There have been concerns about the accuracy of the Modification of Diet in Renal Disease (MDRD) formula in the normal range and in the upper range of stage 3 chronic kidney disease (CKD). Do all of the individuals diagnosed with stage 3 CKD actually have CKD? Only some with eGFR in the 60–90 ml/min per 1.73 $m^2$ range have decrements in GFR and are at risk for progression, but it is difficult to identify these individuals. These cases may be a situation in which cystatin C would be particularly useful. What is needed is a study of measurement of GFR in the general population with simultaneous measurement of creatinine and cystatin C, an area that has not been a high priority in large epidemiologic studies (Figure 1a). In addition, we need a study that evaluates GFR in the general medical population, particularly the elderly, where loss of lean mass that affects creatinine values may be particularly relevant (Figure 1b).

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# Prevention of vascular calcification with bisphosphonates without affecting bone mineralization: a new challenge?

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**Arterial calcification has been found to coexist with bone loss. Bisphosphonates, used as standard therapy for osteoporosis, inhibit experimentally induced vascular calcification, offering perspectives for the treatment of vascular calcification in renal failure patients. However, Lomashvili *et al.* report that the doses of etidronate and pamidronate that are effective in attenuating aortic calcification also decrease bone formation and mineralization in uremic rats, limiting their therapeutic use as anticalcifying agents.**

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In the general population, a strong association between low bone mineral density or osteoporosis and vascular calcification exists. Apparently, demineralization of the bone goes along with the formation of a bone-like structure in the arteries, which is called the calcification paradox. The link between artery calcification and bone diseases is also reported in patients with chronic kidney disease (CKD). Both high- and low-turnover bone disease, manifested as hyperparathyroid and adynamic bone, respectively, may contribute to the onset and/or progression of arterial calcification in renal failure patients. High bone formation and resorption rates represent an increased exchange of calcium and phosphate between the serum and the bone compartments. The continuous supply of these substrates from the bone to the serum can be responsible for the close association between high bone turnover and vascular calcification. On the other hand, in the case of adynamic bone disease, inactive bone remodeling leads to an impaired capacity of the bone to buffer circulating calcium and phosphate, resulting in a decreased rate of incorporation of calcium and phosphate into the bone, which ultimately augments the risk for extraosseous calcification.

In CKD patients, the prevalence of osteoporosis is high and bone mineral density decreases gradually with declining renal function.[1] Osteoporosis may coexist with either high bone turnover or low bone turnover. However, the mechanisms that may explain the concomitant appearance of a decreased bone mineral density and calcified areas in the vessel wall are still poorly understood. Potential mechanisms for this paradox are presented in Figure 1.

In CKD patients, inflammatory markers such as tumor necrosis factor-α and interleukin-6 as well as lipids, particularly oxidized low-density lipoprotein, are proposed to be responsible for the interaction between bone loss and vascular calcification by exerting adverse actions in the bone and the vessel wall. Oxidized lipids promote osteogenic differentiation and mineralization of

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## DISCLOSURE

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## REFERENCES

1. Abrahamson M, Olafsson I, Palsdottir A *et al.* Structure and expression of the human cystatin C gene. *Biochem J* 1990; **268**: 287–294.
2. Turk V, Bode W. The cystatins: protein inhibitors of cysteine proteinases. *FEBS Lett* 1991; **285**: 213–219.
3. Kaseda R, Iino N, Hosojima M *et al.* Megalin-mediated endocytosis of cystatin C in proximal tubule cells. *Biochem Biophys Res Commun* 2007; **357**: 1130–1134.
4. Stevens LA, Schmid CH, Greene T *et al.* Factors other than glomerular filtration rate affect serum cystatin C levels. *Kidney Int* 2009; **75**: 652–660.
5. Muntner P, Winston J, Uribarri J *et al.* Overweight, obesity, and elevated serum cystatin C levels in adults in the United States. *Am J Med* 2008; **121**: 341–348.
6. Knight EL, Verhave JC, Spiegelman D *et al.* Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. *Kidney Int* 2004; **65**: 1416–1421.
7. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 2004; **89**: 2548–2556.
8. Taleb S, Lacasa D, Bastard JP *et al.* Cathepsin S, a novel biomarker of adiposity: relevance to atherogenesis. *FASEB J* 2005; **19**: 1540–1542.
9. Shlipak MG, Sarnak MJ, Katz R *et al.* Cystatin C and the risk of death and cardiovascular events among elderly persons. *N Engl J Med* 2005; **352**: 2049–2060.

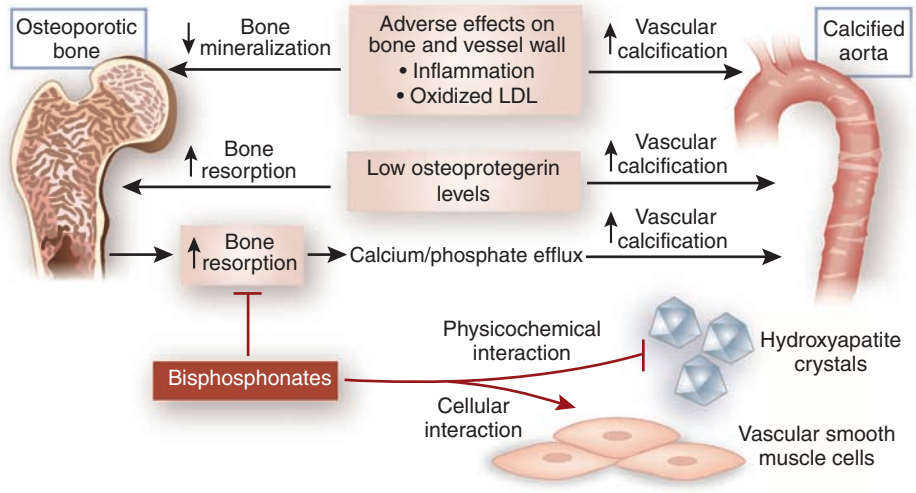


**Figure 1 | Potential mechanisms involved in the calcification paradox.** Bisphosphonates may reduce vascular calcification indirectly by inhibiting bone resorption or directly by either physicochemical interference with hydroxyapatite crystal formation or biological interaction with vascular smooth muscle cell function in the vessel wall. LDL, low-density lipoprotein.

vascular cells,[2] while they reduce bone mineralization.[3] Furthermore, the osteoprotegerin–RANK–RANKL triad may play a role in the calcification paradox. Osteoprotegerin (OPG) knockout mice exhibit a combination of osteoporosis and media calcifications in the aorta,[4] suggesting that OPG, an inhibitor of bone resorption, has a dual effect on bone and arteries, as it reduces bone resorption and inhibits artery calcification.

An increased bone resorption itself is also recognized as a causal factor for the relationship between osteoporosis and arterial calcification. Evidence is provided by studies showing that treatment with different types of bone resorption inhibitors, each with a different mode of action on the osteoclast, prevents the development of vascular calcification. Administration of a V-$H^+$-ATPase inhibitor,[5] an enzyme required for proton secretion by osteoclasts to acidify and dissolve bone mineral, as well as OPG treatment[6] prevented vitamin D-induced arterial calcification in rats. Bisphosphonates also reduced the development of experimentally induced media calcification in rats with chronic renal failure. These non-hydrolyzable pyrophosphate analogues inhibit osteoclast differentiation, recruitment, and activity and are prescribed worldwide for the treatment of osteoporosis. First-class non-nitrogen-containing bisphosphonates such as etidronate are metabolized into compounds that compete with ATP in the cellular energy metabolism, thereby inducing osteoclast apoptosis. Newer nitrogenous bisphosphonates inhibit the intracellular target enzyme farnesyl pyrophosphate synthase. This enzyme is involved in the synthesis of small G proteins that are essential for the control of several cellular processes, such as signal transduction, organization of the cytoskeleton, and cellular protein traffic. Price and co-workers found that alendronate and ibandronate inhibit artery calcification in rats with warfarin-induced vascular calcification and rats with uremia-induced media calcification at doses that inhibit bone resorption.[7,8] These reports have prompted the growing interest in bisphosphonates as a treatment strategy in the prevention of arterial calcification. Studies investigating the effect of bisphosphonates on both the bone turnover and the calcification processes in the vessel wall were lacking. They are, however, highly needed, particularly in patients with advanced CKD, in view of their severe bone and mineral disturbances. Lomashvili *et al.*[9] (this issue) report that etidronate and pamidronate indeed prevent the development of vascular calcification in rats with adenine-induced chronic renal failure, but at the same time bone formation and mineralization are affected. These findings extend previous results of Tamura *et al.*[10] showing that the most effective etidronate dose for the prevention of arterial calcification reduced bone mineral density in 5/6-nephrectomized rats. Both studies suggest that inhibition of arterial calcification by bisphosphonates holds a risk for bone metabolism and that prevention of vessel calcification can thus far not be established without affecting the bone.

The exact mechanism by which bisphosphonates inhibit artery calcification is not entirely understood. One possibility is an indirect effect through inhibition of bone resorption, which reduces the efflux of calcium and phosphate out of the bone, resulting in a decreased availability of the substrates required to form hydroxyapatite in the arterial wall. This is rather unlikely, as various studies did not find a decrease in serum calcium and phosphate levels following bisphosphonate administration. Moreover, the study by Lomashvili *et al.*[9] reports that the prevention of vascular calcification was associated not with an inhibition of bone resorption, but, rather unexpectedly, with the inhibition of bone formation. Alternatively, bisphosphonates may exert a direct effect on the vascular wall, either physicochemically through interference with the mineral formation or biologically at the cellular level through modulation of the transdifferentiation of vascular smooth muscle cells

into an osteoblast-like phenotype, which is a well-known mechanism underlying the calcification process in the artery. Interestingly, Lomashvili *et al.*[9] for the first time examine the isolated effect of bisphosphonates on the development of calcification in rat aorta cultures. Etidronate and pamidronate significantly reduced the calcium content in aortae cultured in medium containing alkaline phosphatase and warfarin to induce calcification, as well as in injured aortae. Additionally, etidronate prevented further progression of manifested calcified areas but could not reverse the calcification process. No effect of etidronate was found on the expression of osteogenic markers in calcifying cultured aortae, indicating that bisphosphonates do not influence osteogenic transdifferentiation of vascular smooth muscle cells. Taken together, these results strongly support the hypothesis that bisphosphonates prevent arterial calcification by blocking the physicochemical formation of apatite crystals in the vessel as they do in the bone of the rat model of Lomashvili *et al.*[9] This is not surprising given the structural resemblance of these agents to the calcification inhibitor pyrophosphate. Yet, as bisphosphonates affect cellular functions, cellular alterations involved in the inhibition of vascular calcification cannot be excluded. Possibly, bisphosphonates may interfere with the formation or transport of matrix vesicles loaded with nascent apatite crystals in osteoblast-like cells in the vessel wall, thereby preventing the development of arterial calcification.

The report by Lomashvili *et al.*[9] indicates that caution is strongly advised for the use of bisphosphonates to treat vascular calcification, especially in patients with CKD, who suffer from an impaired bone turnover inherent to the underlying disease state. As shown, these agents can cause impaired bone mineralization, which eventually may lead to osteomalacia as a consequence of prolonged administration. Moreover, by affecting bone formation, bisphosphonate use may completely block bone turnover, ultimately aggravating adynamic bone, which in turn has been considered a risk factor for vascular calcification.

Despite the relevant contribution of Lomashvili *et al.*[9] to the elucidation of the mechanisms by which bisphosphonates prevent artery calcification, some issues should be taken into account and require further examination. Primarily, etidronate and pamidronate have a relatively low potency regarding the inhibition of bone resorption. High doses are required to exert their inhibitory action on the osteoclast, which simultaneously causes inhibition of hydroxyapatite formation in the bone. Newer bisphosphonates such as risedronate and zoledronate, with a 1000- to 10,000-times-greater potency than etidronate, are used at lower doses and will less likely reduce bone mineralization. Therefore, it is important to investigate whether administration of more potent bisphosphonates can inhibit vascular calcification without reducing bone mineralization. In addition, to determine whether or not bone resorption itself is related to crystal formation in the arteries, the effect of bisphosphonates on bone and arteries should be examined in an animal model with increased bone resorption and apparent bone loss and with concomitant development of vascular calcification—for example, the OPG knockout mouse model. In contrast to the uremic rat model with only slightly increased bone resorption parameters that was used by Lomashvili *et al.*,[9] bisphosphonates will have the opportunity to exert their antiresorbing effect in an osteoporotic animal model.

The therapeutic use of bisphosphonates in CKD patients should be carefully evaluated. Although bisphosphonate treatment was studied in renal-transplant patients, a population at high risk for rapid bone loss, the lack of pharmacokinetic studies in end-stage renal failure patients precludes the use of these drugs in the latter population. A significant proportion of these patients shows low bone turnover. It is not inconceivable that bisphosphonates aggravate adynamic bone disease and thus increase the risk for arterial calcification. Therefore, the effect of bisphosphonates on adynamic bone and its coexistence with vascular calcification need to be investigated. Relatively small clinical studies showed that etidronate stopped the progression of coronary and aortic calcification in hemodialysis patients.[11,12] However, perspectives concerning a therapeutic window for bisphosphonates in the treatment of artery calcification will mainly depend on their action on bone resorption as well as on bone formation, particularly in CKD patients with underlying renal osteodystrophy, covering a wide range of abnormal bone turnover rates.

## DISCLOSURE

All the authors declared no competing interests.

## REFERENCES


1. Rix M, Andreassen H, Eskildsen P *et al.* Bone mineral density and biochemical markers of bone turnover in patients with predialysis chronic renal failure. *Kidney Int* 1999; **56**: 1084–1093.
2. Proudfoot D, Davies JD, Skepper JN *et al.* Acetylated low-density lipoprotein stimulates human vascular smooth muscle cell calcification by promoting osteoblastic differentiation and inhibiting phagocytosis. *Circulation* 2002; **106**: 3044–3050.
3. Parhami F, Tintut Y, Beamer WG *et al.* Atherogenic high-fat diet reduces bone mineralization in mice. *J Bone Miner Res* 2001; **16**: 182–188.
4. Bucay N, Sarosi I, Dunstan CR *et al.* Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes Dev* 1998; **12**: 1260–1268.
5. Price PA, June HH, Buckley JR *et al.* SB 242784, a selective inhibitor of the osteoclastic V-H+ATPase, inhibits arterial calcification in the rat. *Circ Res* 2002; **91**: 547–552.
6. Price PA, June HH, Buckley JR *et al.* Osteoprotegerin inhibits artery calcification induced by warfarin and by vitamin D. *Arterioscler Thromb Vasc Biol* 2001; **21**:1610–1616.
7. Price PA, Faus SA, Williamson MK. Bisphosphonates alendronate and ibandronate inhibit artery calcification at doses comparable to those that inhibit bone resorption. *Arterioscler Thromb Vasc Biol* 2001; **21**: 817–824.
8. Price PA, Roublick AM, Williamson MK. Artery calcification in uremic rats is increased by a low protein diet and prevented by treatment with ibandronate. *Kidney Int* 2006; **70**: 1577–1583.
9. Lomashvili KA, Monier-Faugere M-C, Wang X *et al.* Effect of bisphosphonates on vascular calcification and bone metabolism in experimental renal failure. *Kidney Int* 2009; **75**: 617–625.
10. Tamura K, Suzuki Y, Matsushita M *et al.* Prevention of aortic calcification by etidronate in the renal failure rat model. *Eur J Pharmacol* 2007; **558**: 159–166.
11. Hashiba H, Aizawa S, Tamura K *et al.* Inhibitory effects of etidronate on the progression of vascular calcification in hemodialysis patients. *Ther Apher Dial* 2004; **8**: 241–247.
12. Nitta K, Akiba T, Suzuki K *et al.* Effects of cyclic intermittent etidronate therapy on coronary artery calcification in patients receiving long-term hemodialysis. *Am J Kidney Dis* 2004; **44**: 680–688.