Biomarkers of Bone Physiology & Pathology

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Goals and Objectives

- Provide theoretical framework for interpretation of bone biomarkers
  - Sources of bone biomarkers
  - Review normal bone biology
  - Discuss alterations in bone biology during life phases and in disease conditions
- Review some experimental data from animal models
Bone Remodeling

Bone remodels throughout life, at rates that vary with age and site. The same cells and processes participate in growth, fracture repair and pathological conditions. Most biomarkers are byproducts of bone remodeling.
Cancellous Bone Remodeling

Osteoclasts resorb bone, leaving a scalloped surface

Osteoid mineralizes to become bone

Osteoblasts form osteoid (unmineralized bone matrix)

Histomorphology of bone remodeling
Cortical Bone Remodeling

Like human beings, monkeys have osteonal remodeling of cortical bone.

Haversian osteons in cynomolgus monkey femur shaft.
Sources of Bone Biomarkers

- **Bone cells**
  - Osteocytes
  - Osteoclasts (bone resorbing cells)
  - Osteoblasts (bone forming cells)

- **Bone Matrix**

- **Hormones and other metabolic markers**
Sources of Bone Biomarkers

- **Cell type specific enzymes involved in cell function**
  - TRAP from osteoclasts
  - Alkaline phosphatase from osteoblasts
- **Secretory products of cells**
  - Osteocalcin from osteoblasts
- **Matrix processing or degradation products**
  - Collagen propeptides
  - Collagen telopeptides
- **Regulatory/Metabolic**
  - PTH, calcitonin
  - Ca, P
Bone Structure

Bone structure varies from site to site. Most bone in the body is cortical bone, but vertebrae and long bone metaphyses also contain cancellous bone.

Metabolic activity that produces bone biomarkers takes place on bone surfaces, and cancellous bone has a much higher surface/volume than cortical bone.
Dynamic histomorphometry in normal monkeys

- Circulating biomarkers represent a weighted global average of biomarker production throughout the body.
- Levels of biomarkers in circulation are most highly correlated with tissue volume (TV) referent data.
- Surface-based parameters are comparable across sites, but BFR/TV is 10x lower in cortical vs. cancellous sites.
- Cancellous bone and localized pathological phenomena may be over-represented in biomarker indices.

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Functions of Bone

• Structural Role
  – The skeleton adapts its mass and structure to maintain its integrity during growth and normal usage, using the bone remodeling system
  – After fracture, the skeleton restores a functional structure
  – In the aged, adaptation to loading may fail
  – Adaptive mechanisms may sometimes be activated inappropriately

• Metabolic Role – Calcium Reservoir
  – Skeleton contains 99% of total body Ca, but regulation of Ca is primarily at kidney and gut
  – Mechanisms by which bone participates in calcium regulation are poorly understood
  – Bone remodeling system participates, but role is unclear
Uses and Limitations of Bone Biomarkers

- Bone resorption and formation are coupled, so bone remodeling markers usually reflect overall bone turnover, which is modified by age, sex hormones, calcitropic hormones, and bone-active therapeutic agents.
- Biomarkers reflect global average bone turnover, and are of limited value for site-specific agents.
- Change in bone mass is related to changes in bone balance, which cannot be accurately assessed using bone biomarkers.
- Calcium, phosphorus and their regulatory factors (PTH, calcitonin, vitamin D and its metabolites) are only indirectly indicative of bone status due to the complexity of mineral metabolism.
Using Bone Biomarkers in Animals

- Enzymatic assays (alkaline phosphatase, TRAP) and single amino acid-based assays (hydroxyproline, pyridinoline, deoxypyridinoline) are widely applicable, but not specific for bone.
- Clinical immunoassays are usually applicable in primates, and may be applicable in other species.
- When adapting clinical assays, cross-reactivity and matrix interference are the main issues, and can be usually be assessed by dilutional linearity.
- A few assays have been developed specifically for non-primate species.
Effect of Age on Biomarkers of Bone Metabolism in Rats

Bone biomarker levels decrease with age, and the decline is more marked for the more specific markers.
Both bone biomarker levels and bone formation rate measured histomorphometrically decrease with age.

Effect of Pregnancy and Lactation on Bone Biomarkers in Monkeys

Bone biomarker levels decrease during pregnancy and rise during lactation.

Effect of Pregnancy and Lactation on Bone Mass in Monkeys

Whole Body BMC Change

Serum Estradiol

Bone mass decreases during lactation and recovers only slowly.

Female cynomolgus monkeys become osteopenic when estrogen-deficient. Spine may show bone loss or failure to gain. Femur more consistently shows bone loss.
Bone Biomarkers are Increased in Ovariectomized Cynomolgus Monkeys

Bone biomarkers and histomorphometric indices of bone turnover are increased by estrogen deficiency.
Short-term Bone Efficacy Screening Model Using Bone Biomarkers in Intact Cynomolgus Monkeys

- Short-term studies are needed for proof-of-concept and for dose-ranging for long-term studies.
- The GnRH (chemical) ovariectomy model can be used, but this model is difficult, slow, and expensive.
- Biomarkers respond quickly to bone-active drugs in intact female monkeys, and can be used for rapid screening in relatively small numbers of animals.
- Circadian variations need to be considered in experimental design.
- Short-term models are being developed for additional indications, such as glucocorticoid osteoporosis.
- Biomarker evaluations can also be added to tox studies.
Circadian Rhythms and Sampling Effects on Biomarkers of Bone Metabolism

Circadian variations and sampling effects on biomarkers must be considered in the design of biomarker studies.
Bone Biomarkers in Intact Cynomolgus Monkeys (Short-term Model)

**Bone Resorption Inhibitor**

**Bone Formation Stimulator**

Bone resorption inhibitor response occurs within 24-48 hours, bone formation stimulator response within 1-2 weeks.
Bone Biomarkers in Glucocorticoid-treated Cynomolgus Monkeys

**Bone Resorption Marker**

**Bone Formation Marker**

Conventional glucocorticoids stimulate bone resorption and depress bone formation, and may negatively impact bone growth and/or mass.
Conclusions

- Most bone biomarkers are byproducts of bone remodeling.
- Calcium regulatory indicators have less direct relevance to bone.
- Circulating and excreted bone biomarkers reflect global rates of bone turnover, and may over- or under-represent significant focal or site-specific bone changes.
- Bone resorption and formation are usually coupled, and bone biomarkers lack the precision necessary to assess bone balance.
- Bone turnover and biomarkers generally decrease with age.
- Bone is responsive to estrogen deficiency, causing increased bone turnover and osteopenia in lactation and in the post-menopause.
- Bone biomarkers respond rapidly and robustly to bone active agents, whereas bone mass has a slower and less dramatic response.
- Biomarkers can be useful for proof-of-concept, preliminary efficacy and dose-ranging in dedicated, short studies or as an adjunct to toxicology studies, as well as for monitoring long-term response to therapy.