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Abstract. To understand humans' requirements for magnesium and the effect of magnesium on health, it is important to identify sensitive and population-specific biomarkers of magnesium status. Thus, we assessed the effectiveness of different magnesium status biomarkers through a systematic review of published magnesium supplementation and depletion trials in healthy humans. The methods used in this study included a structured search on Ovid MEDLINE, EMBASE (Ovid) and Cochrane databases up to September 2008, followed by the use of formal inclusion/exclusion criteria, data extraction, validity assessment, and meta-analysis. A total of 20 potential biomarkers of magnesium status were assessed from 21 included publications. The majority of studies included were magnesium supplementation studies. Fewer magnesium depletion studies were identified. Available data analysis suggests that serum/plasma magnesium concentration, red blood cell (RBC) concentration and urinary magnesium excretion responded to dietary manipulation. For other biomarkers with available data, it was not possible to draw any conclusions about their usefulness as magnesium status biomarkers. The lack of data prevented detailed subgroup analysis. In conclusion, although limited data were available, serum/plasma magnesium concentration, RBC magnesium concentration and urinary magnesium excretion appear to be useful biomarkers of magnesium status in the general population. Further high-quality studies are needed to assess the effectiveness of existing and newly developed biomarkers, especially in populations that are vulnerable to magnesium deficiency.

Key words: magnesium, status, systematic review

Magnesium (Mg^{2+}) is the second most abundant cation within the cell. Magnesium plays an essential role in a wide range of fundamental biochemical reactions and cellular functions, including cell cycle, channel regulation, membrane and nucleic acid stability and is a cofactor for hundreds of enzymes [1]. Therefore, it is not surprising that many clinical disorders are associated with magnesium deficiency [2, 3]. Recommendations for magnesium intake are pro-

vided in the "Dietary Reference Intakes (DRIs)" [4] and in the "Apports nutritionnels conseillés pour la population française (ANC)" [5]. The magnesium content of food in the Western countries is consistently decreasing; currently, dietary magnesium in many adults does not meet the recommended intake [6-11]. This low magnesium intake leads to an increased risk of latent magnesium deficiency, which is difficult to diagnose. It is generally accepted that assessments of magnesium status are problematic [12, 13]. The assessment difficulty results from the distribution of magnesium in the body. About half of the total body magnesium is in the bone and

the other half is in the soft tissues. Extracellular magnesium accounts for only about 1 percent of total body magnesium. Therefore, the measurement of blood plasma/serum magnesium does not exactly reflect its intracellular level. In healthy subjects, there is a balance between intestinal magnesium absorption and urinary excretion with the latter being of greater importance. Urinary excretion increases when magnesium intake is in excess, whereas the increased kidney reabsorption conserves magnesium in the case of magnesium deprivation. Therefore, urine magnesium reflects magnesium intake and can provide information on an individual's magnesium status. However, factors affecting kidney filtration, such as diabetes, diuretics, and renal dialysis, strongly influence urinary magnesium, limiting its value in several pathophysiological conditions. Homeostasis of magnesium is mainly maintained via bone stores. Approximately one third of magnesium in bone is freely exchangeable and acts as a reserve for maintaining extracellular magnesium concentrations [13]. For these magnesium metabolism-related evidences, extensive research has been conducted on the evaluation of magnesium in a variety of blood cells and on techniques measuring magnesium concentrations in tissues. Recently published reviews [12, 13] provide an extensive update on the assessment of magnesium status.

The aim of this systematic review is to assess the usefulness of magnesium status biomarkers in healthy humans to determine which biomarkers appropriately reflect changes in magnesium status in response to supplementation or depletion. The methodology of this review is based on the standard methodology developed for the set of reviews within the Eurreca project (EURopean micronutrient RECommendations Aligned) (14) and also related to other minerals [15-18].

Methods

An abbreviated version of the methodology used in this review is provided below with differences from the main methodology noted [14].

Study selection

To be included, a study needed to meet all of the following criteria: 1) an intervention study in humans (including supplementation and/or depletion studies) with no restrictions in study design, including randomised controlled trials (RCTs), controlled clinical trials (CCTs), and before-and-after studies (B/A); 2) report the magnesium status in humans at baseline and after supplementation or depletion; 3) report the daily dose of the supplement and involve magnesium supplementation with magnesium salts that are considered bioavailable; and 4) involve healthy participants who had not recently used mineral or vitamin supplements.

Study inclusion was not limited by the age of the participants and included studies of infants through the elderly. Studies were excluded if they included subjects receiving concomitant therapy for chronic illnesses, nutritional deficiencies other than magnesium, or if the studies' subjects had a condition known to affect magnesium metabolism, such as diabetes, severe kidney diseases, renal dialysis, or alcoholism. Studies were excluded if suitable baseline data were unavailable, if information on the statistical variance of the data was not accessible, and if methods for the status assessment were not found.

Data collection

We searched Ovid MEDLINE (www.ovid.com), EMBASE (Ovid; www.ovid.com), and the Cochrane Library CENTRAL (www.thecochranelibrary.com) databases from inception to September 2008 for magnesium intervention studies using text terms with appropriate truncation and relevant indexing terms. The general structure of the search was "magnesium" and "intervention OR supplementation OR depletion" and "humans."

Titles and abstracts were screened for inclusion by a single reviewer. The full text of all articles collected was screened for inclusion by using an inclusion and exclusion form by two independent reviewers. Data for each included study were extracted into an Access (Microsoft Corp, Redmond, WA) database file by a single reviewer. In unclear cases, studies were discussed with the review team before beginning full data extraction, and, in some cases, study authors were contacted for clarification. When necessary, units of measurement were converted to a standard form to facilitate comparison across studies. Data extraction and synthesis for primary and

secondary measures of interest were undertaken as discussed in the methodology article [14]. To claim whether a biomarker was useful, the following terms indicate the conditions needed to be met: "yes", forest plot showed a significant effect ($p < 0.05$) based on ≥3 studies and ≥50 participants between the intervention and control arms; "unclear but likely to be useful" forest plot showed a significant effect ($p < 0.05$), but the result was based on two studies; "unclear", insufficient data were available.

Results

The flow diagram for this review is shown in *figure 1*. Of the 1,298 titles and abstracts screened after electronic and bibliographic searches, 66 appeared potentially relevant and were collected as full-text articles to be assessed for inclusion. At this stage, studies were excluded for a variety of reasons, including unsuitable study designs, such as nonhuman studies, single-case studies, studies not involving magnesium supplementation or depletion, studies without magnesium status measurements, studies on patients with pathologies known to affect magnesium status, and/or studies on patients receiving medication likely to affect magnesium status.

After analysis of the 66 potentially relevant full-text articles, twenty-seven studies (reported in 21 publications) fulfilled all of the inclusion criteria, and details of the included study characteristics are shown in *table 1*. Studies were excluded if the type of magnesium supplement was not stated, the study design involved multi-nutrient supplementation, the study did not report baseline and/or subsequent magnesium status, no minimal dose was provided, no control group was studied, a short-term study was performed (*e.g.,* only 24 h supplementation), or different population groups were used as supplemented and controls. Studies with altered data also resulted in exclusion. However, the majority of exclusion was due to imprecise data (results presentation or no methodology of status assessment *(figure 1)*. A total of 20 potential biomarkers of magnesium status were assessed within the 27 included studies from 21 articles. The characteristics of the studies included in the analysis are presented in *table 1*. The quality of the included studies varied; 12 RCTs were included in the review, but the method of randomisation was only stated in three studies. The remaining studies included CCT and B/A studies. Methods for checking compliance were reported in only 11 studies. The numbers and reasons for dropouts were reported in eight studies. Full information pertaining to the quality assessment of each study is shown in *table 2*.

Among the identified biomarkers, only serum magnesium, plasma magnesium, RBC magnesium, and urinary magnesium excretion/24 h were reported in more than three studies with a total of more than 50 studied subjects; therefore, it was reliable to evaluate the effectiveness of these biomarkers. Ionised magnesium in serum, plasma or blood was reported in two, one and two studies, respectively. When combined, there were five studies on plasma/serum/blood ionised magnesium with 51 subjects *(figure 1, table 3)*. Saliva magnesium was evaluated in a three-arm supplementation study with 36 included subjects from one publication *(table 3)*. Studies of other markers were limited to one or two studies and, therefore, were not eligible to allow us to decide whether they were effective markers of magnesium status *(table 3)*.

Serum and plasma total magnesium concentration

A large proportion of the studies included in this review measured serum (15 studies) or plasma (7 studies) magnesium concentration. All of the supplementation and depletion studies measuring serum and plasma magnesium were combined for statistical analyses. Serum/plasma magnesium concentration was investigated as a marker of magnesium status in 18 supplementation studies involving 275 participants and four depletion studies involving 47 participants. Combining data from the depletion and supplementation studies (322 subjects), primary analysis revealed an overall significant ($p < 0.02$) response of serum/plasma magnesium concentration to magnesium intake [weighted mean difference (WMD): 0.03 mmol/L; 95% CI: 0.01, 0.06; I^2 96%]. However, the depletion studies did not detect changes in this parameter *(table 3)*. This biomarker was affected by magnesium supplementation *(figure 2)*.
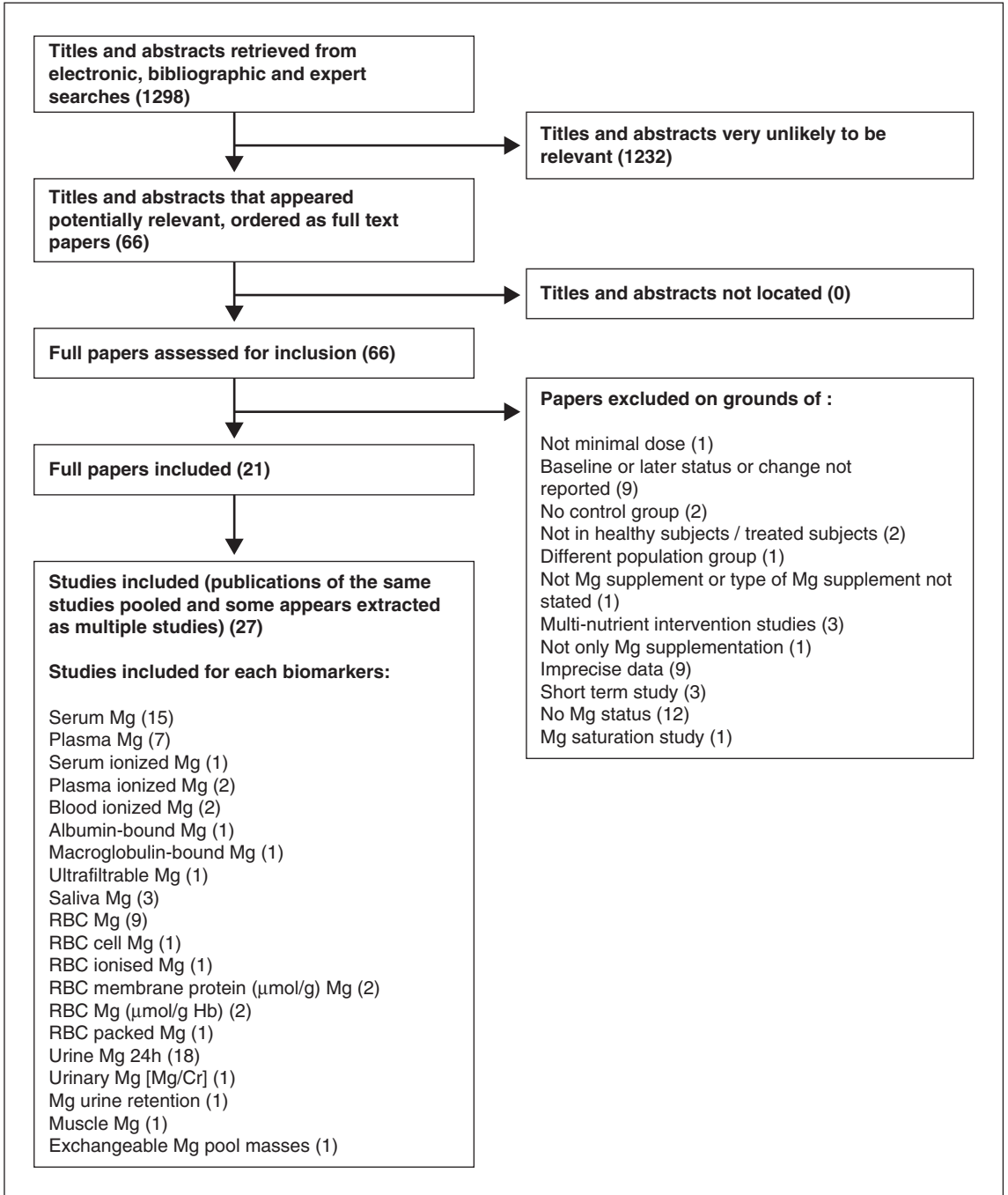
Figure 1. Flow diagram for systematic review of biomarkers of magnesium status. Numbers are in parentheses.

Table 1. Characteristics of included studies.

Study	Population: country(s); age; sex; no. included (treated/control); description	Description of intervention; latest time point; no. in intervention in control at latest time	Micronutrient type	Study design	Biomarker reported
Carpenter et al. 2006 [19]	USA; 8-14; F; 50 (23/27); healthy girls, selected for low Mg intake	300 mg Mg; 12 months; 23/27	Magnesium oxide	RCT parallel	Serum Mg; Urine (Fractional Excretion) FEMg
Dahle et al. 1995 [20]	Sweden; N/A; F; 33 (18/15); pregnancy-induced leg cramps	360 mg Mg; 3 weeks; 18/15	Magnesium oxide	RCT parallel	Serum Mg; Urine Mg
Dimai et al. 1998 [21]	USA; 27-36; M; 24 (12/12); healthy subjects	365 mg Mg; 30 days; 12/12	Magnesium carbonate and magnesium oxide	CCT	Blood ionized Mg; Serum Mg; Urine Mg [Mg/Creatinine]
Fatemi et al. 1991 [22]	USA; 18-48; X; 26; normal volunteers	Residential dietary intervention. Mg depletion achieved via low-Mg diet	depletion study	Before/After	Serum Mg; Mg urine retention
Feillet-Coudray et al. 2002 [23]	France; 24-34; F; 10 (10); healthy woman, selected for low Mg intake	366 mg Mg; 8 weeks; 10	Magnesium pidolate	Before/After	Plasma Mg; Plasma ionized Mg; RBC Mg; Urine Mg, Exchangeable Mg pool masses
Golf et al. 1990 [24]	Germany; 30-60; M; 14 (14); male persons with hyperlipidaemia	360 mg Mg; 8 weeks; 14	Magnesium aspartate hydrochloride	Before/After	Plasma Mg; RBC Mg
Itoh et al. 1997 [25]	Japan; 48-84; X; 41 (23/10); healthy subjects	548 mg Mg (male)/411 mg Mg (female); 4 weeks; 23/10	Magnesium hydroxide	RCT parallel	Serum Mg; Urine Mg
Kisters et al. 1993 [26]	Germany; 38-60; X; 64 (32/32); patients with normal renal function	500 mg Mg; 4 weeks; 32/32	Magnesium hydrogen aspartate	CCT	Plasma Mg; RBC Mg
Lukaski et al. 2002 [27]	USA; 44.9-71; F; 10; postmenopausal women	200 mg Mg; 49 days; 10	Magnesium gluconate	Before/After	Serum Mg; Urine Mg; Muscle Mg; RBC Mg
Lukaski et al. 2002 [27]	USA; 44.9-71; F; 10; postmenopausal women	112 mg Mg; 93 days; 10	By diet	Before/After	Serum Mg; Urine Mg; Muscle Mg; RBC Mg
Mooren et al. 2003 [28]	Germany; 21-30; M; 20 (10/10); healthy volunteers recruited from the sport student population	360 mg Mg; 2 months; 10/10	N/A	RCT parallel	Serum Mg; Blood ionised Mg; RBC Mg; RBC ionised Mg

Table 1. (*Continued*)

Study	Population: country(s); age; sex; no. included (treated/control); description	Description of intervention; latest time point; no. in intervention in control at latest time	Micronutrient type	Study design	Biomarker reported
Nielsen 2004 [29]	USA; 50-78; F; 13; postmenopausal women	200 mg Mg; 6 weeks; 13	Magnesium gluconate	Before/After	Serum Mg; Urine Mg
Nielsen et al. 2003 [30]	USA; 47-78; F; 9; postmenopausal women	300 mg Mg; 11 weeks; 9	Magnesium gluconate	RCT	Serum Mg; Urine Mg; Plasma ionised Mg; RBC Hb, RBC packed Mg; RBC, cell; RBCM (membrane) protein
Nielsen et al. 2007 [31]	USA; 49-71; F; 11; postmenopausal women	314 to 343 mg; 12 weeks; 11	Magnesium gluconate	RCT	Serum Mg; RBC membrane Mg; Urine Mg
Nielsen et al. 2007 [32]	USA; 47-75; F; 13; postmenopausal women	200 mg Mg; 11 weeks; 13 After low Mg diet	Magnesium gluconate	Before/After	Serum Mg; Ultrafiltrable Mg; Albumin-bound Mg; RBC Mg; Macroglobulin-bound Mg;
Paolisso et al. 1992 [33]	Italy; 67-89; X; 12 (12); elderly subjects	390 mg Mg; 4 weeks; 12	Magnesium pidolate	Before/After	Plasma Mg; RBC Mg
Sacks et al. 1998 [34]	USA; 34-44; F; 150 (50/102); healthy nurses, selected for low Mg intake	336 mg Mg; 16 weeks; 48/102	Magnesium lactate	RCT parallel	Urine Mg
Walker et al. 2002 [35]	United Kingdom; 24-29; F; 81 (40/41); woman suffering premenstrual symptoms	200 mg Mg; 1 day; 40/41	Magnesium oxide	RCT crossover	Urine Mg
Walker et al. 2002 [35]	United Kingdom; 24-29; F; 81 (47/41); woman suffering premenstrual symptoms	350 mg Mg; 1 day; 47/41	Magnesium oxide	RCT crossover	Urine Mg
Walker et al. 2002 [35]	United Kingdom; 24-29; F; 81 (33/41); woman suffering premenstrual symptoms	500 mg Mg; 1 day; 33/41	Magnesium oxide	RCT crossover	Urine Mg
Walker et al. 1998 [36]	United Kingdom; N/A; F; 74 (37/37); woman suffering from premenstrual symptoms	200 mg Mg; 1 day; 37/37	Magnesium oxide	RCT crossover	Urine Mg

Table 1. (*Continued*)

Study	Population: country(s); age; sex; no. included (treated/control); description	Description of intervention; latest time point; no. in intervention in control at latest time	Micronutrient type	Study design	Biomarker reported
Walker *et al.* 2003 [37]	United Kingdom; 23-28; X; 21 (12/9); healthy volunteers	300 mg Mg; 8.5 weeks; 12/9	Magnesium amino acid chelate	RCT parallel	Plasma Mg; Saliva Mg; Urine Mg
Walker *et al.* 2003 [37]	United Kingdom; 23-28; X; 21 (10/9); healthy volunteers	300 mg Mg; 8.5 weeks; 10/9	Magnesium citrate	RCT parallel	Plasma Mg; Saliva Mg; Urine Mg
Walker *et al.* 2003 [37]	United Kingdom; 23-28; X; 21 (12/9); healthy volunteers	300 mg Mg; 8.5 weeks; 12/9	Magnesium oxide	RCT parallel	Plasma Mg; Saliva Mg; Urine Mg
Zavaczki *et al.* 2003 [38]	Hungary; N/A; M; 20 (10/10); male with infertily problems	196.8 mg Mg; 90 days; 10/10	Magnesium orotate	RCT parallel	Serum Mg; Serum ionized Mg
Zorbas *et al.* 1999 control [39]	Greece; 22-26; M; 20 (10/10); male athlets	23 mg/kg bw; 52 weeks; 10/10	Magnesium lactate	RCT parallel	Serum Mg; Urine Mg
Zorbas *et al.* 1999 hypokinesia [39]	Greece; 22-26; M; 20 (10/10); male athlets	585 mg-665 mg Mg; 52 weeks; 10/10	Magnesium lactate	RCT parallel	Serum Mg; Urine Mg

Table 2. Validity of included studies.

Studies	Randomization; Method	Dropouts; reasons	Method of checking; results of compliance check	Data problems
Carpenter *et al.* 2006 [19]	Yes; Random Number Table	4/27 for supplemented group; 2/23 for placebo group; moving away, excessive time commitment, difficulty with compliance with treatment	Compliance with treatment was approximately 71% for the placebo group and 74% for the Mg-supplemented group and was confirmed by greater FEMg in the Mg-supplemented subjects at all treatment points	NR
Dahle *et al.* 1995 [20]	Yes; N/A	5/73; acid spillage from urine bottle, hospital admission, failure of attend second visit, admission for premature labor, some data lost for one patient	N/A; N/A	No data on age
Dimai *et al.* 1998 [21]	Yes; N/A	NR	Post study interview - finding of a significant increase in urinary Mg excretion in the test subjects compared to the control group/Full compliance	NR
Fatemi *et al.* 1991 [22]	No; N/A	NR	Twenty-four-hour urine collections for Mg and creatinine determinations were obtained daily to ensure compliance.; N/A	NR
Feillet-Coudray *et al.* 2002 [23]	No; N/A	NR	Unused ampoules were collected periodically	NR
Golf *et al.* 1990 [24]	No; N/A	N/A	Dietician control during study	NR
Itoh *et al.* 1997 [25]	Yes; N/A	8/18 for placebo; study protocol not fulfilled	N/A; N/A	NR
Kisters *et al.* 1993 [26]	No; N/A	NR	N/A; N/A	Data from graphs
Lukaski *et al.* 2002 [27]	No; N/A	NR	N/A; N/A	Data from graphs
Mooren *et al.* 2003 [28]	Yes; N/A	NR	To enhance compliance, individual sessions with skilled counselors were conducted at randomization and at follow-up visits (6 weeks, 3 and 6 months). Phone and mail contacts between counseling sessions were done at the discretion of the individual clinical	NR
Nielsen 2004 [29]	Yes; Latin-square design	2 - not postmenopausal, physician recommendation	Subjects consumed only food and beverages provided by the dietary staff and were chaperoned on all outings from the metabolic unit to ensure compliance with the study protocol.; N/A	NR

Table 2. (*Continued*)

Studies	Randomization; Method	Dropouts; reasons	Method of checking; results of compliance check	Data problems
Nielsen *et al.* 2003 [30]	Yes; N/A	3/3; personal reasons/medical reasons	The magnesium and copper content of 6-d composites of diets and feces were determined by inductively coupled argon plasma emission spectroscopy	NR
Nielsen *et al.* 2007 [31]	Yes; N/A	1 - not tolerated the amount of food; 2 - personal reasons; 1 - illness	Subjects consumed only food and beverages provided by the dietary staff and were chaperoned on all outings from the metabolic unit to ensure compliance with the study protocol. / N/A	NR
Nielsen *et al.* 2007 [32]	No; N/A	NR	Subjects consumed only food and beverages provided by the dietary staff and were chaperoned on all outings from the metabolic unit to ensure compliance with the study protocol. / N/A	NR
Paolisso *et al.* 1992 [33]	Yes; N/A	N/A	N/A; N/A	RCT study but considered as Before/After due to lack of placebo group measurement values
Sacks *et al.* 1998 [34]	Yes; N/A	N/A	Pill count; Good compliance	NR
Walker *et al.* 2002 [35]	Yes; N/A	NR	N/A; N/A	incomplete crossover
Walker *et al.* 1998 [36]	Yes; N/A	NR	N/A; N/A	NR
Walker *et al.* 2003 [37]	Yes; Each participant was issued a unique number after successful screening. To ensure the double-blind nature of the study, the project leader (AFW), who had no direct contact with the volunteers nor with the analysis of the data, undertook the randomization	6; One volunteer dropped out within 24 h of the commencement of the study and 5 others before the end of the study. All withdrawals were for personal reasons associated with the inconveniences of the protocol routine – no withdrawals were due to adverse effects	N/A; N/A	NR

N/A: not available; NR: not reported.

Table 3. Primary outcome data for all biomarkers.

Analysis (study type) and included studies	No. of studies (no. of included participants)	Pooled effect size, WMD (95% CI)	I^2	Appears effective as a biomarker?
Plasma/serum Mg (mmol/L)	22 (322)	0.03 [0.01, 0.06]	96	Yes
Plasma/serum/blood ionised Mg (mmol/L)	5 (51)	0.02 [-0.02, 0.06]	95	No
RBC Mg (mmol/L)	9 (130)	0.16 [0.09, 0.2]	85	Yes
Urinary Mg (mmol/24h)	18 (363)	1.82 [1.29, 2.36]	93	Yes
Saliva Mg (mmol/L)	3 (34)	-0.03 [-0.09, 0.03]	0	Unclear
Albumin bound Mg (mmol/L)	1 (13)	-0.02 [-0.04, 0.00]	-	Unclear
FEMg (%)	1 (23)	0.50 [-1.55, 2.55]	-	Unclear
Macroglobulin-bound Mg (mmol/L)	1 (13)	-0.03 [-0.05, -0.01]	-	Unclear
Mg urine retention (%)	1 (16)	51 [48.23, 53.77]	-	Unclear
Muscle Mg (mmol/100 g dw)	1 (10)	5.50 [2.70,8.30]	-	Unclear
RBC cell (μmol/10^6)	1 (9)	0.01 [0.00, 0.02]	-	Unclear
RBC ionised Mg (mmol/L)	1 (20)	0.05 [-0.22, 0.32]	-	Unclear
RBC Mg (μmol/g Hb)	2 (19)	0.50 [0.14, 0.86]	80	Unclear
RBC packed Mg (mmol/L)	1 (9)	0.11 [0.10, 0.12]	-	Unclear
RBC membrane protein (μmol/g)	2 (20)	0.16 [0.14, 0.18]	0	Unclear
Ultrafiltrable Mg (mmol/L)	1 (13)	0.00 [-0.02, 0.02]	-	Unclear
Urinary Mg (mmol/g creatinine)	1(13)	0.17 [0.15, 0.19]	-	Unclear

A summary of the population subgroup analysis of the effect of magnesium supplementation and depletion on serum/plasma magnesium concentration is given in the *table 4*. Because of the publications available, the data included in this analysis were mostly collected from studies in adults.

Serum, plasma and blood ionised magnesium concentration

Two studies included in this review measured plasma, one measured serum and two measured plasma ionised magnesium concentrations. These five studies were combined for statistical analyses and included 3 RCT, 1 CCT, and 1B/A, with four studies involving supplementation and one involving depletion. The retained studies involved 51 subjects. Neither primary analysis nor any individual study suggested a response of this biomarker to changes in magnesium intake. The primary analysis did not reveal a significant response to changes in dietary magnesium intake (WMD: 0.02 mmol/L; 95% CI: -0.02, 0.06; I^2 95%) *(table 3)*.

Other serum and plasma biomarkers

Only one study on a limited number of subjects was available for each of the other serum and plasma biomarkers: serum ultrafiltrable magnesium, albumin-bound magnesium, and macroglobulin-bound magnesium [31, 32] *(figure 1, table 3)*. Consequently, it was not possible to draw any conclusions regarding the usefulness of these biomarkers.

RBC magnesium concentration

Combining data from three depletion and six supplementation studies (on 130 subjects), primary analysis revealed a significant overall (p<0.0001) response of erythrocyte magnesium concentration to magnesium intake (WMD: 0.16 mmol/L; 95% CI: 0.09, 0.22; I^2 85%) *(figure 3)*.

In some of the selected studies, RBC magnesium was expressed by cell or haemoglobin, and also RBC membrane magnesium or free intracellular ionised magnesium concentrations were measured [28, 30, 32]. The results from these isolated studies were not combined for further analyses with the majority of available studies
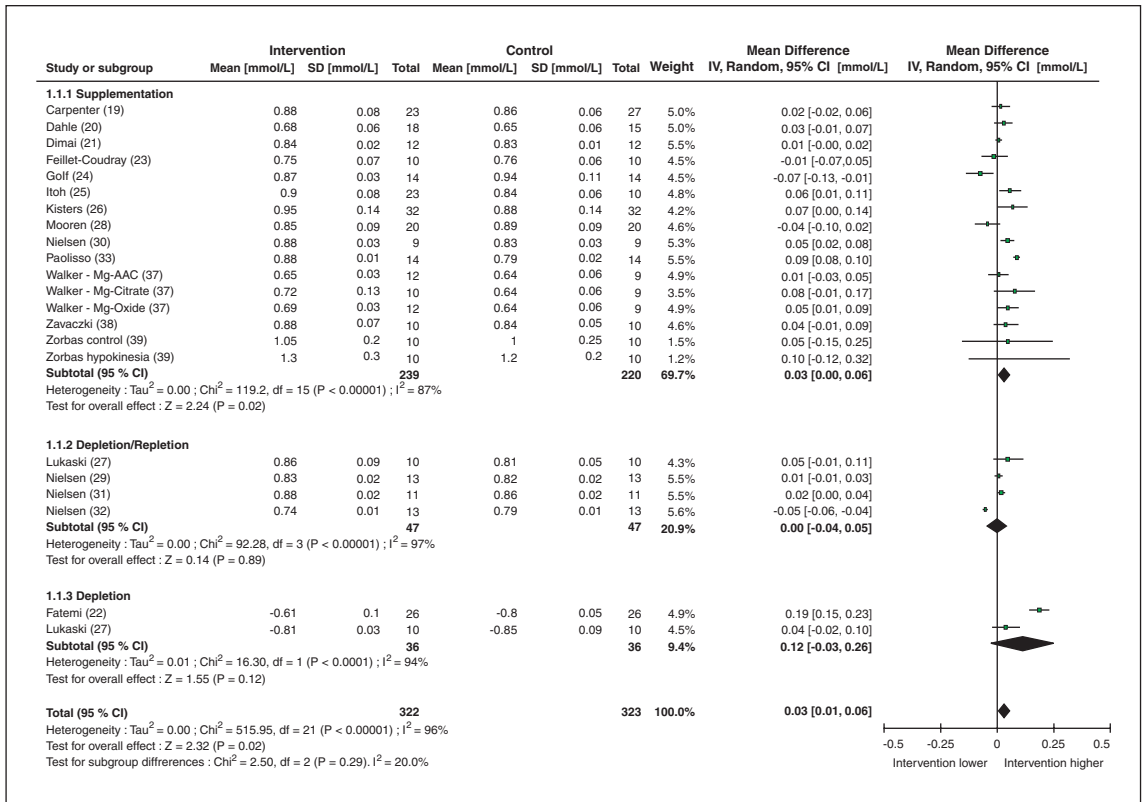
Study or subgroup	Intervention Mean [mmol/L]	SD [mmol/L]	Total	Control Mean [mmol/L]	SD [mmol/L]	Total	Weight	Mean Difference IV, Random, 95% CI [mmol/L]
1.1.1 Supplementation								
Carpenter (19)	0.88	0.08	23	0.86	0.06	27	5.0%	0.02 [-0.02, 0.06]
Dahle (20)	0.68	0.06	18	0.65	0.06	15	5.0%	0.03 [-0.01, 0.07]
Dimai (21)	0.84	0.02	12	0.83	0.01	12	5.5%	0.01 [-0.00, 0.05]
Feillet-Coudray (23)	0.75	0.07	10	0.76	0.06	10	4.5%	-0.01 [-0.07, 0.05]
Golf (24)	0.87	0.03	14	0.94	0.11	14	4.5%	-0.07 [-0.13, -0.01]
Itoh (25)	0.9	0.08	23	0.84	0.06	10	4.8%	0.06 [0.01, 0.11]
Kisters (26)	0.95	0.14	32	0.88	0.14	32	4.2%	0.07 [0.00, 0.14]
Mooren (28)	0.85	0.09	20	0.89	0.09	20	4.6%	-0.04 [-0.10, 0.02]
Nielsen (30)	0.88	0.03	9	0.83	0.03	9	5.3%	0.05 [0.02, 0.08]
Paolisso (33)	0.88	0.01	14	0.79	0.02	14	5.5%	0.09 [0.08, 0.10]
Walker - Mg-AAC (37)	0.65	0.03	12	0.64	0.06	9	4.9%	0.01 [-0.03, 0.05]
Walker - Mg-Citrate (37)	0.72	0.13	10	0.64	0.06	9	3.5%	0.08 [-0.01, 0.17]
Walker - Mg-Oxide (37)	0.69	0.03	12	0.64	0.06	9	4.9%	0.05 [0.01, 0.09]
Zavaczki (38)	0.88	0.07	14	0.84	0.05	10	4.6%	0.04 [-0.01, 0.09]
Zorbas control (39)	1.05	0.2	10	1	0.25	10	1.5%	0.05 [-0.15, 0.25]
Zorbas hypokinesia (39)	1.3	0.3	10	1.2	0.2	10	1.2%	0.10 [-0.12, 0.32]
Subtotal (95 % CI)			**239**			**220**	**69.7%**	**0.03 [0.00, 0.06]**

Heterogeneity : Tau^2 = 0.00 ; Chi^2 = 119.2, df = 15 (P < 0.00001) ; I^2 = 87%
Test for overall effect : Z = 2.24 (P = 0.02)

Study or subgroup	Intervention Mean [mmol/L]	SD [mmol/L]	Total	Control Mean [mmol/L]	SD [mmol/L]	Total	Weight	Mean Difference IV, Random, 95% CI [mmol/L]
1.1.2 Depletion/Repletion								
Lukaski (27)	0.86	0.09	10	0.81	0.05	10	4.3%	0.05 [-0.01, 0.11]
Nielsen (29)	0.83	0.02	13	0.82	0.02	13	5.5%	0.01 [-0.01, 0.03]
Nielsen (31)	0.88	0.02	11	0.86	0.02	11	5.5%	0.02 [0.00, 0.04]
Nielsen (32)	0.74	0.01	13	0.79	0.01	13	5.6%	-0.05 [-0.06, -0.04]
Subtotal (95 % CI)			**47**			**47**	**20.9%**	**0.00 [-0.04, 0.05]**

Heterogeneity : Tau^2 = 0.00 ; Chi^2 = 92.28, df = 3 (P < 0.00001) ; I^2 = 97%
Test for overall effect : Z = 0.14 (P = 0.89)

Study or subgroup	Intervention Mean [mmol/L]	SD [mmol/L]	Total	Control Mean [mmol/L]	SD [mmol/L]	Total	Weight	Mean Difference IV, Random, 95% CI [mmol/L]
1.1.3 Depletion								
Fatemi (22)	-0.61	0.1	26	-0.8	0.05	26	4.9%	0.19 [0.15, 0.23]
Lukaski (27)	-0.81	0.03	10	-0.85	0.09	10	4.5%	0.04 [-0.02, 0.10]
Subtotal (95 % CI)			**36**			**36**	**9.4%**	**0.12 [-0.03, 0.26]**

Heterogeneity : Tau^2 = 0.01 ; Chi^2 = 16.30, df = 1 (P < 0.0001) ; I^2 = 94%
Test for overall effect : Z = 1.55 (P = 0.12)

| **Total (95 % CI)** | | | **322** | | | **323** | **100.0%** | **0.03 [0.01, 0.06]** |

Heterogeneity : Tau^2 = 0.00 ; Chi^2 = 515.95, df = 21 (P < 0.00001) ; I^2 = 96%
Test for overall effect : Z = 2.32 (P = 0.02)
Test for subgroup diffrerences : Chi^2 = 2.50, df = 2 (P = 0.29). I^2 = 20.0%

Figure 2. Serum/plasma magnesium (mmol/L) response to magnesium supplementation or depletion.

that reported RBC magnesium concentrations expressed by RBC volume.

Urinary magnesium

Combining data from 14 RCT and 5 B/A studies *(table 3)*, which included four depletion and 15 supplementation studies, primary analysis revealed an overall significant (p<0.00001) response of urinary magnesium excretion to dietary magnesium intake (WMD: 1.82 mmol/24h; 95% CI: 1.29, 2.36; I^2 93%) *(figure 4)*.

Data from one CCT study expressed urinary magnesium as magnesium/creatinine [21] and from one study calculating urine fractional excretion of magnesium (FEMg) (a parallel RCT with magnesium supplemented girls) [19] have not been incorporated into the statistical analysis because of the difference in the calculation method used and expression of the results; the majority of studies evaluating 24-h Mg excretion. However,

it was not possible to draw any firm conclusions about the effectiveness of these two last parameters, which were considered separately because of the insufficient number of subjects and available studies *(table 3)*.

A summary of the population subgroup analysis of the response of urine magnesium concentration to magnesium supplementation and depletion is provided in the *table 4*. The data included in this analysis were mostly collected from studies in adults. In adults, urinary magnesium appears to be an effective biomarker.

Saliva magnesium

One RCT parallel supplementation study with three different magnesium salts, which included 12 participants per arm, assessed saliva magnesium [37]. There were not enough studies of this biomarker to draw any conclusions about its usefulness *(table 3)*.

Table 4. Secondary outcome data for plasma/serum and urinary biomarkers.

Analysis (study type) and included studies	No. of studies (no. of included participants)	Pooled effect size, WMD (95% CI)	I^2	Appears effective as a biomarker?
Plasma/serum Mg – females (mmol/L)	8 (107)	0.01 [-0.02, 0.05]	95	No
Plasma/serum Mg – mixed (mmol/L)	7 (129)	0.08 [0.04,0.12]	85	Yes
Plasma/serum Mg – males (mmol/L)	6 (76)	-0.01 [-0.04, 0.03]	57	No
Plasma/serum Mg – postmenopausal women (mmol/L)	5 (56)	0.01 [-0.03, 0.05]	97	No
Plasma/serum Mg – adults (mmol/L)	13 (209)	0.04 [0.00, 0.07]	86	No
Urinary Mg – females (mmol/24h)	14 (310)	1.65 [1.18, 2.11]	85	Yes
Urinary Mg – mixed (mmol/24h)	4 (57)	1.54 [0.98, 2.10]	36	Yes
Urinary Mg – postmenopausal women (mmol/24h)	4 (43)	2.31 [1.76, 2.85]	85	Unclear
Urinary Mg – adults (mmol/24h)	13 (310)	1.69 [0.84, 2.54]	94	Yes

Other biomarkers

Urinary magnesium retention was only studied in one B/A study [22] of 26 subjects.

Muscle magnesium was only evaluated in one B/A study on ten magnesium-supplemented postmenopausal women [27]. Exchangeable magnesium pool masses were only evaluated in one B/A study on ten magnesium-supplemented young women [23]. Consequently, it was not possible to draw any conclusions about the usefulness of these biomarkers *(table 3)*.

Discussion

A total of 21 publications with RCTs, CCTs and B/A studies were included in this review. The majority of studies included were magnesium
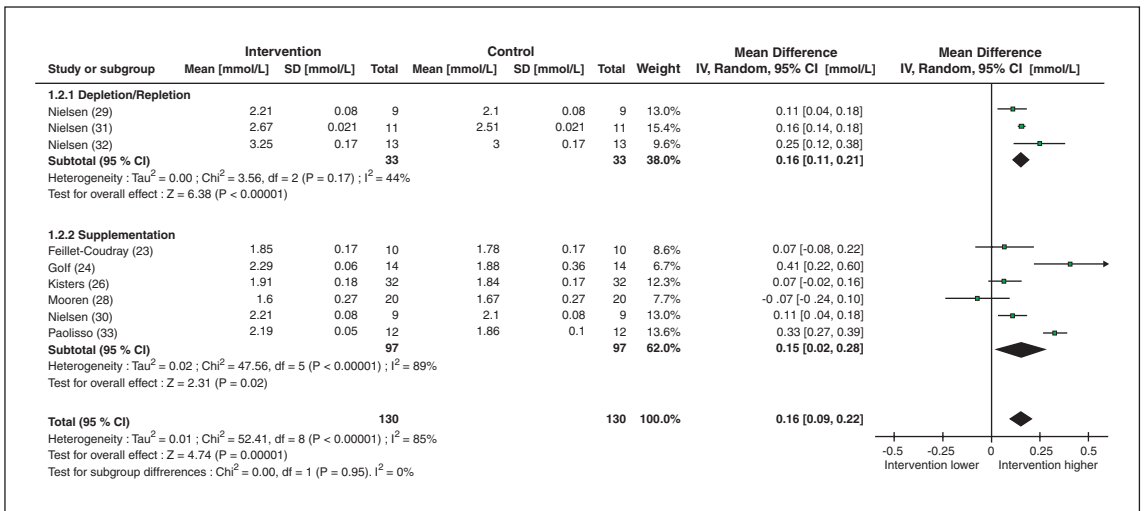

Figure 3. RBC magnesium (mmol/L) response to magnesium supplementation or depletion. Arrowheads indicate direction of response when unable to represent data on most appropriate scale.
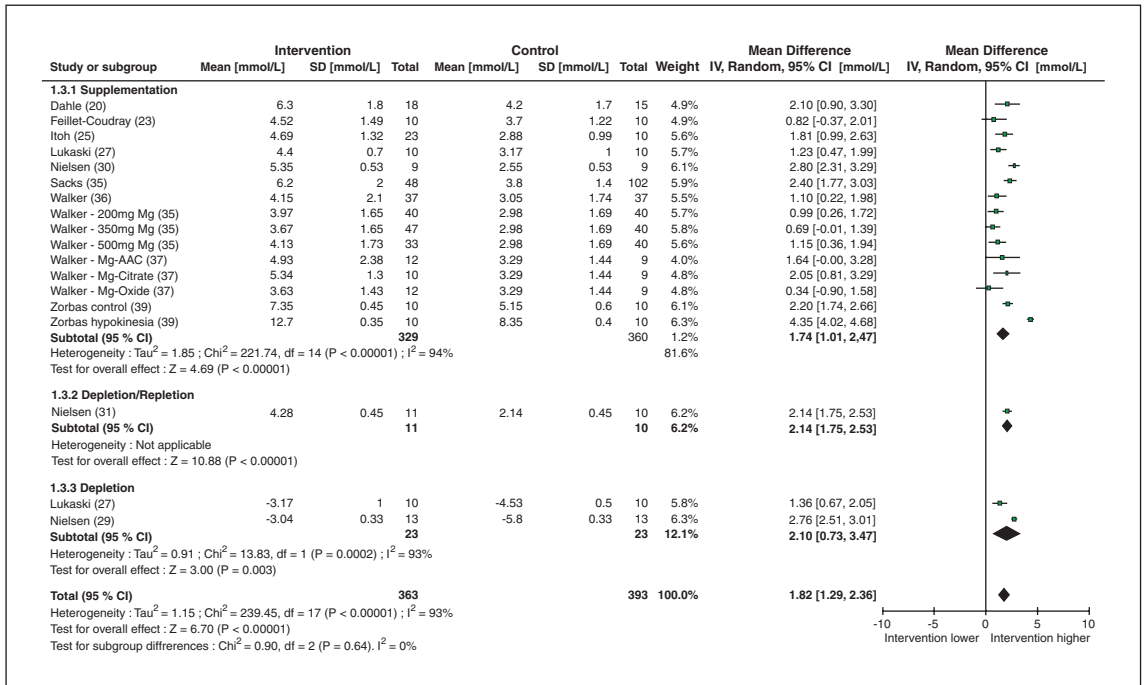
Study or subgroup	Intervention Mean [mmol/L]	SD [mmol/L]	Total	Control Mean [mmol/L]	SD [mmol/L]	Total	Weight	Mean Difference IV, Random, 95% CI [mmol/L]	Mean Difference IV, Random, 95% CI [mmol/L]
1.3.1 Supplementation									
Dahle (20)	6.3	1.8	18	4.2	1.7	15	4.9%	2.10 [0.90, 3.30]	
Feillet-Coudray (23)	4.52	1.49	10	3.7	1.22	10	4.9%	0.82 [-0.37, 2.01]	
Itoh (25)	4.69	1.32	23	2.88	0.99	10	5.6%	1.81 [0.99, 2.63]	
Lukaski (27)	4.4	0.7	10	3.17	1	10	5.7%	1.23 [0.47, 1.99]	
Nielsen (30)	5.35	0.53	9	2.55	0.53	9	6.1%	2.80 [2.31, 3.29]	
Sacks (35)	6.2	2	48	3.8	1.4	102	5.9%	2.40 [1.77, 3.03]	
Walker (36)	4.15	2.1	37	3.05	1.74	37	5.5%	1.10 [0.22, 1.98]	
Walker - 200mg Mg (35)	3.97	1.65	40	2.98	1.69	40	5.7%	0.99 [0.26, 1.72]	
Walker - 350mg Mg (35)	3.67	1.65	47	2.98	1.69	40	5.8%	0.69 [-0.01, 1.39]	
Walker - 500mg Mg (35)	4.13	1.73	33	2.98	1.69	40	5.6%	1.15 [0.36, 1.94]	
Walker - Mg-AAC (37)	4.93	2.38	12	3.29	1.44	9	4.0%	1.64 [-0.00, 3.28]	
Walker - Mg-Citrate (37)	5.34	1.3	10	3.29	1.44	9	4.8%	2.05 [0.81, 3.29]	
Walker - Mg-Oxide (37)	3.63	1.43	12	3.29	1.44	9	4.8%	0.34 [-0.90, 1.58]	
Zorbas control (39)	7.35	0.45	10	5.15	0.6	10	6.1%	2.20 [1.74, 2.66]	
Zorbas hypokinesia (39)	12.7	0.35	10	8.35	0.4	10	6.3%	4.35 [4.02, 4.68]	
Subtotal (95 % CI)			329			360	1.2%	**1.74 [1.01, 2.47]**	
Heterogeneity : Tau² = 1.85 ; Chi² = 221.74, df = 14 (P < 0.00001) ; I² = 94%							81.6%		
Test for overall effect : Z = 4.69 (P < 0.00001)									
1.3.2 Depletion/Repletion									
Nielsen (31)	4.28	0.45	11	2.14	0.45	10	6.2%	2.14 [1.75, 2.53]	
Subtotal (95 % CI)			11			10	6.2%	**2.14 [1.75, 2.53]**	
Heterogeneity : Not applicable									
Test for overall effect : Z = 10.88 (P < 0.00001)									
1.3.3 Depletion									
Lukaski (27)	-3.17	1	10	-4.53	0.5	10	5.8%	1.36 [0.67, 2.05]	
Nielsen (29)	-3.04	0.33	13	-5.8	0.33	13	6.3%	2.76 [2.51, 3.01]	
Subtotal (95 % CI)			23			23	12.1%	**2.10 [0.73, 3.47]**	
Heterogeneity : Tau² = 0.91 ; Chi² = 13.83, df = 1 (P = 0.0002) ; I² = 93%									
Test for overall effect : Z = 3.00 (P = 0.003)									
Total (95 % CI)			363			393	100.0%	**1.82 [1.29, 2.36]**	
Heterogeneity : Tau² = 1.15 ; Chi² = 239.45, df = 17 (P < 0.00001) ; I² = 93%									
Test for overall effect : Z = 6.70 (P < 0.00001)									
Test for subgroup diffrences : Chi² = 0.90, df = 2 (P = 0.64). I² = 0%									

-10 -5 0 5 10
Intervention lower   Intervention higher

Figure 4. Urinary magnesium (mmol/24h) response to magnesium supplementation or depletion.

supplementation studies. Fewer magnesium depletion studies were identified with lower total numbers of participants than supplementation studies. A significant proportion of the analyses conducted for individual biomarkers did not meet the minimum criteria for determining their usefulness, *i.e.,* they had <3 studies or <50 participants contributing data to the meta-analysis according to the methodology used [14]. However, it should be stressed that several of these parameters arise from the application of different methodologies for the equivalent parameters, thereby leading to different expression of the results (*e.g.,* analyses on RBC and urine). With regard to the limited number of these studies, it was not possible to conclude on the potential usefulness of several of these particular assessments. Among the identified biomarkers, only serum magnesium, plasma magnesium, RBC magnesium, and urinary magnesium excretion/24 h were reported in more than three studies with a total of more than 50 studied subjects and thus were reliable for evaluation of biomarker effectiveness. These data were combined as serum/plasma

because, frequently, total serum and plasma magnesium were alternatively measured. Finally, we concluded that serum/plasma magnesium concentration, RBC magnesium concentration, and urinary magnesium excretion responded to dietary manipulation. Consequently, these parameters appear to be useful biomarkers of magnesium status in the general population.

For the other potential biomarkers, special attention was paid to free extracellular ionised magnesium. This fraction of magnesium is generally considered as a more specific marker of magnesium status than total plasma or serum magnesium [12]. Only five studies of this biomarker were eligible according to the review criteria. However, the analyses in these studies were performed on serum, plasma, or blood samples. Also, various equipment (AVL or NOVA8+) were used in the retained studies. As previously discussed, significant differences among the ionised magnesium concentration have been obtained with various analysers [40]. Despite these limitations, we aggregated all the available results on plasma, serum and blood because of the low number of relevant studies. Finally, because

of the available data, it was not possible to conclude if ionised magnesium concentration is likely to be effective or ineffective as biomarkers of magnesium status.

Unfortunately, the studies included in this review on biomarkers of potential interest, i.e., exchangeable pools of magnesium and muscle and saliva magnesium, were only reported by one study, for each of this parameter, with a low number of included subjects. These and other biomarkers of potential interest (bone, platelets, white blood cell magnesium) could also not be analysed because the available studies did not meet sufficient criteria to be included to this review.

Due to the low number of available studies and studies with low number of included subjects, there is not enough information to make a clear decision about magnesium status biomarkers in specific subgroups. The majority of studies have been conducted in adults. After subgrouping, mainly urine magnesium excretion was shown to be significantly modulated by magnesium intake in adults and females *(table 4)*. There was no capacity within the data to conduct subgroup analysis for a range of population groups, especially vulnerable populations, e.g. infants, adolescents, pregnant women, or elderly subjects. In fact, we have identified only one publication for each of following groups: 10-year-old girls [19], pregnant women [20] and elderly subjects [33]. Also, few publications provided an extensive description of the subjects studied. It would be of interest to evaluate the relationship between magnesium status markers and body mass index (BMI). Numerous publications point out the relationship between low magnesium intake and metabolic syndrome, obesity and type 2 diabetes [8, 10, 41-48]. Several studies have reported lower magnesium status in these conditions, and this relationship is currently under debate [49-57]. However, available data did not allowed us to isolate these specific groups for further analyses.

The major limitation for conducting this systematic review is the low number of available studies (preferably RCTs) on healthy subjects. Papers were carefully selected; however, some of selected studies have been conducted on subjects presenting commonly encountered pathophysiological conditions, *i.e.,* pregnancy-induced leg cramps [20], untreated hyperlipidemia [24], premenstrual symptoms [35, 36], male infertility problems [38], intense sport activity [28, 39],

hypokinesia [39]. To our knowledge, all of these subjects did not receive medications or supplements other than magnesium. Because only isolated studies are available for these subjects, it was not possible to relate these conditions to the magnesium status.

Furthermore, it was not possible to draw conclusions about potential relations between biomarker responsiveness and type, dose, or length of supplementation. Various magnesium supplements were used in the selected studies: oxide, aspartate, hydroxide, gluconate, pidolate, citrate, lactate, carbonate and amino acid chelate. The duration of supplementation and the dose used varied in these studies; however, a majority of the included studies used 200 to 400 mg magnesium/day. The use of organic magnesium salts in several of the included studies results from the consideration that these salts are more bioavailable than are nonorganic ones [58]. Because of the diversity of supplements, protocols and subjects, we did not attempt to compare their efficiency in our analysis. It is also noteworthy that we have not included studies with magnesium/vitamin B6 supplementation in our review. Magnesium/vitamin B6 is a common magnesium supplement, but a possible interaction exists between these two compounds. Future analysis of the available data on magnesium/vitamin B6 supplementation should be conducted to evaluate the possible benefits of their interaction.

The data included in this review are mainly from supplemented subjects and few data are available from depleted individuals. A prolonged magnesium depletion study with 101 mg of magnesium/day exhibited adverse heart rhythm changes after 78 days of depletion [32]. Consequently, the implementation of depletion studies should be carefully considered from an ethical point of view.

The present review reveals that even if magnesium supplementation is used for a long time, well-designed intervention RCTs in healthy individuals with respect to their physiological and nutritional conditions are scarce. Although this systematic review offers primary outcome data for each biomarker identified, the limited number of studies included and the limited number of subjects in the population subgroups render it impossible to draw conclusions about potential relations between biomarker responsiveness for a range of population groups. Subsequently, further studies should be conducted on the best way to

evaluate magnesium status in population groups susceptible to magnesium deficiency. In particular, the research should be focused on evaluating intracellular magnesium and body pools of magnesium. It would be of interest to connect these studies with a magnesium balance evaluation [59]. Additionally, it is important to stress that compared to many other minerals, such as selenium, iodine, zinc and copper [15-18], biomarkers for magnesium status are limited to the magnesium measurements in biological fluids, cells and tissues. At present, no indirect biomarkers of magnesium status are identified. The recent progress in identifying genes modulated by magnesium concentration opens interesting perspectives for the research of these biomarkers.

Genetic factors controlling intra- and extracellular Mg levels should be considered in the future research of biomarkers; however, these genetic factors are weakly known. Serum magnesium concentrations have been shown to have a heritable component with heritability estimates of ~30% [60]. The pioneer study by Henrotte *et al.* in 1990 [61] pointed out the importance of genetics for plasma and RBC magnesium levels. This finding was confirmed in a mouse animal model selected for low and high RBC magnesium [62]. Recent advances in the fields of genetics and genomics have allowed for substantial progress in this area by identifying new players of magnesium homeostasis regulation [63-65]. Recently published genome-wide meta-analysis of cohorts identified six genomic regions that contained common variants associated with serum magnesium levels [66]. All of the variants were nominally associated with clinically defined hypomagnesaemia. These data from human genetics will initiate a new era in understanding the relationship between genetics, nutrition and diseases in determining magnesium status.

The selection process of the data included in the present review highlighted that the quality of published data are often poor for the purpose of a systematic review on magnesium status. Several publications did not provide sufficient or clear information on the magnesium intake, on the clinical protocol, and on the assay methodology. These issues should be considered when planning projects and publishing results. Well-designed RCTs of sufficient size with varying doses and duration of supplementation and with evaluation of the magnesium intake using a whole-diet profile (e.g., magnesium/calories ratio) are required

in various population subgroups. With emerging knowledge of genetic factors determining magnesium status, it will be important in future studies to take into account the genetic background of studied subjects. Ultimately, the results of such studies could contribute to the development of evidence-based dietary recommendations that are better targeted to specific populations.

Acknowledgments

Disclosure

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