Oral magnesium supplementation improves vascular function in elderly diabetic patients

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Abstract. Magnesium (Mg) ions directly influence vascular tone and responsiveness and are cofactors for acetylcholine-induced endothelium-dependent relaxation. Alterations in extracellular Mg are able to modify the formation and release of nitric oxide (NO), altering arterial smooth muscle tone. Previous in vivo studies in humans have shown that parenteral or oral Mg supplementation increase endothelial-dependent vasodilation. The aim of the present study was to evaluate the effects of Mg oral supplementation on endothelial function in elderly diabetic and hypertensive subjects. Sixty elderly (≥ 65 years) diabetic patients were recruited (mean age: 71.1 ± 6.1 years; M/F: 35/25). Endothelial function, evaluated by non-invasive flow-mediated dilatation of the brachial artery, as well as anthropometric and laboratory data, including ionized Mg (Mg-ion), were measured in all patients before and after one-month. Thirty patients underwent oral Mg supplementation with 4.5 g/day of Mg pidolate (368 mg/day of Mg ion), while the rest were used as a control group. The usual management of diabetes and hypertension was not changed during the month of study participation for all the patients. In the group of patients that underwent Mg supplementation, Mg-ion concentration significantly increased from 0.42 ± 0.05 mmol/L to 0.49 ± 0.06 mmol/L; p < 0.05. Mg intervention resulted in a significant improvement of the post-ischemic endothelial-dependent flow-mediated dilation (from 3.3 ± 3.6% to 8.4 ± 3.9%; p < 0.05). No significant differences were found, either in ion-Mg or endothelial function, in the control group. In conclusion, the present study suggests that oral Mg improves endothelial function in diabetic elderly subjects.

Keywords: magnesium, endothelium, diabetes mellitus, hypertension, aging, ionized magnesium, endothelial function

Magnesium (Mg), the second most abundant intracellular cation, is involved in a number of important biochemical reactions, including all ATP transfer reactions.

Mg, although not directly involved in the biochemical process of contraction, directly influences vascular tone, baseline tension and vascular responsiveness to vasoconstrictor agents, both via endothelium independent and endothelium dependent pathways. Mg affects calcium ion concentrations and its availability at critical sites, acting as a physiologic calcium channel blocker [1, 2]. Mg is a cofactor for acetylcholine-induced endothelium-dependent relaxation, and alterations in extracellular Mg are able to modify the formation and release of nitric oxide (NO), altering arterial smooth muscle tone [3].

Endothelial function has been consistently found altered in hypertension, and in type 2 diabetes mellitus [4], and alterations of endothelial function...
have been suggested to represent an early indicator of atherosclerosis, associated with an increased incidence of vascular diseases in these conditions [5, 6].

Data showing the positive effect of Mg supplementation in vivo, in humans, have confirmed the link of Mg metabolism to altered endothelial function. Haenni et al., showed that intra-arterial Mg infusion acutely increases endothelial-dependent vasodilation [7], while Shechter et al., demonstrated that a chronic oral Mg supplementation was able to improve endothelial function in patients with coronary artery disease [8].

The present study was designed to evaluate the effects of Mg oral supplementation on brachial artery endothelial function in elderly diabetic hypertensive subjects.

Subjects and methods

Subjects

Sixty elderly (≥ 65 years) diabetic patients were recruited (mean age: 71.1 ± 6.1 years; M/F:35/25) (table 1). Endothelial function, evaluated by non-invasive flow-mediated dilation of the brachial artery, as well as anthropometric and laboratory data, including ionized Mg (Mg-ion), were measured in all patients before and after one-month. During the one-month follow up, thirty patients underwent oral supplementation with Mg pidolate at a dosage of 2.25 g given twice a day, corresponding to 184 mg of Mg ion per dose or 368 mg/day of Mg ion, while the rest of the patients (not supplemented with Mg) (n = 30), were used as a control group (table 1). Mg pidolate was used, based on previous studies reporting adequate bioavailability of this salt. Organic Mg salts, such as Mg pidolate, have been shown to be more bioavailable than inorganic Mg salts [9]. A recent study reported higher serum, urinary and intracellular Mg content after treatment with oral Mg pidolate in hypertensive patients [10].

The study subjects were recruited from those attending the Outpatient Clinic of the Geriatric Unit, at the University Hospital of Palermo, Italy. All participants signed an informed consent after being fully informed of the details of the study, which was approved by the ethical committee of our Institution, and was conducted in accordance with the guidelines of the Declaration of Helsinki for human research. Exclusion criteria included: non-compensated acute disease such as severe congestive heart failure, severe chronic obstructive pulmonary disease, angina pectoris, acute myocardial infarction or ictus in the previous 6 months of the study, severe uncontrolled hypertension (SBP ≥ 180 mmHg), moderate to severe renal or hepatic disease. All the patients were advised not to change any concomitant pharmacological treatment, diet and exercise habits during the one-month study period. Endothelial function was evaluated by flow-mediated dilation of the brachial artery of dominant arm using high-resolution ultrasonography (see below). Subjects fasted for at least

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mg supplemented group</th>
<th>Control group</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>Baseline</td>
<td>After 1 month</td>
</tr>
<tr>
<td></td>
<td>71.0 ± 4.9</td>
<td>71.2 ± 4.6</td>
</tr>
<tr>
<td>M/F</td>
<td>18/12</td>
<td>17/13</td>
</tr>
<tr>
<td>BMI</td>
<td>27.9 ± 1.3</td>
<td>28.1 ± 1.6</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>150 ± 7</td>
<td>148 ± 5</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>82 ± 5</td>
<td>79 ± 5</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>76 ± 4</td>
<td>75 ± 3</td>
</tr>
<tr>
<td>FPG (mg/dL)</td>
<td>141.6 ± 5.2</td>
<td>139.6 ± 5.4</td>
</tr>
<tr>
<td>Mg-tot (mEq/L)</td>
<td>1.82 ± 0.1</td>
<td>1.85 ± 0.1</td>
</tr>
<tr>
<td>Mg-ion (mmol/L)</td>
<td>0.42 ± 0.05</td>
<td>0.49 ± 0.06^</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>3.3 ± 3.6</td>
<td>8.4 ± 3.9^</td>
</tr>
</tbody>
</table>

To convert mg/dL of glucose in mmol/L multiply by 0.5551. ^ means p < 0.01 vs baseline.

BMI: body mass index; BP: blood pressure; FPG: fasting plasma glucose.
10 hours before the study, which was always conducted in the morning between 8 am and 10 am, and performed in a quiet and temperature-controlled room, after 15 minutes of rest. All subjects did not smoke, drink coffee or exercise for at least 24 hours before the study.

**Blood pressure and anthropometric measurements**

The subjects remained in the sitting position for 10 min with a deflated sphygmomanometer cuff of the appropriate size. Cuff size was determined by measuring the circumference of the arm at half the length of the humerus. Blood pressure was measured manually and recorded to the nearest 2 mmHg. The measurement was repeated twice, and the 3 values were averaged for the final reading. Weight and height were measured by standard techniques. Body mass index (BMI) was calculated as body weight in kilograms divided by height squared in meters.

**Ionized Mg measurements**

Blood samples were analyzed for serum total (Mg-tot) and serum ionized Mg (Mg-ion). Mg-tot levels were measured by standard techniques with an automated chemistry analyzer (ISE 900/ISE 1800 modules of Modular Analytics SWA Roche Diagnostics Italia, Monza, Italy). Blood for Mg-ion was drawn into air-evacuated glass tubes containing an inter-cell separating matrix. After clotting and centrifugation, the tubes were inverted and serum was drawn off into a syringe anaerobically, the latter being capped and stored in a refrigerator freezer (0-4°C) for further analysis. A Mg ion-selective electrode (ISE) with a neutral carrier-based membrane (Nova and Stat Profile 8 Ultra analyzers, Waltham, MA, USA) was used to measure serum Mg-ion [11, 12].

**Vascular function protocol**

Endothelial function in the form of endothelium-dependent brachial artery flow-mediated vasodilation (FMD) was measured as previously described [8, 13, 14], before and after one-month supplementation with 4.5 g/day of Mg pidolate (368 mg/day of Mg ion). Briefly, FMD was assessed at the level of the right upper forearm of the subject in the recumbent position in a temperature-controlled room (22°C) after a 10-minute equilibration period. All the exams were performed by a single operator, who was blinded to treatment assignment. An ultrasound system equipped with vascular software for two-dimensional (2D) imaging, color and spectral Doppler, and a high-frequency vascular transducer, was used (Toshiba Sonolayer SSA 270A with doppler ultrasonography). Image resolution was enhanced with broadband (multiple-frequency: 7 to 12 MHz) linear array transducers. Subject were positioned supine with the right arm in a comfortable position with a sphygmomanometer cuff positioned at the level of the upper forearm; the brachial artery was longitudinally imaged and identified 5 cm proximal to the antecubital crease, where the clearest image was obtained. When a reasonable image was obtained, the surface of the skin was marked, and the arm was kept in the same position throughout the study. A clamp was used to keep the ultrasound probe firmly in the same position and to maintain precisely the same image for continuous monitoring during the entire study. After a 2-minute baseline period, a frozen longitudinal image of 3 cm of vessel without color flow was obtained and frozen for 5 seconds. The image was then unfrozen and switched to pulsed-wave Doppler for 5 seconds at a sweep speed at 50 mm/s. A baseline rest image was acquired and blood flow estimated by time-averaging the pulsed Doppler velocity signal. Pneumatic tourniquet placed around the forearm distal to the target artery was inflated after the baseline phase to a pressure of 50 mm Hg above the subject’s systolic blood pressure (or until no blood flow was detected through the brachial artery with the Doppler probe), and this pressure was held for 3 minutes. Increased flow was then induced with sudden cuff deflation. A continuous scan was performed from 30 seconds to 2 minutes after cuff deflation. Diameter and Doppler-flow velocity were measured at 60 and 90 seconds after cuff deflation, and the maximum between the two measurements was considered. The FMD was expressed as: 

\[
\frac{(\text{diameter}_{\text{max}} - \text{diameter}_{\text{baseline}})}{\text{diameter}_{\text{baseline}}} \times 100
\]

The within-session diameter variability in our study was small (CV = 0.9 ± 0.05). Day-to-day variability of baseline diameter was also small (CV = 4.2 ± 1.5%).

**Statistical analyses**

Continuous data are expressed as mean ± SDs. Differences were assessed by paired t tests and with analysis of variance (ANOVA) for repeated measures. Differences were considered to be statistically significant for probability values lower than 0.05. Statistical analyses were performed using Graph Pad version 4.0 software (Graph Pad Software, San Diego, CA, USA).
Results

Clinical and laboratory data are shown in table 1. In the group of patients who underwent Mg supplementation, serum Mg-ion significantly increased from $0.42 \pm 0.05$ mmol/L to $0.49 \pm 0.06$ mmol/L; $p < 0.05$. No significant differences were found in blood pressure, fasting glucose and Mg-tot (table 1) in either group. No significant differences were found in serum ion-Mg in the control group at baseline and after one-month evaluation ($0.43 \pm 0.06$ mmol/L to $0.42 \pm 0.05$ mmol/L; $p = NS$, table 1, figure 1).

In the group of patients who underwent Mg supplementation for one month, the oral Mg intervention resulted in a significant improvement of the post-ischemic endothelial-dependent flow-mediated dilation (from $3.3 \pm 3.6\%$ to $8.4 \pm 3.9\%$; $p < 0.01$), while no significant differences were found in endothelial function in the control group (from $3.4 \pm 3.9\%$ to $3.5 \pm 3.7\%$; $p = NS$, table 1, figure 2).

The supplementation with oral magnesium was generally well tolerated with no significant side effects being reported (i.e. diarrhea, nausea), at the dosage used in the study. None of the participants stopped the supplementation because of new adverse effects.

Discussion

Our group has focused on the critical role of Mg and calcium metabolism in regulating vascular tone, blood pressure homeostasis, and various other cardiovascular and metabolic processes. We have previously shown that type 2 diabetes mellitus, hypertension, metabolic syndrome and aging are conditions frequently associated with a Mg deficient state and with intra and/or extracellular Mg depletions [1, 15, 16]. Decreased circulating Mg-ion concentrations have previously been observed in subjects with diabetes mellitus and in untreated patients with essential hypertension [1, 17, 18].

The results of the present study demonstrate that an oral Mg supplementation resulted in a significant improvement in brachial artery endothelial function in elderly diabetic patients. Our results are in accordance with previous experimental in vitro and in vivo studies that have linked Mg metabolism to altered endothelial function and have indicated that Mg deficiency impairs vasorelaxation. Low Mg promotes endothelial cell dysfunction [19] and endothelium-dependent relaxation induced by extracellular Mg is mediated by NO release by the endothelium [20]. Alterations in the extracellular Mg
concentration are able to modify the basal formation and release of NO, and to alter arterial smooth muscle tone [21]. Hypomagnesaemia following removal of extracellular Mg impaired the release of NO and reduced vasodilation in response to acetylcholine, and normal endothelium-dependent vasodilation was restored by returning Mg to the bathing solution [22].

Previous animal and human studies have shown that oral and/or parenteral Mg supplementation may improve altered endothelial function. In rats receiving a low Mg diet, with low levels of serum Mg, following endothelial injury by balloon catheter, arteries showed decreased endothelium-dependent relaxing responses to acetylcholine when compared to controls, while animals fed a high Mg diet showed normal endothelium-dependent vascular relaxation that did not differ from their respective controls [23]. In humans, chronic oral Mg supplementation in patients with coronary artery disease has been shown to significantly improve brachial artery endothelial function and exercise tolerance [8].

High-frequency ultrasonographic imaging of the brachial artery has been extensively utilized to assess endothelium-dependent flow-mediated vasodilation. This technique allows the evaluation of NO release-dependent vasodilation, which can then be quantitated as an index of endothelial function. It is a non-invasive technique that permits repeated measurements over time to study the effectiveness of various interventions that may affect the vascular tissue [8, 13, 14]. One limitation of this study is that there was no concomitant assessment of endothelium-independent dilation. In our experimental model, there was no change in the control (not supplemented) group, and the single investigator performing the exams was blinded to the treatment assigned.

Although previous studies have linked Mg metabolism to vascular function, this is the first report, to our knowledge, showing in humans: a) that extracellular serum Mg-ion is related to FMD, and b) that an inexpensive oral Mg supplementation may help in restoring endothelial function in diabetic hypertensive elderly subjects. Total Mg

![Figure 2](image_url)

**Figure 2.** Endothelial function measured as percentage change in brachial artery flow-mediated vasodilation (FMD %) in diabetic elderly subjects at baseline and after one month with (black bars) or without (grey bars) oral Mg supplementation with 4.5 g/day of Mg pidolate (368 mg/day of Mg ion).
serum concentrations do not reflect the Mg status or intracellular pool, and intracellular or serum ionized Mg depletion can be seen with normal Mg-tot concentrations [1, 18, 24]. 31P-NMR spectroscopic technique are at present time the gold standard for intracellular measurements of free Mg content in living tissues in situ, but it remains an expensive research-based tool [1], while for routine clinical use, the development of Mg-specific ion-selective electrodes (ISE) has been particularly useful, allowing to measure extracellular free levels of Mg, with a higher sensitivity than Mg-tot, for detecting subclinical Mg deficits in several clinical conditions, such as diabetes and hypertension [1, 17, 18, 24].

The mechanisms explaining the observed beneficial effects of Mg may be multiple. Numerous experimental and clinical data have suggested that Mg deficiency associated to diabetes can induce elevation of intracellular calcium concentrations, formation of oxygen radicals, pro-inflammatory agents and growth factors [1, 2, 25, 26], and that Mg supplementation as a complementary therapy may help to counter these effects [27]. Higher levels of Mg may improve intracellular ATP production and glucose utilization, because Mg is a cofactor of all ATP transfer reactions [1]. The action of Mg as a natural physiological calcium blocker [2] may also contribute to reduce the release of calcium from and into the sarcoplasmic reticulum and reduce vascular resistance. However, while many of these mechanisms remain controversial and in some cases speculative, the beneficial effects related to the consequences of Mg supplementation are apparent.

Our group has previously reported low Mg-ion levels in diabetes and hypertension [1, 12, 16-18] and also in the present study our elderly diabetic patients had baseline Mg-ion levels below normal (table 1), reflecting a Mg deficient state. The beneficial effects of Mg supplementation may be more evident in individuals with a low Mg balance. Diabet es and aging are conditions frequently associated with both extracellular and intracellular Mg depletion [1, 12, 15, 28]. Among the mechanisms that may favor Mg depletion in these conditions, one of the most important is often a low Mg intake [1, 28]. In this regard, dietary habits in the western world have resulted in a daily Mg intake often below the recommended daily allowances [29]. Furthermore, significant correlations between dietary Mg and the incidence of type 2 diabetes, hypertension and cardiometabolic syndrome have been found [30-33]. Since Mg is an inexpensive, natural, and relatively safe element, its possible role as an adjuvant therapy in those subjects who are at high risk of Mg deficiency should be considered. Nevertheless, further studies, with larger populations, are needed to confirm our findings.

In conclusion, our present results demonstrate that oral Mg supplementation significantly improves brachial artery endothelial function in elderly diabetic hypertensive patients.

Perspectives

The present results encourage the use of oral Mg supplementation in elderly subjects with diabetes and hypertension in which Mg deficiency is a common condition and in whom circulating ionized Mg is frequently low.

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Disclosure

None of the authors has any conflict of interest or financial support to disclose.

References


