1. Metabolism

The term metabolism describes the interconversion of chemical compounds in the body, the pathways used by different molecules, their interrelationships, and the mechanisms that control the flow of metabolites through these pathways. Normal metabolism includes adaptation to periods of starvation, exercise, gestation, and lactation. Abnormal metabolism can be caused by a nutritional or enzyme deficiency, abnormal hormone secretion, or the action of drugs or toxins.

Metabolic pathways are divided into three categories:

(1) **Anabolic pathways**: These pathways are involved in the synthesis of larger and more complex compounds from smaller precursors, for example, the synthesis of proteins from amino acids and the synthesis of triacylglycerol and glycogen stores. Anabolic pathways are endothermic.

(2) **Catabolic pathways**: which serve to destroy large molecules, generally involve oxidation processes; they are exothermic, produce reducing equivalents and ATP, mainly via the respiratory chain.

(3) **Amphibolic pathways**: such as the citric acid cycle, appear at the crossroads of metabolism and serve as links between anabolic and catabolic pathways.

2. Glycolysis or Embden-Meyeroff-Parnas pathway

2.1. Introduction

Most tissues have a minimal need for glucose. In the brain, this need is significant. Glycolysis, the main pathway for glucose metabolism, takes place in the cytosol of all cells. It is a unique process because it can be aerobic or anaerobic depending on the availability of oxygen and an electron transport chain. Glucose's ability to provide ATP in the absence of oxygen is crucial because it allows skeletal muscle to function at very high levels when oxygen supply is insufficient, and enables tissues to survive episodes of anoxia.

2.2. Entry of glucose into the cell

The main dietary polysaccharides, starch and glycogen, which are a glucose is a storage form of glucose from plants and animals, respectively. After food intake, the digestion of these plant and animal polysaccharides begins in the mouth, where they are broken down by salivary amylase, leading to the formation of oligosaccharides. Then, in the upper part of the small intestine, the pancreas and the digestive tract, through hydrolytic enzymes (such as pancreatic amylases and oligosaccharidases), break down these oligosaccharides into simple monosaccharides (glucose, fructose, and galactose).(Figure1) Subsequently, these monosaccharides enter enterocytes via membrane transporters. Glucose molecules enter enterocytes via protein transporters, as they are impermeable to the cell membrane, (the membrane is lipophilic).

Glucose absorption through the intestinal lumen occurs via membrane cotransporters coupled to ions(Figure 2),The sodium-glucose cotransporter type 1, or sodium-glucose linked transporter (SGLT-1), carries out active transport (requires ATP). Glucose transporters (GLUTs) are sodium-independent membrane transport proteins that transport monosaccharides, including glucose, fructose, and galactose, and facilitate diffusion (do not require ATP).

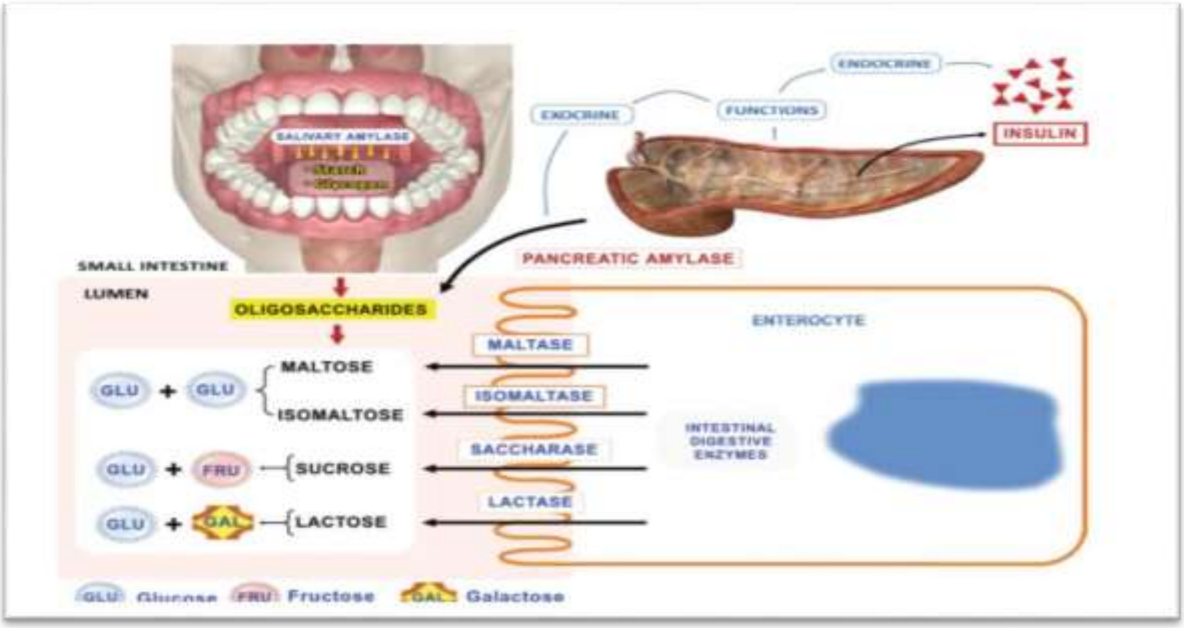

Figure 1: Breakdown of carbohydrates by pancreatic and intestinal enzymes.

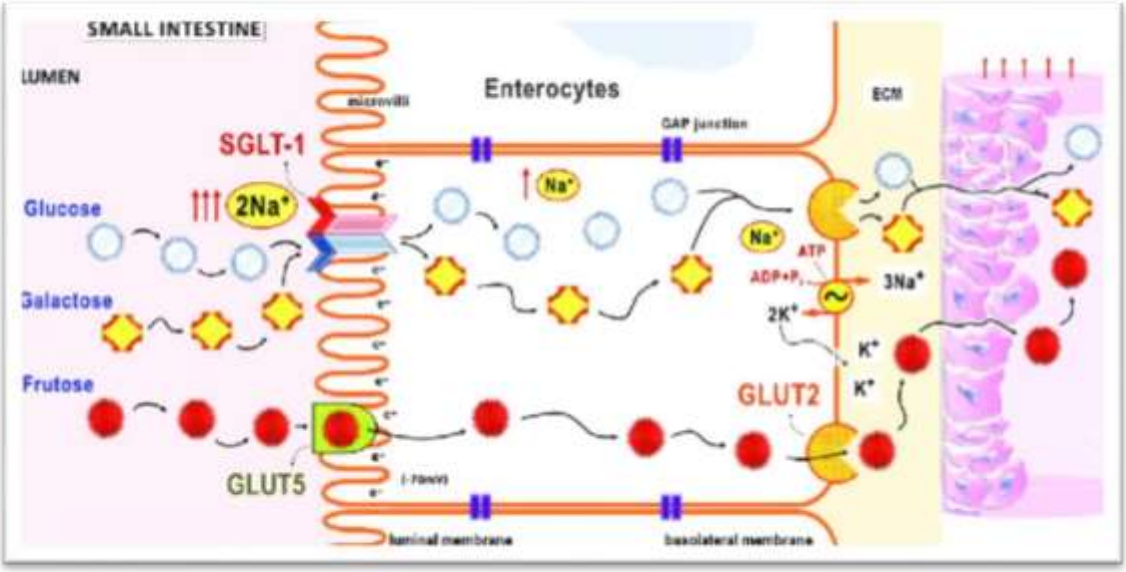

Figure 2:Glucose absorption by enterocytes.

2.3. Stages of glycolysis

2.3.1. Enzymatic steps of the first phase

2.3.1.1. Phosphorylation of glucose by ATP

All the enzymes of glycolysis are located in the cytosol. Glucose enters the glycolytic pathway through its phosphorylation to glucose 6-phosphate, catalyzed by hexokinase, which uses ATP as a phosphate donor. Under physiological conditions, the phosphorylation of glucose to glucose 6-phosphate can be considered irreversible.

2.3.1.2. Transformation of glucose 6-phosphate into fructose 6-phosphate

Glucose 6-phosphate is converted to fructose 6-phosphate by a phosphohexose isomerase, which involves an aldose-ketose isomerization.

2.3.1.3. Phosphorylation of F-6-P to fructose 1,6-bisphosphate

This reaction is followed by another phosphorylation catalyzed by phosphofructokinase (phosphofructokinase-1), yielding fructose 1,6-bisphosphate. The phosphofructokinase-catalyzed reaction can be considered irreversible.

2.3.1.4. Fructose 1,6-bisphosphate cleavage

Fructose 1,6-bisphosphate is cleaved by an aldolase (fructose 1,6-bisphosphate aldolase) into two triose phosphates: glyceraldehyde 3-phosphate and dihydroxyacetone phosphate.

2.3.1.5. Triose phosphate interconversion

Glyceraldehyde 3-phosphate and dihydroxyacetone phosphate are interconverted by an enzyme, phosphotriose isomerase.

3.1.2. Enzymatic steps of the second phase

3.1.2.1. Oxidation of 3-phosphoglyceraldehyde to 1,3-bisphosphoglycerate

Glycolysis continues with the oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate. The enzyme that catalyzes this reaction, glyceraldehyde 3- phosphate dehydrogenase, is NAD-dependent.

3.1.2.2. Phosphate transfer on ADP

In the following reaction, catalyzed by phosphoglycerate kinase, phosphate is transferred from 1,3-bisphosphoglycerate to ADP to form ATP (substrate-level phosphorylation) and 3-phosphoglycerate.

3.1.2.3. Isomerization of 3-phosphoglycerate to 2-phosphoglycerate

3-Phosphoglycerate is isomerized to 2-phosphoglycerate by phosphoglycerate mutase.

3.1.2.4. Dehydration of 2-Phosphoglycerate to phosphoenolpyruvate

The next step is catalyzed by enolase and involves dehydration to form phosphoenolpyruvate.

3.1.2.5. Transfer of phosphate from phosphoenolpyruvate to ADP

Phosphate from phosphoenolpyruvate is transferred to ADP by pyruvate kinase, to generate two ATP molecules per glucose molecule oxidized.

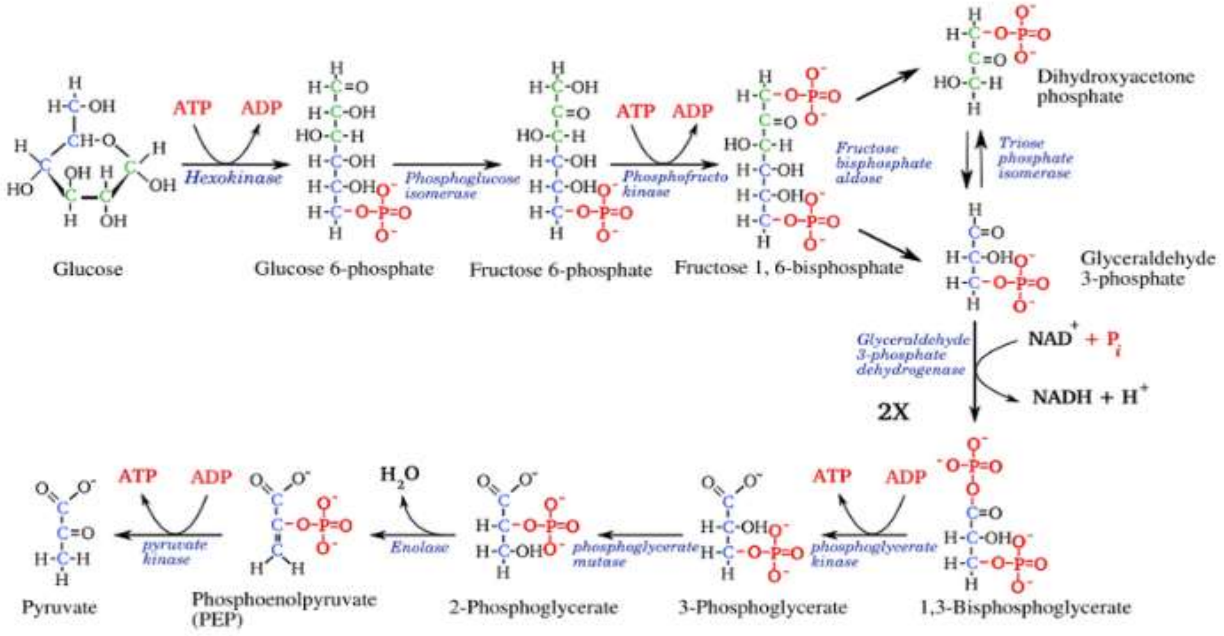

Figure 3: Stages of glycolysis.

4. Energy balance of glycolysis

Glucose + 2 ADP + 2 Pi + 2 NAD^+ $\longrightarrow$ 2 Pyruvate + 2 ATP + 2 $NADH_2$

5. NAD^+ cytosolic regeneration

Pyruvate is then reduced to lactate using $NADH_2$. This reaction is catalyzed by lactate dehydrogenase. The reoxidation of $NADH_2$ via the formation of lactate allows Glycolysis can continue in the absence of oxygen by regenerating sufficient NAD^+ for another reaction cycle catalyzed by glyceraldehyde 3-phosphate dehydrogenase.

6. Shuttles systems of glycolytic NADH transportation

Under aerobic conditions, pyruvate is used in the mitochondria and, after conversion to acetyl CoA, is oxidized to CO_2 through the citric acid cycle. $NADH_2$ is continuously produced in the cytosol by glyceraldehyde 3-phosphate dehydrogenase, an enzyme of the glycolytic pathway. However, under aerobic conditions, $NADH_2$ extramitochondrial molecules do not accumulate and are used in the mitochondria for oxidation by the respiratory chain via shuttles because they cannot penetrate the mitochondrial membrane. The transfer mechanism using the shuttle system:

6.1.Glycerol 3-phosphate shuttle

The transfer of reducing equivalents across the mitochondrial membrane occurs via pairs of substrates linked by appropriate dehydrogenases present on both sides of the mitochondrial membrane. The glycerol 3-phosphate shuttle is composed of two enzymes, cytosolic glycerol 3-phosphate dehydrogenase and mitochondrial glycerol 3-phosphate dehydrogenase (cGPDH and mGPDH, respectively). Electrons are transferred from NAD^+ When dihydroxyacetone phosphate is reduced to glycerol 3-phosphate by cGPDH. On the outer surface of the inner mitochondrial membrane, glycerol 3-phosphate is reoxidized to dihydroxyacetone phosphate by mGPDH, by binding to a prosthetic FAD group (Figure 4).

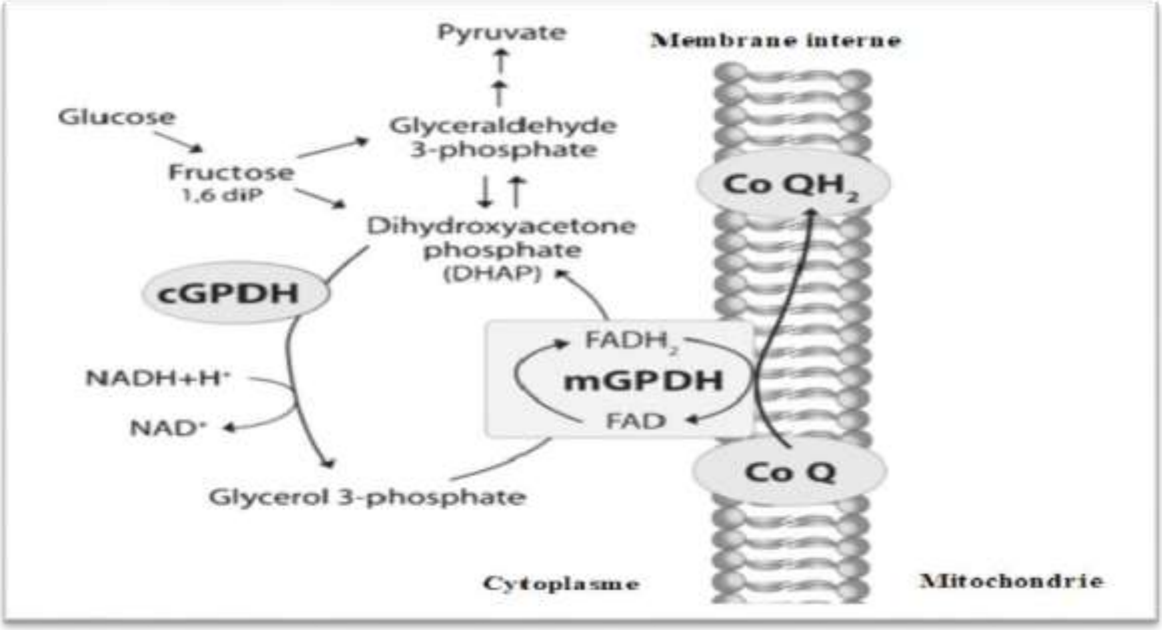

Figure 4: Glycerol 3-phosphate shuttle.

6.1.Malate-Aspartate Shuttle

The malate-aspartate shuttle is composed of two antiporter transporters (aspartate glutamate) and (malate-α-ketoglutarate), which are used for the transfer of the reducing equivalents NADH, H^+ products of glycolysis inside the mitochondria (they do not cross the mitochondrial membrane) (Figure 5). The complexity of this system is due to the impermeability of the mitochondrial membrane to oxaloacetate, which must react with glutamate for transamination into aspartate and α-ketoglutarate before transport across the mitochondrial membrane.

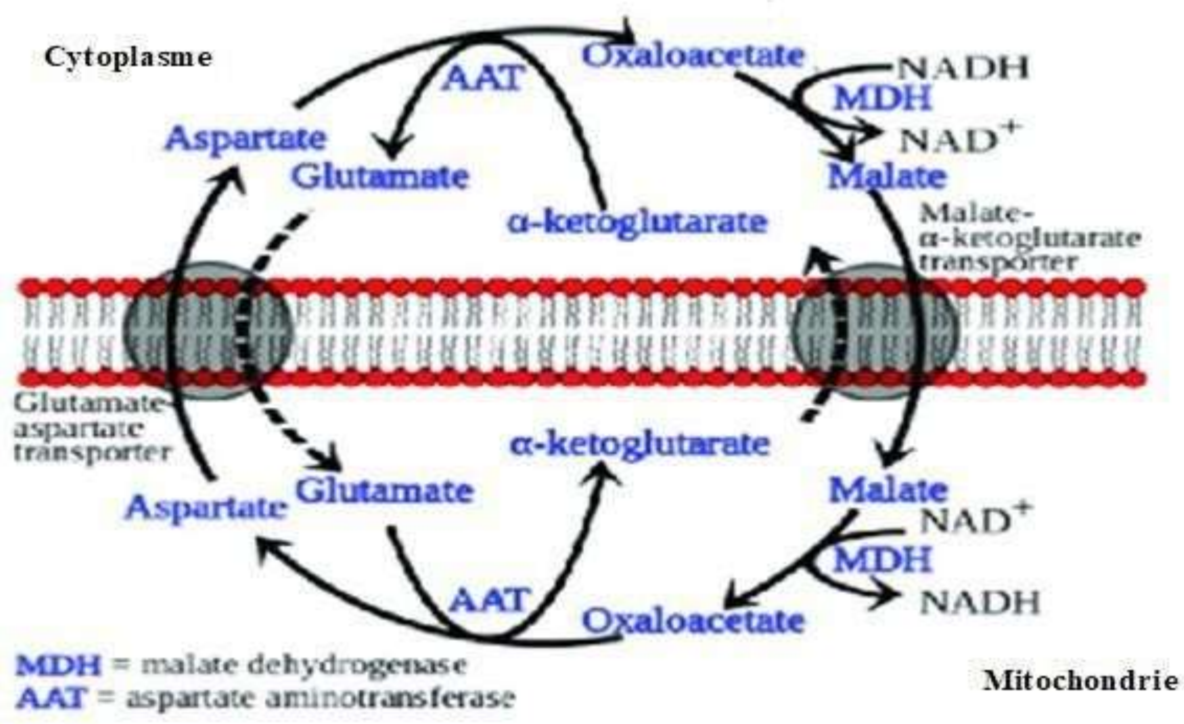

Figure 5: Malate-Aspartate Shuttle.

7.Glycolysis is a source of biosynthetic precursors

- **Glucose 6-phosphate**

Glucose 6-phosphate is an important compound found at the junction of several metabolic pathways: glycolysis, gluconeogenesis, the pentose phosphate pathway (glucuronate synthesis), glycogenesis, glycogenolysis, and ribose synthesis. Thus, in muscle, glucose-6-phosphate can also be formed from glucose-1-phosphate derived from glycogen; finally, it can come from other sugars such as galactose or mannose. In muscle glucose-6 P is degraded via the glycolytic pathway, while in the liver it essentially gives glucose under the action of glucose-6 phosphatase, a microsomal enzyme involved in gluconeogenesis or gluconeogenesis.

- **Pyruvate**

Pyruvate can give rise to other molecules depending on the availability of oxygen (presence or absence), energy requirements, and cellular enzymatic equipment.

- Oxidation of pyruvate to CO_2 and H_2O.
- Reduction of pyruvate to lactate (lactic acid fermentation) by lactate dehydrogenase (Figure 6).
- Transformation of pyruvate to acetaldehyde by pyruvate decarboxylase, followed by the transformation of acetaldehyde to ethanol by alcohol dehydrogenase (alcoholic fermentation) (Figure 6).
- Carboxylation of pyruvate to oxaloacetate (gluconeogenesis reaction).
- Acetyl CoA formation (synthesis of fatty acids and lipids).
- Synthesis of alanine (transamination).

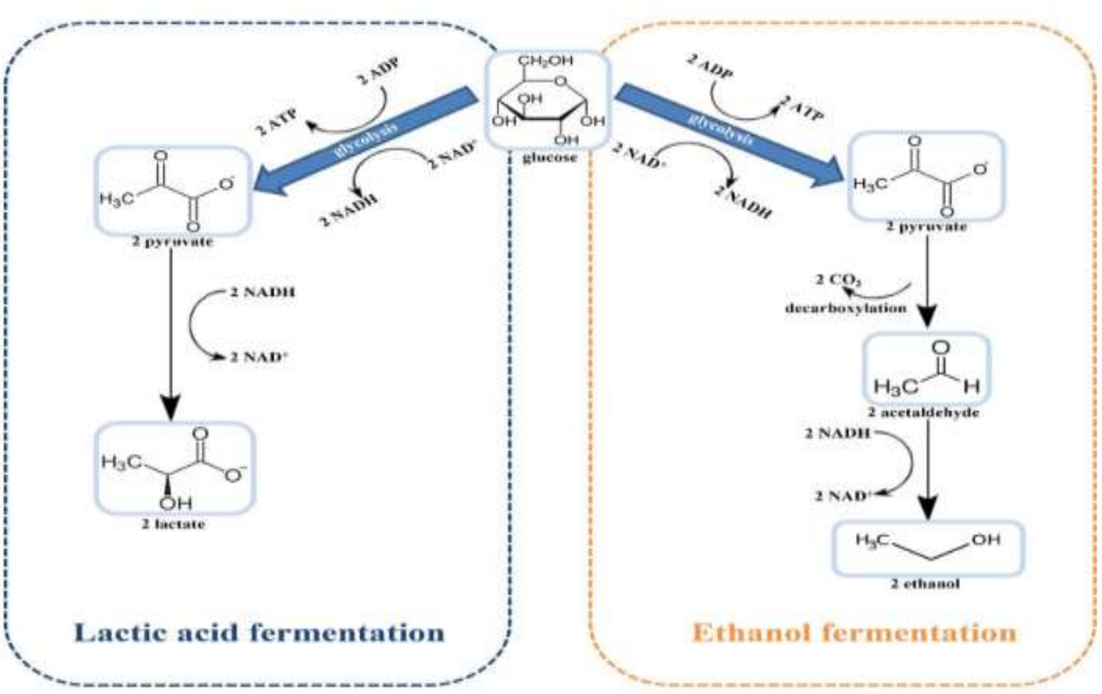

Figure 6: Lactic and alcoholic fermentation.

- **Phosphoenolpyruvate**

- Synthesis of phenylalanine, tyrosine, tryptophan (amino acid synthesis).

- **Dihydroxyacetone phosphate**

- Glycerol-3-phosphate synthesis (synthesis of triglycerides and phospholipids).

8.Entry of other carbohydrates into glycolysis

Food provides an abundance of glycogen and dietary starch (polysaccharides), which are acted upon by hydrolytic enzymes, releasing glucose, which is then phosphorylated into glucose 6-phosphate. Disaccharides (sucrose, lactose, maltose) contain, respectively, a fructose unit, a galactose unit, or a glucose unit linked to a glucose molecule (under the action of specific enzymes secreted by the intestinal mucosa (Figure 7).

The galactose resulting from the hydrolysis of lactose is converted into glucose-6P by a series of three reactions:

- It is phosphorylated by ATP to galactose-1P by the action of galactokinase.
- Phosphogalactose-uridyl transferase catalyzes the transfer of galactosyl from galactose-1P to UDP-glucose with the release of glucose-1P.
- The third reaction, catalyzed by UDP-galactose-4 epimerase, is an epimerization of galactosyl to glucosyl by inversion of the configuration of the hydroxyl in position 4.

Fructose from the hydrolysis of sucrose is converted into glyceraldehyde 3-phosphate through a series of four reactions:

- It is phosphorylated by ATP to fructose-1P under the action of fructokinase.
- Fructose 1 phosphate is cleaved by aldolase 2 (fructose-1-phosphate aldolase) into two triose phosphates: glyceraldehyde and 3-dihydroxyacetone phosphate.
- Phosphorylation of glyceraldehyde to glyceraldehyde-3 phosphate by glyceraldehyde kinase
- Isomerization of 3-dihydroxyacetone phosphate to glyceraldehyde-3-phosphate by phosphotriose isomerase).

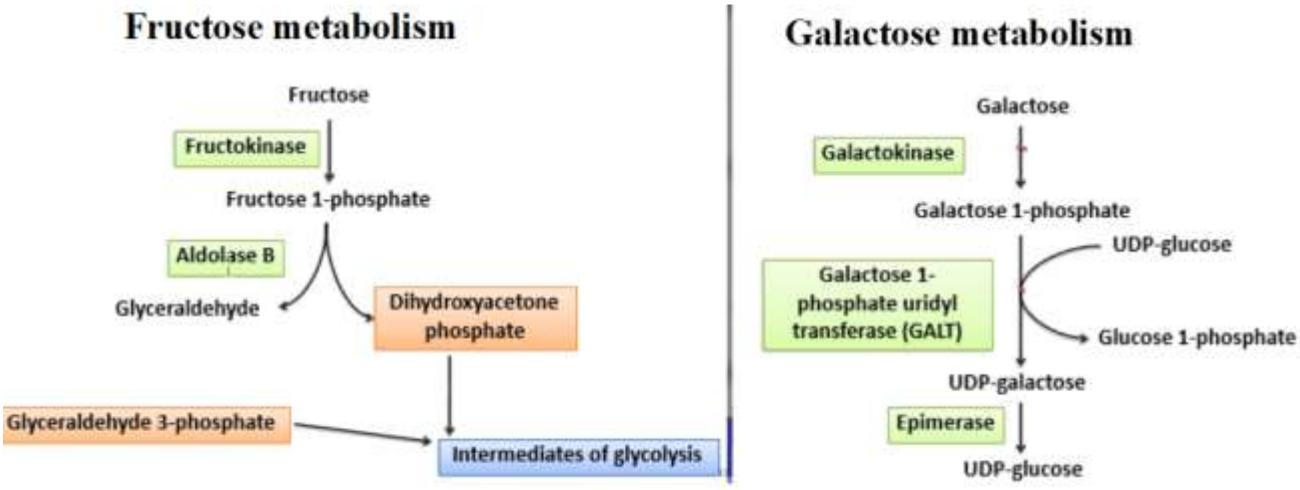

Figure 7: Entry of other carbohydrates into glycolysis

9.Regulation of glycolysis

Glycolysis is regulated by two types of mechanisms: allosteric regulation and hormonal regulation

9.1.Allosteric regulation

In metabolic pathways, the enzymes that catalyze essentially irreversible reactions are potential control sites. Irreversible reactions in glycolysis occur at the level of three enzymes: hexokinase, phosphofructokinase, and pyruvate kinase.

- **Hexokinase**

Hexokinase is allosterically inhibited by its product, glucose 6- phosphate.

- **Glucokinase**

Liver cells also contain an isoenzyme of hexokinase, glucokinase, which has a Km much higher than the normal intracellular concentration of glucose.

- **Phosphofructokinase-1**

Under physiological conditions, it is both inducible and subject to allosteric control. It plays an important role in regulating the rate of glycolysis. PFK1 is an enzyme allosteric. Its activity is inhibited by high concentrations of ATP. The protein has two types of ATP binding sites: the active site and a regulatory site (allosteric site). Inhibition occurs through excess substrate and also through a final product of the reaction (or feedback inhibition), since ATP is a product of glycolysis.

The citrate an intermediate in the Krebs cycle, which can be considered an indicator of energy status, also inhibits this reaction. Conversely, ADP and AMP, phosphate acceptors, are activator of the enzyme. fructose-2,6-bisphosphate (F 2, 6 bi P) is produced by the phosphorylation of fructose 6-phosphate by the active phosphofructokinase 2 (PKF2) PFK1(Figure 8).

Pyruvate kinase

The liver enzyme is tightly regulated; as an allosteric enzyme, it is stimulated by fructose-1,6-bisphosphate. The activity of liver pyruvate kinase is inhibited by ATP and alanine (an amino acid involved in gluconeogenesis).

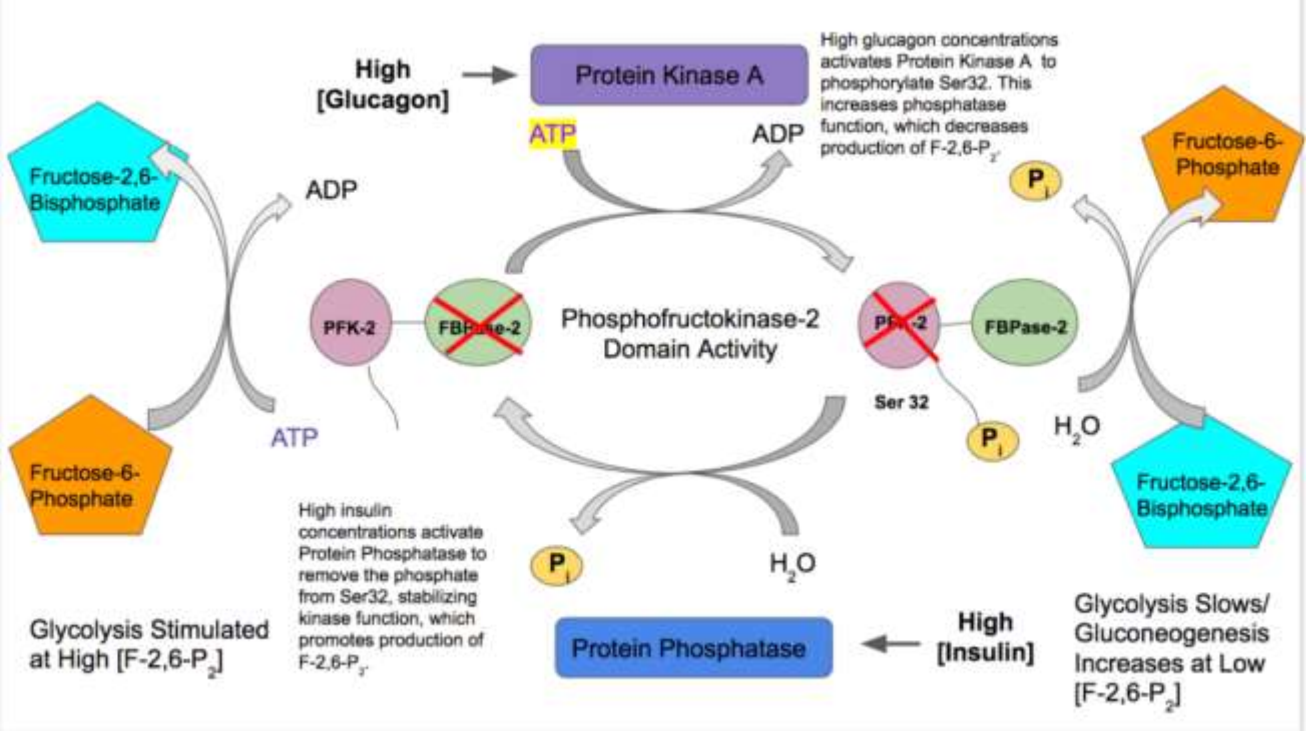

Figure 8:PFK1 Regulation

9.2.Hormonal regulation

Phosphofructokinase-1

It has been shown that, in the liver, glycolysis is regulated by insulin.fructose-2,6 bisphosphate glucagon stimulates phosphofructokinase 1. The phosphorylation of fructose-6-phosphate to fructose-2,6-bisphosphate by phosphofructokinase 2 (PFK2) and the hydrolysis of fructose-2,6- bisphosphate to fructose-6-phosphate by fructose-2,6-bisphosphatase 2 (FBP2) are carried out by the same peptide chain. Thus, in the liver, glucagon, via cAMP-dependent protein kinase, induces the phosphorylation of this protein, which stimulates FBP2 activity and inhibits PFK2. Consequently, glucagon has a negative effect on glycolysis (Figure 8).

Pyruvate Kinase

Several pyruvate kinase isoenzymes, which are distributed differently in tissues, have been characterized in mammals. The hepatic pyruvate kinase enzyme (L-form) is phosphorylated by a cAMP-dependent protein kinase and dephosphorylated by a phosphatase.

Phosphorylation inhibits glycolysis.

10. Cori Cycle

During skeletal muscle exercise, where anaerobic glycolysis predominates, rapid catalysis occurs, driven by lactate dehydrogenase. Lactate is rapidly excreted and enters the bloodstream, where it is largely converted back to pyruvate in the liver via lactate dehydrogenase. The pyruvate not catalyzed to acetyl-CoA can be converted to oxaloacetate, which then enters the gluconeogenesis pathway. This cycle is called the Cori cycle (Figure 9).

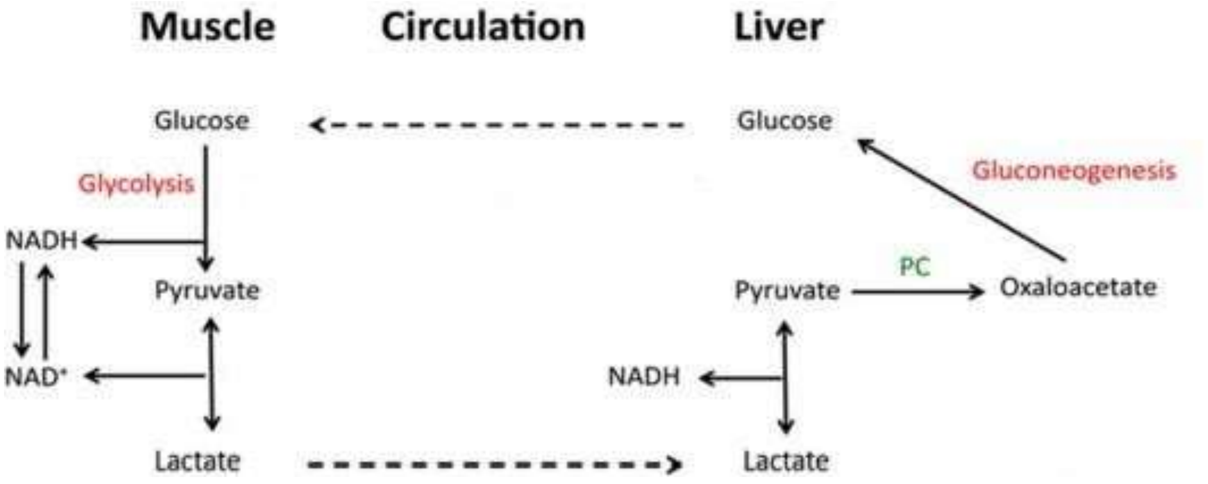

Figure 9: Cori cycle. PC: pyruvate carboxylase.

11. Anaerobic glycolysis

Erythrocytes

Erythrocytes, which lack mitochondria, are entirely dependent on glucose as a metabolic fuel, which they metabolize via anaerobic glycolysis. However, the oxidation of glucose beyond pyruvate, the final stage of glycolysis, requires molecular oxygen and mitochondrial enzyme systems such as the pyruvate dehydrogenase complex, the citric acid cycle, and the respiratory chain.

Muscles

Muscle can contract in an anaerobic environment when deprived of oxygen; glycogen is depleted and lactate is released. The presence of oxygen restores aerobic conditions, and the lactate disappears. However, if the contraction occurs under aerobic conditions, lactate does not accumulate, and pyruvate becomes the primary end product of glycolysis. Pyruvate is oxidized to CO_2 and water. If oxygen becomes scarce, mitochondrial reoxidation of NADH formed during glycolysis does not occur; NADH is then reoxidized by reduction of pyruvate to lactate to promote the continuation of glycolysis.

The overall equation for glycolysis, from glucose to lactate, is as follows:

$$\text{Glucose} + 2\ \text{ADP} + 2\ \text{Pi} \longleftrightarrow \text{Lactate} + 2\ \text{ATP} + 2\ H_2O$$

12.Pathologies related to glycolysis

12.1.Hemolytic anemia

The example of pyruvate kinase deficiency: Red blood cells do not possess mitochondria and depend exclusively on glycolysis for their ATP supply. This leads to impaired glycolysis and insufficient ATP production, necessary for maintaining their function and membrane structure. The membrane becomes deformed, and the red blood cells are prematurely phagocytosed by macrophages, resulting in hemolytic anemia..

12.2.Lactic acidosis

Anaerobic glycolysis is the first step in glucose metabolism and occurs in the cytoplasm of all cells. Under anaerobic conditions, such as during hypoxia, cells are forced to use anaerobic glycolysis as their sole source of energy production. Intracellular lactate concentration increases. If cellular hypoxia is generalized, lactate cannot be converted into pyruvate or glucose (by gluconeogenesis). It then accumulates in the blood with hydrogen ions, leading to the development of lactacidemia.