Biochemistry course

Part 02: metabolic biochemistry

Chapter 01: Carbohydrate metabolism

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Gluconeogenesis

1/Defenition

Gluconeogenesis is a metabolic pathway that enables the synthesis of glucose from non-carbohydrate precursors such as amino acids, lactate, and glycerol. Although often considered the functional counterpart of glycolysis, it is not simply its reverse pathway.

This process occurs primarily in the liver (≈90%) and, to a lesser extent, in the kidney (≈10%), with minor contributions from the intestinal epithelium. Gluconeogenesis takes place across multiple cellular compartments, including the mitochondria**,** cytosol**,** and endoplasmic reticulum**.**

Gluconeogenesis is activated during prolonged fasting, when dietary glucose and glycogen stores are depleted, as well as during intense physical activity**.** Under these conditions, cellular energy status is altered, typically characterized by increased AMP levels and a demand for maintaining blood glucose homeostasis.

2/Precursors (Substrates)

Non-carbohydrate precursors used in gluconeogenesis are of several types:

-Lactate, produced in muscle, is converted into pyruvate by **lactate dehydrogenase**.

-Glucogenic amino acids, derived from the diet or from skeletal muscle protein breakdown. These include **alanine** (accounting for 40–60%), as well as serine, cysteine, threonine, glycine, tyrosine, phenylalanine, and isoleucine.

-Glycerol, released from the breakdown of triglycerides in adipose tissue.

These precursors are first converted into intermediates of glycolysis:

- **Pyruvate** (from lactate and glucogenic amino acids)
- **Dihydroxyacetone phosphate (DHAP)** (from glycerol)

3/ Mechanism and Key Enzymes

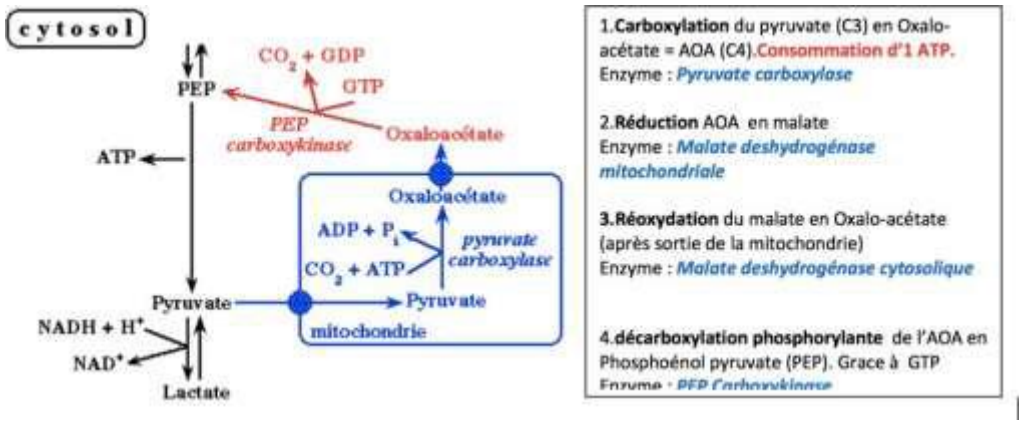
- Gluconeogenesis is not simply the reverse of glycolysis, as several steps in glycolysis are **irreversible**. To allow glucose synthesis to proceed in a thermodynamically favorable manner, these irreversible steps must be bypassed by alternative reactions.
- Of the ten enzymatic reactions involved, **seven are shared with glycolysis** and operate in the reverse direction. However, the **three irreversible steps of glycolysis** are replaced by specific bypass reactions in gluconeogenesis.
- These bypass mechanisms involve **three key enzymes** that are unique to gluconeogenesis and ensure the proper progression of the pathway.

The transition from enolpyruvate to phosphoenolpyruvate (reaction 9 of glycolysis) catalyzed by thephosphoenolpyruvate carboxykinaseis done indirectly. In fact this reaction is bypassed from themalatewhich has the ability to exit the mitochondria via the malate shuttle-aspartateand to be retransformed into oxaloacetate at the cytosol level.oxaloacetatewill itself be transformed intophosphoenolpyruvateby the phosphoenolpyruvatecarboxykinase. - The transition from fructose-1,6-bisphosphate to fructose-6-phosphate catalyzed by the fructose-1,6-bisphosphataseis donedirectly. - The transition from glucose-6-phosphate to glucose catalyzed by glucose-6- phosphatase is direct. It is important to note that this enzyme is only present in the liver, which will therefore be the only organ able to release glucose into the blood.

4/ Stages of Gluconeogenesis

4.1/ Formation of Phosphoenolpyruvate (PEP) from Pyruvate

The first step of gluconeogenesis involves the conversion of **pyruvate into phosphoenolpyruvate (PEP)** via **oxaloacetate**. This process occurs in two distinct stages: a **mitochondrial step** followed by a **cytosolic step**.

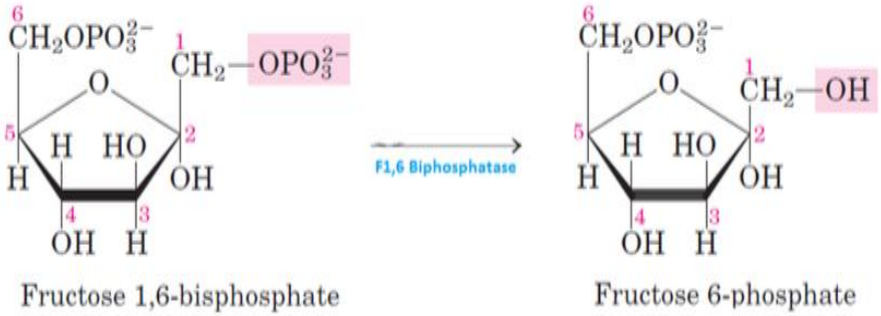


4.2/ Formation of fructose 6-P from fructose 1,6-bisphosphate

This step involves the **hydrolysis of the phosphate group at C1 of fructose-1,6-bisphosphate**, converting it into **fructose-6-phosphate**. This reaction bypasses the third irreversible step of glycolysis.

It is catalyzed by **fructose-1,6-bisphosphatase**, an **allosteric enzyme** that plays a key regulatory role in gluconeogenesis.

This reaction requires the consumption of **one molecule of H_2O**.



Fructose 1,6-bisphosphate

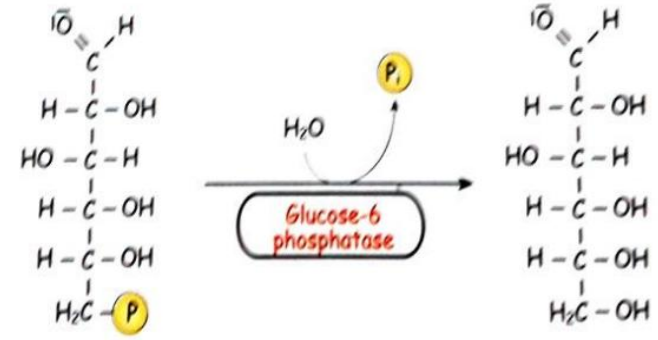
Fructose 6-phosphate

4.3/ Glucose formation from G6P

Glucose-6-phosphate is first transported to the endoplasmic reticulum.

It undergoes the action of the enzyme glucose-6-phosphatase.

The resulting free glucose can then leave the liver or the kidney to be used by glucose-dependent tissues.



Glucose-6-phosphate

Glucose

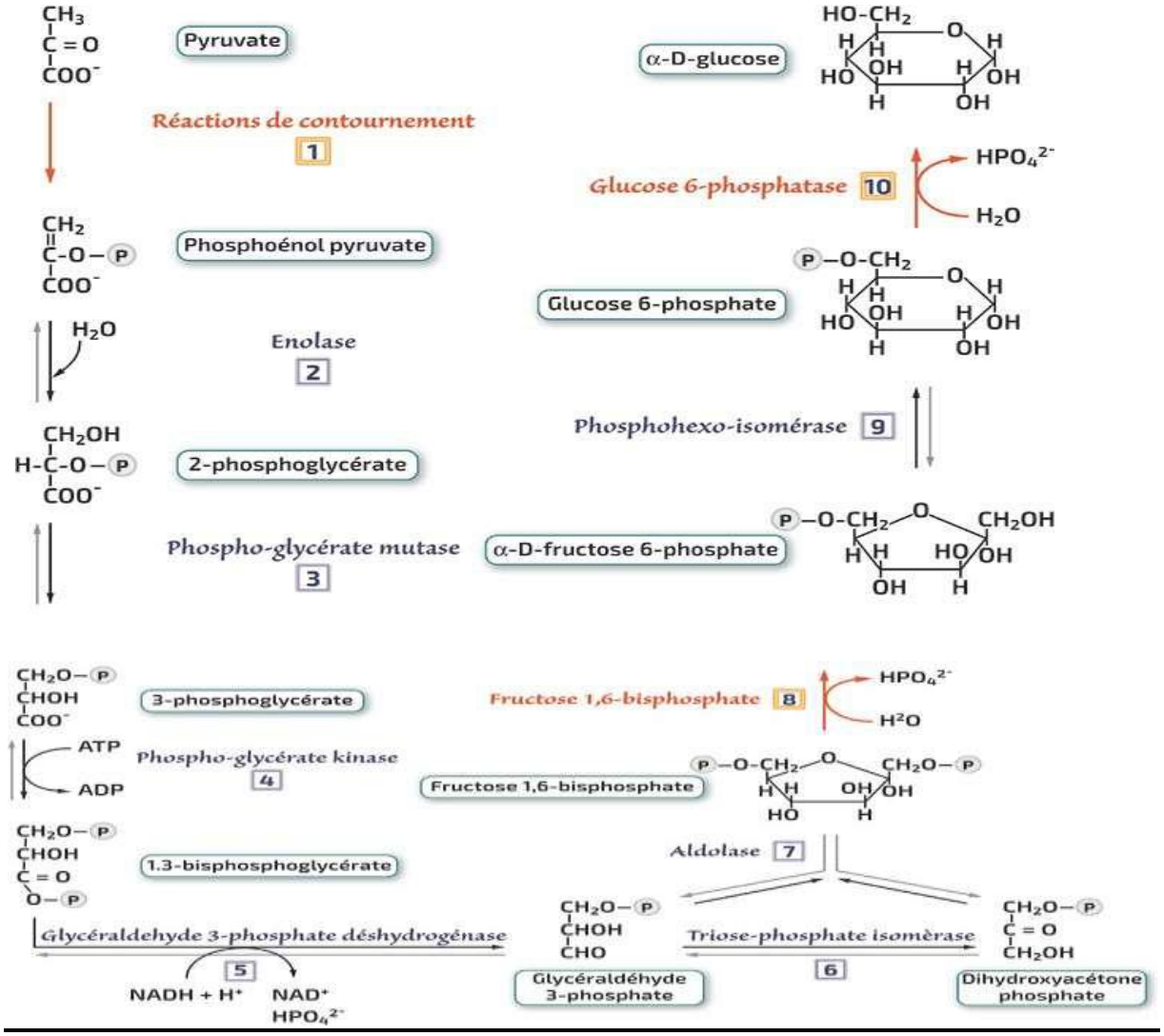


Figure 1. Enzymatic reactions of gluconeogenesis

The balance of the formation of glucose from 2 pyruvates is as follows:

2 pyruvate + 4 ATP+ 2 GTP + 2 NADH,H$^+$	⟶	Glucose + 4 ADP + 2 GDP + 6 Pi + 2 NAD$^+$

5/ Neoglucogenesis from other precursors

5.1/ From muscle lactate

During periods of intense muscular activity (anaerobiosis), oxygen decreases and the Krebs cycle slows down, the muscles have glycolysis as their only source of energy and therefore the formation of pyruvate which is transformed into lactate (by lactate dehydrogenase) to regenerate NAD+.

The lactate produced leaves the muscles and reaches the liver where it is transformed into pyruvate. Pyruvate is transformed in the hepatocyte into glucose by gluconeogenesis. The glucose can then be put back at the disposal of the muscle: this is the Cori cycle.

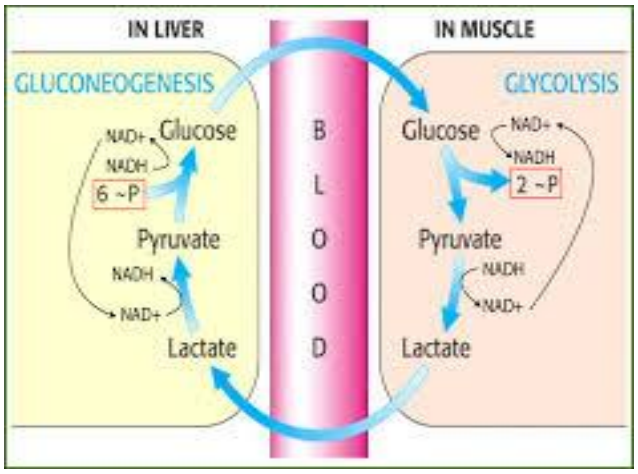
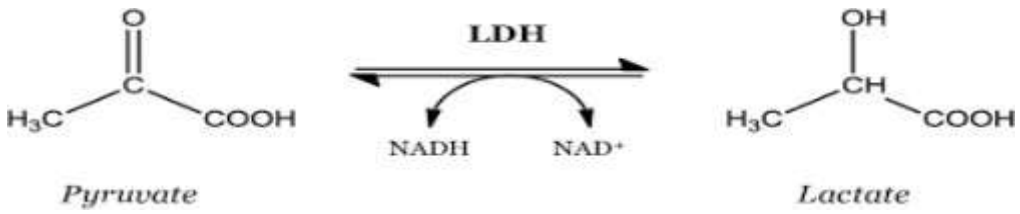


Figure 2. Cori cycle

5.2/ From pyruvate and lactate of globular origin

Anaerobic glycolysis is the only source of energy for red blood cells (which lack mitochondria). Red blood cells produce pyruvate and lactate through lactate dehydrogenase, which regenerates NAD+ for glycolysis.

Red blood cells produce pyruvate and lactate which will be taken up by hepatic gluconeogenesis.



Pyruvate *Lactate*

5.3 /From muscle-derived alanine

Muscle amino acid catabolism is quantitatively unimportant under normal physiological and nutritional conditions. It becomes important in certain nutritional circumstances (e.g. prolonged fasting) or pathological circumstances (unbalanced diabetes mellitus). The amino acid alanine leaves the muscle for the liver where it gives pyruvate by a reaction called transamination catalyzed by ALAT (alanine aminotransferase). Pyruvate is transformed in the hepatocyte into glucose by gluconeogenesis; and the glucose can then be made available to the muscle. This glucose-alanine cycle is called the FELIG cycle.

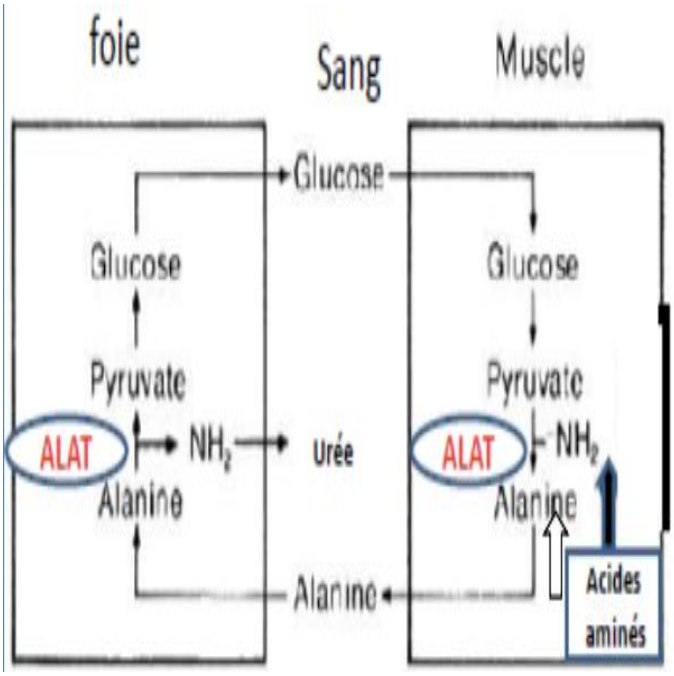


Figure 3. Felig cycle

5.4 /From glycerol

It occurs mainly in the liver from glycerol, for example from the complete hydrolysis of triglycerides. It uses the following pathway:

Glycerol +ATP →**glycerol phosphate**+ADP (**glycerol-phosphate kinase**)

glycerol phosphate + NAD+→ **dihydroxyacetone-phosphate** + NADH,H+(**glycerol phosphate dehydrogenase)**

dihydroxyacetone-phosphate →glyceraldehyde-3-phosphate

From there, the pathway joins that of gluconeogenesis from pyruvate, that is to say:

glyceraldehyde-3-phosphate + dihydroxyacetone-phosphate → fructose-1,6- bisphosphate

-fructose-1,6-bisphosphate + H2O → fructose-6-phosphate + Pi

-fructose-6-phosphate → glucose-6-phosphate

- glucose-6-phosphate + H2O → glucose + Pi

The balance of the formation of glucose from glycerol is as follows:

Consommation	Production
• 2 glycérol • 2 ATP • 2 NAD^+ • 2 H_2O	• 1 glucose • 2 ADP • 2 ($NADH,H^+$) • 2 P_i

5.5 /From glucoforming amino acids

The carbon skeleton of amino acids resulting from protein catabolism is transformed into pyruvate or one of the Krebs cycle intermediates (α ketoglutarate, succinyl-coA, oxaloacetate), or enters the Krebs cycle to exit at the level of malate to join the gluconeogenesis pathway.

6/ Regulation of gluconeogenesis

6.1/ Allosteric regulation

It is carried out at two levels:

- **Phosphofructokinase 1/ Fructose 1,6-bisphosphatase (PFK1/FBP1)**

PFK-1	FBP
- Inhibée par le citrate et l'ATP - Activée par le F2,6-BP, AMP	- activée par le citrate et l'ATP. - Inhibée par le F2,6-BP, AMP

- **Pyruvate dehydrogenase / Pyruvate carboxylase (PDH/PC)**

PDH	PC
- Inhibée par l'Acétyl-CoA, le NADH,H+ et l'ATP.	- Activée par : l'Acétyl-CoA, le NADH,H+ et l'ATP.

6.2 /Hormonal regulation

- In hypoglycemia, the secreted glucagon and adrenaline lower the level of fructose 2,6 bisphosphate which then activates fructose 1,6 bisphosphatase which triggers gluconeogenesis and inhibits glycolysis.

- In hyperglycemia, insulin causes an increase in the concentration of fructose 2,6 bisphosphate which activates phosphofructokinase 1 which triggers glycolysis and inhibits gluconeogenesis.