Regression of vascular calcification in chronic kidney disease – feasible or fantasy? A review of the clinical evidence

Oscar Leonard,¹ Jonas Spaak² & David Goldsmith¹

¹Guy's Hospital, King's Health Partners, London, UK and ²Department of Clinical Sciences, Danderyd University Hospital, Karolinska Institutet, Stockholm, Sweden

Correspondence

Dr David Goldsmith, Guy's Hospital, King's Health Partners, London SE1 9RT, UK.
Tel.: +44 020 7188 7188
Fax: +44 020 7188 5646
E-mail: david.goldsmith@gstt.nhs.uk

Keywords

cardiovascular disease, chronic kidney disease, vascular calcification

Received

7 June 2012

Accepted

23 October 2012

Accepted Article

Published Online

30 October 2012

The complex relationships between cardiovascular, renal, and bone disease are increasingly recognized but not yet clearly understood. Vascular calcification (VC) represents a common end point between these interlinked systems. It is highly prevalent in chronic kidney disease (CKD) and may be responsible for some of the excess cardiovascular events seen in this condition. There is much interest in developing therapeutic agents to stop its development or reverse its progression. Traditionally considered to be due to abnormalities in calcium and phosphate metabolism alone, VC is now known to be the product of active, dynamic processes within the vessel wall. Primary prevention of VC is possible through successful prevention or reversal of progressive renal dysfunction, hypertension and hyperlipidaemia, but is challenging given the increasing global prevalence of these risk factors. Secondary prevention of VC through tight control of calcium and phosphate, can be achieved by dietary or pharmacological means. Both the modification of haemodialysis duration or methods and the use of renal transplantation have an effect. Novel drugs such as cinacalcet were hoped to halt calcification but results have been mixed, and no intervention has yet been shown to reverse calcification reliably. A new range of experimental targets involved in the putative mediatory pathways between bone and vascular disease has emerged. Aiming to manipulate the active mechanisms involved in calcium deposition, these hold hope for reversal of calcification, but are still theoretical or in early animal or human experimentation.

Introduction

Cardiovascular diseases (CVD) are the leading cause of death globally [1]. Hyperlipidaemia, smoking, hypertension and diabetes are well established as the most powerful modifiable risk factors for CVD. In recent years renal dysfunction (chronic kidney disease (CKD)) has emerged as a factor of equal importance [2–5]. The prevalence of CKD, defined by reduced glomerular filtration rate (GFR) or albuminuria, is in the range of 10–13% in the general population [6], with an incidence and prevalence that is increasing at an alarming rate [6]. More than 80% of all cardiovascular deaths can be delayed using changes in life style and commonly prescribed drugs [7]. Despite this, many patients never receive the full benefits from these interventions because of frequent under-diagnosis, late presentation and consequent under-treatment [8, 9]. This is particularly an issue for subjects with CKD.

Cardiovascular disease in chronic kidney disease

Patients with mild to moderate chronic kidney disease are much more likely to develop CVD than to die from end stage renal failure (ESRF) [10]. The detrimental cardiovascular effects of declining renal function have been well described, for instance by Go *et al.* [11], who reported on the association between estimated GFR (eGFR) and the risks of death, cardiovascular events and hospitalization over 2.8 years. The adjusted hazard ratio for death was 1.2 with an eGFR of 45–59 ml min⁻¹ m⁻², 1.8 with eGFR of 30–44 ml min⁻¹ m⁻², 3.2 with eGFR of 15–29 ml min⁻¹ m⁻² and 5.9 with eGFR of <15 ml min⁻¹ m⁻² [11].

In acute coronary syndromes, the single most common cause of death worldwide [1], approximately 40% of the patients have at least moderate kidney dysfunction with an eGFR below 60 ml min⁻¹ 1.73 m⁻² [12]. The 1 year

mortality among these patients is about 25%, compared with 5% in patients with normal renal function [12]. The increased mortality in CKD patients after an acute coronary event is directly related to decreasing renal function, including very mild impairment [2, 13]. This is most likely caused by the range of vascular abnormalities that occur in CKD, for example in abnormal haemostasis and vascular function, but may in part be due to the fact that patients with renal impairment less often receive active treatment, such as early revascularization [14]. There is also a lack of specific guidelines, attributable in part to limited clinical data, as the majority of randomized trials in acute coronary syndromes so far have excluded CKD patients [15].

The cardio-renal concept

The frequent association seen clinically between cardiac and renal disease is sometimes referred to as the cardio-renal syndrome [16]. Conceptually it is most commonly thought of as the link between systolic heart failure and progressive renal dysfunction. However, the cardio-renal syndrome includes a multitude of pathogenic pathways including glucose metabolism, anaemia, inflammation, coagulation, mineral metabolism, sympathetic activation, renin-angiotensin-aldosterone system (RAAS) activation, endothelial dysfunction and lipid disturbances [17]. It is important to recognize that while systolic heart failure directly affects renal function, CKD reciprocally affects cardiac structure and function. Each condition may limit the therapeutic options available to treat the other, provoking debate between the nephrologist and cardiologist over diuretic and RAAS blocker dose in the context of 'heart failure' versus 'kidney failure'.

Vascular disease is a common aetiological factor mediating the cardio-renal syndrome [18]. It progresses from lipid deposition in the vascular wall in early life, through to endothelial dysfunction, hypertension, inflammatory activation, atheroma formation and vascular calcification (VC) in adulthood [19]. In some individuals it causes ischaemic heart disease and heart failure, while in others it predominantly affects the renal arterioles and glomeruli, causing renal dysfunction. Once prevalent in one organ, the disease hastens the development of vascular disease in other [16, 17] partly explaining why kidney disease, even at the very earliest and otherwise asymptomatic stages, is a strong independent risk factors for the development of CVD [20].

Calcification in cardiovascular disease and chronic kidney disease

In the general population VC occurs most commonly in the context of advanced atherosclerosis where microcalcification forms within the vascular smooth muscle cells (VSMCs) of the tunica intima. This process is driven by the

inflammatory and apoptotic processes of atherosclerosis [21] and results in vessel narrowing and dysfunction, increasing the likelihood of a thrombotic event.

Calcification also occurs within the tunica media in association with ageing, usually termed Monckeberg's sclerosis. The exact aetiology is unknown, however this process occurs decades earlier in the CKD population. It was previously thought to be caused by abnormalities in calcium and phosphate concentrations alone [22], but the role of active processes that transform VSMCs to an osteoblastic phenotype is now recognized [23]. Ultrastructural analysis has suggested that the microcalcifications may originate from nanocrystals and they often exhibit a 'core-shell' structure, suggesting that apoptotic cells also play an initiating role [24].

Medial calcification increases arterial stiffness [25] more than other forms of VC, promoting left ventricular hypertrophy (LVH) and fatal arrhythmias [26]. In CKD, medial calcification develops concurrently with classical atherosclerosis [27], which is itself accelerated [28]. The synergistic effect is an excess in cardiovascular mortality, particularly sudden cardiac death [26].

VC is detectable using several methods including plain radiographs of the chest and abdomen [29, 30], while valvular calcification can best be visualized using echocardiography. VC predicts death regardless of the site measured, modality used to visualize it or whether it is measured in CKD patients or the general population [25, 27, 29, 31, 32].

The gold standard non-invasive measure is currently coronary computed tomography (CT) scanning, most commonly multi-detector CT, giving the coronary artery calcium (CAC) score in Agatston units. Calcium scoring is primarily used for risk stratification in asymptomatic patients, while contrast-enhanced CT is primarily used in patients with acute or chronic chest pain. Neither technique can reliably distinguish between intimal or medial calcification. Despite this caveat a high CAC score predicts cardiovascular events in CKD [33], and in the general population where it moderately improves the accuracy of traditional risk factor based risk prediction, such as the Framingham risk score (FRS) [34]. Recent US guidelines for asymptomatic patients advise that a single measurement is reasonable to risk stratify further intermediate FRS individuals [35]. A recent review cautioned against the use of serial measurements due to the relatively high radiation dose (8–18 mSv) [36]. The role of CAC measurement in CKD has not yet been defined and there have been no randomized controlled trials incorporating CAC in risk prediction algorithms in either population.

Reciprocity between calcium in the skeleton, vessels and valves

Ectopic calcification in CKD develops concurrently in the vessels and heart valves [30] and occurs in the context of a

syndrome of metabolic abnormalities. There is a complex relationship between the bone and the vascular systems, in which both parallel and reciprocal changes in mineralization have been observed, the so-called bone-vascular axis [22]. Though this interaction is poorly understood and causal links have not yet been established, CKD is thought to induce a state of low bone turnover, reducing the bone's ability to buffer mineral metabolite (calcium and phosphate) change and promoting VC [37]. Empirically, bone mineral density is inversely associated with CAC score [38] and low bone turnover is associated with CAC progression in haemodialysis patients [39]. Over and above the links between calcium deposition in the vessels and bone there is also a more complex relationship between bone and cardiovascular health which is not well defined. This is illustrated by the fact that left ventricular mass in CKD patients is independently associated with not only aortic calcification, but also lower mean femoral bone mineral density [40].

Several factors unique to renal failure exacerbate VC and are potential therapeutic targets. These factors often co-exist and create what has been referred to as a 'perfect storm' for the development of VC. These are outlined in the Table 1.

Potential therapeutic interventions to prevent, arrest or reverse vascular calcification

Primary prevention

The strong relationship between progressive renal dysfunction and the increased prevalence, severity and progression of VC, means that primary prevention of kidney disease and renal functional loss over time would have a big impact on VC. It would most likely profoundly improve CV survival but, as mentioned, it remains a considerable challenge [41].

Currently the treatment of hypertension and the use of statins are the key interventions in those with CKD, limiting disease progression and reducing cardiovascular events [42–44]. Several large hypertension trials in patients with normal renal function have shown that treatment improves measures arterial stiffness, such as pulse wave velocity [45, 46]. However surprisingly few equivalent studies exist in patients with CKD. Despite this, supportive evidence of efficacy exists from several studies in animal models, where treatment of hypertension reduces vascular calcification [47, 48], and by one recent randomized trial showing that dual RAAS blockade in CKD patients is associated with improved arterial stiffness [49]. In addition optimal treatment of diabetes can slow GFR decline [50], and may help to prevent vessel calcification associated with diabetes. The use of RAAS blockers for albuminuria in diabetes, or proteinuria in non-diabetic disease, is the cornerstone of nephro-protection, and may itself have a beneficial impact on VC [51].

However, given the ageing population and the epidemics of obesity and type II diabetes, there are likely to be many more, not fewer, calcified patients with CKD in the future.

Secondary prevention

The role of CKD mineral bone disease There are CV risks associated with plasma phosphate concentrations in the middle to upper tertiles of the normal range [52]. In both CKD patients and the general population higher plasma phosphate concentrations are associated with the development and progression of CAC, and the development of LVH [52].

Of intense interest presently is whether the vascular toxicity associated with plasma phosphate concentrations is a direct consequence of phosphate acting on VSMCs in the arterial media [53], or a direct effect of the concurrent elevation of fibroblast growth factor-23 (FGF-23) elevation. It is plausible that both plasma phosphate and FGF-23 can be directly detrimental to blood vessel function and structure [52, 54].

This being the case, the focus of prevention shifts to attempts to temper the rise of plasma phosphate with worsening degrees of CKD (i.e. CKD 4–5), and also to attempt to normalize already elevated plasma phosphate concentrations in dialysis patients.

Phosphate control – dietary and oral phosphate binders The mainstay of phosphate control presently is dietary phosphate restriction to about 800–1000 mg phosphate day⁻¹, trying in particular to avoid sources of inorganic phosphate, such as food preservatives and flavourings seen in 'fast-food' meals and soft drinks [55]. These diets can lead to reductions in plasma phosphate and urinary fractional excretion of phosphate but are notoriously hard to persevere with and recidivism is typical [55].

In addition to dietary manipulations there is extensive usage of a range of oral phosphate binding medications [56], which to variable degrees bind phosphate in the gut using calcium, magnesium, aluminium, lanthanum or resin-based salts or compounds. This is a complex area, expertly reviewed recently [56]. There are several studies which support the notion that limitation of excess exogenous calcium, much of it from the calcium-containing oral phosphate binders, can induce relative metabolic and cellular low bone turnover, and thus lead to promotion of ectopic calcium and phosphate deposition in the soft tissues, most prominently in the vasculature. This tendency is worsened by the excessive use of vitamin D compounds, which significantly increase calcium and phosphate absorption from the GI tract [56]. Long term avoidance of a positive calcium and phosphate balance requires closer attention to diet, drugs and dialysis techniques than most patients typically receive currently.

Phosphate control – mode and timing of renal replacement therapy In patients on dialysis treatment

Table 1

The many different influences on vascular calcification, and the relevant biological pathways or pathomechanisms

Parameter	Associations	Possible mechanisms
Phosphate	Higher concentrations associated with: <ul style="list-style-type: none"> • more extensive CAC in CKD patients [96] • death and CV events in CKD patients [97] • death and CV events in the general population [98] • coronary artery occlusion and higher CAC score in normal renal function [99] 	<ul style="list-style-type: none"> • Stimulates VSMC transformation to an osteoblastic phenotype [100] • Stimulates calcification of VSMC extracellular matrix [101] • Promotes of VSMC apoptosis [102] • Inhibits vessel wall macrophage differentiation to osteoclast-like cells, disrupting a potential innate regulatory mechanism [103]
Fibroblast growth factor-23 (FGF-23)	<ul style="list-style-type: none"> • Deficiency associated with VC in animal and human studies • Raised concentrations correlate with CAC in children on haemodialysis [104] and aortic calcification in adults [105] 	<ul style="list-style-type: none"> • Phosphaturic – initially mitigates effect of hyperphosphataemia [106] • Inhibits renal synthesis of 1,25 vitamin D (overall effect unknown) • Possible undefined phosphate-independent inhibitory effect [107] • Raised concentrations in advanced CKD may reflect resistance (e.g. fewer nephrons to respond)
Klotho	A transmembrane protein which acts as an essential co-factor for FGF-23 at its receptor: <ul style="list-style-type: none"> • Knockout mice display a CKD / pro-calcification phenotype • CKD patients have low free urinary klotho [108], and low levels of expression are found in CKD kidneys 	<ul style="list-style-type: none"> • Indirect action via FGF-23 (see above) [109] • In its free form following cleavage of the extracellular domain it has a direct phosphaturic effects on Na-PO₄ channels in the proximal convoluted tubule [108] • Direct effect on VSMCs, suppressing PO₄ entry in to VSMCs [108]
Vitamin D	An evolving picture of sometimes conflicting results: <ul style="list-style-type: none"> • Induces VC in excess, in animal and clinical models [110] • Physiological dose protective in animal CKD model [111] • Deficiency linked to VC, arterial stiffness and CV mortality in CKD [112] • Low and high concentrations associated with VC in children on haemodialysis ('U' shaped relationship) [113] 	<p>A likely mixture of pro-calcific:</p> <ul style="list-style-type: none"> • Direct effect on VSMC • Promoting VC by raising PO₄ and Ca • Over-suppression of PTH leading to adynamic bone disease and low bone turnover <p>And protective:</p> <ul style="list-style-type: none"> • Pleiotropic anti-inflammatory and immunomodulatory effects across the cardiovascular system [113] • Inhibit the production of renin and myocyte proliferation [114] • Prevents hyperparathyroidism and hyperphosphataemia
Matrix GLA-protein (MGP)	<ul style="list-style-type: none"> • Deficiency and under-activated MGP associated with VC in animals [115, 116] 	<p>Precise mechanism not clear but known to:</p> <ul style="list-style-type: none"> • Inhibit bone morphogenetic proteins (BMP) type 2 and 4, thereby blocking induction of VSMC osteoblastic phenotype • Directly bind hydroxyapatite [117]
Warfarin	Commonly prescribed in CKD (e.g. higher incidence of atrial fibrillation [118]): <ul style="list-style-type: none"> • Use results in phenotype similar to that seen in MGP-deficient mice [119] (see above). • Human data link is weaker: a few small, observational studies link use to valvular calcification e.g. [120] • Strongest risk factor for calcific uraemic arteriopathy (calciphylaxis) in haemodialysis patients [121, 122] 	<p>Disrupts vitamin K dependent gamma-carboxylation (activation) of</p> <ul style="list-style-type: none"> • MGP • osteocalcin, a pro-osteoblastic bone protein that is associated with increased bone mineral density
Vitamin K₂	Deficient in dialysis patients [95]. Influenced by warfarin as above. <ul style="list-style-type: none"> • Supplementation reduced VC in animals [123] • Low dietary intake associated with severe aortic valve calcification [124]. • MGP/osteocalcin concentrations improve on supplementation in dialysis patients [95] 	<ul style="list-style-type: none"> • As detailed above, mediated via MGP and osteocalcin
Pyrophosphate	<ul style="list-style-type: none"> • Deficiency in animals and humans leads to massive calcification [125]. • Lower concentrations seen in haemodialysis patients, associated with VC in ESRF [78] • Supplementation inhibited VC in recent animal model [81] 	<ul style="list-style-type: none"> • Directly blocks hydroxyapatite formation within VSMC. • May inhibit nanocrystal formation [24]
Bone morphogenetic protein-7 (BMP-7)	Part of a tumour growth factor beta cytokine family, crucial in development, active in adult life with broadly pro-osteoblastic properties. <p>Down-regulated in renal failure.</p> <ul style="list-style-type: none"> • Supplementation prevents VC in animal CKD model [126] 	<ul style="list-style-type: none"> • Promotes bone formation and skeletal deposition of phosphate [37], ameliorating adynamic bone disease / low bone turnover • Maintains VSMC differentiation and inhibits transformation to an osteoblastic phenotype [127]
Osteoprotegerin (OPG)	Part of the tumour necrosis factor (TNF) superfamily – decoy receptor for receptor activator of nuclear factor kappa B ligand (RANKL) – expressed in endothelial cells, VSMCs and osteoblasts, broadly anti-inflammatory, inhibits osteoclast differentiation. <ul style="list-style-type: none"> • Deficiencies in animal models result in medial VC [128, 129] • Conversely it is positively associated with CAC and cardiovascular events in haemodialysis patients [130] 	<ul style="list-style-type: none"> • Exact pathways of OPG interaction with calcification in the vasculature are unknown, positive association implies OPG may be involved in a compensatory mechanism • RANKL initiates osteoblastic transformation of VSMCs [131] • Inhibits osteoclast formation and activation in bone, preventing resorption • Inhibits pro-apoptotic pathway in VSMCs, reducing the potential for apoptotic initiation of VC [131]
Fetuin-A	An extracellular calcium-regulatory protein which inhibits VC <ul style="list-style-type: none"> • Knockout mice develop extensive VC [132] • CKD patients with lower serum fetuin-A have increased CV mortality [132] • Low fetuin-A in dialysis patients is associated with VC, calcific uraemic arteriopathy and CV mortality [133] 	<ul style="list-style-type: none"> • Inhibits <i>de novo</i> Ca-PO₄ precipitation [134] • Inhibits calcification within VSMCs preventing vesicular-mediated precipitation of Ca-PO₄ [135]
Osteopontin (OPN)	An extracellular phosphoprotein <ul style="list-style-type: none"> • Absent in normal arteries, detectable within the media of calcified vessels in CKD patients [24] • Osteopontin and MGP co-knockout mice develop accelerated calcification vs. MGP knockout mice 	<ul style="list-style-type: none"> • Inhibits hydroxyapatite crystal growth • Promotes osteoclastic function

programmes, the standard 3–4 h dialysis treatment schedules thrice weekly are inefficient at removing plasma phosphate, because of its extensive hydration shell, and the fact that phosphate in the body is mainly intracellular in its location. As plasma phosphate falls through dialysis removal, more intracellular phosphate exits cells to compensate [57].

It is clear that using more sophisticated (haemofiltration, haemodiafiltration) dialysis, or standard dialysis but for 8 h, 6 nights a week, can lead to excellent plasma phosphate control, and to less progression of CAC [57]. However this effect is confounded as better dialysis also corrects a range of other electrolyte, metabolic and hormonal abnormalities associated with CKD, including blood pressure. Sadly this 'gold standard' approach to renal replacement therapy remains the lucky choice for a small minority of contemporary dialysis patients in most countries [58].

Choice of vitamin D analogue In several animal models it has been shown that the choice of synthetic or semi-synthetic vitamin D receptor activator (VDRA) can influence the development of VC. Paricalcitol has been shown in several reports to be less associated with VC in this setting [59]. This may contribute to the survival advantage reported by cross-sectional surveys and registries for patients on paricalcitol as opposed to other VDRA [60], although this remains controversial in the absence of any randomized controlled trial. No differences in VC or survival have ever been shown in man in a suitably sized randomized controlled trial, and many authoritative opinions remain sceptical [61].

Parathyroidectomy and cinacalcet For progressive, biochemically-severe, and often symptomatic secondary hyperparathyroidism, a surgical parathyroidectomy is the treatment of choice. This is particularly true when the usual treatment with vitamin D compounds is ineffective at reducing parathyroid hormone (PTH) concentrations or leads to significant hypercalcaemia or hyperphosphataemia. It has been clearly shown that the high PTH, high calcium, high phosphate milieu associated with advanced secondary hyperparathyroidism is also associated with rapid loss of mineral from bone and reciprocal increases in vessel calcium content. Surgical parathyroidectomy has been shown to arrest VC progression and can lead to dramatic amelioration of calcium deposits in skin, joints and small arteries [62].

Cinacalcet is the first licensed calcium-sensing receptor (CaSR) allosteric activator. Its use has dramatic effects in reducing plasma PTH and calcium concentrations, and a smaller effect in dialysis patients in reducing plasma phosphate concentrations [63]. In animal models these classes of compounds are strikingly successful at preventing or reversing VC. This prompted the ADVANCE trial [64] in which 165 dialysis patients in each of two arms were studied to see the impact of cinacalcet on progressive

coronary artery, aortic and valvular calcification. The overall results were very close to positive (around $P=0.05$) for vessels and valves, and it has been argued that this should be seen as a positive intervention [64, 65]. Recently, the EVOLVE study has reported and this has failed to demonstrate a salutary effect of the use of cinacalcet to reduce PTH concentrations on overall and CV survival rates [66].

Renal transplantation The definitive treatment for renal failure is renal transplantation. It has now been shown very clearly that transplantation does slow down, but does not abolish, established coronary and aortic calcifications in CKD patients. There are however no animal models which have examined calcified animals with advanced CKD who then undergo transplantation. While it is logical to think that the post-transplantation correction of plasma calcium, phosphate, PTH and FGF-23 concentrations would reduce the propensity for VC, there are no animal or clinical data to support this. In the clinical domain Seyahi *et al.* undertook the largest and longest study of post-renal transplantation CAC behaviour, demonstrating that CAC progressed after transplantation [67]. Schanckel *et al.* examined 82 asymptomatic patients and concluded that CAC progression was seen in a quarter of their cohort who was followed-up for just under 2 years [68]. Mazzaferro *et al.* examined 41 renal transplant recipients and a control group composed of 30 dialysis patients. The patients were followed-up for nearly 2 years and progression was observed in 12.2% of the renal transplant recipients [69]. They concluded that renal transplantation slows, but does not halt, CAC progression, and that improvement of CAC is a very rare event. Finally, Bargnoux *et al.* examined CAC progression in 76 patients who were followed-up for 1 year; CAC progressed in 26.3% of their patients and interestingly regressed in 14.5% [70]. Several other factors have been shown in one or more studies to predict post-transplant CAC changes, including lipid concentrations, BP control, osteoprotegerin and phosphate concentrations, and bisphosphonate use.

Manipulating the complex biology of vascular calcification

Sodium thiosulfate

Sodium thiosulfate has recently been suggested as a major improvement in the treatment of the rare but devastating condition calcific uraemic arteriopathy (calciophylaxis) [71, 72]. In this condition the calcification of arterioles and subsequent intimal proliferation leads to luminal obliteration and necrotic skin lesions, with patients commonly dying of sepsis.

Sodium thiosulfate's proposed mechanism is that it greatly enhances the solubility of calcium in deposits and thereby makes it available for haemodialysis clearance. It may also have an antioxidant effect, with enhanced

glutathione generation, nitric oxide and endothelial cell mechanisms. Additionally its metabolite, hydrogen sulphide, might have vasodilatory effects [73]. Sodium thiosulfate is cleared renally and by dialysis, so doses need to be infused after each dialysis (25–50 g intravenously over 1 h). Side effects are limited to a small increase in anion gap because of circulating thiosulfate, as well as reports of anorexia, nausea and vomiting after more rapid high dose infusions.

There is one small study of the use of a prolonged course of sodium thiosulfate targeting CAC in dialysis patients from Korea [74]. Although the findings seemed to support benefit of this intervention, we feel this is urgently in need of being repeated over a longer time period in a bigger more clinically heterogeneous patient cohort. We would not currently recommend its use in this setting outside clinical trials.

Bone morphogenetic protein-7 (animal models)

In a most elegant series of experiments, Hruska's group from St Louis, Missouri, USA showed in two independent and separate studies that renal injury and subsequent CKD directly inhibited skeletal anabolism, and that stimulation of bone formation decreased serum phosphate [37]. Where the serum calcium, phosphate, PTH and calcitriol were maintained normal after renal ablation in mice, and even mild renal injury equivalent to stage 3 CKD, decreased bone formation rates were seen. In low density lipoprotein receptor null (LDLR^{-/-}) mice fed high fat/cholesterol diets, a model of the metabolic syndrome (hypertension, obesity, dyslipidaemia and insulin resistance), the mice have extensive VC, both the intimal and medial. This VC was made worse by CKD and ameliorated by bone morphogenetic protein-7 (BMP-7). These mice also had hyperphosphataemia which responded to BMP-7 treatment. Subsequent examination of their skeletons revealed significant reductions in bone formation rates were associated with high fat feeding, and that the addition of CKD resulted in the low bone turnover or adynamic bone disorder (ABD), while VC was made worse. The effect of CKD in decreasing skeletal anabolism (decreased bone formation rates and reduced number of bone modelling units) occurred despite secondary hyperparathyroidism. The mechanism by which BMP-7 treatment corrected low bone turnover and hyperphosphatemia was thought to be by stimulation of skeletal phosphate deposition, reducing plasma phosphate and thereby removing a major stimulus to VC. This may happen in man [75].

Pyrophosphate

Tissue pyrophosphate release is regulated by three factors. First, the rate-limiting enzyme 'ecto-nucleotide pyrophosphatase phospho-diesterase-1' (ENPP-1), second, the transmembrane transporter 'ANK' encoded by the 'progressive ankylosis' locus and third, the membrane-bound enzyme 'tissue non-specific alkaline phosphatase' (TNAP). Activity

of ENPP-1 determines the production rate of pyrophosphates, and intact ANK secures extracellular availability by specifically transporting pyrophosphates out of the cells. However, TNAP degrades secreted pyrophosphates into single phosphate ions and, thus, an increased enzyme activity would cause local hyperphosphataemia. Both genetic ANK and ENPP-1 deficiencies in mice cause phenotypes of dystrophic calcification. In humans, a loss of function mutation of ENPP-1 has been identified as causing a severe disorder termed idiopathic infantile arterial calcification (IIAC) leading to severe vascular calcification in newborns and small children [76, 77].

A recent report by O'Neill *et al.* concerned the measurement of plasma pyrophosphate concentrations in 115 individuals in CKD stages 4–5 and correlated these concentrations with the degree and change in large artery calcification [78]. This was quantitatively detected at the superficial femoral artery by CT, obtained at baseline and after 1 year. An inverse association was found between baseline calcification score and pyrophosphate concentrations. Also, an inverse, though non-significant association between calcification progression and pyrophosphate concentrations was reported [78]. This paper is of significant relevance because observations like these may pave the way to novel therapeutic pathways and strategies. Taken together, will pyrophosphate measurements have any potential at all to become a relevant risk biomarker for CKD patients in the future? At the moment, there is no definite answer to any of these questions, but this is a potentially exciting interventional pathway [79–81].

Bisphosphonates

Experimental data from animal models and patients on chronic haemodialysis suggest that nitrogen-containing bisphosphonates (NCBPs; e.g. ibandronate, alendronate, risedronate and zoledronate) may limit vascular and valvular calcification [82–84]. Bisphosphonates are primarily used in the management of osteoporosis, binding to hydroxyapatite, preventing osteoclast-mediated bone resorption. NCBPs inhibit farnesylpyrophosphate synthase, an enzyme in the mevalonate pathway further down than HMG-CoA reductase, the site of statin action. Consequently, several pharmacologic effects are common to both NCBPs and statins. NCBPs decrease serum LDL-cholesterol concentrations by approximately 5%, raise HDL-cholesterol by 10–18% [85, 86] and reduce inflammation by inhibiting the secretion of several inflammatory cytokines [87]. However, NCBP inhibition of vascular and valvular calcification may be secondary to prevention of bone resorption and the subsequent release of calcium phosphate from bone. Recent data suggesting that NCBPs and other osteoporosis therapies may slow the progression of aortic stenosis support this hypothesis [88].

There is some suggestion that NCBPs may modulate cardiovascular calcification, including from the recent MESA study. Here NCBPs were associated with decreased

prevalence of cardiovascular calcification in older women, but more prevalent cardiovascular calcification in younger subjects [89].

However, it must be stated very strongly that there are substantial potential risks of bone and other toxicities using NCBPs in CKD which renders this a complex, confusing and challenging area [90]. Most of these compounds are substantially renally-excreted, and thus tend to accumulate significantly in plasma and bone, with prolonged and often excessive skeletal actions. Diagnosis and treatment of osteoporosis, and any impact on associated tendencies to VC, remain challenging and under-researched. Whether the novel anti-resorptive monoclonal antibody denosumab [91], which has no renal excretion or sequelae, or the even newer anti-sclerostin antibodies, can also influence VC is not yet known.

Vitamin K₂ antagonists and warfarin avoidance

It has been suggested that the use of vitamin K antagonists (VKA) in CKD 4–5 and dialysis is associated with tissue calcification (see Table 1). Experimental data suggest that VKA may decrease the activity of matrix-g-carboxyglutamic acid (GLA) protein (MGP), a strong inhibitor of soft tissue calcification [92, 93]. Rennenberg *et al.* [94] showed that the long term use of VKA is associated with enhanced extra-coronary VC also in patients with normal or near-normal kidney function (eGFR > 60 ml min⁻¹). Weijs *et al.* [92] performed a study in which a prospective coronary calcium scan was undertaken in 157 AF patients without significant cardiovascular disease, including 71 (45%) patients who were chronic VKA users. No significant differences in clinical characteristics were found between patients on VKA treatment and non-anticoagulated patients. However, median CAC scores differed significantly between the groups (0 vs. 29 respectively, $P < 0.001$). Mean CAC scores also increased with the duration of VKA use. Multivariable logistic regression analysis revealed that age and VKA treatment were significantly related to increased CAC score.

A large multicentre trial is due to start in which the use of vitamin K₁ supplementation to suppress CKD related over-production of under-carboxylated and under-hydroxylated MGP [95] will be examined. It was originally hoped that vitamin K₂ (menaquinone) could be used in this trial but for regulatory reasons in Germany vitamin K₁ (phyloquinone) will be used (Georg Schlieper, personal communication). It is hoped that the progression of existing CAC in dialysis patients can thus be reduced.

Conclusions

CVD is the chief cause of morbidity and mortality in CKD. VC is common in CKD, worsens with time, presence of diabetes, smoking status and duration of dialysis, and is asso-

ciated with abnormal bone and mineral metabolism parameters, especially plasma phosphate. It is strongly linked with reduced patient survival. There is as yet, however, no consensus on screening for its presence, nor about interventions to retard progression, let alone promote reversal. Many bone-specific or related factors seem associated with its development and progression (see Table 1). It is clear from the most dramatic interventions that normalize phosphate, namely intensive dialysis and renal transplantation, that this cannot regress existing large vessel calcification. This is likely to be because the process of reversal will need to be an active cellular one, involving resorption of calcified lesions in the vessel wall and probably requiring a complex series of pharmacological interventions with agents not yet available. Promoting this process in the vessels, while avoiding simultaneous skeleton demineralization remains a formidable challenge, and is not achievable with the tools currently at our disposal. Thus current clinical practice should focus on prevention and retardation of progression. Interventions such as pyrophosphate, BMP-7, vitamin K₂ and CaSR allosteric activators seem to us to be the most attractive and likely avenues to explore with further well designed randomized controlled trials.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organization for the submitted work. DG has received speaking and advisory honoraria from Amgen, Fresenius, Genzyme and Shire and JS has received speaking and advisory honoraria from MSD, Abbott and Astra-Zeneca. Dr Spaak is coordinator of the Karolinska Cardiorenal Theme Centre, which has received grants from Amgen and Abbott.

Dr Leonard gratefully acknowledges financial support provided by the National Institute of Health Research (NIHR).

REFERENCES

- 1 World Health Organisation, ed. World Health Statistics 2011. Geneva: WHO, 2011.
- 2 Anavekar NS, McMurray JJ, Velazquez EJ, Solomon SD, Kober L, Rouleau JL, White HD, Nordlander R, Maggioni A, Dickstein K, Zelenkofske S, Leimberger JD, Califf RM, Pfeffer MA. Relation between renal dysfunction and cardiovascular outcomes after myocardial infarction. *N Engl J Med* 2004; 351: 1285–95.
- 3 Wright RS, Reeder GS, Herzog CA, Albright RC, Williams BA, Dvorak DL, Miller WL, Murphy JG, Kopecky SL, Jaffe AS. Acute myocardial infarction and renal dysfunction: a high-risk combination. *Ann Intern Med* 2002; 137: 563–70.

- 4 McCullough PA, Soman SS, Shah SS, Smith ST, Marks KR, Yee J, Borzak S. Risks associated with renal dysfunction in patients in the coronary care unit. *J Am Coll Cardiol* 2000; 36: 679–84.
- 5 Santopinto JJ, Fox KA, Goldberg RJ, Budaj A, Pinero G, Avezum A, Gulba D, Esteban J, Gore JM, Johnson J, Gurfinkel EP. Creatinine clearance and adverse hospital outcomes in patients with acute coronary syndromes: findings from the global registry of acute coronary events (GRACE). *Heart* 2003; 89: 1003–8.
- 6 Coresh J, Selvin E, Stevens LA, Manzi J, Kusek JW, Eggers P, Van Lente F, Levey AS. Prevalence of chronic kidney disease in the United States. *JAMA* 2007; 298: 2038–47.
- 7 Emberson JR, Whincup PH, Morris RW, Walker M. Re-assessing the contribution of serum total cholesterol, blood pressure and cigarette smoking to the aetiology of coronary heart disease: impact of regression dilution bias. *Eur Heart J* 2003; 24: 1719–26.
- 8 Li C, Engstrom G, Hedblad B, Berglund G, Janzon L. Blood pressure control and risk of stroke: a population-based prospective cohort study. *Stroke* 2005; 36: 725–30.
- 9 Wolf-Maier K, Cooper RS, Kramer H, Banegas JR, Giampaoli S, Joffres MR, Poulter N, Primatesta P, Stegmayr B, Thamm M. Hypertension treatment and control in five European countries, Canada and the United States. *Hypertension* 2004; 43: 10–7.
- 10 Tonelli M, Wiebe N, Culleton B, House A, Rabbat C, Fok M, McAlister F, Garg AX. Chronic kidney disease and mortality risk: a systematic review. *J Am Soc Nephrol* 2006; 17: 2034–47.
- 11 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.
- 12 Szummer K, Lundman P, Jacobson SH, Lindback J, Stenestrand U, Wallentin L, Jernberg T. Cockcroft-Gault is better than the Modification of Diet in Renal Disease study formula at predicting outcome after a myocardial infarction: data from the Swedish Web-system for Enhancement and Development of Evidence-based care in Heart disease Evaluated According to Recommended Therapies (SWEDEHEART). *Am Heart J* 2010; 159: 979–86.
- 13 Rashidi A, Sehgal AR, Rahman M, O'Connor AS. The case for chronic kidney disease, diabetes mellitus, and myocardial infarction being equivalent risk factors for cardiovascular mortality in patients older than 65 years. *Am J Cardiol* 2008; 102: 1668–73.
- 14 Szummer K, Lundman P, Jacobson SH, Schon S, Lindback J, Stenestrand U, Wallentin L, Jernberg T. Influence of renal function on the effects of early revascularization in non-ST-elevation myocardial infarction: data from the Swedish Web-System for Enhancement and Development of Evidence-Based Care in Heart Disease Evaluated According to Recommended Therapies (SWEDEHEART). *Circulation* 2009; 120: 851–8.
- 15 Tsai TT, Messenger JC, Brennan JM, Patel UD, Dai D, Piana RN, Anstrom KJ, Eisenstein EL, Dokholyan RS, Peterson ED, Douglas PS. Safety and efficacy of drug-eluting stents in older patients with chronic kidney disease: a report from the linked CathPCI Registry-CMS claims database. *J Am Coll Cardiol* 2011; 58: 1859–69.
- 16 Ronco C, Haapio M, House AA, Anavekar N, Bellomo R. Cardiorenal syndrome. *J Am Coll Cardiol* 2008; 52: 1527–39.
- 17 Schiffrin EL, Lipman ML, Mann JFE. Chronic kidney disease. *Circulation* 2007; 116: 85–97.
- 18 Chade AR, Lerman A, Lerman LO. Kidney in early atherosclerosis. *Hypertension* 2005; 45: 1042–9.
- 19 Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. *Circulation* 2004; 109: III-27–III-32.
- 20 Hillege HL, Janssen WM, Bak AA, Diercks GF, Grobbee DE, Crijns HJ, Van Gilst WH, De Zeeuw D, De Jong PE. Microalbuminuria is common, also in a nondiabetic, nonhypertensive population, and an independent indicator of cardiovascular risk factors and cardiovascular morbidity. *J Intern Med* 2001; 249: 519–26.
- 21 Proudfoot D, Shanahan CM. Biology of calcification in vascular cells: intima versus media. *Herz* 2001; 26: 245–51.
- 22 Demer LL, Tintut Y. Vascular calcification: pathobiology of a multifaceted disease. *Circulation* 2008; 117: 2938–48.
- 23 Ketteler M, Rothe H, Kruger T, Biggar PH, Schlieper G. Mechanisms and treatment of extraosseous calcification in chronic kidney disease. *Nat Rev Nephrol* 2011; 7: 509–16.
- 24 Schlieper G, Aretz A, Verberckmoes SC, Kruger T, Behets GJ, Ghadimi R, Weirich TE, Rohrmann D, Langer S, Tordoir JH, Amann K, Westenfeld R, Brandenburg VM, D'Haese PC, Mayer J, Ketteler M, McKee MD, Floege J. Ultrastructural analysis of vascular calcifications in uremia. *J Am Soc Nephrol* 2010; 21: 689–96.
- 25 Sigrist MK, Taal MW, Bungay P, McIntyre CW. Progressive vascular calcification over 2 years is associated with arterial stiffening and increased mortality in patients with stages 4 and 5 chronic kidney disease. *Clin J Am Soc Nephrol* 2007; 2: 1241–8.
- 26 Shamseddin MK, Parfrey PS. Sudden cardiac death in chronic kidney disease: epidemiology and prevention. *Nat Rev Nephrol* 2011; 7: 145–54.
- 27 London GM, Guerin AP, Marchais SJ, Metivier F, Pannier B, Adda H. Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. *Nephrol Dial Transplant* 2003; 18: 1731–40.
- 28 Swaminathan S, Shah SV. Novel inflammatory mechanisms of accelerated atherosclerosis in kidney disease. *Kidney Int* 2011; 80: 453–63.
- 29 Noordzij M, Cranenburg EM, Engelsman LF, Hermans MM, Boeschoten EW, Brandenburg VM, Bos WJ, Kooman JP, Dekker FW, Ketteler M, Schurgers LJ, Krediet RT, Korevaar JC. Progression of aortic calcification is associated with disorders of mineral metabolism and mortality in chronic dialysis patients. *Nephrol Dial Transplant* 2011; 26: 1662–9.
- 30 Bellasi A, Ferramosca E, Muntner P, Ratti C, Wildman RP, Block GA, Raggi P. Correlation of simple imaging tests and

- coronary artery calcium measured by computed tomography in hemodialysis patients. *Kidney Int* 2006; 70: 1623–8.
- 31** Wilson PW, Kauppila LI, O'Donnell CJ, Kiel DP, Hannan M, Polak JM, Cupples LA. Abdominal aortic calcific deposits are an important predictor of vascular morbidity and mortality. *Circulation* 2001; 103: 1529–34.
- 32** Pannier B, Guerin AP, Marchais SJ, Safar ME, London GM. Stiffness of capacitive and conduit arteries: prognostic significance for end-stage renal disease patients. *Hypertension* 2005; 45: 592–6.
- 33** Shantouf RS, Budoff MJ, Ahmadi N, Ghaffari A, Flores F, Gopal A, Noori N, Jing J, Kovesdy CP, Kalantar-Zadeh K. Total and individual coronary artery calcium scores as independent predictors of mortality in hemodialysis patients. *Am J Nephrol* 2010; 31: 419–25.
- 34** Polonsky TS, McClelland RL, Jorgensen NW, Bild DE, Burke GL, Guerci AD, Greenland P. Coronary artery calcium score and risk classification for coronary heart disease prediction. *JAMA* 2010; 303: 1610–6.
- 35** Greenland P, Alpert JS, Beller GA, Benjamin EJ, Budoff MJ, Fayad ZA, Foster E, Hlatky MA, Hodgson JM, Kushner FG, Lauer MS, Shaw LJ, Smith SC Jr, Taylor AJ, Weintraub WS, Wenger NK, Jacobs AK, Anderson JL, Albert N, Buller CE, Creager MA, Ettinger SM, Guyton RA, Halperin JL, Hochman JS, Nishimura R, Ohman EM, Page RL, Stevenson WG, Tarkington LG, Yancy CW. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2010; 56: e50–103.
- 36** McEvoy JW, Blaha MJ, Defilippis AP, Budoff MJ, Nasir K, Blumenthal RS, Jones SR. Coronary artery calcium progression: an important clinical measurement? A review of published reports. *J Am Coll Cardiol* 2010; 56: 1613–22.
- 37** 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.
- 38** Hyder JA, Allison MA, Wong N, Papa A, Lang TF, Sirlin C, Gapstur SM, Ouyang P, Carr JJ, Criqui MH. Association of coronary artery and aortic calcium with lumbar bone density: the MESA Abdominal Aortic Calcium Study. *Am J Epidemiol* 2009; 169: 186–94.
- 39** Barreto DV, Barreto Fde C, Carvalho AB, Cuppari L, Draibe SA, Dalboni MA, Moyses RM, Neves KR, Jorgetti V, Miname M, Santos RD, Canziani ME. Association of changes in bone remodeling and coronary calcification in hemodialysis patients: a prospective study. *Am J Kidney Dis* 2008; 52: 1139–50.
- 40** Chue CD, Wall NA, Crabtree NJ, Zehnder D, Moody WE, Edwards NC, Steeds RP, Townend JN, Ferro CJ. Aortic calcification and femoral bone density are independently associated with left ventricular mass in patients with chronic kidney disease. *PLoS ONE* 2012; 7: e39241.
- 41** Weir MR. Recognizing the link between chronic kidney disease and cardiovascular disease. *Am J Manag Care* 2011; 17 (Suppl 16): S396–402.
- 42** Peralta CA, Norris KC, Li S, Chang TI, Tamura MK, Jolly SE, Bakris G, McCullough PA, Shlipak M. Blood pressure components and end-stage renal disease in persons with chronic kidney disease: the Kidney Early Evaluation Program (KEEP). *Arch Intern Med* 2012; 172: 41–7.
- 43** Baigent C, Landray MJ, Reith C, Emberson J, Wheeler DC, Tomson C, Wanner C, Krane V, Cass A, Craig J, Neal B, Jiang L, Hooi LS, Levin A, Agodoa L, Gaziano M, Kasiske B, Walker R, Massy ZA, Feldt-Rasmussen B, Kraititichai U, Ophascharoensuk V, Fellstrom B, Holdaas H, Tesar V, Wiecek A, Grobbee D, de Zeeuw D, Gronhagen-Riska C, Dasgupta T, Lewis D, Herrington W, Mafham M, Majoni W, Wallendszus K, Grimm R, Pedersen T, Tobert J, Armitage J, Baxter A, Bray C, Chen Y, Chen Z, Hill M, Knott C, Parish S, Simpson D, Sleight P, Young A, Collins R. The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial. *Lancet* 2011; 377: 2181–92.
- 44** Heerspink HJ, Ninomiya T, Zoungas S, de Zeeuw D, Grobbee DE, Jardine MJ, Gallagher M, Roberts MA, Cass A, Neal B, Perkovic V. Effect of lowering blood pressure on cardiovascular events and mortality in patients on dialysis: a systematic review and meta-analysis of randomised controlled trials. *Lancet* 2009; 373: 1009–15.
- 45** Mahmud A, Feely J. Favourable effects on arterial wave reflection and pulse pressure amplification of adding angiotensin II receptor blockade in resistant hypertension. *J Hum Hypertens* 2000; 14: 541–6.
- 46** Anan F, Takahashi N, Ooie T, Yufu K, Hara M, Nakagawa M, Yonemochi H, Saikawa T, Yoshimatsu H. Effects of valsartan and perindopril combination therapy on left ventricular hypertrophy and aortic arterial stiffness in patients with essential hypertension. *Eur J Clin Pharmacol* 2005; 61: 353–9.
- 47** Rahman M, Kim SJ, Kim JS, Kim SZ, Lee YU, Kang HS. Myocardial calcification and hypertension following chronic renal failure and ameliorative effects of furosemide and captopril. *Cardiology* 2010; 116: 194–205.
- 48** Ng K, Hildreth CM, Avolio AP, Phillips JK. Angiotensin-converting enzyme inhibitor limits pulse-wave velocity and aortic calcification in a rat model of cystic renal disease. *Am J Physiol Renal Physiol* 2011; 301: F959–66.
- 49** Frimodt-Moller M, Kamper AL, Strandgaard S, Kreiner S, Nielsen AH. Beneficial effects on arterial stiffness and pulse-wave reflection of combined enalapril and candesartan in chronic kidney disease – a randomized trial. *PLoS ONE* 2012; 7: e41757.
- 50** Shurraw S, Hemmelgarn B, Lin M, Majumdar SR, Klarenbach S, Manns B, Bello A, James M, Turin TC, Tonelli M. Association between glycemic control and adverse outcomes in people with diabetes mellitus and chronic kidney disease: a population-based cohort study. *Arch Intern Med* 2011; 171: 1920–7.
- 51** Armstrong ZB, Boughner DR, Drangova M, Rogers KA. Angiotensin II type 1 receptor blocker inhibits arterial calcification in a pre-clinical model. *Cardiovasc Res* 2011; 90: 165–70.

- 52 Foley RN, Collins AJ, Herzog CA, Ishani A, Kalra PA. Serum phosphorus levels associate with coronary atherosclerosis in young adults. *J Am Soc Nephrol* 2009; 20: 397–404.
- 53 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.
- 54 Faul C, Amaral AP, Oskoue B, Hu MC, Sloan A, Isakova T, Gutierrez OM, Aguillon-Prada R, Lincoln J, Hare JM, Mundel P, Morales A, Scialla J, Fischer M, Soliman EZ, Chen J, Go AS, Rosas SE, Nessel L, Townsend RR, Feldman HI St, John Sutton M, Ojo A, Gadegbeku C, Di Marco GS, Reuter S, Kentrup D, Tiemann K, Brand M, Hill JA, Moe OW, Kuro OM, Kusek JW, Keane MG, Wolf M. FGF23 induces left ventricular hypertrophy. *J Clin Invest* 2011; 121: 4393–408.
- 55 Isakova T, Gutierrez OM, Smith K, Epstein M, Keating LK, Juppner H, Wolf M. Pilot study of dietary phosphorus restriction and phosphorus binders to target fibroblast growth factor 23 in patients with chronic kidney disease. *Nephrol Dial Transplant* 2011; 26: 584–91.
- 56 Hutchison AJ, Smith CP, Brenchley PE. Pharmacology, efficacy and safety of oral phosphate binders. *Nat Rev Nephrol* 2011; 7: 578–89.
- 57 Davenport A. Role of dialysis technology in the removal of uremic toxins. *Hemodial Int* 2011; 15 (Suppl 1): S49–53.
- 58 Raphael KL, Cheung AK. Implications of the frequent hemodialysis network-daily trial. *Semin Dial* 2011; 24: 621–8.
- 59 Guerrero F, Montes de Oca A, Aguilera-Tejero E, Zafra R, Rodriguez M, Lopez I. The effect of vitamin D derivatives on vascular calcification associated with inflammation. *Nephrol Dial Transplant* 2011; 27: 2206–12.
- 60 Teng M, Wolf M, Lowrie E, Ofsthun N, Lazarus JM, Thadhani R. Survival of patients undergoing hemodialysis with paricalcitol or calcitriol therapy. *N Engl J Med* 2003; 349: 446–56.
- 61 Goldsmith DJ, Cunningham J. Mineral metabolism and vitamin D in chronic kidney disease – more questions than answers. *Nat Rev Nephrol* 2011; 7: 341–6.
- 62 Goldsmith DJ, Covic A, Sambrook PA, Ackrill P. Vascular calcification in long-term haemodialysis patients in a single unit: a retrospective analysis. *Nephron* 1997; 77: 37–43.
- 63 Cunningham J, Danese M, Olson K, Klassen P, Chertow GM. Effects of the calcimimetic cinacalcet HCl on cardiovascular disease, fracture, and health-related quality of life in secondary hyperparathyroidism. *Kidney Int* 2005; 68: 1793–800.
- 64 Raggi P, Chertow GM, Torres PU, Csiky B, Naso A, Nossuli K, Moustafa M, Goodman WG, Lopez N, Downey G, Dehmel B, Floege J. The ADVANCE study: a randomized study to evaluate the effects of cinacalcet plus low-dose vitamin D on vascular calcification in patients on hemodialysis. *Nephrol Dial Transplant* 2011; 26: 1327–39.
- 65 Olgaard K, Lewin E, Silver J. Calcimimetics, vitamin D and ADVANCE in the management of CKD-MBD. *Nephrol Dial Transplant* 2011; 26: 1117–9.
- 66 The EVOLVE trial investigators. Effect of cinacalcet on cardiovascular disease in patients undergoing dialysis. *N Engl J Med* 2012; DOI:10.1056/NEJMoa1205624. Epub 3 November 2012.
- 67 Seyahi N, Cebi D, Altiparmak MR, Akman C, Ataman R, Pekmezci S, Serdengeci K. Progression of coronary artery calcification in renal transplant recipients. *Nephrol Dial Transplant* 2011; 27: 2101–7.
- 68 Schanckel K, Robinson J, Bloom RD, Guerra C, Rader D, Joffe M, Rosas SE. Determinants of coronary artery calcification progression in renal transplant recipients. *Am J Transplant* 2007; 7: 2158–64.
- 69 Mazzaferro S, Pasquali M, Taggi F, Baldinelli M, Conte C, Muci ML, Pirozzi N, Carbone I, Francone M, Pugliese F. Progression of coronary artery calcification in renal transplantation and the role of secondary hyperparathyroidism and inflammation. *Clin J Am Soc Nephrol* 2009; 4: 685–90.
- 70 Bargnoux AS, Dupuy AM, Garrigue V, Jaussent I, Gahide G, Badiou S, Szwarc I, Deleuze S, Vernhet H, Cristol JP, Mourad G. Evolution of coronary artery calcifications following kidney transplantation: relationship with osteoprotegerin levels. *Am J Transplant* 2009; 9: 2571–9.
- 71 Hayden MR, Goldsmith DJ. Sodium thiosulfate: new hope for the treatment of calciphylaxis. *Semin Dial* 2010; 23: 258–62.
- 72 Hayden MR, Goldsmith D, Sowers JR, Khanna R. Calciphylaxis: calcific uremic arteriolopathy and the emerging role of sodium thiosulfate. *Int Urol Nephrol* 2008; 40: 443–51.
- 73 Lynn EG, Austin RC. Hydrogen sulfide in the pathogenesis of atherosclerosis and its therapeutic potential. *Expert Rev Clin Pharmacol* 2011; 4: 97–108.
- 74 Adirekkiat S, Sumethkul V, Ingsathit A, Domrongkitchaiporn S, Phakdeekitcharoen B, Kantachavesiri S, Kitiyakara C, Klyprayong P, Disthabanchong S. Sodium thiosulfate delays the progression of coronary artery calcification in haemodialysis patients. *Nephrol Dial Transplant* 2010; 25: 1923–9.
- 75 Freedman BI, Bowden DW, Ziegler JT, Langefeld CD, Lehtinen AB, Rudock ME, Lenchik L, Hruska KA, Register TC, Carr JJ. Bone morphogenetic protein 7 (BMP7) gene polymorphisms are associated with inverse relationships between vascular calcification and BMD: the Diabetes Heart Study. *J Bone Miner Res* 2009; 24: 1719–27.
- 76 Orimo H. The mechanism of mineralization and the role of alkaline phosphatase in health and disease. *J Nippon Med Sch* 2010; 77: 4–12.
- 77 Sage AP, Tintut Y, Demer LL. Regulatory mechanisms in vascular calcification. *Nat Rev Cardiol* 2010; 7: 528–36.
- 78 O'Neill WC, Sigrist MK, McIntyre CW. Plasma pyrophosphate and vascular calcification in chronic kidney disease. *Nephrol Dial Transplant* 2010; 25: 187–91.
- 79 Villa-Bellosta R, Wang X, Millan JL, Dubyak GR, O'Neill WC. Extracellular pyrophosphate metabolism and calcification in vascular smooth muscle. *Am J Physiol Heart Circ Physiol* 2011; 301: H61–8.

- 80** Persy VP, McKee MD. Prevention of vascular calcification: is pyrophosphate therapy a solution? *Kidney Int* 2011; 79: 490–3.
- 81** O'Neill WC, Lomashvili KA, Malluche HH, Faugere MC, Riser BL. Treatment with pyrophosphate inhibits uremic vascular calcification. *Kidney Int* 2011; 79: 512–7.
- 82** Ariyoshi T, Eishi K, Sakamoto I, Matsukuma S, Odate T. Effect of etidronic acid on arterial calcification in dialysis patients. *Clin Drug Investig* 2006; 26: 215–22.
- 83** 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: 817–24.
- 84** Nitta K, Akiba T, Suzuki K, Uchida K, Watanabe R, Majima K, Aoki T, Nihei H. Effects of cyclic intermittent etidronate therapy on coronary artery calcification in patients receiving long-term hemodialysis. *Am J Kidney Dis* 2004; 44: 680–8.
- 85** Adami S, Braga V, Guidi G, Gatti D, Gerardi D, Fracassi E. Chronic intravenous aminobisphosphonate therapy increases high-density lipoprotein cholesterol and decreases low-density lipoprotein cholesterol. *J Bone Miner Res* 2000; 15: 599–604.
- 86** Montagnani A, Gonnelli S, Cepollaro C, Campagna MS, Franci MB, Pacini S, Gennari C. Changes in serum HDL and LDL cholesterol in patients with Paget's bone disease treated with pamidronate. *Bone* 2003; 32: 15–9.
- 87** Corrado A, Santoro N, Cantatore FP. Extra-skeletal effects of bisphosphonates. *Joint Bone Spine* 2007; 74: 32–8.
- 88** Skolnick AH, Osranek M, Formica P, Kronzon I. Osteoporosis treatment and progression of aortic stenosis. *Am J Cardiol* 2009; 104: 122–4.
- 89** Elmariah S, Delaney JA, O'Brien KD, Budoff MJ, Vogel-Claussen J, Fuster V, Kronmal RA, Halperin JL. Bisphosphonate use and prevalence of valvular and vascular calcification in women MESA (The Multi-Ethnic Study of Atherosclerosis). *J Am Coll Cardiol* 2010; 56: 1752–9.
- 90** Toussaint ND, Elder GJ, Kerr PG. Bisphosphonates in chronic kidney disease; balancing potential benefits and adverse effects on bone and soft tissue. *Clin J Am Soc Nephrol* 2009; 4: 221–33.
- 91** Helas S, Goettsch C, Schoppet M, Zeitz U, Hempel U, Morawietz H, Kostenuik PJ, Erben RG, Hofbauer LC. Inhibition of receptor activator of NF-kappaB ligand by denosumab attenuates vascular calcium deposition in mice. *Am J Pathol* 2009; 175: 473–8.
- 92** Weijs B, Blaauw Y, Rennenberg RJ, Schurgers LJ, Timmermans CC, Pison L, Nieuwlaet R, Hofstra L, Kroon AA, Wildberger J, Crijns HJ. Patients using vitamin K antagonists show increased levels of coronary calcification: an observational study in low-risk atrial fibrillation patients. *Eur Heart J* 2011; 32: 2555–62.
- 93** Shea MK, O'Donnell CJ, Vermeer C, Magdeleyns EJ, Crosier MD, Gundberg CM, Ordoas JM, Kritchevsky SB, Booth SL. Circulating uncarboxylated matrix Gla protein is associated with vitamin K nutritional status, but not coronary artery calcium, in older adults. *J Nutr* 2011; 141: 1529–34.
- 94** Rennenberg RJ, de Leeuw PW, Kessels AG, Schurgers LJ, Vermeer C, van Engelshoven JM, Kemerink GJ, Kroon AA. Calcium scores and matrix Gla protein levels: association with vitamin K status. *Eur J Clin Invest* 2010; 40: 344–9.
- 95** Westenfeld R, Krueger T, Schlieper G, Cranenburg EC, Magdeleyns EJ, Heidenreich S, Holzmann S, Vermeer C, Jahnhen-Dechent W, Ketteler M, Floege J, Schurgers LJ. Effect of vitamin k(2) supplementation on functional vitamin K deficiency in hemodialysis patients: a randomized trial. *Am J Kidney Dis* 2012; 59: 186–95.
- 96** Adeney KL, Siscovick DS, Ix JH, Seliger SL, Shlipak MG, Jenny NS, Kestenbaum BR. Association of serum phosphate with vascular and valvular calcification in moderate CKD. *J Am Soc Nephrol* 2009; 20: 381–7.
- 97** Kestenbaum B, Sampson JN, Rudser KD, Patterson DJ, Seliger SL, Young B, Sherrard DJ, Andress DL. Serum phosphate levels and mortality risk among people with chronic kidney disease. *J Am Soc Nephrol* 2005; 16: 520–8.
- 98** Dhirga R, Sullivan LM, Fox CS, Wang TJ, D'Agostino RB Sr, Gaziano JM, Vasan RS. Relations of serum phosphorus and calcium levels to the incidence of cardiovascular disease in the community. *Arch Intern Med* 2007; 167: 879–85.
- 99** Cancela AL, Santos RD, Titan SM, Goldenstein PT, Rochitte CE, Lemos PA, dos Reis LM, Gracioli FG, Jorgetti V, Moyses RM. Phosphorus is associated with coronary artery disease in patients with preserved renal function. *PLoS ONE* 2012; 7: e36883.
- 100** Jono S, McKee MD, Murry CE, Shioi A, Nishizawa Y, Mori K, Morii H, Giachelli CM. Phosphate regulation of vascular smooth muscle cell calcification. *Circ Res* 2000; 87: E10–7.
- 101** Giachelli CM, Speer MY, Li X, Rajachar RM, Yang H. Regulation of vascular calcification: roles of phosphate and osteopontin. *Circ Res* 2005; 96: 717–22.
- 102** Son BK, Kozaki K, Iijima K, Eto M, Kojima T, Ota H, Senda Y, Maemura K, Nakano T, Akishita M, Ouchi Y. Statins protect human aortic smooth muscle cells from inorganic phosphate-induced calcification by restoring Gas6-Axl survival pathway. *Circ Res* 2006; 98: 1024–31.
- 103** Mozar A, Haren N, Chasseraud M, Louvet L, Maziere C, Wattel A, Mentaverri R, Morliere P, Kamel S, Brazier M, Maziere JC, Massy ZA. High extracellular inorganic phosphate concentration inhibits RANK-RANKL signaling in osteoclast-like cells. *J Cell Physiol* 2008; 215: 47–54.
- 104** Srivaths PR, Goldstein SL, Silverstein DM, Krishnamurthy R, Brewer ED. Elevated FGF 23 and phosphorus are associated with coronary calcification in hemodialysis patients. *Pediatr Nephrol* 2011; 26: 945–51.
- 105** Nasrallah MM, El-Shehaby AR, Salem MM, Osman NA, El Sheikh E, Sharaf El Din UA. Fibroblast growth factor-23 (FGF-23) is independently correlated to aortic calcification in haemodialysis patients. *Nephrol Dial Transplant* 2010; 25: 2679–85.

- 106** Shimada T, Kakitani M, Yamazaki Y, Hasegawa H, Takeuchi Y, Fujita T, Fukumoto S, Tomizuka K, Yamashita T. Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. *J Clin Invest* 2004; 113: 561–8.
- 107** Sitara D, Kim S, Razzaque MS, Bergwitz C, Taguchi T, Schuler C, Erben RG, Lanske B. Genetic evidence of serum phosphate-independent functions of FGF-23 on bone. *PLoS Genet* 2008; 4: e1000154.
- 108** Hu MC, Shi M, Zhang J, Quinones H, Griffith C, Kuro-o M, Moe OW. Klotho deficiency causes vascular calcification in chronic kidney disease. *J Am Soc Nephrol* 2011; 22: 124–36.
- 109** Lim K, Lu TS, Molostvov G, Lee C, Lam FT, Zehnder D, Hsiao LL. Vascular Klotho deficiency potentiates the development of human artery calcification and mediates resistance to fibroblast growth factor 23. *Circulation* 2012; 125: 2243–55.
- 110** Mizobuchi M, Ogata H, Koiwa F, Kinugasa E, Akizawa T. Vitamin D and vascular calcification in chronic kidney disease. *Bone* 2009; 45 (Suppl 1): S26–9.
- 111** Mathew S, Lund RJ, Chaudhary LR, Geurs T, Hruska KA. Vitamin D receptor activators can protect against vascular calcification. *J Am Soc Nephrol* 2008; 19: 1509–19.
- 112** Barreto DV, Barreto FC, Liabeuf S, Temmar M, Boitte F, Choukroun G, Fournier A, Massy ZA. Vitamin D affects survival independently of vascular calcification in chronic kidney disease. *Clin J Am Soc Nephrol* 2009; 4: 1128–35.
- 113** Shroff R, Egerton M, Bridel M, Shah V, Donald AE, Cole TJ, Hiorns MP, Deanfield JE, Rees L. A bimodal association of vitamin D levels and vascular disease in children on dialysis. *J Am Soc Nephrol* 2008; 19: 1239–46.
- 114** Levin A, Li YC. Vitamin D and its analogues: do they protect against cardiovascular disease in patients with kidney disease? *Kidney Int* 2005; 68: 1973–81.
- 115** Luo G, Ducky P, McKee MD, Pinero GJ, Loyer E, Behringer RR, Karsenty G. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. *Nature* 1997; 386: 78–81.
- 116** Sweatt A, Sane DC, Hutson SM, Wallin R. Matrix Gla protein (MGP) and bone morphogenetic protein-2 in aortic calcified lesions of aging rats. *J Thromb Haemost* 2003; 1: 178–85.
- 117** Lomashvili KA, Wang X, Wallin R, O'Neill WC. Matrix Gla protein metabolism in vascular smooth muscle and role in uremic vascular calcification. *J Biol Chem* 2011; 286: 28715–22.
- 118** Alonso A, Lopez FL, Matsushita K, Loehr LR, Agarwal SK, Chen LY, Soliman EZ, Astor BC, Coresh J. Chronic kidney disease is associated with the incidence of atrial fibrillation: the Atherosclerosis Risk in Communities (ARIC) study. *Circulation* 2011; 123: 2946–53.
- 119** Price PA, Faus SA, Williamson MK. Warfarin causes rapid calcification of the elastic lamellae in rat arteries and heart valves. *Arterioscler Thromb Vasc Biol* 1998; 18: 1400–7.
- 120** Koos R, Mahnken AH, Muhlenbruch G, Brandenburg V, Pflueger B, Wildberger JE, Kuhl HP. Relation of oral anticoagulation to cardiac valvular and coronary calcium assessed by multislice spiral computed tomography. *Am J Cardiol* 2005; 96: 747–49.
- 121** Coates T, Kirkland GS, Dymock RB, Murphy BF, Brealey JK, Mathew TH, Disney AP. Cutaneous necrosis from calcific uremic arteriolopathy. *Am J Kidney Dis* 1998; 32: 384–91.
- 122** Hayashi M, Takamatsu I, Kanno Y, Yoshida T, Abe T, Sato Y. A case-control study of calciphylaxis in Japanese end-stage renal disease patients. *Nephrol Dial Transplant* 2011; 27: 1580–4.
- 123** Schurgers LJ, Teunissen KJ, Hamulyak K, Knapen MH, Vik H, Vermeer C. Vitamin K-containing dietary supplements: comparison of synthetic vitamin K1 and natto-derived menaquinone-7. *Blood* 2007; 109: 3279–83.
- 124** Geleijnse JM, Vermeer C, Grobbee DE, Schurgers LJ, Knapen MH, van der Meer IM, Hofman A, Witteman JC. Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: the Rotterdam Study. *J Nutr* 2004; 134: 3100–5.
- 125** Lomashvili KA, Cobbs S, Hennigar RA, Hardcastle KI, O'Neill WC. Phosphate-induced vascular calcification: role of pyrophosphate and osteopontin. *J Am Soc Nephrol* 2004; 15: 1392–401.
- 126** 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.
- 127** Lund RJ, Davies MR, Hruska KA. Bone morphogenetic protein-7: an anti-fibrotic morphogenetic protein with therapeutic importance in renal disease. *Curr Opin Nephrol Hypertens* 2002; 11: 31–6.
- 128** Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu W, Lacey DL, Boyle WJ, Simonet WS. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes Dev* 1998; 12: 1260–8.
- 129** Bennett BJ, Scatena M, Kirk EA, Rattazzi M, Varon RM, Averill M, Schwartz SM, Giachelli CM, Rosenfeld ME. Osteoprotegerin inactivation accelerates advanced atherosclerotic lesion progression and calcification in older ApoE^{-/-} mice. *Arterioscler Thromb Vasc Biol* 2006; 26: 2117–24.
- 130** Kurnatowska I, Grzelak P, Kaczmarek M, Stefanczyk L, Nowicki M. Serum osteoprotegerin is a predictor of progression of atherosclerosis and coronary calcification in hemodialysis patients. *Nephron Clin Pract* 2011; 117: c297–304.
- 131** Venuraju SM, Yerramasu A, Corder R, Lahiri A. Osteoprotegerin as a predictor of coronary artery disease and cardiovascular mortality and morbidity. *J Am Coll Cardiol* 2010; 55: 2049–61.
- 132** Ketteler M, Wanner C, Metzger T, Bongartz P, Westenfeld R, Gladziwa U, Schurgers LJ, Vermeer C, Jahnke-Dechent W, Floege J. Deficiencies of calcium-regulatory proteins in dialysis patients: a novel concept of cardiovascular calcification in uremia. *Kidney Int Suppl* 2003; (Suppl. 84): S84–7.

- 133** Rezg R, Barreto FC, Barreto DV, Liabeuf S, Druke TB, Massy ZA. Inhibitors of vascular calcification as potential therapeutic targets. *J Nephrol* 2011; 24: 416–27.
- 134** Heiss A, DuChesne A, Denecke B, Grotzinger J, Yamamoto K, Renne T, Jahnke-Dechent W. Structural basis of calcification inhibition by alpha 2-HS glycoprotein/fetuin-A. Formation of colloidal calciprotein particles. *J Biol Chem* 2003; 278: 13333–41.
- 135** Reynolds JL, Skepper JN, McNair R, Kasama T, Gupta K, Weissberg PL, Jahnke-Dechent W, Shanahan CM. Multifunctional roles for serum protein fetuin-a in inhibition of human vascular smooth muscle cell calcification. *J Am Soc Nephrol* 2005; 16: 2920–30.