GLYCOLYSIS

Generation of ATP from Metabolic Fuels

- **Catabolic process** – degradative pathway
- Energy stored in sugars (carbohydrates) released to perform biological work
- Transforms GLUCOSE to PYRUVATE under ANAEROBIC conditions
- Glucose enters the cell via a specific transporter protein

**Uses:**
- Glucose
- ATP
- ADP + Pi
- NAD⁺ (necessary co-factor)

**Produces:**
- Pyruvate
- ATP
- NADH – can be further oxidized under *aerobic* conditions to make ATP
Reactions of **glycolysis** occur in the **CYTOSOL**

**- THREE FATES OF PYRUVATE**

**- Aerobic conditions**
  - conversion to acetyl CoA (pyruvate dehydrogenase) for use in TCA cycle and oxidative phosphorylation for ATP production

**- Anaerobic conditions**
  - Lactate (animal muscles)
  - Ethanol (yeast)

**- ANABOLIC PROCESS: GLUCONEOGENESIS**
  - Synthesize glucose FROM pyruvate or lactate
  - Increases free glucose concentration
OVERALL REACTION FOR GLYCOLYSIS:

\[
\text{Glucose} + 2 \text{ADP} + 2 \text{NAD}^+ + 2 \text{P}_i \rightarrow 2 \text{Pyruvate} + 2 \text{ATP} + 2 \text{NADH} + 2 \text{H}^+ + 2 \text{H}_2\text{O}
\]

- 10 Step Process – some steps tightly regulated
- Each glucose (6 carbons) split into TWO pyruvates (3 carbons each)
- Two molecules of ATP are produced
- Two molecules of NAD+ are reduced to NADH

- **TWO PHASES:**
  - **INVESTMENT PHASE**
    - First 5 reactions
    - Glucose is activated by phosphorylation
      - “Priming reactions” – need to invest energy to get more out
    - Uses 2 ATP’s per glucose
    - Glucose is converted to TWO molecules of glyceraldehyde 3-phosphate (G3P)
  - **DIVIDEND PHASE**
    - Second set of 5 reactions
    - Each glyceraldehyde 3-phosphate (G3P) \(\rightarrow\) pyruvate
    - Get FOUR ATP’s out
    - Net gain of 2 ATP’s

- Modest return of energy! Will see big return once pyruvates enter TCA cycle and oxidative phosphorylation.

**HANDOUT:**
- Not necessary to memorize structures except glucose and pyruvate
- Know types of enzymes and recognize names of intermediates and enzymes
- Know regulatory steps
- Be able to count ATP’s and follow what is made or used when and where.
INVESTMENT

2 ATP per GLUCOSE
2 NADH per GLUCOSE

DIVIDEND

Table 15.1
The reactions of glycolysis with common enzyme names and reaction type

<table>
<thead>
<tr>
<th>Reaction Number</th>
<th>Reaction</th>
<th>Enzyme</th>
<th>Reaction Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose + ATP → glucose-6-phosphate + ADP</td>
<td>Hexokinase</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Glucose-6-phosphate → fructose-6-phosphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Fructose-6-phosphate + ATP → fructose-1,6-bisphosphate + ADP</td>
<td>Phosphoglucoisomerase</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>Fructose-1,6-bisphosphate → dihydroxyacetone phosphate + glyceraldehyde-3-phosphate</td>
<td>Phosphofructokinase</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Dihydroxyacetone phosphate → glyceraldehyde-3-phosphate</td>
<td>Aldolase</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>Glyceraldehyde-3-phosphate + P, + NAD⁺ → 1,3-bisphosphoglycerate + NADH⁺</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>1.2</td>
</tr>
<tr>
<td>7</td>
<td>1,3-Bisphosphoglycerate + ADP → 3-phosphoglycerate + ATP</td>
<td>Phosphoglycerate kinase</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>3-Phosphoglycerate → 2-phosphoglycerate</td>
<td>Phosphoglycerate mutase</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>2-Phosphoglycerate → phosphoenolpyruvate + H₂O</td>
<td>Enolase</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>Phosphoenolpyruvate + ADP → pyruvate + ATP</td>
<td>Pyruvate kinase</td>
<td>2</td>
</tr>
</tbody>
</table>

* Enzymes are listed by common names.

* Reaction type: (1) oxidation-reduction, (2) phosphoryl group transfer, (3) hydrolysis, (4) nonhydrolytic cleavage (addition or elimination), (5) isomerization-rearrangement, and (6) bond formation coupled to ATP cleavage (see Section 14.2).
Each of these reactions occurs twice because two glyceroldehyde-3-phosphates are produced from one glucose.
REACTIONS OF GLYCOLYSIS IN DETAIL

Investment Phase

In the first five steps of glycolysis, one six-carbon molecule of glucose is split into two 3-carbon compounds. 2 molecules of ATP are required for these reactions.
STEP 1: Glucose to glucose-6-phosphate

- Phosphorylation of glucose by HEXOKINASE
  - KINASE – Enzymes that catalyze the transfer of a phosphoryl group from ATP to an acceptor substrate
  - Type of TRANSFERASE enzyme
  - Regulated but not the committed step
    - Glucose-6-phosphate can form glycogen or other pathways
- ATP COUNT
  - -1 (one ATP used)
  - 1st Investment of Energy

STEP 2: Glucose-6-phosphate to fructose-6-phosphate

ENZYME: Phosphoglucone Isomerase

Type of ISOMERASE – Rearrangement of functional groups to form the isomer

- Convert Glucose-6-phosphate to fructose-6-phosphate
- Not a regulated or committed step
- ATP COUNT:
  - This step – 0
  - Overall count – -1
STEP 3: Fructose-6-phosphate to fructose-1,6-bisphosphate

- **ENZYME**: Phosphofructokinase
  - KINASE – same as first step; TRANSFERASE reaction
  - 2<sup>nd</sup> Investment of energy – one more ATP used

- **ATP COUNT**:
  - This step – -1
  - Overall count – -2

- **KEY CONTROL STEP – IRREVERSIBLE!!**
  - Committed step
  - Note HIGHLY negative $\Delta G^{\circ}$ – means *not* reversible

STEP 4: Fructose-1,6-bisphosphate $\rightarrow$ glyceraldehyde-3-phosphate & dihydroxyacetone phosphate

- **ENZYME**: Aldolase
  - Non-hydrolytic Cleavage reaction (type of lyase)
  - Cleaves glucose molecule into 2 molecules

- **ATP COUNT**:
  - This step – 0
  - Overall count – -2

- **Not a regulatory step**
STEP 5: Dihydroxyacetone phosphate to glyceraldehyde-3-phosphate

- **ENZYME**: Triose phosphate isomerase
  - Isomerization – rearrangement reaction
  - Isomerase enzyme

- **ATP COUNT**:
  - This step – 0
  - Overall count – -2

- Not a regulatory step

Through 1st 5 steps (Investment Phase) we’ve USED 2 ATP molecules
Steps 6-10 → Dividend Phase where the investment pays off!!

**Sum**: Glucose + 2 ATP → 2 glyceraldehyde-3-phosphate +2 ADP + 2 Pi
Dividend Phase

In the second phase of glycolysis, glyceraldehyde-3-phosphate is converted to pyruvate.

These reactions yield 4 molecules of ATP, 2 for each molecule of pyruvate produced.
STEP 6: 2 Glyceraldehyde-3-phosphate to 2 1,3-bisphosphoglycerate

- **ENZYME**: Glyceraldehyde-3-phosphate dehydrogenase
  - DEHYDROGENASE reaction
  - Oxidation – Reduction enzymes (also called oxidoreductases)
  - Reactions generate either NADH, FADH₂ or NADPH
  - This reaction produces NADH

- **ATP COUNT**:
  - This step – 0
  - Overall count – -2
  - +2 NADH produced
  - Not a regulatory step

STEP 7: (2) 1,3-bisphosphoglycerate to (2) 3-phosphoglycerate

- **ENZYME**: Phosphoglycerate Kinase
  - Group Transfer reaction – KINASE reaction (same as 1 and 3)
  - STEP WHERE ATP IS MADE!!

- **ATP COUNT**:
  - This step – +2
  - Overall count – -2 + 2 = 0
  - +2 NADH overall
  - Not a regulatory step
STEP 8: (2) 3-phosphoglycerate to (2) 2-phosphoglycerate

- **ENZYME:** Phosphoglycerate Mutase
  - Isomerization – Rearrangement reaction – Mutase reaction (same as 2 and 5)

- **ATP COUNT:**
  - This step – 0
  - Overall count – -2 + 2 = 0
  - +2 NADH overall

- Not a regulatory step

STEP 9: (2) 2-phosphoglycerate to (2) phosphoenolpyruvate

- **ENZYME:** Enolase
  - Non-hydrolytic cleavage reaction

- **ATP COUNT:**
  - This step – 0
  - Overall count – -2 + 2 = 0
  - +2 NADH overall

- Not a regulatory step
STEP 10: (2) phosphoenolpyruvate to (2) pyruvate

\[
\begin{align*}
\text{COO}^- & \quad \text{PO}_2^- & \text{H}^+ & \text{ADP}^3^- & \text{Mg}^{2+} & \text{K}^+ & \text{COO}^- & \text{CH}_2 \text{PO}_3^- & \text{CH}_3 \\
\text{PEP} & \quad \text{Pyruvate}
\end{align*}
\]

\[\Delta G^{\text{eq}} = -31.7 \text{ kJ/mol}\]

- **ENZYME:** Pyruvate Kinase
  - Group Transfer reaction – KINASE reaction (same as 1 and 3)
  - STEP WHERE ATP IS MADE!!
- **ATP COUNT:**
  - This step: +2
  - Overall count: -2 + 2 + 2 = 2
  - +2 NADH overall
- **This is a REGULATED step – Not Reversible**

**GLYCOLYSIS ANIMATION:**


**Table 15.2**
The ATP and NADH balance sheet for glycolysis

<table>
<thead>
<tr>
<th>Number&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reaction per Glucose</th>
<th>ATP Change per Glucose&lt;sup&gt;b&lt;/sup&gt;</th>
<th>NADH Change per Glucose&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose → glucose-6-phosphate</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Fructose-6-phosphate → fructose-1,6-bisphosphate</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>2 Glyceraldehyde-3-phosphate → 2 1,3-bisphosphoglycerate</td>
<td>0</td>
<td>+2</td>
</tr>
<tr>
<td>7</td>
<td>2 1,3-Bisphosphoglycerate → 2 3-phosphoglycerate</td>
<td>+2</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>2 Phosphoenolpyruvate → 2 pyruvate</td>
<td>+2</td>
<td>0</td>
</tr>
<tr>
<td>Total change</td>
<td></td>
<td>+2</td>
<td>+2</td>
</tr>
</tbody>
</table>

<sup>a</sup>The number corresponds to the reaction number in Table 15.1.

<sup>b</sup>A minus sign indicates loss of ATP by cleavage of a phosphoanhydride bond; a plus sign indicates formation of ATP (from ADP) or NADH (from NAD<sup>+</sup>).

**REGULATION OF GLYCOLYSIS**

- **Glycolysis is a highly regulated process**
  - Need to maintain constant levels of energy in cells
  - Regulation UP and DOWN depends on the cell’s need for ATP and NADH
  - Steps 2, 4-9 have \(\Delta G^{\text{eq}}\) values close to zero, therefore are essentially operating at equilibrium
    - Can go in either direction
    - These steps are common to the GLUCONEOGENESIS pathway
Steps 1, 3 and 10 have large negative $\Delta G^\circ$ values (not at equilibrium) and are the sites of regulation.

**THREE KEY REGULATED STEPS**

1. **Hexokinase** (Step #1)
   a. Regulates entry of free glucose into glycolysis
   b. Controlled by FEEDBACK INHIBITION
      i. Inhibited by product – glucose-6-phosphate
   c. NOT the committed step
   d. Regulates the concentration of glucose-6-phosphate

2. **Phosphofructokinase** (PFK) (Step #3)
   a. Catalyzes phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate (FBP)
   b. KEY REGULATORY POINT OF GLYCOLYSIS
   c. Valve that controls glycolysis
   d. 1st major committed step – can’t go back
   e. PFK is INACTIVE when [ATP] cell is HIGH
      i. Makes good sense – when ATP is high, glycolysis no necessary so turned down at PFK
   f. If [AMP] (low energy precursor of ATP) HIGH, tells cell energy is LOW and to make more ATP
   g. Inhibited by CITRATE – physiological form of citric acid
      i. Citrate formed in TCA cycle from pyruvate
      ii. Therefore, if cellular [citrate] is sufficient, glycolysis is slowed

3. **Pyruvate Kinase** (Step #10)
   a. Regulates formation of pyruvate from phosphoenolpyruvate
   b. Increase [ATP] inhibits pyruvate kinase and slows pyruvate formation

Red blood cells depend on a constant energy supply to maintain structural integrity
   o Remember that they don’t have nuclei or mitochondria
   o Therefore, **glycolysis is the primary source of ATP for red blood cells**
If energy needs are not met, the RBC’s can rupture (called hemolysis) and the blood loss called **hemolytic anemia**.

2nd most common form of **hemolytic anemia** is due to deficiency in **pyruvate kinase**
- Autosomal recessive trait (carriers have no disease)
- Treated with transfusions and/or splenectomies
- No simple treatment

### Table 15.3
The irreversible reactions of glycolysis that are bypassed in gluconeogenesis

<table>
<thead>
<tr>
<th>Number&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reaction</th>
<th>Enzyme</th>
<th>(\Delta G^\circ) (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose + ATP → glucose-6-phosphate + ADP</td>
<td>Hexokinase</td>
<td>−16.7</td>
</tr>
<tr>
<td>3</td>
<td>Fructose-6-phosphate + ATP → fructose-1,6-bisphosphate + ADP</td>
<td>Phosphofructokinase</td>
<td>−14.2</td>
</tr>
<tr>
<td>10</td>
<td>Phosphoenolpyruvate + ADP → pyruvate + ATP</td>
<td>Pyruvate kinase</td>
<td>−31.4</td>
</tr>
</tbody>
</table>

<sup>a</sup>The number corresponds to the reaction number in Table 15.1.

Table 15.3 Concepts in Biochemistry, 3/e
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## ENTRY OF OTHER CARBOHYDRATES INTO GLYCOLYSIS:

### 1. Dietary Starch
- Hydrolyzed in mouth by amylases to glucose monomers
- Hydrolyzed in stomach by acid to glucose monomers
- Glucose absorbed through intestinal walls to blood and transported
  - 1/3 goes to skeletal muscle and heart
  - 1/3 goes to BRAIN – needs 100g glucose/day; can’t use fatty acids
  - 1/3 goes to liver for storage as glycogen

### 2. Disaccharides:
- Maltose → 2 glucose
- Sucrose → fructose and glucose
- Lactose → glucose and galactose

\[
maltose + H_2O \xrightleftharpoons{maltase} 2 \text{ glucose} \\
sucrose + H_2O \xrightleftharpoons{sucrese} \text{ fructose} + \text{ glucose} \\
lactose + H_2O \xrightleftharpoons{lactase} \text{ glucose} + \text{ galactose}
\]
Fructose and galactose enter glycolysis differently!

- **Fructose:**
  - In muscle, hexokinase phosphorylates fructose and enters pathway as fructose-6-phosphate. One step!
  - In liver, multiple steps needed.
    - Fructokinase phosphorylates at position 1
    - Aldolase cleavage
    - Additional phosphorylation
    - Enters as 2 molecules of glyceraldehyde-3-phosphate

- **Galactose:**
  - C4 epimer of glucose
  - Requires 5 reactions to transform it to *glucose-6-phosphate* where it can enter glycolytic pathway

- **Glycerol:**
  - Released during degradation of TAG’s
  - 2 Reactions:
    - Phosphoryl transfer
    - Oxidation
  - Turns glycerol into dihydroxyacetone which isomerizes in glycolysis to glyceraldehyde-3-phosphate

Figure 15-3 Concepts in Biochemistry, 3/e
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