The Circulating Concentration and 24-h Urine Excretion of Magnesium Dose- and Time-Dependently Respond to Oral Magnesium Supplementation in a Meta-Analysis of Randomized Controlled Trials[1–3]

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Abstract

Background: Accurate determination of Mg status is important for improving nutritional assessment and clinical risk stratification.

Objective: We aimed to quantify the overall responsiveness of Mg biomarkers to oral Mg supplementation among adults without severe diseases and their dose- and time responses using available data from randomized controlled trials (RCTs).

Methods: We identified 48 Mg supplementation trials ($n = 2131$) through searches of MEDLINE and the Cochrane Library up to November 2014. Random-effects meta-analysis was used to estimate weighted mean differences of biomarker concentrations between intervention and placebo groups. Restricted cubic splines were used to determine the dose- and time responses of Mg biomarkers to supplementation.

Results: Among the 35 biomarkers assessed, serum, plasma, and urine Mg were most commonly measured. Elemental Mg supplementation doses ranged from 197 to 994 mg/d. Trials ranged from 3 wk to 5 y (median: 12 wk). Mg supplementation significantly elevated circulating Mg by 0.04 mmol/L (95% CI: 0.02, 0.06) and 24-h urine Mg excretion by 1.52 mmol/24 h (95% CI: 1.20, 1.83) as compared to placebo. Circulating Mg concentrations and 24-h urine Mg excretion responded to Mg supplementation in a dose- and time-dependent manner, gradually reaching a steady state at doses of 300 mg/d and 400 mg/d, or after ~20 wk and 40 wk, respectively (all P-nonlinearity $\leq$ 0.001). The higher the circulating Mg concentration at baseline, the lower the responsiveness of circulating Mg to supplementation, and the higher the urinary excretion (all P-linearity < 0.05). In addition, RBC Mg, fecal Mg, and urine calcium were significantly more elevated by Mg supplementation than by placebo (all P-values < 0.05), but there is insufficient evidence to determine their responses to increasing Mg doses.

Conclusions: This meta-analysis of RCTs demonstrated significant dose- and time responses of circulating Mg concentration and 24-h urine Mg excretion to oral Mg supplementation. *J Nutr* doi: 10.3945/jn.115.223453.

Keywords: Mg status, Mg biomarkers, circulating and urine Mg, meta-analysis, randomized controlled trial

Introduction

Magnesium (Mg) is an essential cofactor in hundreds of enzymatic reactions in the human body (1). Mg deficiency or insufficiency, as defined by low circulating Mg concentrations, has been associated with a variety of chronic diseases, especially cardiometabolic diseases (2–4). Mg is found in whole grains, green leafy vegetables, legumes, and nuts (2) but is substantially lost during food refining and processing (3, 4). Mg intake is suboptimal in the US general population (5, 6), particularly among adolescent females, adult females, and the elderly; it is estimated that 70% of the elderly American population has a total Mg intake below the estimated average requirement (7). Mg is currently included in the list of shortfall

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nutrients in the 2015 Dietary Guidelines Advisory Committee Report (8).

Accurate assessment of Mg status is crucial for clinical evaluation of Mg deficiency and associated health endpoints (9). Although accumulating epidemiological evidence suggests associations between low circulating (serum/plasma) Mg concentrations or urinary Mg excretion and cardiometabolic diseases (10, 11), it remains unclear whether and to what extent measurements of circulating or urine Mg concentrations are modifiable. Further, the effects of Mg supplementation on related nutritional biomarkers, such as calcium (Ca^{2+}) and parathyroid hormone (PTH)[11], are unclear.

To comprehensively assess the responsiveness of Mg biomarkers and Mg-related biomarkers to oral Mg supplementation, we conducted a meta-analysis of randomized controlled trials (RCTs), to assess their dose- and time responses to Mg supplementation. In addition, we explored potential sources of between-study heterogeneity by prespecified factors that may influence Mg status responsiveness, such as age, sex, ethnicities, baseline Mg status, cardiometabolic health status [diabetes, hypertension, or cardiovascular diseases (CVD)], Mg formulation, trial sample size, and quality.

Methods

Search strategy. We followed PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines for meta-analyses of RCTs (12). Searches were conducted in MEDLINE and the Cochrane Library up to November 1, 2014. The words "magnesium," "Mg," "supplementation," "supplement," "intervention," "depletion," "randomized controlled trial," "randomized clinical trial," "randomized trial," "controlled trial," and "clinical trial" were used in article texts and MeSH (Medical Subject Headings) terms in searches. We also manually searched additional eligible trials from the references of relevant original or review papers. All searches were limited to articles published in English.

Selection criteria. We included RCTs of oral Mg supplementation in adults, which evaluated Mg biomarkers at baseline and after the intervention. Exclusion criteria are listed as follows: *1)* studies involving pregnant or lactating women; *2)* nonrandomized, open-label, or uncontrolled studies; and *3)* studies of patients with malignancy, severe anemia, severe infectious disease, severe liver or renal diseases, and other severe illnesses, because these disease conditions might directly or indirectly affect normal Mg metabolism. Studies that used combination supplements with Mg in the intervention group were eligible only if the same combined supplements without Mg were included in the control group. Trials comparing multiple micronutrients containing Mg to placebo/blank controls were ineligible.

Study selection. Two authors (X Zhang and Y Song) independently examined the title and abstract of each article to remove irrelevant and duplicated results first. Then, any articles deemed potentially eligible underwent a full-text review, and their eligibilities were assessed based on the same inclusion and exclusion criteria. Any discrepancies were resolved through discussion.

Data extraction. From each included study, we extracted available data on the first author's name, year of publication and country, sex, mean age or age range, number of participants, comorbidities, combination therapy, baseline dietary Mg level, study design, trial duration, formulation and dose of Mg supplements, types of Mg biomarkers assessed, and means and SDs of biomarkers in both Mg and control groups before and after supplementation.

One study compared multiple dose intervention groups with a single placebo or control group. To avoid correlation error and multiple comparisons, we divided the shared control group into 2 independent small groups with the means and SDs weighted by the corresponding sample sizes of intervention groups (13). If repeated measures of Mg biomarker at several time points were reported in a single trial, the values at the end of the study were selected for overall meta-analysis; however, both were included in subgroup analyses when estimates were stratified into separate groups by prespecified factors.

Assessment of risk of bias. Trial quality was evaluated according to the Agency for Healthcare Research & Quality criteria for quality assessment of RCTs (14, 15). The evaluation criteria include adequate sequence generation for randomization, allocation concealment, blinding of outcomes assessors, similarity of groups at baseline, selective reporting, incomplete outcome data, and description of losses and exclusions. Each study was judged to be of either high, low, or unclear risk for each criterion. In order to evaluate the potential confounding effect from trial quality, we also calculated a 5-item Jadad Score (16, 17) and rated each individual trial as being of either low (<4) or high (≥4) quality.

Statistical methods. The primary measures of interest were changes in the concentrations of Mg biomarkers in response to Mg supplementation. The secondary outcomes were changes in the concentrations of indirect but Mg status–related biomarkers, including serum calcium, potassium, PTH, and vitamin D, if available. To evaluate the overall responsiveness of biomarkers, we compared the mean changes between treatment and placebo groups, calculated as weighted mean differences (WMDs) and 95% CIs using a random-effects meta-analysis model (18). Standardized mean differences were estimated only when it was difficult to standardize the measure scales of data from individual studies. We calculated the WMDs for serum and plasma Mg concentrations separately and found similar magnitude and patterns of these responses. Thus, we analyzed and presented circulating Mg concentrations by pooling the results of serum and plasma Mg concentrations from independent trials. Urine Mg was measured as 24-h urine Mg excretion (in mmol/24 h) in this study. We examined between-study heterogeneity by Q test and I^2 statistics, with $I^2 \geq 75\%$ indicating high heterogeneity.

To explore potential sources of heterogeneity and assess robustness of the results, we conducted subgroup analyses stratified by age, sex, ethnicities, baseline Mg status, cardiometabolic health status (diabetes, hypertension, or CVD), Mg formulation, trial sample size, and quality. In addition, sensitivity analyses were conducted by removing 1 study at a time to check if a single study substantially influenced the summary measure of each meta-analysis. We examined possible publication bias by visual inspection of funnel plots and formal tests, including Begg's adjusted rank correlation test and Egger's regression asymmetry test (19, 20).

Restricted cubic spline regression analyses were performed to assess the dose- and time-response relations of biomarkers to Mg supplementation. For each study, we calculated restricted cubic splines with 3 fixed knots at 10%, 50%, and 90% percentiles based on the overall distributions of doses and trial durations of all included studies. Then we combined the estimates to depict dose- and time-dependent linear or nonlinear relations of Mg biomarker responsiveness to Mg supplementation (21, 22).

A 2-tailed $P < 0.05$ was considered statistically significant. Stata (Version 13; StataCorp) was used for all statistical analyses.

Results

Our systematic search initially identified 1766 articles (**Figure 1**). Among them, 38 articles met inclusion criteria, consisting of 48 RCTs assessing 35 Mg biomarkers (**Supplemental Table 1**). A total of 2131 adult participants were studied: 1105 in the randomly assigned Mg supplement groups and 1026 in the placebo groups. Trial durations ranged from 3 wk to 5 y (median: 12 wk). Participants had a median age of 47 y (range: 17–85 y); 56% of
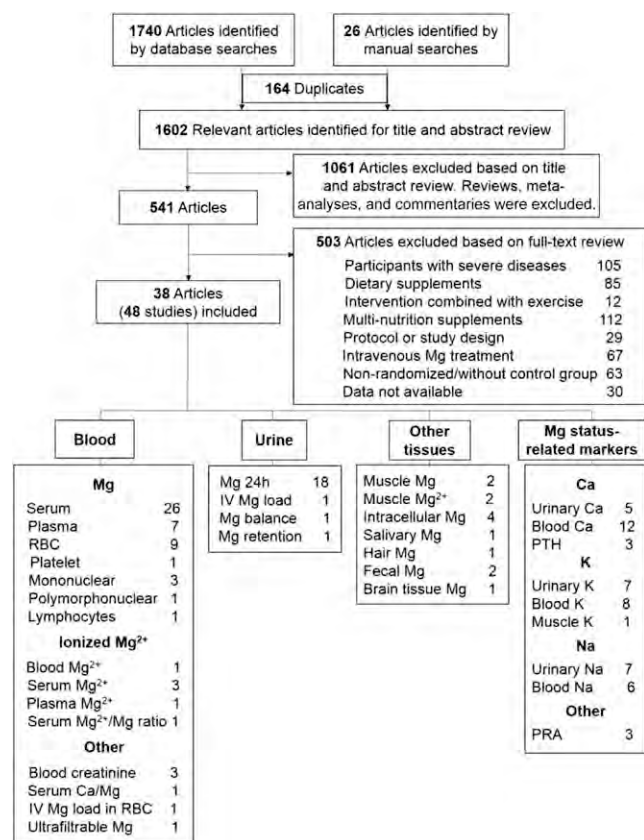

FIGURE 1 Flow chart of study selection of eligible 48 RCTs for the meta-analysis. Mg^{2+}, ionized Mg; Mg^{2+}/Mg, ratio of serum ionized Mg to total serum Mg; PRA, plasma renin activity; PTH, parathyroid hormone; RCT, randomized controlled trial.

participants were women, and 58.4% were considered healthy. Doses of Mg supplements varied widely from 197 to 994 mg elemental Mg/d (median: 360 mg/d). Two major types of Mg salts, organic (50% trials) and inorganic, were administered based on 9 Mg formulations. Among them, the 6 organic formulations were Mg orotate, Mg citrate, Mg aspartate, Mg gluconate, Mg pidolate, and Mg lactate; the 3 inorganic formulations were $Mg(OH)_2$, MgO, and $MgCl_2$.

Of 35 identified biomarkers, serum and plasma Mg were most commonly used (41 trials), followed by 24-h urine Mg (24 trials), RBC Mg (9 trials), and ionized Mg (5 trials). Only a few articles evaluated Mg status in other compartments, such as muscle, intracellular, saliva, hair, feces, and brain tissue (Figure 1). In addition, 16 publications provided data on biomarkers indirectly related to Mg status, including calcium, potassium, and sodium concentrations in serum/plasma or urine, serum Ca/Mg ratio, PTH, and plasma renin activity.

Circulating Mg concentrations. Circulating Mg (serum or plasma) was the most common biomarker assayed (41 trials), accounting for 87% of eligible articles (941 participants in treatment and 953 in control arms). After Mg supplementation at a median dose of 365 mg/d (range: 197–994 mg/d) for 12 wk (range: 3 wk–5 y), circulating Mg concentrations were significantly elevated in the treatment groups in comparison to the placebo groups (WMD: 0.04 mmol/L; 95% CI: 0.02, 0.06) (**Table 1**).

Among participants receiving Mg supplementation with a median dose of 365 mg/d for a median duration of 12 wk, the

overall population distribution shifted from a mean circulating Mg concentration of 0.78 mmol/L at baseline to 0.83 mmol/L at post-treatment (**Figure 2**A).

24-h Urine Mg excretion. Approximately half (55%) of the 24 included trials examined 24-h urine Mg excretion (645 participants in treatment and 716 in control arms). After supplementation with a median dose of 480 mg/d (range: 200–994 mg/d) for a median duration of 3 mo (range: 1 mo-1 y), 24-h urine Mg excretion was significantly elevated (WMD: 1.52 mmol/24 h; 95% CI: 1.20, 1.83) compared to the placebos groups (Table 1). The mean urine Mg excretion in the treatment groups significantly increased by 32% (WMD: 1.24 mmol/24 h; 95% CI: 0.94, 1.54) after treatment compared to baseline (Figure 2B).

RBC Mg. Nine trials examined RBC Mg, with 213 participants receiving Mg supplements and 208 receiving placebos. After Mg supplementation with a median dose of 320 mg/d (range: 250–600 mg/d) for a median duration of 2 mo (range: 3 wk–5 y), RBC Mg was significantly higher in the treatment groups than placebo groups (WMD: 0.12 mmol/L; 95% CI: 0.03, 0.20) (Table 1). A similar dose of 300 mg/d was used in all these 9 trials, which could not allow us to examine the dose-response relation for RBC Mg.

Ionized Mg. Only 5 trials assessed ionized (intracellular) Mg: 3 in serum, 1 in plasma, and 1 in whole blood. Two trials evaluated ionized Mg in muscles. Overall, circulating ionized Mg concentrations were not significantly increased among 111 participants who received Mg supplements at a median dose of 320 mg/d (range: 197–360 mg/d) for 2 mo (range: 1–6.5 mo) compared with 107 participants who received placebos (WMD: 0.004 mmol/L, $P = 0.58$). Two individual studies measured muscle ionized Mg; no significant differences between treatment and placebo groups were observed (23, 24).

Other Mg biomarkers in blood, urine, or other specimens. Because only a few trials examined intravenous Mg load, Mg balance, Mg retention, and mononuclear Mg, insufficient data were available for a meta-analysis of these biomarkers (Table 1).

Mg in other tissues such as intracellular (4 trials), muscle (2 trials), feces (2 trials), hair (1 trial), saliva (1 trial), and brain tissue (1 trial) was also assessed. Among these biomarkers, only fecal Mg concentrations were significantly elevated compared to placebo group after supplementation; the standardized mean difference based on 2 trials was 3.57 (95% CI: 1.59, 5.56) (Table 1). In addition, 2 trials evaluated muscle Mg concentrations, but no significant changes in this biomarker were observed (WMD: −0.20 mmol/L; 95% CI: −0.50, 0.10).

Other Mg status–related biomarkers. Among circulating and urine calcium, sodium, and potassium (Table 1), only urine calcium excretion was significantly increased by Mg supplementation to 0.40 mmol/24 h (95% CI: 0.08, 0.72; $n = 5$ trials). The median Mg dose administered was 360 mg/d (range: 320–485 mg/d) for 16 wk (range: 7 wk–6 mo). Three trials also evaluated the changes of plasma renin activity and plasma PTH; no significant differences between intervention and placebo arms were observed.

For all above analyses, no significant publication bias was detected by either Egger's test or Begg's test (all P values > 0.05).

Dose- and time-dependent responses of circulating and urine Mg concentrations. **Figure 3**A, B shows nonlinear

Biomarkers	Studies, n	Participants, (placebo/treatment), n	Pooled effect sizes, WMD (95% CI)	P values for WMD/SMD	Measures of heterogeneity, I^2, %	P values for heterogeneity[2]
Mg						
Serum Mg, mmol/L	29	(705/683)	0.05 (0.02, 0.07)	<0.0001	98.60	<0.0001
Plasma Mg, mmol/L	12	(236/270)	0.03 (0.01, 0.05)	<0.0001	85.90	<0.0001
Serum/plasma Mg,[3] mmol/L	41	(941/953)	0.04 (0.02, 0.06)	<0.0001	98.10	0.003
RBC Mg, mmol/L	9	(416/426)	0.12 (0.03, 0.20)	0.006	91.50	<0.0001
Mononuclear Mg[4]	4	(292/236)	0.08 (−0.22, 0.38)	0.60	0.00	0.54
Blood ionized Mg, mmol/L	5	(214/222)	0.004 (−0.011, 0.019)	0.58	94.60	<0.0001
24-h Urine Mg, mmol/24 h	24	(1656/1527)	1.52 (1.20, 1.83)	<0.0001	96.90	<0.0001
Muscle Mg, mmol/100 g	2	(118/118)	−0.20 (−0.50, 0.10)	0.08	35.30	0.21
Muscle ionized Mg, mmol/L	2	(50/50)	−0.01 (−0.05, 0.03)	0.32	47.90	0.17
Intracellular Mg[4]	4	(126/136)	−0.39 (−1.51, 0.72)	0.49	86.50	<0.0001
Fecal Mg[4]	2	(80/80)	3.57 (1.59, 5.56)	<0.0001	87.10	<0.0001
Mg status–related markers						
Blood Ca, mmol/L	12	(560/617)	0.01 (−0.01, 0.03)	0.17	82.90	<0.0001
Blood K, mmol/L	8	(340/385)	0.08 (−0.14, 0.31)	0.47	87.20	<0.0001
Blood Na, mmol/L	6	(184/227)	0.13 (−0.73, 0.99)	0.76	34.80	0.18
PTH, pg/mL	3	(134/140)	1.13 (−10.4, 12.7)	0.85	84.80	0.001
PRA[4]	3	(72/113)	0.03 (−0.48, 0.53)	0.91	11.40	0.32
24-h urine Ca, mmol/24 h	5	(428/334)	0.40 (0.08, 0.72)	0.02	81.30	<0.0001
24-h urine K, mmol/24 h	7	(484/425)	2.67 (−1.16, 6.51)	0.17	52.90	0.05
24-h urine Na, mmol/24 h	7	(472/413)	2.54 (−9.80, 14.87)	0.69	77.40	<0.0001

[1] PRA, plasma renin activity; PTH, parathyroid hormone; RCT, randomized controlled trial; SMD, standard mean difference; WMD, weighted mean difference.

[2] P values were calculated for testing the between-study heterogeneity.

[3] Independent trials with either serum or plasma Mg concentrations were pooled together.

[4] The pooled effect was SMD because the units varied among studies.

dose- and time responses of circulating Mg to Mg supplementation (all P-nonlinearity < 0.0001). Based on data from 41 eligible trials, circulating Mg concentrations appeared to increase immediately after supplementation and gradually reached a plateau concentration at a dose of ~300 mg/d (range: 300–994 mg/d) and at a duration of 20 wk (range: 20–52 wk).

As shown in Figure 3C, D, urine Mg excretion increased during the first 5 wk of supplementation, and then slowly reached a maximal steady state at a dose of ~400 mg elemental Mg/d, and after about 40 wk.

Heterogeneity. Between-study heterogeneity was high for circulating Mg, RBC Mg, urine Mg, and ionized Mg; all P values from Q-tests were <0.0001 (all I^2 > 80%) (Table 1). Sensitivity analysis showed that the overall results were robust and no single study affected the meta-analytic estimate by >5%.

Although no significant heterogeneity was observed by specific supplement formulation (Figure 4), circulating Mg concentrations in response to inorganic Mg salt were 4 times higher than the response to organic Mg salt (P-interaction = 0.006). The WMD of circulating Mg for inorganic and organic was 0.07 mmol/L (95% CI: 0.03, 0.10) and 0.015 mmol/L (95% CI: 0.002, 0.029), respectively. In addition, a larger WMD for circulating Mg was observed in the ≥65 y subgroup than <65 y subgroup, although the difference was not statistically significant.

To examine the relation between baseline Mg status and circulating and urine Mg responses, we stratified all eligible trials into 4 subgroups by baseline circulating Mg concentrations (<0.77, 0.77–0.79, 0.80–0.86, and ≥0.87 mmol/L) and 4 subgroups by baseline 24-h urine Mg excretion (<3.15, 3.15–3.63, 3.64–4.62, and ≥4.63 mmol/24 h). We found that high baseline circulating Mg concentrations were associated with less

of an increase in circulating Mg by supplementation and greater 24-h urinary Mg excretion (Figure 5A, C) (both P-linearity < 0.05). Baseline 24-h urine Mg excretion was positively and significantly associated with changes in urine Mg excretion (P-linearity = 0.03) (Figure 5D), but not changes in circulating Mg concentrations in response to supplementation (P = 0.95) (Figure 5B).

Quality of trials. Due to insufficient information in the descriptions of included articles, only 21.1% seemed to have adequate sequence generation and 7.9% had low risk of bias in allocation concealment (Supplemental Figure 1). Sample sizes of included trials were small, ranging from 13 to 155 participants. High-quality studies accounted for 71.0% of the included trials. However, neither study quality nor sample size substantially affected biomarker responses to Mg supplements (Figure 4).

Discussion

In this meta-analysis of 48 RCTs examining a total of 35 Mg biomarkers, circulating and urine Mg, the most frequently measured biomarkers, were significantly increased by Mg supplementation in a dose- and time-response manner. Effect modification by baseline circulating Mg concentrations and inorganic compared to organic formulation of Mg supplement type was observed. To our knowledge, this investigation provides the most comprehensive estimates to date of the relations between Mg biomarkers and Mg supplementation and, for the first time to our knowledge, the time response of Mg biomarkers to supplementation in generally healthy populations.

In our meta-analysis, an elevation of 6% (0.05 mmol/L) in circulating Mg and of 32% (1.29 mmol/24 h) in urine Mg was observed in response to varied doses of oral Mg supplementa-
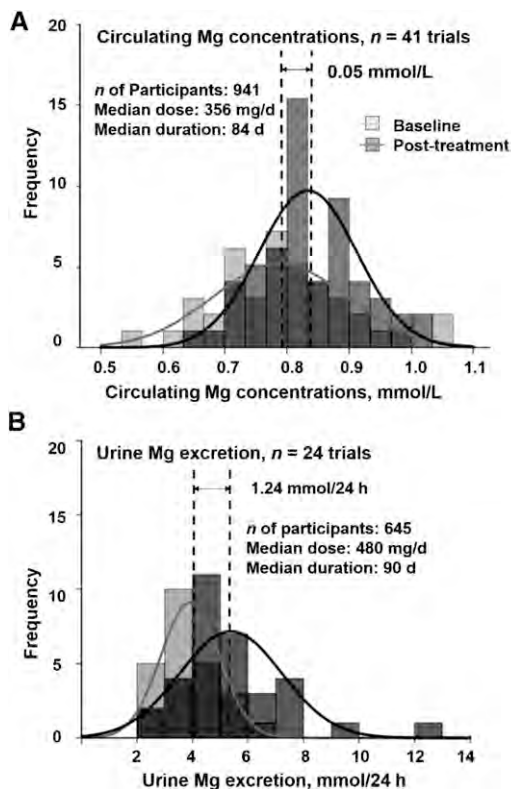
FIGURE 2 Distributions of circulating Mg concentrations (A) and 24-h urine Mg excretion (B) among participants in the treatment groups before and after Mg supplementation.

tion. With the assumption of a conservative cutoff level of serum Mg <0.70 (or <0.75 mmol/L) for defining Mg insufficiency (6), Mg supplementation substantially reduced the prevalence of insufficiency from 26% to 2.1% (or from 39% to 12%). Our analysis of 48 trials builds on and expands on findings of an earlier systematic review (22 trials) in which Mg supplementation increased circulating Mg concentrations by 0.03 mmol/L and urinary Mg excretion by 1.82 mmol/24 h (25). Altogether, this evidence suggests that Mg supplementation may reduce the risk of CVD, because previous meta-analyses of prospective studies have found that higher concentrations of circulating Mg are associated with lower CVD risk (10, 11). In addition, low 24-h urine Mg excretion was independently associated with a 40% higher risk of CVD incidence in a prospective population-based study (26).

Our novel dose- and time-response analyses revealed a maximal response of circulating Mg to oral supplementation at a dose ≥300 mg/d and showed that at least 20 wk of Mg supplementation are required to achieve this steady-state concentration. Consistent with these findings, previous pharmacokinetic research indicated a relatively slow process of Mg equilibrium in humans with a half-life of >1000 h (≈6 wk) (27). Urine Mg achieved its highest levels at doses of ≥400mg/d and required a longer duration of supplementation, 30–40 wk. Give the observed dose- and time-dependent responses, circulating and urine Mg may be clinically useful biomarkers underlying Mg homeostasis in the human body.

Our analysis revealed important heterogeneity by baseline circulating Mg concentrations: we observed that high baseline circulating Mg concentrations were associated with less of an increase in circulating Mg by Mg supplementation and greater 24-h urinary Mg excretion. If baseline circulating Mg

concentrations ≥0.87 mmol/L, a concentration level with the normal range, Mg supplementation did not result in significant changes in circulating Mg concentrations, but substantial increase in urine Mg excretion. In fact, minimal to no responsiveness of circulating Mg concentrations and elevated urinary Mg excretion to Mg supplementation was observed among participants with normal Mg status, as objectively assessed by the gold standard Mg loading test (28, 29). Taken together, the results support the notion that circulating Mg and 24-h urinary excretion Mg may provide meaningful information about underlying Mg status and provide new evidence that circulating Mg concentrations above currently defined clinical thresholds for hypomagnesemia may be appropriate for defining sufficient Mg status.

Our heterogeneity analyses also revealed, for the first time, that circulating Mg concentrations exhibit a significantly greater increase to inorganic Mg supplements than to organic supplements. The formulation of Mg is a key factor in its bioavailability. Previous work showed that organic Mg salts were more bioavailable than inorganic Mg salts supplements in animals (30) due to the limited solubility of inorganic Mg in the intestine (31, 32), a finding that seems to be contrary to our results.
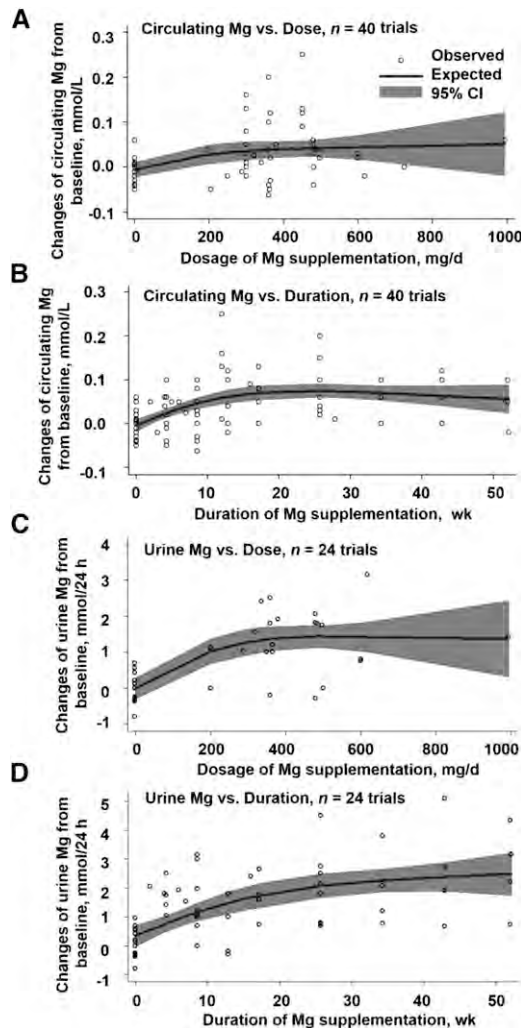

FIGURE 3 Circulating Mg concentrations (A, B) and 24-h urine Mg excretions (C, D) in response to the doses (A, C) or durations (B, D) of Mg supplementation. The relations were fitted by using restricted cubic spline curves and the 95% CIs shown as the gray shaded regions.
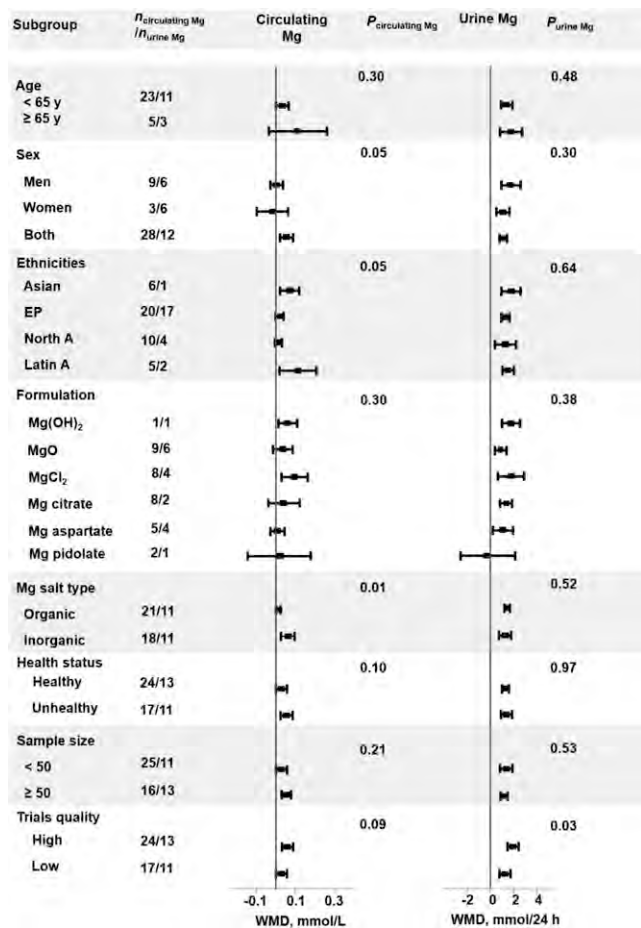
FIGURE 4 Forest plots of WMDs and their 95% CIs of responses of circulating Mg concentrations and 24-h urine Mg excretion to Mg supplementation compared to placebo stratified by age, sex, ethnicities, types of Mg supplements (compound formulation and salt type), cardiometabolic health status of participants, trial sample size, and quality of trials. Closed squares indicate the point estimates of WMDs from random-effects meta-analysis; horizontal bands represent the 95% CIs. Healthy and unhealthy status indicate participants with and without history of diabetes mellitus, cardio-vascular diseases, or hypertension. EP, Europeans; Latin A, Latin Americans; $n_{circulating\ Mg}$, number of the included trials with available data on circulating Mg concentrations; North A, North Americans; $n_{urine\ Mg}$, number of trials with available data on 24-h urine Mg excretion; $P_{circulating\ Mg}$ and $P_{urine\ Mg}$, P values for interactions between Mg supplementation and each of stratified factors on circulating Mg concentrations and 24-h urine Mg excretion, respectively; WMD, weighted mean difference.

However, circulating concentrations of Mg in the inorganic supplement groups were lower than in the organic supplement groups, which may partially explain these findings. Our additional comparisons for different chemical formulations of oral Mg supplements, including $Mg(OH)_2$, MgO, $MgCl_2$, citrate, aspartate, and pidolate Mg, did not show significantly different responses of circulating Mg concentrations and urine Mg excretion. These findings are consistent with evidence from bioavailability experiments, showing that various Mg salts were nearly equivalent in their ability to increase circulating and urine Mg (30, 33, 34).

Ionized Mg has been proposed as a potentially useful biomarker of the biologically active portion of Mg in humans (35). The limited numbers and small sample sizes of included

trials investigating ionized Mg might partially explain the null pooled effect of Mg supplementation on ionized Mg. Also, vastly different assay methods and specimens used in trials may lead to large variance in the measurements of ionized Mg, making these estimates potentially inappropriate for meta-analysis.

Other tissue Mg concentrations such as muscle, saliva, hair, fecal, and brain tissues were also reported in some trials. However, sensitivity of these biomarkers to Mg supplementation requires more data. Co-existence of secondary electrolyte abnormalities may play a key role in the clinical features of Mg depletion (36). For instance, calcium, potassium, and sodium in blood and urine were frequently assessed, and urine
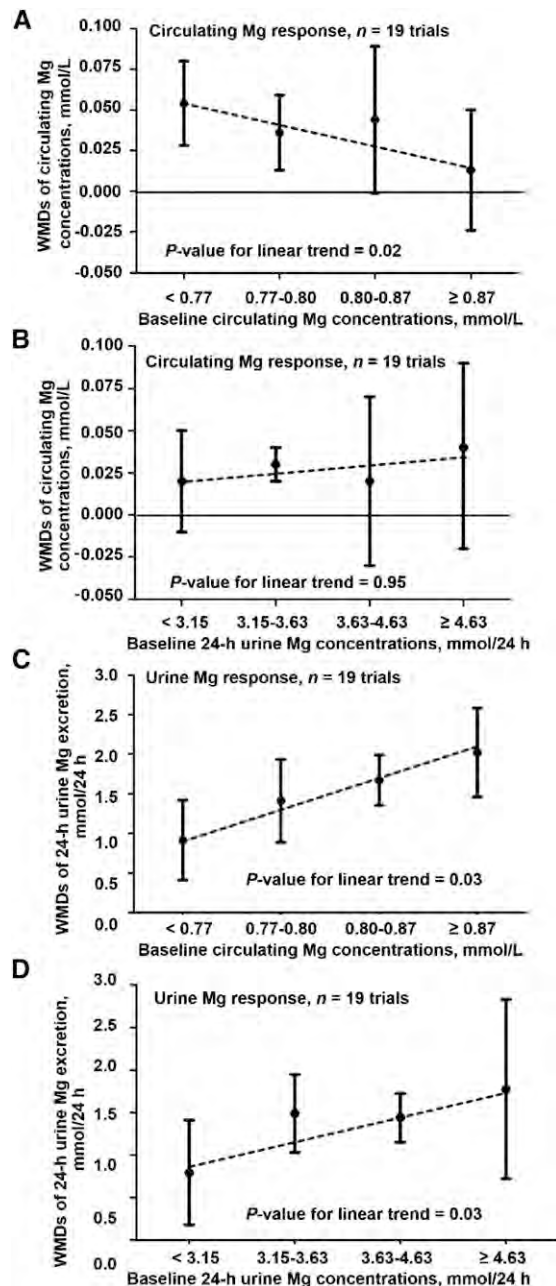

FIGURE 5 Weighted mean differences and their 95% CIs of responses of circulating Mg concentrations (A, B) and 24-h urine Mg excretions (C, D) to Mg supplementation compared to placebo stratified by baseline circulating Mg concentrations (A, C) and urine Mg excretions (B, D). WMD, weighted mean difference.

calcium concentrations appear to be more sensitive to Mg supplementation than others based on the present analysis. Despite some biological evidence suggesting that hypomagnesemia may interfere with the hypocalcemia-induced PTH release (37, 38), our analysis based on 3 RCTs showed that Mg supplementation did not significantly affect PTH concentrations.

Our meta-analysis has several strengths. Our quantitative assessment was based on data from RCTs largely of high quality, which excluded open-label and 1-arm trials, thereby minimizing selection bias and other biases. Our comprehensive search strategy make it unlikely that any major published trials were missed, to our knowledge. The quality of all trials was formally evaluated by Agency for Healthcare Research & Quality criteria and Jada score to assess the influence of overall trial quality on the results. We also systematically reviewed both direct and indirect Mg status–related biomarkers and addressed dose- and time response of Mg biomarkers to Mg supplementation for the first time, to our knowledge.

Several limitations warrant consideration. First, although a large number of randomized trials were included in our meta-analysis, few trials for biomarkers other than circulating and urine Mg were available, such as ionized Mg and Mg in muscle, saliva, and other tissues. Second, the presence of substantial between-study heterogeneity in the main meta-analyses could add uncertainty to estimates. However, we conducted several subgroup analyses stratified by many prespecified factors, such as baseline Mg status, and organic compared to inorganic formulation, which contributed to significant heterogeneity of results. Third, trials with larger sample sizes and longer durations are clearly lacking. Fourth, influence by inadequate sequence generation and allocation concealment as well as compliance could not be assessed due to a lack of relevant information in most of the included RCTs. Finally, as in any meta-analysis of published results, publication bias is possible, although we did not find any evidence of publication bias based on Egger's or Begg's tests.

In conclusion, this meta-analysis of 48 RCTs showed that circulating Mg concentrations and 24-h urine Mg excretion significantly responded to oral Mg supplementation in a dose- and time-dependent manner. High baseline circulating Mg concentrations were associated with less or no changes in circulating Mg concentrations and a greater response of urine Mg excretion by Mg supplementation, consistent with gold-standard Mg loading test findings. Altogether, these findings support the notion that circulating Mg and 24-h urinary excretion Mg may provide meaningful information about underlying Mg status and provide new evidence that a relatively high threshold level of circulating Mg concentrations, well above currently defined clinical thresholds for hypomagnesemia, may be appropriate for defining sufficient Mg status. Our findings also directly inform the design of future Mg supplementation trials. Future well-designed, adequately powered RCTs of Mg supplementation on intermediate and clinical endpoints are warranted to elucidate the potential role of Mg biomarkers for clinical risk assessment and population health.

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