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

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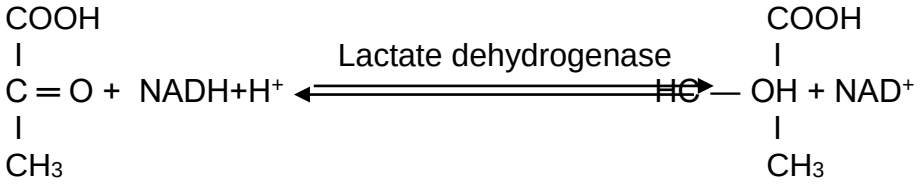
Lecture 4

II.2.3. Anaerobic glycolysis

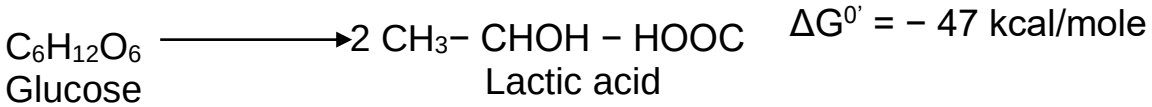
Aerobic oxidation of glucose is the main mechanism of glucose catabolism in most tissues; however, there are some tissues where glucose oxidation takes place under anaerobic conditions. This can happen as:

- A **unique mechanism** of glucose oxidation in red blood cells (they lack mitochondria) and in tissues with low oxygenation such as retina, cornea, skin, internal kidney medullar, neurons, white muscle fibers.
- A **partial mechanism** in tissues with rapid growth rate such as embryonic and cancer tissues (where 50% of glucose metabolism is anaerobic)
- A **temporary mechanism** during periods of low oxygen supply, for example in skeletal muscles under intense and extended effort.

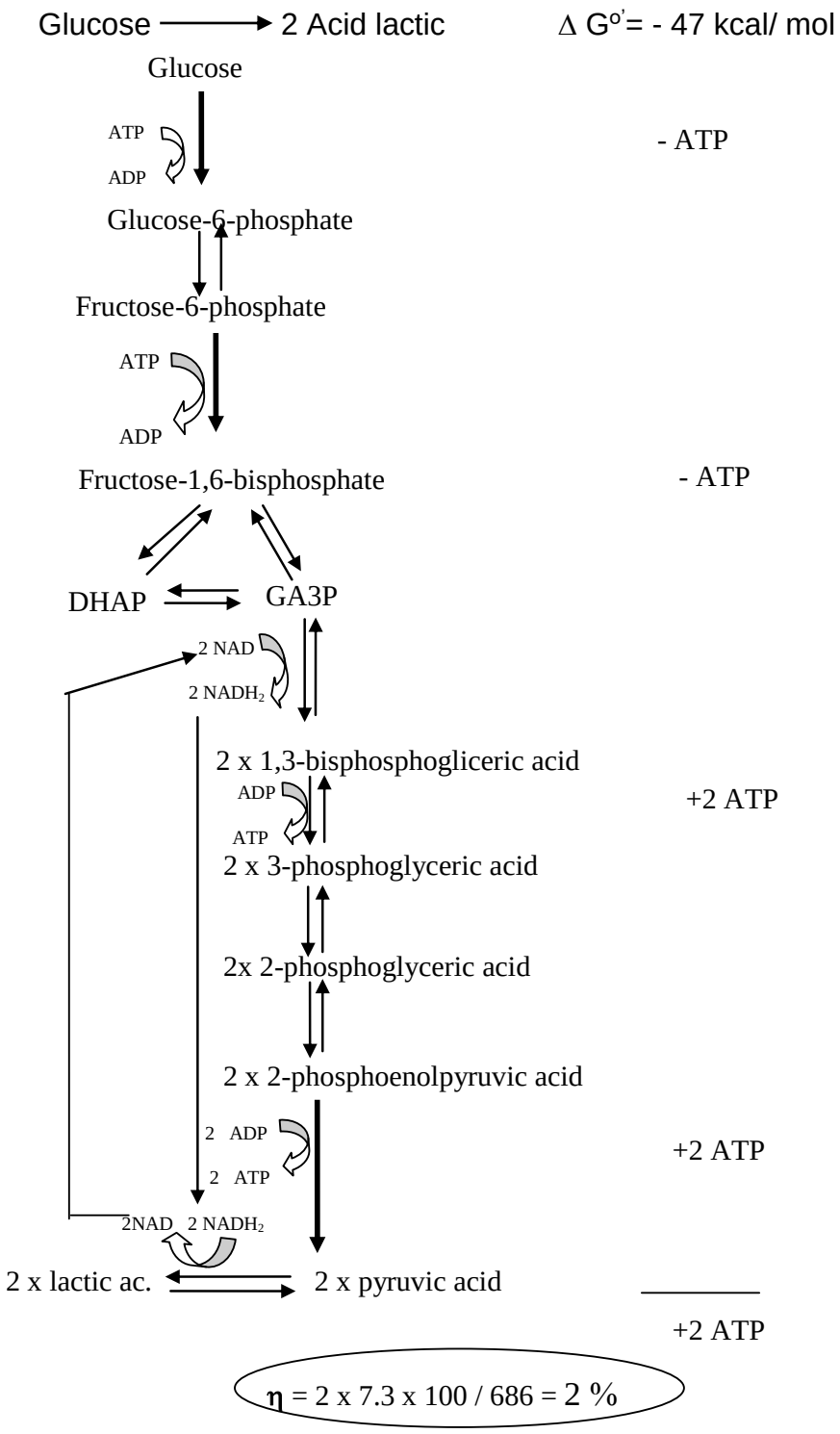
When oxygen is lacking, the coenzymes that are normally taking over the hydrogen during oxidation reactions cannot be regenerated (reoxidized) by releasing the hydrogen towards the oxygen. Therefore, in these conditions the oxidation of glucose will stop to pyruvic acid, which will become the hydrogen acceptor from the coenzyme $NADH+H^+$, being converted into lactic acid (the final product of anaerobic glucose catabolism – anaerobic glycolysis).



In these conditions, the global reaction is:



Only part of the energetic potential of glucose will be released under anaerobic conditions, 47 kcal/mole from the total of 686 kcal/mole. Therefore, the yield of energy stored in macroergic bonds is also lower, only two ATP molecules being produced by anaerobic oxidation of one mole of glucose.

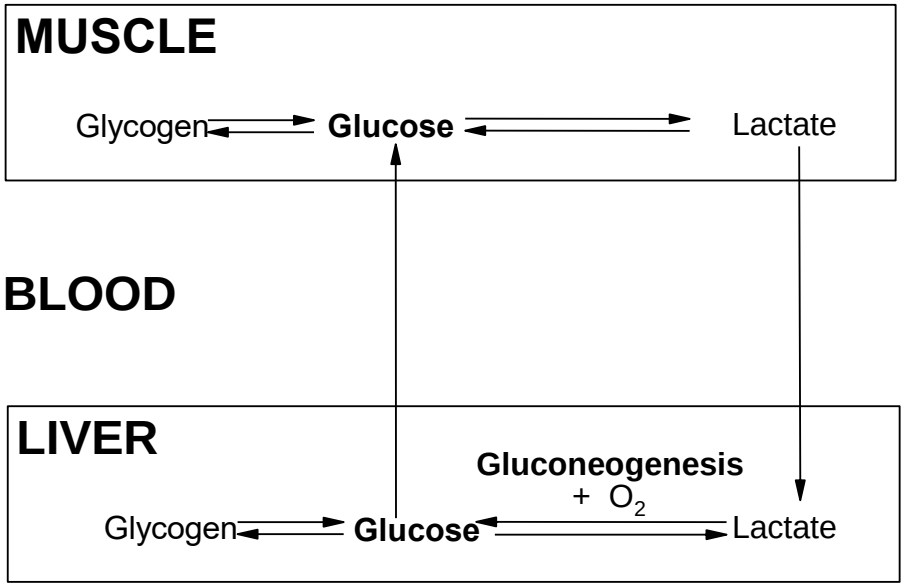


Anaerobic glycolysis energy balance

The energy yield is very low compared to the aerobic oxidation of glucose where 32 ATP molecules are obtained. Therefore, anaerobic oxidation is considered to be a

primitive mechanism of energy production, used by the human body only under certain physiological conditions.

Due to the fact that lactic acid contains a considerable amount of energy, the human body will recuperate it in the liver (an organ much better oxygenated compared to muscle) through a specific metabolic pathway called the **Cori cycle**.



Cori cycle

Regulation of anaerobic glycolysis

Having the same reactions as the Embden-Meyerhof pathway, anaerobic glycolysis will be regulated by the same factors, except for oxygen. With respect to oxygen, the Pasteur effect will be followed: “oxidation (presence of oxygen) inhibits fermentation (anaerobic glycolysis)”. For example, the insufficient oxygenation in the muscle under intense effort will force this tissue to use anaerobic glycolysis in order to obtain the necessary energy for its function. However, in cancer tissues the Pasteur effect is not followed, cancer cells using anaerobic glycolysis regardless of the level of oxygenation.

Pathological conditions related to anaerobic glycolysis

1. **Lactic acidosis** is the most common form of metabolic acidosis. It is produced wither by an increased synthesis or a decreased utilization of lactic acid, mostly caused by a blockage of aerobic oxidation. Other conditions producing lactic acidosis are high altitudes, strenuous physical exercise, pulmonary diseases, severe anemia, CO or CN^- intoxications (blocks the respiratory chain and the hemoglobin), alcohol intoxications, cancer.
2. **Genetic defects of glycolysis enzymes.** The complete deficiency of these enzymes is fatal because red blood cells and neurons obtain energy exclusively by glycolysis. A partial deficiency in pyruvate kinase, which has an incidence of 1:10000, will cause a decrease in the enzymatic function to 5-25% of capacity in red blood cells, generating hemolytic anemia.

II.2.4. Gluconeogenesis

The great majority of the tissues in the human body obtain energy by metabolizing several substances: glucose, fatty acids, amino acids, ketone bodies.

Certain tissues such as red blood cells and neurons use only glucose; for example the human brain consumes 120 grams/day of glucose and needs a constant glycemia level between 70-100 mg% (4-5.5 mM) for optimum functioning.

Dietary carbohydrates will maintain the glycemia level for a few hours after a meal, and then the blood glucose level will be maintained through glucose production in the liver. Liver produces glucose through two mechanisms:

1. **Hydrolysis of glycogen**
2. ***De novo* synthesis of glucose**, using as precursors lactic acid, amino acids and glycerol. This metabolic pathway is called gluconeogenesis and represents the sole source of glucose during starving conditions.

Gluconeogenesis takes place in the liver and the renal cortex (renal proximal tube cells). In absolute terms, the intensity of gluconeogenesis is the same between the two tissues; however the large difference in mass between them ensures that only 10% of all gluconeogenesis processes takes place in the kidney, the large majority occurring in the liver. Recently it has been shown that gluconeogenesis also takes place in enterocytes.

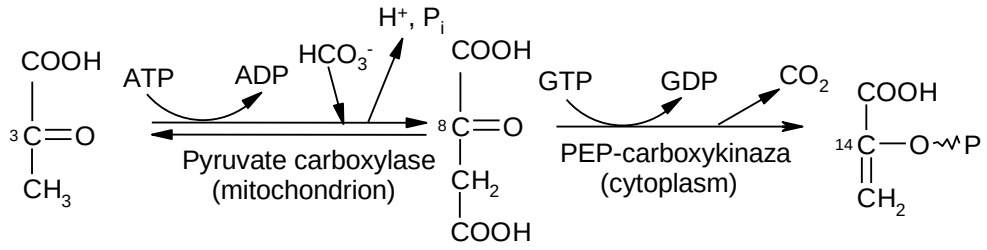
Gluconeogenesis is the opposite process of glucose catabolism and therefore it uses the same reactions as for glycolysis, only in the opposite sense. Only the reversible reactions can be used in this way, while the irreversible ones will be replaced by other specific reactions. The irreversible steps of glycolysis are:

I. Phosphoenolpyruvate ⟶ Pyruvate

II. Fructose–6–phosphate ⟶ Fructose–1,6–bisphosphate

III. Glucose ⟶ Glucose – 6 – phosphate

The first reaction above is replaced with the following:



Pyruvate Oxaloacetate PEP (phosphoenolpyruvic acid)

Due to the fact that oxaloacetate cannot pass through the mitochondrial membrane, specific shuttling systems are used, depending on the specific precursor for gluconeogenesis (Figure 10).

I. The step **PYRUVATE → PHOSPHOENOLPYRUVATE** consists of the following reactions:

1. **Mitochondria**

$$\text{Pyruvate} + CO_2 + \text{ATP} \xrightarrow{\text{Pyruvatecarboxylase (biotin)}} \text{Oxaloacetate} + \text{ADP} + \text{Pi}$$

2. Shuttling the oxaloacetate **from mitochondria into the cytosol**
3. **Cytosol**

$$\text{Oxaloacetate} + \text{GTP} \xrightarrow{\text{Phosphoenolpyruvatecarboxykinase}} \text{Phosphoenolpyruvate} + \text{GDP} + CO_2$$

II. The step **FRUCTOSE-1,6-BISPHOSPHATE → FRUCTOSE-6-PHOSPHATE**

$$\text{Fructose-1,6-bisphosphate} + H_2O \xrightarrow{\text{Fructose–1,6–bisphosphatephosphatase}} \text{Fructose-6-phosphate} + P_i$$

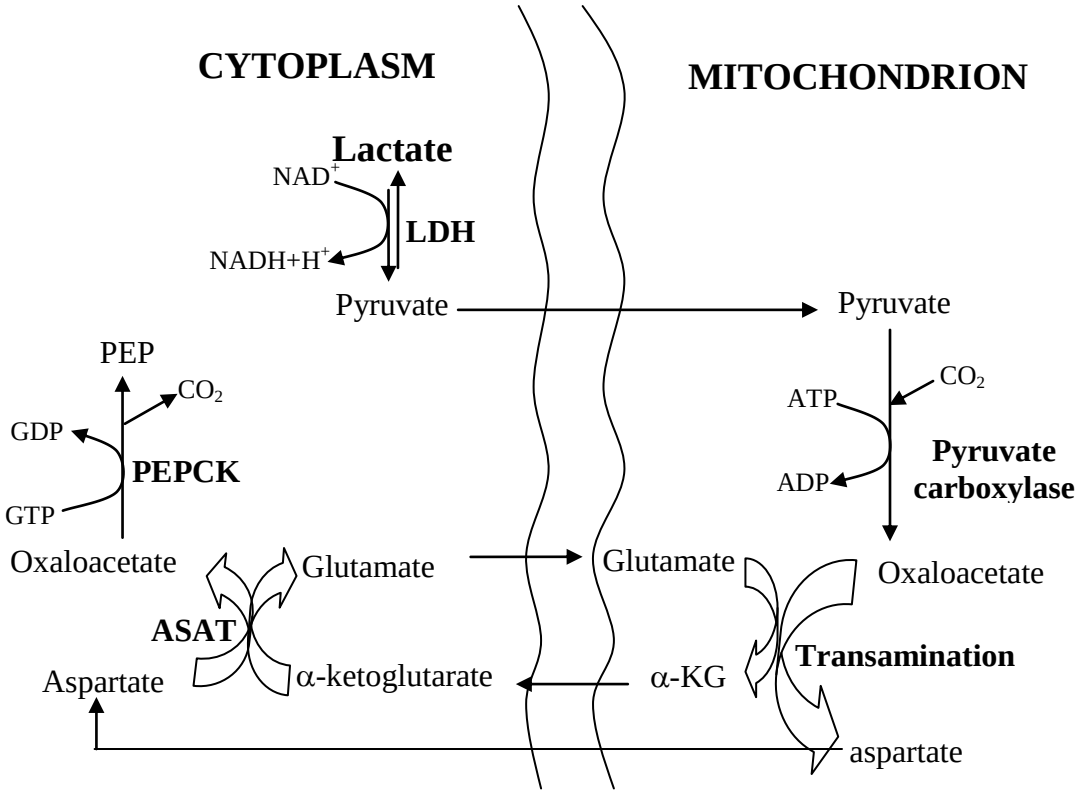
The enzyme is active in the liver, kidney, intestine, skeletal muscles, and is absent in adipose tissue, cardiac and smooth muscle.

III. The step **GLUCOSE-6-PHOSPHATE → GLUCOSE**

$$\text{Glucose-6-phosphate} + H_2O \xrightarrow{\text{Glucose–6P–phosphatase}} \text{Glucose} + P_i$$

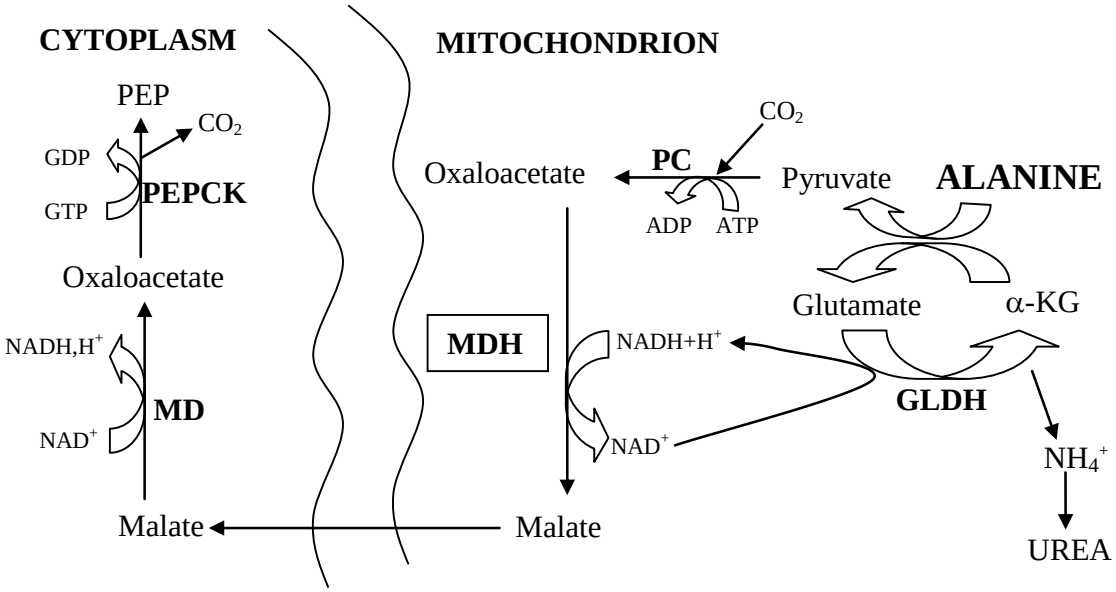
The enzyme is active in liver and kidney which therefore are makers and exporters of glucose in the bloodstream. It is absent in muscle and adipose tissue.

A. Precursor lactate → oxaloacetate-aspartate shuttle



PEPCK – phosphoenolpyruvate carboxykinase
ASAT – aspartate aminotransferase
LDH – lactate dehydrogenase

B. Precursor alanine → oxaloacetate-malate shuttle

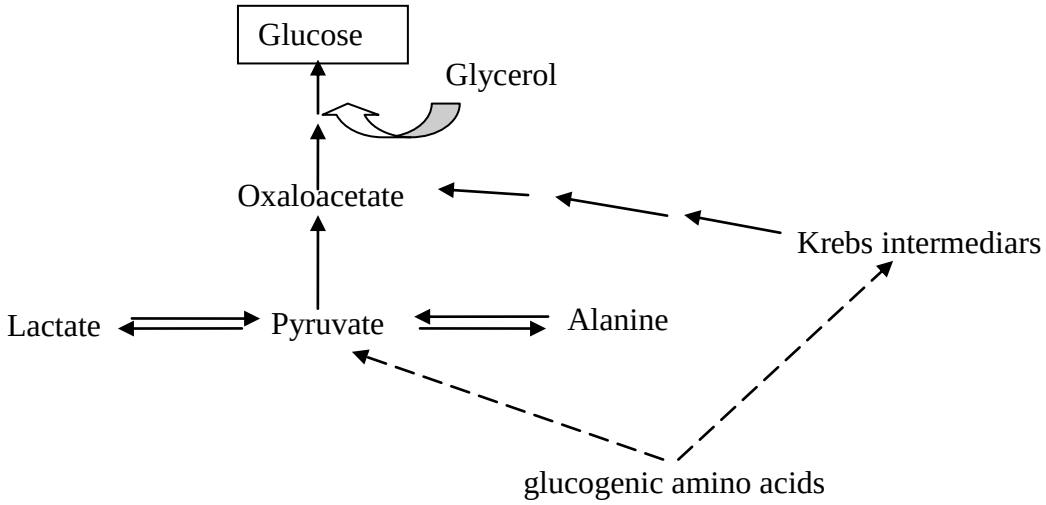


MDH – malate dehydrogenase PC – pyruvate carboxylase
GLDH – glutamate dehydrogenase PEPK – phosphoenolpyruvate carboxykinase

Figure 10. Oxaloacetate shuttling systems

The major substrates for gluconeogenesis are lactate (erythrocytes, muscular effort), glucogenic amino acids (muscular proteolysis), glycerol (lipid hydrolysis), citric acid cycle intermediaries (oxaloacetate, alpha-ketoglutarate, succinyl-CoA, fumarate).

Acetyl-CoA cannot be converted into glucose because there is no reverse reaction for pyruvate → acetyl-CoA. Therefore, substances that produce acetyl-CoA such as fatty acids, ketone bodies, and ethanol cannot be substrates for gluconeogenesis.

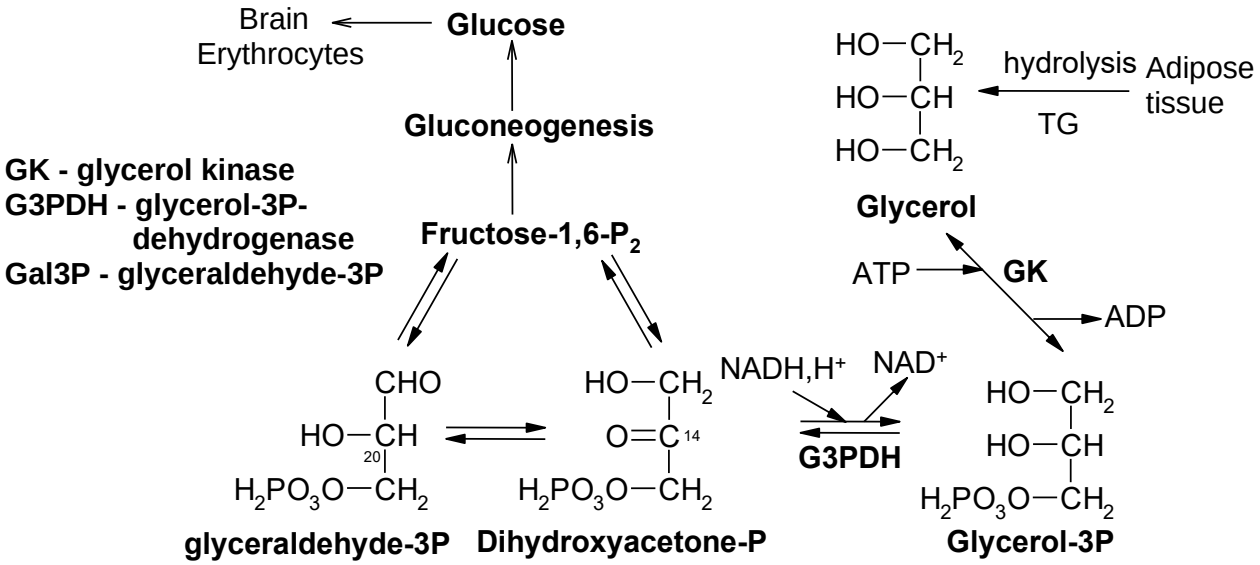


Lactate enters gluconeogenesis as pyruvate, according to the reaction:

$$\text{Lactate} + NAD^+ \xleftrightarrow{\text{Lactatedehydrogenase}} \text{Pyruvate} + NADH,H^+$$

The energy needed for the synthesis of one glucose molecule from 2 lactate molecules is obtained by hydrolyzing 6 ATP molecules, among which 4 ATPs are consumed in the reaction 2pyruvate → 2phosphoenolpyruvate and 2 ATPs are consumed for the reaction 2 3-phopshoenolpyruvic acid → 2 gyceraldehyde-3-phosphate.

Glycerol is obtained by hydrolyzing triglycerides from adipose tissues and enters gluconeogenesis at the level of triose phosphates.



The energy needs for synthesizing one glucose molecule from 2 glycerol molecules is obtained by the hydrolysis of 2 ATPs, which are consumed for the phosphorylation 2glycerol → 2glycerol-3-phosphate.

The **glucogenic amino acids** are the most important precursors for gluconeogenesis during starvation, when amino acids are released in the blood stream through muscle proteolysis, among which alanine is used with maximum intensity by the liver. During catabolism of glucogenic amino acids there are certain citric acid cycle intermediaries that are produced, and these can enter the gluconeogenesis pathway through oxaloacetic acid. According to which of these compound enters gluconeogenesis, the energy needs expressed as ATP molecules will be different. For example:

- 2 alanine molecules are transaminated to form 2 molecules of pyruvic acid which is converted to glucose using 6 ATP molecules and $2NADH+2H^+$.
- 2 molecules of glutamic acid are transaminated into 2 alpha-ketoglutaric acid molecules which will be converted into glucose with the consumption of 2 ATPs and $2NADH+2H^+$.

Regulation of gluconeogenesis

1. Metabolic regulation. Gluconeogenesis occurs during starvation periods when glucose needs to be supplied by endogenous production in order to maintain constant glycemic levels.

$2\ Pyruvate + 6\ ATP + 10\ H_2O \rightarrow Glucose + 6\ ADP + 6\ Pi$

One can notice that gluconeogenesis depends on the availability of substrates and energy. The energy in the form of ATP is obtained either by oxidizing fatty acids during lipolysis which normally happed in starvation, or by the aerobic oxidation of a part of lactic and pyruvic acids through the citric acid cycle and the respiratory chain.

2. Enzymatic regulation. There are two phases for regulation, short term and long term.

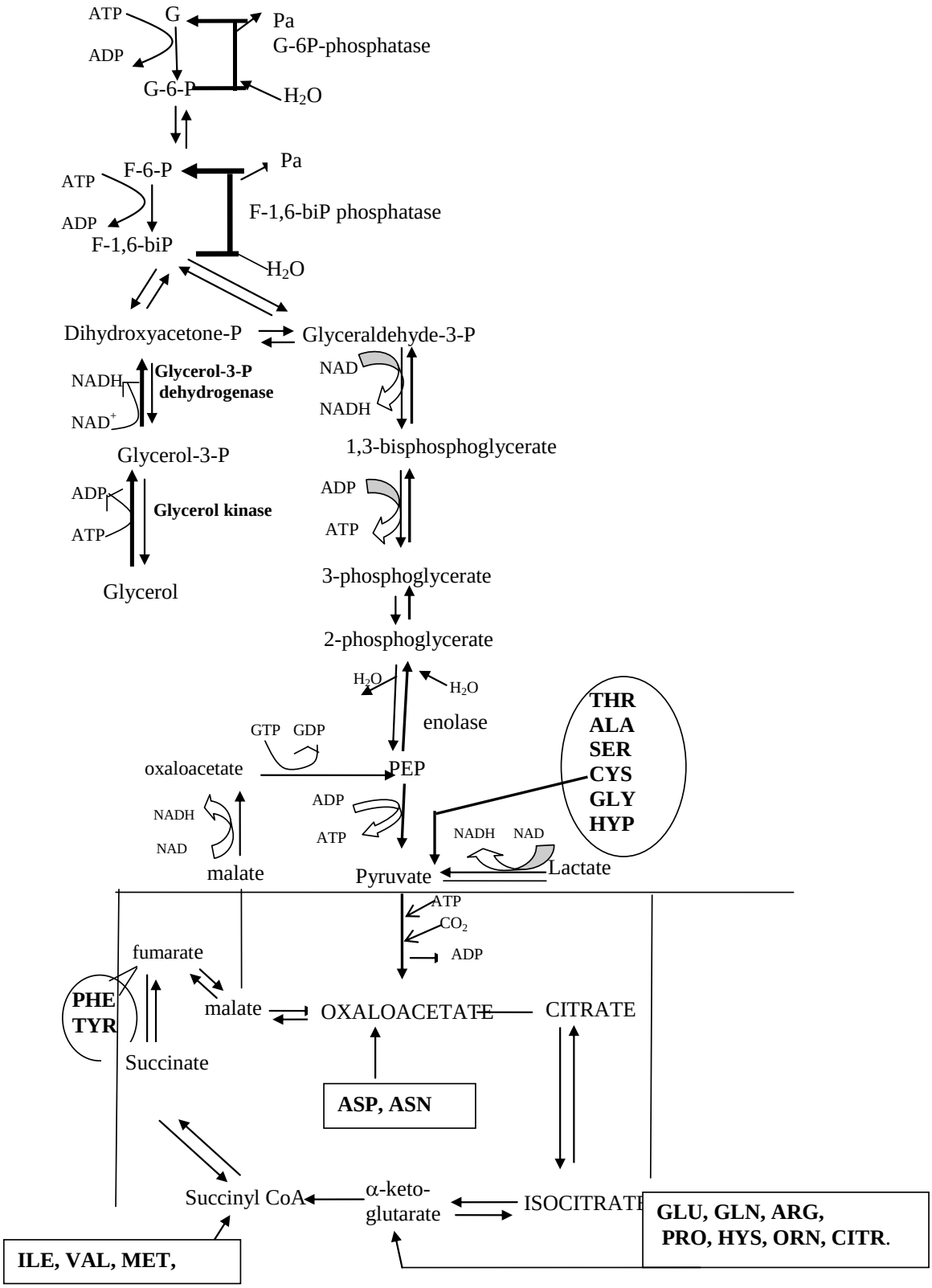
- On **short term**, the hydrophilic hyperglycemic hormones (adrenalin, glucagon) stimulate protein kinase A, an enzyme that phosphorylates key enzymes from the glucose metabolism (the phosphorylated form of these enzymes have gluconeogenesis activity). In addition, there is a feedback allosteric regulation through which the glycolysis intermediary (acetyl-CoA, citrate) and final (ATP) products inhibit the glycolysis enzymes and stimulate the gluconeogenesis enzymes.

- On **long term**, hyperglycemic hormones stimulate the synthesis of gluconeogenesis key enzymes: pyruvate carboxylase, PEP carboxykinase, fructose-1,6-bisphosphatephosphatase and glucose-6-phosphatephosphatase.

II.2.5. Glycogen metabolism

Excess glucose cannot be stored as it is because of its water solubility and increase in osmotic pressure. Therefore, it is deposited as an insoluble polymer – glycogen.

Glycogen is a macromolecule with molecular mass of 10^6-10^7 Da, consisting of 10 to 40 thousands glucose molecules linked through alpha 1-4 glycosidic bonds. The macromolecule has a structure with ramifications; after each linear structure of 10-12 glucose molecules there is a branching formed by an alpha 1-6 glycosidic bond. The ends of the branches are not reducing, ending with the –OH group at position 4.



General schematic representation of gluconeogenesis

The majority of glycogen is found in the liver, making up to 10% of the liver's mass, and also making up to 1% of the mass of skeletal muscles. These two tissues deposit glycogen for different purposes:

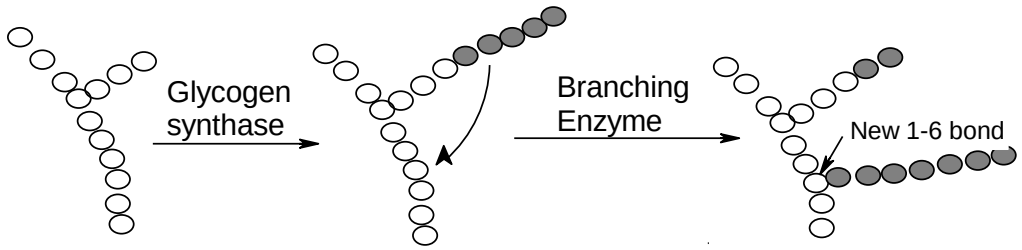
- The liver is synthesizing and depositing glycogen after a meal rich in sugars, which is used for obtaining glucose during starvation periods in order to maintain constant glycemic levels.
- Skeletal muscles deposit glycogen during resting periods and are using it during effort.

As a consequence, the glycogen molecule is always in a dynamic state, growing in size during anabolic states and shrinking during catabolic states.

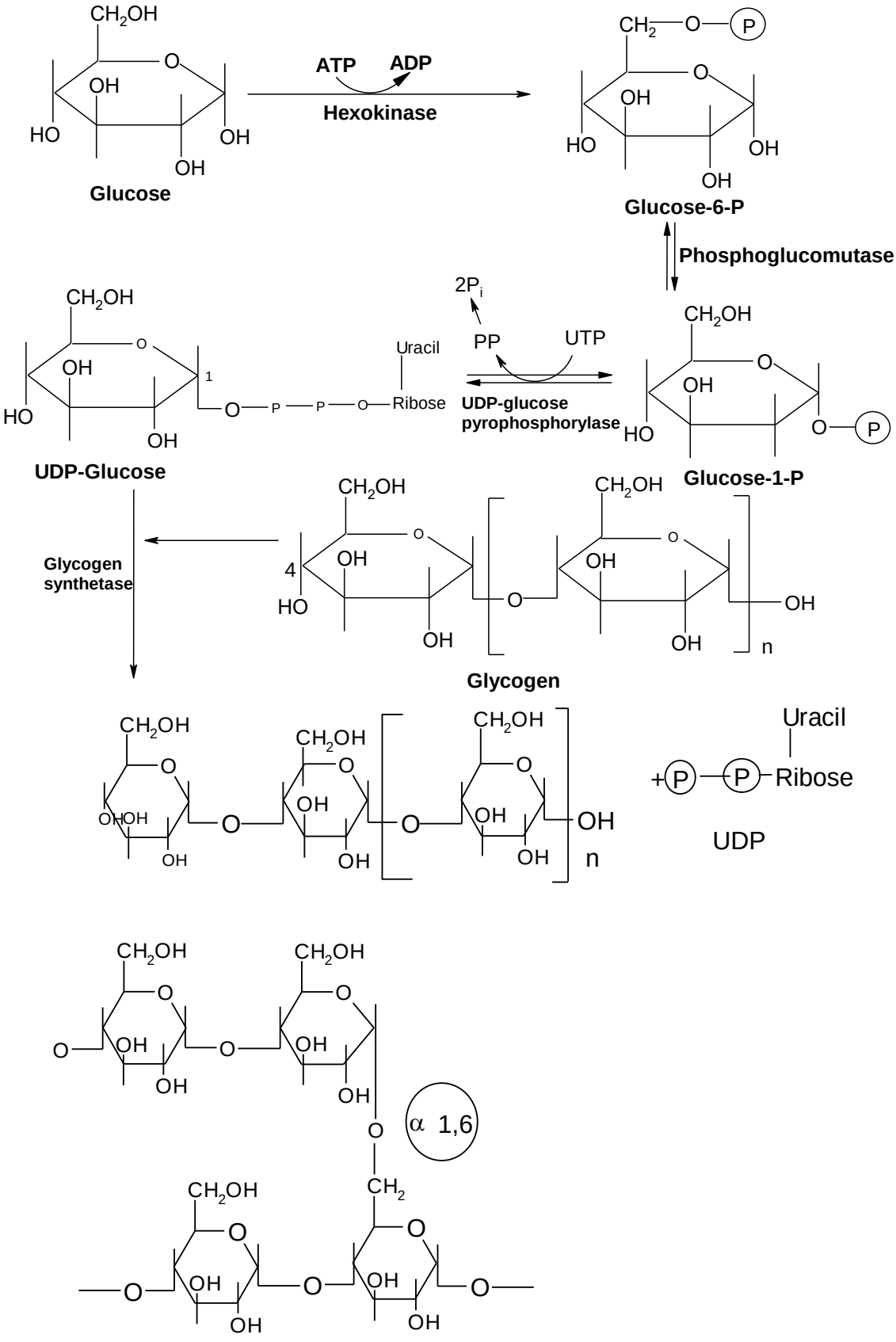
II.2.5.1. Glycogen synthesis

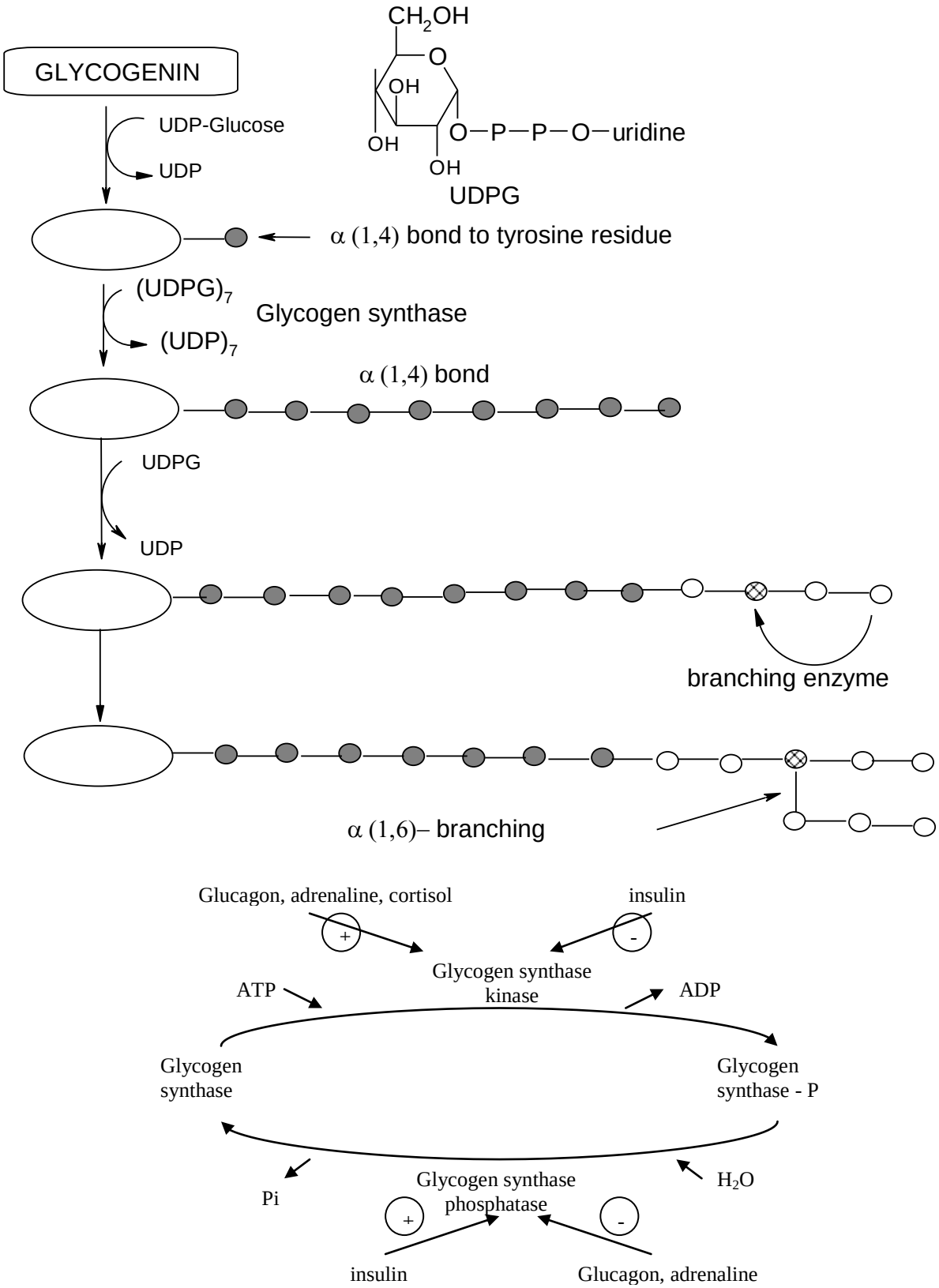
This process takes place by linking one activated glucose molecule in the form of uridine diphosphate glucose (UDP-glucose) to a non-reducing end (C4) of the glycogen molecule. Both synthesis and degradation of glycogen occurs at the surface of a protein support called GLYCOGENIN. This protein has the property of generating a primer glycogen molecule through the synthesis of an 8 glucose molecule primer, linked by alpha 1-4 glycosidic bonds (autoglycosylation reaction). The first glucose residue will bind to glycogenin through a glycosidic bond to an OH group from a tyrosine reside from glycogenin. From then on, glycogen synthesis occurs by binding the OH from C1 of UDP-glucose to the non-reducing end of the last glucose residue in the chain. Therefore, the non-reducing end of glycogen will be elongated each time with a glucose residue.

The main enzyme governing this process is **glycogen synthase** which catalyzes the formation of alpha 1-4 glycosidic bonds when new glucose residues are added. For the branching of the glycogen molecule, there is another enzyme called **glycogen branching enzyme**. This enzyme will transfer a string of 7 glucose molecules from one branch to the C6 of a glucose residue located within the glycogen molecule forming an alpha 1-6 glycosidic bond.



Glycogen synthase is allosterically regulated by phosphorylation-dephosphorylation, the active form of the enzyme being dephosphorylated. This process is catalyzed by a **phosphatase**, activated by insulin. The phosphorylation to the inactive form is catalyzed by a **kinase** activated by glucagon, adrenalin and cortisol.


Glucose
ATP
ADP
Hexokinase
Glucose-6-P
Phosphoglucomutase
UDP-Glucose
Uracil
Ribose
2Pi
PP
UTP
UDP-glucose pyrophosphorylase
Glucose-1-P
Glycogen synthetase
Glycogen
Uracil
Ribose
UDP
α 1,6



Schematic representation of glycogen synthesis and its regulation