Comparison of magnesium status using X-ray dispersion analysis following magnesium oxide and magnesium citrate treatment of healthy subjects*

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Abstract. The magnesium content in food consumed in the Western world is steadily decreasing. Hypomagnesemia is associated with increased incidence of diabetes mellitus, metabolic syndrome, all-cause and coronary artery disease mortality. We investigated the impact of supplemental oral magnesium citrate *versus* magnesium oxide on intracellular magnesium levels ($[Mg^{2+}]_i$) and platelet function in healthy subjects with no apparent heart disease. In a randomized, prospective, double-blind, crossover study, 41 (20 women) healthy volunteers [mean age 53 ± 8 (range 31-75) years] received either magnesium oxide monohydrate tablets (520 mg/day of elemental magnesium) or magnesium citrate tablets (295.8 mg/day of elemental magnesium) for one month (phase 1), followed by a four-week wash-out period, and then crossover treatment for one month (phase 2). $[Mg^{2+}]_i$ was assessed from sublingual cells through x-ray dispersion (normal values 37.9 ± 4.0 mEq/L), serum magnesium levels, platelet aggregation, and quality-of-life questionnaires were assessed before and after each phase. Oral magnesium oxide, rather than magnesium citrate, significantly increased $[Mg^{2+}]_i$ (34.4 ± 3 *versus* 36.3 ± 2 mEq/L, $p<0.001$ and 34.7 ± 2 *versus* 35.4 ± 2 mEq/L, p=0.097; respectively), reduced total cholesterol (201 ± 37 *versus* 186 ± 27 mg/dL, p=0.016 and 187 ± 28 *versus* 187 ± 25 mg/dL, p=0.978; respectively) and low-density lipoprotein (LDL) cholesterol (128 ± 22 *versus* 120 ± 25 mg/dL, p=0.042 and 120 ± 23 *versus* 121 ± 22 mg/dL, p=0.622; respectively). Noteworthy is that both treatments significantly reduced epinephrine-induced platelet aggregation ($78.9 \pm 16\%$ *versus* $71.7 \pm 23\%$, p=0.013 and $81.3 \pm 15\%$ *versus* $73.3 \pm 23\%$, p=0.036; respectively). Thus, oral magnesium oxide treatment significantly improved $[Mg^{2+}]_i$, total and LDL cholesterol compared with magnesium citrate, while both treatments similarly inhibited platelet aggregation in healthy subjects with no apparent heart disease.

Key words: magnesium, nutrition, endothelium, heart disease, hypertension, platelets

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The magnesium content in food consumed in the Western world is steadily decreasing [1, 2]. Hypomagnesemia is common in hospitalized patients [3], especially in the elderly with coronary artery disease (CAD) and/or those with chronic heart failure [4, 5]. Hypomagnesemia is associated with an increased incidence of diabetes mellitus [6], metabolic syndrome [7], all-cause and CAD mortality [8-11].

Magnesium supplementation improves myocardial metabolism, inhibits calcium accumulation and myocardial cell death [12], improves vascular tone [13], peripheral vascular resistance [14], afterload and cardiac output [15], reduces cardiac arrhythmias [16] and improves lipid metabolism [17]. Magnesium also reduces vulnerability to oxygen-derived free radicals [12], improves human endothelial function [18] and inhibits platelet function [19], including platelet aggregation and adhesion [1, 20].

Two, common, oral magnesium supplements in clinical practice are magnesium oxide (an inorganic magnesium salt) and citrate (an organic magnesium salt); however, data regarding the differences in their absorption and efficacy in humans are sparse. Therefore, the aim of the current study was to investigate the impact of supplemental oral magnesium citrate *versus* magnesium oxide on intracellular magnesium levels ($[Mg^{2+}]_i$) and platelet function in healthy subjects with no apparent heart disease.

Material and methods

Study design and population

In a randomized, prospective, double-blind, crossover study, 41 healthy volunteers, without apparent heart disease, were recruited from the Leviev Heart Center Outpatient Clinic of the Sheba Medical Center. All subjects were referred either by primary care physicians or through direct patient request for risk factor evaluation via a primary prevention clinic. No patient was referred because of chest pain. All subjects underwent a full consultation with a cardiologist who performed primary prevention risk management according to the updated National Cholesterol Education Program ATP III and American Heart Association/American College of Cardiology/European Society of Cardiology guidelines. Included in the study were healthy subjects (*i.e.* no history of any chest pain or angina pectoris, myocardial infarction, coronary artery bypass grafting surgery, coronary angiography with percutaneous coronary intervention, or any lesion >50% of the coronary artery luminal diameter, cerebrovascular accident, or peripheral vascular disease), with normal electrocardiograms and echocardiography tests. Exclusion criteria included congestive heart failure, documented CAD or stroke, chest pain or angina pectoris, use of any anti-platelet therapy (including non-steroidal anti-inflammatory drugs) during the last 14 days prior to study inclusion, atrial fibrillation or brady-arrhythmias (resting heart rate <50 beats per minute, sick sinus syndrome or 2nd or 3rd degree atrio-ventricular block), renal failure (blood creatinine >3 mg/dL), chronic liver disease, chronic diarrhea, diabetes mellitus, hyper or hypothyroidism, malignancy, pregnancy, chronic obstructive pulmonary disease, known infectious disease, history of alcohol or drug abuse or refusal to sign the informed consent form. The study was approved by the Institutional Review Board, and all participants gave written, informed consent.

Patients were randomized by a computerized randomization program using SPSS 19.0 software (statistical package for social science, SPSS Inc., USA), to either oral magnesium oxide tablets [Magnox 520^{TM} (magnesium oxide monohydrate, 520 mg/day of elemental magnesium), Naveh Pharma, Israel] or magnesium citrate tablets [Magnesium Diasporal (295.8 mg/day of elemental magnesium) Protina GMBH, Ismaning, Germany] for one month (phase 1), after which there was a four-week wash-out period, followed by crossover treatment for one month (phase 2). Compliance was assessed by pill count.

Subjects were instructed to continue with their regular medications and diet throughout the study. Additionally, subjects were instructed not to add any medications (including over-the-counter medications or herbs), and to record any change in concomitant medications throughout the study period. At entry (day 0) and after one month (day 30) (phase 1), and after two (day 60) and three months (day 90) (phase 2), following an overnight fast, subjects underwent a physical examination, blood tests for measurements of lipids, a blood cell count, electrolytes, serum magnesium, platelet function tests, high-sensitivity C-reactive protein (hs-CRP), and completed quality-of-life and magnesium status questionnaires [21]. In addition, $[Mg^{2+}]_i$ was assessed before and after each phase, from sublingual cells, via X-ray dispersion.

A four-part, magnesium status questionnaire was designed to determine the likelihood of magnesium deficiency amongst the subjects. By adding up the scores from all four parts of the questionnaire, the following general guidelines were ascertained: 0-12 points=low risk; 13-20 points=mild risk; 21-30 points=moderate risk; 31-40 points=high risk; ≥41 points=very high risk of magnesium deficiency [21].

Estimated glomerular filtration rate (eGFR) was calculated at each visit using the abbreviated Modification of Diet in Renal Disease Study equation [22]: eGFR (mL/min/1.73 m^2)=186×(serum creatinine/88.4)$^{-1.154}$×(age)$^{-0.203}$×(0.742 if female).

The blood samples, except those for platelet function, were centrifuged immediately for 15 minutes at 3,000/min. The serum samples were stored at -20 °C and tested at the end of the study. Blood samples for platelet function were assessed immediately after the blood was drawn. All blood samples were evaluated in the same laboratory and by the same operator who was blinded to the patients' clinical status and type of magnesium (oxide or citrate).

Intracellular magnesium levels

Tissue $[Mg^{2+}]_i$ were measured noninvasively in sublingual epithelial cells [15, 19, 23-26] using a FEI Quanta Scanning Electron Microscope (FEI, Corvallis, OR, USA) coupled to a Norman X-ray analyzer. Energy dispersive X-ray analysis of individual epithelial cells was quantified using proven, published, and established normal values (EXA™; IntraCellular Diagnostics, Inc., Medford, OR, USA) (normal mean ± SD values 37.9 ± 4.0 mEq/L). Specimens taken from each patient were kept until the end of the study, when they were sent in one batch to IntraCellular Diagnostics, Inc., Medford, Oregon, USA for analysis by one of the co-authors (BBS), who was blinded to the subjects' magnesium treatment (oxide or citrate). Reported values were the mean of five to 10 cells per patient; a specimen was rejected if variance exceeded 2%. Sublingual epithelial cell $[Mg^{2+}]_i$ correlate well with human atrial $[Mg^{2+}]_i$ [23].

Lipids, blood cell count and electrolytes

Before and after each phase, fasting blood samples were taken for hemoglobin, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), very low-density lipoprotein cholesterol (VLDL-C), triglycerides, fasting glucose and serum magnesium measurements, using standardized auto-analyzer techniques. LDL-C was calculated using the Friedwald formula [27].

Platelet function assessment

Blood for platelet reactivity was drawn with a loose tourniquet through a short, venous catheter into 3.2% sodium citrate-containing tubes. Blood samples, drawn from the study participants before and after each phase, were centrifuged (140×g for 12 minutes), and the upper fraction was collected as platelet-rich plasma. The remaining blood was centrifuged again (1,660×g for 12 minutes) to obtain platelet-poor plasma.

Platelet aggregation was evaluated by a turbidimetric PACKS-4 aggregometer (Helena Laboratories, Beaumont, TX, USA) using adenosine diphosphate (ADP, 5.5 μM), arachidonic acid (AA, 1.6 mM) and epinephrine as platelet agonists.

Statistical analysis

A power calculation assessment based on previous studies [18, 28], demonstrated a 3 mEq/L increase in $[Mg^{2+}]_i$ measured in sublingual epithelial cells, following oral magnesium supplementation. Using a p value of 0.05, and a power of 80%, the total number of patients to be studied for the cross-over design study was estimated to be 40. Values are expressed as mean ± SD for continuous variables and frequencies (%) for categorical variables. Distributions of continuous variables were assessed using the Kolmogorov-Smirnov test. The Wilcoxon-nonparametric test was used to calculate differences over time and the independent t-test was used to compare the treatment groups. All patients randomized into the trial were included in the final analysis by intention-to-treat. *Per* protocol, efficacy analysis and intention-to-treat analyses for safety were performed. All statistical calculations were performed using SPSS 19.0 software (statistical package for social science, SPSS Inc., USA). $P<0.05$ represented statistical significance.

Results

Forty-one subjects (20 women) participated in the study, mean age was 53 ± 5 years (range: 31-75 years) and mean body mass index was 27 ± 6 kg/m^2. Baseline characteristics of the study

population are shown in *table 1*. There were no significant differences between the subjects allocated for the first treatment to magnesium oxide tables (Magnox) and magnesium citrate (Diasporal) as regards gender, risk factors of CAD, concomitant medications, hemodynamic parameters, including resting blood pressure and heart rate, cell blood count, serum electrolytes, renal function tests, eGFR, liver function tests, lipoproteins and platelet aggregation. Lipoproteins of the study participants were within the National Cholesterol Education Program guidelines, and their 10-year Framingham CAD risk was 7.2%.

The baseline self-reported magnesium status questionnaire suggested that the study participants had a very high risk of severe magnesium deficiency (*table 1*). Furthermore, while the study cohort baseline serum magnesium levels were within the normal range, the mean $[Mg^{2+}]_i$ was below the normal range.

One patient who started oral Magnox treatment refused to continue the study and did not come for follow-up visits. For the entire study population, 30-day oral Magnox treatment significantly increased $[Mg^{2+}]_i$ compared to Diasporal *(table 2)* , although serum magnesium levels were almost the same, as were the subjects' magnesium status questionnaires. No significant changes were observed between the two treatment strategies throughout the study period regarding eGFR, serum electrolytes, liver function tests, cell blood count, blood pressure and heart rate.

Magnox, but not Diasporal, significantly reduced total cholesterol (201 ± 37 *versus* 186 ± 27 mg/dL, p=0.0167 and 187 ± 28 *versus* 187 ± 25 mg/dL, p=0.978; respectively) and LDL-C (128 ± 22 *versus* 120 ± 25 mg/dL, p=0.042 and 120 ± 23 *versus* 121 ± 22 mg/dL, p=0.622; respectively) (*table 2*). Additionally, Magnox, but not Diasporal, also significantly reduced hs-CRP (3.1 ± 3.0 *versus* 2.6 ± 2.0 mg/L, p=0.030 and 3.0 ± 4.0 *versus* 3.5 ± 5.0 mg/L, p=0.438; respectively). Furthermore, both oral Magnox and Diasporal treatments significantly reduced epinephrine-induced platelet aggregation (*table 2*). No change in platelet number was seen throughout the study.

About 50% of study participants reported some mild side effects throughout the study, although no significant differences were seen between the two magnesium treatments *(table 3)*. While more patients in the Diasporal group had abdominal pain and diarrhea compared with Magnox, more patients from the Diasporal group had resting leg pain, headache and weakness. Additionally, more patients from the Diasporal group reported that the treatment worsened their general well-being compared with Magnox (25% *versus* 8%; p=0.0320, respectively). Finally, no severe side effects were observed for either of the treatment strategies throughout the study.

Discussion

To the best of our knowledge, this is the first study to compare and contrast the impact of oral magnesium oxide and citrate on $[Mg^{2+}]_i$, demonstrating that a 30-day regime of oral Magnox supplement significantly increases $[Mg^{2+}]_i$ compared to Diasporal in the same apparently healthy volunteers without CAD. Although our study participants were considered to be "healthy", their baseline $[Mg^{2+}]_i$ was relatively low, reflecting a typical Western diet [1, 21], which portends a steadily decreasing amount of magnesium. Data show that the average daily intake of magnesium at the beginning of the 20th century was 410 mg while today it is only 200-300 mg. The rationale behind such reduced mineral consumption, including magnesium, in the contemporary diet is mainly due to industrial food processing and the over-utilization of fields designated for cultivating agricultural produce [21]. The current daily Recommended Dietary Allowance for magnesium is 420 mg for males and 320 mg for females above 31 years, and in stressful situations such as in pregnancy or physical growth, an additional 300 mg daily is recommended. Data from the 1999-2000 National Health and Nutrition Examination Survey suggest that a substantial number of adults in the United States fail to consume recommended daily amounts of magnesium. The diets of adult male and female Caucasians contain significantly more magnesium than those of African-Americans. Magnesium intake is lower among older adults in every racial and ethnic group worldwide. The intake of magnesium is significantly higher among African-American men and male and female Caucasians who take dietary supplements, compared with those who do not. In a population-based study of 30-year-old Israelis, about 60% had a magnesium deficiency [1, 21, 29-33].

Our current study demonstrates that serum magnesium levels, both at baseline and following

Table 1. Baseline characteristics by study group (n=41).

	Magnox (n=21)	Diasporal (n=20)	P value
Males	10 (48%)	11 (55%)	0.636
Females	11 (52%)	9 (45%)	0.636
Work	16 (76%)	15 (75%)	0.929
Risk factors			
Hypertension	0	4 (20%)	0.031
Hyperlipidemia	4 (19%)	6 (30%)	0.414
Smoking	5 (24%)	5 (25%)	0.929
Diabetes type 2	0	1 (5%)	0.300
Family history of premature CAD	5 (24%)	5 (25%)	0.929
Overweight (BMI>25 kg/m^2)	7 (33%)	7 (35%)	0.929
Medications			
Aspirin	1 (5%)	2 (10%)	0.520
Calcium blockers	0	1 (5%)	0.300
ACE-inhibitors	1 (5%)	2 (10%)	0.520
Statins	2 (10%)	5 (25%)	0.188
Vitamins	3 (14%)	2 (10%)	0.675
Hormone replacement therapy	1 (5%)	0	0.323
Others	6 (29%)	4 (20%)	0.523
Age (years)	52 ± 11	54 ± 8	0.503
Magnesium questionnaire	49 ± 19	44 ± 2	0.469
Weight (kg)	77 ± 18	82 ± 2	0.387
Height (meters)	1.69 ± 11	1.71 ± 9	0.396
Body mass index (kg/m^2)	27 ± 6	28 ± 6	0.600
Resting heart rate (beats/min)	67 ± 7	70 ± 1	0.277
Systolic blood pressure (mmHg)	130 ± 19	130 ± 16	0.999
Diastolic blood pressure (mmHg)	78 ± 8	81 ± 10	0.314
Urea (mg/dL)	32 ± 9	32 ± 11	0.927
Creatinine (mg/dL)	1.0 ± 0.1	1.0 ± 0.1	0.272
Fasting glucose (mg/dL)	93 ± 14	92 ± 10	0.812
Potassium (mEq/L)	4.0 ± 0.1	4.0 ± 0.1	0.571
Sodium (mEq/L)	139 ± 2	139 ± 2	0.705
Chloride (mEq/L)	105 ± 2	105 ± 3	0.411
Calcium (mg/dL)	10.0 ± 0.1	10.0 ± 0.1	0.669
Bilirubin (mg/dL)	1.0 ± 0.1	1.0 ± 0.1	0.784
Aspartate aminotransferase (IU/L)	24 ± 6	28 ± 31	0.606
Alanine aminotransferase (IU/L)	25 ± 11	27 ± 29	0.765
Alkaline phosphatase (IU/L)	71 ± 24	73 ± 19	0.714
Albumin (g/dL)	4.0 ± 0.1	4.0 ± 0.1	0.308
hs-CRP (mg/L)	3 ± 3	4 ± 5	0.848
Serum magnesium (mg/dL)	2.0 ± 0.1	2.0 ± 0.1	0.292
Baseline eGFR (MDRD) (mL/min/1.73 m^2)	73 ± 14	77 ± 13	0.312
Total cholesterol (mg/dL)	198 ± 29	182 ± 26	0.07
Triglycerides (mg/dL)	128 ± 90	139 ± 194	0.811
HDL-C (mg/dL)	53 ± 14	55 ± 9	0.54
LDL-C (mg/dL)	128 ± 20	113 ± 20	0.025
VLDL (mg/dL)	26 ± 18	19 ± 9	0.19
White blood cells ($10^3/\mu$L)	7 ± 1	7 ± 2	0.857
Hemoglobin (g/dL)	14 ± 1	14 ± 1	0.422
Platelets ($10^3/\mu$L)	230 ± 71	227 ± 56	0.898

Table 1. (*Continued*)

	Magnox (n=21)	Diasporal (n=20)	P value
Intracellular magnesium levels (mEq/L)	34 ± 3	34 ± 2	0.465
Epinephrine-induced platelet aggregation (%)	80 ± 14	82 ± 17	0.753

Values are presented as mean ± SD or absolute numbers (percentage); ACE= angiotensin-converting enzyme; ADP=adenosine diphosphate; eGFR =estimated glomerular filtration rate using abbreviated Modification of Diet in Renal Disease Study (MDRD) equation; HDL-C=high-density lipoprotein cholesterol; hs-CRP=high-sensitivity C-reactive protein; LDL-C=low-density lipoprotein cholesterol; VLDL=very low-density lipoprotein cholesterol; PLT=platelet.

Table 2. Comparison of the effects of the two magnesium treatments at baseline and at day 30

	Magnox (n=40)			Diasporal (n=40)		
	Baseline	At day 30	P	Baseline	At day 30	P
Magnesium status questionnaire	47 ± 21	45 ± 19	0.511	48 ± 17	48 ± 18	0.986
Weight (kg)	79 ± 19	80 ± 18	0.372	79 ± 19	79 ± 19	0.629
Body mass index (kg/m^2)	27 ± 6	28 ± 6	0.36	27 ± 6	27 ± 6	0.595
Resting heart rate (beats/min)	71 ± 13	69 ± 12	0.259	68 ± 9	68 ± 11	0.964
Systolic blood pressure (mmHg)	131 ± 19	127 ± 18	0.195	126 ± 14	126 ± 16	0.725
Diastolic blood pressure (mmHg)	78 ± 10	76 ± 10	0.445	79 ± 11	77 ± 11	0.264
Urea (mg/dL)	33.2 ± 8.0	33.7 ± 9.0	0.672	32.1 ± 10.0	33.9 ± 9.0	0.129
Creatinine (mg/dL)	1.0 ± 0.1	1.0 ± 0.1	0.107	1.0 ± 0.1	1.0 ± 0.1	0.14
Fasting glucose (mg/dL)	93.0 ± 13.0	94.9 ± 13.0	0.105	92.4 ± 13.0	93.1 ± 14.0	0.679
Potassium (mEq/L)	4.3 ± 0.1	4.3 ± 0.1	0.183	4.3 ± 0.1	4.3 ± 0.1	0.382
Sodium (mEq/L)	139.1 ± 2.0	139.3 ± 2.0	0.560	138.8 ± 2.0	138.9 ± 2.0	0.642
Chloride (mEq/L)	104.7 ± 2.0	105.5 ± 2.0	0.038	105.2 ± 3.0	104.7 ± 3.0	0.127
Calcium (mg/dL)	9.7 ± 0.1	9.6 ± 0.1	0.394	9.8 ± 0.1	9.7 ± 0.1	0.739
Bilirubin (mg/dL)	0.7 ± 0.1	0.7 ± 0.1	0.821	0.6 ± 0.1	0.7 ± 0.1	0.81
hs-CRP (mg/L)	3.1 ± 3.0	2.6 ± 2.0	0.03	3 ± 4.0	3.5 ± 5.0	0.438
Aspartate aminotransferase (IU/L)	24.6 ± 13.0	23.3 ± 10.0	0.114	26.1 ± 23.0	26.9 ± 24.0	0.384
Alanine aminotransferase (IU/L)	25.3 ± 15.0	23.4 ± 12.0	0.101	24.8 ± 23.0	25.4 ± 22.0	0.513
Alkaline phosphatase (IU/L)	72.3 ± 20.0	71.1 ± 19	0.323	71.3 ± 23	70.2 ± 24.0	0.486
Albumin (g/dL)	4.3 ± 0.1	4.3 ± 0.1	0.504	4.4 ± 0.1	4.4 ± 0.1	0.734
Serum magnesium (mg/dL)	2.1 ± 0.1	2.1 ± 0.1	0.989	2.1 ± 0.1	2.0 ± 0.1	0.376
Triglycerides (mg/dL)	185 ± 155	129 ± 73	0.308	144 ± 153	128 ± 61	0.466
Total cholesterol (mg/dL)	201 ± 37	186 ± 27	0.016	187 ± 28	187 ± 25	0.978
HDL-C (mg/dL)	53 ± 11	54 ± 10	0.275	53 ± 11	53 ± 11	0.319
LDL-C (mg/dL)	128 ± 22	120 ± 25	0.042	120 ± 23	121 ± 22	0.622
VLDL (mg/dL)	26 ± 17	25 ± 14	0.785	24 ± 12	25 ± 11	0.521
White blood cells ($10^3/\mu$L)	6.9 ± 2.0	6.8 ± 1.0	0.650	6.6 ± 2.0	6.9 ± 1.0	0.251
Hemoglobin (g/dL)	14.1 ± 1	14.1 ± 1	0.406	14.1 ± 1	14 ± 1	0.282
Platelets ($10^3/\mu$L)	227 ± 66	226 ± 60	0.577	232 ± 63	236 ± 73	0.199
Intracellular magnesium levels (mEq/L)	34.4 ± 3.0	36.3 ± 2.0	0.001	34.7 ± 2.0	35.4 ± 2.0	0.097
Epinephrine-induced aggregation (%)	78.9 ± 16.0	71.7 ± 23.0	0.013	81.3 ± 15.0	73.3 ± 23.0	0.036
eGFR (mL/min/1.73 m^2)	74.9 ± 14	77.1 ± 12	0.14	77 ± 14	75.3 ± 13	0.167

Values are presented as mean ± SD; eGFR =estimated glomerular filtration rate using abbreviated Modification of Diet in Renal Disease Study (MDRD) equation; HDL-C=high-density lipoprotein cholesterol; hs-CRP=high-sensitivity C-reactive protein; LDL-C=low-density lipoprotein cholesterol; VLDL=very low-density lipoprotein cholesterol.

Table 3. Comparison of the side effects between the two magnesium treatment groups at baseline and at day 30

	Magnox (n=40)		Diasporal (n=40)	
	Baseline	**At day 30**	**Baseline**	**At day 30**
Heat sensation	7 (18%)	3 (8%)	5 (13%)	3 (8%)
Headache	6 (15%)	4 (10%)	6 (15%)	7 (18%)
Weakness	12 (30%)	4 (10%)	9 (23%)	8 (20%)
Rest dyspnea	2 (5%)	2 (5%)	1 (3%)	2 (5%)
Effort dyspnea	3 (8%)	0	2 (5%)	1 (3%)
Orthopnea	0	0	0	0
Paroxysmal nocturnal dyspnea	0	0	0	0
Nocturia	0	0	0	1 (3%)
Diarrhea	0	5 (13%)	2 (5%)	3 (8%)
Abdominal pain	0	2 (5%)	2 (5%)	4 (10%)
Dry mouth	8 (20%)	3 (8%)	5 (13%)	4 (10%)
Leg pain	9 (23%)	11 (28%)	8 (20%)	7 (18%)
Leg edema	4 (10%)	2 (5%)	3 (8%)	0
Non-specific chest pain	2 (5%)	3 (8%)	4 (10%)	3 (8%)
Palpitations	5 (13%)	3 (8%)	2 (5%)	2 (5%)
Heart burn	2 (5%)	0	1 (3%)	3 (8%)
Nausea	1 (3%)	0	1 (3%)	3 (8%)
Vomiting	0	0	1 (3%)	1 (3%)
Rash	0	0	1 (3%)	1 (3%)
Itching	0	0	0	0
Depressive mood	0	1 (3%)	0	0
Other	0	0	0	1 (3%)
Summary of side effects				
None	17 (42%)	17 (42%)	24 (60%)	16 (40%)
Mild	21 (53%)	20 (50%)	14 (35%)	17 (42%)
Moderate	0	1 (3%)	2 (5%)	1 (3%)
Severe	0	0	0	0
Did the current medication improve your condition?				
No		2 (5%)		0
Yes		17 (42%)		10 (25%)
Same		19 (48%)		17 (42%)
Worse		3 (8%)		10 (25%)

Values are presented as absolute numbers (percentage).

a 30-day regimen of two different magnesium supplements taken by the same study participants, were within normal values and did not change throughout the study. However, this is not so surprising since only 1% of total body magnesium is found in the serum and therefore its measurement does not reflect the intracellular level. While hypomagnesemia reflects low total body content, normomagnesemia does not necessarily indicate normal or high total body magnesium [3, 34].

While only 30-day oral Magnox, not Diasporal, significantly raised $[Mg^{2+}]_i$, both treatments reduced epinephrine-induced platelet aggregation. This pharmacological effect of magnesium is well known and in accord with previous studies [1, 35-37]. Experimental studies have demonstrated the anti-platelet effects of magnesium, which may prevent the propagation of coronary artery thrombi or re-occlusion of the infarct-related coronary artery after spontaneous or fibrinolysis-induced recanalization [19, 20, 35]. Recently, some studies have demonstrated that magnesium reduces platelet aggregation in healthy volunteers [36-38]. High magnesium levels inhibit blood coagulation [19] and thrombus formation *in vivo* [20], diminish platelet aggregation [39], reduce synthesis of the platelet agonist thrombaxane A_2 [37], and inhibit thrombin-stimulated calcium influx [39].

Our study also demonstrated that a regime of 30-day Magnox, but not Diasporal, significantly reduces total and LDL-C. Magnesium plays an interesting role in lipid regulation, although this role is not yet fully understood [1]. Magnesium is an important cofactor of two enzymes that are essential for lipid metabolism: lecithin-cholesterol acyltransferase and lipoprotein lipase. Rassmussen *et al*. [17] demonstrated a 27% reduction in triglycerides and VLDL, a reduction in apolipoprotein B, and an increase in HDL, after administering 15 mmol of magnesium hydroxide daily to patients with ischemic heart disease over a three-month period. In addition, Davis *et al*. [40] demonstrated a significant improvement in the ratio of HDL to LDL, plus VLDL, by giving 18 mmol of magnesium per day in a four-month clinical trial.

Furthermore, it should be noted that in our study Magnox, not Diasporal, reduced hs-CRP (*table 2*). Low magnesium status has been associated with numerous conditions characterized as having a chronic, inflammatory stress component. Some animal findings indicate that moderate magnesium deficiency, similar to that which commonly occurs in humans, may enhance inflammatory or oxidative stress induced by other factors, including disrupted sleep/sleep deprivation. Recently, Nielsen *et al*. [41] demonstrated that oral magnesium treatment significantly reduced plasma hs-CRP in humans compared to placebo. Interestingly, children consuming a low magnesium diet have higher hs-CRP compared to those who consume a high magnesium diet [42]. Thus, hs-CRP as a marker of inflammation is negatively associated with a low magnesium intake.

Three studies have attempted to estimate the absorption of magnesium oxide using urinary excretion data, but none has evaluated $[Mg^{2+}]_i$ [43-45]. Lindberg *et al*. [43] studied 17 normal volunteers who underwent three, oral loading tests in random order: magnesium citrate (25 mmol=615 mg magnesium), magnesium oxide (25 mmol=615 mg magnesium), or distilled water. The increment in serum magnesium over four hours following loading was higher with the citrate than with the oxide: 0.063 ± 0.135 mmol/L (0.15 ± 0.32 mg/dL) *versus* 0.014 ± 0.007 mmol/L (0.03 ± 0.01 mg/dL), respectively, $p<0.05$. Muhlbauer *et al*. [44], using a similar technique, found that there was lower cumulative magnesium urinary excretion with magnesium oxide capsules than with L-aspartate HCL tablets in eight, healthy volunteers, while plasma magnesium levels remained unchanged after treatment. Altura *et al*. [45] studied 18, male volunteers who were given magnesium-enriched diets followed by 12.34 mmol (304 mg) of magnesium as oxide or phosphate plus oxide. They found that the magnesium oxide preparation improved serum magnesium in those with low basal serum levels, but not in those with normal/high serum levels, while total magnesium again remained the same. Ross *et al*. [46] recently observed the superiority of magnesium oxide absorption over magnesium glycerophosphate in patients with shortened, small bowel-induced malabsorption.

It is well known that magnesium, an alkaline earth metal, stabilizes macromolecule structure and participates as an essential cofactor in many enzymatic reactions. It seems that these tasks require total cellular concentration. Organic magnesium salts, such as citrate, aspartate or glutamate, are largely used as oral magnesium supplements, and are considered to be more bioavailable than inorganic magnesium salts, such as oxide or hydroxide [46, 47]. However, the current study contests this assumption by finding a significant $[Mg^{2+}]_i$ increase following magnesium oxide compared to citrate supplementation.

Furthermore, Lindberg *et al*. [43] noted that approximately 65% of magnesium citrate was complexed as soluble magnesium citrate. Complex molecules with certain metal ions, such as magnesium and citrate, inactivate the metal ions so that they cannot react normally with other elements or ions.

Magnesium citrate, for example, is composed of the metal magnesium bound to the ligand of citrate. A value called "stability constant" is placed on all metal-ligand complexes, and is a measure of that compound's ability to break down under varying pH levels, so that the body can access and use the metal component of that compound. The lower the "stability constant" of a metal-ligand, the more readily it will dissolve in water, facilitating an easier dissociation of the metal from the ligand to which it is bound. The "stability constant" of magnesium citrate is 2.8 compared to 0 (zero) for magnesium oxide [47]. Thus, this may explain the better $[Mg^{2+}]_i$ achieved by Magnox compared with Diasporal oral, magnesium salts.

Finally, both magnesium treatments were tolerated and did not induce serious adverse events,

although more subjects reported that Magnox, rather than Diasporal, improved their general well-being (*table 3*).

Positive elements of the current study lie in its prospective, double-blind, cross-over design, which included 50% women. One could argue that a specific magnesium supplement given the first month could potentially treat the magnesium deficit, and therefore no magnesium deficit would be observed during the second month. However, this study was designed to counteract this potential problem: half the subjects received Magnox during the first month, while the remainder received Diasporal, and *vice versa* during the second month.

While several methods have been used to assess magnesium levels [1, 21], interpretation of measured magnesium is difficult:

(a) *Serum magnesium measurements*. Serum levels are the easiest to obtain, but provide the least reliable index. Since <1% of total body magnesium is in the serum, its measurement does not reflect actual intracellular levels. While hypomagnesemia reflects low total body content, normomagnesemia does not necessarily indicate normal or high total body magnesium [3, 34].

(b) *$[Mg^{2+}]_i$*. The most accurate $[Mg^{2+}]_i$ measurements, which also reflect the intramyocardial muscle cell content, are lymphocytic (more accurate) and erythrocyte (less accurate and cell age-dependent) magnesium levels [4, 48]. During the last two decades, a non-invasive technique, which measures whole-cell magnesium content, (namely the EXA™ test, which measures sublingual, intra-epithelial cell magnesium content), has found a very marked correlation with intramyocardial magnesium content [23]. This assay, used in the current study, is acceptably reproducible, with a coefficiency variance of 2% [23]. The primary limitation of the EXA™ test is that it measures total cellular magnesium content without distinguishing between ionized species. Although magnesium measurements in sublingual cells correlate well with those in cardiac cells, the relationship between changes in sublingual and cardiac levels after an intervention have not been well investigated. Loss of cardiac magnesium, using the EXA™ test in experimental heart failure, prolongs and destabilizes repolarization in dogs [49]. Moreover, in a few prospective randomized, placebo-controlled clinical trials in humans, magnesium supplements have been shown to increase $[Mg^{2+}]_i$ assessed by the EXA™ test, along with improvement of clinical parameters, such as vascular endothelial function [18], systolic blood pressure [26], corrected QT interval [25], exercise duration time [15, 28] and left ventricular ejection fraction [15]. While electrodes for the measurement of free magnesium content are available, there is currently no consensus regarding the normal and abnormal values in various populations, and there is a lack of standardized exits.

(c) *Magnesium retention after oral magnesium or intravenous load test*. This test for measuring magnesium retention is accurate, but requires 24-h urine collection [21, 50].

Nevertheless, our study does have some limitations: (a) X-ray dispersion analysis was used in the current study to assess magnesium status. However, since currently there are very few experiments that use this technique, more studies are needed to determine both the significance of this technology and the inter-method comparisons for measuring $[Mg^{2+}]_i$; (b) The relatively short, 30-day treatment period could obscure the beneficial effects of Diasporal and Magnox on other parameters, such as lipoproteins, blood pressure, quality-of-life, etc. [1]; (c) The Diasporal dose was not identical to that of Magnox, which could explain the null effect on $[Mg^{2+}]_i$; (d) The study cohort was biased towards a generally higher socio-economic representation, including better educated subjects with an awareness of primary care facilities. Primary care physicians send patients to our laboratory for CAD assessment (low *versus* high) to help them decide on treatment management of patients with risk factors for CAD; (e) Stress tests were not performed on all patients; however, the exclusion of CAD was based upon clinical examination, electrocardiography and echocardiography, which were performed for all participants; (f) It should be noted that our study population comprised patients at low risk of CAD, with an estimated 10-year risk of <10%, and not CAD patients, since we wanted to investigate the net effects of oral magnesium supplements on $[Mg^{2+}]_i$ without the interference of other, concomitant medications and potential absorption problems.

In conclusion, oral magnesium oxide monohydrate treatment significantly improves $[Mg^{2+}]_i$, hs-CRP, total and LDL-C compared with magnesium citrate, although both treatments equally inhibited platelet aggregation in healthy subjects with no apparent heart disease.

Disclosure

Burton B. Silver, PhD, is the Research Director and President of IntraCellular Diagnostics, Inc., Medford, Oregon, USA. None of the other authors has any conflict of interest.

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