TCA CYCLE (Citric Acid Cycle)

- The **Citric Acid Cycle** is also known as:
  - **Kreb’s cycle**
    - Sir Hans Krebs
    - Nobel prize, 1953
  - **TCA (tricarboxylic acid) cycle**

- The citric acid cycle requires **aerobic** conditions!!!!
  - Cells have evolved to use oxygen
  - **Oxygen** serves as the **final electron acceptor** as **pyruvate** (from glycolysis) is converted (oxidized) completely to **CO₂** and **H₂O**

- **Pyruvate** converted to **Acetyl-CoA** by **PDH** and then **Acetyl-CoA** enters the TCA cycle

**Energy in the citric acid cycle**

- Energy of the oxidation reactions is largely conserved as **reducing power**

- Coenzymes reduced:
  - NAD⁺/NADH
  - FAD/FADH₂

- Reduced coenzymes used by electron transport chain and oxidative phosphorylation to make ATP
The Tricarboxylic acid (TCA) cycle (citric acid cycle) is **amphibolic** (both *catabolic* and *anabolic*).

**The TCA Cycle Serves Two Purposes:**

1. **Oxidize Acetyl-CoA to CO₂ to produce energy**
   - ATP (GTP)
   - Reducing power of NADH and FADH₂
   - The cycle is involved in the aerobic catabolism of carbohydrates, lipids and amino acids

2. **Supply precursors for biosynthesis of carbohydrates, lipids, amino acids, nucleotides and porphyrins**
   - Intermediates of the cycle are starting points for many biosynthetic reactions
   - The cycle itself is **not** a pathway for a net degradation of any cycle intermediates
   - Cycle intermediates can be shared with other pathways, which may lead to a re-supply or net decrease in cycle intermediates
   - Reactions feeding into the cycle replenish the pool of cycle intermediates

**Fundamental Differences between Glycolysis and TCA Cycle:**

1. Glycolysis is a **linear** pathway; TCA cycle is **cyclic**
2. Glycolysis occurs in the **cytosol** and TCA is in the **mitochondrial matrix**
3. Glycolysis does **not require** oxygen; TCA **requires** oxygen (aerobic)

**Summary of the citric acid cycle**

For each acetyl-CoA that enters the cycle:

1. **Two** molecules of CO₂ are released
2. Coenzymes NAD⁺ and FAD are reduced
3. One GDP (or ADP) is phosphorylated
4. The initial acceptor molecule oxaloacetate is reformed

**Energy conservation by the cycle**

- Energy is conserved in the reduced coenzymes NADH, FADH₂ and one GTP
- NADH, FADH$_2$ can be oxidized to produce ATP by oxidative phosphorylation.
- Energy is also conserved in either ATP or GTP- produced by **substrate-level phosphorylation** (from the thioester bond in succinyl-CoA.)
- The use of many steps in the oxidation of acetyl CoA to CO$_2$ enables conservation of most of the energy as work with little lost as heat.

### Table 16.2

<table>
<thead>
<tr>
<th>Reaction Number$^a$</th>
<th>Reaction</th>
<th>Enzyme</th>
<th>Prosthetic Group</th>
<th>Reaction Type$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acetyl-CoA + oxaloacetate + H$_2$O $\rightarrow$ citrate + CoA</td>
<td>Citrate synthase</td>
<td>3,4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Citrate $\rightarrow$ cis-aconitate $\rightarrow$ isocitrate</td>
<td>Aconitase</td>
<td>Fe–S</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Isocitrate $\rightarrow$ NAD$^+$ $\rightarrow$ α-ketoglutarate + CO$_2$ + NADH + H$^+$</td>
<td>Isocitrate dehydrogenase</td>
<td>1,4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>α-Ketoglutarate + NAD$^+$ + CoA $\rightarrow$ succinyl-CoA + CO$_2$ + NADH + H$^+$</td>
<td>α-Ketoglutarate dehydrogenase complex</td>
<td>Lipoamide, FAD, TPP</td>
<td>1,4</td>
</tr>
<tr>
<td>5</td>
<td>Succinyl-CoA + P$_i$ + ADP or GDP $\rightarrow$ succinate + ATP or GTP + CoA</td>
<td>Succinyl-CoA synthetase</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Succinate + FAD (enzyme bound) $\rightarrow$ fumarate + FADH$_2$ (enzyme bound)</td>
<td>Succinate dehydrogenase</td>
<td>FAD, Fe–S</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Fumarate + H$_2$O $\rightarrow$ l-malate</td>
<td>Fumarase</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>l-Malate + NAD$^+$ $\rightarrow$ oxaloacetate + NADH + H$^+$</td>
<td>Malate dehydrogenase</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ The reaction numbers correspond to the steps in Figure 16.8.
$^b$ Reaction type: 1, oxidation–reduction; 2, phosphoryl group transfer; 3, hydrolysis; 4, nonhydrolytic cleavage (addition or elimination); 5, isomerization–rearrangement; 6, bond formation coupled to ATP cleavage.

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**Figure 16.1** Concepts in Biochemistry, 3/e © 2006 John Wiley & Sons
8 REACTIONS OF THE TCA CYCLE:

1. Formation of Citrate

- Citrate formed from condensation of acetyl CoA and oxaloacetate
- Addition of acetyl to the keto double bond of OAA = aldol condensation
- Only cycle reaction with C-C bond formation
- No energy of ATP hydrolysis needed
- **Synthase** is an enzyme that catalyzes addition to a double bond or elimination to form a double bond without needing ATP hydrolysis
- **Both Hydrolysis Reaction and Non-hydrolytic cleavage (addition or elimination)**
Locoweed is toxic because it accumulates fluoroacetate

- Isomerization of citrate (3° alcohol) to isocitrate (2° alcohol)
- Aconitase contains an iron-sulfur center as a prosthetic group
- Catalyzes a lyase reaction that results in rearrangement of citrate with a tertiary alcohol to isocitrate with a secondary alcohol
  - Non-hydrolytic cleavage (addition or elimination)
  - Goes through an enzyme bound cis-aconitate intermediate
  - Elimination of H₂O from citrate to form C=C bond of cis-aconitate
  - Rearrangement allows the further oxidation of the molecule
3. Isocitrate Dehydrogenase

- First oxidative decarboxylation of isocitrate to $\alpha$-ketoglutarate ($\alpha$-kg)
- Metabolically irreversible reaction
- One of four oxidation-reduction reactions of the cycle
- Also a Non-hydrolytic cleavage reaction (addition or elimination)
- Hydride ion from the C-2 of isocitrate is transferred to NAD$^+$ to form NADH
- Oxalosuccinate is decarboxylated to $\alpha$-ketoglutarate

4. $\alpha$-Ketoglutarate Dehydrogenase Complex

- Second oxidative decarboxylation reaction
- Also a Non-hydrolytic cleavage reaction (addition or elimination)
- $\alpha$-Ketoglutarate converted to Succinyl-CoA
- Similar to pyruvate dehydrogenase complex except a succinyl group is activated, not acetyl
  - Same coenzymes, identical mechanisms
- Succinyl-CoA thioester is VERY high energy
- Generates NADH
- Purpose of step: Collect energy from $\alpha$-ketoglutarate decarboxylation into the high energy succinyl-CoA molecule

\[
\begin{align*}
\text{H}_2\text{C} & \quad \text{COO}^- \\
\text{H}_2\text{C} & \quad \text{C} \quad \text{COO}^- \\
\text{O} & \\
\end{align*}
\]

$\alpha$-Ketoglutarate

\[
\begin{align*}
\text{NAD}^+ & \quad \text{CoA} \\
\text{NADH} & \quad \text{H}^+ \\
\text{CO}_2 & \\
\end{align*}
\]

$\alpha$-Ketoglutarate dehydrogenase

\[
\begin{align*}
\text{H}_2\text{C} & \quad \text{COO}^- \\
\text{H}_2\text{C} & \quad \text{C} \quad \text{SCoA} \\
\text{O} & \\
\end{align*}
\]

Succinyl-CoA

5. Succinyl-CoA Synthetase (Formation of succinate)

- Free energy in thioester bond of succinyl CoA is conserved as GTP (or ATP in plants, some bacteria)
- Enzyme: Succinyl-CoA Synthetase
  - Two forms in higher animals: One prefers ADP the other GDP
  - SUBSTRATE-LEVEL PHOSPHORYLATION = Formation of ATP directly coupled to the reaction (group transfer reaction)
  - Only step where ATP (GTP) is formed directly in the TCA cycle
  - All other ATP is produced by oxidative phosphorylation
    - Oxidative phosphorylation is the oxidation of reduced co-factors NADH and FADH$_2$ to O$_2$ – release of energy drives ATP formation from ADP + Pi

\[
\begin{align*}
\text{H}_2\text{C} & \quad \text{COO}^- \\
\text{H}_2\text{C} & \quad \text{C} \quad \text{SCoA} \\
\text{O} & \\
\end{align*}
\]

Succinyl-CoA

\[
\begin{align*}
\text{GDP} & \quad \text{P} \\
\text{GTP} & \quad \text{CoA} \\
\end{align*}
\]

Succinyl-CoA synthetase

\[
\begin{align*}
\text{H}_2\text{C} & \quad \text{COO}^- \\
\text{H}_2\text{C} & \quad \text{COO}^- \\
\end{align*}
\]

Succinate
6. The Succinate Dehydrogenase (SDH) Complex

- Located on the inner mitochondrial membrane (other components are in the matrix)
- Oxidation-reduction reaction that forms a carbon-carbon double bond
- Succinate is oxidized to fumarate, while FAD is reduced to FADH$_2$
  - NAD$^+$ functions in reactions that interconvert hydroxyl and carbonyl groups
- Dehydrogenation is stereospecific; only the trans isomer is formed
- Also known as Complex II of the electron transport chain – direct feed of electrons from FADH$_2$ into the electron transport chain.

![Chemical Structures](figure_16.10.png)

- Substrate analog malonate is a competitive inhibitor of the SDH complex
- Malonate is a structural analog of succinate
- Malonate binds to the enzyme active site, and is a competitive inhibitor

![Chemical Structures](figure_16.10.png)

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**Figure 16.10**

Structures of succinate, the normal substrate for succinate dehydrogenase, and a competitive inhibitor of succinate dehydrogenase, malonate. Malonate is toxic since it blocks a reaction in the citric acid cycle.
7. **Fumarase**

- Stereospecific *trans* addition of water to the double bond of fumarate to form *L*-malate

![Fumarase Reaction](image)

- Only forms the *L*-isomer
- Non-hydrolytic cleavage reaction

8. **Malate Dehydrogenase**

- Regeneration of oxaloacetate from *L*-malate
- Enzyme: malate dehydrogenase (oxidation – reduction reaction)
- Generates NADH

![Malate Dehydrogenase](image)

OVERALL SUMMARY OF TCA CYCLE:

1. **Oxidation of Acetyl-CoA to CO₂**
   - CO₂ leaves at steps 3 and 4

2. **3 NAD⁺ are reduced to NADH by dehydrogenase reactions**
   - Steps 3, 4, and 8
   - isocitrate dehydrogenase
   - α–ketoglutarate dehydrogenase
   - malate dehydrogenase

3. **1 molecule of FAD reduced to FADH₂**
   - Step 6 – Succinate dehydrogenase

4. **1 phosphoanhydride bond formed in ATP or GTP**
   - Substrate level phosphorylation at step 5: Succinyl-CoA Synthetase
   - Generated from energy stored in CoA thioester
So, per pyruvate:
- 4 NADH (one from pyruvate dehydrogenase complex + 3 TCA)
- 1 ATP or GTP
- 1 FADH$_2$

**ANIMATION:**

- **Fig. 16-2** -- The Reactions of the Citric Acid Cycle

**ENERGY FROM THE TCA CYCLE:**
Reduced Coenzymes Fuel the Production of ATP

- Each acetyl CoA entering the cycle nets:
  1. **3 NADH**
  2. **1 FADH$_2$**
  3. **1 GTP** (or 1 ATP)

- Oxidation of each NADH yields **2.5 ATP**
- Oxidation of each FADH$_2$ yields **1.5 ATP**
- **Complete oxidation** of 1 acetyl CoA = 10 ATP

**Table 16.3**
The ATP, NADH, and FADH$_2$ balance sheet for the pyruvate dehydrogenase complex and the citric acid cycle

<table>
<thead>
<tr>
<th>Reaction Number$^a$</th>
<th>Reaction</th>
<th>ATP (GTP) Change$^b$</th>
<th>NADH Change$^b$</th>
<th>FADH$_2$ Change$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Pyruvate oxidation**
- Pyruvate dehydrogenase complex
- Pyruvate oxidation total

<table>
<thead>
<tr>
<th>Pyruvate oxidation</th>
<th>ATP (GTP) Change</th>
<th>NADH Change</th>
<th>FADH$_2$ Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>+1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

**Citric acid cycle**
- Isocitrate + NAD$^+$ $\rightleftharpoons$ $\alpha$-ketoglutarate + CO$_2$ + NADH + H$^+$
- $\alpha$-Ketoglutarate + NAD$^+$ + CoA $\rightleftharpoons$ succinyl-CoA + CO$_2$ + NADH + H$^+$
- Succinyl-CoA + GDP or ADP + P$_i$ $\rightleftharpoons$ succinate + GTP or ATP + CoASH
- Succinate + FAD $\rightleftharpoons$ fumarate + FADH$_2$
- L-Malate + NAD$^+$ $\rightleftharpoons$ oxaloacetate + NADH + H$^+$

<table>
<thead>
<tr>
<th>Citric acid cycle total</th>
<th>ATP (GTP) Change</th>
<th>NADH Change</th>
<th>FADH$_2$ Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>+1</td>
<td>+3</td>
<td>+1</td>
<td></td>
</tr>
</tbody>
</table>

**Grand Total**
<table>
<thead>
<tr>
<th></th>
<th>ATP (GTP) Change</th>
<th>NADH Change</th>
<th>FADH$_2$ Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>+1</td>
<td>+4</td>
<td>+1</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ The reaction numbers correspond to the steps in Figure 16.8.

$^b$ A $+$ number indicates a production of ATP, NADH, or FADH$_2$. For example, for each pyruvate that is converted to acetyl-CoA by the pyruvate dehydrogenase complex, 1 NADH is formed.

*Table 16-3 Concepts in Biochemistry, 3/e © 2006 John Wiley & Sons*
Glucose degradation via glycolysis, citric acid cycle, and oxidative phosphorylation

**AEROBIC**

TOTAL/glucose = 32 ATP

If anerobic – Lactate is formed from pyruvate after glycolysis by lactate dehydrogenase and the NADH formed is USED. **Therefore, net gain of 2 ATP/glucose, not 32! (Hence 5-10% efficiency)**

- Occurs in muscles during exercise because they go into oxygen debt.
- Soreness due to H\(^+\) from lactic acid
- Metabolism in muscles returns to normal when oxygen replenished

**Regulation of the Citric Acid Cycle**

- Regulation depends on the ENERGY LEVEL of cells – key to keep energy level constant
- When cells have lots of energy (ATP, NADH), the reactions involved in making more are slowed
- The reverse is also true.
- Pathway controlled by:
  1. Small molecule modulators (products of the cycle can inhibit)
  2. Covalent modification of cycle enzymes
  3. Supply of acetyl CoA

**Regulation of the PDH complex**

- Highly regulated
- Regulation of pyruvate dehydrogenase complex controls acetyl CoA supply
- Gatekeeper to aerobic metabolism
- Represents the committed step because pyruvate can still go back to glucose (gluconeogenesis) but acetyl-CoA cannot go back to glucose.
- Inhibitors: Indicators of high energy status
  - NADH, ATP, Acetyl-CoA, Fatty acids (degraded to form acetyl-CoA)
- Stimulators: Indicators of low energy status
  - AMP, NAD⁺, Coenzyme A (CoA-SH)

**Regulation of citrate synthase**
- Inhibitors: NADH, ATP, succinyl-CoA, citrate
- Stimulators: ADP

**Regulation of isocitrate dehydrogenase (ICDH)**
- Inhibitors: NADH and ATP
- Stimulators: NAD⁺, ADP and Ca²⁺

**Regulation of α-ketoglutarate dehydrogenase complex**
- Inhibitors: NADH, ATP and succinyl-CoA
- Stimulators: NAD⁺, ADP, AMP

**POINTS OF REGULATION**

**ANIMATION:**
[http://www.wiley.com/college/fob/anim/ Chapter 16]

- **Fig. 16-14** -- Regulation of the Citric Acid Cycle

<table>
<thead>
<tr>
<th>Table 16.4</th>
<th>The important regulatory enzymes of pyruvate and acetyl-CoA metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme Name</td>
<td>Modulators</td>
</tr>
<tr>
<td>Pyruvate dehydrogenase complex</td>
<td>AMP, NAD⁺, CoA</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>ADP</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>ADP</td>
</tr>
<tr>
<td>α-Ketoglutarate dehydrogenase complex</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 16-4 Concepts in Biochemistry, 3/e
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