# Regulation of calcium metabolism. Endocrine pancreas. Adrenal gland. Stress.

# Calcium metabolism

- Body of adult human (young) contains 1000 – 1100 g of calcium
  - skeleton (98 – 99 %)
  - 1 – 2 % extraosseous, particularly extracellularly
- Very small amounts of calcium intracellularly
  - 55 % ER, the rest namely in mitochondria
  - The cytoplasmic concentration level $10^{-7}$ $mol.L^{-1}$ versus blood plasma level $10^{-3}$ $mol.L^{-1}$ (normal concentration around 2.5 $mmol.L^{-1}$)
  - The necessity of strict regulation - signaling role of calcium ions
    - Muscle contraction, neurotransmission, secretion mechanisms, cell cycle and proliferation, cell death, coagulation, etc.
    - Ligand-gated or voltage-gated channels (type T – transient/type L – long lasting), eventually channels activated mechanically
    - $Ca^{2+}/H^{+}$ ATPase
    - Antiport driven by $Na^{+}$ gradient

# Calcium intake

- Daily intake is about 1.0 g per day and increases during pregnancy, lactation, growth, etc. (up to 1.5 g)
- Under physiological conditions it absorbed about 25 to 40% of received calcium (duodenum - 15%, jejunum - 20%, ileum - 65%)
- Paracelullular/transcelular transport
  - Paracelullar transport – claudin 2 and claudin 12
- Role of 1,25-dihydroxycholecalciferol!
  - Decreased levels of plasma $Ca^{2+}$ increases the synthesis of 1,25-dihydroxycholecalciferol and vice versa
- TRPV6/calbindin, PMCA1 (1:1 $Ca^{2+}$/ATP), $Na^{+}$-$Ca^{2+}$ exchanger (NCX1)
- Inhibited by oxalates and phosphates (formation of insoluble salts)

# TRPV6

- transient receptor potential cation channel, subfamily V, member 6
- Glycosylated transmembrane protein, Mr cca 70 000
- 6 transmembrane domains, N- a C- end intracellularly in cytoplasm
  - Binding sites for calmodulin = $Ca^{2+}$-dependent inactivation
  - Ankyrin repeats – interactions between domains, regulation of the function and channel assembly?
- Strong expression in the small intestine induced by 1,25-dihydroxycholecalciferol
- Furthermore, placenta, exocrine pancreas, mammary gland, salivary glands
  - placental transport of calcium ions between mother and fetus (collateral mineralization of bone tissue)
  - Possible role in the differentiation of keratinocytes
  - Over-expression (or increased expression) in some types of tumors – it is responsible for increased and accelerated cell proliferation and resistance to apoptic signals (prostate, mammary gland)
- Note TRPV5 – renal reabsorption of $Ca^{2+}$

# Calbindin

- Generally, a group of proteins with high affinity for calcium ions
- Originally described as a vitamin D-dependent calcium-binding protein
- High affinity for calcium ions in the cytoplasm
- Kalbindin-D9K *(S100G)*
  - S100 family of mammalian proteins (low molecular weight proteins having two binding sites for calcium ions, a number of intracellular and extracellular functions)
  - Mammalian enterocytes
  - Transport of calcium ions from the apical to basolateral membrane of the enterocyte
  - Stimulation PMCA1 (Plasma membrane Ca2 + ATPase)?
- Note Kalbindin-D28K
  - Neuronal and endocrine tissue, mainly in the cerebellum
  - Uptake (transport) of calcium ions
  - In the brain, independently on 1,25-dihydroxycholecalciferol!
- Calretinin
  - neuronal tissue
  - vitamin D-dependent calcium-binding intracellular protein of the troponin C superfamily involved in calcium signalling
  - In humans, the calretinin protein is encoded by the *CALB2* gene

# Serum calcium

- Concentration 2.5 $mmol.L^{-1}$ , resp. 2.2 – 2.6 $mmol.L^{-1}$ (100 $mg.L^{-1}$)
- The upper limit compatible with life 4 – 5 $mmol.L^{-1}$
- The lower limit 1 $mmol.L^{-1}$
- About 60 % in diffusible form:
  - filtered by the kidneys
  - 50 % ionized – free ($Ca^{2+}$) = biologically active form (1.1 – 1.3 $mmol.L^{-1}$)
  - 10 % in low-molecular complexes (citrates, phosphates, hydrogen carbonates)
- About 40 % in nondiffusible form:
  - Calcium ions bound to proteins
  - Albumin 90 %, globulin 10 %
  - Cannot be filtered
  - Biologically inactive form, BUT may be released at hypocalcemia
  - hypoalbuminemia – fraction bound to albumin decreases (decrease for 10 $g.L^{-1}$ causes no changes in the concentration of ionized calcium)
  - hyperproteinemia (multiple myeloma) – increase in total calcemia, again without changing in the concentration of free calcium
- pH:
  - Alkalosis – amount of ionised calcium decreases
  - Acidosis – amount of ionised calcium increases
  - Competition of $H^+$ and $Ca^{2+}$ for binding sites of albumin

Calcium concentration, both total and free, is characterized by a high physiological variation, depending on age, sex, physiological state (eg, pregnancy), and even season (owing to the seasonal variation of vitamin D, which is directly involved in the regulation of calcium concentration). Therefore, separate reference intervals have been established according to the age and sex of the individual being tested.

***Total calcium reference ranges in males are as follows:***

•Younger than 12 months: Not established
•Age 1-14 years: 9.6-10.6 mg/dL
•Age 15-16 years: 9.5-10.5 mg/dL
•Age 17-18 years: 9.5-10.4 mg/dL
•Age 19-21 years: 9.3-10.3 mg/dL
•Age 22 years and older: 8.9-10.1 mg/dL

***Total calcium reference ranges in females are as follows:***

•Younger than 12 months: Not established
•Age 1-11 years: 9.6-10.6 mg/dL
•Age 12-14 years: 9.5-10.4 mg/dL
•Age 15-18 years: 9.1-10.3 mg/dL
•Age 19 years and older: 8.9-10.1 mg/dL

***Free (ionized) calcium reference ranges in males are as follows*:**

•Younger than 12 months: Not established
•1-19 years: 5.1-5.9 mg/dL
•Age 20 years and older: 4.8-5.7 mg/dL

***Free (ionized) calcium reference ranges in females are as follows:***

•Younger than 12 months: Not established
•1-17 years: 5.1-5.9 mg/dL
•Age 18 years and older: 4.8-5.7 mg/dL

***Calcium (urine) reference ranges are as follows*:***

•Males: 25-300 mg/24-hour urine collection
•Females: 20-275 mg/24-hour urine collection
•Hypercalciuria: >350 mg/specimen
•*Values are for persons with average calcium intake (ie, 600-800 mg/day)

# Phosphorus

- Approximately 500 – 800 g, 85 – 90% in skeleton, 10 – 15% in ECF and soft tissues
  - Approximately two-thirds bound in organic compounds
  - The rest in inorganic phosphorus (phosphates)
- Nucleic acids ATP, cAMP,2,3-diphosphoglycerol, and many others
- Phosphorylation and dephosphorylation of proteins (kinases/phosphatases)
- We receive about 800 - 1400 mg of phosphorus per day (daily intake)
- Especially passive transport in the duodenum and small intestine (60-80%), but also active transport regulated by 1,25-dihydroxycholecalciferol (calcitriol) – NaPi-Iib cotransporter
- Amount of about 3 mg/kg/day enters the bone, but approximately the same amount is released by bone resorption
- Inorganic phosphorus filtered in the glomeruli, but 85-90% resorbed back (reabsorption, proximal tubules $Na^+$dependent cotransporter NaPi P-IIa/IIc, adaptation mechanisms to phosphorus levels in the diet (up-/down regulation, inhibition by PTH)

# Phosphorus

- Reference range:
  - Blood serum 0.7 – 1.5 $mmol.L^{-1}$
  - Urine 15 – 90 $mmol.L^{-1}$
  - hypophosphataemia – < 0,97 $mmol.L^{-1}$ in adults, < 0,6 $mmol.L^{-1}$ in older children, and < 1.2 $mmol.L^{-1}$ in infants, frequently as a result of alkalosis, chronic use of antacids, malabsorption, hypercalcemia, alcoholism
  - hyperphosphatemia – renal failure, healing of extensive fractures, hypervitaminosis D, hyperparathyroidism, acromegaly, etc.
- Note FGF23 (Fibroblast growth factor 23) – regulation of phosphate homeostasis
  - Released by osteocytes in response to increased levels of calcitriol
  - Decreases expression of gene for NaPi transporters (sodium/phosphate cotransporter) in kidney in proximal tubule = increased excretion of phosphate
  - Mutation in gene *FGF23* - autosomal dominant hypophosphatemic rickets (rachitis)
  - Next functions:
    - decrease the synthesis of 1$\alpha$-hydroxylase, and thereby the level of 1,25-dihydroxycholecalciferol in serum
    - Increased expression and production of 24-hydroxylase = lowering of 1,25-dihydroxycholecalciferol in serum
    - Inhibition of PTH – regulation of calcium and phosphorus homeostasis

# Bone physiology

- The compact (cortical) bone - the outer layer of most bones, about 80%
  - The low surface to volume ratio, osteocytes are not active
  - Nutrients supplied via Haversian (central) canals
  - Around each canal concentric layers of collagen - osteons (Haversian systems)
  - The collagen matrix impregnated with bone mineral crystals
    - 20 x 3-7 nm, mostly hydroxyapatites
- Trabecular (spongy) bone - in (inside) cortical bone, 20%
  - High surface to volume ratio
  - High metabolic activity
  - Nutrients diffuse from ECF to trabeculae

# Bone tissue

- 1/3 protein matrix and 2/3 mineral component (= inorganic bone phase, bone mineral)
  - Bone mineral - small crystals of hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ together with minor components – calcium carbonate, calcium fluoride, calcium phosphate
  - Matrix – Approximately 90% of collagen type I and minority of other proteins (osteocalcin, osteonectin, osteopontin, and many others)
- Metabolic activity is provided with bone cells:
  - Osteoblasts (formation of matrix)
  - Osteoclasts (bone resorption)
  - Osteocytes (regulation of bone resorption)
- Permanent rebuilding – bone remodeling
  - Osteoclastic bone resorption versus osteoblastic bone formation
  - A close connection with the metabolism of calcium and phosphorus

# Type I collagen – the most important protein of bone matrix

- Collagen
  - A group of extracellular water-insoluble proteins
  - The basic component of connective tissues
  - 28 types of collagen
- Type I collagen
  - Three polypeptides – two identical $\alpha$1 (*COL1A1*) and one different $\alpha$2 (*COL1A2*)
  - First, the „proforms“ are created (procollagen) that are modified enzymatically to the final form
  - Polypeptides are wound and have a common axis, the total length 1 – 20 $\mu$m
  - Mitation in *COL1A1* (often in only one AA):
    - Ehlers-Danlos syndrome - hypermobility of joints, hyperelasticity of the skin and many others
    - Osteogenesis imperfecta of different types
    - Polymorphism in *COL1A1* gene – increased risk of osteoporosis.

# Osteoblasts

- Origin in mesenchymal stem cells
- Periostum/bone marrow
- Progenitor cells – regulatory transcription factor Cbfa1/Runx2
- Another transcription factor osterix - osteoblast differentiation
- Effect of growth factors
- Individual osteoblasts are interconnected via:
  - Tight junctions – compartmentation of ECF
  - Gap junctions – connection of individual osteoblasts into one functional unit
- Production of type I collagen and several proteins of bone matrix (osteocalcin, osteonectin, osteopontin)+ alkaline phosphatase
- Mineralization of bone matrix
- Production of osteoprotegerin = basic glycoprotein, 401 AAs, binding to RANKL and inhibition of differentiation of osteoclasts

# Osteocytes

- Mature bone, a typical star shape
- Very long lifetime (half-life up to 25 years)
- Lacunae of bone tissue
- Mutual communication
- Metabolically not very active (almost inactive)
- Possible differentiation into osteoblasts
- Role in bone tissue remodeling and repair (production of nerve growth factor NGF)
- Control over the function of osteoblasts and osteoblast? (apoptosis = RANKL = increased production of osteoclasts)
- Role in the mineralization of bone tissue
  - PHEX, DMP-1, MEPE, FGF-23
  - Sclerostin – *SOST,* decrease of bone formation

# Osteoclasts

- Multinucleated cells eroding already formed bone
- Interaction with bone mediated by integrins (transmembrane receptors that are the bridges for cell-cell and cell-extracellular matrix (ECM) interactions) in the projections of plasma membrane (sealing zone)
- Proton pumps ($H^+$-dependent ATPases) subsequently acidify thus isolated area (pH 4)
- Dissolution of hydroxyapatite due to low pH
- Acid proteases destroy collagen (cathepsin K)
- Formation of resorption lacunae
- Products of bone resorption undergo endocytosis and subsequently pass through osteoclasts via transcytosis and are released into the interstitial fluid
- Note
  - MMP-9 – bone microenvironment, expressed in osteoclasts, necessary for the migration of osteoclasts
  - MMP-13 – role in the differentiation of osteoclasts
  - Protein RANKL (Receptor activator of nuclear factor kappa-B ligand) – TNF superfamily, control of the proliferation and differentiation of osteoclasts
  - RANK (Receptor Activator of Nuclear Factor κ B ) – a group of TNF receptors, regulation of differentiation and activation of osteoclasts
  - Osteoprotegerin

# BMP proteins (bone morphogenetic proteins)

- A group of proteins (about 20), for which stimulatory effect on bone tissue has been identified
- Now investigated in connection with their regulatory role in the development of a number of tissues (impaired BMP signaling in many pathological processes, especially cancer)
- In bone (cartilage) metabolism:
  - BMP1 – *BMP1* gene, several isoforms, induces cartilage growth. Identical with procollagen C protease (PCP). Belongs to a group of metalloproteases.
  - BMP2 – TGF-β (transforming growth factor-beta) superfamily, bone growth, but also cardiomyocyte differentiation and transformation of epithelial cells to mesenchymal cells. Increased production of extracellular cartilage matrix
  - BMP3 – negatively affects bone density (antagonist to other BMPs)
  - BMP4 – regulates osteoblast formation of multipotent stem cells, significant role in the differentiation process during early embryonic development. *In vitro* osteogenesis and chondrogenesis
  - BMP5 – trabecular bone and its structure
  - BMP6 – induction of osteogenic precursors, regulation of iron uptake in mammals (hepcidin)
  - BMP7 – homeostasis of bone tissue, kidney development. *In vitro*, BMP7 inhibits proliferation of mesenchymal stem cells and stimulates the production of cartilage tissue
  - BMP8a – homeostasis of bone tissue

# Matrix Gla proteins

- What are proteins with Gla domain?
  - Proteins rich on glutamate moieties that are posttranslationally modified by vitamin K-dependent carboxylation to γ-carboxyglutamate (γ-carboxyglutamic acid)
  - Matrix Gla proteins:
    - coagulation factors VII, IX, X, XIV + prothrombin
    - Vitamin K-dependent protein S
    - Vitamin K-dependent protein Z
    - Transthyretin
    - *Osteocalcin*
    - *Matrix Gla protein*
    - Gas-6 protein (growth arrest-specific protein 6)
    - + some other proteins
  - Gla proteins involved in the coagulation cascade are synthesized by hepatocytes

# Osteocalcin

- = BGLAP (bone gamma-carboxyglutamic acid-containing protein)
- 49 AAs, MR = 5800
- Produced exclusively by osteoblasts - bone / dentin
  - Synthesis is stimulated by 1,25-dihydroxycholecalciferol and depends on the vitamin K
  - Released during bone resoprtion
  - Control of the size and shape of hydroxyapatite
  - Mediates assotiation with bone mineralized matrix via $\alpha$ helical Gla domain
- Encoded by *BGLAP* gene
- Receptor – GPRC6A (G protein-coupled receptor family C group 6 member A)
  - Ligands – some amino acids (the most potent agonist is ornithine; aliphatic, neutral, and basic amino acids), calcium ($Ca^{2+}$), osteocalcin, some steroids = dual sensitivity (extracellular cations and amino acids)
  - Closely related is CASR (calcium-sensing receptor; G protein), however, for its activation higher concentration of calcium ions is necessary
  - Osteocalcin probably modifies, respectively increases response of receptor to calcium ions
  - Expressed in many tissues - lung, liver, spleen, heart, kidney, skeletal muscle, testes, brain, bone tissue
  - „sensing mechanism“ for extracellular calcium ions in osteoblasts
  - In the case of L-ornithine magnesium ions affect positively answer
  - Note – anabolic effect of strontium ions for their anabolic effect on bone tissue is mediated by CASR

# Osteocalcin

- Importance:
  - Stimulation of bone growth
  - Stimulation of the secretion of insulin
  - Stimulation of secretion of adiponectin in adipocytes
  - Regulation of male fertility, probably increases biosynthesis of testosterone
- Marker of the bone metabolism (formation of the bone tissue):
  - Amount of about 20 % of newly synthesized osteocalcin is circulating
  - 9-42 $ng/mL^{-1}$
  - Increased level - osteoporosis, osteomalacia, Paget's disease, rickets, hyperparathyroidism, renal osteodystrophy, thyrotoxicosis, bone metastases, acromegaly, and also fractures
  - Decreased levels - hypoparathyroidism, hypothyroidism
  - Marker of bisphosphonate therapy, or HRT (Hormone Replacement Therapy)
  - ? Marker of the status of vitamin K

# Matrix Gla protein

- Mr = 9600, high percentage of hydrophilic AMK
- BUT very limited solubility in water and aqueous solutions
- In many tissues - mRNA levels highest in the kidneys, lungs, liver, spleen, heart
  - Role in the calcification, especially regulator and inhibitor of calcification of the vascular wall
  - Release into the circulation (circulating fraction)
  - Significant interaction with vitamin K
- Relatively significant fraction of mRNA in the bone and cartilage
  - This production increased depending on the vitamin D
  - Interaction with other protein molecules of bone and cartilage (collagen, proteoglycans, glycosaminoglycans)
  - High affinity to calcium ions
  - Keutel syndrome
    - Diffuse calcification of cartilage and other abnormalities (brachytelephalangy)
    - Calcification of heart valves
    - Mutation in *MPG* gene
  - noncarboxylated MGP - marker of vascular wall calcification
- Protein S?
  - Cofactor for activated protein C
  - Deficiency is associated with osteopenia?
  - Osteoblasts, involved in mineralization of bone tissue

# Osteonectin (SPARC, BM-40)

- Mr = about 40 000, acidic and cysteine-rich glycoprotein
- 3 domains:
  - Highly acidic domain
    - Relatively low affinity to calcium ions
    - Interaction with hydroxyapatite
    - Role in the mineralization of bone tissue
  - Domain rich in cysteine moieties
  - Extracellular $Ca^{2+}$-binding domain
    - Binding of collagen regulated by calcium ions
  - *SPARC* gene
- Expressed in many cells
  - Chondrocytes, fibroblasts, platelets, endothelial cells, epithelial cells, Leydig cells, Sertoli cells, etc.
  - Overexpressed in some types of tumor cells (angiogenesis, cell proliferation, migration)
- Glycoprotein with an affinity for calcium ions and collagen
- Extracellular matrix
- Created by osteoblasts during bone formation
- Initiation of bone mineralization and bone mineral crystals
- Also mediates interaction with the matrix (matrix metalloproteinase increases the formation and modulates their activity)

# Osteopontin

- = bone sialoprotein I (BSP-I, BNSP), ETA-1, SPP1, Ric
- Protein that belongs to the group of SIBLING proteins (small integrin-binding ligand, N-linked glycoprotein)
- Identified in 1986 in osteoblasts
- Extracellular structural protein
  - Approximately 300 AAs (human 314 AAs), 30 glycoside moieties, of which 10 of sialic acid
  - Acidic protein (about 30 – 36 % moieties of glutamic or asparagic acids)
- *SPP1* gene
- Strongly binds to the available calcium ions
  - Inhibitor of mineralization
  - Regulation of bone mineral crystals
  - Important for remodeling the bone tissue – probably plays role in anchoring of osteoclasts onto bone mineral matrix
  - Osteopontin was found in urine – inhibition of crystal formation and kidney stones?
- Next functions:
  - Regulation of cell adhesion and migration
  - Chemotactic property, modulation of immune responses
  - Probable involvement in the myocardial dysfunction (apoptosis of myocytes)
  - Role in the pathological processes (calcification, inflammatory processes, tumor diseases, allergic reactions / asthma)

# SIBLING proteins

- Osteopontin
- Bone sialoprotein (BSP)
- DMP1 (dentin matrix acidic phosphoprotein 1)
  - Protein of extracellular matrix
  - Bone tissue/dentin
  - In undifferentiated osteoblasts = nuclear protein regulating the expression of osteoblast-specific genes
  - During maturation of osteoblasts is phosphorylated and transported to the extracellular matrix
  - Here DMP1 modulates bone mineralization
- DSPP (dentin sialophosphoprotein)
  - Only odontoblasts!
  - Cleavage into three functional proteins:
    - Dentin phosphoprotein (C-terminal end) - regulation of dentin mineralization
    - Dentin sialoprotein (N-terminal end)
    - Dentin glycoprotein (the central part of the molecule)
  - Note dentinogenesis imperfecta type iii.– mutations in the dentin sialoprotein part
- Matrix extracellular phosphoglycoprotein (Osteoblast/osteocyte factor 45, MEPE)
  - Integrin recognition
- Dental enamel
  - Highly organized hydroxyapatite crystals (up to 85 %)
  - Ameloblasts and their interaction with peptides / proteins
  - Enamelin – *ENAM*, mutation – amelogenesis imperfecta
  - Amelogenin – a group of proteins of extracellular matrix
  - Ameloblastin – controls mineralization
  - Tuftelin – acidic phosphorylated glycoprotein
- Dentin
  - Reelin – large extracellular matrix glycoprotein produced by odontoblasts
    - Possible transmission of pain signals

# Bone growth. Formation and bone resorption.

- Bone growth
  - Ossification of membrane (intramembranous) versus cartilage (enchondral)
  - Epiphyses, epiphyseal plates
  - closure of epiphyseal plates
- Permanent degradation and formation of new bone
- Replacement of up to 100% of the calcium in infants per year
- For adults, about 18% per year
- Osteoclasts – osteoblasts
- Permanent remodeling about 5% of bone tissue; affected by a number of factors
- Incorporation of other minerals into bone tissue
  - In particular elements chemically similar to calcium
  - Lead
  - Rapid bone uptake - detoxification mechanism?
  - Fluoride uptake - formation of new bone, the incorporation into the dental enamel (resistance to decay)
  - Large doses can cause discoloration of enamel

# Factors affecting bone remodeling

- Genetic factors
  - 60-80% of the amount of bone tissue is genetically determined
  - The differences between the races (most Negroes, Asians least)
- Mechanical factors
  - Remodeling of bone structure according to the mechanical requirements
  - Physical activity is essential for proper bone development
- Vascular / neural factors
  - Vascularization necessary for proper bone development, especially for ossification
  - Innervation - neuropeptides and their receptors
- Nutritional factors
  - calcium intake
  - Addictions - coffee, smoking, alcohol, excess salt - risk factors for osteopenia
- The function of the endocrine system
- Besides the above mentioned also:
    - Androgens - anabolic effect, stimulation of osteoblasts, bone density modification
    - Estrogens - estrogen receptors found in osteoblasts, osteocytes, and osteoclasts, dual effect - stimulation of of osteoblasts, increased levels of osteoprotegerin, reduction of bone resorption
    - Progesterone - anabolic effect on bone, osteoblasts - receptors - direct / indirect effect (competes with glucocorticoids)
    - Insulin - stimulating the creation of matrix
    - Glucocorticoids - differentiation during development, but postnatally inhibit bone formation, inhibition of of IGF-1 / BMP-2, which are important for osteoblastogenesis
    - Growth hormone - direct effect (stimulation of osteoblasts), the indirect effect via increasing IGF-1/2 = stimulation of osteoblast proliferation and differentiation

# Local remodeling of bone tissue

- Especially growth factors and cytokines
- Growth factors:
  - Polypeptides produced by bone tissue or extraosseously
  - Modulation of growth, proliferation, differentiation
  - IGF-1/2
    - Liver / osteoblasts
    - In high concentration in the bone matrix
    - Stimulation of collagen synthesis
    - Regulation of interactions between osteoblasts and osteoclasts
    - IGF-2 – embryogenesis
  - TGF-β (Transforming growth factor - β)
    - Inhibition of bone resorption by inhibiting osteoclast differentiation and apoptosisStimulace tvorby kostní tkáně, indukce diferenciace a proliferace osteoblastů
    - Inhibition of the synthesis of matrix protease (MMP)
  - BMP
  - PDGF (Platelet - derived growth factor)
    - stimulation of protein synthesis
    - Fibroblast proliferation, neovascularization, collagen synthesis
  - FGF (Fibroblastic growth factor) – mitogenic effect on osteoblasts, mutations in receptors - e.g. Apert syndrome (premature closure of sutures, syndactyly, extension of cranium - turricephaly)
  - EGF (Epidermal growth factor)
  - VEGF (Vascular endothelial growth factor) – stimulation of angiogenesis and proliferation of endothelium, important especially in the early stages of regeneration (fracture)
  - GM-CSF (Granulocyte / macrophage - colony stimulating factor) – osteoclastogenesis, osteopetrosis
  - M-CSF (Macrophage - colony stimulating factor) – osteoclastogenesis first phase, without any direct effect on osteoclasts
  - TNF (Tumor necrosis factor) – stimulation of bone resorption

# Local remodeling of bone tissue

- Cytokines – immune cells, a number of functions (immune response, inflammation, hematopoiesis, autocrine / paracrine effect, pleiotropic effect)
- Interleukin 1
  - Direct stimulation of osteoclastic bone resorption
  - Stimulation of proliferation and differentiation of preosteoblasts
  - Inhibition of apoptosis of osteoclast
- Interleukin 6
  - Stimulation of bone resorption
  - Role in the Paget's disease
  - The initial phase of osteoclastogenesis
- Interleukin 11
  - Bone marrow, stimulation of osteoclastogenesis
- Prostaglandins
  - Especially PGE2
  - Stimulation of bone resorption
  - Experimentally – inhibition of COX2 = inhibition of bone formation in the dependence on the mechanical stress
- Leukotrienes
  - Role in the bone remodeling

# Bone diseases

- Osteogenesis imperfecta
- Osteopetrosis
  - defective osteoclasts
  - Dysfunctional bone resorption
  - Relatively large molecular biological basis
- Rickets / osteomalacia - a lack of accretion of calcium per unit of bone matrix
- Osteoporosis - loss of bone matrix and mineral components

# Markers of bone metabolism

- Markers of bone formation
  - Alkaline phosphatase
    - "Attached" to the outer surface of osteoblasts via glycosylphosphatidylinositol anchor (ectoenzyme)
    - Today the use of ALP discussed and it seems that this marker is unspecific
    - Function - it increases the local concentration of inorganic phosphate (= promoter of mineralization) and simultaneously decreases the local concentration of pyrophosphate (inhibitor of bone mineral formation)
    - Importance in the cardiovascular calcification
  - Osteocalcin
  - N- and C- terminal procollagen propeptides
    - Increase in Paget's disease, and at increased levels of growth hormone
- Markers of bone resorption
  - Proteins containing hydroxyproline
    - Pyridinoline (PYD), deoxypyridindolin (DPD), cross-linking compound of collagen fibers
    - Stabilization of chains of collagen in the extracellular matrix
    - Galactosylhydroxylysine
  - Acid phosphatase
    - = tartarat-resistant acid phosphatase (TRAP) 5b
    - 5b isoform highly specific for osteoclasts

| Marker | Tissue origin | Analytical sample | Analytical method |
|---|---|---|---|
| **Hydroxyproline, total and dialyzable (OH-Pro, OHP);** specific for all fibrilar collagens and a part of collagen proteins, including Ciq and elastin; present in newly synthesized and mature collagen | bone, skin, cartilage, soft tissues | urine | colorimetry, HPLC |
| **Pyridinoline (PYD, Pyr);** high concentrations in cartilage and bone collagen: not present in skin; present only in mature collagen | bone, tendon, cartilage | urine | HPLC, ELISA |
| **Deoxypyrindoline (DPD, d-Pyr);** high concentrations only in bone collagen: not present in cartilage or in skin; present only in mature collagen | bone, dentine | urine | HPLC, ELISA |
| **Cross-linked C-terminal telopeptide of type I collagen (ICTP);** high proportion from bone collagen in type I collagen; can partly originate from newly synthesized collagen | bone, skin | serum | RIA |
| **Cross-linked C-terminal telopeptide of type I collagen (fragments alpha-CTX, beta-CTX);** in type I collagen; probably high proportion from bone collagen | all tissue containing type I collagen | urine, serum | ELISA, RIA, ECLIA |
| **Cross-linked N-terminal telopeptide of type I collagen (fragments NTX);** in type I collagen; big proportion from bone | all tissue containing type I collagen | urine (alpha/beta), serum (only beta) | ELISA, RIA, ICMA |
| **Hydroxylysine-glycosides (Hyl-Glyc);** collagens and collagen proteins; glucogalactosyl- hydroxilysine is highly represented in soft tissue collagens and C1q; galactosil-OHLys is highly represented in bone collagen | bone, skin, soft tissue, serum complement | urine | HPLC, ELISA |
| **Bone sialoprotein (BSP);** synthesized by active osteoblasts and lay in extracellular bone matrix; it seems to express osteoclast activity | bone, dentine, hypertrophic catrilage | serum | RIA, ELISA |
| **Tartarat-resistant acid phosphatase (TR-ACP);** osteoclasts, thrombocytes, erythrocytes | bone, blood | plasma/serum | colorimetry, RIA, ELISA |
| **Free gamma carboxyglutamin acid (GLA);** resulted from bone proteins (e.g. osteocalcin, matrix Gla protein) and from coagulation factor | blood, bone | serum/urine | HPLC |

HPLC – high performance liquid chromatography; ELISA – enzyme-linked immunosorbent assay; RIA – radio immuno assay; ECLIA – electrochemiluminiscence immunoassay; ICMA – immunochemiluminometric assay

# Vitamin D and hydroxycholecalciferols

- Vitamin D = group of related sterols that arise when exposed to UV (290 – 315 nm)
- D3 biologically inactive - conversion by CYP enzymes
- 1,25-dihydroxycholecalciferol = calcitriol is formed also in the placenta, skin keratinocytes, and macrophages
- 25-hydroxycholecalciferol is the major circulating form, bound to plasma DBP
  - 25 $\mu g.L^{-1}$
  - Calcitriol – 30 $ng.L^{-1}$
- Regulation of the synthesis
  - Formation of 1,25-dihydroxycholecalciferol catalyzed by 1$\alpha$-hydroxylase is regulated by feedback by plasma $Ca^{2+}$ and $PO_4^{3-}$
  - Furthermore, it is facilitated by PTH as a response to low plasma levels of calcium
  - At high plasma levels of calcium or high levels of $PO_4^{3-}$ production of relatively biologically inactive 24,25-dihydroxycholecalciferol
  - Prolactin increases activity of 1$\alpha$-hydroxylase
  - Estrogens - increased level of proteins that bind calcitriol (and thus increased levels of total and 1,25-dihydroxycholecalciferol, but not of the free form)
  - The level of 1,25-dihydroxycholecalciferol is lowered in hyperthyroidism and metabolic acidosis
- Note
  - Vitamin D-resistant rickets type I - mutation of the gene for renal hydroxylase
  - Vitamin D-resistant rickets type II – mutation of the gene for receptor for 1,25-dihydroxycholecalciferol

# The mechanism of action

- 1,25-dihydroxycholecalciferol = steroid
- Regulation of protein synthesis of calbindins
- Increasing the number of molecules of $Ca^{2+}H^{+}$ATPase in enterocytes = enhanced transport of calcium ions into the interstitium
- Increase (facilitating) reabsorption of calcium in the kidneys
- Increasing the number of osteoclasts – mobilization of $Ca^{2+}$ and $PO_4^{3-}$
- Receptors
  - In many tissues (skin, lymphocytes, monocytes, skeletal and cardiac muscle, mammary gland, adenohypophysis)
  - Induction of differentiation of immune cells and keratinocytes in the skin
  - In patients with vitamin D deficiency is an increased incidence of infections
  - In other tissues growth regulation because of regulation of growth factors

# Parathyroid glands

- Usually 4 parathyroid glands (high variability)
- 3 x 6 x 2 mm highly vascularized discoid structure
- Parathyroid chief cells (also called parathyroid principal cells or simply parathyroid cells)
  - synthesis and secretion of parathyroid hormone (PTH)
- Oxyphil cell
  - The number increases with age
  - Functions still unknown
  - Production of PTHrP?

# Synthesis and metabolism of PTH

- Linear peptide, 84 AAs, Mr = 9500
- IPTH is created from preproPTH
- 10 – 55 $ng.L^{-1}$
- Half time 10 min, cleavage of fragment from the middle region and C-terminal end in the Kupffer cells and subsequent elimination by the kidneys
- PTH effects directly in bone tissue
  - Increased bone resorption and calcium mobilization = increased plasma levels of calcium
  - Reduction of plasma phosphate - increased phosphate excretion by reducing reabsorption of phosphate in the proximal tubules
  - Increased resorption of calcium ions in the distal tubules
  - Increased creation 1,25-dihydroxycholecalciferol via increased activity of 1$\alpha$-hydroxylase
- Long-term effect - stimulation of osteoblasts and osteoclasts, but the predominant effect on osteoclasts
- Secretion of PTH is regulated by negative feedback by calcium ions (membrane receptor - serpentine G protein-coupled receptor)
  - Increased plasma levels of phosphate = lowering of calcium ions and the inhibition of creation of 1,25-dihydroxycholecalciferol
- 1,25-dihydroxycholecalciferol acts directly in the the parathyroid glands
  - Decrease in the level of mRNA of preproPTH
- The role of magnesium ions - slight stimulate production of PTH
- PTHrP (Parathyroid hormone-related protein)
  - Expressed in a wide range of tissues
  - 140 AAs
  - Significant homology with PTH in the end regions
  - Stimulation of proliferation of chondrocytes and inhibition of mineralization
  - The growth factor for the development of skin, hair follicles, and breast?
  - Excreted in breast milk - significance unclear
  - Cerebral cortex, hippocampus, granular layer of cerebellum

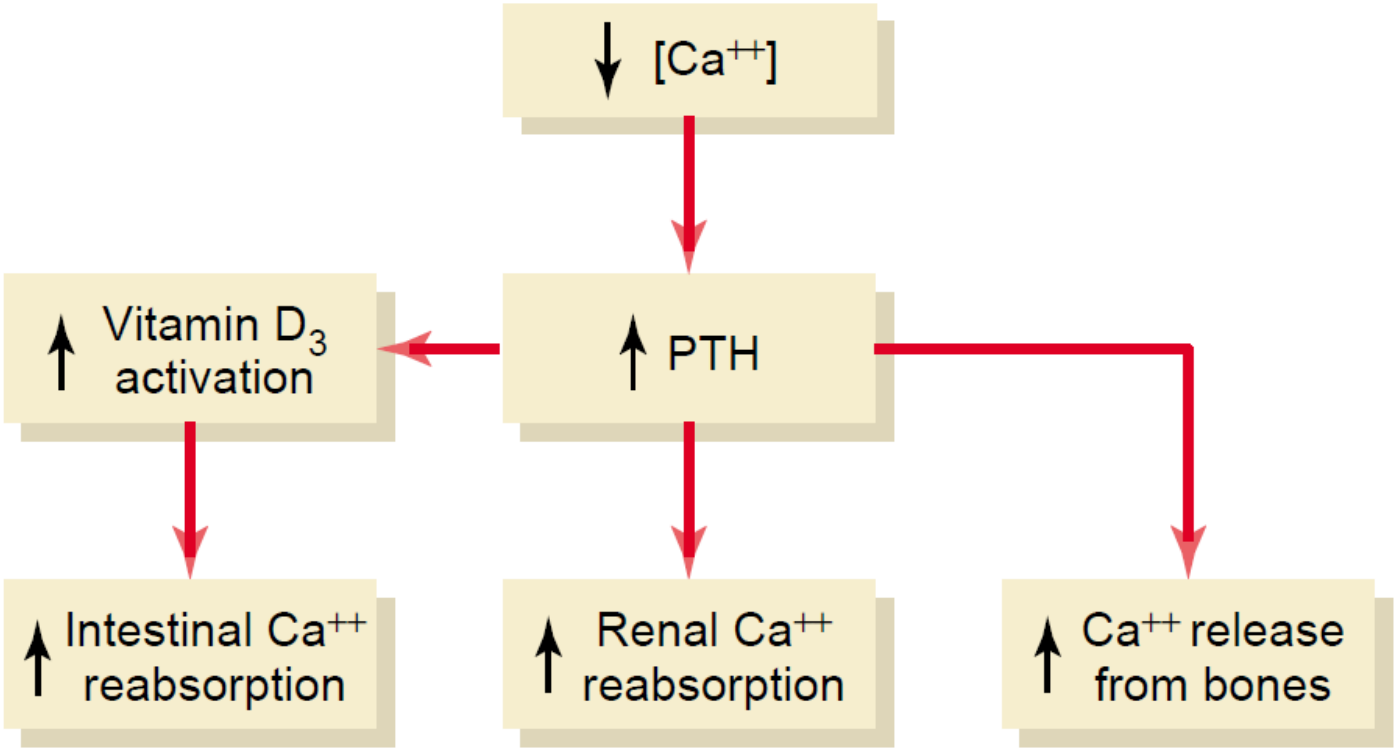



**Figure 29–10**

Compensatory responses to decreased plasma ionized calcium concentration mediated by parathyroid hormone (PTH) and vitamin D.

**Factors That Alter Renal Calcium Excretion**

| ↓ Calcium Excretion | ↑ Calcium Excretion |
|---|---|
| ↑ Parathyroid hormone (PTH) | ↓ PTH |
| ↓ Extracellular fluid volume | ↑ Extracellular fluid volume |
| ↓ Blood pressure | ↑ Blood pressure |
| ↑ Plasma phosphate | ↓ Plasma phosphate |
| Metabolic acidosis | Metabolic alkalosis |
| Vitamin $D_3$ | |

Guyton and Hall Textbook of Medical Physiology. 12th Ed. Elsevier 2006

# The mechanism of action of PTH

- At least three different receptors for PTH (G protein)
- Different expression in different tissues
- For the regulation of calcium metabolism probably the most important PTH1R with affinity to PTHrP, receptor is serpentine
- PTH2R in various tissues (CNC, pancreas, testes, placenta), but without affinity to PTHrP, serpentine receptor
- CPTHR? – ligand = je C-terminal part of PTH
- Pseudohypoparathyreosis
  - Mutations in genes for receptors?
- Parathyroidectomy
  - A steady decline in blood calcium, phosphate level increases simultaneously
  - Tetany, Chvostek sign, Trousseau sign
- Excess of PTH
  - Hypercalcemia, hypophosphatemia, bone demineralization, hypercalciuria, kidney stones
  - Osteitis fibrosa
  - Secondary hyperparathyroidism - when chronically reduced levels of calcium ions in the blood, stimulation of parathyroid, compensatory hypertrophy, hyperphosphatemia
  - Familial hypercalcemia / hypocalcemia

# Calcitonin

- Hormone that decreases level of calcium ions
- Produced by parafollicular cells (C cells, neuroendocrine cells in the thyroid gland)
- Mr = 3500, 32 AAs, significant variability in different species in the primary structure
- *CALC1* gene
- Creating a peptide related with calcitonin gene (CGRP, Calcitonin gene-related peptide)
- Secretion increases at a relatively high level of calcium (higher than 95 $mg.L^{-1}$)
  - Secretion is stimulated by β-adrenergic agonists, dopamine, estrogens
  - Furthermore gastrin, cholecystokinin, glucagon, secretin
  - Probably is secreted also in other tissues
- Half time 10 minutes
- Serpentine receptors in bone tissue and kidneys
  - Inhibition of bone resorption - inhibition of activity osteoclasts
  - BUT only a negligible long-term effects on hypercalcemia (no syndrome of calcitonin deficit, medullary thyroid carcinoma - high levels of calcitonin, but no symptoms)
  - In younger people higher levels - the importance of the development of the skeleton?
  - Protecting mother against excessive loss of calcium in pregnancy
  - Protection against postprandial hypercalcemia (gastin)
- CGRP - Calcitonin gene-related peptide
  - 37 AMK
  - Produced by both central and peripheral neurons
  - Vasodilatation, role in the transmission of pain signals (sensory function)
  - Effector function
  - Probably far more physiological functions
- Use - treatment of Paget's disease

# Endocrine pancreas

- Islets of Langerhans of the pancreas
  - Insulin
  - Glucagon
  - Somatostatin
  - Pancreatic polypeptide
- Especially in the pancreatic tail
- Only 2% of total pancreas
- 1 – 2 millions
- Rich blood supply, the hepatic portal vein
- Cell structure
  - A, B, D, and F cells
  - A – glucagon, they surround B cells, about 20%
  - B – insulin, the most numerous, 60-75% of islet cells
  - D – somatostatin
  - F – pancreatic polypeptide, particularly the rear part of the head of pancreas

# Insulin

- The polypeptide, 2 chains, prepropeptide, which is encoded by *INS*
- Highly conservative structure
- Frederick Sanger 1951
- Chromosome 11
- Several regulatory sequences in the promoter - binding of transcription factors (Par1, Pdx2, Oct1, ISF, Pax4, USF1/USF2, CREB, CREME2A, NeuroD1, and HEB)
- Next mechanisms that regulate insulin „availability“
  - Stability of mRNA
  - Translation processes
  - Posttranslational modifications
- After modifications in the vesicles originating in GA
- These are then transported, exocytosis
- transport across the basal membrane of B cells, fenestrations enable further secretion into the blood
- NSILA – Nonsupressible Insulin-Like Activity, IGF-I/II
- Half time about 5 min
- Binding to insulin receptors and partial internalization
- degraded in endosomes, especially by insulin protease that is internalized together with insulin
- 80% of the secreted insulin is degraded in the liver and kidneys

# Insulin receptor

- Transmembrane, family of receptors with tyrosine kinase activity
- Activation with insulin, IGF-I and IGF-II
- *INSR* gene, alternative splicing that results in IR-A (exon 11+ = + 12 AMK) and IR-B (exon 11-) isoforms
- Tetramer, Mr = 340 000, two alpha and two beta chains are glycosylated
- A large similarity with the receptor for IGF-I, but distinct from receptor IGF-II

- Upon binding ligand ($\alpha$ chain) autophosphorylation of tyrosine residues on the intracellular catalytic TK domain of the $\beta$ chain
- Subsequently recruitment of adapter proteins
  - IRS (insulin receptor substrate proteins) – IRS1 - IRS4
  - SH-2B (SH2 domain-containing adapter protein B)
  - APS
  - PTP1B (protein-tyrosine phosphatase 1B)
- IRS-1 activates PI3 kinase, which catalyzes phosphorylation of PIP2 to form PIP3
- PTEN (phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase) catalyzes the conversion of PIP3 back to PI2
- Binding and activation of protein kinase Akt (protein kinase B, a serine / threonine-specific kinase) follows
- Phosphorylation of other proteins:
  - GSK3 (Glycogen synthase kinase 3) and subsequent activation of glycogen synthase
- Activation of transport of vesicles with GLUT-4, transport and fusion with the plasma membrane and their exposure
- Exposure of GLUT-4 also after physical exertion via mechanism of activated kinase 5´-AMP

# Insulin effects

- very complex
- Rapid/Intermediate/Delayed
- The most famous is the hypoglycemic effect
- Effect on the transport of amino acids and electrolytes
- The result is a „storing“ of sugars, proteins and fats
- Other effects
  - Entry into cells = potassium cause hypokalemia in patients with diabetic acidosis after treatment with insulin
  - Increase in activity of $Na^+K^+$ ATPase (The $Na^+/K^+$ATPase pumps sodium out of cells, while pumping potassium into cells)

# Main effects of insulin I

- Uptake and utilization of Glu by skeletal muscle during physical exertion and postprandially
  - Facilitate the transport of Glu
- „storage“ of Glu in the form of energy in liver
  - Inactivation of hepatic phosphorylase, which cleaves liver glycogen to Glu
  - Increased intake of Glu by increasing the activity of glucokinase - phosphorylated GIu is unable to go back through the plasma membrane
  - Activating enzymes of glycogen synthesis, mainly glycogen synthase
  - Increased conversion of excess GIu to FA
  - Inhibition of gluconeogenesis by inhibiting the relevant liver enzymes
- ! Insulin does not affect the intake of GIu by neurons (passive transport)
- Insulin increases the utilization of GIu by peripheral tissues
- Insulin increases the utilization of Glu by peripheral tissues
  - Increased GIu transport to the liver; glycogen concentration above a certain concentration (5-6%) blocks its further synthesis - Glu is converted to pyruvate and subsequently to acetyl-CoA, which is the starting substrate for the synthesis of FA
  - FA are released from the liver in the form of triglycerides in lipoproteins
  - Insulin activates lipoprotein lipase in the capillary walls of fat tissue - cleavage of triglycerides and release of FA, subsequently are taken into adipocytes and converted to triglycerides
  - Insulin inhibits hormone-sensitive lipase = inhibition of the release of triglycerides from fat tissue into the blood
  - Increased transport of Glu into adipocytes - minor used for the synthesis of FA, but majority for $\alpha$-glycerolphosphate

# Main effects of insulin II

- Insulin and protein metabolism
  - Insulin induces protein synthesis
  - Stimulation of transport (increased capacity of transport systems) of many AMK into cells (especially valine, leucine, isoleucine, tyrosine, and phenylalanine)
  - Increased mRNA translation - creation of proteins
  - Increased DNA transcription
  - Inhibition of protein catabolism – reduction of cellular protein degradation
  - Reduction of hepatic gluconeogenesis - plasma AAs are the substrate!

# Glucose transporters

- Facilitated diffusion, secondary active transport with $Na^+$ in the intestine and kidney
- Muscle, adipose tissue and other - glucose transporters
- 12 transmembrane segments, N- and C- terminal ends intracellularly
- In mammals, 12 different glucose transporters divided into three classes have been described, commonly known 7
  - They differ in affinity to glucose, as well as in expression in various tissues
  - Ex. GLUT-1 - mainly in fetal tissue, in adults especially in Ery and enterocytes and is responsible for maintenance of basal level of Glu to keep cellular processes
- GLUT-4
  - Muscle and fat tissue
  - Significant stimulation with insulin
  - Upon entry of Glu into cells the rate of phosphorylation is further controlled (growth hormone and cortisol in certain tissues inhibit this process)
- Increased entry of Glu mediated by insulin to the liver by the mechanism of increased phosphorylation of Glu

| | Function | $K_m$ (mM)[a] | Major Sites of Expression |
|---|---|---|---|
| **Secondary active transport ($Na^1$-glucose cotransport)** | | | |
| SGLT 1 | Absorption of glucose | 0.1–1.0 | Small intestine, renal tubules |
| SGLT 2 | Absorption of glucose | 1.6 | Renal tubules |
| **Facilitated diffusion** | | | |
| GLUT 1 | Basal glucose uptake | 1–2 | Placenta, blood-brain barrier, brain, red cells, kidneys, colon, many other organs |
| GLUT 2 | B-cell glucose sensor; transport out of intestinal and renal epithelial cells | 12–20 | B cells of islets, liver, epithelial cells of small intestine, kidneys |
| GLUT 3 | Basal glucose uptake | <1 | Brain, placenta, kidneys, many other organs |
| GLUT 4 | Insulin-stimulated glucose uptake | 5 | Skeletal and cardiac muscle, adipose tissue, other tissues |
| GLUT 5 | Fructose transport | 1–2 | Jejunum, sperm |
| GLUT 6 | None | — | Pseudogene |
| GLUT 7 | Glucose 6-phosphate ransporter in endoplasmic reticulum | — | Liver, ? other tissues |

Barret, K.E., Boitano, S., Barman, S.M., Brooks, H.L. Ganong´s Review of Medical Physiology. 23rd Ed. McGraw-Hill Companies 2010

# Lack and excess of insulin

- Diabetes mellitus
  - Experimentally can be induced by substances that destroy the B cells - alloxan, streptozocin
  - Inhibition of insulin secretion
  - Antibodies
  - Polyuria, polydipsia, weight loss despite polyphagia, hyperglycemia, glycosuria, ketosis, acidosis, coma
    - Reduced entry of Glu to peripheral tissues (reduced Glu utilization)
    - Increased release of Glu into the blood from the liver
    - An excess of extracellular Glu and contrary, the lack of intracellular Glu - "starvation in the middle of excess"
    - Subsequently absolute or relative hypersecretion of glucagon
  - Test of glucose tolerance, impaired glucose tolerance
    - Decreased peripheral glucose uptake without altering intestinal absorption and reabsorption in the kidney
    - Impaired glucostatic function of liver (insulin promotes glycogen synthesis and inhibits release of Glu by liver)
      - Inactivation of liver phosphorylase
      - Reduced intake of Glu by hepatocytes (reduction in the activity of glucokinase)
      - Reduced glycogen synthesis
  - The effects of hyperglycaemia
    - blood hyperosmolarity
    - glycosuria
    - Significant loss of water (osmotic diuresis) – polydipsia
    - Non-enzymatic glycosylation of hemoglobin A ($HbA_{1C}$) – marker
  - Intracellular glucose deficit
    - Increased catabolism of fats and proteins – ketoacidosis
    - Probably cause of diabetic hyperphagia

- In particular, the effects of hypoglycemia on the nervous system - why?
  - Limited carbohydrate stores
  - Subsequently intake of Glu
  - Palpitations, sweating, nervousness = activity of autonomic nerves - why?
  - neuroglycopenic symptoms
    - Abnormal mentation, impaired judgement
    - Nonspecific dysphoria, anxiety, moodiness, depression, crying, fear of dying, suicidal thoughts
    - Negativism, irritability, belligerence, combativeness, rage
    - Personality change, emotional lability
    - Fatigue, weakness, apathy, lethargy, daydreaming
    - Confusion, amnesia, dizziness, delirium
    - Staring, "glassy" look, blurred vision, double vision
    - Automatic behavior
    - Difficulty speaking, slurred speech
    - Ataxia, incoordination, sometimes mistaken for "drunkenness"
    - Focal or general motor deficit, paralysis, hemiparesis
    - Paresthesia, headache
    - Stupor, coma, abnormal breathing
    - Generalized or focal seizures
- compensatory mechanisms
  - Inhibition of insulin secretion
  - Secretion of glucagon, adrenaline (increased release of Glu from the liver), growth hormone and cortisol (decrease utilization of Glu in tissues)

# Diabetes mellitus and changes in metabolism

- Metabolism of proteins
  - Increased rate of catabolism of AAs and their increased conversion to Glu in liver
  - Measurement of the ratio of Glu to the nitrogen in urine (D / N ratio)
  - Importance of glucagon and hyperglucagonaemia (stimulation of gluconeogenesis), and adrenal glucocorticoids
  - Negative nitrogen balance (gluconeogenesis versus reduced protein synthesis) associated with decreased resistance to infections
- Lipid metabolism
  - Acceleration fat catabolism with increased production of ketones
  - Reduced synthesis of fatty acids and triglycerides (30-40% versus 5% for DM; reduced conversion of Glu to FA in fat reserves)
  - Increased level of free FA (NEFA, UFA, FFA) – the absence of inhibition of hormone-sensitive lipase in adipose tissue
  - Level of FFA is changed paralelly with level of Glu
  - Catabolism of FA to acetyl-CoA, excess of acetyl-CoA is converted to ketones
    - Formation of acetacetyl-CoA and conversion to acetoacetate and acetone and β-hydroxybutyrate
    - An important source of energy during starvation, but very high levels of ketones, together with reduced utilization of ketones
    - Insulin increases the utilization of ketones in the muscles
    - β-hydroxybutyrate is responsible for acidosis (stimulation of the respiratory center, Kussmaul breathing), subsequently loss of $Na^+$ and $K^+$
    - Coma occurs as a result of dehydration and acidosis (lactic acidosis, hyperosmolar coma)
- Metabolism of cholesterol

  Usually increased plasma levels of mainly VLD and VLDL

# Diabetes mellitus

- Humans, but also other animals
- Many complications
  - Vascular changes
    - Intracellular conversion of glucose to Amadori products
    - They form AGE (Advanced Glycosylation Products) - matrix protein changes
    - Microvascular changes
    - Diabetic retinopathy
    - Diabetic nephropathy
  - Macrovascular changes
    - Accelerated progression of atherosclerosis - a higher rate of some other diseases (IM)
  - Diabetic neuropathy
  - Reduced resistance to infections
- DM type I. = insulin dependent (deficiency of insulin), autoimmune disease? Antibodies against B cells
- DM type II. = resistance to insulin + disorders in insulin secretion depletion of B cells)
- Secondary diabetes - chronic pancreatitis, total pancreatectomy, Cushing's syndrome, acromegaly

# Regulation of insulin secretion

- In healthy subjects, fasting insulin levels is up to 502 $pmol.L^{-1}$
- After ingestion of food up to 10-fold rise occurs
- Approximately amount of 287 mmol of insulin is secreted daily
- Regulation – particularly the feedback via action of blood Glu in the B cells of the pancreas
  - GLUT-2
  - Glu subsequently converted by glucokinase and subsequently ATP is created
  - ATP closes ATP-sensitive $K^+$ channels – it leads to the opening of the $Ca^{2+}$ sensitive channel and calcium ions influx
  - Increased intracellular levels of free calcium ions leads to stimulation of insulin secretion by exocytosis
  - Mechanism mediated by cAMP = increase in the concentration of intracellular $Ca^{2+}$
- Stimulation also some AMK, acetoacetate
  - The probable mechanism mediated by NO (arginine is precursor of NO)
- Insulin and autonomic nervous system
  - $\alpha$2-adrenergic receptors inhibit secretion of insulin
  - $\beta$-adrenergic receptors stimulate secretion of insulin
  - M4 receptors – stimulate secretion of insulin via *n. vagus,* increased intracellular free $Ca^{2+}$ + their release from ER
- GIT hormones
  - Glucagon, secretin, cholecystokinin, gastrin, gastric inhibitory peptide (GIP)
  - Glucagon-like polypeptide 1 (GLP-1)
  - Influx of $Ca^{2+}$ through voltage-gated calcium channels
- Depletion of $K^+$ - reduction of insulin secretion
- Oral hypoglycemics - stimulation of insulin secretion
  - Only for patients with B cells
  - Inhibition of $K^+$ channels
  - biguanides (phenformin and metformin)
    - Reduction of gluconeogenesis
  - Thiazolidinediones
  - They increase peripheral utilization of Glu
    - Mechanism of effect is mediated via PPAR-$\gamma$ receptor
- Note. Depletion of B cells

# Adipokines

- Adipose tissue = endocrine, production of proteins (peptides) with autocrine, paracrine or endocrine effect
- Adipokines – involved in the regulation of energy homeostasis
- Adipokines supporting activity of insulin
  - Leptin – predominantly by adipocytes, regulation of food intake? (adaptive reaction of the body to prolonged starvation)
  - Adiponectin – adipocytes, association with insulin resistance
  - Visfatin – produced predominantly by adipocytes of visceral adipose tissue, activation of the insulin signaling cascade - increases transport of Glu in the myocytes, stimulates lipogenesis and differentiation of adipocytes and reduces production of Glu in hepatocytes
- Adipokines inhibiting activity of insulin
  - TNF-$\alpha$ – its production correlates with the degree of obesity, insulin levels and insulin resistance, direct action on insulin cascade (phosphorylation of insulin receptor substrate), increases the release of FAA
  - Resistin – adipose tissue-specific secretory factor (ADSF) or C/EBP-epsilon-regulated myeloid-specific secreted cysteine-rich protein (XCP1), adipocytes, cells of the immune system, the effect of resistin on insulin resistance is not so great as previously thought
  - IL-6 – adipocytes, immune cells, endothelial cells, myocytes, fibroblasts, secretion pronounced in visceral fat; contributes to the development of insulin resistance

# Glucagon

- A cells of pancreas, linear peptide, Mr = 3485, 29 AAs
- Preproglucagon – 179 AAs
  - A cells, L cells in the lower part of the digestive tract and in the brain
  - In the A cells, glucagon is preproglucagon cleaved to a larger proglucagon fragment MPGF
  - In the L cells is MPGF primarily processed to glicentin
  - A and L cells produce also oxyntomodulin and, in addition, Glicentin-related pancreatic peptide GRPP is preserved (increases level of inzulin, decreases level of glucagon)
  - GLP-1 (7-36) is a potent stimulator of insulin secretion, together with GLP-2 is produced also in brain

# The action of glucagon

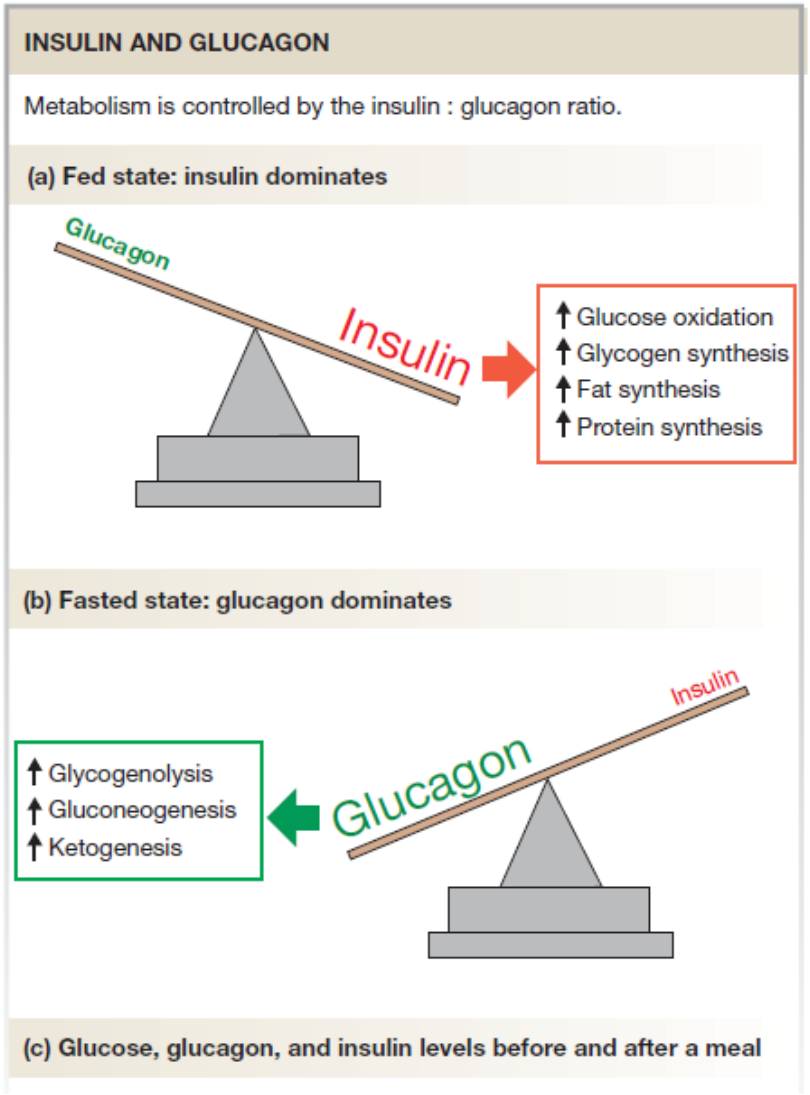
- Glycogenolysis (not in muscle!), stimulation of gluconeogenesis from available AAs, lipolytic and ketogenic effects (lipolytic activity) together with glycogenolysis
- Receptor – Mr = 62000, 485 AAs, serpentine receptor, *GCGR* gene
- In the liver activates adenylate cyclase through Gs = increase in intracellular cAMP concentration
- cAMP via protein kinase A activates phosphorylase (phosphorylase b kinase activation and formation of phosphorylase) - splitting of glycogen to Glu-1P, glycaemia rises after cleavage of P
- Activation of phospholipase C, increasing concentrations of free intracellular calcium ions and stimulation of glycogenolysis
- Protein kinase A decreases metabolism of Glu-6P = increased amount of Glu
- Inhibion of the conversion of Fru-6P to Fru-1,6-PP
- Furthermore, inhibition of storing of triglycerides in the liver
- ! Activation of adipocyte lipase = increased amount of FA in blood
- Positively inotropic effect on the heart (increased amount of cAMP) = increased strenght  of the heart
- Stimulation of GH secretion
- Increased blood circulation (blood flow) in kidneys
- Inhibition of gastric acid secretion
- Inhibition of bile secretion

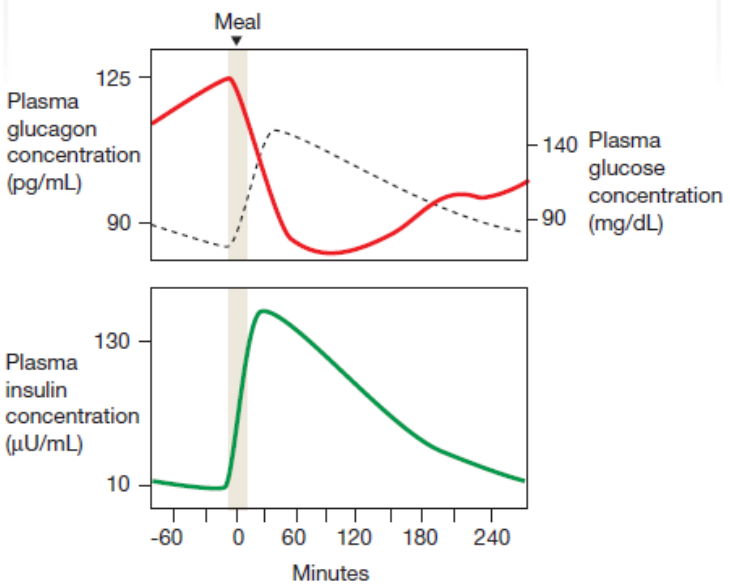
- Half time in the circulating blood 5 -10 min
- Degraded mainly in the liver, but also in other tissues
- Secreted into the portal vein

# Regulation of glucagon secretion

- Increased secretion during hypoglycaemia
- Together with glucagon secretion also GABA is released; GABA inhibits secretion of glucagon in A cells via activation of $GABA_A$ receptors
- Increased secretion after stimulation of the sympathetic innervation of the pancreas ($\beta$-adrenergic receptors, cAMP)
- Stimulated by *n. vagus*
- Inhibition after activation of $\alpha$-adrenergic receptors
- Increased secretion after a meal rich in protein
  - Note protective effect, preventing the hypoglycaemia
- Increased during starvation

# Insulin versus glucagon



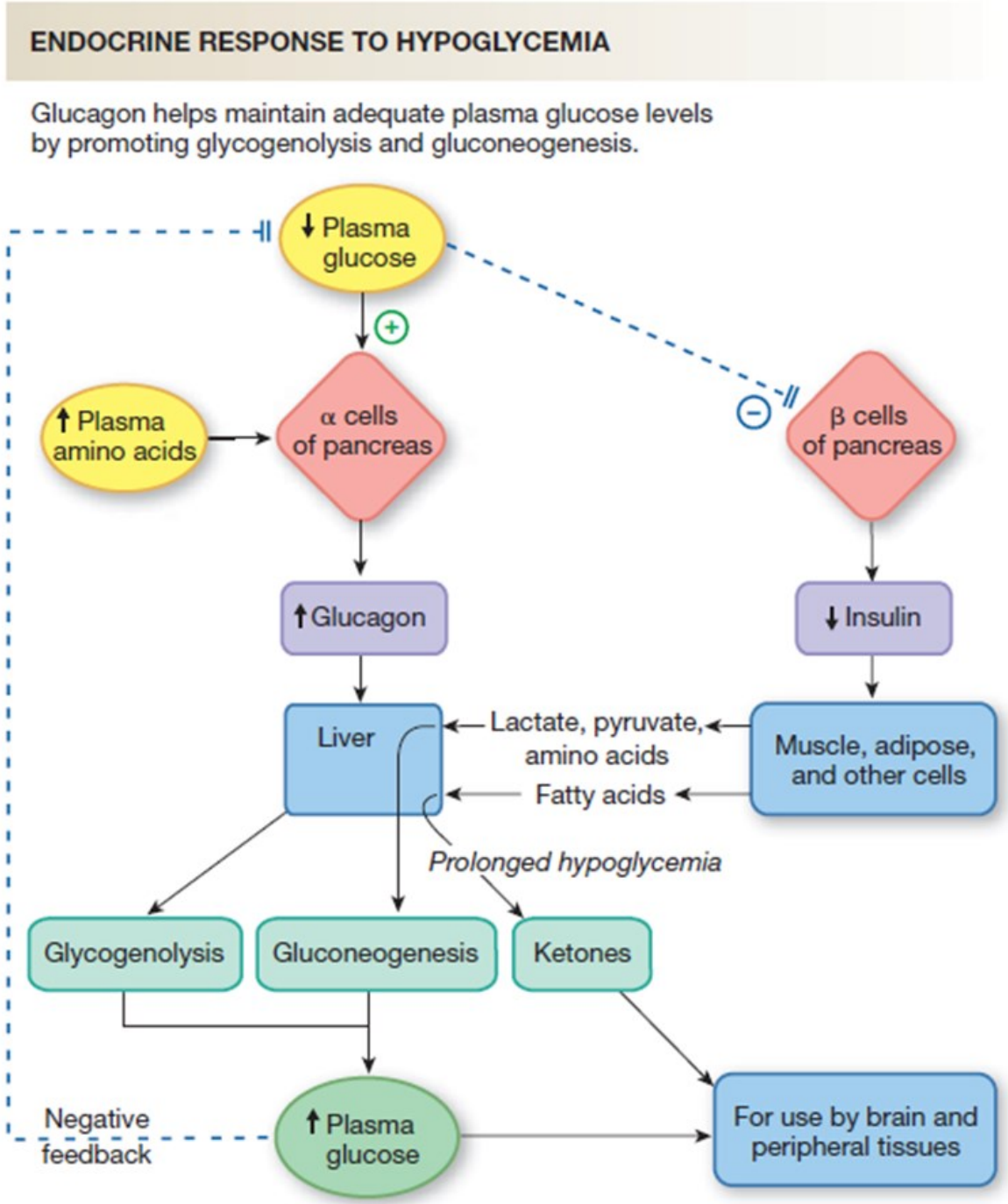




| Condition | Hepatic Glucose Storage (S) or Production (P)[a] | I/G |
|---|---|---|
| **Glucose availability** | | |
| Large carbohydrate meal | 4+ (S) | 70 |
| Intravenous glucose | 2+ (S) | 25 |
| Small meal | 1+ (S) | 7 |
| **Glucose need** | | |
| Overnight fast | 1+ (P) | 2.3 |
| Low-carbohydrate diet | 2+ (P) | 1.8 |
| Starvation | 4+ (P) | 0.4 |

[a]1+ to 4+ indicate relative magnitude.

Courtesy of RH Unger.

Silverthorn, D. U. Human Physiology – an Integrated Approach. 6th. edition. Pearson Education, Inc. 2012.




Silverthorn, D. U. Human Physiology – an Integrated Approach. 6th. edition. Pearson Education, Inc. 2012.

# Somatostatin

- D cells of the buňky Islets of Langerhans
- 14 AAs
- Extremely short half-life (approximately 3 min)
- Secretion is induced (stimulated) by:
  - Increased level of glucose in blood
  - Increased level of some AAs, especially arginine and leucine
  - Increased level of FAA
  - Increased levels of certain hormones of the upper gastrointestinal tract as a response to food intake
- Functions:
  - Decreases secretion of glucagon and insulin, but also pancreatic peptide
    - Reduction of utilization of nutrients - adaptation to their longer availability and utilization?
  - Decreases the motility of the stomach, duodenum and gall bladder
  - Decreases both secretion absorption in the GIT
  - Why? – decreased utilization of nutrients by the tissue, prevention of rapid exhaustion of the food and therefore making it available over longer period of time

# Pancreatic polypeptide

- Linear peptide AMK 36, created and secteted by the F cells
- Related are polypeptide YY (PYY, intestine, gastrointestinal hormone) and neuropeptide Y
- Cholinergic regulation, plasma levels are decreased by atropine
- Secretion increases after eating a diet with high content of proteins, but also after physical exertion, and during acute hypoglycemia
- Level is decreased by somatostatin and i.v. administration of glucose
- Slowing adsorption of nutrients (balancing "peaks"?)
- Elevated levels in *anorexia nervosa* - regulation of food intake?