Gluconeogenesis Cori Cycle. Metabolism Of Glycogen

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Gluconeogenesis

OVERVIEW . Some tissues, such as the brain, red blood cells (RBCs), 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 only 10–18 hours in the absence of dietary intake of carbohydrate. During a prolonged fast, however, hepatic glycogen stores are depleted, and glucose is formed from noncarbohydrate precursors such as lactate, pyruvate, glycerol (derived from the backbone of triacylglycerols), and α-keto acids (derived from the catabolism of glucogenic amino acids). 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 by a special pathway, gluconeogenesis, which requires both mitochondrial and cytosolic enzymes. During an overnight fast, approximately 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 an estimated 40% of the total glucose production.

Gluconeogenic precursors are molecules that can be used to produce a net synthesis of glucose. The most important gluconeogenic prescurors are glycerol, lactate, and the α-keto acids obtained from the metabolism of glucogenic amino acids. [Note: Alanine, which directly gives rise to pyruvate, is an important example of a glucogenic amino acid.]

A. Glycerol Glycerol is released during the hydrolysis of triacylglycerols in adipose tissue and is delivered by the blood to the liver. Glycerol is phosphorylated by

glycerol kinase to glycerol phosphate, which is oxidized by glycerol phosphate dehydrogenase to dihydroxyacetone phosphate, an intermediate of glycolysis. [Note: Adipocytes cannot phosphorylate glycerol because they essentially lack glycerol kinase.]

B. Lactate Lactate is released into the blood by exercising skeletal muscle and by cells that lack mitochondria such as RBCs. In the Cori cycle, bloodborne glucose is converted by exercising muscle to lactate, which diffuses into the blood. This lactate is taken up by the liver and reconverted to glucose, which is released back into the circulation.

C. Amino acids Amino acids derived from hydrolysis of tissue proteins are the major sources of glucose during a fast. The metabolism of the glucogenic amino acids generates α-keto acids. α -Keto acids, such as α-ketoglutarate 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 and amino acids such as lysine and leucine) cannot give rise to a net synthesis of glucose. This is due to the irreversible nature of the pyruvate dehydrogenase (PDH) reaction, which converts pyruvate to acetyl CoA. These compounds give rise instead to ketone bodies and are, therefore, termed ketogenic.]

. REACTIONS UNIQUE TO GLUCONEOGENESIS

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

A. Carboxylation of pyruvate

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 first carboxylated by pyruvate carboxylase to OAA, which is then converted to PEP by the action of PEP-carboxykinase

Pyruvate carboxylase requires a coenzyme: biotin, covalently bound to the ε-amino group of a lysine residue in the enzyme. Hydrolysis of ATP drives the formation of an enzyme–biotin–CO2 intermediate, which subsequently carboxylates pyruvate to form OAA. [Note: HCO3 – is the source of the CO2.] The pyruvate carboxylase reaction occurs in the mitochondria of liver and kidney cells and has two purposes: to provide an important substrate for gluconeogenesis and to provide OAA that can replenish the TCA cycle intermediates that may become depleted, depending on the synthetic needs of the cell. Muscle cells also contain pyruvate carboxylase but use the OAA produced only for the replenishment (anaplerotic) purpose and do not synthesize glucose.

2. Allosteric regulation: Pyruvate carboxylase is allosterically activated by acetyl CoA. Elevated levels of acetyl CoA in mitochondria signal a metabolic state in which the increased synthesis of OAA is required. For example, this occurs during fasting, when OAA is used for the synthesis of glucose by gluconeogenesis in the liver and kidney. Conversely, at low levels of acetyl CoA, pyruvate carboxylase is largely inactive, and pyruvate is primarily oxidized by the PDH complex to produce acetyl CoA that can be further oxidized by the TCA cycle.

B. Transport of oxaloacetate to the cytosol

OAA must be converted to PEP for gluconeogenesis to continue. The enzyme that catalyzes this reaction is found in both the mitochondria and the cytosol in humans. The PEP generated in the mitochondria is transported to the cytosol by a specific transporter, whereas that generated in the cytosol requires the transport of OAA from the mitochondria to the cytosol. However, OAA is unable to be transported across the inner mitochondrial membrane, so it must first be reduced to malate by mitochondrial malate dehydrogenase (MD). Malate can be transported from the mitochondria to the cytosol, where it is reoxidized to OAA by cytosolic MD as nicotinamide adenine dinucleotide (NAD+) is reduced. The NADH produced is used in the reduction of 1,3-

bisphosphoglycerate to glyceraldehyde 3-phosphate, a step common to both glycolysis and gluconeogenesis. [Note: OAA also can be converted to aspartate, which is transported out of the mitochondria.]

C. Decarboxylation of cytosolic oxaloacetate (OAA)

OAA is decarboxylated and phosphorylated to PEP in the cytosol by PEP-carboxykinase (also referred to as PEPCK). The reaction is driven by hydrolysis of guanosine triphosphate ([GTP]. The combined actions of pyruvate carboxylase and PEP-carboxykinase 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.

D. Dephosphorylation of fructose 1,6-bisphosphate

Hydrolysis of fructose 1,6-bisphosphate by fructose 1,6-bisphos-phatase, found in liver and kidney, bypasses the irreversible phosphofructokinase-1 (PFK-1) reaction, and provides an energetically favorable pathway for the formation of fructose 6-phosphate. This reaction is an important regulatory site of gluconeogenesis. 1. Regulation by energy levels within the cell: Fructose 1,6-bisphosphatase is inhibited by elevated levels of adenosine monophosphate (AMP), which signal an "energy-poor" state in the cell. Conversely, high levels of ATP and low concentrations of AMP stimulate gluconeogenesis, an energy-requiring pathway.

2. Regulation by fructose 2,6-bisphosphate: Fructose 1,6-bisphos-phatase is inhibited by fructose 2,6-bisphosphate, an allosteric effector whose concentration is influenced by the insulin to glucagon ratio: when glucagon is high, the effector is not made and, thus, the phosphatase is active. [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

E. Dephosphorylation of glucose 6-phosphate

Hydrolysis of glucose 6-phosphate by glucose 6-phosphatase bypasses the irreversible hexokinase/glucokinase reaction and provides an energetically favorable pathway for the formation of free glucose (Figure 10.6). Liver and kidney are the only organs that release free glucose from glucose 6-phosphate. This process actually requires a complex of two proteins: glucose 6-phosphate translocase, which transports glucose 6- phosphate across the endoplasmic reticular (ER) membrane, and the enzyme glucose 6-phosphatase (found only in gluconeogenic cells), which removes the phosphate, producing free glucose. [Note: These ER-membrane proteins are also required for the final step of glycogen degradation (see p. 130). Type Ia and lb glycogen storage disease, caused by deficiencies in the phosphatase and the transferase, respectively, are characterized by severe fasting hypoglycemia, because free glucose is unable to be produced from either gluconeogenesis or glycogenolysis.] Specific glucose transporters (GLUTs) are responsible for moving free glucose into the cytosol and then into blood. [Note: Glucose 6-phosphate translocase moves inorganic phosphate out of the ER as it moves glucose 6-phosphate in.]

Summary of the reactions of gluconeogenesis

Of the 11 reactions required to convert pyruvate to free glucose, 7 are catalyzed by reversible glycolytic enzymes. The irreversible reactions of glycolysis catalyzed by hexokinase/glucokinase, PFK-1, and PK are circumvented by glucose 6- phosphatase, fructose 1,6-bisphosphatase, and pyruvate carboxylase/PEP-carboxykinase. In gluconeogenesis, the equilibria of the 7 reversible reactions of glycolysis are pushed in favor of glucose synthesis as a result of the essentially irreversible formation of PEP, fructose 6-phosphate, and glucose catalyzed by the gluconeogenic enzymes. [Note: The stoichiometry of gluconeogenesis from pyruvate couples the cleavage of six high-energy phosphate bonds (ATPs) and the oxidation of two NADH with the formation of each molecule of glucose]

REGULATION OF GLUCONEOGENESIS

A. **Glucagon** This peptide hormone from the a cells of pancreatic islets stimulates gluconeogenesis

B. **Substrate availability** The availability of gluconeogenic precursors, particularly glucogenic amino acids, significantly influences the rate of glucose synthesis. Decreased levels of insulin favor mobilization of amino acids from muscle protein and provide the carbon skeletons for gluconeogenesis. The ATP and NADH coenzymes-cosubstrates required for gluconeogenesis are primarily provided by the catabolism of fatty acids

C. **Allosteric activation** by acetyl coenzyme A

D. **Allosteric inhibition** by adenosine monophosphate

Tests.

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 fast.

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

D. It occurs in the cytosol of muscle cells. E. It uses carbon skeletons provided by fatty acid degradation.

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

Correct answer = B. During a fast, glycogen stores are depleted, and gluconeogenesis maintains blood glucose. Gluconeogenesis is an energy-requiring (endergonic) pathway (both ATP and GTP get hydrolyzed) that occurs in liver, with kidney becoming a major glucoseproducing organ in prolonged fasting. It utilizes both mitochondrial and cytosolic enzymes. Gluconeogenesis 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 reaction is physiologically irreversible. It is the carbon skeletons of most amino acids that are gluconeogenic.

2. 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

E. pyruvate →alanine

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

GLYCOGEN METABOLISM

OVERVIEW A constant source of blood glucose is an absolute requirement for human life. Glucose is the greatly preferred energy source for the brain, and the required energy source for cells with few or no mitochondria such as mature red blood cells. Glucose is also essential as an energy source for exercising muscle, where it is the substrate for anaerobic glycolysis. Blood glucose can be obtained from three primary sources: the diet, degradation of glycogen, and gluconeogenesis. Dietary intake of glucose and glucose precursors, such as starch (a polysaccharide), disaccharides, and monosaccharides, is sporadic and, depending on the diet, is not always a reliable source of blood glucose. In contrast, gluconeogenesis can provide sustained synthesis of glucose, but it is somewhat slow in responding to a falling blood glucose level. Therefore, the body has developed mechanisms for storing a supply of glucose in a rapidly

mobilizable form, namely, glycogen. In the absence of a dietary source of glucose, this sugar is rapidly released from liver and kidney glycogen. Similarly, muscle glycogen is extensively degraded in exercising muscle to provide that tissue with an important energy source. When glycogen stores are depleted, specific tissues synthesize glucose de novo, using amino acids from the body's proteins as a primary source of carbons for the gluconeogenic pathway.

STRUCTURE AND FUNCTION OF GLYCOGEN The main stores of glycogen are found in skeletal muscle and liver, although most other cells store small amounts of glycogen for their own use. The function of muscle glycogen is to serve as a fuel reserve for the synthesis of adenosine triphosphate (ATP) during muscle contraction. That of liver glycogen is to maintain the blood glucose concentration, particularly during the early stages of a fast. [Note: Liver glycogen can maintain blood glucose for 10–18 hours.]

Fluctuation of glycogen stores

Liver glycogen stores increase during the well-fed state (and are depleted during a fast. Muscle glycogen is not affected by short periods of fasting (a few days) and is only moderately decreased in prolonged fasting (weeks). Muscle glycogen is synthesized to replenish muscle stores after they have been depleted following strenuous exercise. [Note: Glycogen synthesis and degradation go on continuously. The differences between the rates of these two processes determine the levels of stored glycogen during specific physiologic states.]

SYNTHESIS OF GLYCOGEN (GLYCOGENESIS)

Glycogen is synthesized from molecules of α-D-glucose. The process occurs in the cytosol and requires energy supplied by ATP (for the phosphorylation of glucose) and uridine triphosphate (UTP).

A. Synthesis of a primer to initiate glycogen synthesis. Glycogenin

Glycogen synthase makes the α(1→4) linkages in glycogen. This enzyme cannot initiate chain synthesis using free glucose as an acceptor of a molecule of glucose from UDP-glucose. Instead, it can only elongate already existing chains of glucose and, therefore, requires a primer. A fragment of glycogen can serve as a primer in cells whose glycogen stores are not totally depleted. In the absence of a glycogen fragment, a protein called glycogenin can serve as an acceptor of glucose residues from UDP-glucose. The side-chain hydroxyl group of a specific tyrosine in the protein serves as the site at which the initial glucosyl unit is attached. Because the reaction is catalyzed by glycogenin itself via autoglucosylation, glycogenin is an enzyme. Glycogenin then catalyzes the transfer of the next few molecules of glucose from UDP-glucose, producing a short, α(1→4)-linked glucosyl chain. This short chain serves as a primer that is able to be elongated by glycogen synthase as described below [Note: Glycogenin stays associated with and forms the core of a glycogen granule.]

B. Elongation of glycogen chains by glycogen synthase Elongation of a glycogen chain involves the transfer of glucose from UDP-glucose to the nonreducing end of the growing chain, forming a new glycosidic bond between the anomeric hydroxyl group of carbon 1 of the activated glucose and carbon 4 of the accepting glucosyl residue. [Note: The nonreducing end of a carbohydrate chain is one in which the anomeric carbon of the terminal sugar is linked by a glycosidic bond to another compound, making the terminal sugar nonreducing] The enzyme responsible for making the α(1→4) linkages in glycogen is glycogen synthase. [Note: The UDP released when the new α(1→4) glycosidic bond is made can be phosphorylated to UTP by nucleoside diphosphate kinase (UDP + ATP UTP + ADP]

C. Formation of branches in glycogen

If no other synthetic enzyme acted on the chain, the resulting structure would be a linear (unbranched) chain of glucosyl residues attached by α(1→4)

linkages. Such a compound is found in plant tissues and is called amylose. In contrast, glycogen has branches located, on average, eight glucosyl residues apart, resulting in a highly branched, tree-like structure that is far more soluble than the unbranched amylose. Branching also increases the number of nonreducing ends to which new glucosyl residues can be added (and also, as described later, from which these residues can be removed), thereby greatly accelerating the rate at which glycogen synthesis can occur and dramatically increasing the size of the glycogen molecule. Synthesis of branches: Branches are made by the action of the branching enzyme, ***amylo-α(1→4)→α(1→6)-transglucosidase***. This enzyme removes a set of six to eight glucosyl residues from the nonreducing end of the glycogen chain, breaking an α(1→4) bond to another residue on the chain, and attaches it to a non -terminal glucosyl residue by an α(1→6) linkage, thus functioning as a 4:6 transferase. The resulting new, nonreducing end, as well as the old nonreducing end from which the six to eight residues were removed, can now be further elongated by glycogen synthase.

DEGRADATION OF GLYCOGEN (GLYCOGENOLYSIS)

The degradative pathway that mobilizes stored glycogen in liver and skeletal muscle is not a reversal of the synthetic reactions. Instead, a separate set of cytosolic enzymes is required. When glycogen is degraded, the primary product is glucose 1-phosphate, obtained by breaking α(1→4) glycosidic bonds. In addition, free glucose is released from each α(1→6)–linked glucosyl residue (branch point).

A. *Shortening of chains*

Glycogen phosphorylase sequentially cleaves the α(1→4) glycosidic bonds between the glucosyl residues at the nonreducing ends of the glycogen chains by simple phosphorolysis (producing glucose 1-phosphate) until four glucosyl units remain on each chain before a branch point. [Note: Phosphorylase

contains a molecule of covalently bound pyridoxal phosphate that is required as a coenzyme.] The resulting structure is called a limit dextrin, and phosphorylase cannot degrade it any further

B. *Removal of branches*

Branches are removed by the two enzymic activities of a single bifunctional protein, the debranching enzyme. First, oligo-α(1→4)→α(1→4)-glucantransferase activity removes the outer three of the four glucosyl residues attached at a branch. It next transfers them to the nonreducing end of another chain, lengthening it accordingly. Thus, an α(1→4) bond is broken and an α(1→4) bond is made, and the enzyme functions as a 4:4 transferase. Next, the remaining glucose residue attached in an α(1→6) linkage is removed hydrolytically by amylo-α(1→6)- glucosidase activity, releasing free glucose. The glucosyl chain is now available again for degradation by glycogen phosphorylase until four glucosyl units in the next branch are reached.

C.Conversion of glucose 1-phosphate to glucose 6-phosphate

Glucose 1-phosphate, produced by glycogen phosphorylase, is converted in the cytosol to glucose 6-phosphate by phosphoglucomutase. In the liver, glucose 6-phosphate is transported into the endoplasmic reticulum (ER) by glucose 6-phosphate translocase. There it is converted to glucose by glucose 6-phosphatase. The glucose then is transported from the ER to the cytosol. Hepatocytes release glycogen-derived glucose into the blood to help maintain blood glucose levels until the gluconeogenic pathway is actively producing glucose. [Note: In the muscle, glucose 6-phosphate cannot be dephosphorylated and sent into the blood because of a lack of glucose 6- phosphatase. Instead, it enters glycolysis, providing energy needed for muscle contraction.]

D. Lysosomal degradation of glycogen . glycogen storage disease Type II: ***Pompe disease***

A small amount (1%–3%) of glycogen is continuously degraded by the lysosomal enzyme, α(1→4)-glucosidase (acid maltase). The purpose of this pathway is unknown. However, a deficiency of this enzyme causes accumulation of glycogen in vacuoles in the lysosomes, resulting in the serious glycogen storage disease (GSD) Type II: Pompe disease. [Note: Type II: Pompe disease is the only GSD that is a lysosomal storage disease.]

REGULATION OF GLYCOGENESIS AND GLYCOGENOLYSIS

Because of the importance of maintaining blood glucose levels, the synthesis and degradation of its glycogen storage form are tightly regulated. In the liver, glycogenesis accelerates during periods when the body has been well fed, whereas glycogenolysis accelerates during periods of fasting. In skeletal muscle, glycogenolysis occurs during active exercise, and glycogenesis begins as soon as the muscle is again at rest. Regulation of glycogen synthesis and degradation is accomplished on two levels. First, glycogen synthase and glycogen phosphorylase are hormonally regulated (by phosphorylation/dephosphorylation) to meet the needs of the body as a whole. [Note: Phosphorylation of glycogen phosphorylase is catalyzed by glycogen phosphorylase kinase] Second, these same enzymes are allosterically regulated (by effector molecules) to meet the needs of a particular tissue.

A. Activation of glycogen degradation. The binding of hormones, such as glucagon or epinephrine, to plasma membrane G protein–coupled receptors (GPCRs) signals the need for glycogen to be degraded, either to elevate blood glucose levels or to provide energy for exercising muscle.

B. Inhibition of glycogen synthesis. The regulated enzyme in glycogenesis is glycogen synthase. It also exists in two forms, the active “a” form and the inactive “b” form. However, for glycogen synthase, in contrast to phosphorylase kinase and phosphorylase, the active form is dephosphorylated, whereas the inactive form is phosphorylated. Glycogen synthase a is converted t.o the inactive “b” form by phosphorylation at several sites on the enzyme, with the level of inactivation

proportional to its degree of phosphorylation. Phosphorylation is catalyzed by several different protein kinases that are regulated by cAMP or other signaling mechanisms .

C. Regulation of glycogen synthesis and degradation in the well- fed state: In the well-fed state, glycogen synthase b in both liver and muscle is allosterically activated by glucose 6-phosphate, which is present in elevated concentrations. In contrast, glycogen phosphorylase a is allosterically inhibited by glucose 6-phosphate, as well as by ATP, a high-energy signal in the cell. [Note: In liver, but not muscle, nonphosphorylated glucose is also an allosteric inhibitor of glycogen phosphorylase a.]

GLYCOGEN STORAGE DISEASES (GSD)

These are a group of genetic diseases that are caused by defects in enzymes required for glycogen degradation or, more rarely, glycogen synthesis. They result either in formation of glycogen that has an abnormal structure or in the accumulation of excessive amounts of normal glycogen in specific tissues as a result of impaired degradation. A particular enzyme may be defective in a single tissue, such as liver (resulting in hypoglycemia) or muscle (causing muscle weakness), or the defect may be more generalized, affecting a variety of tissues. The severity of the GSDs ranges from fatal in early childhood to mild disorders that are not life threatening. [Note: Only one GSD is lysosomal because glycogen metabolism occurs primarily in the cytosol.]

Study Questions

Epinephrine and glucagon have which one of the following effects on hepatic glycogen metabolism?

A. Both glycogen phosphorylase and glycogen synthase are activated by phosphorylation but at significantly different rates.

B. Glycogen phosphorylase is inactivated by the resuting rise in calcium, whereas glycogen synthase is activated.

C. Glycogen phosphorylase is phosphorylated and active, whereas glycogen synthase is phosphorylated and inactive.

D. The net synthesis of glycogen is increased.

Correct answer = C. Epinephrine and glucagon both cause increased glycogen degradation and decreased synthesis in the liver through covalent modification (phosphorylation) of key enzymes of glycogen metabolism. Glycogen phosphorylase is phosphorylated and active (“a” form), whereas glycogen synthase is phosphorylated and inactive (“b” form). Glucagon does not cause a rise in calcium.