Lecture : 5 **Dr. Wasan Khairallah Ali**

Gluconeogenesis

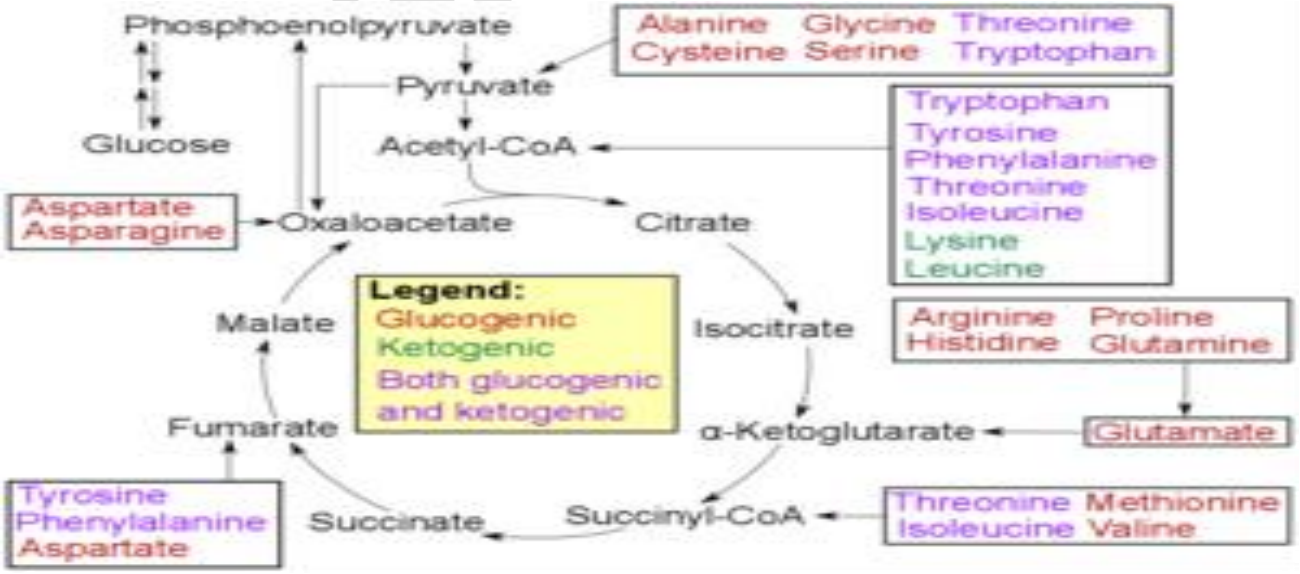
Gluconeogenesis is a process that transforms non-carbohydrate substrates (such as lactate, amino acids, and glycerol) into glucose . Both lactate and alanine are first converted into pyruvate, which then enters the mitochondrion and is carboxylated to oxaloacetate (OAA) by pyruvate carboxylase (PC).

Gluconeogenesis is a metabolic pathway that leads to the synthesis of glucose from pyruvate and other non-carbohydrate precursors, even in non-photosynthetic organisms.

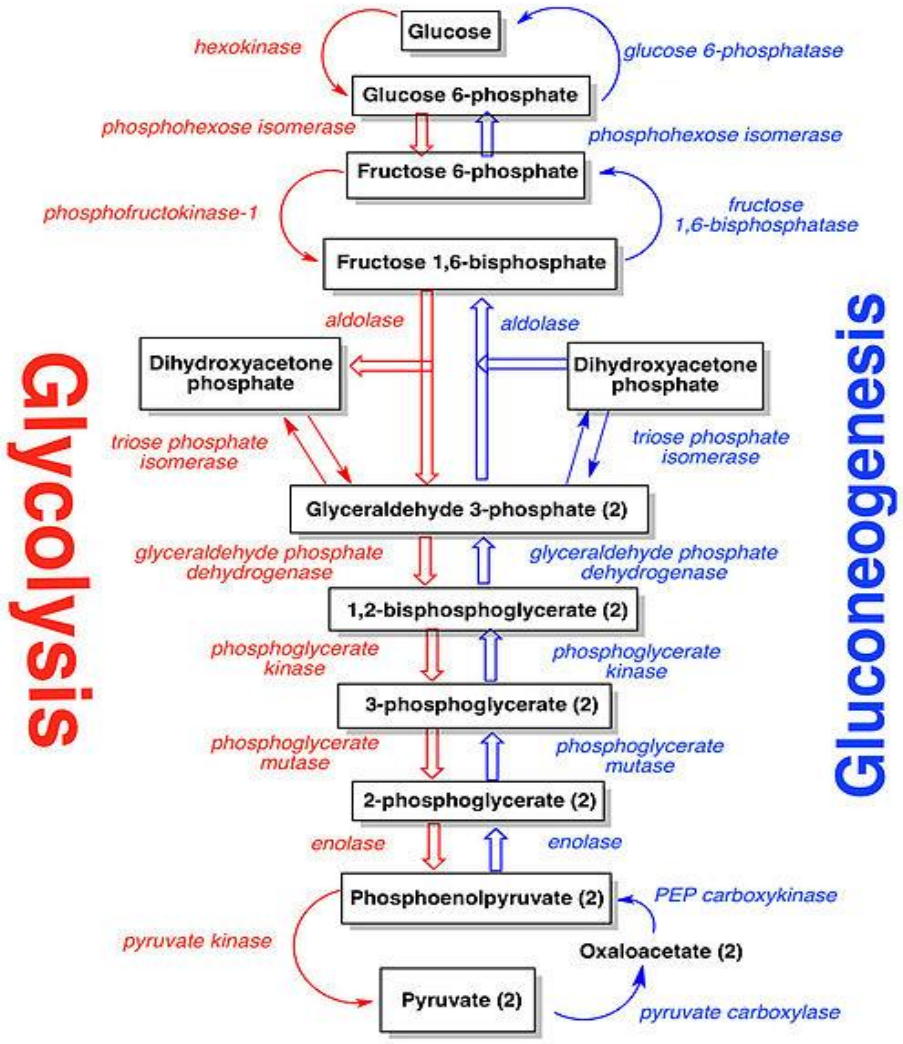
It occurs in all microorganisms, fungi, plants and animals, and the reactions are essentially the same, leading to the synthesis of one glucose molecule from two pyruvate molecules. Therefore, it is in essence glycolysis in reverse, which instead goes from glucose to pyruvate, and shares seven enzymes with it.

Leu and Lys → → acetyl-CoA but not oxaloacetate. **(** inactive in gluconeogenesis).

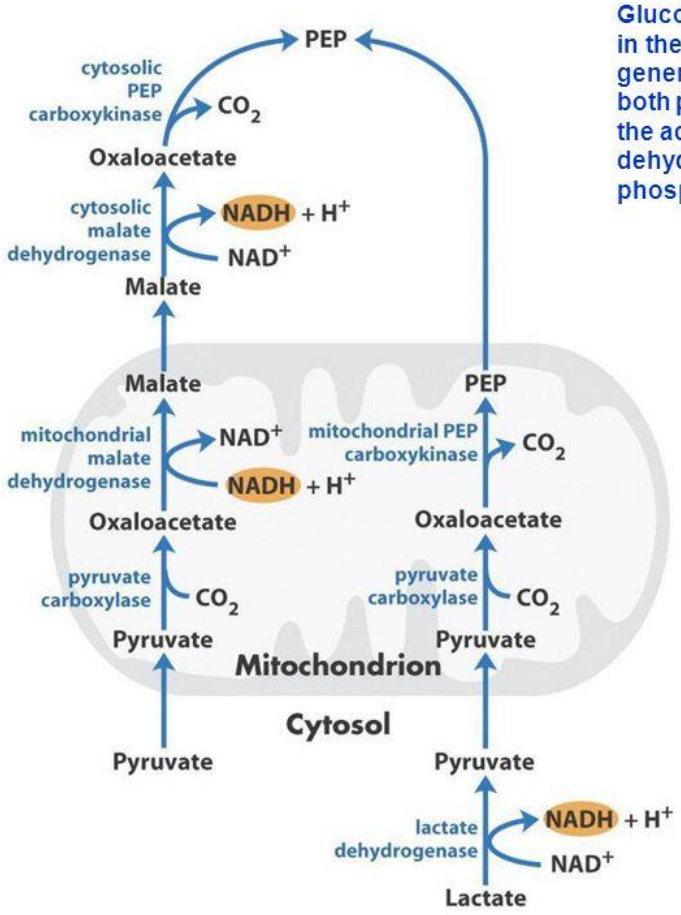
All other amino acids → → oxaloacetate. **(**active in gluconeogenesis).



Gluconeogenesis is much like glycolysis only the process occurs in reverse. However, there are exceptions. In glycolysis there are three highly exergonic steps (steps 1,3,10). These are also regulatory steps which include the enzymes hexokinase, phosphofructokinase, and pyruvate kinase. Biological reactions can occur in both the forward and reverse direction. If the reaction occurs in the reverse direction the energy normally released in that reaction is now required. If gluconeogenesis were to simply occur in reverse the reaction would require too much energy to be profitable to that particular organism. In order to overcome this problem, nature has evolved three other enzymes to replace the glycolysis enzymes hexokinase, phosphofructokinase, and pyruvate kinase when going through the process of gluconeogenesis:



The first step in gluconeogenesis is the conversion of pyruvate to phosphoenolpyruvic acid (PEP). In order to convert pyruvate to PEP there are several steps and several enzymes required. Pyruvate carboxylase, PEP carboxykinase and malate dehydrogenase are the three enzymes responsible for this conversion. Pyruvate carboxylase is found on the mitochondria and converts pyruvate into oxaloacetate. Because oxaloacetate cannot pass through the mitochondria membranes it must be first converted into malate by malate dehydrogenase. Malate can then cross the mitochondria membrane into the cytoplasm where it is then converted back into oxaloacetate with another malate dehydrogenase. Lastly, oxaloacetate is converted into PEP via PEP carboxykinase. The next several steps are exactly the same as glycolysis only the process is in reverse.



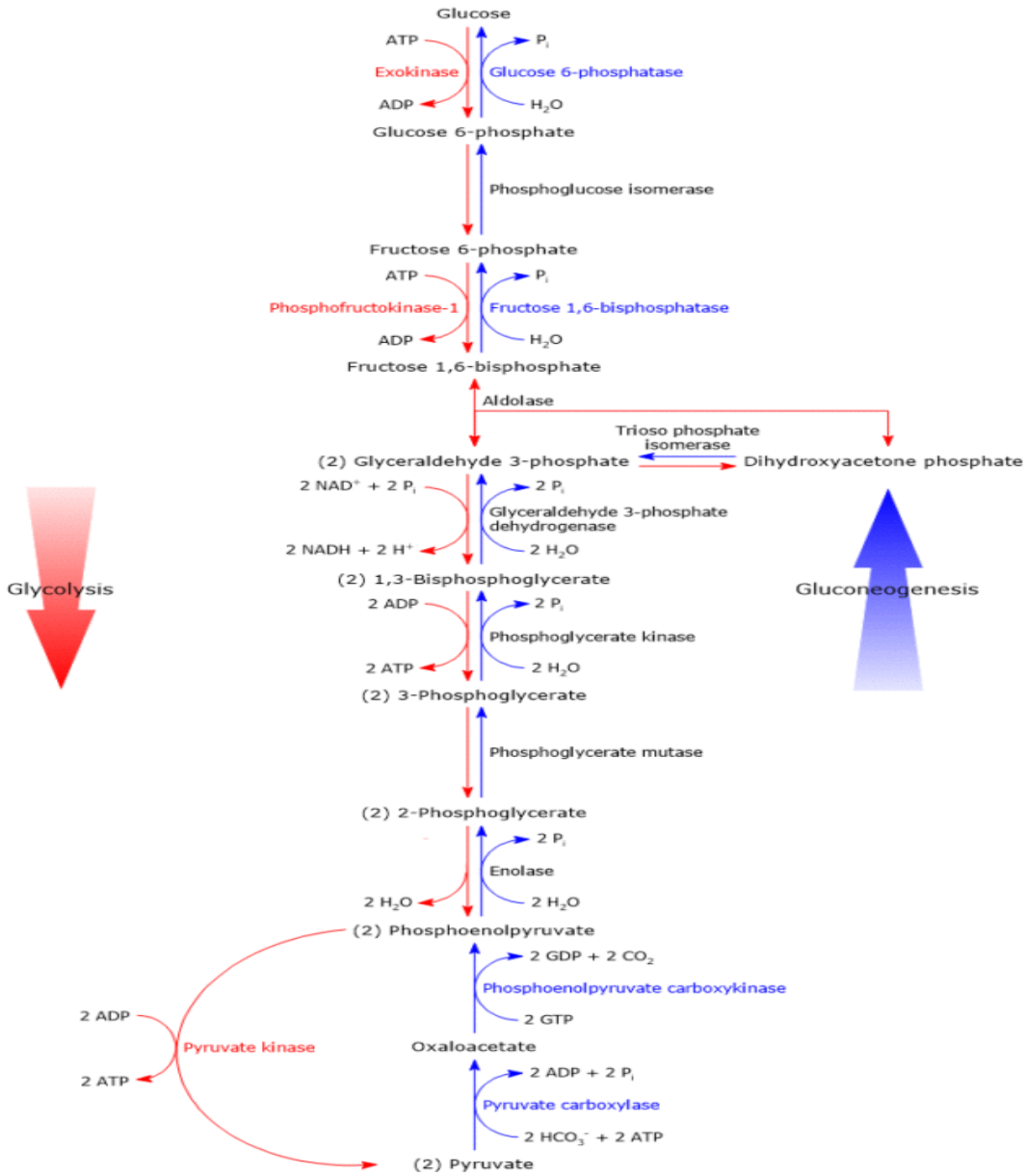
Gluconeogenesis takes place in the cytosol and in the mitochondria. There are two pathways to generate **PEPA** (phosphoenolpyruvic acid). In both pathways **NADH** must be generated to allow the activity of glyceraldehyde-3-phosphate dehydrogenase in the reduction of 3-phosphoglyceric acid.

From PEPA to fructose-1,6-bisphosphate all the steps are shared by glycolysis and gluconeogenesis and are reversible.

10

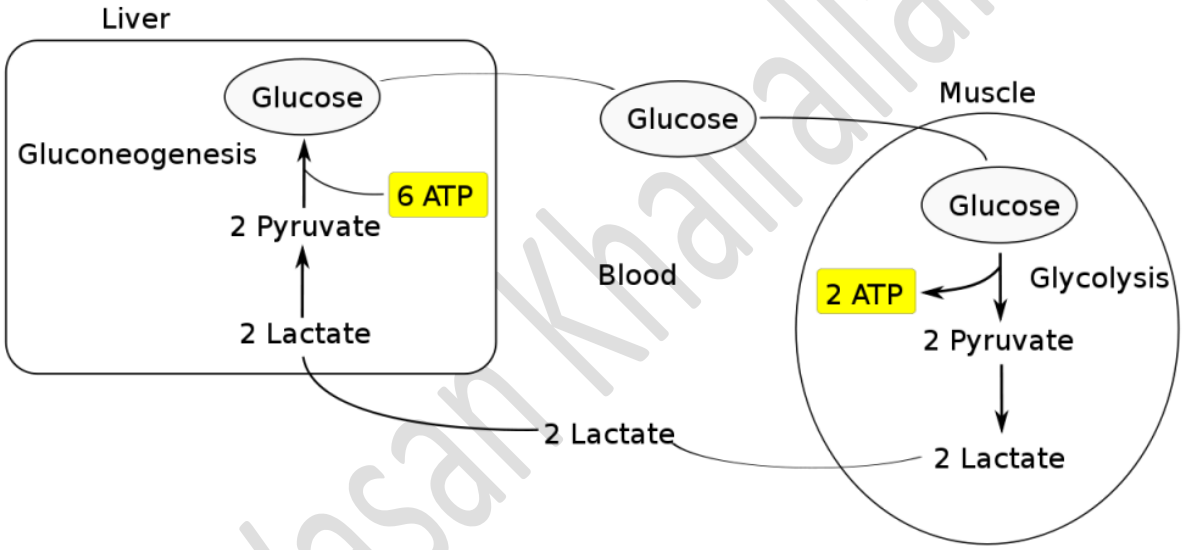
The second step that differs from glycolysis is the conversion of fructose-1,6-bP to fructose-6-P with the use of the enzyme fructose-1,6-phosphatase. The conversion of fructose-6-P to glucose-6-P uses the same enzyme as glycolysis, phosphoglucoisomerase.

The last step that differs from glycolysis is the conversion of glucose-6-P to glucose with the enzyme glucose-6-phosphatase. This enzyme is located in the endoplasmic reticulum.



gluconeogenesis is one of two primary mechanisms – the other being degradation of glycogen (glycogenolysis) – used by humans and many other animals to maintain blood sugar levels, avoiding low levels (hypoglycemia). gluconeogenesis occurs regardless of fasting, low-carbohydrate diets, exercise, etc.

In humans, substrates for gluconeogenesis may come from any non-carbohydrate sources that can be converted to pyruvate or intermediates of glycolysis . For the breakdown of proteins, these substrates include glucogenic amino acids (although not ketogenic amino acids); from breakdown of lipids (such as triglycerides), they include glycerol, odd-chain fatty acids (although not even-chain fatty acids); and from other parts of metabolism they include lactate from the **Cori cycle**

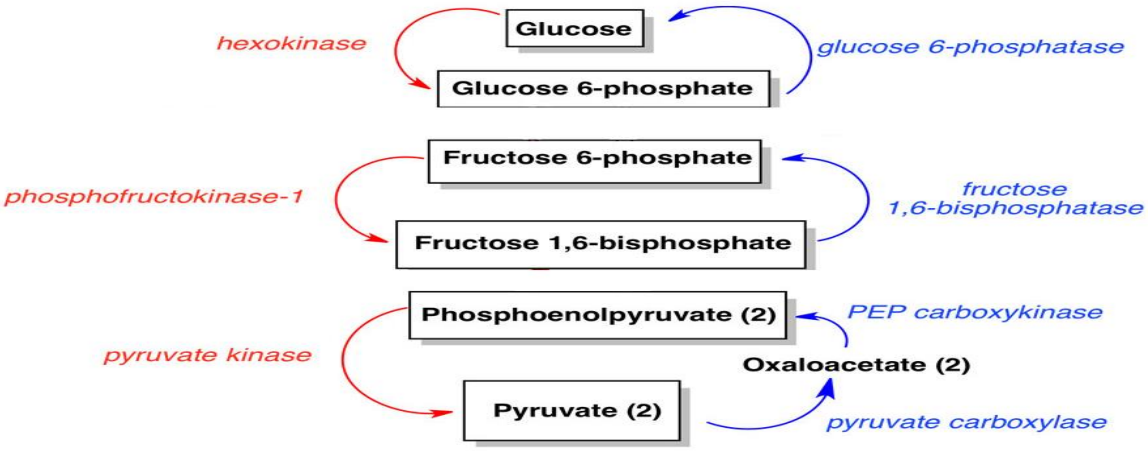


Under conditions of prolonged fasting, acetone derived from ketone bodies can also serve as a substrate, providing a pathway from fatty acids to glucose. Although most gluconeogenesis occurs in the liver, the relative contribution of gluconeogenesis by the kidney is increased in diabetes and prolonged fasting.

The gluconeogenesis pathway is highly endergonic until it is coupled to the hydrolysis of ATP or GTP, effectively making the process exergonic. For example, the pathway leading from pyruvate to glucose-6-phosphate requires 4 molecules

of ATP and 2 molecules of GTP to proceed spontaneously. These ATPs are supplied from fatty acid catabolism via beta oxidation.

In humans the main gluconeogenic precursors are lactate, glycerol (which is a part of the triglyceride molecule), alanine and glutamine. Altogether, they account for over 90% of the overall gluconeogenesis. Other glucogenic amino acids and all citric acid cycle intermediates (through conversion to oxaloacetate) can also function as substrates for gluconeogenesis.



Gluconeogenesis is a pathway consisting of a series of eleven enzyme-catalyzed reactions. The pathway will begin in either the liver or kidney, in the mitochondria or cytoplasm of those cells, this being dependent on the substrate being used. Many of the reactions are the reverse of steps found in glycolysis .

Gluconeogenesis begins in the mitochondria with the formation of oxaloacetate by the carboxylation of pyruvate. This reaction also requires one molecule of ATP, and is catalyzed by pyruvate carboxylase. This enzyme is stimulated by high levels of acetyl-CoA (produced in β-oxidation in the liver) and inhibited by high levels of ADP and glucose.

Oxaloacetate is reduced to malate using NADH, a step required for its transportation out of the mitochondria.

Malate is oxidized to oxaloacetate using NAD+ in the cytosol, where the remaining steps of gluconeogenesis take place.

Oxaloacetate is decarboxylated and then phosphorylated to form phosphoenolpyruvate using the enzyme PEPCK. A molecule of GTP is hydrolyzed to GDP during this reaction.

Glucose-6-phosphate is formed from fructose 6-phosphate by phosphoglucoisomerase (the reverse of step 2 in glycolysis). Glucose-6-phosphate can be used in other metabolic pathways or dephosphorylated to free glucose. Whereas free glucose can easily diffuse in and out of the cell, the phosphorylated form (glucose-6-phosphate) is locked in the cell, a mechanism by which intracellular glucose levels are controlled by cells.

The final gluconeogenesis, the formation of glucose, occurs in the lumen of the endoplasmic reticulum, where glucose-6-phosphate is hydrolyzed by glucose-6-phosphatase to produce glucose and release an inorganic phosphate. Like two steps prior, this step is not a simple reversal of glycolysis, in which hexokinase catalyzes the conversion of glucose and ATP into G6P and ADP. Glucose is shuttled into the cytoplasm by glucose transporters located in the endoplasmic reticulum's membrane.

Why is gluconeogenesis important?

Gluconeogenesis is an essential metabolic pathway for at least two reasons.

1- It ensures the maintenance of appropriate blood glucose levels when the liver glycogen is almost depleted and no carbohydrates are ingested. Especially in cases of fasting or exercising for a long time

Maintaining blood glucose within the normal range, 3.3 to 5.5 mmol/L (60 and 99 mg/dL), is essential because many cells and tissues depend, largely or entirely, on glucose to meet their ATP demands; examples are red blood cells, neurons, skeletal muscle working under low oxygen conditions, the medulla of the kidney, the testes, the lens and the cornea of the eye, and embryonic tissues. For example, glucose requirement of the brain is about 120 g/die .

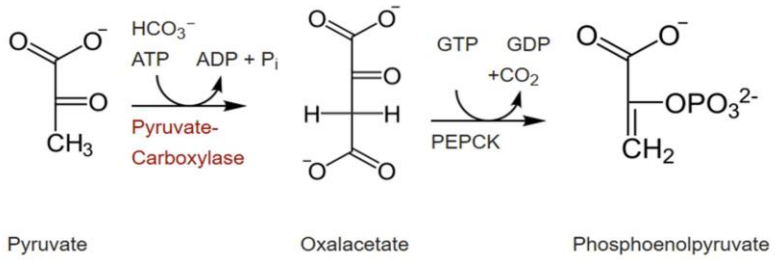
2- If no carbohydrates are ingested, or In the absence of carbohydrates in food, the body can provide glucose from other sources such as proteins or fats. At that point, gluconeogenesis becomes important.

Most important points of Gluconeogenesis Pathway

1-Gluconeogenesis begins in either the mitochondria or cytoplasm of the liver or kidney. First, two pyruvate molecules are carboxylated to form oxaloacetate. One ATP (energy) molecule is needed for this.

Pyruvate → Oxaloacetate → Phosphoenolpyruvate

Pyruvate + HCO_3–+ ATP → Oxaloacetate + ADP + Pi



2-Oxaloacetate is reduced to malate by NADH so that it can be transported out of the mitochondria.

Pyruvate + ATP + GTP + HCO_3– → PEP + ADP + GDP + Pi + CO_2

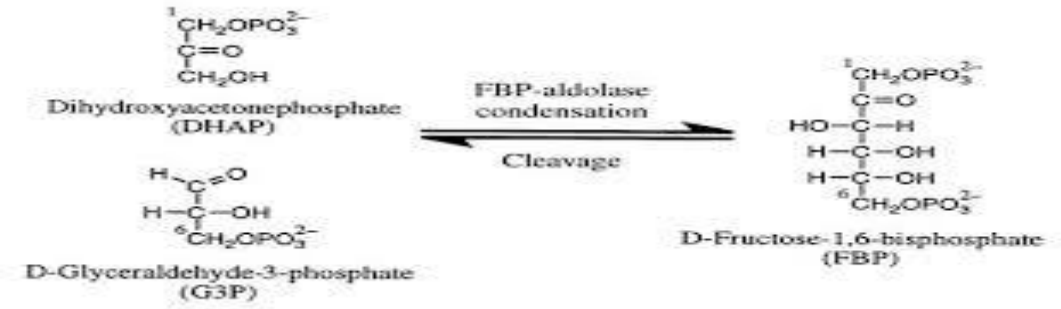
3-Malate is oxidized back to oxaloacetate once it is out of the mitochondria.

Formula: $Malate + NAD^+ \xrightarrow{Malate\ dehydrogenase} Oxaloacetate + NADH + H^+$

4-Oxaloacetate forms phosphoenolpyruvate using the enzyme PEPCK.

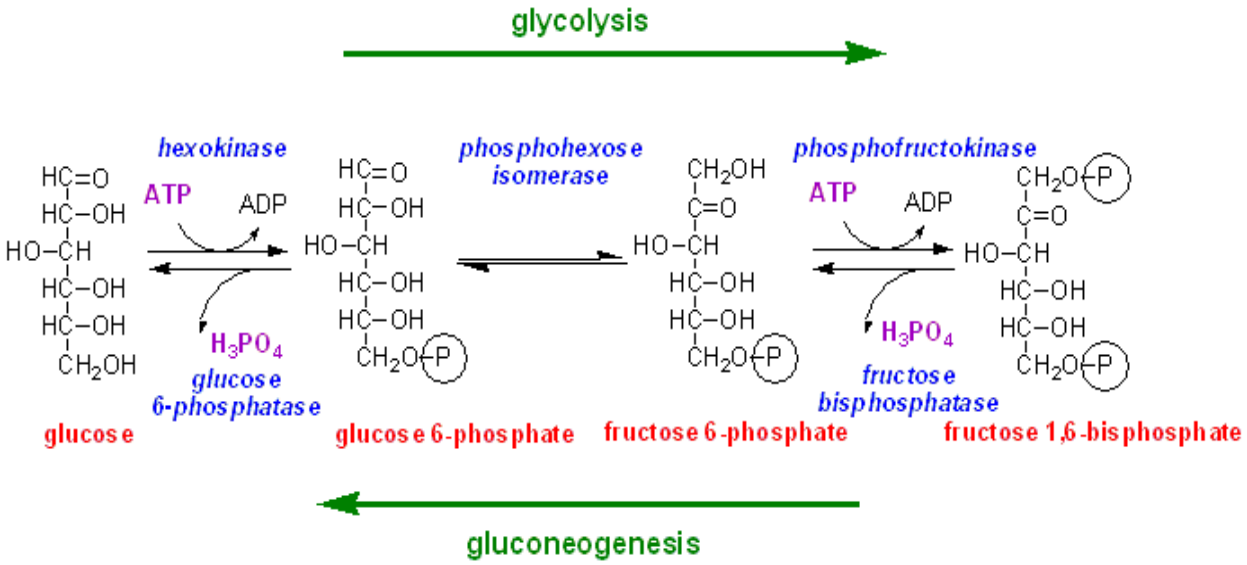
Oxaloacetate + GTP ⇄ PEP + CO_2 + GDP

5-Phosphoenolpyruvate is changed to fructose-1,6-biphosphate, and then to fructose-6-phosphate. ATP is also used during this process, which is essentially glycolysis in reverse.



6-Fructose-6-phosphate becomes glucose-6-phosphate with the enzyme phosphoglucoisomerase.

7-Glucose is formed from glucose-6-phosphate in the cell's endoplasmic reticulum via the enzyme glucose-6-phosphatase. To form glucose, a phosphate group is removed, and glucose-6-phosphate and ATP becomes glucose and ADP.



Regulation

Because it is important for organisms to conserve energy, they have derived ways to regulate those metabolic pathways that require and release the most energy. In glycolysis and gluconeogenesis seven of the ten steps occur at or near equilibrium. In gluconeogenesis the conversion of pyruvate to PEP, the conversion of fructose-1,6-bP, and the conversion of glucose-6-P to glucose all occur very spontaneously which is why these processes are highly regulated. It is important for the organism to conserve as much energy as possible. **When there is an excess of energy available, gluconeogenesis is inhibited. When energy is required, gluconeogenesis is activated.**

The conversion of pyruvate to PEP is regulated by acetyl-CoA. More specifically pyruvate carboxylase is activated by acetyl-CoA. Because acetyl-CoA is an important metabolite in the TCA cycle which produces a lot of energy, when concentrations of acetyl-CoA are high organisms use pyruvate carboxylase to channel pyruvate away from the TCA cycle. **If the organism does not need more energy, then it is best to divert those metabolites towards storage or other necessary processes.**

when energy levels are lower than needed, i.e. a low ATP to AMP ratio, the organism increases glycolysis and decreases gluconeogenesis.

The conversion of glucose-6-P to glucose with use of glucose-6-phosphatase is controlled by substrate level regulation. The metabolite responsible for this type of regulation is glucose-6-P. As levels of glucose-6-P increase, glucose-6-phosphatase increases activity and more glucose is produced. Thus glycolysis is unable to proceed.

Hormonal Control:Gluconeogenesis, like glycolysis, is under tight control of hormones to regulate blood glucose. Stress hormones such as glucagon or cortisol upregulate PEPCK and fructose 1,6-bisphosphatase in order to stimulate gluconeogenesis. However, in a fed, high energy state gluconeogenesis decreases by inhibiting PEPCK and fructose 1,6-bisphosphatase.

How to calculate ATP value

For every molecule of glucose synthesized from two molecules of pyruvate, 4 ATP, 2 GTP, and 2 NADH are used.

In the Mitochondria

Pyruvate + ATP →Oxaloacetate + ADP + P

Oxaloacetate + NADH →Malate + NAD^+

The conversion to malate allows the molecule to be transported out of the mitochondria. Once in the cytoplasm, it is converted back to oxaloacetate.

In the Cytoplasm

Malate + NAD^+ →Oxaloacetate + NADH

Oxaloacetate + GTP →PEP + GDP

From here, it goes through the same intermediates as glycolysis. The last reaction happens in the endoplasmic reticulum.

In the Endoplasmic Reticulum

G6P →glucose (catalyst: glucose-6-phosphatase)

A glucose transporter shuttles the glucose out into the extracellular space.

Gluconeogenesis:

$2\ \text{Pyruvate} + 2\ \text{NADH} + 4\ H^+ + 4\ \text{ATP} + 2\ \text{GTP} + 6\ H_2O \rightarrow \text{Glucose} + 2\ NAD^+ + 4\ \text{ADP} + 2\ \text{GDP} + 6\ \text{Pi}$

(Requires NADH and ATP/GTP as energy sources.)

Glycolysis:

$\text{Glucose} + 2\ NAD^+ + 2\ \text{ADP} + 2\ \text{Pi} \rightarrow 2\ \text{Pyruvate} + 2\ \text{NADH} + 4\ H^+ + 2\ \text{ATP} + 2\ H_2O$

(Produces NADH and ATP.)