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Meet-the-Professor

Handout Booklet

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Bone Biomechanics and Age-dependent Changes

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Bone Biomechanics and Age-dependent Changes
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Significance of the Topic

Risk of bone fracture increases with age (Figure 1). This increase in fracture rate is caused primarily by a decrease in the mechanical integrity of bone with age, resulting from both a **decrease in bone quantity and quality**. Osteoporosis is typically assessed by measuring bone mineral density with dual x-ray absorptiometry, a measure of bone quantity. Treatments for osteoporosis also use bone mineral density as a marker of efficacy. Bone quality is more challenging to measure and is typically characterized by toughness, or the ability of the material to prevent fracture.

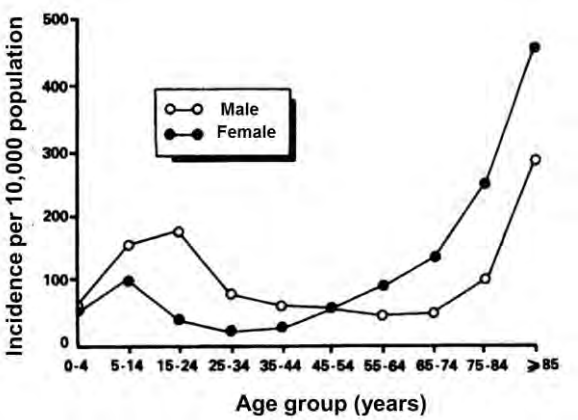

Figure 1: Average annual fracture incidence rate per 10,000 people. From [1].

Learning Objectives

This talk will first outline the different measures used to characterize bone mechanical integrity, the changes in these parameters with age, the structural and compositional alterations underlying the change in mechanics and finally mechanobiological changes with age. The objectives are to:

1. Compare and contrast different mechanical testing methods and their limitations for characterizing mechanical integrity;
2. Identify changes in mechanical properties with age including modulus, strength, and toughness measures (work to fracture, stress intensity)
3. Identify changes in composition and structure that underpin mechanical changes.
4. Identify changes in bone mechanobiology, which play a role in decreasing the mechanical integrity of bone.

Outline

This talk will highlight how mechanical properties change with age and the salient compositional and structural characteristics that underpin the mechanical changes.

Mechanical Properties: The most common way to test whole bone mechanical properties is using three point bending, tension, or torsion tests. All tests require the sample axis to be relatively straight, which is possible in many long bones. We will primarily talk about bending tests. From a three point bending test, the force-displacement curve is obtained (Figure 2) and the stiffness (slope of the linear portion), yield force (force at boundary between elastic and plastic) and ultimate force (maximum force) can be determined. With Bernoulli beam assumptions[1], the following mechanical parameters can be calculated (see [2] for a clear derivation of equations for whole bones in bending and important assumptions):

[1] Using Euler-Bernoulli beam theory assumes: constant cross section for the length of the beam, pure bending (no shear), and homogeneous and isotropic material properties. None of these are accurate for bones, but nonetheless used generalize whole bone

1. **ultimate stress, σ_u** (MPa) – maximum stress the bone can withstand before it breaks. Ultimate strength decreases with age [3–6].
2. **yield stress, σ_y** (MPa) – the stress at the onset of plastic deformation (non-linear part of the curve). Yield strength decreases with age [5, 6]
3. **elastic (Young's) modulus, E** (MPa)– resistance to being deformed elastically (spring constant of the material). Age effects are not clear. Elastic modulus may decrease with age [3, 4, 7], increase with age [8], or have no change with age [5, 9, 10], depending on how and where it is assessed.

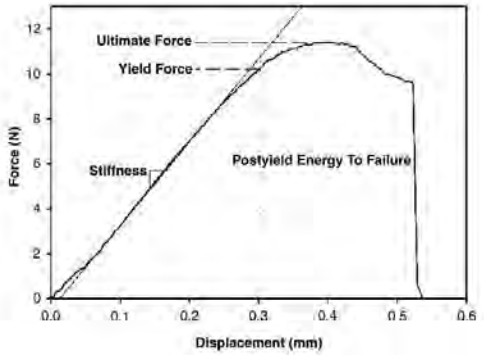

Figure 2: Typical load displacement curve for mechanical test. Adapted from [2].

4. **work to fracture, W_f** (J/m^2)– work per unit area to break the bone. This is calculated by the area under the force/displacement curve divided by 2x the area of the fractured surface [11]. In many publications, this is called 'toughness'. However, in brittle materials, the work to fracture is highly dependent on pre-existing defects in the bone, specimen size and geometry, and is not a material property. This means that extreme care should be taken in comparing work to fracture between studies, bones, and investigators. Work to fracture decreases with age [3–5].

In order to avoid the effects of pre-existing defects in the bone, the sample can be notched to create a dominate flaw. This controls where the fracture occurs and is a better measure of resistance to fracture (toughness). Using this fracture mechanics approach, the following measures of toughness can be found (see [12–14] for equations, Figure 3):

5. **critical stress intensity factor, K_{1c}** (Pa m$^{1/2}$) – stress needed for unstable crack growth, which results in ultimate fracture. K_{1c} is a function of load at fracture, the span width, the crack dimension, and the specimen dimensions. Stress intensity factor assumes only linear elastic deformation and does not take into account any plastic deformation that occurs during fracture. This means the solutions are valid only when the plastic zone is much smaller than the dimensions of the material. Critical stress intensity factor decreases with age [4, 13]
6. **crack initiation toughness, K_i** (Pa m$^{1/2}$) – stress needed to start crack propagation. Crack initiation toughness decreases with age [15]

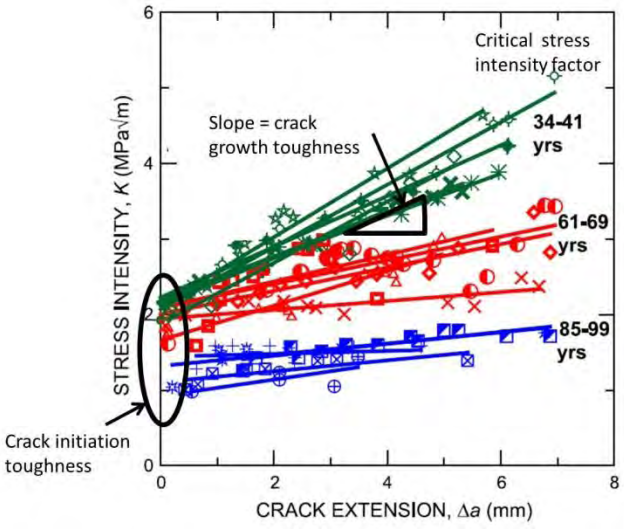

Figure 3: Aging reduces crack initiation toughness, crack growth toughness, and critical stress intensity factor. Adapted from [13]

7. **resistance curve** - Before unstable growth ($K < K_c$), the crack may progress but not cause the bone to fracture. Measuring the stress intensity factor as a function of crack length results in a resistance curve that is an indication of how much energy you need to put into the material to make the crack propagate. The slope of the resistance curve is sometimes called **crack growth toughness**. Crack growth toughness decreases with age [13, 15]

Structural and Compositional Changes with Age

Cortical bone
Cross sectional area – decrease with age [16]
Cortical thickness - thinner with age [17]
Moment of area - increases with age [18]
- decreases with age [16]

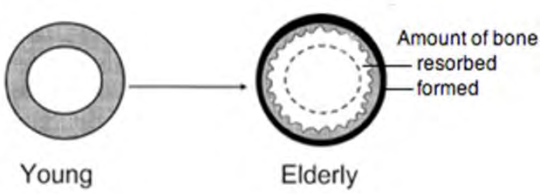

Figure 4: Cortical thickness decreases with age. Adapted from [21].

Trabecular bone
BV/TV – decrease with age [19, 20]
Tb.Th- no change [19], decrease [20]
Tb.N – decrease [19, 20]
SMI – increases (becomes more rod like) [19]
BEWARE: SMI is strongly (inversely) correlated with BV/TV

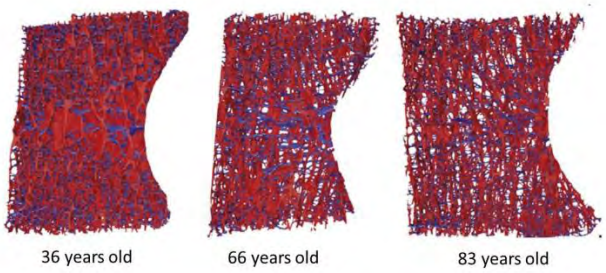

Figure 5 – Changes in trabecular structure with age. Adapted from [19].

Note – There are innumerable studies that show changes in trabecular and cortical bone properties with age. The ones listed here are representative.

Porosity
osteon density – increase with age [6, 15]
vascular porosity – increase with age [5, 22, 23]
lacunar porosity – no change in density, but flatter and larger lacunae with age [24]

Composition
Collagen –
collagen content (total amount of collagen/dry weight of bone) – decrease with age [3, 10]
number of reducible enzymatic crosslinks – decrease with age [25, 26]
non-enzymatic glycation crosslinks (also called advanced glycation endproducts AGEs) – increase with age [5, 6, 10]

Mineral –
Bone density measures:
bone apparent density (pqCT, DXA) - decrease with age [3]
ash content (g mineral/ dry weight) – no change with age [27, 28]
bone mineral density (quantitative backscatter SEM) – no change with age [29]
mineral content (density fractionation g/cc) – more highly mineralized with age [30]
mineral/matrix ratio (Raman) – decrease with age [31]
Ca/P ratio – no change [30]
crystal size – no change [30, 32]
carbonate content – increase with age [31]

Water -
bound water – decrease with age, related to post-yield properties [33, 34]
pore water – increase with age, related to elastic properties [33]

Mechanobiology

Relatively little is known about the mechano-responsiveness of aged bone in humans. Exercise studies indicate moderate benefit of mechanical loading on decreasing bone loss [35–37] However, there are no studies in humans comparing the responsiveness of young and old bone to mechanical load. In mice using the in vivo tibial loading model, old bone responds less to mechanical loading than young bone [38–40]. More research is required to explore effective ways to kick-start the mechano-sensitivity of aged bone.

Remodeling

Reduced estrogen levels increase the rate of remodeling and therefore bone turnover [41]. Osteoclastic activity increases with osteocyte apoptosis [42], which increases with age. In addition, there is increasing evidence that non-enzymatic crosslinks (AGEs) directly affect cellular function through the receptor for AGE (RAGE), a surface receptor on many cell types [43]. In bone, activation of the RAGE receptor inhibits osteoblast proliferation and differentiation [44], reduces matrix production [45], reduces bone formation [46], and increases osteoblast apoptosis [47]. This indicates that crosslinking properties of the matrix not only alter the tissue properties, but directly control cellular function and may play an important role in the decreased bone formation found in osteoporosis [48].

Summary

Compositional changes and structural alterations throughout the hierarchy of bone result in a **decrease in strength and toughness of bone with age**. The decrease in strength is correlated with the reduced total amount of bone. The decrease in toughness seems to be related primarily to collagen changes. Future therapies should address collagen deficits in order to maintain mechanical integrity (primarily toughness) of the bone.

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Bone Quality-Raman, FTIR, SAXS, BSEM: What Do These Acronyms Mean?

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Bone Quality-Raman, FTIR, SAXS, BSEM: What Do These Acronyms Mean?

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Loss of bone mass (BMD) is an important feature related to fractures, accounting for a significant portion of osteoporotic fracture risk [(1)]. Furthermore, BMD has been shown to correlate significantly with bone strength [(2-5)]. On the other hand, BMD alone does not determine whether an individual will sustain a fracture [(6-12)]. There is a considerable overlap between normal and osteoporotic populations [(13)]. As reviewed by Martin [(14)], the strength of bone depends on both its size and shape, and on the strength of the material inside. Both the organic matrix and the mineral contribute to the strength of bone [(15-18)]. Consequently, bone quality (an umbrella term encompassing the structural and material/compositional bone properties) has received considerable attention as a bone strength contributing determinant [(18)]. As a result, bone material properties such as mineral / matrix, the orientation of the mineral particles and the collagen fibers, mineral maturity & crystallinity, and collagen cross-links have come to the forefront as important determinants of bone strength. Moreover, since these properties are dependent on bone turnover, their spatial distribution is of importance.

Techniques such as Backscatter Electron microscopy (BSEM; also referred to in the literature as quantitative backscattered electron imaging (qBEI)), Small Angle X-ray Scattering (SAXS), and Raman (RS) and Fourier transform infrared spectroscopy (FTIR), are well-suited to provide information on the previously mentioned bone quality indices. Specifically, BSEM provides information on the spatial distribution of mineral content, SAXS on the spatial distribution of mineral particle shape and size as well as crystallite and collagen fiber orientation, and RS and FTIR on the spatially resolved mineral and organic matrix content, mineral crystallite maturity and crystallinity, nanoporosity, glycosaminoglycan and lipid content, as well as collagen cross-links.

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

1. Understand the general physical principle of the techniques in discussion, what is actually measured, and what is inferred.
2. Appreciate why the outcomes reported by these techniques are important in the considerations of bone strength
3. Through discussion of clinical examples, understand how the outcomes of these techniques complement the ones from routinely used clinical diagnostic techniques, and provide new information about the pathophysiology of fragile bone.

BSEM/qBEI [(19-21)]: Backscattered electron imaging in scanning electron microscopy (SEM) is a suitable technique to visualize the relative changes of local mineral concentration within cortical and cancellous bone structures. It is based on the detection of electrons backscattered from the surface of bone biopsy sample, struck by the primary electron beam of the SEM. The fraction of the electrons backscattered is a monotonically increasing function of the atomic number Z (Z-contrast) of the respective elements in the sample. Therefore, the BSEM generated grey levels of the image of certain bone area reflects either the presence of a pure element with a certain Z or the presence of a composite of more elements having a certain average atomic number (Zmean)[(22)]. For further quantitative considerations of the mineral content distribution in bone tissue, the BSEM grey levels have to be used as a surrogate for calcium content, something that can be accomplished through calibration against analysis of Ca content as determined by x-ray microprobe analysis (EDX). This inferred Ca content (% wt) can then be converted to amount of hydroxyapatite [(19)]. This greyscale image representing spatial variability in mineral content is then converted into a histogram

providing information on the Bone Mineral Density Distribution (BMDD), which may be considered as a special attribute of bone reflecting bone turnover, mineralization kinetics and average tissue age. For statistical analysis, the following parameters (characteristics of the generated BMDD histogram) have been defined:

CaMEAN, the weighted mean Ca concentration of the bone area obtained from the integrated area under the BMDD curve in units of [wt.% Ca];

CaPEAK, the peak position of the histogram, which indicates the most frequently measured calcium concentration (Ca value with the highest number of pixels) in the bone area in units of [wt.% Ca];

CaWIDTH, the full width at half maximum of the distribution, describing the variation in mineralization density in units of [Δwt.% Ca] (Δ = differences of Ca concentrations);

CaLOW, the percentage of bone area that is mineralized below the 5th percentile of the reference range [23] in units [% bone area], that is below 17.68 wt.% Ca, the parameter corresponds therefore also to the amount of bone area passing primary mineralization;

CaHIGH, the percentage of bone area that is mineralized above the 95th percentile of the reference range [23] in units [% bone area], that is above 25.30 wt.% Ca; the parameter corresponds to the amount of bone area having achieved plateau level of mineralization (amount of fully mineralized bone matrix) and includes also the contribution of highly mineralized cement lines.

SAXS [24]: Small-angle X-ray scattering (SAXS) is based on the elastic scattering of X-rays (wavelength 0.1 ... 0.2 nm) by an inhomogeneous sample, and is recorded at very low angles (typically 0.1 - 10°). It provides information on the microscale or nanoscale structure of particle systems in terms of such parameters as averaged particle sizes, shapes, distribution, and surface-to-volume ratio, as well as mineral particles and collagen fiber alignment. A monochromatic beam of X-rays is shone onto a sample from which some of the X-rays scatter, while most go through without interacting with it. The scattered X-rays form a scattering pattern containing the information on the structure of the sample. Two types of instruments have been developed: i) Point-collimation instruments; these employ pinholes shaping the X-ray beam into a small circular or elliptical spot that illuminates the sample, resulting in scattering that is centro-symmetrically distributed around the primary X-ray beam, thus the scattering pattern in the detection plane consists of circles around the primary beam. Such instruments allow the orientation of non-isotropic systems (such as collagen fibers) to be determined. ii) Line-collimation instruments; these confine the beam in one dimension so that the beam profile is a long but narrow line. This results in a larger sample volume analyzed.

RS & FTIR [25]: They are vibrational spectroscopic techniques that provide chemical information on all tissue components. The common principle underlying both techniques is the transition between vibrational energy states of molecules; infrared transitions arise directly from absorption of energy in the infrared range, while Raman spectra arise from the scattering of visible or ultraviolet photons that have either gained or lost part of their energy upon interaction with the vibrating bonds. Each molecule has its own unique vibrational characteristics and therefore will result in signature FTIR or Raman spectral signatures. Additionally, the neighboring molecular environment influences the vibrational characteristics. The position, intensity, and width of a vibrational band can be used for monitoring a particular functional group or regions of a particular chemical species. The IR and Raman spectra of a given sample differ considerably, as some vibrations are only either IR or Raman active and hence each technique can provide complimentary information regarding the analyzed sample [25]. Since both may be applied coupled to a microscope, information may be obtained with spatial resolution of ~0.6-1 µm for RS and ~7 µm for FTIR. Routinely available information includes:

i) the mineral / matrix ratio, a form of bone density that, unlike other measures of BMD, directly measures and accounts for the amount of the organic matrix as well in the volume analyzed. This parameter has been validated against the golden standard technique of ash-weight measurements (incidentally, the only technique that actually measures directly the amount of mineral and organic

matrix present in bone tissue) in healthy bone [26], is directly proportional to bending stiffness and failure moment, and is a superior predictor of bone-bending stiffness compared to BMD alone [27].

ii) Glycosaminoglycans, part of proteoglycan molecules which play multiple roles involving the modulation of both organic matrix mineralization and remodeling rates, and responsible for tissue water binding [25].

iii) Lipids, which have been reported in the literature as nucleators of collagen fiber mineralization, with a layer of lipids present just behind the first mineral deposited [28,29]. Moreover, oxidized lipids are a substratum involved in AGEs (advanced glycation endproducts) accumulation [30].

iv) Mineral maturity/crystallinity. Both RS and FTIR provide information on the chemical makeup of the poorly crystalline apatitic crystals in bone i.e. the presence of impurities, and, based on comparison to x-ray line broadening analysis, on their shape and size [25].

v) Collagen cross-links (providing the fibrillar matrices with mechanical properties such as tensile strength and viscoelasticity [25]) in a spatially resolved manner. To date, the detection of pyridinoline, deoxypyridinoline, and divalent cross-links is feasible as validated against biochemically characterized standards [31-35].

In case of FTIR determination of mineral maturity/crystallinity and collagen cross-links where deconvolution of composite bands into their constituent peaks is involved, caution should be exercised so that this is achieved through the utilization of spectroscopically acceptable methods (deconvolution or second derivative spectroscopy) [35,36] rather than arbitrary, empirical methods that result in biologically irrelevant mathematical solutions.

When combined with fluorescence microscopy or histology, the outcomes of all 4 analytical techniques may be reported either dependent on bone turnover rate, or independent of it, while when SAXS, RS and FTIR are combined with BSEM (qBEI) images, their results may be reported as a function of mineralization extent.

Clinical cases

All 4 techniques are at their most informative when employed in combination with routinely used clinical outcomes such as BMD, biochemical markers, histology, and histomorphometry. Their utility and how the information gained complements and advances information from routinely used techniques in the clinic such as DXA and biochemical markers will be discussed in the context of the following clinically relevant cases/questions:

i. Strontiun ranelate (SrR) therapy: Upon the introduction of SrR as a potential therapy for osteoporosis, the question arose as to where exactly in bone does strontium incorporates into. Combinatorial application of BSEM and SAXS was able to reveal that it is present in bone packets that were formed during therapy, and that it was incorporated into the apatite crystals in these packets.

ii. Idiopathic osteoporosis (IOP) is a disorder in premenopausal women, in which fragility fractures and/or low bone mineral density (BMD) occur in otherwise healthy women with normal gonadal function [8]. Yet, not all sustain fragility fracture, and the ones that do (IOP-Fx) are not necessarily accounted for by DXA measures and biochemical indices. The question then arises as to whether there is an outcome that distinguishes between the fracturing and non-fracturing IOP patients.

iii. A case study from a patient that sustained an atypical femoral fracture after long-term bone-turnover suppressing therapies will be presented and discussed.

iv. Data will be presented and discussed as to whether outcomes from these techniques can help us distinguish between changes due to healthy aging as opposed to disease, and how they can help us better understand the changes responsible for bone fragility in postmenopausal osteoporosis.

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Communicating Benefits and Risks of Osteoporosis Treatment

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Communicating Benefits and Risks of Osteoporosis Treatment

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Significance of the Topic: Unmet needs in the care of osteoporosis include underdiagnosis, undertreatment, and poor adherence to treatment when it is prescribed. Strategies to improve clinical outcomes include better understanding and more effective communication of the balance of benefits and risks with and without treatment.

Learning Objectives

- Define risk and risk communication
- Identify methods for communicating the balance of benefits and risks with osteoporosis patients
- Include risk communication with shared decision making

Outline

- Patients are sometimes reluctant to take medications to reduce fracture risk due to fear of side effects.
- Patients often have a poor understanding of the potentially serious consequences of fractures and balance of benefits and risks with treatment.
- Skills to effectively communicate the balance of benefits and risks of treatment can be learned.
- Risk may be defined as "probability of harm." However, a definition that is more useful with patient care is "probability of loss of that which we value." (As the ancient Greek philosopher Epictetus once said, "People are disturbed, not by things, but by the view they take of them.")
- Perception of risk varies according to perspective
    - Patients tend to be influenced by anecdotes, advice of friends and relatives, and media reports
    - Physicians are more influenced by data, opinions of thought-leaders, and medical journal reports.
- Risk communication is the study and practice of collectively and effectively understanding risks.
- Potential benefits of effective risk communication
    - Patients have better understanding of
        - Disease consequences (fractures)
        - Benefits (reduced fracture risk) and potential harms of therapy (side effects)
    - Reduced mistrust and fear
    - Better collaboration between physician and patient

    - Possible improved persistence and compliance with therapy
    - Greater reduction of fracture risk?
- Obstacles to effective risk communication include
    - Uncertainty, complexity, and incompleteness of data
    - Distrust of experts-government-industry
    - Selective reporting by news media
    - Anecdotes that generate outrage
    - Psychological and social factors that influence how we process information about risk
    - Statistical illiteracy
- Decision aids are clinical tools (e.g., pamphlets, models, videos) to assist patients in making "close call" decisions that involve consideration of benefits, harms, and scientific uncertainty.
- Models for making clinical decisions include
    - Paternalism, where the physician has all relevant information and is the sole decision maker
    - Independent choice, where the physician presents "the facts" and the patient makes all decisions, and
    - Shared (participatory), where physicians and patient share information, discuss options, and reach collaborative decision
- Components of shared decision making
    - Understanding the risks associated with the condition
    - Understanding the options, including risks, benefits, alternatives, and uncertainties
    - Weighting personal values regarding potential benefits and harms
    - Participating in decision making at the desired level
- "Attentive listening" is a learned skill that is critical for effective risk communication and shared decision making, with core principles that include
    - Perception and cognition (hearing and understanding)
    - Active participation (responding, not just talking)
    - Verbal and non-verbal cues (listening and looking)

Internet Resources

- Therapeutics and Clinical Risk Management
    - http://www.dovepress.com/therapeutics-and-clinical-risk-management-journal
- Center for Risk Communication
    - http://www.centerforriskcommunication.com/home.htm
- Center for Risk Communication Research
    - http://www.comm.riskcenter.umd.edu/
- Harvard Center for Risk Analysis
    - http://www.hcra.harvard.edu/
- Society for Risk Analysis
    - http://www.sra.org/

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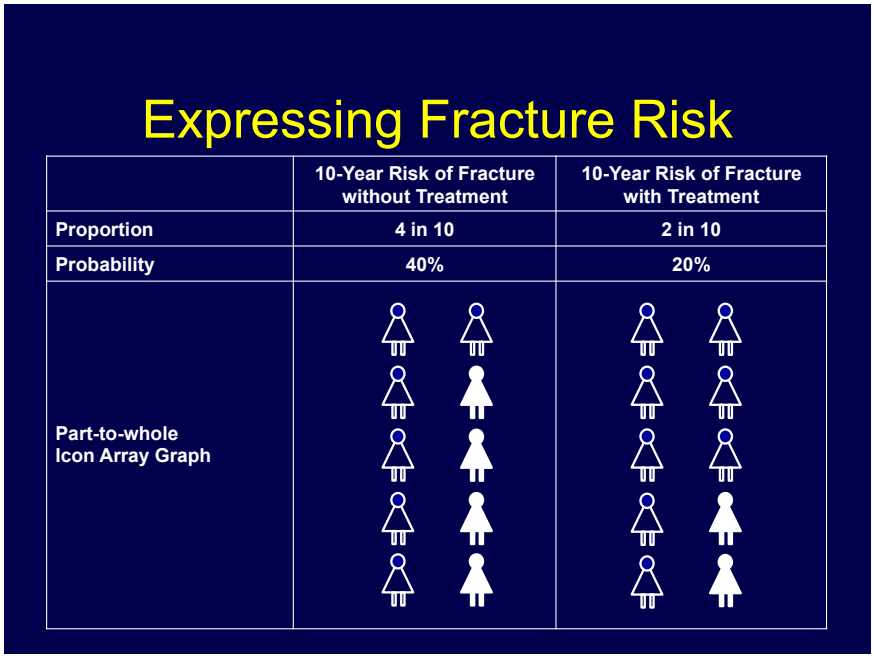
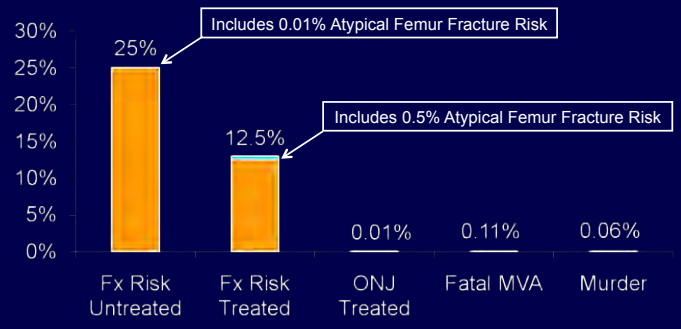
Examples of Decision Aids



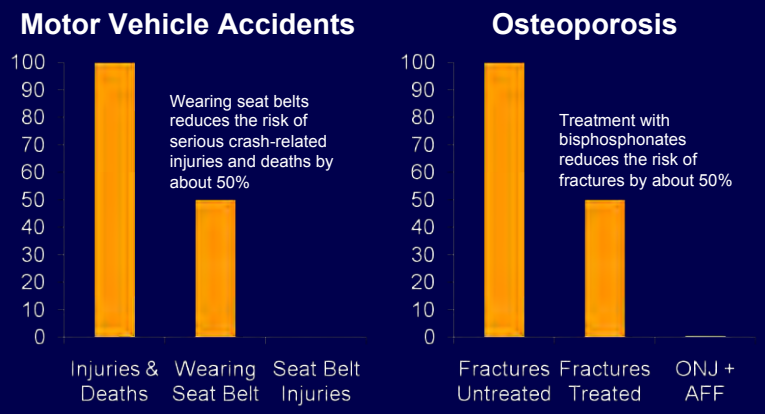
Vertical Bar Graph of 10-Year Probabilities

80 year-old woman with FN T-score = -3.3

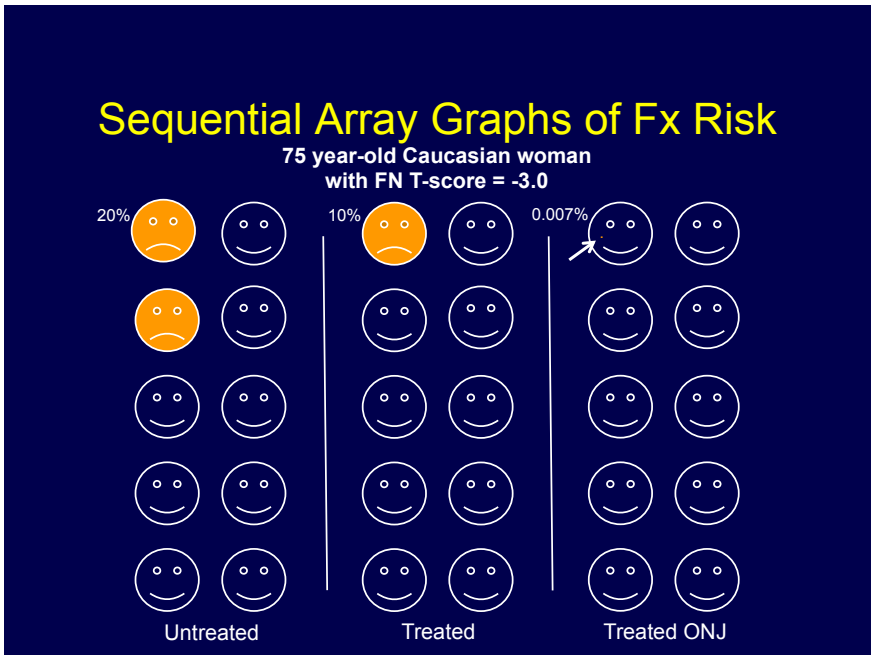


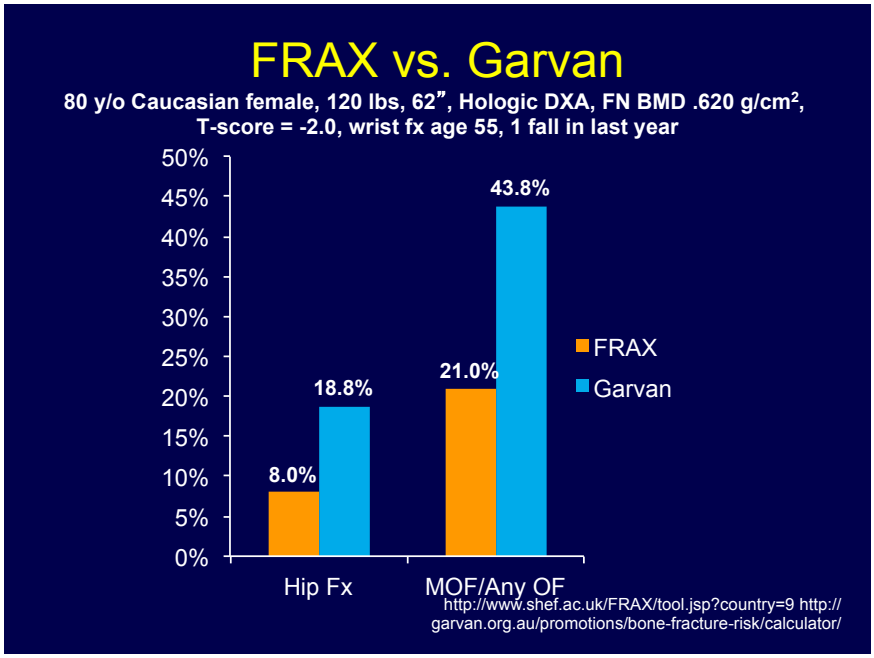
Untreated probability of major osteoporotic fracture calculated by FRAX. ONJ estimate is ~1/100,000 patient-treatment-years from ASBMR Task Force by Khosla S et al. J Bone Miner Res 2007;22:1479–149. AFF estimate untreated is ~0.01/10,000 and treated is ~5/10,000 patient-years from Schilcher J et al. N Engl J Med. 2011;364:1728-1737. Risk estimates assume long-term bisphosphonate therapy resulting in 50% reduction in fracture risk. MVA and murder data from the CDC at http://www.cdc.gov/nchs/data/nvsr/nvsr56/nvsr56_10.pdf.

Benefits and Risks



There are about 2.3 million adults treated in ERs each year for injuries from MVAs and about 2 million osteoporotic fractures each year. The risk of seat belt injuries and serious side effects from osteoporosis treatment is very small in proportion to the benefits. Data from multiple sources.


Sequential Array Graphs of Fx Risk
75 year-old Caucasian woman
with FN T-score = -3.0
20%
10%
0.007%
Untreated
Treated
Treated ONJ


FRAX vs. Garvan
80 y/o Caucasian female, 120 lbs, 62", Hologic DXA, FN BMD .620 g/cm2,
T-score = -2.0, wrist fx age 55, 1 fall in last year
50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
8.0%
18.8%
21.0%
43.8%
FRAX
Garvan
Hip Fx
MOF/Any OF
http://www.shef.ac.uk/FRAX/tool.jsp?country=9 http://
garvan.org.au/promotions/bone-fracture-risk/calculator/

Inflammatory Bone Loss

Deborah Novack, M.D., Ph.D.

Meet-the-Professor: Inflammatory Bone Loss

Deborah Novack, MD, PhD, Associate Professor, Musculoskeletal Research Center, Division of Bone and Mineral Diseases, Washington University School of Medicine, St. Louis, Missouri, USA.

Significance:

The correlation between inflammation and bone loss has been recognized for a long time. The first connections were made between localized inflammation and osteoclast recruitment, such as in the joints of patients with rheumatoid arthritis and in alveolar bone of patients with periodontal disease. Inflammatory cytokines such as TNFα and IL-1β are generated and act locally to recruit osteoclast precursors, enhance their differentiation, and promote their resorptive function. In addition to promoting expression of osteoclastogenic factors by stromal cells, inflammatory cytokines have adverse effects on osteoblasts, decreasing bone formation and contributing to net loss of bone. Clinical studies have also shown that systemic bone loss, ie. osteoporosis, is highly prevalent in patients with inflammatory diseases that do not directly involve the skeleton such as inflammatory bowel disease and systemic lupus erythematosus. Indeed, current theories of aging suggest that persistent low-grade inflammation may represent a common mechanism for age-related changes in most organ systems, including bone. Thus, a fuller understanding of the cells, secreted factors, and signaling pathways activated in inflammatory conditions may inform therapies for bone loss in specific inflammatory diseases as well as in age-related osteoporosis.

Learning Objectives:

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

1. Describe how inflammatory mediators stimulate osteoclast-mediated resorption and inhibit bone formation by osteoblasts.

2. Discuss how the inflammatory environment can alter lineage allocation of early hematopoietic progenitors, leading to enhanced osteoclast formation and osteolysis.

3. Identify the NLR family of proteins as potential modulators of inflammatory bone loss.

Points of Interest:

<u>Role of Inflammatory Mediators in Inflammatory Osteolysis.</u> Rheumatoid arthritis (RA) is the best studied example of inflammatory bone loss. However, there is considerable overlap in the molecular mechanisms between RA and other inflammatory conditions. Furthermore, in addition to localized bone loss at sites of disease, circulating inflammatory factors interact with osteoclast and osteoblast lineage cells systemically to cause bone loss globally (1,2,3).

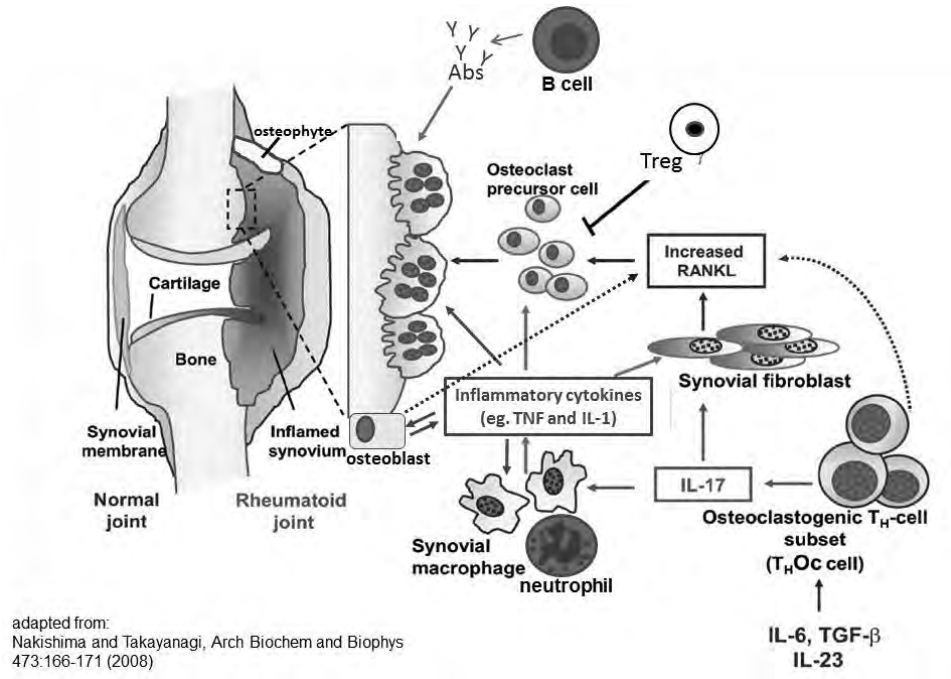


Figure 1. Complex cellular and cytokine networks regulate bone resorption in rheumatoid arthritis (RA). Sites of inflammation, such as the joints in rheumatoid arthritis, are very complex microenvironments in which many different cell types interact, generating and responding to a wide array of secreted and cell surface mediators. Central to the process are the inflammatory cytokines TNFα and IL-1β, which are secreted primarily by synovial macrophages and neutrophils, but also by osteoblasts. These cytokines increase production of RANKL by osteoblasts and synovial fibroblasts, and the sensitivity of osteoclasts and their precursors to this key osteolytic factor is enhanced by their simultaneous direct exposure to TNFα and IL-1β. Osteoblasts are also directly affected by TNFα, which generally reduces their bone forming activity. However, under some conditions, TNFα may be able to enhance osteoblast differentiation of some populations and aberrant bone formation, leading to the generation of osteophytes, a common finding in RA. The central osteolytic cytokine loop is strengthened by inflammatory T cells that produce IL-17, a factor that enhances RANKL production by fibroblasts and osteoblasts. Production of anti-citrullinated protein antibodies (Abs) by B cells also enhances osteoclast formation via direct interaction with vimentin at the osteoclast cell surface. Some T cell subsets restrain osteolysis, both directly via production of osteoclast-inhibitory cytokines (eg. IFNγ and IL-4) and CTLA4 signaling, and indirectly by reducing inflammation.

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Skewing of myeloid progenitors in inflammatory conditions. In addition to affecting osteoclasts and their immediate precursors, the inflammatory state influences early hematopoietic cells, directing them toward myeloid lineages with a high propensity for osteoclastogenesis (4).

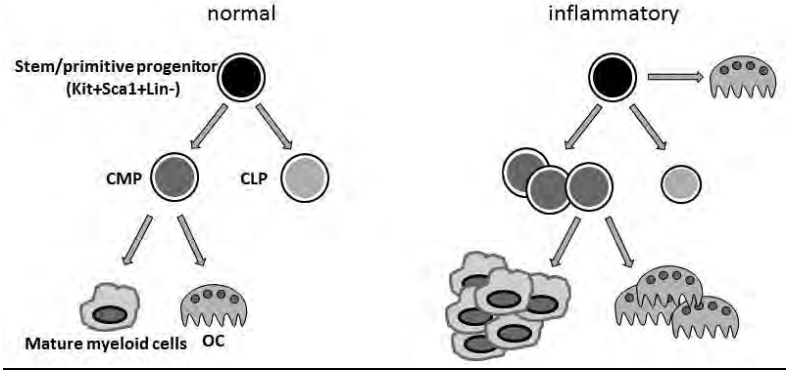

Figure 2. Inflammatory conditions alter the output of hematopoietic stem/progenitor cells. Studies performed on arthritic mice (5) and Scurfy* mice (6) both support a model in which the myeloid and osteoclast output of early hematopoietic cells is increased. Kit+Sca1+Lin- cells contain all stem and early progenitors, and they retain their multilineage potential when isolated from inflamed mice, giving rise to both common myeloid progenitors (CMP) and common lymphoid progenitors (CLP). However, they are more likely to form myeloid cells under limiting cytokine conditions and when transplanted into non-arthritic recipients. More osteoclasts (OC) are also generated from committed myeloid progenitors. In addition, the Kit+Sca1+Lin- population derived from mice in inflammatory conditions seems to give rise to OC directly, in response to M-CSF and RANKL, whereas those from normal mice are not. The mechanisms for inflammation-induced changes in progenitor cells are not well understood. Expression of myeloid inflammatory signature genes such as S100a8 and S100s9 are upgregulated in these multipotential progenitors from arthritic mice. The early progenitors in Scurfy mice were especially sensitive to M-CSF, and its neutralization largely normalized osteoclasts. These studies should broaden our consideration of mechanisms for bone loss in the context of inflammation beyond direct effects on osteoclasts and osteoblasts towards progenitor pools. Our current therapies do little to repair focal bone loss caused by inflammation, and perhaps understanding effects on mesenchymal, as well as hematopoietic, precursors will provide new avenues for therapeutic development.

* Scurfy mice have severe multiorgan inflammation due to a lack of regulatory T cells, leading to high levels of inflammatory (IL-17 and TNFα) and osteoclastogenic (M-CSF and RANKL) cytokines.

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Role of NLR proteins in bone loss. Nucleotide-binding leucine-rich repeat and pyrin domain-containing receptors (NLRPs) are a large family of proteins that bind a variety of ligands associated with tissue damage as well as infection. They are primarily expressed by myeloid lineage cells, and the inflammatory milieu is rich in potential ligands, making them likely players in osteolysis. NLRP3 is a critical component of the inflammasome required for the generation of active IL-1b, and activating mutations cause a spectrum of inflammatory diseases. Mice expressing activated NLRP3 globally or in all myeloid cells have systemic inflammation, a myeloid skewing of bone marrow, and low bone mass (7). Conversely, mice lacking NLRP3 have reduced age-associated bone loss (8), and those lacking the NLRP3 effector caspase-1 are resistant to particle-induced osteolysis (9).

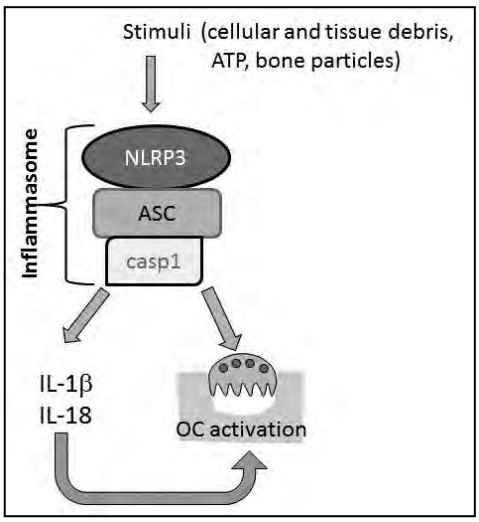

Figure 3. NLRP3 inflammasome promotes osteoclast activation. NLRP3 can be activated by a number of stimuli that might be increased in the context of inflammatory bone loss, including cellular debris, ATP, and fragments of bone matrix. Activation of NLRP3 leads to formation of the inflammasome complex, containing the proteins ASC and caspase-1, and generation of active IL-1b and IL-18 from their inactive "pro" forms. In addition to direct effects of IL-1b and generation of an inflammatory environment on osteoclasts, NLRP3 may directly affect osteoclast differentiation through cytokine-independent mechanisms. Osteoclast lineage cells also express NLRP1, NLRC4, and AIM2 which could play similar roles to NLRP3.

Although members of the NLR family are generally considered as initiators of inflammation, recent studies have shown that some, including NLRP12, act as checkpoint proteins that reduce inflammation. Recently, we have found that NLRP12, a NLR protein without apparent inflammasome function, is expressed in osteoclast precursors and downregulated with differentiation (10). In contrast to NLRP3, it plays a regulatory role and restrains osteoclastogenesis. Interestingly, loss of NLRP12 results in low bone mass, and less RANKL-induced osteolysis in the absence of inflammation. Thus, NLRPs may regulate bone resorption in basal as well as inflammatory conditions.

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Skeletal Aging

Stavros Manolagas, M.D., Ph.D.

Skeletal Aging

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Significance of topic:

Over the past two centuries, life expectancy in developed countries has increased exponentially. It is unclear, however, whether skeletal involution is an inexorable accompaniment of longevity; and if so, whether it can be combated by targeting molecular pathways and mechanisms of aging, so that "bone health span" can increase in tandem with lifespan.

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

- The role of aging in the pathogenesis of osteoporosis
- The hallmarks of aging across all tissues
- The role of redox imbalance, mitochondrial dysfunction, the FoxO transcription factors, and autophagy in skeletal involution
- The contribution of osteocyte dysfunction to skeletal involution and cortical porosity
- The contribution of sex steroid deficiency to the aging of the skeleton

Biologic hallmarks of aging across all tissues:

A. Genomic instability
B. Telomere attrition:
C. Epigenetic alterations:
D. Loss of protein homeostasis or proteostasis (heat shock, ER stress, oxidative stress → protein unfolding → Authophagy, proteasomal degradation, chaperone-mediated folding, aggregation → Aging)
E. Deregulated nutrient sensing
   Insulin and IGF1 signaling (IIS) – the most conserved aging-controlling pathway in evolution – → PI3K, AKT → inhibition of FoxOs
F. Mitochondrial dysfunction
   As cells and organisms age, the efficacy of the respiratory chain system tends to diminish, thus increases electron leakage and ATP generation. There is, however, apparently conflicting evidence regarding the positive, negative, or neutral effects of ROS on aging. This can be reconciled by considering ROS as a stress-elicited survival signal and that the primary effects of ROS will be the

activation of compensatory homeostatic mechanisms. Beyond a certain threshold, ROS levels betray their original homeostatic purpose and eventually aggravate, rather than alleviate, the age-associated damage. In other words, stress signals and defective mitochondrial function generate ROS that, below a certain threshold, induce survival signals to restore cellular homeostasis but, at higher or continued levels, can contribute to aging.

G. Cellular senescence

Because the number of senescent cells increases with aging, it has been widely assumed that senescence contributes to aging. However, this view undervalues what conceivably is the primary purpose of senescence, which is to prevent the propagation of damaged cells and to trigger their demise by the immune system. Therefore, it is possible that senescence is a beneficial compensatory response that contributes to rid tissues from damaged and potentially oncogenic cells. In aged organisms, this turnover system may become inefficient or may exhaust the regenerative capacity of progenitor cells, eventually resulting in the accumulation of senescent cells that may aggravate the damage and contribute to aging.

H. Stem cell exhaustion

The decline in the regenerative potential of tissues is one of the most obvious charactersistics of aging.

I. Altered intercellular communication

i. Inflammaging: smoldering pro-inflammatory phenotype

ii. Other types of intercellular communication

iii. Restoring defective intercellular communication (dietary restriction)

Skeletal aging

Soon after the attainment of peak bone mass – during the third decade of life in humans – the balance between bone formation and bone resorption begins to progressively tilt in favor of the latter, in both women and men. This change begins long before and independently of any changes in sex steroid levels. Nonetheless, at menopause the loss of trabecular bone accelerates. The rate of bone loss in women slows within five to ten years after the menopause and is followed by a slower phase of bone loss that occurs also in men. This later phase affects primarily cortical bone, and a significant portion of it is due to increased endocortical resorption and intracortical porosity.

During the last ten years, genetic evidence from the mouse model has provided a major paradigm shift from the traditional “estrogen-centric” account of the pathogenesis of involutional osteoporosis to one in which age-related (sex monomorphic) mechanisms intrinsic to bone, including mitochondrial dysfunction, oxidative stress, FoxO activation, senescence of mesenchymal stem cells and osteocyte,

and declining autophagy are protagonists and age-related changes in other organs and tissues, such as the ovaries , are contributory.

Specifically, it has been elucidated that similar to humans, sex steroid-sufficient female and male mice experience an age-dependent progressive decline in bone mass and strength that is temporally associated with increased oxidative stress. Importantly, identical changes in oxidative stress are recapitulated in either sex of mice acutely upon loss of sex steroids. Furthermore, the adverse effects of the loss of sex steroids on murine bone can be prevented by the systemic administration of antioxidants or by genetically decreasing H2O2 production in osteoclast mitochondria. In line with a pathogenetic role of oxidative stress on bone formation, reactive oxygen species (ROS) attenuate osteoblastogenesis and shorten the lifespan of osteoblasts. ROS, on the other hand, and in particular, H2O2 are required for osteoclast generation, function, and survival.

Increased ROS generation leads to the activation of FoxOs – transcription factors that promote compensatory adaptations in response to OS and growth factor deprivation. In the osteoblast lineage, the overriding function of FOXOs is to provide an optimal balance among the maintenance of self-renewing stem cells, the replication of lineage-committed intermediates, and the survival of the terminally differentiated progeny, for the purpose of compensatory adaptations to stresses that accumulate in bone with advancing age. Nonetheless, as is the case with several other defense responses against aging, FoxO activation can eventually aggravate the effects of aging on bone and become a culprit of involutional osteoporosis. FoxOs, on the other hand, restrain osteoclastogenesis and bone resorption by attenuating H2O2. Hence, FoxO activation in response to the age associated increase in oxidative stress and/or decline in growth factors may be responsible for the imbalance between bone formation and resorption.

Consistent with the biologic role of osteocytes in the choreography of bone remodeling, emerging evidence indicates that signals arising from apoptotic and old/or dysfunctional osteocytes are seminal culprits in the pathogenesis of involutional, post-menopausal, steroid- and immobilization- induced osteoporosis. In other words, in conditions of overwhelming stress – including age accumulated damage – physiological mechanisms of bone repair are exaggerated and eventually become disease mechanisms.

Notably, whereas acute sex steroid deficiency is a state of high bone turnover with increased resorption and formation, albeit unbalanced, aging is a state of low turnover (at least in trabecular bone) and decreased formation. Consistent with this, genetic evidence from the mouse model has also revealed that increased H2O2 generation with old age in cells of the mesenchymal lineage is a seminal culprit of the loss of cortical bone. In contrast, increased H2O2 generation in cells of the

osteoclast lineage is the culprit of the loss of cortical bone caused by acute sex steroid deficiency, but not old age. Hence, the mechanisms of cortical bone loss in the two conditions are probably distinct.

Recent relevant papers:

1. Lopez-Otin et al.,The hallmarks of aging, Cell, 154: 1194-1217, 20132.

2. Almeida and O'Brien. Basic biology of skeletal aging: role of stress response pathways. J Gerontol A Biol Sci Med Sci. 2013 68:1197-208.

3. Goettsch, et al: NADPH oxidase 4 limits bone mass by promoting osteoclastogenesis. J Clin Invest. 2013, 123(11):4731-8.

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7. Iyer,et al: Sirtuin1 (Sirt1) Promotes Cortical Bone Formation by Preventing beta (β)-Catenin Sequestration by FoxO Transcription Factors in Osteoblast Progenitors. J Biol Chem, 289:24069-78, 2014.

8. Mercken, et al: SRT2104 extends survival of male mice on a standard diet and preserves bone and muscle mass. Aging Cell. 2014 Oct; 13(5):787-96.

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10. Kobayashi, et al: Mitochondrial superoxide in osteocytes perturbs canalicular networks in the setting of age-related osteoporosis. Sci Rep. 2015; 5:9148.

11. Ucer, et al: The effects of androgens on murine cortical bone do not require AR or ERα signaling in osteoblasts and osteoclasts. J Bone Min. Res., 30(7):1138-49., 2015.

Relevant abstracts presented during this meeting:

1. S.S. Ucer, et al: H_2O_2 generated in the mitochondria of osteoclasts is required for the loss of cortical bone mass caused by estrogen or androgen deficiency, but not aging
2. Ha-Neui Kim, et al: Sirtuin1 (Sirt1) activation suppresses osteoclastogenesis by deacetylating FoxOs

Effects of Glucocorticoids on Bone

Hong Zhou, Ph.D.

Effects of Glucocorticoids on Bone

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Basic Meet-the-Professor session, Saturday, October 10, 11:30-12:30

Significance of the Topic: Glucocorticoids are widely used for their unsurpassed anti-inflammatory and immunomodulatory effects. While these beneficial effects can hardly be overestimated, the therapeutic use of glucocorticoids is almost always limited by significant adverse outcomes, such as osteoporosis, diabetes and obesity. In order to understand the pathogenesis of glucocorticoid-induced osteoporosis, it is important to realise that the actions of glucocorticoids on bone and mineral metabolism are strongly dose and time dependent. At both physiological and excessive levels of glucocorticoids, osteoblasts and osteocytes are the major glucocorticoid target cells. At physiology levels, glucocorticoids direct mesenchymal progenitor cells to differentiate towards osteoblasts and thus increase bone formation in a positive way. In contrast with excessive levels of glucocorticoids appear to impact on osteoblast and osteocytes in a negative way in a similar fashion to that seen with therapeutic glucocorticoids.

Prolonged exposure to excessive levels of endogenous or exogenous glucocorticoids is associated with diabetes and obesity. It had been assumed that these adverse effects were mediated by direct effects of glucocorticoids on tissues such as adipose or liver. Recent studies have however indicated that these effects are, at least in part, mediated through the actions of glucocorticoids on osteoblast. In mice, targeted abrogation of glucocorticoid signalling in osteoblasts significantly attenuated the changes in body composition and systemic fuel metabolism seen during glucocorticoid treatment. Heterotopic expression of osteocalcin in the liver of normal mice was also able to protect against the metabolic changes induced by glucocorticoids indicating that osteocalcin was the likely factor connecting bone osteoblasts to systemic fuel metabolism.

Learning Objectives

1. Understand Glucocorticoids signaling pathways
2. Physiological glucocorticoids are required for normal bone development and bone cell function
3. Excess glucocorticoids are detrimental to bone structure and strength through their negative effects on osteoblast and osteocyte function
4. The suppression of osteoblast function and osteocalcin synthesis plays an important role in glucocorticoid-induced diabetes and obesity.

Glucocorticoids, pre-receptors and receptor

Endogenous glucocorticoid (GC) levels are systemically regulated via the hypothalamic-pituitary-adrenal axis. However, glucocorticoid action depends not only on plasma and interstitial fluid hormone concentrations but also on tissue glucocorticoid levels. Within specific tissues, the 11ß-hydroxysteroid dehydrogenase (11ßHSD) enzymes metabolise glucocorticoids at a “pre-receptor” level and can thus control intracellular levels of active glucocorticoids. 11ß-HSD type 1 (11ßHSD1) predominantly catalyses formation of active cortisol (in humans) and corticosterone (in rodents) from inactive cortisone and 11-dehydrocorticosterone, respectively, leading to increased intracellular glucocorticoid concentrations. In contrast, 11ß-HSD type 2 (11ßHSD2) unidirectionally catalyses

conversion of active glucocorticoids to their inactive metabolites (Figure 1).

After moving from the interstitial fluid into the cell cortisol binds to the glucocorticoid receptor (GR). The inactive glucocorticoid cortisone can also be converted to cortisol within the cell by the action of 11ß-HSD1. Cortisol binds to GR in the cytoplasm after which the ligand-bound GR can migrate to the nucleus. The activated GR can either bind to its specific response element (GRE) or bind to other transcription factors such as AP-1 (fos and jun) to transactivate or transrepress gene expression respectively.

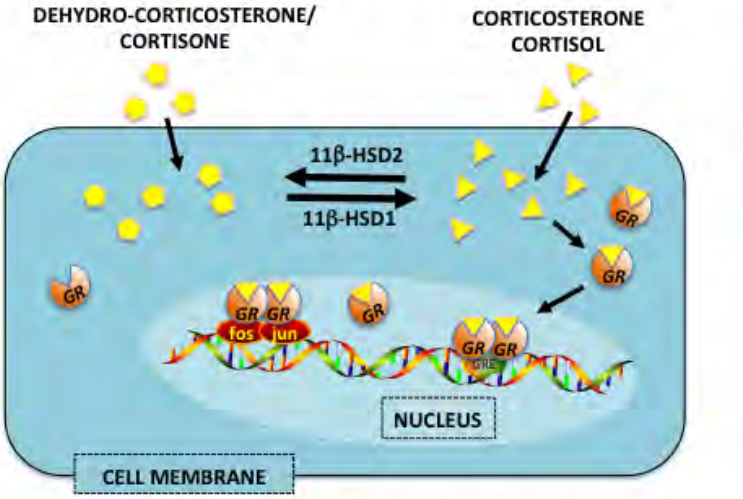


Figure 1. The mechanisms of action of glucocorticoids

Seibel, Cooper & Zhou, Lancet Diabetes Endocrinol 2013; 1:59

Expression of the 11β-HSD enzymes has been extensively characterized in bone. The activity of 11β-HSD1 in primary human osteoblasts is dependent on donor age, with low expression in young donors but high expression in osteoblasts isolated from older donors. An age-related change in expression is paralleled in mice in vivo suggesting that some of the adverse age-related changes in bone could be glucocorticoid mediated.

Endogenous glucocorticoid effects on bone

Major insights into the role of endogenous glucocorticoids within the skeleton have been derived from experiments utilising genetic modified mouse models. Although not expressed naturally in bone cells, the glucocorticoid-inactivating enzyme, 11ßHSD2, has been used as a tool to examine the effects of endogenous glucocorticoids on specific bone cells. The range of osteoblast specific promoters (Figure 2) used to drive either 11ßHSD2 gene expression or to delete the GR gene by Cre expression in the bone lineage ranges.

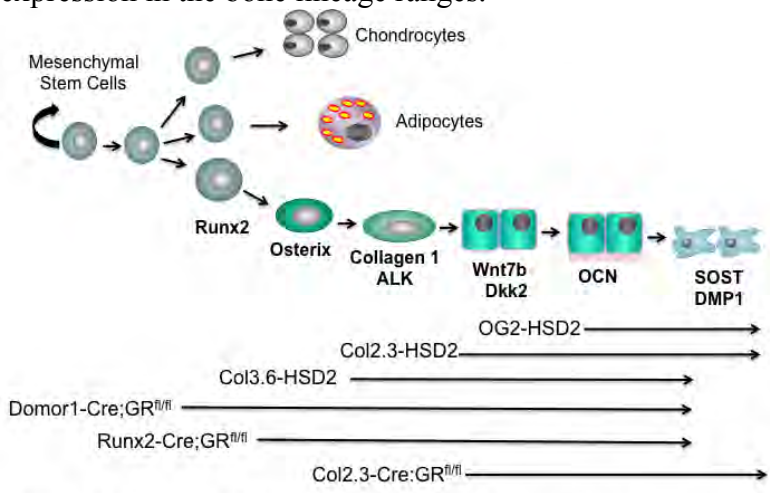
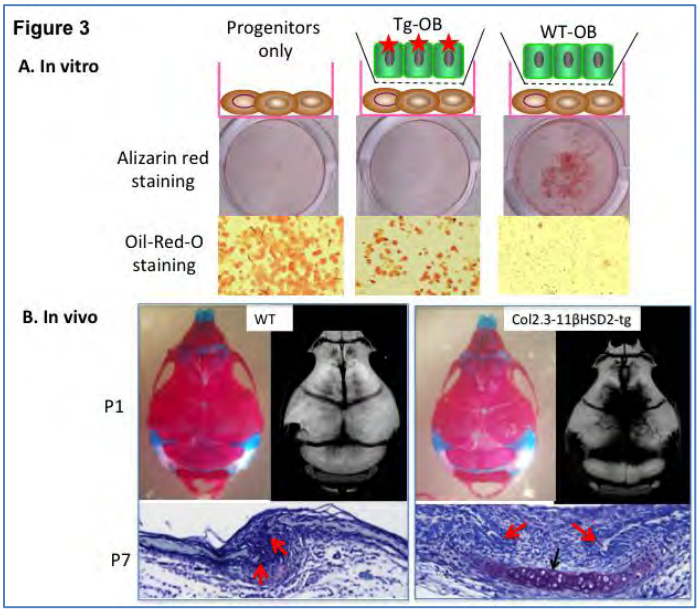


Figure 2. Animal models for investigate glucocorticoid action in bone

Zhou, Cooper and Seibel, Bone Research 2013; 2:107

Glucocorticoids are not only essential for the differentiation of mesenchymal cells into mature osteoblasts but also play an essential role in directing cell lineage commitment of early mesenchymal progenitors towards osteoblast differentiation through regulation of Wnt/ß-catenin signaling in mature osteoblasts.



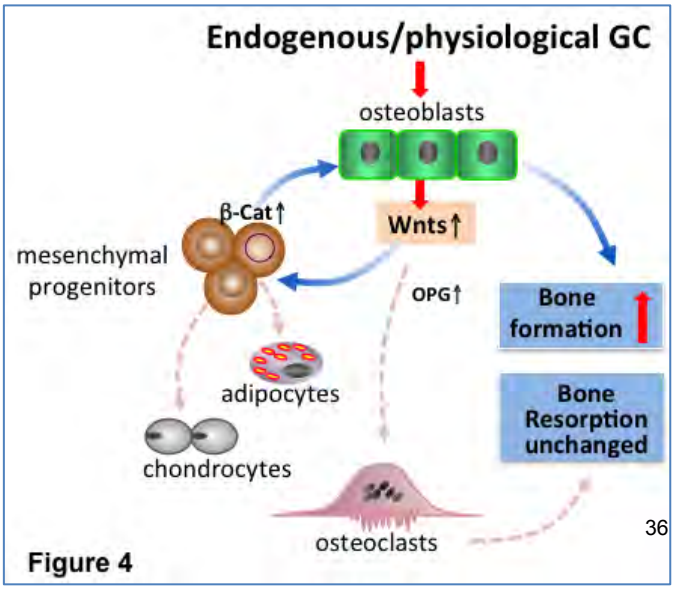
In vitro, calvarial cell cultures derived from Col2.3-11ßHSD2 transgenic mice exhibited greatly reduced osteoblasto-genesis and increased adipogenesis transwell co-culture of Col2.3-11ßHSD2-tg progenitor cells with WT osteoblasts restored osteoblastogenesis.

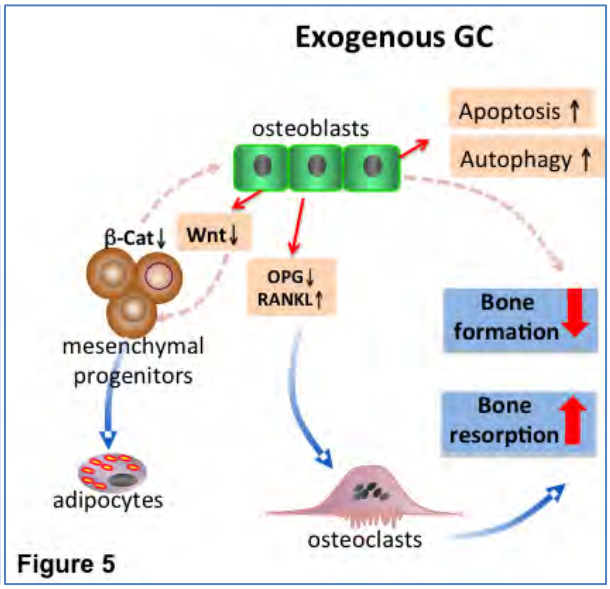
In vivo, embryonic and neonatal Col2.3-11ßHSD2 transgenic mice display a distinct phenotype character-rized by calvarial bone hypoplasia, increased suture patency, ectopic differentiation of cartilage in the sagittal suture and a defect in the postnatal removal of parietal cartilage (Figure 2). These phenotypes are also seen in Col3.6-11ßHSD2 transgenic mice.

Mechanistically, glucocorticoids stimulate mature osteoblasts to produce canonical Wnt proteins, which activate the β-catenin signaling cascade in mesenchymal progenitor cells to differentiate towards osteoblasts and away from chondrocytes and adipocytes. These actions favour bone formation. In addition, β-catenin signaling in osteoblasts and osteocytes promotes osteoprotegerin (OPG) expression which in turn inhibits osteoclast formation resulting in bone resorption being either decreased or unchanged (Figure 4).

Effects of excess glucocorticoid on bone

Glucocorticoids at excess concentrations target negatively impact on osteoblast and osteocytes. These actions include: inhibition of Wnt protein expression in mature osteoblasts which results in mesenchymal progenitor cells differentiating preferentially towards adipocytes and away from osteoblasts; an increase in the RANKL/OPG ratio by stimulation of RANKL and inhibition of OPG expression which together favours increased bone resorption, and increased osteoblast and osteocyte apoptosis which reduces bone formation (Figure 5).





Glucocorticoid Excess and Systemic Fuel Metabolism

The development of insulin resistance and obesity is one of the most common adverse effects of therapeutic GC use in man. While Glucocorticoids are likely to have direct effects on tissues such as liver, adipose tissue and muscle, at least in rodents some of these appear to be mediated via the skeleton. In this context, osteocalcin seems to play a particularly important role, which has been fully appreciated only recently.

In both mice and man, Glucocorticoids profoundly suppress osteoblast function and hence rapidly and profoundly suppresses the synthesis of osteocalcin via transrepression. When GC signalling was disrupted in osteoblasts and osteocytes alone, mice treated with high-dose exogenous GCs maintained higher serum osteocalcin concentrations and were protected from GC-induced insulin resistance and glucose intolerance. Furthermore, heterotopic expression of osteocalcin via gene therapy in GC-treated mice restored insulin sensitivity and glucose disposal to levels seen in untreated mice. Moreover, restoration of normal osteocalcin levels lead to almost complete clearance of hepatic lipid deposits in GC-treated mice.

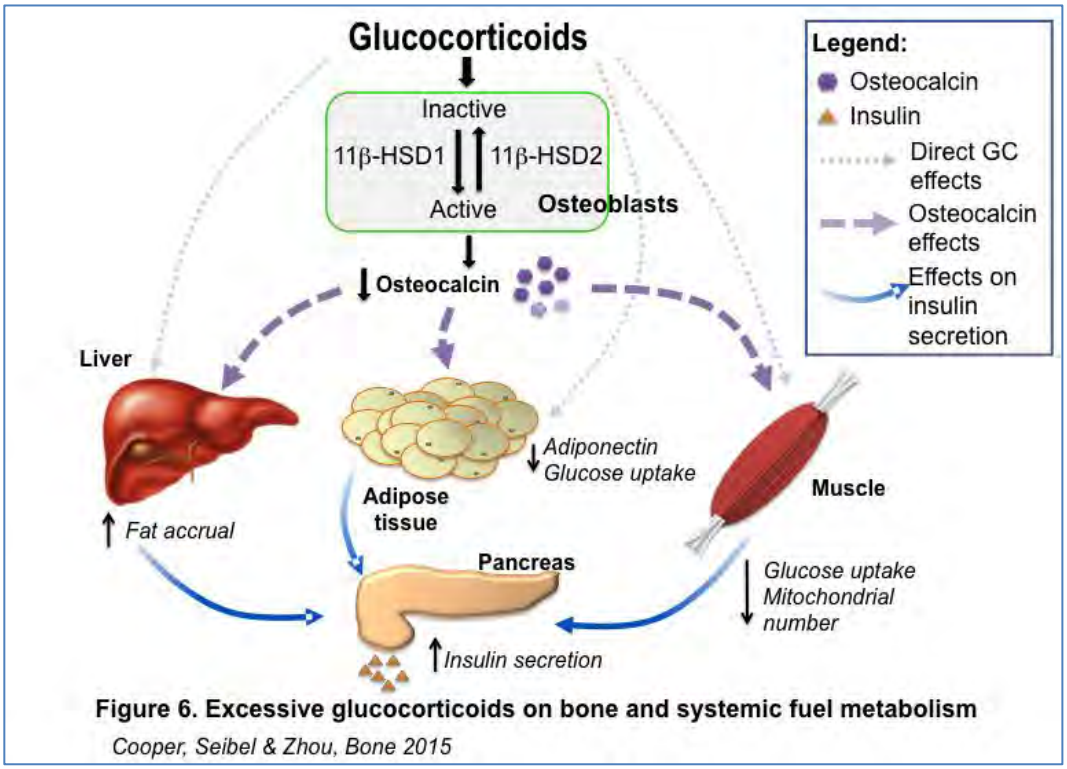


Figure 6. Excessive glucocorticoids on bone and systemic fuel metabolism

Cooper, Seibel & Zhou, Bone 2015

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Primary Hyperparathyroidism: An Update

John Bilezikian, M.D.

Primary Hyperparathyroidism Update

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Significance

When primary hyperparathyroidism (PHPT) became recognized as a common endocrine disorder in the 1970s, due to the widespread introduction of the multichannel screening testing, key questions were raised as to how to deal with a disease that is brought to clinical attention by a test and not necessarily by symptoms. These questions and issues have been highlighted in 4 International Workshops on the management of Primary Hyperparathyroidism, the most recent one of which was held in Florence, Italy, in 2013. With advances in identifying target organ involvement, the need for a more proactive approach to this disease has surfaced. It is also recognized that some, but by no means all, patients demonstrate progression of disease. Finally, in the hands of an expert parathyroid surgeon and successful preoperative localization, removal of the parathyroid adenoma is very straightforward. Minimally invasive parathyroid surgery with measurements of PTH intraoperatively has given further assurance of a curative procedure. On the other hand, the need for surgery can be questioned by physicians when their patients are not symptomatic and cannot be shown to have any significant target organ involvement. At the 4th International Workshop on Asymptomatic Primary Hypeparathyroidism, new knowledge accrued over the previous 5 years was emphasized and considered along with previous information from earlier workshops. As a result, guidelines for the management of asymptomatic PHPT were revised. This MTP session will cover these issues related to the diagnosis and management of asymptomatic PHPT.

Learning Objectives

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

-appreciate the varying clinical presentations of PHPT.
-recognize that complete skeletal and renal evaluation is recommended, taking advantage of newer imaging technology.
-be familiar with current guidelines for surgery and monitoring.

Outline of session with discussion points

Diagnostic criteria for PHPT. The diagnosis of PHPT is very straightforward when the serum calcium and PTH concentration are above normal limits. Second and third generation assays for PTH are equivalently good for clinical purposes. Given high calcium and PTH levels, very few other situations present in this manner (e.g., thiazide or lithium use, FHH). The presence of a non-supressed PTH value is entirely compatible with the diagnosis of PHPT but a "normal" PTH level when hypercalcemia is present continues to be vexing to some clinicians. Metabolic bone disease experts recognize readily that a PTH level that is detectably within the normal range (usually mid-to high normal range) is an abnormal physiological state when hypercalcemia is present.

Presentations of PHPT. PHPT in developed countries is dominated by the mild asymptomatic variant of the disease. In developing countries, particularly India, the disease typically presents, on the other hand, as a symptomatic one. The time-associated epidemiologic transition from symptomatic to asymptomatic PHPT is being seen in some countries like China that has seen in a single decade and in the course of the country's own economic development a transition from symptomatic to asymptomatic disease. This point argues that the incidence of asymptomatic PHPT is very much a function of surveillance and screening, not a comment on a true change in the phenotypic variability of PHPT. Asymptomatic PHPT has probably always existed but routine detection over the past 45 years is due to routine measurement of the serum calcium concentration. Another point that might influence the clinical presentation of symptomatic vs asymptomatic disease may relate to the prevalence of vitamin D deficiency in the population (see below).

In line with a proactive approach to the evaluation of skeletal health, yet another variant of PHPT has surfaced, namely normocalcemic PHPT (NPHPT). In NPHPT, the total and ionized calcium concentrations are consistently normal while the PTH level is consistently elevated. Both 2^{nd} and 3^{rd} generation assays for PTH detect levels above normal. It is important to rule out secondary causes of elevated PTH levels such as vitamin D insufficiency (25-OH D < 30 ng/mL), hypercalciuria, and renal compromise (Clcr < 60cc/min). NPHPT is typically discovered in the context of an evaluation for low bone mass when PTH is measured by many clinicians. Like asymptomatic PHPT, NPHPT is discovered incidentally, but different from asymptomatic PHPT, NPHPT is discovered in a search to investigate a skeletal issue. One might predict that there is another aspect of NPHPT, not necessarily associated with reduced bone mass, if unselected populations are screened. Indeed, this has been demonstrated.

Skeletal Involvement. With dual energy X-ray absorptiometry, skeletal involvement can often be detected in PHPT. The classical pattern of cortical bone loss as seen at the distal 1/3 radius is the most common pattern giving rise to the notion that PTH in PHPT is protective against trabecular bone loss as seen in the lumbar spine. This perception needs to be corrected in view of recent studies that have elucidated trabecular bone involvement by applying new imaging and analytical approaches. By high resolution computed peripheral tomography (HRpQCT- also known as Extreme CT), several groups have demonstrated trabecular abnormalities in PHPT. Resolving the HRpQCT image further and applying another new imaging methodology, trabecular bone score (TBS), the most recent data are now compatible with epidemiological data that have reported an increase in fracture incidence at vertebral (primarily trabecular) and non-

vertebral (primarily cortical) sites for years. Now we have an explanation for these long standing clinical observations. This new knowledge has influenced the most recent guidelines that recommend an evaluation of the trabecular compartment of bone that goes beyond the lumbar spine BMD. Vertebral X-ray, vertebral fracture assessment, and/or TBS are now recommended.

Renal Involvement in PHPT. Renal stones and nephrocalcinosis are still the most common overt complications of PHPT. Recent reports have confirmed a higher incidence of silent kidney stones when imaging studies are conducted in asymptomatic PHPT. In addition, it is now more fully appreciated that a 24-hour urine for biochemical analysis of stone risk factors can give very valuable information in PHPT. The importance, therefore, of conducting a more thorough evaluation of the kidney by imaging (abdominal X-ray, ultrasound or CT) along with a 24-hour urine collection for calcium and other stone risk factors is now being recommended.

Neurocognitive and Cardiovascular Features. These two areas are among the most vexing because it would seem likely that abnormalities may well exist. Yet, it has been difficult to establish specificity to many observations that have been made over the years. Testing abnormal neurocognitive or cardiovascular indices for reversibility under experimental conditions that include appropriate controls continues also to be a major challenge. Not discounting the potential importance of these organ systems as targets of PHPT, it is still premature to make recommendations regarding action to take if either of these systems appears to be involved.

Vitamin D Deficiency. It is generally appreciated that the pathophysiological abnormalities in PHPT are further exacerbated by Vitamin D deficiency. This point has been demonstrated not only in symptomatic cohorts in China and India but also among an asymptomatic cohort in the United States. Perhaps because of the great interest in vitamin D among the general populace and also perhaps because of touted claims of vitamin D's extraskeletal benefits, usage of vitamin D as a supplement appears to be rising. In PHPT, a comparison of recent data indicates that the tendency for 25-OH vitamin D to be low in PHPT is no longer being substantiated. If it is true that 25-OH vitamin D levels are no longer low in PHPT, then one might forecast that an even milder form of PHPT will be appreciated in the years to come. Related to this point, one might predict that the incidence of NPHPT may be on the rise.

Guidelines for surgery. The Florence workshop modified guidelines to include many of the points that we will discuss in the session. The guidelines are shown in Table 1. If a patient meets any one of these criteria, then surgery should be recommended. It is important to state that parathyroid surgery can be performed on individuals who do not meet any guidelines for surgery. This decision could be made by the physician in a situation where the patient has no contraindications to surgery, and both the physician and the patient are attracted to the idea of curing a chronic, but asymptomatic disorder. When the decision for surgery is made, preoperative imaging is performed. Recently, 4-D computed tomography (4D-CT) has gained popularity. In some situations, with 4-D CT, normal parathyroid glands can be identified along with the abnormal one(s). On the other hand, there are patients who meet guidelines for surgery but refuse surgery or have contraindications to it. In addition, there are many patients who do not meet guidelines and do not have surgery. Information on the natural history of PHPT is relevant to this discussion and will be covered.

Monitoring. For those who will be followed without parathyroid surgery, monitoring is strongly recommended. The guidelines for monitoring are shown in Table 2. They will be discussed in the session.

Pharmacological Approaches to PHPT. In patients who do not meet surgical criteria as well as in patients who do, but refuse or are not candidates for surgery, pharmacological approaches can be attractive. They are reserved, though, for one of two situations: to lower the serum calcium when it is > 1mg/dL or to improve bone mineral density. In well-designed, placebo-controlled trials, alendronate has been shown to increase bone density. Serum calcium and PTH levels do not change. With cinacalcet, a calcimimetic, serum calcium typically falls into the normal range, but bone density doesn't change. PTH levels fall by about 20-30%. Cinacalcet is approved by the FDA for the management of PHPT. It also has a role in parathyroid cancer when widespread disease is no longer amenable to surgery.

TABLE 1: 2013 guidelines

Measurement	**Surgery recommended if any 1 of the following is present**
Serum Calcium	>1.0 mg/dl (0.25 mmol/L) above normal
Creatinine Clearance (calculated)	Reduced to < 60 cc/min /1.73 m^2 24-hr urine for calcium >400 mg and increased stone risk by biochemical stone risk analysis
Bone Mineral Density	T-score <-2.5 at spine, hip (total or femoral neck) or 1/3 radius, or vertebral fracture by imaging (X-ray, CT, MRI, or VFA)
Age	< 50 years

TABLE 2: 2013 Guidelines for monitoring asymptomatic PHPT

Measurement	**Frequency**
Serum calcium	Annually
Renal evaluation	Annual estimated Clcr; imaging if the clinical situation calls for it.
Skeletal evaluation	BMD Every 1 – 2 years; further evaluation by X-ray, CT, VFA, TBS if the clinical situation calls for it.

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In Vivo Imaging of Bone Cells

Masaru Ishii, M.D., Ph.D.

ASBMR 2015 Annual Meeting Meet-the-Professor Session
Saturday, October 10, 1130 am -12:30 pm
"In Vivo Imaging of Bone Cells"
Masaru Ishii, M.D., Ph.D., Osaka University (Japan)

Significance of the Topic:

During the last decade, multi-photon fluorescent microscopy has launched a new trend in the field of biology. By using this advanced imaging technique we have established a new system for visualizing in situ behavior of a diversity of living cells within intact tissues and organs. Among them, we succeeded in visualizing the various dynamic phenomena within bone tissues and bone marrow, mysterious places where various kinds of dynamic cellular phenomenon occur although poorly analyzed by conventional methodology such as histological analyses with decalcified bones. We have so far been revealing novel mechanisms controlling migration and function of osteoclasts in situ. Recently we have also focused on the functional association between osteoclasts and osteoblasts, to see the real mode of coupling in live bones.

This novel 'bone histodynametrical' methodology is sure to, collaborating with conventional histomorphometical analyses, contribute to opening a new era in the field of bone and mineral researches in future. In this session, I will present the technical aspects for intravital bone microscopy as well as updated knowledge on bone biology unraveled by this new technology.

Learning objectives

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

- understand the basis of intravital bone imaging by using multiphoton microscopy (including the principle of multi-photon excitation in fluorescent microscopy, successful fixation of bone tissues to be visualized during the experiment, necessary items for developing this imaging system in the attendees' own lab, etc).
- understand how to utilize this advanced imaging technique for revealing the dynamic cellular phenomenon taken place in living bone tissues.
- understand the recent updated information on bone biology derived from this new technology, such as migratory behaviors of osteoclast precursors, dynamic function of mature osteoclasts, and physical coupling between osteoclasts and osteoblasts during bone remodeling. Attendees will realize the revolutionary changes which have already been made or will be brought by in vivo visualization '*histodynametry*' of bones.

Points of Interest

1. General features of intravital multiphoton microscopy

Recent progresses in biomedical imaging techniques have allowed us to visualize a variety of *previously unseen* biological phenomena. In particular, advanced fluorescent microscopy techniques have enabled us to visualize cellular and molecular dynamics in the living body. These new technologies have identified novel therapeutic targets against a wide array of diseases, and have provided novel diagnostic tools for the evaluation of several disease conditions.

Imaging techniques have revolutionized the biological sciences; among them, the development and improved usability of two-photon excitation microscopy have enabled us to visualize biological phenomena that cannot be seen with conventional methods, such as the dynamic behavior of cells deep inside living organs (**Figure 1**). In multi- (normally two-) photon excitation mode, a fluorophore absorbs multiple photons simultaneously. This non-linear optical process can occur only in areas with extremely high photon density, such as the focal plane of optical paths. The limited excitation achieves bright and high-resolution images in regions deep inside tissues and organs. Near-infrared lasers used for multiphoton excitation can penetrate deeper, with less absorption or scattering, than the visible light used with confocal microscopy. In fact, objects can be visualized at depths of 100–1,000 μm with two-photon excitation, whereas conventional microscopy can only access areas at depths of less than 100 μm (1). This property is beneficial for the analysis of live biological systems. The cells observed in a fixed and thin-sectioned sample are dead and no longer moving. The intravital visualization of live dynamic systems often requires the observation of areas deep below the surface, which could be achieved by multiphoton excitation. Moreover, multiphoton excitation with near-infrared light can minimize photo-bleaching and phototoxicity, thereby reducing damage to the imaged tissues and organs (2, 3).

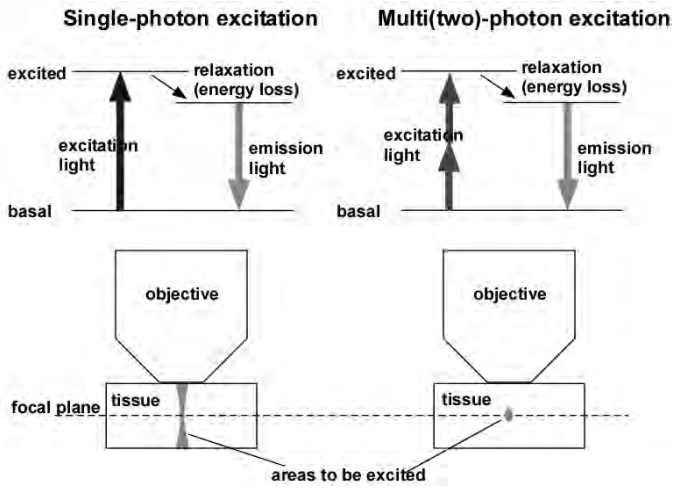

Figure 1. Basic principle of multi(two)-photon microscopy. In multi(two)-photon microscopy, one fluorophore is excited by simultaneous attack by multiple (two) photons, a rare phenomenon only occur in a focal plane, leading to minimized 'unwanted' excitation and high spatial resolution.

In addition to the development of microscope equipment, there were several hurdles to be

overcome for visualization of inside bone tissues. Among them, immobilization of live bone tissues, health maintenance of live animals during experiment, and quantitative analyses of image data were trivial in themselves but bothering and often significant technical issues (see example in **Figure 2**).

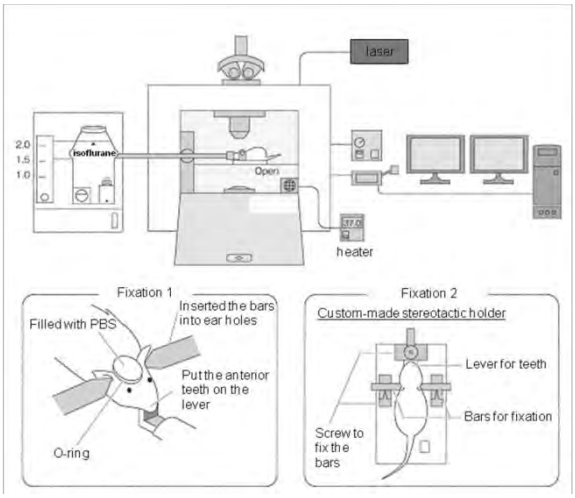

Figure 2. Schematic illustration of intravital two-photon microscopy of mouse skull bone tissues

2. From *Cartoon* to *Real* Biology – shedding light on „dynamic cell world" in vivo

Investigations on biological systems have been so far based on the static histological as well as biochemical analyses and plausible 'cartoon' helped us to understand their dynamic nature in vivo, although the cartoons are sometimes incorrect. Recent imaging technology has shed light on the 'real' dynamic phenomenon that indeed occurs inside the body. By using intravital multi-photon microscopy system, our lab has originally elaborated the system for visualizing the real cellular movements in various tissues and organs in their intact conditions (**Figure 3**).

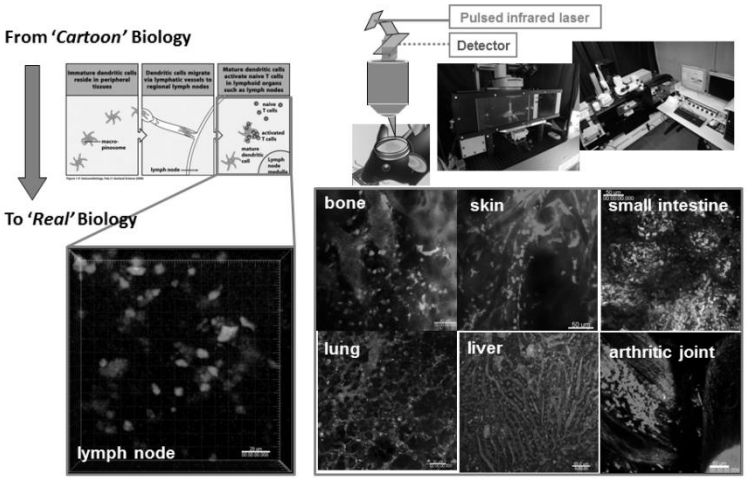

Figure 3. From *Cartoon* to *Real* Biology: Intravital imaging technology sheds light on immune cell dynamics in vivo

3. Use of intravital imaging technique for dissecting bone systems

Bone is a mineralized hard tissue that limits the passage of visible or infrared lasers, and it has long been considered to be extremely difficult to observe intact bone tissues in living animals (12). We have developed a novel imaging system for visualization inside bone cavities with high spatiotemporal resolution. By utilizing this technique, we can demonstrate that osteoclasts, bone-destroying osteoclasts, and their precursors are dynamically migrating under the control of several chemokines and lipid mediators (12-15). By improving bone imaging techniques and originally developing a chemical fluorescent probe (16), we also successfully visualized

bone-resorbing activity of mature osteoclasts lining bone surfaces and identified their real mode of action in situ (15). Despite its hardness, bone is a dynamic and elastic tissue that undergoes continuous remodeling by bone-resorbing osteoclasts and bone-replenishing osteoblasts. Inflammation and hormonal perturbation lead to the aberrant activation of osteoclasts, resulting in several bone-resorptive disorders, chiefly osteoporosis and rheumatoid arthritis. Thus, osteoclasts have emerged as a good therapeutic target against these diseases, and the intravital imaging of bone tissues would be a good tool for the identification of novel target molecules and the development and evaluation of nove l therapeutics (17-20) (**Figure 4**).

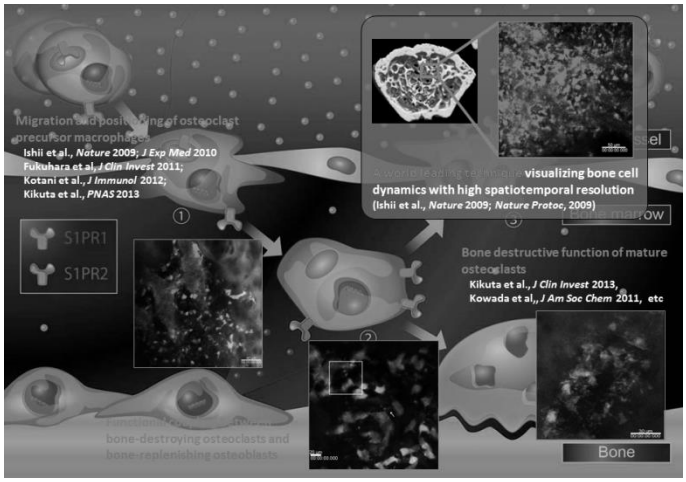

Figure 4. *In vivo* dynamic behaviors of osteoclast, a bone-'destroying' macrophage – visualized by bone intravital microscopy. In vivo bone imaging detects migratory behaviors of osteoclast precursors, mode of resorptive function of mature osteoclasts and coupling between osteoblasts.

Conclusion

Major progress has been made recently in imaging techniques, and several tools for the visualization of live biological systems in situ have become available. These tools must bring a paradigm shift in the field of various fields of biological sciences, and lead to changes in the treatment of several intractable bone diseases in the future.

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Mouse Models and Their Use in Defining Key Osteoporosis Genes

Cheryl Ackert-Bicknell, Ph.D.

Mouse models and their use in defining key osteoporosis genes

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Significance of the Topic

- The mouse is considered among the most important animal models for the study of human diseases (1, 2).
- The mouse genome, while 14% smaller than the human genome, is remarkably comparable to the human genome at the nucleotide level. At the gene level approximately 17,770 mouse genes have a known direct human ortholog (http://www.informatics.jax.org), and organizationally, the mouse and human genomes remain highly syntenic despite a quite long evolutionary distance between them (3).
- Genetic findings in mice are often concordant with genetic findings in humans (4).
- Physiologically and anatomically, mice and humans are remarkably similar. The number of physiologic differences is small compared to the number of similar of physiologic systems that function nearly identically (5).
- Together this makes mice an ideal organism in which to test candidate genes to determine if they impact disease.

Learning Objectives

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

1. Contrast between a forward genetics and a reverse genetics study.
2. Understand homology, synteny, ortholog and conservation and explain what to look for when determining what mouse model to use to test a gene for a role in disease.
3. List the most common different types of mouse models that are used and the pro's and con's of their use.
4. Be able to define "inbred strain," explain the importance of strain background in mouse studies and how that can impact phenotypes. This includes what it means to change strain background.
5. Know where to look for different types of "already published" phenotyping information in online databases.

Types of Genetics studies:

Forward genetics study – This type of study starts with a phenotype or disease and is designed to identify genes and genetic loci associated with alterations in that phenotype or in related phenotypes. This includes Genome Wide Association Studies (GWAS) and quantitative trait loci (QTL) mapping studies. These are usually hypothesis free studies.

Reverse genetic study - One in which a gene is already known and the impact on the phenotype of interest of mutating or eliminating that gene is studied. This includes examining transgenic and spontaneous mutant models. The gene of interest could have been identified from a forward genetics study such as GWAS.

Functional validation – This is a type of reverse genetics study, but it is the logical follow up to a forwards genetic study, as a forward genetic screen establishes association, not causation. Regardless if the association was established using a human or a mouse genetic mapping study, functional validation is required to establish *how* a gene impacts disease. Functional validation is too large of a topic to be covered in one session, but recent functional validation studies for bone research are referenced here (6, 7). Most functional validation studies have focused on protein coding genes. It must be remembered that the causal element for a locus might not be a coding gene at all. Functional validation experiments for intergenic, or non-coding causal variants can be complicated and may require generation of specialized "Humanized mouse models" (8).

Key terminology for comparing mouse and human genomes:

Synteny - This refers to a genomic region that could be as small as a single gene or encompass large segments of chromosomes. Synteny is the degree of similarity in organization between regions that share a common ancestor. This could be done by comparing two separate genomes in two species (i.e. speciation), or at two separate places in the same genome (i.e. when talking about gene duplication). This term is used mostly for describing the organization of two genomes relative to each other and a common ancestor.

Orthologous genes – The "same" gene seen in two different organisms (i.e. mouse and human) such that the gene in each species comes from a common ancestor evolutionarily. Normally, orthologous genes retain the same function in both species. For example, both mice and humans have a gene that codes for an Insulin Receptor (*Insr*) like protein and product of these genes has a similar function in mice and humans. In contrast, humans have gene called *CETP*, which is a cholesterol transporter, but mice lack an orthologous *Cetp* gene (9).

Homology – The similarity of the amino acids or nucleotides for a gene or genetic sequence when comparing two different organisms. The degree of homology between two genes is often used as the basis for determining if two genes might be orthologs.

Conserved sequence – The amount a particular sequence, nucleotide or amino acid has been retained in a group of species that share a common ancestor (Figure 1).

Types of mouse models:

Transgenics – A strain carrying a piece of introduced DNA that has been incorporated into their genome via homologous recombination.

Targeted mutants - A strain in which a gene disruption has been introduced or a gene (or part of a gene) has been replaced or duplicated in their genome by homologous recombination between the exogenous (targeting) DNA and the endogenous (target) gene. This includes knockout and knockin lines.

Chemically induced mutants – A mutation induced by chemical means, such as by N-ethyl-N-nitrosourea (ENU). These mice are usually identified by phenotype and the actual gene(s) mutated may or may not be known, based on the stage of the project. In an ENU screen, mutations are random, not directed at a specific gene (10).

Spontaneous Mutants – A mutation that has arisen by natural means in a gene resulting in an identifiable phenotype. The actual gene(s) mutated may or may not be known, and the nature of the mutation may also be unknown. Examples of commonly used spontaneous mouse mutant models in bone include *grey lethal* (*gl*), which has severe osteopetrosis due to a mutation in the *Ostm1* gene (11) and *osteogenesis imperfect mouse (oim)* mouse, which has a mutation in the *Col1a2* gene (12).

Inbred strains – generation of a strain of mice by interbreeding siblings. By definition, a strain is considered inbred after 20 generations of sibling mating and at the point, statistically, 98.7% of the genome should be homozygous for all alleles (13). Commonly used inbred strains can have very different phenotypes when comparing different strains, but all mice of a particular strain are roughly phenotypically the same (Figure 2).

Congenic strains – a strain of mice in which a region of the genome from one strain has been moved onto the genetic background of another strain by at least 10 generations of backcrossing (13). Some times even moving a small genetic region from one strain to another can have profound effects (Figure 3). By definition, moving a knockout allele made on a hybrid, 129 or other background onto a C57BL/6J (B6) background creates a congenic line. The region transferred is NOT just the knockout allele. Several Mb of genetic material are often transferred and these so called passenger genes can impact the phenotype of interest independent of the knocked-out gene of interest (14).

Web Resources:

Mouse Genome Informatics Database
http://www.informatics.jax.org

International Mouse Strain Resource
http://www.findmice.org

International Mouse Phenotyping Consortium
- This includes the Knockout Mouse Project (KOMP)

http://www.mousephenotype.org

Mouse Phenome Database
http://phenome.jax.org

Ensembl Genome Browser
http://www.ensembl.org

UCSC Genome Browser
https://genome.ucsc.edu

Mouse Genomes Project
http://www.sanger.ac.uk/resources/mouse/genomes/

BioGPS
http://biogps.org

KOMP – skeletal
http://bonebase.org

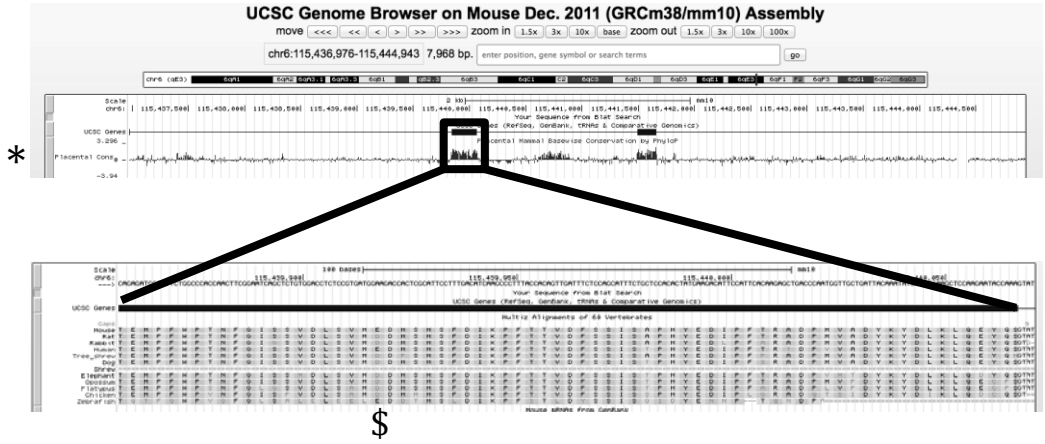

Figure 1. An example of conservation of a gene. The * track is a histogram of the amount a particular base is conserved among placental organisms. While exons are highly conserved, large stretches of non-coding regions maybe too. At the protein level, some amino acids are highly conserved across evolutionary distance and others are not ($). It may be possible to mutate an amino acid that has low conservation with out impacting protein function, depending on what amino acid is substituted in.

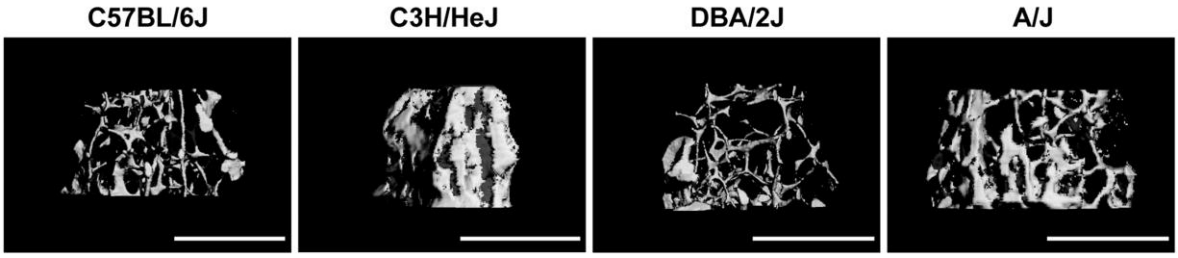

Figure 2. Differences in Bone Volume fraction of the distal femur in four inbred strains of mice. Trabecular bone volume and architecture was measured by uCT in 16 week old female mice (Adapted from (15))

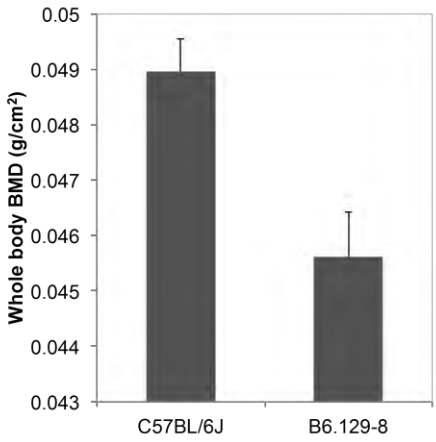

Figure 3. Impact of a congenic region. A small region of chromosome 8 was introgressed from 129S1/SvImJ onto a C57BL/6J background by backcrossing for 10 generations. This region resulted in a lower whole body aBMD in 16 week old female mice, relative to the control (P<0.001).

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Skeletal Tissue Regeneration and Engineering

Frank Luyten, M.D., Ph.D.

Meet-the-Professor Handout:

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Skeletal Biology and Engineering Research Center,
Department of Development and Regeneration
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Belgium

Skeletal Tissue Regeneration and Engineering

Significance of the Topic:

The field of Regenerative Medicine & Tissue Engineering has been growing rapidly with new scientific and technological developments. New treatment approaches have been developed and include cell-based combination products, pushing the boundaries of manufacturing and the regulatory framework. Therefore, clinical developments in this field have evolved into new regulation and legislation for products now categorized as Advance Therapeutic Medicinal Products (ATMPs) in Europe, and Human Cells, Tissues and Cellular and Tissue Based Products (HCTPs) in the US.
The path of clinical development of these novel products, from the bench to the patient and the market, has shown to be quite challenging as they should meet requirements of safety, efficacy and cost-effectiveness as required for any new drug coming to the market in 2015.

In this session, it is the intention to discuss interactively issues and hurdles that may be encountered to translate these innovative therapeutic products and approaches in a safe way into daily clinical practice in a context of worldwide standards of Good Clinical Practice, this for skeletal tissue regeneration and beyond.

Learning Objectives:

- Get acquainted with basic principles of clinical development
- Understand some challenges encountered with the clinical translation of tissue engineered products
- Identify special points to consider in the clinical translation of tissue engineered products

- Recognize some regulatory aspects in the context of regenerative approaches

Clinical Translation of Tissue-Engineered Products

Clinical translation involves a preclinical and clinical dossier.

- **Preclinical studies** include:
    - Some basic research leading to the definition and characterization of the product (identity, dose, potency, purity, safety), in vitro and in vivo
    - small and large animal models of relevance to the problem e.g. cartilage and bone healing
    - studies on mechanism of action, and optimization of the product towards this
    - large animal model(s) representative of the specific patient to be treated with information on feasibility, safety and proof of concept on efficacy.
    - Additional safety studies (bio-distribution, chromosomal aberrations, …)
- **Clinical studies***:* it is highly recommended to contact the regulatory agencies early in the process to discuss the program. It is not possible to copy the process to development of conventional medicinal products to develop and ATMP/HCTP. In addition, most products are applied by specific surgical procedures. Challenges typical for this new class of products include
    - ***Exploratory trials*** to establish safety and proof of concept in a small group of patients. This trial typically involves sequentially escalating doses and looking for biological effects that may reflect the results in the animal models. Feasibility and the early detection of catastrophic safety issues are important goals of these early phase trials.
    - ***Dosing:*** major difference from the logic of conventional drug trials, minimal dosing, threshold and other limitations.
    - ***Defining the comparator:*** what is the currently accepted standard treatment for the specific clinical problem ? Is the standard of care superior to placebo ? Standard of care may not be generally accepted, and may lead to additional research and an additional treatment arm in the clinical studies.
    - ***Randomization:*** how to randomize when decisions are made in the operating theatre ?

- ***Blinding a trial:*** major challenges include the choice of placebo (sham controls ?), and subsequent evaluation and follow up of the patient post surgery.
- ***Standardization of patient care and follow up***: try to minimize the influence of the postoperative management.
- ***Outcome measures:*** patient based outcomes vs so called hard outcomes. Consider surrogate endpoints to be defined, evaluated, validated.

- **Implementation of Clinical Studies:**
  - Developing a Protocol
  - The Investigator Brochure (IB)
  - The Investigational Medicinal Product Dossier (IMPD) and Investigational New Drug application (IND).
  - Informed Consent
  - Case Report Form and Database
  - Institutional Review Board/Ethics Committee
  - Monitoring, Audits and Inspections
  - Sponsor
- **Special Points to consider:**
  - Define the patient: behavior of the product will be influenced by the microenvironment (the patient) and guided by required surgical procedures.
  - Manufacturing Challenges and Upscaling: frequently underestimated leading to products of inferior quality.
  - Exploratory trials in an academic environment and hospital exemption
  - Combination Products require characterization of the starting (raw) materials, but also in depth studies of the combination. Major challenge appears the manufacturing process.

Cases for Discussions:

- Autologous chondrocyte Implantation for the repair of full thickness joint surface lesions of the Knee
- Autologous/Allogenic implants for the repair of non healing long bone defects
  - Injectable cell suspensions

- 3D implants
- Combinations Product: Cells/Growth factors/Scaffolds

References: Clinical Translation by J. Joly et al., in Tissue Engineering: Van Blitterswijk & De Boer, Editors, Elsevier 2015

Treating Osteoporosis in the Elderly: Is the Horse Ever Out of the Barn?

Susan Greenspan, M.D.

Meet-the-Professor: "Treating Osteoporosis in the elderly: Is the Horse Ever Out of the Barn?"

Susan L. Greenspan, MD University of Pittsburgh, Pittsburgh, PA, USA

Significance of the Topic: Osteoporosis is morbid, common and costly especially in the elderly. Each year, 250,000 American suffer from a hip fracture and up to 20% die in the subsequent year, 25% are institutionalized and less than 50% fully recover.[1, 2] The impact of osteoporosis is even more dramatic for the 2 million American's that reside in long-term care (LTC). The prevalence of osteoporosis may be up to 85%.[3] These residents with fractures are hospitalized more often and have 2-3 times the risk for future fracture. A recent review of 60,000 nursing home patients reported that following a hip fracture, 36% died and 53% died or developed new total dependence in 6 months.[4]

Despite these statistics, only 5-36% of LTC residents with osteoporosis are treated.[5-8] The primary reason is the lack of data in this population. The secondary objections are the harmful side effects, unknown side effects, and extra staff time needed for drug administration.

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

1. Understand the results of osteoporosis therapeutic trials that have been performed in healthy community dwelling elderly.
2. Evaluate the data that are available from osteoporosis therapeutic trails in frail elderly.
3. Examine the potential causes for failure of therapy in frail elderly.

Outline:

1. Osteoporosis clinical trial data available in healthy community dwelling elderly women.
    a. Case 1
2. Osteoporosis clinical trial data available in frail elderly.
    a. Case 2
3. Skeletal and non-skeletal factors that may prevent a fracture reduction benefit.

Case 1: 85 year old female, living in the community, comes to see her PCP following her DXA exam. She has heard that her "teeth will fall out and her bones with break" with the osteoporosis drugs. She lives alone, drives, walks her dog daily and has not had a fracture. She has lost "some" height. She takes the "pink" multivitamin that has some calcium and vitamin D. She likes cheese, has milk with cereal and has yogurt daily. The DXA shows a femoral neck T-score= -1.2, spine T-score= -1.7
Medical problems: elevated cholesterol, arthritis in her back.
Meds: "pink" multivitamin, glucosamine chondroitin, Lipitor, fish oil, vitamin C
Weight: 150 lbs., height: 63", BMI: 26.6

Questions:

1. What is her diagnosis and risk?
2. What would you check?
3. Would you treat her?

Discussion:

Risk Assessment: FRAX Percent risk of hip fracture in 10 years (NOF suggest treatment if ≥ 3%)

Femoral Neck T-score	75 years old	80 years old	85 years old	90 years old
-1.1	1.6	2.4	2.9	2.6
-1.2	1.8	2.6	3.0	2.8
-1.3	1.9	2.8	3.2	2.9
-1.4	2.1	3.0	3.5	3.1
-1.5	2.3	3.3	3.7	3.3
-1.6	2.5			
-1.9	3.1			

Studies in healthy elderly: Post-hoc comparison of healthy women in the large clinical trials demonstrated no difference in outcomes or safety in those below and above 75 years of age.

1. Zoledronic acid[9]
2. Teriparatide[10]
3. Denosumab[11]

Case 2: 85 year old female, lives in a long-term care facility. She gets her own meals. She uses a cane for short distances and a walker to get to the dining room. She had "just a little fall" last year but no broken bones. She has fractured her ankle in the past and since it did not heal well, she uses the walker. She has lost roughly 3 inches in height. She just had a DXA but her daughter told her she read on the internet that "those drugs will kill you and they don't help anyway".

Medical problems: Diabetes, HBP, elevated cholesterol, CVD s/p CABG, GERD, hypothyroid, faller, arthritis.

Meds: metformin, HCTZ, lisinopril, atorvastatin, famotidine, calcium plus vitamin D, B vitamin complex, baby ASA, L-thyroxine

Weight: 150 lbs., height: 63", BMI: 26.6
DXA that shows a femoral neck T-score= -1.2, spine T-score= -1.7
FRAX: ?

Questions:

1. What is her diagnosis and risk?
2. What would you check?
3. Would you treat her?

Discussion:

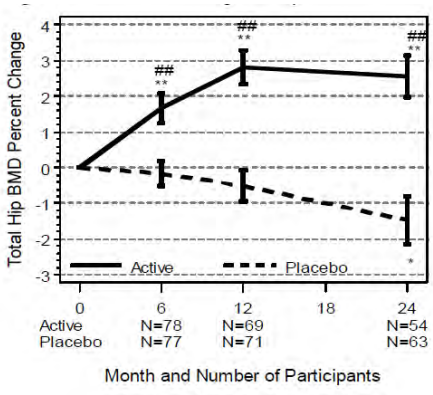
Studies in residents in LTC:

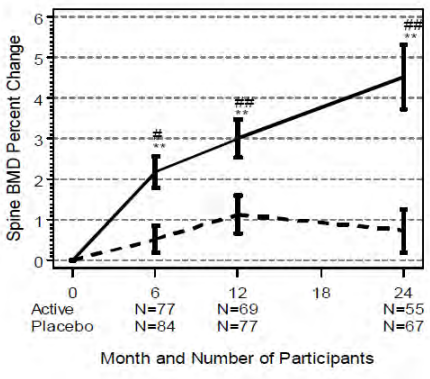
1. Alendronate 10 mg daily versus placebo over 2 years: Hip and spine BMD improved (3.2% and 4.2% respectively) and tolerated well.[12] Although in LTC, participants all relatively healthy, cognitively intact, able to take a *daily pill* and wait 30 minutes before eating.

2. Risedronate HIP Fracture Study: Risedronate 35 mg daily versus placebo in community dwelling elderly that included some in LTC.[13] Hip fracture reduction in those less than 80 years old but not in those over age 80 years old (enrolled based on risk factors).

3. Zoledronic acid 5 mg IV versus placebo given once over 2 years in residents in LTC facilities (skilled and assisted living).[14] Included those with cognitive impairment, limited mobility, frailty, reduced ADLs, poly-pharmacy (including steroids and antiseizure meds). At baseline more with DM, frailty, antiseizure meds, falls in active treatment group.

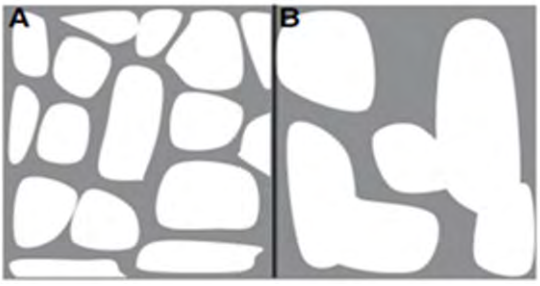
Results: Hip and spine BMD improved, biochemical markers of bone turnover were reduced.[14] Increase in falls in active treatment group that disappeared if adjusted for baseline frailty. No difference in fractures and no clear trend.





Skeletal and non-skeletal factors that may prevent a fracture reduction benefit:

1. Poor trabecular connectivity.[15]



Both A and B have equal bone mass. (A) has thinner trabeculae with more connections and is biomechanically stronger. (B) has thicker trabeculae with fewer connections and is weaker.

2. Impaired cortical or trabecular microstructure.
3. Minimal mobility (few falls due to immobility) and decreased weight bearing.
4. Short life-expectancy.
5. Other?

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Drug Holidays:
When and How?

Robert Josse, M.D.

DRUG HOLIDAYS : WHEN AND HOW

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Objectives:

1. Review the effect of the different anti-osteoporosis therapies
2. Understand the effect of discontinuation of therapy
3. Appreciate the adverse events associated with long term anti-resorptive therapy
4. Review the clinical recommendations for drug holidays

Osteoporosis and the resulting fractures is a major worldwide health burden particularly in the United States and Canada. In North America, osteoporosis accounts for approximately 80% of all fractures. Pain, reduced mobility and long term disability are common sequelae of osteoporotic fragility fractures. Those individuals who have suffered a fracture are at greater risk of a subsequent fracture compared to those with no such history. Moreover, hip and vertebral fractures can be particularly devastating with increased mortality on the order of 20-30% within one year of a hip fracture. Prevention of osteoporotic fragility fractures and aggressive treatment once they have occurred is paramount importance. Therapeutic options supported by high level evidence include aminobisphosphonates, selective estrogen receptor modulators (SERM), hormone therapy, denosumab and the bone formation stimulating therapy, teriparatide. This discussion will focus on the aminobisphosphonates considered to be first line therapy for the treatment of most patients with osteoporosis and fragility fractures. These aminobisphosphonates might also increase survival in ways at least partially independent of their contribution to the decrease in fracture incidence. The anti-fracture efficacy and relative safety of the aminobisphosphonates has been well established in randomized, placebo controlled phase III regulatory trials primarily in postmenopausal women. They are generally of three years duration although some trials have been extended up to five years and beyond (some presenting ten year data). However, clinical trial data for the long term use of aminobisphosphonates in randomized, placebo controlled trials are not available beyond five years of treatment. Moreover, long term therapy generally beyond five years has raised concerns that this prolonged use of these drugs might increase the risk of rare but serious adverse events. This concern has spurned the concept of so-called “drug holidays”, whereby some patients after a variable period of time would have their drug therapy (usually temporarily) discontinued. The concept of a drug holiday for a chronic disease is an unusual one. One does not think of stopping therapy for hypertension, diabetes, hypercholesterolemia, etc., although of course doses of different drugs can be modified.

The aminobisphosphonates, still the principal treatments for osteoporosis, bind to the hydroxyapatite in bone and it can remain there for years. Skeletal binding affinity is greatest with zoledronate, then alendronate, ibandronate and finally least binding with risedronate. With continued drug usage, the amount of the agent deposited in bone continues to accumulate. Once the therapy is discontinued, the drug resident in bone will be slowly released during bone turnover and will continue to provide low concentrations of bisphosphonate that can either be reuptaken into bone and have modest anti-resorptive effect or will be excreted. This is a unique feature of the bisphosphonates whose offset of action is not immediate when the drug is discontinued as opposed to other therapies such as denosumab, SERMs, hormone therapy and teriparatide. One can envisage a positive and negative potential benefits from such a long time residence in bone, i.e., on the beneficial positive side following discontinuation of the bisphosphonate the anti-resorptive and anti-fracture effect may continue for a variable period of time, albeit, with a somewhat lesser efficacy. On the other hand, if adverse effects are associated with the presence of the drug and it lingers in bone for a long time, some of these adverse effects may take a long time to resolve. The major adverse events that have developed with the aminobisphosphonates are osteonecrosis of the jaw (ONJ), atypical femoral fractures (AFF), atrial fibrillation, esophagitis.

In keeping with risk assessment in many chronic disorders, osteoporosis has categorized patients into low, moderate and high risk for (fragility) fracture. Low risk patients (less than 10% ten year fracture probability) do not need specific anti-osteoporosis therapy. Moderate risk patients (10-20% ten year fracture probability) may well be treated and these patients are usually the candidates for a potential drug holiday after three to five years of therapy. Patients at high fragility fracture risk and especially those who have already had fragility fractures are not generally candidates for drug holidays although alternative therapies may be offered to them.

This MTP will discuss the pros and cons of drug holidays in detail.

Antifracture Benefits of Bisphosphonates for Treatment of Postmenopausal Osteoporosis

		Absolute Fracture Risk Reduction			Relative Fracture Risk Reduction			Number Needed to Treat to Prevent One Fracture		
Medication (Clinical Trial)	Y	Vert fx	Non-vert fx	Hip fx	Vert fx	Non-vert fx	Hip fx	Vert fx	Non-vert fx	Hip fx
Alendronate (FIT I)[4]	3	7.1%	2.8%	1.1%	47.1%	18.9%	50.8%	14	36	90
Alendronate (FIT II)[5]	3	1.7%	1.5%	0.2%	44.3%	11.1%	20.7%	60	68	447
Risedronate (VERT NA)[6]†	3	5.0%	3.2%	0.4%	30.7%	38.1%	19.7%	20	31	276
Risedronate (VERT MN)[7]†	3	10.9%	5.1%	0.5%	37.6%	31.9%	18.2%	9	20	203
Risedronate (HIP)[8]	3	NA	1.8%	1.1%	NA	16.1%	28.2%	NA	56	91
Zoledronic acid (HORIZON PFT)[9]	3	7.6%	2.7%	1.1%	70.0%	25.2%	44.0%	13	37	91
Zoledronic acid (HORIZON RFT)[10]	3	NA	3.1%	1.5%	NA	29.0%	42.9%	NA	32	67
Ibandronate (BONE)[11]†	3	4.9%	−0.9%	NA	62%	−11%	NA	20	NA	NA
Alendronate (Men)[12]	2	5.0%	NA	NA	62%	NA	NA	9	NA	NA
Risedronate (GIO)[13]	1	11.0%	NA	NA	70%	NA	NA	9	NA	NA

FIT = Fracture Intervention Trial; GIO = glucocorticoid induced osteoporosis; HIP = Hip Intervention Program; hip fx = hip fracture; NA = not assessed; nonvert fx = nonvertebral fracture; VERT = Vertebral Efficacy with Risedronate Therapy study; vert fx = vertebral fracture; Y = average years of follow-up.

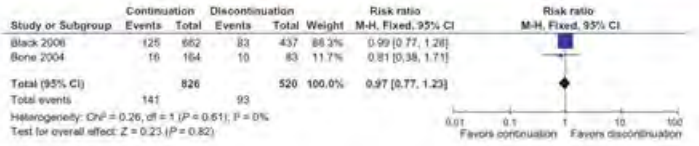
*Widely ranging bone mineral density, age, and previous fracture status in the study populations make direct comparisons of therapies impossible. This table provides a broad overview of the general benefits of the therapies in the respective clinical trial population. For more information about the particular populations studied for each therapy, please refer to the referenced publication.

†Vertebral fracture incidence rate estimated from proportional hazards models.

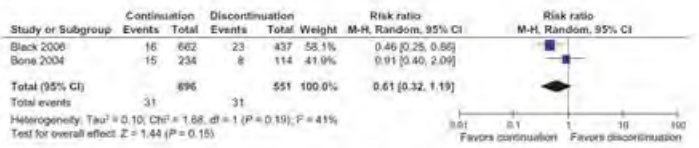
McClung MR et al. *Amer J Med* 2013;126:13-20.

Fracture Risk in Patients who continue bisphospohonate therapy after 5 years vs those who discontinue after 5 years

a. Clinical nonvertebral fractures



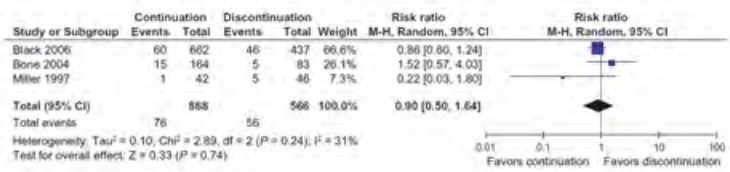
b. Clinical vertebral fractures



Fraser et al. Therapeutics and Clinical Risk Management 2011:7;57–166

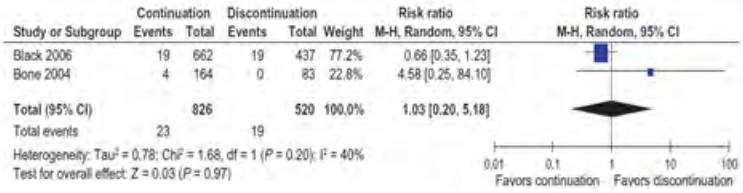
Fracture Risk in Patients who continue bisphosphonate therapy after 5 years vs those who discontinue after 5 years

c. Morphometric vertebral fractures

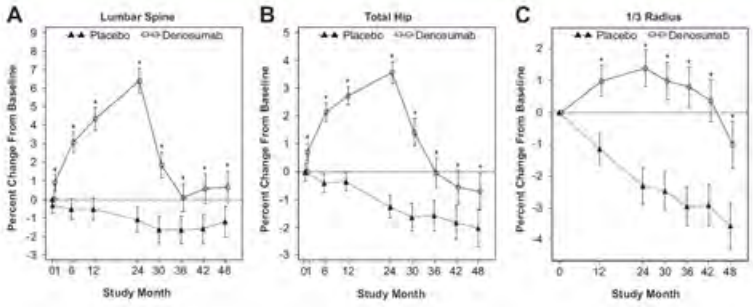


Mortality in Patients who continue bisphosphonate therapy after 5 years vs those who discontinue after 5 years

d. Mortality

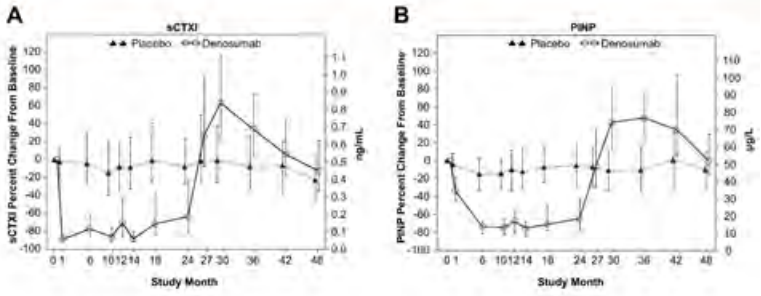


Effects of Denosumab Treatment and Discontinuation on BMD in PM Women with Low Bone Mass



Bone et al. J Clin Endocrinol Metab 2011; 96 (4): 972–980

Effects of Denosumab Treatment and Discontinuation on Bone Turnover Markers in PM Women with Low Bone Mass



Bone et al. J Clin Endocrinol Metab 2011; 96 (4): 972–980

Fracture Prediction After Discontinuation of 4 to 5 Years of Alendronate Therapy

- We found that after discontinuation of 4 to 5 years of alendronate therapy, 22% of women experience fracture during the subsequent 5 years.
- Older age and lower hip BMD at the time of discontinuation strongly predict fracture risk after discontinuation, but neither 1-year change in hip BMD nor 1- or 3-year change in NTX or BAP are associated with the risk of fracture after discontinuation.
- Women with greater total hip bone loss 2 or 3 years after discontinuation may be at increased risk of fracture, but these results need to be confirmed in other studies before routine measurement of BMD after discontinuation of alendronate therapy can be recommended.
- In the meantime, short-term monitoring with BMD, BAP, or NTX after discontinuation of 4 to 5 years of alendronate therapy does not appear to improve fracture prediction

Bauer et al. JAMA Intern Med. 2014 July; 174(7): 1126–1134

For Patients on Long-term Anti-resorptive Treatment, What Are Some Things to Consider?

Consider	Supporting Evidence	Bisphosphonates	Denosumab
Will it still be safe?	Long-term data	AE profile at 5-6 years shows no significant difference in AEs from placebo[1-3]	AE profile through to 8 years similar to that seen in the initial pivotal trial[4]
What happens if my patient stops therapy?	Reversibility	Residual effect; depends on type & length of treatment[7]	Fully reversible[7]
	Duration of action	Slow offset of action[7]	Relatively rapid offset of action[7,8]
	Clearance	Renal; detectable in urine weeks, months or years after stopping[7]	Catabolized in reticuloendothelial system[7]; almost fully cleared by 6 months[8]

AE=Adverse event
BP=Bisphosphonate

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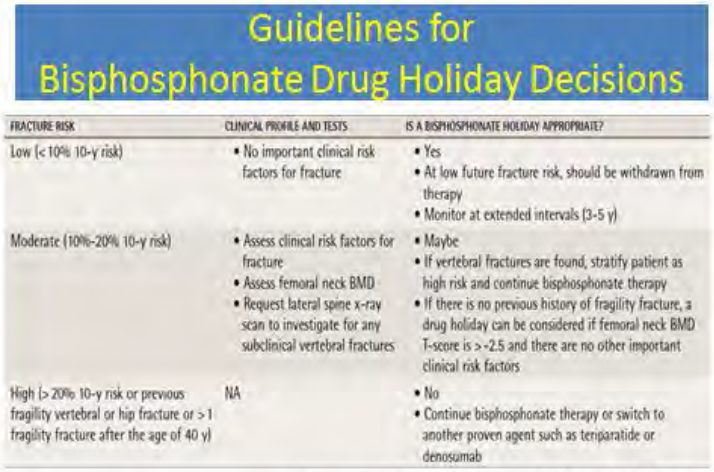
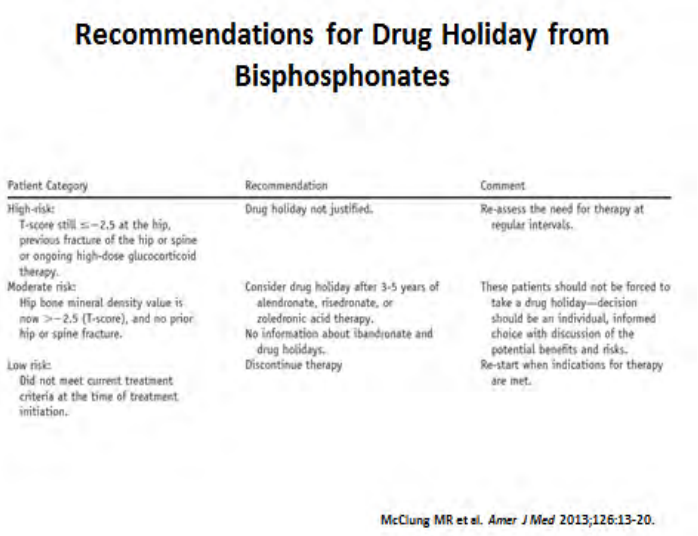


Guidelines for Bisphosphonate Drug Holiday Decisions

FRACTURE RISK	CLINICAL PROFILE AND TESTS	IS A BISPHOSPHONATE HOLIDAY APPROPRIATE?
Low (< 10% 10-y risk)	• No important clinical risk factors for fracture	• Yes • At low future fracture risk, should be withdrawn from therapy • Monitor at extended intervals (3-5 y)
Moderate (10%-20% 10-y risk)	• Assess clinical risk factors for fracture • Assess femoral neck BMD • Request lateral spine x-ray scan to investigate for any subclinical vertebral fractures	• Maybe • If vertebral fractures are found, stratify patient as high risk and continue bisphosphonate therapy • If there is no previous history of fragility fracture, a drug holiday can be considered if femoral neck BMD T-score is > -2.5 and there are no other important clinical risk factors
High (> 20% 10-y risk or previous fragility vertebral or hip fracture or > 1 fragility fracture after the age of 40 y)	NA	• No • Continue bisphosphonate therapy or switch to another proven agent such as teriparatide or denosumab

Brown et al. Can Fam Physician 2014; vol 60: 324-333



Recommendations for Drug Holiday from Bisphosphonates

Patient Category	Recommendation	Comment
High-risk: T-score still ≤ −2.5 at the hip, previous fracture of the hip or spine or ongoing high-dose glucocorticoid therapy.	Drug holiday not justified.	Re-assess the need for therapy at regular intervals.
Moderate risk: Hip bone mineral density value is now ≥ −2.5 (T-score), and no prior hip or spine fracture.	Consider drug holiday after 3-5 years of alendronate, risedronate, or zoledronic acid therapy. No information about ibandronate and drug holidays. Discontinue therapy	These patients should not be forced to take a drug holiday—decision should be an individual, informed choice with discussion of the potential benefits and risks. Re-start when indications for therapy are met.
Low risk: Did not meet current treatment criteria at the time of treatment initiation.		

McClung MR et al. *Amer J Med* 2013;126:13-20.

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Influences on Adaptation to Mechanical Loading

Marjolein van der Meulen, Ph.D.

2015 ASBMR Annual Meeting
Meet-the-Professor: Influences on Adaptation to Mechanical Loading

Presenter:

Marjolein C. H. van der Meulen, PhD
James M. & Marsha McCormick Chair of Biomedical Engineering
Swanson Professor of Biomedical Engineering
Nancy E. and Peter C. Meinig School of Biomedical Engineering
Sibley School of Mechanical & Aerospace Engineering

Significance of the Topic:

Skeletal mass and architecture evolve in response to the mechanical stimuli experienced during the lifetime of the individual, producing a structure that is uniquely suited to bear in vivo functional loads. With aging and the associated hormonal and metabolic changes, however, skeletal bone mass and load-bearing capacity diminish. These changes frequently result in fractures in the aging population during normal function, primarily at corticocancellous sites such as the femoral neck and spine.

Dynamic mechanical loading is an anabolic agent that inhibits bone loss and maintains bone mass and strength following sex hormone deficiency and aging. Adaptation to in vivo loads is fundamental to many biological processes of musculoskeletal biology. Our knowledge of the effects of defined mechanical loads on bone formation is based primarily on cortical bone in healthy animals (Figure 1). The proliferation of in vivo mechanical loading methods using mouse models has yielded important insights into genetic and molecular mechanisms relating to many aspects of bone biology. Several signaling pathways have been identified that regulate mechanotransduction, and more will be learned regarding cellular and molecular control as genetic tools become more prevalent and targeted.

Most loading models are not directly relevant to corticocancellous sites in hormonally-compromised aging individuals who experience fractures clinically, yet these conditions are precisely where mechanical loading holds promise as a treatment. Using in vivo loading of the mouse tibia, we have shown that the anabolic response is greater at cancellous than cortical sites, suggesting that these tissue envelopes and their responses need to be differentiated.

Learning Objectives:

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

- understand the unique adaptive capacity of musculoskeletal tissues to their mechanical environment
- recognize that differences are present in the responses of cortical and cancellous bone tissue
- appreciate the range of factors that influence in vivo bone functional adaptation at the tissue level

Suggested references and relevant reading:

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- Holguin N, Brodt MD, Sanchez ME, Kotiya AA, Silva MJ (2013) Adaptation of tibial structure and strength to axial compression depends on loading history in both C57BL/6 and BALB/c mice. Calcif Tissue Int 93:211-21
- Melville KM, Kelly NH, Surita G, Buchalter DB, Schimenti JC, Main RP, Ross FP, van der Meulen MCH (2015) Deletion of ER-alpha in osteoblast-lineage cells reduces bone mass and enhances bone's response to mechanical loading in female, but not male, mice. J Bone Miner Res 30:1468-80
- Melville KM, Robling AG, van der Meulen MCH (2015) In vivo axial loading of the mouse tibia. Meth Mol Biol 1228: 99-115 (methods)
- Niziolek PJ, Warman ML, Robling AG (2012) Mechanotransduction in bone tissue: The A214V and G171V mutations in Lrp5 enhance load-induced osteogenesis in a surface-selective manner. Bone 51:459-65
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Figures:

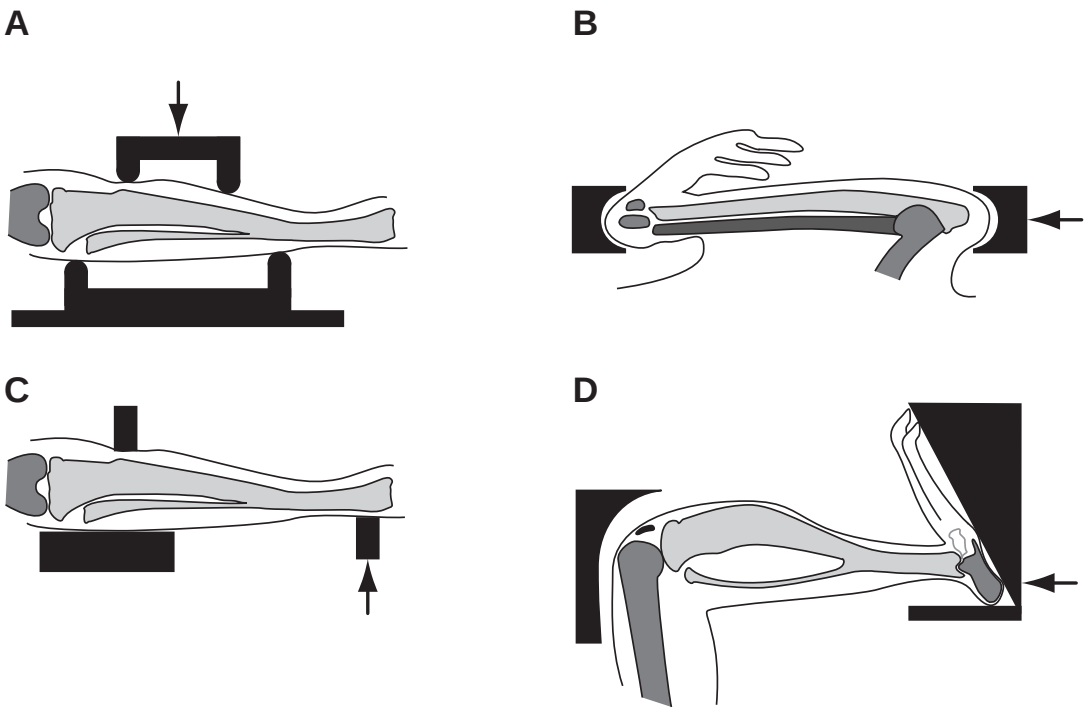

Figure 1: In vivo models to apply controlled loading to cortical bone noninvasively to study bone adaptation: (A) four-point bending of the tibia, (B) compression of the ulna, (C) bending of the tibia and (D) compression of the tibia.

Management of Hypoparathyroidism

Dolores Shoback, M.D.

MEET THE PROFESSOR - MANAGEMENT OF HYPOPARATHYROIDISM 2015

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UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
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Significance of the Topic

Hypoparathyroidism is an uncommon endocrine disorder that can be challenging to diagnose and treat successfully. The disorder most commonly presents after surgery on the thyroid or parathyroid glands or the larynx. Fully 75% of all cases of hypoparathyroidism are postsurgical with approximately 70% of these cases occurring in women. The remaining 25% of patients with this disorder have the condition on the basis of a genetic defect or an acquired disorder that destroys glandular function, or the disorder can be idiopathic or unexplained. In the USA and European Union, hypoparathyroidism is designated an orphan disease. Just 60,000-70,000 patients are estimated as affected by hypoparathyroidism in the USA at the present time. Given that small number of individuals, large studies revealing the natural history and randomized placebo-controlled trials testing the efficacy and safety of different treatment regimens are rare indeed. Thus, until recently, the diagnosis and management of this disorder was largely driven by expert opinion with few trials to guide management and no carefully studied treatment regimens in place.

Considerable interest in the use of full-length parathyroid hormone (PTH) [recombinant human rhPTH (1-84)] and its fragments [PTH(1-34)] has developed in the treatment, indeed in the replacement of the hormone, for this disease state over the last 5 years. Clinical trials and research in this area culminated in the approval (in 1/2015) by the US Food and Drug Administration of rhPTH(1-84) for patients with hypoparathyroidism. This has led to new opportunities to address control of the disorder from the biochemical, systemic physiologic, and quality of life standpoints because new treatment can be introduced and examined for efficacy. In addition, new therapeutic strategies offer clinicians an opportunity to establish better monitoring schedules and take a fresh look at diagnostic and therapeutic benchmarks to improve the management of the disorder.

Learning Objectives

This session will review the diagnosis and management of hypoparathyroidism incorporating results of studies with rhPTH(1-84) in the context of standard disease management with calcium supplements, activated vitamin D (calcitriol) or its analogues, vitamin D3 and magnesium supplements, dietary recommendations and thiazide diuretics and will emphasize the monitoring for safety and long-term complications. By attending this session, the clinician should gain the following.

(1) an understanding of the differential diagnosis of the disorder of hypoparathyroidism and what constitute the risks for the condition in the postoperative setting
(2) a framework for making the diagnosis and monitoring patients with the condition chronically
(3) an approach to treatment and an understanding of the role of medications, supplements, and diet in controlling symptoms and enhancing quality of life in an effort to limit complications
(4) an appreciation of the data available on the use, efficacy, and possible benefits and adverse effects of treatment with rhPTH(1-84) and other PTH analogues based on the clinical trials conducted in patients with hypoparathyroidism

Points of Interest

Differential Diagnosis of Hypocalcemia

- *HYPOPARATHYROIDISM*
- Pseudohypoparathyroidism
- Vitamin D deficiency, resistance, or inadequate 1-hydroxylation
- *Mg depletion or excess*
- Chronic renal failure
- Miscellaneous: pancreatitis, acute hyperphospatemia, tumor lysis, crush injury, rhabdomyolysis, IV bisphosphonate, denosumab therapy (especially in CKD), rapid transfusion of citrated blood, "hungry bone", osteoblastic metastases, *severe (ICU) illness*

italics = low or inappropriately normal PTH levels → suggestive or diagnostic of hypoparathyroidism

Causes of Hypoparathyroidism

- Postsurgical (thyroid, parathyroid, laryngeal)
- Functional: Mg depletion, hyper Mg
- Constitutive activation of CaSRs
- Heterozygous gain of function mutation in CaSR or G alpha 11
- Acquired (activating) CaSR antibodies
- Autoimmune
    - Isolated
    - Autoimmune polyendocrine syndrome 1
- Other GENETIC causes
    - GCM2 mutations
    - PTH mutations
    - Syndromes
        - DiGeorge sequence/CATCH22
        - HDR (hypoPT, renal anomalies, deafness)
        - Kenny-Caffey
        - Sanjad-Sakati
        - Kearns-Sayre and mitochondrial DNA mutations

Destructive: hemochromatosis, thalassemia, metastatic tumor, ^{131}I therapy

Autoimmune Presentations

Age at onset	HP + other associated disorders	Genetics	Auto-AB's	Syndrome
5-20 years	Mucocutaneous candidiasis, Addison's disease	*AIRE*	NALP5, IFN-omega, TPH, AADC, TH, ACA, 21OH	APS-1
Adult	Thyroid autoimmune dis	HLA (?)	CaSR Abs (?)	APS-3
Adult	Other autoimmune disease (not thyroid, AD, CMC)	HLA (?)	CaSR Abs (?)	APS-4
Adult	NONE	HLA (?)	CaSR Abs (?)	Isolated autoimmune HP

Therapeutic approaches

Calcium

- Ca supplements: this is given in divided doses usually 3-4 times per day, initiating at 250 mg elemental Ca per dose (and then adjust upward)
- Use Ca citrate – if achlorhydria or pt is taking a proton pump inhibitor
- Ca carbonate should be used with meals (0.5 – 1.0 G elemental Ca tid)
- Should separate Ca supplements from T4 replacement by at least 60 min
- Count Ca in diet – if the intake is reliable and consistent

Calcitriol

- 0.25 mcg twice daily is the usual starting doses

- 1 alpha calcidol – usual dose about twice that of calcitriol

Magnesium
- Replace Mg if low
- Particularly of concern in patients with *Casr* mutations)

Vitamin D
- Correct low 25 OH vitamin D levels (vitamin D3) to acceptable level (>20 ng/ml or 30 ng/ml)

Thiazide diuretics
- Added after U-Ca checked (to lower U-Ca); should be combined with low salt diet
- Hydrochlorothiazide (25 to 100 mg/day)
- Chlorthalidone (longer duration of action)

Hyperphosphatemia, high Ca x P
- low Phos diet can be considered (no data published)
- P-binders (no data)

9 Studies of PTH (1-34) Treatment of Adults and Children

(1) Winer K et al, 1996: 10 adults; crossover trial of 10 wks/arm using once-daily PTH 1-34 vs twice-daily calcitriol (+Ca); major finding → **PTH DECREASED U-CA**

(2) Winer K et al, 1998: 17 adults; once vs twice-daily treatment with PTH 1-34 in 28 wk crossover study → **normalized S-Ca, S-Mg better with twice daily**

(3) Winer K et al, 2003: 27 adults; twice daily PTH 1-34 vs calcitriol (+Ca), 3 yrs; **URINE CA – better maintained with PTH injections**

(4) Winer K et al, 2008: 14 children; once vs twice daily PTH injections for 28 wks; **TWICE DAILY MORE EFFICIENT with s-Ca and s-Mg nl and U-Ca nl (second half of the day)**

(5) Winer K et al, 2010: 12 children; twice daily PTH 1-34 vs calcitriol (+Ca), 3 yrs → **STABLE BIOCHEMISTRIES and nl growth**

(6) Winer K et al, 2012: 8 adults; crossover study of PTH 1-34 via pump vs 2 daily injections X 3 mos; **PUMP → 50% REDUCTION in U-CA** (vs injections)

(7) Winer K et al, 2014: 12 pts (age 4-20 yrs); In severe congenital HP – compared twice-daily inj vs pump, crossover, 13 wks; → **near nl S-Ca, nl U-Ca with pump**

(8) Gafni R et al, 2012: 5 patients (children and adults); open label (2 or 3 X daily), 18 mos, s-Ca normalized off activated vitamin D; increased bone turnover by markers and by biopsies

(9) Santonati A et al, 2014: 42 adults; 6 mos open-label study; twice-daily injections (20 mcg); Ca & calcitriol supplements decreased substantially, **QOL improved 8/8 domains**; no significant change U-CA

3 Studies with PTH 1-84

• Rubin, Cusano, Bilezikian, and colleagues –
Open-label trial (no placebo group), pts treated QOD with PTH 1-84 (100 ug/dose); 4-yr data in 27 pts → stable S-Ca, less supplements, improved QOL at 5 yrs

• Sikjaer, Rejnmark, Mosekilde, and colleagues -
Randomized 62 pts to PBO vs daily PTH (1-84) at 100 mcg/day as add-on to CA/vitamin D analogue therapy (double-blind) → more hypercalcemia, no effects on QOL, muscle

• Mannstadt and colleagues (pivotal phase III trial)
Randomized, double-blind, placebo-controlled trial for 24 wks in which 134 adults with hypoparathyroidism were randomized; met 1^0 endpoint – maintain S-Ca, reduce Ca, 1,25 D supplements

Monitoring
- Serum Ca, phosphate – every 3 or 6 months or more frequently during medication changes
- Serum creatinine – every 12 months or more frequently as appropriate
- 24 hour urinary Ca, creatinine – every 12 months or more frequently if appropriate
- Renal ultrasound – every 12 months

Cases with Questions – see PDF

Disclosures
The speaker was a PI on the industry sponsored phase 3 trial (by NPS) on the safety and efficacy of rhPTH(1-84) in hypoparathyroidism, and the institutional research foundation received grants to conduct the trial. The speaker has received honoraria (NPS). FDA non-approved uses of the approved medication PTH(1-34) or teriparatide will be discussed during this activity.

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PTH (1-34)

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PTH(1-84)

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Complications

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Neuronal Regulation of Bone Mass

Gerard Karsenty, M.D., Ph.D.

ASBMR 2015 Meet-the-Professor

The Neuronal Regulation of Bone Mass

Gerard Karsenty M.D., Ph.D.

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Significance of the Topic:

One of the most unexpected developments in bone physiology of the last fifteen years has been the demonstration that there is a central control of bone mass. The discovery of this mode of regulation of bone mass came while testing a different hypothesis of endocrinology, namely that there may be a coordinated control of energy metabolism, bone growth and reproduction. The initial testing of this hypothesis revealed that leptin is such a powerful inhibitor of bone mass accrual in mice and humans that its deficiency overcomes the deleterious effect of gonadal failure on the skeleton. Not surprisingly given the way this hormone influences all the physiological functions it targets, this led to the discovery of the central control of bone mass.

The different steps used by leptin signaling to regulate bone mass will be reviewed with a particular emphasis on two aspects.

A. Based on genetic evidence the central regulation of bone mass accrual is one of the most powerful that exists.

B. Serotonergic neurons and serotonin synthesis are a point of convergence of the two main hormones that have been shown so far to contribute to the coordinated regulation of bone growth, energy metabolism and reproduction namely leptin and osteocalcin.

Learning Objectives:

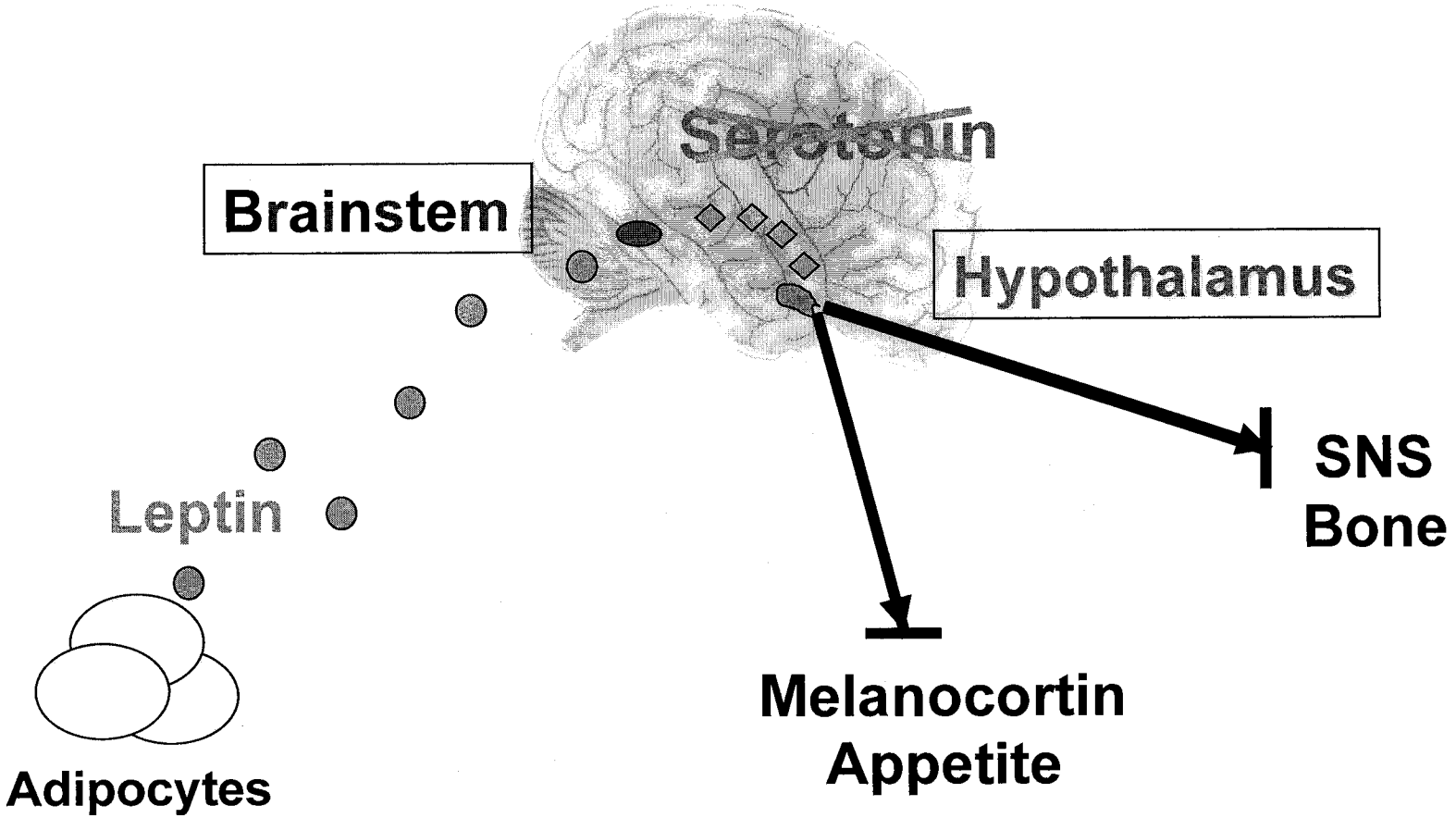
As a result of participating in this session, attendees should be able to:

1. Appreciate the extent of the influence of leptin on the control of bone mass.
2. Realize that leptin regulation of bone mass occurs through a series of inhibitory steps.
3. Appreciate the importance of the sympathetic regulation of bone mass.
4. Appreciate the importance of serotonin synthesis as a focal point on which two hormones converge.

Outline of the Presentation:

- The consequences of the absence of leptin signaling on bone mass accrual.
- What it means in terms of general physiology, given the other functions of leptin.
- The consequence of the absence of leptin signaling in osteoblasts or bone mass accrual.
- The road map used by leptin in the brain to inhibit bone mass accrual.
- The identity and functions of the peripheral mediators of leptin signaling in the brain.
- The importance of serotonergic neurons as a focal point of leptin and osteocalcin signaling.
- The importance of the regulation of bone mass among all functions of leptin.

Coordinated regulation of bone growth and energy intake



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Signaling in Bone Remodeling

Xu Cao, Ph.D.

Handout Material
October 11, 2015 from 11:30am-12:30pm.

Signaling in Bone Remodeling

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Bone is continuously remodeled in the adult skeleton by osteoclast resorption coupled with osteoblast formation. Bone resorption and formation do not occur randomly along the bone surface. Rather, they occur at specific anatomical sites and follow a well-defined sequence of events. During bone resorption, latent TGF-β previously buried in the bone matrix is released and activated to recruit marrow mesenchymal stem cells (MSCs) for subsequent bone formation (1), while IGF-1 released from the bone matrix stimulates osteoblastic differentiation of recruited MSCs by activation of rapamycin (mTOR) signaling pathway (2), thus the spatiotemporal release and activation of matrix TGF-β and IGF-I coupling bone resorption with formation during bone remodeling (3,4) (**Figure 1**).

Dysregulation of TGF-β alters MSCs recruitment and their fate, uncoupling bone remodeling and causing various skeletal disorders. Loss of site-directed recruitment of MSCs are associated with multiple skeletal disorders. For example, Camurati-Engelmann disease (CED) caused by mutations in *TGFB1* that result in premature activation of TGF-β1 disrupts recruitment of MSCs and uncouples bone remodeling (1). Uncoupled bone remodeling also accompanies the onset of osteoarthritis. TGF-β1 is activated in subchondral bone in response to altered mechanical loading in mouse osteoarthritis models (5). High levels of active TGF-β1 induces formation of nestin$^+$ MSC clusters via activation of ALK5-SMAD2/3. MSCs undergoes osteoblast differentiation in these clusters, leading to formation of marrow osteoid islets at the onset of osteoarthritis. Knockout TGFβ type II receptor specifically in nestin$^+$ MSCs by inducible nestin-CreER inhibits migration of nestin$^+$ MSCs and attenuates OA progression. Thus, modulation of TGF-β signaling in recruitment of MSCs may offer potential therapeutic approaches for skeletal diseases (6,7).

A recent study revealed that a specific vessel subtype, CD31hiEmcnhi vessels, couple angiogenesis and osteogenesis. Angiogenesis is essential for bone remodeling and coupled with bone formation in these processes for proper bone homeostasis. We find that preosteoclasts secrete PDGF-BB during bone modeling and remodeling to induce angiogenesis and thus proper osteogenesis (8). Depletion of PDGF-BB in the TRAP+ cell lineage reduces angiogenesis in the bone marrow and periosteum with reduced bone formation. Inhibition of cathepsin K (CTSK) effectively increases preosteoclast numbers and levels of PDGF-BB, stimulating CD31hiEmcnhi vessel and bone formation in OVX mice. PDGF-BB stimulates migration and angiogenesis of endothelia progenitor cells (EPCs) and MSCs. Therefore, preosteoclasts secrete PDGF-BB to recruit EPCs and MSCs to promote angiogenesis to support osteogenesis during bone remodeling. PDGF-BB secreted by preosteoclasts determines the spatiotemporal vessel formation needed for the subsequent bone resorption and new bone formation during bone remodeling (**Figure 2**).

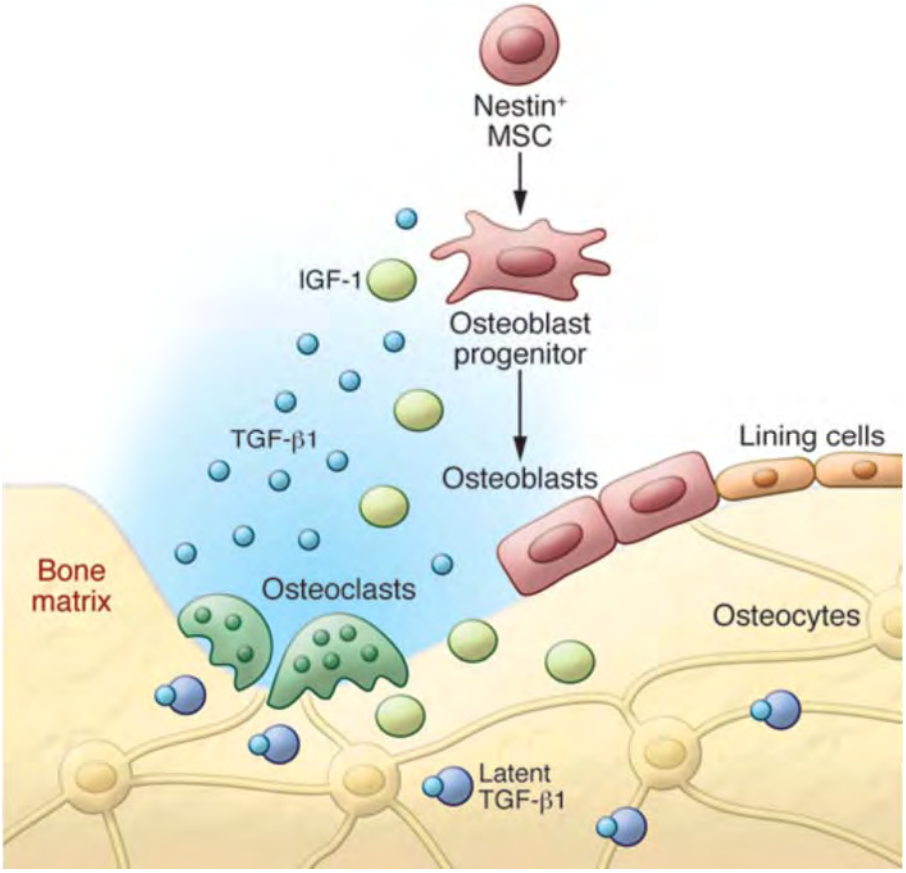

Figure 1. Activation of TGF-β recruits MSCs during bone remodeling. TGF-b1 is released from the bone matrix and activated during osteoclast-mediated bone resorption, creating a gradient. TGF-β1 induces migration of MSCs to the bone remodeling sites to couple bone resorption and formation. The bone-resorptive microenvironment also provides signals such as IGF-1 that direct the cell lineage–specific differentiation of MSCs.

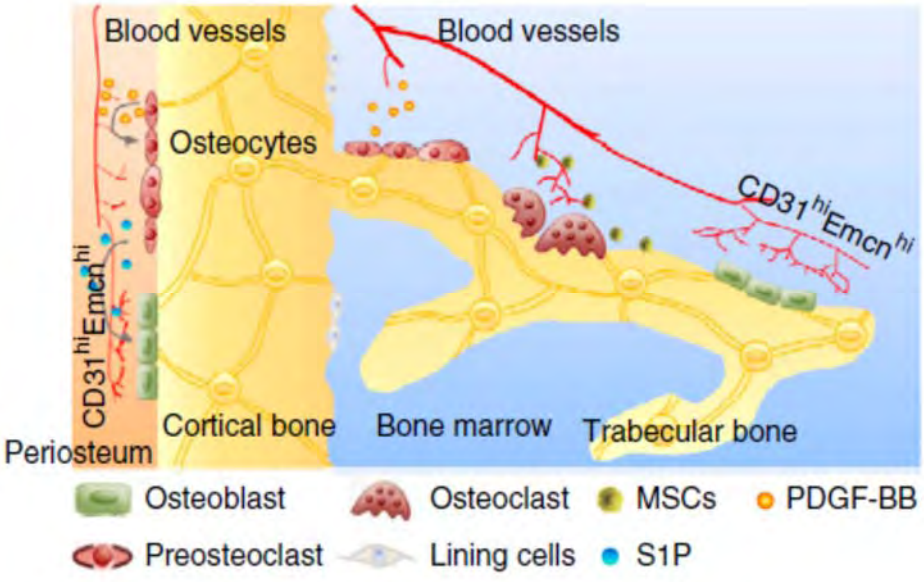

Figure 2. Preosteoclasts secrete PDGF-BB to recruit EPCs and MSCs to promote angiogenesis during its coupling with osteogenesis. PDGF-BB secreted by preosteoclasts determines the temporospatial vessel formation essential for the subsequent bone resorption and new bone formation during bone modeling and remodeling.

Parathyroid hormone (PTH), the systemic hormone that regulates calcium homeostasis, plays a major role in orchestrating bone remodeling by modulating the bone marrow microenvironment and regulating osteogenic signaling pathways. The parathyroid gland, the main production site of the calcium homeostasis regulator PTH, evolved in amphibians and represents the transition of aquatic to terrestrial life. Permanent detection of osteoclasts and bone resorption also emerged as vertebrates transitioned to land, promoting survival by development of lighter cylindrical bones to aid in mobilization and release of calcium from the skeletal matrix. During PTH-mediated osteoclastic bone resorption, growth factors and cytokines are also released from the bone matrix. To protect the integrity of the skeleton in adapting to terrestrial life, PTH regulates bone remodeling by orchestrating signaling of local factors, including TGF-β, Wnts, bone morphogenetic protein (BMP), and IGF-1 (9-13). Thus, the fate of MSCs and other cells in the bone marrow microenvironment are indirectly regulated by PTH to integrate systemic control of bone remodeling (**Figure 3**).

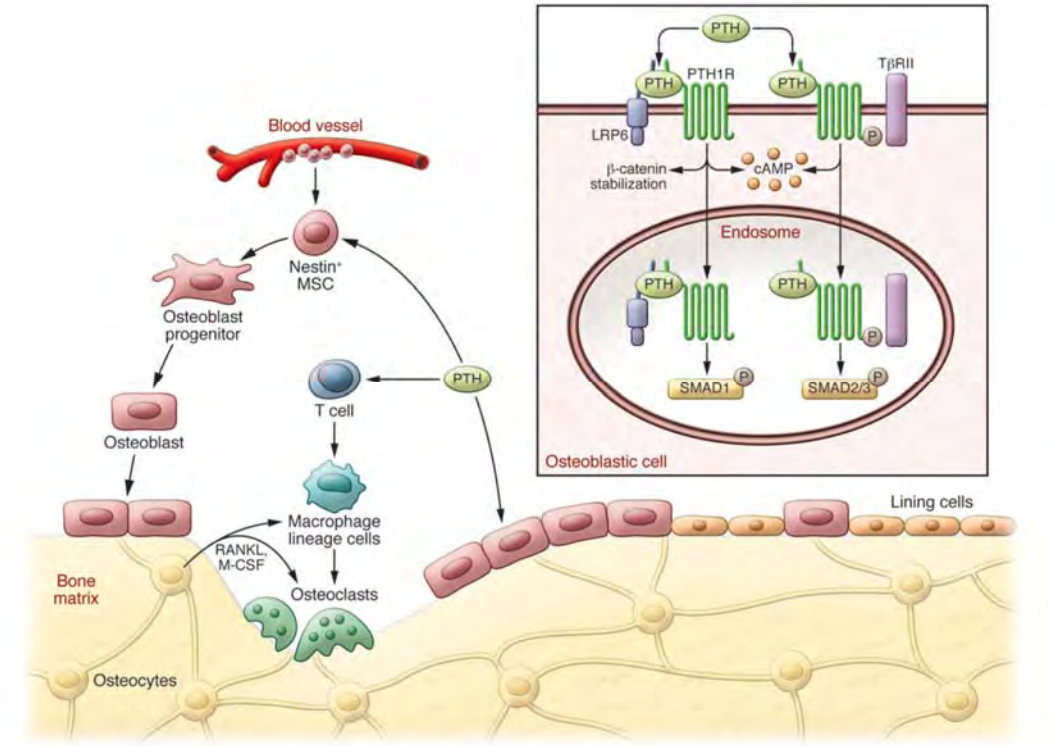

Figure 3. PTH orchestrates signaling of local factors, including (but not limited to) TGF-β, Wnts, and BMP. Thus, PTH regulates cellular activities — including those of MSCs, T cells, and other PTH-responsive cells — in the bone marrow to integrate systemic control of bone remodeling. PTH stimulated bone remodeling expands nestin+ MSC populations, spatially relocates blood vessels closer to sites of new bone formation, and orchestrates the osteogenic bone marrow microenvironment.

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Vitamin D Biology
(Mouse Models)

Geert Carmeliet, M.D., Ph.D.

Vitamin D biology – mouse models

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KU Leuven, Leuven, Belgium

Significance of the Topic

Normal serum calcium levels are a prerequisite for the functioning of several vital cellular processes. The serum calcium concentration is therefore maintained within a very narrow range via the regulation of calcium absorption in the intestine, calcium reabsorption in the kidney, and calcium deposition in or release from the bone. In addition, calcium incorporation in the bone matrix is also required to provide integrity and strength to the skeleton. The active form of vitamin D [1,25$(OH)_2$D] plays an important role in regulating these processes, and the generation of tissue-specific vitamin D receptor (VDR) null mice has provided mechanistic insight in the cell-specific actions of 1,25$(OH)_2$D and their contribution to the physiology of VDR signaling that controls bone and mineral metabolism. Several findings suggest that to secure serum calcium, 1,25$(OH)_2$D acts primarily on the intestine and this pathway indirectly promotes calcium incorporation in bone. Although the intestine is the prime target of 1,25$(OH)_2$D, VDR-mediated actions in the kidney and bone become important to secure serum calcium when dietary calcium acquisition fails. Increased 1,25$(OH)_2$D levels together with low dietary calcium intake will increase bone resorption and decrease bone mineralization in order to maintain serum calcium levels. On the other hand, low levels of vitamin D increases the risk of osteoporosis and bone fractures and severe vitamin D deficiency leads to osteomalacia. Sufficient serum vitamin D levels together with appropriate dietary calcium intake are thus important for skeletal health.

Learning Objectives

Knowledge of the complex hormonal regulation of serum calcium levels

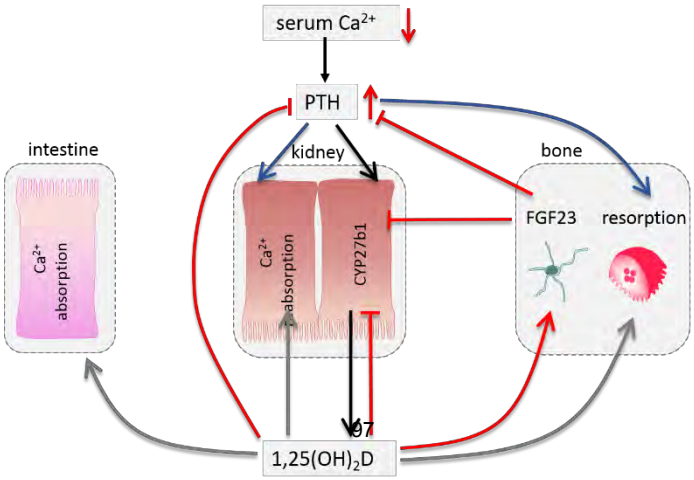
Insight in the contribution of vitamin D receptor (VDR) signaling in calcium homeostasis

Understand the specific role of VDR action in calcium handling tissues

Understand the effect of calcium balance on VDR action.

Overview of hormonal regulation of calcium homeostasis

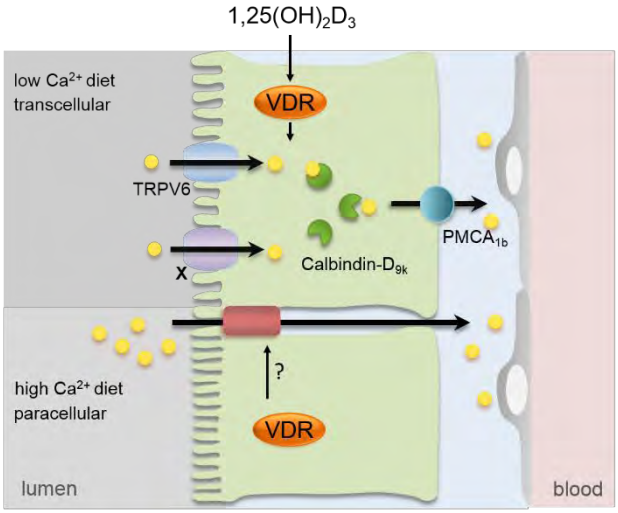
A decrease in serum calcium levels results in increased parathyroid hormone (PTH) levels which



stimulate renal calcium reabsorption and calcium release from the bone by promoting bone resorption. PTH enhances also CYP27B1 activity and thus the formation of active $1,25(OH)_2D$, which on its turn increases intestinal calcium absorption, renal calcium reabsorption and bone resorption. It also stimulates FGF23 secretion by the osteocytes which induces renal phosphate loss and decreases PTH and $1,25(OH)_2D$ levels. Also $1,25(OH)_2D$ negatively regulates PTH levels. These mechanisms thus normalize serum calcium levels.

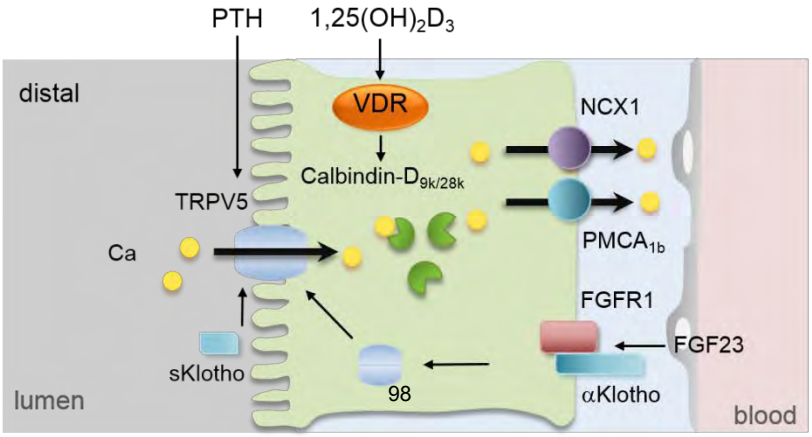
Intestinal VDR activity stimulates intestinal calcium absorption

Intestinal VDR activity promotes intestinal calcium transport especially when dietary calcium intake is low, by increasing the expression of calcium transport proteins involved in active transcellular calcium transport (TRPV6, calbindin-D_{9k} and likely some unidentified proteins). Recent evidence indicates that intestinal $1,25(OH)_2D$ signaling may also regulate the paracellular pathway.



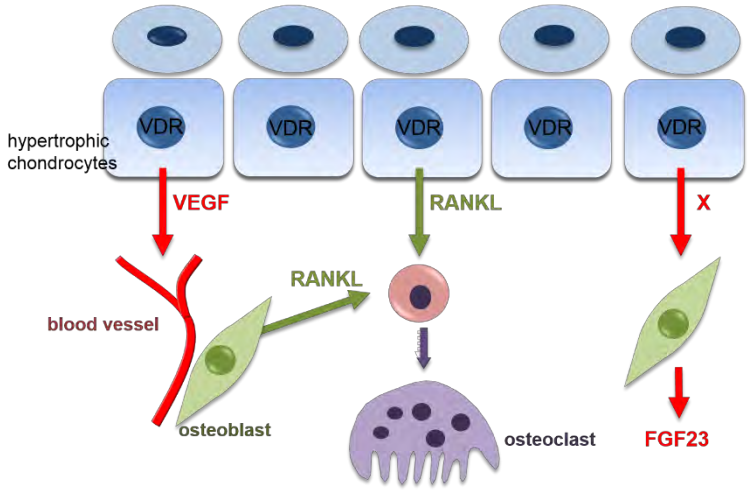
Renal VDR actions

The regulation of renal calcium reabsorption occurs primarily in the distal nephron, where $1,25(OH)_2D$ action stimulates the active transcellular pathway, a process that resembles the active intestinal calcium absorption pathway. In *Vdr* null mice, renal calcium reabsorption is impaired as shown by the inappropriately high urinary calcium levels in light of the hypocalcemia. However, the exact role of $1,25(OH)_2D$ on renal calcium reabsorption requires the generation of kidney-specific *Vdr* null mice.



Paracrine and endocrine effect of VDR in chondrocytes

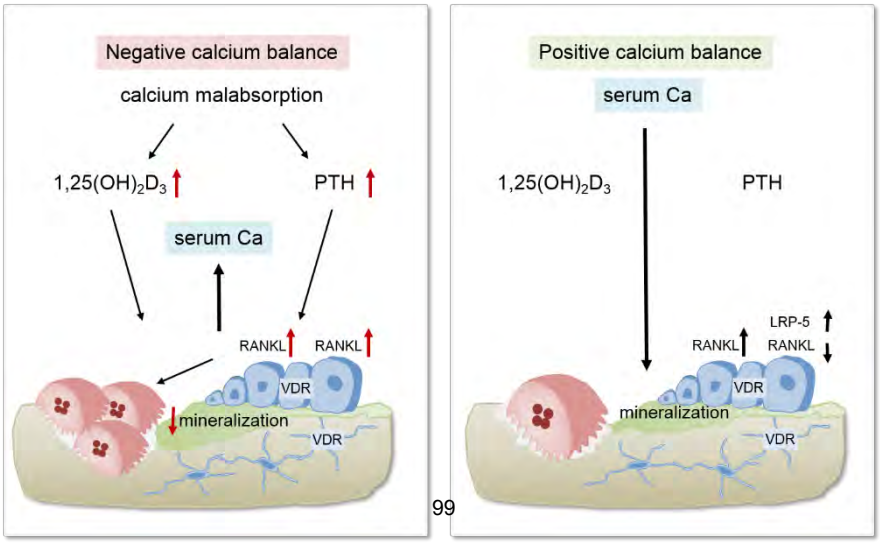
VDR activity in chondrocytes does not regulate chondrocyte behavior directly, but exerts paracrine actions and regulates bone formation by increasing the expression of VEGF and RANKL, thereby regulating angiogenesis and osteoclast formation. In addition, VDR action in chondrocytes stimulates indirectly the expression of FGF23 in osteoblasts and thus regulates phosphate homeostasis and CYP27B1 activity.

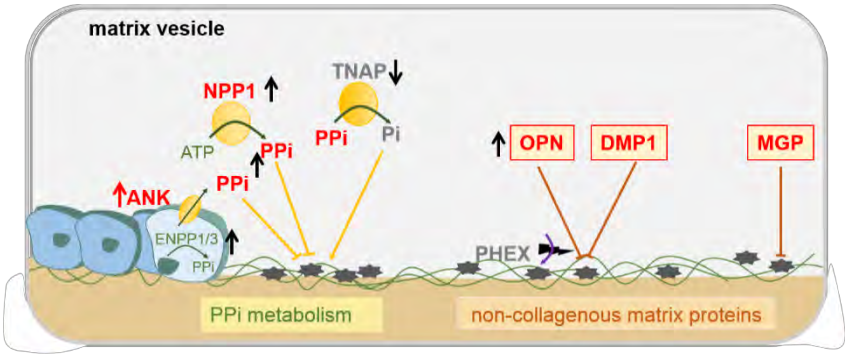


VDR action in osteogenic cells is dependent on calcium balance and differentiation stage

Besides regulating FGF23 expression, VDR activity in osteogenic cells regulates bone metabolism locally by paracrine and autocrine signaling. These effects likely depend on the calcium balance and the differentiation stage of the osteogenic cells. During a positive calcium balance, $1,25(OH)_2D$ signaling in immature osteoblasts increases RANKL expression and decreases bone mass. In mature osteoblasts, VDR overexpression results in increased bone mass by anabolic (increase in LRP-5) and anti-catabolic (decrease in RANKL) actions. VDR signaling in osteocytes has no direct role in bone metabolism.

During a negative calcium balance, $1,25(OH)_2D$ and PTH levels may increase which increase bone resorption and decrease bone matrix mineralization by regulating mineralization inhibitors.





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Calcium and Vitamin D: Current Status

J. Christopher Gallagher, M.D.

Meet the Professor

Calcium and vitamin D status: Current status

Dr Gallagher
Creighton University Medical School, Omaha NE, USA.

Significance

There is controversy about the diagnosis of vitamin D status with different definitions by the IOM (Institute of Medicine)(1) and the Endocrine Society (2**).**

- For osteoporosis, what level of serum 25OHD is clinically important ?
  IOM suggest 20ng/ml (50nmol/L. Endocrine soc. suggest 30ng/ml (75nmol/L).
  Figure 1 (slide)

- For osteomalacia most agree on <~10ng/ml(25nmol/L).
-
- Screening subjects for low serum 25OHD in asymptomatic persons is not supported by evidence of benefit (3).

- What is the role of Ethnicity on serum 25OHD. Do the same criteria apply ? (**slide**)

What is the present role of vitamin D /calcium ? .

For *treatment* most of the world OF's accept that calcium and vitamin D should be given together and be used as adjunctive agents with other Bone protective drugs. Meta analyses show a modest 9 % reduction in all fractures in home-based patients and 16 % reduction in hip fractures in nursing home patients (3).

For *prevention* the use of vitamin D and calcium for bone loss and fractures is more controversial and the US Preventive Services Task force(UPSTF) recommends against their use in independent community people based on present risk benefit data (4).

Despite many association studies linking low serum 25OHD to other diseases and conditions there are no clinical trial studies of vitamin D that have shown benefit (1).

Learning objectives

1 Discuss diagnosis of vitamin D deficiency
2 Use of vitamin D and or calcium in prevention and treatment
3 Risk benefit of calcium and vitamin D

CASE 1

- 72 year old African American women is complaining of generalized weakness, some muscle pains in the lower legs.(late winter)
- Past medical history: Hysterectomy and oophorectomy age 43, diabetes type 2 (8 yr), hypertension (5 yr), reflux esophagitis (7yr)
- Medications: metformin, thiazides, omeprazole.

- Physical findings :
- BMI 35 kg/m2.
- Lab: fasting glucose 160mg/dl, HbA1C 9mg/dl,
- Serum calcium 9.2mg/dl, serum creatinine 1.2mg/dl
- e-creatinine clearance 60ml/min,
- Serum 25OHD 10 ng/ml (N 20-40),
- DEXA total hip T score -1.8.
- X rays knees, marked OA.

DIFFERENTIAL diagnosis
Osteoporosis
Osteomalacia
low normal values

TESTS What other tests are needed ? (slide)
24h urine calcium
Serum phosphate
Serum 1,25(OH)2D,
Serum alkaline phosphatase
Calcium intake ~600mg
Bone biopsy?

FINAL DIAGNOSES:

Treatment options
1) Vitamin D treatment/prevention
daily, weekly, monthly, annual, 50,000 IU weekly x 6-8 weeks
2) Add calcium?

DISCUSSION (slides)

- Definitions of vitamin D deficiency, insufficiency ,sufficiency
- Racial differences in serum 25OHD
- Are there benefits to calcium/D in reducing fractures in this ethnic patient

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CASE 2

A 70-year-old woman comes in for annual review. She was diagnosed with osteoporosis (DEXA spine T score -2.5,hip -2.0) two years ago and started on bisphosphonates. Today she comes in at 3pm for brief physical, repeat DEXA, lab tests.
History she told the doctor that she had fallen 6 months ago on the ice and fractured her radius. She just finished with the orthopedic surgeon and he told her the fracture had healed well. During the last 2 years she had missed about 3-4 doses of her monthly bisphosphonates.
Previous medical history: smoker for 40 years, hysterectomy aged 44 y; high cholesterol for 10 years. Chronic gastritis, Myocardial infarct- 3 years ago.
Medications: statins, bisphosphonates, omeprazole.

Diagnosis

- Osteoporosis
- Previous fracture
- Smoker
- Chronic gastritis
- High cholesterol
- Myocardial infarct
- on bisphosphonates

Measurements (slide)
Height measured on stapediometer shows a decrease of 1cm.
BMI 28kg/m2.
Repeat tests DEXA showed a T score of -2.7 on the spine and -2.2 on the hip; this was not thought to be a significant decrease .She commented on all the recent publicity in the newspapers which quoted experts about people needing to take more vitamin D and calcium to help bones, prevent cancer, diabetes….. She asked if she was getting enough calcium and D since she stopped taking calcium 3 years ago might
Medications
On questioning she takes a daily multivitamin that has vitamin D 400 IU plus calcium 150mg.She used to take more calcium, but her friend told her that experts said 'too much calcium caused heart attacks' (she was taking 1000mg when she had her heart attack). Her 'heart doctor said her heart attack was more likely due to long-term smoking and high cholesterol. Also an early menopause may be a factor

Discussion points
1 Would you give calcium supplements to a woman with a history of heart attack?
2 Or could more vitamin D reduce her risk of another heart attack ?
3 What is the risk benefit of calcium and vitamin D

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Fat-Bone Connection

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Fat-Bone Connection

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Meet the Professor

Significance:

There is now strong experimental and clinical evidence highlighting the importance of the skeleton in the regulation of whole body homeostasis. This is mediated through secretory factors released during skeletal remodeling to enhance insulin sensitivity in muscle and fat cells. But there is more to the connection between bone and fat. Both cell types originate from the same progenitor and hence are 'cousins'. Lineage allocation of MSCs suggests that critical processes beyond the up-regulation of transcription factors, contributes to fate determination; these would include the availability of specific substrates for bone and fat cells, driven by ATP demand. Those cellular events also drive the interaction between bone and fat on a whole body level since energy availability is essential for peak skeletal activity. Yet the relationship between bone and fat is complicated and clearly more complex than the paradigm that obesity protects the skeleton. Part and parcel of this interaction lies in the intercellular signaling between these two tissue types as well as the role of fat tissue as an 'insulator' and 'protector' of the skeleton.

In order to more clearly define bone-fat connections one has to appreciate that not only is the skeleton somewhat heterogeneous (cortical and trabecular, membranous and endocortical bone) but that fat tissue is not all the same. There are basically at least three types of adipose tissue: white (or yellow) brown (pre-formed, interscapular) and beige (brown-like). Moreover there may be a fourth type, that which is present in the bone marrow adjacent to the skeleton. The interactions between these depots and the skeleton are distinct and will be discussed in the session. The importance of delineating specific interactions between bone and fat on a cellular and molecular level will help define how the obesity epidemic may have very different effects on the skeleton and ultimately on osteoporosis risk.

Learning Objectives:

As a result of participating in this session the attendees should:

1-understand the distinct adipose depots and their function

2-determine the clinical relationship between adipose depots and the skeleton

3-clarify the role of bioenergetics and substrate availability on osteogenesis and adipogenesis

Outline:

I. Define the three types of adipose tissue
    a. White
    b. Brown
    c. beige

II. Delineate the uniqueness of the marrow adipose depot
    a. Origin
    b. composition
    c. Function
    d. Relationship to skeletal remodeling
III. Bioenergetics of bone
    a. Substrate use in osteoblasts-
    b. Differences between adipocytes and osteoblasts
IV. Relationship of Fat to bone
    a. Obesity and skeletal micro-architecture
    b. Brown adipose tissue and bone
    c. Beige adipose tissue in WAT and bone
    d. Pathological situations
        i. Anorexia nervosa
        ii. Radiation and chemotherapy
        iii. Rosiglitazone
        iv. Glucocorticoids
        v. Type I and II NIDDM

Cases:

#1 A 56 year old white female presents with a tibial plateau fracture after a skiing accident. She is in good health except for T2DM which she has had for 5 years and is on metformin 500 mg per day and is well controlled. he had a previous radial fracture at age 50 at a time when she entered menopause. Her weight is 180 pounds, she is 5 foot 7 inches. A bone density test reveals a T-score of -0.2 in the spine and +1.1 in the femoral neck. What should be done for her bones?

#2 A 75 year old woman presents with a new hip fracture. Internal fixation is performed to stabilize the bone and a biopsy is obtained (non labeled). Results show a marrow with significant adiposity, few trabecular surfaces and cortical thinness. There is no evidence of increased osteoid. 25OHD level was 30 ng/ml. What is the clinical significance of the marrow adiposity and how does that relate to skeletal fragility?

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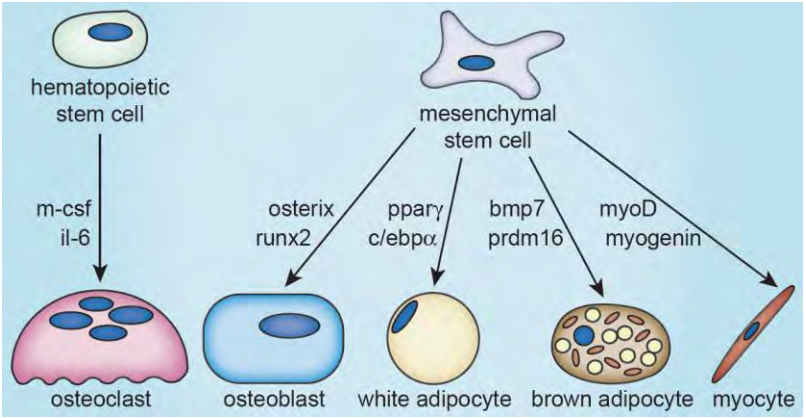
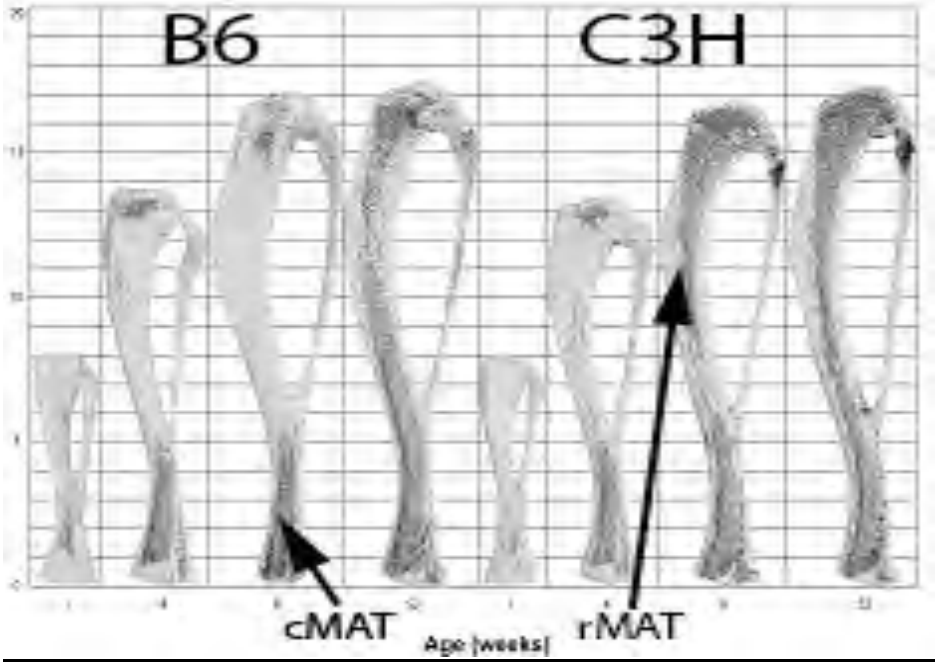
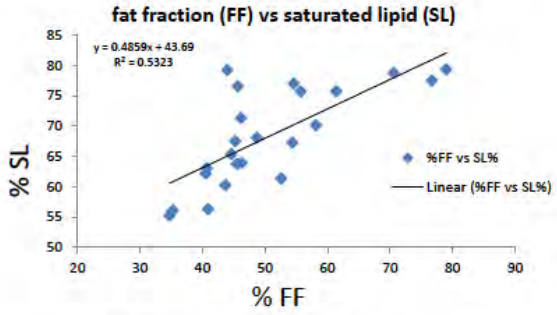
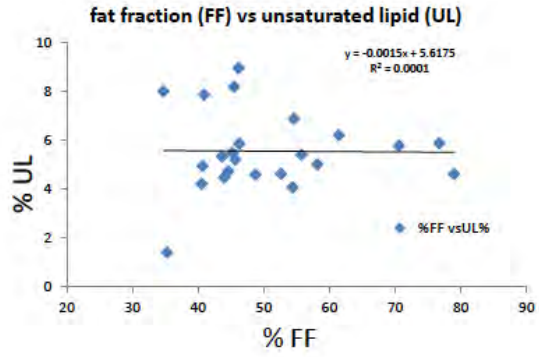
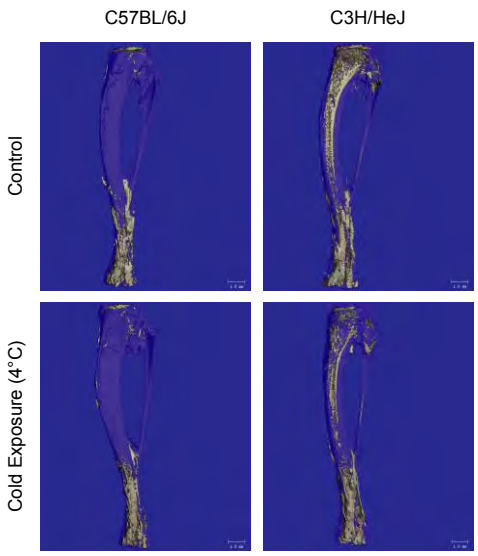


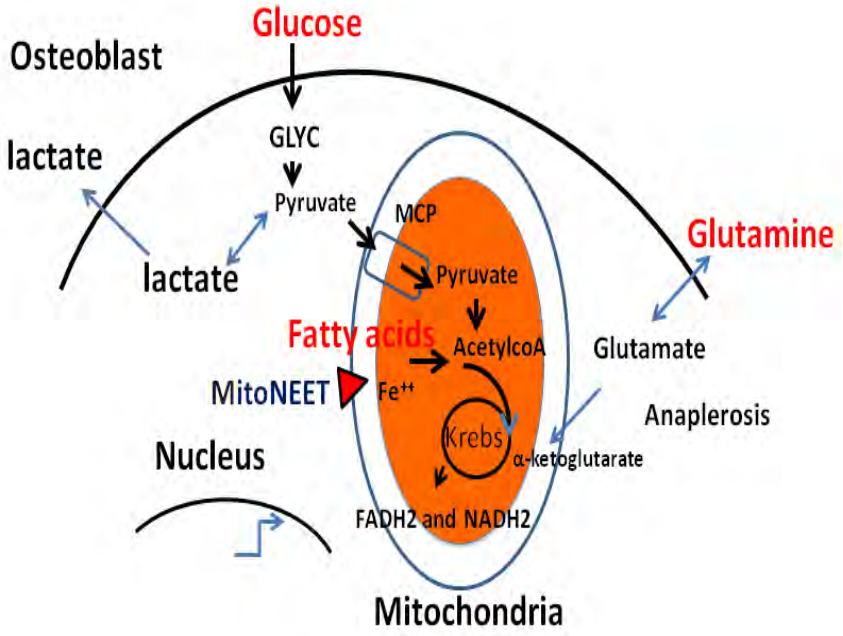


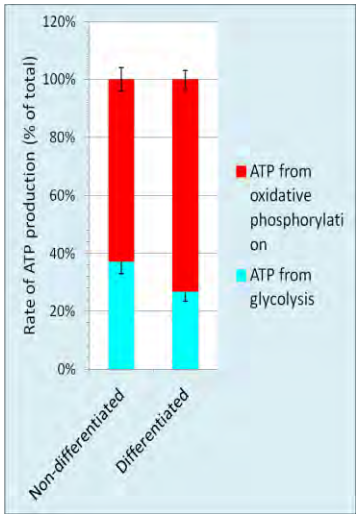
Cancer therapy effect on saturated/unsaturated marrow fat (MF) or fat fraction (FF)

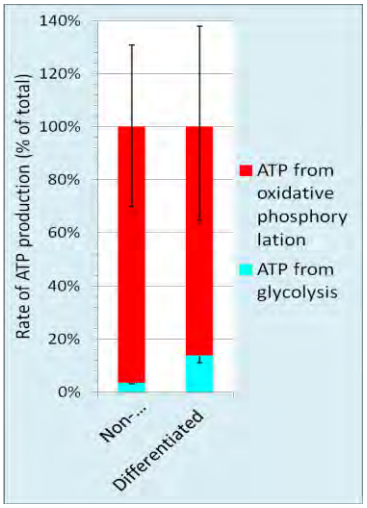












Adipocytes (L) and osteoblast energy utilization (R); Adipocytes use more OxPHos and Obs use more glycolysis

Implementing a Fracture Liaison Service

Piet Geusens, M.D., Ph.D.

Implementing a Fracture Liaison Service (FLS)

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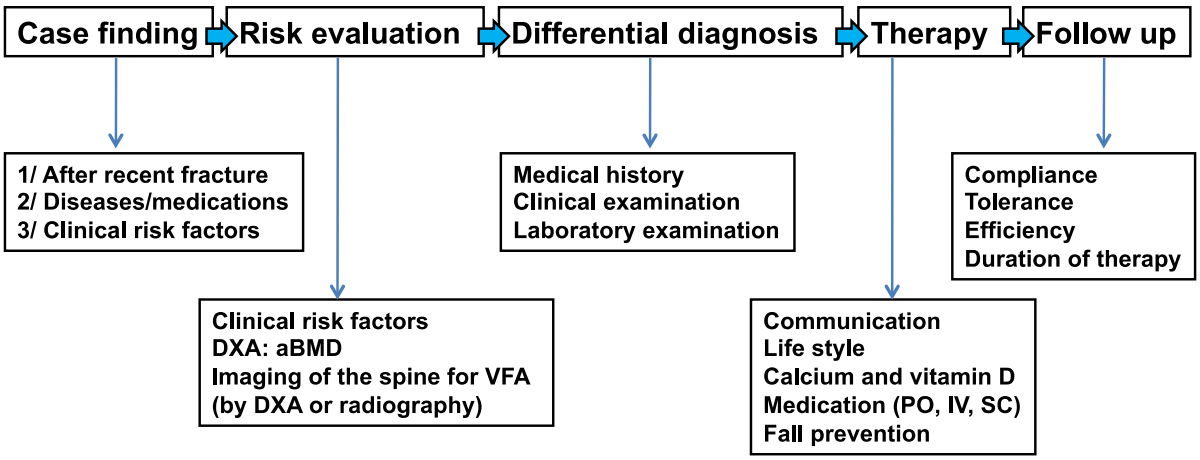
Introduction

After a fracture the risk of a subsequent fracture is doubled (1). However, this subsequent fracture risk is not constant over time. Subsequent fracture risk is particularly high in the first year(s) after any fragility fracture, and thereafter decreases over time (2,3). After the age of 50 years, subsequent fractures account for 40% of all fractures in women and 24% in men (4). Initially, a fracture patient needs acute care, which is supplied by the orthopedic or traumatology surgeon. In difficult cases (e.g. elderly with a hip or other major fracture), geriatricians can help in the pre- and post-operative care, at the so-called ortho-geriatric ward.

Once the acute fracture care has been successful, secondary fracture prevention should be considered (5-7). However, only few patients with a recent fracture have risk assessment (8) and even less are started on preventive medication. In addition, adherence to anti-osteoporotic drugs is low, but presumably higher when started after a recent fracture than when started in other clinical contexts (8).

Fracture prevention in 50+ patients can be presented as a 5-step strategy (figure 1) (9). This scheme will be used to discuss the various aspects of implementing a FLS.

Figure 1. Fracture prevention can be presented as a 5-step strategy.



Learning objectives

As a result of participating in this session, attendees should be able to understand how to organize a FLS in order to:
1/ identify and invite patients with a recent fragility fracture
2/ identify patients at highest risk for subsequent fracture
3/ perform adequate differential diagnosis
4/ initiate appropriate fracture prevention in high-risk patients
5/ organize follow up of treatment

Outline

The setting up and implementation of a FLS is not an easy task. When fully implemented, it requires collaboration between surgeons, internists (rheumatologists, endocrinologists, geriatricians) and general practitioners, a specialist who is responsible to supervise the FLS, a local coordinator (such as a fracture nurse) and, according to local needs and possibilities, a business plan for financing. Furthermore, the FLS strategies need to be based on evidence, which in most countries is incorporated and delivered by national or international guidelines for fracture prevention.

Ganda presented a meta-analysis on the effect of intensity of FLS organization on fracture risk evaluation and treatment (10). He grouped studies into four general models of care: Type A: identification, assessment and treatment of patients as part of the service, Type B: similar to A, without treatment initiation, Type C: alerting patients plus primary care physicians, Type D: patient education only. Meta-regressions revealed a trend towards increased BMD testing ($p=0.06$) and treatment initiation ($p=0.03$) with increasing intensity of intervention.

1/ Case finding: how to identify and contact 50+ patients with a recent fragility fracture?

All patients with a non-traumatic fragility fracture are candidates for evaluation for secondary fracture prevention (finger, toe, skull and road accident fractures excluded) (5-7).
A first and crucial step is the *identification* of patients with a recent fragility fracture (5-7). This requires a close contact with the orthopedic/traumatology surgeon, with regular access to patients and/or to the register of ICD-coded fracture patients in the hospital.
The next step is to *inform and educate* patients with a recent fracture about subsequent fracture risk, the tools of fracture risk evaluation and the possibilities for fracture prevention.
Lastly, patients should be *invited* to the FLS. This can be performed by direct patient contact, in-hospital or out-hospital (at the emergency unit, the ortho-geriatric ward, the plaster consultation), or by mail or by contacting the GP who contacts the patient. Direct contact with the patient has been shown to be the most effective strategy (10). Patient education alone has been shown not to be effective in increasing diagnostic evaluation or therapy (10).

2/ Fracture risk evaluation

Fracture risk evaluation is recommended in all patients with a recent fragility fracture. It contributes to diagnosis (e.g. the presence of osteoporosis, or a subclinical vertebral fracture), to assessment of subsequent fracture risk, and to therapeutic decisions.

Patients with a recent vertebral or hip fracture have a high subsequent fracture risk, and can therefore be proposed treatment without using DXA or VFA, as recommended in several guidelines. However a baseline BMD and imaging of the spine still will be helpful during follow up of changes in BMD and the occurrence of (new) vertebral fractures.

DXA of the lumbar spine and hip is the standard method for measuring BMD. The results of BMD measurements contribute to calculate fracture risk (independent of clinical risk factors), and to therapeutic decisions.

Imaging of the spine allows detecting subclinical vertebral fractures, which are frequent in patients with a recent non-vertebral fracture (in 20%). Imaging can be performed by radiography or DXA. DXA uses much lower irradiation than radiography and has a high negative predictive value for diagnosing radiographic vertebral fractures. The presence, number and severity of vertebral fractures are related to fracture risk and contribute to therapeutic decisions, independent of BMD and other risks.

Fall risks predict falls and fractures, independent of other risks. Fracture risk evaluation is complex and can be evaluated by recent fall history, and by evaluation of muscle force, power and performance, and body balance, in order to decide about fall prevention strategies.

In addition to these methods, fracture risk can be further calculated when other independent risk factors for fractures are included, such as age, gender, BMI, life style, personal and family history of fracture, fall history, use of some medications and underlying diseases. These risk factors are included in algorithms such as *FRAX and the Garvan fracture risk calculator*.

3/ Differential diagnosis

A limited standard laboratory examination allows diagnosis of previously unknown causes of secondary osteoporosis or other metabolic bone diseases (SECOB) (Table 1) (11). These are found in one out of four patients with a recent fracture, when performed systematically in all patients who are able and willing to visit the FLS (11,12). Such newly diagnosed underlying diseases increase the risk of fractures and may influence therapeutic decisions (12).

Table 1. Newly diagnosed contributors to SECOB in patients with a recent fracture seen at the FLS (11).

	Prevalence of newly diagnosed contributors to SECOB					
	Men (n = 144)		Women (n = 482)		Total (n = 626)	
Disorders	n	%	n	%	n	%
MGUS/myeloma	4/1	2.8/0.7	9/0	1.9/0	13/1	2.1/0.2
CKD						
Stage 3	7	4.9	45	9.3	52	8.3
Stage 4	1	0.7	1	0.2	2	0.3
Hyperparathyroidism (HPT)						
1°[a]	1	0.7	16	3.3	17	2.7
2° due to vitamin D deficiency	11	7.6	38	7.9	49	7.8
2° due to CKD	2	1.4	4	0.8	6	1.0
2° due to vitamin D deficiency and CKD	0	0	9	0.8	9	1.4
Hyperthyroidism[b]	8	5.6	31	6.4	39	6.2
Hypogonadism	12	8.3			12	1.9
Total number of new contributors	47		153		200	
Patients with at least one new contributor[c]	40	27.8	126	26.1	166	26.5

4/ Therapy

It is not the aim to go into detail for treatment options. In general, therapeutic measures include:

Adequate information to patient and communication with the GP

Adequate calcium and vitamin D intake

Life style: stop smoking, moderate alcohol intake, exercise

Medication: anti-resorptive and osteo-anabolic medications in high-risk patients to prevent subsequent fracture

Fall prevention in patients with high fall risk to prevent subsequent falls

5/ Follow up

A structured follow up plan is needed for evaluation of tolerance, compliance, adherence, efficiency of treatment and eventual switch of therapy (5,6).

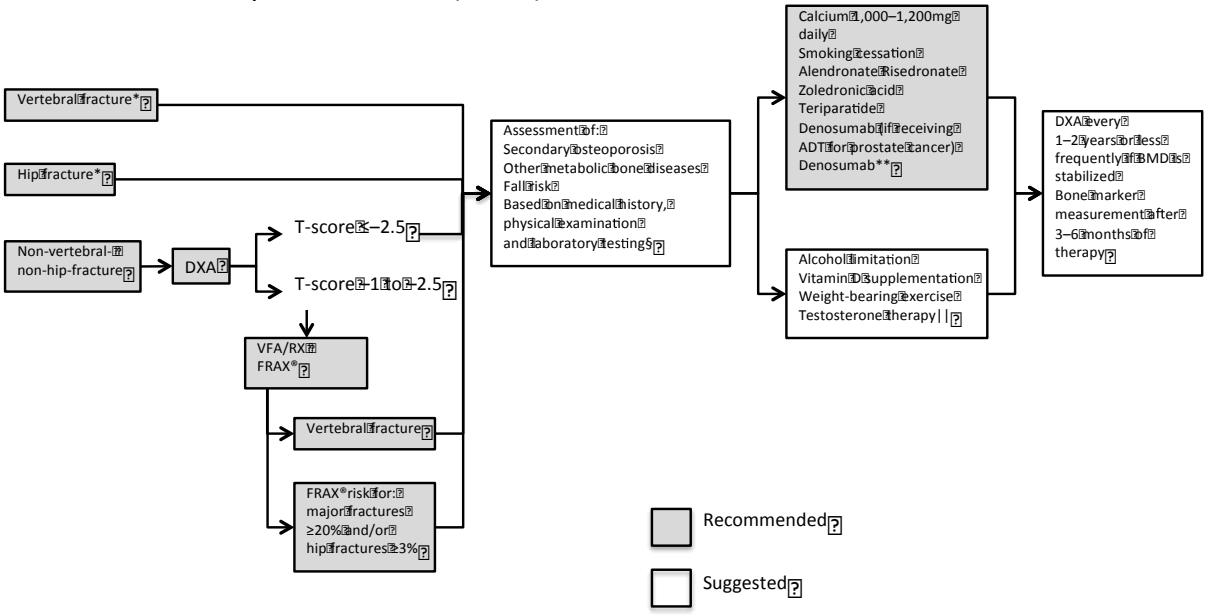
Cases

Propose a FLS pathway in following cases, based on figure 2 and your suggestions:

1/ Women, 65 years old, with a recent wrist fracture after a fall, treated with Paris plaster

2/ Men with a recent hip fracture after a fall from 2 steps, hospitalized for surgery and followed in the ortho-geriatric ward, with good recovery

Figure 2: Example of a five-step evaluation and treatment plan for men aged ≥50 years at high risk of fracture and osteoporosis, based on the 2012 Endocrine Society Clinical Practice Guideline for Osteoporosis in Men (13,14)



*DXA and/or VFA recommended but not necessary for treatment decisions. §Serum calcium, phosphate, creatinine, alkaline phosphatase, liver function, 25-hydroxyvitamin D, total testosterone, complete blood count and 24-h urinary calcium excretion. ||Suggested for men at high risk of fracture with testosterone levels <6.9 nmol/l and contraindications to approved pharmacological agents for osteoporosis. Abbreviations; ADT, androgen deprivation therapy; BMD, bone mineral density; DXA, dual-energy X-ray absorptiometry; RX, lateral radiography of the thoracic and lumbar spine; VFA, vertebral fracture assessment. ** Recently approved in several countries (adapted from 13).

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Mouse Models of Osteoarthritis: Promises and Pitfalls

Martine Cohen-Solal, M.D.

Mouse models of osteoarthritis: promises and pitfalls

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Significance of the Topic:

Cartilage damage which characterizes osteoarthritis is accompanied with bone lesions. Joint integrity results from the balance in the physiological interactions between bone, cartilage and synovium. Several local factors regulate physiological remodelling of cartilage, the disequilibrium of these leading to a higher cartilage catabolism. Cytokines and growth factors secreted by bone cells or their precursors can induce chondrocyte differentiation and apoptosis which suggests their role in the dialogue between both tissues.

Several animal models of OA have been developed in order to assess the mechanism of cartilage loss and chondrocyte functions that encompassed surgical, chemical or genetic approaches. Indeed, the animal models are helpful to investigate the cartilage changes in relation to changes in bone remodelling. Cumulative in vivo evidence show that increased bone resorption occurs at early stage of the development of osteoarthritis. Inhibition of bone resorbing molecules prevents cartilage damage, confirming the role of bone factors in the crosstalk between both tissues. Some participate to the imbalance in cartilage homeostasis and in the pathophysiology of osteoarthritis. Among these numerous molecules, RANKL, OPG, TGFb or the Wnt signalling have an effect in the relation of both bone and cartilage. These local factors are potential candidates for new drug targets for osteoarthritis. Effects of each tissue should be approached in an integrative way in animal models in order to better understand the pathophysiology and to limit the side effects.

Learning Objectives:

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

- Understand the relationship between bone and cartilage remodeling
- Know the different animal models in osteoarthritis, their impact and limitations
- Better understand the role of bone molecules in cartilage remodeling
- Define the role of mechanical factors in the development of cartilage and subchondral bone damage in osteoarthritis.
- Understand the necessity of including joint assessment in the clinical trials for osteoporosis

Points of Interest

Although the cartilage use is the main hallmark of OA, the disease damages the whole joint including bone, synovial tissues and ligaments. In humans, the characterization of each tissue lesion that leads to cartilage degradation in a longitudinal manner is restricted. Lesions developed in the joints at the early stages of OA. Such evaluations have the advantage of providing the localization and the time-course of the tissues alterations. Synovial inflammation, meniscus and bone marrow lesions are good predictors of OA rapid progression at the knee. However, this approach gives only descriptive information and is not fully contributive to the cause of the disease. Therefore, animal models are valuable tools to fully characterize the kinetics of the changes in the tissues. Other advantages are that they can be performed in a short amount of time and that they give access to the mechanism of action and efficacy of new molecules.

The final goal of animal models is to reproduce human OA. Most of them focused in one factor that favors the development of OA such as aging, mechanical stress (surgery), chemical defect (enzyme) or in genetic factors. All of them differ in terms of severity, localization of lesions and pathogenesis. Hence, the choice of the model should be appropriate to the addressed question. The choice should be focused on either the role of tissues or molecules that could trigger OA, the development under a specific genetic background or the use of drugs to prevent the occurrence of

OA. Moreover, the necessity of animal models is driven by the need of preclinical studies in order to evaluate the safety, toxicity and effects of drugs.

Densification of subchondral bone and osteophytes are common features in OA progression. Subchondral bone changes in OA patients might trigger the initiation and allows the progression of cartilage lesions. Subchondral bone thickening appears before cartilage degradation. Osteoclastic activity and structural changes precede cartilage lesions, and can be prevented by bone resorption inhibitors. Manipulation of Wnt signaling in bone can affect the cartilage damage and promote osteoarthritis in mice. Therefore, an in vivo integrative approach using animal models is useful to limit the side effects of treatment for osteoarthritis.

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New Developments in Wnt Signaling and Bone

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New Developments in Wnt Signaling and Bone

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Significance of the Topic:

WNT signaling is one of the most important developmental signaling pathways that controls cell fate decisions and tissue homeostasis. Not surprising, the last decade has provided abundant data implicating the WNT pathway also in bone development and in the regulation of bone mass. Indeed, rare human mutations together with gain-and loss-of-function approaches in mice have clearly demonstrated that flaws in this pathway lead to altered bone mass.

WNT ligands function with an entourage of receptors, co-receptors, agonists and antagonists that either enable or prevent WNT signaling activation. Indeed, the strength of WNT signaling lies in several feedback mechanisms that control proper signaling and thereby proper responses. Even though the WNT signaling cascade in bone has been studied intensively in recent years, not all key aspects of how it regulates bone mass are clear and mechanisms such as the function of specific WNT ligands, agonists and inhibitors or the regulation of signaling specificity between different WNT cascades remain puzzling. Given that WNT signaling can be targeted for drug development, understanding how we can manipulate the different players within the WNT signaling pathways is a major focus for developing new anabolics for treating bone diseases such as osteoporosis.

Learning Objectives:

This session focuses on our current understanding of how WNT signaling influences bone homeostasis. We will recapitulate the exquisite WNT regulatory system and then outline and discuss novel discoveries and explore whether these novel insights provide therapeutic potentials to inhibit bone loss and ultimately rebuild bone.

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

1) To review the complex WNT receptor/ligand system by integrating new information into their existing knowledge.
2) To broaden their familiarity with the complex physical and functional interactions of the canonical and non-canonical WNT signaling cascades in the regulation of bone mass.
3) To acquire insights into the emerging role of this signaling in osteoclastogenesis and in the response of bone to mechanical loading.
4) To gain insights into new developments in Wnt signaling and bone by outlining recent reports on the function of WNT ligands, agonists and antagonist in the differential regulation of trabecular and cortical bone mass.
5) To learn about novels protein families (ZNRF3 and RNF43) that control cell activity by regulating surface expression of WNT receptors and discuss their potential role in the regulation of bone mass.
6) To discuss the novel discovered crosstalk of WNT with the Hippo signaling and its ultimate role in the regulation of bone mass.

An Outline/Points of Interest/Clinical Pearls:

WNTs are secreted cysteine-rich glycoproteins loosely classified as either “canonical” or “non-canonical” depending on whether they activate β-catenin-dependent or -independent signaling events, respectively. In the canonical WNT pathway, activation of the Frizzled-LRP5/6 receptor complex leads to stabilization of cytosolic β-catenin, translocation into the nucleus and subsequent activation of canonical WNT target genes. In the non-canonical WNT signaling WNTs engage Frizzled receptors alone or together with co-receptors such as the receptor tyrosine kinase-like orphans Ror2 or RYK followed by activation of target gene expression. Importantly, WNT ligands function with an entourage of receptors, co-receptors, agonists (R-spondins) and antagonists (Dkk1, Sclerostin, LRP4) that either enable or prevent Wnt signaling activation (Figure 1) [1, 2]. Compelling evidence indicate that WNT signaling plays a major role in bone mass regulation. The excitement

about this signaling pathway started with the findings that in humans loss and gain-of-function mutations in *LRP5* result in skeletal diseases characterized by low bone mass, and high bone mass (HBM), respectively, and that mutations in sclerostin, an inhibitor of WNT–β-catenin signaling encoded by *SOST*, cause sclerosteosis and van Buchem disease both characterized by HBM [1, 3-7]. Numerous studies in genetically modified mouse models have confirmed the phenotype seen in these human mutations and proved that while activation of the canonical WNT pathway results in higher bone mass, its inhibition is associated with decreased bone mass, although the relative impact of distinct components of this signaling machinery on bone formation and bone resorption can differ [1] (Figure 2). In mammals there are nineteen WNT proteins that by engaging various receptor complexes activate different signaling cascades to orchestrate the activity of mesenchymal progenitors, osteoblasts, osteocytes [1, 2, 8]. In addition, canonical WNT signaling also regulates osteoclastogenesis for the most part through down-regulation of the OPG/RANKL ratio and in fact mice lacking β-catenin in mature osteoblasts and osteocytes display a low bone mass phenotype due to increased osteoclastogenesis, while osteoblast function is unaffected [9, 10]. A direct effect of canonical WNT signaling on osteoclastogenesis has also been recently proposed as mice lacking β-catenin in osteoclast precursors develop osteopetrosis because of reduced osteoclast number and activity [11].

Most importantly, a link between canonical WNT signaling in osteocytes and the response of bone to mechanical loading has also been demonstrated [12], suggesting that the osteocytes transduce, via WNT signaling, mechanical stress into biological responses of osteoblasts and osteoclasts through bone modeling and remodeling.

While the role of canonical WNT signaling in postnatal bone homeostasis has been intensively investigated, the function of non-canonical WNT signaling remains elusive[1]. Indeed, it has been shown that in mice Wnt7b regulates osteoblast differentiation and bone formation via the $G\alpha_{q/11}$-Pkcδ non-canonical cascade [13] and that the Wnt5a-Ror2-Jnk and the Wnt16-Jnk signaling are involved in bone homeostasis by regulating RANKL-induced osteoclastic differentiation[14, 15]. Although the general thought is that β-catenin-dependent and -independent pathways activate distinct downstream signaling, it is becoming clear that several WNT ligands can signal through both pathways depending on the cellular context, the nature of receptors and co-receptors as well the presence of specific co-activators and antagonists. Alternatively, given the known interplay between canonical and non-canonical WNT signaling with other signaling pathways, it is possible that alternate pathways contribute to the maintenance of trabecular and cortical homeostasis. Thus, the findings that in mice deletion of Wnt16 affects cortical bone mass but not trabecular bone mass or that deletion of Sfrp4 differentially affect trabecular and cortical bone might be explained by different levels of expression of receptors and co-receptors as well as of by the presence of different negative feedback mechanisms and activation of alternative pathways regulating WNT signaling. These different responses may also be due to the fact that the trabecular and the cortical bone contain different populations of cells (due to the presence of abundant hematopoietic and vascular cells in the trabecular compartment) that might generate a different cellular microenvironment and therefore a different response.

It is well established that WNT activity is tightly regulated by the activity of extracellular antagonists (Dkk1 and Sclerostin among others) as well as by intracellular antagonists [1]. To add another level of complication, WNT signaling strength can be also regulated via the clearance of WNT receptors. Recently, a novel negative feedback mechanism, such as that mediated by the WNT target genes: ZNRF3 and RNF43 have been reported. These two highly homologous E3 transmembrane ubiquitin ligases ubiquitinate Frizzled receptors and therefore promote the turnover of Frizzled and LRP5/6 that in turn results in WNT signaling inhibition [16-18]. Manipulation of these negative regulators therefore, might present a novel avenue to manipulate WNT signaling in bone.

Interaction between WNT signaling and other signaling pathways (including PTH and BMP among others) involved in the regulation of skeletal homeostasis has been reported [1]. Recently, multiple layers of interaction between WNT signaling and the Hippo signaling have been described [19-25]. The Hippo pathway was originally described in *Drosophila* as regulator of cell proliferation and organ size. In mammals, the Hippo signaling has two effector proteins, YAP and TAZ, which act in the nucleus as cofactors for the TEAD transcription factors. In addition to playing a crucial role in controlling cell proliferation, TAZ and YAP are necessary to maintain stem cells pluripotency [26]. A WNT/YAP/TAZ axis in which both TAZ and YAP are part of the destruction complex and thereby can regulate WNT responses, has been recently proposed [19, 20]. In addition, recent studies indicate

that TAZ and YAP are also downstream effectors of non-canonical WNT signaling [24]. Based on these findings we can speculate that these two signaling function together to modulate bone mass. Investigations on the mechanisms by which the WNT-Hippo interaction modulates skeletal homeostasis have just begun and further studies are required to reveal how the fine-tuning of these two important signaling pathways plays a role in skeletal homeostasis.

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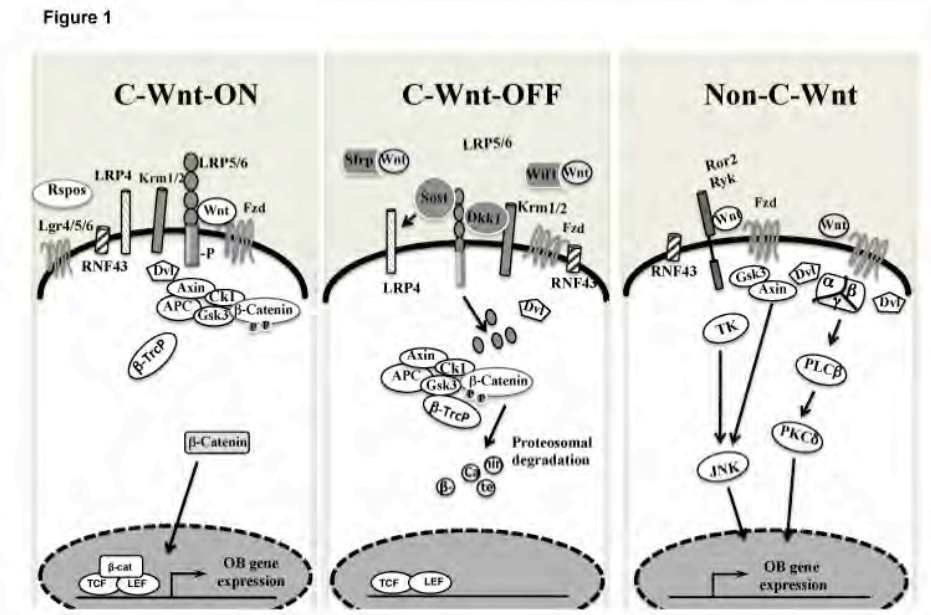

Figure 1. Simplified scheme of canonical and non canonical signaling cascades.

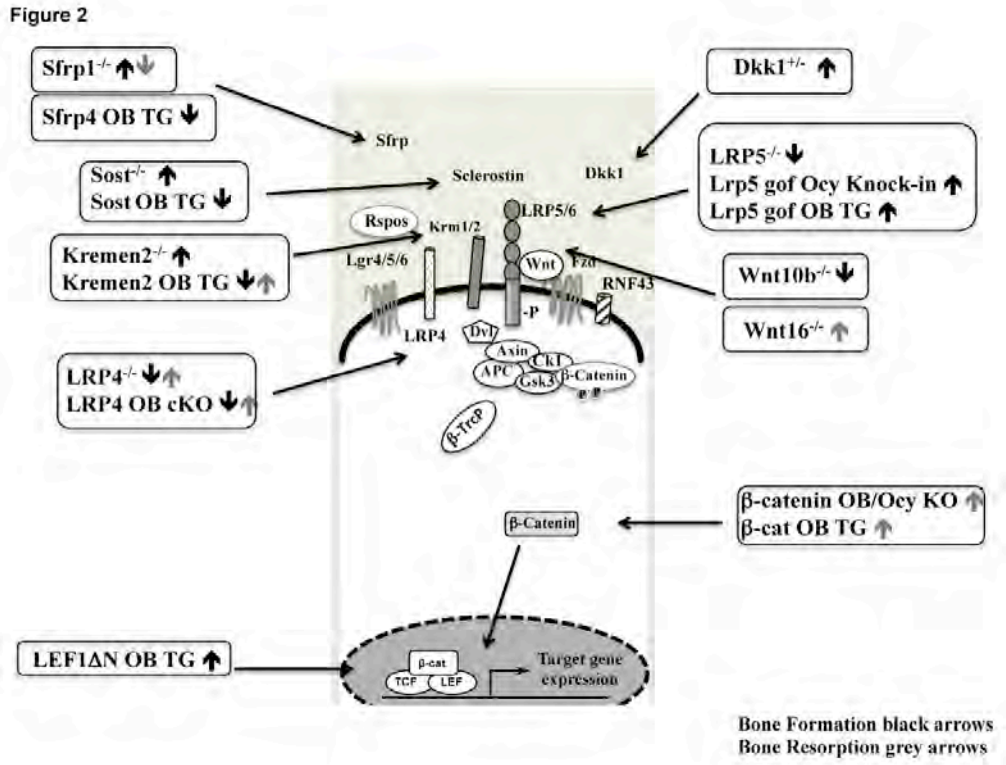

Figure 2. Differential impact of WNT signaling on bone mass.

Role of the Microbiome in Skeletal Biology

Claes Ohlsson, M.D., Ph.D.

Meet-the-Professor

Role of the Microbiome in Skeletal Biology

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Significance of the Topic:

- The gut microbiota (GM), the commensal bacteria living in our intestine, performs numerous useful functions, including modulating host metabolism and immune status.
- Recent studies demonstrate that the GM is also a regulator of bone mass and it is proposed that the effect of the GM on bone mass is mediated via effects on the immune system, which in turn regulates osteoclastogenesis
- The exact mechanism(s) for the effect of the GM on bone mass is unknown.
- The GM might be a novel therapeutic target for osteoporosis and fracture prevention
- Probiotics regulate bone mass in rodents but their impact on the human skeleton is unknown
- The possible role of the GM composition as a biomarker for osteoporosis and fracture risk is unknown
- We propose a new cross-disciplinary GM–bone research field called **'osteo-microbiology'**, bridging the gaps between bone physiology, gastroenterology, immunology, and microbiology.
- Future studies are clearly warranted in this research field to determine if the GM composition might be used as a biomarker for fracture risk prediction and to validate the GM as a possible novel therapeutic target for osteoporosis.

Learning Objectives

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

- Critically evaluate the GM as a **possible novel therapeutic** target for osteoporosis and fracture prevention
- Understand that future studies are warranted to identify the exact **mechanism**(s) for the impact of the GM on bone mass
- Define **Probiotics** and **Prebiotics**
- Participate in the design of a human study to test the hypothesis that treatments affecting the GM **composition** regulate bone mass
- Participate in the design of a human prospective cohort study to determine if the GM composition might be used as a **biomarker** for fracture risk prediction
- Understand the concept of **transplantation** of a beneficial faecal microbiota

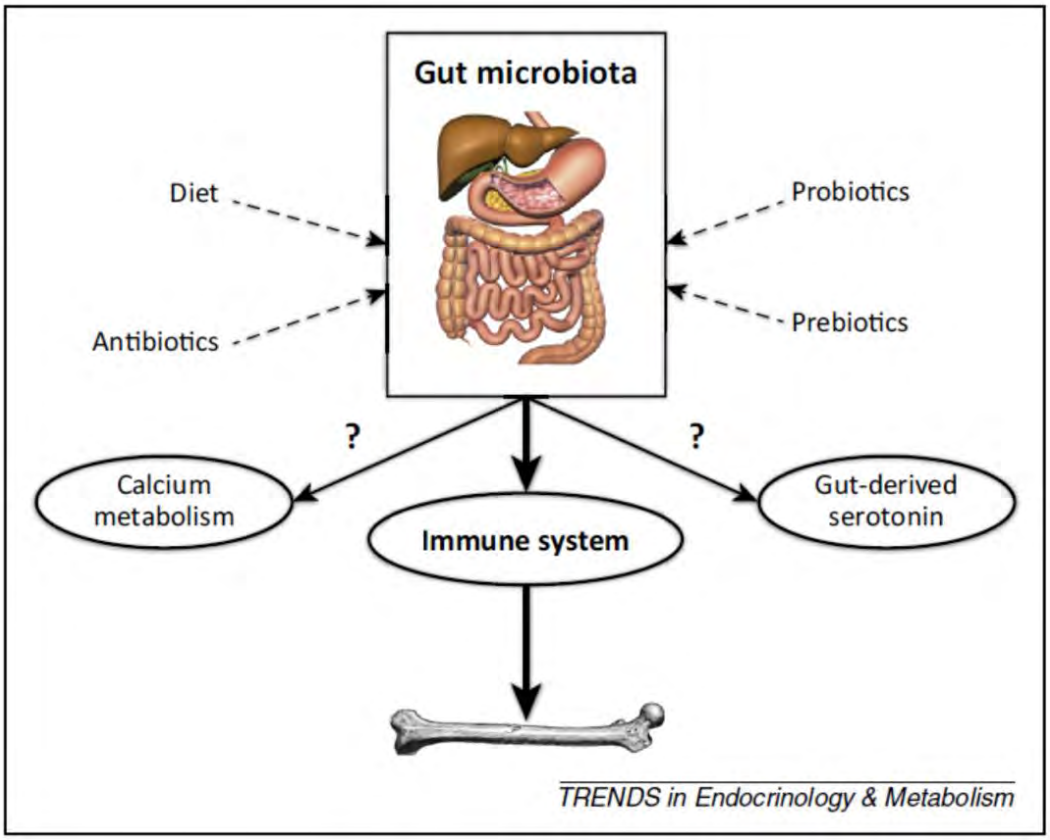

***Fig from**: Ohlsson C, Sjögren K. Effects of the gut microbiota on bone mass. Trends Endocrinol Metab. 2015 Feb;26(2):69-74*

The Gut Microbiota

- Trillions of microbes
- Fetal gut is sterile
- The GM is acquired at birth
- Modulated through life by environmental factors
- Dominated by anaerobic bacteria
- 500-1000 bacterial species
- Firmicutes and Bacteriodetes phyla are most abundant in adults
- The GM composition is efficiently determined by sequencing
- The biomass of GM 1-1.5 kg
- Microbial density increases dramatically from stomach (10^1/g) to colon ($\cong 10^{11}$/g).
- In adult intestine 10^{14} bacteria (= 10 times the number of human cells in the body.)
- The combined bacteria genome, the microbiome, contain more than 5 million genes (100 times more than the human genes)
- The microbiome is plastic
- The GM has a central role for the metabolism of dietary fibers, production of vitamins and it is influenced by the host diet
- The metabolic activity of the gut microbiota equals that of the liver.
- The intestinal microbiota could be considered as an additional organ.
- In elderly reduced GM diversity

To Discuss/Cases

1) Plan an animal experiment to identify the mechanism(s) for the effect of the GM on bone mass

2) Design a human study to test the hypothesis that treatments affecting the **GM composition** regulate bone mass

3) Design a human prospective cohort study to determine if the GM composition might be used as a **biomarker** for fracture risk prediction

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