Gluconeogenesis 10

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I. OVERVIEW

Some tissues, such as the brain, red blood cells (RBC), kidney medulla, lens and cornea of the eye, testes, and exercising muscle, require a continuous supply of glucose as a metabolic fuel. Liver glycogen, an essential postprandial source of glucose, can meet these needs for <24 hours in the absence of dietary intake of carbohydrate (see p. 125). During a prolonged fast, however, hepatic glycogen stores are depleted, and glucose is made from noncarbohydrate precursors. The formation of glucose does not occur by a simple reversal of glycolysis, because the overall equilibrium of glycolysis strongly favors pyruvate formation (that is, the change in standard free energy [ΔG^0] is negative). Instead, glucose is synthesized de novo by a special pathway, gluconeogenesis, which requires both mitochondrial and cytosolic enzymes. [Note: Deficiencies of gluconeogenic enzymes cause hypoglycemia.] During an overnight fast, ~90% of gluconeogenesis occurs in the liver, with the remaining ~10% occurring in the kidneys. However, during prolonged fasting, the kidneys become major glucose-producing organs, contributing ~40% of the total glucose production. [Note: The small intestine can also make glucose.] Figure 10.1 shows the relationship of gluconeogenesis to other essential pathways of energy metabolism.

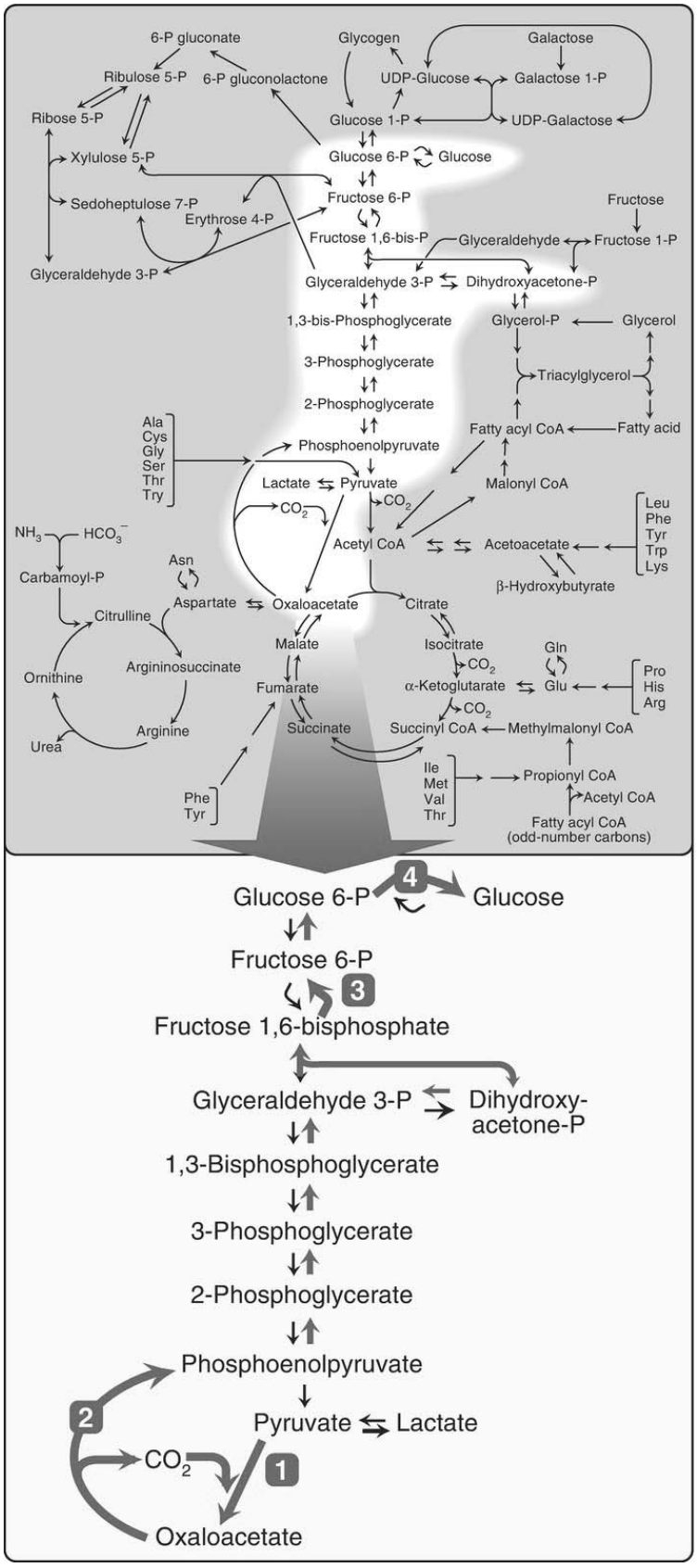

6-P gluconate
Ribulose 5-P
6-P gluconolactone
Ribose 5-P
Xylulose 5-P
Sedoheptulose 7-P
Erythrose 4-P
Glyceraldehyde 3-P
Glycogen
Galactose
UDP-Glucose
Galactose 1-P
Glucose 1-P
UDP-Galactose
Glucose 6-P
Glucose
Fructose 6-P
Fructose
Fructose 1,6-bis-P
Glyceraldehyde
Fructose 1-P
Glyceraldehyde 3-P
Dihydroxyacetone-P
1,3-bis-Phosphoglycerate
Glycerol-P
Glycerol
3-Phosphoglycerate
Triacylglycerol
2-Phosphoglycerate
Fatty acyl CoA
Fatty acid
Ala
Cys
Gly
Ser
Thr
Try
Phosphoenolpyruvate
Lactate
Pyruvate
Malonyl CoA
CO2
Leu
Phe
Tyr
Trp
Lys
NH3
HCO3−
Acetyl CoA
Acetoacetate
β-Hydroxybutyrate
Carbamoyl-P
Asn
Aspartate
Oxaloacetate
Citrate
Citrulline
Malate
Isocitrate
Gln
Pro
His
Arg
Ornithine
Argininosuccinate
Fumarate
α-Ketoglutarate
Glu
Arginine
Urea
Succinate
Succinyl CoA
Methylmalonyl CoA
Ile
Met
Val
Thr
Propionyl CoA
Acetyl CoA
Phe
Tyr
Fatty acyl CoA
(odd-number carbons)
Glucose 6-P
4
Glucose
Fructose 6-P
3
Fructose 1,6-bisphosphate
Glyceraldehyde 3-P
Dihydroxy-
acetone-P
1,3-Bisphosphoglycerate
3-Phosphoglycerate
2-Phosphoglycerate
Phosphoenolpyruvate
2
Pyruvate
Lactate
CO2
1
Oxaloacetate

Figure 10.1 Gluconeogenesis shown as one of the essential pathways of energy metabolism. The numbered reactions are unique to gluconeogenesis. [Note: See Fig. 8.2, p. 92, for a more detailed map of metabolism.] P = phosphate; CO_2 = carbon dioxide.

II. SUBSTRATES

Gluconeogenic precursors are molecules that can be used to produce a net synthesis of glucose. The most important gluconeogenic precursors are glycerol, lactate, and α-keto acids obtained from the metabolism of glucogenic amino acids. [Note: All but two amino acids (leucine and lysine) are glucogenic (see p. 262).]

A. Glycerol

Glycerol is released during the hydrolysis of triacylglycerols (TAG) in adipose tissue (see p. 190) and is delivered by the blood to the liver. Glycerol is phosphorylated by ***glycerol kinase*** to glycerol 3-phosphate, which is oxidized by ***glycerol 3-phosphate dehydrogenase*** to dihydroxyacetone phosphate, an intermediate of glycolysis and gluconeogenesis.

B. Lactate

Lactate from anaerobic glycolysis is released into the blood by exercising skeletal muscle and by cells that lack mitochondria such as RBC. In the Cori cycle, this lactate is taken up by the liver and oxidized to pyruvate that is converted to glucose, which is released back into the circulation (Fig. 10.2).

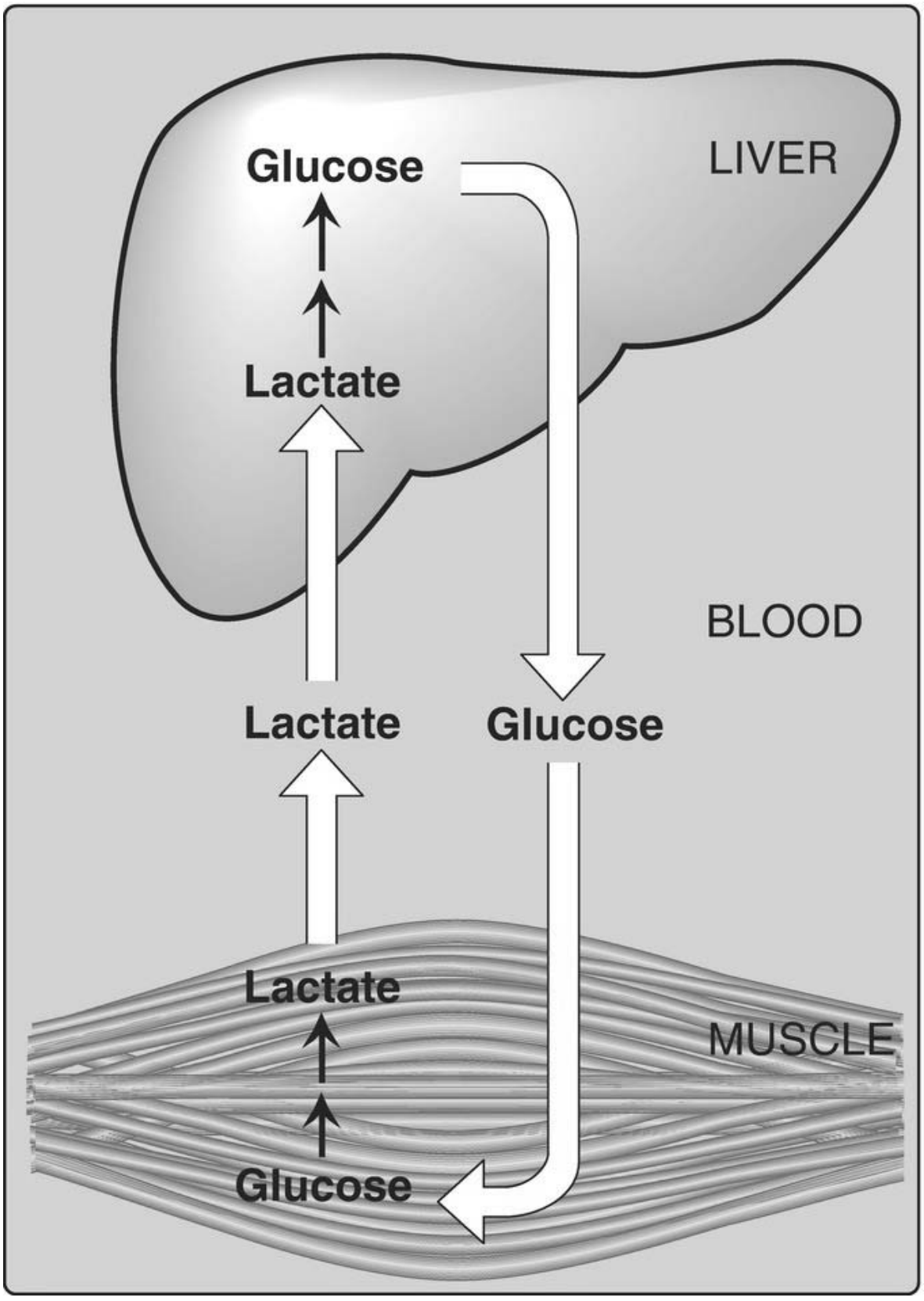

Glucose
LIVER
Lactate
BLOOD
Lactate
Glucose
Lactate
MUSCLE
Glucose

Figure 10.2 The intertissue Cori cycle links gluconeogenesis with glycolysis. [Note: Diffusion of lactate and glucose across membranes is facilitated by transport proteins.]

C. Amino acids

Amino acids produced by hydrolysis of tissue proteins are the major sources of glucose during a fast. Their metabolism generates α-keto acids, such as pyruvate that is converted to glucose, or α-ketoglutarate that can enter the tricarboxylic acid (TCA) cycle and form oxaloacetate (OAA), a direct precursor of phosphoenolpyruvate (PEP). [Note: Acetyl coenzyme A (CoA) and compounds that give rise only to acetyl CoA (for example, acetoacetate, lysine, and leucine) cannot give rise to a net synthesis of glucose. This is because of the irreversible nature of the ***pyruvate dehydrogenase complex*** (***PDHC***), which converts pyruvate to acetyl CoA (see p. 109). These compounds give rise instead to ketone bodies (see p. 195) and are termed ketogenic.]

III. REACTIONS

Seven glycolytic reactions are reversible and are used in the synthesis of glucose from lactate or pyruvate. However, three glycolytic reactions are irreversible and must be circumvented by four alternate reactions that energetically favor the synthesis of glucose. These irreversible reactions, which together are unique to gluconeogenesis, are described below.

A. Pyruvate carboxylation

The first roadblock to overcome in the synthesis of glucose from pyruvate is the irreversible conversion in glycolysis of PEP to pyruvate by ***pyruvate kinase (PK).*** In gluconeogenesis, pyruvate is carboxylated by ***pyruvate carboxylase (PC)*** to OAA, which is converted to PEP by ***PEP-carboxykinase*** (***PEPCK***) (Fig. 10.3).

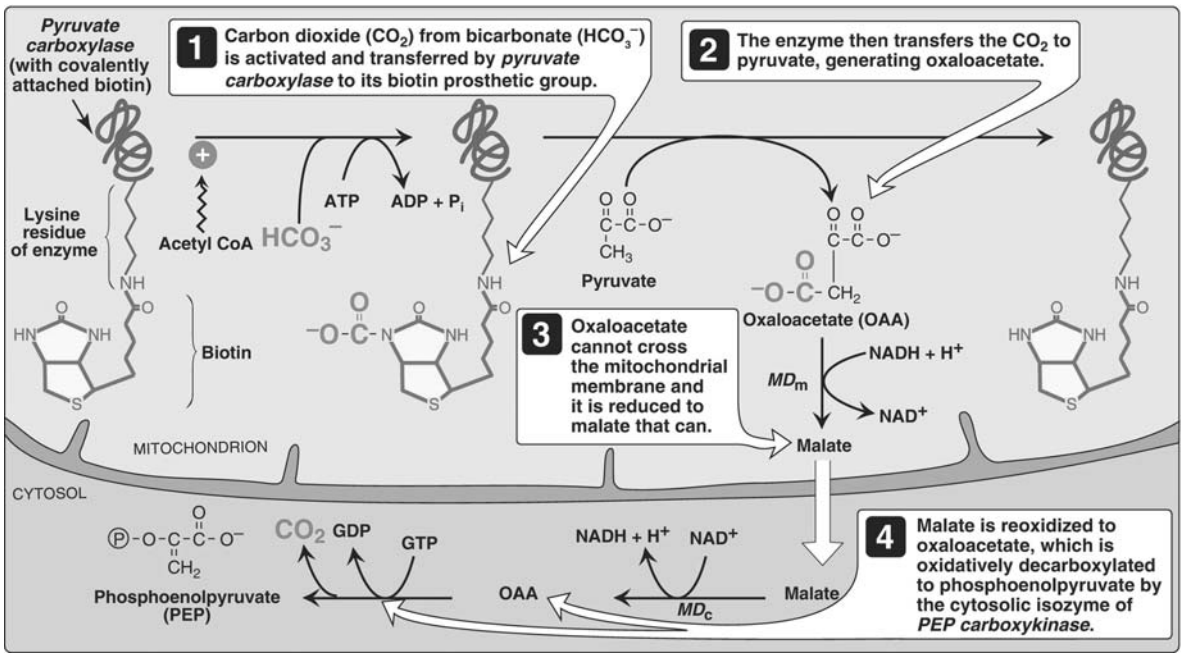


Figure 10.3 PEP synthesis in the cytosol. [Note: The process moves nicotinamide adenine dinucleotide (NADH) reducing equivalents required for gluconeogenesis out of mitochondria into the cytosol.] ***MD***$_m$ and ***MD***$_c$ = mitochondrial and cytosolic isozymes of ***malate dehydrogenase***; GTP and GDP = guanosine tri- and diphosphates; ADP = adenosine diphosphate.

1. Biotin: ***PC*** requires the coenzyme biotin (see p. 385) covalently bound to the ε-amino group of a lysine residue in the enzyme (see Fig. 10.3). ATP hydrolysis drives formation of an enzyme–biotin–carbon dioxide (CO_2) intermediate, which then carboxylates pyruvate to form OAA. [Note: HCO_3^- provides the CO_2.] The ***PC*** reaction occurs in the mitochondria of liver and kidney cells and has two purposes: to allow production of PEP, an important substrate for gluconeogenesis, and to provide OAA that can replenish the TCA cycle intermediates that may become depleted. Muscle cells also contain ***PC*** but use the OAA product only for the replenishment (anaplerotic) purpose and do not synthesize glucose. [Note: Pyruvate carrier protein moves pyruvate from the cytosol into mitochondria.]

PC is one of several ***carboxylases*** that require biotin. Others include ***acetyl CoA carboxylase*** (p. 183), ***propionyl CoA carboxylase*** (p. 194), and

methylcrotonyl CoA carboxylase (p. 266).

2. Allosteric regulation: ***PC*** is allosterically activated by acetyl CoA. Elevated levels of acetyl CoA in mitochondria signal a metabolic state in which increased synthesis of OAA is required. For example, this occurs during fasting, when OAA is used for gluconeogenesis in the liver and kidneys. Conversely, at low levels of acetyl CoA, ***PC*** is largely inactive, and pyruvate is primarily oxidized by the ***PDHC*** to acetyl CoA that can be further oxidized by the TCA cycle (see p. 109).

B. Oxaloacetate transport to the cytosol

For gluconeogenesis to continue, OAA must be converted to PEP by ***PEPCK***. PEP production in the cytosol requires transport of OAA out of mitochondria. However, there is no OAA transporter in the inner mitochondrial membrane, and OAA is first reduced to malate by mitochondrial ***malate dehydrogenase*** (***MD***). Malate is transported into the cytosol and reoxidized to OAA by cytosolic ***MD*** as nicotinamide adenine dinucleotide (NAD^+) is reduced to NADH (see Fig. 10.3). The NADH is used in the reduction of 1,3-bisphosphoglycerate to glyceraldehyde 3-phosphate by ***glyceraldehyde 3-phosphate dehydrogenase*** (see p. 101), a reaction common to glycolysis and gluconeogenesis. [Note: When abundant, lactate is oxidized to pyruvate as NAD^+ is reduced. The pyruvate is transported into mitochondria and carboxylated by ***PC*** to OAA, which can be converted to PEP by the mitochondrial isozyme of ***PEPCK***. PEP is transported to the cytosol. OAA can also be converted to aspartate that is transported into the cytosol.]

C. Cytosolic oxaloacetate decarboxylation

OAA is decarboxylated and phosphorylated to PEP in the cytosol by ***PEPCK***. The reaction is driven by hydrolysis of guanosine triphosphate ([GTP] see Fig. 10.3). The combined actions of ***PC*** and ***PEPCK*** provide an energetically favorable pathway from pyruvate to PEP. PEP is then acted on by the reactions of glycolysis running in the reverse direction until it

becomes fructose 1,6-bisphosphate.

> The pairing of carboxylation with decarboxylation drives reactions that would otherwise be energetically unfavorable. This strategy is also used in fatty acid (FA) synthesis (see p. 184).

D. Fructose 1,6-bisphosphate dephosphorylation

Hydrolysis of fructose 1,6-bisphosphate by ***fructose 1,6-bisphosphatase***, found in the liver and kidneys, bypasses the irreversible ***phosphofructokinase-1*** (***PFK-1***) reaction of glycolysis and provides an energetically favorable pathway for the formation of fructose 6-phosphate (Fig. 10.4). This reaction is an important regulatory site of gluconeogenesis.

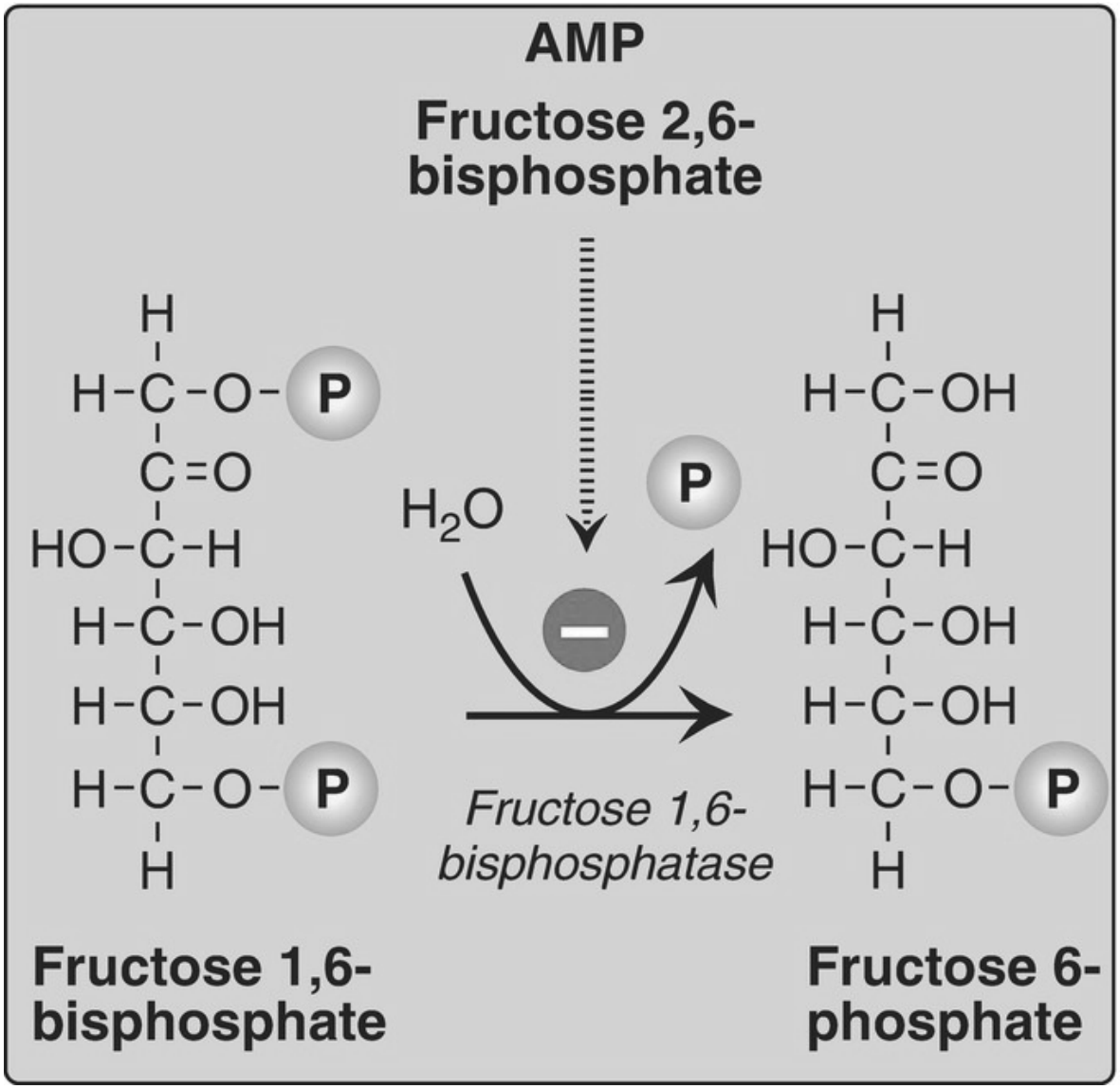


Figure 10.4 Dephosphorylation of fructose 1,6-bisphosphate. AMP = adenosine monophosphate; = phosphate.

1. Regulation by intracellular energy levels: ***Fructose 1,6-bisphosphatase*** is inhibited by a rise in the adenosine monophosphate (AMP)/ATP ratio, which signals a low-energy state in the cell. Conversely, low AMP and high ATP levels stimulate gluconeogenesis, an energy-requiring pathway.
2. Regulation by fructose 2,6-bisphosphate: ***Fructose 1,6- bisphosphatase*** is inhibited by fructose 2,6-bisphosphate, an allosteric effector whose concentration is influenced by the insulin/glucagon ratio. When glucagon

is high, the effector is not made by hepatic ***PFK-2*** (see p. 99), and thus, the ***phosphatase*** is active (Fig. 10.5). [Note: The signals that inhibit (low energy, high fructose 2,6-bisphosphate) or activate (high energy, low fructose 2,6-bisphosphate) gluconeogenesis have the opposite effect on glycolysis, providing reciprocal control of the pathways that synthesize and oxidize glucose (see p. 100).]

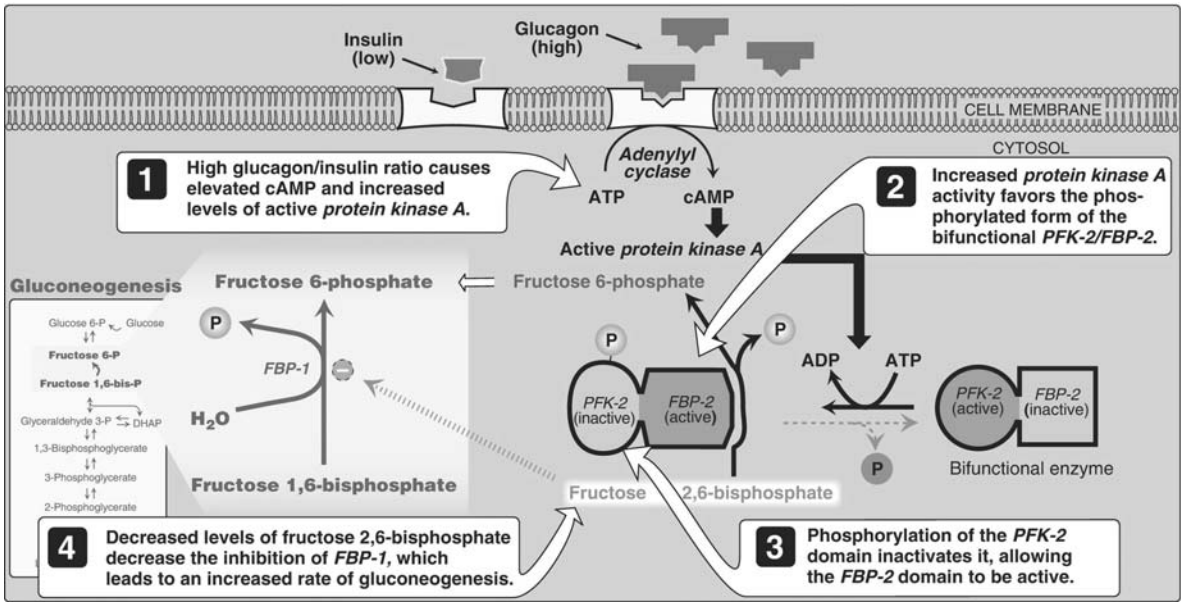


Figure 10.5 Effect of elevated glucagon on the intracellular concentration of fructose 2,6-bisphosphate in the liver. AMP and ADP = adenosine mono- and diphosphates; cAMP = cyclic AMP; ***PFK-2*** = ***phosphofructokinase-2***; ***FBP-2*** = ***fructose 2,6-bisphosphatase***; ***FBP-1*** = ***fructose 1,6-bisphosphatase***; and = phosphate.

E. Glucose 6-phosphate dephosphorylation

Glucose 6-phosphate hydrolysis by ***glucose 6-phosphatase*** bypasses the irreversible ***hexokinase/glucokinase*** reaction and provides an energetically favorable pathway for the formation of free glucose (Fig. 10.6). The liver is the primary organ that produces free glucose from glucose 6-phosphate. This process requires a complex of two proteins found only in gluconeogenic tissue: glucose 6-phosphate translocase, which transports glucose 6-phosphate across the endoplasmic reticular (ER) membrane, and ***glucose 6-phosphatase***, which removes the phosphate, producing free

glucose (see Fig. 10.6). [Note: These ER membrane proteins are also required for the final step of glycogen degradation (see p. 130). Glycogen storage diseases Ia and Ib, caused by deficiencies in the ***phosphatase*** and the translocase, respectively, are characterized by severe fasting hypoglycemia, because free glucose is unable to be produced from either gluconeogenesis or glycogenolysis.] Specific transporters are responsible for moving the free glucose into the cytosol and then into blood.

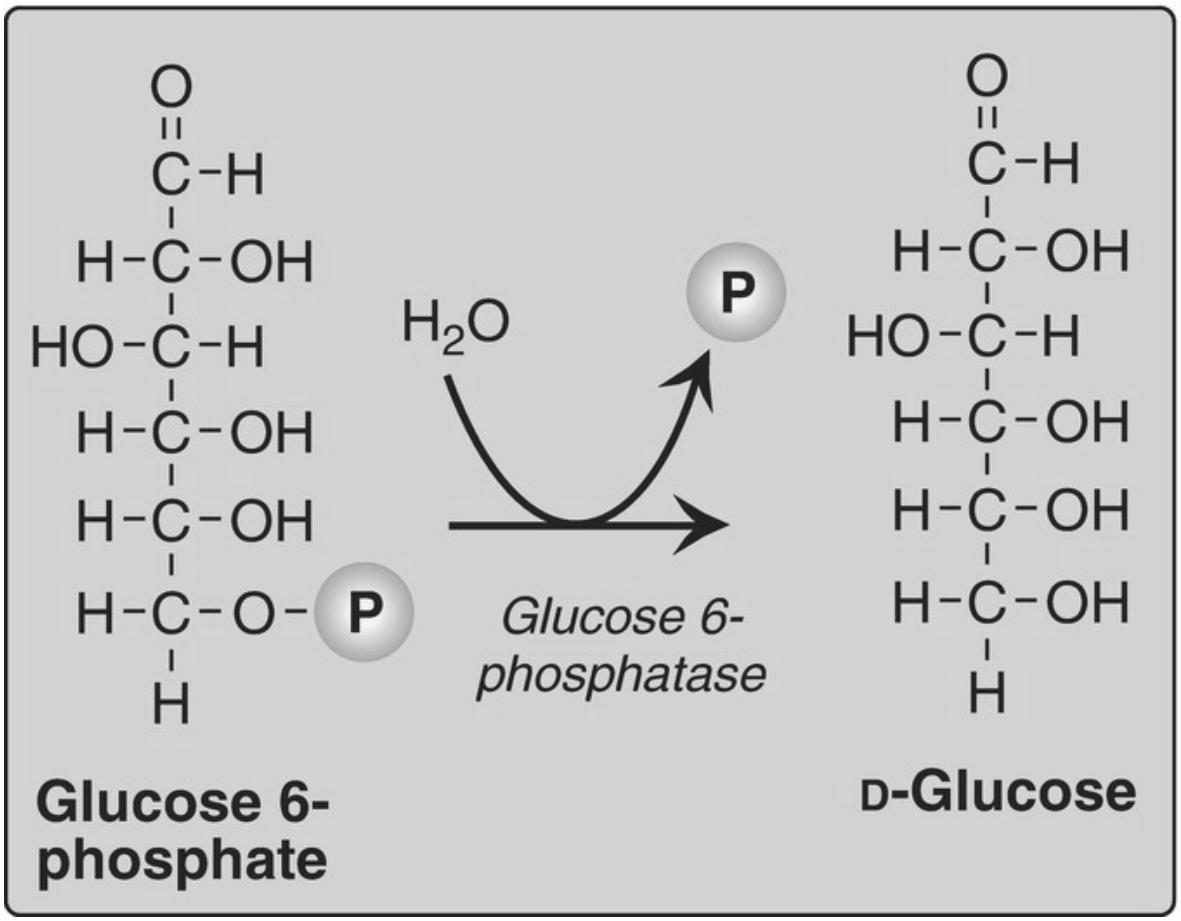


Figure 10.6 Dephosphorylation of glucose 6-phosphate allows release of free glucose from gluconeogenic tissues (primarily the liver) into blood. = phosphate.

F. Summary of the reactions of glycolysis and gluconeogenesis

Of the 11 reactions required to convert pyruvate to free glucose, 7 are

catalyzed by reversible glycolytic enzymes (Fig. 10.7). The 3 irreversible reactions (catalyzed by ***hexokinase/glucokinase***, ***PFK-1***, and ***PK***) are circumvented by reactions catalyzed by ***glucose 6-phosphatase***, ***fructose 1,6-bisphosphatase***, ***PC, and PEPCK.*** In gluconeogenesis, the equilibria of the reversible glycolytic reactions are pushed toward glucose synthesis as a result of the essentially irreversible formation of PEP, fructose 6-phosphate, and glucose by the gluconeogenic enzymes. [Note: The stoichiometry of gluconeogenesis from two pyruvate molecules couples the cleavage of six high-energy phosphate bonds and the oxidation of two NADH with the formation of one glucose molecule (see Fig. 10.7).]

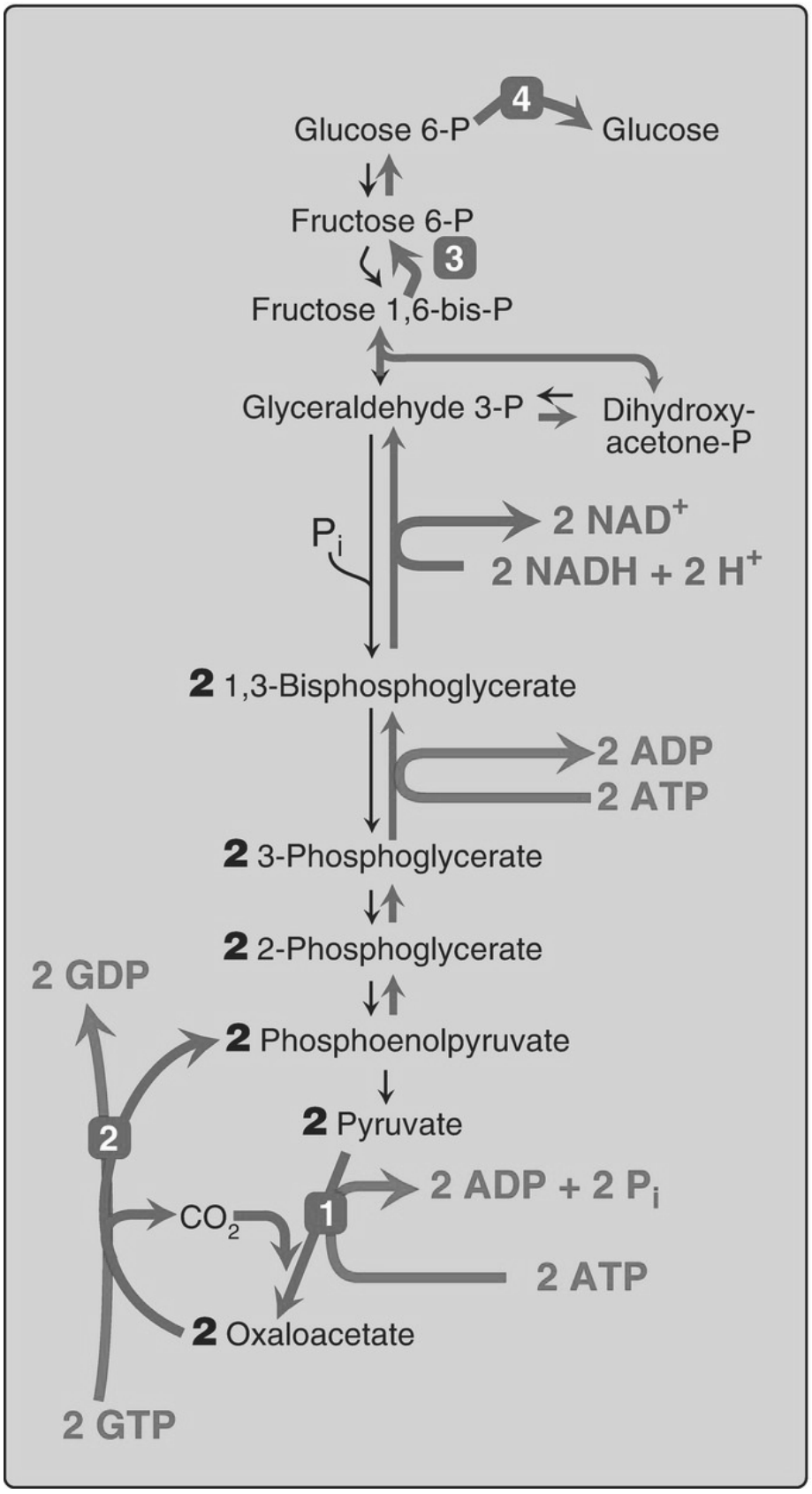

Glucose 6-P
4
Glucose
Fructose 6-P
3
Fructose 1,6-bis-P
Glyceraldehyde 3-P
Dihydroxy-
acetone-P
P_i
2 NAD^+
2 NADH + 2 H^+
2 1,3-Bisphosphoglycerate
2 ADP
2 ATP
2 3-Phosphoglycerate
2 2-Phosphoglycerate
2 GDP
2 Phosphoenolpyruvate
2 Pyruvate
2
CO_2
1
2 ADP + 2 P_i
2 ATP
2 Oxaloacetate
2 GTP

Figure 10.7 Summary of the reactions of glycolysis and gluconeogenesis, showing the energy requirements of gluconeogenesis. The numbered reactions are unique to gluconeogenesis. P = phosphate; GDP and GTP = guanosine di- and triphosphates; NAD(H) = nicotinamide adenine dinucleotide; ADP = adenosine diphosphate.

IV. REGULATION

The moment-to-moment regulation of gluconeogenesis is determined primarily by the circulating level of glucagon and by the availability of gluconeogenic substrates. In addition, slow adaptive changes in enzyme amount result from an alteration in the rate of enzyme synthesis or degradation or both. [Note: Hormonal control of the glucoregulatory system is presented in Chapter 23.]

Glucagon

This peptide hormone from pancreatic islet α cells (see p. 313) stimulates gluconeogenesis by three mechanisms.

1. Changes in allosteric effectors: Glucagon lowers hepatic fructose 2,6-bisphosphate, resulting in ***fructose 1,6-bisphosphatase*** activation and ***PFK-1*** inhibition, thereby favoring gluconeogenesis over glycolysis (see Fig. 10.5). [Note: See pp. 99–100 for the role of fructose 2,6-bisphosphate in glycolysis regulation.]
2. Covalent modification of enzyme activity: Glucagon binds its G protein–coupled receptor (see p. 95) and, via an elevation in cyclic AMP (cAMP) level and ***cAMP-dependent protein kinase A*** activity, stimulates the conversion of hepatic ***PK*** to its inactive (phosphorylated) form. This decreases PEP conversion to pyruvate, which has the effect of diverting PEP to gluconeogenesis (Fig. 10.8).

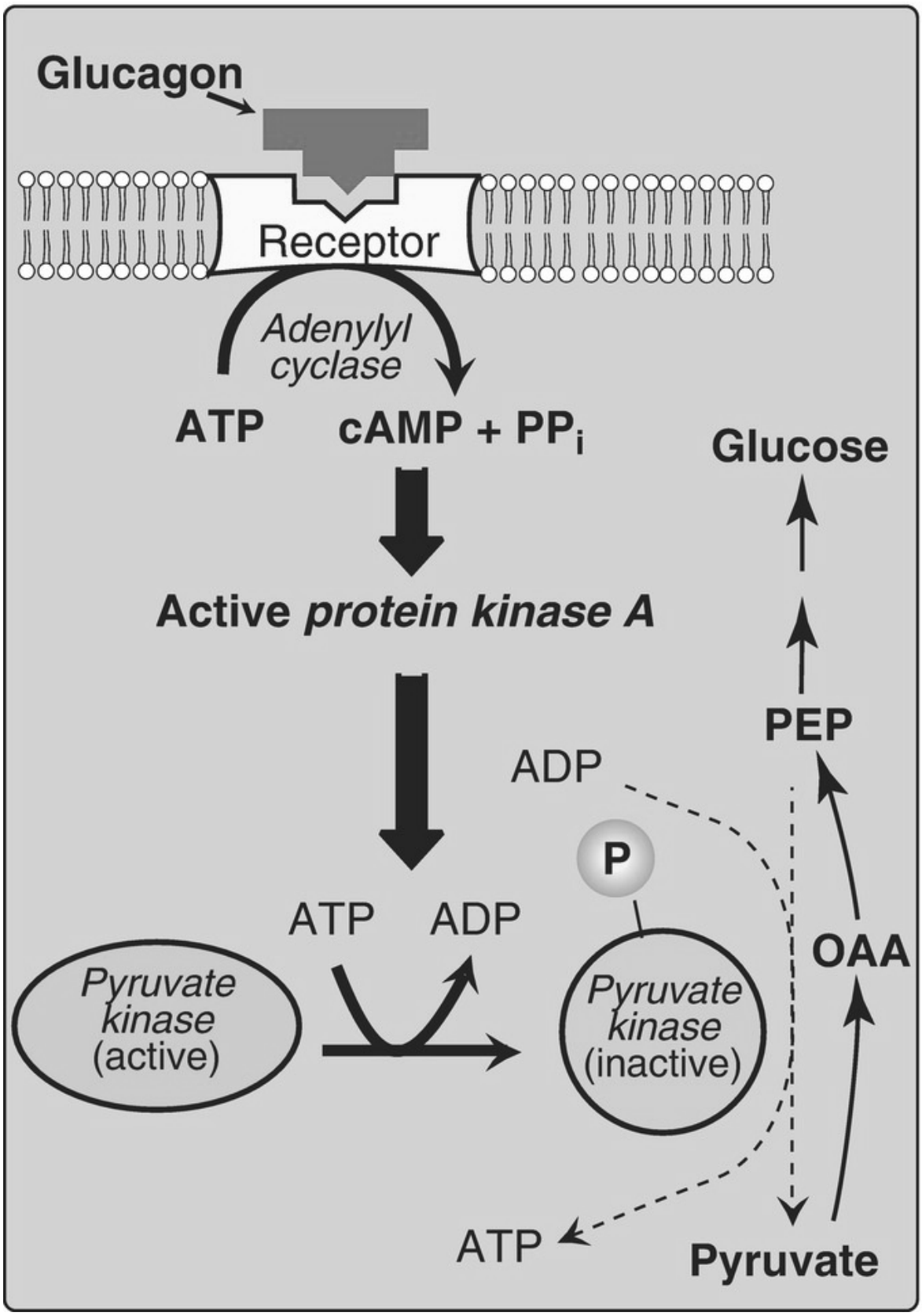

Glucagon
Receptor
Adenylyl cyclase
ATP
cAMP + PPi
Glucose
Active protein kinase A
PEP
ADP
P
ATP
ADP
OAA
Pyruvate kinase (active)
Pyruvate kinase (inactive)
ATP
Pyruvate

Figure 10.8 Covalent modification of ***pyruvate kinase*** results in inactivation of the enzyme. [Note: Only the hepatic isozyme is subject to covalent regulation.] OAA = oxaloacetate; PEP = phosphoenolpyruvate; PP_i = pyrophosphate; = phosphate; AMP and ADP = adenosine mono- and diphosphates; cAMP = cyclic AMP.

3. Induction of enzyme synthesis: Glucagon increases transcription of the gene for ***PEPCK*** via the transcription factor cAMP response element–binding (CREB) protein, thereby increasing the availability of this enzyme as levels of its substrate rise during fasting. [Note: Cortisol (a glucocorticoid) also increases expression of the gene, whereas insulin decreases expression.]

B. Substrate availability

The availability of gluconeogenic precursors, particularly glucogenic amino acids, significantly influences the rate of glucose synthesis. Decreased insulin levels favor mobilization of amino acids from muscle protein to provide the carbon skeletons for gluconeogenesis. The ATP and NADH coenzymes required for gluconeogenesis are primarily provided by FA oxidation.

C. Allosteric activation by acetyl CoA

Allosteric activation of hepatic ***PC*** by acetyl CoA occurs during fasting. As a result of increased TAG hydrolysis in adipose tissue, the liver is flooded with FA (see p. 330). The rate of formation of acetyl CoA by β-oxidation of these FA exceeds the capacity of the liver to oxidize it to CO_2 and water. As a result, acetyl CoA accumulates and activates ***PC***. [Note: Acetyl CoA inhibits the ***PDHC*** (by activating ***PDH kinase***; see p. 111). Thus, this single compound can divert pyruvate toward gluconeogenesis and away from the TCA cycle (Fig. 10.9).]

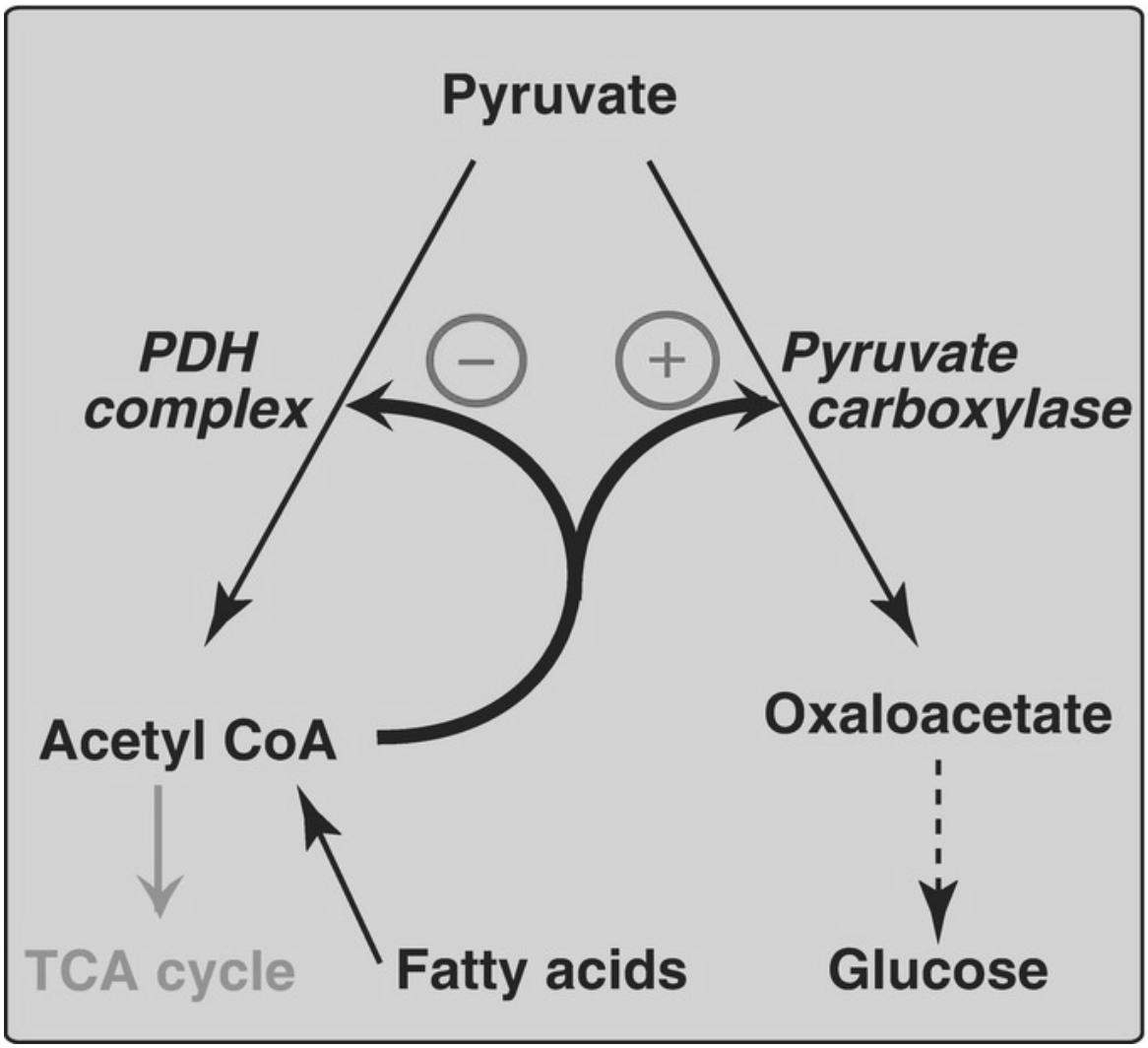


Figure 10.9 Acetyl coenzyme A (CoA) diverts pyruvate away from oxidation and toward gluconeogenesis. ***PDH*** = ***pyruvate dehydrogenase***; TCA = tricarboxylic acid.

D. Allosteric inhibition by AMP

Fructose 1,6-bisphosphatase is inhibited by AMP, a compound that activates ***PFK-1***. This results in reciprocal regulation of glycolysis and gluconeogenesis seen previously with fructose 2,6-bisphosphate (see p. 121). [Note: Thus, elevated AMP stimulates energy-producing pathways and inhibits energy-requiring ones.]

V. CHAPTER SUMMARY

Gluconeogenic precursors include glycerol released during triacylglycerol hydrolysis in adipose tissue, lactate released by cells that lack mitochondria and by exercising skeletal muscle, and α-keto acids (for example, α-ketoglutarate and pyruvate) derived from glucogenic amino acid metabolism (Fig. 10.10). Seven of the reactions of glycolysis are reversible and are used for gluconeogenesis in the liver and kidneys. Three reactions, catalyzed by ***pyruvate kinase***, ***phosphofructokinase-1***, and ***glucokinase***/***hexokinase***, are physiologically irreversible and must be circumvented. Pyruvate is converted to oxaloacetate and then to phosphoenolpyruvate (PEP) by ***pyruvate carboxylase*** (***PC***) and ***PEP-carboxykinase*** (***PEPCK***). ***PC*** requires biotin and ATP and is allosterically activated by acetyl coenzyme A. ***PEPCK*** requires guanosine triphosphate. Transcription of its gene is increased by glucagon and cortisol and decreased by insulin. Fructose 1,6-bisphosphate is converted to fructose 6-phosphate by ***fructose 1,6-bisphosphatase***. This enzyme is inhibited by a high adenosine monophosphate (AMP)/ATP ratio. It is also inhibited by fructose 2,6-bisphosphate, the primary allosteric activator of glycolysis. Glucose 6-phosphate is dephosphorylated to glucose by ***glucose 6-phosphatase***. This enzyme of the endoplasmic reticular membrane catalyzes the final step in gluconeogenesis and in glycogen degradation. Its deficiency results in severe, fasting hypoglycemia.

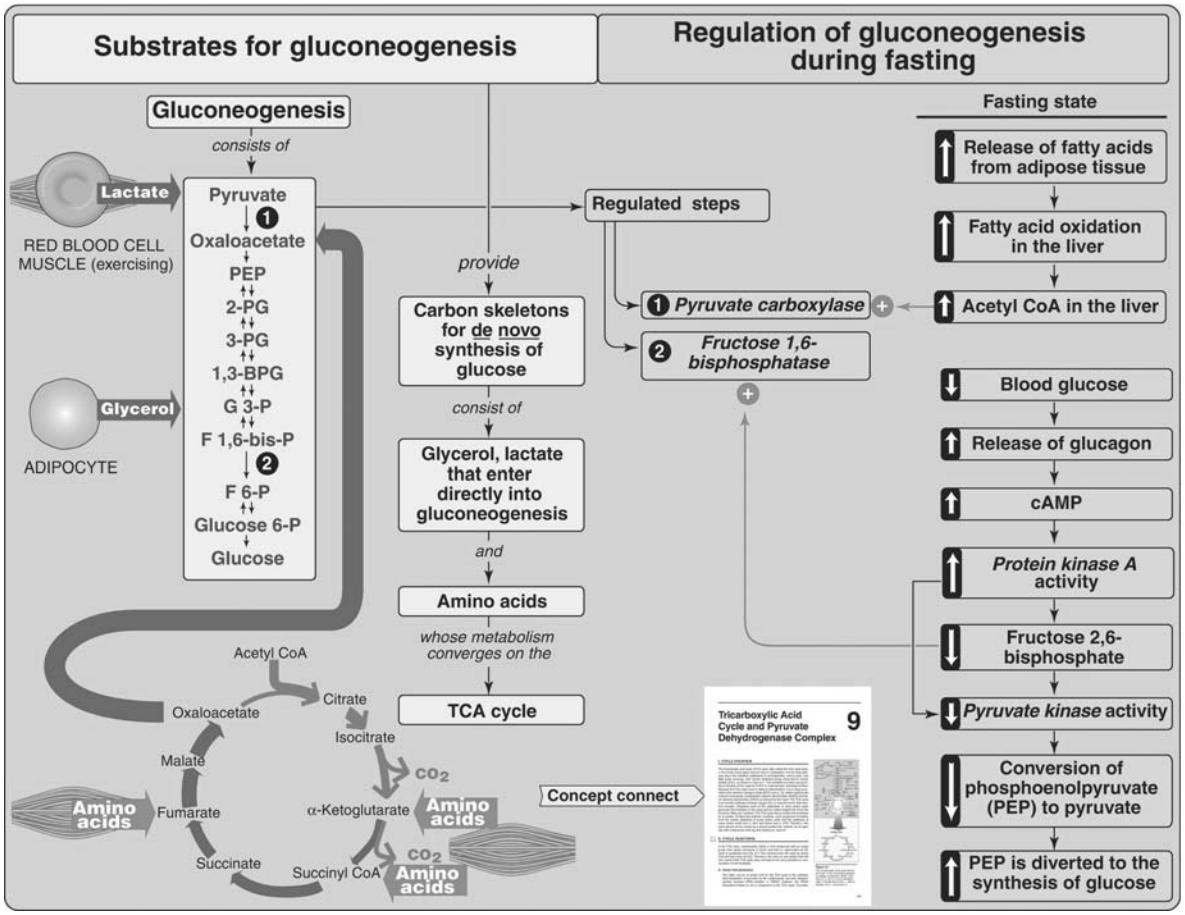


Figure 10.10 Key concept map for gluconeogenesis. TCA = tricarboxylic acid. CoA = coenzyme A; cAMP = cyclic adenosine monophosphate; P = phosphate; (B)PG = (bis)phosphoglycerate; G = glyceraldehyde; F = fructose; CO_2 = carbon dioxide.

Study Questions

Choose the ONE best answer.

0.1. Which one of the following statements concerning gluconeogenesis is correct?

A. It is an energy-producing (exergonic) process.

B. It is important in maintaining blood glucose during a 2-day fast.

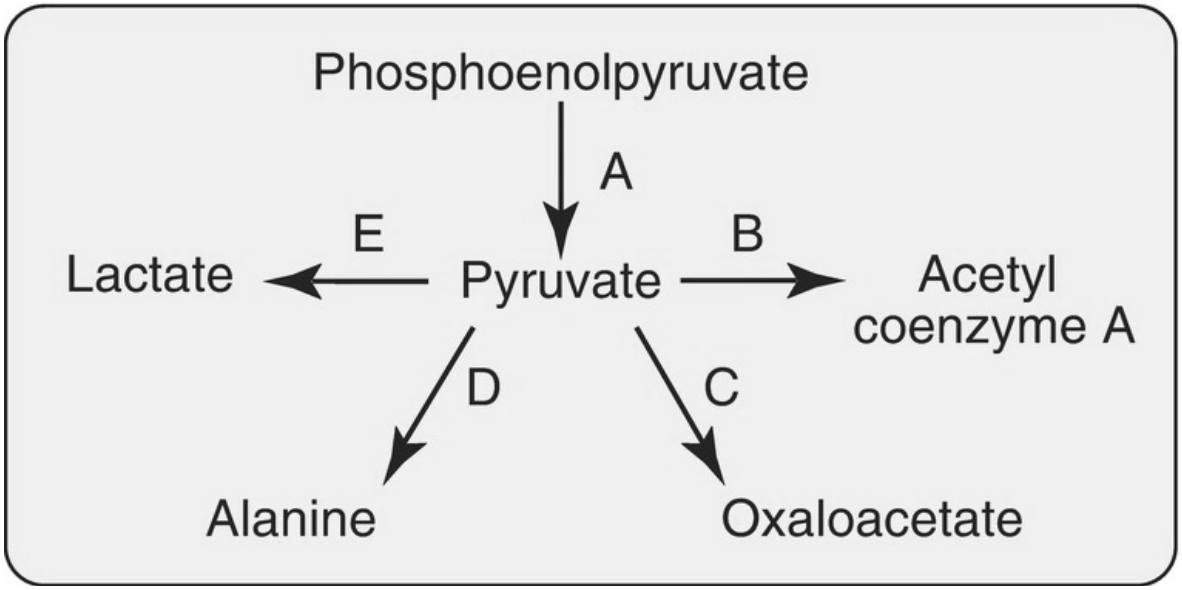
C. It is inhibited by a fall in the insulin/glucagon ratio.

D. It occurs in the cytosol of muscle cells.

E. It uses carbon skeletons provided by fatty acid degradation.

Correct answer = B. During a 2-day fast, glycogen stores are depleted, and gluconeogenesis maintains blood glucose. This is an energy-requiring (endergonic) pathway (both ATP and GTP get hydrolyzed) that occurs primarily in the liver, with the kidneys becoming major glucose producers in prolonged fasting. Gluconeogenesis uses both mitochondrial and cytosolic enzymes and is stimulated by a fall in the insulin/glucagon ratio. Fatty acid degradation yields acetyl coenzyme A (CoA), which cannot be converted to glucose. This is because there is no net gain of carbons from acetyl CoA in the tricarboxylic acid cycle, and the pyruvate dehydrogenase complex is physiologically irreversible. It is the carbon skeletons of most amino acids that are glucogenic.

0.2. Which reaction in the diagram below would be inhibited in the presence of large amounts of avidin, an egg white protein that binds and sequesters biotin?



Correct answer = C. Pyruvate is carboxylated to oxaloacetate by pyruvate carboxylase, a biotin-requiring enzyme. B (pyruvate dehydrogenase complex) requires thiamine pyrophosphate, lipoic acid, flavin and

nicotinamide adenine dinucleotides (FAD and NAD^+), and coenzyme A; D (transaminase) requires pyridoxal phosphate; E (lactate dehydrogenase) requires NADH.

0.3. Which one of the following reactions is unique to gluconeogenesis?

A. 1,3-Bisphosphoglycerate → 3-phosphoglycerate

B. Lactate → pyruvate

C. Oxaloacetate → phosphoenolpyruvate

D. Phosphoenolpyruvate → pyruvate

Correct answer = C. The other reactions are common to both gluconeogenesis and glycolysis.

0.4. Use the chart below to show the effect of adenosine monophosphate (AMP) and fructose 2,6-bisphosphate on the listed enzymes of gluconeogenesis and glycolysis.

Enzyme	Fructose 2,6-bisphosphate	AMP
Fructose 1,6-bisphosphatase		
Phosphofructokinase-1		

Both fructose 2,6-bisphosphate and adenosine monophosphate inhibit fructose 1,6-bisphosphatase of gluconeogenesis and activate phosphofructokinase-1 of glycolysis. This results in reciprocal regulation of the two pathways.

0.5. The metabolism of ethanol by alcohol dehydrogenase produces reduced nicotinamide adenine dinucleotide (NADH) from the oxidized (NAD^+) form. What effect is the fall in the NAD^+/NADH ratio expected to have on gluconeogenesis? Explain.

The increase in NADH as ethanol is oxidized decreases the availability of oxaloacetate (OAA) because the reversible oxidation of malate to