Brief Review

# Magnesium Counteracts Vascular Calcification
## Passive Interference or Active Modulation?

Anique D. ter Braake, Catherine M. Shanahan, Jeroen H.F. de Baaij

***Abstract***—Over the last decade, an increasing number of studies report a close relationship between serum magnesium concentration and cardiovascular disease risk in the general population. In end-stage renal disease, an association was found between serum magnesium and survival. Hypomagnesemia was identified as a strong predictor for cardiovascular disease in these patients. A substantial body of in vitro and in vivo studies has identified a protective role for magnesium in vascular calcification. However, the precise mechanisms and its contribution to cardiovascular protection remain unclear. There are currently 2 leading hypotheses: first, magnesium may bind phosphate and delay calcium phosphate crystal growth in the circulation, thereby passively interfering with calcium phosphate deposition in the vessel wall. Second, magnesium may regulate vascular smooth muscle cell transdifferentiation toward an osteogenic phenotype by active cellular modulation of factors associated with calcification. Here, the data supporting these major hypotheses are reviewed. The literature supports both a passive inorganic phosphate–buffering role reducing hydroxyapatite formation and an active cell-mediated role, directly targeting vascular smooth muscle transdifferentiation. However, current evidence relies on basic experimental designs that are often insufficient to delineate the underlying mechanisms. The field requires more advanced experimental design, including determination of intracellular magnesium concentrations and the identification of the molecular players that regulate magnesium concentrations in vascular smooth muscle cells.

***Visual Overview***—An online visual overview is available for this article. (***Arterioscler Thromb Vasc Biol*. 2017;37: 1431-1445. DOI: 10.1161/ATVBAHA.117.309182.)**

**Key Words:** cardiovascular diseases ■ chronic kidney disease ■ magnesium ■ vascular calcification



Cardiovascular disease is the leading cause of mortality in patients with chronic kidney disease (CKD).[1] Cardiovascular events are 5 to 30× more likely to occur in end-stage renal disease (ESRD) patients compared with the general population of the same age, sex, and race.[2,3] In dialysis patients, arterial stiffness has been identified as an independent risk factor for cardiovascular mortality.[4] An important cause of arterial stiffness in CKD patients is the development of vascular calcifications.

Vascular calcifications are common in CKD. Its prevalence in dialysis patients is >80% and is correlated with reduced glomerular filtration rate.[5–7] The presence of vascular calcification is associated with a systolic increase and a diastolic decrease in blood pressure and an increase in aortic pulse wave velocity of >40%, which causes left ventricular hypertrophy.[4,8] Therefore, vascular calcification is an important prognostic marker for cardiovascular mortality in CKD patients.[9]

Over recent years, an increasing number of observational patient studies report a close relationship between serum magnesium ($Mg^{2+}$) concentration and cardiovascular mortality in ESRD.[10] Although clinical randomized controlled trials are currently not available, experimental studies indicate that this effect is through the prevention of vascular calcification. However, despite a substantial body of in vitro and in vivo studies addressing the role of $Mg^{2+}$ in vascular calcification, the precise mechanisms by which $Mg^{2+}$ acts are subject to debate. In this review, we will evaluate evidence for currently existing hypotheses. We focus on the question of whether $Mg^{2+}$ has its primary effect passively by inorganic phosphate (Pi) binding and hydroxyapatite inhibition or actively by cell-mediated processes involving prevention of osteogenic conversion on the level of the vascular smooth muscle cell (VSMC). However, it is important to note that these processes may not be mutually exclusive. In addition, we provide a detailed overview of studies reporting clinical associations between serum $Mg^{2+}$ and cardiovascular disease.

## Magnesium Homeostasis

### Regulation of Magnesium Homeostasis

In healthy individuals, serum $Mg^{2+}$ concentrations are carefully balanced between 0.7 and 1.1 mmol/L by the coordinate action of the intestine, bone, and kidney.[11] Approximately 30% of the dietary $Mg^{2+}$ intake is absorbed in the small intestine and colon.[12] The bone serves as the body's $Mg^{2+}$ store as 60%

Received on: February 23, 2017; final version accepted on: June 15, 2017.

From the Department of Physiology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands (A.D.t.B., J.H.F.d.B.); Cardiovascular Division, BHF Centre of Research Excellence, James Black Centre, King's College, London, United Kingdom (C.M.S.); and Department of Physiology, Anatomy and Genetics, University of Oxford, United Kingdom (J.H.F.d.B.).

Correspondence to Dr Jeroen H.F. de Baaij, Department of Physiology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands. E-mail jeroen.debaaij@radboudumc.nl

| Nonstandard Abbreviations and Acronyms | |
|---|---|
| ABCC6 | ATP-binding cassette subfamily C member 6 |
| ACP | amorphous calcium-phosphate particle |
| BMP | bone morphogenetic protein |
| CaSR | calcium-sensing receptor |
| CCP | calciprotein particle |
| CKD | chronic kidney disease |
| ESRD | end-stage renal disease |
| FGF | fibroblast growth factor |
| MGP | matrix gla protein |
| Pi | inorganic phosphate |
| PTH | parathyroid hormone |
| RUNX2 | runt-related transcription factor 2 |
| TRPM | transient receptor potential melastatin |
| VSMC | vascular smooth muscle cell |

of the total $Mg^{2+}$ is embedded at the surface of the hydroxyapatite crystals.[13] The kidney is the main organ controlling systemic $Mg^{2+}$ homeostasis, where transport is highly regulated by hormonal and intrarenal factors, including epidermal growth factor, insulin, pH, ATP, and estrogens.[14–18] Daily, 95% of the filtered $Mg^{2+}$ is reabsorbed along the nephron.[11] The largest amount of $Mg^{2+}$ (50%–70%) is reabsorbed paracellularly in the thick ascending limb of the loop of Henle.[19] Fine-tuning of $Mg^{2+}$ reabsorption is achieved in the distal convoluted tubule, where transient receptor potential melastatin type 6 (TRPM6) cation channels mediate apical $Mg^{2+}$ uptake and solute carrier family 41 members 1 and 3 (SLC41A1/A3). $Na^+/Mg^{2+}$-exchangers facilitate basolateral $Mg^{2+}$ extrusion.[20–22]

### Magnesium Balance in CKD

When renal function declines, the fractional excretion of $Mg^{2+}$ is increased to maintain normal serum $Mg^{2+}$ concentrations. Therefore, patients with CKD stages 1 to 3 (glomerular filtration rate >30 mL/min) generally have normal $Mg^{2+}$ concentrations.[23] As renal function further deteriorates during CKD stages 4 and 5, raising fractional excretion eventually fails to compensate for reduced glomerular filtration causing hypermagnesemia, especially if glomerular filtration rate drops <10 mL/min.[24] In a recent cohort of 365 hemodialysis patients, a mean $Mg^{2+}$ concentration of 0.98 mmol/L was measured, which is in the high-normal range of normal serum $Mg^{2+}$ concentrations.[25]

In dialysis patients, the serum $Mg^{2+}$ concentration is largely dependent on the dialysate $Mg^{2+}$ concentration.[26] Dialysates for both peritoneal dialysis and hemodialysis normally contain 0.75 mmol/L $Mg^{2+}$. Given that 30% of serum $Mg^{2+}$ is protein bound, a dialysate $Mg^{2+}$ concentration of 0.75 mmol/L generally results in mild hypermagnesemia (1.0–1.2 mmol/L).[27] The protein-binding properties of $Mg^{2+}$ may cause misinterpretation of measured serum concentrations.[11] The development of acidosis in ESRD potentially decreases the fraction of $Mg^{2+}$ bound to proteins, which in CKD may result in an increased ionized serum $Mg^{2+}$ concentration as renal compensatory mechanisms fail.[28] Measurements of ionized $Mg^{2+}$ therefore provide a more reliable estimation of $Mg^{2+}$ bioavailability; however, this is clinically largely unavailable.[29] Other factors such as diet, diabetes mellitus, and medication may greatly affect serum $Mg^{2+}$ concentrations in CKD patients. For instance, the use of proton pump inhibitors to treat gastric acid production hampers intestinal $Mg^{2+}$ reabsorption and, therefore, has been associated with increased risk of hypomagnesemia.[30,31] Hypomagnesemia is associated with the progression to ESRD in patients with diabetes mellitus type 2 and in patients with diabetic nephropathy.[32,33]

## Magnesium in Cardiovascular Disease

### Cardiovascular Risk

Hypomagnesemia (serum $Mg^{2+}$ concentration <0.7 mmol/L) is a well-established risk factor for cardiovascular disease, events, and mortality in the general population and in CKD patients.[25,34–37] In the general population, dietary $Mg^{2+}$ intake is associated with all-cause mortality, reduced risk of stroke, heart failure, and diabetes mellitus.[38] Moreover, serum $Mg^{2+}$ concentration is inversely associated with a 66% and a 36% increased risk for death from heart failure (<0.7 mmol/L) and coronary heart disease (<0.8 mmol/L), respectively.[39,40] To assess whether $Mg^{2+}$ status is linked to cardiovascular disease, a detailed overview of studies on the association between the circulating $Mg^{2+}$ concentration and cardiovascular disease risk in both healthy and hemodialysis cohorts is provided in Tables 1 and 2, respectively. For both tables, our aim was to assess available evidence on associations between circulating $Mg^{2+}$ concentration and cardiovascular disease outcome. Accordingly, studies on effects of dietary $Mg^{2+}$ and associations between serum $Mg^{2+}$ and indirect measures for cardiovascular disease, such as carotid intima-media thickness and hypertension, were excluded. Our overview of the available clinical association studies, as well as 2 previously published meta-analyses, indicates that serum $Mg^{2+}$ concentration is inversely associated with cardiovascular risk in both healthy cohorts and hemodialysis cohorts.[67,68]

Therefore, the current reference range of 0.7 to 1.1 mmol/L for blood $Mg^{2+}$ concentration is under debate. An international team of $Mg^{2+}$ researchers proposed that the reference values for normal $Mg^{2+}$ concentration may be too low and should be reconsidered because the current range was derived from population studies from the 1970s.[69,70] $Mg^{2+}$ intake is generally insufficient, and $Mg^{2+}$ deficiency-related clinical complications may already arise in low-normal $Mg^{2+}$ values, suggesting that a higher blood $Mg^{2+}$ concentration is beneficial.[69] This notion is supported by data from CKD patients; in the CONTRAST study (Convective Transport Study), the relative risk for mortality in patients with serum $Mg^{2+}$ concentrations <1.14 mmol/L was significantly increased compared with patients with lower serum concentrations.[25] Although $Mg^{2+}$ concentration was negatively associated with cardiovascular risk in a recent Japanese cohort study, it is important to note that concentrations >1.27 mmol/L were found to be associated with increased risk.[34] Interestingly, similar trends were observed in heart failure patients as serum $Mg^{2+}$ concentrations ≥1.05 mmol/L were associated with increased cardiovascular mortality.[71] These studies suggest that depending on the population and the disease state, the optimal $Mg^{2+}$ concentration

**Table 1. The Effects of Serum $Mg^{2+}$ Concentration on Cardiovascular Disease Occurrence in the General Population**

| Author* | Study Type | Cardiovascular Outcome† | No. of Patients (% Women) | Follow-Up | Association Inhibiting Outcome ($P<0.05$) | Associations With Serum $Mg^{2+}$, mmol/L | Associations Increased Serum $Mg^{2+}$, mmol/L | Reference Concentration |
|---|---|---|---|---|---|---|---|---|
| Gartside et al[41] 1995 | Prospective | CHD | 8251 (25) | 10 y | Yes | N/A | ≥0.87 (RR, 0.68; 95% CI, 0.54–0.87) | <0.81 |
| Marniemi et al[42] 1998 | Prospective | Vascular death | 344 (47.1) | 13 y | No | N/A | Highest (RR, 0.90; 95% CI, 0.58–1.38) | Lowest |
| Liao et al[43] 1998 | Prospective | CHD | 13 922 (55.8) | 4–7 y | Yes (women) | N/A | ≥1.8 (Women: RR, 0.55; 95% CI, 0.27–1.14; and Men: RR, 0.84; 95% CI, 0.53–1.31) | ≤0.75 |
| Ford[44] 1999 | Prospective | IHD | 12 340 (59.9) | 19 y | Yes | 0.80–<0.84 (HR, 0.79; 95% CI, 0.58–1.08) | ≥0.89 (HR, 0.69; 95% CI, 0.52–0.90) | <0.80 |
| Leone et al[45] 2006 | Prospective | CV mortality | 4035 (0) | 18 y | Yes | N/A | High (RR, 0.5; 95% CI, 0.3–1.0) | Low |
| Ohira et al[46] 2009 | Prospective | Ischemic stroke | 13 560 (55.4) | 15 y | No | N/A | ≥0.9 (RR, 1.04; 95% CI, 0.82–1.32) | ≤0.75 |
| Khan et al[47] 2010 | Prospective | CVD | 3531 (51.8) | 20 y | No | 0.73–0.77 (HR, 0.99; 95% CI, 0.86–1.37) | 0.81–1.03 (HR, 0.87; 95% CI, 0.69–1.10) | 0.58–0.73 |
| Peacock et al[48] 2010 | Prospective | SCD | 14 232 (54.6) | 12 y | Yes | 0.78–0.8 (HR, 0.97; 95% CI, 0.71–1.33) | ≥0.875 (HR, 0.62; 95% CI, 0.42–0.93) | ≤0.75 |
| Reffelmann et al[49] 2011 | Prospective | CV mortality | 3910 (50.8) | 10.1 y | Yes | ≤0.73 (HR, 1.66; 95% CI, 1.13–2.45) | ≤0.77 (HR, 1.03; 95% CI, 0.72–1.76) | N/A |
| Chiuve et al[50] 2011 | Prospective | SCD | 88 375 (100) | 26 y | Yes | N/A | >0.86 (RR, 0.23; 95% CI, 0.09–0.60) | <0.78 |
| Feng et al[51] 2013 | Cross-sectional | Ischemic stroke | 1493 (36.1) | None | Yes | 0.83–0.88 (RR, 0.65; 95% CI, 0.38–1.10) | ≥0.98 (RR, 0.40; 95% CI, 0.23–0.70) | <0.83 |
| Khan et al[52] 2013 | Prospective | Atrial fibrillation | 3530 (52) | 20 y | Yes | <0.73 (HR, 1.45; 95% CI, 0.99–2.12) | 0.78–0.81 (HR, 1.14; 95% CI, 0.76–1.71) | >0.82 |
| Misialek[53] et al 2013 | Prospective | Atrial fibrillation | 14 290 (53) | 20.6 y | Yes | <0.78 (HR, 1.34; 95% CI, 1.16–1.54) | ≥0.88 (HR, 1.06; 95% CI, 0.91–1.23) | N/A |
| Joosten et al[54] 2013 | Prospective | Fatal and nonfatal IHD | 7664 (51) | 10.5 y | No | <0.77 (HR, 1.06; 95% CI, 0.79–1.43) | >0.85 (HR, 1.07; 95% CI, 0.80–1.45) | N/A |
| Akarolo-Anthony et al[55] 2014 | Case–control | Ischemic stroke | 32 826 (100) | None | Yes | <0.82 (RR, 1.34; 95% CI, 0.82–2.17) | 0.90–<0.95 (RR, 0.75; 95% CI, 0.48–1.16) | 0.95–1.15 |
| Lutsey et al[39] 2014 | Prospective | Heart failure | 14 709 (54.7) | 20.6 y | Yes | 0.25–0.70 (HR, 1.66; 95% CI, 1.42–1.95) | 0.85 (HR, 1.16; 95% CI, 1.01–1.34) | 0.90–1.55 |
| Lee et al[56] 2015 | Cross-sectional | CAC score of >100 | 34 553 (14.6) | None | Yes | <0.78 (OR, 2.10; 95% CI, 1.40–3.15) | >0.95 (OR, 1.30; 95% CI, 0.88–1.93) | N/A |
| Markovits et al[57] 2016 | Retrospective | Atrial fibrillation | 162 162 (64.3) | 25.3 mo | Yes | ≤0.78 (HR, 1.21; 95% CI, 1.07–1.37) | >0.78 (HR, 1.05; 95% CI, 0.92–1.20) | N/A |
| Posadas-Sánchez et al[58] 2016 | Cross-sectional | CAC score of >0 | 1276 (50) | None | Yes | N/A | ≥0.90 (OR, 0.58; 95% CI, 0.374–0.915). Risk reduction per 0.07 increase (OR, 0.84; 95% CI, 0.724–0.986) | <0.8 |
| Kieboom et al[40] 2016 | Prospective | CHD, SCD | 9820 (65.1) | 8.7 y | Yes | ≤0.8 (CHD: HR, 1.36; 95% CI, 1.09–1.69; and SCD: HR, 1.54; 95% CI, 1.12–2.11) | ≥0.89 (HR, 0.69; 95% CI, 0.48–0.98). Risk reduction per 0.1 increase (CHD: HR, 0.82; 95% CI, 0.70–0.96) | N/A |

CAC indicates coronary artery calcification; CHD, coronary heart disease; CI, confidence interval; CV, cardiovascular; CVD, cardiovascular disease; HR, hazard ratio; IHD, ischemic heart disease; mo, months; N/A, not applicable; OR, odds ratio; RR, risk ratio; and SCD, sudden cardiac death.

*Articles were obtained after PubMed search using the following search terms: ("Magnesium"[Mesh] AND "cardiovascular diseases"[mesh] AND ("risk"[Mesh] OR "mortality"[mesh]).

†Studies assessing the effects of dietary $Mg^{2+}$, indirect outcome measures for CVD (eg, hypertension, arterial intima-media thickness), and nonhealthy cohorts were excluded.

may be in the range of 0.9 to 1.2 mmol/L. However, this hypothesis should be further supported by studies defining the optimal $Mg^{2+}$ concentration based on clinical outcomes. This is essential to set a novel clinically relevant reference range for serum $Mg^{2+}$ concentrations.

Determining a clear upper level is important as hypermagnesemia (currently set at >1.1 mmol/L) may result in nausea and vomiting, flushing, and headaches. Severe hypermagnesemia (>3.0 mmol/L) may lead to cardiac complications, such as bradycardia and hypotension.[11] However, the positive association of high serum $Mg^{2+}$ with survival found in CKD patients suggests that a state of mild hypermagnesemia is predominantly protective in this population, possibly through the impact of $Mg^{2+}$ on vascular function. In the following section, we will briefly review the available data on the role of $Mg^{2+}$ in common cardiovascular diseases.

## Arrhythmia

Moderate-to-severe $Mg^{2+}$ deficiency is associated with arrhythmia and atrial fibrillation.[11,52] Reduction in cytosolic $Mg^{2+}$ associated with hypomagnesemia can cause significant alterations in the myocardial action potential.[72] In patients with normal cardiac conduction maintenance $Mg^{2+}$ infusion resulted in prolongation of the electrocardiography P-R interval, A-H interval, atrioventricular refractory period, and sinoatrial conduction time.[73] $Mg^{2+}$ has been widely considered as treatment for arrhythmic disorders, and success of $Mg^{2+}$ treatment has been shown to largely depend on arrhythmia type. For example, $Mg^{2+}$ is beneficial in torsades de pointes and is currently the first line of therapy.[74] Ventricular fibrillation and tachycardia do not respond to $Mg^{2+}$.[75] Although a meta-analysis did not demonstrate beneficial effects of $Mg^{2+}$ treatment on acute atrial fibrillation,[76] a recent editorial calls attention to limitations in sample size, patient selection, and follow-up of the current available studies and therefore emphasizes the need for further trial data to accurately assess a role for $Mg^{2+}$ in improving the management of atrial fibrillation.[77]

## Atherosclerosis and Other Vascular Diseases

Hypomagnesemia is associated with an increased risk for coronary artery disease and carotid atherosclerosis.[40,78] Coronary artery calcification associated with atherosclerosis is a strong predictor of cardiovascular events in the general and the CKD population.[79,80] In CKD, intimal calcifications associated with atherosclerosis are prevalent.[81] Recently, $Mg^{2+}$ status was found to be inversely associated with coronary artery calcification density in ESRD patients, particularly those with high serum Pi concentrations (>1.40 mmol/L).[82] Associations between serum $Mg^{2+}$ concentration and subclinical markers of atherosclerosis and the presence of vascular calcification in CKD patients have been reported extensively.[59,60,62,63,82–84] Although the potential mechanisms remain largely unclear and are beyond the scope of this review, low intracellular $Mg^{2+}$ in vitro is linked with a proinflammatory and a proatherogenic vascular phenotype through increased production of reactive oxygen species, activation of NF-κB (nuclear factor kappa-beta) and cytokines, and proteasome activity in endothelial cells.[78,85,86]

The vasoprotective properties of $Mg^{2+}$ are reinforced by multiple in vivo studies. In low-density lipoprotein receptor$^{-/-}$ and ApoE$^{-/-}$ transgenic mouse models of atherosclerosis, $Mg^{2+}$ supplementation reduced cholesterol and triglyceride levels and atherogenesis in the aortic sinus.[87–89] Endothelial dysfunction in aortas of inbred low serum $Mg^{2+}$ mice has been associated with reduced TRPM7 expression levels, illustrating a potential link between intracellular $Mg^{2+}$ and onset of atherosclerosis.[90] A $Mg^{2+}$-deficient diet in rats led to increased oxidative stress, reduced superoxide dismutase, catalase, and increased collagen synthesis in the arterial wall.[91] Moreover, $Mg^{2+}$-deficient mice demonstrated aortic thinning and structural alterations in collagen and elastin fibers, possibly related to matrix metalloprotease expression and activity.[92] In studies using Abcc$^{-/-}$ and Enpp1$^{asj}$ mice, which develop extensive vascular calcification, $Mg^{2+}$ restriction and supplementation experiments demonstrated a preventive role for $Mg^{2+}$ in the development of ectopic and connective tissue calcification.[93–95] The effects of $Mg^{2+}$ on vascular calcification are discussed in the next section of this review.

## Hypertension

Hypertension is an important contributor to the development of cardiovascular events and is common in CKD because it develops in >80% of patients during stages 4 and 5.[96] The antihypertensive properties of $Mg^{2+}$ are likely attributed to its $Ca^{2+}$ antagonistic properties.[97] Alternative vasodilatory actions of $Mg^{2+}$ are the associated increased production of prostaglandin $I_2$ and nitric oxide in endothelial cells.[98]

Although the role of $Mg^{2+}$ in hypertension has been controversial, a recent meta-analysis of randomized double-blind placebo-controlled trials revealed a significant causal antihypertensive effect of $Mg^{2+}$ supplementation.[99] However, given the modest effect size of 2 mm Hg, the clinical relevance of this effect is questionable. Although the mechanisms are poorly understood, $Mg^{2+}$ supplementation is worldwide the first line of treatment for preeclampsia that is widely advocated by the World Health Organization to prevent early childhood mortality.[100] Despite these results, in the context of CKD, it should be noted that in 14 hemodialysis patients treated with low dialysate $Ca^{2+}$ (1.25 mmol/L), increased $Mg^{2+}$ dialysate from 0.25 to 0.75 mmol/L paradoxically prevented blood pressure drops associated with dialysis.[101] In these patients, postdialysis $Mg^{2+}$ concentrations fell by 35%, whereas intracellular $Ca^{2+}$ fell by 7.7%. The authors propose that given the $Ca^{2+}$-blocking properties of $Mg^{2+}$, subnormal levels of $Mg^{2+}$ in combination with lower extracellular $Ca^{2+}$ may have resulted in reduced cardiovascular contractility, which was reversed by increasing dialysate $Mg^{2+}$ concentration.[101]

## Diabetes Mellitus

Diabetes mellitus is an established and well-known risk factor for cardiovascular disease.[102] Insulin resistance is the main cause of diabetes mellitus type 2 and has been found to be associated with the presence and severity of coronary artery disease.[103] In fact, patients with diabetes mellitus often present with more severe atherosclerosis, characterized by larger and more inflammatory necrotic cores and more extensive lesion

calcification.[104] Diabetes mellitus is strongly associated with hypomagnesemia, of which the potential mechanisms have been reviewed in detail previously.[33] In addition, dietary $Mg^{2+}$ intake was associated with type 2 diabetes mellitus in a recent dose–response meta-analysis.[38] However, any causal relationship between hypomagnesemia and the incidence of cardiovascular disease in diabetes mellitus has yet to be identified.

The link between $Mg^{2+}$ status and cardiovascular disease in humans and the impact of $Mg^{2+}$ interventions on vascular disease in animal models illustrate that $Mg^{2+}$ supplementation should be considered as potential strategy to counteract vascular disease. The field of cardiovascular research now faces the challenge to move forward from association studies toward experimental studies. The many positive effects that were shown in association studies (Table 1) warrant further clinical investigations to elucidate the treatment potential of $Mg^{2+}$. This may be of particular interest for patients with CKD because these patients suffer from disturbed mineral homeostasis and increased cardiovascular risk. In this review, we will further focus on the mechanisms underlying beneficial effects of $Mg^{2+}$ in vascular calcification.

## Vascular Calcification in CKD

### Calcification Milieu

Severe hyperphosphatemia in ESRD patients paradoxically leads to both bone demineralization and vascular calcification.[105] In the course of the disease, high Pi concentrations persistently elevate FGF23 (fibroblast growth factor 23) levels. The resulting defective inhibitory regulation of PTH (parathyroid hormonde) secretion and decreased $1,25[OH)]_2D_3$ (1,25-dihydroxyvitamin D) synthesis results in reduced intestinal $Ca^{2+}$ and Pi absorption and high bone turnover.[106–108] FGF23-specific signaling is regulated by the FGF receptor 1-klotho complex in the distal convoluted tubule of the nephron and the parathyroid.[109,110] However, because klotho expression levels decline over the course of CKD development as functional renal mass decreases, FGF23 signaling is compromised even further.[111] Administration of recombinant α-klotho effectively attenuated CKD progression and CKD-associated cardiac remodeling, highlighting the importance of klotho signaling pathways in CKD and cardiovascular health.[112]

Disturbances in these regulatory axes manifest as CKD-mineral bone disorder, which is characterized by severe hyperphosphatemia and hypercalcemic episodes providing a permissive milieu for vascular calcification (Figure 1). This CKD-induced calcification milieu is associated with loss of proteins that act as local and circulating inhibitors of soft tissue calcification such as fetuin-A and MGP (matrix gla protein).[113] Reduced levels of these proteins are associated with vascular calcification in hemodialysis patients.[114,115] In vivo studies report extensive calcification in knockout mouse models of fetuin-A, MGP, and of local inhibitors native to VSMCs, such as osteoprotegerin and pyrophosphate.[116–119]

### VSMCs: Toward an Osteogenic Phenotype

The development of the calcification milieu and the loss of calcification inhibitors promote the formation of amorphous $Ca^{2+}$-Pi particles (ACPs). Nucleation and maturation of these ACPs into hydroxyapatite crystals in the vessel wall initiate the process of vascular calcification. Ultimately, however, vascular calcification is an active cell-mediated process further potentiated by ACP phagocytosis and increased Pi uptake mediated by sodium-dependent phosphate transporters 1 and 2, during which VSMCs transdifferentiate from a contractile into an osteoblast-like phenotype.[120–123] VSMC transdifferentiation is typically characterized by the expression of genes that are normally restricted to bone tissue, such as *BMP2* (bone morphogenetic protein 2), osterix, *RUNX2* (runt-related transcription factor 2), and alkaline phosphatase.[124] Expression of these osteoinductive genes induces matrix remodeling and mineralization and is accompanied by decreased expression of VSMC lineage markers, such as transgelin and calponin.[125]

Transdifferentiated VSMCs participate in the local spread of calcification by diminished synthesis of calcification inhibitors, the release of $Ca^{2+}$-loaded exosomes (matrix vesicles) and apoptosis. All these factors contribute to the calcification by local $Ca^{2+}$ release and providing ACP nucleation sites in the extracellular matrices of surrounding VSMCs.[126,127] In healthy VSMCs, exosomes are loaded with fetuin-A and MGP and are secreted to maintain vessel compliance.[128] In calcifying VSMCs, however, the presence of calcification inhibitors in these exosomes is depleted and replaced by a protein–lipid complex consisting of phosphatidyl serine and annexin A6, converting the exosome into a potent nucleation site.[129,130] Furthermore, in vitro studies have shown that exposure of VSMCs to artificial ACP and calciprotein particles (CPPs) similar to those found in uremic sera increased exosome secretion, which in turn enhanced calcification.[131,132] Because of the presence of fetuin-A in human serum, CPPs containing ACP have recently shown to form, rather than crystalline, hydroxyapatite.[133] These primary CPPs mature spontaneously into secondary CPPs containing crystalline $Ca^{2+}$-Pi and were found in sera of CKD patients.[134] Exposure of secondary CPP to fixated cells did not result in calcification, demonstrating a role for VSMC in vascular calcification possibly related to exosome secretion.[131]

Similar to exosomes, $Ca^{2+}$-loaded apoptotic bodies released from VSMCs undergoing apoptosis form larger nucleation sites and promote calcification in neighboring cells potentially by causing local $Ca^{2+}$ spikes.[135] Apoptosis plays a role in the initiation and acceleration in VSMC calcification and is induced by an intracellular $Ca^{2+}$ burst after excessively phagocytosed ACP undergoes lysosomal breakdown.[136] Altogether, the calcification milieu, loss of calcification inhibitors, and VSMC transdifferentiation illustrate the dynamic and complex nature of vascular calcification activating a vicious cycle of events amplifying the calcification process.

## Passive Interference: Phosphate Binding and Physiochemical Crystal Inhibition

$Mg^{2+}$-dependent alterations of the calcification milieu may prevent the development of vascular calcification. First, dietary $Mg^{2+}$ can reduce Pi uptake by intestinal Pi binding. Second, $Mg^{2+}$ can passively interfere with hydroxyapatite maturation in the vessel (Figure 2).

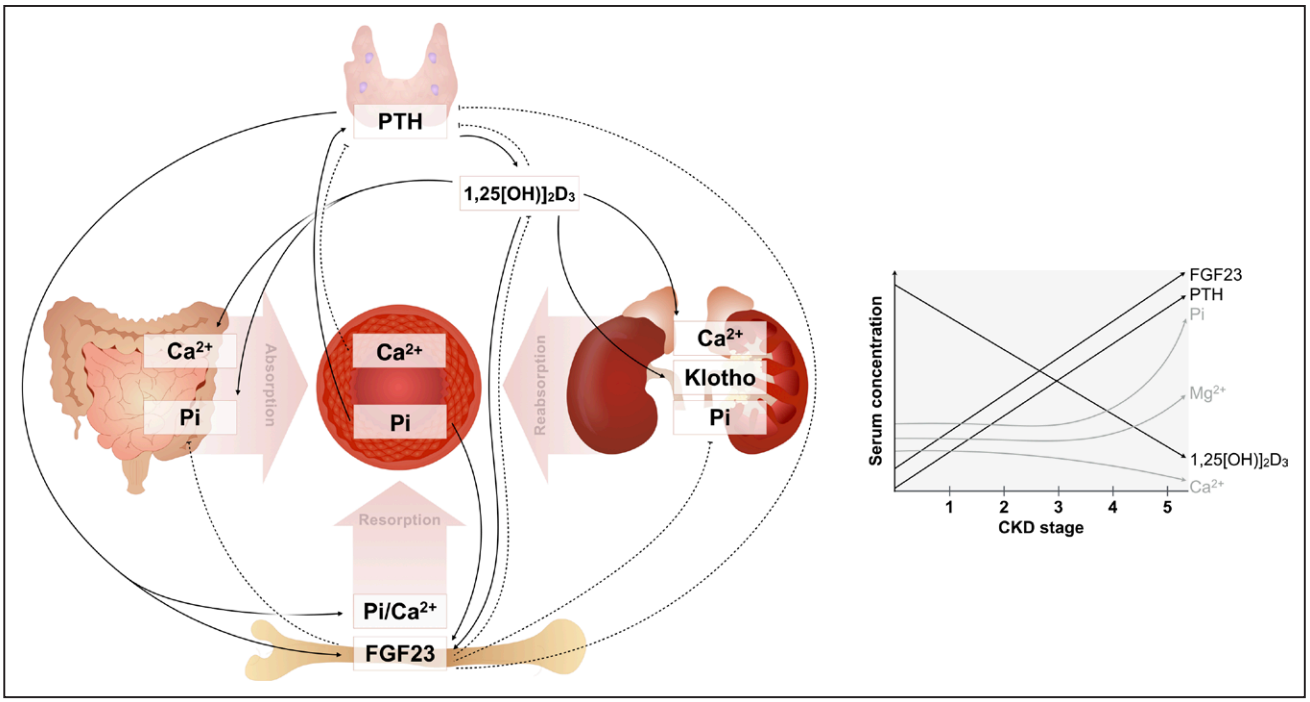


**Figure 1.** Mineral metabolism in chronic kidney disease (CKD). Circulating levels of inorganic phosphate (Pi) and $Ca^{2+}$ are determined by integrated action of the parathyroid, intestine, bone, and kidney. CKD-induced mineral disturbances and diminished klotho result in CKD-mineral bone disorder, which promotes vascular calcification. $1,25(OH)_2D_3$ indicates 1,25-dihydroxyvitamin D3; FGF23, fibroblast growth factor 23; and PTH, parathyroid hormone.

## Magnesium in the Intestines

Reducing Pi load is an important therapeutic strategy to minimalize the risk of cardiovascular complications, including vascular calcifications.[137] $Mg^{2+}$-based Pi binders have been shown to reduce serum Pi concentrations efficiently and were introduced in the early 1980s.[138,139] The introduction of $Mg^{2+}$-based binders was mainly to replace $Ca^{2+}$- or aluminum-based drugs, which can lead to vascular calcification, osteomalacia, dementia, and anemia.[138,140]

Despite the promising first clinical trials testing the use of $Mg^{2+}$-hydroxide and $Mg^{2+}$-carbonate in dialysis patients, concerns rose about hypermagnesemia and gastrointestinal complications.[139,141] Instead, a combination of $Ca^{2+}$-acetate and $Mg^{2+}$-carbonate has been used since and showed similar efficacy in reducing serum Pi concentrations, which was demonstrated in 255 hemodialysis patients.[142] Pi concentrations below the KDIGO (Kidney Disease: Improving Global Outcomes) target of 1.78 mmol/L or lower were achieved in the $Ca^{2+}$-acetate and $Mg^{2+}$-carbonate group after 16 days compared with 30 days in the conventional sevelamer group.[142] Mild hypermagnesemia remained an issue as the serum $Mg^{2+}$ concentration increased by 0.3 mmol/L. Close monitoring of serum $Mg^{2+}$ concentrations in CKD patients and reduced dialysate $Mg^{2+}$ in ESRD patients from 0.75 to 0.5, to 0.25 mmol/L is therefore proposed by the authors as an effective solution to decrease the probability of hypermagnesemia and its potential toxicity.[143] However, the clinical benefit of preventing hypermagnesemia by adjusting dialysate $Mg^{2+}$ concentration in CKD patients is arguable as a negative $Mg^{2+}$ balance increases cardiovascular risk potentially through calcification in this population, as discussed elsewhere in this review.

The promising effects of a $Ca^{2+}$-acetate and $Mg^{2+}$-carbonate binder compared with sevelamer on aortic medial calcification were demonstrated in uremic rats: $Ca^{2+}$-acetate and $Mg^{2+}$-carbonate prevented an increasing serum PTH and aortic calcium content more effectively.[144] Prevention of hyperphosphatemia and medial expression of osteogenic proteins such as BMP-2 and SRY-box 9 (sex-determining region Y box 9) in the media were achieved equally by both $Ca^{2+}$-acetate and $Mg^{2+}$-carbonate and sevelamer.[144] In hemodialysis patients, the use of a $Ca^{2+}$-carbonate/$Mg^{2+}$-carbonate combination correlated with reduced coronary artery calcification in a small clinical pilot study in 2009.[145] Although the size and design of the study are insufficient to admit clinical use, this study served as an indication that $Mg^{2+}$ is an interesting novel and cost-effective treatment option. In addition to the Pi-binding effects of $Mg^{2+}$ in the intestine, the concomitant increase in serum $Mg^{2+}$ concentration may be protective for vascular calcification.[142] Interestingly, the use of sevelamer itself has recently been found to be associated with increased serum $Mg^{2+}$ concentrations.[146] The authors suggest that the beneficial effects of sevelamer on reduced inflammation, inhibition of vascular calcification, and decreased mortality might be partially explained by the higher serum $Mg^{2+}$ concentrations. Follow-up studies should determine whether direct use of $Mg^{2+}$-based Pi binders would be a more efficient treatment option in CKD patients.

## Magnesium in the Circulation

In calcified vessels, hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is the most abundant type of crystal.[147] Reduction or delay of hydroxyapatite formation by magnesium has been proposed

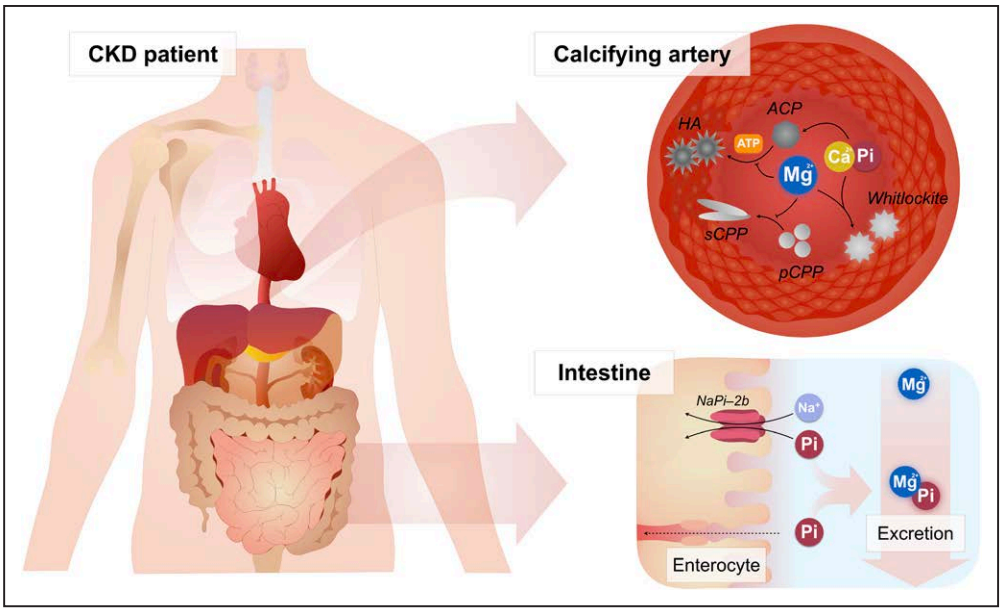


**Figure 2.** Passive interference: phosphate binding and crystal inhibition by $Mg^{2+}$. Elevated blood $Mg^{2+}$ interferes with both amorphous calcium phosphate (ACP) and primary calciprotein particle (CPP) maturation into hydroxyapatite (HA) crystals and secondary CPP (sCPP). $Mg^{2+}$ promotes the formation of the more soluble and smaller whitlockite crystal. In the intestine, $Mg^{2+}$-based inorganic phosphate (Pi) binders promote fecal Pi excretion, reducing Pi uptake via sodium phosphate cotransporter IIb (NaPi-2b) in enterocytes. CKD indicates chronic kidney disease; pCPP, primary CPP.

as a mechanism to halt the calcification process. $Mg^{2+}$ reduces ACP formation and maturation toward hydroxyapatite.[148–150] In aqueous solutions, $Mg^{2+}$ delayed hydroxyapatite maturation with 20 hours, which was determined by the degree of crystallinity.[151] Crystallization of ACP was prevented when the $Mg^{2+}$/$Ca^{2+}$ molar ratio exceeded 0.2 resulting simultaneously in reduced solubility of the crystal.[152] Mechanistically, the stabilizing effect of $Mg^{2+}$ on ACP has been attributed to the capacity of $Mg^{2+}$ to form stronger complexes with Pi than $Ca^{2+}$.[152]

An alternative mechanism is that $Mg^{2+}$ stabilizes extracellular ATP, which is otherwise hydrolyzed at the ACP surface enabling hydroxyapatite formation.[153] $Mg^{2+}$ shields the ACP surface from ATP, thereby preventing its breakdown. Although the effect of $Mg^{2+}$ on ATP has often been neglected, the role of extracellular ATP in vascular calcification has been studied because its hydrolysis is necessary for pyrophosphate synthesis, which is a direct inhibitor of hydroxyapatite formation.[154,155] As noted elsewhere in this review, $Mg^{2+}$ protected against vascular calcification in Abcc6$^{-/-}$ mice.[94] In this model of pseudoxanthoma elasticum, hepatic ABCC6 (ATP-binding cassette subfamily C member 6)–dependent–mediated cellular ATP secretion has been identified as the principal source of circulating pyrophosphate.[156] Pyrophosphate levels are 2.5-fold reduced in pseudoxanthoma patients where ABCC6 is dysfunctional, explaining the underlying mechanism in related mineralization disorders.[156]

The stabilizing effects of $Mg^{2+}$ on ACP nucleation and hydroxyapatite maturation in clinical setting have often been proposed in literature. However, this hypothesis has been poorly addressed in models of vascular calcification. Pasch et al[133] linked $Mg^{2+}$ status to calcification propensity of hemodialysis patients, which is based on the intrinsic capacity of the serum to inhibit the maturation of primary CPP to secondary CPP and found that $Mg^{2+}$ effectively delayed CPP maturation. Of note, secondary CPPs have been shown to induce calcification in vitro.[131]

It is often proposed that $Mg^{2+}$ favors the formation of $Mg^{2+}$-containing whitlockite ($Ca_9Mg(HPO_4)(PO_4)_6$) crystals rather than hydroxyapatite.[157] Whitlockite is smaller, more soluble, and less inflammatory compared with apatite and is only formed when $Mg^{2+}$/$Ca^{2+}$ ratios increase.[157–159] Formation of whitlockite after an increased serum $Mg^{2+}$ concentration may therefore be a mechanism by which $Mg^{2+}$ retards vascular calcification progression. However, $Mg^{2+}$ supplementation to calcifying human VSMCs neither altered cellular apatite architecture nor resulted in the presence of whitlockite.[160] In addition, analysis of iliac arteries of dialysis patients showed the presence of both hydroxyapatite and whitlockite in calcified areas, colocalizing with calcification inhibitors.[161] These findings combined suggest that preventive mechanisms of $Mg^{2+}$ likely involve pathways alternative to the formation of whitlockite.

## Active Modulation: Cell-Mediated Actions of Magnesium in Vascular Calcification

The transdifferentiation of VSMCs toward an osteogenic phenotype is considered a major driving force of vascular calcification.[121] Several groups have shown that this effect is modulated by the intracellular $Mg^{2+}$ concentration, suggesting active modulation of VSMC transdifferentiation by $Mg^{2+}$ (Figure 3).

### Magnesium and Osteogenic Conversion

Multiple studies report that $Mg^{2+}$ supplementation prevents the transcriptional changes in VSMC transdifferentiation and apoptosis, thereby halting the calcification process in both in vitro and ex vivo models of vascular calcification.[162–164] $Mg^{2+}$ supplementation effectively counteracts expression of osteogenic transcription

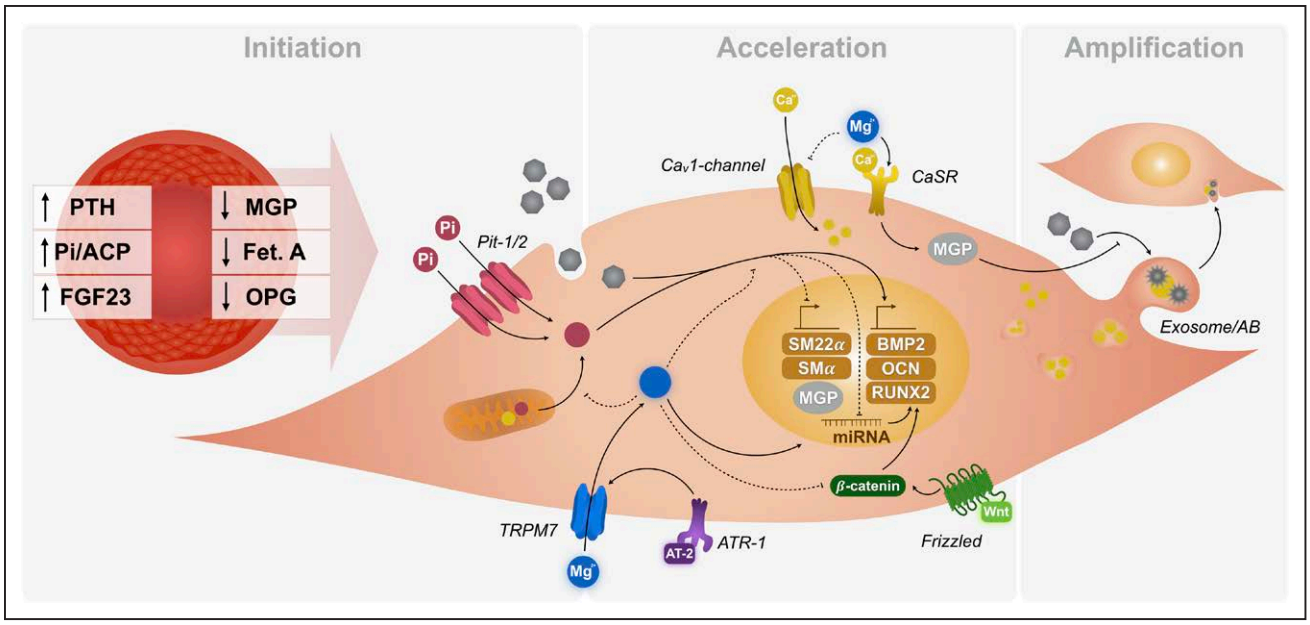


**Figure 3.** Active modulation: $Mg^{2+}$ inhibits vascular smooth muscle cell transdifferentiation. Diminished levels of circulating inhibitors of vascular calcification, elevated levels of inorganic phosphate (Pi), and formation of amorphous $Ca^{2+}$-Pi particle (ACP) in the circulation initiate the transdifferentiation of vascular smooth muscle cell (VSMC). VSMC transdifferentiation is accelerated by the expression of osteogenic genes and amplified by the VSMCs through the release of exosomes and apoptotic bodies. $Mg^{2+}$ potentially prevents this process via different pathways both on the level of initiation and acceleration of VSMC calcification. AB indicates apoptotic body; AT2, angiotensin type 2; ATR-1, angiotensin 2 type 1 receptor; BMP-2, bone morphogenetic protein 2; $Ca_v1$ channel, L-Type calcium channel; CaSR, calcium-sensing receptor; Fet. A, fetuin-A; FGF23, fibroblast growth factor 23; MGP, matrix gla protein; OCN, osteocalcin; OPG, osteoprotegerin; PiT, sodium-dependent inorganic phosphate transporter; PTH, parathyroid hormone; SM22α, transgelin; α-SMA, α–smooth muscle actin; RUNX2, runt-related transcription factor 2; and TRPM7, transient receptor potential melastatin 7.



factors (BMP-2, RUNX2, Msh homeobox 2, SRY-box 9), bone proteins, and genes associated with matrix mineralization (osteocalcin and alkaline phosphatase).[162,165,166] Simultaneously, it was observed that $Mg^{2+}$ prevents the loss of calcification inhibitors (BMP-7, MGP, and osteopontin) that protect against osteogenic conversion. These examples illustrate that $Mg^{2+}$ is actively involved in the prevention of VSMC transdifferentiation to an osteogenic phenotype. However, whether $Mg^{2+}$ directly modulates osteogenic gene expression remains under debate.

Because osteogenic gene expression is a convenient readout for vascular calcification in VSMCs, it has been widely exploited in in vitro studies. Given that inhibition of vascular calcification on any level may delay or even abrogate VSMC transdifferentiation, using osteogenic gene expression as readout is prone to misinterpretation of the mechanisms involved. VSMC calcification is often initiated by Pi- and $Ca^{2+}$-enriched media and adding $Mg^{2+}$ to calcifying VSMCs may have both extracellular and intracellular effects. However, when effective all will result in reduced VSMC transdifferentiation, calcification, and thus in lower osteogenic gene expression. Although this is poorly supported by direct evidence, the experimental bias of measuring osteogenic gene expression has resulted in the predominant hypothesis that intracellular $Mg^{2+}$ reduces vascular calcification, overlooking potential extracellular effects.

The only studies convincingly supporting an intracellular role of $Mg^{2+}$ are the ones that target $Mg^{2+}$ channels. In VSMCs, $Mg^{2+}$ homeostasis is mainly maintained by TRPM7 cation channels, which have been shown to be downregulated in calcification conditions.[165,167] Reduced TRPM7 activity using nonselective inhibitor 2-APB (aminoethoxydiphenyl borate) or a specific siRNA resulted in progressive VSMC transdifferentiation, illustrating a crucial role for intracellular $Mg^{2+}$ in this context.[165,166] Furthermore, angiotensin-2 supplementation prevented osteoinductive expression and calcification in VSMCs by increasing $Mg^{2+}$ influx. This effect was abrogated by blocking $Mg^{2+}$ channel TRMP7 using 2-APB.[168]

Several mechanisms have been proposed by which increased intracellular $Mg^{2+}$ concentrations facilitated by TRPM7 activity could prevent osteoinductive gene expression. First, $Mg^{2+}$ effectively abolished Pi-induced Wnt/β-catenin signaling, which is involved in osteoblast maturation and exercises its osteoinductive effects through increasing RUNX2 expression.[169,170] Second, $Mg^{2+}$ has been implicated in the regulation of miRNAs involved in vascular homeostasis, a variety of which were recently found to be compromised in CKD.[171,172] $Mg^{2+}$ successfully abrogated and even improved deteriorated expression profiles of microRNA-30b, microRNA-133a, and microRNA-223 that regulate RUNX2, Smad1, and osterix expression in calcifying VSMCs.[173] Third, $Mg^{2+}$ is implicated in the modulation of VSMC calcium handling and the activation of the $Ca^{2+}$-sensing receptor (CaSR) important for MGP function, which will be discussed below.

To identify additional mechanisms by which $Mg^{2+}$ prevents calcification, it is relevant to learn from other calcification models. For instance, $Mg^{2+}$ prevented SaOS-2 differentiation into mature osteoblasts in high concentrations (5 mmol/L), as reflected by matrix mineralization and alkaline phosphatase activity.[174] Importantly, however, these results were not reproducible in normal human osteoblasts. Furthermore, in tendon-derived stem cells, $Mg^{2+}$ prevented matrix mineralization, a

process that highly resembles that of VSMCs.[175] The authors proposed a role for $Mg^{2+}$ in mitochondrial export of $Ca^{2+}$ and Pi by the inhibition of mitochondrial transition pores, preventing transmembrane depolarization and matrix mineralization. However, application of these findings to VSMC calcification has not been evaluated to date.

The studies targeting TRPM7 support an intracellular effect of $Mg^{2+}$ and reject the hypothesis that $Mg^{2+}$-dependent regulation of calcification genes is only secondary to extracellular Pi binding and ACP stabilization. However, there is a lack of data on intracellular $Mg^{2+}$ concentrations limiting conclusive confirmation on an active role for $Mg^{2+}$ in this context. Additional studies measuring intracellular $Mg^{2+}$ concentrations are necessary, but are hampered by the poor availability of selective fluorescent $Mg^{2+}$ probes.

### Magnesium and Cellular Calcium Entry

Excessive intracellular $Ca^{2+}$ causes VSMC death and subsequent release of apoptotic bodies, which contribute to matrix calcification by providing ACP nucleation sites.[135,176,177] As a natural $Ca^{2+}$ channel antagonist, $Mg^{2+}$ has the capacity to block $Ca^{2+}$ channels in VSMCs and prevent $Ca^{2+}$ overload.[97,178] As a consequence of $Ca^{2+}$ channel blocking, $Mg^{2+}$ has excellent vasodilatory properties, which in arterioles and venules is already effective at 0.01 to 0.1 mmol/L concentrations and reduces myogenic tone.[97,179] Therefore, a role for $Mg^{2+}$ in preventing intracellular $Ca^{2+}$ bursts, and subsequent apoptosis has been identified as a potential mechanism of action in preventing VSMC calcification.[10]

In VSMCs, $Ca^{2+}$ influx could be regulated by a sensing mechanism. The CaSR is expressed in the parathyroid and the kidney, and there are indications that VSMCs also express functional CaSR.[180] This receptor plays an important role in mineral-bone homeostasis by regulating PTH secretion. In addition to $Ca^{2+}$ channel blocking, $Mg^{2+}$ has been implicated in CaSR activation, possibly functioning as calcimimetic and indirect gatekeeper of $Ca^{2+}$ influx.[181] In contrast to $Ca^{2+}$, $Mg^{2+}$ acts as a partial agonist and activates the CaSR 2 to 3× less potently.[181–183]

Systemically, lower PTH after CaSR activation in the parathyroid results in decreased bone turnover and intestinal $Ca^{2+}$ uptake, but promotes renal Pi reabsorption. In dialysis patients, higher $Mg^{2+}$ concentrations indeed correlate with decreased PTH levels.[184] Although the presence and function of CaSR in VSMCs remain uncertain, vascular calcification has been associated with loss of functional CaSR and MGP in VSMCs.[185–187] In VSMCs, treatment with calcimimetics resulted in the activation of the CaSR, which led to reduced mineralization.[180] In aortas of uremic rats and in bovine VSMCs, the calcimimetic AMG641 decreased medial calcification and increased expression of MGP.[188] Recently, the first in vitro and in vivo evidence suggested that $Mg^{2+}$ supplementation in VSMCs resulted in reduced Pi- and hydroxyapatite-induced calcification through restoring CaSR mRNA and protein levels.[189] However, this study did not examine parameters related to mineral-bone metabolism in response to $Mg^{2+}$ treatment in the in vivo part of their study. Therefore, the role of $Mg^{2+}$ in the regulation of hormones and receptors involved in CKD-mineral bone disorder in its protection against vascular calcification remain to be determined.

## Conclusions

In CKD patients, serum $Mg^{2+}$ concentrations are correlated with cardiovascular morbidity and mortality. Multiple observational studies and several intervention studies identify a direct link between $Mg^{2+}$ and cardiovascular mortality, potentially related to vascular calcification in CKD patients. An increasing number of in vitro, preclinical, and clinical studies demonstrate a protective role for $Mg^{2+}$ in the development of vascular calcification. The current literature supports both a passive Pi-buffering role reducing hydroxyapatite formation and an active cell-mediated role, directly altering osteogenic expression in VSMC. Despite these promising and consistent results among models, absence of large-scale clinical studies impedes clinical implementation of $Mg^{2+}$ supplements in CKD. Well-designed randomized controlled trials in CKD patients are necessary for any definitive conclusions on the preventive effects of $Mg^{2+}$ in vascular calcification.

### Remaining Challenges

Final conclusions about the molecular effects of $Mg^{2+}$ are seriously hampered by the basic experimental setup of many in vitro studies that suffice with simple $Mg^{2+}$ supplementation to calcification medium. This setup does not distinguish between passive chemical and active cell-mediated mechanisms. However, because cellular entrance of $Mg^{2+}$ via TRPM7 has been shown to be necessary for at least some of its protective effects, an active mechanism preventing VSMC transdifferentiation is likely. This review identified a substantial knowledge gap of the role of intracellular $Mg^{2+}$, as the molecular targets linking $Mg^{2+}$ with osteogenic gene expression are unknown. In addition, the effect of $Mg^{2+}$ supplementation on intracellular VSMC $Mg^{2+}$ concentration has never been studied and urgently requires attention. Basic studies toward intracellular $Mg^{2+}$ homeostasis and the molecular players that regulate $Mg^{2+}$ concentrations in VSMCs are lacking and are essential to drive further advances in this field. Several of the mechanisms that have been repeatedly suggested have never been thoroughly studied in the context of vascular calcification, including the relevance of $Mg^{2+}$ on cellular $Ca^{2+}$ fluxes, the role of the CaSR in VSMCs and in particular the chemical impact of $Mg^{2+}$ on ACP maturation. Furthermore, this review highlights the potential experimental bias of measuring osteogenic gene expression as effective inhibition of mineralization by $Mg^{2+}$ through both extracellular and intracellular pathways will all result in reduced VSMC transdifferentiation. Therefore, an additional challenge that the field now faces lies in determining the relative contribution of each effect to the prevention of vascular calcification.

### Clinical Relevance and Implications

In the general population, $Mg^{2+}$ is inversely associated with cardiovascular outcome. Results of these studies strongly reinforce the hypothesis that the current clinical reference ranges (0.7–1.1 mmol/L) for serum $Mg^{2+}$ should be reconsidered, as

**Table 2. The Effects of Serum $Mg^{2+}$ Concentration on Cardiovascular Disease Occurrence in the End-Stage Renal Disease Population**

| Author* | Study Type | Cardiovascular Outcome† | No. of Patients (% Women) | Follow-Up | Association Inhibiting Outcome ($P<0.05$) | Associations With Serum $Mg^{2+}$ (mmol/L) | Associations With Increased Serum $Mg^{2+}$ (mmol/L) | Reference Concentration |
|---|---|---|---|---|---|---|---|---|
| Meema et al[59] 1987 | Prospective | AC | 44 (0) | 27 mo | Yes | 1.1±0.21 in AC compared with 3.02±0.51 in non-AC | N/A | N/A |
| Tzanakis et al[60] 2004 | Cross-sectional | MAC | 56 (39.2) | None | Yes | 1.14±0.12 in MAC vs 1.27±0.095 in non-MAC | >1.23 twice as likely to develop MAC as <1.23 ($\chi^2$=6.98) | N/A |
| Ishimura et al[61] 2007 | Prospective | CV mortality | 515 (40.6) | 51 mo | No | HR, 0.98; 95% CI, 3.13 to 3.086 | N/A | N/A |
| Ishimura et al[62] 2007 | Cross-sectional | VC | 390 (42.1) | None | Yes | 1.10±0.12 in VC vs 1.14±0.14 in non-VC | Presence reduction per 0.4 increase (OR, 0.28; 95% CI, 0.09 to 0.92) | N/A |
| Kanbay et al[63] 2012 | Prospective | Fatal and nonfatal CVE | 283 (50.9) | 38 mo | Yes | HR, 0.21; 95% CI, 0.10 to 0.46 | N/A | N/A |
| Matias et al[64] 2014 | Prospective | VC (SVCS) and CV mortality | 206 (45) | 48 mo | Yes | CV mortality: HR, 0.82; 95% CI, 0.72 to 0.95. SVCS multivariate: β-coefficient, 0.17; 95% CI, 0.08 to 0.30 (cutoff concentration, 1.15) | N/A | N/A |
| Sakaguchi et al[34] 2014 | Prospective | CV mortality | 142 069 (38.1) | 12 mo | Yes | <0.95 (OR, 1.24; 95% CI, 1.08 to 1.42) | ≥1.1–<1.15 (OR, 1.03; 95% CI, 0.85 to 1.23); and ≥1.27 (OR, 1.25; 95% CI, 1.07 to 1.47) | ≥1.15–<1.27 |
| De Roij van Zuijdewijn et al[25] 2015 | Prospective | CV mortality | 365 (38.1) | 3.1 y | Yes | N/A | Risk reduction per 0.1 increase (HR, 0.73; 95% CI, 0.62 to 0.85) | N/A |
| Yu et al[65] 2016 | Prospective | CV mortality | 135 (41.5) | 36 mo | Yes | 17.2% mortality at 0.99±0.10 vs 5.6% at 1.21±0.11, $\chi^2$=4.912 | N/A | N/A |
| Cai et al[35] 2016 | Prospective | CV mortality | 253 (44.7) | 29 mo | Yes | HR, 0.003; 95% CI, 0.000 to 0.055 | N/A | N/A |
| Molnar et al[66] 2017 | Cross-sectional | AAC | 80 (30) | None | Yes | Adjusted $R^2$=0.18, β-coefficient=−12/27; 95% CI, −19.54 to −5.00 | 0.1 increase results in 1.1-point decrease in AAC score | N/A |

AAC indicates abdominal aortic calcification; AC, arterial calcification; CI, confidence interval; CV, cardiovascular; CVE, cardiovascular events; HR, hazard ratio; MAC, mitral annular calcifications; N/A, not applicable; OR, odds ratio; SVCS, simple vascular calcification score; and VC, vascular calcification.

*Articles were obtained after PubMed search using the following search terms: (("Renal Dialysis"[Mesh] OR "Kidney Failure, Chronic"[Mesh]) AND Magnesium"[Mesh]) AND ("Cardiovascular Diseases"[Mesh] OR "calcinosis"[mesh] OR "Survival Analysis"[Mesh]).

†Studies assessing the effects of dietary $Mg^{2+}$, indirect outcome measures for cardiovascular disease (eg, hypertension, arterial intima-media thickness), and predialysis cohorts were excluded.

concentrations of <0.8 mmol/L are associated with increased risk for cardiovascular disease and mortality (Table 1).

In CKD population, the pronounced effects of $Mg^{2+}$ in experimental models of vascular calcifications drive the hypothesis that $Mg^{2+}$ protects against mortality in CKD through the prevention of vascular calcification. However, the clinical role of $Mg^{2+}$ in CKD patients has only been studied in observational cohorts, which focus mostly on total cardiovascular risk (Table 2). The effects of $Mg^{2+}$ supplementation on cardiovascular outcome aside from arrhythmia and preeclampsia have been poorly assessed. Currently, randomized controlled clinical trials using $Mg^{2+}$ supplementation as treatment for vascular calcification are in progress, and their results are eagerly awaited. These large-scale clinical trials will determine the translational value of the many experimental model systems that show a preventive effect of $Mg^{2+}$ on vascular calcification. Nevertheless, further elucidation of the molecular mechanisms may contribute to additional targeted therapeutic options improving $Mg^{2+}$ homeostasis in CKD patients.

## Acknowledgments

We thank Dr Joost G.J. Hoenderop for careful reading of the manuscript.

## Sources of Funding

J.H.F. de Baaij is supported by grants from the Netherlands Organization for Scientific Research (NWO [Nederlandse Organisatie voor Wetenschappelijl Onderzoek/Netherlands Organisation for Scientific Research] Rubicon 825.14.021) and the Dutch Kidney Foundation (Kolff 14OKG17).

## Disclosures

C.M. Shanahan has a consultancy agreement with OPKO Health. The other authors report no conflicts.

## References


1. Foley RN, Parfrey PS. Cardiovascular disease and mortality in ESRD. *J Nephrol*. 1998;11:239–245.
2. Mozaffarian D, Benjamin EJ, Go AS, et al. Heart disease and stroke statistics—2016 update. *Circulation*. 2016;133:447–454.
3. Saran R, Li Y, Robinson B. US Renal Data System 2015 Annual Data Report: epidemiology of kidney disease in the United States. *Am J Kidney Dis*. 2014;67:S1–S434.
4. Guérin AP, London GM, Marchais SJ, Metivier F. Arterial stiffening and vascular calcifications in end-stage renal disease. *Nephrol Dial Transplant*. 2000;15:1014–1021.
5. Braun J, Oldendorf M, Moshage W, Heidler R, Zeitler E, Luft FC. Electron beam computed tomography in the evaluation of cardiac calcification in chronic dialysis patients. *Am J Kidney Dis*. 1996;27:394–401.
6. Goodman WG, Goldin J, Kuizon BD, Yoon C, Gales B, Sider D, Wang Y, Chung J, Emerick A, Greaser L, Elashoff RM, Salusky IB. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med*. 2000;342:1478–1483. doi: 10.1056/NEJM200005183422003.
7. Budoff MJ, Rader DJ, Reilly MP, Mohler ER III, Lash J, Yang W, Rosen L, Glenn M, Teal V, Feldman HI; CRIC Study Investigators. Relationship of estimated GFR and coronary artery calcification in the CRIC (Chronic Renal Insufficiency Cohort) Study. *Am J Kidney Dis*. 2011;58:519–526. doi: 10.1053/j.ajkd.2011.04.024.
8. Floege J, Ketteler M. Vascular calcification in patients with end-stage renal disease. *Nephrol Dial Transplant*. 2004;19(suppl 5):V59–V66. doi: 10.1093/ndt/gfh1058.
9. London GM, Guérin AP, Marchais SJ, Métivier F, Pannier B, Adda H. Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. *Nephrol Dial Transplant*. 2003;18:1731–1740.
10. Massy ZA, Drüeke TB. Magnesium and outcomes in patients with chronic kidney disease: focus on vascular calcification, atherosclerosis and survival. *Clin Kidney J*. 2012;5(suppl 1):i52–i61. doi: 10.1093/ndtplus/sfr167.
11. de Baaij JHF, Hoenderop JGJ, Bindels RJM. Magnesium in man: implications for health and disease. *Physiol Rev*. 2015;95:1–46.
12. Graham LA, Caesar JJ, Burgen AS. Gastrointestinal absorption and excretion of Mg 28 in man. *Metabolism*. 1960;9:646–659.
13. Alfrey AC, Miller NL, Trow R. Effect of age and magnesium depletion on bone magnesium pools in rats. *J Clin Invest*. 1974;54:1074–1081. doi: 10.1172/JCI107851.
14. Thebault S, Alexander RT, Tiel Groenestege WM, Hoenderop JG, Bindels RJ. EGF increases TRPM6 activity and surface expression. *J Am Soc Nephrol*. 2009;20:78–85. doi: 10.1681/ASN.2008030327.
15. Nair AV, Hocher B, Verkaart S, van Zeeland F, Pfab T, Slowinski T, Chen YP, Schlingmann KP, Schaller A, Gallati S, Bindels RJ, Konrad M, Hoenderop JG. Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. *Proc Natl Acad Sci USA*. 2012;109:11324–11329. doi: 10.1073/pnas.1113811109.
16. Li M, Du J, Jiang J, Ratzan W, Su LT, Runnels LW, Yue L. Molecular determinants of Mg2+ and Ca2+ permeability and pH sensitivity in TRPM6 and TRPM7. *J Biol Chem*. 2007;282:25817–25830. doi: 10.1074/jbc.M608972200.
17. de Baaij JH, Blanchard MG, Lavrijsen M, Leipziger J, Bindels RJ, Hoenderop JG. P2X4 receptor regulation of transient receptor potential melastatin type 6 (TRPM6) Mg2+ channels. *Pflugers Arch*. 2014;466:1941–1952. doi: 10.1007/s00424-014-1440-3.
18. Blanchard MG, De Baaij JHF, Verkaart SAJ, et al. Flavaglines stimulate transient receptor potential melastatin type 6 (TRPM6) channel activity. *PLoS One*. 2015;10:e0119028.
19. Quamme GA, Wong NL, Dirks JH, Roinel N, De Rouffignac C, Morel F. Magnesium handling in the dog kidney: a micropuncture study. *Pflugers Arch*. 1978;377:95–99.
20. Voets T, Nilius B, Hoefs S, van der Kemp AW, Droogmans G, Bindels RJ, Hoenderop JG. TRPM6 forms the Mg2+ influx channel involved in intestinal and renal Mg2+ absorption. *J Biol Chem*. 2004;279:19–25. doi: 10.1074/jbc.M311201200.
21. Kolisek M, Nestler A, Vormann J, Schweigel-Röntgen M. Human gene SLC41A1 encodes for the Na+/$Mg^{2}$+ exchanger. *Am J Physiol Cell Physiol*. 2012;302:C318–C326. doi: 10.1152/ajpcell.00289.2011.
22. de Baaij JHF, Arjona FJ, van den Brand M, et al. Identification of SLC41A3 as a novel player in magnesium homeostasis. *Sci Rep*. 2016;6:28565.
23. Dewitte K, Dhondt A, Giri M, Stöckl D, Rottiers R, Lameire N, Thienpont LM. Differences in serum ionized and total magnesium values during chronic renal failure between nondiabetic and diabetic patients: a cross-sectional study. *Diabetes Care*. 2004;27:2503–2505.
24. Coburn JW, Popovtzer MM, Massry SG, Kleeman CR. The physicochemical state and renal handling of divalent ions in chronic renal failure. *Arch Intern Med*. 1969;124:302–311.
25. de Roij van Zuijdewijn CL, Grooteman MP, Bots ML, Blankestijn PJ, Steppan S, Büchel J, Groenwold RH, Brandenburg V, van den Dorpel MA, Ter Wee PM, Nubé MJ, Vervloet MG. Serum magnesium and sudden death in European hemodialysis patients. *PLoS One*. 2015;10:e0143104. doi: 10.1371/journal.pone.0143104.
26. Cunningham J, Rodríguez M, Messa P. Magnesium in chronic kidney disease Stages 3 and 4 and in dialysis patients. *CKJ Clin Kidney J*. 2012;5:i39–i51.
27. Cunningham J, Locatelli F, Rodriguez M. Secondary hyperparathyroidism: pathogenesis, disease progression, and therapeutic options. *Clin J Am Soc Nephrol*. 2011;6:913–921. doi: 10.2215/CJN.06040710.
28. Blumberg D, Bonetti A, Jacomella V, Capillo S, Truttmann AC, Lüthy CM, Colombo JP, Bianchetti MG. Free circulating magnesium and renal magnesium handling during acute metabolic acidosis in humans. *Am J Nephrol*. 1998;18:233–236. doi: 13342.
29. Elin RJ. Assessment of magnesium status for diagnosis and therapy. *Magnes Res*. 2010;23:S194–S198. doi: 10.1684/mrh.2010.0213.
30. Mikolasevic I, Milic S, Stimac D, Zaputovic L, Lukenda Zanko V, Gulin T, Jakopcic I, Klaric D, Gulin M, Orlic L. Is there a relationship between hypomagnesemia and proton-pump inhibitors in patients on chronic hemodialysis? *Eur J Intern Med*. 2016;30:99–103. doi: 10.1016/j.ejim.2016.01.026.
31. Nakashima A, Ohkido I, Yokoyama K, Mafune A, Urashima M, Yokoo T. Proton pump inhibitor use and magnesium concentrations in hemodialysis patients: a cross-sectional study. *PLoS One*. 2015;10:e0143656.
32. Sakaguchi Y, Shoji T, Hayashi T, Suzuki A, Shimizu M, Mitsumoto K, Kawabata H, Niihata K, Okada N, Isaka Y, Rakugi H, Tsubakihara Y. Hypomagnesemia in type 2 diabetic nephropathy: a novel predictor of end-stage renal disease. *Diabetes Care*. 2012;35:1591–1597. doi: 10.2337/dc12-0226.
33. Gommers LM, Hoenderop JG, Bindels RJ, de Baaij JH. Hypomagnesemia in type 2 diabetes: a vicious circle? *Diabetes*. 2016;65:3–13. doi: 10.2337/db15-1028.
34. Sakaguchi Y, Fujii N, Shoji T, Hayashi T, Rakugi H, Isaka Y. Hypomagnesemia is a significant predictor of cardiovascular and non-cardiovascular mortality in patients undergoing hemodialysis. *Kidney Int*. 2014;85:174–181. doi: 10.1038/ki.2013.327.
35. Cai K, Luo Q, Dai Z, et al. Hypomagnesemia is associated with increased mortality among peritoneal dialysis patients. *PLoS One*. 2016;11:e0152488.
36. Li L, Streja E, Rhee CM, Mehrotra R, Soohoo M, Brunelli SM, Kovesdy CP, Kalantar-Zadeh K. Hypomagnesemia and mortality in incident hemodialysis patients. *Am J Kidney Dis*. 2015;66:1047–1055.
37. Guasch-Ferré M, Bulló M, Estruch R, et al; PREDIMED Study Group. Dietary magnesium intake is inversely associated with mortality in adults at high cardiovascular disease risk. *J Nutr*. 2014;144:55–60. doi: 10.3945/jn.113.183012.
38. Fang X, Wang K, Han D, He X, Wei J, Zhao L, Imam MU, Ping Z, Li Y, Xu Y, Min J, Wang F. Dietary magnesium intake and the risk of cardiovascular disease, type 2 diabetes, and all-cause mortality: a dose-response meta-analysis of prospective cohort studies. *BMC Med*. 2016;14:210. doi: 10.1186/s12916-016-0742-z.
39. Lutsey PL, Alonso A, Michos ED, et al. Serum magnesium, phosphorus, and calcium are associated with risk of incident heart failure: the Atherosclerosis Risk in Communities (ARIC). *Am J Clin Nutr*. 2014;100:756–764.
40. Kieboom BCT, Niemeijer MN, Leening MJG, et al. Serum magnesium and the risk of death from coronary heart disease and sudden cardiac death. *J Am Heart Assoc*. 2016;5:e002707.
41. Gartside PS, Glueck CJ. The important role of modifiable dietary and behavioral characteristics in the causation and prevention of coronary heart disease hospitalization and mortality: the prospective NHANES I follow-up study. *J Am Coll Nutr*. 1995;14:71–79.
42. Marniemi J, Järvisalo J, Toikka T, Räihä I, Ahotupa M, Sourander L. Blood vitamins, mineral elements and inflammation markers as risk factors of vascular and non-vascular disease mortality in an elderly population. *Int J Epidemiol*. 1998;27:799–807.
43. Liao F, Folsom AR, Brancati FL. Is low magnesium concentration a risk factor for coronary heart disease? The Atherosclerosis Risk in Communities (ARIC) Study. *Am Heart J*. 1998;136:480–490.

44. Ford ES. Serum magnesium and ischaemic heart disease: findings from a national sample of US adults. *Int J Epidemiol*. 1999;28:645–651.
45. Leone N, Courbon D, Ducimetiere P, Zureik M. Zinc, copper, and magnesium and risks for all-cause, cancer, and cardiovascular mortality. *Epidemiology*. 2006;17:308–314. doi: 10.1097/01.ede.0000209454.41466.b7.
46. Ohira T, Peacock JM, Iso H, Chambless LE, Rosamond WD, Folsom AR. Serum and dietary magnesium and risk of ischemic stroke: the Atherosclerosis Risk in Communities Study. *Am J Epidemiol*. 2009;169:1437–1444. doi: 10.1093/aje/kwp071.
47. Khan AM, Sullivan L, McCabe E, Levy D, Vasan RS, Wang TJ. Lack of association between serum magnesium and the risks of hypertension and cardiovascular disease. *Am Heart J*. 2010;160:715–720. doi: 10.1016/j.ahj.2010.06.036.
48. Peacock JM, Ohira T, Post W, Sotoodehnia N, Rosamond W, Folsom AR. Serum magnesium and risk of sudden cardiac death in the Atherosclerosis Risk in Communities (ARIC) Study. *Am Heart J*. 2010;160:464–470. doi: 10.1016/j.ahj.2010.06.012.
49. Reffelmann T, Ittermann T, Dörr M, Völzke H, Reinthaler M, Petersmann A, Felix SB. Low serum magnesium concentrations predict cardiovascular and all-cause mortality. *Atherosclerosis*. 2011;219:280–284. doi: 10.1016/j.atherosclerosis.2011.05.038.
50. Chiuve SE, Korngold EC, Januzzi JL Jr, Gantzer ML, Albert CM. Plasma and dietary magnesium and risk of sudden cardiac death in women. *Am J Clin Nutr*. 2011;93:253–260. doi: 10.3945/ajcn.110.002253.
51. Feng P, Niu X, Hu J, Zhou M, Liang H, Zhang Y, Tong W, Xu T. Relationship of serum magnesium concentration to risk of short-term outcome of acute ischemic stroke. *Blood Press*. 2013;22:297–301. doi: 10.3109/08037051.2012.759696.
52. Khan AM, Lubitz SA, Sullivan LM, Sun JX, Levy D, Vasan RS, Magnani JW, Ellinor PT, Benjamin EJ, Wang TJ. Low serum magnesium and the development of atrial fibrillation in the community: the Framingham Heart Study. *Circulation*. 2013;127:33–38. doi: 10.1161/CIRCULATIONAHA.111.082511.
53. Misialek JR, Lopez FL, Lutsey PL, Huxley RR, Peacock JM, Chen LY, Soliman EZ, Agarwal SK, Alonso A. Serum and dietary magnesium and incidence of atrial fibrillation in whites and in African Americans–Atherosclerosis Risk in Communities (ARIC) study. *Circ J*. 2013;77:323–329.
54. Joosten MM, Gansevoort RT, Mukamal KJ, van der Harst P, Geleijnse JM, Feskens EJ, Navis G, Bakker SJ; PREVEND Study Group. Urinary and plasma magnesium and risk of ischemic heart disease. *Am J Clin Nutr*. 2013;97:1299–1306. doi: 10.3945/ajcn.112.054114.
55. Akarolo-Anthony SN, Jiménez MC, Chiuve SE, Spiegelman D, Willett WC, Rexrode KM. Plasma magnesium and risk of ischemic stroke among women. *Stroke*. 2014;45:2881–2886. doi: 10.1161/STROKEAHA.114.005917.
56. Lee SY, Hyun YY, Lee KB, Kim H. Low serum magnesium is associated with coronary artery calcification in a Korean population at low risk for cardiovascular disease. *Nutr Metab Cardiovasc Dis*. 2015;25:1056–1061. doi: 10.1016/j.numecd.2015.07.010.
57. Markovits N, Kurnik D, Halkin H, Margalit R, Bialik M, Lomnicky Y, Loebstein R. Database evaluation of the association between serum magnesium levels and the risk of atrial fibrillation in the community. *Int J Cardiol*. 2016;205:142–146. doi: 10.1016/j.ijcard.2015.12.014.
58. Posadas-Sánchez R, Posadas-Romero C, Cardoso-Saldaña G, Vargas-Alarcón G, Villarreal-Molina MT, Pérez-Hernández N, Rodríguez-Pérez JM, Medina-Urrutia A, Jorge-Galarza E, Juárez-Rojas JG, Torres-Tamayo M. Serum magnesium is inversely associated with coronary artery calcification in the Genetics of Atherosclerotic Disease (GEA) study. *Nutr J*. 2016;15:22. doi: 10.1186/s12937-016-0143-3.
59. Meema HE, Oreopoulos DG, Rapoport A. Serum magnesium level and arterial calcification in end-stage renal disease. *Kidney Int*. 1987;32:388–394.
60. Tzanakis I, Virvidakis K, Tsomi A, Mantakas E, Girousis N, Karefyllakis N, Papadaki A, Kallivretakis N, Mountokalakis T. Intra- and extracellular magnesium levels and atheromatosis in haemodialysis patients. *Magnes Res*. 2004;17:102–108.
61. Ishimura E, Okuno S, Yamakawa T, Inaba M, Nishizawa Y. Serum magnesium concentration is a significant predictor of mortality in maintenance hemodialysis patients. *Magnes Res*. 2007;20:237–244.
62. Ishimura E, Okuno S, Kitatani K, Tsuchida T, Yamakawa T, Shioi A, Inaba M, Nishizawa Y. Significant association between the presence of peripheral vascular calcification and lower serum magnesium in hemodialysis patients. *Clin Nephrol*. 2007;68:222–227.
63. Kanbay M, Yilmaz MI, Apetrii M, Saglam M, Yaman H, Unal HU, Gok M, Caglar K, Oguz Y, Yenicesu M, Cetinkaya H, Eyileten T, Acikel C, Vural A, Covic A. Relationship between serum magnesium levels and cardiovascular events in chronic kidney disease patients. *Am J Nephrol*. 2012;36:228–237. doi: 10.1159/000341868.
64. João Matias P, Azevedo A, Laranjinha I, Navarro D, Mendes M, Ferreira C, Amaral T, Jorge C, Aires I, Gil C, Ferreira A. Lower serum magnesium is associated with cardiovascular risk factors and mortality in haemodialysis patients. *Blood Purif*. 2014;38:244–252. doi: 10.1159/000366124.
65. Yu L, Li H, Wang SX. Serum magnesium and mortality in maintenance hemodialysis patients. *Blood Purif*. 2017;43:31–36. doi: 10.1159/000451052.
66. Molnar AO, Biyani M, Hammond I, Harmon JP, Lavoie S, McCormick B, Sood MM, Wagner J, Pena E, Zimmerman DL. Lower serum magnesium is associated with vascular calcification in peritoneal dialysis patients: a cross sectional study. *BMC Nephrol*. 2017;18:129. doi: 10.1186/s12882-017-0549-y.
67. Qu X, Jin F, Hao Y, et al. Magnesium and the risk of cardiovascular events: a meta-analysis of prospective cohort studies. *PLoS One*. 2013;8:e57720.
68. Del Gobbo LC, Imamura F, Wu JH, de Oliveira Otto MC, Chiuve SE, Mozaffarian D. Circulating and dietary magnesium and risk of cardiovascular disease: a systematic review and meta-analysis of prospective studies. *Am J Clin Nutr*. 2013;98:160–173. doi: 10.3945/ajcn.112.053132.
69. Costello RB, Elin RJ, Rosanoff A, Wallace TC, Guerrero-Romero F, Hruby A, Lutsey PL, Nielsen FH, Rodriguez-Moran M, Song Y, Van Horn LV. Perspective: the case for an evidence-based reference interval for serum magnesium: the time has come. *Adv Nutr*. 2016;7:977–993. doi: 10.3945/an.116.012765.
70. Lowenstein FW, Stanton MF. Serum magnesium levels in the United States, 1971-1974. *J Am Coll Nutr*. 1986;5:399–414.
71. Angkananard T, Anothaisintawee T, Eursiriwan S, Gorelik O, McEvoy M, Attia J, Thakkinstian A. The association of serum magnesium and mortality outcomes in heart failure patients: a systematic review and meta-analysis. *Medicine*. 2016;95:e5406. doi: 10.1097/MD.0000000000005406.
72. Agus ZS, Morad M. Modulation of cardiac ion channels by magnesium. *Annu Rev Physiol*. 1991;53:299–307. doi: 10.1146/annurev.ph.53.030191.001503.
73. Kulick DL, Hong R, Ryzen E, Rude RK, Rubin JN, Elkayam U, Rahimtoola SH, Bhandari AK. Electrophysiologic effects of intravenous magnesium in patients with normal conduction systems and no clinical evidence of significant cardiac disease. *Am Heart J*. 1988;115:367–373.
74. Tzivoni D, Banai S, Schuger C, Benhorin J, Keren A, Gottlieb S, Stern S. Treatment of torsade de pointes with magnesium sulfate. *Circulation*. 1988;77:392–397.
75. Ho KM. Intravenous magnesium for cardiac arrhythmias: jack of all trades. *Magnes Res*. 2008;21:65–68.
76. Ho KM, Sheridan DJ, Paterson T. Use of intravenous magnesium to treat acute onset atrial fibrillation: a meta-analysis. *Heart*. 2007;93:1433–1440. doi: 10.1136/hrt.2006.111492.
77. Kotecha D. Magnesium for atrial fibrillation, myth or magic? *Circ Arrhythmia Electrophysiol*. 2016;9:1–4.
78. Maier JAM. Endothelial cells and magnesium: implications in atherosclerosis. *Clin Sci*. 2011;122:397–407.
79. Matsushita K, Sang Y, Ballew SH, Shlipak M, Katz R, Rosas SE, Peralta CA, Woodward M, Kramer HJ, Jacobs DR, Sarnak MJ, Coresh J. Subclinical atherosclerosis measures for cardiovascular prediction in CKD. *J Am Soc Nephrol*. 2015;26:439–447. doi: 10.1681/ASN.2014020173.
80. Yeboah J, McClelland RL, Polonsky TS, Burke GL, Sibley CT, O'Leary D, Carr JJ, Goff DC, Greenland P, Herrington DM. Comparison of novel risk markers for improvement in cardiovascular risk assessment in intermediate-risk individuals. *JAMA*. 2012;308:788–795. doi: 10.1001/jama.2012.9624.
81. Vervloet M, Cozzolino M. Vascular calcification in chronic kidney disease: different bricks in the wall? *Kidney Int*. 2016;91:808–817.
82. Sakaguchi Y, Hamano T, Nakano C, et al. Association between density of coronary artery calcification and serum magnesium levels among patients with chronic kidney disease. *PLoS One*. 2016;11:e0163673. doi: 10.1371/journal.pone.0163673.
83. Tzanakis I, Pras A, Kounali D, Mamali V, Kartsonakis V, Mayopoulou-Symvoulidou D, Kallivretakis N. Mitral annular calcifications in haemodialysis patients: a possible protective role of magnesium. *Nephrol Dial Transplant*. 1997;12:2036–2037.
84. Paula Silva A, Fragoso A, Silva C, et al. Magnesium and mortality in patients with diabetes and early chronic kidney disease. *J Diabetes Metab*. 2014;5:1–6.
85. Ferrè S, Baldoli E, Leidi M, Maier JA. Magnesium deficiency promotes a pro-atherogenic phenotype in cultured human endothelial cells via activation of NFkB. *Biochim Biophys Acta*. 2010;1802:952–958. doi: 10.1016/j.bbadis.2010.06.016.

86. Maier JAM. Low magnesium and atherosclerosis: an evidence-based link. *Mol Aspects Med*. 2003;24:137–146.
87. Cohen H, Sherer Y, Shaish A, Shoenfeld Y, Levkovitz H, Bitzur R, Harats D. Atherogenesis inhibition induced by magnesium-chloride fortification of drinking water. *Biol Trace Elem Res*. 2002;90:251–259. doi: 10.1385/BTER:90:1-3:251.
88. Sherer Y, Shoenfeld Y, Shaish A, Levkovitz H, Bitzur R, Harats D. Suppression of atherogenesis in female low-density lipoprotein receptor knockout mice following magnesium fortification of drinking water: the importance of diet. *Pathobiology*. 2000;68:93–98. doi: 28119.
89. Ravn HB, Korsholm TL, Falk E. Oral magnesium supplementation induces favorable antiatherogenic changes in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol*. 2001;21:858–862.
90. Paravicini TM, Yogi A, Mazur A, Touyz RM. Dysregulation of vascular TRPM7 and annexin-1 is associated with endothelial dysfunction in inherited hypomagnesemia. *Hypertension*. 2009;53:423–429. doi: 10.1161/HYPERTENSIONAHA.108.124651.
91. Shivakumar K, Kumar BP. Magnesium deficiency enhances oxidative stress and collagen synthesis *in vivo* in the aorta of rats. *Int J Biochem Cell Biol*. 1997;29:1273–1278.
92. Pagès N, Gogly B, Godeau G, Igondjo-Tchen S, Maurois P, Durlach J, Bac P. Structural alterations of the vascular wall in magnesium-deficient mice. A possible role of gelatinases A (MMP-2) and B (MMP-9). *Magnes Res*. 2003;16:43–48.
93. Jiang Q, Uitto J. Restricting dietary magnesium accelerates ectopic connective tissue mineralization in a mouse model of pseudoxanthoma elasticum (Abcc6(-/-)). *Exp Dermatol*. 2012;21:694–699. doi: 10.1111/j.1600-0625.2012.01553.x.
94. Gorgels TG, Waarsing JH, de Wolf A, ten Brink JB, Loves WJ, Bergen AA. Dietary magnesium, not calcium, prevents vascular calcification in a mouse model for pseudoxanthoma elasticum. *J Mol Med*. 2010;88:467–475. doi: 10.1007/s00109-010-0596-3.
95. Kingman J, Uitto J, Li Q. Elevated dietary magnesium during pregnancy and postnatal life prevents ectopic mineralization in Enpp1 asj mice, a model for generalized arterial calcification of infancy. *Oncotarget*. 2017;8:38152–38160.
96. U.S. Renal Data System. *USRDS 2010 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States, National Institutes of Health*. Bethesda, MD: National Institute of Diabetes and Digestive and Kidney Diseases; 2010.
97. Altura BM, Altura BT, Carella A, Gebrewold A, Murakawa T, Nishio A. Mg2+-Ca2+ interaction in contractility of vascular smooth muscle: Mg2+ versus organic calcium channel blockers on myogenic tone and agonist-induced responsiveness of blood vessels. *Can J Physiol Pharmacol*. 1987;65:729–745.
98. Howard AB, Alexander RW, Taylor WR. Effects of magnesium on nitric oxide synthase activity in endothelial cells. *Am J Physiol*. 1995;269(3 pt 1):C612–C618.
99. Zhang X, Li Y, Del Gobbo LC, Rosanoff A, Wang J, Zhang W, Song Y. Effects of magnesium supplementation on blood pressure: a meta-analysis of randomized double-blind placebo-controlled trials. *Hypertension*. 2016;68:324–333. doi: 10.1161/HYPERTENSIONAHA.116.07664.
100. McDonald SD, Lutsiv O, Dzaja N, Duley L. A systematic review of maternal and infant outcomes following magnesium sulfate for pre-eclampsia/eclampsia in real-world use. *Int J Gynaecol Obstet*. 2012;118:90–96. doi: 10.1016/j.ijgo.2012.01.028.
101. Kyriazis J, Kalogeropoulou K, Bilirakis L, Smirnioudis N, Pikounis V, Stamatiadis D, Liolia E. Dialysate magnesium level and blood pressure. *Kidney Int*. 2004;66:1221–1231. doi: 10.1111/j.1523-1755.2004.00875.x.
102. Kannel WB, McGee DL. Diabetes and cardiovascular disease. The Framingham study. *JAMA*. 1979;241:2035–2038.
103. Reaven G. Insulin resistance and coronary heart disease in nondiabetic individuals. *Arterioscler Thromb Vasc Biol*. 2012;32:1754–1759. doi: 10.1161/ATVBAHA.111.241885.
104. Yahagi K, Kolodgie FD, Lutter C, Mori H, Romero ME, Finn AV, Virmani R. Pathology of human coronary and carotid artery atherosclerosis and vascular calcification in diabetes mellitus. *Arterioscler Thromb Vasc Biol*. 2017;37:191–204. doi: 10.1161/ATVBAHA.116.306256.
105. Raggi P, Bellasi A, Ferramosca E, Block GA, Muntner P. Pulse wave velocity is inversely related to vertebral bone density in hemodialysis patients. *Hypertension*. 2007;49:1278–1284. doi: 10.1161/HYPERTENSIONAHA.107.086942.
106. Slatopolsky E, Finch J, Denda M, Ritter C, Zhong M, Dusso A, MacDonald PN, Brown AJ. Phosphorus restriction prevents parathyroid gland growth. High phosphorus directly stimulates PTH secretion *in vitro*. *J Clin Invest*. 1996;97:2534–2540. doi: 10.1172/JCI118701.
107. Almaden Y, Canalejo A, Hernandez A, Ballesteros E, Garcia-Navarro S, Torres A, Rodriguez M. Direct effect of phosphorus on PTH secretion from whole rat parathyroid glands *in vitro*. *J Bone Miner Res*. 1996;11:970–976. doi: 10.1002/jbmr.5650110714.
108. Levin A, Bakris GL, Molitch M, Smulders M, Tian J, Williams LA, Andress DL. Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: results of the study to evaluate early kidney disease. *Kidney Int*. 2007;71:31–38. doi: 10.1038/sj.ki.5002009.
109. Urakawa I, Yamazaki Y, Shimada T, Iijima K, Hasegawa H, Okawa K, Fujita T, Fukumoto S, Yamashita T. Klotho converts canonical FGF receptor into a specific receptor for FGF23. *Nature*. 2006;444:770–774. doi: 10.1038/nature05315.
110. Li SA, Watanabe M, Yamada H, Nagai A, Kinuta M, Takei K. Immunohistochemical localization of Klotho protein in brain, kidney, and reproductive organs of mice. *Cell Struct Funct*. 2004;29:91–99.
111. Krajisnik T, Olauson H, Mirza MA, Hellman P, Akerström G, Westin G, Larsson TE, Björklund P. Parathyroid Klotho and FGF-receptor 1 expression decline with renal function in hyperparathyroid patients with chronic kidney disease and kidney transplant recipients. *Kidney Int*. 2010;78:1024–1032. doi: 10.1038/ki.2010.260.
112. Hu MC, Shi M, Gillings N, Flores B, Takahashi M, Kuro-o M, Moe OW. Recombinant α-Klotho may be prophylactic and therapeutic for acute to chronic kidney disease progression and uremic cardiomyopathy. *Kidney Int*. 2017;91:1104–1114.
113. Schoppet M, Shroff RC, Hofbauer LC, Shanahan CM. Exploring the biology of vascular calcification in chronic kidney disease: what's circulating? *Kidney Int*. 2008;73:384–390. doi: 10.1038/sj.ki.5002696.
114. Ketteler M, Bongartz P, Westenfeld R, Wildberger JE, Mahnken AH, Böhm R, Metzger T, Wanner C, Jahnen-Dechent W, Floege J. Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study. *Lancet*. 2003;361:827–833. doi: 10.1016/S0140-6736(03)12710-9.
115. Westenfeld R, Krueger T, Schlieper G, Cranenburg EC, Magdeleyns EJ, Heidenreich S, Holzmann S, Vermeer C, Jahnen-Dechent W, Ketteler M, Floege J, Schurgers LJ. Effect of vitamin K2 supplementation on functional vitamin K deficiency in hemodialysis patients: a randomized trial. *Am J Kidney Dis*. 2012;59:186–195. doi: 10.1053/j.ajkd.2011.10.041.
116. Schafer C, Heiss A, Schwarz A, Westenfeld R, Ketteler M, Floege J, Muller-Esterl W, Schinke T, Jahnen-Dechent W. The serum protein alpha 2-Heremans-Schmid glycoprotein/fetuin-A is a systemically acting inhibitor of ectopic calcification. *J Clin Invest*. 2003;112:357–366. doi: 10.1172/JCI17202.
117. Luo G, Ducy P, McKee MD, Pinero GJ, Loyer E, Behringer RR, Karsenty G. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. *Nature*. 1997;386:78–81. doi: 10.1038/386078a0.
118. Collin-Osdoby P. Regulation of vascular calcification by osteoclast regulatory factors RANKL and osteoprotegerin. *Circ Res*. 2004;95:1046–1057. doi: 10.1161/01.RES.0000149165.99974.12.
119. Johnson K, Polewski M, van Etten D, Terkeltaub R. Chondrogenesis mediated by PPi depletion promotes spontaneous aortic calcification in NPP1-/- mice. *Arterioscler Thromb Vasc Biol*. 2005;25:686–691. doi: 10.1161/01.ATV.0000154774.71187.f0.
120. Boström K, Watson KE, Horn S, Wortham C, Herman IM, Demer LL. Bone morphogenetic protein expression in human atherosclerotic lesions. *J Clin Invest*. 1993;91:1800–1809. doi: 10.1172/JCI116391.
121. Shanahan CM, Cary NR, Salisbury JR, Proudfoot D, Weissberg PL, Edmonds ME. Medial localization of mineralization-regulating proteins in association with Mönckeberg's sclerosis: evidence for smooth muscle cell-mediated vascular calcification. *Circulation*. 1999;100:2168–2176.
122. Moe SM, Duan D, Doehle BP, O'Neill KD, Chen NX. Uremia induces the osteoblast differentiation factor Cbfa1 in human blood vessels. *Kidney Int*. 2003;63:1003–1011. doi: 10.1046/j.1523-1755.2003.00820.x.
123. Pai AS, Giachelli CM. Matrix remodeling in vascular calcification associated with chronic kidney disease. *J Am Soc Nephrol*. 2010;21:1637–1640. doi: 10.1681/ASN.2010040349.
124. Jono S, McKee MD, Murry CE, Shioi A, Nishizawa Y, Mori K, Morii H, Giachelli CM. Phosphate regulation of vascular smooth muscle cell calcification. *Circ Res*. 2000;87:E10–E17.
125. Steitz SA, Speer MY, Curinga G, Yang HY, Haynes P, Aebersold R, Schinke T, Karsenty G, Giachelli CM. Smooth muscle cell phenotypic transition associated with calcification: upregulation of Cbfa1

and downregulation of smooth muscle lineage markers. *Circ Res*. 2001;89:1147–1154.
126. Reynolds JL, Joannides AJ, Skepper JN, McNair R, Schurgers LJ, Proudfoot D, Jahnen-Dechent W, Weissberg PL, Shanahan CM. Human vascular smooth muscle cells undergo vesicle-mediated calcification in response to changes in extracellular calcium and phosphate concentrations: a potential mechanism for accelerated vascular calcification in ESRD. *J Am Soc Nephrol*. 2004;15:2857–2867. doi: 10.1097/01.ASN.0000141960.01035.28.
127. Kapustin A, Shanahan CM. Emerging roles for vascular smooth muscle cell exosomes in calcification and coagulation. *J Physiol*. 2016;7:956–963.
128. Reynolds JL, Skepper JN, McNair R, Kasama T, Gupta K, Weissberg PL, Jahnen-Dechent W, Shanahan CM. Multifunctional roles for serum protein fetuin-a in inhibition of human vascular smooth muscle cell calcification. *J Am Soc Nephrol*. 2005;16:2920–2930. doi: 10.1681/ASN.2004100895.
129. Shroff RC, McNair R, Figg N, Skepper JN, Schurgers L, Gupta A, Hiorns M, Donald AE, Deanfield J, Rees L, Shanahan CM. Dialysis accelerates medial vascular calcification in part by triggering smooth muscle cell apoptosis. *Circulation*. 2008;118:1748–1757. doi: 10.1161/CIRCULATIONAHA.108.783738.
130. Kapustin AN, Davies JD, Reynolds JL, McNair R, Jones GT, Sidibe A, Schurgers LJ, Skepper JN, Proudfoot D, Mayr M, Shanahan CM. Calcium regulates key components of vascular smooth muscle cell-derived matrix vesicles to enhance mineralization. *Circ Res*. 2011;109:e1–e12. doi: 10.1161/CIRCRESAHA.110.238808.
131. Aghagolzadeh P, Bachtler M, Bijarnia R, Jackson C, Smith ER, Odermatt A, Radpour R, Pasch A. Calcification of vascular smooth muscle cells is induced by secondary calciprotein particles and enhanced by tumor necrosis factor-α. *Atherosclerosis*. 2016;251:404–414. doi: 10.1016/j.atherosclerosis.2016.05.044.
132. Kapustin AN, Chatrou ML, Drozdov I, et al. Vascular smooth muscle cell calcification is mediated by regulated exosome secretion. *Circ Res*. 2015;116:1312–1323. doi: 10.1161/CIRCRESAHA.116.305012.
133. Pasch A, Farese S, Gräber S, Wald J, Richtering W, Floege J, Jahnen-Dechent W. Nanoparticle-based test measures overall propensity for calcification in serum. *J Am Soc Nephrol*. 2012;23:1744–1752. doi: 10.1681/ASN.2012030240.
134. Smith ER, Cai MM, McMahon LP, Pedagogos E, Toussaint ND, Brumby C, Holt SG. Serum fetuin-A concentration and fetuin-A-containing calciprotein particles in patients with chronic inflammatory disease and renal failure. *Nephrology*. 2013;18:215–221. doi: 10.1111/nep.12021.
135. Shanahan CM, Crouthamel MH, Kapustin A, Giachelli CM. Arterial calcification in chronic kidney disease: key roles for calcium and phosphate. *Circ Res*. 2011;109:697–711. doi: 10.1161/CIRCRESAHA.110.234914.
136. Kapustin A, Shanahan CM. Targeting vascular calcification: softening-up a hard target. *Curr Opin Pharmacol*. 2009;9:84–89. doi: 10.1016/j.coph.2008.12.004.
137. Tonelli M, Curhan G, Pfeffer M, Sacks F, Thadhani R, Melamed ML, Wiebe N, Muntner P. Relation between alkaline phosphatase, serum phosphate, and all-cause or cardiovascular mortality. *Circulation*. 2009;120:1784–1792. doi: 10.1161/CIRCULATIONAHA.109.851873.
138. Hutchison AJ. Oral phosphate binders. *Kidney Int*. 2009;75:906–914. doi: 10.1038/ki.2009.60.
139. O'Donovan R, Baldwin D, Hammer M, Moniz C, Parsons V. Substitution of aluminium salts by magnesium salts in control of dialysis hyperphosphatemia. *Lancet*. 1986;1:880–882.
140. Moe SM, Chertow GM. The case against calcium-based phosphate binders. *Clin J Am Soc Nephrol*. 2006;1:697–703. doi: 10.2215/CJN.00560206.
141. Morinière P, Vinatier I, Westeel PF, Cohemsolal M, Belbrik S, Abdulmassih Z, Hocine C, Marie A, Leflon P, Roche D. Magnesium hydroxide as a complementary aluminium-free phosphate binder to moderate doses of oral calcium in uraemic patients on chronic haemodialysis: lack of deleterious effect on bone mineralisation. *Nephrol Dial Transplant*. 1988;3:651–656.
142. de Francisco AL, Leidig M, Covic AC, Ketteler M, Benedyk-Lorens E, Mircescu GM, Scholz C, Ponce P, Passlick-Deetjen J. Evaluation of calcium acetate/magnesium carbonate as a phosphate binder compared with sevelamer hydrochloride in haemodialysis patients: a controlled randomized study (CALMAG study) assessing efficacy and tolerability. *Nephrol Dial Transplant*. 2010;25:3707–3717. doi: 10.1093/ndt/gfq292.
143. Hutchison AJ, Wilkie M. Use of magnesium as a drug in chronic kidney disease. *Clin Kidney J*. 2012;5(suppl 1):i62–i70. doi: 10.1093/ndtplus/sfr168.
144. De Schutter TM, Behets GJ, Geryl H, Peter ME, Steppan S, Gundlach K, Passlick-Deetjen J, D'Haese PC, Neven E. Effect of a magnesium-based phosphate binder on medial calcification in a rat model of uremia. *Kidney Int*. 2013;83:1109–1117. doi: 10.1038/ki.2013.34.
145. Spiegel DM, Farmer B. Long-term effects of magnesium carbonate on coronary artery calcification and bone mineral density in hemodialysis patients: a pilot study. *Hemodial Int*. 2009;13:453–459. doi: 10.1111/j.1542-4758.2009.00364.x.
146. Ikee R, Toyoyama T, Endo T, Tsunoda M, Hashimoto N. Impact of sevelamer hydrochloride on serum magnesium concentrations in hemodialysis patients. *Magnes Res*. 2016;29:184–190. doi: 10.1684/mrh.2016.0410.
147. Lee JS, Morrisett JD, Tung CH. Detection of hydroxyapatite in calcified cardiovascular tissues. *Atherosclerosis*. 2012;224:340–347. doi: 10.1016/j.atherosclerosis.2012.07.023.
148. Boistelle R, Lopez-Valero I, Abbona F. [Crystallization of calcium phosphate in the presence of magnesium]. *Nephrologie*. 1993;14:265–269.
149. Eanes ED, Posner AS. Kinetics and mechanism of conversion of noncrystalline calcium phosphate to hydroxyapatite. *Trans N Y Acad Sci*. 1965;28:233–241.
150. Termine JD, Peckauskas RA, Posner AS. Calcium phosphate formation *in vitro*. II. Effects of environment on amorphous-crystalline transformation. *Arch Biochem Biophys*. 1970;140:318–325.
151. Apfelbaum F, Mayer I, Rey C, Lebugle A. Magnesium in maturing synthetic apatite: a Fourier transform infrared analysis. *J Cryst Growth*. 1994;144:304–310.
152. Boskey AL, Posner AS. Magnesium stabilization of amorphous calcium phosphate: a kinetic study. *Mater Res Bull*. 1974;9:907–916.
153. Blumenthal NC, Betts F, Posner AS. Stabilization of amorphous calcium phosphate by Mg and ATP. *Calcif Tissue Res*. 1977;23:245–250.
154. Lomashvili KA, Cobbs S, Hennigar RA, Hardcastle KI, O'Neill WC. Phosphate-induced vascular calcification: role of pyrophosphate and osteopontin. *J Am Soc Nephrol*. 2004;15:1392–1401.
155. Villa-Bellosta R. On vascular calcification and plasma levels of pyrophosphate. *Kidney Int*. 2015;87:239. doi: 10.1038/ki.2014.309.
156. Jansen RS, Duijst S, Mahakena S, Sommer D, Szeri F, Váradi A, Plomp A, Bergen AA, Oude Elferink RP, Borst P, van de Wetering K. ABCC6-mediated ATP secretion by the liver is the main source of the mineralization inhibitor inorganic pyrophosphate in the systemic circulation-brief report. *Arterioscler Thromb Vasc Biol*. 2014;34:1985–1989. doi: 10.1161/ATVBAHA.114.304017.
157. LeGeros RZ, Contiguglia SR, Alfrey AC. Pathological calcifications associated with uremia: two types of calcium phosphate deposits. *Calcif Tissue Res*. 1973;13:173–185.
158. Cheng PT, Grabher JJ, LeGeros RZ. Effects of magnesium on calcium phosphate formation. *Magnesium*. 1988;7:123–132.
159. Lagier R, Baud CA. Magnesium whitlockite, a calcium phosphate crystal of special interest in pathology. *Pathol Res Pract*. 2003;199:329–335. doi: 10.1078/0344-0338-00425.
160. Louvet L, Bazin D, Büchel J, Steppan S, Passlick-Deetjen J, Massy ZA. Characterisation of calcium phosphate crystals on calcified human aortic vascular smooth muscle cells and potential role of magnesium. *PLoS One*. 2015;10:e0115342. doi: 10.1371/journal.pone.0115342.
161. Schlieper G, Aretz A, Verberckmoes SC, et al. Ultrastructural analysis of vascular calcifications in uremia. *J Am Soc Nephrol*. 2010;21:689–696.
162. Kircelli F, Peter ME, Sevinc Ok E, Celenk FG, Yilmaz M, Steppan S, Asci G, Ok E, Passlick-Deetjen J. Magnesium reduces calcification in bovine vascular smooth muscle cells in a dose-dependent manner. *Nephrol Dial Transplant*. 2012;27:514–521. doi: 10.1093/ndt/gfr321.
163. Bai Y, Zhang J, Xu J, Cui L, Zhang H, Zhang S, Feng X. Magnesium prevents β-glycerophosphate-induced calcification in rat aortic vascular smooth muscle cells. *Biomed Rep*. 2015;3:593–597. doi: 10.3892/br.2015.473.
164. Salem S, Bruck H, Bahlmann FH, Peter M, Passlick-Deetjen J, Kretschmer A, Steppan S, Volsek M, Kribben A, Nierhaus M, Jankowski V, Zidek W, Jankowski J. Relationship between magnesium and clinical biomarkers on inhibition of vascular calcification. *Am J Nephrol*. 2012;35:31–39. doi: 10.1159/000334742.
165. Montezano AC, Zimmerman D, Yusuf H, Burger D, Chignalia AZ, Wadhera V, van Leeuwen FN, Touyz RM. Vascular smooth muscle cell differentiation to an osteogenic phenotype involves TRPM7

modulation by magnesium. *Hypertension*. 2010;56:453–462. doi: 10.1161/HYPERTENSIONAHA.110.152058.
166. Louvet L, Büchel J, Steppan S, Passlick-Deetjen J, Massy ZA. Magnesium prevents phosphate-induced calcification in human aortic vascular smooth muscle cells. *Nephrol Dial Transplant*. 2013;28:869–878. doi: 10.1093/ndt/gfs520.
167. He Y, Yao G, Savoia C, Touyz RM. Transient receptor potential melastatin 7 ion channels regulate magnesium homeostasis in vascular smooth muscle cells: role of angiotensin II. *Circ Res*. 2005;96:207–215. doi: 10.1161/01.RES.0000152967.88472.3e.
168. Herencia C, Rodríguez-Ortiz ME, Muñoz-Castañeda JR, Martinez-Moreno JM, Canalejo R, Montes de Oca A, Díaz-Tocados JM, Peralbo-Santaella E, Marín C, Canalejo A, Rodriguez M, Almaden Y. Angiotensin II prevents calcification in vascular smooth muscle cells by enhancing magnesium influx. *Eur J Clin Invest*. 2015;45:1129–1144. doi: 10.1111/eci.12517.
169. Montes de Oca A, Guerrero F, Martinez-Moreno JM, Madueño JA, Herencia C, Peralta A, Almaden Y, Lopez I, Aguilera-Tejero E, Gundlach K, Büchel J, Peter ME, Passlick-Deetjen J, Rodriguez M, Muñoz-Castañeda JR. Magnesium inhibits Wnt/β-catenin activity and reverses the osteogenic transformation of vascular smooth muscle cells. *PLoS One*. 2014;9:e89525. doi: 10.1371/journal.pone.0089525.
170. Shao JS, Cheng SL, Pingsterhaus JM, Charlton-Kachigian N, Loewy AP, Towler DA. Msx2 promotes cardiovascular calcification by activating paracrine Wnt signals. *J Clin Invest*. 2005;115:1210–1220. doi: 10.1172/JCI24140.
171. Brigant B, Metzinger-Le Meuth V, Massy ZA, et al. Serum microRNAs are altered in various stages of chronic kidney disease: a preliminary study. *Clin Kidney J*. 2017;10:30–37.
172. Cui RR, Li SJ, Liu LJ, Yi L, Liang QH, Zhu X, Liu GY, Liu Y, Wu SS, Liao XB, Yuan LQ, Mao DA, Liao EY. MicroRNA-204 regulates vascular smooth muscle cell calcification *in vitro* and in vivo. *Cardiovasc Res*. 2012;96:320–329. doi: 10.1093/cvr/cvs258.
173. Louvet L, Metzinger L, Büchel J, Steppan S, Massy ZA. Magnesium attenuates phosphate-induced deregulation of a microRNA signature and prevents modulation of Smad1 and osterix during the course of vascular calcification. *Biomed Res Int*. 2016;2016:7419524. doi: 10.1155/2016/7419524.
174. Leidi M, Dellera F, Mariotti M, Maier JA. High magnesium inhibits human osteoblast differentiation *in vitro*. *Magnes Res*. 2011;24:1–6. doi: 10.1684/mrh.2011.0271.
175. Yue J, Jin S, Li Y, Zhang L, Jiang W, Yang C, Du J. Magnesium inhibits the calcification of the extracellular matrix in tendon-derived stem cells via the ATP-P2R and mitochondrial pathways. *Biochem Biophys Res Commun*. 2016;478:314–322. doi: 10.1016/j.bbrc.2016.06.108.
176. Proudfoot D, Skepper JN, Hegyi L, Bennett MR, Shanahan CM, Weissberg PL. Apoptosis regulates human vascular calcification *in vitro*: evidence for initiation of vascular calcification by apoptotic bodies. *Circ Res*. 2000;87:1055–1062.
177. Rodenbeck SD, Zarse CA, McKenney-Drake ML, et al. Intracellular calcium increases in vascular smooth muscle cells with progression of chronic kidney disease in a rat model. *Nephrol Dial Transplant*. 2017;32:450–458.
178. Iseri LT, French JH. Magnesium: nature's physiologic calcium blocker. *Am Heart J*. 1984;108:188–193.
179. Zhang J, Berra-Romani R, Sinnegger-Brauns MJ, Striessnig J, Blaustein MP, Matteson DR. Role of Cav1.2 L-type Ca2+ channels in vascular tone: effects of nifedipine and Mg2+. *Am J Physiol Heart Circ Physiol*. 2007;292:H415–H425. doi: 10.1152/ajpheart.01214.2005.
180. Hénaut L, Boudot C, Massy ZA, Lopez-Fernandez I, Dupont S, Mary A, Drüeke TB, Kamel S, Brazier M, Mentaverri R. Calcimimetics increase CaSR expression and reduce mineralization in vascular smooth muscle cells: mechanisms of action. *Cardiovasc Res*. 2014;101:256–265. doi: 10.1093/cvr/cvt249.
181. Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O, Sun A, Hediger MA, Lytton J, Hebert SC. Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid. *Nature*. 1993;366:575–580. doi: 10.1038/366575a0.
182. Riccardi D. Cell surface, Ca2+(cation)-sensing receptor(s): one or many? *Cell Calcium*. 1999;26:77–83. doi: 10.1054/ceca.1999.0066.
183. Ruat M, Snowman AM, Hester LD, Snyder SH. Cloned and expressed rat Ca2+-sensing receptor. *J Biol Chem*. 1996;271:5972–5975.
184. Rodríguez-Ortiz ME, Canalejo A, Herencia C, Martínez-Moreno JM, Peralta-Ramírez A, Perez-Martinez P, Navarro-González JF, Rodríguez M, Peter M, Gundlach K, Steppan S, Passlick-Deetjen J, Muñoz-Castañeda JR, Almaden Y. Magnesium modulates parathyroid hormone secretion and upregulates parathyroid receptor expression at moderately low calcium concentration. *Nephrol Dial Transplant*. 2014;29:282–289. doi: 10.1093/ndt/gft400.
185. Alam MU, Kirton JP, Wilkinson FL, Towers E, Sinha S, Rouhi M, Vizard TN, Sage AP, Martin D, Ward DT, Alexander MY, Riccardi D, Canfield AE. Calcification is associated with loss of functional calcium-sensing receptor in vascular smooth muscle cells. *Cardiovasc Res*. 2009;81:260–268. doi: 10.1093/cvr/cvn279.
186. Schurgers LJ, Spronk HM, Skepper JN, Hackeng TM, Shanahan CM, Vermeer C, Weissberg PL, Proudfoot D. Post-translational modifications regulate matrix Gla protein function: importance for inhibition of vascular smooth muscle cell calcification. *J Thromb Haemost*. 2007;5:2503–2511. doi: 10.1111/j.1538-7836.2007.02758.x.
187. Molostvov G, Hiemstra TF, Fletcher S, Bland R, Zehnder D. Arterial expression of the calcium-sensing receptor is maintained by physiological pulsation and protects against calcification. *PLoS One*. 2015;10:e0138833. doi: 10.1371/journal.pone.0138833.
188. Mendoza FJ, Martinez-Moreno J, Almaden Y, Rodriguez-Ortiz ME, Lopez I, Estepa JC, Henley C, Rodriguez M, Aguilera-Tejero E. Effect of calcium and the calcimimetic AMG 641 on matrix-Gla protein in vascular smooth muscle cells. *Calcif Tissue Int*. 2011;88:169–178. doi: 10.1007/s00223-010-9442-4.
189. Alesutan I, Tuffaha R, Auer T, et al. Inhibition of osteo/chondrogenic transformation of vascular smooth muscle cells by MgCl2 via calcium-sensing receptor. *J Hypertens*. 2016;35:523–532.

## Highlights

- Serum $Mg^{2+}$ concentration is inversely associated with cardiovascular risk in chronic kidney disease.
- $Mg^{2+}$ is protective against vascular calcification.
- $Mg^{2+}$ passively interferes with intestinal inorganic phosphate absorption and crystal formation in the circulation.
- $Mg^{2+}$ actively modulates gene expression in vascular smooth muscle cell and thereby prevents transdifferentiation toward an osteoblastic phenotype.