Original Article

# Oral magnesium supplementation improves endothelial function and attenuates subclinical atherosclerosis in thiazide-treated hypertensive women

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**Background:** Epidemiological studies demonstrate an inverse association between serum magnesium and incidence of cardiovascular disease. Diuretics commonly cause hypomagneseamia.

**Method:** We evaluated effects of magnesium supplementation on blood pressure (BP) and vascular function in thiazide-treated hypertensive women in a randomized, double-blind, clinical trial. Hypertensive women (40–65 years) on hydrochlorothiazide and mean 24-h BP at least 130/80 mmHg were divided into placebo and supplementation (magnesium chelate 600 mg/day) groups. Patients were evaluated for nutritional and biochemical parameters, office and ambulatory blood pressure monitoring, brachial flow-mediated dilatation (FMD), peripheral arterial tonometry, assessment of carotid intima–media thickness, central hemodynamic parameters and pulse wave velocity at inclusion and after 6-month follow-up.

**Results:** The magnesium group had a significant reduction in SBP ($144 \pm 17$ vs. $134 \pm 14$ mmHg, $P = 0.036$) and DBP ($88 \pm 9$ vs. $81 \pm 8$ mmHg, $P = 0.005$) at 6 months, without effect on plasma glucose, lipids, or arterial stiffness parameters. The placebo group showed a significant increase in carotid intima-media thickness ($0.78 \pm 0.13$ vs. $0.89 \pm 0.14$ mm, $P = 0.033$) without change in the magnesium group ($0.79 \pm 0.16$ vs. $0.79 \pm 0.19$ mm, $P = 0.716$) after 6 months. The magnesium group demonstrated a significant increase in variation of FMD vs. the placebo group ($+3.7 \pm 2.1$ vs. $2.4 \pm 1.2\%$, $P = 0.015$). There was a significant correlation between the intracellular magnesium variation and FMD ($r = 0.44$, $P = 0.011$).

**Conclusion:** Magnesium supplementation was associated with better BP control, improved endothelial function and amelioration of subclinical atherosclerosis in these thiazide-treated hypertensive women.

**Keywords:** arterial stiffness, endothelial dysfunction, flow-mediated dilation, hypertension, magnesium, pulse wave velocity

**Abbreviations:** Aix, augmentation index; FMD, flow-mediated dilation; HDL, high-density lipoprotein; homa, homeostatic model assessment; IMT, intima-media thickness; LDL, Low-density lipoprotein; MAP, mean arterial pressure; PAT, peripheral arterial tonometry; PWV, pulse wave velocity

## INTRODUCTION

Many factors have been implicated in the pathogenesis of hypertension, such as activation of the renin–angiotensin–aldosterone system and intracellular changes in ions like calcium, sodium, potassium, and magnesium [1–3]. Magnesium has been the subject of experimental and clinical studies in hypertension [4–8] on the background that epidemiological studies showed significant inverse correlation between serum magnesium levels and incidence of cardiovascular disease [9–11].

Magnesium is the second most abundant intracellular cation and is involved in several important biochemical reactions [12]. Magnesium regulates vascular tone, by influencing formation and release of nitric oxide, and competing against effects of calcium resulting in changes in the vascular smooth muscle tone and contractility [13,14]. In addition, magnesium deficiency has previously been related to oxidative stress, inflammation, endothelial dysfunction, platelet aggregation, insulin resistance, and hyperglycemia [15–19].

Hypomagnesemia has been shown to negatively influence functional and structural vascular alterations in hypertension [20], but the influence on arterial stiffness has not yet been demonstrated been. Hypomagnesemia has been implicated in the pathogenesis of high blood pressure (BP), endothelial dysfunction, dyslipidemia, and inflammation [21–23], with these factors being associated with arterial

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stiffness [24,25]. Furthermore, epidemiological studies indicate a direct relationship between atherosclerosis and low serum magnesium [26], probably associated with insufficient dietary intake of this mineral [27].

In addition to reduced dietary intake of magnesium, drugs, such as thiazide diuretics, also lead to hypomagneseamia. Whether patients treated with such drugs have associated vascular damage remains unclear. Thus, the aim of this study was to evaluate the effects of 6-month magnesium supplementation on functional and structural vascular changes as well as blood pressure control in hypertensive women treated with diuretics.

# METHODS

## Study population

A double-blind randomized clinical trial was carried out to evaluate women with uncontrolled hypertension, aged 40–65 years, on stable monotherapy with hydrochlorothiazide 25 mg at least in the last 4 weeks. Exclusion criteria were BP at least 160/100 mmHg, BMI at least 35 kg/m$^2$, diabetes mellitus, kidney disease, clinical evidence of heart failure, coronary artery disease, and previous stroke. Participants were randomized to receive placebo or 600 mg of magnesium chelate orally twice a day for 6 months. Clinical evaluation, laboratory tests, and vascular studies were performed before and after the 6-month intervention period. The local ethics committee approved the protocol, and all study participants gave their written informed consent. This study was registered at ClinicalTrials.gov (NCT01151683).

## Biochemical evaluation

Venous blood samples were collected after 12 h of fasting. Serum lipids [total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides], and blood glucose were measured with a Technicon DAX 96 Autoanalyser (Miles Inc., Tarrytown, New York, USA). Low-density lipoprotein (LDL) cholesterol was calculated with the Friedewald formula when triglyceride values were less than 400 mg/dl. Serum magnesium was measured by colorimetry and intracellular (intraerythrocyte) magnesium was evaluated by atomic absorption spectrometry. Insulin was measured by radioimmunoassay (in mU/ml) and the homeostatic model assessment-insulin resistance (HOMA-IR) index [fasting glucose (mmol/l) × fasting insulin (mUI/ml)/22.5] and HOMA-β [fasting insulin × 20/(fasting glucose−3.5)] were calculated to estimate insulin sensitivity and secretion [28].

## Ambulatory blood pressure monitoring

The patients underwent 24-h ambulatory BP monitoring in nondominant arm with SpaceLabs 90207 monitor (Spacelabs Inc., Redmond, Washington, USA), validated by the British Hypertension Society and the association for the advancement of medical instrumentation protocol [29]. Readings were taken every 20 min during the day and every 30 min at night. The patients recorded their sleep and wake times during the monitoring. Ambulatory BP monitoring was considered adequate if greater than 70% of measurements were successfully obtained. The percentage decline in nocturnal BP was calculated as follows for SBP and DBP: % BP fall = [(daytime BP − night-time BP)/daytime BP] × 100.

## Assessment of endothelial function

Flow-mediated dilation (FMD) was assessed as a measure of endothelial function. The participant was positioned supine with the arm in a comfortable position, and the brachial artery was imaged above the antecubital fossa. After 10 min of rest, the right brachial artery was scanned in longitudinal section, 5 cm above the antecubital fossa, using a linear array transducer to acquire the baseline diameter of the brachial artery. A cuff was then inflated to at least 50 mmHg above SBP and deflated after 5 min to induce reactive hyperemia. A pulse wave Doppler recording in the artery lumen documented the flow increase, and the maximal diameter after cuff release was registered. FMD was calculated as the maximal percentage change of brachial artery diameter from baseline.

## Peripheral arterial tonometry

Endothelial function was also evaluated in microcirculation with the Endo-Peripheral arterial tonometry system (Itamar Medical Ltd, Caesarea, Israel). During the measurement, the study paricipant remained in a chair with their hands at the level of their heart and fingers hanging freely. Fingertip probes were placed on both index fingers, and pulse wave amplitudes were detected and recorded during the study. After a 5-min baseline measurement, arterial flow was occluded with a cuff on the nondominant arm. After 5 min of occlusion, the cuff was rapidly deflated to allow reactive hyperemia. Pulse wave amplitudes were recorded again for at least 5 min. The software calculated a reactive hyperemia index, which was a ratio of the average pulse wave amplitude measured over 60 s, starting 1 min after cuff deflation, to the average pulse wave amplitude measured at the baseline. The other arm served as a control, and the ratio was corrected for changes in the systemic vascular tone.

## Pulse wave velocity

The same investigator using a Complior device (Alam Medical, Vincennes, France) after the patients had rested for 10 min in supine position in a quiet room with a stable temperature measured carotid-femoral pulse wave velocity (PWV). All measurements were performed between 0800 and 1100 h. During the measurements, speaking or sleeping was not allowed and no meal, caffeine or smoking was allowed within 3 h before measurement. Pulse wave forms were obtained transcutaneously from the right common carotid artery and femoral artery. Aortic PWV was calculated by dividing the distance travelled by the time travelled. The time travelled was obtained by measuring the time difference between the arrival of the pulse wave at the femoral and carotid arteries. The distance travelled was estimated as 80% of the direct tape measure distance between carotid and femoral artery. Carotid-femoral PWV was calculated as distance travelled in meters divided by transit time in seconds (PWV = distance travelled/transit time). The mean of two measurements was calculated and when the difference between them was more than 0.5 m/s,

a third measurement was obtained. All PWV values were adjusted by mean arterial pressure (MAP) to obtain normalized PWV (PWV norm) as 100 × (PWV/MAP).

### Central hemodynamic parameters

Applanation tonometry was performed with the Sphygmocor system (Atcor Medical, Sydney, Australia) with the patient in the sitting position, resting the arm on a rigid surface, and a sensor in the radial artery. Central aortic pressure was calculated from the radial pulse wave analysis with the use of a validated transfer function. Wave reflection parameters, such as augmentation pressure and augmentation index, were also obtained by this method.

### Carotid ultrasound scan

The patient was supine with slight hyperextension and rotation of the neck in the direction opposite the probe. A linear array transducer with a multiple frequency (7–12 MHz) attached to a high-resolution B-mode ultrasound system was used to acquire images by a single sonographer blind to clinical data of study participants. Simultaneous ECG was recorded to assure the timing of end-diastolic images. Manual measurement of intima–media thickness (IMT) was performed in the common carotid artery, at both sides, in a region free of plaque located approximately 20 mm from bulb. At least three values were obtained in different sites of this segment, and the mean value of six measurements (three from each side) was used for analysis.

### Statistical analysis

Continuous variables were expressed as mean ± SD. Baseline differences between the two study groups were evaluated with Student's *t*-tests for continuous variables and Fisher's exact test for categorical variables. For intragroup comparisons between the baseline and study end, paired *t*-test was used. Between-group differences were compared using unpaired *t*-test or Mann–Whitney *U* test where appropriate. To identify possible relationships between the changes in the study parameters, bivariate correlation Pearson coefficients (*r*) were calculated. A *P* value less than 0.05 was considered statistically significant. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 18.0 for Windows (SPSS, Chicago, Illinois, USA).

## RESULTS

Fifty-three hypertensive women treated with thiazides were screened for this study. After initial evaluation, 16 patients were not included because of BP less than 130/80 mmHg and two other patients had fasting plasma glucose higher than 126 mg/dl and were also excluded.

Baseline clinical characteristics in the placebo and magnesium groups are presented in Table 1. The mean age and anthropometric data were not significantly different, and both groups presented intermediate risk prediction for general cardiovascular disease with normal liver and renal function. The magnesium group demonstrated a significant reduction in office SBP and DBP between baseline and 6-month evaluation, and a slight decrease in mean 24-h systolic SBP and DBP but without statistical significance. The same was observed in both daytime and nighttime periods. The changes in SBP and DBP nocturnal fall were not significantly different between the groups (Table 2).

**TABLE 1. Clinical characteristics of the study groups**

| Parameters | Placebo ($n = 18$) | Magnesium ($n = 17$) | *P* value |
|---|---|---|---|
| Age (years) | 57 ± 5 | 54 ± 7 | 0.571 |
| Menopause (*n* %) | 16 (88.9) | 17 (100) | 0.357 |
| Current smoker (*n* %) | 1 (5.5) | 1 (5.9) | 1.000 |
| BMI (kg/m$^2$) | 26.8 ± 3.9 | 29.7 ± 4.1 | 0.311 |
| Waist (cm) | 92.2 ± 10.9 | 98.3 ± 9.9 | 0.259 |
| WHR (units) | 0.84 ± 0.39 | 0.84 ± 0.63 | 0.991 |
| General CV risk (%) | 14.5 ± 7.3 | 14.2 ± 8.5 | 0.228 |
| Creatinine (mg/dl) | 0.78 ± 0.16 | 0.76 ± 0.12 | 0.946 |
| AST (IU/l) | 19.8 ± 0.7 | 19.0 ± 1.5 | 0.809 |
| ALT (IU/l) | 18.6 ± 1.3 | 19.4 ± 1.4 | 0.820 |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CV, cardiovascular; WHR, waist-to-hip ratio.
The values are expressed as mean ± SD.

Fasting plasma glucose, insulin and HOMA indexes were similar at baseline and final evaluation in both groups (Table 3). Total cholesterol was significantly decreased at the end of the study in the magnesium group. In this group, HDL cholesterol was increased and LDL cholesterol was reduced but did not reach statistical significance. There were no significant changes in the lipid profile in the placebo group. With regard to plasma electrolytes, there was a small but significant reduction in serum sodium in the placebo group but not in the magnesium group. Serum potassium and calcium were not significantly different in both groups. Serum magnesium was significantly reduced in the placebo group and did not change in the supplementation group. On the other hand, intracellular and urinary magnesium were significantly increased only in the group receiving supplementation (Fig. 1a–c). Although there was no significant difference in intracellular sodium between the groups, there was a negative correlation between intracellular magnesium and intracellular sodium ($r = -0.48$, $P = 0.023$; Fig. 1d).

Vascular results are shown in Table 4. Carotid-femoral PWV was similar between the groups in baseline and after 6-month supplementation even after adjusting for MAP. Similarly, the central hemodynamic parameters were not significantly different between the groups before and after intervention. The brachial FMD was reduced in the placebo group and increased in the magnesium supplemented study participants although not statistically significant. However, the magnesium group presented significantly higher values and changes of brachial FMD after supplementation when compared with the control group (Fig. 2a). The variation of intracellular magnesium was positively correlated to the variation ($r = 0.40$, $P = 0.015$) and to the end-study values ($r = 0.44$, $P = 0.011$) of brachial FMD (Fig. 2c and d). Reactive hyperemia index obtained by peripheral arterial tonometry was also reduced in the placebo group and increased in the magnesium group but without statistical significance (Fig. 2b). After 6 months of study, the placebo group demonstrated a small but significant increase in carotid IMT and media-to-lumen ratio, which were not changed in the magnesium

**TABLE 2. Office and ambulatory blood pressure levels in placebo and magnesium supplementation groups**

| Parameters | Placebo ($n=18$) | | | Magnesium ($n=17$) | | | Between-group comparison *P* value* |
|---|---|---|---|---|---|---|---|
| | Baseline | Final | *P* value | Baseline | Final | *P* value | |
| Office SBP (mmHg) | 143 ± 16 | 142 ± 18 | 0.635 | 144 ± 17 | 134 ± 14 | 0.036 | 0.240 |
| Office DBP (mmHg) | 86 ± 10 | 86 ± 9 | 0.760 | 88 ± 9 | 81 ± 8 | 0.005 | 0.172 |
| SBP-24 h (mmHg) | 134 ± 11 | 135 ± 13 | 0.168 | 135 ± 11 | 129 ± 11 | 0.074 | 0.322 |
| DBP-24 h (mmHg) | 86 ± 8 | 84 ± 7 | 0.076 | 86 ± 6 | 81 ± 10 | 0.111 | 0.472 |
| PP-24 h (mmHg) | 48 ± 9 | 51 ± 9 | 0.548 | 49 ± 9 | 50 ± 12 | 0.871 | 0.811 |
| Systolic load (%) | 54 ± 27 | 53 ± 29 | 0.191 | 57 ± 32 | 47 ± 34 | 0.190 | 0.678 |
| Diastolic load (%) | 65 ± 23 | 57 ± 23 | 0.221 | 68 ± 20 | 50 ± 32 | 0.109 | 0.597 |
| Daytime SBP (mmHg) | 140 ± 11 | 137 ± 15 | 0.148 | 139 ± 11 | 133 ± 11 | 0.097 | 0.466 |
| Daytime DBP (mmHg) | 91 ± 9 | 87 ± 8 | 0.051 | 90 ± 7 | 85 ± 10 | 0.087 | 0.557 |
| Nighttime SBP (mmHg) | 131 ± 18 | 130 ± 11 | 0.383 | 126 ± 14 | 123 ± 12 | 0.195 | 0.258 |
| Nighttime DBP (mmHg) | 80 ± 9 | 77 ± 7 | 0.299 | 77 ± 8 | 75 ± 9 | 0.231 | 0.605 |
| SBP nocturnal fall (%) | 6.3 ± 7.1 | 5.2 ± 7.3 | 0.878 | 10.1 ± 5.4 | 7.3 ± 5.6 | 0.487 | 0.558 |
| DBP nocturnal fall (%) | 11.8 ± 7.8 | 11.7 ± 6.4 | 0.998 | 14.5 ± 7.1 | 11.5 ± 5.7 | 0.430 | 0.951 |

PP, pulse pressure.
*Student's *t*-test for between groups comparison at the end of the study.
The values are expressed as mean ± SD.

supplemented patients (Fig. 3a and b). Serum magnesium was inversely correlated to mean ($r=-0.37$, $P=0.046$) and maximum ($r=-0.52$, $P=0.004$) carotid IMT (Fig. 3c and d).

## DISCUSSION

The therapeutic value of magnesium supplementation for human hypertension is still unclear, with some studies showing benefit and others failing to demonstrate therapeutic advantage on BP control [30,31]. In the present study, oral magnesium supplementation for 6 months significantly decreased office SBP and DBP in hypertensive women. On the other hand, there was a small and nonstatistically significant lowering of 24-h BP levels after magnesium supplementation. Nevertheless, the reduction of 5 mmHg in SBP and DBP might be clinically relevant concerning the prevention of cardiovascular events such as myocardial infarction and stroke. This finding is in agreement with a recent meta-analysis that demonstrated a small but clinically significant decrease, around 3 mmHg, in SBP and DBP determined by magnesium supplementation [32]. In fact, a previous meta-analysis had already detected dose-dependent BP reductions from magnesium supplementation [33]. Recently, a slight but significant lowering effect of oral magnesium supplementation on 24-h BP levels was observed in patients with mild hypertension and this finding was associated with increase in the intracellular magnesium [34].

Heterogeneity in study design, study participants, and the type of magnesium supplement used could help to explain the different results in several clinical protocols regarding the BP lowering effects of magnesium supplementation. Absorption of magnesium varies from different kinds of supplements. Hence, some forms of administered magnesium may have had lower bioavailability, which

**TABLE 3. Biochemical profile at baseline and study end in the placebo and magnesium supplementation groups**

| Parameters | Placebo ($n=18$) | | | Magnesium ($n=17$) | | | Between-group comparison *P* value |
|---|---|---|---|---|---|---|---|
| | Baseline | Final | *P* value | Baseline | Final | *P* value | |
| Glucose (mg/dl) | 90 ± 12 | 96 ± 11 | 0.428 | 93 ± 8 | 91 ± 10 | 0.270 | 0.346 |
| Insulin (μU/ml) | 12.8 ± 8.9 | 12.8 ± 11.1 | 0.949 | 11.2 ± 3.9 | 10.7 ± 3.8 | 0.733 | 0.596 |
| HOMA-IR | 2.9 ± 2.2 | 2.8 ± 2.6 | 0.832 | 2.6 ± 1.1 | 2.4 ± 0.9 | 0.719 | 0.675 |
| HOMA-β | 176 ± 106 | 139 ± 161 | 0.289 | 141 ± 48 | 184 ± 168 | 0.361 | 0.586 |
| Total cholesterol (mg/dl) | 223 ± 36 | 220 ± 38 | 0.244 | 241 ± 56 | 216 ± 32 | 0.038 | 0.816 |
| HDL-cholesterol (mg/dl) | 54 ± 14 | 50 ± 13 | 0.052 | 58 ± 17 | 62 ± 15 | 0.720 | 0.126 |
| LDL-cholesterol (mg/dl) | 146 ± 32 | 144 ± 32 | 0.541 | 155 ± 39 | 126 ± 31 | 0.058 | 0.253 |
| Triglycerides (mg/dl) | 117 ± 57 | 129 ± 60 | 0.664 | 111 ± 60 | 114 ± 55 | 0.102 | 0.582 |
| Serum Na (mEq/l) | 142 ± 2 | 140 ± 2 | 0.046 | 140 ± 3 | 140 ± 3 | 0.456 | 0.536 |
| Serum K (mEq/l) | 4.2 ± 0.3 | 3.9 ± 0.3 | 0.384 | 4.2 ± 0.4 | 4.1 ± 0.3 | 0.601 | 0.410 |
| Serum Ca (mg/dl) | 9.3 ± 0.5 | 9.6 ± 0.4 | 0.516 | 9.7 ± 0.9 | 9.4 ± 0.5 | 0.317 | 0.253 |
| Serum Mg (mg/dl) | 2.1 ± 0.2 | 1.9 ± 0.2 | 0.048 | 2.1 ± 0.2 | 2.1 ± 0.2 | 0.756 | 0.029 |
| UMCR (g/g) | 0.05 ± 0.02 | 0.05 ± 0.02 | 0.620 | 0.05 ± 0.02 | 0.12 ± 0.07 | 0.057 | 0.018 |
| ic Mg (mg/dl erythrocyte) | 4.27 ± 0.64 | 4.06 ± 0.52 | 0.101 | 4.05 ± 0.61 | 4.75 ± 0.56 | 0.001 | 0.005 |
| ic Na (mg/dl erythrocyte) | 23 ± 9 | 18 ± 6 | 0.504 | 21 ± 9 | 20 ± 3 | 0.343 | 0.585 |

HDL, high density lipoprotein; HOMA Beta, Homeostatic Model Assessment Beta Cell Function; HOMA IR, Homeostatic Model Assessment - Insulin Resistance; ic, intracellular; LDL, low density lipoprotein; UMCR, urinary magnesium creatinine ratio.
*Paired *t*-test for intragroup analysis.
**Student's *t*-test for independent analysis between the groups.
The values are expressed as mean ± SD.

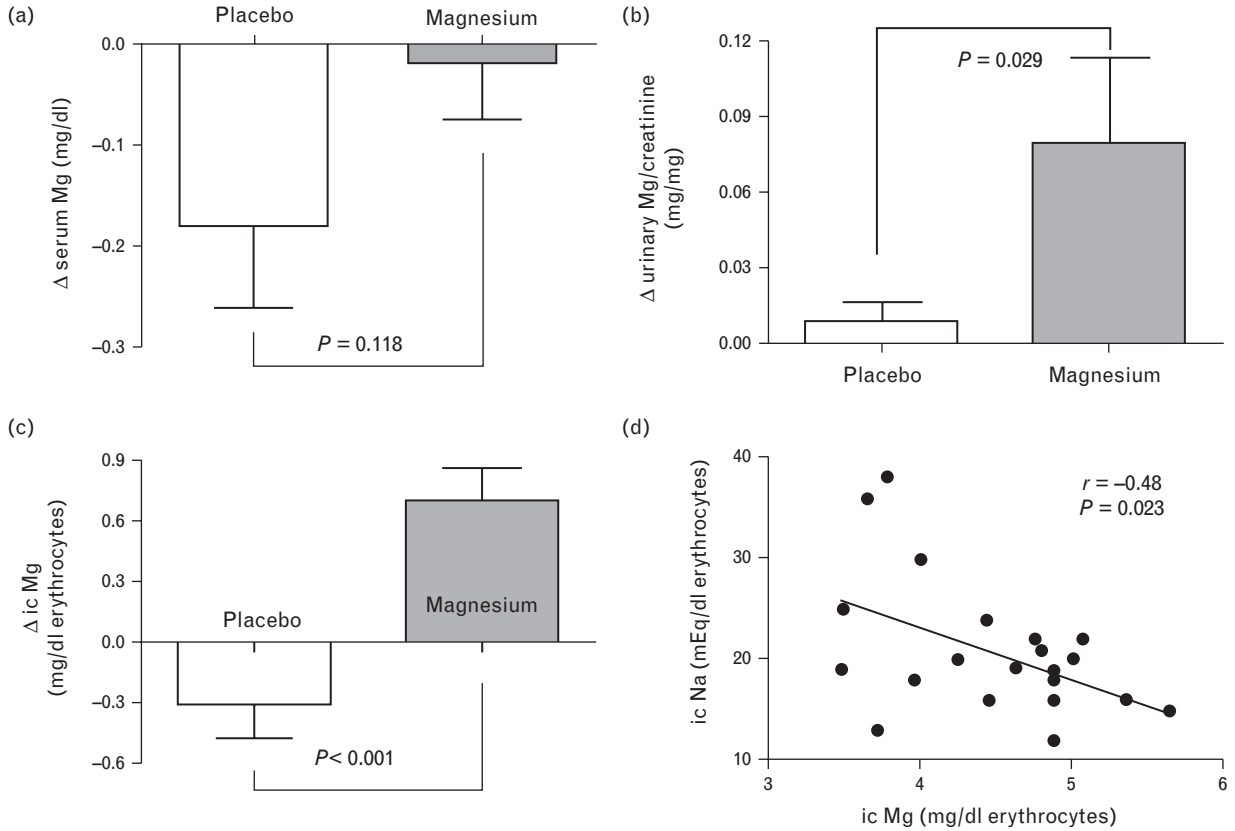


**FIGURE 1** Changes in serum (a), urinary (b), and intracellular (c) magnesium in the study groups. Correlation between intracellular magnesium and sodium (d). ic, intracellular.

might reduce the influence on BP levels. Small studies have found that citrate and chloride forms are more absorbed than magnesium oxide and sulfate. Chelated magnesium is more likely to survive the passage from the stomach to the small intestines intact. Therefore, the chelated magnesium bioavailability seems to be higher than other preparations [35].

Magnesium is involved in the physiological pathways that regulate glucose and lipid metabolism. A growing body of evidence derived from clinical trials shows that oral magnesium supplements improve insulin sensitivity and dyslipidemia in diabetic and nondiabetic individuals [7,31,36]. Insulin resistance is inversely correlated with HDL cholesterol levels and atherogenic changes characterized

**TABLE 4. Comparison of vascular tests between the groups**

| | Placebo ($n = 18$) | | | Magnesium ($n = 17$) | | | |
|---|---|---|---|---|---|---|---|
| **Parameters** | **Baseline** | **Final** | ***P* value** | **Baseline** | **Final** | ***P* value** | **Between-group comparison *P* value*** |
| Brachial FMD (%) | 8.3 ± 5.3 | 5.8 ± 3.7 | 0.064 | 8.3 ± 5.6 | 11.9 ± 6 | 0.109 | 0.008 |
| RHI (units) | 1.96 ± 0.36 | 1.85 ± 0.56 | 0.708 | 1.93 ± 0.30 | 2.09 ± 0.49 | 0.374 | 0.326 |
| PWV (m/s) | 8.24 ± 1.28 | 8.16 ± 0.88 | 0.781 | 8.24 ± 1.12 | 8.08 ± 1.04 | 0.605 | 0.801 |
| PWVnorm (m/s) | 7.92 ± 1.28 | 8.16 ± 1.12 | 0.610 | 7.76 ± 1.20 | 7.68 ± 1.04 | 0.819 | 0.321 |
| AP (mmHg) | 19 ± 6 | 19 ± 4 | 0.960 | 18 ± 5 | 18 ± 7 | 0.920 | 0.597 |
| Aix (%) | 38 ± 9 | 39 ± 2 | 0.721 | 38 ± 4 | 36 ± 7 | 0.260 | 0.158 |
| AIx@75 (%) | 36 ± 9 | 35 ± 3 | 0.798 | 33 ± 3 | 32 ± 6 | 0.595 | 0.263 |
| Aortic SBP (mmHg) | 131 ± 15 | 130 ± 16 | 0.756 | 130 ± 14 | 129 ± 13 | 0.897 | 0.915 |
| Aortic PP (mmHg) | 46 ± 11 | 47 ± 10 | 0.577 | 46 ± 11 | 45 ± 14 | 0.878 | 0.503 |
| Mean carotid IMT (mm) | 0.78 ± 0.13 | 0.89 ± 0.14 | 0.033 | 0.79 ± 0.16 | 0.79 ± 0.19 | 0.716 | 0.282 |
| Max carotid IMT (mm) | 0.99 ± 0.35 | 1.13 ± 0.30 | 0.156 | 0.92 ± 0.19 | 0.93 ± 0.24 | 0.681 | 0.169 |
| Carotid media-lumen ratio (mm) | 0.11 ± 0.01 | 0.13 ± 0.02 | 0.011 | 0.11 ± 0.02 | 0.11 ± 0.03 | 0.917 | 0.036 |

AIx, augmentation index; AIx@75, augmentation index corrected for heart rate of 75 bpm; AP, augmentation pressure; FMD, flow-mediated dilation; IMT, intima–media thickness; PP, pulse pressure; PWV, pulse wave velocity; PWVnorm, pulse wave velocity normalized for mean arterial pressure; RHI, reactive hyperemia index
*Paired *t*-test for intragroup analysis.
**t* test for intergroup analysis.
All values are expressed as mean ± SD.

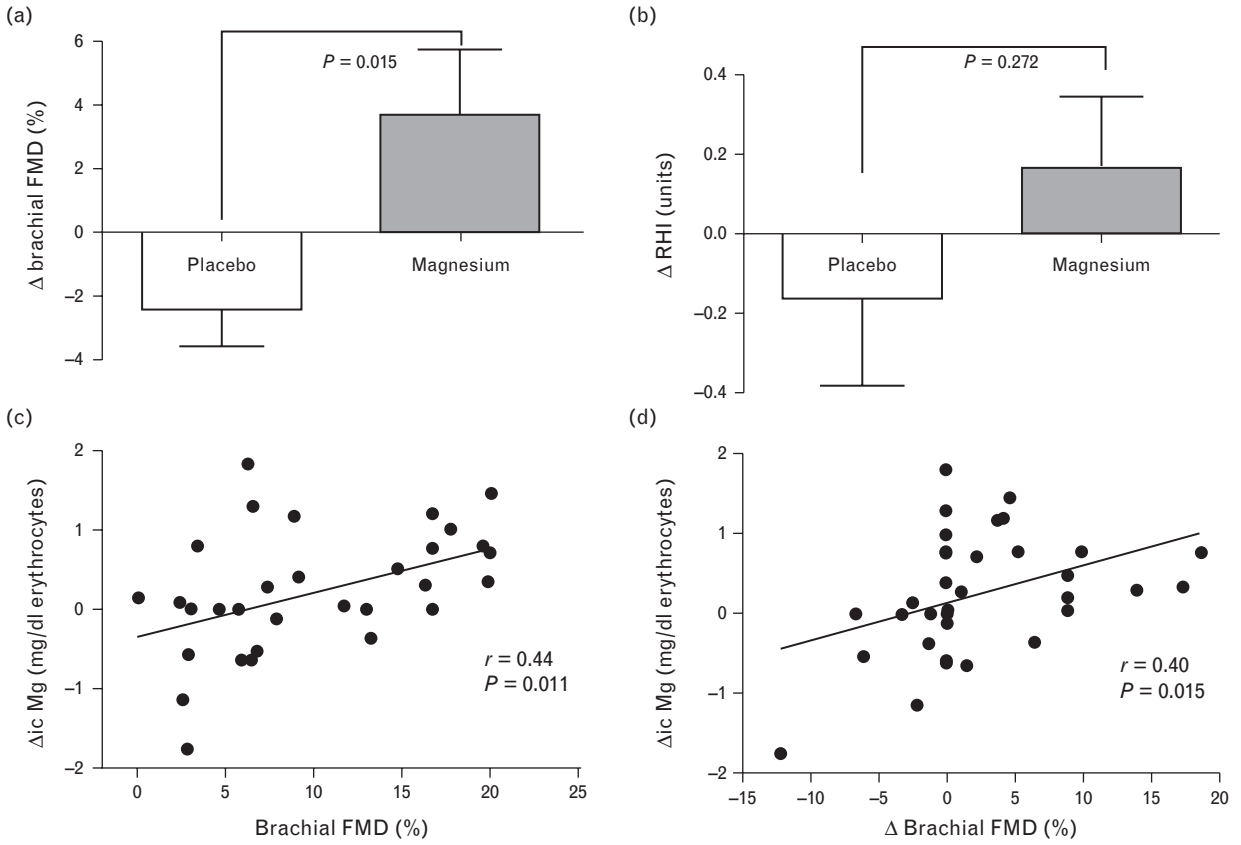


**FIGURE 2** Changes in brachial flow-mediated dilation (a) and in reactive hyperemia index by peripheral artery tonometry (b) in both groups. Correlation of changes in intracellular (ic) magnesium with study end values of brachial FMD (c) and with changes in brachial FMD (d) after 6 months of study. FMD, flow-mediated dialation; ic intracellular; RHI, reactive hyperemia index.

by low HDL cholesterol are mainly seen in insulin-resistant study participants [37]. Magnesium deficiency may be associated to low HDL cholesterol in the prediabetic state and chronic magnesium supplementation can contribute to an improvement in islet B-cell response and insulin action [38], and is useful in the treatment of hyperlipidemia [39] increasing the HDL cholesterol levels [7,40]. Serum magnesium levels have been correlated with total and HDL cholesterol in a large adult study population [41]. Interestingly, the relationship between magnesium status and lipids in healthy individuals may be different from that in patients with chronic conditions, such as obesity, diabetes, and hypertension [42,43]. In this study, patients in the magnesium group demonstrated a beneficial effect with a significant decrease in total cholesterol, and a nonsignificant increase in HDL cholesterol and reduction in LDL cholesterol compared with no significant changes in the lipid profile in placebo group.

There was a small but significant reduction in serum sodium and intracellular sodium in the placebo group, with a negative correlation between intracellular magnesium and intracellular sodium. These findings could indicate a protective effect of magnesium on hyponatremia induced by diuretic treatment. Although the mechanisms were not investigated in the present study, the $Na^+/Mg^{2+}$ antiport has been previously confirmed in mammalian cells [44], and changes in intracellular magnesium could also modify transmembrane calcium movements [45].

Low urinary magnesium excretion has been previously associated with an increased risk of ischemic heart disease [46]. In addition, urinary magnesium excretion can estimate the amount of absorbed magnesium, considering that renal elimination is highly relevant for an adequate magnesium homeostasis. Chronic intervention studies are usually associated to loss of follow-up and low treatment compliance. In the present study, both groups presented normal renal function indicating no influence on magnesium excretion. Therefore, the increase in intracellular and urinary magnesium in the group receiving 6-month supplementation points out the good compliance by these patients. Indeed, the increase in the intracellular ion concentrations has already been associated with BP-lowering effect of oral magnesium supplementation [34].

In this study, magnesium supplementation was not able to change arterial stiffness and wave reflection parameters. In contrast, we recently demonstrated that low intracellular magnesium was associated with more intense wave reflection [47]. Similarly, it has been previously demonstrated that serum magnesium levels were associated with augmentation index although not with PWV. However, the cross-sectional design and the oscillometric method used in that study to evaluate arterial stiffness and pulse wave reflection may be considered as limitations of those findings [48].

Endothelial dysfunction has been suggested to represent an early indicator of atherosclerosis, associated with an increased incidence of vascular diseases. Past studies have

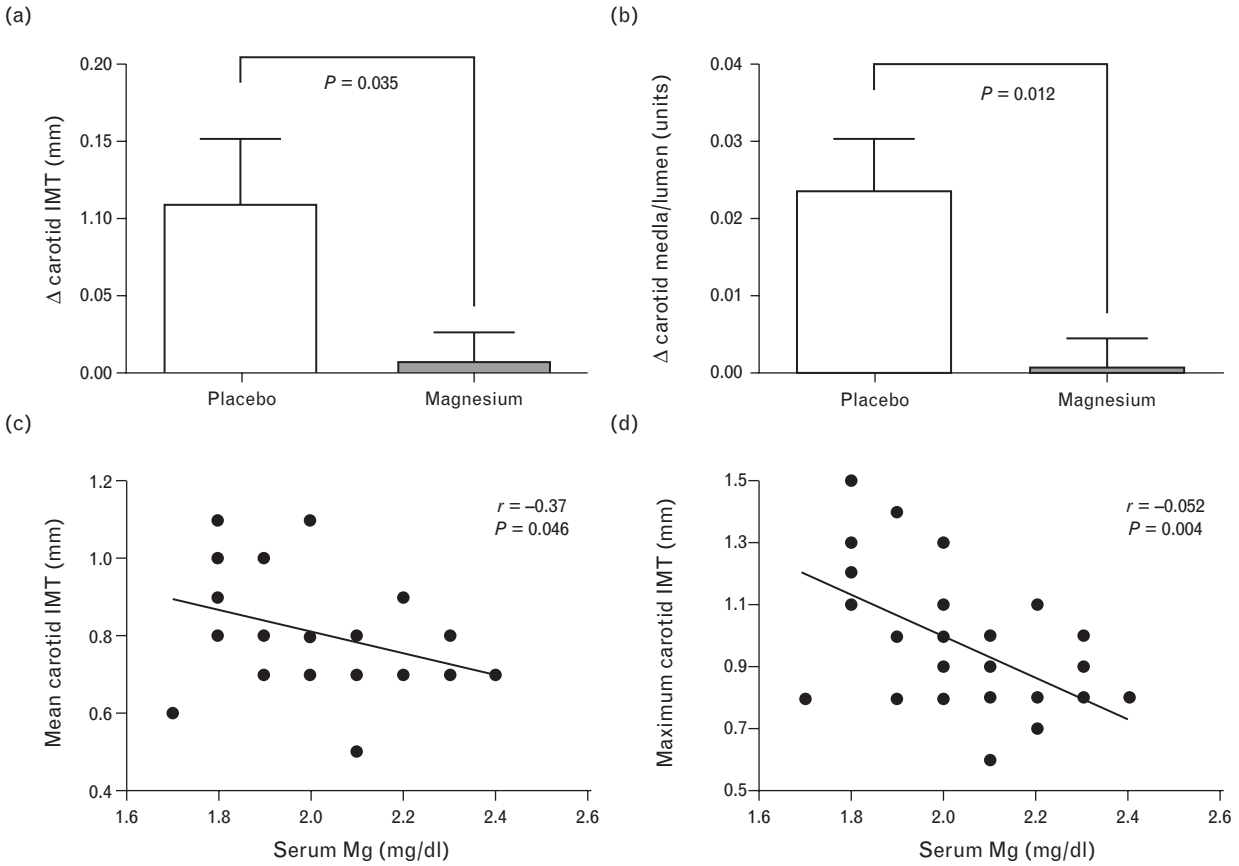


**FIGURE 3** Changes in carotid intima-media thickness (a) and in carotid media-lumen ratio (b) in the placebo and magnesium groups. Correlation of serum magnesium with mean carotid intima-media thickness (c) and with maximum carotid intima-media thickness (d). IMT, intima-media thickness.

demonstrated that low magnesium promotes endothelial cell dysfunction [49] and endothelium-dependent relaxation induced by extracellular magnesium is mediated by nitric oxiderelease by the endothelium [50]. The results of the present study demonstrated the beneficial effects of oral magnesium supplementation on endothelial function, including the positive correlation between variation of intracellular magnesium and brachial FMD. One of the possible mechanisms is that magnesium affects calcium ion concentrations and its availability at critical sites, acting as a physiologic calcium channel blocker [51]. Although not directly involved in the biochemical process of contraction, magnesium influences vascular tone, baseline tension, and vascular responsiveness to vasoconstrictor agents, both via endothelium independent and endothelium dependent pathways [52,53]. Our results are in accordance with previous report that oral magnesium supplementation resulted in a significant improvement in brachial artery endothelial function in elderly diabetic patients [12] and in patients with coronary artery disease [54].

Magnesium deficiency has been introduced as a cardiovascular risk factor, and some studies have shown that hypomagnesemia is associated with atherosclerosis [55,56]. Our data demonstrated an increase in carotid IMT in the placebo group, whereas hypertensive women receiving magnesium supplementation presented no difference. This result might be attributed to higher BP levels in the placebo group. In accordance with these findings, a recent study in hemodialysis patients showed that magnesium oxide for 6 months was able to decrease carotid IMT with an increase in the placebo group [57]. Indeed, the role of magnesium in attenuating the progression of atherosclerosis has been previously demonstrated in hemodialysis patients [58].

Some limitations warrant consideration in the present study. The beneficial effect of magnesium on endothelial function may be considered the main finding but we did not study all biochemical endothelial markers. Moreover, there was no concomitant assessment of endothelium-independent dilation. On the other hand, in our experimental model, all the results were compared with a control, not supplemented group, and there was no improvement in brachial FMD in these patients receiving placebo. Lastly, the single investigator performing the examination was blinded to the treatment assigned.

The significant magnesium effects in our study may relate, in part, to the fact that unlike other studies, we investigated only female patients on thiazide diuretics, who are at particular risk of magnesium wasting. In fact, the recommended dietary allowance for magnesium is different for middle-aged men (420 mg/day) and women (320 mg/day). Considering the study design as a randomized, double-blind, clinical trial, we enrolled only hypertensive women to avoid this sex difference as a confounding factor. Hence, this patient cohort may indeed benefit from magnesium supplementation with respect to

BP control and vascular protection. The effects of sex steroid hormones on magnesium metabolism and actions are not clear yet. Nevertheless, this concern is not relevant in this study as almost all patients were postmenopausal women.

In conclusion, 6-month magnesium supplementation was able to lower BP, attenuate subclinical atherosclerosis, and improve endothelial function in hypertensive women on thiazide treatment. The beneficial effects of magnesium supplementation may be more evident in patients with a negative magnesium balance. As magnesium is an inexpensive, natural, and relatively well tolerated element, its possible role as adjuvant therapy in those study participants who are at high risk of magnesium deficiency should be considered. There is still considerable uncertainty on the clinical utility of magnesium supplements. Therefore, further studies with larger populations are needed to confirm our findings.

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### Conflicts of interest

There are no conflicts of interest.

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## Reviewers' Summary Evaluations

### Reviewer 1

The study is of interest, demonstrating in a controlled manner that magnesium supplementation may improve blood pressure control in treated hypertensive women. However, there are no data in untreated women and/or in males. Further, the design of the study as well as the method used to assess endothelium-mediated vasodilation strongly limit the translation of the study findings to the real life.

### Reviewer 2

This elegant randomized, double-blind study for the first time demonstrates that chronic oral magnesium supplementation reduces blood pressure and ameliorates endothelial function at the level of peripheral conduit arteries in thiazide-treated hypertensive women. These findings convincingly underline the therapeutic value of magnesium supplementation for human hypertension.

Of note, this study investigated only female patients on thiazide diuretics, who are at particular risk of magnesium wasting. Further studied are needed to explore the role of magnesium supplementation to other different populations of hypertensive patients.