Functions of Bone

- Structural
 - Support
 - Protection
 - Movement
- Mineral Storage
 - Calcium
 - Phosphate



Skeletal Problems

- Disease / genetics
 - Osteoporosis (Type I)
 - Multiple myeloma
 - Metastatic bone cancer
 - Rheumatoid arthritis
 - Paget's Disease
- Hormone ablative therapy
- Spinal cord or nerve injury
- Surgery and rehabilitation
- Aging (Type II Osteoporosis)
- Bedrest
- Microgravity







Bone

- Bone composition
 - 70% mineral (Ca²⁺ and PO₄⁻ as hydroxyapatitie)
 - 22% protein (95% Type I collagen + 5% proteoglycans and other materials)
 - 8% water
- Two major types of bone
 - Compact (cortical, i.e., long bones)
 - Mechanical and protective functions
 - Cancellous (spongy, i.e., vertebrae)
 - Metabolic regulation of calcium
- Four types of cells
 - Osteoblasts
 - Osteoclasts
 - Osteocytes
 - Bone lining cells

Bone Cell Types

Osteoblasts	Osteoclasts	Osteocytes	Bone Lining Cells
 Matrix formation Secretes Type I collagen Regulates mineralization Positioned above osteoid matrix Matrix usually polarized but can surround cells Differentiates to become osteocyte 	 Digests bone Large multi- nucleated Exhibits ruffled border and clear zone Exhibits polarity with nuclei away from bone surface High density of Golgi stacks, mitochondria and lysozomal vesicles 	 Born from osteoblasts Maintains bone matrix Occupies lucunae Extends filopodia through canaliculi Forms gap junctions with neighboring cells 	 Flat, elongated cells Generally inactive Cover surfaces of inactive bone Thought to be precursor cells to osteoblasts



Adult Long Bone Structure





Cancellous or Trabecular Bone









Why Remodel Bone

- Allows bone to respond to loads (stresses)
- Maintain materials properties
- Allows repair of microdamage
- Participates in serum Ca²⁺ regulation

Remodeling of Bone

- Resorption accomplished by osteoclasts
 - Form cutting cones to "drill" holes Haversian system
 - Filled in holes become new osteons
- Allows bone to respond to physical stress
- Allows repair of micro damage
- Participates in plasma calcium control
- Random remodelling turns over bone
 - Prevent accumulation of brittle material
 - Skeleton is cycled every 4-5 years

Mechanism of Remodeling

- Basic Multi-cellular Unit (BMU)
 - Becomes "machinery" that remodels bone
 - Forms in response to molecular signal
 - Functions over a period of weeks to months (10 $\mu m/day)$
- BMU burrows through cortical bone
 No net increase/decrease in bone volume
- Bone growth occurs through different mechanism at periosteal (outer) surface

Initiation of BMU

- Develops in response to microcracks
- Signaled by osteocytes in response to loading
- Signaled (or at least influenced) by local hormones, cytokines and growth factors

Phases of Active BMU

- Cellular activation
 - Osteoblasts and osteoclasts are continuously recruited
 - Recruitment occurs at the "cutting" edge
- Resorption
 - Osteoclasts active for ~12 days and then die
 - Causes release of IGF, FGF, etc., which recruits osteoblasts
- Formation and Mineralization
 - Osteoid is formed by osteoblasts
 - Mineralization begins ~13 days later (1μm/day)
 - Rate is same as osteoid formation
 - Mineralization continues after eroded volume filled in





Bone Remodeling – A Coupled Control System



- Osteoblasts have receptors for osteolytic agents
- Osteoclasts in culture are activated by stimulating osteoblasts
- Osteoblasts deposit factors in newly formed matrix



• Factors released as osteoclasts age and die (apoptosis)



Bone Turnover

•Bone Turnover Relative to Development



Bone Strength

- Determinants of strength
 - Geometry
 - Bone mineral density
 - Materials properties
- Bone growth
 - Growth needed during development
 - Growth also needed to withstand increased loads

Relationship of Stress and Strain





Femur Cross Sectional Area with Age



Changes in Bone Mineral Density

(Rooster Ulna)



Candidate Mechanisms of Mechanical Transduction

- 1. Direct cell strain
- 2. Stress-generated potentials
 - Piezoelectric potentials
 - Flow-induced electro-kinetic potentials
 - stress generated potentials (SGPs)
- 3. Fluid flow-induced stress

Osteocyte Network as Sensor

- Osteocytes are abundant (10x more than osteoblasts, osteoclasts are fraction of osteoblasts)
- Syncytium of cells
- Wolff's law (Julius Wolff, put forward in 1892) stated that mechanical stress leads to mass and 3-D structure of bone



Support of Osteocyte-based Mechanism

- Osteocyte network
 - Large surface area in contact with bone
 - 2 orders of magnitude more than any other cell type
 - Provides intracellular and extracellular route of communication
- Isolated turkey ulna stimulated with loading causes significant activation of osteocytes
 - 1 Hz at 500-2,000 (10⁻⁶) strain (Δ I/I)
 - Osteocytes do not appear to respond to static loads
 - Intermittent stresses cause significant changes in osteocytes which suggest function as mechanosensors

Candidate Mechanisms of Mechanical Transduction

- Canaliculi create porosity of likely strategic importance
 - Stress-induced flows are able to reach outer layers of osteons
 - Osteocytes respond to fluid shear *in vitro* of 2-60 dynes/cm² (models predict physiological shears of 8-30 dynes/cm² for peak loads)
- Osteocytes are capable of modifying attachments, matrix proteins, etc.

– May be able to adjust gain of sensor system

Osteocyte Candidate Signaling Molecules

- Nitric Oxide
 - Small molecule, diffuses quickly
 - Produced rapidly
 - Stimulates production of IGF via both autocrine and paracrine mechanisms
- Sclerostin
 - Protein produced only by osteocytes
 - Powerful inhibitor of osteoblasts
 - In new bone (Haversian), osteocytes closer to center of channel produce most sclerostin

Summary of Bone Feedback Control System



Stress vs. Strain

- Most important effect of stress on bone is strain
 - Many activities produced strains of 2,000-3,500 μ E
 - 1E = 1% deformation ($\Delta I/I$)
 - Strain to cause fracture ~25,000 μE
 - Strain model proposes that bone mass is site specific (i.e., system attempts to keep strains below 2,000-3,500 µE)



Stress Shielding

- Occurs with artificial implants
 - Stiffness of implant much greater than natural bone
 - Does not transmit stress uniformly and fully into native bone at interface
 - Bone becomes resorbed at interface and implant becomes "loose"





Porous Material Implant





Effects of Exercise on Bone

• Two types of studies conducted

- Compare trained athletes with sedentary people
 - Athletes and chronic exercisers have higher BMD
 - Competitive runners in 60s have ~40% greater BMD than controls
 - Weight lifters have 10-35% greater spine BMD
 - Tennis players have 30% greater thickness of dominant humerous
- Correlate level of fitness with BMD (Effect not obvious)
- Early life experience is important (Peak BMD)
 - Women who get hip fractures have lower levels of occupational or leisure activity from 15-45 years old
 - Significant associations between hip BMD and early-life exercise both men and women

Model of Stress / Cycle Effect $BMD = [sum(n_i s_i^m)]^{1/2m} \text{ where}$ n = repetitions s = intensity m = 2-6 i = type of activity

- Intensity of load more important than number of cycles (i.e., running with weight better than running longer)
- Static loads have little effect
- Rate of strain also important

Skeletal Response to Exercise



Changes only occur with significant habitual changes in activities over several months

Mechanisms of Exercise Effects

- Increased habitual strain causes an increase in net bone formation
 - Returns bone strain to normal control setpoint
- Studies with intact bone Mechanical loading causes:
 - Formation on periosteal surface without initial resorption
 - Periosteal cell proliferation

Interaction of Age with Exercise

- Increasing age causes deficits in response (I.e., gain of system goes down)
 - Probably caused by multiple factors
 - Women from 60-80 show BMD increase of only 5-8% with exercise
- Increases in BMD with exercise reverts to normal within a few months of terminating training
- Exercise clearly helps maintain bone as system gain or setpoint is reduced

Osteoporosis

 Defined as reduction in bone mass and micro-architecture that leads to susceptibility to fracture



Costs of Osteoporosis

- 10,000,000 cases in U.S. alone
- Affects 1 in 2 women and 1 in 8 men > 50 years old
- Causes 1.5 million fractures/year 700,000 spine, 300,000 hip and 300,000 wrist, 25,000 deaths from complications
- Menopause is the biggest risk factor for disease
- Disease often not diagnosed until after 1 or more fractures have occurred
- Prevalence could rise to 41 million by 2015 from 28 million today
- Cost to health care estimated at \$14 billion (\$38M/day)
- Psychological and social effects of disease are immense

Bone Turnover

•Bone Turnover Relative to Development



Development of Osteoporosis

- Formation takes 3-4 months to replace bone resorbed in 2-3 weeks
- Osteoclast recruitment is increased
- Osteoblast-osteoclast Coupling is interrupted
 - Factors recruiting osteoclasts may not adequately recruit osteoblasts
 - Lack of estrogen may lead to less IGF-1 incorporated into new bone – reduces later osteoblast recruitment
 - Gain or setpoint in mechanosensory feedback
 loop may be reduced

Issue of Peak Bone Mass

- Bone mass peaks in the 20's, starts dropping in the late 30's and accelerates significantly after menopause
- Risk for osteoporosis depends on peak mass and rate of loss
- Peak bone mass depends on
 - Genetics
 - Calcium, diet, exercise, etc. in youth

Osteoporosis Treatments

- Exercise and diet
- Estrogen replacement
- Bisphosphonate therapy
- Osteoprotegerin (OPG)
- Osteogenic agents
- Tissue/cell engineering
- Sclerostin inhibition





Via Rubin *et al* 0.3 G @ 20-90 Hz



Via Rubin *et al (JBMR, 2004, 19:343)* 0.3 G @ 30 Hz

Bone Remodeling – A Coupled Control System



Osteoprotegerin (OPG)

- Seminal paper published in 1997
 - "Osteoprotegerin: A novel secreted protein involved in the regulation of bone density", Simonet et al, *Cell*, 234:137-142
- OPG member of TNF receptor superfamily soluble receptor
- Shown to affect bone density





OPG / RANKL / RANK Receptor

- RANKL and OPG are secreted by osteoblasts and bone marrow stromal cells
- RANKL functions to promote osteoclast formation and activation and inhibit apoptosis



- OPG functions as a decoy receptor to prevent RANKL signaling; ratio of RANKL to OPG dictates bone mass and structural properties
- Current extensive research is elucidating the role of OPG and RANKL in a wide variety of bone-related diseases

Denosumab (OPG mimetic)

- Fully human monoclonal antibody to RANK Ligand
- IgG₂
- High affinity for RANK Ligand (K_d 3 x 10⁻¹² M)
- Does not bind to TNFα, TNFß, TRAIL, or CD40L



Monoclonal Antibody Model

Bekker PJ, et al. *J Bone Miner Res.* 2004;19:1059-1066. Boyle WJ, et al. *Nature.* 2003;423:337-342.

Mechanism of Action for Denosumab



Adapted from Boyle WJ, et al. Nature. 2003;423:337-42.

Phase 2:

Postmenopausal Women With Low BMD Serum C-Telopeptide at Month 12



Rheumatology Meeting. Abstract # 1101.

Phase 2:

Postmenopausal Women With Low BMD Lumbar Spine BMD at Month 12



Note that mean % changes are adjusted for treatment, geography, and baseline BMD.

Cohen SB, et al. Oral Presentation at the 2004 American College of Rheumatology Meeting. Abstract # 1101.