHEA-S levels were numerically higher but did not constitute a statistical difference.

Adipokines and Oxidative Stress Markers

Adipose tissue hormones and oxidative stress markers are presented in Table 4. GLUT-4, an insulin sensitivity marker, was found to be significantly lower in the case group compared to the control group at all measurement times ($p<0.05$).

There is a remarkable pattern in adipokines; Chemerin levels throughout the entire process, and Betatrophin levels at weeks 6 and 12 were surprisingly higher in the control group compared to the case

group (p<0.05). While 8-OHdG levels, one of the oxidative stress parameters, followed a similar course between groups, total antioxidant capacity (TAS) was found to be higher in the control 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. p < 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. $p < 0.05$ 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. $p < 0.05$ was considered statistically significant.

Table 4. Serum 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. $p < 0.05$ was considered statistically significant.

DISCUSSION

In Polycystic Ovary Syndrome (PCOS), characterized by a vicious cycle between insulin resistance and hyperandrogenism, disruptions in magnesium homeostasis, despite often being overlooked, play a critical role in its pathogenesis. The most distinctive aspect that differentiates this study from similar ones in the literature is that it presents, through a holistic approach, the multidimensional regulatory effects of magnesium replacement not only on serum levels but also on metabolic profile, hormonal

balance, and molecular markers such as GLUT-4. The demographic data (age, height, waist-hip ratio) of the case and control groups forming the study cohort exhibited a homogeneous distribution at baseline. There are conflicting data in the literature regarding the effect of Mg on anthropometric measurements in PCOS management. While Farsinejad-Marj et al. (2020) and some obesity studies [15, 16, 17], reported that Mg supplementation provided a significant decrease in BMI and waist circumference, Muneyyirci-Delale et al. (2013) did not observe a significant change over a 12-week period. In our study, although not reaching statistical significance, a numerical downward trend in BMI values was observed in the case group with low Mg levels after treatment. This can be interpreted as Mg supplementation contributing to weight management by supporting metabolic stabilization rather than acting as a weight loss agent alone [18].

The role of trace elements in the etiology of metabolic diseases is increasingly being scrutinized. Magnesium stands out with its key functions in insulin signalization and glucose homeostasis, particularly through tyrosine kinase activity [19]. In our study, in full agreement with the literatüre [20, 21], it was confirmed that both serum and intracellular Mg stores were significantly suppressed in the PCOS group. The increase in Mg levels in the case group after treatment demonstrates the bioavailability of the applied replacement. On the other hand, the low calcium and high PTH levels detected in the case group (Table 3) indicate that chronic magnesium deficiency triggers a compensatory response in the parathyroid axis [22]. This finding suggests that not only Mg but also Ca-PTH balance should be routinely monitored in PCOS patients.

The relationship between magnesium deficiency and atherogenic lipid profile is well-defined [23]. In our study, it is noteworthy that the initially high Total Cholesterol and LDL-Cholesterol levels in the case group regressed to control group levels by the end of the 12th week with Mg replacement. This result strengthens the hypothesis that Mg can improve lipid profile by modulating lipoprotein lipase activity [17].

When examining the hormonal profile of PCOS, it is known that insulin resistance and hyperinsulinemia increase ovarian androgen synthesis [24, 25]. One of the important observations of our study is the increasing trend in SHBG levels in the group receiving Mg treatment. Although statistically marginal, this numerical increase in SHBG may contribute at a clinical level to binding and neutralizing free bioactive androgens. Additionally, the decrease in high Estradiol (E2) levels identified in the case group from the 6th week onwards may indicate Mg's potential regulatory effect on aromatase activity or estrogen metabolism.

The most striking finding of our study at the molecular level is related to GLUT-4. It is known that GLUT-4 expression decreases in adipocytes in PCOS pathogenesis, deepening insulin resistance [26, 27]. Our results showed that deepening magnesium deficiency in the PCOS group coincided with low GLUT-4 levels. The tendency of GLUT-4 levels to increase with treatment supports at the molecular level that Mg supplementation has the potential to break insulin resistance by improving cellular glucose transport.

In terms of adipokine profile, our study presents interesting data that differs from the general consensus in the literature. The most striking finding of our study that diverges from the general opinion in the literature is that Chemerin and Betatrophin levels were detected higher in the healthy control group compared to the PCOS group. These adipokines, which generally increase with insulin resistance [28], being lower in the case group in our study can be explained by the 'adipose tissue dysfunction' and 'exhaustion' hypothesis. It is known that chronic magnesium deficiency suppresses protein synthesis and secretion mechanisms at the cellular level. Therefore, it can be considered that adipocytes in the PCOS group may not have sufficient adipokine synthesis capacity to respond to metabolic needs, while the elevation in the control group actually reflects 'healthy and functional' adipose tissue activity.

The paradoxical increase in 8-OHdG levels, a marker of DNA damage, in the case group after Mg supplementation might initially be interpreted as increased oxidative stress. However, from a biochemical perspective, magnesium is the primary cofactor for DNA repair enzymes (especially base excision repair). The increased 8-OHdG in serum after treatment may reflect the process of clearance, where damaged bases are cut from DNA and released into the bloodstream as a result of activated repair mechanisms, rather than increased cellular damage [29]. Thus, this increase should be considered as a reparative healing response, not a pathological deterioration.

When evaluated in terms of glycemic control; although numerical improvements were observed in glucose, HbA1c, and HOMA-IR levels after Mg supplementation, statistical significance could not be achieved [30]. This suggests that breaking metabolic memory and achieving dramatic improvement in glycemic parameters requires replacement strategies at higher doses or for periods longer than 12 weeks.

In conclusion, this study has revealed that magnesium deficiency in PCOS pathogenesis is not just a consequence; it could be one of the triggers for irregularities in the "oxidative stress–insulin resistance–adipokine" axis. The potential of Mg supplementation to particularly reduce LDL-Cholesterol, support GLUT-4 expression, and increase SHBG levels makes it a valuable adjuvant therapy candidate in PCOS management. Despite limitations such as being conducted under COVID-19 pandemic conditions and sample constraints, the data obtained provides a strong foundation for future large-scale studies.

Declarations

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Van Yüzüncü Yıl University Medical Faculty (decision date: 10.02.2021, decision no: 17; appendix 1-2)

Ethics / Informed Consent Statement: The study was approved by the institutional ethics committee (Approval No: 2021/17/1-2) and conducted in accordance with the Declaration of Helsinki. Written

informed consent was obtained from all participants before inclusion in the study. A copy of the ethics approval document is provided in the supplementary material.

Data Availability Statement: The original contributions presented in this study are included in the article; further inquiries can be directed to the corresponding authors.

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

Author Contributions: Conceptualisation, 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.; visualisation, A.K.Z.; supervision, M.A. and I.Ç.; project administration, A.K.Z.; All authors have read and agreed to the published version of the manuscript.

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