King Saud University
College of Sciences
Biochemistry Department

الأيض (١)
Metabolism (1)
BCH 340

Lecture 5: Gluconeogenesis

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Intended learning outcomes (ILOs)

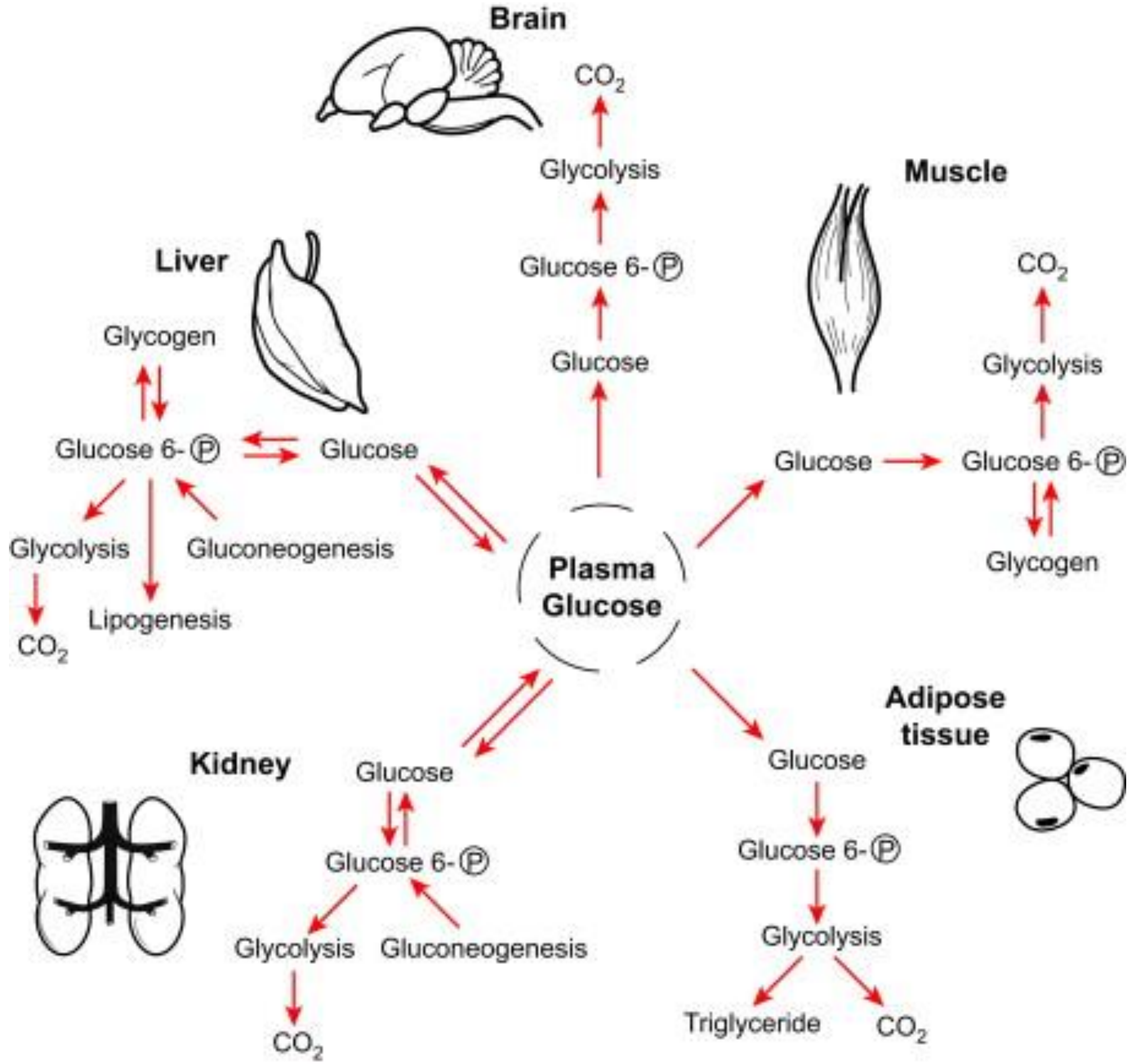
By the end of this lecture, students will be able to:

- Demonstrate a thorough understanding of gluconeogenesis as an anabolic pathway.
- Understand the reciprocal relationship between gluconeogenesis and glycolysis.
- Identify and explain the key regulatory enzymes and factors that control gluconeogenesis.

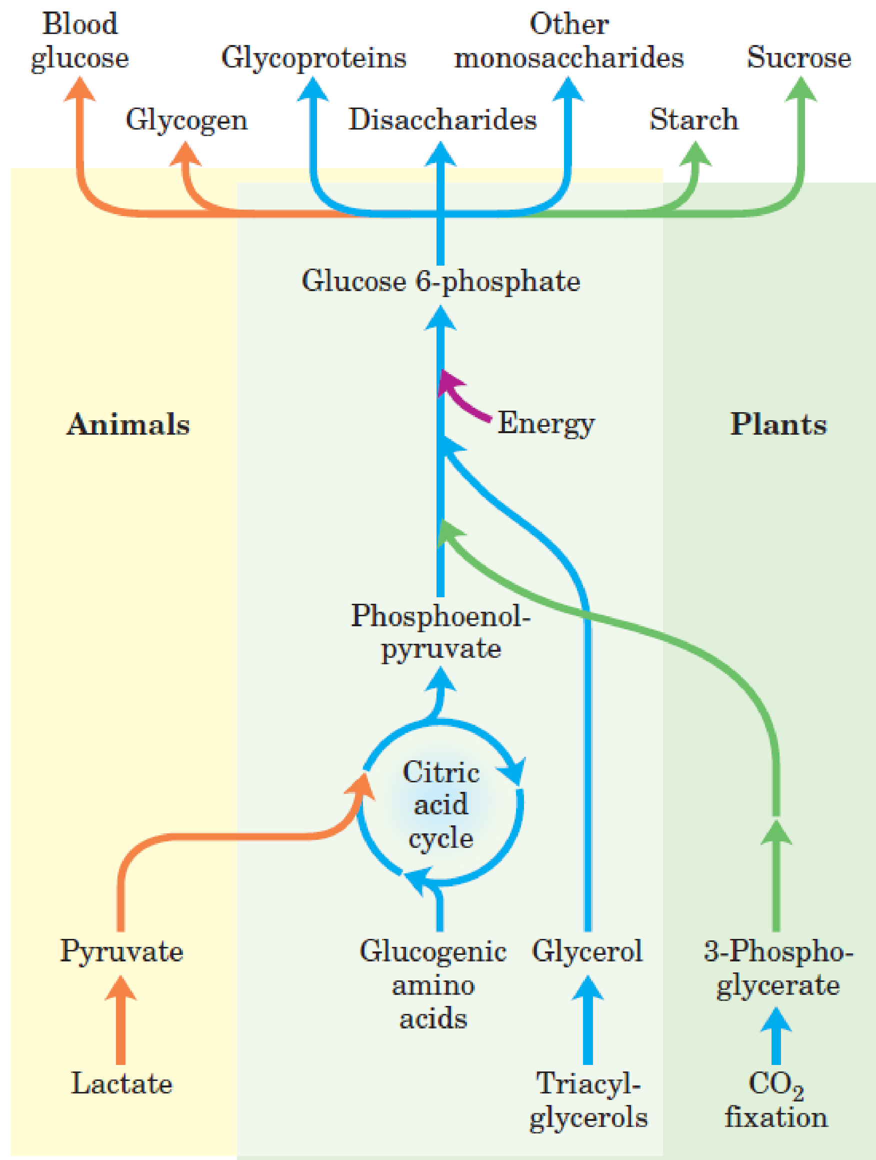
Introduction

- Gluconeogenesis is a metabolic pathway that enables the production of glucose from non-carbohydrate precursors, such as amino acids, lactate, pyruvate, and glycerol.
- Some tissues (such as brain and erythrocytes) depend on glucose as its main energy source and require a constant supply of it.
- Gluconeogenesis is particularly important during periods of fasting, starvation, and low-carbohydrate diets when glucose availability is limited and the liver stores of glycogen are depleted.
- This pathway takes place primarily in the liver (90%) and, to a lesser extent, in the renal cortex of the kidneys.

The flow of glucose to and from plasma to major organs



Carbohydrate synthesis from simple precursors



Importance of gluconeogenesis

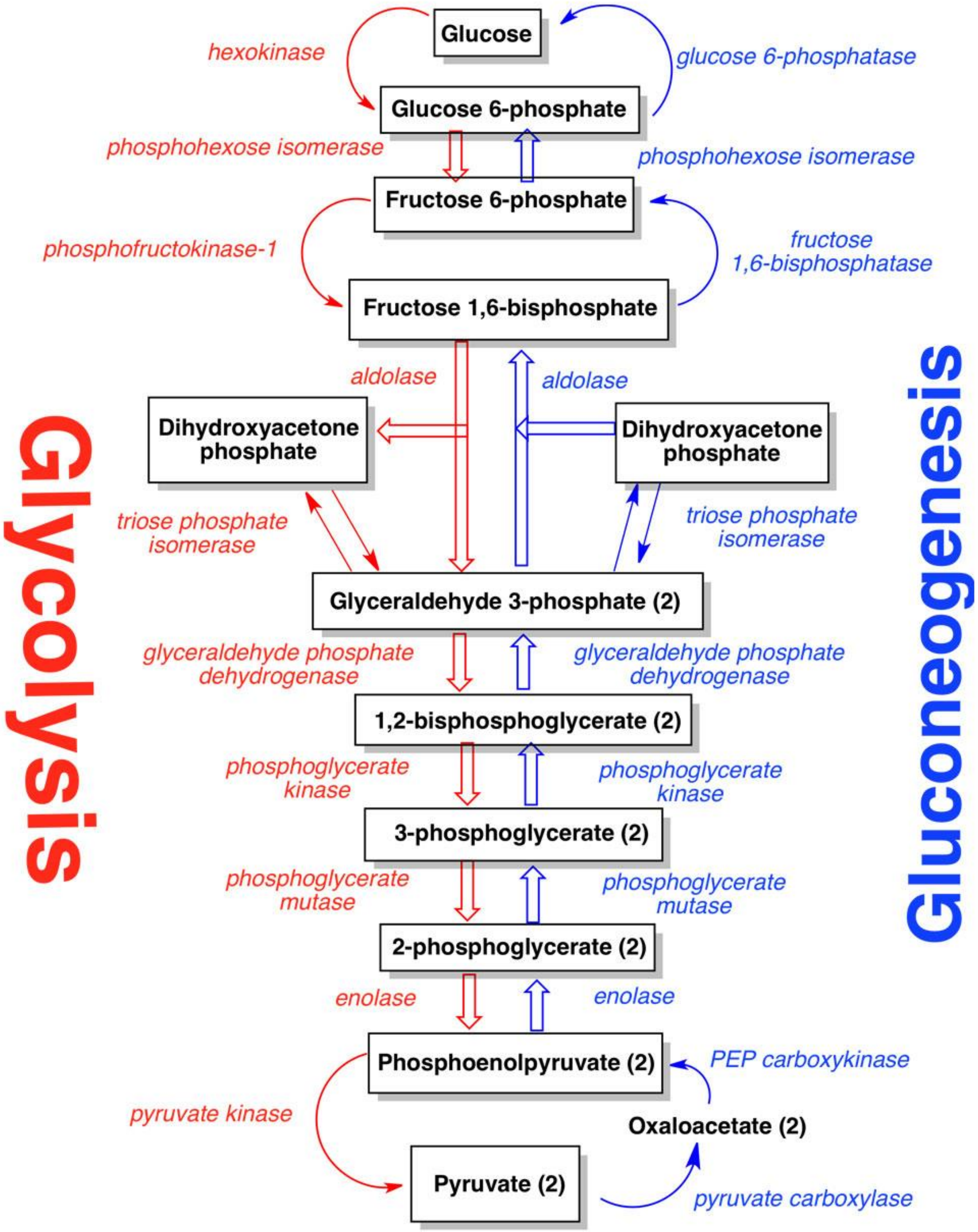
- Beside the vital role of gluconeogenesis in ensuring glucose availability for critical cellular functions, this metabolic pathway has additional roles, including:

I. Control of acid-base balance:

- Production of lactate in excess of its clearance causes metabolic acidosis, however, the synthesis of glucose from lactate is a major route of lactate disposal.

II. Maintenance of amino acid balance:

- Gluconeogenesis provides an alternative pathway for the disposal of excess amino acids when dietary protein intake exceeds the body's immediate needs for protein synthesis.
- It supplements the cell with intermediates that serve as precursors for the synthesis of non-essential amino acid.


Glycolysis
Gluconeogenesis
Glucose
hexokinase
glucose 6-phosphatase
Glucose 6-phosphate
phosphohexose isomerase
phosphohexose isomerase
Fructose 6-phosphate
phosphofructokinase-1
fructose 1,6-bisphosphatase
Fructose 1,6-bisphosphate
aldolase
aldolase
Dihydroxyacetone phosphate
Dihydroxyacetone phosphate
triose phosphate isomerase
triose phosphate isomerase
Glyceraldehyde 3-phosphate (2)
glyceraldehyde phosphate dehydrogenase
glyceraldehyde phosphate dehydrogenase
1,2-bisphosphoglycerate (2)
phosphoglycerate kinase
phosphoglycerate kinase
3-phosphoglycerate (2)
phosphoglycerate mutase
phosphoglycerate mutase
2-phosphoglycerate (2)
enolase
enolase
Phosphoenolpyruvate (2)
PEP carboxykinase
pyruvate kinase
Oxaloacetate (2)
Pyruvate (2)
pyruvate carboxylase

Gluconeogenesis reactions

Gluconeogenesis is NOT a direct reversal of glycolysis:

- Although seven of the ten enzymatic reactions of gluconeogenesis are the reverse of glycolytic reactions. However gluconeogenesis and glycolysis are not identical pathways running in opposite directions.
- Gluconeogenesis bypasses the three essentially irreversible steps in glycolysis catalyzed by:
  - Hexokinase (glucokinase)
  - Phosphofructokinase-1
  - Pyruvate kinase

Gluconeogenesis reactions (cont.)

Unique steps for gluconeogenesis:

- In gluconeogenesis, the following new steps bypass the irreversible reactions of glycolysis:

1. Conversion of pyruvate to PEP:

- The conversion of pyruvate to phosphoenolpyruvate (PEP) is the first committed step of gluconeogenesis.

- This conversion bypasses the non-equilibrium reaction catalyzed by the glycolytic enzyme pyruvate kinase.

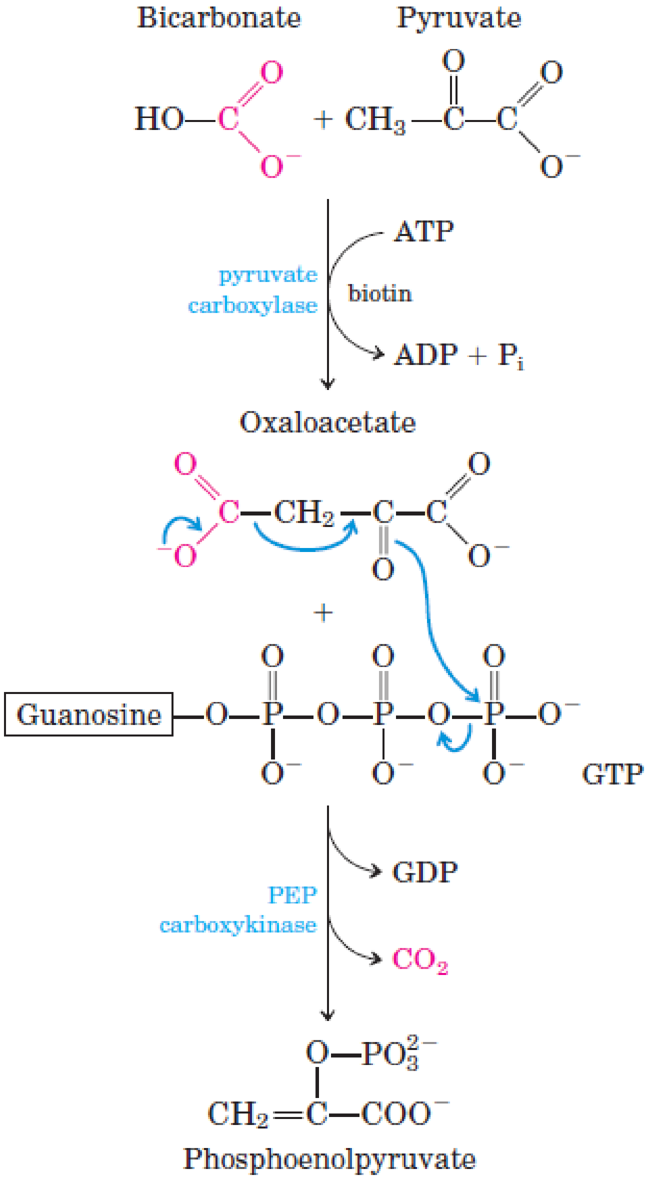
Gluconeogenesis reactions (cont.)

Unique steps for gluconeogenesis (cont.):

1. Conversion of pyruvate to PEP:

- In the mitochondria, pyruvate is converted to oxaloacetate by the action of pyruvate carboxylase, this reaction requires coenzyme biotin and ATP.

- In the cytosol, oxaloacetate is converted to phosphoenolpyruvate (PEP) by the action of PEP carboxykinase.



Gluconeogenesis reactions (cont.)

Unique steps for gluconeogenesis (cont.):

Transportation of oxaloacetate to the cytosol:

- Oxaloacetate (generated in the mitochondria) needs to be transported out of the mitochondria into the cytosol, where it can participate in subsequent steps of the gluconeogenesis pathway.

- Because the mitochondrial membrane has no transporter for oxaloacetate, oxaloacetate must be reduced to malate by mitochondrial malate dehydrogenase, at the expense of NADH.

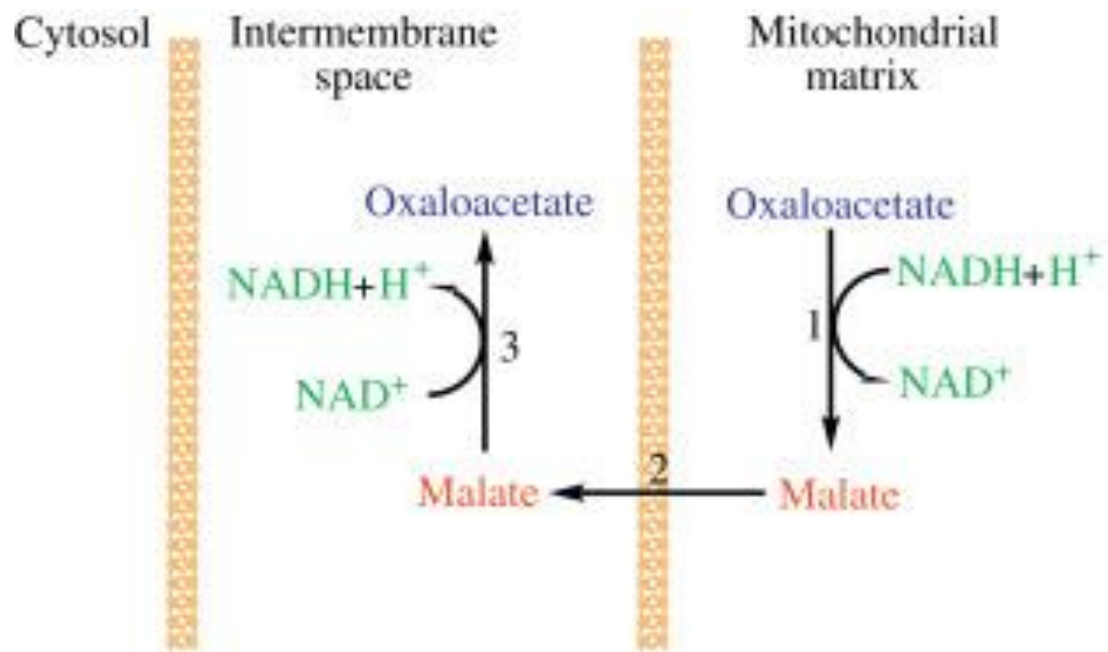
- This transport process is facilitated by a specific transporter protein known as the malate-oxaloacetate shuttle.

Malate-oxaloacetate shuttle

- Malate-oxaloacetate shuttle transports oxaloacetate from mitochondria into the cytosol.

  1 and 3 = malate dehydrogenase

  2 = malate translocase



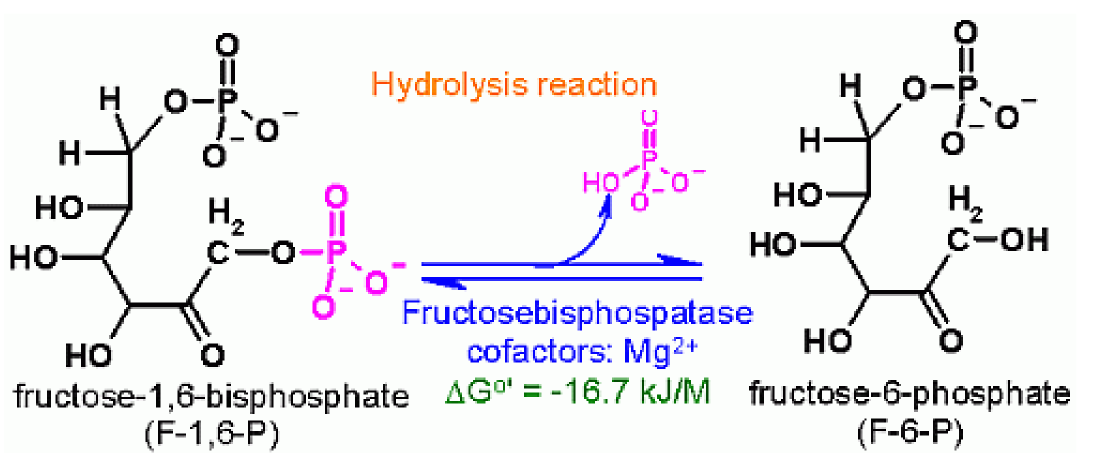
Malate shuttle

Gluconeogenesis reactions (cont.)

Unique steps for gluconeogenesis (cont.):

2. Formation of fructose 6-phosphate:

- Fructose 6-phosphate is generated from fructose 1,6-bisphosphate by the highly exergonic hydrolysis of the phosphate ester at carbon no.1.
- This exergonic hydrolysis is catalyzed by fructose 1,6-bisphosphatase.
- This reaction bypasses the irreversible reaction catalyzed phosphofructokinase-1.

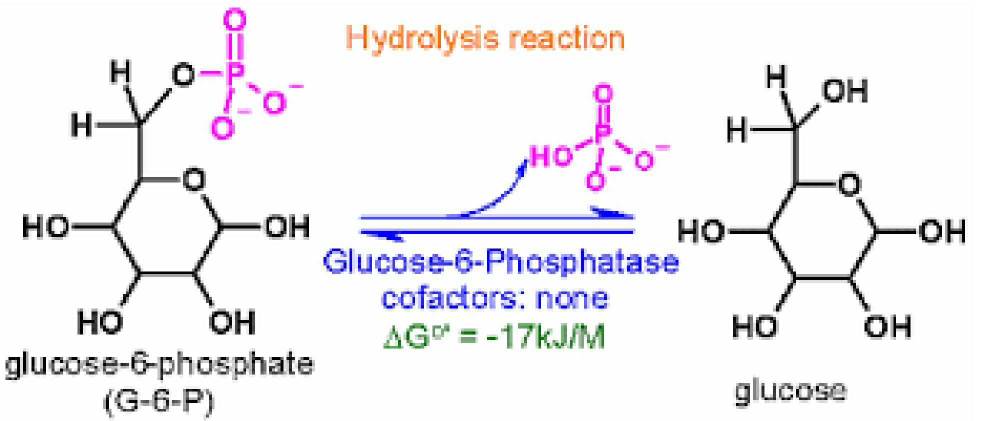


Gluconeogenesis reactions (cont.)

Unique steps for gluconeogenesis (cont.):

3. Formation of glucose:

- In glycolysis, glucose is phosphorylated in the first step by the action hexokinase/glucokinase.
- However, in the final step of gluconeogenesis, glucose 6-phosphatase catalyzes the conversion of glucose-6-phosphate to glucose.
- This Mg^{2+}-activated enzyme is absent in brain and muscle tissues.



Gluconeogenesis energy cost

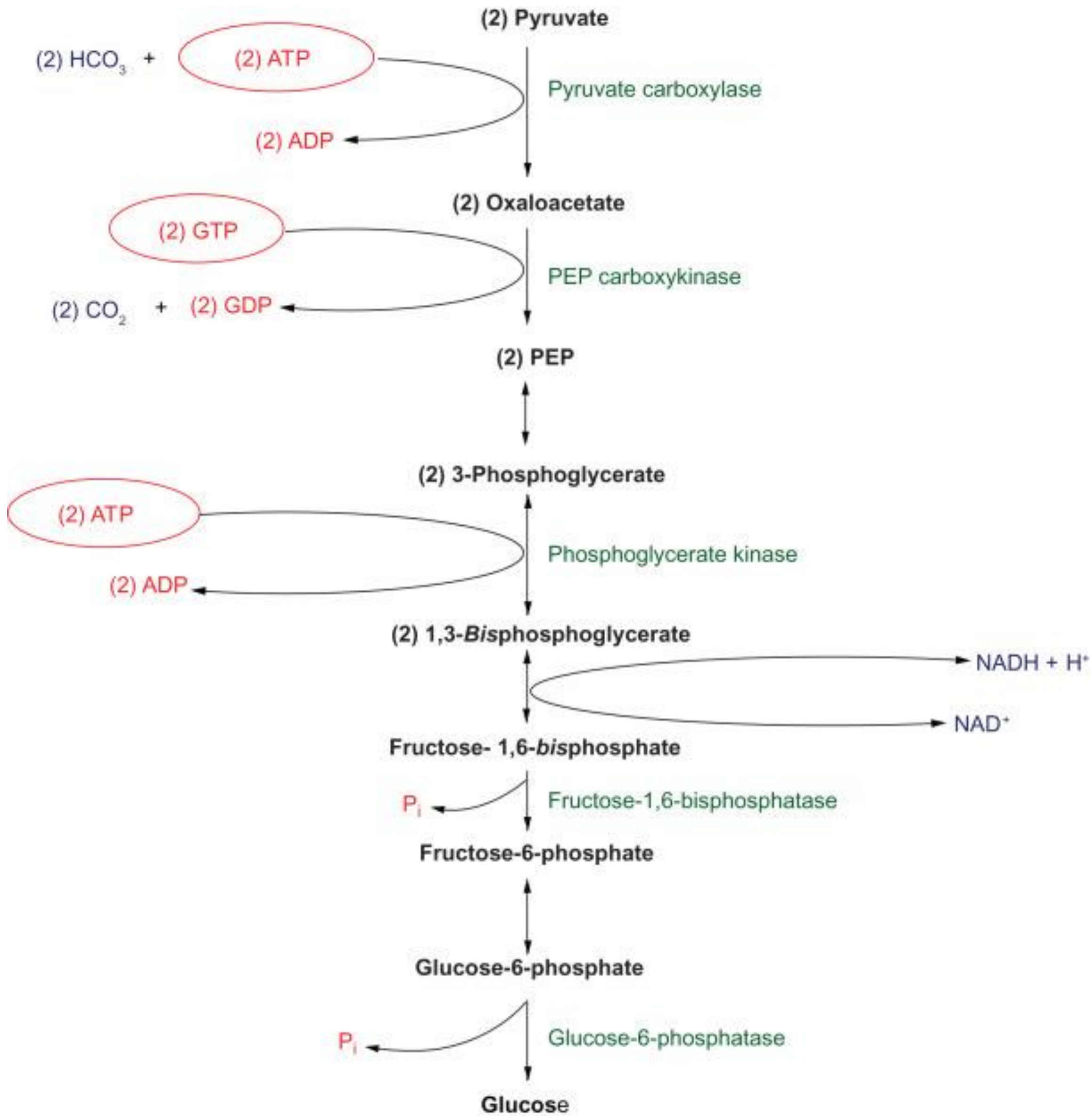
- Gluconeogenesis is an energy-consuming process, requiring ATP and GTP as input to synthesis one molecule of glucose from two molecules of pyruvate.

- It requires four molecules of ATP and two molecules of GTP as input per molecule of glucose synthesized.

- These ATP and GTP molecules are consumed at different steps of the pathway:
  - Pyruvate carboxylase (-2 ATP)
  - PEP carboxykinase (-2 GTP)
  - Phosphoglycerate kinase (-2 ATP)

- Therefore, the total energy consumption in terms of ATP equivalents is 4 ATP + 2 GTP = 6 ATP equivalents.

Gluconeogenesis energy cost (cont.)



Precursors of gluconeogenesis

- Gluconeogenesis is the process by which glucose is synthesized from non-carbohydrate precursors.

- Several precursors can be used to generate glucose via gluconeogenesis, including:
  - Pyruvate
  - Lactate
  - Glycerol
  - Glucogenic amino acids
  - Propionate

Precursors of gluconeogenesis (cont.)

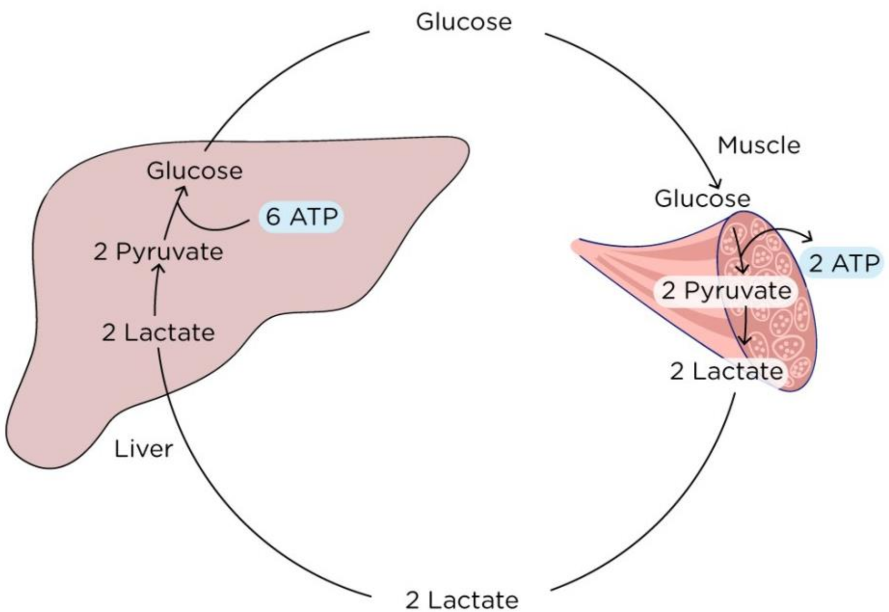
Lactate (Cori cycle):

- Lactate (produced from the anaerobic metabolism of glucose in tissues such as muscles) can serve as a gluconeogenic substrate.

- Lactate released from muscles (during intense exercise) cycles back to the liver where it is converted into pyruvate by lactate dehydrogenase.

- In the liver, pyruvate is used in gluconeogenesis to synthesis glucose.

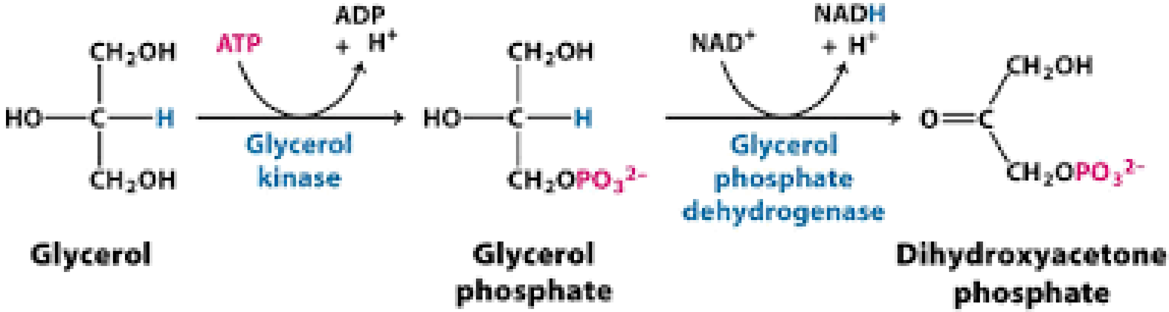
- Glucose is released into the circulation and is taken up by muscle to meet its needs. This pathway is called Cori cycle.



Precursors of gluconeogenesis (cont.)

Glycerol:

- Glycerol (derived from the hydrolysis of triglycerides in adipose tissue) can be converted into glycerol 3-phosphate.
- Glycerol-3-phosphate is then oxidized to dihydroxyacetone phosphate (DHAP) in the liver.
- By the action of by the action of triose isomerase, DHAP can be converted into glyceraldehyde 3-phosphate, an intermediate in gluconeogenesis.
- Two molecules of glycerol is required to synthesis one molecule of glucose by gluconeogenesis pathway.

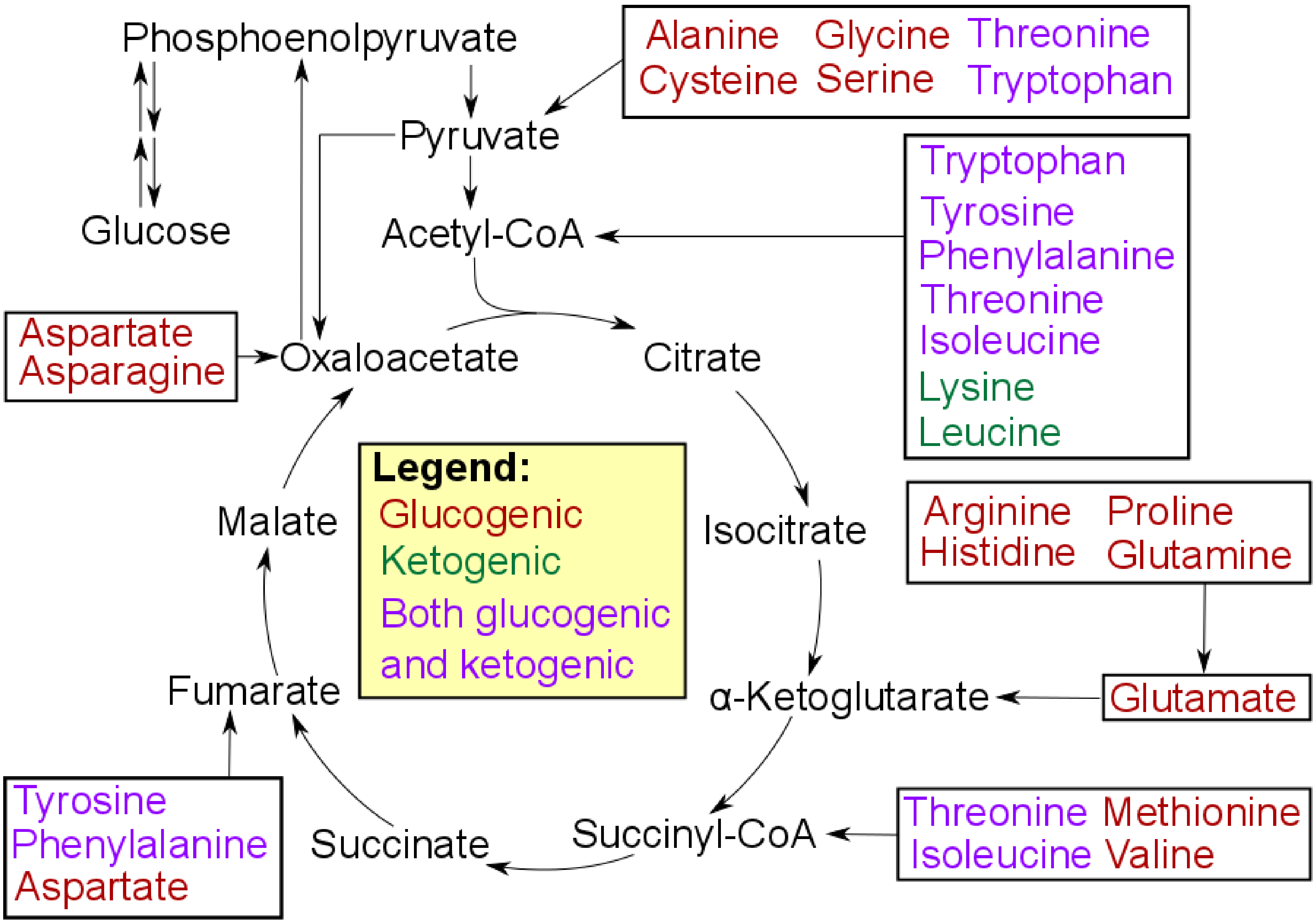


Precursors of gluconeogenesis (cont.)

Glucogenic amino acids:

- Glucogenic amino acids are those amino acids that can serve as precursors for glucose synthesis through gluconeogenesis.
- They include all amino acids except leucine and lysine (they are purely ketogenic).
- In the liver, the production of glucose from glucogenic amino acids involves the conversion of these amino acids to α-keto acids and then to glucose.
- This mechanism predominates during catabolysis, rising as fasting and starvation increase in severity.

Summary of amino acid catabolism



Precursors of gluconeogenesis (cont.)

Glucogenic amino acids (cont.):

Glucose-alanine cycle:

- The glucose-alanine cycle is a metabolic pathway that plays a crucial role in the redistribution of nitrogen and carbons between tissues, particularly between skeletal muscle and the liver.

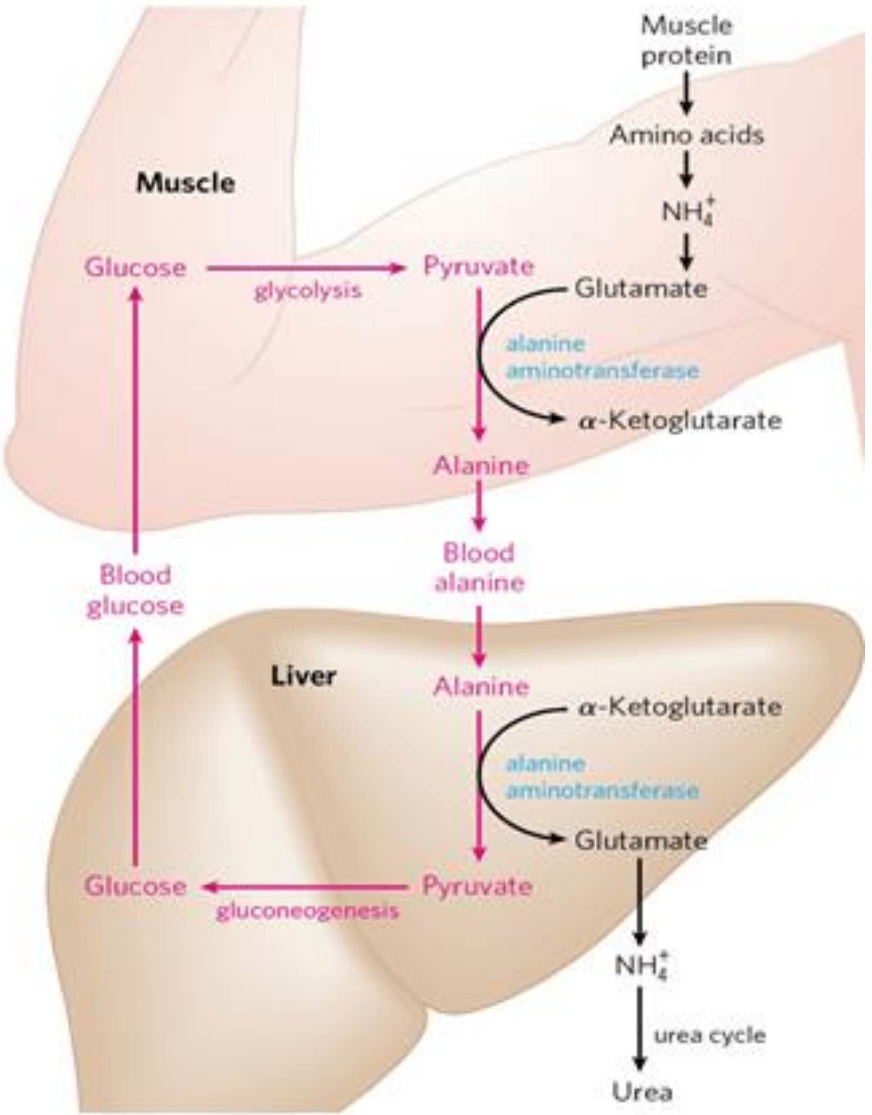
- This cycle allows efficient energy production in skeletal muscle while preventing the accumulation of toxic ammonia by converting nitrogen into a less toxic form (alanine) for transport to the liver for disposal.

- This cycle is particularly important during prolonged exercise or periods of fasting.

Precursors of gluconeogenesis (cont.)

Glucose-alanine cycle (cont.):

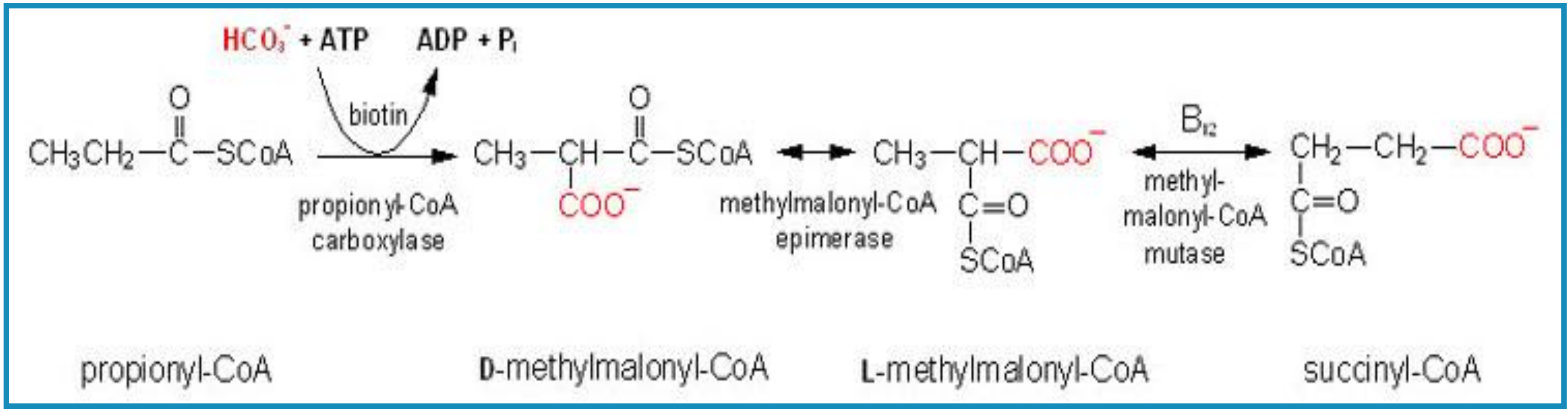
- The cycle involves the conversion of pyruvate generated from glycolysis in muscle tissue into alanine, which is then transported to the liver via the bloodstream.
- In the liver, alanine is converted back into pyruvate, which can be used for gluconeogenesis to produce glucose.
- This glucose is released into the bloodstream and can be taken up by tissues such as skeletal muscle, where it undergoes glycolysis to generate ATP for energy production.



Precursors of gluconeogenesis (cont.)

Propionate:

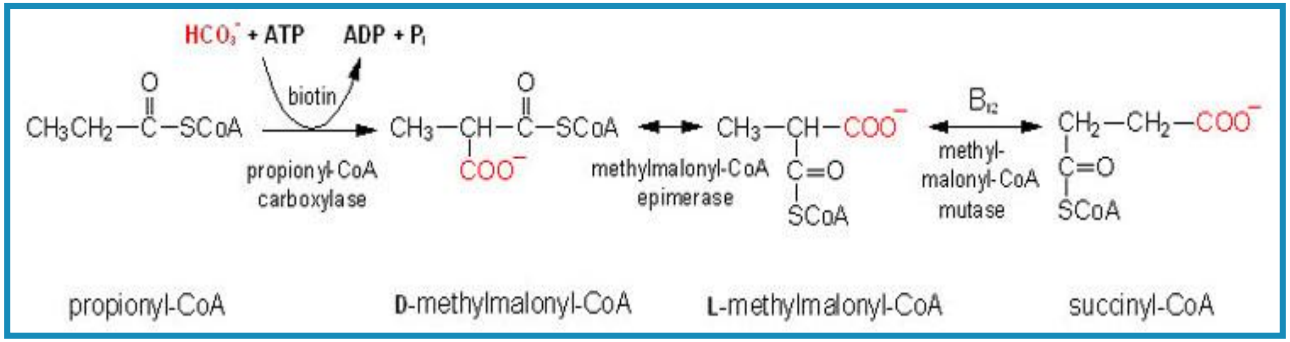
- Propionate, a three-carbon compound (CH_3CH_2COOH) generated from the metabolism of odd-chain fatty acids.
- Propionate can be converted into succinyl-CoA, which can then enter the TCA cycle to generate oxaloacetate, which serves as a precursor for gluconeogenesis.
- The use of propionate as a glucogenic precursor occurs predominantly in ruminants.



Precursors of gluconeogenesis (cont.)

Propionate (cont.):

- First, propionate is activated with ATP and CoA by propionyl-CoA synthetase.
- Propionyl CoA formed undergoes a CO_2 fixation reaction to form D-methylmalonyl-CoA, catalyzed by B_{12}-requiring enzyme propionyl-CoA carboxylase.
- D-Methylmalonyl-CoA is then converted to its isomer, L-methylmalonyl-CoA, by methylmalonyl-CoA racemase.
- Finally, the enzyme, methylmalonyl-CoA isomerase catalyzes the formation of succinyl-CoA.



Regulation of gluconeogenesis

- Gluconeogenesis is tightly regulated to ensure that glucose production is matched to the changes in the energy status and glucose levels in the cell.
- Regulation occurs at multiple levels, including allosteric regulation, hormonal control, and transcriptional regulation.
- It is important to know that both glycolysis and gluconeogenesis are regulated in a reciprocal fashion (i.e. when one pathway is highly active, the other is inhibited as both pathways are regulated with the same effectors, but in opposite direction).

Regulation of gluconeogenesis (cont.)

- **Allosteric regulation:**

- Key regulatory enzymes in gluconeogenesis:
  - Pyruvate carboxylase
  - Phosphoenolpyruvate carboxykinase (PEPCK)
  - Fructose-1,6-bisphosphatase

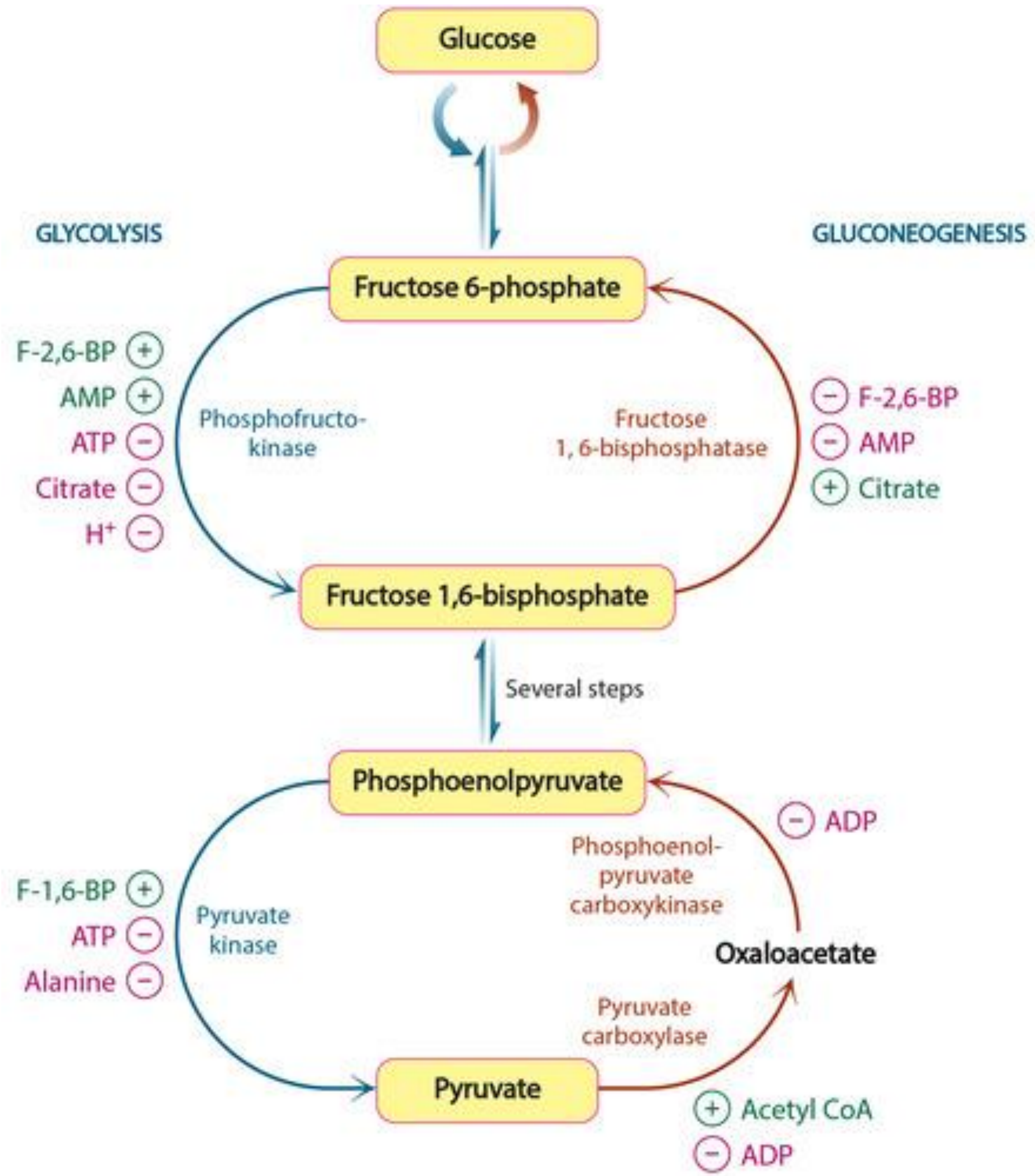
- These enzymes are regulated by allosteric effectors, such as ATP, ADP, and citrate (which reflect the cellular energy status).

- **Hormonal control:**

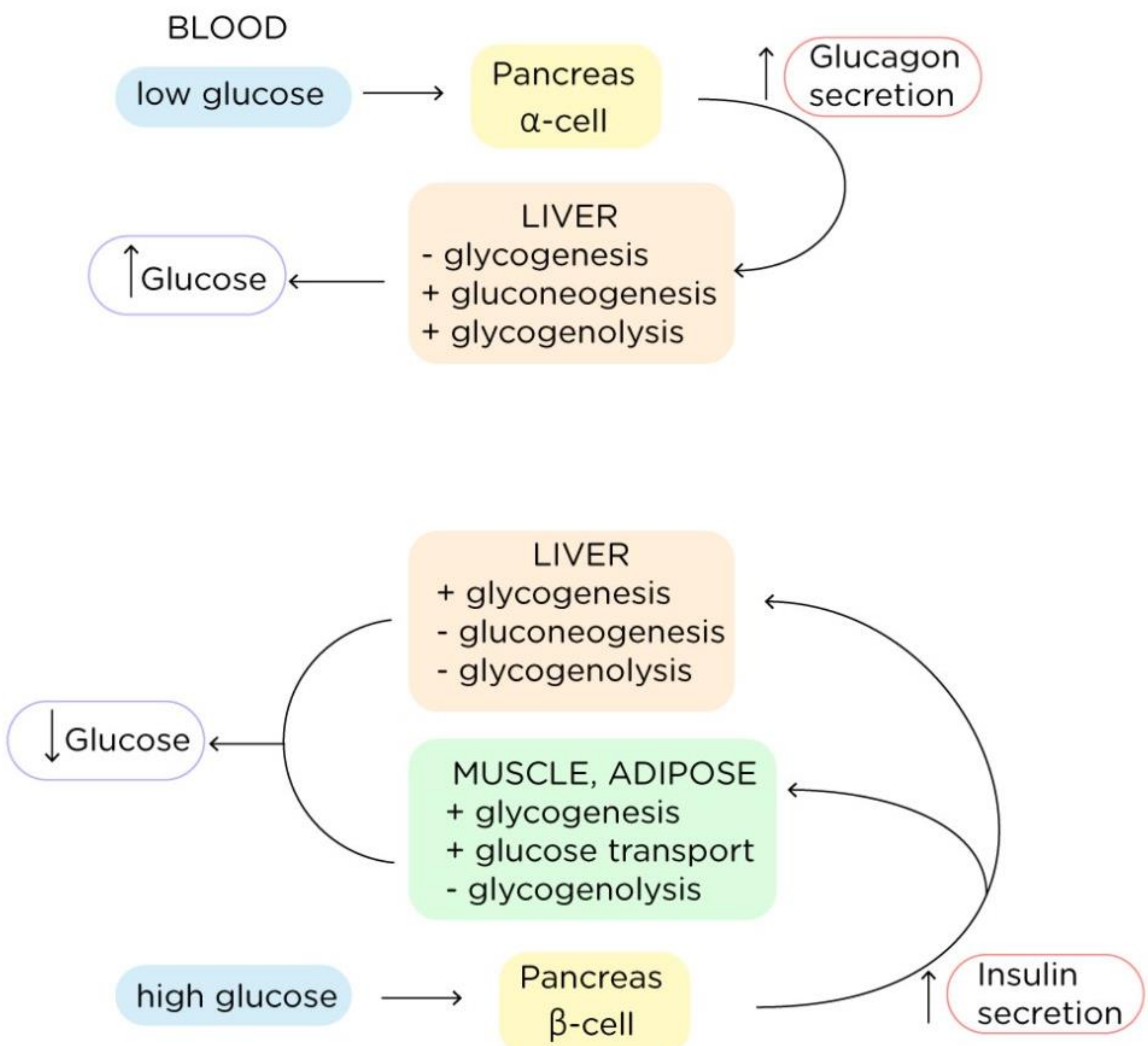
- Glucagon and cortisol stimulate gluconeogenesis during periods of low blood glucose levels and stress.

- Insulin inhibits gluconeogenesis under fed conditions *(insulin stimulates PFK-2 to accumulate fructose 2,6-bisphosphate)*.

Allosteric regulation of gluconeogenesis



Insulin and Glucagon Effects on Glucose Metabolism



Hormonal regulation (cont.)

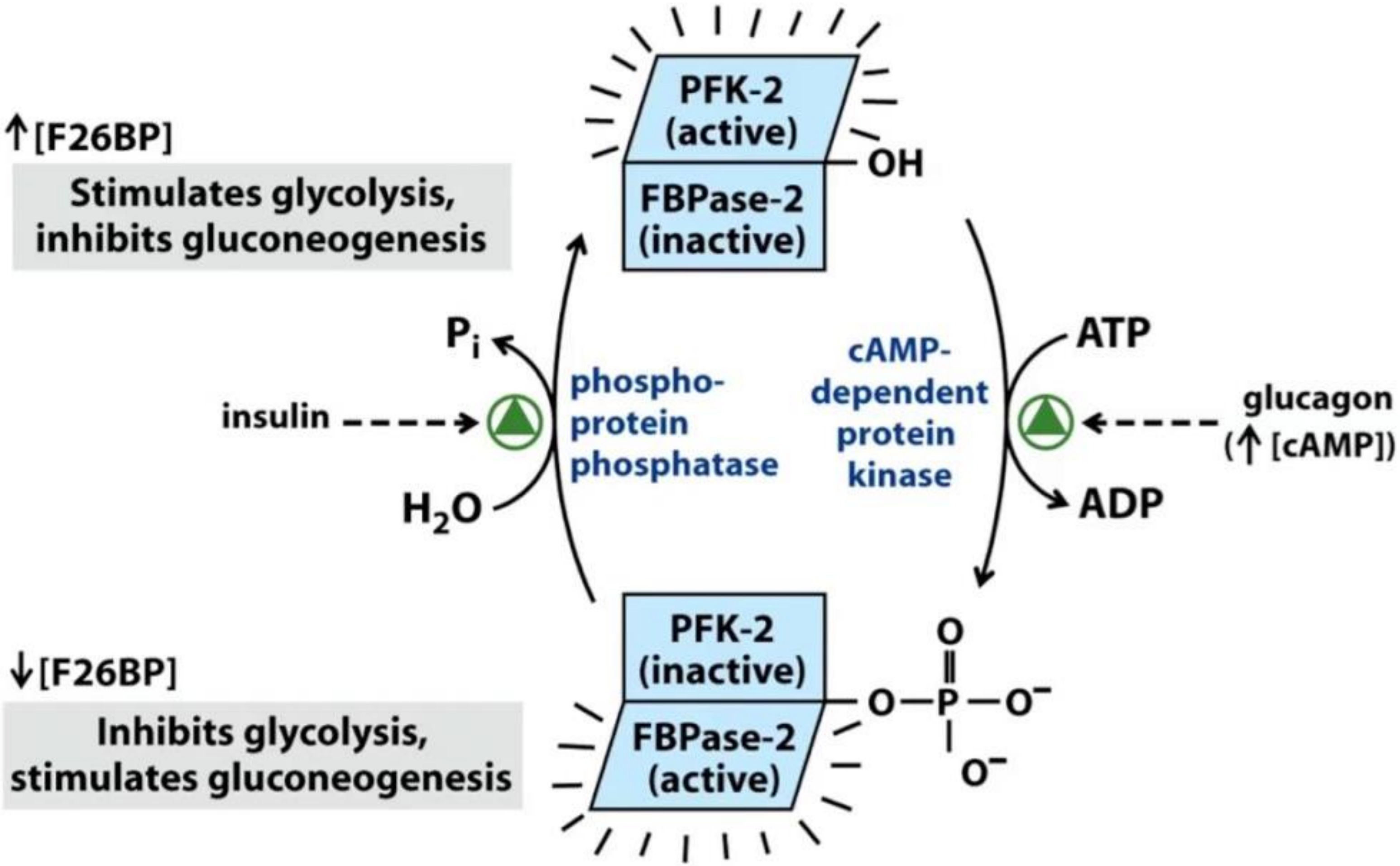


Figure 15-17b
Lehninger Principles of Biochemistry, Fifth Edition

Summary

- Gluconeogenesis is a vital metabolic pathway that allows organisms to synthesize glucose from non-carbohydrate precursors such as pyruvate, lactate, glycerol, amino acids, and propionate.
- It involves a series of enzymatic reactions that essentially reverse glycolysis, with key enzymes and regulatory steps that reciprocate the irreversible steps of glycolysis.
- Gluconeogenesis plays a critical role in maintaining blood glucose homeostasis, providing glucose for tissues with high energy demands, and contributing to overall metabolic flexibility in response to varying nutritional states.