# Physiological and pathological biochemistry of carbohydrates

Digestion and absorption of carbohydrates and its disorders

Basic metabolic pathways of carbohydrates and its disorders

Metabolic disorders of fructose and galactose

Glycogenoses

Regulation of glycemia and its disorders

Complex carbohydrates

mucopolysaccharidoses

Diabetes mellitus as a complex metabolic disease – diabetes biochemistry

Molecular interpretation of late consequences of diabetes

# Carbohydrates

# Carbohydrates

- also **saccharides** (from lat. *saccharum*)

- Organic compound belonging to group of polyhydroxyderivates - aldehydes or ketones Low molecular weight carbohydrates are soluble in water and taste sweet
- Macromolecular carbohydrates are mostly flavourless, their solubility in water is limited (starch, agar) or are totally insoluble (cellulose)

# Carbohydrates

- Consists of carbon, hydrogen and oxygen, they differ in structure and size of molecule
- Basic structural unit - monosaccharide
- Glycosidic bond

- Source of energy for function of brain and muscles
- Primary source of energy in intensive training

- In plants, carbohydrates are formed by assimilation of air $CO_2$ in presence of water and daylight - photosynthesis

- **Daily intake 50 - 60% of overall energetic intake**

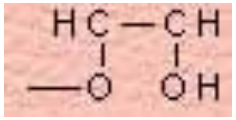
- Amount of energy in 1g = **4 kcal** = **17 kJ**

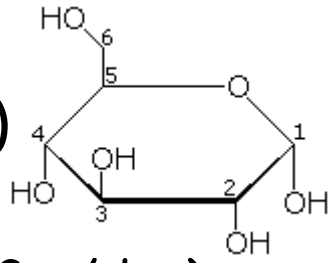
- **Storage glykogen (liver and muscles)**

# Classification of carbohydrates

- Simple carbohydrates
  - Monosaccharides 1 x 6C
  - Disaccharides 2 x 6C
- Complex carbohydrates
  - Polysaccharides 10 and more C

# Simple carbohydrates

- <u>Monosaccharides</u>
  - Occurence: fruit (10 – 12%), honey (35%G, 35%F), vegetable, juices…
  - Sweet taste 




    - **Glucose** (grape, starch sugar, dextrose)
      - » Fastest source of energy
      - » Essential for brain and erytrocytes (150 g/day)
    - **Fructose** (fruit sugar, levulose)
    - **Galactose** (constituent of milk sugar)

# Simple carbohydrates

- Disaccharides
  - **Maltose** (malt sugar) – grain and malt shoots

    **glucose + glucose**

  - **Saccharose** (beet, reed sugar) – sugar beet, sugarcane, maple syrup
    - consumption 100 – 120 g/person/day
    - daily intake 10% max. 10

    **glucose + fructose**

  - **Lactose** (milk sugar) – milk and milk products
    - consumption 10 – 30 g/person/day

    **glucose + galactose**

# Complex carbohydrates

- Polysaccharides
  - **Digestible** (amylose + amylopectin)
    - Consisting of glu units
    - Starch – plant
    - Glycogen - animal
    - Main sources in food: grains and their products (flour, bread, rice, pasta, corn, oat..), potatoes, legumes, vegetable

# Complex carbohydrates

- Polysaccharides
  - **Indigestible** (fibre)
  - Partial to complete resistence to hydrolysis by digestive juices
  - Except for soluble fibre they pass unaltered through small intestine
  - Fermentation by enzymes of large intestine microflora → FA
  - 1 g of fibre = 3 kJ
    - **DDD 25 – 30g ratio R:N 1:3**
      - Division
        - » **Soluble** – pectins, inulin, fructooligosaccharides, slimes, gums, hemicelluloses …
          fruit, oat, malt, legumes, potatoes
        - » **Insoluble** – cellulose. Lignin, hemicelluloses…
          vegetable, bran, whole grain products

# Significance of fibre

- **Soluble**
  - Partial cleavage in small intestine → gels → slowdown in upper part of GIT → elevation of viscosity of intestinal content→ ↓ access of digestive juices to substrates, binding of mineral compounds → ↓ absorption of nutrients and bile acids, velocity slowdown of glu resorption, prebiotic

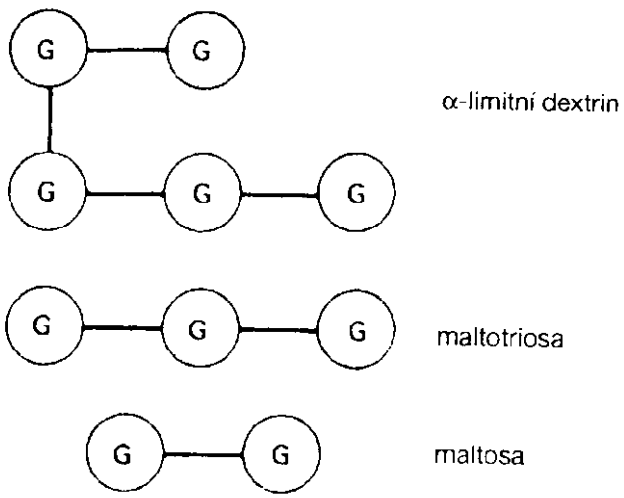
- **Insoluble**
  - ↑ stool contain (dilution and binding of toxic compounds), shortening of transit time → reduction of resorption of toxic compounds, ↓ absorption of some nutrients
    - Fermentation => FA with short chain (acetate, propionate, butyrate) = sources of energy for colonocytes (80%), lower pH

=> Preventive x bowel obstruction, polyps and large intestine tumours, bilestones, reduce blood cholesterol

# Digestion of starch

- Oral cavity
  - Starch – salivary α-amylase (ptyalin)
  - pH optimum 6,7
- Stomach
  - Decreased activity of ptyalin

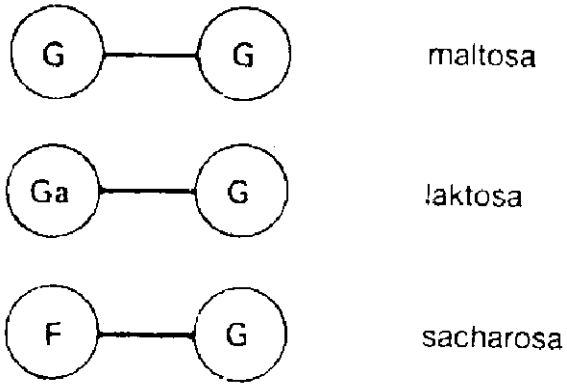
# Digestion of starches




- Small intestine
  - Pancreatic enzymes–
    pancreatic α-amylase
    - Hydrolysis of 1,4 α-bond
      => maltose, maltotriose, glu polymers,
      α-limit dextrines (8 glu)

  - Mucous membranes of small intestine – oligosaccharidases
    - The outside of the brush border
    - α-limit dextrinase – α-limit dextrines
    - Glucoamylase – maltose => glu
      - maltotriose => glu
      - polymers of glu => glu

# Digestion of disaccharides

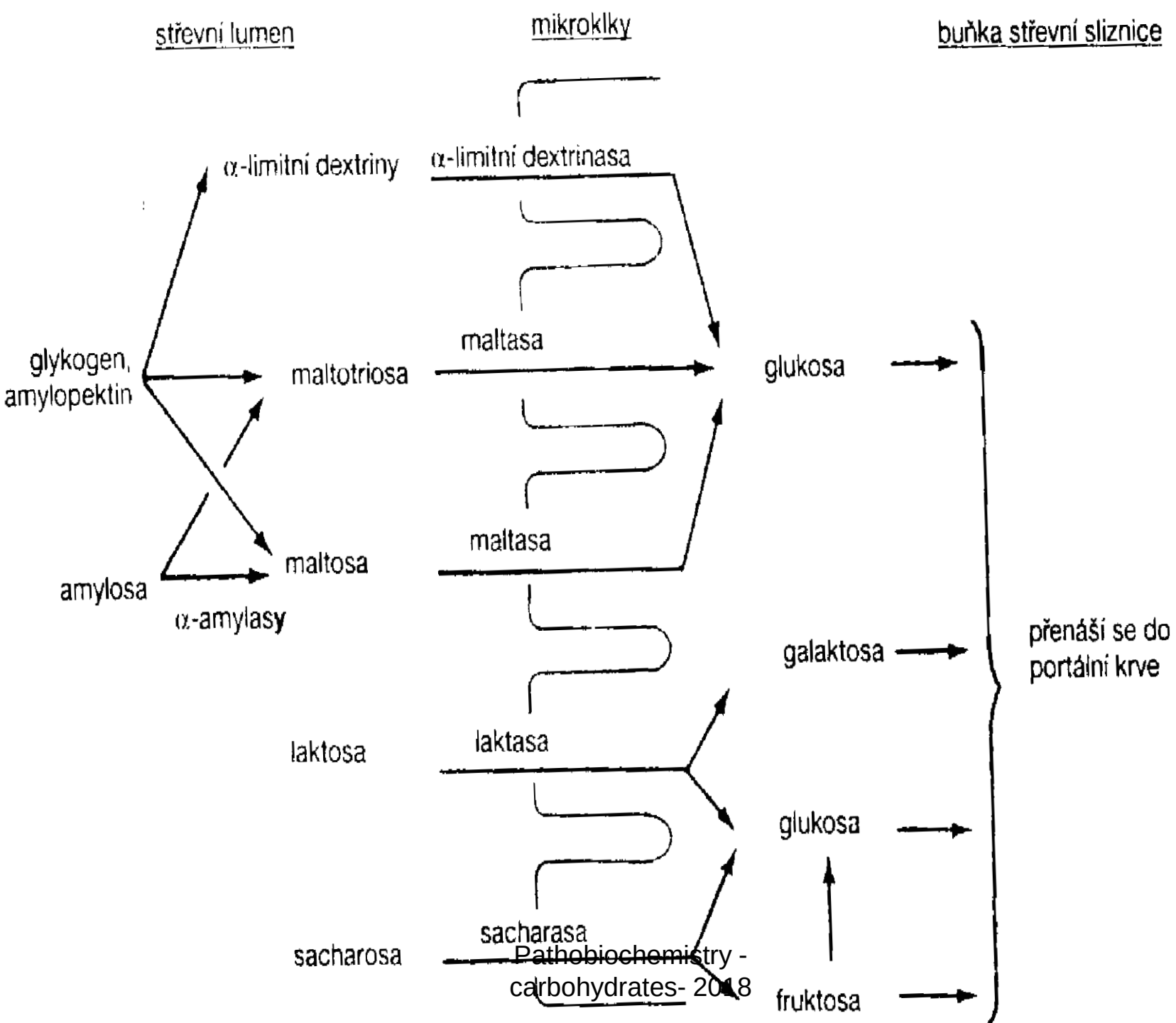



- Small intestine
  - Mucous membrane of small intestine – disaccharidases
    - » Laktose → *lactase* => glu and gal
    - » Maltose → *maltase* => glu and glu
    - » Saccharose → *saccharase* => glu and fru
  - Lack of disaccharidases => diarrhea, flatulence
    - » *Increased amount of osmotically active molecules of oligosaccharides and formation of gases*
  - Lactase – activity declines with age
    - » **lactase intolerance**
  - Digestion and resorption malfunctions in inflammations

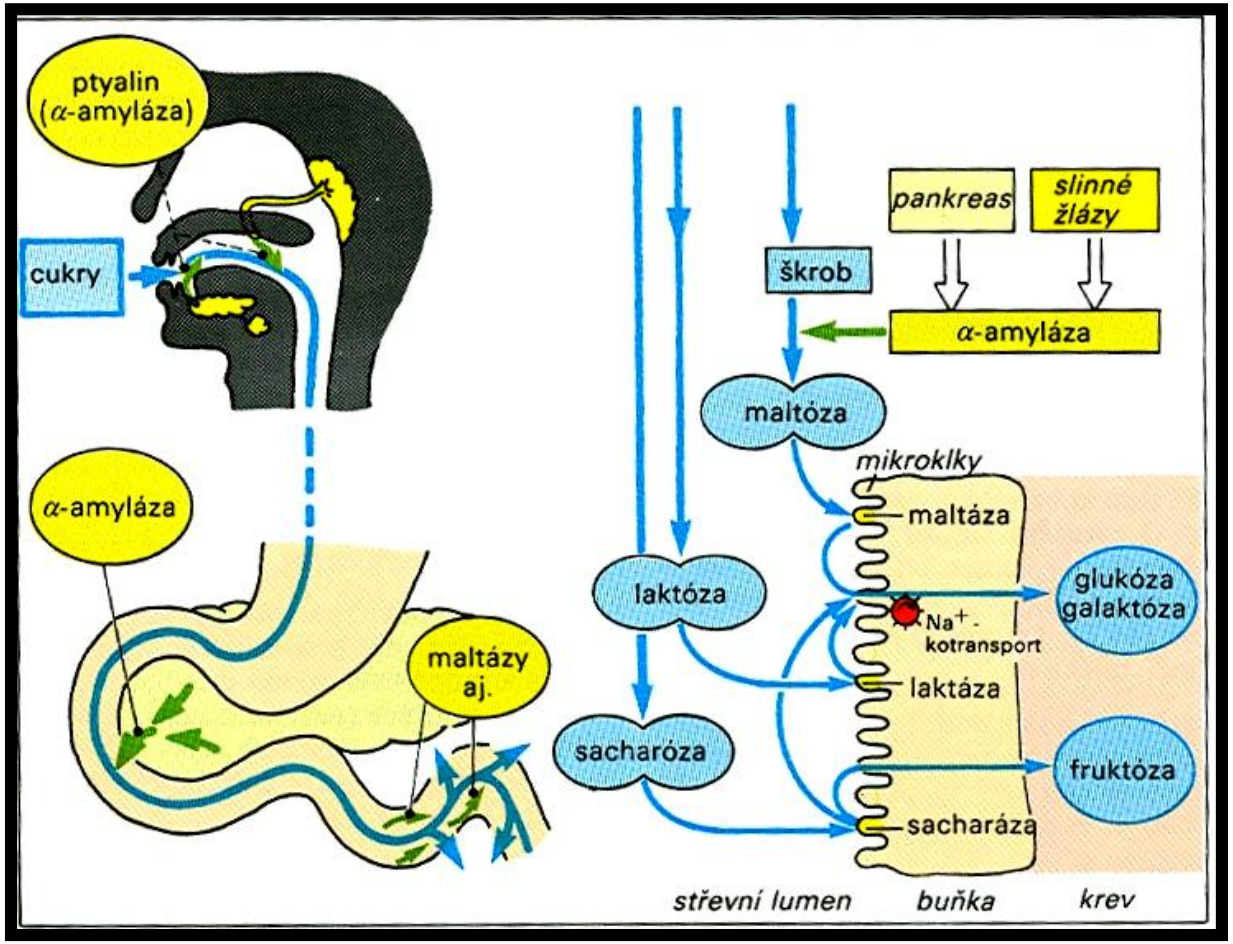
# Absorption of carbohydrates

- Fast – through the wall of small intestine => v. Portae
- Resorption – before the rests of food get into terminal ileum
- Max. speed 120 g/day.
- Site of absorption – the duodenum and proximal jejunum

- Effect of $Na^+$ on carbohydrates transport
  - ↑ c $Na^+$ at mucosal surface of cells → facilitates glu entry into the cell and the other way around
    - Common contransport
  - $Na^+$ - transport down concentration gradient + glu = secondary active transport into ICT, facilitated or simple diffusion into ECT
  - Gal – the mechanism is the same
  - Fru – absorption independent on $Na^+$
    - slower resorption
    - facilitated diffusion
    - part of fru → glu (mucosal cells)


střevní lumen
mikroklky
buňka střevní sliznice
α-limitní dextriny
α-limitní dextrinasa
glykogen,
amylopektin
maltotriosa
maltasa
glukosa
maltasa
maltosa
amylosa
α-amylasy
galaktosa
přenáší se do
portální krve
laktosa
laktasa
glukosa
sacharasa
sacharosa
fruktosa

# Summary of digestion- saccharides




Simple sugars can be resorbed to portal blood and transported to the liver and to other tissues where they serve as a source of energy or storage of energy (glycogen) in liver. Excess intake of carbohydrates is stored in form of fat.

Beginning of carbohydrates digestion - mouth - salivary a-amylase cleaves starches into dextrins, maltotriose, maltose, digestion continues through the esophagus and in the stomach before acidic gastric juice is secreted and salivary amylase is inactivated by its low pH.
pancreatic a-amylase is secreted in duodenum - dextrins to disaccharides which are digested by specific intestinal disaccharidases of intestinal juice into monosaccharides in small intestine
sucrose (by sucrase) = glucose and fructose
lactose (by lactase) = galactose and glucose
maltose (by maltase) = glucose and glucose
Simple carbohydrates are actively absorbed into **enterocytes** - secondary active cotransport of Na+ and delivered down the gradient using a carrier (facilitated diffusion) from cells to portal blood. In case of fructose, only passive transport had been proven and its absorption is faster than of other monosaccharides.

# Carbohydrate metabolism

- Monosaccharides → portal circulation → liver
- 1. step in metabolism of glu,fru, gal – phosphorylation

- Galactose → gal-1-P→ glu-1-P (→ glucose)
  - Share on glycogen synthesis, reversible reactions
  - Gal – formation of glycolipids, mucoproteins
- Fructose → fru-6-P → fru-1,6-diP →
  → fru-1-P → dihydroxyacetone and glyceraldehyde → metabolic pathways of glu
  - glucose, synthesis of glycogen and TAG
    - » Liver - high ability to synthesizeTAG

- **Use of hexose phosphates**
  - Cleavage as an energy substrate in tissues
  - conversion to glycogen (liver, skeletal muscle)
  - conversion to FA and triacylglycerols (TAG) (liver, adipose tissue) – energy reserve
  - Minority part - metabolization in pentose cycle, synthesis of glycoproteins, glycolipids

# Glucose metabolism

- **Glycolysis -anaerobic**
  - degradation of glu under anaerobic conditions to pyruvate or lactate
  - Gain of energy 2 ATP/1 mol of Glu
  - Process consists of several steps
  - Does not take place in mitochondria but in cytoplasm
    - Pyruvate => acetyl-CoA (irreversible reaction)
      => lactate
      => alanine => proteins

# Aerobic glycolysis and Citric acid cycle

- Acetyl-CoA => fats
  => Krebs cycle
- Krebs cycle– oxidation of carbohydrates, fats and some AA
- Krebs cycle => Acetyl-CoA => reduced cofactors, $CO_2$, water and energy
- Aerobic oxidation- respiratory chain– 36-38 mol ATP/1 mol glu

- Process of CC – mitochondria
- Requires $O_2$ delivery
- It doesn't work under anaerobic conditions!!!!!

# Glycogen metabolism

- Glycogenesis
  - Starage glycogen formation from Glu-1-P
  - When? - In excess of glucose
  - Store - liver (100 g), muscle (300-400 g)
  - Glycogen - holds water
- Glycogenolysis
  - Glycogen decomposition
  - When? - In lack of Glu
  - Adrenalin (activation of phosphorylase)

# The physiological importance of glucose

- Glu - the fastest source of energy
  **essential for Ery and brain, nerve cells.**

- Min. need 150 g/24 h.
  - < 150 g/24 h => glukoneogenesis, ketogenesis (energy from FA, formation of ketone bodies in case that production exceeds utilization => ketosis - disturbance of acid-base balance, prevention min. 50 – 100 g sacch./day)

- Blood glucose level – glycemia (3,9 – 6,1 mmol/l)
  - hepatic glucose- liver maintains constant level of glu

- Hypoglycemia => ↑ glucagon, adrenaline => mobilization of Glu formation (glycogenolysis)

- Glu - can not be sythesized from fat only from small amount of glycerol

# Hormonal regulation

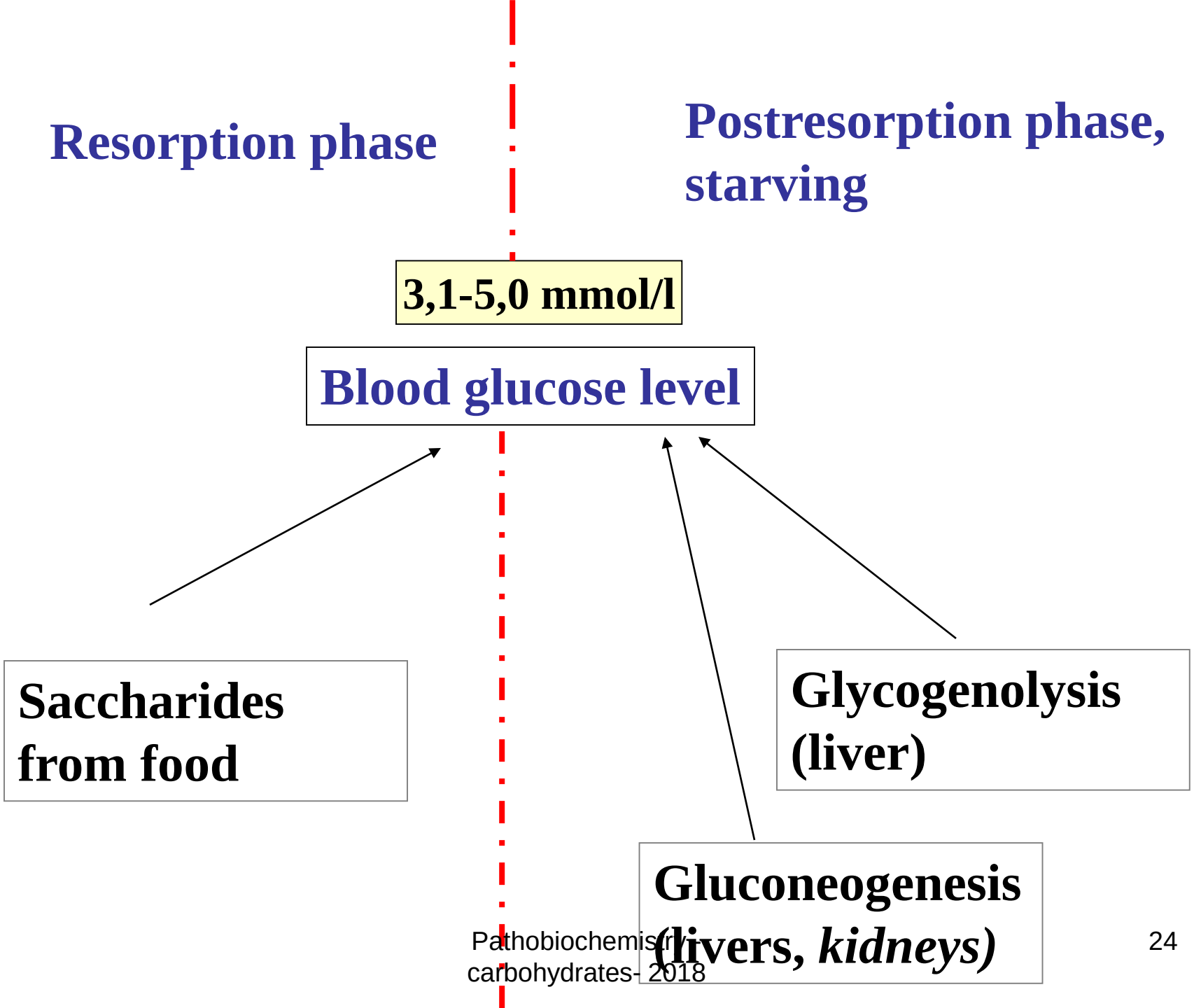
- Insulin
  - Produced by B-cells of Langerhans pankreatic islets
  - Stimulatory effect on glu utilization
  - Secretion regulator – glycemia (gly) level
    - ↑ gly level => ↑ insulin => ↑ utilization of glu to cells => normalization of gly level
  - Is stimulated also by fructose, AA (Arg), glucagon

- Glucagon
  - Produced by A-cells of Langerhans pankreatic islets
  - Activates hepatic phosphorylase => glykogenolysis => ↑ gly level

- Corticosteroids, catecholamines, thyroid hormones, growth hormone

# Gain of glucose under physiological conditions

- Supply from external environment - simple or complex carbohydrates
- from storage – glycogen
- Glukoneogenesis – from AA
  - When? - starvation, low carbohydrate intake, DM, stress
  - Also in reverse order – transamination of glu metabolism products → AA


Resorption phase
Postresorption phase, starving
3,1-5,0 mmol/l
Blood glucose level
Saccharides from food
Glycogenolysis (liver)
Gluconeogenesis (livers, kidneys)

# Glucose in blood

**One of basic priorities of metabolic regulation:**

**blood glucose level can´t drop under 3 mmol/l**

**Hormonal regulation:**

**insulin** (decreases glu level)

**glucagon** (increases glu level)

**adrenalin**

**kortisol**

# Factors determining the glucose level

- <u>The balance between the amount of glu entering the blood and amount of glu that leaves blood</u>
  - Intake of carbohydrates from food
  - The speed of glu entry into muscle cells., adipose tissue cells and other organs
  - 5% glu → glycogen
  - 30 – 40 % glu → fat (when filled stocks with glycogen)
  - The rest → metabolism in muscle and other tissues
  - Liver glycogen during starvation→ glucosa
    - » Prolonged starvation → glykogenu depletion => glukoneogenesis

# Glucose level determining factors

- Balance between amounts of glu entering the blood and glu leaving the blood
  - Intake of saccharides from food
  - Velocity of glu entry to muscle cells, adipocyte cells and other organs
  - 5% of glu → glycogen
  - 30 – 40 % of glu → fat (if glycogen supplies are full)
  - The rest → metabolisation in muscles and other tissues
  - Liver glycogen in starving→ glucose
    - » Long-lasting starving → drawing out of glycogen => gluconeogenesis

# Homeostasis of carbohydrates during exercise

- At rest and after exertion
  - Consumption of FA by skeletal muscle
  - Consumption of glu – brain
- Physical exertion => glycogenolysis => ↑ gly level, gradual reduction during exercise

  => ↑ gluconeogenesis↓ insulin level, ↑ glucagon and adrenalin level
- After physical exertion => gluconeogenesis, decline in output of glu from liver (for liver glycogen refill)

  => ↑ insulin level => Support of glycogen storage

# Entry of glucose to the cells

Glu molecules are strongly polar, can´t diffuse through hydrophobic lipid bilayer membrane (hydrogen bonds between OH groups and water)

## Glucose transporters

-transmembrane proteins facilitate transport of glucose to the cells

- type GLUT (1-14)* or SGLT**

** glucose transporter*

*** sodium-coupled glucose transporter*

## GLUT 1-GLUT 14, identical traits:

~ 500 AA, 12 transmembrane helixes

mechanism:
Facilitated diffusion through membrane (down the concentration gradient, no energy required)

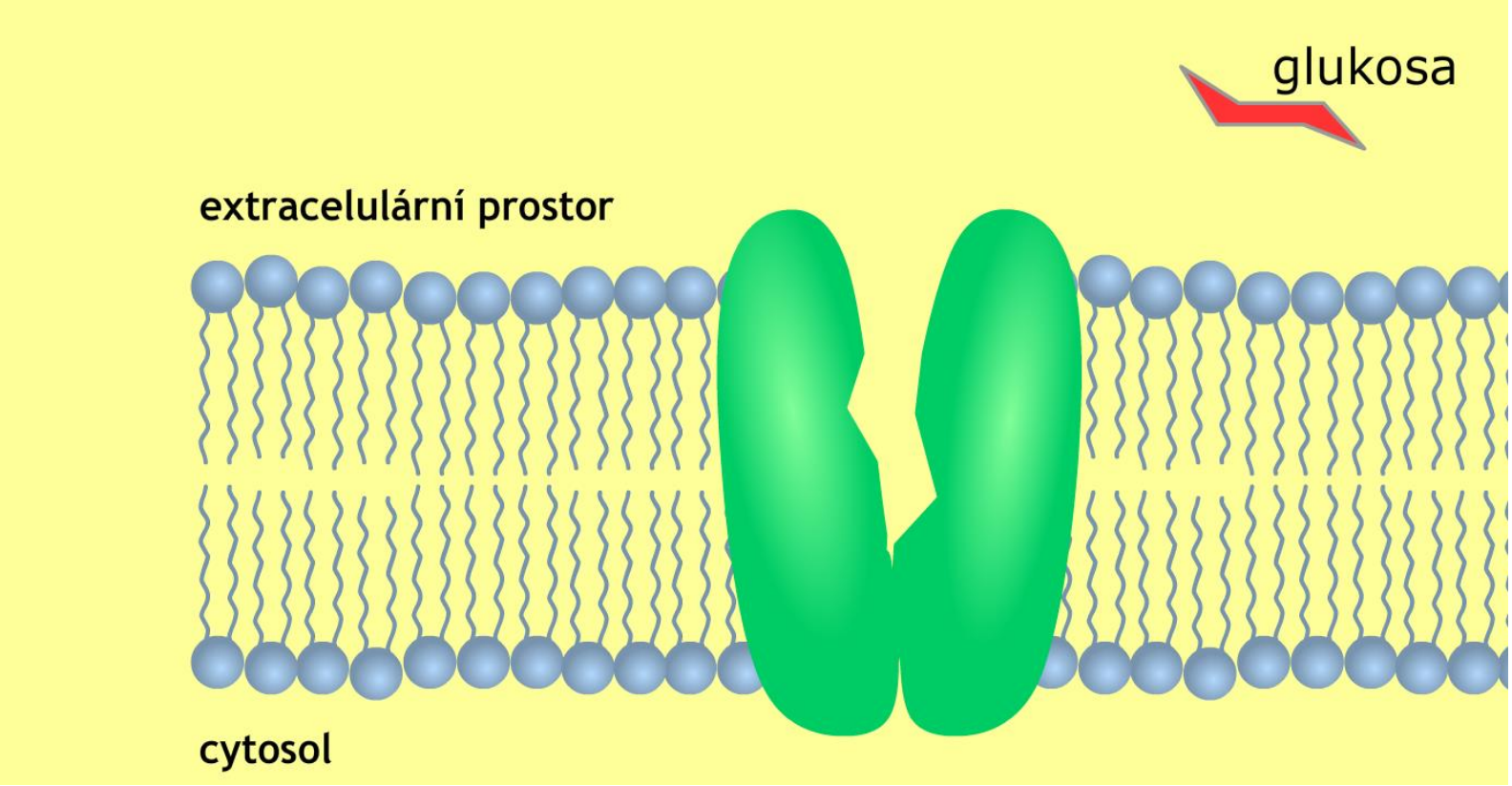
## Why so many types of transporters ?

- they differ in affinity to glucose
- they can by regulated differently
- they occur in different tissues

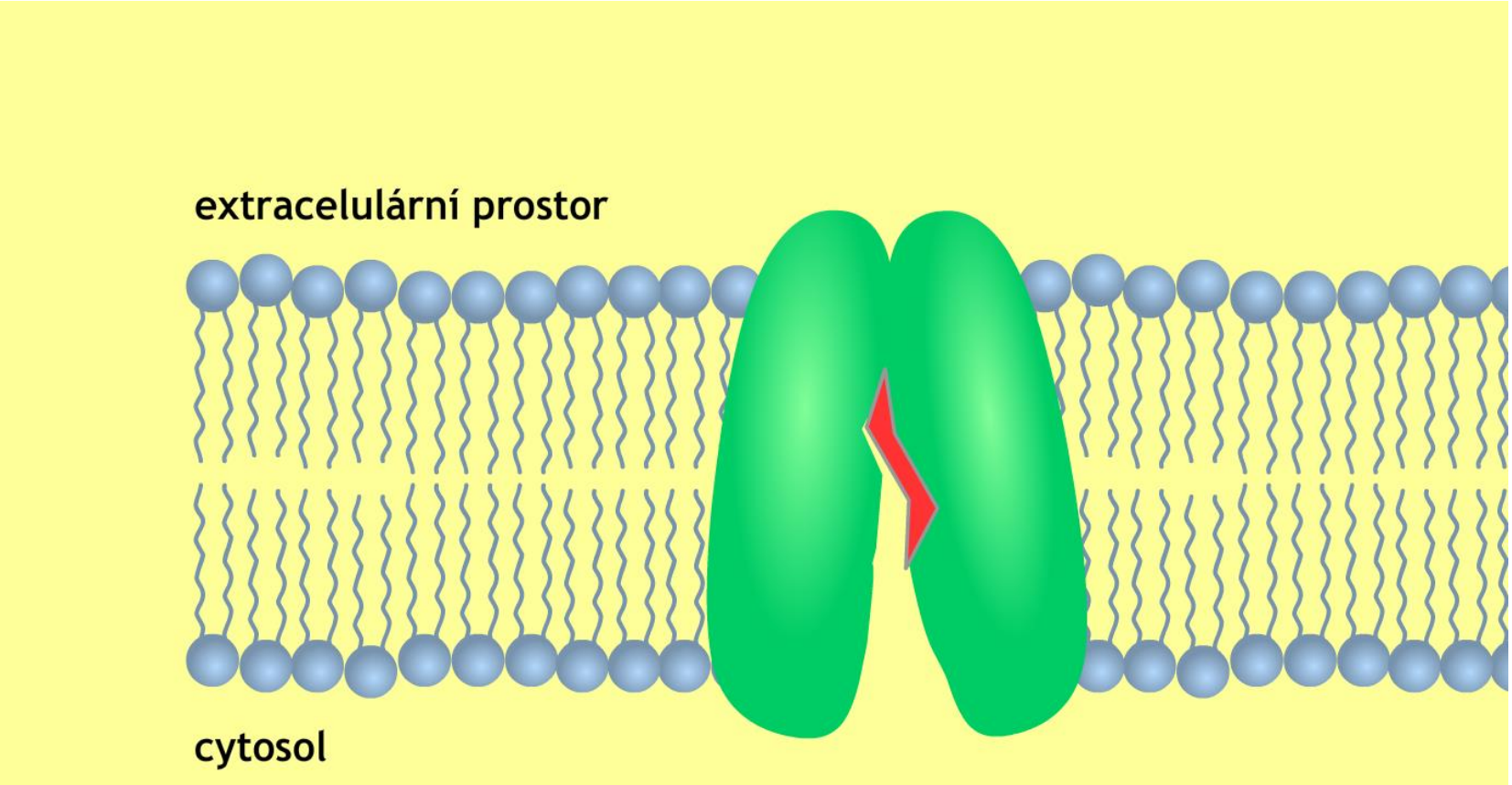
# Glucose transporters of GLUT type

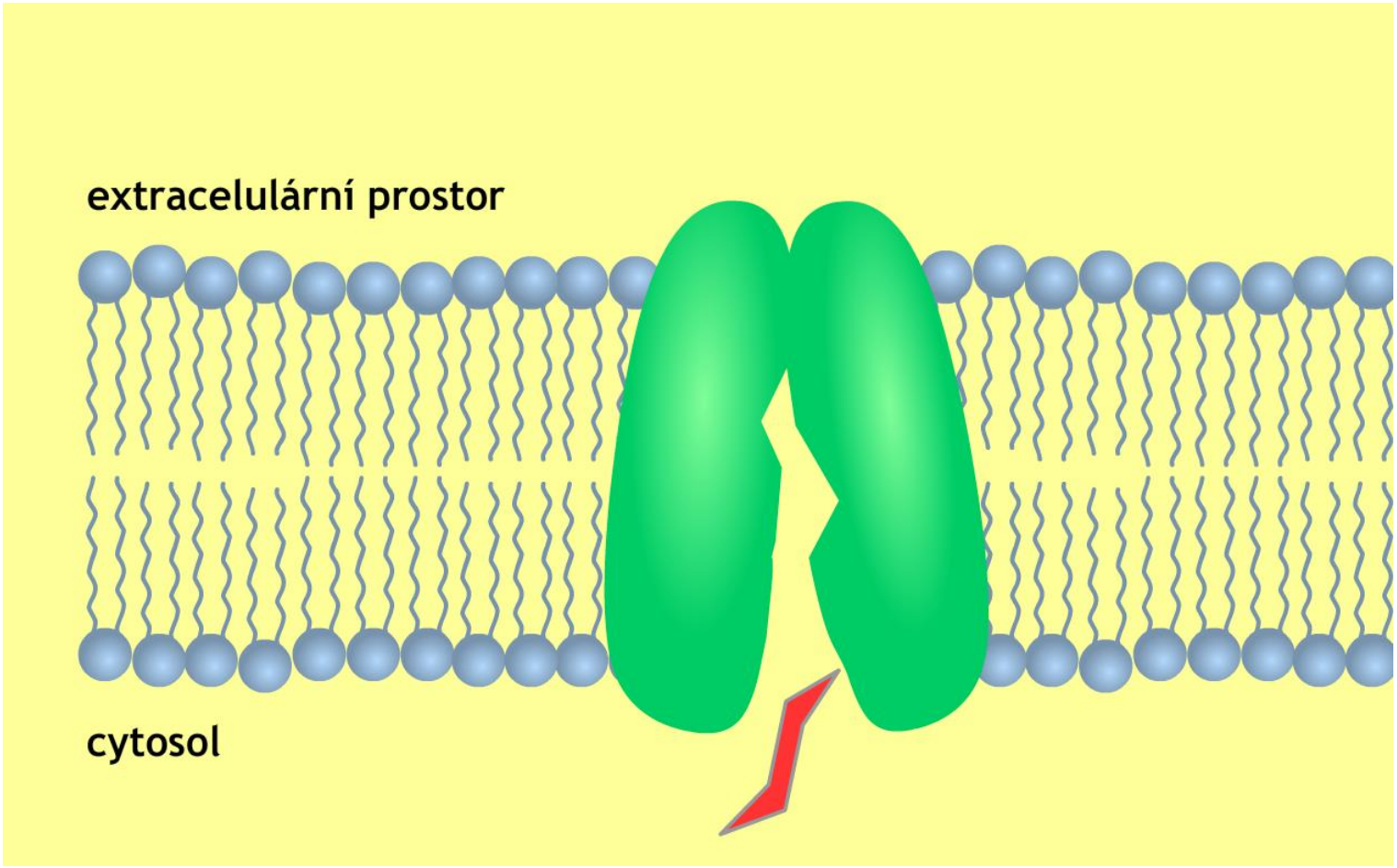
| Type | characteristics |
|---|---|
| GLUT 1 | Majority of cells (Ercs, muscle cells under resting conditions, blood vessels in brain and elsewhere) |
| GLUT 2 | livers, β-cells of pancreas, kidneys |
| GLUT 3 | Nerve cells, placenta and elsewhere |
| GLUT 4 | Muscle, adipocytes – **dependent on insuline** |
| GLUT 5 | Transport of fructose – small intestine and elsewhere |
| GLUT 7 | Intracellular transport in livers |

# Transport of glucose by GLUT




## Mechanism of facilitated diffusion


extracelulární prostor
cytosol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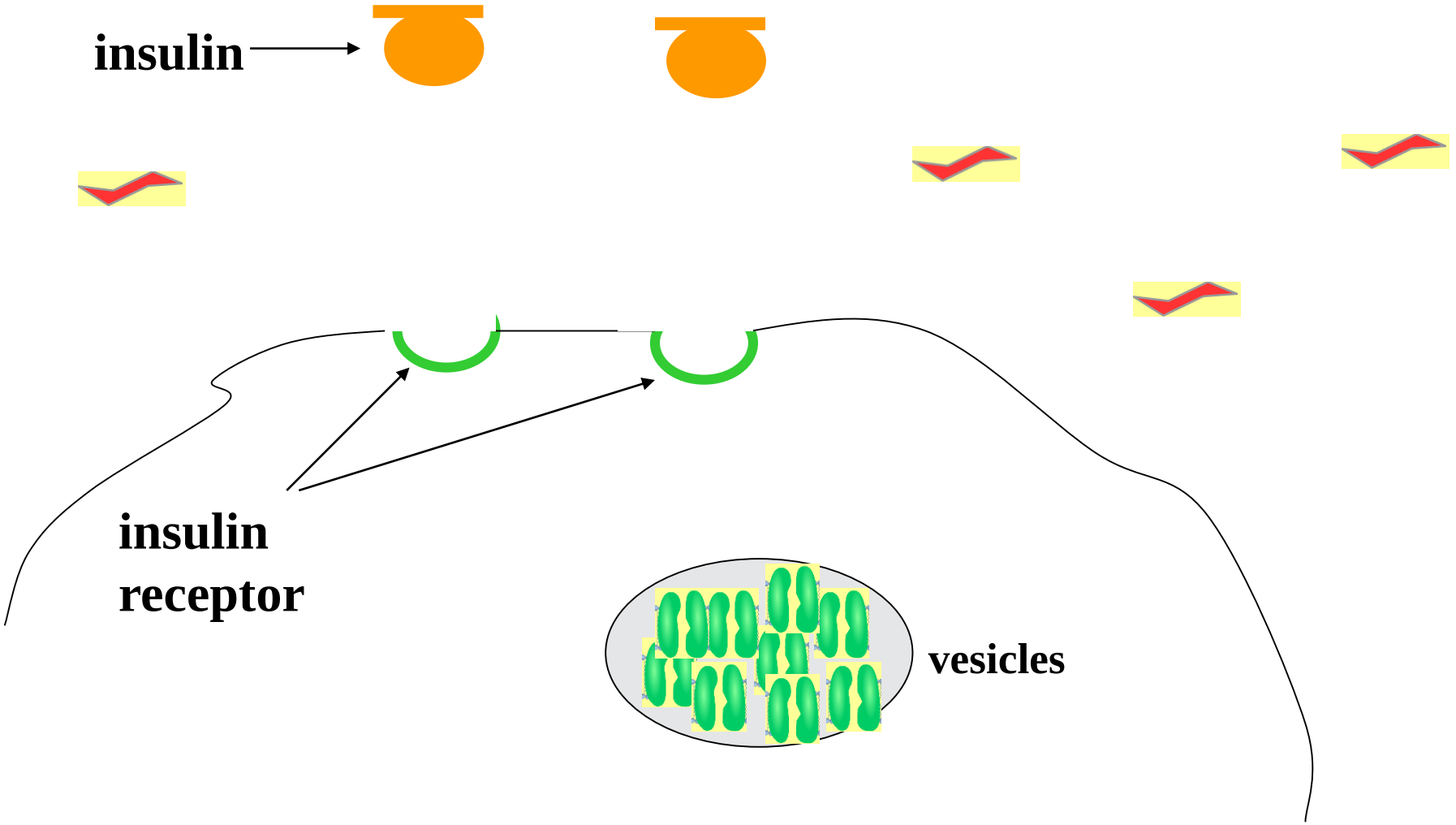
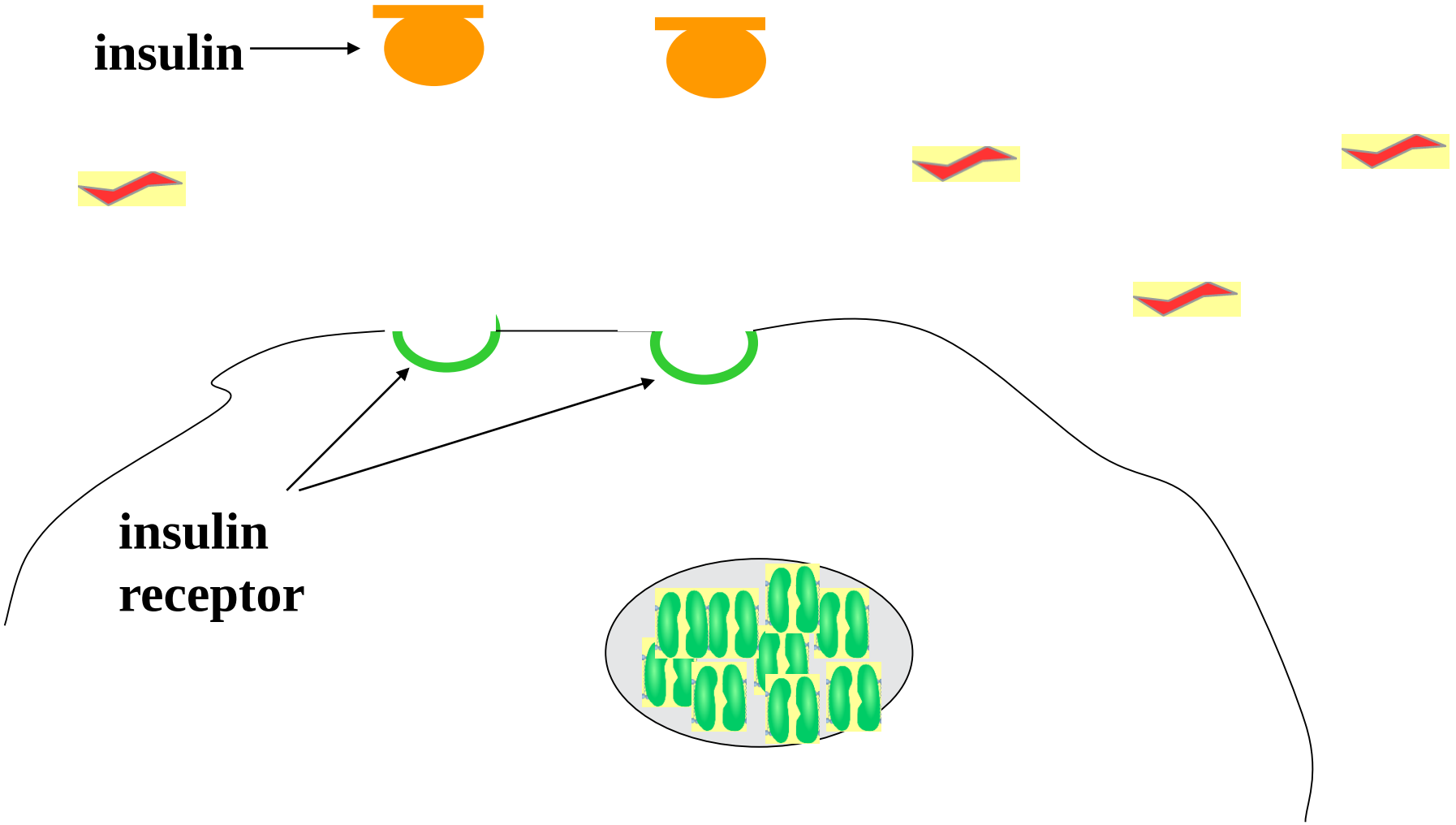
# Receptors GLUT 4 is dependent of insulin

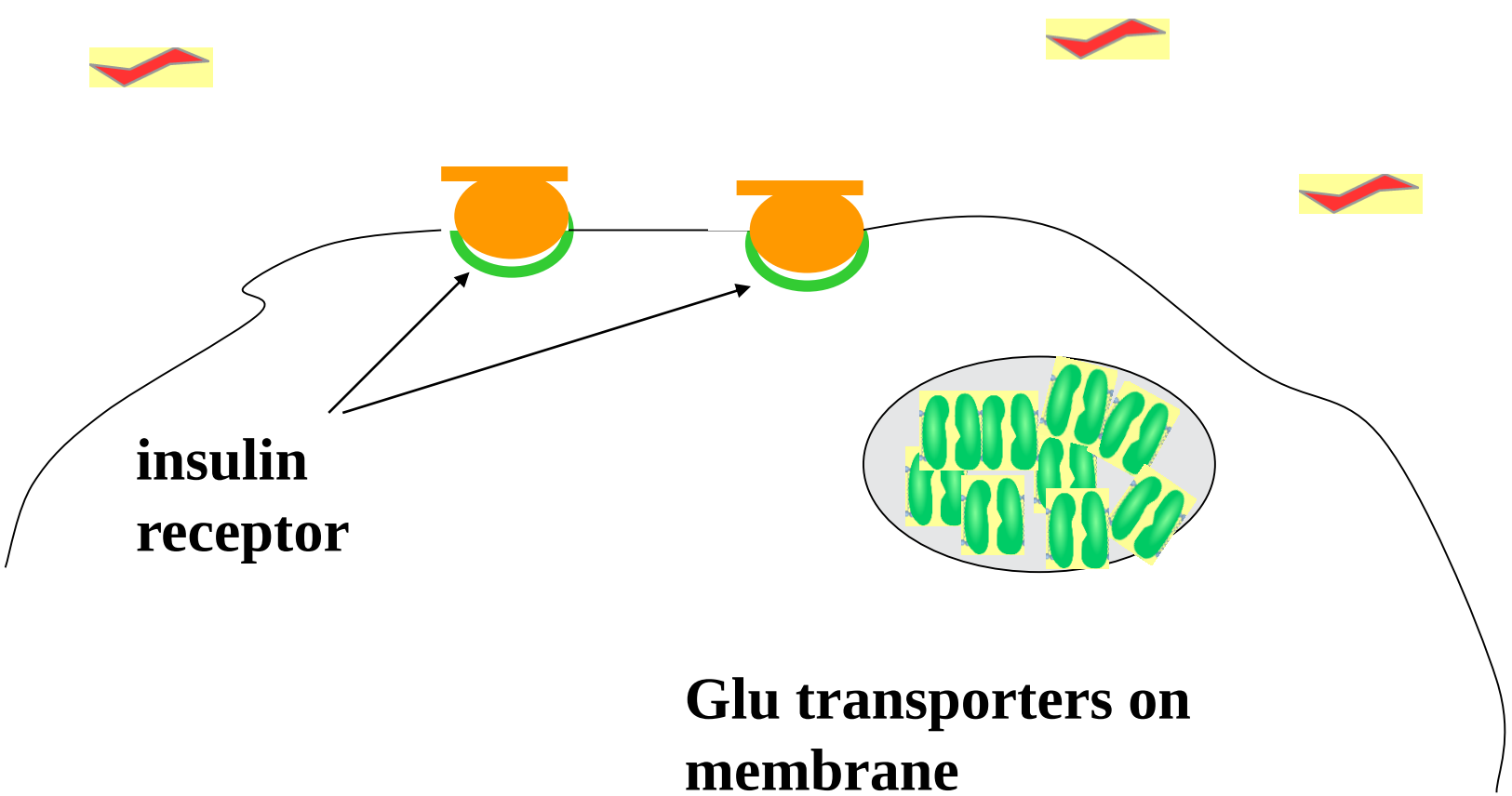


# Binding of insulin on receptor




Transport on membrane

# Transport glu in cell



# Glucose transport into the cells of the intestinal mucosa and the renal tubule (SGLT)

**Mechanism: co-transport with sodium secondary active transport**

at two specific sites linked transporter of glucose and Na + transport thereof coincides (no energy) Na + is then pumped from the cell ATPase (consumption of ATP), glucose is then transported out of the cell via GLUT2

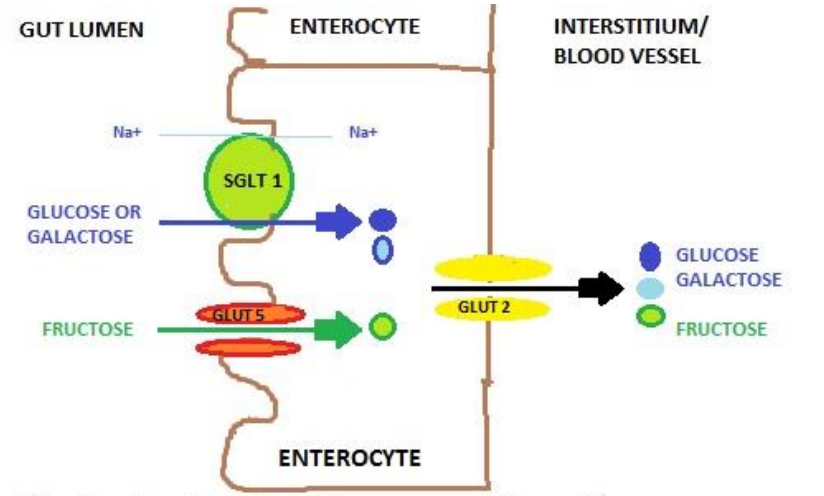



FIG ; showing absorption of hexose sugars from gut lumen.

SGLT 1; It is a cotransporter / symporter that utilises Na+ gradient to symport glucose/ galactose [ ie example of secondary active transport]

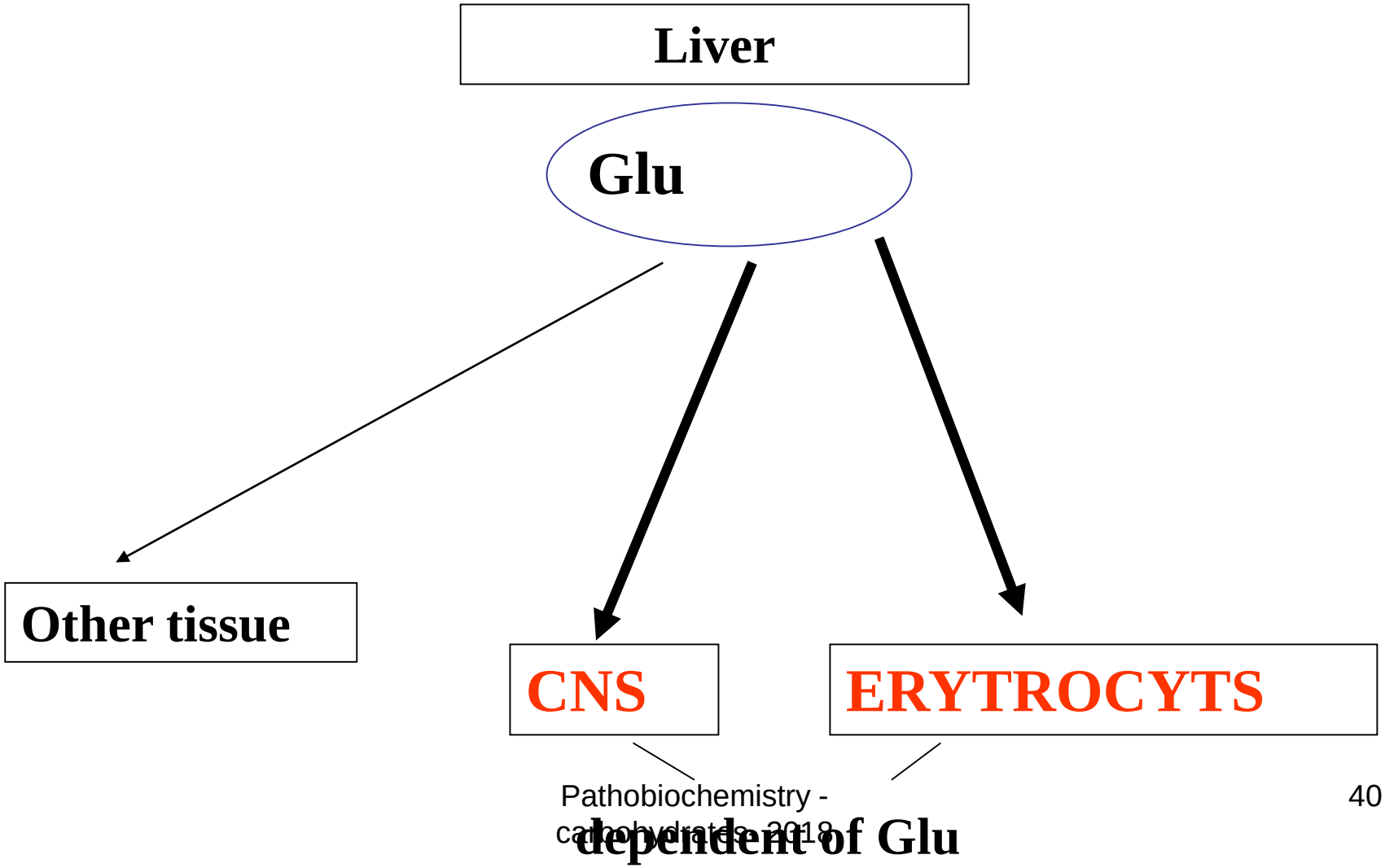
GLUT ; It is responsible for *facillitated diffusion* of glucose across cell membranes.

**Glucose & Galactose* are transported from gut lumen to enterocyte by SGLT 1. so glucose and galactose absorption is example of secondary active transport.

**Fructose* is absorbed into enterocyte by GLUT 5, So fructose absorption is an example of facilitated diffusion.

**Glucose , Galactose & Fructose* are transported out of enterocyte to blood by GLUT2.

# Distribution of Glu to tissue



Glucose is distributed into the tissues as a source of energy

for red blood cells and the CNS glucose is practically the sole and irreplaceable source of energy

Other cells may also metabolize fatty acids
(or amino acids and ketone bodies)

therefore, decreases if the uptake of glucose, the body begins to save glucose and preferably only supplies the CNS and erythrocytes

# Metabolism of glucose

## Glycolysis

- the energy gain profit
- acetyl CoA occurs in most cells

## glycogen synthesis

storage form of glucose

it occurs mostly in the liver and muscles

## pentose cycle

profit pentose

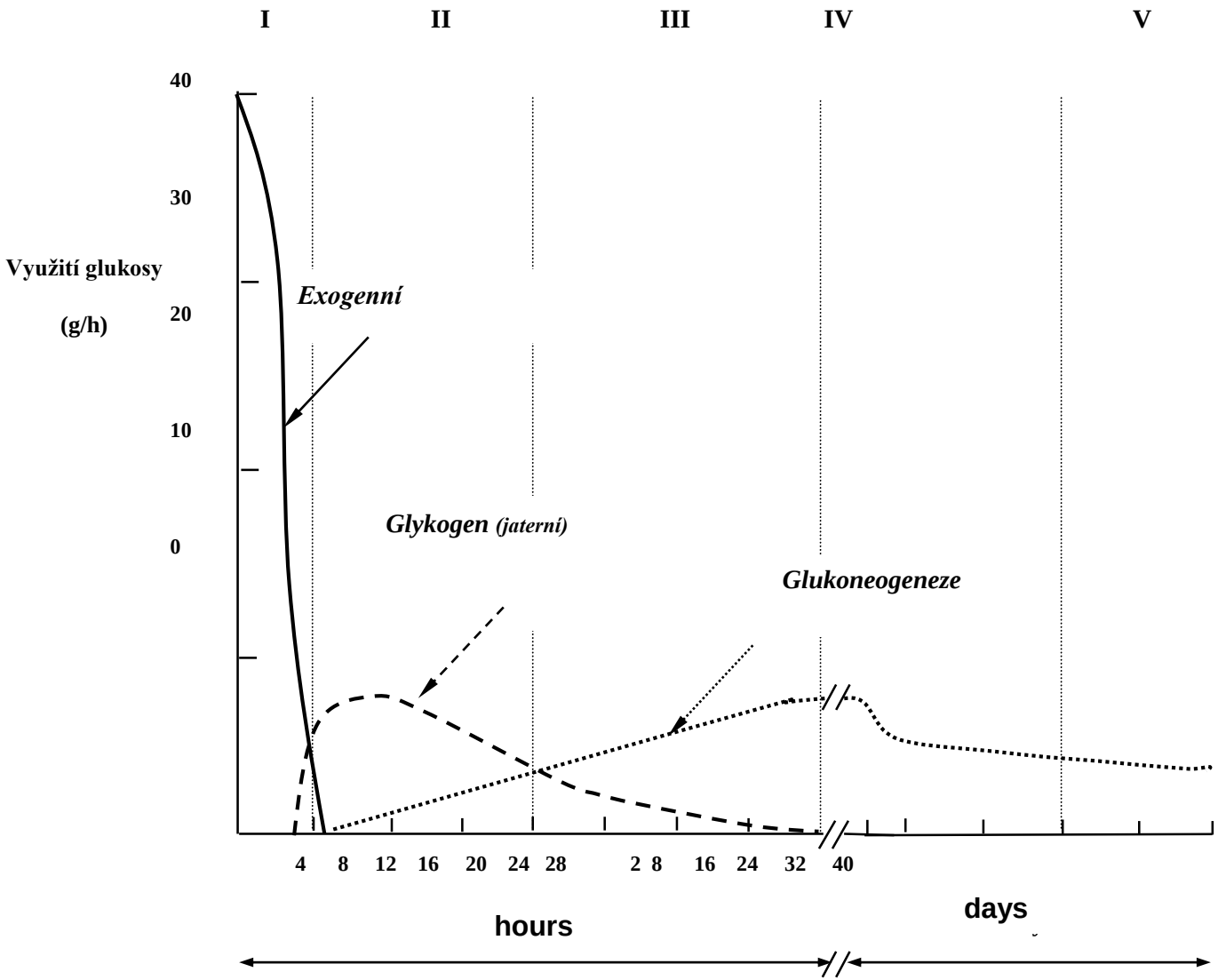
Profit NADPH synthetic reactions

obtains energy

takes place in most c.

# Phase glucose metabolism and hormonal regulation

# Sources of glucose metabolism in various stages



# Metabolism of glucose after a meal (resorptive phase)

**Characteristics:**

**metabolism controlled insulin most tissues uses glucose as an energy source, runs glycolysis glucose is stored "rainy day" (liver, muscle) - the synthesis of glycogen in liver and muscle acetyl CoA generated by glycolysis can be used for the synthesis of fatty acids and consequently lipids ( "fatter after sweet")**

# Glucose metabolism for longer after a meal or in fasted

**Characteristics:**
**metabolic control glucagon (a hormone) organism "saves" glucose, glucose is used mainly CNS and erythrocytes other tissues metabolize other nutrients, especially MK glucose level is replenished glycogen breakdown and gluconeogenesis in the liver**

# Glucose metabolism in short-term stress

•metabolic control stress hormones (adrenaline, noradrenaline) preparation for fight or flight (fight or flight) priority is to supply the muscle glucose in the liver glycogenolysis, glukoneogenze muscle lipolysis, glycogenolysis and glycolysis

TEST

# Glucose in blood

Sources of glucose metabolism in various stages

In describing the metabolism of the two basic metabolic state called absorption (resorptive) and post absorption phase (postresorpční) phase. Absorption phase lasts approximately 4 hours and includes a meal and after. Post absorption phase is the state during and after an overnight fast. Starvation: when food intake is stopped for more than 12 to 14 hours, pass metabolism in starvation phase (= short-from tens of hours to several days, to long = more than two to three weeks). Blood glucose levels in healthy humans is maintained within a very narrow range. In postresorpční stage, blood glucose is maintained in the range of 4.5 to 5.2 mmol / l.

After a meal containing carbohydrates blood glucose levels rise. After 0.5-1 h, reaches the level of

glucose in the blood of healthy people 8-10 mmol / l. Glucose at this stage serves as the main source of energy for

most tissues and is stored as glycogen in the liver.

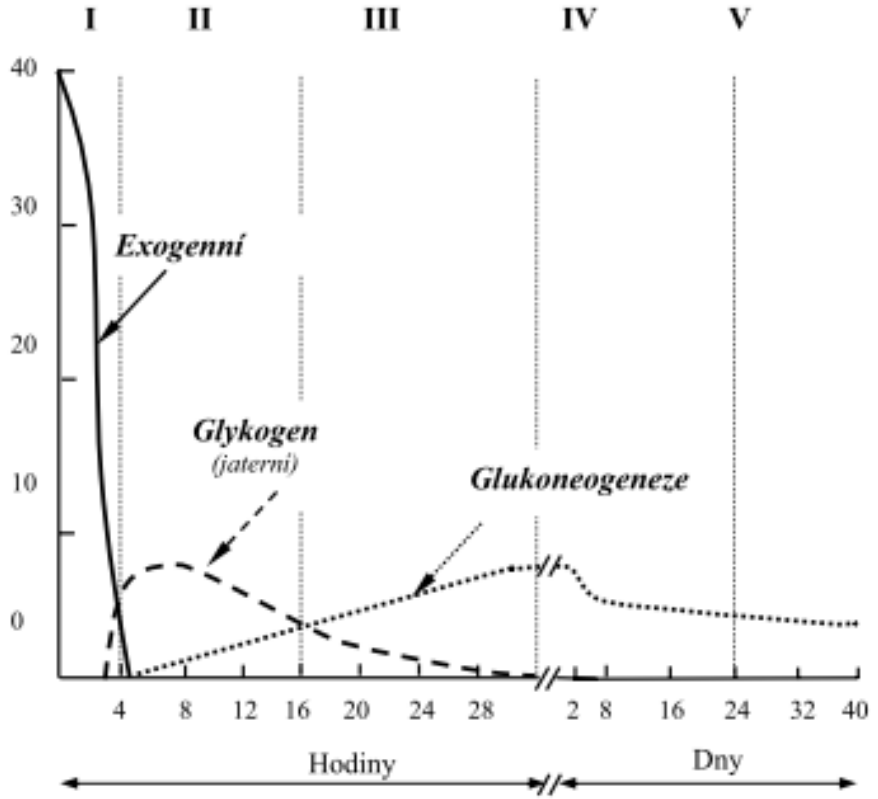
After about 1 hour after meals level begins

glucose to drop, because glucose is consumed catabolism and storage. normoglycemia is

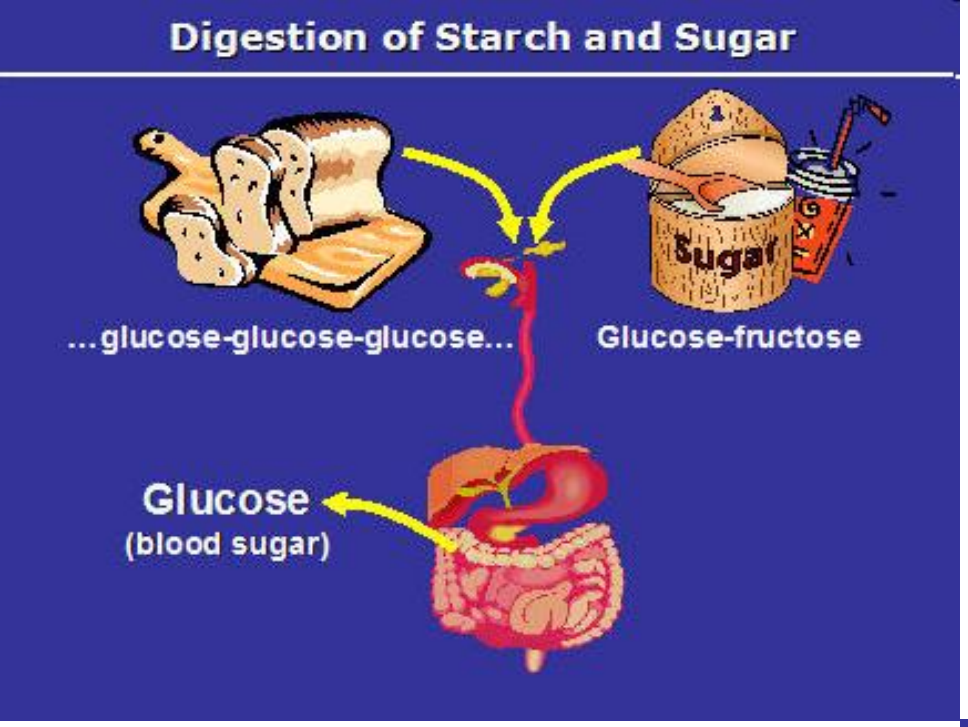
re-established after about 2-4 hours. After this time, the liver, the process of glycogenolysis and glucose

It is released from the liver into the blood. Once glycogen decreases, begin to be dismantled also lipids

in adipose tissue of hormone-sensitive lipase, and in blood they are supplied fatty acids and glycerol. Fatty acids are used as an alternative fuel for certain tissues and glycerol is used for gluconeogenesis.



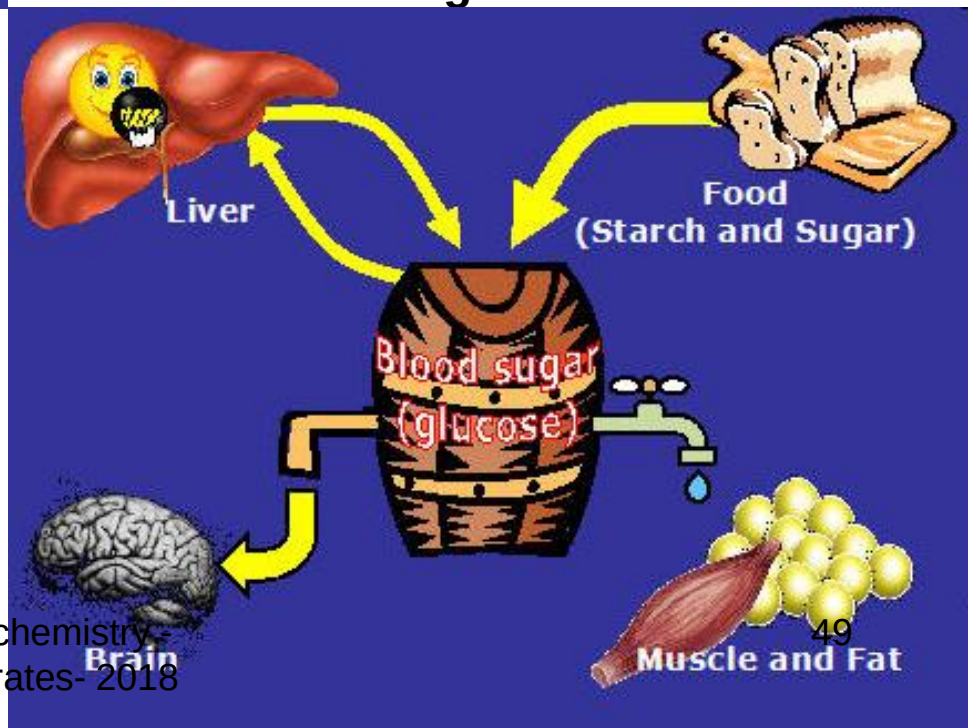

**During the night of fasting is glukosemie maintained both processes - glycogenolysis and gluconeogenesis. After approximately 30 hours of fasting, the glycogen stores in the liver practically exhausted. Gluconeogenesis becomes the sole source of glucose in the blood. Changes in glucose metabolism occurring during the transition from phase to phase saturation starvation are regulated mainly hormones insulin and glucagon. Insulin is increased after a meal, glucagon increases during starvation.**




- 1. To the liver. Here excess sugar from meals is stored to cover sugar shortages between meals and to make fat from excess sugar.
- Transport of sugar goes both to and from the liver. The liver fills the "Sugar Central" between meals.

- 2. To the brain. The brain is completely dependent upon sugar combustion for its supply of energy, in any case under normal conditions. It uses really huge amounts of sugar.

- 3. To muscles and fat tissue. At least 40% of the body is comprised of skeletal muscles. These can use both fats and sugar to supply energy. The rate of sugar uptake and burning follows physical activity; more work; more sugar burned. Muscles do take up and store glucose to cover future activity but they cannot release sugar back to the blood stream or "Sugar Central". Fat tissue stores surplus sugar as fat. About half of this comes from the liver, the rest is made by fat itself.



# Sources glu in various stages of metabolism

| Sources of Glu in phase | I | II | III | IV | V |
|---|---|---|---|---|---|
| Duration of phase | 0-4 h | 4-16 h | 16-32 h | to 24 days | longer than 24 days |
| Main source of Glc | Diet | Glycogen | Glycogen, Gluconeogenesis | Gluconeogenesis | Gluconeogenesis |
| Source of Glc in blood | Diet | Glycogenolysis (gluconeogeneze) | Gluconeogenesis, (glycogenolysis) | Gluconeogenesis (liver, kidney) | Gluconeogenesis (liver, kidney) |
| Tissue-Glc from blood | all | All,no liver Limitation: muscle,adipose t. | All,no liver Limitation: muscle,adipose t. | CNS, Ercs, kidney Limitation: muscle, | Ercs, ledviny Limitation: CNS, |
| Main source of E for CNS | Glc | Glc | Glc, ketone bodies | Glc, ketone bodies | Ketone bodies, Glc |

# Disorders of carbohydrate metabolism

## Blood sugar and hormones

The glucose concentration in the blood (glucose) under physiological conditions is maintained within a narrow range of values from 3.9 to 5.6 mmol / l in fasting and postprandial less than 10 mmol / l. It is strictly regulated by a variety of mechanisms: insulin, which lowers blood glucose and antiinzulin acting hormones - glucagon, catecholamines, glucocorticoids and growth hormone that increases blood glucose. On the regulation of glucose homeostasis is a major part liver. Maintaining constant values of glucose is essential for the activity of the CNS and other tissues and cells (e.g. erythrocytes).

increase in blood glucose levels above 7.77 mmol / l - hyperglycaemia

reduction below 2.5 mmol / l - hypoglycemia

enzymopathies or regulatory disorders (diabetes)

Hypoglycemia - insufficient energy supply to the brain (neuroglycopenia), increased secretion of catecholamines (palpitation, anxiety, tremor, sweating)

Formation: 1. insufficient supply of glucose into the blood circulation

Second from too rapid uptake of circulation

Hypoglycaemia during fasting - sugar metabolism disorders, tumors, endocrine, hepatic cirrhosis, pregnancy, newborns, drug-induced

Hyperglycemia - Diabetes mellitus, endocrine disorders, diseases of the pancreas, liver, severe acute diseases (infection)

# Hereditary disorders of carbohydrate metabolism

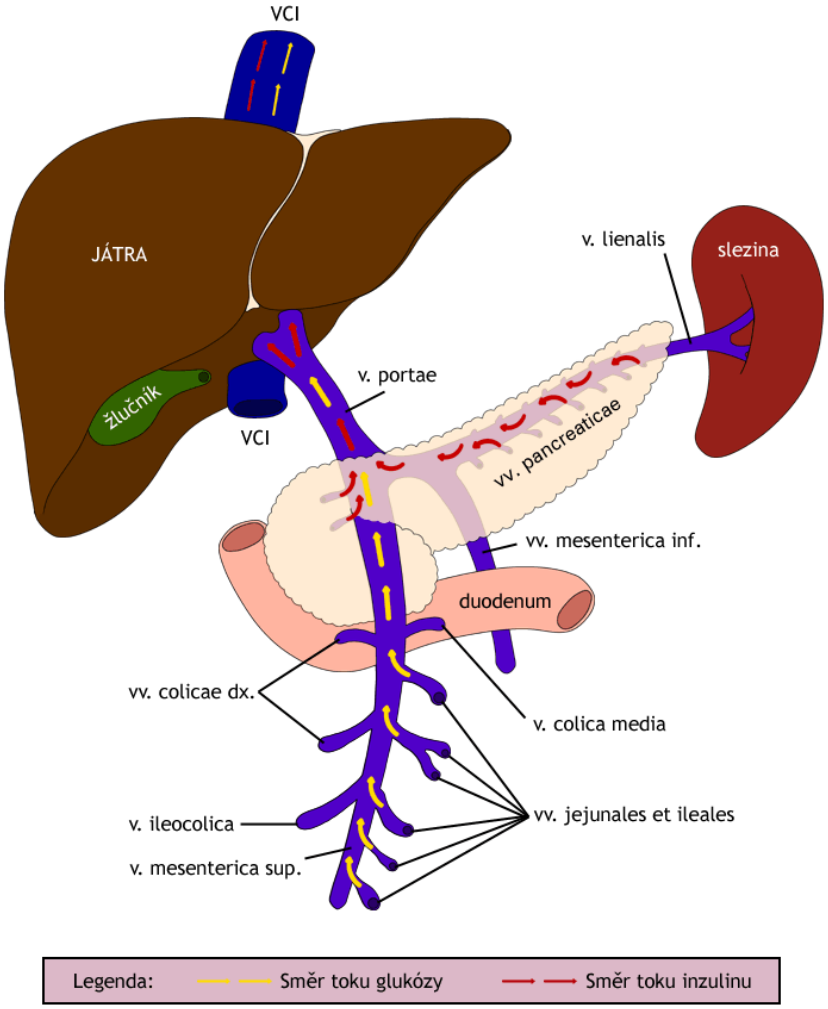
- **Disorders of carbohydrate metabolism („small molecules")**
- Fructose
- Galactose
- **Disorders of metabolism polysaccharides („big molecules")**
- Glycogenosis (also product deficiency)
- **Disorders of protein glykosylation (and lipids)**
- product deficiency
- **Diabetes mellitus** as a complex metabolic disease - diabetes biochemistry
- Molecular interpretation of late effects of diabetes

# Diabetes mellitus

- (Insulin deficiency - increase in plasma glucose concentration, glucose excretion in the urine)
- **Type 1 (insulin-dependent, juvenile type IDDM)**
  polygenic autoimmune disease, the absolute lack of insulin
  genetic predisposition combined with: viral infection, toxins, stress
  (Several years, 5-10% undiagnosed diabetics)
  slow destruction of β-islet cell mediated
  activated T lymphocytes and cytokines - insulitis (lymphocytic infiltration of islet cells, inflammation). Insulitis reduces the number of functional β-cells in the pancreatic buňkách-
  disorders of synthesis and secretion of insulin.

**Type 2 (non-insulin dependent, or adult type, NIDDM)**

combination of hereditary factors and environment
a combination of insulin resistance and relative deficiency of insulin (abnormal insulin receptor antibodies, anti-insulin)
resistance to the action of insulin: reduced number of plasma membrane receptors on target cells, postrecep. blockade of intracellular glucose metabolism (decreased number of receptors, reduced affinity, decreased activity of the complex; abnormal signal transduction or abnormal phosphorylation reaction for the formation of excessive TNF).
gradual loss of the ability of β-cells to respond to glucose, insulin resistance, dysregulation of glucose production in the liver




**Diabetes mellitus (DM) is a chronic disease with high morbidity and mortality, in the last decade was recorded a strong growth of this disease. Currently in the Czech Republic recorded more than 800,000 diabetics. DM includes a heterogeneous group of chronic metabolic diseases whose primary symptom is hyperglycemia. Is due to lack of insulin, its lack of efficacy (some talk about the relative scarcity), or a combination of both.**

**The lack of insulin results in a distortion glucose transport from blood into the cells of the cell membrane, which leads to hyperglycemia and lack of glucose intracellularly. Inadequate utilization of glucose by the cells is replaced by other energy sources. Stimulates gluconeogenesis and glycogenolysis, further increased lipolytic cleavage of triglycerides into fatty acids and glycerol in adipocytes. By degrading fatty acid in β-oxidation is formed acetyl-CoA excess, from that ketone bodies are formed in the liver - acetoacetate, 3-hydroxybutyrate and acetone. Acetoacetate can serve as a source of energy for muscle activity and brain instead of glucose. If the formation of ketone bodies exceeds their utilization by peripheral tissues, ketoacidosis develops. Given that the ketones are soluble and excreted in the urine, occurs ketonuria.**

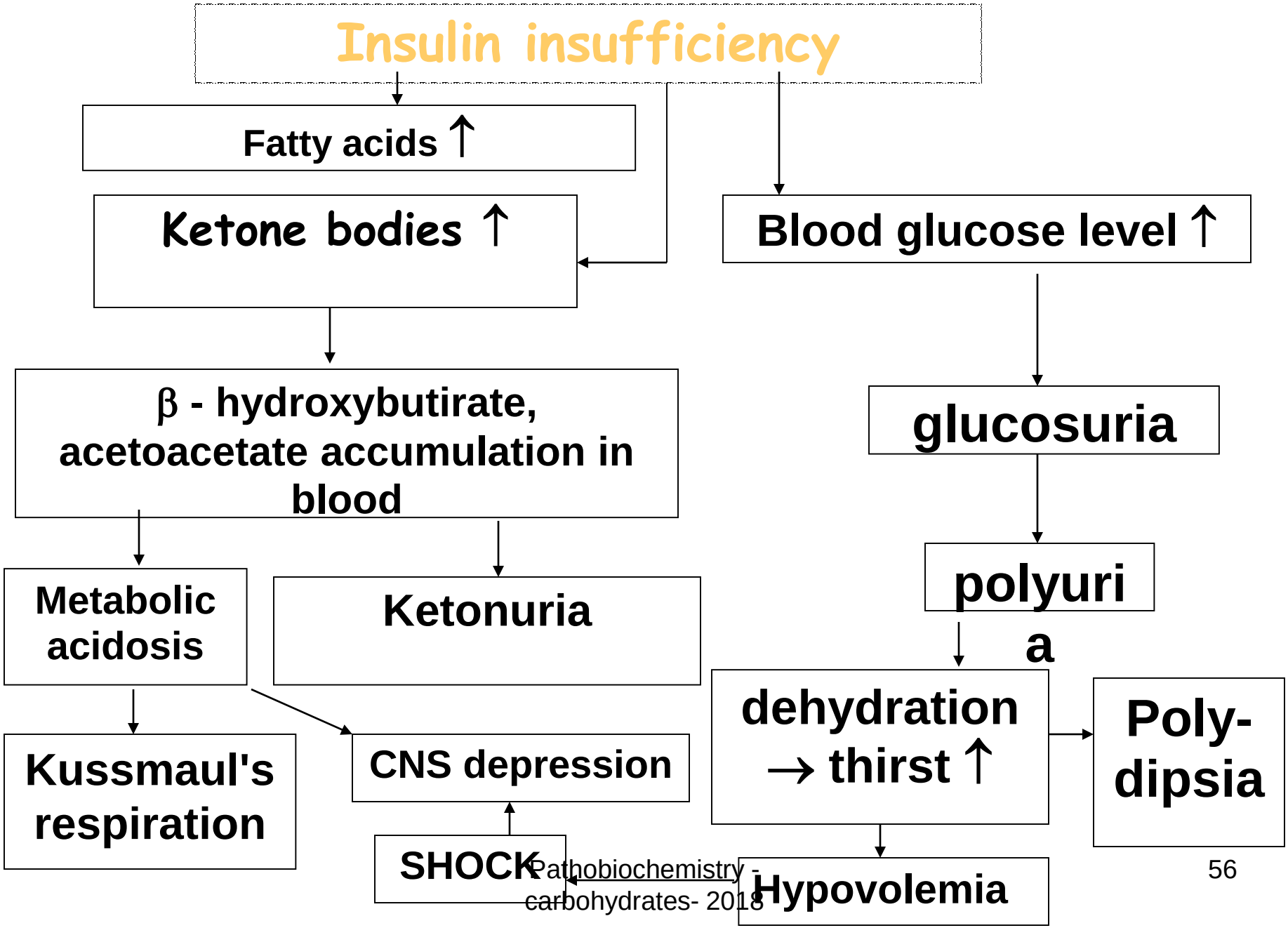
**Excess of glucose also receives into urine and glucosuria develops. Because glucose and ketone bodies are osmotically active, pull down with him larger amounts of water into the urine, which is the basis of polyuria.**
**From the above result and the characteristic symptoms of DM as thirst and polyuria; It also identified lack of appetite and weight loss.**
**Chronic hyperglycemia associated with impaired function of many internal organs, especially the kidneys, eyes, vascular and nervous system.**

TEST

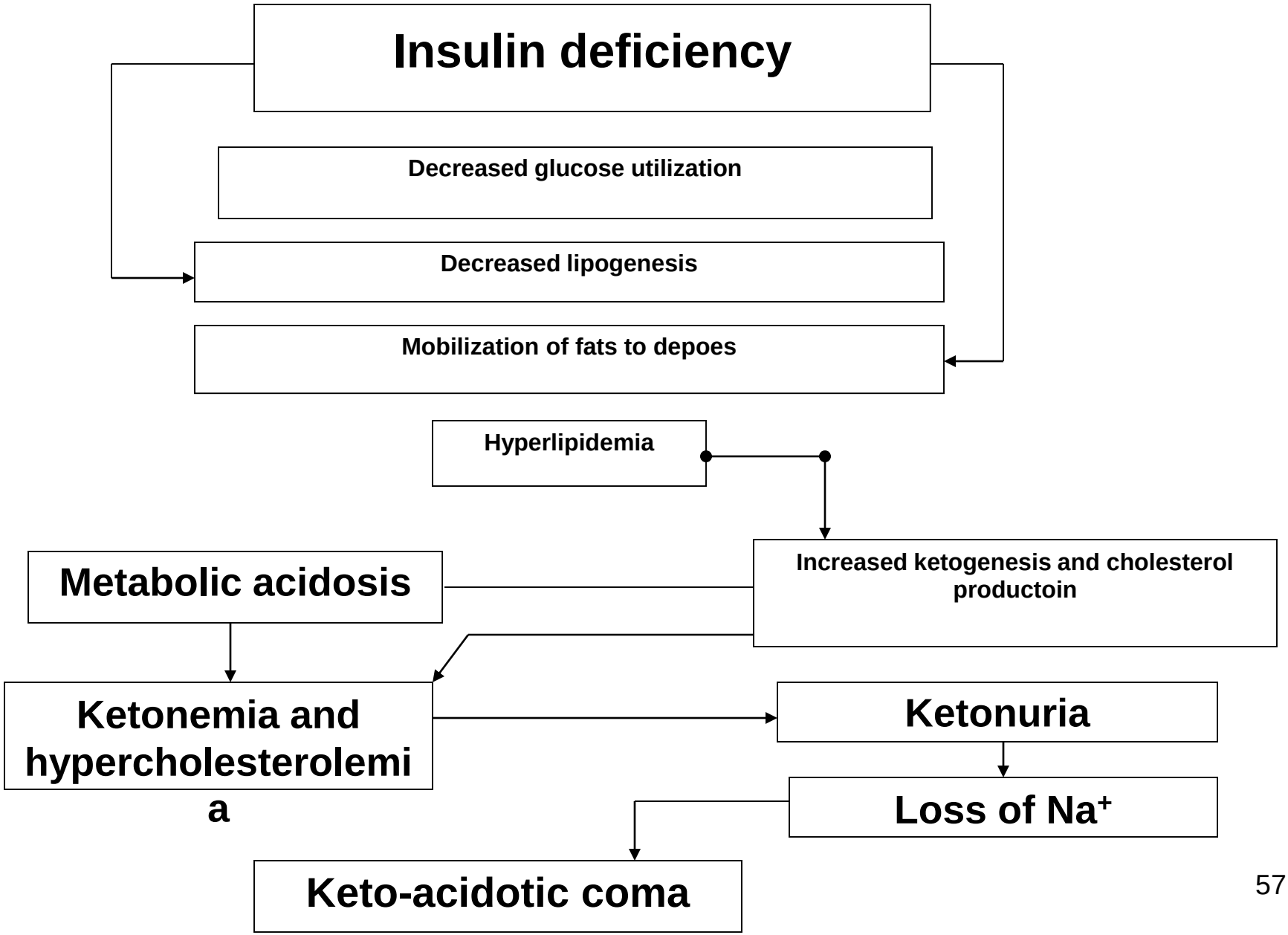
| Differences between types of diabetes | | |
|---|---|---|
| | **Type 1** | **Type 2** |
| **Age** | **usually below 30** | **usually above 30** |
| **Frequency (% of all diabetics)** | **10–20 %** | **80–90 %** |
| **The emergence of symptoms** | **acute or subacute** | **slow** |
| **Obesity** | **Not usual** | **very common** |
| **Inducing factors** | **altered immune response after viral infection** | **obesity, pregnancy, stress** |
| **Pancreatic insulin content** | **absent or trace amount** | **low, normal, high** |
| **Glucagon in plasma** | high, but resumable insulin | high, but resistant to insulin |
| **Antibodies against pancreatic islets** | Present in 85 % cases | less than 5 % |
| **Primary insulin resistance** | minimal | usually pronounced |
| **Response to the insulin treatment** | +++ | + until - |
| **Response to dietary treatment** | slight | always present, but various degrees |
| **Response to treatment with oral antidiabetic agents** | absent | present |
| **Typical acute complications** | ketoacidosis | hyperosmolar coma |
| **Association with HLA** | yes | no |

**Complications: Diabetic ketoacidosis, hypoglycemia, diabetic nephropathy, periodontitis**

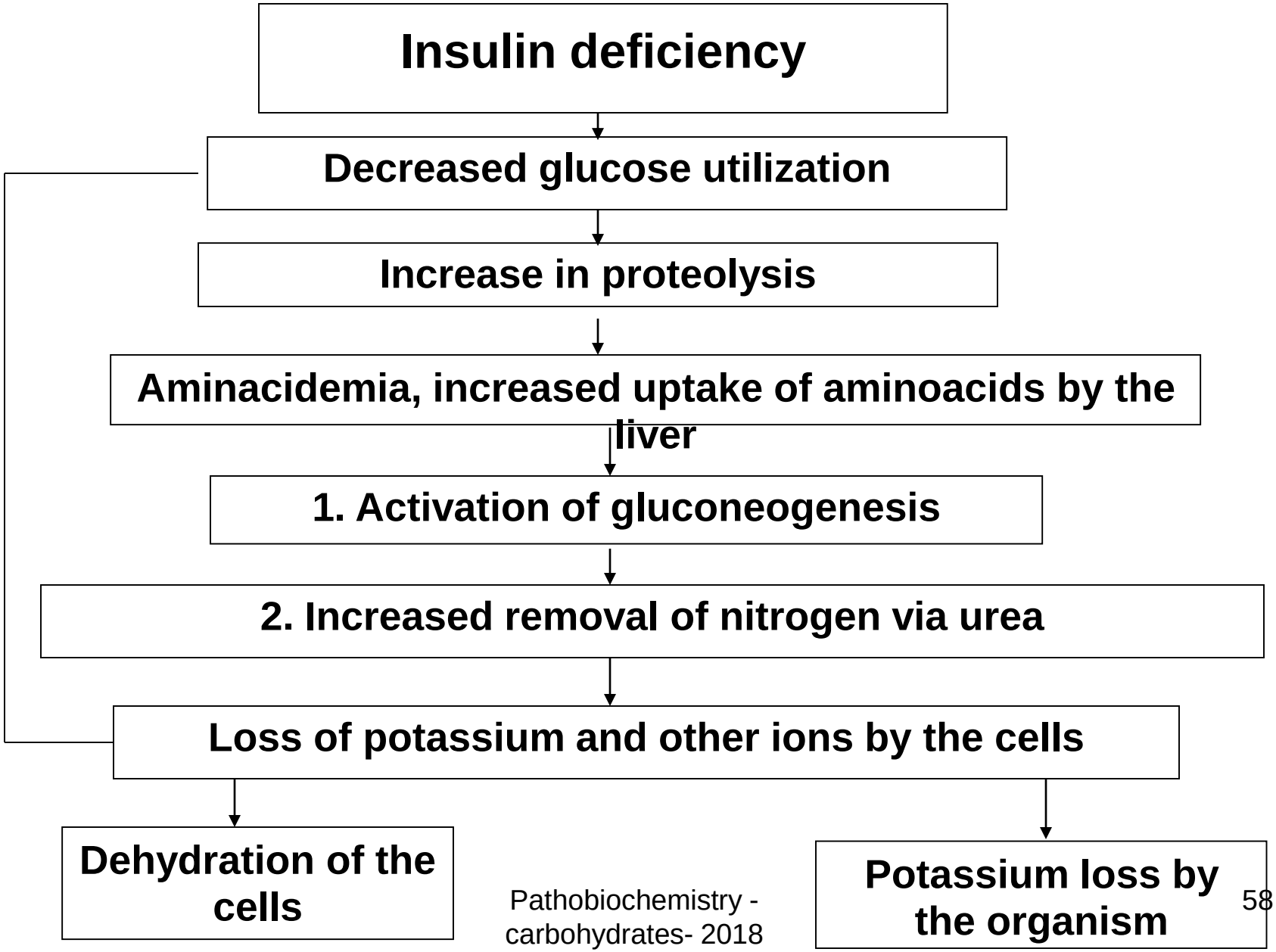
# PATHOGENESIS OF DIABETES MELLITUS



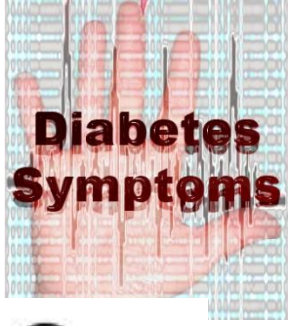
# IMPAIRMENT OF LIPID METABOLISM IN DIABETES MELLITUS



# IMPAIRMENT OF PROTEIN METABOLISM IN DIABETES MELLITUS

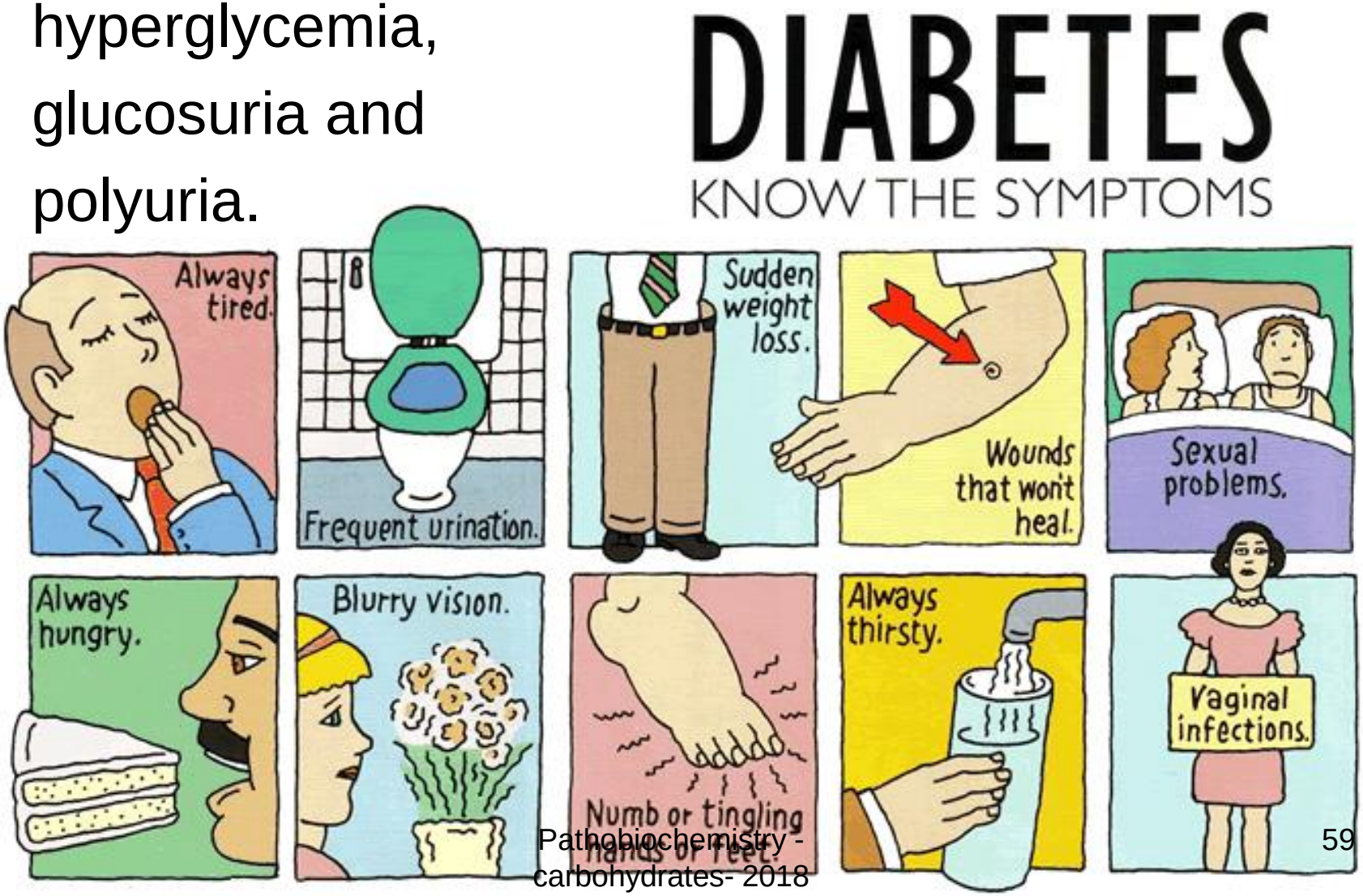


# Symptoms of diabetes mellitus




Major symptoms are:

- hyperglycemia,
- glucosuria and
- polyuria.



# Evaluation of glycemia -fasting

**The reference interval glucose**
**fasting plasma adults: 3.9 to 5.6 mmol / l**

| **deciding value** | |
|---|---|
| **Interpretation** | Glu in plasma - fasting |
| exclusion of diabetes | < 5,6 mmol/l |
| Prediabetes (increase glycemia) | 5,6 – 6,9 mmol/l |
| Diabetes mellitus | ≥ 7,0 mmol/l |

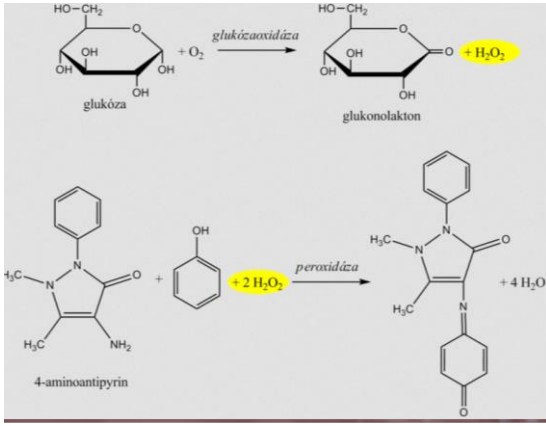
**Glycemia was determined in venous plasma. Glucose values are for the adult population. Diabetes diagnosis must be confirmed by repeated measurements.**

**Evaluations are carried out on the basis of the Czech Society of Clinical Biochemistry and the Czech Diabetes Society (2012) as recommended by WHO www.čskb.cz**

# Determination of glucose concentration in blood

- the examination, that provides basic information on carbohydrate metabolism. Picked up capillary or venous blood, and glucose is determined in whole blood, plasma or serum.
- For determination of glucose in whole blood values are 10-15% lower (depending on hematocrit) in arterial blood is about 10% higher than in venous (arteriovenous difference). To prevent glycolysis added NaF (2.5 mg per 1 ml of whole blood) into containers .
- ***Examination of glucose concentration in blood has the necessary information value only if it´s known the time interval between collection of blood and food intake.***
- Examination of glycemia is performed:
- **Fasting** (blood is drawn at least 8 hours after ingestion) - indicated when searching diabetics and diagnosis of DM;
- **randomly** measured glycemia (blood is collected without giving a time relation to food intake) - is carried out in suspected hypoglycemia or hyperglycemia;
- after a meal - **post-prandial** glycemia (1 hour after a meal containing carbohydrates) - indicated to check the effectiveness of DM treatments;
- as the **glycemic profile** - glycemia is determined several times a day, usually before the main meals, sometimes after meals and at night.

# Methods for glycemia determination




- **Glycemia determination in laboratory conditions**
- To determine the concentration of glucose used different methods.
- Advanced methods are coupled with an enzyme **enzyme**.
- Glucose may be determined by each enzyme,
- that it metabolizes.
-
- **The glucose oxidase reaction**
- Recommended routine method utilizes *enzymatic reactions coupled* ***glucose oxidase*** *(****GOD****, EC 1.1.3.4) and* ***peroxidase*** *(****POD****, EC 1.11.1.7)*. In the first reaction the enzyme **glucose oxidase** catalyzes the oxidation of glucose by atmospheric oxygen to give gluconic acid, which passes the internal ester gluconolactone. It is known that in solution is 36% of glucose in the form of α-anomer and 64% in the form of β-anomer. GOD is highly specific for β-D-glucopyranose. In order to oxidize both anomers, is necessary mutarotation α- to β-anomer, that occurs spontaneously during a sufficiently long incubation. As a byproduct of the reaction, glucose oxidase produces equimolar amounts of **hydrogen peroxide**.
- In a further reaction catalysed by **peroxidase**, formed hydrogen peroxide reacts with a suitable chromogen, which is oxidized to a reactive intermediate, and one with another agent coupled to a constant-soluble dye. An example may be the oxidative coupling of phenol derivative with 4-aminoantipyrine to a red dye, whose absorbance after stabilization of the reaction equilibrium is measured.
- Other methods use **measurements of oxygen depletion** that occurs during the reaction catalyzed by **glucose oxidase** and that we can monitor electrochemically by the oxygen or enzyme electrode.

# Hexokinase reaction

- Hexokinase method is characterized by the high specificity . **Hexokinase** (EC 2.7.1.1) phosphorylates glucose in the presence of ATP to **glucose-6-phosphate**. In the next step, the glucose-6-phosphate is oxidized by **glucose-6-phosphate dehydrogenase** versus NADP + into 6-phosphogluconolactone. Reduction of NADP + into NADPH can be evaluated by direct UV photometry area on the principle of **Warburg optical test**.
- **Determination of glycemia in the outside of laboratory conditions**
- Glycemia amongs the parameters that are often examined without laboratory facilities. Fast approximate determination of glycemia is common in emergency care. Patients treated with insulin are also preferably regularly monitored using a **personal glycemia meter** and then by using the measured values therapy is adjusted. Blood glucose concentrations are among the most common parameters determined examination techniques at the point of patient care (point of care testing, POCT). It should be borne in mind, however, that **POCT** methods, however improve the quality of care and patient comfort, not replace regular medical examination or laboratory control.
- Methods for rapid determination of glycemia use several principles. The starting material is usually a drop of **capillary blood** which is applied to the **test strip**.
- The oldest, but still used strips are based on the same reactions as the photometric measuring glucose concentration. **Capillary blood** migrates several layers of different materials, which makes it separates blood cells and zone only the plasm penetrates into the reaction. The reaction zone contains glucose oxidase, peroxidase and a suitable chromogen. Depending on the concentration of glucose develops differently intense color. The evaluation is done either by visually comparing the color chart or using a glucometer - a dedicated **reflective photometer**. The advantage of the visual evaluation is its independence from any instrumentation. Determination of glycemia using this method is only approximate, however, is quite sufficient especially in emergency care. Glucometers based on reflective photometry are currently pushed reliable electrochemical analyzers.

# Oral glucose tolerance test (OGTT)

| G l ucose tolerance | Glu in plasma after 2 hours after load |
|---|---|
| Normal (exclusion of diabetu mellitu) | < 7,8 mmol/l |
| Impaired glucose tolerance | 7,8 - 11 mmol/l |
| Diabetes mellitus | ≥ 11,1 mmol/l |

**Glucose values are for the adult population.**
**For expressing a diagnosis result must be confirmed repeatedly.**
**The finding of impaired glucose tolerance OGTT is repeated every two years.**

**Evaluations are carried out on the basis of the Czech Society of Clinical Biochemistry and the Czech Diabetes Society (2012) as recommended by WHO www.čskb.cz**

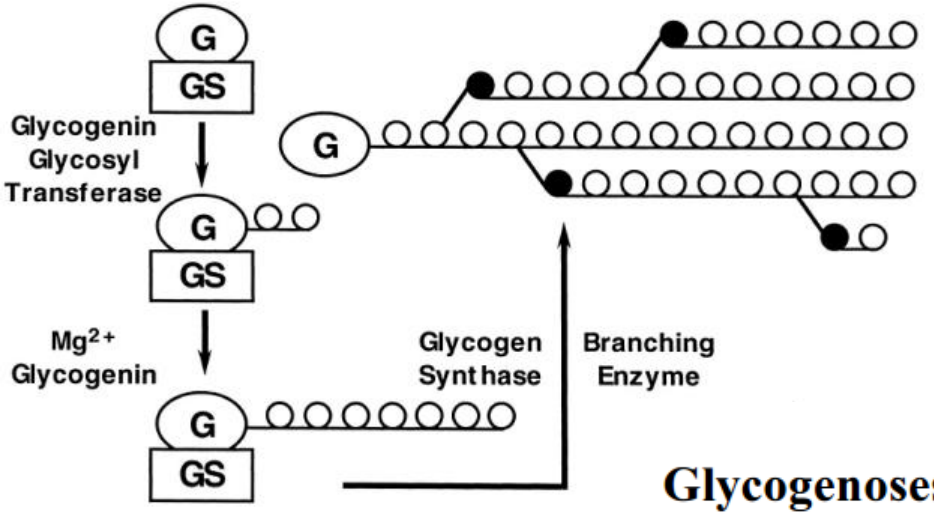
TEST

# 1) Glycogenosis (glycogen storage disease, GSD)

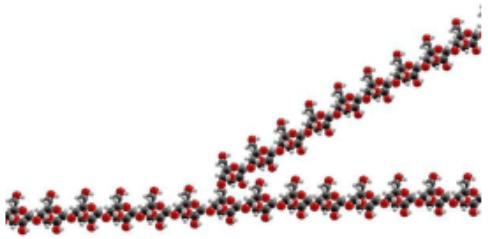
- are inherited metabolic disorders **with a deficiency of an enzyme or transport protein, which result in either abnormal glycogen structure, or an abnormal content in the tissues. Inheritance of all types of GSD is an autosomal recessive**, except there are only two subtypes of GSD IX for which inheritance is bound to the X-chromosome.
- Etiopatogenesis
- The cause of these diseases is zero or **insufficient synthesis of functional proteins (enzymes and transporters) participating in either glycogenolysis or glycogen synthesis**. Depending on the enzyme, glycogenosis can be divided into several types, that differ both clinical course and the biochemical findings and prognosis
- biochemical image
- Most often found in laboratory tests

dominate these symptoms:. hypoglycemia

- hyperlactatemia
- metabolic acidosis
- hyperlipidemia
- hyperuricemia




# Glycogenoses
# Glycogen storage disorders



wikipedia

**Glucose:** primary source of energy for eukaryotic cells

**Glycogen:** macromolecular storage form of glucose
– branched chain polysacharide composed of glucose units.

straight chains **α-1,4 linkages**
branching points **α-1,6 linkages** at intervals of 4-10 glucose residues

Serves as an important source of energy between meals. Especially abundant in the **liver** and in the **muscle**

In the muscle: glycogen β particles- up to 60 000 glucose residues
In the liver: α particles „aggregates" β particles, glycosomes

Synthesis of glycogen: protein „primer" - **glycogenin**

**Glycogenoses:** hereditary enzymopathies that result in storage of abnormal amounts and/or forms of glycogen

# Classification and basic characteristics of glycogenosis

Classification:
Depending accumulation
•by the storage in the cytosol - all except GSD II
•by the storage in **lysosomes** - GSD II
According to organ damage
•**generalized**: II, IV
•**liver**: Ia, Ib, III, VI, IX, 0
•**muscle**: V, VII; red muscle cells may be component type II, III, IX
•with **myocard** impairment : II, III, one of the subtypes IX
•with **renal** impairment: Ia, Ib

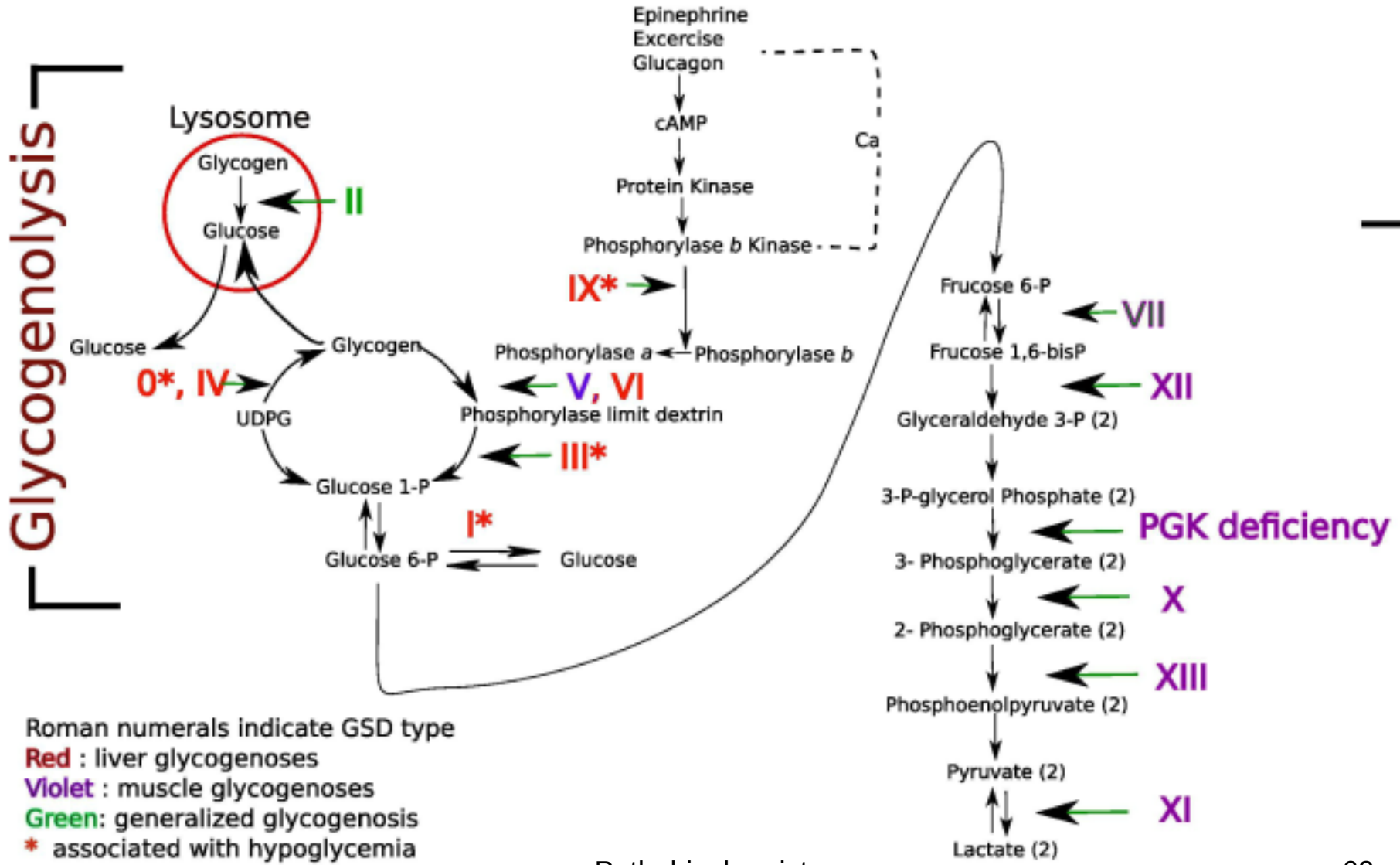
•**muscle glycogen storage disease**
symptoms: muscle weakness and slackness, fatigue, increased exertion pain muscles and attacks myolysis (ev. i hemolysis)
Usually occur after the 20th year of life
Laboratory findings: increased level of CK-MM, AST, ALT, LDH; urine myoglobinuria; since muscular tissue does not influence glucose homeostasis, for the type V, VII (only muscle GSD) hyperlactacidaemia and dyslipidemia aren´t in the results of blood analysis
treatment: symptomatic; demonstrated a beneficial effect of increased protein intake

•**Liver glycogen storage disease**
symptoms : hepatomegalie, menší vzrůst
Laboratory findings: hyperlactacidaemia, dyslipidemia, ketotic hypoglycemia, hyperuricemia

Overview glycogenosis type I-V[1]

| Type of glycogenosis | Defective enzyme | Place of accumulation | main clinical signs |
|---|---|---|---|
| **I – von Gierk** | glucose-6-phosphatase | liver, kidney | slow growth, hypoglycemia, hepatomegaly |
| **II – Pompe** | lysosomal α-glucosidase | muscles, liver | heart failure, hypotonia |
| **III – Corri** | glycogen unbranching enzyme | liver, muscles | Slow growth, muscle weakness, hypoglycemia |
| **IV – Andersen** | glycogen branching enzyme | liver, muscles | failure to thrive, liver failure, muscle weakness |
| **V – McArdle** | phosphorylase | muscles | muscle weakness, cramps |

# Glycogen metabolism and glycogen storage disorders



# Glycogenosis type 0 (aglycogenosis)

- Lack of the enzyme glycogen synthase in the liver (muscle, leukocytes and enterocytes doesn´t missing). Liver glycogen is reduced below 2% normal.

- clinical picture

- Conditions severe hypoglycemia with cramps - lead to brain damage and mental retardation. They occur mainly in the morning, after overnight fasting, are accompanied by ketonemia. After administration of glucose observed prolonged hyperglycemia and increased lactate in serum (liver constitutes glycogen, glucose is metabolised to lactate).

- Urgent diagnosis is essential to child survival.
- Episodes of hypoglycemia can be prevented by frequent administration of protein-rich
- meals

# Glykogenose type Ia (von Gierke's disease)

Impaired activity of **glucose-6-phosphatase** (converts glucose-6-P into glucose, that is if necessary released from the liver into the blood).
AR inherited disease gene on the 17th chromosome.

**Clinical picture**

It begins in infancy, progressive hepatomegaly (liver function is normal, it does not develop cirrhosis) and hypoglycemic convulsions fasting.
During febrile conditions, hypoglycemia is more frequent and is accompanied by lactic acidosis (hyperlactacidemia is consequence of an excess of glucose-6-phosphate, which is in case of inability to hydrolyse to glucose, further metabolized by glycolysis, whose products are lactate and pyruvate) with Kussmaul breathing.

•Characteristic facies "doll face"
•Organism adapts to hypoglycemia - insulin secretion decreases, lipase in adipose tissue is activated → hyperlipoproteinemia occurs → ketone bodies are formed, caused by increased degradation. Ketone bodies and lactate are then involved in acidosis.
•Glucagon administration does not increase glucose but lactate..
•Galactose, fructose and glycerol, also require hepatic G-6-Pase for conversion to glucose → ingestion of sucrose and lactose leads
• to hyperlactacidaemia with only a small rise of blood glucose level.
•Slows the growth and puberty is lagging behind.
•In adulthood xanthomas and nephromegaly may appear, and associated renal failure with hypertension, gout, liver adenomas.
•Laboratory
•fasting hypoglycemia (often only in infants and toddlers)
•hyperlipidemia and hyperlactacidaemia that blocks the secretion of uric acid and makes hyperuricemia

**Diagnostics**

•UZ: hepatomegaly and nephromegaly, hepatic adenomas may occur
•**Liver biopsy**: steatosis and multiplication of glycogen

**Therapy**

•The aim is to prevent states of severe hypoglycemia and MAc
•Diet therapy - The frequent feeding with a restriction of animal fats, lactose, sucrose and fructose
•Calorie requirement is mainly replaced by starches and maltodextrins.
•From toddler age, corn starch is served after every meal.
•At night continuous nutrition through a nasogastric tube is suitable, so as to administrate 30% of daily intake at night.
•In acute metabolic disorder associated with lactic acidosis, i.v. glucose must be administered during infection.
**Supportive pharmacotherapy**: administration of an xantinoxidase inhibitor to prevent gout and uric acid nephropathy
• (but since the uric acid is a powerful antioxidant, the efforts are to keep her blood levels at the upper limit of the normal range);
• when severe hypertriacylglycerolemia – nicotinic acid and fibrates (to reduce the risk of cholelithiasis and pancreatitis).

**Complications**

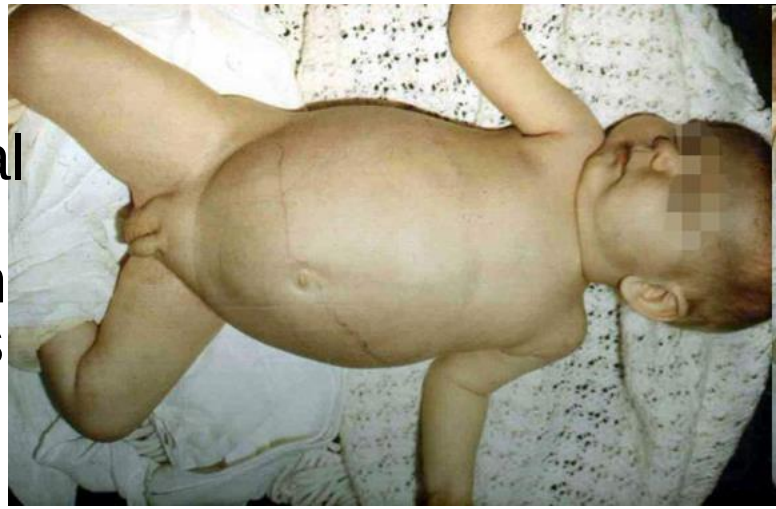
•Hepatic adenomas, osteopenia, anemia, polycystic ovaries, pulmonary hypertension, depression (exhaustive treatment).

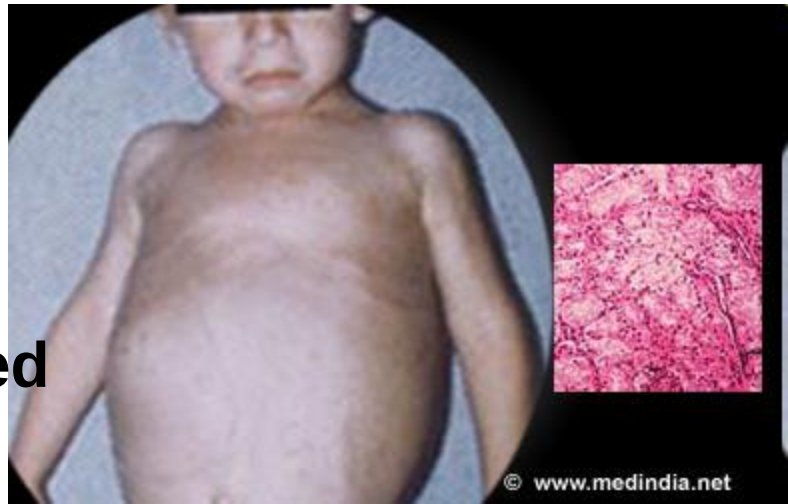
**Prognosis**

•In childhood is good, in adulthood hepatic, renal and cardiovascular complications may develop.

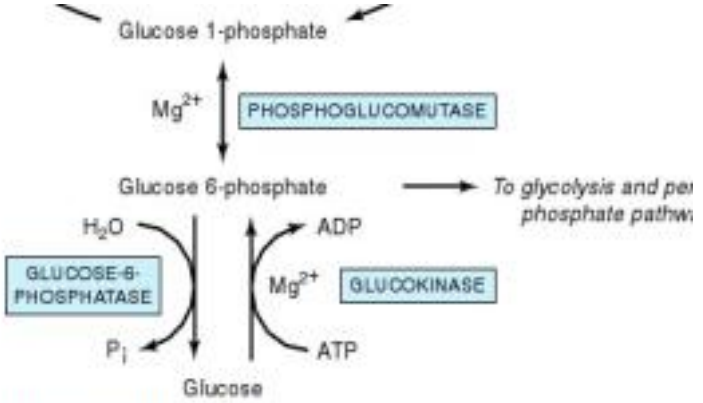
# *Glycogenosis type I – Girke's disease.*

- Girke's disease cause deficit of **glucose-6-phosphatase.** This enzyme provides 90 % of glucose which disengages in liver from glycogen. It play central role in normal glucose homeostasis. Glucose which disengages attached to disintegration of glycogen or is derivated in process of gluconeogenesis obligatory goes over stage of glucose-6-phosphate. Enzyme glucose-6-phosphatase tears away a phosphate group from glucose. There free glucose is formed it goes out in blood. Attached to Girke's disease stage of tearing phosphate group is blocked. There are no free glucose hypoglycemia occur. Hypoglycemia arises. Attached to Girke's disease **glycogen is deponed in liver and kidneys**.



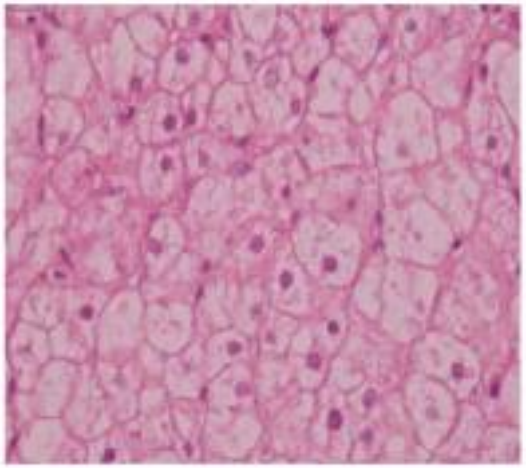


# Type I Glycogen Storage Disease (Glucose 6-Phosphatase Deficiency, von Gierke Disease)




Excessive accumulation of glycogen in liver, kidney and intestinal mucosa

Patients usually present in infancy with hepatomegaly and/or hypoglycaemic seizures, hyperlactacidemia after a short fast



Gout, hyperlipidemia (hypertriglyceridemia), skin xanthomas

Doll-like face, thin extremities, short stature, protuberant abdomen (hepatomegaly), inflammatory bowel disease

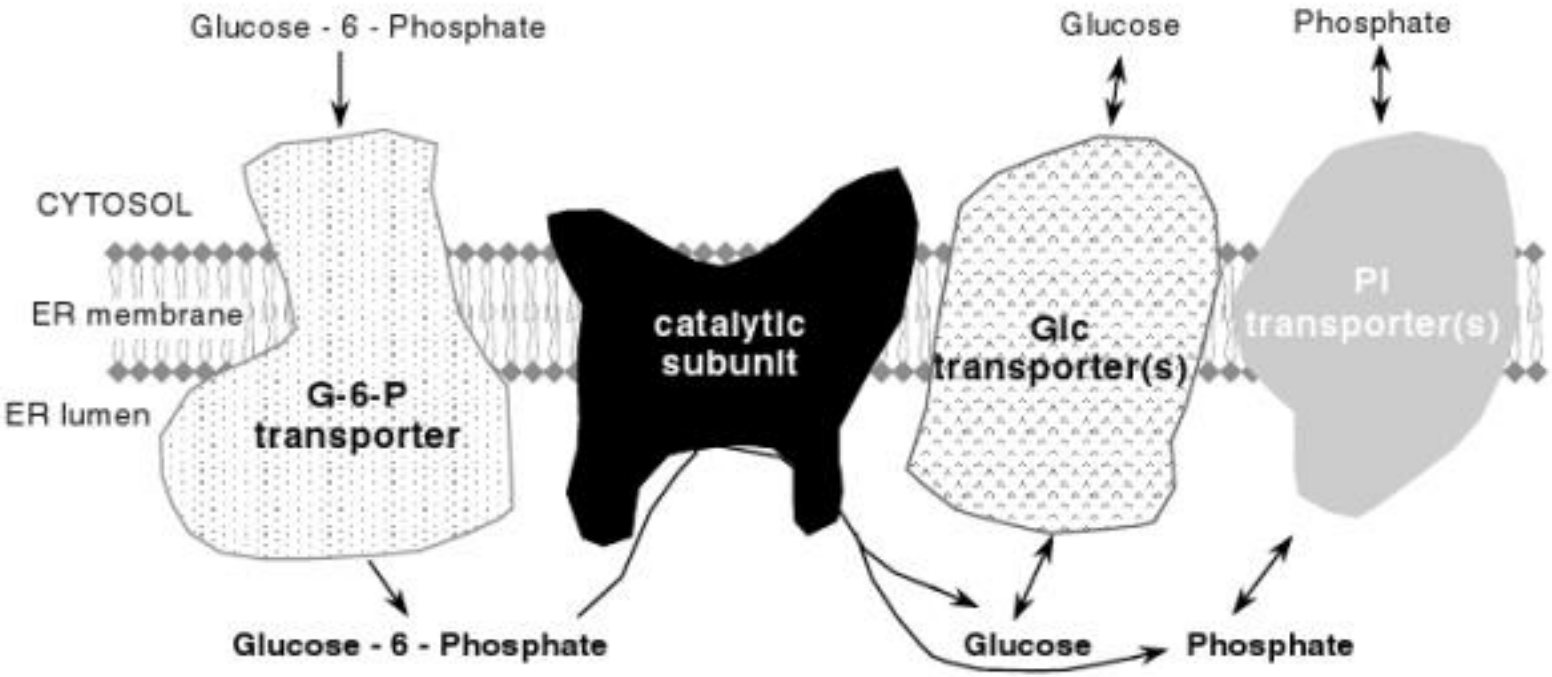
Fibrosis, liver adenomas -cave: malignant transformation, Atherosclerosis

Fasting tolerance improves with age, long-term complications

Treatment : frequent feeding, nocturnal nasogastric drips in infancy, uncooked cornstarch, liver transplantation

Autosomal recessive, overall incidence is 1:10000, frequent in Ashkenazi
The diagnosis is based on clinical presentation, abnormal blood/plasma concentrations of glucose, lactate, uric acid, triglycerides, and lipids, and molecular genetic testing.

## Glucose -6-phosphatase system




Localized to luminal face of ER

Type Ia GSD: deficient activity of phosphatase
Type Ib GSD: a defect in the microsomal membrane transport system of G-6-P
Type Ic GSD: a defect in microsomal phosphate or pyrophosphate transport,

Non-a types associated with neutropenia and inflamarory bowel disease with recurrent bacterial infections and oral ulcers

# Glycogenose type Ib

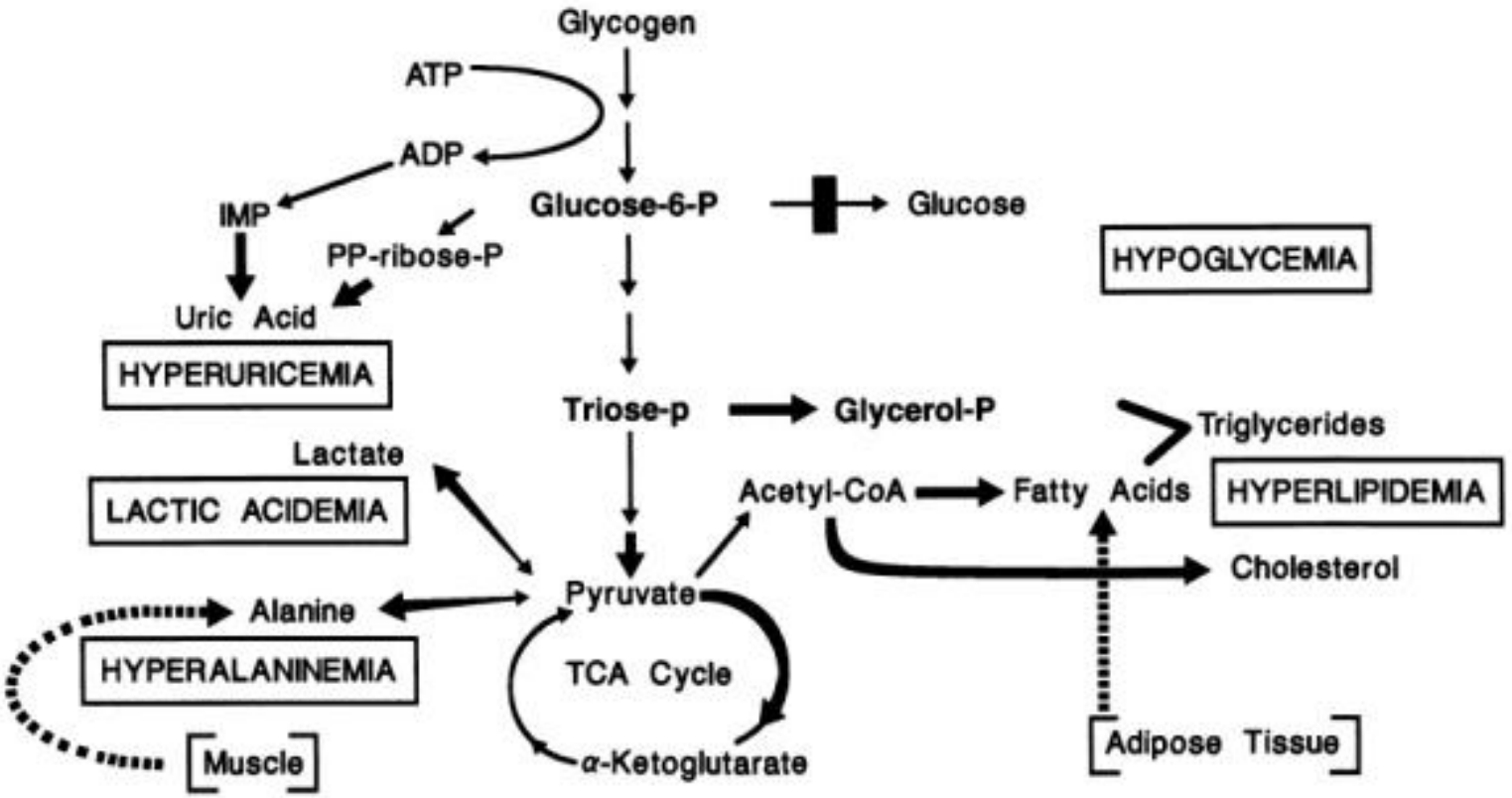
- Defect of glc-6-P translocase (transporter of glucose-6-phosphate across the ER membrane).
- Clinical picture
- It is indistinguishable from Ia.

- Symptoms include - neutropenia with neutrophil dysfunction → frequent respiratory tract infections, urinary tract and skin.
- Most patients have symptoms of inflammatory bowel disease (prolonged diarrhea).

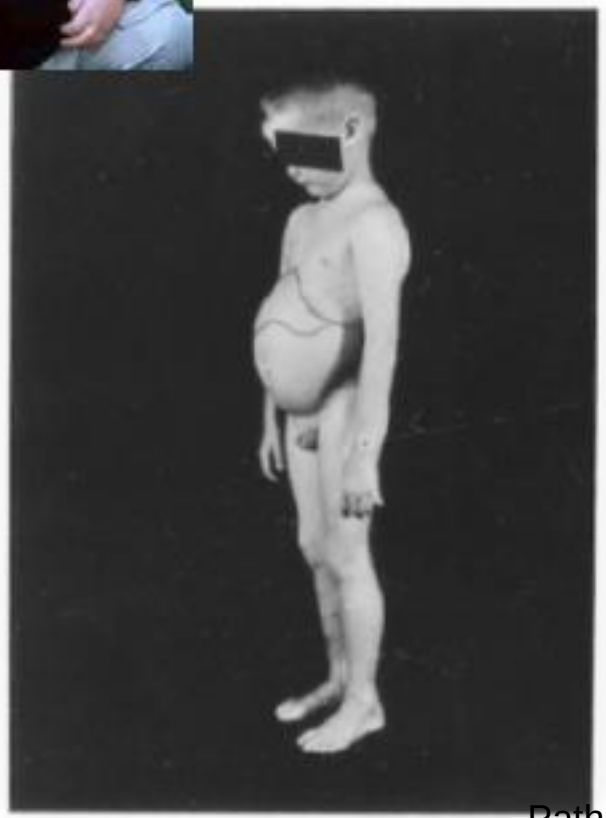
- Pharmacotherapy

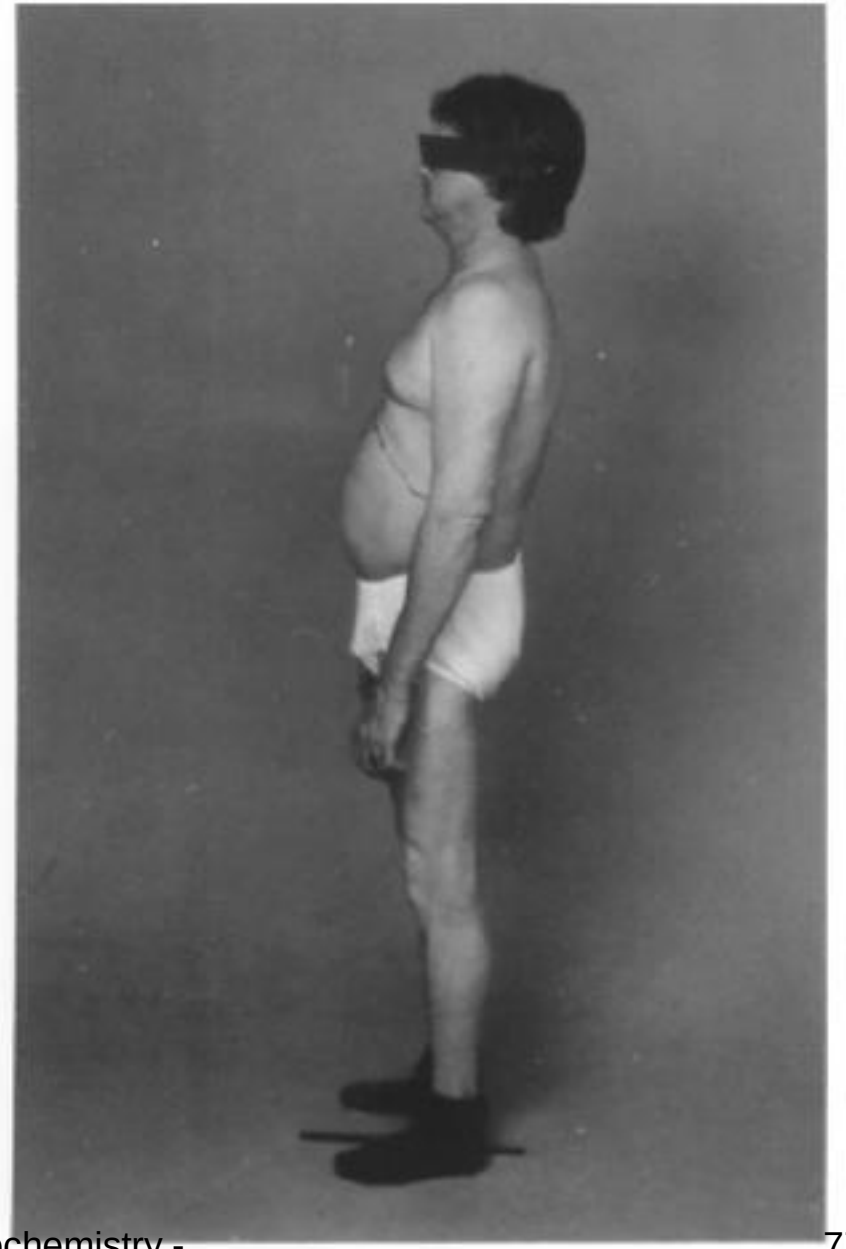
- Prophylaxis with cotrimoxazol; administration of GCSF (granulocyte colony-stimulating factor, factor stimulating the creation of granulocytes) → long term leads to hypersplenism, renal cancer, AML.

# The metabolic consequences of GSD I



http://www.curegsd.org/faces.htm



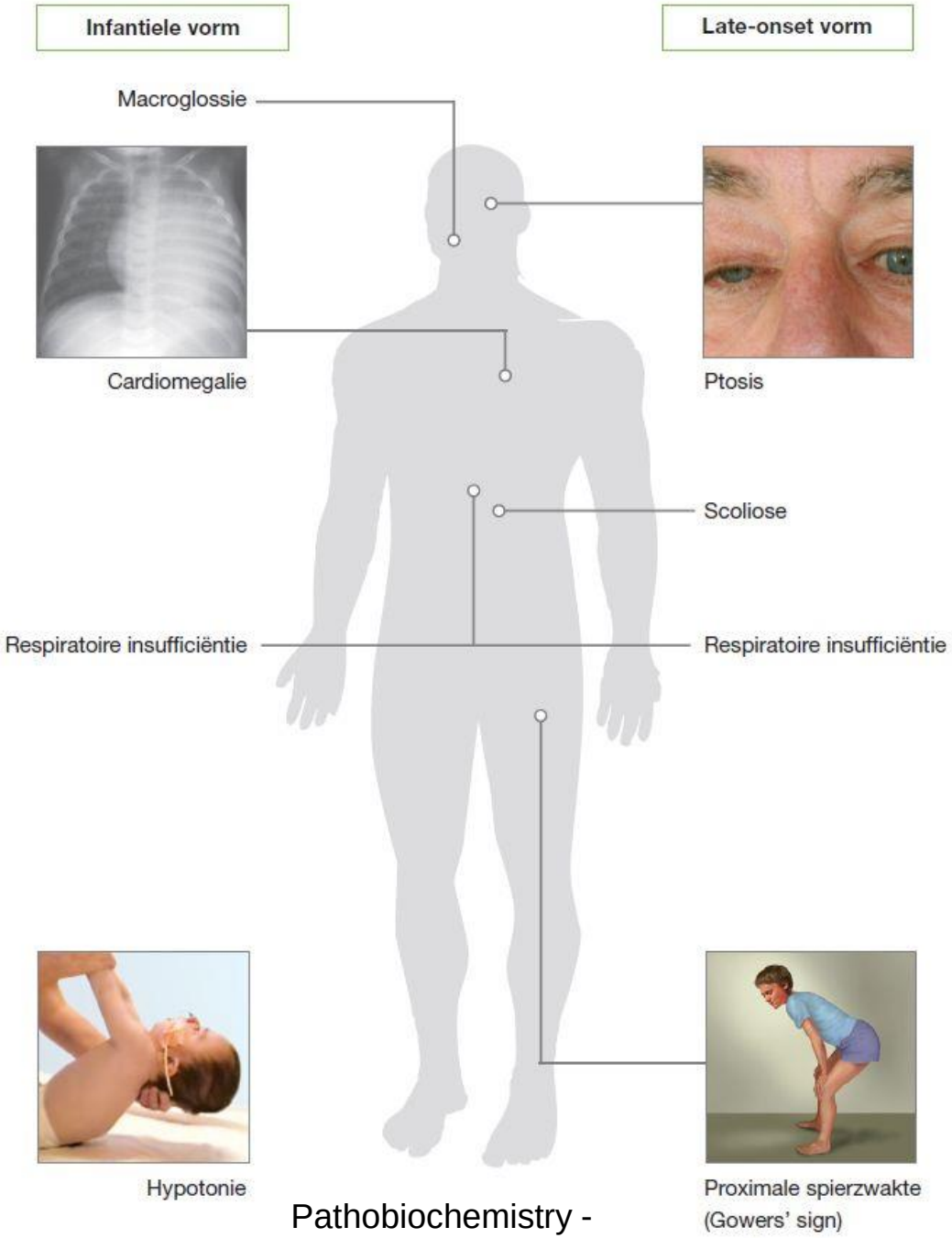


# Glycogenose type II (generalized, Pompe's Disease)

- In 1932, Dutch pathologist dr. J. C. Pompe [2] described this disease. This is a AR hereditary disease caused by the AR gene mutation. This gene encodes acidic lysosomal **α-1,4-glucosidase** (GAA).
- Gene for GAA was localized on the long arm of the 17th chromosome (17q23) [2].
- Due to a deficiency or lack of GAA enzyme activity, lysosomal glycogen accumulation occurs in many tissues, particularly in skeletal muscle and in the myocardium (infants) and to a lesser extent also in endothelial vascular system in the CNS (in particular astrocytes) in the liver and kidneys [2] .
- Incidence: 1:40 000. In the Czech Republic 4 patients are currently diagnosed (assuming considerable underdiagnosis of this disease due to the lack of neonatal screening) [2].
- **Prenatal diagnosis** is possible - finding of abnormal lysosomes in amniocytes.
- **Clinical picture**

- **The classic infantile form (IIa)**
- Affects infants (enzymopathies) - always lethal.
- During the weeks and months the child becomes completely hypotonic - weakly sucks (→ unthrifty), breathing shallowly (→ susceptibility to respiratory infections and sleep apnea).
- Distinct cardiomegaly, high P at EKG, abbreviated PQ and transmission failure.
- Liver slightly enlarged, also described macroglossia.
- Consciousness is not infringed, so does intellect.
- Frequent aspiration pneumonia with atelectasis
- Death of 2 years from respiratory failure.

- **Laboratory finding**
- Elevated liver and muscle enzyme levels in blood (ALT, AST, LDH, CK). The presence of oligosaccharides in urine.


Infantiele vorm
Late-onset vorm
Macroglossie
Cardiomegalie
Ptosis
Scoliose
Respiratoire insufficiëntie
Respiratoire insufficiëntie
Hypotonie
Proximale spierzwakte
(Gowers' sign)

# M.Pompe

Deficiency of lysosomal acid alpha-glucosidase (acid maltase)
Lysosomal storage of glycogen with normal structure

**Infantile type:**
First symptoms in the first months of life: cardiomegaly, muscle weakness, macroglossia
Progressive course, death due to cardiopulmonary failure in the first two years of life

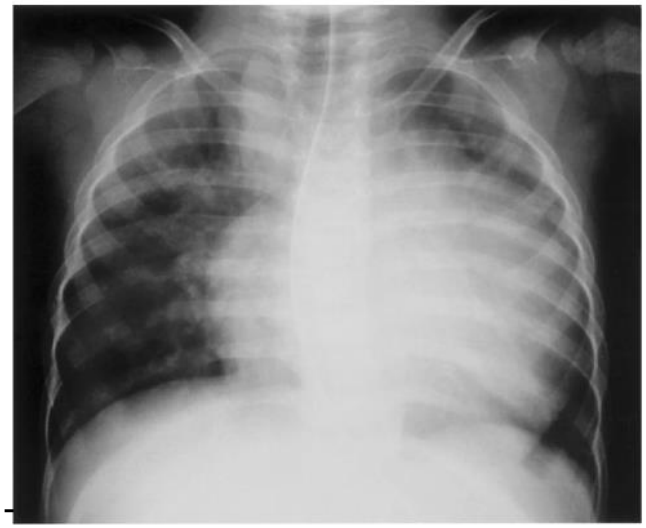
**Adult type**
Slowly progressive proximal myopathy and/or slowly progressive respiratory failure
Heart is not affected

**Intermediate types**

**Diagnostics:**
proof of glycogen storage in tissues
measurement of enzyme activity

**Treatment**
Enzyme supplementation therapy (Myozyme)

**Generalized glycogenosis: Morbus Pompe**



# Late type - juvenile and adult form (IIb)

- It affects older children and adults (enzymopenia)
- Clinically heterogeneous (given by a number of different mutations that can occur in *GAA* gene, there have been already described over 200) → severity is determined by the residual enzyme activity.
- cardiomegaly is smaller, normal ECG, arrhythmias frequently.
- Death usually around the 30th to 40th year of life (according to age manifestation). It may not shorten life expectancy, allows a sedentary job.

- **Symptoms**
- Dominated disability of muscles (muscle weakness, hypotonia) pelvic girdle (difficult standing up) and pharynx (problems with food intake), respiratory muscles are also affected (sleep apnea, dyspnea) → most common cause of death is respiratory failure; however myocardium is not usually affected.

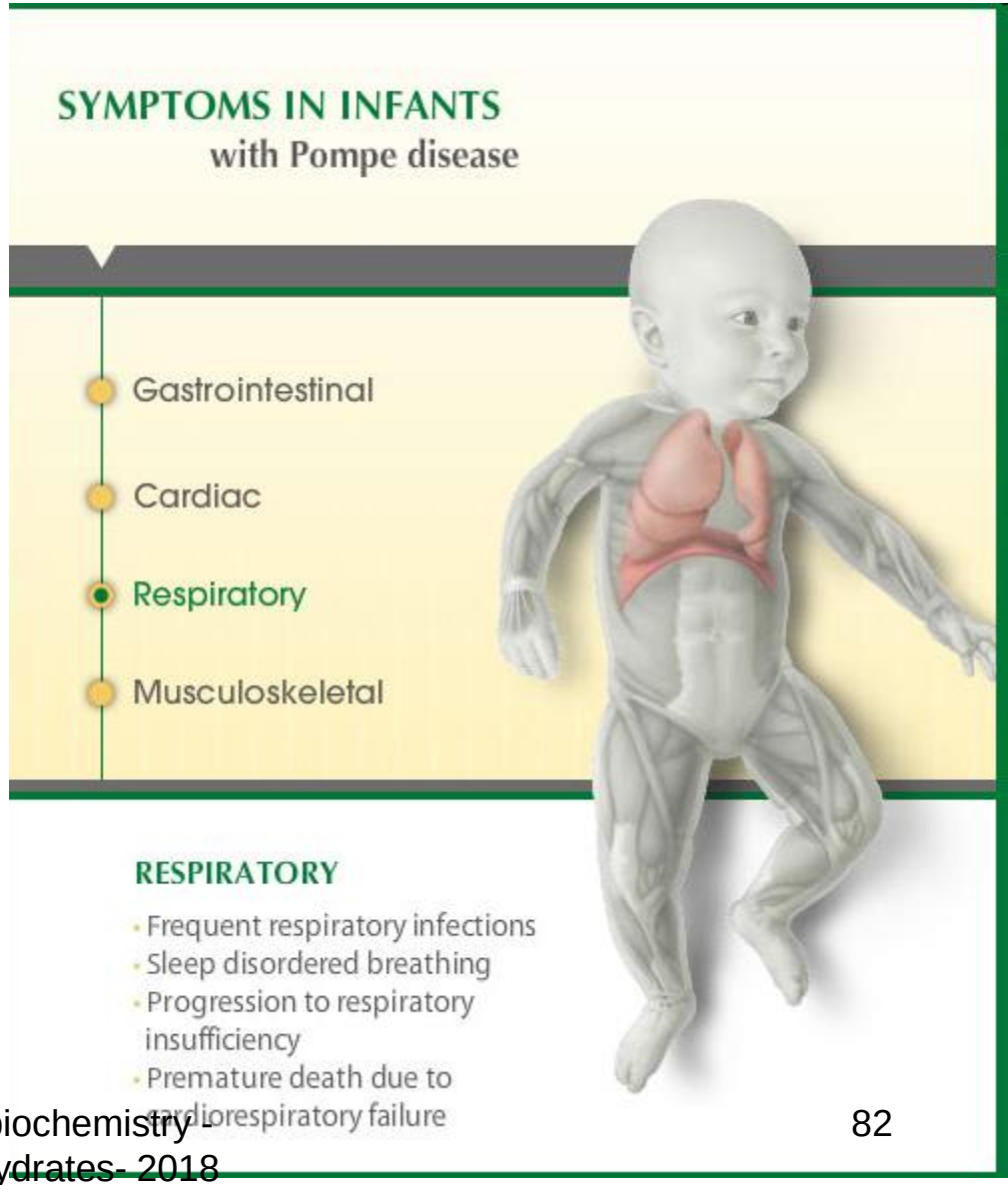
- **Diagnosis, Clinical examination**
- Laboratory findings of *GAA* reduced activity in leukocytes or fibroblasts.
- Molecular-biological identified mutation for a given enzyme.
- Demonstration of glycogen deposits in biopsy specimens (muscle).
- Skin biopsy - electron microscope detectable abnormality of lysosomes.

- **Treatment**

- Only slows the progression; enzyme replacement therapy (ERT - enzyme replacement therapy) by Myozyme® preparation (by infusion), contains *GAA* α-glucosidase precursor, which is by the acidic environment in lysosomes converted to the active enzyme, symptomatic therapy (physiotherapy, medication support, balneotherapy).

# *Type II glycogenosis – Pompe's disease.*

- Illness is related to deficit of lysosomal enzyme – sour maltase, or α-1,4-glucosidase. This enzyme slits glycogene to glucose in digestive vacuoles. Attached to it's deficit glycogen accumulates at first in lysosomes and then in cytosole of hepatocytes and myocytes.



# Glycogen Storage Disorders:

- Type 2- Pompe's disease:
- Normal Glucose
- Do to an accumulation of glycogen in lysosomes.
- **Ancient city of Pompeii was destroyed by Mt. Vesuvius- 79 AD**
- Manifested by massive Cardiomegaly, Hepatomegaly, Macroglossia.
- Fatal If results in CHF.
- Limited therapies in Neonatal Variant.
  - Attempts at enzyme replacement ongoing.

# Glycogenose disease type III (Cori's disease, Forbes disease)

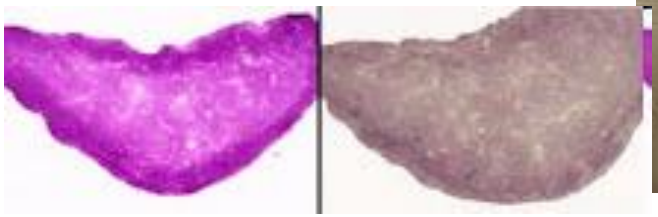
- Rare AR hereditary disease. This is a disorder of enzymes degrading the branching of glycogen (debrancher **amylo-1,6-glucosidase** and **oligo-1,4-glukantransferase**). Produces a similar clinical picture as GSD I, but has a milder course.

**Type III Glycogen Storage Disease (Debrancher Deficiency; Limit Dextrinosis; Cori or Forbes Disease)**

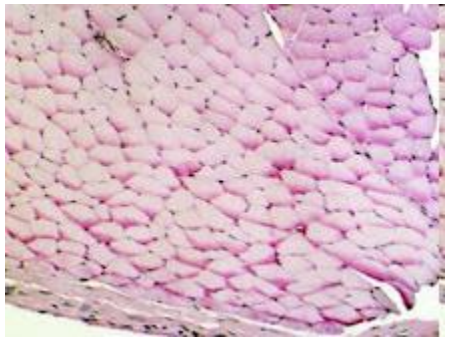
Both liver and muscle are affected: frequent cirrhosis, myopathy, often cardiomyopathy, with fasting ketotic hypoglycemia
about 15% percent of patients have only hepatic presentation
myopathic presentation - also in adulthood
Abnormal glycogen: limit dextrin

# *Type III glycogenosis – Cori's disease, Forbs' disease*

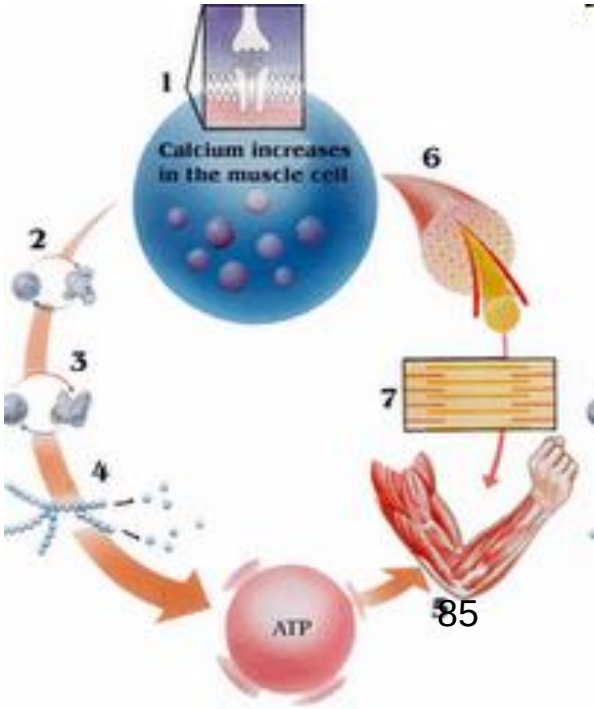
This illness is named limited ecstrinosis. In it's base lies a deficit of amylo-1,6-glucosidase. Degradation of glycogen pauses in sites of branching. Glycogen accumulates in liver and muscles. Cure is diet with big proteins maintenance.



**Glycogen in the Liver (left stained to show glycogen, right normal)**



**Glycogen in Muscle Cells**



# Glykogenose type IV (Andersen's disease)

- Rare AR hereditary disease, previously about 10 cases have been described. Defective enzyme is amylopektinose (branching enzyme) → accumulation of polysaccharide without branching points.
- Infantile type, Symptoms:
- Severe heart and liver diseases (cirrhosis, hepatosplenomegaly, portal hypertension), ascites; rapidly progressive, with the desperate prognosis (death usually occurs due to heart or liver failure in the first year of life)
- Juvenile, adult form
- Atypical;
- manifestations are generalized.

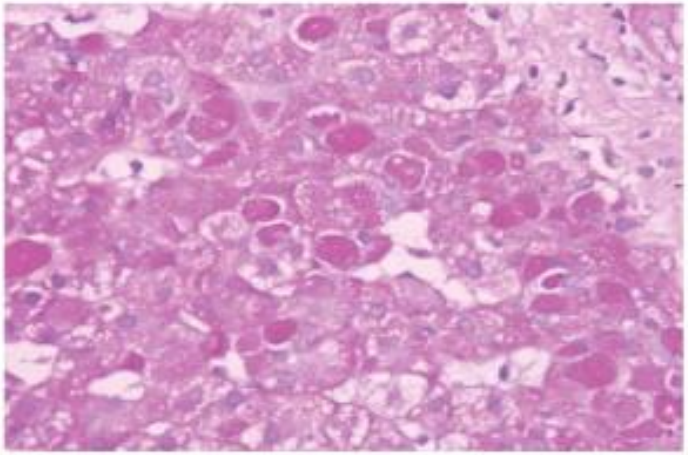
**Type IV (Branching Enzyme Deficiency, Amylopectinosis, or Andersen Disease)**

Abnormal glycogen resembling amylopectin – fewer branching points

presents in infancy with liver failure leading to cirrhosis, rare hypoglycemias, cardiomyopathy

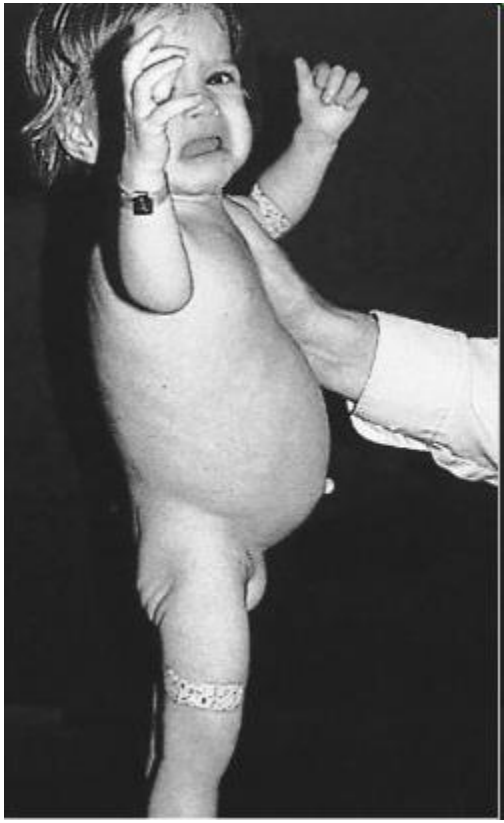
death at 4-5 years withou liver transplantation

Neuromuscular presentation - accumulation of polyglucosan bodies in tissues - myopathy , adult polyglucosan body disease



# Type IV glycogenosis – Anderson's disease.

- It is called by deficit of amilo-1,4,1,6-transglucosidase (branching enzyme).
- As result of this there is derivated anomalous glycogen with very long branches and rare points of branching.
- It is not exposed to degradation and accumulates in liver, heart, kidneys, spleen, lymphatic nods, skeletal muscles.



# Glykogenose type V (McArdler's syndrome)

- Deficiency of myophosphorylase → muscles have an increased glycogen content that forms vacuoles
(up to 4%). Symptoms. Reduced tolerance of physical exertion.

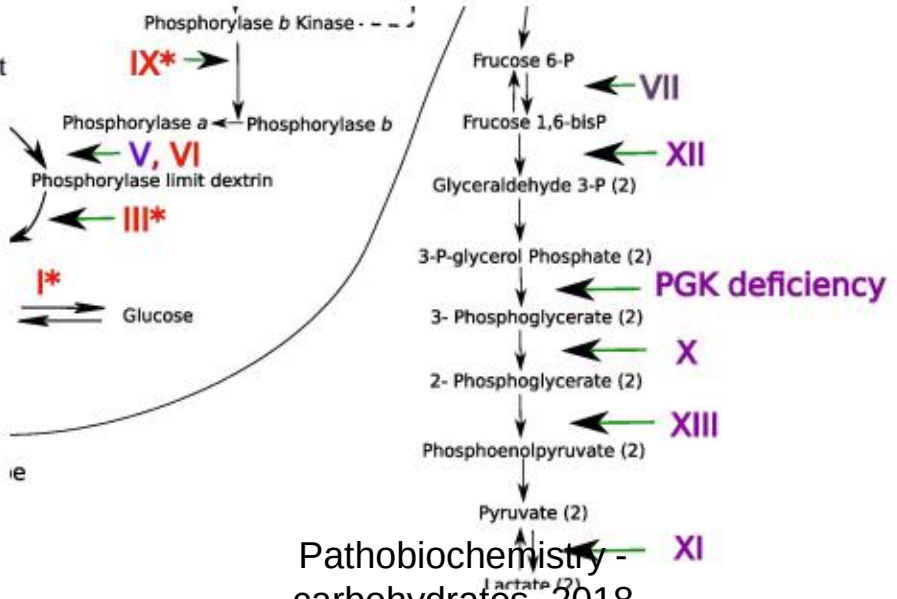
**Muscle glycogenoses (without cardiac involvement)**

*Excercise intolerance, often followed by rhabdomyolysis*

prototypical : GSD V, McArdrle disease, deficiency of myophosphorylase
myalgia and stifness of exercising muscles relieved by rest, often rhabdomyolysis, later in life
may be present muscle wasting

AR inheritance

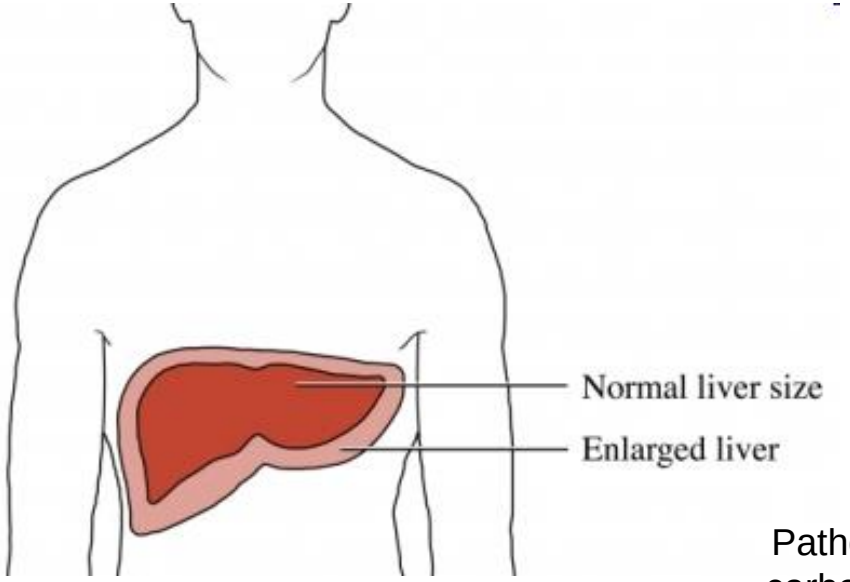
no pharmacological treatment

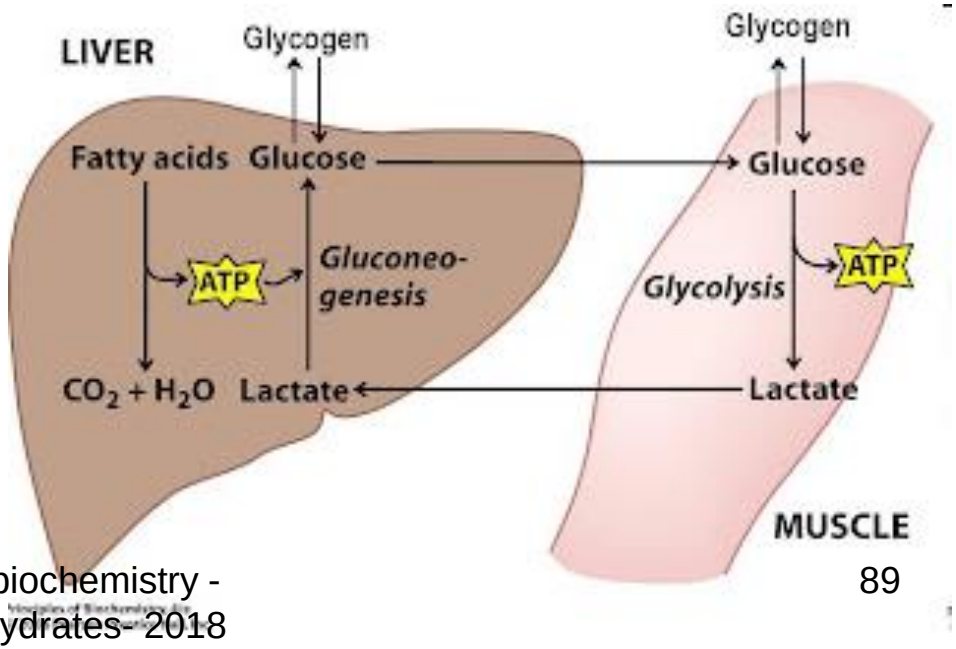


- **Glykogenose type VI (Hers' disease)**

Deficit in liver phosphorylase.

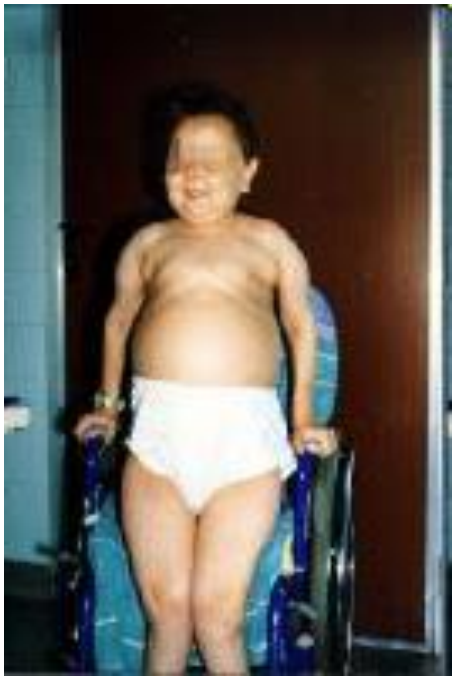
- Illness arises as result of insufficiency of hepatic phosphorylase complex. Glycogen accumulates in liver. Typical sign is hepatomegalia.



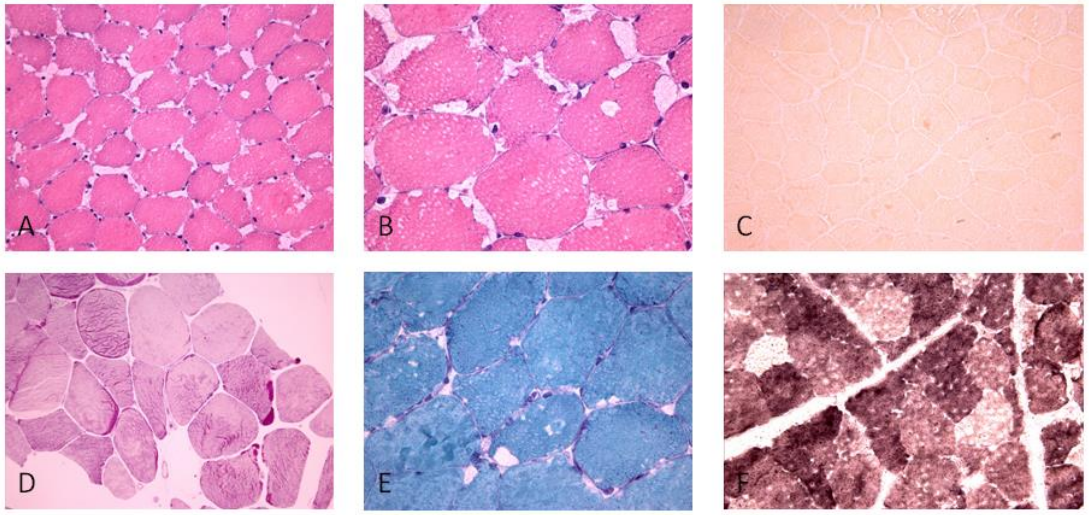



- **Glykogenose type VII (Taruis' disease)**

- Deficit in phosphofructokinase in muscle and erythrocytes.
- Symptoms
- Reduced tolerance of physical exertion, increased muscle glycogen content. There can also occur hemolytic anemia.
- Illness essence is in oppression of muscle phosphofrutkinase. Symptoms are similar to McArdles disease.



Enzyme Histochemistry




Nineteen year old male presented with exercise intolerance, muscle cramps, and episodes of rhabdomyolysis. **A** Hematoxylin and Eosin (H&E) stain of frozen muscle at x400, **B** H&E of frozen muscle at x200, **C** Myophosphorylase stain (absent) at x200, **D** PAS stain on plastic embedded 1-micron section x200, **E** Modified Gomori's Trichrome x400, **F** Myophosphorylase control x200

# Disorders of Fructose Metabolism

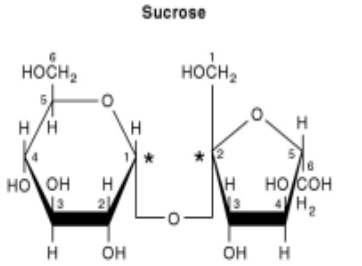
## Fructose

Fructose (β-D-fructofuranose)

Honey, vegetables and fruits

Saccharose

Frucose is the main sugar of seminal fluid




*O*-α-D-Glucopyranosyl-(1 → 2)-β-D-fructofuranoside

raffinose, stachyose, inulin - no role in human nutrition

sorbitol – sugar alcohol, derived from glucose, abundant in fruits. Sorbitol dehydrogenase converts sorbitol to fructose - a source of fructose.

GLUT5 – glucose transporter isoform is probably responsible for fructose transport in the small intestine

Fructose is probably transported into the liver by the same system as glucose and galactose

**Ingestion of large amounts of sucrose (beet or cane sugar) causes, after subsequent decomposition, the increased level of fructose. Fructose is in the liver decomposed significantly faster by glycolysis than glucose, resulting in rapid flow through certain liver metabolic pathways, and consequently there is an increased formation of fatty acids, their esterification and secretion of VLDL - may cause a rise of triglycerides in serum. Excess of contained glucose amplifies this phenomenon.**
**Fructose derived from sucrose can be converted in humans to glucose and lactate prior to entering the portal circulation. Together with glucitol, fructose is contained in the human lens, where it can accumulate in diabetes and can cause a diabetic cataract. It is also found in seminal plasma and secreted into the fetal circulation of ungulates and cetaceans, where it acts as an important source of energy.**

# Inherited disorders of fructose metabolism

Daily intake of fructose in Western diets: 100 g

Metabolised in liver, kidney, intestine

Intravenous fructose in high-doses is toxic: hyperuricemia, hyperlactacidemia, utrastructural changes in the liver.

**Essential fructosuria**

**Hereditary fructose intolerance (aldolase B deficiency)**

**Hereditary fructose 1,6-bisphosphatase deficiency**

Autosomal recessive disorders

**Initially, the degradation of fructose is not glucose dependent, subsequent metabolic steps can enter glycolysis. A possible complication is hereditary fructose intolerance which is caused by the absence of liver aldolase B, which metabolizes fructose-1-phosphate to glyceral and glyceron-3-phosphate, or defect fructose-1,6-bisphosphatase, which causes accumulation of fructose-1-phosphate, leading to inhibition of glycolysis and glycogenolysis, subsequently leads to hypoglycemia. Although there is phosphorylation of fructose, cell lacks ATP and phosphate and can not further degrade. Chronic intake of fructose may cause irreversible destruction of the liver. Treatment consists of reduced fructose diet.**

# Toxicity of fructose

Rapid accumulation of fructose -1-phosphate

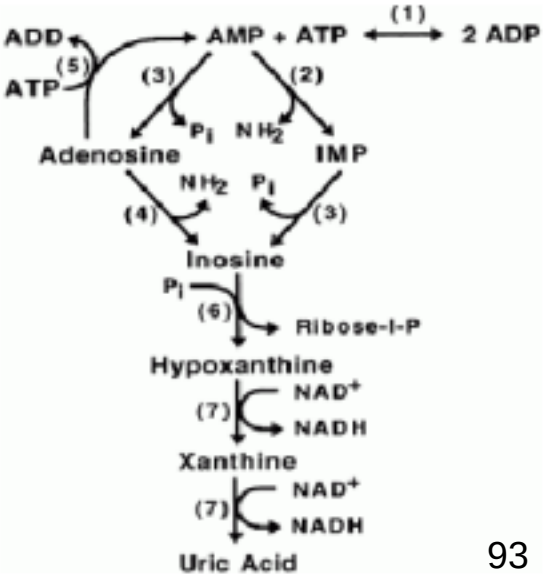
The utilization of F-1-P is limited by **triokinase**

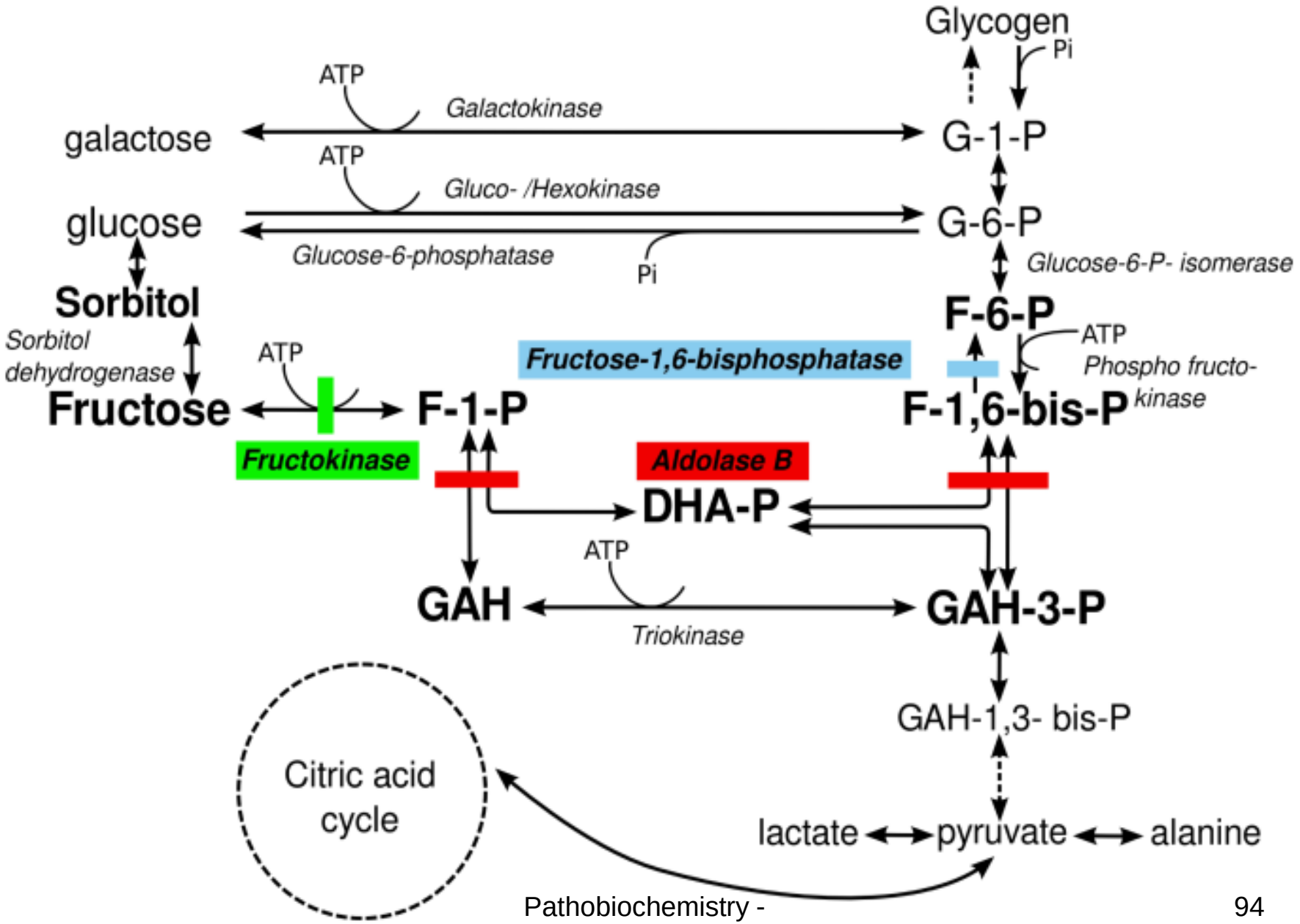
Depletion af ATP

**Hyperuricemia**
Hyperuricemic effect of fructose results from the **degradation of adenine nucleotides (ATP)**.

Adenine dinucleotides → → → uric acid

Increase in lactate




Glycogen
Pi
ATP
Galactokinase
galactose
G-1-P
ATP
Gluco- /Hexokinase
glucose
G-6-P
Glucose-6-phosphatase
Pi
Glucose-6-P- isomerase
Sorbitol
F-6-P
ATP
Phospho fructo-
kinase
Sorbitol
dehydrogenase
ATP
Fructose-1,6-bisphosphatase
Fructose
F-1-P
F-1,6-bis-P
Fructokinase
Aldolase B
DHA-P
ATP
GAH
GAH-3-P
Triokinase
GAH-1,3- bis-P
Citric acid
cycle
lactate
pyruvate
alanine

**1) Essential Benign fructosuria**

fructokinase deficiency, fructose resorbed by intestine in the organism, can't be metabolically used and is excreted in the urine without clinical symptoms

## Essential fructosuria

Deficiency of liver fructokinase

Asymptomatic metabolic anomaly - benign

Hyperfructosemia and hyperfructosuria

# 2) Hereditary fructose intolerance

AR inherited disease, 1:40 000, deficiency of E fructose-6-P aldolase in the liver
pathogenesis: fructose-6-P accumulates in the liver, causes competitive inhibition of phosphorylase and prevents glycogen breakdown to glucose, which causes severe hypoglycemia symptoms at birth are consistent with the classical galactosaemia

Deficiency of **fructoaldolase B** of the liver, kidney cortex (isoenzymes A,B,C)

Severe hypoglycemia upon ingestion of fructose

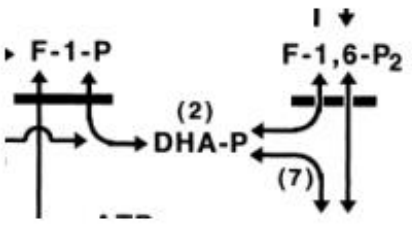
Prolonged fructose intake : poor feeding, vomiting, hepatomegaly jaundice hemorrage, proxima tubular renal syndrome, hepatic failure, death

Strong distaste for fructose containig foods

Fructose -1- phosphate inhibits gluconeogenesis : phosphorylase and aldolase

Patients are healthy on fructose-free food

Diagnostics: (i.v. fructose tolerance test), DNA analysis.



# 3) Hereditary fructose 1,6bisphosphatase deficiency

Fructose 1,6-bisphosphatase catalyzes **the irreversible splitting of fructose 1,6-bisphosphate** into fructose 6-phosphate and inorganic phosphate (P)

Autosomal recessive disorder

**Severe disorder of gluconeogenesis**, gluconeogenetic precursors (amino-acids, lactate, ketones) accumulate after depletion glycogen in the patients

Episodes of hyperventilation, apnea, hypoglycemia, ketosis and lactic acidosis, potentially lethal course

Episodes often triggered by fasting and infection

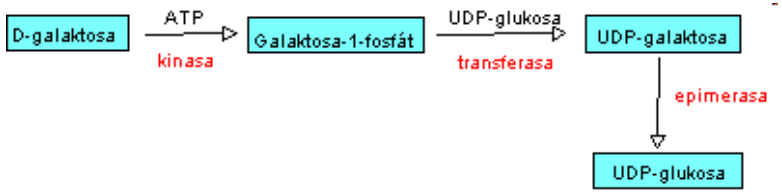
Aversion to sweets does not develop, tolerance to fasting improves with age

# Disorders of Galactose Metabolism

Galactosemia is an increase in the concentration of galactose in the blood serum. Galactosemia may be caused by defects in these enzymes:

galactose-1-phosphate, uridyltransferase

uridyldiphosphate galaktose-4-epimerase and galactokinase.



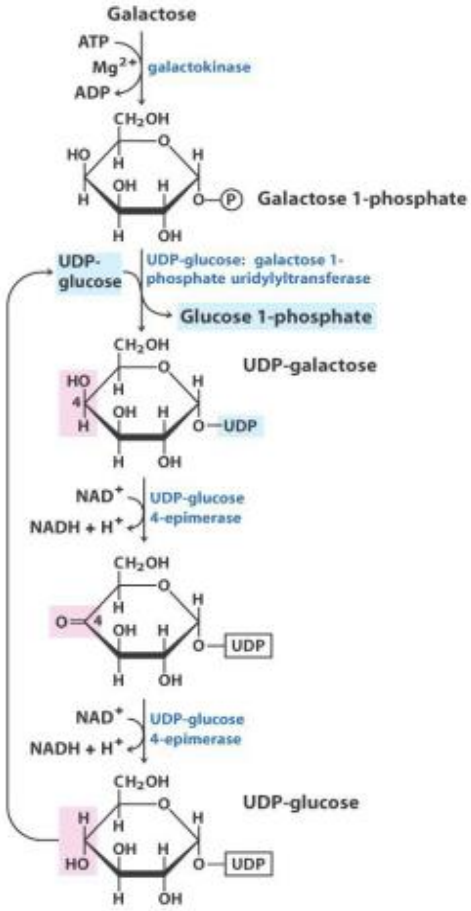

The main sources of galactose are milk and milk products.

Galactose is present as the disaccharide **lactose** (β-D-galactopyranosyl-(1→4)-D-glucose)

**Genetic disorders:**
Galactokinase

Galactose-1-phosphate uridyltransferase

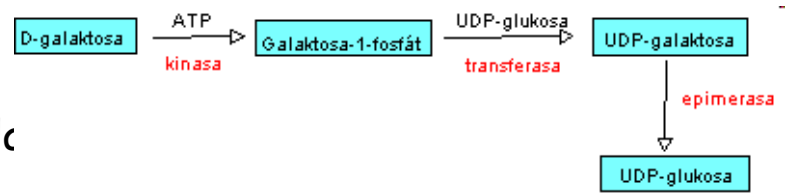
Uridine diphosphate galactose 4-epimerase.



TEST

# Disorders of Galactose Metabolism summary

Galactosemia - increased concentration of galactose in serum - defects: galactose-1-phosphate uridyltransferase, uridyldiphosphatgalaktose-4-epimerase galactokinase.




## 1) Classical galactosemia

AR hereditary disease, 1:50 000, deficiency of galc

which metabolizes galactose-1-phosphate

**Pathogenesis**: galactose-1-phosphate accumulates in the liver, kidneys, brain and in the lens of the eye; it is metabolised by alternative way to galactitol, which is toxic, symptoms after birth, hepatomegaly, progressing jaundice, lethargy, convulsions, acute septic symptoms resemble disease with liver and renal failure, untreated - brain edema and often bilateral cataracts

## 2) Galactokinase deficit

AR hereditary disease, 1: 200,000, deficiency of galactokinase, which catalyzes the conversion of galactose to galactose-1-phosphate

**Pathogenesis**: galactose and galactitol accumulates in the lens and cause its osmotic edema, bilateral cataracts, pseudotumor cerebri

## 3) Uridyldiphosphatgalaktose-4-epimerase deficit

AR inherited disease, uridyldiphosphatgalaktose-4-epimerase deficit, reminiscent of classic galactosemia, psychomotor retardation

# 1) Classical galactosemia

- Serious AR hereditary disease, incidence of 1:50 000
- **Cause:** lack of galactose-1-phosphate-uridyltransferase which metabolizes galactose-1-phosphate
- **Pathogenesis:** galactose-1-phosphate accumulates in the liver, kidney, brain and the eye lens; in alternative way is metabolised to galactitol, which is toxic

- **Clinical picture:** symptoms begin between 4.-9. day
- Vomiting, hepatomegaly, progressing jaundice, lethargy or convulsions
- Symptoms resemble acute septic disease with hepatic and renal failure
- If untreated, brain edema and often bilateral cataracts develop
- In NNPH the symptoms appear after conversion to dairy diet
- **Diagnosis:** proving of elevated concentrations of galactitol in urine and galactose-1-phosphate in erythrocytes
- Confirmation is always necessary on the enzymatic and molecular level
- **Therapy:** at the first suspicion it is necessary to immediately discontinue dairy diet
- In case of confirmation of the diagnosis, lactose lifelong diet is indicated
- **Prognosis:** may not be favorable even if detected in time, because the child has been exposed to galactosis intrauterinary
- Most often speech disorder and hypogonadotropic hypogonadism at girls arises.

# Classical galactosemia: galactose-1-phosphate uridyltransferase deficiency

In the first weeks of life: poor feeding and weight loss, vomiting, diarrhea, lethargy,and hypotonia.

Severe liver dysfunction, hepatomegaly, icterus, vomiting, lethargy bleeding tendencies, septicemia, renal tubular syndrome

Cataracts

Elevated galactose, galactitol, galactose-1-phosphate

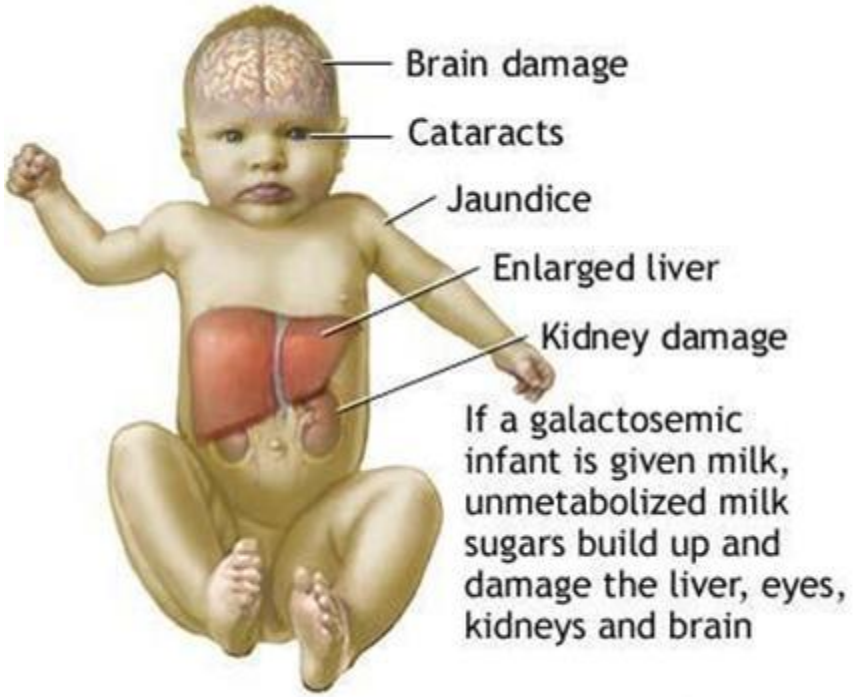
**Long-term complications**
effects on cognitive development,
ovarian failure in females
An ataxic neurologic disease.

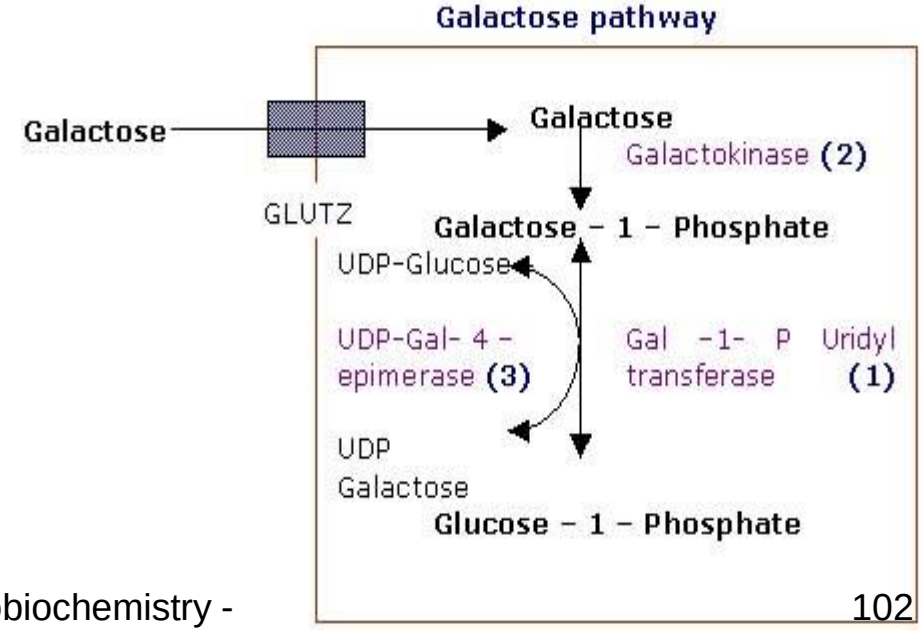
AR, incidence 1:40 000- 60 000,
Neonatal screening for galactose in some countries

Variants (Duarte)

# Galactosemia

- This is hereditary illness. In it's base lies an **blockade of galactose** metabolism. In organism intermediate metabolits accumulate. There are two the main forms of galactosemia on base of transferase insufficiency and on base of galactokinase insufficiency.



# *Deficit of glucose-1-phosphaturidyltransferase.*

- This enzyme converts galactose-1-phosphate in glucose-1-phosphate. Attached to it's insufficiency galactose-1-phosphate and sugar alcohol of galactose (galactit) **accumulates in tissues lens of the eye, liver, brain, kidneys**. Mammal and cow milk contains lactose.
- Therefore the illness symptoms appear with first days of child life.
- Diarrhea, vomiting, dehydrotation occur.
- Liver increases (splenomegalia). Hepatocytes lose ability to conjugate bilirubine. Children become yellowish.
- Affection of kidneys displays in proteinuria, aminoaciduria and acidosis.
- For galactosemia **cataract** is very typical. Their beginnings related to accumulation of osmotic active galactite in vitreous bodies of eyes. Galactite absorb in water, and water breaks tissues.
- Dangerous consequences arise in the brain. This foremost is delay of mental development.
- Mortal end is possible.
- Cure method is diet without galactose.

# 2) Galactokinase deficit

- Rare AR inherited disease, incidence 1: 200 000
- **Cause:** Lack of galactokinase, which catalyzes the conversion of galactose to galactose-1-phosphate
- **Pathogenesis**: galactose and galactitol accumulates in the lens and cause the osmotic edema
- **Clinical picture**: usually bilateral cataracts, pseudotumor cerebri
- **Therapy:** The disease is treatable with diet restricting lactate, cataracts may disappear [1]

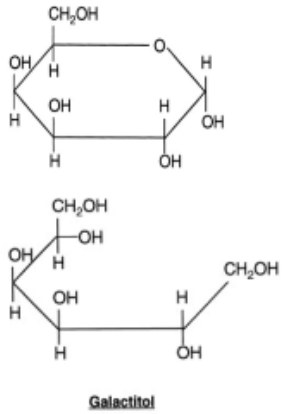
**Attached to this illness variant a process of phosphorilation of galactose is blocked, that is transformation of galactose in galactose-1-phosphat. Illness displays in cataracts. Other symptoms are absent or minor. Cure is diet without galactose.**

## Galactokinase deficiency

Cataracts - usually bilateral and detectable in the early weeks of life

Pseudotumor cerebri

**Galactitol – osmotic oedema of lens**



Galactitol

Treatable by galactose-restricted diet, cataract can resolve

Autosomal recesive, rare condition (cca 1:200 000)

# 3) Uridyldiphosphategalaktose-4-epimerase deficit

- Rare AR hereditary disease
- **Cause**: Lack of uridyldiphosphategalaktose-4-epimerase
- **Clinical picture**:
- Mild form: partial enzyme deficiencies, benign
- Severe form: vomiting, deprivation, hepatopathy neonates reminiscent of classic galactosemia, psychomotor retardation

**Severe form:**

Severe deficiency of epimerase activity

Newborns with vomiting, hepatopthy resembling classical galactosemia.
Mental retardation

**Mild form:**

Partial deficiency of epimerase deficiency
In most patients apparently benign condition

Autosomal recessive

# Hereditary metabolic disorders of complex molecules[1]

- [2] are genetically determined disorders of synthesis, transport and catabolism of macromolecules. They affect cell organelles, where their synthesis or degradation occurs, such as lysosomes and peroxisomes, or transport proteins and then manifest as disturbances of cell transport and processing. It is often cumulative diseases.
- Among macromolecules whose metabolism may be affected belong [1]: sphingolipids, **glycosaminoglycans (mucopolysaccharides),** oligosaccharides, myelin, fatty acid with a long chain etherphospholipides (plasmalogens), phytates and others.

- **Glycoproteinoses**
- **Mucopolysaccharidoses**

# Mucopolysaccharidosis

- **Hereditary disorders of lysosomal enzymes activity** (partial degradation of cellular metabolites, which accumulate intracellularly + are toxic to the organ systems: CNS, eye, bone, visceral organs)
- **Typical disproportionate growth failure with skeletal deformities**
- **mucopolysaccharides are stored in hepatocytes and Kupffer cells, enlarged liver**
- **Mucopolysaccharidoses** are **metabolic disorders** of mucopolysaccharides[1]. They are hereditary disorders of lysosomal enzymes activity (partial degradation of cellular metabolites, which accumulate intracellularly + are toxic to the organ systems: CNS, eye, bone, visceral organs). [2]
- All mucopolysaccharidoses are characterized by disproportionate disorder of growth with skeletal deformities. [1] Various mucopolysaccharides are stored in hepatocytes and Kupffer cells. The liver is enlarged. Fibrosis or cirrhosis are not rare. [3]

**Mucopolysaccharidosis type I. (Hurler syndrome**, gargoylism)

deficiency of **α-L-iduronidase**, exoglycosidase, which cleaves IdUA from non-reducing end of dermatansulphate, heparasulphate, accumulation of **dermatansulphate**, AR heredity, enlargement of the skull, thick hair, the term "Gargoyles" (low brow, broad nose, enlarged lips), blindness, deafness, severe mental retardation, deformity of the chest, hepatosplenomegaly,

**Mucopolysaccharidosis type II. (Hunter syndrome)**

deficiency of **L-iduronosulphatesulphatase** enzyme, accumulation of **heparansulphate** (GR inheritance, men)

**severe form**: faster progression and mortality in 15. year of life, heart failure, macrocephalus, malformed teeth, hepatosplenomegaly, hearing disorders, dementia, cardiomegaly, narrowing of the coronary arteries

**lightweight form**: handicapped can live up to 50 years, slowed growth, flexed posture of the fingers, retinitis pigmentosa, normal intellect, frequent impaired hearing

**Mucopolysaccharidosis type III. (Sanfilippo syndrome)**

accumulation of **heparansulphate**, dominates the affection of CNS, mental retardation, hyperactivity, aggression

**Mucopolysaccharidosis type IV. (Morquio syndrome)**

**keratansulphate** and **chondroitinsulphate** accumulation

significant affection of the skeleton

**Mucopolysaccharidosis type V. (formerly Scheie syndrome)**

mild skeletal manifestations

**Mucopolysaccharidosis type VI. (Maroteaux–Lamy syndrome)**

accumulation of **dermatansulphate**, short stature, systemic organ impairment, skeletal deformity

**Mucopolysaccharidosis type VII.**

AR inheritance, mutation of *β-glucuronidase*

# Glycoproteinoses

- usually AR heredity
- Symptoms are similar to mucopolysaccharidoses, but there neither cummulation of mucopolysaccharides neither nor mucopolysachariduria
- fragments of glycoproteins are present in urine
- leads to lysosomal distention and secondarily induced increased activity of lysosomal enzymes

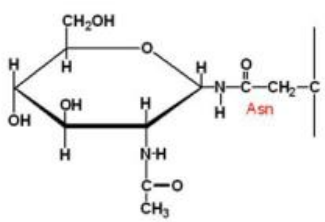
# Glycoproteins

- are proteins that have a central chain with covalently bound oligosaccharides
- the weight proportion of carbohydrates in the molecule is from 1% to 85%
- saccharide units unlike glycosaminoglycans do not alternate regularly
- have predominantly neutral character
- very frequent saccharides are fucose and sialic acid
- have different functions, such as antigens, enzymes
- are a standard part of membranes, have catalytic functions, are carriers of immunological specificity, are part of the mucus and also extracellular matrix
-

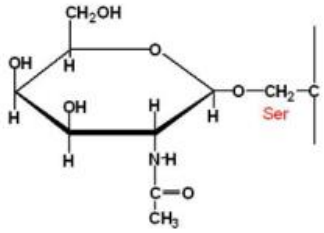
N-glycosylation

O-glycosylation

**Disorders of glycosylation:**
CDGs (previously known as carbohydrate-deficient glycoprotein syndromes)




N-glycosylation
Asn-X-Ser/Thr




O-glycosylation
Thr, Ser

**carrier protein is synthesized on the rough ER, in the GA saccharides are bound to it in two ways:**
**O-glycosidic bond to OH group of serine or threonine protein by N-acetylglucosamine saccharide chain**
**N-glycosidic bond to $NH_2$ group of asparagine protein by N-acetylglucosamine, to which saccharide chain has been transferred from dolicholpyrophosphte carrier degradation in lysosomes by endoglycosidases (fucosidase, aspartylglukosaminidase) and exoglycosidases (galactosidase, neuraminidase, hexosaminidasa, mannosidase)**

# Selective disorders of N-glycosylation

**CDG-Ia, phosphomannomutase 2 deficiency**

Retracted (inverted) nipples, an abnormal distribution of fat, strabismus, cerebellar hypoplasia, facial dysmorphia, convulsions and some level of psychomotor retardation

Stroke-like episodes, peripheral neuropathy, skeletal abnormalities

*Clinical course in three stages*

1. multisystem disease in infancy
2. ataxia and mental retardation in late infancy and childhood
3. stable disability in adulthood

**CDG-Ib, phosphomannose isomerase deficiency**

Phosphomannose isomerase: step in the synthesis of GDP-mannose: fructose-6-P→ mannose-6-P

diarrhea and cyclical vomiting, severe hypoglycemia, failure to thrive, hepatic fibrosis, enteropathy with proteins losses, coagulation disorders, without neurological impairment

**Treatment:** mannose 1g/kg/day (hexokinase: mannose → mannose-6-P)

**CDG-Ic**

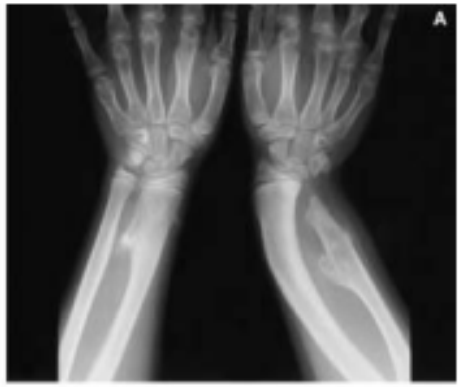
Symptoms of CDG-Ic are similar to those of CDG-Ia but much less severe. Patients have frequent seizures, psychomotor retardation that is milder than in CDG-Ia, pronounced axial hypotonia, and strabismus.

# Disorders *o*-linked glycosylation

A number of rare disorders with highly variable clinical presentation

Examples:

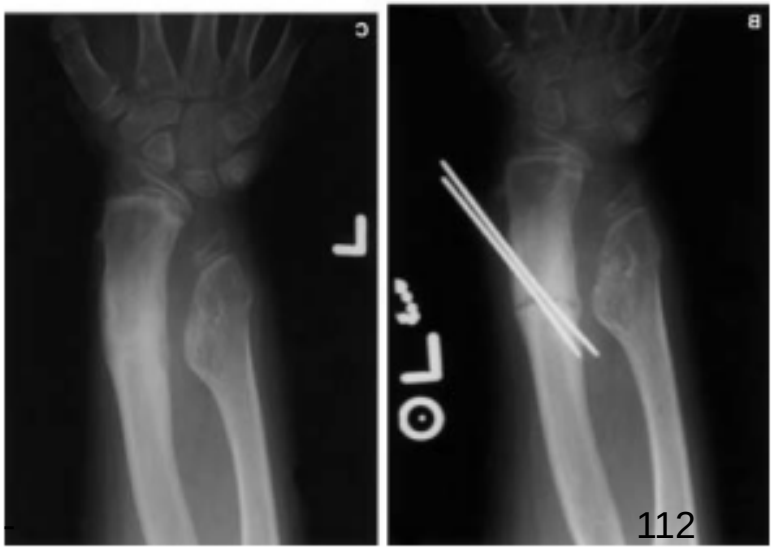
**α-Dystroglycanopathies** – a group of disorder that adds O-mannose-linked glycans to α-dystr
Congeninal muscular dystrophies



**X -linked paroxysmal nocturnal hemoglobinuria**
Defect in synthesis of GPI-anchor (gene PIG-A)

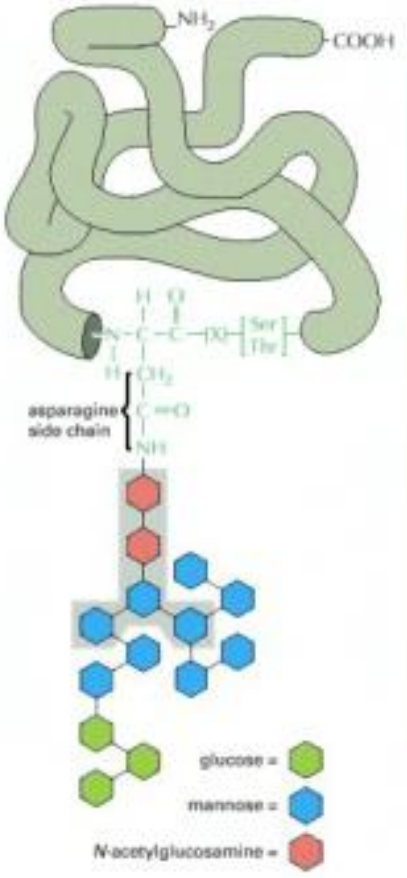
**Walker-Warburg syndrome**
brain and eye malformations, muscular dystrophy
defect in synthesis of mannosylated *O*-linked oligosaccharides

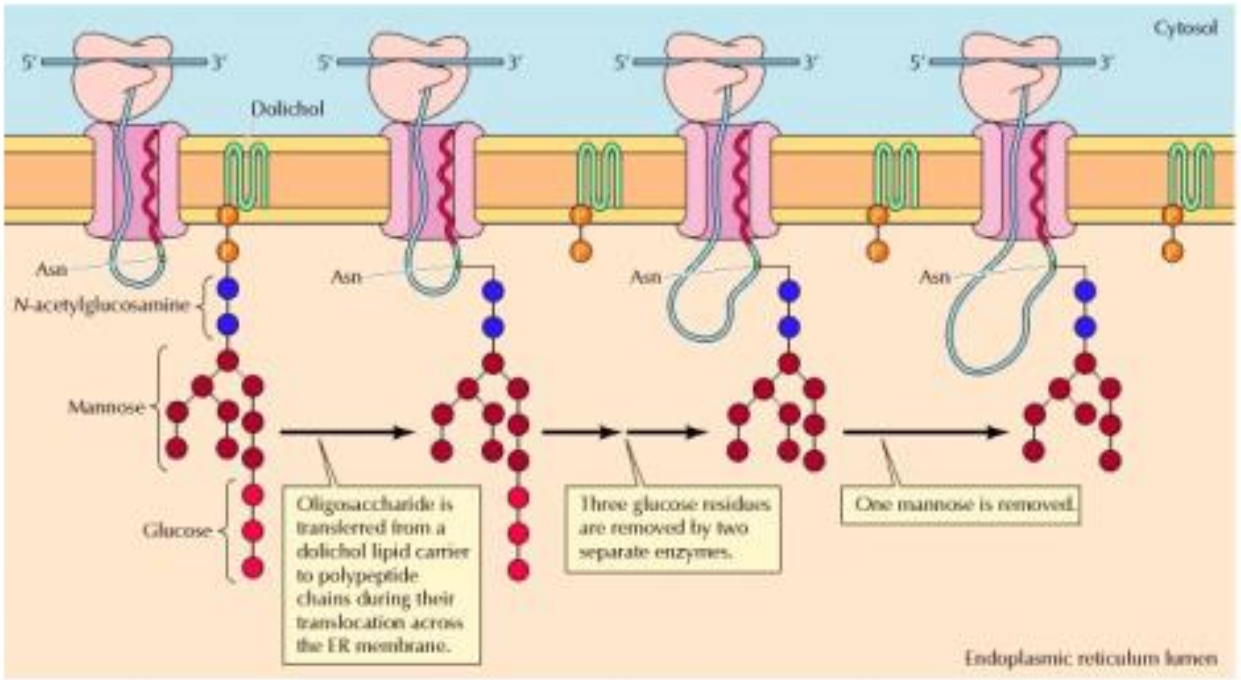
**Hereditary multiple exostoses**
Dominant disorder
deficiency of two glucosyltranferases that function in synhesis of heparan sulfate

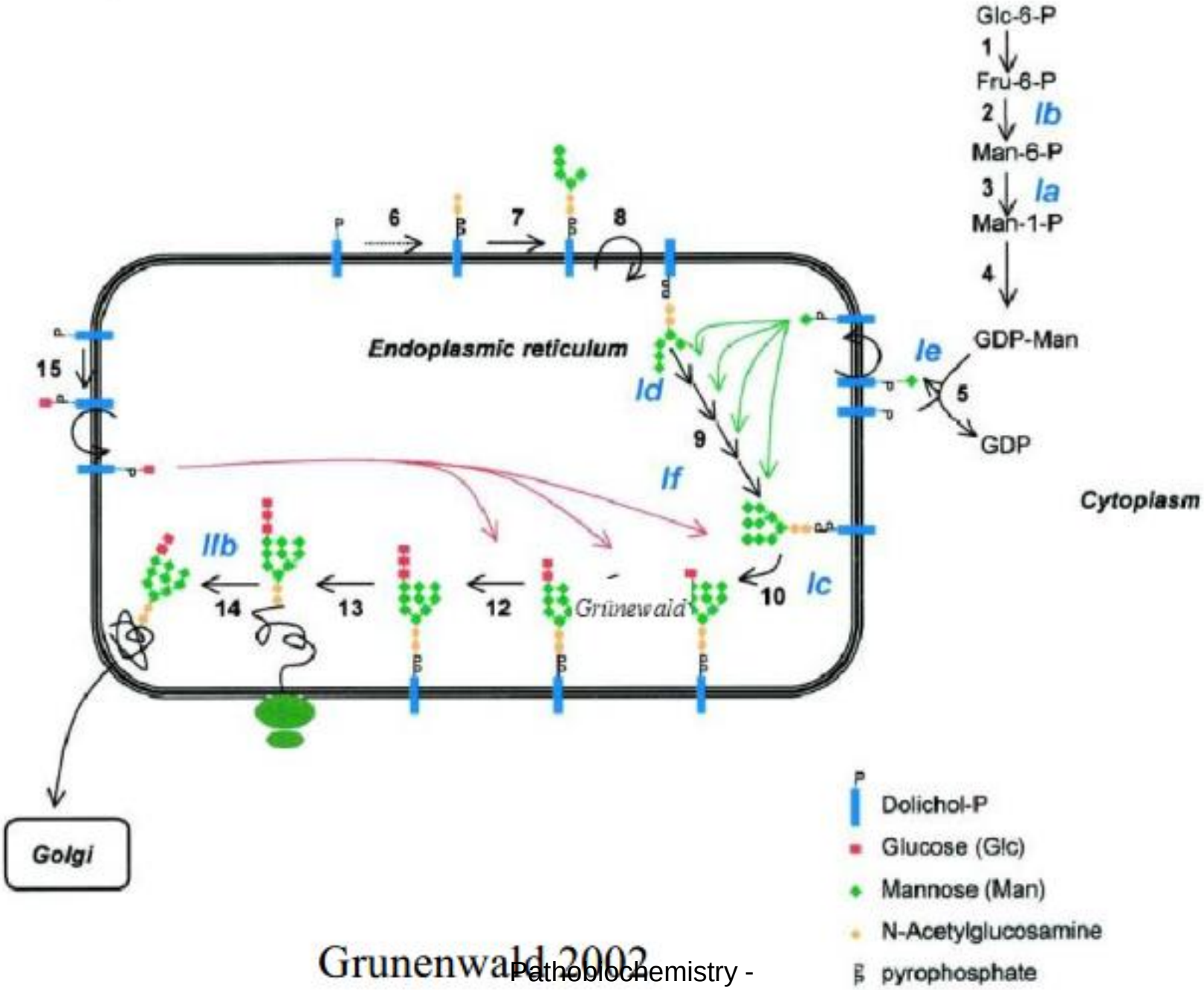


Most Proteins Synthesized in the Rough ER Are Glycosylated by the Addition of a Common N-linked Oligosaccharide

Precursor oligosaccharide is held in the ER membrane by dolichol,






Grunenwald 2002

- **Mucolipidosa I (Sialidosa)**
- deficit of sialidase (neuraminidase)
- **Normosomatic form**
- massive myoclonus (spasmodic twitching of muscles) induced by emotion and movement, red stains on the fundus
- **Dysmorphic form**
- dysmorphia with Hurleroid features
- **Mucolipidose II (Inclusion Disease, I-cell disease)**
- GlcNAc-phosphotransferase mutations
- abnormal transfer of GlcNAc-1-phosphate into newly synthesized lysosomal enzymes, and thus these enzymes are missing Man-6-phosphate, which would send them to the lysosomes
- hydrolases missing in lysosomes, material accumulates therein, which gives rise to an inclusion bodies
- Clinical manifestations as dysmorphia of Hurleroid type
- **Mannosidosa**
- deficit of acid a-mannosidase
- expressive facial dysmorphia
- opacity of the lens, cataracts of the lens
- skeletal disorders
- **Fukosidose**
- deficiency of a-L-fucosidase
- clinically manifests after the first year by the initial hypotonia, mental and motor retardation
- often ends by Abnormal decerebral rigidity before 6 years of age
- mild facial dysmorphia

# Hereditary disorders of sugar metabolism

## Diseases caused by defects in the synthesis of N-glycans

**Congenital disorders of glycosylation** Congenital disorders of glycosylation (CDG) are diseases, whose cause lies in defective enzymes that are involved in the synthesis of oligosaccharide chains of glycoproteins. These diseases include diseases with impaired N- glycosylation, O- glycosylation, combined disorders N-a O- glycosylation, and disorder of lipid glycosylation. The most common cause CDG is defective synthesis of N-glycan. So far is known 21 enzymes in the synthesis of N-glycan that may be defective. The term N-glycan is used for N-linked oligosaccharides and polysaccharides.

- There was discovered over 20 types of congenital disorders of glycosylation.
- It is expected that most CDG will have been discovered.
- Congenital disorders of glycosylation are divided into two groups– I a II, by defect pathways.
- Each of these two groups includes a subset of another, by a defective enzyme.
- **Type I CDG – assembly** disorder including disorders of dolicholphosphate producing
- **Type II CDG** – disrorder of **transport** (processing)

## Glucosa-6-phosphate dehydrogenase deficiency

Glucose-6-phosphate dehydrogenase deficiency (G6PD deficiency) or fabismus or favismus worldwide are among the most common enzyme defects.
G6PD deficiency increases the sensitivity of erythrocytes to oxidative stress.
Clinically, it manifests neonatal jaundice, acute hemolysis and rarely chronic hemolytic anemia.
People with this disease may be asymptomatic in some cases.

It is an X-linked inherited disease that occurs primarily in Africa, Asia, Mediterranean and Middle East.
The number affected is estimated at 400 million people.
There are known various types of genetic mutations in the G6PD gene (Xq28, OMIM: 305900) responsible for different types of G6PD with differently severe clinical manifestations.[1][2]