Figure 8.2
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. Key: Blue text = intermediates of carbohydrate metabolism; brown text = intermediates of lipid metabolism; green text = intermediates of protein metabolism.

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Figure 8.1
Glycolysis, an example of a metabolic pathway.
Glycogen, starch, sucrose

storage

Glucose

oxidation via pentose phosphate pathway

Ribose 5-phosphate

oxidation via glycolysis

Pyruvate
Figure 8.3
Three stages of catabolism.

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Figure 8.4
Comparison of catabolic and anabolic pathways.
Figure 8.5
Some commonly used mechanisms for transmission of regulatory signals between cells.

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What are the levels of glucose in saliva? Is there any correlation between saliva glucose and blood glucose? How can we use saliva glucose levels for health prevention?

Over 80 million Americans and 300 million individuals worldwide are estimated to have diabetes or prediabetes. Already an epidemic, this number is expected to double by 2030 according to the CDC.

To function, your brain cells need two times more fuel than other cells in your body. This is why eating the recommended amount of carbohydrates daily is so important.

Thinking and memory drain glucose from the brain at a higher rate than any other brain functions.
The mean normal blood glucose level in humans is about 5.5 mM (5.5 mmol/L or 100 mg/dL); this level fluctuates throughout the day. Glucose levels are lowest in the morning, (termed "the fasting level"), and rise after meals for an hour or two by a few millimolar. The normal blood glucose level (tested while fasting) for non-diabetics, should be between 70 and 100 mg/dL). Blood sugar levels for those without diabetes and who are not fasting should be below 125 mg/dL. The blood glucose target range for diabetics, according to the American Diabetes Association, should be 70–130 (mg/dL) before meals, and less than 160 mg/dL after meals.

A persistently high level is referred to as hyperglycemia; low levels are referred to as hypoglycemia. Diabetes mellitus is characterized by persistent hyperglycemia from any of several causes, and is the most prominent disease related to failure of blood sugar regulation. Intake of alcohol causes an initial surge in blood sugar, and later tends to cause levels to fall.

The average levels of glucose in saliva is around 0.0017 mmol/l in the normal population and about 0.022 mmol/l in diabetes patients. The global prevalence of diabetes is around 6.4% in adult population. We expect around 438 million people with diabetes for the year 2030.
Glucose can not diffuse directly into cell, there are two mechanisms. **Facilitated transport** (glucose transporters GLUT1-5) Tissue specific GLUT 4 (abundant in adipose tissue and skeletal muscle) insulin regulated. Gradient dependent. GLUT 1,3&4 uptake from blood

**Cotransport** carrier mediated concentration gradient with Na+ monosaccharide (occurs in epithelial cells of the intestine, renal tubes.

GLUT1-14 isoforms
Glycolysis occurs in two stages. First five reactions are energy investment. Formation of fructose at the expense of ATP. Stage 2 is an energy generation stage net of 2 ATP and 2NADH.

GLUT1 erythrocytes, brain
GLUT2 liver, kidney, B cells
Transports from cell to blood
GLUT3 in neurons
GLUT4 adipose skeletal musc
GLUT5 transporter fructose, small intestine and testes

Glycolysis occurs in two stages. First five reactions are energy investment. Formation of fructose at the expense of ATP. Stage 2 is an energy generation stage net of 2 ATP and 2NADH.
Glycolysis employed by all tissues, breakdown of Glucose to provide energy. Pyruvate is the end product in cells with mitochondria and a adequate supply of Oxygen. There are ten reactions in aerobic glycolysis because $O_2$ is required to re-oxidize NADH formed during oxidation of glyceraldehyde 3-P. In anaerobic glycolysis NADH reduces pyruvate to form lactate (there is no net formation of NADH) and therefore can occur in the absence of $O_2$ (red blood cells). Irreversible rxs (three)
<table>
<thead>
<tr>
<th><strong>GLYCOLYSIS</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mostly endergonic reactions</td>
</tr>
</tbody>
</table>

![Glucose molecule](image)

- **Glucose**
- (6 carbons)
**Preparatory phase**

Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate.
Payoff phase

Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH

Glyceraldehyde 3-phosphate (2)

oxidation and phosphorylation

\[ 2 \text{Pi} + 2 \text{NAD}^+ \rightarrow 2 \text{NADH} + H^+ \]

1,3-Bisphosphoglycerate (2)

first ATP-forming reaction (substrate-level phosphorylation)

\[ 2 \text{ADP} \rightarrow 2 \text{ATP} \]

3-Phosphoglycerate (2)

\[ \text{P}_3 \text{OCH}_2 \text{CH}(\text{CO}_2 \text{H}) \]

2-Phosphoglycerate (2)

\[ \text{CH}_2 \text{CH}(\text{CO}_2 \text{H}) \]

Phosphoenolpyruvate (2)

second ATP-forming reaction (substrate-level phosphorylation)

\[ 2 \text{ADP} \rightarrow 2 \text{ATP} \]

Pyruvate (2)
Irreversible phosphorylation reaction. Glucose 6-P does not diffuse out of cell. Commits glucose to further metabolism. Hexokinase catalyze phosphorylation of Glucose. Occurs in most tissues and is one of the three regulatory enzymes (pyruvate kinase and phosphofructokinase (PFK1)). Inhibited by reaction product, glucose 6-P.

Glucokinase: In liver and β cells in the pancreas. Requires higher glucose concentrations for half saturation. Active during carbohydrate rich meal minimizing hyperglycemia during absorptive periods. Increased by carbohydrates and Insulin. Not inhibited by Glucose 6-P.
First irreversible reaction

Phosphorylated on C-6, first ATP consumed

\[ \Delta G^{\circ} = -16.7 \text{ kJ/mol} \]
Low Km = High affinity

High Vm = can phosphorylate large quantities of glucose.

<table>
<thead>
<tr>
<th></th>
<th>Hexokinase</th>
<th>Glucokinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue distribution</td>
<td>Most tissues</td>
<td>Liver and β-cells</td>
</tr>
<tr>
<td>K_m</td>
<td>Low (0.1 mmol/L = 2 mg%*)</td>
<td>High** (10 mmol/L = 200 mg%*)</td>
</tr>
<tr>
<td>V_m</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Inhibition by glucose 6-phosphate</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

*mg% = milligrams glucose per 100 mL plasma; normal fasting blood glucose is 70–90 mg% or about 5 mmol/L.

** The velocity of glucokinase shows a sigmoidal dependence on glucose concentration and thus the term “half-saturation”, rather than K_m, should be used in describing this enzyme.
Hexokinase are more efficient at low substrate conc while Glucokinase are more efficient at high substrate conc.
Glucokinase are also known as hexokinase D it works like a glucose sensor for insulin release. Liver removes most glucose preventing it from entering the systemic circulation minimizing hyperglycemia. This enzyme is regulated by fructose 6-P and glucose.
Figure 8.14
Regulation of glucokinase activity by glucokinase regulatory protein.

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Second reaction

\[ \text{Glucose 6-phosphate} \xrightarrow{\text{Mg}^{2+} \text{ phosphohexose isomerase}} \text{Fructose 6-phosphate} \]

\[ \Delta G^{\circ} = 1.7 \text{ kJ/mol} \]
Figure 8.15
Isomerization of glucose 6-phosphate to fructose 6-phosphate.

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Third Reaction (second irreversible, rate limiting)

Phosphorylated on C-1, consumes ATP
Figure 8.16
Energy investment phase (continued): Conversion of fructose 6-phosphate to triose phosphates.
The irreversible phosphorylation reaction catalyzed by phosphofructokinase 1 (PFK-1) is the most important control point in glycolysis (rate limiting step). Controlled by the concentrations of ATP and fructose 6-P. Abundance of energy +ATP and citrate inhibit PFK-1. Induced by +AMP. The most potent activator of PFK-1 is fructose 2,6-bisphosphate which also inhibit fructose 1,6 biphosphatase (dephosphorylation, page 101) ensuring that both pathways are not active at the same time.
Enzymes and receptors

Importance in metabolism
The extracellular domain contains the binding site for a ligand (a hormone or neurotransmitter).

Intracellular domain that interacts with G-proteins

Seven trans-membrane helices

Figure 8.6
Structure of a typical membrane receptor.

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G proteins, trimeric
Gs and Gi
They have inherent GTPase activity causing rapid hydrolisis of GTP to GDP

cAMP-dependent protein kinase A
see next slide
Protein kinase C not dependent on cAMP

Low levels of blood Glucose

High levels of Glucagon

Gluconeogenesis
Figure 8.17
Effect of elevated insulin concentration on the intracellular concentration of fructose 2,6-bisphosphate in liver.

PFK-2 = phosphofructokinase-2; FBP-2 = Fructose bisphosphate phosphatase-2.

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Fourth and Fifth reactions

Gives this pathway its name lysis

Endergonic phase
Fifth reaction

\[
\begin{align*}
\text{Dihydroxyacetone phosphate} & \quad \text{Glyceraldehyde 3-phosphate} \\
\text{triose phosphate isomerase} & \quad \Delta G^{\circ} = 7.5 \text{ kJ/mol}
\end{align*}
\]

End of Preparatory Phase
G3P the most complex functional group (carbonyl) is labeled C-1 vs C-4
GLYCOLYSIS

<table>
<thead>
<tr>
<th>Mostly endergonic reactions</th>
<th>Mostly exergonic reactions</th>
</tr>
</thead>
</table>

Let's summarize first 5 rx’s
The payoff phase
The Six reaction, first NADH formation

\[ \text{Glyceraldehyde 3-phosphate} + \text{Inorganic phosphate} \rightarrow \text{1,3-Bisphosphoglycerate} \]

\[ \Delta G^{\circ} = 6.3 \text{ kJ/mol} \]
Glycolysis would come to an end for lack of NAD$^+$ If NADH in this step were not continuously reoxidized.
Seventh reaction First ATP formed

\[
\text{1,3-Bisphosphoglycerate} + \text{ADP} \xrightarrow{\text{Rib-Adenine}} \text{3-Phosphoglycerate} + \text{ATP}
\]

\[
\Delta G^{\circ} = -18.5 \text{ kJ/mol}
\]
The formation of 1,3 bisphosphoglycerate is through a substrate level phosphorylation which is coupled directly to the oxidation of the substrate instead of oxidative phosphorylation via the electron transfer chain.

2,3-BPG is found in high concentration in red blood cells and in trace amount in other tissues. These shunt reactions are included in erythrocytes. Most kinase rxs are irreversible, however, this kinase rx is reversible. Since there are two glyceraldehyde 3-P molecules two ATP molecules are formed (from each glucose molecule) replacing the two ATP molecules consumed in earlier rxs.

The next step is a shift of phosphate from carbon 3 to carbon 2 of phosphoglycerate.
Mutase needs to be phosphorylated
Eighth reaction shift in phosphate group to C-2

\[ \Delta G^{\circ} = 4.4 \text{ kJ/mol} \]
Ninth reaction

\[
\begin{align*}
\text{2-Phosphoglycerate} & \rightarrow \text{Phosphoenolpyruvate} \\
\Delta G^{\circ} & = 7.5 \text{ kJ/mol}
\end{align*}
\]

(PEP)
Tenth and final reaction of the pathway, second ATP

Phosphoenolpyruvate + ADP → Pyruvate + ATP

\[ \Delta G^\circ = -31.4 \text{ kJ/mol} \]

Third and last irreversible reaction of the pathway. Exergonic phase
Dehydration to phosphoenolpyruvate (PEP)
The third irreversible rx of glycolysis the formation of pyruvate by pyruvate kinase (hexokinase and PFK-1) This is the second ATP forming rx of the oxidative stage of glycolysis. (another substrate level phosphorylation. In the liver pyruvate kinase is activated by fructose 1,6-bisphosphate.

Phosphorylation by cAMP-dependent protein kinase A due to increase in glucagon (low blood glucose) lead to inactivation of pyruvate kinase inhibiting glycolysis and inducing gluconogenesis (fig 8.7)

Reduction of pyruvate to lactate mainly occurs in red blood cells, lens and cornea, kidney medulla, testes and leucocytes. Lactate formation in muscle NADH/NAD elevated exceeds the capacity oxid.c
Low levels of Glucose in blood induce the secretion of Glucagon from B cells and activate protein Kinase A which (-) PK and stops glycolysis. Glycolytic enzyme deficiency due to a reduced rate of glycolysis and ATP formation in red blood cells 95% show a deficiency in PK second to G6PD in hemolytic anemia.
## GLYCOLYSIS

<table>
<thead>
<tr>
<th>Mostly endergonic reactions</th>
<th>Mostly exergonic reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Summary of Anaerobic Glycolysis

Figure 8.22
Summary of anaerobic glycolysis. Reactions involving the production or consumption of ATP or NADH are indicated. The irreversible reactions of glycolysis are shown with thick arrows. DHAP = dihydroxyacetone phosphate.

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Summary

The image represents a diagram of glycolysis, a metabolic pathway in which glucose is broken down to pyruvate, producing ATP and NADH as products.

- ATP consumption involves the breakdown of glucose to glucose 6-P and subsequent reactions to produce ATP and ADP.
- ATP production follows the reactions that generate 2 ATP molecules from 2 ADP molecules.
- NADH production occurs during the conversion of glyceraldehyde 3-P to 1,3-bis-phosphoglycerate, resulting in 2 NADH + 2H+.
- NADH consumption is seen in the conversion of lactate to pyruvate, utilizing 2 NADH + 2H+ and producing 2 NAD+.

The central reactions involve the conversion of fructose 6-P to fructose 1,6-bis-P, and the production of 2 ATP from 2 ADP.
Figure 8.21
Interconversion of pyruvate and lactate.

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Fructose 2,6-biphosphate is converted back to fructose 6-P by fructose biphosphatase 2 (FBP-2) while fructose 2,6-biphosphate is formed by phosphofructokinase 2 (PFK-2). The kinase and phosphatase activities are on different domains. Insulin and glucagon actions on the kinases.

Well fed state
Glucose is abundant

During starvation,
high glucagon, low insulin

**1** High insulin/glucagon ratio causes decreased cAMP and reduced levels of active protein kinase A.

**2** Decreased protein kinase A activity favors dephosphorylation of PFK-2/FBP-2 complex.

**3** Dephosphorylated PFK-2 is active, whereas FBP-2 is inactive; this favors formation of fructose 2,6-bisphosphate.

**4** Elevated concentration of fructose 2,6-bisphosphate activates PFK-1, which leads to an increased rate of glycolysis.
Three possible catabolic fates of pyruvate from glycolysis

- **Fermentation to alcohol in yeast**
  - 2 Ethanol + 2CO₂
  - Reaction catalyzed by alcohol dehydrogenase

- **Fermentation to lactate in vigorously contracting muscle, erythrocytes, some other cells, and in some microorganisms**
  - 2 Lactate
  - ΔG° = -25.1 kJ/mol

- **Citric acid cycle**
  - 2 Acetyl-CoA
  - Further oxidation to yield 4CO₂ + 4H₂O

**Pyruvate decarboxylase**

**Glucose**
- Glycolysis (10 successive reactions)

**Anaerobic conditions**

**Aerobic conditions**
**Oxidative decarboxylation**

**Carboxylation, substrate for gluconogenesis**

**Microorganisms**

**Lactate dehydrogenase**
- Important in RBC, WBC (and other cells with few or no mitochondria) and in skeletal muscle during intense exercise.
- Physiologically reversible in tissues with a low NADH/NAD⁺, for example, liver and heart muscle.
- Located in the cytosol.

**Pyruvate carboxylase**
- Biotin serves as prosthetic group.
- Activated by acetyl CoA.
- Replenishes intermediates of the TCA cycle.
- Provides substrates for gluconogenesis.
- An irreversible reaction.
- Located in mitochondria.

**Pyruvate dehydrogenase complex**
- Thiamine-PP, lipoic acid, FAD, NAD⁺ and CoA serve as coenzymes.
- Source of acetyl CoA for TCA and fatty acid synthesis.
- An irreversible reaction.
- Located in mitochondria.
Patients with defects in glycolitic enzymes 95% show a defect in pyruvate kinase (PK) and about 4% in glucose phosphate isomerase. They exhibit hemolytic anemia due to erythrocyte destruction.

How many ATP’s are formed from anaerobic and aerobic glycolysis? Each NADH=3ATP
Meal rich in carbohydrates or administration of insulin

Starvation or diabetes

These effects are due to increase or decrease in transcription of specific genes. These effects can result in 10-20 fold increases in enzyme activity.

Rate limiting step enzymes in gluconeogenesis, glucagon activates transcription of PEP carboxykinase, fructose 6-phosphatase, glucose 6-phosphatase (old Lipp page 102)
Fructose 6-phosphate

\[
\text{ATP} \xrightarrow{\text{PFK-1}} \text{ADP} \xleftarrow{} \text{Fructose 1,6-bisphosphate}
\]

\[
\text{Glycolysis} \quad \text{Gluconeogenesis}
\]

\[
\text{P}_{\text{i}} \xrightarrow{} \text{FBPase-1}
\]
Figure 8.25
Key concept map for glycolysis.

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### Table 15-2

**Cytosolic Concentrations of Enzymes and Intermediates of the Glycolytic Pathway in Skeletal Muscle**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Concentration (μM)</th>
<th>Intermediate</th>
<th>Concentration (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldolase</td>
<td>810</td>
<td>Glucose 6-phosphate</td>
<td>3,900</td>
</tr>
<tr>
<td>Triose phosphate isomerase</td>
<td>220</td>
<td>Fructose 6-phosphate</td>
<td>1,500</td>
</tr>
<tr>
<td>Glyceraldehyde 3-phosphate</td>
<td>1,400</td>
<td>Fructose 1,6-bisphosphate</td>
<td>80</td>
</tr>
<tr>
<td>dehydrogenase</td>
<td></td>
<td>Dihydroxyacetone phosphate</td>
<td>160</td>
</tr>
<tr>
<td>Phosphoglycerate kinase</td>
<td>130</td>
<td>Glyceraldehyde 3-phosphate</td>
<td>80</td>
</tr>
<tr>
<td>Phosphoglycerate mutase</td>
<td>240</td>
<td>1,3-Bisphosphoglycerate</td>
<td>50</td>
</tr>
<tr>
<td>Enolase</td>
<td>540</td>
<td>3-Phosphoglycerate</td>
<td>200</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>170</td>
<td>2-Phosphoglycerate</td>
<td>20</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>300</td>
<td>Phosphoenolpyruvate</td>
<td>65</td>
</tr>
<tr>
<td>Phosphoglucomutase</td>
<td>32</td>
<td>Pyruvate</td>
<td>380</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lactate</td>
<td>3,700</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATP</td>
<td>8,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ADP</td>
<td>600</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pi</td>
<td>8,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NAD⁺</td>
<td>540</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NADH</td>
<td>50</td>
</tr>
</tbody>
</table>

How well have you learned?

When a muscle is stimulated to contract aerobically, less lactic acid is formed than when it contracts anaerobically because:

a. glycolysis does not occur to a significant extent under aerobic conditions.
b. muscle is metabolically less active under aerobic than anaerobic conditions.
c. the lactic acid generated is rapidly incorporated into lipids under aerobic conditions.
d. under aerobic conditions in muscle, the major energy-yielding pathway is the pentose phosphate pathway which does not produce lactate.
e. under aerobic conditions most of the pyruvate generated as a result of glycolysis is oxidized by the citric acid cycle rather than reduced to lactate.
The steps of glycolysis between glyceraldehyde 3-phosphate and 3-phosphoglycerate involve all of the following except:

a. ATP synthesis.
b. catalysis by phosphoglycerate kinase
c. oxidation of NADH to NAD$^+$
d. the formation of 1,3-bisphosphoglycerate
e. utilization of Pi.

Glycolysis in the erythrocyte produces pyruvate that is further metabolized to:

a. CO$_2$
b. Ethanol
c. Glucose
d. Hemoglobin
e. lactate