10: Gluconeogenesis

Overview

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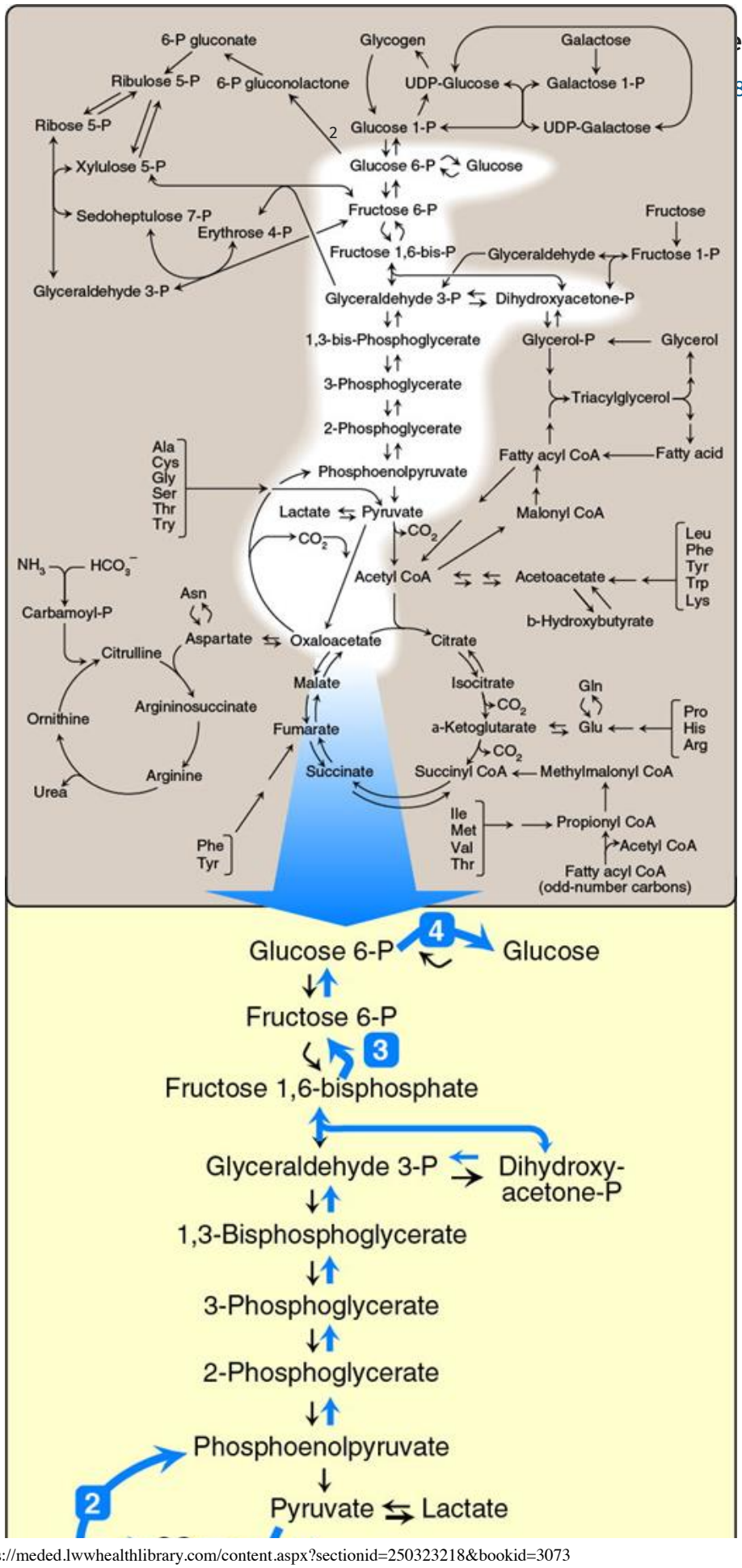
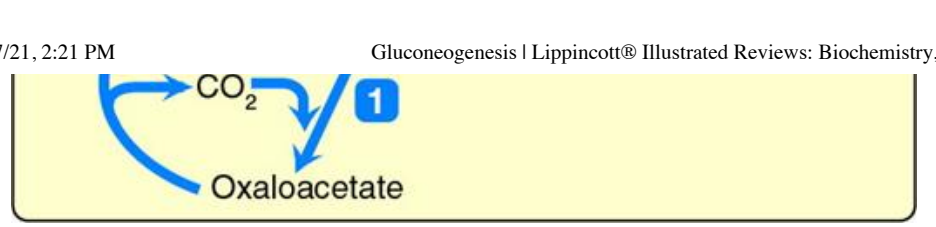
Some tissues, such as the brain, erythrocytes, kidney medulla, lens and cornea of the eye, testes, and exercising skeletal muscle, require a continuous supply of glucose as a metabolic fuel. Liver glycogen, an essential postprandial source of glucose, can meet these needs for <24 hours in the absence of dietary intake of carbohydrate (see p. 137). 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. Instead, glucose is synthesized *de novo* by a special pathway, gluconeogenesis, which requires both mitochondrial and cytosolic enzymes. 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 of 48 hours or longer, the kidneys become major glucose-producing organs, contributing ~40% of the total glucose production. The small intestine can also make glucose. Figure 10.1 shows the relationship of gluconeogenesis to other essential pathways of energy metabolism.

FIGURE 10.1



ergy metabolism.

8.2, for a more detailed map of



Substrates

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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. All but two amino acids (leucine and lysine) are glucogenic.

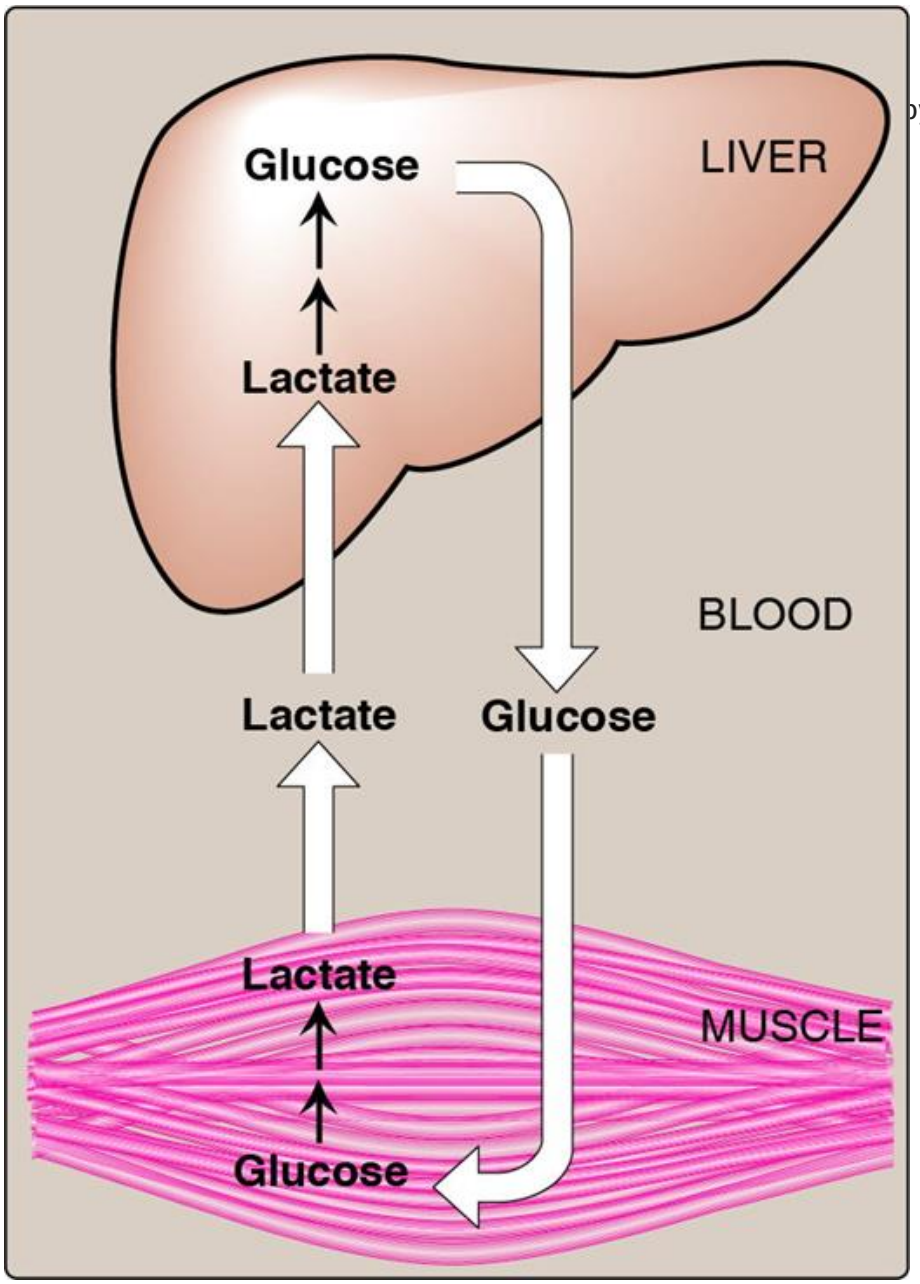
Glycerol

Glycerol is released during the hydrolysis of triacylglycerols (TAGs) in adipose tissue 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.

Lactate

Lactate from anaerobic glycolysis is released into the blood by exercising skeletal muscle and by erythrocytes, cells that lack mitochondria. 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).

FIGURE 10.2



y transport proteins.)

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 [e.g., 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. These compounds give rise instead to ketone bodies and are termed ketogenic.)

Reactions

Listen

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.

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).

Biotin

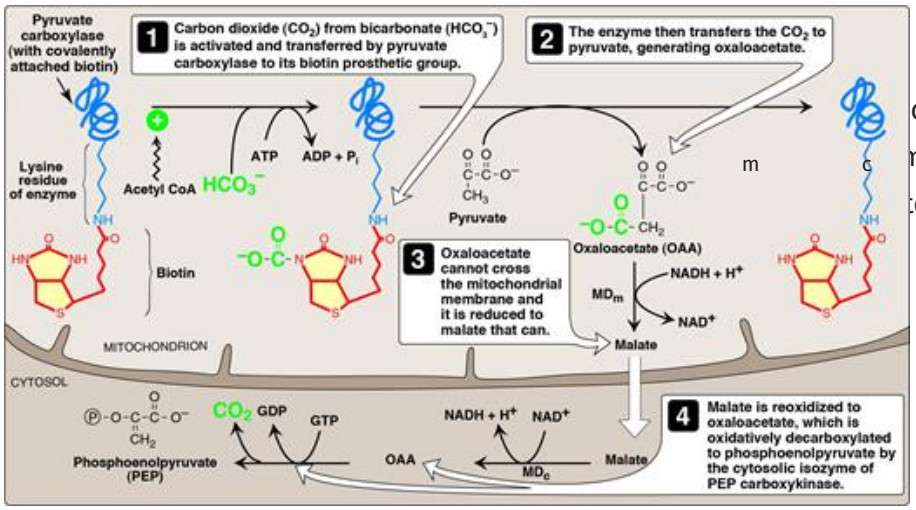
PC requires the coenzyme biotin (see p. 431) covalently bound to the ε-amino group of a lysine residue in the enzyme (see Fig. 10.3). ATP hydrolysis drives formation of an enzyme–biotin–carbon dioxide (CO_2) intermediate, which then carboxylates pyruvate to form OAA. (Note: HCO_3^- provides the CO_2.) 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. 203), propionyl CoA carboxylase (p. 215), and methylcrotonyl CoA carboxylase (p. 295).

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.

FIGURE 10.3



ducing equivalents required for
nitochondrial and cytosolic isozymes
es; ADP = adenosine diphosphate.

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, 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.)

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.

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.

Regulation by intracellular energy levels

Fructose 1,6-bisphosphatase is inhibited by a rise in the ratio of adenosine monophosphate (AMP) to ATP, called the AMP to ATP ratio, which signals a low-energy state in the cell. Conversely, low AMP and high ATP levels stimulate gluconeogenesis, an energy-requiring pathway.

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, 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.)

FIGURE 10.4

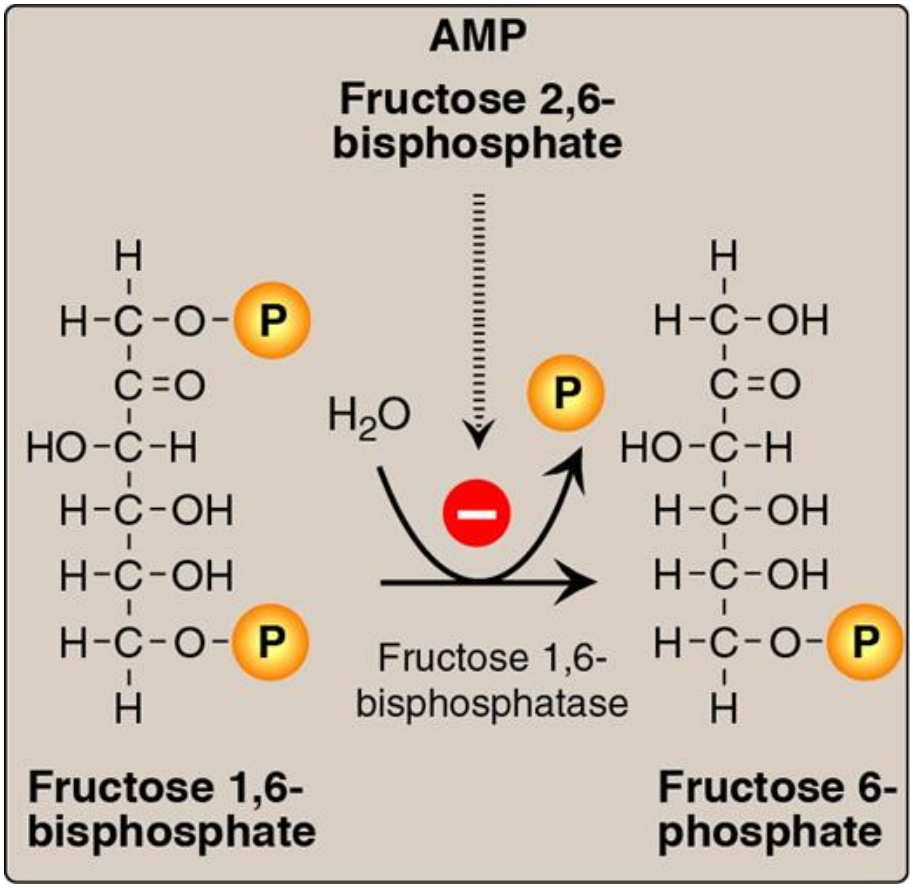
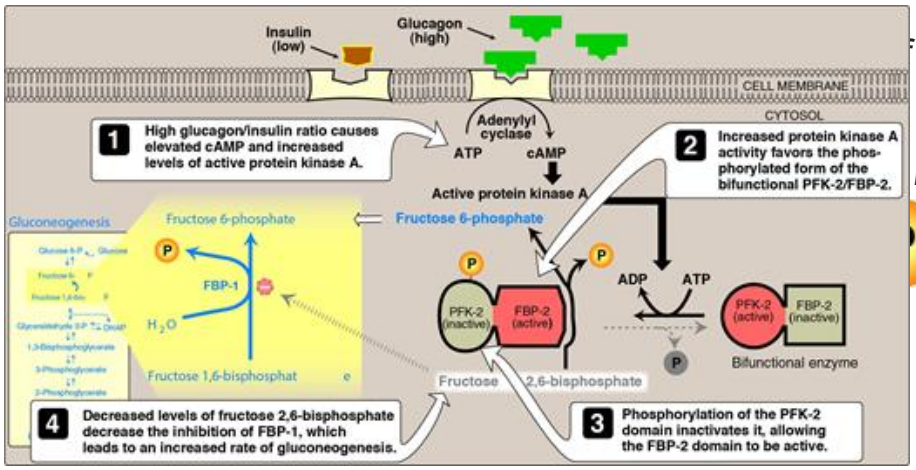


FIGURE 10.5



f fructose 2,6-bisphosphate in the

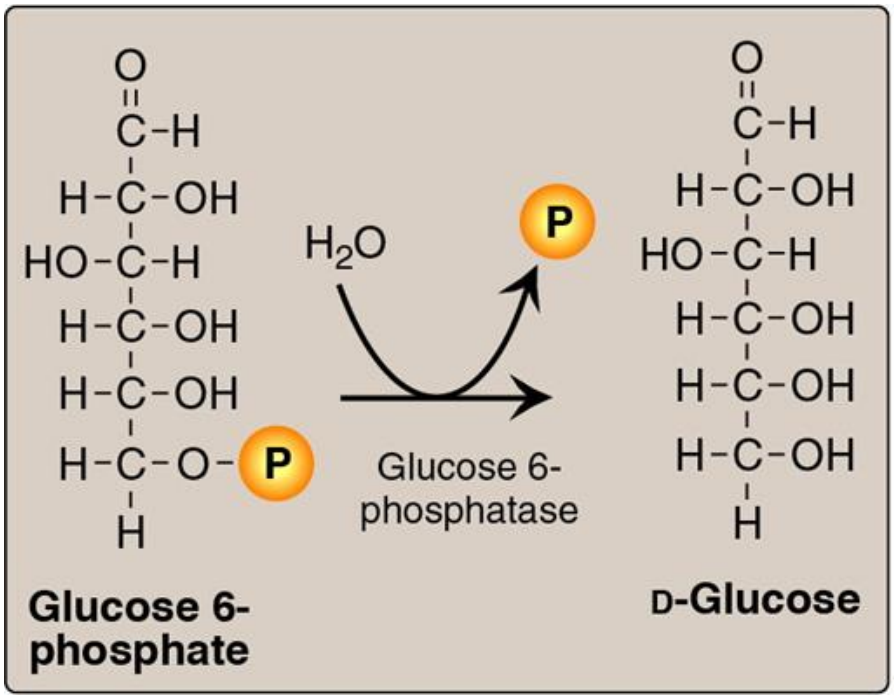
PFK-2 = phosphofructokinase-2; FBP-2

and = phosphate.

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). These ER membrane proteins are also required for the final step of glycogen degradation.

FIGURE 10.6



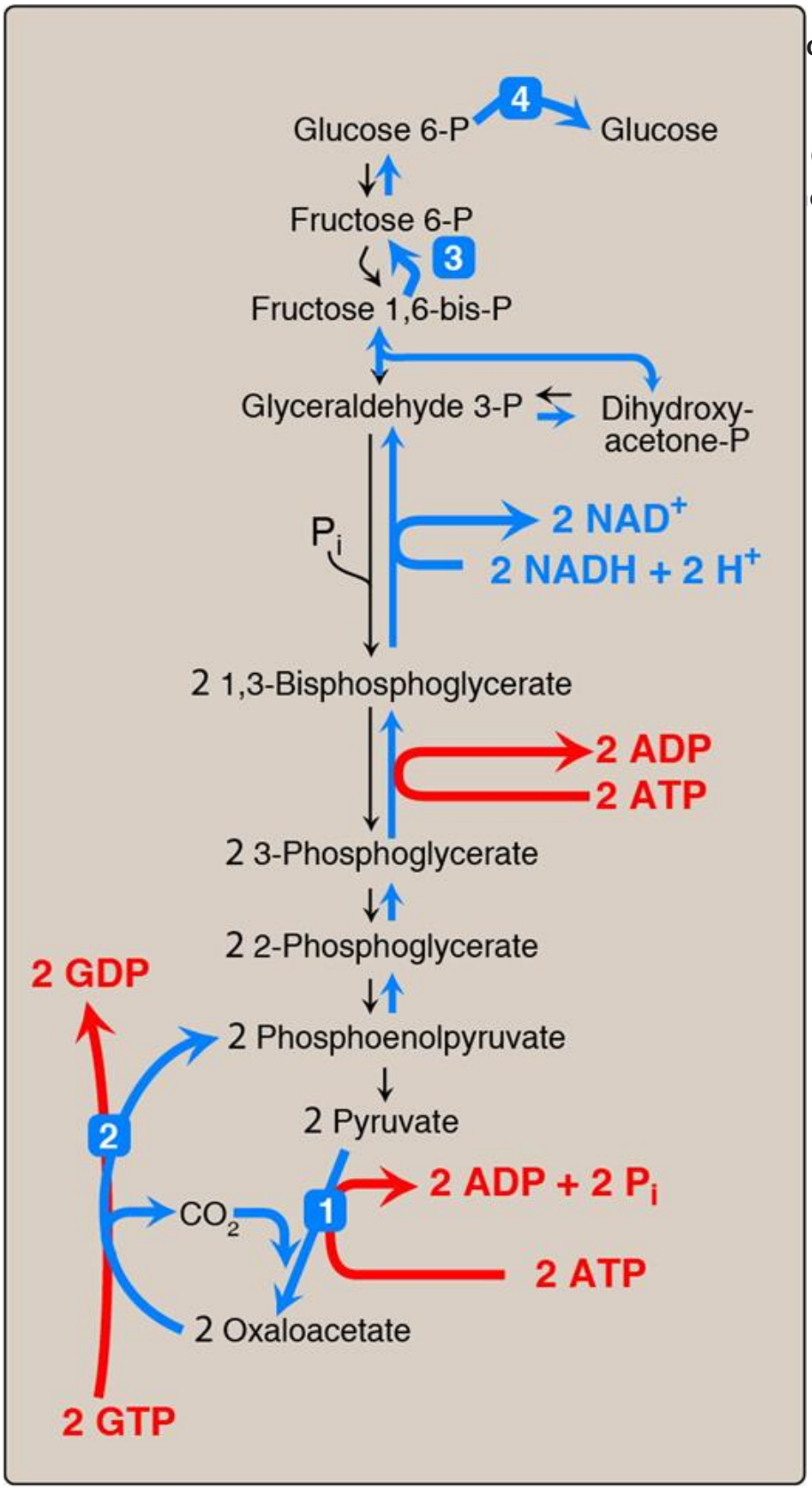
e glucose from gluconeogenic

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.

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].)

FIGURE 10.7



owing the energy requirements of

GDP and GTP = guanosine di- and
osine diphosphate.

Regulation

Listen

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. 347) stimulates gluconeogenesis by three mechanisms.

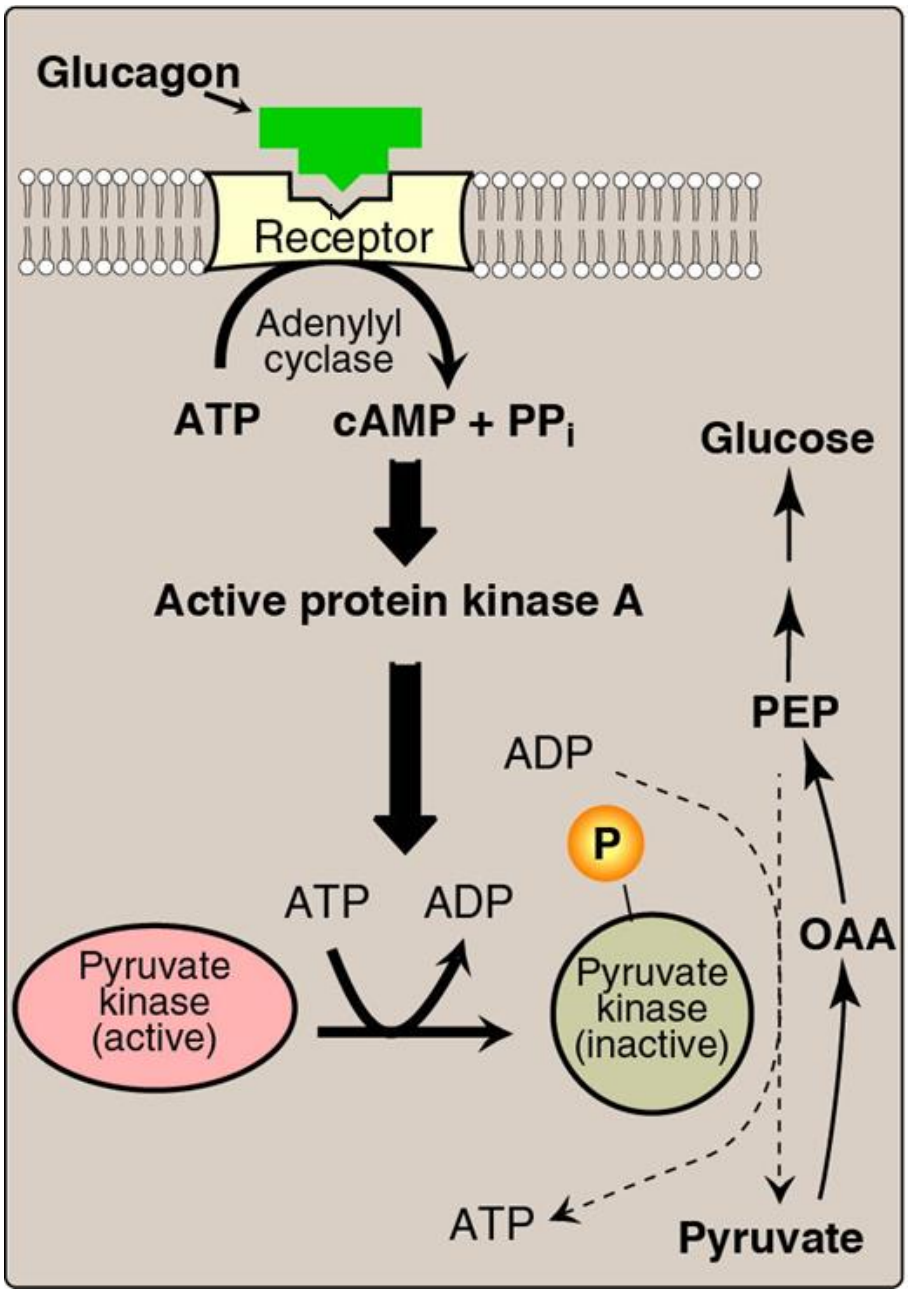
Changes in allosteric effectors

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).

Covalent modification of enzyme activity

Glucagon binds its G protein–coupled receptor 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).

FIGURE 10.8



of the enzyme.

oxaloacetate; PEP =

nd ADP = adenosine mono- and

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. Cortisol, a glucocorticoid, also increases expression of the gene, whereas insulin decreases expression.

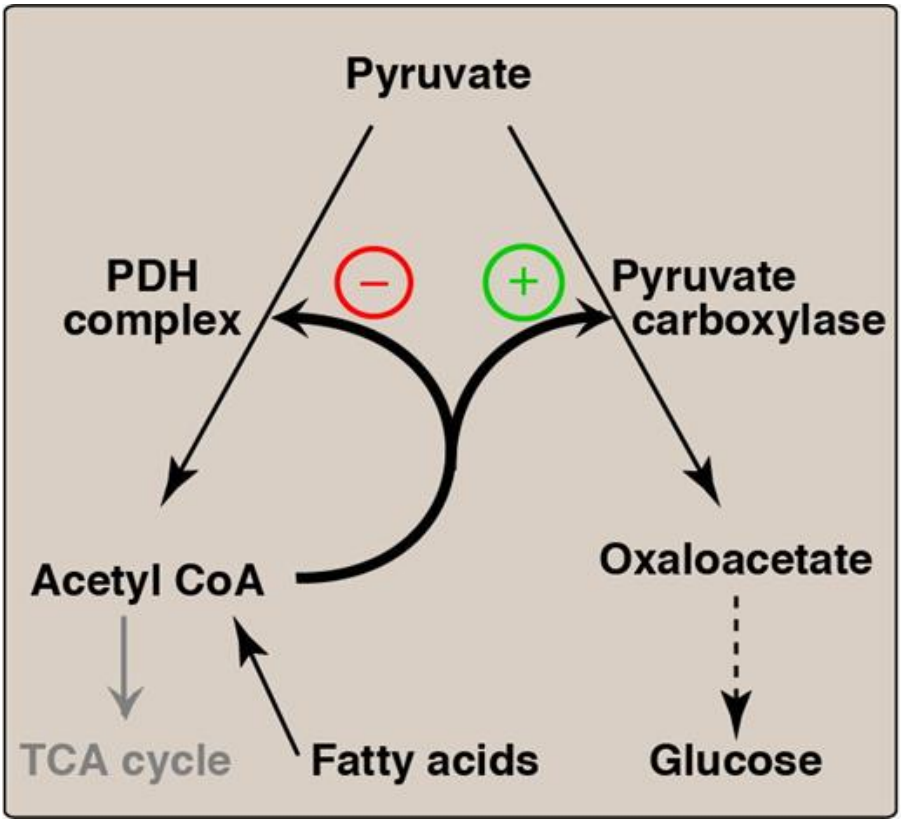
Substrate availability

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.

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. 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]. Thus, this single compound can divert pyruvate toward gluconeogenesis and away from the TCA cycle [Fig. 10.9].)

FIGURE 10.9



nd toward gluconeogenesis.

Allosteric inhibition by AMP

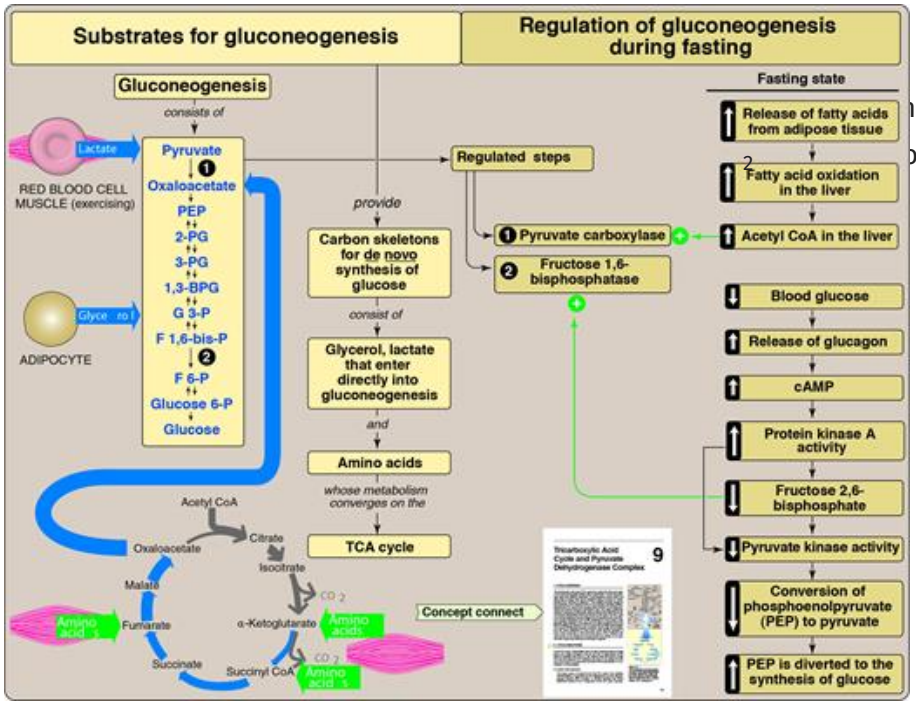
Fructose 1,6-bisphosphatase is inhibited by AMP, a compound that activates PFK-1. This results in reciprocal regulation of glycolysis and gluconeogenesis seen previously with **fructose 2,6-bisphosphate** (see p. 122). Thus, elevated AMP stimulates energy-producing pathways and inhibits energy-requiring ones.

Chapter Summary

Listen

- **Gluconeogenic precursors** include **glycerol** released during TAG hydrolysis in adipose tissue, **lactate** released by cells that lack mitochondria and by exercising skeletal muscle, and **α-keto acids** (e.g., **α-ketoglutarate** and **pyruvate**) derived from glucogenic amino acid metabolism (Fig. 10.10).

FIGURE 10.10



ophosphate; P = phosphate; (B)PG = oxide.

- Seven of the reactions of glycolysis are reversible and are used for gluconeogenesis in the liver and kidneys.
- Three reactions, catalyzed by **PK**, **PFK-1**, and glucokinase/hexokinase, are physiologically irreversible and must be circumvented.
- **Pyruvate** is converted to **OAA** and then to **PEP** by **PC** and **PEPCK**.
- PC requires **biotin** and **ATP** and is allosterically activated by **acetyl CoA** and PEPCK requires **GTP**.
- Fructose 1,6-bisphosphate is converted to fructose 6-phosphate by **fructose 1,6-bisphosphatase**. This enzyme is inhibited by a high AMP/ATP ratio and 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 ER membrane catalyzes the final step in gluconeogenesis and in glycogen degradation. Its deficiency results in severe, fasting hypoglycemia.

Study Questions

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Choose the ONE best answer.

10.1. 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.

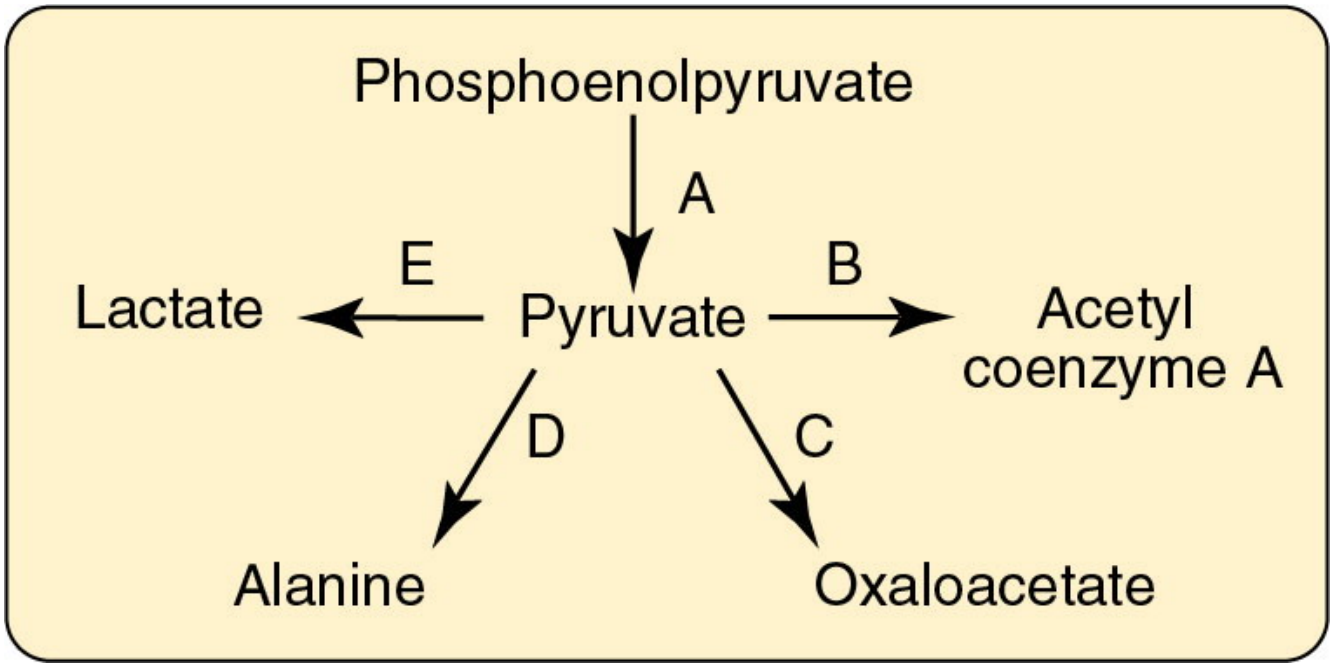
C. It is inhibited by a fall in the insulin/glucagon ratio.

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.

10.2. 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^+, respectively), and coenzyme A; D (transaminase) requires pyridoxal phosphate; E (lactate dehydrogenase) requires NADH.

10.3. 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.

10.4. 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.

10.5. 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 OAA by malate dehydrogenase of the tricarboxylic acid cycle is driven in the reverse direction by NADH. Additionally, the reversible reduction of pyruvate to lactate by lactate dehydrogenase is driven to lactate by NADH. Thus, two important gluconeogenic substrates, OAA and pyruvate, decrease as a result of the increase in NADH during ethanol metabolism. Consequently, gluconeogenesis decreases.

10.6. Given that acetyl coenzyme A cannot be a substrate for gluconeogenesis, why is its production in fatty acid oxidation essential for gluconeogenesis?

Acetyl coenzyme A inhibits the pyruvate dehydrogenase complex and activates pyruvate carboxylase, pushing pyruvate to gluconeogenesis and away from oxidation.