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Title: The acute effect of magnesium supplementation on endothelial function: a randomised cross-over pilot study

Catriona Murphy [1,2], Jennifer Byrne [1,2], Jennifer B. Keogh [1], Michelle L. Headland [1], Peter M. Clifton [1]*

[1]Clinical and Health Sciences, University of South Australia. jenniferbyrne12@hotmail.com
[2]Technological University Dublin, Dublin, Ireland.
*Correspondence: peter.clifton@unisa.edu.au

Abstract: Magnesium (Mg) deficiency may be a catalyst in the process of endothelial dysfunction, an early event in the pathogenesis of atherosclerosis. The aim was to determine the acute effect of an oral Mg supplement compared to control on endothelieal function assessed by flow-mediated-dilatation (FMD). Nineteen participants (39 years, body mass index (BMI) 22.9kg/m^2) completed this randomised cross-over study. Blood pressure (BP) and FMD were measured and blood samples taken before participants drank 200ml water with or without an over the counter Mg supplement (450mg and 300mg for men and women). Measurements were repeated at 60 and 120 minutes. There was a statistically significant two-way interaction between treatment and time on serum Mg ($p = .037$). A difference of -0.085mm in FMD was observed 60 minutes post drink in the control group compared to baseline FMD, and no difference was observed in the supplement group compared to baseline. Despite the non-significant interaction between treatment and time on FMD, the difference seen in the control group and the lack of change in the supplement group at 60 minutes post-drink suggests that Mg may attenuate the reduction in FMD post-prandially.

Keywords: magnesium 1; flow mediated dilatation 2; endothelial function

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide with 31% of all global deaths attributed to CVD [1]. Magnesium (Mg) is emerging as a nutrient of importance in cardiovascular health [2-5]. There is evidence that fruit and vegetable intake is inversely associated with CVD risk [6]. Several nutrients may contribute to this beneficial effect, one of which is Mg [3].

The main underlying cause of CVD is atherosclerosis with endothelial dysfunction as an early event in its pathogenesis [7,8]. Flow mediated dilatation (FMD), an ultrasound measurement of the brachial artery, is a non-invasive technique for measurement of endothelial function and is a useful surrogate measure of the endothelial function in coronary arteries [9,10]. Higher dietary Mg intake and serum Mg concentrations have been inversely associated with endothelial dysfunction and markers of inflammation [11-13]. A recent meta-analysis suggests that chronic Mg supplementation over 6 months may improve endothelial function in overweight or older individuals [16]. However few studies have investigated the effects of an acute dose of Mg on FMD.

The aim of this study was to investigate the acute effect of Mg supplementation on post-prandial FMD in a healthy population to determine its effect on endothelial function associated in the post-prandial state.

2. Materials and Methods

Participants aged 18-75 years, BMI 18 – 35kg/m2 and SBP <140mm, DBP <90mm, were recruited via public advertisement using flyers and social media posts. They were screened for eligibility prior to attending their first visit. Participants were weight stable in the preceding 6 months, and not currently taking cholesterol or antihypertensive medication, non-steroidal anti-inflammatory medications, drugs affecting endothelial function or folate supplementation.

At a screening visit in which participants eligibility for the trial was confirmed, body height and weight measured, blood pressure (BP) measured, and instructions on how to complete a 3-day food diary and 24hr urine collection given. In two subsequent visits measurements of BP & flow mediated dilatation (FMD) were taken and a blood sample collected at time point 0 before consuming a 200ml drink with or without a Mg supplement in a randomized manner.

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These measurements were collected again at 60- and 120- minutes after drink consumption. Supplementation was over the counter Mg citrate supplements (Swisse Wellness, Australia) either 3 x 150mg for men or 2 x 150mg for women. The dose was equivalent to 300mg/day for women and 450mg/day for men, with the aim of meeting the Australian recommended daily intake (RDI) of 400-420mg/day and 310-320mg/day for men and women respectively [14].

This trial was approved by the University of South Australia's Human Research Ethics Committee (approval number: 0000035834) and is registered with the Australian Nez Zealand Clinical Trials Registry (ANZCTR; number 12617000160336). All participants gave written informed consent. An honorarium of $150 was offered to participants once they had completed all study visits.

An online generated random number allocation sequence was used to randomize the order of the visits (www.randomization.com). Randomisation was performed by a research team member that was not completing the FMD measurements and the code was stored on a password protected computer.

At the screening visit, participants body height was measured to the nearest 0.01cm (SECA Hamburg, Germany) whilst barefoot. Body weight was also measured via electronic scales to the nearest 0.05kg (SECA Hamburg, Germany) at each study visit. Participants wore light clothing and wore no footwear.

Participants were asked to complete a 3-day weighed food dairy (one weekend day and two weekdays) prior to their first visit to determine their habitual dietary intakes of Mg. Analysis of the food diaries was conducted via Foodworks Profession Edition 2007 (version 5; Foodworks Professional Edition; Xyris Software, Highgate Hill, Australia). They also collected a 24hr urine sample prior to their first visit. A total weight of the 24hr urine sample was recorded, with a 20-30ml aliquot sent to an accredited laboratory (SA Pathology, Adelaide, South Australia) for analysis of urinary Mg and creatinine concentration.

Blood pressure was measured using an automated sphygmomanometer (SureSigns V3; Philips, North Ryde, Australia) with participants seated. A series of 4 consistent measures were obtained within range of 10 mm with the first reading discarded. The average of the remaining measurements was calculated. Measurements were taken at time point 0, and then at 60 minutes and 120 minutes following the drink at visit 1 and 2.

Flow mediated dilatation (FMD) measurements were performed by a single trained operator (PMC), in a quiet temperature-controlled room. All participants were fasting. Endothelium-dependent FMD of the right brachial artery was measured in the longitudinal plane above the antecubital fossa, with an 8.8-MHz linear array transducer (GE Logiq 5), as previously published [15,16]. The brachial artery diameter was measured before and after forearm ischemia caused by inflation of a sphygmomanometer cuff applied to the right forearm 2cm below the olecranon process, to 200mmHg for 5 minutes. The operator was blinded to participant condition and measures were recorded at time point 0, as well as 60- and 120- minutes post drink ± supplementation consumption. All recorded images were stored offline for analysis at a later point and encoded to ensure blind analysis.

Ultrasound images were recorded at a rate of 30frames/s with screen capture software (Debut Video Capture Software Professional V1.82; NCH Software), and analysed with edge-detection software (Brachial Analyser for Research V6.1.3; Medical Imaging application LLC) to determine artery diameter (mm) values for both baseline and deflation. Baseline was defined as the average 15-s pre inflation, and peak diameter as the maximum diameter post-cuff release. The absolute change in artery diameter (mm) was then calculated as the difference between the two.

Blood samples were collected via cannular insertion into the left brachial vein at each study visit. Samples were collected at baseline, and then 60 and 120 minutes following the drink ± supplement for analysis of Mg. Two x 8ml serum separator clot activator tubes were collected and left to clot for 30min at room temperature. One tube was then centrifuged at 4000RPM for 10 minutes (Universal 32R; Hettich Zentrifugen) and aliquots of serum stored at -80 degrees Celsius, with the other delivered to SA Pathology, Adelaide, South Australia for analysis.

Based on a previous studies [15,16], 32-35 subjects would be required to detect a mean difference in FMD of 0.04mmg (α = 0.05; 80% power) between treatments. A pilot study was conducted (4 males, 17 females) to determine the power needed for a larger study and to explore the protocol to determine its efficacy and if a larger study was needed.

Data was analysed using IBM SPSS software (version 21; IBM, Chicago IL, USA). Significance was set at $p < 0.05$. A two-way ANOVA with repeated measures (with treatment and time as the within subject factors) was used, with and without covariates including SBP, DBP, MAP, age and serum MG and gender as a between subjects' factor. Spearman Rho correlation analyses was used to assess the association between dietary Mg intake and urinary Mg excretion, and the association between dietary Mg intake and baseline FMD. A backwards linear regression was used to explore the association between FMD, age, and dietary variables including saturated fat, polyunsaturated fat, and dietary Mg intake. All data is expressed as mean ± SD or median ± inter-quartile (IQR) as appropriate.

3. Results

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Twenty-one healthy volunteers were enrolled in the study, and 19 completed. Baseline characteristics are outlined in Table 1. FMD, SBP, DBP and MAP were analysed for all completers, however several serum MG values are missing due to blood samples not successfully being collected. Weight was not different between visits.

Table 1. Baseline Characteristics (n = 21)

	Range	Mean/Median	SD/IQR
Age (years)	19 – 75	39	16
Weight (kg)	45.8 – 100.9	59.2 (median)	14.2 (IQR)
Height (cm)	157 – 180	166.6	6.5
BMI (kg/m^2)	17.9 – 31.9	21.1 (median)	5.6 (IQR)
SBP (mmHg)	92 – 134	113.5	13.8
DBP (mmHg)	65 – 90	76.2	7.2
MAP (mmHg)	75 – 101.7	88.5	8.7
24hr urine Mg excretion (mmol/L)	2.03 – 5.23	3.39	0.86
24hr urine Cr excretion (mmol/L)	3.64 – 20.0	8.82 (median)	3.19 (IQR)
Timepoint 0 brachial artery diameter (mm)	3.01 – 5.20	3.8	0.56
Baseline serum mg (mmol)	0.75 – 0.94	0.85	0.047

Dietary intake

Table 2 outlines the usual dietary intake levels of Mg, saturated fat, and PUFA as recorded by 3 day weighed food records, 53% of participants did not meet the RDI for Mg for their respective age and gender, and 26% did not meet the estimated average requirement (EAR) for Mg for their respective age and gender.

Table 2. Usual dietary intake as determined by 3 day weighed food records

Nutrient	Range	Mean	SD
Mg Intake (n=19) mg/day	138 – 510	339	106.7
Male Mg Intake (n=4)	199 – 429	328	51.0
Female Mg Intake (n=15)	138 – 510	343	97.7
Saturated Fat Intake (n=19) g/day	6.7 – 57.9	27.7	13.7
Male Saturated Fat Intake (n=4)	15.3 – 57.9	33.5	17.8
Female Saturated Fat Intake (n=15)	6.7 – 46.4	26.1	12.7
PUFA Intake (n=19) g/day	1.5 – 24.9	12.5	6.2
Male PUFA Intake (n=4)	5.8 – 17.7	10.1	5.2
Female PUFA Intake (n=15)	1.5 – 24.9	13.2	6.4

SD = standard deviation; Mg = Magnesium; PUFA = polyunsaturated fat

Flow Mediated Dilatation

There was no interaction between treatment and time for absolute FMD (F (2,36) = 1.371, p = 0.27). The main effect of treatment showed a difference in absolute FMD between treatments (F (2,18) = 4.948, p = 0.04), which was due to average time point 0 (pre-supplement) differences in absolute FMD of 0.076mm between the supplement and control group (p = 0.02) (**Figure 1**). When baseline measures were excluded from analysis the time*treatment interaction was no longer significant (F (1,18) = 2.078, p = 0.12). There was no significant difference in absolute FMD between time points and no significant effect of age, gender, or serum Mg (between groups or time points) when used as covariates. At time point 60 a difference of -0.085mm was observed in the control group compared to time point 0, with no change observed in the supplement group across the same time frame.

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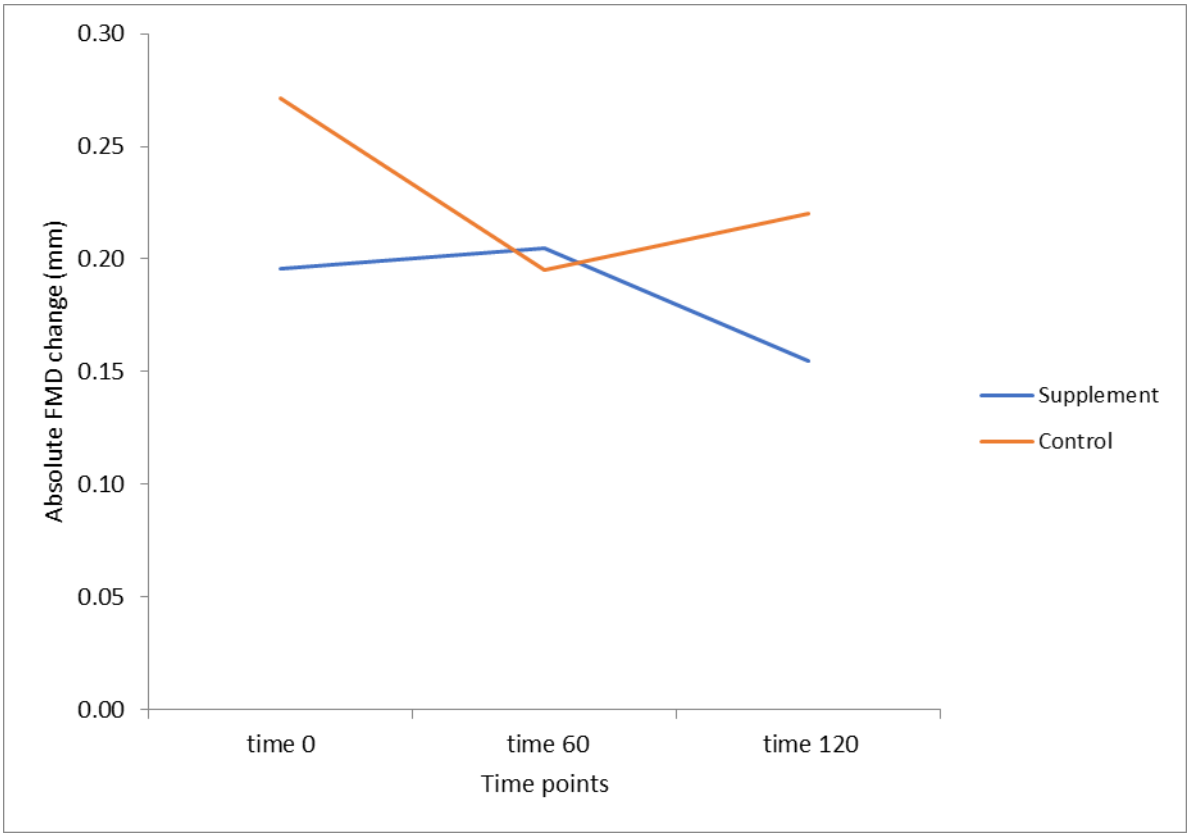
Figure 1. The effect of Treatment*Time Interaction on Absolute FMD



Blood Pressure and Mean Arterial Pressure

There was no interaction between treatment and time on SBP or DBP. No changes were observed between treatments or any time point for either SBP or DBP. Age, gender or differences in serum Mg (between groups or time points) had no impact on SBP or DBP. No interaction between treatment and time was observed for MAP, and furthermore there were no differences in MAP between treatments or time points F (1,18) = 0.509, p = 0.49, F (2,36) = 0.82, p = 0.45 respectively. Age, gender or differences in serum Mg (between groups or time points) had no effect on MAP.

Serum Magnesium

There were no differences in serum Mg concentrations at time 0 or 60 minutes at either of the two visits but was significantly different 120 mins on the intervention visit (mean = 0.090mmol) compared to the control visit (mean = 0.871mmol), p = 0.003. At the intervention visit serum Mg increased from 0.86 ± 0.06mmol pre-intervention, to 0.91 ± 0.06mmol 120 minutes post supplementation (p < 0.001).

Correlations

As there was no significant difference between absolute FMD in the supplement vs control groups over time, an average time point 0 absolute FMD was calculated and used in correlational analysis.

Initial analysis showed a moderate positive correlation between between average dietary Mg intake and 24hr urinary excretion in the cohort (r_s = 0.5, p = 0.029) (Figure 3). There was no significant correlation between average dietary Mg intake and time point 0 absolute FMD (p = 0.435).

Figure 3. Association between Urinary and Dietary Magnesium

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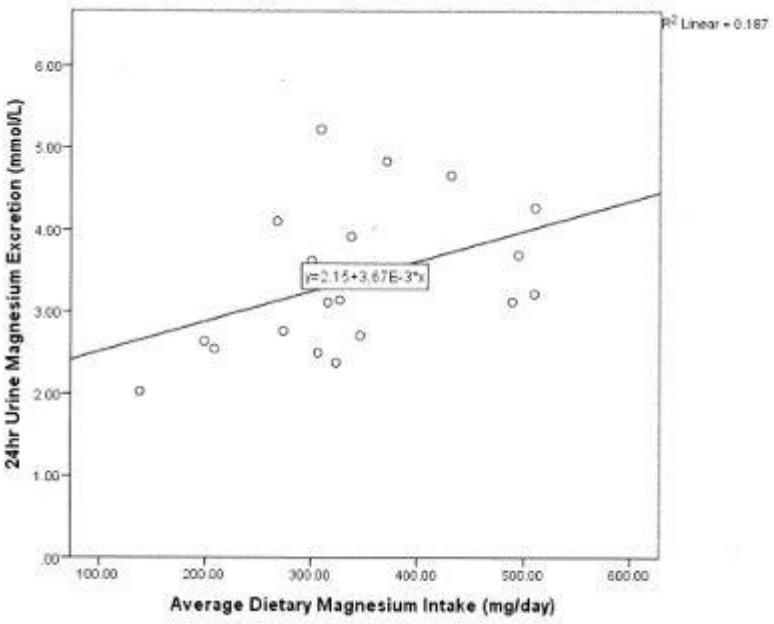

Linear Regression Analysis

Backwards linear regressions were run to predict baseline absolute FMD from age, average dietary Mg, saturated fat and PUFA intake. Age, average dietary Mg, saturated fat, and PUFA did not predict baseline FMD. The addition of age as an independent variable in the model explained 25.8% of the variability of time point 0 absolute FMD, the equivalent to an increase in age of 1 year being associated with a decreased in time point 0 absolute FMD of 0.004mm/year.

4. Discussion

Our findings suggest that FMD was not different at one or two hours after a single dose of Mg in water compared to water alone in healthy adults. It is of interest that Mg appears to attenuate the post-prandial reduction in FMD observed after water and warrants speculation that Mg supplementation potentially attenuates the decrease in endothelial function seen in the post-prandial state. When serum Mg was accounted for as a covariate, the effect of the interaction between intervention and time on absolute FMD approached significance further suggesting that there is a potential acute effect of Mg supplementation on FMD over time and that perhaps a larger sample size would clarify this. Sample size calculations showeb that 79 participants woud be needed in a cross-over study desigb to see a significant difference between a Mg supplement (300mg for women or 450 mg for men) and no supplement as used in the present study. The results of this pilot study suggest a potential role for Mg in the attenuation of post-prandial endothelial dysfunction warranting the need for further research in this area.

Water alone has been shown to impair FMD by 15% in healthy participants [17]. Other minerals and nutrients have also been shown to have an effect on FMD. High salt intakes have acute adverse effects on vascular dilatation in the postprandial state [18] and the addition of potassium to a high-sodium meal attenuates the sodium-induced post-meal reduction in endothelial function as assessed by FMD [19]. A decrease in FMD has been observed 1hr after a 75g glucose drink which is restored within 4hrs [20-22]. This effect can be restored by the antioxidant vitamin C alone and in combination with vitamin E [22,23]. There are few other studies examining the post-prandial effects of Mg. However chronic Mg supplementation for greater than 6 months has been shown to have a beneficial effect on

FMD in individuals > 50 years or in overweight participants although overall there was no effect of Mg found in this meta-analysis [24].

There was a significant effect Mg supplementation on serum Mg over time in the present study which has also been observed in long-term supplementation studies [25]. Del Gobbo [26] found that circulating Mg (per 0.2 mmol/L increment) was associated with a 30% lower risk of CVD. In our study and in others in healthy individuals [27,28] or patients on haemodialysis [29], participants had baseline serum Mg concentrations within normal ranges. There is a need for further studies in participants with low serum Mg concentrations to assess the effects on endothelial function as measured by FMD.

The strengths of this study include that the same operator undertook all of the FMD measurements for the study who was blinded to the treatments at the time of measurement. Limitations of the study incude the small sample size and the lack of a placebo.

5. Conclusion

The difference in FMD in the control group and the lack of change in the supplement group at 60 minutes post-drink suggests that Mg attenuates the reduction in FMD post-prandially. Further research is warranted to investigate the potential beneficial effect of MG supplementation on post-prandial endothelial function.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki. Ethics approval was obtained from the University of South Australia Human Research Ethics Committee (Application No 0000035834) and is registered with the Australian Nez Zealand Clinical Trials Registry (ANZCTR; number 12617000160336).

Informed Consent Statement: Signed informed consent was obtained from all subjects involved in the study.

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Conflicts of Interest: The authors declare no conflict of interest

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