Carbohydrates
Metabolism
Digestion of Dietary Carbohydrates

- The principal sites of dietary carbohydrate digestion are the **mouth** and **intestinal lumen**.
- This digestion is rapid and is catalyzed by **glycoside hydrolases (glycosidases)** that hydrolyze glycosidic bonds.
- The enzymes are primarily **endoglycosidases** that hydrolyze polysaccharides and oligosaccharides, and **disaccharidases** that hydrolyse tri- and disaccharides into their reducing sugar components.
- The **final products** of carbohydrate digestion are the **monosaccharides**, glucose, galactose and fructose, which are absorbed by cells of the small intestine.

A. Digestion of carbohydrates begins in the mouth

- **The major dietary** polysaccharides are of **plant** (starch, composed of amylose and amylopectin) and **animal** (glycogen) origin.
- During mastication, **salivary α-amylase** acts briefly on dietary starch and glycogen, hydrolyzing random **α(1→4) bonds**.
- There are both **α(1→4)- and β(1→4)-endoglucosidases** in nature, but **humans do not produce the latter**. Therefore, we are unable to digest cellulose (has **β(1→4) glycosidic bonds** between glucose residues).
- Because branched amylopectin and glycogen also contain **α(1→6) bonds**, which α-amylase cannot hydrolyze, the digest resulting from its action contains a mixture of short, branched and unbranched oligosaccharides known as **dextrins**. Disaccharides are also present as they are also resistant to amylase.

B. In the stomach:

- Carbohydrate digestion halts temporarily, because the high acidity inactivates salivary **α-amylase**.
B. Digestion of carbohydrates by pancreatic enzymes occurs in the small intestine

- When the acidic stomach contents reach the small intestine, they are neutralized by bicarbonate secreted by the pancreas, and pancreatic α-amylase continues the process of starch digestion.

C. Final carbohydrate digestion by enzymes synthesized by the intestinal mucosal cells

- The final digestive processes occur primarily at the mucosal lining of the upper jejunum, and include the action of several disaccharidases:
  - Isomaltase cleaves the α(1→6) bond in isomaltose
  - Maltase cleaves maltose and maltotriose, each producing glucose,
  - Sucrase cleaves sucrose producing glucose and fructose.
  - Lactase (β-galactosidase) cleaves lactose producing galactose and glucose.
- These enzymes are secreted through, and remain associated with, the luminal side of the brush border membranes of the intestinal mucosal cells.

Absorption and Transport of Glucose into Cells

- The duodenum and upper jejunum absorb the bulk of the dietary sugars. However, different sugars have different mechanisms of absorption.

A. Na⁺-monosaccharide Active cotransporter system Transport

- Galactose and glucose are transported into the mucosal cells by an active, energy-requiring process that requires a concurrent uptake of sodium ions; the transport protein is the sodium-dependent glucose cotransporter 1 (SGLT-1).
- Glucose and Na⁺ bind to different sites on the glucose transporter. Na⁺ moves into the cell down its electrochemical gradient and “drags” glucose with it.
- If Na⁺ in extracellular fluid is low, glucose transport stops.
To maintain a steep Na\(^+\) gradient, this Na\(^+\)-glucose symport is dependent on gradients generated by the Na\(^+\)-K\(^+\) pump that maintains a low intracellular Na\(^+\) concentration.

B. 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.
- The glucose transporters display a tissue-specific pattern of expression:
  1. GLUT-1 is abundant in erythrocytes and blood brain barrier
  2. GLUT-3 is the primary transporter in neurons.
  3. GLUT-4 is abundant in adipose tissue and skeletal muscle.

- GLUT-5 is responsible for absorption of Fructose.
- All three monosaccharides are transported from the intestinal mucosal cell into the portal circulation by yet another transporter, GLUT-2.
Introduction to Metabolism

- In cells, individual enzymatic reactions these reactions rarely occur in isolation, but rather are organized into multistep sequences called **pathways** e.g. Glycolysis
- In a pathway, the **product** of one reaction serves as the **substrate** of the subsequent reaction. Different pathways can also intersect, forming an integrated network of chemical reactions. These are collectively called metabolism, which is 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. **Anabolic** pathways form complex end products from simple precursors, e.g. the synthesis of the polysaccharide, glycogen, from glucose.
- **Cycles** are pathways that regenerate a component.

Catabolic pathways

- Catabolic reactions serve to capture chemical energy in the form of 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. Energy generation by degradation of complex molecules occurs in three stages
- Catabolic pathways are typically oxidative, and require coenzymes such as NAD⁺.
Anabolic pathways

- Anabolic reactions combine small molecules, such as amino acids, to form complex molecules, such as proteins.
- Anabolic reactions require energy (are endergonic), which is generally provided by the breakdown of ATP to ADP and 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.

Metabolic map

A metabolic map can provide the “big picture,” containing the important central pathways of energy metabolism. This map is useful in tracing connections between pathways, visualizing the “movement” of metabolic intermediates, and picturing the effect on the flow of intermediates if a pathway is blocked, for example, by a drug or an inherited deficiency of an enzyme.
Important reactions of intermediary metabolism. Several important pathways to be discussed in later chapters are highlighted. Curved reaction arrows (               ) indicate forward and reverse reactions that are catalyzed by different enzymes. The straight arrows (               ) indicate forward and reverse reactions that are catalyzed by the same enzyme. **Blue text** = intermediates of carbohydrate metabolism; **brown text** = intermediates of lipid metabolism; **green text** = intermediates of protein metabolism.
Glycolysis and Oxidation of Pyruvate

- 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.
- **Pyruvate** is the end product of glycolysis in cells with **mitochondria** and an adequate supply of oxygen. This is called **aerobic glycolysis** because oxygen is required to reoxidize the NADH formed during the oxidation of glyceraldehyde 3-phosphate. Aerobic glycolysis sets the stage for the oxidative decarboxylation of pyruvate to acetyl CoA, a major fuel of the TCA (or citric acid) cycle.
- Alternatively, pyruvate is reduced to **lactate** as NADH is oxidized to NAD\(^+\). This is called **anaerobic glycolysis** because it can occur without the participation of oxygen. Anaerobic glycolysis allows the production of ATP in tissues that lack mitochondria (for example, red blood cells) or in cells deprived of sufficient oxygen.

**Reactions of Glycolysis**

All reactions occur in the **cytosol**. Conversion of glucose to pyruvate occurs in **two stages**.

a. The first five reactions correspond to an **energy investment phase** in which the phosphorylated forms of intermediates are synthesized at the expense of ATP.

b. The subsequent reactions constitute an **energy generation phase** in which a net of **two molecules of ATP** are formed by **substrate-level phosphorylation** per glucose molecule metabolized

1. **Phosphorylation of glucose**

   Phosphorylated sugar molecules do not readily penetrate cell membranes, because:
   - There are no transmembrane carriers for these compounds
   - They are too polar to diffuse through the lipid core of membranes.

   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.
The pathway of glycolysis. At an asterisk: Carbon atoms 1–3 of fructose bisphosphate form dihydroxyacetone phosphate, whereas carbons 4–6 form glyceraldehyde 3-phosphate. The term “bis-,” as in bisphosphate, indicates that the phosphate groups are separated, whereas diphosphate, as in adenosine diphosphate, indicates that they are joined.
<table>
<thead>
<tr>
<th></th>
<th>Glucokinase</th>
<th>Hexokinase</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tissue distribution</strong></td>
<td>Only in liver and pancreatic islets</td>
<td>All tissues except liver and pancreatic islets</td>
</tr>
<tr>
<td><strong>Substrate</strong></td>
<td>Glucose only</td>
<td>Glucose, Fructose and Galactose</td>
</tr>
<tr>
<td><strong>Km (affinity)</strong></td>
<td>High (low affinity)</td>
<td>Low (high affinity)</td>
</tr>
<tr>
<td><strong>Effect of G-6-P on enzyme activity</strong></td>
<td>Not inhibited</td>
<td>Inhibited</td>
</tr>
<tr>
<td><strong>Effect of insulin</strong></td>
<td>Induce enzyme synthesis</td>
<td>Not induced</td>
</tr>
<tr>
<td><strong>Effect of diabetes on enzyme level</strong></td>
<td>Decrease enzyme</td>
<td>No change</td>
</tr>
</tbody>
</table>

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

3. **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 step of glycolysis. PFK-1 is controlled by:

i. **Regulation by energy levels within the cell:** PFK-1 is inhibited allosterically by elevated levels of ATP, which act as an “energy-rich” signal indicating an abundance of high-energy compounds. Elevated levels of citrate, an intermediate in the TCA cycle, also inhibit PFK-1. Citrate inhibition favors the use of glucose for glycogen synthesis. Conversely, PFK-1 is activated allosterically by high concentrations of AMP, which signal that the cell’s energy stores are depleted.

ii. **Regulation by fructose 2,6-bisphosphate:** Fructose 2,6-bisphosphate is the most potent activator of PFK-1 and is able to activate the enzyme even when ATP levels are high. Fructose 2,6-bisphosphate is formed by phosphofructokinase-2 (PFK-2), an enzyme different than PFK-1. Fructose 2,6-bisphosphate is an inhibitor of fructose 1,6-bisphosphatase, an enzyme of gluconeogenesis. The reciprocal actions of fructose 2,6-bisphosphate
on glycolysis (activation) and gluconeogenesis (inhibition) ensure that both pathways are not fully active at the same time, preventing a futile cycle in which glucose would be converted to pyruvate followed by resynthesis of glucose from pyruvate.

4. Cleavage of fructose 1,6-bisphosphate
Aldolase A cleaves fructose 1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. The reaction is reversible and not regulated. Aldolase B, the isoform in the liver and kidney, also cleaves fructose 1-phosphate, and functions in the metabolism of dietary fructose.

5. Isomerization of dihydroxyacetone phosphate
Phosphorozise 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-bisphosphate.

6. Oxidation of glyceraldehyde 3-phosphate
The conversion of two molecules glyceraldehyde 3-phosphate to two molecules 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:
- a. NADH-linked conversion of pyruvate to lactate (anaerobic) that generates no ATP
- b. Oxidation of NADH via the respiratory chain (aerobic) that requires substrate shuttles. This generates 2 X 3 ATP
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 and the energy of this high-energy phosphate drives the synthesis of ATP in the next reaction of glycolysis.

6. 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 directly from oxidation of a substrate rather than from oxidative phosphorylation via the electron transport chain.

**7. Shift of the phosphate group from carbon 3 to carbon 2**

This shift is catalyzed by **phosphoglycerate mutase** and is freely reversible.

**8- 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.

**9. Formation of pyruvate producing ATP**

The conversion of PEP to pyruvate is catalyzed by **pyruvate kinase**, the third **irreversible** reaction of glycolysis.

This is a second example of **substrate-level phosphorylation** that results in the formation of two ATP.

**10. 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**.

**A. 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.

- **The significance of pyruvate to lactate conversion under anaerobic conditions:**

  Under aerobic conditions NADH produced by glyceraldehyde 3-phosphate dehydrogenase is oxidized to NAD⁺ by losing its 2H to the respiratory chain. But,
under anaerobic conditions NADH loses its 2H to pyruvate, transforming it to lactate. This permits glycolysis to proceed under anaerobic conditions.

**B. 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. **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. Liver converts pyruvate to glucose by **gluconeogenesis** or oxidizes it in the TCA cycle. Heart muscle exclusively oxidizes pyruvate to CO\(_2\) and H\(_2\)O via the TCA cycle.

**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.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>ATP (~P) formed/mole of glucose</th>
</tr>
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<tbody>
<tr>
<td>1- Hexokinase</td>
<td>-1 ATP</td>
</tr>
<tr>
<td>2- PFK-1</td>
<td>-1 ATP</td>
</tr>
<tr>
<td>3- Glyceraldehyde 3-P dehydrogenase (aerobic)</td>
<td>+ 2 X 3 ATP</td>
</tr>
<tr>
<td>4- Phosphoglycerate kinase (substrate level)</td>
<td>+ 2 X 1 ATP</td>
</tr>
<tr>
<td>5- Pyruvate kinase (substrate level)</td>
<td>+ 2 X 1 ATP</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
</tr>
<tr>
<td>Aerobic</td>
<td>8 ATP</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>2 ATP</td>
</tr>
</tbody>
</table>

**Regulation of glycolysis**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activator</th>
<th>Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexokinase</td>
<td>---------</td>
<td>G 6-P</td>
</tr>
<tr>
<td>Glucokinase</td>
<td>Insulin (inducer)</td>
<td>---------</td>
</tr>
<tr>
<td>PFK-1</td>
<td>AMP, F 2,6-Bisphosphate</td>
<td>High ATP, Citrate</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>Fructose 1,6-Bisphosphate</td>
<td>ATP</td>
</tr>
</tbody>
</table>
Hormonal Regulation of Glycolysis

- The regulation of glycolysis by allosteric activation or inhibition, or the phosphorylation/dephosphorylation of rate-limiting enzymes, is short term—that is, they influence glucose consumption over periods of minutes or hours. Superimposed on these moment-to-moment effects are slower, and often more profound, hormonal influences on the amount of enzyme protein synthesized. These effects can result in 10-20 fold increases in enzyme activity that typically occurs over hours to days. Reciprocal changes occur in the rate-limiting enzymes of gluconeogenesis.

- 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.

- 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

Pyruvate that resulted from aerobic glycolysis in the cytosol moves to mitochondria where it is oxidatively decarboxylated by pyruvate dehydrogenase complex. This decarboxylation of pyruvate 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. 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.