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# The mechanism of vascular calcification – a systematic review

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## Summary

Calcification of vessels reduces their elasticity, affecting hemodynamic parameters of the cardiovascular system. The development of arterial hypertension, cardiac hypertrophy, ischemic heart disease or peripheral arterial disease significantly increases mortality in patients over 60 years of age. Stage of advancement and the extent of accumulation of calcium deposits in vessel walls are key risk factors of ischemic events.

Vascular calcification is an active and complex process that involves numerous mechanisms responsible for calcium depositions in arterial walls. They lead to increase in arterial stiffness and in pulse wave velocity, which in turn increases cardiovascular disease morbidity and mortality.

In-depth study and thorough understanding of vascular calcification mechanisms may be crucial for establishing an effective vasculoprotective therapy.

The aim of this study was to present a comprehensive survey of current state-of-the-art research into the impact of metabolic and hormonal disorders on development of vascular calcification.

Due to strong resemblance to the processes occurring in bone tissue, drugs used for osteoporosis treatment (calcitriol, estradiol, bisphosphonates) may interfere with the processes occurring in the vessel wall. On the other hand, drugs used to treat cardiovascular problems (statins, angiotensin convertase inhibitors, warfarin, heparins) may have an effect on bone tissue metabolism. Efforts to optimally control calcium and phosphate concentrations are also beneficial for patients with end-stage renal disease, for whom vessel calcification remains a major problem.

**key words:** **vascular calcification • osteoporosis • menopause • estrogens • raloxifene**

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## Background

Vascular calcification (VC) was in the past considered to be an imminent, passive, degenerative process involving advanced arthrosclerotic lesions; however, recent research has revealed numerous similarities with actively controlled processes occurring in the bone tissue [1].

Arterial calcification not only accompanies advanced atherosclerosis, but also appears alone in diabetes or renal failure [2,3]. Recently, intense research is being conducted into intracellular interactions and molecular processes underlying arterial calcification.

The ascending aorta and its arch are mainly composed of elastic fibers that stretch during the systole, contracting to its original length during the diastole. This makes the aorta store energy during the systole and gives it back during the diastole, thus supporting the heart in its action of pumping blood. Loss of aortic wall elasticity caused by calcium depositions results in systolic hypertension, which causes left ventricular myocardial hypertrophy, increased oxygen demand, diastolic dysfunction and valve incompetence.

In coronary vessels, calcification restricts the diastole and changes the physical properties of atherosclerotic plaques, depending on the location and size of depositions [4]. In patients with unstable coronary disease and a history of myocardial infarction, the plaques contain lesions with numerous small calcium deposition foci, while in patients with stable coronary disease the plaques contain a few large calcium deposits [5,6]. It has been proven that small depositions increase the probability of atherosclerotic plaque rupture, especially on their edges, while with individual, large calcification foci such risk may even decrease [7,8].

Calcification in aortal valves often causes life-threatening stenoses. Although it was believed that the accumulation of calcium depositions is a passive, degenerative process with no relation to atherosclerosis, research has shown that it is subject to control by mechanisms similar to calcification of vessel walls.

Studies conducted in the United States have revealed that calcium depositions in arterial walls are reported in nearly 30% of Americans over 45 years of age [9]. Vascular calcification risk factors are similar to those of atherosclerosis: hypertriglyceridemia, increased low-density lipoproteins (LDL), decreased high-density lipoproteins (HDL), obesity, and hypertension [10]. It has also been shown that diabetes and renal failure contribute significantly to higher risk of accumulation of calcium depositions in the vessel wall [11,12]. Despite the reduction of classical risk factors, irrespectively of age, the presence of numerous calcium deposits in the vessel wall makes the probability of coronary incidents as high as that in the patients who demonstrate advanced calcification of the aortal arch [13]. Stiffness of vessel walls caused by calcification is very important in pathogenesis of organ damage, overall morbidity and mortality [14,15]. Myocardial perfusion could be affected in the absence of significant stenoses in arteries, which may be attributable to impaired smooth muscle relaxation, endothelial dysfunction, or both, at the microcirculation level [16].

## Physiology and Pathology of Tissue Mineralization

### Biomineralization

Under physiological conditions, in both serum and tissue fluid there is sufficient concentration of calcium and phosphate ions necessary for the mineralization process. Biomineralization involves different types of cells and their products. The whole system of factors and mechanisms controls and regulates the hydroxyapatite formation – the macromolecular components of organic matter such as collagen, non-collagen proteins (osteonectin, chondrocalcin), the osteoprotegerin/receptor activator factor NF-κB/ receptor activator factor NF-κB ligand system (OPG/RANK/RANKL), phosphoproteins, glycoproteins, proteoglycans and proteolipids. Pyrophosphates, carbonates and magnesium ($Mg^{2+}$) ions also play a regulative role in the biomineralization process [17].

Initiation of the biomineralization process requires the presence of so-called crystallization nucleators, which trigger the formation of primary crystal nucleus and the removal of mineralization inhibitors such as multipass transmembrane protein transporter (ANK), nucleotide pyrophosphatase (NPPS), matrix Gla protein (MGP) [18] or fetuin-A. Interaction of processes that stimulate and inhibit biomineralization occurs on many levels, both intra- and extracellular, and involves different cell structures such as mitochondria and matrix vesicles. Mutual interactions between organic matrix components and calcium and phosphate ions are also crucial here, along with the interaction between these ions and the already-deposited phosphates.

Extracellularly located matrix vesicles contain deposits of calcium and large quantities of alkaline phosphatase (ALP), pyrophosphatase, ATPase, 5'-AMPase, glucose-6-phosphatase and phospholipase $A_2$. Alkaline phosphatase increases the level of inorganic phosphates in the vesicles; it also degrades pyrophosphates [19]. The latter function as inhibitors of calcium phosphate precipitation and hydroxyapatite crystal growth and as production stimulators of osteopontin – another nucleation inhibitor [20,21]. Matrix vesicles contain large amounts of free cholesterol, phospholipids, glycolipids, substantial quantities of phosphatidylserine and the calcium-binding proteins annexin II and V. Annexin V, in the presence of calcium and phosphate ions, binds with phosphatidylserine to form the calcium-phosphate-lipid complex within the membrane of a vesicle. The complexes further become nucleators that cause primary centers of hydroxyapatite to originate out of unstable solutions containing calcium and phosphate ions. In the extracellular space the calcium and phosphate ions continue to be caught and accumulated, so the quantity of the mineral gradually increases.

### Ectopic calcification

During the calcification of vessels there occur processes similar to those in bone tissue biomineralization. In depositions located in both tunica interna and media of the vessel wall, matrix vesicles have been identified [22]. Samples from the walls of calcified vessels as well as *in vitro* studies revealed the expression of macromolecular matrix proteins involved in the regulation of biomineralization (ie, bone morphogenetic protein -2 (BMP-2), osteopontin, MGP, osteonectin, collagen, and osteocalcin) [23–25] (Figure 1).

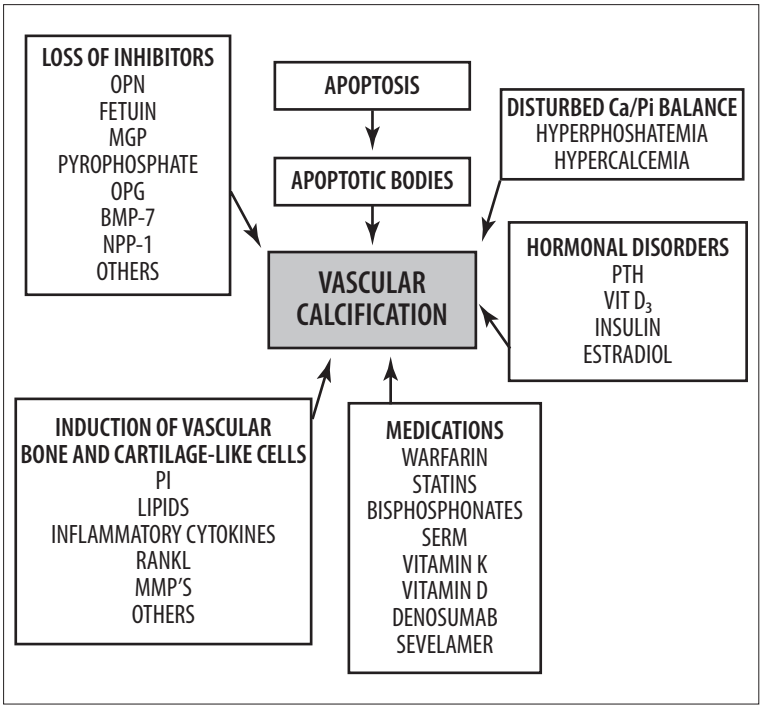


**Figure 1.** Pathogenesis of vascular calcification: *OPN* – osteopontin; *MGP* – γ-carboxyglutamine acid, matrix-GLA protein; *OPG* – osteoprotegerin; *BMP* – bone morphogenic protein; *NPP* – nucleotide pyrophosphatase phosphodiesterase; *Pi* – phosphorus; *RANKL* – receptor activator factor NF-κB ligand; *MMP'S* – metalloproteinases; *PTH* – parathormone; *VIT D$_3$* – vitamin D$_3$; *SERM* – selective estrogen receptor modulators.



The ability of osteogenic transformation and production of matrix vesicles demonstrate pericytes in microvessels and in tunica externa of large arteries, myocytes in tunica media, and myofibroblasts in adventitia [23]. These cells are able to differentiate into many cell types and form the bone, cartilage, adipose, muscle, or even bone marrow tissue [26]. As shown by postmortem studies, vessel walls may contain a typical bone, cartilage or adipose tissue, with bone as the predominating type of metaplasia (to be found in 10–15% of samples), appearing in various morphological forms – from amorphous calcium deposits to mature bone tissue. Similarly as in the bone, calcification in vessel walls is subject to remodeling. Once the calcification activating factors have been eliminated, calcium deposits may even be reduced [27,28]. Statin-based hypolipemic therapy reduces the intensity of calcification of vessel walls and cardiac valves [29]. Using animal models, it has been found that vitamin D$_3$ or warfarin-induced calcification of vessels is subject to regression after the inducing factor is eliminated or its antagonist is administered [30,31]. The bone tissue resorption process most probably involves participation of phagocytes – derivatives of monocytes, with function similar to that of osteoclasts in the bone [31].

## Histoanatomic View of Vascular Calcification

### Classification

The vessel calcification process may be classified according to its histological structure, anatomical localization or etiological criteria (Table 1). Histologically, deposits may have the structure of bone or cartilage tissue, or they may appear in an amorphous form.

Depending on the vessel wall layer, one can speak of (1a) the tunica interna calcification, especially related to atherosclerotic plaque, and atherosclerosis-unrelated (1b) calcification of tunica media (MAC-medial artery calcification, Mönckeberg's-type). The latter group embraces also so-called (1b') calcific uremic arteriolopathy (formerly known as calciphylaxis), affecting the arteriolar vessel walls and leading to the development of organ insufficiency or failure.

Etiologically, calcification can be divided into metastatic and dystrophic. The former type occurs when the concentration of calcium and phosphates is increased, affects healthy tissues and typically accompanies such diseases as hyperthyroidism, neoplasms, milk-alkali syndrome and vitamin D$_3$ overdose. The latter type appears with normal concentration of calcium and phosphate in damaged or necrotic tissues (atherosclerosis, neoplasm, tuberculosis, parasites) [32].

#### *(1a) Intimal atherosclerotic calcification*

This is the most common form of calcium accumulation, particularly in persons demonstrating risk factors of atherosclerosis development, and also in patients suffering from chronic arterial hypertension or osteoporosis. Calcium accumulation is initiated by an increase in atherosclerotic plaque of modified lipid content, pro-inflammatory cytokines, phosphate and lipoprotein complexes, as well as foci of necrosis. A large part of clinical research relates history of long-lasting dyslipidemia to the progression and development of calcification foci [33]. Some studies have indicated the effectiveness of statins in restricting the progression of calcium accumulation in vessel walls [30,34,35]; however, more recent randomized clinical trials have not proved this [36,37]. *In vitro* studies have shown that pro-inflammatory cytokines, oxidized low-density lipoprotein (oxLDL), or other monocyte/macrophage release products promote osteogenesis and the accumulation of calcium deposits [38–40]. On the other hand, increased content of antioxidant factors such as omega-3 fatty acids, or HDL cholesterol results in diminished mineralization characterized by reduced

**Table 1.** Macrovascular calcification: a histoanatomic view.

| Histoanatomic variant | Selected characteristics | Disease example/association |
|---|---|---|
| Atherosclerotic calcification | Cellular necrosis and debris<br>Evolution of fibro-fatty plaque<br>Calcium deposition with lipoproteins, cellular lipid debris<br>Macrophages, T cells, endothelial dysfunction, platelet and myofibroblast activation<br>Cartilage metaplasia/calcified cartilage, endochondrial bone formation<br>Marrow formation with hematopoiesis visualized in advanced disease | Atherosclerosis<br>Hypercholesterolemia<br>Hypertension<br>Inflammation<br>Osteoporosis<br>Similar histology in calcifying fibrotic myocardial infarct |
| Cardiac valve calcification | Interstitial cell activation/inflammation<br>T cells, macrophages, interstitial adipocytes, and myofibroblasts<br>Dystrophic calcium deposition<br>Osteogenesis, intramembranous (nonendochondrial) bone formation<br>Rare cartilage metaplasia/calcified cartilage, infrequent endochondrial bone formation<br>Marrow formation with hematopoiesis visualized in advanced disease | Senile calcific aortic sclerosis<br>Bicuspid aortic valve calcification<br>Bioprosthetic valve calcification<br>Hyperlipidemia<br>Congenital bicuspid valve<br>Rheumatic heart disease |
| Medial artery calcification (Mönckeberg's medial calcific sclerosis) | Adventitial activation/inflammation<br>Macrophages, T cells, myofibroblasts, adipocytes, medial VSMCs and CVCs<br>Matrix vesicles, with osteogenesis resembling intramembranous (nonendochondrial) bone formation<br>No cartilage formation | Type 1 diabetes<br>Type 2 diabetes<br>End-stage renal disease<br>Hyperphosphatemia |
| Vascular calciphylaxis | Amorphous calcium phosphate deposition with widespread organ and soft tissue involvement<br>Serum calcium-phosphorus product >60 mg/dl<br>No osteogenesis<br>No chondrogenesis | End-stage renal disease<br>Acute renal insufficiency with muscle injury<br>Tumor lysis<br>Iatrogenic hiperphosphatemia<br>Warfarin overdose |

*VSMCs* – vascular smooth muscle cells*; CVCs* – calcifying vascular cells (adapted from Vattikuti R, Towler DA: Osteogenic regulation of vascular calcification: an early perspective. Am J Physiol Endocrinol Metab, 2004; 286: E686–96).

expression of ALP. The influence of omega-3 acid primarily results from the activation of mitogen-activated protein kinase (MAPK kinase) or peroxisome proliferator-activated receptor-alpha (PPAR-gamma) pathways [41]. HDL also reduces ALP expression and prevents calcification induction via its influence on Il-1, Il-6, and oxLDL [42].

Under *in vitro* conditions, the calcifying vascular cells (CVCs) (a subpopulation of smooth muscle cells with osteoblastic characteristics that spontaneously form bone mineral) present in atherosclerotic plaques may spontaneously produce mineral that clusters locally as small lumps, histologically resembling atherosclerotic plaque or lesions originating on heart valve cusps [1]. Area density of calcification foci depends on the presence of stimulators and inhibitors of calcium accumulation (Table 2). Increased concentration of transforming growth factor beta (TGF-β), vitamin $D_3$ (Vit. $D_3$) or warfarin – a popular MGP inhibitor – increases the number and size of calcification foci [43,44].

***(1a') Cardiac valve calcification***

Dystrophic calcification most often affects the aortic valve, as a result of long-standing action of mechanical stress and pro-inflammatory factors. It has been proven that the connective tissue of calcified aortal valve stroma demonstrate disturbed organization of elastin, lipid depositions, chronic inflammation, fibrosis, stippled calcium deposits, macrophage and T-lymphocyte infiltration [45,46]. Similar changes in aortal valve stroma were observed in persons who did not exhibit evident features of atherosclerosis [46], which may be a proof that early stages of calcification of valve cusps involve different mechanisms and may anticipate atherosclerosis development in coronary vessels. During the progression of changes, cellular and molecular analysis of valves revealed osteoblastogenesis, the presence of CVCs, presence of chronic inflammation markers, increased expression of osteopontin, BMP, and mature bone tissue in as much as 10% of the samples [47–49]. The substantial contribution of dyslipidemia in heart valve calcification pathogenesis has been proven, thus establishing the favorable influence of atorvastatin [50] and eplerenone (aldosterone receptor inhibitor) on valvular change progression [51]. It has been shown that treatment with 3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors increased the protein expression and functional activity of endothelial nitric oxide synthase (eNOS), improved C-reactive protein (CRP) concentrations, increased nitrite concentrations, and decreased calcification in aortic valves. These changes coincided with inhibition of calcification in the aortic valve and

**Table 2.** Factors involved in arterial calcification.

| Stimulators | Inhibitors |
|---|---|
| Inorganic phosphate | Pyrophosphate |
| TGF-β | Statins |
| 25-hydroxycholesterol | N-3 fatty acids |
| cAMP | Tropoelastin |
| MAP kinase | Bisphosphonates |
| Acetylated LDL | Matrix Gla Protein |
| Homocysteine | BMP-7 |
| Glucose | Osteopontin |
| Endothelin-1 | Osteoprotegerin |
| Elastin degradation products | NPP-1 |
| Pit-1 | Ahsg (Fetuin-A) |
| Leptin | |
| BMP2-Msx2-Wnt | |
| MMPs | |

*TGF* – Transforming growth factor; *cAMP* – cyclic adenosine monophosphate; *MAP* – mitogen-activated protein kinase; *LDL* – low-density lipoprotein; *Pit-1* – sodium dependent phosphate transporter; *NPP* – nucleotide pyrophosphatase phosphodiesterase; *PPi* – inorganic pyrophosphate. *BMP* – bone morphogenic protein; *BMP2-Msx2-Wnt* – wingless signaling pathway; *MMPs* – metalloproteinases (adapted from Guzman RJ: Clinical, cellular, and molecular aspects of arterial calcification. J Vasc Surg, 2007; 45: 57A–63A).

with inhibition of important bone matrix markers critical in osteoblast differentiation [50]. Animal studies showed that eplerenone treatment decreased macrophage accumulation and angiotensin-converting enzyme expression, as well as calcium deposition in the leaflets. Even though it increased aldosterone levels, eplerenone did not affect blood pressure, cholesterol or potassium levels [51]. Additionally, eplerenone improves endothelial function by reducing superoxide formation, increasing NO bioavailability and reducing platelet activation in diabetic rats [52].

Calcium deposits are found in the mitral valve and are most often described in the context of cartilaginous metaplasia of the valvular annulus. It has been observed among coronary artery disease patients that high level of fetuin-A in serum prevents dystrophic calcification of the mitral valve irrespective of co-existent diabetes; it also prevents calcification of the aortal valve; however, this applies only to patients without co-existent diabetes [53]. In 2007, Willens showed that calcification of the mitral valve ring is an excellent predictor of cardiac death, independent of the other risk factors of cardiac events [54].

***(1b) Medial artery calcification (MAC)***

Most intense calcium-phosphate accumulation in the muscle layer of blood vessel wall is observed in patients with diabetes and chronic renal disease [55,56]. It has been established that advanced MAC is connected with increased risk of sudden cardiac death and lower limb amputation due to vascular insufficiency [56–58].

In diabetes, free oxygen radicals induce the development of chronic inflammation in adventitia, leading to the infiltration of connective tissue by macrophages and T-lymphocytes. Hyperglycemia and glycosylation end products (AGEs) increase production of tumor necrosis factor α (TNF-α), BMP-2, osteopontin (OPN), increase expression of msh homeobox homolog 2 (Msx-2) gene. The expression of BMP-2 and Msx-2 particularly arises in adventitial myofibroblasts, while that of OPN arises in muscle cells of tunica media. Inflammatory mediators stimulate proliferation, osteogenic differentiation and migration of myofibroblasts to tunica media, resulting in the thickening, remodeling and calcification of this layer [40,59,60]. The processes leading to calcium accumulation in diabetes are regulated primarily by the wingless signaling pathway (BMP-2/Msx-2/Wnt), typical of intramembranous bone formation [61].

In end-stage renal diseases (ESRD), the excess of calcium and phosphate ions stimulates osteogenic differentiation via the Runx2/Cbfa1 signaling pathway [62]. Inorganic phosphates stimulate vascular smooth muscle cells (VSMCs) to differentiate into CVCs through the activation of sodium-dependent phosphate transporter (PiT-1) with a subsequent cascade of the Runx2/Cbfa1 pathway [63,64]. *In vitro* studies have revealed that (by contrast to normophosphatemia) under the conditions of hyperphosphatemia, BMP-2 intensifies VSMCs calcification, and increases PIT-1 concentration and the expression of PiT-1 mRNA [65].

An important role in the calcification process is attributed to metalloproteinases (MMPs). They are secreted by inflammatory cells in adventitia and tunica media, and cause increase of elastin metabolites which, in turn, activate other monocytes and thus intensify the inflammatory processes [66,67]. Elastin degradation products may become crystallization nucleators that initiate the formation of crystal nuclei [68]. It was observed that metalloproteinase 9 (MMP-9) promotes calcium deposition in warfarin-induced calcification [69], and is responsible for the aortal wall calcification in Marfan's syndrome [70]. Metalloproteinase inhibition breaks the vicious cycle of monocyte activation and restricts vascular calcification.

***(1b') Calciphylaxis (CUA – calcific uremic arteriolopathy)***

Calciphylaxis is a type of calcification of tunica media of the vessel wall and affects small arterioles (<0.6 mm diameter). CUA can be described as patchy medial calcification with intimal proliferation, fibrosis, thrombotic occlusion and consequently, ischemia and necrosis of the skin, subcutaneous tissue, internal organs or muscles. This pathology mainly affects patients with chronic kidney disease (CKD), in particular those to whom warfarin is administered. With an excess of calcium-phosphate products, or lower concentration of precipitation inhibitors, accumulation of amorphous calcium deposition may occur in tissue. Warfarin blocks vitamin K-dependent γ-carboxylation of such proteins as MGP, and Gas-6 – important inhibitors of mineralization [71]. It has been shown that warfarin therapy is associated with aortal stenosis and excessive vascular calcification. Large doses of vitamin K (especially K2) prevent warfarin-induced vascular calcification [71]. It is believed that MGP blocks calcium deposition via 2 mechanisms – directly, forming a complex with fetuin-A; and indirectly, blocking stimulated by BMP-2 osteogenic differentiation [72].

## The Impact of Metabolic Disorders on Vascular Calcification

As shown above, the relation of hypercholesterolemia, lipid oxidation and atherosclerosis with vessel wall calcification is very well documented. Long-lasting dyslipidemia predisposes to the accumulation of calcium deposits in tunica intima, particularly in the regions of atherosclerotic plaques.

Metabolic disorders in hyperglycemia cause both osmotic and oxidative stress, leading to activation of inflammatory cells in the vessel wall (1a,1b). Hyperglycemia causes activation of VSMCs differentiation into osteoblast-like cells and the increased concentration of, among other things, osteopontin, collagen I, alkaline phosphatase, and osteocalcin [73]. Although in diabetes the accumulation of calcium deposits in vessel wall is intensified in both tunica intima and media, MAC is considered to be the most characteristic vessel damage in the course of diabetes [61,73]. In patients with CKD, diabetes enhances vessel wall calcification irrespective of the progression stage of the renal insufficiency [74].

The contribution of hyperphosphatemia in vascular calcification pathogenesis is most obvious in CKD patients; it is also observed in mutation of the fibroblast growth factor 23 (FGF23), which is a powerful inhibitor of tubular phosphate reabsorption [75,76]. Similar effects are produced by mutation in the GALNT3 gene (UDP-N-acetyl-a-D-galactosamine: polypeptide N-acetylglucosaminyltransferase 3). This is an enzyme related to the Golgi apparatus and responsible for O-glycosylation of FGF23, which prevents its proteolytic degradation and enables intact secretion [76]. Mutations in the above-mentioned genes result in the development of hyperphosphatemia, with subsequent vessel wall calcification [77,78]. Recently, randomized clinical trials have confirmed that sevelamer (a polymer that binds phosphates in the small intestine and inhibits their absorption) therapy attenuates the progression of vascular lesions [79,80]. Normalization of calcium and phosphates metabolism may be a major contributor in sevelamer treatment. The other beneficial effects, such as increased serum fetuin-A concentration, lipid and uric acid reduction may also be potential benefits of its administration. Combination therapy with both enalapril (angiotensin convertase inhibitor) and sevelamer had beneficial effects on renal dysfunction and ameliorated secondary hyperparathyroidism, vascular calcification, myocardial hypertrophy and mortality [81]. Therapy using perindopril, another angiotensin convertase inhibitor, improved aortic elastic properties and lowered inflammatory markers levels [82].

Fetuin-A (alpha2-Heremans Schmid glycoprotein; AHSG) is an important circulating inhibitor of calcification *in vivo*, and is downregulated during the acute-phase response, especially in patients with CKD. Fetuin-A concentrations in serum are significantly lower in patients on hemodialysis. Low concentrations of the glycoprotein are associated with raised amounts of CRP, increased calcium deposition and with enhanced cardiovascular and all-cause mortality [83]. Recent studies have emphasized the protective effect of fetuin-A in hemodialysed patients. Age, serum calcium, parathormone (PTH) and fetuin-A are selected as significant variables in the evaluation of predictors of calcification progression [84].

## Hormonal Contribution to The Regulation of Calcification Process

The correlation of osteoporosis with ectopic accumulation of calcium deposits indicates a significant role of calciotropic hormones in the pathogenesis of vascular calcification. Both endothelial cells and VSMCs have vitamin $D_3$ receptors. The influence of calcitriol on vessel homeostasis results from its direct effect on endothelial cells and VSMCs, but also from indirect interaction with other calciotropic hormones and immunomodulation. Activation of vitamin $D_3$ nuclear receptor is followed by the change of expression of more than 150 genes, which results in substantial changes of the cell cycle, reduced proliferation, differentiation and apoptosis of VSMCs [85]. It was found that physiological concentrations of vitamin $D_3$ have a myorelaxation effect, reduce endothelial thrombogenicity, increase fibrinolysis and inhibit inflammatory response [86–88]. Levels of calcitriol that are too high or too low intensify the activity of metalloproteinases (MMP-2, MMP-9), key enzymes for vascular remodeling [89]. Excessive supply of vitamin $D_3$ inflicts intense accumulation of calcium deposits in tunica intima and media, elastin degradation, increased stiffness of vessel walls and left ventricular hypertrophy [90]. Restoration of physiological concentrations of calcitriol results in rapid regression of vascular calcification [91]. Increased concentration of calcitriol, through the suppression of parathormone-like proteins (PTHrP), lead to intensified activation of alkaline phosphatase, which inflicts degradation of pyrophosphates – inhibitors of calcium phosphate precipitation [92,93]; it also results in increased concentration of calcium and phosphate ions, which are important stimulators of matrix vesicle production [94].

### Hormonal disturbances in chronic kidney diseases

Parathormone and PTH-like proteins play an active part in the regulation of vascular calcification (Table 3) [92,93]. Increased PTH concentration and hyperphosphatemia in CKD is considered a significant risk factor for calcium deposit accumulation in the vessel wall [95]. Endothelial and vascular smooth muscle cells are equipped with PTH/PTHrP receptors (PTH1R); increased concentration of PTH-(1-34) attenuates vascular expression of OPN and Msx2, and reduces calcium depositions accumulated in the arterial wall [96]. PTH1 receptor activation provokes alkaline phosphatase suppression, reversible with the receptor antagonist – PTHrP-(7-34) [92,93]. However, regardless of progression stage of CKD, PTH is considered a calcification-promoting factor [41]. PTH inhibits the production and release of osteoprotegerin (OPG), an important osteoprotective factor [97].

Parathormone-like peptides are among the factors that reduce vascular calcification, but we can also find peptides that accumulate in CKD and induce resistance to PTH. PTH-(7-84) binds the PTH1 receptor and, rather than initiating signal transmission, strengthens internalization of the receptor, thus limiting its expression on the cell surface [98]. PTH-(7-84) also inhibits renal production of calcitriol [99].

The majority of clinical trials have focused on vascular calcification in the course of chronic kidney disease, where vitamin $D_3$ concentration is decreased due to reduced number of active nephrons, hyperphosphatemia and developing

**Table 3.** Multiple factors that are deregulated in ESRD and may affect VSMC calcification.

| Factor | Potential Effect |
|---|---|
| P, Ca, Ca x P | VSMC damage, vesicle release, osteogenic differentiation, increased crystal growth |
| PTH/PTHrP | Inhibition of VSMC calcification |
| AGEs | VSMC damage, calcification |
| Inflammation | VSMC osteogenic conversion, decrease in circulating fetuin-A, direct effects on osteo/chondrocytic differentiation of VSMCs |
| Hypertension | VSMC damage, vesicle release, calcium overload |
| Lipids | VSMC osteogenic conversion, inhibition of phagocytosis, increased crystal growth |
| Oxidative stress | Increase in VSMC damage/calcification |
| ABD | Increase in soft tissue calcification. Likely to involve numerous factors affecting both bone and vascular calcification |

*P* – phosphorus; *Ca* – calcium; *PTH* – parathormone; *PTHrP* – parathormone-like proteins; *AGEs* – glycation end products; *VSMCs* – vascular smooth muscle cells; *ABD* – adynamic bone disease.

tissue resistance to calcitriol ($1,25(OH)_2D$) (Table 3). Low serum $1,25(OH)_2D$ levels cause an increase in PTH secretion and the development of secondary hyperparathyroidism. High serum PTH and hyperphosphatemia are known risk factors for increased mortality among patients on permanent dialysis. Treatment with vitamin D receptor activators (VDRAs) improves survival of CKD patients compared to those without this hormone supplementation [100–102]. These relations are independent of PTH levels and calcium and phosphorus products. Treatment with VDRAs inhibits the synthesis of type 1 collagen, reduces cbfa-1 synthesis, stimulates the synthesis of MGP and inhibits BMP-2 production in cultured osteoblastic cells. Vitamin D receptor activation has ameliorating effects on cardiac hypertrophy and inhibits several renin-angiotensin system (RAS) components.

Many studies have presented the key role of the polymorphism of the gene encoding for vitamin D receptor in susceptibility to osteoporosis development. The polymorphisms in the VDR gene are involved in the modulation of vitamin D action and modulate the level of bone mineral density. In 2009 Seremak-Mrozikiewicz et al investigated the frequency of BsmI, ApaI, and TaqI polymorphic variants of the VDR gene in postmenopausal osteoporotic women. They concluded that the presence of T allele of TaqI polymorphism could predict the higher risk of developing osteoporosis in postmenopausal woman, and the presence of A allele (ApaI polymorphism) seems to be connected with osteoporosis susceptibility [103].

It has been shown that very low levels of 25(OH) vitamin D are associated with increased all-cause mortality in patients with and without kidney disease [104]. In patients with 25(OH) vitamin D levels <75 nmol/l, treatment with calcidiol causes significant reduction in PTH levels [105]. Clinical guidelines suggest stringent control of PTH, calcium and phosphate in an attempt to lower the risk of vascular calcification. Kidney Disease Improving Global Outcome (KDIGO) guidelines state that VDRAs should be administered in patients with CKD stage 3 to 5 not on dialysis, in whom serum PTH is rising above the upper limit of normal [106]. Examples of activated vitamin D analogues with this favorable side-effect profile include doxercalciferol, paricalcitol, and alfacalcidol [107].

**Female sex hormones disturbances and vascular calcification**

Female sex hormones also play an important role in bone tissue metabolism: they increase bone density and inhibit osteoclast activity. Estrogens, via their impact on TGF-beta, are osteoblast stimulators; they also stimulate calcitonin production and boost the expression of vitamin $D_3$ receptors in osteoblasts, reduce the production of pro-inflammatory cytokines, and increase OPG gene expression. Estrogens induce the apoptosis of precursors and mature forms of osteoclastic cells. Decreased estrogen concentration in the postmenopausal period is accompanied by increased bone resorption. It is believed that this is connected with increased production of proinflammatory cytokines and weaker activity of endothelial nitric oxide synthase (eNOS), leading to limited production of nitric oxide, which activates osteoblasts and inhibits osteoclasts [108].

Estrogen therapy for postmenopausal women reduces vascular calcification [109,110]. Postmenopausal women with a higher serum estradiol (E2) level had a reduced coronary artery calcium score independent of age and other coronary risk factors. Authors suggest that a higher level of estradiol possibly lowers the calcified-plaque burden of coronary arteries in postmenopausal women [111]. This is due to the indirect effect on calcification risk factors, as well as direct genomic and extragenomic effects on macrophages, endothelial cells and VSMCs. Estradiol has been found to modulate the secretion of matrix proteins (osteopontin, BSP, MGP, RANKL/OPG), and attenuates proliferation and differentiation of VSMCs and the activity of calcifying cells [112,113].

Despite the pleiotropic, cardioprotective effect of estrogens, randomized clinical trials have not confirmed the protective effect of hormone replacement therapy (HRT) on postmenopausal women. The findings of randomized clinical trials such as the Women's Health Initiative (WHI), Heart and Estrogen/progestin Replacement Study (HERS) or Estrogen Replacement and Atherosclerosis Study (ERAS), related also to the effect of HRT on coronary disease, have not provided clear evidence of the positive therapeutic effect of atherogenesis reduction in coronary vessels, or of lowered risk of cardiovascular complications. The trials revealed a temporary increase of risk for cardiovascular complications, especially

in the first year of therapy. The WHI study has shown higher risk of ischemic shock, with no increase of hemorrhagic cerebral stroke; the risk increased substantially with increasing age of women under treatment. Since then, HRT has not been used as prevention of cardiovascular complications on postmenopausal women. Current US Food and Drug Administration (FDA) recommendations limit menopausal hormone treatment to the "shortest duration consistent with treatment goal", with goals generally taken to mean relief of menopausal symptoms and maximal duration as approximately 5 years.

Despite long-term research, the influence of hormone replacement therapy (HRT) on the cardiovascular system is still subject to debate: the effects of HRT depend on the presence of risk factors, the time HRT is started, or type of administered drugs. Research findings suggest that early implementation of postmenopausal estrogen therapy may substantially reduce the risk of cardiovascular events. The Kronos Early Estrogen Prevention Study (KEEPS) was recently conducted to assess the effect of HRT on the cardiovascular system of women in the early stage (up to 3 years) following menopause. One of the aims of this study is the assessment of estradiol effect on coronary artery calcification [114].

**The impact of raloxifene on vascular calcification**

Selective estrogen receptor modulators (SERM), when stimulating the receptor in the cardiovascular system and bone tissue, prove to have an advantageous estrogen-like effect. Raloxifene (Evista) is a non-steroidal selective estrogen receptor modulator (SERM) demonstrating, depending on the target tissue, either agonistic or antagonistic effects. Raloxifene therapy has a beneficial impact on cardiovascular risk factors: increased concentration of HDL-cholesterol, and decreased levels of LDL-cholesterol, fibrinogen, homocysteine, and lower intensity of inflammatory processes. However, the 6-year Raloxifene Use for The Heart (RUTH) trial has not confirmed a positive effect of raloxifene on the progression and complication of coronary heart disease (CHD) in post-menopausal women. It has also been proved that raloxifene lowers the rate of clinical vertebral fractures and estrogen-dependent breast cancer, but also increases the incidence of blood clots and death from stroke. A statistically significant decrease of the cardiovascular event rate was only observed in a group of women under 60 years of age. Raloxifene, used in postmenopausal osteoporosis therapy, affects the synthesis and release of intercellular matrix proteins, proliferation and differentiation of VSMCs [112,113] and has a protective effect on vascular endothelium by, among others, increased expression of eNOS [115]. The mechanisms involving the reducing effect of raloxifene on cardiovascular events rate in women in the early postmenopausal stage have not been fully explored.

**The impact of estradiol and raloxifene on OPG/RANK/RANKL.**

Over the last few years intensive studies have been conducted that assess the OPG/RANK/RANKL system contribution to the vascular calcification process. Osteoprotegerin appears to represent the molecular link between bone resorption and vascular calcification, and may help to understand the high prevalence of atherosclerosis and osteoporosis in postmenopausal women. Serum OPG is potentially an independent predictor of early vascular adverse changes in osteoporotic postmenopausal women [116]. Helas showed that unopposed RANKL activity in osteoprotegerin-deficient mice resulted in osteoporosis and vascular calcification. RANKL inhibition by denosumab reduced vascular calcification in prednisolone-induced osteoporosis in mice, which is further evidence for the link between the bone and vascular systems [117].

Animals subjected to ovariectomy demonstrated a substantial increase of calcium deposit accumulation in the vessel wall, as well as an increase of the OPG/RANKL ratio, primarily resulting from a decrease of RANKL concentration (as opposed to the bone system) [118]. Clinical trials have established that raloxifene therapy significantly lowers the level of RANKL and temporally decreases the level of OPG (its normal level is restored after a year of therapy). The main store of OPG is the bones and cardiovascular system. It is still unclear whether the influence of raloxifene on the concentration of OPG and RANKL in serum is mainly due to its osteoprotective effect, or due to the processes occurring in the vessel wall [119].

## Conclusions

There is a strong correlation between vascular calcification and the occurrence of cardiovascular diseases – VC is recognized as a significant predictive factor for cardiovascular events, including ischemic heart disease and death. Vascular calcification presents as an active and complex process that involves numerous mechanisms responsible for calcium deposition. In-depth study and thorough understanding of such mechanisms may be crucial for establishing an effective vasculoprotective therapy. Due to a great resemblance to the processes occurring in the bone tissue, drugs used for osteoporosis treatment (calcitriol, estradiol, bisphosphonates) may interfere with the processes occurring in the vessel wall. On the other hand, drugs used to treat cardiovascular problems (statins, angiotensin convertase inhibitors, warfarin) may have an effect on bone tissue metabolism.

The increase of osteoporosis parallel to the development of calcification in postmenopausal women proves that the role of female sex hormones is vital to the regulation of deposition and resorption processes in bone tissue. Despite long-term research, the influence of hormone replacement therapy on the cardiovascular system is still subject to debate. The mechanisms involved in the reducing effect of SERM (raloxifene) on cardiovascular events rate in postmenopausal women need to be thoroughly examined.

**Abbreviations**

1,25(OH)2D – 1,25-dihydroxycholecalciferol, calcitriol
5'-AMPase – 5'-nucleotidase
AGEs – glycosylation end products
AHSG – alpha2-Heremans Schmid glycoprotein
ALP – alkaline phosphatase
ANK – multipass transmembrane protein transporter
ATPase – adenosine triphosphatase
BMP-2 – bone morphogenetic protein-2
CHD – coronary heart disease

CKD – chronic kidney disease
CRP – C-reactive protein
CVCs – calcifying vascular cells
CUA – calcific uremic arteriolopathy
eNOS – endothelial nitric oxide synthase
ERAS – Estrogen Replacement and Atherosclerosis Study
ESRD – end stage renal diseases
FGF23 – fibroblast growth factor 23
GALNT3 – UDP-N-acetyl-a-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase 3
HDL – high density lipoproteins
HERS – Heart and Estrogen/progestin Replacement Study
HMG-CoA – 3-Hydroxy-3-methylglutaryl-coenzyme A
HRT – hormone replacement therapy
IHD – ischemic heart disease
KDIGO – Kidney Disease Improving Global Outcome
KEEPS – The Kronos Early Estrogen Prevention Study
LDL – low density lipoproteins
MAC – medial artery calcification
MAPK kinase – mitogen-activated protein kinase
MGP – matrix gla protein
MMPs – metalloproteinases
mRNA – messenger ribonucleic acid
Msx-2 – msh homeo box homolog 2
NPPS – nucleotide pyrophosphatase
OPG/RANK/RANKL – the osteoprotegerin/ receptor activator factor NF-κB/ receptor activator factor NF-κB ligand system
OPN – osteopontin
oxLDL – oxidized low-density lipoprotein
PiT-1 – sodium dependent phosphate transporter
PPAR-gamma – peroxisome proliferator activated receptor-gamma
PTH – parathormone
PTH1R – parathormone receptor
PTHrP – parathormone-like proteins
RAS – renin-angiotensin system
Runx2/Cbfa1 – transcription factor essential for bone formation and osteoblast differentiation
RUTH – Raloxifene Use for The Heart
SERM – selective estrogen receptor modulators
TGF-β – transforming growth factor beta
TNF-α – tumor necrosis factor α
WHI – Women's Health Initiative
Wnt – wingless signaling pathway
VC – vascular calcification
VDRAs – vitamin D receptor activators
VSMCs – vascular smooth muscle cells

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