REVIEW

Osteoporosis and vascular calcifications

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Abstract

In post-menopausal women, aged individuals, and patients with diabetes mellitus or chronic renal disease, bone mineral density (BMD) decreases while the vasculature accumulates arterial calcifications (ACs). AC can be found in the tunica intima and/or in the tunica media. Prospective studies have shown that patients with initially low BMD and/or the presence of fragility fractures have at follow-up a significantly increased risk for coronary and cerebrovascular events and for overall cardiovascular mortality. Similarly, patients presenting with abdominal aorta calcifications (an easily quantifiable marker of vascular pathology) show a significant decrease in the BMD (and an increase in the fragility) of bones irrigated by branches of the abdominal aorta, such as the hip and lumbar spine. AC induction is an ectopic tissue biomineralization process promoted by osteogenic transdifferentiation of vascular smooth muscle cells as well as by local and systemic secreted factors. In many cases, the same regulatory molecules modulate bone metabolism but in reverse. Investigation of animal and *in vitro* models has identified several potential mechanisms for this reciprocal bone–vascular regulation, such as vitamin K and D sufficiency, advanced glycation end-products–RAGE interaction, osteoprotegerin/RANKL/RANK, Fetuin A, oestrogen deficiency and phytooestrogen supplementation, microbiota and its relation to diet, among others. Complete elucidation of these potential mechanisms, as well as their clinical validation via controlled studies, will provide a basis for pharmacological intervention that could simultaneously promote bone and vascular health.

Key Words

- osteoporosis
- bone fractures
- cardiovascular disease
- arterial calcifications
- vascular smooth muscle cells
- osteogenic transdifferentiation

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Introduction

In adults from Westernized societies, the arterial system is the second most mineralized site after the skeleton, and there is increasing evidence of a reciprocal regulation between the calcification of both systems (1, 2). In post-menopausal women and/or aged individuals, particularly if they present with conditions such as diabetes mellitus, chronic renal disease or metabolic syndrome, the arterial wall can acquire a considerable load of calcifications, while bone mineral density (BMD) decreases. Accumulation of arterial calcifications (ACs) in the tunica media contributes to arteriosclerosis with impaired vessel compliance and decreased distal perfusion, while in the tunica intima, ACs are associated with advanced focal atheromatous lesions and a high thrombotic risk. Taken together, while osteoporosis and osteopenia increase the risk of low-stress or fragility bone fractures (3), AC loading increases the risk of stroke, dementia, myocardial infarction, heart failure, renal failure and lower limb amputation (4).

AC induction is an ectopic tissue biomineralization process promoted by osteogenic transdifferentiation of vascular smooth muscle cells (VSMCs) as well as by local and systemic secreted factors (5). This, in turn, depends on the balance between (i) procalcifying factors, such as type 2 bone morphogenic protein (BMP2), RANKL, hyperphosphataemia, hypercalcaemia, oxidative stress, subendothelial accumulation of advanced glycation end-products (AGEs), oestrogen deficiency and/or

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vitamin K deficiency, and (ii) vascular calcification inhibitory factors such as matrix Gla protein (MGP), osteoprotegerin (OPG), Fetuin A (FetA), phosphorylated osteopontin (OPN), pyrophosphate, oestrogens and/or vitamin K sufficiency (2, 6, 7, 8). In many cases, the same regulatory molecules modulate bone metabolism, but in reverse.

Thus, nutritional and/or pharmacological vitamin K deficiency reduces carboxylation in glutamic acid residues both in MGP (blocking its vascular inhibitory effect on BMP2 and on the development of AC) and in osteocalcin (OC) (inhibiting its bone mineralization promoting effect) (8). OPG at the vascular level may prevent the accumulation of AC, while in bone it decreases bone resorption by inhibiting RANK–RANKL interaction (9). Chronic activation of the RAGE receptor (due to AGEs accumulated on the extracellular matrix under conditions of reduced renal clearance, oxidative stress, carbonyl stress and/or hyperglycaemia) stimulates osteogenic transdifferentiation of VSMC in the arterial wall with the generation of AC (6), while in the bone it inhibits both bone formation and remodelling (10). Also, vitamin D levels can affect cardiovascular risk and the probability of bone fractures: hypovitaminosis D not only is associated with low-grade inflammation and risk of atheroma but can also contribute to low bone mass through induction of secondary hyperparathyroidism; hypervitaminosis D is associated with an increase in AC (1, 11), whereas vitamin D sufficiency levels can prevent VSMC osteogenic transdifferentiation induced by chronic RAGE receptor activation by extracellular AGEs (12).

In this review, we provide an update on the analysis of these and other factors that regulate physiological and ectopic tissue mineralization; the pathophysiological consequences of an imbalance in this regulation; clinical evidence for the existence of a bone–vascular axis that controls its biomineralization in a reciprocal way; and the cellular and molecular mechanisms that could support this reciprocal regulation.

Physiological mineralization and pathological consequences of its dysregulation

In humans, physiological mineralization only occurs in certain specialized connective tissues in order to provide organs that meet specific mechanical requirements, such as bones and teeth. Although different mechanisms and cell types are involved in the mineralization of these two groups of organs, in this review we will focus on factors regulating the mineralization of skeletal tissues.

Skeletal mineralization

Bone tissue is a specialized connective tissue possessing a mineralized extracellular matrix that is composed of hydroxyapatite crystals ($Ca10(PO_4)6(OH)_2$) deposited on collagen fibres composed mainly of type 1 collagen (with traces of types 3, 5, 11 and 13) (13).

Various cell types are associated with the bone extracellular matrix. These cells are responsible for bone health and include (i) osteoprogenitor cells, derived from mesenchymal cells, which can give rise to osteoblasts (OBs); (ii) lining cells, which cover the surface of bone not undergoing active remodelling; (iii) OB, the cells involved in bone formation; (iv) osteocytes, the most abundant cell type in bone, derived from OB that have become completely surrounded by a mineralized matrix, and whose functions are of vital importance for the regulation of bone and mineral homeostasis; and (v) osteoclasts (OCs), multinucleated cells derived from granulocyte/monocyte colony-forming units (CFU-GM), responsible for bone resorption.

Bone mineralization involves deposition of hydroxyapatite crystals on the osteoid or unmineralized extracellular matrix (of which >85% is type 1 collagen), and the OB is responsible for regulating this process. Initially, osteoprogenitor cells differentiate into OB as a consequence of molecular stimuli such as IGF1 and 2, the canonical Wnt/b-catenin pathway, Wnt10b, TGFb and/or BMP2/4/7 that modulate the expression of key osteogenic genes such as *RUNX2* and *OSTERIX* (14). OBs are responsible for secreting most of the components of the osteoid, such as collagen fibrils and the amorphous ground substance; they also secrete regulators of the mineralization process such as OC, osteoadherin and bone sialoproteins; as well as the extracellular vesicles rich in tissue non-specific alkaline phosphatase (ALP) that are responsible for modulating the concentration of calcium and phosphate ions and for inducing regulated mineralization of the osteoid.

Another important osteoblastic function in mineral homeostasis is regulation (via cross-talk) of the resorptive action of OC (14). Osteoclastogenesis involves the recruitment of CFU-GM via cytokines such as M-CSF and IL3 to form a pre-OC that expresses receptor activator of NFKB (RANK) on its plasma membrane. Pre-OC RANK can then interact with its ligand (RANKL), expressed by bone marrow stromal cells,

OB and osteocytes, to form a mature and active OC that initiates bone resorption. OB can further modulate this process by secretion of OPG, which acts as a decoy ligand for RANK, preventing it from interacting with RANKL and thus inhibiting OC differentiation. In addition, this pathway can be regulated by different molecules: PTH and vitamin D are pro-resorptive, since they increase the expression of RANKL and inhibit OPG, whereas oestrogen inhibits the resorptive process by decreasing the RANKL/OPG ratio in bone.

Osteopenia and osteoporosis

Osteoporosis and osteopenia are clinical entities characterized by a decrease in BMD with a deterioration of bone microarchitecture, which leads to greater bone fragility with a subsequent increase in fracture risk (15). BMD is usually measured by dual energy X-ray absorptiometry (DEXA), and normalized in S.D. to a young adult control population as the T-score. Osteoporosis in a particular bone site is defined by a T-score below (−2.5), and osteopenia by a T-score between (−1.0) and (−2.5). At the bone tissue level, osteopenia and osteoporosis may occur when osteoclastic activity surpasses osteoblastic formation in a persistent manner. In turn, this may be due to increased resorption and/or decreased bone formation. Osteoporosis is a complex disease with multiple causes, the most frequent being post-menopausal osteoporosis and/or osteoporosis due to ageing (primary osteoporosis). On the other hand, metabolic conditions such as diabetes mellitus (or prolonged treatments with different drugs such as glucocorticoids) can induce secondary osteoporosis, increasing the risk for bone fractures and decreasing bone repair capacity (3, 10).

The strength of a bone depends on (i) the quality of bone material, that is, its mechanical properties (rigidity and tenacity) and (ii) the quality of bone design or architecture, determined by the spatial distribution of its cortical and trabecular components (16). The World Health Organization defines a fragility fracture (osteoporotic fracture) as one that results from a force exerted on the bone that would be insufficient to fracture normal bone. A higher rate of fractures, especially in anatomical locations such as the hip, wrist and vertebrae, causes an increase in morbidity and mortality in the elderly population and in postmenopausal women. This is a serious problem for public health, especially considering that the ageing population in developed countries is expected to grow in the future, due to an increase in life expectancy (3). For example, in Argentina alone, the annual incidence of hip fractures is 21,700 (i.e. one every 24 min), causing more than 8000 deaths, and of the total number of hip fractures that occur in people older than 45 years, more than 70% are due to osteoporosis (3).

An imbalance between bone formation and resorption resulting from a homeostatic alteration between OBs and OCs is characteristic of osteoporosis and appears to be related to the development of AC. It has been observed that patients with lower BMD have a greater risk of developing mineralization of the vasculature (2), which could indicate a common mechanism underlying both conditions.

Main sites of ectopic biomineralization and its clinical consequences

In certain situations, biomineralization in ectopic sites becomes clinically important; this is particularly frequent in the vascular bed, with accumulation of AC. Several imaging techniques have been used to evaluate and quantitate AC (5). Some of these methods are invasive, such as angiography, intravascular ultrasonography (IVUS), virtual histology–IVUS and optical coherence tomography, while the most frequently used techniques for AC evaluation are non-invasive (computed tomography, mammography, DEXA and positron emission tomography with 18F-NaF). AC can be located in different histological locations of the arterial wall; the most commonly observed calcifications are deposited in atheromas of the tunica intima, followed by calcification of the tunica media. This defines two kinds of AC that differ in their origin, histological location and clinical consequences (2). Arteriosclerosis refers to arterial mechanical stiffening that can be due to concentric medial and adventitial fibrosis with medial calcification, elastinolysis and mural thickening and/or to eccentric intimal plaques with calcification, fibrosis and cholesterol-rich lipoprotein deposition (1). Whereas atherosclerosis refers only to the eccentric accumulation of intimal atherosclerotic plaques (i.e. lumen-deforming processes initiating from subendothelial lipoprotein deposits with foam cell formation and subsequent matrix remodelling events that increase the risk for atherothrombosis) (1).

Atherosclerosis

Atherosclerotic calcification occurs within the tunica intima, where AC accumulate eccentrically and localize

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in an atheroma, preferentially located in the branches and curvatures of arteries with turbulent blood flow. The process begins as a consequence of endothelial dysfunction together with an accumulation of lipoproteins in the subendothelium due to the greater permeability of these atheroprone areas. Hyperlipidaemia and biomechanical stress acting on the endothelial cell cause activation of the NFKB pathway with the consequent expression of inflammation marker molecules such as VCAM1, ICAM1, E selectin and TLR2 on the endothelial surface. This induces the release of proinflammatory cytokines (IL6, IL8), growth factors such as PDGF, chemokines such as MCP1 and a lower expression of the enzyme endothelial nitric oxide synthase (eNOS) responsible for the production of nitric oxide (NO), the main vasodilator molecule that also has anti-inflammatory properties (17).

This scenario promotes the recruitment of circulating monocytes, which enter the subendothelium of the intima and differentiate into macrophages, after which they begin to phagocytose extravasated lipoproteins, especially native LDL and its oxidized form (oxLDL), through class A (SRA) and class B (CD36) scavenger-type receptors (18). This intracellular accumulation of lipids causes the macrophage to become a foam cell with a proinflammatory profile, recruiting further monocytes and inducing endothelial dysfunction. The process induces intima thickening and partial occlusion of the arterial lumen. In this microenvironment, VSMC migrate from the tunica media to the intima, proliferate and generate a fibrous cap surrounding a lipid-rich necrotic core, thus forming the fibroatheroma.

Calcifications frequently accumulate in advanced atheromas. Their formation can be induced by the release of vesicles due to macrophage and VSMC apoptosis; these vesicles contain calcium and phosphate that could initiate nucleation centres for the formation of hydroxyapatite-based intimal AC (19). A further mechanism leading to calcification could involve the osteoblastic transdifferentiation of VSMCs due to the loss of osteogenic inhibitors such as MGP, OPG and FetA (which are normally found in the vessel wall) and/or an increase in the expression of procalcifying molecules such as BMP2. Proinflammatory cytokines such as TNFa and IL1b are also related to the phenotypic switch of VSMC (20) and may play an additional role in the accumulation of intimal AC.

Atheromas can remain undetected for decades and have frequently been observed in autopsies of middle-aged adults without apparent symptoms of cardiovascular disease (19). In fact, atheroma progression may not be continuous but rather occurs in stages of relative quiescence followed by phases of rapid growth. This switch can be promoted by external factors such as sleep disruption, stress or a proinflammatory diet.

The most severe consequences of atheromas involve acute events such as acute myocardial infarction or stroke, which usually occur due to rupture of the fibrous external layer of more mature atherosclerotic plaques, rich in lipids and calcifications, inducing thrombotic events by contact of platelets and coagulation factors with thrombogenic material present in the ruptured plaque. Another mechanism has recently been proposed, called 'Surface Erosion', in which loss of endothelium allows granulocytes to adhere to the intimal basement membrane, generating neutrophil extracellular traps (NETs) that propagate inflammation and thrombosis (20). Whatever the mechanism involved, the thrombotic event ends up obstructing blood flow to vital organs.

Thus, the presence of AC in the tunica intima, rather than representing a risk *per se* for the patient, acts as a marker for the existence of mature atheroma plaques with a high thrombotic risk.

Stiffening of the arterial tunica media

Arteriosclerotic calcification can occur in the tunica media vascularis. Concentrically located and diffuse in nature, in this case AC accumulates in the medial muscle layer resulting in a generalized hardening of the vessel with loss of elasticity (1). It is related to conditions such as type 2 diabetes mellitus, metabolic syndrome, chronic kidney disease, hypertension and osteoporosis (20, 21). Several vascular changes contribute to the stiffness of the arterial tunica media: fibrosis or excessive deposits of collagen fibres, accumulation of AC (extracellular nodules of hydroxyapatite associated with collagen and elastin fibres), fragmentation of elastic fibres (elastinolysis) and irreversible cross-linking of collagen and/or elastic fibres due to accumulation of advanced glycation products or AGEs (22).

Typically, VSMCs present in the arterial tunica media undergo a phenotypic switch, from a contractile to an osteogenic phenotype, by mechanisms that are not yet fully elucidated, but in which key players are recognized, such as high levels of circulating calcium and phosphate, LDL, vitamin D, inflammatory cytokines (IL1b, IL6, IL8 and TNFa) and molecules such as BMP2 and VEGF. These factors activate the canonical

Wnt/b catenin signalling pathway, responsible for the regulation of genes associated with bone-forming processes, *RUNX2* being the most important (20).

VSMCs with an osteogenic phenotype secrete abundant quantities of type 1 collagen fibres, altering the composition of the extracellular matrix in the tunica media and providing the substrate for the deposition of hydroxyapatite crystals by the same transdifferentiated VSMCs (1). The most notorious consequence of AC accumulation in the tunica media is the loss of elasticity and compliance of the vessel necessary for its normal function (22).

Stiffening of the arterial wall induces hypertension due to an increase in systolic blood pressure and alters the capillary perfusion of various organs. In the case of the musculoskeletal system, peripheral arterial disease causes an increased risk of lower limb amputations, especially in patients with type 2 diabetes mellitus and/or chronic kidney disease (23).

In addition to its direct cardiovascular consequences, accumulation of AC in the tunica media is also associated with an increase in the risk of bone mineral loss in the femoral neck, leading to hip fractures (1). Tunica media stiffening can also cause disturbances in bone blood flow. In healthy young bones, blood flow follows a centrifugal pattern: perforating arteries give rise to marrow capillaries that irrigate trabecular and cortical bone, which then drain into periosteal venules. However, due to arteriosclerosis of perforating arteries and a generalized age-related reduction in vasodilator response, bone blood flow becomes centripetal (arterial blood tends to enter bone through periosteal arteries, then flows to marrow capillaries and finally drains via perforating veins). This alteration of bone perfusion and blood flow could affect its metabolism, causing a decrease in bone anabolism due to impaired availability of regulating molecules involved in osteoblastic function, such as NO, BMP2 and IGF1 (1).

Clinical evidence for a reciprocal regulation between bone and vascular mineralization

Fifty-five years ago, Dent *et al.* reported for the first time an association between osteoporosis and abdominal aorta calcifications (AACs) in postmenopausal South African Caucasian women (24). Since then, abundant clinical evidence has accumulated confirming the existence of an inverse relationship between skeletal (physiological) and vascular (ectopic) biomineralization. The accumulation of vascular calcifications both in the tunica intima and in the tunica media constitutes an important cardiovascular risk factor, while the degree of decrease in BMD is an important determinant of the probability of fragility fractures.

Vascular calcification and osteoporosis share a genetic basis and molecular signalling mechanisms, as well as common risk factors (diabetes mellitus, menopause, chronic kidney disease, obesity, smoking etc.). The accumulation of vascular calcifications is associated with decreased peripheral blood perfusion, potentially limiting processes such as osteogenesis, which could induce and precede loss of bone mass (25). For example, AAC that induces stiffening of the aortic wall could reduce perfusion via branches of the abdominal aorta and thus decrease bone formation in bones whose irrigation depends on these branches, such as the hip (irrigated by the medial circumflex femoral artery) or the lumbar spine (irrigated by lumbar arteries). Vascular calcifications have also been shown to decrease muscle blood supply and thus contribute to frailty syndrome (26), increasing the individual risk of falls and thus the incidence of bone fractures.

Of the clinical studies designed to evaluate the bidirectional relationship between bone and cardiovascular health reported to date, none have been randomized controlled; they are all observational and, in some cases, show discordant results. In order to standardize results and generate conclusions that have greater clinical validity, the main clinical studies have been consolidated in two recent complementary meta-analyses: one that has assessed the risk of fractures and/or low BMD in patients with AAC accumulation (25) and another that has analysed the prospective probability of adverse cardiovascular events in patients with fragility

Table 1 Presence of abdominal aorta calcifications (AACs) is associated with decreased BMD and increased risk of fragility bone fractures (25).

	(Any/advanced AAC) vs (no/low AAC)	
Bone site	% decrease in BMD (CI)	Fragility fracture relative risk (CI)
Any	N.A.	1.43 (1.37–1.50)[b]
Femoral neck	24% (19–29)[a]	N.A.
Hip	42% (39–45)[a]	1.35 (1.18–1.55)[a]
Lumbar spine	51% (48–55)[a]	N.A.
Vertebral	N.A.	1.57 (1.48–1.66)[b]
Non-vertebral	N.A.	1.27 (1.16–1.38)[a]

[a]$P < 0.05$; [b]$P < 0.02$.
BMD, bone mineral density; N.A., not applicable.

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fractures and/or decreased BMD (27). The main conclusions of both meta-analyses will be described further.

In the first meta-analysis (Table 1), data from 86 clinical trials that included a total of 61,553 patients were evaluated (25). The presence of AAC (any/advanced AAC vs no/low AAC) was significantly associated with both a decrease in BMD in the total hip, femoral neck, lumbar spine and calcaneus (moderate quality of evidence) and with an increased risk of vertebral, non-vertebral and hip prevalent/incident fragility fractures (moderate–high quality of evidence). These results suggest a link between bone metabolism and the presence of vascular calcifications. They show a correlation between the presence of AAC (an easily quantifiable marker of vascular pathology) and a decrease in BMD (and an increase in fragility) of bones irrigated by branches of the abdominal aorta. Its authors propose that AAC detection could be used to optimize the prediction of fractures and for the reclassification of patients.

The second meta-analysis (Table 2) included 28 clinical studies that prospectively followed a total of 1,107,885 patients for a median of 5 years (27). Patients with initially low BMD (in the calcaneus, femoral neck or total hip) showed a 33% increased risk of adverse cardiovascular events (coronary events, cerebrovascular events or cardiovascular mortality) at follow-up. Specifically, each 1-s.d. decrease in BMD was associated with a 44% increase in coronary risk, a 28% increase in cerebrovascular risk and a 22% increase in cardiovascular mortality. Patients with fragility fractures at baseline also showed increased cardiovascular risk at follow-up (47% for cerebrovascular event and 78% for cardiovascular mortality). The overall increase in cardiovascular risk in patients with hip fracture was 48%, while for patients with vertebral fractures it was 26%.

There are several mechanisms that could explain this association between low BMD and increased cardiovascular risk in an individual, but the relative pathophysiological contribution of each one in real life is unknown. For example, it has been shown that chronic low-grade inflammation can induce both a decrease in BMD (28) and an increase in cardiovascular risk (29) of an individual. However, the available evidence indicates that it is the accumulation of vascular calcifications (much more abundant in individuals with low BMD) that can explain most of the increased cardiovascular risk in these patients (25, 27).

The elucidation of relevant mechanisms regulating the bone–vascular axis may provide a basis for pharmacological intervention in a clinical setting. For example, the insulin-sensitizer metformin has been proposed to exert direct osteogenic effects on bone, possibly contributing to prevent diabetic osteopathy (30). When compared to its skeletal effects, the actions of metformin on vascular biomineralization appear to be opposite: metformin treatment significantly reduces the load of coronary AC both in prediabetic patients (31) and in individuals with type 2 diabetes mellitus (32).

Table 2 Low BMD and/or bone fragility fractures (hip/vertebral) are associated with an increase in cardiovascular risk and mortality (27).

Cardiovascular risk for low BMD vs high BMD	
Cardiovascular endpoint	**Hazard ratio (CI)**
Any CVD risk	1.33 (1.27–1.38)[a]
Death due to CVD	1.52 (1.04–2.22)[b]
Coronary heart disease	1.31 (1.25–1.38)[a]
Cerebrovascular conditions	1.22 (1.12–1.34)[a]
Cardiovascular risk per 1-s.d. decrease in BMD	
Cardiovascular endpoint	**Hazard ratio (CI)**
Any CVD risk	1.16 (1.09–1.24)[a]
Death due to CVD	1.22 (1.14–1.30)[a]
Coronary heart disease	1.44 (1.25–1.66)[a]
Cerebrovascular conditions	1.28 (1.18–1.39)[a]
Cardiovascular risk for fragility fracture vs no fracture	
Cardiovascular endpoint	**Hazard ratio (CI)**
Any CVD risk	1.20 (1.06–1.37)[b]
Death due to CVD	1.78 (1.09–2.91)[c]
Coronary heart disease	N.S.
Cerebrovascular conditions	1.47 (1.42–1.54)[a]

[a]$P < 0.0001$; [b]$P < 0.004$; [c]$P < 0.02$.
BMD, bone mineral density; CVD, cardiovascular disease; N.S., not significant.

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In the next section, we will discuss several mechanisms that provide possible links for the inverse and reciprocal relationship in bone–vascular biomineralization, which has been observed and reported in clinical practice over the past few decades.

Possible mechanisms for reciprocal bone–vascular regulation

The study of different animal and *in vitro* models has led to the identification of potential mechanisms for reciprocal bone–vascular regulation, although to date none of these has been clinically validated through controlled studies. Some of the most relevant mechanisms will be briefly described, emphasizing their possible pathophysiological role (Fig. 1).

Vitamin K

Vitamin K is a cofactor for the enzyme gamma-glutamyl-carboxylase, and its function is to facilitate the carboxylation of glutamyl residues in vitamin K-dependent proteins (VKDPs). Adequate levels of vitamin K are required to ensure various physiological processes, such as blood coagulation, bone mineralization and the inhibition of vascular calcifications (33).

Vitamin K is lipid soluble, can be incorporated from the diet and can be present as VK1 (phylloquinone), abundant in green leaves and vegetable oils, or as VK2 (menaquinone), which is found in fermented foods, and is also produced by anaerobic bacteria in our digestive tract. There is strong evidence supporting the positive effect of vitamin K supplementation and increased dietary intake on cognitive performance and on the prevention of cardiovascular disease and of osteoporotic bone fractures (8). On the other hand, vitamin K deficiency (which is usually associated with malabsorption, drug interactions and/or antibiotic use) is associated with an increased risk of ageing-related diseases (8). The recommended daily dietary intake of vitamin K for adequate blood coagulation is 90 mg in women and 120 mg in men. However, these levels are not sufficient to prevent the accumulation of vascular calcifications (34).

MGP is an arterial VKDP, which has an inhibitory effect on vascular calcification exerted via multiple

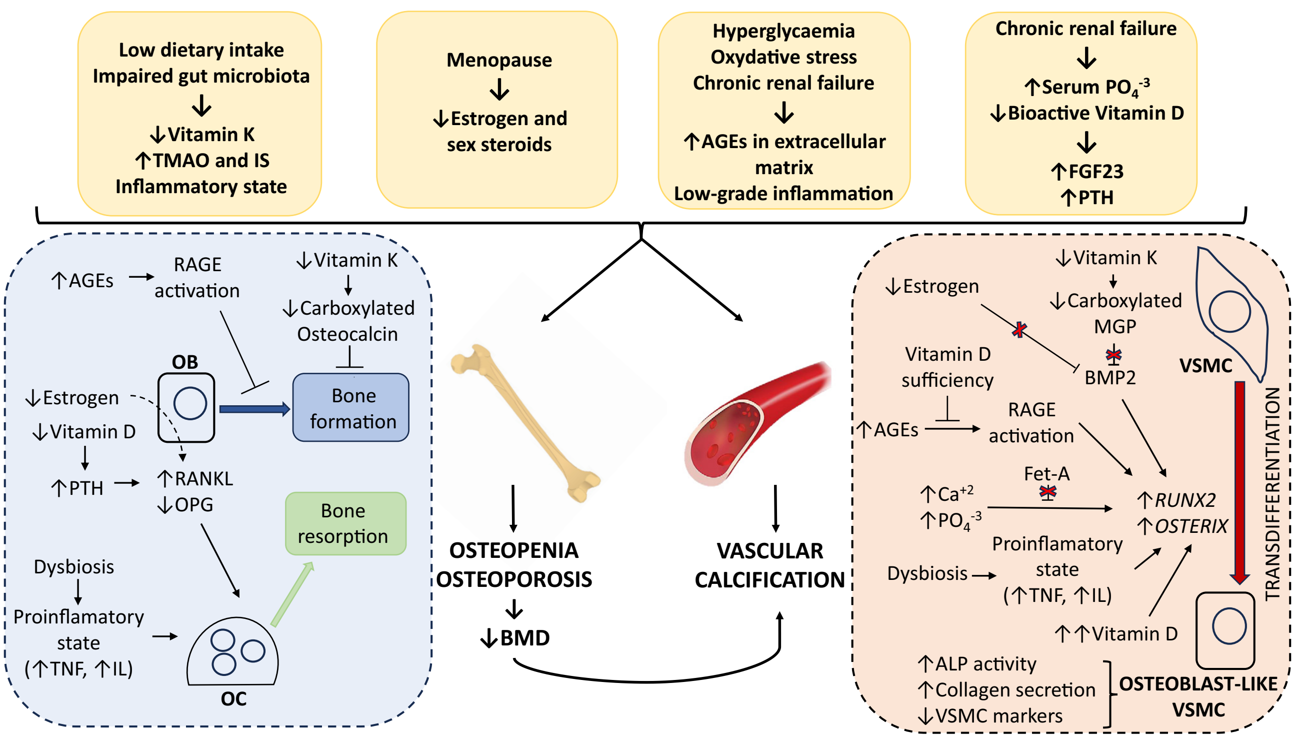

Figure 1
(Dys)regulation of the bone–vascular axis. An increasing body of clinical evidence has confirmed the existence of an inverse relationship between skeletal (physiological) and arterial (ectopic) biomineralization. The study of different animal and *in vitro* models has led to the identification of potential mechanisms for reciprocal bone–vascular regulation, such as vitamin K and D sufficiency, AGE–RAGE interaction, OPG/RANKL/RANK, Fetuin A, oestrogen deficiency and phytooestrogen supplementation, microbiota and its relation to diet, among others.

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mechanisms. The negative charge of MGP provided by its gamma-carboxylated residues allows it to bind and sequester calcium, thus preventing the ectopic growth of hydroxyapatite crystals (33). MGP also induces phagocytosis of these crystals by activated arterial macrophages (35) and interacts with BMP2 to prevent the osteogenic transdifferentiation of VSMC (33). This role is supported by studies carried out in MGP knockout mice, whose survival time does not exceed 8 weeks due to aortic rupture induced by accumulation of AC (33).

OC is another VKDP, synthesized by OBs during the process of bone formation. It possesses three potential gamma-carboxy-glutamic residues. When these sites are fully carboxylated, OC increases its affinity for calcium and hydroxyapatite, and for this reason, it is considered a biomarker of bone formation (33). But when it is in its decarboxylated form, OC is released into the circulation in an endocrine function as a regulator of energy metabolism (33). Several studies have shown that carboxylated OC can also localize to calcified atherosclerotic intimal lesions and in the tunica media of arteriosclerotic vessels, suggesting a potential contribution in stimulating VSMC osteogenic transdifferentiation (35).

Vitamin K antagonists (warfarin, acenocoumarol etc.) are drugs of first choice for long-term anticoagulant therapy and for the prevention of thromboembolic events in individuals with atrial fibrillation. However, their clinical use may be associated both with an increase in the accumulation of coronary and peripheral AC (increasing cardiovascular risk for the patient) (36) and with an increased risk of osteoporotic bone fractures (37). This could be particularly relevant for patients with underlying conditions that predispose to alterations in bone and vascular mineralization, such as chronic kidney disease and diabetes mellitus, since these patients frequently require chronic treatment with oral anticoagulants.

Osteoprotegerin

The OPG/RANK/RANKL system is a fundamental regulator of bone modelling and remodelling. OPG is a soluble protein that functions as an inhibitor of the interaction between RANKL and its receptor RANK, resulting in the inhibition of osteoclastogenesis and, therefore, of bone resorption (14). Recent research suggests that OPG also plays a significant role in the pathogenesis of AC; however, its contribution has not been fully elucidated. OPG is a protein secreted by both OBs and vascular endothelial cells in response to inflammatory stimuli. This suggests that, in addition to its anti-osteoporotic effect, it could have a modulatory role in vascular injury, inflammation and atherosclerosis (9).

OPG knockout mice show osteoporosis and accumulation of AC, and both conditions can be partially reversed by the injection of recombinant OPG (38). This may not be the same for humans: in patients on haemodialysis, higher OPG plasma levels are associated with an increase in radial artery medial calcifications, in carotid intima-media thickness and in carotid intimal calcified atherosclerotic plaques (39), suggesting a role for OPG in the development of AC. However, this could also be reflecting greater endothelial secretion of OPG in areas of atheromatous lesions, as a local mechanism for protection against vascular damage (9, 38).

AGE–RAGE interaction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action or both. Landmark studies such as DCCT and UKPDS have established that persistent hyperglycaemia is one of the main causal factors for chronic complications of diabetes mellitus (40). These complications include diabetic osteopathy with an increase in bone fragility fractures (10), as well as accelerated atherosclerosis, arteriosclerosis and their cardiovascular consequences (40). Chronic hyperglycaemia (as well as systemic oxidative stress, carbonyl stress and/or decreased renal clearance) leads to the accumulation of AGEs on proteins with long half-lives, including collagen of cartilage, bone, blood vessels, tendons and skin (10, 40). Thus, AGEs greatly increase their tissue accumulation in conditions such as diabetes mellitus, metabolic syndrome and chronic renal failure.

Because accumulated AGEs are irreversible and bulky and form uncontrolled intra- and inter-peptide crosslinks, they can directly modify protein and tissue bioactivity (40). For example, in the vascular wall, the accumulation of AGEs on collagen and elastin can increase arterial stiffness, impair NO-mediated vasodilation and induce endothelial dysfunction (41), while the accumulation of AGEs in bone extracellular matrix is related to structural changes that affect the quality of the biomaterial and its biomechanical performance (Fig. 2). Chronic hyperglycaemia and bone accumulation of AGEs synergistically and negatively affect the bone mineralization process, increasing bone fragility (10, 42).

In addition to the effects induced by AGEs on protein bioactivity by direct structural modification, several plasma membrane receptors have been discovered that

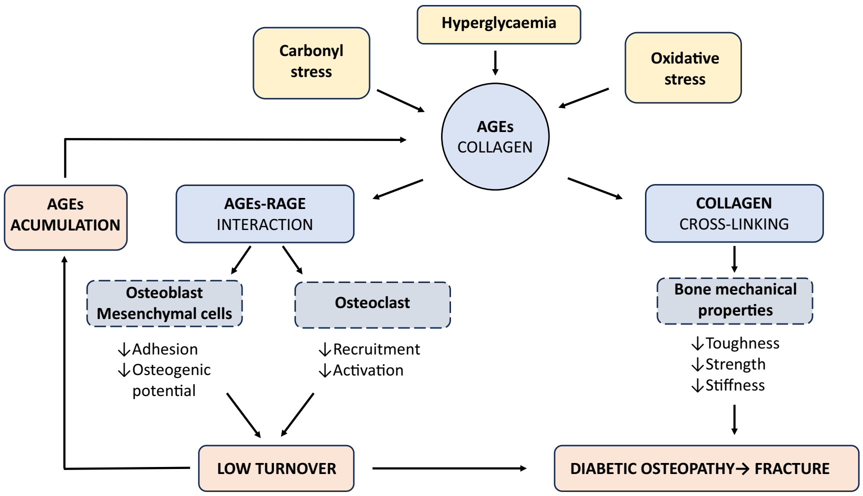

Figure 2
AGE–RAGE and bone metabolism. In ageing individuals (particularly in the context of diabetes mellitus and/or chronic kidney disease) advanced glycation end-products (AGEs) can accumulate on bone collagen, directly altering its biomechanical properties. Bone cells express RAGE, and binding of this receptor to AGEs induces different effects: a decrease in osteoblastic bone formation, a decrease in osteoclastic recruitment and activation and an increase in osteocyte apoptosis. These combined effects can decrease bone turnover which, together with AGEs modification of collagen, significantly reduces bone quality and increases fracture risk.

possess high affinity for AGEs, among which RAGE stands out. AGE–RAGE interaction induces signal transduction mechanisms and changes in cell physiology that are specific to each specific cell type, and RAGE expression has been demonstrated in macrophages, VSMC, endothelial cells, OBs, osteocytes and OCs(among many other cell types) (10).

Research from our laboratory and others has shown that, in bone, AGE–RAGE interaction decreases both osteoblastic formation and osteoclastic resorption; this reduces bone remodelling and favours the accumulation of imperfections, negatively affecting bone quality (Fig. 2).

On the other hand, studies carried out with murine and human VSMC have shown that AGE–RAGE interaction in this cell type induces their transdifferentiation from a contractile myocytic phenotype to a secretory osteogenic phenotype (6, 12), capable of secreting and mineralizing a collagen-rich extracellular matrix. These studies suggest a role for AGE–RAGE signalling in the accumulation of vascular calcifications, particularly in conditions such as diabetes mellitus and chronic renal failure that are associated with vascular accumulation of AGEs (6).

Vitamin D levels

Vitamin D can be obtained from food such as oily fish, eggs and vitamin D-fortified products, or it can be synthesized in the skin after exposure to sunlight. In cases of dietary deficit and particularly in people with low sun exposure (during winter months and/or in geographical areas far from the equator), the resulting vitamin D insufficiency can be reversed by pharmacological supplementation (43). The classic actions of vitamin D are primarily associated with bone and mineral health. vitamin D also regulates a wide spectrum of other so-called non-classical effects, impacting among others on the cardiovascular system.

The skeletal effects of vitamin D are well known (44): vitamin D deficit can cause rickets, osteomalacia and secondary hyperparathyroidism. In particular, sufficiency levels of hydroxylated vitamin D inhibit the synthesis and secretion of PTH and prevent the proliferation of PTH-producing cells: 1,25$(OH)_2$D (systemic or locally produced) upregulates calcium-sensor receptor expression and thus sensitizes parathyroid cells to calcium inhibition. Thus, vitamin D deficiency induces secondary hyperparathyroidism, increasing bone resorption and thus fracture risk.

Epidemiological studies indicate that vitamin D deficiency also increases cardiovascular risk factors, promoting atherogenesis (45). Clinical research has demonstrated an association between serum vitamin D concentrations below 24 ng/mL, and an increased risk of cardiovascular mortality (46). Results from clinical investigations and studies in animal models show that hypovitaminosis D induces endothelial dysfunction, increased expression of pro-inflammatory cytokines such as TNFa, IL1b, IL6 and the Msx2-Wnt signalling pathway (46), and the induction and accumulation of vascular calcifications. vitamin D could also prevent an increase in cardiovascular risk by additional mechanisms: we have recently shown that vitamin D sufficiency levels can prevent the *in vitro* osteogenic transdifferentiation of VSMC, induced by AGE–RAGE interaction (12).

Multiple animal models have shown that hypervitaminosis D can also induce CA accumulation; however, the mechanisms mediating this effect are

unknown (1, 46). This has not been confirmed in clinical studies, since hypervitaminosis D is rarely observed in humans.

Oestrogens and phytoestrogens

Decrease in oestrogen levels at menopause is not only the main cause for primary osteoporosis but also increases cardiovascular risk due to a higher predisposition to develop atherosclerosis. In bone, oestrogen inhibits resorption by decreasing the RANKL/OPG ratio, inducing an increase in BMD (14). At a cardiovascular level, oestrogens are important cardioprotective agents through different mechanisms: they curb the production of inflammatory cytokines, preventing the initiation of inflammatory vascular injury and atherosclerosis (47), and they decrease the burden of AC by promoting autophagy. Clinical studies have shown that in postmenopausal women, oestrogen replacement therapy reduces the risk for cardiovascular disease by decreasing the accumulation of AC (48) while generating an improvement in bone mass (49); chances of success increase when these treatments are initiated early.

Phytoestrogens are a group of plant-derived compounds, structurally and functionally similar to oestrogens. In recent years, interest in their consumption has grown because they may reduce menopausal symptoms, and several studies show that they can be a safe alternative to hormone replacement therapies (50). The consumption of isoflavones from soy, such as genistein, can improve serum lipid profile in postmenopausal women by reducing LDL cholesterol and increasing HDL cholesterol (50), and it can also reduce the accumulation of AC by inhibiting the osteogenic transdifferentiation of VSMC (51). The potential use of phytoestrogens as osteoprotectants in women with postmenopausal osteoporosis has been investigated. In this context, phytoestrogens have been shown to be effective in decreasing RANKL, osteoclastogenesis and inflammatory markers while simultaneously increasing osteoblast-mediated bone formation (52).

Microbiota

Current evidence supports the idea that the intestinal microbiota plays an important role in regulating various physiological and pathological processes related to human health, including the regulation of bone homeostasis and cardiovascular health.

Although the mechanisms by which the microbiota regulates bone metabolism are not precisely known, it has been suggested that metabolites such as short-chain fatty acids (SCFAs), originated from the fermentation of dietary fibre, may interact with the endocrine system and induce the secretion of insulin-like growth factor 1 (IGF1), thus promoting bone growth and development (53).

Dysbiosis is an alteration in microbiota composition which can be induced by conditions such as inflammatory bowel diseases, or simply by Western-style diets (rich in sugar and refined fats, poor in vitamins and minerals). In this situation, a loss of bacterial diversity can be observed at the expense of an increase in microorganisms that favour chronic intestinal inflammation, which in turn can induce chronic low-grade systemic inflammation, contributing to bone loss and atherogenesis (54). In addition, low consumption of nutritional fibre can increase the production of SCFA of bacterial origin, which increases intestinal permeability allowing the absorption of pro-inflammatory bacterial fragments such as lipopolysaccharides (54).

On the other hand, an excessively protein-rich diet (red meat and dairy products) favours the production of metabolites by the microbiota such as trimethylamine N-oxide (TMAO) and indoxyl sulphate (IS), which can be absorbed, act as toxins and also promote the progression of atherosclerosis (55). The increase in TMAO may be particularly relevant in patients with chronic kidney disease and/or diabetes mellitus, contributing to further increase of their cardiovascular risk (55). Interestingly, IS has been linked with both cardiovascular complications (endothelial damage and AC in patients with chronic kidney disease) (56) and alterations in bone and mineral metabolism: it negatively affects bone formation by inducing resistance to PTH and positively correlates with FGF23 levels in patients with chronic kidney disease-related osteodystrophy (57).

Fetuin A

FetA is the most important inhibitor of ectopic calcification and accounts for 50% of all calcification inhibitory activity (2). FetA knockout animals are more susceptible to developing ectopic vascular calcifications (58). FetA participates in calcium metabolism, thanks to its role as a calcium transporter and scavenger; it binds excess calcium and phosphate and then delivers these minerals to bone (59). In extracellular fluids like plasma, calcium and phosphate are super-saturated. Small increases in phosphate (physiological due to enteric absorption, or pathological due to decreased glomerular

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filtration rate or GFR) could increase the formation of amorphous calcium phosphate. However, extra-osseous calcification normally does not occur due to the inhibitory effect of FetA (59). FetA triggers the precipitation of tiny amorphous calcium phosphate crystals that are immediately absorbed by the protein, thus resulting in the formation of spherical nanoparticles (30–150 nm) (59) called primary calciprotein monomers (CPPs). Thus, primary CPPs prevent ectopic precipitation of hydroxyapatite and transport calcium and phosphate to bone for storage (59). However, if they are mistakenly directed towards the vascular wall, they can induce arteriosclerosis, calcification and arterial stiffness. Primary CPP can also evolve into larger secondary CPP by undergoing a phase transition from an amorphous to crystalline state (59). Secondary CPP, when coming into contact with VSMC, induce their osteogenic transdifferentiation (59). VSMC internalize secondary CPP, then secrete calcium- and phosphate-rich matrix vesicles, which act as nucleation sites to generate extracellular accumulation of AC (1, 59).

Several clinical studies have shown that elevated serum levels of phosphate and of secondary CPP correlate positively with age and inversely with the GFR (60). About 15% of the adult US population suffers from chronic kidney disease (CKD), and this is associated with serious mineral and bone disorders (MBDs), which in turn induce an increase in atherosclerosis, arteriosclerosis, cardiovascular mortality, frailty and bone fractures. In CKD–MBD, there are complex alterations of mineral homeostasis (7): renal insufficiency (i.e. GFR <60 mL/min) leads to high plasma phosphate, which in turn induces an increase in FGF23, decreasing the hydroxylation of vitamin D, and this reduction in bioactive vitamin D generates secondary hyperparathyroidism with increased bone resorption and fragility. Serum calcium and phosphate overload is captured by FetA, generating increases in primary and then secondary CPP, which cause accumulation of AC, thus inducing a significant increase in cardiovascular risk.

Conclusions and perspectives

Over more than 50 years, a large and increasing body of clinical evidence has confirmed the existence of an inverse relationship between skeletal (physiological) and arterial (ectopic) biomineralization. Arterial calcifications can be found in the tunica intima and in the tunica media. Prospective studies have shown that patients with low BMD and/or the presence of fragility fractures have at follow-up a significantly increased risk for coronary and cerebrovascular events and for overall cardiovascular mortality. Similarly, patients presenting with AACs show a significant decrease in the BMD (and an increase in the fragility) of bones irrigated by branches of the abdominal aorta, such as the hip and lumbar spine.

The study of different animal and *in vitro* models has led to the identification of potential mechanisms for reciprocal bone–vascular regulation, such as vitamin K and D sufficiency, AGE–RAGE interaction, OPG/RANKL/RANK, FetA, oestrogen deficiency and phytoestrogen supplementation, microbiota and its relation to diet. Complete elucidation of these potential mechanisms, as well as their clinical validation via controlled studies, will provide a basis for pharmacological intervention that could simultaneously promote bone and vascular health.

Declaration of interest

All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported. Dr Antonio McCarthy is a Senior Editor of *Endocrine Connections*. Dr McCarthy was not involved in the review or editorial process for this paper, in which he is listed as an author.

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