Gluconeogenesis

- Gluconeogenesis is the process of synthesizing glucose from noncarbohydrate precursors. It meets the needs of the body for glucose when insufficient carbohydrate is available from the diet or glycogen reserves.
- Some tissues, such as the brain, RBCs, kidney medulla, lens and cornea of the eye, testes, and exercising muscle require a continuous supply of glucose as a fuel.
- Liver glycogen can meet these needs for only 10–18 hours in the absence of dietary intake of carbohydrate.
- Glucose is also important in maintaining the level of intermediates of TCA cycle even when fatty acids are the main source of acetyl-CoA in the tissues.
- In addition, gluconeogenesis clears lactate produced by muscle and erythrocytes and glycerol produced by adipose tissue.
- **Gluconeogenesis is not a simple reversal of glycolysis**; but is a special pathway that requires both mitochondrial and cytosolic enzymes.
- During an overnight fast, about 90% of gluconeogenesis occurs in the liver, with the kidneys providing 10% of the newly synthesized glucose molecules. However, during prolonged fasting, the kidneys become major glucose-producing organs, contributing an estimated 40% of the total glucose production.

Substrates for Gluconeogenesis

Gluconeogenic precursors are molecules that can be used to produce a net synthesis of glucose. They include:

1. lactate, pyruvate,
2. glycerol (derived from the backbone of triacylglycerols, and
3. α-ketoacids (derived from the catabolism of glucogenic amino acids.
4. Intermediates of glycolysis and TCA cycle.

Reactions Unique to Gluconeogenesis

- **Seven** of the reactions of glycolysis are reversible and are used in the synthesis of glucose from lactate or pyruvate. However, **three** of the reactions are irreversible.
- The irreversible reactions (energy barrier or exergonic reactions) that obstruct a simple reversal of glycolysis are:
  1- From PEP to pyruvate (pyruvate kinase)
  2- From Fructose 1, 6-bisphosphate to Fructose 6-P (PFK-1)
  3- From Glucose 6-P to Glucose (Hexokinase or Glucokinase)
- These irreversible glycolysis reactions must be circumvented by **four alternate reactions** that are unique to gluconeogenesis.
Reactions Unique to Gluconeogenesis

I- Gluconeogenesis from lactate and pyruvate

1- conversion of pyruvate to phosphoenol pyruvate (PEP)

A. Carboxylation of pyruvate
The first “roadblock” to overcome in the synthesis of glucose from pyruvate is the irreversible conversion in glycolysis of PEP to pyruvate by pyruvate kinase. In gluconeogenesis, pyruvate is first carboxylated by pyruvate carboxylase to OAA. This reaction occurs in the mitochondria of liver and kidney cells and aims to:
  1. provide an important substrate for gluconeogenesis
  2. provide OAA to replenish the TCA cycle intermediates that may become depleted, depending on the synthetic needs of the cell.

B. Transport of oxaloacetate to the cytosol
Oxaloacetate, formed in the mitochondria, must be transported to the cytosol where the other enzymes of gluconeogenesis are located. However, OAA cannot directly cross the inner mitochondrial membrane. Thus, OAA can be transported by:
  1. Reduction to malate by mitochondrial malate dehydrogenase. Malate can be transported from the mitochondria to the cytosol, where it is reoxidized to oxaloacetate by cytosolic malate dehydrogenase as NAD$^+$ is reduced. The NADH produced is used in the reduction of 1,3-BPG to glyceraldehyde 3-phosphate, a step common to both glycolysis and gluconeogenesis.
2. Conversion to citrate by citrate synthase. Then, citrate is transported to the cytosol where it is converted back to OAA by ATP-citrate lyase.

3. Transamination to aspartate.

C. Decarboxylation of cytosolic oxaloacetate
Oxaloacetate is decarboxylated and phosphorylated to PEP in the cytosol by PEP-carboxykinase. The reaction is driven by hydrolysis of GTP. The combined actions of pyruvate carboxylase and PEP carboxy kinase provide an energetically favorable pathway from pyruvate to PEP. Then, PEP is acted on by the reactions of glycolysis running in the reverse direction until it becomes fructose 1,6-bisphosphate.

2- Dephosphorylation of fructose 1,6-bisphosphate
Hydrolysis of fructose 1,6-bisphosphate by fructose 1,6-bisphosphatase bypasses the irreversible phosphofructokinase-1 reaction, and provides an energetically favorable pathway for the formation of fructose 6-phosphate. This reaction is an important regulatory site of gluconeogenesis.

3- Dephosphorylation of glucose 6-phosphate
Hydrolysis of glucose 6-phosphate by glucose 6-phosphatase bypasses the irreversible hexokinase reaction. Liver and kidney are the only organs that release free glucose from glucose 6-phosphate. This process actually requires two proteins: glucose 6-phosphate translocase, which transports glucose 6-phosphate across the endoplasmic reticulum (ER) membrane, and the ER enzyme, glucose 6-phosphatase (found only in gluconeogenic cells), which removes the phosphate, producing free glucose. These proteins are also required for the final step of glycogen degradation. Specific transporters are responsible for releasing free glucose and phosphate back into the cytosol and, for glucose, into blood.

II- Gluconeogenesis from Glycerol
- Glycerol is released during the hydrolysis of triacylglycerols in adipose tissue, and is delivered by the blood to the liver. Glycerol is phosphorylated by glycerol kinase to glycerol phosphate, which is oxidized by glycerol phosphate dehydrogenase to dihydroxyacetone phosphate—an intermediate of glycolysis.
- Adipocytes cannot phosphorylate glycerol because they essentially lack glycerol kinase.
III- **Gluconeogenesis from Amino acids**

- Amino acids derived from hydrolysis of **tissue proteins** are the major sources of glucose during fasting or starvation. **Glucogenic** amino acids, via transamination or deamination, give rise to α-Ketoacids. These α-ketoacids can enter the TCA cycle and form oxaloacetate that is a direct precursor of phosphoenol pyruvate.
- **Acetyl CoA** and compounds that give rise only to acetyl CoA (e.g. fatty acids, acetoacetate and Ketogenic amino acids) cannot give rise to a net synthesis of glucose. This is due to the **irreversible** nature of the **pyruvate dehydrogenase** reaction, which converts pyruvate to acetyl CoA. These compounds give rise instead to ketone bodies.

IV- **Gluconeogenesis from Propionate**

Propionate is a major precursor of glucose in **ruminants**. In nonruminants, including humans, propionate arises from the β-oxidation of odd-chain fatty acids that occur in ruminant lipids, as well as the oxidation of isoleucine and the side chain of cholesterol, and is a minor substrate for gluconeogenesis. Methylmalonyl-CoA isomerase is a vitamin B12-dependent enzyme, and in deficiency methylmalonic acid is excreted in the urine (**methylmalonicaciduria**).
**Cori cycle**

Lactate is released into the blood by exercising skeletal muscle, and by cells that lack mitochondria, such as red blood cells. In the Cori cycle, blood borne glucose is converted by exercising muscle to lactate, which diffuses into the blood. This lactate is taken up by the liver and reconverted to glucose, which is released back into the circulation.

**Energy requirements of gluconeogenesis**

A- Six moles of high energy compounds (ATP or GTP) are utilized:

i. Two moles of ATP for converting pyruvate to oxaloacetate.

ii. Two moles of GTP for converting oxaloacetate to PEP

iii. Two moles of ATP for converting 3-phosphoglycerate to 1,3-bis phosphoglycerate

B- Energy in the form of reducing equivalent (NADH) is required for converting 1,3-bis phosphoglycerate to glyceraldehyde 3-P
Opposing pathways of glycolysis and gluconeogenesis
Regulation of Gluconeogenesis

The moment-to-moment regulation of gluconeogenesis is determined primarily by the circulating level of glucagon, and by the availability of gluconeogenic substrates. In addition, slow adaptive changes in enzyme activity result from an alteration in the rate of enzyme synthesis or degradation, or both.

I- Hormonal regulation

A. Glucagon

It is released from pancreatic islets α cells in response to hypoglycemia and stimulates gluconeogenesis by three mechanisms.

1. Changes in allosteric effectors: Glucagon lowers fructose 2,6-bisphosphate, resulting in activation of fructose 1,6-bisphosphatase and inhibition of phosphofructokinase-1, thus favoring gluconeogenesis over glycolysis.

2. Covalent modification of enzyme activity: Glucagon, via protein kinase activity, stimulates the conversion of hepatic pyruvate kinase to its inactive (phosphorylated) form. This decreases the conversion of PEP to pyruvate, which has the effect of diverting PEP to the synthesis of glucose.

3. Induction of enzyme synthesis: Glucagon as well as epinephrine and cortisol increase the transcription of the gene for PEP-carboxykinase, thereby increasing the synthesis of this enzyme as levels of its substrate rise during fasting.

Note: Insulin causes decreased transcription of the mRNA for this enzyme.

B. Insulin

It inhibits gluconeogenesis by lowering the transcription of the genes coding for the four key enzymes of gluconeogenesis.

II- Allosteric regulation

- During fasting, elevated acetyl CoA activates hepatic pyruvate carboxylase. As a result of increased lipolysis in adipose tissue, the liver is flooded with fatty acids. The rate of formation of acetyl CoA by β-oxidation of these fatty acids exceeds the capacity of the liver to oxidize it to CO$_2$ and H$_2$O. As a result, acetyl CoA accumulates and leads to activation of pyruvate carboxylase.

- Acetyl CoA inhibits pyruvate dehydrogenase (by activating PDH kinase).

- Thus, this single compound can divert pyruvate toward gluconeogenesis and away from the TCA cycle.

- Fructose 1,6-bisphosphatase is inhibited by AMP—a compound that activates phosphofructokinase-1. This results in a reciprocal regulation of glycolysis and gluconeogenesis seen previously with fructose 2,6-bisphosphate. Elevated AMP thus stimulates pathways that oxidize nutrients to provide energy for the cell.
III- **Substrate availability**

- The availability of **gluconeogenic** precursors, particularly glucogenic amino acids, significantly influences the rate of hepatic glucose synthesis.
- Decreased levels of **insulin** favor mobilization of amino acids from muscle protein, and provide the carbon skeletons for gluconeogenesis.
- In addition, **ATP** and **NADH**, coenzymes-cosubstrates required for gluconeogenesis, are primarily provided by the **catabolism of fatty acids.**