Diabetes mellitus

Practicals – experimental diabetes mellitus in laboratory animal

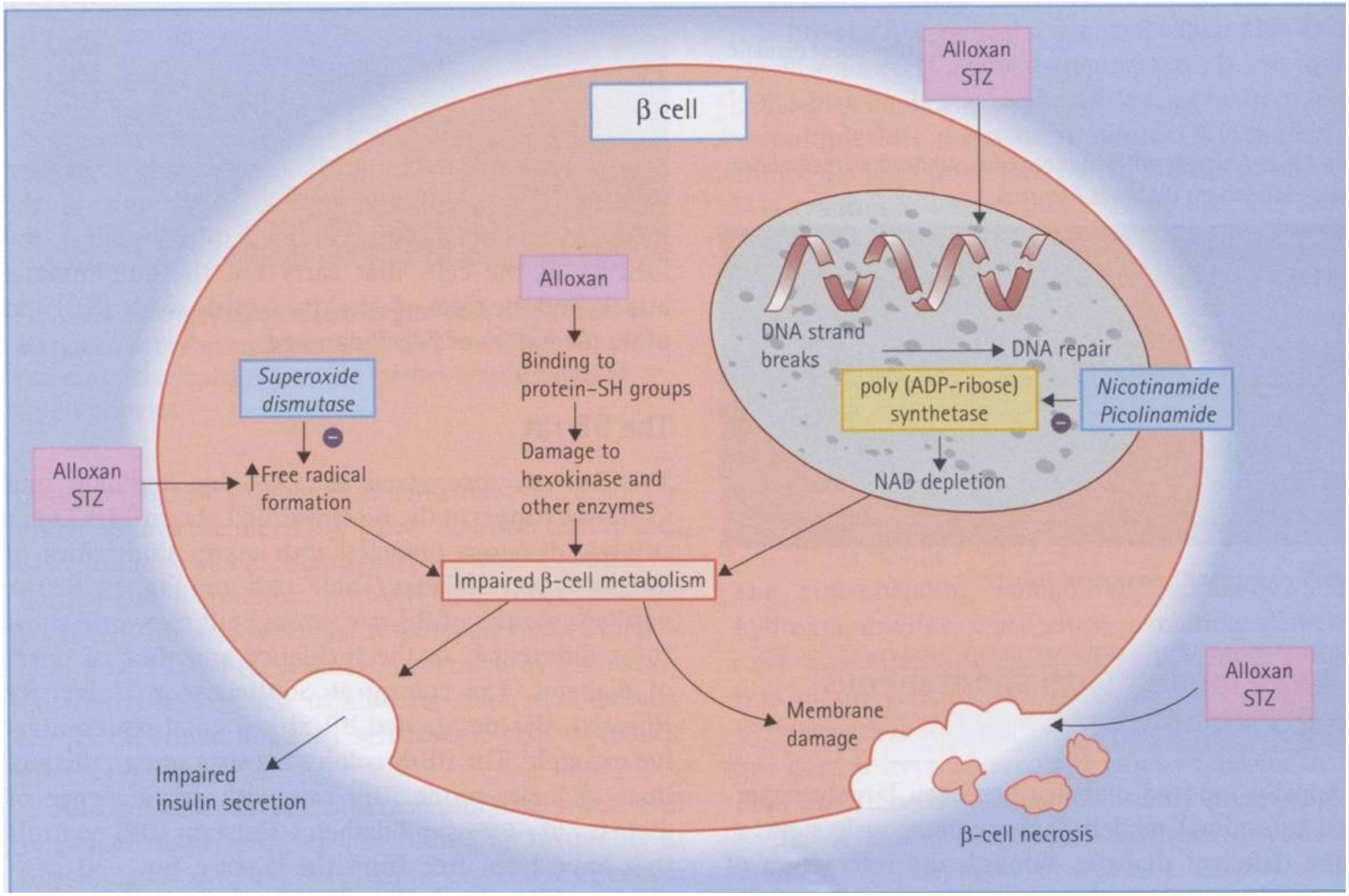
Animal models of type 1 diabetes

- 1889 – pancreas removal
  - Minkowski and Von Mering
    - diabetic syndrome in dog
- Banting and Best
  - insulin discovery and testing
- exp. diabetes induced in various species
  - β-cells toxins and viruses
- specific strains in which insulin-deficient diabetes develops spontaneously

Chemically induced insulin-deficient diabetes

- alloxan
  - first known diabetogenic chemical agent (1943)
    - islet-cells necrosis in rabbit
  - high doses
    - β-cells necrosis
  - acts on membrane and interior
    - inhibits insulin release
    - is taken up into the β-cell
      - glucokinase, PARP
      - free radicals
  - practical problems *in vivo*
    - instability at physiological pH
    - dosage variation with age and species
    - toxicity on other organs
- streptozotocin (STZ)
  - induces severe diabetes
    - i.v. or i.p.
  - most commonly used model
  - β-cell necrosis within 1-2 days
  - insulin falls to 10-30%
  - hyperglycemia 20-30 mmol/l
  - at dosage 50 mg/kg severe ketosis does not develop
    - survival without insulin replacement
  - may share cytotoxic mechanisms with alloxan

Mechanisms of alloxan and STZ action



Animal models with spontaneous T1DM

- BB rat (BioBreeding)
  - defects in immunity
    - inflitration of islets with T lymphocytes
    - autoantibodies against GAD (glutamic acid decarboxylase) are present
    - severe hypoinsulinemia and hyperglycemia
  - two main T1DM susceptibility genes
    - 8 additional loci
- NOD mouse
  - non-obese diabetic
  - autoimmunie diabetes
  - diabetes develops as a result of insulitis
  - NOD mice will develop spontaneous diabetes when left in a sterile environment
  - affects 80 % of females and 20 % of males

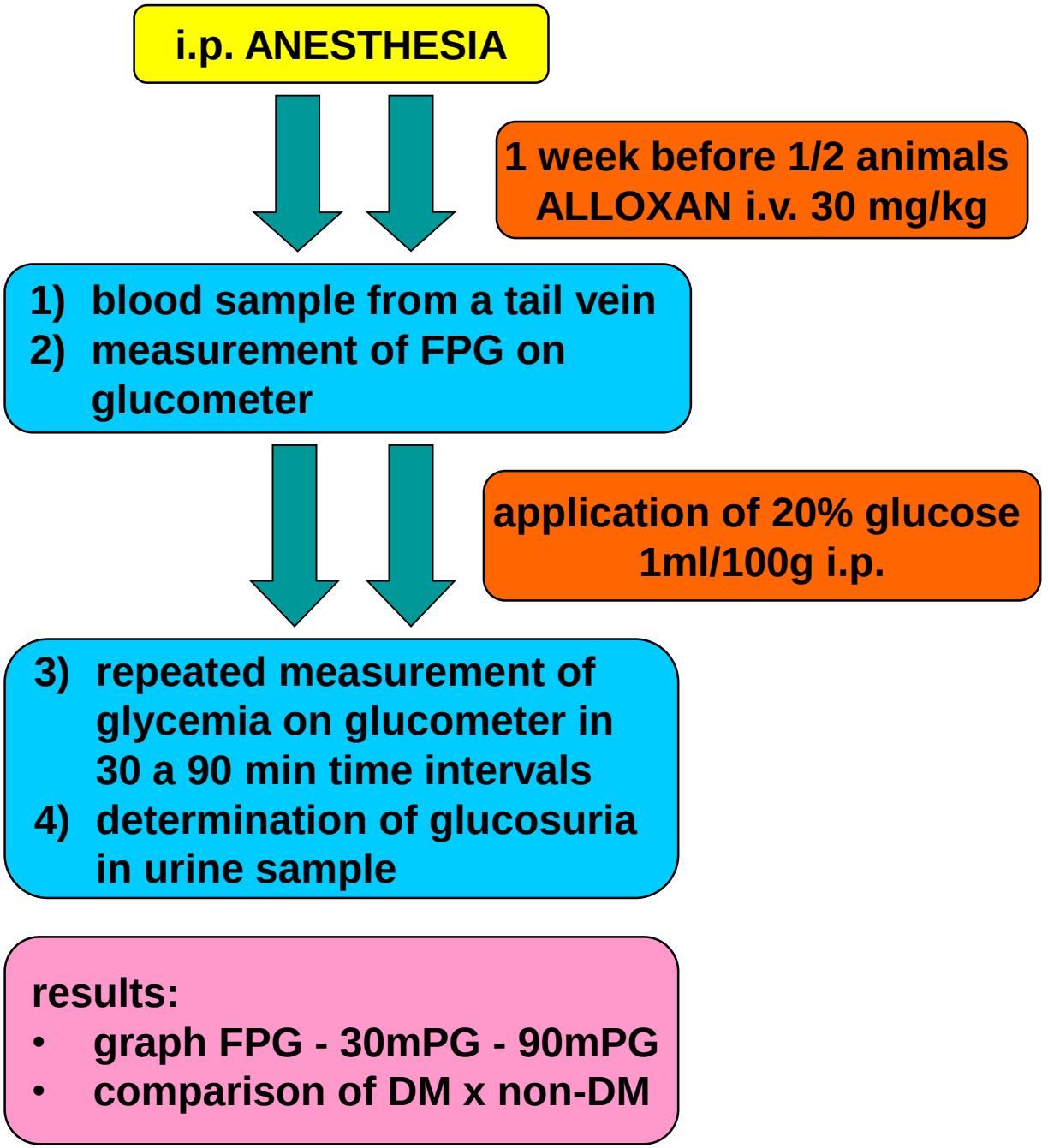
Summary of rodent models of T1DM

Induction mechanism	Model	Main features	Possible uses
Chemical Induction	High dose streptozotocin	Simple model of hyperglycaemia.	New formulations of insulin
	Alloxan		Transplantation models.
	Multiple low dose streptozotocin	Model of induced insulitis.	Treatments that may prevent beta cell death
Spontaneous autoimmune	NOD mice	Beta cell destruction due to an autoimmune process	Understanding genetics of type 1 diabetes
	BB rats		Understanding mechanism of type 1 diabetes
	LEW.1AR1/-iddm rats		Treatments that may prevent beta cell death
			Treatments that may manipulate autoimmune process
Genetically induced	AKITA mice	Beta cell destruction due to ER stress. Insulin dependent.	New formulations of insulin
			Transplantation models.
			Treatments to prevent ER stress
			(could also be used in type 2 diabetes research)
Virally-induced	Coxsackie B virus	Beta cell destruction induced by viral infection of beta cells	Establish potential role of viruses in the development of type 1 diabetes
	Encephalomyocarditis virus		
	Kilham rat virus		
	LCMV under insulin promoter		

Summary of rodent models of T2DM

Induction mechanism	Model	Main features	Possible uses
Obese models (monogenic)	$Lep^{ob/ob}$ mice	Obesity-induced hyperglycaemia	Treatments to improve insulin resistance
	$Lepr^{db/db}$ mice		Treatments to improve beta cell function
	ZDF Rats		
Obese models (polygenic)	KK mice	Obesity-induced hyperglycaemia	Treatments to improve insulin resistance
	OLETF rat		Treatments to improve beta cell function
	NZO mice		Some models show diabetic complications
	TallyHo/Jng mice		
	NoncNZO10/LtJ mice		
Induced obesity	High fat feeding (mice or rats)	Obesity-induced hyperglycaemia	Treatments to improve insulin resistance
	Desert gerbil		Treatments to improve beta cell function
	Nile grass rat		Treatments to prevent diet-induced obesity
Non-obese models	GK rat	Hyperglycaemia induced by insufficient beta cell function/mass	Treatments to improve beta cell function
			Treatments to improve beta cell survival
Genetically induced models of beta cell dysfunction	hIAPP mice	Amyloid deposition in islets	Treatments to prevent amyloid deposition
			Treatments to improve beta cell survival
	AKITA mice	Beta cell destruction due to ER stress.	Treatments to prevent ER stress
			Treatments to improve beta cell survival

Practicals


i.p. ANESTHESIA
1 week before 1/2 animals ALLOXAN i.v. 30 mg/kg
1) blood sample from a tail vein
2) measurement of FPG on glucometer
application of 20% glucose 1ml/100g i.p.
3) repeated measurement of glycemia on glucometer in 30 a 90 min time intervals
4) determination of glucosuria in urine sample
results:
• graph FPG - 30mPG - 90mPG
• comparison of DM x non-DM

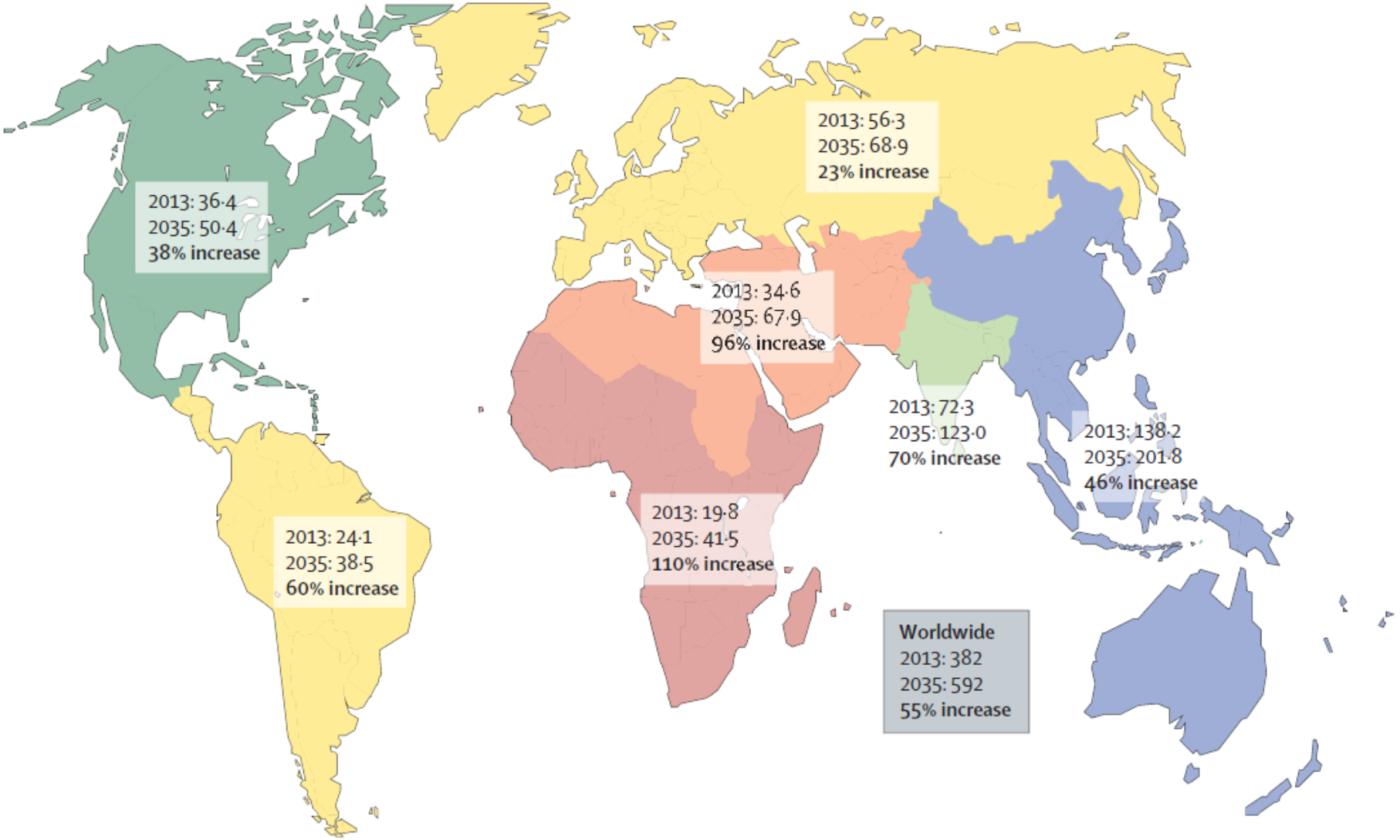
Oral glucose tolerance test (oGTT)

- tool used for diagnosis of
  - diabetes mellitus
    - presence of diabetes in the family,
    - in obese patients and in hypertesion,
    - patients with glycemia 6.1 – 7.0 mmol/l twice in the row
  - gestational diabetes
    - early (< 12th week of pregnancy in women with at least 2 risk factors
      - age > 30 years
      - presence of diabetes in the family
      - macrosomia
      - obesity
      - diabetes mellitus in previous pregnancy
      - glycosuria
      - hypertension in previous pregnancy
      - repeated abortions
  - "prediabetes"
    - impaired glucose tolerance (IGT)
      - 2-h PPG ≥7.8 - <11.1 mmol/l during oGTT
    - impaired fasting glucose (IFG)
      - FPG ≥5.6 – <7 mmol/l
- procedure
  - FPG, drinking glucose solution (75 g + 250 ml water) within 5 – 10 min, glycemia measurement after 60 and 120 min

Definition of DM

- DM is a group of metabolic disorders characterized by **hyperglycemia** as a reason of impaired effect of insulin
  - absolute
  - relative
    - insulin resistance
    - impaired insulin secretion (gluco- and lipotoxicity)
- **chronic hyperglycemia** leads to cell & tissue damage (**complications**)
  - retina
  - kidney
  - nerves

Diabetes prevalence



Diagnosis of DM

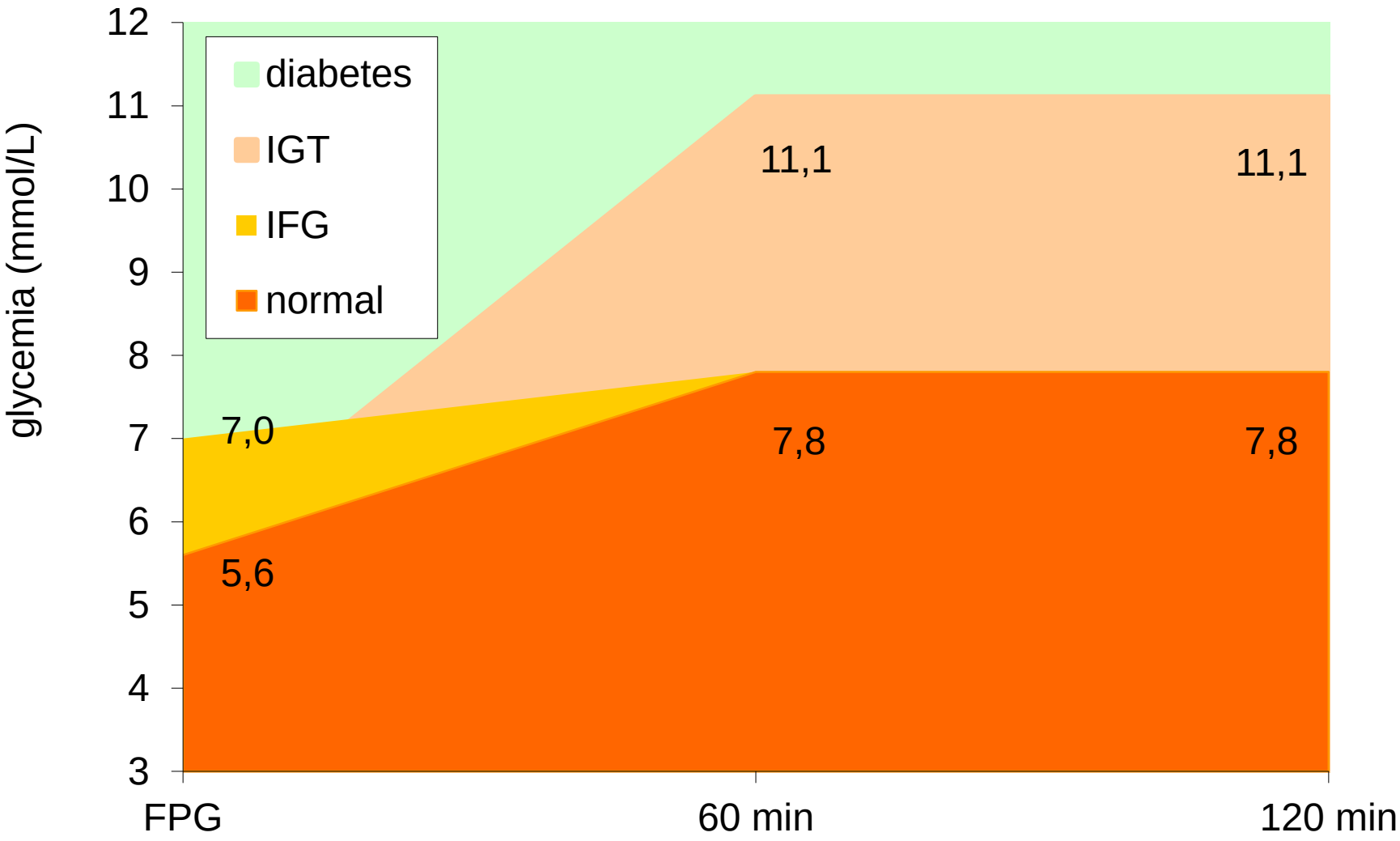
- classical symptoms of diabetes + random plasma glycemia ≥11.1 mmol/l
  - any time of the day
  - symptoms include polyuria, polydipsia and rapid loose of weight
- FPG (fasting plasma glucose) ≥7.0 mmol/l
  - fasting means at least 8 h from the last meal
- 2-h PG (postprandial glucose) ≥11.1 mmol/l during oGTT
  - according to WHO standard load of 75 g of glucose

Interpretation of glycemia

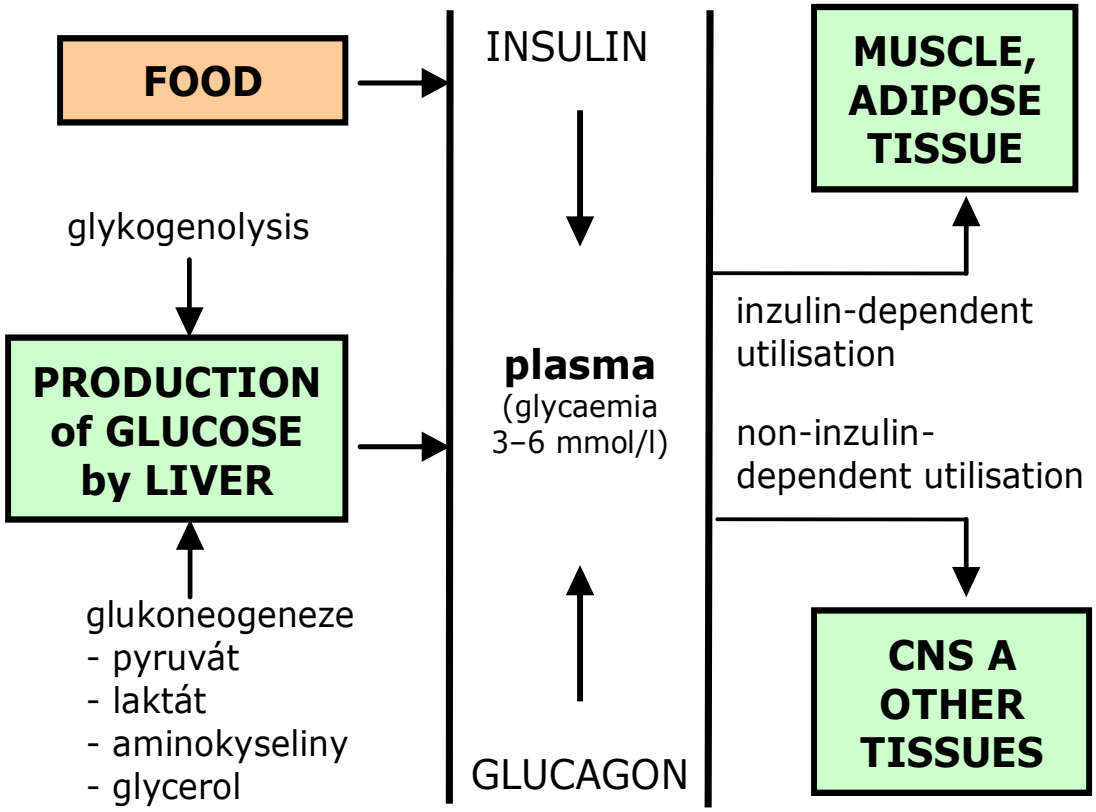
- FPG:
  - <6.1 mmol/l = normal glycemia
  - 6.1-7.0 mmol/l = IFG (impaired fasting glucose)
  - ≥7.0 mmol/l = diabetes

- oGTT – 2h PG:
  - <7.8 mmol/l = normal glucose tolerance
  - 7.8-11.1 mmol/l = IGT (impaired glucose tolerance)
  - ≥11.1 mmol/l = diabetes

oGTT interpretation

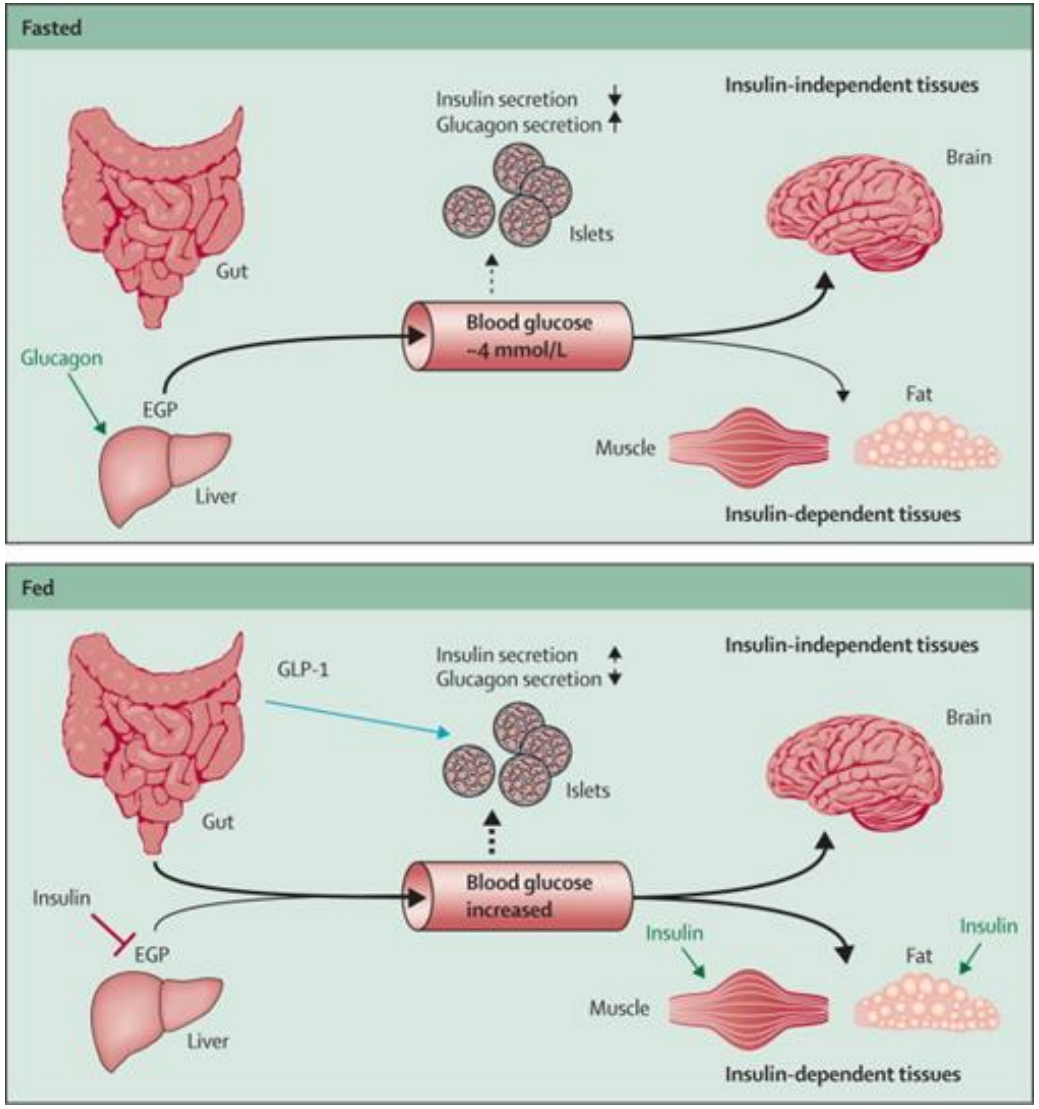


Regulation of glycemia

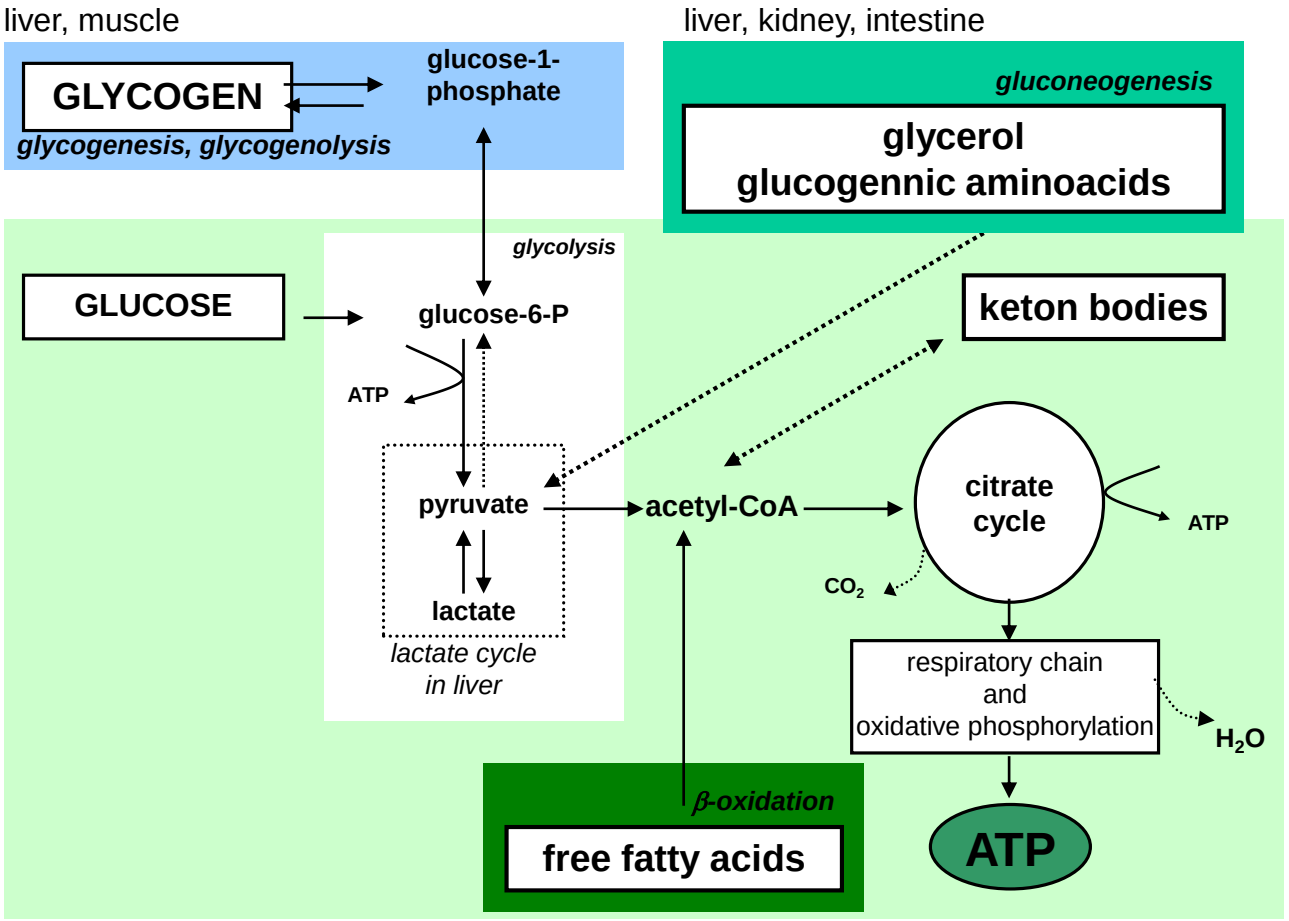


- humoral
  - principal
    - insulin
    - glucagon
  - auxiliary
    - glucocorticoids
    - adrenalin
    - growth hormone
- neural
  - sympaticus
    - hyperglycemia
  - parasympaticus
    - hypoglycemia

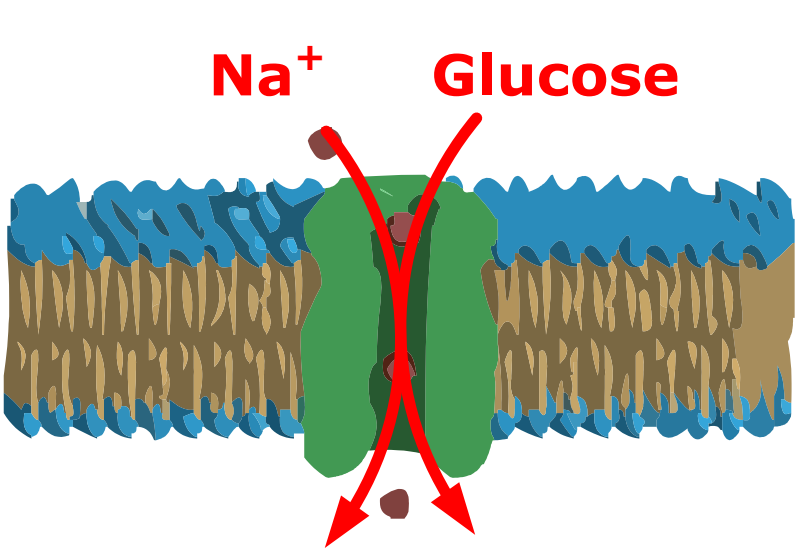
Normal glucose homeostasis

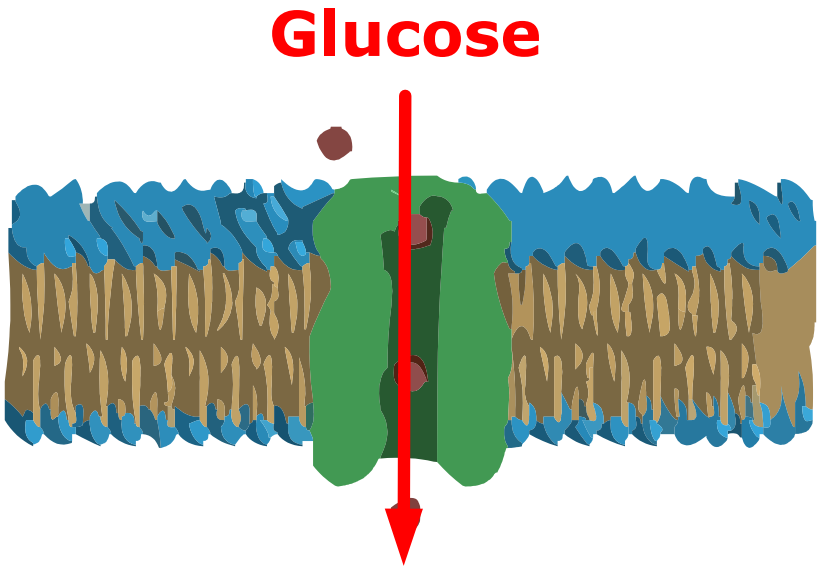


Mutual interchange of substrates in intermediate metabolism



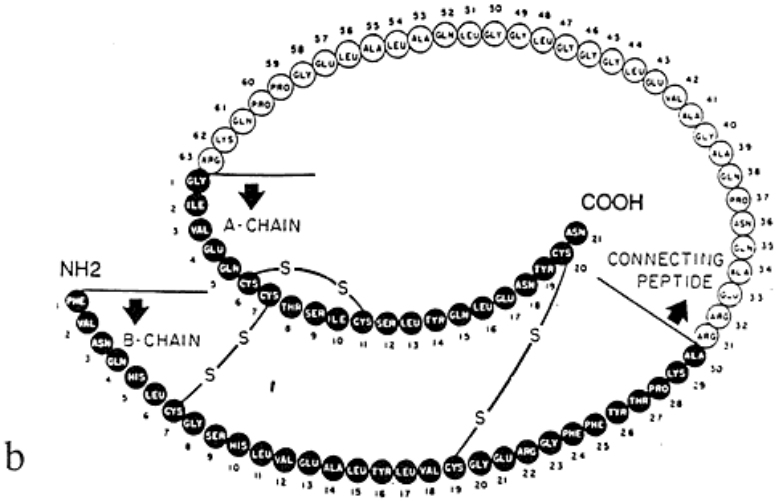
Question – how does glucose enter the cell???





Insulin

- preproinsulin → proinsulin → insulin + C-peptide
- exocytosis into portal circulation
  - 50% degraded during first pass through liver
- total daily production 20 - 40 U
  - 1/2 basal secretion, 1/2 stimulated
- basal secretion pulsatile
  - 5 - 15 min intervals
- stimulated – glucose, amino acids, FFA, GIT hormones
  - early phase (ready insulin)
  - late phase (synthesis de novo)



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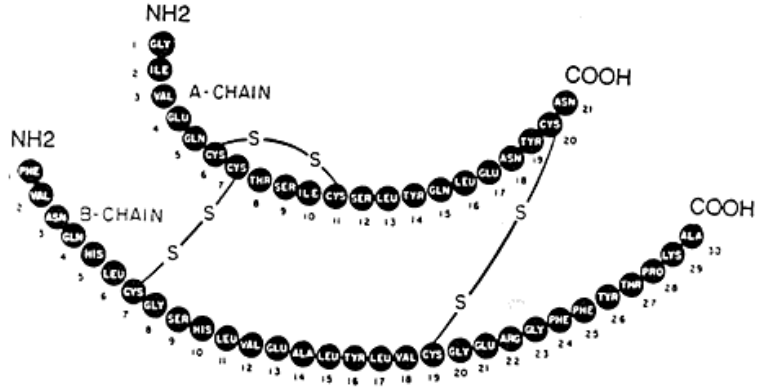

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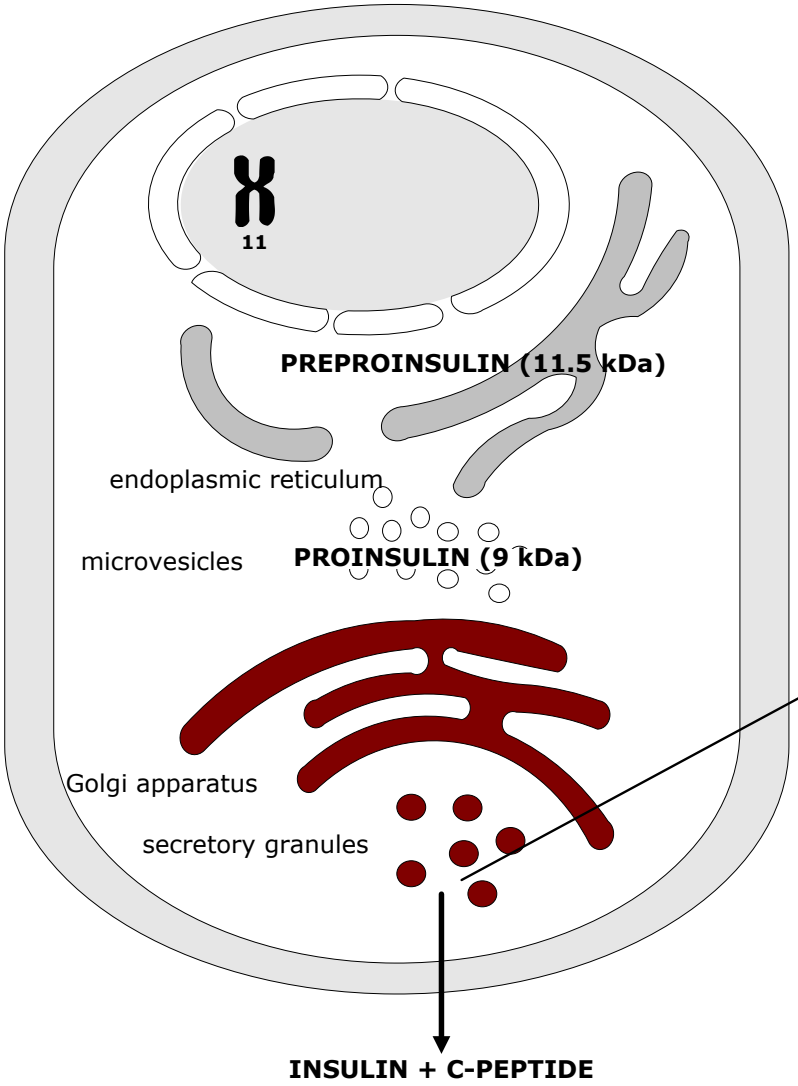
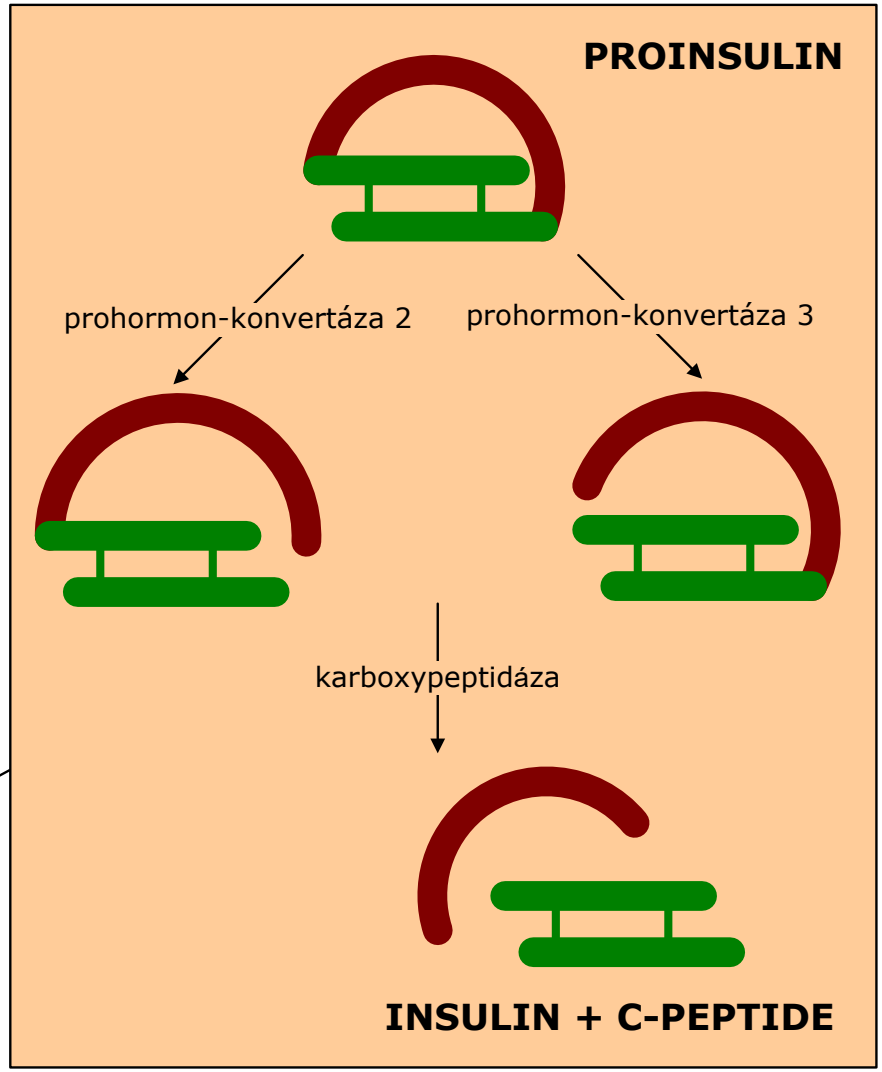
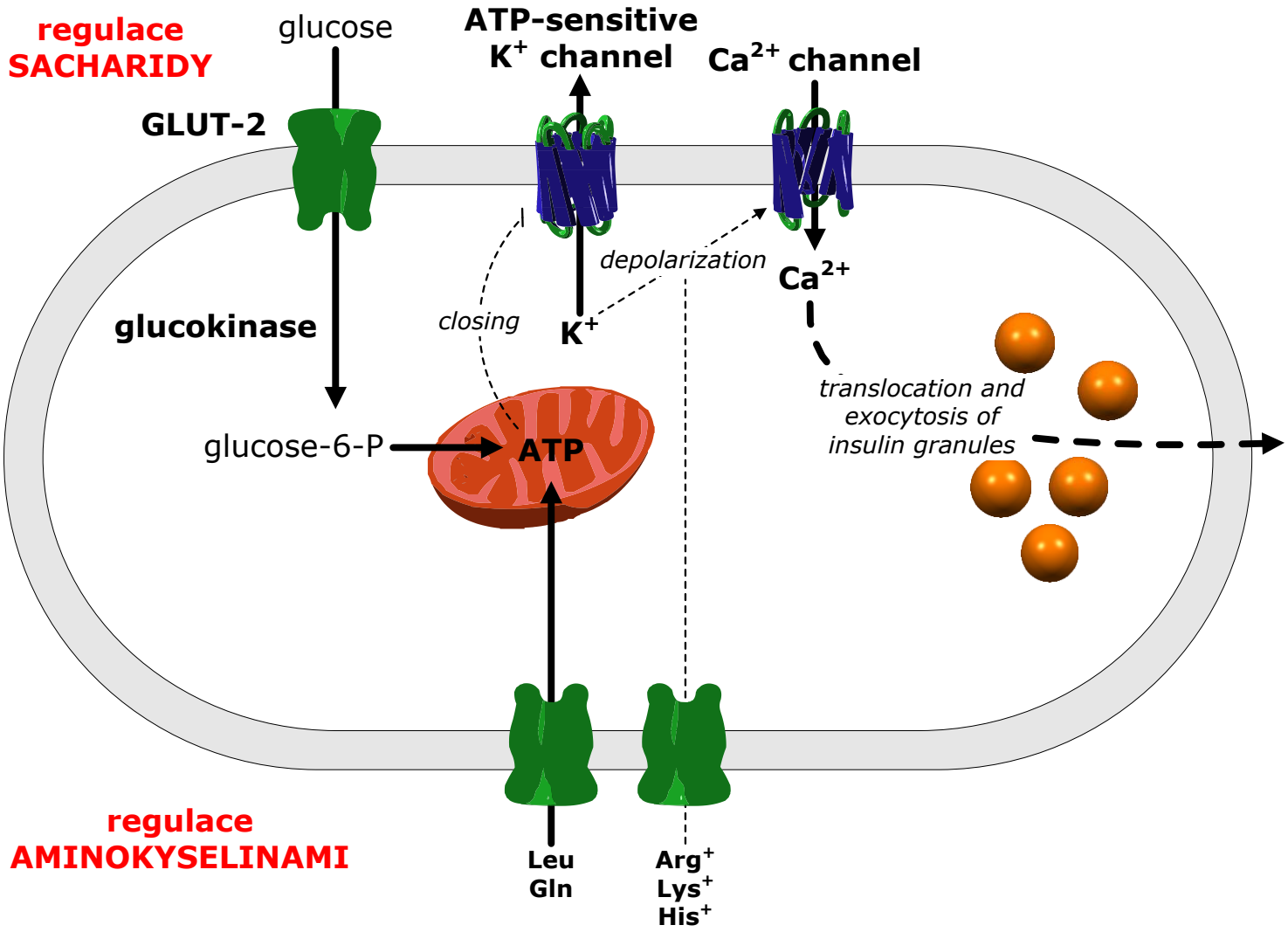
Synthesis of insulin

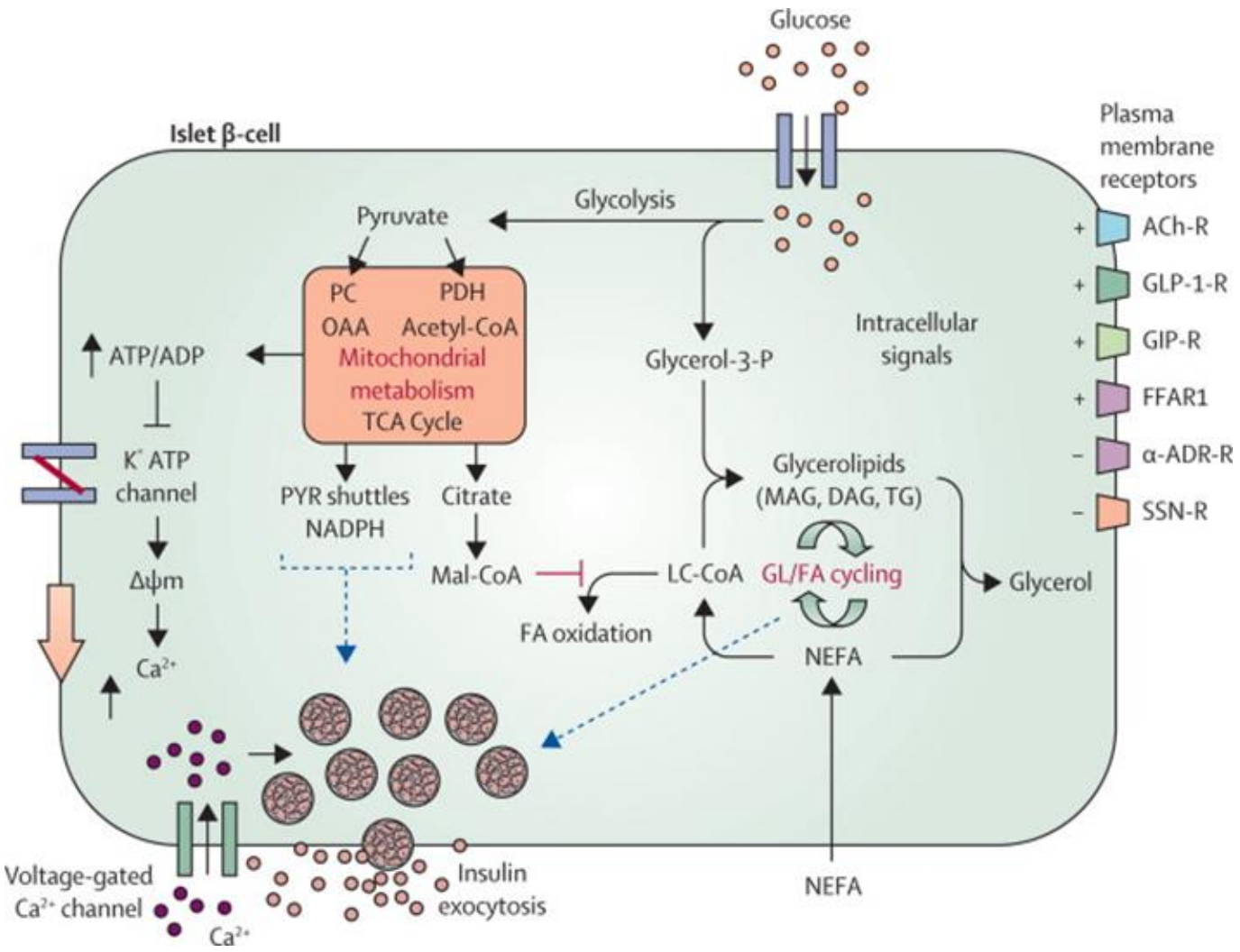




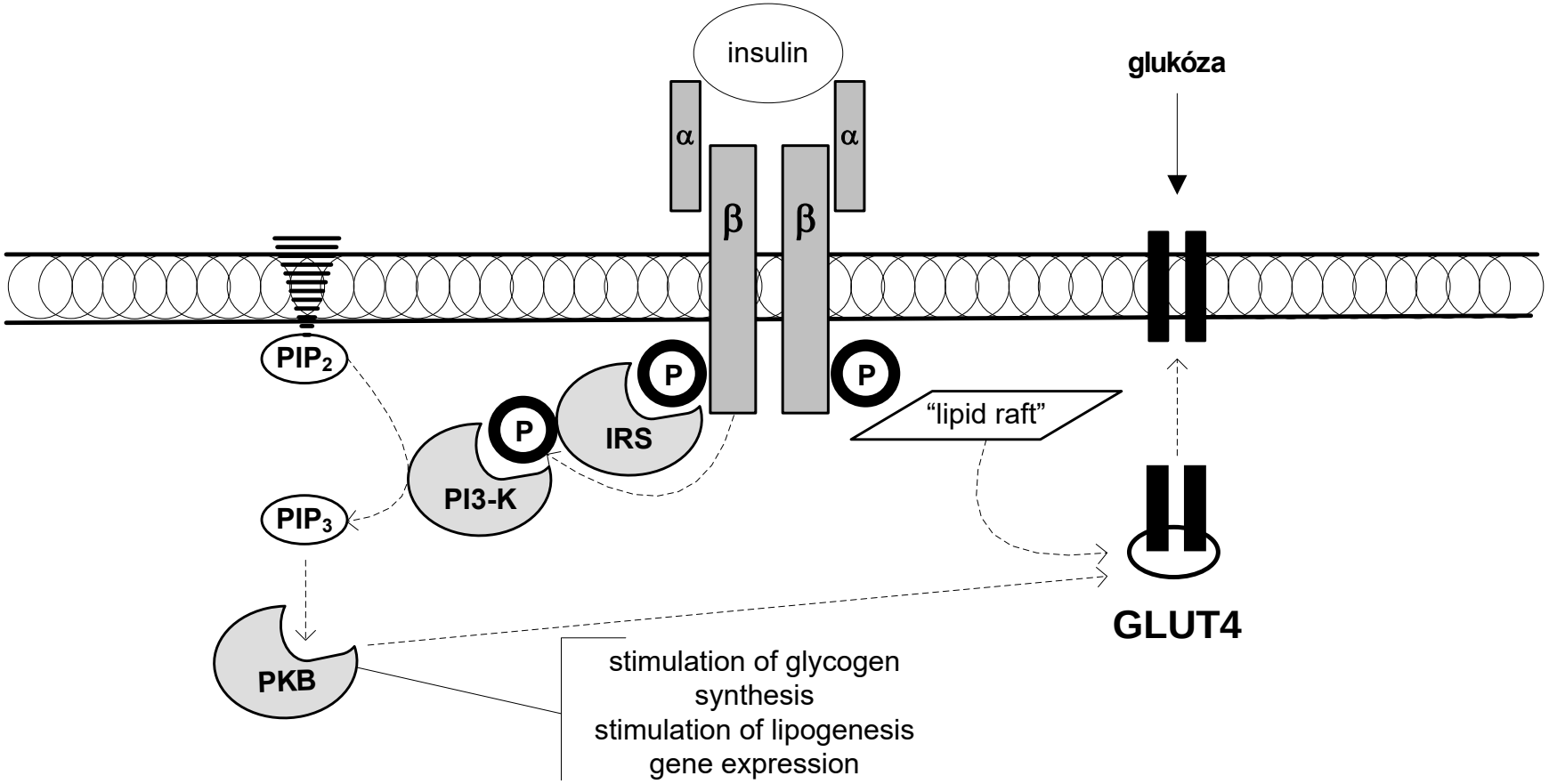
Relationship glycemia – insulin secretion



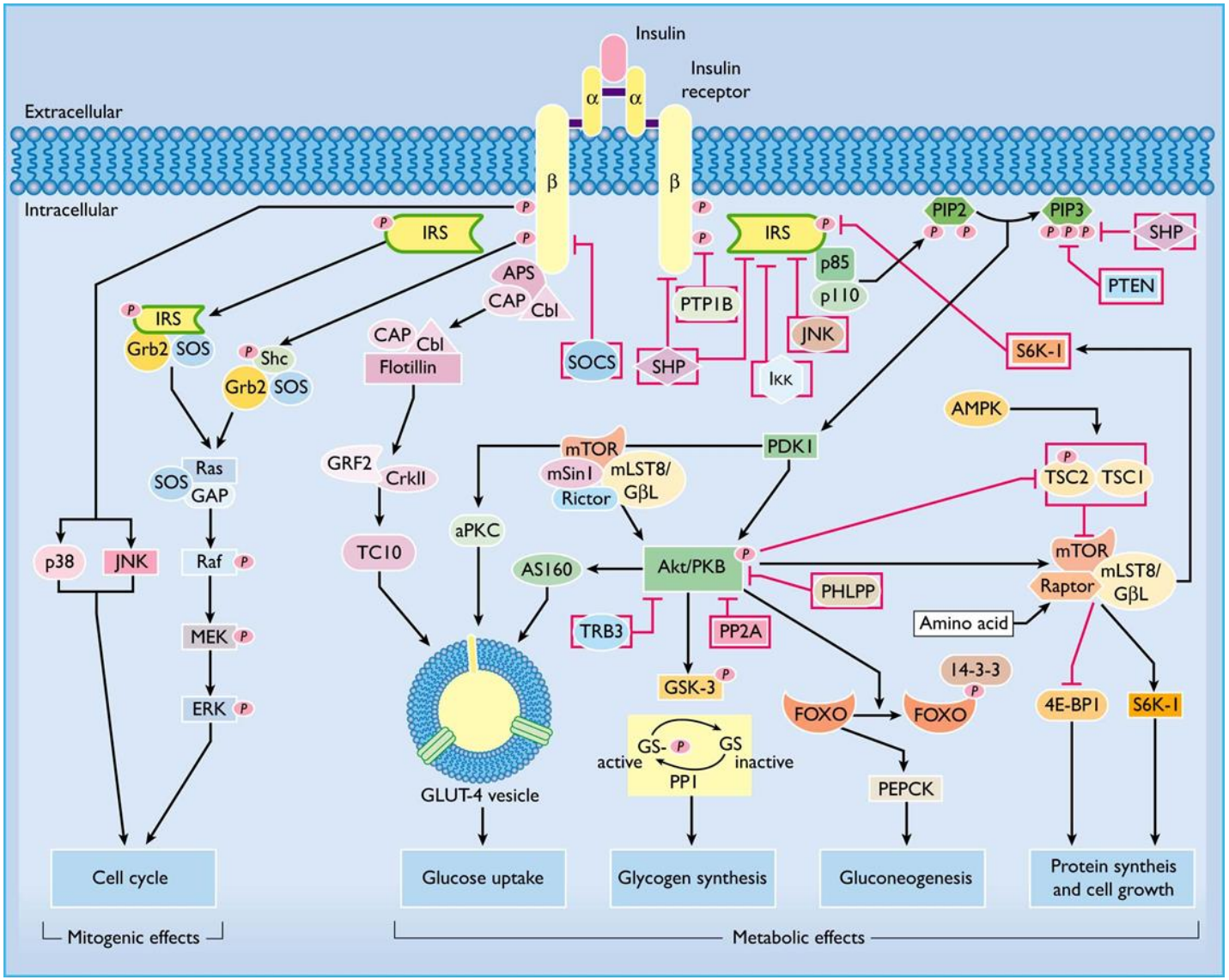
Islet β-cell metabolic activation



Intracellular cascade of insulin receptor



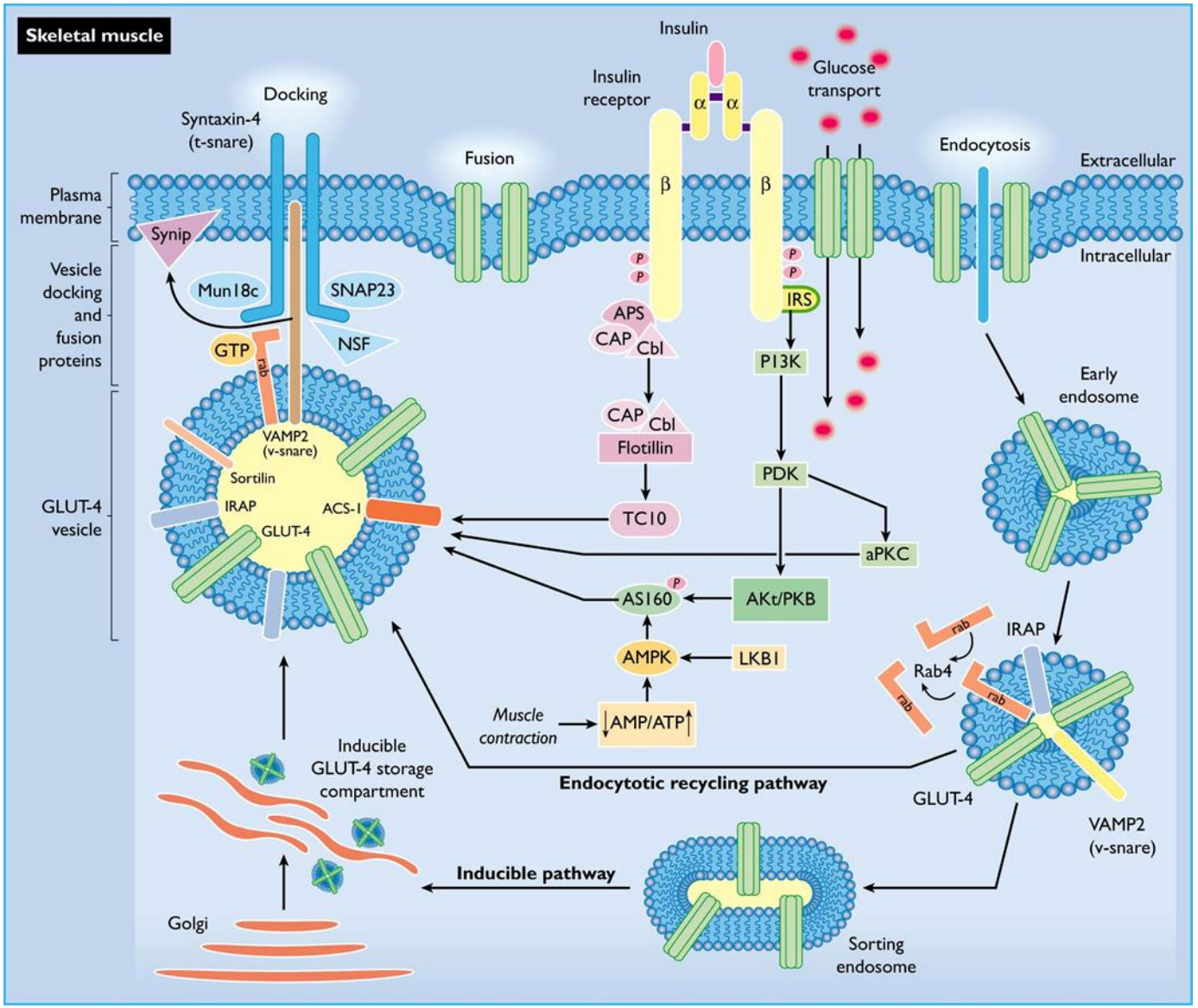
Intracellular insulin signalling



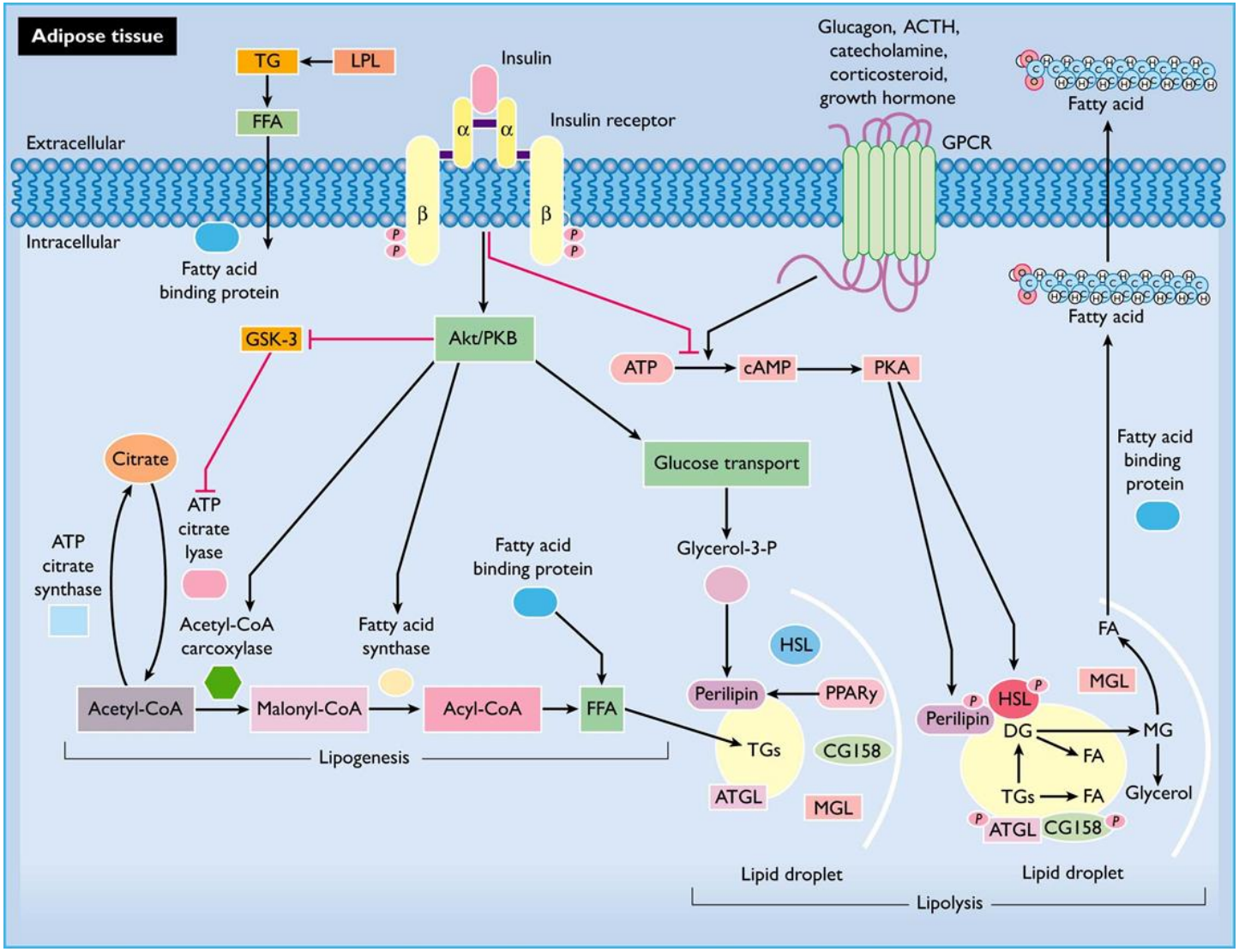
Classification of tissues according to insulin action:

- insulin-sensitive
  - muscle, adipose tissue
    - facilitated diffusion by GLUT4
    - integration into cytoplasmic membrane regulated by insulin
  - liver
    - stimulation of glycogenolysis
    - inhibition of gluconeogenesis
- insulin-non-sensitive
  - others (incl. muscle, adipose tissue, liver)
  - transport of glucose depends on
    - concentration gradient
    - density of transporters (GLUT1-4,8-10)
    - rate of glycolysis

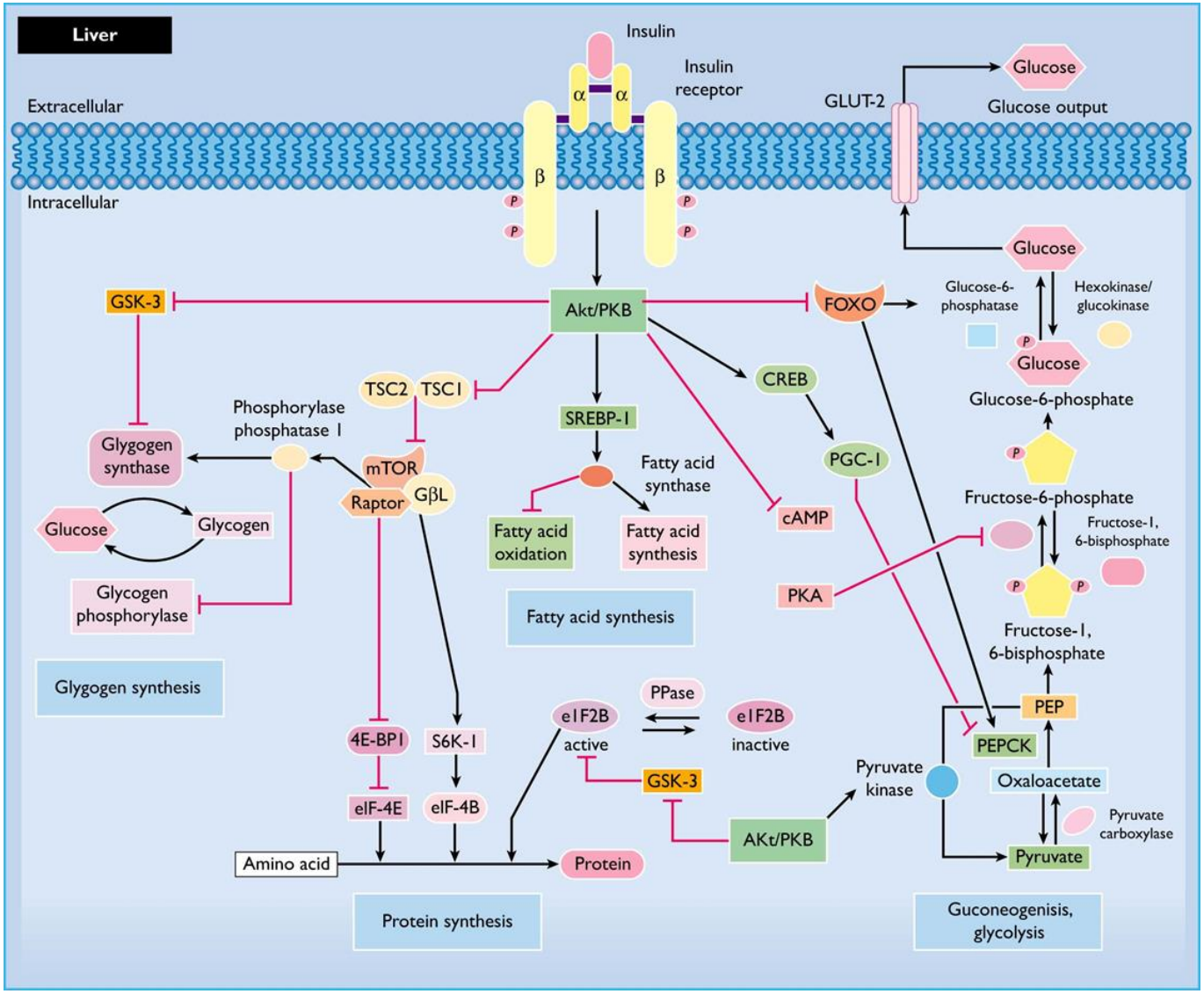
Insulin action in the muscle



Insulin action in adipose tissue



Insulin action in the liver



Diabetes mellitus

- heterogeneous syndrome characterized by hyperglycemia due to deficiency of insulin action (as a result of complete depletion or peripheral resistance)

- prevalence of DM in general population 5%, over the age of 65 already 25%

Causes of insulin deficiency

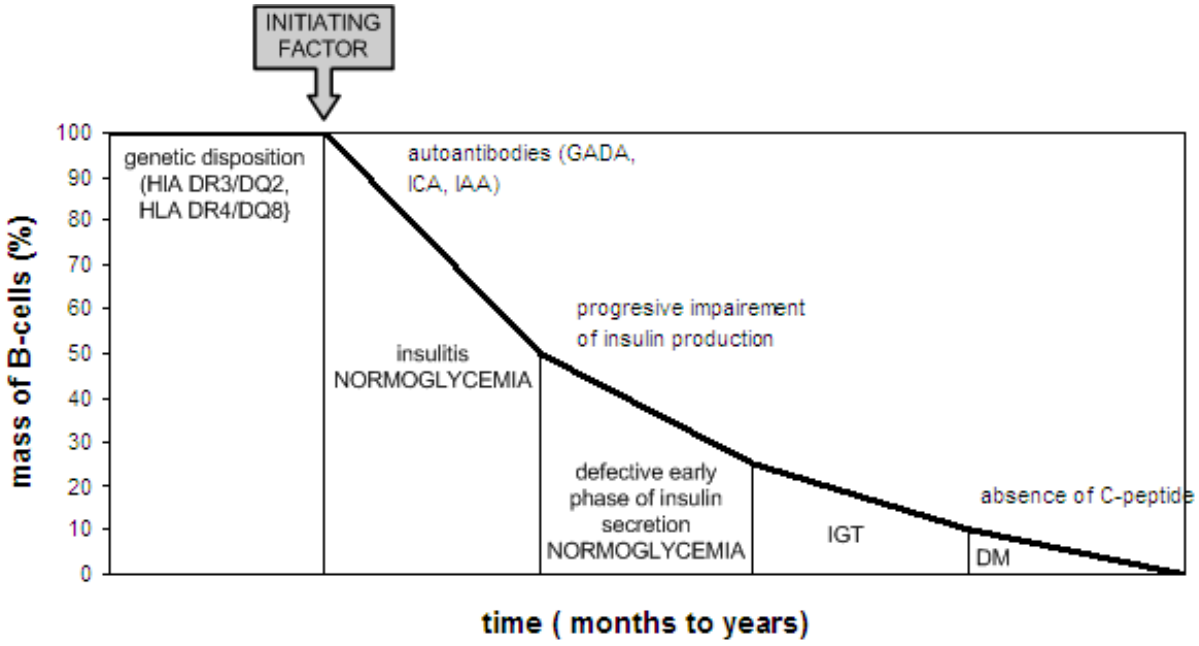
- **absolute**
  - destruction of the β-cells of the islets of Langerhan´s
- **relative**
  - insulin
    - abnormal molecule of insulin (mutation)
    - defective conversion of preproinsulin to insulin
    - circulating antibodies against insulin or receptor
  - insulin resistance in peripheral tissue
    - receptor defect
    - post-receptor defect

Classification of DM

I. DIABETES MELLITUS
Diabetes mellitus of type 1 (T1DM)
Diabetes mellitus of type 2 (T2DM)
Gestational diabetes mellitus
Other specific types - genetic defects of β cell function (MODY) - genetic abnormalities of insulin receptor - exocrine pancreas disorders - endocrinopathies - iatrogenic - rare genetic syndromes
II. IMPAIRED GLUCOSE TOLERANCE (IGT)
- with obesity - without obesity

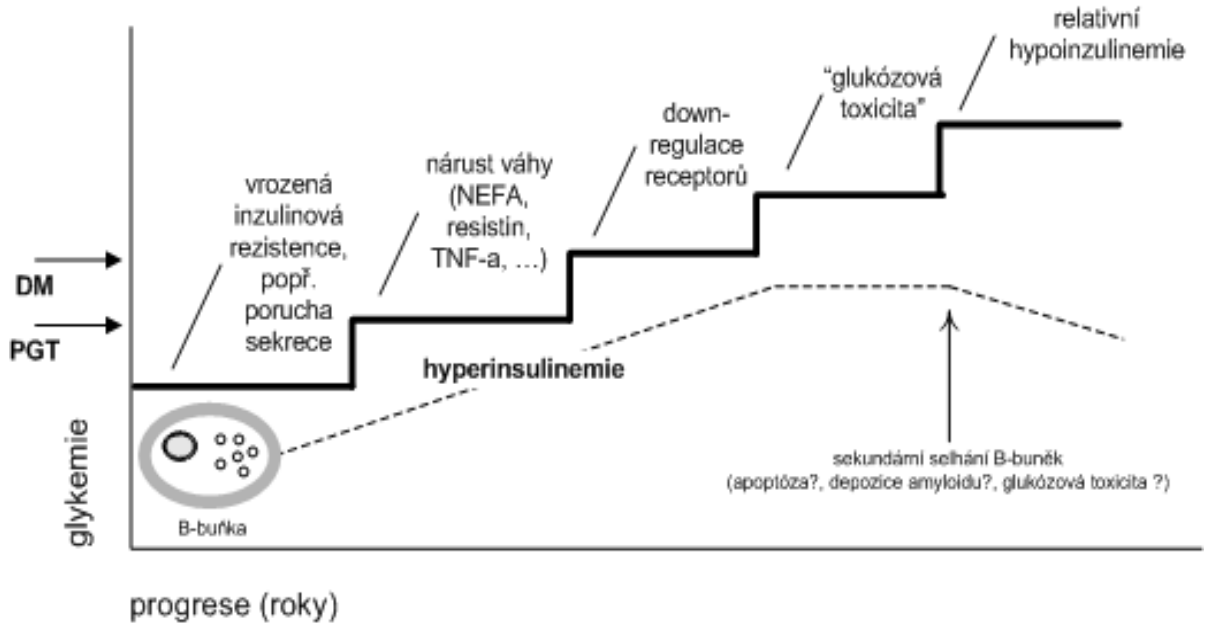
Type 1 DM (formerly IDDM)

- selective destruction of β cells of LO in genetically predisposed individuals
  - chrom. 6 - HLA (DR3-DQ2 a DR4-DQ8), chrom. 11 - inzulin gene
  - initiation by infection (viruses)
- autoimmunity mediated by T-lymphocytes (antibodies against β cells (ICA, GAD) though)
- manifestation typically in childhood
- absolute dependence on exogenous supplementation by insulin

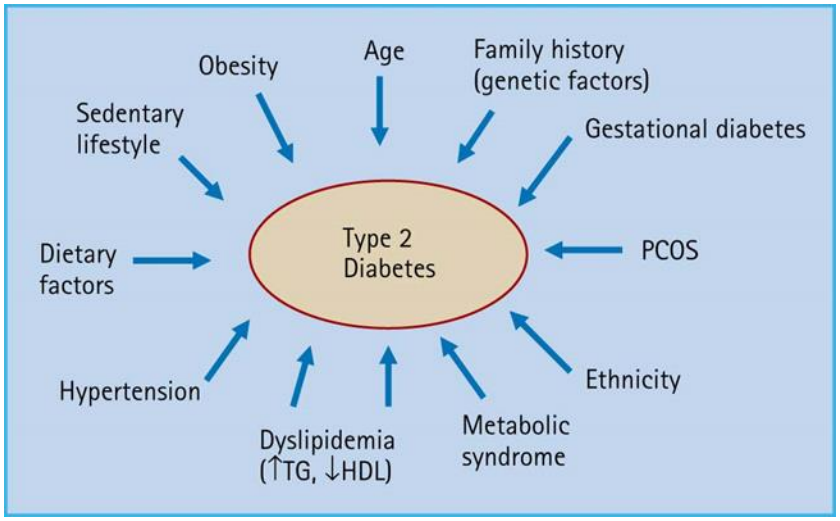


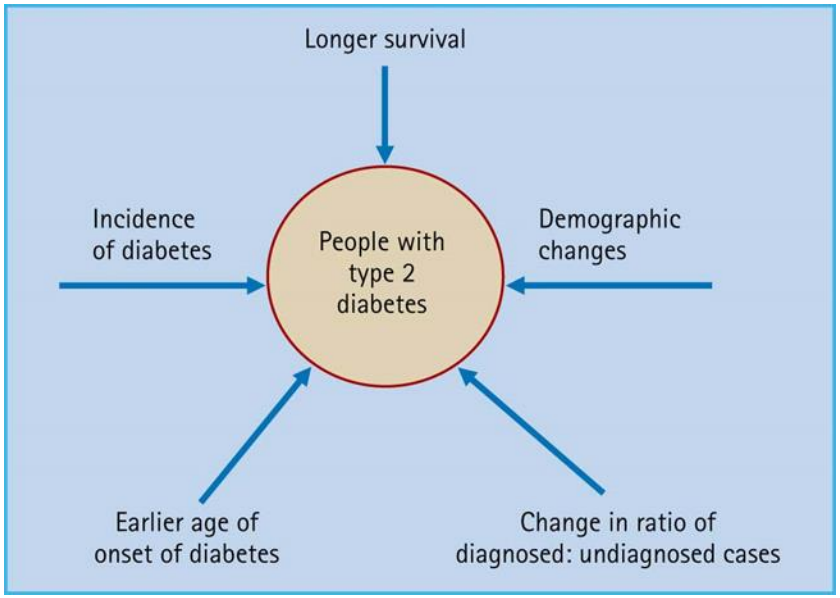
Type 2 DM (formerly NIDDM)

- imbalance between secretion and affect of insulin
- genetic predisposition – polygenic
  - insulin resistance
  - impairment of secretion
- clinically manifested T2DM has concomitant insulin resistance and impairment of secretion
  - due to epigenetic factors
  - typically in older adults
- 90% of subjects is obese – **metabolic syndrome!!!**



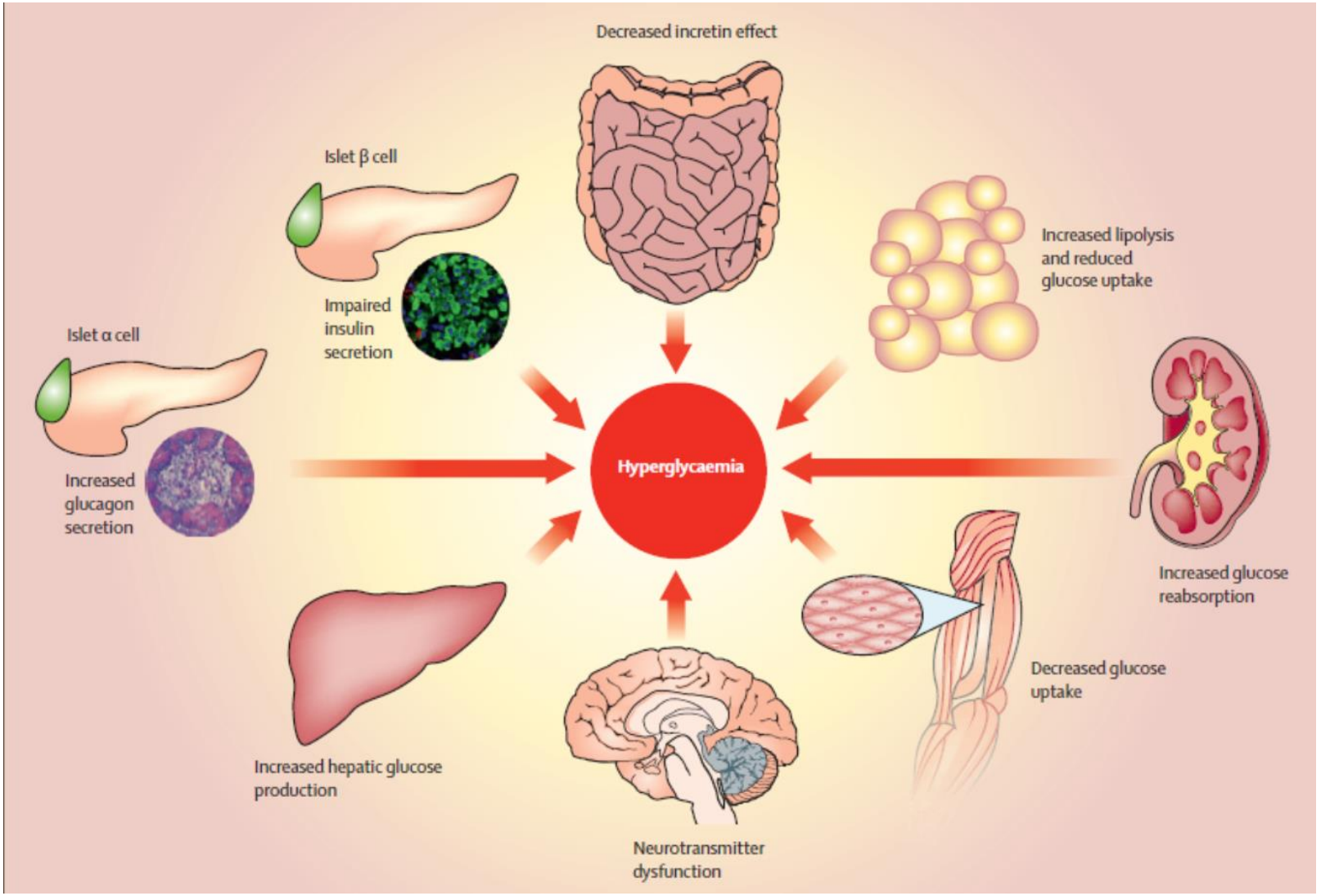
Epidemiology of T2DM



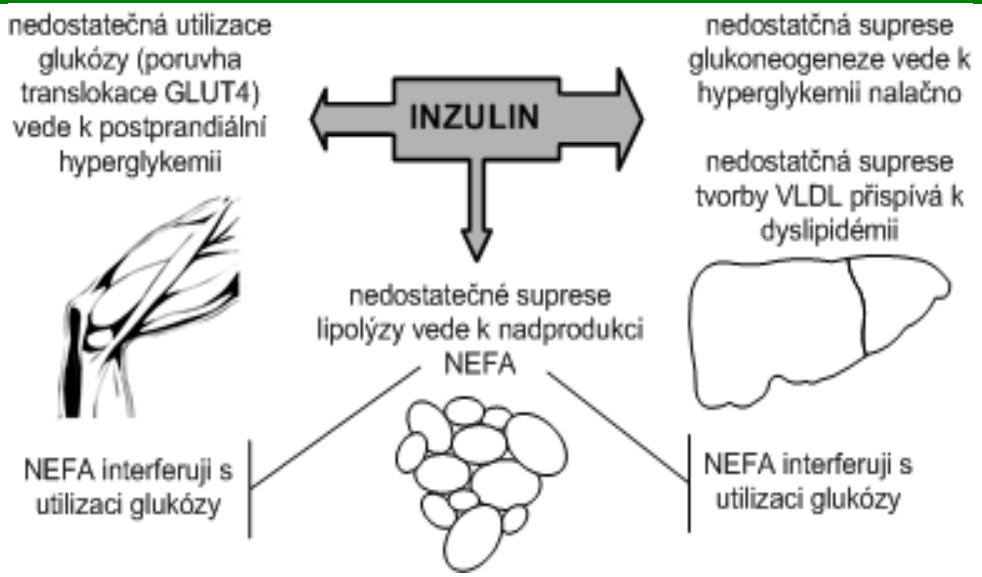


- another risk factors
  - sleep restriction
    - 57% nárůst rizika DM (10 let pozorování, 70 000 žen)
  - léky
  - environmental pollutants
  - Low birthweight and fetal malnutrition

The ominous octet

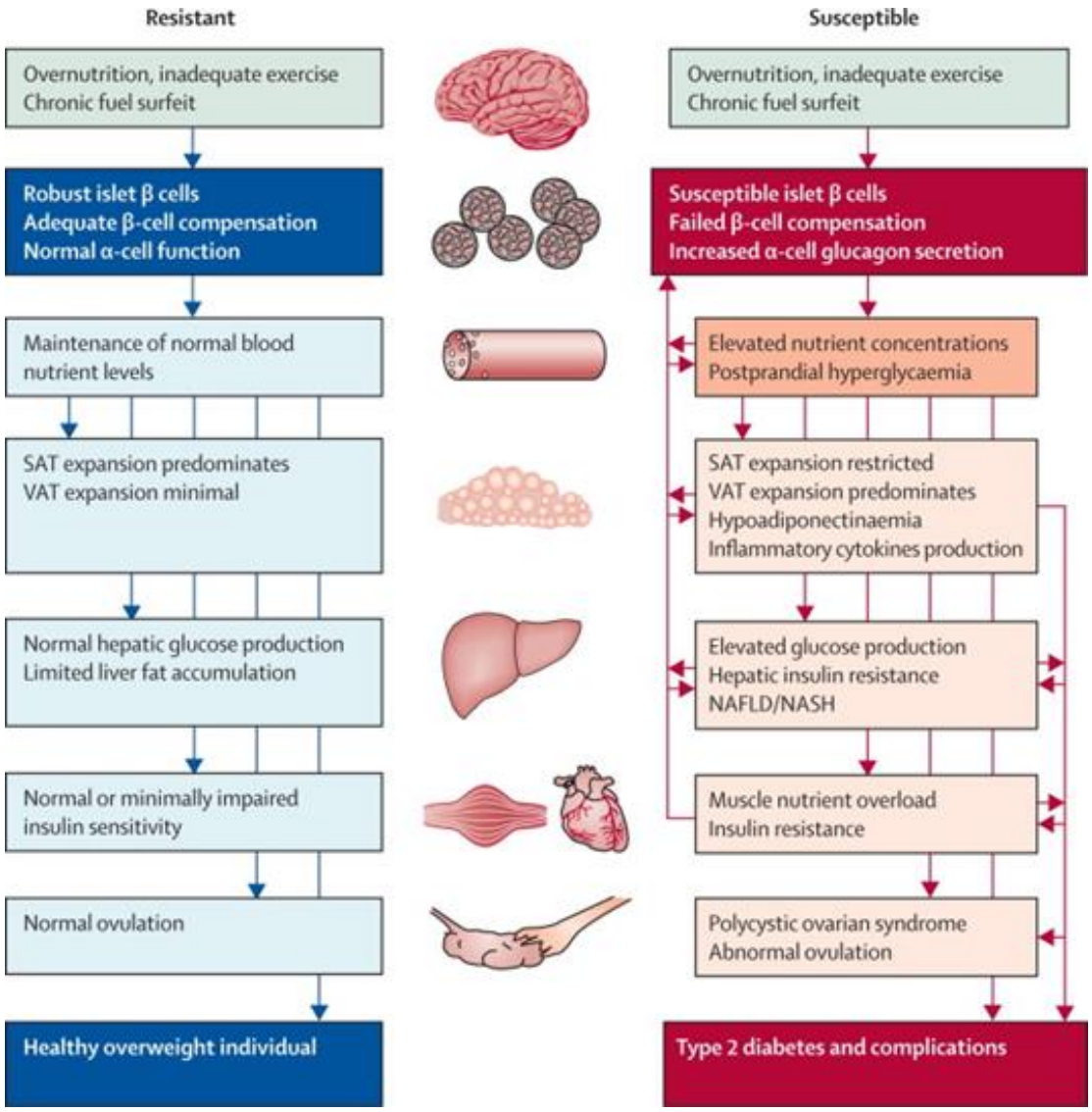


Insulin resistance

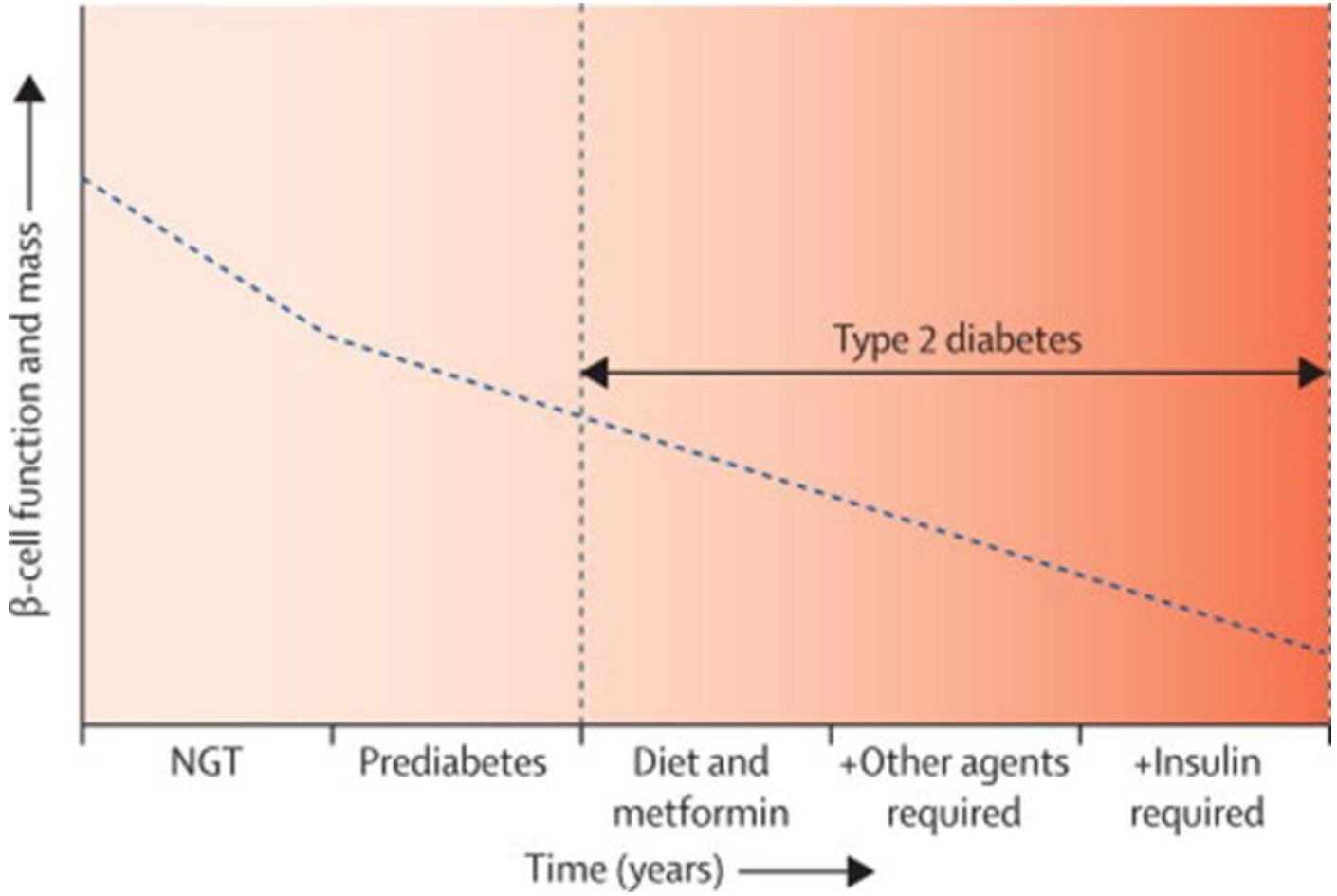


- physiologic amount of insulin does not cause adequate response
- compensatory hyperinsulinism
- further worsening by down-regulation of insulin receptors

Pathway to T2DM



Natural history of T2DM



Maturity-onset diabetes of the young (MODY1-6)

- group of monogenic conditions with autosomal dominant inheritance
- childhood, adolescence or early adulthood onset
- genetically determined β-cells dysfunction
  - but long-term measurable C-peptide without signs of autoimmunity
- 1% of diabetic patients

- two subgroups
  - mutations in glucokinase (MODY2)
    - glucokinase = glucose sensor (production and releasing of insulin is slowing)
    - mild form without considerable risk of complications
  - mutations in the genes encoding transcription factors (remaining 5 types)
    - severe β-cells defects progressively leading to diabetes with serious complications
    - Affected glucose-stimulated production and release of insulin and also proliferation and differentiation of β-cells

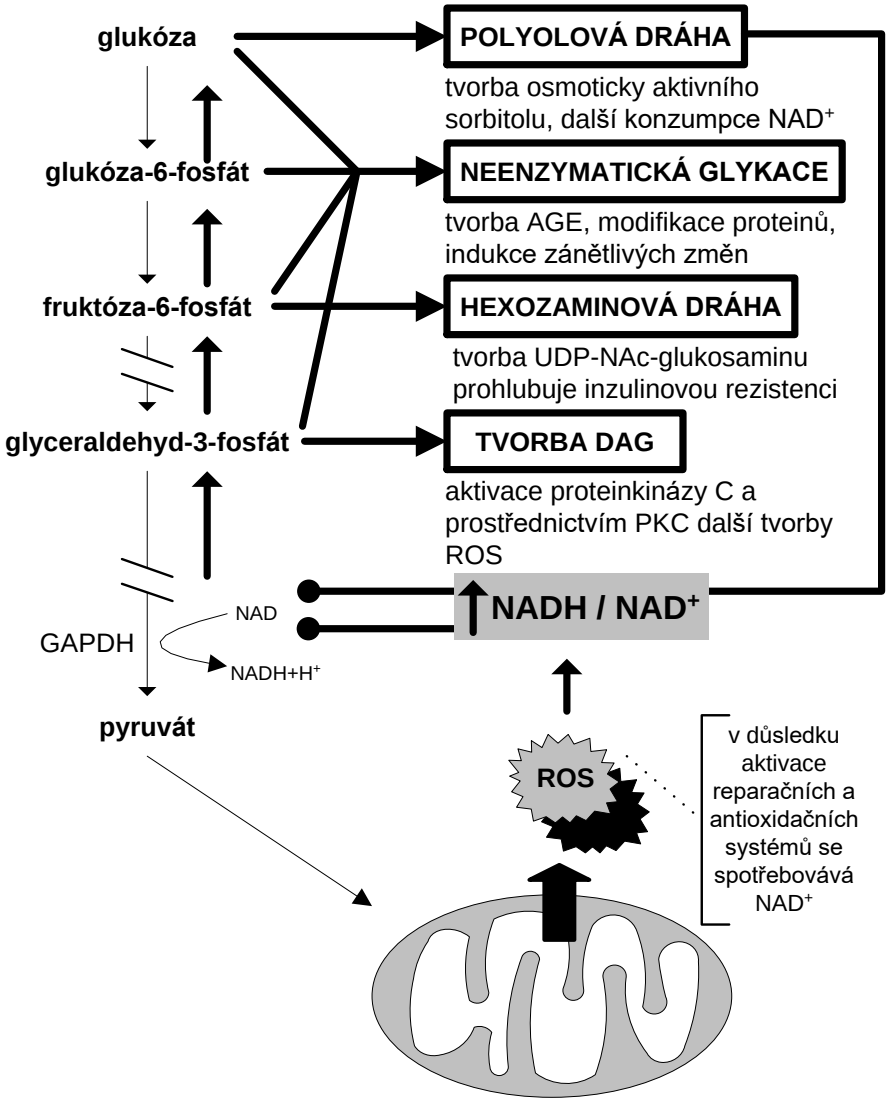
Main characteristics of T1DM and T2DM and MODY

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

Clinical presentation of manifest DM

- due to the increase of blood osmolality, osmotic diuresis and dehydration
  - classical
    - polyuria
    - thirst
    - polydipsia
    - weight loss
    - temporary impairment of visus
    - cutaneous infections
- acute
  - hyperglycemic coma
    - ketoacidotic
    - non-ketoticidotic
  - hyperosmolar nonketoacidotic hyperglycemia
  - lactate acidosis

Complications of DM



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