Bone Development and Metabolism

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Molecular Endocrinology Group

- Skeletal physiology
  - Bone structure
  - Bone development
    - Chondrocytes
    - Osteoblasts
  - Bone remodelling
    - Osteocytes
    - Osteoclasts

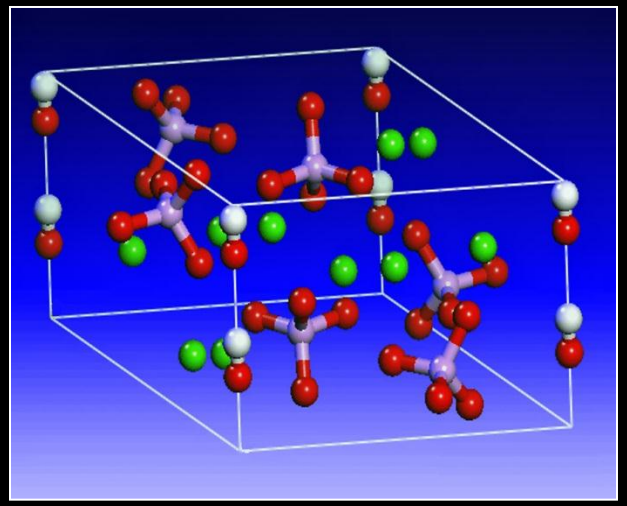
- Skeletal pathology
  - Osteoporosis
  - Paget’s disease of bone

Skeletal Physiology

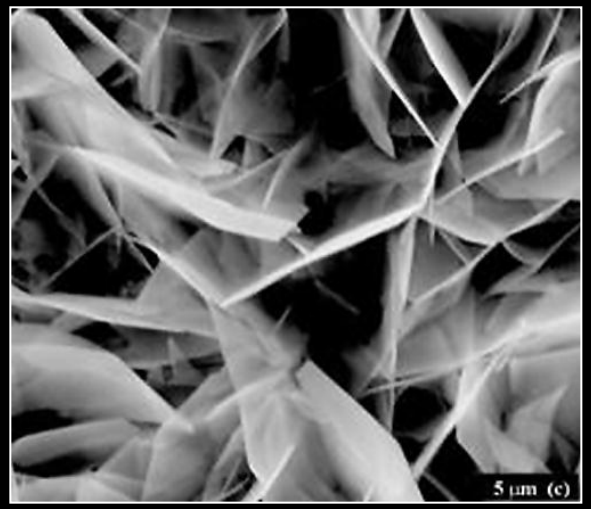
Bone Structure

Bone must be stiff yet flexible and light yet strong

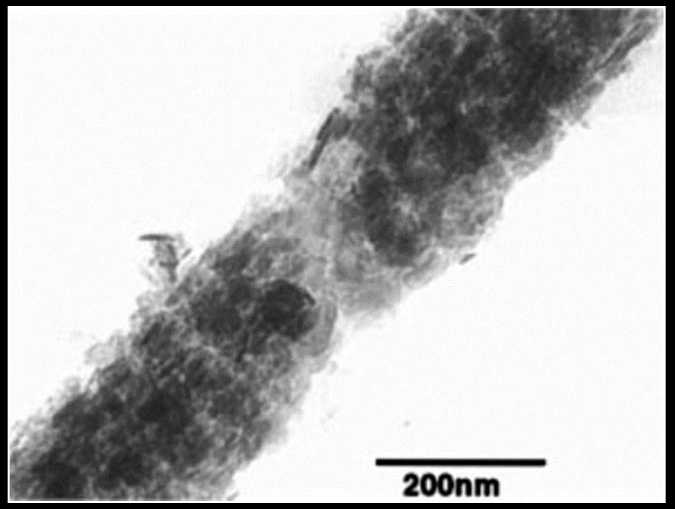
Bone mineral



Hydroxyapatite
$Ca_{10}(PO_4)_6(OH)_2$

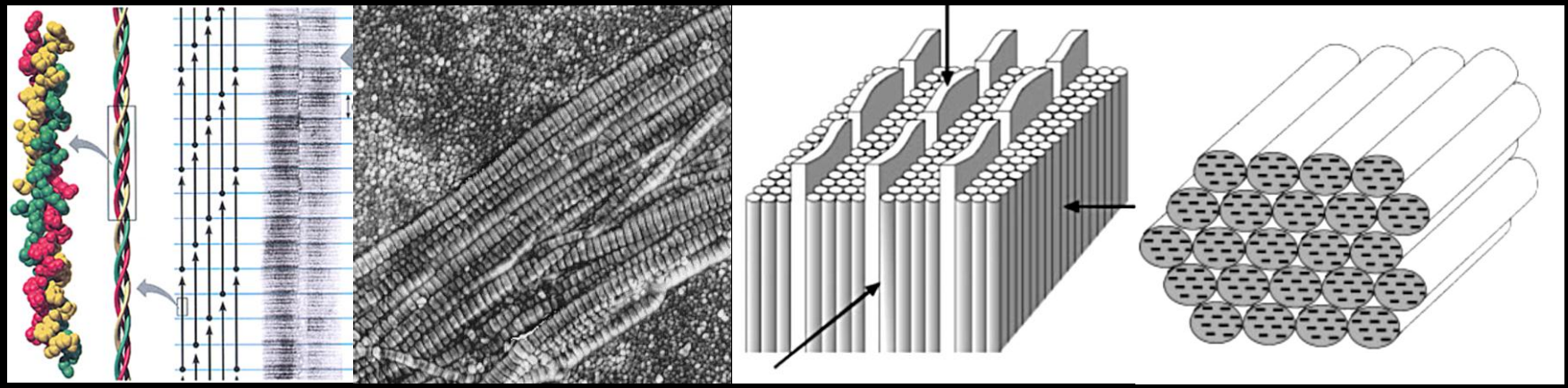


Roof tile like crystals
(4 x 50 x 25nm)



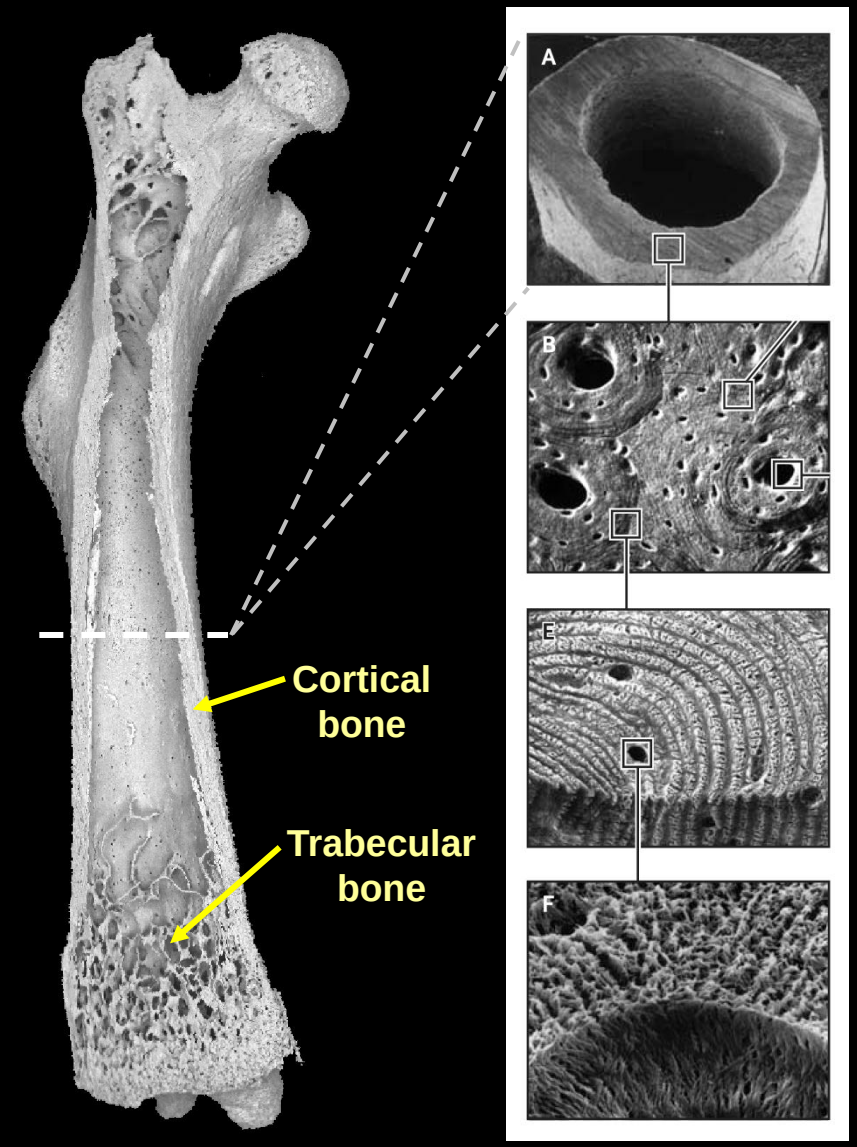
Crystals pack into
Type 1 collegen

Bone matrix



- Matrix component
  - Type I collagen rich Osteoid
  - Type I collagen molecule (1.5nm in diameter)
    - Triple helicle collagen molecule 300nm long
  - Collagen fibril (100nm in diameter)
    - Collagen packs in an array with mineral crystals
  - 200 non-collagenous proteins
    - <10% of total protein
- Human bone is 60% mineralised
  - Increased mineralisation increases stiffness but reduces flexibility

Macro and microstructure of cortical bone



Ovelapping parallel osteon structure
- Result of completed remodelling cycles

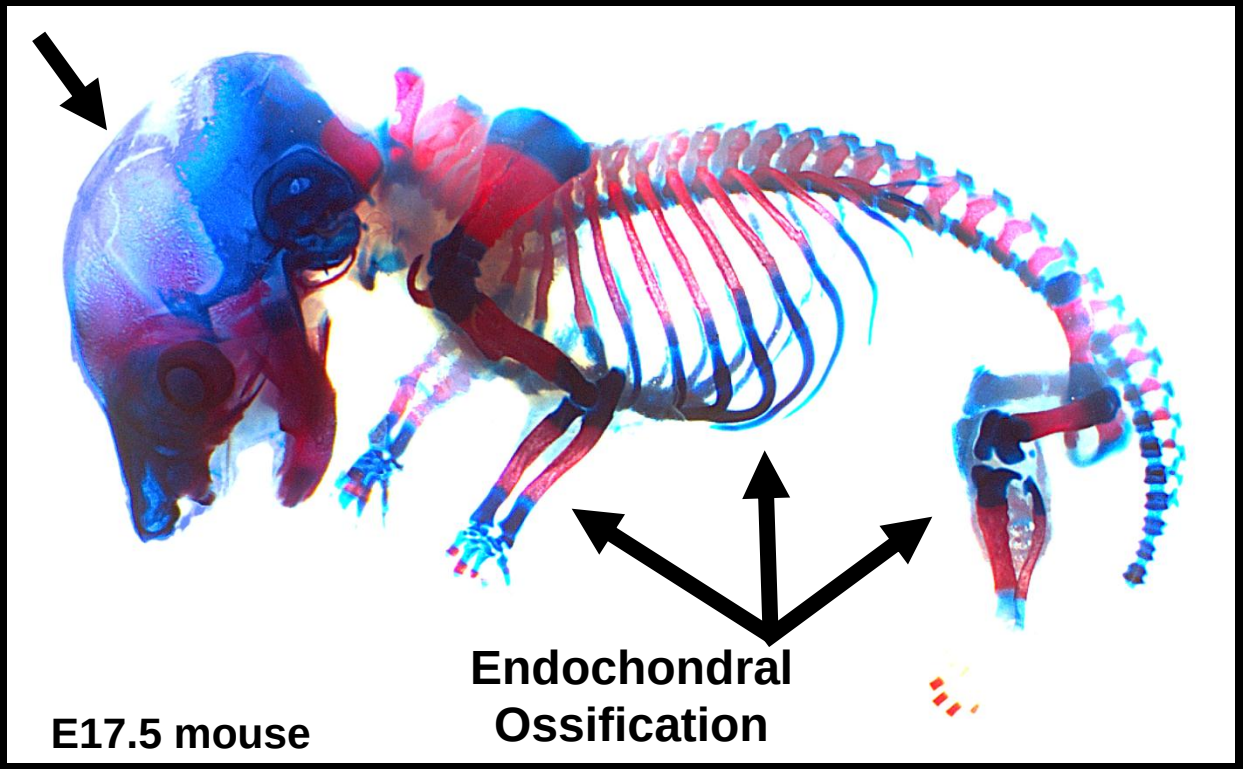
Osteon structure limits fracture propagation
- Concentric lamellae
- Alternately loose and dense packing
- Collagen fibres orientated in various directions

(Seeman E et al 2008 NEJM 354:2250–2261)

Bone development

Skeletal development

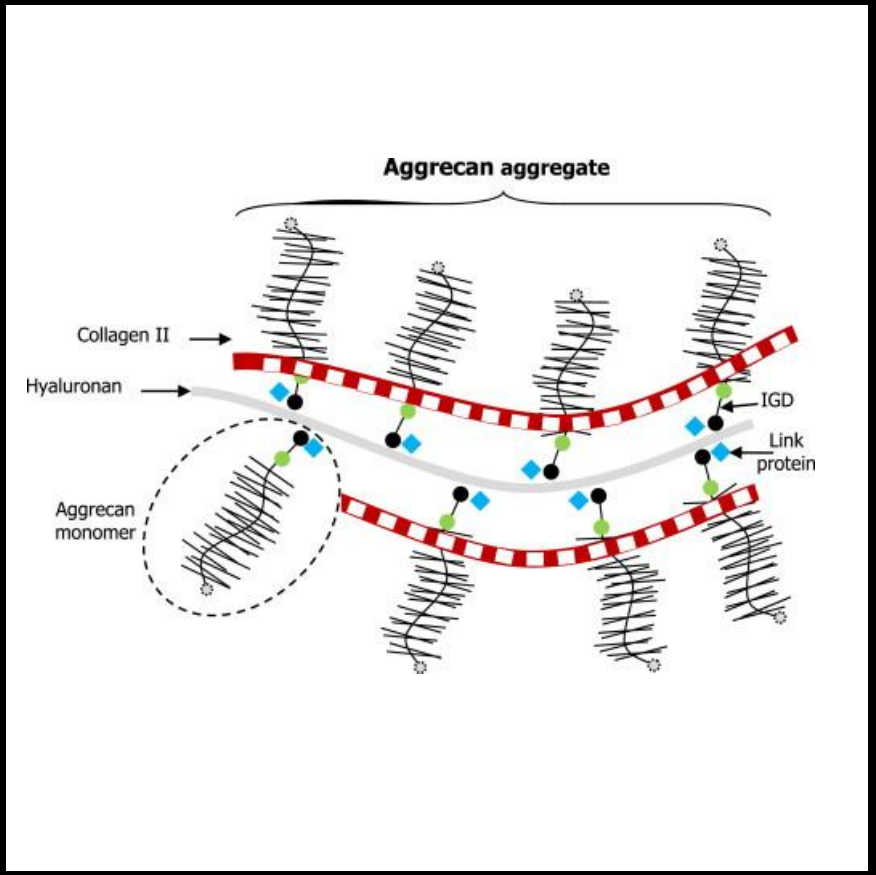
Intramembranous Ossification



Long bone form by endochondral ossification

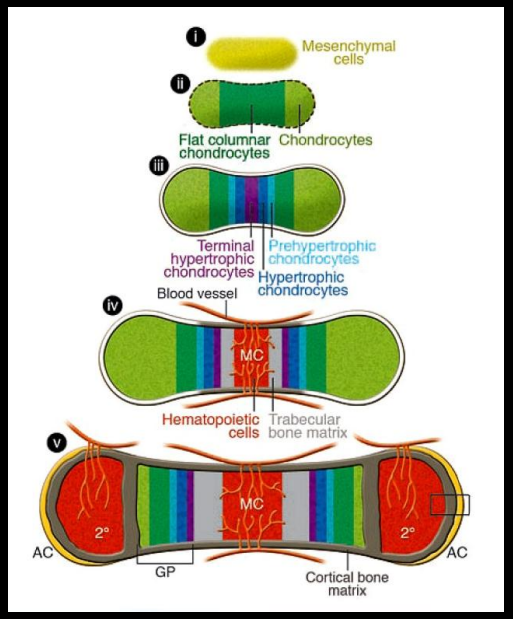
Craniofacial bones by intramembranous ossification

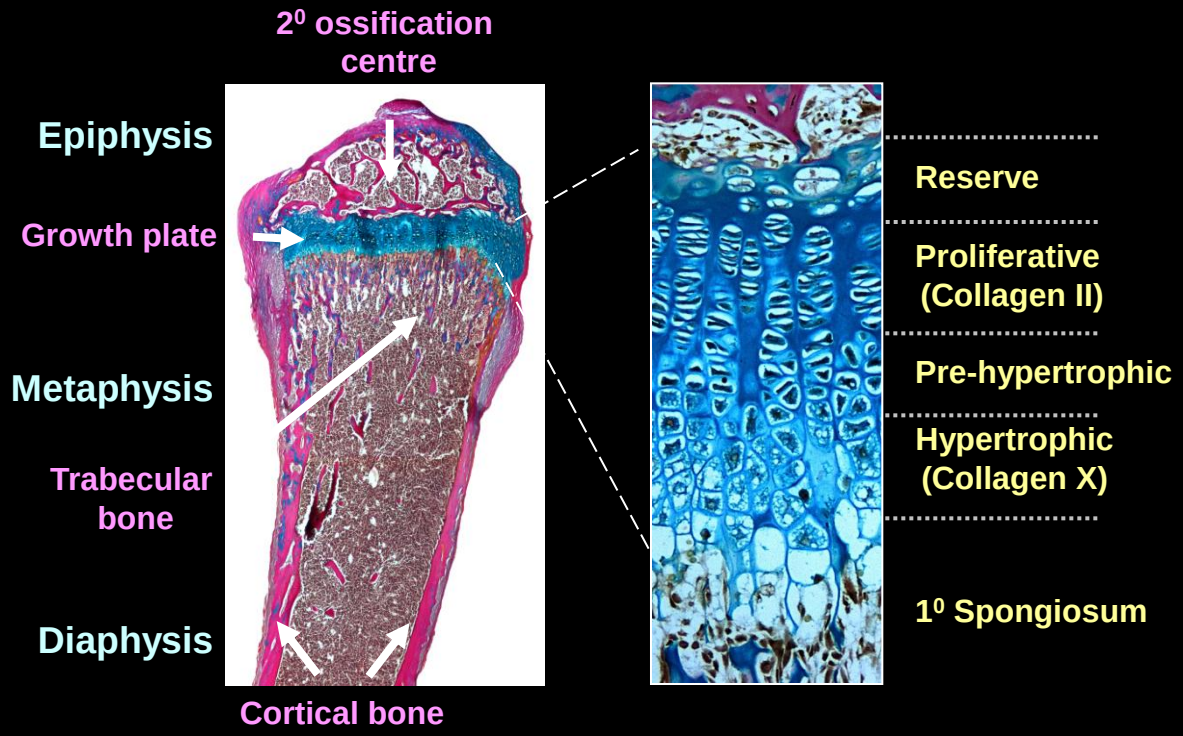
Chondrocytes



Cartilage formation

Endochondral ossification





Sox9	Master transcriptional regulator in chondrocyte
FGF/FGFRs	Inhibit chondrocyte proliferation and differentiation
Indian hedgehog (Ihh)	Promotes chondrocyte proliferation and induced PTHrP
PTHrP/PTHR1	Inhibit chondrocyte differentiation

(Zuscik MJ et al 2008 J. Clin. Invest. 118:429–438)

Achondroplasia

FGF/FGFR3 signalling inhibits chondrocyte proliferation and differentiation

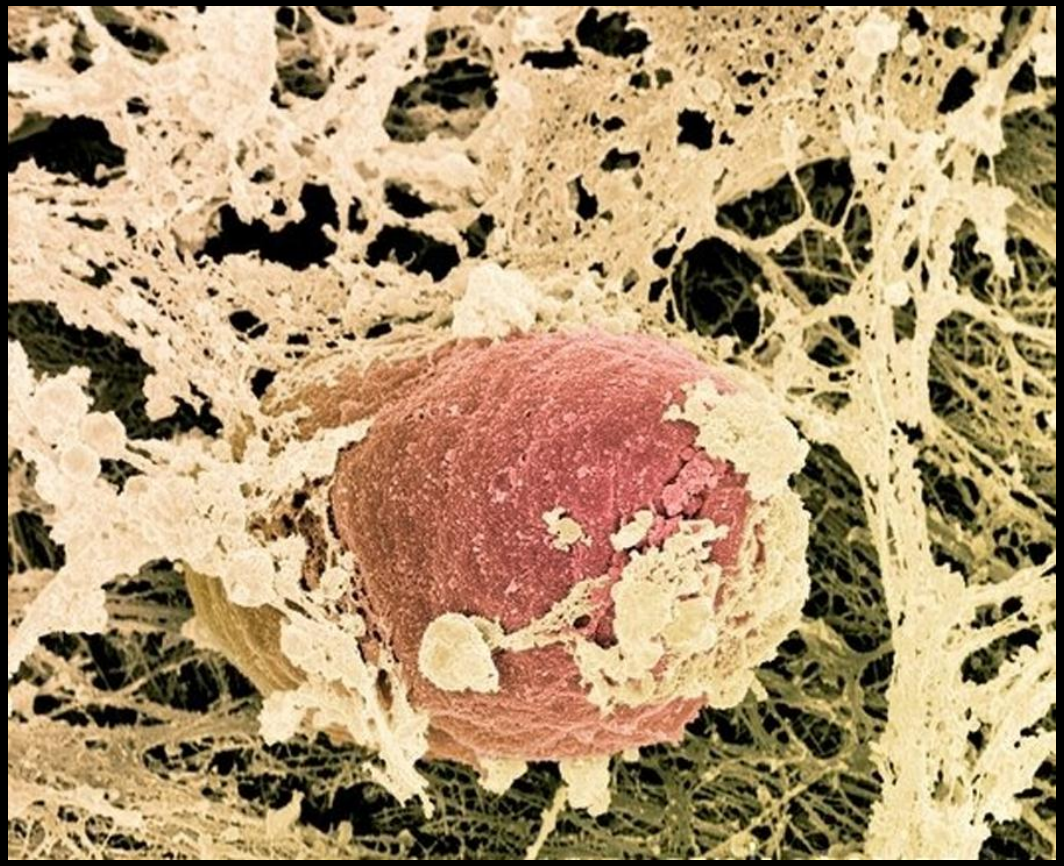
Most common form of dwarfism (1:250 000)

Gain of function mutation FGFR3 (Gly380Arg)

- 95% have the same point mutation
- 80% of these are new mutations
- Macrocephaly, frontal bossing, midface hypoplasia, small chest, rhizomelia

(Day TF et al (2008) J Bone Joint Surg 90:19-24; Horton WA et al (2007) Lancet.370:162-72)

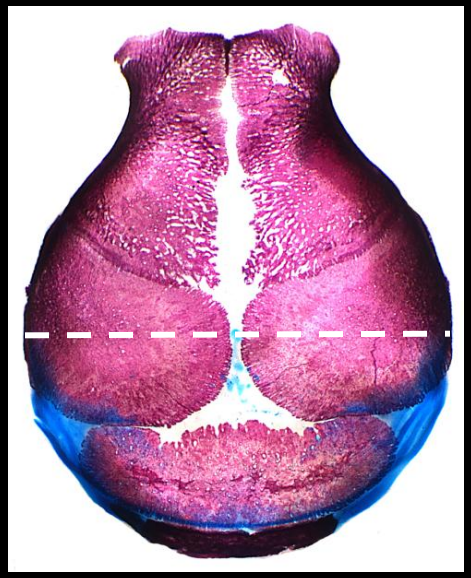
Osteoblasts

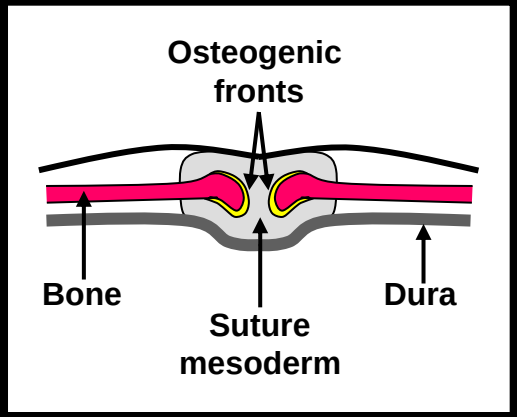


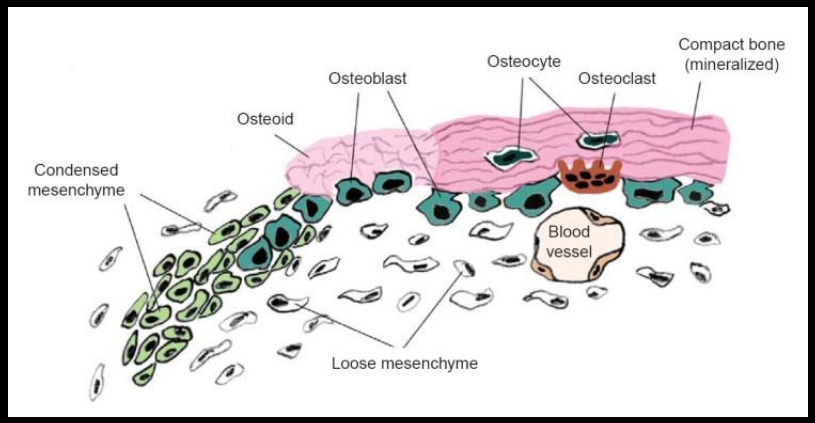
Bone formation

Intramembranous ossification

Craniofacial skeleton forms by intramembranous ossification







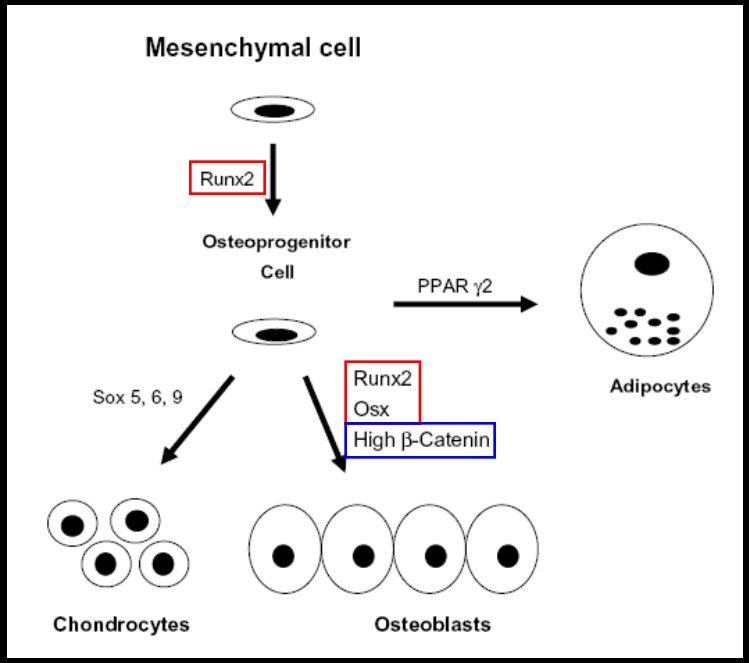
Mesenchymal cells differentiate into osteoblasts

Bone is formed directly without a cartilage scaffold

(Hartmann C (2006) Trends in Cell Biol 16:151-8)

Osteoblastogenesis

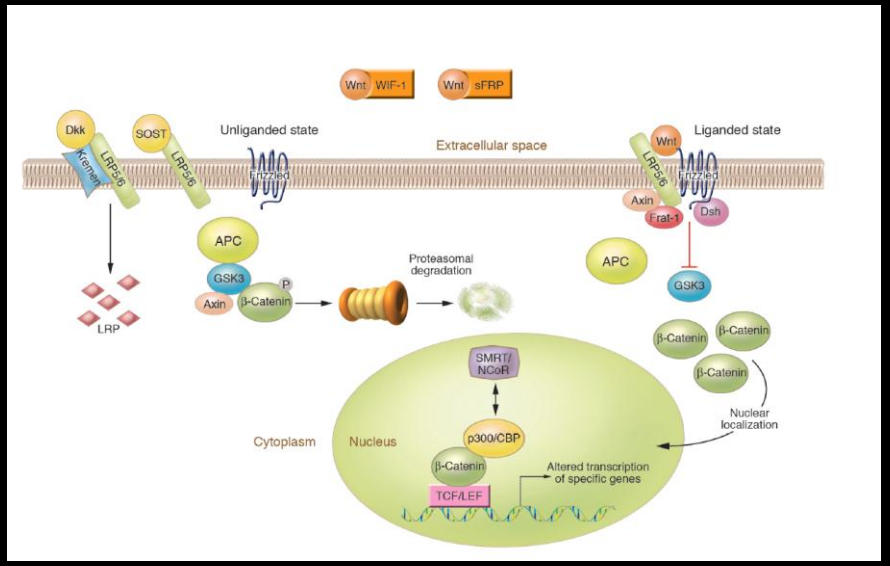
Osteoblasts, chondrocytes and adipocytes all derive from mesenchymal cells



Key transcription regulators: **Runx2, Osterix and β-Catenin**
Paracrine factors: **Wnt, BMPs and FGFs**
Systemic hormones: **GH/IGF1, GCs, E2, PTH and $1,25(OH)_2D$**

Wnt signalling regulates bone mass

Promote osteoblast differentiation, proliferation, and mineralisation



On absence of Wnt

- GSK3/APC/Axin targets β-catenin for degradation by phosphorylation

Wnt binds Frizzled with co-receptors LRP5/6 and inhibits GSK3

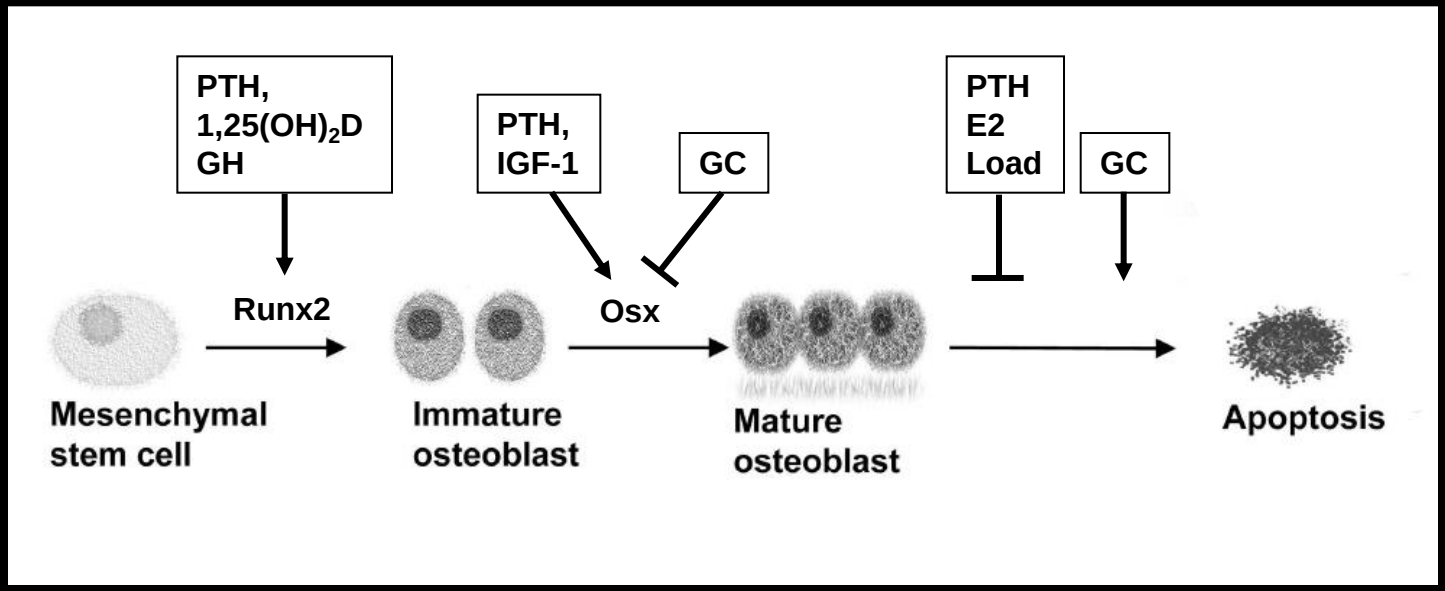
- Preventing β-catenin degradation
- β-catenin enters nucleus regulating target genes

Negative regulation of Wnt signalling

- Wnt binding (WIF-1 and sFRP)
- LRP5/6 degradation (Sclerostin (SOST) and Dickkopf (Dkk))

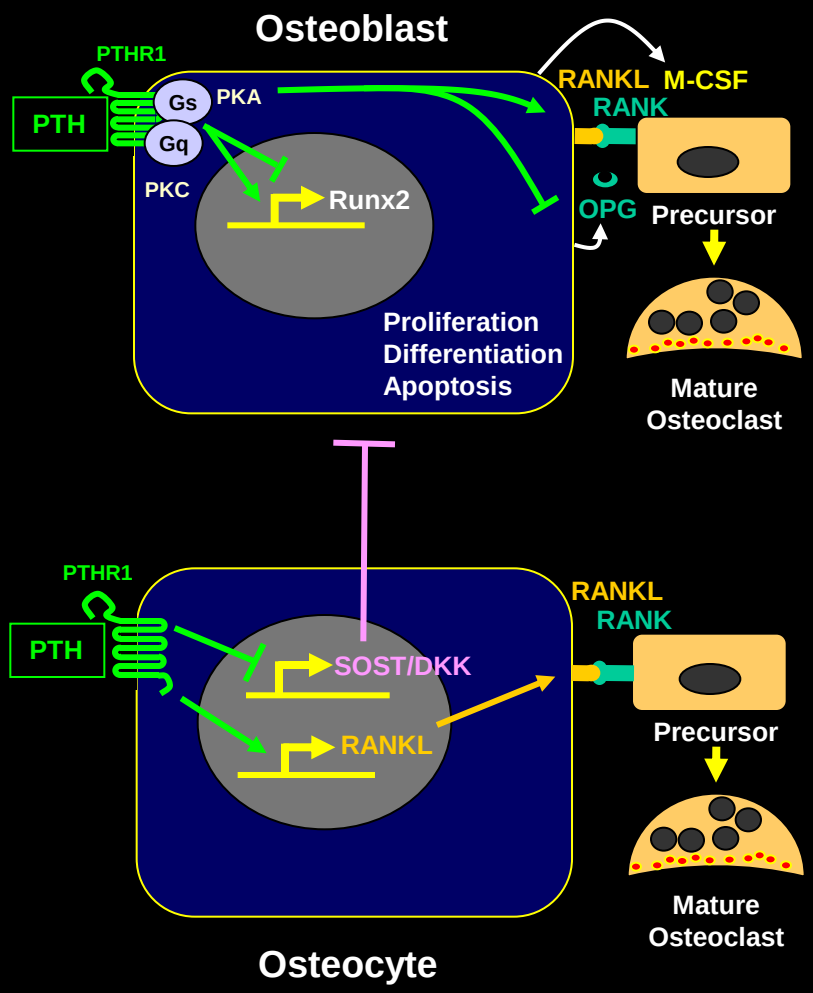
(Krishnan V eta l (2006) J. Clin. Invest. 116:1202–1209)

Endocrine regulation of osteoblasts



PTH has anabolic and catabolic actions

Continuous PTH results in net cortical resorption
Intermittent PTH results in net trabecular formation



Increases osteoclast differentiation indirectly
In osteoblasts (↑ MCSF/RANKL and ↓ OPG)
In osteocytes (↑ RANKL)

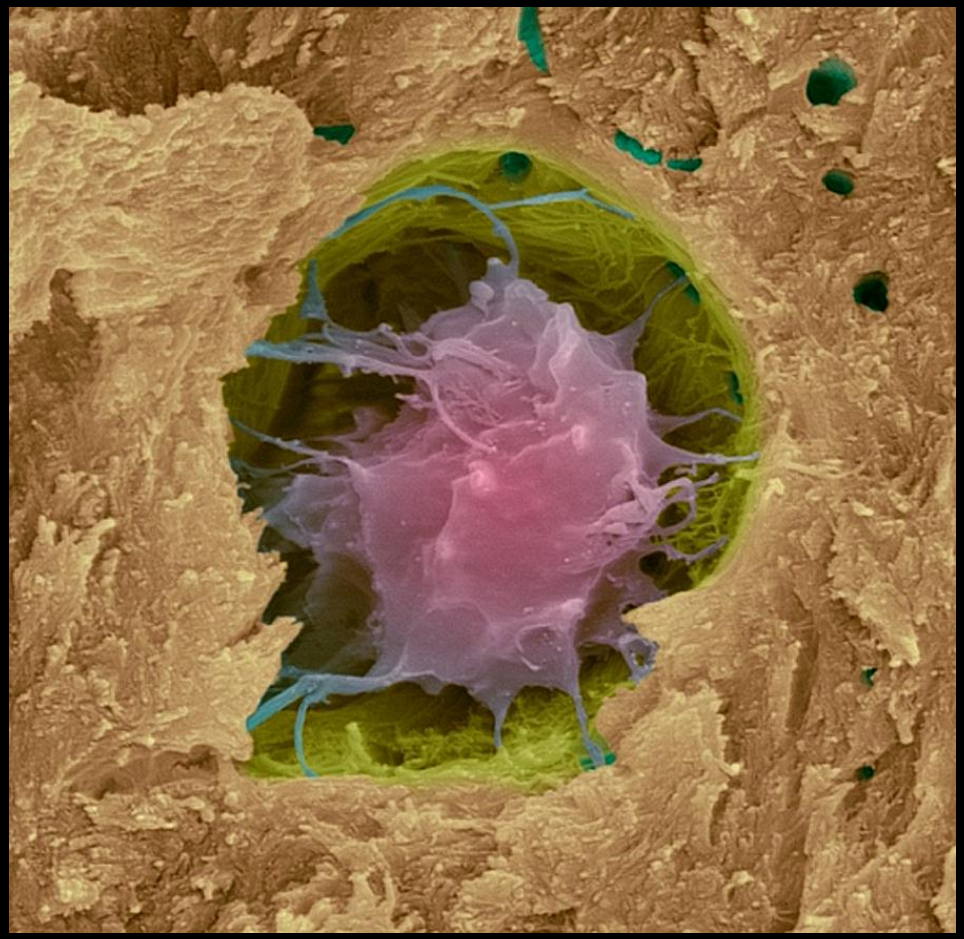
Regulates pre-osteoblast maturation
In pre-osteoblasts
- Continuous PTH ↓ Runx2
- Intermittent PTH ↑ Runx2

In osteocytes
- PTH ↓ SOST/DKK (↑ Wnt signalling)

Other paracrine mechanisms
- PTH ↑ IGF-1 and FGF release

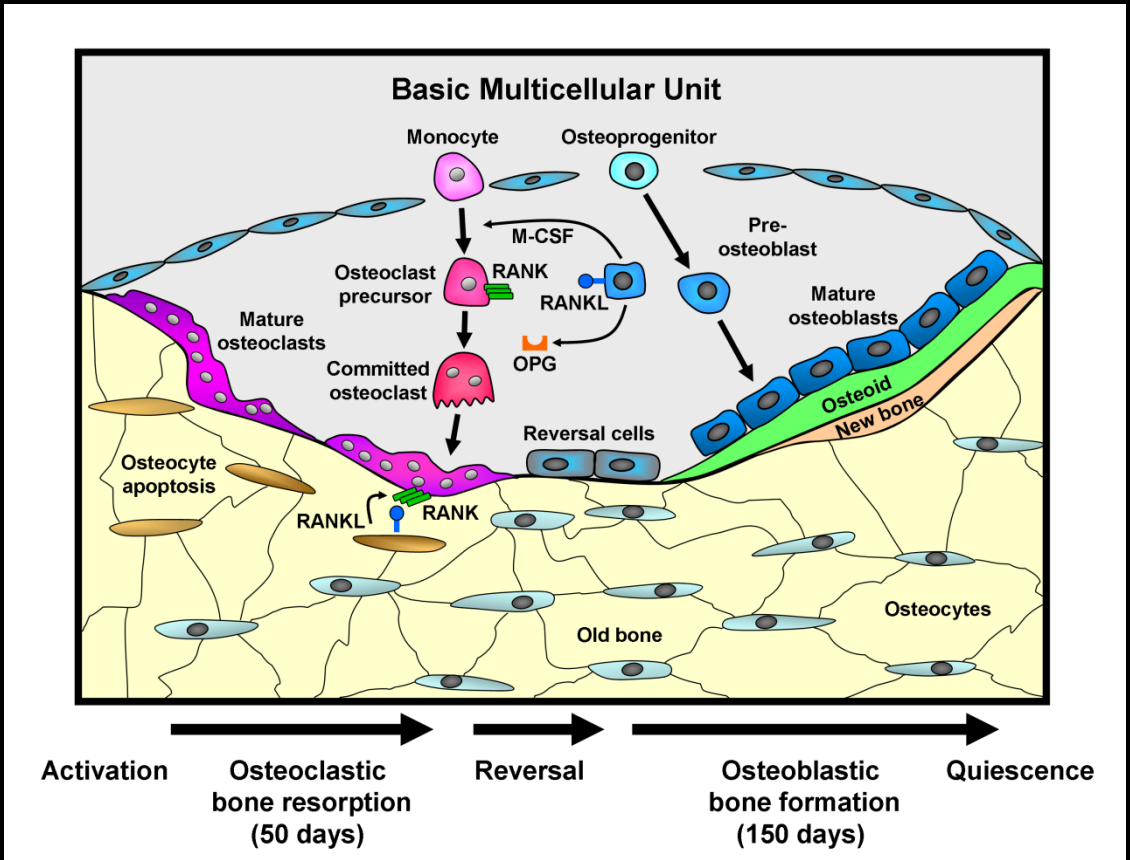
Maintenance of adult bone

Osteocytes



Mechanosensor & regulator of bone remodeling

The bone remodelling cycle



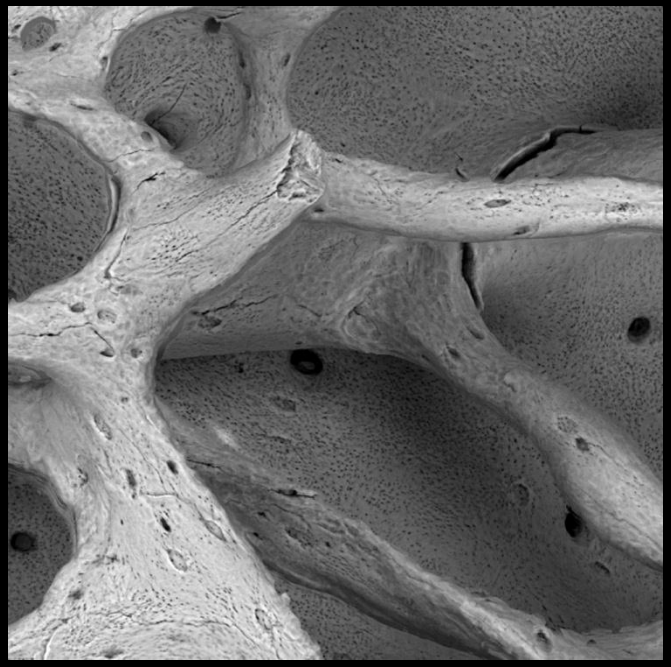
Ostoclastic bone resorption followed by ostoblastic bone formation

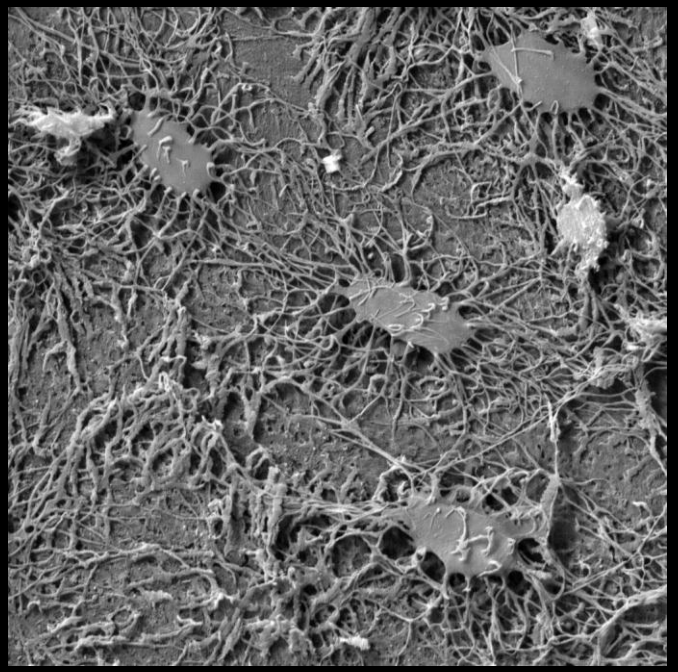
- Maintain homeostasis of Ca^{2+} and PO_4^{3-}
- Repair damaged matrix and micro-fractures
- Adapt to mechanical stress and strain

Resorption and formation are coupled temporally and spatially

Uncoupling leads to osteoporosis or osteopetrosis

Osteocytes orchestrate bone remodeling





Ostrocytes make up 90-95% of all adult bone cells
- Osteoblasts 5%, osteoclasts 1-2%
- Osteocyte surface area >100x that of the bone itself

Osteocytes form a complex network of connected processes
- Mechanical load sensors regulating bone resorption and formation
- Endocrine organ regulating phosphate (FGF23)
- Endocrine organ regulating metabolism (osteocalcin) ?

Osteocytes regulate bone turnover

Bone resorption

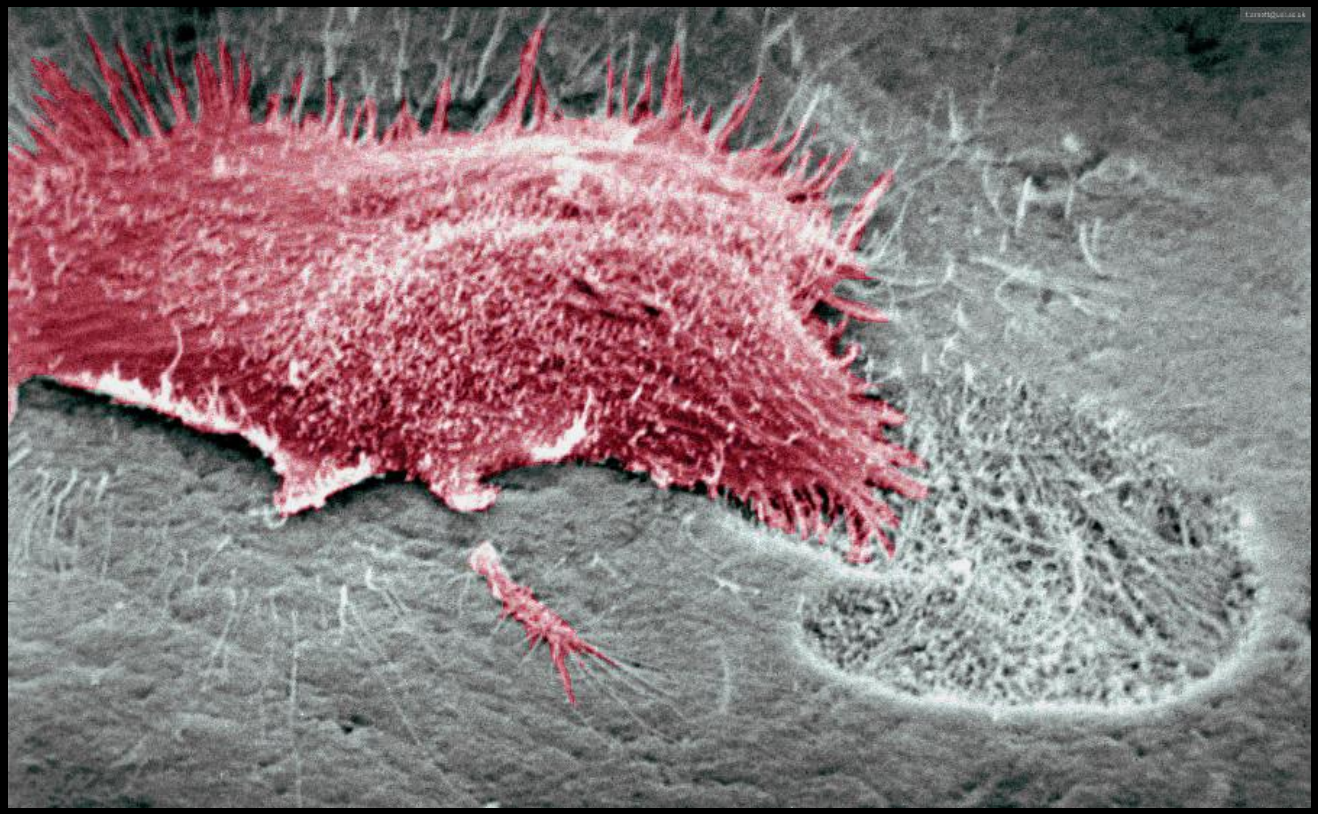
- During bone loading osteocytes inhibit osteoclast resorption (↑ TGFβ?)
- Unloading, hypoxia or apoptotosis initiates resorption (↑ RANKL)

Regulation of bone formation and mineralisation

- Osteocyte Sclerostin binds LRP5 (↓ Wnt signaling and bone formation)
- Osteocyte Dmp1 and Phex increases phosphate (↓ FGF23)
  - Osteocyte Mepe inhibits phosphate resorption

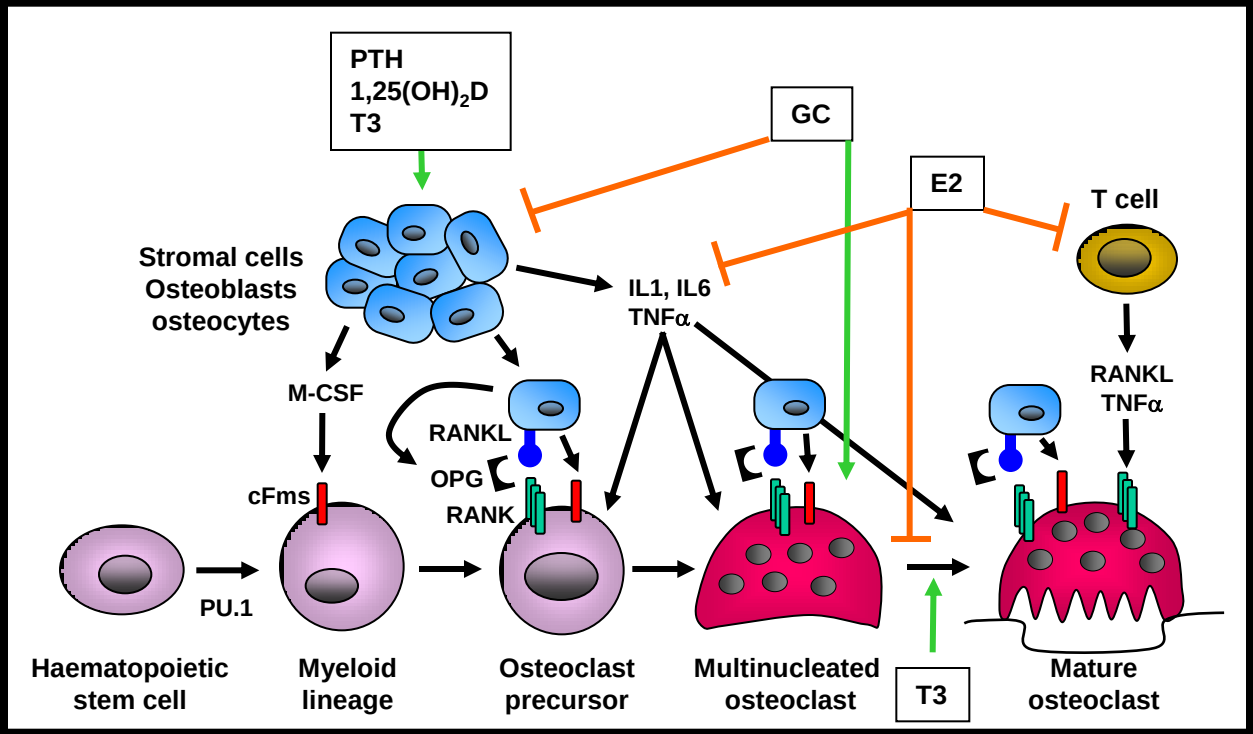
(Bonewald LF (2008) ASBMR Primer 22-27; Xiong J (2012) JBMR 27:499-505)

Osteoclasts



Bone resorption

Osteoclastogenesis

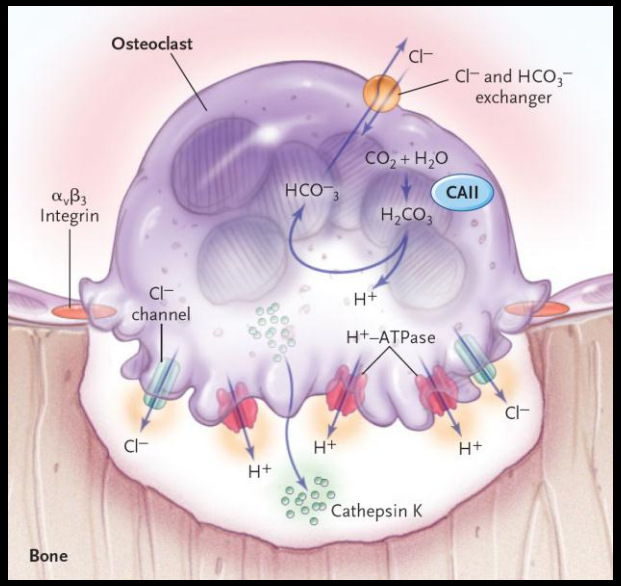


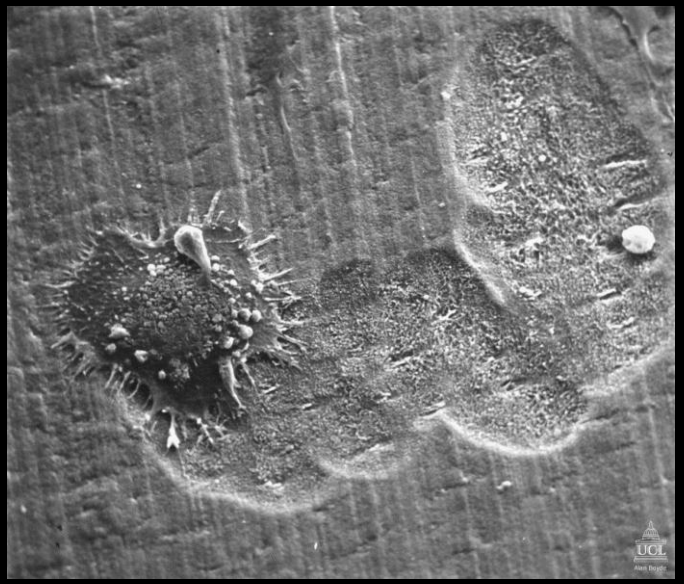
Osteoclasts derive from the myeloid lineage and are multinucleated cell
- M-CSF regulates proliferation, survival and differentiation of precursors
- RANKL is key osteoclastogenic cytokine sufficient for differentiation
- OPG is a decoy receptor (physiological inhibitor of RANKL/RANK signaling)

PTH, $1,25(OH)_2D$ and pro-inflammatory cytokines
- increase RANKL expression and suppress OPG

(Ross FP (2008) ASBMR Primer. 16-22)

Osteoclast function





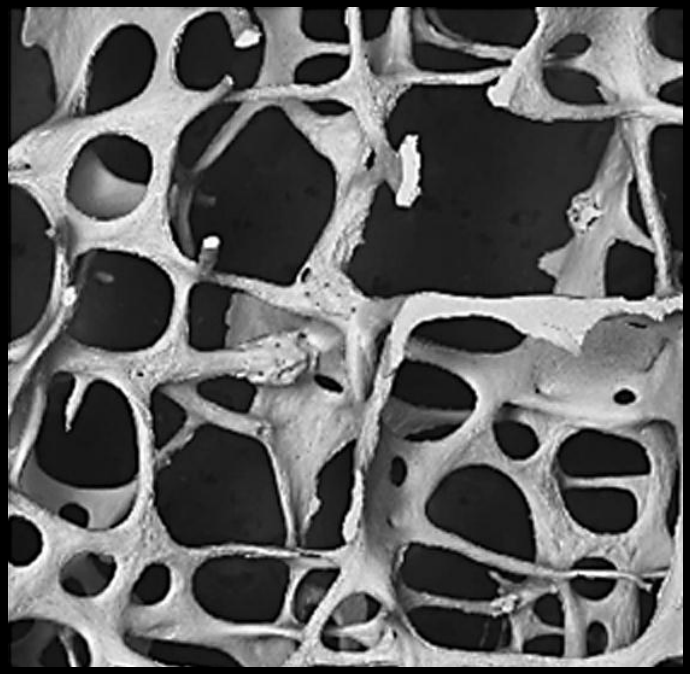
Active osteoclasts are polarised cells

- **Attach to the bone surface via integrin $\alpha_v\beta_3$ (Sealing zone)**
  - **Requires action of small GTPases (inhibited by bisphosphonates)**
- **Form ruffled membrane adjacent to bone surface**
- **Secrete hydrogen and chloride ions that dissolve bone mineral**
  - **H^+ generated by CAII; H^+-ATPase and CLCN7 secrete H^+ and Cl^-**
- **Matrix metalloproteinases and Cathepsin K degrade the collagen matrix**

Skeletal Pathology

Osteoporosis

Normal bone

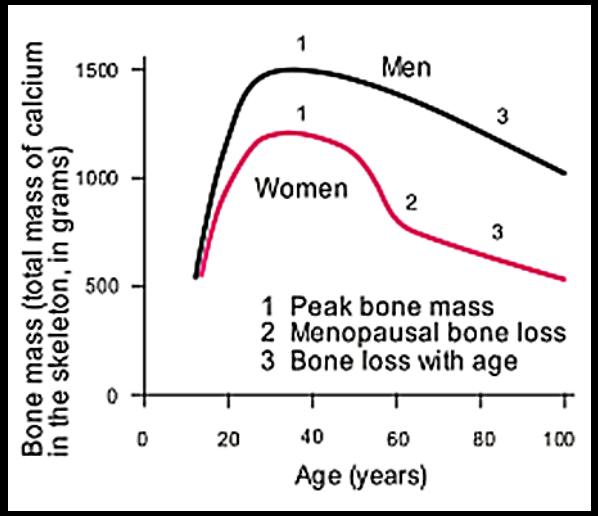


Osteoporotic bone

Low bone mass
Micro-architectural deterioration
Fragility fractures

Osteoporosis

Affects 50% of women and 1 in 5 men over 50 years old
Costs the European Community €31 billion per annum



Peak bone mass

- Achieved at 20 and 30 years of age (major genetic component)
- Estrogens is critical in both male and females for peak bone mass
- Physical exercise, alcohol XS, smoking, eating disorders, systemic illness

Progressive loss of bone mass occurs from 45 years of age

- More rapid loss in women due to estrogens deficiency at menopause

Commonest fractures

- Female: Hip, vertebra and Colles'
- Male: Hip and vertebra

Age related osteoporosis

Increased bone resorption relative to formation

Mechanism

- Estrogens deficiency at the menopause
  - Increased expression of skeletal cytokines especially IL-6
  - Reduced expression of OPG and thus increased osteoclastogenesis
- Decreased cutaneous vitamin D synthesis and 1α-hydroxylase activity
  - Decreased $1,25(OH)_2D$
  - Reduced intestinal Ca^{2+} absorption and increased renal losses
- Reduced calcium increases PTH
  - Increases osteoclastic resorption

Risk factors for fracture

- Low BMD, advanced age, postmenopausal fracture, 1st degree relative with fracture, smoking, low BMI, vitamin D deficiency, premature menopause, alcohol excess, history of falls, institutionalisation and immobility

Diagnosis of osteoporosis

- Fragility fracture and decreased bone mineral density (BMD)

Investigation

- Ca^{2+}, Pi, ALP, Cre, PTH, 25-OH-vitD, DEXA, Urinary NTX

Secondary Osteoporosis

Endocrine

- Thyrotoxicosis (increased bone turnover)
- Hyperprolactinemia (reduced gonadotrophins and sex hormones)
- Primary hyperparathyroidism (Increased resorption)
- Hypogonadism (increased resorption)
- Cushing's Syndrome (impaired bone formation)

Nutritional

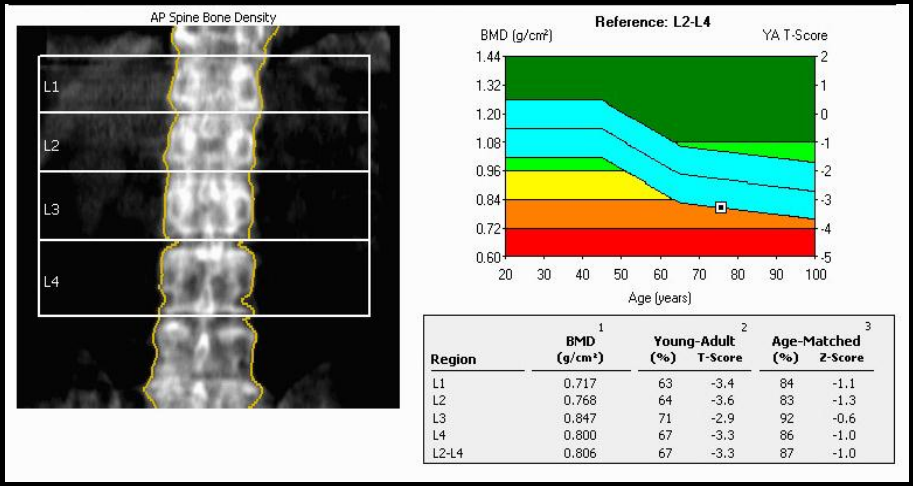
- Vitamin D deficiency (impaired mineralisation)
- Coeliac disease (impaired mineralisation)
- Chronic liver disease

Iatrogenic

- High dose glucocorticoids (Glucocorticoid induced osteoporosis)
- GnRH agonists (Prostate cancer)
- Aromatase inhibitors (Breast cancer)
- Thyroid hormone excess (Excessive replacement or Thyroid cancer)
- Anticoagulants and Anticonvulsants
- Immunosuppression (inhibits calcineurin and NFAT)
- Thiazolidinediones (PPARγ agonists) (↓ osteoblastogenesis ↑adipogenesis)

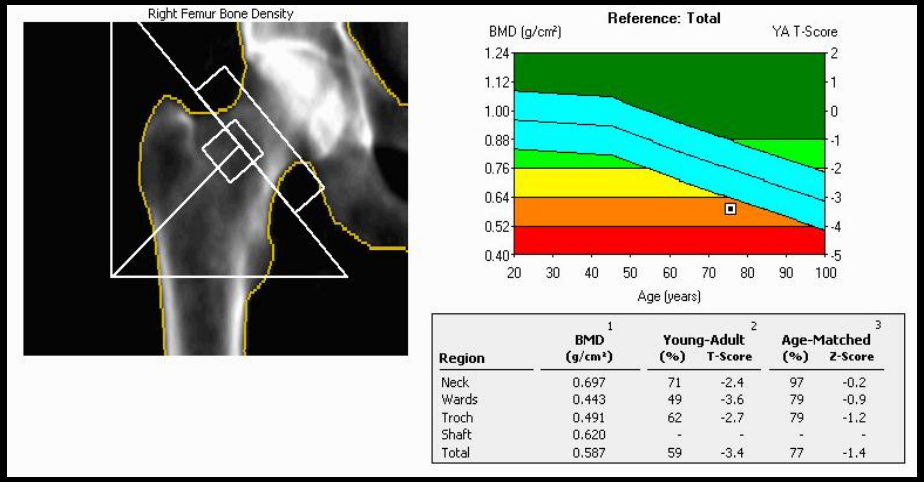
Dual-energy X-ray absorptiometry

Lumbar spine



Region	BMD [1] (g/cm²)	Young-Adult [2] (%)	Young-Adult [2] T-Score	Age-Matched [3] (%)	Age-Matched [3] Z-Score
L1	0.717	63	-3.4	84	-1.1
L2	0.768	64	-3.6	83	-1.3
L3	0.847	71	-2.9	92	-0.6
L4	0.800	67	-3.3	86	-1.0
L2-L4	0.806	67	-3.3	87	-1.0

Right hip



Region	BMD [1] (g/cm²)	Young-Adult [2] (%)	Young-Adult [2] T-Score	Age-Matched [3] (%)	Age-Matched [3] Z-Score
Neck	0.697	71	-2.4	97	-0.2
Wards	0.443	49	-3.6	79	-0.9
Troch	0.491	62	-2.7	79	-1.2
Shaft	0.620	-	-	-	-
Total	0.587	59	-3.4	77	-1.4

DXA results are compared to age, sex and ethnically matched data

Bone mineral density is normally distributed in the population

Results are interpreted according to the standard deviation from the mean of
a) Sex matched peak bone mass (T-score)
b) Sex and age matched BMD (Z-score)

WHO diagnostic criteria

Osteoporosis

T score ≤ -2.5 lumbar spine, femoral neck or total hip

Osteopenia

T score ≤ -1.0 lumbar spine, femoral neck or total hip

Treatment of age related osteoporosis

Treatment is indicated if prior fragility fracture or T-score ≤ -2.5

Simple advice
- **Weight bearing exercise, smoking and alcohol**

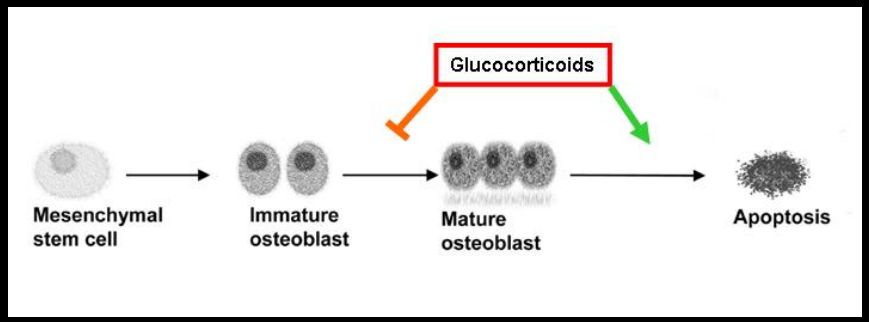
Optimise vitamin D status maintain >80nmol/l
- **Calcium and vitamin D supplementation**

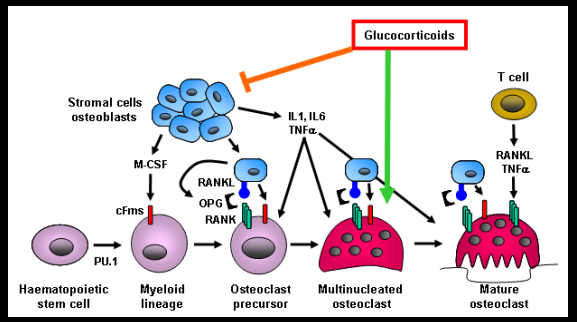
Antiresorptive agents
- **Selective estrogens receptor modulators (↑OPG, ↓IL6)**
- **Bisphosphonates (impaired sealing zone attachement)**
- **Denosumab (monoclonal ab to RANKL) (OPG like activity)**

Anabolic agents
- **Strontium ranelate (?)**
- **Teriparatide (PTH 1-34) (Intermittent PTH)**

Glucocorticoid induced osteoporosis (GIO)

Commonest iatrogenic cause of osteoporosis
(Predisolone >7.5mg/d for >3/12)





Rapid bone loss in first year slow loss thereafter
- **Decreased osteoblastogenesis and increased apoptosis**
- **Decreased osteoclastogenesis but prolonged survival**
- **Increased osteocyte apoptosis**

Fractures in 30-50% of chronically treated patients
- **BMD correlate far less well with fracture risk in GIO**

Treatment
- **Bisphosphonates: Considered in if glucocorticoids required for >3months**
- **Teriparatide: Increases BMD more than Alendronate in GIO**

Paget's Disease of Bone

Paget's disease of bone

(Localised disorder of bone remodelling)

Bone Scan

Tibia X-ray

- Focally abnormal bone remodelling
  - Osteoclast abnormality
    - Increased osteoclast numbers
  - Osteoblast abnormality
    - Disorganised rapid bone formation
- Chronic effects
  - Replacement by sclerotic bone
  - Bone marrow cavity replaced by vascular fibrous connective tissue
  - Increase in bone size and bone deformity
- Increased markers of formation and resorption
  - Bone alkaline phosphatase
  - P1NP
  - uNTX

Paget's disease

Commonest metabolic bone disease after osteoporosis
- Single site (Monostotic); Multiple sites (Polyostotic)

Aetiology
- Predominantly unknown (Restricted geographic distribution)
- More common in women
- Family history in 15% (Sequestome-1 (SQSTM1), RANK and OPG)
- Reason for decline in frequency is unknown

Clinical features
- Bone pain, joint pain, deformity, fracture and increased temperature
- Deafness (may be conductive or sensorineural)
- Abnormal x-ray

Diagnosis
- Raised alkaline phosphatase
- X-ray (osteolysis, osteosclerosis and bone expansion)
- ^{99}Tc bone scan is far more sensitive than plain X-ray

Paget's disease

Complications

- Osteoarthritis due to deformity
- Cranial nerve palsy and spinal stenosis
- Hypercalcaema if immobilised with active disease
- Osteosarcoma (very rare)

Treatment: Bone pain is the indication for treatment

- Simple analgesia (NSAIDs)
- Physio/hydrotherapy
- Bisphosphonates: reduce pain, do not prevent #, deformity or deafness
  - Zolendronic acid 5mg iv (Alk Phos normalises in 90%)
- Ensure patients are vitamin D and calcium replete
- Surgery for severe deformity or osteoarthritis

Follow up

- Alkaline phosphatase
- ^{99}Tc bone scan (if AlkP raised)

References

General

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Learning objectives

1. Contrasting endochondral and intramembranous ossification
2. Understand the role of chondrocyte, osteoblasts, osteoclasts and osteocytes
3. Describe the bone remodelling cycle
4. Contrast the affect of intermittent and continuous PTH on the skeleton
5. Define age related osteoporosis and list the common risk factors
6. Describe the causes of secondary osteoporosis
7. Describe DXA BMD analysis with particular reference to the T and Z scores
8. List the main medications used to treat osteoporosis and describe their action
9. Compare and contrast the uses of PTH and the bisphosphonates
10. Describe the skeletal consequences of long term glucocorticoid treatment
11. Describe the indications for treatment of Paget's disease and the mechanism of action of the therapy