

Biology of Bone Biomarkers

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Overview

- Biomarkers of bone cellular activity are commonly measured as part of an assessment of bone health, often in combination with bone histology/histomorphometry and/or bone imaging. Bone biomarkers provide insight into the state of bone formation and bone resorption (bone turnover) in the skeleton; they are not indicators of tissue damage. The biologic origin of each of the commonly measured non-clinical biomarkers will be reviewed in the context of bone growth and homeostasis. Some roles of other biomarkers, particularly hormones, on bone biology will also be reviewed.

Bone Growth, Turnover & Biomarkers

- In younger rodents and very young larger animals, growth is the primary process in bone.
- In older rodents and adult larger animals, turnover is the primary process in bone.
- In bone growth and bone turnover, cells which form bone (osteoblasts) and cells which resorb bone (osteoclasts) are active.
- Bone biomarkers are divided into two categories: bone formation markers and bone resorption markers.
- Bone biomarkers are enzymes or products of these cells which are released into the circulation and can be measured in either blood or urine.

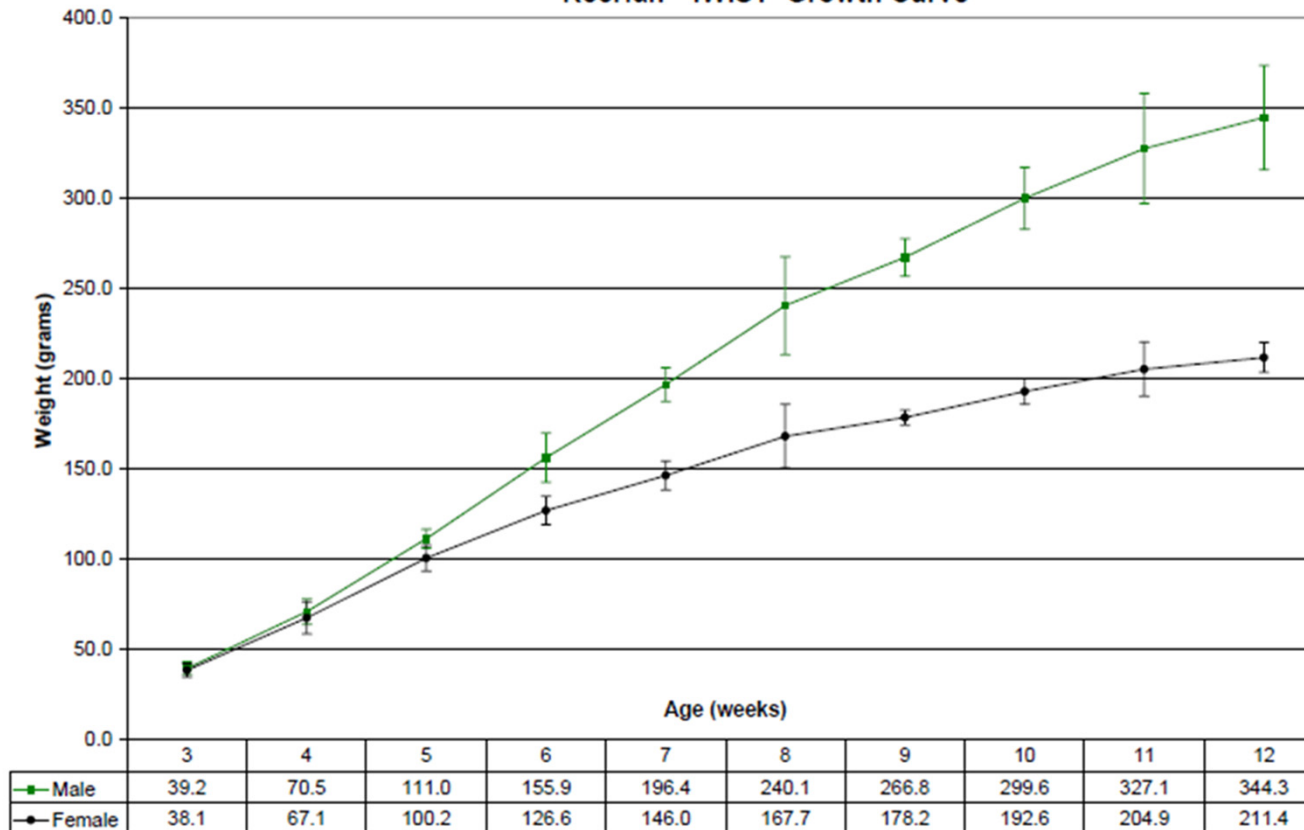
Bone Modeling

- Bone Modeling: Bone formation where there has not been previous bone resorption.
- Longitudinal bone growth
 - Occurs at growth plates (endochondral ossification)
 - Very rapid growth at first, then growth rate slows
 - Rate/duration differs by sex in some species
 - Continues until about 6 months of age in rats
 - Growth plates stay 'open' in rodents
- Bone shaping
 - Necessary to achieve correct bone shape and structure
 - Formation of cortical bone shell
 - Control amount of metaphyseal trabecular bone
- Osteoblasts and osteoclasts are both active during bone modeling (growth and shaping)

Wistar Rat Growth Curve

Harlan Laboratories, Inc.
 Combined data from multiple barriers
 2009

RccHan™:WIST Growth Curve



Growth data must be used as a guideline only.
 Data can be subject to differences in maintenance of rats.
 Growth chart incl. mean +/- 2 SD's representative of population distribution

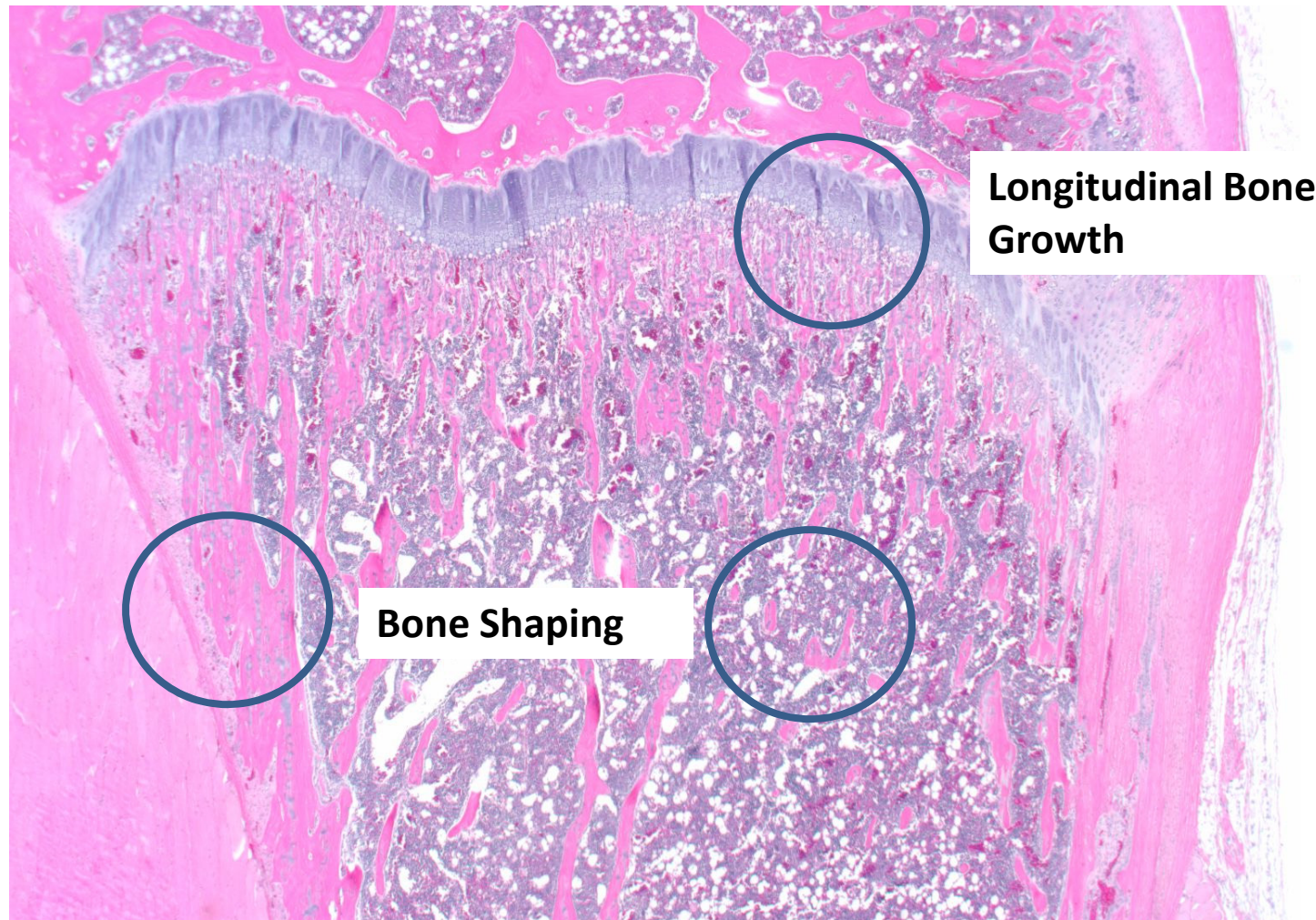
Maintained on Teklad Global Rodent Diet
 2018S
 (18% protein)



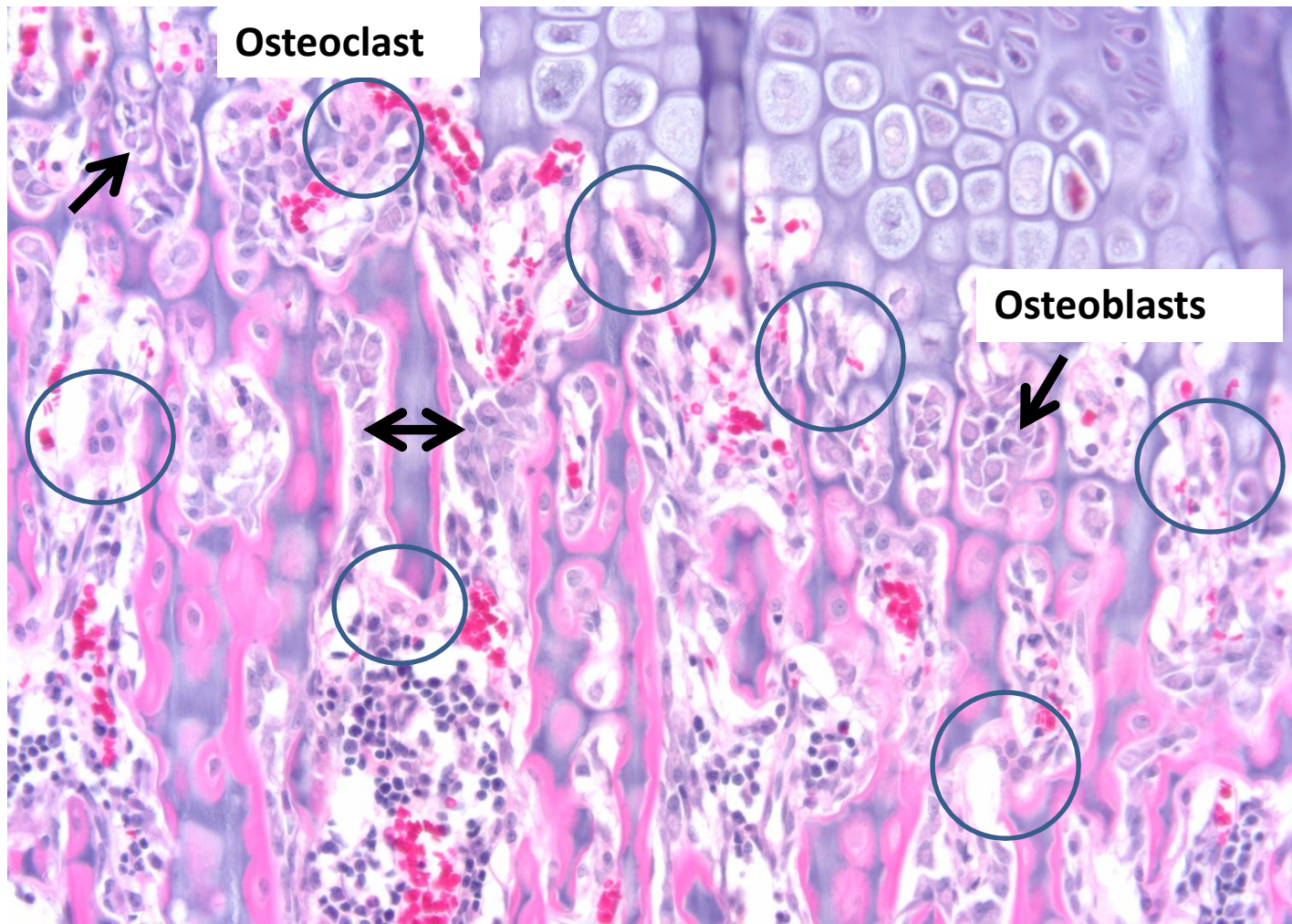
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Bone Modeling in a Young Rat



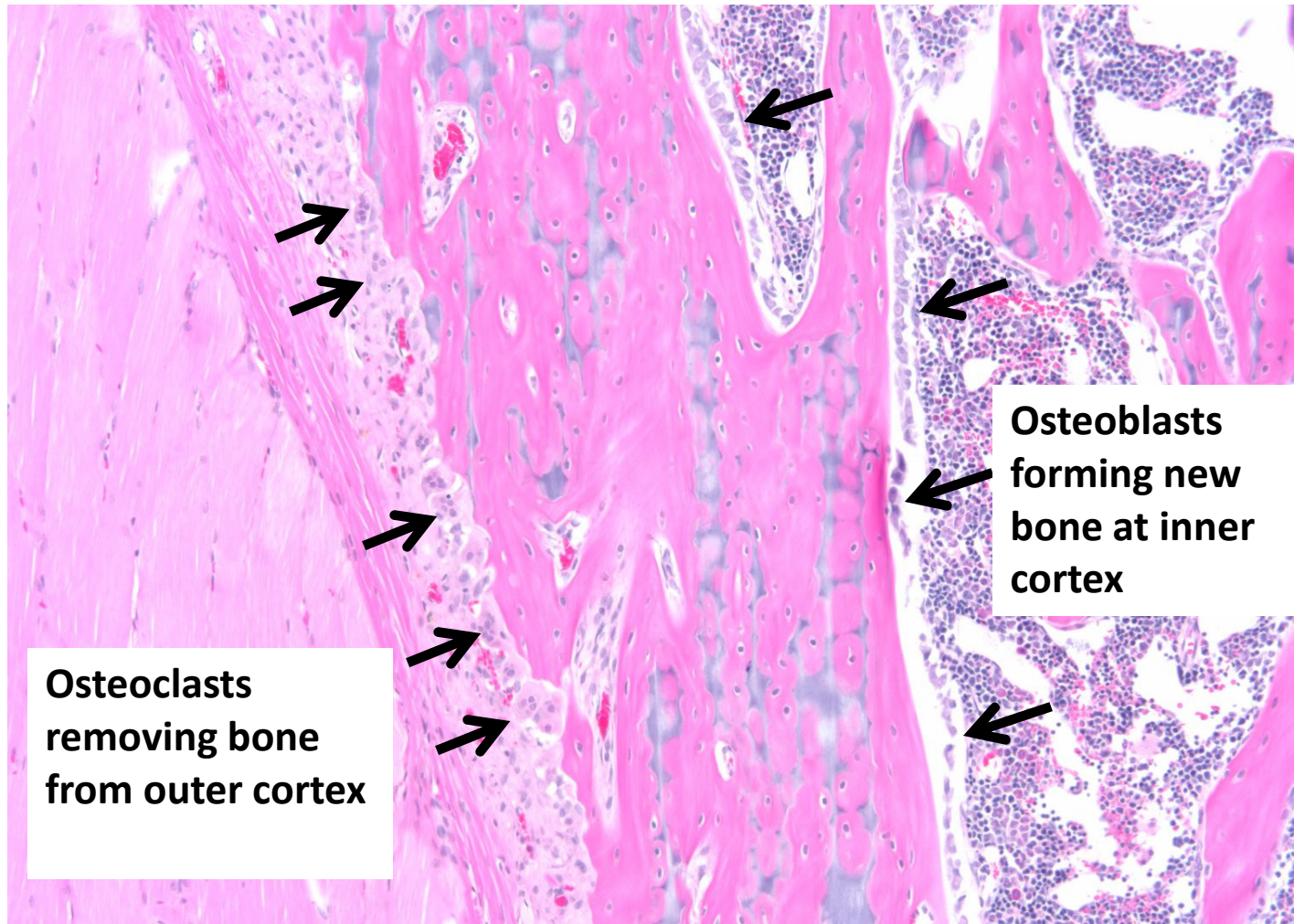
Longitudinal Bone Growth (Endochondral Ossification)



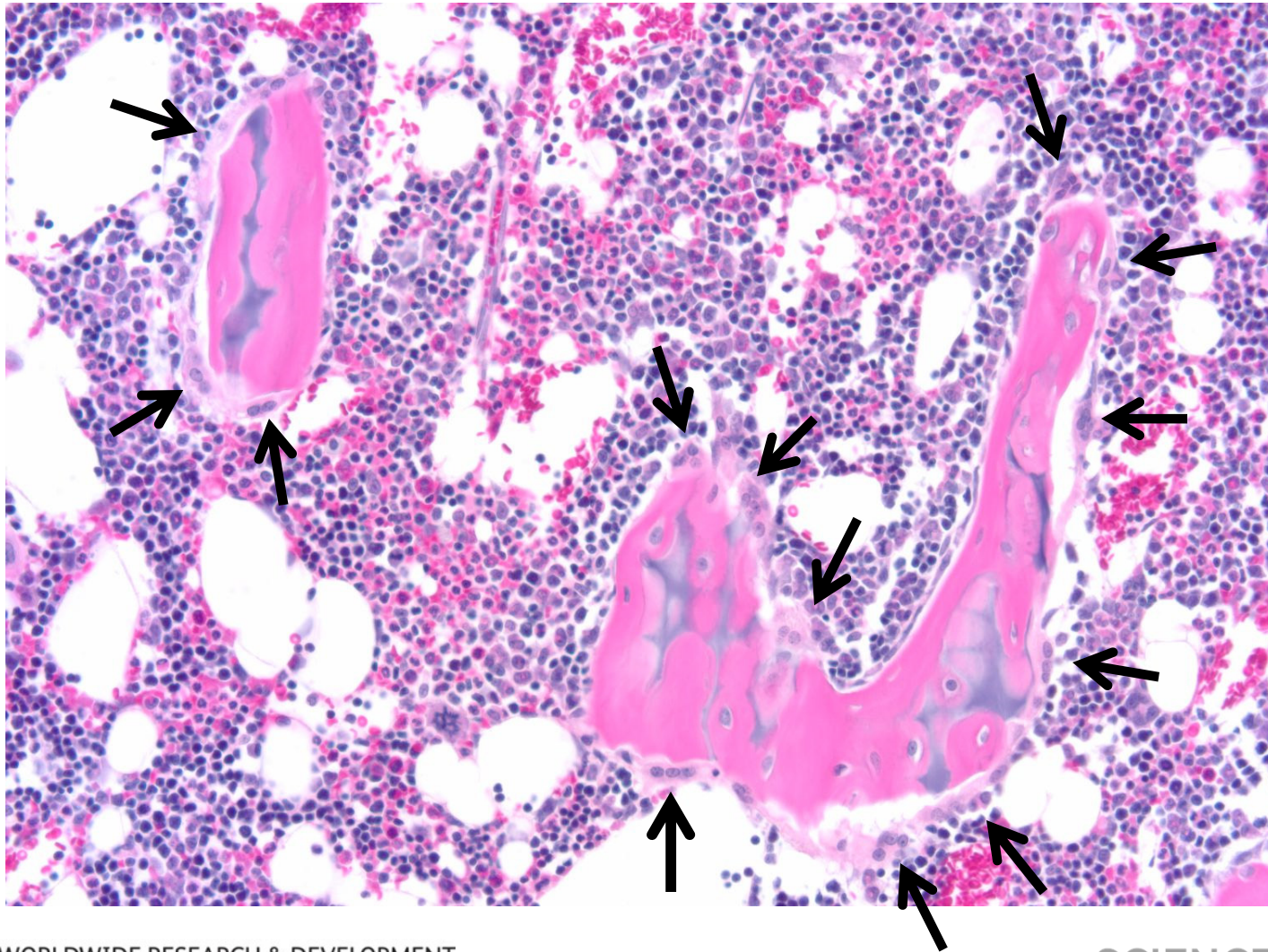
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Bone Shaping to Form Cortical Shell



Osteoclasts removing trabecular bone



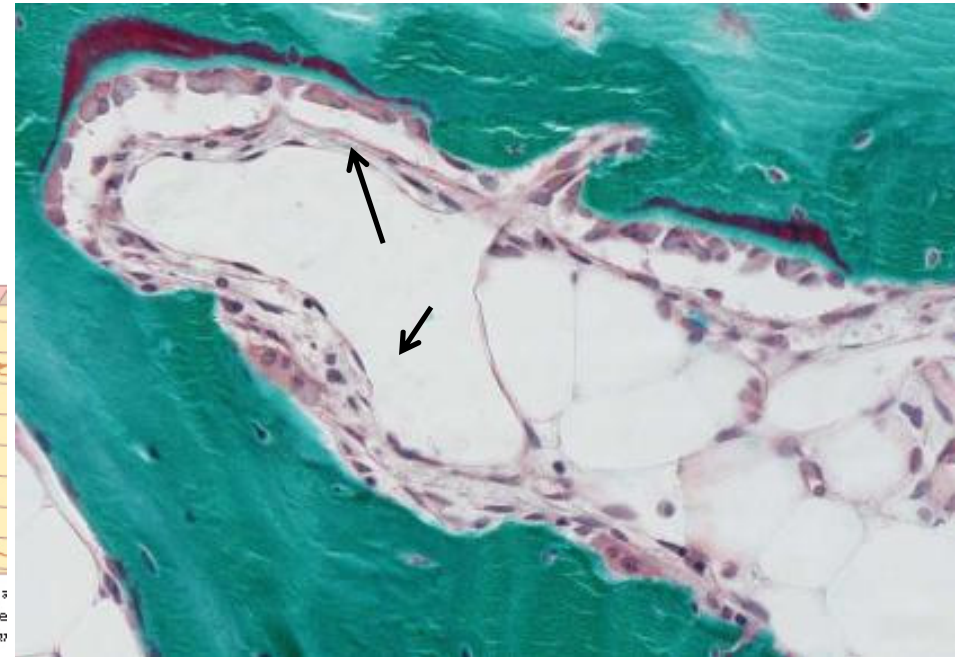
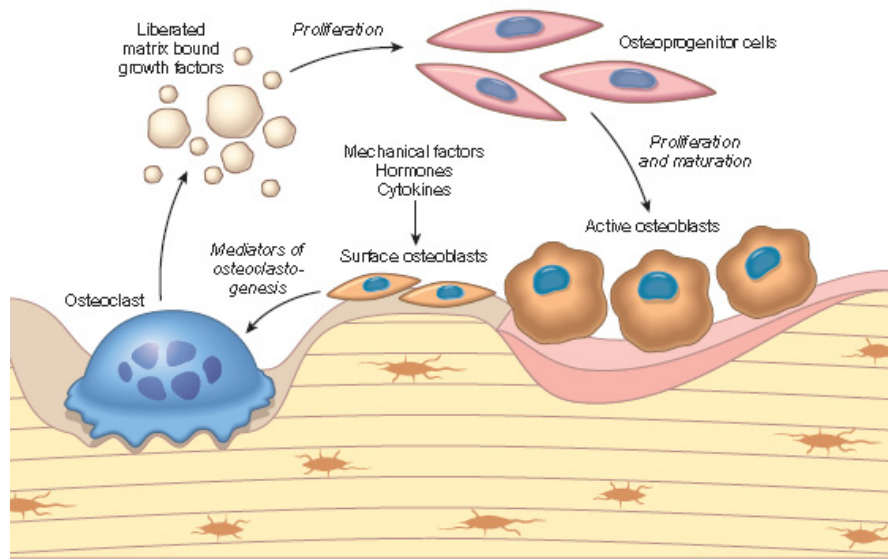
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Bone Turnover

- Accomplished by BMU-based remodeling in most species
 - BMU = Basic Multicellular Unit
 - Osteoclast and osteoblast functions are coupled in location and timing
 - Targeted remodeling
 - Response to microdamage or change in mechanical load
 - Stochastic remodeling
 - Response to homeostatic signals (ex. Ca^{++} demand)
- Once the rapid period of bone growth in young animals is over, most bone cell activity occurs in BMUs

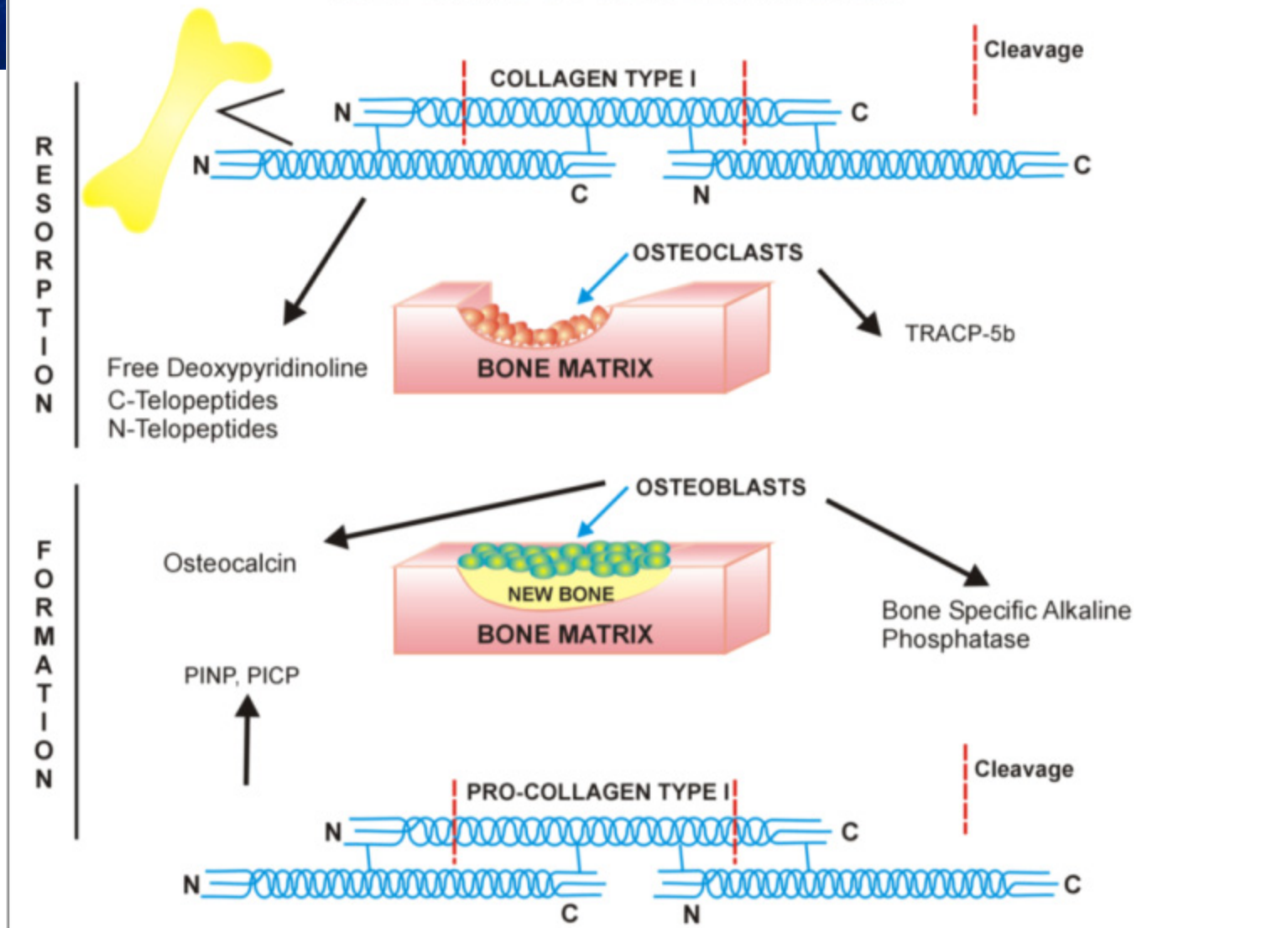
BMU-based Bone Remodeling



4.1 Bone resorption and formation are coupled processes that are controlled by systemic factors and local cytokines some of which are deposited in the bone matrix. Cytokines, growth factors, and signal-transducing molecules are key to the actions of osteoblasts and osteoclasts (From Rosenberg, A.E. (2005). Bones, joints, and soft tissue tumors. In: *Robbins and Cotran's Pathologic Basis of Disease*, 7th edn. Elsevier Saunders, New York. Figure 26-4, p. 1277).

Bone resorption and formation are coupled processes (A) where the reciprocal actions of osteoblasts and osteoclasts are controlled by systemic factors as well as local cytokines and growth factors, some of which are deposited directly in the bone matrix. (B) Basic multicellular unit (BMU) for coupled bone resorption and formation from the proximal tibia epiphysis of a cynomolgus monkey showing an active resorption surface with two multinuclear osteoclasts on the lower section of trabecula opposite active formation surfaces with cuboidal osteoblasts and red-staining osteoid on the upper section of trabecula. Note the canopy of thin tissue (arrows) separating the BMU from the marrow space. Processing conditions: undecalcified section, modified Goldner's trichrome stain. Figure (A) reproduced from Rosenberg (2005) *Bones, joints, and soft tissue tumors*, In: *Robbins and Cotran's Pathologic Basis of Disease*, 7th Ed. (V. Kumar, A.K. Abbas, and N. Fausto, eds). Elsevier Saunders, Fig. 26-4, p. 1277, with permission. Figure (B) reproduced from Gunson, Gropp, & Varola (2013). *Bone and Joints*, In: *Haschek & Rousseaux's Handbook of Toxicologic Pathology*, 3rd Ed. (W. M. Haschek, C. G. Rousseaux, and M.A. Wallig, eds) Academic Press, Fig 63-7, p. 2777, with permission.

SCHEMATIC OF BONE TURNOVER



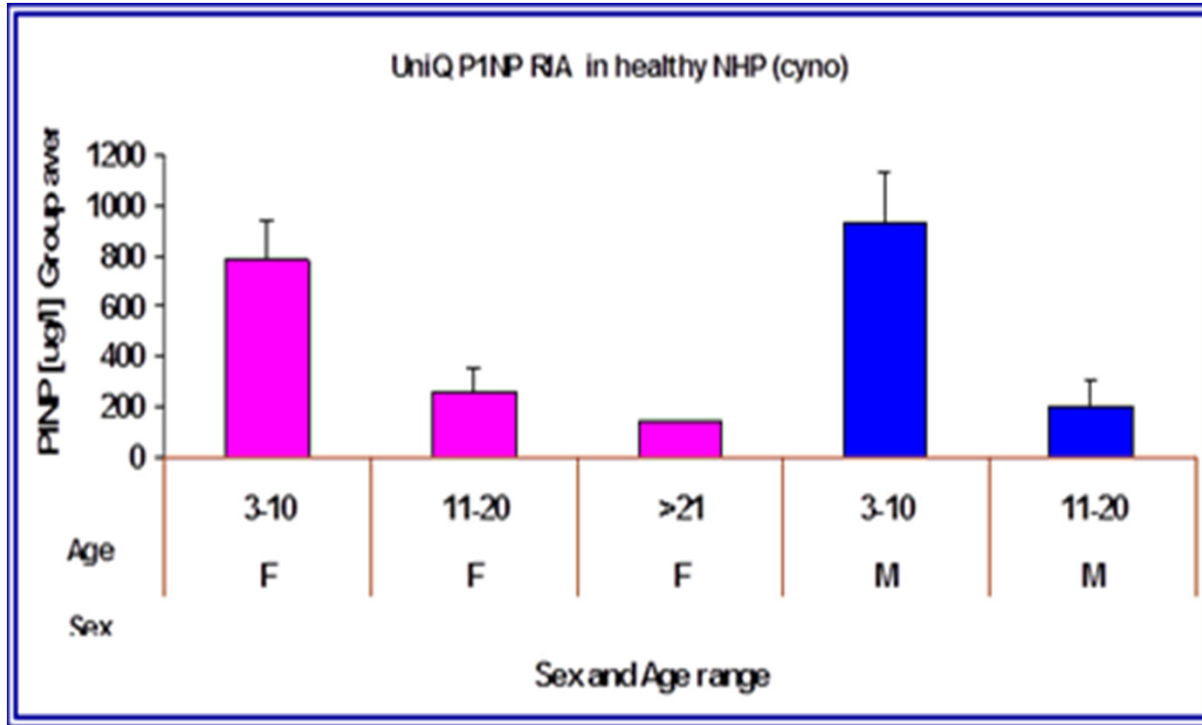
Schematic diagram of bone turnover illustrating the generation of various bone biomarkers for resorptive and formative processes. Reproduced from Haschek & Rousseaux Handbook of Toxicologic Pathology, 3rd Ed. Academic Press 2013, p. 2782.



Bone Formation Biomarkers

- Alkaline Phosphatase (ALP)
 - Bone Specific ALP
 - Can use total ALP as a surrogate
 - Keep in mind other endogenous sources (liver)
 - High levels in growing animals, decreases as bone growth slows
- Osteocalcin
 - Matrix protein
- Pro-collagen Type I Telopeptides
 - By-products of Type I Collagen synthesis
 - Amino (N)-terminal fragment (PINP)
 - Carboxy (C)-terminal fragment (PICP)

Change in PINP levels in aging Cynomolgus monkeys



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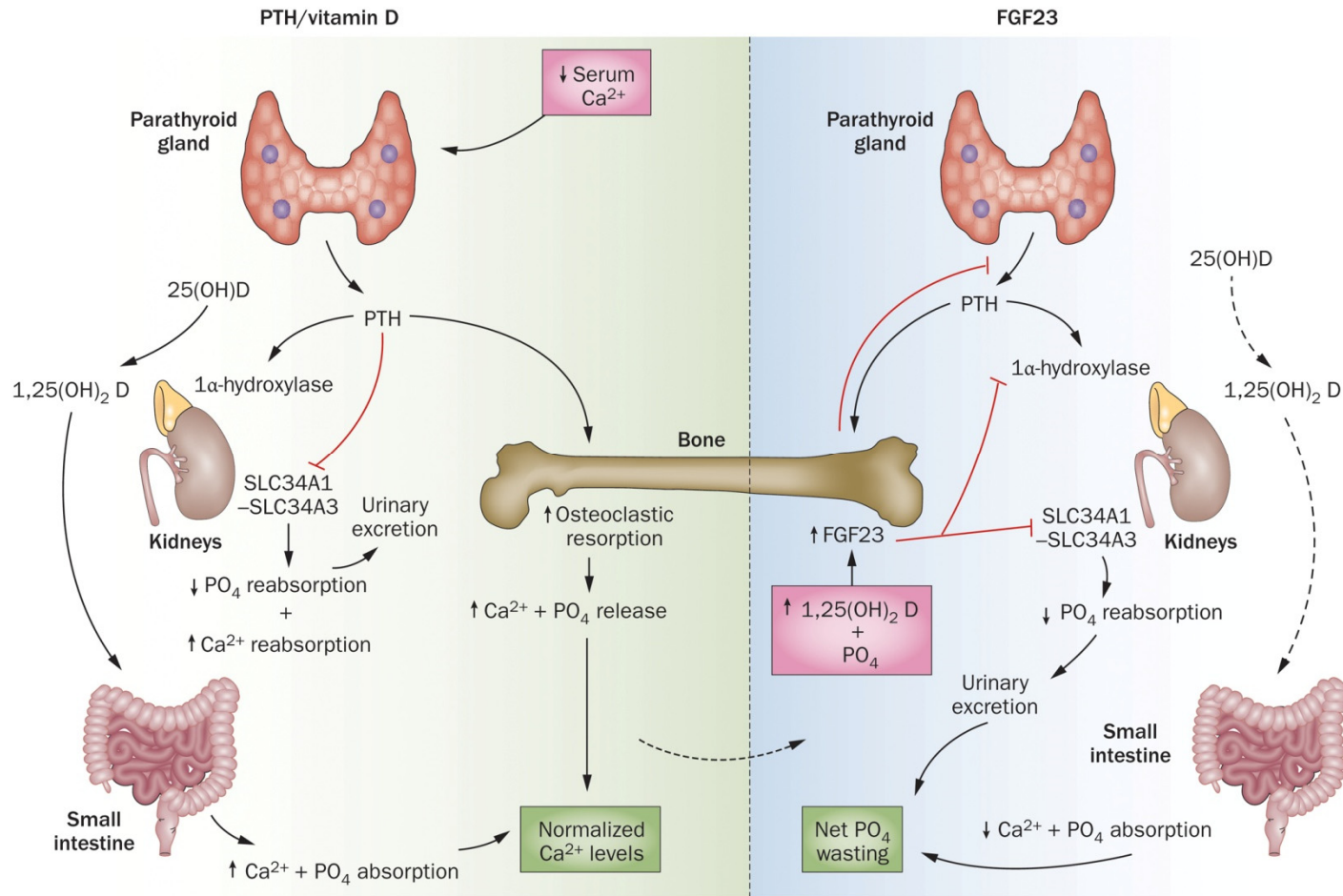
Bone Resorption Biomarkers

- Tartrate-Resistant Acid Phosphatase (TRAP)
 - TRAP5b is specific for osteoclasts
- N- or C-terminal telopeptide fragments of Type I Collagen
 - CTX-I
 - NTX-I
 - ICTP (produced by action of MMPs)
- Hydroxypyridinium crosslinks of collagen
 - Formed during extracellular collagen maturation
 - Deoxypyridinoline (DPD) (bone and dentin)
 - Pyridinoline (PYD) (bone, cartilage, tendons, ligaments and blood vessels)

Mineral and Hormone Biomarkers

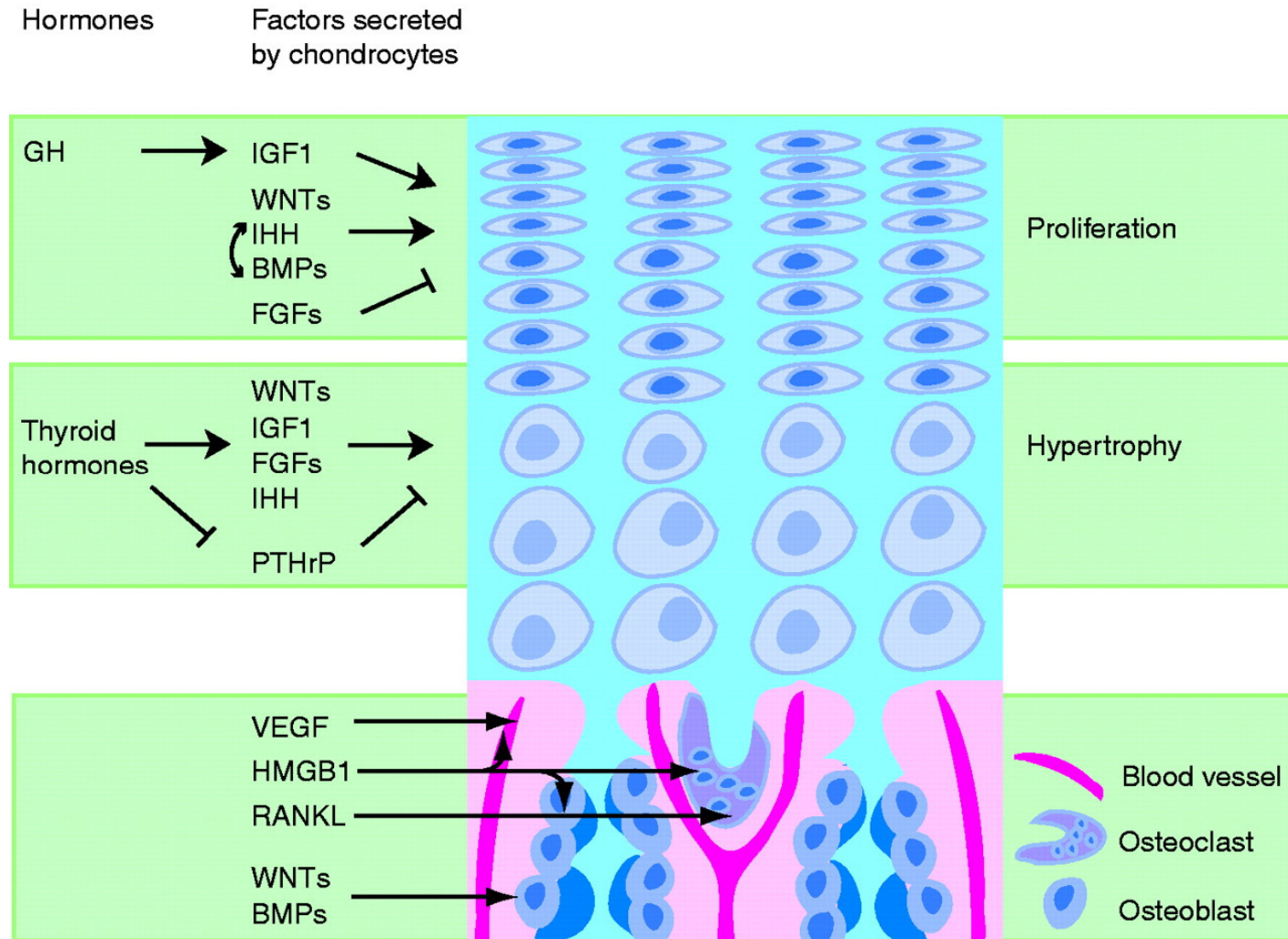
- Calcium
 - Ionized Ca^{++}
 - Regulated by PTH & Calcitonin
 - Continuously high PTH levels lead to bone loss (catabolic)
 - Intermittently high PTH levels lead to bone gain (anabolic)
 - Intestinal absorption stimulated by 1,25 dihydroxy-Vitamin D
 - Effects of vitamin D on bone are many and intense research is on-going.
- Phosphorus
 - Regulated by FGF23
- IGF1
 - Growth Hormone/IGF1 axis
 - Systemic and local IGF1
 - Lower levels lead to growth retardation

The regulation of calcium and phosphate homeostasis by PTH, vitamin D and FGF23



DiGirolamo, D. J. *et al.* (2012) The skeleton as an endocrine organ
Nat. Rev. Rheumatol. doi:10.1028/nrrheum.2012.157

Overview of the effects of factors secreted by chondrocytes on growth plate cell function and invasion.



Mackie E J et al. *J Endocrinol* 2011;211:109-121



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Key Points

Bone cellular activity is a balance between bone formation and bone resorption

Bone modeling is the predominant processes in rapidly growing animals (esp. rodents)

Bone remodeling (turnover) is the predominant process in older (and larger) animals

Bone biomarkers are be divided into two categories: markers of bone formation and markers of bone resorption

Bone biomarkers include by-products of Type I collagen synthesis or breakdown, bone matrix components, and enzymes.

Bone is integral in the maintenance and regulation of calcium and phosphorus; hormonal regulators of Ca and P have profound effects on bone.

Longitudinal bone growth is sensitive to many influences via the growth hormone/IGF1 axis.



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