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GLUCONEOGENESIS

lacture

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Gluconeogenesis, which occurs mainly in the liver, is the synthesis of glucose from compounds that are not carbohydrates.The major precursors for gluconeogenesis are lactate, amino acids (which form pyruvate or TCA cycle intermediates), and glycerol (which forms DHAP). Even–chain length fatty acids do not produce any net glucose.

Gluconeogenesis involves several enzymatic steps that do not occur in glycolysis; thus, glucose is not generated by a simple reversal of glycolysis. Pyruvate carboxylase converts pyruvate to oxaloacetate in the mitochondrion. Oxaloacetate is converted to malate or aspartate, which travels to the cytosol and is reconverted to oxaloacetate. Phosphoenolpyruvate carboxykinase converts oxaloacetate to phosphoenolpyruvate. Phosphoenolpyruvate forms fructose 1,6-bisphosphate by reversal of the steps of glycolysis. Fructose 1,6-bisphosphatase converts fructose 1,6-bisphosphate to fructose-6-phosphate, which is converted to glucose-6-phosphate. Glucose-6-phosphatase converts glucose-6-phosphate to free glucose, which is released into the blood. Gluconeogenesis occurs under conditions in which pyruvate dehydrogenase, pyruvate kinase, PFK1, and glucokinase are relatively inactive. The low activity of these enzymes prevents futile cycles from occurring and ensures that, overall, pyruvate is converted to glucose. The synthesis of 1 mole of glucose from 2 moles of lactate requires energy equivalent to about 6 moles of ATP.

Reactions of gluconeogenesis

1. Conversion of pyruvate to phosphoenolpyruvate

(1) Pyruvate (produced from lactate, alanine, and other amino acids) is first converted to oxaloacetate by pyruvate carboxylase, a mitochondrial enzyme that requires biotin and ATP. Oxaloacetate cannot directly cross the inner mitochondrial membrane. Therefore, it is converted to malate or to aspartate, which can cross the mitochondrial membrane and be reconverted to oxaloacetate in the cytosol.

(2) Oxaloacetate is decarboxylated by phosphoenolpyruvate carboxykinase to form phosphoenolpyruvate.This reaction requires GTP.

(3) Phosphoenolpyruvate is converted to fructose 1,6-bisphosphate by reversal of the glycolytic reactions.

2. Conversion of fructose 1,6-bisphosphate to fructose-6-phosphate

Fructose-1,6-bisphosphate is converted to fructose-6-phosphate in a reaction that releases inorganic phosphate and is catalyzed by fructose-1,6-bisphosphatase. Fructose-6-phosphate is converted to glucose 6- phosphate by the same isomerase used in glycolysis.

3. Conversion of glucose-6-phosphate to glucose

Glucose-6-phosphate releases inorganic phosphate, which produces free glucose that enters the blood. The enzyme is glucose 6-phosphatase. Glucose-6-phosphatase is involved both in gluconeogenesis and glycogenolysis

Regulatory enzymes of gluconeogenesis

a. Pyruvate dehydrogenase

(1) Decreased insulin and increased glucagon stimulate the release of fatty acids from adipose tissue.

(2) Fatty acids travel to the liver and are oxidized, producing acetyl-CoA, NADH, and ATP, which cause inactivation of pyruvate dehydrogenase.

(3) Because pyruvate dehydrogenase is relatively inactive, pyruvate is converted to oxaloacetate, not to acetyl-CoA.

b. Pyruvate carboxylase

(1) Pyruvate carboxylase, which converts pyruvate to oxaloacetate, is activated by acetyl-CoA (which is generated from fatty acid oxidation within the mitochondria).

(2) Note that pyruvate carboxylase is active in both the fed and fasting states.

c. Phosphoenolpyruvate carboxykinase (PEPCK)

(1) PEPCK is an inducible enzyme.

(2) Transcription of the gene encoding PEPCK is stimulated by binding of proteins (CREB, for cyclic AMP response element–binding protein) that are phosphorylated in response to Camp and by binding of glucocorticoid–protein complexes to regulatory elements in the gene.

(3) Increased production of PEPCK mRNA leads to increased translation, resulting in higher PEPCK levels in the cell.

d. Pyruvate kinase

(1) Glucagon, via cAMP and protein kinase A, causes pyruvate kinase to be phosphorylated and inactivated.

(2) Because pyruvate kinase is relatively inactive, phosphoenolpyruvate formed from oxaloacetate is not reconverted to pyruvate but, in a series of steps, forms fructose-1,6-bisphosphate, which is converted to fructose-6-phosphate.

e. Phosphofructokinase 1

(1) PFK1 is relatively inactive because the concentrations of its activators, AMP and F-2,6-P, are low and its inhibitor, ATP, is relatively high.

f. Fructose 1,6-bisphosphatase

(1) The level of F-2,6-P, an inhibitor of fructose 1,6-bisphosphatase, is low during fasting. Therefore, fructose 1,6-bisphosphatase is more active.

(2) Fructose 1,6-bisphosphatase is also induced in the fasting state.

g. Glucokinase

(1) Glucokinase is relatively inactive because it has a high Km for glucose, and under conditions that favor gluconeogenesis, the glucose concentration is low. Therefore, free glucose is not reconverted to glucose-6-phosphate

Precursors for gluconeogenesis

Lactate, amino acids, and glycerol are the major precursors for gluconeogenesis in humans.

1. Lactate is oxidized by NAD1 in a reaction catalyzed by LDH to form pyruvate, which can be converted to glucose. The sources of lactate include red blood cells and exercising muscle.

2. Amino acids for gluconeogenesis come from degradation of muscle protein.

a. Amino acids are released directly into the blood from muscle, or carbons from amino acids are converted to alanine and glutamine and released.

(1) Alanine is also formed by transamination of pyruvate that is derived by the oxidation of glucose.

(2) Glutamine is converted to alanine by tissues such as gut and kidney.

b. Amino acids travel to the liver and provide carbon for gluconeogenesis. Quantitatively, alanine is the major gluconeogenic amino acid.

c. Amino acid nitrogen is converted to urea.

3. Glycerol, which is derived from adipose triacylglycerols, reacts with ATP to form glycerol- 3-phosphate, which is oxidized to DHAP and converted to glucose

Energy requirements for gluconeogenesis

1. From pyruvate

a. Conversion of pyruvate to oxaloacetate by pyruvate carboxylase requires one ATP.

b. Conversion of oxaloacetate to phosphoenolpyruvate by phosphoenolpyruvate carboxykinase requires one GTP (the equivalent of one ATP).

c. Conversion of 3-phosphoglycerate to 1,3-bisphosphoglycerate by phosphoglycerate kinase requires one ATP.

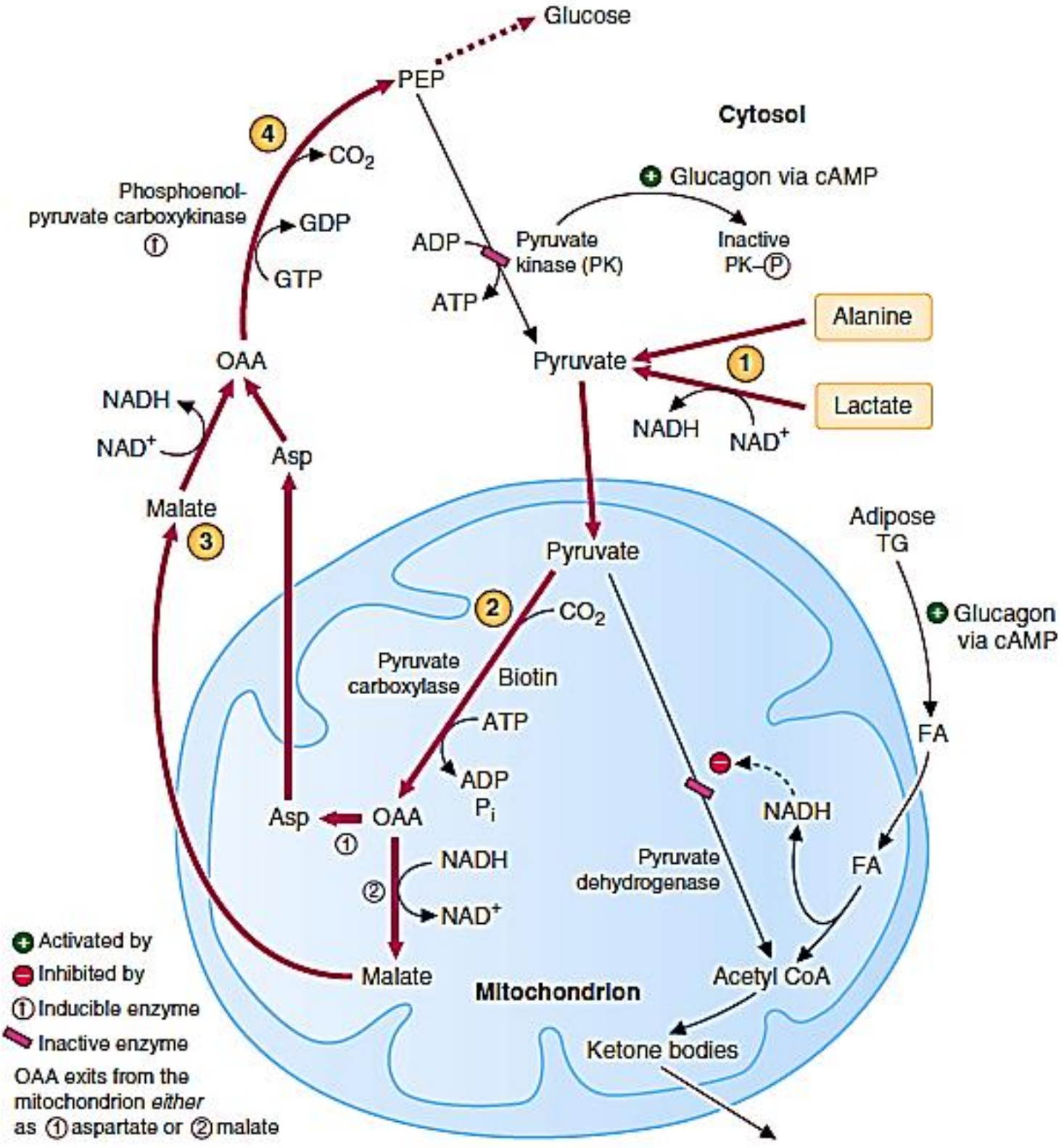
d. Since 2 moles of pyruvate are required to form 1 mole of glucose, 6 moles of high-energy phosphate are required for the synthesis of 1 mole of glucose.

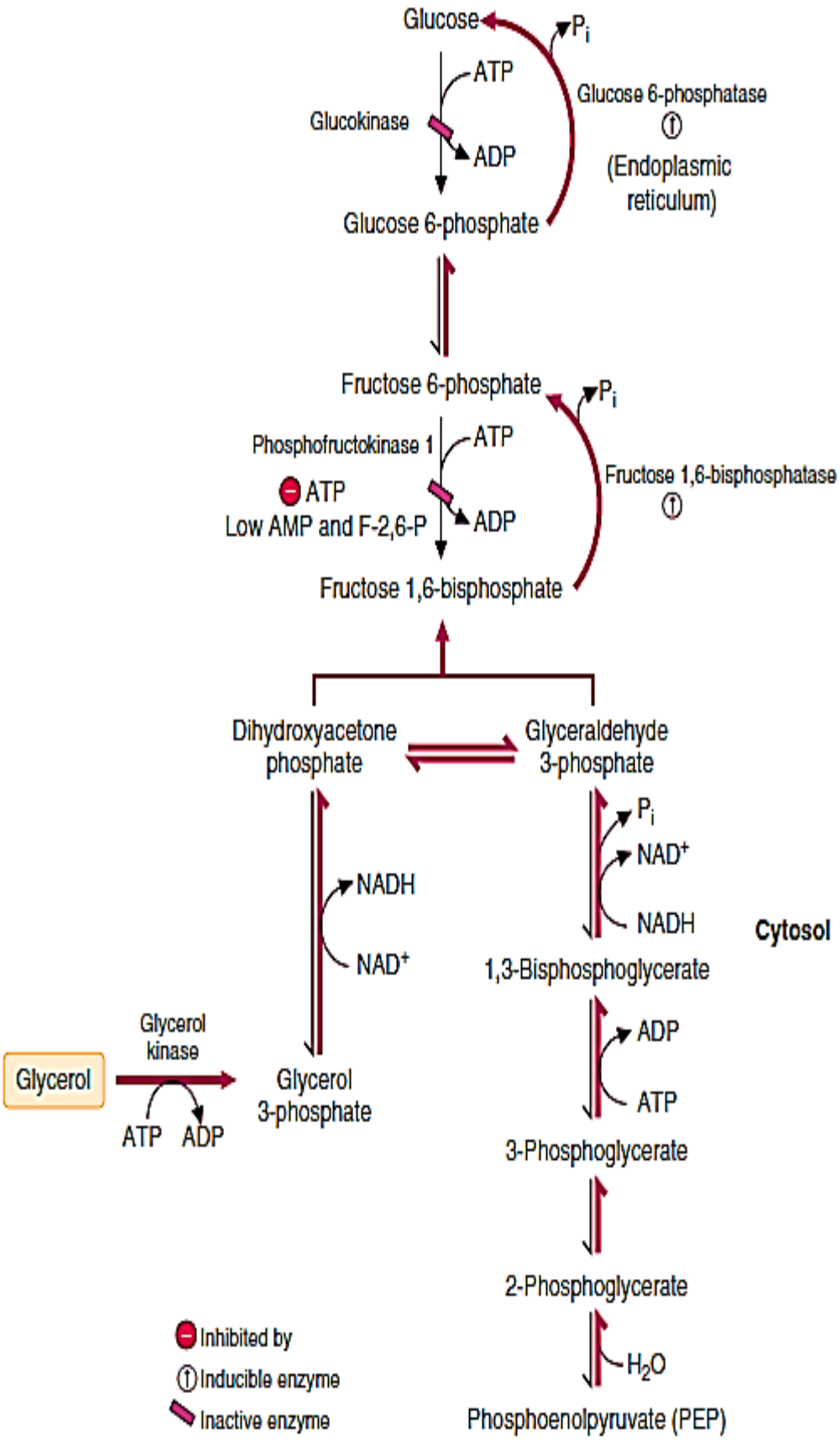
2. From glycerol

Glycerol enters the gluconeogenic pathway at the DHAP level.

(1) Conversion of glycerol to glycerol-3-phosphate, which is oxidized to DHAP, requires one ATP.

(2) Since 2 moles of glycerol are required to form 1 mole of glucose, 2 moles of high-energy phosphate are required for the synthesis of 1 mole of glucose.


Glucose
PEP
Cytosol
4
CO_2
Phosphoenol-pyruvate carboxykinase
①
GDP
GTP
ADP
ATP
Pyruvate kinase (PK)
Glucagon via cAMP
Inactive PK–Ⓟ
Alanine
Lactate
OAA
Pyruvate
1
NADH
NAD^+
NADH
NAD^+
Asp
Malate
3
Pyruvate
Adipose TG
2
CO_2
Glucagon via cAMP
Pyruvate carboxylase
Biotin
ATP
ADP
P_i
FA
Asp
OAA
①
NADH
②
NAD^+
NADH
Pyruvate dehydrogenase
FA
Malate
Mitochondrion
Acetyl CoA
Ketone bodies
Activated by
Inhibited by
① Inducible enzyme
Inactive enzyme
OAA exits from the mitochondrion *either* as ① aspartate or ② malate


Glucose
P_i
ATP
Glucokinase
ADP
Glucose 6-phosphatase
(Endoplasmic reticulum)
Glucose 6-phosphate
Fructose 6-phosphate
P_i
Phosphofructokinase 1
ATP
ATP
Low AMP and F-2,6-P
ADP
Fructose 1,6-bisphosphatase
Fructose 1,6-bisphosphate
Dihydroxyacetone phosphate
Glyceraldehyde 3-phosphate
P_i
NAD^+
NADH
NADH
NAD^+
Cytosol
1,3-Bisphosphoglycerate
ADP
ATP
3-Phosphoglycerate
2-Phosphoglycerate
H_2O
Phosphoenolpyruvate (PEP)
Glycerol
Glycerol kinase
ATP
ADP
Glycerol 3-phosphate
Inhibited by
Inducible enzyme
Inactive enzyme