Highlights of ASBMR 2011

Clifford J Rosen MD

Epidemiology
Heart Failure is a Major Risk Factor for Fracture Independent of BMD

Majumdar, 1031

Hyponatremia Causes Bone Loss and Sarcopenia

Reduced extracellular hyponatremia increases osteoclastogenesis

Chronic Hyponatremia in elders increases fracture risk

Infusions of DDAVP in rats at low and high dose vs controls x 3 months

Spine and femur BMD decreased 25% in the rats

In aged rats, this was accompanied by a 3.66% decrease over two weeks in thigh muscle Mass, reduced myocyte number and interstitial fibrosis

1169; Sharma,
MrOS: Men with the highest cytokines had a 2-4.35 fold had an increased risk of Fractures; But men with the highest IL-10 had a 40-60% lower risk of fractures.

<table>
<thead>
<tr>
<th>Table: Multivariable* adjusted hazard ratio (95% CI) for hip and clinical spine fractures across quartiles (Q) of cytokine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hip Fractures</strong></td>
</tr>
<tr>
<td>Quartiles</td>
</tr>
<tr>
<td>TNF-α Ref</td>
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<tr>
<td>TNF-β Ref</td>
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<tr>
<td>TNF-β Ref</td>
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<td>CRP Ref</td>
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<td>IL-6 Ref</td>
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<td>IL-6 Ref</td>
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<tr>
<td>IL-10 Ref</td>
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**Clinical Spine Fractures**

| Quartiles | Q1 | Q2 | Q3 | Q4 | P trend |
| TNF-α Ref | 1.52 (0.7, 3.5) | 2.25 (1.0, 5.1) | 2.33 (1.1, 5.1) | 0.004 |
| TNF-β Ref | 0.87 (0.3, 2.3) | 3.66 (1.6, 8.6) | 4.35 (1.7, 11.2) | <0.0001 |
| TNF-β Ref | 0.70 (0.3, 1.9) | 2.46 (1.1, 5.4) | 4.02 (1.6, 9.1) | <0.0001 |
| CRP Ref | 0.69 (0.3, 1.5) | 0.69 (0.3, 1.5) | 1.15 (0.6, 2.4) | 0.63 |
| IL-6 Ref | 1.37 (0.7, 2.9) | 1.52 (0.7, 3.2) | 1.94 (0.9, 4.1) | 0.037 |
| IL-6 Ref | 0.70 (0.3, 1.5) | 1.01 (0.5, 2.1) | 1.00 (0.5, 2.0) | 0.60 |
| IL-10 Ref | 0.50 (0.3, 1.0) | 0.33 (0.2, 0.7) | 0.26 (0.1, 0.6) | <0.001 |

*Ref=reference, adjusted for age, race, site, height, weight, smoking, alcohol intake, physical activity, calcium intake, diabetes, osteoarthritis, stroke, COPD, CHD, NSAIDS, statins, corticosteroids, fracture history, total hip BMD.

Cauley, 1019

Women Who Fracture Despite Osteoporosis Meds: Who are they?

<table>
<thead>
<tr>
<th>Baseline characteristic</th>
<th>Odds ratio (95% CI)</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Age (per 10-year increase)</td>
<td>1.18 (1.03-1.35)</td>
<td>0.018</td>
</tr>
<tr>
<td>History of stroke</td>
<td>1.67 (1.09-2.88)</td>
<td>0.031</td>
</tr>
<tr>
<td>History of Parkinson’s disease</td>
<td>2.86 (1.05-7.74)</td>
<td>0.036</td>
</tr>
<tr>
<td>History of diabetes</td>
<td>2.74 (1.61-4.68)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prior fracture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wrist</td>
<td>1.46 (1.09-1.95)</td>
<td>0.011</td>
</tr>
<tr>
<td>Spine</td>
<td>2.00 (1.38-2.88)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rib</td>
<td>2.08 (1.49-2.90)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cortisone use (vs. never use)</td>
<td></td>
<td>0.006</td>
</tr>
<tr>
<td>Current</td>
<td>0.94 (1.29-2.92)</td>
<td>0.002</td>
</tr>
<tr>
<td>Past</td>
<td>1.15 (0.87-1.51)</td>
<td>0.32</td>
</tr>
<tr>
<td>Alcohol intake (&gt;20 vs &lt;20 drinks/week)</td>
<td>8.02 (2.81-22.85)</td>
<td>&lt;0.0001</td>
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GLOW: 46,000 women; Diez-Perez et al 1024
FRAX underestimates Fracture Risk in Diabetics

Figure. Calibration plots for FRAX major osteoporotic and hip fracture predictions by risk category
(low, moderate, high).

Canadian Data Base: 3518 diabetics and 36,058 controls

Kanis, McCloskey et al

High BMI when adjusted for BMD is a risk factor for fracture in Women

27 population based cohorts, nearly 300,000 women; 1.4 million years f/u

Johansson 1036
A Link Between Obesity and Osteoporosis?

Study of Fractures in Men

- 5,994 men recruited at 6 clinical centers in US
  - Baseline: April 2000 - March 2002

- Rib fractures were identified as a common fracture in older men, and one that is associated with an increased risk of additional fractures

- In the US most fractures in older men occur in overweight or obese men

Nielsen, JBMR. 2010; Barrett-Connor, BMJ. 2010
SC Fat is directly related to CBA and PMI

Gilsanz, 2009

100 adolescent girls: CT vBMD

Gilsanz et al., 2009 JCEM

Visceral Fat is negatively associated with trabecular BMD by QCT, and positively with Muscle CSA

Bredella, 2010 Obesity International
Ovariectomy: A Profound Increase in Intra-Abdominal fat and Greater Release of FFAs

+/+ B6  

Igfbp2 +/-  

Sham  

OVX  

Glycerol, NEFA, TG lipase  

OVX: Trabecular Bone Loss  

Fig. 1: The skeleton is a lipid storage organ.
Environmental scanning electron microscopy (ESEM) scan from inside a mouse distal tibia. Bone marrow adipocytes appear as large, light spheres. It has long been recognized that the skeleton is a lipid storage organ: “Good news puts fat on the bones” (The Bible: Proverbs 15:30) bar: 0.25 mm
New Approaches to Rx

Fig. 2: The skeleton contributes to dietary fat clearance

12 weeks-old male C57BL/6J wild-type mice received a lipid gavage (olive oil) with tracer amounts of $^{14}$H-oleate. Fatty acid organ uptake 2 h after gavage was determined by scintillation counting. (A) Liver and brown adipose tissue (BAT) display the highest specific uptake of all organs analyzed. Parts of the skeleton (indicated in red) display specific uptake comparable to white adipose tissues, the major specialized lipid storage organ. epIMAT: epididymal white adipose tissue, subIMAT: subcutaneous IMAT (B) However, when calculated for total organ uptake, the skeleton (mean from parts analyzed in A) takes up about 10% of total $^{14}$H-oleate, demonstrating that the skeleton substantially contributes to the clearance of dietary fatty acids.
Delivery System for RNAi-Based Anabolic Therapy:
DOTAP-based cationic liposomes attached to six repetitive sequences of aspartate-serine-serine (DSS)6 for delivering siRNAs to specifically approach bone formation surfaces (Figure 1 and Figure 2). Using the system, we encapsulated an osteogenic siRNA targeting casein kinase-2 interacting protein-1 (CKIP-1), a protein that interacts with and augments the osteoblast-suppressing activity of the ubiquitin ligase Smurf1.

Zhang et al 1027

LLP2A-Alendronate, a Novel Compound That Directs Mesenchymal Stem Cells to Bone, Prevents Bone Losses Induced By Aging or Ovariectomy

Attach a synthetic high affinity and highly specific peptidomimetic ligand (LLP2A) against integrin α4β1 that expressed on the MSC surface, to a bisphosphonate, Ale.

Guan et al, 1025
[1078] Transdifferentiation of Hematopoietic Stem Cells into Mesenchymal Stem Cells for Use in Bone Regeneration

OCT4, SOX2, MYC and KLF4 placed in a lentiviral vector and transduced CD34+ cells

At one week OCT4MYC+ CD34+ cells were MSC like

Then passaged for 2-3 weeks

Flow cytometry-
-no hematopoietic markers,
-CD29, CD44, CD73, CD90, CD105 and CD 166+

These cells can be induced to differentiate into osteoblasts, adipocytes, chondrocytes

Baylink 1078

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**Origin of Adipocytes**

**Bone Marrow**

Adapted from Bouxsein and Rosen Nature Reviews 2006
Gonadal Fat Pad- Cell Sorting and Isolation of APs That Generate Mature Adipocytes with HSC markers

Figure 1. Flow cytometry isolation of resident adipogenic progenitors from adipose stroma. Hematopoietic lineage (Lin−) stroma was prepared from mouse gonadal fat by magnetic bead separation. (A) Lin− cells were simultaneously labeled with anti-CD29, PE/C7, anti-CD34, PE, anti-Sca-1, PerCP/Cy5.5, and anti-CD29 APC/Cy7 antibodies. The cells were sorted into the indicated subpopulations on a MoFlo XDP using the isolation scheme reported by Friedman and coworkers [18]. (B) Cells from the indicated populations were plated under colony forming unit-thro- bit conditions until they reached confluence. They were then treated with adipogenic inducers for 10 days and stained with oil red O to reveal triglyceride droplets. Longer periods of induction did not increase the number of lipid-containing cells. Our results substantiate the ability of Lin−CD29−CD34−Sca-1−CD24+stromal cells to undergo adipogenic conversion in culture as described by the Friedman group. Abbreviations: FSC, forward scatter; SSC, side scatter; Lin−, hematopoietic lineage.

The Adaptive Response of Cancellous Bone to In Vivo Loading in Mice Is diminished in Adulthood

Willie, 1106
Parathyroid Hormone 1-84 Accelerates Fracture Healing in Pubic Bones of Elderly Osteoporotic Women

Six-five patients (mean age: 82.8 years) had plain x-rays and a computer tomography (CT) scan to verify fractures and were scanned for osteoporosis. Twenty-one patients received a once daily injection of 100µg PTH 1-84 starting within two days after admission to the hospital. Forty-four patients without PTH treatment served as a control group. All patients received 1000 mg Calcium and 800 IU Vitamin D. CT scans were repeated every fourth week until radiographic evidence of cortical bridging was confirmed. Functional outcome was assessed using a pain Visual Analogue Scale (VAS) and a Timed Up and Go (TUG) test.

Results: In all 21 patients treated with PTH 1-84 pelvic fractures were healed at a mean of 7.8 weeks, whereas in patients with no PTH treatment fractures had healed after 12.6 weeks (p<0.001). At week 8 all fractures in the treatment group were healed and four fractures in the control group (healing rate 100% versus 9.1%; p<0.001). Both the VAS and TUG improved statistically significant (p<0.001) compared to control.

PTH enhances fracture healing in pelvic fractures and improves functional outcomes

Holzer, 1199

Effect of Weekly Teriparatide in Patients with Osteoporosis

Methods: To examine the anti-fracture efficacy and safety of weekly subcutaneous injection of 200 units of teriparatide (56.5 µg) in Japanese patients (65-95 years old) with primary osteoporosis, a randomized, double-blind, placebo-controlled trial was conducted in 578 patients with 1-5 prevalent vertebral fractures and low BMD (L2-L4 BMD <80% of young adult mean). Patients were randomly assigned to receive weekly teriparatide injection (n=290; 13 men) or placebo (n=288; 10 men) for 72 weeks, with daily supplements of calcium 610 mg and vitamin D 400 IU. The primary endpoint was new vertebral fractures.

Results: As compared with placebo, teriparatide reduced the risk of new vertebral fractures, with a cumulative incidence of 3.1% in the teriparatide group, versus 14.5% in the placebo group (p<0.0001), and a relative risk reduction of 79.9% (95% confidence interval, 54.7-91.1%). BMD increases at 72 weeks were significant in the teriparatide group compared to the placebo group (L2-L4: 6.7% vs. 0.3%, P<0.0001; total hip: 3.1% vs. 0.1%, P<0.000). Serum osteocalcin increased and urinary NTX decreased in the teriparatide group. Transient headache, malaise and nausea after injection were more frequent in the teriparatide group than in the placebo group, but were generally mild and tolerable.

Conclusion: Weekly injection of teriparatide (56.5 µg) is safe and effective in reducing vertebral fracture in patients with primary osteoporosis with prevalent vertebral fracture.

Nakamura, 1201
PTH treatment leads to reduced cortical BMD due to increased cortical porosity  Bogado et al

20 postmenopausal women treated with 20 ug1-34PTH HR-pQCT 0,3,6, 12 months;

<table>
<thead>
<tr>
<th>Month 9</th>
<th>Month 6</th>
<th>Month 12</th>
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<tbody>
<tr>
<td>CV vBMD</td>
<td>-0.46</td>
<td>-1.02</td>
</tr>
<tr>
<td>Cl Po</td>
<td>0.73</td>
<td>0.70</td>
</tr>
<tr>
<td>sCTX</td>
<td>1.41</td>
<td>0.32</td>
</tr>
<tr>
<td>P1NP</td>
<td>95.45</td>
<td>91.53</td>
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</table>

* p < 0.05 versus baseline, † p < 0.01 versus baseline

Acute Decline in Serum Sclerostin in Response to PTH Infusion in Healthy Men  M00120

Elaine Yu, Massachusetts General Hospital, USA; Ruchit Kumbhani, MGH Endocrine Unit, USA; Erica Siwila-Sackman, MGH Endocrine Unit, USA; Benjamin Leder, Massachusetts General Hospital Harvard Medical School, USA.

Serum sclerostin declines during PTH infusion
Sclerostin Inhibition Prevents Low Bone Mass Associated with Type 2 Diabetes Mellitus in Rats

Circulating Sclerostin Demonstrates a Circadian Rhythm in Young Healthy Men   SU 0389

N= 6men with normal BMD
Serotonin regulates hypothalamic entrainment of internal and external environmental signals.
**FoxO1 is a Transcriptional Mediator of the Regulation of Bone Formation by Circulating Serotonin**

-Gut serotonin inhibits bone formation by blocking Lrp5 thru the Htr1b receptor

-serotonin upregulates the activity of FoxO1 and the expression of FoxO1 transcriptional targets that control cell cycle

-Removal of one allele of Htr1b increases bone mass

-Lrp5 deficient mice have high FoxO1 and high serotonin

Removal of one allele of FoxO1 corrects the high bone mass of Htr1b-/- mice

CREB is the transcriptional partner of FoxO1 and regulates its response to serotonin; thus serotonin activates FoxO1 by disrupting the FoxO1-CREB interaction leading to inhibition of proliferation

Kode, 1205

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**Serotonin and Bone as Proposed by Karsenty et al**

[Diagram of serotonin and bone interaction]
The Serotonin Transporter

SSRIs and Bone

14 month changes in volumetric BMD of Radius and Tibia upon anti-depressant drug administration in young depressive pts -Winterhalder

40 patients (34 female, 6 male) –SSRI(26) or non-SSRI (14)- x age-37 pQCT measurements at baseline and 12 months-radius and tibia

RESULTS
1-weight increase more in non-SSRI (TCA,NSRI) group
2-no change in bone parameters in SSRI group
3-Non-SSRI group increase in trabecular vBMD radius

Conclusion
There is slight bone gain with non-SSRIs used to treat depression
SSRI therapy had no effect on trabecular or cortical bone mass
Muscle and Bone Loss after Botox in Rat; Bouvard et al SU 0082

25-4mo old rats randomized to IM BTX or placebo

Followed for 28 days

Recovery began at day 28

25OHD
How Vitamin D Deficiency Affects the Nature of Fracture Propagation in Human Bone

Cortical structure indices in vitamin D deficient bone demonstrated significantly increased cortical porosity, mean osteon diameter and osteocyte lacunar volume accompanied by significantly decreased cortical thickness. The osteon density remained unchanged in osteomalacic bone in comparison to the normal cases. These structural features correlated with significantly decreased crack growth toughness, while alterations to the collagenous environment in osteomalacic bone correlated with significantly decreased crack initiation values.

Busse 1108

S-25(OH)D and all cause mortality

Mortality (%)

Odds ration 1.81

Odds ration 1.6

≤
10
20
30
40
50
60
70
80
90
100
110
120
130
≥140

S-25(OH)D (nmol/l)

All, N = 247,574
Women, N = 161,428
Men, N = 86,146
No relation of serum 25OHD to:

(O.Th, OS/BS or OV/BV), or bone formation rate, or age on 25-OHD (range 10-55 ng/ml).

- Retrospective analysis of bone histomorphometric data from 93 otherwise healthy post-menopausal women who participated in a study of the effect of age & menopause on bone structure & turnover.

Osteoprobe as a measure of material indices

Micro-indentation to measure distance
Validation of a Novel Microindenter for Bone Material Strength Measurement

Osteoprobe
21 cases and 12 controls (fractures)
Adjusted for age

Methods:
5 indentations on a patient
5 indentations on a phantom
Case Presentation

- 60 year old woman with a new radial fracture presents to your office
- T-score L-S: -1.4; FN: -1.5
- Asymptomatic without family history
- 25OHD level was 18 ng/ml; Calcium supplements- 1500 mg per day
- PTH-30 pg/ml; 1,25OHD: 22 pg/ml
- ANY MORE TESTS? WOULD YOU TREAT? WITH WHAT
TRPM8 is the cold thermal receptor in bone, is highly expressed in Obs and is circadian regulated.

Total Body Fat Mass is inversely correlated with vBMD, bone geometry strength indices.