





The Potential of Magnesium Sulfate to Change Serum Lysyl Oxidase and Nitrite Levels in Patients with Atherosclerosis: A Double-Blind Clinical Trial Study

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Abstract

Background: Lysyl oxidase (LOX) and magnesium contribute to vascular development and stability. This research describes how magnesium sulfate ($MgSO_4$) regulates the serum levels of LOX and nitric oxide in patients with moderate coronary artery disease (CAD).

Methods: This was a randomized double-blind placebo-controlled clinical trial with 76 patients with moderate CAD divided into four groups. The subjects were randomly assigned to groups that took either capsules containing placebo or $MgSO_4$ (300 mg) daily for 6 months. Thus, the experiment included Mg-treated groups with one and two vessels with atherosclerotic plaque (Mg-VR1 and Mg-VR2) and placebo-treated groups with one and two vessels with atherosclerotic plaque (placebo-VR1 and placebo-VR2). Every 3 months, LOX, homocysteine, nitrite, and lipid profile levels were measured. Women and men with moderate CAD who were more than 55 and 50 years old, respectively, were included.

Results: Total cholesterol and triglyceride (TG) levels significantly decreased 6 months after intervention ($P < 0.01$). Decrease of serum homocysteine levels was seen in all groups during the study, but the reduction in the placebo groups was greater than the Mg-treated groups ($P < 0.001$). Three months after treatment with $MgSO_4$, LOX had stayed at high levels, and it then returned to baseline in the 6-month follow up ($P < 0.001$). The rise in nitrite levels in the placebo-VR2 group was significantly greater than in the $MgSO_4$ -VR2 group ($P < 0.001$).

Conclusion: magnesium sulfate may prevent the progression of arteriosclerosis through modulating LOX and homocysteine levels and preventing the increase of nitrite levels.

Trial Registration: Identifier: IRCT20151028024756N3; <https://www.irct.ir/trial/29097>.

Keywords: Atherosclerosis, Lysyl oxidase, Magnesium sulfate, Nitrite, CAD

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Introduction

Endothelial dysfunction plays an important role in atherosclerosis plaque formation and coronary artery disease (CAD) (1,2). During CAD, endothelial dysfunction and high LDL-C (low-density lipoprotein cholesterol) concentrations reduce nitric oxide (NO) bioavailability and the expression of an important enzyme called lysyl oxidases (LOX) in endothelial cells (3,4).

Studies suggest that LOX downregulation causes endothelial dysfunction and instability and rupture of atherosclerotic plaques (4-6). In addition, reduction of

NO bioavailability increases oxidative stress leading to endothelial dysfunction (1,7).

It has been demonstrated that serum Mg levels are inversely associated with the risk of CAD (8). The results have shown that 53% of cardiac patients are hypomagnesaemic (9,10). In fact, Mg deficiency can promote oxidative and nitrosative stress and, consequently, endothelial dysfunction in CAD patients. Based on previous reports, Mg^{2+} treatment can potentially protect people against CAD by enhancing endothelium-dependent vasodilation and reducing vascular resistance



and oxidative stress (8).

Although different mechanisms have been proposed for the cardioprotective role of Mg, its exact mechanism of effect is unclear. Therefore, further investigations are required to explore other possible functions of this important element. In previous works, the effect of Mg on LOX, as a key enzyme involved in endothelial dysfunction, has not been studied. Thus, our research aimed to investigate the treatment effects of MgSO₄ on serum LOX levels in patients with moderate CAD. We also studied the effect of Mg administration on serum nitrite and homocysteine levels, as possible mechanisms through which magnesium may control LOX levels.

Materials and Methods

Trial design

This double-blind randomized placebo-controlled clinical trial has a parallel design with a 1:1 allocation ratio (38 people in the placebo group and 38 participants in the Mg-treated group). This investigation was carried out to evaluate the effect of MgSO₄ on serum LOX, homocysteine, and nitrite levels in patients with moderate CAD. This randomized study has been approved by the Iranian Registry of Clinical Trials with the code IRCT20151028024756N3. The trial diagram is depicted in Figure 1.

Participants

In this trial, 76 male and female patients aged more than 55 and 50 years, respectively, with moderate CAD and at

least one major coronary artery with less than 60% stenosis were included. All subjects underwent angiography via the femoral artery. Subjects with hypertension and hyperlipidemia and smokers were also included.

Pregnant women, patients who were taking magnesium and calcium dietary supplements, those with renal failure (creatinine levels ≥ 1.3 mg/dL in women and ≥ 1.5 in men), increased hepatic enzymes, recent infections, chronic inflammatory diseases, cerebrovascular accidents (CVAs), cancer, and drug sensitivity and patients with hyperthyroidism and hypothyroidism were excluded from this study. All subjects were selected from the angiography center of Shaheed Mohammadi hospital, Hormozgan University of Medical Sciences, Bandar Abbas.

Study protocol, Interventions and sample size calculation

The participants were randomly asked to use either capsules containing MgSO₄ (300 mg) or placebo capsules daily for 6 months along with their routine treatments, including metoprolol, aspirin, clopidogrel, nitrate, atorvastatin, losartan, and metformin. The MgSO₄ supplement and placebo were made by Niak Pharmaceuticals Company (Gorgan, Iran). Both capsules had the same color and were oval in shape, with the placebo capsule containing wheat bran instead of MgSO₄. Evaluation of pericardial arteries was done by angiography. According to the number of atherosclerotic vessels, the patients were assigned to four groups, Mg-treated participants with one atherosclerotic vessel (Mg-VR1, n=14), Mg-treated participants with

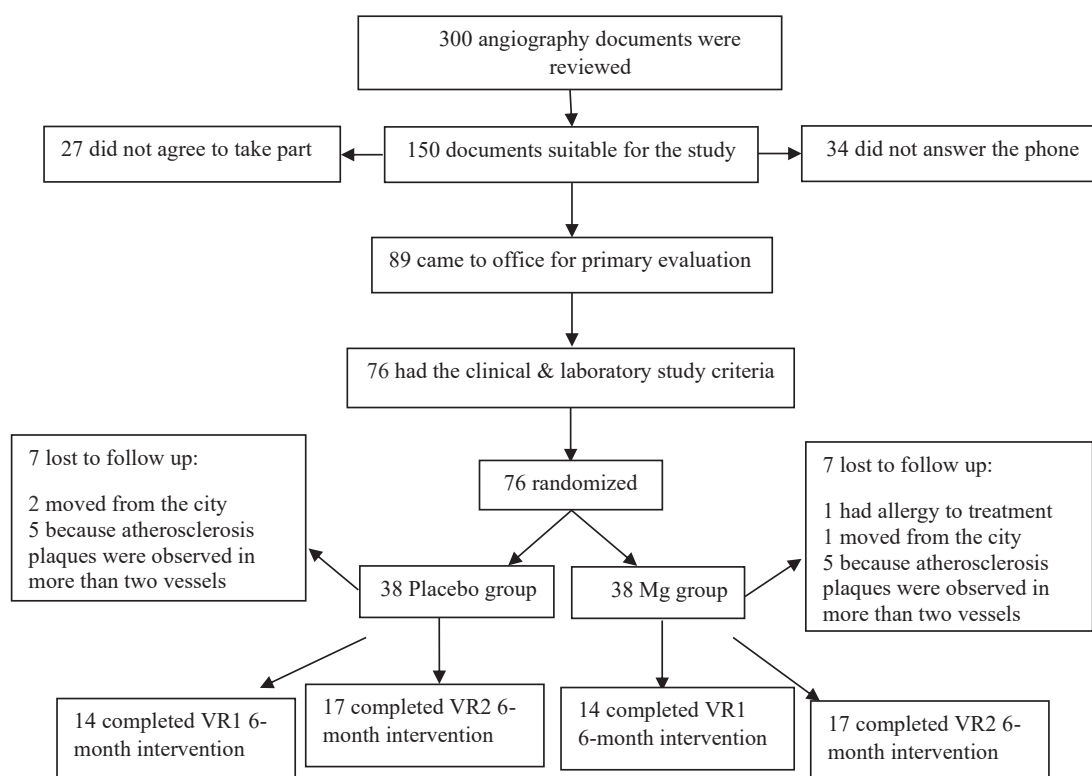


Figure 1. Study follow diagram

two atherosclerotic vessels (Mg-VR2, n=17), and two groups of placebo-treated participants, with one and two atherosclerotic vessels (placebo-VR1, n=14 and placebo-VR2, n=17) (Figure 1). In this study, computer-generated random numbers were used to generate the random allocation sequence, and block randomization was used with block size=2 (the two groups consisted of patients with one atherosclerotic vessel and patients with two atherosclerotic vessels). Neither the participant nor the investigator was aware of the capsule contents, but one of our colleagues had access to the data and patient information. In this study, the formula for determining the sample size was $n = 2SD (z\alpha / 2 + Z\beta) ^2 / d^2$ ($z\alpha / 2 = 1.96$, $Z\beta = 0.84$, $d = 0.05$, $SD = 0.012$).

Exclusion and inclusion criteria

Women and men with moderate CAD aged more than 55 years and 45 years, respectively, were included in the study, even if they had hypertension or hyperlipidemia or were smokers.

Pregnant women, patients who were taking magnesium and calcium dietary supplements, those with renal failure (serum creatinine levels ≥ 1.3 mg/dL in women and ≥ 1.5 mg/dL in men), increased hepatic enzymes, recent infections, chronic inflammatory diseases, CVAs, cancer, and drug sensitivity and patients with hyperthyroidism or hypothyroidism were excluded from this study.

Study outcomes

The primary outcomes, including angiography, lipid profile, and homocysteine, were measured at the baseline and 3 and 6 months following magnesium therapy. The secondary outcome of the study was the levels of nitrite, LOX, serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), creatinine (Cr), and blood urea nitrogen (BUN), measured at the baseline and at the 3 and 6 months following magnesium therapy.

Biochemical measurements

Fasting blood samples were collected three times, before intervention and at the 3- and 6-month follow-ups. Samples were centrifuged at 1500 g for 10 minutes. Serum was separated and maintained at -80 °C for biochemical assessment. Serum total cholesterol, triglycerides (TG), Cr, BUN, and homocysteine were measured by enzymatic methods (Pars Azmun kit, Iran). Very low-density lipoprotein cholesterol (VLDL-C) concentration was calculated. Serum LOX was determined by an ELISA assay (ZellBio GmbH, Germany, Cat. No: ZB-15122C-H9648). Serum nitrite levels were assessed using a standard kit (Promega Corporation, USA, G2930). Also, liver function tests, including SGPT and SGOT, were carried out using available commercial kits (Pars Azmun kit, Iran).

Statistical analysis

The findings were reported as mean \pm SD, and Kolmogorov-Smirnov test was used to check the normality of all variables. Comparisons between groups and the possible differences between the means of each group during the intervention were analyzed using one-way ANOVA and Tukey's post-hoc test. SPSS software version 13 was used for the analyses, and $P < 0.05$ was taken as statistically significant.

Results

In the present study, 76 patients were selected according to the number of atherosclerotic vessels and were randomly divided into Mg and placebo groups: M-treated group with one atherosclerotic vessel (Mg-VR1, n=14), MgSO₄-treated group with two atherosclerotic vessels (Mg-VR2, n=17) and two placebo-treated groups with one or two atherosclerotic vessels (placebo-VR1, n=14 and placebo-VR2, n=17, respectively (Figure 1).

Effects of MgSO₄ on lipid profile, BUN, Cr, and liver enzymes

Measurements at baseline

The baseline total cholesterol, TG, and VLDL-C concentration are shown in Figure 2. Total cholesterol, TG, and VLDL-C levels were higher in participants with two atherosclerotic vessels compared with one atherosclerotic plaque at the beginning of the study ($P < 0.001$) (Figure 2). We did not observe any change in the serum levels of BUN, Cr, SGOT, and SGPT at baseline (Table 1).

Measurements after Mg treatment

The effects of 3 and 6 months Mg administration on serum analyses in different groups are shown in Figure 2 and Table 2 and 3. Total cholesterol was significantly decreased 6 months after intervention in both placebo and Mg-treated groups ($P < 0.001$). In addition, TG decreased at the end of 6 months in the placebo groups ($P < 0.01$), but the plasma TG levels of Mg-VR2 patients increased after 3 months and then decreased at 6 months follow up ($P < 0.001$).

In patients with one atherosclerotic vessel, MgSO₄ was able to reduce VLDL-C levels 3 months after intervention ($P < 0.001$), but in placebo groups, this reduction took place after 6 months ($P < 0.01$). Therefore, Mg treatment decreased VLDL-C faster compared with the placebo-VR1 group (Figure 2).

Following Mg administration, we observed no change in the serum levels of BUN, Cr, SGOT, and SGPT (Tables 2 and 3).

Effects of MgSO₄ on homocysteine levels

Measurement at baseline

The baseline homocysteine concentrations are shown in

Table 1. Demographic information in MgSO₄ (Mg) treated moderate coronary artery disease patients with one (Mg-VR1) and two (Mg-VR2) atherosclerotic vessels as well as in placebo-treated patients with one (placebo-VR1) and two (placebo-VR2) atherosclerotic vessels before intervention

Variables	Mg-treated		Placebo		P value
	VR1 (n=14)	VR2 (n=17)	VR1 (n=14)	VR2 (n=17)	
Age (y)	60.07 ± 3.2	60.4 ± 2.1	57.06 ± 2	61.05 ± 1.6	0.3
Gender (F/M)	7/7	8/9	6/8	8/9	-
BMI (kg/m ²)	25.9 ± 1.6	23.8 ± 0.7	25.1 ± 1.1	26.1 ± 0.8	0.41
SBP (mm Hg)	121.4 ± 4.1	127.8 ± 3.5	130.3 ± 3.5	130 ± 3.03	0.29
DBP (mm Hg)	72.1 ± 1.3	75.8 ± 2.3	78.6 ± 2.1	79.2 ± 1.9	0.07
HR	71.3 ± 1.57	69.2 ± 1.7	68.8 ± 2.5	71.7 ± 1.9	0.71
SGOT (U/L)	19.9 ± 1.8	23.1 ± 2.7	20.4 ± 1.1	22.4 ± 2.1	0.22
SGPT (U/L)	25.6 ± 4.3	28.1 ± 3.6	23.1 ± 3.7	26.3 ± 4.3	0.24
BUN (mg/dL)	14.5 ± 0.7	15.5 ± 0.5	14.6 ± 0.5	15.3 ± 0.7	0.28
Cr (mg/dL)	0.8 ± 0.01	0.9 ± 0.02	0.8 ± 0.03	0.8 ± 0.02	0.4

BMI (body mass index), SBP (systolic blood pressure), DBP (diastolic blood pressure), HR (heart rate), Cr (creatinine), BUN (blood urea nitrogen), SGPT (serum glutamate-pyruvate transaminase), SGOT (glutamate oxaloacetate transaminase). Data were presented as mean ± SD and analyzed using one-way ANOVA.

Table 2. Study variables in MgSO₄ (Mg) treated moderate coronary artery disease patients with one (Mg-VR1) and two (Mg-VR2) atherosclerotic vessel as well as in placebo treated patients with one (placebo-VR1) and two (placebo-VR2) atherosclerotic vessels after 3 months of intervention

Variables	Mg-treated		Placebo		P value
	VR1 (n=14)	VR2 (n=17)	VR1 (n=14)	VR2 (n=17)	
SGOT (U/L)	20.3 ± 1.2	21.1 ± 1.5	21.1 ± 1.2	21.2 ± 1.1	0.42
SGPT (U/L)	21.6 ± 1.8	22.5 ± 2.4	22.7 ± 3.7	21.5 ± 2.1	0.32
BUN (mg/dL)	12.2 ± 0.7	13.2 ± 0.6	13.6 ± 1.1	13.3 ± 1.1	0.3
Cr (mg/dL)	0.8 ± 0.02	0.8 ± 0.04	0.7 ± 0.03	0.8 ± 0.03	0.45

Cr (creatinine), BUN (blood urea nitrogen), SGPT (serum glutamate-pyruvate transaminase), SGOT (glutamate oxaloacetate transaminase). Data was presented as mean ± SD and analyzed using one-way ANOVA.

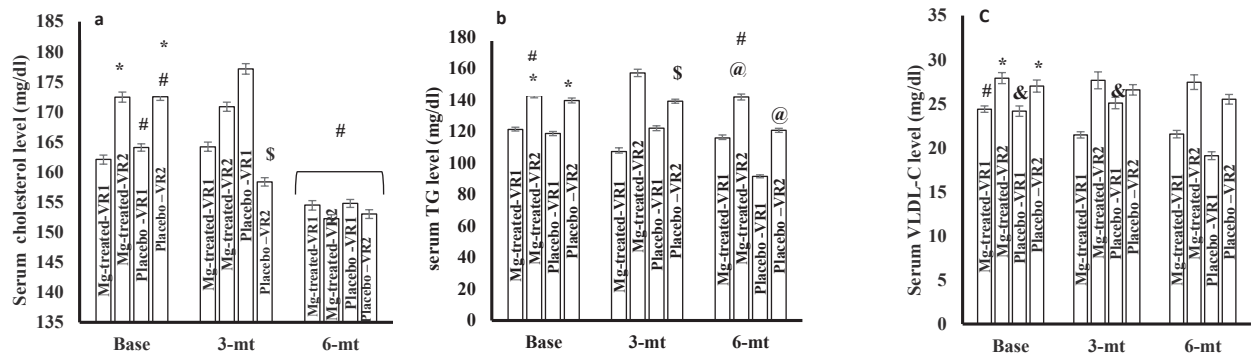


Figure 2. Serum cholesterol (a), triglyceride (TG, b) and very low density lipoprotein cholesterol (VLDL-C, c) levels in four groups of moderate coronary artery disease (CAD): 1) MgSO₄-treated CAD participants with one atherosclerotic vessel (Mg-VR1), 2) Mg- treated CAD patients with two atherosclerotic vessels (Mg-VR2), 3) placebo-treated CAD patients with one atherosclerotic vessel (placebo-VR1), and 4) placebo-treated CAD patients with two atherosclerotic vessels (placebo-VR2). n = 14 for one atherosclerotic vessel in each group and n = 17 for two atherosclerotic vessels in each group. Data were presented as mean ± SD and analyzed using one-way ANOVA and Tukey's post-hoc test. * P < 0.001 significant differences compared with the VR1 groups before intervention (base). # P < 0.001 significant difference compared with the same group 3 months after intervention (3-mt). \$ P < 0.001 significant difference compared with the placebo-VR1 group after 3 months (3-mt). @ P < 0.01 significant difference compared with the VR1 group 6 months after intervention (6-mt). & P < 0.01 significant difference compared with the placebo-VR1 group 6 months after intervention (6-mt).

Figure 3a. The serum levels of homocysteine were higher in VR2 patients compared to VR1 patients in both the Mg and placebo groups (P < 0.001) (Figure 3a).

Measurement after Mg treatment

Homocysteine variations after 3 and 6 months MgSO₄

treatment and its comparison with placebo groups are shown in Figure 3a.

Decrease of serum homocysteine levels was seen in all groups during the study but the reduction was greater in the placebo groups compared to the Mg- treated groups after 3 months (P < 0.001) (Figure 3a).

Table 3. Study variables in MgSO₄ (Mg)-treated moderate coronary artery disease patients with one (Mg-VR1) and two (Mg-VR2) atherosclerotic vessels and placebo-treated patients with one (placebo-VR1) and two (placebo-VR2) atherosclerotic vessels after 6 months of intervention

Variables	Mg-treated		Placebo		P value
	VR1 (n=14)	VR2 (n=17)	VR1 (n=14)	VR2 (n=17)	
SGOT (U/L)	20.1 ± 1.4	19.3 ± 1.1	24 ± 2.6	21.1 ± 0.7	0.2
SGPT (U/L)	22.5 ± 3.1	22.7 ± 2.1	29.5 ± 6.7	23.2 ± 2.1	0.2
BUN (mg/dL)	12.1 ± 0.8	12.1 ± 0.8	13.6 ± 0.9	11.8 ± 0.7	0.4
Cr (mg/dL)	0.8 ± 0.04	0.9 ± 0.03	0.8 ± 0.03	0.9 ± 0.03	0.46

Cr (creatinine), BUN (blood urea nitrogen), SGPT (serum glutamate-pyruvate transaminase), SGOT (glutamate oxaloacetate transaminase). Data were presented as mean ± SD and analyzed using one-way ANOVA.

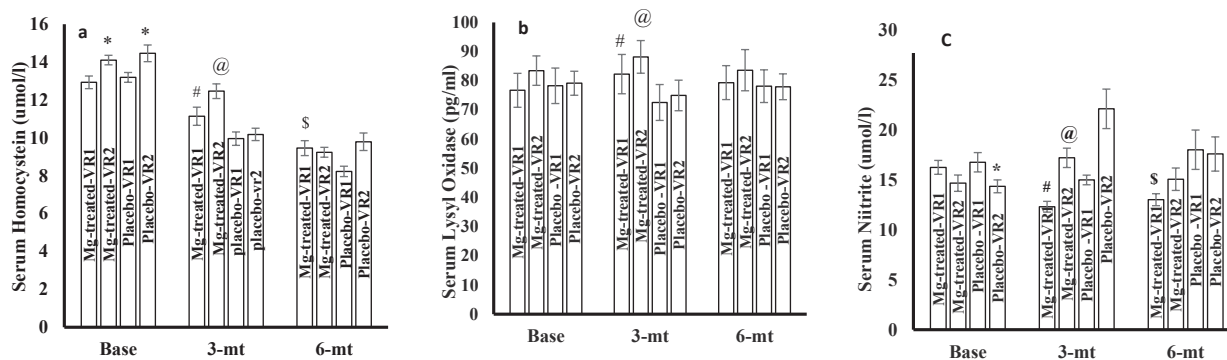


Figure 3. Serum homocysteine (a), lysyl oxidase (b) and nitrite (c) levels in MgSO₄ (Mg)-treated moderate coronary artery disease patients with one atherosclerotic vessel (Mg-VR1), Mg-treated moderate coronary artery disease patients with two atherosclerotic vessels (Mg-VR2), placebo-treated moderate coronary artery disease patients with one atherosclerotic vessel (placebo-VR1) and placebo-treated moderate coronary artery disease patients with two atherosclerotic vessels (placebo-VR2). $n = 14$ for one atherosclerotic vessel in each group and $n = 17$ for two atherosclerotic vessels in each group. Data were presented as mean ± SD and analyzed using one-way ANOVA and Tukey's post-hoc test. * $P < 0.001$ significant difference compared with VR1 groups before intervention (baseline). # $P < 0.001$ significant difference compared with the placebo-VR1 group 3 months after intervention (3-mt). \$ $P < 0.001$ significant difference with the placebo-VR1 group 6 months after intervention (6-mt). @ $P < 0.001$ significant difference compared with the placebo-VR2 group 3 months after intervention (3-mt)

Effects of MgSO₄ on serum LOX levels

Measurement at baseline

The baseline LOX concentrations are shown in Figure 3b. There was no significant difference in LOX levels among the four groups (Figure 3b).

Measurement after Mg treatment

LOX changes after 3 and 6 months MgSO₄ treatment are shown in Figure 3b.

Three months after treatment, serum LOX was found to be higher in the VR1 and VR2 Mg-treated groups than in the placebo groups, so Mg was able to increase LOX levels ($P < 0.001$). Moreover, the LOX levels returned to baseline after 6 months in the Mg-treated groups (Figure 3b).

Effects of MgSO₄ on serum levels of nitrite

Measurement at baseline

The baseline serum nitrite concentrations are shown in Figure 3c. Nitrite levels were lower in the VR2 patients compared with the VR1 group in both Mg- and placebo-treated groups ($P < 0.001$) (Figure 3c).

Measurement after Mg treatment

Alteration in serum levels of nitrite after 3 and 6 months treatment with MgSO₄ are shown in Figure 3c.

A rise of nitrite levels was seen in the VR2 groups in both intervention and placebo groups after 3 months, and it then returned to the baseline levels after 6 months ($P < 0.001$). This enhancement of nitrite levels in the placebo-VR2 group was significantly greater than the Mg-VR2 group after 3 months ($P < 0.001$), indicating Mg had prevented the increase in nitrite levels in VR2 patients (Figure 3c).

Discussion

In this study, we found that Mg was able to maintain LOX levels and reduce VLDL-C, homocysteine and nitrite, especially in the 3 months after intervention. The LOX enzyme may be considered an effective modifier of Mg's influence on cardiovascular disorders. These observations demonstrated that Mg can prevent the decrease of LOX levels and explain the beneficial effects of this element in CAD.

Maintaining the elasticity of the vascular system via regulation of collagen and elastin turnover is one way Mg protects vessels against vessel stiffness (11). On the other hand, the development of vessel walls is induced by LOX (12). Thus, it is reasonable to assume that Mg and this enzyme have a common pathway to regulate vessel wall development and elasticity. The impairment of the NO system usually accompanies cardiovascular diseases

(CVDs). The reduction of NO production is associated with endothelial dysfunction and atherosclerosis (13,14). Our data showed that Mg was able to maintain LOX levels and decrease nitrite levels. Nitrite assay is considered an index of NO synthesis (15). Previous studies have demonstrated that elastic fiber formation occurs via NO production. Therefore, decrease in NO synthesis could be responsible for the reduction of vessel elasticity and induction of plaque formation (16). It is suggested that NO accelerates the expression of LOX, which induces filament cross-links (17). In addition, Mg stimulates NO production and endothelial NOS (eNOS) activity, so it could be postulated that in this way, Mg can induce cardiovascular protection (11). One study indicated that Mg alters LOX function, but the underlying mechanism needs further investigation (11). As a vasodilator, NO contributes to vessel function (18). In physiological conditions, NO production by eNOS accounts for the biological function of vessel strength. However, NO production by inducible NOS (iNOS) accelerates oxidative stress, leading to initiation and progression of cardiovascular problems (19,20). In addition, increase the activity of iNOS, and eNOS impairment appears to play a fundamental role in the progression of atherosclerosis (19, 21). We observed that nitrite levels were significantly greater in the placebo-VR2 group than the Mg-VR2 group after 3 months. This means Mg may prevent nitrite levels from increasing by inhibiting iNOS expression in patients with high severity of atherosclerosis, modulating LOX activity and improving CAD.

Epidemiological studies have shown that a rise in plasma homocysteine can induce atherosclerotic plaque formation as an independent risk factor. Hyperhomocysteinemia accelerates thrombogenicity, oxidative stress status, and endothelial dysfunction (22,23). Even slightly increased serum homocysteine levels can be a risk factor for the development of coronary disease (24). Moreover, optimal homocysteine levels are below 10 to 12 $\mu\text{mol/L}$, and the initial threshold level for hyperhomocysteinemia can be 15 $\mu\text{mol/L}$ (25). In our study, subjects with two atherosclerotic vessels showed significantly higher basal concentrations of homocysteine than subjects with one atherosclerotic vessel, indicating that homocysteine level is associated with the degree of CAD. In line with our research, the result of a large cohort study in patients undergoing coronary angiography has revealed that elevated homocysteine is significantly associated with the degree of CAD (22). In our research, three and 6 months after intervention, the level of homocysteine significantly decreased both in patients receiving placebo and MgSO_4 compared to baseline levels. However, the reduction was greater in placebo groups than Mg-treated groups after 3 months. In line with the current study, Mg treatment has been reported to reduce homocysteine level in pregnancy-induced hypertension syndrome (26). It is assumed that

Mg administration can modulate homocysteine levels in CAD, because the major enzymes of homocysteine metabolism pathway are Mg-dependent (26). Our study demonstrated that in 3 months of treatment, MgSO_4 was able to maintain LOX at high levels, and after 6 months, the LOX levels returned to baseline. It has been shown that disturbance in LOX expression and activity is associated with cardiovascular problems and the normal activity of LOX is necessary for vascular hemostasis (3,27). The data showed that LOX deficiency leads to alternation of endothelial function and vascular wall remodeling (28). Moreover, in the initial phase of CAD, the downregulation of LOX promotes cardiovascular complications (3). Additionally, overactivity of LOX has been reported in hypertension and chronic CAD (29). In the present work, the enhancement of LOX levels in the initial phase of Mg treatment may lead to the amelioration of atherosclerosis. Hyperhomocysteinemia has been shown to interfere with post-translational modifications of collagen, directly by inhibiting LOX and indirectly by downregulation of mRNA expression of LOX and other genes that are involved in collagen cross-linking (30). Based on our data, serum homocysteine levels in the magnesium groups showed a slower decrease compared to the placebo groups, especially after 3 months. In fact, in the group that received magnesium for 3 months, the reduction of homocysteine level kept it in the optimum range. At the same time, LOX was found to be higher in the VR1 and VR2 Mg-treated groups than in the placebo groups, suggesting that Mg was able to maintain LOX levels. Based on these results, we assume Mg can modulate LOX levels by controlling serum homocysteine concentration. Also, participants (both in placebo and Mg groups) received their routine treatments, including atorvastatin, aspirin, clopidogrel, metoprolol, nitrate, losartan, and metformin, so we assume the reduction of homocysteine levels is related to these drugs in the placebo and mg groups.

Hyperlipidemia is an early event that accelerates CAD development and can alter the composition and permeability of the endothelial barrier. Hypercholesterolemia can alter the endothelial extracellular matrix and induce endothelial dysfunction by significant downregulation of vascular LOX expression (3,27). Our results demonstrate that cholesterol and TG levels were significantly decreased 6 months after intervention in both the placebo and Mg-treated groups. Also, in patients with one atherosclerotic vessel, Mg decreased VLDL-C faster compared with placebo-VR1. Also we assume that Mg can appropriately maintain LOX levels in patients with a lower degree of CAD by lowering the VLDL levels.

In order to show the safety of Mg-treatment, liver and kidney function tests were also conducted. Serum Cr, BUN, SGOT, and SGPT activity showed no significant

difference between the Mg-treated and placebo-treated groups, and liver and kidney function were both within normal range. Therefore, based on our results, magnesium administration for 6 months does not show any toxic effect on the kidney or liver.

Conclusion

Our findings showed that Mg administration in CAD patients for 6 months was able to modulate LOX levels and decrease serum levels of some factors that affect the expression and activity of LOX, including VLDL-C, homocysteine, and nitrite. Therefore, Mg may be considered as an auxiliary supplement to improve some risk factors of CAD by modulating LOX levels.

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Authors' Contribution

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Competing Interests

The authors declare that they have no competing interests.

Consent for Publication

All of the authors have consented to publication.

Data Availability Statement

The datasets are available from the corresponding author on reasonable request.

Ethical Approval

The study was approved by the Ethics Committee of Hormozgan University of Medical Sciences with the reference number IR.HUMS.REC.1397.308, approval date 2019-02-17. Informed written consent to participate in the study was obtained from all subjects.

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References

- Matsuzawa Y, Lerman A. Endothelial dysfunction and coronary artery disease: assessment, prognosis, and treatment. *Coron Artery Dis.* 2014;25(8):713-24. doi: [10.1097/mca.000000000000178](https://doi.org/10.1097/mca.000000000000178).
- Bernhard B, Gräni C. Sex differences in the manifestation and evolution of coronary artery plaques. *Int J Cardiovasc Imaging.* 2021;37(9):2773-5. doi: [10.1007/s10554-021-02265-5](https://doi.org/10.1007/s10554-021-02265-5).
- Rodríguez C, Martínez-González J, Raposo B, Alcudia JF, Guadall A, Badimon L. Regulation of lysyl oxidase in vascular cells: lysyl oxidase as a new player in cardiovascular diseases. *Cardiovasc Res.* 2008;79(1):7-13. doi: [10.1093/cvr/cvn102](https://doi.org/10.1093/cvr/cvn102).
- Martínez-González J, Varona S, Cañes L, Galán M, Briones AM, Cachofeiro V, et al. Emerging roles of lysyl oxidases in the cardiovascular system: new concepts and therapeutic challenges. *Biomolecules.* 2019;9(10):610. doi: [10.3390/biom9100610](https://doi.org/10.3390/biom9100610).
- Onoda M, Yoshimura K, Aoki H, Ikeda Y, Morikage N, Furutani A, et al. Lysyl oxidase resolves inflammation by reducing monocyte chemoattractant protein-1 in abdominal aortic aneurysm. *Atherosclerosis.* 2010;208(2):366-9. doi: [10.1016/j.atherosclerosis.2009.07.036](https://doi.org/10.1016/j.atherosclerosis.2009.07.036).
- Wei S, Gao L, Wu C, Qin F, Yuan J. Role of the lysyl oxidase family in organ development (review). *Exp Ther Med.* 2020;20(1):163-72. doi: [10.3892/etm.2020.8731](https://doi.org/10.3892/etm.2020.8731).
- Jeddi S, Yousefzadeh N, Kashfi K, Ghasemi A. Role of nitric oxide in type 1 diabetes-induced osteoporosis. *Biochem Pharmacol.* 2022;197:114888. doi: [10.1016/j.bcp.2021.114888](https://doi.org/10.1016/j.bcp.2021.114888).
- Larsson SC, Burgess S, Michaëlsson K. Serum magnesium levels and risk of coronary artery disease: Mendelian randomisation study. *BMC Med.* 2018;16(1):68. doi: [10.1186/s12916-018-1065-z](https://doi.org/10.1186/s12916-018-1065-z).
- DiNicolantonio JJ, Liu J, O'Keefe JH. Magnesium for the prevention and treatment of cardiovascular disease. *Open Heart.* 2018;5(2):e000775. doi: [10.1136/openhrt-2018-000775](https://doi.org/10.1136/openhrt-2018-000775).
- Sun X, Zhuang X, Huo M, Feng P, Zhang S, Zhong X, et al. Serum magnesium and the prevalence of peripheral artery disease: the Atherosclerosis Risk in Communities (ARIC) study. *Atherosclerosis.* 2019;282:196-201. doi: [10.1016/j.atherosclerosis.2018.12.004](https://doi.org/10.1016/j.atherosclerosis.2018.12.004).
- Kostov K, Halacheva L. Role of magnesium deficiency in promoting atherosclerosis, endothelial dysfunction, and arterial stiffening as risk factors for hypertension. *Int J Mol Sci.* 2018;19(6):1724. doi: [10.3390/ijms19061724](https://doi.org/10.3390/ijms19061724).
- Remus EW, O'Donnell RE Jr, Rafferty K, Weiss D, Joseph G, Csiszar K, et al. The role of lysyl oxidase family members in the stabilization of abdominal aortic aneurysms. *Am J Physiol Heart Circ Physiol.* 2012;303(8):H1067-75. doi: [10.1152/ajpheart.00217.2012](https://doi.org/10.1152/ajpheart.00217.2012).
- Pereira BP, do Vale GT, Ceron CS. The role of nitric oxide in renovascular hypertension: from the pathophysiology to the treatment. *Naunyn Schmiedebergs Arch Pharmacol.* 2022;395(2):121-31. doi: [10.1007/s00210-021-02186-z](https://doi.org/10.1007/s00210-021-02186-z).
- Chen JY, Ye ZX, Wang XF, Chang J, Yang MW, Zhong HH, et al. Nitric oxide bioavailability dysfunction involves in atherosclerosis. *Biomed Pharmacother.* 2018;97:423-8. doi: [10.1016/j.biopha.2017.10.122](https://doi.org/10.1016/j.biopha.2017.10.122).
- Dehghani A, Saberi S, Nematbakhsh M. Nitric oxide synthases blockade; L-NAME and estradiol alter renal blood flow response to angiotensin 1-7 in ovariectomized rats. *Indian J Physiol Pharmacol.* 2015;59(3):266-74.
- Rafieian-Kopaei M, Setorki M, Dousti M, Baradaran A, Nasri H. Atherosclerosis: process, indicators, risk factors and new hopes. *Int J Prev Med.* 2014;5(8):927-46.
- Sugitani H, Wachi H, Tajima S, Seyama Y. Nitric oxide stimulates elastin expression in chick aortic smooth muscle cells. *Biol Pharm Bull.* 2001;24(5):461-4. doi: [10.1248/bpb.24.461](https://doi.org/10.1248/bpb.24.461).

18. Dehghani A, Saberi S, Nematbakhsh M. Role of Mas receptor antagonist A799 in renal blood flow response to Ang 1-7 after bradykinin administration in ovariectomized estradiol-treated rats. *Adv Pharmacol Sci*. 2015;2015:801053. doi: [10.1155/2015/801053](https://doi.org/10.1155/2015/801053).
19. Soskić SS, Dobutović BD, Sudar EM, Obradović MM, Nikolić DM, Djordjević JD, et al. Regulation of inducible nitric oxide synthase (iNOS) and its potential role in insulin resistance, diabetes and heart failure. *Open Cardiovasc Med J*. 2011;5:153-63. doi: [10.2174/1874192401105010153](https://doi.org/10.2174/1874192401105010153).
20. Nahavandi S, Ahmadi S, Sobhani SA, Abbasi T, Dehghani A. A high dose of estrogen can improve renal ischemia-reperfusion-induced pulmonary injury in ovariectomized female rats. *Can J Physiol Pharmacol*. 2021;99(12):1241-52. doi: [10.1139/cjpp-2021-0130](https://doi.org/10.1139/cjpp-2021-0130).
21. Tran N, Garcia T, Aniq M, Ali S, Ally A, Nauli SM. Endothelial nitric oxide synthase (eNOS) and the cardiovascular system: in physiology and in disease states. *Am J Biomed Sci Res*. 2022;15(2):153-77.
22. Schaffer A, Verdoia M, Cassetti E, Marino P, Suryapranata H, De Luca G. Relationship between homocysteine and coronary artery disease. Results from a large prospective cohort study. *Thromb Res*. 2014;134(2):288-93. doi: [10.1016/j.thromres.2014.05.025](https://doi.org/10.1016/j.thromres.2014.05.025).
23. Sreckovic B, Sreckovic VD, Soldatovic I, Colak E, Sumarac-Dumanovic M, Janeski H, et al. Homocysteine is a marker for metabolic syndrome and atherosclerosis. *Diabetes Metab Syndr*. 2017;11(3):179-82. doi: [10.1016/j.dsx.2016.08.026](https://doi.org/10.1016/j.dsx.2016.08.026).
24. Zhang L, Yuan XF, Li Q, Fan L, Chen Z, Zhang B, et al. [Correlation between serum homocysteine level, MTHFR gene polymorphism and patients with hematological diseases complicated with coronary heart disease]. *Zhongguo Shi Yan Xue Ye Xue Za Zhi*. 2022;30(1):305-9. doi: [10.19746/j.cnki.issn.1009-2137.2022.01.050](https://doi.org/10.19746/j.cnki.issn.1009-2137.2022.01.050). [Chinese].
25. Vuckovic BA, Cabarkapa VS, Ilic TA, Salatic IR, Lozanov-Crvenkovic ZS, Mitic GP. Clinical significance of determining plasma homocysteine: case-control study on arterial and venous thrombotic patients. *Croat Med J*. 2013;54(5):480-8. doi: [10.3325/cmj.2013.54.480](https://doi.org/10.3325/cmj.2013.54.480).
26. Ma L, Li L, Han P, Meng F, Jiao C, Zhang H. Effect of the drug combination of magnesium sulfate and phentolamine on homocysteine and C-reactive protein in the serum of patients with pregnancy-induced hypertension syndrome. *Exp Ther Med*. 2019;17(5):3682-8. doi: [10.3892/etm.2019.7385](https://doi.org/10.3892/etm.2019.7385).
27. Rodríguez C, Raposo B, Martínez-González J, Casaní L, Badimon L. Low density lipoproteins downregulate lysyl oxidase in vascular endothelial cells and the arterial wall. *Arterioscler Thromb Vasc Biol*. 2002;22(9):1409-14. doi: [10.1161/01.atv.0000033818.21748.99](https://doi.org/10.1161/01.atv.0000033818.21748.99).
28. Rodríguez C, Martínez-González J. The role of lysyl oxidase enzymes in cardiac function and remodeling. *Cells*. 2019;8(12):1483. doi: [10.3390/cells8121483](https://doi.org/10.3390/cells8121483).
29. Martínez-Revelles S, García-Redondo AB, Avendaño MS, Varona S, Palao T, Orriols M, et al. Lysyl oxidase induces vascular oxidative stress and contributes to arterial stiffness and abnormal elastin structure in hypertension: role of p38MAPK. *Antioxid Redox Signal*. 2017;27(7):379-97. doi: [10.1089/ars.2016.6642](https://doi.org/10.1089/ars.2016.6642).
30. Thaler R, Agsten M, Spitzer S, Paschalis EP, Karlic H, Klaushofer K, et al. Homocysteine suppresses the expression of the collagen cross-linker lysyl oxidase involving IL-6, Fli1, and epigenetic DNA methylation. *J Biol Chem*. 2011;286(7):5578-88. doi: [10.1074/jbc.M110.166181](https://doi.org/10.1074/jbc.M110.166181).

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