Biol Trace Elem Res (2018) 181:207–215
DOI 10.1007/s12011-017-1056-5

Magnesium Supplementation and the Effects on Wound Healing and Metabolic Status in Patients with Diabetic Foot Ulcer: a Randomized, Double-Blind, Placebo-Controlled Trial

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Received: 4 April 2017 / Accepted: 15 May 2017 / Published online: 24 May 2017

Abstract Hypomagnesemia is associated with the development of neuropathy and abnormal platelet activity, both of which are risk factors for diabetic foot ulcer (DFU). This study was carried out to evaluate the effects of magnesium administration on wound healing and metabolic status in subjects with DFU. This randomized, double-blind, placebo-controlled trial was performed among 70 subjects with grade 3 DFU. Subjects were randomly divided into two groups (35 subjects each group) to receive either 250 mg magnesium oxide supplements or placebo daily for 12 weeks. Pre- and post-intervention wound depth and appearance were scored in accordance with the "Wagner-Meggitt's" wound assessment tool. Fasting blood samples were taken at baseline and after the 12-week intervention to assess related markers. After the 12-week treatment, compared with the placebo, magnesium supplementation resulted in a significant increase in serum magnesium (+0.3 ± 0.3 vs. −0.1 ± 0.2 mg/dL, $P < 0.001$) and significant reductions in ulcer length (−1.8 ± 2.0 vs. −0.9 ± 1.1 cm, $P = 0.01$), width (−1.6 ± 2.0 vs. −0.8 ± 0.9 cm, $P = 0.02$), and depth (−0.8 ± 0.8 vs. −0.3 ± 0.5 cm, $P = 0.003$). In addition, significant reductions in fasting plasma glucose (−45.4 ± 82.6 vs. −10.6 ± 53.7 mg/dL, $P = 0.04$), serum insulin values (−2.4 ± 5.6 vs. +1.5 ± 9.6 μIU/mL, $P = 0.04$), and HbA1c (−0.7 ± 1.5 vs. −0.1 ± 0.4%, $P = 0.03$) and a significant rise in the quantitative insulin sensitivity check index (+0.01 ± 0.01 vs. −0.004 ± 0.02, $P = 0.01$) were seen following supplementation of magnesium compared with the placebo. Additionally, compared with the placebo, taking magnesium resulted in significant decrease in serum high-sensitivity C-reactive protein (hs-CRP) (−19.6 ± 32.5 vs. −4.8 ± 11.2 mg/L, $P = 0.01$) and significant increase in plasma total antioxidant capacity (TAC) concentrations (+6.4 ± 65.2 vs. −129.9 ± 208.3 mmol/L, $P < 0.001$). Overall, magnesium supplementation for 12 weeks among subjects with DFU had beneficial effects on parameters of ulcer size, glucose metabolism, serum hs-CRP, and plasma TAC levels. Clinical trial registration number: http://www.irct.ir: IRCT201612225623N96

Keywords Magnesium supplementation · Wound healing · Metabolic status · Diabetic foot

Introduction

The diabetic foot ulcer (DFU) is defined as manifestations of an invasion and multiplication of microorganisms in soft tissues or bone anywhere below the malleoli in a person with type 2 diabetes mellitus (T2DM) [1]. Development of foot ulcers is primarily due to diabetic neuropathy and peripheral vascular disease [2]. It is reported that up to 25% of diabetic subjects are at risk of developing DFU during their lifetime and poor wound healing is an important reason for morbidity and mortality [3]. Several studies have suggested that hyperglycemia, insulin resistance, dyslipidemia, increased inflammation, and reactive oxygen (ROS)/nitrogen species play a main function in the pathogenesis of DFU [4, 5].

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Hypomagnesemia due to low magnesium intake and increased magnesium loss is common in poorly controlled diabetes [6]. Previous studies have shown that low serum magnesium levels are associated with DFU [7, 8]. Hypomagnesemia has been associated with the development of neuropathy and abnormal platelet activity [8, 9], which both are risk factors for the development of DFU [10]. In addition, the beneficial effects of magnesium supplementation on metabolic profiles and biomarkers of inflammation and oxidative stress have previously been reported among patients without DFU. We have previously demonstrated that 250 mg/day magnesium administration as magnesium oxide for 6 weeks among subjects with gestational diabetes (GDM) had beneficial effects on metabolic profiles and biomarkers of inflammation and oxidative stress [11]. Furthermore, in another study, magnesium supplementation led to significant decreases in mean fasting glucose, triglyceride levels, and insulin resistance in normal-weight subjects; however, no significant effect had been found on mean HDL cholesterol levels [12]. However, magnesium administration for 16 weeks has been found to not affect lipid profiles among subjects with T2DM [13] and inflammatory factors among healthy middle-aged overweight women [14].

Improvement of indices of insulin metabolism, lipid profiles, biomarkers of inflammation, and oxidative stress by magnesium might be due to their effects on the acetyl-CoA carboxylase enzyme that catalyzes the formation of malonyl-CoA and is implicated in physiological insulin secretion [15], and inhibiting nuclear factor-kappa B (NF-kappa B) [16]. As there is evidence that taking magnesium supplements may accelerate wound healing and has an anti-diabetic effect, we hypothesized that magnesium supplementation might help subjects with DFU to heal their wound faster and have a better effect on metabolic profiles, and biomarkers of inflammation and oxidative stress. The aim of the current study, therefore, was to evaluate the effects of magnesium supplementation on wound healing and metabolic status in subjects with DFU.

Methods

Trial Design and Participants

The current randomized double-blind placebo-controlled clinical trial, registered in the Iranian website for registration of clinical trials as http://www.irct.ir: IRCT201612225623N96, was carried out among 70 subjects with grade 3 DFU according to "Wagner-Meggitt's" criteria aged 40–85 years who were referred to the Naghavi Hospital in Kashan, Iran, from December 2016 to February 2017. This trial was performed in accordance with the Declaration of Helsinki and informed consent was taken from all subjects. Grade 3 DFU is defined as deep ulcer with abscess or osteomyelitis [1]. The main exclusion criteria for the study were as follows: pregnant and breastfed patients; taking magnesium, multivitamin-mineral and antioxidant supplements, and anti-inflammatory agents; and change in consuming medications throughout the study and patients with history of diseases which influence the development of DFU including chronic trauma.

Study Design

At first, all individuals were matched for gender, type and dosage of medications, duration of diabetes mellitus, percentage of plantar ulcer and non-plantar, pre-treatment body mass index (BMI) (<25 and ≥25 kg/m^2), and age (<55 and ≥55 years). Then, all subjects were randomly divided into two groups to take either 250 mg/day magnesium supplements as magnesium oxide or placebo (n = 35 in each groups) for 12 weeks. In addition, all subjects underwent a similar treatment protocol for the diabetic foot, based on the Infectious Diseases Society of America [17]. Magnesium supplements and the placebo were manufactured by the 21st Century Pharmaceutical Company (AZ, USA) and Barij Essence Pharmaceutical Company (Kashan, Iran), respectively. Subjects were requested not to change their ordinary physical activity and not to take any nutritional supplements during the 12-week treatment. Compliance to the magnesium intake was evaluated through quantification of serum magnesium values. The use of magnesium supplementation and the placebo during the study was checked by asking subjects to return the medication containers and receiving brief daily cell phone reminders to take the supplements. All subjects completed 3-day food records and three physical activity records at study baseline, weeks 3, 6, and 9 of the intervention, and end of the trial. Daily macro- and micro-nutrient intakes were analyzed by nutritionist IV software (First Databank, San Bruno, CA). In the current study, physical activity was described as metabolic equivalents (METs) in hours per day [18].

Assessment of Anthropometric Measures

Weight of participants was determined in an overnight fasting status using a standard scale (Seca, Hamburg, Germany) at the onset of the study and 12 weeks after treatment. Height was measured using a non-stretched tape measure (Seca, Hamburg, Germany) to the nearest 0.1 cm. BMI was calculated as weight in kilograms divided by height in meters squared.

Assessment of Outcomes

Wound healing and parameters of glucose metabolism were considered as the primary outcome variables and lipid

profiles, and markers of inflammation and oxidative stress were considered as the secondary outcome variables.

Clinical Assessment

Mean ulcer area was estimated as the product of longest measured length times longest perpendicular to length [19]. Ulcer volume was estimated as the estimated area times the deepest ulcer depth [19]. Depth ulcer was recorded as superficial or deep in addition to recording a measured depth using a sterile blunt nasal probe [19]. Infection was diagnosed if edema, erythema, discharge, regional lymph node enlargement, pain, or fever was present [17]. Participants were considered positive for infection if at least two of the above listed features were present. According to their location, ulcers were defined as plantar or non-plantar. Each foot was evaluated and graded according to the Wagner classification [17].

Biochemical Assessment

At the baseline and 12 weeks after the intervention, 10 mL blood samples were obtained from each patient at Kashan reference laboratory, Kashan, Iran, affiliated to Kashan University of Medical Sciences in an early morning after an overnight fast. Serum insulin concentrations were measured using available enzyme-linked immunosorbent assay (ELISA) kit (DiaMetra, Milano, Italy) with intra- and inter-assay coefficient variances (CVs) of 2.5 and 4.3%, respectively. The homeostasis model of assessment-insulin resistance (HOMA-IR), β cell function (HOMA-B), and the quantitative insulin sensitivity check index (QUICKI) were determined according to the suggested formulas [20]. Hemoglobin A1c ($HbA1_C$) levels in the whole blood were assayed by Glycomat kit (BiocodeHycel, Massy, France) using the method of exchange chromatography. Enzymatic kits (Pars Azmun, Tehran, Iran) were used to quantify magnesium, fasting plasma glucose (FPG), serum triglycerides, VLDL, total, LDL, and HDL cholesterol concentrations. All inter- and intra-assay CVs for FPG and lipid fractions were less than 5%. Erythrocyte sedimentation rate (ESR by the Westergren method) was obtained at the study baseline and 12 weeks after starting intervention. Serum high-sensitivity C-reactive protein (hs-CRP) concentrations were quantified by a commercial ELISA kit (LDN, Nordhorn, Germany). The plasma nitric oxide (NO) concentrations were assessed using the Griess method [21]. Plasma total antioxidant capacity (TAC) concentrations by the method of ferric reducing antioxidant power developed by Benzie and Strain [22], total glutathione (GSH) using the method of Beutler et al. [23], and malondialdehyde (MDA) concentrations by the thiobarbituric acid reactive substance spectrophotometric test [24] were evaluated. All inter- and intra-assay CVs for NO, TAC, GSH, and MDA concentrations were less than 5%.

Sample Size

To estimate the sample size, we used a randomized clinical trial sample size formula where type 1 (α) and type 2 errors (β) were 0.05 and 0.20 (power = 80%), respectively. Based on a previous study [11], we used a standard deviation of 1.62 and a difference in mean (d) of 1.2, considering HOMA-IR as the key variable. The calculation indicated 29 persons were needed in each group. Assuming a dropout of 5 persons per group, the final sample size was determined to be 35 persons per group.

Randomization

Randomization assignment was carried out using computer-generated random numbers. Randomization and allocation were concealed from the researchers and subjects until the final analyses were completed. The randomized allocation sequence, enrolling patients and allocating them to interventions, was conducted by a trained staff at the clinic.

Statistical Analysis

To ensure the normal distribution of variables, the Kolmogorov-Smirnov test was used. The analyses were carried out based on intention-to-treat (ITT) principle. To detect differences in anthropometric measures as well as in dietary intakes between the two groups, we applied independent samples Student's t test. To determine the effects of magnesium intake on ulcer size, glucose metabolism, lipid fractions, biomarkers of inflammation, and oxidative stress, we used one-way repeated measures analysis of variance. Differences in proportions were evaluated by chi-square test or Fisher's exact tests. To control confounding variables including baseline values, age, and baseline BMI, we used analysis of covariance. P values <0.05 were considered statistically significant. All statistical analyses were done by the use of the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, IL, USA).

Results

Among subjects in the placebo group, 4 subjects [withdrawn due to personal reasons (n = 4)] and in the magnesium group, 5 subjects [withdrawn due to personal reasons (n = 5)] were excluded (Fig. 1). At the end, 61 subjects with DUF [magnesium (n = 30) and placebo (n = 31)] completed the trial. However, as the analysis was based on the ITT principle, all 70 persons (35 persons in each group) were included in the final analyses.

Gender distribution, mean age, height, weight and BMI at baseline and end of treatment, METs at baseline and end of

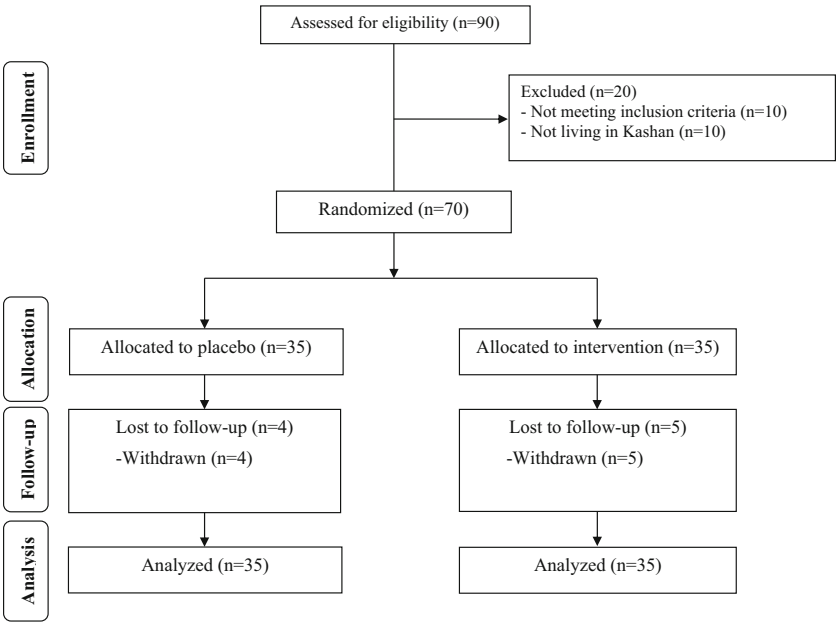

Fig. 1 Summary of patient flow diagram

trial, and insulin and metformin therapy were not statistically different between the two groups (Table 1).

Based on the 3-day dietary records obtained at baseline, end of treatment, and throughout the trial, no significant differences were observed between the two groups in terms of dietary intakes of energy, carbohydrates, proteins, fats, saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, cholesterol, crude fiber, total dietary fiber, magnesium, and manganese (data not shown).

After the 12-week treatment, compared with the placebo, magnesium supplementation resulted in a significant increase in serum magnesium (+0.3 ± 0.3 vs. −0.1 ± 0.2 mg/dL, $P < 0.001$) and significant reductions in ulcer length (−1.8 ± 2.0 vs. −0.9 ± 1.1 cm, $P = 0.01$), width (−1.6 ± 2.0 vs. −0.8 ± 0.9 cm, $P = 0.02$), and depth (−0.8 ± 0.8 vs.

Table 1 General characteristics of study participants

	Placebo group ($n = 35$)	Magnesium group ($n = 35$)	P^a
	Gender (%)		
Male	24 (68.6)	22 (62.9)	0.80[b]
Female	11 (31.4)	13 (37.1)	
Age (years)	59.0 ± 10.1	60.1 ± 11.1	0.65
Height (cm)	168.6 ± 7.2	168.3 ± 8.0	0.88
Weight at study baseline (kg)	74.9 ± 13.9	79.4 ± 12.3	0.15
Weight at end of trial (kg)	75.0 ± 14.1	79.5 ± 12.2	0.16
Weight change (kg)	0.1 ± 1.6	0.1 ± 0.6	0.62
BMI at study baseline (kg/m^2)	26.2 ± 4.1	28.2 ± 5.2	0.08
BMI at end of trial (kg/m^2)	26.3 ± 4.2	28.2 ± 5.2	0.09
BMI change (kg/m^2)	0.1 ± 0.5	0.01 ± 0.2	0.62
MET-h/day at study baseline	26.0 ± 2.5	26.8 ± 2.2	0.17
MET-h/day at end of trial	25.9 ± 2.6	26.9 ± 2.3	0.09
MET-h/day change	−0.1 ± 0.6	0.1 ± 0.8	0.18
Insulin therapy (%)	35 (100.0)	35 (100.0)	1.00[b]
Metformin therapy (%)	31 (88.6)	31 (88.6)	1.00[b]

Data are means ± SDs

METs metabolic equivalents

[a] Obtained from independent *t* test

[b] Obtained from Pearson chi-square test

−0.3 ± 0.5 cm, $P = 0.003$) (Table 2). In addition, significant reductions in FPG (−45.4 ± 82.6 vs. −10.6 ± 53.7 mg/dL, $P = 0.04$) and serum insulin values (−2.4 ± 5.6 vs. +1.5 ± 9.6 μIU/mL, $P = 0.04$) and a significant rise in QUICKI (+0.01 ± 0.01 vs. −0.004 ± 0.02, $P = 0.01$) were seen following supplementation of magnesium compared with the placebo. Additionally, compared with the placebo, taking magnesium resulted in significant decrease in serum hs-CRP (−19.6 ± 32.5 vs. −4.8 ± 11.2 mg/L, $P = 0.01$) and significant increase in plasma TAC concentrations (+6.4 ± 65.2 vs. −129.9 ± 208.3 mmol/L, $P < 0.001$). There were no significant changes in HOMA-B, lipid profiles, and other biomarkers of inflammation and oxidative stress between the two groups.

There was a significant difference in baseline levels of ulcer depth ($P = 0.03$), plasma TAC ($P < 0.001$), and MDA ($P < 0.001$) between the two groups. Therefore, we adjusted the analysis for baseline values of biochemical parameters, age, and baseline BMI. When we adjusted the analysis for baseline values of biochemical parameters, age, and baseline BMI, FPG ($P = 0.18$), HbA1c ($P = 0.10$), and plasma TAC levels ($P = 0.09$) became non-significant, while plasma MDA ($P = 0.01$) became statistically significant, and other findings did not change (Table 3).

Discussion

In this study, we evaluated effects of magnesium supplementation on wound healing, parameters of glucose metabolism, lipid profiles, and biomarkers of inflammation and oxidative stress among subjects with DFU. Our findings demonstrated that magnesium supplementation for 12 weeks among subjects with DFU had beneficial effects on parameters of ulcer size, glucose metabolism, serum hs-CRP, and plasma TAC levels, but did not had any effect on lipid profiles and other biomarkers of inflammation and oxidative stress. It must be

Table 2 Wound healing, metabolic profiles, and biomarkers of inflammation and oxidative stress at baseline and after the 12-week intervention in patients with diabetic foot ulcer

	Placebo group ($n = 35$)			Magnesium group ($n = 35$)			P^a
	Baseline	End of trial	Change	Baseline	End of trial	Change	
Magnesium (mg/dL)	2.0 ± 0.2	1.9 ± 0.2	−0.1 ± 0.2	2.1 ± 0.3	2.3 ± 0.2	0.3 ± 0.3	<0.001
Ulcer length (cm)	3.6 ± 1.6	2.7 ± 1.9	−0.9 ± 1.1	3.6 ± 2.7	1.8 ± 2.9	−1.8 ± 2.0	0.01
Ulcer width (cm)	2.9 ± 1.4	2.1 ± 1.5	−0.8 ± 0.9	3.3 ± 2.8	1.7 ± 2.9	−1.6 ± 2.0	0.02
Ulcer depth (cm)	1.3 ± 0.6	0.9 ± 0.5	−0.3 ± 0.5	1.7 ± 1.1	0.9 ± 1.4	−0.8 ± 0.8	0.003
FPG (mg/dL)	209.8 ± 66.9	199.2 ± 75.8	−10.6 ± 53.7	226.3 ± 90.8	180.9 ± 72.5	−45.4 ± 82.6	0.04
Insulin (μIU/mL)	17.1 ± 9.7	18.6 ± 9.7	1.5 ± 9.6	17.8 ± 7.2	15.5 ± 7.2	−2.4 ± 5.6	0.04
HOMA-IR	8.7 ± 5.6	9.5 ± 5.4	0.8 ± 5.3	10.3 ± 6.5	9.1 ± 6.2	−1.2 ± 3.4	0.06
HOMA-B	49.8 ± 31.0	68.9 ± 62.7	19.1 ± 61.2	53.3 ± 36.9	66.6 ± 51.1	13.3 ± 61.7	0.69
QUICKI	0.28 ± 0.01	0.28 ± 0.01	−0.004 ± 0.02	0.28 ± 0.01	0.29 ± 0.02	0.01 ± 0.01	0.01
HbA1c (%)	7.8 ± 0.6	7.7 ± 0.6	−0.1 ± 0.4	8.3 ± 1.9	7.6 ± 1.3	−0.7 ± 1.5	0.03
Triglycerides (mg/dL)	163.5 ± 95.3	161.4 ± 107.9	−2.1 ± 33.5	168.0 ± 84.0	155.7 ± 77.9	−12.3 ± 71.1	0.44
VLDL cholesterol (mg/dL)	32.7 ± 19.1	32.3 ± 21.6	−0.4 ± 6.7	33.6 ± 16.8	31.1 ± 15.6	−2.5 ± 14.2	0.44
Total cholesterol (mg/dL)	159.5 ± 51.7	163.8 ± 54.9	4.3 ± 23.5	152.0 ± 55.5	158.7 ± 52.9	6.7 ± 55.3	0.81
LDL cholesterol (mg/dL)	91.2 ± 45.0	93.6 ± 49.9	2.5 ± 22.2	80.4 ± 44.5	87.9 ± 43.8	7.5 ± 46.7	0.57
HDL cholesterol (mg/dL)	35.6 ± 6.7	37.8 ± 9.1	2.2 ± 5.7	38.0 ± 8.8	39.7 ± 11.3	1.7 ± 10.5	0.77
hs-CRP (mg/L)	43.2 ± 26.8	38.4 ± 22.0	−4.8 ± 11.2	41.7 ± 32.6	22.2 ± 28.9	−19.6 ± 32.5	0.01
ESR (mm/h)	53.1 ± 23.9	45.7 ± 24.3	−7.4 ± 14.6	51.1 ± 33.5	41.2 ± 30.1	−9.9 ± 23.7	0.59
NO (μmol/L)	43.4 ± 4.9	43.5 ± 5.2	0.1 ± 6.5	46.6 ± 4.5	45.2 ± 5.2	−1.4 ± 4.6	0.26
TAC (mmol/L)	1083.6 ± 231.7	953.7 ± 223.1	−129.9 ± 208.3	870.4 ± 70.3	876.7 ± 76.6	6.4 ± 65.2	<0.001
GSH (μmol/L)	540.4 ± 99.7	536.8 ± 118.5	−3.5 ± 156.7	545.2 ± 82.1	523.5 ± 67.6	−21.7 ± 106.1	0.57
MDA (μmol/L)	3.1 ± 0.6	3.0 ± 0.7	−0.1 ± 0.7	2.6 ± 0.2	2.4 ± 0.2	−0.2 ± 0.3	0.29

All values are means ± SDs

ESR erythrocyte sedimentation rate, *FPG* fasting plasma glucose, *GSH* total glutathione, *HOMA-IR* homeostasis model of assessment-estimated insulin resistance, *HOMA-B* homeostasis model of assessment-estimated B cell function, *hs-CRP* high-sensitivity C-reactive protein, *MDA* malondialdehyde, *NO* nitric oxide, *QUICKI* quantitative insulin sensitivity check index, *TAC* total antioxidant capacity

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

Table 3 Adjusted changes in metabolic profile of the patients with diabetic foot ulcer

	Placebo group (n = 35)	Magnesium group (n = 35)	P[a]
Magnesium (mg/dL)	−0.1 ± 0.03	0.3 ± 0.03	<0.001
Ulcer length (cm)	−0.9 ± 0.3	−1.8 ± 0.3	0.02
Ulcer width (cm)	−0.8 ± 0.2	−1.6 ± 0.2	0.04
Ulcer depth (cm)	−0.4 ± 0.1	−0.8 ± 0.1	0.005
FPG (mg/dL)	−18.5 ± 9.8	−37.5 ± 9.8	0.18
Insulin (μIU/mL)	1.5 ± 1.2	−2.4 ± 1.2	0.02
HOMA-IR	0.6 ± 0.7	−1.0 ± 0.7	0.10
HOMA-B	19.3 ± 9.8	13.1 ± 9.8	0.66
QUICKI	−0.004 ± 0.003	0.008 ± 0.003	0.01
HbA1c (%)	−0.2 ± 0.1	−0.6 ± 0.1	0.10
Triglycerides (mg/dL)	−3.4 ± 9.3	−10.9 ± 9.3	0.57
VLDL cholesterol (mg/dL)	−0.7 ± 1.9	−2.2 ± 1.9	0.57
Total cholesterol (mg/dL)	5.7 ± 6.6	5.2 ± 6.6	0.95
LDL cholesterol (mg/dL)	4.7 ± 5.7	5.1 ± 5.7	0.96
HDL cholesterol (mg/dL)	1.8 ± 1.4	2.1 ± 1.4	0.86
hs-CRP (mg/L)	−3.5 ± 3.3	−21.0 ± 3.3	<0.001
ESR (mm/h)	−6.5 ± 3.1	−10.7 ± 3.1	0.35
NO (μmol/L)	−0.8 ± 0.8	−0.5 ± 0.8	0.79
TAC (mmol/L)	−95.7 ± 25.7	−27.8 ± 25.7	0.09
GSH (μmol/L)	−10.4 ± 16.5	−14.9 ± 16.5	0.84
MDA (μmol/L)	0.1 ± 0.1	−0.3 ± 0.1	0.01

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

ESR erythrocyte sedimentation rate, *FPG* fasting plasma glucose, *GSH* total glutathione, *HOMA-IR* homeostasis model of assessment-estimated insulin resistance, *HOMA-B* homeostasis model of assessment-estimated B cell function, *hs-CRP* high-sensitivity C-reactive protein, *MDA* malondialdehyde, *NO* nitric oxide, *QUICKI* quantitative insulin sensitivity check index, *TAC* total antioxidant capacity.

[a] Obtained from ANCOVA

kept in mind that all patients were instructed to reduce ulcer trigger factors by the use of off-loading, insole, and appropriate shoes based on available guidelines. However, we believe that this would not influence our findings because individuals in both intervention and non-intervention groups were taking identical advices. On average, the compliance of these instructions in the current study was high, such that more than 90% of advices were conducted throughout the study in both groups. Nevertheless, this should be taken into account in the interpretation of our findings. It must be considered that in the current study, observed changes in ulcer size in the magnesium group compared with placebo group were clinically significant. On the other hand, the percentage of those who was treated after intervention was significantly different between the two groups (56.7% for magnesium vs. 28.1% for placebo group, P = 0.02).

This research demonstrated that magnesium administration in subjects with DFU for 12 weeks resulted in a significant improvement in parameters of wound healing compared with the placebo. Data on the effects of magnesium supplementation on wound healing in human studies are limited. Several studies have reported that magnesium concentrations are lower in patients with T2DM [25, 26] and DFU [7, 8]. One of the causes of low magnesium in these patients is increased renal excretion due to hyperglycemia, glycosuria, and insulin resistance [27]. In addition, low intracellular magnesium concentrations negatively influence the transportation of cellular glucose, tyrosine kinase activity, post-receptor insulin action, and secretion of insulin from the pancreas [28]. Hypomagnesemia in DFU patients can worsen the glycemic control, and both micro- and macrovascular complications of diabetes are strongly associated with hyperglycemia and/or uncontrolled glycemia [10].

Our data supported that magnesium supplementation compared with the placebo in subjects with DFU for 12 weeks decreased FPG, serum insulin, and HbA1c and increased QUICKI, but did not influence HOMA-IR, HOMA-B, and lipid profiles. In line with our study, 365 mg/day magnesium supplementation among hypomagnesemic chronic kidney disease subjects with pre-diabetes and obesity for 12 weeks improved parameters of insulin metabolism [29]. In a meta-analysis study by Veronese et al. [30], it was observed that magnesium supplementation improved glucose parameters in people

with diabetes and also improves insulin-sensitivity parameters in those at high risk of diabetes. However, taking magnesium at dosage of 300 mg/day as magnesium oxide for 12 weeks did not affect insulin sensitivity in normomagnesemic non-diabetic overweight subjects [31]. In addition, no significant difference in levels of serum total, LDL, and HDL cholesterol, triglycerides, or serum and erythrocyte magnesium was seen following supplementation with 384 mg/day of sustained-release magnesium chloride for 6 weeks among patients with T2DM [32]. Considering low serum magnesium levels among DFU patients [7] and observed beneficial effects of magnesium supplementation on lipid profiles in previous studies, we hypothesized that magnesium supplementation might help DFU patients to control their lipid profiles. In a study, supplementation with magnesium oxide at a dosage of 300 mg/day for 3 months resulted in significant reductions in serum triglycerides, total, and LDL cholesterol and a significant increase in HDL cholesterol levels in hypomagnesemic diabetic children [33]. In a meta-analysis study, magnesium supplementation could produce a favorable effect on triglycerides, LDL, and HDL cholesterol among patients with T2DM [34]. Significant variation was observed among diabetic or non-diabetic subjects, as well as hypomagnesemia or normomagnesemic populations, and also with ≤3 or >3 months of treatment duration. The results of above meta-analysis study demonstrated a positive effect of magnesium supplementation on diabetic dyslipidemia, with a more pronounced effect in hypomagnesemia subjects. In the current study, majority patients had normal lipid profiles. Meta regression analysis showed an inverse association between magnesium dose and lipid profiles [34]. From the meta-analysis data [34], it was evident that significant beneficial effects could be obtained with an elemental magnesium dose of 300–400 mg. However, we have previously observed beneficial effects of magnesium supplementation at dosage of 250 mg/day for 6 weeks on glucose metabolism, serum triglycerides, and VLDL cholesterol levels among GDM women [11]. In the current study, we used the dosage of 250 mg magnesium oxide. Insulin resistance in DFU may be associated with an acceleration of arteriosclerotic changes of the greater arteries resulting in decreased blood flow, further contributing to a limb threatening ischemic condition [35]. In addition, low intake of magnesium induces changes in biochemical pathways that can increase the risk of hypertension and cardiovascular disease (CVD) and T2DM [36]. The important role of magnesium in the etiology of CVD pathology has been pointed out by a considerable number of experimental [37], epidemiological [38], and clinical studies [39]. Increased magnesium intake appears to have a beneficial effect on heart disease and its risk factors, including diabetes mellitus [40] and heart disease [38]. Therefore, improvement in insulin metabolism has been suggested as effective means to delay vascular complications in DFU subjects. Magnesium intake may stimulate the acetyl-CoA carboxylase enzyme that catalyzes the formation of malonyl-CoA, which is implicated in physiological insulin secretion [15]. Furthermore, taking magnesium may competitively inhibit the voltage-dependent calcium channel, which is known to play a role in insulin secretion [41].

We found that compared with the placebo, magnesium administration for 12 weeks among subjects with DFU was associated with a significant decrease in serum hs-CRP and a significant increase in plasma TAC values, but unchanged plasma NO, GSH, and MDA concentrations. Reports from animal studies have shown that the inflammatory response is an early consequence of magnesium deficiency [42, 43]. In humans, low serum magnesium levels have been associated with hs-CRP levels [44, 45]. In a meta-analysis study by Dibaba et al. [46], it was indicated that dietary magnesium intake was significantly and inversely associated with serum CRP values. Several cross-sectional studies have shown inverse relationships between magnesium intake and some inflammatory markers, including hs-CRP [47, 48]. We have previously shown that supplementation with 250 mg magnesium oxide/day significantly decreased serum hs-CRP levels among GDM for 6 weeks [11]. In addition, in adults older than 51 years with poor-quality sleep, supplementation with 320 mg magnesium/day as magnesium citrate for 7 weeks decreased plasma CRP in subjects with baseline values above 3 mg/L [49]. Subjects with high inflammatory stress or low magnesium levels might have better and faster response to magnesium supplementation. In another study, the lower antioxidant capacity found in moderate magnesium deficiency in patients with unexplained chronic fatigue [50]. However, supplementation with 440 mg magnesium as magnesium oxide three times per week for 6 months in hemodialysis subjects did not influence CRP values [51]. Furthermore, serum levels of MDA were enhanced in non-diabetic rats treated with magnesium sulfate and cisplatin for 10 days [52]. Anti-inflammatory effects of magnesium may be due to the effects of its antagonism to calcium [53], inactivation of *N*-methyl-sd-aspartate receptors, and inhibiting NF-kappa B [16]. In addition, magnesium intake may increase TAC levels through decreasing ROS production [54] and increasing glutathione-peroxidase activity [55].

This study had few limitations. In the current study, we did not measure any direct dynamic test such as glucose tolerance test or hyperinsulinemic clamp in the current study. In addition, due to limited funding, we did not evaluate the effects of magnesium supplementation on

other biomarkers of systemic inflammation and oxidative stress.

Overall, magnesium supplementation for 12 weeks among subjects with DFU had beneficial effects on parameters of ulcer size, glucose metabolism, serum hs-CRP, and plasma TAC levels, but did not affect lipid profiles and other biomarkers of inflammation and oxidative stress.

Acknowledgments The current study was funded by a grant from the Vice-chancellor for Research, KUMS, and Iran. The authors gratefully acknowledge Dr. Fariba Kolahdooz, who reviewed the manuscript and offered critical comments.

Authors' Contributions ZA contributed in conception, design, statistical analysis, and drafting of the manuscript. RR, FP, MM-H, FB, and HA contributed in data collection and manuscript drafting. All authors approved the final version for submission.

Compliance with Ethical Standards

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

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