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REVIEW

Osteoporosis and Cardiovascular Disease

Brittle Bones and Boned Arteries, Is There a Link?

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Both osteoporosis and cardiovascular disease (CVD) are major public health problems leading to increased morbidity and mortality. Although traditionally viewed as separate disease entities that increase in prevalence with aging, accumulating evidence indicates that there are similar pathophysiological mechanisms underlying both diseases. In addition to menopause and advanced age, other risk factors for CVD such as dyslipidemia, oxidative stress, inflammation, hyperhomocystinemia, hypertension, and diabetes have also been associated with increased risk of low bone mineral density (LBMD). Elevated LDL and low HDL cholesterol are associated with LBMD, altered lipid metabolism is associated with both bone remodeling and the atherosclerotic process, which might explain, in part, the co-existence of osteoporosis and atherosclerosis in patients with dyslipidemia. Similarly, inflammation plays a pivotal role in both atherosclerosis and osteoporosis. Elevated plasma homocysteine levels are associated with both CVD and osteoporosis. Nitric oxide (NO), in addition to its known atheroprotective effects, appears to also play a role in osteoblast function and bone turnover. Supporting this notion, in a small randomized controlled trial, nitroglycerine (an NO donor) was found to be as effective as estrogen in preventing bone loss in women with surgical menopause. Statins, agents that reduce atherogenesis, also stimulate bone formation. Furthermore, bisphosphonates, used in the treatment of osteoporosis, have been shown to inhibit atherogenesis. Intravenous bisphosphonate therapy significantly decreases serum LDL and increases HDL in postmenopausal women.

The exciting possibilities of newer pharmacological agents that effectively treat both osteoporosis and CVD hold considerable promise. However, it is important to emphasize that the current evidence linking both of these diseases is far from conclusive. Therefore, additional research is necessary to further characterize the relationship between these two common illnesses.

Key Words: Osteoporosis; cardiovascular disease; aging; atherosclerosis; bone mineral density; risk factors.

Introduction

Cardiovascular disease (CVD) and osteoporosis are major public health problems that frequently coexist and account for significant morbidity and mortality in the aging population. Although CVD *(1)* and osteoporosis *(2)* are traditionally viewed as unrelated disorders of senescence, other risk factors are also prevalent in both disorders (Table 1). Accumulating evidence indicates a pathophysiological link between the two diseases *(3–9)*. In a recent analysis from the Rotterdam study, low bone mineral density (LBMD) was associated with increased risk of peripheral arterial disease *(10)*. An age-independent association between progressive atherosclerotic calcification and bone loss has also been observed in a prospective study *(8)*. An association between mortality due to stroke *(9)* and CVD *(6)* and LBMD has also been observed.

Increased coronary calcium is a frequent and consistent feature of coronary atherosclerosis *(11)*. Vascular remodeling and plaque formation accompany early stages of atherosclerosis, luminal narrowing only occurs after a significant increase in the total burden of atherosclerotic plaques. Studies have consistently confirmed that coronary calcium is a marker and measure of atherosclerotic plaque burden *(12)*. Coronary artery calcification was associated with reduced survival rates, suggesting that extent of coronary calcium may be an independent prognostic marker *(13)*. Early stages of atherosclerosis are characterized by changes in vascular

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Table 1
Risk Factors Common for Both Cardiovascular Disease and Low Bone Density

Risk factors proposed	Vascular system	Bone
Age	Associated with ↑ vascular disease	Associated with ↓ BMD
Diabetes mellitus	Increased risk of macrovascular and microvascular disease in both type I and type 2 diabetes. Diabetes itself is considered a CVD.	Type 1 diabetes is associated with LBMD. Association of type 2 diabetes with L BMD is less clear ↑ risk of fracture in postmenopausal diabetic women
Hypertension	An established risk factor for CVD	Associated with increased bone loss at the femoral neck in postmenopausal elderly white women. Associated with ↓ BMD of proximal radius in white men
Inflammation	Endothelial dysfunction, Increased coagulation, plaque instability, Vitamin D production, ↑ vascular calcification	↑ Bone loss: ↑ NO production, osteoblast apoptosis, osteoclast activity
Dyslipidemia	Endothelial dysfunction, diminished NO production, plaque formation, ↑ cell adhesion; ox-LDL ↑ CVC activity, HDL ↓ CVC activity and cytokine formation.	Associated with ↓ BMD; OX-LDL inhibits osteoblastic differentiation
Homocystienemia	Endothelial dysfunction, diminished NO-bioavailability, lipid peroxidation, smooth muscle cell proliferation, increased platelet aggregation, enhanced tissue factor activity, reduced von Willebrand factor secretion, inhibition of tissue plasminogen activator	Hyerhomocysteinemia associated with LBMD
Estrogen deficiency	Diminished NO production, ↑ free radical production, ↑ VSMC proliferation, hypercoagubality, ↑ inflammation, ↑ vascular reactivity	↑ Bone resorption, cytokine activity, ↓ TGF-β, ↓ vitamin D
Sedentary lifestyle	Associated with increased vascular dysfunction	Associated with LBMD
Genetic predisposition (common candidate genes)	Osteoprotegerin, estrogen receptor-alpha (ER alpha) gene, Apolipoprotein E, vitamin D receptor, matrix Gla protein, peroxisome proliferator activated receptor gamma, methylenetetra-hydrofolate reductase (MTHFR), Il-6, LDLRP polymorphism	Osteoprotegerin, estrogen receptor-alpha (ER alpha) gene, Apolipoprotein E, vitamin D receptor, matrix Gla protein, peroxisome proliferator activated receptor gamma, methylenetetrahydrofolate reductase (MTHFR), Il-6 polymorphism

CVC, calcifying vascular cells.

compliance, for example, in a recent study, a measure of arterial stiffness (brachial–ankle pulse wave velocity) was significantly associated with reduced bone mineral density (BMD). This finding suggests an association between increased bone loss and early stages of atherosclerosis *(14)*, supporting earlier studies suggesting such an association with generalized arterial calcification (observed in the later stage of atherosclerosis).

Atherosclerotic calcification is a regulated process with many cellular mechanisms similar to bone formation and resorption. The concept of mismatch in osteoclastogenesis and osteoblastogenesis in osteoporosis resulting in enhanced bone resorption can be applied to the process of vascular calcification, in reverse. However, many other concurrent processes modulate the milieu of bone and vascular tissue to result in the apparent paradox of simultaneous progression of atherosclerosis/calcification and demineralization of bone. Many of the established atherogenic factors such as estrogen deficiency, dyslipidemia, oxidative stress, decreased nitric oxide (NO) availability, inflammatory cytokines, homocysteinemia, and sedentary lifestyle negatively affect osteogenesis and mineralization *(5–8)*. Human, animal, and in vitro studies reveal that some of these factors may also modulate atherosclerosis/vascular calcification. In this review, we will emphasize the pathophysiological mechanisms underlying both diseases, highlighting the basis to suggest a common soil hypothesis for the co-occurrence of osteoporosis and atherosclerosis.

Mechanisms

Arterial calcification, like osteogenesis, involves complex interaction of various cells that produce matrix vesicles and subsequent mineralization. Recent evidence suggests the presence of osteoblast-like cells in the vasculature capable of calcifying vascular tissue, the calcifying vascular

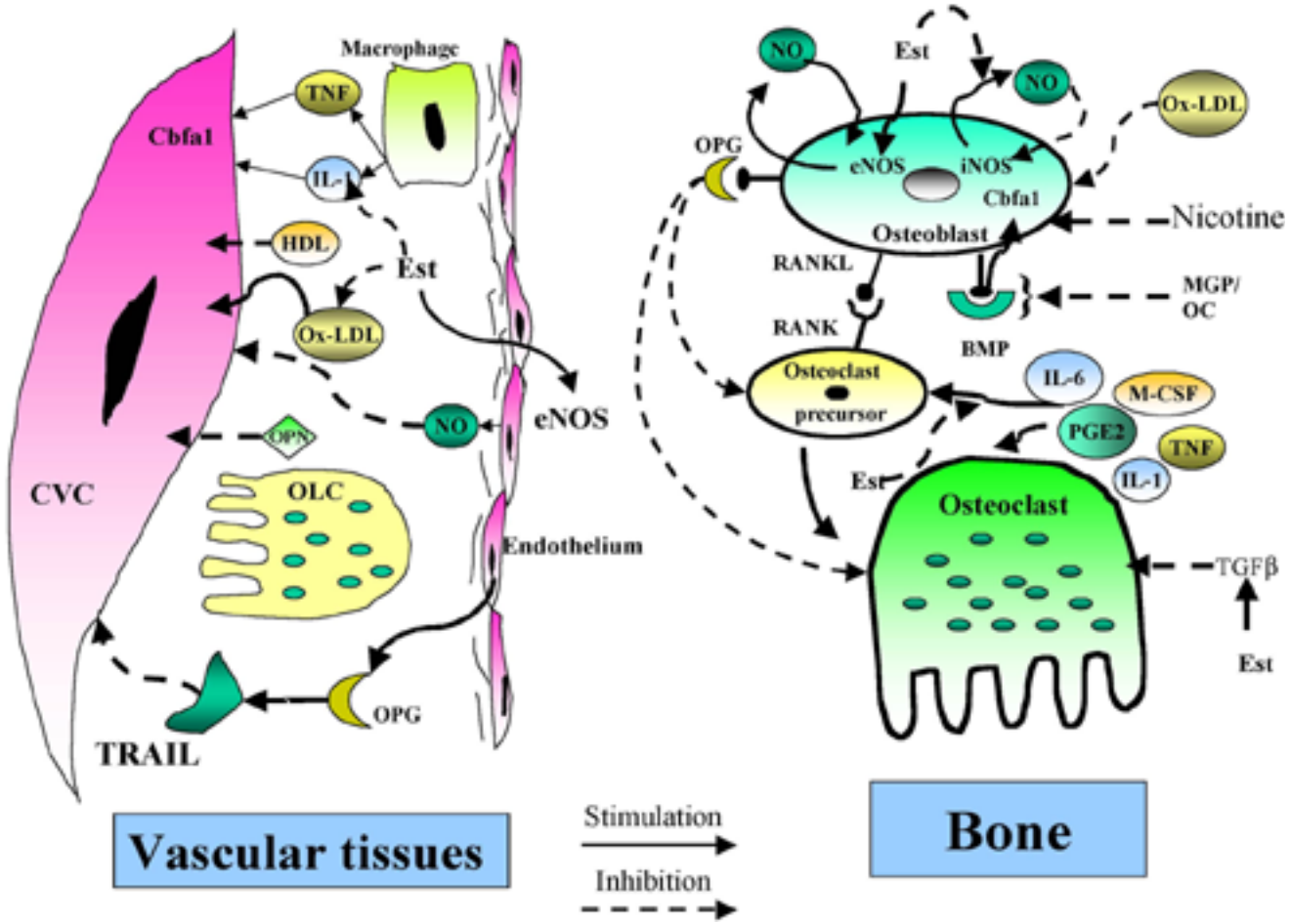

Fig. 1. Common cellular mechanisms underlying both vascular disease and osteoporosis. NO = nitric oxide; eNOS = endothelial nitric oxide synthase; INOS = inducible nitric oxide synthase; Est = estrogen; Ox-LDL = oxidized-low-density lipoprotein; HDL = high-density lipoprotein; OPG = osteoprotegerin; RANKL = receptor-activated nuclear factor-kappa B ligand; RANK = receptor-activated nuclear factor-kappa B; BMP = bone morphogenetic protein; MGP = matrix Gla protein; OC = osteocalcin; IL-6 = interleukin-6; M-CSF = macrophage-colony-stimulating factor; IL-1 = interleukin-1; TNF = tumor necrosis factor; PGE2 = prostaglandin E2; TGFβ = transforming growth factor-beta; OPN = osteopontin; CVC = calcifying vascular cell; OLC = osteoclast-like cell; Cbfa-1 = core-binding factor-α1; TRAIL = tumor necrosis factor-related ligand.

cells (CVCs) *(15)* (Fig. 1). Resident and recruited macrophages, monocytes, and lymphocytes play key roles in atherosclerotic calcification and osteoporosis. Paracrine regulators of bone metabolism such as matrix Gla protein (MGP), osteocalcin (OC), bone morphogenetic protein (BMP), osteopontin (OPN), osteonectin (ON), osteoprotegerin (OPG), receptor-activated nuclear factor-kappa B ligand (RANKL), and inflammatory cytokines are also present in atherosclerotic arteries *(16)*. In addition, osteoclast-like cells (OLCs) have been demonstrated in calcified arteries *(17)*. Thus, the vascular microenvironment possesses mechanisms to maintain mineral homeostasis similar to that of bone tissues (Fig. 1).

OPG/RANK/RANKL System

OPG, a cytokine that is a soluble/decoy receptor of the tumor necrosis factor (TNF) receptor superfamily, produced by bone marrow stromal cells, osteoblasts, vascular smooth muscle cells (VSMCs), and endothelial cells is a potent inhibitor of osteoclast formation *(18)*. Soluble or cell-bound RANKL expressed by osteoblast precursors and lymphocytes interact with RANK to initiate a series of cellular events to promote osteoclast fusion, formation, and activation leading to bone resorption *(19)*. OPG binds to RANKL and thereby inhibits osteoclast activation (Fig. 1). OPG knockout mice exhibit osteoporosis and also develop aortic and renal arterial calcification. Transgenic OPG restoration reverses these abnormalities, whereas intravenous OPG administration reverses only the osteoporotic phenotype *(20)*. Similarly, a single subcutaneous injection of OPG significantly reduced bone resorption in postmenopausal women for up to 6 wk *(21)*.

OPG is expressed by VSMCs in normal aortas, whereas in advanced atherosclerotic lesions OPG lines the calcific deposits, and RANKL is present in the extracellular matrix

around these calcium deposits *(16)*. The aortic calcified lesions contain many multinucleated cells positive for markers for osteoclasts [tartarate-resistant acid phosphatase (TRAP) and cathepsin K^+] and negative for macrophage markers (F4/80). These findings indicate the presence of osteoclasts or their equivalents, i.e., OLCs *(20)* that may mediate the inhibitory actions of OPG on vascular calcification. However, in a prospective cohort study of elderly White women with osteoporosis, increased OPG levels were associated with diabetes, stroke and increased CVD mortality *(22)*. These findings suggest that increased OPG may be a compensatory response to accentuated bone loss and enhanced vascular calcification, and that OPG may serve as a marker for both osteoporosis and CVD. The mechanisms underlying the paradoxical effects of OPG in bone and vascular tissue are unclear; however, the opposing actions may be due to site-specific and differential expression of RANK/RANKL in these tissues. Absence of OPG may prevent apoptosis of T-lymphocytes and CVCs favoring calcification *(23)* (Fig. 1).

Gla Proteins

γ-Carboxyglutamic acid (Gla)-containing proteins, including matrix Gla protein (MGP) and osteocalcin (OC), are key mediators and inhibitors of osteoid formation. MGP is a secretory protein with widespread tissue expression including bone and vascular wall. Depletion of the vitamin K-dependent Gla proteins by chronic warfarin treatment results in increased mineralization and growth-plate closure in rats *(24)*. Similar features are observed in human embryos exposed to anticoagulants in early pregnancy *(25)*. Knockout mice that lack MGP develop rapid lethal and extensive calcification of arteries *(26)*. These models of MGP depletion clearly confirm the antimineralization role of MGP.

MGP is constitutively expressed in normal human aortas and is up-regulated in atherosclerotic plaques *(27)*, suggesting a possible a mechanism to limit vascular osteogenesis. Furthermore, MGP inhibits bone morphogenetic protein (BMP)-induced chondrocyte differentiation and absence of this inhibition leads to osteogenic differentiation of the vascular mesenchyme leading to increased calcification *(28)*. In contrast, others propose that MGP binding of hydroxyapatite prevent further nucleation and growth of crystals *(29)*. It is likely that both of these mechanisms contribute to vascular calcification.

Osteocalcin (OC) an abundant protein in the bone, also inhibits calcification (Fig. 1). Mice with deletion of OC gene exhibit enhanced bone formation *(30)*. Interestingly, these mice do not display any vascular calcification, suggesting that OC does not play a dominant role in this process. In humans, OC parallels MGP expression in both normal and atherosclerotic vessels *(16)*. Serum OC is also elevated in women with atherosclerosis and osteoporosis *(31,32)*. Thus, the Gla proteins play an integral role in both vascular wall and bone.

Bone Morphogenetic Protein (BMP) and Osteopontin (OPN)

BMPs belong to the transforming growth factor-β (TGF-β) superfamily of proteins. Fifteen BMPs are currently identified, BMP-2 and BMP-4 are osteoinductive molecules *(33)*. The osteogenic effects of BMPs have been demonstrated in vitro and in vivo *(34,35)*. BMP-2/4 initiates the commitment of pluripotent mesenchymal cells to an osteoblastic lineage. BMPs stimulate the expression of a key molecule in osteoblast differentiation termed core-binding factor-α1 (Cbfa-1) *(36)* (Fig. 1). Indeed, local application of BMP stimulates osteogenesis and bone gap healing in humans *(37)*. Given its osteoinductive properties, vascular expression of BMPs should favor calcification. Indeed, human atherosclerotic lesions have enhanced expression of BMP-2 and Cbfa-1, while they are absent in normal arteries *(38)* (Fig. 1). The pattern of expression of these proteins provides more insight into some plausible mechanisms; for example, Cbfa-1 is synthesized in regions with calcification and absent in MGP-expressing areas *(38)*. This may be explained by the inhibitory effect of MGP on BMP-Cbfa-1 pathway. Thus, spatial and temporal expression of BMPs may modulate the vascular milieu toward calcification.

Osteopontin (OPN), a multifunctional glycoprotein, is expressed in various cell types including bone and vascular tissue. In bone, OPN is produced by osteoclasts and osteoblasts and inhibits matrix mineralization *(39)*. Recent studies suggest that OPN is an important regulator of osteoclast activity. OPN-knockout mice are resistant to ovariectomy- and PTH-induced bone resorption *(40,41)*. Furthermore, OPN expression is up-regulated in calcified atheromatous lesions *(16)* presumably as a compensatory response to attenuate the mineralization process. OPN likely plays a pivotal role in vascular inflammation and may be directly involved in atherogenesis. In fact, transgenic mice overexpressing OPN develop medial thickening and enhanced neointimal formation after vascular injury *(42)*. Thus, OPN possibly modulates mineralization and inflammatory responses independently.

Role of Nitric oxide (NO)

NO, a pleiotrophic molecule, plays a key role in bone and vascular function. NO is produced by a group of enzymes NO synthase (NOS), which catalyze the conversion of L-arginine to NO and citrulline. Constitutively expressed, cNOS produces NO in small amounts on demand in a calcium-dependent fashion. In contrast, the inducible form, iNOS, is calcium-independent and produces large amounts in a sustained fashion *(43)*. cNOS is present in the endothelium lining vascular tissues and osteocytes. iNOS can be induced in endothelium, VSMC, inflammatory cells, osteocytes *(44)*, and chondrocytes, by a variety of cytokines *(45)*. cNOS responds by producing NO to a variety of physiological stimuli such as shear stress, mechanical loading, estrogen, statins,

and growth factors in both bone and vascular tissue *(46–52)*. NO has a biphasic effect on bone and vascular tissue (Fig. 1). This molecule has many atheroprotective functions such as inhibiting platelet function, VSMC proliferation, cell adhesion, and vascular dilatation *(43)*. Absence of cNOS in the endothelium of mice leads to hypertension, endothelial dysfunction, and enhanced vascular neointimal formation; in addition, these mice exhibit reduced bone mineral density (BMD), cortical thinning, and reduced osteoblasts number and function *(53)*. High-dose exogenous estrogen in ovariectomized eNOS-knockout mice failed to show the typical anabolic stimulation. These studies suggest that cNOS plays a critical role in osteoblast function and bone turnover. Clinical studies also suggest that NO may inhibit resorptive activity of osteoclasts *(54)*. In a randomized controlled trial, nitroglycerin, a NO donor, was as effective as estrogen in preventing bone loss in surgically induced postmenopausal women *(55)*. In contrast, in a mouse model of inflammation-induced osteoporosis, excess NO produced by the inducible NO pathway, inhibits osteoblast growth and differentiation *(56)* (Fig. 1). In mice lacking iNOS, inflammation-induced bone loss was less severe compared to the wild type *(57)*. Chronic inflammatory conditions are associated with elevated levels of cytokines and high levels of NO *(58)* and inhibition of NO production, reversed the cytokine-induced bone loss *(59)*. Thus, in bone and vascular tissue, the biological response to NO is dose-dependent. iNOS accentuates bone loss whereas deficiency of cNOS accelerates atherosclerosis and osteoporosis.

Role of Lipids and Oxidative Stress

Epidemiological and interventional studies have established dyslipidemia as a major risk factor for atherosclerosis. LDL cholesterol is regarded as an essential risk factor for atherogenesis, whereas HDL protects against this process *(60)*. Evidence now exists linking BMD and dyslipidemia; elevated LDL and reduced HDL are associated with low BMD in postmenopausal women *(61)*. Although it may be argued that these processes occur in parallel with senescence, recent studies suggest otherwise. Both bone and vascular tissue share similar pathological features. As in atherosclerosis, increased amounts of lipids get accumulated beneath the vascular intima and perivascular area in bones. Oxidized-LDL (Ox-LDL) plays a pivotal role in decreasing BMD as it does in promoting atherosclerosis *(62)*. In a mouse model of diet-induced atherosclerosis, there was an inverse relationship between serum LDL levels and BMD at the femur *(63)*. Consistent with this notion, administration of high-fat diet reduced bone mineralization in mice *(62)*. The differential effects of lipids have been systematically examined in in vitro models. Ox-LDL enhances CVC, but inhibits osteoblast differentiation *(64)* (Fig. 1). Ox-LDL through activation of PPARγ influences gene expression and LDL-uptake in resident inflammatory cells such as monocytes/macrophages *(65)*. Interestingly, activation of PPARγ results in the suppression of terminal differentiation of marrow cells to osteoblasts *(66)*. In contrast, HDL decreases cytokine and LDL-induced osteogenic differentiation of CVCs *(67)* (Fig. 1). Thus, HDL and LDL regulate osteoblastic differentiation/calcification in bone and vascular tissue.

Epidemiological studies also suggest that higher intake of specific fatty acids, such as γ-linoleic acid, is associated with a reduced coronary atherosclerosis *(68)*. Similar beneficial effects on bone have been reported *(69)*, and animals with fatty acid deficiency develop osteoporosis and vascular calcification. The beneficial effects of these fatty acids and their derivatives, eicosanoids, may be related to their effects on NO synthesis anti-inflammatory actions, modulation of prostaglandin metabolism, or changes in local growth factors. Thus, lipids modulate bone remodeling and the atherosclerotic process in opposite directions, and these effects may help explain the coexistence of osteoporosis and atherosclerosis in people with dyslipidemia.

Cytokines and Inflammation

Inflammation plays a pivotal role in the biology of atherosclerosis. Markers of inflammation are increased and correlate with the severity of the atherosclerotic process *(70)*. Markers of inflammation such as C-reactive protein (CRP), IL-6, TNF-α, and MCP-1 have, in varying degrees, been proposed as CVD risk factors. The vascular inflammatory response is a complex process that leads to thrombus formation, angiogenesis, neointimal thickening, and atherosclerosis. A wide array of cytokines is elaborated in the vascular wall by endothelial, VSMCs, and macrophages/monocytes such as IL-1, TNF-α, and IL-6. This leads to augmented expression of adhesion molecules on leukocytes (e.g., CD11b) and endothelial cells [e.g., P-selectin, intracellular adhesion molecule (ICAM-1)]. These cytokines also stimulate transcription of genes encoding chemoattractant factors, such as MCP-1 and macrophage stimulatory factor, which attract monocytes to the vessel wall. Indeed, inflammatory cells such as macrophages are frequently found in atherosclerotic lesions and play a crucial role in vascular calcification *(71)*.

Monocytes and macrophages are known to stimulate osteogenic differentiation of CVCs through specific cellular interactions. Indeed, TNF-α and IFN-γ induce expression of alkaline phosphatase in vascular tissue promoting osteogenic activity (Fig. 1). Furthermore, both these cytokines are potent stimulators of 1,25-dihydroxyvitamin D in macrophages *(72)*. The causal role of these cytokines was studied in a rodent model of atherosclerosis; neutralization of IL-1 activity with IL-1ra or TNF-α with a binding protein abrogated *(73)*, whereas injections of IL-6 promoted, the atherosclerotic process *(74)*. Proinflammatory cytokines have a negative influence on vascular function and are key mediators in atherogenesis.

Interestingly, the changes in cytokine profile in atherogenesis are similar in osteoporosis. Inflammatory cytokines are powerful stimulators of bone resorption. In a study of human postmenopausal osteoporosis, serum IL-6 was the single most important predictor of bone loss *(75)*. In fact, the levels of IL-1, IL-6, and TNF-α directly correlated to the bone resorptive action of monocytes from post-menopausal women, and this action was neutralized by IL-1 and anti-TNF-α antibody *(76)*. Surgical or natural menopause is permissive for the expression of many cytokines such as M-CSF, GM-CSF, IL-6, IL-1, and TNF-α. These cytokines produced by the macrophages, lymphocytes, and stromal cells in the bone regulate both osteoclast and osteoblast function. They stimulate osteoclast precursor proliferation and differentiation. IL-1 and IL-6 also enhances the resorptive activity of osteoclasts *(77)*. A combination of diminished transforming growth factor-β (TGF-β) and increased levels of IL-1, RANKL, and M-CSF delays osteoclast cell death *(78)* (Fig. 1). The prolonged activity of osteoclasts results in an imbalance between osteogenesis and resorption leading to osteoporosis. Thus, cytokines play a key role in both osteoporosis and atherosclerosis; the polymorphic responses in the vascular wall and bone is a result of complex interaction between cells, the local microenvironment, and the temporal pattern of expression of the cytokines.

Homocystienemia

Epidemiological data indicate an association between elevated plasma homocysteine and CVD *(79)*. Proposed mechanisms of vascular injury by homocysteine are multiple and include endothelial dysfunction, diminished NO-bioavailability, lipid peroxidation, smooth muscle cell proliferation, increased platelet aggregation, enhanced tissue factor activity, increased plasminogen activator inhibitor-1, and inhibition of tissue plasminogen activator. These actions favor augmented vascular response to injury in atherosclerosis. Interestingly, sex hormone deficiency is associated with elevated homocysteine. In a European case-control study, post-menopausal women had higher post-methionine load of plasma homocysteine *(80)*. Both estrogen replacement and tamoxifen decrease plasma homocysteine levels *(81)*. There is an increased prevalence of osteoporosis in people with homocystinuria *(82)*. A recent study examined the association of polymorphism in methylenetetrahydrofolate reductase (MTHFR) and BMD. Polymorphism of MTHFR correlates with hyperhomocysteinemia and LBMD *(83)*. This clearly suggests that the genotype of MTHFR is one of the genetic risk factors for LBMD, although the exact nature of this relationship and whether or not the effect is mediated via changes in homocystiene level remains to be seen.

Hypertension

High blood pressure is a well-recognized risk factor for atherosclerotic CVD. Studies have suggested an association between increased bone loss and elevated blood pressure *(84,85)*. Significant hypercalciuria was observed in the hypertensive cohorts compared to normotensives in these studies. Recently, a negative correlation between osteocalcin and blood pressure has also been reported *(86)*, suggesting altered skeletal metabolism in these patients. In fact, rodent models of hypertension have reduced bone mineral content and significant hypercalciuria *(87,88)*. Altered renal handling of calcium and changes in calcitropic hormones and microvasculature have been suggested to play a role in hypertension associated bone loss *(88)*.

Diabetes Mellitus

Diabetes mellitus (DM) is an established risk factor for atherosclerosis. Macro- and microvascular disease is common in patients with type 1 and type 2 DM and associated with intimal and medial calcification of arteries. However, the association between osteoporosis and DM is equivocal. Reduced bone mass in type 1 DM is a consistent feature *(89,90)*. Osteocalcin levels were also reduced in patients with type 1 DM *(91)*. The onset of type 1 DM temporally overlaps with the period of continued bone deposition; studies have reported osteopenia at the time of diagnosis of type 1 DM, suggesting that osteoporosis may not be a late complication of type 1 DM. Intensive insulin therapy and improved metabolic control have been shown to stabilize bone loss *(92)*. Patients with type 1 DM not only suffer from enhanced bone loss, but also are prone to higher rates of hip fractures *(93,94)*. The mechanisms that trigger bone loss in these patients are not understood. Poor metabolic control has been suggested to adversely affect skeletal metabolism *(95,100)*. Recent studies in rodent models of type 1 DM suggest that advanced glycation end products (AGEs) through their interaction with the receptor for AGEs (RAGE) inhibits osteoblast function *(96)*. Consistent with human studies, mice with insulin deficiency exhibit diminished expression of osteocalcin and many transcription factors that regulate osteoblastic differentiation (Cbfa1), runt domain factor-2 (Runx-2). Interestingly, these abnormalities were reversed with insulin replacement *(97)*; this is similar to the effects of insulin therapy on bone in type 1 DM. It would be interesting to examine if similar mechanisms operate in patients with type 1 DM.

In contrast to type 1 DM, available data suggesting an association of reduced BMD in type 2 DM is equivocal. Type 2 DM has been associated with increased *(98–100)*, unchanged *(101)*, or decreased *(102,103)* BMD. Clinically, patients with type 2 DM have higher fracture rates compared to non-diabetics *(104)*. The apparent paradox of normal or elevated BMD and increased fracture rates may be explained by the presence of independent risk factors for fracture in diabetic patients and the possibility of decreased bone strength and quality features, which are not measured

by conventional dual-energy X-ray absorptiometry (DXA). Changes in calcium, vitamin D, insulin and IGF-1, cytokines, and altered neural and vascular function may all lead to diabetes-related bone disease in type 2 DM. Thus, DM adversely affects bone metabolism and vascular function and may play a causal role in both these disease entities.

Pharmacological Agents: Statins and Bisphosphonates

The beneficial actions of statins and bisphosphonates on the vascular wall and bone support a link between atherosclerosis and osteoporosis. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are widely used pharmacological agents to treat dyslipidemia. Bisphosphonates are agents commonly used in the prevention and treatment of osteoporosis. Statins lower cholesterol by reduced production and enhanced clearance of LDL particles. In addition to lowering cholesterol, they also reduce cellular isoprenoid intermediates such as dolichol, ubiquinone, farnesol, and geranylgeraniol (Fig. 2). Reduction in the latter components inhibits isoprenylation and activity of small GTP binding proteins of the Ras/ Rho family *(105)*. These low-molecular-weight G-proteins are involved in cell proliferation, differentiation, apoptosis, migration, contraction, and regulation of gene transcription. Thus, inhibition of these proteins can critically affect various cellular processes. The anchoring of these small G-proteins to cell membranes requires prenylation; Ras proteins are farnesylated, whereas Rho proteins are geranylgeranylated. Small G-proteins exist in an inactive GDP-bound cytosolic form, and upon cellular activation they exchange GTP and translocate to the active-membrane form. Lack of protein isoprenylation leads to cytosolic sequestration and loss of biological activity.

Bisphosphonates are stable analogs of inorganic pyrophosphate that avidly bind to mineral surfaces in vivo and inhibit osteoclast recruitment, differentiation, activity, and survival to prevent bone resorption *(106)*. Bisphosphonates act downstream from the site of action of statins (Fig. 2), inhibiting enzymes in the mevalonate pathway, such as farnesyl diphosphate synthase and squalene synthase *(105)*. This pathway is again involved in regulating the activity of Ras/Rho proteins. It is postulated that inhibition of protein prenylation might indeed be related to the anti-resorptive actions of bisphosphonates. Thus, it is clear that these two different classes of drugs affect the mevalonate pathway, although with different potencies to mediate their effects. It would be logical to assume that they may modulate osteoporosis and atherosclerosis.

Bisphosphonates have long been known to inhibit atherogenesis *(107–109)*. Bisphosphonate administration not only suppresses the formation but also reduces the extent of established atherosclerotic lesions in rabbits. High doses of a new bisphosphonate derivative, SR-9223, have been reported to reduce serum cholesterol by about 33% and decreased deposition of cholesterol in the aorta in different animal models *(110)*. Furthermore, oral etidronate therapy decreased the carotid intimal thickness in patients with type 2 diabetes and osteopenia, with no change in lipid profile *(111)*. In contrast, chronic bisphosphonate significantly decreased serum LDL and increased HDL in post-menopausal women *(112)*. The anti-atherogenic actions of bisphosphonates have been attributed to direct effects on the vascular wall. They sensitize macrophages to undergo apoptosis, prevent foam cell formation by inhibiting the uptake of LDL and affect cell replication/function by inhibition of ras/rho family of proteins *(113)*. Thus, bisphosphonates clearly inhibit atherosclerosis and osteoporosis.

Statins have multiple actions above and beyond cholesterol lowering. Cardiovascular benefits of statins have conventionally been attributed to reduction of LDL cholesterol. However, subanalyses of the large clinical trials suggest that some of the beneficial effects of statins are independent of their classical actions on lipoproteins *(105)*. Statins modulate various aspects of atherogenesis. Statins are known to stabilize atherosclerotic plaques by decreasing metalloproteases, Ox-LDL, and macrophage activity *(105)*. Furthermore, statins improve endothelial function by enhancing NO formation via increasing the expression of cNOS *(105)*. Recent evidence indicates that statins also possess anti-inflammatory properties and affect many of the events in the inflammatory cascade. Statin therapy reduces MCP-1/IL-6 expression, monocyte infiltration, cytokine, and adhesion molecule expression *(105)*. In several human and animal studies, various statins have been shown to inhibit uptake of Ox-LDL and formation of superoxide anion *(105)*. Thus, statins modulate atherogenesis by its pleiotropic effects.

Statins have also been shown to stimulate bone formation in several studies. In vitro, statins increase the number of osteoblasts and the amount of new bone formation in mouse calvaria. Oral administration of simvastatin to rats increased trabecular bone volume and the rate of new bone formation *(114)*. These effects were correlated to enhanced BMP-2 expression in statin-treated murine osteoblasts. Inhibition of Rho-kinase, a downstream signaling molecule to Rho-enhanced osteocalcin expression in human osteoblasts *(115)*. All of these findings illustrate the positive effects of statins on bone remodeling. However, the clinical support for the effect of statins on BMD and the association of statin and fracture risk have been examined and found to be inconclusive *(105)*.

Finally, both bisphosphonates and statins affect atherosclerosis and bone resorption, and the selectivity to either bone or vascular wall seems to be determined by the pharmacokinetic properties of these agents. The fact that these agents positively modulate these pathological entities suggests a common link in their pathogenesis.

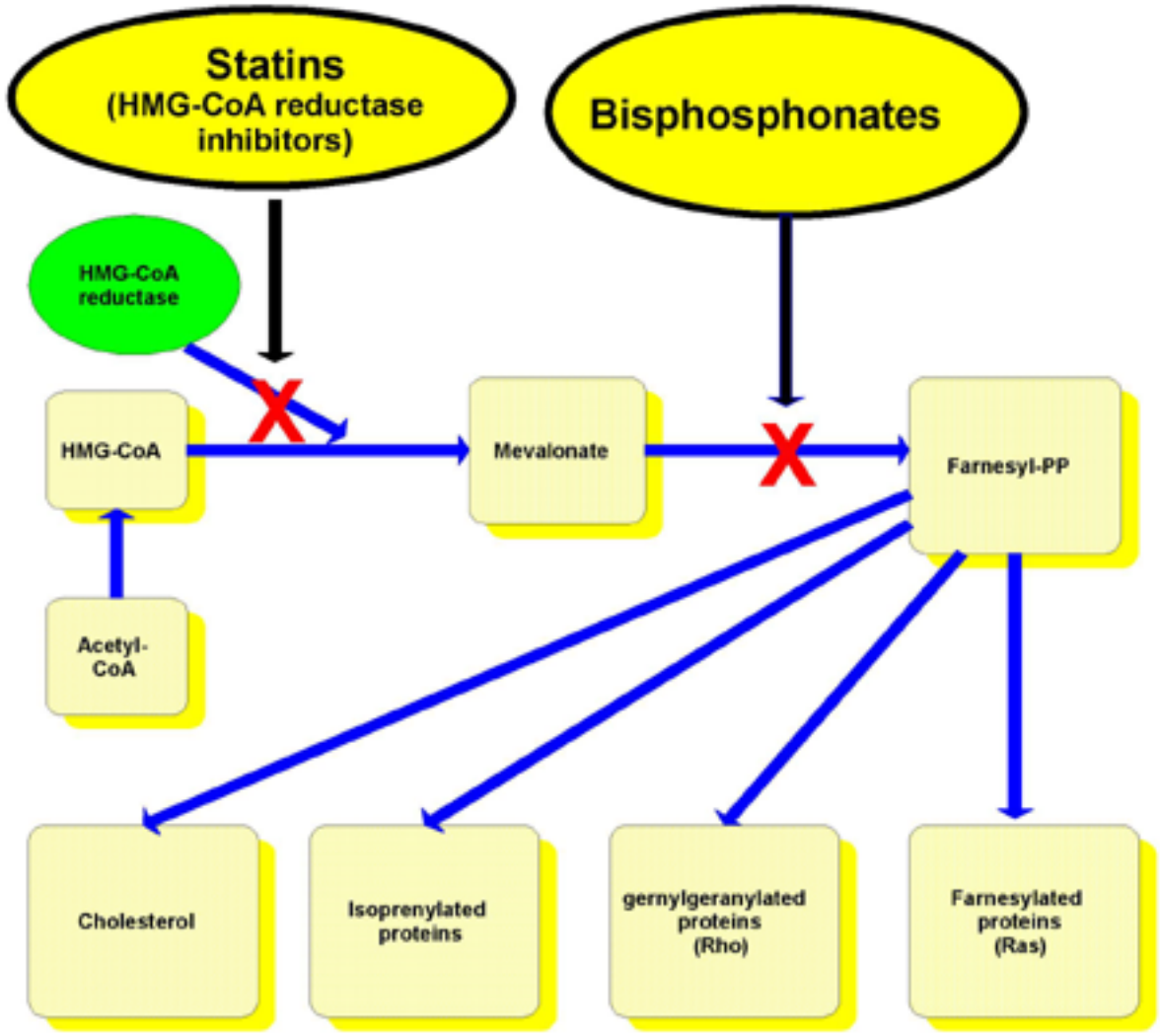

Fig. 2. Site of action of statins and bisphosphonates on the mevalonate pathway.

Summary

Accumulating evidence suggests a link between osteoporosis and atherosclerosis. Both human studies and various animal models suggest that complex interactions of various common factors promote both vascular disease and bone loss in a simultaneous fashion. Traditional CVD risk factors such as dyslipidemia, oxidative stress, lack of estrogen, hyperhomocysteinemia, inflammation, hypertension, and diabetes also regulate bone remodeling. Therapeutic agents currently used in the treatment of either of these entities have salutary beneficial effects on the other, suggesting common mechanisms for both diseases. Future studies are necessary to define and further characterize the relationship between CVD and osteoporosis. Finally, enhanced understanding of the mechanisms of vascular and bone mineralization might lead to the development of therapeutic agents that effectively target both these processes.

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