Notes

3

CARBOHYDRATE METABOLISM

3.1 INTRODUCTION

All living cells require energy to carry out various cellular activities. This energy is stored in the chemical bonds of organic molecules (e.g. carbohydrates, fats, proteins) that we eat as food. These organic molecules are broken down by enzymatic reactions in cells to generate energy in the form of adenosine triphosphate (ATP). The ATP generated by these pathways in cells is used to drive fundamental cellular processes. The food we consume is mainly comprised of proteins, polysaccharides (carbohydrates) and fats. These are first broken down into smaller units: proteins into amino acids, polysaccharides into sugars, and fats into fatty acids and glycerol. This process of digestion occurs outside the cell. The amino acids, simple sugars and fatty acids then enter the cell and undergo oxidation by glycolysis (in the cytosol) and the citric acid cycle (in the mitochondria) to generate ATP (from ADP and P_i).

OBJECTIVES

After reading this lesson you will be able to

- describe glycolysis, Citric Acid Cycle
- explain Glycogenesis and Glycogenolysis
- describe the Hormonal regulation of blood sugar level

3.2 GLYCOLYSIS (EMBDEN-MEYERHOF PATHWAY)

Definition

In glycolysis pathway glucose is converted to pyruvate (aerobic condition) or lactate (anaerobic condition), along with production of a small quantity of energy.

Site of reaction: All the reaction steps take place in the cytoplasm.

Importance of the glycolysis pathway:

- It is the only pathway that is taking place in all the cells of the body.

- Glycolysis is the only source of energy in erythrocytes.

- In strenuous exercise, when muscle tissue lacks enough oxygen, anaerobic glycolysis forms the major source of energy for muscles.

- The glycolytic pathway may be considered as the preliminary step before complete oxidation.

- The glycolytic pathway provides carbon skeletons for synthesis of non-essential amino acids as well as glycerol part of fat.

- Most of the reactions are reversible.

Notes

Steps of glycolytic pathway

1. Glucose is phosphorylated to glucose -6-phosphate. The enzyme is hexokinase, which splits ATP into ADP and the Pi is added on to the glucose. The energy released by hydrolysis of ATP is utilised for the forward reaction. Hexokinase is the key glycolytic enzyme and the reaction is irreversible.

2. Glucose-6-phosphate is isomerised to fructose-6-phosphate by phosphohexose isomerase.

3. Fructose-6-phosphate is further phosphorylated to fructose-1,6-bisphosphate. The enzyme is phosphofructokinase, it is an important key enzyme and the reaction is irreversible.

4. Fructose-1, 6-bisphosphate is cleaved into two 3 carbon atoms; one glyceraldehyde-3-phosphate and another molecule of dihydroxyacetone phosphate. The enzyme is aldolase. Dihydroxyacetone phosphate is isomerised to glyceraldehyde-3-phosphate by the enzyme phophotriose isomerase.

5. Glyceraldehyde-3-phosphate is dehydrogenated and simultaneously phosphorylated to 1,3-bis-phosphoglycerate with the help of NAD^+. The enzyme is glyceraldehyde-3-phosphate dehydrogenase.

6. 1, 3-bis-phosphoglycerate is converted to 3-phosphoglycerate by the enzyme 1, 3-bis-phosphoglycerate kinase. Here one molecule of ATP is formed and this reaction is an example for Substrate level phosphorylation.

7. 3-phosphoglycerate is isomerised to 2-phosphoglycerate by shifting the phosphate group from 3rd to 2nd carbon atom. The enzyme is phosphoglucomutase.

8. 2-phosphoglycerate is converted to phosphoenol pyruvate by the enzyme enolase. One water molecule is removed. A high energy phosphate bond is produced. This enzyme requires Mg^{++} and inhibited by fluoride.

9. Phosphoenol pyruvate is dephosphorylated to pyruvate, by pyruvate kinase. One molecule of ATP is generated. This step is irreversible.

10. In anaerobic condition pyruvate is reduced to lactate by lactate dehydrogenase.In aerobic conditions pyruvate enters citric acid cycle for complete oxidation. The lactate from anaerobic cycle enters cori's cycle.
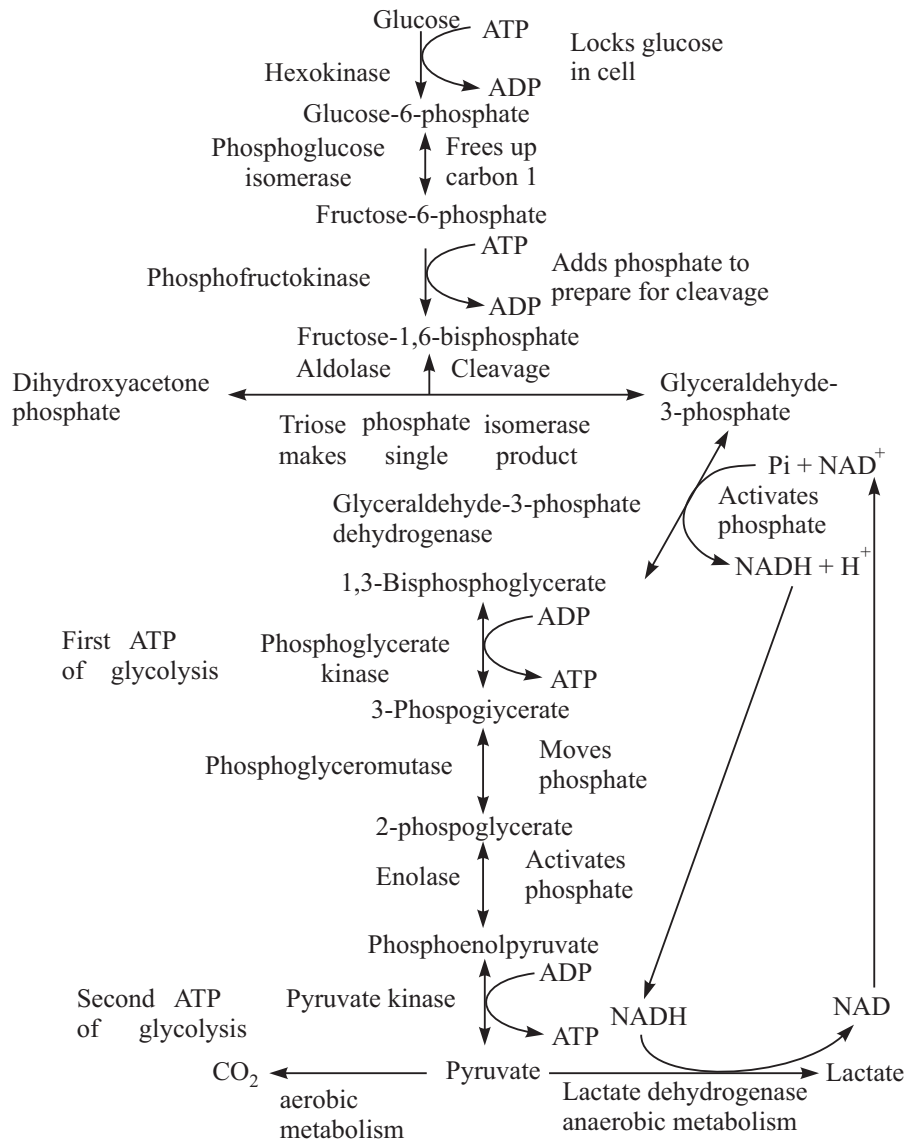
Glucose — ATP
Locks glucose in cell

Hexokinase → ADP

Glucose-6-phosphate

Phosphoglucose isomerase — Frees up carbon 1

Fructose-6-phosphate
— ATP

Phosphofructokinase → ADP
Adds phosphate to prepare for cleavage

Fructose-1,6-bisphosphate
Aldolase — Cleavage

Dihydroxyacetone phosphate ← → Glyceraldehyde-3-phosphate

Triose phosphate isomerase makes single product

Glyceraldehyde-3-phosphate dehydrogenase

$Pi + NAD^+$
Activates phosphate

$NADH + H^+$

1,3-Bisphosphoglycerate
— ADP

First ATP of glycolysis — Phosphoglycerate kinase → ATP

3-Phospogiycerate

Phosphoglyceromutase — Moves phosphate

2-phospoglycerate

Enolase — Activates phosphate

Phosphoenolpyruvate
— ADP

Second ATP of glycolysis — Pyruvate kinase → ATP   NADH   NAD

CO_2 ← Pyruvate → Lactate
aerobic metabolism   Lactate dehydrogenase anaerobic metabolism

Fig. 3.1

Energy yield from glycolysis

Aerobic conditions

Number of ATPs gained per glucose molecule is 8 ATPs

Anaerobic conditions

Number of ATPs gained per glucose molecule is 2 ATPs

MODULE

Biochemistry

3.3 CITRIC ACID CYCLE: (KREB'S CYCLE)

Under aerobic conditions the end product of glycolysis is pyruvic acid. The next step is the formation of acetyl coenzyme A (acetyl CoA) - this step is technically not a part of the citric acid cycle, but is shown on the diagram on the top left.

Acetyl CoA, whether from glycolysis or the fatty acid spiral, is the initiator of the citric acid cycle. In carbohydrate metabolism, acetyl CoA is the link between glycolysis and the citric acid cycle. The initiating step of the citric acid cycle occurs when a four carbon compound (oxaloacetic acid) condenses with acetyl CoA (2 carbons) to form citric acid (6 carbons).

The whole purpose of a "turn" of the citric acid cycle is to produce two carbon dioxide molecules. This general oxidation reaction is accompanied by the loss of hydrogen and electrons at four specific places. These oxidations are connected to the electron transport chain where many ATP are produced.

Notes

Step 1

The acetic acid subunit of acetyl CoA is combined with oxaloacetate to form a molecule of *citrate*. The acetyl coenzyme A acts only as a transporter of acetic acid from one enzyme to another. After Step 1, the coenzyme is released by hydrolysis so that it may combine with another acetic acid molecule to begin the Krebs cycle again.

Step 2

The citric acid molecule undergoes an isomerization. A hydroxyl group and a hydrogen molecule are removed from the citrate structure in the form of water. The two carbons form a double bond until the water molecule is added back. Only now, the hydroxyl group and hydrogen molecule are reversed with respect to the original structure of the citrate molecule. Thus, *isocitrate* is formed.

Step 3

In this step, the isocitrate molecule is oxidized by a NAD molecule. The NAD molecule is reduced by the hydrogen atom and the hydroxyl group. The NAD binds with a hydrogen atom and carries off the other hydrogen atom leaving a carbonyl group. This structure is very unstable, so a molecule of CO_2 is released creating *alpha-ketoglutarate*.

Step 4

In this step, coenzyme A, returns to oxidize the alpha-ketoglutarate molecule. A molecule of NAD is reduced again to form NADH and leaves with another hydrogen. This instability causes a carbonyl group to be released as carbon

dioxide and a thioester bond is formed in its place between the former alpha-ketoglutarate and coenzyme A to create a molecule of *succinyl-coenzyme A* complex.

Step 5

A water molecule sheds its hydrogen atoms to coenzyme A. Then, a free-floating phosphate group displaces coenzyme A and forms a bond with the succinyl complex. The phosphate is then transferred to a molecule of GDP to produce an energy molecule of GTP. It leaves behind a molecule of *succinate*.

Step 6

In this step, succinate is oxidized by a molecule of FAD (Flavin adenine dinucleotide). The FAD removes two hydrogen atoms from the succinate and forces a double bond to form between the two carbon atoms, thus creating *fumarate*.

Step 7

An enzyme adds water to the fumarate molecule to form *malate*. The malate is created by adding one hydrogen atom to a carbon atom and then adding a hydroxyl group to a carbon next to a terminal carbonyl group.

Step 8

In this final step, the malate molecule is oxidized by a NAD molecule. The carbon that carried the hydroxyl group is now converted into a carbonyl group. The end product is *oxaloacetate* which can then combine with acetyl-coenzyme A and begin the Krebs cycle all over again.

Summary of Krebs Cycle

In summary, three major events occur during the Krebs cycle. One GTP (guanosine triphosphate) is produced which eventually donates a phosphate group to ADP to form one ATP; three molecules of NAD are reduced; and one molecule of FAD is reduced. Although one molecule of GTP leads to the production of one ATP, the production of the reduced NAD and FAD are far more significant in the cell's energy-generating process. This is because NADH and $FADH_2$ donate their electrons to an electron transport system that generates large amounts of energy by forming many molecules of ATP.

Yield of ATP

At this point the yield of ATP is 4 moles per mole of Glucose as it passes through the Krebs cycle.

- This is not much more than the 2 moles which would have been produced from glycolysis.

- However, NADH and FADH2 are energy rich molecules

- Their oxidation is highly exergonic and is coupled with the production of ATP from ADP

- Oxidation of 1 mole NADH produces 3 moles ATP

- Oxidation of 1 mole FADH2 produces 2 moles ATP

- Thus total ATP yield = $(10 \times 3) + (2 \times 2) + 4 = 38$ moles ATP per mole Glucose
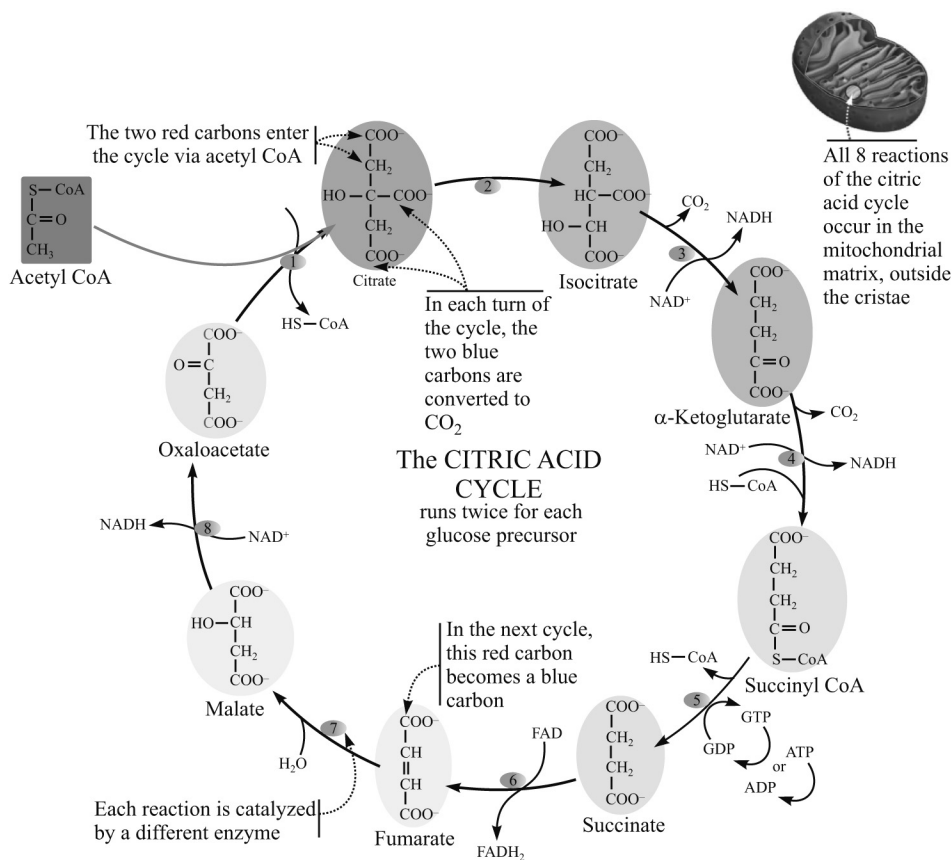
Notes

Fig. 3.2: Glycogenesis and Glycogenolysis

Biosynthesis of Glycogen

The goal of glycolysis, glycogenolysis, and the citric acid cycle is to conserve energy as ATP from the catabolism of carbohydrates. If the cells have sufficient supplies of ATP, then these pathways and cycles are inhibited. Under these conditions of excess ATP, the liver will attempt to convert a variety of excess molecules into glucose and/or glycogen.

Glycogenesis

Glycogenesis is the formation of glycogen from glucose. Glycogen is synthesized depending on the demand for glucose and ATP (energy). If both are present in relatively high amounts, then the excess of insulin promotes the glucose conversion into glycogen for storage in liver and muscle cells.

In the synthesis of glycogen, one ATP is required per glucose incorporated into the polymeric branched structure of glycogen. actually, glucose-6-phosphate is the cross-roads compound. Glucose-6-phosphate is synthesized directly from glucose or as the end product of gluconeogenesis.
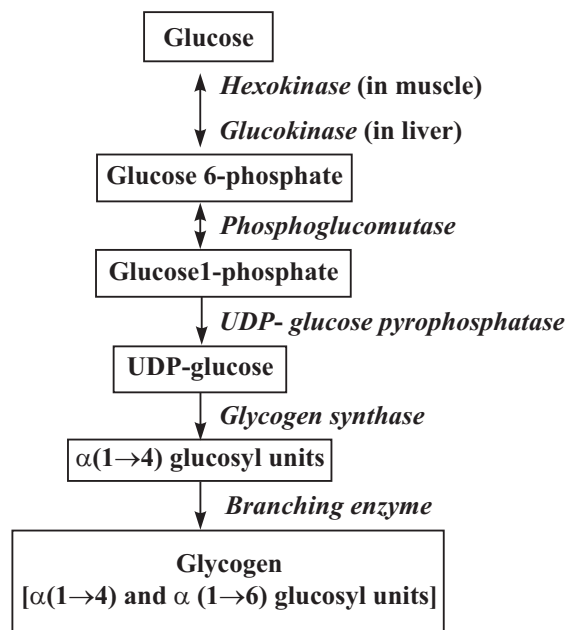
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                    ┌─────────────┐
                    │   Glucose   │
                    └─────────────┘
                       ↕  Hexokinase (in muscle)
                          Glucokinase (in liver)
              ┌───────────────────────┐
              │ Glucose 6-phosphate   │
              └───────────────────────┘
                       ↕  Phosphoglucomutase
              ┌───────────────────────┐
              │  Glucose1-phosphate   │
              └───────────────────────┘
                       ↓  UDP- glucose pyrophosphatase
                    ┌─────────────┐
                    │ UDP-glucose │
                    └─────────────┘
                       ↓  Glycogen synthase
              ┌───────────────────────┐
              │ α(1→4) glucosyl units │
              └───────────────────────┘
                       ↓  Branching enzyme
        ┌───────────────────────────────────┐
        │             Glycogen              │
        │ [α(1→4) and α (1→6) glucosyl units]│
        └───────────────────────────────────┘
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Fig. 3.3: Steps of glycogensis

Glycogenolysis

In glycogenolysis, glycogen stored in the liver and muscles, is converted first to glucose-1- phosphate and then into glucose-6-phosphate. Two hormones which control glycogenolysis are a peptide, glucagon from the pancreas and epinephrine from the adrenal glands.

Glucagon is released from the pancreas in response to low blood glucose and epinephrine is released in response to a threat or stress. Both hormones act upon enzymes to stimulate glycogen phosphorylase to begin glycogenolysis and inhibit glycogen synthetase (to stop glycogenesis).
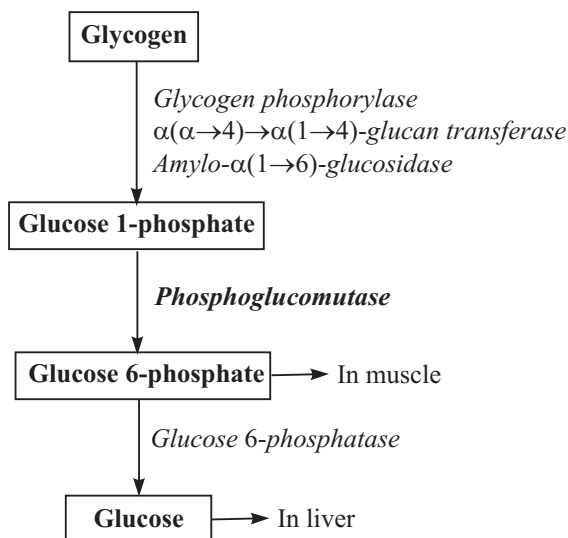
Glycogen is a highly branched polymeric structure containing glucose as the basic monomer. First individual glucose molecules are hydrolyzed from the

chain, followed by the addition of a phosphate group at C-1. In the next step the phosphate is moved to the C-6 position to give glucose 6-phosphate, a cross road compound.

Glucose-6-phosphate is the first step of the glycolysis pathway if glycogen is the carbohydrate source and further energy is needed. If energy is not immediately needed, the glucose-6-phosphate is converted to glucose for distribution in the blood to various cells such as brain cells.

Glycogen

Glycogen phosphorylase
α(α→4)→α(1→4)-*glucan transferase*
Amylo-α(1→6)-*glucosidase*

Glucose 1-phosphate

Phosphoglucomutase

Glucose 6-phosphate	⟶ In muscle

Glucose 6-*phosphatase*

Glucose	⟶ In liver

Fig. 3.4: Steps of glycogenolysis

Gluconeogenesis

Gluconeogenesis is a metabolic pathway that results in the generation of glucose from non-carbohydrate carbon substrates such as pyruvate, lactate, glycerol, and glucogenic amino acids.

The vast majority of gluconeogenesis takes place in the liver and, to a smaller extent, in the cortex of kidneys. This process occurs during periods of fasting, starvation, or intense exercise and is highly endergonic. Gluconeogenesis is often associated with ketosis.

Entering the pathway

Several non-carbohydrate carbon substrates can enter the gluconeogenesis pathway. One common substrate is lactic acid, formed during anaerobic respiration in skeletal muscle. Lactate is transported back to the liver where it is converted into pyruvate by the Cori cycle using the enzyme lactate

dehydrogenase. Pyruvate, the first designated substrate of the gluconeogenic pathway, can then be used to generate glucose. All citric acid cycle intermediates, through conversion to oxaloacetate, amino acids other than lysine or leucine, and glycerol can also function as substrates for gluconeogenesis. Amino acids must have their amino group removed by transamination or deamination before entering the cycle directly (as pyruvate or oxaloacetate), or indirectly via the citric acid cycle.
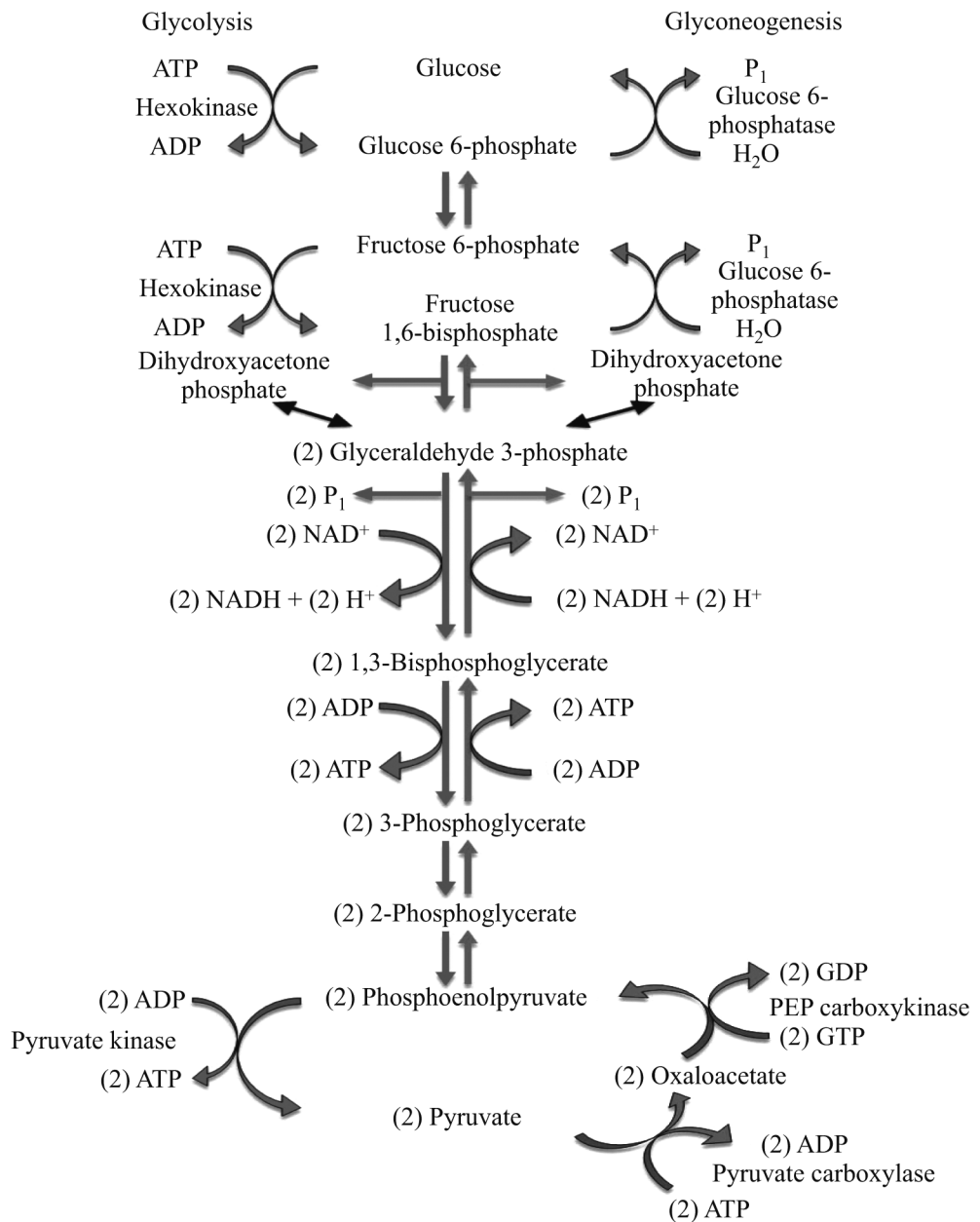

Fig. 3.5

Carbohydrate Metabolism

Fatty acids cannot be converted into glucose in animals, the exception being odd-chain fatty acids which yield propionyl CoA, a precursor forsuccinyl CoA. In plants, specifically in seedlings, the glyoxylate cycle can be used to convert fatty acids (acetate) into the primary carbon source of the organism. The glyoxylate cycle produces four-carbon dicarboxylic acids that can enter gluconeogenesis. Glycerol, which is a part of alltriacylglycerols, can also be used in gluconeogenesis. In organisms in which glycerol is derived from glucose (e.g., humans and other mammals), glycerol is sometimes not considered a true gluconeogenic substrate, as it cannot be used to generate new glucose.

Gluconeogenesis is a pathway consisting of eleven enzyme-catalyzed reactions. The pathway can begin in the mitochondria or cytoplasm, depending on the substrate being used. Many of the reactions are reversible steps found in glycolysis.

Gluconeogenesis begins in the mitochondria with the formation of oxaloacetate through carboxylation of pyruvate at the expense of one molecule of ATP. This reaction is catalyzed by pyruvate carboxylase, which is stimulated by high levels of acetyl-CoA(when fatty acid oxidation is high in the liver) and inhibited by high levels of ADP.

Oxaloacetate must then be reduced into malate using NADH in order to be transported out of the mitochondria.

In the cytoplasm, malate is oxidized to oxaloacetate using NAD+, where the remaining steps of gluconeogenesis occur. Oxaloacetate is then decarboxylated and phosphorylated to produce phosphoenolpyruvate by phosphoenolpyruvate carboxykinase. One molecule of GTP is hydrolyzed to GDP in the course of this reaction.

The next steps in the reaction are the same as reversed glycolysis. However, fructose-1,6-bisphosphatase converts fructose-1,6-bisphosphate to fructose-6-phosphate. The purpose of this reaction is to overcome the large negative ΔG.

Glucose-6-phosphate is formed from fructose-6-phosphate by phospho gluco-isomerase. Glucose-6-phosphate can then be used for glucose generation or in other metabolic pathways. Free glucose is not generated automatically because glucose, unlike glucose-6-phosphate, tends to freely diffuse out of the cell.

The final reaction of gluconeogenesis, the formation of glucose, is carried out in the lumen of the endoplasmic reticulum. Glucose-6-phosphate is hydrolyzed by glucose-6-phosphatase to produce glucose. Glucose is then shuttled into the cytosol by glucose transporters located in the membrane of the endoplasmic reticulum.

Notes

Notes

Regulation

While most steps in gluconeogenesis are the reverse of those found inglycolysis, three regulated and strongly exergonic reactions are replaced with more kinetically favorable reactions. Hexokinase/glucokinase, phosphofructokinase, and pyruvate kinase enzymes of glycolysis are replaced with glucose-6-phosphatase, fructose-1,6-bisphosphatase, and PEP carboxykinase. This system of reciprocal control allow glycolysis and gluconeogenesis to inhibit each other and prevent the formation of afutile cycle.

The majority of the enzymes responsible for gluconeogenesis are found in the cytoplasm; the exceptions are mitochondrial pyruvate carboxylase, and, in animals, phosphoenol-pyruvate carboxykinase. The latter exists as an isozyme located in both the mitochondrion and the cytosol. As there is no known mechanism to transport phosphoenolpyruvate from the mitochondrion into the cytosol, the cytosolic enzyme is believed to be the isozyme important for gluconeogenesis. The rate of gluconeogenesis is ultimately controlled by the action of a key enzyme, fructose-1,6-bisphosphatase, which is also regulated through signal ransduction bycAMP and its phosphorylation.

Most factors that regulate the activity of the gluconeogenesis pathway do so by inhibiting the activity or expression of key enzymes. However, both acetyl CoA and citrate activate gluconeogenesis enzymes (pyruvate carboxylase and fructose-1,6-bisphosphatase, respectively). Due to the reciprocal control of the cycle, acetyl-CoA and citrate also have inhibitory roles in the activity of pyruvate kinase.

Insulin and Glucagon: Control of Blood Glucose

One of the most important and tightly regulated responses in the human body is the concentration of blood glucose (blood sugar). Glucose is the major breakdown product of cellular metabolism. As such, it is required both as an energy source and as a source of carbon for making organic molecules. Blood glucose concentrations are regulated by negative feedback pathways that are modulated by two separate hormones: insulin and glucagon. Both of these hormones are produced in special cells called islet cells, or islets of Langerhans – are found in clusters throughout the pancreas. Islet cells make up a very small percentage of the pancreas (about 1-2%); the remainder of the organ is an exocrine gland producing digestive enzymes and bicarbonate ion. This tiny number of endocrine cells is exceedingly important. Each islet contains two kinds of cells: alpha cells, which produce glucagon, and beta cells, which produce insulin.

Insulin vs. Glucagon

Normally, blood glucose concentrations in human blood should range between 70-110 milligrams (mg/ml). Insulin and glucagon operate in an antagonistic (opposing) manner. The result is a precise control of blood glucose levels within this range.

The insulin pathway is activated when blood glucose levels are too high. High blood glucose levels (e.g., occurring after the stomach has digested a food high in sugar) stimulate beta cells in the pancreas to release insulin. Insulin causes an increased uptake of glucose from the blood; promotes conversion of glucose into triglycerides in the liver, fat and muscle cells; and increases the cellular rate of glycolysis – breaking glucose into smaller components that can be used for synthesis of other compounds.

The glucagon pathway is activated when blood glucose levels are too low. Low blood glucose levels (e.g., due to exercise combined with not eating for several hours) stimulate the alpha cells in the pancreas to produce glucagon. Glucagon causes the liver to convert stored glycogen into glucose, then release the glucose into the blood (a process called glycogenolysis). The two hormones, insulin and glucagon, each regulate the other. A decrease in insulin (as well as low glucose levels) stimulates the secretion of glucagon, while an increase in insulin (as well as an increase in blood glucose) suppresses glucagon secretion. This results in a continuous cycle, with insulin and glucagon constantly monitoring blood glucose levels and regulating their secretion to maintain these levels as nearly constant as possible.

The main function of insulin is removal of excess blood glucose. Because all cells use glucose as an energy source and as a raw material for making other organic compounds, all cells except brain cells are targets for insulin. Since the function of glucagon is opposite that of insulin, it stimulates the addition of glucose to the bloodstream. Thus, it targets cells with high concentrations of energy stored as glycogen, including the liver and skeletal muscles. It also stimulates glucose production from fats, so adipose tissue cells are another target of glucagon.

Lactose intolerance

Lactose intolerance is a common digestive problem where the body is unable to digest lactose, a type of sugar mainly found in milk and dairy products. The body digests lactose by using an enzyme called lactase to break down lactose into two simpler sugars called glucose and galactose, which can then be easily absorbed into the bloodstream. Enzymes are proteins that cause chemical reactions to occur.

In cases of lactose intolerance, the body does not produce enough of the lactase enzyme so lactose stays in the digestive system, where it is fermented by bacteria (in the same way that yeast is fermented to produce beer). It's this fermentation process that causes the symptoms associated with lactose intolerance.

Levels of lactase often fall as people grow older and some health conditions can also reduce the production of lactase.

Symptoms of lactose intolerance include

● a bloated stomach

● flatulence (wind)

● diarrhoea

Treating lactose intolerance

Limiting intake of food and drink containing lactose is the main treatment for lactose intolerance.

Depending on a person's levels of intolerance, they may also require additional calcium and vitamin D supplements to keep the bones strong and healthy.

Advice from a dietitian may sometimes be helpful in determining the best diet for a person.

Lactase substitutes are also available. These are drops that you can add to your meals or drinks to improve your digestion of lactose.

Diabetes Mellitus

Diabetes mellitus (often referred to simply as *diabetes*) is a group of metabolic diseases characterized by high blood glucose levels. The term comes from two Greek words: "diabetes" comes from a verb that means "to pass through" and refers to the frequent, copious urination that is a characteristic of the disease; the word "meli" is Greek for "honey" so the term "mellitus" refers to the presence of high levels of glucose (sugar) in the blood. In addition to urination, other classic symptoms of diabetes are increased thirst and hunger. The diabetic's blood contains more glucose than can be taken up by the cells so this excess glusose is therefore released in the urine (a diagnostic characteristic of diabetes is sugar in the urine). The presence of sugar results in more water being drawn into the urine to balance the osmotic pressure, leading to copious urination.

INTEXT QUESTIONS 3.1

1. In glycolysis pathway glucose is aerobically converted to .... and anaerobically ....

2. All the reaction steps take place in ....

3. Glycolysis is the only source of energy in .... cells

4. Number of ATPs gained per glucose molecule in Aerobic conditions of glycolysis is ....

5. Number of ATPs gained per glucose molecule in Anaerobic conditions of glycolysis is ....

6. Krebs Cycle yield .... ATP per mole Glucose

Notes

WHAT HAVE YOU LEARNT

- In glycolysis pathway glucose is converted to pyruvate in aerobic condition or lactate in anaerobic condition. All the reaction steps take place in the cytoplasm.

- Glycolysis is the only pathway that is taking place in all the cells of the body and is the only source of energy in erythrocytes.

- In Glycolyis, Aerobic conditions yields 8 ATPs and in Anaerobic conditions yields 2 ATPs per glucose molecule

- Citric acid cycle produces two carbon dioxide molecules. And oxidations are connected to the electron transport chain where many ATP are produced.

- A total of 38 moles ATP per mole Glucose is yielded in Krebs Cycle

- Glycogenesis is the formation of glycogen from glucose. Glycogen is synthesized depending on the demand for glucose and ATP (energy).

- In glycogenolysis, glycogen stored in the liver and muscles, is converted first to glucose-1- phosphate and then into glucose-6-phosphate.

- Two hormones which control glycogenolysis are a peptide, glucagon from the pancreas and epinephrine from the adrenal glands.

- Gluconeogenesis is a metabolic pathway that results in the generation of glucose from non-carbohydrate carbon substrates such as pyruvate, lactate, glycerol, and glucogenic amino acids.

- Blood glucose concentrations in human blood should range between 70-110 milligrams per milliliter (mg/mL). Insulin and glucagon operate in an antagonistic (opposing) manner.

Notes

TERMINAL QUESTIONS

1. Explain glycolysis
2. Explain krebs cycle
3. Explain glycogenesis
4. What is the hormone control of blood sugar

ANSWERS TO INTEXT QUESTIONS

3.1

1. Pyruvate & Lactate
2. Cytoplasm
3. Erythrocytes
4. 8 ATPs
5. 2 ATPs
6. 38 moles