Introduction to glucose breakdown and synthesis

Carbohydrates, one of the main nutrients of heterotrophic organisms, are found in every cell of our body, where they perform a number of functions - a **source of energy** for cells, a source of **carbon atoms** for the synthesis of substances, ' "reserve" form of chemical energy (glycogen) or ""structural function"" (proteoglycans).

Glucose

Glucose (Glc) is a **universal energy substrate'** ***- the oxidation of one gram of glucose yields approximately 17 kJ, i.e. 4 kcal. The fact that our cells can get energy from it even in the absence of*** O_2 ***is of considerable importance. This distinguishes glucose from other nutrients. Some cells, e.g. erythrocytes or CNS cells, even strictly depend on glucose. The*** *pyruvate dehydrogenase reaction'* ***(PDH) is*** *irreversible* and therefore glucose cannot be synthesized from fatty acids. Excess carbohydrates, on the other hand, can be converted by our body through acetyl-CoA into fatty acids and subsequently into triacylglycerols (TAG). Glucose consumption

Glycemia indicates blood glucose concentration. Its normal fasting level is ``3.3-5.6 mmol/l*, but after a meal it can temporarily rise to 7.0 mmol/l. Under physiological circumstances, glucose is not excreted in the urine. If the blood glucose exceeds the value of* ***10 mmol/l'*** *(the so-called* renal threshold *for glucose), glucose appears in the definitive urine - we then speak of* ***[[Glucose in the urine|glycosuria]]****.*

Glucose is found in food either free or as part of disaccharides or polysaccharides. Only free glucose is absorbed from the digestive tract into the blood.

Sources of Blood Glucose

1. Carbohydrates from '*food* (digestion of carbohydrates in the GIT), or conversion of other nutrients from food into glucose.
2. **Decomposition of liver glycogen** - used between meals. Liver glycogen stores are enough for about 24 hours.
3. **Gluconeogenesis from C_3 and C_4** substances (lactate, glycerol, most amino acids) is a source of glucose during long-term fasting or under pathological conditions. During long-term starvation, the share of energy drawn from glucose in the total consumption of the organism is only 20%, the greater part of the energy is obtained by oxidation of lipids.

Blood Glucose Recipients

1. Consumption by '*glucose-dependent* tissues such as brain, erythrocytes - independent of insulin.
2. Consumption by tissues that are **not dependent on glucose'*****, and can therefore also use other energy substrates, e.g. skeletal muscles -*** *dependent on insulin*.
3. **Glycogen synthesis** in the liver, muscles and other tissues.
4. **Excess *glucose is*** *transformed* ***into*** *fatty acids* ***and*** *TAG* - storage mainly in adipose tissue.
5. Formation of many important substances (other monosaccharides, monosaccharide derivatives, etc.).

Carbohydrates are metabolized in the form of ``phosphoric esters*. The key substance in carbohydrate metabolism,* ***glucose-6-phosphate'*** *(Glc-6-P), represents the link of many metabolic pathways: glycolyses, gluconeogenesis, pentose cycle, glycogenesis and glycogenolysis. At the same time, it keeps glucose in the cells, as this derivative does not pass through the cell membrane. By phosphorylating glucose, the concentration gradient of glucose between the extracellular and intracellular spaces is still maintained, which facilitates the further entry of glucose into the cell.*

Glycolysis is used by higher organisms as the main way of breaking down carbohydrates, while the pentose cycle is used as an additional way.

Mechanism of glucose transport across the cell membrane

Glucose can be transported across the membrane by two mechanisms:

- by **facilitated diffusion** using **GLUT** transporters;
- by **secondary active transport'** through **SGLT** transporters.

A number of glucose transporters operating on the principle of "facilitated diffusion" are found in cell membranes. This is a passive process, during which glucose molecules are transferred along their concentration gradient with the help of the transporter ``GLUT 1-7 (GLUcose Transporter)*. Of these, only GLUT 4 depends on insulin levels. After binding to its receptor, insulin increases the number of GLUT 4 transporters in the membrane of the respective cells.* GLUT 2 and SGLT 1/2

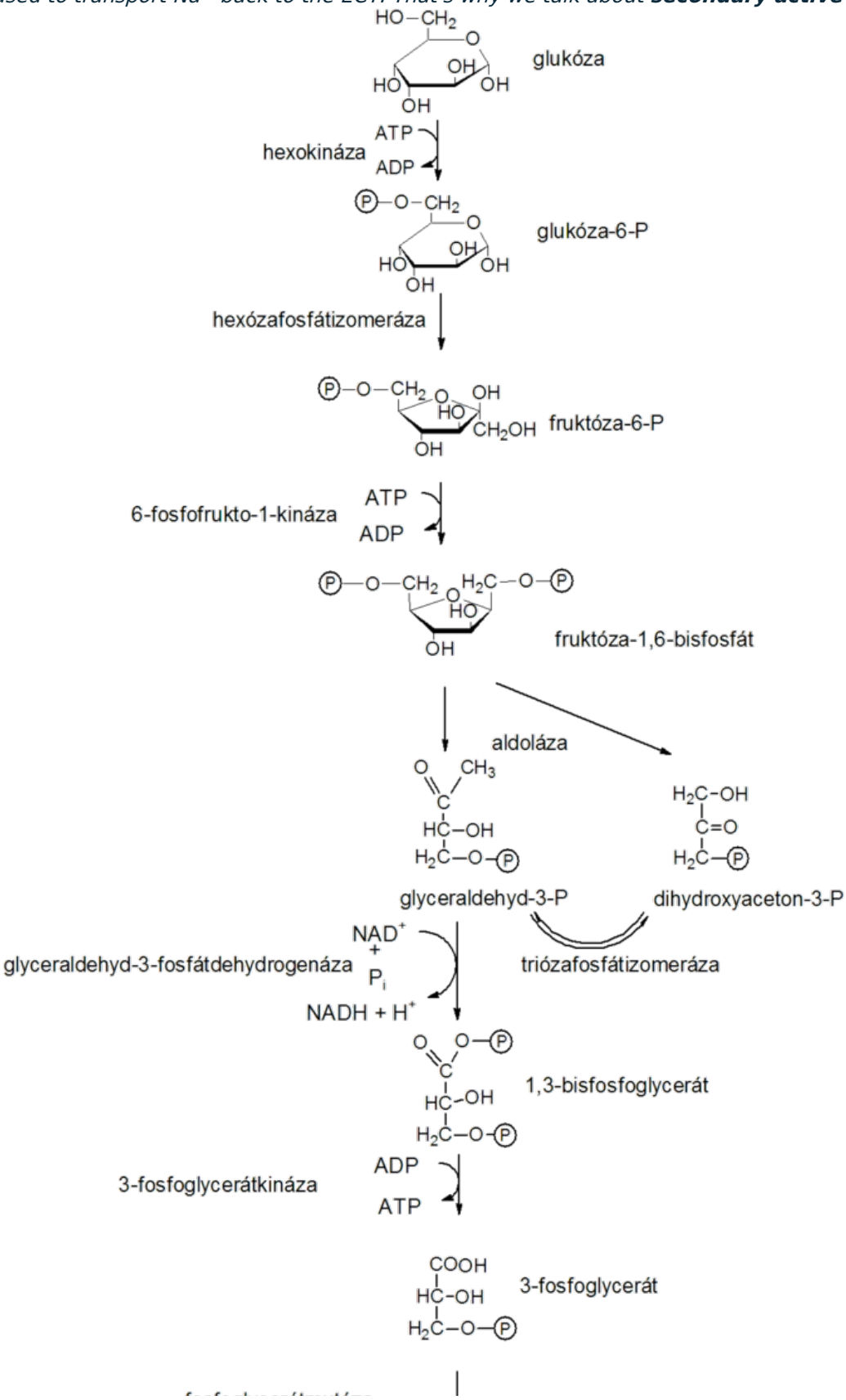
Here is a basic overview of GLUT transporters:

- **GLUT 1** and **3** - serve to maintain basal glucose uptake by tissues whose metabolism is dependent on glucose

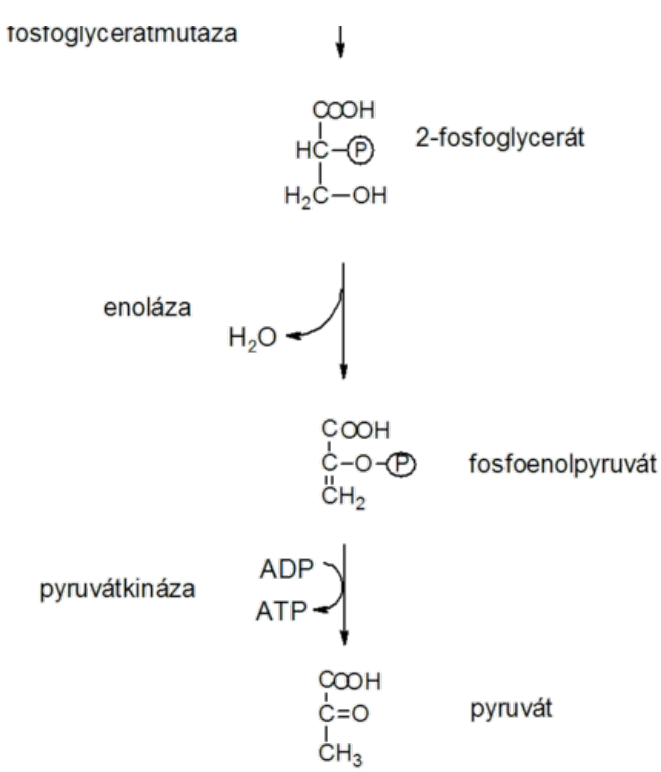
(brain, erythrocytes, but also kidneys and placenta).

- **GLUT 2** - located on the membrane of β-cells of the pancreas and hepatocytes, also enables the transfer of glucose from the resorptive epithelia (proximal kidney tubule, intestinal enterocytes) into the blood.
- **GLUT 4** - is a glucose transporter in the so-called *insulin-dependent tissues* - skeletal muscle, myocardium and adipose tissue. Its exposure on the membrane conditions the presence of higher levels of insulin in the blood - the carriers are prepared in vesicles, and after the binding of insulin to the receptor, the vesicles fuse with the cell membrane. This happens especially after a meal, when the mentioned tissues are responsible for the metabolism of up to 80% of glucose from the blood. In the period between meals, on the contrary, they do not absorb it and save it for tissues dependent on it.

In enterocytes and cells of the proximal tubule of the kidney, glucose is absorbed from the lumen by ``active transport*. Glucose transport is provided by cotransport with Na^+. Glucose molecules go against their concentration gradient into the cell. Energy will be provided by Na^+ ions, which pass into the cell along its concentration gradient. This transfer is made possible by the so-called* SGLT-1 and 2 *(Sodium-Glucose Transporter). ATP - Na^+/K^+-ATPase is used to transport Na^+ back to the ECT. That's why we talk about* ***secondary active transport*****.** __NOTOC_



Glycolysis (or



the ``Embden-Meyerhof-Parnas pathway*) is a basic metabolic process taking place in the cytoplasm of all cells of the human body. It ranks among catabolic pathways. Glycolysis produces two three-carbon molecules from one glucose molecule - pyruvate (Pyr) or lactate (Lac). Glycolysis fulfills many functions, for example the gain of energy or the formation of acetyl-CoA as a substrate for the synthesis of lipids.*

Glycolysis takes place under both ``aerobic *and* ``*anaerobic conditions*. Under aerobic conditions, two molecules of pyruvate, two molecules of ATP and two molecules of NADH are produced. Under anaerobic conditions, pyruvate undergoes another reaction that regenerates the cofactor NAD^+ - the product is then lactate.

Glycolysis reaction

The entire glycolysis can be summarized as an equation:

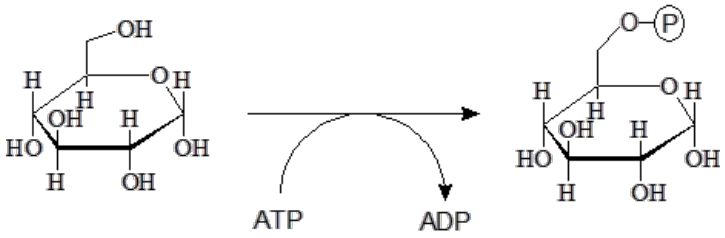
Glucose + 2 NAD^+ + 2 ADP + 2 P_i → 2 pyruvate + 2 NADH + 2 ATP + 2 H_2O

Glycolysis is divided into several phases:

1. Investment of energy and simultaneous activation of glucose molecules.
2. Cleavage of a hexose into two trioses.
3. Oxidation of triose and simultaneous energy gain.
4. Conversion of pyruvate to lactate (under anaerobic conditions).

In the following overview, we will describe her individual reactions:

1. Phosphorylation of glucose



After glucose molecules enter the cells, their immediate phosphorylation occurs. This reaction converts the neutral glucose molecule into an anion. Glucose modified in this way can be further metabolized and at the same time no longer "passes through the cell membrane". It is thus captured in the cytosol, where it is further metabolized.

Glucose + ATP → Glc-6-P + ADP

In addition to one macroergic bond of the ATP molecule, the reaction also requires enzyme catalysis mediated by one of the two isozymes - "hexokinase" or "glucokinase".

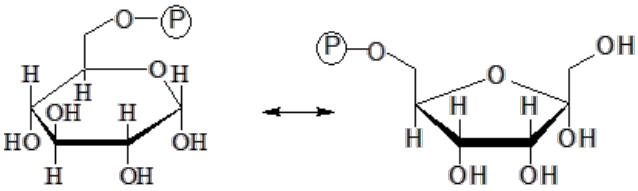
Glucokinase (or hexokinase type IV) is localized only in hepatocytes and pancreatic β-cells, while hexokinase is in all tissues. In addition to localization, they also differ in their physico-chemical properties. Glucokinase has a high ``K_M **value (10 mmol/l) and is therefore only activated at higher glucose concentrations.**

It is mainly used after a meal, when the concentration of glucose in the portal blood is high and it needs to be taken up by the liver (e.g. for glycogen synthesis). At the same time, β-cells of the pancreas respond to higher blood glucose levels by increasing insulin secretion.

'*Hexokinase **is always almost fully active under physiological conditions, as its*** K_M is only 0.1 mmol/l (compare with the physiological range of glycemia 3, 3–5.6 mmol/l). Its activity is thus regulated by a different mechanism, and that is the inhibition by its own product – Glc-6-P. Simply put, hexokinase produces as much Glc-6-P as the cell is able to utilize in its pathways. Once Glc-6-P starts to accumulate, hexokinase inhibition occurs. In addition to the phosphorylation of glucose, hexokinase also enables the phosphorylation of fructose.

2. Isomerization of Glc-6-P to Fru-6-P

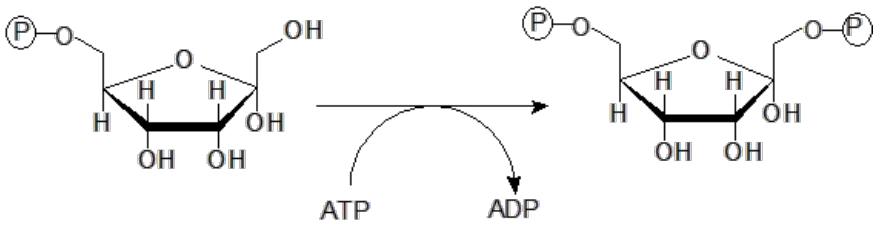
Isomerization of Glc-6-P to Fru-6-P is a reversible reaction catalyzed by ``hexosaphosphate isomerase*.*



3. Phosphorylation of Fru-6-P under ATP consumption to Fru-1,6-bisP

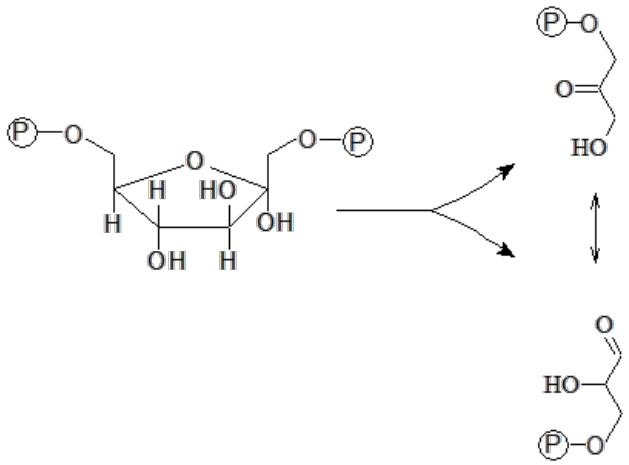
Phosphorylation of Fru-6-P to Fru-1,6-bisP is catalyzed by the enzyme ``6-phosphofructo-1-kinase*. It is a key allosteric regulatory enzyme of glycolysis.*

During the three steps so far, two molecules of ATP have been invested for one molecule of glucose.



4. Aldol cleavage of Fru-1,6-bisP into two phosphorylated trioses

Fru-1,6-bisP is subsequently split into two phosphorylated trioses – glyceraldehyde-3-P (Gra-3-P, aldose) and dihydroxyacetone-3-P (DHA-3-P, ketose). Catalysis is provided by *aldolase* belonging to the class of lyases. We distinguish between its two isozymes - *aldolase A and B*.

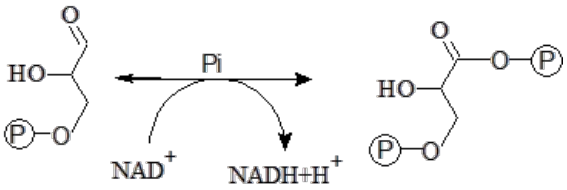


5. Isomerization of trioses

Glyceraldehyde-3-P and dihydroxyacetone-3-P can be converted into each other by the enzyme ``triose phosphate isomerase*. This reaction is of great importance, because only glyceraldehyde-3-P is involved in the next reaction of glycolysis, and this isomerization continuously replenishes its cytosolic pool.*

6. Oxidation of glyceraldehyde-3-P to 1,3-bisphosphoglycerate

This reaction is the only oxidation reaction in all of glycolysis. The oxidation is catalyzed by ``glyceraldehyde-3-phosphate dehydrogenase*. The reaction produces 1,3-bisphosphoglycerate (an energy-rich compound) and a reduced cofactor – NADH+H^+. The reaction is exergonic – P_i binds to the newly formed COO– group by oxidation via a macroergic anhydride bond.*



7.Conversion of 1,3-bisphosphoglycerate to 3-phosphoglycerate

1,3-bisphosphoglycerate is hydrolyzed to 3-phosphoglycerate by ``phosphoglycerate kinase*. At the same time, substrate phosphorylation will occur (phosphorylation at the substrate level) - ATP is formed from ADP.*

8. Isomerization of 3-phosphoglycerate to 2-phosphoglycerate

Isomerization is catalyzed by ``phosphoglycerate mutase**.**

9. Dehydration of 2-phosphoglycerate to phosphoenolpyruvate (PEP)

Dehydration of 2-phosphoglycerate is catalyzed by the enzyme "enolase". The reaction leads to the formation of the macroergic compound phosphoenolpyruvate, which contains an ester-bound phosphate group.

10. Conversion of phosphoenolpyruvate to pyruvate

P_i cleavage takes place first, then the unstable enol-pyruvate isomerizes to the more stable keto-pyruvate. A large amount of free energy is released during this transformation. This reaction is therefore strongly exergonic and practically irreversible. The released energy is used to synthesize ATP from ADP – substrate phosphorylation.

The reaction is catalyzed by the regulatory enzyme "pyruvate kinase".

During the 4th-10th reactions produced **two molecules of ATP'** per one three-carbon fragment (Pyr). The energy balance of the entire glycolysis is thus +2 moles of ATP per 1 mole of glucose (−2 ATP consumed, +4 ATP created).

Metabolic fates of pyruvate

Pyruvate is the branch point of glycolysis. The fate of pyruvate depends on the oxidation state of the cell – NADH must be reoxidized to NAD^+.

Under ``aerobic conditions*, pyruvate is transported to the matrix of the ``mitochondria*, where it is converted to acetyl-CoA via the pyruvate dehydrogenase reaction, which can participate, for example, in the Krebs cycle. The reduced cofactor NADH cannot simply transfer to the matrix mitochondria, where it should be involved in processes in the respiratory chain, because the mitochondrial membrane is impermeable to it. That is why it is used to reduce some substances – for example, cytoplasmic oxaloacetate to malate or dihydroxyacetone-P to glycerol-3-P. The resulting products already pass through the inner mitochondrial membrane and thus transport reducing equivalents into the mitochondrion. We are talking about the so-called *shuttle mechanism* or simply shuttles. For the transfer of NADH, there are two different shuttles in the cell - "glycerol-phosphate" and "malate-aspartate". In the mitochondrion, the above reactions take place in the opposite direction:

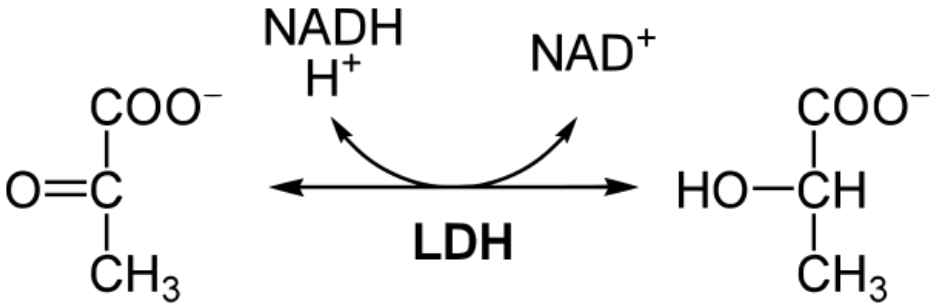
Malate + NAD^+ → oxaloacetate + $NADH+H^+$

Glycerol-3-P + FAD → dihydroxyacetone-P + $FADH_2$

The obtained reduced cofactors can subsequently enter the mitochondrial *respiratory chain*, where they are regenerated - ATP is simultaneously produced by aerobic phosphorylation. The return of oxaloacetate (OAA) back to the cytosol is not direct. It first requires a **transamination to aspartate'** catalyzed by aspartate aminotransferase (AST)**. The opposite reaction takes place in the cytosol - OAA is formed.**

Under ``anaerobic conditions *(e.g. an intensively working muscle with insufficient oxygen supply) or in erythrocytes, pyruvate is transformed into ``lactate*, which is subsequently released from the cell into the bloodstream. At the same time, NAD^+ regeneration occurs. The reaction is catalyzed by the enzyme lactate dehydrogenase (LDH):

Pyruvate + NADH + H^+ → lactate + NAD^+



The NAD^+ created by this reaction is a coenzyme for glyceraldehyde-3-phosphate dehydrogenase, without which glycolysis would stop. The resulting lactate can either participate in the Cori cycle or be oxidized in tissues with aerobic metabolism (heart, liver) to CO_2 and H_2O. Accumulation of lactate causes a drop in pH, which causes muscle pain and fatigue.

At this point it should be recalled that aerobic glycolysis produces much more ATP per 1 mole of glucose than anaerobic glycolysis.

2,3-BPG shunt

In ``erythrocytes*, a branch of glycolysis called the 2,3-BPG shunt plays an important role. 1,3-bisphosphoglycerate is converted to 2,3-bisphosphoglycerate. This intermediate product no longer contains macroergically bound phosphate, and during its further conversion to 3-phosphoglycerate, no ATP is synthesized – only inorganic phosphate is released. The erythrocyte thus obtains less ATP during this course of glycolysis. But the significance of the detour lies in the fact that **2,3-bisphosphoglycerate reduces the affinity of hemoglobin to oxygen**', i.e. it participates in the regulation of oxygen transport to hemoglobin.*

Regulation of glycolysis

The regulatory points in glycolysis are three enzymes:

1. 6-phosphofructo-1-kinase;
2. pyruvate kinase;
3. hexokinase.

These enzymes catalyze irreversible exergonic reactions.

6-phosphofructo-1-kinase (PFK-1)

Phosphofructokinase, an allosteric enzyme regulated by several activators and inhibitors, is the main regulatory point of glycolysis:

1. **Increasing the ATP / AMP ratio leads to inhibition of glycolysis**

   Glycolysis is a process leading to the production of ATP.
   ATP is a substrate and at the same time an allosteric inhibitor of this enzyme. AMP, on the other hand, acts as an enzyme activator. With an excess of ATP, further consumption of glucose as a nutrient stops.

2. **Citrate inhibits glycolysis**

   When fatty acids are oxidized, the resulting acetyl-CoA inhibits PDH.
   The resulting pyruvate goes into carboxylation to oxaloacetate. If there is enough acetyl-CoA and oxaloacetate at the same time, citrate is synthesized, which accumulates in front of the enzyme isocitrate dehydrogenase. **Citrate escapes into the cytoplasm** where it blocks the regulatory enzyme of glycolysis. It signals that there are enough substrates of the Krebs cycle in the mitochondrion, and therefore there is no need to create more.

3. **Fructose-2,6-bisphosphate (Fru-2,6-P)**

   Fructose-2,6-bisphosphate, an activator of glycolysis, acts as an extended arm of insulin - its concentration increases if the insulin / glucagon ratio is increased. It arises from fructose-6-P by a reaction catalyzed by *6-phosphofructokinase-2 (PFK-2)*.

4. **Glycolysis is activated by insulin and inhibited by counterregulatory hormones**

   Increased insulin/glucagon ratio decreases intracellular cAMP concentration; this results in a predominance of dephosphorylation events. A decrease in the ratio and the action of other counterregulatory hormones will, on the contrary, cause an increase in the concentration of cAMP - phosphorylation events prevail. 6-phosphofructo-1-kinase is active in the dephosphorylated form.

5. **Acid pH inhibition**

   6-phosphofructo-1-kinase is inhibited by protons. Both pyruvate and lactate are relatively strong acids and their significant accumulation could endanger the cell. Therefore, their increased concentrations lead to the inhibition of the regulatory enzyme through protons.

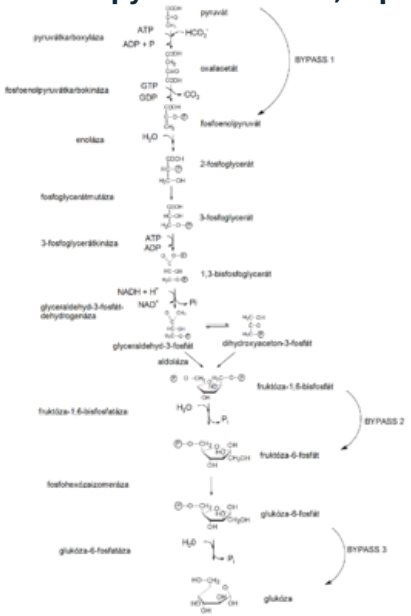
The remaining enzymes are of minor importance, so we will only describe them very briefly. As we have already mentioned, hexokinase is inhibited by its product – Glc-6-P, and activated by insulin. Pyruvate kinase is regulated by covalent modifications under the influence of the insulin / glucagon ratio.

Clinical Correlations

Relatively common enzymatic defects include "congenital pyruvate kinase deficiency". Mainly erythrocytes are affected, in which less ATP is formed. This results in their lower ability to maintain the integrity of their membrane (active membrane transport) and their shape. The consequence is their increased breakdown - hemolytic anemia develops. In case of insufficient oxygenation of tissues **(hypoxia)**, **lactate** is formed to an increased extent, which, as a relatively strong acid, causes acidification of the body - this drop in pH is called **[[metabolic acidosis|lactic acidosis]]**.

Gluconeogenesis is the process of creating glucose molecules from substances of a non-carbohydrate nature. Precursors are mainly **three- and four-carbon substances**' - e.g. lactate, pyruvate, glycerol, alanine, glycine and other glucogenic amino acids or propionate (mainly in ruminants). Gluconeogenesis is localized both in the matrix mitochondria and in the cytosol, mainly in liver cells, kidney tubular cells and enterocytes. Thanks to gluconeogenesis, we can survive even longer starvation, because glycogen reserves are exhausted within approximately 24 hours of starvation. However, it is activated already in the morning after a night's fasting.

The process of gluconeogenesis could take place simply by reversing the glycolysis. However, some reactions of glycolysis are irreversible and must be bypassed in gluconeogenesis with the use of different enzymes. We are talking about the so-called *bypasses 1, 2 and 3*. The three irreversible reactions in glycolysis are catalyzed by three kinases: **pyruvate kinase, 6-phosphofructo-1-kinase and hexokinase/glucokinase**.



Gluconeogenesis reaction

In the following overview, we will describe the individual reactions of gluconeogenesis:

1. Bypass 1 – conversion of pyruvate to phosphoenolpyruvate

The first step is the transport of pyruvate into the mitochondrial matrix. This is followed by its carboxylation to oxaloacetate catalyzed by ``pyruvate carboxylase *with simultaneous consumption of ATP (the cofactor is carboxybiotin). Oxaloacetate is then transported from the mitochondrion to the cytosol (transamination to Asp or reduction to malate), where the enzyme* phosphoenolpyruvate carboxykinase *converts it to phosphoenolpyruvate (GTP is consumed).*

2. Reactions 3–8 are essentially the reverse form of glycolysis and take place in the cytosol

3. Bypass 2 – conversion of Fru-1,6-bisP to Fru-6-P

Fructose-1,6-bisphosphatase hydrolyzes Fru-1,6-bisP to Fru-6-P. This reaction represents a key regulatory step in gluconeogenesis.

4. Fru-6-P isomerizes to Glc-6-P

5. Bypass 3 – conversion of Glc-6-P to free glucose

Glucose-6-phosphatase hydrolyzes Glc-6-P to free glucose – thus catalyzes the cleavage of phosphate. This enzyme is bound to the membranes of the smooth endoplasmic reticulum. Glc-6-P is transported to the ER by the enzyme translocase. This separation into the ER serves to ensure that the resulting glucose is not immediately rephosphorylated to Glc-6-P. Free glucose is then released into the blood, where it can serve as a

source of energy.

Energy balance of gluconeogenesis

Gluconeogenesis is an energy-intensive process – it consumes six macroergic phosphates per one molecule of glucose. In summary, we can express it by the following equation:

$$\mathbf{2\ Pyr + 4\ ATP + 2\ GTP + 2\ NADH + 4\ H_2O \rightarrow Glc + 4\ ADP + 2\ GDP + 6\ P_i + 2\ NAD^+ + 2\ H^+}$$

Substrates for gluconeogenesis

Lactate

Lactate, one of the main sources of carbon atoms in the process of gluconeogenesis, is formed during **anaerobic glycolysis'** from pyruvate by a reaction catalyzed by **lactate dehydrogenase'** (LDH). Its main producers are working muscle cells and erythrocytes. From them, lactate is released into the bloodstream, which takes it to the liver, where it is converted into glucose. Glucose is then released into the blood, from where the aforementioned cells can obtain it again. With this, we closed the so-called **Cori cycle**.

Pyruvate

Pyruvate can be produced by many peripheral tissues. At this point, we will describe the so-called *glucose-alanine cycle*, which takes place between muscle cells and the liver. After pyruvate is formed in muscle cells, it undergoes transamination to form alanine. It is released into the blood, which transports it to the liver, where alanine is converted back into pyruvate by transamination, which can be involved in gluconeogenesis. The resulting glucose is transferred by blood to the muscles and the whole cycle is closed.

Glucose-alanine cycle

Glucogenic amino acids

The carbon skeletons of all amino acids **except leucineu and lysineu** can be a source of carbon atoms for the process of gluconeogenesis. Alanine and glutamine are the main ones. The exact mechanism of their involvement is beyond the scope of this tutorial. The main source of glucogenic amino acids is muscle proteins.

Glycerol

Glycerol obtained during the **hydrolysis of triacylglycerols'** can be used as a substrate for gluconeogenesis. The first step is its phosphorylation to glycerol-3-P by glycerol kinase. This is followed by its dehydrogenation to dihydroxyacetone-P catalyzed by glyceraldehyde-3-phosphate dehydrogenase, which gives rise to an intermediate of gluconeogenesis.

Energy

Energy for gluconeogenesis is mainly obtained from **β-oxidation of fatty acids** - during starvation, fatty acids are released from the stored triacylglycerols of adipose tissue and are metabolized in the liver.

Regulation of gluconeogenesis

Gluconeogenesis is a metabolic pathway that is activated mainly during **starvation'** or in **pathological conditions** (stress due to infection, polytrauma, etc.).

Gluconeogenesis regulatory enzymes are those that bypass the irreversible reactions of glycolysis:

1. **Pyruvate carboxylase** - it is activated by acetyl-CoA originating, for example, from β-oxidation of fatty acids.
2. **PEP carboxykinase, Fru-1,6-bisphosphatase'** and **Glc-6-phosphatase** - they are regulated by the same influences as glycolysis reactions, only in the opposite direction. For example, Fru-1,6-bisphosphatase is activated by citrate, while AMP or Fru-2,6-bisP shows an inhibitory effect.

In addition to the activity of regulatory enzymes, an important factor determining the effectiveness of gluconeogenesis is the supply of its substrates, which are produced, for example, by proteolysis or lipolysis.

Counterregulatory hormones (glucocorticoids, glucagon or catecholamines) enhance gluconeogenesis, insulin, on the contrary, inhibits it.