Chapter 13
Carbohydrate Metabolism

Chapter Objectives:
- Learn about Blood glucose.
- Learn about the glycolysis reaction pathways and the regulation of glycolysis.
- Learn about the fates of pyruvate under various conditions.
- Learn about the citric acid cycle.
- Learn about the electron transport chain and oxidative phosphorylation.
- Learn about glycogen metabolism, gluconeogenesis, and the hormonal control of carbohydrate metabolism.

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The Digestion of Carbohydrates
- After digestion is completed, glucose, fructose, and galactose are absorbed into the bloodstream through the lining of the small intestine and transported to the liver.
- In the liver, fructose and galactose are rapidly converted to glucose or to compounds that are metabolized by the same pathway as glucose.

Blood Sugar Levels
- Glucose is the most plentiful monosaccharide in blood. The term blood sugar usually refers to glucose.
- In adults, the normal blood sugar level measured after a fast of 8-12 hours is 70-110 mg/100 mL (in clinical reports the units are in mg/dL).
  - The blood sugar level reaches a maximum of about 140-160 mg/100 mL about 1 hour after a carbohydrate-containing meal, and returns to normal after 2-2.5 hours.
- Hypoglycemia occurs when blood sugar levels are below the normal fasting level.
  - Mild hypoglycemia leads to dizziness and fainting as brain cells are deprived of energy.
  - Severe hypoglycemia can result in convulsions and shock.
Blood Sugar Levels

- **Hyperglycemia** occurs when blood sugar levels are above the normal fasting level.
  - If blood glucose levels are above about 180 mg/100 mL, the sugar is not completely reabsorbed by the kidneys, and glucose is excreted in the urine.
  - The blood glucose level at which this occurs is the **renal threshold**, and the condition when glucose appears in the urine is called **glucosuria**.
  - Prolonged hyperglycemia at a glucosuric level indicates a problem with the body’s normal ability to control blood sugar levels.

Blood Sugar Levels and the Liver

- The liver is the key organ involved in regulating blood glucose levels.
  - When blood glucose levels rise after a meal, the liver removes glucose from the bloodstream, and converts it to glycogens or triglycerides for storage.
  - When blood glucose levels are low, the liver converts stored glycogen to glucose, and synthesizes new glucose from noncarbohydrate sources (**gluconeogenesis**).

Glycolysis

- **Glycolysis** is a series of ten reactions, with a net result of converting a glucose molecule into two molecules of pyruvate.
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**A Summary of the Glycolysis Pathway**

Glycolysis Reactions

- All of the enzymes in the glycolysis pathway are found in cellular cytoplasm.
- The net result of adding all of these reactions together gives the equation:

  \[
  \text{glucose} + 2\text{P}_i + 2\text{ADP} + 2\text{NAD}^+ \rightarrow 2\text{pyruvate} + 2\text{ATP} + 2\text{NADH} + 4\text{H}^+ + 2\text{H}_2\text{O}
  \]
- There is a net gain of 2 moles of ATP for every mole of glucose that is converted to pyruvate.
- Other sugars are also digested in glycolysis:
  - Fructose enters glycolysis as dihydroxyacetone phosphate and glyceraldehyde-3-phosphate.
  - Galactose is isomerized to form glucose-6-phosphate.

The Regulation of Glycolysis

- The glycolysis pathway is regulated by three enzymes:
  - hexokinase — catalyzes the conversion of glucose to glucose-6-phosphate and initiates the glycolysis pathway.
    - The enzyme is inhibited by a high concentration of glucose-6-phosphate (feedback inhibition).
  - phosphofructokinase — catalyzes the irreversible conversion of fructose 6-phosphate to fructose 1,6-bisphosphate
    - As an allosteric enzyme, it is inhibited by high concentrations of ATP and citrate, and activated by high concentrations of ADP and AMP.
  - pyruvate kinase — catalyzes the conversion of 3-phospho-enolpyruvate to pyruvate.
    - This is an allosteric enzyme that is inhibited by high concentrations of ATP.
    - When the glycolysis pathway is operating, so are the citric acid cycle and the electron transport chain, which produce large amounts of ATP.
      - If ATP use decreases, the concentration of ATP increases. The ATP binds to phosphofructokinase and pyruvate kinase, slowing down their activity, and thus slowing the glycolysis pathway.
      - When ATP concentrations are low, ADP and AMP concentrations are high, which activates the phosphofructokinase, accelerating the glycolysis pathway.

**Glycolysis Animation**
The Fates of Pyruvate

• There are three things that can happen to pyruvate after glycolysis:
  – oxidation to acetyl CoA under aerobic conditions
  – reduction to lactate under anaerobic conditions
  – reduction to ethanol under anaerobic conditions

Reduction to Lactate

• Under anaerobic conditions (restricted O₂ supply), such as those that accompany strenuous or long-term muscle activity, the cellular supply of oxygen is not adequate for the reoxidation of NADH to NAD⁺.

• Under these conditions, the cells begin reducing pyruvate to lactate as a means of regenerating NAD⁺:

\[
\text{CH}_3-\text{C} = \text{COO}^- + \text{NADH} + \text{H}^+ \xrightarrow{\text{lactate dehydrogenase}} \text{CH}_3-\text{CH} = \text{COO}^- + \text{NAD}^+
\]

• Adding this equation to the net results of glycolysis produces the equation for lactate fermentation:

\[
\text{C}_6\text{H}_12\text{O}_6 + 2\text{ADP} + 2\text{P} \rightarrow 2\text{CH}_3-\text{CH} = \text{COO}^- + 2\text{ATP}
\]

Oxidation to Acetyl CoA

• Under aerobic conditions (a plentiful supply of oxygen), pyruvate is oxidized in the mitochondria to form acetyl CoA:

\[
\text{CH}_3-\text{C} = \text{COO}^- + \text{CoA} \rightarrow \text{S} + \text{H} + \text{NAD}^+ \xrightarrow{\text{pyruvate dehydrogenase complex}} \text{CH}_3-\text{C} = \text{S} + \text{CoA} + \text{NADH} + \text{CO}_2
\]

• Most of the acetyl CoA formed can enter the citric acid cycle on its way to complete oxidation to CO₂.

• Some acetyl CoA serves as a starting material for fatty acid biosynthesis.

• NAD⁺ is regenerated when NADH transfers its electrons to O₂ in the electron transport chain.
Reduction to Ethanol

- Several organisms, including yeast, regenerate NAD\(^+\) under anaerobic conditions by **alcoholic fermentation**, by decarboxylation (removing CO\(_2\)) of pyruvate to produce acetaldehyde:

  \[
  \text{pyruvate} \rightarrow \text{acetaldehyde} + \text{CO}_2 + \text{H}_2\text{O} + \text{NAD}^+ \rightarrow \text{NADH} + \text{H}^+ \rightarrow \text{ethanol} + \text{NAD}^+ \]

  - The CO\(_2\) thus produced causes beer to foam and wine and champagnes to bubble.

- Acetaldehyde is then reduced by NADH to form ethanol (also regenerating NAD\(^+\) for glycolysis):

  \[
  \text{acetaldehyde} + \text{NADH} + \text{H}^+ \rightarrow \text{ethanol} + \text{NAD}^+ \]

The Citric Acid Cycle

- Stage III in the oxidation of fuel molecules begins when the two-carbon acetyl units of acetyl CoA enter the **citric acid cycle**.

  - The citric acid cycle is also known as the **TCA cycle** (tricarboxylic acid cycle) because of the three carboxylic acid groups in citric acid, and the **Krebs cycle** after Hans A. Krebs, who deduced the reaction sequence in 1937.
The Citric Acid Cycle

- The citric acid cycle is the principal process for generating the reduced coenzymes NADH and FADH$_2$, which are necessary for the reduction of oxygen and ATP synthesis in the electron transport chain.
  - The citric acid cycle also functions as a source of intermediates for biosynthesis of other important molecules (e.g., some amino acids).
- The reactions of the citric acid cycle occur within the matrix of the mitochondria.
- There are eight reactions in the cycle. Of particular importance are the reactions where NADH (Steps 3, 4, and 8) and FADH$_2$ (Step 6) are produced.

A Summary of the Citric Acid Cycle

- The reactions of the citric acid cycle can be added to give the net equation:
  acetyl CoA + 3NAD$^+$ + FAD + GDP + P$_i$ + 2H$_2$O →
  2CO$_2$ + CoA-S-H + 3NADH + 2H$^+$ + FADH$_2$ + GTP

Some important features of the citric acid cycle:
1. Acetyl CoA is the fuel of the citric acid cycle.
2. The operation of the cycle requires a supply of the oxidizing agents NAD$^+$ and FAD from the electron transport chain.
   - Because oxygen is the final acceptor of electrons in the electron transport chain, the continued operation of the cycle depends ultimately on an adequate supply of oxygen.
3. Two carbon atoms enter the cycle as an acetyl unit, and two carbon atoms leave the cycle as two molecules of CO$_2$. However, the carbon atoms leaving the cycle correspond to carbon atoms that entered in the previous cycle; there is a one-cycle delay between the entry of two carbon atoms as an acetyl unit and their release as CO$_2$.
4. In each complete cycle, four oxidation-reduction reactions produce three molecules of NADH (Steps 3, 4, and 8) and one molecule of FADH$_2$ (Step 6).
5. One molecule of the high-energy phosphate compound guanosine triphosphate (GTP) is generated (Step 5).

Regulation of the Citric Acid Cycle

- The rate at which the citric acid cycle operates is precisely adjusted to meet cellular needs for ATP.
  - Citrate synthetase (Step 1) is an allosteric enzyme that is inhibited by ATP and NADH and activated by ADP.
  - Isocitrate dehydrogenase (Step 3) is an allosteric enzyme that is inhibited by NADH and activated by ADP.
  - The α-ketoglutarate dehydrogenase complex (Step 4) is a group of allosteric enzymes that is inhibited by succinyl CoA, NADH, the products of the reaction that it catalyzes, and ATP.
- The rate at which the citric acid cycle operates is reduced when cellular ATP levels are high, and stimulated when ATP supplies are low (and ADP levels are high).
The Electron Transport Chain

- The reduced coenzymes NADH and FADH$_2$ are end products of the citric acid cycle.
- In the final stage of food oxidation, the hydrogen ions and electrons carried by these coenzymes combine with oxygen to form water:

$$4H^+ + 4e^- + O_2 \rightarrow 2H_2O$$

- This series of reactions is called the electron transport chain. It involves a number of enzymes and cofactors located within the inner membrane of the mitochondria.
- Electrons from the reduced coenzymes are passed from one electron carrier to another within the membrane in assembly-line fashion, until they are combined with the final electron acceptor, O$_2$.

The Reactions of the Electron Transport Chain

- The electron transport chain is found in the inner membrane of the mitochondria.
- A summary of the reactions of the ETC:
  - The first electron carrier is an enzyme similar to FAD called flavin mononucleotide (FMN).
  - Two electrons and one H$^+$ from NADH pass to FMN, then to an iron-sulfur protein, and then to coenzyme Q (CoQ).
- CoQ is also the entry point for the two electrons and two H$^+$ ions from FADH$_2$. As NADH and FADH$_2$ release their H$^+$ and electrons, NAD$^+$ and FAD are regenerated for reuse in the citric acid cycle.
- Four of the five remaining electron carriers are cytochromes (cyt), which are iron-containing enzymes.
- In the final step, an oxygen atom accepts the electrons and combines with two H$^+$ ions to form water.
Oxidative Phosphorylation

• As electrons move along the electron transport chain, about 52.6 kcal/mol of free energy is released.

• Some of this energy is conserved by the synthesis of ATP from ADP and $P_i$. Because this synthesis of ATP (a phosphorylation reaction) is linked to the oxidation of NADH and FADH$_2$, it is called oxidative phosphorylation. It takes place at three different locations along the electron transport chain (see next slide).

Energy Changes in the Electron Transport Chain

- During oxidative phosphorylation:
  – the conversion of NADH to NAD$^+$ generates 2.5 molecules of ATP from ADP.
  – the conversion of FADH$_2$ to FAD generates 1.5 molecules of ATP.

- Every molecule of acetyl CoA entering the citric acid cycle produces:
  – 3 molecules of NADH
  – 1 molecule of FADH$_2$
  – GTP (equivalent to ATP)

The Chemiosmotic Hypothesis

- The mechanism by which the cell couples the oxidations of the electron transport chain and the synthesis of ATP involves a flow of protons ($H^+$).

- The chemiosmotic hypothesis proposes that the flow of electrons through the electron transport chain causes $H^+$ ions to be “pumped” from the matrix across the inner membrane and into the space between the inner and outer mitochondrial membranes, creating a difference in $H^+$ concentration and electrical potential across the membrane.

  – As a result, protons flow back through the membrane through a channel formed by the enzyme $F_1$-ATPase.

  – The flow of protons through this enzyme is believed to drive the phosphorylation reaction, and provides energy for ATP synthesis.
The Chemiosmotic Hypothesis

- NADH produced in the cytoplasm during glycolysis does not pass through the mitochondrial membrane to the site of the electron transport chain.
- Brain and muscle cells employ a transport mechanism that passes electrons from cytoplasmic NADH through the membrane to FAD molecules inside the mitochondria, forming FADH$_2$. This cytoplasmic NADH generates 1.5 molecules of ATP.
- Liver, heart, and kidney cells have a more efficient shuttle, where one cytoplasmic NADH results in one mitochondrial NADH, and 2.5 molecules of ATP.
- The complete aerobic catabolism of 1 mol of glucose in the liver results in 32 mol of ATP, mostly from oxidative phosphorylation.

The Complete Oxidation of Glucose

- Only 2 mol of ATP is produced per mole of glucose by lactate fermentation and alcoholic fermentation. Complete aerobic oxidation of glucose is thus 16 times more efficient than either of these processes.
- The total energy available in glucose is:
  \[ C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O; \Delta G^\circ = -686 \text{ kcal/mol} \]
- ATP synthesis:
  \[ 32ADP + 32P_i \rightarrow 32ATP + 32H_2O; \Delta G^\circ = +234 \text{ kcal/mol} \]
- Overall reaction:
  \[ C_6H_{12}O_6 + 6O_2 + 32ADP + 32P_i \rightarrow 6CO_2 + 38H_2O + 32 \text{ ATP}; \Delta G^\circ = -452 \text{ kcal/mol} \]

Energy from One Mole of Glucose

<table>
<thead>
<tr>
<th>Reaction</th>
<th>ATP Yield</th>
<th>ATP Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycolysis</td>
<td>1 each</td>
<td>2 ADP + 2 ATP</td>
</tr>
<tr>
<td>Glucose + 6 NADH + 6H$_2$O</td>
<td>1 each</td>
<td>6 ADP + 6 ATP</td>
</tr>
<tr>
<td>2 pyruvates + 2 NADH + 2H$_2$O</td>
<td>2 each</td>
<td>4 ADP + 4 ATP</td>
</tr>
<tr>
<td>2 acetyl-CoA + 2 NADH + 2H$_2$O</td>
<td>2 each</td>
<td>4 ADP + 4 ATP</td>
</tr>
<tr>
<td>Oxidation of 2 pyruvates to 2 acetyl-CoA</td>
<td>4 each</td>
<td>8 ADP + 8 ATP</td>
</tr>
<tr>
<td>Citric acid cycle, one turn</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 CO$_2$ + 6 H$_2$O + 6 ADP + 6 P + 6 NADH + 6FADH$_2$</td>
<td>6 each</td>
<td>6 ATP + 6 NADH + 6FADH$_2$</td>
</tr>
<tr>
<td>1 NADH + 1 FADH$_2$ in the oxidation of pyruvate</td>
<td>1 each</td>
<td>1 ATP</td>
</tr>
<tr>
<td>3 NADH + 3 FADH$_2$ in the citric acid cycle</td>
<td>3 each</td>
<td>3 ATP</td>
</tr>
<tr>
<td>6 NADH + 6 FADH$_2$ in the citric acid cycle</td>
<td>6 each</td>
<td>6 ATP</td>
</tr>
<tr>
<td>Total yield of ATP</td>
<td></td>
<td>32 ATP</td>
</tr>
</tbody>
</table>

Energy Efficiency in Living Organisms

- Thus, glucose oxidation liberates 686 kcal/mol, whereas the synthesis of 32 mol of ATP stores 234 kcal/mol. The efficiency of the energy storage is:
  \[ \frac{\text{energy available}}{\text{energy stored}} \times 100 = \frac{234 \text{ kcal/mol}}{686 \text{ kcal/mol}} \times 100 = 34.1\% \]

ATP Calculations, Summary

<table>
<thead>
<tr>
<th>Catabolic step</th>
<th>Energy production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycolysis (1 glucose $\rightarrow$ 2 pyruvates)</td>
<td>2 NADH (cytoplasmic) $\rightarrow$ 2 ATP</td>
</tr>
<tr>
<td>Liver, heart, kidneys</td>
<td>5 ATP</td>
</tr>
<tr>
<td>Brain, muscle</td>
<td>(3 ATP)</td>
</tr>
<tr>
<td>Oxidation of 2 pyruvates to 2 acetyl-CoA</td>
<td>3 NADH (mitochondrial) $\rightarrow$ 3 ATP</td>
</tr>
<tr>
<td>Citric acid cycle, one turn</td>
<td>1 NADH $\rightarrow$ 1.5 ATP</td>
</tr>
<tr>
<td>1 FADH$_2$ $\rightarrow$ 1 ATP</td>
<td></td>
</tr>
<tr>
<td>10 ATP</td>
<td></td>
</tr>
<tr>
<td>2 $\times$ 10 = 20 ATP</td>
<td></td>
</tr>
<tr>
<td>Citric acid cycle, two turns</td>
<td>producer</td>
</tr>
<tr>
<td>6CO$_2$ + 38H$_2$O + 32 ATP</td>
<td></td>
</tr>
<tr>
<td>Total glucose molecule = 32 ATP (30 ATP)</td>
<td></td>
</tr>
</tbody>
</table>
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Glycogen Synthesis

• Excess glucose is converted into glycogen in a process called glycogenesis.
  – Glycogen is stored primarily in the liver and muscle tissue, although some glycogenesis can occur in all cells.
  – The liver can store about 110 g of glycogen, and the muscles can store about 245 g.
• This anabolic process results in a bonding of glucose units to a growing glycogen chain. The energy is provided by the hydrolysis of uridine triphosphate (UTP; uracil + ribose + three phosphates).

\[
(glucose)_n + P_i \rightarrow (glucose)_{n-1} + \text{glucose 1-phosphate}
\]

Glycogen Breakdown

• Glycogenolysis is the breakdown of glycogen back into glucose.
  – Glycogenolysis can occur in the liver (and kidney and intestinal cells) but not in muscle tissue because one essential enzyme (glucose 6-phosphatase) is missing.
  – The first step in glycogen breakdown is the cleaving of the $\alpha(1\rightarrow4)$ linkages, catalyzed by glycogen phosphorylase. Glucose units are released from the glycogen chain as glucose 1-phosphate:

\[
glucose 1\text{-phosphate} \leftrightarrow \text{glucose 6-phosphate}
\]

• In the final step, glucose 6-phosphate is hydrolyzed to free glucose by the enzyme glucose 6-phosphatase (found only in liver, kidney, and intestinal cells):

\[
\text{glucose 6-phosphate} + H_2O \rightarrow \text{glucose} + P_i
\]

• Glycogen 6-phosphate can then be catabolized by the glycolysis pathway.

Glycogen in Muscles and the Liver

• Muscle cells lack glucose 6-phosphatase and cannot form free glucose from glycogen.
  – They can carry out the first two steps of glycogenolysis to produce glucose 6-phosphate.
  – This form of glucose is the first intermediate in the glycolysis pathway, which produces energy.
  – Muscles therefore only use glycogen for energy production.
  – In the liver, glycogen is broken down all the way to form free glucose, which is released into the blood during muscular activity and between meals.
  – This glucose is used to maintain a relatively constant level of blood glucose.
Gluconeogenesis

- The supply of glucose in the form of liver and muscle glycogen can be depleted by about 12-18 hours of fasting, and in a shorter time as a result of heavy work or strenuous exercise. Nerve tissue, including the brain, would be deprived of glucose if the only source was glycogen.

- **Gluconeogenesis** is the process of synthesizing glucose from noncarbohydrate materials.
  - When carbohydrate intake is low, and when glycogen stores are depleted, the carbon skeletons of lactate, glycerol (derived from the hydrolysis of fats), and certain amino acids are used to synthesize pyruvate, which is then converted to glucose:

  \[
  \text{lactate, certain amino acids, glycerol} \rightarrow \text{pyruvate} \rightarrow \text{glucose}
  \]

The Cori Cycle

- About 90% of gluconeogenesis occurs in the liver.
  - Very little takes place in the brain, skeletal muscle, or heart, even though these tissues have a high demand for glucose.
  - This allows the liver to maintain blood glucose levels so that tissues needing glucose can extract it from the blood.

- Gluconeogenesis involving lactate is especially important under anaerobic conditions.
  - During exercise, lactate levels increase in muscle tissue, and some diffuses into the blood.
  - This lactate is transported to the liver, where lactate dehydrogenase converts it back into pyruvate:

\[
\text{CH}_3-\text{CH} \rightarrow \text{COO}^- + \text{NAD}^+ \rightleftharpoons \text{CH}_3-\text{C} \rightarrow \text{COO}^- + \text{NADH} + \text{H}^+
\]

  - The pyruvate is then converted to glucose by the gluconeogenesis pathway, and enters the blood.
  - Thus, the liver increases a low blood glucose level and makes glucose available to the muscles.
  - This cyclic process of transport of lactate from muscle to liver, the resynthesis of glucose by gluconeogenesis, and the return of glucose to muscle tissue is called the **Cori cycle**.
Regulation of Carbohydrate Metabolism

It is important that metabolic pathways be responsive to cellular conditions that energy is not wasted in producing unneeded materials.

Besides the regulation of enzymes at key control points, the body also uses three important regulatory hormones:
- epinephrine
- glucagon
- insulin

**Insulin**

- **Insulin** is a polypeptide hormone (51 aa’s) made in the β-cells of the pancreas. When carbohydrates are consumed, blood glucose levels rise, and the pancreas releases insulin into the bloodstream:
  - This enhances the absorption of glucose from the blood into the cells of active tissues such as skeletal and heart muscles.
  - Insulin also increases the rate of synthesis of glycogen, fatty acids, and proteins.
  - Insulin stimulates glycolysis.
  - As a result, blood glucose levels begin to decrease within one hour, and return to normal in three hours.

**Glucagon**

- **Glucagon** is a polypeptide hormone (29 aa’s) made in the α-cells of the pancreas.
  - Glucagon activates the breakdown of glycogen in the liver, thereby increasing blood glucose levels, thus counteracting the effect of insulin.
  - Insulin and glucagon work in opposition to each other, and blood sugar levels depend in part of the biochemical balance between these hormones.

**Epinephrine**

- **Epinephrine** (also known as adrenaline) is a hormone and a neurotransmitter.
  - It stimulates glycogen breakdown in muscles, and to a smaller extent in the liver.
  - This glycogenolysis reaction provides energy for a sudden burst of muscular activity as a response to pain, anger, or fear (the “fight-or-flight” response)
  - Epinephrine also increases heart rate, constricts blood vessels, and dilates air passages.

**Hormonal Control of Glycogen**

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Source</th>
<th>Effect on Glycogen</th>
<th>Impact on Blood Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>β-cells of pancreas</td>
<td>Increases formation</td>
<td>Lowers blood glucose levels</td>
</tr>
<tr>
<td>Glucagon</td>
<td>α-cells of pancreas</td>
<td>Activates breakdown of liver glycogen</td>
<td>Raises blood glucose levels</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>Adrenal medulla</td>
<td>Stimulates breakdown</td>
<td>Raises blood glucose levels</td>
</tr>
</tbody>
</table>