

# SELECTED ABSTRACTS



## SOFT TISSUE CALCIFICATION

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### **Ectopic Calcification**

#### **Gathering Hard Facts about Soft Tissue Mineralization**

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*American Journal of Pathology*. 1999;154:671-675.)

Ectopic calcification is defined as inappropriate biomineralization occurring in soft tissues. Ectopic calcifications are typically composed of calcium phosphate salts, including hydroxyapatite, but can also consist of calcium oxalates and octacalcium phosphate as seen in kidney stones. In uremic patients, a systemic mineral imbalance is associated with widespread ectopic calcification, referred to as metastatic calcification. In the absence of a systemic mineral imbalance, ectopic calcification is typically termed dystrophic calcification. Often, these sites show evidence of tissue alteration and/or necrosis. Dystrophic mineralization is commonly observed in soft tissues as a result of injury, disease, and aging. Although most soft tissues can undergo calcification, skin, kidney, tendons, and cardiovascular tissues appear particularly prone to developing this pathology. In addition, a number of prosthetic devices are prone to ectopic calcification, as discussed below. Recent insights into the mechanisms regulating ectopic calcification have come from studies of cardiovascular calcification, including that by Kim et al in this issue of the *Journal*, and thus will be the major focus of this article. The reader is referred to other reviews for information about additional tissue-specific ectopic calcifications.

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### **Basic calcium phosphate crystals stimulate the endocytotic activity of cells--inhibition by anti-calcification agents.**

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*Biochem Biophys Res Commun*. 2003 Dec 26;312(4):1053-9

Pathological calcifications are associated with many medical conditions including diabetes, breast cancer, and crystals-associated osteoarthritis. The deposition of calcium-containing crystals on cells induces detrimental cellular effects and speeds up the progression of associated diseases. We carried out the present study to test the hypotheses that calcium-containing crystals may stimulate the influx of other molecules existing in the extracellular fluid disturbing normal molecular signaling and that anti-calcification agent will inhibit such endocytotic process. We found that basic calcium phosphate (BCP) crystals greatly stimulated the endocytotic activity of cells by rendering the cells more permeable and that the anti-calcification agent phosphocitrate and several others inhibited the crystals-mediated endocytosis. This is the first study reporting that the endocytotic activity of cells is affected by BCP crystals and that such endocytotic activity can be inhibited by anti-calcification agents. Since calcium-containing crystals are associated with many human diseases and in many circumstances are associated with apoptotic bodies, extracellular and matrix vesicles where DNA fragments, small peptides, and minerals are released into extracellular space, the findings reported here are important for our understanding of the complex biological effects and the potential pathological role of calcium-containing crystals in crystals-associated diseases, and for the development of disease modifying agents as well.

## Molecular Mechanisms of Vascular Calcification

### Lessons Learned from the Aorta

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*Arterioscler Thromb Vasc Biol.* 2006;26:1423-1430.

**Abstract**—Vascular calcification increasingly afflicts our aging and dysmetabolic population. Once considered a passive process, it has emerged as an actively regulated form of calcified tissue metabolism, resembling the mineralization of endochondral and membranous bone. Executive cell types familiar to bone biologists, osteoblasts, chondrocytes, and osteoclasts, are seen in calcifying macrovascular specimens. Lipidaceous matrix vesicles, with biochemical and ultrastructural “signatures” of skeletal matrix vesicles, nucleate vascular mineralization in diabetes, dyslipidemia, and uremia. Skeletal morphogens (bone morphogenetic protein-2 (BMP) and BMP4 and Wnts) divert aortic mesoangioblasts, mural pericytes (calcifying vascular cells), or valve myofibroblasts to osteogenic fates. Paracrine signals provided by these molecules mimic the epithelial–mesenchymal interactions that induce skeletal development. Vascular expression of pro-osteogenic morphogens is entrained to physiological stimuli that promote calcification. Inflammation, shear, oxidative stress, hyperphosphatemia, and elastinolysis provide stimuli that: (1) promote vascular BMP2/4 signaling and matrix remodeling; and (2) compromise vascular defenses that limit calcium deposition, inhibit osteo/chondrogenic trans-differentiation, and enhance matrix vesicle clearance. In this review, we discuss the biology of vascular calcification. We highlight how aortic fibrofatty tissue expansion (adventitia, valve interstitium), the adventitial-medial vasa, vascular matrix, and matrix vesicle metabolism contribute to the regulation of aortic calcium deposition, with greatest emphasis placed on diabetic vascular disease.

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## Calcification in atherosclerosis: Bone biology and chronic inflammation at the arterial crossroads

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Dystrophic or ectopic mineral deposition occurs in many pathologic conditions, including atherosclerosis. Calcium mineral deposits that frequently accompany atherosclerosis are readily quantifiable radiographically, serve as a surrogate marker for the disease, and predict a higher risk of myocardial infarction and death. Accelerating research interest has been propelled by a clear need to understand how plaque structure, composition, and stability lead to devastating cardiovascular events. In atherosclerotic plaque, accumulating evidence is consistent with the notion that calcification involves the participation of arterial osteoblasts and osteoclasts. Here we summarize current models of intimal arterial plaque calcification and highlight intriguing questions that require further investigation. Because atherosclerosis is a chronic vascular inflammation, we propose that arterial plaque calcification is best conceptualized as a convergence of bone biology with vascular inflammatory pathobiology.

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## Vascular calcification mechanisms

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*J Am Soc Nephrol.* 2004 Dec;15(12):2959-64.

Vascular calcification is highly correlated with cardiovascular disease mortality, especially in patients with ESRD or diabetes. In addition to the devastating effects of inappropriate biomineralization seen in cardiac valvulopathies, calciphylaxis, and idiopathic arterial calcification, vascular calcification is now recognized as a marker of atherosclerotic plaque burden as well as a major contributor to loss of arterial compliance and increased pulse pressure seen with age, diabetes, and renal insufficiency. In recent years, several mechanisms to explain vascular calcification have been identified including (1) loss of inhibition, (2) induction of bone formation, (3) circulating nucleation complexes, and (4) cell death. Alterations in calcium (Ca) and phosphorus (P) balance as seen in patients with ESRD promotes vascular calcification via multiple mechanisms and may explain the alarmingly high levels of cardiovascular disease deaths in these patients. Strategies to control Ca and P levels in patients with ESRD have met with early success in preventing progression of vascular calcification. Whether or not vascular calcification can be reversed is not yet known, but exciting new studies suggest that this may be possible in the future.

## Reduced bone mineral density is associated with breast arterial calcification

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J Clin Endocrinol Metab. 2008 Jan;93(1):208-11.

**BACKGROUND:** Arterial calcification, a marker of atherosclerosis, results from a complex process of biomineralization resembling bone formation. Breast arterial calcification (BAC) has been associated with angiographic and clinical cardiovascular disease. The purpose of this study was to determine the association between reduced bone mineral density (BMD) and BAC, which may share a common pathophysiology. **METHODS:** We conducted a retrospective study of 228 women (55% Hispanic, mean age 64 +/-10 yr) who had both mammography and BMD evaluation at Columbia University Medical Center from 2001-2003. Each mammogram was reviewed for the presence of BAC using standardized methods. BMD was measured using dual-energy x-ray absorptiometry and categorized as normal, low bone density (osteopenia), or osteoporosis as defined by the World Health Organization. Univariate and multivariate logistic regression analyses were performed to evaluate the association between reduced BMD and BAC. **RESULTS:** The prevalence of BAC, low bone density (osteopenia), and osteoporosis was 39, 42, and 29%, respectively. Women with BAC were significantly more likely to be older, Hispanic, and postmenopausal and have osteoporosis as compared with women without BAC. In age-adjusted analyses, women with BAC were more likely to have reduced BMD (odds ratio 3.0,  $P < 0.01$ ) as compared with women without BAC. Furthermore, osteoporosis was strongly associated with the presence of BAC (odds ratio 3.5,  $P < 0.01$ ). **CONCLUSION:** These data suggest that osteoporosis and arterial calcification are strongly and independently correlated. Reduced BMD may identify women at risk of vascular disease.

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## Association between systemic calcified atherosclerosis and bone density.

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*Calcif Tissue Int.* 2007 May;80(5):301-6.

Both atherosclerosis and osteoporosis are responsible for significant morbidity and mortality, are independent predictors of cardiovascular disease (CVD) events, and may share common regulatory mechanisms as well as histopathology. Multiple reports of weak or null relationships between traditional CVD risk factors and calcified atherosclerosis have heightened interest in novel predictors of arterial calcium. One such hypothesis is for an inverse relationship between bone mineral density (BMD) and calcified coronary atherosclerosis. Although contrary findings have been reported, the majority of cross-sectional and all prospective studies have demonstrated a significant inverse association between arterial calcium deposits and BMD. The few studies that include men are equivocal, and, to date, no study has investigated the relationship between BMD and systemic arterial calcium. The aim of this study was to test the hypothesis that lumbar BMD is significantly associated with the presence of arterial atherosclerotic calcium in the carotid, coronary, and iliac vascular beds as well as the aorta.

## **Incidence and significance of prostatic stones in men with chronic prostatitis/chronic pelvic pain syndrome.**

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*Urology. 2007 Aug;70(2):235-8.*

**OBJECTIVES:** Prostatic calcification is common in asymptomatic elderly men. However, young men with chronic pelvic pain syndrome (CPPS) often have significantly calcified prostates. We studied the incidence and significance of prostatic calcification in men with CPPS. **METHODS:** From July 2005 to August 2006, 130 new patients with CPPS were seen at our clinic. Of these 130 patients, 47 underwent transrectal ultrasonography. Prostatic calcification correlated with symptoms (National Institutes of Health chronic prostatitis symptom index score), examination findings, and culture results. The variables were compared using the Student t test, Wilcoxon unpaired test, or chi-square test. **RESULTS:** The 47 men who had undergone transrectal ultrasonography had symptoms identical to those who had not but were older (range 46.1 to 41.6 years,  $P = 0.02$ ) and had had symptoms longer (median 60 versus 12 months,  $P = 0.0001$ ). Of the 47 patients, 22 (47%) had significant calcification. The symptoms with or without calcification were identical (chronic prostatitis symptom score 23.7 versus 23.9). Men with calcification had had symptoms longer (median 84 versus 27 months,  $P = 0.05$ ) but were similar in age (49 versus 45 years,  $P = 0.21$ ) and had a similar prostate size (21.7 cm<sup>3</sup> for both groups). Men with calcification were less likely to have pelvic floor tenderness (50% versus 85%,  $P = 0.03$ ) but were more likely to have bacteria in the prostatic fluid ( $P = 0.05$ ) and had a higher median white blood cell count (3.5 versus 0 white blood cells per high power field,  $P = 0.058$ ). **CONCLUSIONS:** Prostatic calcification is common in patients with CPPS and is associated with greater inflammation, bacterial colonization, and symptom duration. Pelvic floor spasm is more common in patients without calcification. This might be an important parameter with which to stratify clinical trials.

## **Proinflammatory Activation of Macrophages by Basic Calcium Phosphate Crystals via Protein Kinase C and MAP Kinase Pathways**

### **A Vicious Cycle of Inflammation and Arterial Calcification?**

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*(Circ Res. 2005;96:1248-1256.)*

**Abstract**—Basic calcium phosphate (BCP) crystal deposition underlies the development of arterial calcification. Inflammatory macrophages colocalize with BCP deposits in developing atherosclerotic lesions and in vitro can promote calcification through the release of TNF alpha. Here we have investigated whether BCP crystals can elicit a proinflammatory response from monocyte-macrophages. BCP microcrystals were internalized into vacuoles of human monocyte-derived macrophages in vitro. This was associated with secretion of proinflammatory cytokines (TNF $\alpha$ , IL-1 $\alpha$ , and IL-8) capable of activating cultured endothelial cells and promoting capture of flowing leukocytes under shear flow. Critical roles for PKC, ERK1/2, JNK, but not p38 intracellular signaling pathways were identified in the secretion of TNF alpha, with activation of ERK1/2 but not JNK being dependent on upstream activation of PKC. Using confocal microscopy and adenoviral transfection approaches, we determined a specific role for the PKC-alpha isozyme. The response of macrophages to BCP crystals suggests that pathological calcification is not merely a passive consequence of chronic inflammatory disease but may lead to a positive feed-back loop of calcification and inflammation driving disease progression.