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Oral Magnesium Supplementation Improved Lipid Profile but Increased Insulin Resistance in Patients with Diabetic Nephropathy: a Double-Blind Randomized Controlled Clinical Trial

Mehdi Sadeghian[1,2] · Leila Azadbakht[2,3] · Noushin Khalili[4,5] · Mojgan Mortazavi[6] · Ahmad Esmaillzadeh[1,3,7]

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Abstract

Low serum magnesium concentrations were associated with development of renal failure. We aimed to determine whether magnesium supplementation improves renal function, insulin resistance, and metabolic profiles in patients with diabetic nephropathy. A total of 80 hypomagnesemic patients diagnosed with type 2 diabetes and early-stage nephropathy were recruited. Subjects received either daily magnesium oxide or placebo for 12 weeks. Biochemical and anthropometric variables were measured. Physical activity and dietary intakes were also recorded. This study was approved by the ethics committee of Isfahan University of Medical Sciences and was registered on the Iranian Registry of Clinical Trials website (IRCT registration no. IRCT201404271485N12). Serum magnesium levels were not changed significantly. Although the supplementation did not influence glycemic indices, patients in the magnesium group had greater insulin resistance compared with the placebo group after intervention (0.3 ± 2.3 μIU/mL vs. -0.04 ± 2.05, $P = 0.04$). No significant changes were observed in serum total cholesterol, triglycerides, HDL, LDL, and total cholesterol/HDL cholesterol ratio. Furthermore, magnesium did not affect inflammation, serum levels of creatinine, and blood urine nitrogen. However, a marginal decrease in microalbuminuria (-3.1 ± 2.2 mg/L vs. -14 ± 9.9, $P = 0.09$) was observed. Oral magnesium supplementation slightly improved microalbuminuria but resulted in increased insulin resistance in patients with diabetic nephropathy.

Keywords Diabetic nephropathy · Insulin resistance · Magnesium · Microalbuminuria · Renal failure

Introduction

Diabetes is a public health problem that is growing worldwide particularly in developing countries [1]. In Iran, it has been estimated that 8% of adult population are affected [2]. Diabetic nephropathy, the most prevalent micro-vascular complication of diabetes, is the leading cause of renal diseases in these patients [3].

To manage renal function in diabetic nephropathy, several strategies including supplementation of vitamin D, zinc, thiamin, and turmeric have been applied [4–8]. Earlier studies have shown lower magnesium status in these patients [9, 10]. Low serum magnesium concentrations were associated with development of renal failure and increased serum creatinine levels and were a strong predictor of end-stage renal disease [11]. On the other hand, people with diabetes and normal renal function had lower levels of serum magnesium compared with healthy subjects. Some studies have shown that magnesium supplementation might improve glycemic control, insulin resistance, lipid profiles, and endothelial function [12–15]. Reduced serum level of magnesium in diabetic

✉ Ahmad Esmaillzadeh
a-esmaillzadeh@sina.tums.ac.ir

1 Food Security Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

2 Research Committee of Community Nutrition, School of Nutrition and Food Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

3 Department of Community Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, P.O. Box 14155-6117, Tehran, Iran

4 Endocrine and Metabolism Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

5 Isfahan Kidney Diseases Research Center, Department of Nephrology, Isfahan University of Medical Sciences, Isfahan, Iran

6 Department of Internal Medicine, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

7 Tehran University of Medical Sciences, International Campus, Tehran, Iran

patients was considered one of the main reasons of development of end-stage renal disease (ESRD) in cohort studies [11]. Moreover, hypomagnesemia is associated with diabetic complications such as albuminuria [9]. Other studies have reported an inverse correlation of serum magnesium with serum creatinine among patients with diabetes and near-normal function of kidney [16]. Meta-analysis of previous clinical trials on patients with diabetes or at high risk of diabetes showed that oral magnesium supplementation with a median dose of 360 mg/day may improve glucose parameters and increase plasma levels of high-density lipoprotein (HDL) cholesterol, although the long-term benefits and safety remain to be determined [17, 18]. Earlier studies were conducted on diabetic patients with normal renal function, while we are aware of no study examining the effect of magnesium supplementation in patients with early-stage nephropathy. Considering earlier literature, we hypothesized that magnesium supplementation might have a renal protective effect [11] through its effect on improvement of glycemic control and tubular injury [14, 19] and prohibition of depletion of myo-inositol cells [20]. This study aimed to examine the effect of magnesium supplementation on renal function, insulin resistance, and metabolic profiles in patients with diabetic nephropathy.

Patients and Methods

Participants We conducted this randomized placebo-controlled trial during February 2015 to April 2015. This study was performed among 80 diabetic patients with early stage of nephropathy. First, we screened more than 2000 active records to identify individuals that met our inclusion criteria. After phone calls to those who met our inclusion criteria ($n = 120$), 80 patients agreed to participate in the study. After stratification for age, sex, type and dose of medication, and BMI, subjects were randomly assigned to receive either magnesium supplements ($n = 40$) or placebo ($n = 40$) for 12 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. To be enrolled in the study, participants had to be (1) aged more than 18 years old, (2) diagnosed with type 2 diabetes (fasting plasma glucose ≥ 126 mg/dL or taking oral glucose-lowering agents or insulin), and (3) in the early-stage nephropathy (urine albumin of 30–300 mg/dL and glomerular filtration rate (GFR) of ≥ 90 mL/min). Patients demonstrated features of other primary kidney diseases, arrhythmia, tetany, hypothyroidism, hyperthyroidism, urinary tract infection, and any infection-related fever, liver disease, cancers, and inflammatory diseases, and those who used cigarettes or any other tobacco products were not included in the study. We also did not include individuals who had lost or gained weight more than 4 kg during the last 3 months as well as those that were taking any type of magnesium-containing supplements. The study was conducted according to the guidelines provided by the Declaration of Helsinki. Written informed consent was provided by each participant before the study. The consent form was reviewed and approved by the ethics committee.

Study Design Subjects were randomly assigned to receive either one tablet of magnesium oxide or placebo daily for 12 weeks. Each magnesium supplement contained 250 mg magnesium oxide (250 mg elemental magnesium and 47 mg calcium carbonate) and was manufactured by the 21 century company (Arizona, USA). We used the formulation MgO as it induced less diarrhea in previous reports [21]. Placebo contained lactose, starch, calcium stearate, and calcium phosphate and was manufactured by Razi industrial town (Isfahan, Iran). Placebos and supplements were similar in appearance (color, shape, size, and packaging). Magnesium dose was based on an earlier study on the effects of vitamin and mineral supplementation in patients with type 2 diabetes [22]. The upper limit of magnesium from supplement is reported to be 350 mg/day for both adolescents and adults [23]. Participants were asked not to alter their routine physical activity or usual dietary intakes throughout the study. They were also requested to report any changes in their medication dose and type. All measurements were performed a day before the start of the study and at the end of 12 weeks of intervention. To examine the compliance to the supplements, we quantified serum magnesium concentrations. In addition, participants were requested to bring their medication containers and to report the number of unused tablets at the end of the intervention. To ensure that the subjects maintained their usual diet and activity levels throughout the intervention, each participant was requested to record daily physical activities and dietary intakes for 4 separate non-consecutive days (one in every 3 weeks). To minimize the bias, 4 different days of the week (including one holiday and three working days) were considered. In addition, participants were not aware of the day they had to record their dietary intakes or activities until the early morning of a given day. Household measurements were used to convert the portion sizes participants reported to the grams per day. Nutrient intakes of subjects were calculated using Nutritionist IV software (First Databank, San Bruno, CA, USA) modified for Iranian foods. Physical activity records were processed manually, and considering the type of physical activity and the times spent for each activity, we calculated metabolic equivalents (METs)-h per day for each participant using published standards [8].

Biochemical Assessment Urinary albumin-to-creatinine ratio (UACR) was considered the primary outcome and other metabolic profiles were defined as the secondary outcome. In

order to quantify biochemical indicators, a venous blood sample of 10 mL was taken after a12-h overnight fasting at the Aseman laboratory at study baseline and week 12 of intervention. Participants did not avoid taking medications until the night before blood sample collection. Fasting plasma glucose (FPG) levels were measured on the day of blood sampling. To separate 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, HDL cholesterol, BUN, creatinine, and urinary microalbumin concentrations (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 and 3 to 7% for renal indicators. ELISA kit (Cobas Integra 800 Autoanalyzer, Roche Diagnostics, Germany) was used to assay serum insulin levels. The intra- and inter-assay CVs for serum insulin were 1.9% and 2.6%, respectively. HOMA-IR, HOMA-B, and QUICKI were calculated on the basis of suggested formulas [24]. Serum high-sensitivity C-reactive protein (hs-CRP) was quantified using an ELISA kit (LDN, Nordhorn, Germany) with intra- and inter-assay CVs of 2.6% and 4.5%, respectively. Reagents for HbA1c measurement were obtained from Biosystems S.A. Costa Brava 30, Barcelona, Spain, with intra- and inter-assay CVs of 5.9% and 6.6%, respectively.

Assessment of Variables Anthropometric measurements were taken by a trained dietitian at the Endocrine Research Center. Body weight, height, and waist and hip circumferences were measured at study baseline and 12 weeks after intervention. Body weight was measured in minimal clothing and without shoes over a night fasting, using a digital scale with 0.1-kg accuracy (Seca, Hamburg, Germany). To measure height and waist and hip circumferences, a non-stretch tape measure (Seca) to the nearest 0.1 cm was used. Waist circumference was measured in the slimmest area and hip circumference in the largest part of the hip with no pressure on the body surface, while individuals were at the end of a normal exhalation. BMI was calculated as weight in kilogram divided by height in meters squared. Data on socio-economic status, age, and sex were self-reported at baseline.

Statistical Analysis Sample size was estimated considering type 1 (α) and type 2 errors (β) of 0.05 and 0.10 (power = 90%), respectively, and urinary albumin-to-creatinine ratio (UACR) as a key variable. The SD of albumin-to-creatinine ratio was 81 mg/mmol and the mean difference (d) was 70 mg/mmol according to a former study [25]. Therefore, the sample size was estimated to be 29 participants in each group using the suggested formula for parallel clinical trials. To consider probable dropouts during the study, we recruited 40 patients in each group. To determine if the distribution of variables were normal, Kolmogorov-Smirnov test was performed, and histograms of each variable were generated to visually assess the distribution. We used intention-to-treat analysis of the primary study endpoint which was used for all the patients assigned randomly. ANOVA of mixed-model repeated measures was applied to examine the effects of magnesium supplementation on insulin metabolism, lipid profiles, inflammatory factor, and biomarkers of renal function. Considering the assumption that missing values are randomly missing, we did not impute these values because the mixed-model analysis without any ad hoc imputation is the same power as analysis using mixed methods (http://www.rti.org/rtipress). To avoid potential bias, all analyses were controlled for baseline values of age and weight in order to not affect the magnitude of the change in dependent variables. We used chi-square test for categorical variables and 1-factor ANOVA for continuous variables to define the effect of magnesium supplementation on renal outcomes. P values less than 0.05 were considered significant. All statistical analyses were done using SPSS version 17.

Results

The process of assessing and selecting the studies are illustrated in Fig. 1. Five subjects dropped out (two in the MgO group and three in the placebo group) throughout the study. Totally, the supplements were tolerated well. However, 7 patients (5% in the MgO group and 12% in the placebo group) reported adverse effects including rashes on skins and lips ($n = 1$, 2%), headache ($n = 2$, 5%), stomachache ($n = 2$, 5%), and kidney pain ($n = 2$, 5%). As we used ITT (intention to treat), all participants in the study were included in the analysis. Results of the Kolmogorov-Smirnov test are illustrated in Fig. 2 in the Appendix**.** Normality histograms of each variable are shown in Figs. 3, 4, 5, 6 in the Appendix.

General characteristics of the study population that received magnesium supplements or placebo are shown in Table 1. There was no significant difference in mean age between the two groups. Distribution of participants in terms of gender was not significantly different. Differences for mean BMI, waist circumference, hip circumference, and duration of diabetes were not significant between the two groups. Furthermore, distribution of subjects in terms of marital status and physical activity was not significantly different.

Dietary intake of study participants throughout the study are provided in Table 2. We did not find any significant difference in mean energy intake, macronutrients, cholesterol, dietary fiber, and magnesium between the two groups.

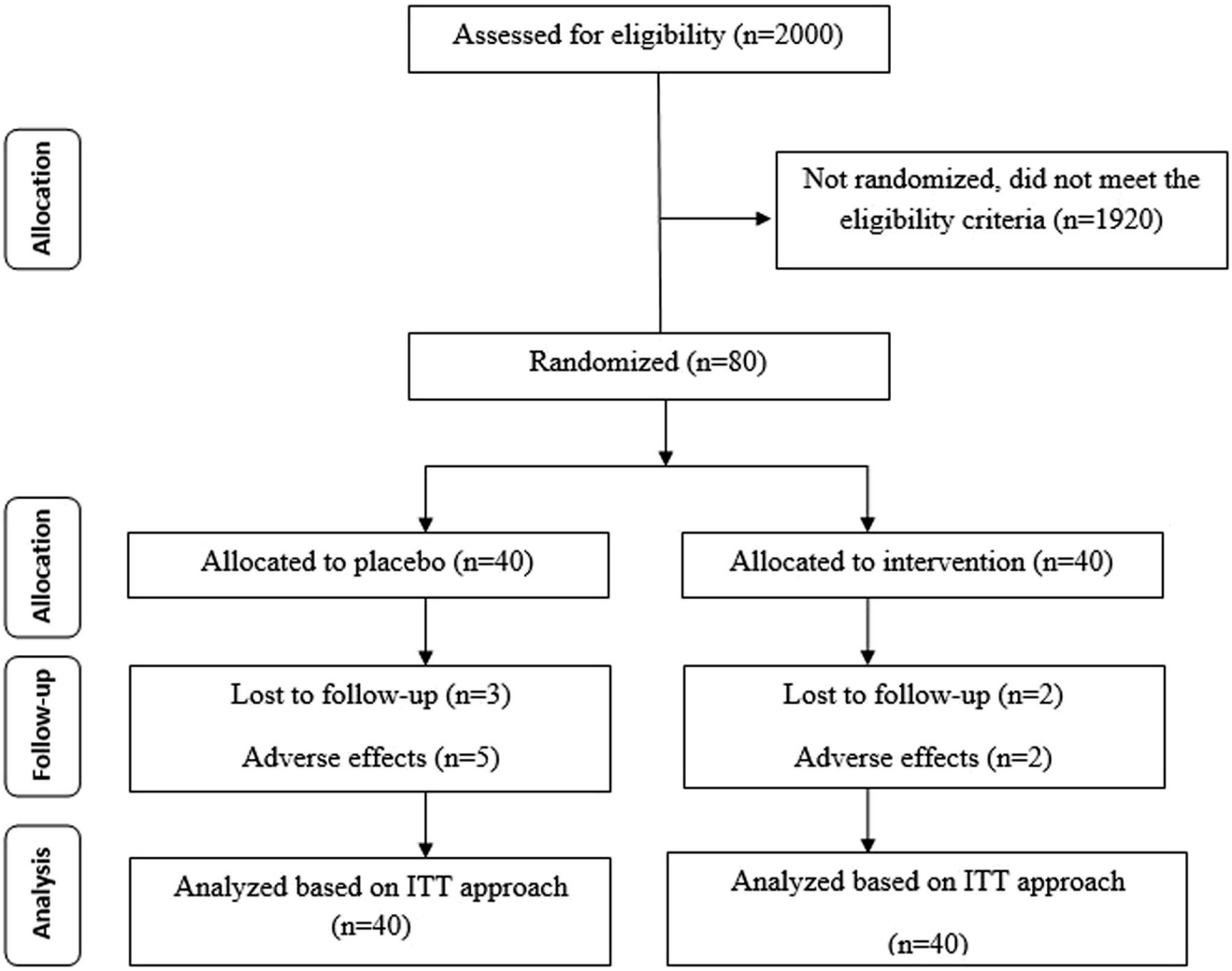

Fig. 1 Process of assessing and selecting the studies

Comparison of serum magnesium levels, glycemic indicators, lipid profiles, and biomarkers of renal function between the magnesium and placebo groups is shown in Table 3. Magnesium supplementation did not result in a significant change in serum magnesium levels. Although the supplementation for 12 weeks did not influence glycemic indices, patients in the magnesium group had greater insulin resistance compared with those in the placebo group after intervention. No significant changes were observed in serum total cholesterol, triglycerides, HDL, LDL, and total cholesterol/HDL cholesterol ratio. Furthermore, magnesium supplementation did not affect hs-CRP, serum levels of creatinine, and BUN. However, a marginal decrease in microalbuminuria was observed after supplementation.

When the findings were controlled for baseline levels of corresponding variables, total dietary fiber intake, use of insulin injection, and oral hypoglycemic agents (OHAs), a significant decrease was observed in serum levels of triglyceride and VLDL (Table 4).

Discussion

We found that oral magnesium supplementation in patients with diabetes in the first stages of nephropathy resulted in a slight decrease in albuminuria; however, it was associated with increased insulin resistance. To the best of our knowledge, this is the first study describing the influence of magnesium supplementation on metabolic, renal, and inflammatory outcomes in patients with diabetic nephropathy.

In the current study, after a 12-week supplementation with magnesium in subjects with diabetes in early stages of nephropathy, a slight decrease was found in microalbuminuria. Earlier observational studies have investigated the relationship between serum magnesium levels and renal outcomes in patients with diabetic nephropathy. Hypomagnesaemia was significantly associated with progression of ESRD [11] and prevalence of microalbuminuria [26] in patients with type 2 diabetic nephropathy. A significant inverse association was also

Table 1 General characteristics of patients with diabetic nephropathy who received either magnesium supplements or a placebo. Values are means ± SDs

	Placebo group ($n = 40$)	Magnesium group ($n = 40$)	P value
Age (year)	42.8 ± 8.4	41.2 ± 8.8	0.76
Female (%)	67.5	65.9	0.53
Height (cm)	158.2 ± 7.4	162.9 ± 9.3	0.01
Weight at study baseline (kg)	77.7 ± 13.2	82.1 ± 12.1	0.12
Weight at end of trial (kg)	77.1 ± 13.6	80.7 ± 12.5	0.27
Weight change (kg)	− 0.6 ± 2.2	− 0.3 ± 2.3	0.52
BMI at study baseline (kg/m^2)	30.9 ± 4.4	31.2 ± 5.5	0.84
BMI at end of trial (kg/m^2)	30.6 ± 4.6	30.4 ± 5.4	0.89
BMI change (kg/m^2)	− 0.1 ± 0.6	− 0.2 ± 1.0	0.64
Waist circumference at study baseline (cm)	101.5 ± 11.2	104.6 ± 10.9	0.22
Waist circumference at end of trial (cm)	103.7 ± 13.0	104.9 ± 11.6	0.70
Waist circumference change (cm)	2.6 ± 5.4	1.6 ± 5.9	0.51
Hip circumference at study baseline (cm)	107.8 ± 11.4	110.0 ± 11.6	0.38
Hip circumference at end of trial (cm)	105.8 ± 10.4	106.8 ± 10.0	0.70
Hip circumference change (cm)	− 1.8 ± 3.9	− 2.6 ± 7.6	0.59
Disease duration (year)	12.8 ± 7.5	13.2 ± 8.6	0.82
Married (%)	90.0	90.2	0.38
Physical activity (%)	37.5	26.8	0.21

BMI, body mass index

reported between serum magnesium levels and serum creatinine concentrations among patients with diabetes and near-normal function of the kidney [16]. In addition, no significant difference in microalbuminuria was reported in patients with type 2 diabetes plasma magnesium levels of < 0.75 mmol/L versus ≥ 0.75 mmol/L [13]. In a long-term cohort study, no significant association was observed between serum magnesium concentrations and renal outcomes [27]. Although there was a significant inverse correlation between ionized serum magnesium levels and serum triglyceride concentrations as well as HbA_{1c} in patients with diabetes and microalbuminuria (20 < urea < 200 μg/min) or clinical proteinuria (urea > 200 μg/min), no correlation was found in patients with normoalbuminuria (urea < 20 μg/min) [9]. In addition, findings describing the association between magnesium depletion and microalbuminuria are inconsistent. Microalbuminuria did not predict serum concentrations of magnesium in a previous study [10]. However, patients with clinical proteinuria had altered magnesium homeostasis with respect to controls [28]. Studies that have examined magnesium supplementation in subjects with diabetes [21] and pre-diabetes [29] with normal renal function reached controversial findings. Rodriguez-Moran et al. [14] showed an improved metabolic control and insulin sensitivity after supplementation with $MgCl_2$ in type 2 diabetes; however, they observed an increased serum levels of insulin. Farvid et al. [22] did not observe any significant

Table 2 Dietary intakes of patients with diabetic nephropathy who received either magnesium supplements or a placebo. Values are means ± SDs.

	Placebo group ($n = 40$)	Magnesium group ($n = 40$)	P value
Energy (kcal/day)	1639.6 ± 472.1	1794.9 ± 470.4	0.22
Carbohydrates (g/day)	236.3 ± 64.5	253.7 ± 72.0	0.35
Protein (g/day)	57.9 ± 19.1	63.3 ± 19.0	0.29
Fat (g/day)	53.9 ± 20.0	61.8 ± 24.5	0.19
SFAs (g/day)	14.6 ± 5.4	16.0 ± 5.0	0.34
PUFAs (g/day)	18.6 ± 9.5	23.0 ± 11.6	0.13
MUFAs (g/day)	14.0 ± 5.9	15.9 ± 8.6	0.35
Cholesterol (mg/day)	163.6 ± 83.8	170.9 ± 81.1	0.74
TDF (g/day)	14.2 ± 5.5	17.5 ± 7.2	0.06
Magnesium (mg/day)	210.5 ± 160.3	195.5 ± 77.7	0.65

TDF, total dietary fiber; *SFA*, saturated fatty acid; *PUFA*, polyunsaturated fatty acid; *MUFA*, monounsaturated fatty acid

Table 3 Metabolic and lipid profiles, inflammatory factors, and biomarkers of renal function at baseline and after 12 weeks of intervention in patients with diabetic nephropathy who received either magnesium supplements or a placebo. Values are means ± SDs

	Placebo group ($n = 40$)			Magnesium group ($n = 40$)			P value[a]
	Week 0	Week 12	Change	Week 0	Week 12	Change	
Magnesium (mg/dL)	2.3 ± 0.3	2.4 ± 0.3	0.07 ± 3	2.2 ± 0.3	2.3 ± 0.4	0.1 ± 0.5	0.77
FPG (mg/dL)	166.6 ± 45.9	156.8 ± 53.0	− 7.9 ± 39.2	163 ± 46.3	162.8 ± 62.0	− 1.4 ± 58.5	0.47
HbA1c (% (mmol/mol))	7.4 ± 1.2 (57 ± 13.1)	7.8 ± 1.4 (62 ± 15.3)	0.29 ± 0.1 (3.2 ± 1.1)	7.6 ± 1.4 (60 ± 15.3)	7.7 ± 1.6 (61 ± 17.5)	0.09 ± 1.3 (1 ± 14.2)	0.36
Insulin (μIU/mL)	8.3 ± 5.6	10.0 ± 5.7	1.4 ± 4.5	5.8 ± 5.1	10.1 ± 7.6	3.8 ± 6.1	0.07
HOMA-IR	3.6 ± 2.9	3.8 ± 2.7	0.2 ± 2.5	2.4 ± 2.4	4.3 ± 3.8	1.9 ± 4.0	0.04*
HOMA-B	33 ± 23.2	74.4 ± 151.2	41.4 ± 147.9	28.8 ± 42.6	59.2 ± 114.8	30.4 ± 114.8	0.73
QUICKI	0.3 ± 0.05	0.3 ± 0.03	− 0.01 ± 0.05	0.4 ± 0.1	0.3 ± 0.05	− 0.4 ± 0.1	0.20
Total cholesterol (mg/dL)	191.9 ± 31.6	182.0 ± 20.5	− 8.0 ± 25.0	182.7 ± 27.6	188.1 ± 36.3	5.2 ± 33.0	0.04*
LDL cholesterol (mg/dL)	115 ± 23.1	104.1 ± 20.4	− 8.8 ± 20.1	106 ± 26.3	108.7 ± 35	2.4 ± 31.8	0.057
Triglycerides (mg/dL)	161.9 ± 74.5	152.8 ± 43.7	− 7.3 ± 54.1	158.6 ± 69.0	169.4 ± 64.8	8.9 ± 52.7	0.16
VLDL cholesterol (mg/dL)	32.4 ± 14.9	30.5 ± 8.7	− 1.5 ± 10.7	31.8 ± 13.8	33.9 ± 12.9	1.7 ± 10.6	0.16
HDL cholesterol, mg/dL	44.5 ± 6.6	47.4 ± 6.5	2.3 ± 8.2	44.9 ± 7.9	45.5 ± 5.8	0.6 ± 9.0	0.33
Total/HDL cholesterol ratio	4.4 ± 1.0	3.9 ± 0.8	− 0.5 ± 1.1	4.2 ± 1.0	4.2 ± 1.2	0.05 ± 1.5	0.10
hs-CRP (μmol/mL)	3.3 ± 3.1	2.3 ± 2.2	− 0.8 ± 2.0	2.5 ± 3.0	2.3 ± 2.5	− 0.4 ± 1.7	0.17
Serum creatinine (mg/dL)	0.91 ± 1.2	0.93 ± 1.1	0.02 ± 0.1	1.0 ± 2.1	0.93 ± 1.9	− 0.07 ± 0.1	0.78
BUN (mg/dL)	18.4 ± 6.3	15.6 ± 3.6	− 2.3 ± 4.6	18.3 ± 6.7	16.6 ± 5.3	− 1.3 ± 4.9	0.37
Microalbuminuria (mg/L)	82.6 ± 44.1	79.5 ± 39.3	− 3.1 ± 2.2	92.1 ± 56.0	78.1 ± 42.6	− 14 ± 9.9	0.09
GFR (mL/min)	101.3 ± 54.1	99.8 ± 48.1	− 2.1 ± 4.8	102 ± 59.5	114.5 ± 68.1	15.3 ± 13.2	0.73
Insulin injection (%)	60.5	65.4		39.5	34.6		
Insulin injection (unit)	18.3 ± 29.7	17.1 ± 28.9	− 1.2 ± 20.0	17.6 ± 27.2	15.6 ± 25.4	− 2.0 ± 16.8	0.85
OHA use (%)	11.1	27.2		88.9	72.8		
OHA use (number)	3.2 ± 1.9	2.8 ± 2.4	− 0.4 ± 2.1	3.3 ± 2.0	3.1 ± 2.2	− 0.2 ± 2.1	0.71

FPG, fasting plasma glucose; *HOMA-IR*, homeostasis model assessment as an index of insulin resistance; *HOMA-B*, homeostasis model of assessment-estimated β-cell function; *QUICKI*, quantitative insulin sensitivity check index; *VLDL*, very-low-density lipoprotein; *HDL*, high-density lipoprotein; *hs-CRP*, high-sensitivity C-reactive protein; *BUN*, blood urine nitrogen; *GFR*, glomerular filtration rate; *OHA*, oral hypoglycemic agents

[a] *P* values represent the time × group interaction (computed by mixed-model repeated-measure ANOVA)

increment in serum magnesium concentrations among patients with diabetic nephropathy whom they provided 100 mg magnesium plus 15 mg zinc. Overall, it seems that further studies are required to investigate the effects of magnesium supplementation on renal function as well as on glycemic control in subjects with diabetes.

Findings from our study revealed that supplementation with 250 mg MgO had no significant effect on serum creatinine, BUN concentrations, and GFR as well as serum levels of hs-CRP in patients at the first stages of diabetic nephropathy; however, there was a slight decrease in urinary microalbumin excretion. Lack of finding a significant effect of magnesium supplementation on these variables might be attributed to the non-significant increase in serum levels of magnesium after 12 weeks of intervention. Despite a relatively good compliance to magnesium supplements, it is not clear why serum magnesium levels did not increase after supplementation. No evidence is available indicating if magnesium absorption alters in patients with diabetic nephropathy. Duration of intervention may play an important role in this regard. Perhaps, long-term intervention is required to influence serum magnesium concentrations. In a previous 16-week supplementation with magnesium in subjects with diabetes, Rodriguez et al. [14] observed a decrement in serum magnesium levels at the first 4 weeks of intervention but it was increased gradually in the 12 following weeks. Due to limited funding, we were unable to continue the study for a long time. Earlier studies have indicated reduced tubular reabsorption of magnesium in people with diabetes and renal failure [9]. This might also help us explain our findings. Dose and type of

Table 4 Adjusted changes in metabolic variables of patients with diabetic nephropathy who received either magnesium supplements or a placebo. Values are means ± SEs. Values are adjusted for TDF, baseline levels, insulin, and OHA dose

	Placebo group ($n = 40$)	Magnesium group ($n = 40$)	P value[a]
Magnesium (mg/dL)	0.1 ± 0.07	0.098 ± 0.07	0.97
FPG (mg/dL)	− 7.2 ± 8.8	− 4.2 ± 8.4	0.80
HbA1c (% (mmol/mol))	0.2 ± 0.2 (2.2 ± 2.2)	0.04 ± 0.2 (0.4 ± 2.2)	0.54
Insulin (μIU/mL)	2.5 ± 1.0	3.7 ± 0.9	0.37
HOMA-IR	0.8 ± 0.6	1.4 ± 0.6	0.45
HOMA-B	56.6 ± 29.4	23.6 ± 28.8	0.44
QUICKI	− 0.04 ± 007	− 0.03 ± 0.07	0.75
Total cholesterol (mg/dL)	− 5.7 ± 5.4	1.7 ± 5.2	0.34
LDL cholesterol (mg/dL)	− 5.4 ± 5.2	− 2.8 ± 5	0.73
Triglycerides (mg/dL)	− 17.3 ± 8.9	15.9 ± 8.6	0.009
VLDL cholesterol (mg/dL)	− 3.6 ± 1.8	3.4 ± 1.7	0.008
HDL cholesterol (mg/dL)	3.4 ± 1.3	1.0 ± 1.2	0.21
Total/HDL cholesterol ratio	− 0.4 ± 0.2	− 0.09 ± 0.2	0.25
hs-CRP (μmol/mL)	− 1.1 ± 0.3	− 0.2 ± 0.3	0.04
Serum creatinine (mg/dL)	0.06 ± 0.3	− 0.08 ± 0.2	0.80
BUN (mg/dL)	− 2.7 ± 0.7	− 1.7 ± 0.7	0.28
Microalbumin (mg/L)	− 42.5 ± 9.2	− 54.5 ± 8.9	0.37
GFR (mL/min)	− 3.2 ± 4.4	10.3 ± 6.2	0.27

FPG, fasting plasma glucose; *HOMA-IR*, homeostasis model assessment as an index of insulin resistance; *HOMA-B*, homeostasis model of assessment-estimated β-cell function; *QUICKI*, quantitative insulin sensitivity check index; *VLDL*, very-low-density lipoprotein; *HDL*, high-density lipoprotein; *hs-CRP*, high-sensitivity C-reactive protein; *BUN*, blood urine nitrogen; *GFR*, glomerular filtration rate

[a] Calculated by mixed-model repeated-measure ANOVA (time × group interaction)

elemental magnesium used in this study might also provide a reason. Comparing two dosages of 41.4 mmol/day vs. 20.1 mmol/day of magnesium oxide, it was shown that the latter dosage did not affect metabolic control in patients with type 2 diabetes [21]. While a 5% solution of $MgCl_2$ (50 g per 1000 mL) had beneficial effects on insulin sensitivity and serum magnesium concentrations after a 16-week intervention [14]. Discrepant results were reported from small studies that compared the bioavailability of magnesium oxide with organic compounds (i.e., magnesium citrate). Walker et al. [30] could not find any significant difference in bioavailability between both magnesium preparations. Although Kappeler et al. [31] indicate MgO is poorly absorbed from the intestine, no statistically significant difference was observed between Mg citrate and Mg oxide when comparing intracellular magnesium concentrations. Moreover, a recent study conducted by Zghoul et al. [32] showed that oral Mg-L-lactate replacement therapy after 3 months of treatment with an average dose of 336 mg daily in hypomagnesemic patients with type 2 diabetes could not significantly change hs-CRP levels in spite of increased levels of serum and intracellular magnesium. They also reported that serum magnesium reached to the normal levels (0.74 mEq/L) only in obese patients; however, this is a lower level of cut-point considering normal range of serum magnesium.

We found that serum levels of insulin as well as HOMA-IR and HOMA-B were elevated in both placebo and supplemented groups. Controversial findings have been reported regarding the association of low serum magnesium levels with both insulin sensitivity [25] and impaired insulin response [33]. An experimental study indicated that insulin resistance in subjects with diabetes not only impairs glucose uptake, but also impairs the insulin-induced fall in plasma magnesium [34]. It must be taken into account that some participants in the current study were receiving insulin or glibenclamide, insulin-stimulating oral glucose-lowering agent, which might affect our findings. Although the placebo arm received probably bioactive substances including calcium stearate and calcium phosphate, it occurred to a fairly limited extent to which biological effects would be expected concerning elevated insulin resistance. In addition, serum magnesium levels of most patients enrolled in the current study were at the normal levels. This might also explain why we could not find any significant effect of magnesium supplementation in this study.

Magnesium supplementation could not significantly change lipid profile. However, there was a significant decrease in serum levels of triglyceride and VLDL in the MgO group after controlling for confounding factors. Several studies have shown that fecal excretion of fat is increased through dietary

intake of divalent cations, such as magnesium [35, 36]. Therefore the decreased levels of serum TG and VLDL may be in part due to inhibition of absorption. Moreover, different OHAs have different effects on serum lipid levels in patients with diabetes including rosiglitazone which increase apolipoprotein B levels [37]. Thus, the use of anti-hyperglycemic medications may be another reason that prevented the lowering effects of magnesium in levels of lipids.

The current study had a number of limitations. Although the simplest test for assessment of magnesium status is serum total magnesium test [38], measurements are not representative of magnesium status. As magnesium is a predominantly intracellular cation, depletion of intracellular magnesium could be present despite normal serum concentrations [7]. Some investigators recommended the use of erythrocyte or white blood cell content of magnesium to evaluate dietary intake [32]. A recent study determined the feasibility of assessing intracellular magnesium using epithelial cells [39]. Since we were not able to measure intracellular magnesium levels due to financial constraints, it may lead to less accurate assessment. The other potential limitation is that patients in our study received various doses and types of antidiabetic agents including insulin and sulfonylureas which influence pancreatic insulin secretion. Inconsistent results reported different bioavailability of magnesium compounds; however, future studies are recommended to investigate organic form of magnesium supplements. Although higher dosages of magnesium were previously supplemented in patients with diabetes [18], its use in patients with diabetic nephropathy should be done cautiously due to renal dysfunction. In addition to design and control of potential confounders, calculating dietary magnesium is a noted strength of our study.

Oral supplementation with magnesium had negative effect on insulin sensitivity. Although we observed a slight decrease in microalbuminuria, magnesium supplementation did not have any effect on microalbuminuria after adjusting for potential confounding variables. Therefore, the findings of the current study suggest that magnesium should be prescribed with caution in diabetic patients with nephropathy. More prolonged interventions with higher doses are suggested for future studies.

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Authors' contribution MS, PS, FS, and AE contributed in the 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

Transparency Declaration The lead author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported. The reporting of this work is compliant with CONSORT guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned (IRCT registration no. IRCT201404271485N12) have been explained.

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

Novelty Statement To manage renal function in diabetic nephropathy, several strategies included have been applied, but this is the first study which examined the effect of magnesium supplementation on renal function, insulin resistance, and metabolic profiles in patients with diabetic nephropathy.

Appendix

	Kolmogorov-Smirnov[a]		
	Statistic	df	Sig.
Magnesium.pre	.104	67	.069
Magnesium.post	.106	67	.061
Creatinine.pre	.098	67	.184
Creatinine.post	.077	67	.200*
BUN.pre	.104	67	.072
BUN.post	.095	67	.200*
HS.CRP.pre	.088	67	.200*
HS.CRP.post	.104	67	.070
HbA1C.pre	.098	67	.180
HbA1C.post	.096	67	.200*
FBS.pre	.060	67	.200*
FBS.post	.094	67	.200*
Cholesterol.pre	.064	67	.200*
Cholesterol.post	.063	67	.200*
Triglyceride.pre	.107	67	.054
Triglyceride.post	.103	67	.078
HDL.pre	.059	67	.200*
HDL.post	.094	67	.200*
LDL.pre	.055	67	.200*
LDL.post	.076	67	.200*
VLDL.pre	.103	67	.075
VLDL.post	.091	67	.200*

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Fig. 2 Results of Kolmogorov-Smirnov test

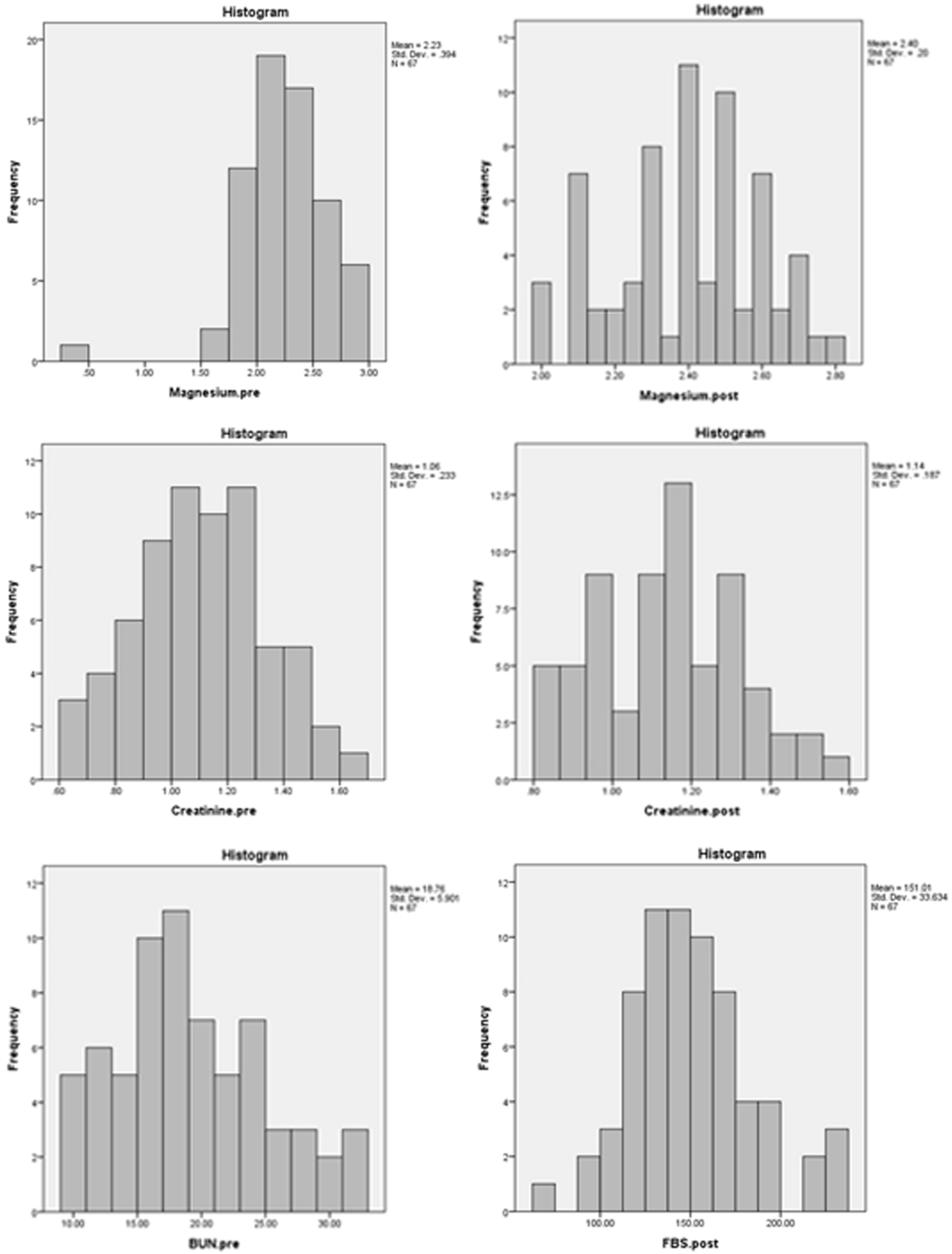

Fig. 3 Normality histograms

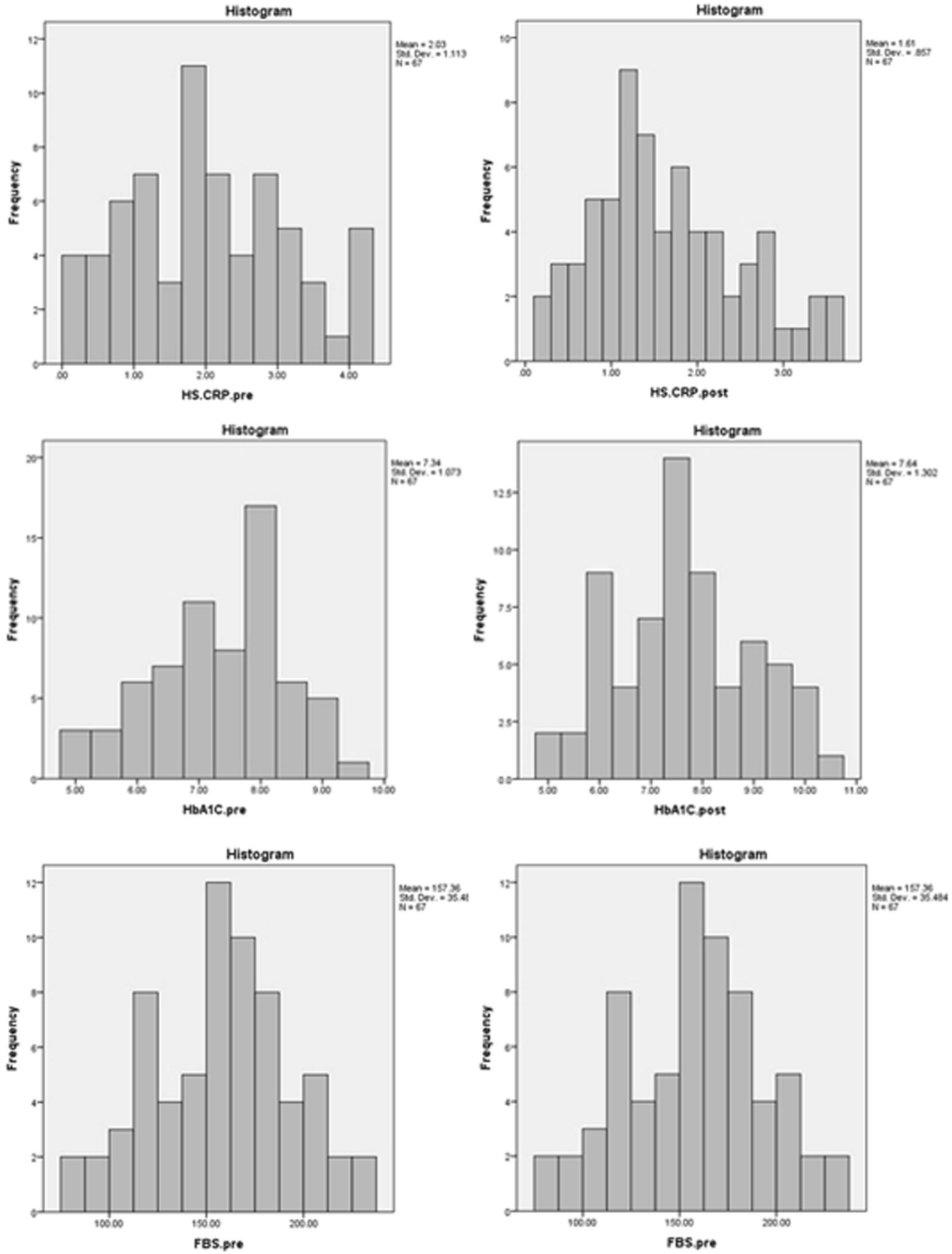

Fig. 4 Normality histograms

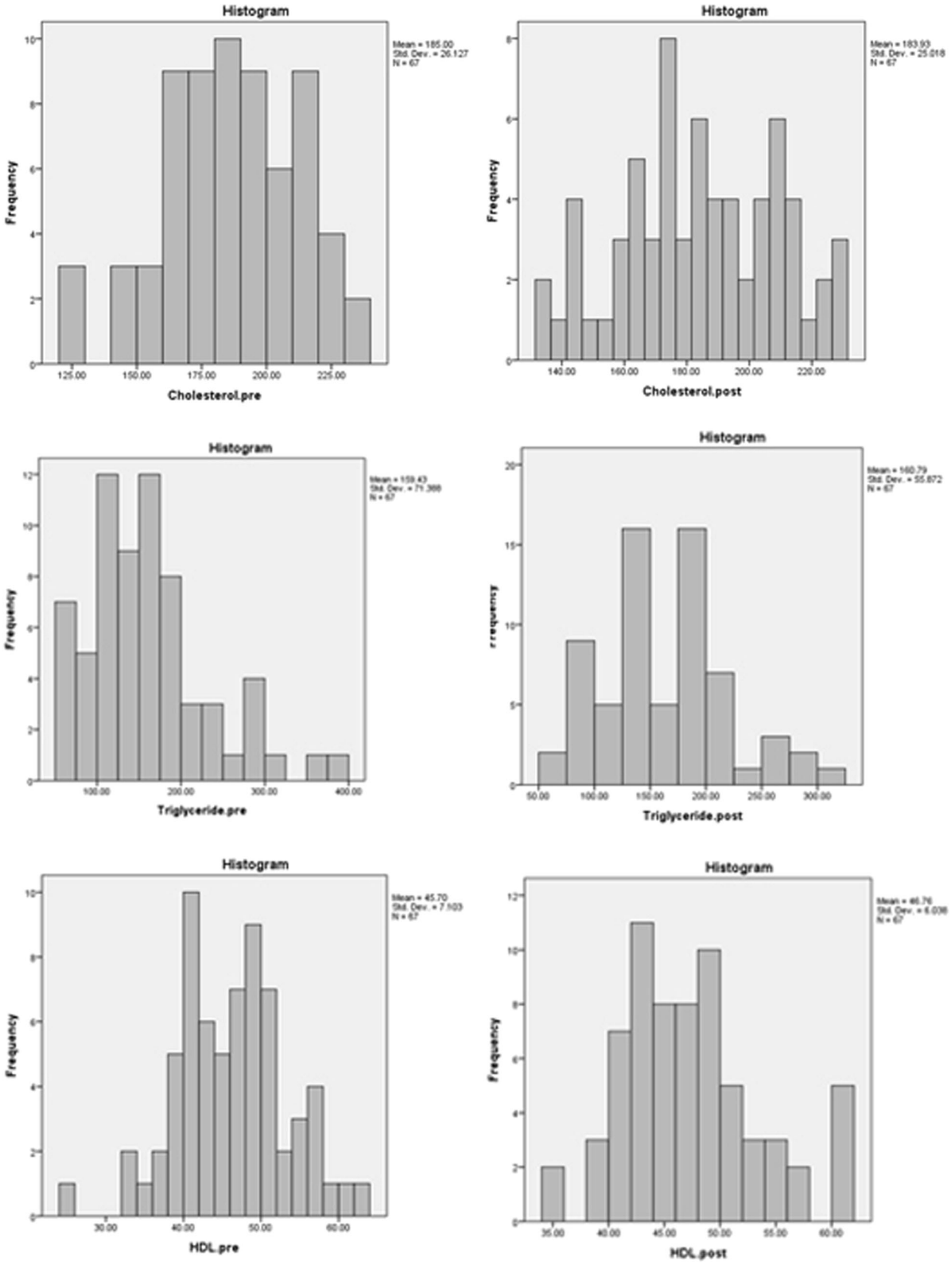

Fig. 5 Normality histograms

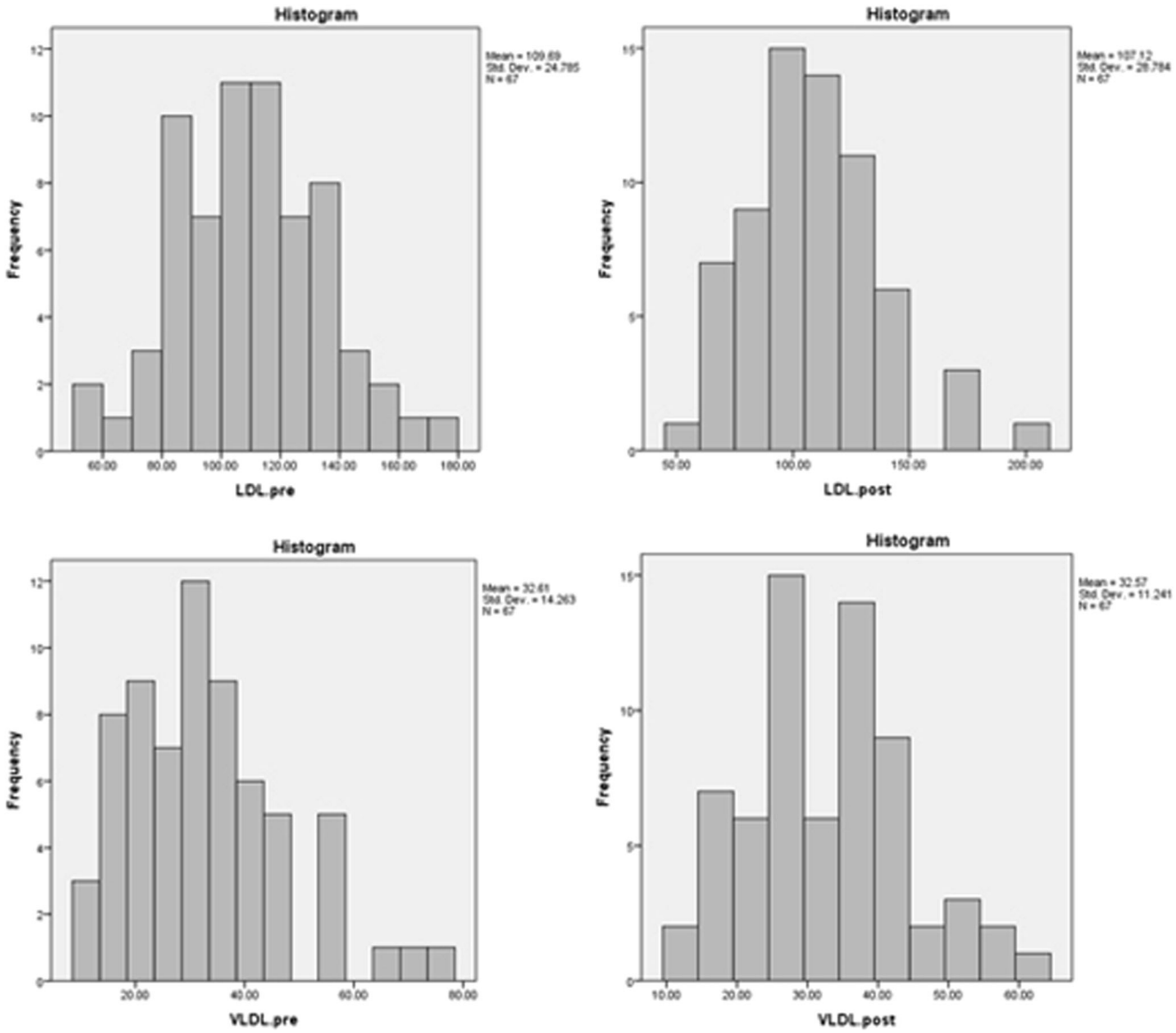

Fig. 6 Normality histograms

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