Gluconeogenesis
- Liver glycogen is depleted after an overnight fast.

- The requirement for glucose is met by de novo synthesis from non-carbohydrate precursors such as lactate and alanine. This process is gluconeogenesis.

- Gluconeogenesis is essentially glycolysis in reverse, except that the irreversible steps are bypassed by additional ones.
Two enzymes are required to bypass the pyruvate kinase step:

\[
\begin{align*}
\text{Pyruvate carboxylase} & : COO^- + C=O + HCO_3^- \rightarrow COO^- + C=O + CH_2COO^- + ATP \\
& \quad \text{ADP} + Pi
\end{align*}
\]
Pyruvate Carboxylase:

- Contains biotin covalently linked to a lysine residue. Biotin participates in the transfer of bicarbonate.

- Pyruvate carboxylase reaction is irreversible.

- Acetyl CoA is an allosteric activator

- OAA synthesis increases TCA cycle activity. Accumulation of Acetyl CoA signals the need for more OAA. If TCA is inhibited, OAA undergoes gluconeogenesis.
Pyruvate carboxylase reaction occurs in the mitochondrion. OAA is then converted to malate (what’s the enzyme?) and malate exits the mitochondrion via the malate -α-kg transporter. Malate is reconverted to OAA in the cytosol. (These reactions are part of a “shuttle” - which one?)
Following the pyruvate carboxylase reaction, OAA is converted to PEP:

\[
\begin{align*}
\text{COO-} & \quad \text{PEP} & \quad \text{COO-} \\
\text{C=O} & \quad \text{Carboxykinase} & \quad \text{C-OPO}_3^{2-} \\
\text{CH}_2 & \quad \text{GTP} & \quad \text{CH}_2 \\
\text{COO-} & \quad \text{GDP} & \quad \text{OAA} \\
\text{OAA} & \quad \text{CO}_2 & \quad \text{PEP}
\end{align*}
\]
During fasting, production of glucagon by the pancreas leads to increased synthesis of PEP carboxykinase in liver (example of hormonal induction).

Glucagon elevates c-AMP, which triggers transcription of the PEP carboxykinase gene.

After several hours of starvation, levels of PEP carboxykinase rise, raising the rate of gluconeogenesis.
• **Insulin acts in opposition to glucagon, leading to a reduction in the synthesis of PEP carboxykinase; the rate of gluconeogenesis is decreased.**

• **The reactions that convert PEP to F-1,6BP are simply a reverse of the near-equilibrium reactions of glycolysis (enolase, phosphoglycerate kinase, phosphoglucomutase, G-3-P d’hase).**
The PFK reaction is bypassed by the irreversible reaction catalyzed by Fructose 1,6 Bisphosphatase:

- $\text{H}_2\text{O} 
- \text{F-1,6 Bisphosphate} 
- \text{Pi} 
- \text{F-6-PO}_4$
**Fructose 1,6 Bisphosphatase:**

- Activated by citrate and inhibited by AMP; just the opposite of PFK-1.

- Allosterically inhibited by F-2,6BP, which is an activator of PFK-1.

- Thus, we have reciprocal regulation of glycolysis and gluconeogenesis.
After the near-equilibrium reaction of F-6-P to G-6-P (G-6-P isomerase), the final enzyme required for gluconeogenesis is Glucose-6-phosphatase:
Glucose-6-Phosphatase:

- Has two components: one is a transport protein embedded in the endoplasmic reticulum membrane that is specific for glucose-6-P, the other is a non-specific hydrolase located in the lumen of the endoplasmic reticulum.

- G-6-P'tase is only found in the ER of liver and kidney; thus only these tissues are gluconeogenic.
Futile Cycles:

- Many tissues contain only a partial set of gluconeogenesis enzymes.
- Muscle contains PEP carboxylase and F-1,6BP’tase but muscle is not gluconeogenic.
- In tissues running both the PFK and F-1,6P’tase reactions, the net balance is the hydrolysis of ATP.
- Now termed substrate cycles, it is believed that these perform an important regulatory role.
Reciprocal Regulation of Glycolysis and Gluconeogenesis:

**Glycolysis**
- $\oplus$ F-2,6BP
- $\ominus$ ATP
- $\ominus$ Citrate
- $\ominus$ Glucagon (inhibition of pyruvate kinase)
- $\ominus$ Acetyl CoA (inhibition of pyruvate kinase & pyruvate d’hase)

**Gluconeogenesis**
- $\ominus$ F-2,6BP
- $\ominus$ AMP
- $\oplus$ Citrate
- $\oplus$ Glucagon (c-AMP induction of PEPCK)
- $\oplus$ Acetyl CoA (stimulation of pyruvate carboxylase)
Reciprocal Regulation of Glycolysis and Gluconeogenesis (cont’d):

In response to elevated glucagon (during a fast) c-AMP dependent protein kinase phosphorylates PFK-2 and inactivates it. Less F-2,6BP is made; hence the rate of gluconeogenesis is increased and the rate of glycolysis decreased.