



Introduction to Metabolism



- ✦ In cells, enzymatic reactions are organized into multistep sequences called pathways,
- ✦ Glycolysis, gluconeogenesis, etc.
- ✦ In a pathway: the product of one reaction serves as the substrate of the subsequent reaction.

Introduction to Metabolism

- ✦ **Metabolism**: the sum of all the chemical changes occurring in a cell, a tissue, or the body.
- ✦ Most pathways can be classified as either **catabolic** (degradative) or **anabolic** (synthetic).
- ✦ Catabolic reactions break down complex molecules, such as proteins, polysaccharides, and lipids, to a few simple molecules, for example, CO_2 , NH_3 (ammonia), and water.
- ✦ Anabolic pathways form complex end products from simple precursors, for example, the synthesis of the polysaccharide, glycogen, from glucose

Metabolic Map



- ✦ Each metabolic pathway is composed of **multienzyme sequences**, and each enzyme, in turn, may exhibit important **catalytic** or **regulatory** features.

Catabolic Pathways




- ✦ Catabolic reactions serve to capture chemical energy in the form of adenosine triphosphate (ATP) from the degradation of energy-rich fuel molecules.
- ✦ Catabolism also allows molecules in the diet (or nutrient molecules stored in cells) to be converted into building blocks needed for the synthesis of complex molecules.

Anabolic pathways



- ✦ Anabolic reactions combine small molecules, such as amino acids, to form complex molecules, such as proteins
- ✦ Require energy (endergonic), which is generally provided by the breakdown of ATP to adenosine diphosphate (ADP) and inorganic phosphate (Pi).
- ✦ Anabolic reactions often involve chemical reductions in which the reducing power is most frequently provided by the electron donor NADPH.

- 
- ✦ Catabolism is a convergent process – that is, a wide variety of molecules are transformed into a few common end products.
 - ✦ By contrast, anabolism is a divergent process in which a few biosynthetic precursors form a wide variety of polymeric or complex products.

GLYCOLYSIS



- ✦ The glycolytic pathway is employed by all tissues for the breakdown of glucose to provide energy (in the form of ATP) and intermediates for other metabolic pathways.
- ✦ Glycolysis is at the hub of carbohydrate metabolism because virtually all sugars – whether arising from the diet or from catabolic reactions in the body can ultimately be converted to glucose.

Glucose Transport into Cells




- ✦ Glucose cannot diffuse directly into cells, but enters by one of two transport mechanisms:
 1. Na^+ -independent, **facilitated diffusion transport system**
 2. **Na^+ -monosaccharide cotransporter system.**

1. Na⁺-independent facilitated diffusion transport



- ✦ This system is mediated by a family of 14 glucose transporters in cell membranes.
- ✦ They are designated GLUT-1 to GLUT-14 (glucose transporter isoforms 1-14).

- 
- ✦ These transporters exist in the membrane in two conformational states.
 - ✦ Extracellular glucose binds to the transporter, which then alters its conformation, transporting glucose across the cell membrane.

Glut proteins are tissue specific !!

- ✦ The glucose transporters display a tissue-specific pattern of expression. For example,
- ✦ GLUT-1 is abundant in erythrocytes and blood brain barrier, but is low in adult muscle,
- ✦ GLUT-2 is present in liver and kidney (GLUT-2 is also found in pancreatic β cells)
- ✦ GLUT-3 is the primary glucose transporter in neurons.
- ✦ GLUT-4 is abundant in adipose tissue and skeletal muscle.

Glut proteins have specialized functions !!!



- ✦ In facilitated diffusion, glucose movement follows a concentration gradient, that is, from a high glucose concentration to a lower one.
- ✦ For example, GLUT-1, GLUT-3, and GLUT-4 are primarily involved in glucose uptake from the blood.
- ✦ In contrast, GLUT-2, which is found in the liver and kidney, can either transport glucose into these cells when blood glucose levels are high, or transport glucose from these cells when blood glucose levels are low (for example, during fasting).
- ✦ GLUT-5 is unusual in that it is the primary transporter for fructose (instead of glucose) in the small intestine and the testes.

2. Na⁺-monosaccharide cotransporter system




- ✦ This is an energy-requiring process that transports glucose “against” a concentration gradient – that is, from low glucose concentrations outside the cell to higher concentrations within the cell.
- ✦ This system is a carrier-mediated process in which the movement of glucose is coupled to the concentration gradient of Na⁺, which is transported into the cell at the same time.
- ✦ The carrier is a sodium-dependent-glucose transporter or SGLT.
- ✦ This type of transport occurs in the epithelial cells of the intestine and renal tubules

Reactions of Glycolysis



- ✦ The conversion of glucose to pyruvate occurs in **two** stages.

Reactions of Glycolysis



- ✦ The first five reactions of glycolysis correspond to an **energy investment phase** in which the phosphorylated forms of intermediates are synthesized at the expense of ATP.
- ✦ The subsequent reactions of glycolysis constitute an energy generation phase in which a net of two molecules of ATP are formed by substrate-level phosphorylation per glucose molecule metabolized.

Energy Investment Phases



A. Phosphorylation of glucose



- ✦ Phosphorylated sugar molecules can not penetrate cell membranes.

Why ??

- ✦ Because there are no specific transmembrane carriers for these compounds,
- ✦ Because they are too polar to diffuse through the lipid core of membranes.

A. Phosphorylation of glucose



- ✦ The **irreversible** phosphorylation of glucose, therefore, effectively traps the sugar as cytosolic glucose 6-phosphate, thus committing it to further metabolism in the cell.
- ✦ Mammals have several isozymes of the enzyme hexokinase that catalyze the phosphorylation of glucose to glucose 6-phosphate.

Hexokinase and Glucokinase



1. **Hexokinase:** In most tissues, the phosphorylation of glucose is catalyzed by hexokinase, one of three regulatory enzymes of glycolysis (others phosphofructokinase and pyruvate kinase).
 - ✓ Hexokinase has broad substrate specificity and is able to phosphorylate several hexoses in addition to glucose.
 - ✓ Hexokinase is inhibited by the reaction product, glucose 6-phosphate, which accumulates when further metabolism of this hexose phosphate is reduced.
 - ✓ Hexokinase has a low K_m (and, therefore, a high affinity) for glucose. This permits the efficient phosphorylation and subsequent metabolism of glucose even when tissue concentrations of glucose are low.
 - ✓ Hexokinase, however, has a low V_{max} for glucose and, therefore, cannot sequester (trap) cellular phosphate in the form of phosphorylated hexoses, or phosphorylate more sugars than the cell can use.

Hexokinase and Glucokinase



2. Glucokinase: In liver parenchymal cells and β cells of the pancreas, glucokinase is the predominant enzyme responsible for the phosphorylation of glucose.

- ✓ In β cells, glucokinase functions as the glucose sensor, determining the threshold for insulin secretion.
- ✓ In the liver, the enzyme facilitates glucose phosphorylation during hyperglycemia.
- ✓ Despite the popular but misleading name glucokinase, the sugar specificity of the enzyme is similar to that of other hexokinase isozymes.

Glucokinase differs from hexokinase in several important properties !!!




For example, it has a much higher K_m , requiring a higher glucose concentration for half-saturation.


Thus, glucokinase functions only when the intracellular concentration of glucose in the hepatocyte is elevated, such as during the brief period following consumption of a carbohydrate-rich meal, when high levels of glucose are delivered to the liver via the portal vein.

Glucokinase has a high V_{max} , allowing the liver to effectively remove the flood of glucose delivered by the portal blood.

This prevents large amounts of glucose from entering the systemic circulation following a carbohydrate-rich meal, and thus minimizes hyperglycemia during the absorptive period.

- 
- ✦ Glucokinase activity is not directly inhibited by glucose 6-phosphate as are the other hexokinases, but rather is indirectly inhibited by fructose 6-phosphate (which is in equilibrium with glucose 6-phosphate, a product of glucokinase), and is indirectly stimulated by glucose (a substrate of glucokinase) via the following mechanism.
 - ✦ Glucokinase regulatory protein (GKRP) in the liver regulates the activity of glucokinase through reversible binding.

B. Isomerization of glucose 6-phosphate



- ✦ The isomerization of glucose 6-phosphate to fructose 6-phosphate is catalyzed by phosphoglucose isomerase.
- ✦ The reaction is readily reversible and is not a rate-limiting or regulated step.

C. Phosphorylation of fructose 6-phosphate

- ✦ The irreversible phosphorylation reaction catalyzed by phospho-fructokinase-1 (PFK-1) is the most important control point and the rate-limiting and committed step of glycolysis.
- ✦ PFK-1 is controlled by the available concentrations of the substrates ATP and fructose 6-phosphate.

D. Cleavage of fructose 1,6-bisphosphate



- ✦ **Aldolase** cleaves fructose 1,6-bisphosphate to
 - dihydroxyacetone phosphate
 - glyceraldehyde 3-phosphate.
- ✦ The reaction is reversible and not regulated.

E. Isomerization of dihydroxyacetone phosphate



- ✦ Triose phosphate isomerase interconverts dihydroxyacetone phosphate and glyceraldehyde 3-phosphate.
- ✦ Dihydroxyacetone phosphate must be isomerized to glyceraldehyde 3-phosphate for further metabolism by the glycolytic pathway.
- ✦ This isomerization results in the net production of two molecules of glyceraldehyde 3-phosphate from the cleavage products of fructose 1,6- biphosphate.


Energy Generating Phases



F. Oxidation of glyceraldehyde 3-phosphate

- ✦ The conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate by glyceraldehyde 3-phosphate dehydrogenase is the **first oxidation-reduction reaction** of glycolysis
- ✦ Because there is only a limited amount of NAD^+ in the cell, the NADH formed by this reaction must be **reoxidized** to NAD^+ for glycolysis to continue.
- ✦ Two major mechanisms for oxidizing NADH are:
 - 1) the NADH-linked conversion of pyruvate to lactate (anaerobic),
 - 2) oxidation of NADH via the respiratory chain (aerobic). T

1. Synthesis of 1,3-bisphosphoglycerate (1,3-BPG):

- 
- ✦ The oxidation of the aldehyde group of glyceraldehyde 3-phosphate to a carboxyl group is **coupled to the attachment of Pi to the carboxyl group**.
 - ✦ The high-energy phosphate group at carbon 1 of 1,3-BPG conserves much of the free energy produced by the oxidation of glyceraldehyde 3-phosphate.
 - ✦ The energy of this high-energy phosphate drives the synthesis of ATP in the next reaction of glycolysis.

Synthesis of 2,3-bisphosphoglycerate (2,3-BPG) in red blood cells:

- ✦ Some of the 1,3-BPG is converted to 2,3-BPG by the action of **bisphosphoglycerate mutase**.
- ✦ 2,3-BPG, which is found in only trace amounts in most cells, is present at high concentration in red blood cells (increases O₂ delivery).
- ✦ 2,3-BPG is hydrolyzed by a **phosphatase** to 3-phosphoglycerate, which is also an intermediate in glycolysis.
- ✦ In the red blood cell, glycolysis is modified by inclusion of these “shunt” reactions.

G. Synthesis of 3-phosphoglycerate producing ATP

- ✦ When 1,3-BPG is converted to 3-phosphoglycerate, the high-energy phosphate group of 1,3-BPG is used to synthesize ATP from ADP.
- ✦ This reaction is catalyzed by **phosphoglycerate kinase**, which, unlike most other kinases, is physiologically reversible.
- ✦ Because two molecules of 1,3-BPG are formed from each glucose molecule, this kinase reaction replaces the two ATP molecules consumed by the earlier formation of glucose 6-phosphate and fructose 1,6-bisphosphate.
- ✦ This is an example of **substrate-level phosphorylation**, in which the energy needed for the production of a high-energy phosphate comes from a substrate rather than from the electron transport chain.

H. Shift of the phosphate group from carbon 3 to carbon 2



- ✦ The shift of the phosphate group from carbon 3 to carbon 2 of phosphoglycerate by **phosphoglycerate mutase** is freely reversible.

I. Dehydration of 2-phosphoglycerate



- ✦ The dehydration of 2-phosphoglycerate by **enolase** redistributes the energy within the 2-phosphoglycerate molecule, resulting in the formation of phosphoenolpyruvate (PEP), which contains a high-energy enol phosphate.
- ✦ The reaction is reversible despite the high-energy nature of the product.

J. Formation of pyruvate producing ATP



- ✦ The conversion of PEP to pyruvate is catalyzed by **pyruvate kinase**, the third irreversible reaction of glycolysis.
- ✦ The equilibrium of the pyruvate kinase reaction favors the formation of ATP.
- ✦ This is another example of **substrate-level phosphorylation**.

K. Reduction of pyruvate to lactate



- ✦ Lactate, formed by the action of **lactate dehydrogenase**, is the final product of **anaerobic glycolysis** in eukaryotic cells.
- ✦ The formation of lactate is the major fate for pyruvate in lens and cornea of the eye, kidney medulla, testes, leukocytes and red blood cells, because these are all poorly vascularized and/or lack mitochondria.

1. Lactate formation in muscle:

- ✦ In exercising skeletal muscle, NADH production (by glyceraldehyde 3-phosphate dehydrogenase and by the three NAD⁺-linked dehydrogenases of the citric acid cycle) exceeds the oxidative capacity of the respiratory chain.
- ✦ This results in an elevated NADH/NAD⁺ ratio, favoring reduction of pyruvate to lactate.
- ✦ Therefore, during intense exercise, lactate accumulates in muscle, causing a drop in the intracellular pH, potentially resulting in cramps.
- ✦ Much of this lactate eventually diffuses into the bloodstream, and can be used by the liver to make glucose.

2. Lactate consumption:

- ✦ The direction of the lactate dehydrogenase reaction depends on the relative intracellular concentrations of pyruvate and lactate, and on the ratio of NADH/NAD^+ in the cell.
- ✦ For example, in liver and heart, the ratio of NADH/NAD^+ is lower than in exercising muscle. These tissues oxidize lactate (obtained from the blood) to pyruvate.
- ✦ In the liver, pyruvate is either converted to glucose by gluconeogenesis or oxidized in the TCA cycle.
- ✦ Heart muscle exclusively oxidizes lactate to CO_2 and H_2O via the citric acid cycle.

3. Lactic acidosis:



- ✦ Elevated concentrations of lactate in the plasma, termed lactic acidosis, occur when there is a collapse of the circulatory system, such as in myocardial infarction, pulmonary embolism, and uncontrolled hemorrhage, or when an individual is in shock.
- ✦ The failure to bring adequate amounts of oxygen to the tissues results in impaired oxidative phosphorylation and decreased ATP synthesis.
- ✦ To survive, the cells use anaerobic glycolysis as a backup system for generating ATP, producing lactic acid as the endproduct.

L. Energy yield from glycolysis



- ✦ Despite the production of some ATP during glycolysis, the end products, **pyruvate** or **lactate**, still contain most of the energy originally contained in glucose.
- ✦ The **TCA cycle** is required to release that energy completely

1. Anaerobic glycolysis



- ✦ Two molecules of ATP are generated for each molecule of glucose converted to two molecules of lactate.
- ✦ There is no net production or consumption of NADH.

2. Aerobic glycolysis



- ✦ The direct consumption and formation of ATP is the same as in anaerobic glycolysis – that is, a net gain of two ATP per molecule of glucose.
- ✦ Two molecules of NADH are also produced per molecule of glucose.
- ✦ Ongoing aerobic glycolysis requires the oxidation of most of this **NADH by the electron transport chain**, producing approximately three ATP for each NADH molecule entering the chain.
- ✦ [Note: NADH cannot cross the inner mitochondrial membrane, and substrate shuttles are required]

HORMONAL REGULATION OF GLYCOLYSIS



- ✦ The regulation of glycolysis by allosteric activation or inhibition, or the phosphorylation/dephosphorylation of rate-limiting enzymes, is short-term (?)
- ✦ These effects can result in 10-fold to 20-fold increases in enzyme activity that typically occur over hours to days.

Hormonal Regulation Of Glycolysis

- ✦ Regular consumption of meals **rich in carbohydrate** or administration of **insulin** initiates an increase in the amount of glucokinase, phosphofructokinase, and pyruvate kinase in liver.
- ✦ These changes reflect an increase in gene transcription, resulting in increased enzyme synthesis.
- ✦ High activity of these three enzymes favors the conversion of glucose to pyruvate, a characteristic of the well-fed state.

Hormonal Regulation Of Glycolysis



- ✦ Conversely, gene transcription and synthesis of glucokinase, phosphofructokinase, and pyruvate kinase are decreased when plasma **glucagon** is high and **insulin** is low, for example, as seen in fasting or diabetes.

ALTERNATE FATES OF PYRUVATE



- A. Oxidative decarboxylation of pyruvate
- B. Carboxylation of pyruvate to oxaloacetate
- C. Reduction of pyruvate to ethanol (microorganisms)

A. Oxidative decarboxylation of pyruvate



- ✦ Oxidative decarboxylation of pyruvate by **pyruvate dehydrogenase** complex is an important pathway in tissues with a high oxidative capacity, such as cardiac muscle.
- ✦ Pyruvate dehydrogenase irreversibly converts pyruvate, **the end product of glycolysis**, into **acetyl CoA**, a major fuel for the TCA cycle and the building block for fatty acid synthesis.

B. Carboxylation of pyruvate to oxaloacetate



- ✦ Carboxylation of pyruvate to oxaloacetate (OAA) by **pyruvate carboxylase** is a biotin-dependent reaction.
- ✦ This reaction is important because it replenishes the citric acid cycle intermediates, and provides substrate for gluconeogenesis

C. Reduction of pyruvate to ethanol (microorganisms)

✦ The conversion of pyruvate to ethanol occurs by the two reactions:

1. The decarboxylation of pyruvate by **pyruvate decarboxylase** occurs in yeast and certain other microorganisms, but not in humans.

The enzyme requires thiamine pyrophosphate as a coenzyme, and catalyzes a reaction similar to that described for pyruvate dehydrogenase



**THANKS
FOR
LISTENING,
ANY
QUESTIONS?**

References



- ◆ Biochemistry, Lippincott's Illustrated Reviews, 5th Edition, Richard A. Harvey