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Bone: a new endocrine organ at the heart of chronic kidney disease and mineral and bone disorders

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Recent reports of several bone-derived substances, some of which have hormonal properties, have shed new light on the bone–cardiovascular axis. Deranged concentrations of humoral factors are not only epidemiologically connected to cardiovascular morbidity and mortality, but can also be causally implicated, especially in chronic kidney disease. FGF23 rises exponentially with advancing chronic kidney disease, seems to reach maladaptive concentrations, and then induces left ventricular hypertrophy, and is possibly implicated in the process of vessel calcification. Sclerostin and DKK1, both secreted mainly by osteocytes, are important Wnt inhibitors and as such can interfere with systems for biological signalling that operate in the vessel wall. Osteocalcin, produced by osteoblasts or released from mineralised bone, interferes with insulin concentrations and sensitivity, and its metabolism is disturbed in kidney disease. These bone-derived humoral factors might place the bone at the centre of cardiovascular disease associated with chronic kidney disease. Most importantly, factors that dictate the regulation of these substances in bone and subsequent secretion into the circulation have not been researched, and could provide entirely new avenues for therapeutic intervention.

Introduction

For decades, it has been acknowledged that bone is affected by chronic kidney disease (CKD). The clinical findings of bone deformations and increased incidence of bone pain and fractures, originally named Von Recklinghausen disease (after a German pathologist who described it in 1892) and now known as osteitis fibrosa cystica, led to early recognition of CKD-induced bone disease, referred to as renal osteodystrophy.[1] The traditionally described cause of this disorder was secondary hyperparathyroidism, driven by phosphate retention and a progressive lack of active vitamin D, leading to hypocalcaemia.[2] This conceptual framework was additionally based on pioneering findings from bone biopsy of patients with advanced CKD, which showed grossly abnormal bone architecture, comprising primarily hyperdynamic (ie, high-turnover) bone disease, the histological hallmark of hyperparathyroidism.[3] The skeleton was therefore thought to be merely one of the tissues passively affected by the metabolic consequences of CKD.

This perception of bone involvement in CKD has now substantially changed after the description of two important epidemiological findings. The first was the severely increased risk of cardiovascular disease associated with CKD.[4] The second finding was the consistent association between any type of bone disease at any stage of CKD and cardiovascular morbidity.[5,6] The inference from these new insights was that cardiovascular disease in CKD might be mediated or modified by bone disease. Importantly, however, this suggestion is based on epidemiological association only, and no studies have prospectively tested this hypothesis. Because the skeleton is the most important reservoir for several minerals (including calcium, phosphate, and magnesium), disturbed skeletal handling and homoeostasis of these minerals have been thought to be the link between diseased bone and cardiovascular disease, especially cardiovascular calcification. Indeed, evidence exists of a decreased so-called buffer capacity for peak loads of phosphate and calcium in cases of low bone turnover or adynamic bone disease, which could favour the deposition of these minerals in many soft tissues, particularly the arterial wall and cardiac valves.[7] Conversely, increased release of these ions from bone in the setting of secondary hyperparathyroidism could also have the same harmful effects, although the quantitative effects of these mechanisms are unclear. Moreover, emerging insights have suggested that hypercalcaemia and hyperphosphataemia in conjunction can induce a phenotypic change of vascular smooth-muscle cells into bone-forming cells, genotypically strikingly resembling osteoblasts.[8] This frequent coexistence of bone, mineral, and cardiovascular disorders (mineral bone disorders [MBD]), in the setting of CKD, gives rise to the now widely accepted notion of CKD-MBD as a specific entity.[9]

New data about the role of bone in CKD on fundamental biological pathways, (eg, mineral homoeostasis, energy and glucose metabolism, and CKD-related pathology of the cardiovascular system) have been reported, and seem to suggest a further conceptual shift, pointing to the skeleton as an active inducer of pathology as a deregulated endocrine gland in kidney disease. In this Review, we describe recent research that either substantiates or refutes this new understanding of bone in CKD.

Our search of the scientific literature suggested a small number of hormones or circulating factors that met our prespecified criteria. We identified FGF23, the Wnt inhibitors sclerostin and DKK1, and osteocalcin as factors that are mainly bone derived and potentially have distant effects. Although less obviously bone derived, the bone morphogenic proteins BMP2 and BMP7 originate at least partly from this tissue and have clear distant effects, and are probably relevant in CKD.

FGF23

Arguably the first, but certainly the most prominent, bone-derived factor that is clearly abnormally elevated in

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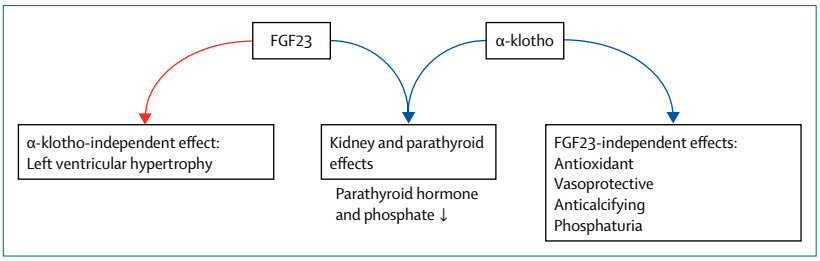

Figure 1: **The effects of FGF23 and α-klotho**

CKD is FGF23. Although the kidney, the liver, and coronary arteries have been suggested as sources of this factor,[10,11] overwhelming data point to bone (particularly the osteocyte) as the main production site. A unique feature of FGF23 is its low affinity for heparan sulphate (a proteoglycan found on cell surfaces or extracellular matrix), enabling it to escape from capture in bone.[12] A cofactor, α-klotho, is needed for FGF23 to bind to receptors,[13] and the absence of α-klotho in bone therefore possibly accounts for the lack of paracrine effects of FGF23 in skeletal tissue. Conversely, the expression of α-klotho in the parathyroid gland and distal convoluted tubule of the kidney, where it heterodimerises with the FGFR1 receptor, yields a high-affinity receptor for FGF23,[13] qualifying FGF23 as a true bone-derived hormone. An intricate association between FGF23 and α-klotho exists, with some physiological functions needing both factors for FGF23 signal transduction across FGFR1. However, FGF23 and α-klotho also seem to have pathophysiological effects independent from each other (figure 1). Details about the FGF23-independent effects of α-klotho are beyond the scope of this Review.

The physiological role of FGF23 is to maintain balance of phosphorus and vitamin D; its concentration increases as the glomerular filtration rate falls, because the kidney has a key role in excreting excess phosphate. Reduced ultrafiltration and excretion of phosphate due to a decreased glomerular filtration rate is balanced by decreased tubular reabsorption of phosphate due to the action of FGF23, which is an important physiological homoeostatic mechanism, tending to keep plasma concentrations of phosphate close to normal. Other physiological actions of FGF23 are to reduce production and secretion of parathyroid hormone, and interference with metabolism of vitamin D, leading to a fall in the concentration of 1,25-dihydroxycholecalciferol (the active form of vitamin D).[14] Possibly related to the effect of FGF23 on activation of vitamin D are intriguing clinical and experimental findings that point to an important role of calcium as an inducer of FGF23 secretion.[15–17] This suggested role of calcium possibly explains the lack of FGF23-lowering potency of therapy with calcium-based phosphate binder.[18] The physiological importance of FGF23 was shown in a rat model of CKD,[19] in which blocking of FGF23 was associated with increased vascular calcification and mortality. In human beings, impaired glycosylation of FGF23, resulting from mutations in the *GALNT3* gene, leads to augmented FGF23 degradation.[20] These changes to the FGF23 molecule abolish its phosphaturic-activating and vitamin-D-activating effects and lead to hyperphosphataemia and calcification of soft tissues.[20]

Several lines of evidence suggest that in CKD, FGF23 evades its physiological regulation to some extent. Although even in physiology the precise regulation of FGF23 has not been elucidated, phosphorus balance is conceivably an important determinant.[21] In early-stage CKD, however, increased concentrations of FGF23 coincide with slight hypophosphataemia, suggesting that this increase is maladaptive, due to an unidentified CKD-related factor.[22] Another argument for maladaptive increments of FGF23 comes from the non-consistent effects of oral phosphate-binding therapy on FGF23 concentrations, even when phosphorus uptake or plasma concentrations are adequately reduced.[18] An additional reason to assume that CKD itself, separate from phosphate balance or concentrations, induces FGF23 is the finding that after kidney transplantation, FGF23 concentrations fall for several months, despite pronounced hypophosphataemia in many patients.[23] At longer post-transplantation follow-up, FGF23 is positively associated with phosphate concentration again, suggesting disappearance of the maladaptive increase with time.[24] In further investigations of the effects of CKD (and not phosphate concentrations) on FGF23, almost all circulating FGF23 was intact in patients undergoing peritoneal dialysis,[25] by contrast with healthy people.[17] This finding suggests that CKD inhibits FGF23 catabolism.

However, besides being maladaptive (especially in early stages of CKD), some evidence suggests that FGF23 concentrations rise because of a relative resistance to its renal effects, possibly due to downregulation of tissue α-klotho, a cofactor for FGF23 signalling.[26]

The mechanisms that underlie the maladaptive component of increased FGF23 concentrations suggest that bone is the cause. Findings from human bone biopsy studies showed that expression of FGF23 by osteocytes, although highly correlated with circulating concentrations, was increased in very early-stage CKD.[27] Importantly, its upregulation strongly correlated with several indices of local mineralisation status in bone. The cause of this early upregulation of FGF23 in CKD is unclear, but disturbances in local FGF23 regulatory factors (eg, DMP1 and PHEX) might be implicated,[28] and abnormal function of DMP1, an FGF23 inhibitor, has also been suggested.[27] In addition to increased production by osteocytes, a decrease in local catabolism of FGF23 by osteocytes could be involved in increased release of FGF23 from bone.[29]

Both epidemiological and experimental data strongly suggest that (maladaptive) increases in FGF23 concentrations are directly involved in the dismal clinical outcome in CKD. Findings from observational studies

consistently showed increased mortality with elevated concentrations of FGF23 in all stages of CKD, including after transplantation.[30–32] An even more important result from these studies was that the hazard ratio (HR) for FGF23 not only exceeded that for phosphate, but that correction for potential confounders, including phosphate, did not mitigate the strength of the HR, suggesting causality of FGF23 for these outcomes. The lack of confounding by phosphate might be partly accounted for by non-phosphate-related maladaptive increases of FGF23 by local mechanisms in bone.

Because the leading cause of morbidity and mortality in CKD is cardiovascular disease, the obvious site for direct FGF23 toxicity would be the cardiovascular system. Indeed, as for mortality, increased prevalence of left ventricular hypertrophy is strongly associated with increased FGF23 concentrations.[33] Findings from a landmark study[34] lent support to this epidemiological link and provided compelling mechanistic evidence for direct induction of left ventricular hypertrophy by Fgf23 in rodents. Importantly, these direct myocardial effects were independent of α-klotho.[34] Less convincing are the effects of FGF23 on calcification of the arterial wall, which is another prominent comorbidity in CKD. Epidemiological data are conflicting, with some studies showing no association between FGF23 and arterial calcification,[35] and others suggesting a positive association.[36–38] Investigators of one study[39] noted an inverse association between FGF23 and cardiovascular disease, and no association with calcification. This finding is in line with the presumed protective effects of FGF23 on calcification of the arterial wall in experimental conditions,[40,41] but again contradicts studies in which no FGF23 signalling in the arterial wall was reported.[35,42] In addition to the (disputed) potential role of FGF23 in vascular calcification, indirect evidence suggests that the protein negatively affects endothelial function. In a community-based study,[43] FGF23 was associated with cardiovascular events. Although this association remained significant after multivariable adjustment, it was attenuated when other indices of endothelial function were modelled. Moreover, reduction in FGF23 concentrations caused by therapy with oral phosphate binders was associated with an improvement in flow-mediated vasodilatation, a marker of endothelial function.[44]

In conclusion, increases in FGF23 concentrations are pronounced in CKD, but the direct mechanisms that induce this rise are mostly obscure. The epidemiological associations between FGF23 and poor outcomes are very consistent, but potential causal mechanisms have not been established.

Sclerostin and DKK1: Wnt inhibitors and their effects

Wnt inhibitors are secreted factors that interact with Wnt receptors or Wnt ligands and attenuate Wnt signalling activity (panel). In this Review we focus on sclerostin and DKK1. Both proteins are soluble Wnt inhibitors,[48] have already undergone some experimental and clinical investigations about their role in CKD-MBD, and can be measured in human blood with use of ELISA techniques. However, when blood sclerostin measurements in CKD cohorts are compared, substantial discrepancies between assays need to be taken into account,[49] although generally concentrations are increased in CKD. Whether these increased concentrations in CKD result from increased production, renal retention, or both, has not been established.[50,51]

Despite some homologies in action, DKK1 and sclerostin both have distinct biological effects and are differently regulated.[52] Both proteins use a different receptor to inhibit the formation of the frizzled-LRP5-LRP6 receptor complex.[46] Experimental findings suggest that sclerostin might exert more selected regulatory actions, whereas DKK1 might be a pan-Wnt inhibitory blocking protein, because it interferes with various Wnt classes.[48] Accordingly, striking differences exist between the two corresponding rodent knockout models *Sost*$^{-/-}$ (*SOST* is the gene encoding sclerostin) and *Dkk1*$^{-/-}$. By contrast with the fairly benign phenotype with predominant skeletal changes in *Sost*$^{-/-}$ knockouts, *Dkk1*$^{-/-}$ mice die prematurely from severe developmental defects.[53] Sclerostin seems to be a typical Wnt inhibitor because it is almost exclusively bone derived, and is secreted by osteocytes and to some extent by osteoclast precursors.[54]

Wnt signalling is of great importance for skeletal health. Besides being an inhibitor of the Wnt-catenin pathway (figure 2), sclerostin acts by other modes—eg, suppressive effects on bone morphogenic proteins.[55,56] The clinical relevance of Wnt signalling for bone can easily be established in human disorders such as sclerostosis and Van Buchem's disease, and in genetically modified rodent models.[57–60] Although reduced activity of sclerostin and DKK1 leads to increased bone mass and strength, the opposite occurs in animal models with overexpression of both sclerostin and Dkk1.[61,62] In addition to direct effects of sclerostin on local Wnt

Panel: What is Wnt signalling?

Wnt signalling pathways play a key part in many diverse biological processes, such as cell proliferation, growth, migration, and differentiation.[45,46] Wnt signalling encompasses at least three different complex pathways, including the Wnt-β-catenin pathway (also called the canonical pathway). The canonical Wnt pathway involves interaction of several Wnt ligands and inhibitors with a transmembrane receptor complex (frizzled family receptor), including the coreceptors LRP5 and LRP6.[46,47] With activation of this receptor complex, cytoplasmatic-β-catenin degradation through phosphorylation is reduced. Hence, β-catenin accumulates intracellularly and allows more β-catenin to translocate into the nucleus where it assists in the activation of various target genes (figure 2). Wnt signalling (including soluble inhibitors) has a key role in bone physiology and regulation of bone cellular activities and mineralisation processes.[48]

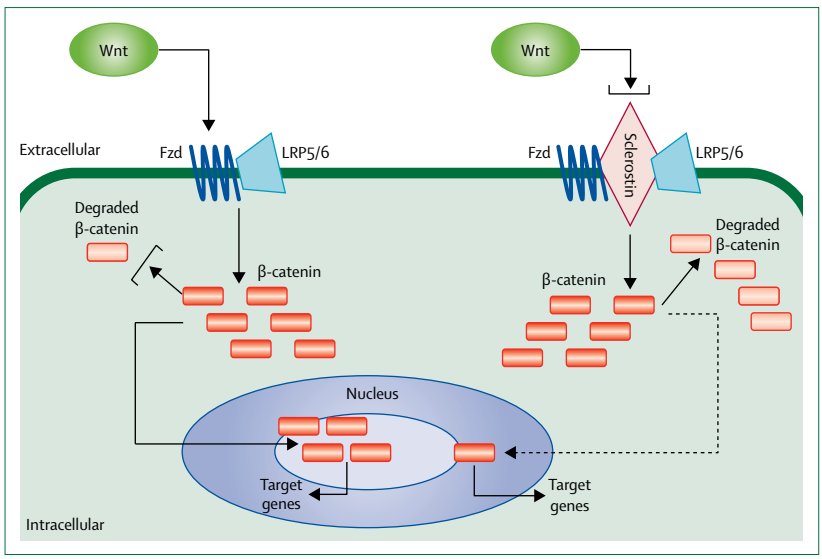

Figure 2: **Inhibition of Wnt signalling**
Within the canonical pathway, Wnt ligands interact with a transmembrane receptor complex including frizzled (Fzd) and LRP5/6. Activation of the receptor complex stabilises cytosolic β-catenin by blocking degradation processes. Hence, more β-catenin can enter the nucleus and assist activation of target genes. Wnt inhibitors such as sclerostin interfere with Wnt-receptor complex activation and finally reduce intranuclear β-catenin activity by stimulating phosphorylation degradation.

pathways, the effects of parathyroid hormone on bone also depend on sclerostin activity, as shown in animal models.[63] All these data point to the importance of balanced activity of Wnt signalling for bone health. In CKD, this activity is probably disturbed because of typically increased circulating concentrations of sclerostin.[64] However, the effects of these increased concentrations are not straightforward; on the one hand, reduced osteocytic Wnt-β-catenin signalling was an early finding during the course of experimental CKD-MBD,[65] but on the other, activity of Wnt-catenin pathways is increased in monocytes from patients with CKD.[66]

Besides affecting bone metabolism, Wnt signalling has a crucial role in human atherosclerosis,[47] and therefore increased concentrations of bone-derived Wnt inhibitors (eg, sclerostin) in CKD could interfere with this process. Missense mutations in *LRP6* are indeed associated with premature coronary artery disease.[67] Additionally, findings from experimental studies have shown a wide range of effects of Wnt activation on cell function for vascular smooth muscle, including protection against apoptosis.[68] In view of the crucial role of apoptosis of vascular smooth-muscle cells in vascular calcification, circulating Wnt inhibitors also affect this process. In addition to bone-derived sclerostin, recent research findings suggest that in tissue from calcified aortic valves of patients undergoing haemodialysis greater upregulation of sclerostin mRNA was detectable than in tissues from non-calcified controls.[51] Sclerostin expression has also been shown in calcifying vascular smooth-muscle cells in vitro.[69] Occurrence of sclerostin in calcified tissue is also detectable in patients with calciphylaxis—a severe form of uraemic small-vessel calcification with consecutive ulcer development.[70]

Disturbed Wnt signalling could be particularly relevant in adynamic bone disease, a frequently encountered subtype of bone disease in CKD that is characterised by low bone turnover, and is partly mediated by fairly low concentrations of parathyroid hormone or resistance to parathyroid hormone.[71] However, in CKD-MBD potential interactions between parathyroid hormone and Wnt signalling are incompletely understood. Preliminary results from a study[50] of 60 patients undergoing dialysis showed a negative correlation between serum concentrations of sclerostin and parathyroid hormone. Moreover, sclerostin showed strong negative associations with parameters of bone turnover, pointing towards a role of high sclerostin concentrations in induction of adynamic bone disease, or vice versa. By contrast, no such association was reported for DKK1. Furthermore, experimental evidence lends support to a crosstalk between parathyroid hormone and Wnt signalling actions on bone metabolism, mediated particularly by sclerostin: Although in-vivo overexpression of Dkk1 in mice did not blunt the osteoanabolic effect of exogenous parathyroid hormone,[62] sclerostin overexpression did reduce parathyroid-hormone-associated bone gain.[63] To what extent is this crosstalk between parathyroid hormone and sclerostin preserved under uraemic conditions, and do high sclerostin concentrations contribute to uraemic skeletal resistance to parathyroid hormone? If sclerostin induces uraemic resistance to parathyroid hormone, it could be another feature of disturbed bone metabolism in CKD, and might account for the simultaneous occurrence of elevated concentrations of parathyroid hormone and sclerostin in CKD.

Romosozumab (Amgen and VLB Pharma, CA, USA), a humanised monoclonal antibody against sclerostin, can increase bone mineral density in postmenopausal osteoporosis,[72] and might provide new methods to improve sclerostin-induced disturbances in Wnt signalling in CKD. However, caution is warranted because sclerostin in the vasculature might also act as an antimineralisation substance (as it does in bone), which would raise safety concerns in patients with a high propensity for vascular calcification, such as those with advanced CKD.

As for FGF23, reasonable evidence points to a severely deregulated metabolism of the Wnt inhibitors sclerostin and DKK1 in CKD. Because Wnt signalling is important in both bone disease and vascular biology, deranged regulation of both sclerostin and DKK1 is very likely to affect at least these tissues. However, the exact roles of sclerostin and DKK1 are largely unresolved, and the therapeutic potential of intervention in this system is unexplored but promising.

Osteocalcin

Osteocalcin, also known as bone gla protein, is a 6 kD calcium-binding bone-matrix protein produced by osteoblasts, and is one of the most abundant non-collagen proteins in bone. It is regulated by the two major mineral and bone-controlling hormones, vitamin D and parathyroid hormone. Osteocalcin expression by the *BGLAP* gene and production are directly stimulated by vitamin D through a vitamin-D-responsive element located in the promoter region of *BGLAP*. Parathyroid hormone also stimulates osteocalcin production through its binding to the receptor PTH1R and activation of the cAMP-dependent protein-kinase-A intracellular signalling pathway. No receptor for osteocalcin has been identified, but the protein might modulate activities of the G-coupled protein receptor GPRC6A, which is widely expressed.[73] Osteocalcin acts as a regulator of bone mineralisation, affecting hydroxyapatite size and shape, and is a potential modulator of osteoblast and osteoclast activities through its vitamin-K-dependent γ-carboxylated form.[74] Uncarboxylated (or under-carboxylated) forms of osteocalcin that are not bound to hydroxyapatite are released into the bloodstream, where they accumulate with fragments derived from direct proteolysis of osteocalcin after bone resorption, to constitute circulating osteocalcin.[74] Although the measurement of circulating osteocalcin by different assays is not standardised and is hampered by the heterogeneity of circulating osteocalcin fragments, uncarboxylated osteocalcin is estimated to account for up to 50% of total osteocalcin in serum samples from normal people.[75] Concentrations of circulating uncarboxylated forms of osteocalcin are affected by vitamin K status[75] and directly by the degree of bone remodelling, as reported in both patients with CKD and those undergoing haemodialysis, for whom circulating concentration of total osteocalcin is positively correlated with histological parameters of bone remodelling.[76,77]

The role of circulating uncarboxylated forms of osteocalcin was first described by Lee and colleagues,[78] who suggested that in mice these forms act as hormones to increase insulin secretion and sensitivity, and protect mice from adiposity. In support of this, results from several (but not all) epidemiological studies of this topic have shown an inverse link between total osteocalcin concentrations in serum samples and glycaemia, glucose metabolism, and measures of adiposity.[79] However, few of these studies have evaluated the concentrations of uncarboxylated forms of osteocalcin, and they have not shown such associations between uncarboxylated forms of osteocalcin and glucose or adiposity,[79] by contrast with data from murine models. Differences in osteocalcin genetics (one gene in human beings compared with three genes in mice), concentrations, and metabolism between human beings and mice could all account for this discrepancy.[79] The complex association between vitamin K status and osteocalcin could be another explanation. Vitamin K supplementation decreases the concentrations of uncarboxylated forms of osteocalcin,[79,80] but has shown variable effects on glucose metabolism in the small number of human studies reported,[79] and no solid data about the effect the of oral vitamin K antagonist, warfarin, on energy metabolism are available.

Because osteocalcin is a product of osteoblasts, and after Lee and colleagues[78] proposed that uncarboxylated osteocalcin is released by osteoclastic resorption of osteogenic matrix, Confavreux and colleagues[81] used another approach to study the effects of osteocalcin on glucose metabolism. They reported that the resection of osteocalcin-secreting osteoid osteomas in two patients led to a postoperative decrease of total osteocalcin by 62% and 30%, respectively, with proportionate increases in blood glucose, without modifying markers of bone turnover.[81] However, concentrations of uncarboxylated osteocalcin were not recorded in this study. Schafer and colleagues[82] reported that parathyroid hormone (1–84) increased and alendronate reduced concentrations of uncarboxylated osteocalcin, and that these changes were associated with changes in bodyweight, fat mass, and serum concentrations of adiponectin, but the investigators did not find linkage between changes in uncarboxylated osteocalcin and changes in concentrations of glucose and insulin.[82] Moreover, a post-hoc analysis of three large, randomised, placebo-controlled trials, did not show any evidence that bone antiresorptive therapy had a clinically important effect on fasting glucose, weight, or diabetes risk in postmenopausal women.[83] So, contrary to predictions from mouse models, additional evidence is needed to show whether decreased bone turnover plays an important part in glucose metabolism in human beings.

In the setting of CKD, the ratio of uncarboxylated osteocalcin to total osteocalcin in serum samples was reported to be high in 172 patients with CKD stages 3–5, suggesting an elevation in uncarboxylated forms of osteocalcin; this ratio correlated with CKD stage and albumin-to-creatinine ratio in urine.[84] The factors affecting circulating concentrations osteocalcin in CKD have not been explored, but the abnormal metabolism of vitamin K reported in these patients might have a role.[84] Indeed, data from animal models of CKD showed that warfarin treatment resulted in significantly increased uncarboxylated osteocalcin and significantly decreased carboxylated osteocalcin; importantly, high dietary intake of vitamin K1 resulted in an increase in the carboxylation of osteocalcin in the setting of CKD.[85] Increased serum concentrations of uncarboxylated osteocalcin, which were associated with bone metabolism markers, were also inversely associated with indices of glucose metabolism (eg, glucose concentration, HbA_{1c}, and glycated albumin concentration) in 189 patients undergoing haemodialysis.[86] Concentrations of N-terminal mid-fragment osteocalcin were also reported to be high in patients with CKD stages 2–4, and were correlated with adiponectin concentrations, but not with bone parameters, as assessed by high-resolution peripheral quantitative CT.[87]

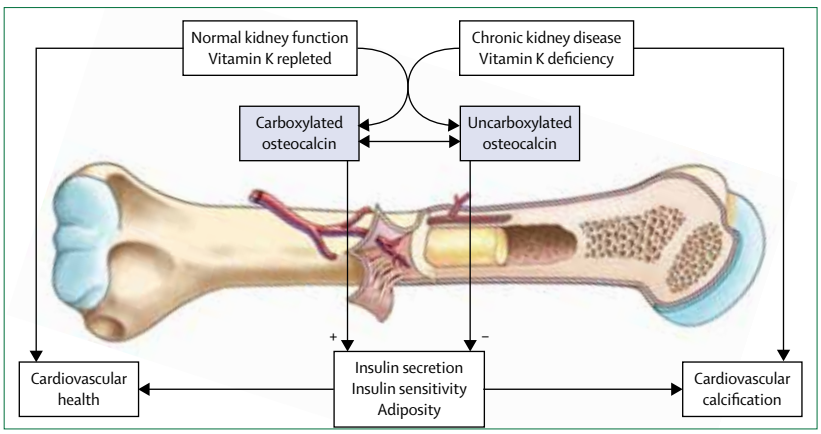

Figure 3: **Osteocalcin in chronic kidney disease**
Under conditions of normal kidney function and the absence of vitamin K deficiency, the proportion of uncarboxylated osteocalcin is low, stimulating insulin secretion and improving insulin sensitivity and energy handling. In the setting of chronic kidney disease or vitamin K deficiency, the amount of uncarboxylated osteocalcin increases, and as such negatively affects insulin secretion and sensitivity, promoting cardiovascular disease. Chronic kidney disease has a direct calcifying effect, not mediated by bone, and additionally vitamin K deficiency can also negatively affect the carboxylation status of matrix gla protein (not shown).

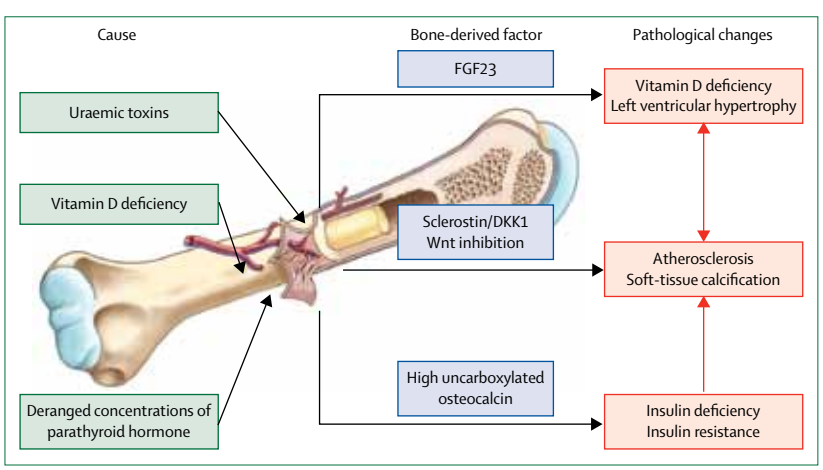

Figure 4: **The possible role of bone in CKD-MBD**
Hormonal changes associated with CKD or poorly defined uraemic toxins (green boxes) induce changes in bone metabolism, leading to a maladaptive increase of several humoral factors (blue boxes) from bone into the circulation. These circulating factors directly induce pathological changes (red boxes). The red arrows show the amplifying effects of pathological changes induced by diseased bone. CKD=chronic kidney disease. MBD=bone, mineral, and cardiovascular disorders.

Supplementation with vitamin K2 induced a dose-dependent and time-dependent decrease in circulating uncarboxylated osteocalcin in addition to other vitamin-K-dependent γ-carboxylated molecules in patients undergoing haemodialysis.[88] However, the investigators of this study did not report whether this modification was associated with effects on glucose metabolism or measures of adiposity.

Figure 3 summarises a conceptual view of interrelations between osteocalcin and glucose metabolism in health and CKD. Several aspects need additional experimental support, and the clinical implications of these associations are unclear.

Search strategy and selection criteria

We searched Embase and Medline, using "bone" and "skeleton" as obligate terms, with the following MESH terms or abstract words in all combinations of a minimum of two: "cardiovascular", "vascular", "glucose", "factor", "heart", "cardiac", and "metabolism". We assessed the abstracts of the retrieved results and conditionally selected papers based on three criteria. First, the paper had to address a mainly bone-derived circulating factor; second, the regulation of that factor had to be probably disturbed in CKD; and third, arguments that the specific factor could have distant detrimental effects should be present. Abstracts describing factors meeting these criteria were thought to be of possible relevance. Of the papers identified by this selection, we manually screened citations for relevance based on these criteria. Because of the nature of this Review (ie, substantiation of a new notion), the selection of search and selection criteria inevitably remained arbitrary.

Bone morphogenetic proteins

The capacity of bone extracts to induce ectopic bone formation has been recognised since 1965.[89] However, the first bone morphogenetic protein was isolated only two decades later and named osteogenin.[90] The number of bone morphogenetic proteins has since increased substantially,[91] and these proteins are characterised by both the presence of a conserved C-term domain with seven cysteine residues, and by the capacity to induce several biological functions in several species.[92] Bone morphogenetic proteins have a role in several clinical disorders ranging from vascular calcification[93] to obesity, diabetes, and cancer.[94] Accordingly, the family of bone morphogenetic proteins now includes substances that are not exclusively bone derived and are not invariably characterised by bone-forming properties. As part of the superfamily of proteins that interact with the receptor transforming growth factor β, bone morphogenetic proteins can be regarded as cytokines with growth and differentiation properties. Proteins in this family act through a common receptor which, through complex activations of the intracellular Smad signalling pathway, subsequently elicit different effects in different tissues.[95,96] Bone morphogenetic proteins can be generally regarded as local (auto or paracrine) modulators of the inflammatory effects of transforming growth factor β.[97]

In recognition of their biological properties, even though not mainly produced in bone, some bone morphogenetic proteins seem to be linked with the CKD-MBD syndrome. In particular, BMP2 and BMP7 have both been extensively studied in relation to experimental vascular calcification.[98,99] BMP2 stimulates the osteoregulatory gene *MSX2* and enhances phosphate uptake through upregulation of the type III sodium-dependent phosphate co-transporter in vascular smooth-muscle calcification.[100] BMP2 expression is increased in

the vessel wall in response to inflammation,[101,102] and these calcifying effects are inhibited by MGP (which binds to BMP2) and SMAD6.[103–105] BMP2 is thus regarded as an important induction factor of calcification. Clinically, serum concentrations of BMP2 are increased in patients with CKD and are positively associated with vascular stiffness.[106] Intriguingly, although BMP2 is associated with decreased expression of smooth-muscle cell markers in vascular smooth-muscle calcification (which promotes the osteoblastic-like phenotype), BMP7 promotes the phenotype of vascular smooth-muscle calcification, and thus represents an inhibitor of calcification possibly implicated in bone and vascular disease. In different experimental models of renal disease, a significant reduction of BMP7 expression occurs in renal tissue,[107–110] and circulating BMP7 concentrations are reported to be low.[111] Furthermore, BMP7 inhibits vascular calcification in experimental atherosclerosis[112] and adynamic bone lesions and vascular calcification in experimental models of CKD and metabolic syndrome.[113] BMP2 and BMP7 have been suggested to have anabolic effects through interaction with SOST and DKK1 in bone[55] and to protect podocytes from profibrotic stimuli.[114] Besides these local cytokine-like effects, BMP2 and BMP7 might have systemic effects in view of their reported association with calciprotein particles and arterial stiffness in patients with CKD.[115] In summary, CKD could be a disorder of BMP2 and BMP7 dysregulation, potentially involved in the pathological processes of calcification in orthotopic and ectopic tissues.

Discussion

In this Review we have summarised several new insights that point to a putative active role of skeletal tissue as an endocrine regulator of energy metabolism and mineral homoeostasis in addition to the induction and propagation of cardiovascular disease. The conceptual framework that connects bone disease to systemic pathological changes might accordingly evolve, from bone as a mere target of uraemia, through to a diseased tissue unable to handle fluctuations in phosphorus and calcium concentrations appropriately, to a maladapted endocrine gland from which active substances enter the systemic circulation and induce additional morbidity (figure 4).

If this controversial and unproven notion is true, it would be a huge cause of morbidity and mortality in view of the high prevalence of CKD, affecting up to 40% of people older than 60 years.[116] The importance of crosstalk between bone and the cardiovascular system could in fact go beyond the setting of CKD, because age-related osteoporosis itself emerges as a risk factor for CVD.[117,118] A malign cycle of crosstalk between dysfunctional diseased bone and pathological changes in cardiovascular tissue might operate, supported by the finding that people with subclinical cardiovascular disease are at higher risk of subsequent bone loss and fractures.[119] These associations between bone and vascular systems suggest that the proportion of the general population affected by bone-driven, so-called age-related morbidity is probably severely underestimated. Besides the unexpectedly high number of people at risk from their affected skeletons, this actual risk might furthermore exceed the outcomes of the systems described in detail in this Review, because we have focused mainly on bone-derived factors. Several bone-regulating factors that are also expressed outside skeletal tissue seem to be already dysregulated in early CKD as well,[120] and even bone-forming circulating cells (possibly as a result of entry into the bloodstream after microtrauma) have been implicated in the development of cardiovascular disease.[121]

Although detailed research has focused on the clinical outcomes of these bone-derived substances, the local factors that dictate their production and secretion in CKD are an important gap in present knowledge and continuing research. In the case of FGF23, its epidemiological meaning and biochemical outcomes have been the subject of intense research and have partly been elucidated, but the factors that dictate its production, epigenetic regulation, secretion, and subsequent metabolic fate are still largely obscure.[122]

We postulate that bone, affected by CKD, and possibly also by age-related osteoporosis, predisposes to accelerated cardiovascular disease. This mechanism is probably mediated by bone-derived factors (eg, FGF23, sclerostin, osteocalcin, and possibly DKK1) that affect structure, metabolism, and function of crucial cardiovascular tissues, and also more indirectly by interfering in insulin secretion and sensitivity by osteocalcin. These direct detrimental effects from bone are amplified by additional factors with biological effects on (ectopic) bone formation, such as the bone morphogenetic proteins. Although this hypothesis definitely needs additional support, an intensified focus of research on factors that dictate the production and release from bone of humoral factors that can induce pathological changes at distant sites, including metastatic bone formation, could open an entire new avenue for clinical interventions for an unmet medical need.

Contributors

All authors contributed to the concept and design of the Review, and read and revised subsequent versions. MGV, VMB, ZAM, and SM did the literature search. MGV, VMB, ZAM, and SM initially wrote the Review. MGV, VMB, and ZAM prepared the figures. MGV had final responsibility for the decision to submit for publication.

Declaration of interests

MGV reports personal fees from Amgen, personal fees from Astellas, grants and personal fees from AbbVie, grants and personal fees from Shire, grants and personal fees from Sanofi, grants from Pfizer, grants from Dutch Kidney Foundation, and non-financial support from Alexion, outside the submitted work. ZAM reports grants from Baxter, grants and personal fees from FMC, grants and personal fees from Amgen, grants and personal fees from Sanofi/Genzyme, personal fees from Chugai, personal fees from Abbott, and personal fees from Vifor, outside the submitted work. VMB reports research grants from Amgen

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References

1 Moe S, Drüeke T, Cunningham J, et al, and Kidney Disease: Improving Global Outcomes (KDIGO). Definition, evaluation, and classification of renal osteodystrophy: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* 2006; **69:** 1945–53.

2 Slatopolsky E, Martin K, Hruska K. Parathyroid hormone metabolism and its potential as a uremic toxin. *Am J Physiol* 1980; **239:** F1–12.

3 Malluche HH, Mawad HW, Monier-Faugere MC. Renal osteodystrophy in the first decade of the new millennium: analysis of 630 bone biopsies in black and white patients. *J Bone Miner Res* 2011; **26:** 1368–76.

4 Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004; **351:** 1296–305.

5 Raggi P, Giachelli C, Bellasi A. Interaction of vascular and bone disease in patients with normal renal function and patients undergoing dialysis. *Nat Clin Pract Cardiovasc Med* 2007; **4:** 26–33.

6 Tankó LB, Christiansen C, Cox DA, Geiger MJ, McNabb MA, Cummings SR. Relationship between osteoporosis and cardiovascular disease in postmenopausal women. *J Bone Miner Res* 2005; **20:** 1912–20.

7 London G, Coyne D, Hruska K, Malluche HH, Martin KJ. The new kidney disease: improving global outcomes (KDIGO) guidelines—expert clinical focus on bone and vascular calcification. *Clin Nephrol* 2010; **74:** 423–32.

8 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.

9 Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). *Kidney Int Suppl* 2009; **113:** S1–130.

10 Zanchi C, Locatelli M, Benigni A, et al. Renal expression of FGF23 in progressive renal disease of diabetes and the effect of ace inhibitor. *PLoS One* 2013; **8:** e70775.

11 van Venrooij NA, Pereira RC, Tintut Y, et al. FGF23 protein expression in coronary arteries is associated with impaired kidney function. *Nephrol Dial Transplant* 2014; published online Jan 23. DOI:10.1093/ndt/gft523.

12 Goetz R, Ohnishi M, Kir S, et al. Conversion of a paracrine fibroblast growth factor into an endocrine fibroblast growth factor. *J Biol Chem* 2012; **287:** 29134–46.

13 Urakawa I, Yamazaki Y, Shimada T, et al. Klotho converts canonical FGF receptor into a specific receptor for FGF23. *Nature* 2006; **444:** 770–74.

14 Razzaque MS, Lanske B. The emerging role of the fibroblast growth factor-23-klotho axis in renal regulation of phosphate homeostasis. *J Endocrinol* 2007; **194:** 1–10.

15 David V, Dai B, Martin A, Huang J, Han X, Quarles LD. Calcium regulates FGF-23 expression in bone. *Endocrinology* 2013; **154:** 4469–82.

16 Quinn SJ, Thomsen AR, Pang JL, et al. Interactions between calcium and phosphorus in the regulation of the production of fibroblast growth factor 23 in vivo. *Am J Physiol Endocrinol Metab* 2013; **304:** E310–20.

17 Vervloet MG, van Ittersum FJ, Büttler RM, Heijboer AC, Blankenstein MA, ter Wee PM. Effects of dietary phosphate and calcium intake on fibroblast growth factor-23. *Clin J Am Soc Nephrol* 2011; **6:** 383–89.

18 Adema AY, de Borst MH, Ter Wee PM, Vervloet MG, and the NIGRAM Consortium. Dietary and pharmacological modification of fibroblast growth factor-23 in chronic kidney disease. *J Ren Nutr* 2013; published online Nov 9. DOI:10.1053/j.jrn.2013.09.001.

19 Shalhoub V, Shatzen EM, Ward SC, et al. FGF23 neutralization improves chronic kidney disease-associated hyperparathyroidism yet increases mortality. *J Clin Invest* 2012; **122:** 2543–53.

20 Frishberg Y, Ito N, Rinat C, et al. Hyperostosis-hyperphosphatemia syndrome: a congenital disorder of O-glycosylation associated with augmented processing of fibroblast growth factor 23. *J Bone Miner Res* 2007; **22:** 235–42.

21 Gutiérrez OM, Wolf M, Taylor EN. Fibroblast growth factor 23, cardiovascular disease risk factors, and phosphorus intake in the health professionals follow-up study. *Clin J Am Soc Nephrol* 2011; **6:** 2871–78.

22 Isakova T, Wahl P, Vargas GS, et al. Fibroblast growth factor 23 is elevated before parathyroid hormone and phosphate in chronic kidney disease. *Kidney Int* 2011; **79:** 1370–78.

23 Evenepoel P, Meijers BK, de Jonge H, et al. Recovery of hyperphosphatoninism and renal phosphorus wasting one year after successful renal transplantation. *Clin J Am Soc Nephrol* 2008; **3:** 1829–36.

24 Rao M, Jain P, Ojo T, Surya G, Balakrishnan V. Fibroblast growth factor and mineral metabolism parameters among prevalent kidney transplant patients. *Int J Nephrol* 2012; **2012:** 490623.

25 Shimada T, Urakawa I, Isakova T, et al. Circulating fibroblast growth factor 23 in patients with end-stage renal disease treated by peritoneal dialysis is intact and biologically active. *J Clin Endocrinol Metab* 2010; **95:** 578–85.

26 Hu MC, Shiizaki K, Kuro-o M, Moe OW. Fibroblast growth factor 23 and Klotho: physiology and pathophysiology of an endocrine network of mineral metabolism. *Annu Rev Physiol* 2013; **75:** 503–33.

27 Pereira RC, Juppner H, Azucena-Serrano CE, Yadin O, Salusky IB, Wesseling-Perry K. Patterns of FGF-23, DMP1, and MEPE expression in patients with chronic kidney disease. *Bone* 2009; **45:** 1161–68.

28 Martin A, Liu S, David V, et al. Bone proteins PHEX and DMP1 regulate fibroblastic growth factor Fgf23 expression in osteocytes through a common pathway involving FGF receptor (FGFR) signaling. *FASEB J* 2011; **25:** 2551–62.

29 Farrow EG, Yu X, Summers LJ, et al. Iron deficiency drives an autosomal dominant hypophosphatemic rickets (ADHR) phenotype in fibroblast growth factor-23 (Fgf23) knock-in mice. *Proc Natl Acad Sci USA* 2011; **108:** E1146–55.

30 Gutiérrez OM, Mannstadt M, Isakova T, et al. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. *N Engl J Med* 2008; **359:** 584–92.

31 Isakova T, Xie H, Yang W, et al, and the Chronic Renal Insufficiency Cohort (CRIC) Study Group. Fibroblast growth factor 23 and risks of mortality and end-stage renal disease in patients with chronic kidney disease. *JAMA* 2011; **305:** 2432–39.

32 Baia LC, Humalda JK, Vervloet MG, Navis G, Bakker SJ, de Borst MH, and the NIGRAM Consortium. Fibroblast growth factor 23 and cardiovascular mortality after kidney transplantation. *Clin J Am Soc Nephrol* 2013; **8:** 1968–78.

33 Jovanovich A, Ix JH, Gottdiener J, et al. Fibroblast growth factor 23, left ventricular mass, and left ventricular hypertrophy in community-dwelling older adults. *Atherosclerosis* 2013; **231:** 114–19.

34 Faul C, Amaral AP, Oskouei B, et al. FGF23 induces left ventricular hypertrophy. *J Clin Invest* 2011; **121:** 4393–408.

35 Scialla JJ, Lau WL, Reilly MP, et al, and the Chronic Renal Insufficiency Cohort Study Investigators. Fibroblast growth factor 23 is not associated with and does not induce arterial calcification. *Kidney Int* 2013; **83:** 1159–68.

36 Nakayama M, Kaizu Y, Nagata M, et al. Fibroblast growth factor 23 is associated with carotid artery calcification in chronic kidney disease patients not undergoing dialysis: a cross-sectional study. *BMC Nephrol* 2013; **14:** 22.

37 Schoppet M, Hofbauer LC, Brinskelle-Schmal N, et al. Serum level of the phosphaturic factor FGF23 is associated with abdominal aortic calcification in men: the STRAMBO study. *J Clin Endocrinol Metab* 2012; **97:** E575–83.

38 Desjardins L, Liabeuf S, Renard C, et al, and the European Uremic Toxin (EUTox) Work Group. FGF23 is independently associated with vascular calcification but not bone mineral density in patients at various CKD stages. *Osteoporos Int* 2012; **23:** 2017–25.

39 Moldovan D, Moldovan I, Rusu C, Kacso I, Patiu IM, Gherman-Caprioara M. FGF-23, vascular calcification, and cardiovascular diseases in chronic hemodialysis patients. *Int Urol Nephrol* 2014; **46:** 121–28.

40 Lim K, Lu TS, Molostvov G, et al. Vascular Klotho deficiency potentiates the development of human artery calcification and mediates resistance to fibroblast growth factor 23. *Circulation* 2012; **125:** 2243–55.

41 Zhu D, Mackenzie NC, Millan JL, Farquharson C, MacRae VE. A protective role for FGF-23 in local defence against disrupted arterial wall integrity? *Mol Cell Endocrinol* 2013; **372:** 1–11.

42 Lindberg K, Olauson H, Amin R, et al. Arterial klotho expression and FGF23 effects on vascular calcification and function. *PLoS One* 2013; **8:** e60658.

43 Ärnlöv J, Carlsson AC, Sundström J, et al. Serum FGF23 and risk of cardiovascular events in relation to mineral metabolism and cardiovascular pathology. *Clin J Am Soc Nephrol* 2013; **8:** 781–86.

44 Yilmaz MI, Sonmez A, Saglam M, et al. Comparison of calcium acetate and sevelamer on vascular function and fibroblast growth factor 23 in CKD patients: a randomized clinical trial. *Am J Kidney Dis* 2012; **59:** 177–85.

45 Kühl M, Sheldahl LC, Park M, Miller JR, Moon RT. The Wnt/Ca^{2+} pathway: a new vertebrate Wnt signaling pathway takes shape. *Trends Genet* 2000; **16:** 279–83.

46 Monroe DG, McGee-Lawrence ME, Oursler MJ, Westendorf JJ. Update on Wnt signaling in bone cell biology and bone disease. *Gene* 2012; **492:** 1–18.

47 Marinou K, Christodoulides C, Antoniades C, Koutsilieris M. Wnt signaling in cardiovascular physiology. *Trends Endocrinol Metab* 2012; **23:** 628–36.

48 Ke HZ, Richards WG, Li X, Ominsky MS. Sclerostin and dickkopf-1 as therapeutic targets in bone diseases. *Endocr Rev* 2012; **33:** 747–83.

49 Stevens KK, Beattie EC, Delles C, Jardine AG. Phosphate impairs endothelial function—a mechanism for increased cardiovascular risk? ERA-EDTA 50th Congress; Istanbul, Turkey; May 18–21, 2013. SO-018.

50 Cejka D, Herberth J, Branscum AJ, et al. Sclerostin and Dickkopf-1 in renal osteodystrophy. *Clin J Am Soc Nephrol* 2011; **6:** 877–82.

51 Brandenburg VM, Kramann R, Koos R, et al. Relationship between sclerostin and cardiovascular calcification in hemodialysis patients: a cross-sectional study. *BMC Nephrol* 2013; **14:** 219.

52 Gifre L, Ruiz-Gaspà S, Monegal A, et al. Effect of glucocorticoid treatment on Wnt signalling antagonists (sclerostin and Dkk-1) and their relationship with bone turnover. *Bone* 2013; **57:** 272–76.

53 Mukhopadhyay M, Shtrom S, Rodriguez-Esteban C, et al. Dickkopf1 is required for embryonic head induction and limb morphogenesis in the mouse. *Dev Cell* 2001; **1:** 423–34.

54 Poole KE, van Bezooijen RL, Loveridge N, et al. Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. *FASEB J* 2005; **19:** 1842–44.

55 Kamiya N. The role of BMPs in bone anabolism and their potential targets SOST and DKK1. *Curr Mol Pharmacol* 2012; **5:** 153–63.

56 Krause C, Korchynskyi O, de Rooij K, et al. Distinct modes of inhibition by sclerostin on bone morphogenetic protein and Wnt signaling pathways. *J Biol Chem* 2010; **285:** 41614–26.

57 Boyden LM, Mao J, Belsky J, et al. High bone density due to a mutation in LDL-receptor-related protein 5. *N Engl J Med* 2002; **346:** 1513–21.

58 Babij P, Zhao W, Small C, et al. High bone mass in mice expressing a mutant *LRP5* gene. *J Bone Miner Res* 2003; **18:** 960–74.

59 Balemans W, Patel N, Ebeling M, et al. Identification of a 52 kb deletion downstream of the *SOST* gene in patients with van Buchem disease. *J Med Genet* 2002; **39:** 91–97.

60 Morvan F, Boulukos K, Clément-Lacroix P, et al. Deletion of a single allele of the *Dkk1* gene leads to an increase in bone formation and bone mass. *J Bone Miner Res* 2006; **21:** 934–45.

61 Winkler DG, Sutherland MK, Geoghegan JC, et al. Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. *EMBO J* 2003; **22:** 6267–76.

62 Yao GQ, Wu JJ, Troiano N, Insogna K. Targeted overexpression of Dkk1 in osteoblasts reduces bone mass but does not impair the anabolic response to intermittent PTH treatment in mice. *J Bone Miner Metab* 2011; **29:** 141–48.

63 Kramer I, Loots GG, Studer A, Keller H, Kneissel M. Parathyroid hormone (PTH)-induced bone gain is blunted in SOST overexpressing and deficient mice. *J Bone Miner Res* 2010; **25:** 178–89.

64 Thambiah S, Roplekar R, Manghat P, et al. Circulating sclerostin and dickkopf-1 (DKK1) in predialysis chronic kidney disease (CKD): relationship with bone density and arterial stiffness. *Calcif Tissue Int* 2012; **90:** 473–80.

65 Sabbagh Y, Graciolli FG, O'Brien S, et al. Repression of osteocyte Wnt/β-catenin signaling is an early event in the progression of renal osteodystrophy. *J Bone Miner Res* 2012; **27:** 1757–72.

66 Al-Chaqmaqchi HA, Moshfegh A, Dadfar E, et al. Activation of Wnt/β-catenin pathway in monocytes derived from chronic kidney disease patients. *PLoS One* 2013; **8:** e68937.

67 Mani A, Radhakrishnan J, Wang H, et al. *LRP6* mutation in a family with early coronary disease and metabolic risk factors. *Science* 2007; **315:** 1278–82.

68 Mill C, George SJ. Wnt signalling in smooth muscle cells and its role in cardiovascular disorders. *Cardiovasc Res* 2012; **95:** 233–40.

69 Zhu D, Mackenzie NC, Millán JL, Farquharson C, MacRae VE. The appearance and modulation of osteocyte marker expression during calcification of vascular smooth muscle cells. *PLoS One* 2011; **6:** e19595.

70 Kramann R, Brandenburg VM, Schurgers LJ, et al. Novel insights into osteogenesis and matrix remodelling associated with calcific uraemic arteriolopathy. *Nephrol Dial Transplant* 2013; **28:** 856–68.

71 Cannata-Andía JB, Rodriguez García M, Gómez Alonso C. Osteoporosis and adynamic bone in chronic kidney disease. *J Nephrol* 2013; **26:** 73–80.

72 McClung MR, Grauer A, Boonen S, et al. Romosozumab in postmenopausal women with low bone mineral density. *N Engl J Med* 2014; **370:** 412–20.

73 Wellendorph P, Bräuner-Osborne H. Molecular cloning, expression, and sequence analysis of GPRC6A, a novel family C G-protein-coupled receptor. *Gene* 2004; **335:** 37–46.

74 Neve A, Corrado A, Cantatore FP. Osteocalcin: skeletal and extra-skeletal effects. *J Cell Physiol* 2013; **228:** 1149–53.

75 Gundberg CM, Nieman SD, Abrams S, Rosen H. Vitamin K status and bone health: an analysis of methods for determination of undercarboxylated osteocalcin. *J Clin Endocrinol Metab* 1998; **83:** 3258–66.

76 Coen G, Mazzaferro S, Bonucci E, et al. Bone GLA protein in predialysis chronic renal failure. Effects of 1,25(OH)2D3 administration in a long-term follow-up. *Kidney Int* 1985; **28:** 783–90.

77 Malluche HH, Faugere MC, Fanti P, Price PA. Plasma levels of bone Gla-protein reflect bone formation in patients on chronic maintenance dialysis. *Kidney Int* 1984; **26:** 869–74.

78 Lee NK, Sowa H, Hinoi E, et al. Endocrine regulation of energy metabolism by the skeleton. *Cell* 2007; **130:** 456–69.

79 Booth SL, Centi A, Smith SR, Gundberg C. The role of osteocalcin in human glucose metabolism: marker or mediator? *Nat Rev Endocrinol* 2013; **9:** 43–55.

80 Sokoll LJ, Booth SL, O'Brien ME, Davidson KW, Tsaioun KI, Sadowski JA. Changes in serum osteocalcin, plasma phylloquinone, and urinary γ-carboxyglutamic acid in response to altered intakes of dietary phylloquinone in human subjects. *Am J Clin Nutr* 1997; **65:** 779–84.

81 Confavreux CB, Borel O, Lee F, et al. Osteoid osteoma is an osteocalcinoma affecting glucose metabolism. *Osteoporos Int* 2012; **23:** 1645–50.

82 Schafer AL, Sellmeyer DE, Schwartz AV, et al. Change in undercarboxylated osteocalcin is associated with changes in body weight, fat mass, and adiponectin: parathyroid hormone (1-84) or alendronate therapy in postmenopausal women with osteoporosis (the PaTH study). *J Clin Endocrinol Metab* 2011; **96:** E1982–89.

83 Schwartz AV, Schafer AL, Grey A, et al. Effects of antiresorptive therapies on glucose metabolism: results from the FIT, HORIZON-PFT, and FREEDOM trials. *J Bone Miner Res* 2013; **28:** 1348–54.

84 Holden RM, Morton AR, Garland JS, Pavlov A, Day AG, Booth SL. Vitamins K and D status in stages 3–5 chronic kidney disease. *Clin J Am Soc Nephrol* 2010; **5:** 590–97.

85 McCabe KM, Booth SL, Fu X, et al. Dietary vitamin K and therapeutic warfarin alter the susceptibility to vascular calcification in experimental chronic kidney disease. *Kidney Int* 2013; **83:** 835–44.

86 Okuno S, Ishimura E, Tsuboniwa N, et al. Significant inverse relationship between serum undercarboxylated osteocalcin and glycemic control in maintenance hemodialysis patients. *Osteoporos Int* 2013; **24:** 605–12.

87 Bacchetta J, Boutroy S, Guebre-Egziabher F, et al. The relationship between adipokines, osteocalcin and bone quality in chronic kidney disease. *Nephrol Dial Transplant* 2009; **24:** 3120–25.

88 Westenfeld R, Krueger T, Schlieper G, et al. Effect of vitamin K2 supplementation on functional vitamin K deficiency in hemodialysis patients: a randomized trial. *Am J Kidney Dis* 2012; **59:** 186–95.

89 Urist MR. Bone: formation by autoinduction. *Science* 1965; **150:** 893–99.

90 Sampath TK, Muthukumaran N, Reddi AH. Isolation of osteogenin, an extracellular matrix-associated, bone-inductive protein, by heparin affinity chromatography. *Proc Natl Acad Sci USA* 1987; **84:** 7109–13.

91 Wang EA, Rosen V, Cordes P, et al. Purification and characterization of other distinct bone-inducing factors. *Proc Natl Acad Sci USA* 1988; **85:** 9484–88.

92 Hogan BL. Bone morphogenetic proteins: multifunctional regulators of vertebrate development. *Genes Dev* 1996; **10:** 1580–94.

93 Rennenberg RJ, Schurgers LJ, Kroon AA, Stehouwer CD. Arterial calcifications. *J Cell Mol Med* 2010; **14:** 2203–10.

94 Kim M, Choe S. BMPs and their clinical potentials. *BMB Rep* 2011; **44:** 619–34.

95 Kingsley DM. The TGF-β superfamily: new members, new receptors, and new genetic tests of function in different organisms. *Genes Dev* 1994; **8:** 133–46.

96 Heldin CH, Miyazono K, ten Dijke P. TGF-β signalling from cell membrane to nucleus through SMAD proteins. *Nature* 1997; **390:** 465–71.

97 Miyazono K. Signal transduction by bone morphogenetic protein receptors: functional roles of Smad proteins. *Bone* 1999; **25:** 91–93.

98 Hruska KA, Mathew S, Saab G. Bone morphogenetic proteins in vascular calcification. *Circ Res* 2005; **97:** 105–14.

99 Shao JS, Cai J, Towler DA. Molecular mechanisms of vascular calcification: lessons learned from the aorta. *Arterioscler Thromb Vasc Biol* 2006; **26:** 1423–30.

100 Li X, Yang HY, Giachelli CM. BMP-2 promotes phosphate uptake, phenotypic modulation, and calcification of human vascular smooth muscle cells. *Atherosclerosis* 2008; **199:** 271–77.

101 Parhami F, Morrow AD, Balucan J, et al. Lipid oxidation products have opposite effects on calcifying vascular cell and bone cell differentiation. A possible explanation for the paradox of arterial calcification in osteoporotic patients. *Arterioscler Thromb Vasc Biol* 1997; **17:** 680–87.

102 Fukui N, Zhu Y, Maloney WJ, Clohisy J, Sandell LJ. Stimulation of BMP-2 expression by pro-inflammatory cytokines IL-1 and TNF-α in normal and osteoarthritic chondrocytes. *J Bone Joint Surg Am* 2003; **85-A** (suppl 3)**:** 59–66.

103 Zebboudj AF, Shin V, Boström K. Matrix GLA protein and BMP-2 regulate osteoinduction in calcifying vascular cells. *J Cell Biochem* 2003; **90:** 756–65.

104 Boström K, Tsao D, Shen S, Wang Y, Demer LL. Matrix GLA protein modulates differentiation induced by bone morphogenetic protein-2 in C3H10T1/2 cells. *J Biol Chem* 2001; **276:** 14044–52.

105 Galvin KM, Donovan MJ, Lynch CA, et al. A role for smad6 in development and homeostasis of the cardiovascular system. *Nat Genet* 2000; **24:** 171–74.

106 Dalfino G, Simone S, Porreca S, et al. Bone morphogenetic protein-2 may represent the molecular link between oxidative stress and vascular stiffness in chronic kidney disease. *Atherosclerosis* 2010; **211:** 418–23.

107 Vukicevic S, Basic V, Rogic D, et al. Osteogenic protein-1 (bone morphogenetic protein-7) reduces severity of injury after ischemic acute renal failure in rat. *J Clin Invest* 1998; **102:** 202–14.

108 Hruska KA, Guo G, Wozniak M, et al. Osteogenic protein-1 prevents renal fibrogenesis associated with ureteral obstruction. *Am J Physiol Renal Physiol* 2000; **279:** F130–43.

109 Wang S, Chen Q, Simon TC, et al. Bone morphogenic protein-7 (BMP-7), a novel therapy for diabetic nephropathy. *Kidney Int* 2003; **63:** 2037–49.

110 Zeisberg M, Hanai J, Sugimoto H, et al. BMP-7 counteracts TGF-β1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. *Nat Med* 2003; **9:** 964–68.

111 Mathew S, Davies M, Lund R, Saab G, Hruska KA. Function and effect of bone morphogenetic protein-7 in kidney bone and the bone-vascular links in chronic kidney disease. *Eur J Clin Invest* 2006; **36** (suppl 2)**:** 43–50.

112 Davies MR, Lund RJ, Hruska KA. BMP-7 is an efficacious treatment of vascular calcification in a murine model of atherosclerosis and chronic renal failure. *J Am Soc Nephrol* 2003; **14:** 1559–67.

113 Davies MR, Lund RJ, Mathew S, Hruska KA. Low turnover osteodystrophy and vascular calcification are amenable to skeletal anabolism in an animal model of chronic kidney disease and the metabolic syndrome. *J Am Soc Nephrol* 2005; **16:** 917–28.

114 Tossidou I, Schiffer M. TGF-β/BMP pathways and the podocyte. *Semin Nephrol* 2012; **32:** 368–76.

115 Smith ER, Ford ML, Tomlinson LA, Rajkumar C, McMahon LP, Holt SG. Phosphorylated fetuin-A-containing calciprotein particles are associated with aortic stiffness and a procalcific milieu in patients with pre-dialysis CKD. *Nephrol Dial Transplant* 2012; **27:** 1957–66.

116 Eckardt KU, Coresh J, Devuyst O, et al. Evolving importance of kidney disease: from subspecialty to global health burden. *Lancet* 2013; **382:** 158–69.

117 Crepaldi G, Maggi S. Epidemiologic link between osteoporosis and cardiovascular disease. *J Endocrinol Invest* 2009; **32** (suppl)**:** 2–5.

118 Lampropoulos CE, Papaioannou I, D'Cruz DP. Osteoporosis—a risk factor for cardiovascular disease? *Nat Rev Rheumatol* 2012; **8:** 587–98.

119 den Uyl D, Nurmohamed MT, van Tuyl LH, Raterman HG, Lems WF. (Sub)clinical cardiovascular disease is associated with increased bone loss and fracture risk; a systematic review of the association between cardiovascular disease and osteoporosis. *Arthritis Res Ther* 2011; **13:** R5.

120 Thompson B, Towler DA. Arterial calcification and bone physiology: role of the bone-vascular axis. *Nat Rev Endocrinol* 2012; **8:** 529–43.

121 Fadini GP, Rattazzi M, Matsumoto T, Asahara T, Khosla S. Emerging role of circulating calcifying cells in the bone-vascular axis. *Circulation* 2012; **125:** 2772–81.

122 Wolf M. Update on fibroblast growth factor 23 in chronic kidney disease. *Kidney Int* 2012; **82:** 737–47.