Metabolic bone diseases

Osteoporosis

Clinical Biochemistry

Dept. Biochemistry 2008 (J.S.)

The bone

The bone is a tissue exhibiting very high metabolic activity, though it doesn't seem so at first glance.

The **unremitting bone remodelation**, both osteoresorption and bone formation, continues after the growth of bones is finished.

The bulkiness and consistency of bones depends on the balance between resorption and formation.

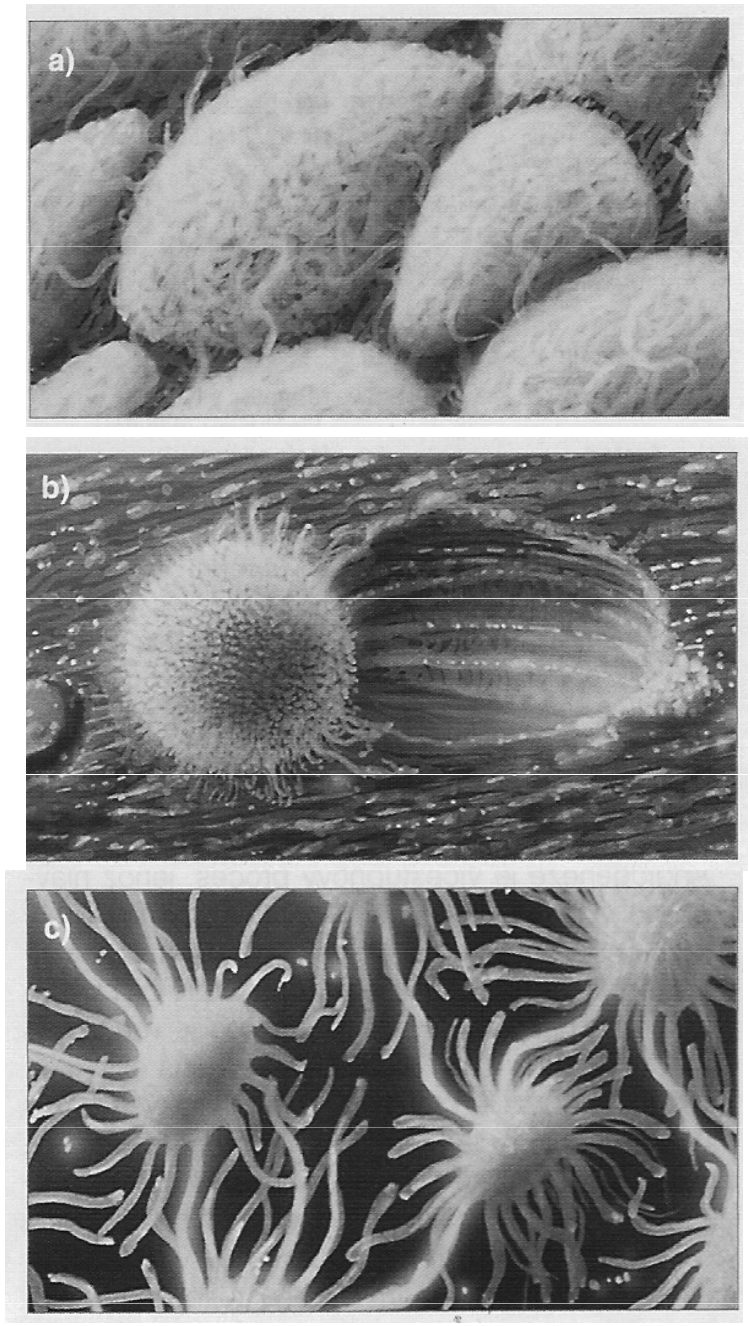
Bone cells: **osteoclasts** (modified macrophages, bone resorption), **osteoblasts** (a type of fibroblasts, bone formation), and **osteocytes** (transformed aged osteoblasts) affect the activity of previous cell types by **paracrine secretion** of interleukins, tumour necrosis factor (TNF), osteoprotegerin, prostaglandins and other various growth factors.

Bone cells

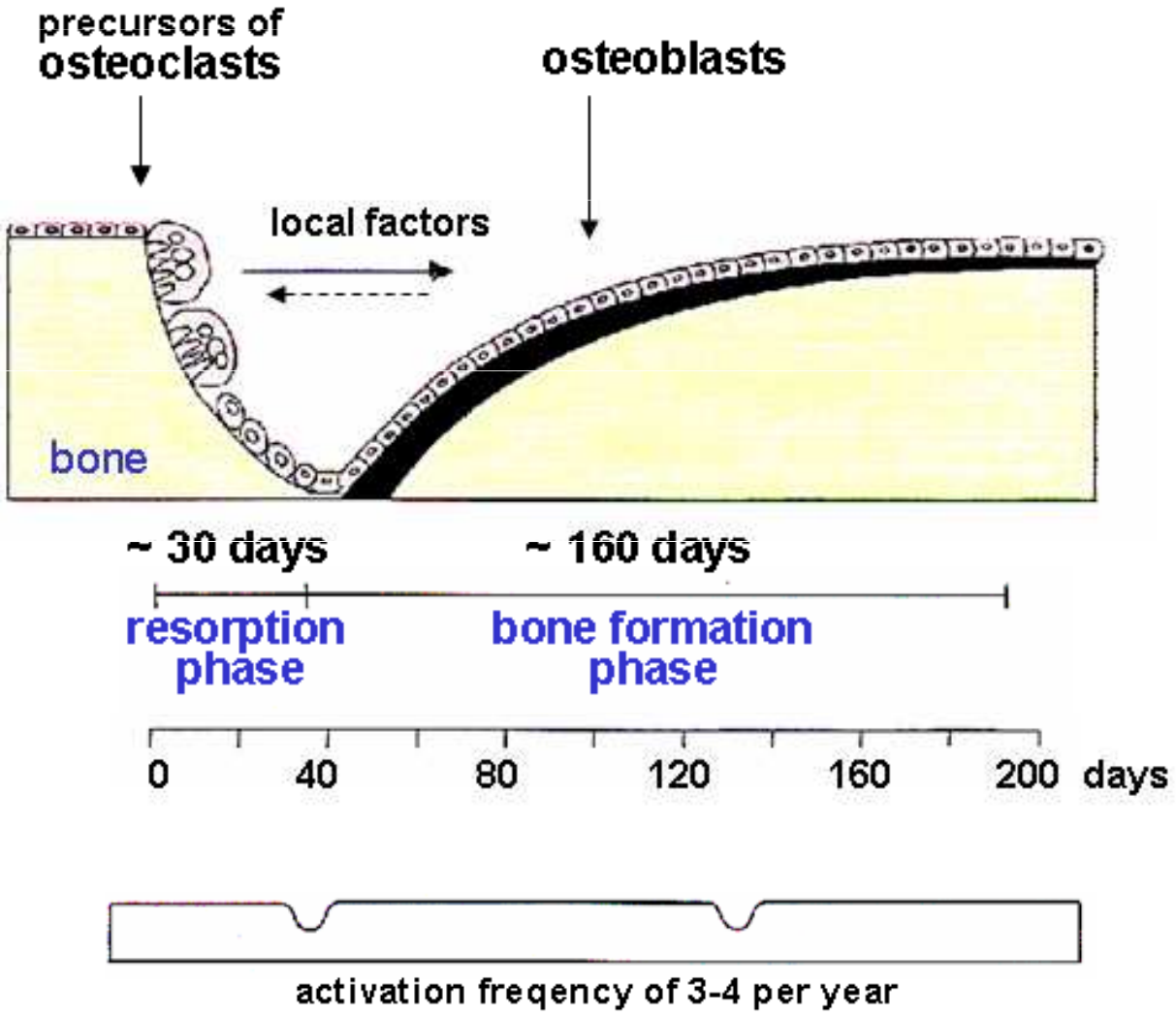
Osteoblasts occur on the surfaces of growing or remodelated bones, less frequently inside adult bones. They synthesize and insert osteoid (extracellular matrix), deposit the bone minerals, and consecutively settle and transform into osteocytes. The surface of osteoblasts binds molecules of alkaline phosphatase that support mineralization of the matrix.

Osteoclasts occur at sites of active bone resorption, in resorption pits. They include many lysosomes exhibit high activity of acid phosphatase. Osteoclasts liquidate fragments of collagen and other organic components through phagocytosis.

Osteocytes are prevailing cellular elements of mature bone. They are dispersed in lacunas and form a cellular net by means of contacts between their projections. Life span of osteocytes is estimated to about 25 years, extinct cells initiate bone resorption.



Control of bone remodelation



Bone resorption

stimulated by	parathyrin calcitriol
inhibited by	calcitonin estrogens

Bone formation

stimulated by	(parathyrin) calcitriol
inhibited by	androgens estrogens glucocorticoids

Bone remodelling

takes place only on the surface of bone and in closely coordinated local packets. The cells involved in a particular remodelling event are referred to as a basic multicellular unit or **bone metabolic unit.**

In a typical remodelling cycle, resorption takes ~7-10 days, whereas formation requires 2-3 months. Overall, ~10% of bone is replaced each year. However, **remodelling occurs exclusively on bone surfaces**.

Trabecular bone makes up only ~20% of the skeletal mass, but 80% of the surface is cancellous bone. Because of this, cancellous bone is more metabolically active and more rapidly remodelled than cortical bone.

Approximately 25% of cancellous bone is renewed each year, compared with only ~3% of cortical bone.

Composition of bones

Water ~ **25 %** in compact bone (12 % cementum, 10 % dentin, 1 % tooth enamel)

Organic components **30 %**

Mineral components **40 %**

In dry bone tissue organic components 40 – 45 %
mineral components 60 – 55 %

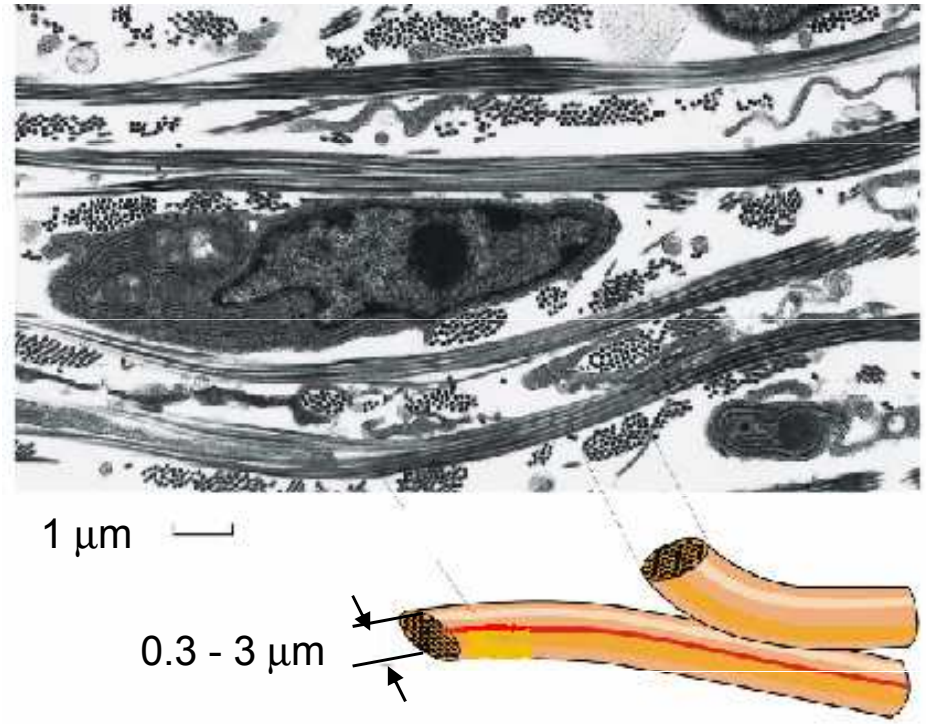
Extracellular matrix:

organic components - **collagen type I**
osteocalcin
sialoprotein, proteoglycans, citrate,

mineral components – **calcium phosphates** on the whole **85 %**
(hydroxylapatite $3Ca_3(PO_4)_2.Ca(OH)_2$
octacalcium phosphate $Ca_8(HPO_4)_8.5H_2O$
amorphous calcium phosphate $Ca_3(PO_4)_2$)

$CaCO_3$	10 %
CaF_2	0.3 %
$CaCl_2$	0.2 %
$Mg_3(PO_4)_2$	1 %
alkaline salts	2 %

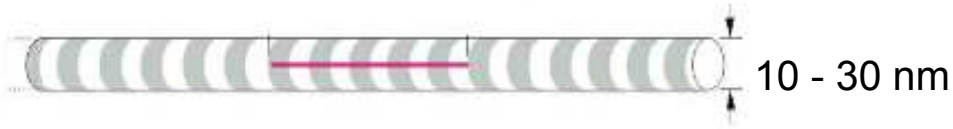
Collagen fibrils



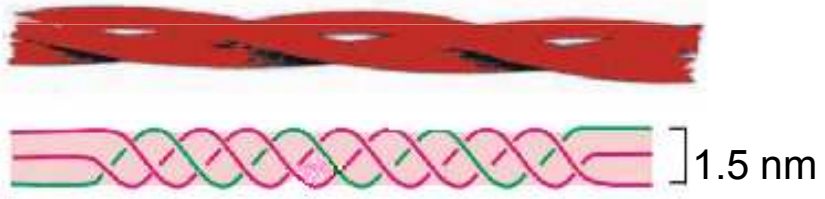
electron optical picture
of soft connective tissue

microfibrils – either a felty tangle or arranged bundles of protofibrils interacting with proteoglycans

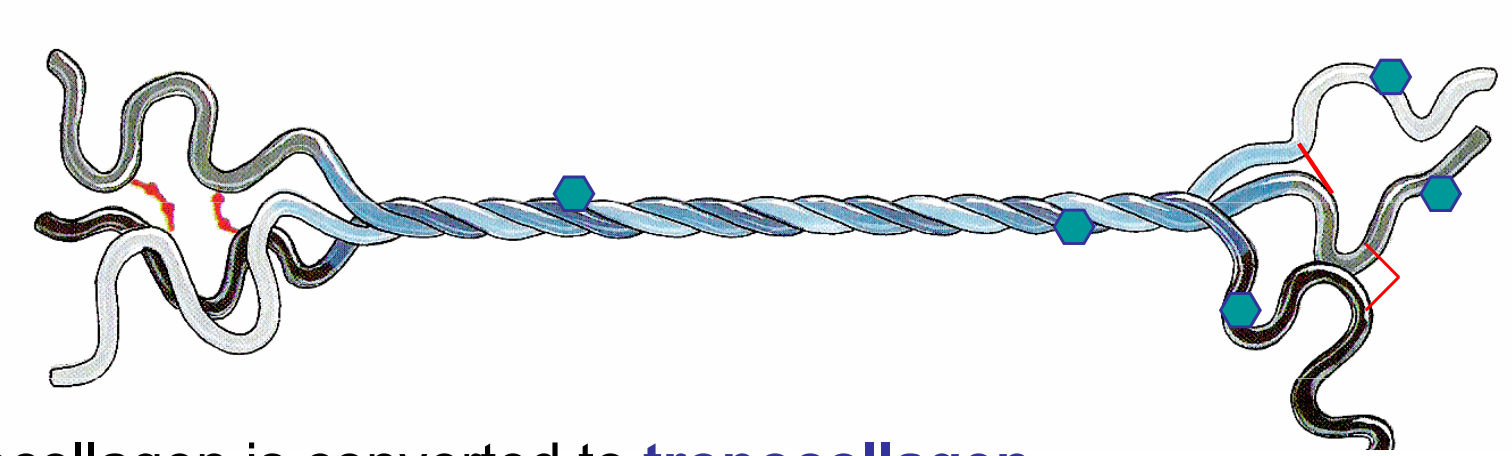
protofibrils are formed through aggregation of tropocollagen units



tropocollagen units 300 nm x 1.5 nm
(triple helix, in type I collagen
two chains α_1I and one chain α_2I)



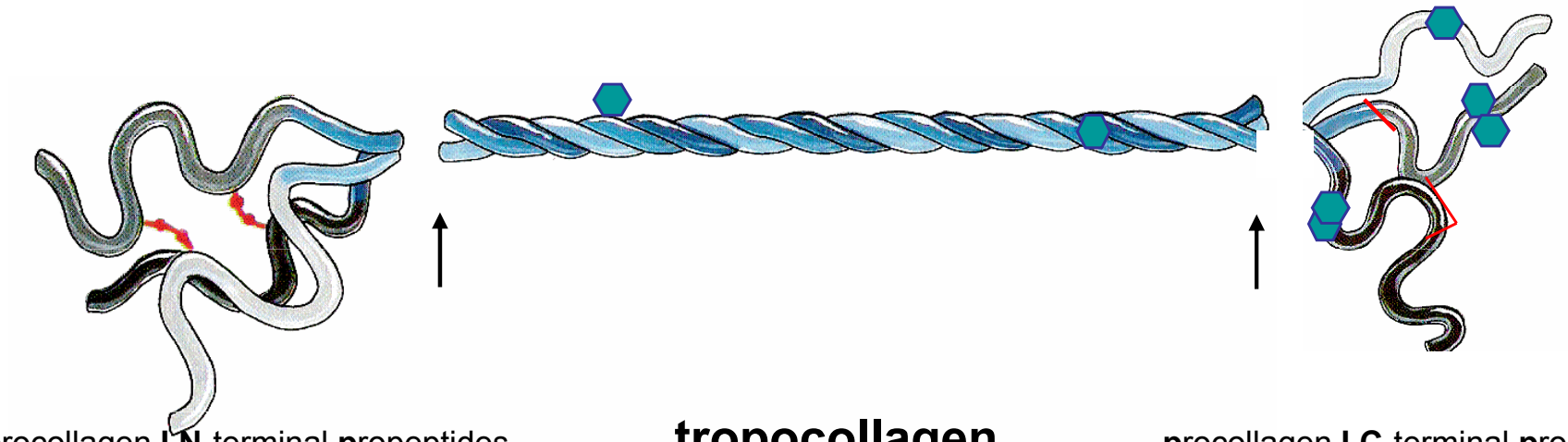
Procollagen - the middle parts wind in the triple helix



Procollagen is converted to **tropocollagen**

Specific procollagen peptidases catalyze the removal of globular (and stabilized by disulfide bonds) **N-terminal and C-terminal propeptides** by hydrolysis within the non-helical segments.

The abbreviations PINP and PICP are used for those propeptides.



procollagen **I** **N**-terminal **p**ropeptides

tropocollagen

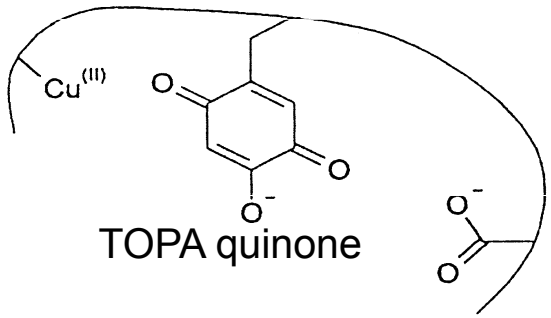
procollagen **I** **C**-terminal **p**ropeptides

Stabilization (maturing, ripening) of collagen – formation of covalent crosslinks

In crosslink formation, the decisive role belongs to the side chains of **lysine** or non-glycosylated **hydroxylysine** residues.

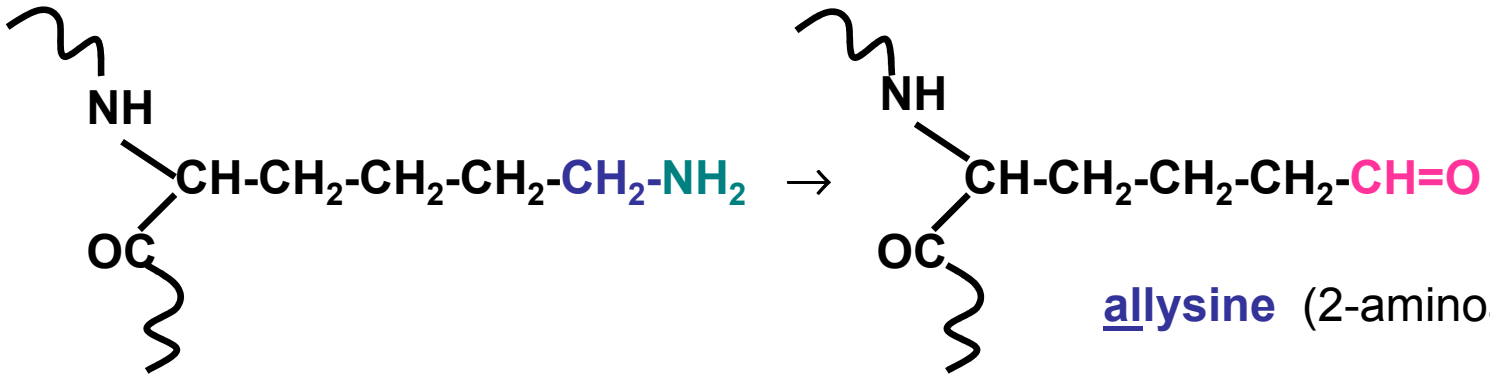
The initial reaction is the **oxidative deamination** of lysine or hydroxylysine side chains in the non-helical ends of fibril-forming tropocollagens, catalyzed by a specific extracellular aminooxidase **lysyl oxidase.**

Lysyl oxidase is copper-containing enzyme, coenzyme TOPA quinone:



TOPA quinone

Residues of amino acid called **allysine** with the reactive **aldehyde group** are the products of this reaction.



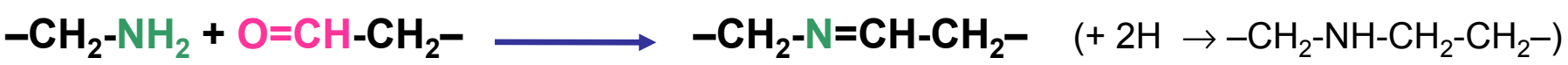
allysine (2-aminoadipaldehydate)

Interchain covalent crosslinks

originate in non-catalyzed reactions between the side chains of allysine and lysine residues.

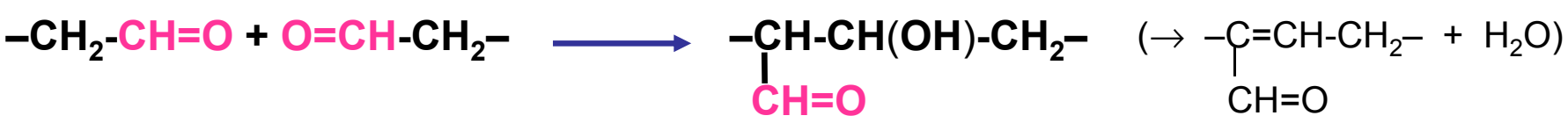
Simple covalent bridges join only **two** adjacent polypeptide chains, two types of those crosslinks are possible:

– **Aldimine type**, when the aldehyde group of allysine reacts with the ε-amino group of lysine - the product is an aldimine (Schiff base):



The product is unstable, it is stabilized slowly by hydrogenation and the crosslink is then called **lysinonorleucine** bridge.

– **Aldol type**, when two aldehyde groups of allysine react with each other (aldol condensation). One aldehyde group remains free in the bridge, so that it can take part in another reaction.

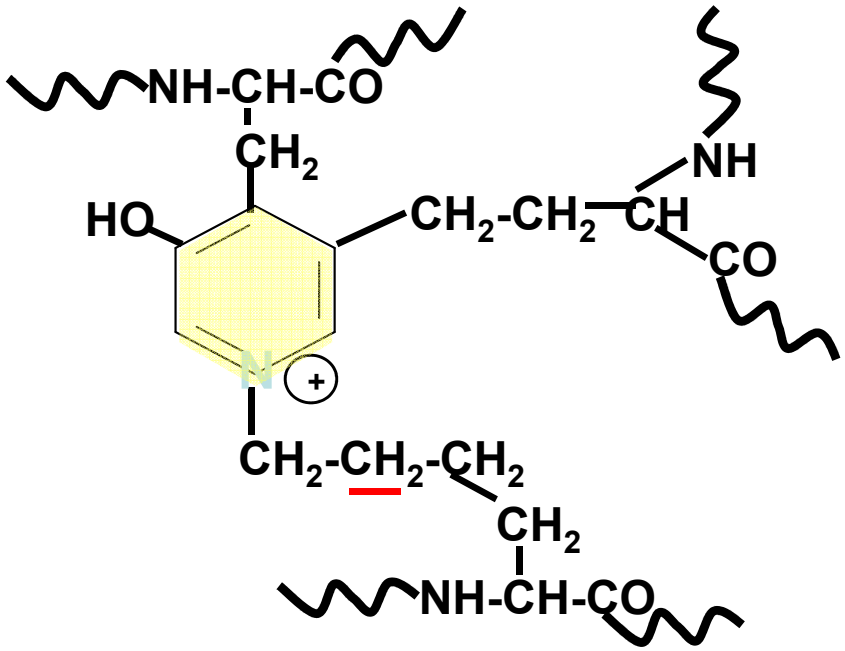


The resulting aldol is unstable, but it can be stabilized by elimination of water (a double bond is formed) to the **dehydroallysinealdehyde** bridge..

The **hydroxypyridinium type** of crosslink is formed when an aldimine bridge reacts with the aldehyde group of another allysine side chain so that the heterocyclic pyridinium ring is closed (**three** polypeptide chains are bound).

In each tropocollagen unit, only four of all lysine residues (two near the N-ends and other two near the C-ends of the chains) can take part in formation of those hydroxypyridinium crosslinks.

Therefore, hydroxypyridinium crosslinks join N-ends of chains with C-ends of adjacent tropocollagen units.



The hydroxypyridine ring is a very stable structure. It can be determined even after hydrolysis of all peptide bonds in collagen fragments, e.g., excreted into the urine, as **deoxypyridinoline** or **pyridinoline**. Those two structures differ in the highlighted position, the hydroxyl is present, when hydroxylysine was one of the reactants.

METABOLIC BONE DISEASES

Generalized defects in bone mineralization, frequently associated with abnormal calcium or phosphate metabolism, are sometimes grouped together under the term metabolic bone diseases.

Three major types of imbalances in new bone formation and bone resorption:

- **osteoporosis** – characterized by a reduction of bone mass which is not accompanied by changes in the ratio of mineral to osteoid,

  Histologically, a decrease in the cortical thickness of bone occurs and the number and size of trabeculae in cancellous bone is reduced.

- **osteomalacia (and rickets)** – characterized by defective mineralization of the organic matrix of bone, most often resulting from vitamin D deficiency,

- **osteodystrophy** – characterized by enhanced bone resorption compensated partly by pathological bone remodelling.
  It occurs predominantly either as renal osteodystrophy in chronic renal failure (mainly from secondary hyperparathyroidism that may result in osteitis fibrosa) or as Paget´s disease in its osteosclerotic and osteolytic form.

OSTEOPOROSIS

is a very common skeletal disorder that represents a major global health and social problem. It is characterized by reduced bone mass and degeneration of bone tissue, resulting in an increased risk for fractures.

Osteoporotic fractures are characteristically of the hip, wrist, and spinal column, although in old age other sites can be affected.
Vertebral fractures are less easily recognised, patients with spinal fractures may present with sever pain or be identified by height loss due to curvature of the spine.

Osteoporosis is asymptomatic and there is no clinical evidence of its presence (bone loss) until a fracture has occurred.

Prior to fracture, bone loss is slow and the disorder may be present for some years before it is diagnosed. Early recognition is important.

Bone mineral density (**BMD**) **measurement** can predict the risk of future fractures and this is currently the basis for defining osteoporosis.

Osteoporosis

Is a syndrome that can be subdivided into **three types**:

Type I – post-menopausal osteoporosis – it affects women in the first 5–15 years after the cessation of ovarian function (estrogen deficiency) and is characterized by a rapid loss especially in trabecular bone and by fractures mainly localized at the spine and wrist.

Type II – senile osteoporosis – affects people 75 years old or more, with women twice as likely to be affected. It is characterized by borth cortical and trabecular bone loss. Fractures may occur in all the skeletal sites, but the pathognomonic fracture for type II osteoporosis is the hip fracture.

Type III osteoporosis includes all **secondary causes**. Among these, osteoporosis induced by glucocorticoids (Cushing's syndrome), hyperparathyroidism, renal osteopathies, malabsorption, chronic liver disease, rheumatoid arthritis, immobilization, etc..

Approx. 80% of an individual's peak bone mass is genetically determined with the remaining 20% being influenced by environmental and lifestyle factors.

Enhancing **positive lifestyle factors** and minimising negative ones will encourage the attainment of peak bone mass and minimise bone loss later in life:

During childhood, adequate calcium intake and physical exercise play an important role in maximising bone mass.

In adulthood, a balanced diet rich in calcium and vitamin D combined with weight bearing exercise (eg. walking) are important preventative measures against osteoporosis.

Negative influences include among others

advancing age,
premature ovarian failure, early menopause, hysterectomy,
smoking,
alcohol abuse,
the use of certain medications
(eg. corticosteroids, prolonged heparin administration, anticonvulsives).

In osteoporosis, results of **routine chemical investigations** (Ca^{2+}, phosphates, ALP) are **usually all normal**.

The precise bone Ca accretion and resorption rates can be determined, but they require the use of Ca isotopes (usually radioactive ones) and usually long-term admission to metabolic wards.

The state of the skeleton can be evaluated by a variety of techniques, including **histomorphometry** and densitometry.

Histomorphometry is invasive, expensive, has a long turnaround time, and is limited to a single skeletal site (iliac crest).

Densitometry is precise and non-invasive but slow to reveal changes.

In studying bone turnover, we have to rely upon indices of the turnover of the organic matrix rather than the mineral phase of bone.

Biochemical markers of bone remodelling represent a non-invasive means of providing direct information. Markers respond to intervention more rapidly than does densitometry.

Because the process of resorption is shorter than the process of formation, resorption markers respond faster to changes than do formation markers.

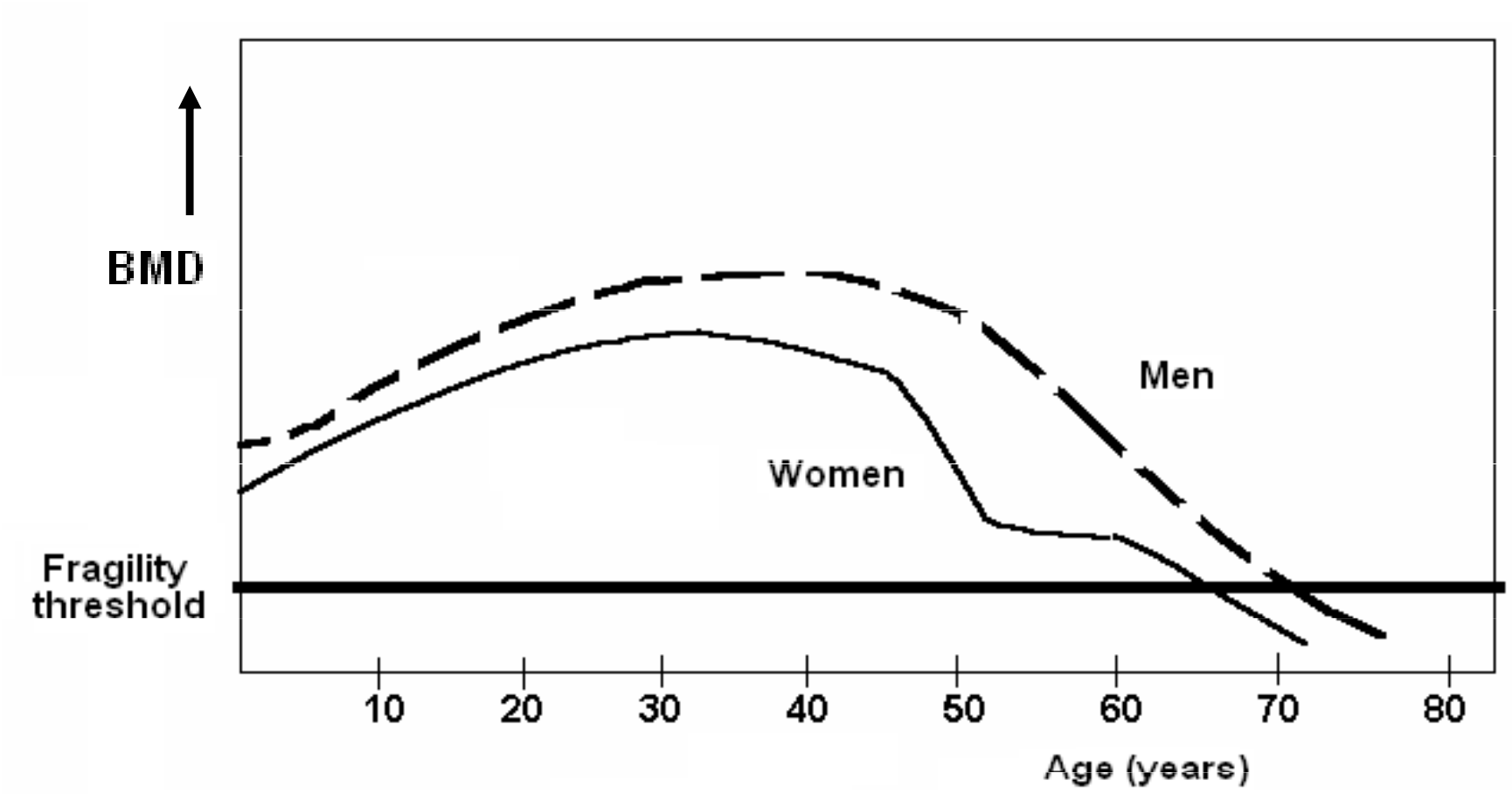
Bone mineral density (BMD) is usually measured by **dual energy X-ray absorptiometry** (DEXA), a method which enables measurements to be undertaken at almost any skeletal site comparatively cheaply.

A BMD not more than 1 standard deviation below the mean for 35 % young, normal women (**< 1 T-score**) is within the **normal range.**

T-score in the range **1 - 1,5** is taken as **osteopenia**.

According to WHO criteria, **T-score more than 2.5** (a bone mineral density of more than 2.5 standard deviations below the mean for young, normal women) indicates that the patient has **osteoporosis**, is at risk of fracture, and that the treatment which can halt the progression should be started.

Bone mineral density (BMD) depends on age



Obr. 1 Denzitometrický záznam 50-ročnej ženy. Osteoporóza zistená pri screeningu.

I. Ortopedická klinika
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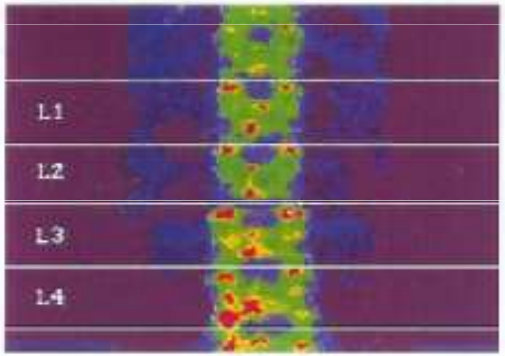
PATIENT ID:
NAME:

SCAN: 1.3z 24.10.95
ANALYSIS: 1.3z 24.10.95



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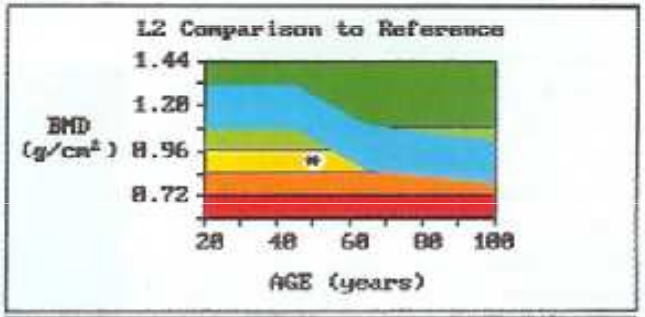


L2 BMD (g/cm²)[1]	0.988 ± 0.03
L2 % Young Adult[2]	76 ± 3
L2 % Age Matched[3]	88 ± 3

Age (years).....	50	Large Standard.....	271.79	Scan Mode.....	Fast
Sex.....	Female	Medium Standard.....	202.20	Scan Type.....	DPX-L
Weight (Kg).....	62.0	Small Standard.....	143.32	Collimation (mm).....	1.00
Height (cm).....	163	Low keV Air (cps)...	718947	Sample Size (mm).....	1.2x 1.2
Ethnic.....	White	High keV Air (cps)..	445921	Current (uA).....	3000
System.....	7071	Rvalue (%Fat).....	1.348(21.3)		

REGION	BMD[1] g/cm²	Young Adult[2] %	Young Adult[2] Z	Age Matched[3] %	Age Matched[3] Z
L1	0.874	77	-2.14	82	-1.58
L2	0.908	76	-2.43	80	-1.87
L3	0.968	81	-1.93	85	-1.37
L4	1.063	89	-1.14	94	-0.58
L1-L2	0.890	77	-2.16	82	-1.60
L1-L3	0.921	79	-2.08	83	-1.52
L1-L4	0.961	81	-1.82	86	-1.26
L2-L3	0.942	79	-2.15	83	-1.59
L2-L4	0.987	82	-1.78	87	-1.22
L3-L4	1.016	85	-1.53	90	-0.97

1 - See appendix E on precision and accuracy. Statistically 68% of repeat scans will fall within 1 SD.
2 - Germany AP Spine Reference Population, Ages 20-45. See Appendices.
3 - Matched for Age, Weight(males 50-100kg; females 35-80kg), Ethnic.

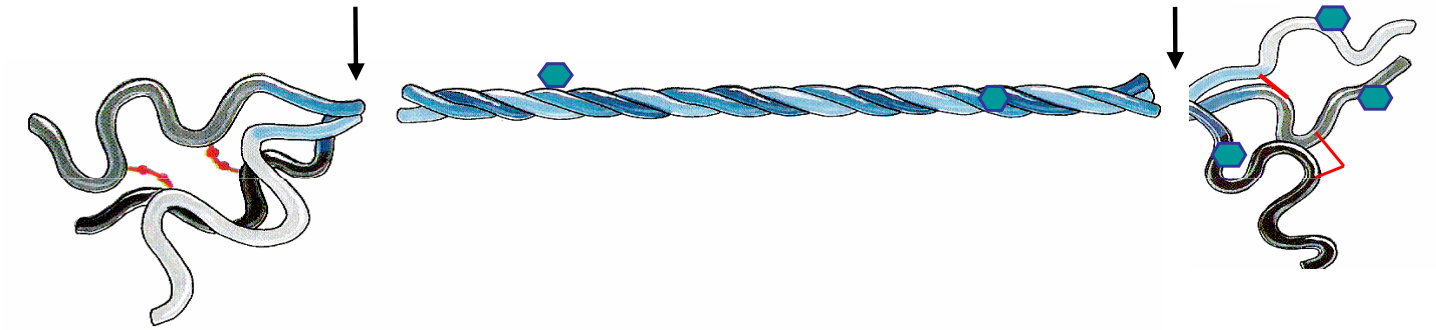
Biomarkers of bone formation

Catalytic concentration of the **bone isoenzyme of alkaline phosphatase** (ALP) in serum – this isoenzyme is a marker of osteoblast activity, of which it is an ectoenzyme. Total activity of ALP can serve only as a very rough estimate due to prevalence of other isoenzymes.

Concentration of **osteocalcin** (bone-Gla protein, BGP, OC) in serum – osteocalcin is a modulator of bone remodelation secreted by osteoblasts, its production is induced by calcitriol.

Osteocalcin is the main non-collagen protein in the extracellular matrix of bones.

Concentration of **N-terminal** or **C-terminal propeptides of procollagen I** (PINP and PICP, procollagen extension peptides) in serum.



procollagen I N-terminal propeptide (PINP) tropocollagen **procollagen I C-terminal propeptide (PICP)**

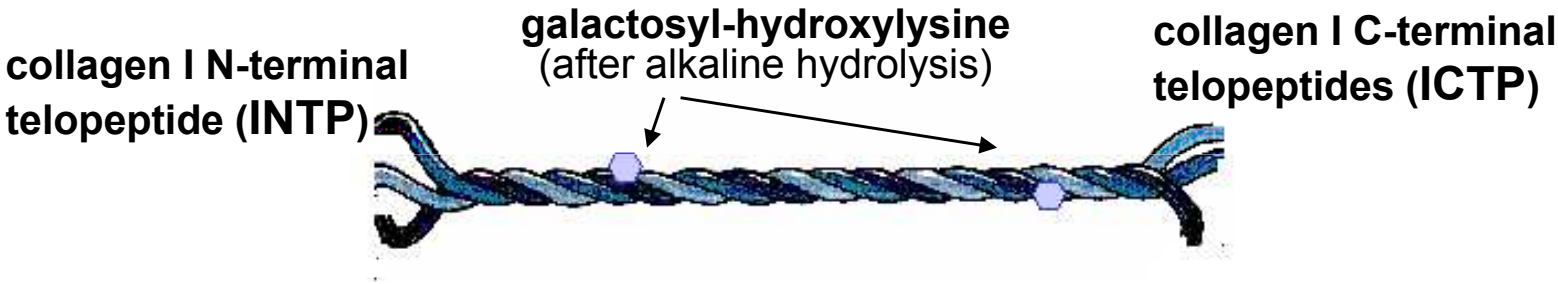
Biomarkers of bone resorption

Catalytic concentration of **bone isoenzyme of acid phosphatase** (ACP, known also as tartrate-resistant, TRAcP) in serum. This isoenzyme (one of the six AcP isoenzymes) is secreted from osteoclasts during active bone resorption.

Concentration of **type I collagen crosslinked C-terminal telopeptides** (ICTP) in serum or urinary excretion of the **C-terminal octapeptide** (CTx) from C-terminal sequences of tropocollagen I, and urinary excretion of **N-terminal telopeptides of collagen I** (INTP, NTx) – N-terminal non-helical sequences of tropocollagen I that bind through Dpd crosslinks to the helical region of another tropocollagen molecule.

Urinary excretion of **deoxypyridinoline** (Dpd) and **pyridinoline** (Pyd)

Urinary excretion of **galactosyl hydroxylysine** (GH, Hyl glycosides).



Determination of **hydroxyproline** urinary excretion of (free or total Hyp)) was in common use for years, but currently it is taken as an obsolete test for bone resorption.