Osteoporosis—a risk factor for cardiovascular disease?

Christos E. Lampropoulos, Ioanna Papaioannou and David P. D'Cruz

Abstract | Osteoporosis is a serious health problem worldwide that is associated with an increased risk of fractures and mortality. Vascular calcification is a well-defined independent risk factor for cardiovascular disease (CVD) and mortality. Major advances in our understanding of the pathophysiology of osteoporosis and vascular calcification indicate that these two processes share common pathogenetic mechanisms. Multiple factors including proteins (such as bone morphogenetic proteins, receptor activator of nuclear factor κB ligand, osteoprotegerin, matrix Gla protein and cathepsins), parathyroid hormone, phosphate, oxidized lipids and vitamins D and K are implicated in both bone and vascular metabolism, illustrating the interaction of these two, seemingly unrelated, conditions. Many clinical studies have now confirmed the correlation between osteoporosis and vascular calcification as well as the increased risk of CVD in patients with osteoporosis. Here, we explore the proposed mechanistic similarities between osteoporosis and vascular calcification and present an overview of the clinical data that support the interaction between these conditions.

Lampropoulos, C. E. *et al. Nat. Rev. Rheumatol.* **8**, 587–598 (2012); published online 14 August 2012; doi:10.1038/nrrheum.2012.120

Introduction

Osteoporosis is a serious public health concern with an estimated worldwide incidence of over 200 million.[1] Osteoporosis mainly affects females; 80% of individuals with osteoporosis in the USA are women.[2] Approximately 30% of postmenopausal women in developed countries have osteoporosis and at least 40% of women and 15–30% of men will sustain a fracture; the risk of a further fracture is increased by 50–100%.[1] The worldwide annual incidence of hip fracture is 1.7 million.[3] Women with hip fractures have 10–20% higher mortality than would be expected for their age, and osteoporosis accounts for more days in hospital in women ≥45 years of age than any other disease, including diabetes mellitus, myocardial infarction and breast cancer.[4] By the year 2050, the annual incidence of hip fracture is estimated to increase by 240% in women and 310% in men, to 6.3 million in total, owing to the increase in the elderly population of the world and the increased incidence of falls in these individuals.

Vascular calcification is an independent risk factor for cardiovascular disease (CVD). Calcification of any artery or cardiac valve increases the risk of cardiovascular events and mortality threefold to fourfold and is accepted as a predictor of coronary heart disease (CHD).[5] In coronary arteries, calcium deposits can weaken vasomotor responses and alter the stability of atherosclerotic plaques. Patients with unstable angina or myocardial infarction tend to have lesions comprising multiple small calcium deposits whereas those in patients with stable angina are associated with few large deposits.[6] Coronary artery calcification is readily detected by CT and calcification scores can be used in clinical studies to estimate the risk of future cardiovascular events. Likewise, aortic calcification is an independent predictor of cardiovascular events.[7] Vascular calcification reduces arterial elasticity resulting in substantial morbidity and mortality from hypertension, aortic stenosis, cardiac hypertrophy, myocardial infarction and lower-limb ischaemia. In this Review, we describe the common pathogenetic mechanisms between osteoporosis and vascular calcification, and also discuss clinical data investigating the incidence of vascular calcification and risk of cardiovascular events in patients with osteoporosis.

Pathophysiology of osteoporosis

Osteoporosis is caused by an imbalance between bone formation and resorption. The major cell types responsible for these two processes are osteoblasts and osteoclasts, respectively.

Osteoblastogenesis

Osteoblasts are mononuclear cells that produce osteoid (composed mainly of type I collagen) and are responsible for mineralization of the osteoid matrix. Osteoblasts arise from immature osteoprogenitor cells in the periosteum and bone marrow that express the master regulatory transcription factor RUNX2 (runt-related transcription factor 2, also known as CBF-α1). Osteoprogenitor cells differentiate following stimulation by multiple growth factors including bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs; mainly FGF18), platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β). Following differentiation into

Competing interests
The authors declare no competing interests.

Department of Internal Medicine, General Hospital of Nafplio, Kolokotroni and Asklipiou Streets, 21100 Nafplio, Greece (**C. E. Lampropoulos**). Branch of Public Hygiene, Ministry of Health, 17 Aristotelous Street, 10187 Athens, Greece (**I. Papaioannou**). Lupus Research Unit, The Rayne Institute, St Thomas' Hospital, 4 Lambeth Palace Road, London SE1 7EH, UK (**D. P. D'Cruz**).

Correspondence to:
D. P. D'Cruz
david.d'cruz@kcl.ac.uk

Key points

- Osteoporosis and vascular calcification share common pathogenetic mechanisms, involving bone morphogenetic proteins, the RANKL–RANK–OPG pathway, MGP and vitamin K
- Patients with osteoporosis have higher levels of vascular calcification than those with normal bone mineral density
- Clinical evidence reveals that osteoporosis is associated with cardiovascular events and increased mortality; moreover, vascular calcification is related to an increased risk of fracture
- In patients with osteoporosis, cardiac and/or vascular calcification can be easily detected by use of simple screening tests, such as ultrasonography of the heart and carotid arteries, or thoracic and abdominal radiography

osteoblasts, the cells express osteogenic markers including transcription factor Sp7 (formerly known as osterix), Col1A1, bone sialoprotein 2 (BSPII), macrophage-colony stimulating factor 1 (M-CSF), bone-specific alkaline phosphatase (ALP), osteocalcin (also known as bone Gla protein), osteopontin (OPN), RUNX2, Wnt–β-catenin signalling mediators, *N*-terminal propeptide of type I collagen and SPARC (also known as osteonectin). The bone-forming activity of mature osteoblasts is stimulated by insulin-like growth factor II (IGF-II) and TGF-β. Osteoblasts that are trapped in the bone matrix become osteocytes and cease to generate osteoid and mineralized matrix; instead, these cells act in a paracrine manner on active osteoblasts, and also seem to inhibit osteoclast formation and bone resorption.[8,9]

Osteoclastogenesis

Osteoclasts are derived from the monocyte–macrophage cell lineage and strongly express tartrate-resistant acid phosphatase type 5 (TRAP) and cathepsin K. The phenotype of terminally differentiated mature osteoclasts is characterized by expression of markers such as TRAP and the calcitonin receptor. At the site of bone resorption, osteoclasts form a specialized cell membrane, termed the 'ruffled border', which increases the resorbent surface area. TRAP secreted from the ruffled border dephosphorylates OPN and stimulates osteoclast migration and bone resorption. Calcium and phosphate ions released upon dissolution of hydroxyapatite (the major component of the mineralized matrix) are absorbed into small vesicles and released into extracellular fluid. Markers of bone resorption include serum *C*-telopeptide of type I collagen and urinary *N*-telopeptide of type I collagen. The activity of osteoclasts is regulated by hormones including parathyroid hormone (PTH), calcitonin and IL-6; soluble factors such as M-CSF (its deficiency induces osteopetrosis);[10] transcription factors such as c-Fos, NFATc1 and NFκB; and the protein receptor activator of nuclear factor κB ligand (RANKL; also known as TNF superfamily member 11 [TNFSF11]).[11] Bone resorption also involves synthesis of cysteine proteinases such as cathepsin K and matrix metalloproteinases (MMPs). MMP-9 and MMP-14 provide a stimulus for migration of osteoclasts to bone surfaces. Oestrogen deficiency increases bone resorption whereas poor intake of vitamin K, low plasma concentrations of vitamin K and undercarboxylation of osteocalcin are associated with low bone mineral density (BMD) and increased risk of fracture.[12–15]

Pathophysiology of vascular calcification

Vascular calcification can be classified into four types: intimal, medial, cardiac valve and calcific arteriolopathy, of which intimal calcification is the most common form and is caused by dyslipidaemia and inflammatory factors such as lipoproteins and cytokines. Medial calcification is associated with age, diabetes mellitus and chronic kidney disease (CKD), and results in higher cardiovascular mortality and amputation risk in these patients, compared with those without calcified vessels.[16,17] In medial calcification elastin is degraded into metabolites that activate cell-dependent calcium deposition, whereas an intact elastin matrix stabilizes the vascular smooth muscle cell (SMC) phenotype *in vivo*.[18] Mitral annular calcification, the most common form of cardiac valve calcification (followed by aortic), correlates positively with atherosclerosis and cardiovascular mortality independently of CHD severity.[19] Calcific arteriolopathy is a severe and life-threatening form of vascular calcification that usually occurs in patients with advanced CKD or hyperparathyroidism, leading to cutaneous necrosis and panniculitis. Mortality reaches 80% due to progressive skin ischaemia and sepsis.[20] Mesenteric and pulmonary tissues can also be involved in rare cases.

Atherosclerosis is an inflammatory disorder. Endothelial risk factors, such as dyslipidaemia , hypertension, diabetes mellitus or inflammatory cytokines derived from excess adipose tissue, augment the expression of molecules that promote the adhesion of leukocytes to the inner surface of the arterial wall and their communication with endothelial cells and SMCs. As a major consequence of this inflammation, SMCs migrate from the tunica media into the intima, proliferate and transform into osteoblast-like cells and induce calcification and atherosclerosis.[21] Monocytes and dendritic cells are also involved in this process, secreting MMPs in response to various oxidative and inflammatory signals.

Examination of human calcified vessels revealed indicators of osteogenesis (ALP, osteocalcin, BSPII, collagen II), osteoblast transcription factors (RUNX2, Sp7, MSX2) and the chondrocyte transcription factor SOX9.[22] Knockdown of *RUNX2* considerably reduces calcification in vascular SMCs.[23] Intimal atherosclerotic plaque calcification involves endochondral ossification, whereas tunica medial calcification is a process more akin to intramembranous bone formation as mineralization initially occurs at matrix vesicles associated with extracellular matrix fibrils, no cartilaginous precursor is required and BMP-2 is a central feature of the mineralization process.[24] Aortic valve calcification is associated with an osteoblast phenotype as increased levels of mRNA encoding OPN, BSPII, osteocalcin and RUNX2 have been detected in calcified valves.[25] Cultures of human aortic valve interstitial cells treated with osteogenic media supplemented with BMP-2, BMP-4, BMP-7 and TGF-β for 21 days resulted in osteoblastic differentiation of valve interstitial cells, as shown by increased

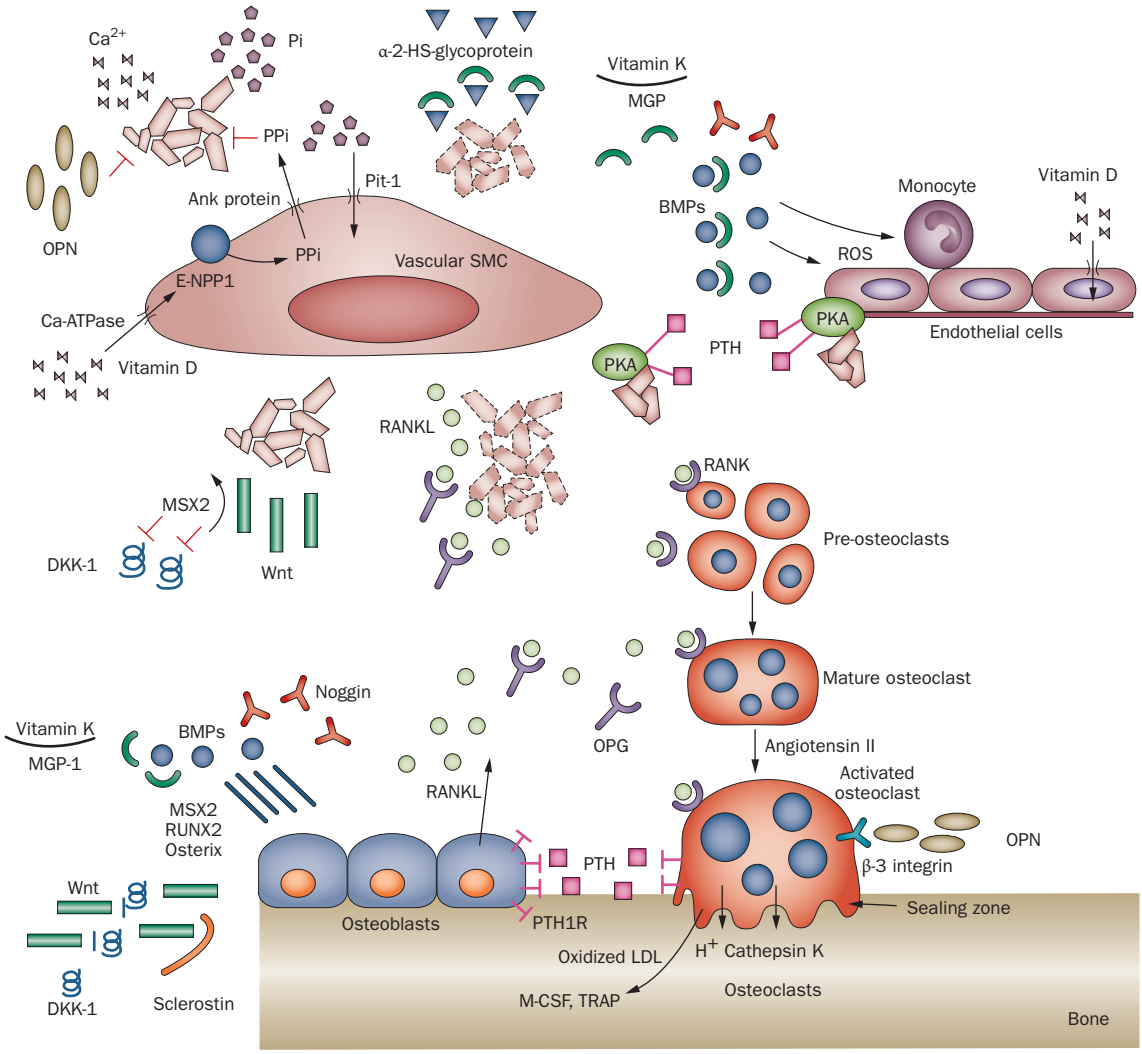

Figure 1 | Common pathogenetic mechanisms in vascular calcification and osteoporosis. BMPs participate in osteoblasts differentiation while they simultaneously produce ROS and increase the adhesiveness of monocytes on the vascular wall. Their action is blocked by MGP, a vitamin K-dependent protein, which also inhibits vascular mineralization as a co-factor of α-2-HS-glycoprotein (also known as fetuin-A). RANKL is a key factor of osteoclast maturation and also acts as an anticalcifying molecule. OPG prevents the interaction of RANKL with its receptor. Wnt signaling, which is important for osteoblast differentiation, is inhibited by sclerostin and DKK-1. On the vascular wall, Wnt is upregulated by the transcription factor MSX2, which blocks the inhibitory effect of DKK-1, resulting in increased vascular calcification. Phosphate, which penetrates the SMC wall through the Pit-1 co-transporter, directly stimulates vascular calcification whereas pyrophosphate acts as an inhibitor of calcification. OPN binds calcium and hydroxyapatite ions, thereby inhibiting crystal formation and vascular calcification; it interacts with integrin receptors resulting in osteoclast activation. PTH inhibits osteoblast activation and increases bone resorption; via PKA activation it induces osteoblastic differentiation and mineralization of vascular cells. Vitamin D increases the entry of calcium into vascular cells, resulting in calcification. Oxidized LDL cholesterol induces the expression of potent mediators of osteoclastic differentiation. Finally, angiotensin II participates in osteoclast activation. Abbreviations: BMP, bone morphogenetic protein; Ca^{2+}, calcium; DKK-1, Dickkopf-1; E-NPP1, ectonucleotide pyrophosphatase/phosphodiesterase family member 1; M-CSF, macrophage-colony stimulating factor 1; MGP, matrix Gla protein; OPG, osteoprotegerin; OPN, osteopontin; Pi, inorganic phosphate; PPi, pyrophosphate; PKA, protein kinase A; PTH, parathyroid hormone; PTH1R, parathyroid hormone 1 receptor; RANKL, receptor activator of nuclear factor κB ligand; ROS, reactive oxygen species; RUNX2, runt-related transcription factor 2; SMC, smooth muscle cell; TRAP, tartrate-resistant acid phosphatase type 5.

activity and expression of ALP.[26] Microvascular pericytes and human aortic valve interstitial cells can differentiate into osteoblasts, chondrocytes and adipocytes, contributing to the development and progression of vascular calcification.[27] The mineral within calcified plaques in the vasculature is hydroxyapatite. Low intake of vitamin K (an essential cofactor of activation of uncarboxylated matrix Gla protein [MGP]) and high levels of uncarboxylated MGP are strongly associated with increased risk of cardiovascular calcification and mortality.[28]

Common pathogenetic mechanisms

Factors implicated in the pathogenesis of both osteoporosis and vascular calcification include proteins, hormones, elements, lipids and vitamins (Figure 1). The

Table 1 | Pathogenetic factors shared by osteoporosis and vascular calcification

Factor	Role in bone metabolism	Role in vascular calcification
BMPs	Induce osteoblastic differentiation and bone formation	Proinflammatory and pro-oxidant effects on arterial wall cells
RANKL	Promotes differentiation and activation of osteoclasts	Reduces the extent of calcification and plaque vulnerability
OPG	Inhibits osteoclastic bone resorption by blocking activity of RANKL	Inhibits vascular calcification and acts as a marker of cardiovascular disease
Wnt signalling pathway	Stimulates osteoblastic bone formation	Promotes vascular calcification
MGP	Inhibits bone mineralization indirectly, by blocking BMP-2, and directly	Inhibits vascular calcification
Vitamin K deficiency	Reduces bone mineral density	Increased risk of vascular calcification and coronary heart disease
Phosphate	Stimulates bone mineralization	Directly stimulates vascular calcification
Cathepsin K	Degrades bone matrix components	Promotes atherogenesis
OPN	Activates osteoclasts	Inhibits vascular calcification
PTH	Inhibits osteoblast activity and increases bone resorption	Induces vascular calcification by promoting osteoblastic differentiation and mineralization of vascular cells
Vitamin D	Maintains bone mass by promoting intestinal absorption of calcium	Induces vascular calcification at high doses
Dyslipidaemia	Promotes bone resorption by suppressing osteoblast differentiation	Increases risk of vascular calcification
Renin–angiotensin–aldosterone system	Activates osteoclasts	Promotes atherosclerosis

Abbreviations: BMP, bone morphogenetic protein; MGP, matrix Gla protein; OPG, osteoprotegerin; OPN, osteopontin; PTH, parathyroid hormone; RANKL, receptor activator of nuclear factor κB ligand.

mechanisms common to both processes are discussed in the following sections and summarized in Table 1.

Bone morphogenetic proteins

BMPs, members of the TGF-β superfamily, induce the differentiation of mesenchymal cells towards the osteoblastic lineage and thereby increase collagen synthesis. These proteins also inhibit the expression of collagenase-3 by osteoblasts, resulting in reduced collagen degradation and maintenance of bone mass.[29] BMP-2 induces osteoblastic differentiation through induction of the transcription factor MSX2. BMP-6 mediates the stimulatory effects of glucocorticoids on osteoblastic cell differentiation, as glucocorticoid treatment results in an important increase in levels of BMP-6 mRNA and protein expression.[30] In bone formation, BMP-2 and BMP-7 induce expression of RUNX2 and Sp7; moreover, they promote transcription of noggin, which binds BMPs with high affinity and inhibits their biological effects, probably in an autoregulatory mechanism to limit the activity of BMPs in osteoblasts.

BMPs exert proinflammatory and pro-oxidant effects in systemic arteries. Studies confirm a striking upregulation of BMPs in atherosclerotic lesions. BMP-2 is expressed by vascular endothelial cells and SMCs and is regulated by proinflammatory stimuli such as TNF and hydrogen peroxide.[31] NFκB signalling has a central role in the regulation of BMP-2 expression. Indeed, endothelial NFκB activation and increased expression of BMP-2 and TNF have been demonstrated in hyperhomocysteinaemia.[32] BMP-2 causes endothelial dysfunction and substantial production of NADPH oxidase-derived reactive oxygen species (ROS) in endothelial cells, resulting in endothelial activation and increased monocyte adhesiveness. In a mouse model of diabetes, upregulation of Bmp-2 and Msx2 is associated with increased vascular calcification, and a high-fat diet upregulates the expression of Msx1 and Msx2 in perivascular adventitial cells.[33] BMP-4 is more abundant in pulmonary arteries than in vessels of systemic circulation and causes considerable endothelial dysfunction in systemic arteries, with vasoconstriction, hypertension and atherosclerotic plaque development, whereas pulmonary arteries are completely protected.[34] Upregulation of BMP-4 has been linked to atherosclerosis and hypertension, whereas disruption of BMP-4 signalling is associated with pulmonary hypertension. BMP antagonists (including follistatin, noggin and MGP) are expressed in systemic arterial endothelial cells, regulating BMP activity in the vascular wall. In mice with CKD, therapy with BMP-7 resulted in a considerable reduction in aortic calcification and correction of hyperphosphataemia. Nevertheless, the size of atherosclerotic lesions was not further decreased. BMP-7 is associated with reduction of osterix expression.[35]

RANKL–RANK–OPG pathway

RANKL is produced by stromal cells and osteoblasts and is the key factor for differentiation of monocyte–macrophage osteoclast precursors into multinucleated osteoclasts and activation of mature osteoclasts. RANKL activates the antiapoptotic serine/threonine kinase Akt (also known as protein kinase B) through a signalling complex involving Src kinase and TNF receptor-associated factor 6 (TRAF6). Binding of RANKL to its receptor on osteoclast precursors leads to activation of NFκB and NFATc1, which are required for osteoclast differentiation. NFκB activation is stimulated almost immediately whereas NFATc1 stimulation begins 24–48 h after binding. RANKL generates ROS that include oxygen ions, free radicals and peroxides, both inorganic and organic, which are crucial for osteoclastogenesis. RANKL signalling also induces caspase-3, an enzyme involved in apoptotic events; osteoclasts fail to differentiate in response to RANKL when caspase-3 activity is inhibited.[36] Osteoprotegerin (OPG, also known as TNF receptor superfamily member 11B [TNFRSF11B]) binds to RANKL, preventing its interaction with RANK (also known as TNFRSF11A) and inhibits osteoclast differentiation and suppresses expression of cathepsin K and TRAP.[37] OPG also stimulates the expression of tissue

inhibitor of metalloproteinases-1 (TIMP-1), which seem to directly stimulate the bone-resorbing activity of mature osteoclasts.[38] OPG is stimulated *in vitro* by oestrogens; furthermore, lack of oestrogens induces a decrease in OPG.[39]

RANKL is overexpressed in vulnerable atherosclerotic lesions that are prone to rupture;[40] it seems to be an anticalcifying molecule and probably capable of reducing plaque vulnerability. Soluble RANKL predicted the risk of CVD over a 15-year follow-up period in a study of 909 patients.[41] In humans, OPG is positively associated with vascular calcification as well as with impaired pulse wave velocity (a measure of arterial stiffness).[42] OPG was found to be independently related to severity and 10-year progression of carotid artery disease in a study of 826 patients;[43] moreover, it is associated with the presence and severity of CHD, diabetes mellitus, stroke, cardiovascular morbidity and mortality, atherosclerosis and extent of vascular calcification in elderly women.[44] OPG is a receptor for the cytotoxic TNF-related apoptosis inducing ligand (TRAIL; also known as TNFSF10) and might inhibit TRAIL-induced apoptosis of vascular cells.[45] By contrast, mice with OPG deficiency develop early-onset osteoporosis and vascular calcification, and OPG treatment inhibits this process.[46]

Wnt signalling pathway

Wnt signalling is crucial for osteoblast differentiation and bone formation and the Wnt–β-catenin pathway is particularly important for bone metabolism.[47] Sclerostin (a soluble factor secreted by osteocytes) negatively regulates Wnt signalling by binding to the Wnt co-receptor LRP5.[48] Dickkopf-related protein 1 (DKK-1) is another inhibitor of Wnt signalling, the expression of which was increased in 66 patients with osteoporosis before treatment with zoledronic acid and decreased, after therapy, to levels observed in healthy individuals.[49] Increased expression of DKK-1 seems also to have a role in the pathogenesis of osteoporosis caused by glucocorticoid use or oestrogen deficiency, in cancer and in multiple myeloma-related bone disease.[50–52] Glucocorticoids inhibit osteoblastogenesis, reduce osteoblast function and induce apoptosis of osteoblasts and osteocytes.[53] These agents also enhance the expression of DKK-1 and prevent soluble Wnt protein from binding to its receptor complex.[54] Additionally, in mice glucocorticoids stimulate mature osteoclasts, decrease their apoptosis and reduce the expression of IGF-I, an important regulator of osteoblast activity.[55]

In *in vitro* studies in mice, Msx2 upregulates Wnt3a and Wnt7a and blocks the inhibitory effect of Dkk-1, resulting in increased vascular calcification. The osteogenic and atherogenic action of Msx2 is reversed after Dkk-1 treatment.[56]

Matrix Gla protein

MGP belongs to the family of mineral-binding γ-carboxyglutamic acid-containing proteins; it inhibits mineralization directly, as a part of a complex with α-2-HS-glycoprotein, and indirectly, by interfering with binding of BMP-2 to its receptor and thereby inhibiting BMP-2-induced osteogenic differentiation. α-2-HS-glycoprotein (also known as fetuin-A) is an important inhibitor of ectopic calcification; it is produced by the liver and contains a TGF-β receptor II-like domain. α-2-HS-glycoprotein forms stable spheres with calcium and phosphorus. Low levels of α-2-HS-glycoprotein are associated with increased vascular calcification, cardiovascular mortality and mitral annular calcification in patients with CKD on haemodialysis or with CHD.[57,58] In *in vitro* studies, MGP seems to be a potent inhibitor of extracellular matrix calcification as mice deficient in *Mgp* display premature mineralization of long bones and severe vascular calcification.[59] *MGP* also seems to be a specific target of Fra-1, a Fos-related antigen that activates bone matrix formation and might lead to osteosclerosis.[60]

In normal vasculature, MGP is strongly expressed on endothelial cells and SMCs whereas RUNX2 is present at very low levels. During progression of vascular calcification, MGP expression is lost whereas RUNX2 expression increases in atherosclerotic lesions.[61] By contrast, other studies correlate arterial calcification with increased MGP expression, perhaps in a feedback attempt to reduce calcium deposits. The inhibitory effect of MGP on BMP-2 depends on the degree of MGP γ-carboxylation rather than the amount of MGP: lack of function from insufficient γ-carboxylation is the factor that increases the risk of calcification. Low serum levels of uncarboxylated MGP are positively associated with increased total coronary artery calcium score, aortic calcification and left-ventricular dysfunction in patients with symptomatic aortic stenosis.[62,63] Glucocorticoids are a well-known risk factor for atherosclerosis and osteoporosis. Dexamethasone induces calcification by downregulating inhibitors of calcification such as MGP, OPN and vascular calcification-associated factor. Oestrogens also seem to participate in the vascular calcification process via the BMP–MGP regulatory system. *In vitro* studies with human aortic endothelial cells showed that oestrogen replacement therapy blocks the BMP osteogenic pathway by increasing *MGP* mRNA expression.[64] Oestrogen receptors have been reported to be expressed on osteoblasts, osteoclasts, vascular endothelial cells and SMCs, suggesting a direct effect of oestrogens on vascular endothelial and bone cells.[65] Consistent with these observations, hormone replacement therapy in postmenopausal women improves brachial arterial endothelial function and increases BMD.[66]

Vitamin K

Numerous studies have demonstrated the importance of vitamin K in bone health and its protective effect on bone mass. This role is mediated through the vitamin K-dependent γ-carboxylation of bone proteins such as MGP. Low dietary intake and low circulating serum levels of vitamin K are associated with low BMD and increased risk of hip fractures.[67–69]

Conditions causing a relative deficiency of functional vitamin K could increase vascular calcification and the risk of CHD because of incomplete γ-carboxylation and reduced function of MGP.[70] High dietary intake of

vitamin K (from green, leafy vegetables, vegetable oils, meat, cheese and eggs) or vitamin K supplementation have been shown to reduce the progression of vascular calcification, protect against CHD and improve arterial elasticity.[71–73] The Western diet (characterized by high levels of processed food and low levels of vegetables) contains insufficient vitamin K to ensure complete MGP carboxylation in the healthy adult population, resulting in suboptimal protection against calcification. Hence, low dietary vitamin K intake is an obvious risk factor for vascular calcification, especially when combined with calcium supplementation, where vitamin K could be essential to neutralize the increased calcification tendency.[74] The anticoagulant warfarin interferes with the availability of bioactive vitamin K and subsequently with MGP function; it inhibits the formation of Gla residues in MGP and can cause rapid calcification of elastic lamellae of arterial media. Consistent with these mechanisms, warfarin has been associated with increased calcification of cardiac valves and coronary arteries.[75,76]

Phosphate

Phosphate is a central element in bone structure. Hypophosphataemia causes defective cartilage and bone formation, whereas hyperphosphataemia stimulates mineralization in chondrocytes and osteoblasts. Hyperphosphataemia is an independent risk factor for CVD. Phosphate directly stimulates vascular calcification, via elevated calcium–phosphate product, and is a signalling molecule involved in osteoblastic differentiation.[77] Inorganic phosphate causes matrix calcification whereas inorganic pyrophosphate—produced by ectonucleotide pyrophosphatase/phosphodiesterase family member 1 (E-NPP 1) and transported by Ank protein—acts as an inhibitor of calcification. The extent of calcification depends on the relative concentrations of inorganic phosphate and inorganic pyrophosphate. In CKD, increased serum levels of phosphorus and PTH are correlated positively with increased risk of cardiovascular mortality.[78] The generation of phosphate by β-glycerophosphate and its uptake through Pit-1 (a type III sodium-dependent phosphate co-transporter expressed in human SMCs) is essential for the calcification of SMCs. Elevated phosphate upregulates osteochondrogenic differentiation markers such as RUNX2 and OPN. *In vitro*, transient calcium elevations sensitize vascular SMCs to phosphorus by increasing the expression of Pit-1 mRNA. Consequently, vascular SMCs acquire an osteoblast and chondrocyte phenotype, expressing the cartilage transcription factor SOX9 and the cartilage extracellular matrix protein collagen II.[79] Treatment with a phosphate binder could inhibit the development of vascular calcification and this hypothesis is being tested for its potential role in clinical practice.

Cathepsins

The cysteine protease cathepsin K has an essential role in osteoclast function and in degradation of protein components of bone matrix, such as type I and type II collagen, elastin and osteonectin. Cathepsin K is produced by bone-resorbing macrophages and synovial fibroblasts. Serum levels of cathepsin K are increased in patients with rheumatoid arthritis and correlate with radiological destruction.[80] Cathepsin K inhibitors, such as odanacatib, are being investigated for possible use in the treatment of osteoporosis.[81]

In mice, cathepsin L1 participates directly in atherogenesis by mediating the degradation of internal elastic lamina by SMCs, the migration and accumulation of SMCs in intimal lesions, and the transmigration of peripheral blood monocytes and lymphoctes into the lesions. Furthermore, deficiency of cathepsin L1 reduces diet-induced atherosclerosis.[82] Disruption of cathepsin K reduces atherosclerosis progression and induces plaque fibrosis, resulting in increased plaque stability.[83]

Osteopontin

OPN is an extracellular structural protein synthesized by a range of tissues and stimulated by calcitriol ($1,25[OH]_2D3$). It has a high content of aspartic acid residues that bind calcium and hydroxyapatite ions, inhibiting crystal formation. OPN can also act through binding to several integrin receptors, especially integrin β-3; binding to this receptor leads to a decrease in cytosolic calcium concentration, which is associated with osteoclast activation, and induces expression of carbonic anydrase 2, which creates the acidic environment essential to resorption of ectopic calcification. In experiments in mice deficient for the gene encoding Opn, administration of recombinant OPN rescued the defective resorption of ectopic bone that had been implanted in muscles.[84]

OPN is an inhibitor of vascular calcification.[85] *Mgp*-deficient mice that are also deficient for the gene encoding Opn show more extensive vascular calcification than mice deficient in *Mgp* alone.[86] OPN is highly expressed in calcified, atherosclerotic lesions of patients with diabetes mellitus and chronic renal failure, probably reflecting a compensatory mechanism of reducing mineralization. Serum levels of OPN and OPG are elevated in patients with carotid stenosis and CHD, and increase with increasing disease activity.[87] OPN directly inhibits calcification of cultured bovine aortic SMCs and aortic valves *in vivo*.[88]

Parathyroid hormone

PTH has paradoxical effects on bone turnover: chronically elevated PTH secretion inhibits osteoblast activity and increases bone resorption, whereas intermittent PTH administration increases bone formation.[89] PTH exerts its effects on osteoblasts through binding to the PTH1 receptor and involving intracellular signalling pathways, such as protein kinase A (PKA) and mitogen activated protein kinase (MAPK) pathways, and PTH-responsive transcription factors, such as cyclic AMP-responsive element-binding protein, AP1 and RUNX2. PTH induces the expression of MGP on osteoblasts via PKA and extracellular signal-regulated kinase (ERK)–MAPK signalling pathways; this action is mediated by Sp-family and RUNX2 transcription factors.

In the vasculature, PTH activates PKA, inducing calcification independently of calcium or phosphorus.[90] Both

Table 2 | Clinical evidence linking osteoporosis and vascular calcification

Study	Patient population	Findings
Hyder *et al.* (2009)[114]	946 women (mean age 65.5 years) and 963 men (mean age 64.1 years)	Low BMD is associated with more extensive arterial artery calcification in women and men, and with more extensive coronary artery calcification in women
Choi *et al.* (2009)[115]	467 subjects (128 men, 339 women)	Low BMD is associated with higher coronary calcium score and atherosclerotic plaque burden in women
Hak *et al.* (2000)[116]	236 women, 45–57 years of age at baseline, followed for 9 years	Progression of aortic calcification is associated with metacarpal bone loss in women during menopause
Adragao *et al.* (2009)[117]	38 patients undergoing haemodialysis	Low bone volume is a risk factor for coronary calcification in patients undergoing haemodialysis
Tanko *et al.* (2003)[118]	963 women 60–85 years of age	Low BMD is related to advanced atherosclerosis
Reddy *et al.* (2008)[119]	228 women (mean age 64 years)	Osteoporosis is strongly associated with the presence of breast arterial calcification
Uyama *et al.* (1997)[120]	30 postmenopausal women 67–85 years of age	Osteoporosis is associated with severity of carotid atherosclerosis in postmenopausal women
Sumino *et al.* (2008)[121]	175 postmenopausal women	Increased carotid intima–media thickness is associated with low lumbar spine BMD in postmenopausal women
Sumino *et al.* (2007)[122]	85 postmenopausal women	Osteoporosis is associated with impaired brachial arterial endothelial function in postmenopausal women
Seo *et al.* (2009)[123]	152 postmenopausal women	Osteoporosis is associated with increased arterial stiffness and presence of coronary artery atherosclerosis in postmenopausal women

Abbreviation: BMD, bone mineral density.

primary and secondary hyperparathyroidism induce aortic valve calcification, which resolves in parallel with normalization of PTH levels. PKA activation by TNF or by cyclic AMP analogues induces osteoblastic differentiation and mineralization of vascular cells.[91] Forskolin, an activator of PKA signalling through adenylate cyclase and production of cyclic AMP, causes vascular calcification via effects of phosphate transport proteins and pyrophosphate generating enzymes. In animal studies, treatment with forskolin induces expression of osteoblastic differentiation markers (Opn, ALP, BSPII and osteocalcin) and the transcription factor Runx2.[92]

Vitamin D

Vitamin D participates in calcium metabolism by promoting its intestinal absorption, whereas deficiency of vitamin D is a secondary cause of osteoporosis. Moreover, novel studies have implicated vitamin D in the pathogenesis of vascular calcification. In animal studies with rats, administration of high doses of vitamin D led to vascular calcification.[93,94] Exogenous vitamin D is carried in blood by lipoproteins rather than the usual binding protein of endogenous vitamin D; thus, LDL cholesterol could carry exogenous vitamin D to the artery wall in high concentrations.[95] Both endothelial cells and vascular SMCs express high-affinity receptors for the biologically active form of vitamin D_3. Vitamin D metabolites seem to have multiple effects on SMCs, including enhanced expression of calcium ATPase, increasing the entry of calcium into the cell, elevated cytosolic levels of free calcium and effects on arterial tone. Evidence exists of an enzymatically active 25-hydroxyvitamin D_3-1α-hydroxylase system in human vascular SMCs that can be upregulated by PTH and oestrogenic compounds.[96]

Dyslipidaemia

Dyslipidaemia is a major risk factor for vascular calcification. In mice, oxidized lipids inhibit osteoblastic differentiation in vascular tissues and reduce BMD.[97] Oxidized LDL cholesterol induces the expression of M-CSF and TRAP (potent mediators of osteoclastic differentiation) and suppresses the terminal differentiation of stromal cells into osteoblasts.[98,99] Accumulation of oxidized lipids in the subendothelial space of arteries promotes vascular calcification, and in skeletal bone arteries this accumulation inhibits bone mineral formation.[100] Increased levels of LDL cholesterol and reduced levels of HDL cholesterol have been associated with osteoporosis in postmenopausal women.[101] Statins seem to increase bone mineralization in mice and in patients with osteoporosis whilst they are associated with decreased risk of bone fractures.[102–104]

Renin–angiotensin–aldosterone system

An activated renin–angiotensin system is a well-known promoting factor of atherosclerosis.[105] An *in vitro* study showed that angiotensin II activates osteoclasts, leading to osteoporosis.[106] In a rat model of heart failure, aldosterone levels were directly correlated with elevated PTH levels and increased excretion of calcium, and these changes were attenuated by spironolactone.[107] Conversely, angiotensin-converting enzyme (ACE) inhibitors seem to reduce the risk of bone fractures and increase BMD.[108]

Clinical implications

Current data, described above, support the hypothesis that a common metabolic pathway links osteoporosis and vascular calcification. These two conditions develop gradually with progression of age, suggesting

Table 3 | Clinical evidence linking vascular calcification with fracture risk

Study	Patient population	Findings
Szulc *et al.* (2008)[124]	781 men ≥50 years of age	Increasing severity of abdominal aortic calcification is associated with increasing risk of fracture in older men
Schulz *et al.* (2004)[125]	2,348 postmenopausal women	Presence of aortic calcification is associated with increased risk of fracture in the spine and hip
Bagger *et al.* (2006)[126]	2,662 postmenopausal women followed for 7.5 years	Increasing severity of aortic calcification is associated with increasing risk of hip fracture
Naves *et al.* (2008)[127]	624 men and women >50 years of age	Presence of severe aortic calcification is associated with risk of vertebral fracture
Rodriguez-Garcia *et al.* (2009)[136]	193 patients undergoing haemodialysis	Presence of vascular calcification is associated with a higher rate of vertebral fractures

Table 4 | Clinical evidence linking osteoporosis with cardiovascular events

Study	Patient population	Findings
Farhat *et al.* (2007)[129]	2,310 men and women 68–80 years of age	Low BMD is associated with increased incidence of cardiovascular disease in women and in white men
Farhat *et al.* (2006)[130]	3,075 men and women 68–80 years of age	Cardiovascular disease is related to low BMD in men and women
Trivedi & Khaw (2001)[131]	Men 65–76 years of age	Low BMD is related to higher mortality from cardiovascular disease in elderly men
Jorgensen *et al.* (2001)[132]	251 men and women ≥60 years of age	Risk of stroke is increased in women with low BMD
Kiel *et al.* (2001)[133]	364 women and 190 men	Greater magnitude of bone loss is associated with more severe progression of aortic calcification, during 25 years of follow-up
Marcovitz *et al.* (2005)[134]	209 men and women	Osteoporosis independently predicts coronary heart disease
Tanko *et al.* (2005)[135]	2576 postmenopausal women (mean age 66.5 years)	Risk of cardiovascular events is increased in postmenopausal women with osteoporosis
Collins *et al.* (2009)[137]	5,781 men ≥65 years of age	Peripheral arterial disease is associated with low BMD and increased risk of fracture in men
Laroche *et al.* (2003)[138]	25 men and women with unilateral lower limb arterial disease, mean age 62.3 years	Unilateral peripheral arterial disease is associated with low BMD in the affected leg
Pennisi *et al.* (2004)[139]	36 patients with peripheral vascular disease and 30 healthy individuals	Low BMD is associated with atherosclerosis of peripheral vessels in men and women
Vogt *et al.* (1997)[140]	1,292 elderly women (mean age 71 years)	Reduced blood flow to the extremities is associated with decreased BMD
Wong *et al.* (2005)[141]	3,998 men and women 65–92 years of age	Risk of peripheral arterial disease is increased in men and women with osteoporosis
van Diepen *et al.* (2008)[142]	16,294 patients ≥65 years of age	Heart failure is associated with a fourfold increased risk of hip fractures

Abbreviation: BMD, bone mineral density.

an inevitable and age-dependent association. A possible explanation for this connection is that vascular calcification affects bone metabolism by reducing blood flow or by limiting physical activity, leading in turn to bone loss. Studies in rats demonstrated that bisphosphonates, at doses comparable to those that inhibit bone resorption, inhibit calcification of arteries and valves without affecting serum levels of calcium or phosphate.[109] This effect is attributable to the protective action of bisphosphonates on the vessel wall; that is, by sensitizing macrophages to undergo apoptosis and by preventing the formation of foam cells via inhibition of LDL cholesterol uptake.[110] In hypertensive rats, bisphosphonates reduced atherosclerosis and vascular SMC proliferation.[111] In a prospective clinical study, bisphosphonates inhibited the progression of plaques and abdominal aortic calcification (AAC) score in women with osteoporosis whereas vascular calcification progressed in healthy women who did not receive treatment, suggesting a protective role for bisphosphonates against atherosclerosis.[112]

A study that quantitatively measured AAC by use of CT failed to correlate osteoporosis with calcification after adjustment for age or for multiple variables (age, BMI and smoking status);[113] however, many investigators have reported an independent association between these two processes (Table 2). The MESA abdominal aortic calcium study, for example, showed that, after adjustment for age and risk factors, lower BMD is associated with greater coronary artery calcium score among women and with greater AAC score in both sexes.[114] In another study, which used multidetector row CT to evaluate coronary arteries, coronary calcium score and atherosclerotic plaque burden were found to be associated with low BMD in both premenopausal and postmenopausal women, independently of cardiovascular risk factors and age.[115] In a 9-year study of 236 women, loss of bone mass during menopause was significantly greater in those with progression of aortic calcification than those without.[116] In patients with renal failure undergoing haemodialysis, a significant negative correlation has been noted between the rate of bone turnover, as determined from bone biopsy samples, and cardiac calcification score.[117] Low BMD in the hip seems to be a marker of advanced atherosclerosis in elderly women.[118] Breast arterial calcification, which is known to be associated with CVD, is also strongly associated with osteoporosis.[119] Carotid intima–media thickness (IMT), a well-known cardiovascular risk factor, has been related to osteoporosis in many studies.[120,121] Women with osteoporosis have impaired brachial arterial endothelial function compared with healthy individuals, as depicted by flow-mediated vasodilatation after reactive hyperaemia.[122] Arterial stiffness, measured by brachial–ankle pulse wave velocity, is associated with osteoporosis and coronary artery atherosclerosis, as determined by multidetector CT.[123]

A number of clinical studies have also demonstrated associations between vascular calcification and risk of osteoporotic fracture (Table 3), and between osteoporosis and cardiovascular events (Table 4). In the MINOS Study, which followed 781 men aged ≥50 years for 10 years, higher AAC scores were associated with a twofold to threefold increase in risk of fractures, regardless of BMD or history of falls.[124] In a group of 2,348 healthy

postmenopausal women, degree of aortic calcification (as measured by use of CT) was significantly and age-independently associated with bone loss; furthermore, women with calcification were five times more likely to experience a spine fracture and three times more likely to have a hip fracture than those without calcification.[125] In a population-based cohort study of 2,662 healthy postmenopausal women, advanced aortic calcification was associated with lower BMD and a 2.3-fold increased risk of proximal femur fractures after 7.5 years of observation.[126] An increased risk of fractures, especially vertebral fractures, and the rate of BMD decline have also been positively associated with progression of aortic calcification.[127] Conversely, the population-based Framingham Study failed to connect hip fracture risk with vascular calcification in 2,499 middle-aged adults over 21 years of observation.[128] In clinical studies, low BMD is associated with CVD and increased mortality in elderly individuals.[129–131] Women with osteoporosis have a 4.8-fold higher risk of stroke compared with women with normal BMD.[132] In another study of the Framingham cohort, women with lower BMD had greater progression of AAC over a 25-year follow-up period.[133] In women with substantial CHD (defined as >50% luminal narrowing of a major vessel), osteoporosis seems to be an independent predictor of the disease.[134] Accelerated bone loss from the hip was associated with increased risk of cardiovascular mortality in a study of 2,576 postmenopausal women.[135] In 193 patients undergoing haemodialysis, vascular calcification and vertebral fractures were positively associated with mortality.[136] An independent correlation has been identified between BMD, increased risk of fracture and peripheral arterial disease[137–141] A population-based cohort study of 16,294 patients showed that heart failure is associated with factors that contribute to accelerated bone loss and an increased risk of fractures, in particular a fourfold increase of hip fracture;[142] this finding might not be surprising as patients with heart failure are likely to be less mobile.

Several studies have raised the issue of the safety of supplementation with calcium or vitamin D, or both. In particular, a re-analysis of the Women's Health Initiative calcium–vitamin D supplementation study published in 2011 showed that use of calcium supplements confers an increased risk of cardiovascular events (including myocardial infarction and stroke).[143] Previous studies also reflect uncertainty about the safety of this approach.[144–148] Although the Women's Health Initiative study has many limitations and has generated a lot of controversy, the message is clear that the usage and safety of calcium and vitamin D supplements, especially in older people, must be reassessed.

Conclusions

Osteoporosis, vascular calcification and cardiovascular events seem to be closely related, seemingly independently of age. Many pathogenetic mechanisms have been implicated in this paradoxical phenomenon. Although data are now questioning the safety of supplementation with calcium and vitamin D, other treatments for osteoporosis seem to reduce vascular calcification in animal models. Further studies are needed to establish the relationships and to determine if osteoporosis is a cardiovascular risk factor. Nevertheless, on the basis of available data it might be prudent to assess the risk of CVD in patients with osteoporosis by use of simple screening tests, such as ultrasonography of the heart and carotid arteries or thoracic and abdominal radiography, to detect cardiac and/or vascular calcification.

Review criteria

The literature was reviewed by searching the MEDLINE database without limits on date of publication. Search terms included "osteoporosis", "vascular calcification", "pathophysiology" and "cardiovascular disease". Selected references are full-text papers published in English language. Reference lists of selected papers were explored for additional information.

1. Reginster, J. Y. & Burlet, N. Osteoporosis: a still increasing prevalence. *Bone* **38** (Suppl.), S4–S9 (2006).
2. Sweet, M. G., Sweet, J.M., Jeremiah, M.P. & Galazka, S. S. Diagnosis and treatment of osteoporosis. *Am. Fam. Physician* **79**, 193–200 (2009).
3. Sambrook, P. & Cooper, C. Osteoporosis. *Lancet* **367**, 2010–2018 (2006).
4. Cummings, S.R. & Melton, L. J. Epidemiology and outcomes of osteoporotic fractures. *Lancet* **359**, 1761–1767 (2002).
5. Greenland, P. *et al.* ACCF/AHA 2007 clinical expert consensus document on coronary artery calcium scoring by computed tomography in global cardiovascular risk assessment and in evaluation of patients with chest pain: a report of the American College of Cardiology Foundation Clinical Expert Consensus Task Force (ACCF/AHA Writing Committee to Update the 2000 Expert Consensus Document on Electron Beam Computed Tomography). *Circulation* **115**, 402–426 (2007).
6. Demer, L. L. & Tintut, Y. Vascular calcification: pathobiology of a multifaceted disease. *Circulation* **117**, 2938–2948 (2008).
7. Wilson, P. W. *et al.* Abdominal aortic calcific deposits are an important predictor of vascular morbidity and mortality. *Circulation* **103**, 1529–1534 (2001).
8. Tan, S. D. *et al.* Osteocytes subjected to fluid flow inhibit osteoclast formation and bone resorption. *Bone* **41**, 745–751 (2007).
9. Eriksen, E. F. Cellular mechanisms of bone remodeling. *Rev. Endocr. Metab. Disord.* **11**, 219–227 (2010).
10. Radi, Z. A., Guzman, R. E. & Bell, R. R. Increased connective tissue extracellular matrix in the op/op model of osteopetrosis. *Pathobiology* **76**, 199–203 (2008).
11. Teitelbaum, S. L. Osteoclasts: what do they do and how do they do it? *Am. J. Pathol.* **170**, 427–435 (2007).
12. Feskanich, D. *et al.* Vitamin K intake and hip fractures in women: a prospective study. *Am. J. Clin. Nutr.* **69**, 74–79 (1999).
13. Szulc, P., Chapuy, M. C., Meunier, P. J. & Delmas, P. D. Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture in elderly women. *J. Clin. Invest.* **91**, 1769–1774 (1993).
14. Luukinen, H. *et al.* Strong prediction of fractures among older adults by the ratio of carboxylated to total serum osteocalcin. *J. Bone Miner. Res.* **15**, 2473–2478 (2000).
15. Knapen, M. H., Nieuwenhuijzen Kruseman, A. C., Wouters, R. S. & Vermeer, C. Correlation of serum osteocalcin fractions with bone mineral density in women during the first 10 years after menopause. *Calcif. Tissue Int.* **63**, 375–379 (1998).
16. London, G. M. *et al.* Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. *Nephrol. Dial. Transplant.* **18**, 1731–1740 (2003).
17. Guzman, R. J. Clinical, cellular and molecular aspects of arterial calcification. *J. Vasc. Surg.* **45** (Suppl. A), A57–A63 (2007).
18. Smith, E. R. *et al.* Elastin degradation is associated with progressive aortic stiffening and all-cause mortality in predialysis chronic kidney disease. *Hypertension* **59**, 973–978 (2012).
19. Willens, H. J. *et al.* The relation between mitral annular calcification and mortality in patients undergoing diagnostic coronary angiography. *Echocardiography* **23**, 717–722 (2006).

20. Ross, E. A. Evolution of treatment strategies for calciphylaxis. *Am. J. Nephrol.* **34**, 460–467 (2011).
21. Libby, P. & Theroux P. Pathophysiology of coronary artery disease. *Circulation* **111**, 3481–3488 (2005).
22. Shao, J. S., Cheng, S. L., Sadhu, J. & Towler, D. A. Inflammation and the osteogenic regulation of vascular calcification: a review & perspective. *Hypertension* **55**, 579–592 (2010).
23. Nakano-Kurimoto, R. *et al.* Replicative senescence of vascular smooth muscle cells enhances the calcification through initiating the osteoblastic transition. *Am. J. Physiol. Heart Circ. Physiol.* **297**, 1673–1684 (2009).
24. Vattikuti, R. & Towler, D. A. Osteogenic regulation of vascular calcification: an early perspective. *Am. J. Physiol. Endocrinol. Metab.* **286**, 686–696 (2004).
25. Rajamannan, N. M. *et al.* Human aortic valve calcification is associated with an osteoblast phenotype. *Circulation* **107**, 2181–2184 (2003).
26. Osman, L., Yacoub, M. H., Latif, N., Amrani, M. & Chester, A. H. Role of human valve interstitial cells in valve calcification and their response to atorvastatin. *Circulation* **114**, 1547–1552 (2006).
27. Farrington-Rock, C. *et al.* Chondrogenic and adipogenic potential of microvascular pericytes. *Circulation* **110**, 2226–2232 (2004).
28. Rennenberg, R. J. *et al.* Calcium scores and matrix Gla protein levels: association with vitamin K status. *Eur. J. Clin. Invest.* **40**, 344–349 (2010).
29. Gazzerro, E., Rydziel, S. & Canalis, E. Skeletal bone morphogenetic proteins suppress the expression of collagenase-3 by rat osteoblasts. *Endocrinology* **140**, 562–567 (1999).
30. Boden, S. D. *et al.* Glucocorticoid-induced differentiation of fetal rat calvarial osteoblasts is mediated by bone morphogenetic protein-6. *Endocrinology* **138**, 2820–2828 (1997).
31. Csiszar, A. *et al.* Bone morphogenetic protein-2 induces proinflammatory endothelial phenotype. *Am. J. Pathol.* **168**, 629–638 (2006).
32. Ungvari, Z. *et al.* Increased superoxide production in coronary arteries in hyperhomocysteinemia: role of tumor necrosis factor-α, NAD(P)H oxidase and inducible nitric oxide synthase. *Arterioscler. Thromb. Vasc. Biol.* **23**, 418–424 (2003).
33. Towler, D. A., Bidder, M., Latifi, T., Coleman, T. & Semenkovich, C. F. Diet-induced diabetes activates an osteogenic gene regulatory program in the aortas of low density lipoprotein receptor-deficient mice. *J. Biol. Chem.* **273**, 30427–30434 (1998).
34. Csiszar, A., Labinskyy, N., Jo, H., Ballabh, P. & Ungvari, Z. Differential proinflammatory and prooxidant effects of bone morphogenetic protein-4 in coronary and pulmonary arterial endothelial cells. *Am. J. Physiol. Heart Circ. Physiol.* **295**, H569–H577 (2008).
35. Mathew, S. *et al.* The mechanism of phosphorus as a cardiovascular risk factor in CKD. *J. Am. Soc. Nephrol.* **19**, 1092–1105 (2008).
36. Szymczyk, K. H., Freeman, T. A., Adams, C. S., Srinivas, V. & Steinbeck, M. J. Active caspase-3 is required for osteoclast differentiation. *J. Cell. Physiol.* **209**, 836–844 (2006).
37. Kearns, A. E., Khosla, S. & Kostenuik, P. J. Receptor activator of nuclear factor κB ligand and osteoprotegerin regulation of bone remodeling in health and disease. *Endocr. Rev.* **29**, 155–192 (2008).
38. Sobue, T. *et al.* Tissue inhibitor of metalloproteinases 1 and 2 directly stimulate the bone-resorbing activity of isolated mature osteoclasts. *J. Bone Miner. Res.* **16**, 2205–2214 (2001).
39. Hofbauer, L. C. *et al.* Estrogen stimulates gene expression and protein production of osteoprotegerin in human osteoblastic cells. *Endocrinology* **140**, 4367–4370 (1999).
40. Sandberg, W. J. *et al.* Enhanced T-cell expression of RANK ligand in acute coronary syndrome: possible role in plaque destabilization. *Arterioscler. Thromb. Vasc. Biol.* **26**, 857–863 (2006).
41. Kiechl, S. *et al.* Soluble receptor activator of nuclear factor-κB ligand and risk for cardiovascular disease. *Circulation* **116**, 385–391 (2007).
42. Shargorodsky, M. *et al.* Osteoprotegerin as an independent marker of subclinical atherosclerosis in osteoporotic postmenopausal women. *Atherosclerosis* **204**, 608–611 (2009).
43. Kiechl, S. *et al.* Osteoprotegerin is a risk factor for progressive atherosclerosis and cardiovascular disease. *Circulation* **109**, 2175–2180 (2004).
44. Browner, W. S., Lui, L. Y. & Cummings, S. R. Association of serum osteoprotegerin levels with diabetes, stroke, bone density, fractures and mortality in elderly women. *J. Clin. Endocrinol. Metab.* **86**, 631–637 (2001).
45. Emery, J. G. *et al.* Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL. *J. Biol. Chem.* **273**, 14363–14367 (1998).
46. Morony, S. *et al.* Osteoprotegerin inhibits vascular calcification without affecting atherosclerosis in *Ldlr*$^{-/-}$ mice. *Circulation* **117**, 411–420 (2008).
47. Krishnan, V., Bryant, H. U. & MacDougald, O. A. Regulation of bone mass by Wnt signaling. *J. Clin. Invest.* **116**, 1202–1209 (2006).
48. Veverka V. *et al.* Characterization of the structural features and interactions of sclerostin: molecular insight into a key regulator of Wnt-mediated bone formation. *J. Biol. Chem.* **284**, 10890–10900 (2009).
49. Voskaridou E. *et al.* Serum Dickkopf-1 is increased and correlates with reduced bone mineral density in patients with thalassemia-induced osteoporosis. Reduction post-zoledronic acid administration. *Haematologica* **94**, 725–728 (2009).
50. Wang F. S. *et al.* Knocking down dickkopf-1 alleviates estrogen deficiency induction of bone loss: a histomorphological study in ovariectomized rats. *Bone* **40**, 485–492 (2007).
51. Politou, M. *et al.* Serum concentrations of Dickkopf-1 protein are increased in patients with multiple myeloma and reduced after autologous stem cell transplantation. *Int. J. Cancer* **119**, 1728–1731 (2006).
52. Voorzanger-Rousselot, N. *et al.* Increased Dickkopf-1 expression in breast cancer bone metastases. *Br. J. Cancer* **97**, 964–970 (2007).
53. Yao, W. *et al.* Glucocorticoid excess in mice results in early activation of osteoclastogenesis and adipogenesis and prolonged suppression of osteogenesis: a longitudinal study of gene expression in bone tissue from glucocorticoid-treated mice. *Arthritis Rheum.* **58**, 1674–1686 (2008).
54. Ohnaka, K., Tanabe, M., Kawate, H., Nawata, H. & Takayanagi, R. Glucocorticoid suppresses the canonical Wnt signal in cultured human osteoblasts. *Biochem. Biophys. Res. Commun.* **329**, 177–181 (2005).
55. Jia, D., O'Brien, C. A., Stewart, S. A., Manolagas, S. C. & Weinstein, R. S. Glucocorticoids act directly on osteoclasts to increase their life span and reduce bone density. *Endocrinology* **147**, 5592–5599 (2006).
56. Shao, J. S. *et al.* Msx2 promotes cardiovascular calcification by activating paracrine Wnt signals. *J. Clin. Invest.* **115**, 1210–1220 (2005).
57. Ketteler, M. *et al.* Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study. *Lancet* **361**, 827–833 (2003).
58. Ix, J. H. *et al.* Association of fetuin-A with mitral annular calcification and aortic stenosis among persons with coronary heart disease: data from the Heart and Soul Study. *Circulation* **115**, 2533–2539 (2007).
59. Suttamanatwong, S. *et al.* Sp proteins and Runx2 mediate regulation of matrix Gla protein (MGP) expression by parathyroid hormone. *J. Cell. Biochem.* **107**, 284–292 (2009).
60. Eferl, R. *et al.* The Fos-related antigen Fra-1 is an activator of bone matrix formation. *EMBO J.* **23**, 2789–2799 (2004).
61. Jono, S. *et al.* Matrix Gla protein is associated with coronary artery calcification as assessed by electron-beam computed tomography. *Thromb. Haemost.* **91**, 790–794 (2004).
62. Ueland, T. *et al.* Undercarboxylated matrix Gla protein is associated with indices of heart failure and mortality in symptomatic aortic stenosis. *J. Intern. Med.* **268**, 483–492 (2010).
63. Schurgers, L. J. *et al.* The circulating inactive form of matrix Gla protein is a surrogate marker for vascular calcification in chronic kidney disease: a preliminary report. *Clin. J. Am. Soc. Nephrol.* **5**, 568–575 (2010).
64. Osako, M. K. *et al.* Estrogen inhibits vascular calcification via vascular RANKL system: common mechanism of osteoporosis and vascular calcification. *Circ. Res.* **107**, 466–475 (2010).
65. Nofer, J. R. Estrogens and atherosclerosis: insights from animal models and cell systems. *J. Mol. Endocrinol.* **48**, R13–R29 (2012).
66. Sumino, H. *et al.* Relationship between brachial arterial endothelial function and lumbar spine bone mineral density in postmenopausal women. *Circ. J.* **71**, 1555–1559 (2007).
67. Booth, S. L. *et al.* Vitamin K intake and bone mineral density in women and men. *Am. J. Clin. Nutr.* **77**, 512–516 (2003).
68. Hart, J. P. *et al.* Electrochemical detection of depressed circulating levels of vitamin K1 in osteoporosis. *J. Clin. Endocrinol. Metab.* **60**, 1268–1269 (1985).
69. Hodges, S. J, Akesson, K., Vergnaud, P., Obrant, K. & Delmas, P. D. Circulating levels of vitamins K1 and K2 decreased in elderly women with hip fracture. *J. Bone Miner. Res.* **8**, 1241–1245 (1993).
70. Cranenburg, E. C. *et al.* The circulating inactive form of matrix Gla protein (ucMGP) as a biomarker for cardiovascular calcification. *J. Vasc. Res.* **45**, 427–436 (2008).
71. Geleijnse, J. M. *et al.* Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: The Rotterdam Study. *J. Nutr.* **134**, 3100–3105 (2004).
72. Gast, G. C. *et al.* A high menaquinone intake reduces the incidence of coronary heart disease. *Nutr. Metab. Cardiovasc. Dis.* **19**, 504–510 (2009).
73. Shea, M. K. *et al.* Vitamin K supplementation and progression of coronary artery calcium in older men and women. *Am. J. Clin. Nutr.* **89**, 1799–1807 (2009).
74. Bolland, M. J. *et al.* Vascular events in healthy older women receiving calcium supplementation: randomized controlled trial. *BMJ* **336**, 262–266 (2008).

75. Schurgers, L. J., Aebert, H., Vermeer, C., Bültmann, B. & Janzen, J. Oral anticoagulant treatment: friend or foe in cardiovascular disease? *Blood* **104**, 3231–3232 (2004).
76. Price, P. A., Faus, S. A. & Williamson, M. K. Warfarin causes rapid calcification of the elastic lamellae in rat arteries and heart valves. *Arterioscler. Thromb. Vasc. Biol.* **18**, 1400–1407 (1998).
77. Hruska, K. A., Mathew, S., Lund, R., Qiu, P. & Pratt, R. Hyperphosphatemia of chronic kidney disease. *Kidney Int.* **74**, 148–157 (2008).
78. Hagström, E. *et al.* Plasma parathyroid hormone and the risk of cardiovascular mortality in the community. *Circulation* **119**, 2765–2771 (2009).
79. Li, X., Yang, H. Y. & Giachelli, C. M. Role of the sodium-dependent phosphate cotransporter, Pit-1, in vascular smooth muscle cell calcification. *Circ. Res.* **98**, 905–912 (2006).
80. Skoumal, M. *et al.* Serum cathepsin K levels of patients with longstanding rheumatoid arthritis: correlation with radiological destruction. *Arthritis Res. Ther.* **7**, R65–R70 (2005).
81. Perez-Castrillon, J. L., Pinacho, F., De Luis, D., Lopez-Menendez, M. & Laita, A. D. Odanacatib, a new drug for the treatment of osteoporosis: review of the results in postmenopausal women. *J. Osteoporos.* http://dx.doi.org/10.4061/2010/401581.
82. Kitamoto, S. *et al.* Cathepsin L deficiency reduces diet-induced atherosclerosis in low-density lipoprotein receptor-knockout mice. *Circulation* **115**, 2065–2075 (2007).
83. Lutgens, E. *et al.* Disruption of the cathepsin K gene reduces atherosclerosis progression and induces plaque fibrosis but accelerates macrophage foam cell formation. *Circulation* **113**, 98–107 (2006).
84. Asou, Y. *et al.* Osteoponotin facilitates angiogenesis, accumulation of osteoclasts, and resorption in ectopic bone. *Endocrinology* **142**, 1325–1332 (2001).
85. Scatena, M., Liaw, L. & Giachelli, C. M. Osteopontin: a multifunctional molecule regulating chronic inflammation and vascular disease. *Arterioscler. Thromb. Vasc. Biol.* **27**, 2302–2309 (2007).
86. Speer, M. Y. *et al.* Inactivation of the osteopontin gene enhances vascular calcification of matrix Gla protein-deficient mice: evidence for osteopontin as an inducible inhibitor of vascular calcification *in vivo*. *J. Exp. Med.* **196**, 1047–1055 (2002).
87. Kadoglou, N. P. *et al.* The relationship between serum levels of vascular calcification inhibitors and carotid plaque vulnerability. *J. Vasc. Surg.* **47**, 55–62 (2008).
88. Wada, T., McKee, M. D., Steitz, S. & Giachelli, C. M. Calcification of vascular smooth muscle cell cultures: inhibition by osteopontin. *Circ. Res.* **84**, 166–178 (1999).
89. Suzuki, A., Sekiguchi, S., Asano, S. & Itoh, M. Pharmacological topics of bone metabolism: recent advances in pharmacological management of osteoporosis. *J. Pharmacol. Sci.* **106**, 530–535 (2008).
90. Huang, M. S., Sage, A. P., Lu, J., Demer, L. L. & Tintut, Y. Phosphate and pyrophosphate mediate PKA-induced vascular cell calcification. *Biochem. Biophys. Res. Commun.* **374**, 553–558 (2008).
91. Tintut, Y., Patel, J., Parhami, F. & Demer, L. L. Tumor necrosis factor-α promotes *in vitro* calcification of vascular cells via the cAMP pathway. *Circulation* **102**, 2636–2642 (2000).
92. Tintut, Y., Parhami, F., Boström, K., Jackson, S. M. & Demer, L. L. cAMP stimulates osteoblast-like differentiation of calcifying vascular cells: potential signaling pathway for vascular calcification. *J. Biol. Chem.* **273**, 7547–7553 (1998).
93. Mizobuchi, M., Finch, J. L., Martin, D. R. & Slatopolsky, E. Differential effects of vitamin D receptor activators on vascular calcification in uremic rats. *Kidney Int.* **72**, 709–715 (2007).
94. Bas, A., Lopez, I., Perez, J., Rodriguez, M. & Aquilera-Tejero, E. Reversibility of calcitriol-induced medial artery calcification in rats with intact renal function. *J. Bone Miner. Res.* **21**, 484–490 (2006).
95. Demer, L. L. A skeleton in the atherosclerosis closet. *Circulation* **92**, 2029–2032 (1995).
96. Somjen, D. *et al.* 25-hydroxyvitamin D_3-1α-hydroxylase is expressed in human vascular smooth muscle cells and is upregulated by parathyroid hormone and estrogenic compounds. *Circulation* **111**, 1666–1671 (2005).
97. Parhami, F. *et al.* Atherogenic high-fat diet reduces bone mineralization in mice. *J. Bone Miner. Res.* **16**, 182–188 (2001).
98. Parhami, F., Garfinkel, A. & Demer, L. L. Role of lipids in osteoporosis. *Arterioscler. Thromb. Vasc. Biol.* **20**, 2346–2348 (2000).
99. Tintut, Y., Morony, S. & Demer, L. L. Hyperlipidemia promotes osteoclastic potential of bone marrow cells *ex vivo*. *Arterioscler. Thromb. Vasc. Biol.* **24**, 6–10 (2004).
100. Parhami, F. *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.* **17**, 680–687 (1997).
101. Yamaguchi, T. *et al.* Plasma lipids and osteoporosis in postmenopausal women. *Endocr. J.* **49**, 211–217 (2002).
102. Mundy, G. *et al.* Stimulation of bone formation *in vitro* and in rodents by statins. *Science* **286**, 1946–1949 (1999).
103. Edwards, C. J., Hart, D. J. & Spector, T. D. Oral statins and increased bone-mineral density in postmenopausal women. *Lancet* **355**, 2218–2219 (2000).
104. Meier, C. R., Schlienger, R. G., Kraenzlin, M. E., Schlegel, B. & Jick, H. HMG-CoA reductase inhibitors and the risk of fractures. *JAMA* **283**, 3205–3210 (2000).
105. Shapiro, Y., Boaz, M., Matas, Z., Fux, A. & Shargorodsky, M. The association between the rennin–angiotensin–aldosterone system and arterial stiffness in young healthy subjects. *Clin. Endocrinol. (Oxf.)* **68**, 510–512 (2008).
106. Shimizu, H. *et al.* Angiotensin II accelerates osteoporosis by activating osteoclasts. *FASEB J.* **22**, 2465–2475 (2008).
107. Law, P. H., Sun, Y., Bhattacharya, S. K., Chhokar, V. S. & Weber, K. T. Diuretics and bone loss in rats with aldosteronism. *J. Am. Coll. Cardiol.* **46**, 142–146 (2005).
108. Lynn, H., Kwok, T., Wong, S. Y., Woo, J. & Leung, P. C. Angiotensin converting enzyme inhibitor use is associated with higher bone mineral density in elderly Chinese. *Bone* **38**, 584–588 (2006).
109. Price, P. A., Faus, S. A. & Williamson, M. K. Biphosphonates alendronate and ibandronate inhibit artery calcification at doses comparable to those that inhibit bone resorption. *Arterioscler. Thromb. Vasc. Biol.* **21**, 817–824 (2001).
110. Luckman, S. P. *et al.* Nitrogen-containing biphosphonates inhibit the mevalonate pathway and prevent post-translational prenylation of GTP-binding proteins, including Ras. *J. Bone Miner. Res.* **13**, 581–589 (1998).
111. Su, J. Z., Fukuda, N., Kishioka, H., Hu, W. Y. & Kanmatsuse, K. Etidronate influences growth and phenotype of rat vascular smooth muscle cells. *Pharmacol. Res.* **46**, 7–13 (2002).
112. Kanazawa, I. *et al.* Effects of treatment with risedronate and alfacalcidol on progression of atherosclerosis in postmenopausal women with type 2 diabetes mellitus accompanied with osteoporosis. *Am. J. Med. Sci.* **339**, 519–524 (2010).
113. Chow, J. T. *et al.* Abdominal aortic calcification, BMD, and bone microstructure: a population-based study. *J. Bone Miner. Res.* **23**, 1601–1612 (2008).
114. Hyder, J. A. *et al.* Association of coronary artery and aortic calcium with lumbar bone density. The MESA Abdominal Aortic Calcium Study. *Am. J. Epidemiol.* **169**, 186–194 (2009).
115. Choi, S. H. *et al.* Lower bone mineral density is associated with higher coronary calcification and coronary plaque burdens by multidetector row coronary computed tomography in pre- and postmenopausal women. *Clin. Endocrinol.* **71**, 644–651 (2009).
116. Hak, A. E., Pols, H. A., van Hemert, A. M., Hofman, A. & Witteman, J. C. Progression of aortic calcification is associated with metacarpal bone loss during menopause: a population-based longitudinal study. *Arterioscler. Thromb. Vasc. Biol.* **20**, 1926–1931 (2000).
117. Adragao, T. *et al.* Low bone volume—a risk factor for coronary calcifications in hemodialysis patients. *Clin. J. Am. Soc. Nephrol.* **4**, 350–455 (2009).
118. Tanko, L. B., Bagger, Y. Z. & Christiansen, C. Low bone mineral density in the hip as a marker of advanced atherosclerosis in elderly women. *Calcif. Tissue Int.* **73**, 15–20 (2003).
119. Reddy, J., Bilezikian, J. P., Smith, S. J. & Mosca, L. Reduced bone mineral density is associated with breast arterial calcification. *J. Clin. Endocrinol. Metab.* **93**, 208–211 (2008).
120. Uyama, O., Yoshimoto, Y., Yamamoto, Y. & Kawai, A. Bone changes and carotid atherosclerosis in postmenopausal women. *Stroke* **28**, 1730–1732 (1997).
121. Sumino, H. *et al.* Relationship between carotid atherosclerosis and lumbar spine bone mineral density in postmenopausal women. *Hypertens. Res.* **31**, 1191–1197 (2008).
122. Sumino, H. *et al.* Relationship between brachial arterial endothelial function and lumbar spine bone mineral density in postmenopausal women. *Circ. J.* **71**, 1555–1559 (2007).
123. Seo, S. K. *et al.* Bone mineral density, arterial stiffness and coronary atherosclerosis in healthy postmenopausal women. *Menopause* **16**, 937–943 (2009).
124. Szulc, P., Kiel, D. P. & Delmas, P. D. Calcifications in the abdominal aorta depicts fractures in men: MINOS study. *J. Bone Miner. Res.* **23**, 95–102 (2008).
125. Schulz, E., Arfai, K., Liu, X., Sayre, J. & Gilsanz, V. Aortic calcification and the risk of osteoporosis and fractures. *J. Clin. Endocrinol. Metab.* **89**, 4246–4253 (2004).
126. Bagger, Y. Z., Tanko, L. B., Alexandersen, P., Qin, G. & Christiansen, C. Radiographic measure of aorta calcification is a site-specific predictor of bone loss and fracture risk at the hip. *J. Intern. Med.* **259**, 598–605 (2006).
127. Naves, M., Rodriguez-Garcia, M., Diaz-Lopez, J. B., Gomez-Alonso, C. & Cannata-Andia, J. B. Progression of vascular calcifications is associated with greater bone loss and increased bone fractures. *Osteoporos. Int.* **19**, 1161–1166 (2008).

128. Samelson, E. J. *et al.* Vascular calcification in middle-age and long term risk of hip fracture: the Framingham Study. *J. Bone Miner. Res.* **22**, 1449–1454 (2007).
129. Farhat, G. N. *et al.* The association of bone mineral density measures with incident cardiovascular disease in older adults. *Osteoporos. Int.* **18**, 999–1008 (2007).
130. Farhat, G. N. *et al.* Volumetric and areal bone mineral density measures are associated with cardiovascular disease in older men and women: the health, aging and body composition study. *Calcif. Tissue Int.* **79**, 102–111 (2006).
131. Trivedi, D. P. & Khaw, K. T. Bone mineral density at the hip predicts mortality in elderly men. *Osteoporos. Int.* **12**, 259–265 (2001).
132. Jorgensen, L., Engstad, T. & Jacobsen, B. Bone mineral density in acute stroke patients, low bone mineral density may predict first stroke in women. *Stroke* **32**, 47–51 (2001).
133. Kiel, D. P. *et al.* Bone loss and the progression of abdominal aortic calcification over a 25 year period: the Framingham Heart Study. *Calcif. Tissue Int.* **68**, 271–276 (2001).
134. Marcovitz, P. A. *et al.* Usefulness of bone mineral density to predict significant coronary artery disease. *Am. J. Cardiol.* **96**, 1059–1063 (2005).
135. Tanko, L. B. *et al.* Relationship between osteoporosis and cardiovascular disease in postmenopausal women. *J. Bone Miner. Res.* **20**, 1912–1920 (2005).
136. Rodriguez-Garcia, M. *et al.* Vascular calcifications, vertebral fractures and mortality in haemodiaysis patients. *Nephrol. Dial. Transplant.* **24**, 239–246 (2009).
137. Collins, T. C. *et al.* Peripheral arterial disease is associated with higher rates of hip bone loss and increased fracture risk in older men. *Circulation* **119**, 2305–2312 (2009).
138. Laroche, M. *et al.* Bone mineral decrease in the leg with unilateral chronic occlusive arterial disease. *Clin. Exp. Rheumatol.* **21**, 103–106 (2003).
139. Pennisi, P. *et al.* Low bone density and abnormal bone turnover in patients with atherosclerosis of peripheral vessels. *Osteoporos. Int.* **15**, 389–395 (2004).
140. Vogt, M. T., Cauley, J. A., Kuller, L. H. & Nevitt, M. C. Bone mineral density and blood flow to the lower extremities: the study of osteoporotic fractures. *J. Bone Miner. Res.* **12**, 283–289 (1997).
141. Wong, S. Y. *et al.* Bone mineral density and the risk of peripheral arterial disease in men and women: results from Mr and Ms Os, Hong Kong. *Osteoporos. Int.* **16**, 1933–1938 (2005).
142. van Diepen, S., Majumdar, S. R., Bakal, J. A., McAlister, F. A. & Ezekowitz, J. A. Heart failure is a risk factor for orthopaedic fracture: a population-based analysis of 16,294 patients. *Circulation* **118**, 1946–1952 (2008).
143. Bolland, M. J., Grey, A., Avenell, A., Gamble, G. D. & Reid, I. R. Calcium supplements with or without vitamin D and risk of cardiovascular events: reanalysis of the Women's Health Initiative limited access dataset and meta-analysis. *BMJ* **342**, d2040 (2011).
144. Hsia, J. *et al.* Calcium/vitamin D supplementation and cardiovascular events. *Circulation* **115**, 846–854 (2007).
145. Lewis, J. R., Calver, J., Zhu, K., Flicker, L. & Prince, R. L. Calcium supplementation and the risks of atherosclerotic vascular disease in older women: results of a 5-year RCT and a 4.5-year follow-up. *J. Bone Miner. Res.* **26**, 35–41 (2011).
146. Wang, T. K. *et al.* Relationships between vascular calcification, calcium metabolism, bone density, and fractures. *J. Bone Miner. Res.* **25**, 2777–2785 (2010).
147. Bolland, M. J. *et al.* Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. *BMJ* **341**, c3691 (2010).
148. Bolland, M. J. *et al.* Vascular events in healthy older women receiving calcium supplementation: randomised controlled trial. *BMJ* **336**, 262–266 (2008).

Author contributions

C. E. Lampropoulos and I. Papaioannou researched data for the article, all authors provided a substantial contribution to discussions of the content, C. E. Lampropoulos wrote the article, and C. E. Lampropoulos and D. P. D'Cruz reviewed and/or edited the article before submission.