Gluconeogenesis

• **Gluconeogenesis** is process of *synthesizing glucose from non-carbohydrate precursors*, such as lactate, pyruvate, glucogenic amino acids, glycerol, and propionate (in ruminants).

• **Liver and kidney** are the major tissues involved??
  • since they contain the necessary enzymes.

• **Gluconeogenesis** uses several enzymes of *glycolysis*, but it is **not a simple reversal of glycolysis**. It is a special pathway that requires both mitochondrial and cytosolic enzymes → the pathway is **partly mitochondrial and partly cytoplasmic**.

• The **three** irreversible steps in glycolysis are overcome by **four enzymes** which are designated as **the key enzymes of gluconeogenesis**.
Enzymes that overcome the irreversible steps in glycolysis

- **Pyruvate kinase reaction**
  - Pyruvate carboxylase
  - Mitochondria

- **Phosphofructokinase reaction**
  - Phosphoenolpyruvate carboxy-Kinase
  - Cytoplasm

- **Glucokinase (Hexokinase) reaction**
  - Fructose-1, 6-bisphosphatase
  - Cytoplasm
  - Glucose-6-phosphatase
  - Cytoplasm
Reactions Unique to Gluconeogenesis

- Glucose
  - hexokinase
  - glucose 6-phosphatase
  - Glucose 6-phosphate
    - phosphofructokinase-1
    - fructose 1,6-bisphosphatase
    - Fructose 6-phosphate
      - phosphofructokinase-1
      - PEP carboxykinase
      - Fructose 1,6-bisphosphate
    - PEP carboxykinase
    - Phosphoenolpyruvate (2)
    - PEP carboxykinase
    - Oxaloacetate (2)
  - Pyruvate kinase
  - Pyruvate (2)
    - pyruvate carboxylase
Substrates for gluconeogenesis

Gluconeogenic precursors are molecules that can be used to produce glucose. They include:

1. lactate, pyruvate.

2. glycerol (derived from the backbone of triacylglycerols.

3. α-ketoacids derived from the catabolism of glucogenic amino acids.

4. Intermediates of glycolysis and TCA cycle.
Reactions of gluconeogenesis

1. Conversion of pyruvate to phosphoenolpyruvate (PEP) (by 2 enzymes):
   A. Carboxylation of pyruvate

   In mitochondria: **Pyruvate carboxylase**, in the presence of ATP, biotin, and CO$_2$ converts pyruvate to oxaloacetate.

   ![Chemical Reaction Diagram]

   **Significance of the above reaction:** It provides OAA:

   1. an important substrate for **gluconeogenesis**
   2. to replenish the **TCA cycle intermediates** that may become depleted, depending on the synthetic needs of the cell.
B. Transport of oxaloacetate to cytosol

Oxaloacetate cannot cross the **inner** mitochondrial membrane. It can be transported to cytosol by three ways:

1- **Reduction to malate** by mitochondrial **malate dehydrogenase**. Malate can be transported to the cytosol, where it is reoxidized to oxaloacetate by cytosolic malate dehydrogenase.

   Produced **NADH** is used in the **reduction of 1,3-BPG → GAP**, 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**.
Malate aspartate shuttle

Transport of oxaloacetate to cytosol by “malate shuttle”.

Because oxaloacetate cannot penetrate mitochondrial membrane

PEP Carboxykinase

phosphoenolpyruvate

PEP Carboxykinase

Malate dehydrogenase

Oxaloacetate

NADH + H^+

NAD^+

Malate

Pyruvate carboxylase

CO_2 + ATP

ADP + Pi

Oxaloacetate

NADH + H^+

NAD^+

Malate dehydrogenase

Malate

Cytosol
C. Decarboxylation of cytosolic oxaloacetate

- *Phosphoenolpyruvate carboxykinase*, converts oxaloacetate to PEP.
- GTP is required in this reaction, and CO$_2$ is liberated.
2- **Reversal of glycolysis:** In the cytosol (the site of glycolysis), PEP undergoes further reactions catalysed by the reversible glycolytic enzymes, to form **F-1,6- bisphosphate**.

3- **Dephosphorylation of fructose 1,6- bisphosphate to fructose 6-phosphate:** by **fructose 1,6-bisphosphatase**.

* This reaction is an important **regulatory site** of gluconeogenesis.
4- Dephosphorylation of glucose 6-phosphate to glucose:

by *glucose 6-phosphatase* (present only in liver and kidney).

*glucose 6-phosphatase* is also required for the **final step** of glycogen degradation (**glycogenolysis**).
2. Gluconeogenesis from lactate:

- Lactate $\xrightarrow{LDH}$ Pyruvate $\xrightarrow{}$ Oxaloacetate
- Glucose $\xleftarrow{}$ Reversal of glycolysis $\xleftarrow{}$ PEP
Energy requirement for gluconeogenesis

✓ The overall reaction

\[
2 \text{Pyruvate} + 4 \text{ATP} + 2 \text{GTP} + 2 \text{NADH} + 2 \text{H}^+ + 6 \text{H}_2\text{O} \rightarrow \text{Glucose} + 4 \text{ADP} + 2 \text{GDP} + 6 \text{Pi} + 2 \text{NAD}^+
\]

✓ The synthesis of one glucose molecule from two pyruvate molecules consumes 4 ATP and 2 GTP (equivalent to 6 ATP) and requires two molecules of NADH.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Energy used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate carboxylase</td>
<td>2 ATP</td>
</tr>
<tr>
<td>Phosphoenolpyruvate carboxykinase (PEPCK)</td>
<td>2 GTP (ATP)</td>
</tr>
<tr>
<td>Phosphoglycerate Kinase</td>
<td>2 ATP</td>
</tr>
<tr>
<td>Glyceraldehyde -3-phosphate dehydrogenase</td>
<td>2 NADH (6 ATP equivalents)</td>
</tr>
</tbody>
</table>
Energy requirement for gluconeogenesis
• **Glycerol** is released during **lipolysis** in adipose tissue, then is delivered by the blood to the **liver, where it is phosphorylated** by **glycerol kinase** to **glycerol-Phosphate that** is oxidized by **glycerol phosphate dehydrogenase** to DAHP (an intermediate of glycolysis).

• **Adipocytes** cannot phosphorylate glycerol because they essentially lack **glycerol kinase**.
3. **Gluconeogenesis from amino acids:**

- Fasting or starvation → hydrolysis of tissue proteins → amino acids.
- **Glucogenic amino acids** → transamination or deamination → \( \alpha \)-Ketoacids → TCA cycle & form oxaloacetate → PPP → glucose
- Fatty acids, acetoacetate & **Ketogenic amino acids** → Acetyl CoA that cannot → glucose due to the **irreversibility** of pyruvate dehydrogenase reaction. These compounds → ketone bodies.
4. Gluconeogenesis from Propionate:

- Propionate is a minor precursor of glucose in humans, but a major source in ruminants.
- Propionate is formed from the oxidation of odd chain fatty acids (from dietary vegetables) as well as the oxidation of isoleucine.
- It enters the gluconeogenesis pathway via citric acid cycle after being converted to succinyl-CoA → PPP → glucose.
Thiokinase

Propionate → Propionyl-CoA → D-Methylmalonyl-CoA

Propionyl-CoA → Succinyl-CoA

Methylenetetrahydrofolate → B12 coenzyme → L-Methylmalonyl-CoA
Cori cycle (Lactic acid cycle):

- Lactate, produced by anaerobic glycolysis in exercising skeletal muscle and RBCs, is released into the blood.
- From blood, lactate is taken up by liver and converted to glucose (by gluconeogenesis), which is released back into the circulation then taken by the muscle for utilization as fuel.
Regulation of gluconeogenesis

1. Allosteric regulation

- Acetyl CoA
  - Pyruvate carboxylase
  - Pyruvate Dehydrogenase

Acetyl CoA increases during starvation due to increased FAs oxidation. This single compound divert pyruvate away from TCA cycle and toward gluconeogenesis.

- AMP & Fructose 2,6 Bisphosphate
  - Fructose 1,6 bisphosphatase
  - Phosphofructokinase-1

2. Hormonal regulation

- Insulin
  - Synthesis of regulatory enzymes

- Cortisol, Epinephrine, Glucagon, Starvation & Diabetes mellitus
  - Synthesis of regulatory enzymes
3. Availability of gluconeogenic substrates

**Glucogenic amino acids**

In diabetes & excess cortisol intake (these conditions favor protein catabolism and mobilization of amino acids from muscle providing the carbon skeleton for gluconeogenesis)

**Glycerol**

during fasting & diabetes (due to increased lipolysis in adipose tissue)

**ATP and NADH**

are primarily provided by the catabolism of fatty acids
Physiological significance of gluconeogenesis

**Maintenance of blood glucose level under conditions of fasting & starvation**

**Removal of metabolic waste products**
(glycerol, lactate) from the blood

**Continuous supply of glucose to Brain & RBCs**
Where it is an essential energy source

Severe hypoglycemia can leads to coma and death.