The Citric Acid Cycle
Catabolism of proteins, fats, and carbohydrates in the three stages of cellular respiration

- Stage 1: oxidation of fatty acids, glucose, and some amino acids yields acetyl-CoA.
- Stage 2: oxidation of acetyl groups in the citric acid cycle to form NADH and FADH$_2$.
- Stage 3: electrons are funneled into a chain of electron carriers reducing O$_2$ to H$_2$O. This electron flow drives the production of ATP.
Only a Small Amount of Energy Available in Glucose is Captured in Glycolysis

\[
\text{Glycolysis} \quad \Delta G^\circ = -146 \text{ kJ/mol}
\]

GLUCOSE

\[
\text{Full oxidation (+ 6 O}_2) \quad \Delta G^\circ = -2,840 \text{ kJ/mol}
\]

6 CO\textsubscript{2} + 6 H\textsubscript{2}O
Cellular Respiration

- process in which cells consume O\textsubscript{2} and produce CO\textsubscript{2}
- provides more energy (ATP) from glucose than glycolysis
- also utilizes energy stored in lipids and amino acids
- evolutionary origin: developed about 2.5 billion years ago
- used by animals, plants, and many microorganisms
- occurs in three major stages:
  1. acetyl CoA production
  2. acetyl CoA oxidation
  3. electron transfer and oxidative phosphorylation
Respiration: Stage 3

NADH, FADH$_2$ (reduced e$^-$ carriers)

Stage 3 Electron transfer and oxidative phosphorylation

Respiratory (electron-transfer) chain

2H$^+$ + $\frac{1}{2}$O$_2$ → H$_2$O

ADP + P$_i$ → ATP
In Eukaryotes, Citric Acid Cycle Occurs in Mitochondria

- Glycolysis occurs in the cytoplasm

- Citric acid cycle occurs in the mitochondrial matrix†

- Oxidative phosphorylation occurs in the inner mitochondrial membrane

† Except succinate dehydrogenase, which is located in the inner membrane
Anatomy of a mitochondrion

• The mitochondrial pool of coenzymes and intermediates is functionally separate from the cytosolic pool

• Cristae (convolutions) of the inner membrane provide a very large surface area

• The inner membrane of a single liver mitochondrion may have more than 10,000 sets of electron-transfer systems (respiratory chains) and ATP synthase molecules
Oxidation of Acetyl-CoA in Citric Acid Cycle
Acetyl-coenzyme A (acetyl-CoA)
The Citric Acid Cycle

Krebs Cycle
Tricarboxylic Acids Cycle
TCA cycle
The Citric Acid Cycle

1. Condensation
   - Acetyl-CoA + CoA-SH → Citrate

2a. Dehydration
   - Citrate → Aconitate

2b. Hydration
   - Aconitate → Isocitrate

3. Oxidative decarboxylation
   - Isocitrate → α-Ketoglutarate

4. Oxidative decarboxylation
   - α-Ketoglutarate → Succinyl-CoA

5. Substrate-level phosphorylation
   - Succinyl-CoA → Succinyl-CoA + GDP + Pi

6. Dehydrogenation
   - Succinate → FADH2

7. Hydration
   - Malate → Fumarate

8. Dehydrogenation
   - Oxaloacetate → NADH

- Malate dehydrogenase
- Succinate dehydrogenase
- α-ketoglutarate dehydrogenase complex
- Succinyl-CoA synthetase
- Oxidative decarboxylation
- Substrate-level phosphorylation
Sequence of Events in the Citric Acid Cycle

• Step 1: C-C bond formation to make citrate
• Step 2: Isomerization via dehydration, followed by hydration
• Steps 3-4: Oxidative decarboxylations to give 2 NADH
• Step 5: Substrate-level phosphorylation to give GTP
• Step 6: Dehydrogenation to give reduced FADH$_2$
• Step 7: Hydration
• Step 8: Dehydrogenation to give NADH
1) The Citrate Synthase Reaction

- The only cycle reaction with C-C bond formation
- Essentially irreversible process

\[
\begin{align*}
\text{Acetyl-CoA} + \text{Oxaloacetate} & \rightarrow \text{Citrate} \\
\Delta G^{\circ} & = -32.2 \text{ kJ/mol}
\end{align*}
\]
1) The Citrate Synthase Reaction

Step 1: C-C bond formation to make citrate
2) Isomerization of Citrate

- Thermodynamically unfavorable reaction
- Equilibrium contains less than 10% of isocitrate
- Isocitrate is rapidly consumed in the next reaction

\[ \Delta G^\circ = 13.3 \, \text{kJ/mol} \]
2) Isomerization of Citrate

Step 2: Isomerization via dehydration, followed by hydration
3) Dehydrogenation of Isocitrate

- Oxidation of an alcohol to ketone - transfer of 2e\textsuperscript{-} and a proton and hydride to NAD\textsuperscript{+}
- Production of a molecule of NADH
- Production of a molecule of CO\textsubscript{2}

\[
\Delta G' = -8.4 \text{ kJ/mol}
\]
3) Dehydrogenation of Isocitrate

Steps 3-4: Oxidative decarboxylations to give 2 NADH
4) Oxidation of $\alpha$-ketoglutarate

- **Enzyme:** $\alpha$-ketoglutarate dehydrogenase complex
- **Similar to pyruvate dehydrogenase complex**
- **Three enzymes homologous to E1, E2 and E3**
- **Same coenzymes, identical mechanisms**

$$\Delta G^{\circ} = -33.5 \text{ kJ/mol}$$
4) Oxidation of $\alpha$-ketoglutarate

Step 5: Substrate-level phosphorylation to give GTP
5) Substrate-Level Phosphorylation

- Succinyl-CoA – high energy thioester bond
- Produces GTP, which can be converted to ATP
- Phosphate $P_i$ is first bound to carboxy group of succinate than to His group of the enzyme and finally to GDP to form GTP

\[ \Delta G'^\circ = -2.9 \text{ kJ/mol} \]
5) Substrate-Level Phosphorylation

Step 6: Dehydrogenation to give reduced FADH$_2$
6) Dehydrogenation of Succinate

- Dehydrogenation of succinate to fumarate
- Formation of double bond
- Stereospecificity

\[ \Delta G^\circ = 0 \text{ kJ/mol} \]
6) Dehydrogenation of Succinate

- Enzyme bound to the **mitochondrial membrane**
- Covalently bound FAD is reduced to FADH$_2$
- Contains three Fe-S clusters
- FADH$_2$ passes electrons further via Fe-S clusters to **coenzyme Q** of the **electron transport chain** of mitochondrial membrane where
- Reduced coenzyme (QH$_2$) is used to make ATP (process of respiration – **oxidative phosphorylation**)

![Diagram of electron transport chain](image-url)
6) Dehydrogenation of Succinate

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Step 7: Hydration

- Condensation
- Dehydration
- Dehydrogenation
- Hydration
- Oxidative decarboxylation
- Substrate-level phosphorylation
- Oxidative decarboxylation
- GTP (ATP)
- Succinate dehydrogenase
- Succinyl-CoA synthetase
- α-ketoglutarate dehydrogenase complex
- α-Ketoglutarate
- Isocitrate
- Fumarase
7) Hydration of Fumarate to Malate

- Fumarase is highly stereospecific
- Hydration of *trans* double bond (no *cis* specificity)
- Adds OH\(^-\) to fumarate - carbanion intermediate
- Then adds H\(^+\) to the carbanion
- Reversible reaction (only L-malate)

\[ \Delta G^\circ = -3.8 \text{ kJ/mol} \]
7) Hydration of Fumarate to Malate
8) Oxidation of Malate to Oxaloacetate

- Thermodynamically unfavorable reaction
- Oxidation occurs because oxaloacetate concentration is very low as it is continuously used to make citrate

\[ \Delta G^{\circ} = 29.7 \text{ kJ/mol} \]
8) Oxidation of Malate to Oxaloacetate

Step 8: Dehydrogenation to give NADH
Products from One Turn of the Cycle

- Acetyl-CoA
- Citrate
- Isocitrate
- Oxaloacetate
- Malate
- Fumarate
- Succinate
- Succinyl-CoA
- FADH$_2$
- NADH
- CO$_2$
- α-Ketoglutarate
- GTP (ATP)
Products from One Turn of the Cycle

\[
\text{Acetyl-CoA} + 3\text{NAD}^+ + \text{FAD} + \text{GDP} + P_i + 2 \text{H}_2\text{O} \rightarrow 2\text{CO}_2 + 3\text{NADH} + \text{FADH}_2 + \text{GTP} + \text{CoA} + 3\text{H}^+
\]

- One acetyl enters in a form of Acetyl-CoA
- Carbon is oxidized to CO\(_2\)
- Electrons from oxidation are captured on 3 NADH and 1 FADH\(_2\)
- Production of one GTP (ATP)
- One molecule of oxaloacetate reacts to one citrate
- One molecule of oxalacetátu is regenerated
- Oxaloacete can circle infinitely
## Products from One Turn of the Cycle

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Number of ATP or reduced coenzyme directly formed</th>
<th>Number of ATP ultimately formed*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Isocitrate → 2 α-ketoglutarate</td>
<td>2 NADH</td>
<td>5</td>
</tr>
<tr>
<td>2 α-Ketoglutarate → 2 succinyl-CoA</td>
<td>2 NADH</td>
<td>5</td>
</tr>
<tr>
<td>2 Succinyl-CoA → 2 succinate</td>
<td>2 ATP (or 2 GTP)</td>
<td>2</td>
</tr>
<tr>
<td>2 Succinate → 2 fumarate</td>
<td>2 FADH$_2$</td>
<td>3</td>
</tr>
<tr>
<td>2 Malate → 2 oxaloacetate</td>
<td>2 NADH</td>
<td>5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>20</strong></td>
</tr>
</tbody>
</table>

*This is calculated as 2.5 ATP per NADH and 1.5 ATP per FADH$_2$. A negative value indicates consumption.
Citric acid cycle is central to the metabolism

- Not only a place of oxidation and energy conservation
- Precursors for variety of products
- **Anaplerotic** reactions – replenishing substrates
Role of the Citric Acid Cycle in Anabolism

Glucose

Phosphoenolpyruvate (PEP)

Serine
Glycine
Cysteine
Phenylalanine
Tyrosine
Tryptophan

Aspartate
Asparagine

Pyrimidines

Malate

PEP carboxylase

Pyruvate carboxylase

Pyruvate

Acetyl-CoA

Citrate

α-Ketoglutarate

Succinyl-CoA

Porphyrim, heme

Glutamine
Proline
Arginine

Glutamate

Fatty acids, sterols

Purines
Anaplerotic Reactions

- these reactions **replenish metabolites** for the cycle
- four-carbon intermediates are formed by **carboxylation** of three-carbon precursors

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Tissue(s)/organism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate + HCO$<em>3^-$ + ATP $\xrightleftharpoons</em>{pyruvate carboxylase}^{pyruvate carboxylase}$ oxaloacetate + ADP + P$_i$</td>
<td>Liver, kidney</td>
</tr>
<tr>
<td>Phosphoenolpyruvate + CO$<em>2$ + GDP $\xrightleftharpoons</em>{PEP carboxykinase}^{PEP carboxykinase}$ oxaloacetate + GTP</td>
<td>Heart, skeletal muscle</td>
</tr>
<tr>
<td>Phosphoenolpyruvate + HCO$<em>3^-$ $\xrightleftharpoons</em>{PEP carboxylase}^{PEP carboxylase}$ oxaloacetate + P$_i$</td>
<td>Higher plants, yeast, bacteria</td>
</tr>
<tr>
<td>Pyruvate + HCO$<em>3^-$ + NAD(P)H $\xrightleftharpoons</em>{malic enzyme}^{malic enzyme}$ malate + NAD(P)$^+$</td>
<td>Widely distributed in eukaryotes and bacteria</td>
</tr>
</tbody>
</table>
Biosynthetic precursors produced by an incomplete citric acid cycle in anaerobic bacteria

\[ \text{CO}_2 \] \rightarrow \text{PEP or pyruvate} \rightarrow \text{Oxaloacetate} \rightarrow \text{Malate} \rightarrow \text{Fumarate} \rightarrow \text{Succinate} \rightarrow \alpha\text{-Ketoglutarate} \rightarrow \text{Biosynthetic products (amino acids, nucleotides, heme, etc.)} \]
Role of the Citric Acid Cycle in Anabolism
Central molecules in metabolism

- **Pyruvate** and **Acetyl-CoA**

Vertebrates **cannot** metabolize **Acetyl-CoA** to sugars

Vertebrates can make sugars from pyruvate

Oxidation of fat (fatty acids) leads only to **Acetyl-CoA**

Vertebrates therefore **cannot** change fats to sugars
Glyoxylate Cycle

• **Pyruvate** to Acetyl-CoA is **one way** reaction

• In gluconeogenesis – pyruvate is converted to **oxalacetate** - to phosphoenolpyruvate - to carbohydrates

• Reactions of Citric Acid Cycle – **do not** produce oxalacetate from acetyl-CoA

  \[(\text{Oxalacetate} + \text{acetyl-CoA} \rightarrow 2\text{CO}_2 + \text{oxaloacetate})\]

• **Glyoxalate cycle** makes **succinate** from **2** acetyl-CoA

• **Succinate** enters the Citric Acid Cycle to produce oxalacetate
Glyoxylate Cycle in Glyoxysomes

1. Oxaloacetate
   - Oxaloacetate to Malate
   - Malate dehydrogenase (NADH)
2. Malate
   - Malate dehydrogenase (NAD^+)
   - Malate synthase
3. Glyoxylate
   - Glyoxylate lyase
4. Acetyl-CoA
   - Acetyl-CoA
5. Citrate
   - Citrate synthase
6. Isocitrate
   - Aconitase
7. Succinate

Chemical reactions:
- Oxaloacetate + NAD^+ → Malate + NADH
- Malate + NAD^+ → Glyoxylate + NADH
- Glyoxylate + CoA → Acetyl-CoA
- Acetyl-CoA + CoA → Citrate
Relationship between the glyoxylate and citric acid cycles

- Glyoxylate cycle proceed simultaneously with the citric acid cycle
- Intermediates pass between these cycles (compartments)
- Conversion of succinate to malate by citric acid cycle and malate to oxalacetate in cytosole
Learning objectives

- Role of acetyl-CoA
- Reactions od Citric acid cycle
- Products of the oxidation of acetyl in Citric acid cycle
- Role of Citric acid cycle in catabolism
- Role of Citric acid cycle in anabolism
- Anaplerotic reactions
- Glyoxylate cycle
- Conversion of acetyl into sugars