Long-term magnesium supplementation improves arterial stiffness in overweight and obese adults: results of a randomized, double-blind, placebo-controlled intervention trial[1–3]

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ABSTRACT

Background: Epidemiologic studies have suggested a protective effect of magnesium intake on cardiovascular disease risk. However, intervention trials of magnesium supplementation on blood pressure and conventional cardiometabolic risk markers are inconsistent. Effects on vascular function markers related to cardiovascular disease risk have rarely been studied.

Objective: The objective was to evaluate the effects of long-term magnesium supplementation on arterial stiffness.

Design: We performed a 24-wk, randomized, double-blind, placebo-controlled intervention study. Fifty-two overweight and slightly obese individuals (30 men and 22 postmenopausal women, mean ± SD age: 62 ± 6 y) were randomly allocated to receive either 3 times daily magnesium (3 × 117 mg or 350 mg/d) or placebo capsules. Twenty-four-hour urine collections and 24-h ambulatory blood pressure assessments were performed at the start and end of the study. Carotid-to-femoral pulse wave velocity (PWV_{c-f}) was assessed at baseline, after 12 wk, and at week 24.

Results: Serum magnesium concentrations did not differ after 12 wk but tended to increase after 24-wk magnesium supplementation compared with placebo by 0.02 mmol/L (95% CI: 0.00, 0.04 mmol/L; $P = 0.09$). Twenty-four-hour urinary magnesium excretion increased by 2.01 mmol (95% CI: 1.22, 2.93 mmol; $P < 0.001$) at week 24. PWV_{c-f} was not changed after 12 wk (0.0 m/s; 95% CI: −0.6, 0.5 m/s; $P = 0.90$) but was improved in the magnesium compared with the placebo group by 1.0 m/s (95% CI: 0.4, 1.6 m/s; $P = 0.001$) after 24 wk. Office and 24-h ambulatory blood pressure levels were not changed. No adverse events were observed.

Conclusion: Our data indicate that a daily magnesium supplement of 350 mg for 24 wk in overweight and obese adults reduces arterial stiffness, as estimated by a decrease in PWV_{c-f}, suggesting a potential mechanism by which an increased dietary magnesium intake beneficially affects cardiovascular health. This trial was registered at clinicaltrials.gov as NCT02235805. *Am J Clin Nutr* 2016;103:1260–6.

INTRODUCTION

A meta-analysis of 16 prospective cohort studies involving 313,041 subjects found an inverse association between dietary magnesium and cardiovascular disease (CVD)[7] risk (1). Each 200-mg/d increment in dietary magnesium intake was associated with a 22% lower risk of ischemic heart disease. Another approach to estimate dietary magnesium intake is to measure 24-h urinary magnesium excretion. In a prospective cohort study involving 7664 adults, low urinary magnesium excretion was associated with an increased risk of ischemic heart disease (2). Results of these epidemiologic studies underline the need for well-designed intervention trials to examine a potential causal role of magnesium intake in the prevention of CVD. However, randomized controlled trials (RCTs) with cardiovascular events as endpoints are missing, whereas results from intervention studies investigating the effects of magnesium intake on blood pressure (3) and other conventional cardiometabolic risk markers (4–8) are inconsistent and showed in general no clear effects. An alternative approach is to investigate the effect of magnesium supplementation on noninvasive vascular function

markers that are related to cardiovascular disease risk. Arterial stiffness is a validated marker to demonstrate cardiovascular health benefits (9). In this respect, carotid-to-femoral pulse wave velocity (PWV$_{c-f}$), a noninvasive method that measures the propagation of the forward pressure wave traveling along the aorta, is considered the gold-standard method for quantifying arterial stiffness (10). A recently published meta-analysis of individual participant data from 16 studies involving a total of 17,635 participants showed that PWV$_{c-f}$ was a predictor of ischemic heart disease, CVD risk and CVD mortality, independent of other established cardiovascular disease risk factors (11). To date, one RCT has addressed the effect of oral magnesium supplementation on arterial stiffness but found no effect (8). Of note, this study was carried out with only 14 healthy young men and lasted only 8 wk, which may have been too short to induce changes in arterial stiffness. Therefore, the objective of this randomized, double-blind, placebo-controlled intervention trial was to investigate the effects of magnesium supplementation for a 24-wk period in a large group of subjects. The trial was performed in overweight and slightly obese middle-aged and elderly adults, because they may be expected to have an increased arterial stiffness at baseline (12), allowing for improvement by the intervention.

METHODS

Study population

Apparently healthy overweight and slightly obese men and women were recruited via posters in university and hospital buildings or by advertisements in local newspapers. In addition, volunteers who had participated in earlier studies were approached. To avoid any possible variations in the study outcomes due to hormonal effects, only postmenopausal (≥2 y after last menstruation) women were included. Volunteers were invited for a screening visit if they met the following inclusion criteria: aged 45–70 y, stable body weight (weight gain or loss <3 kg within 3 mo before the screening visit), nonsmoker, willing to abstain from dietary supplements 1 mo before and during the study, no current use of proton pump inhibitors or medication known to affect lipid or glucose metabolism, no diabetes, not receiving antihypertensive medication, and no participation in another biomedical study within 1 mo before the screening visit. Fifty-two overweight and slightly obese adults were included. They had a BMI (in kg/m^2) between 25 and 35; fasting serum triacylglycerol concentrations ≤4.5 mmol/L; serum creatinine concentrations <116 μmol/L for men and <101 μmol/L for women; no indications for treatment with cholesterol-lowering medications according to the Dutch Cholesterol Consensus (13); no active CVD such as congestive heart failure or any cardiovascular event in the past, such as an acute myocardial infarction or cerebrovascular accident; no active inflammatory disease; and no drug or alcohol abuse. All study participants gave written consent before entering the study. The study was conducted according to the guidelines laid down in the Declaration of Helsinki, approved by the Medical Ethics Committee of Maastricht University Medical Center, and registered on 8 September 2014 at clinicaltrials.gov as NCT02235805.

Study design and products

The study had a randomized, double-blind, placebo-controlled, parallel design with a 24-wk experimental period. Subjects were allocated to receive either magnesium or placebo capsules with a computer-generated randomization scheme stratified by sex. Study participants were requested to take 1 capsule after breakfast, 1 capsule after lunch, and 1 capsule after dinner each day for 24 wk. On the days of blood sampling, the first capsule was taken after all measurements were completed. Each capsule provided 116.7 mg magnesium [Magnesium Citrate Complex (Mg 16%); AMT Laboratories Inc.]. Thus, daily magnesium intake provided by the 3 capsules was 350 mg. Placebo capsules contained starch (Amylum Solani). All capsules were kindly provided by Laboratorium Medisan B.V. DRcaps (Capsugel) acid-resistant hypromellose capsules were used. The capsules were prepared in 1 batch. A total daily dose of 350 mg was administered as this is considered the tolerable upper intake level of supplemental magnesium for adults (14). Magnesium citrate was deliberately chosen, because this type of supplement has a higher bioavailability than other magnesium formulations (15). Capsules were provided in blister strips that contained 15 capsules. The blisters were number-coded so as to blind both study subjects and investigators. Our research dietitian provided 6 blister strips to the volunteers every 4 wk. Participants were requested to return all blisters at the next visit, including any unused capsules that were counted as a measure of compliance. Study volunteers maintained their habitual diet, physical activity levels, and consumption of alcohol throughout the total study period.

Measurements were performed at the start of the study, after 12 wk, and at the end of the study. Twenty-four-hour ambulatory blood pressure was monitored (Mobil-O-Graph; I.E.M.), and all subjects collected 24-h urine samples at baseline (day −3) and at week 24 (day 165). Blood pressure recordings were taken every 15 min during daytime (from 0700 to 2300) and every 30 min at night (from 2300 to 0700) on the nondominant arm. Subjects were asked to maintain their normal daily activities during all the recording periods and to avoid intensive exercise. The first recording was discarded, and a 24-h mean ambulatory blood pressure was calculated, as well as daytime (from 0700 to 2300) and nighttime (from 2300 to 0700) means. Variabilities were quantified as the SD of 24-h, daytime, and nighttime values. Nocturnal blood pressure reductions were calculated as continuous variables: [(mean daytime blood pressure − mean nighttime blood pressure) ÷ mean daytime blood pressure] × 100 (16). For 24-h urine collection, study volunteers were instructed to discard the first morning urine samples and to collect all urine for the following 24 h. A separate container was used to collect all urine during the night and the first morning urine sample of the next morning after waking. Participants were requested to empty their bladder completely before going to sleep. In addition, they completed at the start and end of the study a food-frequency questionnaire (FFQ) to estimate food intake from the previous 4 wk. FFQs were immediately checked by a research dietitian in the presence of the participants. Energy and nutrient intakes were calculated with the Dutch Food Composition Tables (17). Individuals were requested to record daily in study diaries any signs or symptoms of illnesses, use of medication, consumption of alcohol, any deviations of the study protocol, and any other complaints.

Blood sampling and analyses

Fasting blood samples were taken at the start of the study (days −3 and 0), at week 12 (day 84), and at the end of the study

(days 165 and 168) from a forearm vein by venipuncture. On the days preceding blood sampling, participants were requested not to consume alcohol or to perform any strenuous physical exercise. On the morning of blood sampling, subjects arrived after an overnight fast (no food or drink after 2000, except for water) at the Metabolic Research Unit Maastricht research facilities by public transport or by car to standardize measurements as much as possible. After blood sampling, sodium fluoride–containing Vacutainer tubes (Becton Dickinson) and EDTA-coated Vacutainer tubes (Becton Dickinson) were immediately kept on ice and centrifuged within 30 min. To obtain plasma, plasma separator tubes were centrifuged at $1300 \times g$ for 15 min at 4°C. Blood drawn in Vacutainer serum tubes (Becton Dickinson) was first allowed to clot for ≥ 30 min at 21°C. To obtain serum, serum separator tubes were centrifuged at $1300 \times g$ for 15 min at 21°C. After centrifugation, plasma and serum samples were immediately portioned into aliquots and stored at −80°C until analysis at the end of the study.

Serum and urinary magnesium and calcium concentrations were determined photometrically with Magnesium and Calcium Gen.2 (COBAS; Roche Diagnostics GmbH) by the Central Diagnostic Laboratory Maastricht University Medical Center. Sodium and potassium concentrations were assessed with the indirect ion-selective electrode method Na-K-Cl for Gen.2 (COBAS; Roche Diagnostics GmbH). In addition, hematologic variables were determined, including hemoglobin, red blood cell count, hematocrit, white blood cell count, differential leukocyte count, and platelet count. Technicians were not aware of the treatments of the participants.

Clinical measurements

Height was measured during the screening with a wall-mounted stadiometer. Weight and the waist-to-hip circumference ratio as a measure of body fat distribution were determined before the start of the vascular measurements at the start of the trial (day 0), at week 12, and at the end of the trial (day 168). After placement of the tonometer leads and an acclimatization period of 30 min in the supine position, office blood pressure [systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse pressure] and heart rate were measured with a semicontinuous blood pressure monitoring device (Omron M7; CEMEX Medische Techniek). The first measurement was discarded, and the mean of the last 3 measurements is reported.

Vascular measurements were performed in a quiet, darkened, temperature-controlled room at 22°C. Radial artery pulse wave analysis was performed in triplicate with a tonometer (SphygmoCor v9; AtCor Medical) that was applied to the radial artery near the wrist of the arm. The central arterial waveform was derived from the peripheral waveform with a validated transfer function. The central augmentation index adjusted for heart rate (CAIxHR75) was defined as the difference between the first peak and second peak of the arterial waveform, expressed as a percentage of the pulse pressure and corrected for heart rate. With the same tonometer, PWV_{c-f} was determined, in triplicate, by measuring the arrival of the pulse wave and the delay to the R-wave of the electrocardiogram at the carotid and femoral artery. PWV_{c-f} was calculated automatically by the program of the manufacturer after entering 80% of the direct straight carotid-to-femoral distance (18).

Statistical analyses

Results are presented as means $\pm$ SDs unless otherwise indicated. Before the start of the study, it was calculated that the statistical power to detect a true difference of ≥ 1.2 m/s in PWV_{c-f} with 25 participants/treatment group was 80%. For these power calculations, an α of 0.05 and a within-subject variability of 1.5 m/s in PWV_{c-f} that we found in a previous trial were used (19). Differences in baseline values between both treatment groups were tested with an unpaired Student's t test. A 1-factor ANCOVA, with use of the baseline measurements of the outcome variables as covariates, was conducted to evaluate differences in responses between magnesium and placebo treatments. A $P < 0.05$ was considered statistically significant. Analyses were performed with SPSS 23.0 for Mac OS X (SPSS Inc.).

RESULTS

Study participants

A Consolidated Standards of Reporting Trials flow diagram of participants throughout the study is shown in **Supplemental Figure 1**. After screening, 52 subjects were eligible for participation and started the trial. One male subject from the magnesium group dropped out in week 6 because of personal reasons. A total of 51 individuals (29 men and 22 postmenopausal women) completed the trial. FFQ data at the start of the study were missing for 1 woman from the placebo group. Baseline characteristics of the adults who completed the study are shown in **Table 1**. Age of the study participants was 62 ± 6 y, and their BMI was 29.6 ± 2.8.

Energy and nutrient intakes, as estimated with FFQs, did not change during the study (**Supplemental Table 1**). BMI (29.3 ± 2.6 compared with 29.9 ± 3.1; $P = 0.40$) and fat distribution (waist-to-hip circumference ratio; 0.95 ± 0.07 compared with 0.96 ± 0.09; $P = 0.59$) were comparable at baseline between the magnesium and placebo groups (Table 1). At the end of the study, these values were respectively 29.4 ± 2.9 and 0.95 ± 0.07 compared with 30.2 ± 3.4 and 0.97 ± 0.08 for the magnesium and placebo groups. Changes were not different between the 2 groups. No effects on hematologic variables were observed.

As evidenced from capsule count, overall compliance ranged between 86% and 102% and was on average 99% and 98% for the magnesium and placebo groups, respectively. No serious adverse events were reported in individuals' diaries. Only 1 woman from the magnesium group reported mild headache and mild gastrointestinal complaints for 7 d during week 11 of the study.

Vascular function markers and blood pressure

After 12 wk of supplementation, changes in PWV_{c-f} were not statistically different between the 2 treatment groups (**Table 2**). At the end of the study, PWV_{c-f} was significantly improved in the oral magnesium supplementation group compared with the placebo group by 1.0 m/s (95% CI: 0.4, 1.6 m/s; $P = 0.001$). No effects were observed on CAIxHR75.

The effects of dietary magnesium supplementation on blood pressure are presented in Table 2 and **Supplemental Table 2**. Office SBP, DBP, pulse pressure, and heart rate did not change after 12 wk and 24 wk of supplementation. There were also no significant effects on mean 24-h, mean daytime, and mean

TABLE 1
Baseline characteristics of the overweight and slightly obese adults who completed the study[1]

	Magnesium group (n = 26)	Placebo group (n = 25)
Men/women, n	14/12	15/10
Age, y	62 ± 5[2]	62 ± 6
BMI, kg/m²	29.3 ± 2.6	29.9 ± 3.1
Waist circumference, cm	100 ± 9	104 ± 11
Waist-to-hip circumference ratio	0.95 ± 0.07	0.96 ± 0.09
Total cholesterol, mmol/L	6.08 ± 0.88	5.70 ± 0.81
TAG, mmol/L	1.34 ± 0.63	1.37 ± 0.50
Glucose, mmol/L	5.53 ± 0.52	5.49 ± 0.60
Serum creatinine, μmol/L	80.1 ± 11.3	82.1 ± 13.3
Brachial SBP, mm Hg	130 ± 15	126 ± 14
Brachial DBP, mm Hg	82 ± 8	81 ± 7
HR, beats/min	58 ± 7	61 ± 6

[1]DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure; TAG, triacylglycerol.

[2]Mean ± SD (all such values).

nighttime ambulatory blood pressure levels. Variabilities and nocturnal reductions in SBP and DBP did not differ between the 2 treatment groups.

Serum and urinary minerals

Serum magnesium concentrations did not differ at 12 wk but tended to increase after oral magnesium supplementation compared with the placebo treatment by 0.02 mmol/L (95% CI: 0.00, 0.04 mmol/L; $P = 0.09$) at the end of the study. Magnesium intake resulted in increased serum potassium concentrations of 0.24 mmol/L (95% CI: 0.07, 0.42 mmol/L; $P = 0.007$) at 12 wk, whereas no significant differences were observed compared with placebo after 24 wk. No effects on serum calcium and sodium concentrations were found (**Table 3**). Serum mineral concentrations were within normal range at baseline, at week 12, and at the end of the trial (20).

The effects of 24-wk magnesium citrate supplementation on urinary variables are shown in Table 3. Twenty-four-hour urinary magnesium excretion increased by 2.01 mmol (95% CI: 1.22, 2.93 mmol; $P < 0.001$). In fact, all individuals from the magnesium group had increased 24-h urinary magnesium concentrations at the end of the trial. No differences in other urinary minerals were observed.

DISCUSSION

In this RCT with overweight and slightly obese men and postmenopausal women, we found a decrease in PWV_{c-f} after 24 wk of daily supplementation with 350 mg Mg, indicating an improvement in arterial stiffness. The PWV_{c-f}, which is considered the current gold-standard method for measuring arterial stiffness (10), was reduced by 1.0 m/s. Results of longitudinal epidemiologic studies have estimated that the risk of cardiovascular events decreases by 14% when PWV_{c-f} improves by 1.0 m/s (21), underlining the potential clinical relevance of these findings.

To our knowledge, this is the first human intervention trial to demonstrate an improvement in arterial stiffness after magnesium supplementation. Effects of magnesium supplementation (368 mg/d) on arterial stiffness have been examined in only one other intervention study, but no effects were reported (8). However, arterial stiffness was estimated by a method that is not considered the gold standard (22) and correlated poorly with PWV_{c-f} (23). Moreover, the number of participants was limited, and the trial lasted only 8 wk (8). In fact, we also did not observe an improvement in PWV_{c-f} at week 12 of the trial, whereas PWV_{c-f} was improved after 24 wk of magnesium supplementation.

Blood pressure is a major determinant of arterial stiffness. In fact, a systematic review of observational studies showed that blood pressure was an independent predictor of the PWV response in 90% of the published studies involving both outcomes (24). In contrast, we found an improved PWV_{c-f} without any change in blood pressure levels or variability. A lack of effect on

TABLE 2
Vascular function and blood pressure measurements at baseline and after a 12-wk and 24-wk magnesium or placebo treatment in a randomized controlled trial with overweight and slightly obese adults[1]

	Magnesium group (n = 26)			Placebo group (n = 25)			Treatment effect	
	Baseline	12 wk	24 wk	Baseline	12 wk	24 wk	Δ 12 wk	Δ 24 wk
Vascular function								
PWV_{c-f}, m/s	8.8 ± 1.4[2]	8.7 ± 1.5	8.3 ± 1.5	8.6 ± 1.2	8.6 ± 1.3	9.1 ± 1.3	0.0 (−0.6, 0.5)[3]	−1.0 (−1.6, −0.4)*
CAIxHR75, %	24 ± 9	25 ± 8	24 ± 8	23 ± 11	24 ± 10	22 ± 11	0.2 (−2.2, 2.6)	0.2 (−2.5, 2.8)
Office BP								
Brachial SBP, mm Hg	130 ± 15	127 ± 15	126 ± 14	126 ± 14	124 ± 12	123 ± 12	−1 (−4, 3)	0 (−4, 4)
Brachial DBP, mm Hg	82 ± 8	80 ± 9	79 ± 8	81 ± 7	80 ± 7	79 ± 7	0 (−2, 3)	0 (−3, 3)
PP, mm Hg	48 ± 11	46 ± 9	48 ± 8	45 ± 9	44 ± 7	45 ± 8	−1 (−3, 2)	1 (−2, 3)
HR, beats/min	58 ± 7	58 ± 9	57 ± 6	61 ± 6	61 ± 5	60 ± 6	−1 (−3, 2)	−1 (−3, 1)
Ambulatory BP								
24-h SBP, mm Hg	124 ± 11	NA	123 ± 11	125 ± 12	NA	125 ± 13	NA	−1 (−5, 4)
24-h DBP, mm Hg	78 ± 8	NA	78 ± 9	80 ± 8	NA	79 ± 9	NA	0 (−3, 3)
24-h PP, mm Hg	46 ± 6	NA	46 ± 6	46 ± 9	NA	46 ± 8	NA	0 (−3, 3)
24-h HR, beats/min	67 ± 8	NA	67 ± 8	70 ± 6	NA	69 ± 7	NA	1 (−3, 4)

[1]Treatment effect: *$P < 0.01$. BP, blood pressure; CAIxHR75, central augmentation index adjusted for heart rate; DBP, diastolic blood pressure; HR, heart rate; NA, not available; PP, pulse pressure; PWV_{c-f}, carotid-to-femoral pulse wave velocity; SBP, systolic blood pressure.

[2]Mean ± SD (all such values).

[3]Mean change with 95% CI in parentheses obtained from a 1-factor ANCOVA with baseline value as covariate.

TABLE 3
Serum and urinary minerals at baseline and after a 12-wk and 24-wk magnesium or placebo treatment in a randomized controlled trial with overweight and slightly obese adults[1]

	Magnesium group (n = 26)			Placebo group (n = 25)			Treatment effect	
	Baseline	12 wk	24 wk	Baseline	12 wk	24 wk	Δ 12 wk	Δ 24 wk
Serum minerals, mmol/L								
Magnesium	0.84 ± 0.05[2]	0.87 ± 0.05	0.86 ± 0.04	0.85 ± 0.05	0.86 ± 0.04	0.85 ± 0.05	0.01 (−0.01, 0.04)[3]	0.02 (0.00, 0.04)*
Potassium	4.49 ± 0.29	4.69 ± 0.29	4.58 ± 0.28	4.47 ± 0.38	4.44 ± 0.39	4.47 ± 0.30	0.24 (0.07, 0.42)**	0.10 (−0.04, 0.23)
Calcium	2.35 ± 0.07	2.36 ± 0.09	2.36 ± 0.06	2.35 ± 0.09	2.34 ± 0.06	2.35 ± 0.08	−0.01 (−0.02, 0.05)	0.00 (−0.03, 0.04)
Sodium	140.5 ± 2.3	140.9 ± 1.9	140.8 ± 2.1	140.8 ± 2.2	141.2 ± 1.6	140.7 ± 1.9	−0.1 (−1.0, 0.7)	0.2 (−0.8, 1.3)
Urinary minerals, mmol/24 h								
Magnesium	4.67 ± 1.15	NA	6.55 ± 1.15	4.32 ± 1.44	NA	4.28 ± 2.17	NA	2.01 (1.22, 2.93)***
Potassium	79.1 ± 25.4	NA	79.9 ± 21.5	83.6 ± 27.3	NA	80.3 ± 21.9	NA	3.1 (−7.7, 13.9)
Calcium	4.38 ± 1.68	NA	4.58 ± 2.27	4.07 ± 2.81	NA	3.95 ± 2.93	NA	0.45 (−0.65, 1.55)
Sodium	150.4 ± 46.1	NA	149.2 ± 69.3	160.2 ± 55.7	NA	158.8 ± 57.4	NA	−5.1 (−37.4, 27.1)

[1] Treatment effect: *P < 0.10, **P < 0.01, ***P < 0.001. NA, not available.
[2] Mean ± SD (all such values).
[3] Mean change with 95% CI in parentheses obtained from a 1-factor ANCOVA with baseline value as covariate.

blood pressure is in agreement with the findings of a meta-analysis of 20 RCTs that involved a total of 1220 adult participants. In that meta-analysis, no overall effect of magnesium supplementation on SBP and DBP was reported (3). However, significant heterogeneity between included studies was found, and subgroup analyses suggested a beneficial effect of magnesium supplementation on blood pressure at total daily doses >370 mg, which is slightly above the daily supplement of 350 mg we have used (25). In contrast to the PWV$_{c-f}$, we observed no effects on CAIxHR75. The CAIxHR75 is a noninvasive measurement of the arterial pressure waveform that depends on the tone of peripheral resistance arteries, whereas PWV$_{c-f}$ is a direct indicator of arterial stiffness depending on the longer-term structural remodeling of large elastic arteries (10). Previous trials showed that vascular stiffness and wave reflection indexes do not necessarily change in parallel (26, 27). Therefore, it is possible that long-term magnesium supplementation has an impact on the aorta but not on peripheral muscular arteries. Also, other longer-term dietary interventions have been shown to improve markers of arterial stiffness without any apparent effects on brachial blood pressure levels (28), possibly through improvements in structural characteristics of large elastic arterial walls (10). In fact, compared with muscular arteries, a much more pronounced age-related decline in arterial wall properties of the aorta has been found, allowing for improvement of this large elastic artery by interventions. These differences are probably related to differences in elastin-collagen smooth-muscle proportions (29).

Although we did not address underlying mechanisms, magnesium may improve arterial structure by blocking the deposition of calcium in the human arterial wall (30, 31). Serum and urinary calcium concentrations did not differ after magnesium supplementation, whereas serum potassium concentrations were higher after 12 wk. Other studies have reported that magnesium supplementation for 4 wk (32) and 6 wk (33) also increased serum potassium concentrations. However, we observed no effects on serum potassium concentrations after 24 wk of oral magnesium supplementation, suggesting that it takes a few months for potassium concentrations to reach a new steady state. Also, serum concentrations of sodium did not change significantly. Therefore, it is not likely that the observed effects on PWV$_{c-f}$ are due to homeostatic changes in these minerals. Alternative possible mechanisms to explain the beneficial effects of magnesium may relate to the postulated actions of magnesium on vascular tone, endothelial function, inflammation, and oxidative stress (34).

Intervention studies have shown that oral magnesium bioavailability varies between different formulations (15). Magnesium citrate supplementation in our study tended to increase serum magnesium concentrations by 0.02 mmol/L, whereas 24-h urinary magnesium excretion increased by 2.01 mmol. These findings are in agreement with those of a recent meta-analysis of magnesium supplementation studies. Increases of 0.05 mmol/L (95% CI: 0.02, 0.07 mmol/L) in serum and of 1.52 mmol (95% CI: 1.20, 1.83 mmol) in urinary magnesium were observed (35), when total daily doses of magnesium varied between 197 and 884 mg (median: 360 mg). Increases in serum and urinary magnesium concentrations were more pronounced at higher intakes and longer study duration. Because 2 mmol is equivalent to 49 mg Mg, the observed urinary increment appears to be rather low. This may be attributable to a lower rate of gastrointestinal absorption than the 30%, which is

usually assumed for magnesium absorption from the gut (36, 37), possibly because of the relatively high magnesium intake in our trial (38). All subjects who received the magnesium capsules had higher urinary magnesium concentrations at the end of the study. This indicates that the compliance was excellent, as also evidenced from capsule count. Urinary magnesium excretion did not increase in the previous RCT that found no effect of magnesium supplementation on arterial stiffness (8). In that study, magnesium pidolate was administered to healthy young men, aged 23–33 y, with a family history of the metabolic syndrome. These results could not relate to total daily magnesium dose, because they also used ∼350 mg as we did. Thus, except for subject characteristics and study duration, the magnesium formulation used may also at least partly explain the lack of effects in that study. We deliberately chose to supply magnesium citrate because this type of supplement has a higher bioavailability than other formulations (15).

In conclusion, our data indicate that an oral magnesium intervention for 24 wk in overweight and slightly obese adults results in a clinically relevant reduction in arterial stiffness, suggesting a potential mechanism by which an increased magnesium intake beneficially affects cardiovascular health outcomes.

REFERENCES

1. Del Gobbo LC, Imamura F, Wu JH, de Oliveira Otto MC, Chiuve SE, Mozaffarian D. Circulating and dietary magnesium and risk of cardiovascular disease: a systematic review and meta-analysis of prospective studies. Am J Clin Nutr 2013;98:160–73.
2. Joosten MM, Gansevoort RT, Mukamal KJ, van der Harst P, Geleijnse JM, Feskens EJ, Navis G, Bakker SJ, Group PS. Urinary and plasma magnesium and risk of ischemic heart disease. Am J Clin Nutr 2013; 97:1299–306.
3. Jee SH, Miller ER 3rd, Guallar E, Singh VK, Appel LJ, Klag MJ. The effect of magnesium supplementation on blood pressure: a meta-analysis of randomized clinical trials. Am J Hypertens 2002; 15:691–6.
4. Guerrero-Romero F, Tamez-Perez HE, Gonzalez-Gonzalez G, Salinas-Martinez AM, Montes-Villarreal J, Trevino-Ortiz JH, Rodriguez-Moran M. Oral magnesium supplementation improves insulin sensitivity in non-diabetic subjects with insulin resistance: a double-blind placebo-controlled randomized trial. Diabetes Metab 2004;30:253–8.
5. Paolisso G, Sgambato S, Gambardella A, Pizza G, Tesauro P, Varricchio M, D'Onofrio F. Daily magnesium supplements improve glucose handling in elderly subjects. Am J Clin Nutr 1992;55:1161–7.
6. Marken PA, Weart CW, Carson DS, Gums JG, Lopes-Virella MF. Effects of magnesium oxide on the lipid profile of healthy volunteers. Atherosclerosis 1989;77:37–42.
7. Chacko SA, Sul J, Song Y, Li X, LeBlanc J, You Y, Butch A, Liu S. Magnesium supplementation, metabolic and inflammatory markers, and global genomic and proteomic profiling: a randomized, double-blind, controlled, crossover trial in overweight individuals. Am J Clin Nutr 2011;93:463–73.
8. Cosaro E, Bonafini S, Montagnana M, Danese E, Trettene MS, Minuz P, Delva P, Fava C. Effects of magnesium supplements on blood pressure, endothelial function and metabolic parameters in healthy young men with a family history of metabolic syndrome. Nutr Metab Cardiovasc Dis 2014;24:1213–20.
9. Cohn JN, Quyyumi AA, Hollenberg NK, Jamerson KA. Surrogate markers for cardiovascular disease: functional markers. Circulation 2004;109:IV31–46.
10. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson I, Struijker-Boudier H, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J 2006;27: 2588–605.
11. Ben-Shlomo Y, Spears M, Boustred C, May M, Anderson SG, Benjamin EJ, Boutouyrie P, Cameron J, Chen CH, Cruickshank JK, et al. Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. J Am Coll Cardiol 2014; 63:636–46.
12. Wildman RP, Mackey RH, Bostom A, Thompson T, Sutton-Tyrrell K. Measures of obesity are associated with vascular stiffness in young and older adults. Hypertension 2003;42:468–73.
13. Nederlandse Huisarten Genootschap. Multidisciplinaire richtlijn cardiovasculair risicomanagement, Herziening 2011. Houten (Netherlands): Bohn Stafleu van Loghum; 2011.
14. Food and Nutrition Board, Institute of Medicine. Magnesium. Dietary Reference Intakes: calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington (DC): National Academies Press; 1997. p. 190–249.
15. Walker AF, Marakis G, Christie S, Byng M. Mg citrate found more bioavailable than other Mg preparations in a randomised, double-blind study. Magnes Res 2003;16:183–91.
16. Muxfeldt ES, Cardoso CR, Salles GF. Prognostic value of nocturnal blood pressure reduction in resistant hypertension. Arch Intern Med 2009;169:874–80.
17. Nederlands Voedingsstoffenbestand (NEVO). Dutch food consumption database. Bilthoven (Netherlands): National Institute for Public Health and the Environment; 2013.
18. Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank J, De Backer T, Filipovsky J, Huybrechts S, Mattace-Raso F, Protogerou A, et al. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. J Hypertens 2012;30:445–8.
19. Joris PJ, Mensink RP. Beetroot juice improves in overweight and slightly obese men postprandial endothelial function after consumption of a mixed meal. Atherosclerosis 2013;231:78–83.
20. Heil W, Ehrhardt V. Reference ranges for adults and children: preanalytical considerations. 9th ed. Mannheim (Germany): Roche Diagnostics; 2008.
21. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol 2010;55:1318–27.
22. Gunarathne A, Patel JV, Hughes EA, Lip GY. Measurement of stiffness index by digital volume pulse analysis technique: clinical utility in cardiovascular disease risk stratification. Am J Hypertens 2008;21: 866–72.
23. Salvi P, Magnani E, Valbusa F, Agnoletti D, Alecu C, Joly L, Benetos A. Comparative study of methodologies for pulse wave velocity estimation. J Hum Hypertens 2008;22:669–77.
24. Cecelja M, Chowienczyk P. Dissociation of aortic pulse wave velocity with risk factors for cardiovascular disease other than hypertension: a systematic review. Hypertension 2009;54:1328–36.
25. Kass L, Weekes J, Carpenter L. Effect of magnesium supplementation on blood pressure: a meta-analysis. Eur J Clin Nutr 2012;66:411–8.
26. Vlachopoulos C, Dima I, Aznaouridis K, Vasiliadou C, Ioakeimidis N, Aggeli C, Toutouza M, Stefanadis C. Acute systemic inflammation increases arterial stiffness and decreases wave reflections in healthy individuals. Circulation 2005;112:2193–200.
27. Kelly RP, Millasseau SC, Ritter JM, Chowienczyk PJ. Vasoactive drugs influence aortic augmentation index independently of pulse-wave velocity in healthy men. Hypertension 2001;37:1429–33.
28. Pase MP, Grima NA, Sarris J. The effects of dietary and nutrient interventions on arterial stiffness: a systematic review. Am J Clin Nutr 2011;93:446–54.
29. van der Heijden-Spek JJ, Staessen JA, Fagard RH, Hoeks AP, Boudier HA, van Bortel LM. Effect of age on brachial artery wall properties differs from the aorta and is gender dependent: a population study. Hypertension 2000;35:637–42.
30. Iseri LT, French JH. Magnesium: nature's physiologic calcium blocker. Am Heart J 1984;108:188–93.

31. Shechter M, Kaplinsky E, Rabinowitz B. The rationale of magnesium supplementation in acute myocardial infarction: a review of the literature. Arch Intern Med 1992;152:2189–96.

32. Cohen N, Alon I, Almoznino-Sarafian D, Zaidenstein R, Weissgarten J, Gorelik O, Berman S, Modai D, Golik A. Metabolic and clinical effects of oral magnesium supplementation in furosemide-treated patients with severe congestive heart failure. Clin Cardiol 2000;23: 433–6.

33. Bashir Y, Sneddon JF, Staunton HA, Haywood GA, Simpson IA, McKenna WJ, Camm AJ. Effects of long-term oral magnesium chloride replacement in congestive heart failure secondary to coronary artery disease. Am J Cardiol 1993;72:1156–62.

34. Cunha AR, Umbelino B, Correia ML, Neves MF. Magnesium and vascular changes in hypertension. Int J Hypertens 2012;2012: 754250.

35. Zhang X, Del Gobbo LC, Hruby A, Rosanoff A, He K, Dai Q, Costello RB, Zhang W, Song Y. The circulating concentration and 24-h urine excretion of magnesium dose- and time-dependently respond to oral magnesium supplementation in a meta-analysis of randomized controlled trials. J Nutr 2016;146:595–602.

36. Joosten MM, Gansevoort RT, Mukamal KJ, Kootstra-Ros JE, Feskens EJ, Geleijnse JM, Navis G, Bakker SJ, Group PS. Urinary magnesium excretion and risk of hypertension: the prevention of renal and vascular end-stage disease study. Hypertension 2013;61:1161–7.

37. Bohn T, Walczyk T, Davidsson L, Pritzkow W, Klingbeil P, Vogl J, Hurrell RF. Comparison of urinary monitoring, faecal monitoring and erythrocyte analysis of stable isotope labels to determine magnesium absorption in human subjects. Br J Nutr 2004;91:113–20.

38. Bohn T. Dietary factors influencing magnesium absorption in humans. Curr Nutr Food Sci 2008;4:53–72.