

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.)

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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 (Mg²⁺) 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 Mg²⁺ in vascular calcification, the precise mechanisms by which Mg²⁺ acts are subject to debate. In this review, we will evaluate evidence for currently existing hypotheses. We focus on the question of whether Mg²⁺ 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 Mg²⁺ and cardiovascular disease.

Magnesium Homeostasis

Regulation of Magnesium Homeostasis

In healthy individuals, serum Mg²⁺ 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 Mg²⁺ intake is absorbed in the small intestine and colon.¹² The bone serves as the body's Mg²⁺ store as 60%

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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

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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	calcioprotein 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 Mg²⁺ 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
Chiuvè 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 Mg²⁺, 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- κ B (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 anti-hypertensive 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 mmHg, 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 Mg²⁺ 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 Mg²⁺ status and cardiovascular disease in humans and the impact of Mg²⁺ interventions on vascular disease in animal models illustrate that Mg²⁺ 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 Mg²⁺. 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 Mg²⁺ 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 hormone) secretion and decreased 1,25[OH]₂D₃ (1,25-dihydroxyvitamin D) synthesis results in reduced intestinal Ca²⁺ 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 Ca²⁺-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 Ca²⁺-loaded exosomes (matrix vesicles) and apoptosis. All these factors contribute to the calcification by local Ca²⁺ 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 Ca²⁺-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, Ca²⁺-loaded apoptotic bodies released from VSMCs undergoing apoptosis form larger nucleation sites and promote calcification in neighboring cells potentially by causing local Ca²⁺ spikes.¹³⁵ Apoptosis plays a role in the initiation and acceleration in VSMC calcification and is induced by an intracellular Ca²⁺ 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

Mg²⁺-dependent alterations of the calcification milieu may prevent the development of vascular calcification. First, dietary Mg²⁺ can reduce Pi uptake by intestinal Pi binding. Second, Mg²⁺ can passively interfere with hydroxyapatite maturation in the vessel (Figure 2).

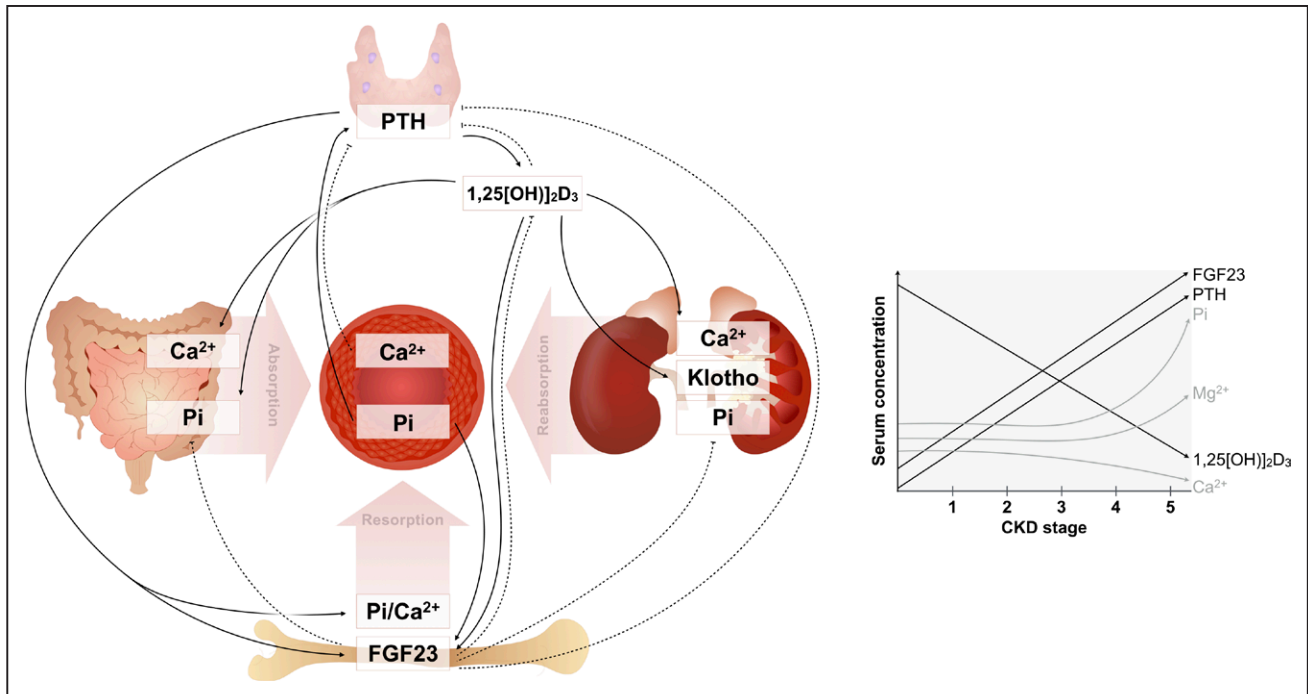


Figure 1. Mineral metabolism in chronic kidney disease (CKD). Circulating levels of inorganic phosphate (Pi) and Ca^{2+} are determined by integrated action of the parathyroid, intestine, bone, and kidney. CKD-induced mineral disturbances and diminished klotho result in CKD-mineral bone disorder, which promotes vascular calcification. $1,25(\text{OH})_2\text{D}_3$ 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 minimize 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 ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) is the most abundant type of crystal.¹⁴⁷ Reduction or delay of hydroxyapatite formation by magnesium has been proposed

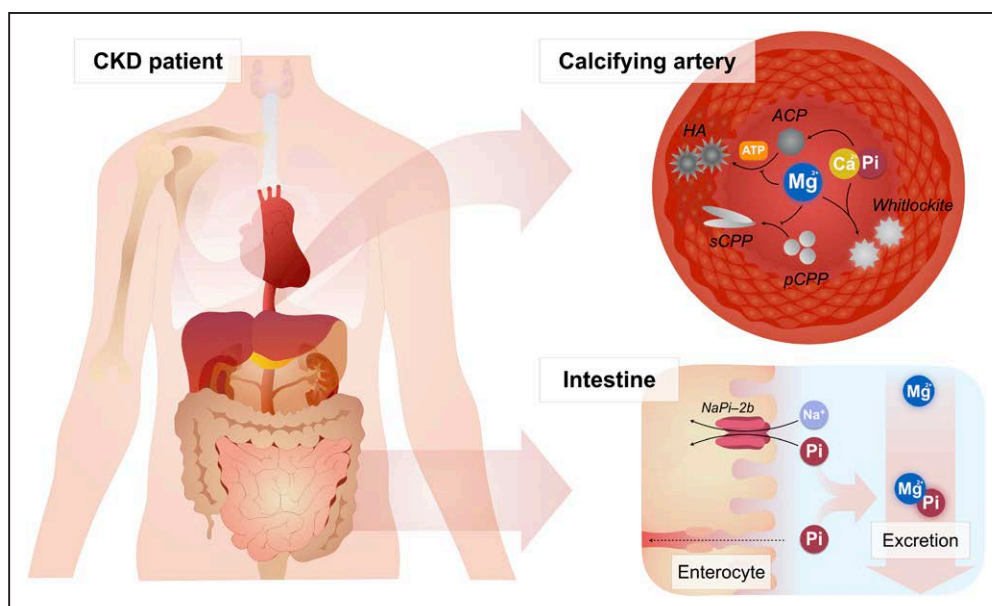


Figure 2. Passive interference: phosphate binding and crystal inhibition by Mg^{2+} . Elevated blood Mg^{2+} interferes with both amorphous calcium phosphate (ACP) and primary calciprotein particle (CPP) maturation into hydroxyapatite (HA) crystals and secondary CPP (sCPP). Mg^{2+} promotes the formation 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 al¹³³ 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

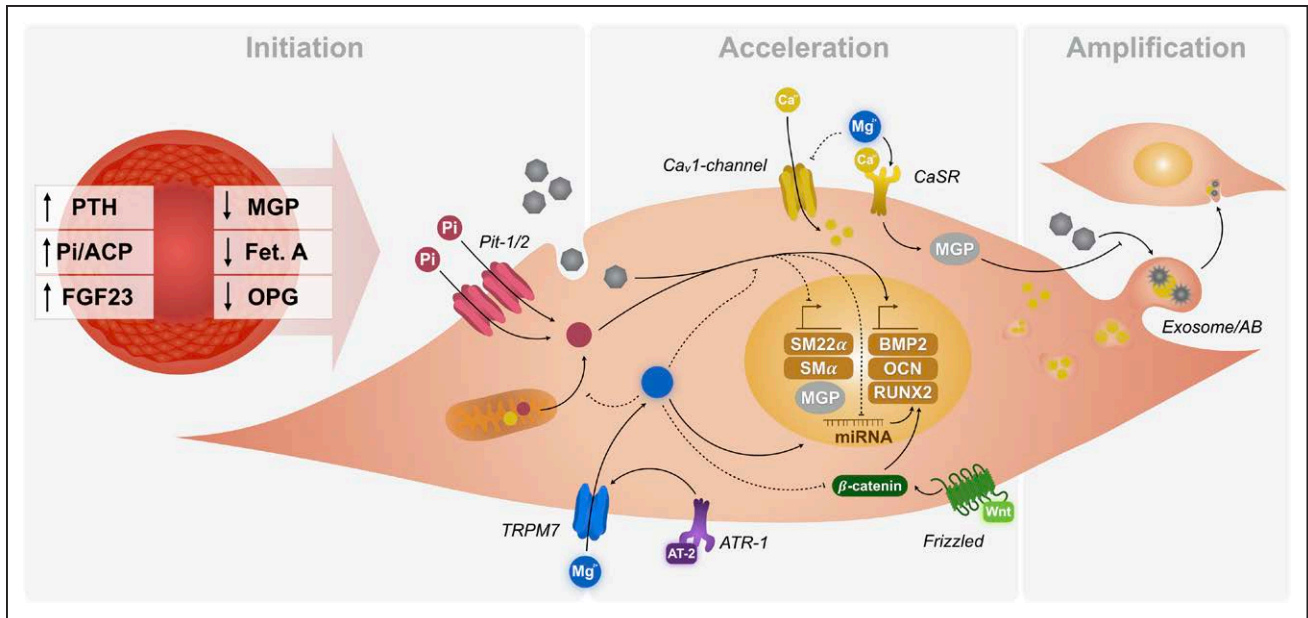


Figure 3. Active modulation: Mg^{2+} inhibits vascular smooth muscle cell transdifferentiation. Diminished levels of circulating inhibitors of vascular calcification, elevated levels of inorganic phosphate (Pi), and formation of amorphous Ca^{2+} -Pi particle (ACP) in the circulation initiate the transdifferentiation 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; SM22 α , 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 TRPM7 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 Mg²⁺ in mitochondrial export of Ca²⁺ 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 Mg²⁺ and reject the hypothesis that Mg²⁺-dependent regulation of calcification genes is only secondary to extracellular Pi binding and ACP stabilization. However, there is a lack of data on intracellular Mg²⁺ concentrations limiting conclusive confirmation on an active role for Mg²⁺ in this context. Additional studies measuring intracellular Mg²⁺ concentrations are necessary, but are hampered by the poor availability of selective fluorescent Mg²⁺ probes.

Magnesium and Cellular Calcium Entry

Excessive intracellular Ca²⁺ 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 Ca²⁺ channel antagonist, Mg²⁺ has the capacity to block Ca²⁺ channels in VSMCs and prevent Ca²⁺ overload.^{97,178} As a consequence of Ca²⁺ channel blocking, Mg²⁺ 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 Mg²⁺ in preventing intracellular Ca²⁺ bursts, and subsequent apoptosis has been identified as a potential mechanism of action in preventing VSMC calcification.¹⁰

In VSMCs, Ca²⁺ 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 Ca²⁺ channel blocking, Mg²⁺ has been implicated in CaSR activation, possibly functioning as calcimimetic and indirect gatekeeper of Ca²⁺ influx.¹⁸¹ In contrast to Ca²⁺, Mg²⁺ 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 Ca²⁺ uptake, but promotes renal Pi reabsorption. In dialysis patients, higher Mg²⁺ 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 Mg²⁺ 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 Mg²⁺ treatment in the in vivo part of their study. Therefore, the role of Mg²⁺ 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 Mg²⁺ concentrations are correlated with cardiovascular morbidity and mortality. Multiple observational studies and several intervention studies identify a direct link between Mg²⁺ 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 Mg²⁺ 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 Mg²⁺ supplements in CKD. Well-designed randomized controlled trials in CKD patients are necessary for any definitive conclusions on the preventive effects of Mg²⁺ in vascular calcification.

Remaining Challenges

Final conclusions about the molecular effects of Mg²⁺ are seriously hampered by the basic experimental setup of many in vitro studies that suffice with simple Mg²⁺ supplementation to calcification medium. This setup does not distinguish between passive chemical and active cell-mediated mechanisms. However, because cellular entrance of Mg²⁺ 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 Mg²⁺, as the molecular targets linking Mg²⁺ with osteogenic gene expression are unknown. In addition, the effect of Mg²⁺ supplementation on intracellular VSMC Mg²⁺ concentration has never been studied and urgently requires attention. Basic studies toward intracellular Mg²⁺ homeostasis and the molecular players that regulate Mg²⁺ 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 Mg²⁺ on cellular Ca²⁺ fluxes, the role of the CaSR in VSMCs and in particular the chemical impact of Mg²⁺ on ACP maturation. Furthermore, this review highlights the potential experimental bias of measuring osteogenic gene expression as effective inhibition of mineralization by Mg²⁺ 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, Mg²⁺ 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 Mg²⁺ should be reconsidered, as

Table 2. The Effects of Serum Mg²⁺ 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 (χ 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 ⁶⁴ 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, χ 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 Mg²⁺, 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 Mg²⁺ in experimental models of vascular calcifications drive the hypothesis that Mg²⁺ protects against mortality in CKD through the prevention of vascular calcification. However, the clinical role of Mg²⁺ in CKD patients has only been studied in observational cohorts, which focus mostly on total cardiovascular risk (Table 2). The effects of Mg²⁺ supplementation on cardiovascular outcome aside from arrhythmia and preeclampsia have been poorly assessed. Currently, randomized controlled clinical trials using Mg²⁺ 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 Mg²⁺ on vascular calcification. Nevertheless, further elucidation of the molecular mechanisms may contribute to additional targeted therapeutic options improving Mg²⁺ homeostasis in CKD patients.

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Highlights

- Serum Mg²⁺ concentration is inversely associated with cardiovascular risk in chronic kidney disease.
- Mg²⁺ is protective against vascular calcification.
- Mg²⁺ passively interferes with intestinal inorganic phosphate absorption and crystal formation in the circulation.
- Mg²⁺ actively modulates gene expression in vascular smooth muscle cell and thereby prevents transdifferentiation toward an osteoblastic phenotype.