Al-Mustaqbal University

جامعة المستقبل
AL MUSTAQBAL UNIVERSITY

College of Medical and Health Techniques

Medical Laboratories Techniques Departments

Biochemistry Lectures for 2nd Year Students

First Semester

(2 Credit Hrs. Theory + 4 Credit Hrs. Practice / Week = 4 Credit Unit

Academic Year: 2024 - 2025

Course Organizers:

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Lecture No. 9

Date: Dec., 8th , 2024

Carbohydrate Metabolism - Gluconeogenesis

Objectives:

The reader will be able to know and answer questions on gluconeogenesis and malate shuttle topics.

Gluconeogenesis:

Gluconeogenesis occurs in all animals, plants, fungi, and microorganisms. The reactions are essentially the same in all tissues and all species. The important precursors used for glucose generation in animals are three-carbon compounds such as lactate, pyruvate, and glycerol, as well as certain amino acids as shown in **Figure-1.**

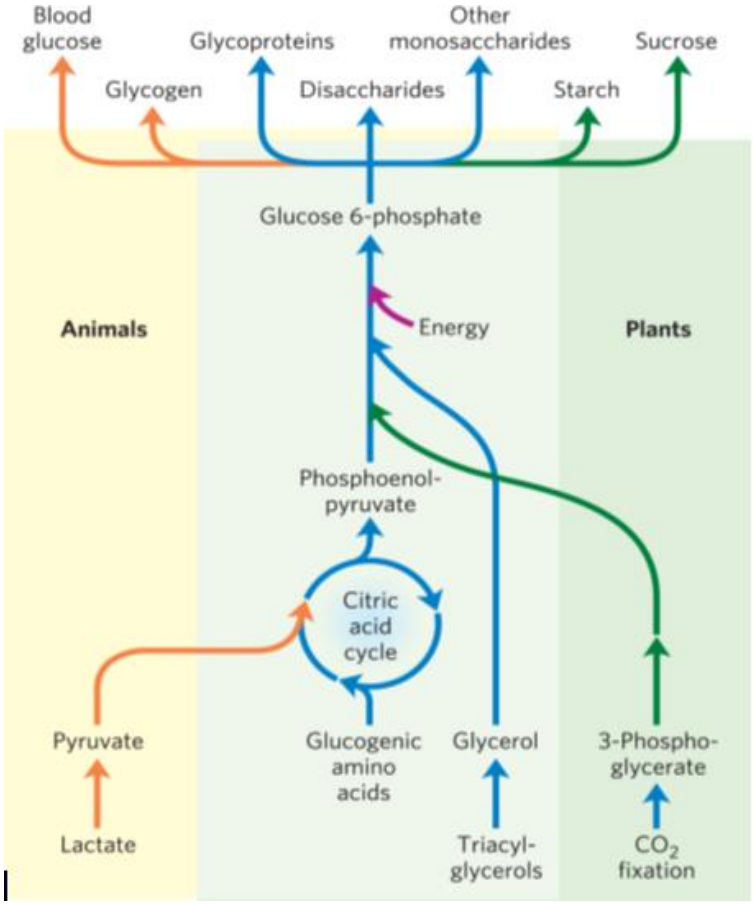

Fig. 1: Carbohydrate generation from simple precursors.

In mammals, gluconeogenesis takes place mainly in the liver, and to a lesser extent in the renal cortex and in the epithelial cells that line the small intestine. The glucose produced passes into the blood to supply other tissues. After vigorous exercise, lactate produced by anaerobic glycolysis in skeletal muscle returns to the liver and is converted to glucose, which moves back to muscle and is converted to glycogen — a circuit called the Cori cycle as mentioned in lecture No.8.

In plant seedlings, stored fats and proteins are converted, via paths that include gluconeogenesis, to the disaccharide sucrose for transport throughout the

developing plant. Glucose and its derivatives are precursors for the synthesis of plant cell walls, nucleotides and coenzymes, and a variety of other essential metabolites. In many microorganisms, gluconeogenesis starts from simple organic compounds of two or three carbons, such as acetate, lactate, and propionate, in their growth medium.

Definition and Site of Occurrence: Gluconeogenesis is the process by which glucose molecules are produced from non-carbohydrate precursors. These include lactate, glucogenic amino acids, glycerol part of fat and propionyl-CoA derived from odd chain fatty acids. It occurs mainly in the liver, and to a lesser extent in the renal cortex. The pathway is partly mitochondrial and partly cytoplasmic.

Gluconeogenesis involves several enzymes of glycolysis, but it is not a reversal of glycolysis. The irreversible steps in glycolysis are circumvented by four enzymes catalyzed the irreversible steps of gluconeogenesis which are designated as the key enzymes of gluconeogenesis, see **Figure-2.**

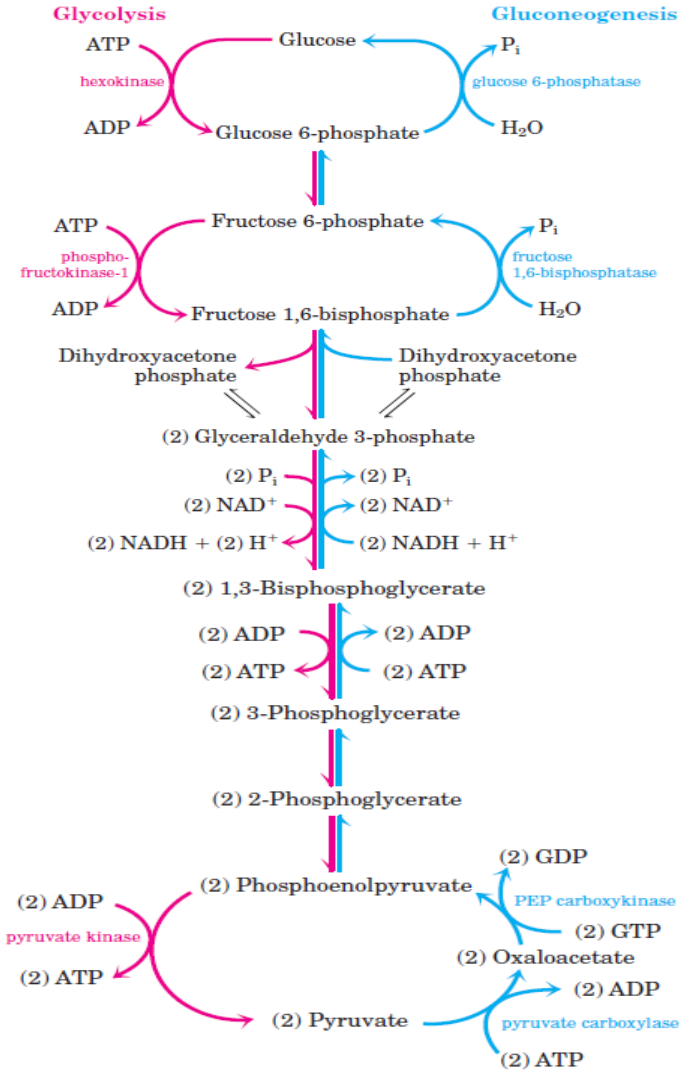

Fig. 2: Opposing pathways of gluconeogenesis and glycolysis in liver
The reactions of glycolysis are shown on the left side in red color; the opposing pathway of gluconeogenesis is shown on the right in blue color.

Malate Aspartate Shuttle

The previous reactions of gluconeogenesis take place in mitochondria. So, oxaloacetate is generated inside the mitochondria. This oxaloacetate has to be transported from mitochondria to cytosol, because further reactions of gluconeogenesis are taking place in cytosol. This is achieved by the malate aspartate shuttle. Oxaloacetate is first converted to malate, which transport across the membrane and reaches cytoplasm. Malate is then re-converted to oxaloacetate. Malate dehydrogenase is present in both mitochondria and cytoplasm as shown in **Figure-3.**

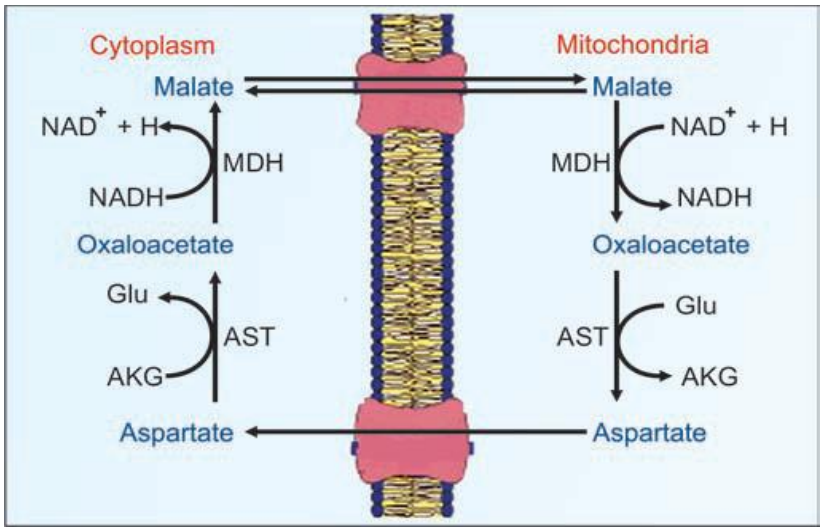

Fig. 3. Malate-aspartate shuttle. MDH = Malate dehydrogenase. AST = Aspartate amino transferase. Glu= Glutamic acid. AKG = Alpha keto glutaric acid

When alanine is the substrate for gluconeogenesis, the malate shuttle predominantly operates, because NADH is also required in the cytoplasm for the gluconeogenesis to continue. When lactate is the substrate for gluconeogenesis, the aspartate shuttle operates, because sufficient NADH is available in the cytoplasm by the LDH reaction.

Irreversible Reactions of Gluconeogenesis:

Phosphoenolpyruvate Carboxykinase

In the cytoplasm, phosphoenolpyruvate carboxykinase (PEPCK) enzyme then converts oxaloacetate to phosphoenol pyruvate by removing a molecule of CO_2. Guanosine triphosphate (GTP) or inosine triphosphate (ITP) donates the phosphate, see **Figure-4.**

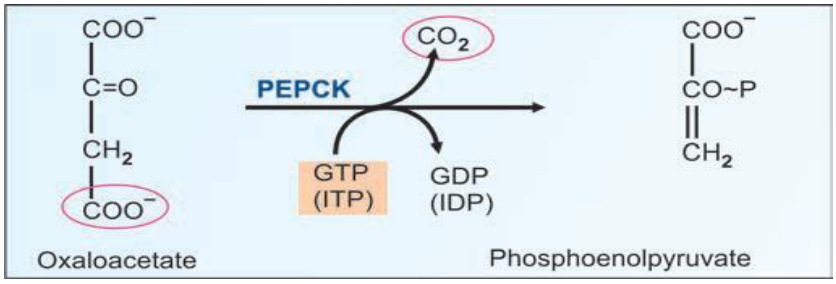

Fig. 4. Phosphoenolpyruvate carboxykinase

The net effect of these two reactions is the conversion of pyruvate to phosphoenol pyruvate. This circumvents the irreversible step in glycolysis catalyzed by pyruvate kinase (step 9 of glycolysis).

Partial Reversal of Glycolysis

The phosphoenol pyruvate undergoes further reactions catalyzed by the glycolytic enzymes to form fructose-1,6-bisphosphate (see glycolysis steps 8,7,6,5 and 4). All these reactions are freely reversible.

Fructose-1,6-bisphosphatase

Fructose-1,6-bisphosphate is then acted upon by fructose-1,6-bisphosphatase to form fructose-6-phosphate. This will bypass the step of PFK reaction (see step 3 of glycolysis).

Fructose-1,6-bisphosphatase

Fructose-1,6-bisphosphate ----------► Fructose-6 phosphate + Pi

Then fructose-6-phosphate is isomerized to glucose-6-phosphate by the freely reversible reaction catalyzed by hexosephosphate isomerase (second step in glycolysis).

Glucose-6-phosphatase Reaction

The glucose-6-phosphate is hydrolyzed to free glucose by glucose-6-phosphatase according to the following equation.

Glucose-6-phosphate + H_2O ----------► Glucose + Pi

Glucose-6-phosphatase is active in liver. It is present in kidney and intestinal mucosa to a lesser extent, but is absent in muscle.

Significance of Gluconeogenesis

1. Only liver can replenish blood sugar through gluconeogenesis, because glucose-6-phosphatase is present mainly in liver. So, liver plays the major role in maintaining the blood glucose level.
2. During starvation gluconeogenesis maintains the blood glucose level. The stored glycogen is depleted within the first 12–18 hours of fasting. On prolonged starvation, the gluconeogenesis is speeded up and protein catabolism provides the substrates, namely glucogenic amino acids.

Energy requirement: The reactions catalyzed by pyruvate carboxylase, phosphoenol pyruvate carboxy kinase and phosphoglycerate kinase require one ATP each; so, 3 ATPs are used by 1 pyruvate residue to produce one-half molecule of glucose; or 6 ATP molecules are utilized to generate one glucose molecule from two pyruvate molecules by gluconeogenesis whereas glucose oxidized to pyruvate by glycolysis generate two ATP molecules, therefore, gluconeogenesis is enhanced by ATP.

2 pyruvate ----------► 2 oxaloacetate = 2 ATP

2 oxaloacetate ----------► 2 phosphoenol pyruvate (2GTP) = 2 ATP

2 × 3-phosphoglycerate ----------► 2 × 1,3-bisphosphoglycerate = 2 ATP

Regulation of Gluconeogenesis

Gluconeogenesis and glycolysis are reciprocally regulated so that one pathway is relatively inactive when the other is active. The regulatory steps are:

Pyruvate Carboxylase

It is an allosteric enzyme, acetyl-CoA is an activator of pyruvate carboxylase so that generation of oxaloacetate is favored when acetyl-CoA level is sufficiently high.

Fructose-1,6-bisphosphatase

Citrate is an activator while fructose-2,6-bis-phosphate and AMP are inhibitors. All these three effectors have an exactly opposite effect on the phosphofructokinase (PFK) (Fig. 5).

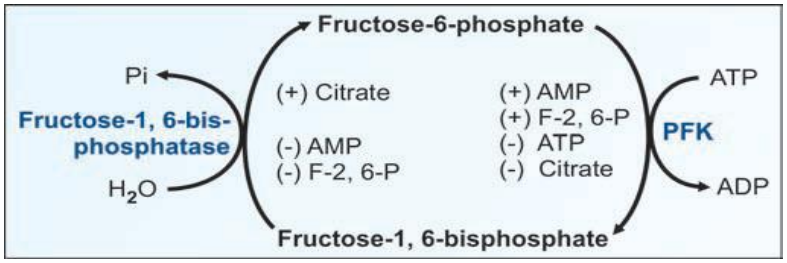

Fig. 5. Reciprocal regulation of PFK (glycolytic enzyme) and Fructose-1,6-bisphosphatase (gluconeogenic enzyme)

Hormonal Regulation of Gluconeogenesis

1. Glucagon and glucocorticoids hormones increase gluconeogenesis (Fig. 6).

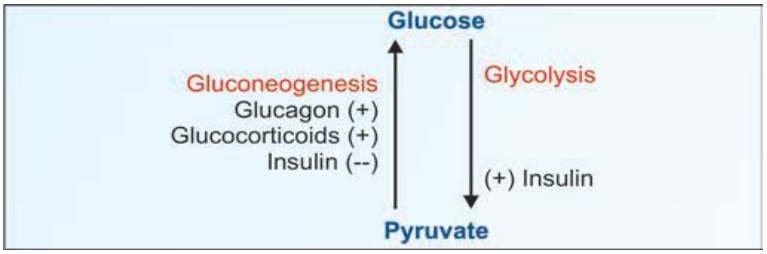

Fig. 6. Hormonal regulation of gluconeogenesis

2. Glucocorticoids induce the synthesis of hepatic amino transferases thereby providing substrate for gluconeogenesis.
3. The high glucagon-insulin ratio also favors induction of synthesis of gluconeogenic enzymes (Phosphoenolpyruvate carboxy kinase. PEPCK, Fructose-1,6-bisphosphatase and glucose-6-phosphatase).
4. At the same time, synthesis of glycolytic enzymes HK, PFK and PK are depressed.
5. Insulin inhibits gluconeogenesis.