Citric Acid Cycle (CAC; Tricarboxyl Acid Cycle; Krebs Cycle) — Definition and Glyoxylate Cycle

See online here

As the final stage of food utilization, the citric acid cycle is primarily responsible for many anabolic and katabolic processes of the human body. As the ‘hub of metabolism’, it is a very popular subject in medical examinations. The following article will help you to better understand its functions, the different reaction steps, and their regulation.

Definition of the Citric Acid Cycle

The citric acid cycle is also referred to as the Krebs cycle or tricarboxylic acid cycle (TCA) and is a cyclic metabolic process. It takes place in the matrix of the mitochondria and plays an important role for anabolism and catabolism.

Per reaction cycle, 1 acetyl-CoA is transformed into 2 CO₂. The resulting energy is fixated as 3 NADH+H⁺, 1 FADH₂, and 1 GTP. In the respiratory chain, the electrons of NADH+H⁺ and FADH₂ are used for ATP-synthesis.
*Note:* ATP is not produced in the citric acid cycle itself.

## Functions of the Citric Acid Cycle

The citric acid cycle is referred to as the ‘hub of the intermediate metabolism’ since it has a central role for a lot of metabolic pathways. Its most important function, however, is the **retrieval of electrons for the respiratory chain** by oxidation of acetyl-CoA.

The needed acetyl-CoA is created in the **beta-oxidation of fatty acids** and the **oxidative decarboxylation of pyruvate**, which forms during glycolysis. Also, some amino acids like isoleucine, leucine, and tryptophan can be degraded to acetyl-CoA.

In addition, the citric acid cycle has the following additional functions:

- It is the final path of the **degradation of amino acids**, which cannot be degraded to acetyl-CoA or pyruvate.
- It produces **substances for re-synthesis of amino acids** (e.g. oxaloacetate for aspartate)
- Reaction products from the citric acid cycle are diverted and fed into other metabolic pathways: citrate for **fatty acid synthesis**, oxaloacetate for **gluconeogenesis**, or succinyl-CoA for the formation of delta-aminolevulinic acid as the basic substance for **heme synthesis**.

## Electrochemical Gradient

![Electrochemical Gradient](image)

**The ETC creates an electrochemical gradient.**

## Individual Reaction Steps of the Citric Acid Cycle

Even if it is hard to learn the individual steps and the respective structural formulas in detail, it is definitely worth it since they are often asked for in oral and written exams.
Step 1: Acetyl-CoA + Oxaloacetate → Citrate

Citrate synthase catalyzes the transfer of acetyl-CoA to oxaloacetate with the formation of citrate. H₂O is inserted and the coenzyme A is split off. Hereby, the high-energy thioester-bond of acetyl-CoA is hydrolyzed.

Step 2: Citrate → Isocitrate

Acinotase hydratase, also called acinotase, transforms citrate to isocitrate. Via shifting of one OH group, the tertiary alcohol becomes a secondary one. The intermediate substance of this isomerization is referred to as cis-acinotate.

Step 3: Isocitrate → alpha-Ketoglutarate

Isocitrate dehydrogenase catalyzes the NAD+-dependent oxidation of isocitrate. In this process, the unstable intermediate substance oxalosuccinate forms, which then spontaneously decarboxylizes to become succinyl-CoA. In this reaction step, the first oxidation reaction and the first decarboxylation of the citric acid cycle occur – with the formation of 1 NADH+H⁺ and the release of CO₂.

Step 4: Alpha-Ketoglutarate → Succinyl-CoA

Alpha-ketoglutarate dehydrogenase is a large enzymatic complex that is very similar to the pyruvate dehydrogenase. For the oxidative decarboxylation of alpha-
ketogluarate to succinyl-CoA, it needs the following cofactors: thiamine pyrophosphate, liponamide, coenzyme A, FAD, and NAD⁺. Again, CO₂ and 1 more NADH⁺ form for the respiratory chain.

In case of a lack of thiamine (e.g. caused by malnutrition due to alcoholism), Wernicke’s encephalopathy occurs since both alpha-ketoglutarate and pyruvate dehydrogenases rely on thiamine as a cofactor. If both enzymes do not work correctly, glutamate can accumulate and the utilization of glucose is diminished which then results in cerebral cell damage.

**Step 5: Succinyl-CoA → Succinate + CoA + GTP**

The enzyme succinyl-CoA synthetase catalyzes the hydrolysis of the high-energy thioester-bond of succinyl-CoA. Coenzyme A is split off which leads to the formation of succinate. The released energy is used to synthesize 1 GTP – also referred to as phosphate-level phosphorylation.

If one phosphate group of GTP is transferred to ADP, ATP results: GTP + ADP → GDP + ATP. However, this reaction itself is not part of the citric acid cycle.

**Step 6: Succinate → Fumarate + FADH₂**

The FAD-dependent succinate dehydrogenase performs the oxidation of succinate to fumarate. This happens with the formation of a double bond and the release of 1 FADH₂.

An important aspect is that the succinate dehydrogenase is the only enzyme of the citric acid cycle that does not freely float in the matrix space but is anchored to the inner membrane of the mitochondrion. Thus, it can directly supply the respiratory chain with the electrons of FADH₂ and is therefore called complex II.

**Step 7: Fumarate + H₂O → Malate**

The fumarate hydratase – also called fumarase – catalyzes the addition of water to fumarate, which results in malate.

**Step 8: Malate → Oxaloacetate**

The NAD+-dependent malate dehydrogenase oxidizes malate to oxaloacetate, which can be used as a substrate for step 1 of the citric acid cycle. In this process, 1 NADH⁺⁺ is formed for the respiratory chain.

**All in all, the energetic yield of one cycle is:** 3 NADH⁺⁺, 1 FADH₂, 1 GTP. The two 2 CO₂ molecules that are also formed during the cycle are, in this context, useless waste products.

**Energy Balance of the Citric Acid Cycle**

In the respiratory chain, the mentioned yield of the citric acid cycle results in the following energy values:

- 1 NADH⁺⁺ is transformed to ca. 2,5 ATP.
- 1 FADH₂ is transformed to ca. 1,5 ATP.

So, per cycle of the citric acid cycle, the following fixated energy is produced: 7,5 ATP
out of 3x NADH+H⁺ + 1,5 ATP out of 1 FADH2 + 1 ATP out of 1 GTP (since they are energetically interchangeable) – this results in a sum of ca. 10 ATP.

In the literature, the energetic yield of NADH+H⁺ and FADH2 used to be overrated so that one should not be confused when there is talk of a sum yield of 12 molecules of ATP.

Mnemonic for the Citric Acid Cycle

The following mnemonic helps to remember the steps of the citric acid cycle:

Can intelligent Karen solve some foreign Mafia operations?

- Can = Citrate
- Intelligent = Isocitrate
- Karen = Alpha-Ketoglutarate
- Solve = Succinyl-CoA
- Some = Succinate
- Foreign = Fumarate
- Mafia = Malate
- Operations = Oxaloacetate

Regulation of the Citric Acid Cycle

The citric acid cycle is mainly regulated by the following three factors:

1. The supply of the substrates, which include the cofactors NAD⁺ and FAD
2. The formation of products
3. The inhibition via feedback

The following table illustrates how far the individual enzymes of the citric acid cycle are activated or inhibited.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activation by</th>
<th>Inhibition by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate synthase</td>
<td>ADP, oxaloacetate, acetyl-CoA (thus, high activity of pyruvate dehydrogenase)</td>
<td>Citrate, NADH+H⁺, ATP, succinyl-CoA</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>ADP, Ca²⁺</td>
<td>ATP, NADH+H⁺</td>
</tr>
<tr>
<td>Alpha-ketoglutarate dehydrogenase</td>
<td>Ca²⁺</td>
<td>Succinyl-CoA, NADH+H⁺</td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>Succinate</td>
<td>Oxaloacetate</td>
</tr>
</tbody>
</table>

Besides pyruvate dehydrogenase as a link between glycolysis and the citric acid cycle, isocitrate dehydrogenase seems to have the greatest influence on the activity of the citric acid cycle.

Since the citric acid cycle also synthesizes intermediate products that are needed for other metabolic pathways, it has to be ensured that individual reactions of the cycle occur, even if the whole cycle is inhibited. Thus, there is no key enzyme for the citric acid cycle. Hormones have no direct influence on the enzymes of the citric acid cycle.

Anaplerotic Reactions

Anaplerotic reactions are metabolic pathways which supply the citric acid cycle so that it does not lack the necessary substrates. For illustration: If the body heavily
performs gluconeogenesis and thus needs a lot of oxaloacetate out of the citric acid cycle, the cycle would lack the oxaloacetate for the first reaction step (acetyl-CoA + oxaloacetate → citrate).

For this life-threatening situation not to occur, there are the anaplerotic reactions. For the exams, the pyruvate carboxylation reaction is relevant: \( \text{pyruvate} + \text{CO}_2 + \text{ATP} \leftrightarrow \text{oxaloacetate} + \text{ADP} + \text{P}. \) So, the enzyme pyruvate carboxylase catalyzes the transformation of pyruvate and carbon dioxide to oxaloacetate under ATP usage. This ensures that oxaloacetate is constantly present as a substrate for the citric acid cycle.

**Glyoxylate Cycle**

**Overview of the glyoxylate cycle**

**Isocitrate lyase**
Malate synthase

Input 4 carbons (2-Acetyl-CoA)
Releases 0 CO₂ molecules
Produces one extra oxaloacetate
Two oxidations
1 NADH, 1 FADH₂ 1 (extra) oxaloacetate per turn of cycle
Net synthesis of glucose from Acetyl-CoA

The Citric Acid Cycle as the Amphibolic Center of the Intermediate Metabolism

First of all, we have to ask: “What does amphibolic mean?” Amphibolic is a term used to refer to metabolic pathways that are both catabolic and anabolic.
**Amino acid metabolism:** A lot of amino acids are degraded to substrates of the citric acid cycle. In the same way, they serve as substrates for the synthesis of other amino acids, e.g., for the synthesis of nonessential amino acids like glutamate and aspartate. *Glutamate* is created via a transamination out of alpha-ketoglutarate and *aspartate* via a transamination out of oxaloacetate.

**Carbohydrate metabolism:** On one hand, glycolysis leads to the citric acid cycle via the link of the pyruvate dehydrogenase. On the other, oxaloacetate is a substrate for gluconeogenesis. Here, one should never forget that, due to the irreversibility of the *pyruvate dehydrogenase reaction*, acetyl-CoA itself can never serve as a substrate for gluconeogenesis.

**Fatty acids and steroids:** With acetyl-CoA, beta-oxidation produces the basic substrate of the citric acid cycle and, simultaneously, citrate serves for the synthesis of fatty acids and cholesterol or steroids.

One must not forget that the synthesis of *porphyrins or heme* also depends on the citric acid cycle, i.e., its intermediate product succinyl-CoA.

**Cell Respiration**

Most ATP is made during chemiosmosis actual yields vary.
Cellular respiration occurs in 4 phases:

1. **Glycolysis**
   - Glucose to 2 ATP
   - 2 NADH

2. **Pyruvate Oxidation**
   - 2 NADH
   - 1 NADH
   - 2 FADH₂

3. **Krebs Cycle**
   - 6 NADH
   - 2 FADH₂

4. **Chemiosmosis**
   - 2 ATP
   - 5 ATP
   - 3 ATP

**ATP Yields / Glucose is Contentious**
- 2.5 ATP per NADH
- 1.5 ATP per FADH
- 2 ATP per glycolysis

**Efficiency**
- Glucose = 686 kcal/mol
- ATP = 7.3 kcal/mol

\[
\text{Efficiency} = \frac{7.3 \text{kcal/mol} \times (30 \text{ATP})}{686 \text{kcal/mol}} = 32\%
\]
Mitochondria are considered the power plant of the cell.

Review Questions

Solutions can be found below the references.

1. Some enzymes of the citric acid cycle use NAD\(^+\) as coenzymes. Which constellation is correct?

A. Isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase
B. Isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase, malate-dehydrogenase
C. All dehydrogenases of the citric acid cycle
D. Aconitase, succinate dehydrogenase, alpha-ketoglutarate dehydrogenase
E. Isocitrate dehydrogenase, succinyl-CoA synthetase, succinate dehydrogenase
2. Which one is not a cofactor of alpha-ketoglutarate dehydrogenase?
   
   A. NAD+  
   B. FAD  
   C. Coenzyme A  
   D. Mg2+  
   E. Thiamine

3. Which statement concerning the citric acid cycle is wrong?
   
   A. The citric acid cycle takes place in the matrix space of the mitochondria.  
   B. Alpha-ketoglutarate dehydrogenase is the key enzyme of the citric acid cycle.  
   C. There is no ATP formed in the citric acid cycle.  
   D. Hormones do not directly regulate the citric acid cycle.  
   E. It is also referred to as Krebs cycle.

References


Correct Answers: 1B, 2D, 3B

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