Calcium Homeostasis

Anti-osteoporotic drugs

Calcium

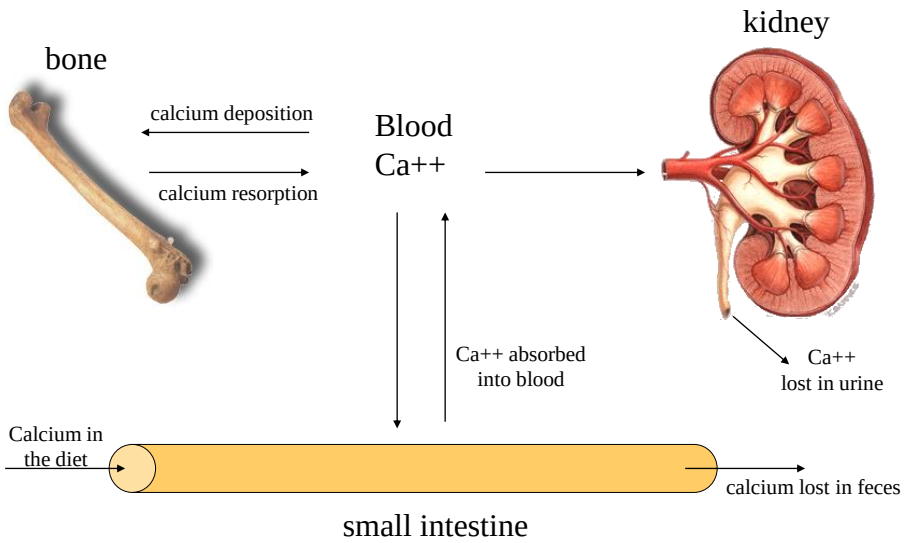
- Functions
  - Major constituent of bone – Supportive
  - Nerve conduction - Responsible for exocytosis of secretory granules in neuronal synapses
  - Muscle contraction
  - Blood clotting
  - Cofactor of many enzymes implicated in signal transduction
    (Serves as second messenger in many cells)
- Blood: Ca++ level usually 10 mg/100 ml (so 500 mg total in plamsa = 0.5 g)
- Ca++ levels change
  - after a large meal
  - during a growth spurt
  - during pregnancy or lactation
  - During disease
- Hyper or hypo condition – detrimental to health

HOMEOSTASIS

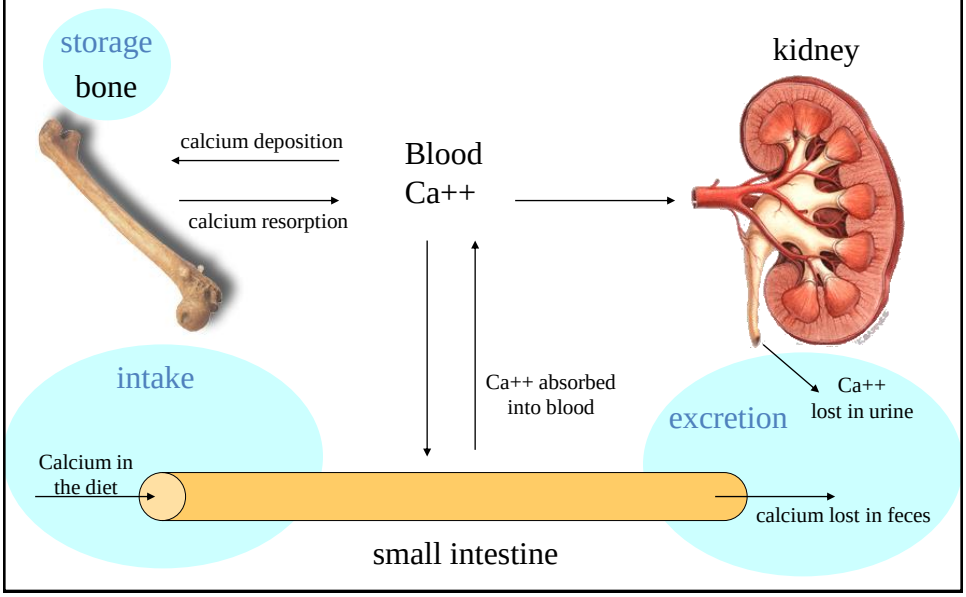
Calcium homeostasis

- Regulation of calcium levels occurs in three different organs:
  - Bone
  - Kidney
  - Small intestine
- Via Hormonal / Molecular control
  - Calcitonin
  - Parathyroid hormone
  - Vitamin D3

Calcium homeostasis



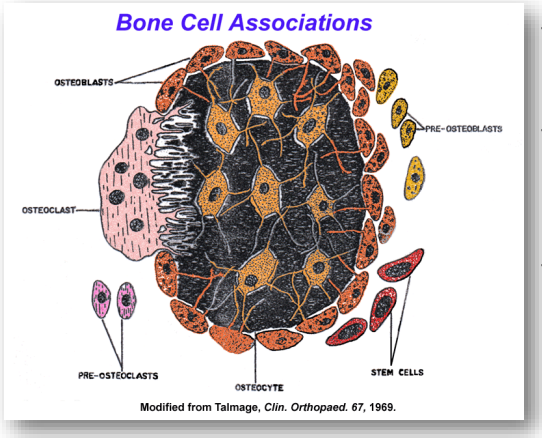
Calcium homeostasis



Bone

Bone is a complex tissue including

- Organic - Collagen
- Inorganic - hydroxyapatite a complex calcium salt including phosphate, carbonate, magnesium, & other ions.
- Cellular components.
  - osteoblasts that cover the surface of bone & help deposit bone mineral
  - osteocytes, also bone builders, which occupy interior locations in bone that eventually are walled off by mineral deposition
  - osteoclasts, large multinucleate cells, which are probably modified macrophages, that act to break down & remodel bone



Calcium cycling in bone tissue

- Bone formation
  - Osteoblasts
    - Synthesize a collagen matrix that holds Calcium Phosphate in crystallized form
    - Once surrounded by bone, become osteocyte
- Bone resorption
  - Osteoclasts
    - Change local pH, causing Ca++ and phosphate to dissolve from crystals into extracellular fluids
    - Bone breakdown

Osteoblasts are responsible for deposition of Ca in bone from plasma
Osteoclasts are responsible for resorption of Ca from bone to plasma

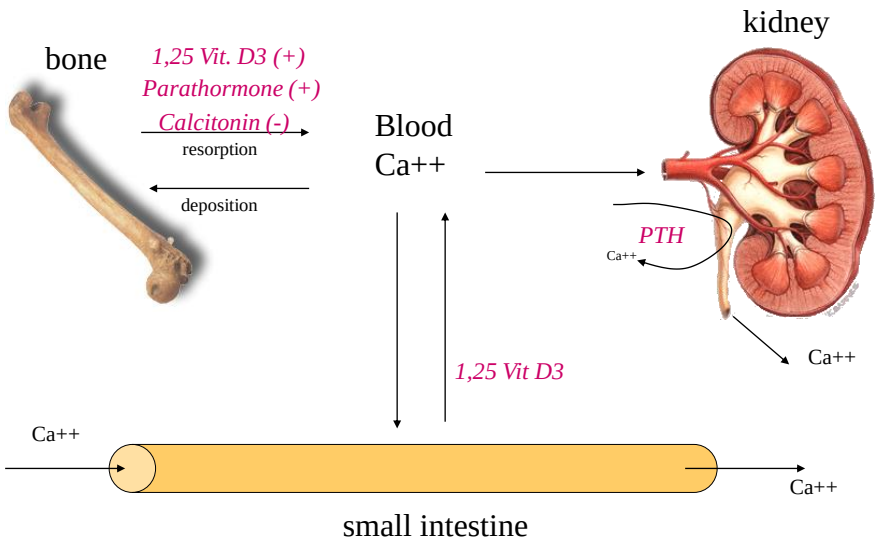
Hormonal Regulators

- **Calcitonin (CT)**
- **Parathormone (PTH)**
- **1,25 Vitamin D3**

Hormonal Regulators

- **Calcitonin (CT)**
  - Lowers Ca++ in the blood
  - Inhibits osteoclasts
- **Parathormone (PTH)**
  - Increases Ca++ in the blood
  - Decreases excretion of Ca++ from the kidneys
  - Stimulates osteoclasts
- **1,25 Vitamin D3**
  - Increases Ca++ in the blood
  - Increase Ca++ uptake from the gut
  - Stimulates osteoclasts

Calcium homeostasis



Estrogens

- Other hormonal regulators of Ca++ homeostasis: **Estrogens**
  - stimulate osteoblast activity , limits osteoclast activity
  - **Increase Ca++ storage in bone**

- **Clinical aspects**: -Osteoporosis
  - decalcification and loss of bone matrix from the skeleton
  - maximum bone mass is achieved in women at age 35
  - in the 30 years after menopause, women lose 30-50% of their bone mass
  - Treatment:
    - estrogen replacement
    - increased Ca++ in the diet (slow down Ca++ turnover from bone)
    - exercise (especially weight bearing activities)
      - stimulates bone deposition
      - raquet arm of tennis players is 35% more dense than other arm

Osteoporosis

Osteoporosis

- Progressive bone disease that is characterized by a
  - decrease in bone mass and density (BMD)
  - Deterioration of bone micro-architecture
- The disease may be classified as
  - primary type 1 or postmenopausal osteoporosis
    - The form of osteoporosis most common in women after menopause is referred to as, which is attributable to the decrease in estrogen production after menopause.
  - Primary type 2 osteoporosis or senile osteoporosis
    - occurs after age 75 and is seen in both females and males at a ratio of 2:1.
  - Secondary osteoporosis
    - may arise at any age and affect men and women equally
    - this form results from chronic predisposing medical problems or disease, or
    - prolonged use of medications such as glucocorticoids, when the disease is called steroid- or glucocorticoid-induced osteoporosis.
- Osteoporosis itself has no symptoms
- Main consequence is the increased risk of bone fractures - fragility fractures.
- Typical fragility fractures occur in the vertebral column, rib, hip and wrist

Mechanism

- The underlying mechanism in all cases of osteoporosis is an imbalance between bone resorption and bone formation
- In normal bone, matrix remodeling of bone is constant
  - up to 10% of all bone mass may be undergoing remodeling at any point in time.
- Bone is resorbed by osteoclast cells (which derive from the bone marrow), after which new bone is deposited by osteoblast cells.
- The three main mechanisms by which osteoporosis develops are
  - an inadequate bone mass (the skeleton develops insufficient mass and strength during growth),
  - excessive bone resorption,
  - and inadequate formation of new bone during remodeling.
  - An interplay of these three mechanisms underlies the development of fragile bone tissue.

Who Gets Osteoporosis?

- Calcium/vitamin D
  - Low intake
  - Low sun exposure
- Age - Slow down of bone build up (Senile osteoporosis)
- Hormone imbalance
  - Estrogen deficiency
  - Testosterone deficiency
  - Post-menopausal syndrome
  - Hyperthyroidism
  - Hyperparathyroidism
- Family history/genetics
- Poor Lifestyle
  - Lack of exercise
  - Smoking
  - Alcohol
  - Low body weight/anorexia
- Disease
  - Liver and renal disease (Vitamin D synthesis affected)
  - Malignancies (metastatic disease; multiple myeloma can present as osteopenia!)
- Medications
  - Glucocorticoids
  - antiepileptics
  - heparin
  - Prednisone

Fracture Reduction

- Goal: prevent fracture, not just treat Bone Mineral Density
- Osteoporosis treatment options
  - Calcium and vitamin D
  - Calcitonin
  - Parathyroid Hormone
  - Estrogen replacement
  - **Selective Estrogen Receptor Modulators - Raloxifene**
  - **Bisphosphonates – Alendronate, Teriparatide**

Classification

- Antiresorptive agents
  - SERM - Raloxifene
  - Bisphophonates - Alendronate
- Bone forming agents
  - Recombinant PTH - Teriparatide

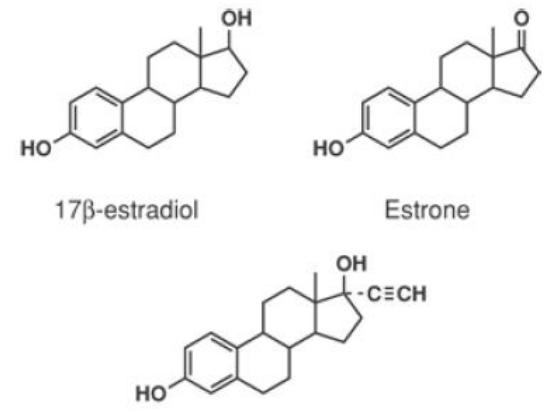
Estrogen – Antiresorptive agent

- Postmenopausal or estrogen deficiency increases risk for osteoporosis
- Estrogen replacement effective in conservation of bone and protection against osteoporotic fracture after menopause

- Mechanism
  - Reduction in bone resorption
    - Suppresses the proliferation and differentiation of osteoclasts
    - Increases osteoclast apoptosis.
  - Estrogen improves calcium absorption

- Side effects: increased risks of heart disease and breast cancer found in chronic treatment

Estrogens



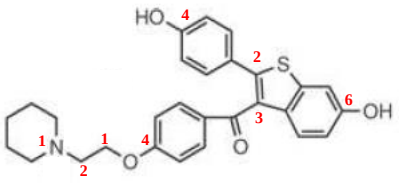
Selective Estrogen Receptor Modulators (SERMs) - Raloxifene

- Raloxifene (marketed as Evista by Eli Lilly and Company) is an oral selective estrogen receptor modulator (SERM) that has estrogenic actions on bone and anti-estrogenic actions on the uterus and breast.
- Decrease bone resorption like estrogen
- No increased risk cancer (decrease risk breast cancer)

- Mechanism
  - Reduction in bone resorption
    - Suppresses the proliferation and differentiation of osteoclasts
    - Increases osteoclast apoptosis.

Raloxifene

- Raloxifene possesses agonist activity in certain tissues (e.g. , bone and cardiovascular) and antagonist activity in others (e.g. , breast and uterus)
- Raloxifene, the first SERM approved for the prevention of osteoporosis in postmenopausal women, acts as an estrogen agonist on receptors in osteoblasts and osteoclasts but as an antagonist at breast and uterine estrogen receptors.
- This selective action means that this agent does not increase the risk of endometrial or breast cancer

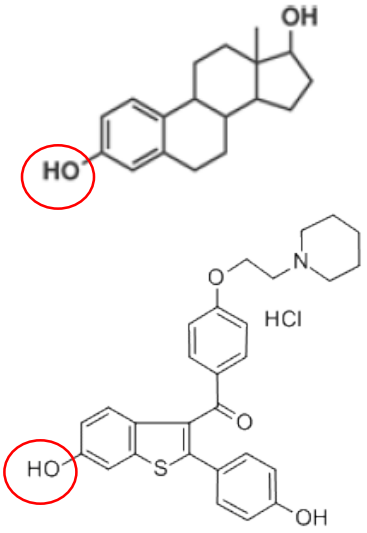


Raloxifene hydrochloride (Evista)

[6-hydroxy-2-(4-hydroxyphenyl)-benzothiophen-3-yl] - [4-[2-(1-piperidyl)ethoxy]phenyl] -methanone

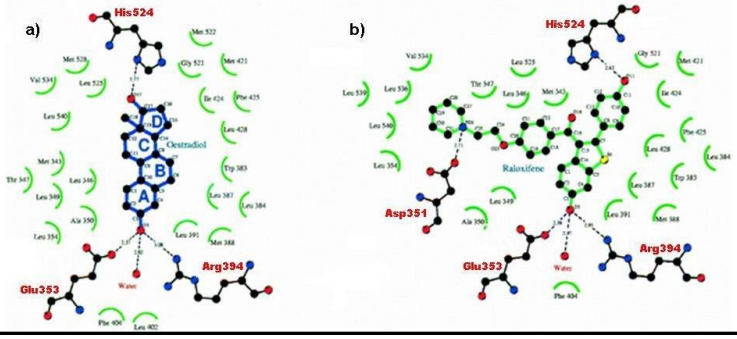
SAR

- Raloxifene belongs to the triaryl class of SERMs.
- The orientation of the three aryl rings in a propeller type of arrangement is important for tight receptor binding (mimics estrogens) and selectivity
- In this class of agents, the benzothiazole ring phenol is critical for binding to the estrogen receptor, because it mimics the essential 3-phenolic group in estrogens

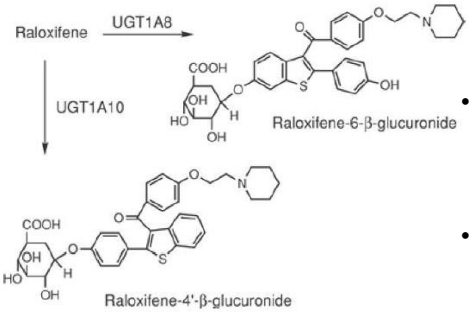


SAR and binding to estrogen receptor

- In the triaryl class of agents, the benzothiazole ring phenol is critical for binding to the estrogen receptor, because it mimics the essential 3-phenolic group in estrogens
- The free phenol OH mimics the 17-OH of estrogens
- The orientation of the three aryl rings in a propeller type of arrangement also is important for tight receptor binding and selectivity
- The third arm of raloxifene makes additional interactions in the estrogen receptors on the bone but not in the breast and uterus

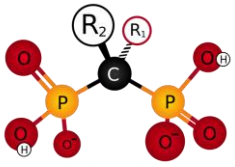


Raloxifene metabolism

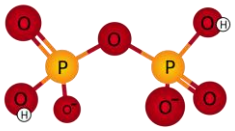


- Raloxifene is rapidly absorbed following oral administration, with an estimated **60% absorption,** but it **has a very low bioavailability (2%)** , associated with extensive phase II metabolism.
- Metabolism of raloxifene occurs to a great extent via glucuronide conjugation catalyzed by uridine diphosphate glucuronosyltransferase (UGT)
- In addition there is efflux by intestinal cells of the resulting glucuronide occurs via P-glycoprotein
- The combination of rapid metabolism and efflux can account for the low bioavailability.

Bisphoshonates



Bisphosphonate

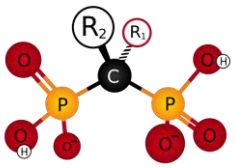


Pyrophosphate
(present in bone, ATP etc)

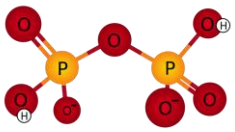
- All bisphosphonate drugs share a common P-C-P "backbone"
  - The two PO3 (phosphonate) groups covalently linked to carbon

- The bisphosphonates are synthetic in origin and are designed to mimic pyrophosphate, where the oxygen in P-O-P is replaced with a carbon atom to create a nonhydrolyzable backbone
- Because pyrophosphate is a normal constituent of bone, these analogues have high affinity for the bone and selectively bind to the hydroxyapatite portion of the bone, from where they are ingested by the osteoclasts.

Bisphoshonates



Bisphosphonate

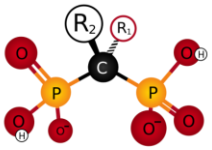


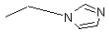
Pyrophosphate

- All bisphosphonate drugs share a common P-C-P "backbone"
  - The two PO3 (phosphonate) groups covalently linked to carbon

- Bisphosphonate-based drugs' specificity comes from the two phosphonate groups (and possibly a hydroxyl at R 1) that work together to coordinate calcium ions.
- Bisphosphonate molecules preferentially "stick" to calcium and bind to it, thereby preventing decalcification of bone – bone resorption. (unlike Ca bound to pyrophosphate)
- Besides this, the bisphosphonates effectively inhibit osteoclast proliferation, decrease osteoclast activity, reduce osteoclast life span
- By these mechanisms, the bisphosphonates are able to limit bone turnover and allow the osteoblasts to form well-mineralized bone without opposition

Classification



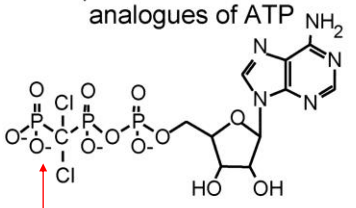
Agent	R_1 side chain	R_2 side chain
Etidronate	-OH	$-CH_3$
Clodronate	-Cl	-Cl
Tiludronate	-H	-S-  -Cl
Pamidronate	-OH	$-CH_2-CH_2-NH_2$
Neridronate	-OH	$-(CH_2)_5-NH_2$
Olpadronate	-OH	$-(CH_2)_2N(CH_3)_2$
Alendronate	-OH	$-(CH_2)_3-NH_2$
Ibandronate	-OH	$-CH_2-CH_2N(CH_3)(CH_2)_4-CH_3$
Risedronate	-OH	
Zoledronate	-OH	

- *Bis* refers to the fact that there are two such groups in the molecule.
- The long *side-chain* (**R2** in the diagram) determines the chemical properties, the mode of action and the strength of bisphosphonate drugs.
- The short *side-chain*(R1), often called the 'hook', mainly influences chemical properties and pharmacokinetics.
- Classification
  - Non-nitrogen bisphosphonates
    Etidronate, Clodronate, tiludronate
  - nitrogen-containing bisphosphonates **Alendronate**, neridronate, ibandronate, pamidronate, risedronate, and zoledronate

Mechanism – Role of the central Carbon substituents

Non–nitrogen-containing BPs

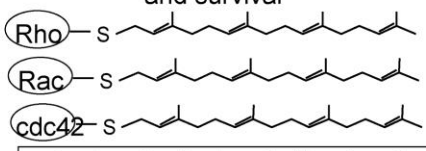
Incorporated into intracellular analogues of ATP



clodronate, etidronate, tiludronate

Nitrogen-containing BPs

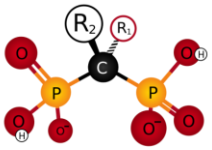
Inhibit the prenylation and function of GTP-binding proteins required for osteoclast formation, function, and survival

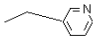
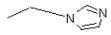


alendronate, risedronate, ibandronate, pamidronate, zoledronate

- Form a nonfunctional molecule that competes with ATP in the bone cellular energy metabolism.
- The osteoclast initiates apoptosis and dies, leading to an overall decrease in the breakdown of bone

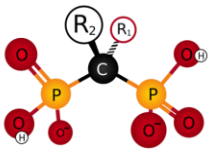
SAR

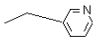
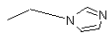


Agent	R_1 side chain	R_2 side chain
Etidronate	-OH	$-CH_3$
Clodronate	-Cl	-Cl
Tiludronate	-H	-S-  -Cl
Pamidronate	-OH	$-CH_2-CH_2-NH_2$
Neridronate	-OH	$-(CH_2)_5-NH_2$
Olpadronate	-OH	$-(CH_2)_2N(CH_3)_2$
Alendronate	-OH	$-(CH_2)_3-NH_2$
Ibandronate	-OH	$-CH_2-CH_2N$ 
Risedronate	-OH	
Zoledronate	-OH	

- The SAR studies have concluded that a hydroxyl substituent (R1)
  - maximizes the affinity of the agent for the hydroxyapatite Ca as well as
  - improves the antiresorptive character of the agent and
  - influences chemical properties and pharmacokinetics.

SAR



Agent	R_1 side chain	R_2 side chain
Etidronate	-OH	$-CH_3$
Clodronate	-Cl	-Cl
Tiludronate	-H	-S-  -Cl
Pamidronate	-OH	$-CH_2-CH_2-NH_2$
Neridronate	-OH	$-(CH_2)_5-NH_2$
Olpadronate	-OH	$-(CH_2)_2N(CH_3)_2$
Alendronate	-OH	$-(CH_2)_3-NH_2$
Ibandronate	-OH	$-CH_2-CH_2N$ 
Risedronate	-OH	
Zoledronate	-OH	

- The character of the R2 substituent varies widely and clearly has a significant influence on the potency of this class of compounds
- The R2 amino–substituted bisphosphonates (pamidronate, alendronate) are more potent than non nitrogen containing bisphosphonates (etidronate and clodronate)
- The R2 four-carbon amino linear chain for alendronate is more potent than the R2 three-carbon derivative pamidronate and the R2 six-carbon analogue neridronate.
- Alkylation of the amine functional group improves potency as is demonstrated by compounds with branched amine substituents at R2 (e.g. olpadronate and ibandronate) and those that contain rings at R2

Bone forming agents - Teriparatide

- Teriparatide is recombinant human parathyroid hormone, PTH 1-34, the biologically active portion of the endogenously produced preprohormone.
- Unlike the bisphosphonates, which are classified as bone restorative agents, teriparatide is the first approved bone- forming agent .
- Bone formation is possible because of the ability of this agent to increase the number of osteoblasts.

Bone forming agents - Teriparatide

- Bone formation is possible because of the ability of this agent to increase the number of osteoblasts.
- Although teriparatide enhances the function of both osteoclasts and osteoblasts, the exposure incidence dictates its effect on the skeleton.
- If administered once daily or intermittently, teriparatide preferentially enhances osteoblastic function and bone format ion occurs.
- Continuous exposure to endogenous PTH may result in poor skeletal composition because of enhanced osteoclast-mediated bone resorption