Brazilian Journal of Medical and Biological Research (2007) 40: 435-442
ISSN 0100-879X

Bone metabolism and vascular calcification

C.F. Danilevicius, J.B. Lopes and R.M.R. Pereira

Laboratório de Metabolismo Ósseo (LIM-17), Disciplina de Reumatologia, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brasil

Abstract

Osteoporosis and atherosclerosis are chronic degenerative diseases which have been considered to be independent and whose common characteristic is increasing incidence with age. At present, growing evidence indicates the existence of a correlation between cardiovascular disease and osteoporosis, irrespective of age. The morbidity and mortality of osteoporosis is mainly related to the occurrence of fractures. Atherosclerosis shows a high rate of morbidity and especially mortality because of its clinical repercussions such as angina pectoris, acute myocardial infarction, stroke, and peripheral vascular insufficiency. Atherosclerotic disease is characterized by the accumulation of lipid material in the arterial wall resulting from autoimmune and inflammatory mechanisms. More than 90% of these fatty plaques undergo calcification. The correlation between osteoporosis and atherosclerosis is being established by studies of the underlying physiopathological mechanisms, which seem to coincide in many biochemical pathways, and of the risk factors for vascular disease, which have also been associated with a higher incidence of low-bone mineral density. In addition, there is evidence indicating an action of antiresorptive drugs on the reduction of cardiovascular risks and the effect of statins, antihypertensives and insulin on bone mass increase. The mechanism of arterial calcification resembles the process of osteogenesis, involving various cells, proteins and cytokines that lead to tissue mineralization. The authors review the factors responsible for atherosclerotic disease that correlate with low-bone mineral density.

Correspondence
R.M.R. Pereira
Disciplina de Reumatologia, FMUSP
Av. Dr. Arnaldo, 455, Sala 3107
01246-903 São Paulo, SP
Brasil
Fax: +55-11-3061-7490
E-mail: rosamariarp@yahoo.com or cam-isa@bol.com.br

Research supported by FAPESP (No. 03/09313-0).

Received September 18, 2006
Accepted February 22, 2007

Key words
• Osteoporosis
• Vascular calcification
• Atherosclerosis
• Low-bone mineral density
• Arterial calcification

Introduction

Osteoporosis and atherosclerosis have long been considered to be independent diseases, whose common characteristic is their increasing incidence with age (1,2). At present, growing evidence indicates the existence of a correlation between cardiovascular disease and osteoporosis/fractures, related or not to age (3-5). Some studies have shown a direct and individual relationship between these two diseases and increased mortality rates (3).

Osteoporosis and atherosclerosis are chronic degenerative diseases with a high incidence in the general population and represent two of the major public health problems (6,7). With the growth of the elderly population this number will increase over the next decades. According to the World

Health Organization, the percentage of people older than 60 years rose from 8 to 10% between 1950 and 1998 and will possibly reach 20% by 2050. These figures are even higher in developing countries where the elderly population will show an increase of at least 9-fold by 2050 (8).

The morbidity and mortality of osteoporosis is mainly related to the occurrence of fractures, particularly hip and vertebral fractures, with fractures at other sites such as the wrist, ribs, humerus, and phalanx also being observed (9,10). Vertebral fractures are mostly asymptomatic, and are usually detected by radiology or due to intense pain and restriction of habitual activities which are the reason for hospitalization and for an increased number of visits to outpatient clinics and medical offices (11). Hip fractures are extremely symptomatic and in most cases require surgical treatment, hospitalization, and prolonged bed rest (12).

Atherosclerosis shows a high rate of morbidity and especially of mortality because of clinical repercussions such as angina pectoris, acute myocardial infarction, stroke, and peripheral vascular insufficiency (13,14). Cardiovascular diseases continue to be the main cause of death in the world. In addition, they are responsible for a high rate of dependence for the execution of habitual tasks, often with high rates of hospitalization for prolonged periods of time (13).

Atherosclerotic disease is characterized by the accumulation of lipid material in the arterial wall resulting from autoimmune and inflammatory mechanisms (15). More than 90% of these fatty plaques undergo calcification (16). Some studies have demonstrated a direct relationship between the degree of calcification of the atherosclerotic plaque and mortality due to cardiovascular events (17).

Due to its inflammatory physiopathology, atherosclerosis has been correlated with low-bone mineral density in some immunological diseases. Ramsey-Goldman and Manzi (18) reported an association between osteoporosis and cardiovascular disease in patients with a diagnosis of systemic lupus erythematosus. Bezerra et al. (19), studying 30 premenopausal women with Takayasu arteritis, demonstrated an association between low-bone mineral density values and the severity of arterial calcification, a finding that contributed to the idea of an association between bone metabolism and cardiovascular disease.

Some of the physiopathological mechanisms underlying osteoporosis and atherosclerosis seem to coincide in many biochemical pathways. Risk factors for vascular disease, such as dyslipidemia, systemic arterial hypertension, diabetes mellitus, and hyperhomocystinemia, have been associated with a higher incidence of low-bone mineral density. In addition, there is evidence indicating an action of antiresorptive drugs on the reduction of cardiovascular risks and an effect of statins, antihypertensives and insulin on bone mass increase. The mechanism of arterial calcification resembles the process of osteogenesis, involving various cells, proteins and cytokines that lead to tissue mineralization (7).

Ectopic bone tissue has been identified in calcified plaques and bone-specific cells have been found in the arterial wall, with evidence of transdifferentiation of endothelial cells into osteoblasts (20). Osteoclast-like cells have also been demonstrated in calcified arteries (21).

Mediators of bone mineral metabolism and vascular calcification

Local and serum lymphocytes, monocytes and macrophages play an important role in osteoporosis and vascular calcification. Chemical mediators of bone metabolism such as matrix Gla protein (MGP), osteocalcin, bone morphogenetic protein (BMP), osteopontin (OPN), osteonectin, osteoprotegerin (OPG), receptor activator of nuclear

factor kappa B ligand (RANKL), and inflammatory cytokines are also present in atherosclerotic arteries (7).

Matrix Gla proteins

The so-called Gla proteins, which contain γ-carboxyglutamic acid, include osteocalcin and MGP. These proteins are expressed in different human tissues, mainly bone and vascular cells, and are mediators and inhibitors of osteoid formation (7). Osteocalcin is a Gla protein synthesized mainly by osteoblasts and, when carboxylated, it binds to hydroxyapatite in bone, leading to bone mineralization. However, osteocalcin does not seem to play a dominant role in the process of vascular calcification (22). On the other hand, experimental studies on MGP-knockout mice have shown the formation of extensive and lethal arterial calcifications, a finding confirming the inhibitory role of this protein in vascular calcification. These animals also presented osteopenia, fractures, short stature, and erratic mineralization of the growth plates (23). Recent evidence indicates that this protein inhibits mesenchymal differentiation into osteogenic cell lines by blocking the action of BMP, a potent factor of bone maturation. The absence of this inhibition leads to the differentiation of vascular mesenchyme into bone cells, thus increasing calcification (24).

Bone morphogenic protein

Another protein related to bone metabolism is BMP, which belongs to the transforming growth factor beta (TGF-ß) superfamily. BMP-2 is one of the most extensively studied proteins in this group. This protein is expressed in myofibroblasts and may play its role in the mechanism of vessel wall calcification by stimulating the expression of a key molecule in osteoblastic differentiation, i.e., core binding factor alpha-1 (Cbfa-1/Runx2) (25), or by inducing apoptosis of vascular smooth muscle cells, a critical event that leads to the onset of vascular calcification (26). In humans, atherosclerotic lesions show an increased expression of BMP-2 and Cbfa-1 compared to normal arteries (27). Since MGP inhibits BMP, Cbfa-1 is only synthesized in regions where MGP is not expressed (7). The effect of MGP on BMP-2 depends, in addition to its concentration, on the degree of γ-carboxylation of MGP. Thus, loss of MGP function might be a risk factor for vascular calcification. In fact, MGP isolated from calcified atherosclerotic plaques of mice shows incomplete γ-carboxylation (28).

Osteopontin

OPN is another matrix protein that functions as an important inhibitor of calcification (28). OPN binds to osteoclasts through αvß3 integrin, which leads to cell activation and a consequent increase in bone resorption (29). Steitz et al. (30) suggested that this binding also promotes resorption of ectopic calcification. Studies on mice have shown that regression of arterial calcification was associated with the accumulation of OPN around osteoclast-like cells, when porcine aortic valves were subcutaneously implanted into mice carrying OPN homozygous wild-type alleles, compared to OPN homozygous null and OPN heterozygous alleles (30).

Osteoprotegerin/receptor activator of nuclear factor kappa-B/receptor activator of the nuclear factor kappa-B ligand system

After the discovery of OPG, a protein of the tumor necrosis factor (TNF) receptor family (31), the association between osteoporosis and vascular calcification became even more evident (32). OPG is a soluble cytokine produced by bone marrow stromal cells, immune system cells, lungs, liver, intestine, osteoblasts, vascular smooth muscle cells, and endothelial cells (31). Various

cytokines, peptides, hormones, and drugs modulate the expression and production of this protein. Cytokines such as TNF-α, interleukin 1a (IL-1a), IL-18 and TGF-ß, BMPs and steroid hormones are upper-regulators of OPG mRNA levels (33). On the other hand, substances such as parathormone, prostaglandin E2 and basic fibroblast growth factor, as well as drugs such as glucocorticosteroids and cyclosporin A, suppress the expression of OPG (34).

OPG acts by competing with receptor activator of nuclear factor kappa-B (RANK), a surface molecule of osteoclasts and dendritic cells, binding to RANKL present on osteoblasts and activated T lymphocytes. RANKL is involved in bone remodeling through a mechanism of osteoclast activation, as well as in the survival of dendritic cells and lymph node organogenesis (32). OPG-knockout mice present osteoporosis and calcification of the vascular wall of the aorta and renal arteries. These abnormalities were reversed after transgenic OPG restoration, whereas intravenous administration of the protein only reversed the osteoporotic phenotype (21). Similarly, in humans, Bekker et al. (35), analyzing bone mineral density in menopausal women, showed that subcutaneous injection of a single dose of OPG markedly reduced bone resorption in these women after 6 weeks. In addition, some investigators have suggested that estrogen therapy is associated with an increase in OPG levels (36).

Min et al. (21) demonstrated that OPG is normally expressed in arteries but RANK and RANKL are not detected in the arterial walls of wild-type adult mice. Differently, RANKL and RANK transcripts are detected in the calcified arteries of OPG null mice. Furthermore, RANK transcript expression coincides with the presence of multinuclear osteoclast-like cells. These findings indicate that the OPG/RANK/RANKL signaling pathway may play an important role in calcification processes.

Yano et al. (37) also observed an increase in serum OPG in postmenopausal women with osteoporosis when compared to those with normal bone mass, and OPG levels were higher in patients with more severe osteoporosis. This paradoxical increase of OPG levels in patients with osteoporosis and vascular disease has been interpreted as an incomplete mechanism of regulation of the progression of these diseases. A recent study involving women with osteoporosis has shown a significant correlation between increased serum OPG levels, diabetes mellitus, stroke, and mortality due to cardiovascular disease (38). All of these findings suggest that OPG might be used as a marker of disease, with the increase in the levels of this protein being a compensatory response to bone mass loss and vascular damage (7).

Inflammatory mediators and osteoporosis and vascular calcification

The increase in the serum levels of some cytokines during the atherosclerotic process confirms the inflammatory etiology of this disease. Markers of inflammation such as C-reactive protein, IL-6 and TNF-α can be considered risk factors and some are directly related to the severity of atherosclerosis (39). Most inflammatory cytokines, such as IL-1, TNF-α, and IL-6, are produced in the vascular wall by the endothelium, smooth muscle cells and macrophages. These cytokines increase the expression of adhesion molecules on leukocytes (CD11b) and endothelial cells (P-selectin and intracellular adhesion molecule 1), in addition to stimulating the transcription of genes responsible for the production of chemotactic factors (7). Macrophages and monocytes, which are frequently found in atherosclerotic plaques, induce the osteogenic differentiation of cells in the vessel wall, provoking vascular calcification (40).

The effects of inflammation on osteoporosis are similar. Inflammatory cytokines are

potent stimulators of bone resorption (7). The bone resorptive potential of monocytes was found to be directly correlated with serum IL-1, IL-6, and TNF-α levels in postmenopausal women, and this action was inhibited by anti-TNF-α and anti-IL-1 antibodies. In addition, these cytokines stimulate the proliferation and differentiation of osteoclast precursors (41). The reduction of TGF-ß levels, together with the increase of IL-1, RANKL, and monocyte colony-stimulating factor, delay osteoclast apoptosis, with a consequent imbalance between bone formation and resorption which leads to loss of bone mass (42).

Other substances related to inflammatory processes that link osteoporosis and atherosclerosis are homocysteine and nitric oxide. Several mechanisms of vascular injury have been proposed for homocysteine, including a reduction in nitric oxide, endothelial dysfunction, increased platelet aggregation, and proliferation of vascular smooth muscle cells, among others (7).

Arterial hypertension, diabetes, dyslipidemia, and osteoporosis

Other well-known risk factors for atherosclerotic disease that have been related to bone metabolism are systemic arterial hypertension and diabetes mellitus (7). Some epidemiological studies have shown an association between increased blood pressure and low-bone mineral density, as well as higher urinary calcium excretion, in hypertensive patients compared to normotensive individuals (43). Furthermore, hypotensive drugs, such as thiazide diuretics and angiotensin-converting enzyme inhibitor, have been associated with an increase in bone mineral density (44). The presence of vascular disease is a common finding in diabetic patients and is almost always associated with calcifications of the middle and intimal layers. However, the association with osteoporosis is conflicting (7), although low-bone mass is a frequent characteristic of patients with type 1 diabetes (45). In contrast to type 1 diabetes, type 2 diabetes shows no specific correlation with osteoporosis (7).

Like hypertension and diabetes mellitus, dyslipidemia is one of the main risk factors for atherosclerotic disease. The low-density lipoprotein (LDL) fraction of cholesterol plays a fundamental role in the genesis of fatty plaques in the arterial wall, whereas high-density lipoprotein (HDL) protects against the occurrence of these plaques (46). At the same time that hyperlipidemia promotes calcification of the vessel wall, it inhibits osteoblastic differentiation in bone tissue (47). An increase of LDL levels and a reduction of HDL levels have been associated with low-bone mineral density in postmenopausal women (48). Oxidized LDL induces the expression of monocyte colony-stimulating factor, a potent stimulator of osteoclastic differentiation, thus promoting bone resorption by recruiting osteoclast precursor cells (49). At the same time, this lipid molecule acts in the suppression of terminal differentiation of stromal cells into osteoblasts (50). HDL, on the other hand, inhibits cytokines responsible for the osteogenic differentiation of vascular cells (51).

Statins and bisphosphonates

Statins have proven efficacy in the treatment of dyslipidemia, reducing cardiovascular mortality, with regression of coronary calcification especially due to a reduction of LDL cholesterol levels in these patients (52). In addition, these drugs are known to stabilize atherosclerotic plaques by reducing metalloproteases, oxidized LDL and macrophage activity (53). These hypolipidemic agents have also been related to increased bone mineralization in mice (54) and in patients with osteoporosis (55), with a reduction in the incidence of fractures (56). Some observational studies have shown a greater reduction in the incidence of fractures in

patients using statins compared to those taking other classes of hypolipidemic agents (56).

Another class of drugs with a possible anti-atherogenic action is that of the bisphosphonates, which are primarily inhibitors of bone resorption and are used in the treatment of osteoporosis (57). Experimental studies using animal models of vascular calcification have demonstrated that bisphosphonates completely inhibit arterial and cardiac calcification in mice (58). The protective effect of bisphosphonates has been attributed to their direct action on the vessel wall by sensitizing macrophages to undergo apoptosis, preventing foam cell formation by inhibiting the uptake of LDL and affecting cell replication (59). Also, a recent study showed that bisphosphonates induce inflammation and rupture of atherosclerotic plaques in apolipoprotein-E null mice (60).

The fact that these drugs act both on osteoporosis and on vascular calcification suggests that these diseases share common physiopathological pathways (7). The priority is to establish to what extent treatment for atherosclerosis is beneficial or not for osteoporosis and vice versa, as well as to determine the exact mechanisms shared by the two diseases (49). Thus, further studies are necessary to elaborate efficient and simultaneous strategies to reverse such common diseases that affect the general population and have a great impact on public health.

References

1. Wildner M, Peters A, Raghuvanshi VS, Hohnloser J, Siebert U. Superiority of age and weight as variables in predicting osteoporosis in postmenopausal white women. *Osteoporos Int* 2003; 14: 950-956.
2. Larson MG. Assessment of cardiovascular risk factors in the elderly: the Framingham Heart Study. *Stat Med* 1995; 14: 1745-1756.
3. von der Recke P, Hansen MA, Hassager C. The association between low bone mass at the menopause and cardiovascular mortality. *Am J Med* 1999; 106: 273-278.
4. Bagger YZ, Tanko LB, Alexandersen P, Qin G, Christiansen C. Radiographic measure of aorta calcification is a site-specific predictor of bone loss and fracture risk at the hip. *J Intern Med* 2006; 259: 598-605.
5. Sinnott B, Syed I, Sevrukov A, Barengolts E. Coronary calcification and osteoporosis in men and postmenopausal women are independent processes associated with aging. *Calcif Tissue Int* 2006; 78: 195-202.
6. Kiel DP, Kauppila LI, Cupples LA, Hannan MT, O'Donnell CJ, Wilson PW. Bone loss and the progression of abdominal aortic calcification over a 25 year period: the Framingham Heart Study. *Calcif Tissue Int* 2001; 68: 271-276.
7. McFarlane SI, Muniyappa R, Shin JJ, Bahtiyar G, Sowers JR. Osteoporosis and cardiovascular disease: brittle bones and boned arteries, is there a link? *Endocrine* 2004; 23: 1-10.
8. United Nations Population Division. *World population prospects: The 1998 revision*. New York: Population Division of the Department of Economic and Social Affairs of the United Nations; 1998.
9. Becker C, Crow S, Toman J, Lipton C, McMahon DJ, Macaulay W, et al. Characteristics of elderly patients admitted to an urban tertiary care hospital with osteoporotic fractures: correlations with risk factors, fracture type, gender and ethnicity. *Osteoporos Int* 2006; 17: 410-416.
10. Guggenbuhl P, Meadeb J, Chales G. Osteoporotic fractures of the proximal humerus, pelvis, and ankle: epidemiology and diagnosis. *Joint Bone Spine* 2005; 72: 372-375.
11. Miazgowski T. The prospective evaluation of the osteoporotic vertebral fractures incidence in a random population sample. *Endokrynol Pol* 2005; 56: 154-159.
12. O'Neill TW, Roy DK. How many people develop fractures with what outcome? *Best Pract Res Clin Rheumatol* 2005; 19: 879-895.
13. Poole-Wilson P. The prevention of cardiovascular disease worldwide: whose task and WHO's task? *Clin Med* 2005; 5: 379-384.
14. Koizumi J, Shimizu M, Miyamoto S, Takeda R, Ohka T, Kanaya H, et al. Risk evaluation of coronary heart disease and cerebrovascular disease by the Japan Atherosclerosis Society Guidelines 2002 using the cohort of the Holicos-PAT study. *J Atheroscler Thromb* 2005; 12: 48-52.
15. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation* 2002; 105: 1135-1143.
16. Tintut Y, Demer LL. Recent advances in multifactorial regulation of vascular calcification. *Curr Opin Lipidol* 2001; 12: 555-560.
17. Wayhs R, Zelinger A, Raggi P. High coronary artery calcium scores pose an extremely elevated risk for hard events. *J Am Coll Cardiol* 2002; 39: 225-230.
18. Ramsey-Goldman R, Manzi S. Association of osteoporosis and cardiovascular disease in women with systemic lupus erythematosus. *Arthritis Rheum* 2001; 44: 2338-2341.
19. Bezerra MC, Calomeni GD, Caparbo VF, Gebrim ES, Rocha MS, Pereira RM. Low bone density and low serum levels of soluble RANK ligand are associated with severe arterial calcification in patients with Takayasu arteritis. *Rheumatology* 2005; 44: 1503-1506.
20. Tintut Y, Parhami F, Tsingotjidou A, Tetradis S, Territo M, Demer LL. 8-Isoprostaglandin E2 enhances receptor-activated NFkappa B ligand (RANKL)-dependent osteoclastic potential of marrow hematopoietic precursors via the cAMP pathway. *J Biol Chem* 2002; 277:

14221-14226.
21. Min H, Morony S, Sarosi I, Dunstan CR, Capparelli C, Scully S, et al. Osteoprotegerin reverses osteoporosis by inhibiting endosteal osteoclasts and prevents vascular calcification by blocking a process resembling osteoclastogenesis. *J Exp Med* 2000; 192: 463-474.
22. Ducy P, Desbois C, Boyce B, Pinero G, Story B, Dunstan C, et al. Increased bone formation in osteocalcin-deficient mice. *Nature* 1996; 382: 448-452.
23. Luo G, Ducy P, McKee MD, Pinero GJ, Loyer E, Behringer RR, et al. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. *Nature* 1997; 386: 78-81.
24. Bostrom K, Tsao D, Shen S, Wang Y, Demer LL. Matrix GLA protein modulates differentiation induced by bone morphogenetic protein-2 in C3H10T1/2 cells. *J Biol Chem* 2001; 276: 14044-14052.
25. Lee KS, Kim HJ, Li QL, Chi XZ, Ueta C, Komori T, et al. Runx2 is a common target of transforming growth factor beta1 and bone morphogenetic protein 2, and cooperation between Runx2 and Smad5 induces osteoblast-specific gene expression in the pluripotent mesenchymal precursor cell line C2C12. *Mol Cell Biol* 2000; 20: 8783-8792.
26. Hruska KA, Mathew S, Saab G. Bone morphogenetic proteins in vascular calcification. *Circ Res* 2005; 97: 105-114.
27. Engelse MA, Neele JM, Bronckers AL, Pannekoek H, De Vries CJ. Vascular calcification: expression patterns of the osteoblast-specific gene core binding factor alpha-1 and the protective factor matrix gla protein in human atherogenesis. *Cardiovasc Res* 2001; 52: 281-289.
28. Abedin M, Tintut Y, Demer LL. Vascular calcification: mechanisms and clinical ramifications. *Arterioscler Thromb Vasc Biol* 2004; 24: 1161-1170.
29. Miyauchi A, Alvarez J, Greenfield EM, Teti A, Grano M, Colucci S, et al. Recognition of osteopontin and related peptides by an alpha v beta 3 integrin stimulates immediate cell signals in osteoclasts. *J Biol Chem* 1991; 266: 20369-20374.
30. Steitz SA, Speer MY, McKee MD, Liaw L, Almeida M, Yang H, et al. Osteopontin inhibits mineral deposition and promotes regression of ectopic calcification. *Am J Pathol* 2002; 161: 2035-2046.
31. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997; 89: 309-319.
32. Schoppet M, Preissner KT, Hofbauer LC. RANK ligand and osteoprotegerin: paracrine regulators of bone metabolism and vascular function. *Arterioscler Thromb Vasc Biol* 2002; 22: 549-553.
33. Bezerra MC, Carvalho JF, Prokopowitsch AS, Pereira RM. RANK, RANKL and osteoprotegerin in arthritic bone loss. *Braz J Med Biol Res* 2005; 38: 161-170.
34. Hofbauer LC, Shui C, Riggs BL, Dunstan CR, Spelsberg TC, O'Brien T, et al. Effects of immunosuppressants on receptor activator of NF-kappaB ligand and osteoprotegerin production by human osteoblastic and coronary artery smooth muscle cells. *Biochem Biophys Res Commun* 2001; 280: 334-339.
35. Bekker PJ, Holloway D, Nakanishi A, Arrighi M, Leese PT, Dunstan CR. The effect of a single dose of osteoprotegerin in postmenopausal women. *J Bone Miner Res* 2001; 16: 348-360.
36. Hofbauer LC, Schoppet M, Schuller P, Viereck V, Christ M. Effects of oral contraceptives on circulating osteoprotegerin and soluble RANK ligand serum levels in healthy young women. *Clin Endocrinol* 2004; 60: 214-219.
37. Yano K, Tsuda E, Washida N, Kobayashi F, Goto M, Harada A, et al. Immunological characterization of circulating osteoprotegerin/osteoclastogenesis inhibitory factor: increased serum concentrations in postmenopausal women with osteoporosis. *J Bone Miner Res* 1999; 14: 518-527.
38. Browner WS, Lui LY, Cummings SR. Associations of serum osteoprotegerin levels with diabetes, stroke, bone density, fractures, and mortality in elderly women. *J Clin Endocrinol Metab* 2001; 86: 631-637.
39. Libby P. Inflammation in atherosclerosis. *Nature* 2002; 420: 868-874.
40. Tintut Y, Patel J, Territo M, Saini T, Parhami F, Demer LL. Monocyte/macrophage regulation of vascular calcification *in vitro*. *Circulation* 2002; 105: 650-655.
41. Miyaura C, Kusano K, Masuzawa T, Chaki O, Onoe Y, Aoyagi M, et al. Endogenous bone-resorbing factors in estrogen deficiency: cooperative effects of IL-1 and IL-6. *J Bone Miner Res* 1995; 10: 1365-1373.
42. Pfeilschifter J, Koditz R, Pfohl M, Schatz H. Changes in proinflammatory cytokine activity after menopause. *Endocr Rev* 2002; 23: 90-119.
43. Tsuda K, Nishio I, Masuyama Y. Bone mineral density in women with essential hypertension. *Am J Hypertens* 2001; 14: 704-707.
44. Lynn H, Kwok T, Wong SY, Woo J, Leung PC. Angiotensin converting enzyme inhibitor use is associated with higher bone mineral density in elderly Chinese. *Bone* 2006; 38: 584-588.
45. Tuominen JT, Impivaara O, Puukka P, Ronnemaa T. Bone mineral density in patients with type 1 and type 2 diabetes. *Diabetes Care* 1999; 22: 1196-1200.
46. Castelli WP, Garrison RJ, Wilson PW, Abbott RD, Kalousdian S, Kannel WB. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. *JAMA* 1986; 256: 2835-2838.
47. Parhami F, Jackson SM, Tintut Y, Le V, Balucan JP, Territo M, et al. Atherogenic diet and minimally oxidized low density lipoprotein inhibit osteogenic and promote adipogenic differentiation of marrow stromal cells. *J Bone Miner Res* 1999; 14: 2067-2078.
48. Yamaguchi T, Sugimoto T, Yano S, Yamauchi M, Sowa H, Chen Q, et al. Plasma lipids and osteoporosis in postmenopausal women. *Endocr J* 2002; 49: 211-217.
49. Parhami F, Garfinkel A, Demer LL. Role of lipids in osteoporosis. *Arterioscler Thromb Vasc Biol* 2000; 20: 2346-2348.
50. Lecka-Czernik B, Moerman EJ, Grant DF, Lehmann JM, Manolagas SC, Jilka RL. Divergent effects of selective peroxisome proliferator-activated receptor-gamma 2 ligands on adipocyte versus osteoblast differentiation. *Endocrinology* 2002; 143: 2376-2384.
51. Parhami F, Basseri B, Hwang J, Tintut Y, Demer LL. High-density lipoprotein regulates calcification of vascular cells. *Circ Res* 2002; 91: 570-576.
52. Callister TQ, Raggi P, Cooil B, Lippolis NJ, Russo DJ. Effect of HMG-CoA reductase inhibitors on coronary artery disease as assessed by electron-beam computed tomography. *N Engl J Med* 1998; 339: 1972-1978.
53. McFarlane SI, Muniyappa R, Francisco R, Sowers JR. Clinical review 145: Pleiotropic effects of statins: lipid reduction and beyond. *J Clin Endocrinol Metab* 2002; 87: 1451-1458.
54. Mundy G, Garrett R, Harris S, Chan J, Chen D, Rossini G, et al. Stimulation of bone formation *in vitro* and in rodents by statins. *Science* 1999; 286: 1946-1949.
55. Edwards CJ, Hart DJ, Spector TD. Oral statins and increased bone-mineral density in postmenopausal women. *Lancet* 2000; 355: 2218-2219.
56. Meier CR, Schlienger RG, Kraenzlin ME, Schlegel B, Jick H. HMG-CoA reductase inhibitors and the risk of fractures. *JAMA* 2000; 283:

3205-3210.
57. Ylitalo R, Oksala O, Yla-Herttuala S, Ylitalo P. Effects of clodronate (dichloromethylene bisphosphonate) on the development of experimental atherosclerosis in rabbits. *J Lab Clin Med* 1994; 123: 769-776.
58. Price PA, Faus SA, Williamson MK. Bisphosphonates alendronate and ibandronate inhibit artery calcification at doses comparable to those that inhibit bone resorption. *Arterioscler Thromb Vasc Biol* 2001; 21: 817-824.
59. Luckman SP, Hughes DE, Coxon FP, Graham R, Russell G, Rogers MJ. Nitrogen-containing bisphosphonates inhibit the mevalonate pathway and prevent post-translational prenylation of GTP-binding proteins, including Ras. *J Bone Miner Res* 1998; 13: 581-589.
60. Shimshi M, Abe E, Fisher EA, Zaidi M, Fallon JT. Bisphosphonates induce inflammation and rupture of atherosclerotic plaques in apolipoprotein-E null mice. *Biochem Biophys Res Commun* 2005; 328: 790-793.