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Vascular calcification and osteoporosis: inflammatory responses to oxidized lipids

Linda L Demer

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In the early 1900s, cholesterol deposition in atherosclerosis was considered a passive, degenerative, inevitable and end-stage process of ageing. After decades of research, it is now recognized as an active, regulated, treatable and preventable disorder related to deposition and oxidation of lipoprotein components. Similarly, in the past few decades, vascular calcification also has been considered a passive, degenerative, inevitable, and end-stage process of ageing. But, after recent clinical and laboratory findings, there is increasing recognition that vascular calcification is an active, regulated process related to oxidized lipids that may be treatable and preventable.

The fact that complete bone tissue forms within the atherosclerotic artery wall has been known since at least the 1800s. In 1863, Virchow observed that vascular calcium deposits were not mere calcification, but ossification.[1] In 1908, investigators reported red marrow elements in bone tissue within atherosclerotic plaque.[2,3] Experimental models of atherosclerosis also have cartilage and marrow within plaque.[4]

As an overview, vascular calcification in general, and coronary calcification in particular, increase with ageing, are present in almost all subjects over age 65, are more frequent in diabetics, less common in African-Americans, and extremely common in end-stage renal disease. Current studies of coronary calcification utilize electron beam computed tomographic scanning (EBCT). This method has been described as an 'inaccurate' predictor of stenosis severity. While this is not incorrect, it may leave the wrong impression since EBCT is accurate when used to predict the presence of significant coronary artery disease, and the degree of plaque burden.[5]

Mechanism of vascular calcification

The mechanism of vascular calcification is under investigation. There may be more than one mechanism, since atherosclerosis itself occurs by several different mechanisms, and also because atherosclerotic calcification, which primarily involves the intimal layer of the arteries, appears to be in a different category than medial calcification (also known as Monckeberg's medial calcinosis), which is particularly common in diabetic patients.[6,7] Such a determination is difficult, though, because EBCT does not identify the layer of the artery affected.

Investigators in the 1980s recognized bone-like features of vascular calcification including the mineral hydroxyapatite and matrix vesicles. Similarities between artery and bone at the molecular level were identified by Giachelli *et al.*[8] who discovered a bone matrix protein, osteopontin, expressed in immature vascular cells. Schor *et al.* demonstrated that microvascular pericytes were capable of producing mineralization *in vitro*.[9] The possibility that atherosclerotic calcification occurs by the same molecular mechanism as embryonic bone formation was proposed by Bostrom *et al.* who demonstrated expression of the potent embryonic bone differentiation factor, BMP-2, in human calcified plaque.[10] A variety of bone proteins were then found in atherosclerotic lesions.[11–16]

Vascular calcification can be studied in tissue culture models. A subpopulation of cells from the aortic medial layer spontaneously produce bone mineral (hydroxyapatite) in tissue culture.[10] These calcifying vascular cells (CVC) recapitulate the sequence of molecular events defining osteoblastic differentiation including co-ordinate expression of alkaline phosphatase, collagen I, osteopontin, osteonectin, and osteocalcin.[17,18] Key mechanisms of vascular calcification include genetic determination, inflammatory mediators, apoptosis, matrix components, and homeobox genes.[19–24]

Clinical significance of vascular calcification

Measurement of vascular calcification

Aortic calcification is often measured by visual indexes of simple lateral roentgenography, though it is more accurately measured by quantitative computed tomographic (CT) scanning, since apparent density depends on penetration and technique. Coronary calcification is most often measured by EBCT, which differs from ordinary CT scanning in that images are acquired rapidly enough to minimize heart motion artefacts. Some groups are now attempting to validate routine CT scanning coupled with ECG gating to substitute for the more expensive EBCT. Coronary calcification is also measured by intravascular ultrasound, which detects mineral by sound reflection at the inner edge of the mineral deposit.

Departments of Medicine and Physiology, UCLA School of Medicine, Box 951679, 10833 LeConte Ave, Los Angeles, CA 90095–1679, USA. E-mail: Ldemer@mednet.ucla.edu

Coronary events

The degree of coronary calcification by EBCT is a sensitive and specific predictor for future cardiac events. The earliest evidence for this was limited by the need to include 'soft' endpoints to achieve sufficient numbers of events,[25] such as interventional or surgical treatment, which could be influenced by the results of the EBCT scan. Other early studies suggested that there was not a relationship with hard endpoints at early follow-up.[26] As follow-up time has increased, the correlation between EBCT results and the hard endpoints, myocardial infarction and coronary death, has remained positive.[27] However, patients with the least circumferential extent of coronary calcification, measured by intravascular ultrasound, have more occasions of acute coronary syndrome.[28] These findings are difficult to reconcile with those of EBCT, suggesting that the circumferential extent of calcification is not related to calcium score by EBCT, and that the circumferential versus longitudinal distribution have different implications.

Plaque rupture

Cardiac events are often the result of plaque rupture or ulceration.[29] Plaque disruption may be prevented or promoted by calcium deposits because they may strengthen the plaque against circumferential mechanical stress,[30] but they also introduce solid shear stress concentration where the non-distensible mineral interfaces with distensible tissue. Under mechanical stress induced by balloon angioplasty, calcified plaque is more likely to rupture than non-calcified plaque,[31] and the rupture occurs along the interface between the calcium deposit and soft tissue.[32] Thus, the ratio of surface area to volume in calcium deposits may determine whether they are harmful or protective. The presence of calcium deposits also correlates with adverse outcomes,[33] and restenosis[34] in coronary interventional procedures.

Loss of the Windkessel effect in aortic calcification

Mineralization of the aorta may have greater significance than of the coronaries. The normal aorta, with its multiple layers of elastin, is highly resilient. This resilience serves a pump function, known as the Windkessel effect. During systole, the aorta distends which reduces the work of the heart by reducing afterload. During diastole, the aorta recoils, with an energy that propels blood throughout the vasculature, particularly into the coronary tree, which depends on this diastolic aortic recoil for most of its perfusion. When the aorta calcifies and becomes rigid, it loses its Windkessel function,[35] the work of the heart increases,[36] and coronary flow is reduced[37] leading to left ventricular hypertrophy, congestive heart failure and coronary insufficiency in patients with coronary disease.[38–41] Congestive heart failure and myocardial infarction are major health problems in the over 65 age group. Aortic calcification, present in the vast majority of these individuals,[42] is considered a factor in both.[43–46]

Cardiac valve calcification

Calcific valvular stenosis is responsible for significant cardiovascular morbidity and mortality. For decades, valvular stenosis was considered independent of atherosclerosis and its risk factors. However, it is now known that cardiac valvular calcification shares risk factors with atherosclerosis[47] and it has many features of bone.[48–50] In addition, its progression is reduced in response to lipid lowering therapy.[51]

Accelerated vascular calcification in dialysis patients

In haemodialysis patients, vascular calcification develops early and progresses rapidly,[52] paralleling their high rate of premature cardiovascular disease.[53] These patients often receive treatment with vitamin D and warfarin. At high doses, vitamin D promotes vascular calcification,[39,54] and warfarin, a widely-used anti-coagulant, blocks vitamin K dependent carboxylation[55] which is critical for the function of some proteins in the clotting cascade and two involved in mineralizing tissue, osteocalcin and matrix GLA protein (MGP). Matrix GLA protein is expressed in the artery wall, and it regulates *in vitro* vascular calcification,[56] and mice deficient in MGP develop complete ossification of the aorta and all its branches. There is now evidence that MGP binds and inhibits bone morphogenetic protein (BMP-2),[57,58] the level of which determines the lineage to be taken by mesenchymal progenitor cells. Hence, in the MGP null mouse, where BMP-2 activity would be unopposed, the high level of activity would be expected to direct medial cells along an osteoblastic rather than smooth muscle lineage, thus accounting for the phenotype. Since MGP function depends on gamma-carboxylation, warfarin treatment may interfere with its protective function in the vasculature. This raises the question of whether warfarin treatment contributes to the accelerated vascular calcification in dialysis patients.[59]

Vascular calcification and osteoporosis

Osteoporosis treatment efficacy is often assessed by bone densitometry of the lumbar vertebrae. In this technique, an X-ray beam is projected through the abdominal wall and lumbar spine. Since the amount of beam attenuation corresponds with the amount of calcium mineral in the beam path, the density of mineral in the vertebrae can be calculated.[60] However, it is often unappreciated that this beam path includes the abdominal aorta, a prominent and early site of vascular calcification. Thus, a treatment that increased only aortic calcification would also increase lumbar densitometric beam attenuation, potentially leading to its incorrect identification as successful osteoporosis treatment.

In general, postmenopausal women are advised to take calcium supplements to prevent or treat osteoporosis, implying that bone loss is due to insufficient dietary calcium. Yet, in many patients with osteoporosis, loss of bone tissue from the skeleton occurs at the same time as formation of bone in the artery wall. This paradox suggests that dietary calcium is not the limiting factor. The association of osteoporosis with vascular calcification has been reported widely,[61–66] and it may[67] or may not[68–70] be explained by their mutual correlation with ageing. In rodents, vascular calcification and osteoporosis co-exist under at least three conditions: deficiency of osteoprotegerin, an osteoclast inhibitory factor,[71] deficiency of dietary essential fatty acids[72] and hyperlipidaemia.

Lipids and biomineralization

In vitro and *in vivo* studies show that oxidized lipids not only promote mineralization of vascular cells but they also inhibit mineralization of bone cells.[73] Low density lipoprotein (LDL) levels correlate with both coronary and aortic valve calcification progression,[74] and LDL proteins accumulate in calcified aortic valves.[75] Hyperlipidaemia is associated with rapid progression of coronary calcification,[76] and lipid-lowering therapy reduces progression of both coronary and valvular calcification.[51,77] Oxidized lipids induce osteoblastic differentiation in vascular cells *in vitro*,[78] and hyperlipidaemia reduces bone mineral density *in vivo* in mice.[79]

The paradox of simultaneous osteolysis and ectopic ossification

One possible unifying theme explaining this paradox is that accumulation and oxidation of lipid deposits in tissue may mimic chronic infection and stimulate immune responses that promote hardening of soft tissue and the softening of hard tissue. The bacterial cell wall contains lipids, and they are modified by oxidizing factors released by phagocytic cells, such as superoxide radical and nitric oxide from macrophages. Thus, oxidized lipids in general may trigger the immune system to respond as it does to persistent bacterial infection. It is well known that the immune response to longstanding infection or inflammation in bone is osteolysis,[80] which would dissolve a substrate for bacterial infectious growth. It is also well known that the immune response to longstanding infection or inflammation in soft tissue is heterotopic bone formation around the site, which would wall off any infectious organism. Tuberculous granulomata result from this process. Thus, lipid accumulation and oxidation may lead to a reversal of the normal regional control of biomineralization, promoting calcification of soft tissue and osteolysis of bone, accounting for the paradox of bone formation in the arteries of patients who are losing bone from their skeletons.

Additional epidemiological considerations

In a case-control study from Thailand, serum biomarkers of osteoporosis and coronary heart disease were compared. No statistically significant difference was found in bone turnover markers between 118 coronary artery disease patients versus control subjects.[81] Since a reduction in osteogenesis is not always accompanied by changes in turnover, however, such measures may not detect an association between coronary disease and reduced bone formation. If accurate markers of bone differentiation/formation are developed in the future, this type of study, comparing degree of vascular calcification with serum markers of bone differentiation or formation may help determine whether such a relation exists.

From a mechanical standpoint, whether calcium deposits in arteries are circumferential versus longitudinal may influence stability. These assessments are difficult to make by EBCT because of resolution limitations, and they are difficult by intravascular ultrasound because calcium deposits reflect the echoes allowing assessment only of the edge of the deposit closest to the tranducer. Beckman *et al.* compared the extent of cirumferential calcification (not longitudinal), and found that patients with acute coronary syndromes had less cirumferential extent than those with stable angina.[82] Although there are potential confounding effects in this study, it raises the possibility that circumferential calcification has a stabilizing effect.

Although long-term warfarin use in atrial fibrillation, by reducing function of MGP, would be expected to promote arterial calcification, clinical events are generally reduced in treated patients. The likely reason, of course, is warfarin's direct effect on blood coagulation and its contribution to thrombosis, the major event in atrial fibrillation. It also remains possible that calcification could stabilize plaque as well as reduce clot formation.

It remains controversial whether coronary heart disease risk factor profiles (e.g. the Framingham score) have a greater predictive value if the extent of arterial calcification is included. Arad *et al.* determined the areas under the receiver-operator characteristics curves as 0.84 and 0.86 for predicting non-fatal myocardial infarctions and deaths from coronary calcification score.[83] Overall, the view is that calcification scores make a small improvement in predictive value.

Coronary calcification and osteoporosis have been associated with presence of infectious agents, such as *Chlamydia pneumoniae* and *Helicobacter pylori*, as well as markers of chronic infection, such as C-reactive protein. While this most likely suggests that arterial calcification is a chronic inflammatory process, it remains possible that inflammatory processes in bone alter the serum bone regulatory factor levels, resulting in indirect effects on vascular calcification.

References

[1] Virchow R. *Cellular Pathology: As Based upon Physiological and Pathological Histology* (translated by Frank Chance, 1971). An unabridged and unaltered republication of the English translation originally published in Dover; New York, 1863, pp. 404–08.

[2] Buergher L, Oppenheimer A. Bone formation in sclerotic arteries. *J Exper Med* 1908;**10:**354–67.

[3] Bunting CH. The formation of true bone with cellular (red) marrow in a sclerotic aorta. *J Exper Med* 1906;**8:**365–76.

[4] Haust MD, Moore RH. Spontaneous lesions of the aorta in the rabbit. In: Roberts JC, Straus R (eds). *Comparative Atherosclerosis: The Morphology of Spontaneous and Induced Atherosclerotic Lesions in Animals and Its Relation to Human Disease.* New York: Harper and Row, 1965, p. 268.

[5] Sangiorgi G, Rumberger JA, Severson A *et al.* Arterial calcification and not lumen stenosis is highly correlated with atherosclerotic plaque burden in humans: a histologic study of 723 coronary artery segments using nondecalcifying methodology. *J Am Coll Cardiol* 1998;**31:**126–33.

[6] Lehto S, Niskanen L, Suhonen M, Ronnemaa T, Laakso M. Medial artery calcification. A neglected harbinger of cardiovascular complications in non-insulin dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol* 1995;**16:**978–83.

[7] Nicolosi AC, Pohl LL, Parsons P, Cabria RA, Olinger GN. Increased incidence of radial artery calcification in patients with diabetes mellitus. *J Surg Res* 2002;**102:**1–5.

[8] Giachelli C, Bae N, Lombardi D, Majesky M, Schwartz S. Molecular cloning and characterization of 2B7, a rat mRNA which distinguishes smooth muscle cell phenotypes *in vitro* and is identical to osteopontin (secreted phosphoprotein I, 2aR). *iochem Biophys Res Commun* 1991;**177:**867–73.

[9] Schor AM, Allen TD, Canfield AE, Sloan P, Schor SL. Pericytes derived from the retinal microvasculature undergo calcification *in vitro*. *J Cell Sci* 1990;**97:**449–61.

[10] Bostrom K, Watson KE, Horn S, Wortham C, Herman IM, Demer LL. Bone morphogenetic protein expression in human atherosclerotic lesions. *J Clin Invest* 1993;**91:**1800–09.

[11] Hirota S, Imakita M, Kohri K *et al.* Expression of osteopontin messenger RNA by macrophages in atherosclerotic plaques. A possible association with calcification. *Am J Pathol* 1993;**143:**1003–08.

[12] Ikeda T, Shirasawa T, Esaki Y, Yoshiki S, Hirokawa K. Osteopontin mRNA is expressed by smooth muscle-derived foam cells in human atherosclerotic lesions of the aorta. *J Clin Invest* 1993;**92:**2814–20.

[13] O'Brien ER, Garvin MR, Stewart DK *et al.* Osteopontin is synthesized by macrophage, smooth muscle, and endothelial cells in primary and restenotic human coronary atherosclerotic plaques. *Arterioscler Thromb* 1994;**14:**1648–56.

[14] Shanahan CM, Cary NRB, Metcalfe JC, Weissberg PL. High expression of genes for calcification–regulating proteins in human atherosclerotic plaques. *J Clin Invest* 1994;**93:**2393–402.

[15] Iimura T, Oida S, Takeda K, Maruoka Y, Sasaki S. Changes in homeobox-containing gene expression during ectopic bone formation induced by bone morphogenetic protein. *Biochem Biophys Res Commun* 1994;**201:**980–87.

[16] Fitzpatrick LA, Severeson A, Edwards WD, Ingram RT. Diffuse calcification in human coronary arteries: Association of osteopontin with atherosclerosis. *J Clin Invest* 1994;**94:**1597–604.

[17] Watson KE, Bostrom K, Ravindranath R, Lam T, Norton B, Demer LL. TGF-beta 1 and 25-hydroxycholesterol stimulate osteoblast-like vascular cells to calcify. *J Clin Invest* 1994;**93:**2106–13.

[18] Tintut Y, Parhami F, Bostrom K, Jackson SM, Demer LL. cAMP stimulates osteoblast-like differentiation of calcifying vascular cells. Potential signaling pathway for vascular calcification. *J Biol Chem* 1998;**273:**7547–53.

[19] Qiao JH, Xie PZ, Fishbein MC *et al.* Pathology of atheromatous lesions in inbred and genetically engineered mice. Genetic determination of arterial calcification. *Arterioscler Thromb* 1994;**14:**1480–97.

[20] Tintut Y, Patel J, Parhami F, Demer LL. Tumor necrosis factor-alpha promotes *in vitro* calcification of vascular cells via the cAMP pathway. *Circulation* 2000;**102:**2636–42.

[21] Proudfoot D, Skepper JN, Hegyi L, Bennett MR, Shanahan CM, Weissberg PL. Apoptosis regulates human vascular calcification *in vitro*: evidence for initiation of vascular calcification by apoptotic bodies. *Circ Res* 2000;**87:**1055–62.

[22] Canfield AE, Farrington C, Dziobon MD *et al.* The involvement of matrix glycoproteins in vascular calcification and fibrosis: an immunohistochemical study. *J Pathol* 2002;**196:**228–34.

[23] Watson KE, Parhami F, Shin V, Demer LL. Fibronectin and collagen I matrixes promote calcification of vascular cells *in vitro*, whereas collagen IV matrix is inhibitory. *Arterioscler Thromb Vasc Biol* 1998;**18:**1964–71.

[24] Towler DA, Bidder M, Latifi T, Coleman T, Semenkovich CF. Diet-induced diabetes activates an osteogenic gene regulatory program in the aortas of low density lipoprotein receptor-deficient mice. *J Biol Chem* 1998;**273:**30427–34.

[25] Arad Y, Newstein D, Cadet F, Roth M, Guerci AD. Association of multiple risk factors and insulin resistance with increased prevalence of asymptomatic coronary artery disease by an electron-beam computed tomographic study. *Arterioscler Thromb Vasc Biol* 2001; **21:**2051–58.

[26] Detrano RC, Wong ND, Doherty TM *et al.* Coronary calcium does not accurately predict near-term future coronary events in high-risk adults. *Circulation* 1999;**99:**2633–38.

[27] Keelan PC, Bielak LF, Ashai K *et al.* Long-term prognostic value of coronary calcification detected by electron-beam computed tomography in patients undergoing coronary angiography. *Circulation* 2001;**104:**412–17.

[28] Beckman JA, Ganz J, Creager MA, Ganz P, Kinlay S. Relationship of clinical presentation and calcification of culprit coronary artery stenoses. *Arterioscler Thromb Vasc Biol* 2001;**21:**1618–22.

[29] Farb A, Burke AP, Tang AL *et al.* Coronary plaque erosion without rupture into a lipid core. A frequent cause of coronary thrombosis in sudden coronary death. *Circulation* 1996;**93:**1354–63.

[30] Huang H, Virmani R, Younis H, Burke AP, Kamm RD, Lee RT. The impact of calcification on the biomechanical stability of atherosclerotic plaques. *Circulation* 2001;**103:**1051–56.

[31] Honye J, Mahon DJ, Jain A *et al.* Morphological effects of coronary balloon angioplasty *in vivo* assessed by intravascular ultrasound imaging. *Circulation* 1992;**85:**1012–25.

[32] Fitzgerald PJ, Ports TA, Yock PG. Contribution of localized calcium deposits to dissection after angioplasty. An observational study using intravascular ultrasound. *Circulation* 1992;**86:**64–70.

[33] Ellis SG, DeCesare NB, Pinkerton CA *et al.* Relation of stenosis morphology and clinical presentation to the procedural results of directional coronary atherectomy. *Circulation* 1991;**84:**644–53.

[34] Tohma M, Yamaguchi T. Risk factors for later restenosis after successful coronary angioplasty: Mitsui Memorial Hospital experience. *J Cardiol* 1991;**21:**43–52.

[35] Maeta H, Hori M. Effects of a lack of aortic 'Windkessel' properties on the left ventricle. *Jpn Circ J* 1985;**49:**232–37.

[36] Watanabe H, Ohtsuka, S, Kakihana M, Sugishita Y. Coronary circulation in dogs with an experimental decrease in aortic compliance. *J Am Coll Cardiol* 1993;**21:**1497–506.

[37] Ohtsuka S, Kakihana M, Watanabe H, Sugishita Y. Chronically decreased aortic distensibility causes deterioration of coronary perfusion during increased left ventricular contraction. *J Am Coll Cardiol* 1994;**24:**1406–14.

[38] Simonson E, Nakagawa K. Effect of age on pulse wave velocity and aortic ejection time in healthy men and in men with coronary artery disease. *Circulation* 1960;**22:**126–29.

[39] Niederhoffer N, Borbryshev YV, Lartaud-Idjouadiene I, Giummelly P, Atkinson J. Aortic calcification produced by vitamin D3 plus nicotine. *J Vasc Res* 1997;**34:**386–98.

[40] Dart AM, Lacombe F, Yeoh JK *et al.* Aortic distensibility in patients with isolated hypercholesterolaemia, coronary artery disease, or cardiac transplant. *Lancet* 1991;**338:**270–73.

[41] Bouthier JD, De Luca N, Safar ME, Simon AC. Cardiac hypertrophy and arterial distensibility in essential hypertension. *Am Heart J* 1985;**109:**1345–52.

[42] Newman AB, Naydeck BL, Sutton-Tyrrell K, Feldman A, Edmundowicz D, Kuller LH. Coronary artery calcification in older adults to age 99: prevalence and risk factors. *Circulation* 2001; **104:**2679–84.

[43] Mitchell JR, Adams JH. Aortic size and aortic calcification: a necropsy study. *Atherosclerosis* 1977;**27:**437–46.

[44] Baedenkopf WG, Daoud AS, Love BM. Calcification in the coronary arteries and its relationship to arteriosclerosis and myocardial infarction. *Am J Roentgenol* 1964;**92:**865–71.

[45] Iribarren C, Sidney S, Sternfeld B, Browner WS. Calcification of the aortic arch: Risk factors and association with coronary heart disease, stroke, and peripheral vascular disease. *JAMA* 2000;**283:**2810–15.

[46] Stefanadis C, Wooley CF, Bush CA, Koibash AJ, Boudoulas H. Aortic distensibility abnormalities in coronary artery disease. *Am J Cardiol* 1987;**49:**1300–304.

[47] Pohle K, Maffert R, Ropers D *et al.* Progression of aortic valve calcification: association with coronary atherosclerosis and cardiovascular risk factors. *Circulation* 2001;**104:**1927–32.

[48] Feldman T, Glagov S, Carroll JD. Restenosis following successful balloon valvuloplasty: Bone formation in aortic valve leaflets. *Cathet Cardiovasc Diagn* 1993;**29:**1–7.

[49] O'Brien KD, Kuusisto J, Reichenbach DD *et al.* Osteopontin is expressed in human aortic valvular lesions. *Circulation* 1995;**92:**2163–68.

[50] Mohler ER 3rd, Gannon F, Reynolds C, Zimmerman R, Keane MG, Kaplan FS. Bone formation and inflammation in cardiac valves. *Circulation* 2001;**103:**1522–28.

[51] Novaro GM, Tiong IY, Pearce GL, Lauer MS, Sprecher DL, Griffin BP. Effect of hydroxymethylglutaryl coenzyme a reductase inhibitors on the progression of calcific aortic stenosis. *Circulation* 2001; **104:**2205–09.

[52] Goodman WG, Goldin J, Kuizon BD *et al.* Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med* 2000;**342:**1478–83.

[53] Varghese K, Cherian G, Abraham UT, Hayat NJ, Johny KV. Predictors of coronary disease in patients with end stage renal disease. *Ren Fail* 2001;**23:**797–806.

[54] Jono S, Nishizawa Y, Shioi A, Morii H. 1,25-dihydroxyvitamin D3 increases *in vitro* vascular calcification by modulating secretion of endogenous parathyroid hormone-related peptide. *Circulation* 1998;**98:**1302–06.

[55] Nash G. Vitamin K in anticoagulation therapy. *Lancet* 2001;**357:**638.

[56] Canfield AE, Doherty MJ, Kelly V *et al.* Matrix GLA protein is differentially expressed during the deposition of a calcified matrix by vascular pericytes. *FEBS Lett* 2000;**487:**267–71.

[57] 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:**14044–52.

[58] Zebboudj AF, Imura M, Bostrom K. Matrix GLA protein, a regulatory protein for bone morphogenetic protein-2. *J Biol Chem* 2002; **277:**4388–94.

[59] Goodman WG. Vascular calcification in chronic renal failure. *Lancet* 2001;**358:**1115–16.

[60] Kinoshita H, Tamaki T, Hashimoto T, Kasagi F. Factors influencing lumbar spine bone mineral density assessment by dual-energy X-ray absorptiometry: comparison with lumbar spinal radiogram. *J Orthop Sci* 1998;**3:**3–9.

[61] Sugihara N, Matsuzaki M. The influence of severe bone loss on mitral annular calcification in postmenopausal osteoporosis of elderly Japanese women. *Jpn Circ J* 1993;**57:**14–26.

[62] Ouchi Y, Akishita M, de Souza AC, Nakamura T, Orimo H. Age-related loss of bone mass and aortic/aortic valve calcification—reevaluation of recommended dietary allowance of calcium in the elderly. *Ann N Y Acad Sci* 1993;**676:**297–307.

[63] Banks LM, Lees B, MacSweeney JE, Stevenson JC. Effect of degenerative spinal and aortic calcification on bone density measurements in post-menopausal women: Links between osteoporosis and cardiovascular disease? *Eur J Clin Invest* 1994;**12:**813–17.

[64] Broulik PD, Kapitola J. Interrelations between body weight, cigarette smoking and spine mineral density in osteoporotic Czech women. *Endocr Regul* 1993;**27:**57–60.

[65] Boukhris R, Becker KL. Calcification of the aorta and osteoporosis. *JAMA* 1972;**219:**1307–11.

[66] Dent CE, Engelbrecht HE, Godfrey RC. Osteoporosis of lumbar vertebrae and calcification of abdominal aorta in women living in Durban. *BMJ* 1968;**4:**76–79.

[67] Aoyagi K, Ross PD, Orloff J, Davis JW, Katagiri H, Wasnich RD. Low bone density is not associated with aortic calcification. *Calcif Tissue Int* 2001;**69:**20–24.

[68] Jie KG, Bots ML, Vermeer C, Witteman JC, Grobbee DE. Vitamin K status and bone mass in women with and without aortic atherosclerosis: a population-based study. *Calcif Tissue Int* 1996; **59:**352–56.

[69] 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:**271–76.

[70] Hak AE, Pols HA, van Hemert AM, Hofman A, Witteman JC. Progression of aortic calcification is associated with metacarpal bone loss during menopause: a population-based longitudinal study. *Arterioscler Thromb Vasc Biol* 2000;**28:**1926–31.

[71] Bucay N, Sarosi I, Dunstan CR *et al.* Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes Dev* 1998;**12:**1260–68.

[72] Kruger MC, Horrobin DF. Calcium metabolism, osteoporosis and essential fatty acids: A review. *Prog Lipid Res* 1997;**36:**1331–51.

[73] Parhami F, Morrow AD, Balucan J *et al.* Lipid oxidation products have opposite effects on calcifying vascular cell and bone cell differentiation. A possible explanation for the paradox of arterial calcification in osteoporotic patients. *Arterioscler Thromb Vasc Biol* 1997;**17:**680–87.

[74] Pohle K, Maffert R, Ropers D *et al.* Progression of aortic valve calcification: Association with coronary atherosclerosis and cardiovascular risk factors *Circulation* 2001;**104:**1927–32.

[75] O'Brien KD, Reichenbach DD, Marcovina SM, Kuusisto J, Alpers CE, Otto CM. Apolipoproteins B, (a), and E accumulate in the morphologically early lesion of 'degenerative' valvular aortic stenosis. *Arterioscler Thromb Vasc Biol* 1996;**16:**523–32.

[76] Tamashiro M, Iseki K, Sunagawa O *et al.* Significant association between the progression of coronary artery calcification and dyslipidemia in patients on chronic hemodialysis. *Am J Kidney Dis* 2001;**38:**64–69.

[77] 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:**1972–78.

[78] Parhami F, Jackson SM, Tintut Y *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:**2067–78.

[79] Parhami F, Tintut Y, Beamer WG, Gharavi N, Goodman W, Demer LL. Atherogenic high-fat diet reduces bone mineralization in mice. *J Bone Miner Res* 2001;**16:**182–88.

[80] Chole RA, Hughes RM, Faddis BT. Keratin particle-induced osteolysis: a mouse model of inflammatory bone remodeling related to cholesteatoma. *J Assoc Res Otolaryngol* 2001;**2:**65–71.

[81] Poungvarin N, Leowattana W, Mahanonda N, Bhuripanyo K, Pokum S, Worawattananon P. Biochemical markers of bone turnover in angiographically-demonstrated coronary artery disease patients and health Thais. *J Med Assoc Tha* 2000;**83:**S13–18.

[82] Beckman JA, Ganz J, Creager MA, Ganz P, Kinlay S. Relationship of clinical presentation and calcification of culprit coronary artery stenoses. *Arterioscler Thromb Vasc Biol* 2001;**21:**1618–22.

[83] Arad Y, Spadaro LA, Goodman K, Newstein D, Guerci AD. Prediction of coronary events with electron beam computed tomography. *J Am Coll Cardiol* 2000;**36:**1253–60.