

Editorial Comment

Magnesium: a renewed player of vascular ageing in diabetic CKD patients?

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Chronic kidney disease (CKD) patients are prone to a higher cardiovascular morbidity and mortality compared with the general population [1]. Consistent results from the basic and clinical research showed that cardiovascular susceptibility in CKD is due, partially at least, to a considerable acceleration of the vascular ageing processes in the context of *chronic kidney disease-mineral bone disorder syndrome* (CKD-MBD) [2]. This premature cardiovascular senescence is mainly characterized by altered endothelial reactivity, followed by pathologic calcification of cardiac valves and medial layer in the arteries [3]. The consequent increase of arterial stiffness worsens the cardiac afterload, contributing to the onset of left ventricular hypertrophy and to the progressive increase of pulse pressure (PP) leading to a significant reduction of diastolic tissue perfusion. The metabolic pathways linked to this premature ageing involve the dysregulation of several systems such as inflammation, oxidative stress, insulin resistance and mineral metabolism [3]. The alterations of calcium (Ca) and phosphate (P) homeostasis in renal patients, mainly driven by a derangement of Klotho/fibroblast growth factor 23 (FGF23)—parathormone (PTH)—vitamin D axis, are considered pivotal triggers and regulators of vascular ageing rather than mere biomarkers of CKD-MBD syndrome [3]. Thus, P overload and hyperparathyroidism are well known targets of medical treatments, such as P binders, vitamin D and calcimimetics, although with still limited evidence-based advantages in terms of survival in renal patients [3, 4]. The tough hedge that is still keeping nephrologists far from a conclusive and winning approach against vascular ageing is reasonably related to the still partial comprehension of the molecular pathways involved in a so complex, multifactorial and extreme process. New biomarkers and new actors are thus needed to orient the scientific knowledge towards further intervention strategies against CKD-MBD.

In the present issue of the *Clinical Kidney Journal*, Fragoso *et al.* [5] present the first observational data linking magnesium (Mg) and FGF23 levels to PP in 80 pre-dialysis type 2 CKD diabetic patients. This elegant cross-sectional analysis observed that a PP higher than 50 mmHg was independently associated with lower Mg

and higher FGF23 levels [OR 0.550 (95% CI 0.305–0.727) and 1.239 (95% CI 1.001–2.082), respectively]. Consistent with previous results, Mg levels were also negatively correlated with PTH (P=0.0001), c-term FGF23 (P=0.0001), Ca levels (P=0.026) and HOMA-IR (P=0.003) as well.

A growing body of evidence is supporting a cardinal role of Mg in several diseases linked to vascular ageing in CKD-MBD and diabetes. More specifically, low Mg levels were associated with low insulin resistance, poor endothelial reactivity, higher oxidative stress, increased intima-media thickness, vascular calcification, progression of CKD and mortality [6–9]. Although the common clinical experience depicted how Mg was directly involved in the altered Ca and PTH homeostasis, as in those cases of symptomatic hypocalcaemia secondary to severe hypomagnesaemia [10], the specific hormonal regulation of Mg balance and the specific pathways linking Mg to the pathophysiology of the mineral and vascular systems are still incompletely understood. Of note, altered Mg levels were associated with diabetes [7], menopause [11] and CKD [8], three conditions that share a common phenotype of accelerated vascular ageing, characterized by Monckeberg calcification and impaired bone metabolism (ranging from adynamic bone disease in diabetes to high bone turnover in osteoporotic menopausal women and certain CKD patients). Nevertheless, the maintenance of adequate intracellular Mg levels appears essential for life *per se*, considering that Mg is a fundamental co-enzyme of vital biochemical reactions as Krebs cycle and glycolysis [7] and it is essential for the maintenance of RNA and DNA stability [12]. Thus Mg may represent a cardinal biomarker and regulator of vascular ageing in humans, with special emphasis in CKD diabetic patients. Furthermore, nephrologists are called to a growing comprehension of Mg physiology, as a consequence of the renewed adoption of Mg as a P binder, now available in a single formulation together with calcium-acetate [13].

The data by Fragoso *et al.* [5] confirm the growing interest that nephrology community should point towards Mg metabolism with particular emphasis on CKD-MBD diabetic patients.

Magnesium balance in healthy and renal patients

Magnesium is an important element for several physiologic processes in humans, as the maintenance of bone health and the well functioning of the nervous system, energy metabolism and the synthesis of proteins, DNA and RNA [14]. The Mg storage in healthy adults amounts to 25 g (2000 mEq), 99% of whom are intracellularly distributed. As intracellular cation, Mg is mainly stored at the bone level (60–65%) and to a lesser extent into skeletal muscles (25–30%) and in other soft tissues (10–15%). Only 5–10% of the intracellular Mg is free, while the remaining is bound to proteins, citrates, RNA and DNA. Consequently, circulating Mg represents only 1% of the total body contents, ranging between 0.62 and 1.02 mmol/L, where the 60% of this circulates as the biologically active free cation, while the remaining 40% is protein bound or complexed as salts. A neutral balance of Mg requires a daily intake of 0.5–0.7 mEq/Kg of Mg, which is mainly present in cereals, nuts, legumes and green vegetables. Ingested Mg is thereafter absorbed in the proximal portion of the small intestine via a paracellular route and to a lesser extent in the colon via transcellular transport, probably involving the epithelial transient receptor potential melastin 6 (TRPM6) [15]. The rate of magnesium absorption ranges from 25 to 80%, accordingly to different nutritional intakes [14]. In normal subjects, the kidneys filter ~70–80% of the plasmatic Mg, 95% of which are reabsorbed, mainly in the thick ascending limb of the loop of Henle (TAL) [15]. In the proximal tubule and in the TAL Mg is reabsorbed together with Ca via a passive paracellular transport mediated by claudin-16 and claudin-19. Notably Ca and Mg, as activators of the calcium sensing receptor (CaSR), can reduce Mg excretion at this site via the lysosomal degradation of claudin-16. On the opposite, the reabsorption of Mg and Ca in the distal convoluted tubule (DCT) is driven by active transcellular processes, via the TRMP6 channel expressed in the first segment of DCT (DCT1) and via the transient receptor potential vanilloid 5 (TRPV5) in the late DCT (DCT2). Also at this site of the nephron, CaSR seems to interact with Mg handling. A crosstalk between CaSR and the potassium basolateral channel Kir4.1 may influence the TRPM6 activity in the DCT1, while the increased urinary Ca levels in TRPV5-null mice downregulated the aquaporin 2 water channel via the activation of the luminal CaSR, protecting from calcium deposits [15].

Although a specific hormonal regulation of Mg balance has not been clearly shown, several hormones and acid-base balance seem involved in Mg handling. Insulin can directly stimulate the renal Mg resorption, favouring the translocation of TRPM6 in the cell surface of the DCT [16]. Oestrogens normalized the renal TRMP6 mRNA levels previously reduced by Mg restriction in mouse models [17]. Chronic metabolic acidosis decreased renal TRPM6 expression leading to hypermagnesaemia, while an opposite effect was induced by metabolic alkalosis [18]. Furthermore, moving from bench to bedside, Mg balance needs to be cautiously interpreted considering the several concomitant treatments that may influence Mg excretion as thiazide and loop diuretics, acetazolamide [18], proton pump inhibitors [19], chemotherapies [20] and calcineurin inhibitors [21].

The trend of Mg levels in CKD is still controversial. Until a glomerular filtration rate (GFR) higher than 30 mL/min Mg levels are commonly maintained in the normal range.

Although the total pool of Mg can be increased along with the further reduction of GFR, the ionic Mg is more commonly normal, due to Mg bound with phosphate and other ions. Not severe hypermagnesaemia (<2 mmol/L) is frequent and asymptomatic in dialysis patients. Notably, the levels of Mg commonly assessed by the available assays should be cautiously interpreted especially in CKD, considering that circulating Mg represents the 1% of the organic Mg pool and that ionized and intracellular Mg levels could be more representative of the active Mg state [7]. As it is speculated for the circulating P levels [3], the significant variation of the circulating Mg concentrations could represent a late event in respect to faster and more relevant variations of intracellular and skeletal Mg levels. Actually, in the study by Fragoso *et al.* [5], higher circulating Mg levels, although within the normal range in CKD patients, were associated with a significant vascular damage, represented by higher P. Thus, further investigations on Mg pathophysiology could lead to a reevaluation of the 'normal' Mg levels or to a more appropriate Mg assessment for clinical purposes in renal patients.

Magnesium and mineral metabolism

Although the skeletal system represents the principal Mg store in humans, where it is concentrated into the hydration shell surrounding hydroxyapatite crystals [14], the relation of Mg with other skeletal ions (Ca and P) and with the regulatory hormones of mineral metabolism (PTH, Vitamin D, FGF23/Klotho) is still incompletely understood.

Old studies observed that a high Mg content in the bones was associated with osteomalacic mineralization defects probably due to the interference with Ca solubility or by the precipitation of Mg with pyrophosphate [14]. On the contrary hypomagnesaemia was associated with reduced bone mineral density [15]. The action of Mg on PTH looks different in the presence of chronic-severe hypomagnesaemia and in the presence of normal-high Mg levels. Chronic and severe Mg deficiency is classically associated with severe hypocalcaemia secondary to impaired PTH secretion and to peripheral resistance to PTH action [10]. In those cases, the administration of Mg rapidly restored normal levels of calcium through the normalization of PTH synthesis and action. On the opposite *in vitro* and *in vivo* studies observed that high Mg concentrations inhibited PTH secretion. It is well known that magnesium can inhibit PTH secretion via the direct activation of CaSR, but this action seems limited in the presence of low Ca levels [22]. Furthermore, the incubation of parathyroid gland with high Mg media induced the expression of vitamin D receptor (VDR), Klotho and FGF23 receptor [22]. Notably the reduction of PTH secretion at moderately elevated Mg levels did not lead to a PTH over-suppression [22]. Notably, low Mg levels and low Mg dietary intake was associated with osteoporosis, a classic high bone turnover condition [11]. Magnesium supplementation improved the hormonal assets and the urinary markers of bone turnover in osteoporotic postmenopausal women [11]. Magnesium carbonate is now available as P binder in a unique formulation with calcium acetate (CaMg). In the CALMAG study, CaMg was comparable to sevelamer-hydrochloride (HCl) in the control of P levels in dialysis patients [13]. Furthermore, CaMg reduced the FGF23 levels similarly to sevelamer-HCl [23], contrarily to the previous data reporting a

lower reduction of FGF23 levels in normophosphatemic CKD patients receiving Ca acetate compared with those treated with sevelamer. The mechanisms linking Ca/Mg P binder to a more potent FGF23 suppression compared with the calcium alone, P binders are still incompletely understood.

It could be speculated that while severe hypomagnesaemia leads to symptomatic relative hypoparathyroidism, the maintenance of normal-high Mg levels could be adjuvant in the control of secondary hyperparathyroidism via the activation of CaSR and via the optimization of the regulatory molecules acting on VDR and FGF23/Klotho axis. Fragoso *et al.* [5], consistent with previous results, observed that higher Mg levels were associated with a more favourable mineral asset, characterized by lower PTH, FGF23 and calcium levels. However, the cross-sectional design limits any conclusion on a causal relation of Mg with FGF23/Klotho and, more extensively, with mineral metabolism, which still requires further investigation to be clearly elucidated. Furthermore, whether Mg supplementation may improve bone health in CKD and osteoporotic patients needs to be more extensively explored.

Magnesium: a possible link between glucose metabolism and CKD-MBD?

Low Mg levels are an established risk factor for insulin resistance, metabolic syndrome and diabetes [7]. However, recent insights support the role of Mg as relevant trigger of insulin resistance rather than its mere biomarker. In fact, Mg deficiency can impair insulin sensitivity via the direct inhibition of tyrosine kinase [7]. The consequent insulin resistance and hyperglycaemia can increase the urine Mg output through the downregulation of TRPM6 and hyperfiltration [16]. Thus low Mg levels could be an active sustainer of a vicious cycle contributing to the progressive worsening of insulin resistance towards diabetes. Dietary Mg intake was associated with a higher risk of metabolic syndrome and diabetes [7]. Conflicting results have been reported about the potential benefits of Mg supplementation on glycaemic control. The meta-analysis by Song *et al.* [24] observed that 4–16 weeks of supplements with oral Mg, at a median dose of 360 mg/day, was effective in reducing plasma fasting glucose levels, however with no impact on glycated haemoglobin.

The results by Fragoso *et al.* [5] move in the same direction, showing a negative association between Mg and insulin sensitivity assessed by HOMA-IR in diabetic CKD patients. Notably, the association between HOMA-IR and PP lost significance in the multivariate analysis, where Mg and FGF23 levels remained the only independent predictors of PP. Thus, Mg metabolism could represent a considerable link between glucose metabolism and vascular ageing. Not surprisingly hypomagnesaemia was associated with several diabetic complications as retinopathy, foot ulcerations and nephropathy. Low Mg levels resulted an independent predictor of GFR decline in several studies [8]. Furthermore, reduced Mg levels are considered important inducers of vascular damage through several mechanisms, together with diabetes and CKD, as inflammation, oxidative stress, vasoconstriction and hypertension. Low Mg levels have been likewise associated to the vascular damages common to diabetes and CKD, as increased intima-media thickness, arterial stiffness and vascular calcifications (VC) [6].

Magnesium is a well-known P binder; thus, its dietary administration *in vivo*, as well as its presence in the culture media *in vitro*, can hamper the onset of P-induced VC. However, recent insights have shown that Mg can protect arterial walls from the P-induced VC also through active processes. The *in vitro* study by Montezano *et al.* [25], conducted on VSMC and aortas of rodents, observed that Mg negatively regulated the osteogenic differentiation of vascular smooth muscle cells (VSMC) through the activity of transient receptor potential melastin (TRPM7) and increased expression of protective proteins as matrix-GLA protein, bone morphogenic protein 7 and osteopontin. These results have been recently confirmed by Louvet *et al.* [26] in human VSMC *in vitro*. Surprisingly, the protective action of Mg was hampered in dead cells exposed to very high Mg concentrations, showing that the protection of Mg requires an active cellular role to be effective. Nonetheless, Mg could be protective through the direct activation of the CaSR in the VSMC and through the downregulation of inflammatory and oxidative stressors.

Over the last decades a strict crosstalk between bones and vascular system has been described. Alterations in bone handling, from low to high bone turnover, appeared both related to higher VC and worse survival in CKD. Diabetic patients are characteristically prone to osteopenia and to low PTH levels [27], the latter probably due to a reduced PTH secretion [28]. This relative hypoparathyroidism in diabetic ESRD patients was associated with an accelerated progression of VC [29]. Furthermore, a lower response of diabetic patients to the phosphaturic action of the FGF23/Klotho system after dietary P load has been reported [30]. In addition, it has been documented an impaired response of FGF-23 to oral phosphate in patients with type 2 diabetes, a possible mechanism of atherosclerosis, and poor renal response to FGF23 were associated with a higher CVD and mortality risk in humans [31]. Thus, FGF23 resistance could make diabetic patients even more susceptible to P overload and to the consequent higher risk of VC. Furthermore, lower FGF23 levels were associated with higher HOMA-IR as well [32]. Low Mg content in bones has been repeatedly reported in diabetic rats where it was reversible through the administration of Mg [33] and insulin [34]. Even more, Mg supplementation restored PTH levels in diabetic patients probably correcting the altered PTH secretion [27].

Data by Fragoso *et al.* [5], highlighting Mg and FGF23 as two independent predictors of arterial stiffness in CKD diabetic patients, are consistent with the evidence mentioned above. Thus, it can be argued that hypomagnesaemia or, even more strikingly, the reduced levels of intracellular Mg may be actively detrimental for CKD-MBD in diabetes, contributing to low bone turnover, renal FGF23 resistance, poor insulin sensitivity and lower protection against inflammation and oxidative stress in arteries leading to higher VC. Whether Mg supplementation could improve CKD-MBD in diabetic patients still needs to be investigated. Furthermore, our considerations are far from encouraging the maintenance of a marked hypermagnesaemia in renal patients, which could be toxic [14] and associated to a higher all-cause mortality probably also through relative hypoparathyroidism [9].

Conclusions

The study by Fragoso *et al.* [5] represents a relevant step forward in our epidemiological knowledge about the

important roles played by Mg and FGF23 in the arena of vascular ageing in diabetes and CKD-MBD. However, further investigations are required to better elucidate whether Mg supplementation could improve vascular ageing in diabetic CKD patients and to identify the optimal circulating Mg levels in this particular population.

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