Metabolism of calcium and phosphates. Regulation of bone remodelling. Osteoporosis.

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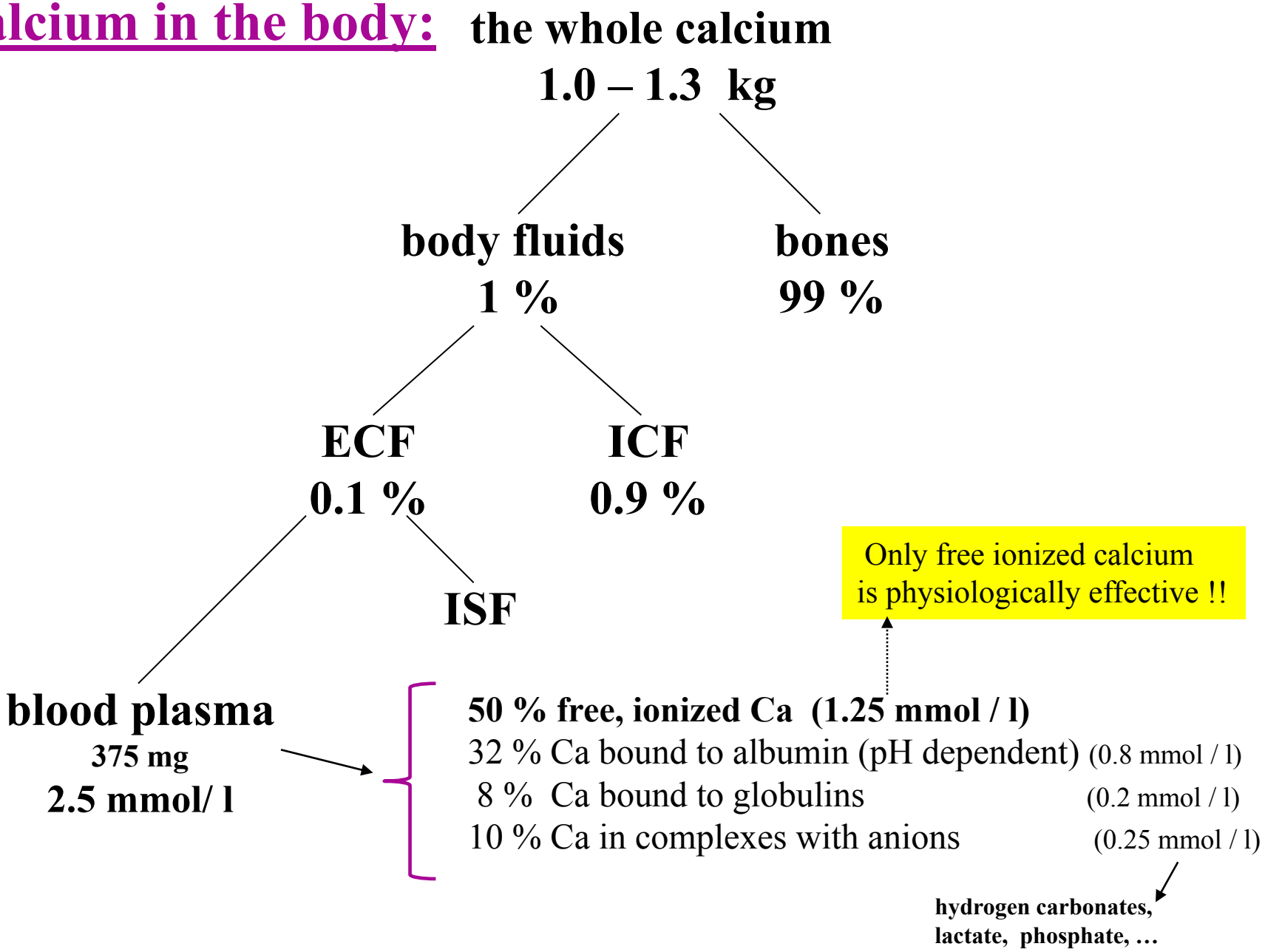
Calcium in the body:



Biological effects of calcium:

- signal transduction into cells (Ca – calmodulin complex)
- building material (bones, teeth, calcifications)
- neuromuscular irritability (hypocalcemia increase irritability, hypercalcemia increase contractility)
- blood clotting

Daily need of calcium:

- daily need of calcium is approx. 1g (≈ 25 mmol), older people and pregnant women 1.5g
- (0.5l of milk or 65g of cheese or 250ml of yoghurt contains approx. 0.5g of calcium)
- in childhood we can absorb 50% from daily intake, in adulthood only 10-40% (depend on need and vitamin D levels)

Sources of calcium:

- **appropriate sources**
  - dairy products from semi-skimmed milk
  - fermented milk products (acidity improves absorption)
  - some vegetable (cauliflower, endive, broccoli, Brussels sprout)
  - marginal sources – poppy seeds, nuts, sardines, tap water (in Brno approx. 2-2.5 mmol Ca/l – 10% of daily need)
- **inappropriate sources**
  - spinach (formation of insoluble calcium oxalate)
  - processed cheese (high content of phosphates → formation of insoluble calcium phosphates salts)
  - high content of phosphates represent also Coca-Cola and similar beverages
  - leafy vegetable with high content of magnesium (ideal ratio is 2:1)

Metabolism of bones:

1/ osteoblasts
formation of bones

2/ osteoclasts
resorption of bones

- healthy bone has both processes in ballance
- under pathological conditions predominates usually increased resorption

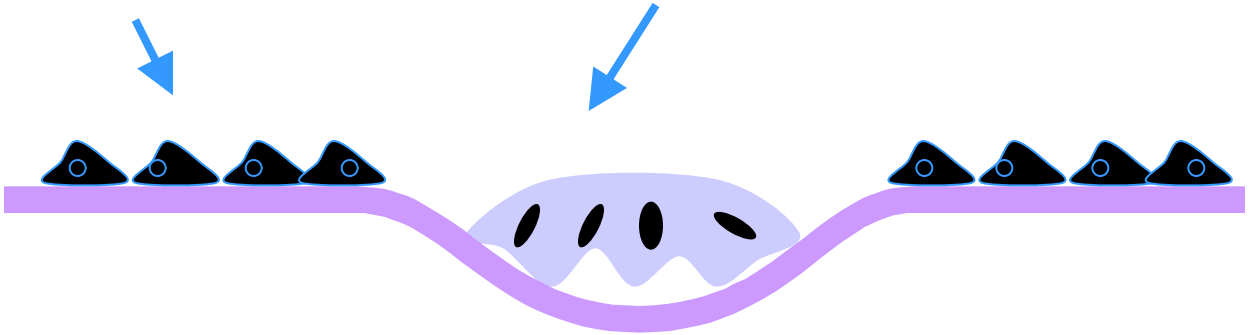
Bone remodelling :

- complex process of coordinated activity of bone cells – osteoblasts, osteoclasts and osteocytes
- functions:
  - adaptation of bone to changing mechanical load
  - reparation of small mechanical injuries, which accumulation can cause bone ageing
  - replacement of old bone tissue by new one, mechanically more appropriate

Bone resorption:

border cells covering the bone

osteoclasts resorb the bone

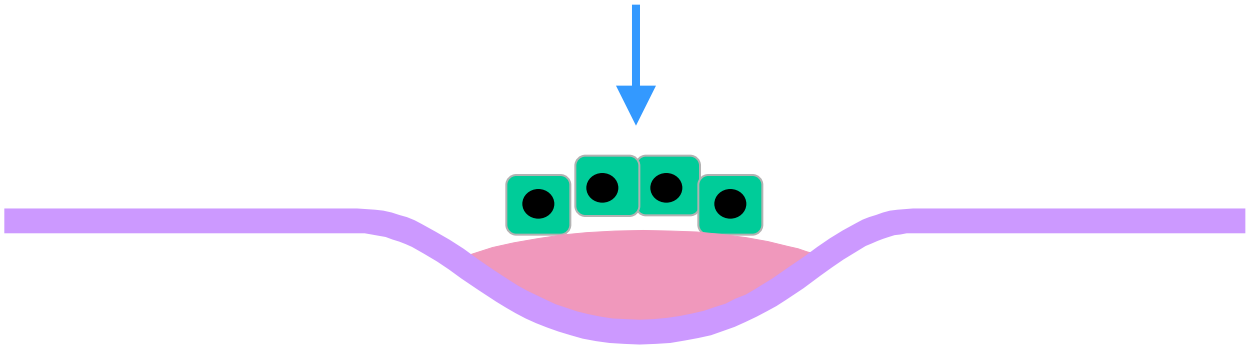


the activation of bone resorption

~ 20 days

Bone formation:

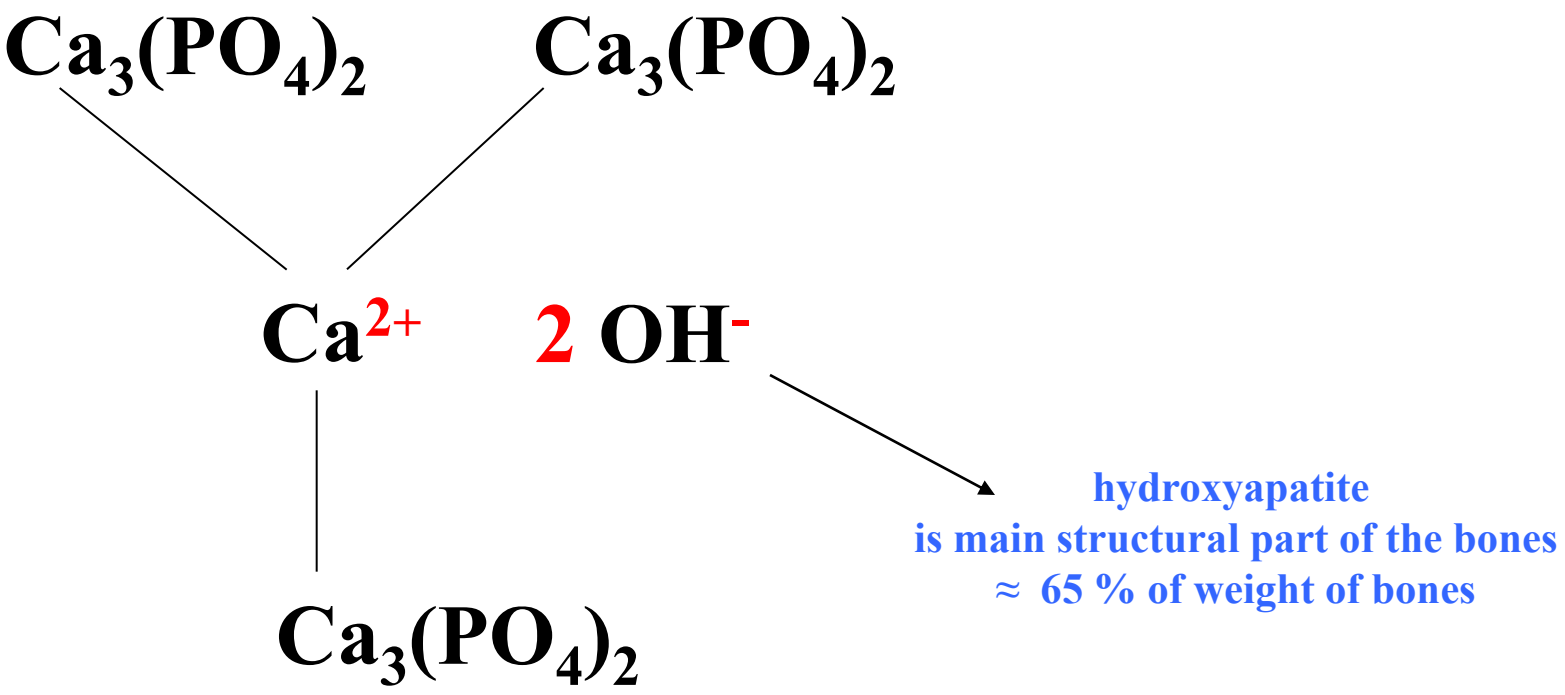
osteoblasts placed a new osteoid



return osteoformation
~ 160 days

newly deposited osteoid is mineralizing for several month

Hydroxyapatite :

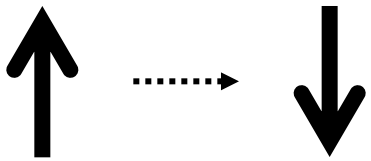


$3\ Ca_3(PO_4)_2 \bullet Ca(OH)_2$
hydroxyapatite

Solubility product (K_S) :

		K_S
calcium phosphate	**$Ca_3(PO_4)_2$**	$2 \cdot 10^{-30}$
hydroxyapatite	**$Ca_5(PO_4)_3OH$**	$2{,}3 \cdot 10^{-59}$
fluorapatite	**$Ca_5(PO_4)_3F$**	$3{,}1 \cdot 10^{-60}$

$$[Ca^{2+}]^3 \bullet [\ PO_4^{3-}\ ]^2 \ = \ K_S$$



Inorganic phosphate (P_i) in serum:

$[P_i] = 1$ mmol / l

$$\frac{[HPO_4^{2-}]}{[H_2PO_4^{-}]} = 4 : 1 \qquad (\text{pH} = 7.40)$$

$[PO_4^{3-}] = 0$!!

$$[Ca^{2+}]^3 \bullet [PO_4^{3-}]^2 = K_S$$

Inorganic phosphate (P_i) in bones:

$$HPO_4^{2-} + OH^- \rightarrow PO_4^{3-} + H_2O$$

$$H_2PO_4^{2-} + 2\ OH^- \rightarrow PO_4^{3-} + 2\ H_2O$$

the formation of insoluble bone mineral → alkaline reaction
(remember ALP !!)

↑ $[\ PO_4^{3-}\ ]$

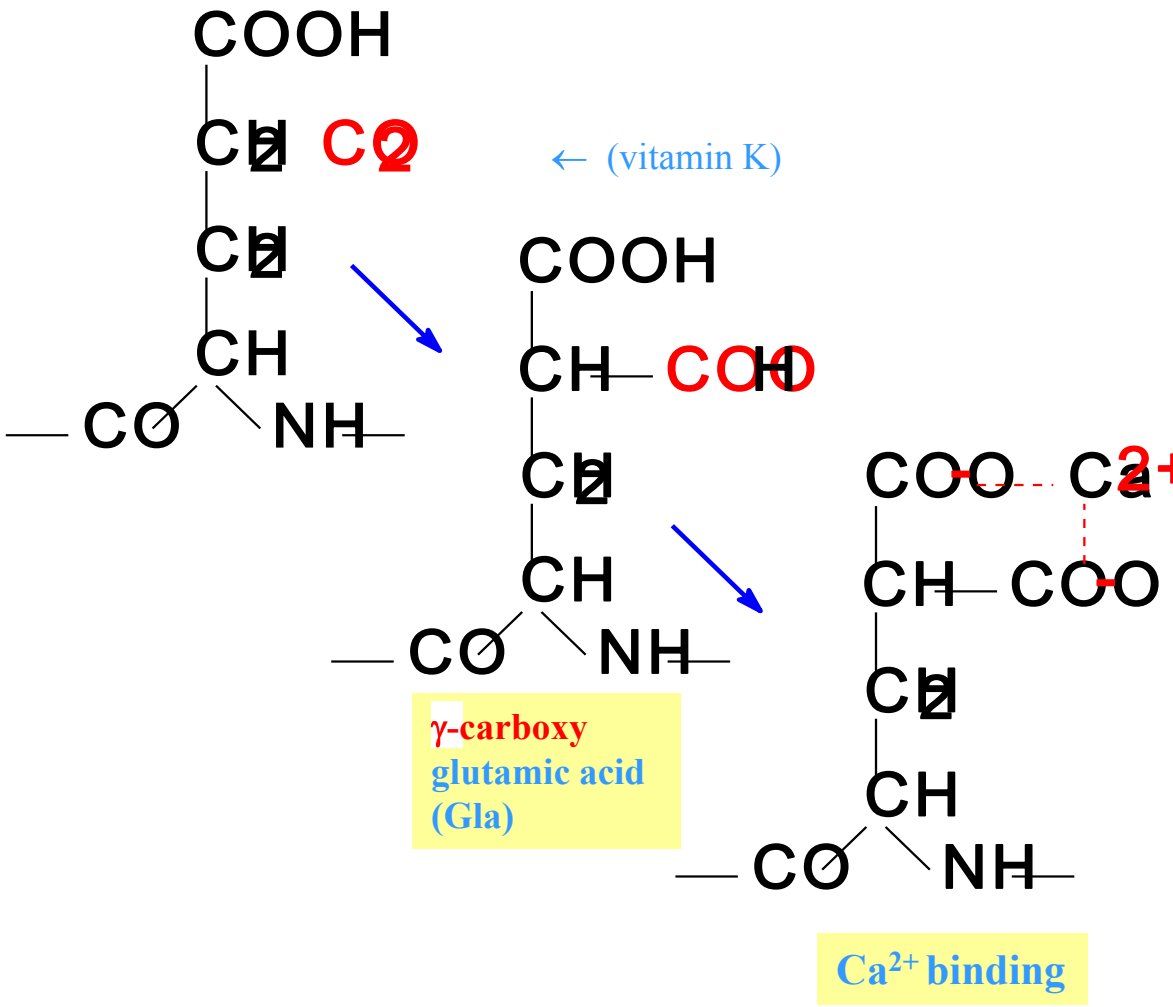
$$[Ca^{2+}]^3 \bullet [\ PO_4^{3-}\ ]^2 = K_S$$

in the opposite in bone resorption

Osteocalcin:

= BGP = bone gamma carboxyglutamic acid-containig protein

- contains 3 carboxyglutamates for calcium binding
- regulates bone mineralisation



Calcium homeostasis :

1. parathyrin (PTH, parathormone)
2. calcitonin (thyreocalcitonin)
3. calcitriol

1. parathyroid hormone (PTH)

- most important regulator of extracellular level of Ca^{2+}
- formed in parathyroid glands, effective is 34-N-terminal end of the prohormone
- secretion is tonic (cave hyperplasia!) and pulsatile
- pulsatile secretion depends on calcemia, is also regulated by vitamin D3

Sensor of calcemia :

- situated in parathyroid glands

receptor $\rightarrow$ G_q – protein $\rightarrow$ increase of calcemia

in plasma cause increased influx of Ca^{2+} into cells

$\rightarrow$ increased intracellular level of Ca^{2+} here has

inhibitive effect (by contrast to others cells!)

Parathyrin - effects:

defence against hypocalcemia

- bone:
  - ↑ **releasing of calcium** and phosphorus from bones by effecting osteoclasts (through osteoblasts!)
- kidney:
  - ↑ **reabsorption of calcium** from glomerular filtrate, ↓ **reabsorption of phosphates** (Ks!)
- ↑ synthesis of 1,25-vitamin D and this way increase an absorption of calcium from small intestine

Parathyrin – effects on bone:

- quick – in minutes
- slow – hours to days, continue even after decrease of PTH levels in plasma
- stimulates receptors of osteoblasts, they activate **osteoclasts** sequentially
- **osteoblasts** themselves are subdued at first, after several days PTH support their growth and osteoid formation
- PTH affects also **osteocytes** (mobilisation of calcium via osteocytic osteolysis)
- long-term permanent stimulation by PTH cause increased amount and activity of osteoclasts, low dosages of PTH intermittently applied increase bone formation!! (changed cellular signalling)

2. calcitonin

- (thyreocalcitonin, 32 AA, C-cells of thyroid gland)
- antagonist of PTH, effect is stimulated by estrogens
- narrow significance for regulation – protection against sudden increase of calcemia (under physiological condition has minimal effect)
- secretion is regulated by calcemia (sensor similar to parathyroid glands)
- subdue bone resorption by inhibition of osteoclasts, support formation of bone matrix (therapy of osteoporosis)
- inhibits resorption of calcium and phosphates in kidneys → increase calciuria and phosphaturia
- analgesic effects on bone pain

3. calcitriol

7-dehydrocholesterol (liver)

↓

calciol (skin, UV)

↓

25 - calcidiol (liver, 25-hydroxylase)

↓

1,25 - calcitriol (kidney, 1-hydroxylase)

calcidiol is main metabolite of vitamin D in plasma (< 10 µmol/l, seasonal differences, $t_{1/2} \approx$ 20-30 d, bond to vitamin D-binding protein)

↗

inhibition: ↑ calcitriol and calcitonin
abundance of ingested calcium

stimulation: PTH during hypocalcemia
somatotropin, prolactin

Calcitriol – effects in calcium metabolism:

- enterocytes
  - increase absorption, transport through enterocytes and releasing to plasma
  - increase also absorption of phosphates
- kidneys
  - increase resorption of calcium in renal tubules

Calcitriol – effects in calcium metabolism II

- bones
  - ➢ complex effects, maintain balance between formation and resorption of bones
  - ➢ during hypocalcemia increases resorption of bones by coordinated activity of osteoblasts and osteoclasts
  - ➢ under favourable conditions increases incorporation of calcium into bones
- interaction with PTH
  - – calcitriol inhibits the synthesis and secretion of PTH – it serves as negative feedback on calcitriol synthesis (PTH stimulates the synthesis of calcitriol)

Calcitriol – other effects:

- **receptors** are situated **in many tissues** (heart, vessels, stomach, liver, brain, ...)
- regulates cellular differentiation and proliferation
- inhibits cellular growth
- stimulate the secretion of insulin
- inhibits the production of renin
- cells of immune system have a receptor for vitamin D, some of them even produce calcitriol

  → vitamin D has immunomodulatory effect!

calcitriol – other effects II:

- deficit of calcitriol increase a risk of many diseases:
  - ***autoimmune diseases*** (DM type I, sclerosis multiplex, rheumatiod arthritis)
  - ***tumours*** (colorectal, prostatic and breast cancer)
  - ***cardiovascular diseases***
  - ***DM type II***
  - ***psychiatric diseases*** (schizophrenia, depression)

- in Europe have lack of vitamin D 30% of population, among older people it is even 75%

Additional regulators of bone metabolism

1. estrogens
2. growth hormone/somatotropin
3. thyroid hormones
4. glucotropic hormones cortisol and insulin
5. local factors (system RANK/OPG, Wnt/sclerostin)

1. estrogens

- complex effect
- decrease the effect of PTH and thyroid hormones
- inhibit the releasing of cytokines from osteoblasts (and so decrease the activity of osteoclasts)
- the effect on regulation of calcitonin and calcitriol is assumed
- deficit of estrogens increase the production of TNF alfa, IL-1 a IL-6 which have pro-resorptive effect

2. growth hormone

- it stimulates 1α- hydroxylase (vitamin D)
- increase bone turnover with predominance of osteoformation
- influences also absorption of calcium
- stimulates proliferation of osteoblasts

3. thyroid hormones

- important for bone development during fetal life, for bone remodelling in childhood and for remodelling cycles in adulthood (hyperthyreosis accelerates them, hypothyreosis decelerates)
- necessary for formation and maturation of bone cells
- they potentiate one another with growth hormone
- stimulate production of IGF-1 (growth factor)

4a. insulin

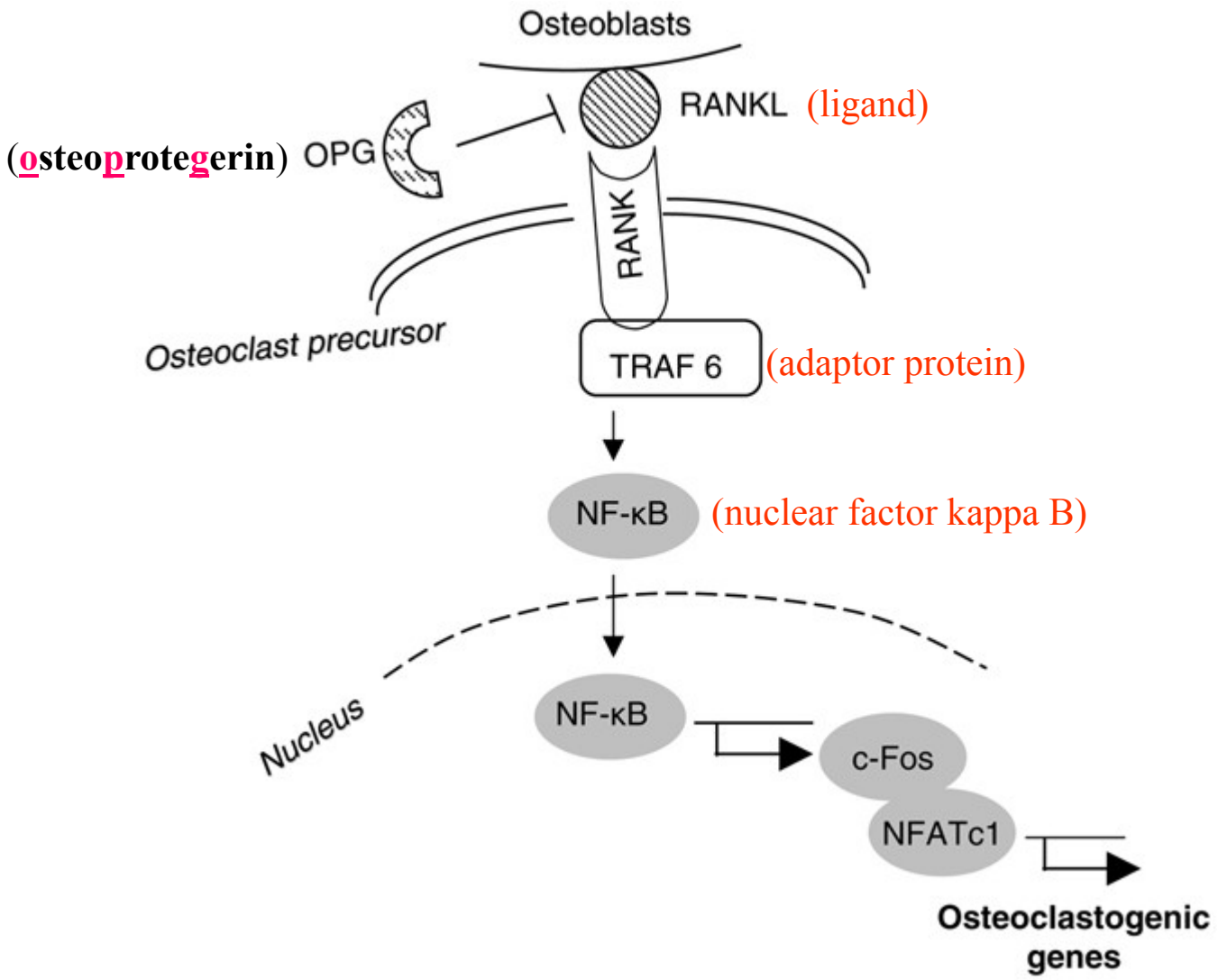
- anabolic hormone
- supports osteoblastogenesis
- inhibits the activity of osteoclasts
- influences biomechanical qualities of bones
- has synergic effect with other hormones
- diabetics (type I) are in higher risk of osteoporosis

4b. cortisol

- decreases the absorption of calcium from small intestine
- decreases the formation of collagen in bones
- influences formation and functions of osteoblasts in a negative way
- limiting dose for osteoporosis development is 7.5 mg of prednison/d, osteoporosis can develop after several months of drug administration

5a. system RANK/OPG

(receptor activator NF-κB)



5b. sclerostin and Wnt

- activation of Wnt signal pathway leads to increased proliferation and diferentiation of osteoblasts
- main inhibitor of this pathway is sclerostin – glycoprotein produced by osteocytes
- sclerostin defend Wnt from binding on its receptor and blocks bone formation

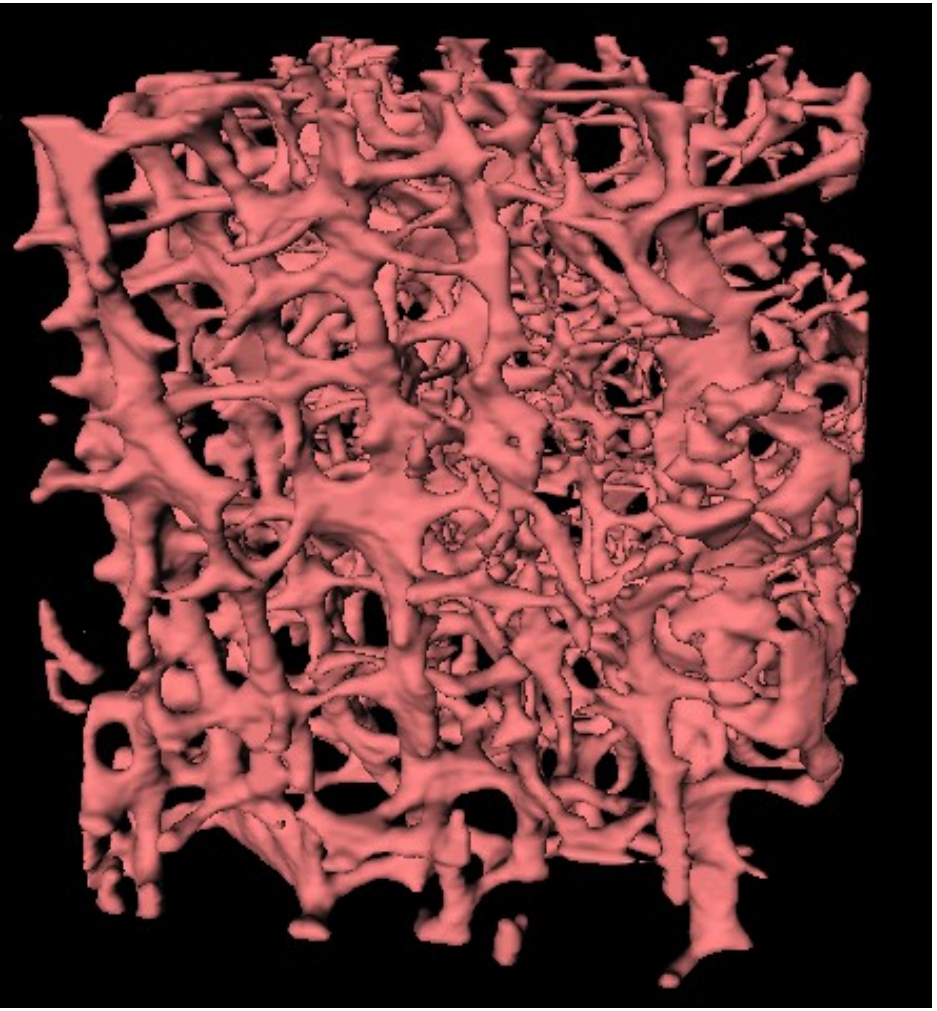
OSTEOPOROSIS

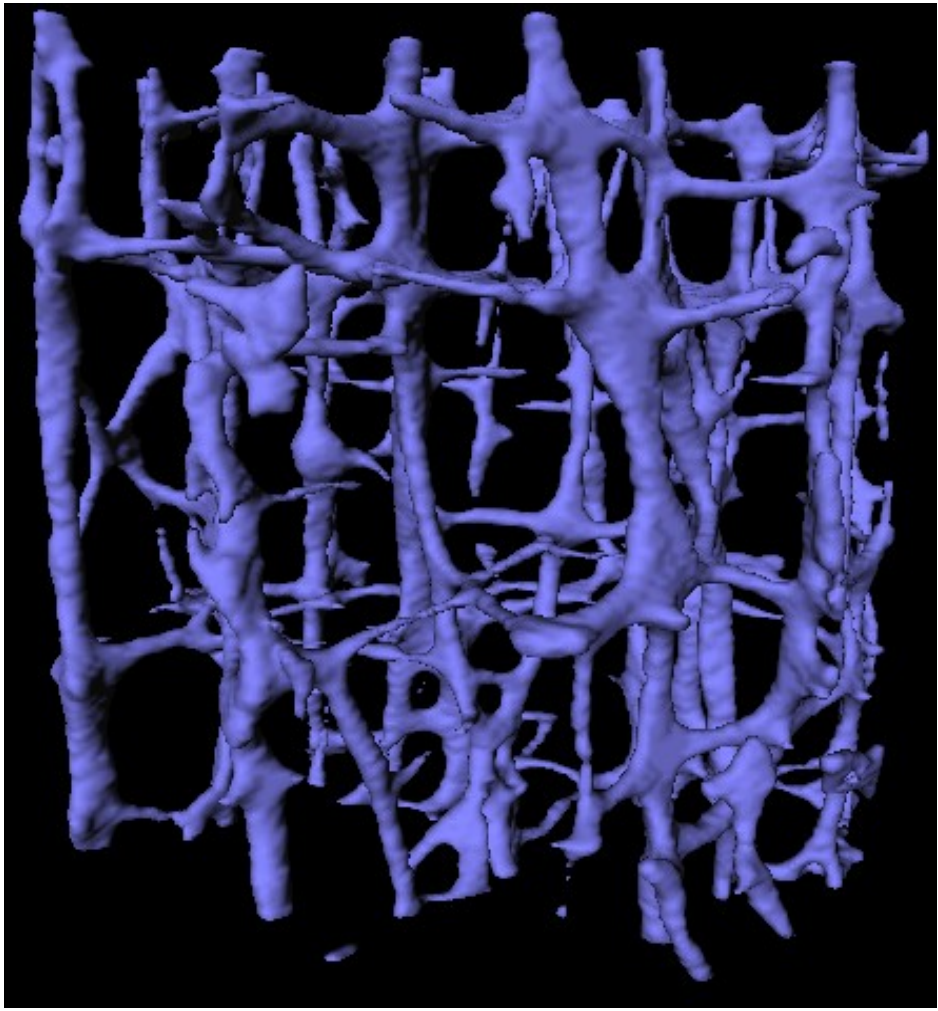
Osteoporosis :

- systemic skeleton disease
- decrease in bone density
- disruption of microarchitecture of bone tissue
- increase in bone fragility
- higher risk of fractures

decrement of bone tissue is **proportional**!! (= decrement of minerals and proteins equally)

(in contrast to osteomalacia = defect in bone mineralisation, but organic matrix is untouched)





in Czech republic suffer from osteoporosis every 3rd woman and every 5th man

Common places of osteoporotic fractures:

- spine*
- hip*
- distal radius*
- proximal humerus

*places of BMD measurement

Risk factors of osteoporosis:

- female gender
- advancing age
- Caucasian race
- family history (especially in men)

noninfluencable factors

- low BMI
- smoking and alcohol consuming
- inadequate nutrition
- previous fractures
- immobilisation
- use of glucocorticoids and other medicaments
- endokrinopathies

influencable factors

picture of typical patient in high risk of osteoporosis

Classification of osteoporosis:

1/ primary

- juvenile
- in adults → postmenopausal
  → senile (involutional)

2/ secondary

Secondary osteoporosis:

- endocrinopathies (hyperparathyreosis, m. Cushing, thyreotoxicosis)
- systemic inflammatory diseases (rheumatoid arthritis)
- nutrition disorders, asthenic habitus (BMI under 19)
- renal osteodystrophy (→ secondary hyperparathyreosis)
- inactivity
- tumours (breast, ovarian, prostate, testicular, thyroid cancer)
- drugs (corticosteroids, antiepileptics, heparin, loop diuretics, SSRI, inhibitiors of aromatase)

Diagnostics of osteoporosis

1. anamnesis and clinical investigation
2. bone mineral density (BMD) measurement
3. laboratory tests

BMD measurement

- BMD is important and quantifiable risk factor of osteoporosis
- BMD is expressed in:
  - absolute values (g of mineral per cm^2)
  - standard deviation (SD)

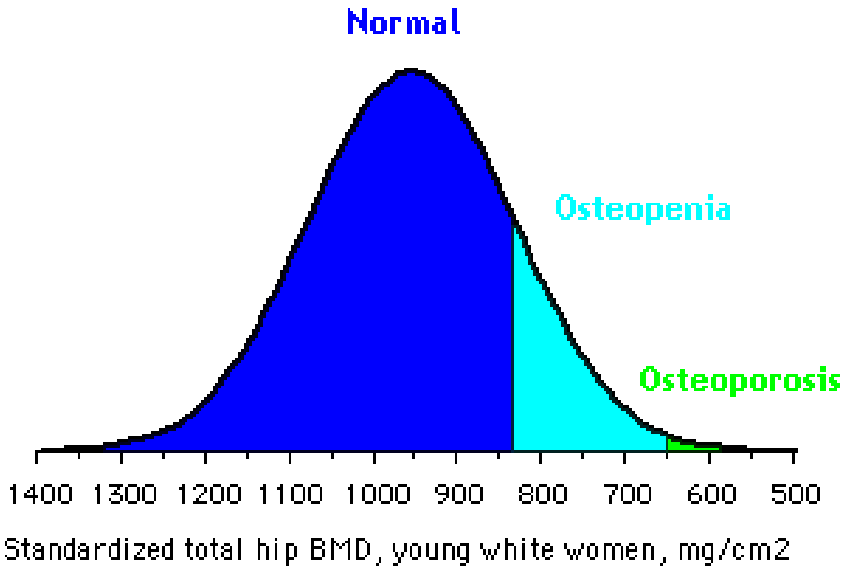
    → T-score and Z-score – they express how is the value of BMD different from mean

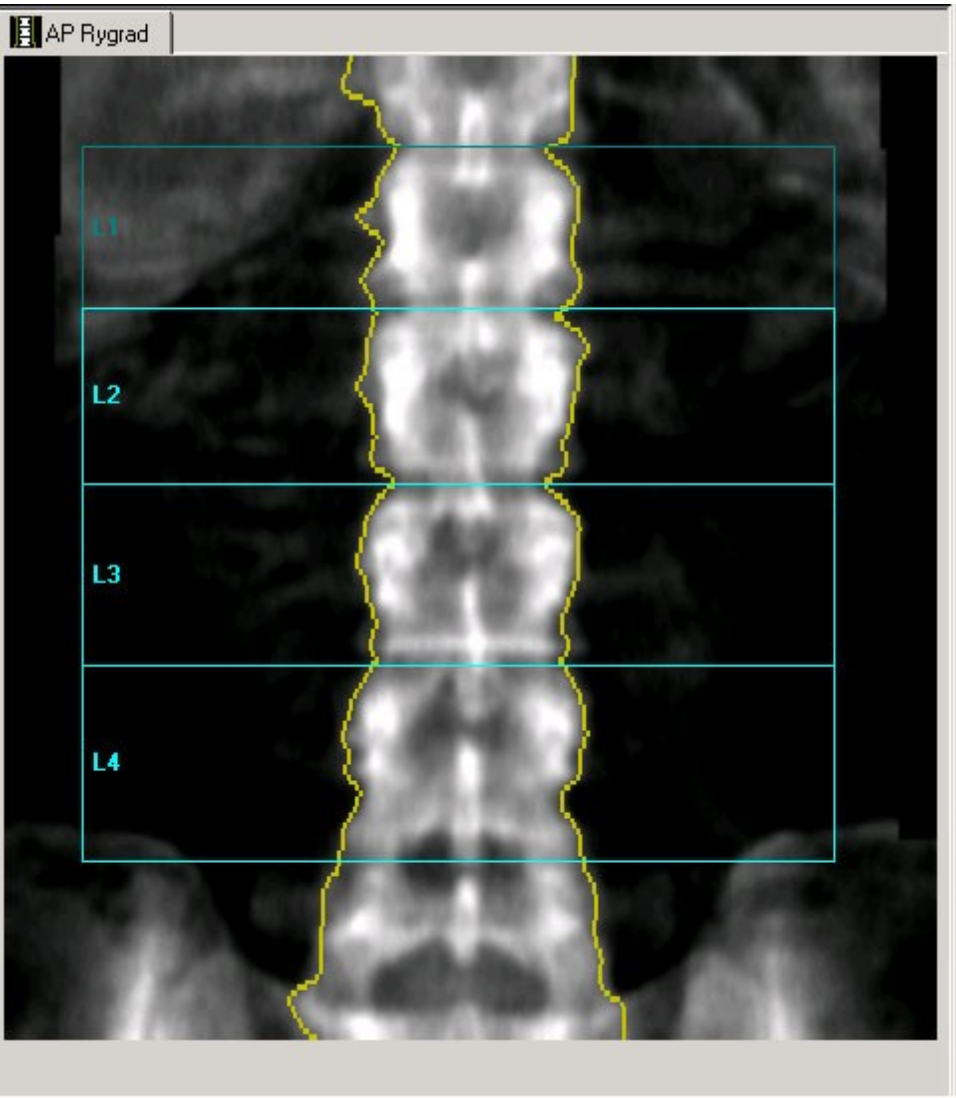
T-score vs. Z-score

- T-score is comparison of patient‘s BMD to mean BMD of healthy human between the ages of twenty and thirty, of the same gender and race
  - used more often, correlates with risk of fracture
- Z-score is comparison of patient‘s BMD to mean BMD of healthy human of the same age group, gender and race
  - shows future development of BMD in patients
  - normal distribution in statistics = Z distribution

Diagnosis of osteoporosis (WHO) :

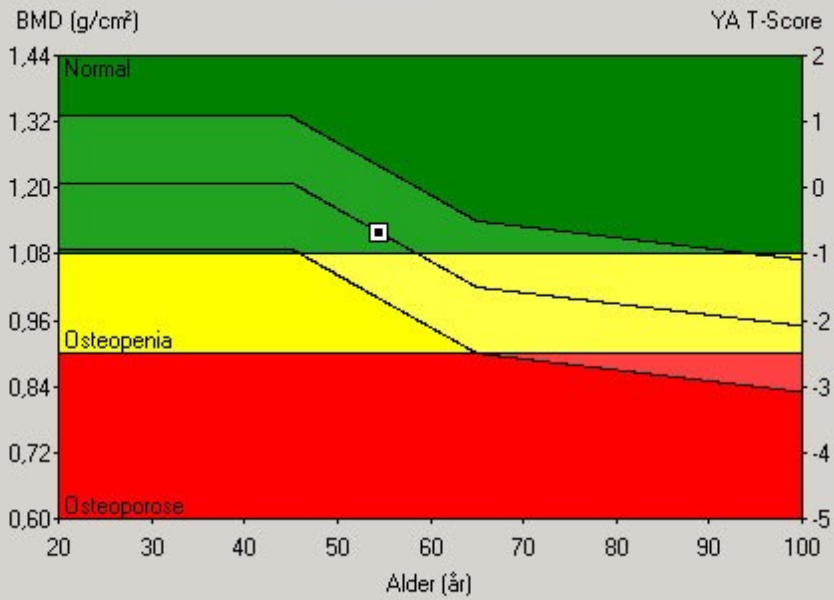
BMD (T-score, SD)	diagnosis
-1 and more	normal
-1 to -2.5	osteopenia
-2.5 and less	osteoporosis
-2.5 and less + fx	severe osteoporosis





Densitometri | Reference | Trend | Information

Reference:L2-L4

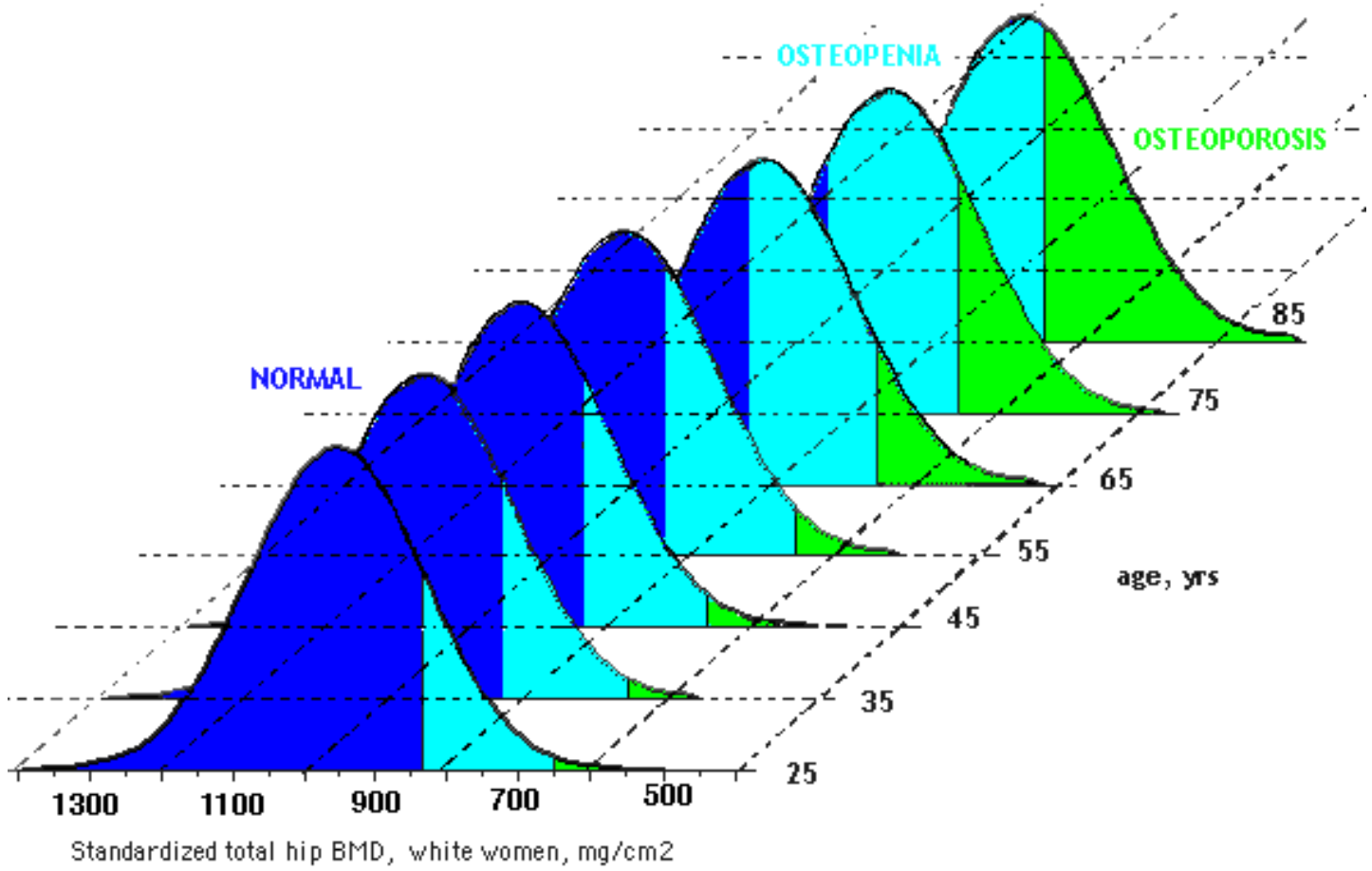


Reference

Region	BMD (g/cm²)	YA T-Score	AM Z-Score
L1	1,153	0,2	0,9
L2	1,160	-0,3	0,3
L3	1,136	-0,5	0,1
L4	1,065	-1,1	-0,5
L1-L2	1,157	-0,1	0,6
L1-L3	1,149	-0,2	0,5
L1-L4	1,124	-0,5	0,2
L2-L3	1,148	-0,4	0,2
L2-L4	1,116	-0,7	0,0
L3-L4	1,097	-0,9	-0,2

https://www.sundhed.dk/borger/sygdomme-a-aa/hormoner-og-stofskifte/illustrationer/billeddiagnostik/rygsoejle-dxa-skanning-normalbillede/

BMD, age and osteoporosis:



Laboratory tests

1. basic tests
2. biochemical markers of bone turnover
3. tests within the scope of different diagnosis of secondary osteoporosis and other metabolic diseases of skeleton (indications depend on anamnesis)

Basic tests

- calcium and phosphates in plasma
- creatinin (renal function)
- ALP
- calciuria (for 24 hours)
- vitamin D (total, izoforms)

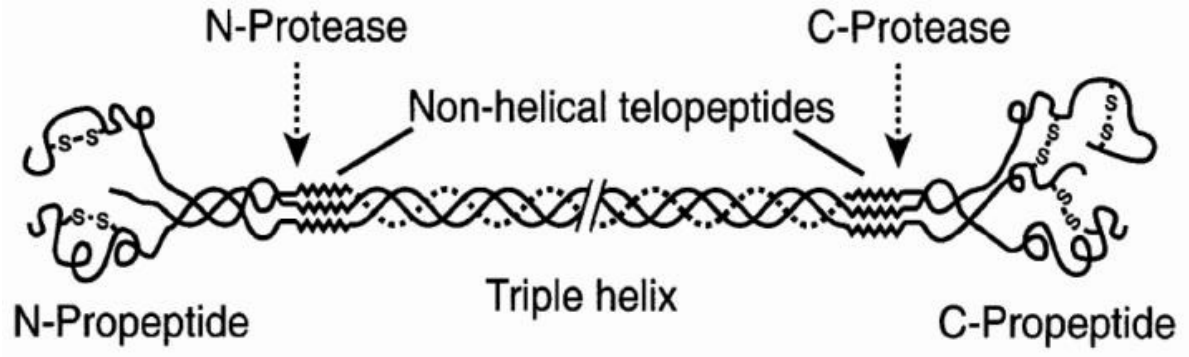
Assessment of bone turnover

- markers of resorption
  - pyridinoline (PYR) and deoxypyridinoline (DPD) in urine
  - hydroxyproline a hydroxylysine in urine
  - tartrate-resistant acid phosphatase 5b (TRAP5b)

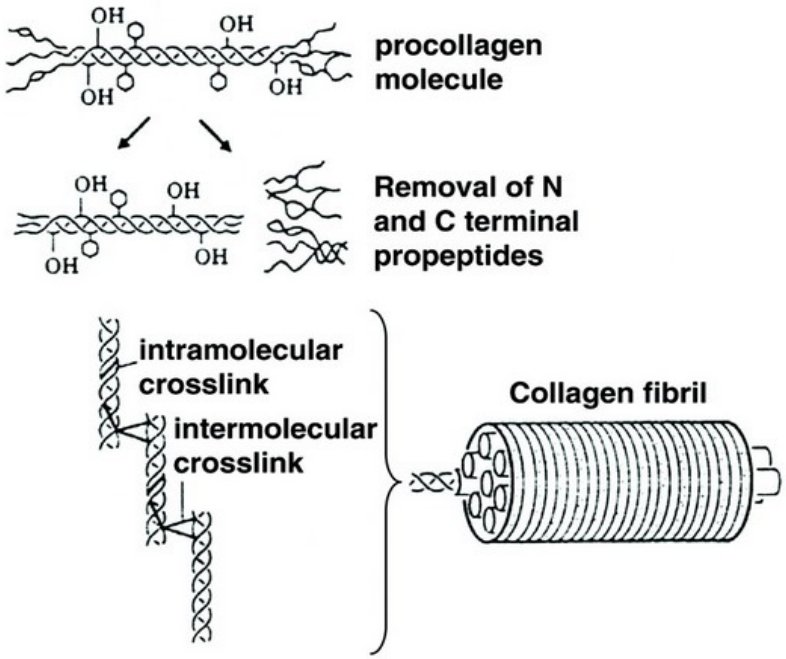
  less frequent
  - C-terminal telopeptide of type I collagen (CTx or ICTP) in serum
  - N-terminal telopeptide of type I collagen (NTx or INTP) in serum
  - (sclerostin)
- markers of formation
  - bone isoenzyme of alkaline phosphatase (bALP) in serum
  - osteocalcin in serum

    released from osteoblasts
  - procollagen type I N-terminal propeptide (P1NP) in serum
  - procollagen type I C-terminal propeptide (P1CP) in serum

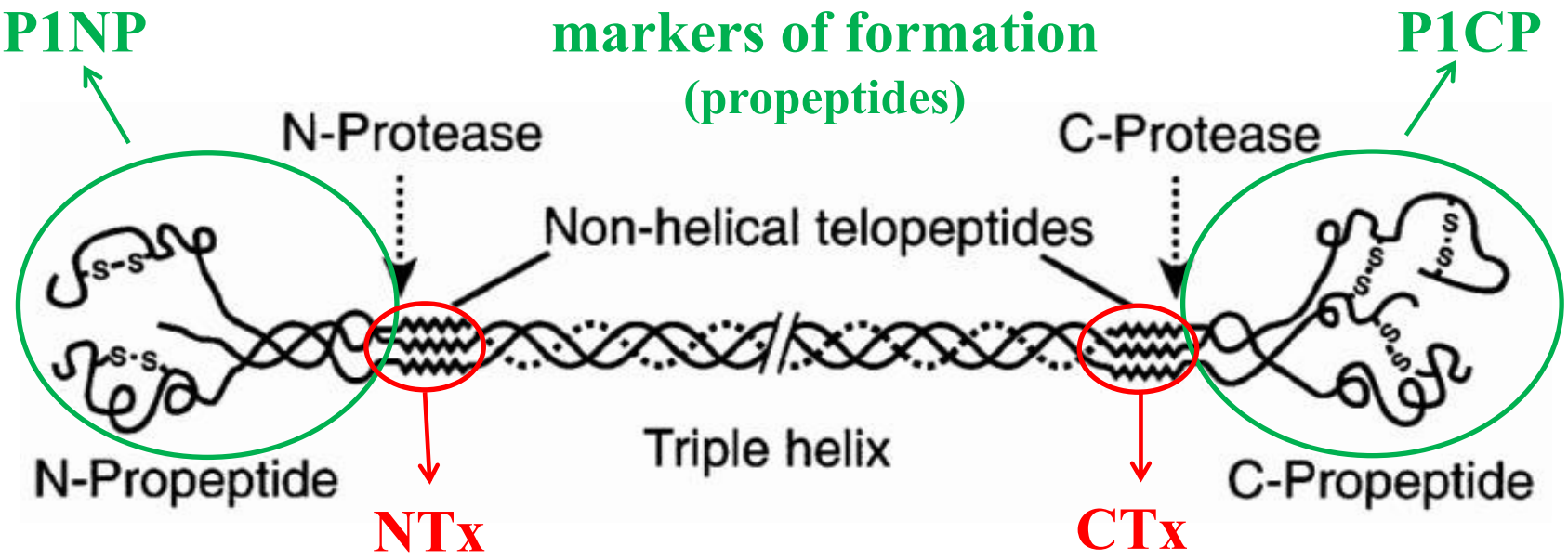
Procollagen type 1 - structure



Collagen 1 – cross links



Markers of bone formation and resorption:



markers of resorption
(cross-linking telopeptides)

Clinical evidence of markers:

- **evaluation of bone remodelling severity**
- speed of bone density decrease („fast vs. slow bone losers“)
- prediction of fracture risk independently from BMD value
- **monitoring of treatment** (they react quickly by contrast to BMD)
- NOT for differential diagnosis (most metabolic diseases of skeleton cause quantitative, not qualitative changes of bone remodelling)

Treatment of osteoporosis

1. nutrition, lifestyle, exercise
2. calcium + vitamin D
3. bisphosphonates
4. *strontium ranelate
5. *HRT – hormone replacement therapy
6. SERM – selective estrogen receptor modulator
7. *calcitonin
8. teriparatide and PTH
9. denosumab – monoclonal antibody against RANKL
10. romosozumab - monoclonal antibody against sclerostin

*less frequently used for treatment

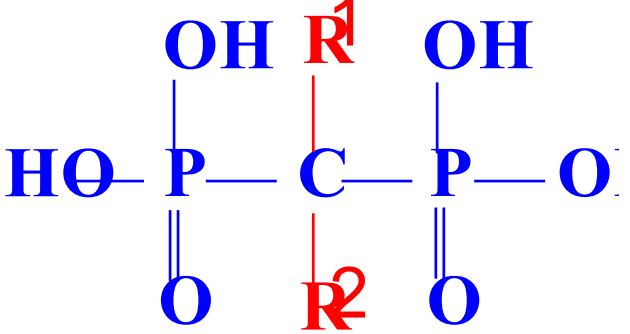
1. Nutrition, lifestyle, exercise

- important for every patient – minimisation of fracture risk
- **varied diet** with enought calcium and vitamins
- low phosphates and sodium intake (sodium increase renal elimination of calcium!)
- appropriate BMI (both extremes are negative)
- **low consumption of alcohol, stop smoking**
- **EXERCISE!!!** (walking, hiking, cycling, swimming, pilates, yoga)
- falls prevention

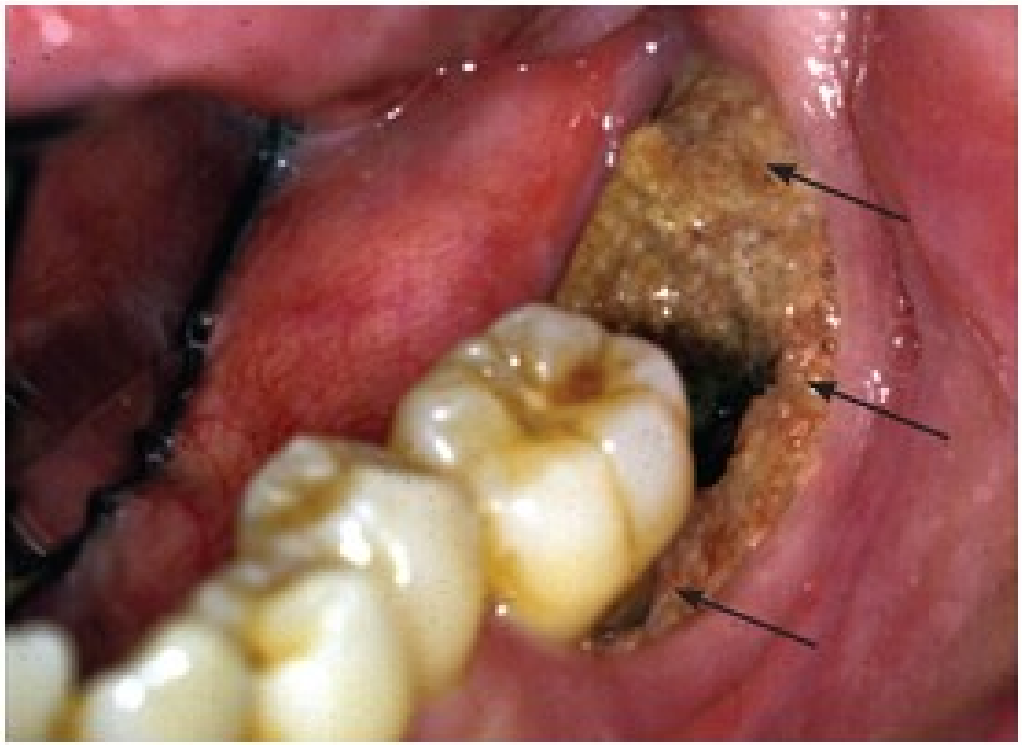
2. Calcium + vitamin D

- **automatically administered**
- calcium dose is 800 – 1200mg as calcium carbonate, citrate or lactate
- vitamin D dose is 800 – 1000 IU as calciol, exist also formulations with active form of vitamin D (1,25-dihydroxyvitamin D3)

3. Bisphosphonates



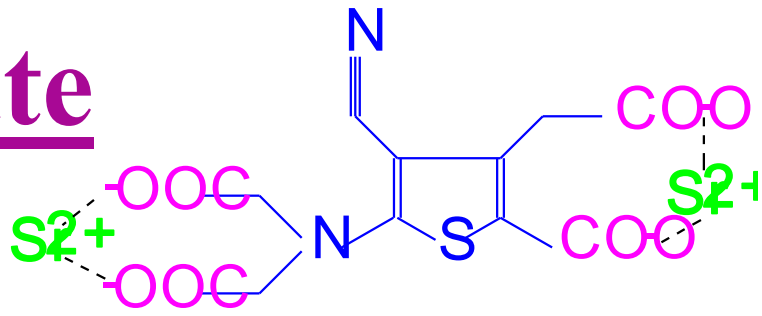
- most often treatment used not only of osteoporosis, but also in oncology and other branches
- mechanism of action – they bind on bone surface and **interfere with osteoclasts‘ enzymatic activity**, disarray cytoskeletal structure and increase their **apoptosis**
- effect continues months to years after treatment termination
- side effects – mostly gastrointestinal discomfort, osteomyelitis and necrosis of jaw bone



Bisphosphonate-related osteonecrosis of the jaw at extraction site of tooth. Necrotic, nonhealing exposed bone extends up the ramus and to the buccal aspect of tooth.

http://www.aafp.org/afp/2012/0615/p1134.html

4. Strontium ranelate



- dual effect – **it stimulates formation of bone and protects against decrease of BMD**
- improve mechanical characteristics of bone
- side effects – contraindication is the anamnesis of venous thrombosis and the presence of risk factors of thrombosis or cardiovascular diseases, because higher incidence of heart attack was proved
- dual effect was disputed lately
- approved only as a last possibility when other treatment is impossible due some reasons

5. Hormone replacement therapy

- artificial estrogens which balance hormone levels after menopause
- due to higher risk of breast cancer and cardiovascular diseases (thrombosis, heart attack, stroke) is the only indication for their use climacteric syndrome (**premature or surgically induced menopause**)

- phytoestrogens – plant derived compounds included in food supplements, effect on osteoporosis was not proved, but they can improve menopausal symptoms (hot flashes, night sweats)

6. Selective estrogen receptor modulators

- effect is different in different receptors:
  - estrogen agonists in bone and cardiovascular system (improve lipid profile in blood)
  - estrogen antagonists in breast and uterus

- appropriate mostly for younger women with higher risk of spinal fractures and breast cancer
- from this group only ramoxifen approved specifically for osteroporosis treatment

7. Calcitonin

- defend from bone resorption by direct effect on osteoclasts
- salmon calcitonin is used
- presently is not very used for treatment of osteoporosis
- is used for short-term treatment of Paget disease and hypercalcemia because of bone metastases (here is the advantage its analgesic effect)

8. Teriparatide and PTH

- teriparatide – terminal sequence of PTH with the highest biological effects
- core of its effect is intermittent administration of small doses, which has osteoanabolic effects on trabecular and cortical bone
- it changes the regulation of gene expression and system RANK/OPG
- highly effective but expensive ☹ (very strict indication criteria)
- subcutaneous administration can discourage patients from usage

9. Denosumab

- specific monoclonal antibody against RANKL (act as osteoprotegerin)
- effective and safe form of treatment without severe contraindications
- administration is once per six months ☺
- rare side effect is necrosis of jaw bone (similar to bisphosphonates)

10. Romosozumab

- specific monoclonal antibody against sclerostin
- it is simply inhibitor of inhibitor → inhibits the bond of sclerostin and supports osteoformation via Wnt signalling pathway
- increase BMD more then bisphosphonates and PTH
- clinical studies are still in progress
- subcutaneous administration

Thank you for your attention.