

# Magnesium supplementation improves indicators of low magnesium status and inflammatory stress in adults older than 51 years with poor quality sleep\*

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**Abstract.** Low magnesium status has been associated with numerous conditions characterized as having a chronic inflammatory stress component. Some animal findings indicate that a moderate magnesium deficiency, similar to which apparently commonly occurs in humans, may enhance inflammatory or oxidative stress induced by other factors, including disrupted sleep/sleep deprivation. Thus, an experiment was performed with 100 adults (22 males and 78 females) aged  $59 \pm 8$  years (range 51 to 85 years) with poor sleep quality revealed by a Pittsburg Sleep Quality Index (PSQI) score higher than five. The participants were randomly assigned to two groups matched by gender, age, and overall PSQI score. After baseline assessment (week one) of body mass index (BMI), diet, blood and urine biochemical variables, and sleep quality, one group was given a 320 mg magnesium/day supplement as magnesium citrate and the other group a sodium citrate placebo for seven weeks. Final assessments were made five and seven weeks (which were combined for statistical analysis to reduce intra-individual variation) after supplement initiation for the 96 participants who completed the study as designed. Based on food diaries, 58% of the participants were consuming less than the US Estimated Average Requirement (EAR) for magnesium. Consuming less than the EAR was associated with a significantly higher BMI and plasma C-reactive protein (CRP) concentration. Only 40 participants had plasma CRP concentrations higher than 3.0 mg/L (an indication of chronic inflammatory stress). Overall PSQI scores improved ( $10.4 \rightarrow 6.6$ ,  $p < 0.0001$ ) and erythrocyte magnesium increased ( $4.75 \rightarrow 5.05$  pg/cell,  $p = 0.01$ ) regardless of magnesium or placebo supplementation. Magnesium vs placebo supplementation did not significantly affect serum magnesium when all participants were included in the analysis. When only the 37 participants with serum magnesium concentrations  $< 1.8$  mg/dL (indication of deficient magnesium status) were analyzed, magnesium supplementation, but not the placebo, increased serum magnesium concentrations. Magnesium supplementation vs placebo decreased plasma CRP in participants with baseline values  $> 3.0$  mg/L. The findings show that many individuals have a low magnesium status associated with increased chronic inflammatory stress that could be alleviated by increased magnesium intake. Because dietary magnesium

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intake did not change during the experimental period, another factor, possibly a placebo effect, improved sleep quality, which resulted in increased erythrocyte magnesium. This factor prevented the determination of whether magnesium deficiency contributes to poor sleep quality. The findings, however, suggest an association between magnesium status and sleep quality that needs further study to definitively determine whether a low magnesium status is a cause or an effect of poor sleep quality.

**Key words:** magnesium deficiency, inflammatory stress, sleep, magnesium intake, magnesium supplementation

Over 75 years ago, findings were obtained that suggested magnesium deficiency results in an inflammatory response [1]. Evidence obtained in the past 25 years, mostly from animal experiments, has confirmed that severely limiting magnesium intake to less than 10% of the requirement results in an inflammatory response characterized by the release of inflammatory cytokines and acute phase proteins, and excessive production of free radicals and oxidative stress [2]. The United States (US), National Health and Nutrition Examination Survey (NHANES) 2005-2006 data indicated that the usual magnesium intake from food of about 60% of all adults does not meet the American Estimated Average Requirement (EAR) of 255-265 mg/day for females and 330-350 mg/day for males [3, 4]. However, severe human magnesium deficiency caused by low dietary intake is unlikely. Based on dietary surveys, most people have intakes that meet at least 50% of the EAR [3]. Moderate to marginal or sub-clinical (~ 50% to < 100% of requirement) magnesium deficiency alone apparently does not affect variables associated with chronic inflammatory stress in animal models [5, 6]. However, some animal findings indicate that moderate magnesium deficiency can enhance the inflammatory or oxidative stress induced by other factors [7-9].

One factor that may increase inflammatory stress is disrupted sleep/sleep deprivation [10]. Inadequate sleep duration has been associated with increases in several inflammatory biomarkers including plasma C-reactive protein (CRP) [10]. Sleep quality also has been associated with increased morning concentrations of inflammatory biomarkers IL-6 in healthy adults, elderly women and spousal Alzheimer's caregivers, and circulating IL-1 $\beta$  in women but not men [10]. Magnesium intake has been found to be inversely related to elevated circulating CRP concentrations [11-15]. Thus, subclinical magnesium deficiency through exacerbating a low grade inflammation may be a factor in sleep disruption or deprivation. The possibility that magnesium depriva-

tion affects sleep quality is supported by a few human and animal studies. In a placebo-controlled, randomized cross-over experiment with 12 older (aged 60 to 80 years) participants, magnesium supplementation significantly reversed electroencephalogram (EEG) changes, including decreased slow wave sleep, that occur during aging [16]. Another study found that 27 patients with parasomnias displayed hypomagnesemia and nocturnal EEG abnormalities occurring during slow wave sleep [17]. Magnesium treatment of alcohol-dependent patients (who often have magnesium metabolism disturbances) significantly decreased sleep onset latency and improved subjective sleep quality as assessed by the Pittsburgh Sleep Quality Index (PSQI) [18]. In rats, magnesium deficiency significantly increased wakefulness at the expense of slow wave sleep; magnesium supplementation restored sleep organization to its original pattern [19]. In addition to a chronic inflammatory stress relationship, sleep architecture and magnesium may have a biochemical relationship. It has been suggested that magnesium regulates sleep because it is an N-methyl-D-aspartate antagonist and a  $\gamma$ -aminobutyric acid (GABA) agonist [16, 19]. Sleep architecture, especially slow wave sleep, apparently is closely associated with the glutamatergic and GABAergic system.

The lack of controlled studies using a relatively large number of participants was the impetus for an experiment to determine whether magnesium supplementation improved sleep behavior (quantity, quality, and disturbance), and whether this was associated with a change in inflammatory stress measured by plasma CRP concentrations. In addition, because it has been suggested that people who have a missense variant of Thr-1482 to isoleucine (Ile) in the transient receptor potential melastatin 7 (TRPM7) gene may be at increased risk for magnesium deficiency [20], the possible influence of this polymorphism on the response to magnesium supplementation was determined.

## Subjects and methods

### Subjects

Male and female adults older than 51 years (age group with increased sleep disorders and likelihood of low magnesium status [21]) with sleep complaints were recruited for the study until 100 had completed the eight-week experimental protocol. Applicants for the study were not accepted for the study if they were consuming supplements providing 100 mg of magnesium or greater per day or consuming sleep medications. Because they were likely to have sleep disorders not related to magnesium status, applicants with a body mass index (BMI) greater than  $40 \text{ kg/m}^2$ , respiratory tract disease, chronic obstructive pulmonary disease, or using oxygen or continuous positive airway pressure were not accepted. Individuals on angiotension converting enzyme inhibitors for blood pressure control, other magnesium-retaining drugs, or potassium-sparing drugs were not accepted because of the possibility these drugs would cause retention of magnesium and potassium upon magnesium supplementation that could lead to heart rhythm changes.

Eligible applicants were invited to an information meeting that explained the purpose of the study, procedures involved, and expectations of the participants. After consenting, the applicants had blood drawn for complete blood count and liver and kidney functions tests and completed the PSQI. Applicants invited to participate in the study had blood results in the normal range and a global PSQI score of greater than five (indicator of poor sleep quality). Based on a study that used the PSQI to determine the effect of magnesium supplementation on sleep of alcohol-dependent patients [18], a power analysis

indicated that to detect a significant effect of magnesium with 0.90 power and an alpha of 0.05 would require 50 participants per placebo and magnesium-supplemented group. Seventy-eight females and 22 males gave written informed consent to participate in the experimental protocol that was approved by the Institutional Review Board of the University of North Dakota, followed the guidelines of the Department of Health and Human Services and the Helsinki Doctrine regarding the use of human subjects, and registered in Clinical Trials.gov as protocol NCT00833092. Beginning or baseline average ages, PSQI scores, BMIs and plasma CRP concentrations of the participants with no detected health problem or drug use are given in Table 1. The number of subjects consuming less than the EAR is also indicated because this was a factor in some of the responses to magnesium supplementation.

### Experimental protocol

The experiment was an eight-week, double-blind, placebo-controlled, supplementation trial. Following baseline assessment during week one of blood and urine biochemical variables, BMI, diet, and sleep quality, the participants were randomly assigned to two groups matched by gender and global PSQI score. One group was given a 320 mg/day magnesium supplement as magnesium citrate provided in five capsules, each containing an analyzed 64 mg of elemental magnesium. The other group was given five capsules containing a sodium citrate placebo. The capsules provided about 1.75 g citrate/day. The placebo and magnesium supplements were made by Gallipot, Inc. (St. Paul, Mn) from magnesium citrate and sodium citrate supplied by Dr. Paul Lohmann, Inc. (Islandia, NY). The parti-

**Table 1.** Baseline (mean  $\pm$  SEM) age, Pittsburgh Sleep Quality Index (PSQI), Body Mass Index (BMI), and plasma C-reactive protein (CRP) of subjects grouped by food diary magnesium intakes.

Magnesium intake	Sex	N	Age <sup>1</sup>	PSQI	BMI	CRP, mg/L
< EAR (265 mg/d)	Female	44	59.4 $\pm$ 1.2 (51-85)	11.1 $\pm$ 0.5	29.1 $\pm$ 0.8	2.74 (2.38, 3.14) <sup>2</sup>
$\geq$ EAR (265 mg/d)	Female	34	58.6 $\pm$ 1.1 (52-76)	10.5 $\pm$ 0.4	27.2 $\pm$ 0.8	1.84 (1.57, 2.16)
< EAR (350 mg/d)	Male	14	57.9 $\pm$ 2.7 (51-81)	8.9 $\pm$ 0.6	31.1 $\pm$ 1.0	3.12 (2.44, 3.99)
$\geq$ EAR (350 mg/d)	Male	8	62.4 $\pm$ 2.2 (52-68)	8.9 $\pm$ 0.9	28.4 $\pm$ 1.6	1.30 (0.94, 1.79)
< EAR	All	58	All Subjects	10.6 $\pm$ 0.4	29.6 $\pm$ 0.6 <sup>3</sup>	2.92 <sup>4</sup> (2.50, 3.19)
$\geq$ EAR	All	42	59.2 $\pm$ 0.8 (51-85)	10.2 $\pm$ 0.4	27.4 $\pm$ 0.7	1.55 (1.50, 1.98)

<sup>1</sup> Range of ages given in parentheses.

<sup>2</sup> Geometric mean (-1 SE, +1 SE).

<sup>3</sup> Significantly higher ( $p < 0.03$  based on t-test) than all subjects with intakes  $>$ EAR.

<sup>4</sup> Significantly higher ( $p < 0.009$  based on t-test of ln (CRP) values than all subjects with intakes  $>$ EAR.

cipants were instructed to consume two capsules with a morning meal, one with the noon meal, and two during an evening meal. Final assessments of blood and urine biochemical variables, BMI, diet, and sleep quality were made five and seven weeks after supplementation initiation, which were averaged for statistical analysis to reduce intra-individual variation.

### Diet and sleep quality determinations

During baseline and weeks five and seven, the participants kept a three-day food diary that included one weekend day as instructed by a dietician. Estimated average daily magnesium intake was calculated by using U.S. Department of Agriculture food composition data [22].

Sleep quality was measured by the PSQI, which is a self-rated questionnaire [23]. The PSQI has 19 individual items that generate 7 component scores for subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medications, and daytime dysfunction. The sum of scores for these seven components yields one global score. In this study, use of sleep medications did not influence the global score because their use was forbidden. The global score has a possible range of 0 to 21. In this study, a global PSQI score of greater than five was considered a sign of poor sleep quality.

### Biochemical variables determinations

Magnesium and calcium in 24-hour urine samples diluted 1:10 were determined directly by inductively coupled argon plasma emission spectroscopy (ICP) (Model 3100 XL, Perkin Elmer, Waltham, MA). Concurrent analyses of Seronorm Urine (SERO AS, Billingstad, Norway) yielded means (mg/L)  $\pm$  SD of  $107 \pm 5$  and  $69.9 \pm 2.3$  compared with certified values of  $107 \pm 4$  and  $70.1 \pm 2.5$ , respectively, for calcium and magnesium. Urine citrate was determined by enzymatic assay by using a commercially available kit (Cat. # 10 139 076-035, R-Biopharm/Boehringer Mannheim, Marshall, MI) that determined citric acid. Citrate values were obtained by using its molar mass of 189.1 g/mol. Quality control determinations indicated intra assay variation of  $0.33 \pm 0.01$  (SD) mg/dL and inter assay variation of  $1.69 \pm 0.07$  mg/dL. Urine creatinine was determined by using Kit # 04810716190 for the Cobas Integra Analyzer (Roche Diagnostics, Indianapolis, IN).

Blood was processed within 90 minutes to obtain serum or plasma. Blood was allowed to clot for

20 minutes before centrifuging at 2,000 RPM for 10 minutes to obtain serum. Complete blood counts were determined by using the Cell-Dyn 3700 System (Abbott Laboratories, Santa Clara, CA). Serum total cholesterol, HDL-cholesterol, triglycerides, and glucose were determined by using standard methods of the Cobas Integra Analyzer (Roche Diagnostics, Indianapolis, IN). LDL-cholesterol was calculated by subtracting HDL-cholesterol and VLDL-cholesterol (triglycerides  $\div$  5) from total cholesterol. Ionized magnesium in heparinized plasma normalized to pH 7.4 was determined by using an ion-selective electrode (Nova-CRT-8 Analyzer, Nova Biomedical, Waltham, MA). Quality control determinations indicated an intra assay variation of  $1.31 \pm 0.02$  (SD). High sensitivity CRP was determined by using a commercially available kit (Immulite 1000, Cat. # LKCR1, Diagnostics Products Corp., Los Angeles, CA). Quality control determinations found intra assay variation of (mean  $\pm$  SD) of  $0.17 \pm 0.01$ ,  $0.70 \pm 0.02$ , and  $9.32 \pm 0.41$  mg/dL. The threshold for elevated CRP was defined  $\geq 3.0$  mg/L, a concentration the American Heart Association designated as being associated with high cardiovascular risk [24], and used by others to associate elevated CRP with low magnesium intakes [11-13].

Calcium and magnesium in serum samples diluted 1:10 were directly determined by using ICP (Model 3100 XL, Perkin Elmer, Waltham, MA). Concurrent analyses of UTAK Serum (UTAK Laboratories, Valencia, CA) yielded means (mg/dL)  $\pm$  SD of  $7.62 \pm 1.2$  and  $1.67 \pm 0.11$  compared with certified values of  $8.3 \pm 2.1$  and  $1.9 \pm 0.5$ , respectively, for calcium and magnesium. Magnesium in digested red blood cells was determined by using ICP (Model 3100 XL, Perkin Elmer, Waltham, MA). The digestion procedure consisted of placing 2 mL of red blood cells in a glass tube with 2 mL of HNO<sub>3</sub> and allowing to stand at room temperature for four hours before placing the tube in a heating block to heat contents to near dryness (not allowed to completely dry). Then, another 2 mL of HNO<sub>3</sub> was added to the tube and the contents heated to near dryness, cooled to room temperature, and brought up to a 4 mL volume with 2% HNO<sub>3</sub>.

### Genotyping assay

Genomic DNA was extracted from blood samples by using a DNA isolation kit (Qiagen, Valencia, CA). The genotypes of participants were determined for the TRPM7 gene, and allelic discrimination of the rs8042919 polymorphism in the TRM7 gene was assessed by using the TaqMan genotyping

assay (Assay ID: C-25756319-10; Applied Biosystems, Foster City, CA). The final volume for each reaction was 25 µL, consisting of 12.5 µL TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 1.25 µL primers/TaqMan probes, and 20 ng genomic DNA. The PCR conditions were: 95 °C - 10 m for an initial denaturation step; 45 cycles at 95 °C - 15 s, and at 60 °C - 60 s. Fluorescence was measured with 7300 Real-Time PCR system, genotypes were determined by using 7300 System SDS Software (Applied Biosystems, Foster City, CA).

### Data analysis

Statistical analyses were done by using SAS Version 9.2 (SAS Institute, Cary, NC). Baseline data comparisons were made by the t-test. Treatment comparisons were made by repeated measures analysis of variance followed by Tukey contrasts when appropriate. Sleep component comparisons were made by Chi-square analysis. CRP data were highly skewed and were logarithmically transformed so their distribution would more closely approximate a normal distribution. Results for CRP are reported as geometric mean with a ± 1 SE interval. A p ≤ 0.05 was considered significant. Statistical analyses did not include three participants who did not adhere to protocol guidelines for the consumption of supplements and medications. A fourth subject was not included because she became ill with an inflammatory condition during week seven that required antibiotic and anti-inflammatory medication.

### Results

The food diaries indicated that dietary (non-supplemented) magnesium intakes did not change during the experimental period. The mean intakes ± SEM (mg/day) for periods one, two and three, respectively, were 287 ± 12, 281 ± 13 and 278 ± 15 for the placebo group and 280 ± 11, 287 ± 14 and 283 ± 13 for the magnesium-supplemented group. Based on the mean of the three sets of food diaries for each individual, 58% of the participants were consuming less than the EAR for magnesium (44 of 78 women and 14 of 22 men; *table 1*). This number is similar to the 60% number found by NHANES 2005-2006 for adults [1]. The low magnesium intake was associated with a significantly higher BMI and plasma CRP concentration at baseline (*table 1*). Only 40 of the participants had a plasma CRP concentration higher than 3.0 mg/L at baseline. Genotyping analysis of TRPM7 gene found 80 participants were thr1482 homozygous; 18 participants were Thr1482Ile heterozygous; and only two participants had the Ile1482 homozygous polymorphism.

The data in *table 2* indicate that most participants followed protocol guidelines for consumption of supplements. Urinary citrate excretion (mg/24 hr) tended (p = 0.07) to increase upon consumption of the supplements, which contained citrate. Urinary magnesium increased in participants supplemented with 320 mg magnesium/day but not in participants receiving the placebo. At the end of the study, the magnesium-supplemented participants excreted

**Table 2.** Effect of treatment on urinary excretion of magnesium, calcium and citrate.

Treatment	N	Period	Magnesium		Calcium		Citrate	
			mg/24hr	mg/mg Cr	mg/24hr	mg/mg Cr	mmol/dL	mmol/mmol Cr
Placebo	49	Baseline	91 <sup>a</sup> (86, 96) <sup>1, 2</sup>	0.084 <sup>a</sup> (0.079, 0.087)	138 (127, 150)	0.127 (0.117, 0.137)	1.78 (1.64, 1.94)	1.85 (1.69, 2.02)
Placebo	49	End	83 <sup>a</sup> (79, 87)	0.077 <sup>a</sup> (0.073, 0.081)	115 (106, 125)	0.107 (0.099, 0.116)	2.17 (1.99, 2.36)	2.28 (2.09, 2.49)
+300 mg Mg/d	46	Baseline	83 <sup>a</sup> (79, 87)	0.082 <sup>a</sup> (0.078, 0.087)	127 (117, 137)	0.126 (0.117, 0.136)	1.42 (1.31, 1.54)	1.60 (1.47, 1.74)
+300 mg Mg/d	46	End	120 <sup>b</sup> (114, 126)	0.115 <sup>b</sup> (0.110, 0.121)	138 (128, 150)	0.133 (0.123, 0.144)	1.59 (1.46, 1.72)	1.73 (1.59, 1.88)
Analysis of variance of ln values – p values								
Treatment			0.009	0.0002	0.54	0.19	0.001.	0.01
Week			0.009	0.01	0.56	0.48	0.07	0.10
Treatment x Week			< 0.0001	< 0.0001	0.10	0.17	0.61	0.44

<sup>1</sup> Values not followed by the same letter are significantly different from each other as determined by Tukey's contrasts.

<sup>2</sup> Values presented are geometric means (-1 SE, +1 SE).

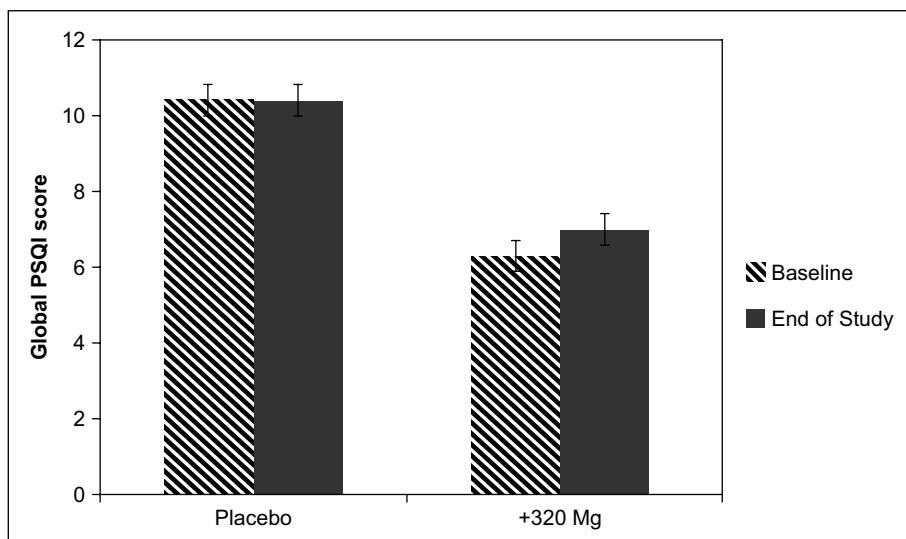
significantly more magnesium, and slightly, but not significantly ( $p = 0.10$  for diet x week interaction), more calcium than participants consuming the placebo.

*Figure 1* shows that regardless of treatment, global PSQI scores decreased from baseline to the end of the study. The decreases were from a mean of 10.4 to 7.0 in the magnesium-supplemented participants and 10.4 to 6.3 in the placebo group. Analyses of the seven components of the global PSQI, which were similar at the beginning of the study, found a significant change in only the number of sleep disturbances at the end of the study (*figure 2*). More magnesium-supplemented than placebo participants had sleep disturbance component scores of 2 or more *vs* 0-1 at the end of the study.

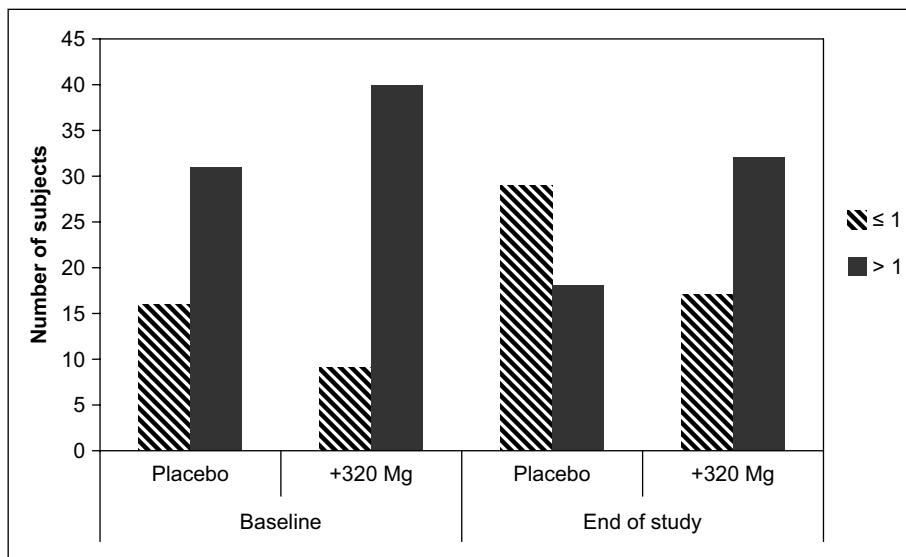
When all participants were included in the analysis, serum total and ionized magnesium and serum calcium concentrations were not significantly affected by treatment (*table 3*). In addition, when all participants were included in the analysis, blood cell counts, cholesterol, cholesterol fractions, triglycerides, and glucose were not affected by treatment (data not shown). However, erythrocyte magnesium expressed per cell or per gram of hemoglobin increased between baseline and the end of the study regardless of treatment (*table 3*).

To determine whether differences in inflammatory stress or magnesium status had an effect on

the response to the magnesium supplementation, analyses were performed on data obtained from participants with indicators suggesting a low magnesium status or low-grade inflammation. Sleep quality responses were not different from any participants when the 37 participants with baseline serum magnesium concentrations less than 1.8 mg/dL (an indication of deficient magnesium status) were included in the analysis (data not shown). However, in these 37 participants, both serum calcium and total magnesium increased from baseline to the end of the study regardless of treatment (*table 4*). The increase resulted in the mean being in the normal range ( $\geq 1.8$  mg/dL) for the magnesium-supplemented participants; however, their increase was not significantly different from that of the participants given the placebo. Ionized magnesium increased slightly, but not significantly ( $p = 0.10$ ) across both groups. Although total and ionized magnesium were lower, the percentage of serum magnesium that was ionized was significantly higher (74.6 *vs* 68.9,  $p < 0.0001$ , *t*-test) in these participants than in those with baseline serum magnesium greater than 1.8 mg/dL. Although the increase from baseline to the end of the study in erythrocyte magnesium appeared to be the same as when all participants were included in the analysis, this was not significant; apparently this was caused by the increased variability with a smaller number participants.



**Figure 1.** Effect of treatment on global Pittsburgh Sleep Quality Score (PSQI). The global PSQI score decreased significantly ( $p < 0.0001$ ) from baseline to eight weeks regardless of treatment.



**Figure 2.** Effect of treatment on the number of subjects with a PSQI Sleep Disturbances score of  $\leq 1$  or  $> 1$ . More magnesium-supplemented than placebo subjects had sleep disturbances scores greater than one at the end of the study (Chi-square  $p = 0.008$ ).

**Table 3.** Effect of treatment on serum (mean  $\pm$  SEM) total and ionized magnesium, erythrocyte (RBC) magnesium, and serum calcium in all subjects.

Treatment	N	Period	Serum Magnesium			RBC Magnesium		Serum Ca mg/dL
			Total, mg/dL	Ionized, mg/dL	Ionized, %	pg/cell	$\mu\text{g/g Hb}$	
Placebo	47	Baseline	1.85 $\pm$ 0.02	1.33 $\pm$ 0.01	72.3 $\pm$ 0.8	4.78 $\pm$ 0.09	157 $\pm$ 3	8.8 $\pm$ 0.08
Placebo	47	End	1.86 $\pm$ 0.02	1.35 $\pm$ 0.02	73.5 $\pm$ 0.7	4.94 $\pm$ 0.12	162 $\pm$ 4	8.7 $\pm$ 0.07
+300 mg Mg/d	49	Baseline	1.87 $\pm$ 0.03	1.31 $\pm$ 0.01	70.0 $\pm$ 0.8	4.72 $\pm$ 0.11	155 $\pm$ 4	8.7 $\pm$ 0.08
+300 mg Mg/d	49	End	1.92 $\pm$ 0.03	1.33 $\pm$ 0.02	69.9 $\pm$ 0.7	5.15 $\pm$ 0.14	168 $\pm$ 4	8.8 $\pm$ 0.07

©Analysis of variance - p values

Treatment	0.15	0.20	0.0001	0.53	0.57	0.79
Week	0.22	0.14	0.44	0.01	0.02	0.79
Treatment x Week	0.44	0.99	0.36	0.26	0.28	0.36

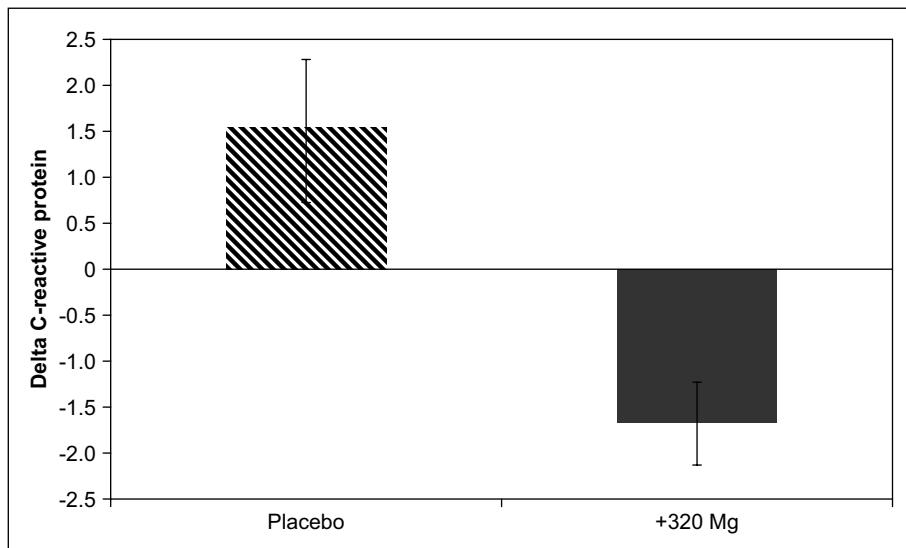
The magnesium supplementation was beneficial to the 36 participant who had plasma CRP concentrations higher than 3.0 mg/L (an indication of chronic inflammatory stress) at baseline. *Figure 3* shows that the magnesium-supplemented participants showed a decline of 1.6 mg/L while the participants consuming the placebo showed an increase of 1.5 mg/L between baseline and the end of the study; the difference between the two groups was significant ( $p < 0.002$ ). *Figure 4* shows the changes in CRP as a ratio between baseline and the end of the study. The difference between the magnesium-

supplemented mean (0.81) and the placebo mean (1.23) was significant ( $p < 0.008$ ).

The participants who were heterozygous for the Thr1482Ile polymorphism in the TRPM7 gene did not exhibit any magnesium status characteristics different than those with the normal TRPM7 gene. Only two participants (both female and on the placebo treatment) had a homozygous 1482Ile polymorphism, which is not a sufficient number to make any conclusive statements about an effect on magnesium status. However, these two participants had serum total magnesium (1.63 and 1.72 mg/dL),

**Table 4.** Effect of treatment on serum (mean  $\pm$  SEM) total and ionized magnesium, erythrocyte (RBC) magnesium, and serum calcium in subjects with baseline serum magnesium concentrations less than 1.8 mg/dL.

Treatment	N	Period	Serum magnesium			RBC magnesium		Serum Ca
			Total, mg/dL	Ionized, mg/dL	Ionized, %	pg/cell	$\mu\text{g/g Hb}$	mg/dL
Placebo	17	Baseline	1.69 $\pm$ 0.02	1.27 $\pm$ 0.02	75.3 $\pm$ 1.3	4.94 $\pm$ 0.21	160 $\pm$ 7	8.43 $\pm$ 0.11
Placebo	17	End	1.73 $\pm$ 0.02	1.30 $\pm$ 0.03	75.1 $\pm$ 1.2	4.94 $\pm$ 0.21	159 $\pm$ 7	8.52 $\pm$ 0.11
+300 mg Mg/d	20	Baseline	1.69 $\pm$ 0.02	1.25 $\pm$ 0.01	74.1 $\pm$ 1.1	4.70 $\pm$ 0.19	154 $\pm$ 6	8.36 $\pm$ 0.10
+300 mg Mg/d	20	End	1.82 $\pm$ 0.03	1.29 $\pm$ 0.02	71.1 $\pm$ 1.1	5.22 $\pm$ 0.19	170 $\pm$ 6	8.74 $\pm$ 0.13
Analysis of variance – p values								
Treatment			0.07	0.44	0.03	0.91	0.78	0.49
Week			0.001	0.10	0.19	0.20	0.27	0.04
Treatment x Week			0.12	0.93	0.24	0.20	0.19	0.21

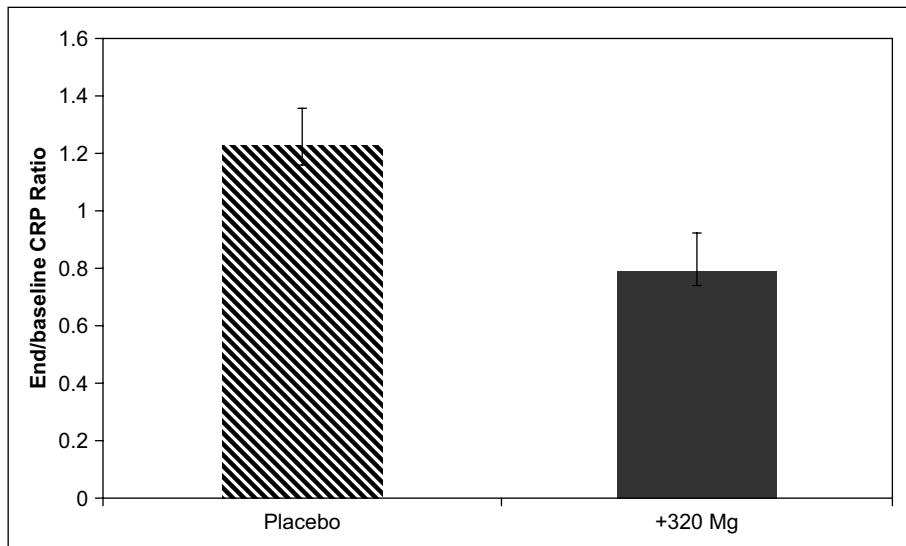
**Figure 3.** Effect of treatment on change in plasma C-reactive protein (CRP, mg/dL) between baseline and the end of the study. Compared to the placebo group, the decrease in CRP was significant ( $p = 0.001$ ).

percent ionized magnesium (80% and 84%), and urinary magnesium excretion (68 and 48 mg/day) that indicated a deficient magnesium status with dietary intakes of 238 and 289, respectively.

## Discussion

CRP apparently readily responds by increasing with sleep deprivation [25], but sleep quality apparently is not as consistently associated with an increase in chronic inflammation [10, 26]. Finding that only 40%

of the participants in the present study reporting poor sleep quality, as assessed by the PSQI, had CRP values of 3.0 mg/L or greater suggests that some of the participants in the present study, who scored high on the PSQI (poor sleep quality), were not sleep-deprived. Their poor sleep quality was apparently caused by factor(s) other than an inadequate number of hours of sleep, which often causes an increase in CRP. This may have influenced the finding that magnesium supplementation *vs* a placebo did not significantly affect sleep quality. Sleep quality improved from a mean of 10.4 during



**Figure 4.** Effect of treatment on the ratio end of study/baseline C-reactive protein (CRP) concentration. The difference between the magnesium-supplemented and placebo group was significant ( $p = 0.008$ ) ratio.

the study for all participants such that the average global PSQI score (6.6) was only slightly above the lowest score for poor quality sleep (5.0). The reason for this improvement is unclear but one possibility is that the increase was the result of a placebo effect.

The placebo effect could explain some of the findings in this study. Sleep disorders are associated with decreased erythrocyte magnesium [27, 28]. Thus, if the placebo effect improved sleep, erythrocyte magnesium may increase. This is consistent with the finding of increased erythrocyte magnesium in both groups at the end of the study. The urinary excretion results suggest that the increased erythrocyte magnesium was reflected by increased magnesium retention. The magnesium-supplemented group had the expected increase in urinary magnesium, most likely because more magnesium was available for absorption. In contrast, the placebo group showed a numerically decreased, but not significant according to Tukey's contrast, in urinary magnesium excretion from baseline to the end of the study, which hints at an increased retention of magnesium because dietary magnesium intake did not change substantially during the study with this group. Citrate also might have had an influence on the findings because it was the only known factor besides magnesium to which the participants were differently exposed during the study. The findings suggest that

further studies, in which magnesium is supplemented in another form to participants with sleep disturbances, that elevate inflammatory stress are needed to determine whether magnesium deficiency contributes to morbidity and mortality associated with chronic inflammation in participants with poor quality sleep or sleep deprivation.

Although the present study did not definitively show that improved magnesium status improved sleep quality, it did show that magnesium supplementation improved magnesium status in participants who had a low magnesium status, based on serum magnesium concentrations.

The present study also confirmed that a low dietary magnesium intake is associated with increased circulating CRP [11-15], which is a marker of inflammatory stress. In addition, an association between low magnesium status, indicated by dietary intake and BMI, was found, which is consistent with reports that a low magnesium status is associated with chronic inflammation indicators, or with diseases with a chronic inflammation component, in obesity [29-32]. The finding that magnesium supplementation decreased plasma CRP concentrations in people with elevated CRP (< 3.0 mg/L) while those on placebo also showed an increase supports the suggestion that the magnesium deficiency that occurs in the population according to NHANES data [1] contributes to chronic inflammatory stress.

The increase in the CRP ratio between baseline and the end of the study (instead of remaining close to 1.0) in the placebo group while the ratio decreased in the magnesium-supplemented group indicates that the unknown factor improving sleep quality and increasing erythrocyte magnesium did not alleviate chronic inflammatory stress indicated by a CRP value over 3.0 mg/L.

Genotyping the participants did not help in determining which subjects were more likely to be magnesium-deficient or have more severely changed magnesium status indicators with specific deficient intakes of magnesium. However, the percentage of participants with heterogeneous Thr1482Ile (18%) and with homozygous 1482Ile (2%) genotypes were similar to those reported Dai *et al.* (25.5% and 1.6% respectively) [20]. The two participants with the homozygous genotype exhibited serum and urine magnesium values that support the contention that people with this genotype may be more susceptible to magnesium deficiency.

## Conclusion

A study, in which 22 males and 78 females older than 51 years with poor sleep quality participated, found 58% were consuming less than the EAR for magnesium and 37% had serum magnesium concentrations below 1.8 mg/dL, which indicates a significant number of older adults may have subclinical magnesium deficiency. The low magnesium status indicated by dietary intakes less than the EAR was associated with increased plasma CRP and BMI. The finding that magnesium citrate supplementation compared to a sodium citrate placebo decreased plasma CRP in participants with values above 3.0 mg/dL indicates that subclinical magnesium deficiency may exacerbate conditions that result in chronic inflammatory stress. Whether magnesium deficiency contributes to chronic inflammatory stress induced by some forms of poor sleep quality was not established in the present study because some unknown factor, possibly a placebo effect, resulted in improved sleep quality in all participants during the study. However, a change in erythrocyte magnesium and different urinary magnesium excretion and the CRP response to magnesium *vs* placebo suggest that there is an association between magnesium and sleep quality that needs further study using different supplements and participants with sleep changes resulting in inflammatory stress to determine its nature.

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## Disclosure

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

## References

- Kruse HD, Orent ER, McCollum EV. Studies on magnesium deficiency in animals. Symptomatology resulting from magnesium deprivation. *J Biol Chem* 1932; 96: 519-39.
- Mazur A, Maier JAM, Rock E, Gueux E, Nowacki W, Rayssiguier Y. Magnesium and the inflammatory response: potential physiopathological implications. *Arch Biochem Biophys* 2007; 458: 48-56.
- Moshfegh A, Goldman J, Ahuja J, Rodes D, LaComb R. *What we eat in America, NHANES 2005-2006: Usual intakes from food and water compared to 1997 Dietary Reference Intakes for vitamin D, calcium, phosphorus, and magnesium*. US Department of Agriculture, Agricultural Research Service, 2009. Available at: <http://www.ars.usda.gov/ba/bhnrc/fsrg>. Accessed November 2009.
- Food and Nutrition Board, Institute of Medicine. Magnesium. In: *Dietary Reference Intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride*. Washington, DC: National Academy Press, 1997: 190-249.
- Vormann J, Günther T, Höllriegel V, Schümann K. Pathobiological effects of graded magnesium deficiency in rats. *Z Ernährungswiss* 1998; 37 (Suppl. 1): 92-7.
- Kramer JH, Mak IT, Phillips TM, Weglicki WB. Dietary magnesium intake influences circulating proinflammatory neuropeptide levels and loss of myocardial tolerance to postischemic stress. *Exp Biol Med* 2003; 228: 655-73.
- Hegsted DM, Vitale JJ, McGrath H. The effect of low temperature and dietary calcium upon magnesium requirement. *J Nutr* 1956; 58: 175-88.

8. Chaudhary DP, Boparai RK, Bansal DD. Implications of oxidative stress in high sucrose low magnesium diet fed rats. *Eur J Nutr* 2007; 46: 383-90.
9. Adrian M, Chanut E, Laurant P, Gaume V, Berthelot A. A long-term moderate magnesium-deficient diet aggravates cardiovascular risks associated with aging and increases mortality in rats. *J Hypertens* 2008; 26: 44-52.
10. Simpson N, Dinges DF. Sleep and inflammation. *Nutr Rev* 2007; 65: S244-52.
11. King DE, Mainous III AG, Geesey ME, Woolson RF. Dietary magnesium and C-reactive protein levels. *J Am Coll Nutr* 2005; 24: 161-71.
12. Bo S, Durazzo M, Guidi S, Carello M, Sacerdote C, Silli B, Rosato R, Cassader M, Gentile L, Pagano G. Dietary magnesium and fiber intakes and inflammatory and metabolic indicators in middle-aged subjects from a population-based cohort. *Am J Clin Nutr* 2006; 84: 1062-9.
13. King DE, Mainous III AG, Geesey ME, Ellis T. Magnesium intake and serum C-reactive protein levels in children. *Magnes Res* 2007; 20: 32-6.
14. Song Y, Li TY, van Dam RM, Manson JE, Hu FB. Magnesium intake and plasma concentrations of markers of systemic inflammation and endothelial dysfunction in women. *Am J Clin Nutr* 2007; 85: 1068-74.
15. Chacko SA, Song Y, Nathan L, Tinker L, De Boer IH, Tylavsky F, Wallace R, Liu S. Relations of dietary magnesium intake to biomarkers of inflammation and endothelial dysfunction in an ethnically diverse cohort of postmenopausal women. *Diabetes Care* 2010; 33: 304-10.
16. Held K, Antonijevic IA, Kunzel H, Uhr M, Wetter TC, Golly IC, Steiger A, Murck H. Oral Mg (2+) supplementation reverses age-related neuroendocrine and sleep EEG changes in humans. *Pharmacopsychiatry* 2002; 35: 135-43.
17. Popoviciu L, Delast-Popoviciu D, Delast-Popoviciu R, Bagathai I, Bicher G, Buksa C, Covaci S, Szalay E. Parasomnias (non-epileptic nocturnal episodic manifestations) in patients with magnesium deficiency. *Rev Roum Neurol Psychiatr* 1990; 28: 19-24.
18. Hornyak M, Haas P, Veit J, Gann H, Riemann D. Magnesium treatment of primary alcohol-dependent patients during subacute withdrawal: an open pilot study with polysomnography. *Alcohol Clin Exp Res* 2004; 28: 1702-9.
19. Depoortere H, Francon D, Llopis J. Effects of a magnesium-deficient diet on sleep organization in rats. *Neuro-psychobiology* 1993; 27: 237-45.
20. Dai Q, Shrubsole MJ, Ness RM, Schlundt D, Cai Q, Smalley WE, Li M, Shyr Y, Zheng W. The relation of magnesium and calcium intakes and a genetic polymorphism in the magnesium transporter to colorectal neoplasia risk. *Am J Clin Nutr* 2007; 86: 743-51.
21. International Longevity Center Workshop Report. Sleep, Health and Aging. New York, NY, International Longevity Center - USA; 2003: 25 pp.
22. Nutrient Data Laboratory. *USDA Nutrient Database for Standard Reference, Release 20*. U.S. Department of Agriculture, Agricultural Research Service 2007. Available at: <http://www.ars.usda.gov/ba/bhnrc/ndl>, accessed July 2009.
23. Buysse DJ, Reynolds III CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatric Res* 1989; 28: 193-213.
24. Pearson TA, Mensah GA, Alexander RW, Anderson AL, Cannon 3<sup>rd</sup> RO, Criqui M, Fadl YY, Fortmann SP, Hony Y, Myers GL, Rifai N, Smith Jr SC, Taubert K, Tracy RP, Vinicor F. AHA/CDC scientific statement. Markers of inflammation and cardiovascular disease. *Circulation* 2003; 107: 499-511.
25. Meier-Ewert HK, Ridker PM, Rifai N, Regan MM, Price NJ, Dinges DF, Mullington JM. Effect of sleep loss on C-reactive protein, an inflammatory marker of cardiovascular risk. *J Am Coll Card* 2004; 43: 678-83.
26. Prinz PN, Bailey SL, Woods DL. Sleep impairments in healthy seniors: roles of stress, cortisol, and interleukin-1 beta. *Chronobiol Int* 2000; 17: 391-404.
27. Popoviciu L, Bagathai J, Buksa C, Delast-Popoviciu D, Bicher G, Delast-Popoviciu R, Covaci S, Szalay E. Clinical and polysomnographic researches in patients with sleep disorders associated with magnesium deficiencies. In: Lasserre B, Durlach J, eds. *Magnesium – a relevant ion*. London: John Libbey, 1991: 353-65.
28. Osada N, Suzuki N, Nakayama M, Yokoyama Y, Yamamoto A, Oya M, Murabayashi T, Yamamoto M, Omiya K, Itoh H, Murayama M. Erythrocyte magnesium and prostaglandin dynamics in chronic sleep deprivation. *Clin Cardiol* 1997; 20: 265-8.
29. Rodríguez-Morán M, Guerrero-Romero F. Elevated concentrations of TNF-alpha are related to low serum magnesium levels in obese subjects. *Magnes Res* 2004; 17: 189-96.
30. Corica F, Allegra A, Lentile R, Buemi M. Magnesium concentrations in plasma, erythrocytes, and platelets in hypertensive and normotensive obese subjects. *Am J Hypertens* 1997; 10: 1311-3.
31. Lee S, Park HK, Son SP, Lee CW, Kim U, Kim HJ. Effects of oral magnesium supplementation on insulin sensitivity and blood pressure in normomagnesemic nondiabetic overweight Korean adults. *Nutr Metab Cardiovasc Dis* 2009; 19: 781-8.
32. Huerta MG, Roemmich JN, Kington ML, Bovbjerg V, Weltman AL, Holmes VF, Patrie JT, Rogol AD, Nadler JL. Magnesium deficiency is associated with insulin resistance in obese children. *Diabetes Care* 2005; 28: 1175-81.