Diabetes mellitus

Regulation of glucose metabolism
Insulin a ins. sensitivity vs. resistance
Classification of DM
PP of primary types of DM – T1DM and T2DM
Acute and chronic complications of DM

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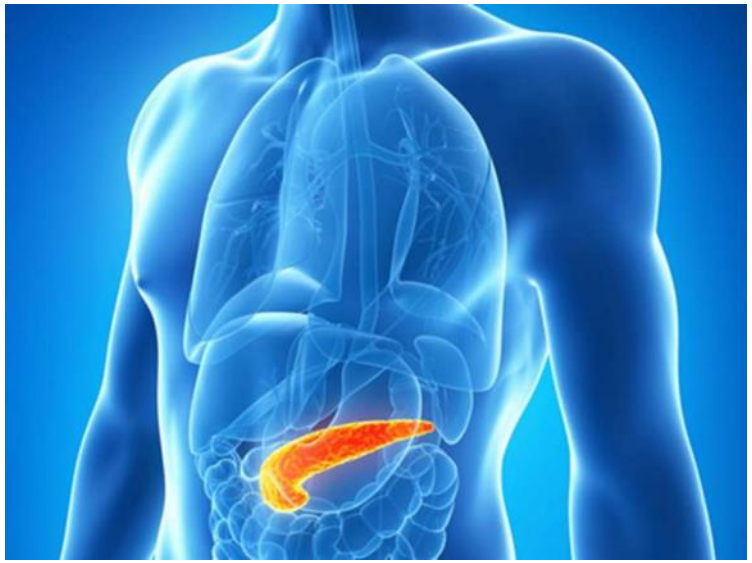
Definition of diabetes mellitus (DM)

- DM is a group of metabolic disorders characterized by **hyperglycemia** resulting from a lack of insulin effect
  - due to either defect in insulin secretion or insulin action

- **chronic hyperglycemia** leads to long-term cell, tissue & organ damage = **diabetic complications**
  - retina
  - kidney
  - nerves



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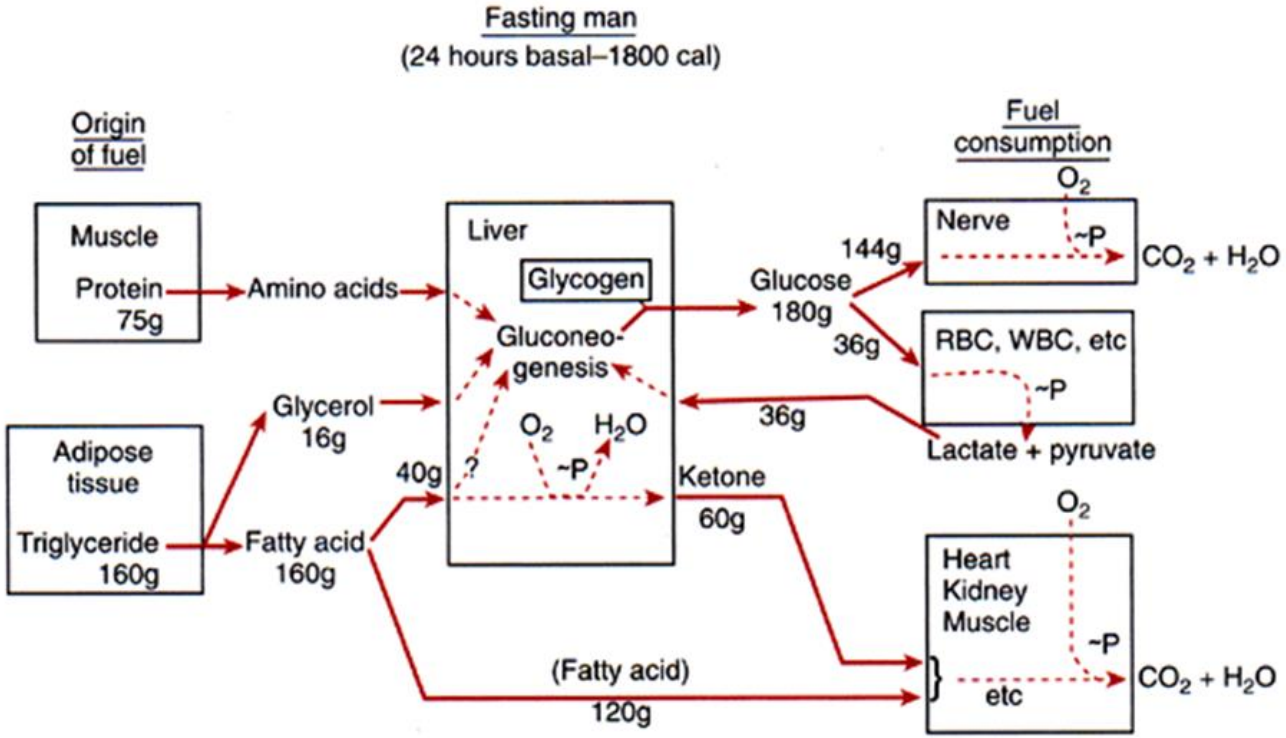
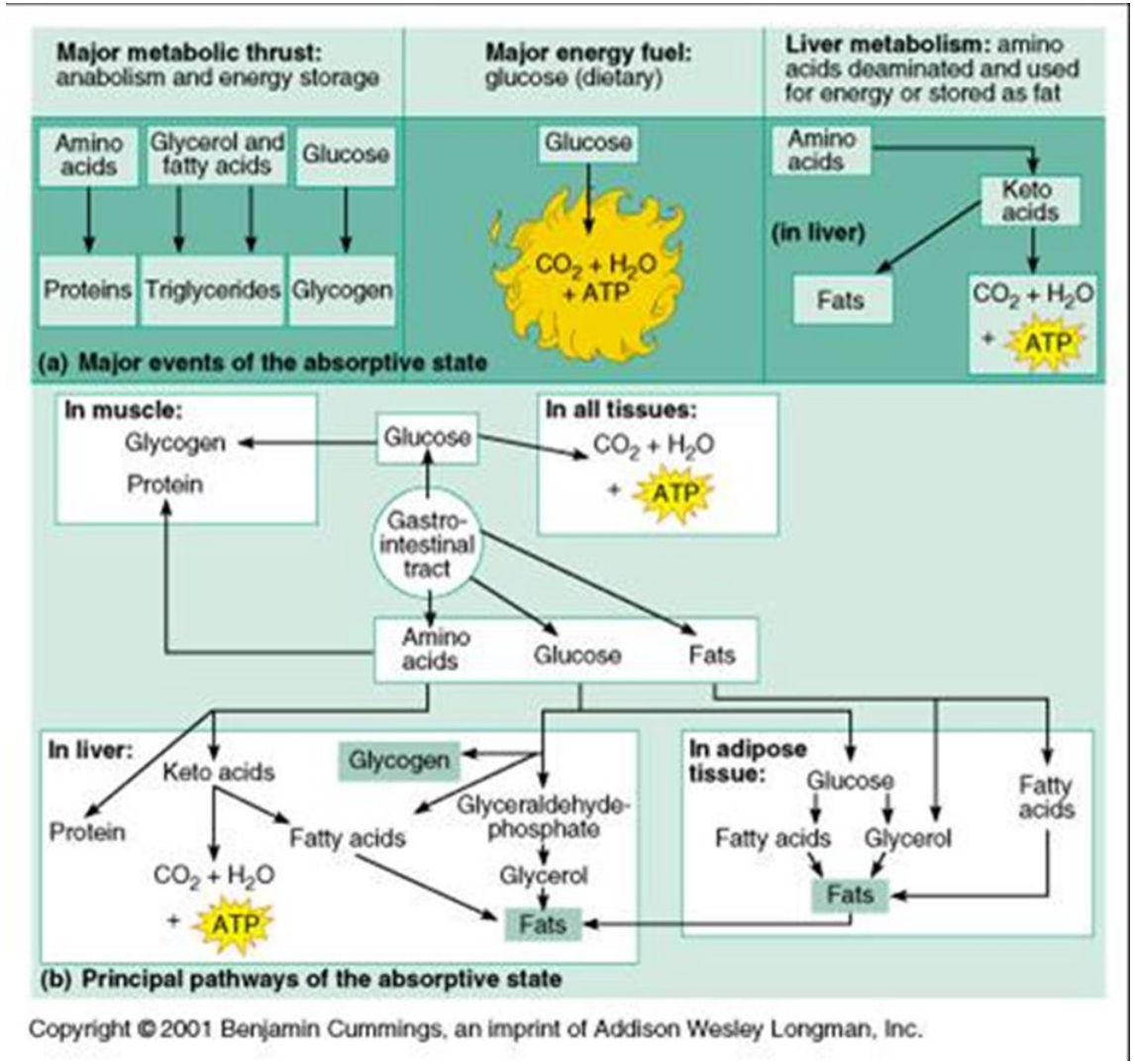
Fasting vs. absorptive state





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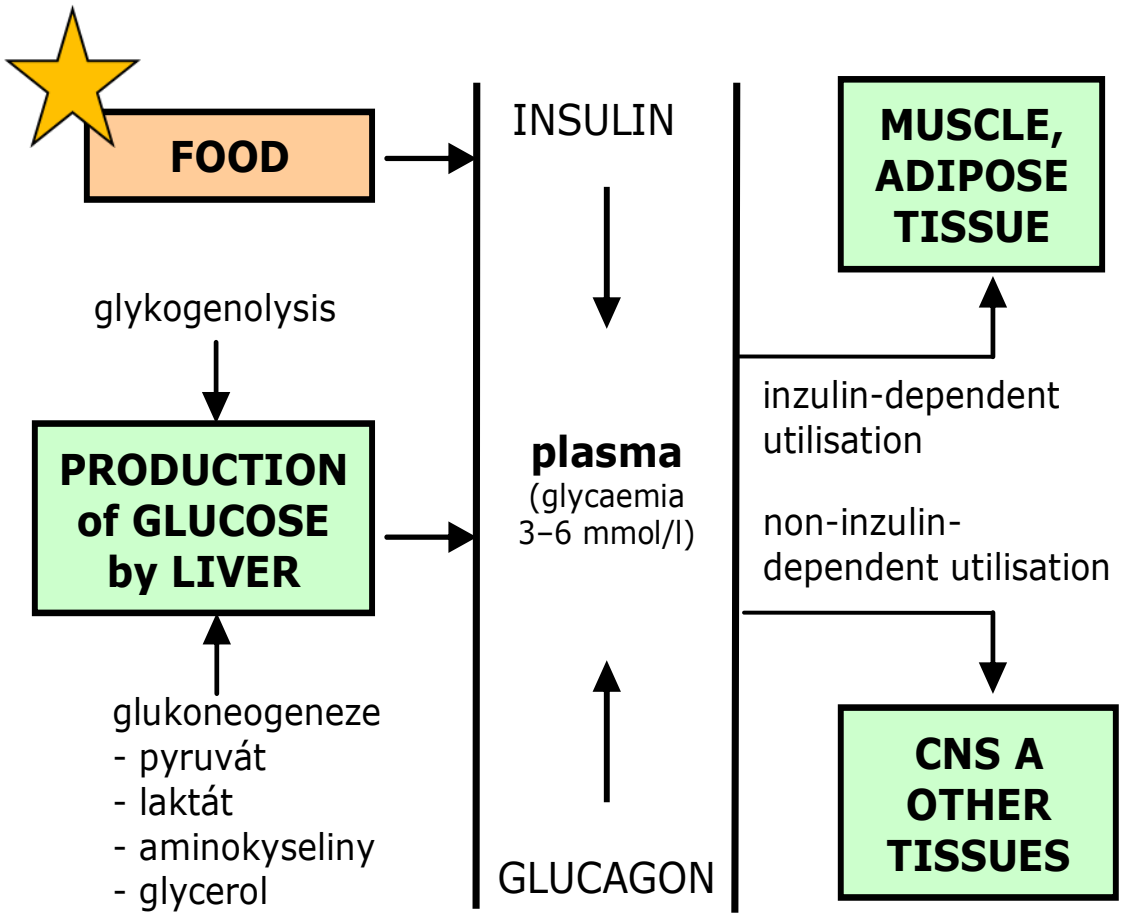
Regulation of glycemia



- humoral
  - principal
    - **insulin**
    - **glucagon**
  - auxiliary
    - glucocorticoids
    - adrenalin
    - growth hormone
- neural
  - sympaticus
    - hyperglycemia
  - parasympaticus
    - hypoglycemia

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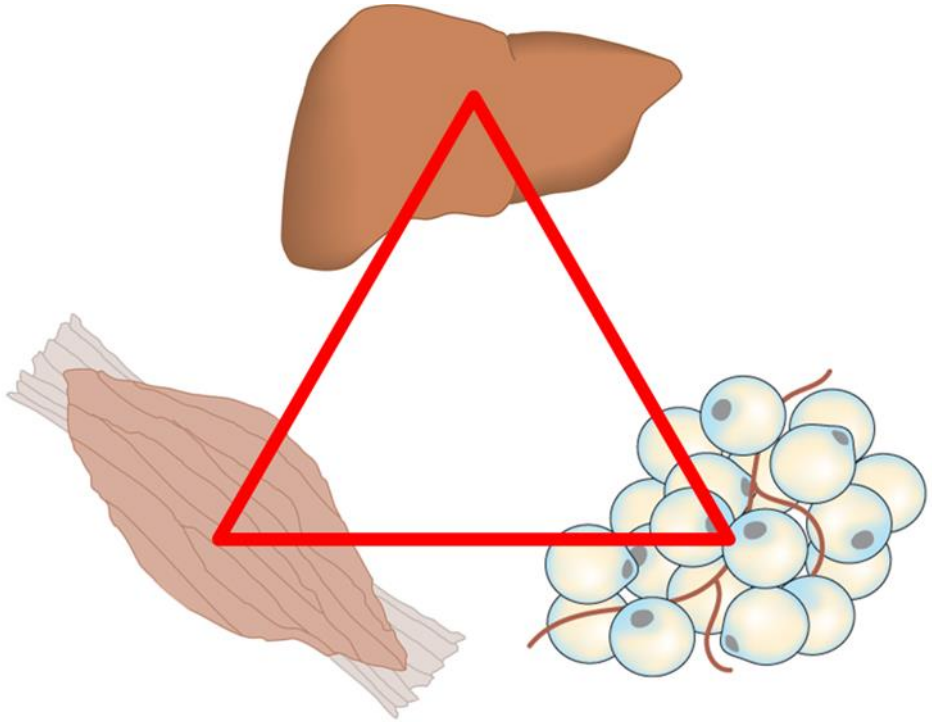
What happens (in healthy man) after meal = insulin orchestrates alocation and utilisation of nutrients

diabetic “triumvirate”



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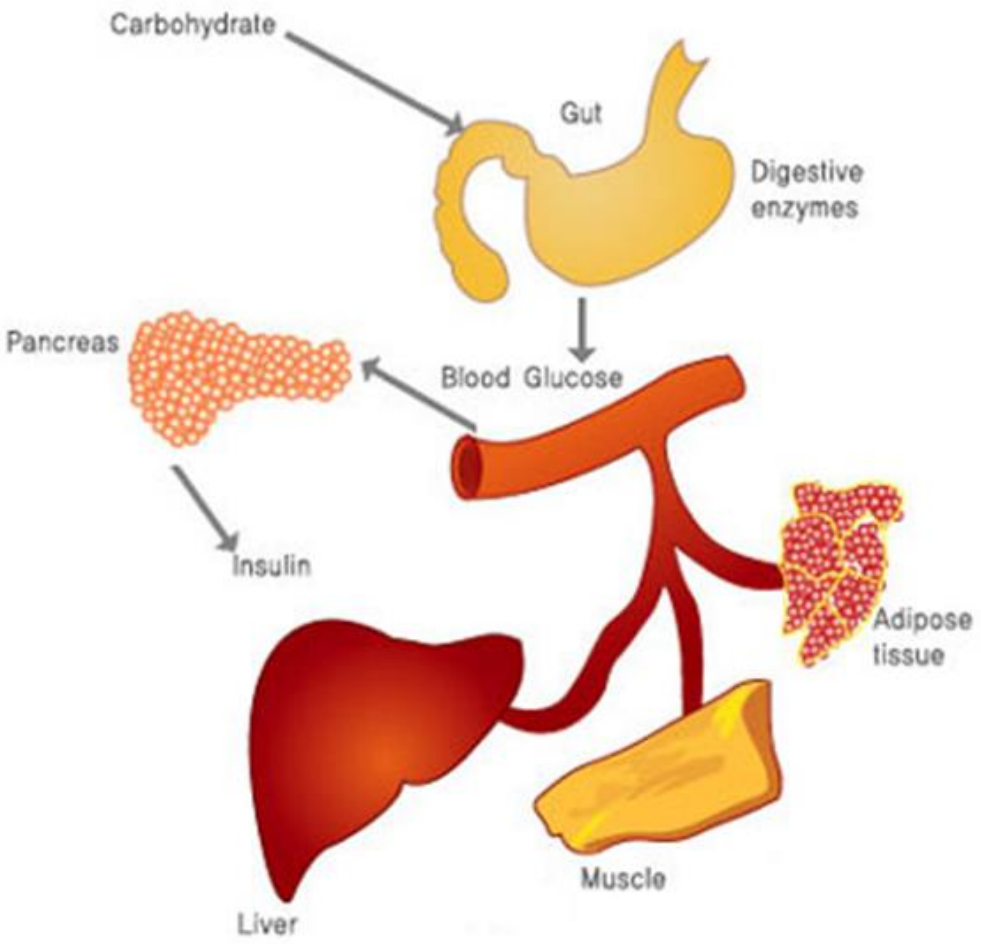
What happens (in healthy man) after meal = insulin orchestrates alocation and utilisation of nutrients



- liver
  - stimulation of glycogen formation (up to~ 5% of liver weight)
    - ↑ hexokinase, phosphophructokinase, glycogensynthase
    - ↓ G-6-P-kinase
  - inhibition of gluconeogenesis
    - ↓ PEPCK
  - fat formation
    - ↑ synthesis of FFA and VLDL
  - proteosynthesis
    - ↑ transport of AA
  - inhibition of ketogenesis
- muscle
  - translocation of GLUT4
  - formation of glycogen
  - proteosynthesis
    - ↑ transport of AA
- adipose tissue
  - translocation of GLUT4
    - Glc → glycerol
  - stimulation of adipogenesis
    - ↑ activity of LPL
      - hydrolysis of VLDL and resynthesis of TAG
    - ↓ hormone-sensitive lipase
- brain
  - insulin participates in the control of appetite/satiety

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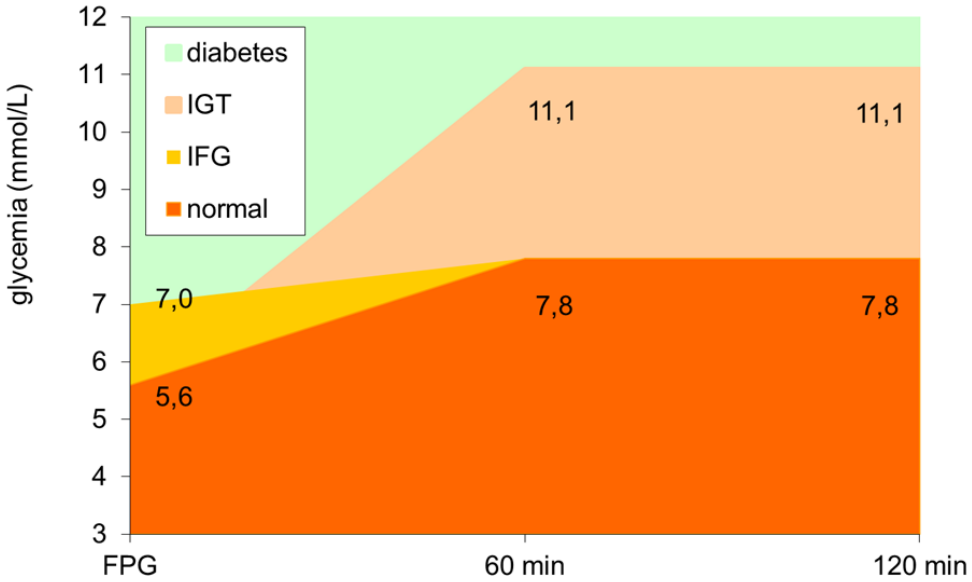
Diagnosis of DM

- diabetes
  - classical symptoms + random plasma glycemia ≥11.1 mmol/l (venous plasma)
    - random = any time of the day
    - symptoms include polyuria and polydipsia
  - FPG (fasting plasma glucose) ≥7.0 mmol/l
    - fasting means at least 8 h from the last meal
  - 2-h PG (postprandial glucose) ≥11.1 mmol/l during oGTT
    - oGTT: according to the WHO consists of FPG examination followed by a standard load of 75g of glucose (diluted in water) and examination of glycemia in 60th and 120th minute
- impaired glucose tolerance (IGT)
  - excluded <7.8 mmol/l
  - 2-h PG ≥7.8 - <11.1 mmol/l during oGTT
- impaired fasting glucose (IFG)
  - diabetes excluded by FPG ≤5.6 mmol/l
  - FPG ≥5.6 – <7 mmol/l



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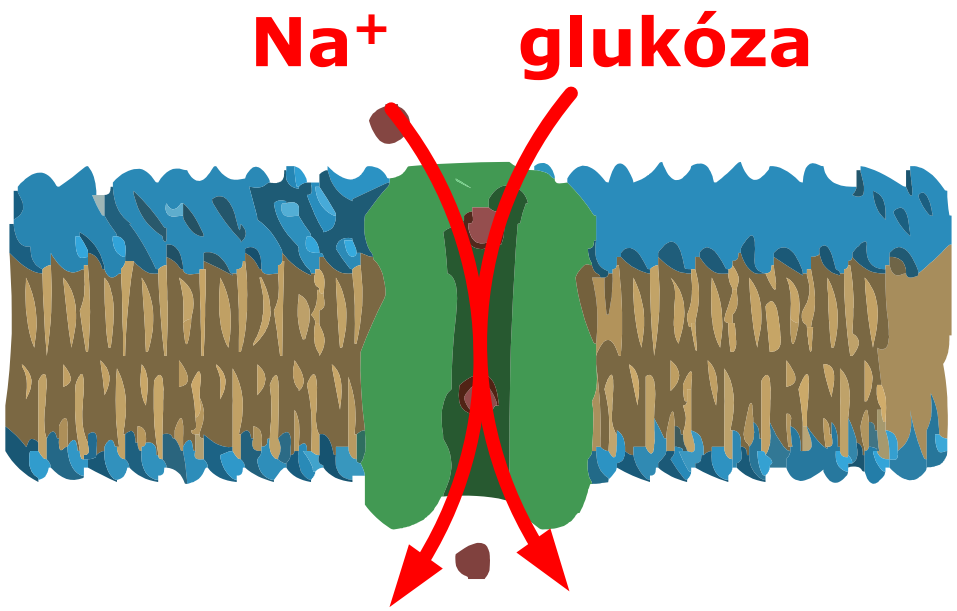
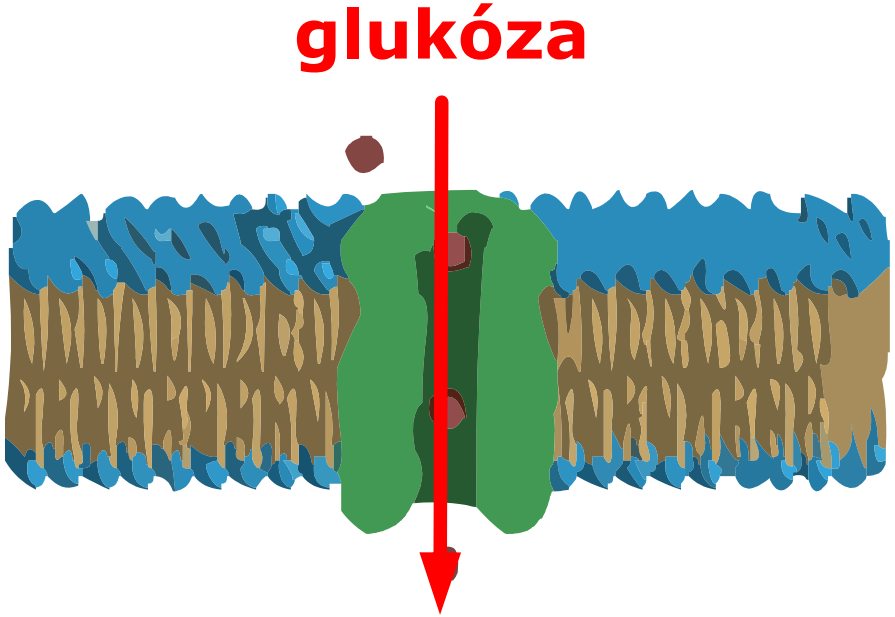
Q1: The way glucose enters the cell??





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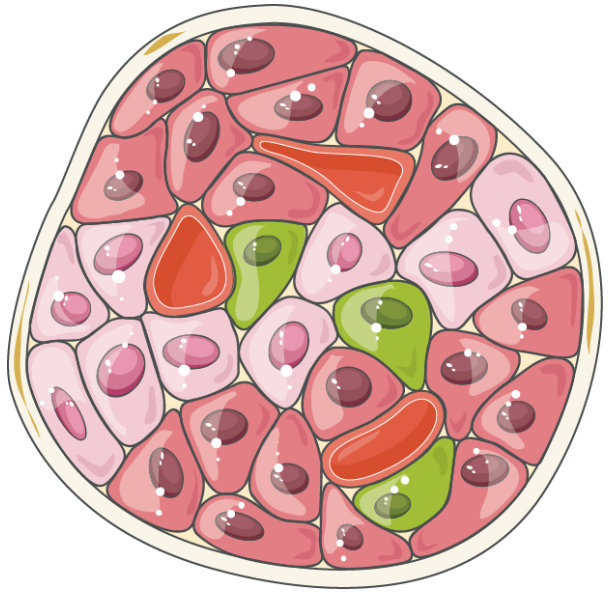

INSULIN, INSULIN SENSITIVITY VS. RESISTANCE

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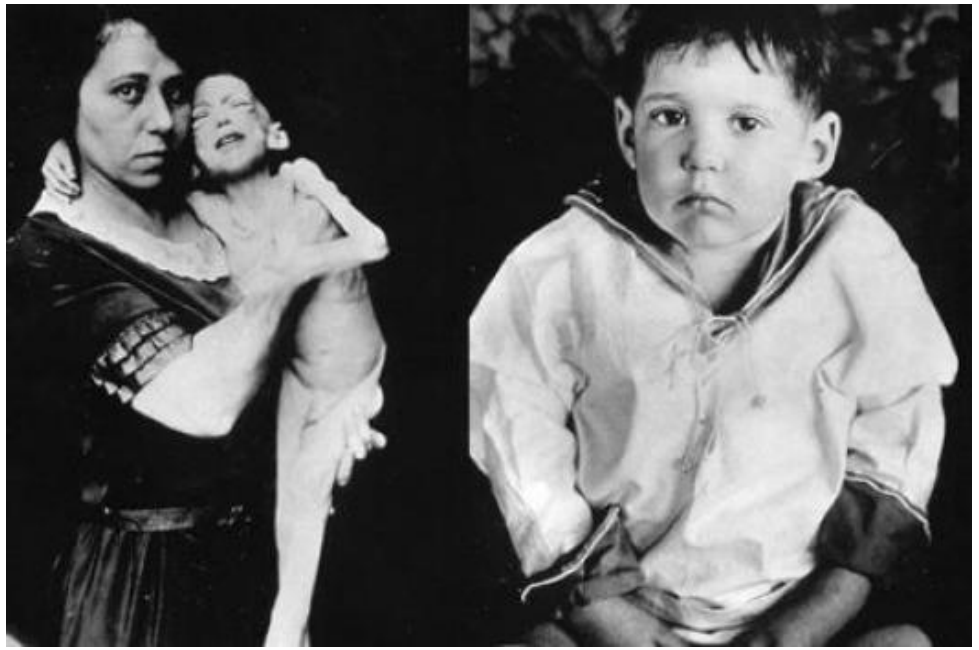
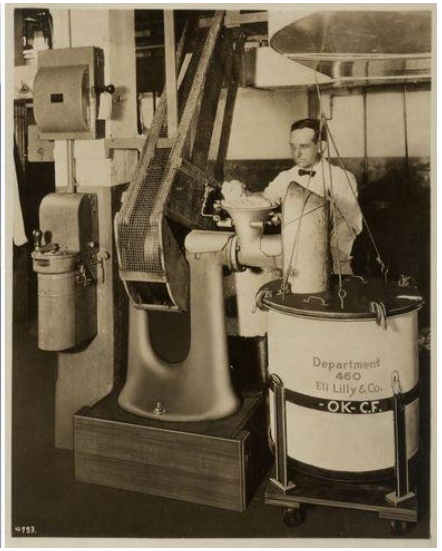
Insulin – world diabetes day

- 14/11 (od 1991)
- birthday of the man who co-discovered insulin, Frederick Banting
- Banting discovered insulin in 1922 alongside Charles Best under the directorship of John McLeod and with assistance of James Collip
- The Nobel Prize in Physiology or Medicine 1923 was awarded jointly to Frederick Grant Banting and John James Rickard Macleod "for the discovery of insulin"





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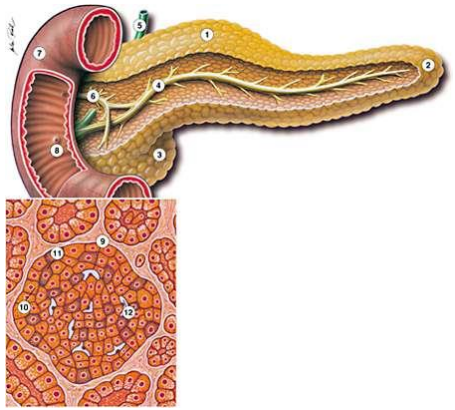
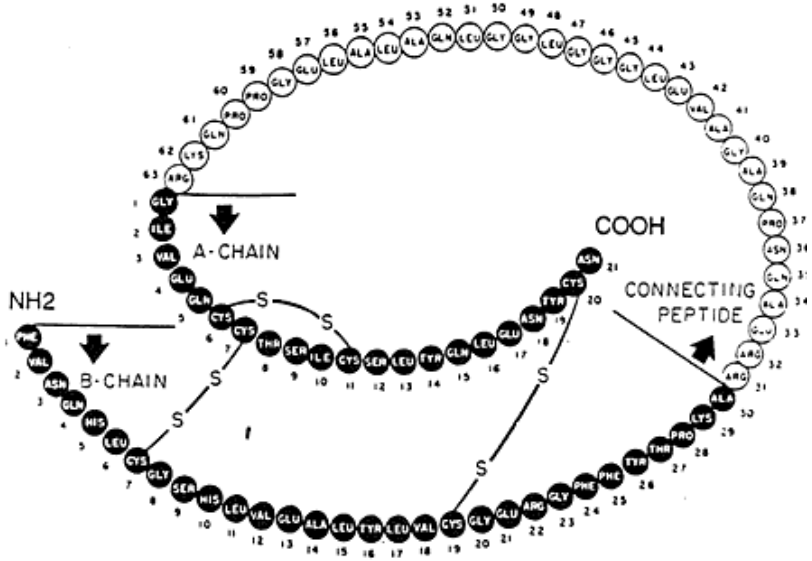
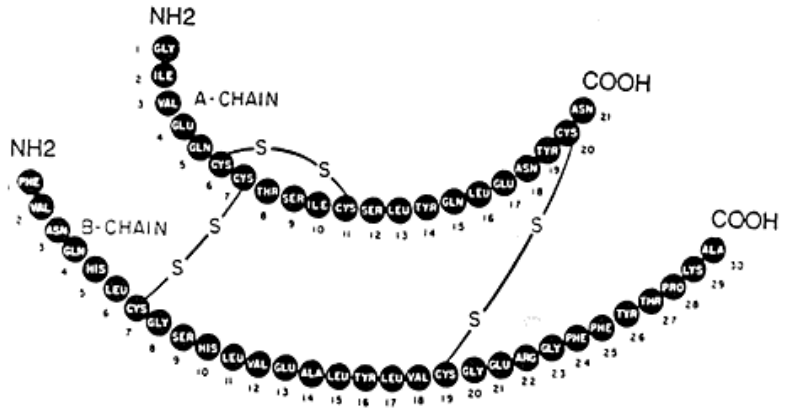
Insulin

- exocytosis from B-cells of islets of Langerhans into portal circulation
  - 50% degraded during first pass through liver
  - parallel cleavage of the C-peptide
- total daily production in healthy subject ~20-40 U
  - 1/2 **basal** (postabsortive) secretion
    - pulsatile (5 - 15 min intervals)
  - 1/2 **stimulated** (postprandial)
    - early phase (ready insulin)
      - Glc/K_{ATP}-dependent
    - late phase (synthesis de novo)
      - other secretagogues
- stimulation of secretion
  - <<<glucose
  - <<amino acids
  - <GIT hormones (incretins)
  - FFA
    - variable stimulation (length of chain & (un)saturation)!!
    - since insulin is acting also as peripheral “satiety” signal, reaching the satiety is delayed after fatty meal







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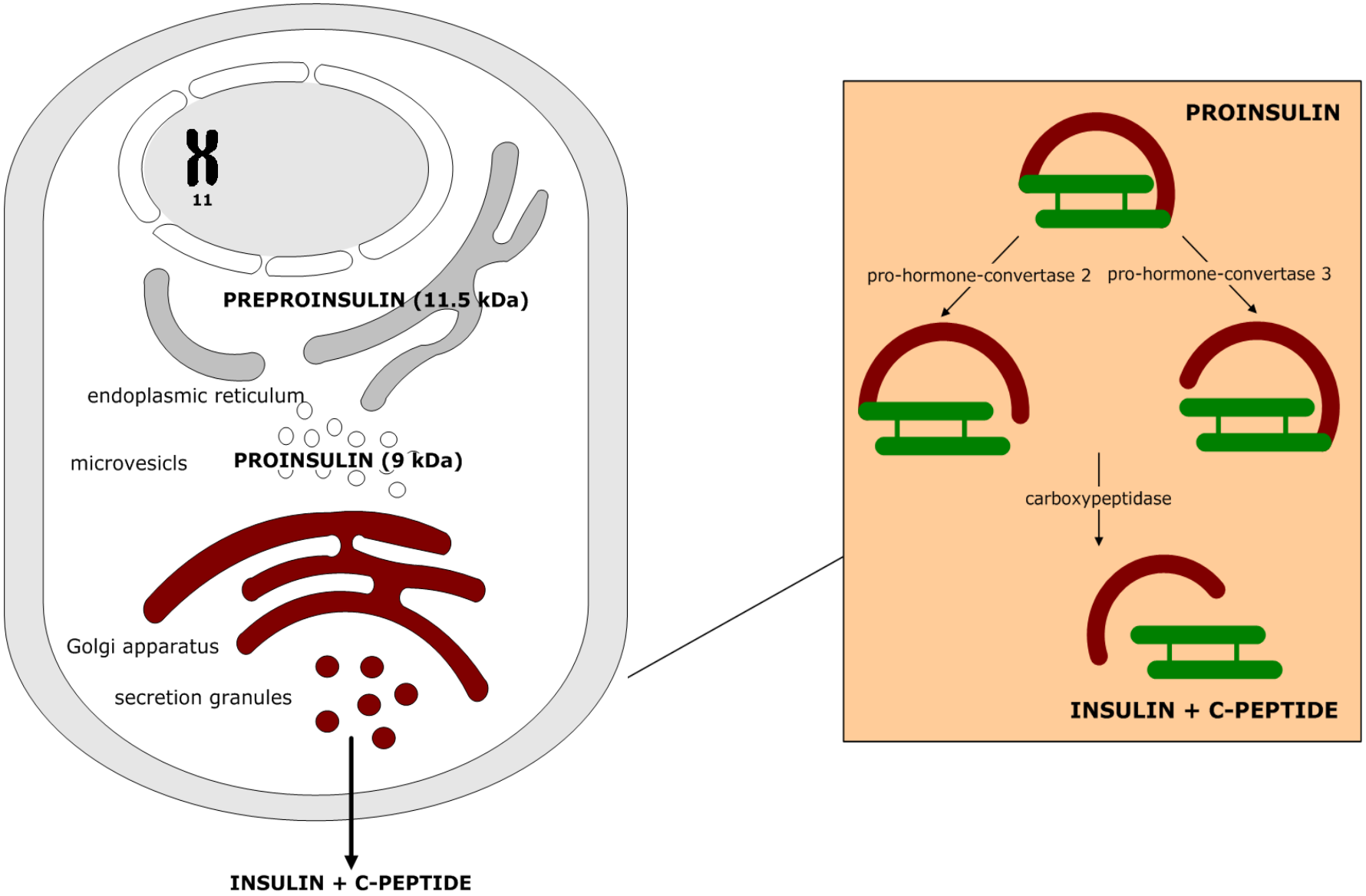
Insulin synthesis



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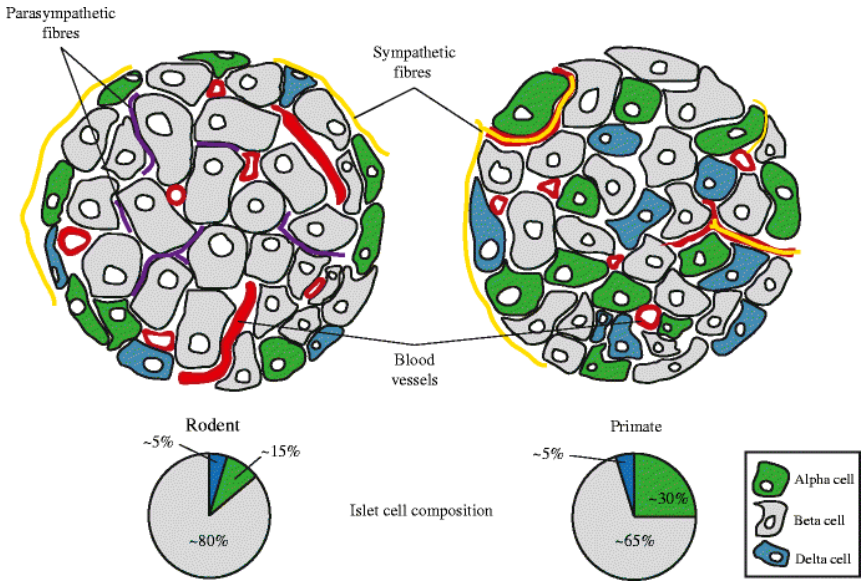
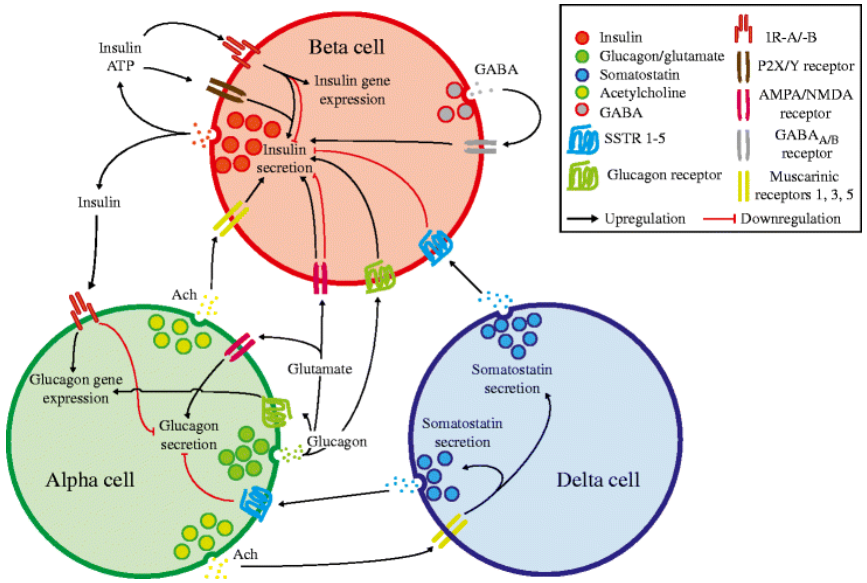
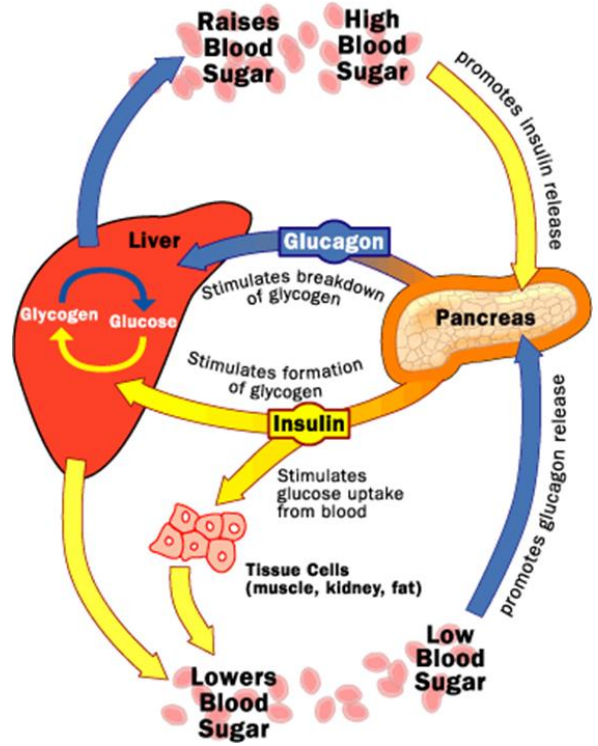
Langerhans islets - architecture

- The pancreatic islet blood flow is 5–10 times higher than that of the exocrine pancreas, and can be selectively enhanced whenever the need for insulin secretion is increased
- B-A-D flow hypothesis
  - that is why contra-regulation insulin/glucagon works so well







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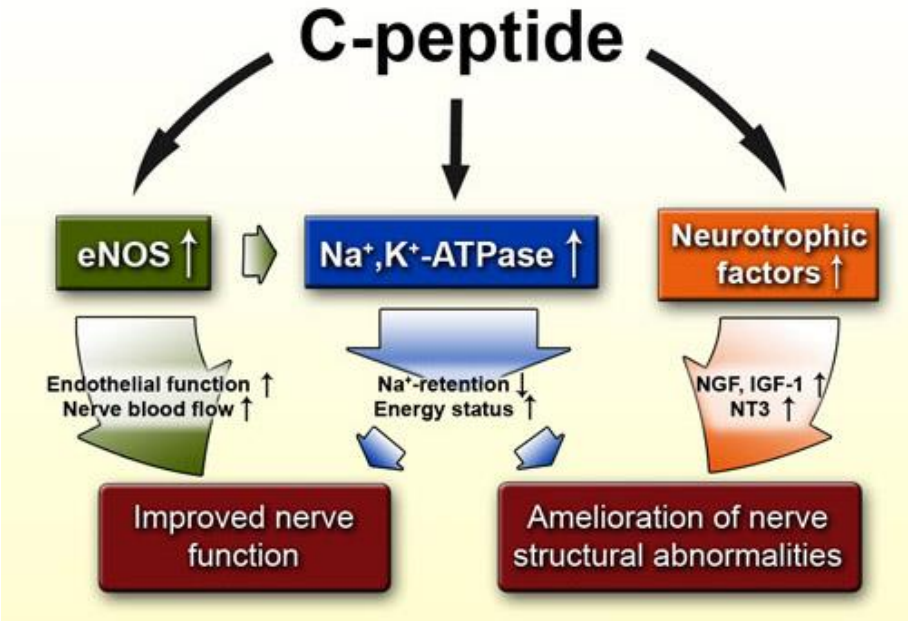
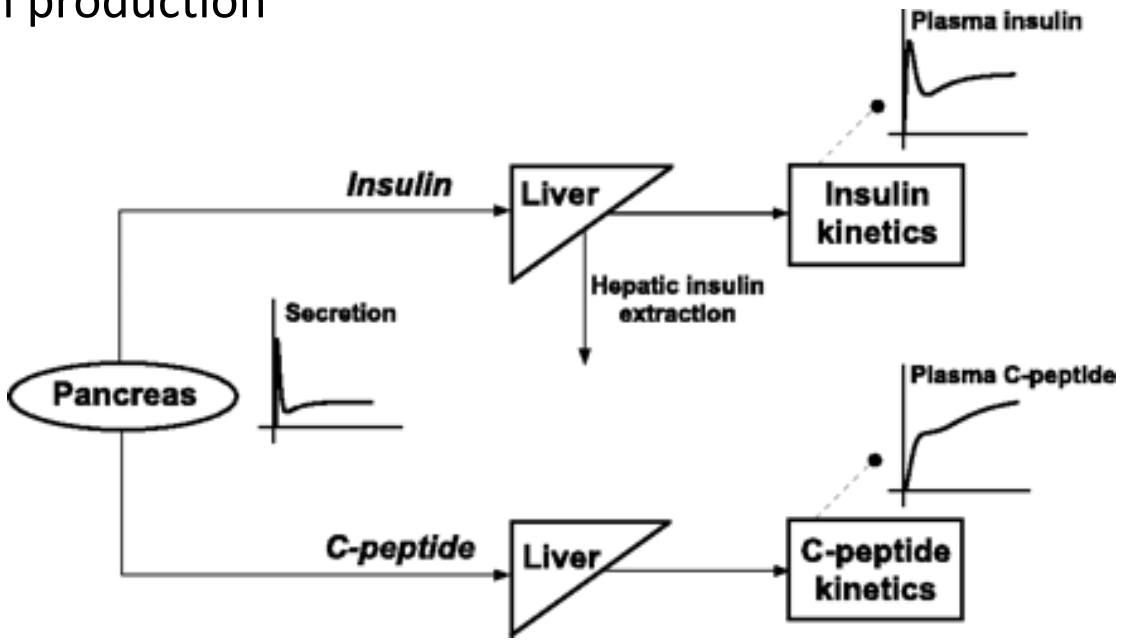
C peptide

- activity
  - certain beneficial vascular effects (nitric oxide)
- mainly diagnostic use
  - equimolar to insulin
  - unlike insulin, C-peptide is not degraded from portal blood in liver
  - the systemic concentration reflects endogenous insulin production





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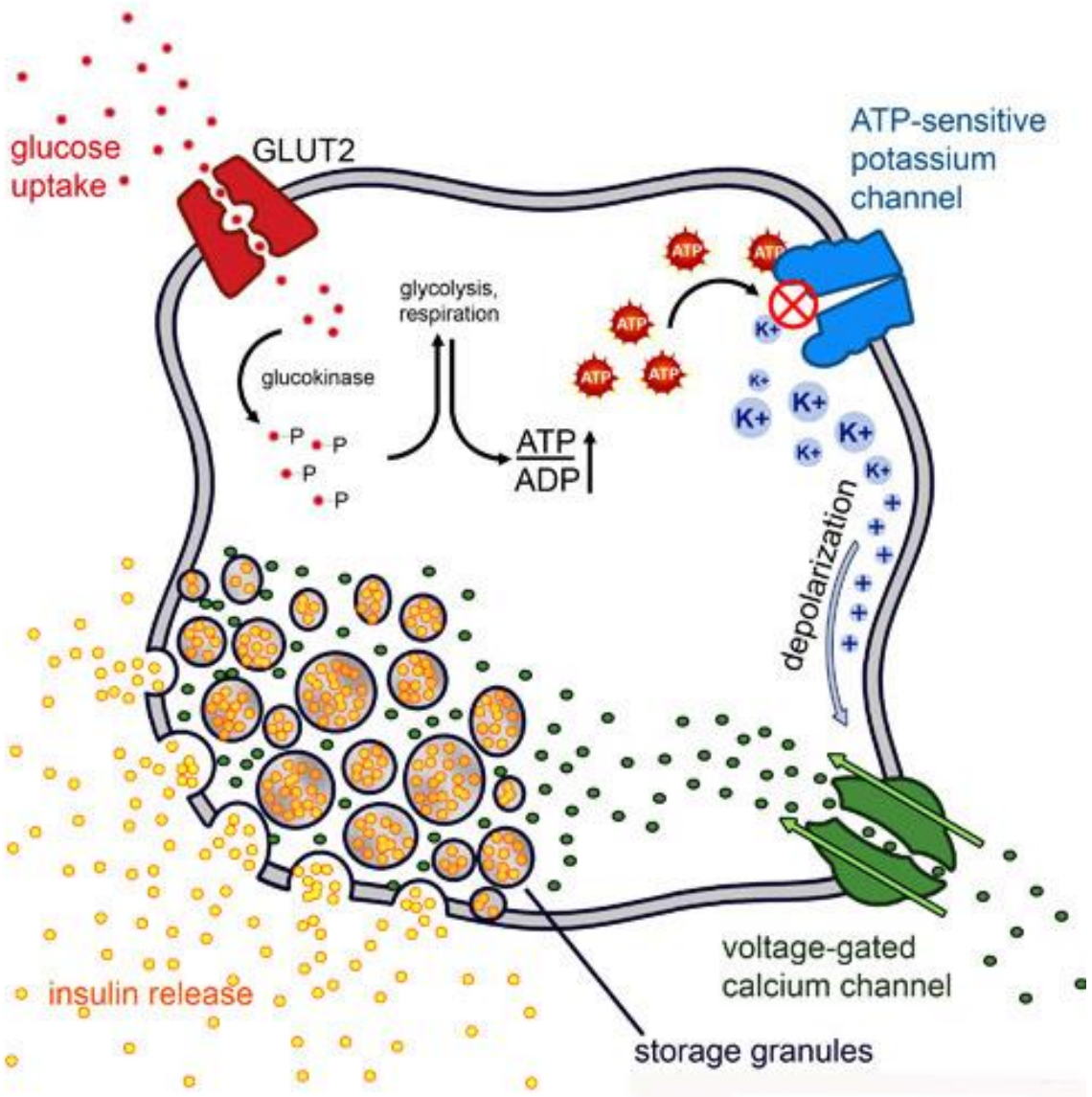
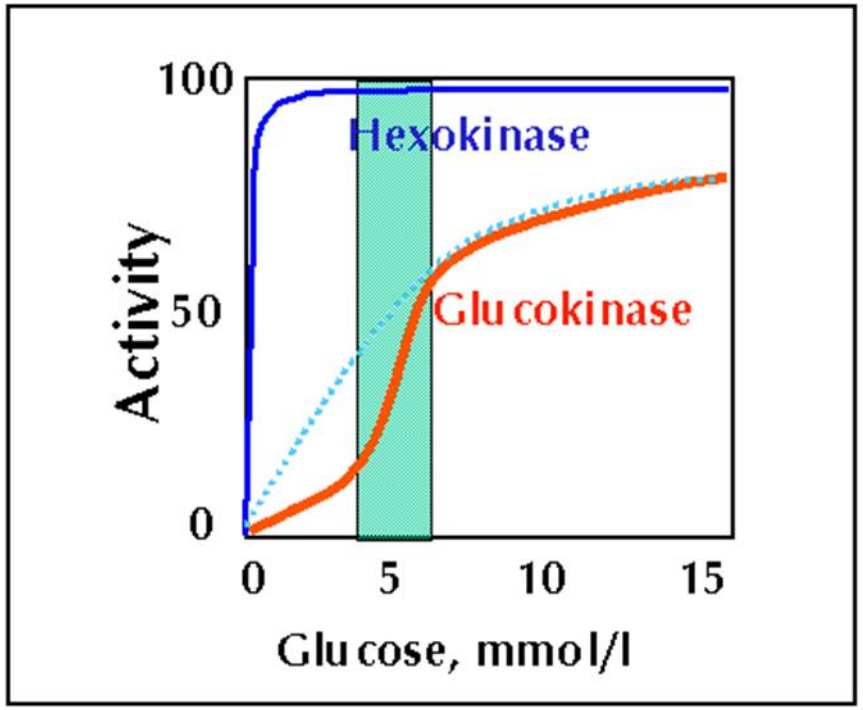
Coupling: glycemia – insulin secretion



Hexokinase vs. glucokinase



- **hexokinase** (ubiquitously with exception of liver and pancreatic b-cells)
  - activity increases with increased glucose but activity is inhibited by increased G6P
  - levels of enzyme are constitutive
  - only generates ATP when energy is required
- **glucokinase** (hepatocytes and b-cells)
  - is not normally active because its Km is lower than normal blood glucose levels
  - eating food increases glu in blood, activates glucokinase which converts glu to glycogen and fatty acids
  - activity increases with increased glucose but is not inhibited by increased glu6PO4
  - the levels of the protein are regulated by insulin
  - rate of reaction is driven by substrate-glucose not by demand for product-G6P
    - allows all glu available to be converted to G6P and then if excess present, it is converted to glycogen and from there to triglycerides and fatty acids

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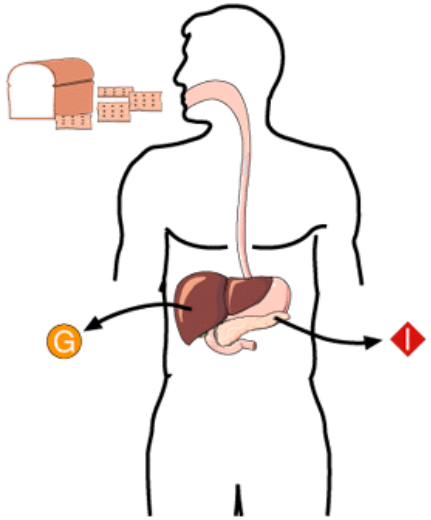
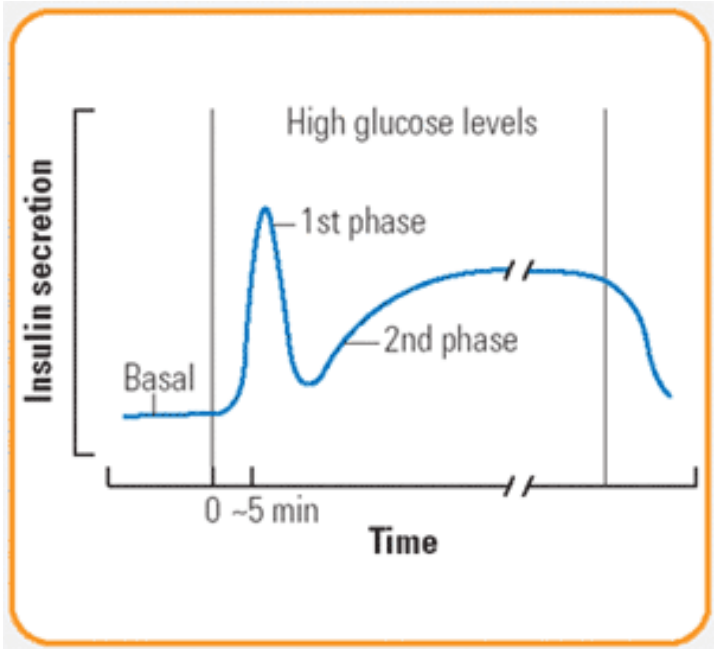
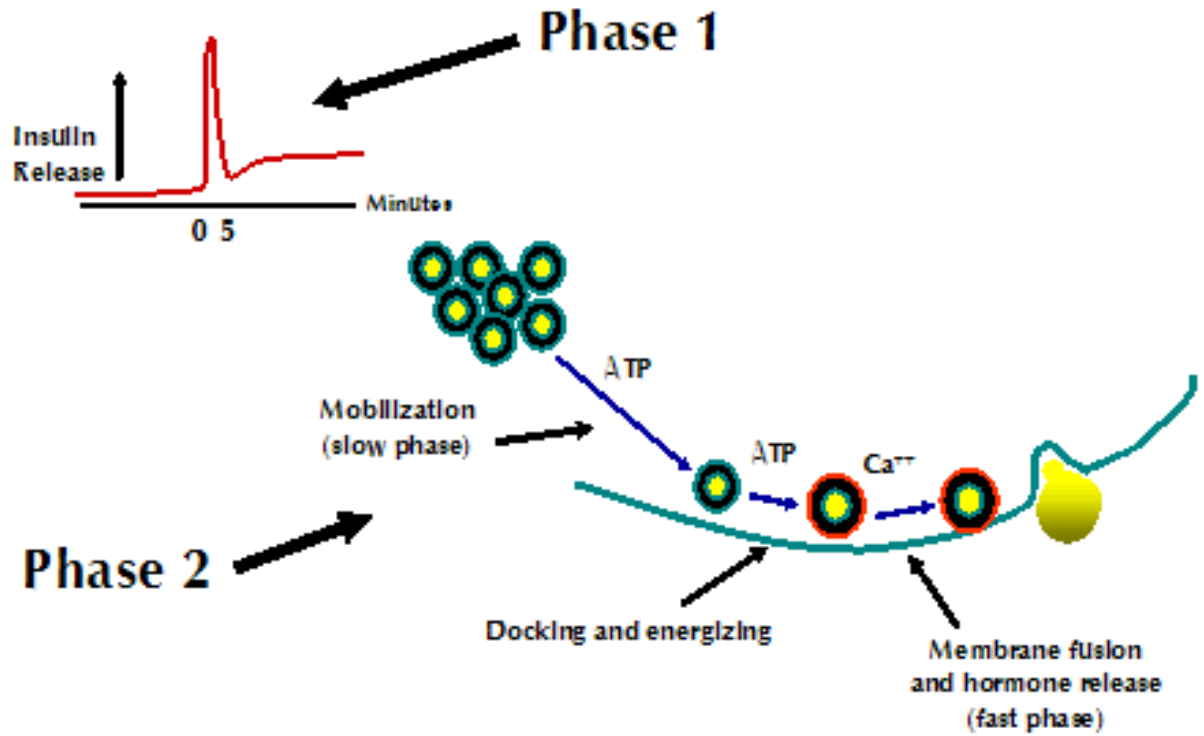




Insulin Secretion is Biphasic



- in vivo not so obvious
  - 1. phase – Glc/K_{ATP}-dependent
  - 2. phase – other secretagogues

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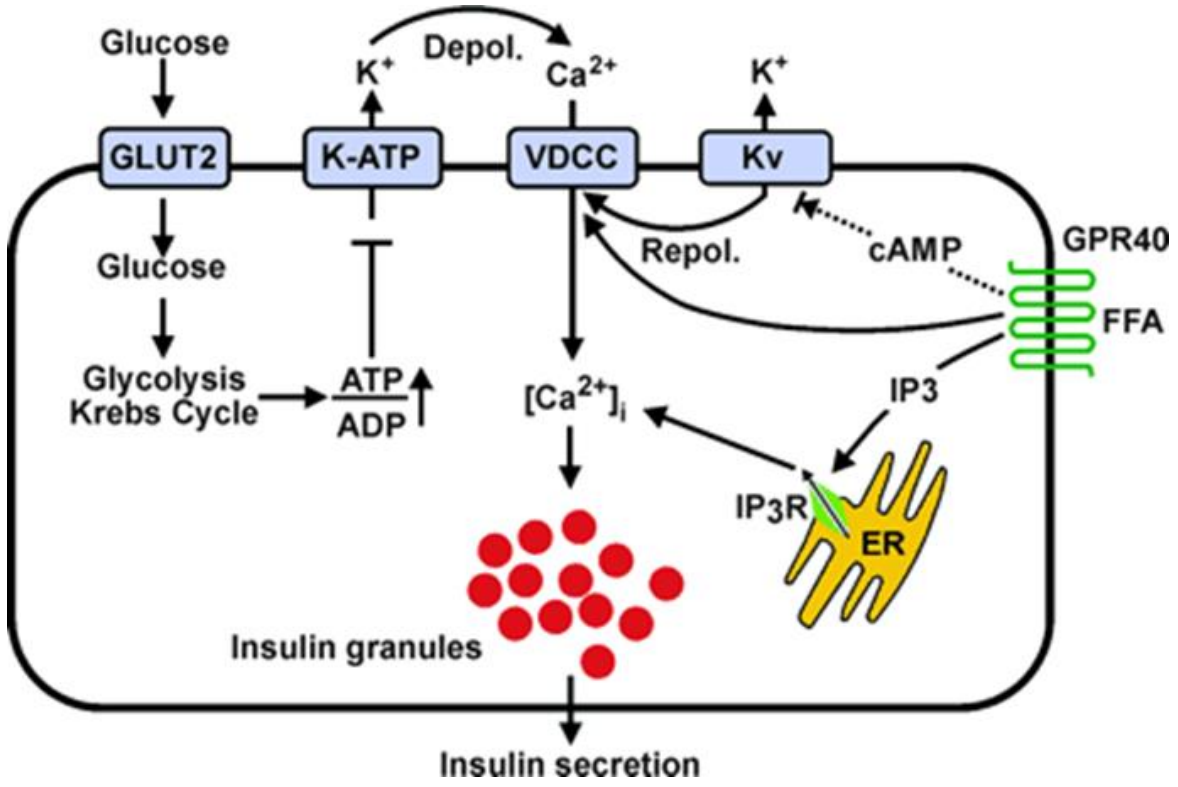
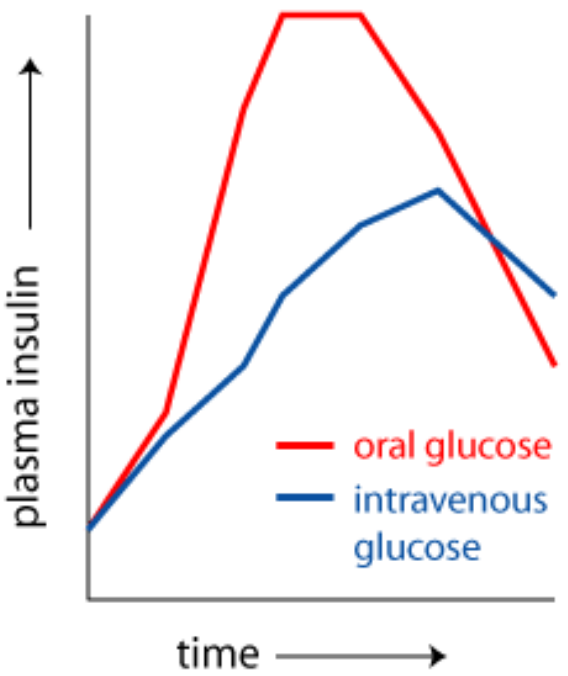
NEFA and insulin secretion

- NEFA can enter cells (incl. B-cells)
  - directly by diffusion across the membrane (short-chain FA) → metabolism (oxidation) → ATP …. insulin secretion
  - via receptor (GPR40) → see the figure
- however, long term exposure to NEFA, esp. long-chain saturated (e.g. palmitate), suppress secretion of insulin and damages B-cells



Incretins – enteroinsular axis

- GIT hormones produced by endocrine cells of small intestine stimulating insulin secretion even **before elevation of blood glucose**
  - Ins-secretion after oral Glc >> after i.v. Glc
    - hypoglycemia – if the patient still conscious then better to give Glc per os
- "forward" regulatory mechanism – anticipation of increase of Glc
- 2 major incretin hormones
  - GIP (glucose-dependent insulinotropic peptide or gastric inhibitory peptide)
  - GLP-1 (glucagon-like peptide-1)
- treatment of T2DM [= delayed effect of Glc on Ins stimulation] by incretin analogues
    - GLP-1 analogue - exenatide (GLP-receptor agonist)
    - DPP-4 inhibitors (dipeptyl peptidase 4 - proteolytic degradation of incretins) - gliptins
  - improvement of Glc-stimulated Ins secretion after meal
  - supression of postprandial glucagon release
  - delayed gastric emptying
  - protection of β-cells from apoptosis



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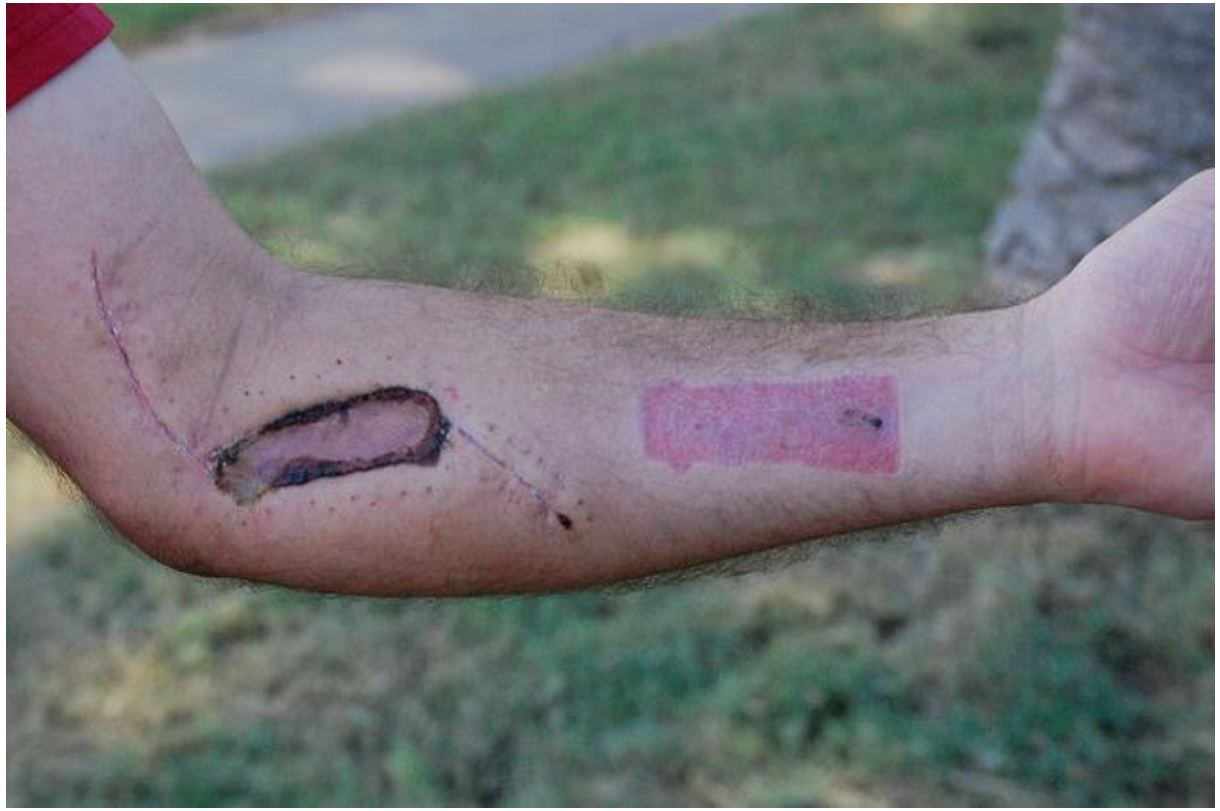
Gila monster



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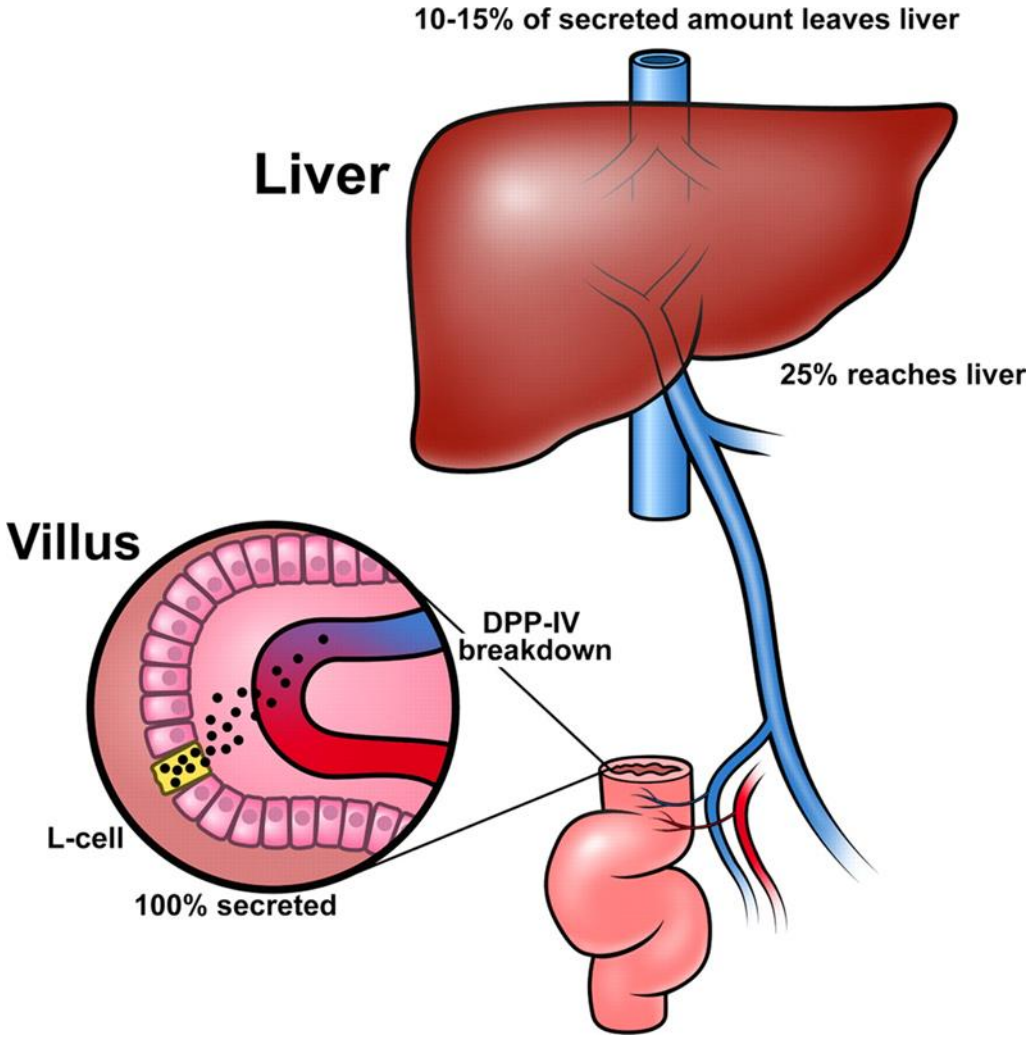
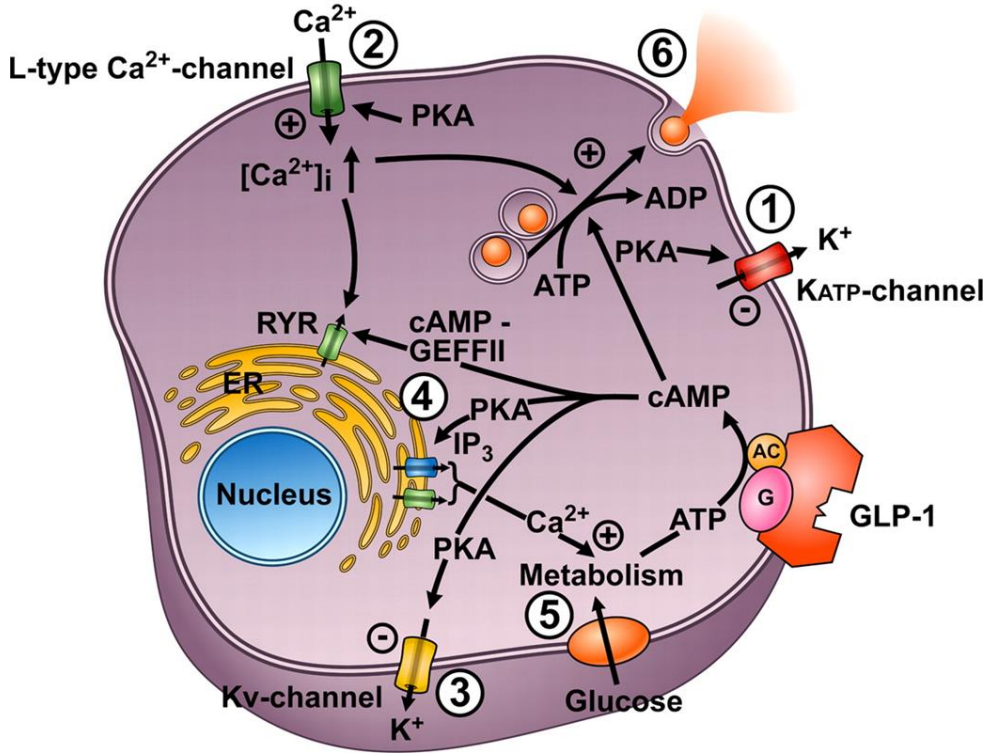
Effect of GLP-1 – anticipation of need to rise insulin





Jens Juul Holst Physiol Rev 2007;87:1409-1439

Physiological Reviews

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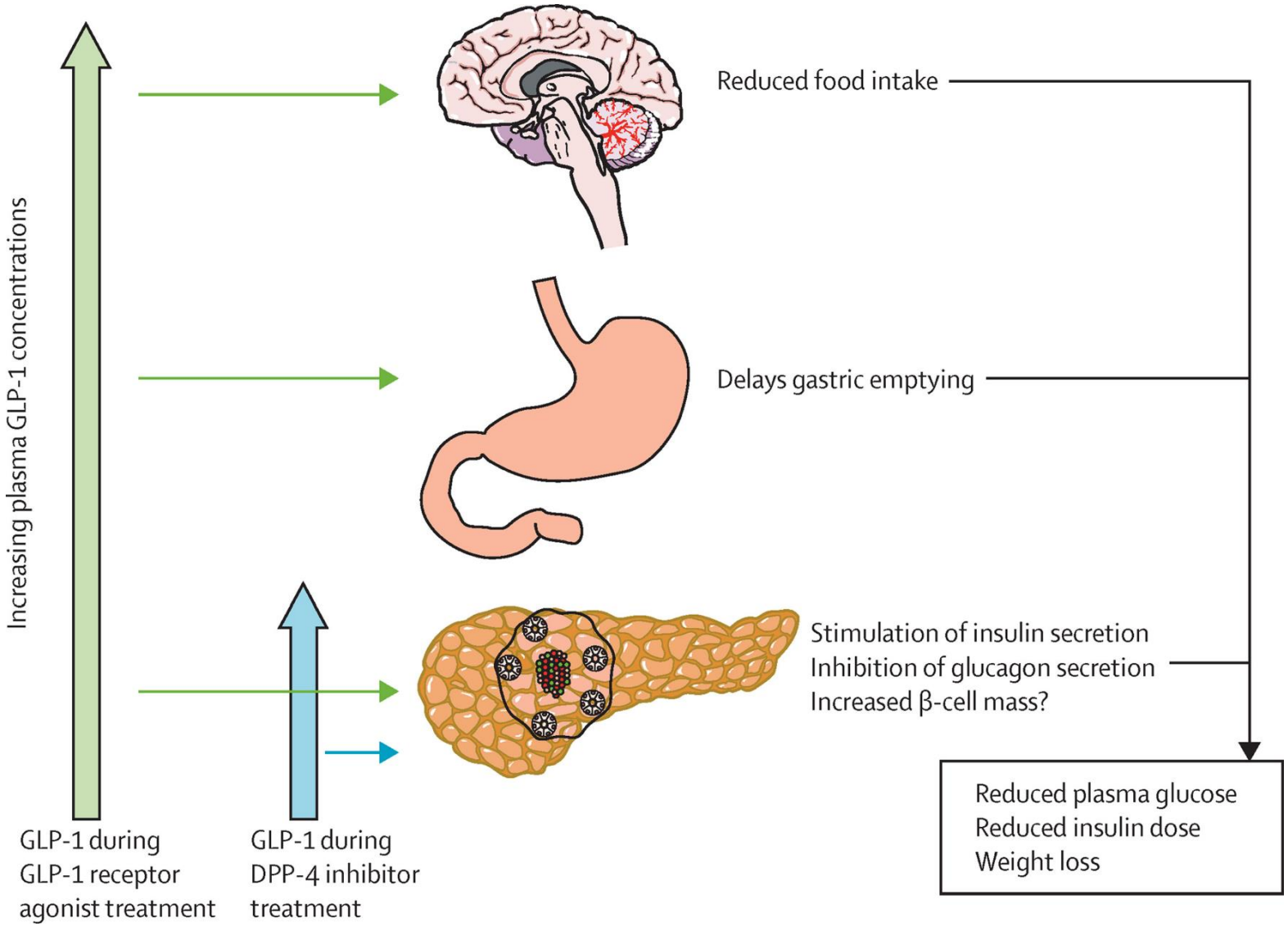
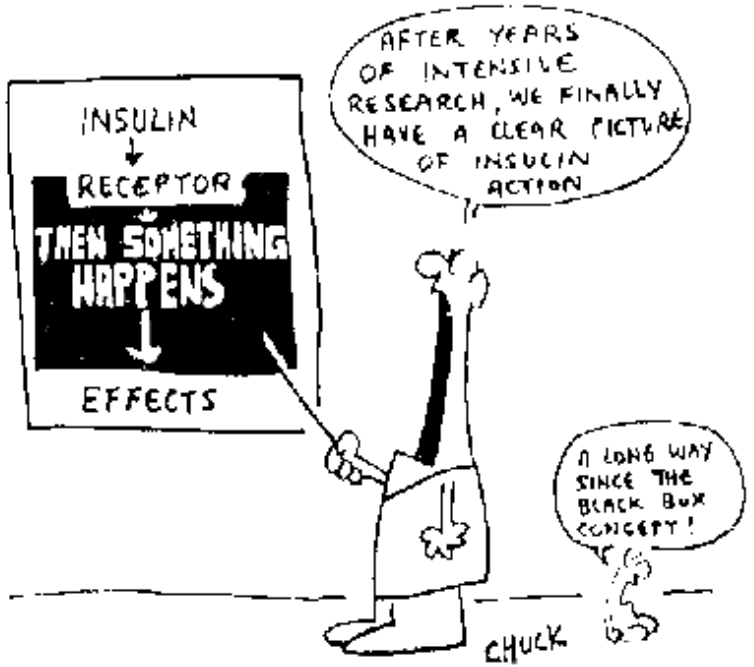
Incretins have systemic effects too





INSULIN SIGNALLING

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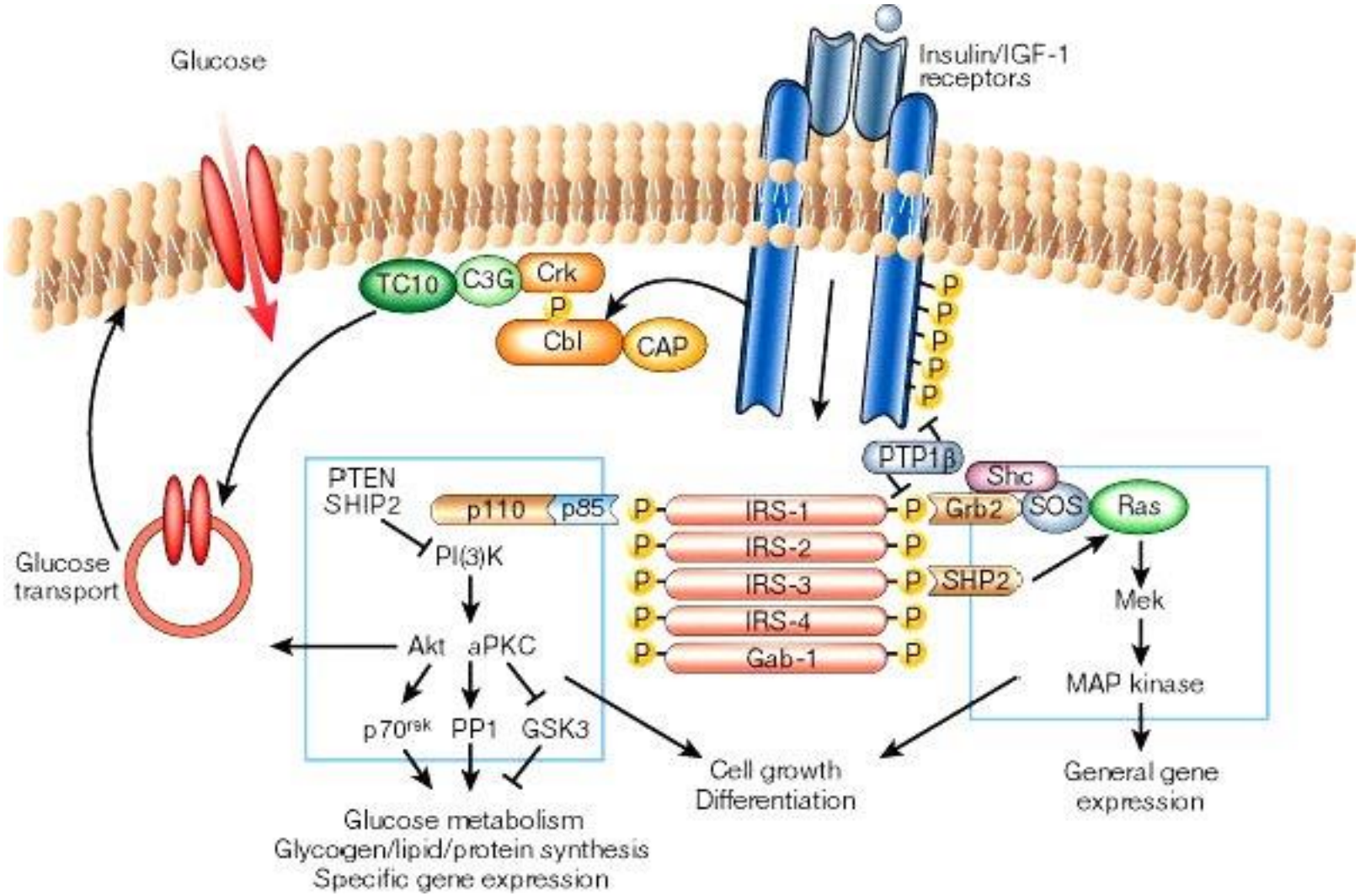
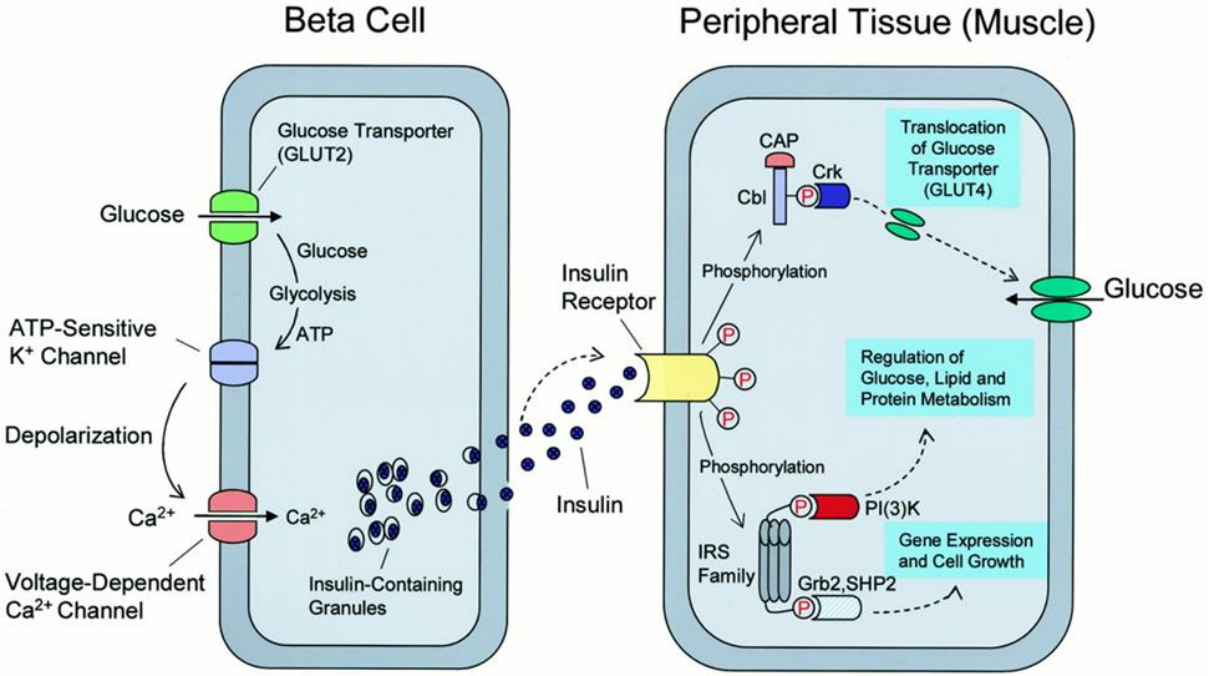
Insulin receptor



Insulin receptor made simple



- insulin receptor is a **tyrosinkinase** type (2 α and 2 β subunits) receptor
- signal transduction consists of series of phosphorylation events
  - intracellular proteins, other kinases and finaly enzymes
    - i.e. their activation or inhibition
      - activation of anabolic pathways (i.e. glycegenogenesis, lipogenesis)
      - inhibition of catabolic pathways (e.g. lipolysis, glycogenolysis) and gluconeogenesis
- two main effects happen in insulin-dependent tissues
  - (1) ↑ glucose uptake
    - by translocation of GLUT4 in sceleatl muscle and adipose tissue
  - (2) metabolic: IRS → PI-3-K → PDK → PKB (=Akt)
    - → GSK (glycogen-synthase-kinase) → ↑ glycogen synthesis
    - → cAMP phosphodiesterase → inhibition of lipolysis
    - ↓ gluconeogenesis
- ubiquitously (3) ↑ gen. expression (mitogenic effect)
  - MAPK → transcription factors

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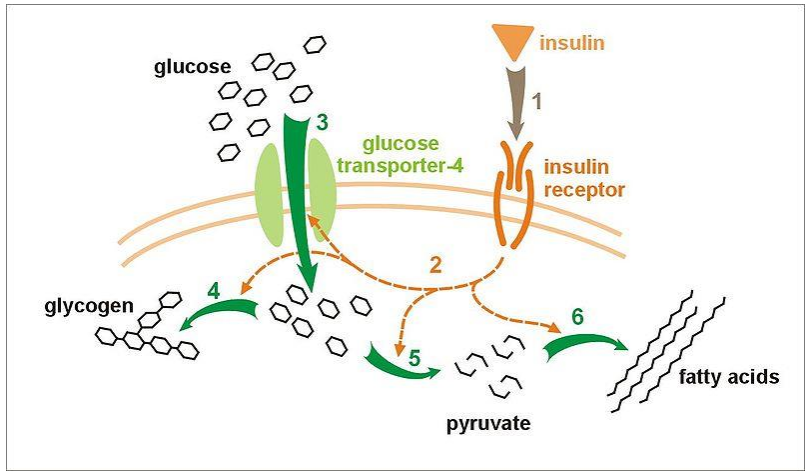
Classification of tissues according to insulin action:

- **insulin-sensitive**
  - **skeletal and heart muscle**
  - **adipose tissue**
    - in both glucose uptake facilitated by **GLUT4**, which becomes integrated into cell membrane after insulin receptor activation



  - **liver**
    - metabolic actions

- **insulin-insensitive**
  - **all others incl. muscle, adipose and liver**
    - glucose uptake is realized by facilitated diffusion by **GLUT1, 2, 3, 5, …** permanently localized in the cell membrane
    - transport of glucose depends solely on
      - concentration gradient
      - type and density of GLUTs

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CLASSIFICATION OF DM, T1DM A T2DM

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Pathophysiology of diabetes mellitus

- **heterogeneous** syndrome characterized by **hyperglycemia** due to **deficiency of insulin action** as a result of
  - absolute insulin deficiency
    - **destruction of the β-cells of the islets of Langerhans**
  - relative deficiency of insulin secretion and/or action
    - abnormal molecule of insulin (mutation of insulin gene)
    - defective conversion of preproinsulin to insulin
    - circulating antibodies against insulin or its receptor
    - **insulin resistance in peripheral tissues + secondary failure of β-cells of the islets of Langerhans**
      - receptor defect
      - **post-receptor defect**
- prevalence of DM in general population 5%, over the age of 65 already 25%

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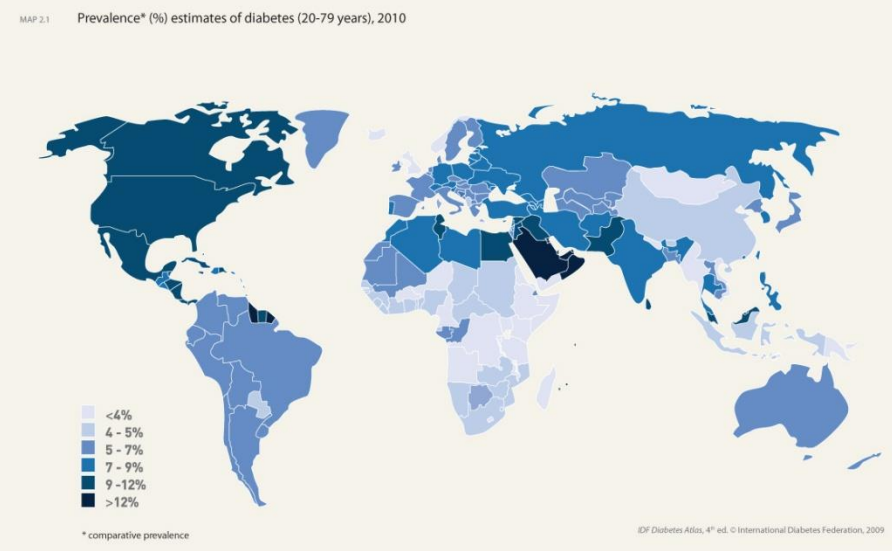
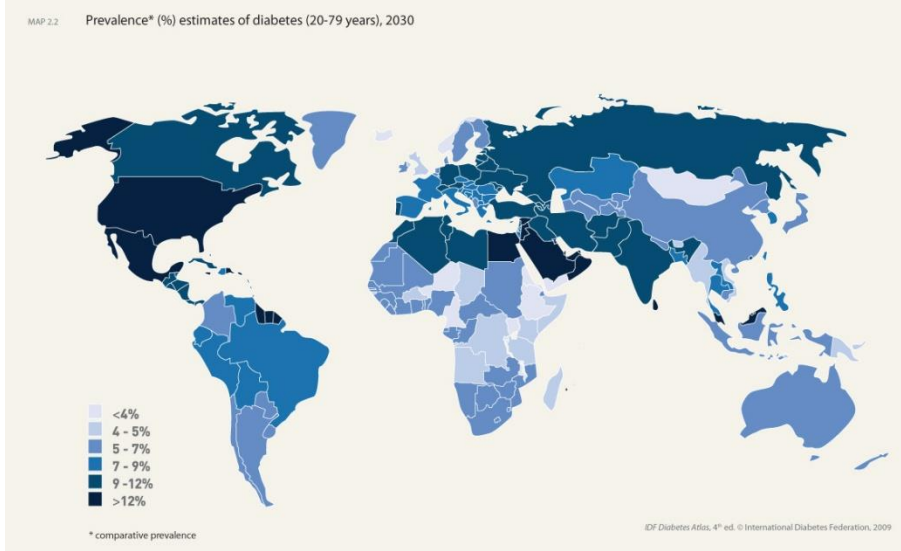
Prevalence (%) of diabetes (population 20-79 years)

2010 – 4.3 bil. (from a total of 7 bil.)
285 mil. diabetics
0.75 mil. diabetics in Czech rep.

2030 – 5.6 bil. (from a total of 8.5 bil.) 30%
438 mil. diabetics 54%
1.2 mil. diabetics in Czech Rep. 60%





[*IDF Diabetes Atlas*, 4th ed. ©International Diabetes Federation, 2009]

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Classification of DM

1. Diabetes mellitus type 1 (T1DM) ~5%

2. Diabetes mellitus type 2 (T2DM) ~90%

3. Other specific types:

a. genetic defects of B-cell
- monogenic DM (MODY1 - 6)
- mutation of mitochondrial DNA

b. genetic defects leading to insulin resistance
- type A insulin resistance, leprechaunismus, Rabson-Mendenhal syndrome, lipoatrophic DM

c. diseases of exocrine pancreas
- pancreatitis, tumor, cystic fibrosis, hemochromatosis

d. endokrinopathies
- Cushing syndrome, acromegaly, pheochromocytoma, hyperthyreosis

e. iatrogenic DM (i.e. drugs and toxins)

f. other genetic syndromes associated with DM
- Down, Klinefelter, Turner syndromes, …

4. Gestational diabetes mellitus

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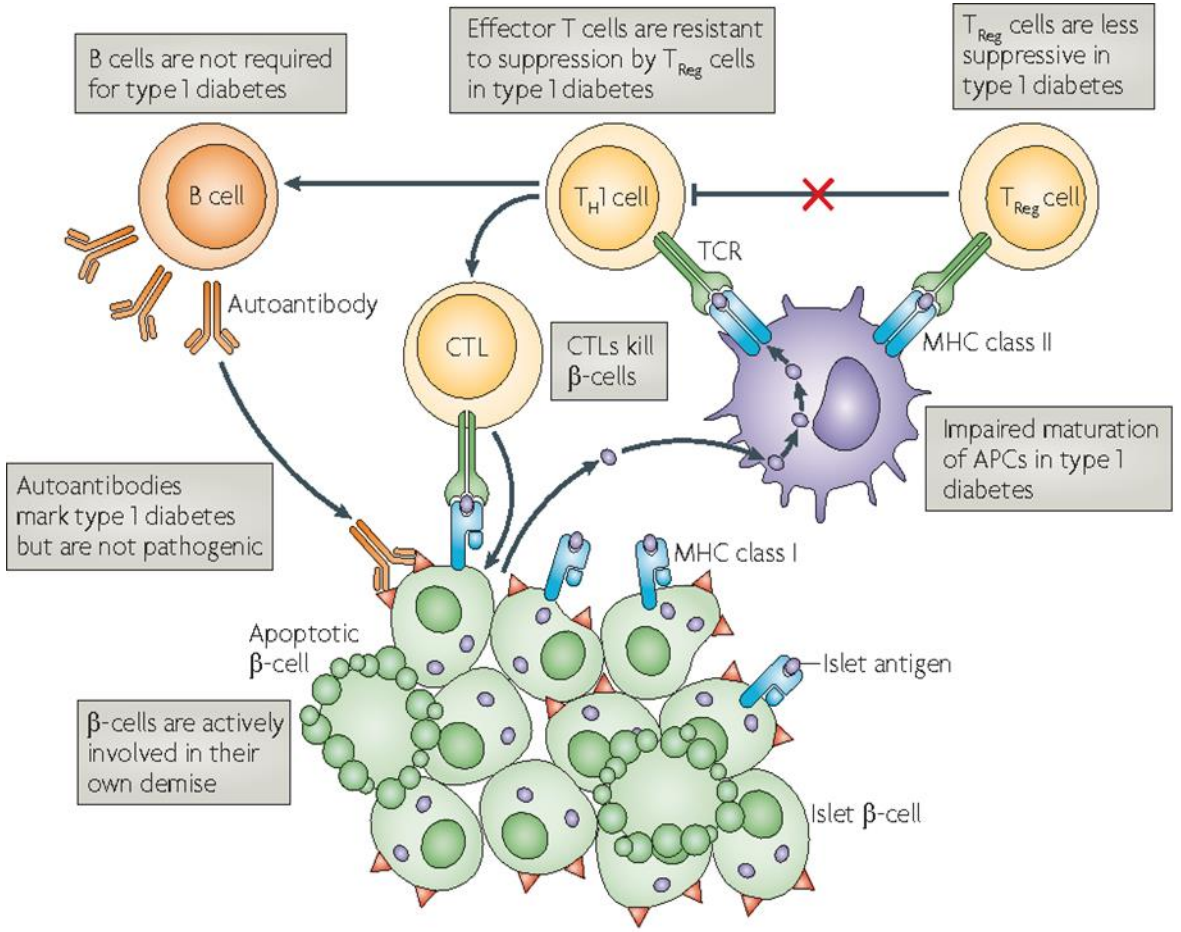
T1DM (formerly IDDM)

- selective autoimmune destruction of β cells of IofL in genetically predisposed individuals
- genetic susceptibility
  - chromosome 6 – MHC class III
    - DR3-DQ2 and DR4-DQ8
  - chromosome 11 - insulin gene
    - promotor polymorphism (variable length)
  - in both cases genetic background leads to insufficient deletion of autoreactive T-lymphocytes in thymus and therefore **suboptimal central immune (auto)tolerance**
- cytotoxic autoimmunity mediated by T-lymphocytes
  - there are also antibodies against β cell structures (ICA, GAD, IAA), but they are rather markers of autoimmunity than causal agents
- common association of T1DM with other autoimmune diseases
  - celiac disease
  - thyreopathy,
  - Addison syndrome



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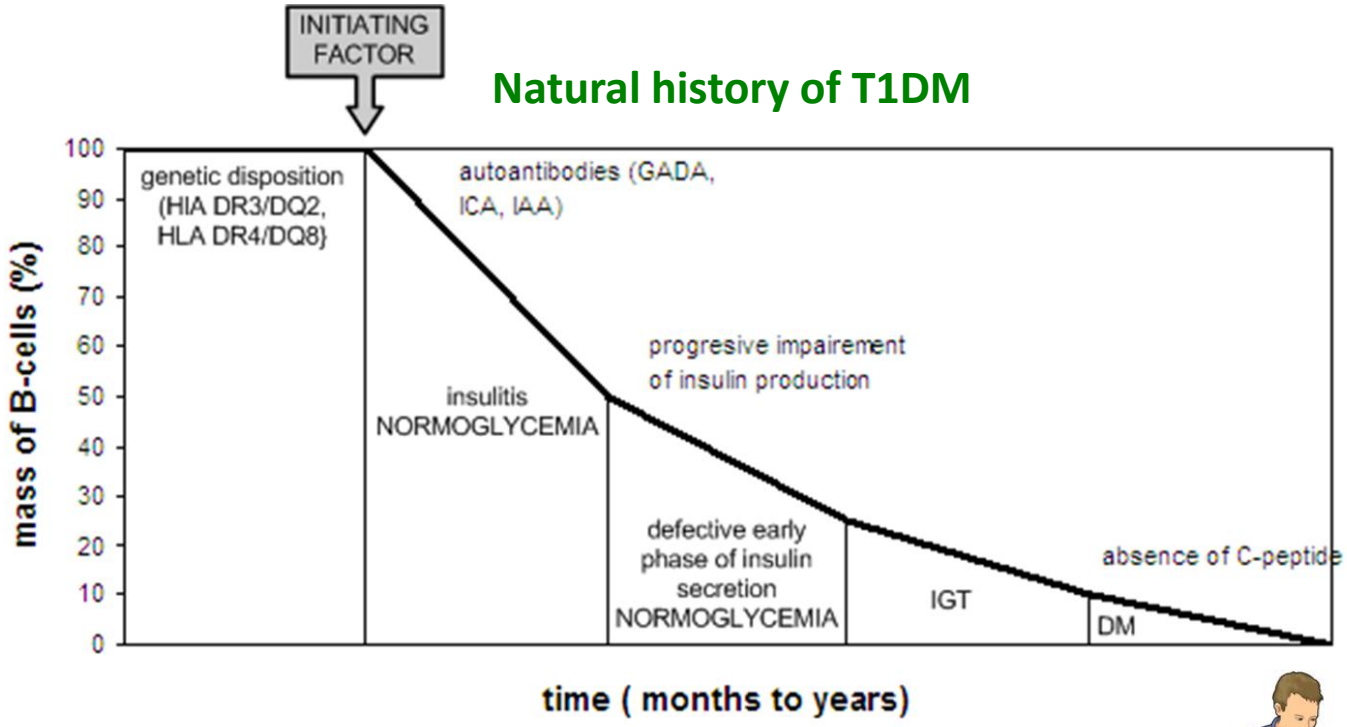
T1DM

- autoimmunity has to be triggered by various factors
  - infection
    - viruses
      - rubella, measles, coxsackie B, CMV, EBV, enteroviruses, retro-viruses
    - mechanism is unclear
      - cytolytic (⊗ sequestration of antigens
      - formation of neoantigens
      - molecular mimicry or superantigens
  - environmental factors (according to the epidemiologic evidence)
    - diet – early exposition proteins of cow's milk
      - bovine insulin
    - vitamin D – reason for **northern-southern geographical gradient**?
    - toxins (diet, water, bacteria)
    - gluten???
- manifestation typically in childhood
- absolute dependence on exogenous supplementation by insulin



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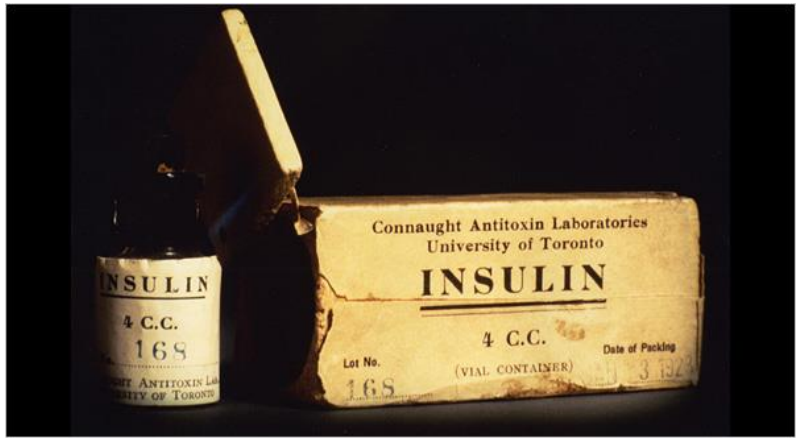
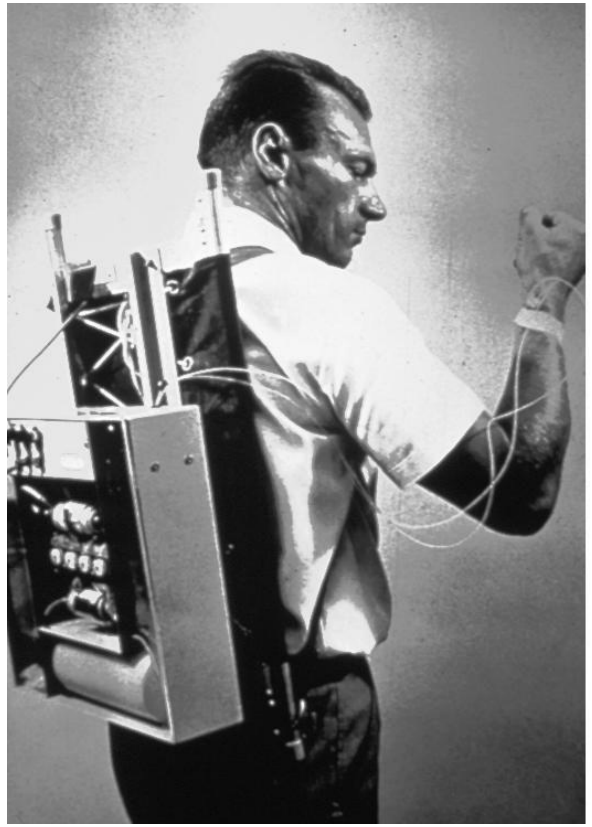
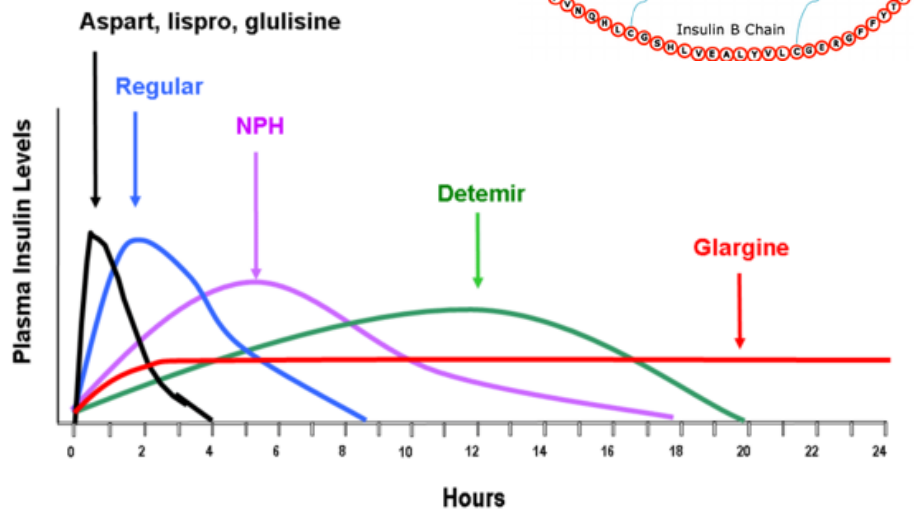
Insulin treatment historically



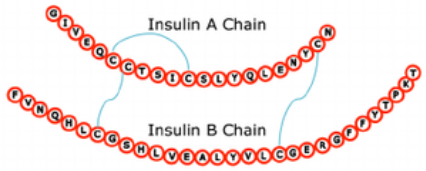


2 tuny prasečích slinivek ⇒ cca 100g inzulinu

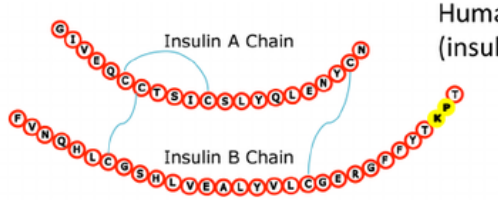
Insulin treatment nowadays (analogues)



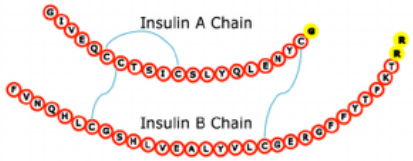
Human Insulin
MW 5808



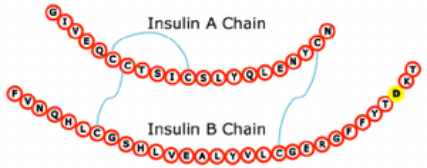
Humalog
(insulin lispro)



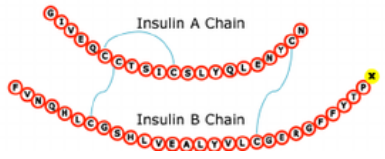
Insulin glargine
(Lantus®)
Avg MW 6063



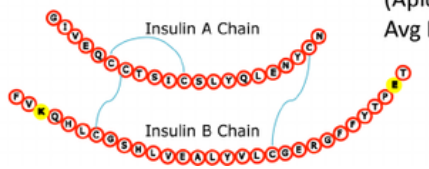
Insulin aspart
(Novalog®)
Avg MW 5826



Insulin detemir
(Levemir®)
Avg MW 5917



Insulin glulisine
(Apidra®)
Avg MW 5823



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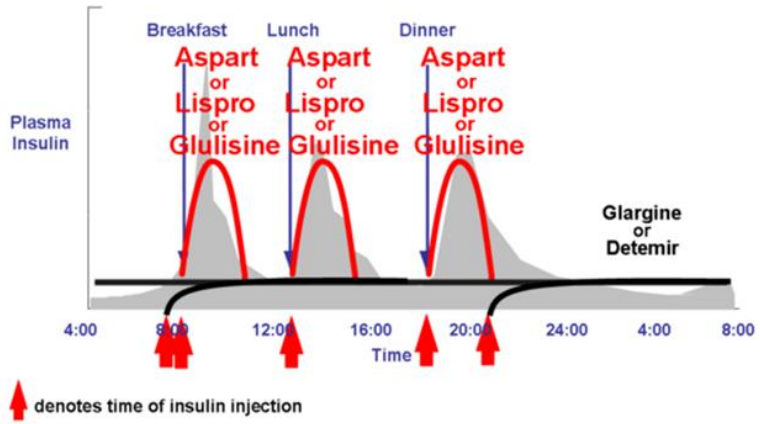
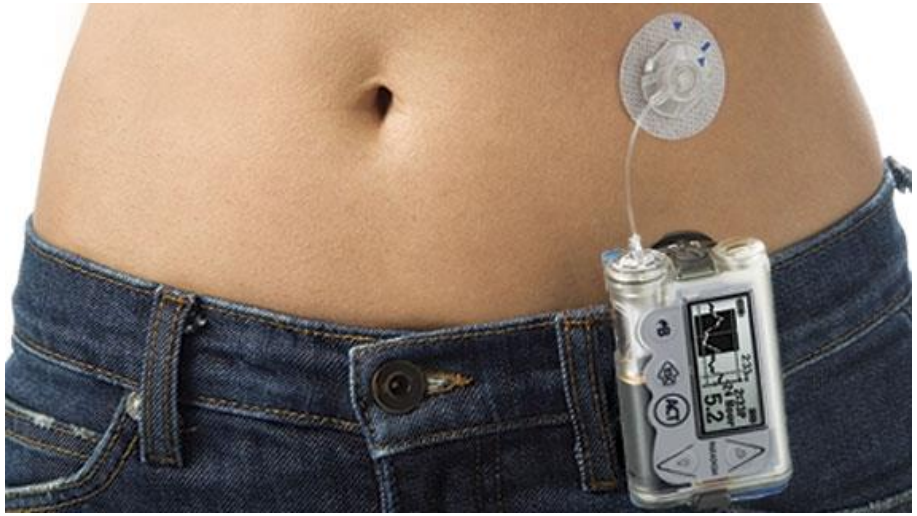
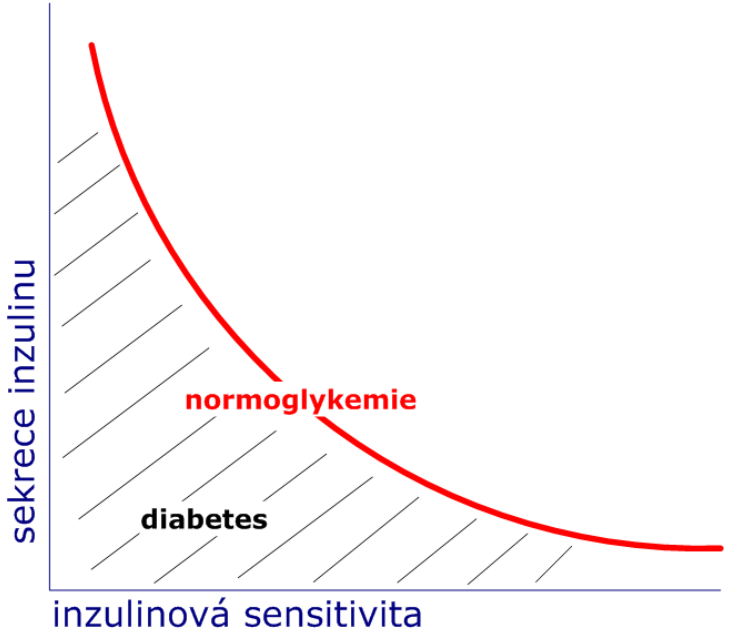
Insulin treatment nowadays (analogues)

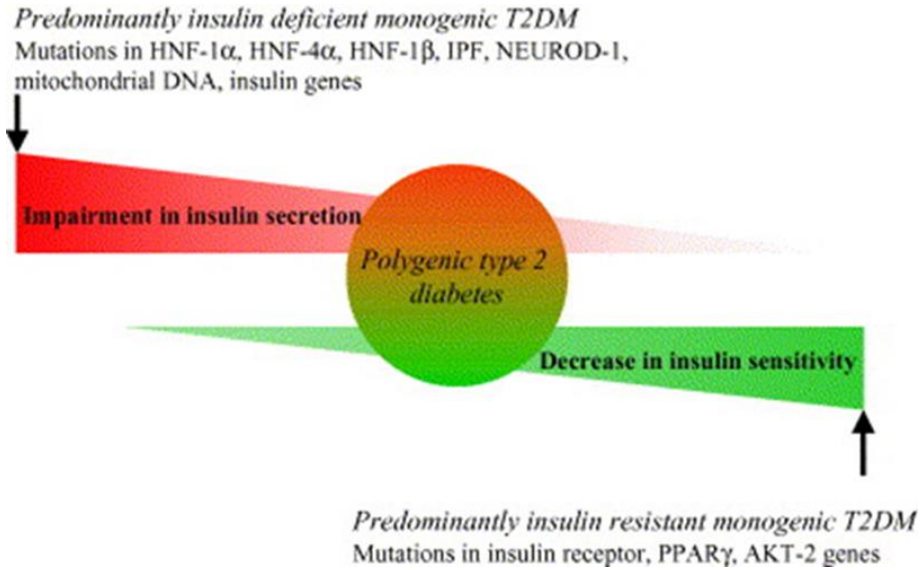




Insulin resistance … T2DM

- insulin sensitivity (= given effect of dose of insulin on individual‘s glycaemia) is a continuous trait with distinct interindividual variability, it can be assessed by:
  - hyperinsulinemic euglycemic clamp
  - calculated indexes (based on relationship between glycaemia and insulin during fasting or oGTT)
    - e.g. HOMA, QUICKI, …
- insulin sensitivity changes (= **insulin resistance**) in many situations
  - physiologically in pregnancy
  - pathologically in obesity, inflammation etc.
- **should increasing insulin resistance always lead to compensatory increase of insulin secretion than glycaemia would stay stable**
  - however capacity to compensatory increase secretion of insulin by beta-cells is apparently limited
- main pathophysiologic feature of T2DM is an imbalance between insulin secretion and its effect
  - **in the time of clinical manifestation there are both insulin resistance and impairment of insulin secretion**
- what is “chicken” and what is “egg”??
  - insulin resistance
    - genetic predisposition (polygenic) – thrifty phenotype
    - acquired factors
      - competition of GIs with NEFA!!! (diet)
      - effect of adipokines from adipose tissue (obesity)
      - ↓ mobilization of GLUT4 in physical inactivity
      - down-regulation of ins. receptor due to hyperinsulinemia
  - impairment of secretion
    - inherited factors
      - fewer B-cells (~20-40%)
      - defect of 1. phase of Ins secretion (~80% reduction)
    - acquired factors
      - – gluco- and lipotoxicity for B-cells
- **90% of subjects are obese** – metabolic syndrome!!!





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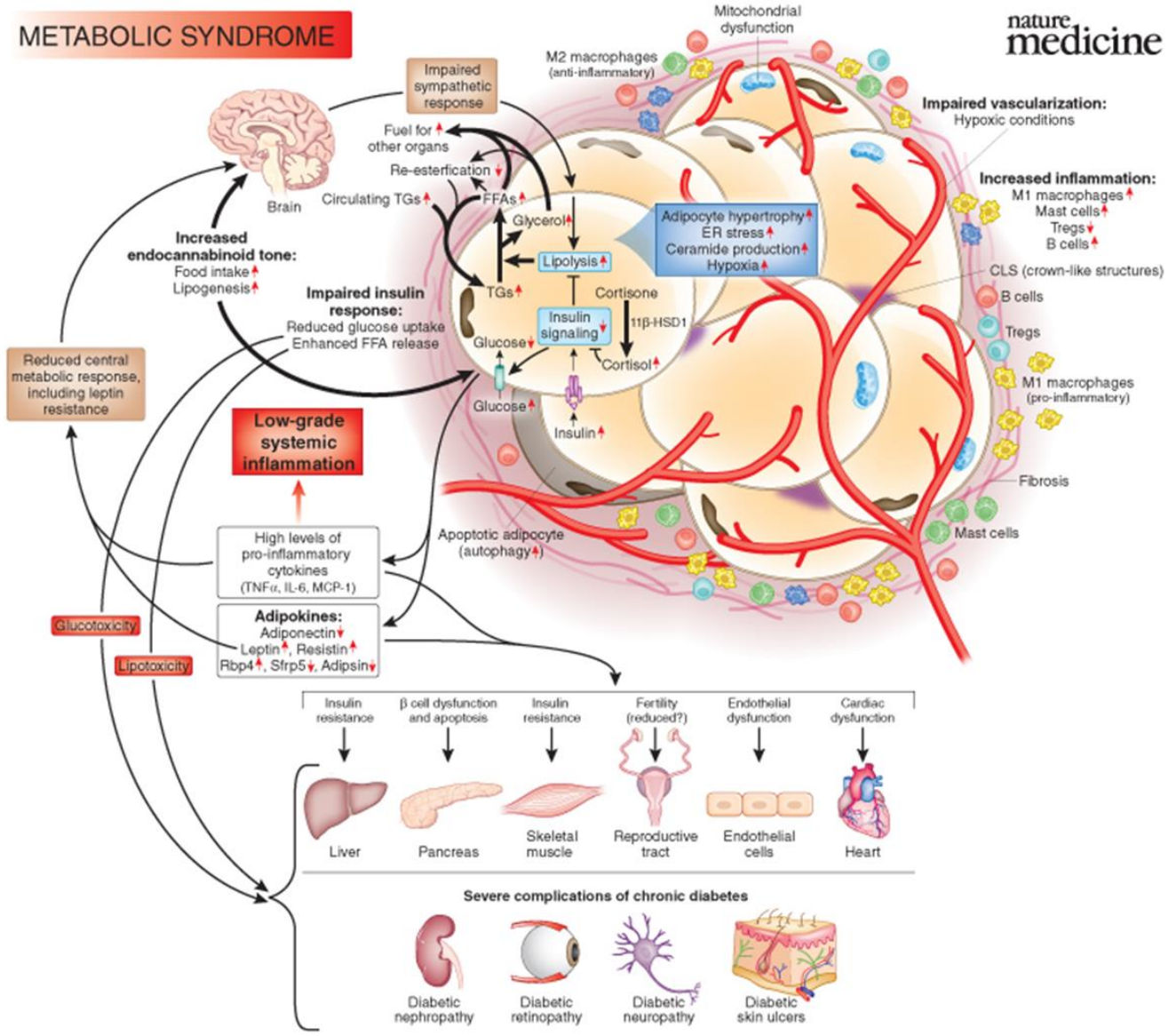
Metabolic syndrome



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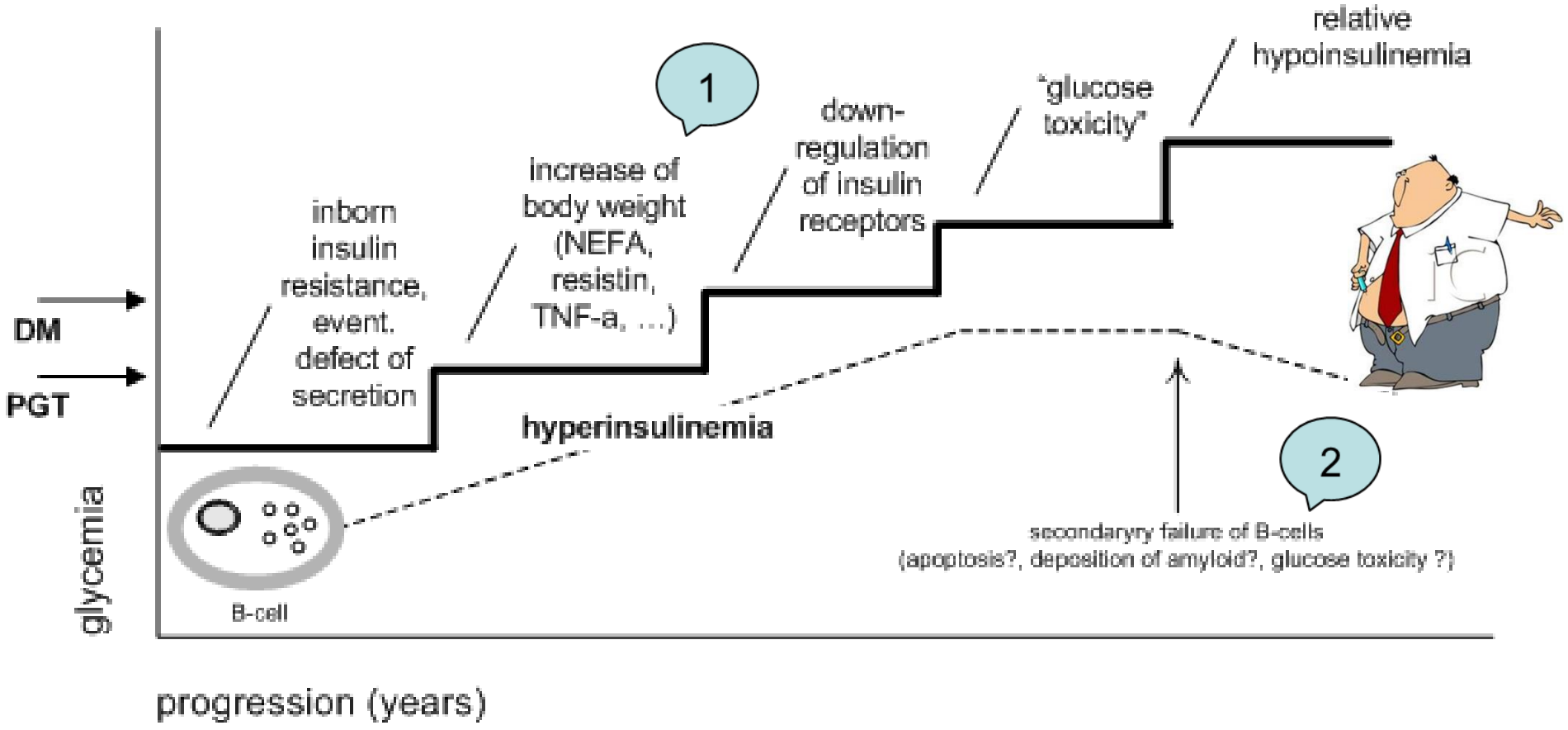
Natural history of T2DM



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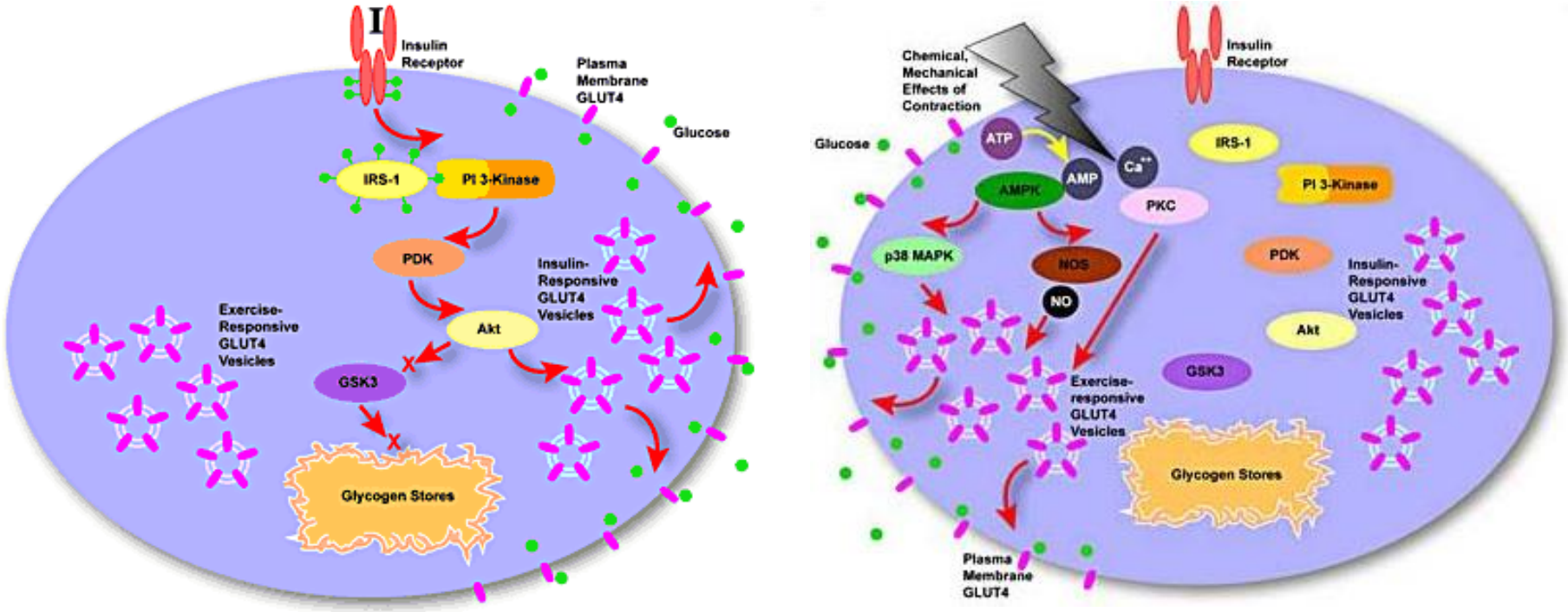
1 Insulin- and “sport”-dependent translocation of GLUT4



- 2 intracellular “pools” of GLUT4
  - insulin-dependent (see cascade of Ins-receptor)
  - Ca^{2+} / NO / AMPK?-dependent
    - this mechanism is responsible for improvement of insulin sensitivity in physically active subjects

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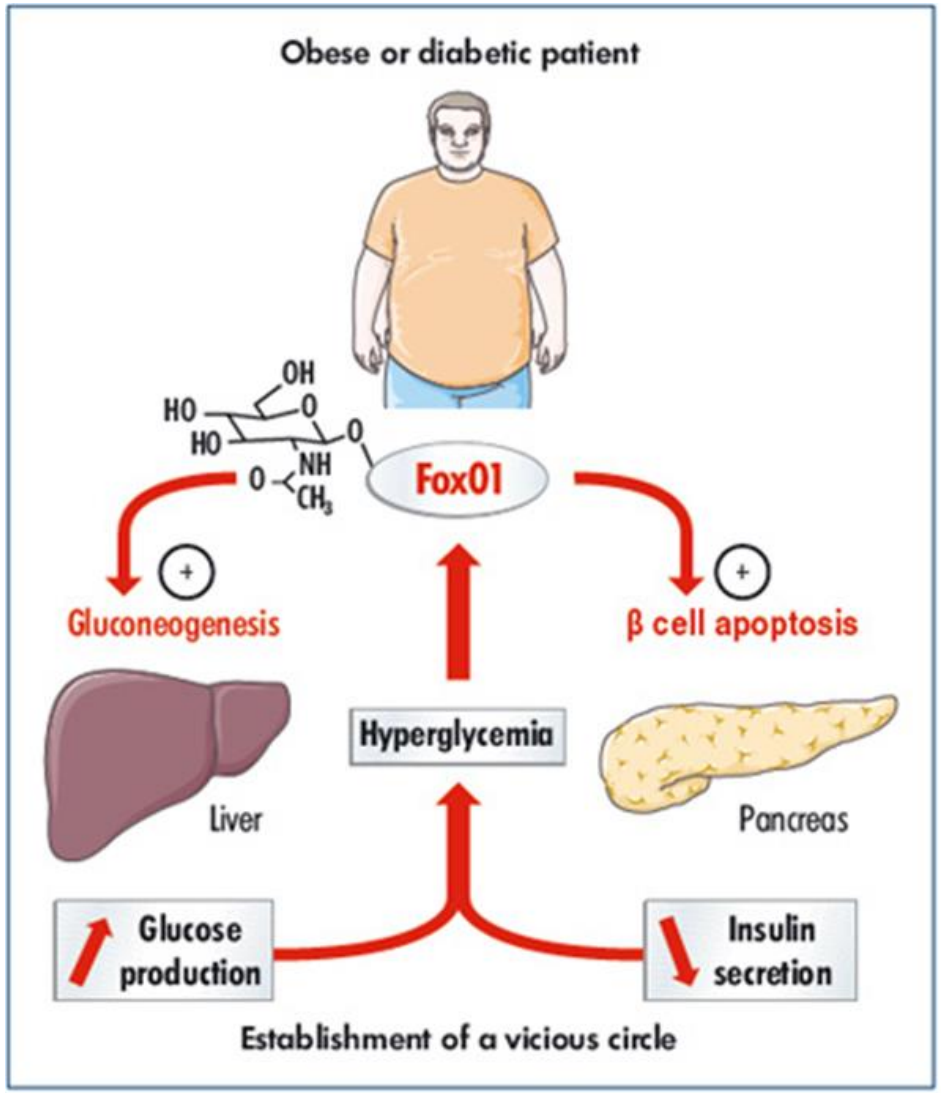
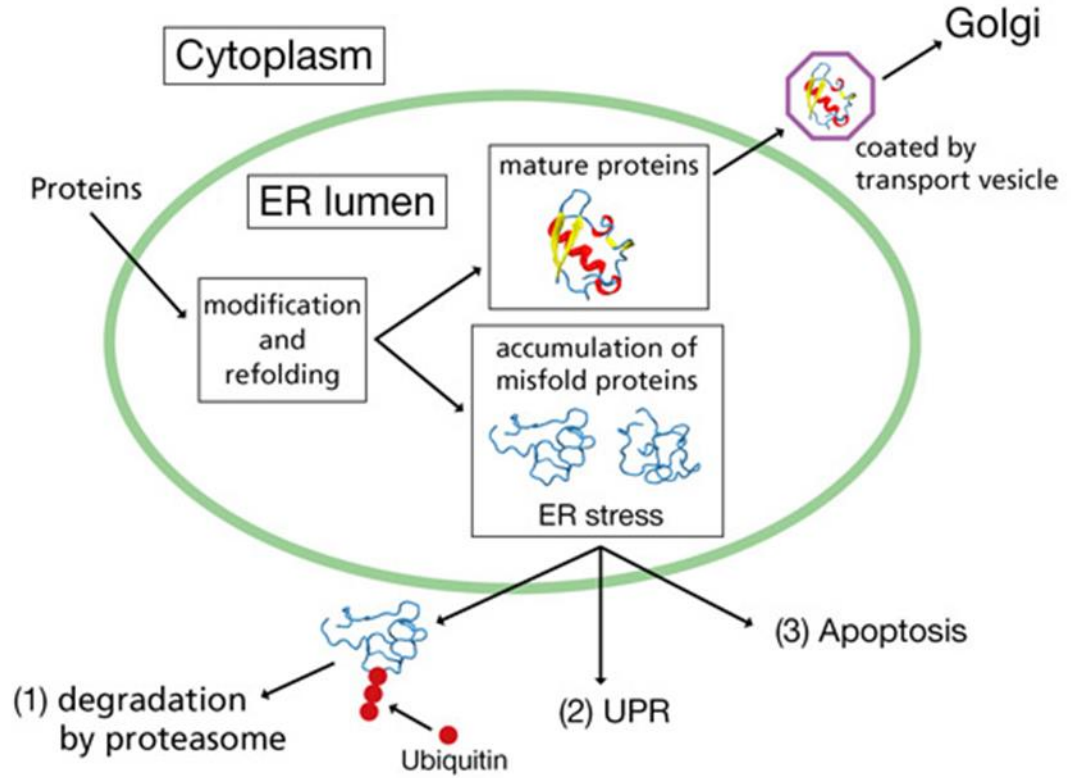
2 Secondary failure of β cells

- hyperglycemia induces:
  - oxidative stress
  - endoplasmic reticulum (ER) stress
- high concentration of NEFA causes lipotoxicity
  - short term increase of NEFA stimulates secretion of insulin
  - long term exposure to NEFA, esp. long-chain saturated (e.g. palmitate), suppress secretion of insulin and damages B-cells
    - ↑ ceramide → **apoptosis**



ER stress → Unfolded protein response

- The unfolded protein response (UPR) is activated in response to an accumulation of unfolded or misfolded proteins in the lumen of ER
  - incl. insulin in β-cells
- UPR has two primary aims:
  - initially to restore normal function of the cell by halting protein translation and activate the signaling pathways that lead to increasing the production of molecular chaperones involved in protein folding
  - if these objectives are not achieved within a certain time lapse or the disruption is prolonged, the UPR aims to apoptosis



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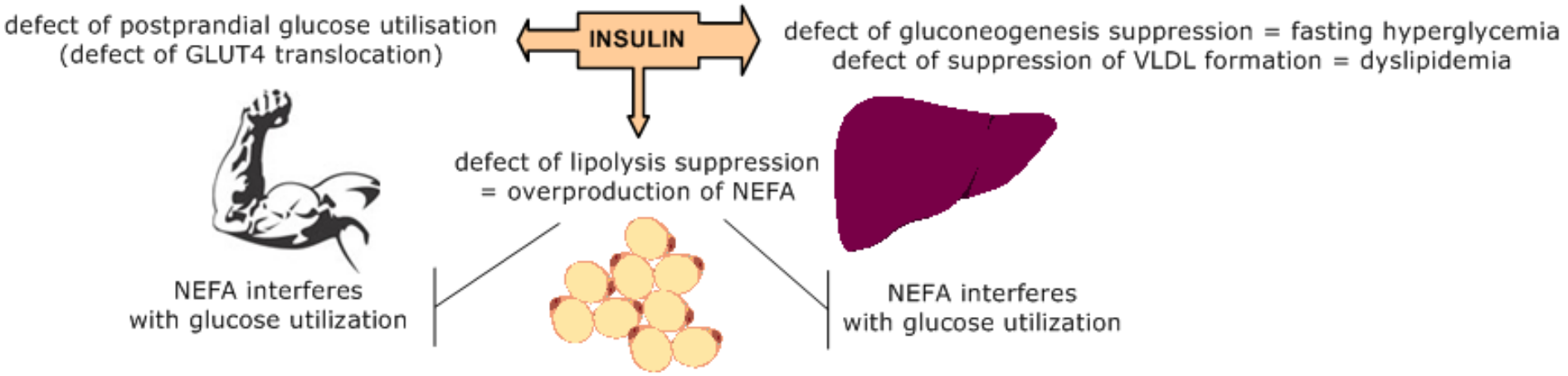
Overt T2DM



- manifest T2DM is characterized by (variable degree of):
  - fasting hyperglycemia (due to gluconeogenesis)
    - **insulin resistance in liver**
  - postprandial hyperglycemia (due to decreased peripheral glucose uptake
    - **insulin resistance in muscle and adipose tissue**
  - mixed dyslipidemia
    - increased plasma NEFA (due to unsuppressed lipolysis)
      - **insulin resistance in adipose tissue**
    - pro-atherogenic dyslipidemia (due to stimulated VLDL production in liver)
      - **substrate effect**

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Main characteristics – comparison - of T1DM and T2DM

	T1DM	T2DM
onset	childhood	adults
genetic disposition	yes (oligogenic)	yes (polygenic)
clinical manifestation	often acute	mild or none
autoimmunity	yes	No
insulin resistance	no	yes
dependence on insulin	yes	No
obesity	no	yes

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Other types of DM

- **LADA** (Latent Autoimmune Diabetes in Adults) = **slow-onset T1DM**
  - diagnosis in > 30yrs of age, clinically similar to T2DM (slow onset)
  - initially on diet and pills, no ketoacidosis
  - later insulin dependent (during months – 1 year)
  - positive antibodies (= autoimmunity), low or no C-peptide
  - negative family history of T2DM
- **MODY** (Maturity-onset diabetes of the young) – cca 5% T2DM
  - **monogenic** diabetes with familiar clustering and well defined (Mendelian) inheritance (usually AD), early manifestation (childhood or adolescence) and without obesity
  - 6 types (MODY1-6)
  - pathophysiology: genetically conditioned **dysfunction of β-cells** but long-term measurable C-peptide without the signs of autoimmunity
    - MODY due to **glucokinase** mutations (MODY2)
      - glucokinase = "glucose sensor" (impaired insulin secretion)
      - milder form without the complication risk
    - MODY due to **transcription factor** mutations (other 5 types)
      - severe defects of β-cells progressively leading to diabetes with complications
      - impairment of glucose-stimulated insulin secretion and proliferation and differentiation of β-cells

MODY	lokus	gen	produkt	prim. defekt	závaž	
		HNF4A	hepatocyte nuclear factor-4α	pankreas	vysoká	časté
		GCK	glukokináza	pancreas/játra	mírná	vzácně
		TCF1 (HNF1A)	hepatocyte nuclear factor-1α	pancreas/ledviny	vysoká	časté
		IPF1	insulin promoter factor-1	pancreas	vysoká	?
5	17q	*TCF2 (HNF4B)*	hepatocyte nuclear factor-1β	pancreas/ledviny	vysoká	renální
6	2q32	*NEUROD1*	NEUROD1	pankreas	vysoká	?

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Acute manifestation and long-term consequences (complications) of diabetes

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Q2: Effect of rising plasma glucose ???

OSMOLARITA = 2 Na^+ + urea + glukóza

275 - 295 = 2 x 140 + 2.5 + 5

> 300 = 2 x 140 + 2.5 + **35**

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Clinical presentation of DM

- due to the mild increase of blood osmolarity, osmotic diuresis and dehydratation
  - classical
    - polyuria, thirst, polydipsia
    - tiredness
    - temporary impairment of vision
  - others
    - recurrent infections
    - perio-/parodontitis

- extreme hyperglycemia (>40 mmol/l, osmolarity >350 mosmol/l)
  - **ketoacidosis/coma**
    - ↑ ketone bodies, metabolic acidosis an d hyperglycemia
  - **non-ketoticidotic hyperglycemic coma**
    - hyperglycemia, dehydration and pre-renal uremia
  - **lactic acidosis/coma**
    - either complication of therapy (biguanides / type of peroral antidiabetics)
    - associated with hypoxic states (sepsis, shock, heart failure, …)

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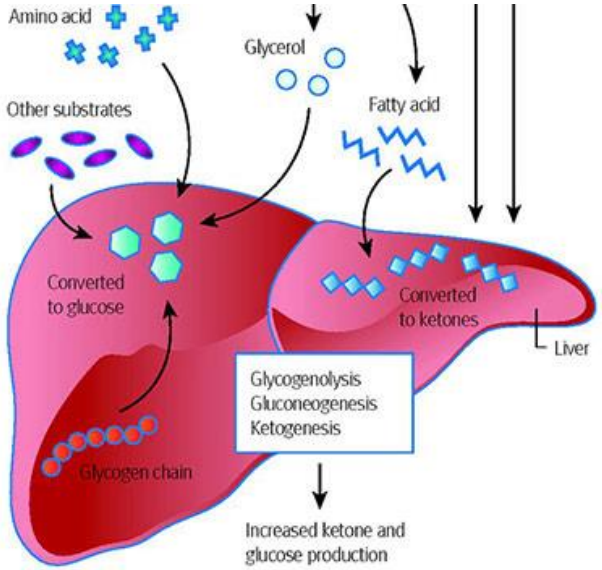
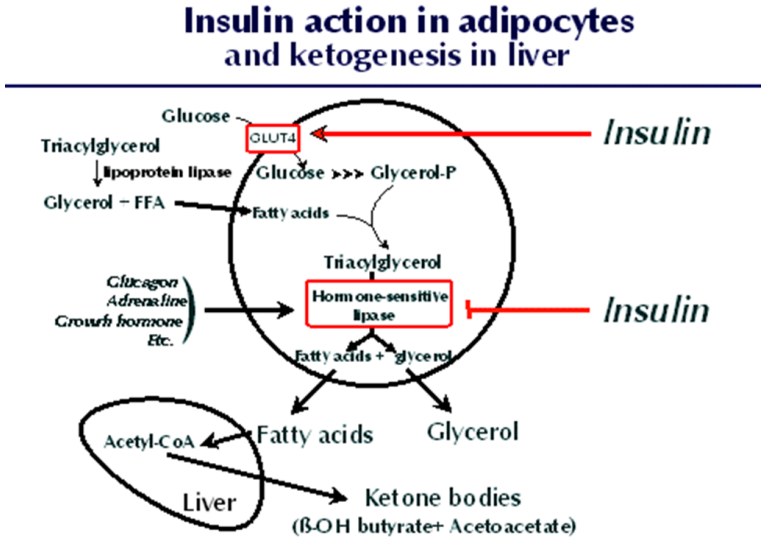
Diabetic ketoacidosis

- Excessive thirst
- Frequent urination
- Nausea and vomiting
- Abdominal pain
- Weakness or fatigue
- Shortness of breath
- Fruity-scented breath
- Confusion





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Late complications of DM

- microvascular
  - diabetic retinopathy
  - diabetic nephropathy
    - diabetic kidney disease (DKD)
  - diabetic neuropathy
    - sensoric
    - motoric
    - autonomous
- macrovascular
  - accelerated atherosclerosis (CAD, peripheral and cerebrovascular vascular disease)
- combined
  - diabetic foot (ulcerations, amputations and Charcot´s joint)
- others
  - periodontitis
  - cataract
  - glaucoma

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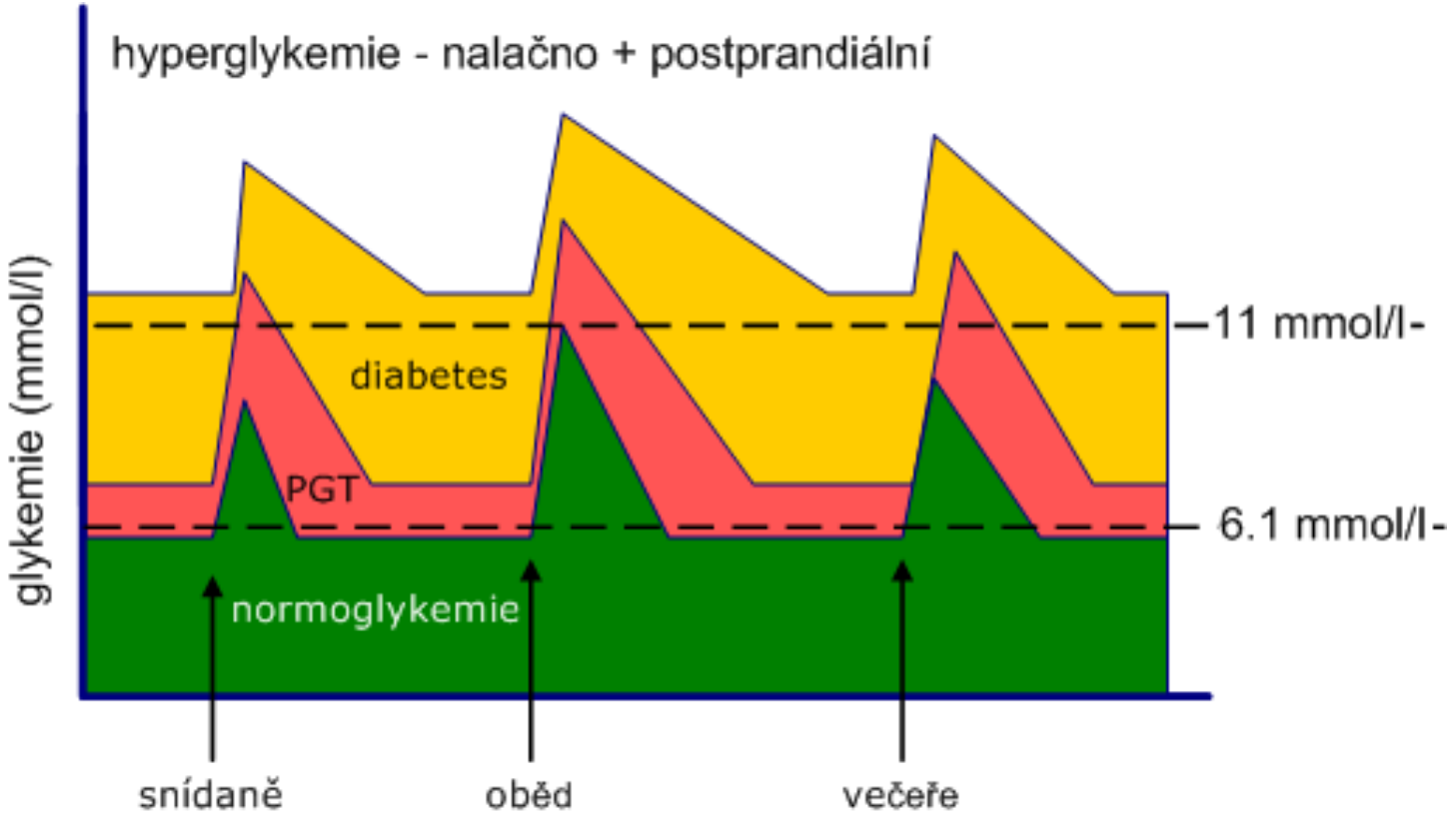
Chronic hyperglycemia



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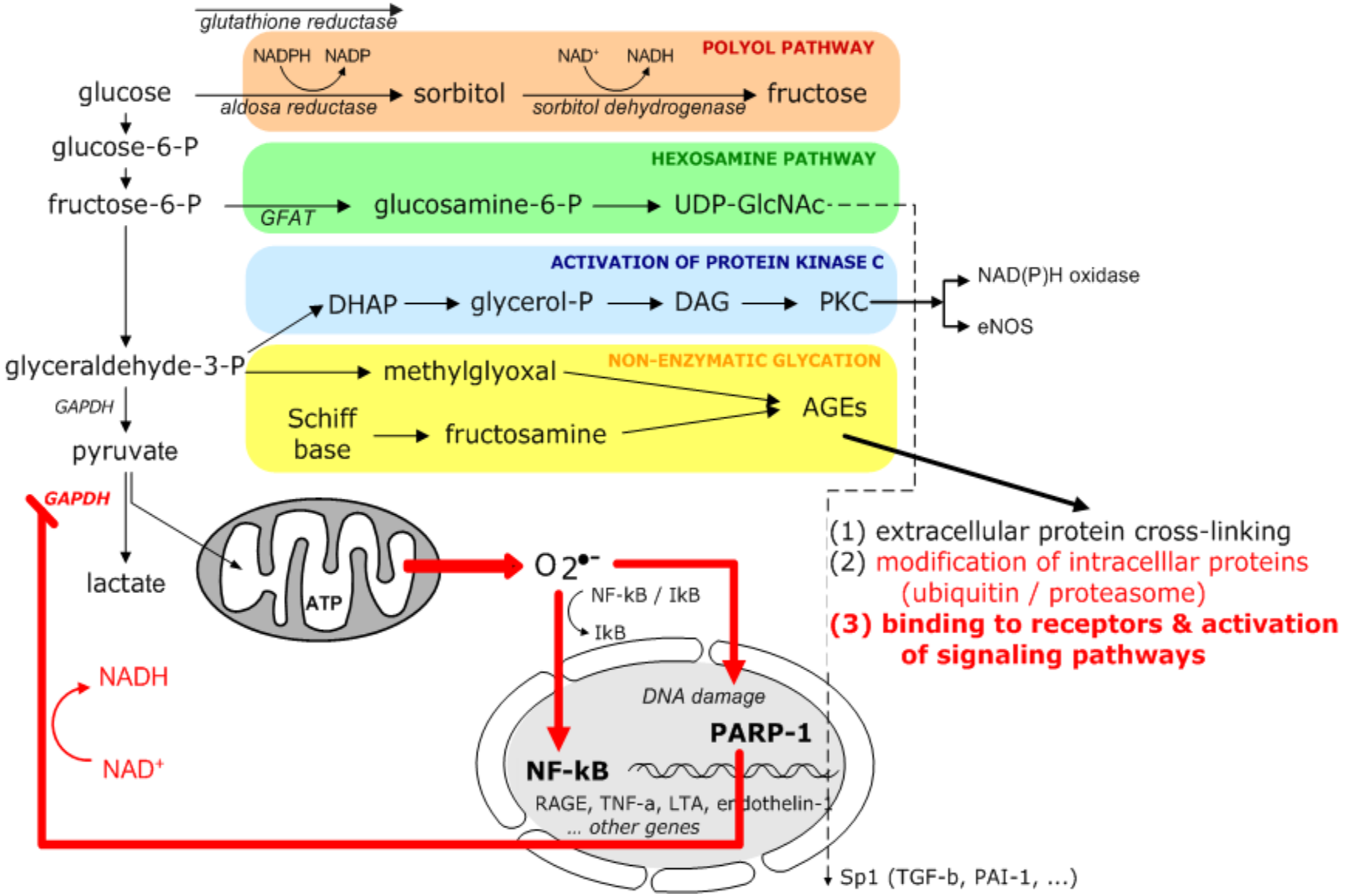
Pathogenesis of complications



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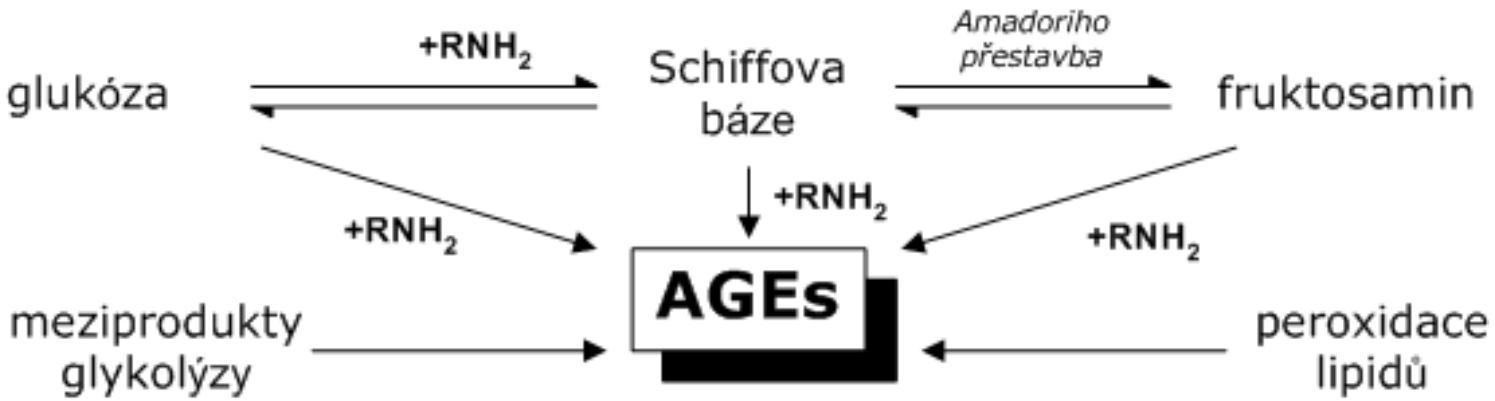
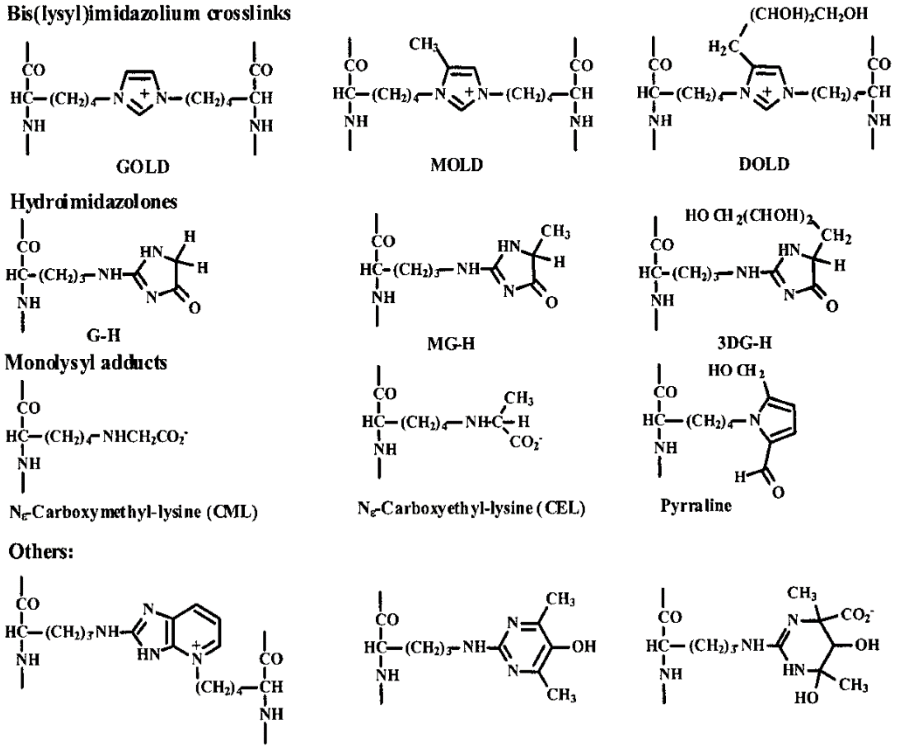
Advanced glycation end products (AGEs)





- cross-linking of extracellular proteins
- modification of intracellular proteins and DNA
  - ubiquitin/proteasom
- binding to pattern-recognition receptors and activation of signaling pathways

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Maillard reaction in food – AGEs in diet

- AGEs are similar to products of Maillard reaction (MRP) formed during thermal processing of food
  - sugar + protein
- Louis Camille Maillard (1878 - 1936)
  - original description of reactions during cooking (“browning”) leading to formation of MRPs (=AGEs)
    - MRP influence taste and visual characteristics, smell, shelve life
  - biologic properties of MRP
    - positive – antioxidants
      - melanoidins, polyphenols
    - negative – carcinogens
      - acrylamid

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Pathophysiology of DKD

Diabetes

Genetic / Epigenetic factors
- Susceptibility genes

Metabolic Stimulus
- Hyperglycemia
- Insulin resistance
- ROS

Haemodynamic Stimulus
- Hypertension
- Fluid/Salt Balance

Microalbuminuria

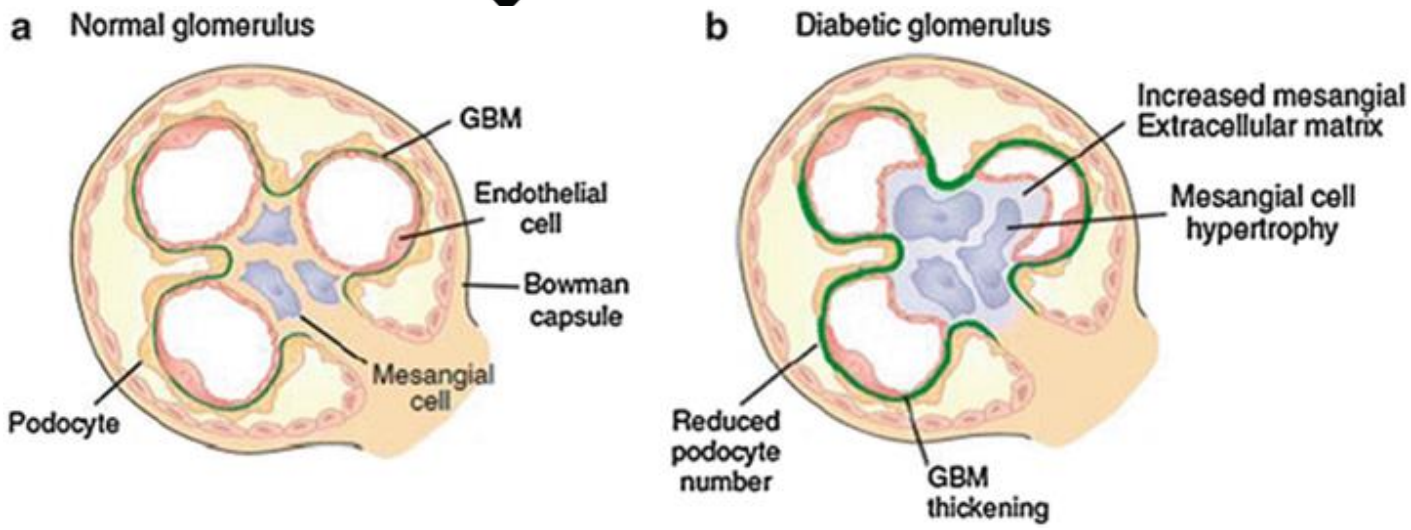
Signalling Pathway activation
- TGFβ1, CTGF, VEGF,
- PKC, Ang II
- AGEs
- NFκB pathway
- miRNA responses

→

Renal consequences
- Infiltrating immune cells
- Matrix accumulation
- Endothelial dysfunction
- Podocyte loss
- Basement membrane thickening
- Proteinuria
- Renal tubule damage

Macroalbuminuria

ESRD



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