ASBMR 2012 Annual Meeting

Meet-the-Professor

Handout Booklet

October 12-15, 2012

Minneapolis Convention Center

Minneapolis, Minnesota, USA

Table of Contents

This booklet contains handouts supplied by the professors by the printing date of 09/18/12 and are intended to be a supplement to the material being presented in the session. Please be sure to complete an evaluation form of the Meet-the-Professor sessions and provide feedback and suggestions for the Meet-the-Professor Handout Booklets for the future.

Osteoporosis in the “Old Old”

Cathleen Colon-Emeric, M.D., M.S.

Osteoporosis in the "Oldest Old"

Cathleen Colón-Emeric, MD, MHS
Duke University and Durham VA GRECC, colon001@mc.duke.edu

Significance of the Topic

- Rapidly aging population world-wide
- Highest fall and fracture rates
- Under-represented in clinical trials
- Complex clinical decision-making due to co-morbidities, lower life expectancy, polypharmacy

Learning Objectives: As a result of participating in this session, attendees should be able to

1. Describe important issues that arise when making osteoporosis treatment decisions with the oldest old, including co-morbidities, functional status, and remaining life expectancy.
2. Summarize the available evidence supporting the treatment of osteoporosis in select "oldest-old" individuals, including dedicated trials, subgroup analyses, and decision/cost analyses.
3. Discuss non-pharmacologic interventions for preventing fractures in the oldest old.

Discussion Outline

I. Illustrative Cases
   a. What is "old"?
   b. What factors need to be considered in making clinical decisions for the oldest old?

II. What data is available to inform clinical decisions in the oldest old?
   a. RCTs specifically attempting to enroll elderly subjects
   b. Subgroup analyses and meta-analyses from large registration trials
   c. RCTs in subjects with common co-morbid conditions of aging
   d. Time to efficacy onset analyses
   e. Decision/Cost analyses
   f. Comparative effectiveness studies

III. Deciding which oldest-old patients should be treated
   a. Geriatric fracture risk factors not considered in FRAX or Garvan
   b. Estimating fall risk
   c. Estimating life expectancy
   d. Understanding functional status, goals of care

IV. Beyond the Bisphosphonates – other fracture prevention considerations for the oldest old
   a. Preventing falls
   b. Environmental modifications
   c. Addressing weight loss, under-nutrition
   d. Addressing functional decline

Illustrative Cases:

Case 1. 95 year old Caucasian woman, living completely independently in the community. She has no significant medical history and takes no medications. She has fallen twice in the last 6 months, during her exercise class and at church. Her T score on screening DXA is -3.0 at the total hip, and she has a parental history of hip fracture.

Would you treat her?

Case 2. 91 year old Caucasian woman, who is a resident of a skilled nursing facility. She has been on glucocorticoids (prednisone 10 mg daily) for > 10 years for temporal arteritis. Other medical conditions include steroid induced diabetes, chronic kidney disease stage 3, and mild vascular dementia. She has prior vertebral fractures. She is no longer independently ambulatory and requires assistance with transfers. She had been on alendronate 70 mg weekly for 5 years, when she suffered a non-traumatic pelvic fracture during a transfer. She cannot transfer onto the table to complete a DXA. Serum calcium and 25(OH)D are normal.

What do you do?

Case 3. 88 year old Caucasian male Veteran with a history of stage 4 esophageal cancer, living at home with assistance from his wife, currently undergoing XRT. Other medical problems include significant COPD, CAD, and ischemic cardiomyopathy with Class 3 symptoms. He becomes dehydrated and falls, fracturing his hip. The Housestaff want to schedule him for zoledronic acid.

Do you approve the drug?

Tables and Figures

Table 1. Summary of available data to inform osteoporosis treatment decisions in the oldest old

Type of Data	Brief Description	Conclusions/Key Points
RCTs specifically attempting to enroll elderly subjects	• RCT zoledronic acid after hip fracture, mean age 75, 14% over 85[1] • RCT aldendronate in long-term care, 12% SNF/residential[2] • RCT risedronate elderly women with high fall risk +/- osteoporosis[3]	• Similar efficacy and harm results as observed in younger populations, limited to those with osteoporosis • Healthy volunteer bias
Subgroup analyses and meta-analyses from large registration trials	• Post-hoc analysis of FIT (alendronate) by age[4] • Pooled analysis of women >80 yrs in risedronate trials[5] • Post-hoc analysis FPT (teriparatide) by age[6] • Post-hoc analysis of HORIZON (zoledronic acid) by age[7] • Post-hoc analysis of MORE (raloxifene) by age	• Similar relative risk reduction for most fracture types o Exceptions: raloxifene, vert fx? • Higher absolute risk reduction • Similar harm • Healthy volunteer bias
RCTs in subjects with common co-morbid conditions of aging	• Post-hoc analysis of BPs in CKD[8,9] • Meta-analysis of BP efficacy in Parkinson's, stroke, and dementia[10,11] • Subgroup analysis of BP efficacy in Diabetes[12] • Subgroup analysis of BP and denosumab efficacy in prostate cancer[13,14]	• Treatment efficacy appears to be maintained despite the presence of co-morbidities common in older adults
Time to efficacy onset analyses	• Systematic review of literature[15]	• Clinical vertebral fx 6 mo • Non-vertebral fx 12 mo • Hip fx 24-36 months
Decision/Cost analyses	Cost-effectiveness analyses using Markov models or similar methods[16,17]	• Cost-effectiveness tends to improve with advancing age • Even in 90 year olds at lowest quartile of life expectancy, BP cost effective
Comparative effectiveness studies	• AHRQ Comparative Effectiveness Evidence Synthesis[18]	• "In general, a high level of evidence suggests that BPs are at least as effective for older people as for younger"

Figure 1. Absolute risk of fracture in risedronate trials by age and treatment group. Adapted from Boonen et al., Journal of the American Geriatrics Society, 2004;52:1832-9

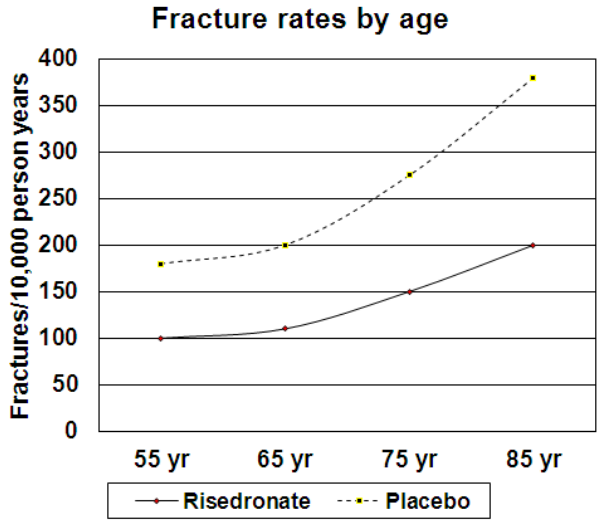


Table 2. Life-expectancy in years at different ages for the median, healthiest (25th percentile) and sickest (75th percentile) patients. Calculated from 2004 U.S. life tables.

Age	Life Expectancy 25th percentile (yrs) Women	Men	Life Expectancy 50th percentile (yrs) Women	Men	Life Expectancy 75th percentile(yrs) Women	Men
70	22.2	18.0	16.2	12.4	10.1	6.7
75	17.9	14.2	12.8	9.3	7.2	4.9
80	13.9	10.8	9.7	6.7	4.9	3.3
85	10.3	7.9	7.1	4.7	3.2	2.2
90	7.4	5.8	5.1	3.2	2.0	1.5

Table 3. Incremental cost per quality adjusted life year of a strategy of treating a white woman with osteoporosis for 5 years with generic alendronate, according to age and life expectancy. Adapted from Pham et al., Journal of the American Geriatrics Society, 2011;59(9):1642-9.

Age (yrs)	25th percentile ($)	50th percentile ($)	75th percentile ($)
50	42,275	26,256	17,653
55	38,243	25,501	14,918
60	23,956	14,589	7472
65	18,421	10,324	4244
70	12,134	5149	69
75	4622	Cost saving	Cost saving
80	2407	Cost saving	Cost saving
85	5307	Cost saving	Cost saving
90	13,909	3599	458

Box 1. SOF Frailty Index: predicts falls, disability and death in older women[19]

2 or more of the following:

1. Weight loss
2. Inability to rise from chair 5 times without using arms
3. Self-reported reduced energy level

Table 4. Summary of effective fall prevention interventions[20]

Intervention	**Relative Risk Reduction**
Multiple risk factor reduction	25%
Exercise interventions, Tai Chi	17-34%
Withdrawal of psychoactive medications	66%
Cataract surgery	34%
Home safety evaluation for high risk or visually impaired	22%
Vitamin D supplements (NH residents and insufficient community-dwellers)	25%

Box 2. Useful fall prevention resources

- Age Page: Preventing Falls and Fractures (NIA) http://www.nia.nih.gov/HealthInformation/Publications/falls.htm
- Home safety checklists (CDC) http://www.cdc.gov/ncipc/pub-res/toolkit/Falls_ToolKit/DesktopPDF/English/booklet_Eng_desktop.pdf
- Fall risk assessment tools and other materials (VA) http://www.patientsafety.gov/CogAids/FallPrevention/index.html#page=page-3

References

1. Lyles K, Colón-Emeric C, Magaziner J, et al. Zoledronic acid and clinical fracture and mortality after hip fracture. *New Engl J Med.* 2007;357:1799-1809.
2. Greenspan S, Schneider D, McClung M, et al. Alendronate improves bone mineral density in elderly women with osteoporosis residing in long-term care facilities. A randomized, double-blind, placebo-controlled trial. *Ann Int Med.* 2002;136(10):742-746.
3. McClung M, Gueusens P, Miller P, et al. Effect of risedronate on the risk of hip fracture in elderly women. Hip Intervention Program Study Group. *New Engl J Med.* 2001;344(5):333-340.
4. Hochberg MC, Thompson DE, Black DM, et al. Effect of alendronate on the age-specific incidence of symptomatic osteoporotic fractures. *Journal of Bone & Mineral Research.* Jun 2005;20(6):971-976.
5. Boonen S, McClung MR, Eastell R, et al. Safety and efficacy of risedronate in reducing fracture risk in osteoporotic women aged 80 and older: implications for the use of antiresorptive agents in the old and oldest old. *Journal of the American Geriatrics Society.* Nov 2004;52(11):1832-1839.
6. Boonen S, Marin F, Mellstrom D, et al. Safety and efficacy of teriparatide in elderly women with established osteoporosis: bone anabolic therapy from a geriatric perspective.[see comment]. *Journal of the American Geriatrics Society.* May 2006;54(5):782-789.
7. Boonen S, Black DM, Colón-Emeric CS, et al. Efficacy and Safety of a Once-Yearly Intravenous Zoledronic Acid 5 mg for Fracture Prevention in Elderly Postmenopausal Women with Osteoporosis Aged 75 and Older. *Journal of the American Geriatrics Society.* 2010;58(2):292-299.
8. Miller PD, Roux C, Boonen S, et al. Safety and efficacy of risedronate in patients with age-related reduced renal function as estimated by the Cockcroft and Gault method: a pooled analysis of nine clinical trials. *Journal of Bone & Mineral Research.* Dec 2005;20(12):2105-2115.
9. Jamal SA, Bauer DC, Ensrud KE, et al. Alendronate Treatment in Women With Normal to Severely Impaired Renal Function: An Analysis of the Fracture Intervention Trial. *Journal of Bone and Mineral Research.* 2007;22(4):503-508.
10. Sato Y, Honda Y, Iwamoto J. Risedronate and ergocalciferol prevent hip fracture in elderly men with Parkinson disease. *Neurology.* March 20, 2007 2007;68(12):911-915.
11. Iwamoto J, Matsumoto H, Takeda T. Efficacy of risedronate against hip fracture in patients with neurological diseases: a meta-analysis of randomized controlled trials. *Current Medical Research and Opinion.* 2008;24(5):1379-1384.
12. Keegan TH, Schwartz AV, Bauer DC, et al. Effect of alendronate on bone mineral density and biochemical markers of bone turnover in type 2 diabetic women: the fracture intervention trial. *Diabetes Care.* Jul 2004;27(7):1547-1553.
13. Smith MR, Egerdie B, Toriz NH, et al. Denosumab in Men Receiving Androgen-Deprivation Therapy for Prostate Cancer. *New England Journal of Medicine.* 2009;361(8):745-755.
14. Satoh T, Kimura M, Matsumoto K, et al. Single infusion of zoledronic acid to prevent androgen deprivation therapy-induced bone loss in men with hormone-naive prostate carcinoma. *Cancer.* 2009;115(15):3468-3474.
15. Inderjeeth C, Chan K, Kwan K, Lai M. Time to onset of efficacy in fracture reduction with current anti-osteoporosis treatments. *Journal of Bone and Mineral Metabolism.*1-11.
16. Pham AN, Datta SK, Weber TJ, Walter LC, Colón-Emeric CS. Cost-Effectiveness of Oral Bisphosphonates for Osteoporosis at Different Ages and Levels of Life Expectancy. *Journal of the American Geriatrics Society.* 2011;59(9):1642-1649.
17. Johnell O, Jonsson B, Jonsson L, Black D. Cost effectiveness of alendronate (fosamax) for the treatment of osteoporosis and prevention of fractures. *Pharmacoeconomics.* 2003;21(5):305-314.
18. Crandall C, Newberry S, Diamant A, et al. Treatment to prevent fractures in men and women with low bone density or osteoporosis: update of a 2007 report. In: AHRQ, ed. Vol 53. Rockville, MD: Comparative Effectiveness Review; 2012.
19. Ensrud Ke ESKTBC, et al. COmparison of 2 frailty indexes for prediction of falls, disability, fractures, and death in older women. *Archives of Internal Medicine.* 2008;168(4):382-389.
20. Gillespie L, Gillespie W, Robertson M, Lamb S, Cumming R, Rowe B. Interventions for preventing falls in elderly people. *Cochrane Database of Systematic Reviews.* 2004;3

Osteocytes

Jian Q. Feng, M.D., Ph.D.

Osteocyte-the Essential Cell for Bone Mineralization

Jerry Feng MD, PHD

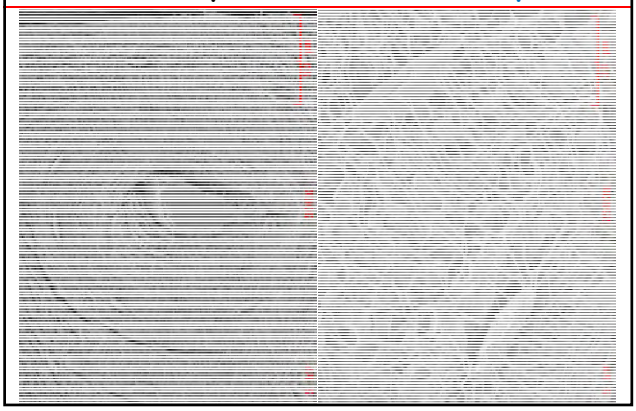
Biomedical Sciences
Baylor College of Dentistry, Texas A&M Health Science Center

Texas A&M Health Science Center
Baylor College of Dentistry

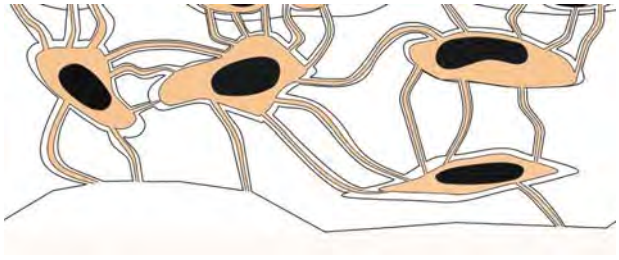
Bone

Osteoblasts Osteoclasts

Osteocytes make up over 90-95% of all bone cells; Osteocytes are mechanosensory cells



The Lacuno-Canalicular System

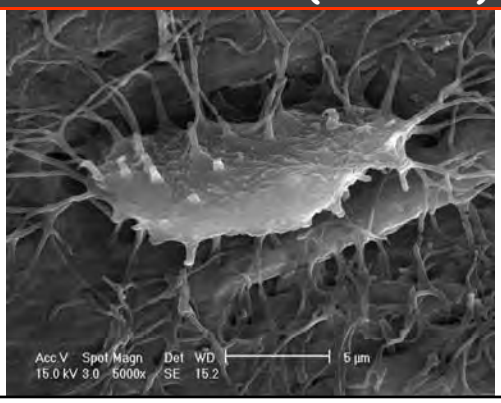


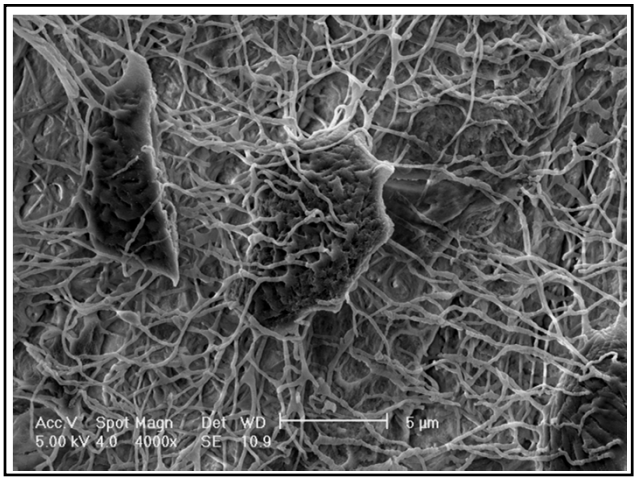
As a mechanosensor

Our View on Osteocytes

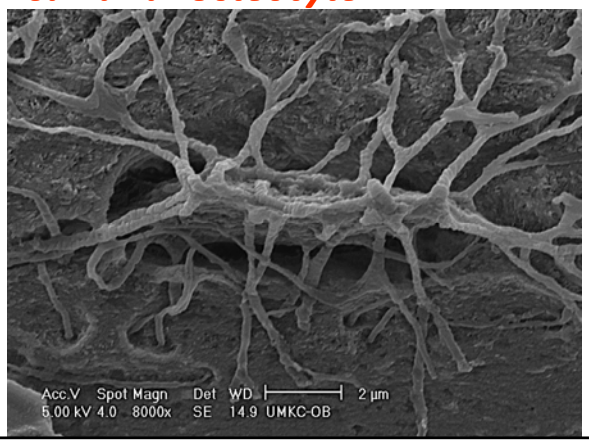
Osteocytes are Unique in Their Morphologies

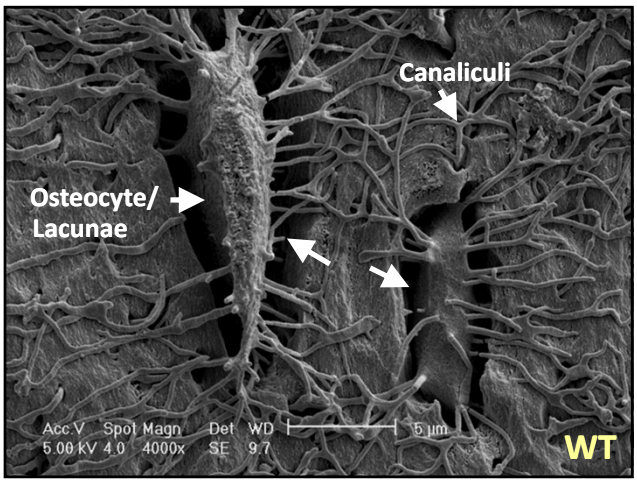
Acid etching-Resin-cast Osteocyte in Alveolar Bone (3-wk-old)





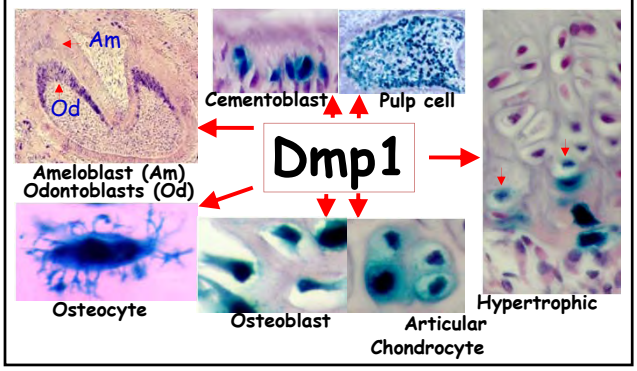
Calvarial Osteocyte



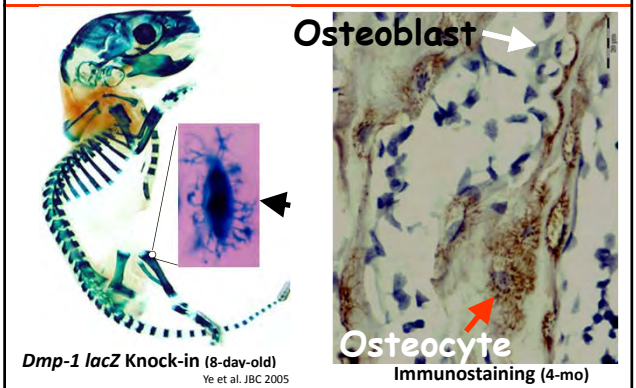


DMP1 & Osteocyte

Dmp1 is Highly Expressed in All Skeletons and Teeth



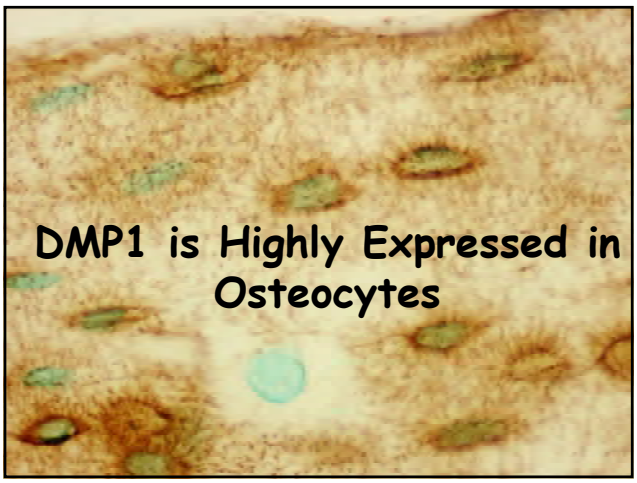
DMP1 is Mainly Expressed in Osteocytes During Postnatal Development



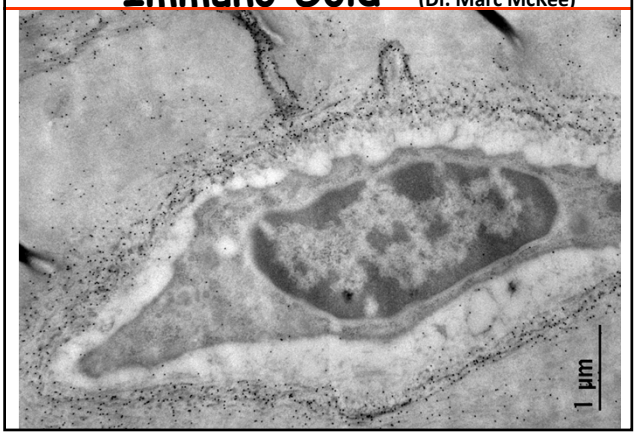
Dmp-1 lacZ Knock-in (8-day-old)
Ye et al. JBC 2005

Immunostaining (4-mo)

DMP1 is Highly Expressed in Osteocytes



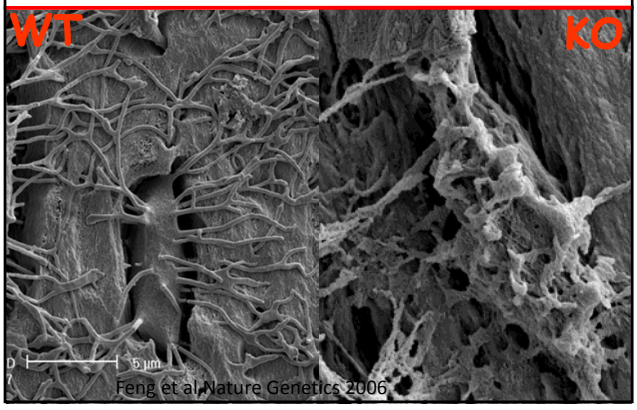
Immuno Gold (Dr. Marc McKee)



Dmp1 Null Newborns Appear Normal

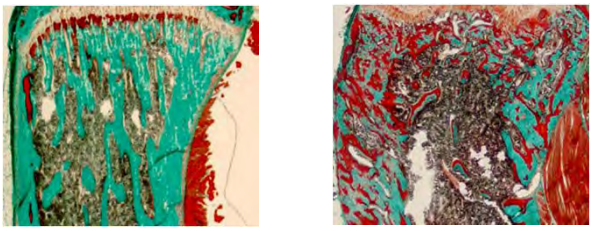
Dmp1-KO leads to Bone/Mineralization Abnormalities

Defects of Osteocytes in Dmp1 KO



Feng et al Nature Genetics 2006

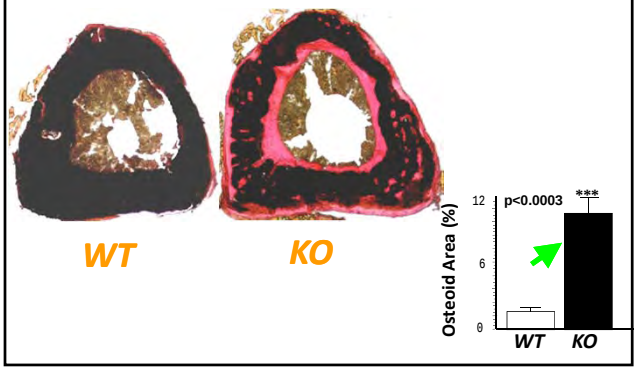
DMP1 KO Mice Exhibits a Rickets/Osteomalacia-like Phenotype



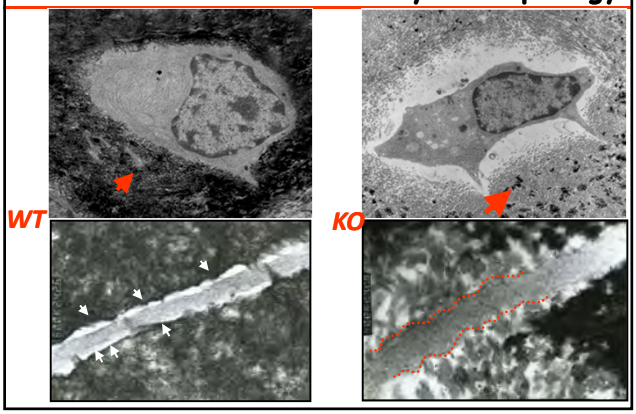
WT KO

Osteoid in red; Calcified tissue in Green Ling et al. JBMR 2005

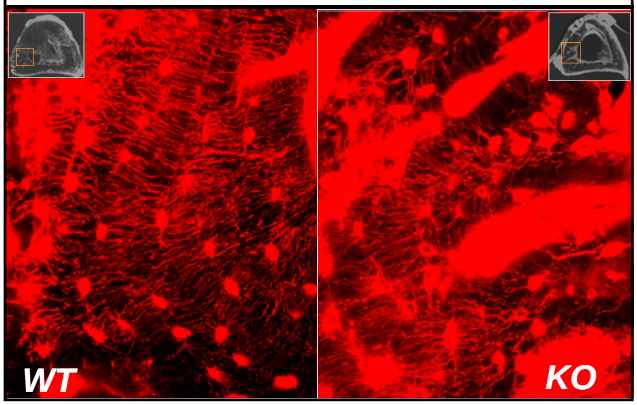
Defects in Matrix Mineralization (von Kossa)



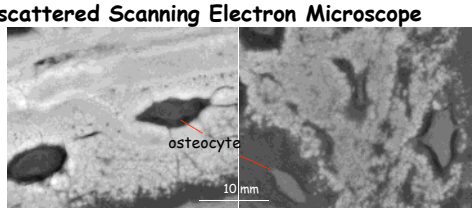
Transmission Electron Microscopy is Used to Visualize Mineral and Osteocyte Morphology



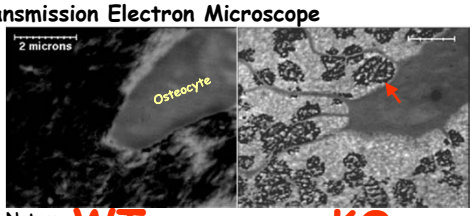
Procion Red Labeling is Used to Visualize Osteocyte Lacunae and Canaliculi



Backscattered Scanning Electron Microscope



Transmission Electron Microscope

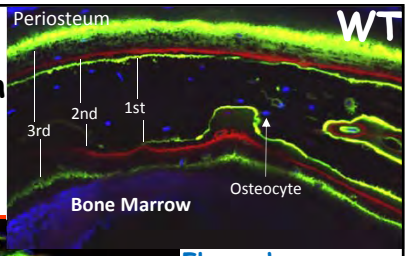


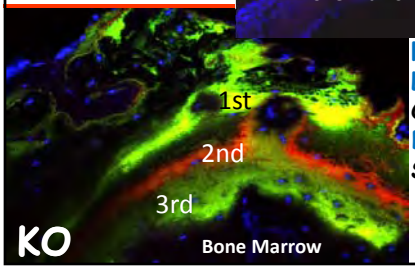
Feng et al. Nature Genetics 06

WT KO

Defects in Mineralization is Linked to Osteocytes

Feng et al. Nature Genetics 06





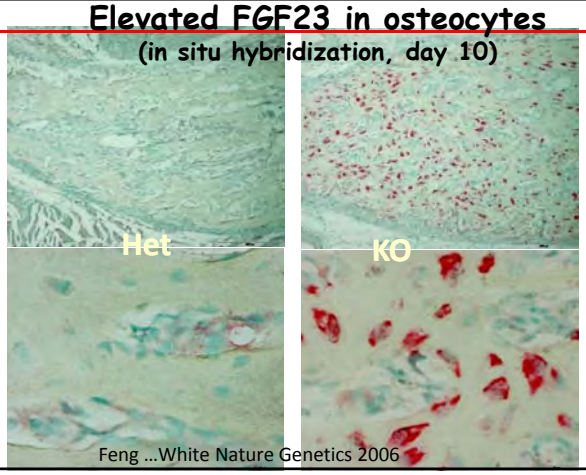
Fluorochrome Labeling Combining with DAPI Nucleus Staining

Calcein - green;
Alizarin – Red;
DAPI - Blue

Osteocytes, as endocrine cells, regulate Pi homeostasis

Elevated FGF23 in osteocytes

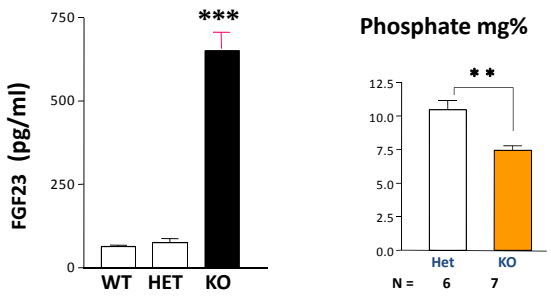
(in situ hybridization, day 10)



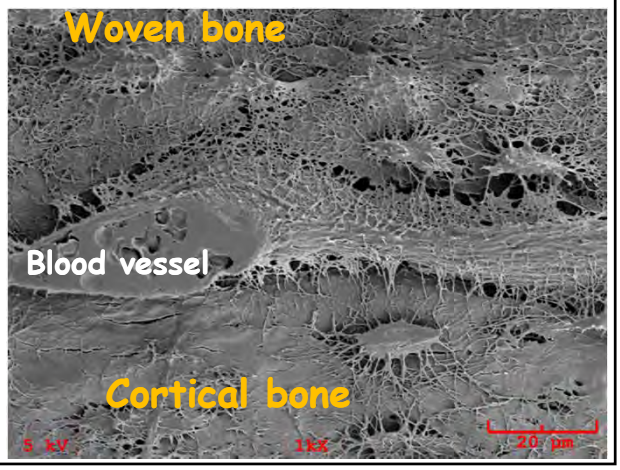
Feng …White Nature Genetics 2006

Changes of FGF23 and Pi

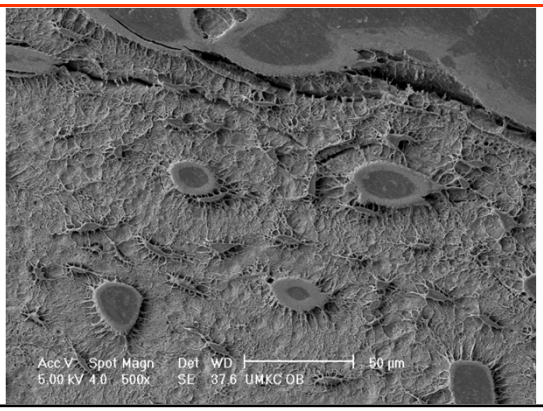
N = 4, *P < 0.05; ***P < 0.001

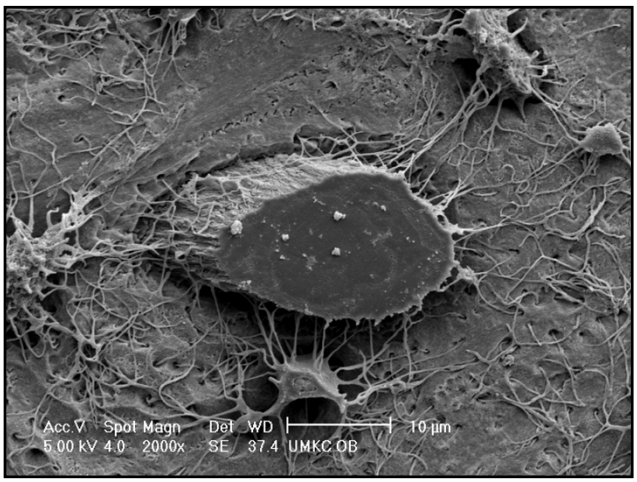


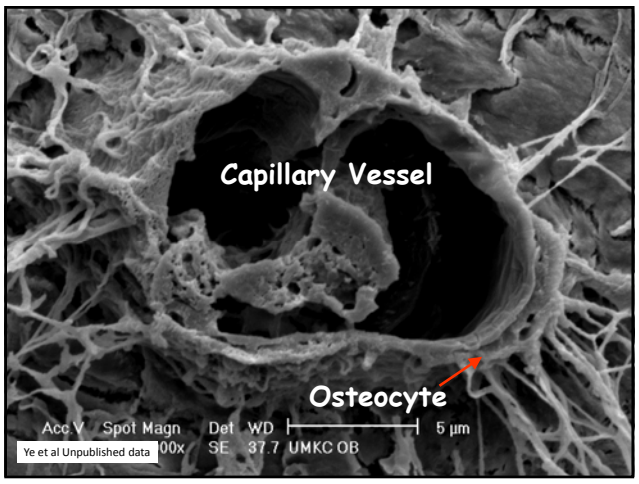
Feng et al. Nature Genetics 2006



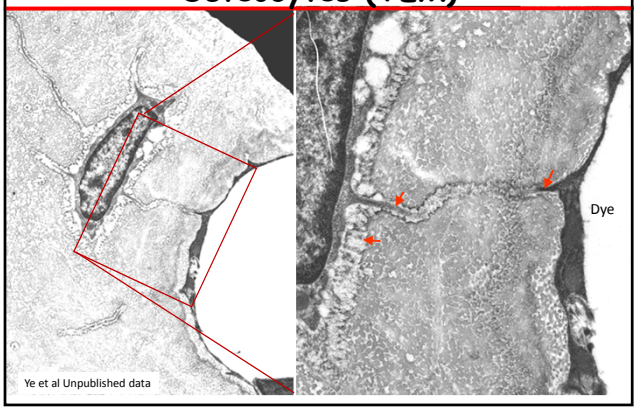
Osteocytes and Blood Vessel



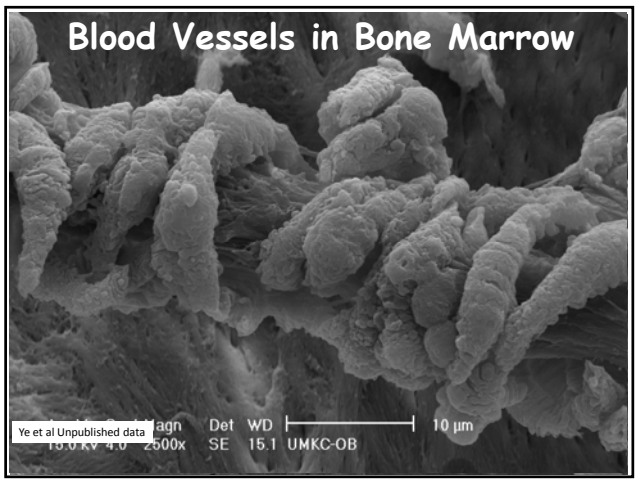




Connections between Blood Vessels and Osteocytes (TEM)



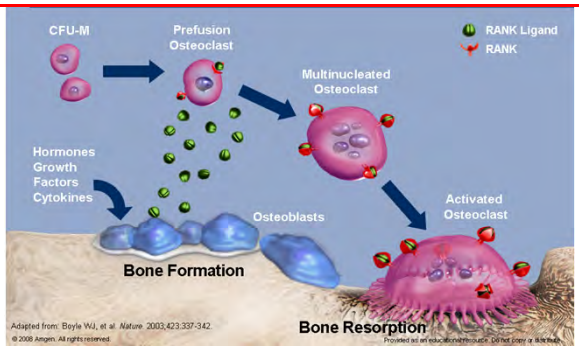
Blood Vessels in Bone Marrow



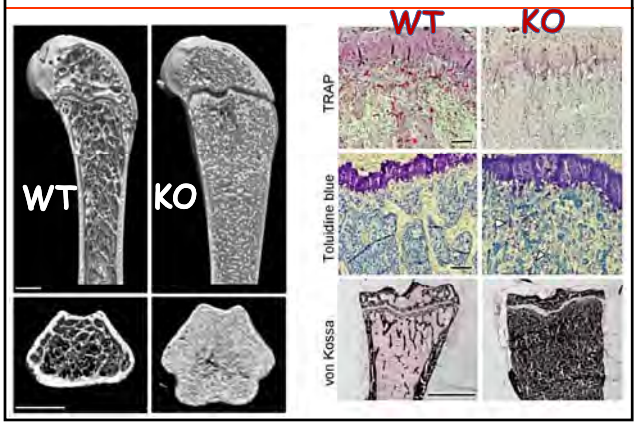
Osteocyte Regulation of Osteoclast via RANKL

Nature Medicine Oct. 2011
Hiroshi Takayanagi Lab in Japan

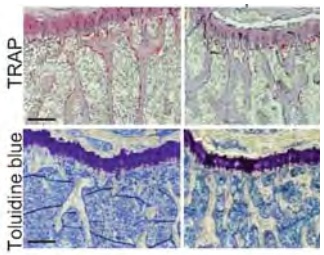
Osteoblasts Controls Osteoclasts via RANKL



Global Deletion of RANKL

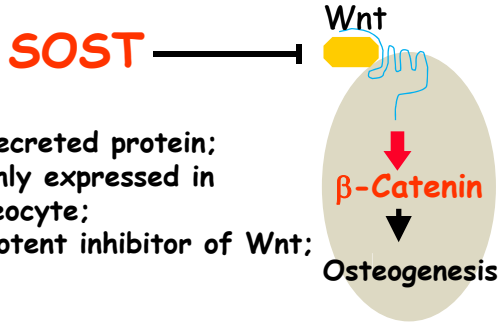


Osteocyte Deletion of RANKL by Dmp1-Cre



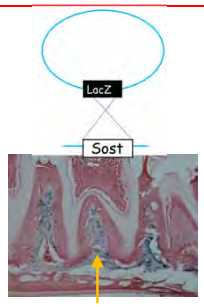
Evidence for osteocyte regulation of bone homeostasis through RANKL expression
Tomoki Nakashima, Mikihito Hayashi, Takanobu Fukunaga, Kosaku Kurata, Masatsugu Oh-hora, Jian Q Feng, Lynda F Bonewald, Tatsuhiko Kodama, Anton Wutz, Erwin F Wagner, Josef M Penninger & Hiroshi Takayanagi
Nature Medicine, 09-2011

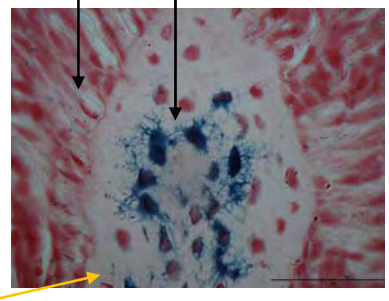
Osteocytes and Sost



- A secreted protein;
- Highly expressed in osteocyte;
- A potent inhibitor of Wnt;

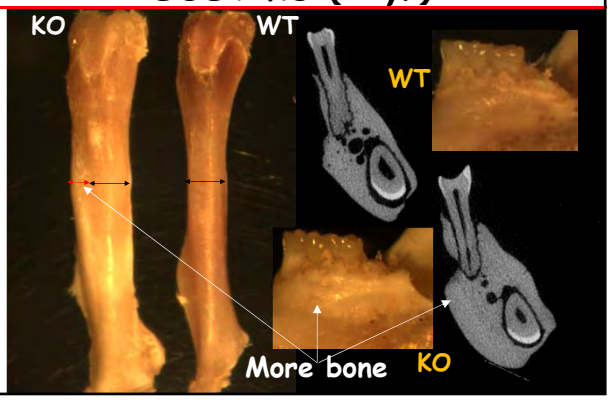
In Sost-LacZ Knock-in Mice, lacZ is Highly Expressed in Osteocytes but not in PDL



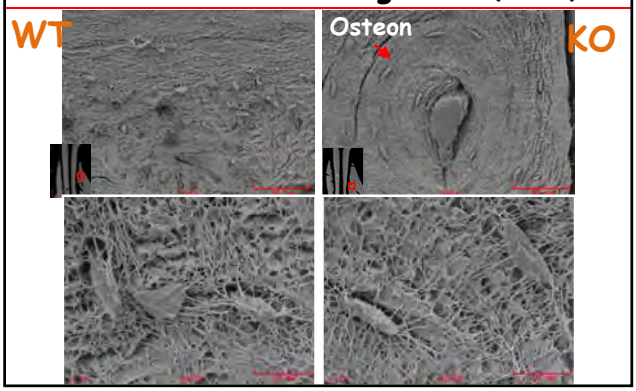


Alveolar Bone

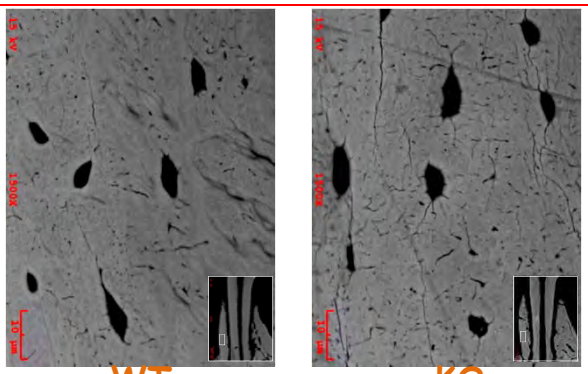
Massive Increase in Bone Volume in SOST KO (2-yr)



Osteon in Sost KO Alveolar Bone under Views of Acid Etching SEM (500x)



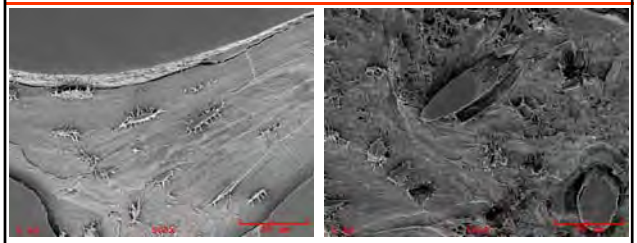
Osteocytes Under Views of Backscattered SEM (1,500x)



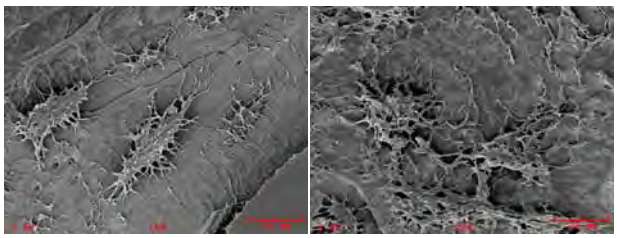
Osteocytes and Osteoarthritis

Yin Xiao
Institute of Health and Biomedical Innovation
Queensland University of Technology
Brisbane, Queensland 4059
Australia

A Switch from Lamella Bone to Woven Bone in OA

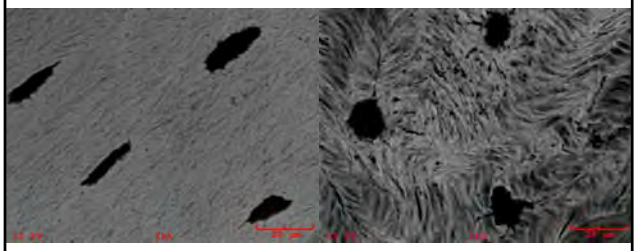


Morphological Changes in Osteocytes



Control OA

Morphological Changes in Osteocytes



Control OA

Arthritis and TGFβ Signaling

Regis O’Keefe, M.D.

Significance of the Topic

In the 2005 National Health Survey, arthritis emerged as the number one cause of disability in the United States[1]. In 2008, 27 million Americans were afflicted by OA[2,3], with forecasts indicating that 25% of the adult population in the United States, or nearly 67 million people, will have physician-diagnosed disease by 2030[4].

Joint injuries are highly associated with the development of osteoarthritis. Full or partial meniscectomy is widely accepted to be a central pathogenic mechanism of joint degeneration, with an estimated 6-fold increase in the risk of developing radiographic OA within 21 years following injury or surgery[5]. Joint arthroscopy has become a much more common procedure. The Centers for Disease Control and Prevention performed National Surveys of Ambulatory Surgery in 1996 and in 2006[6]. In 1996 15% of orthopaedic ambulatory surgeries were knee arthroscopy; this increased to 51% in 2006, with a large increase in the numbers of middle-aged patients undergoing this procedure[6].

Because of the high incidence of osteoarthritis, joint arthroplasty is currently among the most common and costly medical/surgical procedures in the health care system. The 2006 National Health Discharge Survey reported that the rate of knee replacements for those aged 45–64 years more than doubled from 13.1 per 10,000 population in 2000 to 27.3 per 10,000 population in 2006[7]. In addition to the well documented impact of OA on physical function[8] and quality of life[9], there is a significant financial burden, with aggregate annual medical expenditures exceeding $185 billion in 2008[10]. This does not include lost wages or the economic impact of disability, which are reported to range between $100 billion[11] and $300 billion[1] annually.

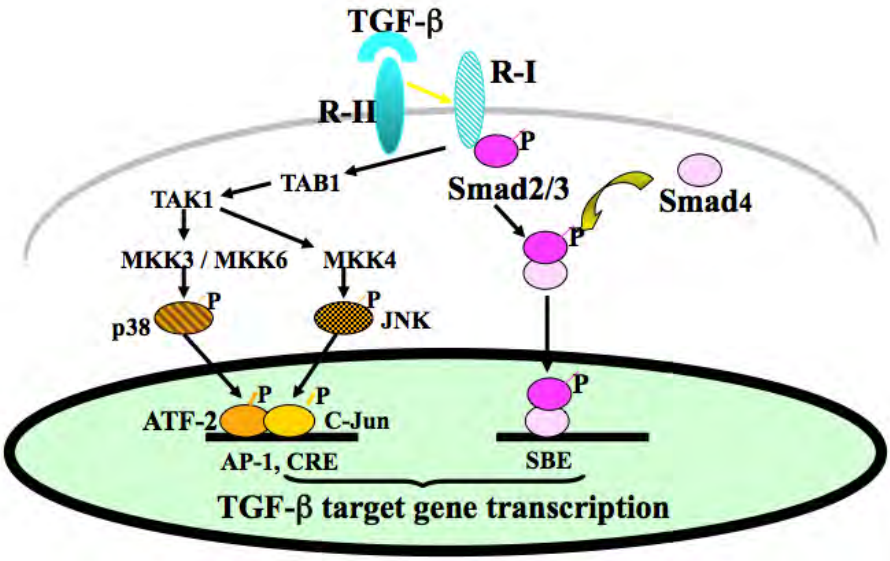
Over the next 20 years, an additional 5-7-fold increase in the number of total knee replacement surgeries is expected. Thus, there is enormous need for preventative therapies and/or anabolic agents for the treatment of osteoarthritis.

Learning Objectives

1. Understanding how TGF-beta signals through the Smad and MAP kinase pathways and regulates chondrocyte maturation;
2. Defining experimental models of osteoarthritis and quantitative methods used to assess osteoarthritis in murine models.
3. Understanding unique aspects of articular chondrocyte gene expression and the changes in gene expression/phenotype that occurs in osteoarthritis.
4. Defining the growth factors and signaling pathways associated with OA
5. Understanding the role of TGF-beta in osteoarthritis and potential therapeutic targets.

Overview and Points of Interest
TGF-beta signaling Pathways and Chondrocyte maturation

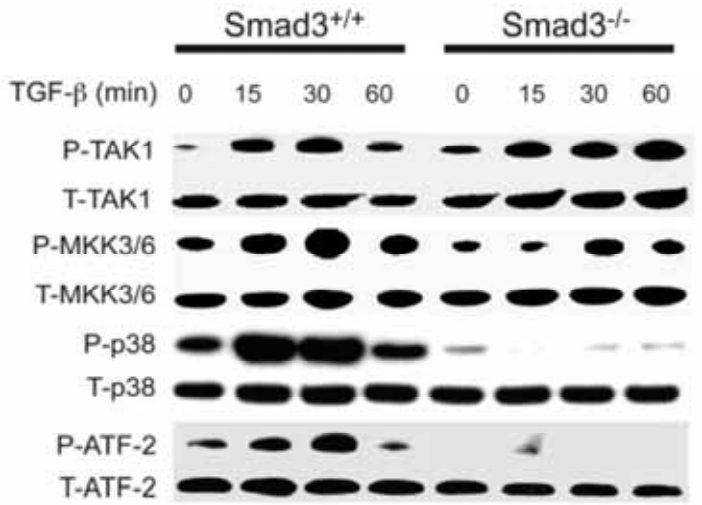
TGF-beta signals through both the Smad pathway and the MAP kinase pathways[12,13]. TGF–beta signaling also interacts with other signaling pathways, including the BMP and Wnt/beta-catenin pathways [12,14-17],[18].



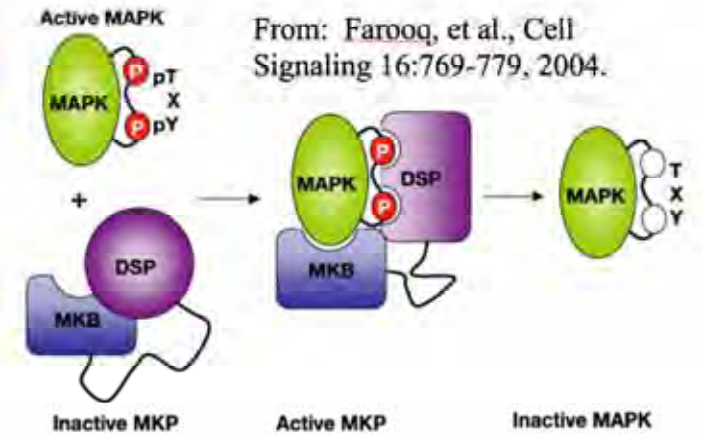
TGF-beta maintains chondrocytes in a immature, prehypertrophic phenotype. Both the Smad and MAP kinase pathways mediate the inhibition of chondrocyte maturation by TGF-beta[12,13].

In this experiment isolated chondrocytes treated with TGF-beta have reduced expression of the maturation marker *type X collagen*. The ability of TGF-beta to block maturation is partially inhibited by infection with retroviral vectors expressing dominant negative ATF-2 and dominant negative Smad3 alone. However, when both dominant negative vectors are expressed, the inhibitory effect of TGF-beta on type X collagen expression is completely blocked (From Ionescu et al., Exp. Cell Res. 228: 198-207, 2003).

There is crosstalk between the TGF-beta mediated Smad and MAP kinase signaling pathways. Interestingly, in Smad3$^{-/-}$ chondrocytes TGF-beta leads to phosphorylation of the MAP-kinase-kinase-kinase TAK1 and to phosphorylation of the MAP-kinase-kinases MEKK3 and MEKK6. However, in the absence of Smad3, there is no phosphorylation of p38 MAP kinase (see figure below from Li, et al., Arthritis and Rheumatism 2010;62:2359-69).



Isolated mouse sternal chondrocytes from WT and Smad3$^{-/-}$ mice were treated with TGF-beta and Western blot performed on protein extracts (from Li, et al., Arthritis and Rheumatism 2010;62:2359-69).

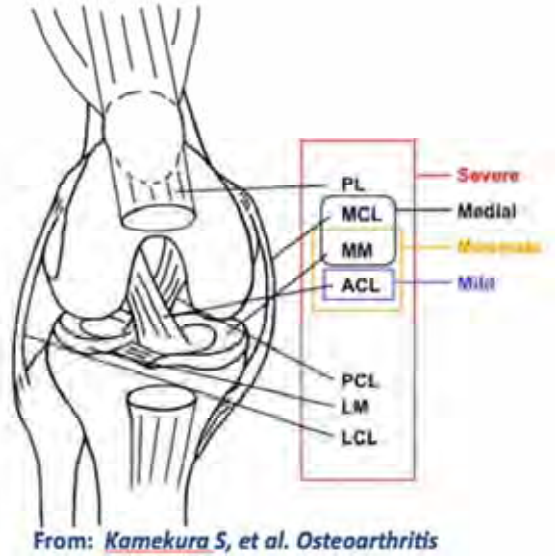


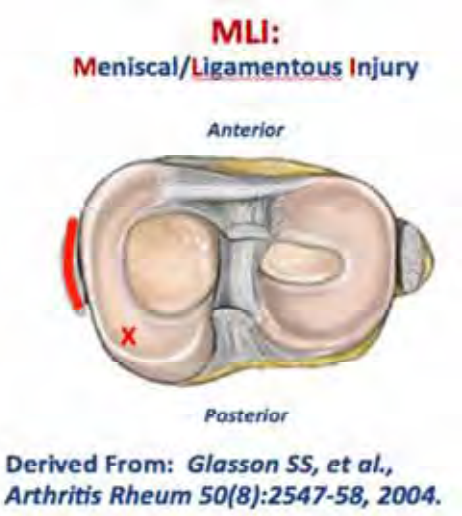
A schematic shows MAP Kinase Phosphatases (MKP) and regulation of p38 MAP kinase phosphorylation. MKPs inactivate (dephosphorylate) p38 MAP Kinase

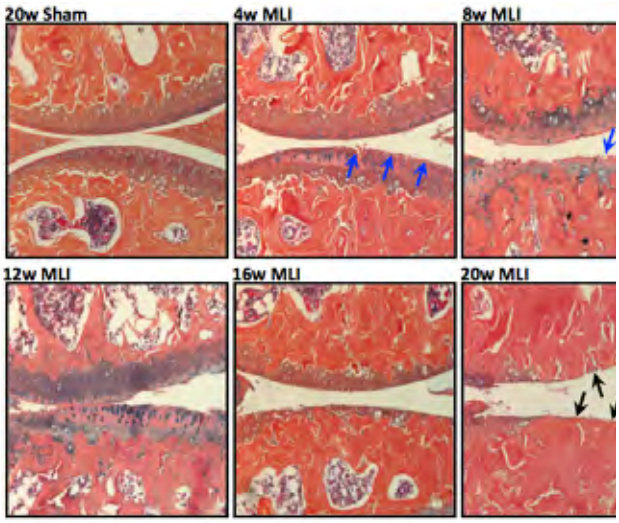
The experiment shows that without Smad3, p38 MAP Kinase is inactive (non-phosphorylated). P38 MAP kinases undergo transition between an inactive state (unphosphorylated) and an active state (phosphorylated) based upon the balance of activity of the upstream kinase (MKK3/6) and a family of phosphatases that de-phosphorylate active p38 MAP kinase and lead to inactivation of the kinase. The MAP Kinase Phosphatase (MKP) de-phosphorylate p38 MAP Kinase and are shown in the schematic.

Smad3 associates with MKP-1 and leads to its inactivation. In the absence of Smad3, MKP-1 has increased activity and rapidly inactivates p38 MAP Kinase. Thus, in the absence of Smad3, MKP1 activity is high and p38 is in an inactive state despite activation of the upstream kinases, TAK1 and MKK3/6 by TGF-beta [12].

Experimental Models of Osteoarthritis



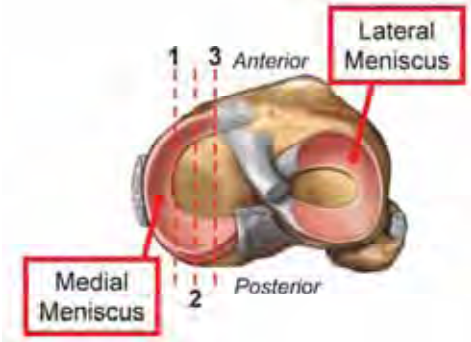


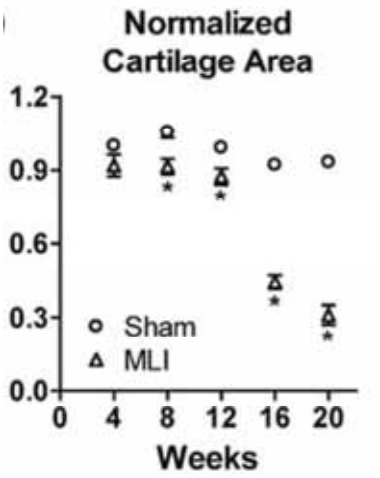


Histology of mouse knee following MLI in 20 week sham and in mice 4, 8, 12, 16, and 20 weeks. There is progressive arthritic change including loss of cartilage, subchondral bone sclerosis, and osteophyte formation.

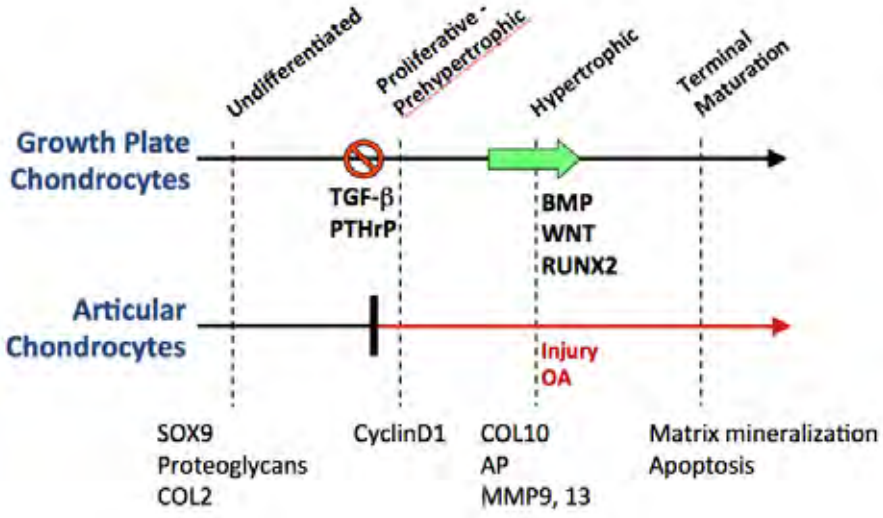
From Sampson et al. J Orthop Res. 2011 Aug;29(8):1145-51 [19]

Histomorphometry to determine cartilage area.





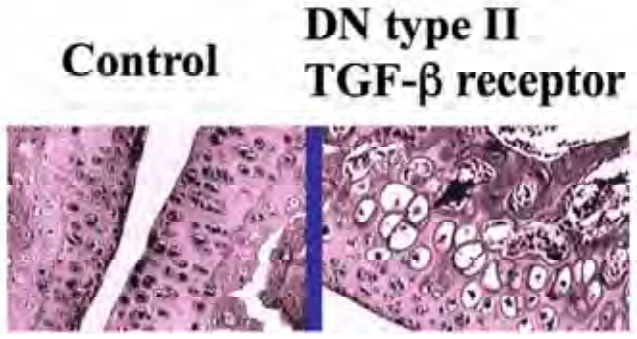
Altered Gene Regulation and Changes in Phenotype in Articular Chondrocytes in



Osteoarthritis

Articular chondrocytes express genes found in immature chondrocytes involved in endochondral ossification and associated with the development and maintenance of a hyaline cartilage matrix. This includes *Sox9, col2,* and *aggrecan*. Articular chondrocytes typically have reduced expression of *MMPs*. *Prg4* is a gene that is expressed primarily in the superficial zone of the articular cartilage and its protein product, lubricin, is involved in joint lubrication [20].

TGF-beta/Smad3 was recently shown to reduce Runx2 expression. In the absence of Smad3 chondrocytes had reduced expression of *col2* and increased expression of *MMP13*, resulting in a catabolic state and cartilage degeneration. The activation of the p38 MAP kinase pathway mediated increased Runx2 and MMP13 activation, suggesting disparate effects of TGF-beta on matrix genes are mediated by the Smad and MAP kinase pathways [21].



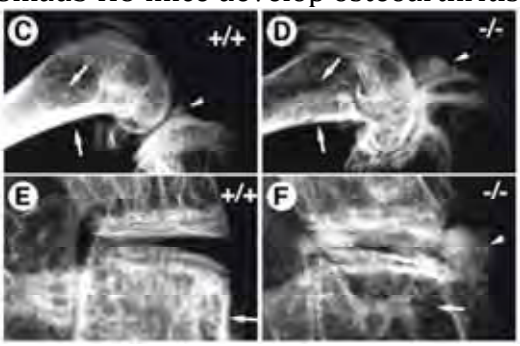
From Serra et al., J. Cell Biol. 139:541-52, 1997.

The expression of a truncated type II TGF receptor in cartilage that acted as a dominant negative was associated with the development of chondrocyte hypertrophy in the joint cartilage and expression of type X collagen and other markers of maturation [22].

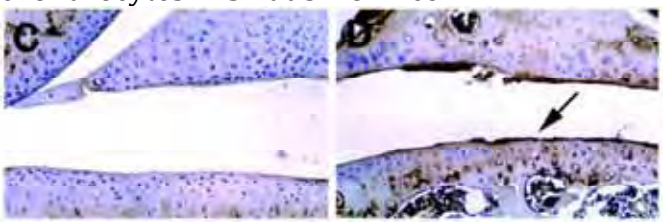
Defining the growth factors and signaling pathways associated with experimental osteoarthritis

Genetic experiments in mice have shown that perturbation of multiple growth factors/pathways, signaling molecules, matrix components, and transcription factors result in the development of an osteoarthritis phenotype. Growth factor pathways that have been associated with the OA phenotype include TGF-beta, Wnt/beta catenin, and Indian hedgehog [3,23,24] [22,25,26].

Smad3 KO mice develop osteoarthritis



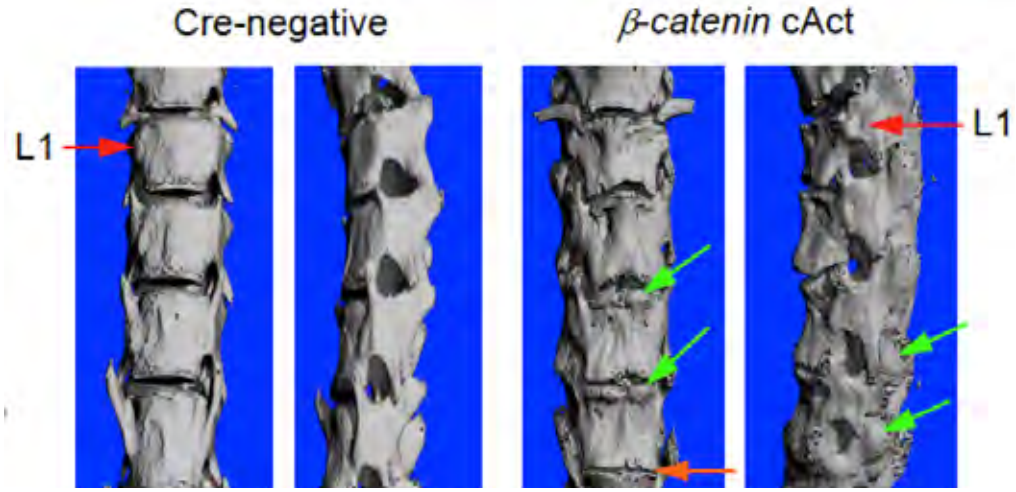
Type X collagen is increased in articular chondrocytes in Smad3 KO mice



From: Yang, et al., J Cell Biol. 153:35-46, 2001

The Smad signaling pathway has been implicated as a regulator of the articular chondrocyte phenotype. Runx2 is a particularly important downstream transcription factor. Heterozyous Runx2$^{+/-}$ mice have reduced cartilage degeneration in a knee injury model [27]. TGF-beta loss of function and Wnt/beta catenin gains of function regulate articular chondrocytes phenotype and the development of OA through Runx2 [3]. HIF-2 alpha is another transcription factor involved in the development of OA [28].

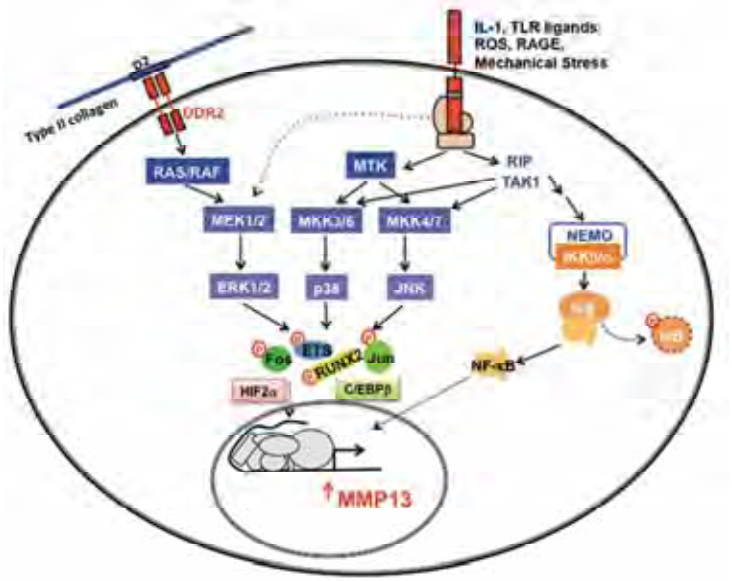
Beta-catenin gain of function results in OA changes in the intervertebral disc and spine.



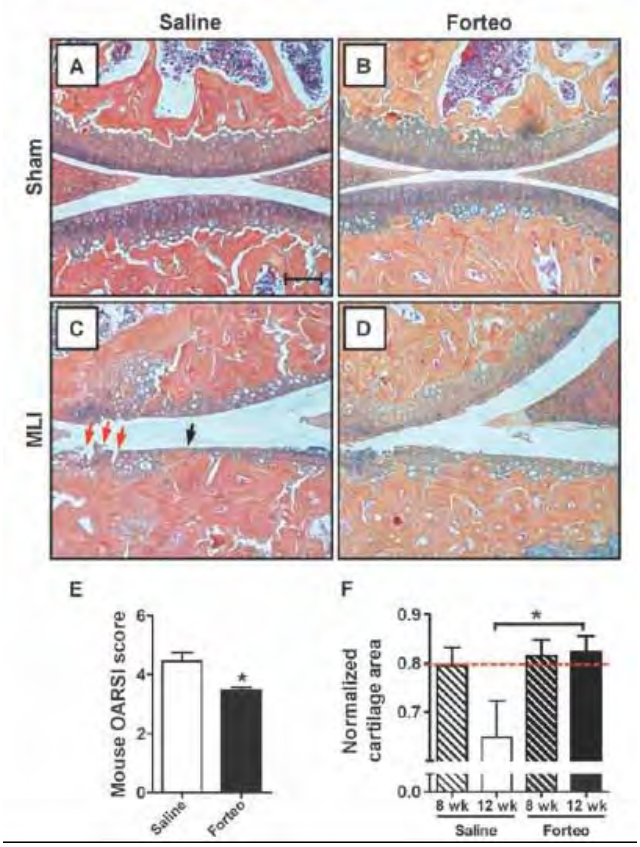
From: Wang, et al., Arthritis Rheum. 2012 Aug;64(8):2611-23

Understanding the role of TGF-beta in osteoarthritis and potential therapeutic targets

From Goldring, Ther Adv Musculoskelet Dis. 2012 Aug;4(4):269-85.



Signaling pathways involved in the pathogenesis of OA converge on MMP13 [29]. MMP13 gene deletion inhibits the development of osteoarthritis that occurs in mice with beta-catenin gain of function. In addition a pharmacologic inhibitor of MMP13 also inhibits the development of OA in beta-catenin gain of function[23]. TGF-beta, which suppresses OA, reduces MMP13 [30].



PTH1-34 (Forteo) binds to the PTHR1 receptor and suppresses maturation/hypetrophy in growth plate chondrocytes. PTHR1 is increased in OA leading to the hypothesis that Forteo might suppress the expression of genes associated with chondrocyte maturation and the development of OA. Forteo suppressed the loss of cartilage in a mouse MLI model of knee injury[31]. Furthermore when Forteo was administered to mice with MLI after the establishment of OA, there was evidence of regeneration of cartilage[3131].

References

1. Rep MMMW. Prevalence and most common causes of disability among adults--United States, 2005. MMWR Morb. Mortal. Wkly. Rep. 58, 421-426. In. Atlanta: CDC; 2009:421-6.
2. Helmick CG, Felson DT, Lawrence RC, et al. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part I. Arthritis Rheum 2008;58:15-25.
3. Chen CG, Thuillier D, Chin EN, Alliston T. Chondrocyte-intrinsic Smad3 represses Runx2-inducible MMP-13 expression to maintain articular cartilage and prevent osteoarthritis. Arthritis and rheumatism 2012.
4. Hootman JM, Helmick CG. Projections of US prevalence of arthritis and associated activity limitations. Arthritis Rheum 2006;54:226-9.
5. Roos H, Lauren M, Adalberth T, Roos EM, Jonsson K, Lohmander LS. Knee osteoarthritis after meniscectomy: prevalence of radiographic changes after twenty-one years, compared with matched controls. Arthritis Rheum 1998;41:687-93.
6. Kim S, Bosque J, Meehan JP, Jamali A, Marder R. Increase in outpatient knee arthroscopy in the United States: a comparison of National Surveys of Ambulatory Surgery, 1996 and 2006. The Journal of bone and joint surgery American volume 2011;93:994-1000.
7. DeFrances CJ, Lucas CA, Buie VC, Golosinskiy A. 2006 National Hospital Discharge Survey; 2008.
8. Corti MC, Rigon C. Epidemiology of osteoarthritis: prevalence, risk factors and functional impact. Aging Clin Exp Res 2003;15:359-63.
9. Katon W, Lin EH, Kroenke K. The association of depression and anxiety with medical symptom burden in patients with chronic medical illness. Gen Hosp Psychiatry 2007;29:147-55.
10. Kotlarz H, Gunnarsson CL, Fang H, Rizzo JA. Insurer and out-of-pocket costs of osteoarthritis in the US: evidence from national survey data. Arthritis Rheum 2009;60:3546-53.
11. Bitton R. The economic burden of osteoarthritis. Am J Manag Care 2009;15:S230-5.
12. Li TF, Gao L, Sheu TJ, et al. Aberrant hypertrophy in Smad3-deficient murine chondrocytes is rescued by restoring transforming growth factor beta-activated kinase 1/activating transcription factor 2 signaling: a potential clinical implication for osteoarthritis. Arthritis and rheumatism 2010;62:2359-69.
13. Ionescu AM, Schwarz EM, Zuscik MJ, et al. ATF-2 cooperates with Smad3 to mediate TGF-beta effects on chondrocyte maturation. Experimental cell research 2003;288:198-207.
14. Dao DY, Yang X, Chen D, Zuscik M, O'Keefe RJ. Axin1 and Axin2 are regulated by TGF- and mediate cross-talk between TGF- and Wnt signaling pathways. Ann N Y Acad Sci 2007;1116:82-99.
15. Dong Y, Drissi H, Chen M, et al. Wnt-mediated regulation of chondrocyte maturation: modulation by TGF-beta. J Cell Biochem 2005;95:1057-68.
16. Dong YF, Soung do Y, Chang Y, et al. Transforming growth factor-beta and Wnt signals regulate chondrocyte differentiation through Twist1 in a stage-specific manner. Mol Endocrinol 2007;21:2805-20.

17. Gunnell LM, Jonason JH, Loiselle AE, et al. TAK1 regulates cartilage and joint development via the MAPK and BMP signaling pathways. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2010;25:1784-97.
18. Li TF, Chen D, Wu Q, et al. Transforming growth factor-beta stimulates cyclin D1 expression through activation of beta-catenin signaling in chondrocytes. J Biol Chem 2006;281:21296-304.
19. Sampson ER, Beck CA, Ketz J, et al. Establishment of an index with increased sensitivity for assessing murine arthritis. Journal of orthopaedic research : official publication of the Orthopaedic Research Society 2011;29:1145-51.
20. Taniguchi N, Carames B, Ronfani L, et al. Aging-related loss of the chromatin protein HMGB2 in articular cartilage is linked to reduced cellularity and osteoarthritis. Proceedings of the National Academy of Sciences of the United States of America 2009;106:1181-6.
21. Rhee DK, Marcelino J, Baker M, et al. The secreted glycoprotein lubricin protects cartilage surfaces and inhibits synovial cell overgrowth. The Journal of clinical investigation 2005;115:622-31.
22. Serra R, Johnson M, Filvaroff EH, et al. Expression of a truncated, kinase-defective TGF-beta type II receptor in mouse skeletal tissue promotes terminal chondrocyte differentiation and osteoarthritis. The Journal of cell biology 1997;139:541-52.
23. Wang M, Tang D, Shu B, et al. Conditional activation of beta-catenin signaling in mice leads to severe defects in intervertebral disc tissue. Arthritis and rheumatism 2012;64:2611-23.
24. Wu Q, Kim KO, Sampson ER, et al. Induction of an osteoarthritis-like phenotype and degradation of phosphorylated Smad3 by Smurf2 in transgenic mice. Arthritis Rheum 2008;58:3132-44.
25. Yang X, Chen L, Xu X, Li C, Huang C, Deng CX. TGF-beta/Smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage. The Journal of cell biology 2001;153:35-46.
26. Lin AC, Seeto BL, Bartoszko JM, et al. Modulating hedgehog signaling can attenuate the severity of osteoarthritis. Nature medicine 2009;15:1421-5.
27. Kamekura S, Kawasaki Y, Hoshi K, et al. Contribution of runt-related transcription factor 2 to the pathogenesis of osteoarthritis in mice after induction of knee joint instability. Arthritis Rheum 2006;54:2462-70.
28. Wang M, Shen J, Jin H, Im HJ, Sandy J, Chen D. Recent progress in understanding molecular mechanisms of cartilage degeneration during osteoarthritis. Annals of the New York Academy of Sciences 2011;1240:61-9.
29. Goldring MB. Chondrogenesis, chondrocyte differentiation, and articular cartilage metabolism in health and osteoarthritis. Ther Adv Musculoskelet Dis 2012;4:269-85.
30. Blaney Davidson EN, Remst DF, Vitters EL, et al. Increase in ALK1/ALK5 ratio as a cause for elevated MMP-13 expression in osteoarthritis in humans and mice. J Immunol 2009;182:7937-45.
31. Sampson ER, Hilton MJ, Tian Y, et al. Teriparatide as a chondroregenerative therapy for injury-induced osteoarthritis. Sci Transl Med 2011;3:101ra93.

Bone Drugs in Children

Gordon Klein, M.D., MPH

MEET THE PROFESSOR SESSION: BONE DRUGS IN CHILDREN

Gordon L Klein MD MPH

University of Texas Medical Branch

Galveston, Texas USA

SIGNIFICANCE:

Currently there are no FDA-approved uses for drugs to either prevent or treat bone loss in children. As a result there are no universally agreed-upon ways to manage bone loss problems in children, nor is it established which conditions result in bone loss and would thus require evaluation and treatment.

In particular there is no agreement as to whether the bisphosphonates should be used in children given the uncertainty of their effects on growth and calcium status, in addition to the concerns about possible atypical fractures or osteonecrosis of the jaw . Furthermore, other drugs, such as recombinant human growth hormone and oxandrolone have had an anabolic effect on bone but have only been used under very restricted circumstances. Finally, the use of recombinant human parathyroid hormone in children will not receive FDA approval despite the demonstrated benefits of this drug in adults because of its association with an increased incidence of osteogenic sarcoma in rats. Consensus is needed in the development of criteria that might provide either reassurance that the incidence of this condition is no greater in children than in adults or rationale for permanent restriction on its use in children.

LEARNING OBJECTIVES:

As a result of participating in this session attendees should be able to:

1. Identify the drugs in which there is some pediatric experience in the treatment or prevention of bone loss.
2. Understand the limitations which attend the widespread use of these drugs.
3. Understand the benefits that each of these drugs can confer to the pediatric patient
4. Recognize the need for standardization of conditions of use

OUTLINE OF DISCUSSION:

1. Enumeration of the drugs to be discussed
   a) Anti-resorptives: the bisphosphonates

      How have they been used? Osteogenesis imperfecta (OI), burns, spinal cord injury, oral vs intravenous

      What are the benefits? OI: decreased bone pain, fracture incidence, increased BMD
      Burns: acute administration only: prevention of early bone loss
      Spinal cord injury: limited data but appears to increase bone mass

      What are the adverse events? No growth failure reported, no atypical fractures, no
      Osteonecrosis of the jaw in the above conditions. Yet a great

deal of caution attends the possible use of these drugs. Oral bisphosphonate data insufficient for efficacy in children.

What are the potential uses? More data needed for spinal cord injury; conditions to consider: all those with increased resorption, including Crohn's disease, rheumatoid arthritis and other inflammatory conditions; other conditions of disuse/immobilization.

What should be the criteria for use? Demonstrated increased bone resorption either by biomarkers or histomorphometry. Subject to consensus development.

b) Anabolic agents: recombinant human growth hormone (rhGH)

How has it been used in treating bone loss? So far has been used in two conditions: Turner's syndrome and burns.

What are the benefits? In Turner's syndrome rhGH plus estrogen replacement is associated with higher cortical volumetric bone density. In burns rhGH works through IGF-1 and increases bone mineral content and bone area proportionately with no effect on bone density. Bone is bigger and biomechanically stronger.

What are the adverse effects? In pediatric burns patients no increase in intensive care unit mortality, no hyperglycemia, no premature growth plate fusion reported. Caution: at a dose of 0.2 mg/kg/day for 12 months, rhGH stimulates bone resorption.

What are the potential uses? Any condition in which there is demonstrated adynamic bone.

c) Anabolic agents: oxandrolone

How has it been used to treat bone loss? So far use limited to burns. Use in conjunction with rhGH in Turner's syndrome ineffective in increasing BMD.

What are the benefits? As with rhGH oxandrolone stimulates IGF-1 and increases both BMC and bone area without a demonstrable effect on BMD. The bone becomes bigger and biomechanically stronger. The advantage of oxandrolone over rhGH is that it is given orally.

What are the adverse effects? In its limited use in burn patients there have been no growth problems or virilization reported, though both have been looked for.

What are the potential uses? As with rhGH , any condition in which there is demonstrated adynamic bone

d) Anabolic agents: recombinant human parathyroid hormone (rhPTH):

Any direct knowledge of pediatric use other than for hypoparathyroidism?

2. Proposal of a potential pediatric consensus development conference on bone drug use
Discussion.

REFERENCES:

Bisphosphonates

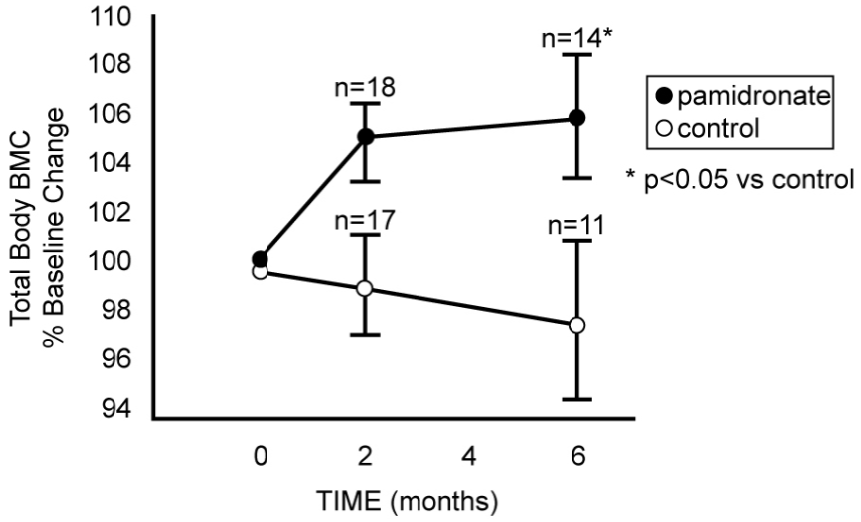
1. Cheung MS, Glorieux FH. Osteogenesis imperfect: update on presentation and management. *Rev Endocr Metab Disord* 2008; 9: 153-60.
2. Vuorines I, Toivaron-Salo S, Hero M, Makitie O. Zoledronic acid treatment in children with osteogenesis imperfecta . *Horm Res Paediatr* 2011; 75: 346-53.
3. Klein GL, Wimalawansa SJ, Kulkarni G, Sherrard DJ, Sanford AP, Herndon DN. The efficacy of acute administration of pamidronate on the conservation of bone mass following severe burn injury. *Osteoporos Int* 2005; 16: 631-5.
4. Przkora R, Herndon DN, Sherrard DJ, Chinkes DL, Klein GL. Pamidronate preserves bone mass for at least 2 years following acute administration for pediatric burn injury. *Bone* 2007; 41: 297-302.
5. Ooi HL, Briody J, McQuade M, Munns CF. Zoledronic acid improves bone mineral density in pediatric spinal cord injury. *J Bone Miner Res* 2012; 27: 1536-40.

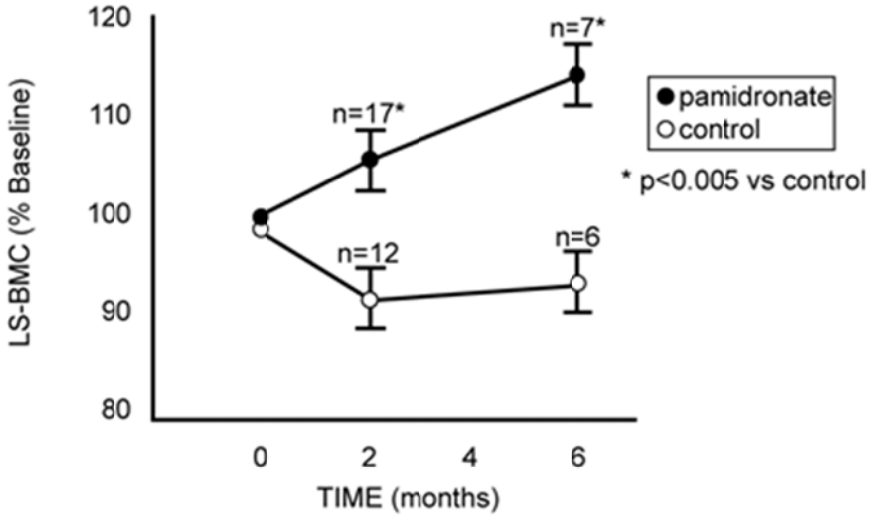
rhGH

1. Branski LK, Herndon DN, Barrow RE, Kulp GA, Klein GL, Suman OE, Przkora R, Meyer W3rd, Huang T, Lee JO, Chinkes DL, Mlcak RP, Jeschke MG. Randomized controlled trial to determine the efficacy of long-term growth hormone treatment in severely burned children. *Ann Surg* 2009; 250: 514-23.
2. Soucek O, Lebl J, Snadjderova M, Kouloskova S, Rocek M, Hlavka Z, Cinek O, Rittweger J, Sumnik Z. Bone geometry and volumetric bone mineral density in girls with Turner syndrome of different pubertal stages. *Clin Endocrinol (Oxf)* 2011; 74: 445-52.

Oxandrolone

1. Porro LJ, Herndon DN, Rodriguez NA, Jennings K, Klein GL, Mlcak RP, Meyer WJ, Lee JO, Suman OE, Finnerty CC. Five-year outcomes after oxandrolone administration in severly burned children: a randomized clinical trial of safety and efficacy. *J Am Coll Surg* 2012; 214: 489-502.





Klein et al, Osteoporos Int 2005

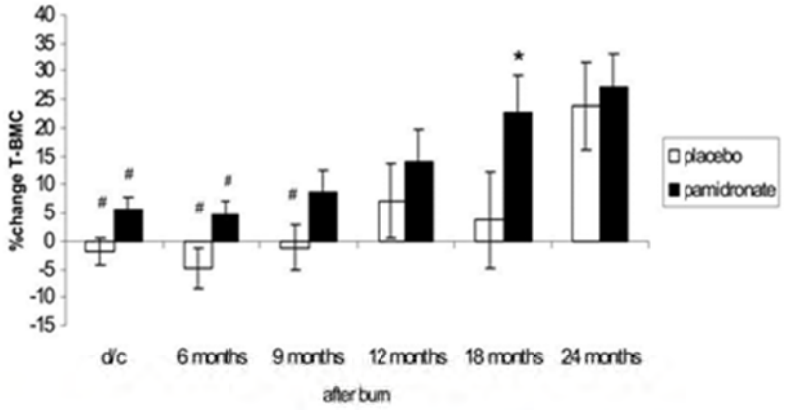


Figure 1
Percent change in total bone mineral content from baseline to 24 months after burn (d/c: Hospital discharge). Values are means ± SEM.
* Significant difference between placebo and pamidronate, $p<0.05$.
Significant time effect within group when compared to changes at 24 months, $p<0.05$.

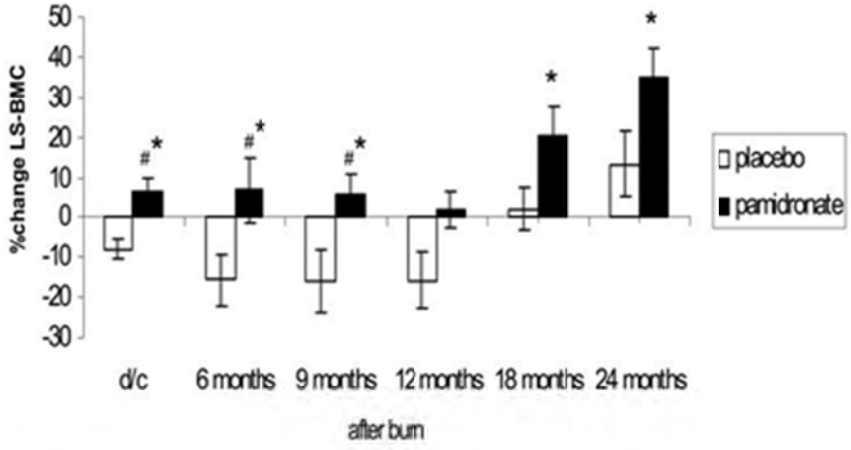


Figure 2
Percent change in lumbar spine bone mineral content (d/c: Hospital discharge). Values are presented as means ± SEM. * Significant difference between placebo and pamidronate, $p<0.05$. Significant time effect within group when compared to changes at 24 months, $p<0.05$.

Przkora et al, Bone 2007

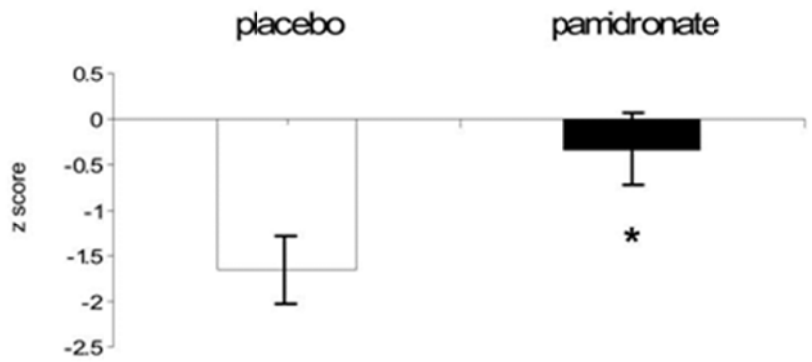


Figure 5
Lumbar spine bone mineral density Z scores at 2 years after burn.
Note Z scores represent the number of standard deviations from normal, healthy age- and sex-matched children. Values are displayed as means ± SEM.
* Significant difference between placebo and pamidronate, $p<0.05$.

Przkora et al, Bone 2007

Mechanical Loading

Angela Cheung, M.D., Ph.D.

Mark Johnson, Ph.D.

Meet-the-Professor Session

Mechanical Loading

Mark L. Johnson, Ph.D.
Professor and Chair
Department of Oral Biology
UMKC School of Dentistry
650 East 25th Street
Kansas City, MO 64108
816-235-2453
johnsonmark@umkc.edu

Angela M. Cheung, MD, PhD, FRCPC, CCD
Lillian Love Chair in Women's Health,
Director, Osteoporosis Program
University Health Network
200 Elizabeth Street, 7 EN 221
Toronto ON M5G 2C4
416-340-4301
angela.cheung@uhn.ca

In vivo Loading Models

The rodent forearm and tibia are widely used in the bone field as a means of investigating the response of bone to in vivo mechanical loading. There are a number of considerations when using these models that the investigator should be aware of when planning their experimental design:

a) Loading regimen, which is largely determined by endpoints to be measured
b) Age and sex of animal to be used
c) Genetic background and choice of appropriate controls

1) Analysis of the Load:Strain Relationship

When applying in vivo loads the critical factor is the global strain that is achieved, not the amount of load *per se*. It is important to apply the same global strain across animal groups, especially when genetically altered mice are being used that may have differences in bone mass, biomaterial properties and bone architecture as a result of the genetic alteration. However, it is also important to remember that measuring strain using a strain gage (see below) estimates an average surface strain and so Finite Element Analysis (FEA) models are often employed to estimate more local strains at various points along the loaded bone (for more discussion of FEA modeling, see: Lu, Y., et al. Med Engin Phys 34:350-356, 2012. PMID 21903442).

To determine the global strain, a strain gage study needs to be first performed. This can be done either ex vivo or in vivo, but for ease the ex vivo approach is much simpler. Ideally, freshly isolate forearms (ulnae) or tibias are used, but if necessary bones can be isolated, carefully stripped of the surrounding muscle and wrapped in a PBS soaked gauze and stored at -20 to-80^{o}C until strain gaging. If in vivo strain gaging is to be performed then properly anaesthetized mice (or rats) are used and the animal is euthanized before waking up from the anesthesia.

For strain gaging analysis the gages are applied to the ulnae (at 2 mm distal to the ulna mid-shaft on the medial surface and at a site 5 mm distal to the end of the olecranon process on the lateral surface). For tibia the strain gages are most easily applied to the lateral surface of the tibial shaft proximal to the fibula junction. Strain measurements show that there are compressive and tensile strains on the medial and lateral surfaces, respectively. After application of strain gages, loading is conducted at 2Hz (or whatever frequency you wish to load at), using a haversine waveform for 15 cycles. Loading is conducted at several load levels and strains in the last five cycles are averaged.

Figure 1: Ex vivo Strain Gaging. Image showing a Vishay strain gage glued to the medial surface of a right forearm mouse ulna. For ex vivo strain measurements the muscle can be fully stripped off of the forearm for ease of application of the strain gage.

Tip: The bone surface needs to be cleaned and dried with EtOH and acetone in order to get good bonding of the strain gage to the bone.

Strain equipment: We use uniaxial strain gages (EA-06-015DJ-120 -option, Vishay Micro-Measurements; Raleigh, NC). The glue of choice is the Bond 200 kit from Measurements Group. We also use an electronic bridge conditioner Model 7000-32-SM (Vishay Micro-Measurements; Raleigh, NC) and StrainSmart software (Vishay Micro-Measurements; Raleigh, NC) to obtain the strain measurements.

2) Loading Regimens

Strains ranging from 1000-4000 microstrain are commonly reported in the literature to initiate an in vivo bone formation response (see the recent paper by Sugiyama et al. JBMR 27(8):1784-1793, 2012, which is an excellent study of bone response to loading as a function of applied strain). Several other considerations are frequency and duration of loading, use of rest insertion, and the number and frequency of loading sessions. Your choice with regard to these options in large part depends upon the endpoint you wish to examine in your study.

3) Loading Paradigms versus Endpoint Considerations

In vivo loading studies come in a wide variety of formats. When considering all of the variables that comprise a loading paradigm, perhaps the most important and first consideration should be the endpoint you plan to measure. If you intend to use dynamic histomorphometry to assess the endpoints associated with new bone formation then loading multiple sessions performed over several days to several weeks is likely to be the most appropriate choice. If you want to measure changes in gene expression by in situ hybridization, immunohistochemistry or other appropriate method, then a short duration loading session followed by sample collection a few hours to a few days after the applied load may be more appropriate.

For new bone formation studies we routinely use a 3 week loading schedule with session each Monday-Wednesday-Friday. Calcien is injected on Friday of the second week and Alizarin red is injected and the Friday of the third week. Animals are sacrificed on the Monday of the 4th week. This gives 7 days between the two injections for rate calculations. The "Gold Standard" reference for histomorphometry is: Parfitt AM, et al J Bone Miner Res **2**(6)**:**595-610, 1987. Many other protocol regimens have been used by various investigators and can be found in the literature.

Important consideration: The age of the animal is very critical. Loading mice, for example, at 8-12 weeks of age introduces the potential confounder of a growing skeleton. We routinely do not load until 16 weeks of age so that growth effects are minimal. However, loading at an older age also means that we resort to the three week loading regimen described above. Loading at a younger age generally only requires a single week loading regimen with injections at days 5 and 2 before sacrifice. Loading aged mice, >16 months of age, introduces another variable in that the non-loaded bones may have little or virtually no labeled surfaces.

4) Some Final Comments

Because each animal can serve as its own control (loaded right limb vs. non-loaded left limb in our studies) group sizes of 5-6 are generally sufficient. If you are loading for the purpose of harvesting tissue, then larger group sizes and pooling of samples may be required. For example for RNA isolation from periosteum loading of 15 mice and then pooling 5 periosteum from 5 mice may be required to get one sample for RT-PCR or Arrays (hence you have 3 replicate groups).

Oftentimes the creation of transgenic and knockout lines of mice results in mixed genetic backgrounds and depending upon the particular experiment the choice of an appropriate control group of mice to load for comparison purposes is critical. Loading of non-transgenic/knockout littermates controls are generally the best controls in these instances, but sometimes it may be necessary to maintain parental lines with the correct mixed genetic background for your loading studies. If the proper controls are used for a loading study, then concerns over genetic background that may confound the interpretation of results are no longer valid. In all cases, strain gaging to determine the load:strain relationship (see subsection A, above) must be done!

In Vitro Loading of Bone Cells

The approaches that are most commonly used for the study of mechanical loading in vitro can generally be grouped into fluid flow shear stress (FFSS) and mechanical stretch (MS) model systems. Which one is correct is subject to constant debate and even between investigators who prefer FFSS or MS there is debate as to which type of FFSS or MS most accurately reflects the loading that is likely to occur in vivo. For example within the FFSS community there has been debate about the use of continuous vs. pulsatile vs. oscillatory type of fluid flow and within the MS aficionados debate over uniaxial vs. equiaxial/biaxial stretch (strain) can be found in the literature. It is important to appreciate that different types of in vivo loading using these approaches can yield different results and so consistency in your choice of method is critical if you want to make comparisons across experiments.

Another important consideration is that responses are often cell type dependent. Cell lines vs. primary cells and osteoblastic vs. osteocytic cells all can yield differing results depending upon the type of FFSS or MS applied. Another consideration is the magnitude of load to apply. For example, in our recent study of the 2T3 osteoblastic cell line compared to the MLO-Y4 osteocytic cell line we observed major differences in the relative sensitivity of these cell lines to FFSS in terms of PGE_2 release and β–catenin nuclear translocation (Kamel, M.A., et al. Bone 47:872-881, 2010. PMC2952691). This is not to say that other response endpoints might show no differences, so the message is to test the endpoint you wish to measure directly if you are making comparisons between cell types.

1) Fluid Flow Shear Stress (FFSS)

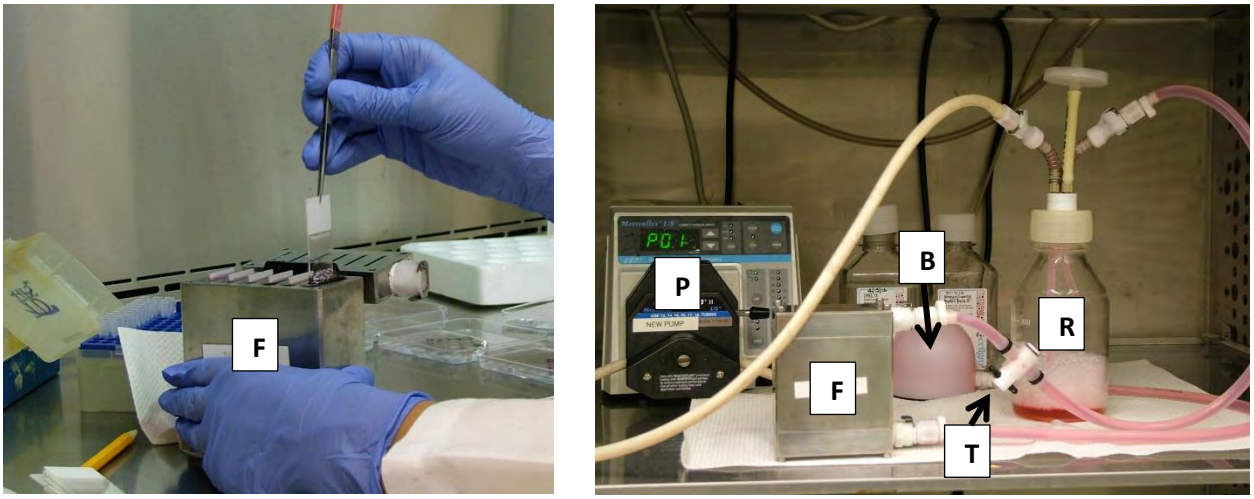
A detailed protocols manual is available from the ASBMR for purchase that was developed as part of the July 16, 2012 Pre-Meeting Workshop held in conjunction with the 2012 Special Topics Meeting on Bone and Skeletal Muscle Interactions.

Many different types of custom and commercial systems have been described in the literature. The basic concept is the generation of shear across a layer of cells by the movement of fluid at a defined rate and physical space so that shear can be calculated from simple engineering principles. For example the most commonly used fluid flow apparatuses involve a parallel plate design. In these systems cells are grown on a glass slide (often a glass microscope slide coated with collagen or other extracellular matrix protein/material) and the cells are stimulated by passing fluid over their surfaces. In order to achieve shear, the apparatus design guides the movement of fluid between the cell coated glass plate and a covering glass plate or holding block. As such the surface area of the plates and the distance or height separating the two plates, between which fluid is pumped are fixed and the amount of shear is a direct function of the rate at which the fluid is passed over the cells (between the two

plates). Another key variable is the viscosity of the fluid, but generally this is also a constant as cell culture media is what is being pumped through the system.

For studies in our laboratory we use the Streamer Gold System (Flexcell International Corp., Hillsborough, NC). This system holds 6 standard microscope slides and the dimensions are predetermined so that the amount of FFSS is all controlled through the associated software and simply changing the speed of pumping by the peristaltic pump. While ease of operation is one of this unit's hallmarks, it does require larger volumes of media (350+ ml) and higher numbers of cells, which may be an issue in some applications. We have also recently begun using a rotating disc FFSS system developed by Donahue and colleagues that applies shear to one side of a transwell cup (Methods in Molecular Biology, Volume 455, "Osteoporosis: Methods and Protocols" Humana Press, edited by: Jennifer J. Westerndorf, Chapter 23, pages 335-345, 2008). This system uses smaller volumes of media 25+ ml and we routinely reduce the volume further by placing a Delrin plug in the lower chamber that cuts the total volume in half. This two-chamber system also allows one to perform cell-cell-communication type studies by plating cells on both sides of the transwell membrane, applying FFSS to one side and measuring a response in the non-sheared cells on the other side of the membrane. The two-chamber system uses much smaller numbers of cells, which may be an advantage or disadvantage depending upon cell availability and your experimental endpoints.

Figure 1. Streamer Gold Fluid Flow system (FlexCell International Corp.)



Left Panel: Loading microscope slides into fluid flow chamber. **Right Panel:** Fluid flow system assembled. Fluid is pumped into the bottom and out through the top of the fluid flow chamber. We have inserted a T-valve stopcock in the exit line (return line to the media reservoir in order to permit collection of media during the application of FFSS. **Legend:** F: Fluid Flow Chamber, R: Media Reservoir; P: Peristaltic Pump, B: Baffle, T: T-valve stopcock.

2) Post Fluid Flow Cell Harvesting and Analysis

All fluid flow shear stress systems lend themselves to a variety of endpoint determinations post flow. The starting cell numbers are much higher when using the Streamer Gold System and consequently

the yields of protein and RNA are greater, but both units basically work well for the same types of measurements. Immunostaining of the cells is straightforward as both the glass slides and the transwell membranes can be used directly, although the background can be higher with the transwell cup membranes depending upon the fluorescent dye being used. This can be solved by using confocal microscopy. For RNA and protein we generally directly lyse the cells off of the slides or membrane with a minimal volume into appropriately sized centrifuge tubes.

TIP: For microscope slides we place two slides back-to-back (cell monolayer out) in a 50 ml tubes and then pipet/scrap the cells off using 1 ml of lysis buffer. For transwell cup membranes, first cut the membrane out of the well with a scalpel and place it in a 1 ml to 50 ml tube for cell harvesting depending upon volume.

3) Substrate Stretching (Mechanical Stretch)

It is clear from the literature that different cell types respond differently to mechanical stretch versus fluid flow shear stress. Therefore, the choice of in vitro loading model to use should in part be based upon a consideration of which form is the most biologically relevant. With regard to mechanical stretch, uniaxial strain is most appropriate for cells that in vivo are loaded along one axis, while equiaxial/biaxial strain is best applied to cells that in vivo would be loaded along multiple axes. We use the FlexCell system for applying mechanical stretch and uniaxial versus equiaxial/biaxial stretch is achieved easily by the use of the appropriate loading posts. The membranes that the cells are plated on allow easy post stretch visualization using immunostaining and microscopy or lysis of the cells for protein and/or RNA methods of analysis. For microscopic types of visualizations the use of confocal microscopy greatly improves image quality.

TIP: Use as little sealing grease as possible as post stretch applications can get messy if you overdo the grease. You can actually use a pea sized drop of grease on the loading posts and NO grease on the gaskets.

4) Final comments

It has often been pointed out in the literature that the magnitude of shear or stretch that is required to initiate a response in vitro is far greater than the mechanical load induced strains achieved in vivo. This is especially true when one examines the older literature regarding osteocytes. However recent studies show that the in vivo magnitude of load is 10 fold or more higher than originally predicted and as such the level of strain achieved with the in vitro models is closer to physiologic that originally thought (Bonivtch, A.R. et al. “Tissue strain amplification at the osteocyte lacuna: a microstructural finite element analysis” J. Biomech. 40(10): 2199-2206, 2007).

Mechanical stimuli and bone health: what is the evidence?

Angela M. Cheung and Lora Giangregorio

Purpose of review
With the recent emergence of associations of bisphosphonate therapy with atypical fractures and osteonecrosis of the jaw, there is renewed interest among clinicians and patients for nonpharmacological approaches to bone health. Here, we review the new studies published in the past year or two that advance our knowledge of the effect of mechanical stimuli on bone health.

Recent findings
Physical activity is associated with serum sclerostin levels; the most physically active individuals have the lowest serum sclerostin levels. Observational trials suggest that physical activity participation results in higher bone mass, but clinical trials suggest that the effects of exercise on areal bone mineral density are small, and vary with the site measured and the type of exercise. Based on current data, it may be best to combine progressive resistance training with interventions such as walking or aerobic dancing if the desire is to improve both spine and hip in postmenopausal women. Low-magnitude high-frequency whole body vibration does not improve bone mineral density and bone structure in postmenopausal women.

Summary
Physical activity and exercise are important for the maintenance of musculoskeletal health as we age. Future studies need to investigate the effects of exercise in older populations with rheumatological diseases and those with a history of fragility fractures.

Keywords
bone density, exercise, whole body vibration

INTRODUCTION

Bone is a living tissue that has the ability to respond to mechanical stimuli such as physical activity and exercise. Wolff's law [1] states that bone will adapt to the loads it is placed under, providing the 'use it or lose it' tenet of bone physiology. Recent data suggest that even passive mechanical stimuli, such as whole body vibration (WBV) therapy, may be beneficial to bone health. In this article, we will review new studies published on the effects of physical activity, exercise and WBV therapy on bone health and highlight those which may be of interest to rheumatologists. We will also review data from a recent study suggesting that the effects of mechanical loading on bone may be mediated by the Wnt/β-catenin signaling pathway.

PHYSICAL ACTIVITY OR EXERCISE AND BONE

We define physical activity as '... any bodily movement produced by skeletal muscles that results in energy expenditure... and can be categorized into occupational, sports, conditioning, household, or other activities' and exercise as '... a subset of physical activity that is planned, structured, and repetitive and has as a final or an intermediate objective the improvement or maintenance of physical fitness' [2].

Physical activity and sclerostin

Sclerostin is a protein produced by osteocytes, which plays a key role in regulating formation of bone. It functions as a Wnt antagonist, blocking the Wnt/β-catenin signaling pathway. When activated,

Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada

Correspondence to Angela M. Cheung, MD, PhD, 200 Elizabeth Street, 7 Eaton North, Room 221, Toronto, Ontario M5G 2C4, Canada. Tel: +1 416 340 4301; fax: +1 416 340 4105; e-mail: angela.cheung@uhn.ca

Curr Opin Rheumatol 2012, 24:561–566
DOI:10.1097/BOR.0b013e3283570238

KEY POINTS

- Older adults should engage in challenging balance exercises to prevent falls and combine strength training with weight-bearing aerobic exercise to prevent bone loss. The effects are not limited to postmenopausal women.
- Exercise programs that emphasize walking over balance training or strength training may not be the best approach for preventing falls or fractures.
- Exercise may also have an effect on pQCT-based assessments of volumetric BMD, but the studies in this area are limited in number and sample size.
- Exercise-mediated alterations in the Wnt/β-catenin signaling pathway may facilitate the maintenance of bone mass.
- Low magnitude whole body vibration therapy alone does not affect bone density or structure in healthy postmenopausal women with low bone mass.

Wnt-signaling leads to increased osteoprogenitor cell populations and decreased apoptosis of mature osteoblasts. Inhibitors of sclerostin, therefore, may be expected to increase osteoblastic bone formation [3▪]. Currently, there are phase 2 and 3 studies examining the safety and effectiveness of sclerostin inhibitors as drug therapies for increasing bone density and strength, and reducing fractures. A recent cross-sectional observational study [4▪▪] of 161 healthy men and premenopausal women aged 19–64 years shows that serum sclerostin levels increase with age in both men and women and are positively correlated with BMI and bone mineral content and negatively correlated with osteocalcin (a bone formation marker) and calcium. The investigators found that people in the most physically active quartile had significantly lower sclerostin levels compared with those in the least active quartile. To test the hypothesis that mechanical loading modulates bone formation by affecting the Wnt/β-catenin signaling pathway, interventional studies will need to be performed to explore whether exercise affects sclerostin levels.

Physical activity or exercise and areal bone mineral density

Few epidemiological studies have investigated the long-term associations between physical activity during adulthood and areal bone mineral density (aBMD) later in life. [aBMD by dual-energy X-ray absorptiometry (DXA) is the standard test for assessing skeletal strength in the clinical setting.] There are two studies over this past year that examined this association, prospectively. The Canadian Multicentre Osteoporosis study [5▪], a prospective population-based study, examined 2855 men and 6442 women at baseline and year 5 and found that those who increased their physical activity from baseline to year 5 increased their aBMD slightly with a concomitant decrease in BMI. The Tromso Study [6], a population-based study in Norway, examined 1766 women and 1451 men aged 20–54 years at baseline who were followed up 22 years later. After adjusting for age, height, weight and smoking status at baseline, there was a positive linear relationship between aBMD and physical activity levels in both men and women ($P < 0.05$). This linear trend was consistent at the hip (total hip, femoral neck and trochanter area) as well as the forearm (distal and ultradistal). Data from a subsample of the participants (2436 men and women under the age of 70) revealed that 6% were sedentary at both baseline and follow-up and 71% were moderately active or active at both baseline and follow-up. In this subgroup, those who were moderately active or active at both baseline and follow-up had higher aBMD than those who were sedentary at both time points ($P \leq 0.01$). These results suggest that physical activity undertaken during adulthood has a positive effect on bone and is associated with a higher aBMD later in life.

In randomized trials of exercise interventions, point estimates of the effect of exercise on aBMD vary from −0.7 to 3.22% [7▪▪,8,9]. However, not all exercise programs are created equal. The most recent Cochrane review [7▪▪] on the effects of exercise on aBMD in postmenopausal women reported that the overall effects of exercise on percentage change in aBMD of the spine [mean difference 0.85%; 95% confidence interval (CI) 0.62–1.07%] and trochanter (mean difference 1.03%; 95% CI 0.56–1.49%) were in favour of exercise, but the overall effects on femoral neck (mean difference −0.08%; 95% CI −1.08 to 0.92%) or total hip aBMD (mean difference 0.41%; 95% CI −0.64 to 1.45%) were not significant. Subgroup analyses were performed to determine the effects of specific types of exercise and the following represent some key points:

(1) High-force dynamic exercise, such as jogging, jumping or dancing, resulted in a between-group difference in favour of exercise at the hip (mean difference 1.55%; 95% CI 1.41–1.69%), but not at the lumbar spine (mean difference −1.20%; 95% CI −4.45 to 2.05%);
(2) Low-force dynamic exercise, such as walking or Tai Chi, resulted in a between-group difference in favour of exercise at the lumbar spine (mean difference 0.87%; 95% CI 0.26–1.48%), but no

effect at the femoral neck (mean difference −1.20%; 95% CI −4.45 to 2.05%);
(3) Progressive resistance training resulted in significant between-group differences in favour of exercise at both the lumbar spine (mean difference 0.86%; 95% CI 0.58–1.13%) and neck of femur (mean difference 1.03%; 95% CI 0.24–1.82%);
(4) No significant benefit of low load, high repetition resistance training was reported.

Therefore, it appears that to improve both lumbar spine and femoral neck aBMD, progressive resistance training may be ideal, alone or in combination with other interventions.

The effect of exercise on aBMD is not limited to women. A recent randomized trial of men aged 55–75 years reported a net gain in femoral neck and lumbar spine aBMD of approximately 2% after 18 months of thrice weekly progressive resistance training and weight-bearing exercise [10▪▪]. Furthermore, the addition of exercise to a weight loss program in older obese adults prevented the diet-induced loss of aBMD and increase in bone turnover markers that occurred in the diet-alone group [11▪▪].

Physical activity or exercise and bone strength

aBMD by DXA only accounts for a portion of whole bone strength, and it has been argued that DXA-based exercise studies seriously underestimate the effects of mechanical loading on bone [12]. Bone size, shape and structure, as well as the material properties of collagen, are also important determinants of whole bone strength [13]. With novel bone imaging techniques such as peripheral quantitative computed tomography (pQCT) and high-resolution pQCT (HRpQCT) we can differentiate between cortical and trabecular compartments of bone and understand their relative contribution to bone strength *in vivo*. Research examining how physical activity or exercise modifies these two bone compartments has emerged.

A recent study [14] examining 234 postmenopausal women (mean age 62 years) using pQCT found that leisure physical activity was positively associated with cortical bone mass and geometry, indices of bending and torsional strength, but was not associated with trabecular bone parameters. The Osteoporotic Fractures in Men (MrOS) study [15] investigated 1171 men aged at least 65 years and found that men in the most physically active quartile had the highest bone strength index (a measure of bone compressive strength) and bone bending strength, when compared with men in the least physically active quartile. Most interestingly, they found that the association between bone strength and physical activity was primarily attributable to greater total bone area and not to the increase in volumetric BMD (vBMD) or aBMD. Men with the greatest muscle power also had the greatest bone strength [15], consistent with the theory that muscle pulling on bone is an important stimulus for maintaining bone strength. Taken together, it appears that physical activity has the potential to modify bone strength and muscle strength and perhaps influence bone fragility as well.

A systematic review and meta-analysis of six randomized controlled trials examining the effects of structured exercise in postmenopausal women found that lower extremity exercises resulted in small (0.9%) but significant improvements in trabecular vBMD of the distal tibia ($P = 0.0006$) and in cortical vBMD of the tibial shaft ($P = 0.0007$) [16▪▪]. Specifically, only studies with longer durations of exercise (12 months) and those in early postmenopausal women showed significant changes in cortical vBMD at the tibial shaft. However, most of the studies in this review were limited by the small sample sizes and the short duration of the exercise regimens. The magnitude of the effect of exercise on vBMD in this meta-analysis was similar to the magnitudes in previous meta-analyses of exercise studies using aBMD as the outcome.

Physical activity or exercise and fractures

There are no prospective randomized controlled trials of exercise that have used fractures as a primary endpoint. Earlier observational studies suggest that in both sexes, moderate physical activity is associated with a 45% (95% CI, 31–56%) lower risk of hip fracture, whereas vigorous physical activity lowers hip fracture risk by 38% (95% CI, 31–44%) [17]. A more recent study [18] examined hip fracture risk in the European Prospective Investigation of Cancer-Norfolk cohort and found that moderate activities at home and during leisure time were associated with a reduction in risk of hip fracture in women (hazard ratios 0.51 and 0.55, *P* value 0.02 and 0.03, respectively). However, in men, only activities during leisure time were associated with lower hip fracture risk (hazard ratio = 0.58; *P* for trend <0.001), whereas activities at home increased the risk of any fracture (hazard ratio = 1.25; *P* for trend = 0.008). In both men and women, walking for any length of time for leisure or transport was associated with a reduction of fracture risk. Overall, a U-shaped association was evident between home activities and fracture risk especially in women; increased risk of fracture was found with low and high levels of home activities, whereas the lowest fracture risk was found in participants who were in the middle of the

distribution. This study suggests that the association with fracture risk may vary with the domain of physical activity and by sex.

It is possible that exercise, particularly unsupervised exercise, might increase the risk of fractures, especially in those who are at high risk. A recent 15-year population-based study [19] revealed that active individuals had a moderately increased risk of wrist fracture compared with inactive individuals; the risk was higher in the winter, and most often associated with a fall. In addition, whether exercise improves outcomes after a fracture is unclear. A Cochrane review [20] which included seven randomized controlled trials with a total of 488 women with vertebral fractures did not find a clear benefit of exercise for reducing fractures and falls or for improving aBMD; no trials measured falls or fractures as an outcome. Only one study measured aBMD, and no significant effect on aBMD was reported after 1 year of home-based exercise. Although some trials did report benefits for some quality of life, pain and physical function outcomes, others had contradictory results. Pooled analyses of two trials suggest that exercise interventions in individuals with vertebral fractures may improve Timed Up and Go test performance, and one trial reported improved walking speed after a 3-month exercise intervention, but the improvements were equivalent to performing the tests approximately 1 and approximately 2 s better, respectively. Moreover, a few fractures were reported that were directly attributed to the intervention, suggesting that caution is warranted when providing exercise instruction to individuals with vertebral fractures.

Although the target of exercise programs may be to improve BMD in the hopes of preventing fractures, it may be of greater value to focus on improved posture or fall prevention in older adults with osteoporosis. Interestingly, a recent meta-analysis of exercise trials in older adults revealed that the exercise regimens that were most effective for preventing falls were those that included challenging balance exercises, had at least 50 h of exercise over the trial period and did not include a walking program [21▪▪]. The maintenance of upright posture was emphasized in an epidemiologic study [22▪▪] that revealed that height loss greater than 5 cm was associated with an almost 50% increased risk of hip fracture, nonvertebral fracture and mortality, and this was after controlling for aBMD and history of vertebral fracture.

WHOLE BODY VIBRATION AND BONE

WBV has received much attention as a potential anti-osteoporotic intervention over the past decade. Various animal studies [23–25] have found significant improvements in bone formation rate, BMD, trabecular structure and cortical thickness. In humans, WBV therapy involves standing on a motorized oscillating platform that produces vertical accelerations, which are transmitted from the feet to the weight-bearing muscles and bones [26]. The intensity is expressed as vibration frequency (1 Hz = 1 oscillation/s), peak-to-peak displacement (in millimeters) and peak acceleration or magnitude [in units of acceleration due to gravity (g); $1\,g = 9.81\ m/s^2$] [26]. There are many WBV platforms available worldwide and they can be categorized on the basis of magnitude [high ($\geq 1\,g$) or low ($<1\,g$)] and type of vertical movement (synchronous or side-alternating) [26,27].

WBV therapy is hypothesized to exert osteogenic effects by changing the flow of bone fluid as a result of direct bone stimulation and transduction of mechanical signals. This osteogenic bone effect may occur via osteocytes and Wnt-β-catenin signaling, or via indirect bone stimulation through skeletal muscle activation, possibly by means of the stretch reflex [27,28]. WBV therapy has also been hypothesized to be beneficial for older adults with diminished mechanical loading of the skeleton due to muscle loss and reduced mobility as it might mimic the mechanical signals typically generated by postural muscle contractions or such low-intensity activities as walking [23,28].

Whole body vibration and bone mineral density and bone structure

In the past 2 years, there have been five systematic reviews and/or meta-analyses on the effect of WBV on BMD, all with similar conclusions [29,30▪▪,31,32▪,33▪]. Our group published one in 2010 and included eight randomized trials (five in postmenopausal women, one in young adults and two in children and adolescents) and found statistically significant but clinically small improvements in hip aBMD in postmenopausal women, statistically significant and larger improvements in spine aBMD in children and adolescents, but no difference in young adults [29]. Our results were limited by the small number of studies, small sample sizes, low study quality and often inconsistent results. There was also heterogeneity in the designs of the WBV platforms and the protocols for their use. To shed some light on this issue, von Stengel *et al.* [34▪] compared the 12-month effects of different WBV devices on BMD. They randomized 108 postmenopausal women (mean age 65.8 years) to one of three groups: thrice weekly 15 min of 12.5 Hz 12 mm rotational WBV, thrice weekly 15 min of 35 Hz 1.7 mm vertical WBV and a control group that involved two blocks of 10 low-intensity gymnastics

sessions. They found a small effect on spine BMD and no difference on hip BMD for rotational WBV and no effect for vertical WBV when compared to control.

To clarify the effect of WBV on BMD and bone structure in postmenopausal women, our group conducted a 1-year randomized trial in 202 postmenopausal women (mean age 60 years) comparing two high frequencies (30 and 90 Hz) and low magnitude (0.3 g) WBV versus control (the Vibration Study) [35▪▪]. We chose low-magnitude high-frequency WBV because stronger osteogenic effects were observed with low-magnitude high-frequency WBV in animal studies, and because high magnitude WBV has been linked to adverse effects in occupational settings. Women were asked to stand on the vibrating platform for 20 min a day. They were also assessed for calcium and vitamin D intake and supplemented according to current guidelines. Disappointingly, there was no difference between the WBV groups and the control group in aBMD, vBMD or any of the HRpQCT structural parameters after 1 year. Adherence ranged from 65 to 79%.

Other groups have also recently shown no effect of WBV on BMD in postmenopausal women. The Erlangen Longitudinal Vibration Study randomized 151 postmenopausal women (mean age 68.5 years) to one of three groups (training, training plus WBV, and control) and found no difference in hip or spine aBMD with WBV (frequency 25 Hz and amplitude 1.7 mm) when compared to control after 18 months [36▪]. However, the investigators did find a significant difference in falls between the WBV and the control group (0.7 versus 1.5 fall/person). Beck and Norling [37] investigated the effect of 8 months of twice weekly supervised WBV (15 min of 30 Hz and 0.3 g, or two sessions of 3 min of 12.5 Hz and 1 g) in 47 postmenopausal women (mean age 71.5 years) and found no differences between WBV and control groups on anthropometrics, BMD, muscle strength and balance. Verschueren *et al.* [38▪▪] conducted a two-by-two factorial-design trial involving 113 institutionalized older women (mean age 79.6 years) who were randomly assigned to either a WBV (<2.2 g) or a no-training group, receiving either a conventional dose (880 IU/day) or a high dose (1600 IU/day) of vitamin D_3. After 6 months, there was no difference between the WBV group and the no-training group in terms of hip aBMD, dynamic muscle strength, isometric strength and muscle mass.

No randomized trial has explored the effect of WBV on fractures, although some investigated its effect on balance and falls, as well as muscle function. However, the studies on balance and falls, and muscle function and morphology are again limited by small sample sizes, short duration, heterogeneity in the WBV protocol and irregularities in study design, similar to the WBV studies on BMD. In the Vibration Study, some women reported shin and plantar foot pain, whereas others reported exacerbation of headaches and inner ear problems with WBV, although there were no statistically significant differences between the WBV and the control groups [35▪▪]. Most of the WBV studies have excluded individuals with joint replacement because of a concern of loosening of hardware. Based on current data, low-magnitude WBV therapy does not increase BMD or improve bone structure in postmenopausal women. Future studies will need to better explore the effect of WBV on muscle function and falls in this population. In addition, based on our systematic review and meta-analysis [29], WBV may be more effective in other populations such as children and adolescents with compromised bone health. Currently, there are randomized controlled trials under way in adolescents [39], patients with spinal cord injury [40] and institutionalized elderly persons [41].

CONCLUSION

Physical activity and exercise are important for the maintenance of musculoskeletal health as we age. Based on current data, the best type of exercise for postmenopausal women to improve both spine and hip BMD is progressive resistance training, either alone or in combination with other interventions. In older adults, physical activity and exercise only have minimal effects on BMD; perhaps fall prevention and strength training should be the focus for this population. Low-magnitude WBV does not appear to improve BMD and bone structure in postmenopausal women. Future studies need to investigate the effect of exercise in older populations with compromised bone health such as those with rheumatological diseases and those with a history of fragility fractures.

Acknowledgements

We thank Ms. Hajera Khaja for her assistance in the preparation of this manuscript for submission.

Conflicts of interest

The authors have no conflicts of interest to declare.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 592).

1. Wolff J. The law of bone remodeling. Berlin Heidelberg New York: Springer; 1986.

2. Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. Public Health Rep 1985; 100:126–131.
3. ▪ Lewiecki EM. Sclerostin: a novel target for intervention in the treatment of osteoporosis. Discov Med 2011; 12:263–273.

This review article discusses why sclerostin is a novel target for osteoporosis therapies.

4. ▪▪ Amrein K, Amrein S, Drexler C, *et al.* Sclerostin and its association with physical activity, age, gender, body composition, and bone mineral content in healthy adults. J Clin Endocrinol Metab 2012; 97:148–154.

This study is the first study to examine the relationship between sclerostin and physical activity and found that physically active individuals have lower serum sclerostin levels.

5. ▪ Langsetmo L, Hitchcock CL, Kingwell EJ, *et al.* Physical activity, body mass index and bone mineral density-associations in a prospective population-based cohort of women and men: the Canadian Multicentre Osteoporosis Study (CaMos). Bone 2012; 50:401–408.

This Canadian prospective population-based cohort study found that individuals who increased their physical activity have slightly higher aBMD at 5 years.

6. Morseth B, Emaus N, Wilsgaard T, *et al.* Leisure time physical activity in adulthood is positively associated with bone mineral density 22 years later. The Tromso study. Eur J Epidemiol 2010; 25:325–331.
7. ▪▪ Howe TE, Shea B, Dawson LJ, *et al.* Exercise for preventing and treating osteoporosis in postmenopausal women. Cochrane Database Syst Rev 2011; 7:CD000333.

This is the most up-to-date Cochrane review of the effects of exercise on aBMD in postmenopausal women.

8. Bonaiuti D, Shea B, Iovine R, *et al.* Exercise for preventing and treating osteoporosis in postmenopausal women. Cochrane Database Syst Rev 2002;(3):CD000333.
9. Kelley GA. Exercise and regional bone mineral density in postmenopausal women: a meta-analytic review of randomized trials. Am J Phys Med Rehabil 1998; 77:76–87.
10. ▪▪ Kukuljan S, Nowson CA, Sanders KM, *et al.* Independent and combined effects of calcium-vitamin D3 and exercise on bone structure and strength in older men: an 18-month factorial design randomized controlled trial. J Clin Endocrinol Metab 2011; 96:955–963.

This is one of the few well conducted randomized controlled trials examining the effects of exercise in older men.

11. ▪▪ Shah K, Armamento-Villareal R, Parimi N, *et al.* Exercise training in obese older adults prevents increase in bone turnover and attenuates decrease in hip bone mineral density induced by weight loss despite decline in bone-active hormones. J Bone Miner Res 2011; 26:2851–2859.

This study illustrates why exercise training is important for attenuating bone loss with weight loss.

12. Jarvinen TL, Kannus P, Sievanen H. Have the DXA-based exercise studies seriously underestimated the effects of mechanical loading on bone? J Bone Miner Res 1999; 14:1634–1635.
13. Cheung AM, Detsky AS. Osteoporosis and fracture: missing the bridge? JAMA 2008; 299:1468–1470.
14. Hamilton CJ, Thomas SG, Jamal SA. Associations between leisure physical activity participation and cortical bone mass and geometry at the radius and tibia in a Canadian cohort of postmenopausal women. Bone 2010; 46:774–779.
15. Cousins JM, Petit MA, Paudel ML, *et al.* Muscle power and physical activity are associated with bone strength in older men: the Osteoporotic Fractures in Men study. Bone 2010; 47:205–211.
16. ▪▪ Polidoulis I, Beyene J, Cheung AM. The effect of exercise on pQCT parameters of bone structure and strength in postmenopausal women: a systematic review and meta-analysis of randomized controlled trials. Osteoporos Int 2012; 23:39–51.

This systematic review and meta-analysis quantifies the volumetric BMD changes with exercise in postmenopausal women.

17. Moayyeri A. The association between physical activity and osteoporotic fractures: a review of the evidence and implications for future research. Ann Epidemiol 2008; 18:827–835.
18. Moayyeri A, Besson H, Luben RN, *et al.* The association between physical activity in different domains of life and risk of osteoporotic fractures. Bone 2010; 47:693–700.
19. Rikkonen T, Salovaara K, Sirola J, *et al.* Physical activity slows femoral bone loss but promotes wrist fractures in postmenopausal women: a 15-year follow-up of the OSTPRE study. J Bone Miner Res 2010; 25:2332–2340.
20. Giangregorio LM, MacIntyre N, Thabane L, *et al.* Exercise for improving outcomes after osteoporotic vertebral fracture (Protocol). Art. No.: CD008618. 2010.
21. ▪▪ Sherrington C, Tiedemann A, Fairhall N, *et al.* Exercise to prevent falls in older adults: an updated meta-analysis and best practice recommendations. N S W Public Health Bull 2011; 22:78–83.

This recent meta-analysis of exercise regimens found that challenging balance exercises in conjunction with other exercises are most effective for preventing falls. They also found that walking may not be the most effective type of exercise to prevent falls.

22. ▪▪ Hillier TA, Lui LY, Kado DM, *et al.* Height loss in older women: risk of hip fracture and mortality independent of vertebral fractures. J Bone Miner Res 2012; 27:153–159. doi: 10.1002/jbmr.558.

This epidemiologic study showed that height loss of greater than 5 cm was associated with an increased risk of hip fracture, nonvertebral fracture and mortality, and this was independent of aBMD and a history of vertebral fractures.

23. Rubin C, Turner AS, Bain S, *et al.* Anabolism. Low mechanical signals strengthen long bones. Nature 2001; 412:603–604.
24. Tiny vibrations may help muscle, bone, and balance. Harv Womens Health Watch 2006; 14:7.
25. Prisby RD, Lafage-Proust MH, Malaval L, *et al.* Effects of whole body vibration on the skeleton and other organ systems in man and animal models: what we know and what we need to know. Ageing Res Rev 2008; 7:319–329.
26. Rauch F, Sievanen H, Boonen S, *et al.* Reporting whole-body vibration intervention studies: recommendations of the International Society of Musculoskeletal and Neuronal Interactions. J Musculoskelet Neuronal Interact 2010; 10:193–198.
27. Judex S, Rubin CT. Is bone formation induced by high-frequency mechanical signals modulated by muscle activity? J Musculoskelet Neuronal Interact 2010; 10:3–11.
28. Ozcivici E, Luu YK, Adler B, *et al.* Mechanical signals as anabolic agents in bone. Nat Rev Rheumatol 2010; 6:50–59.
29. Slatkovska L, Alibhai SM, Beyene J, *et al.* Effect of whole-body vibration on BMD: a systematic review and meta-analysis. Osteoporos Int 2010; 21:1969–1980.
30. ▪▪ Wysocki A, Butler M, Shamliyan T, *et al.* Whole-body vibration therapy for osteoporosis: state of the science. Ann Intern Med 2011; 155:680–686; W206-W213.

This review is an up-to-date balanced summary of the evidence of the effect of whole body vibration therapy on bone.

31. Mikhael M, Orr R, Fiatarone Singh MA. The effect of whole body vibration exposure on muscle or bone morphology and function in older adults: a systematic review of the literature. Maturitas 2010; 66:150–157.
32. ▪ Lau RW, Liao LR, Yu F, *et al.* The effects of whole body vibration therapy on bone mineral density and leg muscle strength in older adults: a systematic review and meta-analysis. Clin Rehabil 2011; 25:975–988.

This recent review also summarizes the effect of whole body vibration therapy on BMD and muscle strength.

33. ▪ del Pozo-Cruz B, Adsuar JC, Parraca JA, *et al.* Using whole-body vibration training in patients affected with common neurological diseases: a systematic literature review. J Altern Complement Med 2012; 18:29–41.

This recent review summarizes the effect of whole body vibration therapy in patients with neurological disorders.

34. ▪ Von Stengel S, Kemmler W, Bebenek M, *et al.* Effects of whole-body vibration training on different devices on bone mineral density. Med Sci Sports Exerc 2011; 43:1071–1079.

This study compares the effects of different vibration platforms on aBMD at the spine and hip.

35. ▪▪ Slatkovska L, Alibhai SM, Beyene J, *et al.* Effect of 12 months of whole-body vibration therapy on bone density and structure in postmenopausal women: a randomized trial. Ann Intern Med 2011; 155:668–679; W205.

This recent randomized controlled trial examining the effects of low-magnitude high-frequency whole body vibration therapy on BMD and bone structure in postmenopausal women found no effect on either after 1 year of therapy.

36. ▪ von Stengel S, Kemmler W, Engelke K, *et al.* Effects of whole body vibration on bone mineral density and falls: results of the randomized controlled ELVIS study with postmenopausal women. Osteoporos Int 2011; 22:317–325.

This study also found no effect on spine and hip aBMD with whole body vibration therapy.

37. Beck BR, Norling TL. The effect of 8 mos of twice-weekly low- or higher intensity whole body vibration on risk factors for postmenopausal hip fracture. Am J Phys Med Rehabil 2010; 89:997–1009.
38. ▪▪ Verschueren SM, Bogaerts A, Delecluse C, *et al.* The effects of whole-body vibration training and vitamin D supplementation on muscle strength, muscle mass, and bone density in institutionalized elderly women: a 6-month randomized, controlled trial. J Bone Miner Res 2011; 26:42–49.

This randomized controlled trial examined the effect of whole body vibration therapy in institutionalized elderly women and found no effect on aBMD or muscle strength after 6 months of therapy.

39. Lam T. Improving low bone mass with vibration therapy in adolescent idiopathic scoliosis (AIS) [clinical trial]. http://www.clinicaltrials.gov/ct2/show/NCT01108211. [Accessed 14 September 2011]
40. Craven BC. Effectiveness of vibration and standing versus standing alone for the treatment of osteoporosis for people with spinal cord injury [clinical trial]. http://www.clinicaltrials.gov/ct2/show/NCT00150683. [Accessed 14 September 2011]
41. Kiel DP, Hannan MT. 'VIBES': low magnitude mechanical stimulation to improve bone mineral density [clinical trial]. http://www.clinicaltrials.gov/ct2/show/NCT00396994. [Accessed 14 September 2011]

Idiopathic Osteoporosis in Premenopausal Women

Elizabeth Shane, M.D.

PREMENOPAUSAL OSTEOPOROSIS

Elizabeth Shane, M.D.
Meet The Professor

INTRODUCTION

Osteoporosis is most commonly diagnosed in postmenopausal women and older men. However, premenopausal women may present with either low bone mass or fractures and few data exist to guide their management. Today I will discuss how to diagnose, evaluate and manage osteoporosis in premenopausal women.

DEFINITION OF OSTEOPOROSIS IN PREMENOPAUSAL WOMEN

Measurement of bone mineral density (BMD) by dual energy X-ray absorptiometry (DXA) is used to diagnose osteoporosis in postmenopausal women and elderly men. The World Health Organization (WHO) defines osteoporosis in postmenopausal women as a BMD value at the spine, hip, or forearm 2.5 or more SD (standard deviations) below the young adult mean (T-score ≤ -2.5), with or without the presence of a fragility fracture. The term "osteopenia" refers to T scores that are above -2.5 but below -1.0 (normal).

In premenopausal women, the prevalence of fractures is orders of magnitude lower than postmenopausal women. Therefore the predictive relationship between BMD and fracture risk is much different than in older women. Young women with low BMD but without other risk factors for fracture are usually at very low short-term risk of fracture.

- For these reasons, BMD measurements *alone* should not be used to diagnose "osteoporosis" in premenopausal women.
- In addition, it is preferable:
    - To avoid using the T score to categorize BMD in premenopausal women
    - To avoid using labels such as "osteopenia" or "osteoporosis" in premenopausal women with low T scores
- Instead, it is preferable:
    - To use the Z score - which compares a young woman's BMD to the mean of an age-, gender-, and ethnicity-matched reference population - to categorize BMD in premenopausal women
    - To use less "loaded" terms such as "low bone density" or bone density that is "below expected for age".
- A Z score that is below -2.0 can be considered as "low bone density" or bone density that is "below expected for age".

When is it appropriate to apply the term "osteoporosis" to a premenopausal woman?

- When there is a history of low-trauma (fragility) fractures, regardless of the BMD measurement
- When a history of secondary causes of osteoporosis (e.g., glucocorticoid therapy, hypogonadism, or hyperparathyroidism) accompanies the low BMD.

INDICATIONS FOR BMD TESTING IN PREMENOPAUSAL WOMEN

Routine BMD screening of premenopausal or perimenopausal women is not currently recommended (10). However, in some clinical situations, BMD testing is an integral part of good clinical management. Recommendations for BMD testing in premenopausal women are noted in **Table 1**.

A key point is that BMD should be measured in any premenopausal women with a history of one or more fragility fractures.

Table 1. Guidelines for BMD Testing in Premenopausal Women

- History of fragility fracture
- Diseases or conditions associated with low bone mass or bone loss
    - Premenopausal estrogen deficiency (e.g., anorexia nervosa, hyperprolactinemia, prolonged amenorrhea)
    - Chronic obstructive pulmonary disease
    - Cystic fibrosis
    - Hyperparath yroidism
    - Rheumatoid arthritis
    - Inflammatory bowel disease
    - Celiac disease
- Medications that cause bone loss
    - Glucocortico ids
    - Depot progesterone
    - GnRH agonists
    - Aromatase inhibitors
    - Antiepileptic drugs (phenobarbital, phenytoin, carbamazepine, valproate)
- If pharmacologic therapy of osteoporosis is being considered
- Being monitored for effectiveness of pharmacologic therapy for osteoporosis

EPIDEMIOLOGY

The Importance of Peak Bone Mass:

A premenopausal woman's BMD depends the amount of bone she formed during adolescence, or her "peak bone mass". Peak bone mass is defined as the maximum BMD achieved by age 30, as measured by DXA. In healthy girls, the most rapid period of bone mass accrual occurs between ages 11 and 14. Although ~95% of peak bone mass is acquired by the late teens, there are small gains from ages 20 to 29. When interpreting BMD measurements in premenopausal women under age 30, one must always consider the possibility that peak bone mass has not yet been achieved.

A premenopausal woman may have low BMD because her peak bone mass is below average because of genetic predisposition, or because she had an illness, medication exposure, or life style choice (alcohol, tobacco, low calcium intake, lack of exercise) that negatively affected accrual of bone mass during adolescence.

Based on the definition of a Z-score, approximately 2.5% of the population will have a Z-score more than 2 SD below the mean. However, the clinical significance of isolated low bone density in young women is unknown, since this diagnosis is not clearly related to a defect in bone strength or quality, nor is it necessarily related to an increased short-term risk of fracture.

Some premenopausal women with low BMD, particularly those with a known secondary cause of osteoporosis, may indeed have abnormal bone strength that can lead to an increased risk of fracture. Premenopausal women with low bone density and no known secondary cause may or may not have decreased bone strength.

Premenopausal Bone Loss

In most healthy premenopausal women, BMD is relatively stable until the preimenopause, although recent studies have documented some bone loss at the proximal femur and forearm. Women with subclinical ovulation disturbances or perimenopausal symptoms are more likely to sustain detectable bone loss before menopause.

Fractures in Premenopausal Women

Although premenopausal women generally have much lower rates of fracture than postmenopausal women, young women with fractures tend to have lower BMD than controls without fractures.

This also applies to women who sustain stress fractures, such as professional ballet dancers, military recruits, and female athletes. Other factors associated with stress fractures in premenopausal women include higher exercise intensity, higher body mass index, tobacco use and poor physical conditioning.

Vertebral fractures may be much more common than appreciated in premenopausal women with autoimmune disorders on high doses of glucocorticoids. Importantly, they may occur despite normal BMD measurements.

Relationship Between Premenopausal and Postmenopausal Osteoporosis and Fractures

A history of premenopausal or perimenopausal fracture significantly increases the risk of a postmenopausal fracture, suggesting that the risk of sustaining fractures is a life-long trait, probably reflecting the interaction of an individual's bone mass, bone quality, fall frequency, and neuromuscular protective response to falls.

PATHOGENESIS OF PREMENOPAUSAL BONE LOSS AND OSTEOPOROSIS

Factors that influence the rate and degree of premenopausal bone loss include age, weight changes, BMI, calcium intake, physical activity, alcohol consumption, family history of osteoporosis, smoking, and number of pregnancies.

In most premenopausal women with low BMD, there is a “secondary cause” of osteoporosis, such as an underlying disorder or exposure to medication that has either interfered with acquisition of peak bone mass during adolescence or caused excessive bone loss thereafter **(Table 2)**.

Table 2: Secondary causes of osteoporosis in premenopausal women

Anorexia nervosa
Gastrointestinal malabsorption (eg. celiac disease, postoperative states)
Vitamin D and/or calcium deficiency
Hyperthyroidism
Hyperparathyroidism
Cushing’s syndrome
Hypogonadism
Hypercalciuria
Rheumatoid arthritis and other inflammatory conditions
Alcoholism
Renal Disease
Liver disease
Osteogenesis imperfecta
Marfan’s syndrome
Homocystinuria

Medications

Glucocorticoids
Immunosuppressants (cyclosporine)
Antiseizure medications (particularly phenobarbital and phenytoin)
GnRH agonists (when used to suppress ovulation)
Heparin
Cancer chemotherapy
Depot medroxyprogesterone acetate
Excess thyroid hormone

Pregnancy and lactation associated bone loss

Whether significant bone loss occurs during pregnancy is controversial. In contrast, lactation is associated with losses of 3-10% at the spine and hip over the first 3-6 months, likely related to both maternal calcium losses during milk production and estrogen deficiency. Bone loss reverses after weaning. Recovery from lactation associated bone loss may continue to occur for 18 months or longer after the cessation of lactation. Neither parity (number of births) nor lactation have been associated with osteoporosis or increased fracture risk in postmenopausal women.

Knowledge of BMD changes that occur during and after lactation affect how the physician interprets bone density results. When bone density measurements are obtained around the time of a pregnancy or lactation, it may be difficult to separate physiologic from pathophysiologic changes.

Idiopathic Osteoporosis in Premenopausal Women

Young women with no known secondary causes of osteoporosis or disturbances in calcium metabolism are said to have “idiopathic osteoporosis” (IOP). Idiopathic osteoporosis primarily affects Caucasians, men and women equally. Presentation may occur during pregnancy or lactation. The mean age at diagnosis is in the mid-thirties. Fractures are usually multiple, occur over a 5 to 10 year period and involve sites rich in cancellous bone, such as the vertebrae. The hip is affected in approximately 10% of cases. There are few data on the biochemical features of IOP, although hypercalciuria, subclinical estogen deficiency and increased bone resorption markers have been reported. Abnormalities of osteoblast function and decreased IGF-1 have been found in some studies, but not others. In a bone biopsy study of women with IOP, decreased bone formation was the most prominent finding.

EVALUATION OF WOMEN WITH PREMENOPAUSAL OSTEOPOROSIS

Premenopausal women who have a low trauma fracture or a very low bone density should have a thorough evaluation to identify potential secondary causes. A secondary cause of osteoporosis can be found in a substantial proportion of premenopausal women with osteoporosis. Identification of a contributing condition is key, as the correct diagnosis helps to guide management.

History and physical exam should focus on ruling out the conditions listed in **Table 2**.
Laboratory evaluation may help to diagnose secondary causes of osteoporosis such as renal or liver disease, hyperthyroidism, hyperparathyroidism, Cushing’s syndrome, early menopause, celiac disease and other forms of malabsorption, idiopathic hypercalciuria, or, rarely, connective tissue disorders. Laboratory tests that can be helpful in the diagnosis of secondary causes of premenopausal osteoporosis are listed in **Table 3**.

Table 3: Laboratory evaluation for premenopausal osteoporosis

Initial laboratory tests
Complete chemistry profile (including alkaline phosphatase)
Complete blood count
Calcium, phosphorus, magnesium
25 hydroxyvitamin D
1,25 dihydroxyvitamin D
Intact PTH
24 hour urine for calcium and creatinine
Celiac screen
TSH

Additional laboratory tests if indicated
24 hour urine for free cortisol
Estradiol, LH, FSH, prolactin
Serum protein electrophoresis/urine protein electrophoresis

Erythrocyte sedimentation rate, C-reactive protein
Rheumatoid factor
Ferritin and carotene levels
Iron and total iron binding capacity
Serum tryptase and histamine levels
Homocysteine
Skin biopsy for connective tissue disorders
COL1A genetic testing for osteogenesis imperfecta
Serum and urine markers of bone turnover
Premenopausal women with no identifiable etiology after extensive evaluation for secondary causes are said to have idiopathic osteoporosis. In patients with a history of low trauma fractures without a known cause, bone biopsy may be indicated to identify other sources of bone fragility and to guide therapeutic interventions (89).

MANAGEMENT

Lifestyle Modifications

There are no official guidelines for management of premenopausal low bone mass or osteoporosis. However, good clinical judgement should be applied until more is known and better recommendations can be formulated.

Table 4: Management of Premenopausal Osteoporosis
Thorough history and physical exam
Laboratory evaluation for secondary causes
Calcium 1000 - 1200 mg daily
Vitamin D3 400-800 IU daily
Weight bearing exercise
Changes in habits (avoidance of smoking, excess alcohol, poor nutrition)
Treatment of secondary causes
Pharmacologic treatment in severe cases

Lifestyle modifications should be encouraged for all women with low bone mass since peak bone mass may improve well into the fourth decade:

- Adequate calcium intake (1000 – 1200 mg elemental calcium daily)
- Adequate vitamin D intake (400-800 IU vitamin D3 daily)
- Regular physical activity, particularly weight-bearing exercise
- Cessation of smoking
- Maintain normal body weight
- Avoid excessive dieting and wide swings in weight
- Avoid excess alcohol, caffeine and phosphorus containing drinks.

A recent study of 16 premenopausal women with IOP treated only with increased dietary calcium and physical activity revealed significant increases in lumbar spine and femoral neck BMD after 2 or 3 years and no new fractures.

Management of Secondary Causes of Low Bone Density

When a secondary cause of osteoporosis is detected in premenopausal women, treatment should be targeted to that disease or abnormality. Examples of specific approaches that have been shown to lead to increases in BMD include:

- Institution of a gluten-free diet in young women with celiac disease.
- Parathyroidectomy in young women with primary hyperparathyroidism.
- Discontinuation of medroxyprogesterone acetate.
- Oral contraceptives for women with oligo- or amenorrhea, on gonadotropin-releasing hormone (GnRH) therapy with perimenopausal bone loss

- The selective estrogen receptor modulator, raloxifene, in premenopausal women receiving GnRH agonist therapy for uterine leiomyomas.

In contrast, combination estrogen-progestin therapy is not effective in increasing BMD in young women with anorexia nervosa. Instead, weight gain and resumption of normal menstrual function are necessary for recovery of bone mass. Oral bisphosphonates have also been successful in increasing BMD in patients with anorexia.

- Lumbar spine BMD increased by 4.9% after 9 months of risedronate 5 mg daily.
- Femoral neck BMD increased by 4.4% and lumbar spine by 3.5% with10 mg alendronate daily, significantly greater than a control group treated with nutritional support.

Pharmacologic Therapies

- **Selective Estrogen Receptor Modulators (SERMs)**
  - SERMs such as raloxifene and tamoxifen should not be used in menstruating women as they block estrogen action on bone, leading to further bone loss
- **Calcitonin**
  - There are very few published studies on the safety or efficacy of salmon calcitonin in young women. However, in a double-blind study of 120 perimenopausal women treated with 100 IU intranasal calcitonin or placebo spray daily, there was no improvement in BMD or bone markers.
- **Bisphosphonates**
  - Bisphosphonates carry a Category C rating for safety in pregnancy due to toxic effects produced in pregnant rats. Bisphosphonates cross the placenta and accumulate in fetal bones in an experimental rat model. There have been no reports of adverse effects of bisphosphonates on the fetuses of humans or animals. However, only small case series and case reports have been published. The long half-life of bisphosphonates in bone makes the use of these agents in reproductive age women a concern.
  - In premenopausal women with low BMD alone without fractures or known secondary causes for fractures, such as glucocorticoids, bisphosphonates are generally not indicated.
- **Teriparatide**
  - Teriparatide has been shown to prevent bone loss in premenopausal women on GnRH agonists for endometriosis and some premenopausal women were included in the recent RCT of teriparatide in GIOP (Saag, NEJM, 2007).
  - Teriparatide has the advantage of not being retained in the skeleton. However, its effects may dissipate after cessation unless followed by bisphoshponate therapy.

Management of Premenopausal Women at High Risk for Rapid Bone Loss or Fractures

There are some groups of premenopausal women for whom aggressive therapy with anti-osteoporosis agents may be necessary.

- **Glucocorticoid-Induced Osteoporosis**
  - Bisphosphonates are efficacious for prevention and treatment of glucocorticoid-induced bone loss. However, relatively few premenopausal women have been included in the large registration trials. A few studies of premenopausal women have generally noted preservation of bone mass, particularly at the spine. The American College of Rheumatology guidelines recommend pharmacologic therapy for prevention and treatment of glucocorticoid-induced osteoporosis in premenopausal women taking at least 5 mg of prednisone or equivalent per day but they urge caution in the use of bisphosphonates in young women.
- **Chemotherapy-Induced Osteoporosis**
  - Premenopausal women receiving chemotherapy for breast cancer represent another group at risk for rapid bone loss, primarily related to induction of premature menopause. Prospective studies demonstrate bone loss at 1 year of 4–8% in the spine and 2–4% at the hip in premenopausal women who become menopausal after receiving adjuvant chemotherapy. Tamoxifen, which maintains bone mass in postmenopausal women with breast cancer, has the opposite effect in women who remain premenopausal during therapy. Intravenous pamidronate

(60 mg every 3 months) successfully prevented bone loss in premenopausal women with chemotherapy-induced amenorrhea.

- **Osteogenesis Imperfecta**
  - A final group of premenopausal women at increased risk for fractures are those with osteogenesis imperfecta (OI). Given the very high morbidity of this inherited collagen disorder, bisphosphonates recommended even in children. Both oral alendronate and intravenous pamidronate have been used successfully in children and adults with OI.

SUGGESTED REFERENCES

Reviews

1. Leib ES, Lewiecki EM, Binkley N, Hamdy RC. Official positions of the International Society for Clinical Densitometry. J Clin Densitom 2004;7(1):1-6.
2. Leib ES. Treatment of low bone mass in premenopausal women: when may it be appropriate? Curr Osteoporos Rep 2005;3(1):13-8.
3. Gourlay ML, Brown SA. Clinical considerations in premenopausal osteoporosis. Arch Intern Med 2004;164(6):603-14.
4. Diagnosis of osteoporosis in men, premenopausal women, and children. J Clin Densitom 2004;7(1):17-26.
5. Lewiecki EM. Premenopausal bone health assessment. Curr Rheumatol Rep 2005;7(1):46-52
6. Mora S, Gilsanz V. Establishment of peak bone mass. Endocrinol Metab Clin North Am 2003;32(1):39-63
7. Khan AA, Syed Z. Bone densitometry in premenopausal women: synthesis and review. J Clin Densitom 2004;7(1):85-92.

Epidemiology

8. Sowers MR, Galuska DA. Epidemiology of bone mass in premenopausal women. Epidemiol Rev 1993;15(2):374-98.
9. Bainbridge KE, Sowers MF, Crutchfield M, Lin X, Jannausch M, Harlow SD. Natural history of bone loss over 6 years among premenopausal and early postmenopausal women. Am J Epidemiol 2002;156(5):410-7.
10. Slemenda C, Longcope C, Peacock M, Hui S, Johnston CC. Sex steroids, bone mass, and bone loss. A prospective study of pre-, peri-, and postmenopausal women. J Clin Invest 1996;97(1):14-21.
11. Thompson PW, Taylor J, Dawson A. The annual incidence and seasonal variation of fractures of the distal radius in men and women over 25 years in Dorset, UK. Injury 2004;35(5):462-6.
12. Hosmer WD, Genant HK, Browner WS. Fractures before menopause: a red flag for physicians. Osteoporos Int 2002;13(4):337-41.
13. Wu F, Mason B, Horne A, et al. Fractures between the ages of 20 and 50 years increase women's risk of subsequent fractures. Arch Intern Med 2002;162(1):33-6.
14. Fiorano-Charlier C, Ostertag A, Aquino JP, de Vernejoul MC, Baudoin C. Reduced bone mineral density in postmenopausal women self-reporting premenopausal wrist fractures. Bone 2002;31(1):102-6.

Stress Fractures

15. Lappe J, Davies K, Recker R, Heaney R. Quantitative ultrasound: use in screening for susceptibility to stress fractures in female army recruits. J Bone Miner Res 2005;20(4):571-8.
16. Joy EA, Campbell D. Stress fractures in the female athlete. Curr Sports Med Rep 2005;4(6):323-8.
17. Korpelainen R, Orava S, Karpakka J, Siira P, Hulkko A. Risk factors for recurrent stress fractures in athletes. Am J Sports Med 2001;29(3):304-10.
18. Lauder TD, Dixit S, Pezzin LE, Williams MV, Campbell CS, Davis GD. The relation between stress fractures and bone mineral density: evidence from active-duty Army women. Arch Phys Med Rehabil 2000;81(1):73-9.

Secondary Causes

19. Borba VZ, Matos PG, da Silva Viana PR, Fernandes A, Sato EI, Lazaretti-Castro M. High prevalence of vertebral deformity in premenopausal systemic lupus erythematosus patients. Lupus 2005;14(7):529-33
20. Kumagai S, Kawano S, Atsumi T, et al. Vertebral fracture and bone mineral density in women receiving high dose glucocorticoids for treatment of autoimmune diseases. J Rheumatol 2005;32(5):863-9.

21. Peris P, Guanabens N, Martinez de Osaba MJ, et al. Clinical characteristics and etiologic factors of premenopausal osteoporosis in a group of Spanish women. Semin Arthritis Rheum 2002;32(1):64-70.
22. Ward KD, Klesges RC. A meta-analysis of the effects of cigarette smoking on bone mineral density. Calcif Tissue Int 2001;68(5):259-70.
23. Bacon L, Stern JS, Keim NL, Van Loan MD. Low bone mass in premenopausal chronic dieting obese women. Eur J Clin Nutr 2004;58(6):966-71
24. Stenson WF, Newberry R, Lorenz R, Baldus C, Civitelli R. Increased prevalence of celiac disease and need for routine screening among patients with osteoporosis. Arch Intern Med 2005;165(4):393-9.
25. Armagan O, Uz T, Tascioglu F, Colak O, Oner C, Akgun Y. Serological screening for celiac disease in premenopausal women with idiopathic osteoporosis. Clin Rheumatol 2005;24(3):239-43.
26. Scholes D, LaCroix AZ, Ichikawa LE, Barlow WE, Ott SM. Change in bone mineral density among adolescent women using and discontinuing depot medroxyprogesterone acetate contraception. Arch Pediatr Adolesc Med 2005;159(2):139-44.
27. Kaunitz AM, Miller PD, Rice VM, Ross D, McClung MR. Bone mineral density in women aged 25-35 years receiving depot medroxyprogesterone acetate: recovery following discontinuation. Contraception 2006;74(2):90-9.

Pregnancy and Lactation

28. Black AJ, Topping J, Durham B, Farquharson RG, Fraser WD. A detailed assessment of alterations in bone turnover, calcium homeostasis, and bone density in normal pregnancy. J Bone Miner Res 2000;15(3):557-63.
29. Naylor KE, Iqbal P, Fledelius C, Fraser RB, Eastell R. The effect of pregnancy on bone density and bone turnover. J Bone Miner Res 2000;15(1):129-37.
30. Kovacs CS. Calcium and bone metabolism in pregnancy and lactation. J Clin Endocrinol Metab 2001;86(6):2344-8.
31. Rizzoli R, Bonjour JP. Pregnancy-associated osteoporosis. Lancet 1996;347(9011):1274-6.
32. Di Gregorio S, Danilowicz K, Rubin Z, Mautalen C. Osteoporosis with vertebral fractures associated with pregnancy and lactation. Nutrition 2000;16(11-12):1052-5.
33. Peris P, Guanabens N, Monegal A, et al. Pregnancy associated osteoporosis: the familial effect. Clin Exp Rheumatol 2002;20(5):697-700.

Idiopathic Osteoporosis

34. Khosla S, Lufkin EG, Hodgson SF, Fitzpatrick LA, Melton LJ, 3rd. Epidemiology and clinical features of osteoporosis in young individuals. Bone 1994;15(5):551-5.
35. Heshmati HM, Khosla S. Idiopathic osteoporosis: a heterogeneous entity. Ann Med Interne (Paris) 1998;149(2):77-81.
36. Reed BY, Zerwekh JE, Sakhaee K, Breslau NA, Gottschalk F, Pak CY. Serum IGF 1 is low and correlated with osteoblastic surface in idiopathic osteoporosis. J Bone Miner Res 1995;10(8):1218-24.
37. Pepene CE, Seck T, Diel I, Minne HW, Ziegler R, Pfeilschifter J. Concentration of insulin-like growth factor (IGF)-I in iliac crest bone matrix in premenopausal women with idiopathic osteoporosis. Exp Clin Endocrinol Diabetes 2004;112(1):38-43.
38. Rubin MR, Schussheim DH, Kulak CA, et al. Idiopathic osteoporosis in premenopausal women. Osteoporos Int 2005;16(5):526-33.
39. Donovan MA, Dempster D, Zhou H, McMahon DJ, Fleischer J, Shane E. Low bone formation in premenopausal women with idiopathic osteoporosis. J Clin Endocrinol Metab 2005;90(6):3331-6.
40. Peris P, Monegal A, Martinez MA, Moll C, Pons F, Guanabens N. Bone mineral density evolution in young premenopausal women with idiopathic osteoporosis. Clin Rheumatol 2006

Management

41. Jamal SA, Ridout R, Chase C, Fielding L, Rubin LA, Hawker GA. Bone mineral density testing and osteoporosis education improve lifestyle behaviors in premenopausal women: a prospective study. J Bone Miner Res 1999;14(12):2143-9
42. Jones G, Scott F. Low bone mass in premenopausal parous women: identification and the effect of an information and bone density feedback program. J Clin Densitom 1999;2(2):109-15.
43. Mein AL, Briffa NK, Dhaliwal SS, Price RI. Lifestyle influences on 9-year changes in BMD in young women. J Bone Miner Res 2004;19(7):1092-8.
44. Winzenberg TM, Shaw K, Fryer J, Jones G. Calcium supplementation for improving bone mineral density in children. Cochrane Database Syst Rev 2006(2):CD005119.

45. Wallace BA, Cumming RG. Systematic review of randomized trials of the effect of exercise on bone mass in pre- and postmenopausal women. Calcif Tissue Int 2000;67(1):10-8.
46. Peris P, Monegal A, Martinez MA, Moll C, Pons F, Guanabens N. Bone mineral density evolution in young premenopausal women with idiopathic osteoporosis. Clin Rheumatol 2006.
47. Mitwally MF, Gotlieb L, Casper RF. Prevention of bone loss and hypoestrogenic symptoms by estrogen and interrupted progestogen add-back in long-term GnRH-agonist down-regulated patients with endometriosis and premenstrual syndrome. Menopause 2002;9(4):236-41.
48. Golden NH, Lanzkowsky L, Schebendach J, Palestro CJ, Jacobson MS, Shenker IR. The effect of estrogen-progestin treatment on bone mineral density in anorexia nervosa. J Pediatr Adolesc Gynecol 2002;15(3):135-43.
49. Miller KK, Grieco KA, Mulder J, et al. Effects of risedronate on bone density in anorexia nervosa. J Clin Endocrinol Metab 2004;89(8):3903-6
50. Golden NH, Iglesias EA, Jacobson MS, et al. Alendronate for the treatment of osteopenia in anorexia nervosa: a randomized, double-blind, placebo-controlled trial. J Clin Endocrinol Metab 2005;90(6):3179-85.
51. O'Sullivan SM, Grey AB, Singh R, Reid IR. Bisphosphonates in pregnancy and lactation-associated osteoporosis. Osteoporos Int 2006;17(7):1008-12.
52. Shapiro CL, Manola J, Leboff M. Ovarian failure after adjuvant chemotherapy is associated with rapid bone loss in women with early-stage breast cancer. J Clin Oncol 2001;19(14):3306-11.
53. Fuleihan Gel H, Salamoun M, Mourad YA, et al. Pamidronate in the prevention of chemotherapy-induced bone loss in premenopausal women with breast cancer: a randomized controlled trial. J Clin Endocrinol Metab 2005;90(6):3209-14.

Nephrolithiasis

Murray Favus, M.D.

Howard Fink, M.D., MPH

Meet the Professor: Nephrolithiasis

Howard A. Fink, M.D., U.S. Veterans Medical Center, Minneapolis, MN, USA
Murray J. Favus, M.D., the University of Chicago, Chicago, IL, USA

Significance of the topic: Nephrolithiasis is a common chronic disorder with the potential for severe complications such as bleeding, infection, obstruction and kidney damage that may result in multiple hospitalizations and surgical procedures. Kidney stones are the ninth most common reason for visits to emergency rooms. 13% of men and 7% of women will have at least one kidney stone event during their adult years, and 50% of single stone formers will relapse within 5 to 7 years of the initial stone event. Kidney stones are common, and there are a number of medical and nutritional therapies suggested to reduce stone recurrence. However, studies of most therapies and dietary interventions are inadequate to draw clear conclusion about efficacy. Therefore, treating physicians must be familiar with the appropriate interventions and their efficacy to be effective in reducing recurrence. The Meet-The-Professor Session will provide a literature review of the current medical and nutritional therapies to assess the strength of evidence for each intervention.

Learning Objectives

As a result of participating in this session, attendees should be able to:

1. Weigh the evidence from the available trials and make therapeutic decisions regarding selection of pharmaceutical agents; and nutritional interventions;
2. Assess the strength of evidence for prevention of stone recurrence using dietary interventions;
3. Review the impact of dietary salt intake on hypercalciuria, is a major risk factor for kidney stone recurrence, and impact on calcium balance and potential effect on bone mass.

Points of interest include: the attached tables A and B that summarize dietary interventions and pharmacologic therapies for kidney stone recurrence; and a case report and summary of a key publication which address management of calcium metabolism with dietary salt control.

References are attached.

Table A. Summary of evidence for prevention of kidney stones: Dietary interventions (KQ 2)		
Interventions, Studies (Study Quality)	Stone Recurrence Results	Strength of Evidence*
Increased Fluid Intake vs. No Treatment 2 RCTs (fair) in patients with single past calcium stone42,46	Symptomatic: No results reported. Composite: Reduced risk (12 vs. 27%; RR, 0.45 [CI, 0.24 to 0.84], n=1 trial) and increased time to recurrence (39 vs. 25 mo., p=0.016, n=1 trial). Radiographic: No reduced risk (8 vs. 56%; RR, 0.15 [CI, 0.02 to 1.07], n=1 trial).	Symptomatic: Insufficient Composite: Low Radiographic: Low
Increased Oligomineral Water Intake vs. Increased Tap Water Intake 1 RCT (fair) in patients with recurrent calcium stones.54	Symptomatic: No results reported. Composite: No results reported. Radiographic: No reduced risk (17 vs. 23%; RR, 0.73 [CI, 0.48 to 1.09]).	Symptomatic: Insufficient Composite: Insufficient Radiographic: Low
Reduced Soft Drink Intake vs. Control 1 RCT (fair) in men with high soft drink intake and 1 or more past stones55	Symptomatic: Reduced risk (34 vs. 41%; RR, 0.83 [CI, 0.71 to 0.98]), particularly in participants whose most frequently consumed soft drink was acidified by phosphoric acid and not citric acid (30% vs. 46%; RR, 0.65 [CI, 0.49 to 0.87], p=0.02 for interaction). Composite: No results reported. Radiographic: No results reported.	Symptomatic: Low Composite: Insufficient Radiographic: Insufficient
Multicomponent Diet (Borghi 2002) vs. Control Diet† 1 RCT (fair) in patients with recurrent calcium stones51	Symptomatic: No results reported. Composite: Reduced risk (20 vs. 38%; RR, 0.52 [CI, 0.29 to 0.95]). Radiographic: No results reported.	Symptomatic: Insufficient Composite: Low Radiographic: Insufficient
Multicomponent Diet (Hiatt 1996) vs. Control Diet‡ 1 RCT (fair) in patients with single past calcium stone57	Symptomatic: No results reported. Composite: Increased risk (24 vs. 4%; RR, 5.88 [CI, 1.39 to 24.92]). Radiographic: No results reported.	Symptomatic: Insufficient Composite: Low Radiographic: Insufficient
Tailored Diet vs. Empiric Diet 1 RCT (fair) in patients with single past calcium stone53	Symptomatic: No results reported. Composite: Reduced risk (6 vs. 19%; RR, 0.32 [CI, 0.14 to 0.74]). Radiographic: No results reported.	Symptomatic: Insufficient Composite: Low Radiographic: Insufficient
Decreased Animal Protein Diet vs. Control Diet 1 RCT (fair) in patients with 1 or more past calcium stones56	Symptomatic: No results reported. Composite: No reduced risk (48 vs. 48%; RR, 1.00 [CI, 0.52 to 1.91]). Radiographic: No results reported.	Symptomatic: Insufficient Composite: Low Radiographic: Insufficient
Increased Fiber Diet vs. Control Diet 1 RCT (fair) in patients with 1 or more past calcium stones56	Symptomatic: No results reported. Composite: No reduced risk (63 vs. 48%; RR, 1.18 [CI, 0.66 to 2.12]). Radiographic: No results reported.	Symptomatic: Insufficient Composite: Low Radiographic: Insufficient

CI = 95 percent confidence interval; KQ = Key Question; RCT = randomized controlled trial; RR = relative risk.

*Strength of evidence was rated using the following grades: (1) high confidence indicated that further research is very unlikely to change the confidence in the estimate of effect, meaning that the evidence reflects the true effect; (2) moderate confidence denoted that further research may change our confidence in the estimate of effect and may change the estimate; (3) low confidence indicated that further research is very likely to have an important impact on the confidence in the estimate of effect and is likely to change the estimate, meaning there is low confidence that the evidence reflects the true effect; and (4) insufficient, indicating that the evidence was unavailable or did not permit a conclusion.

†Borghi 2002 multicomponent diet (high calcium, low protein and low sodium intake) versus control diet (low calcium intake).
‡Hiatt 1996 multicomponent diet (low animal protein and high fiber intake) versus control diet.

Table B. Summary of evidence for prevention of stone recurrence: Pharmacological interventions (KQ 4)		
Interventions, Studies (Study Quality)	Stone Recurrence Results	Strength of Evidence*
Thiazide Diuretic vs. Placebo or Control 7 RCTs (fair) in patients with recurrent calcium stones41,47-50,52,58	Symptomatic: No reduced risk (24 vs. 23%; RR, 1.04 [CI, 0.39 to 2.80], n=1 trial reporting), but reduced risk of lithotripsy (8 vs. 26%, p=0.03, n=1 trial). Composite: Reduced risk (25 vs. 49%; RR, 0.53 [CI, 0.41 to 0.68], n=6 trials). Radiographic: No results reported.	Symptomatic: Low Composite: Moderate Radiographic: Insufficient
Citrate vs. Placebo or Control 6 RCTs (1 good, 5 fair) in patients with recurrent calcium stones43-45,59,63,64	Symptomatic: No results reported. Composite: Reduced risk (11 vs. 52%; RR, 0.25 [CI, 0.14 to 0.44], n=4 trials). Radiographic: No reduced risk (69 vs. 73%; RR, 0.95 [CI, 0.62 to 1.44], n=1 trial).	Symptomatic: Insufficient Composite: Moderate Radiographic: Low
Allopurinol vs. Placebo or Control 4 RCTs (fair) in patients with recurrent calcium stones37-40	Symptomatic: No reduced risk (10 vs. 29%; RR, 0.36 [CI, 0.11 to 1.19], n=1 trial) but increased time to recurrent stone (33 vs. 27 months, p<0.05, n=1 trial). Composite: Reduced risk (33 vs. 55%; RR, 0.59 [CI, 0.42 to 0.84], n=2 trials). Radiographic: No reduced risk (7 vs. 6%; RR, 1.07 [CI, 0.16 to 7.10], n=1 trial).	Symptomatic: Low Composite: Moderate Radiographic: Low
Acetohydroxamic Acid vs. Placebo or Control 3 RCTs (fair) in patients with chronic urea-splitting urinary tract infections and recurrent struvite stones60-62	Symptomatic: No results reported. Composite: No results reported. Radiographic: No reduced risk (13 vs. 20%; RR, 0.81 [CI, 0.18 to 3.66], n=2 trials).	Symptomatic: Insufficient Composite: Insufficient Radiographic: Low
Magnesium vs. Placebo 1 RCT (fair) in patients with recurrent calcium stones47	Symptomatic: No results reported. Composite: No reduced risk (29 vs. 45%; RR, 0.65 [CI, 0.37 to 1.16]). Radiographic: No results reported.	Symptomatic: Insufficient Composite: Low Radiographic: Insufficient
Thiazide Diuretic plus Citrate vs. Thiazide 1 RCT (fair) in patients with recurrent calcium stones58	Symptomatic: No results reported. Composite: No reduced risk (RR, 0.94 [CI, 0.52 to 1.68]). Radiographic: No results reported.	Symptomatic: Insufficient Composite: Low Radiographic: Insufficient
Thiazide Diuretic plus Allopurinol vs. Thiazide 1 RCT (fair) in patients with recurrent calcium stones41	Symptomatic: No results reported. Composite: No reduced risk (RR, 0.79 [CI, 0.18 to 3.49]). Radiographic: No results reported.	Symptomatic: Insufficient Composite: Low Radiographic: Insufficient

CI = 95 percent confidence interval; KQ = Key Question; RCT = randomized controlled trial; RR = relative risk.

*Strength of evidence was rated using the following grades: (1) high confidence indicated that further research is very unlikely to change the confidence in the estimate of effect, meaning that the evidence reflects the true effect; (2) moderate confidence denoted that further research may change our confidence in the estimate of effect and may change the estimate; (3) low confidence indicated that further research is very likely to have an important impact on the confidence in the estimate of effect and is likely to change the estimate, meaning there is low confidence that the evidence reflects the true effect; and (4) insufficient, indicating that the evidence was unavailable or did not permit a conclusion.

References

1. Pearle MS, Calhoun EA, Curhan GC. Urologic diseases in America project: urolithiasis. J Urol. 2005 Mar;173(3):848-57. PMID: 15711292.
2. Stamatelou KK, Francis ME, Jones CA, et al. Time trends in reported prevalence of kidney stones in the United States: 1976-1994. Kidney International. 2003 May;63(5):1817-23. PMID: 12675858.
3. Penniston KL, McLaren ID, Greenlee RT, et al. Urolithiasis in a rural Wisconsin population from 1992 to 2008: narrowing of the male-to-female ratio. J Urol. 2011 May;185(5):1731-6. PMID: 21420112.
4. Scales CD, Jr., Curtis LH, Norris RD, et al. Changing gender prevalence of stone disease. J Urol. 2007 Mar;177(3):979-82. PMID: 17296391.
5. Lieske JC, Pena de la Vega LS, Slezak JM, et al. Renal stone epidemiology in Rochester, Minnesota: an update. Kidney International. 2006 Feb;69(4):760-4. PMID: 16518332.
6. Boyce CJ, Pickhardt PJ, Lawrence EM, et al. Prevalence of urolithiasis in asymptomatic adults: objective determination using low dose noncontrast computerized tomography. J Urol. 2010 Mar;183(3):1017-21. PMID: 20092842.
7. Uribarri J, Oh MS, Carroll HJ. The first kidney stone. Ann Intern Med. 1989 Dec 15;111(12):1006-9. PMID: 2688503.
8. Saigal CS, Joyce G, Timilsina AR. Direct and indirect costs of nephrolithiasis in an employed population: opportunity for disease management? Kidney International. 2005 Oct;68(4):1808-14. PMID: 16164658.
9. Moe OW. Kidney stones: pathophysiology and medical management. Lancet. 2006 Jan 28;367(9507):333-44. PMID: 16443041.
10. Wagner CA, Mohebbi N. Urinary pH and stone formation. J Nephrol. 2010 Nov-Dec;23 Suppl 16:S165-9. PMID: 21170875.
11. Levy FL, Adams-Huet B, Pak CY. Ambulatory evaluation of nephrolithiasis: an update of a 1980 protocol. American Journal of Medicine. 1995 Jan;98(1):50-9. PMID: 7825619.
12. Attanasio M. The genetic components of idiopathic nephrolithiasis. Pediatr Nephrol. 2011 Mar;26(3):337-46. PMID: 20563734.
13. Curhan GC, Willett WC, Knight EL, et al. Dietary factors and the risk of incident kidney stones in younger women: Nurses' Health Study II. Arch Intern Med. 2004 Apr 26;164(8):885-91. PMID: 15111375.
14. Curhan GC, Willett WC, Rimm EB, et al. A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones. New England Journal of Medicine. 1993 Mar 25;328(12):833-8. PMID: 8441427.
15. Curhan GC, Willett WC, Speizer FE, et al. Comparison of dietary calcium with supplemental calcium and other nutrients as factors affecting the risk for kidney stones in women. Ann Intern Med. 1997 Apr 1;126(7):497-504. PMID: 9092314.
16. Taylor EN, Stampfer MJ, Curhan GC. Dietary factors and the risk of incident kidney stones in men: new insights after 14 years of follow-up. J Am Soc Nephrol. 2004 Dec;15(12):3225-32. PMID: 15579526.
17. Taylor EN, Curhan GC. Fructose consumption and the risk of kidney stones. Kidney International. 2008 Jan;73(2):207-12. PMID: 17928824.
18. Mollerup CL, Vestergaard P, Frokjaer VG, et al. Risk of renal stone events in primary hyperparathyroidism before and after parathyroid surgery: controlled retrospective follow up study. BMJ. 2002 Oct 12;325(7368):807. PMID: 12376441.
19. Taylor EN, Stampfer MJ, Curhan GC. Obesity, weight gain, and the risk of kidney stones. JAMA. 2005 Jan 26;293(4):455-62. PMID: 15671430.
20. Taylor EN, Stampfer MJ, Curhan GC. Diabetes mellitus and the risk of nephrolithiasis. Kidney International. 2005 Sep;68(3):1230-5. PMID: 16105055.
21. Kramer HM, Curhan G. The association between gout and nephrolithiasis: the National Health and Nutrition Examination Survey III, 1988-1994. American Journal of Kidney Diseases. 2002 Jul;40(1):37-42. PMID: 12087559.
22. Ciacci C, Spagnuolo G, Tortora R, et al. Urinary stone disease in adults with celiac disease: prevalence, incidence and urinary determinants. J Urol. 2008 Sep;180(3):974-9. PMID: 18639267.
23. Fink HA, Akornor JW, Garimella PS, et al. Diet, fluid, or supplements for secondary prevention of nephrolithiasis: a systematic review and meta-analysis of randomized trials. European Urology. 2009 Jul;56(1):72-80. PMID: 19321253.
24. Pearle MS, Roehrborn CG, Pak CY. Meta-analysis of randomized trials for medical prevention of calcium oxalate nephrolithiasis. Journal of Endourology. 1999 Nov;13(9):679-85. PMID: 10608521.
25. Escribano J, Balaguer A, Pagone F, et al. Pharmacological interventions for preventing complications in idiopathic hypercalciuria. Cochrane Database of Systematic Reviews. 2009(1):CD004754. PMID: 19160242.
26. Kairaitis L, Caring for Australians with Renal I. The CARI guidelines. Kidney stones: prevention of recurrent calcium nephrolithiasis. Nephrology. 2007 Feb;12 Suppl 1:S11-20. PMID: 17316271.
27. Mattle D, Hess B. Preventive treatment of nephrolithiasis with alkali citrate--a critical review. Urological Research. 2005 May;33(2):73-9. PMID: 15875173.
28. Becker G, Caring for Australians with Renal I. The CARI guidelines. Kidney stones: cystine stones. Nephrology. 2007 Feb;12 Suppl 1:S4-10. PMID: 17316277.
29. Becker G, Caring for Australians with Renal I. The CARI guidelines. Kidney stones: uric acid stones. Nephrology. 2007 Feb;12 Suppl 1:S21-5. PMID: 17316272.
30. Turk C KT, Petrik A, Sarica K, Straub M, Steitz C. Guidelines on Urolithiasis. March 2011. www.uroweb.org/professional-resources/guidelines/.
31. Schulz KF, Grimes DA. Allocation concealment in randomised trials: defending against deciphering. Lancet. 2002 Feb 16;359(9306):614-8. PMID: 11867132.

32. Higgins JP, Green S. Cochrane Handbook for Systematic Reviews of Interventions, V. 5.1.0. In: Collaboration TC, editor. 5.1.0 ed; 2011.
33. Santaguida P.R. PR. McMaster Quality Assessment Scale of Harms (McHarm) for primary studies: manual for use of the McHarm. Hamilton, Canada: McMaster University; 2011.
34. RevMan RMcpV. Copenhagen:The Nordic Cochrance Centre of the Cochrane Collaboration. 2008.
35. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. BMJ. 2003 Sep 6;327(7414):557-60. PMID: 12958120.
36. Owens DK, Lohr KN, Atkins D, et al. AHRQ series paper 5: grading the strength of a body of evidence when comparing medical interventions--Agency for Healthcare Research and Quality and the Effective Health Care Program. Journal of Clinical Epidemiology. 2010 May;63(5):513-23. PMID: 19595577.
37. Ettinger B, Tang A, Citron JT, et al. Randomized trial of allopurinol in the prevention of calcium oxalate calculi. New England Journal of Medicine. 1986 Nov 27;315(22):1386-9. PMID: 3534570.
38. Smith MJ. Placebo versus allopurinol for renal calculi. Journal of Urology. 1977 Jun;117(6):690-2. PMID: 875139.
39. Robertson WG PM, Sepby PL, Williams RE, Clark P, Chisholm GD. A multicentre trial to evaluate three treatments for recurrent idiopathic calcium stone disease - a preliminary report. Plenum Press. 1986.
40. Miano L, Petta S, Galatioto GP, et al. A placebo controlled double-blind study of allopurinol in severe recurrent idiopathic renal lithiasis. In: Schwille PO, Smith LH, Robertson WG, Vahlensieck W, eds. Urolithiasis and Related Clinical Research. New York, Plenum Press; 1985:521-4.
41. Borghi L, Meschi T, Guerra A, et al. Randomized prospective study of a nonthiazide diuretic, indapamide, in preventing calcium stone recurrences. Journal of Cardiovascular Pharmacology. 1993;22 Suppl 6:S78-86. PMID: 7508066.
42. Sarica K, Inal Y, Erturhan S, et al. The effect of calcium channel blockers on stone regrowth and recurrence after shock wave lithotripsy. Urological Research. 2006 Jun;34(3):184-9. PMID: 16463053.
43. Barcelo P, Wuhl O, Servitge E, et al. Randomized double-blind study of potassium citrate in idiopathic hypocitraturic calcium nephrolithiasis. Journal of Urology. 1993 Dec;150(6):1761-4. PMID: 8230497.
44. Ettinger B, Pak CY, Citron JT, et al. Potassium-magnesium citrate is an effective prophylaxis against recurrent calcium oxalate nephrolithiasis. Journal of Urology. 1997 Dec;158(6):2069-73. PMID: 9366314.
45. Soygur T, Akbay A, Kupeli S. Effect of potassium citrate therapy on stone recurrence and residual fragments after shockwave lithotripsy in lower caliceal calcium oxalate urolithiasis: a randomized controlled trial. Journal of Endourology. 2002 Apr;16(3):149-52. PMID: 12028622.
46. Borghi L, Meschi T, Amato F, et al. Urinary volume, water and recurrences in idiopathic calcium nephrolithiasis: a 5-year randomized prospective study. Journal of Urology. 1996 Mar;155(3):839-43. PMID: 8583588.
47. Ettinger B, Citron JT, Livermore B, et al. Chlorthalidone reduces calcium oxalate calculous recurrence but magnesium hydroxide does not. Journal of Urology. 1988 Apr;139(4):679-84. PMID: 3280829.
48. Ala-Opas M, Elomaa I, Porkka L, et al. Unprocessed bran and intermittent thiazide therapy in prevention of recurrent urinary calcium stones. Scandinavian Journal of Urology & Nephrology. 1987;21(4):311-4. PMID: 2832935.
49. Laerum E, Larsen S. Thiazide prophylaxis of urolithiasis. A double-blind study in general practice. Acta Medica Scandinavica. 1984;215(4):383-9. PMID: 6375276.
50. Ahlstrand, ed. Prophylactic treatment of calcium stone formers with hydrochlorothiazide and magnesium. 1995; Edsbruk. Akademitryck AB.
51. Borghi L, Schianchi T, Meschi T, et al. Comparison of two diets for the prevention of recurrent stones in idiopathic hypercalciuria. New England Journal of Medicine. 2002 Jan 10;346(2):77-84. PMID: 11784873.
52. Scholz D, Schwille PO, Sigel A. Double-blind study with thiazide in recurrent calcium lithiasis. J Urol; 1982. p. 903-7.
53. Kocvara R, Plasgura P, Petrik A, et al. A prospective study of nonmedical prophylaxis after a first kidney stone. BJU International. 1999 Sep;84(4):393-8. PMID: 10468751.
54. Di Silverio F, Ricciuti GP, D'Angelo AR, et al. Stone recurrence after lithotripsy in patients with recurrent idiopathic calcium urolithiasis: Efficacy of treatment with Fiuggi water. European Urology; 2000. p. 145-8.
55. Shuster J, Jenkins A, Logan C, et al. Soft drink consumption and urinary stone recurrence: a randomized prevention trial. Journal of Clinical Epidemiology. 1992 Aug;45(8):911-6. PMID: 1624973.
56. Dussol B, Iovanna C, Rotily M, et al. A randomized trial of low-animal-protein or high-fiber diets for secondary prevention of calcium nephrolithiasis. Nephron. 2008;110(3):c185-94. PMID: 18957869.
57. Hiatt RA, Ettinger B, Caan B, et al. Randomized controlled trial of a low animal protein, high fiber diet in the prevention of recurrent calcium oxalate kidney stones. American Journal of Epidemiology. 1996 Jul 1;144(1):25-33. PMID: 8659482.
58. Fernández-Rodríguez A, Arrabal-Martín M, García-Ruiz MJ, et al. The role of thiazides in the prophylaxis of recurrent calcium lithiasis. Actas urologicas espanolas; 2006. p. 305-9.
59. Hofbauer J, Hobarth K, Szabo N, et al. Alkali citrate prophylaxis in idiopathic recurrent calcium oxalate urolithiasis--a prospective randomized study. British Journal of Urology. 1994 Apr;73(4):362-5. PMID: 8199822.
60. Griffith DP, Khonsari F, Skurnick JH, et al. A randomized trial of acetohydroxamic acid for the treatment and prevention of infection-induced urinary stones in spinal cord injury patients. Journal of Urology. 1988 Aug;140(2):318-24. PMID: 3294442.
61. Griffith DP, Gleeson MJ, Lee H, et al. Randomized, double-blind trial of Lithostat (acetohydroxamic acid) in the palliative treatment of infection-induced urinary calculi. European Urology. 1991;20(3):243-7. PMID: 1726639.

62. Williams JJ, Rodman JS, Peterson CM. A randomized double-blind study of acetohydroxamic acid in struvite nephrolithiasis. New England Journal of Medicine. 1984 Sep 20;311(12):760-4. PMID: 6472365.
63. Premgamone A, Sriboonlue P, Disatapornjaroen W, et al. A long-term study on the efficacy of a herbal plant, Orthosiphon grandiflorus, and sodium potassium citrate in renal calculi treatment. Southeast Asian Journal of Tropical Medicine & Public Health. 2001 Sep;32(3):654-60. PMID: 11944733.
64. Lojanapiwat B, Tanthanuch M, Pripathanont C, et al. Alkaline citrate reduces stone recurrence and regrowth after shockwave lithotripsy and percutaneous nephrolithotomy. International Braz J Urol. 2011 Sep-Oct;37(5):611-6. PMID: 22099273.
65. Trochim W. Regression to the Mean. The Research Knowledge Base. In: Methods SR, editor. 2nd Edition ed; 2006.
66. Curhan GC, Willett WC, Speizer FE, et al. Twenty-four-hour urine chemistries and the risk of kidney stones among women and men. Kidney International. 2001 Jun;59(6):2290-8. PMID: 11380833.

Case Report: Kidney Stone and Low Bone Mass

60. year old woman seen in the University of Chicago Endocrine Bone Clinic in June, 2010
CC: low bone mass

1994 - rib fracture with coughing
2002 - right metatarsal fracture in a fall
Stable height and posture

2004 – LMP and onset of symptomatic menopause. Estrogen replacement 2005-2007
Calcium intake from foods plus Calcium tablets = 3,250 mg daily
Vitamin D intake 1,000 unit tablet; calcium supplements = 2,300 units daily

DEXA Scans: BMDs	2006	2008	2010	2012
Lumbar spine L1-L4	1.103	1.068 (-3.2%)	1.020 (-4.5%)	0.989 (-3.0%)
Mean total hip	0.947	0.922 (-2.6%)	0.878 (-4.8%)	0.851 (-3.1%)

2003 passed a kidney stone primarily composed of calcium but no further details.
No family history of osteoporosis or kidney stones.

Laboratory Tests:
Serum

Date	sCa	sPhos	sMg	sCreat
7/25/12	9.50	3.42	2.25	0.78

Urine

Date	Vol	Ca mg/24 hr	Ca Ca/Kg	Ca Ca/Cr	Cit	UNa	
7/25/12	2.69	153	2.53	200.7	604	32	
7/13/11	2.71	152	2.51	172.8	705	24	
12/28/10	3.10	261	4.35	229.5	1046	65	
9/1/10	2.61	201	3.40	212.1	855	94	
6/8/10	2.66	335	5.53	300.7	754	181	

Questions/Comments:
1. What is the cause of the bone loss?
2. How are the bone loss and hypercalciuria related?
3. Discuss the reduction in urine calcium with low salt diet

Sodium and Bone Health: Impact of Moderately High and Low Salt Intakes on Calcium Metabolism in Postmenopausal Women

Birgit Teucher, Jack R Dainty, Caroline A Spinks, Gosia Majsak-Newman, David J Berry, Jurian A Hoogewerff, Robert J Foxall, Jette Jakobsen, Kevin D Cashman, Albert Flynn and Susan J Fairweather-Tait

ABSTRACT: High salt intake is a well-recognized risk factor for osteoporosis because it induces calciuria, but the effects of salt on calcium metabolism and the potential impact on bone health in postmenopausal women have not been fully characterized. This study investigated adaptive mechanisms in response to changes in salt and calcium intake in postmenopausal women. Eleven women completed a randomized cross-over trial consisting of four successive 5-wk periods of controlled dietary intervention, each separated by a minimum 4-wk washout. Moderately low and high calcium (518 versus 1284 mg) and salt (3.9 versus 11.2 g) diets, reflecting lower and upper intakes in postmenopausal women consuming a Western-style diet, were provided. Stable isotope labeling techniques were used to measure calcium absorption and excretion, compartmental modeling was undertaken to estimate bone calcium balance, and biomarkers of bone formation and resorption were measured in blood and urine. Moderately high salt intake (11.2 g/d) elicited a significant increase in urinary calcium excretion (p _ 0.0008) and significantly affected bone calcium balance with the high calcium diet (p _ 0.024). Efficiency of calcium absorption was higher after a period of moderately low calcium intake ($p < 0.05$) but was unaffected by salt intake. Salt was responsible for a significant change in bone calcium balance, from positive to negative, when consumed as part of a high calcium diet, but with a low calcium intake, the bone calcium balance was negative on both high and low salt diets. **J Bone Miner Res 2008;23:1477–1485. Published online on April 14, 2008; doi: 10.1359/JBMR.080408**

Table 1. Mean (±SD) Urinary Excretion of Sodium, Calcium, Phosphorus, and Potassium According to Dietary Intervention

	Sodium (mg/d)	*Calcium (mg/d)*	*Phosphorus (mg/d)*	*Potassium (mg/d)*
Habitual diet	2686 (818)*	164 (91)*	721 (171)*	2830 (889)*
−Ca−Na diet	1388 (205)[a]	123 (80)[†]	678 (100)[‡]	2898 (551)
+Ca−Na diet	1415 (109)[a]	159 (87)	585 (131)	2944 (537)
−Ca+Na diet	3728 (618)[b]	141 (79)[†§]	698 (116)[‡]	2969 (618)
+Ca+Na diet	3828 (241)[b]	192 (102)[§]	607 (124)	2982 (430)

* These values are descriptive only and were not included in the statistical analysis; habitual diet was not controlled and can therefore not be compared with the controlled intervention diet.

† Calcium is the model variable responsible for the significantly different calcium excretion on low compared with high calcium diets ($p < 0.0001$).

‡ Calcium is the model variable responsible for the significantly higher phosphorus excretion during low compared with high calcium diets ($p < 0.001$).

§ Sodium is the model variable responsible for the significantly different calcium excretion on high sodium diets compared with low calcium diets ($p < 0.0001$).

[a,b] Sodium is the model variable responsible for differences in sodium excretion; values with different superscripts are significantly different from each other; in all cases ($p < 0.0001$).

Table 2. Summary of calcium kinetic data for the Low and High Ca diets

Diet	V_a	V_u	V_f	V_F	V_{O+}	V_{O-}	V_{bal}
Low Ca diet							
Low salt	140 (31)	102 (71)	112 (133)	542 (125)	455 (129)	565 (156)	−71 (93)
High salt	122 (24)	123 (81)	115 (57)	501 (38)	469 (155)	585 (185)	−115 (70)
High Ca diet							
Low salt	284 (41)	112 (143)*	82 (168)	1078 (83)	437 (139)	346 (88)	90 (46)[†]
High salt	287 (78)	153 (139)*	146 (111)	1110 (72)	361 (144)	372 (121)	−12 (84)[†]

All data are mean (SD) in units of milligrams calcium per day. V_a is the rate of calcium absorbed from the diet, V_u is the rate of urinary calcium excretion, V_f is the rate of endogenous calcium loss in feces, V_F is the rate of dietary calcium loss in feces, V_{O+} is the rate of calcium deposited in the bone, and V_{O-} is the rate of calcium resorbed from bone.

* Significant difference ($p = 0.0008$) in urinary Ca excretion.

† Significant difference ($p = 0.0240$) in Ca bone balance.

Table 3. Biochemical Markers of Bone Resorption (NTX, Dpyr, Pyr) and Bone Formation (OC, B-ALP) After Each Dietary Intervention [Mean (±SD)]

	NTX (nM BCE/d)	*Dpyr (nmol/d)*	*Pyr (nmol/d)*	*OC (ng/ml)*	*B-ALP (U/liter)*
−Ca−Na diet	578 (185)*†	193 (46)[§]	350 (115)[††]	32.8 (12.5)	28.3 (8.9)
+Ca−Na diet	378 (152)[†]	144 (46)[¶]	295 (103)[‡‡]	30.8 (11.5)	25.5 (7.5)
−Ca+Na diet	550 (260)[‡]	199 (51)**	358 (138)[§§]	31.1 (14.7)	27.6 (10.4)
+Ca+Na diet	445 (234)	171 (35)	341 (102)[¶¶]	30.7 (15.7)	28.8 (12.6)

*† $p = 0.0017$; †† $p = 0.0077$; all others NS.

§¶ $p = 0.0009$; ¶** $p = 0.002$; all others NS.

††‡‡ $p = 0.0001$; ‡‡§§ $p = 0.0039$; §§¶¶ $p = 0.001$; all others NS.

NTX, urinary type 1 collagen cross-linked-N-telopeptides (marker of bone resorption); Dpyr, urinary deoxypyridinoline (marker of bone resorption); Pyr, urinary pyridinoline (marker of bone resorption); OC, serum osteocalcin (marker or bone formation); B-ALP, bone alkaline phosphatase (marker of bone formation).

Screening for Osteoporosis

Susan Greenspan, M.D.

Screening for Osteoporosis

Susan L. Greenspan, MD
Professor of Medicine
University of Pittsburgh

ASBMR October 2012

Significance of Topic

- 50% of women and 20% of men have an osteoporotic fracture after age 50
- 2 million fractures annually
- 350,000 hip fractures with mortality of 20%
- Cost -- $19 billion annually

Significance Continued

Bone mineral density (BMD)

- Is the best early predictor of fracture risk
- Correlates with fracture risk
  - Better than cholesterol correlates with heart attack
  - Similar to blood pressure for predicting stroke

Learning Objectives

As a result of participating in this session, attendees should be able to:

- Know screening guidelines for osteoporosis
- Understand strategies available for osteoporosis screening and treatment
- Consider approach for rescreening patients

Case

- History: 67 year old woman
  - Slipped on sidewalk 5 years ago and fractured her ankle
  - Her PCP started her on calcium, vitamin D and once weekly bisphosphonate
  - Her PCP has moved and she comes to you for follow-up
  - She had a heel ultrasound at the pharmacy and was told she was "normal"
  - She stopped therapy – concerned her "jaw would fall apart"

Questions

- What does the patient have?
  - Osteoporosis
  - Low bone mass/osteopenia
  - Normal
  - Don't know
- What should we do?
  - Calcium/vitamin D
  - Anti-osteoporosis medications
  - Further testing

NOF Guidelines for Pharmacologic Therapy in Postmenopausal Women and in Men Aged ≥50

Fracture	A vertebral or hip fracture
T-score	T-score ≤-2.5 at femoral neck or spine
FRAX® assessment If T-score is between -1.0 and -2.5	10-year probability of a major fracture ≥20% 10-year probability of a hip fracture ≥3%

National Osteoporosis Foundation. *Clinician's Guide to Prevention and Treatment of Osteoporosis.* 2008.

7

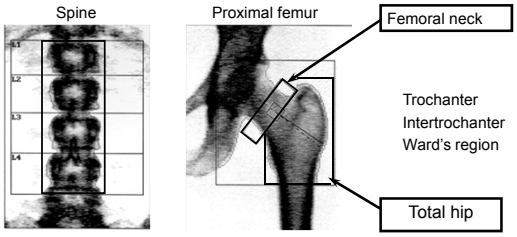
Who Should Have a Bone Density Test?

- Screening
  - All women age 65 and older[1,2]
  - All men age 70 and older[1]
- Test younger postmenopausal women and men age 50-69[1]
  - Fracture after age 50
  - Risk factors for osteoporosis*
- Monitoring
  - Treatment effect or untreated patient in whom evidence of bone loss would lead to treatment

*Risk factors to consider include family history of osteoporosis, low body weight, smoking, premature menopause, other diseases and medications

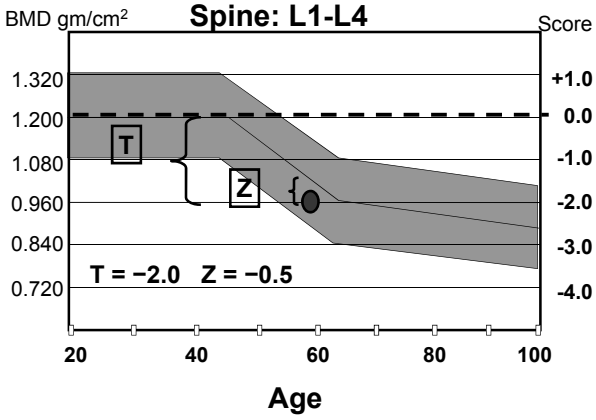
[1]NOF Clinician's Guide to Prevention and Treatment of Osteoporosis 2010. www.nof.org.
[2]US Preventive Services Task Force. *Ann Intern Med.* 2011;154:356-64.

DXA Measurements of Spine and Hip



To classify patient, use the lowest value of either:

- Spine (L1-L4)
- Femoral neck
- Total hip



10

DXA-Based Diagnosis of Osteoporosis: Premenopausal Women and Men Aged < 50

- Z-scores <-2.0 defined as low bone mineral density for chronological age or below the expected range for age

Peripheral Sites

ADVANTAGES

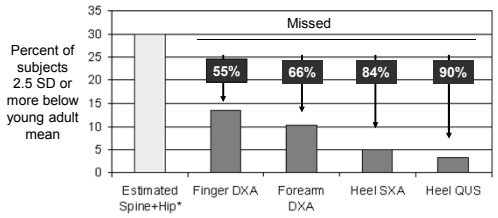
- Portable
- Less expensive than central DXA
- Ultrasound does not involve radiation

LIMITATIONS

- Cannot be used for diagnosis with WHO criteria
- Cannot be used for monitoring (sites less likely to change)
- 30% false negatives

NORA Study

200,160 ambulatory women age 50 and older



*Estimated from NHANES III

Siris E, et al. *JAMA*. 2001;286:2815-22.

Case Continued

- Bone mineral density test:
  - Spine T-score: -2.3
  - Hip T-score: -1.7
  - Femoral neck T-score: -1.9

Questions

- What does the patient have?
  - Osteoporosis
  - Low bone mass/osteopenia
  - Normal
  - Don't know
- What should we do?
  - Calcium/vitamin D
  - Anti-osteoporosis medications
  - Further testing

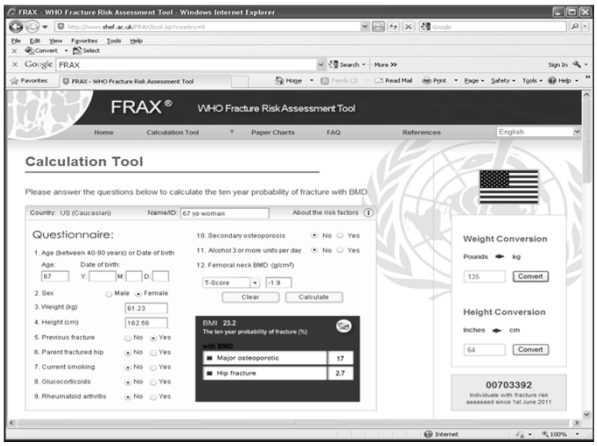
FRAX®

- Statistically robust fracture risk prediction tool developed by WHO for worldwide use
- Combines BMD and clinical risk factors to predict fracture risk better than either alone
- Predicts the 10-year probability of
  - Major osteoporotic fracture (hip, spine, wrist, or humerus)
  - Hip fracture

FRAX Risk Factors

- Age
- Gender
- BMI
- Previous fracture (adult)
- Parent fractured hip
- Current smoking
- Glucocorticoids (>3 months, ≥prednisone 5mg daily)
- Rheumatoid arthritis (confirmed)
- Secondary: type I (insulin dependent) diabetes, osteogenesis imperfecta in adults, untreated long-standing hyperthyroidism, hypogonadism or premature menopause (<45 years), chronic malnutrition, or malabsorption and chronic liver disease
- Alcohol 3 or more units/d

Kanis JA. 2002: 359; 1929-1936



Case Continued

- FRAX score:
  - 10 year major osteoporotic risk: 17%
  - 10 year hip fracture risk: 2.7%

Question

- Should we treat her?
  - Yes
  - No
  - Don't know

FRAX Clinical Statements: Underestimates Risk

- Impaired functional status
- Smoking dose/duration
- Falls
- Multiple fractures
- Severity of vertebral fracture
- Parental history of non-hip fracture
- Glucocorticoids dose/duration
- Inhaled glucocorticoids

ISCD 2010 Position Statement on FRAX

FRAX BMD Statements

- Only femoral neck BMD or T-score recommended
- FRAX under/over estimate risk when spine T-score lower or higher (>1 SD discrepancy) than femoral neck T-score
- FRAX with BMD predicts risk better than clinical risk factors alone
- Cannot be used for monitoring Rx response
- Variability of hip fracture rates worldwide

ISCD 2010 Position Statement on FRAX

Question

- Are we missing anything else?

Case Continued

- Clinical history
  - Height loss of 1.5-2 inches

Measure Height Yearly in Women and Men ≥ 50

Order spine imaging if low bone mass and:

- Kyphosis
- Height loss from peak (>1.6" in women; >2.4" in men)
- Prospective height loss (>0.8" in women; >1.2" in men)[1]

Vertebral Fracture Assessment (VFA) by DXA

or

Lateral spine x-rays

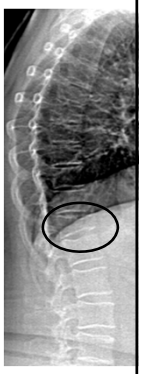


Image courtesy of M Lewiecki

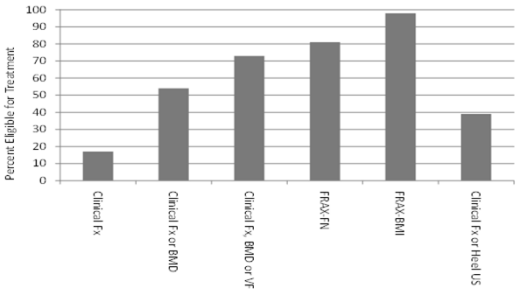
[1]Adapted from ISCD Position Statements. www.iscd.org.

Case continued

- Lateral vertebral x-rays demonstrate a T10 moderate vertebral compression fracture

FRAX or Fiction: How to Screen for Rx

180 long term care residents over age 65
46% with vertebral fractures; 74% silent, missed by FRAX



Greenspan SL, JAGS, 2012

Screening Bone Mass Measurement Costs

Technique	Radiation (mRem)	Cost
DXA spine/hip	10	~$60
DXA forearm	5	~$30
QCT spine	1000	~$200
Heel ultrasound	0	N/A
Vertebral Fracture Assessment (DXA)	35	~$30
Thoracic/lumbar spine x-rays	600-759	~$40

Question

- Are we missing anything else?

Common Medical Causes of Bone Loss, Osteoporosis, and Fractures

Diseases	Conditions	Drugs
Hypogonadism (premature)	Anorexia	Glucocorticoids
Diabetes	Alcoholism	Excess thyroxine
Depression	Hypercalciuria	Antiepileptics
COPD	Vitamin D deficiency	Depo-Provera
GI diseases (malabsorption)		Androgen deprivation therapy (ADT)
Rheumatoid arthritis		Aromatase inhibitors
Cholestatic liver disease		SSRIs
Hyperthyroidism		Proton pump inhibitors
Hyperparathyroidism		

Evaluation of the Patient With Osteoporosis

Careful history and examination

- Laboratory testing
  - Chemistry (Ca, Phos, Cr, Alk Phos, LFTs, protein)
  - CBC
  - TFTs if symptoms, elderly, or on T4
  - 24-hour urine Ca (and Cr)
  - **25-hydroxy vitamin D**

Identified 92% of new diagnoses at cost of $56-$79 per patient (Medicare rates)

Data reanalyzed from Tannenbaum C, et al. *J Clin Endocrinol Metab*. 2002;87:4431-7 using current definition of vitamin D deficiency (personal communication: Luckey MM).

Case continued

- Work-up for secondary causes:
  - 25 hydroxy vitamin D 21 ng/dL
  - 24 hour urinary calcium 125 mg/24 hours

Follow-up Testing Interval and Transition to Osteoporosis

•4957 women >67 years (Study of Osteoporotic Fractures cohort)
•Baseline femoral neck or total hip T-score (lowest used)
•No hip or vertebral fractures
•No osteoporosis treatment
•Followed 15 years
•Primary outcome: time for 10% patients transition to osteoporosis

Baseline BMD	T-score	Interval Between Baseline and Osteoporosis (years)	95% CI
Normal	>-1.0	16.8	11.5-24.6
Mild osteopenia	-1.01 to -1.49	17.3	13.9-21.5
Moderate osteopenia	-1.50 to -1.99	4.7	4.2-5.2
Advanced osteopenia	-2.00 to -2.49	1.1	1-1.3

Gourlay MG, NEJM, 2012

Follow-up Testing continued

- Limitations of study
  - No adjustment for risk factors
  - No adjustment of spine T-score
  - 50% SOF women excluded from analysis (baseline osteoporosis, hip or clinical vertebral fracture, received Rx, too few DXAs for longitudinal f/u
  - SOF cohort mainly white
- Strengths: long f/u, large cohort, repeated BMD

Question

- Could we use FRAX if she was on therapy for osteoporosis?
  - Yes
  - No
  - Don't know

Does Therapy Invalidate FRAX?

- Manitoba Bone Density Program
  - Retrospectively calculated FRAX
  - 37,000 women >50 years with BMD
  - F/U 5.3 years
- Results:
  - No Rx: predicted and observed Fx rate concurrent
  - Rx
    - Major osteoporotic fracture: concurrent
    - Hip fracture: rate 39% lower (similar to alendronate trial)

Leslie WD , JBMR, 2012

Use of FRAX When Patients on Therapy

- When you don't have past information to calculate a FRAX before therapy
  - If patient does not meet criteria, helps decision to stop therapy
- May help decision regarding bisphosphonate holiday

Back to Patient

- To treat or not to treat…
  - 67 years old
  - ~4 years bisphosphonate therapy
  - Vertebral compression and ankle fractures
  - Low vitamin D level (to be treated with vitamin D 50,000 units once weekly for 3 months)

38

Cost-effectiveness of Screening Strategies for Osteoporosis

- Model: cost-effectiveness model, over 100 different screening strategies modeled
  - Instruments: DXA, ultrasound, questionnaire SCORE (Simple Calculated Osteoporosis Risk Estimation)
  - Screening initiation for postmenopausal women at ages 55, 60, 65, 70, 75,and 80 years
  - Treatment: oral bisphosphonate cost $108/yr, 50% adherence, 5 year on/off treatment plan
  - Outcome: hip, spine or wrist fracture

Nayak S, Roberts M, Greenspan S, AIM, 2011

Cost-effective of Screening Continued

- Screening superior to not screening at all ages
- Best strategy at all ages (including 55 and older) was DXA with T≤-2.5 (FN or spine) for Rx with 5 year f/u
  - Incremental cost-effectiveness ratio of less than $50,000 per quality-adjusted life-year (QALY)
- Other strategies also cost-effective

Summary

- Screening:
  - Women over 65, men over 70 (NOF guidelines)
- Height loss: check for vertebral fractures
- Peripheral/QCT misses osteoporosis
- Screening/Treatment
  - T-score $\leq$-2.5 spine, total hip, femoral neck
  - Hip or vertebral fracture
  - FRAX score

Summary continued

- Follow-up screening (patients with no hip/vertebral factures, no treatment)
  - Normal to mild osteopenia: ~15 years
  - Moderate osteopenia: ~5 years
  - Advanced osteopenia/osteoporosis: 1-2 years
- Screening cost-effective in women age 55 and older

Case #2: Would You Screen This Patient?

- 35-year-old premenopausal woman
  - Regular menstrual periods
  - Low diet in calcium
  - Avoids the sun, fair skin
  - Sedentary, little exercise
  - Significant family history: mom fractured hip age 65
  - Fractured ankle playing soccer in junior high school
  - No height loss
  - BMI 30

Case #3: Would You Screen This Patient?

- 55-year-old UPS delivery man
  - Prostate cancer on androgen deprivation therapy for 2 years
  - Diet low in calcium
  - Outside on job and plays golf
  - Negative family history
  - Height loss ~ 1 inch
  - No fractures

Case #4: Would You Screen This Patient?

- 81-year-old woman resides in long term care facility
  - Walks with walker
  - Takes 1 calcium supplement and multivitamin for years
  - Has fallen and fractured wrist in past
  - Height loss of 2 inches
  - Other medical problems include: HBP, CVD, DM, osteoarthritis, anemia, hypothyroidism

Case #5: Would You Screen This Patient?

- 60 year old postmenopausal women with long history of asthma
  - Current exacerbation treated with prednisone, now reduced to 5 mg daily at 3 months
  - Difficulty weaning prednisone
  - Takes calcium supplement and vitamin D 2000 IU/day
  - Walks or goes to gym for 45 minutes, 4-5 times per week
  - Height loss ~1.5 inches
  - Fractured toe during summer wearing sandals

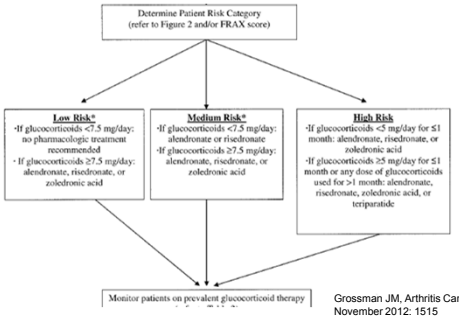
American College of Rheumatology 2010 GIOP Guidelines for Screening/Treatment

Risk Category	Risk of Major Osteoporotic Fracture using FRAX*
Low	< 10%
Medium	10-20%
High	> 20%

*FRAX: Enter yes if the patient is currently exposed to oral glucocorticoids or has been exposed to oral glucocorticoids for more than 3 months at a dose of prednisone of 5mg daily or more (or equivalent doses of other glucocorticoids)

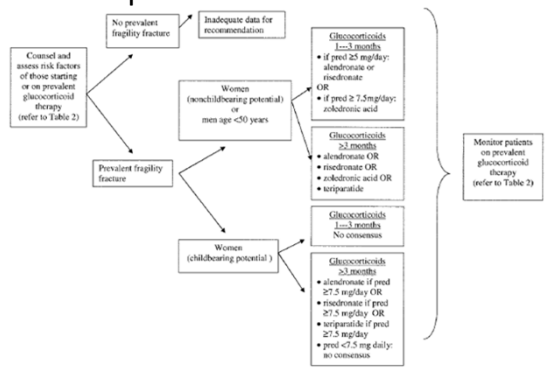
Grossman JM, Arthritis Care & Rheumatism, November 2012: 1515

American College of Rheumatology 2010 GIOP Guidelines for Screening/Treatment



Grossman JM, Arthritis Care & Rheumatism, November 2012: 1515

ACR 2010 GIOP Guidelines for Premenopausal Women and Men < 50



References

(1) National Osteoporosis Foundation. National Osteoporosis Foundation. Clinician's Guide to Prevention and Treatment of Osteoporosis . Washington, DC: Copyright NOF; 2008.

(2) U.S.Preventive Services Task Force. Screening for osteoporosis : US preventive services task force recommendation statement. Ann Intern Med 2011;154(356):364.

(3) Siris ES, Miller PD, Barrett-Connor E, Faulkner KG, Wehren LE, Abbott TA, et al. Identification and fracture outcomes of undiagnosed low bone mineral density in postmenopausal women. JAMA 2001;286(22):2815-22.

(4) Kanis JA. Diagnosis of osteoporosis and assessment of fracture risk. Lancet 2003;359:1929-36.

(5) International Society for Clinical Densitometry, International Osteoporosis Foundation. 2010 Official Positions on FRAX. 2010. (pamphlet)

(6) Greenspan SL, Perera S, Nace D, Zukowski KS, Ferchak M, Lee CJ, et al. FRAX or Fiction: Determining optimal screening strategies for treatment of osteoporosis in residents in long-term care facilities. J Am Geriatr Soc 2012;60(4):684-90.

(7) Tannenbaum C, Clark J, Schwartzman K, Wallenstein S, Lapinski R, Meier D, et al. Yield of laboratory testing to identify secondary contributors to osteoporosis in otherwise healthy women. J Clin Endocrinol Metab 2002;87:4431-7.

(8) Gourlay ML, Fine JP, Preisser JS, May RC, Li C, Lui L-Y, et al. Bone density testing interval and transition to osteoporosis in older women. N Engl J Med 2012;366(3):225-33.

(9) Leslie WD, Lix LM, Johansson H, Oden A, McCloskey E, Kanis JA, et al. Does osteoporosis therapy invalidate FRAX for fracture prediction? J Bone Min Res 2012;27(6):1243-51.

(10) Nayak S, Roberts MS, Greenspan SL. Cost-effectiveness of different screening strategies for osteoporosis in postmenopausal women. Ann Intern Med 2011;155(11):751-61.

(11) Grossman JM, Gordon R, Ranganath VK, Deal C, Caplan L, Chen W, et al. American college of rheumatology 2010 recommendations for the prevention and treatment of glucocorticoid-induced osteoporosis. Arthritis Care & Research 2010;62(11):1515-26

50

Subchondral Bone and Osteoarthritis

David Burr, Ph.D.

Subchondral Bone and Osteoarthritis

David B Burr, PhD
Indiana University School of Medicine
Indianapolis, IN, USA

Significance of the Topic

Osteoarthritis (OA) is characterized by progressive alterations of joint structure involving articular cartilage degeneration, subchondral bone remodeling, increased subchondral bone density, and inflammation of the joint. Initiation of cartilage damage, which occurs with age in nearly everyone, does not inevitably progress to full thickness cartilage loss, or clinically symptomatic OA. Therefore, prevention of the progression of cartilage loss and the developing subchondral sclerosis, rather than simply prevention of initiation, is the critical step in controlling OA.

The classical view of the pathogenesis of OA is that subchondral sclerosis is associated with, and perhaps causes age-related joint degeneration. But recent observations have demonstrated that OA is associated with early loss of bone owing to increased bone remodelling, followed by slow turnover leading to densification of the subchondral plate and complete loss of cartilage. Subchondral densification is a late event in OA that involves only the subchondral plate and calcified cartilage; the subchondral cancellous bone beneath the plate may remain osteopenic. In experimental models, inducing subchondral sclerosis without allowing the prior stage of increased bone remodelling to occur does not lead to progressive OA. Therefore, both early-stage increased remodelling and bone loss, and the late-stage deceleration of remodelling and subchondral densification are important components of the pathogenic process that leads to OA. The apparent paradoxical observations that OA is associated with both increased remodelling and osteopenia, as well as decreased remodelling and sclerosis, are consistent when the spatial and temporal differences that occur during joint degeneration are taken into consideration.

Treatments to prevent or reverse joint degeneration are not available currently. To identify viable treatments, it is necessary understand the progression of disease. This is particularly true for OA, which is multi-phasic, involves different tissues that respond very differently to mechanical factors and to therapeutic/pharmaceutical treatments, and which is likely to be pathogenically diverse.

Learning Objectives

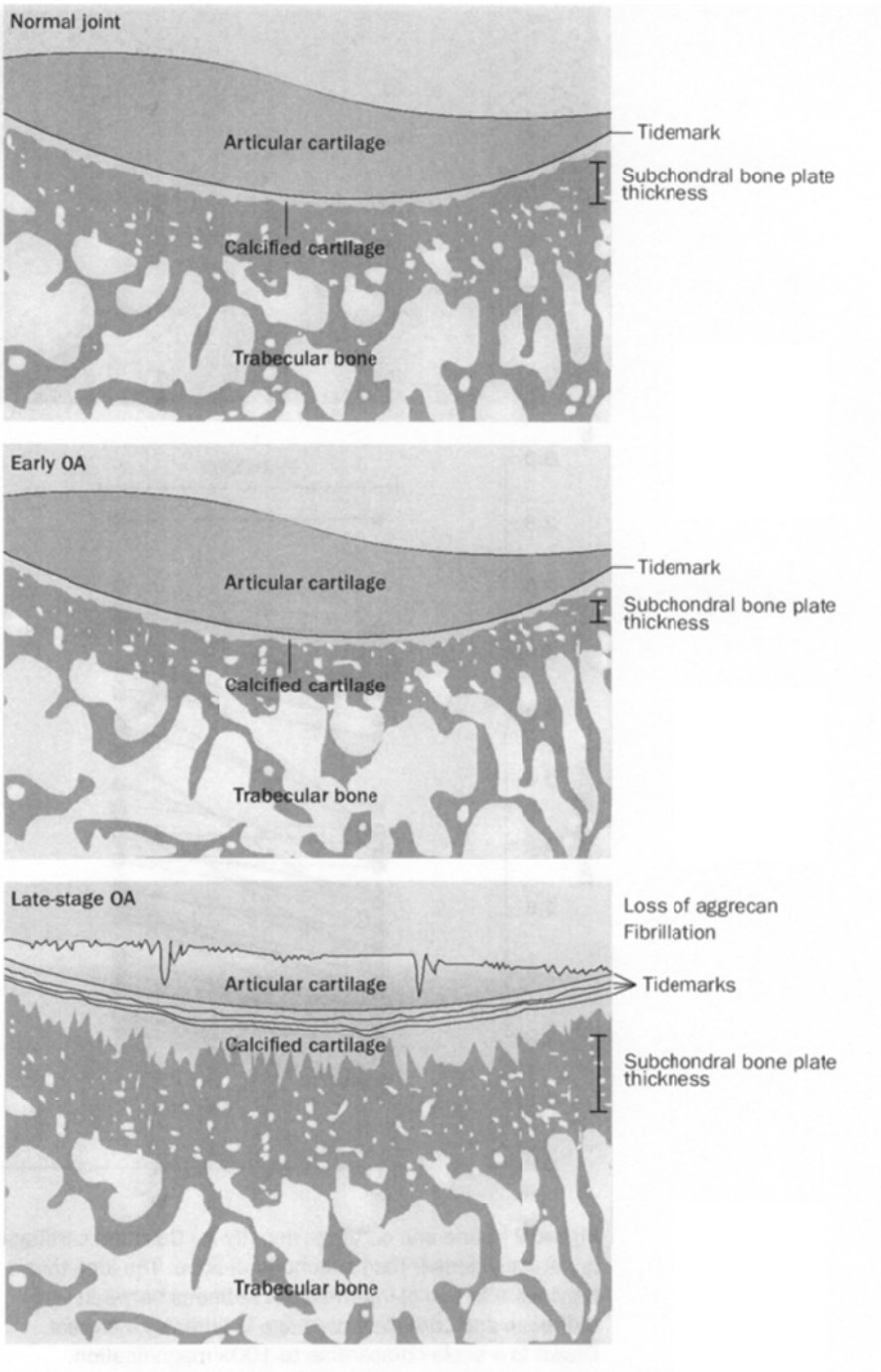
1. As a result of participating in this session, attendees should be able to:
2. Define the different phases of developing osteoarthritis (OA)
3. Define the role that the subchondral mineralized tissues play in the progression of OA
4. Identify the architectural, physiological and mechanical differences between subchondral cortical bone and subchondral cancellous bone, and their roles in the pathogenesis of OA
5. Discuss the potential value of various bone-targeting agents for the prevention of progressive OA

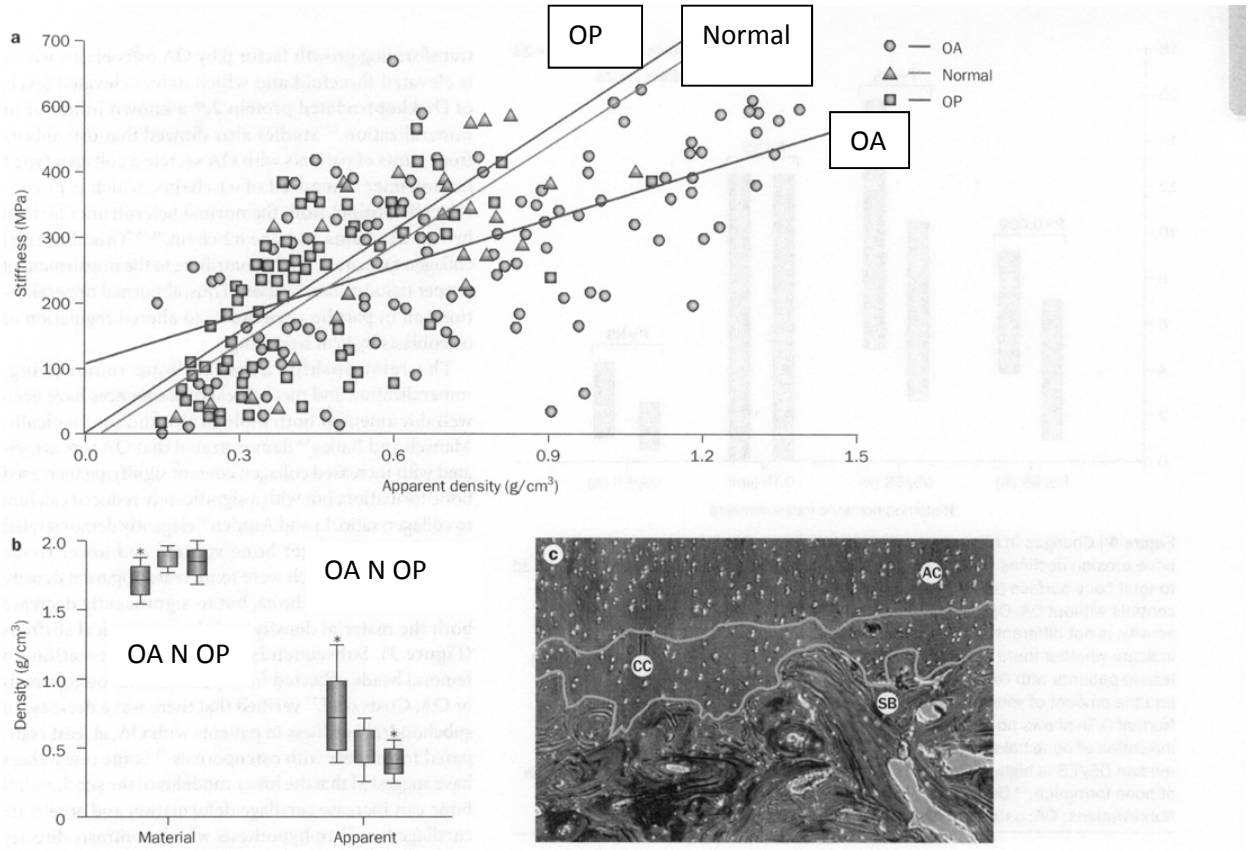
Key points

- Subchondral cortical bone and subchondral trabecular bone are architecturally, physiologically and mechanically different, and respond differently in osteoarthritis (OA)
- The initiation and the progression of OA are distinct pathophysiological processes
- The early stages of OA are characterized by increased vascularity and reduced bone density
- Late-stage OA is characterized by decreased bone resorption without a decrease in bone formation, and by the development of subchondral sclerosis
- Increased bone remodelling is a necessary pre-condition for OA, and increased subchondral bone density alone does not lead to OA
- Antiresorptive agents that suppress bone remodelling during late stages of OA have not proven, and are not likely, to be effective treatments

Stages of Progressive Joint Degeneration in OA

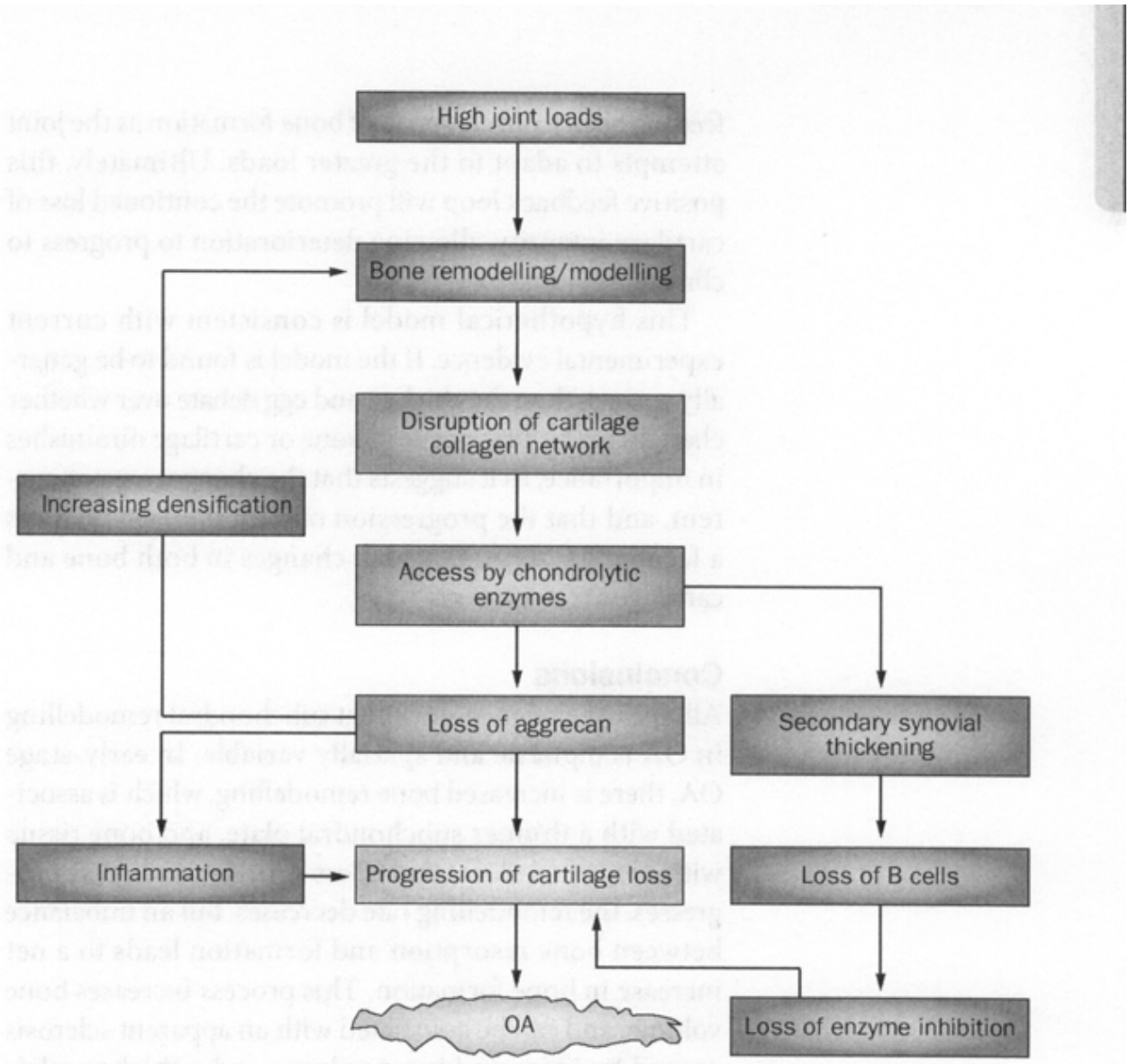
From Burr and Gallant, 2012





The Relationship Between Subchondral Bone Density and its Mechanical Properties

From Li and Aspden, 1997



Hypothetical Model for OA Pathogenesis

From Burr and Gallant, 2012

References

Pathogenesis and Mechanism

1. Burr DB. Subchondral bone in the pathogenesis of osteoarthritis. Mechanical aspects. In: *Osteoarthritis* (Brandt KD, Doherty M, Lohmander LS., eds.). 2nd edition, pp. 125-133 (2003).
2. Burr DB, Gallant M.A. Bone remodelling in osteoarthritis. *Nat Rev Rheumatol* Aug 7, 2012 doi:10.1038/nrrheum.2012.130 (2012).
3. Chan TF, Couchourel D, Abed E, Delalandre A, Duval N, LaJeunesse D. Elevated Dickkopf-2 levels contribute to the abnormal phenotype of human osteoarthritic osteoblasts. *J Bone Miner Res* **26,** 1399-1410 (2011).
4. Li B, Aspden RM. Composition and mechanical properties of cancellous bone from the femoral head of patients with osteoporosis and osteoarthritis. *J Bone Miner Res* **12,** 641–51 (1997)
5. Pan J. *et al.* Elevated cross-talk between subchondral bone and cartilage in osteoarthritic joints. *Bone* **51,** 212-217 (2012).
6. Sniekers YH. *et al.* A role for subchondral bone changes in the process of osteoarthritis; a micro-CT study of two canine models. *BME Musculoskel Dis* **9**, 20-30 (2008).

Treatment

1. Kadri A, Ea HK, Bazille C, Hannouche D, Lioté F, Cohen-Solal ME Osteoprotegerin inhibits cartilage degradation through an effect on trabecular bone in murine experimental osteoarthritis. *Arth Rheum* **58**, 2379-2386 (2008).
2. Kadri A. *et al.* Inhibition of bone resorption blunts osteoarthritis in mice with high bone remodeling. *Ann Rheum Dis* **69**,1533-1538 (2010).
3. Karsdal MA *et al.* Should subchondral bone turnover be targeted when treating osteoarthritis? *Osteoarth Cart* **16,** 638-646 (2008).
4. Saag KG. Bisphosphonates for osteoarthritis prevention: "Holy Grail" or not? *Ann Rheum Dis* **67,** 1358-1359 (2008).

Mechanical Signal and Bone Formation

Yi-Xian Qin, Ph.D.

ASBMR 2012 Meet-the-Professor
Mechanical Signal and Bone Formation
Yi-Xian Qin, Ph.D.
Department of Biomedical Engineering
Stony Brook University – State University of New York
Stony Brook, New York
Email: Yi-Xian.Qin@StonyBrook.edu

Significance of the Topic

The ability of musculoskeletal tissues to respond to changes in its functional milieu is one of the most intriguing aspects of this living organ, and certainly contributes to its success as a structure. Such ability to rapidly accommodate changes in its functional environment ensures that sufficient skeletal mass is appropriately placed to withstand the rigors of functional activity, an attribute described as Wolff's Law [5,6]. The mechanical signals define the structure and function of almost every tissue in the human body, especially in musculoskeletal tissues, where loading has been known to mediate adaptation. This adaptive capability of bone suggests that biophysical stimuli may be able to provide a site-specific, exogenous treatment for controlling both bone mass and morphology. The premise of a mechanobiological influence on skeletal morphology has become a basic tenet of bone physiology [2]. Absence of functional loading results in the loss of bone mass [3,4], while exercise or increased activity results in increased bone mass [1]. To define the formal relationship between the mechanical milieu and the adaptive response will prove instrumental in devising a mechanical intervention for skeletal disorders such as osteoporosis, designing biomechanical means to accelerate fracture healing, and promoting bony ingrowth.

Mechanotransduction has demonstrated translational potentials for tissue adaptation and regeneration under *in vivo* and *in vitro* cellular conditions. While poor bone quantity and quality are principal factors in osteoporosis, consequences of the disease are exacerbated by a functional and age-related decrease in muscle strength and postural stability, markedly increasing the risk of falling and injury. Yet the mechanism(s) through which cells sense mechanical stimulation and convert that stimulus into a biochemical response is less defined. While the majority of treatment strategies for osteoporosis are pharmaco-centric, the objective of this discuss will be focused on the potential of exercise in general, and dynamic mechanical signals in particular, as the basis of a non-drug strategy to prevent bone loss and restore function of the musculoskeletal system. A review of the current status of mechanobiology, focusing on appropriate animal models, cellular activities, and the potential mechanotransduction pathways, as well as their interactions, will be discussed. Finally, future directions in musculoskeletal research related to mechanotransduction and tissue regeneration will be explored.

Learning objectives:
As a result of participating in this session, attendees should be able to discuss:
1. What we have learned and current status of mechanical stimulation mediated bone adaptation.
2. What are the suitable *in vivo* models to evaluate general and specific hypothesis of mechanotransduction?
3. Cellular and potential molecular pathways of tissue response to mechanical loading.
4. Role of mechanotransduction in tissue regeneration and fracture repair.
3. Future directions of translational research potential.

Key points of discussion:
1. Overview of current status of mechanobiology in tissue and cells.
2. *In vivo* evidences of tissue formation and mitigation of bone loss influenced by mechanical stimulation.
3. Disuse animal model and microgravity induced bone loss, and countermeasurement.
4. Role of stem cells in response to dynamic and passive loading.
5. Tissue response to specific mechanical signals, stress and strain, fluid flow and fluid shear stress, and "sensing mechanism."
6. Cellular pathways related to loading induced bone adaptation.

References

1. Judex, S. and R. F. Zernicke. High-impact exercise and growing bone: relation between high strain rates and enhanced bone formation. J.Appl.Physiol. 88:2183-2191, 2000.
2. Martin, R. B. and D. B. Burr. Structure, Function and Adaptation of Compact Bone. New York. 1989.
3. Rubin, C. T. and L. E. Lanyon. Regulation of bone formation by applied dynamic loads. J.Bone Joint Surg.[Am.]. 66:397-402, 1984.
4. Turner, C. H. Site-specific skeletal effects of exercise: importance of interstitial fluid pressure. Bone. 24:161-162, 1999.
5. Wolff J. Das Gesetz Der Transformation Der Knochen. Berlin. 1892.
6. Wolff J. The Law Of Bone Remodeling. Berlin. 1986.

Role of T Cells in Osteoporosis and PTH Function

Roberto Pacifici, M.D.

Role of T Cells in Osteoporosis and PTH Function

Roberto Pacifici, M.D.
Garland Herndon Professor of Medicine
Director, Division of Endocrinology, Metabolism and Lipids
Emory University School of Medicine
Atlanta, GA 30322

SIGNIFICANCE OF THE TOPIC

Studies from our laboratory have revealed that T cells play a pivotal role in the mechanism of action of estrogen and PTH in bone. We have also shown that T cells are required for PTH to expand Hemopoietic stem cells. We will discuss the overall role of T cells in bone biology and the specific effects of estrogen deficiency and T cells in bone. We will also address methods and techniques required to study the interactions of bone cells and immune cells.

LEARNING OBJECTIVES

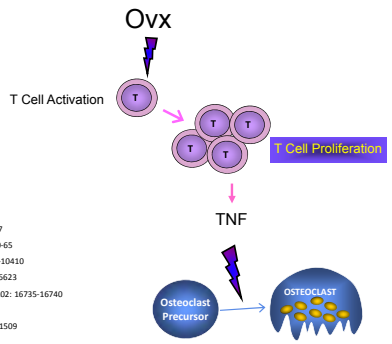
As a result of participating in this session, attendees should be able to discuss about:

1. Mechanisms by which T cells lead to bone loss in estrogen deficient mice.
2. Mechanisms by which T cells lead to bone loss in mice treated with continuous PTH, a model of hyperparathyroidism.
3. Mechanisms by which T cells stimulate bone formation and promote bone accretion in mice treated with intermittent PTH.
4. Mechanisms by which T cells regulate hemopoietic stem cell expansion.

POINTS OF INTEREST

More than a decade has passed since the publication of the first report implicating T cells in the bone loss induced by ovariectomy (ovx). More recently, strong evidence has emerged that T cells play an unexpected role in the mechanism of action of PTH. It has been a period of extraordinary progress in the understanding of the regulatory network that links the hemopoietic and the mesenchymal compartments of the bone marrow, the interactions between the immune system and bone, and the role of lymphocytes as mediators of the effects of calciotrophic hormones in bone. Collectively this body of knowledge has led to the firm establishment of “Osteoimmunology” as a novel discipline and promising area of investigation. In this session we will review advances and controversies and discuss research priorities for the future.

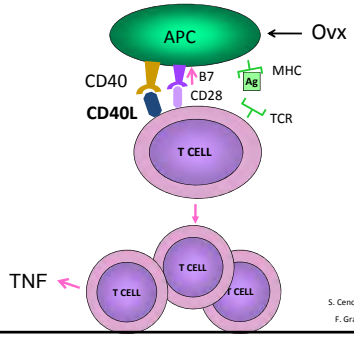
Ovx increases T cell activation and proliferation



S. Cenci et al 2000 JCI 106: 1229-1237
C. Roggia et al, 2001 PNAS 98: 13960-65
S. Cenci et al PNAS 2003. 100:10405-10410
Y. Gao et al 2004 PNAS 101: 16618-16623
M. Robbie-Ryan et al 2005 PNAS 102: 16735-16740
Y. Gao et al JCI 2007 117: 122-132
F. Grassi et al PNAS 2007 104:15087-1509

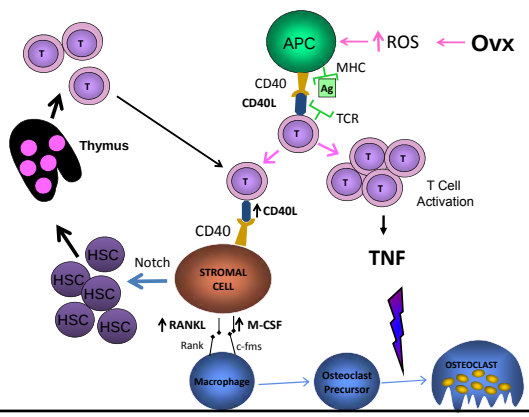
Co-stimulatory molecules involved in ovx-induced T cell activation

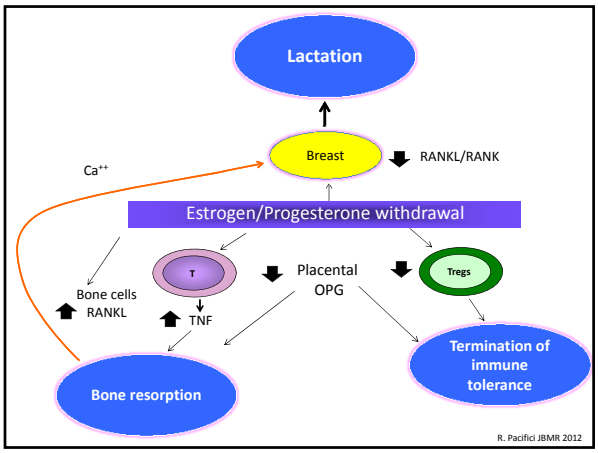
APC = Antigen Presenting Cells

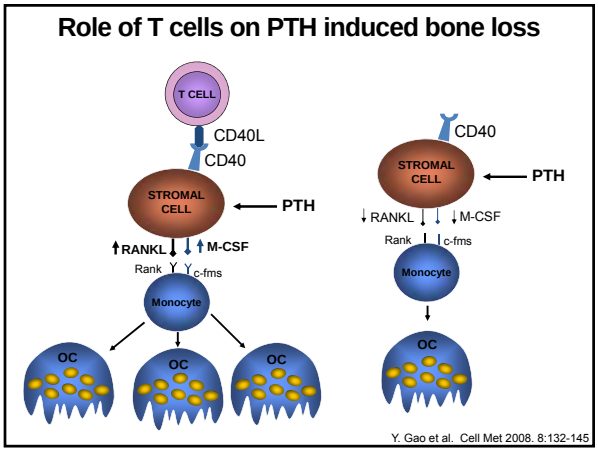


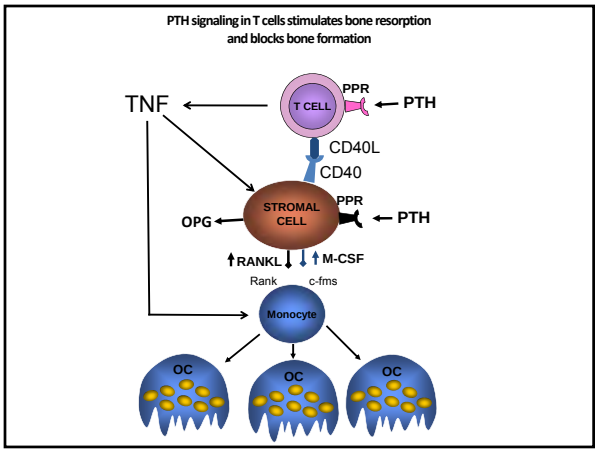
S. Cenci et al PNAS 2003. 100:10405-10410
F. Grassi et al PNAS 2007 104:15087-1509

Current Model

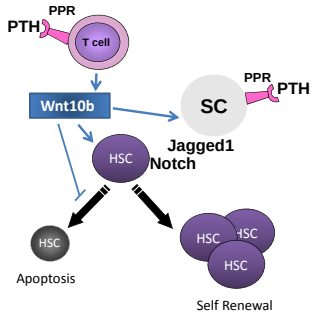



Lactation
Breast
RANKL/RANK
Ca++
Estrogen/Progesterone withdrawal
T
Tregs
Placental
OPG
Bone cells
RANKL
TNF
Termination of immune tolerance
Bone resorption
R. Pacifici JBMR 2012

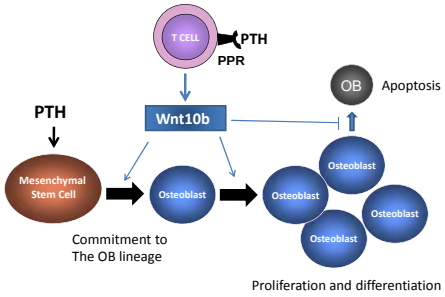

Role of T cells on PTH induced bone loss
T CELL
CD40L
CD40
STROMAL CELL
PTH
RANKL
M-CSF
Rank
c-fms
Monocyte
OC
Y. Gao et al. Cell Met 2008. 8:132-145


PTH signaling in T cells stimulates bone resorption and blocks bone formation
TNF
T CELL
PPR
PTH
CD40L
CD40
STROMAL CELL
OPG
RANKL
M-CSF
Rank
c-fms
Monocyte
OC

T cells are required for PTH to expand HSCs



Mechanism of action of iPTH induced bone anabolism



Glucocorticoid-induced Osteoporosis

Marc Hochberg, M.D., MPH

Glucocorticoid-induced Osteoporosis

Marc C. Hochberg, MD, MPH
Professor of Medicine and Epidemiology and Public Health
University of Maryland School of Medicine
Baltimore, Maryland, U.S.A.

Learning Objectives: As a result of participating in this session, attendees should be able to 1) understand the recommendations for the management of glucocorticoid-induced osteoporosis published by the American College of Rheumatology (ACR), International Osteoporosis Foundation (IOF) and European Calcified Tissue Society (ECTS); and 2) apply these recommendations in real world case scenarios.

Significance: Glucocorticoid-induced osteoporosis (GIOP) is one of the major complications associated with chronic glucocorticoid therapy. There is a rapid decline in bone mineral density (BMD), particularly in the cancellous bone of the lumbar spine, within 3-6 months after initiation of glucocorticoid therapy (1). Furthermore, glucocorticoid therapy is associated with an increased risk of fracture, even when used at low doses (2,3). Hence, it is incumbent on physicians who treat patients with glucocorticoids to be familiar with treatment options for the prevention and management of GIOP.
This session will review recently published recommendations for the prevention and management of GIOP from the ACR and IOF-ECTS as well as controversies raised by the Professional Practice Committee of the American Society for Bone and Mineral Research (ASBMR) (4-6). Finally, we will note any recent updates; e.g. work from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (7). We will review these recommendations and then discuss their application through the use of real world case scenarios.

References:
1. Laan RF, van Riel PL, van de Putte LB, van Erning LJ, van't Jof MA, Lemmens JA: Low-dose prednisone induces rapid reversible axial bone loss in patients with rheumatoid arthritis. A randomized, controlled study. Ann Intern Med 1993;119:963-8.
2. Van Staa TP, Leufkens HG, Abenhaim L, Zhang B, Cooper C: Use of oral corticosteroids and risk of fractures. J Bone Miner Res 2000;15:993–1000
3. Kanis JA, Johansson H, Oden A, Johnell O, de Laet C, Melton LJ III, et al: A meta-analysis of prior corticosteroid use and fracture risk. J Bone Miner Res 2004;19:893–99.
4. Grossman JM, Gordon R, Ranganath VK, Deal C, Caplan L, Chen W, et al: American College of Rheumatology 2010 recommendations for the prevention and treatment of glucocorticoid-induced osteoporosis. Arthritis Care Res 2010;62:1515-26.
5. Lekamwasam S, Adachi JD, Agnusdel D, Bilezikian J, Boonen S, Borgstrom F, et al: A framework for the development of guidelines for the management of glucocorticoid-induced osteoporosis. Osteoporos Int 2012 March 21. [Epub ahead of print]
6. Hansen KE, Wilson HA, Zapalowski C, fink HA, Minisola S, Adler RA: Uncertainties in the prevention and treatment of glucocorticoid-induced osteoporosis. J Bone Miner Res 2011;26:1989-96.
7. Rizzoli R, Adachi JD, Cooper C, Dere W, Devogelaer JP, Diez-Perez A, et al: Management of glucocorticoid-induced osteoporosis. Calcif Tissue Int 2012;91:225-43. Epub 2012 Aug 10.

Table 1. Recommendations on counseling for lifestyle modification and assessment of patients starting glucocorticoids at any dose with an anticipated duration ≥3 months	
Recommendation	**Level of evidence**
Vitamin D supplementation*	A
Weight-bearing activities	C
Smoking cessation	C
Avoidance of excessive alcohol intake (>2 drinks per day)	C
Nutritional counseling on calcium and vitamin D intake	C
Fall risk assessment	C
Baseline dual x-ray absorptiometry	C
Serum 25-hydroxyvitamin D level	C
Baseline height	C
Assessment of prevalent fragility fractures	C
Consider radiographic imaging of the spine or vertebral fracture assessment for those initiating or currently receiving prednisone ≥5 mg/day or its equivalent	C
Calcium intake (supplement plus oral intake) 1,200–1,500 mg/day*	A

* Recommendations for calcium and vitamin D supplementation are for any dose or duration of glucocorticoids, rather than a duration of >3 months.

Table 2. Recommended monitoring for patients receiving prevalent glucocorticoid therapy for a duration of ≥3 months

Recommendation	Level of evidence
Consider serial bone mineral density testing	C
Consider annual serum 25-hydroxyvitamin D measurement	C
Annual height measurement	C
Assessment of incident fragility fracture	C
Assessment of osteoporosis medication compliance	C

Table 3. Pharmacologic recommendations for postmenopausal women and men age ≥50 years starting glucocorticoid therapy with an anticipated duration of ≥3 months, or prevalent glucocorticoid therapy of a duration of at least 3 months (unless otherwise noted)

Recommendations	Level of evidence
Low-risk patient	
Alendronate for ≥7.5 mg/day prednisone	A
OR	
Risedronate for ≥7.5 mg/day prednisone	A
OR	
Zoledronic acid for ≥7.5 mg/day prednisone*	B
Medium-risk patient	
Alendronate for any dose of glucocorticoids	A
OR	
Risedronate for any dose of glucocorticoids	A
OR	
Zoledronic acid for ≥7.5 mg/day prednisone*	B

Table 3. Pharmacologic recommendations for postmenopausal women and men age ≥50 years starting glucocorticoid therapy with an anticipated duration of ≥3 months, or prevalent glucocorticoid therapy of a duration of at least 3 months (unless otherwise noted)	
Recommendations	**Level of evidence**
High-risk patient[†]	
Alendronate	A
OR	
Risedronate	A
OR	
Zoledronic acid[*]	B
OR	
Teriparatide[‡]	B

* Head-to-head comparison data available in the Discussion section.

† Any anticipated dose or duration of glucocorticoids justifies initiating prescription therapy for high-risk patients.

‡ For ≥5 mg/day prednisone with a duration ≤1 month and for any dose of glucocorticoids with a duration >1 month. Head-to-head comparison data available in the Discussion section.

Table 4. Treatment of Postmenopausal Women and Men Over Age 50			
	Low risk	Medium risk	High risk
ASBMR PPC[a]	Prednisone <7.5 mg/d: If no therapy given, monitor closely for prevalent fracture and decline in BMD Prednisone ≥7.5 mg/day: Bisphosphonate[b]	Bisphosphonate[b]	Bisphosphonate[b] or teriparatide
Comparison to ACR[c]	Agreement	ACR recommended zoledronate only in patients taking ≥7.5 mg of prednisone daily	ACR recommended teriparatide only in patients taking glucocorticoids >1 month, or ≥5 mg daily for <1 month

a The consensus of the ASBMR Professional Practice Committee.

b Treatment with alendronate, risedronate, or zoledronate, which are all Food and Drug Administration approved for the treatment of glucocorticoid-induced osteoporosis.

c American College of Rheumatology 2010 guidelines for the prevention and treatment of glucocorticoid-induced osteoporosis.

Table 5. Treatment of Premenopausal Infertile Women and Men Under Age 50			
	No prevalent fracture	Prevalent fracture	
		Prednisone ≤3 months	Prednisone >3 months
ASBMR PPC[a]	Consider therapy if Z-score −2.0 or significant decline in BMD related to glucorticoid therapy	Bisphosphonate[b]	Bisphosphonate[b] or teriparatide
Comparison to ACR guidelines[c]	ACR committee found inadequate data for this subgroup	ACR committee recommended zoledronate only if prednisone dose ≥7.5 mg/d	Agreement

a The consensus of the ASBMR Professional Practice Committee.

b Treatment with alendronate, risedronate, or zoledronate, which are all Food and Drug Administration approved for the treatment of glucocorticoid-induced osteoporosis.

c American College of Rheumatology 2010 guidelines for the prevention and treatment of glucocorticoid-induced osteoporosis.

Table 6. Treatment of Premenopausal Fertile Women			
	No prevalent fracture	Prevalent fracture	
		Prednisone ≤3 months	Prednisone >3 months
ASBMR PPC[a]	Consider therapy if Z-score −2.0 or lower, or significant decline in BMD related to ongoing glucocorticoid therapy	Little data to support therapy	Preference for short-acting drugs like teriparatide or denosumab instead of bisphosphonates[b]
Comparison to ACR guidelines[c]	ACR committee found inadequate data for this subgroup	Agreement	No consensus if prednisone dose <7.5 mg/d; alendronate, risedronate, or zoledronate (not teriparatide) if prednisone dose ≥7.5 mg/d

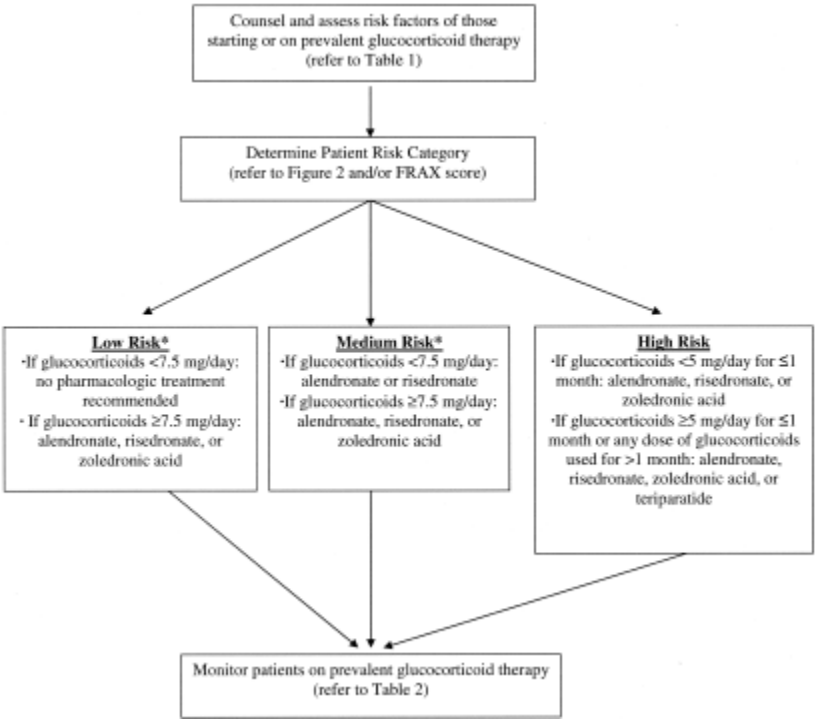
Contraception is recommended for all fertile women, regardless of which therapy is chosen.

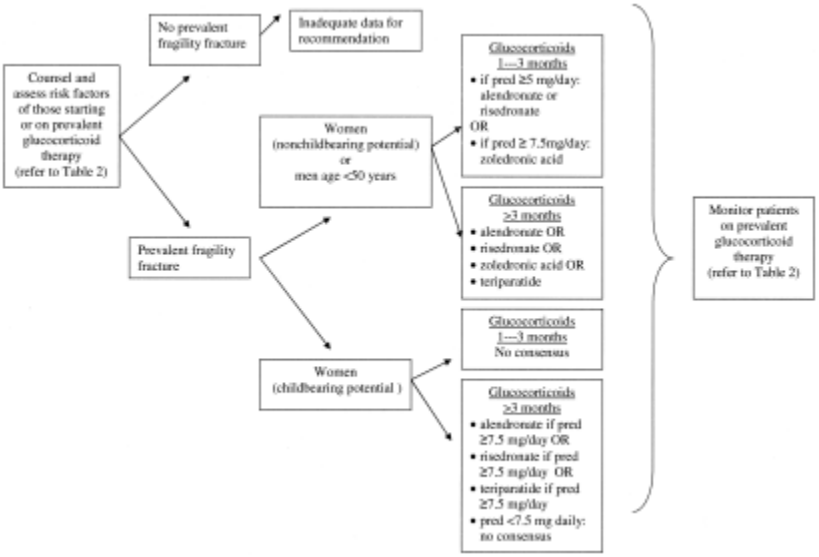
a The consensus of the American Society for Bone and Mineral Research Professional Practice Committee.

b Treatment with alendronate, risedronate, or zoledronate, which are all Food and Drug Administration approved for the treatment of glucocorticoid-induced osteoporosis.

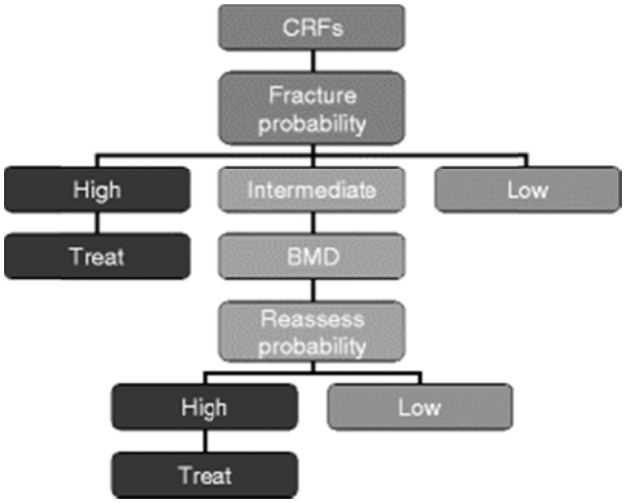
c American College of Rheumatology 2010 guidelines for the prevention and treatment of glucocorticoid-induced osteoporosis.

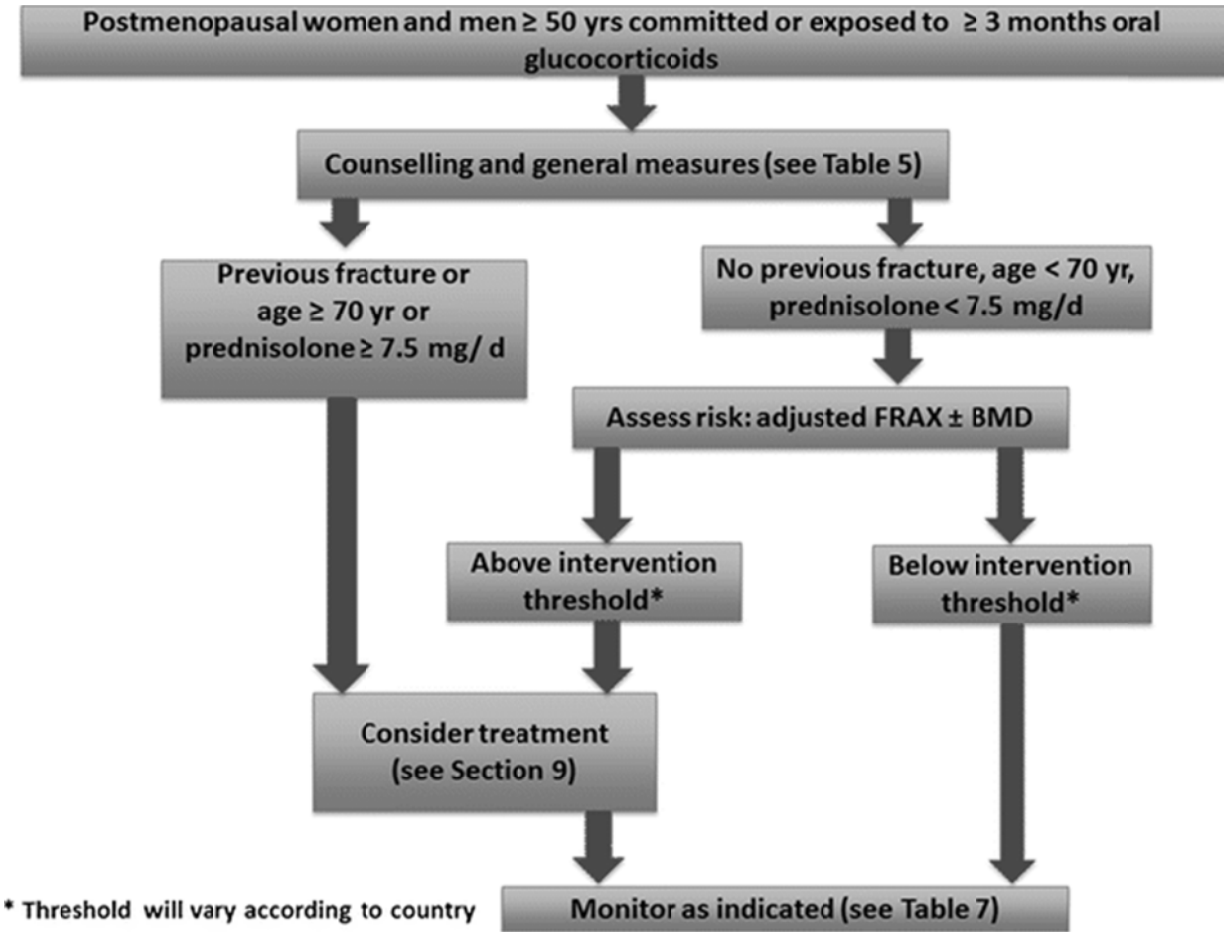
American College of Rheumatology Recommendations

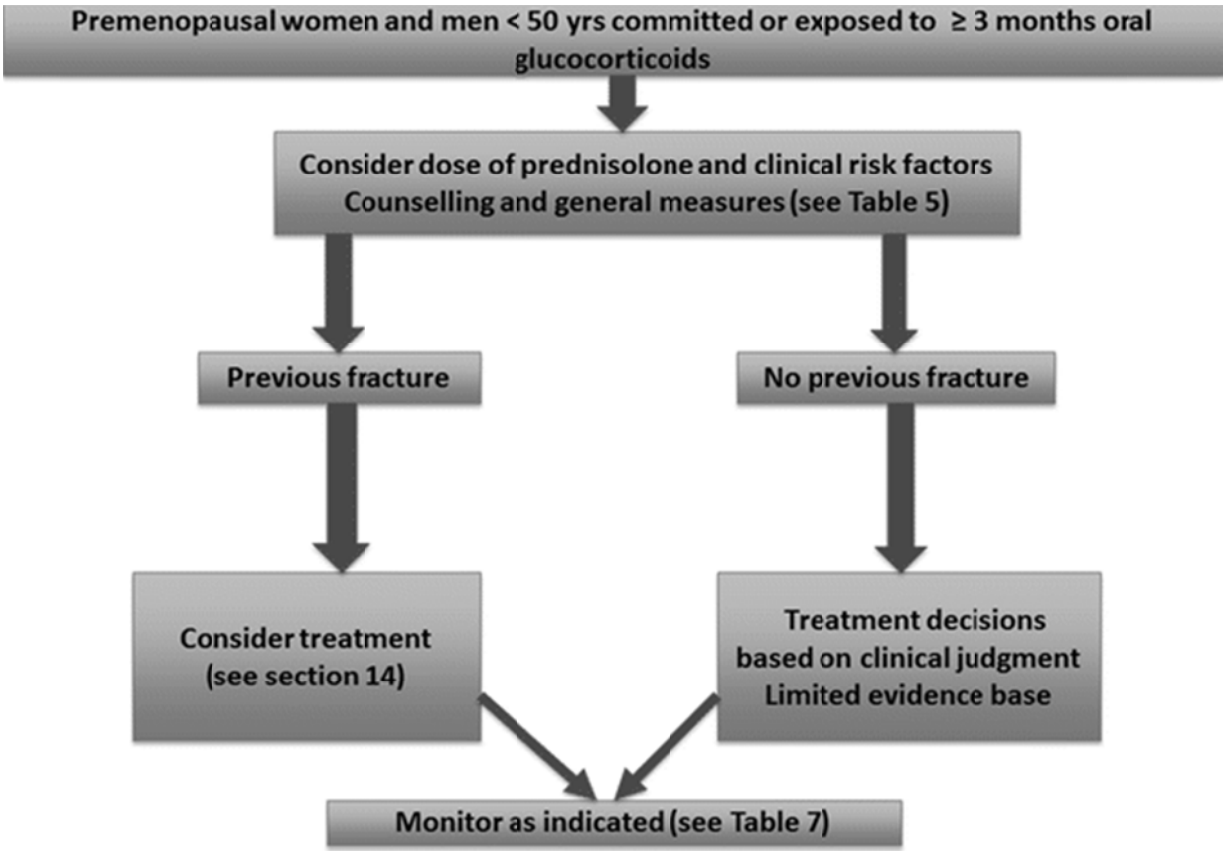




International Osteoporosis Foundation Recommendations






Premenopausal women and men < 50 yrs committed or exposed to ≥ 3 months oral glucocorticoids
Consider dose of prednisolone and clinical risk factors
Counselling and general measures (see Table 5)
Previous fracture
No previous fracture
Consider treatment (see section 14)
Treatment decisions based on clinical judgment Limited evidence base
Monitor as indicated (see Table 7)

Female Athletic Triad, Eating Disorders and Low Bone Mass

Catherine Gordon, M.D.

Female Athlete Triad, Eating Disorders and Low Bone Mass

Catherine M. Gordon, MD, MSc
Director of Adolescent Medicine, Hasbro Children's Hospital and Professor of Pediatrics, Brown University
Research Associate, Boston Children's Hospital, Harvard Medical School
USA

Significance of Topic:

Many experts consider the menstrual cycle to be a vital sign (1-2). Obtaining a menstrual history should be as important as obtaining a pulse or blood pressure in the clinical evaluation of a young woman, as amenorrhea may signify estrogen deficiency which negative ramifications for bone health and the achievement of peak bone mass. Low energy availability, with or without an eating disorder, amenorrhea, and osteoporosis, alone or in combination, pose significant health risks to physically active girls and women (3).

Learning Objectives:

As a result of participating in the session, attendees should be able to:
1) Understand the effect of hypothalamic amenorrhea on bone mass in young women
2) Be familiar with components of the Female Athlete Triad
3) Feel comfortable with a diagnostic plan and management strategy for adolescents and young women with athletic amenorrhea and/or an eating disorder

The **female athlete triad*** refers to the inter-relatedness of:

- Disordered eating
- Amenorrhea
- Osteoporosis

* term first coined by the American College of Sports Medicine in 1992

A **more recent definition of the Triad** refers to the interrelationships among (pos paper):

- Energy availability
- Menstrual function
- Bone mineral density

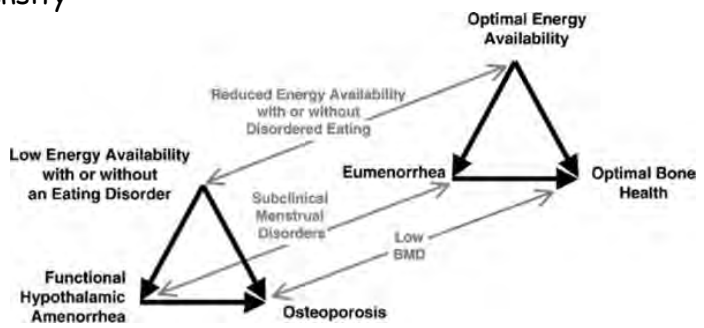


FIGURE 1 -Female athlete triad. The spectrums of energy availability, menstrual function, and bone mineral density along which female athletes are distributed (narrow arrows). An athlete's condition moves along each spectrum at a different rate, in one direction or the other, according to her diet and exercise habits. Energy availability, defined as dietary energy intake minus exercise energy expenditure, affects bone mineral density both directly via metabolic hormones and indirectly via effects on menstrual function and thereby estrogen (thick arrows).

The Female Athlete Triad. Medicine & Science in Sports & Exercise. 39(10):1867-1882, October 2007. DOI: 10.1249/mss.0b013e318149f111

The young female athlete, driven to excel in her chosen sport and pressured to fit a specific athletic image to achieve her goals, is at risk for developing patterns of disordered eating. These patterns may lead to menstrual dysfunction and subsequent inadequate bone accrual, compromised peak bone mass, and/or premature osteoporosis (2-3).

Amenorrhea: Defined as absence of 3 or more consecutive cycles in young women who are already menstruating. Female athletes training before puberty may experience delayed menarche (or 'primary amenorrhea'). Periods of oligomenorrhea (cycles greater than 35 days in length) are also common in young athletes. After the 1st gynecologic year, cycles are generally 45 days in length or less (1). It is a myth that menstrual cycles are almost always irregular and 'anything goes' with respect to menstrual cycles during the teenage years! Concern should be raised if a clinician's history reveals the absence of 3 or more cycles. Diagnosed beyond athletic/hypothalamic amenorrhea should be considered (4)(Table 1).

How common is the Triad?
Incidence of amenorrhea in athletes is 3-66% (3, 5-9), with significant variation noted among different studies and cases series. Disordered eating is reported in 15-62% of young female athletes (3, 10-12). The incidence of osteoporosis in adults who had the Triad during adolescence and young adulthood is unknown.

What athletes are most at risk? Those in whom a lean physique is considered an advantage (gymnast, figure skater, ballet dancer, distance runner). Interestingly, the prevalence of menstrual irregularities can vary among different athletic disciplines (Table 2).

What factors are associated with irregular menses in athletes? Low body weight and weight loss; low body fat (that may be sports-related); presence of eating disorder; delayed menarche; prior menstrual irregularity; adolescent age group; young age at which training began; nonporous; stress; type of sport (highest in runner and lower in swimmer and cyclists); level of training; diet (low-calorie, vegetarian, high fiber); hereditary?

Does exercise have a significant effect on bone in amenorrheic athlete?
Progressive increase in exercise, especially if accompanied by weight loss, leads to an increased incidence of luteal phase defects and anovulation, and some girls develop hypoestrogenic amenorrhea which can be associated with bone loss and an increased incidence of stress fractures (2-3,13). Energy availability promotes bone health and development indirectly by preserving the menstrual cycle. Estrogen production is preserved which restrains bone resorption and directly stimulates the production of hormones that promotion bone formation. BMD is often above average for age, given all of the above. Low energy availability removes estrogen's restraint on bone resorption and suppresses hormones that promote bone formation (2-3).

In gymnasts, losses of height velocity at puberty and short stature have been reported with potential inherent negative effects on bone health (14).

What factors are associated with low bone mass in athletes?
Low weight, low percentage body fat, low estrogen, delayed puberty, duration of amenorrhea (present and past history), low use of oral contraceptives and other estrogens, low calcium intake, higher fiber intake, increased cortisol, eating disorders, family history of osteoporosis, lack of mechanical load.

* Note that an athlete's BMD reflects her cumulative history of energy availability and menstrual status, as well as genetic endowment and exposure to other nutritional, behavioral, and environmental factors. Therefore, it is important to consider current BMD, as well as how it is moving along the bone accretion curve.

What factors are associated with stress fractures?

Warren et al. noted an increased risk of scoliosis and stress fractures in ballet dancers with amenorrhea, among other menstrual disturbances (15). Risk factors include low bone density; low calcium intake and other dietary insufficiencies; training errors; and bone geometry (e.g., narrow tibial width, shorter tibial length)(15-16)

Friedl 1992 (13): 2312 duty women surveyed regarding stress fractures. 1630 (70.5%) returned; mean age 26.1 ± 5.8 yr. History of stress fracture ranged from 31.6 in non-black smoker with episodes amenorrhea and family history of osteoporosis to 8% for black non-smoker with normal menses and negative family history.

	Odds ratio of stress fracture < 25 yr
Amenorrhea history (6 mo – no menses)	2.24
Smoker	1.96
White/Asian	1.78
Family history	1.66

What impact does anorexia nervosa have on bone health?

- Women with anorexia nervosa have a spinal BMD that is 25% below that predicted for age and gender (17-18).
- Trabecular losses predominate, but cortical losses observed, as well (as reflected by total body + hip BMD measures)(17-19).
- Demineralization can rapid (decreased formation + increased resorption – older teens; decreased bone turnover in younger teens)(18-19).
- Hallmark finding in this eating disorder is decreased bone formation.
- Increased marrow fat observed in peripheral (20), as well as axial skeleton (21). Mesenchymal stem cells appear to differentiate into adipocytes in lieu of osteoblasts, explaining the characteristic low bone formation seen in these patients.
- Hormonal profile predisposes to decreased formation, increase resorption and increased marrow fat (including *decreased* estrogens, androgens, insulin-like growth factor I, and leptin and *increased* adiponectin, ghrelin, and cortisol)(19,22).

What adolescent athletes should be evaluated?

First, consider definitions of delayed development and amenorrhea.

Many elite female athletes have delayed puberty and primary amenorrhea.

In an adolescent girl, delayed puberty is defined as no pubertal development at age 13 years. Primary amenorrhea is classically defined as the absence of menarche by age 16 years. However, the age of 15 appears to be a more valid and data-based benchmark, representing the 95-98th percentile for menarche (1,4,23).

Management:

- A multi-disciplinary treatment team should include a physician or other health care professional; registered dietician; and for athletes with eating disorders, a mental health

practitioner. Other valuable team members can include an athletic trainer, exercise physiologist, and the athlete's coach, parents and other family members.

- The primary aim of treatment is to increase energy availability by increasing energy intake and/or reducing exercise energy expenditure.
- Regular work with a skilled dietician is key to successful management of these patients. My patients typically see a nutritionist at least monthly and often, more often, early into the diagnosis. Note that nutritional counseling and monitoring are sufficient intervention for many athletes.
  Monitoring of BMI, percentage ideal body weight, and intake of calcium, vitamin D and protein should take place by the dietician and physician.
- However, patients with eating disorders require psychotherapy. Athletes with eating disorders should be required to meet established criteria to continue athletic participation, and their training and competition may need to be modified.

<u>Pharmacologic Options</u>:
Note that no pharmacologic agent completely restores bone loss or corrects metabolic abnormalities that impair health and performance in athletes with 'stress' or functional hypothalamic amenorrhea.

Potential treatments include (24-34):
Estrogen/progestin, including combined oral contraceptive pills (OCPs):

- Conjugated estrogen therapy in postmenopausal doses
- Combined oral contraceptive pills (ranging from 20 – 35 mcg EE)
- Overall, estrogen/progestin replacement, prescribed as monotherapy, has yielded disappointing results.

DHEA:

- Monotherapy or as combined DHEA + estrogen replacement therapy (ERT)

Testosterone:

- Oral or transdermal

IGF-I:

- Monotherapy or as combined IGF-I + OCP

Transdermal estrogen
Bisphosphonates

- Should be used with caution in young women of reproductive age

<u>What about the patient with persistent amenorrhea despite reaching her 'ideal body weight'?</u>

- Start with progestin challenge (medroxyprogesterone acetate, 10 mg daily as oral dose for 10 days).
- Assess psychological status and stress level. Some young women are extremely stressed about their weight gain. In some cases of persistent amenorrhea despite adequate weight gain, receipt of 3-4 months of a 20 mcg EE OCP can be helpful, prescribed as a short-term intervention
- Continue to keep menstrual calendar
- Follow closely in clinic to make sure that eating issues have remained 'in remission'

Table 1: Differential Diagnosis of Amenorrhea/Menstrual Disorders?
(adapted from Emans and Laufer, Pediatric and Adolescent Gynecology 2012; 6th edition)

CNS Causes

Hypogonadotropic hypogonadism (low to normal FSH + LH)

- Chronic disease, especially which is associated with malnutrition (Crohn's, CF, celiac disease) or hyperprolactinemia (including renal disease)
- Kallman's syndrome
- CNS tumors
- Lawrence-Moon-Biedl, Prader-Willi
- Pituitary causes (tumor, infiltrative disease, hemochromatosis, head trauma, postpartum necrosis, "empty sella," irradiation, surgery
- Endocrinopathies, including hypothyroidism diabetes mellitus, Cushing syndrome (including iatrogenic from steroid therapy)
- Depression
- Drugs
- Physiologic delay

Thyroid

- Hypothyroidism, hyperthyroidism

Adrenal

- Cushing syndrome, primary adrenal insufficiency (Addison's), late onset congenital adrenal hyperplasia (e.g., 21-hydroxylase deficiency)

Ovaries

Hypergonadotropic hypogonadism (high LH and FSH)

- Gonadal dysgenesis
- Radiation or chemotherapy, surgery
- Autoimmune oophoritis (normal karyotype and ovarian insufficiency; associated with - autoimmune polyglandular syndrome – hypothyroidism, primary adrenal insufficiency, hypoparathyroidism, myasthenia gravis, pernicious anemia vitiligo; variable course, with spontaneous resumption of menses in some)
- Resistant ovary syndrome
- Other – galactosemia, myotonia dystrophica, Trisomy 21, sarcoidosis, ataxia telangiectasia, ovarian hemorrhage, torsion, removal or destruction; non-autoimmune oophoritis

Uterus

- Pregnancy
- Congenital anomalies (mullerian agenesis, androgen insensitivity)
- Asherman's syndrome

Vagina, Cervix and Hyman

- Congenital anomalies, including agenesis, imperforate hymen, tranverse septum

Table 2: Prevalence of Menstrual Irregularities (Oligomenorrhea and Amenorrhea) in Different Athletic Disciplines
(Warren & Perlroth J Endocrinol 2001;170:3-11).

Table 1 The prevalence of menstrual irregularities (oligomenorrhea and amenorrhea) in different athletic disciplines

	Study	Number of subjects	Percentage with irregularities
General population	Petterson *et al.* (1973)	1862	1·8
	Singh (1981)	900	5·0
Weight-bearing sports			
Ballet	Abraham *et al.* (1982)	29	79·0
	Brooks-Gunn *et al.* (1987)	53	59·0
	Feicht *et al.* (1978)	128	6–43
	Glass *et al.* (1987)	67	34·0
Running	Shangold & Levine (1982)	394	24·0
	Sanborn *et al.* (1987)	237	26·0
Non-weight-bearing sports			
Cycling	Sanborn *et al.* (1987)	33	12·0
Swimming	Sanborn *et al.* (1987)	197	12·0

From Constantini & Warren MP (1994) with permission.

References:

1. Hillard PJA, Nelson LM. Adolescent girls, the menstrual cycle, and bone health. J Ped Endocrinol Metab 2003;16:673-681.
2. Gordon CM. Clinical practice: Functional hypothalamic amenorrhea. N Engl J Med 2010; 363:365-371.
3. Nattiv A, Loucks AB, Monroe MM, Sanborn CF, Sundgot-Borgen J, Warren MP. Med Sci Sports Exerc 2007;1867-1882.
4. Emans SJ, Laufer MR (eds). *Pediatric and Adolescent Gynecology*, 6th edition, Wolters/Kluwer and Lippincott, Williams and Wilkins, Philadelphia, 2012.
5. Frisch 1981; Frisch RE. Nutrition, fatness, puberty and fertility. Compr Ther. 1981;7:15-23.
6. Warren MP. The effects of exercise on pubertal progression and reproduction in girls. J Clin Endocrinol Metab 1980;51:1150.
7. Warren MP, Perlroth NE. Intense exercise and female reproduction. J Endocrinol 2001; 170:3-11.
8. Nichols JF, Rauch MJ, Lawson MJ, Barkai HS. Prevalence of the female athlete triad syndrome among high school athletes. Arch Pediatr Adolesc Med 2006; 160:137-142.
9. Torstveit MK, and Sundgot-Borgen J. The female athlete triad exists in both elite athletes and controls. Med Sci Sports Exerc 2005;37:1449-1459.
10. Loucks AB. Effects of exercise training on the menstrual cycle: existence and mechanisms. Med Sci Sports Exerc 1990;22:275-280.
11. Cannovo S, Curto L, Rimarchi F. Exercise-related female reproductive dysfunction. J Endocrinol Invest 2001;24:823-832.
12. Cobb KL, Bachrach LK, Greendale G, et al. Disordered eating, menstrual irregularity, and bone mineral density in female runners. Med Sci Sports Exerc 2003;35:711-719.
13. Friedl KE, Nuovo JA. Factors associated with stress fractures in young Army women: indications for further research. Military Med 1992;157:334-338.
14. Theintz GE, Howald H, Weiss U, Sizonenko PC. Evidence for a reduction of growth potential in adolescent female gymnasts. J Pediatr 1993;122:306-13.
15. Frusztajer NT, Dhuper S, Warren MP, Brooks-Gunn and Fox RP. Nutrition and the incidence of stress fractures in ballet dancers. Am J Clin Nutr 1990;51:779-783.
16. Ackerman KE, Nazem T, Chapko D, Russell M, Mendes N, Taylor AP, Bouxsein ML, Misra M. Bone microachitecture is impaired in adolescent amenorrheic athletes compared with eumenorrheic athletes and nonathletic controls. J Clin Endocrinol Metab. 2011;96:3123-33.
17. Mazess RB, Barden HS, Ohlrich ES. Skeletal and body composition effects of anorexia nervosa. Am J Clin Nutr. 1990;52:438-41.
18. Bachrach LK, Guido D, Katzman DK, et al. Decreased bone density in adolescent girls with anorexia nervosa. Pediatrics 1990;86:440-447.
19. Gordon CM, Goodman E, Emans SJ, Grace E, Becker KA, Rosen CJ, Gundberg CM, Leboff MS. Physiologic regulators of bone turnover in young women with anorexia nervosa. J Pediatr. 2002;141:64-70.
20. Ecklund K, Vajapeyam S, Feldman HA, Buzney CD, Mulkern RV, Kleinman PK, Rosen CJ, Gordon CM. Bone marrow changes in adolescent girls with anorexia nervosa. J Bone Miner Res. 2010;25:298-304.
21. Bredella MA, Fazeli PK, Miller KK, Misra M, Torriani M, Thomas BJ, Ghomi RH, Rosen CJ, Klibanski A. J Clin Endocrinol Metab. 2009;94:2129-36.
22. Misra M. Effects of hypogonadism on bone metabolism in female athletes and young adults, Nat Rev Endocrinol. 2012;8:395-404.

23. Gordon CM, Welt C, Rebar RW, Hillard PJA, Matzuk MM, Nelson LM (eds). *The Menstrual Cycle and Adolescent Health*. Blackwell Publishing, Boston, 2008.
24. Hergenroeder AC, Smith EO, Shypailo R, et al. Bone mineral changes in young women with hypothalamic amenorrhea treated with oral contraceptives, medroxyprogesterone or placebo over 12 months. Am J Obstet Gyn 1997;176:1017-1025.
25. Klibanski A, Biller BM, Schoenfeld DA, et al. The effects of estrogen administration o trabecular bone loss in young women with anorexia nervosa. J Clin Endocrinol Metab 1995;80:898-904.
26. Golden NH, Lanzkowsky L, Schebendach J, Palestro CJ, Jacobson MS, Shenker IR. The effect of estrogen-progestin treatment on bone mineral density in anorexia nervosa. J Pediatr Adolesc Gynecol. 2002;15:135-43.
27. Gordon CM, Grace E, Emans SJ, Feldman HA, Goodman E, Becker KA, Rosen CJ, Gundberg CM, LeBoff MS. Effects of oral dehydroepiandrosterone on bone density in young women with anorexia nervosa: a randomized trial. J Clin Endocrinol Metab. 2002;87:4935-41.
28. Grinspoon S, Baum H, Lee K, Anderson E, Herzog D, Klibanski A. Effects of short-term recombinant human insulin-like growth factor I administration on bone turnover in osteopenic women with anorexia nervosa. J Clin Endocrinol Metab. 1996;81:3864-70.
29. Grinspoon S, Thomas L, Miller K, Herzog D, Klibanski A. Effects of recombinant human IGF-I and oral contraceptive administration on bone density in anorexia nervosa. J Clin Endocrinol Metab. 2002;87:2883-91.
30. Miller KK, Grieco KA, Klibanski A. Testosterone administration in women with anorexia nervosa. J Clin Endocrinol Metab. 2005;90:1428-33.
31. Misra M, Katzman D, Miller KK, Mendes N, Snelgrove D, Russell M, Goldstein MA, Ebrahimi S, Clauss L, Weigel T, Mickley D, Schoenfeld DA, Herzog DB, Klibanski A. Physiologicestrogen replacement increases bone density in adolescent girls with anorexia nervosa. J Bone Miner Res. 2011;26:2430-8.
32. Golden NH, Iglesias EA, Jacobson MS, Carey D, Meyer W, Schebendach J, Hertz S, Shenker IR. Alendronate for the treatment of osteopenia in anorexia nervosa: a randomized, double-blind, placebo-controlled trial. J Clin Endocrinol Metab. 2005;90:3179-85.
33. Miller KK, Grieco KA, Mulder J, Grinspoon S, Mickley D, Yehezkel R, Herzog DB, Klibanski A. Effects of risedronate on bone density in anorexia nervosa. J Clin Endocrinol Metab. 2004;89:3903-6.
34. Miller KK, Meenaghan E, Lawson EA, Misra M, Gleysteen S, Schoenfeld D, Herzog D, Klibanski A. Effects of risedronate and low-dose transdermal testosterone on bone mineral density in women with anorexia nervosa: a randomized, placebo-controlled study. J Clin Endocrinol Metab. 2011;96:2081-88.

The Pathology of Common (or not so common) Bone Lesions in Humans and Mouse Models

Brendan Boyce, M.D.

The Pathology of Common (or not so common) Bone Lesions in Humans and Mouse Models

Brendan F. Boyce M.D.

Significance of the Topic:

To be able to understand the effects of over- or under-production of hormones, cytokines and growth factors on the skeleton and the pathogenesis of acquired, congenital and inherited diseases, it is important to be aware of the processes of normal bone modeling and remodeling and how these can become disrupted in common and rare bone diseases. Genetic mouse models and human genetic studies during the past 2 decades have significantly improved understanding of the mechanisms controlling skeletal development and bone remodeling and the pathologic basis of many diseases affecting the skeleton.

Learning Objectives: As a result of participating in this session, attendees should be able to describe the histologic features of normal endochondral ossification and bone remodeling as well as the pathologic features of common bone diseases and how these affect bone remodeling.

Points of Interest

General considerations about bone modeling and remodeling in humans and mice.

1. The processes of bone modeling and remodeling in humans and mice is similar.
2. Growth plates do not close in mice, but they do in humans at various times in teenagers. Therefore, there is continuous modeling of metaphyseal bone in mice until they are many months old. Growth plates close in osteocalcin -/- mice and in rats treated long-term with heparin, indicating that the function of heparin-binding proteins, such as osteocalcin, to maintain open growth plates in rodents can be inhibited pharmacologically.
3. Modeling in metaphyses involves bone being formed by osteoblasts (OBs) on cartilaginous trabeculae at growth plates and subsequently being removed by osteoclasts (OCs) in the primary spongiosa. The bone trabeculae that remain after resorption then have new bone laid down on them by OBs. This bone remains in the secondary spongiosa of the metaphysis and is remodeled continuously in a process that appears to be require RANKL released by osteocytes and possibly other cells.
4. The trabecular bone in the central parts of vertebral bodies is remodeled continuously in mice in a process more similar to what happens in adult human trabecular bone than occurs in the metaphyses of long bones of mice, despite the presence of open growth plates at the ends of each bone.
5. During the first 4-6 weeks after birth, mice like growing babies, have a period of rapid growth of their skeleton with high rates of bone formation and resorption at growth plates. If there is a congenital or genetically induced defect in OC formation or function, this is the time when it will be most obvious. It will result in osteopetrosis, a disorder characterized by dense metaphyseal trabecular bone and long bones with club-shaped, rather than funnel-shaped ends. This shape occurs in osteopetrosis because osteoclasts do not remove bone laid down by osteoblasts on the periosteal surfaces below growth plates
6. Drugs, such as bisphosphonates and RANKL inhibitors, prevent resorption of bone formed in the primary and secondary spongiosae of growing mice and children. Administration of them in these circumstances induces a characteristic form of localized osteopetrosis seen radiologically and histologically as a band of dense bone extending from the growth plate into the metaphysis. The presence of this can be used as an indication that the drug is working effectively in clinical situations and in animal models.
7. Drugs or animal models associated with increased bone formation in mice result in osteosclerosis of varying degrees, i.e., trabecular number and/or thickness increase in a more uniform fashion than that seen in osteopetrosis without the persistence of unresorbed cartilage in the centers of the trabeculae.

Pathology of common bone diseases in humans and associated mouse models.

Most of the common bone diseases impact normal bone remodeling

1. Osteoporosis:
    a. Most common in postmenopausal women as a result of sex steroid deficiency. Bone loss occurs most rapidly during the first 5-10 years after menopause, associated with increased remodeling; resorption exceeds formation and there is increased production of cytokines, including TNF, IL-1, IL-6. These cytokines induce expression of RANKL by osteoblastic cells leading to bone loss. Ovariectomy in mice and rats mimics the effects of menopause-associated sex steroid deficiency in long bone metaphyses and vertebral bodies. Cytokine inhibitors prevent the increase in resorption in mice and the associated bone loss.
    b. Next most common is age-related osteoporosis, affecting women and men and characterized by reduced bone formation and normal resorption. Also occurs in aging mice and rats. There are numerous murine genetic models of age-associated osteoporosis and of high bone mass. Some of these result from failure of bone loss to occur as a consequence of knockout of specific genes or of over-expression of genes that encode inhibitors of bone loss.

2. Osteomalacia:
    a. Commonest cause is Vitamin D-deficiency due to dietary insufficiency coupled with low Vitamin D intake in food. Early pathologic changes reflect the effects of hypocalcemia, which results in secondary hyperparathyroidism and increased bone resorption and formation. This is followed by defective mineralization of osteoid and eventually in osteomalacia. Osteomalacia is diagnosed by presence of extensive, thickened osteoid seams and low bone mineralization rate, measured following double fluorochrome labeling. Tetracycline is the only fluorochrome that can be given to humans. Tetracycline and calcein are used commonly to assess mineralization in mice and rats. Von Kossa or Goldner's trichrome staining methods are used commonly to visualize calcified bone and uncalcified osteoid in human and rodent bone samples. Osteoid can also be visualized in samples of bone that have been immersed overnight in silver nitrate solution and then decalcified using the method of Tripp and McKay. The silver nitrate passes through the osteoid into the underlying calcified matrix to which it binds and is not removed during decalcification. It can be seen as a band of black staining matrix under the osteoid in H&E-stained decalcified sections. There are no reliable biochemical tests for osteomalacia, but serum levels of alkaline phosphatase typically are increased in advanced cases in humans. Increased PTH production by the parathyroid glands can maintain blood calcium levels within the normal range by increasing calcium reabsorption from the kidney and absorption from the gut and inducing increased osteoclastic resorption.
    b. Osteomalacia can also be induced by inhibitors of mineralization, such as aluminum hydroxide, which is administered to patients with chronic renal failure to reduce phosphate absorption from the gut, and fluoride, which is present in some sparkling waters and was used as a treatment to stimulate bone formation in osteoporosis in the 1970s to 1990s, particularly in Europe.

3. Hyperparathyroidism. Characterized by increased bone resorption and formation, which are typically in balance when Vitamin D levels are normal, and 'fibrosis' of the bone marrow close by the trabecular surfaces where resorption is increased, and is called osteitis fibrosa. The cells in the 'fibrosis' are alkaline phosphatase-positive and in the osteoblast, rather than the fibroblast lineage. Hyperparathyroidism occurs in patients with excessive production of PTH by their parathyroid glands as a result of a parathyroid adenoma, a benign tumor of one or more of the glands (primary hyperparathyroidism), or in response to low blood calcium levels in patients with Vitamin D deficiency or chronic renal failure (secondary hyperparathyroidism). In chronic renal failure, the failing kidneys are unable to adequately excrete phosphate into the urine and as a result serum phosphate rises, associated with low blood calcium in part because calcium reabsorption from the kidneys is also impaired.

Continuous administration of PTH to rodents results in increased bone resorption and formation with associated marrow fibrosis. Intermittent administration of PTH stimulates new bone formation by a number of mechanisms and leads to increased bone mass in rodents and humans. It is used to stimulate bone formation is patients with established osteoporosis who have not responded to anti-resorptive therapy; treatment is restricted to a period of 2 years.

4. Paget's disease. Characterized by foci of excessive bone resorption by very large osteoclasts at any site in the skeleton in adult typically more than 50 years old. Results in radiologically obvious lytic lesions, which subsequently become filled with new bone and ultimately become sclerotic. Can occur in children due to activating mutations in the RANK gene or mutations in the osteoprotegerin gene. These mutations have also been reported in adults, but in most of adults the etiology remains unclear. Previous paramyxovirus infections in childhood have been implicated in the pathogenesis of Paget's disease in adult. At least 2 animal models have been developed, one based on a point mutation in the ubiquitin-associated domain of SQSMT1 (which encodes sequestosome 1/p62, a scaffold protein that plays a key role in RANKL signaling and has been implicated in the pathogenesis in ~10% of patients) and another on over-expression of measles virus nucleocapsid gene (which has also been implicated in the pathogenesis) in osteoclast precursors.

5. Rheumatoid arthritis (RA). An autoimmune disease affecting multiple tissues and especially synovial joints in ~1% of the population. Characterized by inflamed, swollen and painful joints. Most patients have high serum and joint levels of TNF and 50-60% respond positively to anti-TNF treatments, which have significantly altered the natural history of the disease for most patients by preventing joint pain and destruction. Several animal models have been developed and some closely resemble RA with synovial joint destruction and systemic bone loss. The synovial membrane is thickened due to an increase in the number of synoviocytes and infiltration of the underlying synovial soft tissue by lymphocytes, plasma cells and macrophages. RANKL is secreted by synoviocytes and T and B lymphocytes. Macrophages are a major source of TNF, IL-1 and IL-6, all of which contribute to increase inflammatory cell infiltration in an auto-amplifying process involving NF-κB, which positively regulates formation of osteoclasts and immune cells.

6. Psoriatic arthritis affects ~25% of the 7.5 million Americans with psoriasis, a chronic inflammatory skin disease. TNF drives the inflammation in the joints of many of the affected patients, although the inflammation and joint swelling tend to be less than in RA and new bone growth around joints is greater. Only one or two animal models of the disease has been developed.

7. Osteoarthritis. The most common joint disease affecting 10% of the general population and about 40% of people older than 70 years. It is associated with aging, wear and tear, obesity, and joint strain or damage. Genetic and environmental influences are involved in the pathogenesis with genetic factors estimated to account for about 50% of the risk of developing osteoarthritis in the hip or knee. The etiology not clear in most patients, but severe joint injury, e.g. to younger athletes is associated with high prevalence in later years. It is characterized by erosion of the joint articular cartilage with sclerosis of the underlying bone in which lytic cystic lesions can develop. Spurs of new bone, called osteophytes, develop around the edges of the joints and further limit joint movement. Several animal models developed by damaging menisci or joint ligaments or by genetic manipulation of mice are available.

8. Acute and chronic osteomyelitis. Typically bacterial in origin and arises by blood-borne infection from extra-osseous sites. It affects children and adults with and without immunodeficiency or following injury or surgical intervention. In children, it occurs typically in the metaphyses where bone remodeling is most active with an associated rich blood supply. Bones affected by acute osteomyelitis have an inflammatory infiltrate consisting mainly of neutrophils, but there is typically chronic inflammation around it. It can advance to chronic osteomyelitis, which has a plasma cell and lymphocytic infiltrate

with associated increased bone resorption and reactive new bone formation. This histologic picture could be mistaken histologically for hyperparathyroidism.

9. Other bone diseases include a variety of chronic inflammatory and neoplastic conditions that typically cause osteolytic lesions, but they can also cause sclerotic lesions. Some chronic histiocytic lesions, such as Langerhans' granulomatosis, can begin as lytic lesions and later become sclerotic. The commonest malignant tumor affecting the skeleton is metastatic carcinoma from the breast, lungs, prostate, kidney and thyroid. Animal models have been developed for a number of metastatic bone diseases.

Bone-Vascular Axis

Dwight Towler, M.D., Ph.D.

Arterial calcification and bone physiology: role of the bone–vascular axis

Bithika Thompson and Dwight A. Towler

Abstract | Bone never forms without vascular interactions. This simple statement of fact does not adequately reflect the physiological and pharmacological implications of the relationship. The vasculature is the conduit for nutrient exchange between bone and the rest of the body. The vasculature provides the sustentacular niche for development of osteoblast progenitors and is the conduit for egress of bone marrow cell products arising, in turn, from the osteoblast-dependent haematopoietic niche. Importantly, the second most calcified structure in humans after the skeleton is the vasculature. Once considered a passive process of dead and dying cells, vascular calcification has emerged as an actively regulated form of tissue biomineralization. Skeletal morphogens and osteochondrogenic transcription factors are expressed by cells within the vessel wall, which regulates the deposition of vascular calcium. Osteotropic hormones, including parathyroid hormone, regulate both vascular and skeletal mineralization. Cellular, endocrine and metabolic signals that flow bidirectionally between the vasculature and bone are necessary for both bone health and vascular health. Dysmetabolic states including diabetes mellitus, uraemia and hyperlipidaemia perturb the bone–vascular axis, giving rise to devastating vascular and skeletal disease. A detailed understanding of bone–vascular interactions is necessary to address the unmet clinical needs of an increasingly aged and dysmetabolic population.

Thompson, B. & Towler, D. A. *Nat. Rev. Endocrinol.* advance online publication 3 April 2012; doi:10.1038/nrendo.2012.36

Introduction

Bone never forms without vascular interactions.[1,2] Whilst anatomically apparent, the physiological and pharmacological implications of this dynamic relationship are under-appreciated. The vasculature is, of course, the conduit for nutrient exchange between the skeleton and the rest of the body; this vasculature is required to provide rapid access to the skeletal calcium 'bank' needed when physiological demands are urgent[3]—be it for deposits or withdrawals—but also for the delivery of metabolic substrate to the basic multicellular unit underlying the bone–forming functions of osteoblasts.[4] The vasculature also provides the niche for development of osteoprogenitors.[5] Via its conduit functions, the vasculature enables egress of bone marrow cell products arising, in turn, from the osteoblast-dependent haematopoietic niche.[6] A comprehensive understanding of bone–vascular interactions during development and disease is required to better address the burgeoning clinical needs of our aging, dysmetabolic population.[7]

Remarkably, the inextricable interdependence of vascular physiology, skeletogenesis, bone remodelling and mineral metabolism has, in general, escaped widespread appreciation. Arteriosclerosis—the stiffening of conduit arteries from any cause, including medial calcification and fibrosis in addition to atherosclerosis—contributes to disorders that increase morbidity and mortality, such as musculoskeletal disease. The lower extremities bear the brunt of this disease burden, which is best manifested in the increased risks of hip fracture,[8] limb ischaemia[9,10] and amputation.[9,11,12] In a preclinical model of critical limb ischaemia, Napoli *et al.* have begun to capitalize upon this physiology, coupling parathyroid hormone (PTH)-mediated bone anabolism with treatment with granulocyte colony stimulating factor as a strategy to mobilize endothelial progenitor cells and improve limb recovery.[13] In humans, several groups have demonstrated that marrow-derived progenitors with 'osteogenic signatures' portend and participate in arterial function,[14,15] valve calcification[16] and fracture repair.[17] Anabolic crosstalk between osteoblasts and endothelial cells has been manipulated as a successful strategy to enhance bone regeneration following injury.[18,19] Thus, a bone–vascular axis has emerged, in which the vasculature supports musculoskeletal functions, and bone-derived cell types and endocrine and/or paracrine cues affect vascular health.

This Review provides an overview of the intricacies of the bone–vascular axis, emphasizing interactions during disease rather than during development (two outstanding reviews detail these interactions during skeletal morphogenesis[2,20]). Dysmetabolic states, such as diabetes mellitus, dyslipidaemia and uraemia, that compromise vascular health—and thus skeletal health via heterogeneous arteriosclerotic calcification processes—are emphasized.[21,22] The article recounts new data demonstrating that calciotropic hormones regulate vascular calcification,[23–26] and that the dysmetabolic milieu

Competing interests
The authors declare no competing interests.

Department of Internal Medicine, Division of Endocrinology, Metabolism and Lipid Research, Washington University School of Medicine, Campus Box 8127, 660 South Euclid Avenue, St Louis, MO 63110, USA (**B. Thompson**, **D. A. Towler**).

Correspondence to:
D. A. Towler
dtowler@im.wustl.edu

Key points

- Clinically important and actively regulated processes control tissue mineralization in the skeleton and the arterial vasculature
- Bidirectional communication between the vasculature and bone—conveyed by cellular, endocrine and metabolic messengers—is critical to maintenance of bone health and vascular health
- Dysmetabolic states, such as diabetes mellitus, uraemia and hyperlipidaemia, perturb the bone–vascular axis and give rise to vascular and skeletal disease
- As understanding of the bone–vascular axis continues to improves, so too will our capacity to meet the clinical needs of patients with metabolic bone and cardiovascular disorders

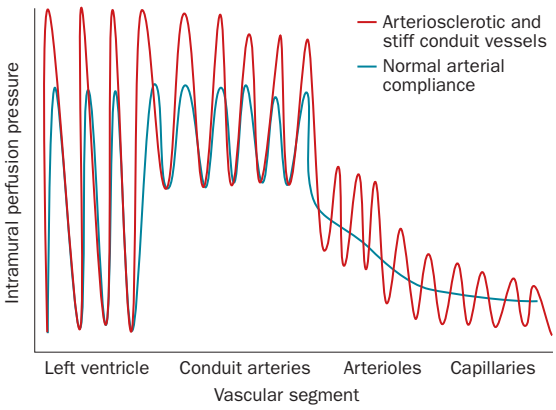


Figure 1 | Consequences of arterial stiffening and impaired Windkessel physiology. During systole, some kinetic energy is stored as potential energy in the elastic conduit arteries. This stored energy permits not only coronary perfusion but also smooth distal capillary perfusion during diastole (blue tracing). With arteriosclerotic stiffening (red tracing), less potential energy is stored during systole, giving rise to impaired, pulsatile and erratic flow during diastole (two-thirds of the cardiac cycle).[210] Systolic blood pressure is also increased. The topic has been reviewed elsewhere.[81]

impairs calciotropic hormone signals vital to the preservation of normal bone and vascular mineral homeostasis.[27–29] Also discussed are the mechanisms whereby cellular, biochemical and hormonal cues produced by the skeleton systemically affect vascular health and vessel conduit function. The Review ends by pointing to emerging therapeutic opportunities afforded by an improved understanding of the bone–vascular axis.

Vascular calcification and human health

Vascular calcification has afflicted human beings for at least 5 millennia. Ötzi, the Tyrolean Ice Mummy who succumbed to homicide ~5,300 years ago—500 years before Stonehenge was erected—had considerable deposits of arterial calcium in his abdominal aorta.[30] Only in the past few decades have the physiological consequences of arterial calcification become evident—a clinical appreciation afforded by the greater longevity enjoyed by modern humans. Coronary artery calcium scores identify individuals at the greatest risk of progressive cardiovascular disease among patients with otherwise intermediate risk.[31] Tibial artery calcium (TAC) scores outperform ankle–brachial indices in portending amputation risk in patients with peripheral arterial disease (PAD).[9] Furthermore, assessment of patients with type 2 diabetes mellitus (T2DM) with plain radiographs revealed that the presence of arterial medial calcification was a greater contributor to amputation risk than atherosclerotic calcification in this patient population.[12] The presence and extent of calcific aortic valve disease is the single best predictor of clinical progression in patients with asymptomatic, mild or moderate,[32] or severe[33] calcific aortic stenosis.

London and colleagues used plain pelvic and femoral radiographs to phenotype vascular calcification in a setting of end-stage renal disease (ESRD) requiring renal replacement therapy; the researchers observed that arterial calcification clearly portended mortality.[34] Although the calculated 5-year Kaplan-Meier survival rate was <50% for patients with ESRD affected by atherosclerotic calcification or medial calcification, the fortunate one-third of patients lacking clinically significant arterial calcification enjoyed ~90% survival during the same period.[34] After adjustment for age, time on dialysis, sex, ethnicity, presence of diabetes mellitus, non-dialysis chronic kidney disease (CKD) status, hypertension, tobacco use, existence of prior parathyroid surgery and BMI, patients with atherosclerotic calcification and medial calcification had fivefold and 16-fold increases, respectively, in the relative risk of mortality compared with those without vascular calcification.[12] In the past year, a study in which CT-based volumetric scoring of carotid artery calcification load was used, intracranial carotid calcification was linked to the extent of MRI-detected central nervous system (CNS) white matter lesions and extracranial carotid calcification was linked to overt CNS infarcts.[35] Clearly, arterial calcific vasculopathy is a harbinger of cardiovascular disease.

How can arterial calcification convey such clinically significant risks? Of course, thromboembolic events and fixed reductions in vessel lumen size induce tissue ischaemia, and the calcified Stary type Vb plaque,[36,37] characteristic of atherosclerotic calcification, is a culprit lesion in acute coronary syndromes.[37,38] Aortic valve sclerosis distorts and stiffens valve leaflets, not only causing stenosis with attendant myocardial workload demand but also precluding the efficient valve leaflet coaptation that prevents regurgitant flow.[39,40] Additionally, conduit vessel stiffening (which arises from either atherosclerotic or medial mineralization)[41] impairs Windkessel physiology,[42] which depends upon the rubbery elasticity of conduit vessels and is necessary for smooth distal tissue perfusion (Figure 1).[43]

With each cardiac cycle, a portion of the kinetic energy elaborated during ventricular systole is stored as potential energy throughout the vascular tree;[42] this energy is released during diastole and helps maintain uniformity of flow in distal capillary beds during the cardiac cycle.[43] Moreover, with arterial stiffening, the attendant increase in pulse wave velocity interacts with an impedance mismatch along the vascular tree to elevate systolic blood pressure, increasing myocardial workload as well as end-organ barotrauma (Figure 1).[43,44] Thus, the presence, extent and histoanatomic type of arterial calcification carries ominous predictions with respect to cardiovascular and CNS disease, mortality and amputation risk.[22]

At this point, the differences denoted when using the terms arteriosclerosis and atherosclerosis should be highlighted. Arteriosclerosis refers to arterial mechanical stiffening from any cause, be it from concentric medial and adventitial fibrosis with medial calcification, elastinolysis and mural thickening, as occurs in T2DM; eccentric intimal–medial atherosclerotic plaques with calcification, fibrosis and cholesterol-laden lipoprotein deposition; or neointima formation and mural fibrosis, as occurs in chronic allograft vasculopathy in transplanted organs. Atherosclerosis specifically refers to the intimally oriented, eccentric, lumen-deforming processes initiating from subendothelial lipoprotein deposits. Cholesterol-laden foam cell formation, endothelial dysfunction, and matrix remodelling events increase the risk of acute atherothrombosis with atherosclerosis. Although atherosclerosis decreases arterial compliance by causing arteriosclerosis, not all arteriosclerosis arises from atherosclerotic processes.

Vascular calcification: pathobiology

Vascular calcification was once considered only a passive process of dead and dying cells; however, data from a multitude of laboratories worldwide have clearly demonstrated that vascular calcification is an actively regulated form of extracellular matrix biomineralization.[21] Virchow's initial pathological description of atherosclerosis presciently identified the contributions of perturbed lipid metabolism, inflammation and osteo-fibrogenic differentiation to the biology of vascular calcium accrual.[45,46] Of note, vascular ossification—presence of true ectopic bone replete with marrow elements—can be seen in up to 15% of calcified arterial lesions.[47] At the molecular level, however, the signature of active osteogenic processes is found in virtually all calcified arterial segments.[48] Seminal studies from the laboratories of Linda Demer and H. Clark Anderson first identified the osteogenic 'fingerprints' in calcifying atherosclerotic and medial lesions.[48] Alkaline phosphatase, tissue-nonspecific isozyme (TNAP)—a highly characteristic and important ectoenzyme required for bone mineralization[49]—was localized to mineralizing arterial segments.[50,51] Moreover, the powerful bone morphogen BMP-2 was shown to be expressed in calcifying atherosclerotic plaques of human vessels.[52] Oxylipids derived from oxidized LDL (oxLDL) were subsequently identified as potent inducers of endothelial BMP-2,[53,54] TNF[55] and other macrophage-derived signals that drive mineralization of calcifying vascular cells (CVCs) *in vitro* and *in vivo* (see below).[55] However, in atherosclerotic mice, inhibition of arterial BMP-2 signalling with matrix Gla protein (MGP) reduces vascular calcium and lesion size.[56]

By histoanatomic and clinical criteria, at least five types of arterial calcification can be identified (Table 1): atherosclerotic calcification, medial artery calcification, calcific aortic valve disease (also known as calcific aortic stenosis), calcific uraemic arteriolopathy (also known as calciphylaxis) and the vascular calcification of ESRD (also known as CKD–MBD, chronic kidney disease mineral and bone disorder). The different types of arterial calcification have been discussed in-depth in previous reviews.[22,57] For the purposes of this Review, only atherosclerotic calcification, medial calcification and vascular calcification of CKD–MBD will be briefly discussed because of their immediate relevance to skeletal physiology and bone–vascular interactions.

Atherosclerotic calcification

Atherosclerotic calcification represents the prototypic lesion described by Virchow.[45,46] In the lexicon of cardiovascular pathology, this is the type Vb plaque,[58] characterized by an eccentric, lumen deforming, outward remodelling lesion possessing a fibrous cap, cholesterol-laden macrophages and lipoprotein deposits, intensive focal inflammatory cell infiltration and localized elastinolysis (Figure 2).[37,38] Juxtaposed mineralizing apoptotic bodies and mineralizing matrix vesicles are elaborated by neighbouring vascular smooth muscle cells (VSMCs) and CVCs undergoing chondroid metaplasia.[37,38] Stippled fibrous cap calcification is also noted, and such lesions appear to be precursors to the ruptured plaques of acute coronary syndromes.[37,38] In bone, biomineralization occurs via either endochondral ossification or membranous ossification processes programmed by chondrocytes and osteoblasts.[59–61] Endochondral ossification is characterized by the mineralization of a chondrocyte-derived skeletal template, following chondrocyte hypertrophy, apoptosis, vascular invasion and concomitant osteoprogenitor recruitment with subsequent osteoblast-mediated bone formation.

Of note, Runx2 (also known as Cbfa1)—a master transcriptional regulator absolutely essential for both endochondral and membranous ossification processes[62]—is upregulated in the vessel walls of *apoE*$^{-/-}$ mice undergoing atherosclerotic calcification.[63] Elegant studies by the Giachelli laboratory have demonstrated the 'transdifferentiation' of arterial VSMCs to cells of the chondrocyte and osteoblast lineage during vascular calcification (Figure 3).[64–66] Utilizing a transgenic mouse model in which cells originally derived from the VSMC lineage are forever 'tagged', these researchers demonstrated that locally-derived VSMCs ended up as vascular chondrocytes and osteoblasts in the mineralizing segments.[65] VSMC transdifferentiation in MGP$^{-/-}$ mice occurred in the absence of augmented MSX-2, Wnt or Sp7 (also known as Osx) signalling.[65]

What then triggers osteochondrogenic transdifferentiation of VSMCs? Oxidative stress signalling, oxylipids and phosphate (see below) have important pathophysiological roles in this process (Figure 3).[67] Oxysterols (derived from oxLDL) and hydrogen peroxide upregulate Runx2 expression[68] and osteochondrogenic transdifferentiation of VSMCs.[69] Activation of TNAP in these VSMCs is critical to matrix mineralization responses elicited by oxLDL and hydrogen peroxide, and inhibitors of TNAP limit atherosclerotic calcification by VSMCs.[70] Intriguingly, hypercholesterolaemia and oxylipids derived from oxLDL also suppress bone formation[71,72] and bone anabolic responses to PTH.[29,73] Moreover, PTH responses were restored by administration of HDL mimetics.[29,73] These data reveal a metabolic

Table 1 | Common histoanatomic types of vascular calcification

Type	Characteristics and settings	Pathobiology
Calcific aortic valve disease (CAVD; also known as senile calcific aortic stenosis)	Calcification of aortic valve leaflets Advanced age, bicuspid aortic valve, hypercholesterolaemia, the metabolic syndrome, T2DM, hypertension	Fibrofatty expansion of valve fibrosa, splitting of elastic laminae, oxylipid deposition Both osteogenic mineralization and amorphous calcium phosphate nodules deposited True ectopic ossification—woven bone formation—in ~13% of specimens, but ectopic osteogenic gene expression seen in all settings Local interstitial cells (VICs) and circulating osteoprogenitors contribute to valve osteogenic cell populations
Atherosclerotic intimal calcification (AIC)	Calcification of atherosclerotic plaques; eccentric, lumen-deforming, oriented by patchy intimal lipoprotein deposits Same risk factors as CAVD	Focal fibrofatty atherosclerotic plaque calcification with VSMC chondroid metaplasia and endochondral mineralization observed at the base of lesions CVCs related to pericytes also contribute to calcification, which is also driven by oxylipids from oxLDL Lipid core and fibrous microcalcifications, focal elastinolysis noted
Arterial medial calcification (AMC, sometimes MAC; also known as Mönckeberg's medial calcific sclerosis)	Calcification of the arterial tunica media; concentric, extensive, almost confluent Common in T2DM, autonomic neuropathy, ESRD (CKD5)	Circumferential calcification of the tunica media with lipidaceous matrix vesicles, elastinolysis and early expression of membranous mineralization programs (later endochondral) Inflammation-driven, adventitial to medial BMP–Wnt signalling directs initial osteogenic programming of vascular multipotent mesenchymal progenitors (pericytes, CVCs)
Vascular calcification of chronic kidney disease (also known as CKD–MBD)	CKD5 with any of the above Major perturbations in calcium phosphate homeostasis, and reductions in serum fetuin and pyrophosphate levels	VSMC apoptosis and osteochondrogenic metaplasia driven by hyperphosphataemia, worsened by iatrogenic hypoparathyroidism and low-turnover bone disease Occurs most often in settings of antecedent medial and atherosclerotic disease processes that initiated before uraemia
Calcific uraemic arteriolopathy (CUA; also known as calciphylaxis)	CKD4 or CKD5 with dermal arteriolar medial calcification and dermal fat necrosis, usually of pannus, buttocks, or lower extremities Patients are usually receiving warfarin	Arteriolar (vessels with generally <50 μm diameter) medial calcification with fibroproliferative occlusion leads to tissue necrosis Dermal fat, lung and mesentery most affected Warfarin impairs MGP gamma-carboxylation and functions, including BMP-2 and BMP-4 inhibition and fetuin-dependent clearance of mineralizing matrix vesicles

Abbreviations: CKD4, chronic kidney disease stage 4; CKD5, chronic kidney disease stage 5; CKD–MBD, chronic kidney disease mineral and bone disorder; CVC, calcifying vascular cell; ESRD, end-stage renal disease; MAC, medial arterial calcification; MGP, matrix Gla protein; oxLDL, oxidized LDL; T2DM, type 2 diabetes mellitus; VIC, valve interstitial cell; VSMC, vascular smooth muscle cell.

milieu that can simultaneously engender atherosclerosis and osteoporosis. Thus, inflammatory oxylipids and oxLDL accumulating at sub-intimal venues drive atherosclerotic intimal calcification by activating osteogenic BMP-2 and Runx2 transdifferentiation of VSMCs.[67,74] Recruitment of CVCs[75]—mural multipotent mesenchymal cells related to microvascular pericytes[76]—provides an additional source of osteoprogenitors.[52]

Arterial medial calcification

Arterial medial calcification (sometimes called Mönckeberg's medial calcific sclerosis) is particularly common in arteriosclerosis associated with T2DM.[10–12,77] Medial calcification is a concentric process that occurs in the tunica media and is associated with biomineralization initiating along mural elastin fibres (Figure 2).[78,79] Traditionally, medial calcification was thought to be clinically inconsequential. This idea was succinctly articulated in a pathology textbook published in 1984, in which medial calcification was referred to as a disorder "of relatively little clinical significance", which "accounts for roentgenographic densities in the vessels of the extremities of aged individuals, but it is to be remembered that the lesions do not produce narrowing or occlusion of the vascular lumen".[80] Yet, as subsequently highlighted, medial artery calcification impairs vessel mechanics and the Windkessel physiology necessary for smooth distal tissue perfusion[81] and is the best predictor of risk of lower extremity amputation in patients with T2DM.[12] Similar to the membranous ossification of craniofacial bone—in which osteoblast-mediated biomineralization occurs in a matrix based on type I collagen without a preceding cartilage template[61]—medial artery calcification does not involve overt chondrogenesis during disease initiation;[82] however, chondrogenesis is sometimes seen in advanced disease with true ectopic ossification.[83] Electron microscopy first identified vesicles positive for TNAP associated with fragmented elastin in human arteries with medial artery calcification.[50]

Additional insights into the pathobiology of disease initiation and progression have been forthcoming from detailed study of preclinical disease models.[22] When fed high-fat diets characteristic of westernized societies, male *Ldlr*$^{-/-}$ mice develop obesity, T2DM and dyslipidaemia,[22] with progressively severe medial artery calcification and subsequent atherosclerotic calcification as plaques begin to accumulate.[82] At the earliest phases of disease, arterial *Msx2* and *Msx1* (genes encoding osteoblast homeodomain transcription factors essential for membranous bone formation in the skull)[84] are upregulated in the aortas of these animals.[85] Immunohistochemistry and *in situ* hybridization demonstrated the expression of MSX-2 in aortic valve interstitial myofibroblasts, arterial adventitial myofibroblasts and a subset of cells within the

tunica media.[25,48,85] Data from our laboratory and others have contributed to the characterization of adventitial myofibroblasts as pericyte-like CVCs with multi-lineage mesenchymal cell potential (Figure 3).[86]

As in atherosclerotic calcification, inflammation is again key in medial artery calcification;[22] diabetes mellitus with or without dyslipidaemia upregulates adventitial TNF[87] production by monocytes and macrophages,[55] which in turn induces pro-calcific MSX-2 activity and paracrine Wnt signalling cascades that promote mural calcification and fibrosis (Figure 3).[82] Along with BMP-2, a powerful bone morphogen secreted by inflamed endothelial cells,[88] MSX-regulated Wnt family members produced by myofibroblasts and endothelial cells support the osteogenic differentiation and collagenous matrix mineralization by mesenchymal progenitors,[89–91] be it in bone[92] or within the arterial wall.[25,91] Thus, the paracrine polypeptide milieu of osteogenic morphogens activates osteoblast gene regulatory programmes in multipotent mural mesenchymal cells such as CVCs and adventitial myofibroblasts.[93] Adventitial-to-medial biological signals drive concentric involvement of arterial vessels in diabetes-associated medial calcification—which is quite a distinct process from the eccentric atherosclerotic calcification processes organized by sub-intimal oxylipid deposits (Figure 2). In diabetic arteriosclerosis, neoangiogenesis arising from the vasa vasorum in the inflamed adventitial–medial junction circumferentially upregulates mural BMP–Wnt signalling, and spawns additional adventitial myofibroblasts that can be allocated and/or programmed to produce osteogenic and fibrogenic phenotypes (Figure 3).[94,95] Not surprisingly, surgical stripping of arterial adventitia reduces medial calcification in preclinical disease models.[96]

Of note, data from several laboratories have demonstrated that similar processes contribute to aortic valve calcification directed by valve interstitial cells.[97] Activation of MSX and Wnt signalling cascades is observed in calcifying human aortic valves,[98] and *apoE*$^{-/-}$ mice lacking the Wnt receptor LRP-5 are protected from valve calcium accrual.[99] The behaviour and molecular phenotype of the three calcifying vascular mesenchymal cell populations—CVCs, adventitial myofibroblasts and valve interstitial cells—closely resembles that of the pericyte, the proliferative microvascular smooth muscle cell that maintains capillary integrity during neoangiogenesis.[100] Thus, as occurs in atherosclerotic calcification, medial calcification and valve calcium accrual are driven in great part by osteogenic cells derived from vascular residents (Figure 3).[21] However, exciting new data have led to the identification of a circulating CD45^{+}, osteocalcin-positive osteoprogenitor (COP) cell that elaborates BMP family members and 'homes' to sites of vascular injury.[16] As initially formulated by Eghbali-Fatourechi *et al.* in their elegant studies of human bone growth and fracture repair,[17] circulating osteoprogenitors may also participate in vascular mineralization processes in T2DM[101] as well as in true ectopic vascular ossification.[102] Therefore, strategies that inhibit vascular BMP–Wnt signalling or reduce COP cell populations may help alleviate the burden of arteriosclerotic disease in T2DM.

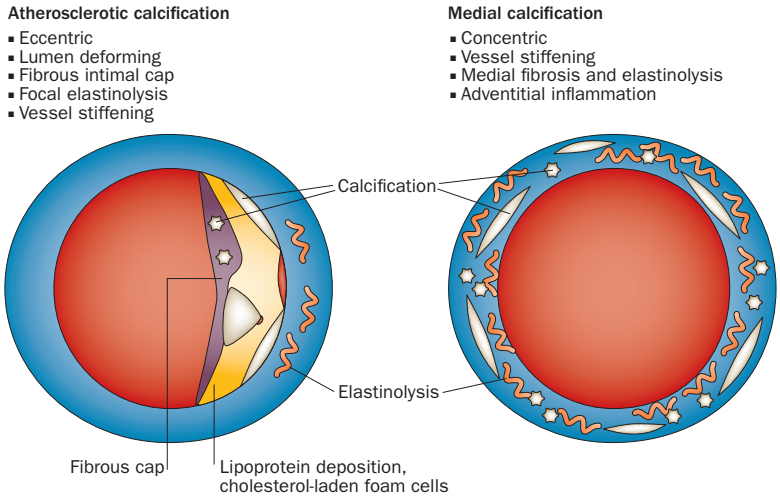


Figure 2 | Atherosclerotic versus medial arterial calcification. Both atherosclerotic calcification and medial calcification stiffen arterial conduit vessels, impairing Windkessel physiology. The eccentric remodelling of atherosclerotic calcification also reduces lumen diameter and predisposes individuals to acute thrombosis.

Vascular calcification in CKD–MBD

In 2005, the KGIDO (International Group on Kidney Disease: Improving Global Outcomes) codified the clinical entity CKD–MBD, the mineral and bone disorder of chronic kidney disease that encompasses the vascular calcification of CKD.[103,104] In CKD–MBD, the clinical link between bone disease and vascular disease arising from primary perturbations in calcium phosphate homeostasis is now formally recognized. Diabetes mellitus and hypertension—two diseases that independently promote arteriosclerotic calcification—are responsible for approximately 60% of patients with ESRD requiring renal replacement therapy;[105] however, the vast majority of patients with CKD from any cause experience increased cardiovascular-related mortality and calcific vasculopathy.[34,106] Whilst antecedent diabetes mellitus, hypertension, dyslipidaemia and the metabolic syndrome continue to contribute to arteriosclerosis,[107] hyperphosphataemia, reduced Klotho expression and impaired soft-tissue calcification defences are key pathophysiological components of CKD–MBD.[108,109] Indeed, even in the setting of normal renal function, serum phosphate levels track severity and extent of coronary artery calcium.[110,111]

Hyperphosphataemia and hypercalcaemia increase the production of VMSC matrix vesicles—~100 nm diameter phosphatidylserine-rich and annexin-rich, bilaminate spheroids resembling the mineralizing vesicles of chondrocytes.[112] Reynolds *et al.* demonstrated that VSMCs produce these matrix vesicles as one mechanism to clear extracellular matrix calciprotein complexes (Figure 3).[112,113] However, these same matrix vesicles can serve to nucleate and propagate extracellular matrix mineralization in the absence of cell-mediated pinocytotic uptake.[113] Matrix vesicle uptake by VSMCs requires serum fetuin,[113] a hepatocyte-derived calcium-binding protein that maintains calcium solubility in the supersaturated serum and interstitial fluid compartments.[114] Dialysis reduces serum fetuin levels, as does inflammation.[115]

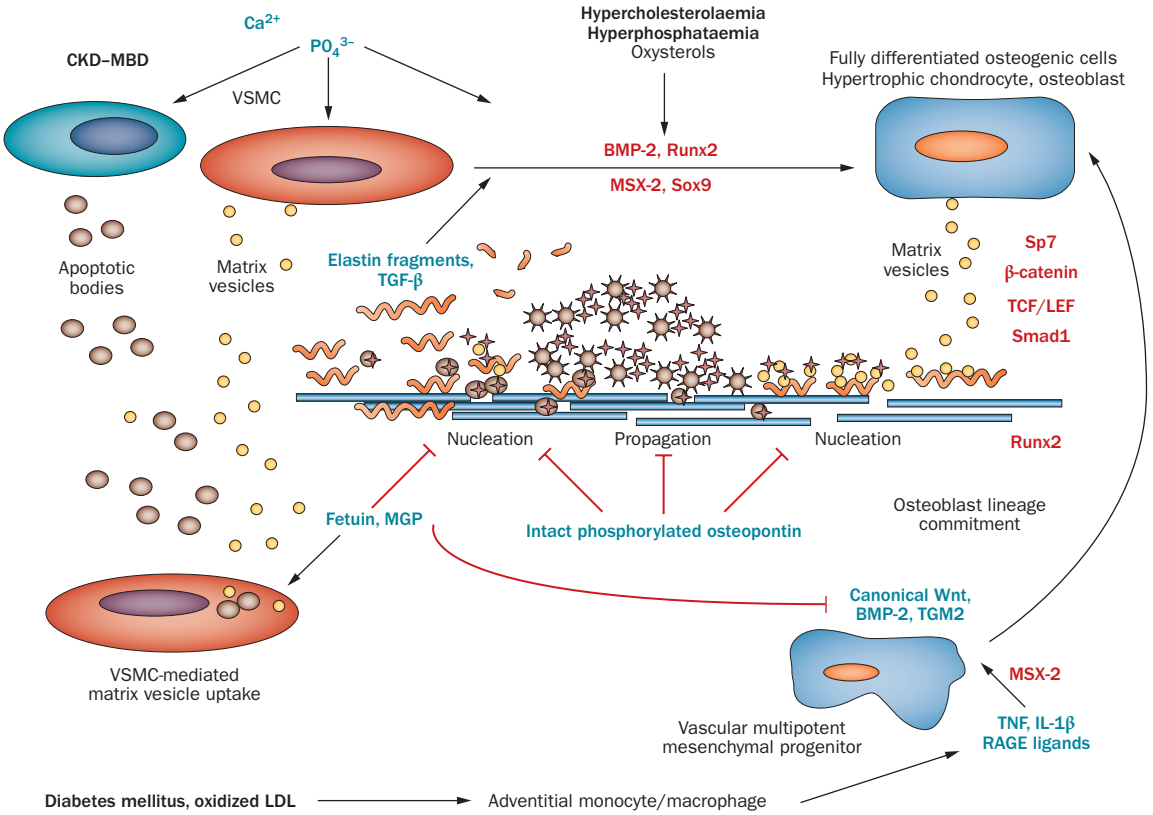


Figure 3 | Vascular osteogenic cell origins, functions and phenotypes in arterial calcification. Vascular mineralization is regulated by processes overlapping yet distinct from those that control skeletal bone formation. Osteogenic progenitors can arise from 'transdifferentiation' of VSMCs, or osteogenic lineage allocation of multipotent mesenchymal progenitors. Healthy VSMCs also have an important role in limiting vascular calcium accrual via fetuin-dependent and MGP-dependent pinocytotic uptake of matrix vesicles. Metabolic and inflammatory insults induce vascular changes that impair normal VSMC function and viability and induce osteogenic differentiation of vascular mesenchymal cells. Not shown are the circulating osteoprogenitors that may contribute to the 'vascular ossification'—true bone formation replete with marrow elements—that can be seen in ~15% of calcified vascular segments. Extracellular factors are blue and intracellular transcriptional regulators are red. Abbreviations: CKD–MBD, chronic kidney disease mineral and bone disorder; MGP, matrix Gla protein; RAGE ligands, ligands for receptor for advanced glycation end products; TGM2, protein-glutamine gamma-glutamyltransferase 2, also known as tissue transglutaminase;[211,212] VSMCs, vascular smooth muscle cells. Permission to modify obtained from the American Society of Nephrology © Mizobuchi, M. *et al. J. Am. Soc. Nephrol.* **20**, 1453–1464 (2009).[108]

In addition to increasing matrix vesicle release, phosphate also increases BMP-2 expression by VSMCs and upregulates VSMC Runx2 and MSX-2 via PiT-1 signalling.[65,116] BMP-2, in turn, further enhances phosphate uptake and osteogenic programming of VSMCs in a feedforward vicious cycle.[117] Thus, similar to oxLDL and hydrogen peroxide, elevated serum phosphate levels can reprogram the VMSC phenotype to support osteogenic mineral deposition.[66] Furthermore, sustained hyperphosphataemia simultaneously induces VSMC apoptosis, removing the first-line cell-mediated mechanism for clearing vascular calciprotein complexes.[118] The ensuing vascular mineral deposition is an exuberant medial calcification that is almost always superimposed on antecedent atherosclerotic and medial calcification arising from diabetes mellitus, dyslipidaemia and other causes. Finally, with the massive increase in mural extracellular matrix calcium, the second-line defences for handling vascular extracellular matrix calcium, the cells of the monocyte and macrophage lineage, are recruited.[119] Vascular calcium phosphate deposition elicits innate immune responses by monocytes or macrophages,[120] increasing production of TNF[121] and downstream activation of osteogenic BMP, MSX, Wnt and Runx2 signalling[116,121]—yet another feedforward vicious cycle in vascular mineralization. Thus, patients with CKD suffer the 'perfect storm' of vascular calcification.

Perhaps not surprisingly, therefore, statin-based strategies focused upon LDL cholesterol reduction have generally failed to reduce cardiovascular-related morbidity and mortality in ESRD.[122] This past year, a combined approach using a statin and the cholesterol-absorption inhibitor ezetimibe reduced major atherosclerotic disease end points by 20%, including the risk of non-haemorrhagic stroke, but failed to significantly reduce the risk of myocardial infarction or associated death.[123] Strategies that emphasize phosphate binders as a primary approach to control hyperphosphataemia have met with early success, as long as the binding was not calcium-based.[124–126] As highlighted by Raggi *et al.*,

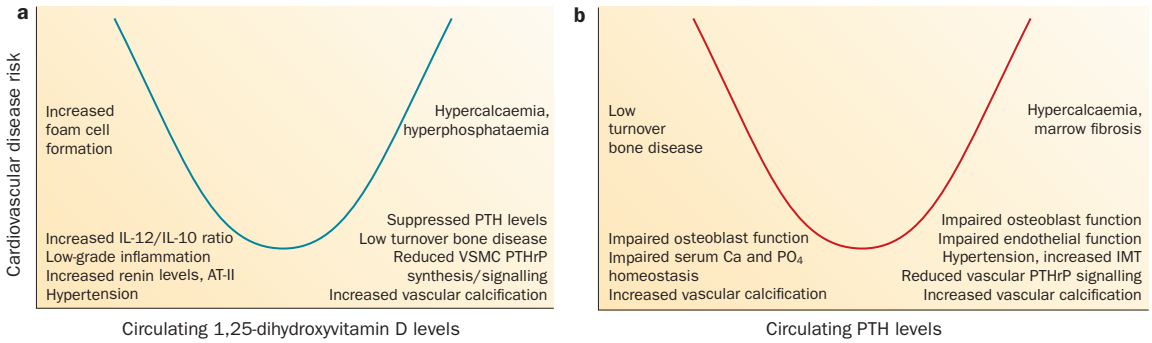


Figure 4 | The biphasic relationship between cardiovascular disease and calciotropic hormones. As in all key endocrine systems, a 'sweet spot' exists that represents the optimal set point for calciotropic hormone levels and vascular health. **a** | Both calcitriol excess and deficiency have been associated with cardiovascular disease,[141,213] and also observed in children with chronic kidney disease.[214] **b** | Similar cardiovascular problems arise with either excesses[197] or insufficiencies[127,128] in PTH. Abbreviations: AT-II, angiotensin II; IMT, intima–media thickness; PTH, parathyroid hormone; PTHrP, parathyroid hormone-related protein; VSMC, vascular smooth muscle cell.

the use of calcium-based phosphate binders substantially contributes to vascular calcium load in ESRD.[127] This effect may be directly related to the impaired capacity of the uraemic skeleton to rapidly handle serum calcium transients. Indeed, London *et al.* demonstrated that patients with the most extensive vascular calcification exhibited the lowest levels of bone formation as assessed by histomorphometric evaluation, which was reflected in inappropriately normal or low PTH values.[128] As compared to sevelamer—the first phosphate binder that does not contain calcium or aluminium—calcium-based phosphate binders suppress PTH, reduce bone mass and increase vascular calcium load in ESRD.[127] Because of the biphasic relationships between PTH, vitamin D and vascular health in ESRD, a rigorous focus upon the role and endocrine physiology of calciotropic hormones and calcium phosphate homeostasis should provide new hope to patients with CKD.[129]

Regulation of vascular calcification

Vascular calcification is clearly an actively regulated form of extracellular calcified matrix metabolism; however, remarkably few studies have been undertaken to investigate the regulation of vascular calcification by calciotropic hormones.[130] The prototypic calciotropic hormones are PTH, vitamin D and its metabolites, PTH-related protein (PTHrP), calcitonin and estrogens including estradiol. Estrogen signalling via non-genotropic signalling mechanisms acutely activates endothelial nitric oxide synthase in caveolae—an acute vasodilatory response that is theoretically protective.[131] Estrogen exposure in women was shown to reduce the incidence of arterial calcification as revealed from mammography.[132] In the Rancho Bernardo Study, estrogen use in postmenopausal women was associated with reduced coronary artery calcification;[133] however, estrogen replacement therapy worsened cardiovascular end points in older women in the Women's Health Initiative,[134] and estrogens including low-dose oral contraceptives are associated with PAD.[135] No published studies have examined the actions of calcitonin on arteriosclerosis, but the calcitonin gene-related peptide 1, encoded by the calcitonin gene, is a vasodilator.[136]

Vitamin D insufficiency is associated with PAD[137] as well as other cardiovascular diseases, including congestive heart failure.[138] Additionally, vitamin D inhibits foam cell formation and macrophage activation in patients with T2DM.[139] However, vitamin D replacement has not been shown to improve any primary cardiac end point—although the largest studies have not used uniform preparations of vitamin D.[140] In ESRD, a biphasic, U-shaped, bimodal response to calcitriol levels has clearly emerged with respect to vascular disease (Figure 4a).[141] Whilst vitamin D insufficiency and reduced levels of 1,25-dihydroxyvitamin D in ESRD engender more-severe secondary hyperparathyroidism, excessive 1,25-dihydroxyvitamin D dosing can induce low-turnover bone disease and hypoparathyroidism, and increase vascular calcium deposition. As London *et al.* first established, patients with ESRD who had the most extensive and severe arterial calcification exhibited the lowest bone formation rates and PTH levels.[128]

Vitamin D intoxication induces widespread vascular calcification and nephrocalcinosis, and rodents with vitamin D intoxication are commonly used as models of vascular calcification thus highlighting the bimodal relationship in ESRD.[142,143] The calcium-sensing receptor is expressed in VSMCs as well as in parathyroid glands,[144] and signalling with this agonist to control secondary hyperparathyroidism in uraemic rat models results in reduced vascular calcium accrual.[145] Of note, in cultured VSMCs, calcitriol induces calcification in part by suppressing PTHrP production and inducing TNAP.[146] Via paracrine actions, PTHrP relaxes smooth muscle cells, inhibits fibroproliferative responses, prevents TNAP induction and inhibits calcium deposition by signalling via the PTH/PTHrP receptor (PTH1R).[146] Indeed, adenoviral delivery of the obligatory paracrine form of PTHrP inhibits neointima formation in a porcine model of stent-induced coronary restenosis.[147,148] The role of PTHrP–PTH1R signals in the VSMC in the setting of cardiovascular arteriosclerosis has not been exhaustively studied to date, in part because *Pth1r*$^{-/-}$ mice die *in utero* owing to massive cardiomyocyte apoptosis.[149] Nevertheless, the postnatal vasculopathy arising from

vitamin D intoxication may involve lesioning of protective paracrine PTHrP–PTH1R signals in VSMCs in addition to hyperphosphataemia and hypercalcaemia.[145,146]

This past decade, our group examined the impact of PTH1R signalling on arteriosclerotic calcification in the *Ldlr*$^{-/-}$ mouse model.[23–25] Bone anabolic responses that increased skeletal calcium accrual were accompanied by reductions in aortic calcium accrual.[23,24] Additionally, arterial expression of osteogenic genes was downregulated whilst skeletal expression of these same genes was increased.[24,25] PTH1R is highly expressed in VSMCs, and is very susceptible to homologous desensitization upon tonic exposure to PTH or PTHrP.[150] Of note, sustained pharmacologic vascular exposure to either PTHrP or PTH—mimicking the setting of hyperparathyroidism—induces arterial tachyphylaxis to acute PTH or PTHrP agonist administration *in vitro*.[150]

In order to address the potential VSMC-autonomous role of PTH1R signalling in arteriosclerotic vascular responses, transgenic mice were generated and evaluated.[23] In these animals, a ligand-independent, constitutively active form of PTH1R, PTH1R(H223R),[151] is expressed in VSMCs under the control of a VSMC-specific promoter, the SM22 promoter.[23] As compared with nontransgenic male siblings, male SM22-PTH1R(H223R);*Ldlr*$^{-/-}$ mice exhibited reduced aortic calcification, aortic fibrosis and aortic oxidative stress.[23] Arteriosclerotic Wnt–β-catenin signalling and type I collagen gene expression were concomitantly reduced.[23] Aortic wall thickness was also decreased, and *ex vivo* vessel mechanical compliance (distensibility) was increased. Importantly, Sebastian *et al.* independently demonstrated that pulsatile administration of $PTH_{1–34}$ reduces arterial mineralization in a rat model of uraemia, although fibrosis and vessel mechanics were not evaluated.[26]

Our *in vivo* data with the SM22-PTH1R(H223R) transgene demonstrate that sustained activation of PTH1R in VSMCs actually reduces vascular oxidative stress and pro-calcific and pro-fibrotic signals that drive arteriosclerotic disease.[23] Of note, dysmetabolic states associated with macrovascular disease, such as diabetes mellitus, dyslipidaemia and uraemia, induce tissue resistance to PTH1R activation.[27,29,152] These findings converge to prompt a major reconsideration in our thinking as concerns the pathobiology of arteriosclerosis in T2DM and other dysmetabolic states, including primary and secondary hyperparathyroidism.

How do these findings begin to change our view? The negative impact of dysmetabolic states (dyslipidaemia, diabetes mellitus, uraemia) on PTH1R signalling can be seen as inducing insufficiency in endocrine or paracrine PTHrP actions that help preserve vascular health. The increase in carotid intima–media thickness associated with primary hyperparathyroidism[153] might be viewed, in part, as being the consequence of homologous vascular desensitization,[150,154] which occurs in response to tonically elevated PTH levels and impaired capacity of paracrine production of PTHrP in VSMCs to restrain proliferative[155] and calcific[146] arteriosclerotic responses (Figure 4b). However, via its bone anabolic actions, PTH signalling upregulates circulating intact osteopontin[24] —an inhibitor of vascular mineralization—and supports the haematopoietic niche, including cellular elements such as endothelial progenitor cells that programme vascular healing responses. Furthermore, PTH upregulates the expression of MGP,[156,157] an important negative regulator of matrix mineralization and BMP-2 and BMP-4 signalling in the vasculature.[56] Whether MGP participates in PTH inhibition of vascular myofibroblast BMP-2 signalling[25] remains to be evaluated. The relative contributions of direct versus indirect actions of PTH1R on vascular health are undergoing additional scrutiny.

Arteriosclerosis and skeletal health

Atherosclerosis, calcification, mural hypertrophy and fibrosis, and elastin matrix senescence cause arteriosclerosis, the age-associated vascular stiffening that impairs the Windkessel physiology necessary for smooth distal tissue perfusion. With aging, vascular remodelling processes can increase wall thickness and regionally reduce conduit artery lumen patency, thus worsening arterial compliance and its clinical impact.[43] In the musculoskeletal system, the lower extremities experience the brunt of arteriosclerotic disease. Claudication and amputation are the most salient manifestations that reduce mobility and increase morbidity, but hip fracture is also increased with PAD.[8] Multiple studies have now established that the presence and extent of arteriosclerotic calcification conveys lower extremity amputation risk. Medial arterial calcification in T2DM conveys a threefold increased risk of amputation.[12]

By use of CT scanning and the Agatston method to quantify TAC, Guzman *et al.* established that TAC scoring outperforms ankle–brachial indices in predicting lower extremity amputation risk.[9] Applying the conventional ankle–brachial indices method to characterize PAD, Collins *et al.* showed that PAD increased the rate of femoral neck bone loss and increased the risk of hip fracture in men.[8] They concluded that "further research should examine the biological mechanisms underlying the association between reduced limb blood flow and fractures".[8] The increasing prevalence of T2DM and associated PAD will contribute to the future arteriosclerotic disease burden in our society.

Atherosclerotic deposits can and do affect bone marrow arteries. However, the physiological responses of bone to arteriosclerosis are multifactorial. In 1985, Brenneise and Squier examined the relationship between atherosclerotic disease and blood flow in rhesus monkeys maintained on high-fat atherogenic diets.[158] These diets induced the formation of extensive calcified atherosclerotic carotid plaques, as well as atherosclerotic changes in arteries perfusing skeletal muscle. However, atherogenic diets failed to induce substantial atherosclerotic changes in the principal nutrient arteries of maxillary and mandibular bone—and did not alter osseous lumen area, intraosseous vessel wall thickness, vessel lumen area or lumen tissue area.[158] Nevertheless, atherosclerosis reduced osseous blood flow by 80% in the anterior mandible, posterior mandible and maxilla.[158]

How can this situation occur? These seminal findings indicated that macrovascular Windkessel function and endothelial function, which are necessary for regulating bone tissue perfusion, are severely impaired with atherosclerosis and arteriosclerosis.[81] Our group has described and characterized a novel mouse model of arteriosclerotic vascular stiffening—the osteopontin (OPN)-null mouse on the LDLR-deficient background. Even in the absence of atherogenic diets, male *Opn*$^{-/-}$;*Ldlr*$^{-/-}$ mice exhibit aortic adventitial fibrosis and vascular stiffening. Use of fluorescence microsphere perfusion assays demonstrated significantly reduced lower extremity bone (femur) blood flow in arteriosclerotic *Opn*$^{-/-}$;*Ldlr*$^{-/-}$ animals.[159] By contrast, blood flow to the kidneys was not significantly altered as assessed with this assay. Thus, arteriosclerosis and atherosclerosis impair skeletal blood flow.[158,159]

Of note, in healthy young long bone, diaphyseal blood flow is primarily centrifugal, with flow originating from the marrow compartment (supplied via the nutrient artery) to the trabeculae and bone cortex (Figure 5).[160] However, with aging, flow becomes increasingly centripetal, with the greatest extent of diaphyseal cortex being perfused by periosteal arteries.[161] This age-dependent change in the 'vector' of blood flow may become more exaggerated owing to the enhanced sensitivity of the nutrient artery to vasoconstrictors as compared to vasodilators.[162] With aging, the vasodilator response in bone nutrient arteries becomes progressively reduced.[163] Blood flow is a critical determinant of the bone formation rate at the basic multicellular unit (BMU) within the adult skeleton.[4] The interactions between age-dependent changes in this cortical bone flow vector and arteriosclerotic conduit vessel stiffening have not been systematically examined. As osteoblast-mediated bone formation is directly related to perfusion,[4] these age-dependent changes are predicted to contribute to reduced skeletal anabolism in the absence of intervention.

Certainly, perfusion-dependent and diffusion-dependent nutrient supplies must be rate-limiting contributors to the impairment of bone physiology with arteriosclerotic disease. Indeed, Prisby *et al.* highlighted that it is the proximity of the bone-forming BMU to the microvasculature—not the mass of the skeletal microvasculature—that is critical in maintaining bone anabolism.[164] Vascular endothelial growth factor (VEGF), a prototypic angiogenic factor produced by osteoblasts, was shown to be critically important in this process; inhibition of VEGF prevented both microvascular alignment with the BMU and bone anabolic responses to PTH.[164] Moreover, the sub-intimal vascular accumulation of oxLDL in bone impairs anabolic responses to PTH.[27] However, it has become increasingly evident that the vasculature itself provides paracrine and juxtacrine cues that regulate osteoblast functions.[165] Ephrin-B2, BMP-2, RANKL and nitric oxide are but a few of the potent osteotropic signals expressed by endothelial cells.[165–171] Furthermore, the vasculature also provides the sustentacular niche for osteoblast progenitors,[172] and is the conduit for egress of bone-marrow-derived formed elements from the osteoblast-regulated haematopoietic niche.[173] Napoli *et al.* demonstrated the capacity of endocrine pharmacotherapy targeting skeletal osteoblasts and bone marrow to enhance ischaemic limb recovery in mice.[13] Thus, multiple bone–vascular interactions may contribute to impaired skeletal anabolism with arteriosclerosis. The important preclinical 'proof of principle' supplied by Napoli *et al.*[13] provides compelling pharmacologic and physiological evidence for an emerging bone–vascular axis that regulates cardiovascular and skeletal health.

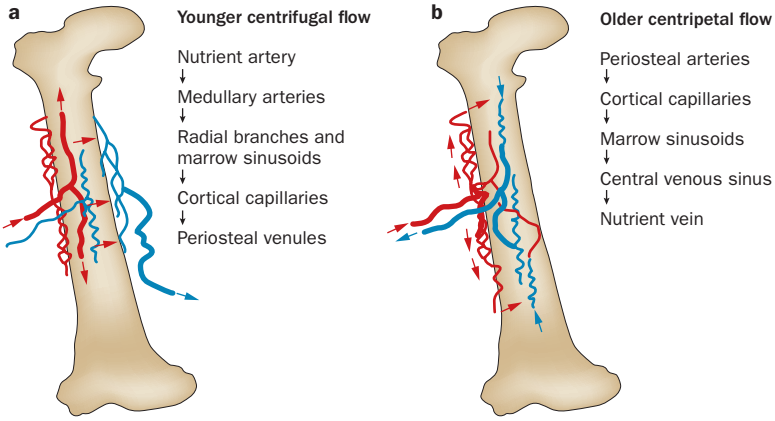


Figure 5 | Age-dependent changes in cortical blood flow of long bones. **a** | Healthy cancellous bone has a marrow flow of about 20 ml/min/100 g via the nutrient, ascending and descending medullary arteries; this flow helps maintain a fairly high intramedullary pressure that drives centrifugal flow through cortical bone (~5 ml/min/g).[160] **b** | With aging[161] and arteriosclerosis,[158,159] perfusion is altered, with blood supply to the aging cortex increasingly provided from periosteal conduit vessels.[161] Age-related and exercise-related changes in vasodilatation responses of nutrient arteries can also affect the extent of centrifugal versus centripetal cortical blood flow,[163,215] as the nutrient arteries are more responsive to vasoconstrictors.[162]

Vascular health and the bone–vascular axis

In the preceding sections the impact of vascular disease on skeletal function has been emphasized. However, in the past decade it has became clear that bone is in fact an endocrine organ,[174] capable of producing phosphaturic hormones such as DMP-1 and FGF-23, which are relevant to the pathobiology of vascular disease.[175] FGF-23 is an osteocyte-derived hormone that enhances phosphate excretion by the kidney via downregulation of proximal tubule sodium phosphate symporters.[176] Hyperphosphataemia and vascular calcification were observed in *Fgf23*$^{-/-}$ mice—mitigated by simultaneous reductions in the enzyme CYP27B1.[177] Of note, FGF-23 directly suppresses CYP27B1.[178] Thus, the vascular toxicity arising from deficiency in this bone-derived hormone is due to endogenous calcitriol intoxication with hyperphosphataemia.[179] With declining renal function, post-prandial elevations in FGF-23 are amongst the earliest changes observed,[180] and probably serve as a bone-derived defence mechanism to limit the toxicities of hyperphosphataemia in conjunction with PTH.[175] With progressive uraemia, end-organ responses to FGF-23 are diminished owing to reduced Klotho co-factor expression by the kidney and parathyroid glands.[181–185]

Of note, both FGF-23–Klotho[185,186] and DMP-1[187] signalling have been demonstrated to exert direct

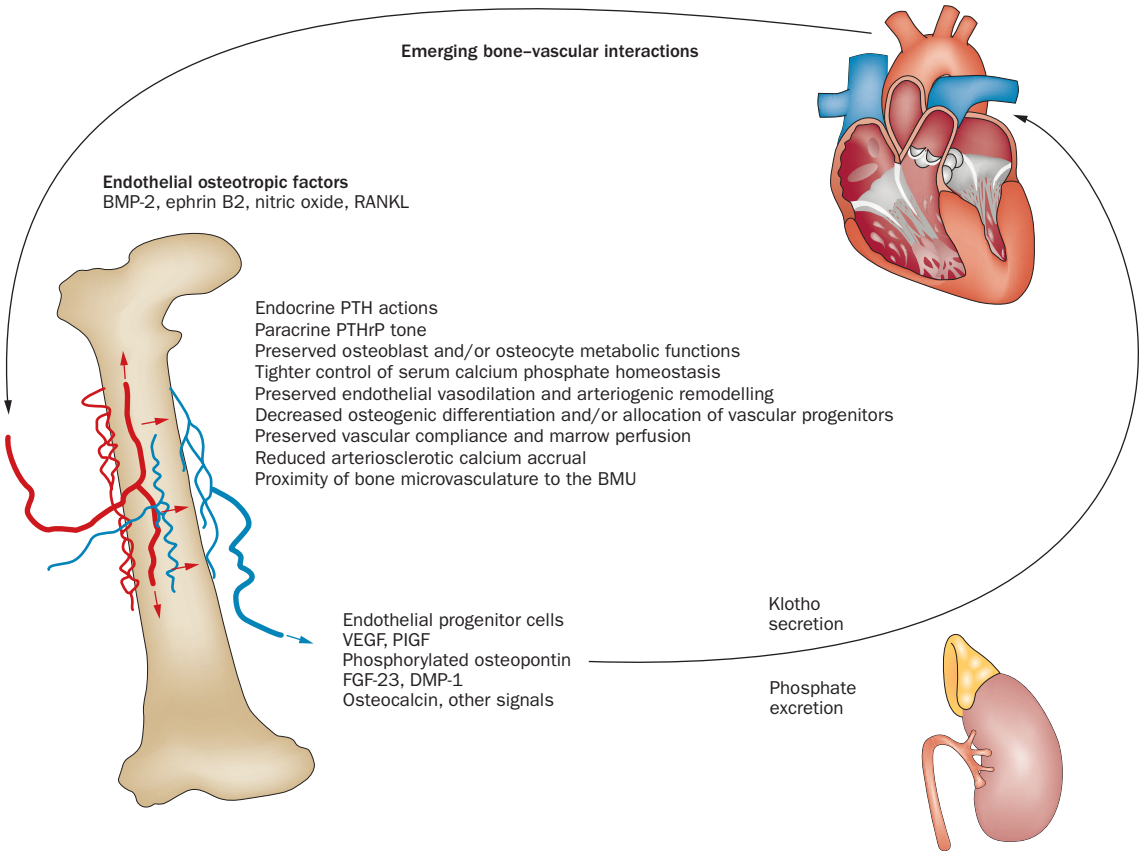


Figure 6 | Clinical promises and pitfalls of the emerging bone–vascular axis. A bidirectional endocrine relationship exists between bone and the vasculature that mutually benefits bone and vascular health. The kidney is an important intermediary in this process, via regulation of phosphate excretion[182] and expression of Klotho.[109,200] Importantly, PTH1R signalling maintains bone formation; sustains haematopoietic niche function[216] and endothelial progenitor cell mass;[13] promotes intact osteoblast osteopontin[24] and osteocyte FGF-23[198,200] secretion; supports renal Klotho production;[200] and suppresses aortic osteofibrogenic Wnt/β-catenin signalling[23,146] and vascular calcium accrual.[23,26] PTH1R signalling also reduces aortic[23] and skeletal[217] oxidative stress, and maintains the proximity of the microvasculature to the BMU during bone formation.[164] Declining renal function and tissue resistance to PTH1R signalling are key features in the perturbation of the bone–vascular axis in the setting of disease. Age-related changes in marrow composition and the vector of bone perfusion (Figure 5) may also functionally perturb the bone–vascular axis. Abbreviations: BMU, basic multicellular unit; PlGF, placental growth factor; PTH1R, PTH/PTHrP receptor; RANKL, receptor activator of nuclear factor κB ligand; VEGF, vascular endothelial growth factor. See text for details.

beneficial vascular actions. The kidney, therefore, emerges as a particularly important intermediary in the bone–vascular axis via hormonally regulated phosphate excretion and Klotho production (Figure 6). Osteopontin, a very potent inhibitor of matrix mineralization in its phosphorylated form,[188] is secreted into the circulation by skeletal osteoblasts and its levels increase in response to bone anabolic stimuli such as PTH.[24] As osteoblast-derived osteopontin is highly phosphorylated[189] and stable to proteolysis,[190] this circulating pool of osteopontin may also serve as an important defence against vascular mineral accrual, as first posited by Jono *et al.*[188]

Studies by Lee *et al.* have highlighted the important role of bone-derived osteocalcin as a Gla-dependent hormone controlling β-cell longevity.[191] With respect to arteriosclerosis, the capacity of osteocalcin to support adiponectin production by adipocytes may be of particular importance.[191] Adiponectin suppresses the inflammatory responses elicited by TNF[192,193]—an important stimulus for vascular calcification, as highlighted above.[82] Moreover, adiponectin-deficient mice develop arterial calcification that is mitigated by adenoviral-mediated vascular restoration.[194] Idelevich *et al.* have provided new evidence that osteocalcin may also directly enhance osteochondrogenic mineralization of VSMCs;[143] this finding has yet to be confirmed in atherosclerotic disease models. Nevertheless, given that bone-marrow-derived cell types can either promote or limit vascular calcium accrual, two arms of the bone–vascular axis clearly emerge: one being cell-mediated, via the paracrine actions of osteoblasts on the haematopoietic niche and cell egress; and the other being humoral, via endocrine signals that derive from osteoblasts or osteocytes and regulate renal phosphate excretion, PTH production and vascular proximity to the BMU (Figure 6).

In conclusion, with respect to diseases of the bone–vascular axis, three relationships can now be envisioned (Figure 7). Certainly, the metabolic milieu and genetics

can independently cause arteriosclerotic and bone disease. Additionally, primary changes in arteriosclerotic vessel functions arising from the metabolic milieu and genetics can cause bone disease via alterations in perfusion. However, new understanding of the bone–vascular axis indicates that primary disease changes in bone and bone marrow function arising from the metabolic milieu and genetics may, in turn, cause vascular disease. Under this last and novel view, a healthy skeleton and marrow helps preserve vascular health and function. The relative contributions of bone-derived cellular versus endocrine signals to changes in vascular physiology could be particularly important with respect to uraemia, aging and diabetes mellitus.[195] Indeed, Faul *et al.* have shown in the past year that FGF-23—which is secreted by osteoblasts and osteocytes in response to the phosphate retention that characterizes CKD—ultimately promotes left ventricular hypertrophy.[196] A better understanding of how bone-derived endocrine cues and marrow-derived cell types interact to regulated vascular health is clearly necessary.

Conclusions

Preclinical and clinical studies performed over the past two decades have converged to highlight the presence and critical role of a bone–vascular regulatory axis in human health. Not unlike the famous legend of the three blind men describing the elephant, the perspectives of experts in endocrinology, cardiology, developmental biology, orthopaedics, biochemistry, genetics, pathology, engineering and haematology often emphasize different features of the bone–vascular axis. Fortunately, the assembly of these multiple viewpoints is providing an increasingly sharper image.

Calciotropic hormones have direct and indirect vasculotropic actions deserving consideration; both excesses[197] and insufficiencies[128] in calciotropic hormones such as PTH engender vascular disease. The inability to tightly regulate calcium phosphate homeostasis results in vascular toxicity. Cellular and endocrine signals arising from bone and marrow have an impact on vascular physiology and function, and are maintained in part by PTH-dependent signals.[13,182,198] Age-dependent and disease-dependent changes in vascular physiology affect bone health and fracture risk. Inflammation, oxidative stress and oxylipid signals reciprocally regulate bone and vascular mineralization, and impair PTH1R signalling important to bone and vascular health.[29,67,199] The kidney is an important intermediary in the bone–vascular axis via hormonally regulated phosphate expression and Klotho expression.[182,200] Declining renal function and tissue resistance to PTH1R signalling represent key features in the disease-associated perturbation of the bone–vascular axis (Figure 6). Endocrine regulation of the bone–vascular axis is feasible; for example, strategies that modulate PTH1R signalling, end-organ responsiveness and calcium phosphate homeostasis offer opportunities to improve bone health and preserve vascular health in patients with diabetes mellitus, dyslipidaemia and uraemia associated with PAD.[13,23,29,127,201,202]

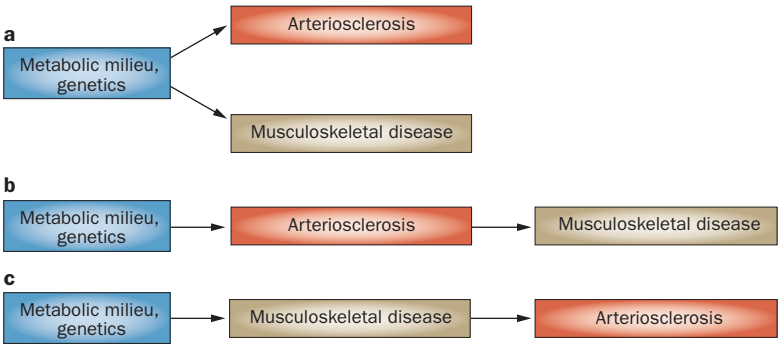


Figure 7 | Relationships between the metabolic milieu, genetics, arteriosclerosis and musculoskeletal disease. **a** | Oxylipids simultaneously drive arteriosclerotic calcification, suppress bone formation and increase osteoclastogenesis; parallel progression of arteriosclerosis and musculoskeletal disease takes place. **b** | However, via vessel stiffening and reductions in endothelium-dependent control of bone perfusion, arteriosclerosis can negatively affect bone anabolic responses necessary for skeletal homeostasis and fracture repair. **c** | Finally, osteoblasts, osteocytes and bone marrow produce cellular elements and hormonal cues that prevent arteriosclerotic remodelling and preserve vascular health; therefore, primary disease of bone may give rise to, or at least exacerbate, arteriosclerotic disease. Factors that affect the metabolic milieu include aging, diabetes mellitus, dyslipidaemia, uraemia and inflammation.

On a cautionary note, however, our understanding of the relationships between bone and vascular mineral homeostasis is rudimentary. As clinicians, we are coming to appreciate the reciprocal relationships between bone health and atherosclerosis as detected by vascular calcification;[203–206] however, the endocrine regulation of this relationship might change with age. For example, aminobisphosphonate therapy for osteoporosis decreases the risk of aortic valve and thoracic aorta calcification in women aged >75 years but increases risk in women under age 55 years.[207] Furthermore, although helpful to bone in some contexts, oral calcium supplementation in patients with renal insufficiency[127] and advanced age[208,209] may have a negative effect on cardiovascular health. As our understanding of the bone–vascular axis continues to improve, so too will our capacity to meet the clinical needs of patients with musculoskeletal and vascular diseases.

Review criteria

PubMed was searched for articles published in 1970–2011, emphasizing literature from the past decade. Except for two didactic abstracts, only full-text papers published in English were considered for inclusion. Primary search terms included "vascular calcification", "valve calcification", "arterial calcification", "atherosclerotic calcification", "arteriosclerosis", "diabetic arteriosclerosis", "medial calcification", "Monckeberg's sclerosis", "coronary artery calcification", "calcific aortic stenosis", "calcific aortic valve disease", "valve sclerosis", "calciphylaxis", "calcific uremic arteriolopathy", "calcific vasculopathy", "peripheral arterial disease", "lower extremity amputation", "calcium metabolism hyperphosphatemia", "osteoporosis", "fracture", "fracture repair", "bone vascularization", "bone perfusion", "bone blood flow", "bone formation", "ossification", "avascular necrosis", "osteonecrosis", "Windkessel physiology", and "CKD–MBD".

1. Zelzer, E. *et al.* Skeletal defects in VEGF(120/120) mice reveal multiple roles for VEGF in skeletogenesis. *Development* **129**, 1893–1904 (2002).
2. Maes, C. *et al.* Osteoblast precursors, but not mature osteoblasts, move into developing and fractured bones along with invading blood vessels. *Dev. Cell* **19**, 329–344 (2010).
3. Qing, H. *et al.* PTHR1 in osteocytes plays a major role in perilacunar remodeling through the activation of "osteoclastic" genes in osteocytes [abstract]. *J. Bone Miner. Res.* **25** (Suppl. 1), a1082 (2010).
4. Reeve, J. *et al.* Skeletal blood flow, iliac histomorphometry, and strontium kinetics in osteoporosis: a relationship between blood flow and corrected apposition rate. *J. Clin. Endocrinol. Metab.* **66**, 1124–1131 (1988).
5. Bianco, P. Bone and the hematopoietic niche: a tale of two stem cells. *Blood* **117**, 5281–5288 (2011).
6. Eash, K. J., Greenbaum, A. M., Gopalan, P. K. & Link, D. C. CXCR2 and CXCR4 antagonistically regulate neutrophil trafficking from murine bone marrow. *J. Clin. Invest.* **120**, 2423–2431 (2010).
7. Towler, D. A. The osteogenic-angiogenic interface: novel insights into the biology of bone formation and fracture repair. *Curr. Osteoporos. Rep.* **6**, 67–71 (2008).
8. Collins, T. C. *et al.* Peripheral arterial disease is associated with higher rates of hip bone loss and increased fracture risk in older men. *Circulation* **119**, 2305–2312 (2009).
9. Guzman, R. J. *et al.* Tibial artery calcification as a marker of amputation risk in patients with peripheral arterial disease. *J. Am. Coll. Cardiol.* **51**, 1967–1974 (2008).
10. Soor, G. S., Vukin, I., Leong, S. W., Oreopoulos, G. & Butany, J. Peripheral vascular disease: who gets it and why? A histomorphological analysis of 261 arterial segments from 58 cases. *Pathology* **40**, 385–391 (2008).
11. Everhart, J. E., Pettitt, D. J., Knowler, W. C., Rose, F. A. & Bennett, P. H. Medial arterial calcification and its association with mortality and complications of diabetes. *Diabetologia* **31**, 16–23 (1988).
12. Lehto, S., Niskanen, L., Suhonen, M., Rönnemaa, T. & Laakso, M. Medial artery calcification. A neglected harbinger of cardiovascular complications in non-insulin-dependent diabetes mellitus. *Arterioscler. Thromb. Vasc. Biol.* **16**, 978–983 (1996).
13. Napoli, C. *et al.* Therapeutic targeting of the stem cell niche in experimental hindlimb ischemia. *Nat. Clin. Pract. Cardiovasc. Med.* **5**, 571–579 (2008).
14. Gössl, M., Mödder, U. I., Atkinson, E. J., Lerman, A. & Khosla, S. Osteocalcin expression by circulating endothelial progenitor cells in patients with coronary atherosclerosis. *J. Am. Coll. Cardiol.* **52**, 1314–1325 (2008).
15. Gössl, M. *et al.* Coronary endothelial dysfunction in humans is associated with coronary retention of osteogenic endothelial progenitor cells. *Eur. Heart J.* **31**, 2909–2914 (2010).
16. Egan, K. P., Kim, J. H., Mohler, E. R. 3rd & Pignolo, R. J. Role for circulating osteogenic precursor cells in aortic valvular disease. *Arterioscler. Thromb. Vasc. Biol.* **31**, 2965–2971 (2011).
17. Eghbali-Fatourechi, G. Z. *et al.* Circulating osteoblast-lineage cells in humans. *N. Engl. J. Med.* **352**, 1959–1966 (2005).
18. Shen, X. *et al.* Prolyl hydroxylase inhibitors increase neoangiogenesis and callus formation following femur fracture in mice. *J. Orthop. Res.* **27**, 1298–1305 (2009).
19. Wan, C. *et al.* Activation of the hypoxia-inducible factor-1α pathway accelerates bone regeneration. *Proc. Natl Acad. Sci. USA* **105**, 686–691 (2008).
20. Zelzer, E. & Olsen, B. R. Multiple roles of vascular endothelial growth factor (VEGF) in skeletal development, growth, and repair. *Curr. Top. Dev. Biol.* **65**, 169–187 (2005).
21. Demer, L. L. & Tintut, Y. Vascular calcification: pathobiology of a multifaceted disease. *Circulation* **117**, 2938–2948 (2008).
22. Shao, J. S., Cheng, S. L., Sadhu, J. & Towler, D. A. Inflammation and the osteogenic regulation of vascular calcification: a review and perspective. *Hypertension* **55**, 579–592 (2010).
23. Cheng, S. L. *et al.* Activation of vascular smooth muscle parathyroid hormone receptor inhibits Wnt/beta-catenin signaling and aortic fibrosis in diabetic arteriosclerosis. *Circ. Res.* **107**, 271–282 (2010).
24. Shao, J. S., Cheng, S. L., Charlton-Kachigian, N., Loewy, A. P. & Towler, D. A. Teriparatide (human parathyroid hormone (1–34)) inhibits osteogenic vascular calcification in diabetic low density lipoprotein receptor-deficient mice. *J. Biol. Chem.* **278**, 50195–50202 (2003).
25. Shao, J. S. *et al.* Msx2 promotes cardiovascular calcification by activating paracrine Wnt signals. *J. Clin. Invest.* **115**, 1210–1220 (2005).
26. Sebastian, E. M., Suva, L. J. & Friedman, P. A. Differential effects of intermittent PTH(1–34) and PTH(7–34) on bone microarchitecture and aortic calcification in experimental renal failure. *Bone* **43**, 1022–1030 (2008).
27. Huang, M. S. *et al.* Hyperlipidemia impairs osteoanabolic effects of PTH. *J. Bone Miner. Res.* **23**, 1672–1679 (2008).
28. Huang, M. S. *et al.* Atherogenic phospholipids attenuate osteogenic signaling by BMP-2 and parathyroid hormone in osteoblasts. *J. Biol. Chem.* **282**, 21237–21243 (2007).
29. Sage, A. P. *et al.* Hyperlipidemia induces resistance to PTH bone anabolism in mice via oxidized lipids. *J. Bone Miner. Res.* **26**, 1197–1206 (2011).
30. Murphy, W. A. Jr *et al.* The iceman: discovery and imaging. *Radiology* **226**, 614–629 (2003).
31. Polonsky, T. S. *et al.* Coronary artery calcium score and risk classification for coronary heart disease prediction. *JAMA* **303**, 1610–1616 (2010).
32. Rosenhek, R. *et al.* Mild and moderate aortic stenosis. Natural history and risk stratification by echocardiography. *Eur. Heart J.* **25**, 199–205 (2004).
33. Rosenhek, R. *et al.* Predictors of outcome in severe, asymptomatic aortic stenosis. *N. Engl. J. Med.* **343**, 611–617 (2000).
34. London, G. M. *et al.* Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. *Nephrol. Dial. Transplant.* **18**, 1731–1740 (2003).
35. Bos, D. *et al.* Calcification in major vessel beds relates to vascular brain disease. *Arterioscler. Thromb. Vasc. Biol.* **31**, 2331–2337 (2011).
36. Stary, H. C. Natural history and histological classification of atherosclerotic lesions: an update. *Arterioscler. Thromb. Vasc. Biol.* **20**, 1177–1178 (2000).
37. Burke, A. P., Kolodgie, F. D., Farb, A., Weber, D. & Virmani, R. Morphological predictors of arterial remodeling in coronary atherosclerosis. *Circulation* **105**, 297–303 (2002).
38. Ward, M. R., Pasterkamp, G., Yeung, A. C. & Borst, C. Arterial remodeling. Mechanisms and clinical implications. *Circulation* **102**, 1186–1191 (2000).
39. Rajamannan, N. M. Calcific aortic stenosis: lessons learned from experimental and clinical studies. *Arterioscler. Thromb. Vasc. Biol.* **29**, 162–168 (2009).
40. Rajamannan, N. M., Bonow, R. O. & Rahimtoola, S. H. Calcific aortic stenosis: an update. *Nat. Clin. Pract. Cardiovasc. Med.* **4**, 254–262 (2007).
41. Greenwald, S. E. Ageing of the conduit arteries. *J. Pathol.* **211**, 157–172 (2007).
42. Westerhof, N., Lankhaar, J. W. & Westerhof, B. E. The arterial Windkessel. *Med. Biol. Eng. Comput.* **47**, 131–141 (2009).
43. O'Rourke, M. F. Arterial aging: pathophysiological principles. *Vasc. Med.* **12**, 329–341 (2007).
44. Safar, M. E. & Boudier, H. S. Vascular development, pulse pressure, and the mechanisms of hypertension. *Hypertension* **46**, 205–209 (2005).
45. Virchow, R. & Chance, F. *Cellular pathology, as based upon physiological and pathological histology. Twenty lectures delivered in the Pathological Institute of Berlin during the months of February, March and April, 1858* (R. M. De Witt, New York, 1860).
46. Virchow, R. Cellular pathology. As based upon physiological and pathological histology. Lecture XVI—Atheromatous affection of arteries. 1858. *Nutr. Rev.* **47**, 23–25 (1989).
47. Mohler, E. R. 3rd *et al.* Bone formation and inflammation in cardiac valves. *Circulation* **103**, 1522–1528 (2001).
48. Tyson, K. L. *et al.* Osteo/chondrocytic transcription factors and their target genes exhibit distinct patterns of expression in human arterial calcification. *Arterioscler. Thromb. Vasc. Biol.* **23**, 489–494 (2003).
49. Hessle, L. *et al.* Tissue-nonspecific alkaline phosphatase and plasma cell membrane glycoprotein-1 are central antagonistic regulators of bone mineralization. *Proc. Natl Acad. Sci. USA* **99**, 9445–9449 (2002).
50. Tanimura, A., McGregor, D. H. & Anderson, H. C. Calcification in atherosclerosis. I. Human studies. *J. Exp. Pathol.* **2**, 261–273 (1986).
51. Tanimura, A., McGregor, D. H. & Anderson, H. C. Calcification in atherosclerosis. II. Animal studies. *J. Exp. Pathol.* **2**, 275–297 (1986).
52. Boström, K. *et al.* Bone morphogenetic protein expression in human atherosclerotic lesions. *J. Clin. Invest.* **91**, 1800–1809 (1993).
53. Zhang, M. *et al.* Atorvastatin downregulates BMP-2 expression induced by oxidized low-density lipoprotein in human umbilical vein endothelial cells. *Circ. J.* **72**, 807–812 (2008).
54. Cola, C., Almeida, M., Li, D., Romeo, F. & Mehta, J. L. Regulatory role of endothelium in the expression of genes affecting arterial calcification. *Biochem. Biophys. Res. Commun.* **320**, 424–427 (2004).
55. Tintut, Y. *et al.* Monocyte/macrophage regulation of vascular calcification *in vitro*. *Circulation* **105**, 650–655 (2002).
56. Yao, Y. *et al.* Inhibition of bone morphogenetic proteins protects against atherosclerosis and vascular calcification. *Circ. Res.* **107**, 485–494 (2010).
57. Towler, D. A. & Demer, L. L. Thematic series on the pathobiology of vascular calcification: an introduction. *Circ. Res.* **108**, 1378–1380 (2011).
58. Stary, H. C. *et al.* A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation* **92**, 1355–1374 (1995).
59. Chung, U. I., Kawaguchi, H., Takato, T. & Nakamura, K. Distinct osteogenic mechanisms of bones of distinct origins. *J. Orthop. Sci.* **9**, 410–414 (2004).

60. Cohen, M. M. Jr. The new bone biology: pathologic, molecular, and clinical correlates. *Am. J. Med. Genet. A* **140**, 2646–2706 (2006).
61. Abzhanov, A., Rodda, S. J., McMahon, A. P. & Tabin, C. J. Regulation of skeletogenic differentiation in cranial dermal bone. *Development* **134**, 3133–3144 (2007).
62. Karsenty, G. Transcriptional control of skeletogenesis. *Annu. Rev. Genomics Hum. Genet.* **9**, 183–196 (2008).
63. Aikawa, E. *et al.* Multimodality molecular imaging identifies proteolytic and osteogenic activities in early aortic valve disease. *Circulation* **115**, 377–386 (2007).
64. Steitz, S. A. *et al.* Smooth muscle cell phenotypic transition associated with calcification: upregulation of Cbfa1 and downregulation of smooth muscle lineage markers. *Circ. Res.* **89**, 1147–1154 (2001).
65. Speer, M. Y. *et al.* Smooth muscle cells give rise to osteochondrogenic precursors and chondrocytes in calcifying arteries. *Circ. Res.* **104**, 733–741 (2009).
66. Speer, M. Y., Li, X., Hiremath, P. G. & Giachelli, C. M. Runx2/Cbfa1, but not loss of myocardin, is required for smooth muscle cell lineage reprogramming toward osteochondrogenesis. *J. Cell. Biochem.* **110**, 935–947 (2010).
67. Demer, L. & Tintut, Y. The roles of lipid oxidation products and receptor activator of nuclear factor-kappaB signaling in atherosclerotic calcification. *Circ. Res.* **108**, 1482–1493 (2011).
68. Richardson, J. A. *et al.* Oxysterol-induced osteoblastic differentiation of pluripotent mesenchymal cells is mediated through a PKC- and PKA-dependent pathway. *J. Cell. Biochem.* **100**, 1131–1145 (2007).
69. Byon, C. H. *et al.* Oxidative stress induces vascular calcification through modulation of the osteogenic transcription factor Runx2 by AKT signaling. *J. Biol. Chem.* **283**, 15319–15327 (2008).
70. Narisawa, S. *et al.* Novel inhibitors of alkaline phosphatase suppress vascular smooth muscle cell calcification. *J. Bone Miner. Res.* **22**, 1700–1710 (2007).
71. Parhami, F. *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.* **17**, 680–687 (1997).
72. Parhami, F. *et al.* Atherogenic high-fat diet reduces bone mineralization in mice. *J. Bone Miner. Res.* **16**, 182–188 (2001).
73. Sage, A. P., Lu, J., Tintut, Y. & Demer, L. L. Hyperphosphatemia-induced nanocrystals upregulate the expression of bone morphogenetic protein-2 and osteopontin genes in mouse smooth muscle cells *in vitro*. *Kidney Int.* **79**, 414–422 (2011).
74. Sage, A. P., Tintut, Y. & Demer, L. L. Regulatory mechanisms in vascular calcification. *Nat. Rev. Cardiol.* **7**, 528–536 (2010).
75. Tintut, Y. *et al.* Multilineage potential of cells from the artery wall. *Circulation* **108**, 2505–2510 (2003).
76. Collett, G. D. & Canfield, A. E. Angiogenesis and pericytes in the initiation of ectopic calcification. *Circ. Res.* **96**, 930–938 (2005).
77. Nelson, R. G. *et al.* Lower-extremity amputations in NIDDM. 12-yr follow-up study in Pima Indians. *Diabetes Care* **11**, 8–16 (1988).
78. Leskinen, Y., Salenius, J. P., Lehtimäki, T., Huhtala, H. & Saha, H. The prevalence of peripheral arterial disease and medial arterial calcification in patients with chronic renal failure: requirements for diagnostics. *Am. J. Kidney Dis.* **40**, 472–479 (2002).
79. Towler, D. A. Vascular calcification: A perspective on an imminent disease epidemic. *IBMS BoneKEy* **5**, 41–58 (2008).
80. Robbins, S. L., Cotran, R. S. & Kumar, V. *Pathologic Basis of Disease* (Saunders, Philadelphia, 1984).
81. O'Rourke, M. F. & Hashimoto, J. Mechanical factors in arterial aging: a clinical perspective. *J. Am. Coll. Cardiol.* **50**, 1–13 (2007).
82. Al-Aly, Z. *et al.* Aortic Msx2-Wnt calcification cascade is regulated by TNF-α-dependent signals in diabetic Ldlr$^{-/-}$ mice. *Arterioscler. Thromb. Vasc. Biol.* **27**, 2589–2596 (2007).
83. Qiao, J. H., Mertens, R. B., Fishbein, M. C. & Geller, S. A. Cartilaginous metaplasia in calcified diabetic peripheral vascular disease: morphologic evidence of enchondral ossification. *Hum. Pathol.* **34**, 402–407 (2003).
84. Satokata, I. *et al.* Msx2 deficiency in mice causes pleiotropic defects in bone growth and ectodermal organ formation. *Nat. Genet.* **24**, 391–395 (2000).
85. Towler, D. A., Bidder, M., Latifi, T., Coleman, T. & Semenkovich, C. F. Diet-induced diabetes activates an osteogenic gene regulatory program in the aortas of low density lipoprotein receptor-deficient mice. *J. Biol. Chem.* **273**, 30427–30434 (1998).
86. Canfield, A. E. *et al.* Role of pericytes in vascular calcification: a review. *Z. Kardiol.* **89** (Suppl. 2), 20–27 (2000).
87. Zhang, L. *et al.* Diabetes-induced oxidative stress and low-grade inflammation in porcine coronary arteries. *Circulation* **108**, 472–478 (2003).
88. Csiszar, A. *et al.* Regulation of bone morphogenetic protein-2 expression in endothelial cells: role of nuclear factor-kappaB activation by tumor necrosis factor-α, H2O2, and high intravascular pressure. *Circulation* **111**, 2364–2372 (2005).
89. Rawadi, G., Vayssière, B., Dunn, F., Baron, R. & Roman-Roman, S. BMP-2 controls alkaline phosphatase expression and osteoblast mineralization by a Wnt autocrine loop. *J. Bone Miner. Res.* **18**, 1842–1853 (2003).
90. Cheng, S. L., Shao, J. S., Charlton-Kachigian, N., Loewy, A. P. & Towler, D. A. MSX2 promotes osteogenesis and suppresses adipogenic differentiation of multipotent mesenchymal progenitors. *J. Biol. Chem.* **278**, 45969–45977 (2003).
91. Shao, J. S., Cai, J. & Towler, D. A. Molecular mechanisms of vascular calcification: lessons learned from the aorta. *Arterioscler. Thromb. Vasc. Biol.* **26**, 1423–1430 (2006).
92. Cheng, S. L., Shao, J. S., Cai, J., Sierra, O. L. & Towler, D. A. Msx2 exerts bone anabolism via canonical Wnt signaling. *J. Biol. Chem.* **283**, 20505–20522 (2008).
93. Boström, K. I., Rajamannan, N. M. & Towler, D. A. The regulation of valvular and vascular sclerosis by osteogenic morphogens. *Circ. Res.* **109**, 564–577 (2011).
94. Orrico, C. *et al.* Dysfunctional vasa vasorum in diabetic peripheral artery obstructive disease with critical lower limb ischaemia. *Eur. J. Vasc. Endovasc. Surg.* **40**, 365–374 (2010).
95. Hayden, M. R. & Tyagi, S. C. Vasa vasorum in plaque angiogenesis, metabolic syndrome, type 2 diabetes mellitus, and atherosclerosis: a malignant transformation. *Cardiovasc. Diabetol.* **3**, 1 (2004).
96. Buján, J. *et al.* Modifications induced by atherogenic diet in the capacity of the arterial wall in rats to respond to surgical insult. *Atherosclerosis* **122**, 141–152 (1996).
97. Miller, J. D. *et al.* Dysregulation of antioxidant mechanisms contributes to increased oxidative stress in calcific aortic valvular stenosis in humans. *J. Am. Coll. Cardiol.* **52**, 843–850 (2008).
98. Caira, F. C. *et al.* Human degenerative valve disease is associated with up-regulation of low-density lipoprotein receptor-related protein 5 receptor-mediated bone formation. *J. Am. Coll. Cardiol.* **47**, 1707–1712 (2006).
99. Rajamannan, N. M. The role of Lrp5/6 in cardiac valve disease: experimental hypercholesterolemia in the ApoE$^{-/-}$/Lrp5$^{-/-}$ mice. *J. Cell. Biochem.* **112**, 2987–2991 (2011).
100. Hellberg, C., Ostman, A. & Heldin, C. H. PDGF and vessel maturation. *Recent Results Cancer Res.* **180**, 103–114 (2010).
101. Fadini, G. P. *et al.* Widespread increase in myeloid calcifying cells contributes to ectopic vascular calcification in type 2 diabetes. *Circ. Res.* **108**, 1112–1121 (2011).
102. Pignolo, R. J. & Shore, E. M. Circulating osteogenic precursor cells. *Crit. Rev. Eukaryot. Gene Expr.* **20**, 171–180 (2010).
103. Hruska, K. A., Choi, E. T., Memon, I., Davis, T. K. & Mathew, S. Cardiovascular risk in chronic kidney disease (CKD): the CKD-mineral bone disorder (CKD–MBD). *Pediatr. Nephrol.* **25**, 769–778 (2010).
104. Moe, S. M., Drüeke, T., Lameire, N. & Eknoyan, G. Chronic kidney disease-mineral-bone disorder: a new paradigm. *Adv. Chronic Kidney Dis.* **14**, 3–12 (2007).
105. Foley, R. N. & Collins, A. J. End-stage renal disease in the United States: an update from the United States Renal Data System. *J. Am. Soc. Nephrol.* **18**, 2644–2648 (2007).
106. London, G. M. Cardiovascular calcifications in uremic patients: clinical impact on cardiovascular function. *J. Am. Soc. Nephrol.* **14** (Suppl. 4), S305–S309 (2003).
107. Ishimura, E. *et al.* Different risk factors for vascular calcification in end-stage renal disease between diabetics and nondiabetics: the respective importance of glycemic and phosphate control. *Kidney Blood Press. Res.* **31**, 10–15 (2008).
108. Mizobuchi, M., Towler, D. & Slatopolsky, E. Vascular calcification: the killer of patients with chronic kidney disease. *J. Am. Soc. Nephrol.* **20**, 1453–1464 (2009).
109. Moe, S. M. *et al.* The pathophysiology of early stage chronic kidney disease-mineral bone disorder (CKD–MBD) and response to phosphate binders in the rat. *J. Bone Miner. Res.* **26**, 2672–2681 (2011).
110. Foley, R. N., Collins, A. J., Herzog, C. A., Ishani, A. & Kalra, P. A. Serum phosphate and left ventricular hypertrophy in young adults: the coronary artery risk development in young adults study. *Kidney Blood Press. Res.* **32**, 37–44 (2009).
111. Foley, R. N., Collins, A. J., Herzog, C. A., Ishani, A. & Kalra, P. A. Serum phosphorus levels associate with coronary atherosclerosis in young adults. *J. Am. Soc. Nephrol.* **20**, 397–404 (2009).
112. Reynolds, J. L. *et al.* Human vascular smooth muscle cells undergo vesicle-mediated calcification in response to changes in extracellular calcium and phosphate concentrations: a potential mechanism for accelerated vascular calcification in ESRD. *J. Am. Soc. Nephrol.* **15**, 2857–2867 (2004).
113. Reynolds, J. L. *et al.* Multifunctional roles for serum protein fetuin-a in inhibition of human vascular smooth muscle cell calcification. *J. Am. Soc. Nephrol.* **16**, 2920–2930 (2005).

114. Jahnen-Dechent, W., Heiss, A., Schäfer, C. & Ketteler, M. Fetuin-A regulation of calcified matrix metabolism. *Circ. Res.* **108**, 1494–1509 (2011).
115. Ciaccio, M. *et al.* Changes in serum fetuin-A and inflammatory markers levels in end-stage renal disease (ESRD): effect of a single session haemodialysis. *Clin. Chem. Lab. Med.* **46**, 212–214 (2008).
116. Villa-Bellosta, R., Levi, M. & Sorribas, V. Vascular smooth muscle cell calcification and SLC20 inorganic phosphate transporters: effects of PDGF, TNF-α, and Pi. *Pflugers Arch.* **458**, 1151–1161 (2009).
117. Li, X., Yang, H. Y. & Giachelli, C. M. BMP-2 promotes phosphate uptake, phenotypic modulation, and calcification of human vascular smooth muscle cells. *Atherosclerosis* **199**, 271–277 (2008).
118. Shroff, R. C. *et al.* Dialysis accelerates medial vascular calcification in part by triggering smooth muscle cell apoptosis. *Circulation* **118**, 1748–1757 (2008).
119. Nadra, I. *et al.* 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? *Circ. Res.* **96**, 1248–1256 (2005).
120. Boström, K. Proinflammatory vascular calcification. *Circ. Res.* **96**, 1219–1220 (2005).
121. Koleganova, N. *et al.* Arterial calcification in patients with chronic kidney disease. *Nephrol. Dial. Transplant.* **24**, 2488–2496 (2009).
122. Fellström, B. C. *et al.* Rosuvastatin and cardiovascular events in patients undergoing hemodialysis. *N. Engl. J. Med.* **360**, 1395–1407 (2009).
123. Baigent, C. *et al.* The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial. *Lancet* **377**, 2181–2192 (2011).
124. Block, G. A., Raggi, P., Bellasi, A., Kooienga, L. & Spiegel, D. M. Mortality effect of coronary calcification and phosphate binder choice in incident hemodialysis patients. *Kidney Int.* **71**, 438–441 (2007).
125. Moe, S. M. & Chertow, G. M. The case against calcium-based phosphate binders. *Clin. J. Am. Soc. Nephrol.* **1**, 697–703 (2006).
126. Raggi, P., Vukicevic, S., Moysés, R. M., Wesseling, K. & Spiegel, D. M. Ten-year experience with sevelamer and calcium salts as phosphate binders. *Clin. J. Am. Soc. Nephrol.* **5** (Suppl. 1), S31–S40 (2010).
127. Raggi, P. *et al.* Decrease in thoracic vertebral bone attenuation with calcium-based phosphate binders in hemodialysis. *J. Bone Miner. Res.* **20**, 764–772 (2005).
128. London, G. M. *et al.* Arterial calcifications and bone histomorphometry in end-stage renal disease. *J. Am. Soc. Nephrol.* **15**, 1943–1951 (2004).
129. Demer, L. & Tintut, Y. The bone–vascular axis in chronic kidney disease. *Curr. Opin. Nephrol. Hypertens.* **19**, 349–353 (2010).
130. Findlay, D. M. & Martin, T. J. Receptors of calciotropic hormones. *Horm. Metab. Res.* **29**, 128–134 (1997).
131. Chambliss, K. L. *et al.* Estrogen receptor α and endothelial nitric oxide synthase are organized into a functional signaling module in caveolae. *Circ. Res.* **87**, E44–E52 (2000).
132. Ferreira, J. A., Pompei, L. M., Fernandes, C. E., Azevedo, L. H. & Peixoto, S. Breast arterial calcification is a predictive factor of cardiovascular disease in Brazilian postmenopausal women. *Climacteric* **12**, 439–444 (2009).
133. Barrett-Connor, E. & Laughlin, G. A. Hormone therapy and coronary artery calcification in asymptomatic postmenopausal women: the Rancho Bernardo Study. *Menopause* **12**, 40–48 (2005).
134. Rossouw, J. E. *et al.* Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. *JAMA* **297**, 1465–1477 (2007).
135. Van Den Bosch, M. A. *et al.* The RATIO study: oral contraceptives and the risk of peripheral arterial disease in young women. *J. Thromb. Haemost.* **1**, 439–444 (2003).
136. Bukoski, R. D. & Kremer, D. Calcium-regulating hormones in hypertension: vascular actions. *Am. J. Clin. Nutr.* **54** (Suppl.), 220S–226S (1991).
137. Holick, M. F. Sunlight and vitamin D: both good for cardiovascular health. *J. Gen. Intern. Med.* **17**, 733–735 (2002).
138. Brewer, L. C., Michos, E. D. & Reis, J. P. Vitamin D in atherosclerosis, vascular disease, and endothelial function. *Curr. Drug Targets* **12**, 54–60 (2011).
139. Oh, J. *et al.* 1,25$(OH)_2$ vitamin D inhibits foam cell formation and suppresses macrophage cholesterol uptake in patients with type 2 diabetes mellitus. *Circulation* **120**, 687–698 (2009).
140. Neuhouser, M. L. *et al.* Multivitamin use and risk of cancer and cardiovascular disease in the Women's Health Initiative cohorts. *Arch. Intern. Med.* **169**, 294–304 (2009).
141. Querfeld, U. & Mak, R. H. Vitamin D deficiency and toxicity in chronic kidney disease: in search of the therapeutic window. *Pediatr. Nephrol.* **25**, 2413–2430 (2010).
142. Price, P. A., Williamson, M. K., Nguyen, T. M. & Than, T. N. Serum levels of the fetuin-mineral complex correlate with artery calcification in the rat. *J. Biol. Chem.* **279**, 1594–1600 (2004).
143. Idelevich, A., Rais, Y. & Monsonego-Ornan, E. Bone Gla protein increases HIF-1α-dependent glucose metabolism and induces cartilage and vascular calcification. *Arterioscler. Thromb. Vasc. Biol.* **31**, e55–e71 (2011).
144. Alam, M. U. *et al.* Calcification is associated with loss of functional calcium-sensing receptor in vascular smooth muscle cells. *Cardiovasc. Res.* **81**, 260–268 (2009).
145. Henley, C. *et al.* 1,25-Dihydroxyvitamin = D3 but not cinacalcet HCl (Sensipar/Mimpara) treatment mediates aortic calcification in a rat model of secondary hyperparathyroidism. *Nephrol. Dial. Transplant.* **20**, 1370–1377 (2005).
146. Jono, S., Nishizawa, Y., Shioi, A. & Morii, H. Parathyroid hormone-related peptide as a local regulator of vascular calcification. Its inhibitory action on *in vitro* calcification by bovine vascular smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.* **17**, 1135–1142 (1997).
147. Fiaschi-Taesch, N. *et al.* Mutant parathyroid hormone-related protein, devoid of the nuclear localization signal, markedly inhibits arterial smooth muscle cell cycle and neointima formation by coordinate up-regulation of p15Ink4b and p27kip1. *Endocrinology* **150**, 1429–1439 (2009).
148. Fiaschi-Taesch, N., Takane, K. K., Masters, S., Lopez-Talavera, J. C. & Stewart, A. F. Parathyroid-hormone-related protein as a regulator of pRb and the cell cycle in arterial smooth muscle. *Circulation* **110**, 177–185 (2004).
149. Qian, J. *et al.* Midgestational lethality in mice lacking the parathyroid hormone (PTH)/PTH-related peptide receptor is associated with abrupt cardiomyocyte death. *Endocrinology* **144**, 1053–1061 (2003).
150. Nyby, M. D. *et al.* Desensitization of vascular tissue to parathyroid hormone and parathyroid hormone-related protein. *Endocrinology* **136**, 2497–2504 (1995).
151. Schipani, E., Kruse, K. & Jüppner, H. A constitutively active mutant PTH–PTHrP receptor in Jansen-type metaphyseal chondrodysplasia. *Science* **268**, 98–100 (1995).
152. Langub, M. C. *et al.* Administration of PTH-(7–84) antagonizes the effects of PTH-(1–84) on bone in rats with moderate renal failure. *Endocrinology* **144**, 1135–1138 (2003).
153. Walker, M. D. *et al.* Carotid vascular abnormalities in primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **94**, 3849–3856 (2009).
154. Maeda, S. *et al.* Targeted overexpression of parathyroid hormone-related protein (PTHrP) to vascular smooth muscle in transgenic mice lowers blood pressure and alters vascular contractility. *Endocrinology* **140**, 1815–1825 (1999).
155. Song, G. J. *et al.* EBP50 inhibits the anti-mitogenic action of the parathyroid hormone type 1 receptor in vascular smooth muscle cells. *J. Mol. Cell. Cardiol.* **49**, 1012–1021 (2010).
156. Suttamanatwong, S., Franceschi, R. T., Carlson, A. E. & Gopalakrishnan, R. Regulation of matrix Gla protein by parathyroid hormone in MC3T3-E1 osteoblast-like cells involves protein kinase A and extracellular signal-regulated kinase pathways. *J. Cell. Biochem.* **102**, 496–505 (2007).
157. Gopalakrishnan, R., Suttamanatwong, S., Carlson, A. E. & Franceschi, R. T. Role of matrix Gla protein in parathyroid hormone inhibition of osteoblast mineralization. *Cells Tissues Organs* **181**, 166–175 (2005).
158. Brenneise, C. V. & Squier, C. A. Blood flow in maxilla and mandible of normal and atherosclerotic rhesus monkeys. *J. Oral Pathol.* **14**, 800–808 (1985).
159. Shao, J. S. *et al.* Vascular calcification and aortic fibrosis: a bifunctional role for osteopontin in diabetic arteriosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **31**, 1821–1833 (2011).
160. McCarthy, I. The physiology of bone blood flow: a review. *J. Bone Joint Surg. Am.* **88** (Suppl. 3), 4–9 (2006).
161. Bridgeman, G. & Brookes, M. Blood supply to the human femoral diaphysis in youth and senescence. *J. Anat.* **188**, 611–621 (1996).
162. Brinker, M. R., Lippton, H. L., Cook, S. D. & Hyman, A. L. Pharmacological regulation of the circulation of bone. *J. Bone Joint Surg. Am.* **72**, 964–975 (1990).
163. Prisby, R. D. *et al.* Aging reduces skeletal blood flow, endothelium-dependent vasodilation, and NO bioavailability in rats. *J. Bone Miner. Res.* **22**, 1280–1288 (2007).
164. Prisby, R. *et al.* Intermittent PTH(1–84) is osteoanabolic but not osteoangiogenic and relocates bone marrow blood vessels closer to bone forming sites. *J. Bone Miner. Res.* **26**, 2583–2596 (2011).
165. Bouletreau, P. J. *et al.* Hypoxia and VEGF up-regulate BMP-2 mRNA and protein expression in microvascular endothelial cells: implications for fracture healing. *Plast. Reconstr. Surg.* **109**, 2384–2397 (2002).
166. Zhao, C. *et al.* Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis. *Cell Metab.* **4**, 111–121 (2006).
167. Bochenek, M. L., Dickinson, S., Astin, J. W., Adams, R. H. & Nobes, C. D. Ephrin-B2 regulates

endothelial cell morphology and motility independently of Eph-receptor binding. *J. Cell Sci.* **123**, 1235–1246 (2010).
168. Kanazawa, I. *et al.* Adiponectin and AMP kinase activator stimulate proliferation, differentiation, and mineralization of osteoblastic MC3T3-E1 cells. *BMC Cell Biol.* **8**, 51 (2007).
169. Armour, K. E. *et al.* Defective bone formation and anabolic response to exogenous estrogen in mice with targeted disruption of endothelial nitric oxide synthase. *Endocrinology* **142**, 760–766 (2001).
170. Collin-Osdoby, P. *et al.* Receptor activator of NF-kappa B and osteoprotegerin expression by human microvascular endothelial cells, regulation by inflammatory cytokines, and role in human osteoclastogenesis. *J. Biol. Chem.* **276**, 20659–20672 (2001).
171. Dominguez, J. M. 2nd, Prisby, R. D., Muller-Delp, J. M., Allen, M. R. & Delp, M. D. Increased nitric oxide-mediated vasodilation of bone resistance arteries is associated with increased trabecular bone volume after endurance training in rats. *Bone* **46**, 813–819 (2010).
172. Bianco, P., Sacchetti, B. & Riminucci, M. Osteoprogenitors and the hematopoietic microenvironment. *Best Pract. Res. Clin. Haematol.* **24**, 37–47 (2011).
173. Brunner, S. *et al.* Parathyroid hormone effectively induces mobilization of progenitor cells without depletion of bone marrow. *Exp. Hematol.* **36**, 1157–1166 (2008).
174. Fukumoto, S. & Martin, T. J. Bone as an endocrine organ. *Trends Endocrinol. Metab.* **20**, 230–236 (2009).
175. Gutierrez, O. *et al.* Fibroblast growth factor-23 mitigates hyperphosphatemia but accentuates calcitriol deficiency in chronic kidney disease. *J. Am. Soc. Nephrol.* **16**, 2205–2215 (2005).
176. Bergwitz, C. & Jüppner, H. Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23. *Annu. Rev. Med.* **61**, 91–104 (2010).
177. Sitara, D. *et al.* Genetic ablation of vitamin D activation pathway reverses biochemical and skeletal anomalies in Fgf-23-null animals. *Am. J. Pathol.* **169**, 2161–2170 (2006).
178. Fukumoto, S. & Yamashita, T. FGF23 is a hormone-regulating phosphate metabolism–unique biological characteristics of FGF23. *Bone* **40**, 1190–1195 (2007).
179. Stubbs, J. R. *et al.* Role of hyperphosphatemia and 1,25-dihydroxyvitamin D in vascular calcification and mortality in fibroblastic growth factor 23 null mice. *J. Am. Soc. Nephrol.* **18**, 2116–2124 (2007).
180. Isakova, T. *et al.* Postprandial mineral metabolism and secondary hyperparathyroidism in early CKD. *J. Am. Soc. Nephrol.* **19**, 615–623 (2008).
181. Shimizu, H. *et al.* Indoxyl sulfate downregulates renal expression of Klotho through production of ROS and activation of nuclear factor-kB. *Am. J. Nephrol.* **33**, 319–324 (2011).
182. Farrow, E. G. & White, K. E. Recent advances in renal phosphate handling. *Nat. Rev. Nephrol.* **6**, 207–217 (2010).
183. Canalejo, R. *et al.* FGF23 fails to inhibit uremic parathyroid glands. *J. Am. Soc. Nephrol.* **21**, 1125–1135 (2010).
184. Adijiang, A. & Niwa, T. An oral sorbent, AST-120, increases Klotho expression and inhibits cell senescence in the kidney of uremic rats. *Am. J. Nephrol.* **31**, 160–164 (2010).
185. Hu, M. C. *et al.* Klotho deficiency causes vascular calcification in chronic kidney disease. *J. Am. Soc. Nephrol.* **22**, 124–136 (2011).
186. Saito, Y. *et al.* Klotho protein protects against endothelial dysfunction. *Biochem. Biophys. Res. Commun.* **248**, 324–329 (1998).
187. Wacker, M. *et al.* Dentin matrix protein 1 null mice, a model of human autosomal recessive hyphosphatemic rickets, exhibit decreases in cardiac, skeletal, and vascular smooth muscle function [abstract]. *J. Bone Miner. Res.* **25** (Suppl. 1), a1157 (2010).
188. Jono, S., Peinado, C. & Giachelli, C. M. Phosphorylation of osteopontin is required for inhibition of vascular smooth muscle cell calcification. *J. Biol. Chem.* **275**, 20197–20203 (2000).
189. Christensen, B. *et al.* Cell type-specific post-translational modifications of mouse osteopontin are associated with different adhesive properties. *J. Biol. Chem.* **282**, 19463–19472 (2007).
190. Scatena, M., Liaw, L. & Giachelli, C. M. Osteopontin: a multifunctional molecule regulating chronic inflammation and vascular disease. *Arterioscler. Thromb. Vasc. Biol.* **27**, 2302–2309 (2007).
191. Lee, N. K. *et al.* Endocrine regulation of energy metabolism by the skeleton. *Cell* **130**, 456–469 (2007).
192. Whitehead, J. P., Richards, A. A., Hickman, I. J., Macdonald, G. A. & Prins, J. B. Adiponectin—a key adipokine in the metabolic syndrome. *Diabetes Obes. Metab.* **8**, 264–280 (2006).
193. Son, B. K. *et al.* Adiponectin antagonizes stimulatory effect of tumor necrosis factor-α on vascular smooth muscle cell calcification: regulation of growth arrest-specific gene 6-mediated survival pathway by adenosine 5'-monophosphate-activated protein kinase. *Endocrinology* **149**, 1646–1653 (2008).
194. Luo, X. H. *et al.* Development of arterial calcification in adiponectin-deficient mice: adiponectin regulates arterial calcification. *J. Bone Miner. Res.* **24**, 1461–1468 (2009).
195. Lomonte, C. *et al.* Serum parathyroid hormone and phosphate influence the levels of circulating $CD34^+$ cells in uremia. *J. Nephrol.* **23**, 693–698 (2010).
196. Faul, C. *et al.* FGF23 induces left ventricular hypertrophy. *J. Clin. Invest.* **121**, 4393–4408 (2011).
197. Yu, N. *et al.* Increased mortality and morbidity in mild primary hyperparathyroid patients. The Parathyroid Epidemiology and Audit Research Study (PEARS). *Clin. Endocrinol. (Oxf.)* **73**, 30–34 (2010).
198. Rhee, Y. *et al.* Parathyroid hormone receptor signaling in osteocytes increases the expression of fibroblast growth factor-23 *in vitro* and *in vivo*. *Bone* **49**, 636–643 (2011).
199. Sage, A. P. *et al.* Hyperlipidemia induces resistance to PTH bone anabolism in mice via oxidized lipids. *J. Bone Miner. Res.* **26**, 1197–1206 (2011).
200. López, I. *et al.* Direct and indirect effects of parathyroid hormone on circulating levels of fibroblast growth factor 23 *in vivo*. *Kidney Int.* **80**, 475–482 (2011).
201. Kosch, M. *et al.* Impaired flow-mediated vasodilation of the brachial artery in patients with primary hyperparathyroidism improves after parathyroidectomy. *Cardiovasc. Res.* **47**, 813–818 (2000).
202. Teng, M. *et al.* Survival of patients undergoing hemodialysis with paricalcitol or calcitriol therapy. *N. Engl. J. Med.* **349**, 446–456 (2003).
203. Golestani, R. *et al.* Abdominal aortic calcification detected by dual X-ray absorptiometry: a strong predictor for cardiovascular events. *Ann. Med.* **42**, 539–545 (2010).
204. Sennerby, U. *et al.* Cardiovascular diseases and risk of hip fracture. *JAMA* **302**, 1666–1673 (2009).
205. von Mühlen, D., Allison, M., Jassal, S. K. & Barrett-Connor, E. Peripheral arterial disease and osteoporosis in older adults: the Rancho Bernardo Study. *Osteoporos. Int.* **20**, 2071–2078 (2009).
206. Bagger, Y. Z. *et al.* Links between cardiovascular disease and osteoporosis in postmenopausal women: serum lipids or atherosclerosis *per se*? *Osteoporos. Int.* **18**, 505–512 (2007).
207. Elmariah, S. *et al.* Bisphosphonate use and prevalence of valvular and vascular calcification in women MESA (The Multi-Ethnic Study of Atherosclerosis). *J. Am. Coll. Cardiol.* **56**, 1752–1759 (2010).
208. Bolland, M. J. *et al.* Vascular events in healthy older women receiving calcium supplementation: randomised controlled trial. *BMJ* **336**, 262–266 (2008).
209. Bolland, M. J., Grey, A., Avenell, A., Gamble, G. D. & Reid, I. R. Calcium supplements with or without vitamin D and risk of cardiovascular events: reanalysis of the Women's Health Initiative limited access dataset and meta-analysis. *BMJ* **342**, d2040 (2011).
210. Yoshimura, T. *et al.* Impaired peripheral circulation in lower-leg arteries caused by higher arterial stiffness and greater vascular resistance associates with nephropathy in type 2 diabetic patients with normal ankle–brachial indices. *Diabetes Res. Clin. Pract.* **80**, 416–423 (2008).
211. Johnson, K. A., Polewski, M. & Terkeltaub, R. A. Transglutaminase 2 is central to induction of the arterial calcification program by smooth muscle cells. *Circ. Res.* **102**, 529–537 (2008).
212. Faverman, L., Mikhaylova, L., Malmquist, J. & Nurminskaya, M. Extracellular transglutaminase 2 activates beta-catenin signaling in calcifying vascular smooth muscle cells. *FEBS Lett.* **582**, 1552–1557 (2008).
213. Towler, D. A. Calciotropic hormones and arterial physiology: "D"-lightful insights. *J. Am. Soc. Nephrol.* **18**, 369–373 (2007).
214. Shroff, R. *et al.* A bimodal association of vitamin D levels and vascular disease in children on dialysis. *J. Am. Soc. Nephrol.* **19**, 1239–1246 (2008).
215. Behnke, B. J. *et al.* Influence of ageing and physical activity on vascular morphology in rat skeletal muscle. *J. Physiol.* **575**, 617–626 (2006).
216. Kronenberg, H. M. PTH regulates the hematopoietic stem cell niche in bone. *Adv. Exp. Med. Biol.* **602**, 57–60 (2007).
217. Jilka, R. L. *et al.* Decreased oxidative stress and greater bone anabolism in the aged, when compared to the young, murine skeleton with parathyroid hormone administration. *Aging Cell* **9**, 851–867 (2010).

Acknowledgments
D. A. Towler was supported by NIH grants HL88651, HL69299, and HL81138, and the Barnes-Jewish Hospital Foundation.

Author contributions
D. A. Towler researched the data for the article and wrote the article. Both authors reviewed and/or edited the manuscript before submission.

PTH and Marrow Microenvironment

Laurie McCauley, D.D.S., Ph.D.

Meet the Professor:

Laurie K. McCauley, DDS, PhD
William K. and Mary Anne Najjar Professor
Department of Periodontics and Oral Medicine
Professor, Department of Pathology
University of Michigan

PTH and the Marrow Microenvironment

PTH has long been known to directly target osteoblasts and kidney cells and indirectly target osteoclasts, but more recently PTH has been found to also directly target osteocytes and lymphocytes, and indirectly target other cells of hematopoietic origin. The anabolic actions of PTH in bone cannot effectively be replicated using osteoblast differentiation models *in vitro* suggesting other cells in the bone marrow microenvironment may be instrumental in the anabolic actions of PTH. This session will focus on PTH actions (direct and indirect) on various 'non-bone' cell types found in the marrow microenvironment.

Outline:

I. PTH brief background – '*setting the stage*'

II. PTH mediates cytokines that impact hematopoietic cells

III. Effect of PTH on osteoclasts

IV. Myeloid/macrophages and PTH

V. B cells and PTH

VI. T cells and PTH

VII. PTH support of the hematopoietic stem cell (HSC) niche

VIII. Evidence for/against therapeutic application of PTH

IX. Effect of PTH on hematopoietic lineage cell mobilization

X. Conclusions and future directions

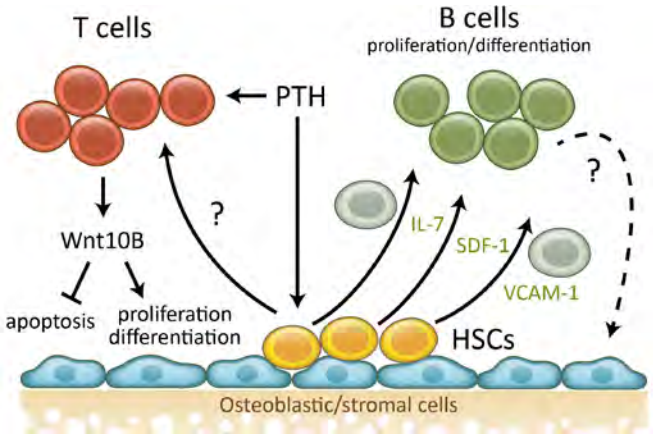
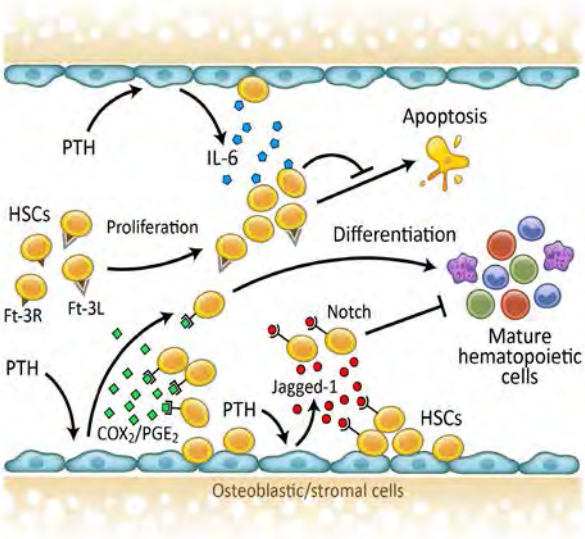


Figure 1: Interaction between osteoblastic/stromal cells and lymphocytes mediated by PTH. In response to direct stimulation by PTH, bone marrow T lymphocytes secrete the Wnt ligand Wnt10b. After binding to its receptors on osteoblastic/stromal cells, Wnt10b activates canonical Wnt/β-catenin signaling which stimulates osteoblast proliferation/differentiation and inhibits apoptosis. PTH also stimulates osteoblastic/stromal cells, however, whether these PTH stimulated osteoblastic/stromal cells can affect T lymphocytes is unclear. PTH stimulated osteoblastic/stromal cells support bone marrow B lymphopoiesis. Differentiation of HSCs to B lymphocytes requires cell-cell contact with osteoblastic/stromal cells and this process is mediated via VCAM-1, SDF-1, and IL-7 signaling induced by PTH. Less is known about the effect of B cells on osteoblastic/stromal cells.

Figure 2: PTH stimulates osteoblastic/stromal cells to increase Jagged1, cyclooxygenase (COX) 2/PGE2 and IL-6 levels in the HSC niche. Jagged1 with binding to the Notch receptor on HSCs increases HSC numbers and inhibits differentiation of HSC/progenitor cells. On the other hand, COX-2/PGE2 specifically increases short-term HSCs without altering long-term HSC or inhibiting their lineage specific differentiation. Flt-3 ligand (Flt-3L), a stem cell factor which is enriched in the HSC niche, increases hematopoietic progenitor cell proliferation. IL-6 produced in response to PTH action on stromal cells supports the Flt-3L mediated HSC expansion by inhibiting apoptosis of responsive cells.



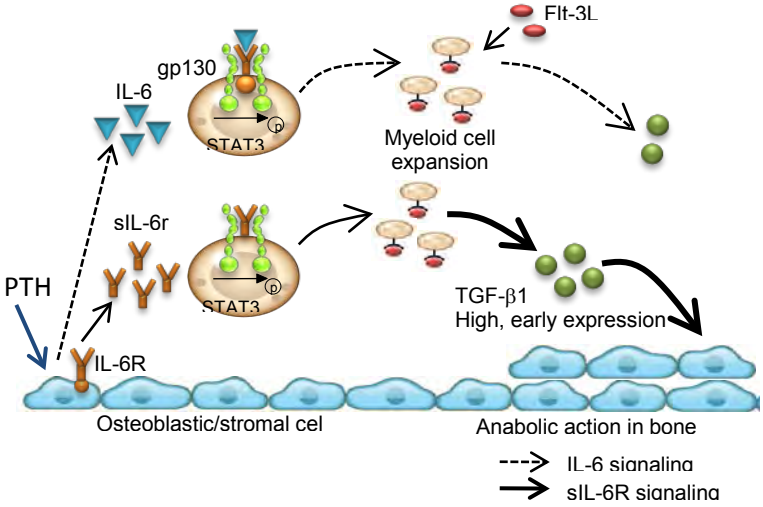


Figure 3: sIL-6R signaling mediates PTH actions in both hematopoietic and skeletal systems. PTH binds to receptors on osteoblast/stromal cells. In response, osteoblasts produce IL-6 and sIL-6R whose signaling phosphorylates STAT3 and results in myeloid cell expansion. sIL-6R/gp130/STAT3 mediated hematopoietic cell expansion positively impacts PTH anabolic action in bone by stimulating TGF-β1 in myeloid cells. IL-6 contributes to PTH mediated hematopoietic cell expansion but is not essential for PTH anabolic actions in bone. sIL-6R signaling in myeloid cells results in higher and more rapid TGF-β1 expression than IL-6/gp130/STAT3 signaling. TGF-β1 has been previously shown to recruit mesenchymal stem cells to bone surfaces and support bone formation.

Outstanding questions:

1. *What is the relationship between PTH actions on cells in the marrow and anabolic actions of PTH?*
2. *What cells in the marrow have PTH/PTHrP receptors and what is the nature of these receptors?*
3. *How do 'PTH-informed' hematopoietic lineage cells interact with osteoblasts, osteocytes, osteoclasts?*
4. *What is the entire repertoire of PTH-mediated osteoblast/stromal/osteocyte derived factors that impact hematopoietic cells and which ones are critical regulators of the bone marrow microenvironment?*
5. *What are the temporal/cell stage dependent actions of PTH relative to cells in the bone marrow?*

References

1. Rao, L.G., Murray, T.M., and Heersche, J.N. (1983). Immunohistochemical demonstration of parathyroid hormone binding to specific cell types in fixed rat bone tissue. *Endocrinology*, 113:805-810.

2. Yamamoto, I., Potts, J.T., Jr., and Segre, G.V. (1983). Circulating bovine lymphocytes contain receptors for parathyroid hormone. *J Clin Invest*, 71:404-407.

3. Lee, S.K., and Lorenzo, J.A. (1999). Parathyroid hormone stimulates TRANCE and inhibits osteoprotegerin messenger ribonucleic acid expression in murine bone marrow cultures: correlation with osteoclast-like cell formation. *Endocrinology*, 140:3552-3561.

4. Fu, Q., Jilka, R.L., Manolagas, S.C., and O'Brien, C.A. (2002). Parathyroid hormone stimulates receptor activator of NFkappa B ligand and inhibits osteoprotegerin expression via protein kinase A activation of cAMP-response element-binding protein. *J Biol Chem*, 277:48868-48875.

5. Horowitz, M.C., Coleman, D.L., Flood, P.M., Kupper, T.S., and Jilka, R.L. (1989). Parathyroid hormone and lipopolysaccharide induce murine osteoblast-like cells to secrete a cytokine indistinguishable from granulocyte-macrophage colony-stimulating factor. *J Clin Invest*, 83:149-157.

6. Weir, E.C., Lowik, C.W., Paliwal, I., and Insogna, K.L. (1996). Colony stimulating factor-1 plays a role in osteoclast formation and function in bone resorption induced by parathyroid hormone and parathyroid hormone-related protein. *J Bone Miner Res*, 11:1474-1481.

7. Feyen, J.H., Elford, P., Di Padova, F.E., and Trechsel, U. (1989). Interleukin-6 is produced by bone and modulated by parathyroid hormone. *J Bone Miner Res*, 4:633-638.

8. de la Mata, J., Uy, H.L., Guise, T.A., Story, B., Boyce, B.F., Mundy, G.R., and Roodman, G.D. (1995). Interleukin-6 enhances hypercalcemia and bone resorption mediated by parathyroid hormone-related protein in vivo. *J Clin Invest*, 95:2846-2852.

9. Onyia, J.E., Libermann, T.A., Bidwell, J., Arnold, D., Tu, Y., McClelland, P., and Hock, J.M. (1997). Parathyroid hormone (1-34)-mediated interleukin-6 induction. *J Cell Biochem*, 67:265-274.

10. Greenfield, E.M., Gornik, S.A., Horowitz, M.C., Donahue, H.J., and Shaw, S.M. (1993). Regulation of cytokine expression in osteoblasts by parathyroid hormone: rapid stimulation of interleukin-6 and leukemia inhibitory factor mRNA. *J Bone Miner Res*, 8:1163-1171.

11. Lorenzo, J.A., Sousa, S.L., and Leahy, C.L. (1990). Leukemia inhibitory factor (LIF) inhibits basal bone resorption in fetal rat long bone cultures. *Cytokine*, 2:266-271.

12. Maciel, F.M., Sarrazin, P., Morisset, S., Lora, M., Patry, C., Dumais, R., and de Brum-Fernandes, A.J. (1997). Induction of cyclooxygenase-2 by parathyroid hormone in human osteoblasts in culture. *J Rheumatol*, 24:2429-2435.

13. Tetradis, S., Pilbeam, C.C., Liu, Y., and Kream, B.E. (1996). Parathyroid hormone induces prostaglandin G/H synthase-2 expression by a cyclic adenosine 3',5'-monophosphate-mediated pathway in the murine osteoblastic cell line MC3T3-E1. *Endocrinology*, 137:5435-5440.

14. Li, X., Qin, L., Bergenstock, M., Bevelock, L. M., Novack, D.V., and Partridge, N.C. (2007). Parathyroid hormone stimulates osteoblastic expression of MCP-1 to recruit and increase the fusion of pre/osteoclasts. *J Biol Chem*, 282:33098-33106.

15. Blair, H.C., Julian, B.A., Cao, X., Jordan, S.E., and Dong, S.S. (1999). Parathyroid hormone-regulated production of stem cell factor in human osteoblasts and osteoblast-like cells. *Biochem Biophys Res Commun*, 255:778-784.

16. Brunner, S., Theiss, H.D., Murr, A., Negele, T., and Franz, W.M. (2007). Primary hyperparathyroidism is associated with increased circulating bone marrow-derived progenitor cells. *Am J Physiol Endocrinol Metab*, 293:E1670-1675.

17. Turner, R.T., Iwaniec, U.T., Marley, K., and Sibonga, J.D. (2010). The role of mast cells in parathyroid bone disease. *J Bone Miner Res*, 25:1637-1649.

18. Mazo, I.B., and von Andrian, U.H. (1999). Adhesion and homing of blood-borne cells in bone marrow microvessels. *J Leukoc Biol*, 66:25-32.

19. Berry, J.E., Pettway, G.J., Cordell, K.G., Jin, T., Datta, N.S., and McCauley, L.K. (2008). JunB as a potential mediator of PTHrP actions: new gene targets Ephrin B1 and VCAM-1. *Oral Dis*, 14:713-726.

20. Itoh, K., Udagawa, N., Matsuzaki, K., Takami, M., Amano, H., Shinki, T., Ueno, Y., Takahashi, N., and Suda, T. (2000). Importance of membrane- or matrix-associated forms of M-CSF and RANKL/ODF in osteoclastogenesis supported by SaOS-4/3 cells expressing recombinant PTH/PTHrP receptors. *J Bone Miner Res*, 15:1766-1775.

21. Udagawa, N., Takahashi, N., Jimi, E., Matsuzaki, K., Tsurukai, T., Itoh, K., Nakagawa, N., Yasuda, H., Goto, M., Tsuda, E., et al. (1999). Osteoblasts/stromal cells stimulate osteoclast activation through expression of osteoclast differentiation factor/RANKL but not macrophage colony-stimulating factor: receptor activator of NF-kappa B ligand. *Bone*, 25:517-523.

22. Demiralp, B., Chen, H.L., Koh, A.J., Keller, E.T., and McCauley, L.K. (2002). Anabolic actions of parathyroid hormone during bone growth are dependent on c-fos. *Endocrinology*, 143:4038-4047.

23. Koh, A.J., Demiralp, B., Neiva, K.G., Hooten, J., Nohutcu, R.M., Shim, H., Datta, N.S., Taichman, R.S., and McCauley, L.K. (2005). Cells of the osteoclast lineage as mediators of the anabolic actions of parathyroid hormone in bone. *Endocrinology*, 146:4584-4596.

24. Ma, Y.L., Bryant, H.U., Zeng, Q., Schmidt, A., Hoover, J., Cole, H.W., Yao, W., Jee, W.S., and Sato, M. (2003). New bone formation with teriparatide [human parathyroid hormone-(1-34)] is not retarded by long-term pretreatment with alendronate, estrogen, or raloxifene in ovariectomized rats. *Endocrinology*, 144:2008-2015.

25. Pierroz, D.D., Bonnet, N., Baldock, P.A., Ominsky, M.S., Stolina, M., Kostenuik, P.J., and Ferrari, S.L. (2010). Are osteoclasts needed for the bone anabolic response to parathyroid hormone? A study of intermittent parathyroid hormone with denosumab or alendronate in knock-in mice expressing humanized RANKL. *J Biol Chem*, 285:28164-28173.

26. Samadfam, R., Xia, Q., and Goltzman, D. (2007). Co-treatment of PTH with osteoprotegerin or alendronate increases its anabolic effect on the skeleton of oophorectomized mice. *J Bone Miner Res*, 22:55-63.

27. Wu, X., Pang, L., Lei, W., Lu, W., Li, J., Li, Z., Frassica, F.J., Chen, X., Wan, M., and Cao, X. (2010). Inhibition of Sca-1-positive skeletal stem cell recruitment by alendronate blunts the anabolic effects of parathyroid hormone on bone remodeling. *Cell Stem Cell*, 7:571-580.

28. Shurtz-Swirski, R., Shkolnik, T., and Shasha, S.M. (1995). Parathyroid hormone and the cellular immune system. *Nephron*, 70:21-24.

29. Yamamoto, I., Bringhurst, F.R., Potts, J.T., Jr., and Segre, G.V. (1988). Properties of parathyroid hormone receptors on circulating bovine lymphocytes. *J Bone Miner Res*, 3:289-295.

30. Perry, H.M., 3rd, Chappel, J.C., Bellorin-Font, E., Tamao, J., Martin, K.J., and Teitelbaum, S.L. (1984). Parathyroid hormone receptors in circulating human mononuclear leukocytes. *J Biol Chem*, 259:5531-5535.

31. Atkinson, M.J., Hesch, R.D., Cade, C., Wadwah, M., and Perris, A.D. (1987). Parathyroid hormone stimulation of mitosis in rat thymic lymphocytes is independent of cyclic AMP. *J Bone Miner Res*, 2:303-309.

32. Klinger, M., Alexiewicz, J.M., Linker-Israeli, M., Pitts, T.O., Gaciong, Z., Fadda, G.Z., and Massry, S.G. (1990). Effect of parathyroid hormone on human T cell activation. *Kidney Int*, 37:1543-1551.

33. Kaneko, T., Osono, E., Hayama, N., Lino, Y., and Terashi, A. (1997). T-cell activation modified by parathyroid hormone (PTH) in patients with end-stage renal disease. *Clin Nephrol*, 48:353-358.

34. Alexiewicz, J.M., Gaciong, Z., Klinger, M., Linker-Israeli, M., Pitts, T.O., and Massry, S.G. (1990). Evidence of impaired T cell function in hemodialysis patients: potential role for secondary hyperparathyroidism. *Am J Nephrol*, 10:495-501.

35. Tzanno-Martins, C ., F utata, E ., J orgetti, V ., and Duarte, A.J. (2000). R estoration of i mpaired T -cell proliferation after parathyroidectomy in hemodialysis patients. *Nephron*, 84:224-227.

36. Shasha, S .M., K ristal, B ., S teinberg, O., and S hkolnik, T. (1989). E ffect of par athyroidectomy on T c ell functions in patients with primary hyperparathyroidism. *Am J Nephrol*, 9:25-29.

37. Kotzmann, H., Koller, M., Abela, C., Clodi, M., Riedl, M., Graninger, W., Niederle, B., and Luger, A. (1998). Effects of parathyroid hormone and serum calcium on the phenotype and function of mononuclear cells in patients with primary hyperparathyroidism. *Eur J Clin Invest*, 28:353-358.

38. Gaciong, Z., Alexiewicz, J.M., Linker-Israeli, M., Shulman, I.A., Pitts, T.O., and Massry, S.G. (1991). Inhibition of i mmunoglobulin pr oduction b y par athyroid hor mone. I mplications i n c hronic r enal f ailure. *Kidney Int*, 40:96-106.

39. Alexiewicz, J.M., Klinger, M., Pitts, T.O., Gaciong, Z., Linker-Israeli, M., and Massry, S.G. (1990). Parathyroid hormone inhibits B cell proliferation: implications in chronic renal failure. *J Am Soc Nephrol*, 1:236-244.

40. Gaciong, Z., A lexiewicz, J .M., an d M assry, S .G. (1991). I mpaired i n vivo an tibody production in C RF r ats: role of secondary hyperparathyroidism. *Kidney Int*, 40:862-867.

41. Li, Y., Toraldo, G., Li, A., Yang, X., Zhang, H., Qian, W.P., and Weitzmann, M.N. (2007). B cells and T cells are c ritical f or t he pr eservation of bone homeostasis and at tainment of peak b one m ass i n v ivo. *Blood*, 1 09:3839-3848.

42. Zhu, J., Garrett, R., Jung, Y., Zhang, Y., Kim, N., Wang, J., Joe, G.J., Hexner, E., Choi, Y., Taichman, R.S., et al . (2007). O steoblasts s upport B -lymphocyte c ommitment and di fferentiation f rom he matopoietic s tem c ells. *Blood*, 109:3706-3712.

43. Calvi, L.M., Adams, G.B., Weibrecht, K.W., Weber, J.M., Olson, D.P., Knight, M.C., Martin, R.P., Schipani, E., Divieti, P., Bringhurst, F.R., et al. (2003). Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature*, 425:841-846.

44. Jung, Y., Wang, J., Schneider, A., Sun, Y.X., Koh-Paige, A.J., Osman, N.I., McCauley, L.K., and Taichman, R.S. (2006). Regulation of SDF-1 (CXCL12) production by osteoblasts; a possible mechanism for stem cell homing. *Bone*, 38:497-508.

45. Wu, J.Y., Purton, L.E., Rodda, S.J., Chen, M., Weinstein, L.S., McMahon, A.P., Scadden, D.T., and Kronenberg, H.M. (2008). Osteoblastic regulation of B lymphopoiesis is mediated by Gs{alpha}-dependent signaling pathways. *Proc Natl Acad Sci U S A*, 105:16976-16981.

46. Peschon, J .J., Morrissey, P.J., G rabstein, K .H., R amsdell, F .J., M araskovsky, E ., G liniak, B .C., P ark, L .S., Ziegler, S.F., Williams, D.E., Ware, C.B., et al. (1994). Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. *J Exp Med*, 180:1955-1960.

47. von F reeden-Jeffry, U ., Vieira, P ., Lucian, L. A., McNeil, T ., Burdach, S.E., a nd Murray, R . (1995). Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. *J Exp Med*, 181:1519-1526.

48. Theill, L.E., Boyle, W.J., and Penninger, J.M. (2002). RANK-L and RANK: T cells, bone loss, and mammalian evolution. *Annu Rev Immunol*, 20:795-823.

49. McCauley, L.K., Rosol, T.J., Capen, C.C., and Horton, J.E. (1989). A comparison of bone turnover in athymic (nude) and euthymic mice: biochemical, histomorphometric, bone ash and in vitro studies. *Bone*, 10:29-34.

50. Stojceva-Taneva, O., Fadda, G.Z., Smogorzewski, M., and Massry, S.G. (1993). Parathyroid hormone increases cytosolic calcium of thymocytes. *Nephron*, 64:592-599.

51. McCauley, L.K., Rosol, T.J., Merryman, J.I., and Capen, C.C. (1992). Parathyroid hormone-related protein binding to human T-cell lymphotropic virus type I-infected lymphocytes. *Endocrinology*, 130:300-306.

52. Rifas, L. (2006). T-cell cytokine induction of BMP-2 regulates human mesenchymal stromal cell differentiation and mineralization. *J Cell Biochem*, 98:706-714.

53. Rifas, L., Arackal, S., and Weitzmann, M.N. (2003). Inflammatory T cells rapidly induce differentiation of human bone marrow stromal cells into mature osteoblasts. *J Cell Biochem*, 88:650-659.

54. Rifas, L., and Arackal, S. (2003). T cells regulate the expression of matrix metalloproteinase in human osteoblasts via a dual mitogen-activated protein kinase mechanism. *Arthritis Rheum*, 48:993-1001.

55. Hory, B.G., Roussanne, M.C., Rostand, S., Bourdeau, A., Drueke, T.B., and Gogusev, J. (2000). Absence of response to human parathyroid hormone in athymic mice grafted with human parathyroid adenoma, hyperplasia or parathyroid cells maintained in culture. *J Endocrinol Invest*, 23:273-279.

56. Pettway, G.J., Schneider, A., Koh, A.J., Widjaja, E., Morris, M.D., Meganck, J.A., Goldstein, S.A., and McCauley, L.K. (2005). Anabolic actions of PTH (1-34): use of a novel tissue engineering model to investigate temporal effects on bone. *Bone*, 36:959-970.

57. Terauchi, M., Li, J.Y., Bedi, B., Baek, K.H., Tawfeek, H., Galley, S., Gilbert, L., Nanes, M.S., Zayzafoon, M., Guldberg, R., et al. (2009). T lymphocytes amplify the anabolic activity of parathyroid hormone through Wnt10b signaling. *Cell Metab*, 10:229-240.

58. Whitfield, J.F. (2005). Parathyroid hormone (PTH) and hematopoiesis: new support for some old observations. *J Cell Biochem*, 96:278-284.

59. Larizza, P., and Chirico, G. (1952). Experimental study on the effect of prolonged administration of parathyroid extracts on hemopoiesis. *Haematologica*, 36:653-670.

60. Rixon, R.H., Whitfield, J.F., and Youdale, T. (1958). Increased survival of rats irradiated with x-rays and treated with parathyroid extract. *Nature*, 182:1374.

61. Rixon, R.H., and Whitfield, J.F. (1961). The radioprotective action of parathyroid extract. *Int J Radiat Biol*, 3:361-367.

62. Rixon, R.H., and Whitfield, J.F. (1972). Hypoplasia of the bone marrow in rats following removal of the parathyroid glands. *J Cell Physiol*, 79:343-352.

63. Swierenga, S.H., MacManus, J.P., Braceland, B.M., and Youdale, T. (1976). Regulation of the primary immune response in vivo by parathyroid hormone. *J Immunol*, 117:1608-1611.

64. Gallien-Lartigue, O., and Carrez, D. (1974). [In vitro induction of the S phase in multipotent stem cells of bone marrow by parathyroid hormone]. *C R Acad Sci Hebd Seances Acad Sci D*, 278:1765-1768.

65. Adams, G.B., Martin, R.P., Alley, I.R., Chabner, K.T., Cohen, K.S., Calvi, L.M., Kronenberg, H.M., and Scadden, D.T. (2007). Therapeutic targeting of a stem cell niche. *Nat Biotechnol*, 25:238-243.

66. Weber, J.M., Forsythe, S.R., Christianson, C.A., Frisch, B.J., Gigliotti, B.J., Jordan, C.T., Milner, L.A., Guzman, M.L., and Calvi, L.M. (2006). Parathyroid hormone stimulates expression of the Notch ligand Jagged1 in osteoblastic cells. *Bone*, 39:485-493.

67. Pirih, F.Q., Michalski, M.N., Cho, S.W., Koh, A.J., Berry, J.E., Ghaname, E., Kamarajan, P., Bonnelye, E., Ross, C.W., Kapila, Y.L., et al. (2010). Parathyroid hormone mediates hematopoietic cell expansion through interleukin-6. *PLoS One*, 5:e13657.

68. Tetradis, S., Pilbeam, C.C., Liu, Y., Herschman, H.R., and Kream, B.E. (1997). Parathyroid hormone increases prostaglandin G/H synthase-2 transcription by a cyclic adenosine 3',5'-monophosphate-mediated pathway in murine osteoblastic MC3T3-E1 cells. *Endocrinology*, 138:3594-3600.

69. Kawaguchi, H., Raisz, L.G., Voznesensky, O.S., Alander, C.B., Hakeda, Y., and Pilbeam, C.C. (1994). Regulation of the two prostaglandin G/H synthases by parathyroid hormone, interleukin-1, cortisol, and prostaglandin E2 in cultured neonatal mouse calvariae. *Endocrinology*, 135:1157-1164.

70. Varnum-Finney, B., Xu, L., Brashem-Stein, C., Nourigat, C., Flowers, D., Bakkour, S., Pear, W.S., and Bernstein, I.D. (2000). Pluripotent, cytokine-dependent, hematopoietic stem cells are immortalized by constitutive Notch1 signaling. *Nat Med*, 6:1278-1281.

71. Stier, S., Cheng, T., Dombkowski, D., Carlesso, N., and Scadden, D.T. (2002). Notch1 activation increases hematopoietic stem cell self-renewal in vivo and favors lymphoid over myeloid lineage outcome. *Blood*, 99:2369-2378.

72. Brunner, S., Zaruba, M.M., Huber, B., David, R., Vallaster, M., Assmann, G., Mueller-Hoecker, J., and Franz, W.M. (2008). Parathyroid hormone effectively induces mobilization of progenitor cells without depletion of bone marrow. *Exp Hematol*, 36:1157-1166.

73. Rubin, M.R., Manavalan, J.S., Dempster, D.W., Shah, J., Cremers, S., Kousteni, S., Zhou, H., McMahon, D.J., Kode, A., Sliney, J., et al. (2010). Parathyroid hormone stimulates circulating osteogenic cells in hypoparathyroidism. *J Clin Endocrinol Metab*, 96:176-186.

74. Coppolino, G., Bolignano, D., De Paola, L., Giulino, C., Mannella, A., Riccio, M., Mascaro, M.A., Lombardi, G., Fuiano, G., Lombardi, L., et al. (2011). Parathyroid Hormone and Mobilization of Circulating Bone Marrow-Derived Cells in Uremic Patients. *J Investig Med*.

75. Ballen, K.K., Shpall, E.J., Avigan, D., Yeap, B.Y., Fisher, D.C., McDermott, K., Dey, B.R., Attar, E., McAfee, S., Konopleva, M., et al. (2007). Phase I trial of parathyroid hormone to facilitate stem cell mobilization. *Biol Blood Marrow Transplant*, 13:838-843.

76. Novince CM, Koh AJ, Michalski MN, Marchesan JT, Wang J, Jung Y, Berry JE, Eber MR, Rosol TJ, Taichman RD, McCauley LK. (2011) Proteoglycan 4, a novel immunomodulatory factor, regulates parathyroid hormone actions on hematopoietic cells. Am J Pathol 179:2431-2442.

76. Ballen K. Mendizabal AM, Cutler C, Politikos I, Jamieson K, Shpall EJ, Dey BR, Attar E, McAfee S, Delaney C, McCarthy P, Ball ED, Kamble R, Avigan D, Maziarz RT, Ho VT, Koreth J, Alvea E, Soiffer R, Wingard JR, Boussiotis V, Spitzer TR, Antin JH (2012) Phase II trial parathyroid hormone after double umbilical cord blood transplantation. *Biol Blood Marrow Transplant*: In press: available on-line.

77. Bromberg O, Frisch BJ, Weber JM, Porter RL, Civitelli R, Calvi LM (2012) Osteoblastic N-cadherin is not required for microenvironmental support and regulation of hematopoietic stem and progenitor cells. Blood 120:303-313.

78. Calvi LM, Bromberg O, Rhee Y, Weber JM, Smith JN, Basil MJ, Frisch BJ, Bellido T. (2012) Osteoblastic expansion induced by parathyroid hormone receptor signaling in murine osteocytes is not sufficient to increase hematopoietic stem cells. Blood 119:2489-2499.

79. Cho SW, Pirih FQ, Michalski M, Eber M, Ritchie K, Sinder B, Oh S, Lee J, Kozloff K, Danciu T, Wronski T, McCauley LK (2012) Soluble interleukin-6 receptor signaling mediates hematopoietic and skeletal actions of parathyroid hormone. In review.

Medication-induced Osteoporosis

Cyrus Cooper, D.M., FRCP, MedSci

Medication-Induced Osteoporosis

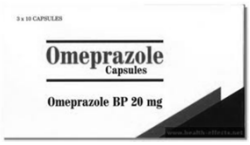
Cyrus Cooper, FMedSci

Professor of Rheumatology and Director, MRC Lifecourse Epidemiology Unit, University of Southampton; and

Professor of Musculoskeletal Science, University of Oxford, UK

Outline

- PPI/ H2RA
- Anti-depressants
- Glitazones
- CTIBL



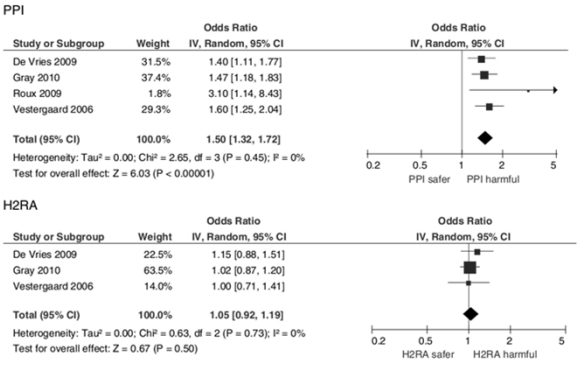
Current PPI use and risk of fracture

		Fracture site		
		Any	*Hip*	*Vertebral*
Current use		**1.15***	**1.22***	**1.40***
		(1.10-1.20)	(1.10-1.37)	(1.11-1.78)
Defined daily dose				
Low	< 1.0	**1.09***	1.13	1.05
		(1.03-1.16)	(0.98-1.31)	(0.76-1.45)
Medium	1.0 – 1.75	**1.18***	**1.27***	**1.61***
		(1.12-1.25)	(1.12-1.45)	(1.23-2.11)
High	> 1.75	**1.19***	**1.45***	1.54
		(1.03-1.37)	(1.06-1.99)	(0.80-2.95)

Contrast: Current PPI use versus past PPI use

De Vries et al., OI 2009

PPI, H2RA and spine fractures



Kwok et al., Bone 2011

Concomitant BP/PPI use and risk of fracture

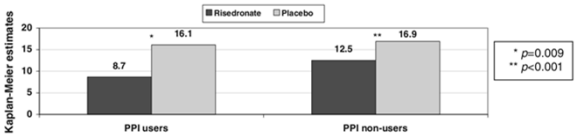
		Fracture site		
		Any	Hip	Vertebral
Current use		**1.13***	**1.24***	1.18
		(1.03-1.24)	(1.04-1.48)	(0.92-1.48)
Defined daily dose				
Low	< 1.0	0.90	0.99	0.84
		(0.77-1.05)	(0.74-1.33)	(0.56-1.27)
Medium	1.0 – 1.75	**1.20***	**1.35***	**1.35***
		(1.07-1.36)	(1.08-1.69)	(1.01-1.82)
High	> 1.75	1.32	1.23	1.09
		(0.92-1.89)	(0.58-2.59)	(0.41-2.94)

Contrast: BP + PPI versus BP alone

De Vries et al., OI 2009

PPI use, new vertebral fracture and BMD in risedronate trials

Incidence new vertebral fracture for current vs no PPI use



BMD % change over 36 months

PPI use	Lumbar spine (L1-L4) BMD		Femoral neck BMD	
	Risedronate	Placebo	Risedronate	Placebo
PPI user	6.32	0.45	2.50	-0.99
PPI non-user	5.92	1.10	2.12	-0.84

Roux et al., OI 2012

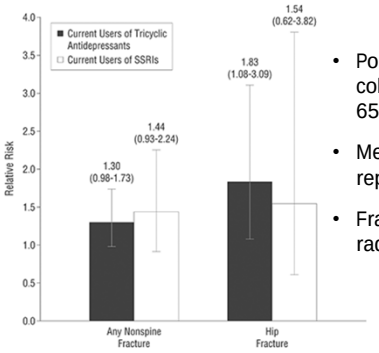
Possible mechanisms

- ASM used in patients likely to be less compliant with BP?
  - No effect of compliance adjustment in GPRD
  - Not explain ASM effect in BP non-users
- An effect on falling?
  - Om and ranitidine assoc with dizziness
  - But wrist fractures not increased
  - Slow offset of risk on stopping ASM
  - Not explain increased risk vert fracture
- Reduced calcium absorption- conflicting studies
- Reduced BMD?
  - Unlikely as risk not increase with duration of drug, and other studies suggest no BMD association

Outline

- PPI/ H2RA
- Anti-depressants
- Glitazones
- CTIBL

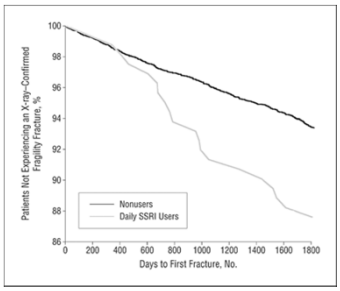
Study of Osteoporotic Fractures Cohort



- Population based cohort 8127 women > 65 yrs old
- Medication by self-report
- Fractures validated radiographically

Ensrud et al., AIM 2003

SSRI use and risk of fracture



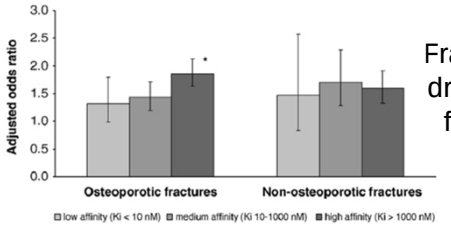
- Population-based cohort 5008, aged > 50 yrs, followed 5 years

Table 2. SSRI Use and Risk of Fragility Fracture

Variable	Multivariate Adjusted Hazard Ratio (95% Confidence Interval)
Daily SSRI use	2.1 (1.3-3.4)
Daily dose of SSRI use	1.5 (1.1-2.1)
Recurrent daily SSRI use	2.1 (1.1-4.0)

Abbreviation: SSRI, selective serotonin reuptake inhibitor.

Richards et al., AIM 2007



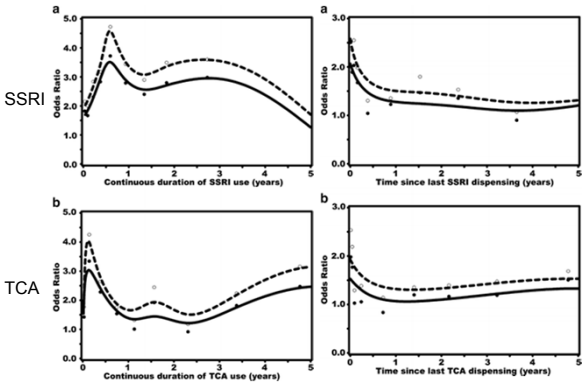
Fracture and drug affinity for 5-HTT

Verdel et al., Bone 2010

5-HT transporter inhibition	Cases (n=6,763)	Controls (n=26,341)	Adjusted OR	95% CI
Low	46	102	1.74	1.14–2.35
Medium	132	241	1.92	1.53–2.40
High	234	358	2.31	1.94–2.76
Unclassified	10	24	1.44	0.67–3.04

Van den Brand et al., OI 2009

Antidepressants and odds fracture over time



Van den Brand et al., OI 2009

Antidepressant use and bone loss

Mean Annualized Rate of Bone Loss at Total Hip, Femoral Neck, and Trochanter by Category of Antidepressant Use

Mean annualized change In Bone Mineral Density by Location, % (95% CI)	Antidepressant Use Category		
	Nonusers (n = 2406)	TCA Users (n = 118)	SSRI Users (n = 198)
Total hip			
Age-adjusted model	-0.49 (-0.54 to -0.43)	-0.44 (-0.68 to -0.20)	-0.77 (-0.96 to -0.58)†
Multivariable model*	-0.47 (-0.53 to -0.42)	-0.47 (-0.70 to -0.24)	-0.82 (-1.00 to -0.64)‡
Femoral neck			
Age-adjusted model	-0.24 (-0.31 to -0.17)	-0.31 (-0.62 to -0.01)	-0.57 (-0.82 to -0.03)†
Multivariate model*	-0.23 (-0.29 to -0.16)	-0.32 (-0.62 to -0.02)	-0.60 (-0.84 to -0.36)†
Trochanter			
Age-adjusted model	-0.49 (-0.57 to -0.42)	-0.47 (-0.80 to -0.14)	-0.89 (-1.16 to -0.63)†
Multivariable model*	-0.48 (-0.55 to -0.41)	-0.47 (-0.79 to -0.15)	-0.93 (-1.18 to -0.68)‡

2722 women, population-based cohort (SOF)

Diem et al., AIM 2007

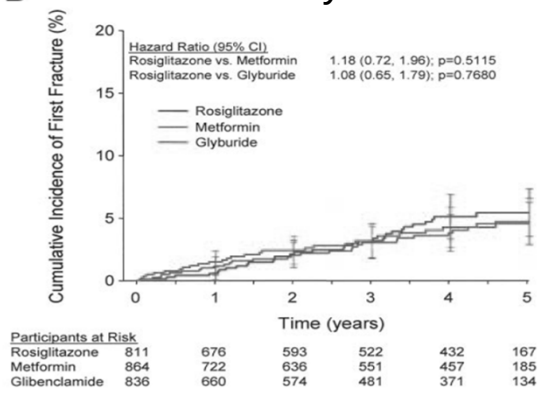
Discussion

- SSRI and TCA associated with increase in falls and fractures
- SSRI appears to impair bone
- Rapid onset/ offset of risk
- Confounding by indication and channelling bias

Outline

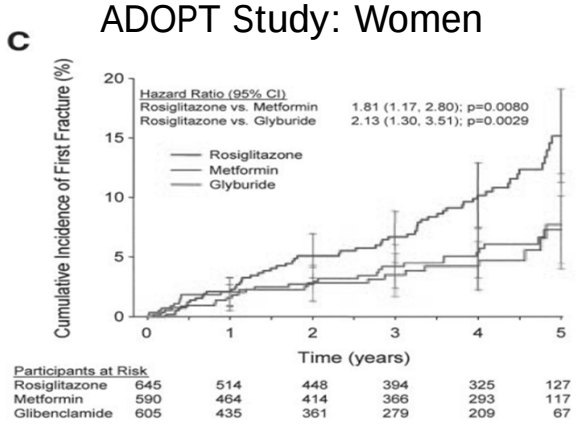
- PPI/ H2RA
- Anti-depressants
- Glitazones
- CTIBL

ADOPT Study: Men

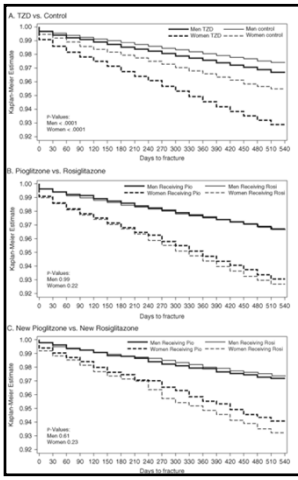


Kahn et al., 2008

ADOPT Study: Women



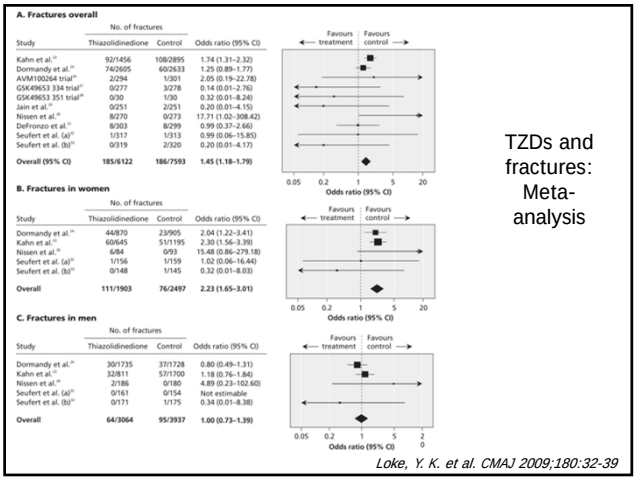
Kahn et al., 2008



Pioglitazone, rosiglitazone and fractures

Cohort study
69047 TZD
75352 control
Claims database

Aubert et al., 2010



TZDs and fractures: Meta-analysis

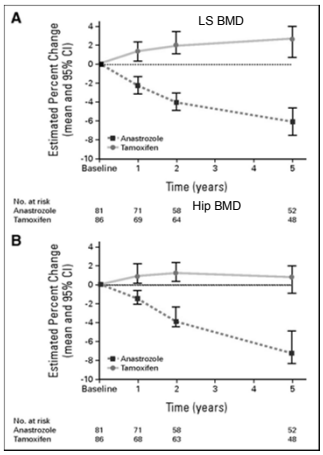
Loke, Y. K. et al. CMAJ 2009;180:32-39

Discussion

- Glitazones associated with increased fracture risk
  - ? Adipocyte, IGF-1, estrogen
- Many RCT/ cohorts/ case-control
- Probably women > men
- Distal fractures (but may reflect age)
- Consider as risk factor for fracture

Outline

- PPI/ H2RA
- Anti-depressants
- Glitazones
- CTIBL

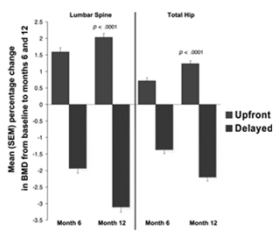


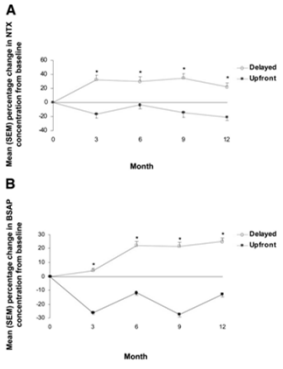
Arimidex, Tamoxifen, Alone or in Combination Trial (ATAC)
Anastrazole 1mg/d
Tamoxifen 20 mg/d
Combination stopped at 33 months

- Anastrazole vs tamoxifen during active treatment:
  - (number [annual rate]: 375 [2·93%] *vs* 234 [1·90%];
  - incidence rate ratio [IRR] 1·55 [1·31–1·83], p<0·0001)

Lancet Oncology 2008

CTIBL prevention

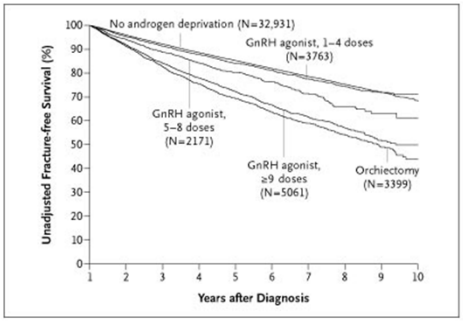




- 1677 from two trials
- Zol 4mg every 6 months
- Within 14 days or if BMD drop or fracture

Brufsky et al, The Oncologist 2008

Androgen deprivation therapy



- 3.7% prostate cancer 1991; 30.9% 1999
- Increase in bone resorption markers
- Bone loss, fractures

Shahinian et al, NEJM 2005

Conclusions

- Range of drugs associated with adverse bone outcomes is increasing
- Conflicting evidence regarding concommitent PPI and bisphosphonate use
- Be alert to use of PPI, glitazones, antidepressants, hormonal cancer therapies
- Guidelines only for breast cancer; and consider use of FRAX secondary osteoporosis

Mechanical Sensor and Osteocytes

Jean Jiang, Ph.D.

Mechanical Sensor and Osteocytes

Jean X. Jiang, Ph.D.
Professor
University of Texas Health Science center
San Antonio, TX 78229-3900

Significance of the topic:

The skeleton adapts to mechanical usage and mechanical loading promotes bone formation and remodeling. Although most bone cells are involved in mechanosensing, it is widely accepted that osteocytes are the principal mechanosensory cells. Osteocytes are embedded inside the bone mineral matrix and have stellate morphology with small cell body and long dendritic processes. The long dendritic processes of osteocytes form a network connecting the neighboring osteocytes and the cells on the bone surface, such as osteoblasts and osteoclasts. In last decade or so, the osteocyte has been perceived as the center of bone remodeling by coordinating both osteoblast and osteoclast function, and also as the initiator of bone remodeling by sensing the bone matrix. Osteocyte cell body and processes are surrounded by fluid-filled space, forming an extensive lacuno-canalicular network. The osteocyte dendritic processes and the cell body are surrounded by fluid filled spaces termed as canaliculi and lacuna, respectively. The canaliculi around the dendrites are narrow when compared to that of the lacunar space surrounding osteocyte cell body. Various studies suggest that flow of interstitial fluid driven by extravascular pressure is a likely stress-related factor that transmits mechanical stimulation to bone cells. Dendritic processes of osteocytes are postulated as the mechanical sensory region on osteocytes. The mechanisms by which osteocytes sense and respond to mechanical loading in osteocytes are active research focuses in multiple laboratories.

Learning Objectives:

As a result of participating in this session, attendees should be able to understand the current knowledge and research in

(1) Current models of mechanical stimulation on osteocytes.

(2) Mechanosensory areas of osteocytes, including the involvement of cell body, dendritic processes and cilia and primary approaches being used.

(3) Critical mechanosensory molecules involved, including integrins, glycocalyx, etc.

(4) Roles of osteocytic connexin channels in mechanostransduction.

(5) Signaling mechanisms activated by mechano-stimulation.

(6) Relevance to physiology and pathology of the bone tissues.

(7) Challenges and future research directions.

References:

Fritton, S. P. and S. Weinbaum. (2009). Fluid and solute transport in bone: flow-induced mechanotransduction. Annu.Rev.Fluid Mech. 41, 347-374.

Riddle, R.C. and Donahue, H.J. (2009). From streaming-potential to shear stress: 25 years of bone cell mechanotransduction. J. Orthop. Res. 27, 143-149.

Hughes, J.M. and Petit, M.A. (2010) Biological underpinnings of Frost's mechanostat thresholds: The important role of osteocytes. J. Musculosketet. Neuronal. Interact. 10, 128-135.

Temiyasathit, S., and Jacob, C.R. (2010). Osteocyte primary cilium and its role in bone mechanotransduction. Ann. N. Y. Acad. Sci. 1192, 422-428.

Burra, S., Nicolella. D.P., Fran cis, W.L ., Freitas, C.J., M ueschke, N.J., P oole, C., a nd Jiang, J.X. (2010) Dendr itic process of osteocytes is a mechanotransducer that induces the opening of hemichannels. *Proc. Natl. Acad. Sci.* 107, 13648-13653.

Wu, D., Ganatos, P., Spray, D.C. , and Weinbaum, S. (2011). On the electrophysiological response of bone cells us ing a Stokesian fluid stimulus probe for selivery of quantifiable localized picoNewton level forces. J. Biomech. 44, 1702-1708.

Bonewald, L.F. (2012). The amazing osteocyte. J. Bone Miner. Res. 26, 229-238.

Thompson, W.R., Rubin, C.T., and Rubin, J. (2012) Mechanical regul ation of signaling pathways in bone. Gene 503, 179-193.

Batra, N., Burra, S., Siller-Jackson, A.J., Gu, S., Xi a, X., Weber, G., DeSimone, D., Bonewald, L.F., Lafer, E.M., Sprague, E., Schwartz, M.A., and Ji ang, J.X. (2012) Mechanical stress activates integrin α5β1 induc es opening of connexin 43 hemichannels. *Proc. Nat. Acad. Sci.* 109, 3359-3364.

Fibrocytes and Marrow Fibrosis

Ernestina Schipani, M.D., Ph.D.

Fibrocytes and Marrow Fibrosis

Ernestina Schipani M.D., Ph.D.

Indiana University School of Medicine

USA

SIGNIFICANCE OF THE TOPIC

We have recently identified in normal bone marrow a novel population that carries both hematopoietic and mesenchymal markers. We will discuss how this novel population may modulate bone marrow and bone homeostasis. Moreover, we will also talk about how the marrow precursors of this novel population may contribute to the reactive stroma of malignant tumors. Overall, the topic is relevant to both bone and bone marrow physiology and cancer biology.

LEARNING OBJECTIVES

As a result of participating in this session, attendees should be able to discuss about:

1) Cellular heterogeneity of the bone marrow stroma
2) Fibrocytes
3) Fibrocyte-like cells in the bone marrow
4) Contribution of bone marrow fibrocyte precursors to the reactive stroma of tumors

POINTS OF INTEREST

The bone marrow is comprised of both hematopoietic and mesenchymal populations. Hematopoietic cells derive from self-renewing hematopoietic stem cells (HSCs), and constitute the vast majority of the adult bone marrow's cellularity, whereas the mesenchymal population is thought to originate from mesenchymal stem cells (MSCs) [1]. The presence of non-hematopoietic stem cells in the bone marrow was first suggested by the German pathologist Cohneim about 130 years ago, who proposed that bone marrow can be the source of fibroblasts contributing to wound healing in numerous peripheral tissues [2]. In the early 1970's, the pioneering work of Friedenstein and colleagues demonstrated that the rodent bone marrow had fibroblastoid cells with clonogenic potential *in vitro* [3, 4]. Over the years, numerous laboratories have confirmed and expanded these findings by showing that cells isolated according to Friedenstein's protocol were also present in the human bone marrow, and by demonstrating that these cells could be sub-passaged and differentiated *in vitro* into a variety of cells of the

mesenchymal lineages. Friedenstein had thus isolated from the bone marrow what later on would be renamed “mesenchymal stem cell” or MSC.

The current model proposes that there are at least two types of stem cells in the bone marrow: hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). HSCs would give rise to hematopoietic cell types and to cells that resorb bone (osteoclasts), whereas MSCs would differentiate into a variety of mesenchymal lineages such as chondrocytes, adipocytes and osteoblasts, at least *in vitro*.

Another cell type of bone marrow origin, referred to as “fibrocyte”, has been recently characterized [5-9]. Fibrocytes are collagen-producing cells of the peripheral blood, and comprise 0.1-0.5% of the circulating population of “non-erythrocytic cells”. They were first identified as cells that, upon isolation from blood and subsequent *in vitro* culture, exhibited mixed morphological and molecular characteristics of hematopoietic stem cells, monocytes and fibroblasts. They are present in wounds, at sites of pathological fibroses, and in the reactive stroma of tumors [9-12]. It is not clear whether fibrocytes exist in circulation as such, though it is more likely that they represent the obligate intermediate stage of differentiation into mature mesenchymal cells of a bone marrow-derived precursor of the monocyte lineage that circulates and becomes a “fibrocyte” only at specific tissue sites under permissive conditions [9]. Fibrocytes are thought to derive from the hematopoietic lineage since they express cell surface antigens such as CD34, CD45, and CD11b, though they produce matrix proteins such as collagen type I; moreover, their bone marrow origin has been extensively documented in transplant models [9]. They also constitutively secrete extracellular matrix degrading enzymes, primarily MMP9 [6, 13], which promotes endothelial cell invasion, and several pro-angiogenic factors including VEGF, bFGF, IL-8 and PDGF [6, 10, 14]. Collectively, these findings suggest that the fibroblast population of bone marrow origin contributing to wound healing proposed 130 years ago by the German pathologist Cohneim could indeed be formed, at least in part, by fibrocytes.

We have recently identified in normal bone marrow a novel population that, like fibrocytes, carries both hematopoietic and mesenchymal markers [15] (Figure 1). We will refer to it as “bone marrow fibrocyte-like cells”, since it shares similarities with fibrocytes, whose presence in the bone marrow has not been reported before.

In particular, a double mutant mouse expressing constitutively active PTH/PTHrP receptor (PPR*) and green fluorescent protein (GFP) under the control of the type I collagen promoter (PPR*Tg/ GFP) was generated. Confocal microscopy and flow cytometry revealed the presence of a cell population expressing GFP [GFP(+)] that was also positive for the hematopoietic marker CD45 in the bone marrow of both PPR*Tg/GFP and controls. This population was expanded in PPR*Tg/GFP. Existence of cells expressing both type I collagen and CD45 in the adult bone marrow was confirmed by immunohistochemistry and fluorescence-activated cell sorting (FACS). Analysis of total RNA extracted from sorted GFP(+)CD45(+) showed that these cells produced not only type I collagen, but also PTH/PTHrP receptor and RANK mRNAs, which further proved their features of being both mesenchymal and hematopoietic lineages. Of note, PPR*Tg is a mouse model of bone marrow fibrosis [16].

In this Meet-the-Professor section, we will discuss whether and how this novel population may contribute to bone marrow homeostasis and to the development and/or progression of marrow fibrosis.

In particular, it has been proposed that fibrocytes contribute to wound healing and to pathological fibroses by producing both a collagenous matrix and a variety of pro-angiogenic/pro-fibrotic factors. It is possible that also the bone marrow fibrocyte-like cells we have recently identified contribute to the pathogenesis of marrow fibrosis by producing pro-angiogenic/pro-fibrotic cytokines, in addition to matrix proteins. In this regard, we would like to re-emphasize that is quite intriguing that number of bone marrow fibrocyte-like cells is increased in PPR*Tg, i.e. in a model of marrow fibrosis.

Moreover, potential contribution of the bone marrow precursors of our novel population to the reactive stroma of tumors and their differentiation into myofibroblasts will be also discussed.

In particular, myofibroblasts, which are critical mediator of fibrosis, are generated by numerous sources, including resident mesenchymal cells, epithelial and endothelial cells in processes termed epithelial/endothelial mesenchymal transition (EMT, EndMT), and probably also bone marrow fibrocytes, as briefly discussed above [17, 18] (Figure 2). The presence of myofibroblasts is typically revealed by their expression of both alpha-smooth muscle actin (alphaSMA) and vimentin, which is a marker of fibroblasts, whereas smooth muscle cells, which are positive for alphaSMA, lack significant vimentin staining; conversely, fibrocytes are positive for vimentin, but not for alphaSMA. Myofibroblasts, which are a subset of “CAFs” or “carcinoma

associated fibroblasts", are present, as fibrocytes, both at sites of chronic wounding and in the stroma of advanced carcinomas [19].

As aforementioned, upon Tgfbeta1 treatment human CD34 (+) alphaSMA (-) fibrocytes can differentiate into CD34 (-) alpha SMA (+) myofibroblasts in vitro [20]. Several histopathological studies have correlated the loss of CD34 (+) stromal cells at tumor sites with malignant potential, and other studies have associated the loss of CD34 expression with an increase in alphaSMA production [21] [8] [6]. It is possible that each of these changes could be indeed the consequence of fibrocytes differentiating into myofibroblasts. Both cancerous cells and cells at the interface of malignant lesions produce high amount of Tgfbeta1; moreover, hypoxic regions of tumors contain elevated levels of endothelin-1 (ET-1) [9]. Therefore, the apparent loss of CD34 (+) cells and the concomitant increase in the number of CD34 (-) alphaSMA (+) cells in the stroma surrounding malignant lesions may indicate an increased differentiation of CD34 (+) fibrocytes into mature CD34 (-) myofibroblasts triggered by the presence of excessive levels of Tgfbeta1 and ET1. Since fibrocytes, but not myofibroblasts, have antigen-presenting capabilities [9], it is tempting to speculate that fibrocytes could be important for local immunosurveillance, whereas their differentiation into myofibroblasts would favor a more invasive phenotype of malignant tumors.

Bone marrow has been shown to be a source of myofibroblasts both in physiological and in pathological conditions [18, 22]; these findings are in agreement with the working model that myofibroblasts originate, at least in part, from fibrocytes, whose bone marrow origin has been well documented [9].

It is possible that in analogy to the fibrocyte precursors also the bone marrow precursors of our novel population may circulate and differentiate into myofibroblasts within a tumor stroma.

Figure 1

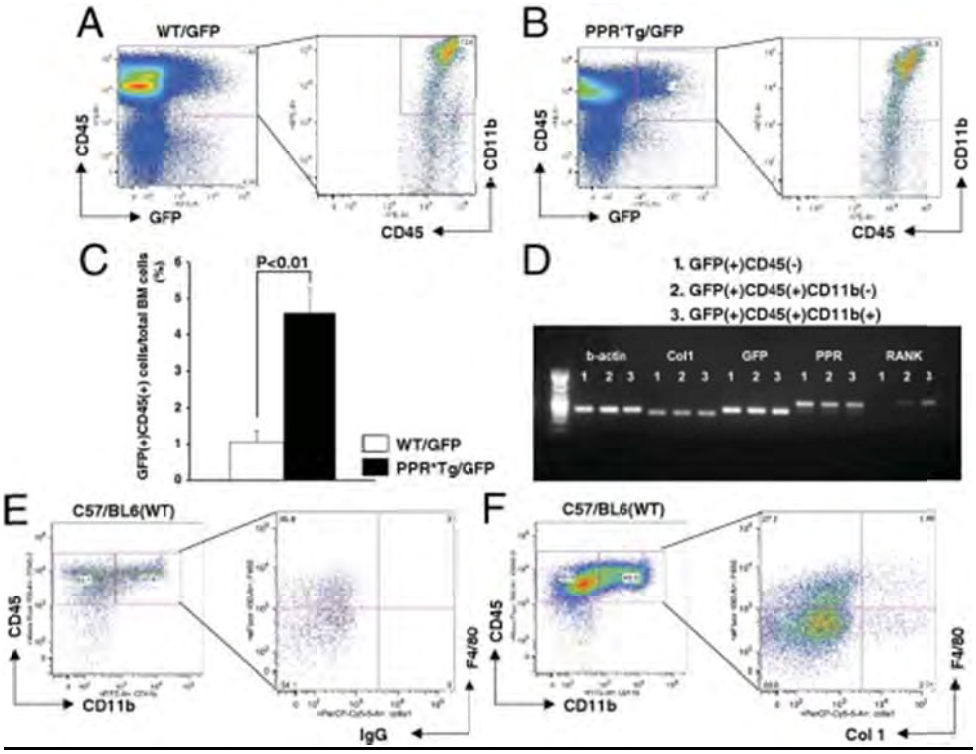


Figure 2

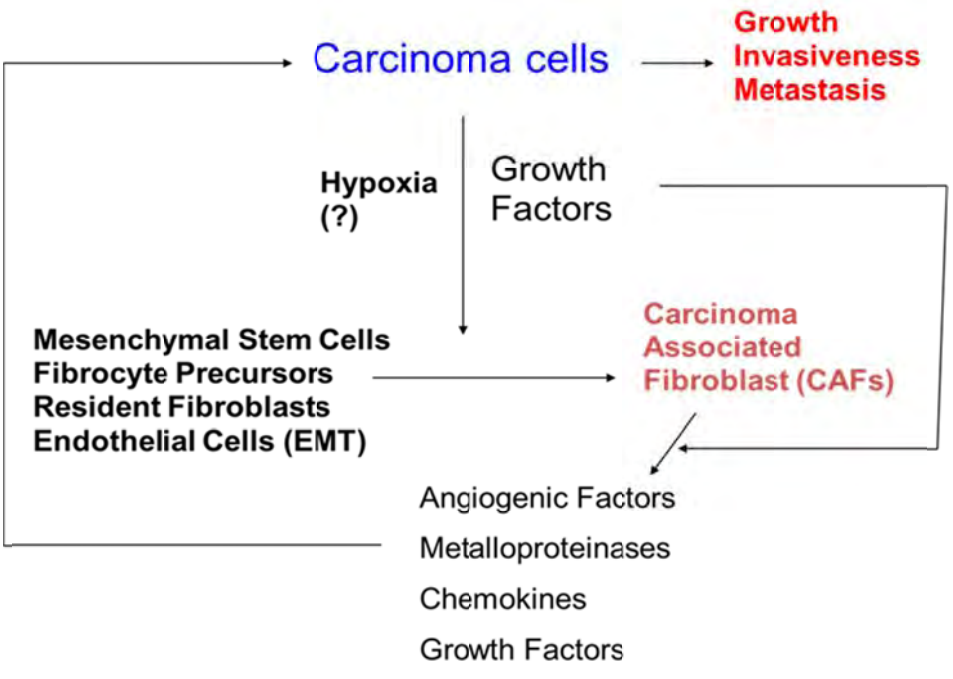


Figure Legends

Figure 1

(A-C) Identification and characterization of GFP(+)CD45(+) cells in WT and PPR*Tg mice. BM cells were isolated as described in Materials and Methods from WT/GFP (A) and PPR*Tg/GFP (B) mice, and analyzed for the expression of GFP, CD45 and CD11b. Note the expansion of GFP(+)CD45(+) cell population in PPR*Tg (B left panel) compared to WT (A left panel). Right panels in A and B show that the majority of the GFP(+)CD45(+) cells are positive for CD11b.
(C) Comparison of percentage of GFP(+)CD45(+) cells per total BM cells between WT/GFP (n=4) and PPR*Tg/GFP (n=5) mice. The results of 4 independent experiments were summed and the average values are shown. Student t test was performed to validate the significance of difference. P*<0.01
(D) GFP(+)CD45(-) cells, GFP(+)CD45(+)CD11b(-) cells and GFP(+)CD45(+)CD11b(+) cells were sorted from BM of WT/GFP mice, and total RNA was collected. RT-PCR was performed for beta actin (product size: 154bp), Col I (133bp), GFP (159bp), PPR (225bp) and RANK (243bp). The MspI digest of pBR322 DNA was used as molecular weight standards.
(E) Detection of CD45(+)CD11b(+) Col1(+) F4/80(+) and CD45(+)CD11b(+) Col1(+) F4/80(-) cells in the BM of WT mice. Shown are CD45+CD11b+ cells in lineage-negative gated BM (left panels). These can be further subdivided based on their expression of F4/80 and Col 1 (lower right panel). Isotype control for Col 1 is shown in upper right panel.
From [15]

Figure 2

Carcinoma-associated fibroblasts (CAFs) play a major role in organizing the tumor microenvironment and in modulating tumor growth, invasiveness and metastatization. From [23]

References

1. Schipani, E. and H.M. Kronenberg, Adult mesenchymal stem cells. Stembook, 2008. doi/10.3824/stembook.1.38..1.
2. Prockop, D., Marrow stromal cells as stem cells for nonhematopoietic tissues. Science, 1997. 276: p. 71-74.
3. Friedenstein, A., R. Chailakhjan, and K. Lalykina, The development of fibroblast colonies in monolayer cutures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet, 1970. 3: p. 393-403.
4. Friedenstein, A., Stromal mechanisms of bone marrow: cloning in vitro and retransplantation in vivo. Haematol Blood Transf, 1980. 25: p. 19-29.
5. Bucala, R., et al., Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. Mol Med, 1994. 1(1): p. 71-81.
6. Quan, T.E., et al., Circulating fibrocytes: collagen-secreting cells of the peripheral blood. Int J Biochem Cell Biol, 2004. 36(4): p. 598-606.
7. Quan, T.E. and R. Bucala, Culture and analysis of circulating fibrocytes. Methods Mol Med, 2007. 135: p. 423-34.
8. Barth, P.J. and C.C. Westhoff, CD34+ fibrocytes: morphology, histogenesis and function. Curr Stem Cell Res Ther, 2007. 2(3): p. 221-7.
9. Bellini, A. and S. Mattoli, The role of the fibrocyte, a bone marrow-derived mesenchymal progenitor, in reactive and reparative fibroses. Lab Invest, 2007. 87(9): p. 858-70.
10. Metz, C.N., Fibrocytes: a unique cell population implicated in wound healing. Cell Mol Life Sci, 2003. 60(7): p. 1342-50.
11. Phillips, R.J., et al., Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. J Clin Invest, 2004. 114(3): p. 438-46.
12. Wynn, T.A., Cellular and molecular mechanisms of fibrosis. J Pathol, 2008. 214(2): p. 199-210.
13. van Deventer, H.W., et al., C-C chemokine receptor 5 on pulmonary fibrocytes facilitates migration and promotes metastasis via matrix metalloproteinase 9. Am J Pathol, 2008. 173(1): p. 253-64.
14. Hartlapp, I., et al., Fibrocytes induce an angiogenic phenotype in cultured endothelial cells and promote angiogenesis in vivo. Faseb J, 2001. 15(12): p. 2215-24.
15. Ohishi, M., et al., A novel population of cells expressing both hematopoietic and mesenchymal markers is present in the normal adult bone marrow and is augmented in a murine model of marrow fibrosis. Am J Pathol, 2012. 180(2): p. 811-8.
16. Ohishi, M., et al., Osteoprotegerin abrogated cortical porosity and bone marrow fibrosis in a mouse model of constitutive activation of the PTH/PTHrP receptor. Am J Pathol, 2009. 174(6): p. 2160-71.
17. Wels, J., et al., Migratory neighbors and distant invaders: tumor-associated niche cells. Genes Dev, 2008. 22(5): p. 559-74.
18. Direkze, N.C., et al., Multiple organ engraftment by bone-marrow-derived myofibroblasts and fibroblasts in bone-marrow-transplanted mice. Stem Cells, 2003. 21(5): p. 514-20.
19. De Wever, O., et al., Stromal myofibroblasts are drivers of invasive cancer growth. Int J Cancer, 2008. 123(10): p. 2229-38.
20. Hong, K.M., et al., Differentiation of human circulating fibrocytes as mediated by transforming growth factor-beta and peroxisome proliferator-activated receptor gamma. J Biol Chem, 2007. 282(31): p. 22910-20.
21. Barth, P.J., et al., CD34+ fibrocytes, alpha-smooth muscle antigen-positive myofibroblasts, and CD117 expression in the stroma of invasive squamous cell carcinomas of the oral cavity, pharynx, and larynx. Virchows Arch, 2004. 444(3): p. 231-4.
22. Direkze, N.C., et al., Bone marrow contribution to tumor-associated myofibroblasts and fibroblasts. Cancer Res, 2004. 64(23): p. 8492-5.
23. Giaccia, A.J. and E. Schipani, Role of carcinoma-associated fibroblasts and hypoxia in tumor progression. Current topics in microbiology and immunology, 2010. 345: p. 31-45.

Genetic Skeletal Diseases

Brendan Lee, M.D., Ph.D.

Genetics of Human Skeletal Dysplasias
Brendan Lee, M.D., Ph.D.,
Baylor College of Medicine & Howard Hughes Medical Institute

Human skeletal dysplasias are disorders that result from errors in bone, cartilage, and joint development. A complex series of signaling pathways, including the FGF, TGFβ, BMP, WNT, Notch, and Hedgehog pathways, are essential for proper skeletogenesis, and human skeletal
dysplasias are often a consequence of primary or secondary dysregulation of these pathways. Although these pathways interact to regulate bone, cartilage, and joint formation, human genetic phenotypes point to the predominant action of specific components of these pathways. Mutations in the genes with a role in metabolic processing within the cell, the extracellular matrix, and transcriptional regulation can lead to dysregulation of cell–cell and cell–matrix signaling that alters tissue patterning, cell differentiation, proliferation, and apoptosis. We propose a morphogen rheostat model to conceptualize how mutations in different metabolic processes can lead to the integration of differential signaling inputs within a temporal and spatial context to generate apparently divergent skeletal phenotypes.

Reproduced from D. Baldridge, O. Shchelochkov, B. Kelley, and **B. Lee**. Signaling Pathways in Human Skeletal Dysplasias. ***Annual Review of Genomics and Human Genetics***, 11:189-217 (2010).

As a result of participating in this session, attendees should be able to appreciate the contribution of human genetic studies of the skeletal dysplasias for elucidating basic mechanisms of skeletal development and homeostasis.

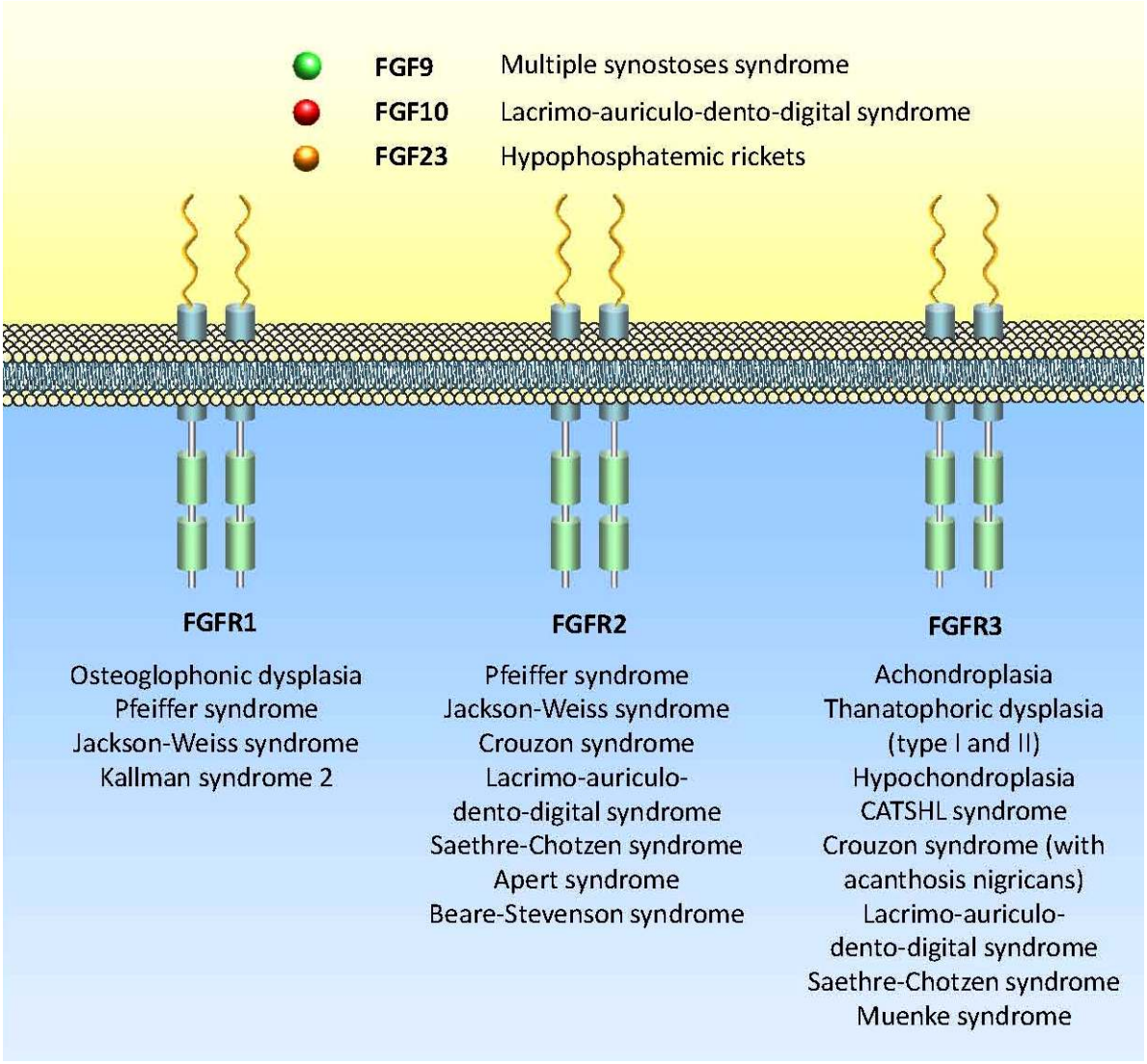


Figure 1. FGF signaling components and the human skeletal dysplasias they cause when mutated. Ligands such as FGF9, FGF10, and FGF23, bind and activate membrane bound receptors, including FGFR1, FGFR2, and FGFR3. This leads to activation of downstream mediators of FGF signaling, including MAP kinase, PI3 kinase, and phospholipase C gamma. Note that there is significant overlap of the clinical phenotype observed in syndromes caused by mutations in FGF signaling pathway. CATSHL: camptodactyly, tall stature, and hearing loss syndrome.

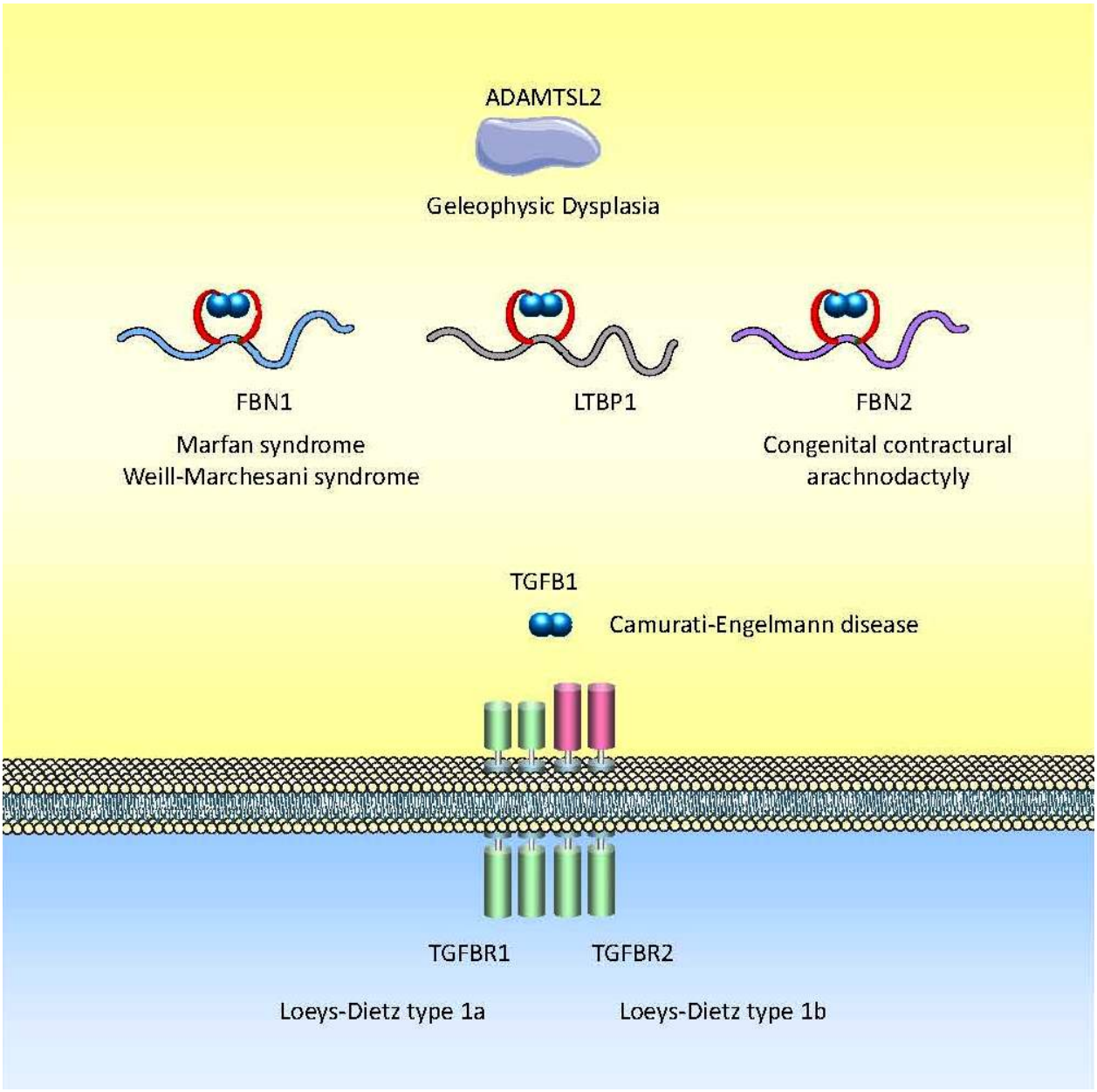


Figure 2. Genes that code for members of the TGF signaling pathway and the human skeletal dysplasias that are caused by mutations in these genes. The ligand TGF 1, which is coded for by the gene *TGFB1* and represented by a blue sphere, forms dimers in the extracellular space. This morphogen is usually kept in an inactive state because it is non-covalently bound by its own propeptide, shown in red, and by proteins such as latent-TGF -binding protein-1 (LTBP-1, encoded by *LTBP1*), or fibrillin 1 or 2 (encoded by *FBN1* or *FBN2*). ADAMTS-like protein 2, coded by the *ADAMTSL2* gene, can bind to LTBP-1 and thereby regulate TGF signaling. Once released, the TGF 1 ligand can bind to its cell-surface receptors, coded by the genes *TGFBR1* and *TGFBR2,* which in turn activate downstream signaling by phosphorylation of the Smad family of transcription factors.

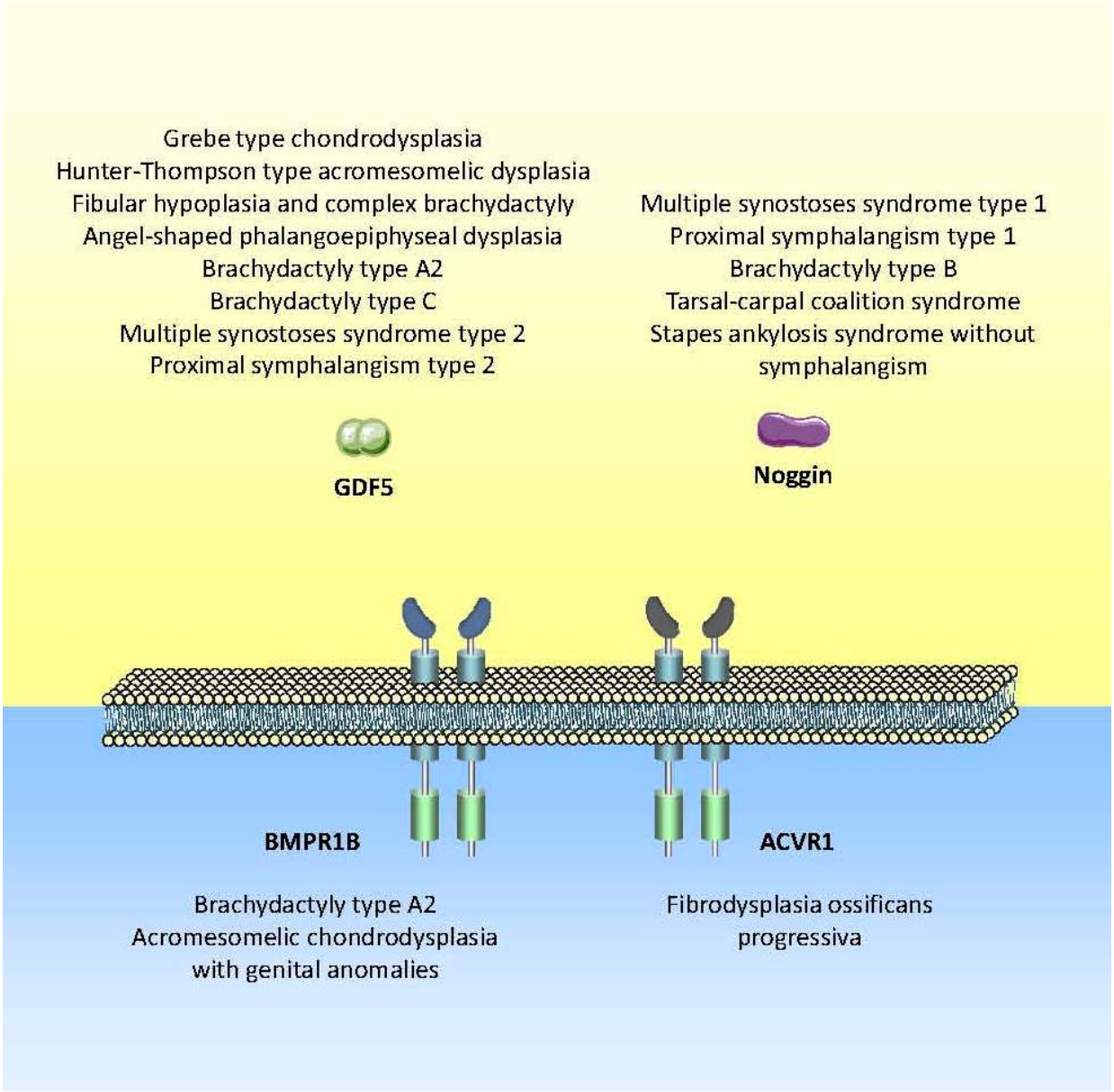


Figure 3. BMP signaling pathway genes and the human skeletal dysplasias they cause when mutated. Similar to the TGF signaling pathway, BMP ligands, such as GDF5, are dimers, and they bind to membrane-bound receptors, such as the type I receptors coded by the genes *BMPR1B* and *ACVR1*. This activates downstream signals, including both Smad-dependent and Smad-independent pathways. The protein noggin, which is coded for by the gene NOG, is an inhibitor of BMP signaling that binds and sequesters BMP ligands in the extra-cellular space.

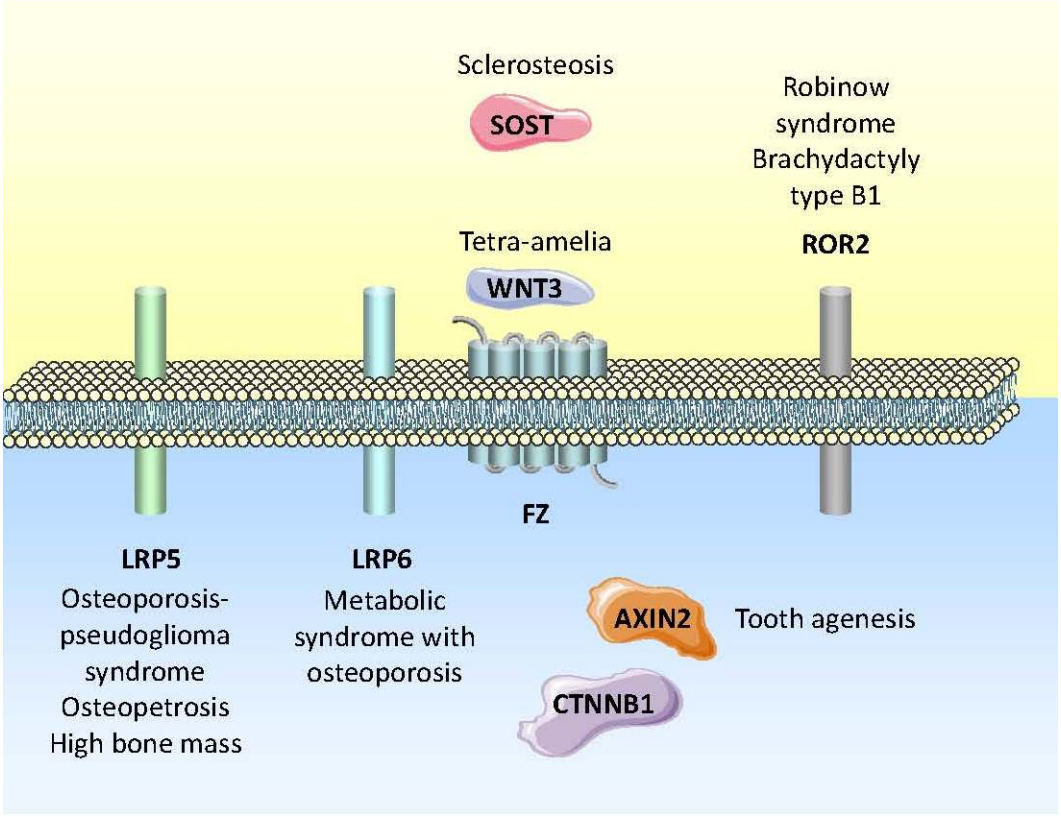


Figure 4. Genes that code for WNT signaling pathway members and the skeletal dysplasias that are caused by mutations in these genes. Canonical WNT signaling involves the binding of ligands, such as WNT3, to the Frizzled family of receptor proteins, coded by the *FZD* genes. These seven-transmembrane-containing receptors are regulated by co-receptors, including LRP5 and LRP6. When WNT signaling is activated, the downstream protein catenin beta-1 (encoded by *CTNNB1*) is not degraded, and is allowed to enter the nucleus, where it activates the transcription of WNT target genes. Additional regulators of this pathway include sclerostin, which is an extra-cellular inhibitor that can bind to LRP5 or LRP6, and the intracellular inhibitor AXIN2, which normally degrades catenin beta-1 when WNT signaling is NOT activated. In addition, the transmembrane protein ROR2 can act as an inhibitory co-receptor the WNT pathway.

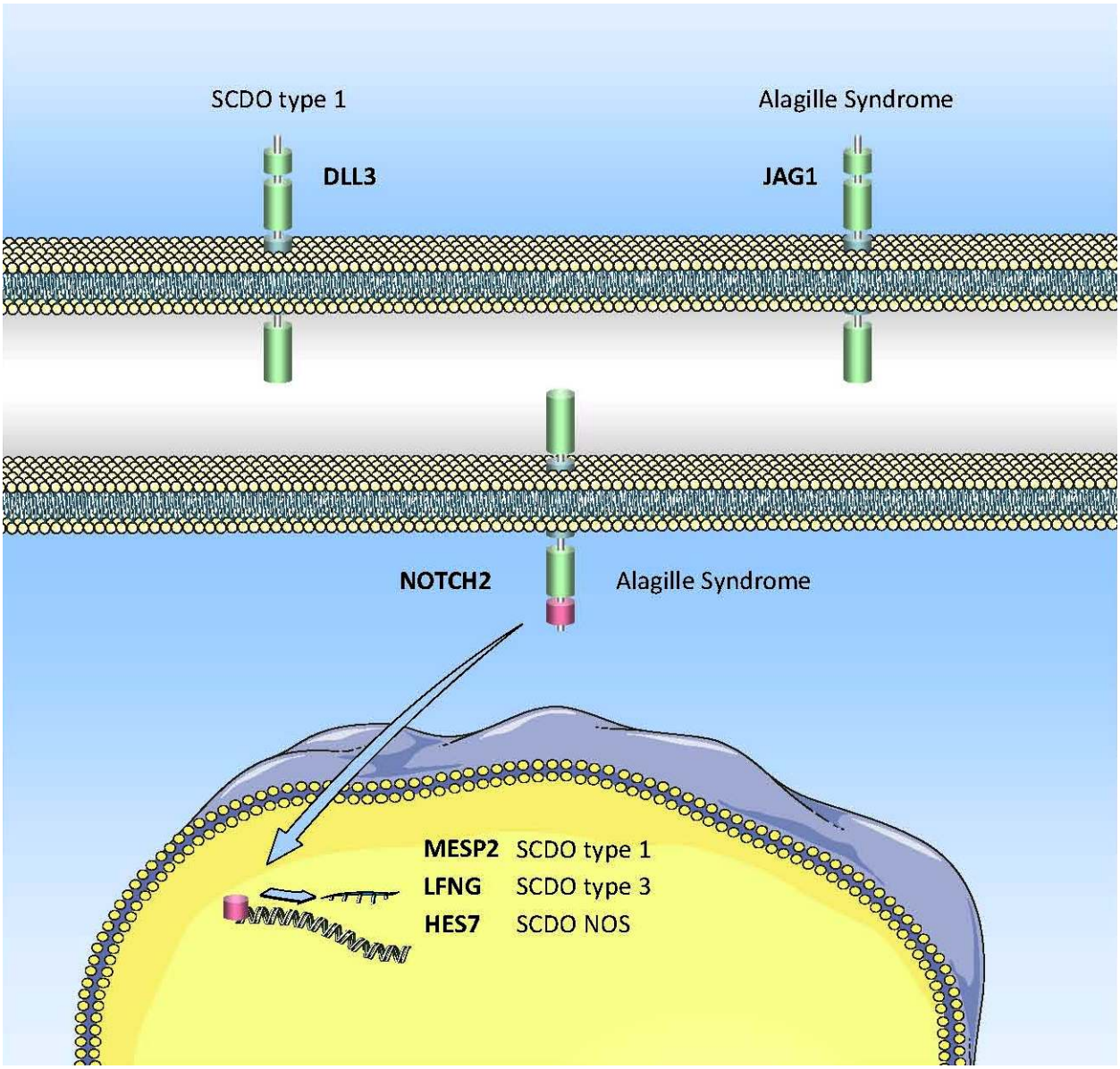


Figure 5. Notch signaling pathway genes and the human skeletal dysplasias they cause when mutated. Ligands such as those encoded by the genes *DLL3* or *JAG1*, which are found on the cell surface of a neighboring cell, can activate the membrane bound Notch receptors, including Notch2. When this pathway is activated, the intracellular portion of the Notch receptor can translocate to the nucleus where it activates transcription of downstream target genes, such as *MESP2*, *LFNG*, and *HES7*. Some of the proteins coded for by these genes, in particular *MESP2*, can in turn regulate upstream portions of the Notch signaling pathway, for example by causing decreased expression of the gene *DLL1*, which encodes a Notch ligand. SCDO: Spondylocostal dysostosis.

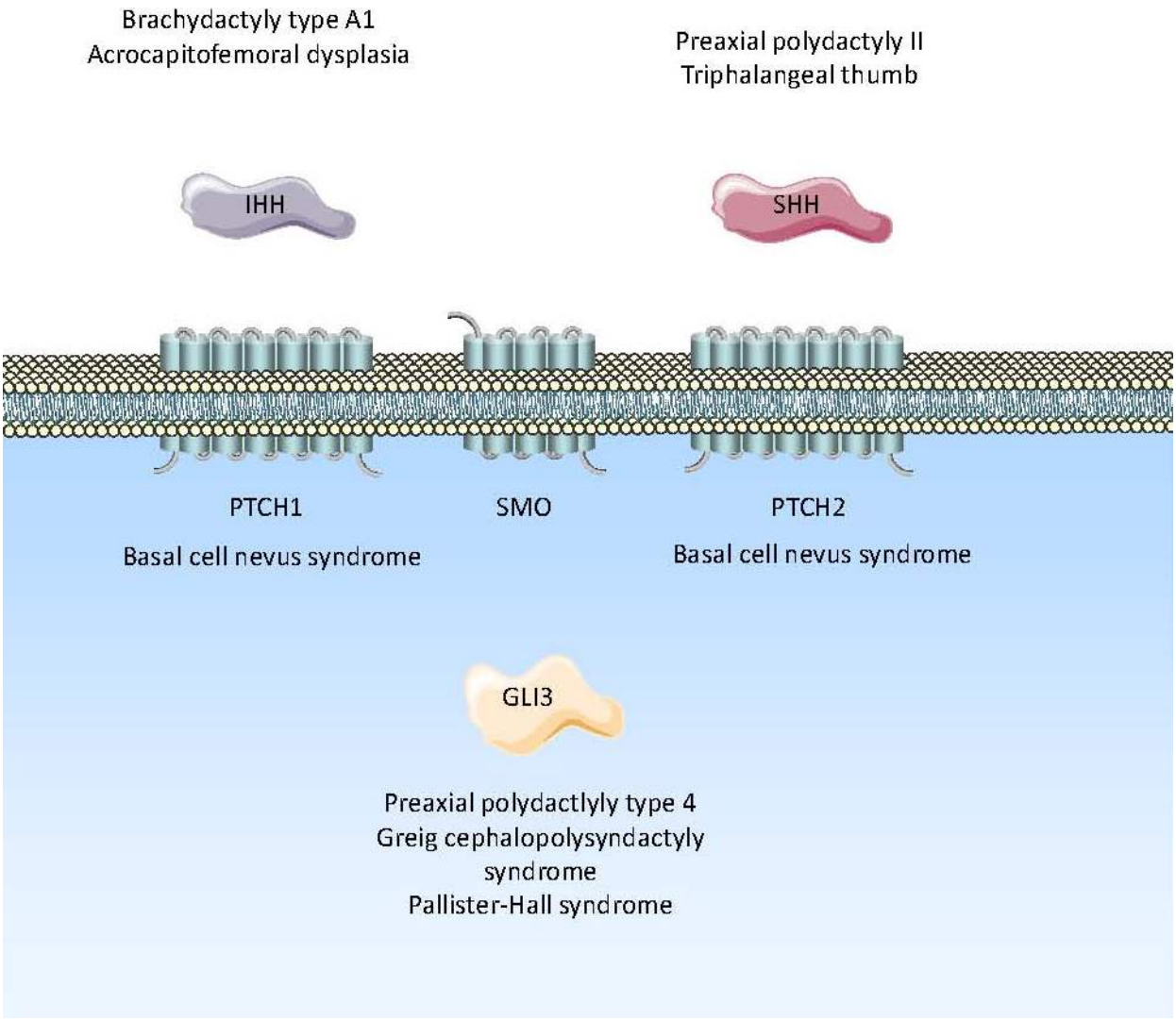


Figure 6. Genes that code for hedgehog pathway members and the skeletal dysplasias that are caused by mutations in these genes. *IHH* and *SHH* code for ligands that bind and activate the patched receptor, which is encoded by the genes *PTC1* or *PTC2*. Activated patched leads to release of smoothened, coded for by the gene *SMO*, and subsequent conversion of the GLI family of transcriptional regulators, including the protein encoded by *GLI3*, from repressors to activators of downstream target genes of the hedgehog pathway.

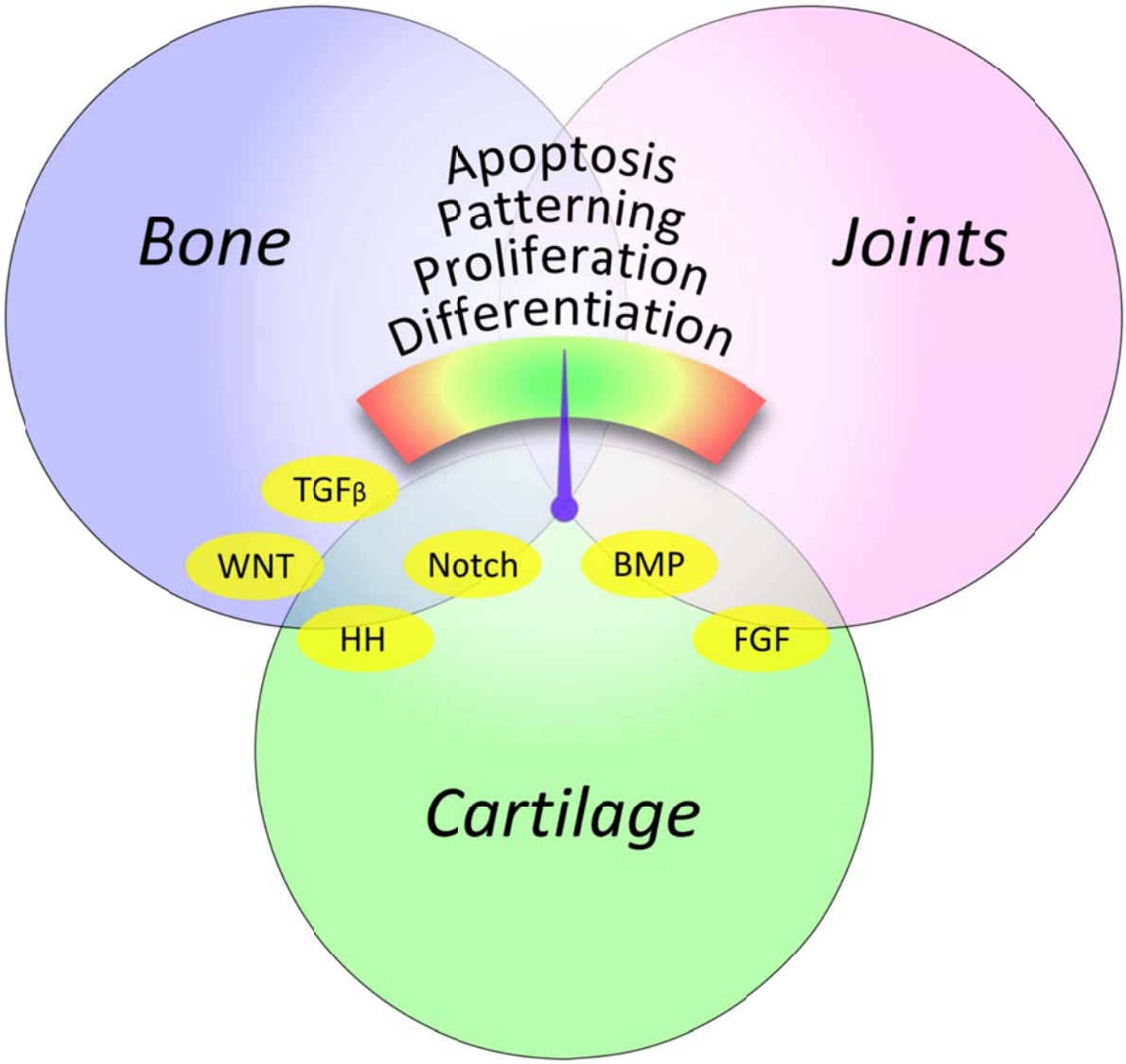


Figure 7. Integration of each of the classical signaling pathways, represented by yellow ovals, is accomplished at the cellular level of each tissue of the skeleton, including the bone, cartilage, and joints, represented by large circles. The predominant tissue that is affected by disruption of each pathway is represented by the location of the pathway on the diagram. For example, FGF signaling mainly affects the growth plate cartilage of the skull and long bones, but also can affect joints as see in FGF9 mutations and multiple synostoses syndrome. The signaling pathways are integrated into a morphogen rheostat, represented in the center of the figure, which serves to buffer differential inputs to ultimately control basic cellular processes such as cell differentiation, proliferation, apoptosis, and tissue patterning.

Breast and Prostate Cancer & Osteoporosis

Pamela Taxel, M.D.

Catherine Van Poznak, M.D.

Breast and Prostate Cancer & Osteoporosis
ASBMR, Minneapolis, Minnesota
Monday, October 15, 2012 @ 1:30 p.m. - 2:30 p.m.

Catherine Van Poznak, MD
University of Michigan School of Medicine
Ann Arbor, Michigan USA

Pamela Taxel, MD
University of Connecticut Medical Center
Farmington, Connecticut USA

Significance

Breast cancer and prostate cancers are common diagnoses, with each of these cancers being diagnosed in over 200,000 individuals annually. The vast majority of these cancers are diagnosed in an aging population and median age of breast cancer (BCA) diagnosis is 61 and prostate cancer (PCA) is 67. Most BCA and PCA are diagnosed early through screening and are treated with curative intent. As of January 2012, it is estimated that there are over 3.6 million breast cancer (BCA) and prostate cancer (PCA) survivors in the United States. Common therapies for these cancers involve endocrine manipulations that may increase the risk of bone loss, and fracture. Bone health in the care of those affected by BCA and PCA is a growing public health concern. This session will review concerns for the detection, prevention and management of osteoporosis in those diagnosed with early stage BCA and PCA. Management of bone metastases will not be the focus of this session.

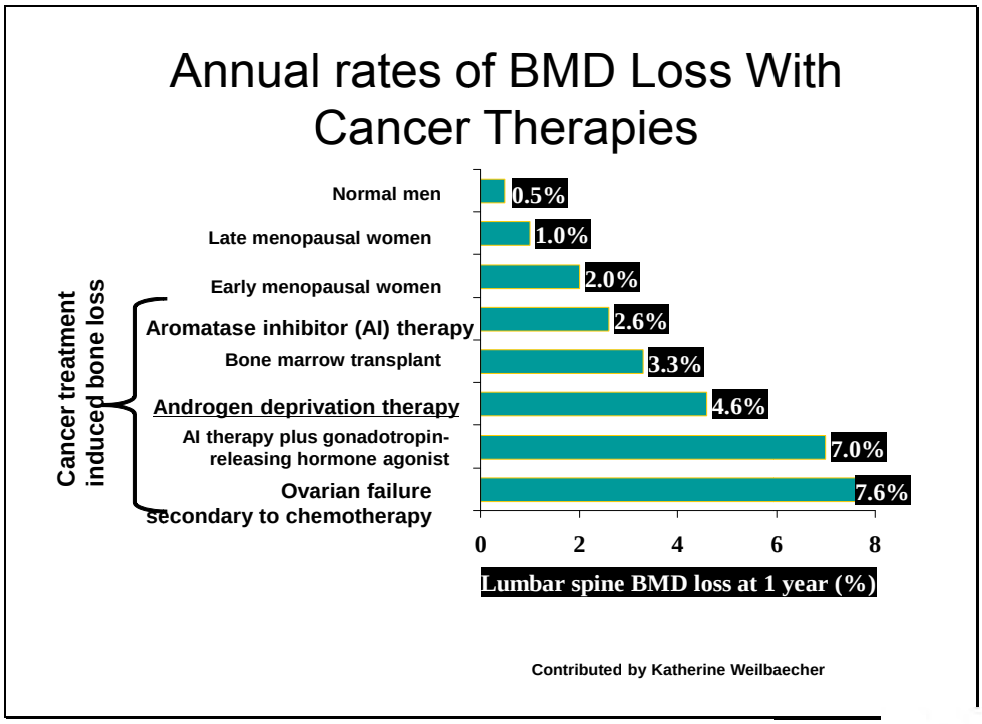
Learning Objectives: As a result of participating in this session, attendees should be able to:

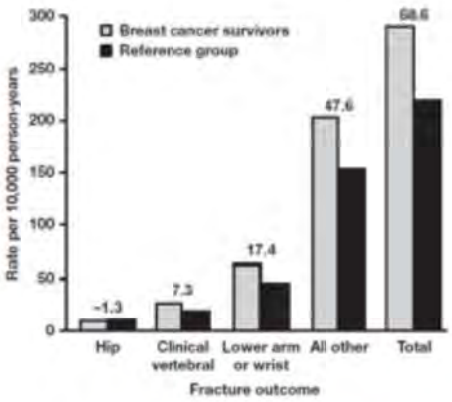
- To identify therapies used in the management of breast and prostate cancer that can accelerate bone loss and increase the risk of fracture
- To develop a strategy for evaluation of bone health including history, physical exam, laboratory data and to assess the risk of bone loss and fracture in women with breast and men with prostate cancer
- To review clinical trials examining osteoclast inhibitors in the management of non-metastatic breast and prostate cancer

Points of Interest:

Cancer Term Definitions:

- Adjuvant: Treatment after surgery, used with curative intent
- ADT: Androgen Deprivation Therapy
- CRPC: Castrate Resistant Prostate Cancer (not the focus of this presentation)
- ER: Estrogen Receptor; PR: Progesterone Receptor
- Metastatic: Distant disease is present and goals of care are palliative (non-curative)
- Neo-adjuvant: Treatment prior to surgery, typically to down stage the tumor, prior to surgery

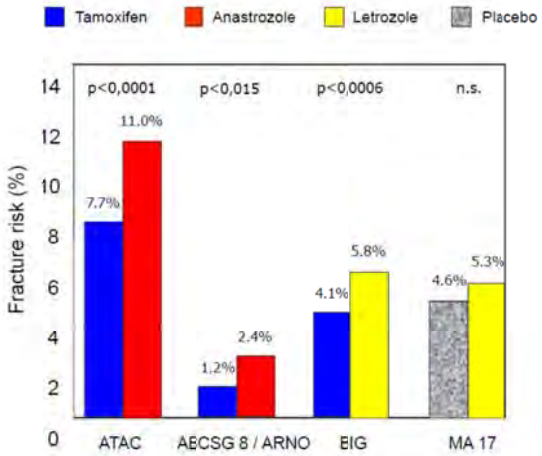




Chen et al Arch Int Med 2005

Breast Cancer:

- Chemot herapy (systemic effects)
  - Indications for adjuvant chemotherapy and bone effects
    - Tum or size, lymph node status
    - Mole cular profiling
  - Risks of premature menopause
    - Age
    - Drug s used
  - Supportive medications commonly used (steroids, PPI, GCSF, antidepressants)
- Radiatio n therapy: Local therapy which increases risk of rib fractures <2%
  - Lumpectomy: external beam radiation therapy (EBRT), brachytherapy (mammosite) or other
  - Post mastectomy radiation (EBRT): tumor > 5 cm, lymph nodes involved > 4, margins (+)
- Endocri ne therapy: Indications and bone effects
  - Ovarian ablation (chemical or surgical)
    - Ooph orectomy (BRCA mutation carriers before age)
  - Endocrine therapy for Estrogen Receptor (ER) and/or Progesterone Receptor (PR) (+) breast cancer; duration of therapy 5-10 years (Burstein J. Clin Onc 2010)
    - Tam oxifen
    - Aromatase inhibitors
      - Anastrozole (arimidex)
      - Exemestane (aromasin)
      - Letrozole (femara)



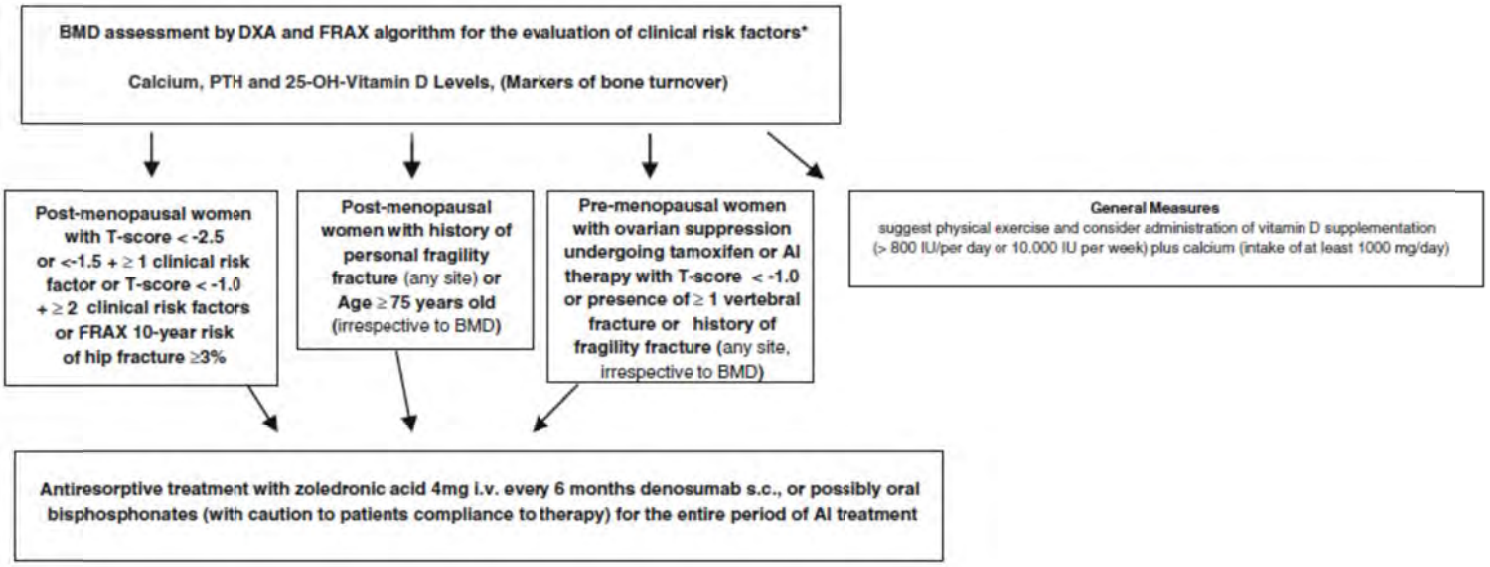
Hadji Semin Oncol 2007

- Menop ausal symptoms
  - Exogenous estrogen (any form) generally to be avoided

- HABITS (Holmberg Lancet 2004)
  - ER(+)& ER(-) cancers had an increased risk of recurrence with HRT
- WHI (Anderson Lancet Oncology 2012)
  - Estrogen was associated with a lower incidence of invasive breast cancer HR 0·77, 95% CI 0·62–0·95; p=0·02

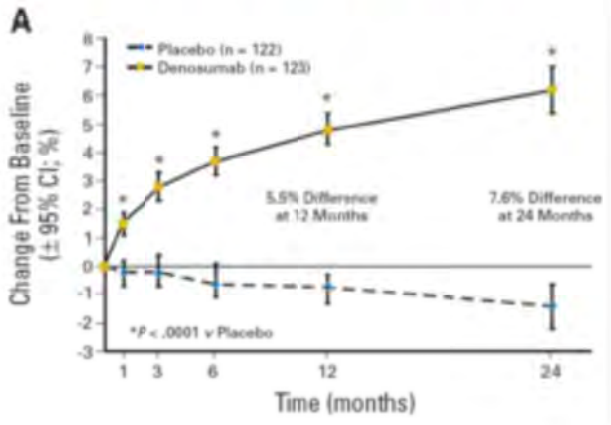
o Lubricants, Hot flash management

- Osteoporosis risk assessment
  - o Surveillance Epidemiology, and End Results (SEER) – Medicare research suggests that < 20% of women with breast cancer have bone mineral density testing (Synder JCO 2009) (Earl JCO 2003)
  - o Consensus Guidelines (Hillner JCO 2003) (Reid Can Treat Rev 2008) (Rizzoli OI 2011 – diagram below)

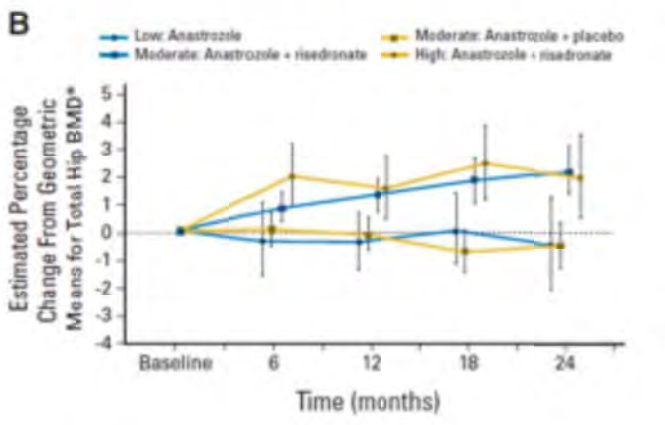


- Foundation: calcium, vitamin D, weight bearing exercise, lifestyle
- Threshold to treat with pharmacologic intervention
- Drug options

Women with osteopenia on an aromatase inhibitor +/- denosumab: Ellis JCO 2008



Women with osteopenia on an aromatase inhibitor +/- risedronate: Van Poznak, JCO 2010



Osteoporosis Rx & Breast Cancer

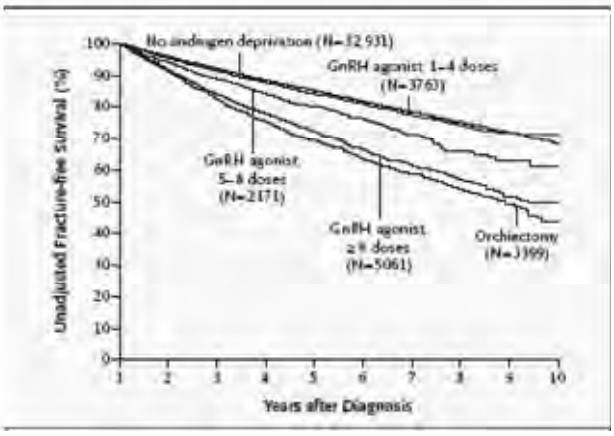
Drug Class	FDA treat or prevent Postmenopausal Osteoporosis	BCA patient may have special considerations
Hormonal	Estrogen +/- Progesterone	Generally, considered contra-indicated
	SERM (Raloxifene)	Consider
	Calcitonin	FDA change 2012
	Parathyroid hormone (PTH) (Teriparatide)	Generally, considered contra-indicated
Bisphosphonate	Alendronate, Ibandronate (oral or IV), Risedronate, Zoledronic acid (5mg)	Typically, the drugs of choice
RANKL Inhibition	Denosumab (60 mg subcutaneously Q6m)	Consider

Prostate Cancer

- Radiation Therapy
  - Can be either external beam radiation or radioactive tumor seeding (brachytherapy)
  - Commonly part of initial treatment in newly diagnosed men (28%) with or without Androgen Deprivation therapy.
- Endocrine therapy
  - Approx. 70 % of prostate cancers are androgen-dependent and respond to some form of hormonal ablation therapy. (Schally AV)
  - Who receives endocrine therapy
    - High risk adjuvant
      - Radical prostatectomy with lymph node positive disease (locally advanced).
      - High risk local disease (↑ Gleason score).
    - Neoadjuvant with RT /brachytherapy
    - Biochemical Relapse
      - After failure of 1° therapy with RT or prostatectomy for increasing PSA
  - LHRH-agonists (analogs) - inhibit pituitary gonadotrophins (FSH and LH) leading to decreased testosterone production from testis.
  - Anti-androgens - are competitive inhibitors of the androgen receptor, blocking testosterone action and are typically used in conjunction with LHRH-agonists (Combined androgen blockade) or as monotherapy (Pronzato P).

- Bone Loss and Fracture Risk: increases with increased duration of ADT

Unadjusted Fracture-free Survival in men with P Ca, according to ADT.



Shahanian VB et al. NEJM, 2005, 352;154.

- Biochemical and DXA Evaluation of Men on Androgen Deprivation Therapy
  - History, Physical Examination and DXA at initial visit (? LVA)
  - Laboratory Evaluation: to rule out other 2^{o} causes of bone loss
    - eg. CBC, TSH, Calcium, Albumin, 25-OH vitamin D
    - Bone Turnover Markers (sCTX, BSAP, P1NP, uNtx)

- Whom to Treat?
  - Calcium and Vitamin D
    - Limited data on optimal levels
    - 1200 mg/day of calcium, 600-1000 IU Vitamin D/day

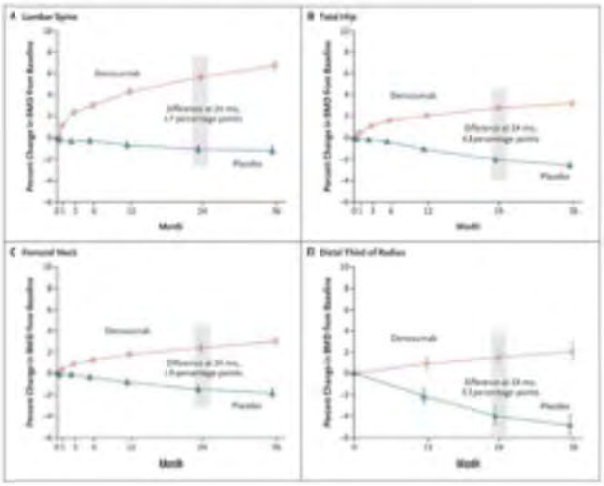
  - Consideration of Medical Therapy
    - Based on BMD and risk factors
    - Use of FRAX Algorithm

- Approved Therapies in Men on ADT
  - Oral bisphosphonates
  - IV Zoledronic Acid (if Osteoporosis)
  - Denosumab

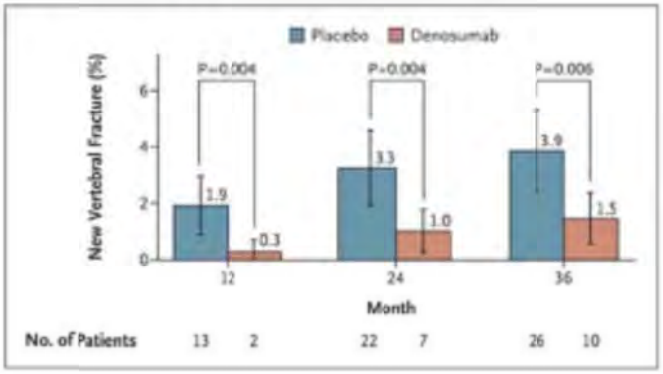
Oral BPs and ADT (Adler R . Maturitas, 2011; 68; 143-47.)

Author	Trial	Baseline	Change in BMD	Comments
Bruder (2006)	Open label – alendronate 70 mg or no Rx q week *n* = 46	Osteopenia or osteoporosis in all but 5	S −1.3%, +1.4% TH −0.9%, +1%	Not randomized, variable duration
Greenspan 2007	Alendronate 70 mg weekly × 1 year *n* = 112	91% osteopenia or osteoporosis	S −1.4% +3.7% FN −2.7%, +1.6%	RCT
Ishizaka (2007)	Open label, no control risedronate 2.5 mg daily for 6 months *n* = 61	Normal to osteoporosis, 11 with bone metastases. Mean UDR *T*-score was −2.6	S +4.9% FN −0.1% UDR +1.1%	Majority responded, regardless of baseline BMD
Izumi (2009)	Open label RIS 2.5 mg daily for BMD < 90% normal young mean, *n* = 56		S −2.2%, +2.5%	
Planas (2009)	RCT, *n* = 71 alendronate 70 mg weekly for 1 year	*T*-score < −2	S 0%, +0.5% TH −1.2%, +1.3%	
Taxel (2010)	RCT *n* = 40 risedronate 35 mg q week For 6 months	Mean *T*-scores normal	S −1.5%, +1.7% TH −2.2%, +0.3%	Treatment started with onset of ADT

Mean Percent Changes from Baseline Bone Mineral Density (BMD) Values during the Study Period, According to Skeletal Site and Study Group: Smith M et al. N Engl J Med 2009;361:745-755



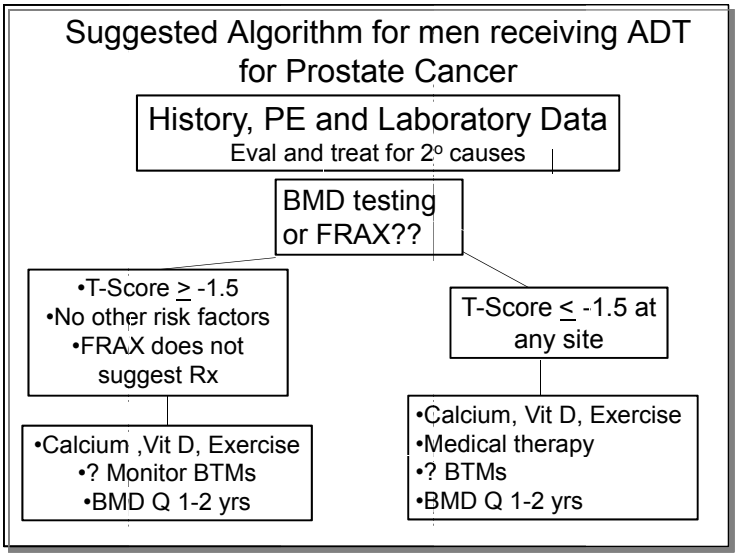
Cumulative Incidence of New Vertebral Fracture at 12, 24, and 36 Months, According to Study Group: Smith M et al. N Engl J Med 2009;361:745-755



- Monitori ng of Treatment
  - BTMs- ?
  - BMDs - ? frequency

SUMMARY

- Women with Breast Cancer & Men with Prostate Cancer receiving systemic therapies are at increased risk of bone loss and consequent fractures.
- Bisphos phonates, SERMs and denosumab can prevent bone loss.
- Need for further prospective studies to determine which individuals are at highest risk for bone loss and fractures (esp non-vertebral fractures).



REFERENCES

Breast Cancer References

Exogenous estrogens after Breast Cancer diagnosis

- Holmber g L, Anderson H; HABITS steering and data monitoring committees. HABITS (hormonal replacement therapy after breast cancer – is it safe?), a randomised comparison: trial stopped. *Lancet* 2004;363(9407):453-455
- Antoine C, Liebens F, Carly B, Pastijn A, Neusy S, Rozenberg S. Safety of hormone therapy after breast cancer: a qualitative systematic review.Hum Reprod. 2007 Feb;22(2):616-22. Epub 2006 Oct 18
- Anderson GL, Chlebowski RT, Aragaki AK, Kuller LH, Manson JE, Gass M, Bluhm E, Connelly S, Hubbell FA, Lane D, Martin L, Ockene J, Rohan T, Schenken R, Wactawski-Wende J. Conjugated equine oestrogen and breast cancer incidence and mortality in postmenopausal women with hysterectomy: extended follow-up of the Women's Health Initiative randomised placebo-controlled trial. Lancet Oncol. 2012 May;13(5):476-86.

Adjuvant Endocrine therapy for Breast Cancer: Guideline with bone data reviewed

- Burstein HJ, Prestrud AA, Seidenfeld J, et al. American Society of Clinical Oncology clinical practice guideline: update on adjuvant endocrine therapy for women with hormone receptor-positive breast cancer. J Clin Oncol. 2010 Aug 10;28(23):3784-96. Epub 2010 Jul 12.

Screening & management of bone loss in Breast Cancer

- Snyder CF, Frick KD, Kantsiper ME, et al. Prevention, screening, and surveillance care for breast cancer survivors compared with controls: changes from 1998 to 2002.J Clin Oncol. 2009 Mar 1;27(7):1054-61
- Earle CC, Burstein HJ, Winer EP, Weeks JC. Quality of non-breast cancer health maintenance among elderly breast cancer survivors.J Clin Oncol. 2003 Apr 15;21(8):1447-51.
- Hillner et al. American Society of Clinical Oncology 2003 Update on the Role of Bisphosphonates and Bone Health Issues in Women With Breast Cancer. J. Clin Onc 2003, doi: 10.1200/JCO.2003.08.017
- Reid et al. Guidance for the management of breast cancer treatment-induced bone loss: A consensus position statement from a UK Expert Group. Cancer Treatment Reviews (2008) 34, S3– S18
- Rizzoli et al. Guidance for the prevention of bone loss and fractures in postmenopausal women treated with aromatase inhibitors for breast cancer: an ESCEO position paper. Osteoporos Int. 2011. DOI 10.1007/s00198-011-1870-0
- Hadji P, Hartenfels M, Kyvernitakis J, Hars O, Baumann KH, Kalder M. Recommendations for antiresorptive therapy in postmenopausal patients with breast cancer: Marburg AIBL Guideline Evaluation Study (MAGES).Breast Cancer Res Treat. 2012 Jun;133(3):1089-96.

Osteoclast inhibition during adjuvant aromatase inhibition in breast cancer

- Lester JE, Dodwell D, Purohit OP, Gutcher SA, Ellis SP, Thorpe R, et al. Prevention of anastrozole-induced bone loss with monthly oral ibandronate during adjuvant aromatase inhibitor therapy for breast cancer.Clin Cancer Res. 2008 Oct 1;14(19):6336-42.
- Van Poznak C, Hannon RA, Mackey JR, Campone M, Apffelsaedt JP, Clack G, et al. Prevention of Aromatase Inhibitor-Induced Bone Loss Using Risedronate: The SABRE Trial. J Clin Oncology. 2010;28(6):967-75.
- Markopoulos C, Tzoracoleftherakis E, Polychronis A, Venizelos B, Dafni U, Xepapadakis G, et al. Management of anastrozole-induced bone loss in breast cancer patients with oral risedronate: results from the ARBI prospective clinical trial. Breast Cancer Res. 2010;12(2):R24.
- Ellis GK, Bone HG, Chlebowski R, Paul D, Spadafora S, Smith J, et al. Randomized trial of denosumab in patients receiving adjuvant aromatase inhibitors for nonmetastatic breast cancer. J Clin Oncol. 2008; 26(30):4875-82

Prostate References

1. Howlader N, SEER Cancer Statistics Review,1975-2008.
2. U.S. Cancer Statistics Working Group, 2010, United States Cancer Statistics: 1999–2007 Incidence and Mortality Web-based Report. Atlanta (GA): Department of Health and Human Services, Centers for Disease Control and Prevention, and National Cancer Institute.
3. Schally AV, 2007, Luteinizing hormone-releasing hormone analogues and hormone ablation for prostate cancer: state of the art. BJU Int.100 Suppl 2:2-4.
4. Gooren L, 2003 Androgen deficiency in the aging male: benefits and risks of androgen supplementation.J Steroid Biochem Mol Biol. 2003 Jun;85(2-5):349-55.
5. Bischoff-Ferrari HA, Orav EJ, Dawson-Hughes B. 2008 Additive benefit of higher testosterone levels and vitamin D plus calcium supplementation in regard to fall risk reduction among older men and women. Osteoporos Int.19(9):1307-14.
6. Pronzato P, Rondini M. 2005 Hormonotherapy of advanced prostate cancer. Ann Oncol. 2005 May;16 Suppl 4:iv80-84. Review.
7. Datta M, Schwartz GG. Calcium and Vitamin D Supplementation During Androgen Deprivation Therapy for Prostate Cancer: A Critical Review.Oncologist. 2012 Jul 25.
8. Bishr M, Saad F.Preventing bone complications in prostate cancer.Curr Opin Support Palliat Care. 2012 Sep;6(3):299-303.

9. Schröder F, Crawford ED, Axcrona K, Payne H, Keane TE. Androgen deprivation therapy: past, present and future. BJU Int. 2012 Jun;109 Suppl 6:1-12.
10. Smith MR, McGovern FJ, Zietman AL, Fallon MA, Hayden DL, Schoenfeld DA, Kantoff PW, Finkelstein JS 2001 Pamidronate to prevent bone loss during androgen-deprivation therapy for prostate cancer. N Engl JMed. 345:948-55.
11. Smith MR, Malkowicz SB, Brawer MK, Hancock ML, Morton RA, Steiner MS. 2011 Toremifene decreases vertebral fractures in men younger than 80 years receiving androgen deprivation therapy for prostate cancer. Urol. 186(6):2239-44.
12. Smith, MR., Blair Egerdie B, Hernández Toriz, N, et. al. for the Denosumab HALT Prostate Cancer Study Group. 2009 Denosumab in Men Receiving Androgen-Deprivation Therapy for Prostate Cancer N Engl J Med; 361:745-755.
13. Adler RA, Hastings FW, Petkov VI.2010 Treatment thresholds for osteoporosis in men on androgen deprivation therapy: T-score versus FRAX.Osteoporos Int.21(4):647-53.
14. Michaelson MD, Kaufman DS, Lee H, McGovern FJ, Kantoff PW, Fallon MA, Finkelstein JS, Smith MR. Randomized controlled trial of annual zoledronic acid to prevent gonadotropin-releasing hormone agonist-induced bone loss in men with prostate cancer.J Clin Oncol. 2007 Mar 20;25(9):1038-42.

Paget's Disease

Ethel Siris, M.D.

Paget's Disease

Ethel Siris, M.D.
Columbia University
New York, New York
USA

Paget's Disease of Bone

Ethel S. Siris, M.D. and G. David Roodman, MD

Paget's disease of bone is a localized disorder of bone remodeling. The process is initiated by increases in osteoclast-mediated bone resorption, with subsequent compensatory increases in new bone formation, resulting in a disorganized mosaic of woven and lamellar bone at affected skeletal sites. This structural change produces bone that is expanded in size, less compact, more vascular, and more susceptible to deformity or fracture than is normal bone (1). Clinical signs and symptoms will vary from one patient to the next depending on the number and location of affected skeletal sites, as well as on the degree and extent of the abnormal bone turnover. It is believed that most patients are asymptomatic, but a substantial minority may experience a variety of symptoms, including bone pain, secondary arthritic problems, bone deformity, excessive warmth over bone from hypervascularity, fracture and a variety of neurological complications caused in most instances by compression of neural tissues adjacent to pagetic bone.

ETIOLOGY

Although Paget's disease is the second most common bone disease after osteoporosis, the factors involved in its pathogenesis are just beginning to be clarified. Both genetic and environmental factors have been implicated in the pathophysiology of Paget's disease. Paget's disease occurs commonly in families and can be transmitted vertically in an autosomal dominant pattern.

Fifteen to 30% of Paget's disease patients have positive family histories of the disorder (2-4), and familial aggregation studies in a United States population (5) suggest that the risk of a first-degree relative of a pagetic subject developing the condition is 7 times greater than is the risk for someone who does not have an affected relative.

Multiple genetic loci have been linked to familial Paget's disease, and three genes have been identified. Recent genome wide association studies have identified several susceptibility loci for Paget's disease. These include variants in the CSF-1 gene, the RANK gene, PML gene and three other genes (6, 7). An insertion mutation in the RANK gene has been reported (8) but this mutation is rarely found in patients with familial Paget's disease (9).

The most frequent mutations linked to Paget's disease are in a gene on 5q35-QTER, which encodes an ubiquitin binding protein, sequestasome-1 (SQSTM1/p62) (10). Mutations in SQSTM1 occur in 30% of patients with familial Paget's disease with the P392L mutation being the most frequent (11). Mutations in the SQSTM1 gene have been associated with the severity of Paget's disease. Mutation carriers had an earlier age of onset and more commonly required surgery and bisphosphonate therapy (12). Sequestasome-1 plays an important role in the NFκB signaling pathway. Patients with SQSTM1 mutations can have a variable clinical phenotype, including no evidence of Paget's disease in at least 1 or 2 individuals, and no gene dose effect can be seen between heterozygotes and homozygotes individuals. Recent studies have reported that the P392L mutation in p62 is either a predisposing mutation for Paget's disease or can result in Paget's disease in experimental animal models (13, 14). Human osteoclast precursors transfected with the $p62^{P392L}$ do not form osteoclasts characteristic of Paget's disease, and transgenic mice with the $p62^{P392L}$ mutation targeted to the osteoclast lineage develop progressive osteopenia and not Paget's disease. However, mice in which the normal p62 gene has been replaced with $p62^{P392L}$ either do not develop Paget's disease (13) or develop pagetic like lesions predominantly in their femurs (14).

There is a restricted geographic distribution for the occurrence of Paget's disease. Paget's disease is most common in Europe, North America, Australia, and New Zealand in persons of Anglo-Saxon descent and is extremely uncommon in Asia, Africa, and Scandinavia.

Some recent studies have reported an apparent decline in the frequency and severity of Paget's disease in both Great Britain and New Zealand (15, 16). The basis for this decline is unknown, but the changes are too rapid to be explained by genetic factors and cannot be explained by migration patterns of persons with

a predisposition to Paget's disease.

For more than 30 years, studies have suggested that Paget's disease may result from a chronic paramyxoviral infection. This is based on ultrastructural studies by Rebel and coworkers (17) who demonstrated that nuclear and, less commonly, cytoplasmic inclusions that were similar to nucleocapsids from paramyxoviruses were present in osteoclasts from Paget's disease patients. Mills and Singer (18) also reported that the measles virus nucleocapsid antigen was present in osteoclasts from patients with Paget's disease, but not from patients with other bone diseases. In some specimens, both measles virus and respiratory syncytial virus nucleocapsid proteins were demonstrated by immunocytochemistry on serial sections. Gordon and colleagues (19), using *in situ* hybridization studies, found canine distemper virus nucleocapsid protein in 11 of 25 Paget's disease patients, and Mee and coworkers (20), using highly sensitive *in situ* PCR techniques, found that osteoclasts from 12 of 12 English patients with Paget's disease expressed canine distemper virus nucleocapsid transcripts.

Kurihara et al (21) provided evidence for a pathophysiologic role for measles virus in the abnormal osteoclast activity in Paget's disease both in vitro and in vivo. Transfection of the measles virus nucleocapsid gene into normal human osteoclast precursors resulted in formation of osteoclasts that expressed many of the abnormal characteristics of pagetic osteoclasts. However, other workers have been unable to confirm the presence of measles virus or CDV in pagetic osteoclasts (22). Kurihara et al also targeted the measles virus nucleocapsid gene to cells in the osteoclast lineage in transgenic mice, and found that 29% of these mice develop localized bone lesions that are similar to lesions seen in patients with Paget's disease (23). More recently, these investigators reported that mice expressing the MVNP gene and the p62^{P392L} mutation develop exuberant pagetic lesions (24). They further demonstrated that many of the effects of MVNP seen in these mice were moderated by IL-6 (24).

Among the many questions that still remain to be explained to understand the contributions of environmental and genetic factors to Paget's disease are: 1) Since paramyxoviral infections such as measles virus occur worldwide, why does Paget's disease have a very restricted geographic distribution? 2) How does the virus persist in osteoclasts in patients who are immunocompetent for such long periods of time, since measles virus infections generally occur in children rather than adults, and Paget's disease is usually diagnosed in patients over the age of 55? 3) Why does Paget's disease remain so highly localized in patients after diagnosis? 4) What is the explanation for the variable phenotypic presentation of patients with familial Paget's disease, especially that some of these patients who carry the mutated gene do not have Paget's disease even though they are over 70 years of age?

PATHOLOGY

The initiating lesion in Paget's disease is an increase in bone resorption due to an abnormality in the osteoclasts found at affected sites. Pagetic osteoclasts are more numerous than normal and contain substantially more nuclei than do normal osteoclasts, with up to 100 nuclei per cell. In response to the increase in bone resorption, numerous osteoblasts are recruited to pagetic sites where active and rapid new bone formation occurs. It is generally believed that the osteoblasts are intrinsically normal (25, 26).

In the earliest phases of Paget's disease, increased bone resorption dominates, and lytic changes are seen on radiographs. After this, there is a combination of increased resorption and relatively tightly coupled new-bone formation, produced by the large numbers of osteoblasts present at these sites. During this phase, and presumably because of the accelerated nature of the process, the new bone that is made is abnormal. Newly deposited collagen fibers are laid down in a haphazard rather than a linear fashion, creating more primitive woven bone. The end product is the so-called mosaic pattern of woven bone plus irregular sections of lamellar bone linked in a disorganized way by numerous cement lines representing the extent of previous areas of bone resorption. The bone marrow becomes infiltrated by excessive fibrous connective tissue and by an increased number of blood vessels, explaining the hypervascular state of the bone. Bone matrix is typically normally mineralized, and tetracycline labeling shows increased calcification rates. It is not unusual, however, to find areas of pagetic biopsies in which widened osteoid seams are apparent, perhaps reflecting inadequate calcium/phosphorus products in localized areas where rapid bone turnover heightens mineral demands.

In time, the hypercellularity at a locus of affected bone may diminish, leaving the end product of a sclerotic, pagetic mosaic without evidence of active bone turnover, so-called burned out Paget's disease. Typically, all phases of the pagetic process can be seen at the same time at different sites in a particular subject. The chaotic architectural changes that occur in pagetic bone contribute to the loss of structural integrity. Figure 1 compares the appearances of normal and of pagetic bone by scanning electron microscopy.

BIOCHEMICAL INDICES IN PAGET'S DISEASE

Measurements of biochemical markers of bone turnover are useful clinically in the assessment of the extent and severity of disease in the untreated state and for monitoring the response to treatment (27). Increases in serum or urine levels of biomarkers of bone resorption such as the C-and N-terminal telopeptides of collagen, CTX and NTX, reflect the increases in osteoclast mediated bone resorption. Secondary increases in osteoblastic activity are associated with elevated levels of bone formation markers including serum total alkaline phosphatase (SAP), bone specific alkaline phosphatase and procollagen type-1 N-terminal propeptide (P1NP). In untreated patients, the values of serum CTX or urine NTX and SAP rise in proportion to each other, reflecting the preserved coupling of resorption and formation. The magnitude of the increase in markers offers an estimate of the extent or severity of the abnormal bone turnover, with higher levels reflecting a more active, ongoing localized metabolic process. Active monostotic disease may have lower SAP values than polyostotic disease. Lower values (e.g., <3 times the upper limit of normal) may indicate fewer pagetic sites or a lesser degree of increased bone turnover at affected sites. However, mild elevations in a patient with limited and highly localized disease (e.g., the proximal tibia) may still be associated with symptoms and clear progression of disease at that site. Even a so-called "normal" SAP (e.g. at the upper limit of the normal range) may not truly be normal for the pagetic patient. To be confident that the SAP reflects quiescent disease, a result in the middle of the normal range is probably required.

Potent bisphosphonates are capable of normalizing the biochemical markers, an indication of a remission of the bone-remodeling abnormality, in a majority of patients and bringing the markers to near normal in most others so that monitoring the markers is helpful in assessing treatment effects. CTX or NTX may become normal in days to a few weeks after bisphosphonate therapy is initiated. It is often adequate, however, to monitor SAP alone, with a baseline measure pre-treatment, a post treatment test one to three months after treatment is completed and at 6-12 month intervals thereafter to determine duration of the effect of that treatment course.

Serum calcium is typically normal in untreated Paget's' disease, but secondary hyperparathyroidism and transient decreases in serum calcium can occur in some patients being treated with potent bisphosphonates. This results from the early suppression of bone resorption in the setting of not yet reduced new-bone formation (28). As restoration of coupling occurs with time, PTH levels fall. The problem can be largely avoided by being certain that such patients are and remain replete in both calcium and vitamin D.

CLINICAL FEATURES

Paget's disease affects both men and women, with most series describing a slight male predominance. It is rarely observed to occur in individuals younger than age 25 years, it is thought to develop as a clinical entity after the age of 40 in most instances, and it is most commonly diagnosed in people over the age of 50. In a survey of over 800 selected patients in the US, 600 of whom had symptoms, the average age at diagnosis was 58 years (29). It seems likely that many patients have the disorder for a period of time before any diagnosis is made, especially because it is often an incidental finding.

Paget's disease may be monostotic, affecting only a single bone or portion of a bone (Fig. 2), or may be polyostotic, involving two or more bones. Sites of disease are often asymmetric. Clinical observation suggests that in most instances, sites affected with Paget's disease when the diagnosis is made are the only ones that will show pagetic change over time. Although progression of disease within a given bone may occur, the sudden appearance of new sites of involvement years after the initial diagnosis is uncommon.

The most common sites of involvement include the pelvis, femur, spine, skull, and tibia. The humerus, clavicle, scapula, ribs, and facial bones are less commonly involved, and the hands and feet are only rarely affected. It is believed that most patients with Paget's disease are asymptomatic and that the disorder is most often diagnosed when an elevated SAP is noted on routine screening or when a radiograph taken for an unrelated problem reveals typical skeletal changes. The development of symptoms or complications of Paget's disease is influenced by the particular areas of involvement, the interrelationship between affected bone and adjacent structures, the extent of metabolic activity, and presence or absence of disease progression within an affected site.

SIGNS AND SYMPTOMS

Bone pain from a site of pagetic involvement, experienced either at rest or with motion, is probably the most common symptom. Pagetic bone associated with a high turnover state has an increased vascularity, leading to a sensation of warmth of the skin overlying bone (eg skull or tibia) that some patients perceive as an unpleasant sensation. Small transverse lucencies along the expanded cortices of involved weight-bearing bones or advancing, lytic, blade-of-grass lesions sometimes cause pain.

A bowing deformity of the femur or tibia can cause clinical problems. A bowed limb is typically shortened, resulting in specific gait abnormalities that can lead to abnormal mechanical stresses. Clinically severe secondary arthritis can occur at joints adjacent to pagetic bone (e.g., the hip, knee, or ankle).

Back pain may result from enlarged pagetic vertebrae. Vertebral compression fractures can occur since the bone is of suboptimal quality. Lumbar spinal stenosis with neural impingement may arise, producing radicular pain and possibly motor impairment. Degenerative changes in the spine that are unrelated to Paget's disease may also contribute to a patient's symptoms. Kyphosis may occur, or there may be a forward tilt of the upper back, particularly when a compression fracture or spinal stenosis is present. Paget's disease in the thoracic spine may rarely cause direct spinal cord compression with motor and sensory changes. Several cases of apparent direct cord compression have been documented to have resulted from a vascular steal syndrome, whereby hypervascular pagetic bone "steals" blood from the neural tissue (30).

Paget's disease of the skull may be asymptomatic, but common complaints in up to one third of patients with diffuse skull involvement may include an increase in head size with or without frontal bossing or deformity, or headache, sometimes described as a band-like tightening around the head. Hearing loss may occur as a result of isolated or combined conductive or neurosensory abnormalities; cochlear damage from pagetic involvement of the temporal bone with loss of bone density in the cochlear capsule may be an important component (31). Cranial nerve palsies (such as in nerves II, VI, and VII) occur rarely. With extensive skull involvement, a softening of the base of the skull may produce flattening and basilar invagination, so that the odontoid process begins to extend upward as the skull sinks downward upon it. Rarely basilar invagination can produce direct brainstem compression or an obstructive hydrocephalus and increased intra-cranial pressure caused by blockage of cerebrospinal fluid flow. Pagetic involvement of the facial bones may cause facial deformity, dental problems, and, rarely, narrowing of the airway.

Fracture through pagetic bone can occur, particularly in long bones with active areas of advancing lytic disease; the most common sites are the femoral shaft or subtrochanteric area (32). Increased vascularity of high trunover pagetic bone (i.e., with a moderately increased SAP) may lead to substantial blood loss in the presence of fractures due to trauma. Fractures also may occur in the presence of areas of malignant degeneration, a rare complication of Paget's disease. Far more common are the small fissure fractures along the convex surfaces of bowed lower extremities, which may be asymptomatic, stable, and persistent for years, but sometimes a more extensive transverse lucent area extends medially from the cortex, typically with symptoms of discomfort, and may lead to a clinical fracture with time. These painful lesions warrant treatment and careful radiographic follow-up over time. Fracture through pagetic bone usually heals normally, although some groups have reported as high as a 10% rate of nonunion.

Neoplastic degeneration is a relatively rare event, occurring with an incidence of less than 1%. This lesion typically presents as severe new pain at a pagetic site and has a grave prognosis. The majority of the tumors are classified as osteogenic sarcomas, although both fibrosarcomas and chondrosarcomas are also seen. The most common site of sarcomatous change appears to be the pelvis, with the femur and humerus next in frequency (33).Typically osteosarcomas are osteolytic, although these lesions involve cells of osteoblastic lineage (34).

Benign giant-cell tumors also may occur in bone affected by Paget's disease. These may present as localized masses at the affected site. Radiographic evaluation may disclose lytic changes. Biopsy reveals clusters of large osteoclast-like cells, which some authors believe represent reparative granulomas (35). These tumors usually show a remarkable sensitivity to high dose glucocorticoids, and the mass will shrink or even disappear after treatment with prednisone or dexamethasone (36), although some will grow back after treatment ends. Anecdotal evidence also suggests possible shrinkage of benign giant cell tumors in pagetic bone with thalidomide.

DIAGNOSIS

When Paget's disease is suspected, the diagnostic evaluation should include a careful medical history, including family history of the condition and symptom history, and a focused physical examination. The physical exam should note the presence or absence of warmth, tenderness, or bone deformity in the skull, spine, pelvis, and extremities, as well as evidence of loss of range of motion at major joints or leg length discrepancy.

Laboratory tests include measurement of SAP and in some cases a marker of bone resorption, as described earlier. It is also reasonable to assure normalcy of serum calcium and 25-hydroxy-vitamin D. Radiographic studies (bone scans and conventional radiographs) complete the initial evaluation. Bone biopsy is not usually indicated, as the characteristic radiographic and laboratory findings are diagnostic in most instances.

Bone scans are the most sensitive means of identifying possible pagetic sites but are nonspecific, and

also can be positive in nonpagetic areas that have degenerative changes or, more ominously, metastatic disease. Plain radiographs of bones noted to be positive on the bone scan provide the most specific information, because radiographic findings are usually characteristic to the point of being pathognomonic. Enlargement or expansion of bone, cortical thickening, coarsening of trabecular markings, and typical lytic and sclerotic changes may be found. Radiographs also show the condition of the joints adjacent to involved sites, identify fissure fractures, indicate the degree to which lytic or sclerotic lesions predominate, and demonstrate the presence or absence of deformity or fracture.

Repeated scans or radiographs are usually unnecessary in observing patients over time, unless new symptoms develop or current symptoms become significantly worse. The possibility of an impending fracture or, rarely, of sarcomatous change should be borne in mind in these situations. Although imaging studies such as CT or MRI scans are not usually required in routine cases, a CT scan may be helpful in the assessment of a fracture where radiographs are not sufficient, and MRI scans are useful in assessing the possibility of sarcoma, giant cell tumor or metastatic disease at a site of Paget's disease. Anecdotal data suggest that positron emission tomography (PET) scans of sclerotic lesions in patients with Paget's disease may help distinguish pagetic lesions from bone metastases, as the former are likely to be minimally-to non-metabolic as compared with marked hyper-metabolic changes seen with bone metastases (37).

The characteristic x-ray and clinical features of Paget's disease usually eliminate problems with differential diagnosis. However, an older patient may occasionally present with severe bone pain, elevations of the serum alkaline phosphatase and urinary N-telopeptide, a positive bone scan, and less-thancharacteristic radiographic areas of lytic or blastic change. Here the possibility of metastatic disease to bone or some other form of metabolic bone disease (e.g., osteomalacia with secondary hyperparathyroidism) must be considered. Old radiographs and laboratory tests are very helpful in this setting, as normal studies a year earlier would make a diagnosis of Paget's disease less likely. A similar dilemma occurs when someone with known and established Paget's disease develops multiple painful new sites; here, too, the likelihood of metastatic disease must be carefully considered, and bone biopsy for a tissue diagnosis may be indicated.

TREATMENT

Specific antipagetic therapy consists of those agents capable of suppressing the activity of pagetic osteoclasts. Currently approved agents available by prescription in the United States include six bisphosphonate compounds: orally administered etidronate, tiludronate, alendronate, and risedronate and intravenously administered pamidronate and zoledronic acid; and parenterally administered synthetic salmon calcitonin. Several of these are discussed briefly below. A more detailed review of these agents including more information on dosing regimens, clinical trial results and side effects has been published (38).

Other symptomatic treatments for Paget's disease, including analgesics, anti-inflammatory drugs, use of orthotics or canes and selected orthopedic and neurosurgical interventions, have important roles in management in many patients.

Two logical indications for medical treatment of Paget's disease are to relieve symptoms and to prevent future complications. It has been shown that suppression of the pagetic process by any of the available agents can effectively ameliorate certain symptoms in the majority of patients. Bone aches or pain, excessive warmth over bone, headache due to skull involvement, low-back pain secondary to pagetic vertebral changes, and some syndromes of neural compression (e.g., radiculopathy and some examples of slowly progressive brainstem or spinal cord compression) are the most likely to be relieved. Pain due to a secondary arthritis from pagetic bone involving the spine, hip, knee, ankle, or shoulder may or may not respond to antipagetic treatment. Filling in of osteolytic blade-of-grass lesions in weight-bearing bones has been reported in some treated cases with either calcitonin or bisphosphonates. On the other hand, a bowed extremity or other bone deformity will not change after treatment, and clinical experience indicates that deafness is unlikely to improve, although limited studies suggest that progression of hearing loss may be slowed (39) or even, in one case with pamidronate, reversed (40).

A second indication for treatment is to prevent the development of late complications in those patients deemed to be at risk, based on their sites of involvement and evidence of active disease, as shown by elevated levels of bone turnover markers. Admittedly, it has not been proven that suppression of pagetic bone turnover will prevent future complications. However, there is a restoration of normal patterns of new bone deposition in biopsy specimens after suppression of pagetic activity. It is also clear that active, untreated disease can continue to undergo a persistent degree of abnormal bone turnover for many years, with the possibility of severe bone deformity over time. Indeed, substantial (e.g., 50%) but incomplete suppression of elevated indices of bone turnover with older and less effective therapies has been associated with disease progression (41); with potent

bisphosphonates, however, indices become normal after treatment for extended periods in the majority of patients and approach normal in most of the rest.

Some treatment guides recommend, therefore, that the presence of asymptomatic but active disease (i.e., SAP above normal) at sites where the potential for later problems or complications exists (e.g., weight-bearing bones, areas near major joints, vertebral bodies, extensively involved skull) is an indication for treatment (38). The need for treatment in this setting may be particularly valid in patients who are younger, for whom many years of coexistence with the disorder is likely. However, even in the elderly, one can justify treatment if a degree of bone deformity is present that might create serious problems in the next few years. Others argue that the evidence does not support such use; in the PRISM clinical trial -with a median of only three years of observation -disease suppression with bisphosphonates failed to reduce short term complications or improve quality of life (42).

Although controlled studies are not available to prove efficaciousness in this situation, the use of a potent bisphosphonate before elective surgery on metabolically active pagetic bone also is recommended (43). The goal is to reduce the hypervascularity associated with moderately active disease (e.g., a threefold or more elevation in SAP) to minimize blood loss at operation.

Recommendations for the management of Paget's disease have been published as guideline or management documents by consensus panels in the US (38), UK (44) and Canada (45).

Bisphosphonates

Studies with etidronate (46), tiludronate (47), alendronate (48), risedronate (49), pamidronate (50) and zoledronic acid (also referred to as zoledronate) (51) have all demonstrated the efficacy of these agents in suppressing the localized bone turnover abnormality and in improving many symptoms in patients with Paget's disease, and all of them are available and approved for use in the United States.

In most instances the drug of choice, based on the efficaciousness of the agent and patient preferences regarding an intravenous or an oral regimen, is intravenous zoledronate or oral risedronate. Generic alendronate, 40 mg per day for 6 months (with the potential to repeat after a drug free interval) and generic pamidronate, with several possible dosing approaches based on the patient's status (38), are also available at lower cost but with less convenient dosing regimens. Etidronate (400 mg per day for 6 months with repeated 6 month on, 6 month off cycles as needed) and tiludronate (3 months of 400 mg per day) are much less frequently used as they are of lesser potency than the other four bisphosphonates and may typically reduce elevated bone turnover markers by about 50% rather than achieve biochemical remission in the majority of patients.

Risedronate is prescribed as a daily oral dose of 30 mg for 2 months note that this is a different dosing regimen than that for osteoporosis. The pill is taken after an overnight fast upon arising each morning with 8 oz. of plain water. The patient must remain upright and take nothing else by mouth for 30 minutes, after which he or she should eat. A follow-up measurement of SAP one to two months after completing the course is useful; if the value is not yet normal or near normal, a third or fourth month of risedronate could be offered with a good likelihood of normalcy or near normalcy of indices thereafter. In the pivotal clinical trial 80% of the patients had achieved a normal SAP six months after initiation of two months of treatment, with a period of subsequent disease suppression of up to 18 months (49). Periodic measurement of SAP (every 6-12 months) should be done and retreatment is suggested, if indicated, if and when SAP rises above normal or increases by >25% of the nadir value if full remission was not achieved.

Zoledronic acid at a dose of 5 mg is administered as a single 15 minute intravenous infusion. In the pivotal clinical trial comparing one 5 mg infusion of zoledronic acid with 2 months of 30 mg per day oral risedronate, a normal SAP was achieved by 89% of zoledronic acid subjects compared with 58% of risedronate subjects (51). A period of biochemical remission after the first zoledronic acid infusion of up to 18 months following the end of the initial observation period of 6 months was also noted in this study population. In practice, if a patient has a very high SAP pre-treatment that fails to come to normal or near normal by a few months after the infusion, a second infusion can be provided. For patients who enter a biochemical remission or near remission after one (or two) doses, 6-12 month follow up SAP measurements are suggested, and once the SAP begins to rise above normal or >25% above nadir levels if remission was not achieved, and if treatment is again indicated based on symptoms or concerns about complications, another dose can be provided. Again, note that treating at variable intervals based on biochemical remission and relapse differs from the regimen that is used when zoledronic acid is given for osteoporosis.

Secondary resistance to the effects of a given bisphosphonate (failure to achieve remission or a similar reduction in turnover markers with repeated courses of treatment) has been noted anecdotally with etidronate and has been reported with pamidronate (52) in some patients. Changing treatment at that point to a different

bisphosphonate appears to be effective (52).

It is important to emphasize the need for full repletion of both calcium and vitamin D prior to and during treatment with potent bisphosphonates to avoid hypocalcemia and secondary hyperparathyroidism. Calcium and vitamin D repletion should be maintained thereafter in these patients as a general principle.

Side effects with alendronate and risedronate include upper gastrointestinal symptoms consistent with esophageal irritation in a minority of individuals. Excessive doses of etidronate (e.g., more than 6 months without a drug free interval before re-treating) can infrequently induce a transient mineralization defect and osteomalacia. The first ever dose of either pamidronate or zoledronic acid in a patient who has not previously received a nitrogen containing bisphosphonate can be associated with a flu-like reaction for 1-2 days after treatment with fever, headache, myalgia and arthralgia, ameliorated by using acetaminophen or an NSAID; this reaction is unlikely to occur with subsequent doses. Finally, relatively rare cases of uveitis or iritis have been described with nitrogen containing bisphosphonates. In such patients, either etidronate or tiludronate can be given, as these compounds do not contain the nitrogen atom.

Osteonecrosis of the jaw has recently been described as a complication typically following dental extractions in patients receiving relatively high doses of potent bisphosphonates given primarily for management of bone metastases. At least seven patients with Paget's disease have also been reported to have had this complication, most of whom were given very high doses for prolonged periods of time outside the usual prescribing guidelines (53). This topic is discussed in detail elsewhere in the Primer.

Calcitonin

Synthetic salmon calcitonin is available as a subcutaneous injection. It is less effective than the nitrogen containing bisphosphonates and is most useful in the rare patient who is intolerant of all bisphosphonates or if bisphosphonate therapy is contra-indicated. The usual starting dose is 100 U (0.5 ml; the drug is available in a 2 ml vial)), generally self-injected subcutaneously, initially on a daily basis. Symptomatic benefit may be apparent in a few weeks, and the biochemical benefit (typically about a 50% reduction from baseline in SAP) is usually seen after 3 to 6 months of treatment. After this period, many clinicians reduce the dose to 50 to100 U every other day or 3 times weekly. Escape from the efficacy of salmon calcitonin may sometimes occur after a variable period of benefit. The main side effects of parenteral salmon calcitonin include, in a minority of patients, the development of nausea or queasiness, with or without flushing of the skin of the face and ears. Intranasal calcitonin is not indicated for use in Paget's disease, but anecdotal experience suggests it may relieve some symptoms and lower elevated bone turnover markers in patients with mild disease.

Other Therapies

Analgesics such as acetaminophen, aspirin and non-steroidal anti-inflammatory agents (NSAIDs) may be tried empirically with or without antipagetic therapy to relieve pain. In particular, pain from pagetic arthritis (i.e., osteoarthritis caused by deformed pagetic bone at a joint space) is often helped by some of these agents.

Surgery on pagetic bone (54) may be necessary in the setting of established or impending fracture. Elective joint replacement, more complex with Paget's disease than with typical osteoarthritis, is often very successful in relieving refractory pain. Rarely, osteotomy is performed to alter a bowing deformity in the tibia. Neurosurgical intervention is sometimes required in cases of spinal cord compression, spinal stenosis, or basilar invagination with neural compromise. Although medical management may be beneficial and adequate in some instances, all cases of serious neurological compromise require immediate neurological and neurosurgical consultation to allow the appropriate plan of management to be developed.

REFERENCES

1. Kanis JA 1998 Pathophysiology and Treatment of Paget's Disease of Bone. 2nd edition. Martin Dunitz Ltd., London, UK.
2. Siris ES, Canfield RE, Jacobs TP 1980 Paget's disease of bone. Bull NY Acad Med 56:285-304.
3. Morales-Piga AA, Rey-Rey JS, Corres-Gonzalez J, Garcia-Sagredo IM, Lopez-Abente G 1995 Frequency and characteristics of familial aggregation of Paget's disease of bone. J Bone Miner Res 10:663-670.
4. McKusick VA 1972 Heritable disorders of connective tissue (5th edition). CV Mosby, St. Louis, Missouri, USA, 718-723.
5. Siris ES, Ottman R, Flaster E, Kelsey JL 1991 Familial aggregation of Paget's disease of bone. J Bone Miner Res 6:495-500.
6. Albagha OM, Visconti MR, Alonso N, Langston AL, Cundy T, Dargie R, Dunlop MG, Fraser WD, Hooper MJ, Isaia G, Nicholson GC, del Pino Montes J, Gonzalez-Sarmiento R, di Stefano M, Tenesa A, Walsh JP, Ralston SH.

2010 Genome-wide association study identifies variants at CSF1, OPTN and TNFRSF11A as genetic risk factors for Paget's disease of bone.. Nat Genet. Jun; 42(6):520-4. Epub 2010 May. PMID: 20436471
7. The Genetic Determinants of Paget's Disease (GDPD) Consortium, Albagha OM, Wani SE, Visconti MR, Alonso N, Goodman K, Brandi ML, Cundy T, Chung PY, Dargie R, Devogelaer JP, Falchetti A, Fraser WD, Gennari L, Gianfrancesco F, Hooper MJ, Van Hul W, Isaia G, Nicholson GC, Nuti R, Papapoulos S, Montes JD, Ratajczak T, Rea SL, Rendina D, Gonzalez-Sarmiento R, Di Stefano M, Ward LC, Walsh JP, Ralston SH. 2011 Genome-wide association identifies three new susceptibility loci for Paget's disease of bone. Nat Genet. 2011 May 29 [Epub ahead of print] PMID: 21623375.
8. Sparks AB, Peterson SN, Bell C, Loftus BJ, Hocking L, Cahill DP, Frassica FJ, Streeten EA, Levine MA, Fraser CM, Adams MD, Broder S, Venter JC, Kinzler KW, Vogelstein B, Ralston SH 2001 Mutation screening of the TNFRSF11A gene encoding receptor activator of NF kappa B (RANK) in familial and sporadic Paget's disease of bone and osteosarcoma. Calcif Tissue Int 68:151-155.
9. Hocking L, Slee F, Haslam SI, Cundy T, Nicholson G, van Hul W, Ralston SH 2000 Familial Paget's disease of bone: Patterns of inheritance and frequency of linkage to chromosome 18q. Bone 26:577-580.
10. Laurin N, Brown JP, Morissette J, Raymond V 2002 Recurrent mutation of the gene encoding sequestosome 1 (SQSTM1/p62) in Paget disease of bone. Am J Hum Genet 2002 70:1582-1588.
11. Hocking LJ, Herbert CA, Nicholls RK, Williams F, Bennett ST, Cundy T, Nicholson GC, Wuyts W, Van Hul W, Ralston SH 2001 Genomewide search in familial Paget disease of bone shows evidence of genetic heterogeneity with candidate loci on chromosomes 2q36, 10p13, and 5q35. Am J Hum Genet. 69:1055-1061.
12. Visconti MR, Langston AL, Alonso N, Goodman K, Selby PL, Fraser WD, Ralston SH. 2010 Mutations of SQSTM1 are associated with severity and clinical outcome in paget disease of bone. J Bone Miner Res. Nov; 25(11):2368-73. PMID: 20499339.
13. Kurihara N, Hiruma Y, Zhou H, Subler MA, Dempster DW, Singer FR, Reddy SV, Gruber HE, Windle JJ, Roodman GD 2007 Mutation of the Sequestosome 1 (p62) gene increases osteoclastogenesis but does not induce Paget's disease. J Clin Invest 117:133-142
14. Daroszewska A, van 't Hof RJ, Rojas JA, Layfield R, Landao-Basonga E, Rose L, Rose K, Ralston SH. 2011 A point mutation in the ubiquitin-associated domain of SQSMT1 is sufficient to cause a Paget's disease-like disorder in mice. Hum Mol Genet. May 16 [Epub ahead of print]. PMID: 21515589
15. Cooper C, Schafheutle K, Dennison E, Kellingray S, Guyer P, Barker D. 1999 The epidemiology of Paget's disease in Britain: Is the prevalence decreasing? J Bone Miner Res 14:192-197.
16. Cundy T,McAnulty K, Wattie D, Gamble G, Rutland M, Ibbertson HK 1997 Evidence for secular changes in Paget's disease. 20:69-71
17. Rebel A, Malkani K, Basle M, Bregeon C 1997 Is Paget's disease of bone a viral infection? Calcif Tissue Res. 22 Suppl: 283-286.
18. Mills BG, Singer FR, Weiner LP, Suffin SC, Stabile E, Holst P 1984 Evidence for both respiratory syncytial virus and measles virus antigens in the osteoclasts of patients with Paget's disease of bone. Clin Orthop 183:303-311.
19. Gordon MT, Mee AP, Sharpe PT 1994 Paramyxoviruses in Paget's disease. Semin Arthritis Rheum. 23:232-234.
20. Mee AP, Dixon JA, Hoyland JA, Davies M, Selby PL, Mawer EB 1998 Detection of canine distemper virus in 100% of Paget's disease samples by in situ-reverse transcriptase-polymerase chain reaction. Bone. Aug; 23:171-175.
21. Kurihara N, Reddy SV, Menaa C, Anderson D, Roodman GD 2000 Osteoclasts expressing the measles virus nucleocapsid gene display a pagetic phenotype. J Clin Invest 2000 105:607-614.
22. Ooi CG, Walsh CA, Gallagher JA, Fraser WD 2000 Absence of measles virus and canine distemper virus transcripts in long-term bone marrow cultures from patients with Paget's disease of bone. Bone 27:417-421.
23. Kurihara N, Zhou H, Reddy SV, Garcia-Palacios V, Subler MA, Dempster DW, Windle JJ, Roodman GD 2006 Expression of measles virus nucleocapsid protein in osteoclasts indures Paget's disease–like bone lesions in mice. J Bone Miner Res 21**:**446–455.
24. Kurihara N, Hiruma Y, Yamana K, Michou L, Rousseau C, Morissette J, Galson DL, Teramachi J, Zhou H, Dempster DW, Windle JJ, Brown JP, Roodman GD. 2011 Contributions of the measles virus nucleocapsid gene and the SQSTM1/p62(P392L) mutation to Paget's disease. Cell Metab. Jan 5; 13(1):23-34. PMID: 21195346.
25. Rebel A, Basle M, Pouplard A, Malkani K. Filmon R, Lepatezour A 1980 Bone tissue in Paget's disease of bone: ultrastructure and immunocytology. Arthritis Rheum 23:11041114.
26. Singer FR, Mills BG, Gruber HE, Windle JJ, Roodman GD 2006 Ultrastructure of bone cells in Paget's disease of bone. J Bone Miner Res 21, Suppl 2:P51-P54.
27. Shankar S, Hosking DJ 2006 Biochemical assessment of Paget's disease of bone. J Bone Miner Res 21, Suppl 2:P22-P27
28. Siris ES, Canfield RE 1994 The parathyroids and Paget's disease of bone. In: Bilezikian J, Levine M, Marcus R, eds. The Parathyroids. New York, NY, USA: Raven Press, 823-828.
29. Siris ES 1991 Indications for medical treatment of Paget's disease of bone. In: Singer FR, Wallach S, eds. Paget's disease of bone: clinical assessment. present and future therapy. Elsevier, New York, USA 44-56.

30. Herzberg L, Bayliss E 1980 Spinal cord syndrome due to non-compressive Paget's disease of bone: a spinal artery steal phenomenon reversible with calcitonin. Lancet 2:13-15.
31. Monsell EM 2004 The mechanism of hearing loss in Paget's disease of bone. Laryngoscope 114:598-606.
32. Barry HC 1980 Orthopedic aspects of Paget's disease of bone. Arthritis Rheum 23:1128-1130.
33. Wick MR, Siegal GP, Unni KK, McLeod RA, Greditzer HB 1981 Sarcomas of bone complicating osteitis deformans (Paget's disease). Am J Surg Pathol 5:47-59.
34. Hansen MF, Seton M, Merchant A 2006 Osteosarcoma in Paget's disease of bone. J Bone Miner Res 21, Suppl 2:P58-P63.
35. Upchurch KS, Simon LS, Schiller AL, Rosenthal DI, Campion EW, Krane SM 1983 Giant cell reparative granulomas of Paget's disease of bone: a unique clinical entity. Ann Intern Med 98:35-40.
36. Jacobs TP, Michelsen J, Polay J, D' Adamo AC, Canfield RE 1979 Giant cell tumor in Paget's disease of bone: familial and geographic clustering. Cancer 44:742-747.
37. Sundaram M 2006 Imaging of Paget's disease and fibrous dysplasia of bone. J Bone Miner Res 21, Suppl 2:P28-P30.
38. Siris ES, Lyles KW, Singer FR, Meunier PJ: 2006 Medical management of Paget's disease of bone: Indications for treatment and review of current therapies. J Bone Miner Res 21: Suppl 2, P94-98.
39. EI-Sammaa M, Linthicum FH, House HP, House JW 1986 Calcitonin as treatment for hearing loss in Paget's disease. Am J Otol 7:241-243.
40. Murdin L, Yeoh LH 2005 Hearing loss treated with pamidronate. J R Soc Med 98:272-274.
41. Meunier PI, Vignot E 1995 Therapeutic strategy in Paget's disease of bone. Bone 17:489S-49IS.
42. Langston AL, Campbell MK, Fraser WD, MacLennan GS, Selby PL, Ralston SH 2010 Randomized trial of intensive bisphosphonate treatment versus symptomatic management in Paget's disease of bone. J Bone Miner Res 25:20-31.
43. Kaplan FS 1999 Surgical management of Paget's disease. J Bone Miner Res 14S2:34-38.44. Selby PL, Davie MWJ, Ralston SH, Stone MD 2002 Guidelines on the management of Paget's disease of bone. Bone 31:366-373.
44. Selby PL, Davie MWJ, Ralston SH, Stone MD 2002 Guidelines on the management of Paget's disease of bone. Bone 31:366-373.
45. Drake WM, Kendler DL, Brown JP 2001 Consensus statement on the modern therapy of Paget's disease of bone from a Western Osteoporosis Alliance Symposium. Clin Ther 2001; 23:620-626.
46. Canfield R, Rosner W, Skinner J, McWhorter J, Resnick L, Feldman F, Kammerman S, Ryan K, Kunigonis M, Bohne W 1977 Diphosphonate therapy of Paget's disease of bone. J Clin Endocrinol Metab 44:96-106.
47. McClung MR, Tou CPK, Goldstein NH, Picot C 1995 Tiludronate therapy for Paget's disease of bone. Bone 17:493S-496S.
48. Siris E, Weinstein RS, Altman R, Conte JM, Favus M, Lombardi A, Lyles K, McIlwain H, Murphy WA Jr, Reda C, Rude R, Seton M, Tiegs R, Thompson D, Tucci JR, Yates AJ, Zimering M 1996 Comparative study of alendronate vs. etidronate for the treatment of Paget's disease of bone. J Clin Endocrinol Metab 81:961-967.
49. Miller PD, Adachi JD, Brown JP, Khairi RA, Lang R, Licata AA, McClung MR, Ryan WG, Singer FR, Siris ES, Tenenhouse A, Wallach S, Bekker PJ, Axelrod DW 1997 Risedronate vs. etidronate: durable remission with only two months of 30 mg risedronate. J Bone Miner Res 12:S269
50. Harinck HI, Papapoulos SE, Blanksrna HJ, Moolenaar AJ, Vermeij P, Bijvoet OL. 1987 Paget's disease of bone: Early and late responses to three different modes of treatment with aminohydroxypropylidene bisphosphonate (APD). Br Med J 295:13011305.
51. Reid IR, Miller P, Lyles K, Fraser W, Brown J, Saidi Y, Mesenbrink P, Su G, Pak J, Zelenakas K, Luchi M, Richardson P, Hosking D 2005 A single infusion of zoledronic acid improves remission rates in Paget's disease: a randomized controlled comparison with risedronate. N Engl J Med 353:898-908.
52. Gutteridge DH, Ward LC, Stewart GO, Retallack RW, Will RK, Prince RL, Criddle A, Bhagat CI, Stuckey BG, Price RI, Kent GN, Faulkner DL, Geelhoed E, Gan SK, Vasikaran S 1999 Paget's disease: acquired resistance to one aminobisphosphonate with retained response to another. J Bone Miner Res 14S2:79-84.
53. Khosla S, Burr D, Cauley J et al. 2007 Bisphosphonate-associated osteonecrosis of the jaw: report of a task force of the American Society for Bone and Mineral Research. J Bone Miner Res 22:1479-1491.
54. Parvizi J, Klein GR, Sim FH 2006 Surgical management of Paget's disease of bone. J Bone Miner Res 21, Suppl 2:P75-P82.