6. Gluconeogenesis pathway

Gluconeogenesis is an endogenous metabolic pathway in which non carbohydrate substances are converted to glucose. The term gluconeogenesis means the generation (genesis) of new (neo) glucose. This biochemical pathway occurs principally in the liver and is essential for survival during prolonged starvation. This process is essential for long-term muscle work, and when the body consumes more fats and proteins than carbohydrates. It also plays a major role in the disposal of lactate and the maintenance of glucose during exercise. The liver plays the central role in this process, while it is believed that the kidneys contribute to 25 % of total glucose synthesis at most.

The production of glucose from other metabolites is necessary to maintain the glucose level in the blood as a fuel source by the brain, erythrocytes, kidney medulla and testes, since glucose is the sole energy source for these organs (Figure18). During starvation, pancreatic -cells release glucagon in response to low levels of glucose. Glucagon stimulates fat mobilization from adipose tissue to skeletal muscle for β-oxidation to release energy. Glycerol released from lipolysis, amino acids from muscle protein breakdown, and lactate generated from muscle and red blood cells are transported through the circulation to the liver where they serve as substrates for gluconeogenesis and production of glucose. The glucose produced provides fuel for erythrocytes and the brain. The adipose tissue needs glucose, which is also necessary for the synthesis of triacylglycerls.

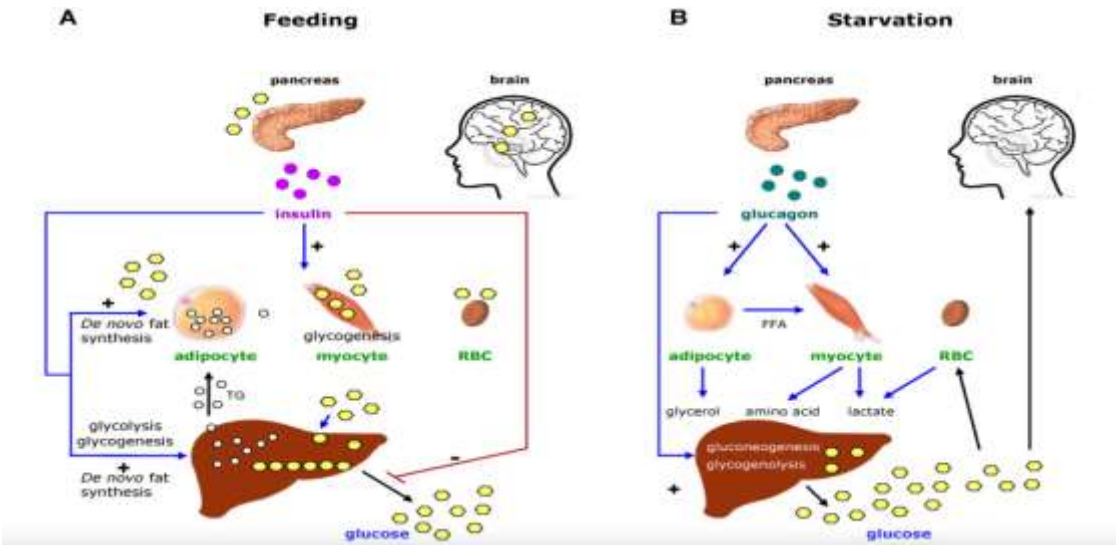

Figure 18: Hormonal regulation of fuel during starvation and feeding.

FFA: free fatty acids; **RBC**: red blood cell; **TG**: triglycerides.

6.1. Enzymes of Gluconeogenesis

Seven reversible steps in gluconeogenesis are catalyzed by the same enzymes used in glycolysis. Gluconeogenesis requires four additional enzymes which bypass the three irreversible reactions catalyzed by the glycolytic enzymes pyruvate kinase, phosphofructokinase and hexokinase. These four gluconeogenic enzymes are pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1, 6-bisphosphatase and glucose-6-phosphatase. The gluconeogenic pathway needs to:

- Conversion of pyruvate to PEP by the enzymes: pyruvate carboxylase (PC), mitochondrial malate deshydrogenase, cytosolic malate deshydrogenase and phosphoenolpyruvate carboxykinase (PCK),
- Dephosphorylation of fructose 1, 6-bisphosphate by Fructose 1, 6 biphosphatase,
- Dephosphorylation of glucose 6-phosphate by Glucose 6 phosphatase

The first two reactions occur in the mitochondrial matrix, whereas the following reactions take place in the cytosol. The reactions of gluconeogenesis are presented in Figure 19.

6.1.1. Conversion of pyruvate to PEP

6.1.1.1. Pyruvate carboxylase

Pyruvate carboxylase (PC) is a mitochondrial enzyme in the ligase class that catalyzes the irreversible carboxylation of pyruvate to oxaloacetate in the metabolic pathway of gluconeogenesis. The reaction is dependent on biotin, adenosine triphosphate (ATP) and magnesium. Acetyl-CoA is the allosteric effector of PC in humans. PC catalyzes the following reaction:

$$\text{Pyruvate} + CO_2 + \text{ATP} \longrightarrow \text{oxaloacetate} + \text{ADP} + \text{Pi}$$

6.1.1.2. Mitochondrial malate deshydrogenase

Oxaloacetate is not able to cross the inner mitochondrial membrane; however, malate can permeate it, which influences the manner in which gluconeogenesis takes place. Oxaloacetate is reduced to malate by using NADH and mitochondrial malate dehydrogenase. Mitochondrial malate deshydrogenase catalyzes the following reaction:

$$\text{Malate} + \text{NADH}, H^+ \longrightarrow \text{oxaloacetate} + \text{NAD}+$$

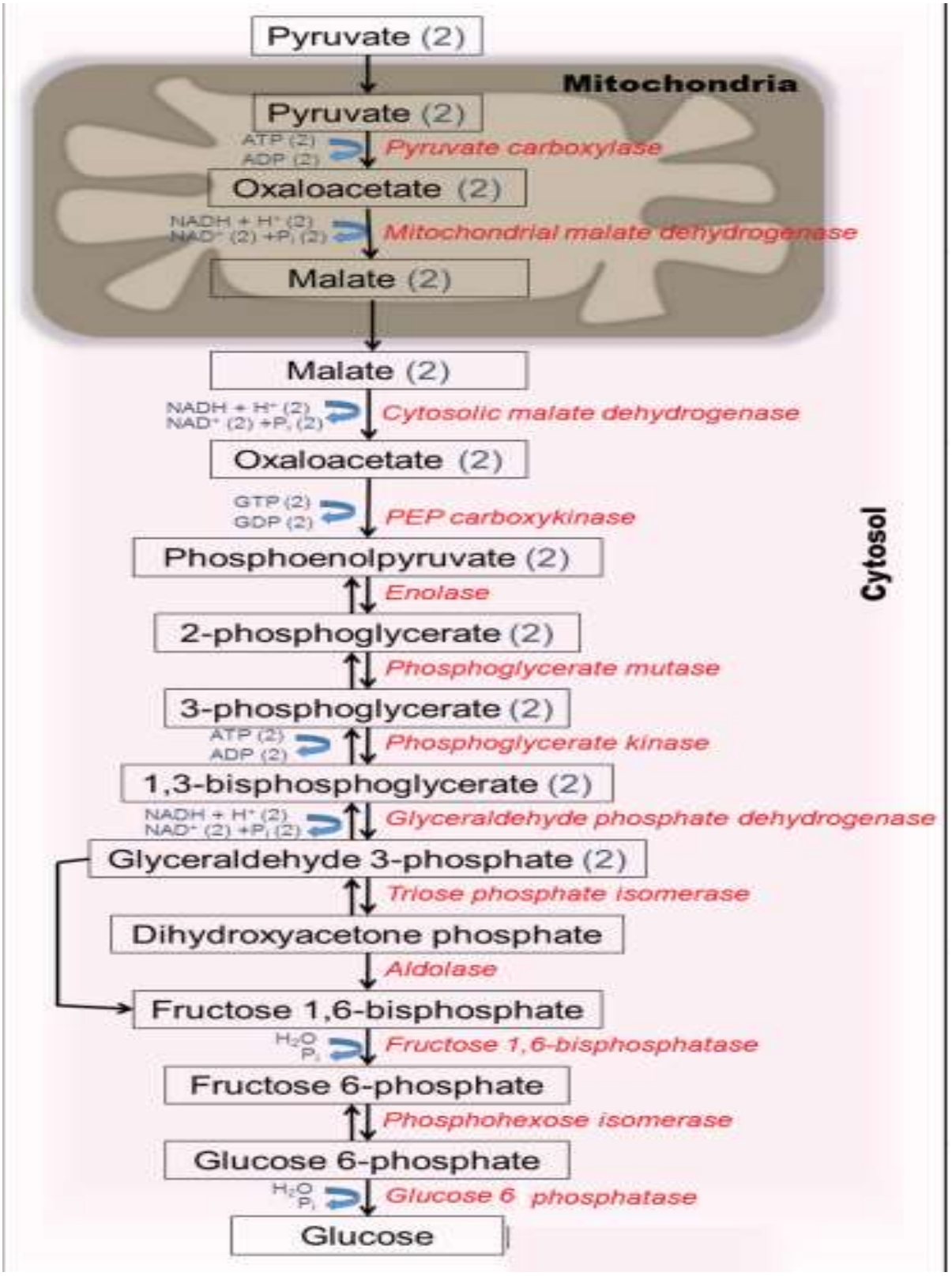

Figure 19: Stages of gluconeogenesis

6.1.1.3. Cytosolic malate deshydrogenase

After this step, the remaining steps of gluconeogenesis process occur in the cytosol. In the next step, malate is oxidized to oxaloacetate using NAD^+ and the cytosolic isozyme of malate dehydrogenase. Cytosolic malate deshydrogenase catalyzes the following reaction:

$$\text{Oxaloacetate} + NAD^+ \longrightarrow \text{Malate} + NADH, H^+$$

6.1.1.4. Pyruvate carboxykinase

Pyruvate carboxykinase (PCK) is an enzyme in the lyase family that converts oxaloacetate into phosphoenolpyruvate and carbon dioxide. (PCK) catalyzes the following reaction:

$$\text{Oxaloacetate} + GTP \longrightarrow \text{Phosphoenolpyruvate} + GDP + CO_2$$

6.1.2. Dephosphorylation of fructose 1, 6-bisphosphate

6.1.2.1. Fructose 1, 6 biphosphatase

Fructose 1, 6 biphosphatase (FBPase) is a cytosolic enzyme that catalyzes the dephosphorylation of fructose 1, 6-bisphosphate to fructose 6-phosphate and inorganic phosphate in gluconeogenesis. Fructose 1, 6 biphosphatase catalyzes the following reaction:

$$\text{Fructose 1, 6-bisphosphate} + H_2O \longrightarrow \text{fructose 6-phosphate} + Pi$$

6.1.3. Dephosphorylation of glucose 6-phosphate

6.1.3.1. Glucose 6 phosphatase

Glucose 6 phosphatase is an enzyme situated in the endoplasmic reticulum and hydrolyzes glucose 6-phosphate to produce glucose and inorganic phosphate. Only the liver and kidney contain all the enzymes necessary for gluconeogenesis, including glucose-6-phosphatase, which enables the release of glucose. Glucose 6 phosphatase catalyzes the following reaction:

$$\text{Glucose 6-phosphate} + H_2O \longrightarrow \text{glucose} + Pi$$

6.2. Precursors

The major substrates for gluconeogenesis are lactate, glycerol, and gluconeogenic amino acids (Figure 20).

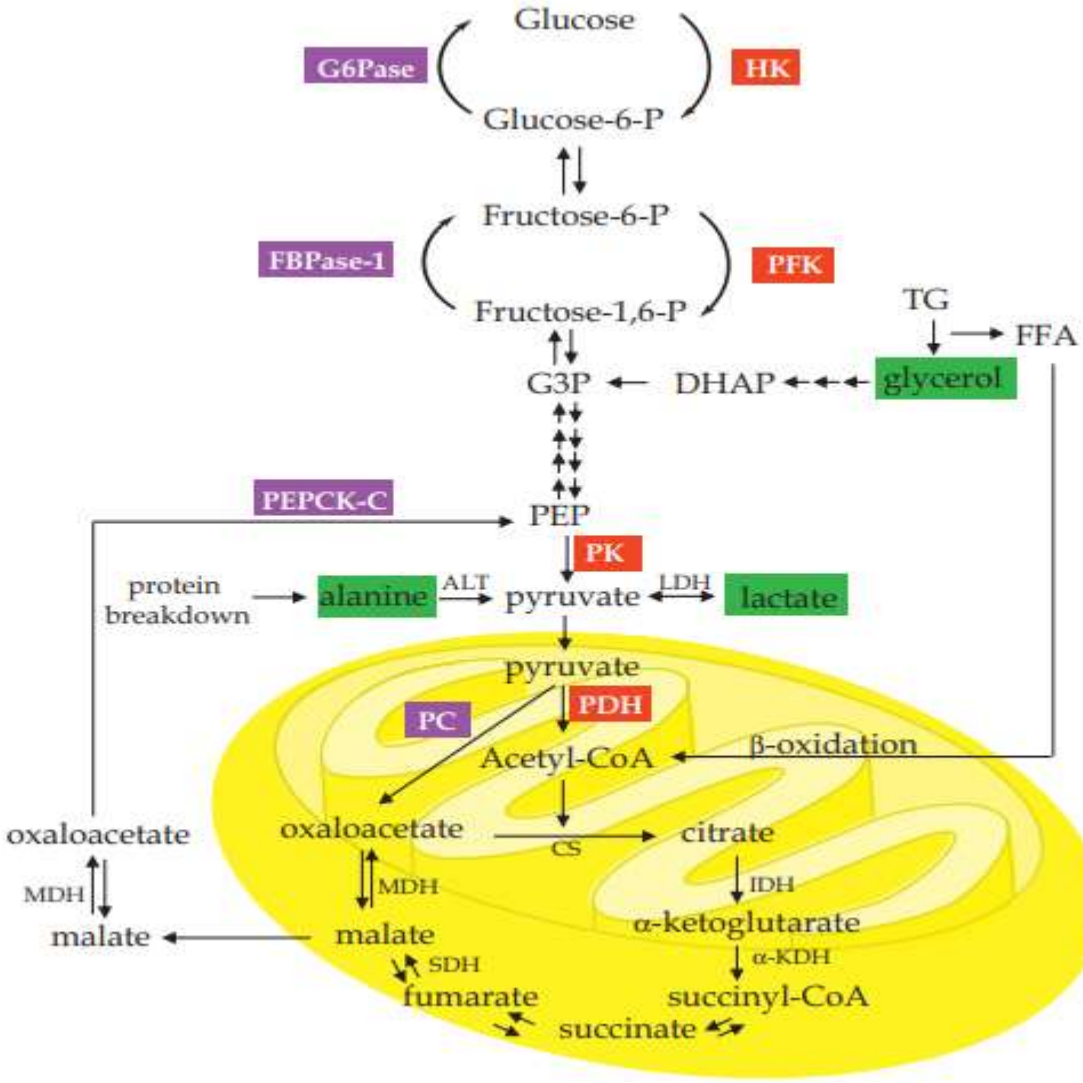

Figure 20: Main precursors for gluconeogenesis**.**

MDH: mitochondrial malatedehydrogenase; **PEP**: phosphoenolpyruvate; **PEPCK**: phosphoenolpyruvate carboxykinase; **FBPase**: fructose-1, 6-bisphosphatase; **G6Pase**: glucose-6-phosphatase; **PFK**: phosphofructokinase; **HK**: hexokinase. **TG**: triglycerides; **DHAP**: dihydroxyacetonephosphate; **ALT**: alanine aminotransferase; **CS**: citrate synthase; **G3P**: glyceraldehyde-3-phosphate; **IDH**: isocitrate dehydrogenase; **KDH**: α-ketoglutarate dehydrogenase; **LDH**: lactate dehydrogenase; **PDH**: pyruvate dehydrogenase complex; **PK**: pyruvate kinase; **SDH**: succinate dehydrogenase.

6.2.1. Lactate and Cori cycle

The main source of lactate is anaerobic glycolysis of red blood cells and the skeletal muscle. This cycle (glucose → pyruvate → lactate → pyruvate → glucose) is known as the Cori cycle (Figure 21). Lactate, formed by the oxidation of glucose in skeletal muscles and by erythrocytes through the proceses of anaerobic glycolysis, is transported to the liver and kidney, where it reforms glucose, which again become available via the circulation for oxidation in the tissues.

The conversion of lactate to glucose begins with the oxidation of lactate, by the action of *lactate dehydrogenase*, to pyruvate. In the presence of ATP, pyruvate carboxylase and CO_2 convert pyruvate to oxaloacetate. The enzyme, phosphoenolpyruvate carboxykinase transfers oxaloacetate to phosphoenolpyruvate in the presence of GTP and by elimination of CO_2.

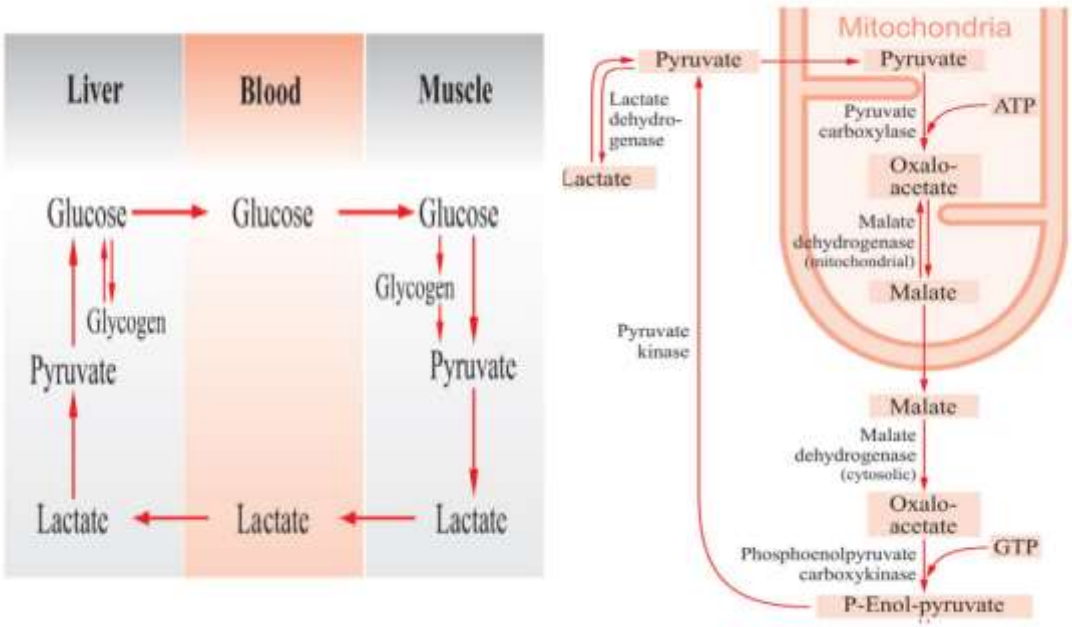

Figure 21: Cori cycle

6.2.2. Glycerol

The contribution of glycerol to gluconeogenesis is directly correlated with its release from the adipose tissue together with fatty acids. Glycerol, a product of the continual lipolysis, diffuses out of the tissue into the blood. It is converted back to glucose by gluconeogenic mechanisms in the liver and kidney. Thus, a continuous cycle exists in which glucose is transported from the liver to adipose tissue and, hence, glycerol is returned to be synthesized into glucose by the liver. Glycerokinase, which requires ATP, catalyzes the conversion of glycerol to glycerol phosphate. Glycerokinase is present in liver and kidney. The enzyme glycerol phosphate dehydrogenase oxidizes glycerol phosphate to the dihydroxacetone phosphate, the component of glycolysis, which enters the glycolytic pathway as a substrate for triose phosphate isomerase (Figure 22).

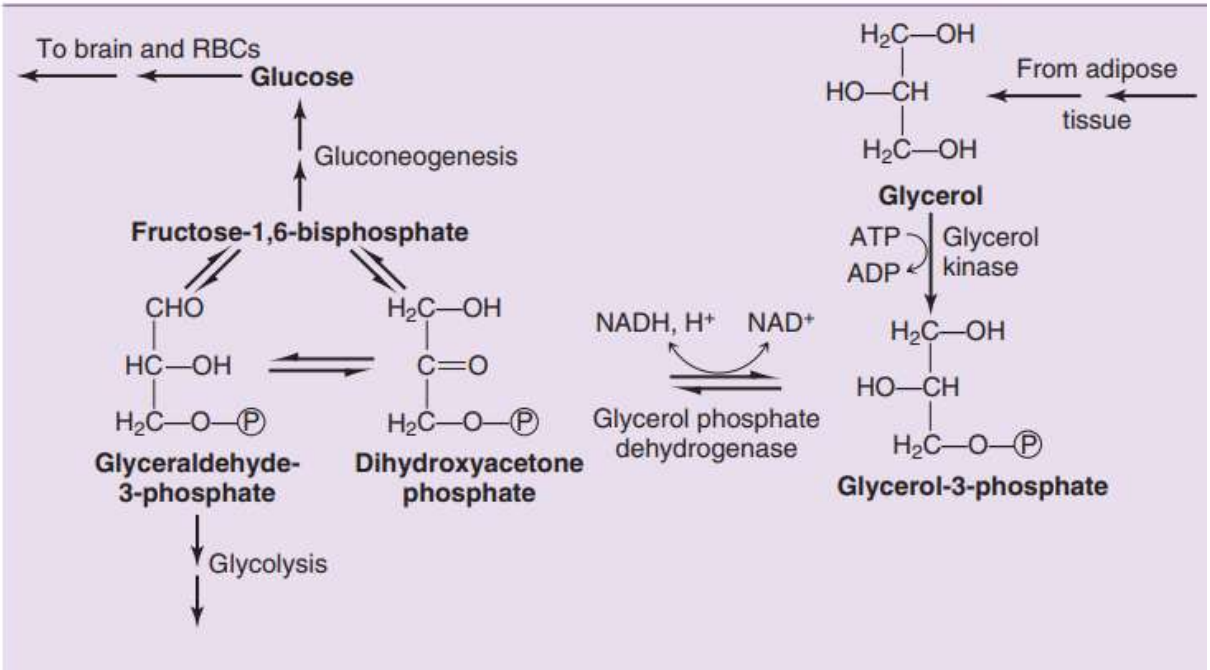

Figure 22: Glycerol enters gluconeogenesis (and glycolysis) at the level of the triose phosphates. **RBC:** Red blood cell

6.2.3. Gluconeogenic amino acids

Gluconeogenic amino acids are mostly those that can be converted via pyruvate or intermediates of citric-acid cycle to oxaloacetate, which is the initial substrate of gluconeogenesis. The most important amino acids are alanine and glutamine, which are released from muscles during starvation, exercise. As most of the nitrogen produced during amino acid catabolism is converted into ammonia.

6.2.3.1. Alanine

Glucose-alanine cycle (Figure 23) represents a cycling glucose from the liver to the muscles and alanine from muscles to liver, effecting a net transfer of amino nitrogen from muscle to liver and free energy from liver to muscle. At the level of muscles, pyruvate, formed by glycolysis, transforms to alanine by the action of alanine transaminase (ALT) or glutamate pyruvate transaminase (GPT). The reaction is freely reversible. At the level of hepatocytes alanine transfers to pyruvate by the action of the same enzyme.

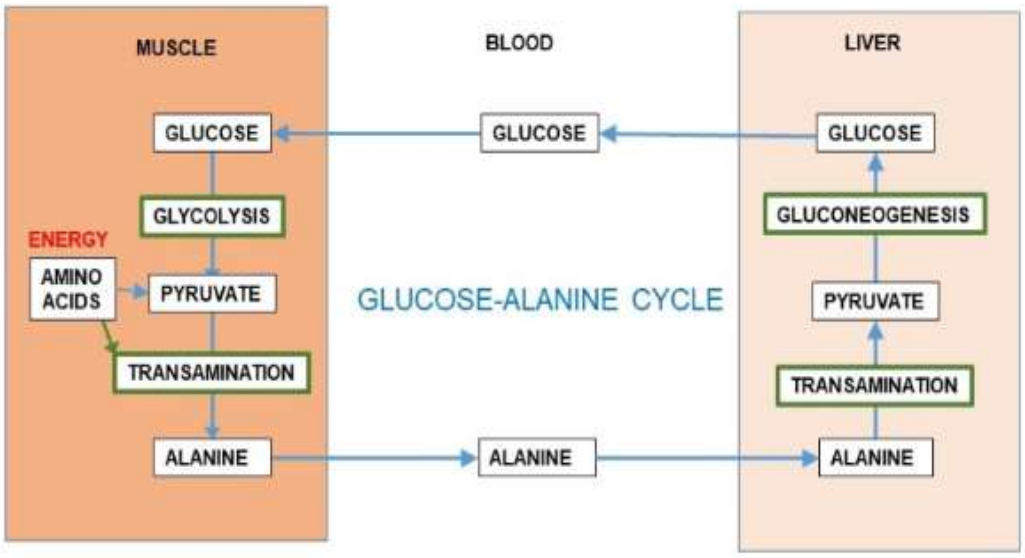

Figure 22 3: Glucose-alanine cycle.

6.2.3.1. Glutamate

The synthesis of glucose in the kidney cortex is directly related to the loss of ketone bodies in the urine. During periods of fasting, the kidney excretes large amounts of ketone bodies (weak acids), but produces urine that is near neutrality. The relative acidity of the tubular urine is maintained at about pH 6.0 by the generation of ammonia from the metabolism of glutamine that has been mobilized from the muscle during starvation. Glutamine is converted into glutamate by glutaminase and glutamate to a-ketoglutarate by glutamate dehydrogenase; this generates two molecules of ammonia that are released into the urine to maintain the neutrality of the urine. The a-ketoglutarate, produced by the removal of the two amino groups of glutamine, enters the citric acid cycle, is oxidized to malate, and then proceeds to glucose via gluconeogenesis. Thus, ammoniagenesis in the kidney is linked to gluconeogenesis (Figure 24).

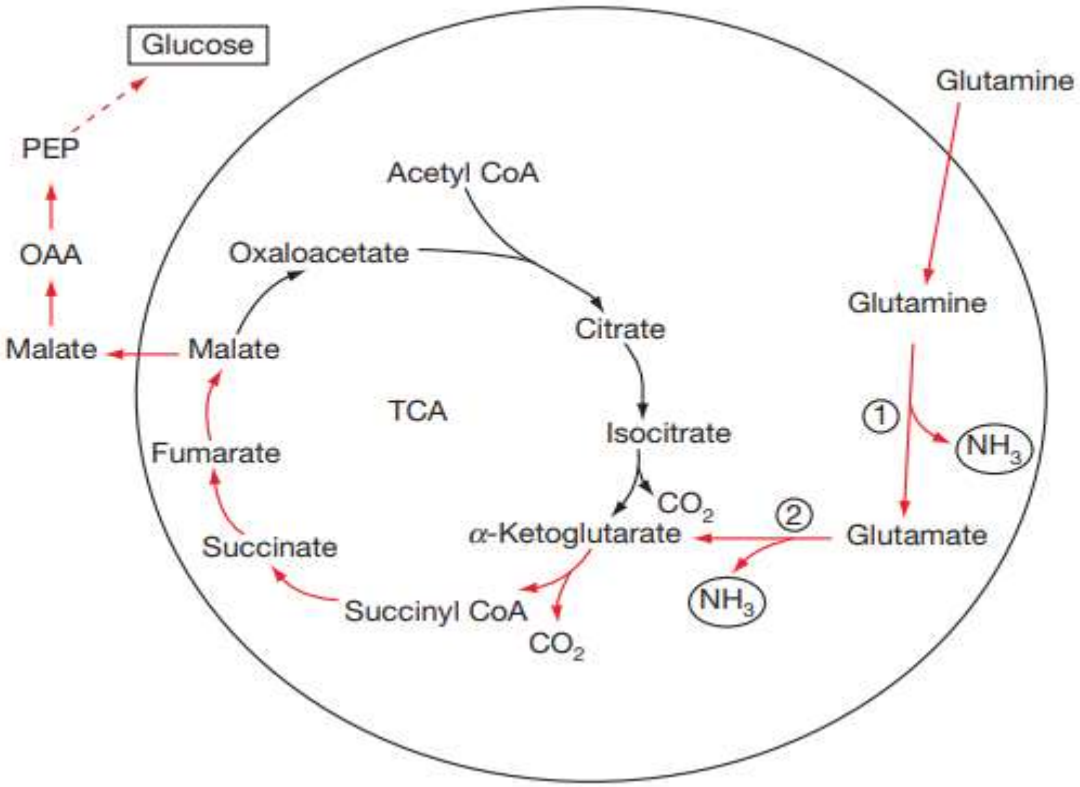

Figure 24: Gluconeogenesis from glutamine in the kidney

6.2.4. Propionate

Propionate is a major hepatic gluconeogenic substrate. Certain glucogenic amino acids (namely isoleucine, valine, threonine, and methionine), the terminal 3 carbons of odd-chain fatty acids undergoing mitochondrial b-oxidation can also enter hepatic gluconeogenesis at the level of propionyl-CoA.

6.4. Regulation

6.4.1. Allosteric regulation

PC from most sources is allosterically activated by acetyl-CoA and competitively inhibited by ADP. Fructose 1, 6-bisphosphatase is activated by high cytoplasmic ATP and citrate levels. This reaction bypasses the regulatory reaction of glycolysis catalyzed by phosphofructokinase, which is inhibited by glucagon, ATP, and citrate. Fructose 1, 6-bisphosphatase (FBPase) is mainly expressed in the liver and is allosterically inhibited by fructose 2, 6-bisphosphate, which is produced by the bifunctional enzyme phosphofructokinase 2/fructose-2, 6-bisphosphatase (PFK 2 / FBPase 2). fructose 2, 6-bisphosphate is a powerful

allosteric activator of phosphofructokinase 1 (PFK-1), the enzyme that controls one of the most critical steps of glycolysis (Figure 25).

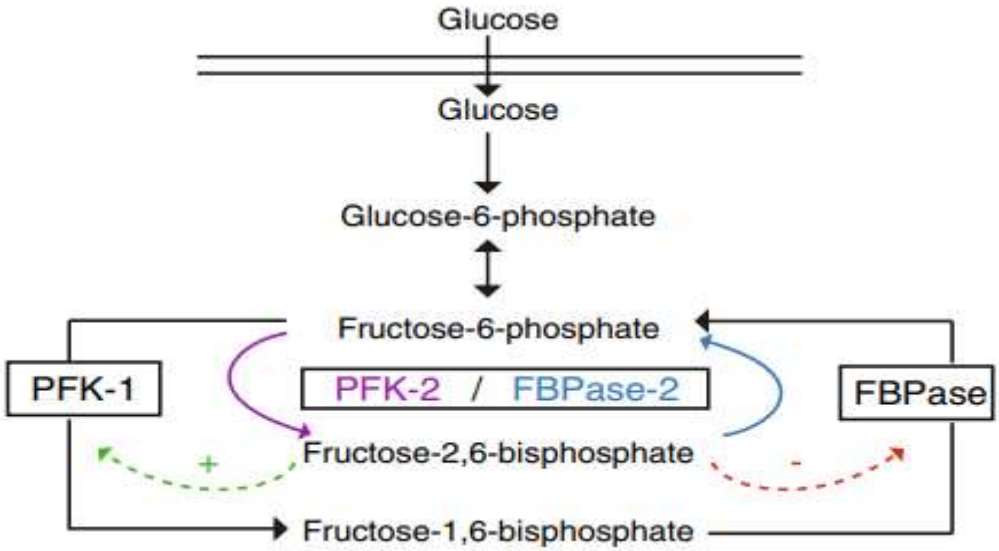

Figure 25: Allosteric regulation of glycolysis and gluconeogenesis.

6.4.2. Covalent modification or hormonal regulation

Insulin and glucagon are the most important hormones regulating hepatic gluconeogenesis. The activity of bifunctional enzyme phosphofructokinase 2/fructose-2, 6-bisphosphatase 2 is a key mechanism for regulating glycolysis and gluconeogenesis through synthesis or hydrolysis of fructose-2, 6-bisphosphate.

Fructose-2, 6-bisphosphate is both synthesized and degraded by the bifunctional enzyme phosphofructokinase 2/fructose-2,6-bisphosphatase, which can act as a kinase (it synthesizes fructose-2,6-bisphosphate using ATP as a source of phosphate) or as a phosphatase (it converts fructose 2,6-bisphosphate into fructose-6-phospahte plus inorganic phosphate).

- High glucagon/insulin ratio (fasting state): Glucagon triggers the production of cAMP, which activates protein kinase A (PKA). PKA phosphorylates the enzyme, which inhibits its kinase domain and activates its phosphatase domain (Fructose 2,6 biphosphatase 2). This leads to the breakdown of fructose 2, 6 biphosphate, this results in a decrease in the concentration of fructose-2, 6-bisphosphate, thereby inhibiting the activity of phosphofructokinase (inhibiting glycolysis) and simulating the activity of fructose-1, 6-bisphosphatase (stimulating gluconeogenesis) (Figure 26).

- Low glucagon/insulin ratio (fed state): insulin signaling triggers the dephosphorylation of the enzyme. In its dephosphorylated state, the kinase domain (phosphofructokinase 2) becomes active while the phosphatase domain is inhibited. This promotes the synthesis of fructose 2, 6 biphosphate from fructose-6-phosphate, thereby activating glycolysis and inhibiting gluconeogenesis.

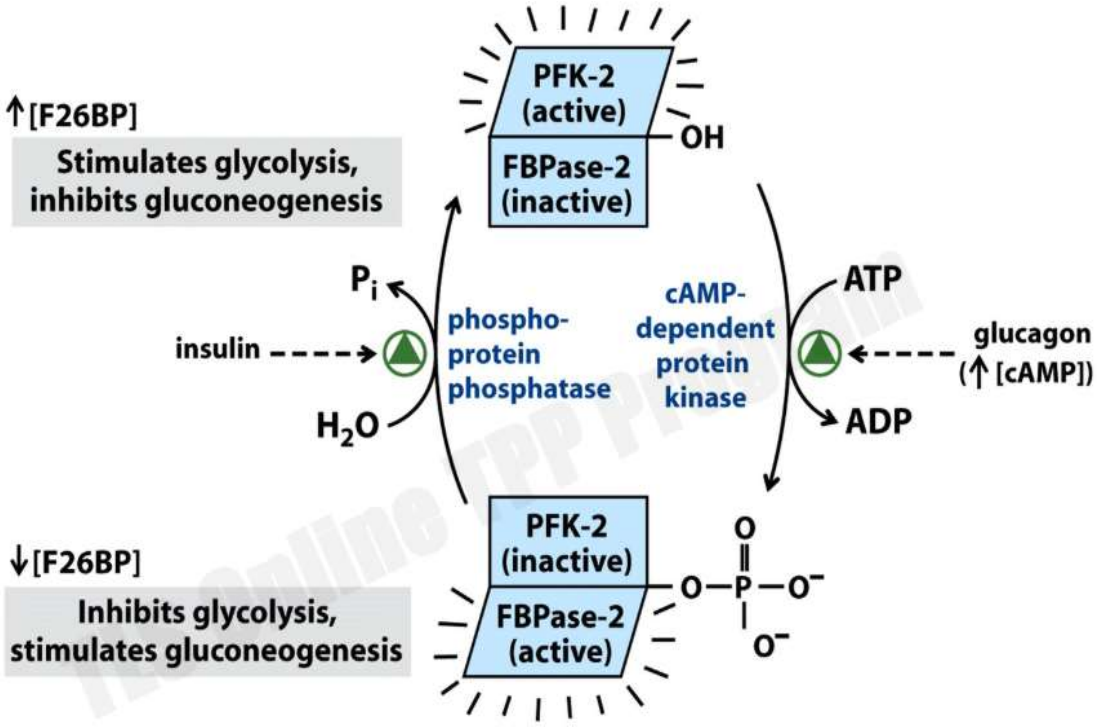

Figure 26: Allosteric regulation of glycolysis and gluconeogenesis.