BIOCHEMISTRY **1st Year, 2nd Semester**

Prof. Catalin MARIAN, MD PhD Dr. Habil.
Email: cmarian@umft.ro

Lecture 3

II. CARBOHYDRATE METABOLISM

II.1. Digestion and absorption of carbohydrates

Carbohydrates from food (300-500 grams/day) are mainly composed of 50% starch, 40% sucrose, 5-10% lactose and other saccharides in small amounts. During digestion, the oligo- and poly-saccharides are enzymatically hydrolyzed into glucose, fructose and galactose, these being mono-saccharides capable of passing through the enterocyte cell membrane. The hydrolysis is realized by enzymes from the class of hydrolases called amylases. There are two types of amylases: salivary (secreted by salivary glands) and pancreatic (secreted by the exocrine part of the pancreas).

The digestion process begins in the oral cavity where the salivary amylase will hydrolase part of the glycosidic bonds within poly-saccharides such as the alpha-1,4 glycosidic bonds found in starch. Because of the short time spent in the oral cavity, the action of salivary amylases is limited and the hydrolysis products will be dextrines, maltotriose, maltose. The salivary amylase is inactivated by the strong acid pH in the stomach.

The pancreatic amylase will continue the hydrolysis of alpha-1,4 glycosidic bonds in the intestine, and in the intestinal villi the intestinal enzymes alpha-1,6 glucosidase and maltase will hydrolyze the last glycosidic bonds from dextrins, invertase from sucrose, and beta-galactosidase or lactase from lactose, resulting the main mono-saccharides **glucose**, fructose and galactose.

A low beta-galactosidase activity can lead to a pathological condition called milk intolerance or lactose intolerance.

Absorption

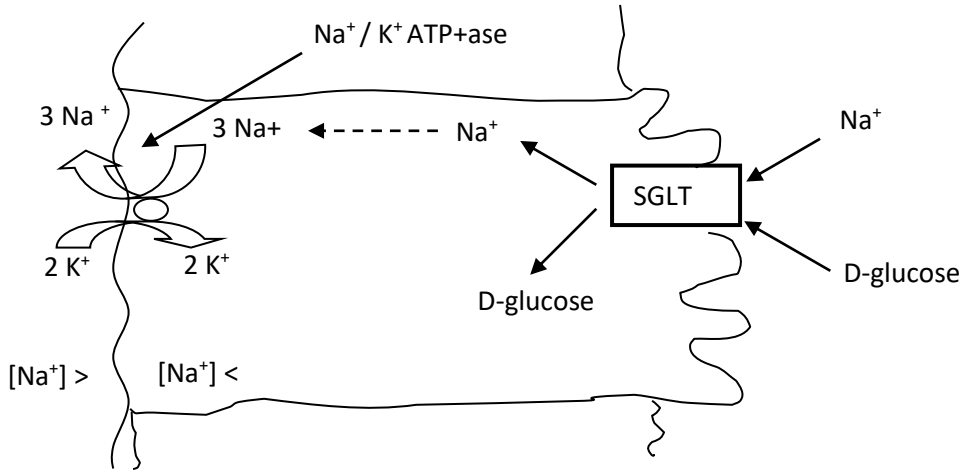
The hydrophilic mono-saccharides cannot pass the hydrophobic cell membranes by themselves, needing specific transporter systems. For glucose, which is the most important mono-saccharide in the human body, there are several situations needing trans membrane transport:

- passing from the intestinal lumen into the enterocyte
- passing from enterocytes into the bloodstream
- passing from the bloodstream into other tissues
- reabsorption of glucose from primary urine in the proximal tube cells in the kidney.

There two types of glucose transporters for all these processes:

a) Sodium-glucose linked transporter (SGLT), also called Sodium-dependent glucose cotransporter, which transport glucose along with sodium against a concentration

gradient across cell membranes. This system is used in the intestinal absorption and renal glucose reabsorption.



b) Glucose specific transporter (GLUT), transports glucose according to the concentration gradient. There are five glucose transporters, according to their different localization, expression and affinity for glucose. The properties of the main glucose transporters are presented in Table 3.

Properties of glucose transporters

Type	Tissue distribution	Mono-saccharide transported	K_M (mM/l) for glucose	Sensibility to insulin
GLUT 1	Most tissue	Glucose Galactose	1	-
GLUT 2	Liver cells, beta pancreatic cells	Glucose Galactose Fructose	15-20	-
GLUT 3	Most tissues, neurons	Glucose Galactose	1	-
GLUT 4	Skeletal and cardiac muscle, adipose tissue	Glucose	5	+
GLUT 5	Small intestine	Fructose	-	-

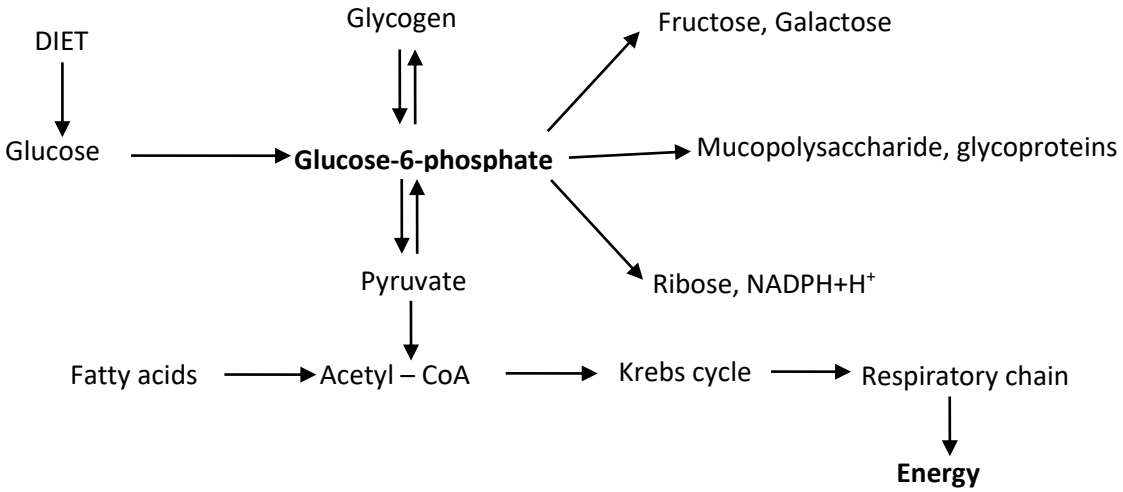
The main 2 types of GLUT are: GLUT4 (muscle) with high affinity for glucose (K_M = 5) and GLUT2 (liver, intestine, kidney) with low affinity for glucose (K_M = 15-20). These differences have important physiological consequences. As such, the normal glucose concentration in blood (5 mM/L) is equal to the K_M of GLUT4 and 4 times lower than the K_M of GLUT2. Therefore, the glucose transport will occur at maximum capacity in cells having GLUT4, being independent of blood glucose levels and only depending on the number of transporter molecules. On the contrary, in cells with GLUT2, the

transport will be dependent on the blood glucose levels, occurring at higher rates under high glycemic conditions.

II.2. Glucose metabolism

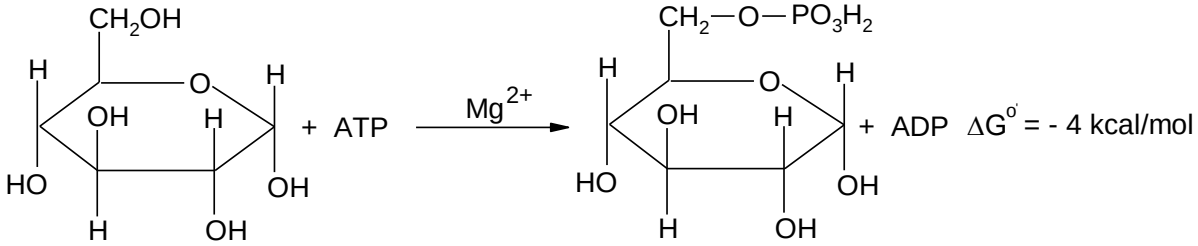
Glucose is the most important carbohydrate in the human body and is metabolized in all cells. However, the way it is metabolized depends on the cell type and the physiological state of the organism.

The main use of glucose is as a substrate for energy. It can be directly used for energy production, or it can be stored as glycogen or lipids for later use of energy. When energy is not needed, glucose can also be used for the synthesis of other compounds.



II.2.1. Glucose phosphorylation

Regardless of the metabolic pathway to follow, the first step in glucose metabolism is the activation reaction by phosphorylation to glucose-6-phosphate.



There are four isozymes for hexokinase, labeled I to IV, with different affinity for glucose.

Table 4. Types of hexokinases

Type	Location	Specificity	Association with glucose transporters	Regulation
I,II,III K_M= 50 µ M	Kidney, brain, skeletal and cardiac muscle, adipose tissue, intestine	Besides glucose, they can phosphorylate other mono-saccharides such as mannose, glucosamine, etc.	Associated with GLUT 4, thus functioning at maximum rate. All the glucose that enters the muscle will be phosphorylated and there will be no free glucose in the muscle cell.	Negative feedback by the reaction product, glucose–6 phosphate
IV (**Glucokinase**) K_M= 2,5 m M	Liver, pancreatic beta cells	Absolute specificity for glucose	Associated with GLUT 2, thus not all glucose will entering the liver cell will be phosphorylated, part of it remaining as free glucose.	It is dependent on the nutrition state. The enzyme synthesis is controlled through positive feedback by insulin.

Postprandial, a large quantity of glucose reaches the liver through the portal vein, the blood glucose concentration reaching here 300-400 mg%. These concentrations surpass the K_M of liver glucokinase and so the enzyme will start phosphorylating glucose until the glucose concentration decreases below the glucokinase K_M value. Therefore, the liver represents a filter for the glucose from food, and because glucokinase is not inhibited by the end product (glucose-6 phosphate), it will act upon glucose until the glucose concentration will decrease sufficiently.

II.2.2. Glucose as a source of energy

Glucose is a source of energy for all tissue, and for some of them, such as red blood cells and neurons, it is the only source of energy. Energy can be obtained from glucose through oxidation reactions coupled with phosphorylation reactions as part of the respiratory chain during aerobic conditions, or through substrate phosphorylation.

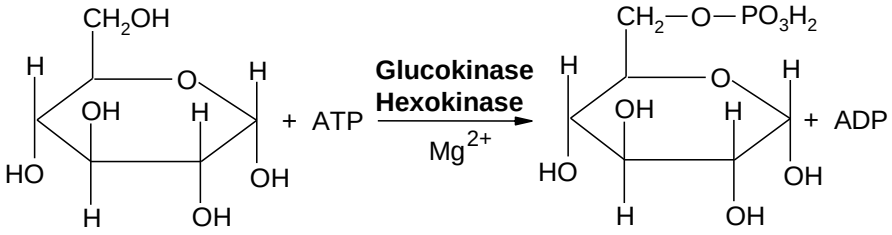
II.2.2.1. Oxidation of glucose to pyruvic acid in the cytosol – the Embden Meyerhof pathway

This is a metabolic pathway that can be considered the sum of two processes:

- activation of glucose to fructose-1,6-bisphosphate
- oxidation of fructose-1,6-bisphosphate to pyruvic acid.

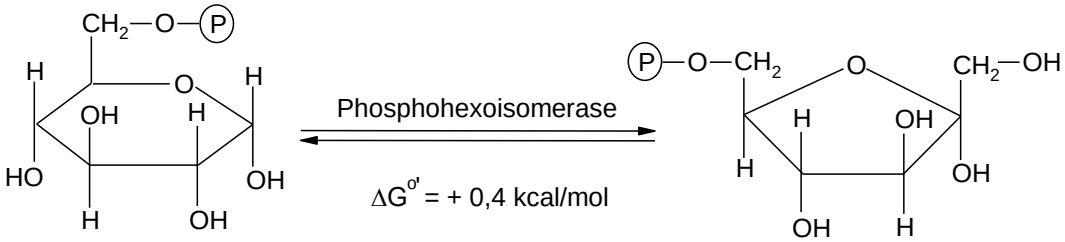
1. Activation of glucose consists of the following reactions:

a. Glucose phosphorylation



$\Delta G^{o'}$= - 4 kcal/mol

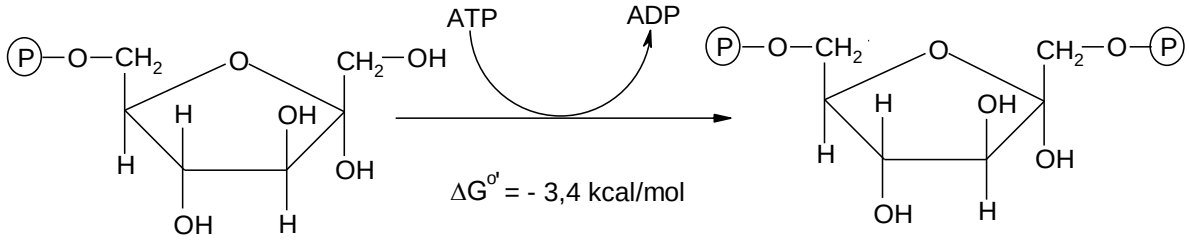
b. Glucose-6 phosphate isomerization



$\Delta G^{o'}$ = + 0,4 kcal/mol

Glucose-6-P Fructose-6-P

c. Fructose-6 phosphate phosphorylation



$\Delta G^{o'}$ = - 3,4 kcal/mol

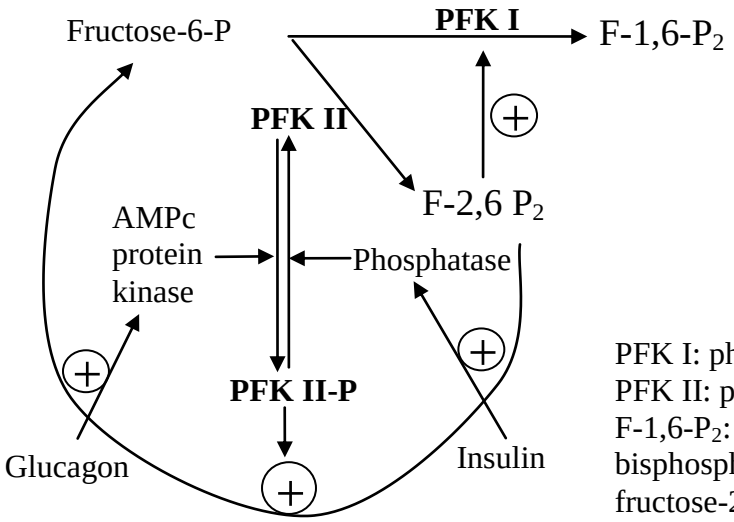
Fructose-6-P Fructose-1,6-P_2

This reaction controls the rate of the Embden-Meyerhof pathway.

There is another enzyme, phosphofructokinase II, that also acts upon fructose-6 phosphate, converting it to fructose-2,6-bisphosphate, which is a strong activator for phosphofructokinase I. Phosphofructokinase II is an enzyme regulated by phosphorylation-dephosphorylation, with a double function:

- the dephospfotylated form has kinase activity, phosphorylating F-6-P → F-2,6-P_2 (the active form for glycolysis)
- the phosphorylated form has a phosphatase activity, hydrolyzing F-2,6-P_2 → F-6-P + P_i

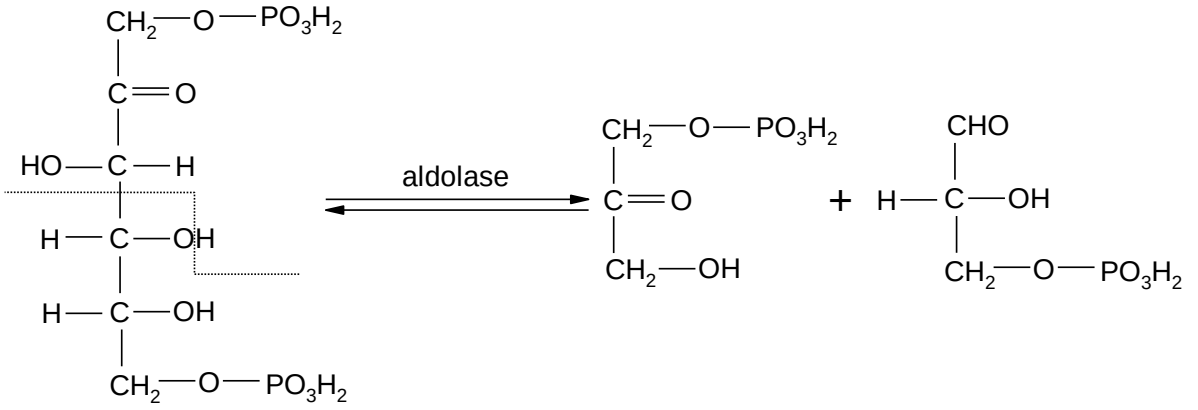
Phosphofructokinase II is controlled hormonally, being activated by insulin and inhibited by glucagon, and allosterically regulated, being inhibited by ATP and citrate, and activated by ADP, AMP, K^+, NH_4^+, glucose-1,6-bisphosphate.



PFK I: phosphofructokinase I,
PFK II: phosphofructokinase II
F-1,6-P_2: fructose-1,6-bisphosphate, F-2,6-P_2: fructose-2,6- bisphosphate

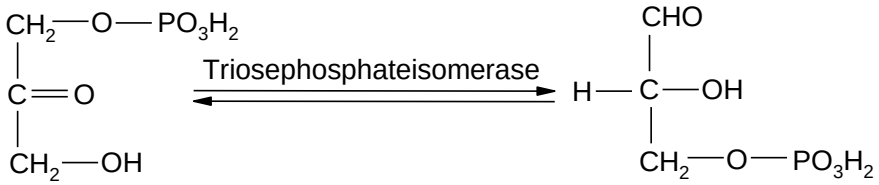
2. Oxidation of fructose-1,6-bisphosphate to pyruvic acid

a. Splitting fructose-1,6-bisphosphate into triose-phosphates



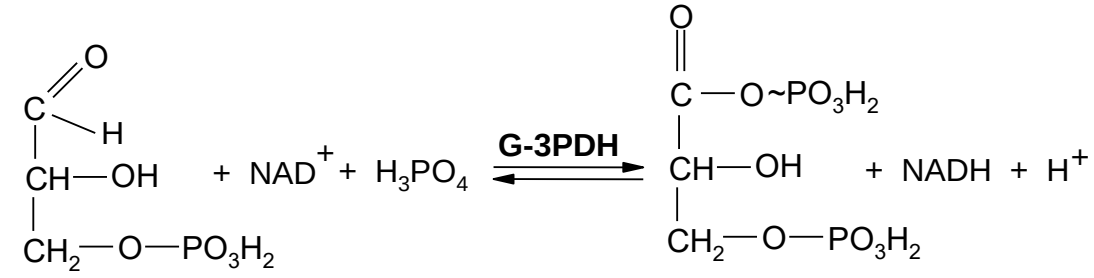
Fructose-1,6-diphosphate Phosphodihydroxyacetone Phosphoglyceraldehyde

The two compounds obtained are isomers found in an equilibrium reaction:



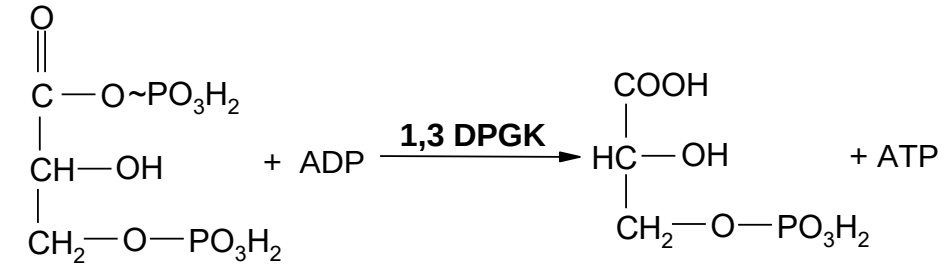
Although the equilibrium is strongly favored towards the left, only glyceraldehyde-3-phosphate will be used further, ensuring that all dihydroxyacetone phosphate will eventually be converted into glyceraldehyde-3-phosphate. Therefore, one can notice that during glucose oxidation, two molecules of glyceraldehyde-3-phosphate can be obtained from one molecule of fructose-1,6-bisphosphate.

b. Oxidation of glyceraldehyde-3-phosphate to 3-phosphoglyceric acid. This reaction constitutes a possibility for obtaining ATP during anaerobic oxidation of glucose and it occurs in two steps:



Glyceraldehyde-3-phosphate 1,3 bisphosphogliceric acid

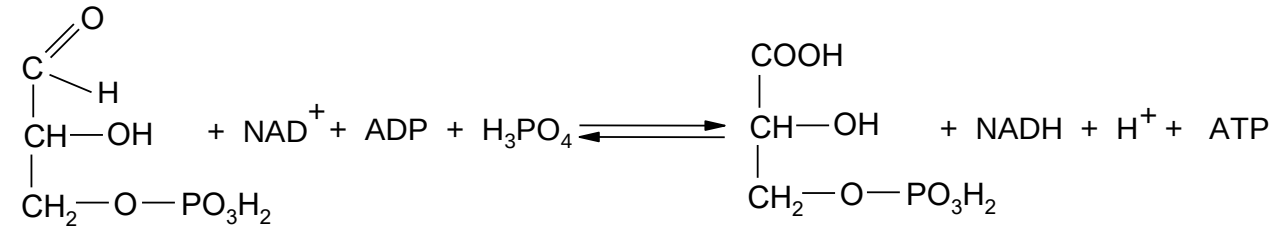
G-3PDH - Glyceraldehyde-3-phosphate-dehydrogenase



1,3 bisiphosphogliceric acid 3-phosphogliceric acid

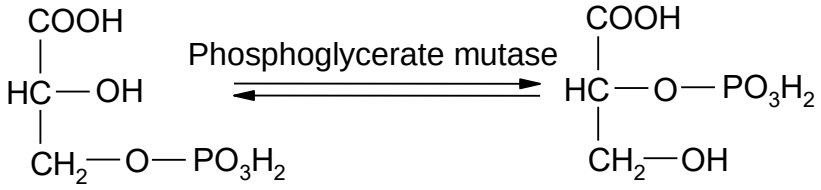
1,3 DPGK - phosphoglicerate kinaza

The global reaction is:



Glyceraldehyde-3-phosphate 3-phosphogliceric acid

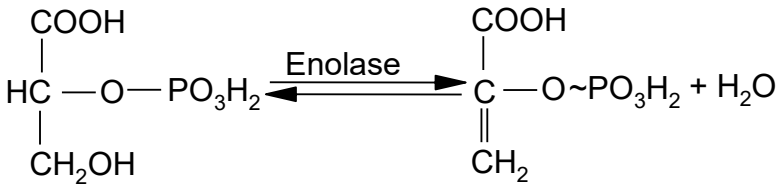
c. Isomerization of 3-phosphoglyceric acid to 2-phosphoglyceric acid



3-phosphogliceric acid 2-phosphogliceric acid

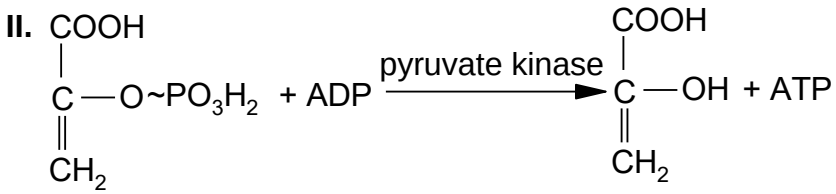
d. Transformation of 2-phosphoglyceric acid into pyruvic acid. This reaction also constitutes a possibility for obtaining ATP during anaerobic oxidation of glucose and it occurs in two steps:

I.



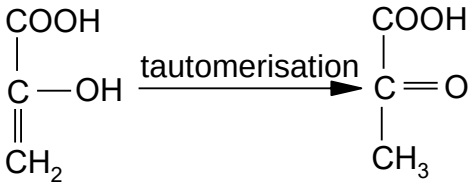
2-phosphogliceric acid phosphoenolpyruvic acid (PEP)

II.



PEP enolpyruvic acid

The enolpyruvic acid will be spontaneously converted into pyruvic acid by tautomerization, thus the reactions become irreversible.



enolpyruvic acid pyruvic acid

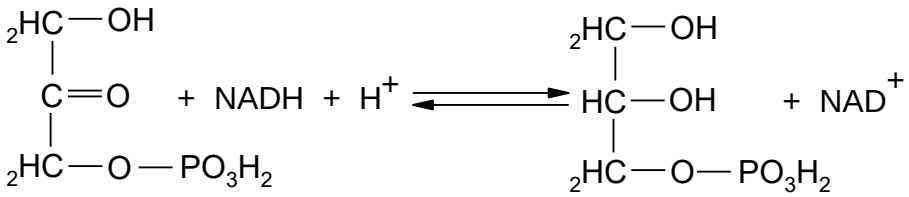
The enzyme **enolase** is specifically inhibited by the **fluoride ion F^-**, and so this ion is added to blood when collecting it for glucose blood level determination. In the blood collected for this analysis, the fluoride ion is blocking the oxidation of glucose in the time period between collection and analysis, so that an accurate determination of glucose concentration can be performed.

Role of the Embden-Meyerhof pathway

The main role of this pathway is to produce energy. Through this pathway, one mole of glucose is oxidized to obtain two moles of pyruvic acid, a net gain of two moles of ATP, and two moles of NADH,H^+ are also generated. Unloading the two NADH,H^+ in the respiratory chain will produce an additional 5 moles of ATP, which will bring the energy producing potential of the Embden-Meyerhof pathway to 7 moles of ATP in aerobic conditions.

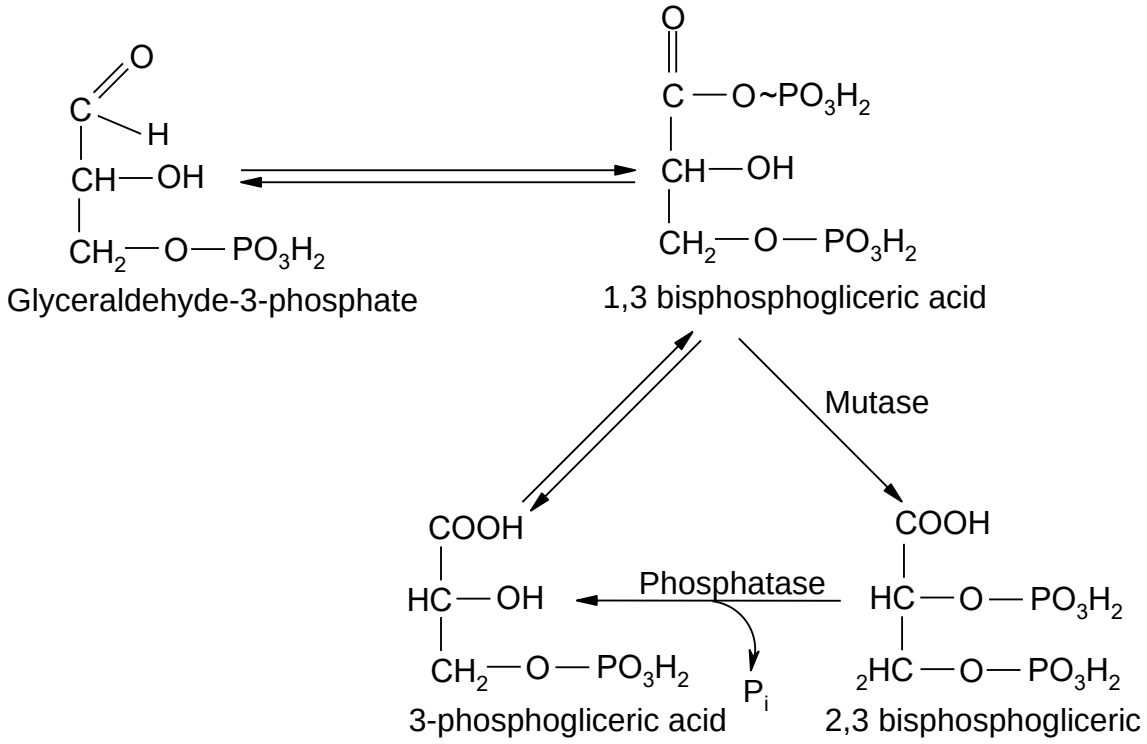
Some of the intermediary compounds obtained can be used in other syntheses:

1. The reversible reactions of this pathway (with the exception of three of them) can be used in the reverse direction in order to make glucose (gluconeogenesis), under adequate metabolic conditions.
2. The pyruvic acid can be used in a transamination reaction to produce alanine.
3. The 3-phosphoglyceric acid can be a precursor for serine synthesis.
4. Dihydroxyacetone phosphate can be reversibly converted by reduction into glycerol phosphate, which is the active form needed fr the synthesis of triacylglycerols and glycerophospholipids.



Dihydroxyacetone-phosphate Glycerol-3-phosphate

5. The 1,3-bisphosphoglyceric acid can be converted into 2,3-bisphosphoglyceric acid under the action of a bisphosphoglycerate mutase. The resulted molecules is found in large quantities in red blood cells, approximately in equimolar quantities with hemoglobin and it bounds to it, having an allosteric activity on the oxyhemoglobin dissociation by reducing the affinity of hemoglobin for oxygen. This phenomenon plays a role in tissue oxygenation. The 2,3-bisphosphoglyceric acid can be converted by a phosphatase into 3-phosphoglyceric acid, thus creating a functional molecule on the expense of a substrate phosphorylation reaction, which could generate ATP.



Regulation of the Embden-Meyerhof pathway

The functioning of the pathway is conditioned by the availability of reactants, the functioning of the enzymes controlling the irreversible reactions, and the action of hormones involved in the carbohydrate metabolism.

Regarding the reactants, the pathway depends foremost on the availability of glucose, and then on the availability of oxygen and NAD^+.

Regarding the enzymes, the main enzyme controlling this pathway's rate is phosphofructokinase. This enzyme is allosterically regulated by the action of negative effectors (ATP-negative feedback through the end product of energetic processes, citrate) or positive effectors (ADP and AMP, fructose-1,6-bisphosphate in liver and glucose-1,6-bisphosphate in other tissues).

Regarding hormones, the hyperglycemic hormones (glucagon, adrenaline, glucocorticoids) will inhibit the pathway, and insulin (hypoglycemic hormone) will stimulate the pathway. The hormones' action takes place upon the enzymes catalyzing the irreversible reactions (phosphofructokinase, hexokinase, pyruvate kinase), thus controlling the rate of the pathway. The hormones either stimulate or inhibit the synthesis of these enzymes, or have a positive or negative action on the regulation of these enzymes through phosphorylation-dephosphorylation.

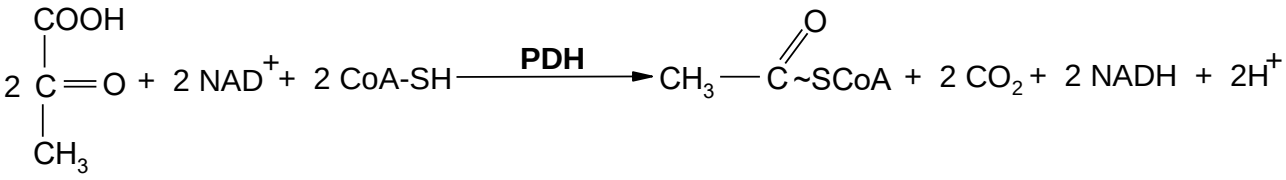
II.2.2.2. Oxidation of pyruvic acid to acetyl-CoA

This process occurs in the mitochondria and the pyruvic acid produced in the cytosol through the Embden-Meyerhof pathway needs to be transported across the mitochondrial membrane. This is done by an active antiport transport in which when one pyruvic acid molecule enters the mitochondrion, one HO^- ion exits. Inside mitochondria, pyruvic acid undergoes an oxidative decarboxylation resulting acetyl-CoA, CO_2 and

hydrogen transferred to $NADH,H^+$. This process is catalyzed by a multi-enzymatic complex called pyruvate dehydrogenase, containing three dehydrogenase enzymes, each having a different cofactor and catalyzing a distinct intermediary step:

- E1 – TPP thiamine pyrophosphate dehydrogenase
- E2 – lipoate dihydrolipoyl transacetylase
- E3 – FAD dihydrolipoyl dehydrogenase.

The global reaction is the following, considering that 2 molecules of pyruvic acid are obtained from one molecule of glucose:

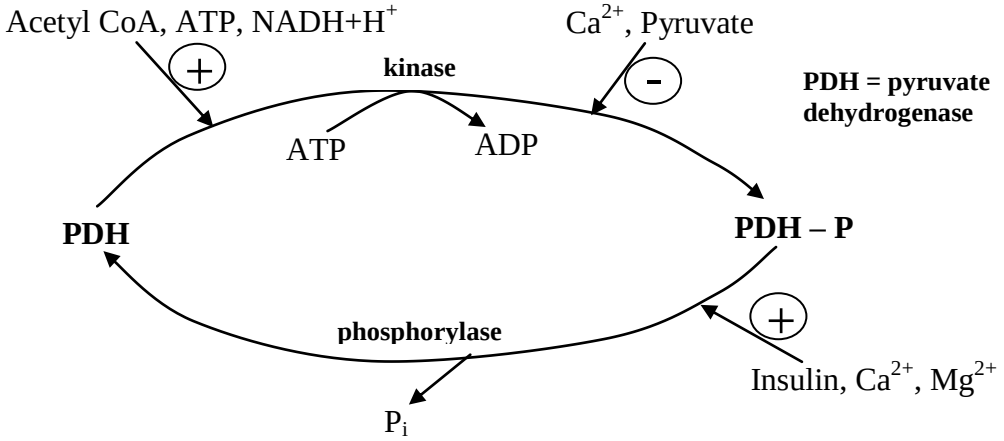


Energy balance

During this phase there are two macroergic thiol bonds formed between the two acetyl-CoA molecules obtained and two $NADH,H^+$ molecules, which can be oxidized in the respiratory chain to form 5 ATP molecules.

Regulation of this phase

The pyruvate dehydrogenase is allosterically regulated, pyruvic acid being a positive effector, and acetyl-CoA and $NADH,H^+$ are negative effectors. This enzyme is active in its dephosphorylated state and inactive when phosphorylated, the enxymes catalyzing these transformations also being allosterically and hormonally regulated. Insulin stimulates the activity of pyruvate dehydrogenase.



Related pathology

In order to function properly, pyruvate dehydrogenase needs sufficient amounts of cofactors derived from vitamins such as pantothenic acid, niacin, riboflavin, thiamine,

lipoic acid. Any severe deficiency of these vitamins will lead to a reduced activity of the enzyme, thus increasing the blood concentration of pyruvate which will lead to an increase of lactic acid concentration, producing lactic acidosis.

The genetic deficiency of pyruvate dehydrogenase affecting over 60% of its enzymatic activity will produce microencephalitis, optic atrophy, motor dysfuction, mental retard.

Arsenate is a strong poison because it can bond to lipoic acid (enzymatic cofactor) thus blocking the activity of pyruvate dehydrogenase. It reacts similarly with the sulfhydryl groups from the immature keratin from hair and nails, this being used in forensic medicine for identifying arsenic poisoning.

II.2.2.3. Oxidation of acetyl-CoA in the citric acid cycle

This takes place according to the general equation:

$$2CH_3—\overset{\overset{O}{\|}}{C}\sim S—CoA + 2 \times 3\ NAD^+ + 2\ FAD + 2\ GDP + 2P_i + 2 \times 2H_2O \longrightarrow$$

$$\longrightarrow 2 \times 2\ CO_2 + 2 \times 3\ NADH+3H^+ + 2\ FADH_2 + 2\ GTP + 2\ CoA\text{-}SH$$

2 GTP molecules are equivalent to 2 ATP molecules.

Under aerobic conditions, oxidation of the reducing equivalents will generate:

2×3 moles $NADH+H^+ \longrightarrow 2 \times 3 \times 2.5$ ATP = 15 ATP
2×1 moles $FADH_2 \longrightarrow 2 \times 1.5$ ATP = 3 ATP

TOTAL ATP = 2 ATP + 15 ATP +3 ATP = 20 ATP

II.2.2.4. Oxidation of hydrogen in the respiratory chain, a process coupled with ATP synthesis

This step is also called oxidative phosphorylation in the respiratory chain and it oxidizes the hydrogen obtained from the other three steps of complete oxidative catabolism of glucose according to the general equation:

1 moles $NADH+H^+ \longrightarrow$ 2,5 ATP
1 moles $FADH_2 \longrightarrow$ 1,5 ATP

II.2.2.5. Global energy balance of the complete aerobic oxidation of glucose

The energy resulted during the oxidative catabolism of glucose is stored in the macroergic bonds of ATP, formed either by phosphorylation of substrates or by phosphorylation in the respiratory chain. The energy balance for the oxidation of one mole of glucose is:

Step I	
Activation of glucose to fructose 1,6-bisphosphate	- 2 ATP
Oxidation of fructose 1,6-bisphosphate to pyruvic acid	+ 9 ATP
Step II	
Oxidation of pyruvic acid to acetyl-CoA	+ 5 ATP
Step III	
Oxidation of acetyl-CoA in the citric cycle	+ 20 ATP
Total	+ 32 ATP

Tables below are presenting the contributions according to reaction type.

a. Oxidative phosphorylation of substrates

Transformation	Catabolic step	Total ATP obtained
2 glyceraldehyde-3-P →2 3-P-glyceric acid	E-M pathway	2
2 2-P-glyceric acid→ 2 pyruvic acid	E-M pathway	2
2 alpha-ketoglutaric acid →2 succinic acid	E-M pathway	2
Total		**6**

b. Oxidative phosphorylation in the respiratory chain

Transformation	Catabolic step	Reduced Coenzyme generated	Total ATP generated in the respiratory chain
2 glyceraldehyde-3-P →2 3-P-glyceric acid	E-M pathway	2 $NADH+2H^+$	5
2 pyruvic acid → 2 acetyl-CoA	Oxidative decarboxylation of pyruvic acid	2 $NADH+2H^+$	5
2 isocitric acid → 2 alpha-ketoglutaric acid	Citric cycle	2 $NADH+2H^+$	5
2 alpha-ketoglutaric acid → 2 succinic acid	Citric cycle	2 $NADH+2H^+$	5
2 succinic acid →2 fumaric acid	Citric cycle	2 $FADH_2$	3
2 malic acid →2 oxaloacetic acid	Citric cycle	2 $NADH+2H^+$	5
Total			**28**

Total ATP generated = 6 + 28 = 34 moles ATP.

ATP consumed

Transformation	Metabolic path	ATP consumed
Glucose phosphorylation	E – M pathway	1
Fructose-6-P phosphorylation	E – M pathway	1
Total		**2**

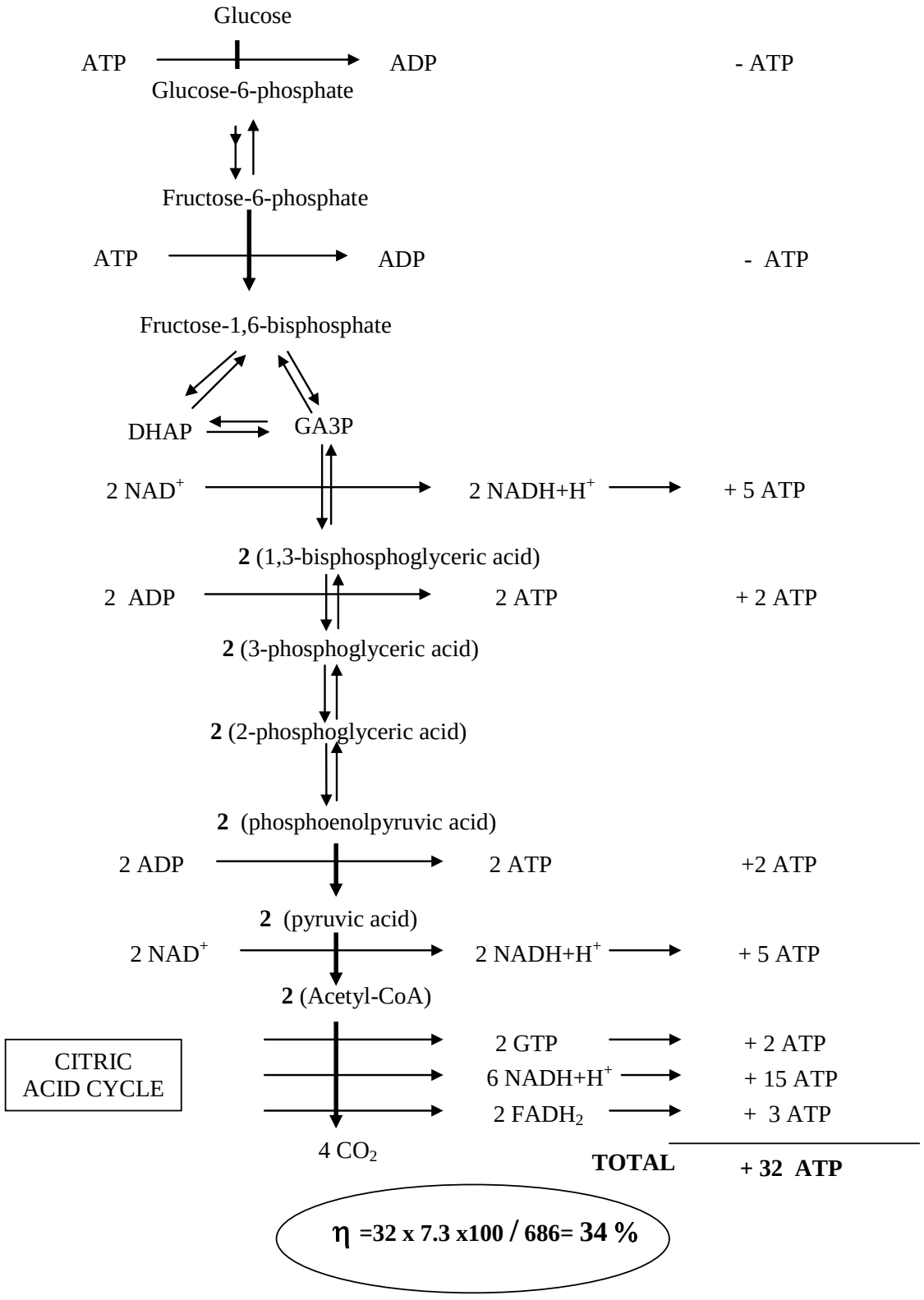
General balance=34 moles ATP generated-2 moles ATP consumed=32 moles ATP

The **yield (η)** of energy from glucose oxidation that is stored in ATP macroergic bonds is calculated as follows:

Total energy released: $\Delta G^{0'}$ = - 686 kcal/mole

The energy used in forming the ATP macroergic bonds: 32 x 7,3 = 233,6 kcal/mole.

$$\eta = \frac{32x7,3}{686} x100 = 34\ \%$$



General schematic representation of the aerobic oxidation of glucose (complete catabolism)

Regulation of the complete oxidative catabolism of glucose

Glucose catabolism is dependent primarily on the physiological state of the organism, being intense immediately after a meal when large quantities of glucose from food are available, and having a reduced intensity late after a meal when the glucose quantity is low and the body uses alternative sources for energy such as fatty acids.

On the short term, the control of glucose catabolism is done by enzymatic regulation, primarily of the activity of phosphofructokinase I. This enzyme is:

- Inhibited by **ATP**, citrate, low blood pH (high concentrations of lactic and pyruvic acid) and high glycemic hormones (glucagon, cortisol, adrenalin)
- Activated by ADP, AMP and insulin (hypoglycemic hormone).