

BIOCHEMISTRY #1

مجموعة التفريغ السريع

MISS

Subject: *BONE METABOLISM*

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Bone Metabolism

The objectives of this lecture:

- Discusses the biochemical structure of bone tissue, the collagen matrix (organic) and the hydroxyapatite cement (inorganic).
 - List bone matrix proteins and describe their function.
 - Describe the Composition of calcified tissues, calcification in bones and teeth and formation of hydroxyapatite.
 - Understand the role of alkaline phosphatase, calcium and phosphate and vitamin D: 1, 25-Dihydroxy-vit-D in bone formation and remodeling.
 - Review calcium and phosphate homeostasis
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Bone:

1. Inorganic constituent (67%):

Main component is “Hydroxyapatite $3\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ”.

-These are **phosphate salts, mainly salts of calcium**.

-It is composed of *calcium, phosphorus* and *hydroxyl group*.

-When it is crystalized, it will harden the tissue and the bone become harder.

-It will be **crystalized on specific proteins**, like collagen type 1 and other proteins.

-Once it's crystalized and calcified → the bone become hard.

There is some amorphous calcium phosphate

2. Organic (33%) component is called osteoid :

Consist of:

a. Type I collagen (28%)

b. Non-collagen proteins (5%) like:

i. Proteoglycans

ii. Glycosaminoglycan's

iii. Sialoproteins

iv. Gla-containing proteins (gamma carboxyglutamate)

v. Phosphoproteins

vi. Bone specific proteins: osteocalcin, osteonectin

vii. Growth factors -as proteins- and cytokines (Trace)

Apatite

Apatite is calcium phosphate salts or hydroxylated ones.

Hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$: is the major Apatite form; because of the hydroxyl group, specially the H^+ on the hydroxyl group.

It will help in binding to calcium and calcification process.

Other apatite forms:

- Chlorapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{Cl})_2$.
- Bromapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{Br})_2$.
- fluorapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{F})_2$:

Fluorapatite (or fluoroapatite)

Represent large portion of dentin and enamel in the teeth.

It is more resistant to acid attack than is hydroxyapatite.

For this reason, toothpaste typically contains fluoride (e.g. sodium fluoride, sodium monofluorophosphate).

Bone calcification

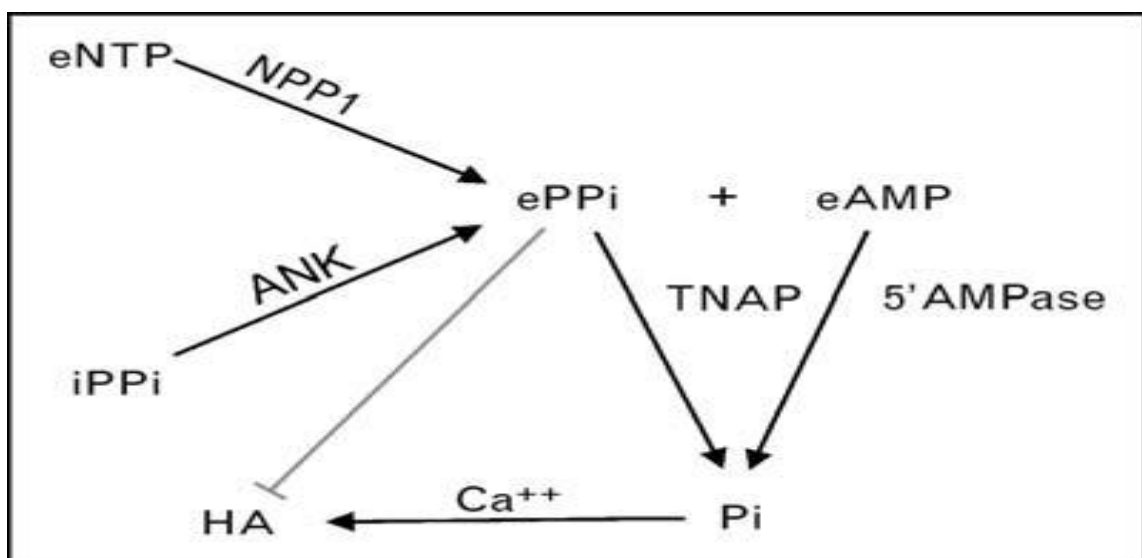
- What is involved in calcification is the crystallization of hydroxyl apatite on collagen type 1.
- Collagen is the most important base for hydroxyapatite to start crystallization on it.
- It occurs in the extracellular spaces.
- Osteoblasts help in triggering and promoting the crystallization process; because it produce some other factors and proteins that will help in the process of calcification.
- The rate of crystallization depends on Ca^{2+} and PO_4 . We are going to see how the body will control the concentration of Ca^{2+} and PO_4 , and the mechanism of keeping the concentrations available for crystallization and calcification.

- There are some inhibitors of calcification; because we don't want an always formation of bones, sometimes we need to resorp. Such as proteoglycans.

This is the role of alkaline phosphates in the process of calcification.

The most important thing for calcification is the availability of phosphorus and calcium.

Alkaline phosphates is an enzyme that will provide a lot of inorganic phosphate to be available for formation of carboxyapatite and start the process of crystallization and calcification.



- ✓ So as you see here from many sources the **TNAP (=tissue non-specific alkaline phosphatase)** it will hydrolyze different substrates that have **PHOSPHATE** such as (eAMP, eNTP, etc.). So these enzymes will help to provide the pyrophosphate, and the alkaline phosphatase will hydrolyze these pyrophosphates from other nucleotides and produce an inorganic phosphate.
- ✓ In the presence of an inorganic phosphate -in assistance of alkaline phosphatase- and Ca ions, hydroxyapatite will be produced.

So the role of the alkaline phosphate is to provide more inorganic phosphate from different substrates in the bone tissue, with the presence of calcium that is controlled by vitamin D and PTH (parathyroid hormone) and other factors

So to sum up >>>>

Inorganic phosphate + calcium ions + hydroxyl groups = hydroxyapatite.

- ❖ Remember that the presence of pyrophosphate is an inhibitor for hydroxyapatite formation, so it must be hydrolyzed.
-

Now let's talk about type 1 collagen:

It's a fibrous protein dominant in bone tissue and composed of 3 polypeptide chains (two alpha 1 (I) and one alpha 2(I)) each one comes from different gene. The three of them will combined with each other and linked in a specific helical structure and form a right handed helical (triple helix).

Each one is composed of 1050 amino acid, and in each amino acid the 3^{ed} one is GLYCINE. In addition to that there is proline and lysine amino acids and there are also some modifications of these lysine and proline.

The importance of having these glycine every 3^{ed} amino acid in this 1050 amino acid chain is its size. So there will be no spaces when the 3 polypeptide chains twists on each other and thus they will provide a very strong structure of collagen fibrils. Suppose it wasn't glycine, let's say it was valine for example, that will create a very elastic and flexible form of collagen and thus we will have elastic and flexible bone structure.

So having glycine as 33 % in amino acid composition of these polypeptide chains is very important to give strong structure.

Now modifications of lysine and proline is very important; because they will participate in the covalent linkages between collagen fibrils to form collagen fibers. That will also give the collagen molecule a strength to be structural molecule in the bone.

Collagen requires vitamin C as a coenzyme for one of the hydroxylases (the proline hydroxylase or lysine hydroxylase) in order to modify these amino acids.

If the vitamin C is deficient → very weak collagen molecule will presence; because there will be no covalent linkages between hydroxylysine and lysine or hydroxyproline and proline.

There are more than 50 types of collagen depending on the genes that will produce the polypeptide chain.

In general: Every collagen molecule is a triple helix.

The production of collagen type 1:

As we have mentioned before, collagen type one consists of two alpha 1(I) and one alpha 2(I) polypeptide chain.

1. **Preprocollagen** (inside the cell):

It is synthesized **in the ribosomes in the (RER)**.

It has a **signal peptide** as well as **registration regions** at the C terminal.

The registration regions are very important; because it will be used as a marker for bone formation and bone remodeling.

If there are any bone disease one could look at these collagen components and use them as markers for diagnosing.

2. **Pro-alpha chains:**

It is formed after a signal peptide has been removed from the previous step which is the preprocollagen.

Signal peptide is removed by an enzyme called **SIGNAL PEPTIDASE** in the lumen of endoplasmic reticulum.

3. **Procollagen:**

Occurs inside the lumen of endoplasmic reticulum.

The hydroxylation and glycosylation will take place for the lysine and proline, and as we mentioned vitamin C is very important.

Then this procollagen after it has been modified by hydroxylation and glycosylation, it will be removed outside the cell and will be converted to

4. **TROPOCOLLAGEN**

The registration peptides will be cleaved by specific peptidases at the C-terminal. Then the

5. **Collagen fibrils** will be formed after multiple tropocollagen molecules linked to each other.

So, tropocollagen molecule signal peptides have been removed, registration regions have been removed, modification in terms of hydroxylation and glycosylation take place and removed outside. There, many tropocollagen molecules will be linked to each other to make what is called **collagen fibril**.

6. Many bundles of collagen fibrils will link to each other to form **collagen fibers**, which are found in bones and used as a matrix for crystallization of hydroxyapatite and forming the bone.

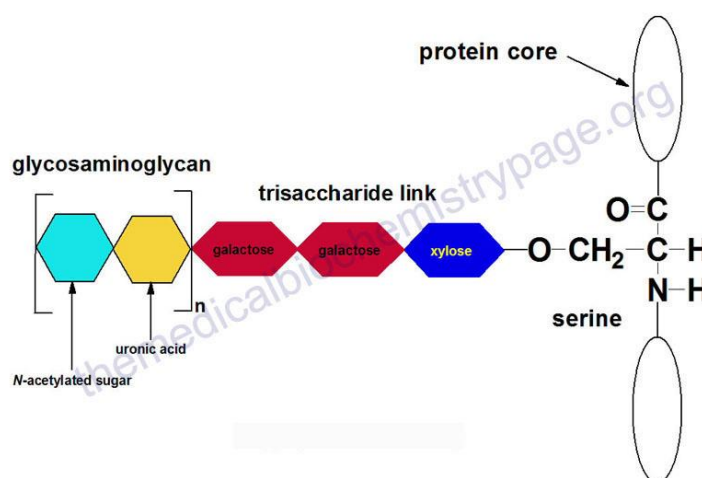
What is the importance of collagen type one to bone?

Apatite will be crystalized and use it as a substrate.

These are different proteins which are non-collagen protein as components of bone and as you see proteoglycans, glycosaminoglycans (only remember the structure of them, we are not supposed to remember the details).

Proteoglycan as you see is composed of a protein called core protein.

From it there are brush-like structures coming outside that protein. Brush-like structures are trisaccharides as you see xylose and galactose in glycoside bond with serine (as one of the components of protein core) and attached to them glycosaminoglycans.



These structures which are found in bone are important for binding with cations such as Ca, Na, K and they can bind with water.

So because of this they could act as natural inhibitor of crystallization and formation of hydroxyapatite because they bind with Ca and prevent crystallization of Ca with phosphorus and hydroxyl group to form hydroxyapatite.

The functions of proteoglycans:

- Major component of ECM.
- Bind cations (Na, K, and Ca) and H₂O.
- Act as lubricant

This part of proteoglycans, which is composed of repeating units of sugars and those sugars are glucuronic acid and glucose amine sugar, is negatively charged, bind with cations and act as a natural inhibitor of crystallization.

Bone sialoprotein

It's a protein helps in process of mineralization, formation of apatite crystals and calcification. Also this protein will help direct, redirect or inhibit apatite growth; because not always we want to form bones but sometimes the body need the bones to be resorped.

Osteocalcin

It is a protein produces from osteoblasts, has carboxyglutamic acid, it binds with Ca and used as **marker for bone formation**. It is **also important for mineralization and Ca homeostasis**.

Osteonectin

It is another protein produced from osteoclasts.

It is important for mineralization and increase the production and activity of some metalloproteinase (These are proteases enzymes that will attack any invading cancer cells within the body).

Ca and P homeostasis:

We want to see how these minerals are maintained or produced.

Bones, intestine and the kidney are the organs that are important or participate in the process of Ca homeostasis.

The availability of Ca in the blood is low → bone resorption will be increased by the process of PTH. More Ca will be produced and the reabsorptions of Ca by kidney by the effect of PTH will increase Ca concentration.

At the same time PTH will prevent Phosphorus reabsorption; because we don't want excess Phosphorus. Also PTH will activate hydroxylase enzyme in the kidney and produce the active form of vitamin D.

Once vitamin D has been activated it will increase bone resorption, more Ca will be released into the blood, also Ca and P absorption will be increased as a result of vitamin D.

So all these factors will help in maintaining Ca and Phosphorus concentrations in normal range by the effects of bone resorption or intestinal absorption or by effect on kidney reabsorption.

Estrogen and calcitonin have an effect of vitamin D on bone reabsorption.

This how calcium and phosphorus are maintained in blood by the effect of PTH, Vitamin D, calcitonin and estrogen.

Bone remodeling

We require specific cells. These specific cells are:

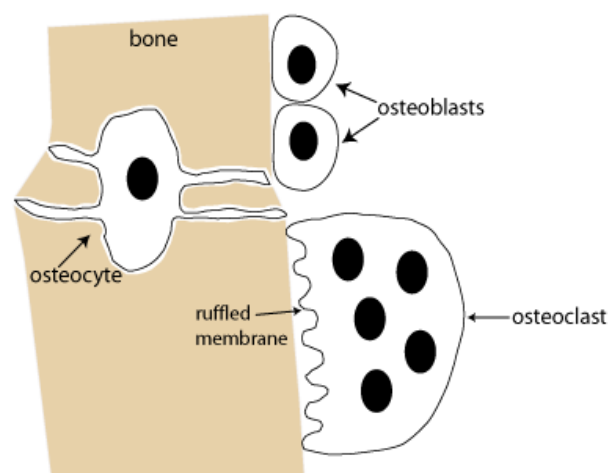
- 1- Osteoblast: responsible for formation or building the Bones.
- 2- Osteoclast: resorption or breaking bones down.

We are going to see how these cells are integrated to do their job in forming and resorption.

Osteoclasts:

(Very big and multinucleated cells with ruffled membrane) it will produce some acids that will dissolve bone and degrade some proteins.

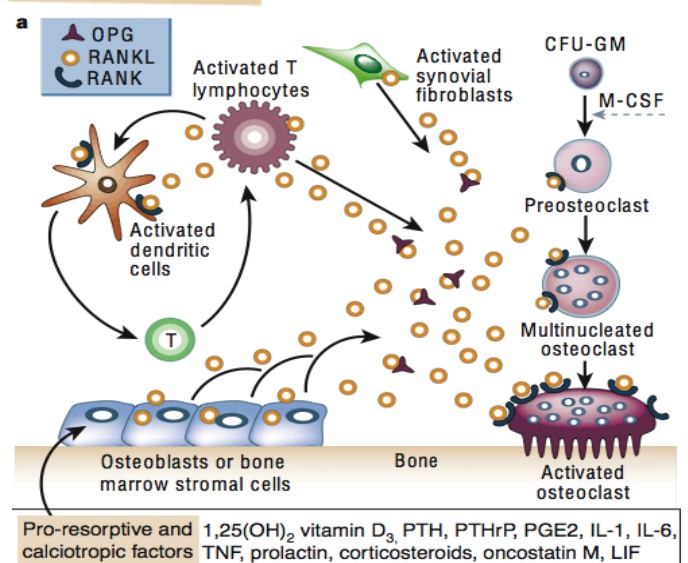
Also these are bone cells which are osteocytes.



Osteogenesis

Osteoblast will produce important protein called **RANKLs** that activate **osteoclasts** for remodeling.

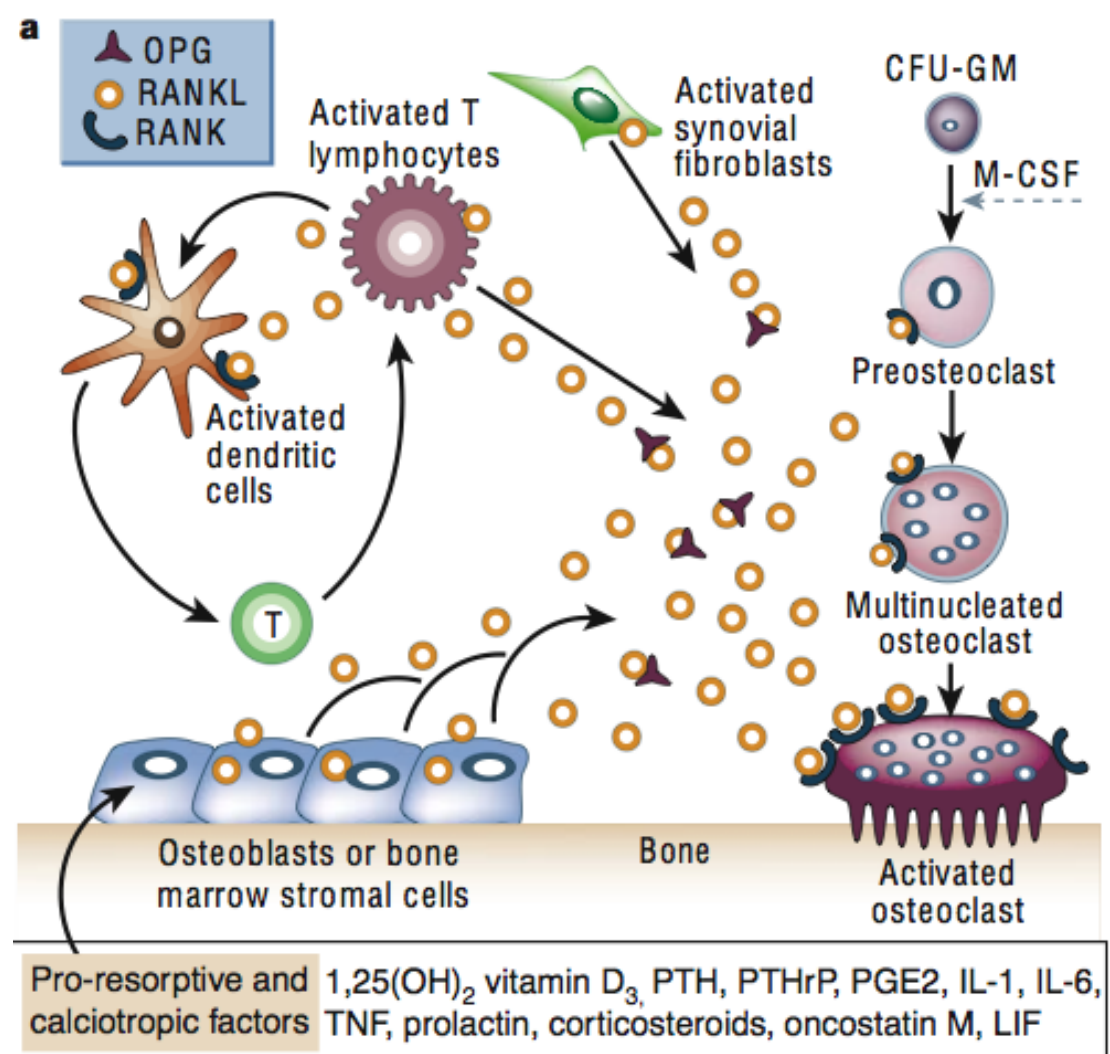
Once **RANKLs** are produced they bind specific receptors on osteoclast.



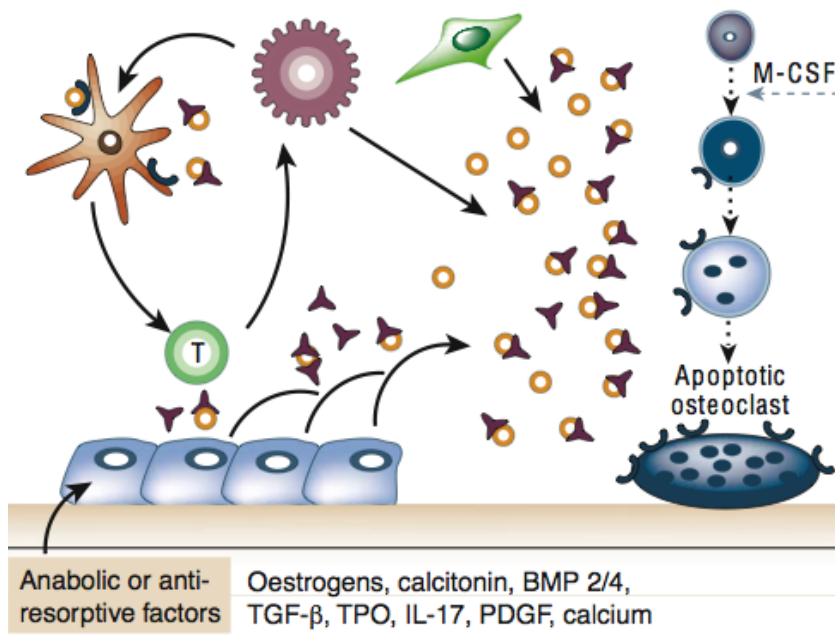
(RANKLs receptors resulting in activation of osteoclast precursor cells (in bone marrow) and grow resulting in formation of the ruffled membrane, thus beginning of reabsorption process.

Another protein called osteo-protegrin produced from T-lymphocyte or other activated cells. It is an important protein that controls remodeling; because it reduce the concentrations of RANKLs.

So once the body does not want a lot of osteoclast, the expression of osteo-protegrin will increase and stabilize binding to RANKLs. Thus decrease the rate of synthesis of osteoclast

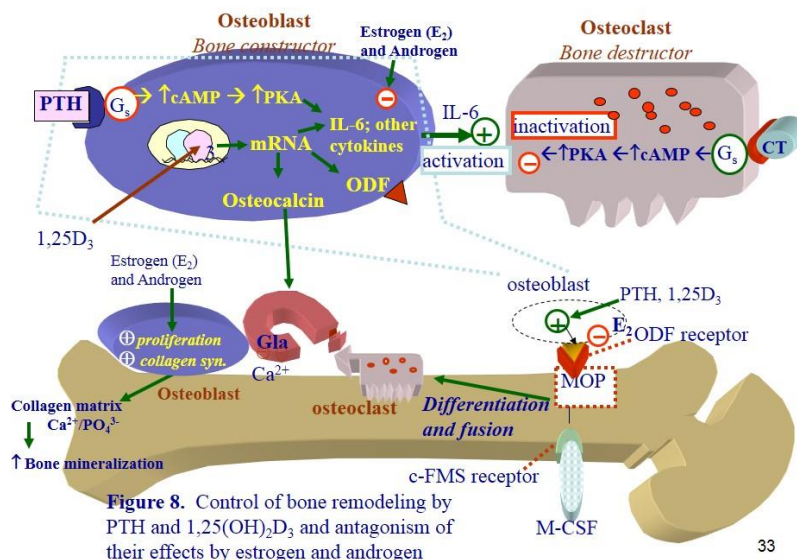


As you see here vitamin D, PTH and other activating factors will help to produce these protein and activation.



And this one shows the anti-reabsorptive factors and how it acts like estrogen, calcitonin and others which will cause production of osteo-protective protein that stabilize RANKLs preventing them from binding to the precursors of osteoclast and thus osteoclast will not be formed or activated and reabsorption will stop.

As you know our bones under dynamic process of formation and deformation.



This is to show you the interaction between osteoblast and the production of RANKLs and how they activate osteoclasts. Also, how vitamin D will activate osteoblast and how these interaction will help osteoclast differentiation into

osteoblast. The same thing here for the effect of estrogen on mineralization and proliferation of collagen

Biochemical Markers of Bone Turnover	
Bone Formation	Bone Resorption
<u>Products of active OB:</u> <ul style="list-style-type: none"> ▶ Alkaline phosphatase (TAP, BAP) ▶ Osteocalcin (OC) ▶ Procollagen type I propeptides (PINP, PICP) 	<u>Degradation products of bone collagen:</u> <ul style="list-style-type: none"> ▶ Hydroxyproline (OHP) ▶ Pyridinium crosslinks (PYD, DPD) ▶ Crosslinked telopeptides of type I collagen (NTX, CTX, ICTP) <u>Non-collagenous proteins of bone matrix:</u> <ul style="list-style-type: none"> ▶ Bone sialoprotein ▶ Osteopontin ▶ Osteocalcin fragments (urine) <u>Osteoclast enzymes:</u> <ul style="list-style-type: none"> ▶ Tartrate-resistant acid phosphatase (TRACP 5b) ▶ Cathepsin K

These are some of biochemical markers that help in diagnoses bone diseases and from understanding the structure of the bone in terms of collagen and other proteins you could take biopsies which will tell you about the pathology.

Among those markers for bone **formation** alkaline phosphatase, osteocalcin and procollagen type 1 propeptide are very important.

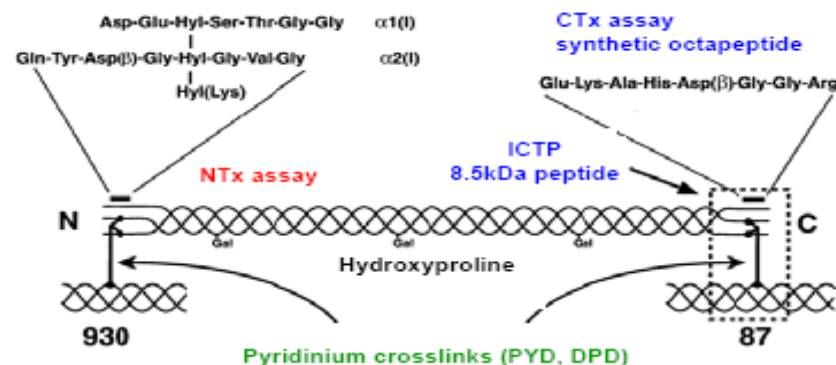
For **degradation**: hydroxyproline, pyridinium crosslinks between collagen fibrils, sialoprotein, titrate resistant acid phosphatase, cathepsin K they are important markers for bone reabsorption.

The next 2 tables have got no comment or explanation from the doctor enjoy with them ^_^

Collagen Related Markers of Bone Resorption

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

Molecular Origin of Markers of Collagen Degradation



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)

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