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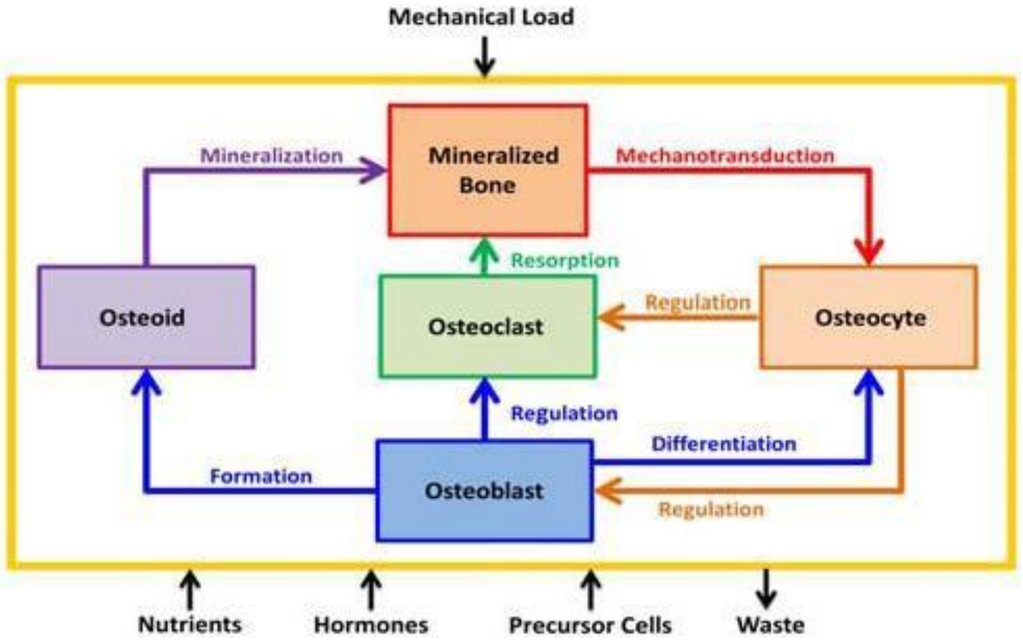
Pathophysiology of musculoskeletal system

Bone properties

- Bones
  - stiff
  - do not bend when loaded.
  - flexible - absorb the energy imposed by loading as potential energy by elastic then plastic deformation.
  - Structural failure may occur if bones deform too little or too much.
- High remodeling reduces the mineral content of bone, resulting in loss of stiffness.

Bone remodelling



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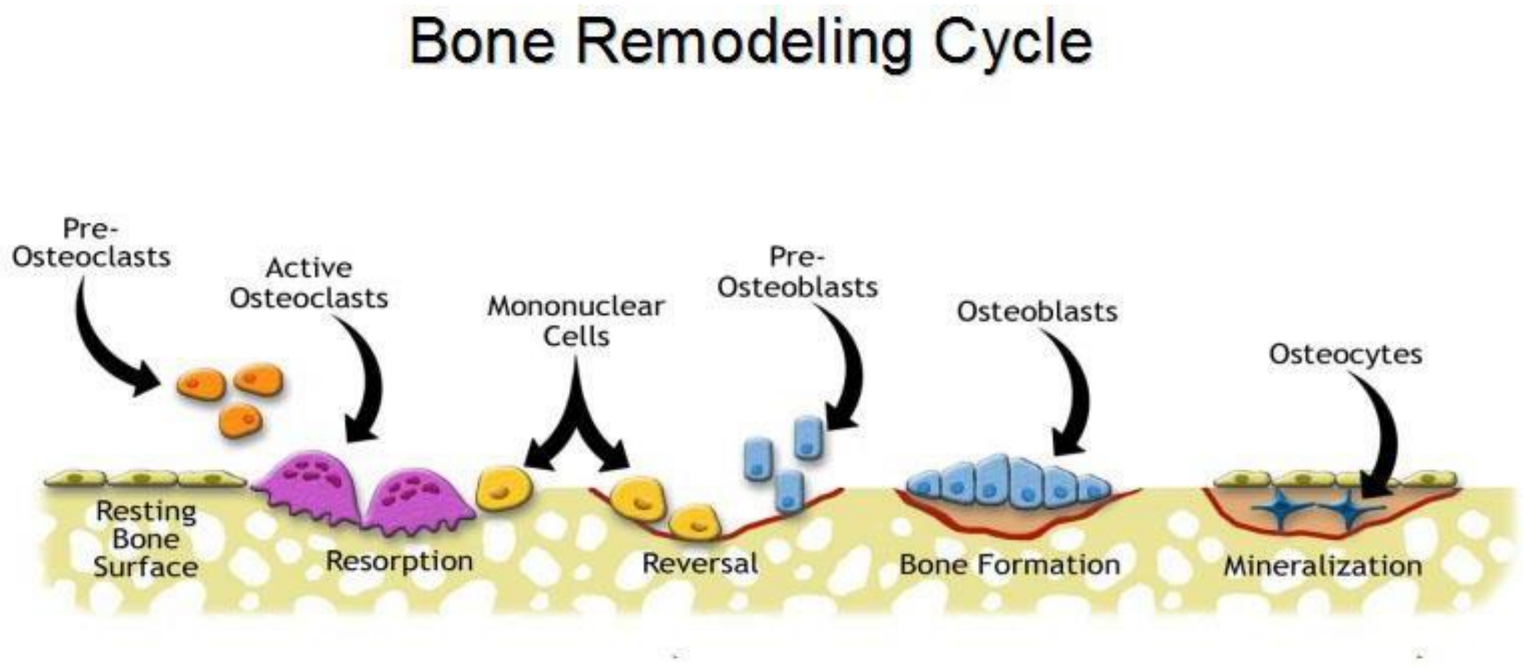
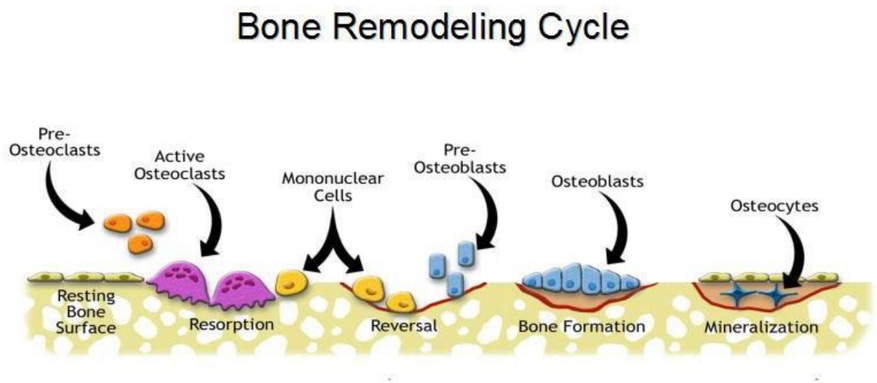
Bone remodeling

- tightly coordinated
- requires the synchronized action of osteoclasts, osteoblasts, bone-lining cells and osteocytes
- in a microanatomical structure separated from the bone marrow cavity by a canopy of cells but accessible through microcapillaries
- process starts with the retraction of bone-lining cells covering the bone surface and the recruitment of osteoclast precursors to this remodeling site.



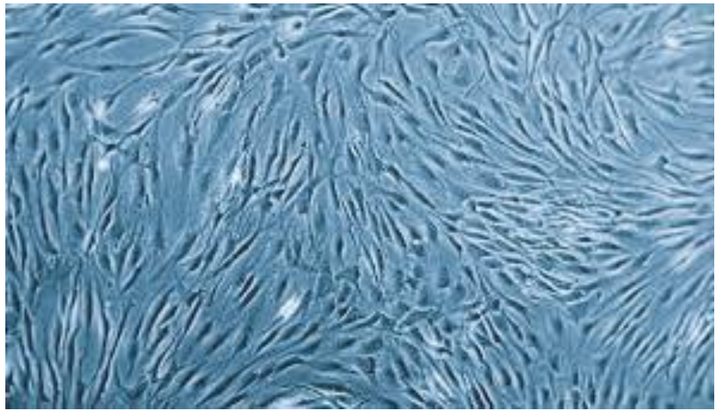
Bone remodeling

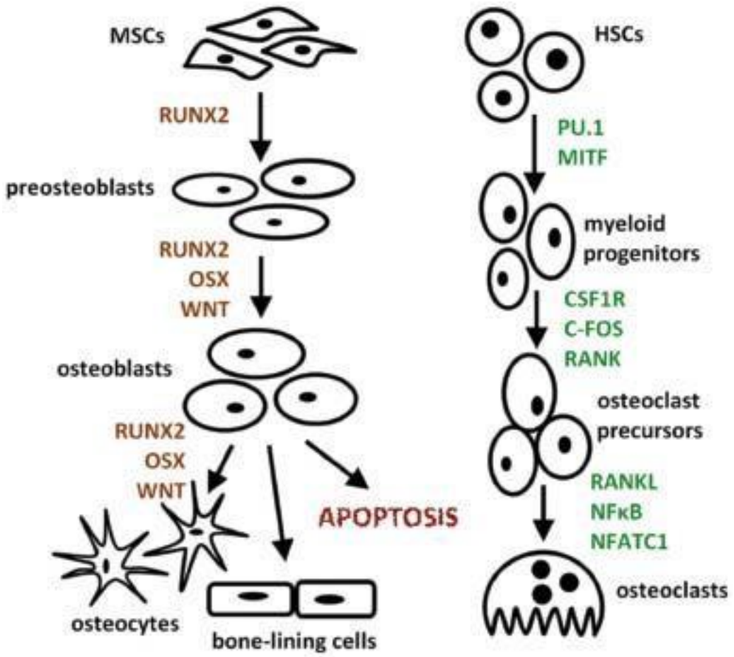
- ***Osteoclast activation***
- ***Resorbtion phase-*** due to osteoclast activation- short period
- ***Reverse phase-*** bone surface is covered by mononuclear cell
- ***Formation phase-*** osteoblast production in bone matrix - long.



Bone formation

- starts with the differentiation of osteoblasts and laying down of the organic osteoid, consisting mainly of collagen type I.
- completed after osteoblast-mediated mineralization of the organic matrix.
- resting bone surface covered by bone-lining cells belonging to the osteoblast lineage is re-established

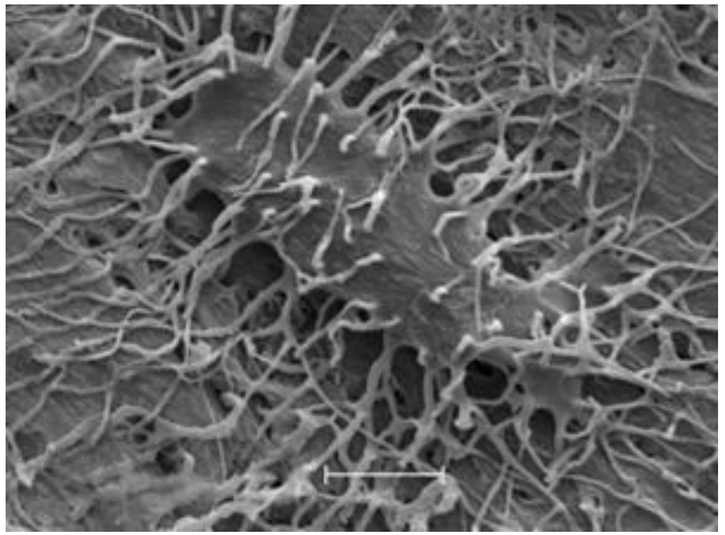


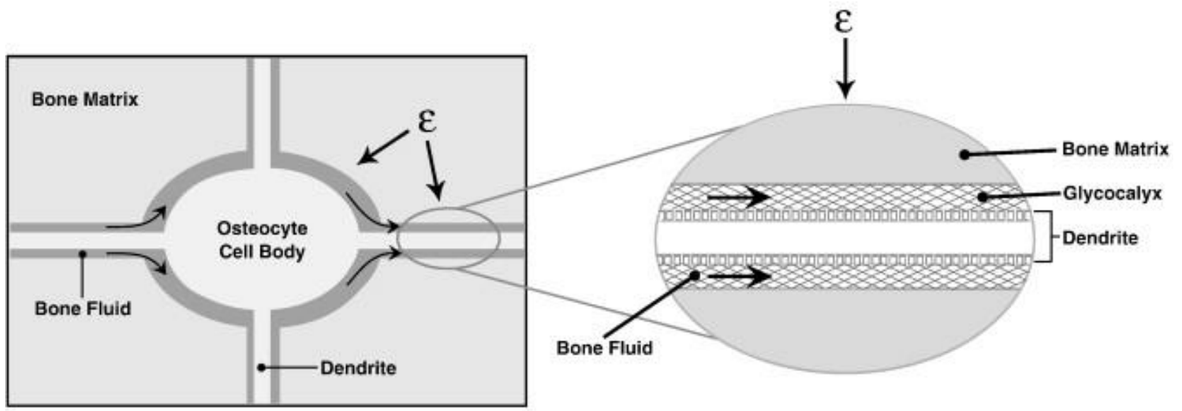


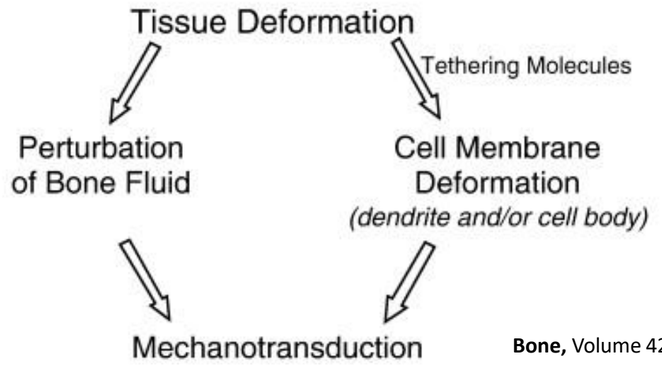
The osteoblast lineage derives from MSCs under the control of the transcriptional regulator RUNX2. The multipotent differential capacity of MSCs can also give rise to chondrocyte, adipocyte, myocyte and other cell lineages, utilizing lineage-specific transcription factors SOX9, PPARγ2 and MYOD/MYF5, respectively. RUNX2 is indispensible in all stages of osteoblast differentiation. After reaching maturity, three different potential fates await osteoblasts. Cells that become entombed within the bone matrix are called osteocytes, bone-lining cells cover all bone surfaces while the remainder undergo apoptosis

Osteocytes

- terminally differentiated osteoblasts entombed within the bone matrix
- account for almost 95% of all cells in the mature bone tissue – form a network of canaliculi within the mineralized bone.
- mechanosensing cells - detect mechanical strain and associated bone microdamage
- respond by initiating bone resorption and the regulation of bone remodeling.



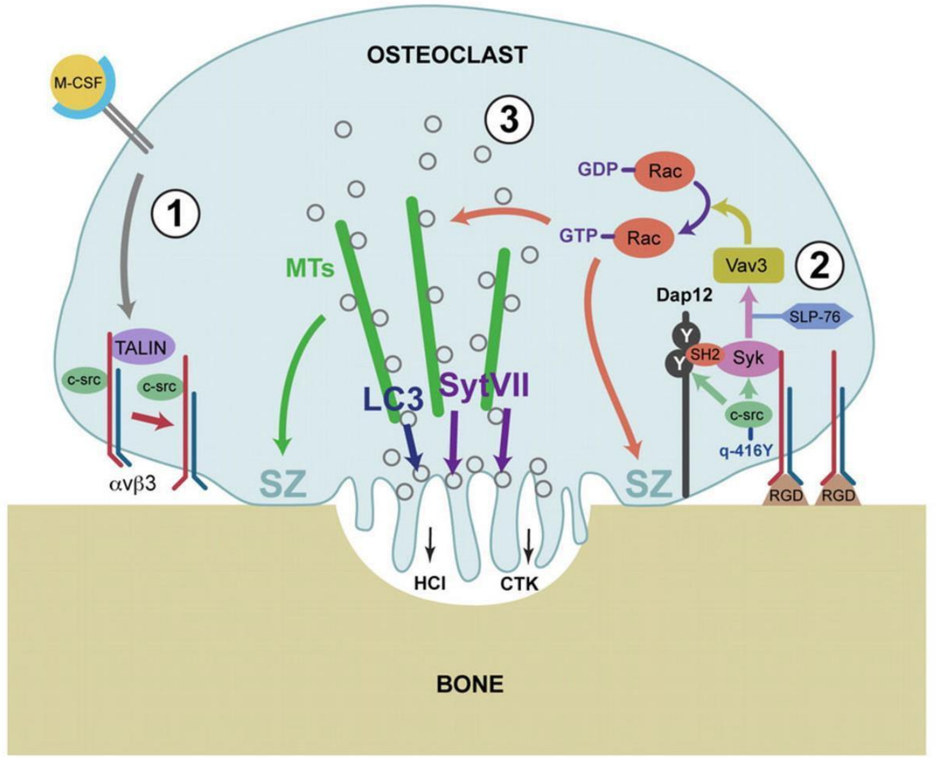




Bone, Volume 42, Issue 4

Mature osteoclasts

- large, multinucleated, short-lived, highly active cells attached to the bone surface
- responsible for the dissolution of the minerals and enzymatic degradation of the remaining organic matrix.
- after osteoclast-mediated resorption is complete, collagen remnants are removed
- resorption lacunae is prepared for subsequent osteoblast-mediated bone formation in a process that is still poorly understood.

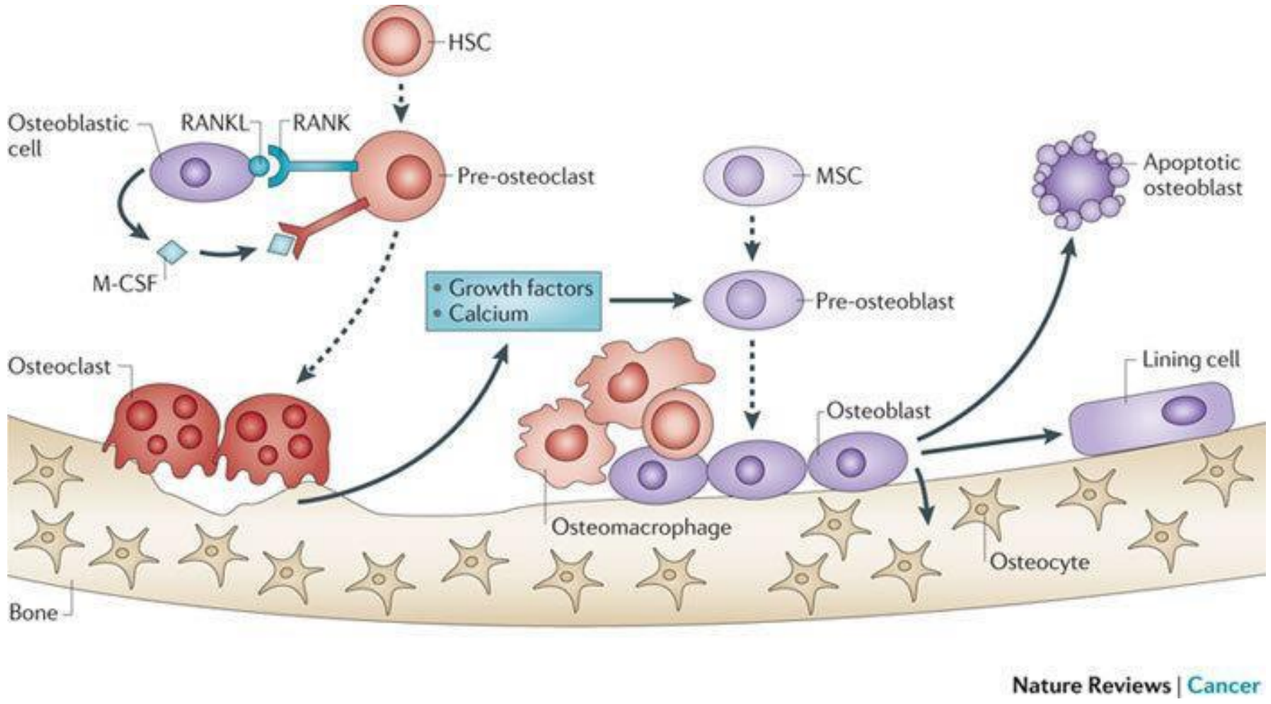


Proposed mechanism organizing the cytoskeleton of resorbing osteoclasts. 1). M-CSF occupying its receptor, c-fms, stimulates inside-out αvβ3 activation by inducing talin association with the β3 cytoplasmic domain that binds c-src constitutively. 2). Clustering of the integrin by RGD ligand increases avidity as well as affinity by outside-in activation. The liganded integrin activates c-src as evidenced by Y416 phosphorylation. Activated c-src tyrosine phosphorylates ITAM proteins that recruit Syk to the integrin by binding Syk-SH2 domains. c-src activates β3-associated Syk that phosphorylates Vav3 in the context of SLP-76. Vav3 then shuttles Rac-GDP to its activated GTP-associated state. 3). Rac-GTP prompts association of lysosome-derived secretory vesicles with microtubules (MTs) that deliver them to the bone-apposed plasma membrane into which they insert under the influence of Syt VII and LC3. Rac-GTP and MTs also promote sealing zone (SZ) formation. Secretory vesicle fusion focally expands the plasma membrane forming the ruffled border and eventuating in discharge of cathepsin K (CTK) and HCl into the resorptive microenvironment.

orthobullets.com/basic-science/9002/bone-cells

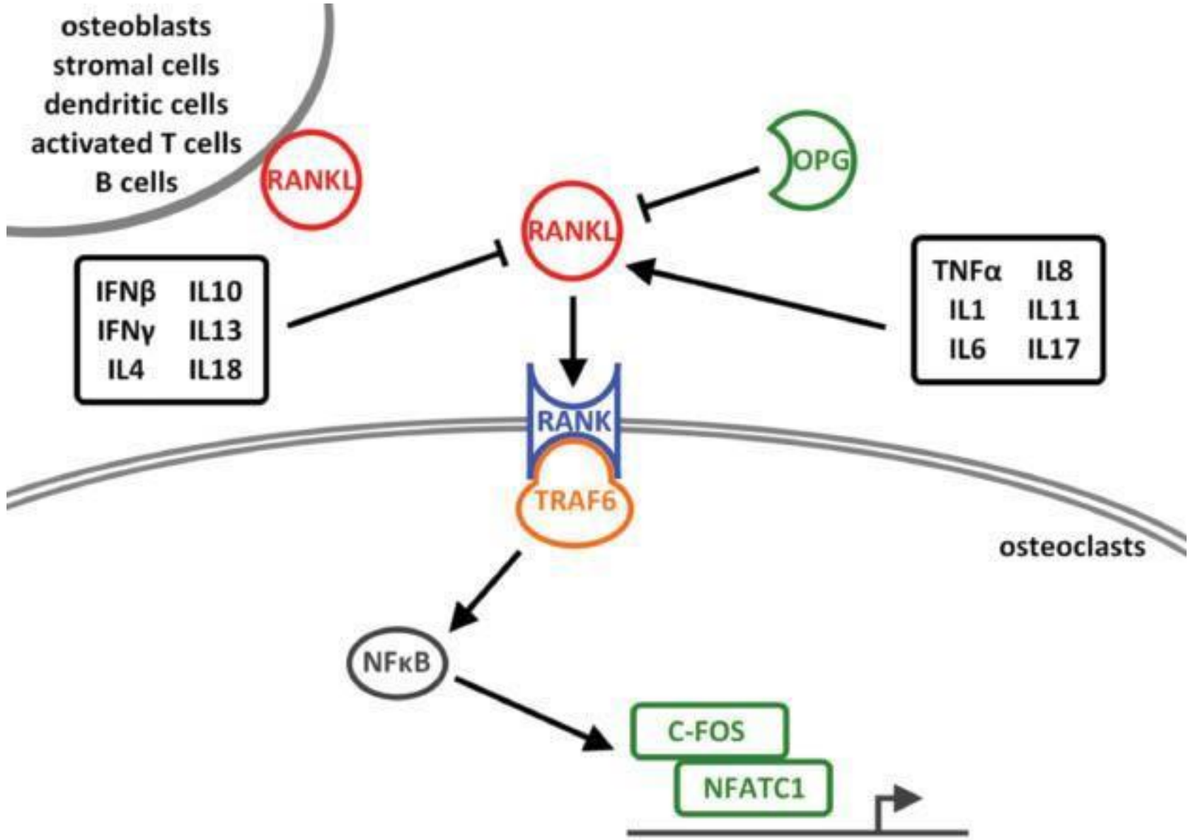
RANK/RANKL/OPG system

- one of the most important regulators of bone resorption and remodeling
- RANK, located on the surface of osteoclasts and their precursors, and its ligand RANKL are essential for the formation, differentiation, activity and survival of osteoclasts.
- RANKL is produced by cells of the osteoblast lineage as well as other cell types in both soluble and membrane-bound forms.
- The binding of RANKL to RANK, results in the activation of transcription factors NFκB and NFATC1 and the expression of osteoclastogenic genes.
- OPG, secreted by osteoblasts and a few other cell types, functions as a decoy receptor by binding to RANKL, thereby preventing the activation of RANK.
- inhibition of RANKL leads to the rapid arrest of osteoclast formation, activation and survival, is crucial for the suppression of bone resorption and maintenance of bone mass



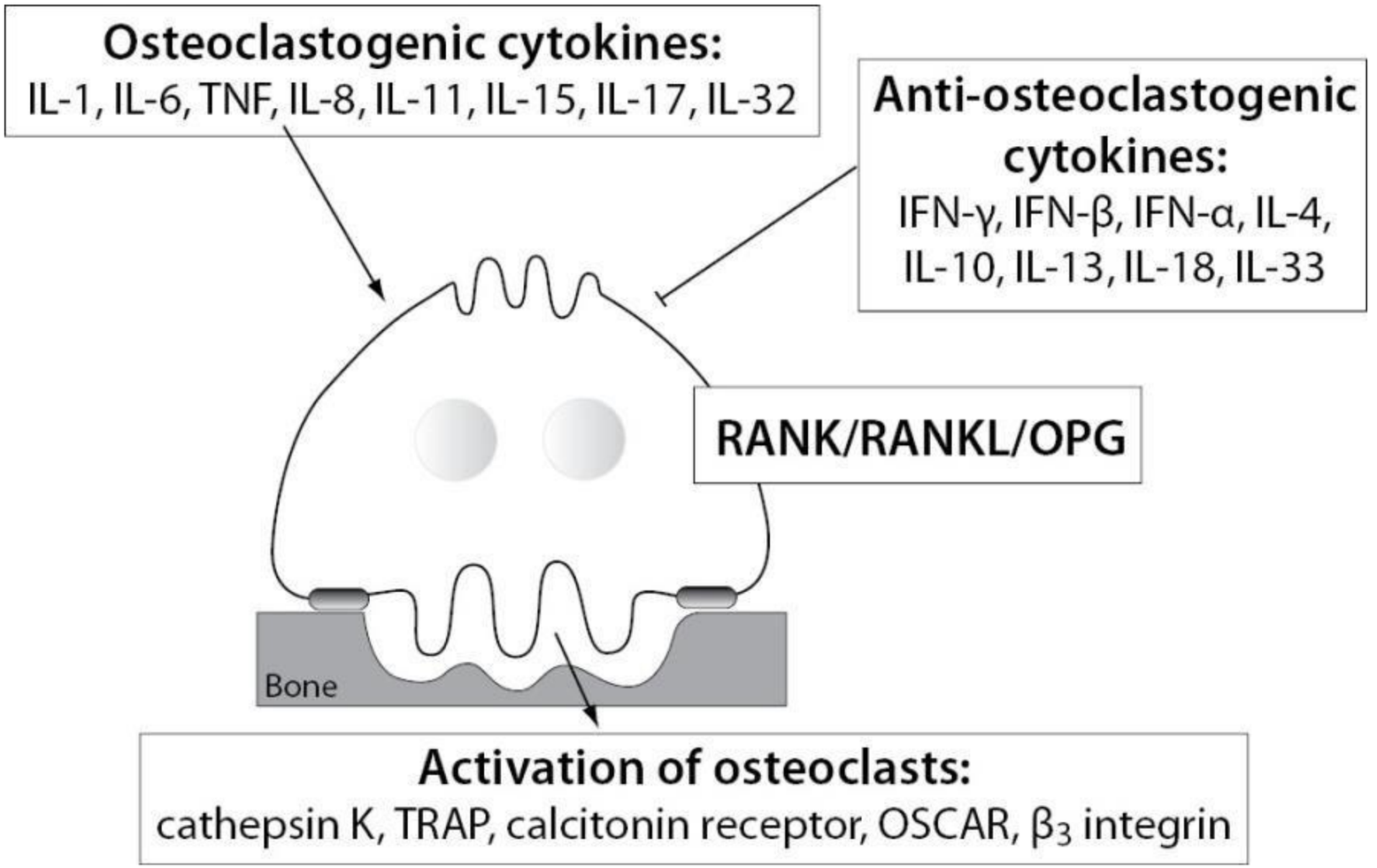
Other modulators of RANK/RANKL/OPG system

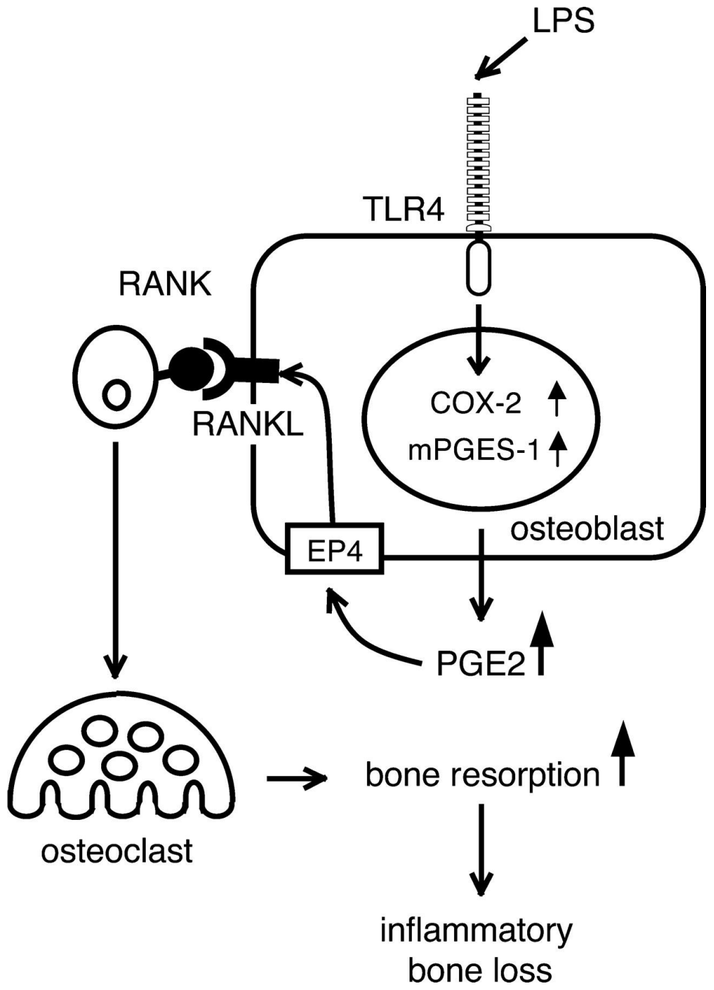
- Pro-inflammatory cytokines secreted by different immune cells
  - including activated T cells, B cells, macrophages, mast cells and natural killer cells
- TNFα, IL1, IL6, IL8, IL11 and IL17
  - osteoclastogenic cytokines promoting RANKL-mediated osteoclast differentiation and activity,
- IFNγ, IL4, IL10, IL13 and IL18
  - anti-osteoclastogenic cytokines IFNβ, inhibit osteoclasts through the RANK/RANKL/OPG system.
- Certain cytokines can exert opposite effects on osteoclasts (e.g., IL7 and IL23)



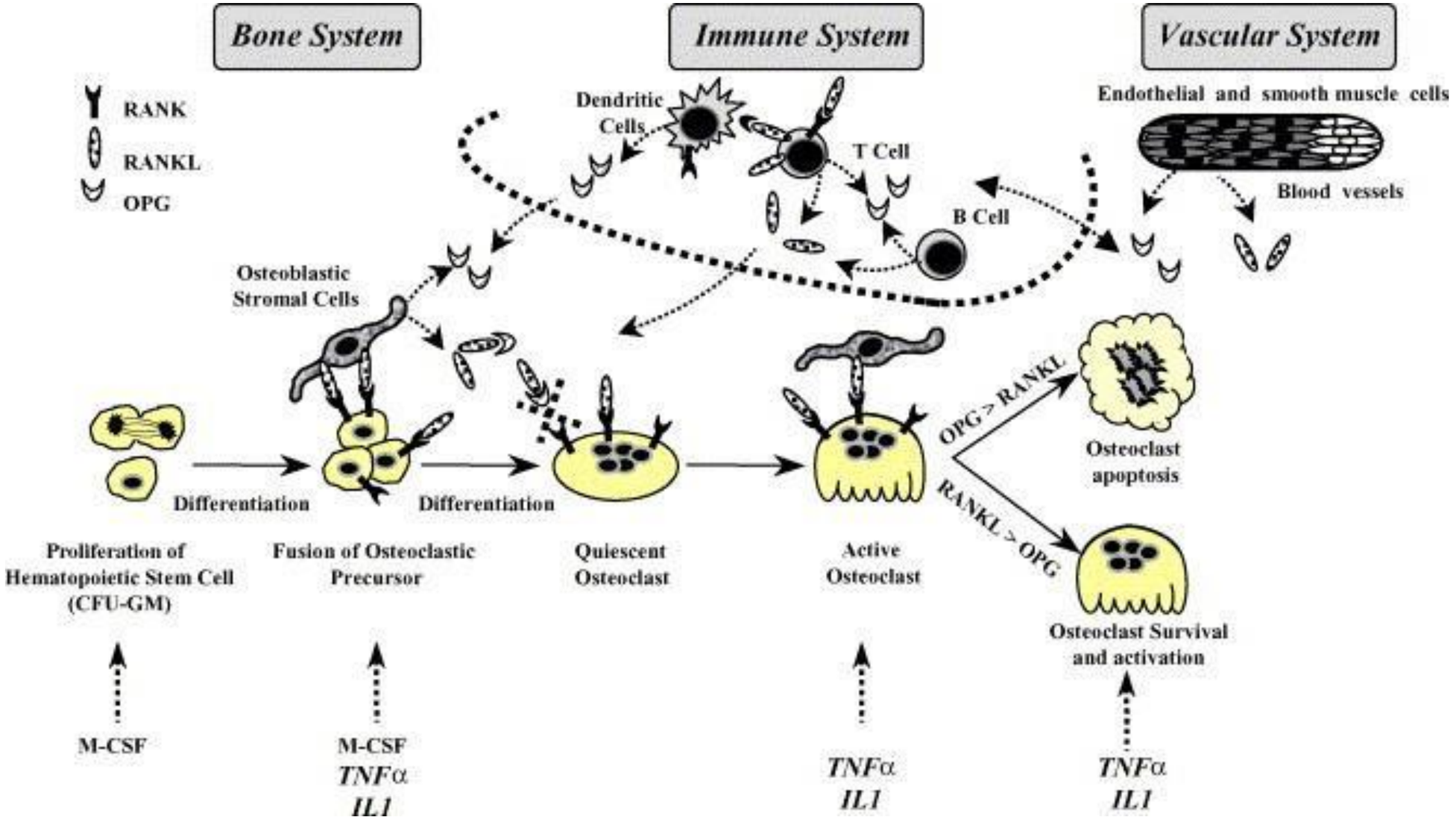
RANK/RANKL/OPG system.The RANK/RANKL/OPG system is essential for the formation and differentiation of osteoclasts, their resorptive activity and survival. The binding of RANKL to RANK results in the recruitment of TRAF6, which activates various protein kinase pathways and transcription factors like NFκB. The activated NFκB up-regulates the expression of C-FOS, which subsequently interacts with NFATC1 to induce the expression of osteoclastogenic genes. Conversely, OPG prevents the activation of RANK by binding RANKL .

Cytokines and prostaglandins



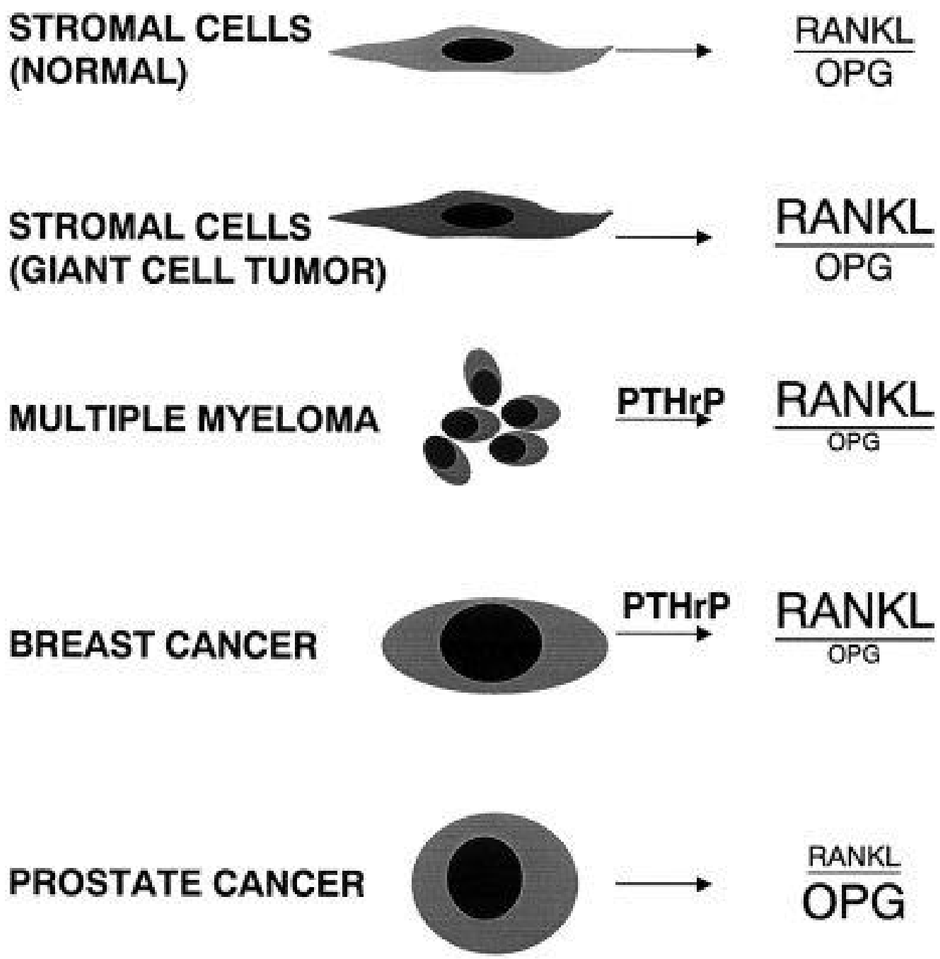


Osteo-immunomodulatory complex



Osteoclasts activation

- Under pathologic conditions, inflammatory and malignant cells can increase osteo-clastogenesis by producing soluble or membrane-bound M-CSF and RANKL as well as PTH-related protein (PTHrP), cytokines, and prostaglandins.



Parathyroid Hormone Relation Peptide (PTHrP)

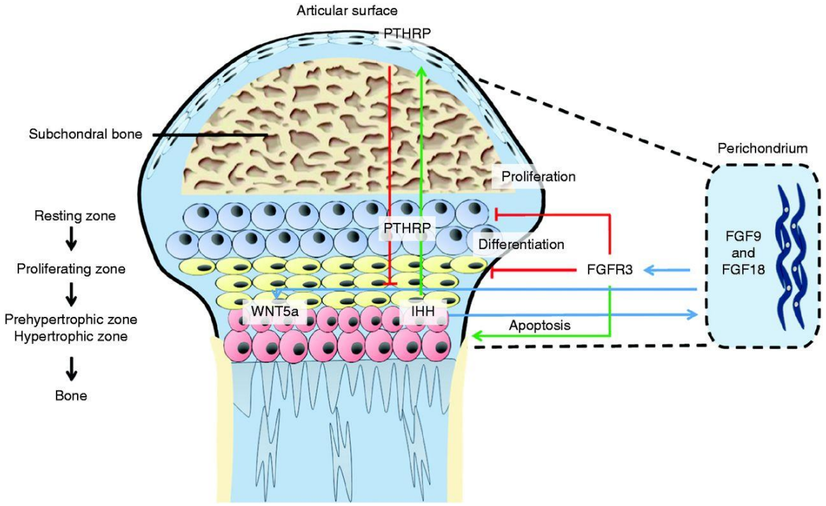
- PTHrP was discoverde as mediator of syndrome ***"humoral hypercalcemia of malignancy" (HHM***).
- During the syndrome inn different type of cancer (in absebce of metastases) similar compounds to PTH are produceds which is related to:
- ***Hypercalcemia***
- ***Hypophosphatemia***
- ***Increased cAMP exctretion by urine***
- The effects are similar to those caused by PTH; no PTH levels are detected.

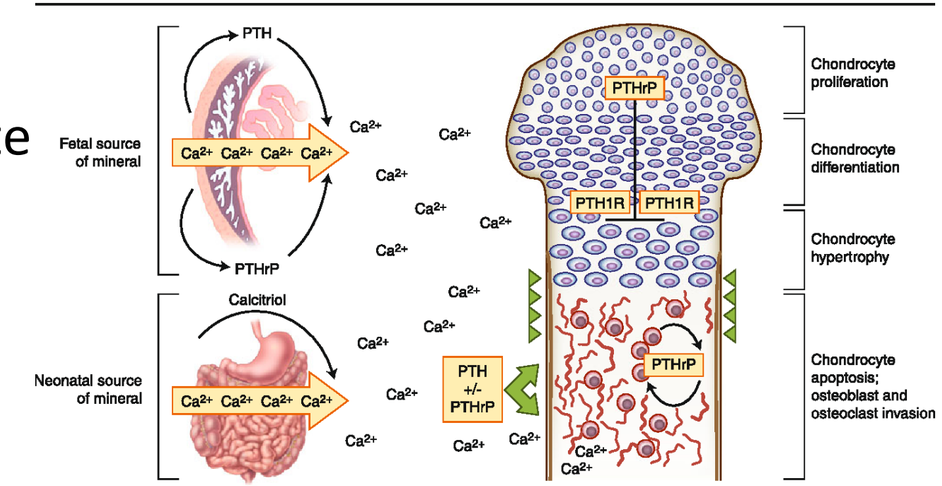
Effects of PTHrP

Cell growth, differentiation and apoptosis in many fetal and adult tissues. In the absence of PTHrP there is a reduction in chondrocyte proliferation with emphasis on chondrocyte differentiation and apoptosis

Normal development of cartilaginous growth plate. In the fetal period, PTH plays a dominant anabolic role in trabecular bone development. PTHrP regulates growth plate development.

Postnatally, PTHrP, as a paracrine/autocrine regulator, assumes an anabolic role for bone homeostasis, whereas PTH primarily maintains Ca ++ levels in the ECT through bone resorption.

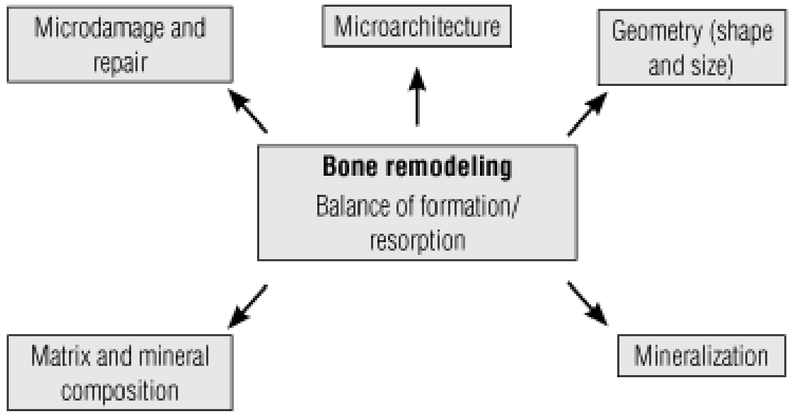




Development 2008 135: 1947-1956

Gene	Mutation	Disease
RANK	18 bp duplication	Familial expansile osteolysis
	27 bp duplication	Early onset Paget's disease
	15 bp duplication	Expansile skeletal hyperphosphatasia
RANKL	Deletion of amino acids 145-177	Autosomal recessive osteopetrosis
	A single nucleotide change (596T-A) in exon 8 of both alleles	Autosomal recessive osteopetrosis
	Deletion of two nucleotides (828_829delCG)	Autosomal recessive osteopetrosis
OPG	Deletion making OPG inactive	Juvenile Paget's disease
	20 bp deletion resulting in premature termination of OPG translation	Juvenile Paget's disease

Bone - pathophysiology



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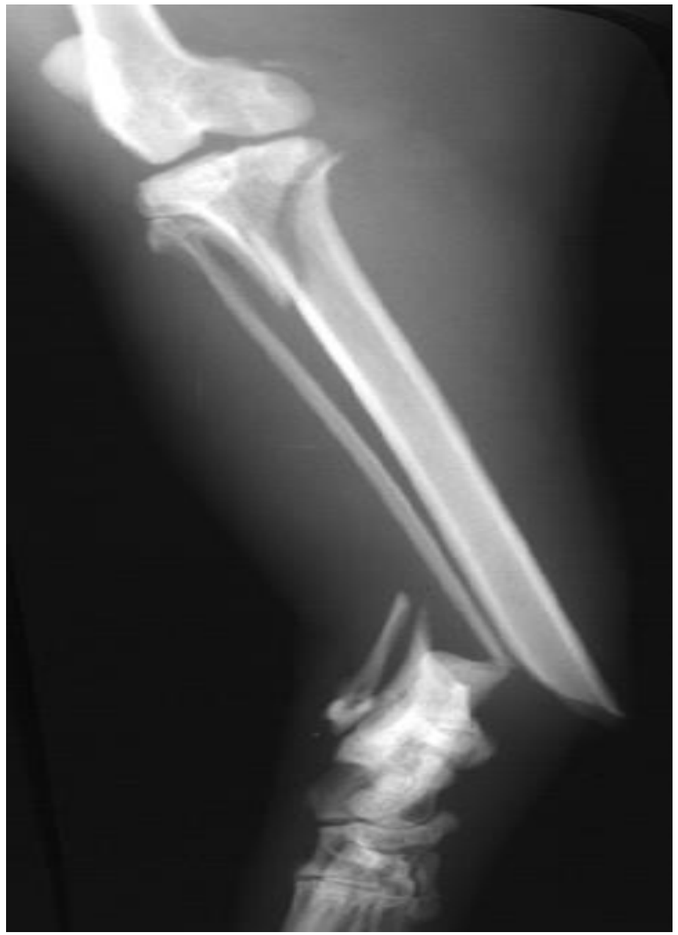
Skeletal fragility

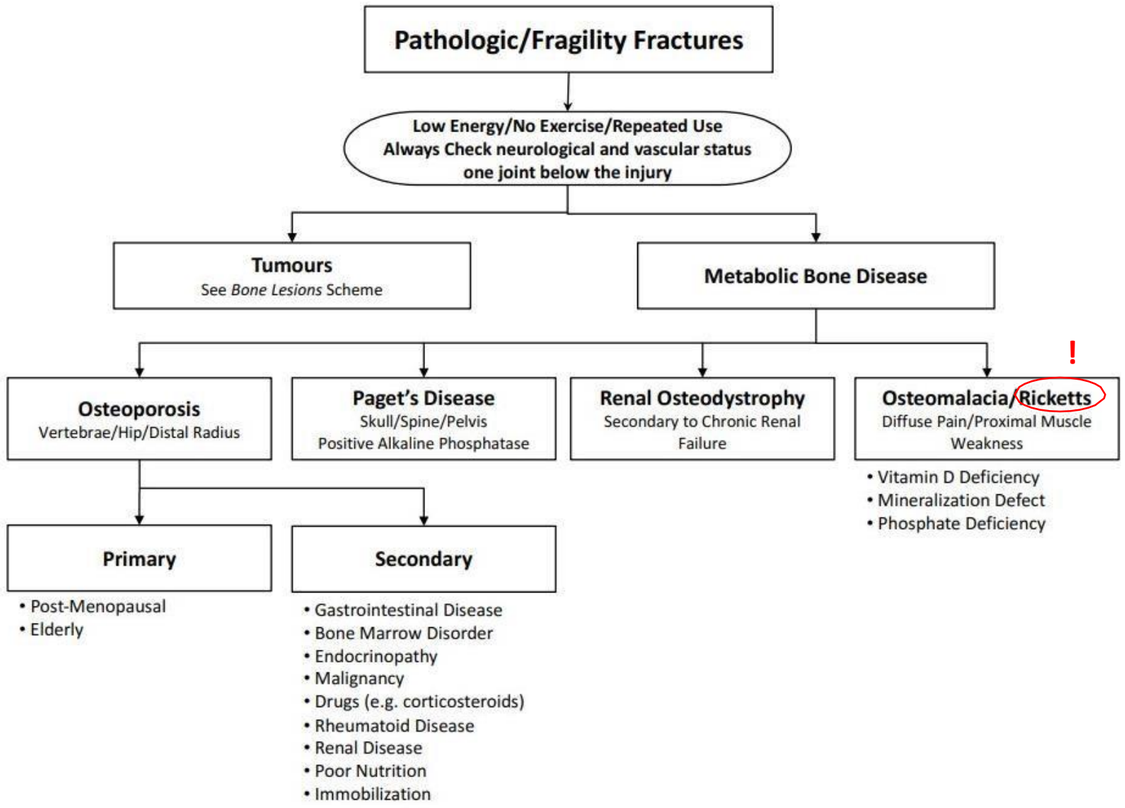
Skeletal fragility can result from:

- failure to produce a skeleton of optimal mass and strength during growth;
- excessive bone resorption resulting in decreased bone mass and microarchitectural deterioration of the skeleton;
- and an inadequate formation response to increased resorption during bone remodeling.



Pathologic Fractures

- Tumors
  - primary
  - secondary (metastatic) (most common)
- Metabolic
  - osteoporosis (most common)
  - Paget's disease
  - hyperparathyroidism



Toronto Notes for Medical Students, Inc. (2009). *Toronto Notes 209: Comprehensive Medical Reference and Review for MCCQE I & USMLE II*. McGraw-Hill: Toronto, Ontario.

Pathological fracture in pediatric patient

- a fracture associated with
  - minimal trauma
  - location of the fracture is unusual
  - abnormal process in the bone is seen in the radiographs
- cause of changes to the normal biomechanics of bone
  - Intrinsic processes
    - bone tumours (both benign and malignant),
    - metabolic diseases
    - osteogenesis imperfecta,
    - infection
  - extrinsic processes
    - internal fixation,
    - biopsy tracts and
    - radiation

Common predisposing benign and malignant lesions by age (adapted with permission from Arkader A, Dormans JP. Pathologic fractures associated with tumors and unique conditions of the musculoskeletal system. In: Beaty JH, Skaggs DL, Flynn JM, Waters K, eds. Rockwood and Wilkins' fractures in children. Seventh ed. Philadelphia: Lippincott Williams & Wilkins, 2010:120–191)

Age (yrs)	Benign lesions	Malignant lesions
0 to 5	Osteomyelitis Eosinophilic granuloma Hand-Schuller-Christian disease	Metastatic tumours (neuroblastoma, Wilm's tumour) Leukaemia Ewing sarcoma Fibrosarcoma Eosinophilic granuloma/ Letterer-Siwe disease
5 to 10	Unicameral bone cyst (UBC) Aneurysmal bone cyst (ABC) Nonossifying fibroma (NOF) Osteochondroma Fibrous dysplasia Enchondroma/Ollier disease Neurofibromatosis/Congenital pseudarthrosis of the tibia	Leukaemia Osteogenic sarcoma Ewing sarcoma
10 to 20	Unicameral bone cyst Aneurysmal bone cyst Nonossifying fibroma Osteochondroma Fibrous dysplasia Chondroblastoma Giant cell tumour Osteoid osteoma	Leukaemia Lymphoma Osteogenic sarcoma Ewing sarcoma

Bone remodelling defects

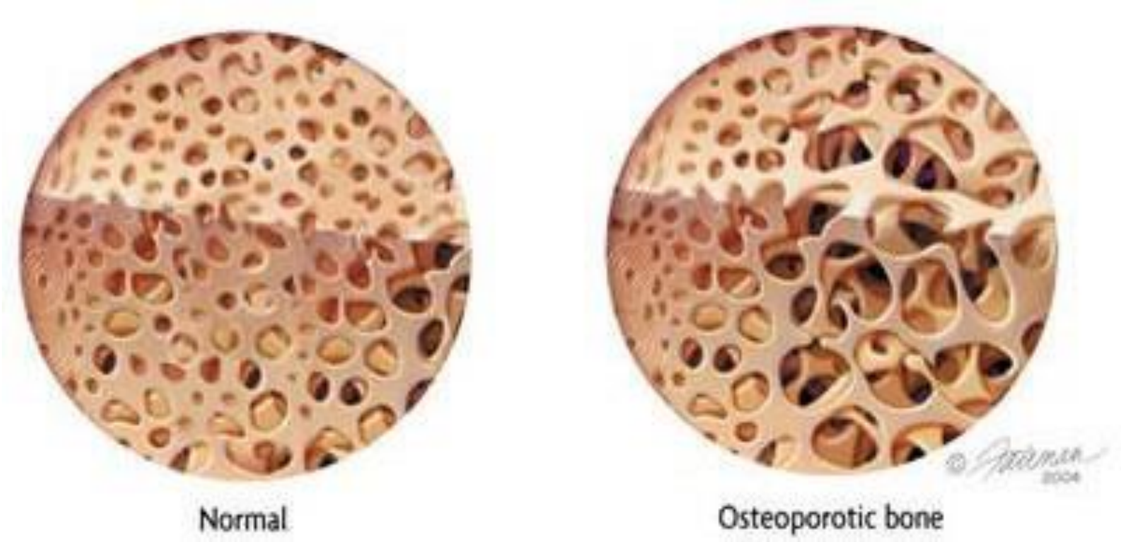
- Osteoporosis
- Osteodystrophy
- Rachitis/osteomalacia
- Paget`s disease

Bone remodelling defects

- Osteoporosis
- Osteodystrophy
- Rachitis/osteomalacia
- Paget`s disease

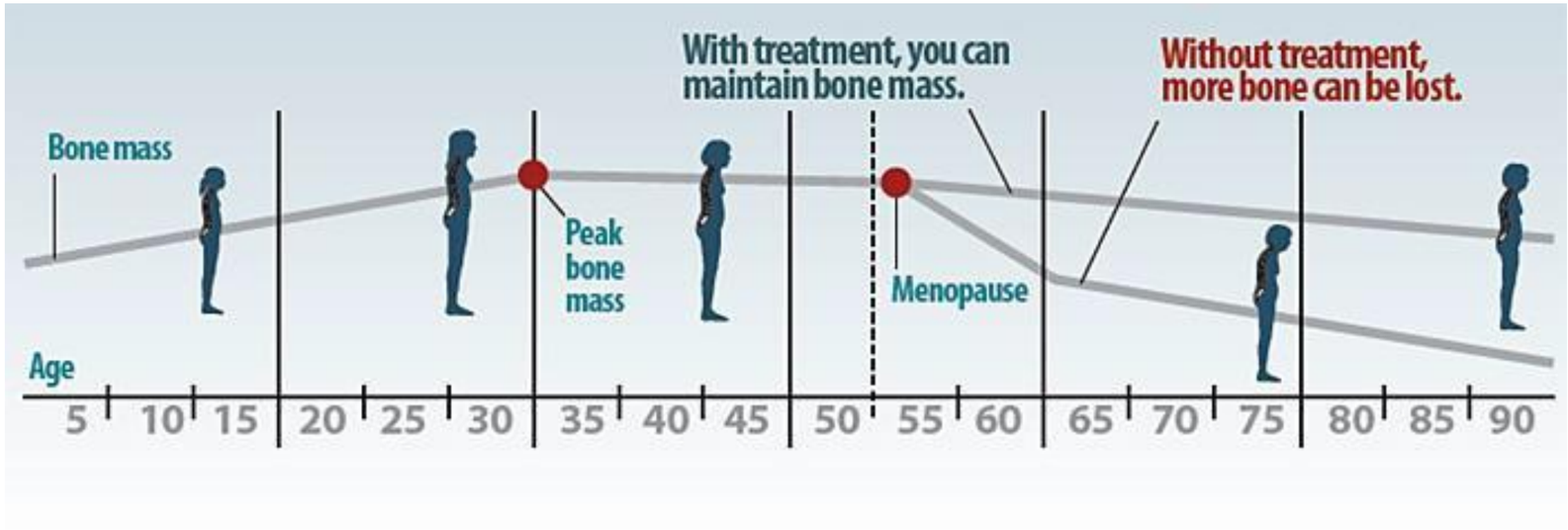
Metabolic bone diseases

- Osteoporosis remains the most common metabolic abnormality of bone. It has been described as “a silent epidemic” affecting one in two women and one in five men, older than 50 years of age, during their lifetime.
- It is now defined as a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone resulting in fractures with little or no trauma.



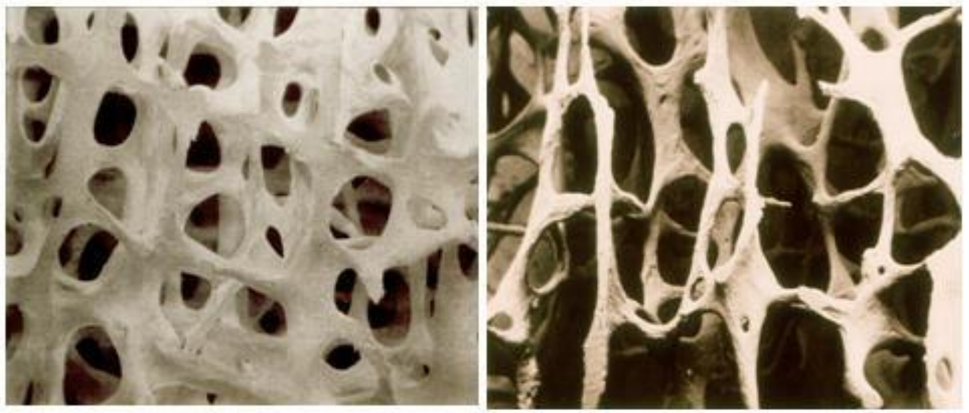
Osteoporosis

- The bone mass of an individual in later life is a result of the peak bone mass accrued during intrauterine life, childhood, and puberty, as well as the subsequent rate of bone loss.
- Although genetic factors strongly contribute to peak bone mass, environmental factors in intrauterine life, childhood, and adolescence modulate the genetically determined pattern of skeletal growth.



Osteoporosis

- is a skeletal disease characterised by low bone mass and microarchitectural deterioration with a resulting increase in bone fragility and hence susceptibility to fracture.
- Caucasin population: about 50% of women and 20% of men older than 50 years will have a fragility fracture in their remaining lifetime.



Healthy bone

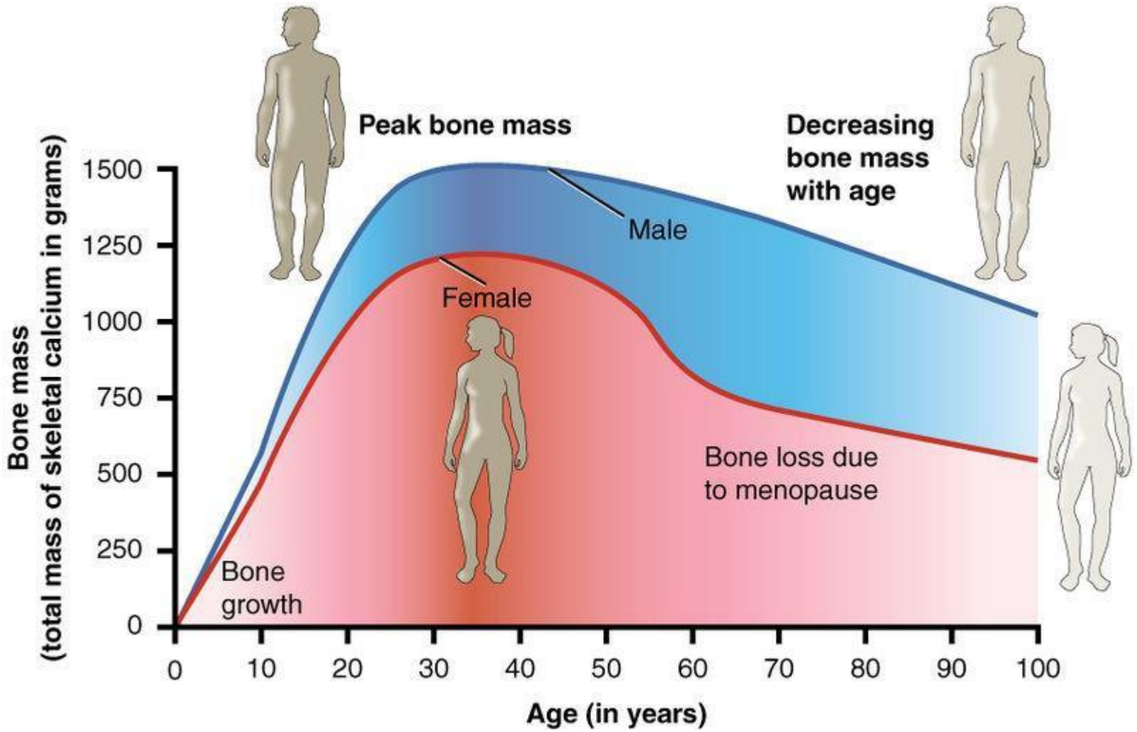
Osteoporotic bone

Etiopathogenesis of osteoporosis

- complex interactions among local and systemic regulators of bone cell function.
- The heterogeneity of osteoporosis may be due to
  - differences in the production of systemic and local regulators,
  - changes in receptors,
  - signal transduction mechanisms,
  - nuclear transcription factors, and
  - enzymes that produce or inactivate local regulators.
- Since the first human osteoporosis study indicated an association among bone mass, fragility, and polymorphisms in the *vitamin D receptor* (*VDR*) gene, more than 30 candidate genes have been reported that might influence skeletal mass and fragility.
- Since osteoporosis is a complex, polygenic disorder, the contributions of specific gene polymorphisms are likely to be relatively small, but may still be clinically important.

Osteoporosis - causes

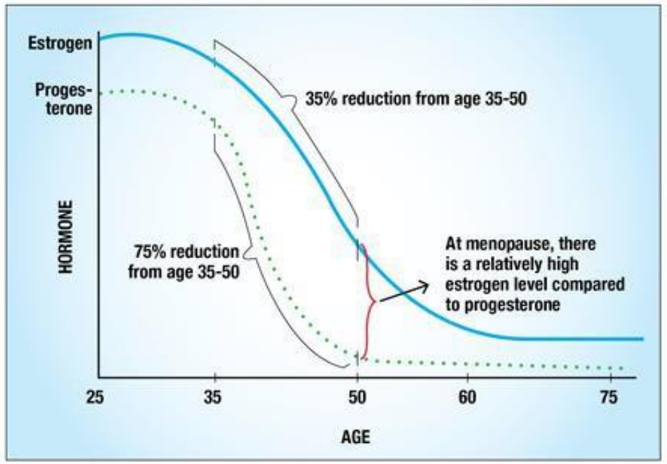
- Estrogene deficiency
- Glucocorticoids excess
- Vitamin K2 deficiency
- Immobilization

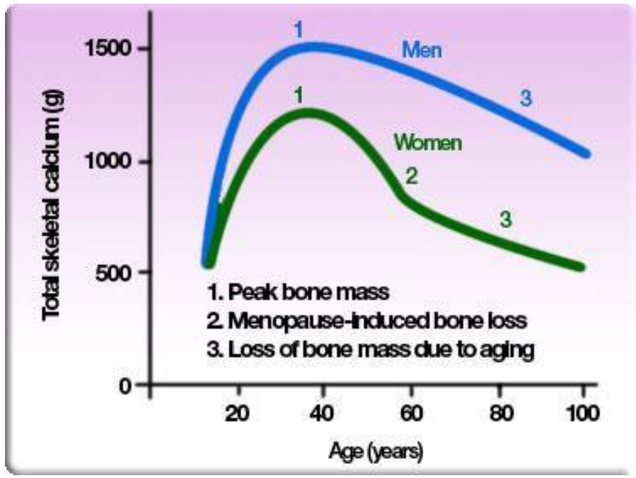


Anatomy & Physiology, Connexions Web site. http://cnx.org/content/col11496/1.6/, Jun 19, 2013.

Estrogen influence on bone state

- Estrogen is critical for
  - epiphyseal closure in puberty in both sexes and
  - regulates bone turnover in men as well as women.
- Estrogen has a greater effect than androgen in inhibiting bone resorption in men, although androgen may still play a role.
- Estrogen may also be important in the acquisition of peak bone mass in men.
- Osteoporosis in older men is more closely associated with low estrogen than with low androgen levels.

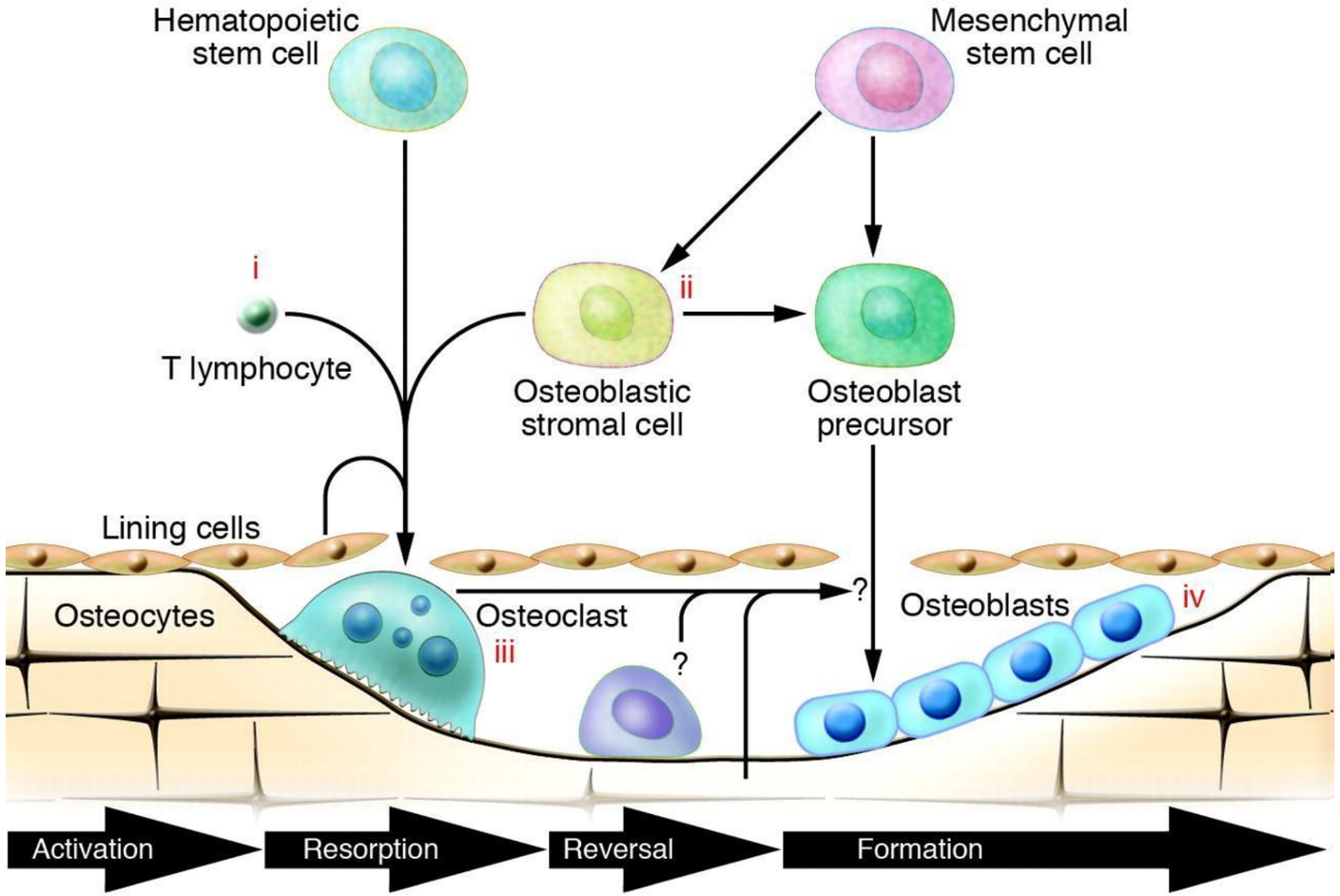




Central role of estrogen deficiency - today

- An increase in bone resorption, and not impaired bone formation, appears to be the driving force for bone loss in the setting of estrogen deficiency.
- The rapid and continuous bone loss that occurs for several years after the menopause indicate an impaired bone formation response, since in younger individuals going through the pubertal growth spurt, even faster rates of bone resorption can be associated with an increase in bone mass.
- However, the increased bone formation that normally occurs in response to mechanical loading is diminished in estrogen deficiency, suggesting estrogen is both anti-catabolic and anabolic.

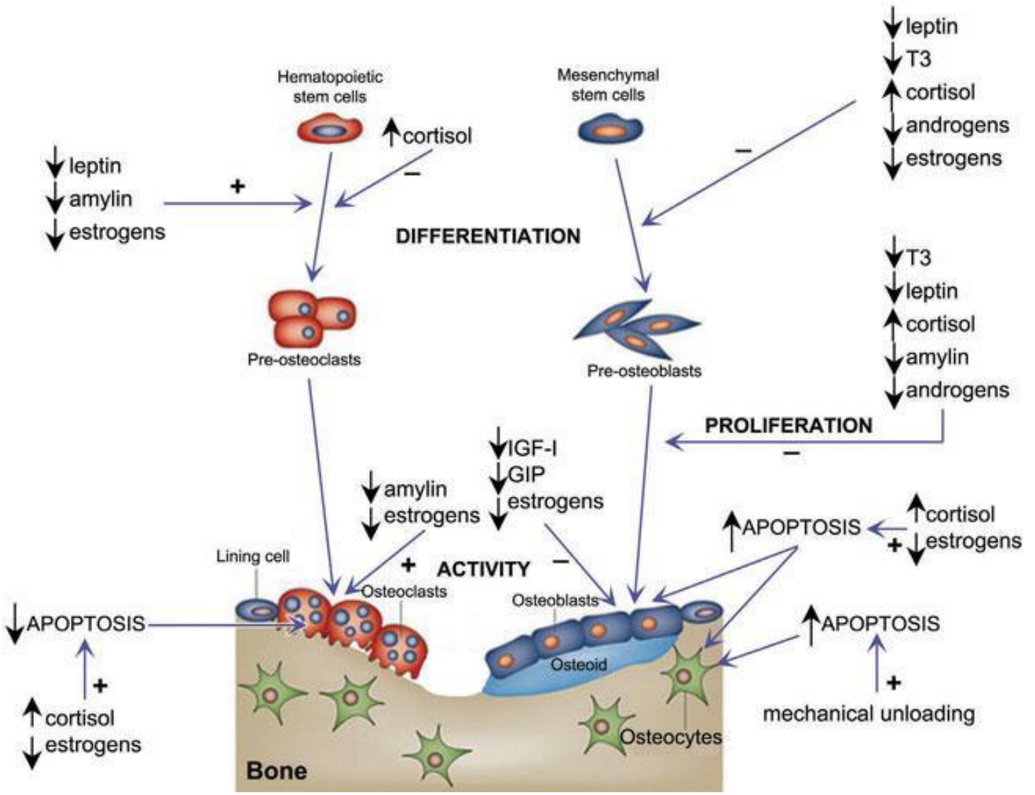
Remodelling of bones. Estrogen action places (i)



Raisz, L. G. J. Clin. Invest. 2005;115:3318-3325

Osteoporosis induced by cortisol

- Cortisol modifies proliferative and metabolic activities of bone cells
- Cortisol inhibits osteoblastogenesis
- Reduces half-life time of osteoblasts which is leading to decreased bone formation



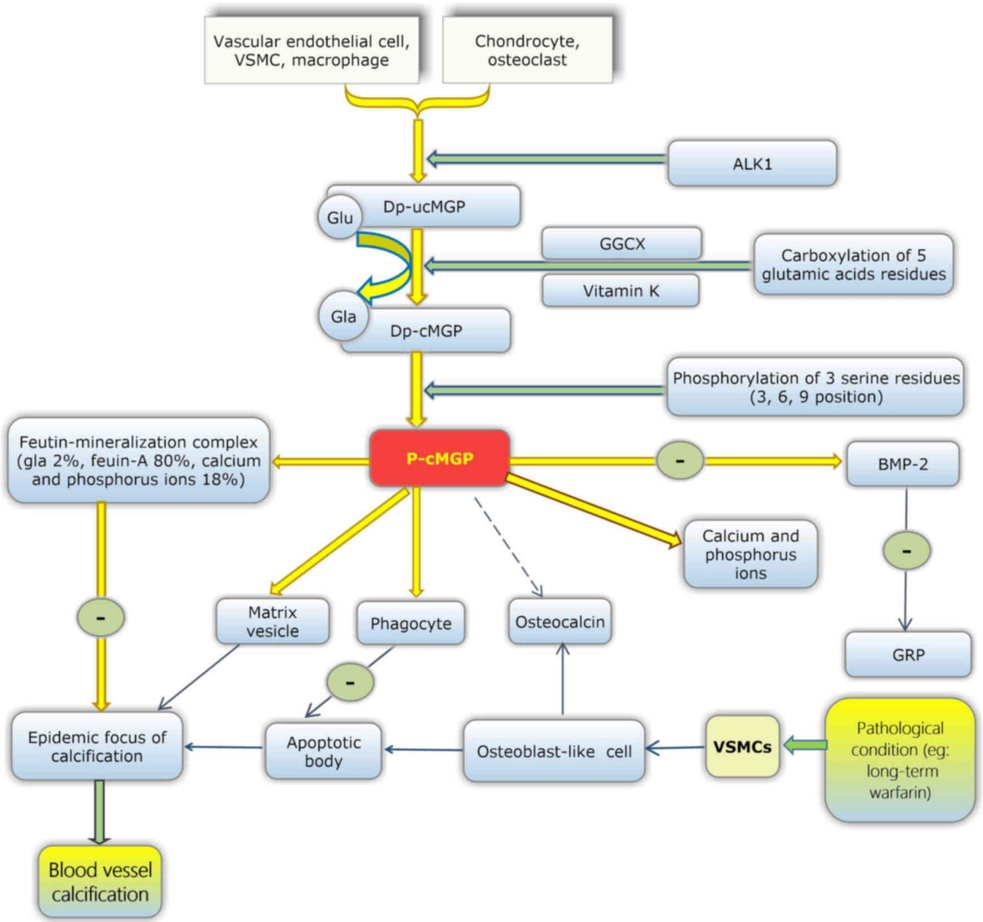
Common adverse effects of glucocorticoid therapy- **glucocorticoid-induced osteoporosis**

- Glucocorticoid-induced osteoporosis is the most common type of iatrogenic osteoporosis and a frequent cause of secondary osteoporosis.
- An estimated 50% of patients taking glucocorticoids for longer than 6 months will develop secondary osteoporosis.
- The absolute risk for glucocorticoid-induced osteoporosis is higher in patients aged 65 years or older given their baseline age-related fracture risk, although the relative risk of fracture related to glucocorticoid use may be even higher in patients under 65.

Vitamin K and bones

- cofactor for γ-carboxylase, enzyme which catalyses conversion of specific residuals of glutamic acid to Gla residuals
- γ-carboxylation of proteins of bone matrix which contain Gla as MGP (= matrix Gla protein) and osteokalcin
- Uncompleted γ-carboxylation of osteocalcin and MGP during vitamin K decrease lead to osteoporosis and high risk of fractures.
- stimulates synthesis of osteoblastic markers and bone deposition.
- decreases bone reabsorbtion by inhibition of osteclasts formation and by decrease of their resorbtion activity.

- Vitamin K_2 treatment induces osteoclast apoptosis, but inhibits osteoblasts apoptosis which is leading to increased bone formation.
- Vitamin K_2 supports osteocalcin expression (increases its mRNA) which can be further modulated by 1, 25-$(OH)_2$ vitamin D_3.



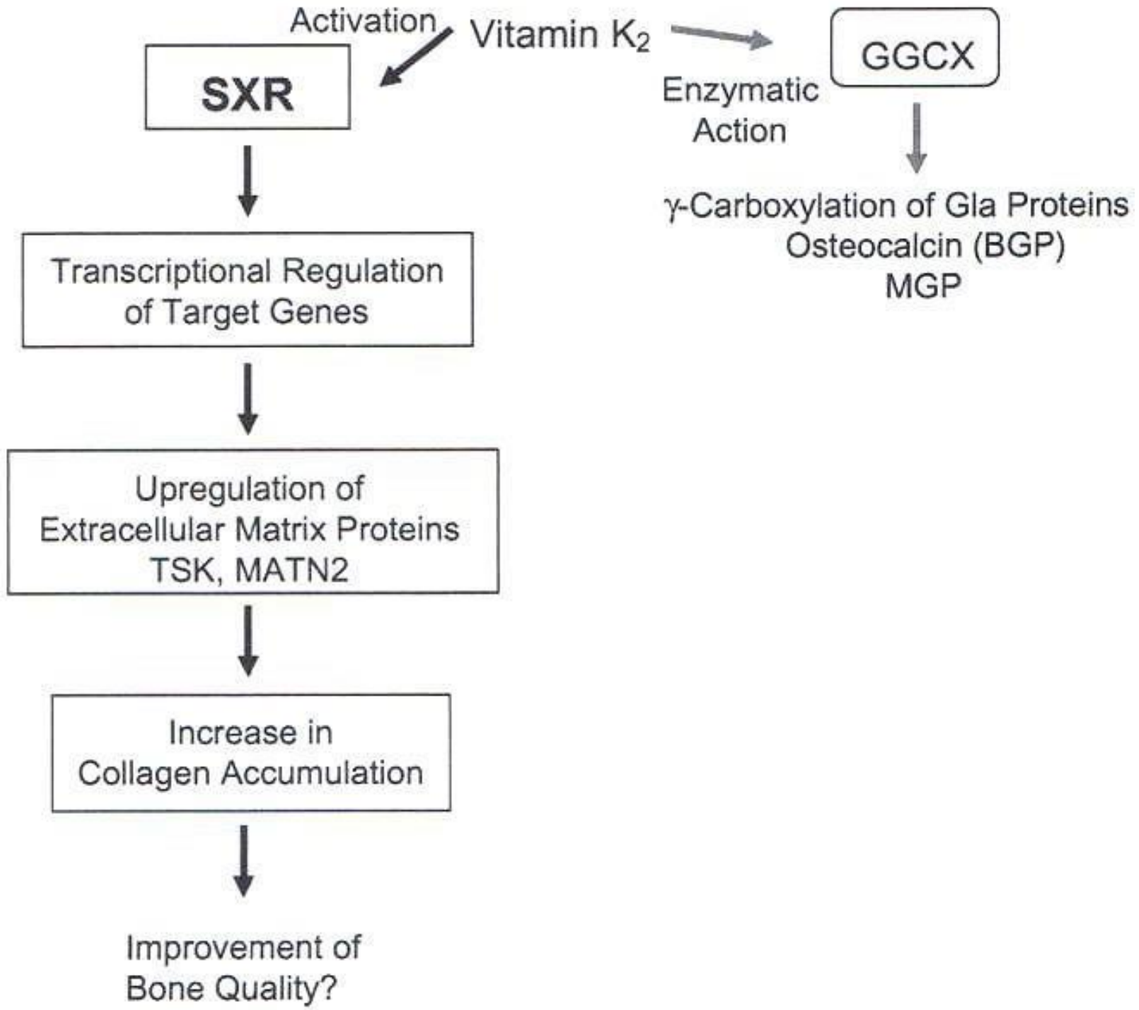
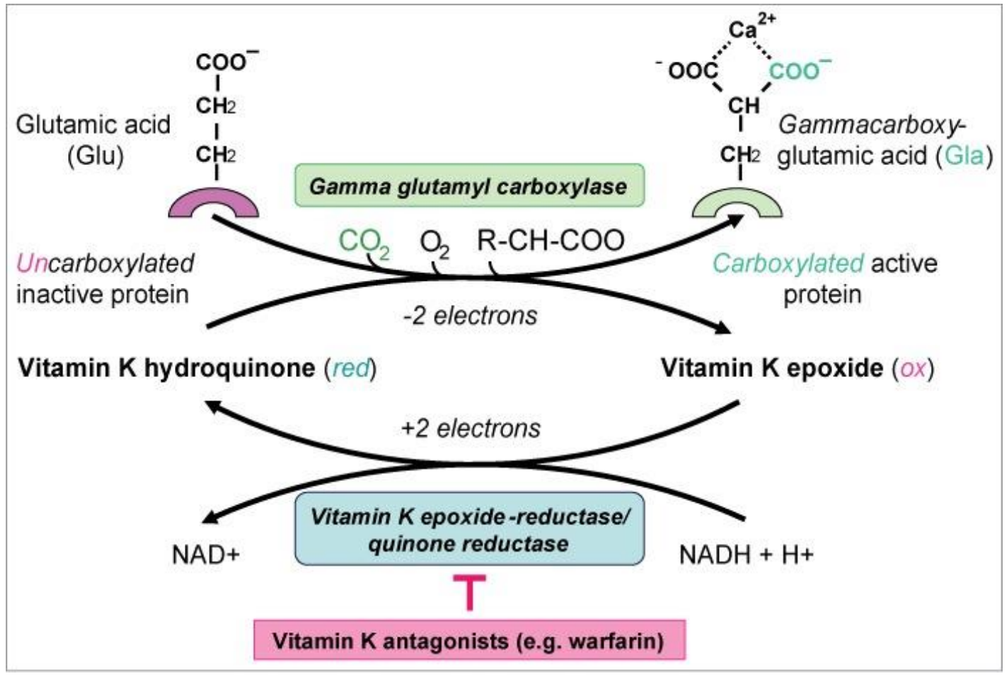


Fig. 3. SXR- and vitamin K_2-dependent regulatory mechanisms of bone metabolism in osteoblastic cells. SXR promotes collagen accumulation in osteoblastic cells by regulating the transcription of its target genes including those encode extracellular matrix proteins. Vitamin K_2 plays a role in the posttranslational modification of Gla proteins by functioning as a coenzyme of γ-glutamyl carboxylase (GGCX) and also acts as a potent SXR ligand in bone metabolism



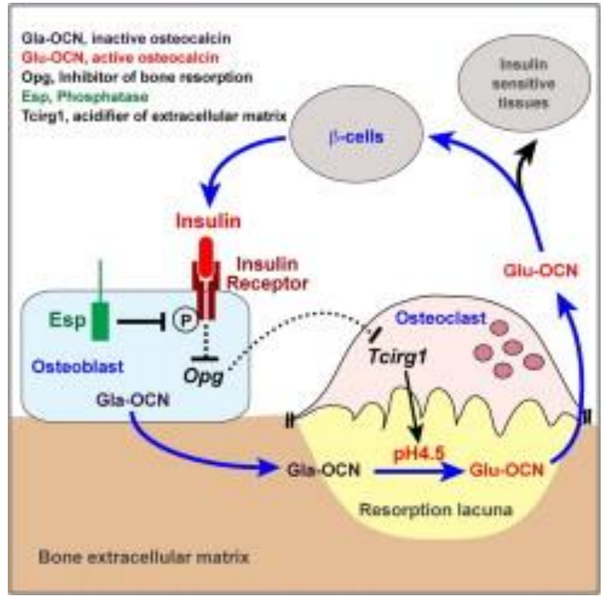
Dermato-endocrinology, 01 Jan 2014, 6(1):e968490

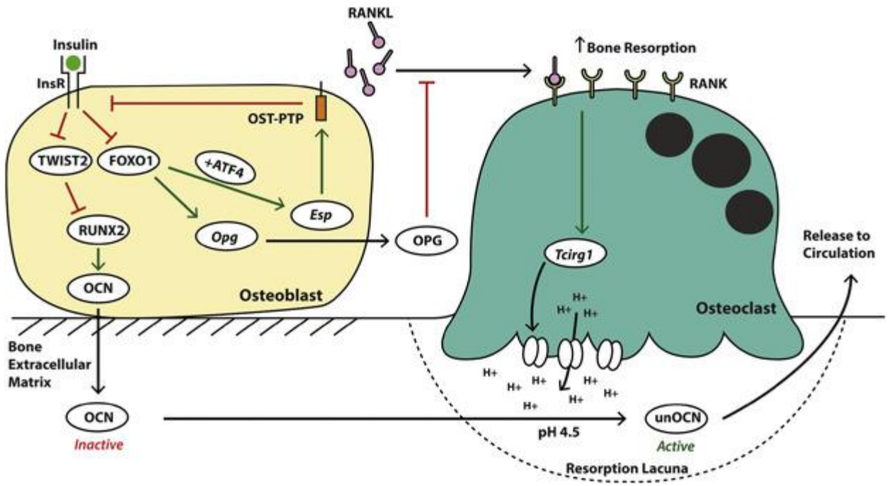
Vitamin K_2 is transcription regulator od specific bone genes, functioning using SXR which will lead to increase of osteoblastic markers expression. SXR originally identifies as xenobiotic sensor…

Vitamin K dependent Gla protein	Function
Liver	Hepatic carboxylation
Clotting factors II, VII, X and XII	Haemostasis (procoagulant activity)
Protein C, S and Z	Haemostasi (anticoagulant activity)
Various tissues	Extra hepatic carboxylation
Osteocalcin	Calcium and bone metabolism
Matrix-Gla-Protein	Inhibitor of vascular calcification (cartilaginous tissue, vascular wall of the vascular smooth muscle cells)
Growth-arrest specific gene 6 (Gas6)	Cell growth (endothelium, smooth muscle cells), apoptosis, phagocytosis (?)
Transmembrane GLA-protein	Signal transduction to phosphatidylserine (?)
Periostin	Bone metabolism, cell migration, angiogenesis (?)
Other: carboxylase, transthyretin, Gla-rich-Protein (GRP)	To date mainly unknown

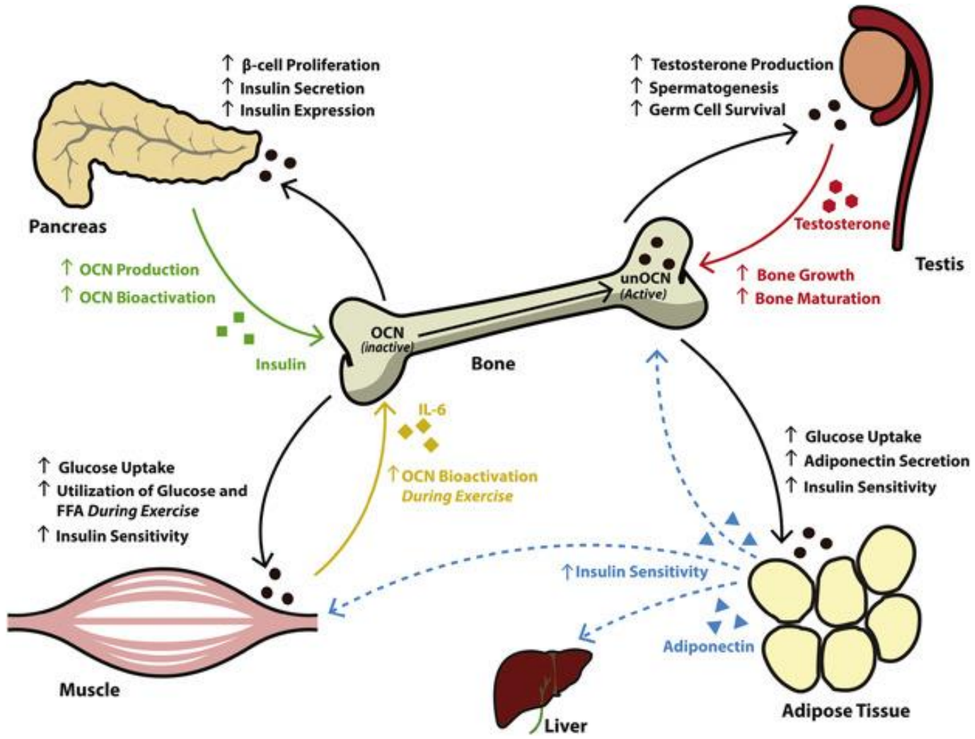
Osteocalcin (OCN)

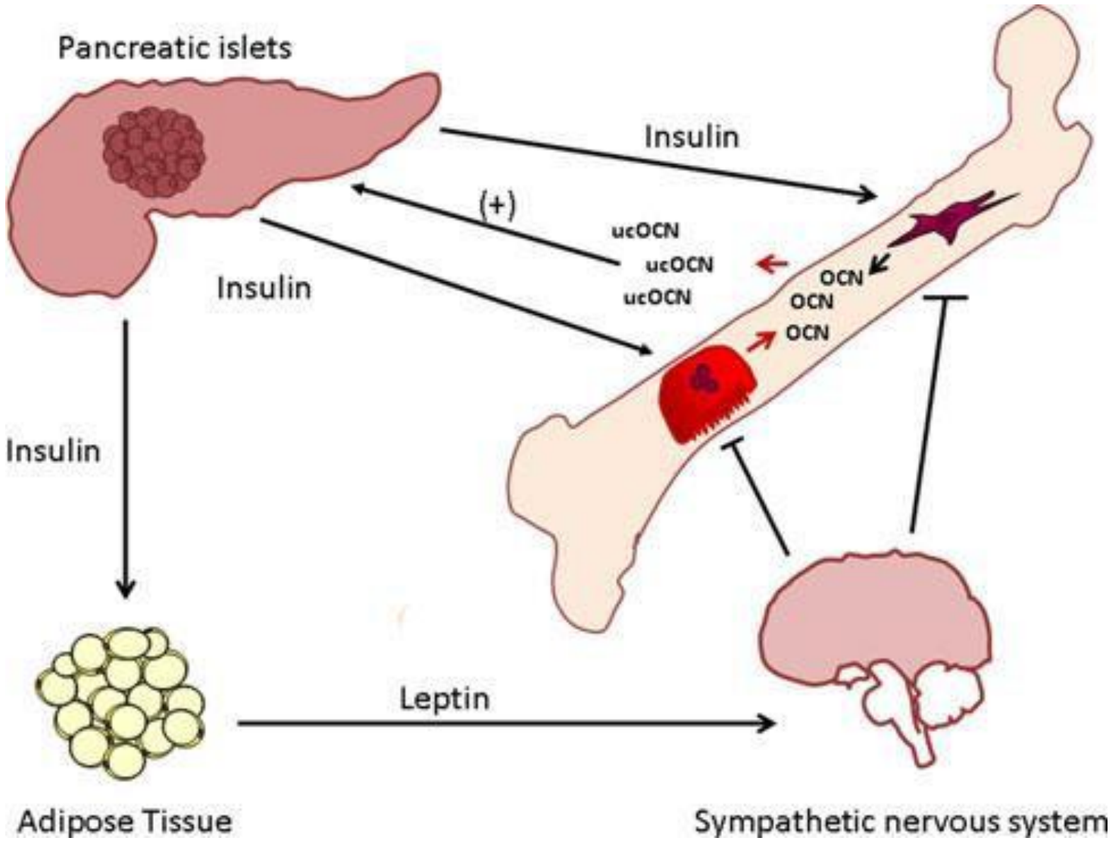
- the most abundant non-collagen bone matrix protein
- has been widely used as a biochemical serum marker of bone formation
- a hormone that regulates glucose homeostasis, energy expenditure, male fertility, brain development, and cognition.
- OCN is regulated by insulin signaling in OBs and, in a feed-forward loop,
- OCN stimulates pancreatic β-cell proliferation and insulin secretion and improves insulin sensitivity in peripheral tissues.
- newly assigned metabolic role for the skeleton raises important questions as to the normal physiological and pathophysiological regulation of glucose metabolism by the skeleton





J Cell Physiol.2018;233:3769–3783.





J Cell Physiol.2018;233:3769–3783.

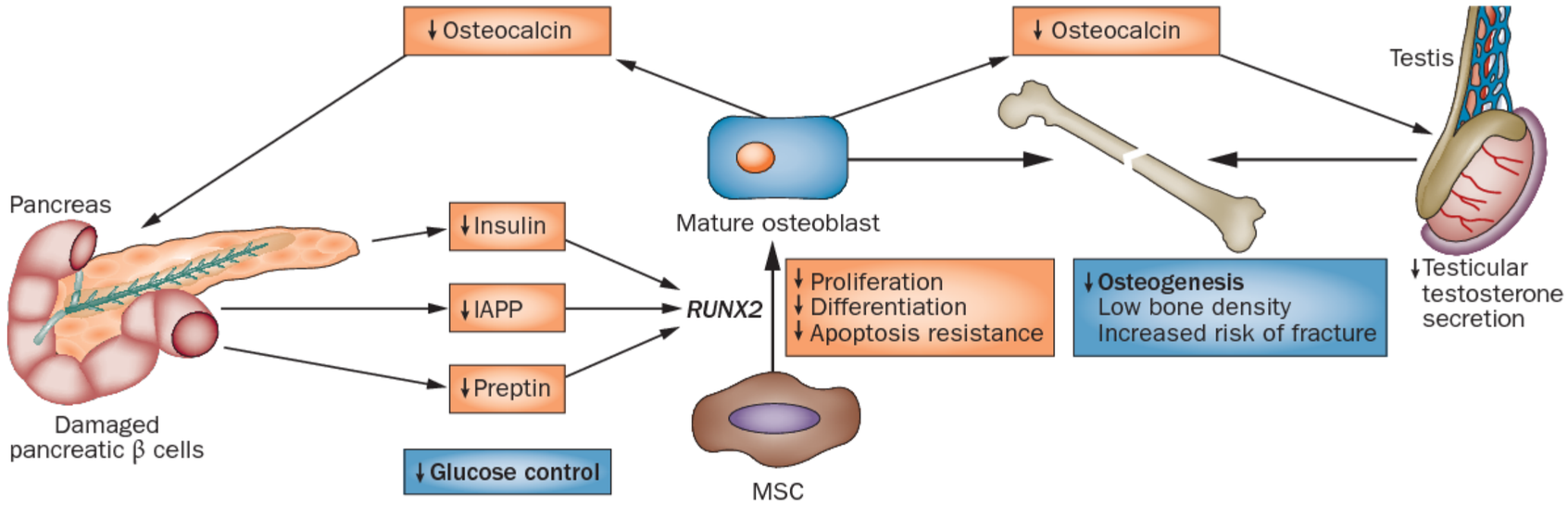
Expected reciprocal regulation of endocrine function of adipose tissue and bone:
Carboxylated osteocalcin (OCN) is produced by osteoblasts and is subsequently bound to the hydroxyapatite mineral of mature bone.
During bone resorption controlled by osteoclasts, it is released into the circulation uncarboxylated osteocalcin ucOCN from which it significantly promotes pancreatic insulin production. Insulin increases the expression of OCN by osteoblasts and at the same time promotes its decarboxylation by osteoclasts. Insulin also has a positive effect on leptin secretion by adipocytes, leading to inhibition of bone production and resorption by the hypothalamic effect of leptin. The production of ucOCN is thus reduced and the orexigenic effects of ucOCN on insulin production by the pancreas are modulated.

Sweet bone—osteoporotic fractures in diabetes mellitus

Impaired osteogenesis in T1DM. Pancreatic β-cell destruction in patients with T1DM prevents secretion of insulin, IAPP and preptin, thereby reducing their effects on the RUNX2 gene.

This reduction decreases proliferation and differentiation of MSCs into osteoblasts and their resistance to apoptosis—preventing osteogenesis and bone mass

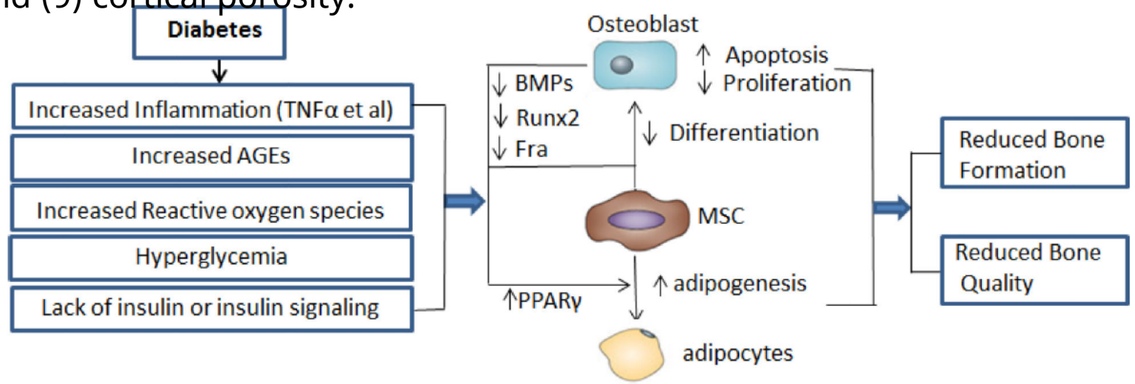
Type 1 diabetes mellitus (T1DM) affects the skeleton more severely than type 2 diabetes mellitus (T2DM)



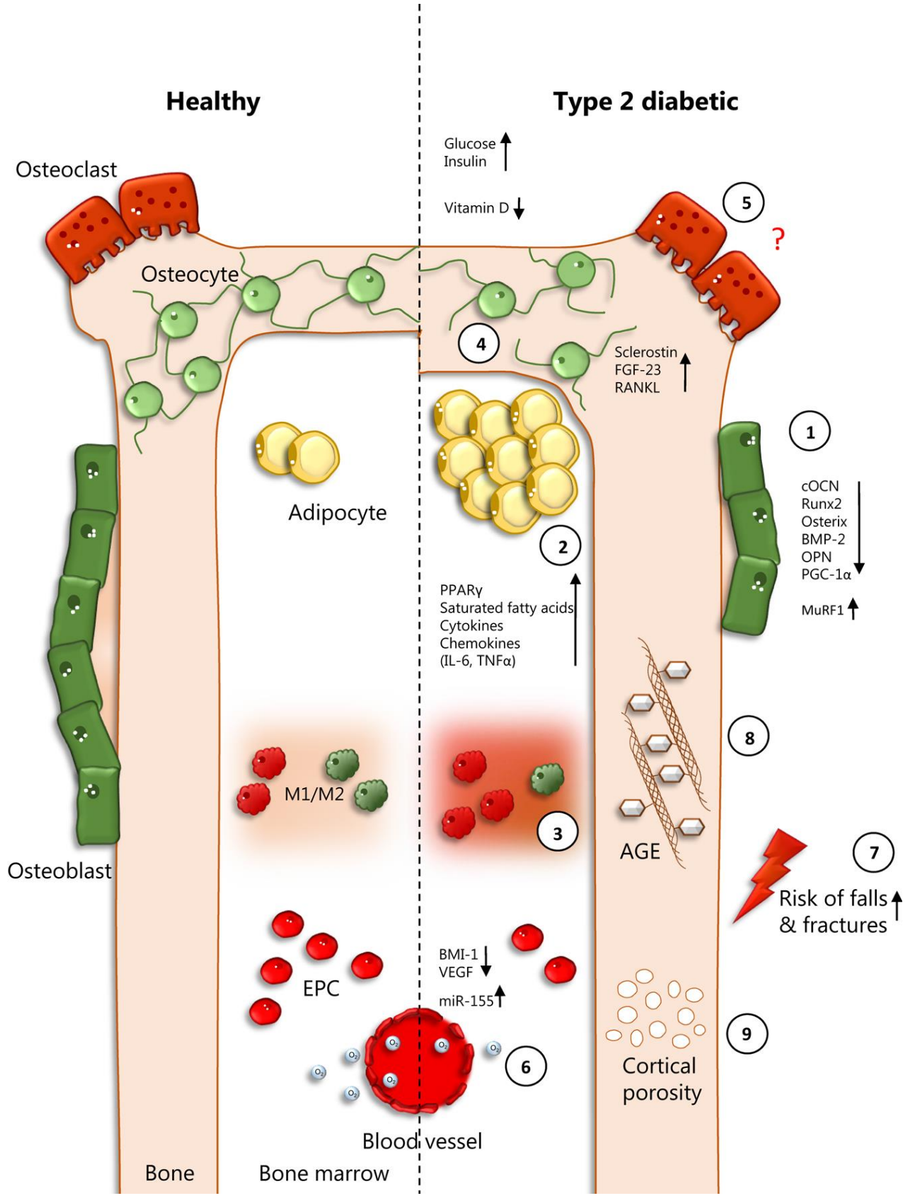
Nature Reviews Endocrinology **volume 8**, pages297–305 (2012)

DM bone

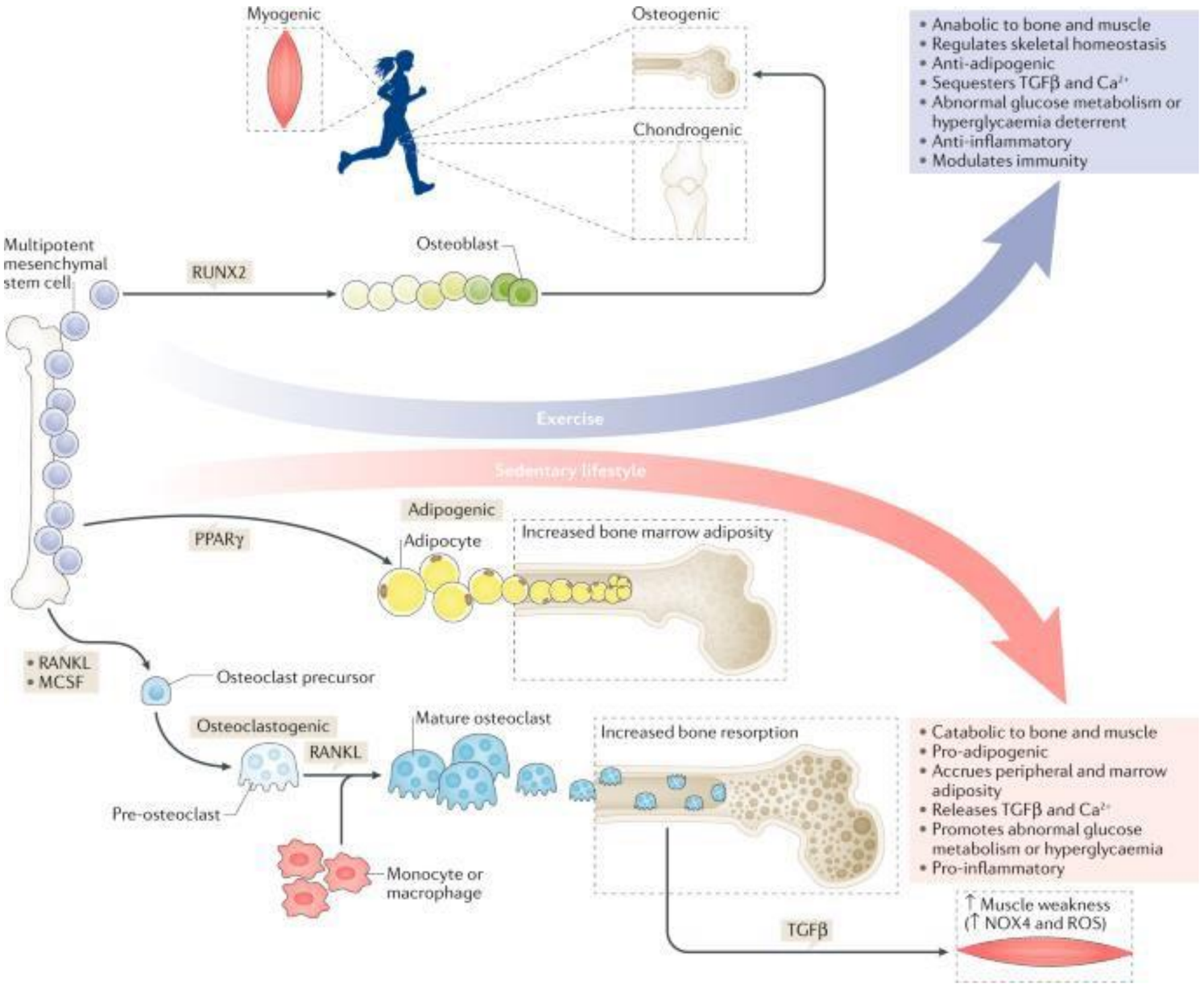
T2DM negatively affects trabecular bone mass, while cortical bone mass is increased. (1) The number and function of bone-forming osteoblast is reduced. In addition, vitamin D serum levels are decreased, which alters calcium and phosphate homeostasis. (2) Osteoblasts derive from MSC that favor differentiating into fat-storing adipocytes in T2DM leading to bone marrow adiposity and increased expression of cytokines and chemokines as well as to an elevated amount of free unsaturated fatty acids. (3) This results in increased inflammation leading to accumulation of pro-inflammatory M1 macrophages and reduced switch into anti-inflammatory M2 macrophages. (4) The network of osteocytes is reduced due to an increased apoptosis rate. They increase their expression of sclerostin, an inhibitor of osteoblast function, and RANKL, a promoter of osteoclastogensis. FGF-23, a phosphaturic hormone, is additionally increased. (5) Effects on osteoclasts are controversial in the literature, but T2DM is generally accepted to reduce bone turnover and thus also osteoclast function. (6) The amount of endothelial progenitor cells (EPC) is reduced in T2DM leading to vessel permeability. In addition, T2DM causes microhypoxia in bone niche, which in turn increases inflammation. (7) T2DM patients have an increased risk of falls and fractures due to reduced bone quality indicated by (8) an increased formation of advanced glycation end-products (AGEs) and (9) cortical porosity.



Current Osteoporosis Reports, 13 (5), 327-335.



Endocrine Connections 2019; 8, 3; 10.1530/EC-18-0456



Nature Reviews Endocrinology **volume 15**, pages333955–355(2019)

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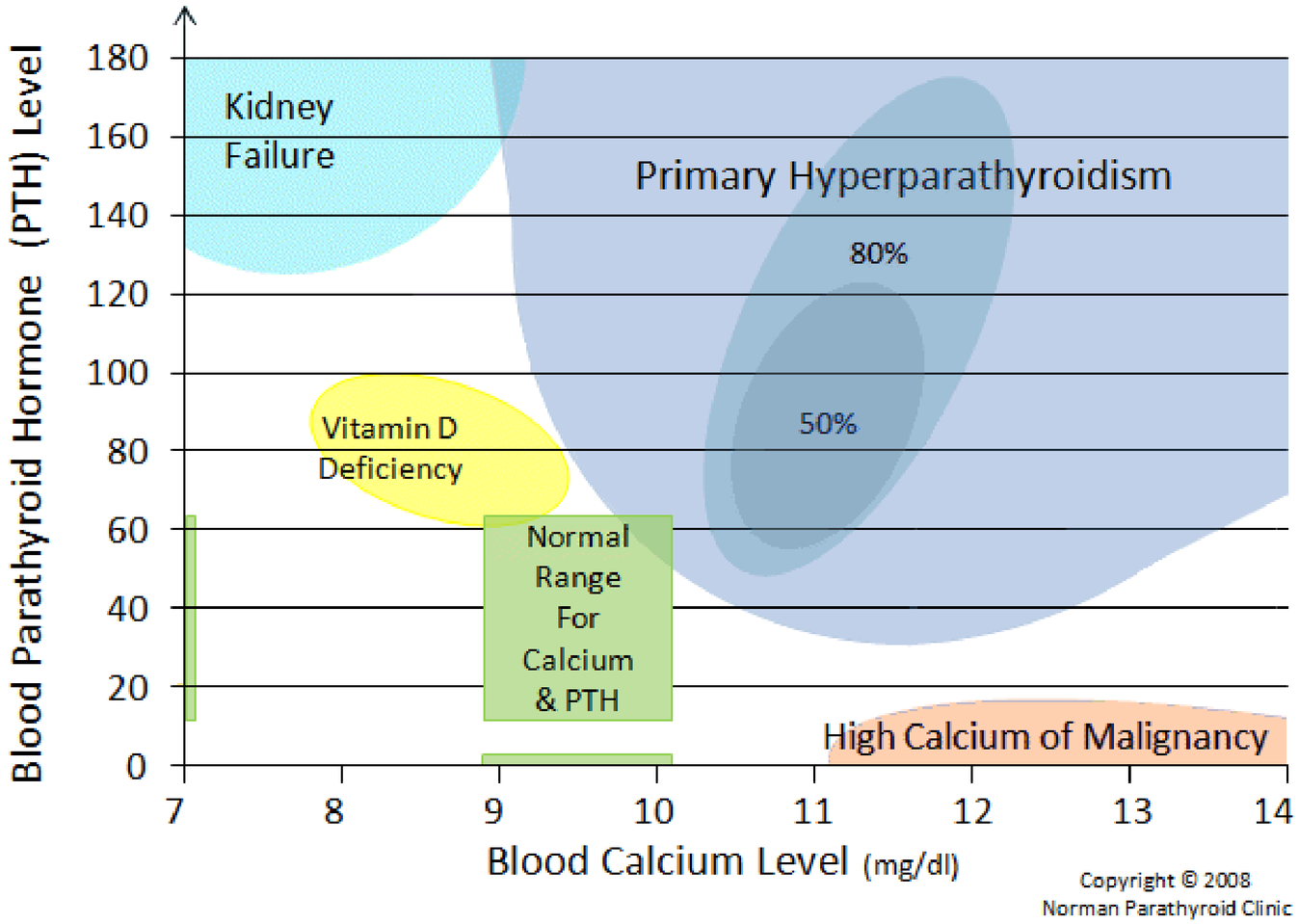
Bone remodelling defects

- Osteoporosis
- Osteodystrophy
- Rachitis/osteomalacia
- Paget`s disease
- Rare diseases

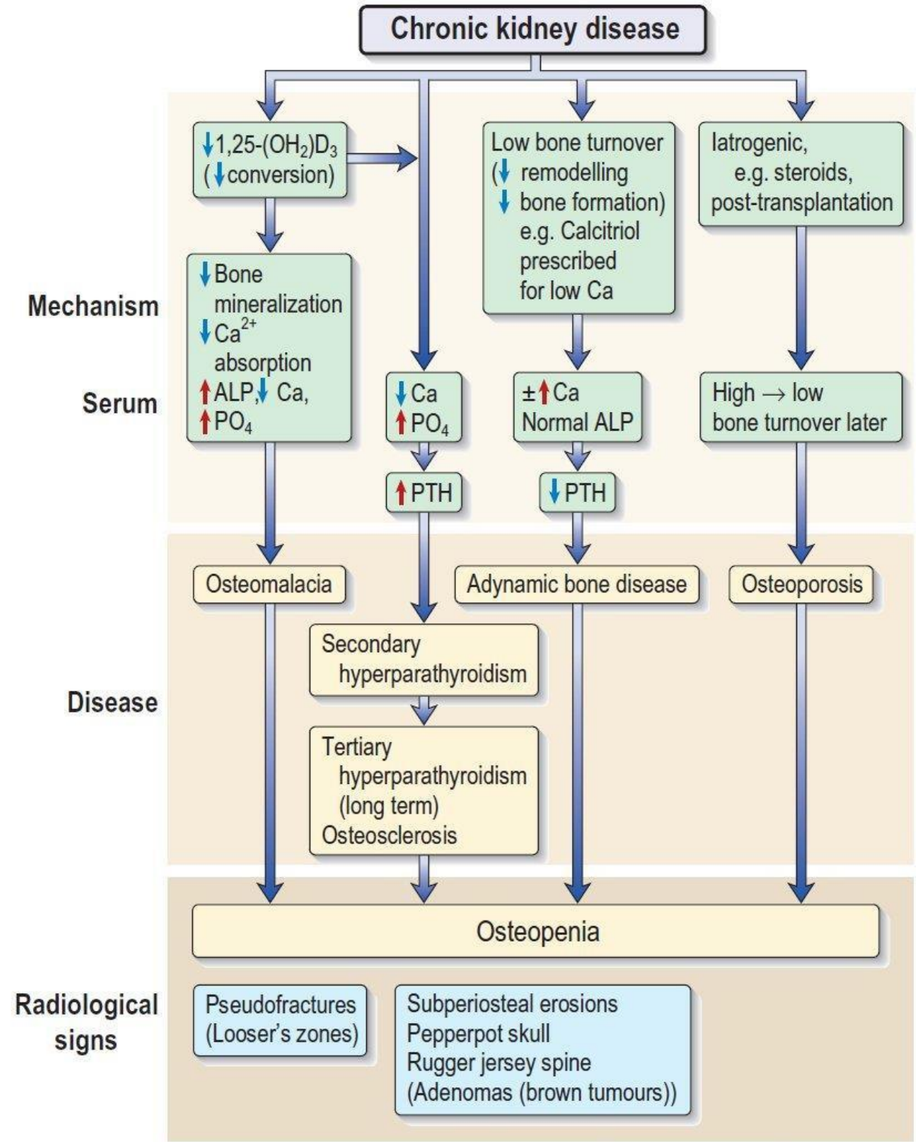
Osteodystrophy

- Primary hyperparathireoidism
- Symptoms: chronic hypecalcaemia, nephrocalcinosis, osteodystrophy as a manifestation of excessive bone remodeling.

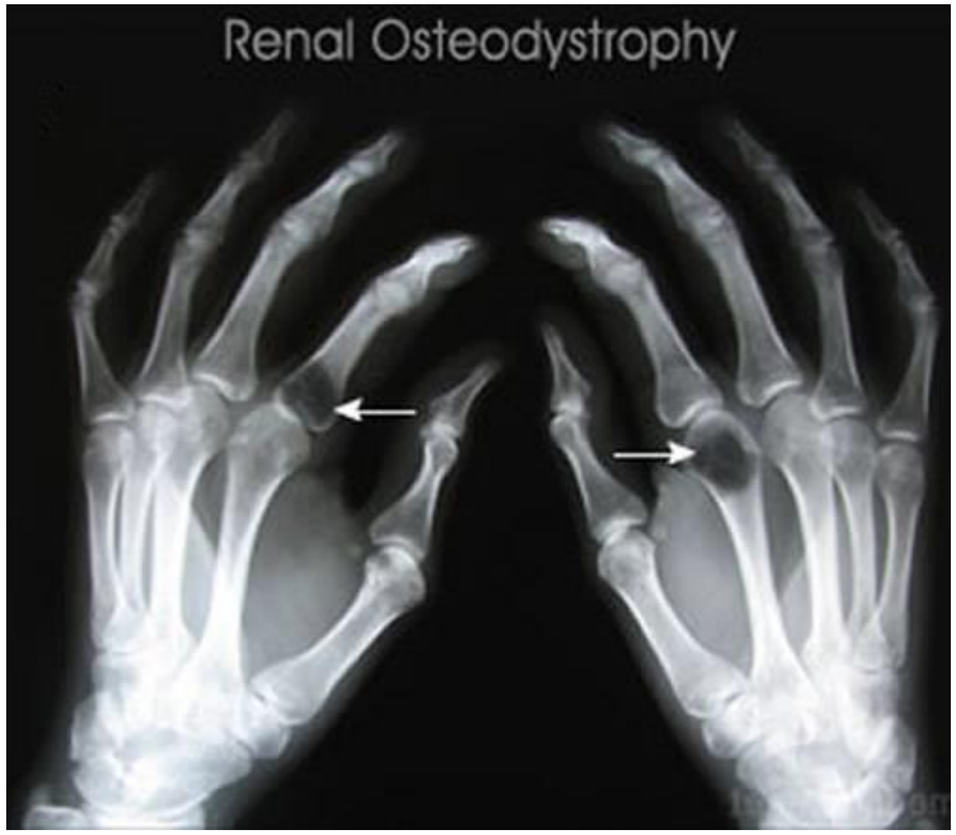


Osteodystrophy

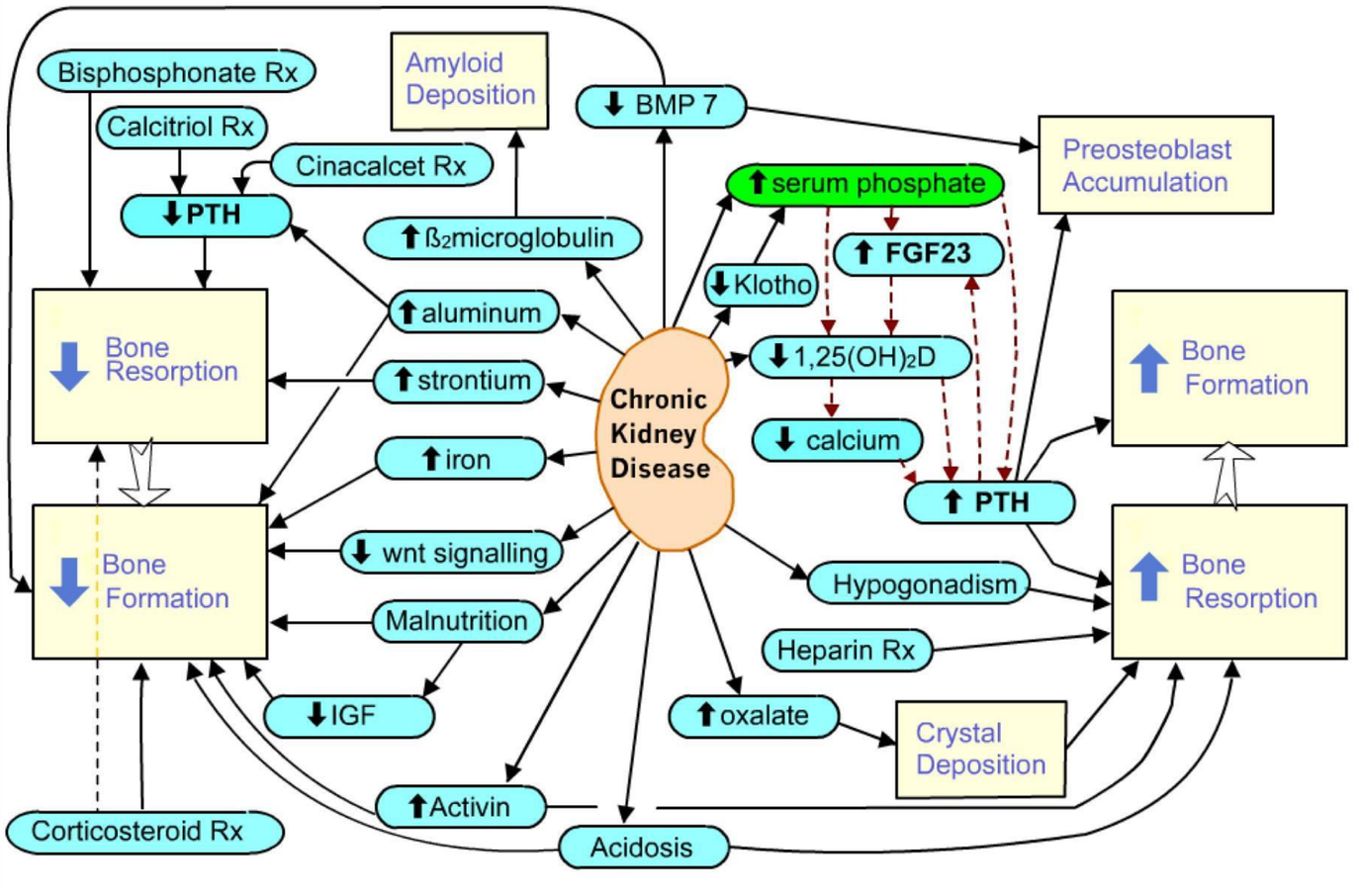
- *Secondary hyperparathyroidism - usually in chronic kidney disease with a tendency to develop chronic renal failure due to the inability of the kidneys to resorb calcium-renal osteodystrophy as a manifestation of excessive bone remodeling.*
- Other causes-usually nutritional: calcium and phosphate deficiency in the diet, excess phosphate in the diet.



Renal osteodystrophy: Pathogenesis and radiological features of renal bone disease. ALP, alkaline phosphatase.



Wheeless` Textbook of Orthopaedics



Renal Spondyloarthropathy

- seen in hemodialysis patients with chronic renal failure
- typically invovles **three adjacent vertebrae** with intervening discs;
  - changes include
  - subluxation, degeneration, and narrowing of disc;
    - although the process may resemble infection, it probably represents crystal or amyloid deposition;
    - bone disease is a major complication of uremia and persists and sometimes worses even after the initiation of hemodialysis;
    - when bone disease becomes severe, spontaneous fractures may occur, esp in the ribs, pelvis, and hips;
    - uremic pts with advanced hyperparathyroidism appear prone to non-traumatic aseptic necrosis of the hips;
    - 20% of pts with renal osteodystrophy also show osteosclerosis, most frequently in the spine, but may also occur in long bones;
    - osteomalacia is commonly seen in patients on hemodyalysis therapy for chronic renal failure;

Bone remodelling defects

- Osteoporosis
- Osteodystrophy
- Rachitis/osteomalacia
- Paget`s disease

Osteomalacia and rickets

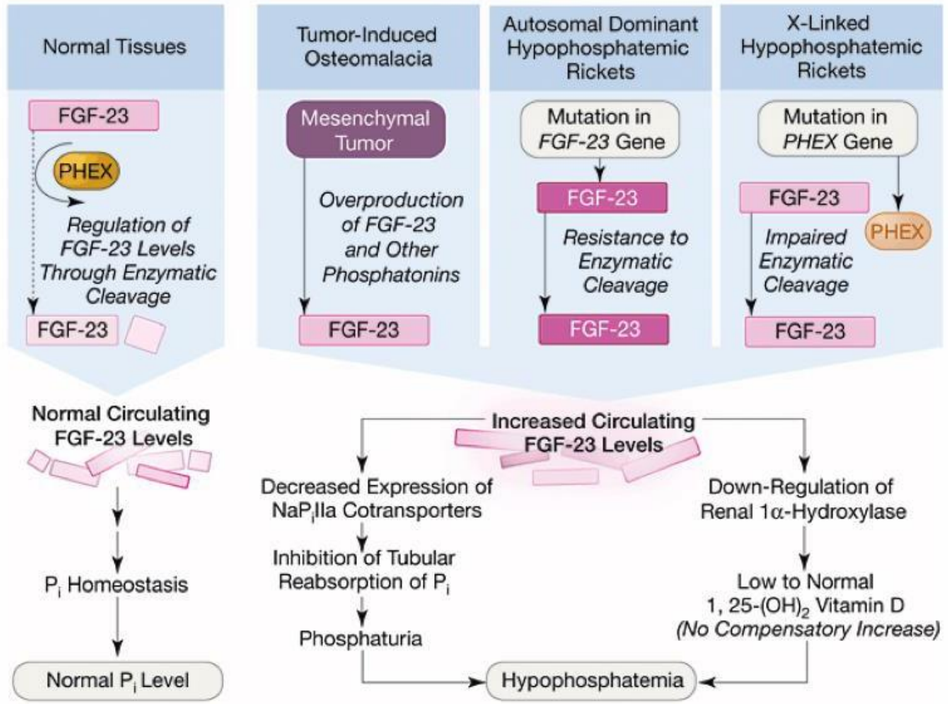
- Classically, the deficiency of vitamin D, essential for the absorption of calcium, has been the major cause of rickets in the child and osteomalacia in the adult
- resulting in absence or delay in the mineralization of growth cartilage or newly formed bone collagen.

Osteomalacia and rickets

- A consequence of a low serum phosphate and normal serum calcium.
- Two such conditions are *x-linked hypophosphatemic rickets/osteomalacia* and *oncogenic osteomalacia.*
- When present, the signs of rickets and osteomalacia in the low serum phosphate states are indistinguishable from the classic hypocalcemic states.

X-linked hypophosphatemic osteomalacia

- The condition is characterized by low tubular reabsorption of phosphate in the absence of secondary hyperparathyroidism.
- X-linked hypophosphatemia occurs in about 1 in 25,000 and is considered the most common form of genetically induced rickets.



Jan de Beur, S. M. JAMA 2005;294:1260-1267. With Permission.

Oncogenic osteomalacia

- Oncogenic osteomalacia is a paraneoplastic syndrome in which a bone or soft tissue tumor or tumor-like lesion induces hypophosphatemia and low vitamin D levels that reverse when the inciting lesion is resected.

Oncogenic osteomalacia

- ***Phosphotonin***
  - a humoral factor,
  - has been identified in clinical and experimental studies as being responsible for the serum biochemical changes.
  - causes hyperphosphaturia by inhibiting the reabsorption of phosphate by the proximal renal tubules.

- Fibroblast growth factor 23, phosphate-regulating gene with homologies to endopeptides located on the ‘x’ chromosome (PHEX) and matrix extracellular phosphoglycoprotein (MEPE) are candidates proposed for the production of phosphatonin and the altered pathophysiology in oncogenic osteomalacia.

Bone remodelling defects

- Osteoporosis
- Osteodystrophy
- Rachitis/osteomalacia
- Paget`s disease

Gene	Mutation	Disease
RANK	18 bp duplication	Familial expansile osteolysis
	27 bp duplication	Early onset Paget's disease
	15 bp duplication	Expansile skeletal hyperphosphatasia
RANKL	Deletion of amino acids 145-177	Autosomal recessive osteopetrosis
	A single nucleotide change (596T-A) in exon 8 of both alleles	Autosomal recessive osteopetrosis
	Deletion of two nucleotides (828_829delCG)	Autosomal recessive osteopetrosis
OPG	Deletion making OPG inactive	Juvenile Paget's disease
	20 bp deletion resulting in premature termination of OPG translation	Juvenile Paget's disease

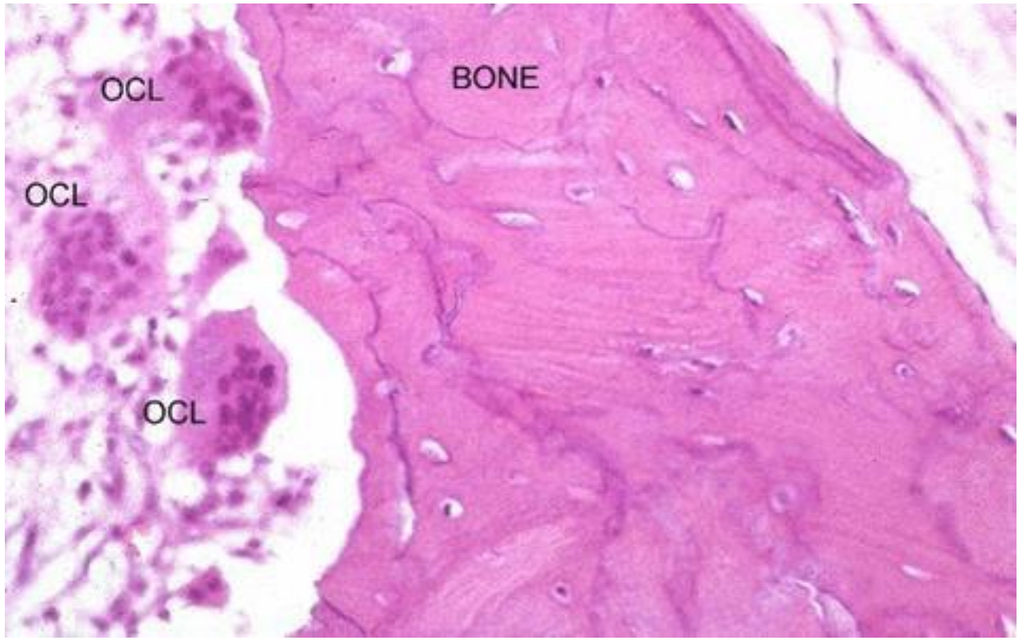
Paget's Disease

Laboratory findings
- •elevated serum ALP
- •elevated urinary collagen cross-links
- •elevated urinary hydroxyproline (collagen breakdown marker)
- •increased urinary N-telopeptide, alpha-C-telopeptide, and deoxypyridinoline
- •normal calcium levels

- abnormal bone remodeling
  - •active interplay between excessive bone resorption and abnormal new bone formation

- Pathophysiology causes
  - genetic predisposition
  - slow virus infection (intra-nuclear nucleocapsid-like structure)
    - •paramyxovirus
    - •respiratory syncytial virus

•Epidemiology
- peak incidence in the 5th decade of life
- common in Caucasians
- males = females
- location
  - monostotic or polyostotic
  - common sites include femur > pelvis > tibia > skull > spine

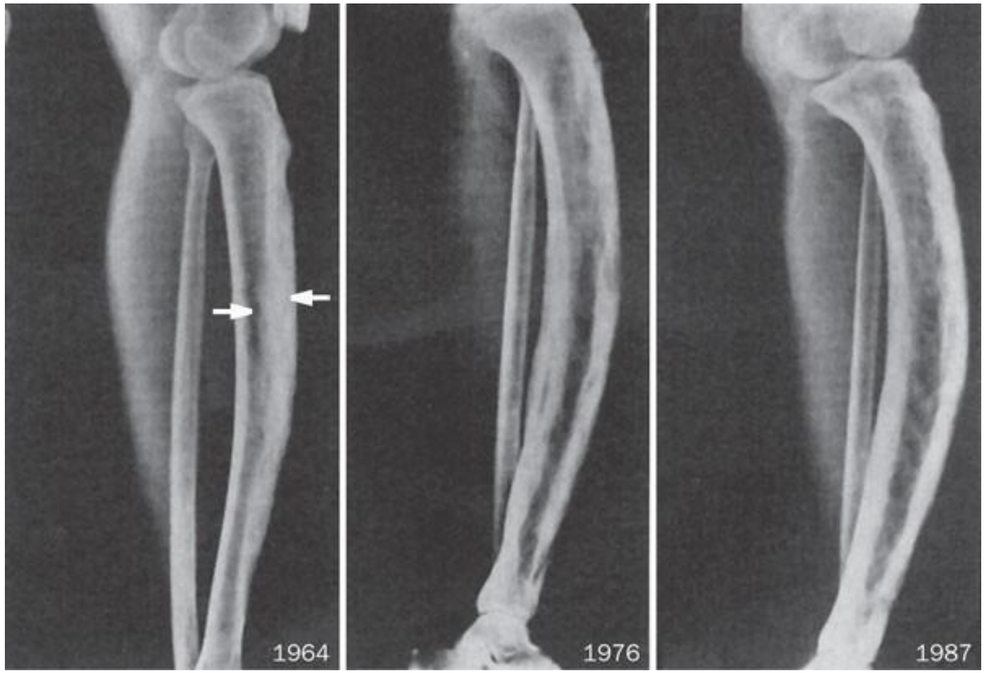


- Signs and symptoms
  - Majority asymptomatic
  - Skull: deformity with emlargement, hearing loss, dizziness
  - Spine and pelvis: bone pain, spinal stenosis, nerve compression
  - Long bones: defformities with increased fracture risk

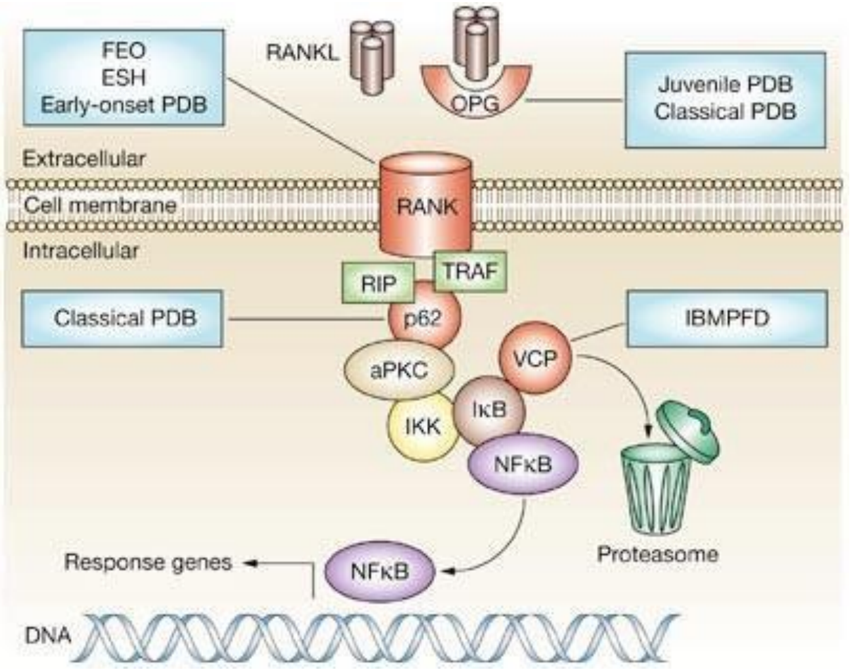
Paget's Disease - genetics

Genetics
- •inheritance
  - •most cases are spontaneous
  - •hereditary
    - •familial clusters have been described with ~40% autosomal dominant transmission
- •genetics
  - •most important is 5q35 QTER (ubiquitine binding protein sequestosome 1) SQSTM1 (p62/Sequestosome)
    - •tend to have severe Paget disease
  - •also insertion mutation in TNFRSF11A for gene encoding RANK

IBM = inclusion body myopathy
FEO = Familial expansile osteolysis



Nature Reviews Rheumatology **volume 5**, pages483–489(2009)



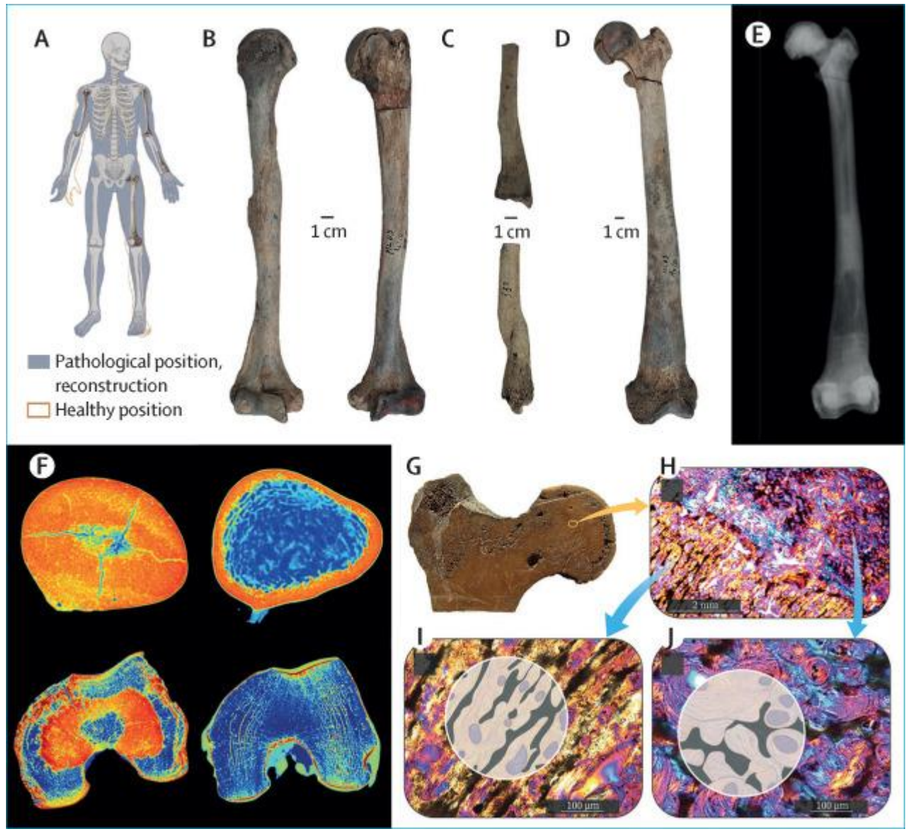
Nature Clinical Practice Rheumatology **volume 2**, pages270–277(2006)

Bone remodelling defects

- Osteoporosis
- Osteodystrophy
- Rachitis/osteomalacia
- Paget`s disease
- Rare diseases

Osteopetrosis

- a rare genetic bone disorder caused by a malfunction of the osteoclasts that leads to increased bone density.
- The three primary types are
  - autosomal recessive osteopetrosis,
  - intermediate autosomal recessive osteopetrosis,
  - and autosomal dominant osteopetrosis.
- Autosomal dominant osteopetrosis is the most frequent and less severe type, begins in adolescence, predominantly affects the axial skeleton and the long bones symmetrically, and is accompanied by an increased fracture rate due to the instability of affected bone.
- Autosomal dominant osteopetrosis has a general worldwide prevalence of one in 20 000 births.



Neolithic individual with osteopetrosis and the diagnostic methods applied

Bones showed a marked, generalised sclerosis with obliteration of the medullary cavity. They were remarkable for their heavy weight. A distinct characteristic of osteopetrosis is the flaring of the metaphyses in long bones.

www.thelancet.com/diabetes-endocrinology Vol 8 October 2020

Osteopetrosis

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  increased
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  - intermediate a
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- Autosoma s t
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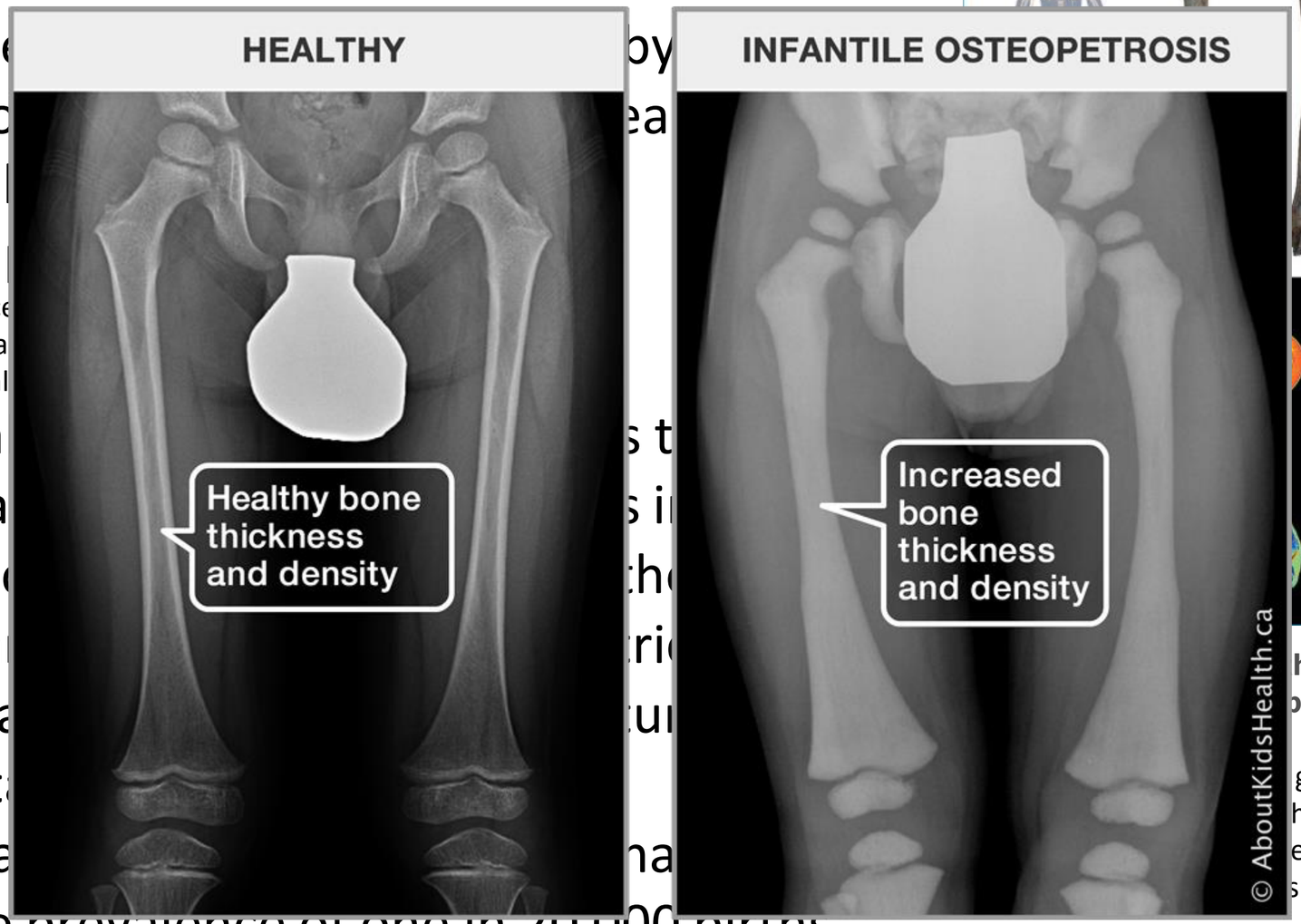

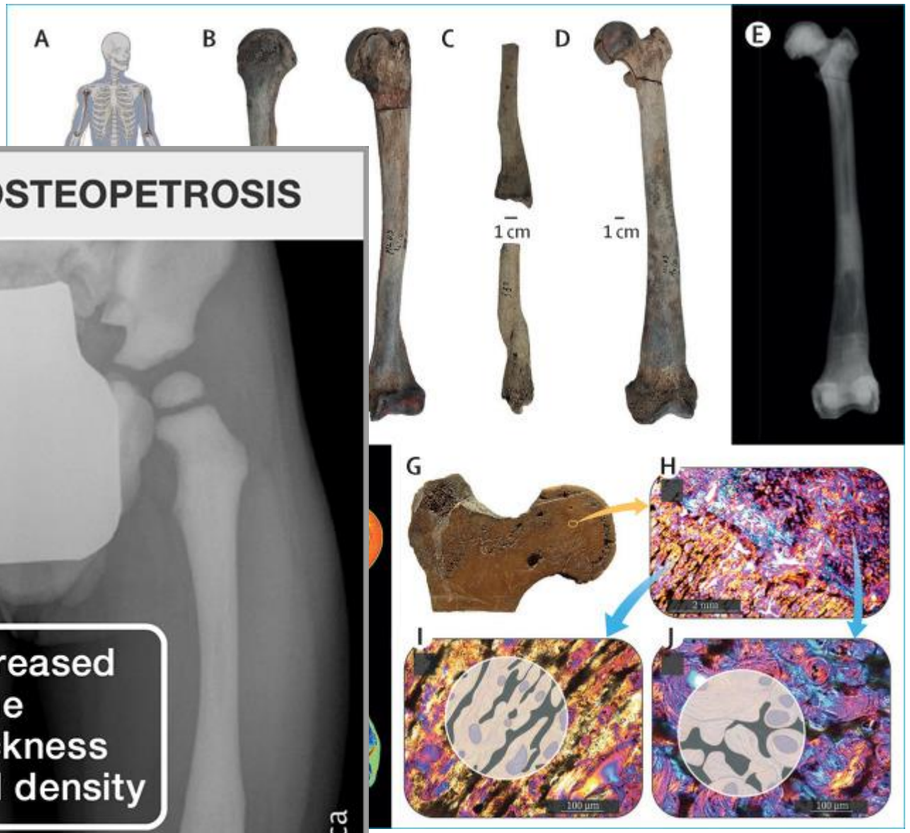

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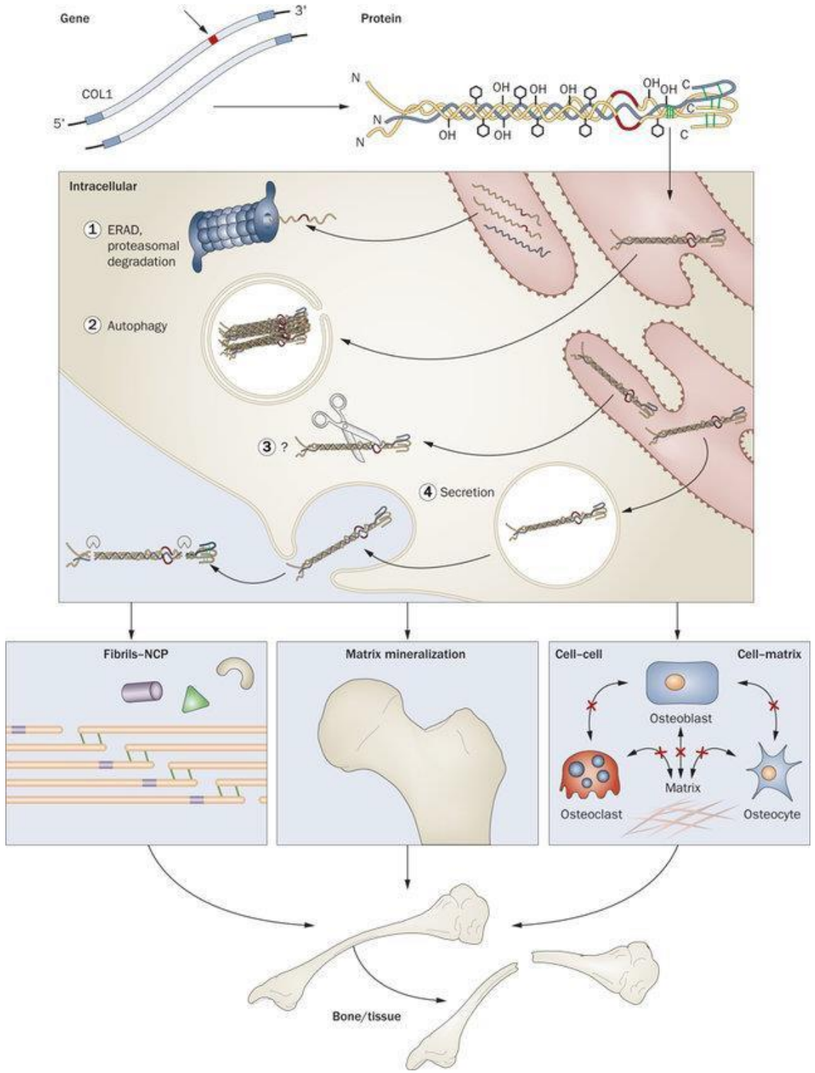
Collagen abnormalities



Nature Reviews Endocrinology 7(9):540-57

AD osteogenesis imperfecta bone dysplasia:

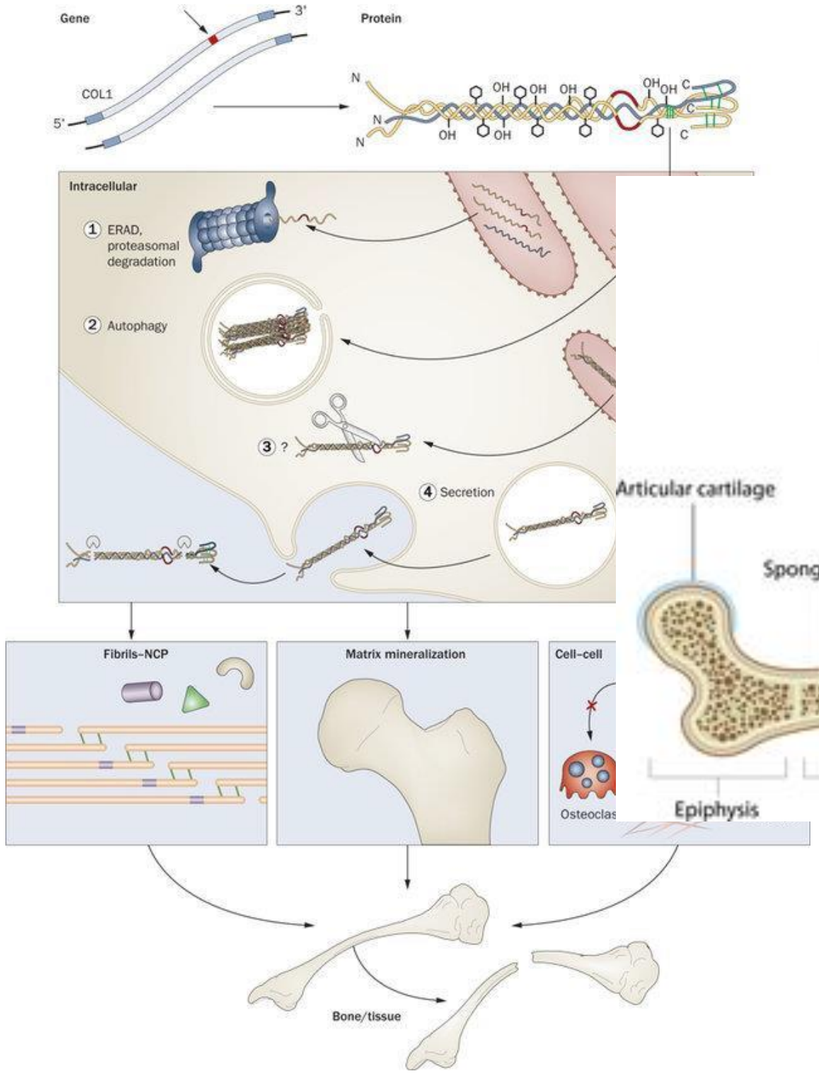
Mutations in either COL1A1 or COL1A2 are translated into collagen α-chains with abnormal structure, which delay folding of the heterotrimer and result in excess post-translational modification of the collagen helical region.

Mutant procollagen chains unable to incorporate into heterotrimers are

- retrotranslocated into the cytosol and degraded by the ERAD pathway (1);
- fully misfolded heterotrimers with structural defects generate supramolecular aggregates that are eliminated by autophagy (2);
- mutant molecules with triple helical mutations are degraded through an unidentified pathway (3).
- abnormal procollagen can be secreted, processed and incorporated in the extracellular matrix (4).

The secreted mutant collagen affects fibril structure and interactions of noncollagenous proteins with matrix, as well as matrix mineralization and osteoblast development and cell–cell and cell–matrix crosstalk. The overall result is bone deformity and fragility

Collagen abnormalities



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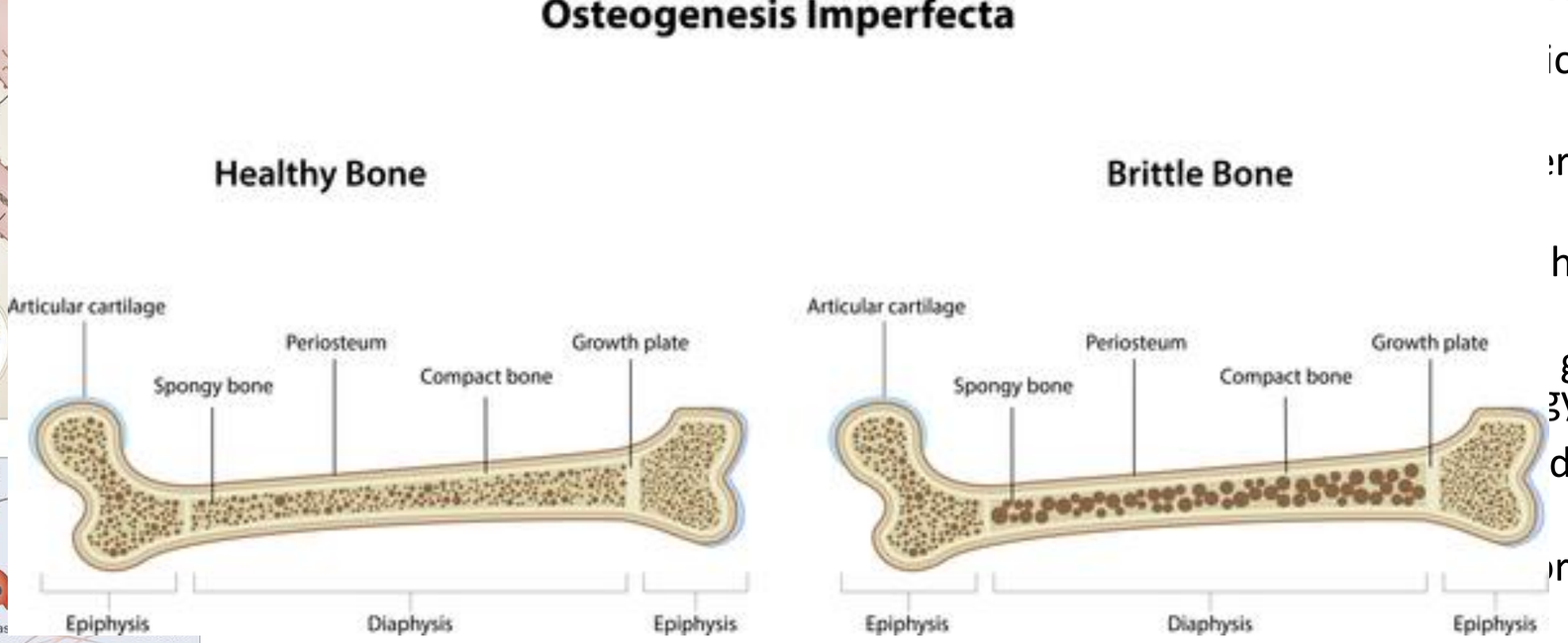

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Nature Reviews Endocrinology 7(9):540-57

Noncollagen genes in which mutations cause osteogenesis imperfecta variants

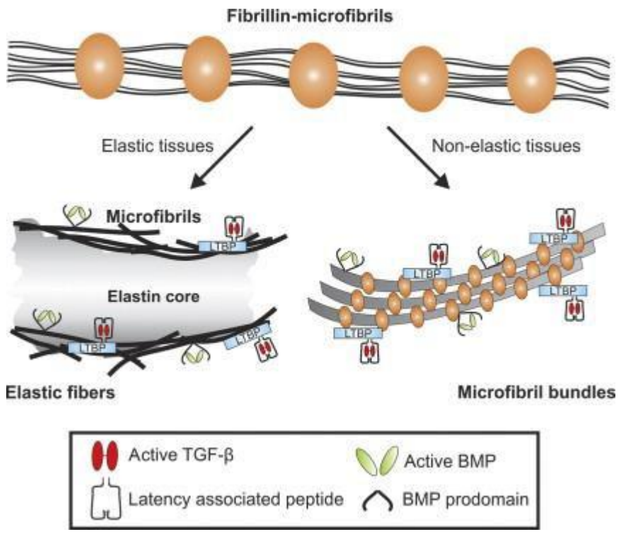
Gene	Protein		Phenotype	Bone collagen abnormalities
CRTAP	CRTAP	P3H1 complex	AR, bone fragility, reduced mineral density	α1(I)P986 and α2(I)P707 under prolyl-3-hydroxylation, high HP/LP (*CRTAP*, *LEPRE1*), low HP/LP (*PPIB*)
LEPRE1	P3H1,prolyl 3-hydroxylase			
PPIB	CYPB, cyclophilin B			
FKPB10	FKBP65		AR, Bruck syndrome: bone fragility, joint contractures	Lack of telopeptide hydroxylysines produces skin-like cross-links
PLOD2	LH2, lysyl hydroxylase 2			
SERPINH1	HSP47, heat-shock protein 47		AR, bone fragility (type III OI)	High HP/LP and abnormal arrangement of cross-linking bonds
SERPINF1	PEDF, pigment epithelium-derived factor		AR, bone fragility, low bone mass and wide osteoid seams	Defective mineralization, no other collagen abnormalities detected
BMP1	Procollagen type I C-propeptidase		AR, bone fragility, high mineral density	Defective C-propeptide removal, potential cross-linking defects
IFITM5	Bril, osteoblast-specific small transmembrane protein		AD, bone fragility, hyperplastic callus (type V OI)	None reported

OI osteogenesis imperfecta, *AR* autosomal recessive, *AD* autosomal dominant, *HP* hydroxylysylpyridinoline, *LP* lysylpyridinoline

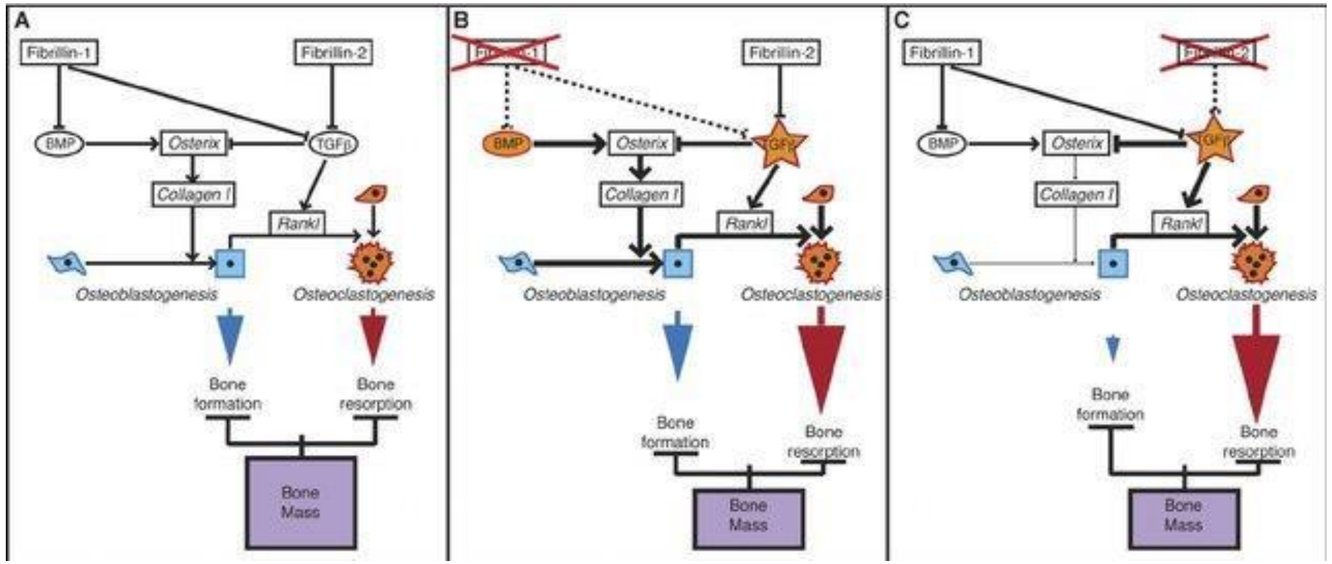
Calcified Tissue International 2013

Marfan syndrome

- Fibrillin is the major component of beaded microfilaments possessing elasticity



Reference Module in Biomedical Sciences, 2017



The Journal of Cell Biology 190(6):949-51

Name	M.W.	Structure	Domains	Site of expression	Normal function	Pathology
Fibrillin-1	~320 kDa	Extracellular microfibrils	cbEGF-like, TB, hybrid domains	Mature skin, embryonic tissues, aorta	Proper assembly of elastic fibers	Marfan, Weill–Marchesani syndrome
Fibrillin-2	~350 kDa	Extracellular microfibrils	cbEGF-like, TB, hybrid domains	Developing skin and other embryonic tissues, developing digits	Proper assembly of elastic fibers, bone formation	Mild skin pathology, Beals syndrome, distal arthrogryposis
Fibrillin-3	~350 kDa	Extracellular microfibrils	cbEGF-like, TB, hybrid domains	Embryonic tissues	Assembly of elastic fibers	Unknown

Source: cb EGF-like calcium-binding EGF-like domain, TB TGFβ binding domain.
Abbreviations: cb, calcium binding; EGF, epidermal growth factor; TB, binding sites for TGFβ.

Joints

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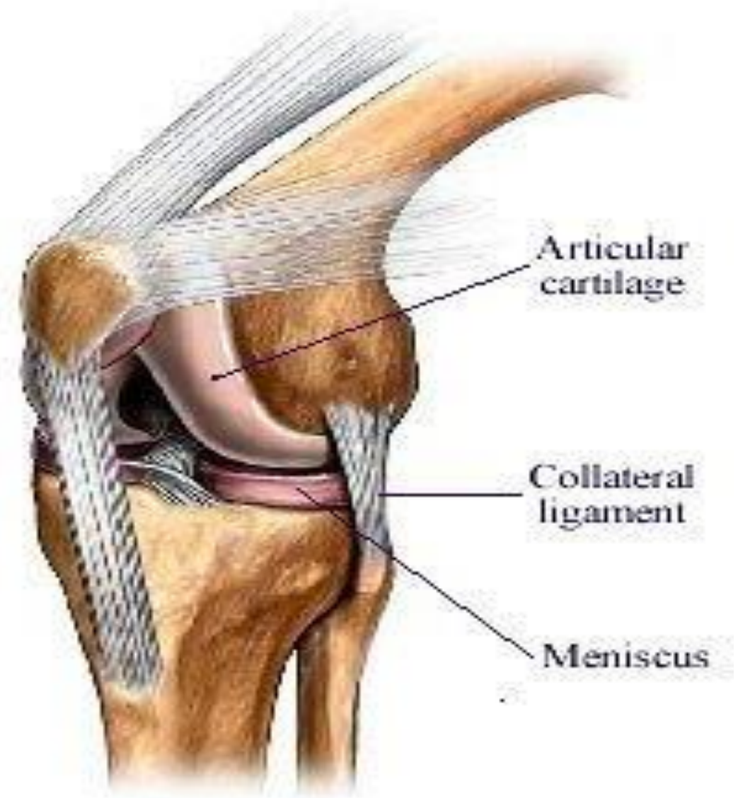
Articular diseases

- irreversible destruction of the cartilage, tendon, and bone that comprise synovial joints
  - rheumatoid arthritis (RA) and
  - osteoarthritis (OA).

- While cartilage is made up of proteoglycans and type Ⅱcollagen, tendon and bone are composed primarily of type Icollagen.



Articular diseases

- rheumatoid arthritis (RA) and
- osteoarthritis (OA).

Rheumatoid Arthritis

- The prevalence of rheumatoid arthritis in most Caucasian populations approaches 1% among adults 18 and over and increases with age, approaching 2% and 5% in men and women, respectively, by age 65
- The incidence also increases with age, peaking between the 4th and 6th decades
- Both prevalence and incidence are 2-3 times greater in women than in men
- Monozygotic twins 13.5% vs dizygotic twins 3.5%

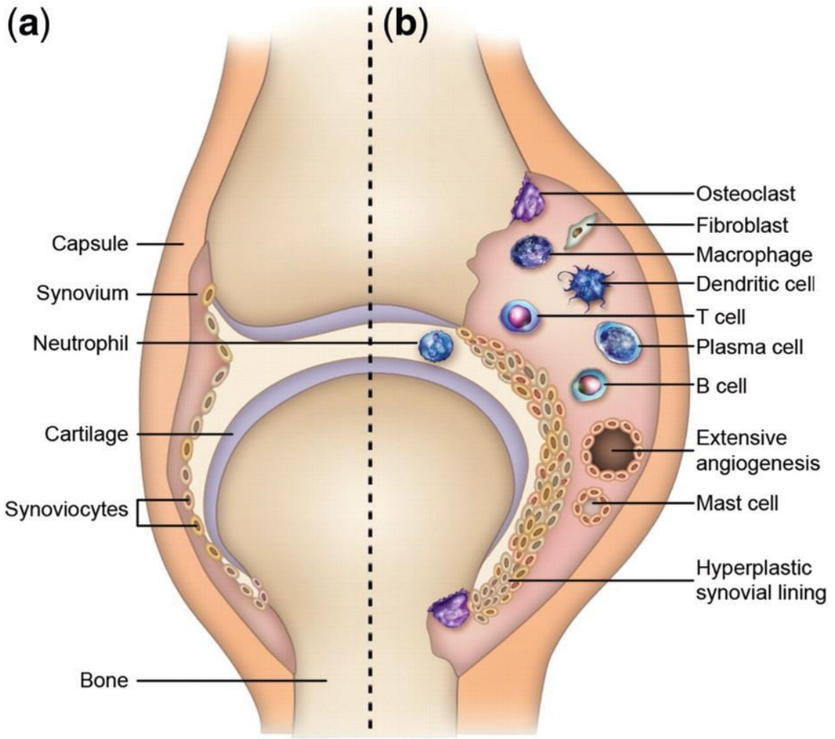
“One must from time to time attempt things that are beyond one’s capacity.”

—Pierre-Auguste Renoir

Rheumatoid Arthritis

- Rheumatoid arthritis is an autoimmune disease affecting the joints, tendons, and bones, resulting in inflammation and destruction of these tissues.

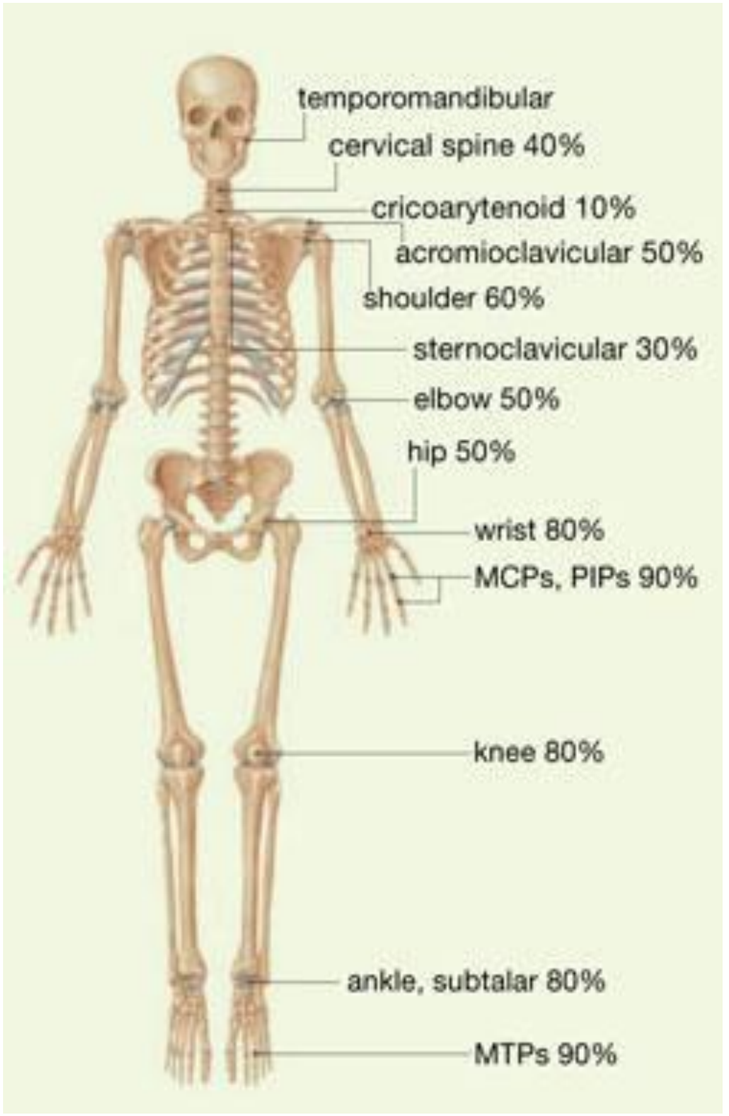
- The term ‘arthritis’ is used to denote clinically apparent soft tissue swelling or fluid (not bony overgrowth alone).



Rheumatoid arthritis

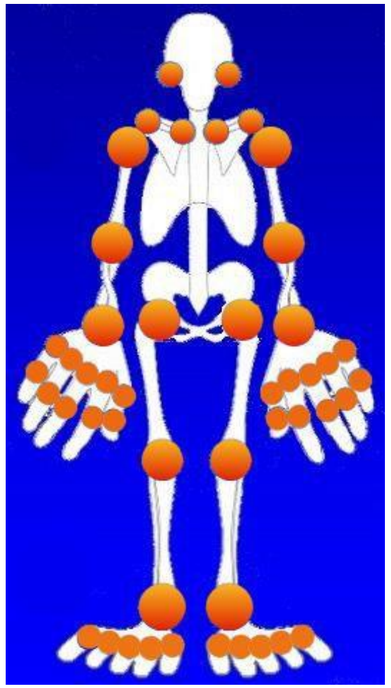
- characterised by a symmetric polyarthritis usually involving the small joints of the hands and feet.

- Extra-articular involvement of organs such as the skin, heart, lungs, and eyes can be significant.



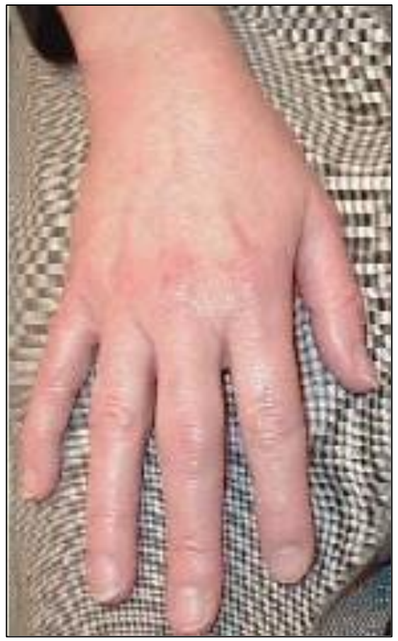
Rheumatoid Arthritis

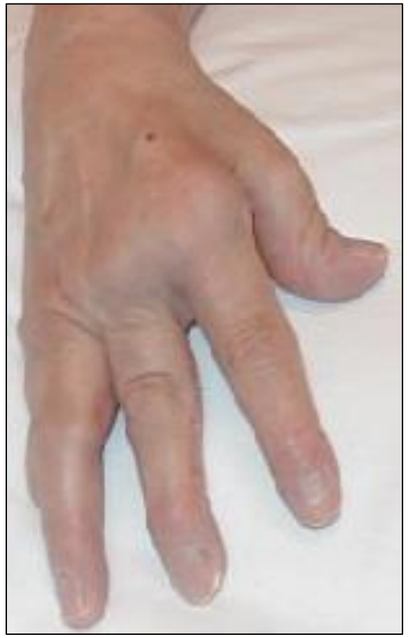
- Description
  - Morning stiffness
  - Arthritis of 3 or more joints
  - Arthritis of hand joints
  - Symmetric arthritis
  - Rheumatoid nodules
  - Serum rheumatoid factor
  - Radiographic changes

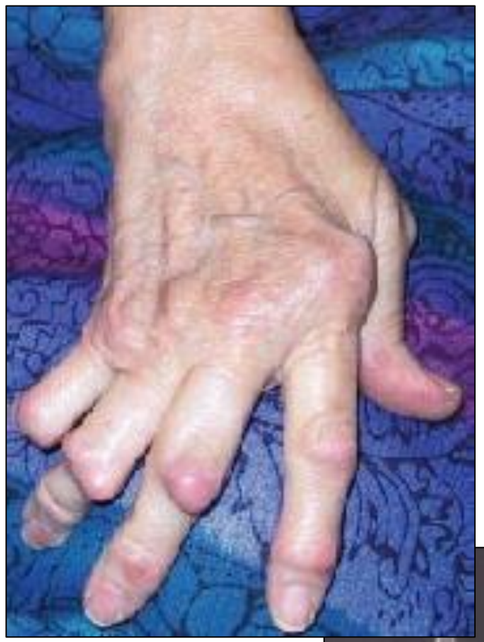


- having rheumatoid arthritis – positive 4 of 7 criteria, with criteria 1-4 present for at least 6 weeks

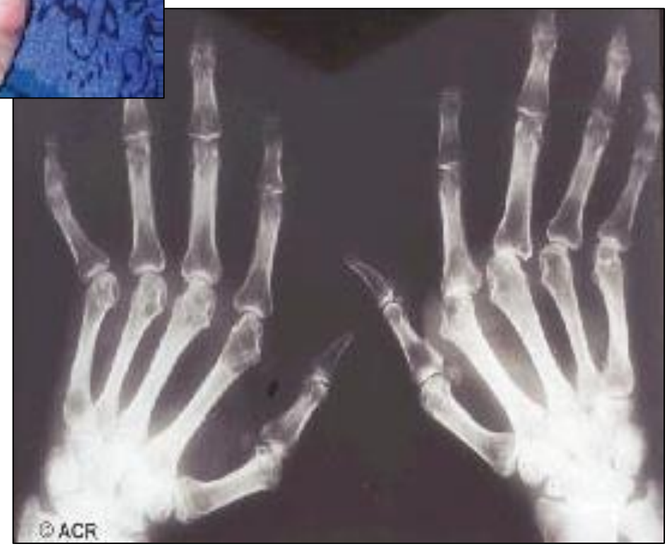
Clinical Presentation of RA







Severe RA



Early RA

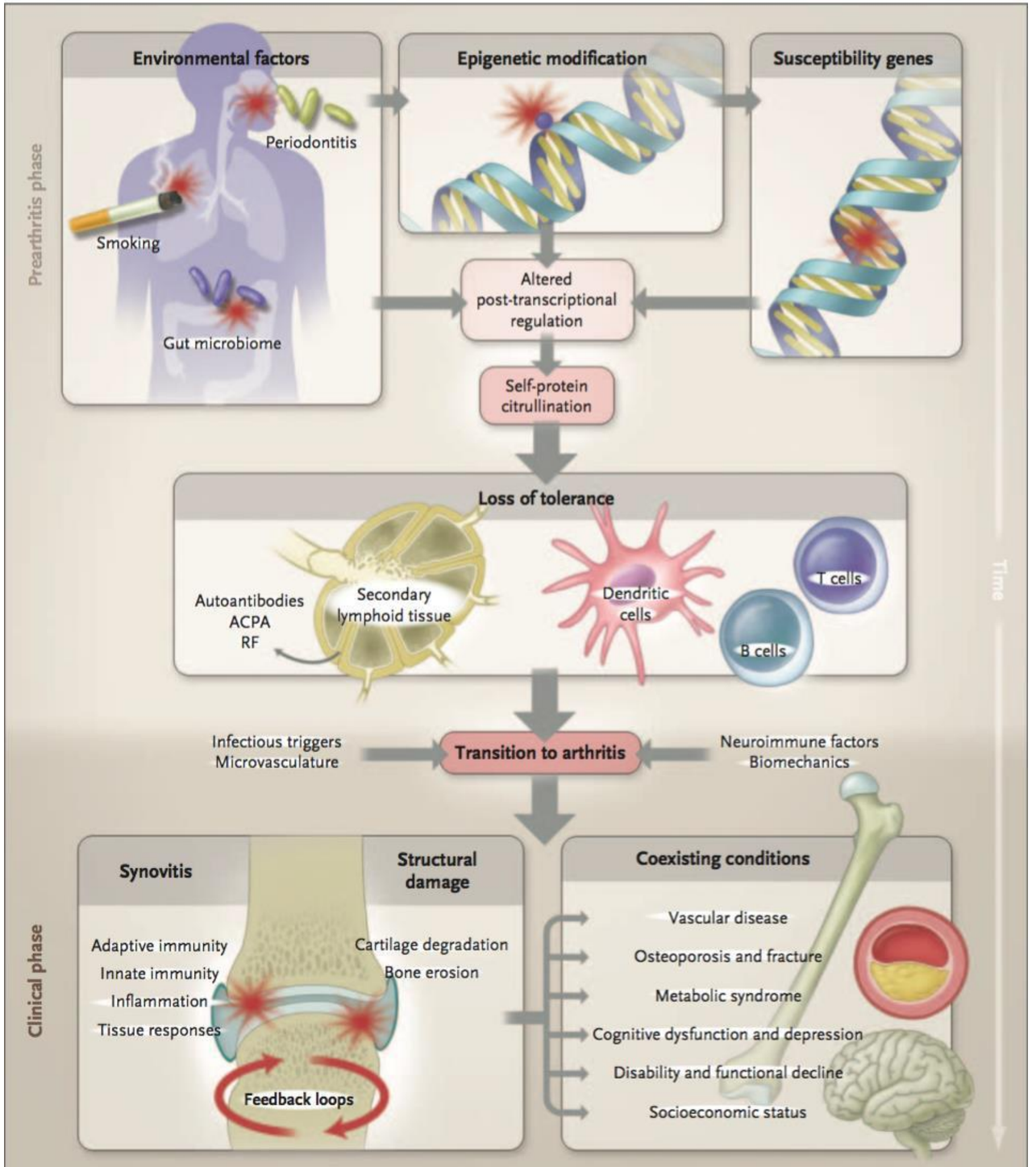
Intermediate

Latinis KM, et al. *The Washington Manual™ Rheumatology Subspecialty Consult.* Philadelphia, Pa: Lippincott Williams & Wilkins; 2004.

Rheumatoid Arthritis

- Pathogenesis of RA is attributed to a complex interaction between genetic and environmental factors and the repeated activation of innate and adaptative immunite system evolves into the breakdown of immune tolerance, aberrant autoantigen presentation and antigen-specific T and B cells activation.

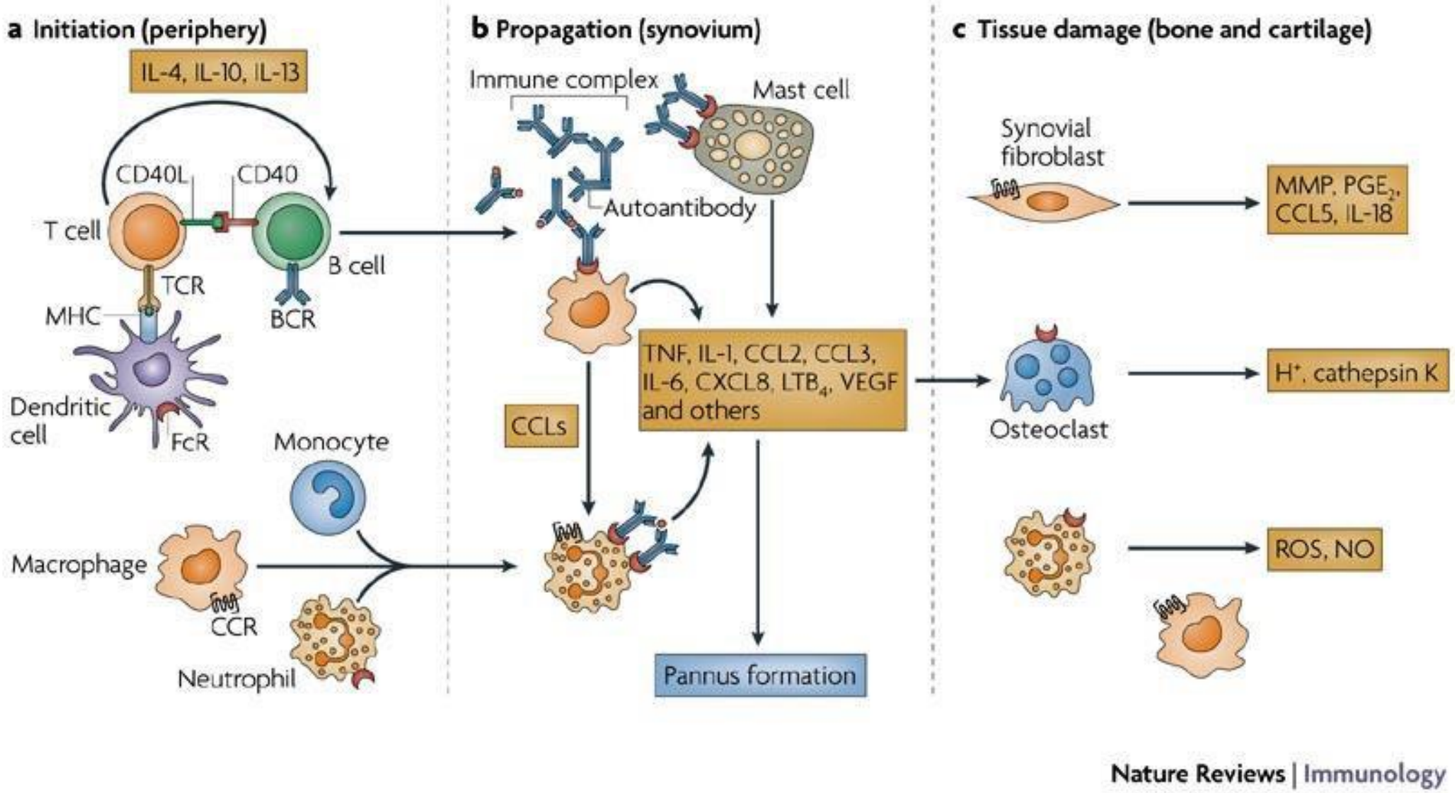
- Genetic factors have an important role in the susceptibility to rheumatoid arthritis
  - HLA-DRB



McInnes and Schett, *NEJM* 2011

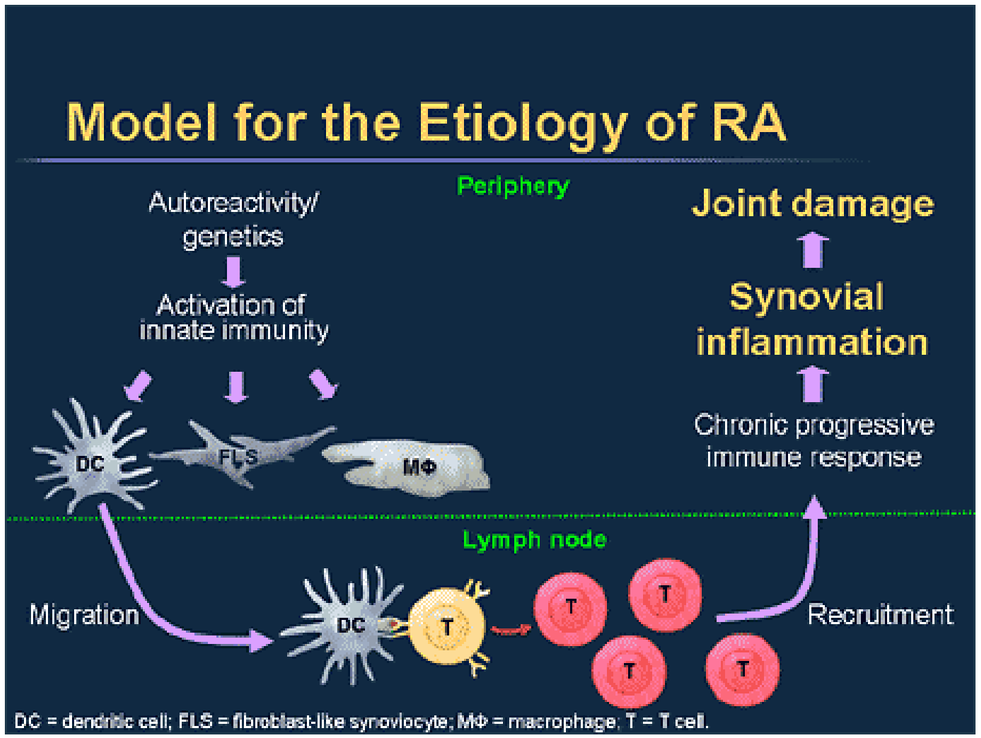
RA without clinical arthritis

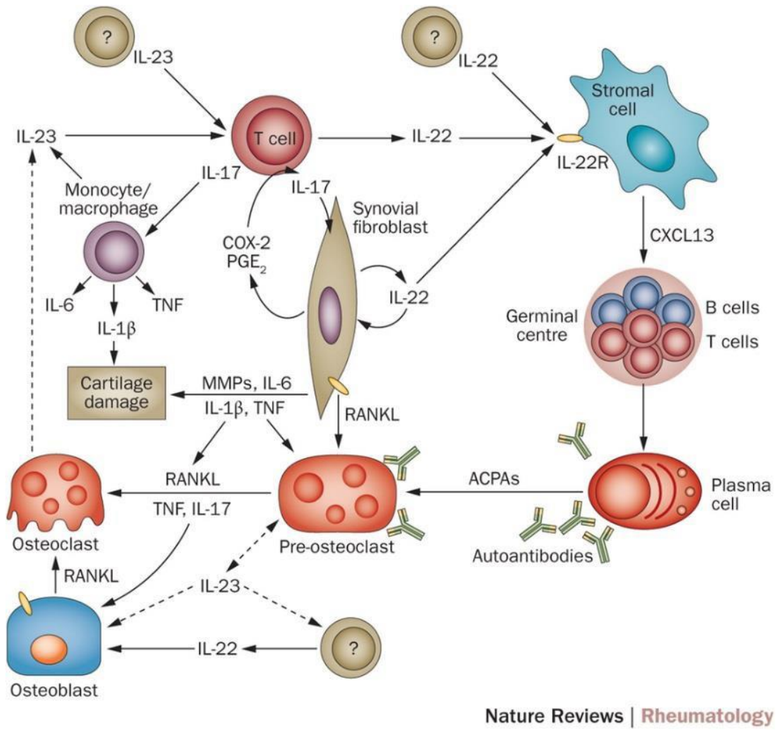
- An initial phase, characterised by systemic autoimmunity without synovial inflammation, may be followed by a shorter phase during which asymptomatic synovitis is present.



RA progression

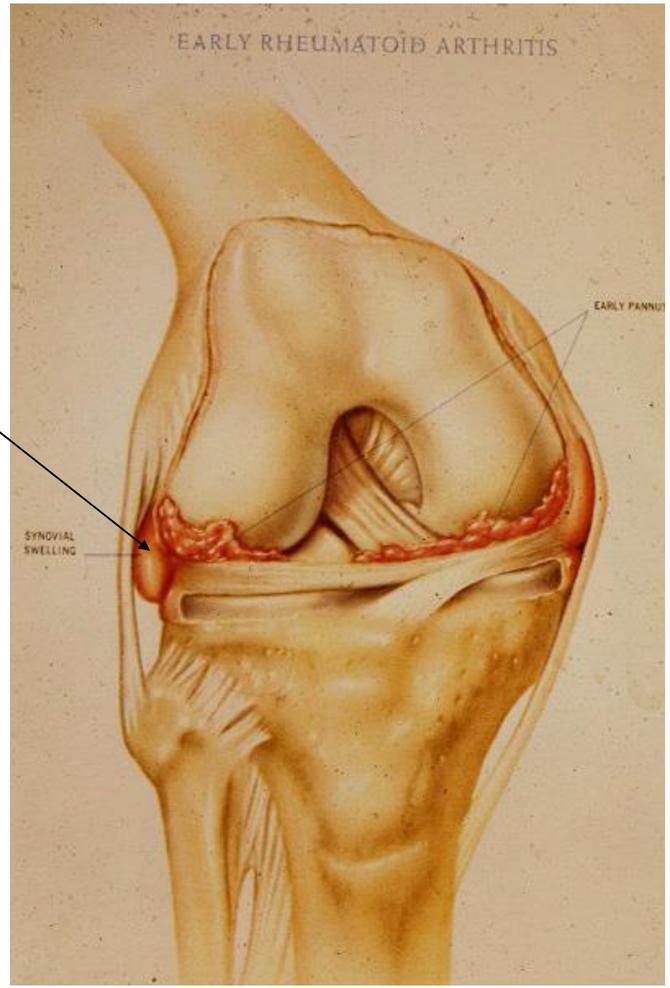
- events culminate in synovial inflammation, hyperplasia and bone destruction leading to joint swelling and deformity and to systemic inflammation.





RA progression

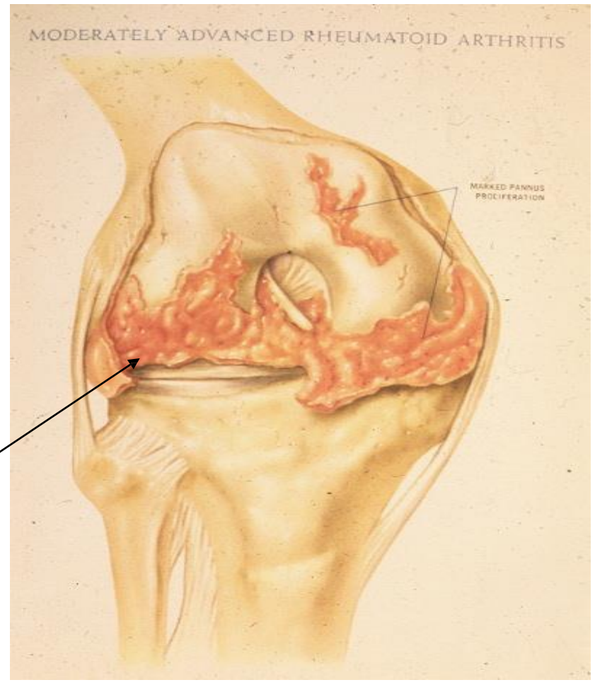
- **Early Pannus**
  - **Granulation, inflammation at synovial membrane, invades joint, softens and destroys cartilage**



RA progression

Mod advanced Pannus

joint cartilage disappears, underlying bone destroyed, surfaces collapse



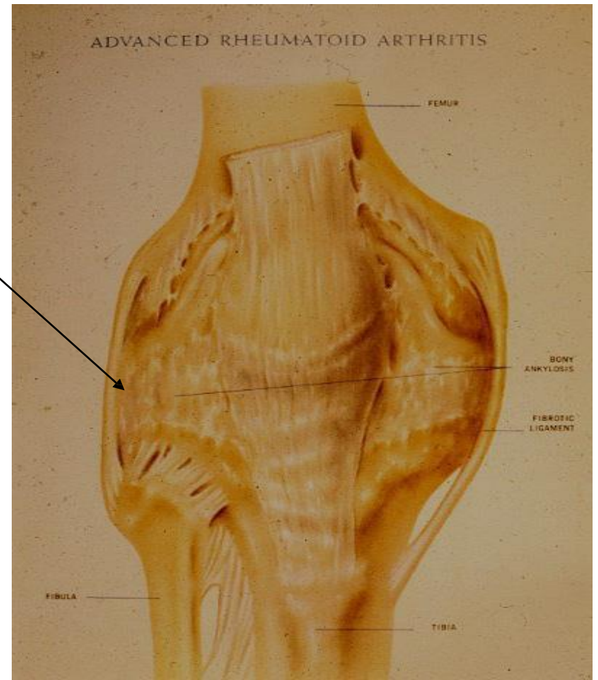
joint

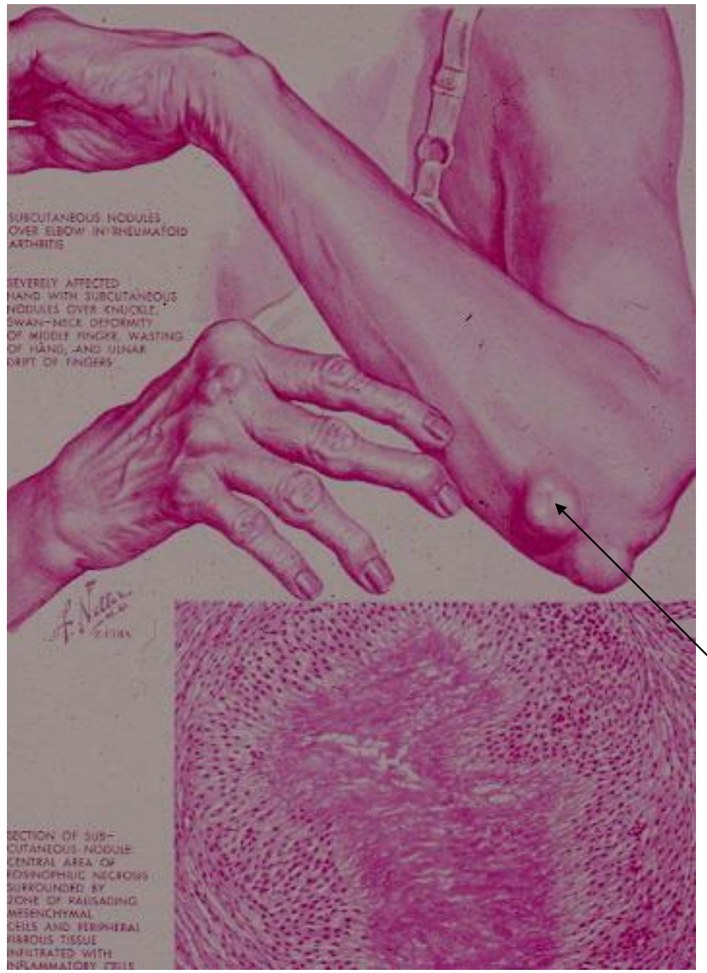
Fibrous Ankylosis

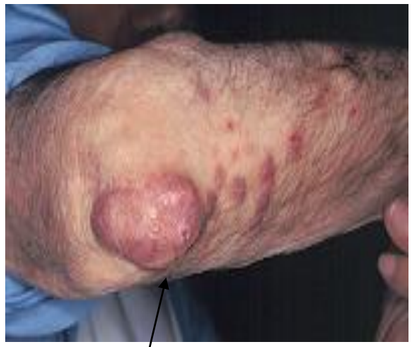
Fibrous connective tissue replaces pannus; loss of joint otion

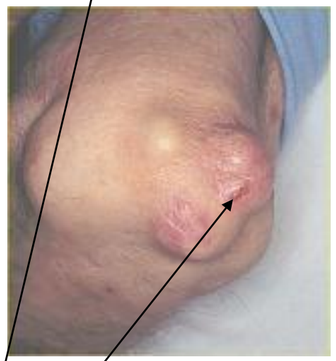
Bony Ankylosis

Eventual tissue and joint calcification









Subcutaneous nodules

(disappear and appear without warning)

Diagnostic Tools in Rheumatoid Arthritis

- Rheumatoid factor
- Anti-CCP antibodies
- Plain X-ray
- MRI
- Ultrasound

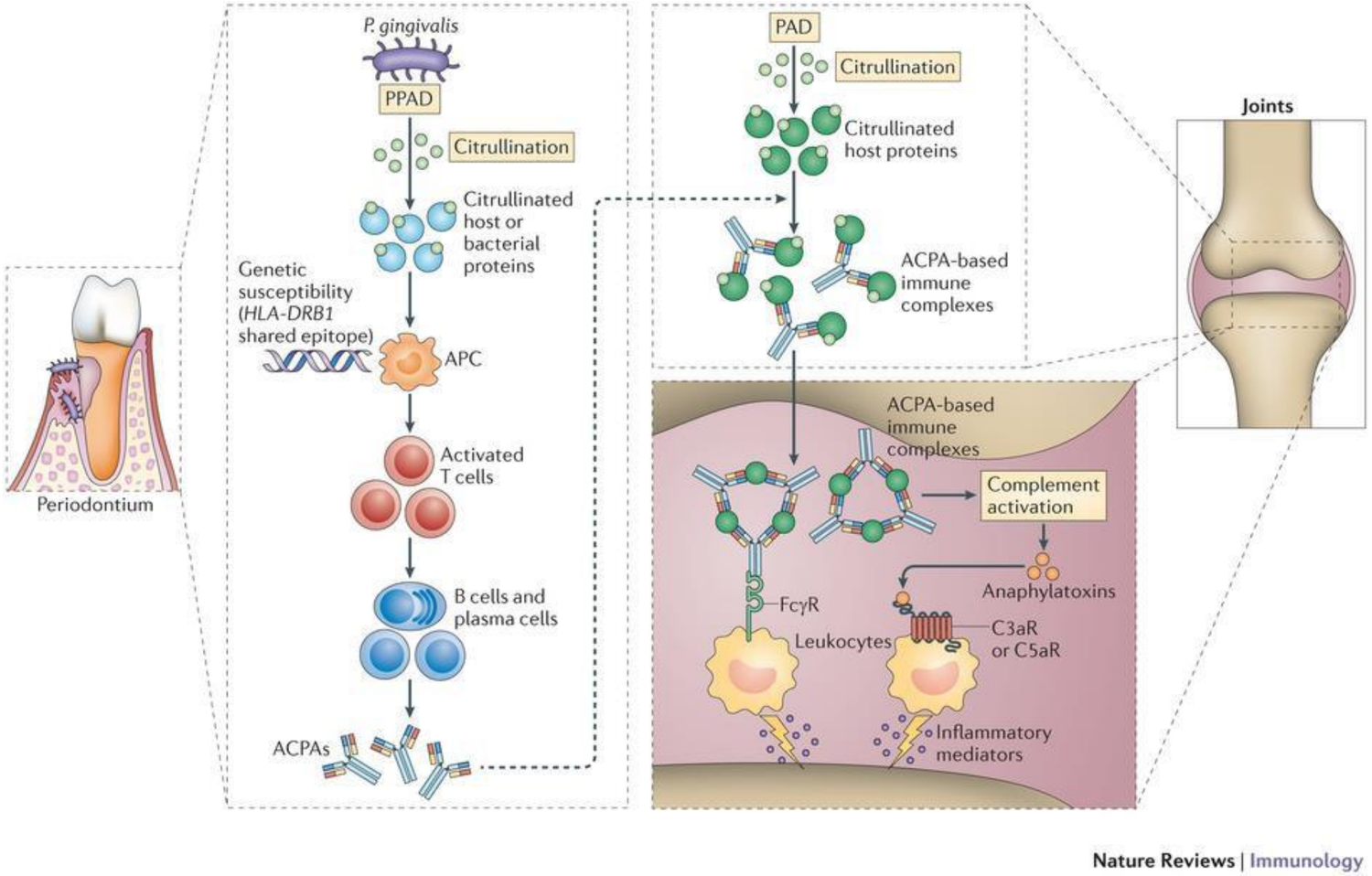
Rheumatoid Factor

- Antibody directed against the Fc portion of IgG
- Present in approximately 80% of RA patients
  - Sensitivity for RA is ~80%
  - Specificity is 85-95%
- May be involved in disease pathogenesis
- Higher levels tend to be associated with poorer prognosis
- Found in other conditions, especially Hepatitis C

Anti-Cyclic Citrullinated Peptide (CCP) Antibodies in RA

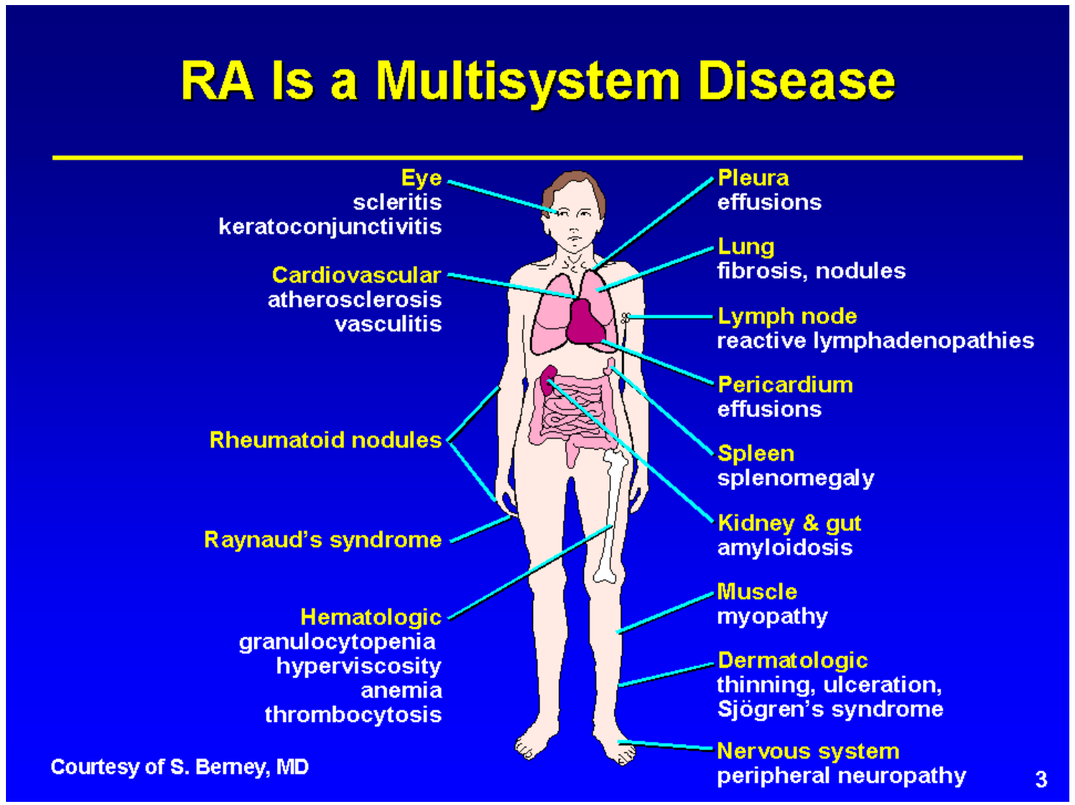
- Anti-citrulline Abs produced in RA synovium
- Early RA Diagnosis
  - sensitivity 48%; specificity 96%
  - seen in 2% of pts with other autoimmune diseases and infections (vs. 14% for RF)
  - less than 1% of healthy controls
- Predicts erosive disease PPV - 63% and NPV - 90%
- Present years before the onset of symptoms. 34% of blood samples obtained 2.5 yr before onset of symptoms (vs. 1.8% of controls)

Anti-Cyclic Citrullinated Peptide (CCP) Antibodies in RA



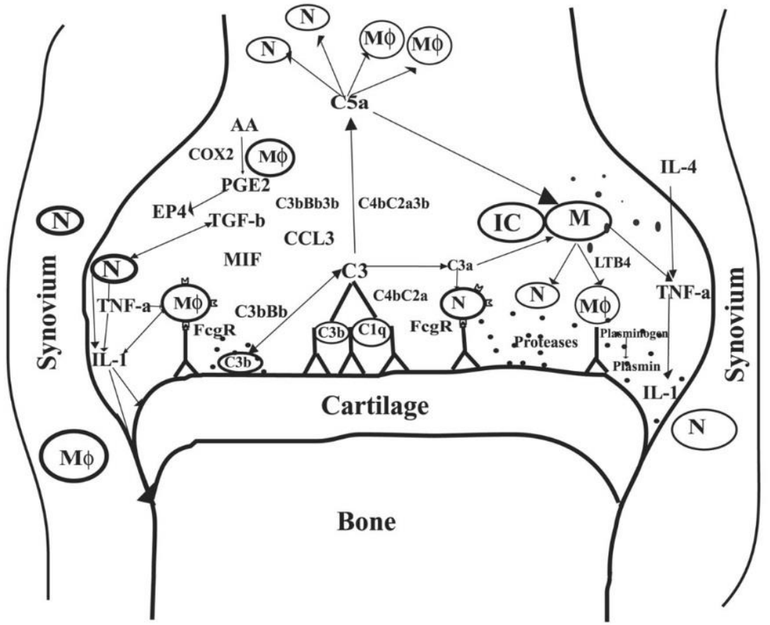
Complications of Rheumatoid Arthritis

- Complications include:
  - Carpal tunnel syndrome, Baker's cyst, vasculitis, subcutaneous nodules, Sjögren's syndrome, peripheral neuropathy, cardiac and pulmonary involvement, Felty's syndrome, and anemia



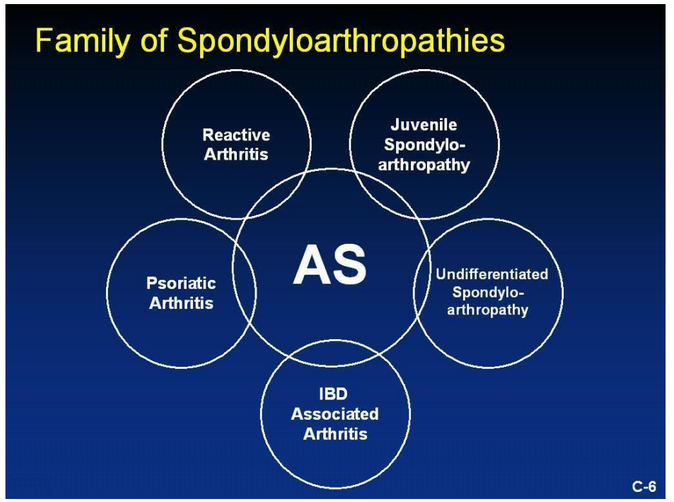
Lupus joints

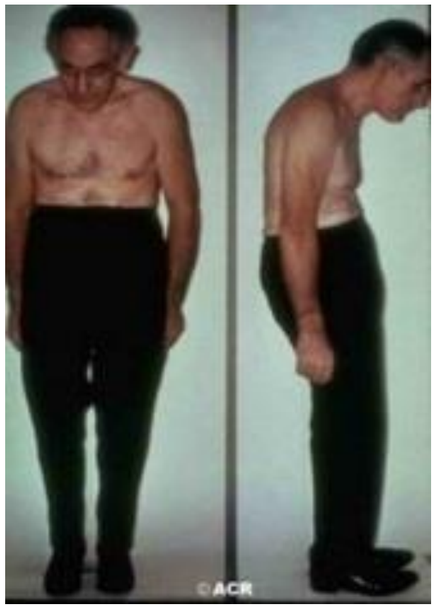
- Almost everyone with SLE has joint pain or inflammation.
- Any joint can be affected, but the most common spots are the hands, wrists, and knees.
- Usually the same joints on both sides of the body are affected
- The soft tissues around the joints are often swollen, but there is usually no excess fluid in the joint.
- Many SLE patients describe muscle pain and weakness, and the muscle tissue can swell.



Seronegative Spondyloarthropathy

- Consist of a group of related disorders that include Reiter's syndrome, ankylosing spondylitis, psoriatic arthritis, and arthritis in association with inflammatory bowel disease
- Occurs commonly among young men, with a mean incidence between ages 25 and 34
- The prevalence is about 1%
- The male-to-female ratio approaches 4 to 1 among adult Caucasians
- Genetic factors play an important role in the susceptibility to each disease





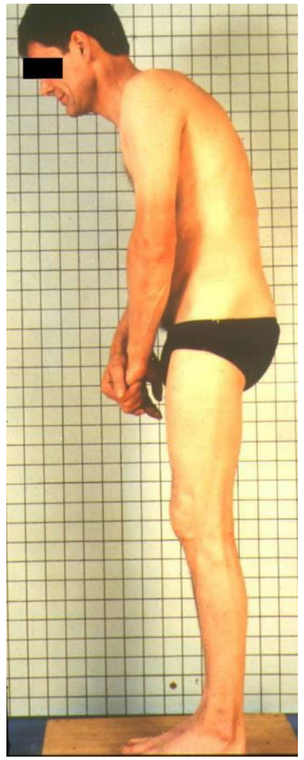
Seronegative Spondyloarthropathy

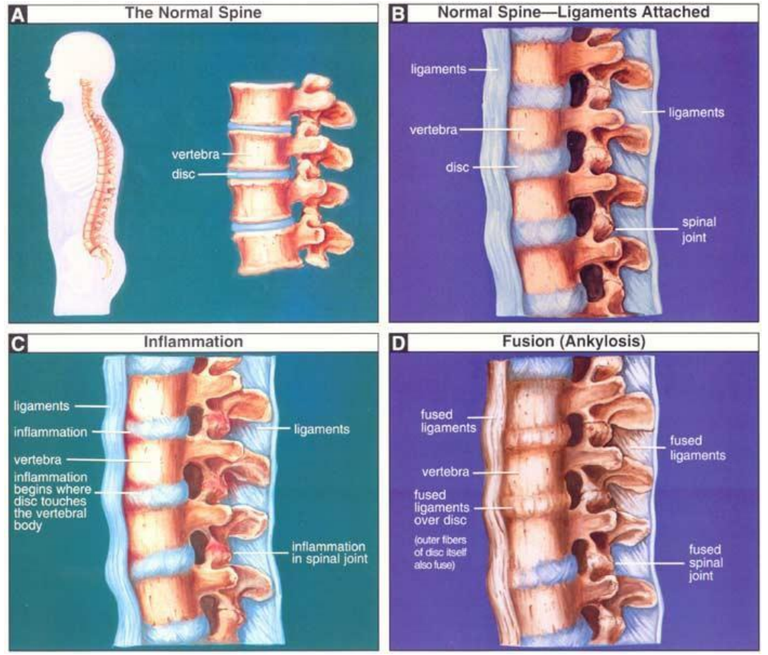
- The spondyloarthropathies share certain common features, including the absence of serum rheumatoid factor, an oligoarthritis commonly involving large joints in the lower extremities, frequent involvement of the axial skeleton, familial clustering, and linkage to HLA-B27

- These disorders are characterized by inflammation at sites of attachment of ligament, tendon, fascia, or joint capsule to bone (enthesopathy)

Ankylosing Spondylitis

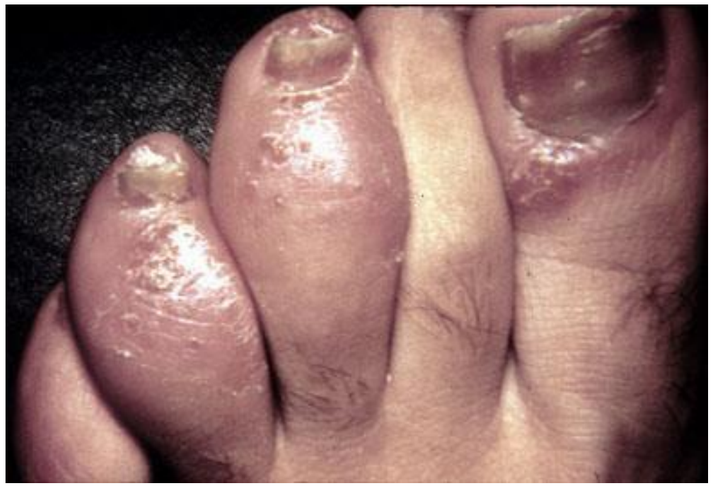
- Chronic disease that primarily affects the spine and may lead to stiffness of the back.
- The joints and ligaments become inflamed. The joints and bones may fuse.
- The effects are inflammation and chronic pain and stiffness in the lower back that usually starts where the lower spine is joined to the pelvis or hip.
- Diagnosis: X-rays, and blood tests for HLA-B27 gene

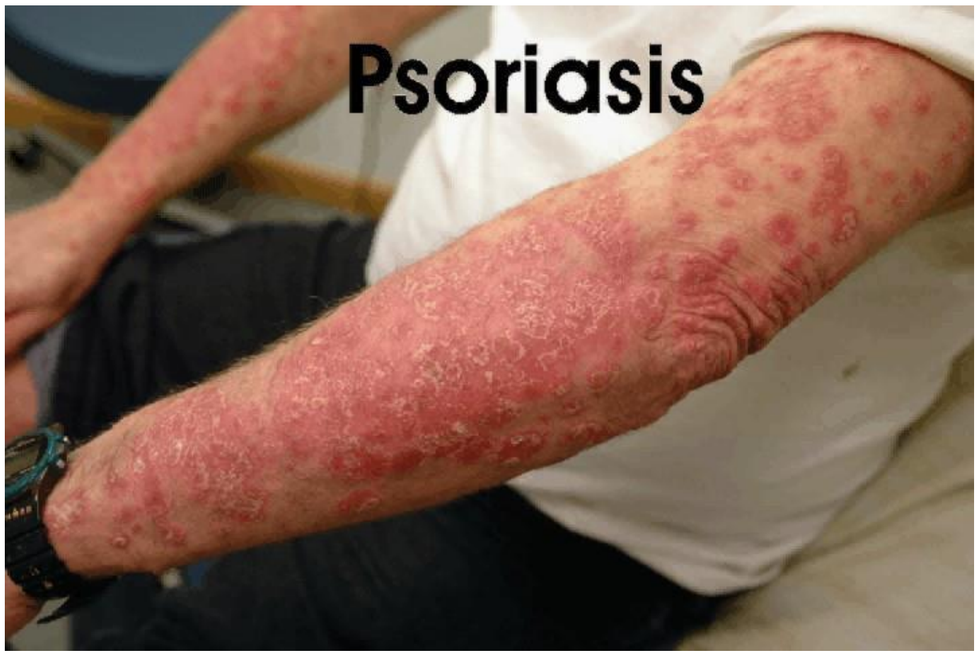




Psoriatic Arthritis

- Causes pain and swelling in some joints and scaly skin patches on some areas of the body.
- The symptoms are:
  - About 95% of those with psoriatic arthritis have swelling in joints outside the spine
  - Silver or grey scaly spots on the scalp, elbows, knees and/or lower end of the spine.
  - Pain and swelling in one or more joints
  - Swelling of fingers/toes that gives them a "sausage" appearance.

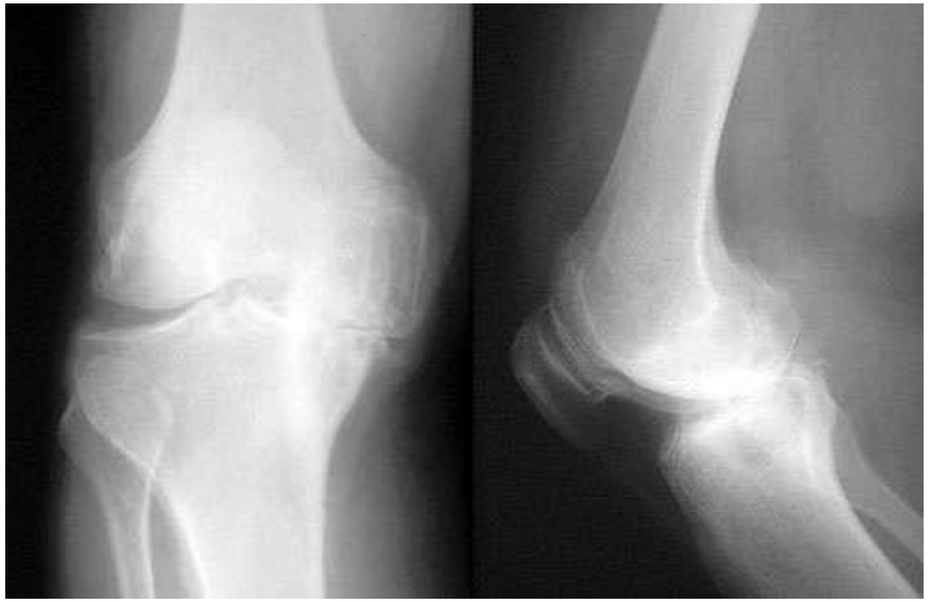




Articular diseases

- rheumatoid arthritis (RA) and
- osteoarthritis (OA).

Osteoarthritis



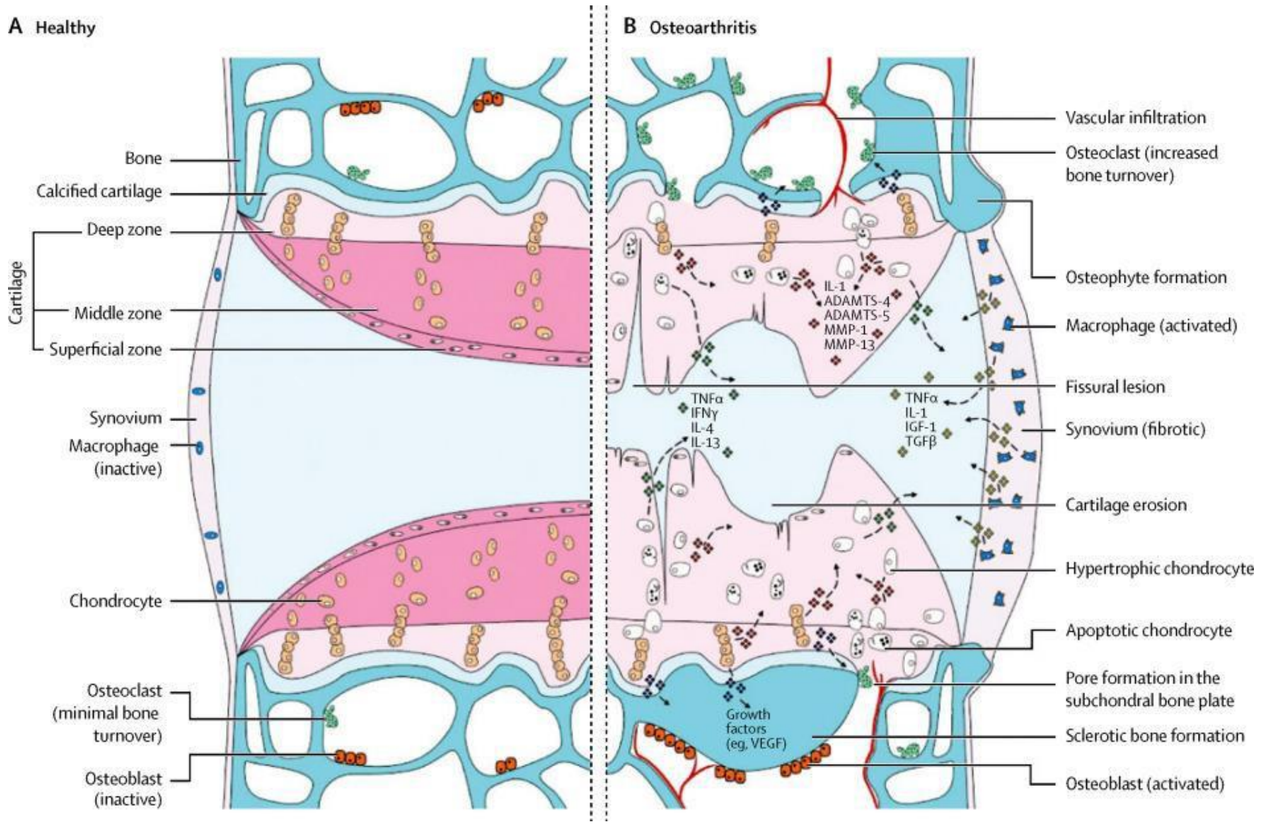
- major source of pain, disability, and socioeconomic cost worldwide
- epidemiology - complex and multifactorial, with genetic, envirnmental, and biomechanical components
- characterized by progressive loss of cartilage and reactive changes at the margins of the joint and in the subchondral bone

Risk factors for osteoarthritis

- Joint biomechanics are dictated by anatomical and functional factors
  - Anatomical factors include joint morphology
  - With respect to functional factors, poor quadriceps function
  - Sport
- Age
  - reduction in regenerative capacity and accumulation of risk factors
- Injury
- Obesity
  - load on weight-bearing joints,
  - increased joint susceptibility through inflammatory adipokines
- Genetics

Pathogenesis

- Osteoarthritis - once viewed as a disease of purely mechanical cartilage degradation,
- but it is now known to be a complex condition affecting the whole joint, in which activation of matrix proteases has a pivotal role

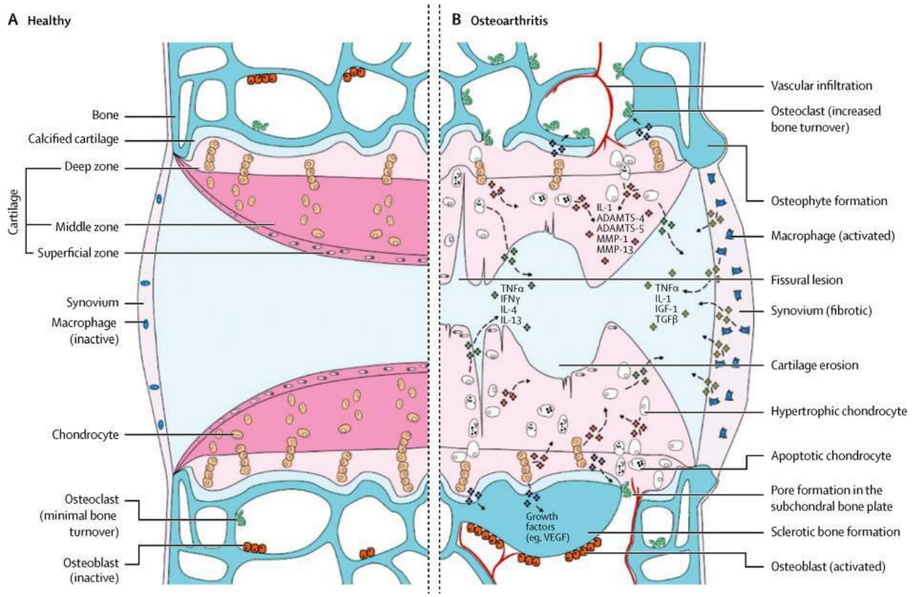


THE LANCET, VOLUME 386, ISSUE 9991, P376-387, JULY 25, 2015

Cartilage, subchondral bone, and synovium probably all have key roles in disease pathogenesis, and an association with systemic inflammation could also be present.

Synovium

- Synovitis is a common feature of osteoarthritis, even in early disease. In established osteoarthritis, proliferation of synoviocytes and tissue hypertrophy are notable, with increased vascularity.
- Synoviocytes synthesise lubricants such as hyaluronic acid and lubricin.
  - These contribute to optimum joint function but show reduced lubricating capacity in subsets of patients with osteoarthritis.
- Synoviocytes, like chondrocytes and osteoblasts, also release inflammatory mediators and degradative enzymes.



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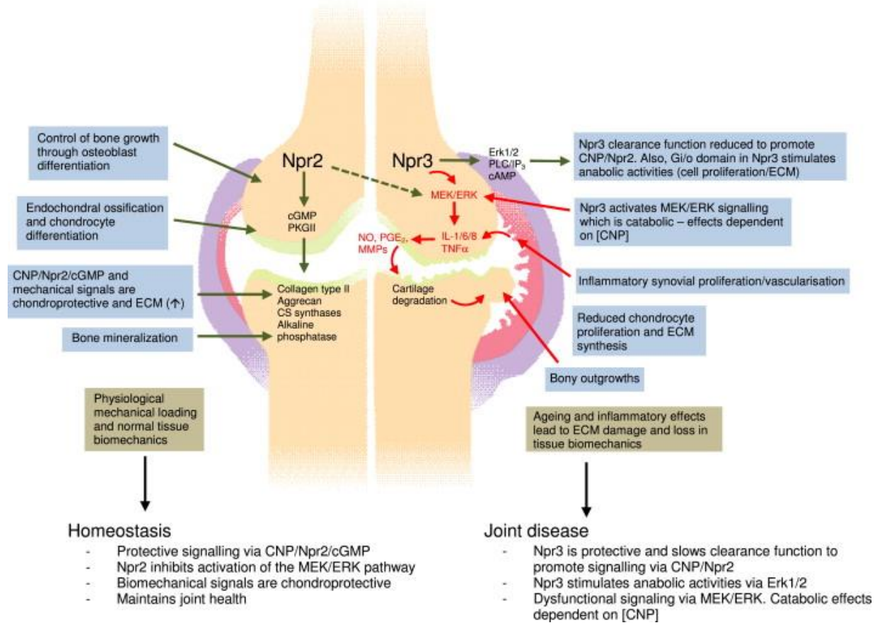
Cartilage

- main structural protein - type II collagen, which provides a meshwork that receives stabilisation from other collagen types and non-collagenous proteins and provides cartilage with tensile strength.
- Aggrecan and other proteoglycans are embedded within this framework, and draw water into the cartilage, providing compressive resistance.
- Cartilage architecture and biochemical composition are strictly regulated by chondrocytes in response to changes
  - they produce several inflammatory response proteins, such as cytokines, including interleukin 1β, interleukin 6, and tumour necrosis factor (TNF) α, and matrix-degrading enzymes including the metalloproteinases and a disintegrin and metalloproteinase with thrombospondin-like motifs (ADAMTS).

Subchondral bone

- Subchondral cortical bone forms an interface between the calcified cartilage below the tidemark and the underlying trabecular bone.
- Pronounced changes from normal are seen in the structure and composition of both the cortical plate and trabecular bone in osteoarthritis.
- Features of endochondral ossification are reinitiated in osteoarthritis and the tidemark advances, with associated vascular penetration. This process is accompanied by the formation of osteophytes and subchondral cysts.



Osteoarthritis and Cartilage 22 (2014)

Degenerative Joint Disease

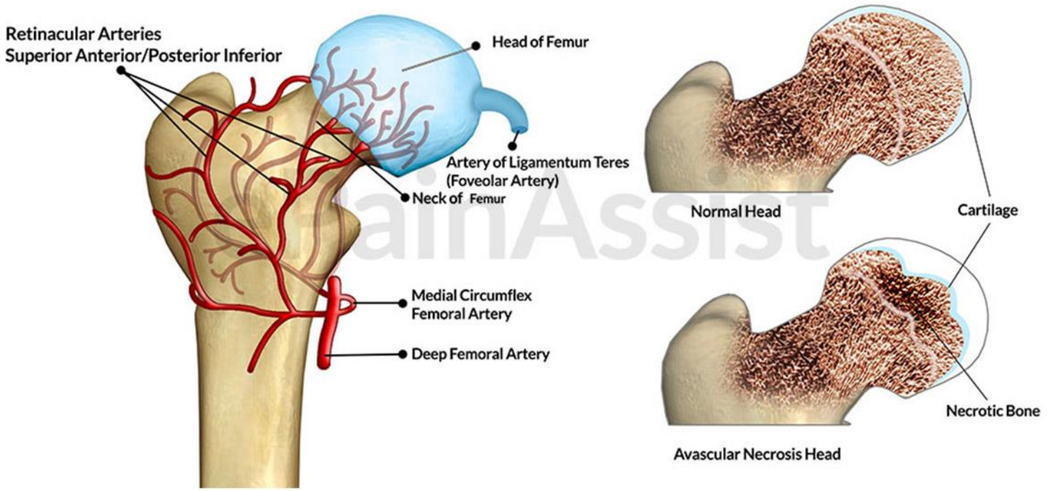
- In early disease, pain occurs only after joint use and is relieved by rest
- As the disease progresses, pain occurs with minimal motion or even at rest
- Nocturnal pain is commonly associated with severe disease

Image of the knee joint with arthritis clearly present

Osteonecrosis

- may be idiopathic or secondary to numerous diseases

- spontaneous occurrences lack an obvious etiology,
- most cases occur secondary to trauma
- non-traumatic has been associated with
  - corticosteroid usage,
  - alcoholism,
  - infection,
  - hyperbaric events,
  - storage disorders,
  - marrow infiltrating diseases,
  - coagulation defects
  - some autoimmune diseases.



E.g., in trauma, the normal vascular supply to the femoral head is damaged, leading to ONFH.

Disease Models & Mechanisms

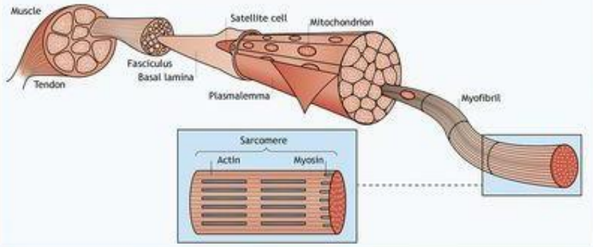
Skeletal muscle in health and disease

Jennifer Morgan and Terence Partridge

The Company of Biologists

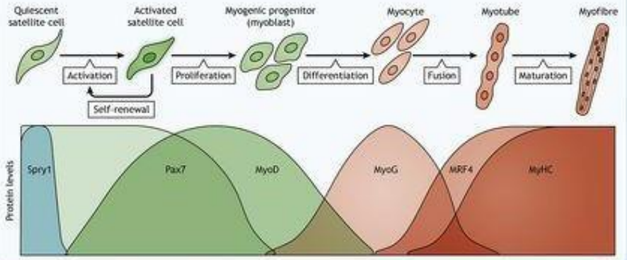
Structure of skeletal muscle

Skeletal muscle fibres are multinucleated cells that are specialized to perform muscle contraction. Growth and repair of muscle fibres occurs by fusion of precursor cells derived from satellite cells. These, in normal uninjured muscle, lie in a dormant state between the plasmalemma of the muscle fibre and the overlying basement membrane, but are rapidly activated by injury of the host fibre and, to some extent, nearby fibres. Acutely injured muscle is efficiently repaired by this process but fails progressively in the context of the repeated chronic injury that characterizes muscular dystrophies such as Duchenne muscular dystrophy.



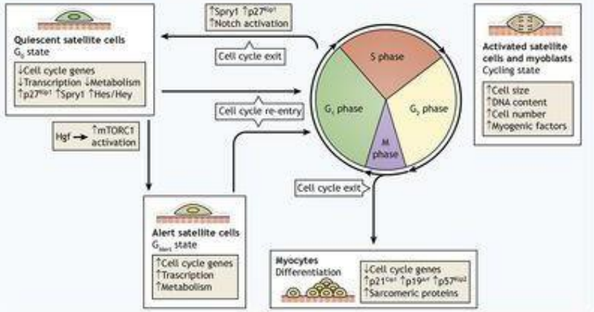
Satellite cell differentiation

The expression of key myogenic regulators drives the activation and differentiation of satellite cells that fuse to form myotubes and muscle fibres. Satellite cells may become activated either during normal muscle growth or in response to injury. Activated satellite cells can return to quiescence as a source of future myogenic cells (self-renewal). Alternatively, they may proliferate and differentiate, ultimately giving rise to myofibres, within which the contractile elements of skeletal muscle are expressed.



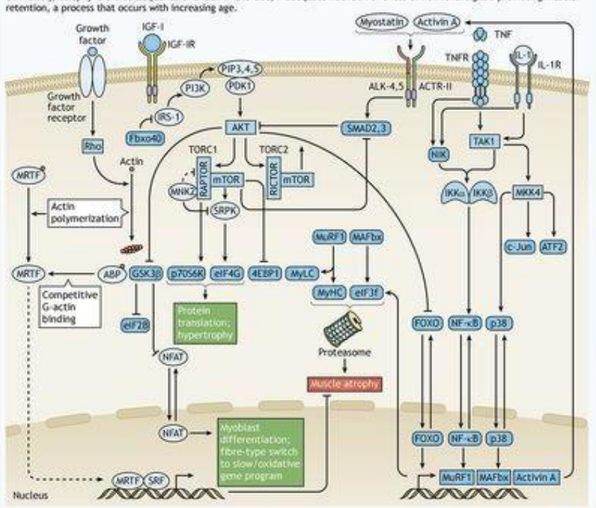
Activation of satellite cells

Activation and differentiation of satellite cells occurs through regulation of their cell cycle. In resting adult muscles, satellite cells are not actively dividing (dormant or G_0 state). However, mitogenic signals released upon muscle injury can cause quiescent satellite cells to re-enter the cell cycle and start to divide.

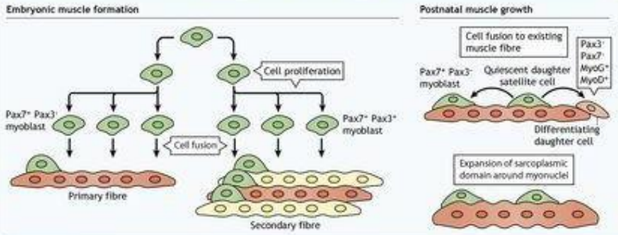


Molecular regulation of muscle size: hypertrophy and atrophy

A variety of environmental factors contribute to increases (hypertrophy) or decreases in muscle size (atrophy). Hypertrophy is supported by mechanical exercise and nutrition (particularly through the intake of amino acids, which induce the mTOR pathway). Conversely, atrophy is the consequence of a lack of exercise, inadequate nutrition or a loss of hormonal signals promoting muscle retention, a process that occurs with increasing age.

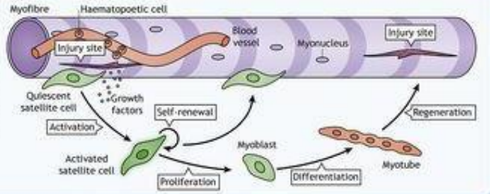


Embryonic muscle formation and postnatal muscle growth



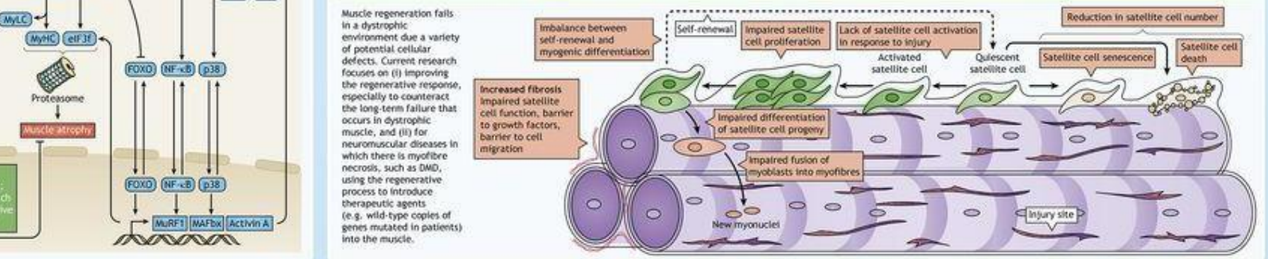
Muscle regeneration

Satellite cells in the region of muscle damage proliferate predominantly by symmetrical division to generate a mass of cells, which either fuse with one another, or with the damaged fibres, to repair the muscle at the site of injury.



Cellular phenotypes of impaired regeneration in dystrophic muscle

Muscle regeneration fails in a dystrophic environment due a variety of potential cellular defects. Current research focuses on (i) improving the regenerative response, especially to counteract the long-term failure that occurs in dystrophic muscle, and (ii) for neuromuscular diseases in which there is myofibre necrosis, such as DMD, using the regenerative process to introduce therapeutic agents (e.g. wild-type copies of genes mutated in patients) into the muscle.



Problems and the future

Muscle regeneration is effective following acute injury, but is inadequate in some muscular dystrophies.

Better models to study muscular dystrophies are required. Although the mdx mouse is a useful model for many purposes, it does not adequately reproduce the limited muscle regeneration or extensive fibrosis that occur in DMD patients.

The multifactorial influences on skeletal muscle regeneration *in vivo* portray the complexity of the system; this should be borne in mind when designing experiments and analysing data.

Abbreviations: 4EBP1, Eukaryotic translation initiation factor 4E-binding protein 1; ABP, Actin-binding protein; ACTR-II, Activin receptor type II; AKT, Serine/threonine-protein kinase AKT; ALK-4,5, Activin receptor-like kinase 4,5; ATF2, Activating transcription factor 2; DMD, Duchenne muscular dystrophy; c-Jun, Proto-oncogene c-Jun; eIF3f, Eukaryotic translation initiation factor 3 subunit F; eIF4G, Eukaryotic translation initiation factor 4 gamma; Fbxo40, F-box only protein 40; FOXO, Forkhead box protein O; GSK3β, Glycogen synthase kinase-3β; Hes, Hairy and enhancer of split; Hey, Hairy and enhancer o split-related protein; Hgf, Hepatocyte growth factor; IGF-I, Insulin-like growth factor I; IGF-IR, IGF-I receptor; IKKα, Inhibitor of nuclear factor kappa-B kinase subunit α; IKKβ, Inhibitor of nuclear factor kappa-B kinase subunit β; IL-1, Interleukin-1; IL-1R, Interleukin-1 receptor; IRS-1, Insulin receptor substrate 1; MAFbx, Muscle atrophy F-box protein; MKK4, Mitogen-activated protein kinase kinase 4; MNK2, MAP kinase-interacting serine/threonine-protein kinase 2; MRTF, Myocardin-related transcriptional factor; mTORC1, mammalian target of rapamycin complex 1; MuRF1, Muscle-specific RING finger protein 1; MyoD, Myoblast determination protein; MyoG, Myogenin; MyHC, Myosin heavy chain; MyLC, Myosin light chain; NFAT, Nuclear factor of activated T-cells; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B-cells; NIK, NF-κB-inducing kinase; p19^Arf, Tumor suppressor ARF; p21^Cip1, Cyclin-dependent kinase inhibitor p21; p27^Kip1, Cyclin-dependent kinase inhibitor p27; p38, Mitogen-activated protein kinases p38; p57^Kip2, Cyclin dependent kinase inhibitor p57; p70S6K, p70 ribosomal S6 kinase; Pax3, Paired box 3; Pax7, Paired box 7; PDK1, Phosphoinositide-dependent kinase-1; PI3K, Phosphoinositide 3-kinase; PIP3,4,5, Phosphatidylinositol (3,4,5)-trisphosphate; RAPTOR, Regulatory-associated protein of TOR; RICTOR, Rapamycin-insensitive companion of TOR; SMAD2,3, SMAD family member 2,3; Spry1, Sprouty1; SRF, Serum response factor; SRPK, SRSF protein kinase; TAK1, Transforming growth factor-beta-activated kinase 1; TNF, Tumor necrosis factor; TNFR, Tumor necrosis factor receptor; TORC1,2, Target of rapamycin complex 1, 2.

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Jennifer Morgan, and Terence Partridge Dis. Model. Mech. 2020;13:dmm042192

Disease Models & Mechanisms

Muscles

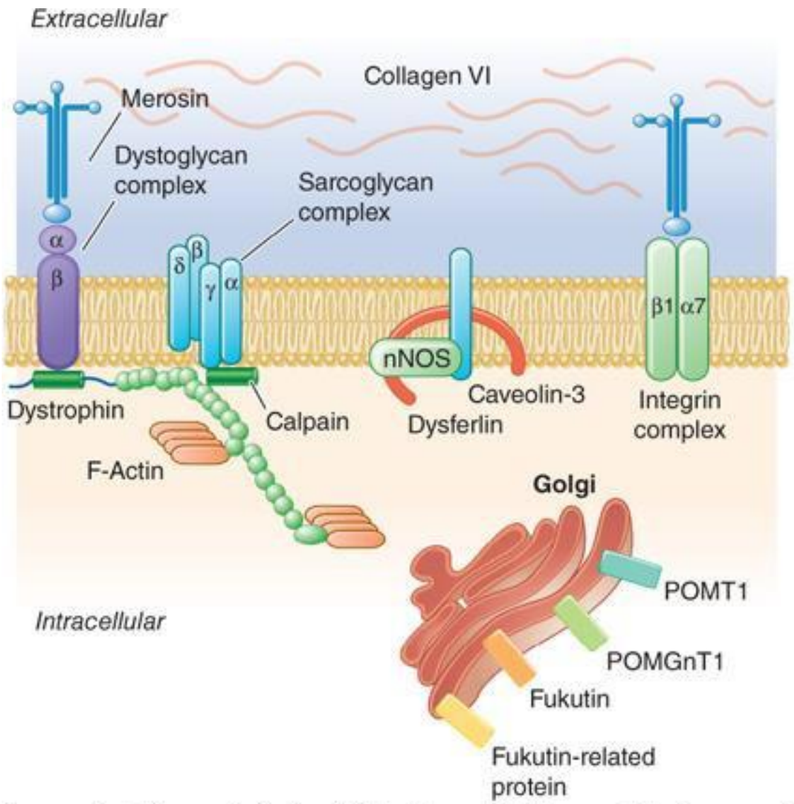
Familial	Acquired
Pre-junctional (peripheral neuropathies): • Charcot-Marie-Tooth • Fredrich's ataxia • Spinal muscular atrophy	Pre-junctional: • Motor neurone disease • Multiple sclerosis • Guillain-Barré syndrome • Peripheral neuropathies e.g. diabetes mellitus
Junctional: • Congenital myasthenia gravis	Junctional: • Myasthenia gravis • Eaton-Lambert syndrome

Post-junctional:
• Dystrophies:
  ◦ Duchenne
  ◦ Becker's
• Myotonias:
  ◦ Myotonic dystrophy
  ◦ Myotonia congenital
• Hyper, hypokalaemic periodic paralysis
• Congenital myopathies
• Metabolic/ mitochondrial disorders
• Malignant hyperthermia susceptibility
	Post-junctional:
	• Inflammatory myopathies
	• Critical illness polyneuropathy and myopathy

DUCHENNE MUSCULAR DYSTROPHY

- -linked recessive disorder, sometimes also called *pseudohypertrophic muscular dystrophy*
- incidence of ~1 per 5200 live-born males
- by age 5 years, muscle weakness is obvious by muscle testing
- muscle biopsy shows muscle fibers of varying size as well as small groups of necrotic and regenerating fibers
- connective tissue and fat replace lost muscle fibers
- caused by a mutation of the gene that encodes dystrophin,

Dystrophin



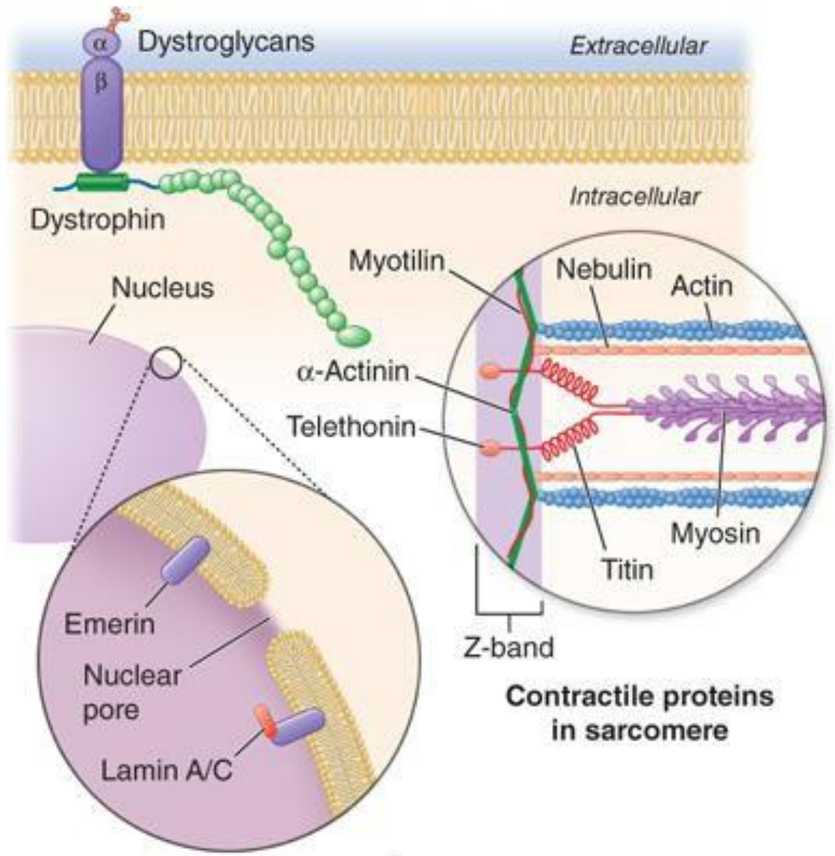
- a 427-kDa protein localized to the inner surface of the sarcolemma of the muscle fiber
- dystrophin gene is >2000 kb in size and thus is one of the largest identified human genes
- localized to the short arm of the X chromosome at Xp21.
- the most common gene mutation is a deletion
- the size varies but does not correlate with disease severity

Source: D. L. Kasper, A. S. Fauci, S. L. Hauser, D. L. Longo, J. L. Jameson, J. Loscalzo: Harrison's Principles of Internal Medicine, 19th Edition.
www.accessmedicine.com

BECKER MUSCULAR DYSTROPHY

- less severe form of X-linked recessive muscular dystrophy results from allelic defects of the same gene responsible for Duchenne dystrophy.
- Becker muscular dystrophy is ~10 times less frequent than Duchenne
- proximal muscles, especially of the lower extremities, are prominently involved
- as the disease progresses, weakness becomes more generalized
- mental retardation may occur in Becker dystrophy, but it is not as common as in Duchenne
- Genetic testing reveals deletions or duplications of the dystrophin gene in 65% of patients with Becker dystrophy
- in ~95% of patients with Becker dystrophy, the DNA deletion does not alter the translational reading frame of messenger RNA. These “in-frame” mutations allow production of some dystrophin

Muscular dystrophy associated proteins



- emerin and lamin A/C are constituents of the inner nuclear membrane. Several dystrophy-associated proteins are represented in the sarcomere including titin, nebulin, calpain, telethonin, actinin, and myotilin

Table 2. Defects caused by the different muscular dystrophies

Muscular dystrophy	Gene	Protein	Where protein is expressed in skeletal muscle	Cellular phenotype of disease	Therapeutic targets
Duchenne and Becker muscular dystrophy (DMD and BMD)	*DMD*	Dystrophin	Myofibre sarcolemma; satellite cells	Myofibre degeneration; satellite cell exhaustion; impaired satellite cell self-renewal	Dystrophin restoration by gene therapy (Aguti et al., 2018) or exon skipping (Cirak et al., 2011) in animal models and clinical trials
Laminin alpha-2 deficiency (MDC1A)	*LAMA2*	Laminin alpha-2	Extracellular matrix	Myofibre degeneration; impaired regeneration	Expression of linker proteins (mini-agrin) in mice (Reinhard et al., 2017); anti-apoptotic agents (Meinen et al., 2011) in mice
Collagen VI-deficient congenital muscular dystrophy (CMD)	*COL6A1* *COL6A2* *COL6A3*	Collagen VI	Extracellular matrix	Myofibre degeneration; defective autophagy; impaired satellite cell self-renewal	Reactivation of autophagy in clinical trial (Castagnaro et al., 2016); anti-apoptotic agents in mice (Palma et al., 2009)
Dystroglycanopathy	*POMT1* *POMT2* *FKTN* *FKRP* *LARGE* *POMGNT1* *ISPD*	Protein-O-mannosyl-transferase 1; protein-O-mannosyl-transferase 2; fukutin; fukutin-related protein; like-acetylglucosaminyltransferase; O-linked mannose beta-1,2-N-acetyl-glucosaminyl-transferase; isoprenoid synthase domain-containing protein	Myofibre sarcolemma	Impaired satellite cell proliferation	Restore glycosylation in mice (Cataldi et al., 2018); *FKRP* gene therapy in mice (Vannoy et al., 2018)
SEPN1 (also known as *SELENON*)-related myopathy	*SEPN1*	Selenoprotein N	Endoplasmic reticulum	Reduced satellite cell number; impaired muscle regeneration	Antioxidants *in vitro* (Arbogast et al., 2009)
LMNA-related CMD (L-CMD)	*LMNA*	Lamin A/C	Nuclear envelope	Skeletal muscle atrophy; impaired satellite cell differentiation	Trans-splicing gene therapy to reduce mutated transcript, *in vitro* and mouse model (Azibani et al., 2018)
Emery-Dreifuss muscular dystrophy (EDMD)	*EMD*	Emerin	Nuclear envelope	Impaired satellite cell proliferation	mTOR inhibitors (reviewed in Chiarini et al., 2019)
Sarcoglycanopathy LGMD2D LGMD2E LGMD2C LGMD2F	*SGCA* *SGCB* *SGCG* *SGCD*	Alpha-sarcoglycan; beta-sarcoglycan; gamma-sarcoglycan; delta-sarcoglycan	Myofibre sarcolemma	Reduced satellite cell number	Gene therapy to restore beta-sarcoglycan in mice (Pozsgai et al., 2017); endoplasmic reticulum quality control *in vitro* (Soheili et al., 2012)
Calpainopathy LGMD2A	*CAPN3*	Calpain 3	Myofibrils; differentiating myoblasts	Impaired satellite cell proliferation and differentiation	Genome editing *in vitro* (Selvaraj et al., 2019)
Dysferlinopathy LGMD2B	*DYSF*	Dysferlin	Myofibre sarcolemma	Impaired satellite cell differentiation	Exon skipping in mouse model (Malcher et al., 2018); membrane stabilization in mouse model (Sreetama et al., 2018)
Facioscapulo-humeral muscular dystrophy	*DUX4*	Double homeobox 4	Nucleus: hypo-methylation of the D4Z4 region of chromosome 4	Myoblast apoptosis	Silencing *DUX4* by gene therapy to deliver targeted microRNA in mouse model (Wallace et al., 2018); scapulothoracic arthrodesis (Eren et al., 2019)
Myotonic dystrophy Type 1 Type 2	*DMPK* *CNBP*	Dystrophia myotonica protein kinase; CCHC-type zinc finger nucleic acid-binding protein	Nucleus: expansion of CTG in untranslated region	Reduced satellite cell number; impaired satellite cell proliferation; myoblast senescence	*DMPK* mRNA knockdown *in vitro* (Seow et al., 2012; reviewed in Overby et al., 2018); Mexiletine (Nguyen and Campbell, 2016); adding muscleblind-like protein 1 (reviewed in Konieczny et al., 2017)
Oculopharyngeal muscular dystrophy (OPMD)	*PABPN1*	Poly(A)-binding protein nuclear 1	Nucleus	Impaired satellite cell proliferation and differentiation; increased number of satellite cells in affected muscles	Myoblast transplantation clinical trial (Perié et al., 2014); modulation of endoplasmic reticulum stress in a mouse model (Malerba et al., 2019); knockdown of protein *in vitro* (Abu-Baker et al., 2019)
Carey-Fineman-Ziter syndrome	*MYMK*/ *TMEM8C*	Myomaker	Cell membrane; Golgi apparatus	Defect in myoblast fusion	None as yet
Early-onset myopathy, areflexia, respiratory distress and dysphagia (EMARDD)	*MEGF10*	Multiple epidermal growth factor-like domains protein 10	Cell membrane	Dysregulation of myogenesis; impaired satellite cell proliferation, self-renewal and quiescence	Selective serotonin reuptake inhibitors *in vitro* and in *Drosophila* and zebrafish models (Saha et al., 2019)
POGLUT1 muscular dystrophy	*POGLUT1*	Protein O-glucosyl-transferase 1	Endoplasmic reticulum	Reduced satellite cell number	None as yet
X-linked myotubular myopathy	*MTM1*	Myotubularin	Cytoplasm	Reduced satellite cell number	Gene therapy to deliver short hairpin RNA to knock down dynamin 2 in a mouse model (Tasfaout et al., 2018)
PAX7-related myopathy	*PAX7*	Paired box 7	Satellite cell nucleus	Satellite cell exhaustion	None as yet

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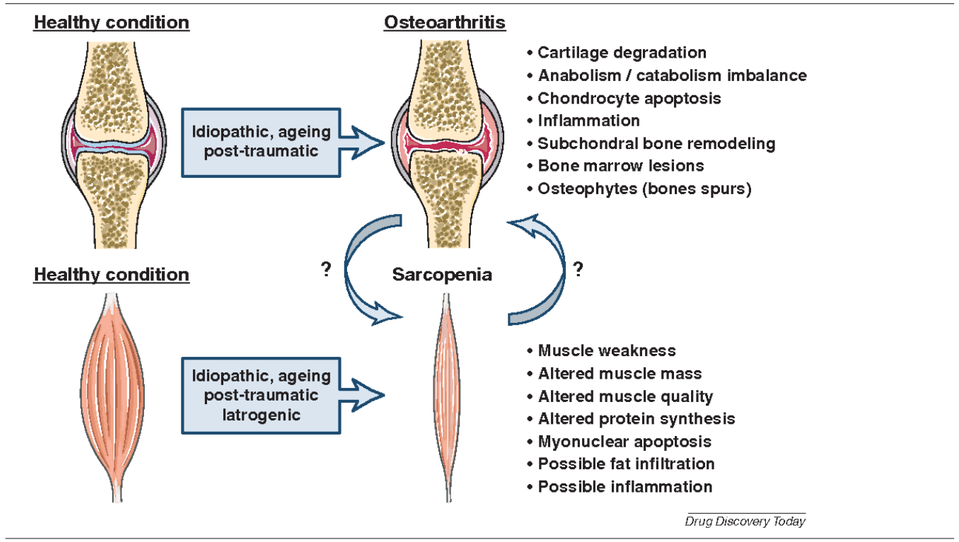
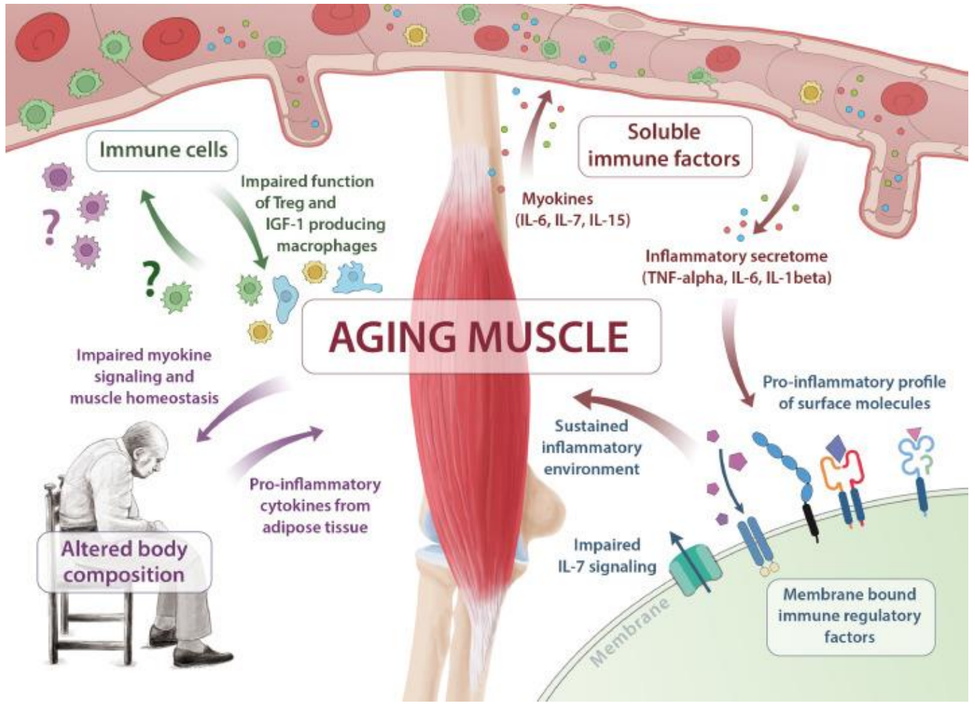
Sarcopenia

Aging of skeletal muscle is central in the pathogenesis of immune senescence and sarcopenia. Multiple pathways are affected, including insufficient myokine signalling (IL-6, IL-7, IL-15), shifting of membrane bound immune regulatory factors towards a pro-inflammatory profile, impaired immune cell function and altered body composition.





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Thank you for your attention