Bone Metabolism
MSS/Biochemistry, fall-2017

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Learning objectives

• Describe the biochemical structure of bone composition: inorganic, collagen and noncollagen proteins
• List bone matrix proteins and functions.
• Review calcium and phosphate homeostasis in relation to PTH, calcitonin and vitamin D
• Know the biochemical functions of osteoblast and osteoclast bone cells
• Understand the role of alkaline phosphatase, calcium, phosphate, PTH, calcitonin and vitamin D in biomineralization
• List Bone biomarkers and their clinical significance
Bone

- Inorganic (67%): Hydroxyapatite $3 \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$
- Organic (33%) component is called osteoid
  - Type I collagen (28%)
  - Non-collagen structural proteins (5%)
Apatite

- Apatite is a group of phosphate minerals:
  - hydroxyapatite, Ca$_{10}$(PO$_4$)$_6$(OH)$_2$ : major component of tooth enamel and bone mineral.
  - fluorapatite, Ca$_{10}$(PO$_4$)$_6$(F)$_2$
  - chlorapatite, Ca$_{10}$(PO$_4$)$_6$(Cl)$_2$
  - bromapatite, Ca$_{10}$(PO$_4$)$_6$(Br)$_2$.
- Fluorapatite (or fluoroapatite) is more resistant to acid attack than is hydroxyapatite.
- For this reason, toothpaste typically contains a source of fluoride anions (e.g. sodium fluoride, sodium monofluorophosphate).
Collagen

Synthesis of collagen

Chains

Procollagen

Fibril

640 Å

Fibre
Intracellular processing
Extracellular processing

Secreted Procollagen Molecules

PROCOLLAGEN

PRO-PEPTIDE CLEAVAGE

FIBRIL SELF-ASSEMBLY

CROSS-LINK FORMATION
Non collagenous proteins

<table>
<thead>
<tr>
<th>protein</th>
<th>function</th>
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<tbody>
<tr>
<td><strong>Bone sialoprotein (BSP)</strong></td>
<td>nucleus for the formation of the first apatite crystals.</td>
</tr>
<tr>
<td><strong>Osteocalcin</strong> <em>(Bone gamma-carboxyglutamic acid-containing protein)</em></td>
<td>bone mineralization</td>
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<tr>
<td><strong>Osteonectin</strong></td>
<td>mineralization</td>
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</table>
Ca\(^{2+}\) and PO\(_4^{3-}\) matrix

Figure 1. Control of blood Ca\(^{2+}\) and PO\(_4^{3-}\) matrix.
Figure 1. Control of blood Ca\(^{2+}\) and PO\(_4\)\(^{3-}\) matrix

INTESTINE

Absorption

Ca\(\cdot\)PO\(_4\)

BLOOD

KIDNEY

BONE
**Figure 1.** Control of blood 
Ca$^{2+}$ and PO$_4^{3-}$ matrix

PTH, in response to low serum Ca, increases plasma Ca by increasing bone resorption, and renal reabsorption of Ca
Figure 1. Control of blood $\text{Ca}^{2+}$ and $\text{PO}_4^{3-}$ matrix

PTH prevents hyperphosphatemia, which could be caused by the PTH effect on bone resorption, by inhibiting renal reabsorption of phosphate.
Figure 1. Control of blood Ca$^{2+}$ and PO$_4^{3-}$ matrix

PTH activates the hydroxylation of 25(OH)D$_3$ to the active 1,25(OH)$_2$D$_3$ form
**Figure 1.** Control of blood Ca\(^{2+}\) and PO\(_4\)^{3-}\) matrix

1,25(OH)\(_2\)D\(_3\), in response to low serum Ca, increases plasma Ca by increasing intestinal absorption, bone resorption, and renal reabsorption of Ca.
Figure 1. Control of blood \( \text{Ca}^{2+} \) and \( \text{PO}_4^{3-} \) matrix

\( 1,25(\text{OH})_2\text{D}_3 \) increases intestinal and renal absorption of phosphate to help promote bone mineralization
Figure 1. Control of blood Ca^{2+} and PO_{4}^{3-} matrix

calcitonin (CT) can counteract the effect of PTH on bone resorption

estrogen (E2) counteracts effects of PTH and 1,25(OH)\_2D\_3 on bone resorption
Figure 1. Control of blood \( \text{Ca}^{2+} \) and \( \text{PO}_4^{3-} \) matrix

Caclcitonin (CT) can counteract the effect of PTH on bone resorption

Estrogen (E2) counteracts effects of PTH and \( 1,25(\text{OH})_2\text{D}_3 \) on bone resorption
Osteoblast and Osteoclast Function

**Osteoblasts**
- Bone formation
- Synthesis of matrix proteins
  - Type I collagen
  - Osteocalcin
  - Others
- Mineralization
- Activation of osteoclasts via RANKL production

**Osteoclasts**
- Bone resorption
  - Degradation of proteins by enzymes
  - Acidification
- RANK is activated by RANKL, and this leads to cells differentiation to osteoclasts
Mineralization Modulators

- **Promoters:** local mineralization promoters synthesized by osteoblasts
  - tissue-nonspecific alkaline phosphatase (TNAP)
  - phosphatase orphan 1 (PHOSPHO1).
- **Inhibitors:** local inhibitors by osteoblasts and osteocytes
  - inorganic pyrophosphate (PPi)
  - organic non-collagenous proteins or peptides of the extracellular matrix, such as osteopontin
- **Modulators** act in a paracrine/autocrine manner,
  - fibroblast growth factor 23 (FGF23) regulates systemic phosphate levels by creating bone–kidney–parathyroid feedback loops.
• Pi/PPi ratio controls the local promoters and inhibitors.

• Pi/PPi ratio is regulated by circulating effects of FGF23 on phosphate homeostasis.
Regulatory intercellular and intracellular signaling pathways operated by the osteoblast, to control the local Pi/PPi balance required for physiological bone mineralization.

Right side: formation of bone matrix; (A) accumulation of Ca\(^{2+}\) and Pi within MVs initiates HA formation. (B) and (C) extracellular HA growth, propagation and deposition into bone matrix. Left side: inhibition of mineralization by a low Pi/PPi ratio; (a) and (b) PPi formed within the cell is transported to the ECM. (c) PPi and also OPN inhibit mineralization. (d) TNAP upregulates mineralization by degrading PPi and by dephosphorylation of OPN. (e) In turn, Pi and PPi downregulate TNAP activity.
Negative regulation of phosphate homeostasis by FGF23

FGF23 is negatively regulated by bone signaling mechanisms (DMP1 and PHEX), and is positively regulated by systemic factors (serum Pi, 1,25(OH)$_2$D$_3$, PTH). A-H: intestine–bone–kidney–parathyroid gland feedback loops. (A) High absorbed dietary Pi stimulates FGF23 secretion. (B) 1,25(OH)$_2$D$_3$ also stimulates FGF23 secretion. (C) In turn, FGF23 suppresses kidney production of 1,25(OH)$_2$D$_3$, resulting in (D) decreased potential 1,25(OH)$_2$D$_3$-dependent Pi absorption by the intestine. (E) PTH also up-regulates FGF23 synthesis and secretion, and in turn (F) PTH synthesis is decreased by FGF23. (G) As a result, potential PTH-dependent inhibition of renal Pi reabsorption is decreased. (H) Overall elevated levels of FGF23 result in inhibited renal Pi reabsorption (phosphaturic action). (M) Local bone FGF23 excess results in inhibited mineralization. (P) The sum consequence of FGF23 action is to decrease excess serum Pi to a physiological range.
# Biochemical Markers of Bone Turnover

## Bone Formation

- **Products of active OB:**
  - Alkaline phosphatase (TAP, BAP)
  - Osteocalcin (OC)
  - Procollagen type I propeptides (PINP, PICP)

## Bone Resorption

- **Degradation products of bone collagen:**
  - Hydroxyproline (OHP)
  - Pyridinium crosslinks (PYD, DPD)
  - Crosslinked telopeptides of type I collagen (NTX, CTX, ICTP)

- **Non-collagenous proteins of bone matrix:**
  - Bone sialoprotein
  - Osteopontin
  - Osteocalcin fragments (urine)

- **Osteoclast enzymes:**
  - Tartrate-resistant acid phosphatase (TRACP 5b)
  - Cathepsin K
Deoxypyridinoline cross-linking in bone collagen.
Molecular Origin of Markers of Collagen Degradation

Asp-Glu-Hyl-Ser-Thr-Gly-Gly α1(I)
Gln-Tyr-Asp(β)-Gly-Hyl-Gly-Val-Gly α2(I)
Hyl(Lys)

CTx assay
synthetic octapeptide
Glu-Lys-Ala-His-Asp(β)-Gly-Gly-Arg

ICTP
8.5kDa peptide

NTx assay

Hydroxyproline

Pyridinium crosslinks (PYD, DPD)

Hydroxypyridinium crosslinks (PYD, DPD; HPLC, EIA)
Crosslinked telopeptides*: ICTP (CTX-MMP, carboxyterminal type I collagen telopeptide; RIA)
CTx (Linear octapeptide derived from carboxyterminal type I collagen telopeptide; ELISA)
NTx (Aminoterminal crosslinked type I collagen telopeptide; ELISA)
### Collagen Related Markers of Bone Resorption

<table>
<thead>
<tr>
<th>Marker</th>
<th>Tissue of origin</th>
<th>Analytical specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyproline (Hyp)</td>
<td>Bone, cartilage, skin, soft tissue</td>
<td>Urine</td>
</tr>
<tr>
<td>Pyridinoline (PYD)</td>
<td>Bone, cartilage, tendon, blood vessels</td>
<td>Urine Serum</td>
</tr>
<tr>
<td>Deoxypyridinoline (DPD)</td>
<td>Bone, dentin</td>
<td>Urine Serum</td>
</tr>
<tr>
<td>Carboxy-terminal crosslinked telopeptide of type I collagen (ICTP, CTX-MMP)</td>
<td>Bone, skin</td>
<td>Serum</td>
</tr>
<tr>
<td>Carboxy-terminal crosslinked telopeptide of type I collagen (CTX-I)</td>
<td>All tissues containing type I collagen</td>
<td>Urine (α/β) Serum (β only)</td>
</tr>
<tr>
<td>Amino-terminal crosslinked telopeptide of type I collagen (NTX-I)</td>
<td>All tissues containing type I collagen</td>
<td>Urine Serum</td>
</tr>
</tbody>
</table>
Metabolic Bone Disorders

- Osteoporosis
- Paget’s disease of bone
- Osteomalacia in adults and Rickets in children
- Osteogenesis imperfecta
Osteoporosis

• Osteoporosis is a disease where **decreased bone strength** increases the risk of a broken bone

• The underlying mechanism in all cases of osteoporosis is an **imbalance between bone resorption and bone formation**.
Osteoporosis Labs Blood tests

- PTH
- 25 OH vitamin D
- PO4
- 24 hour urine calcium
Osteomalacia

- Osteomalacia is the **softening of the bones** caused by impaired bone metabolism primarily due to: inadequate levels of available phosphate, calcium, and vitamin D,

- Biochemical findings
  - low vitamin D concentration in blood serum.
  - Low serum and urinary calcium
  - Low serum phosphate.
  - Elevated serum alkaline phosphatase (due to an increase in compensatory osteoblast activity)
  - Elevated parathyroid hormone (due to low calcium)
Osteogenesis imperfecta (OI)

- Osteogenesis imperfecta (OI) is the result of a mutation in one of the two genes that carry instructions for making type 1 collagen (the major protein in bone and skin).
- The mutation may result in either a change in the
  - structure of type 1 collagen molecules, (quality)
  - in the number of collagen molecules made (quantity).
- Either of these changes results in weak bones that fracture easily.