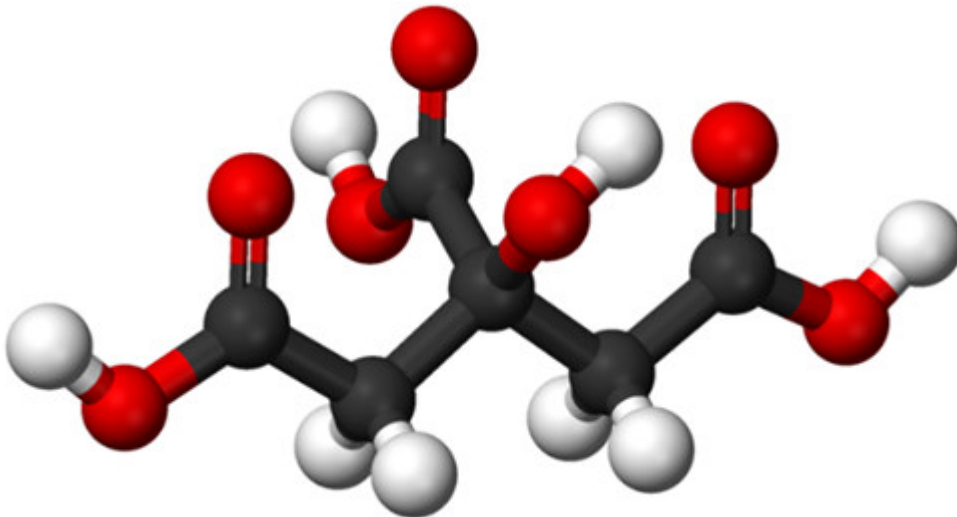


Citric Acid Cycle (CAC; Tricarboxyl Acid Cycle; Krebs Cycle) – Definition and Glyoxylate Cycle

[See online here](#)

As the final stage of food utilization, the citric acid cycle is primarily responsible for many anabolic and catabolic 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.





Video Course: Carbohydrate Metabolism

In this course, Dr. Kevin Ahern will guide you through the complex process of carbohydrate metabolism. He will comprehensively cover the step-by-step reactions of various metabolic pathways.

Definition of the Citric Acid Cycle

The citric acid cycle is also referred to as the Krebs cycle or tricarboxylic acid cycle (TCA), and it 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” because 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.

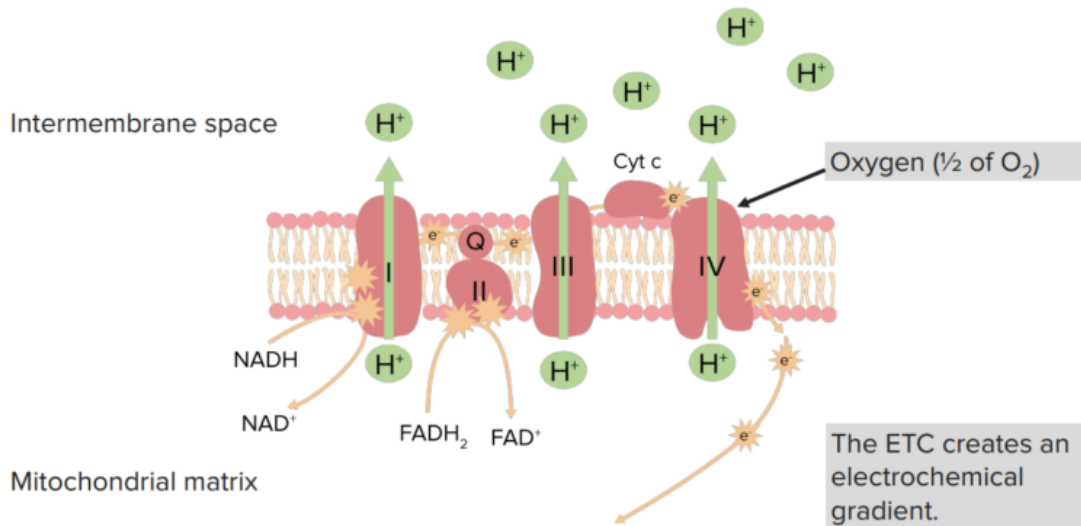
In addition, the citric acid cycle has the following functions:

- It is the final path of the **degradation of amino acids**, which cannot be degraded to acetyl-CoA or pyruvate.
- It produces **substances for resynthesis 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



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 because they are often asked for in oral and written exams.

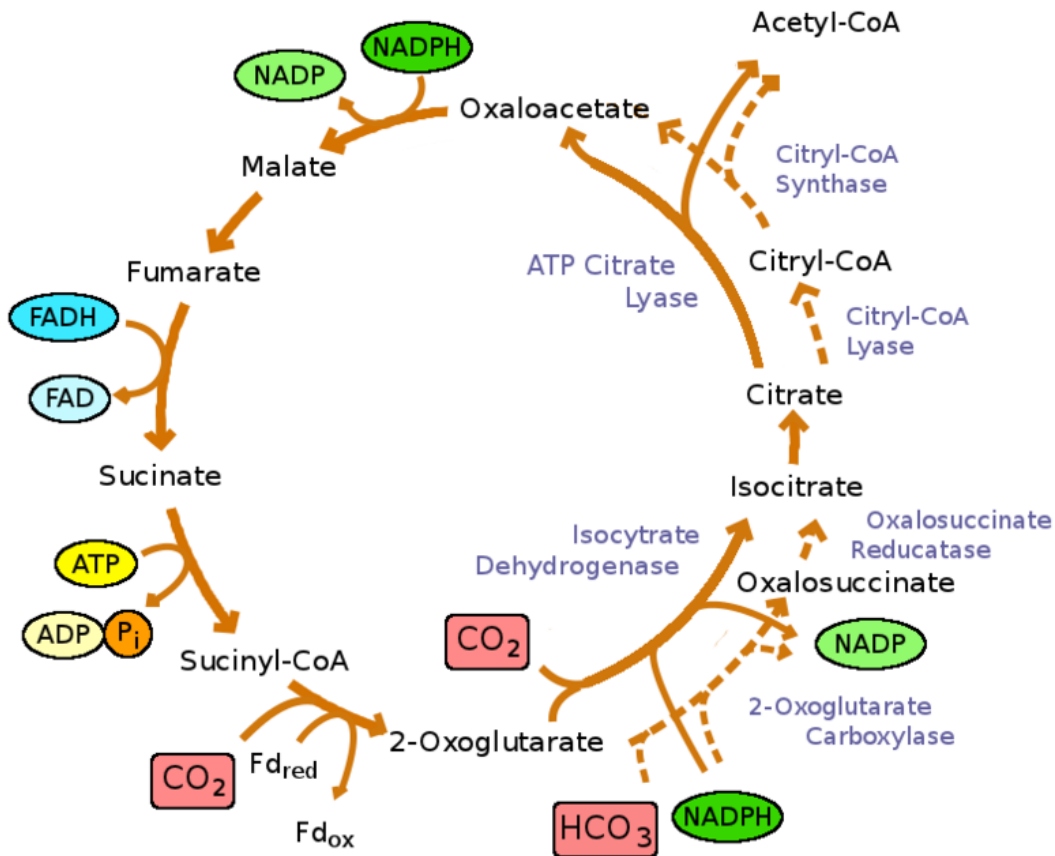


Image: "The reductive TCA cycle. Dotted lines denote alternative enzymes pathways." by Andrewgross. License: [CC BY-SA 3.0](https://creativecommons.org/licenses/by-sa/3.0/)

Step 1: Acetyl-CoA + Oxaloacetate → Citrate

Citrate synthase catalyzes the transfer of acetyl-CoA to oxaloacetate with the formation of citrate. H_2O is inserted and coenzyme A is split off. As a result, the high-energy thioester-bond of acetyl-CoA is hydrolyzed.

Step 2: Citrate → Isocitrate

Aconitate hydratase, also called aconitase, transforms citrate to isocitrate. Via shifting of one OH group, the tertiary alcohol becomes a secondary one. The intermediate substance formed by this isomerization is referred to as cis-aconitate.

Step 3: Isocitrate → α -Ketoglutarate

Isocitrate dehydrogenase catalyzes the NAD^+ -dependent oxidation of isocitrate. In this process, the unstable intermediate substance oxalosuccinate forms, which then spontaneously **decarboxylates** 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 **CO_2** .

Step 4: α -Ketoglutarate → Succinyl-CoA

α -ketoglutarate dehydrogenase is a large enzymatic complex that is very similar to pyruvate dehydrogenase. For the **oxidative decarboxylation** of α -ketoglutarate to

succinyl-CoA, the following cofactors are needed: thiamine pyrophosphate, liponamide, coenzyme A, FAD, and NAD^+ . Again, CO_2 and **1 more $\text{NADH} + \text{H}^+$** form for the respiratory chain.

In case of a **lack of thiamine** (e.g., caused by malnutrition due to alcoholism), **Wernicke's encephalopathy** occurs because both α -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 \rightarrow 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: $\text{GTP} + \text{ADP} \rightarrow \text{GDP} + \text{ATP}$. **However, this reaction itself is not part of the citric acid cycle.**

Step 6: Succinate \rightarrow Fumarate + FADH_2

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 FADH_2** .

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 FADH_2 and is therefore called complex II.**

Step 7: Fumarate + H_2O \rightarrow Malate

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

Step 8: Malate \rightarrow 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 $\text{NADH} + \text{H}^+$** is formed for the respiratory chain.

All in all, the energetic yield of one cycle is 3 $\text{NADH} + \text{H}^+$, 1 FADH_2 , 1 GTP. The two 2 CO_2 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 $\text{NADH} + \text{H}^+$** is transformed to approximately **2.5 ATP**.
- **1 FADH_2** is transformed to approximately **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 about 10 ATP.

In the literature, the energetic yield of NADH + H⁺ and FADH₂ used to be overestimated so 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 one 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 the individual enzymes of the citric acid cycle are activated or inhibited.

Enzyme	Activation by	Inhibition by
Citrate synthase	ADP, oxaloacetate, acetyl-CoA (thus, high activity of pyruvate dehydrogenase)	Citrate, NADH + H ⁺ , ATP, succinyl-CoA
Isocitrate dehydrogenase	ADP, Ca ²⁺	ATP, NADH + H ⁺
Alpha-ketoglutarate dehydrogenase	Ca ²⁺	Succinyl-CoA, NADH + H ⁺
Succinate dehydrogenase	Succinate	Oxaloacetate

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.

Because 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.**

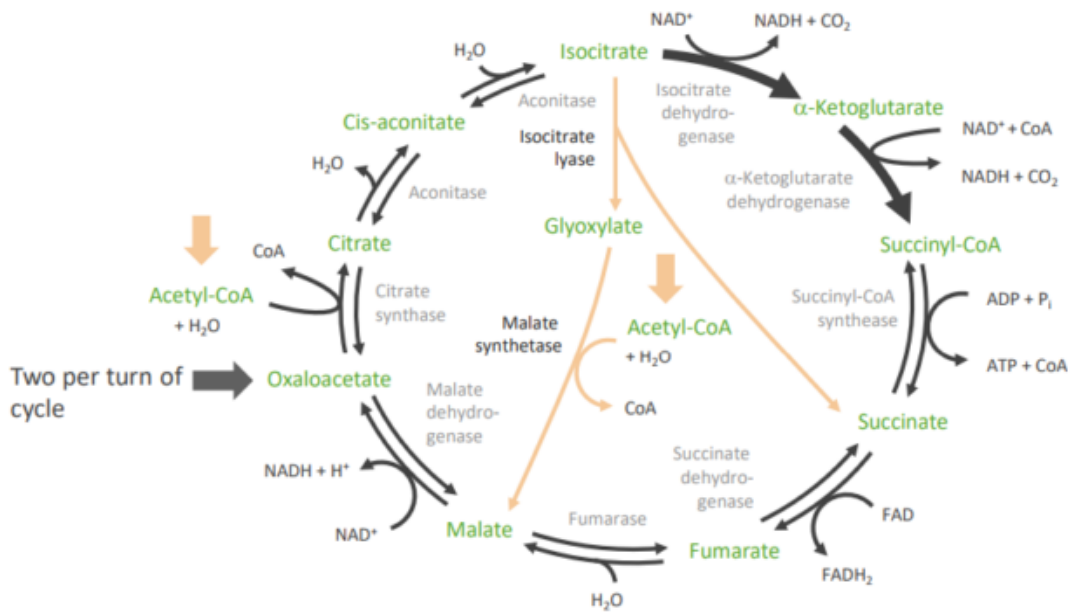
Anaplerotic Reactions

Anaplerotic reactions are **metabolic pathways that supply the citric acid cycle so it does not lack the necessary substrates.** For illustration: If the body heavily performs

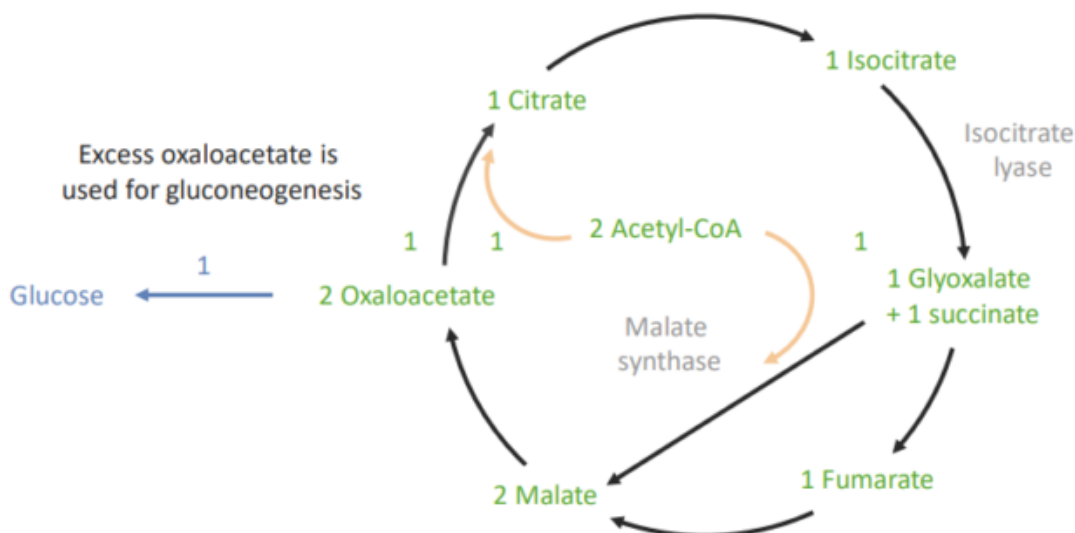
gluconeogenesis and thus needs a lot of oxaloacetate from the citric acid cycle, the cycle would lack oxaloacetate for the first reaction step (acetyl-CoA + oxaloacetate → citrate).

For this life-threatening situation not to occur, there are the anaplerotic reactions. For the exams, the pyruvate carboxylation reaction is relevant: **pyruvate + CO₂ + ATP ↔ oxaloacetate + ADP + 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

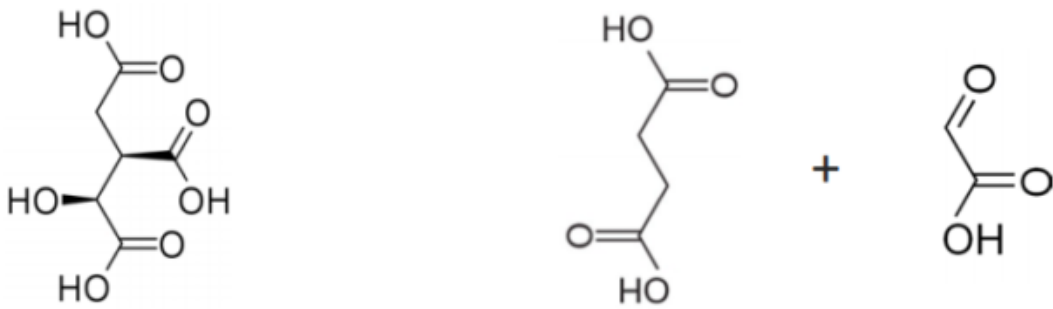


Overview of the glyoxylate cycle



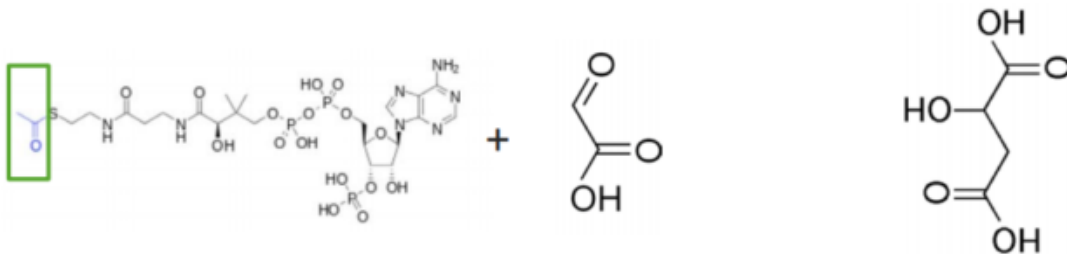
Isocitrate lyase

Isocitrate \longrightarrow Succinate + Glyoxylate



Malate synthase

Acetyl-CoA + Glyoxylate \longrightarrow L-Malate

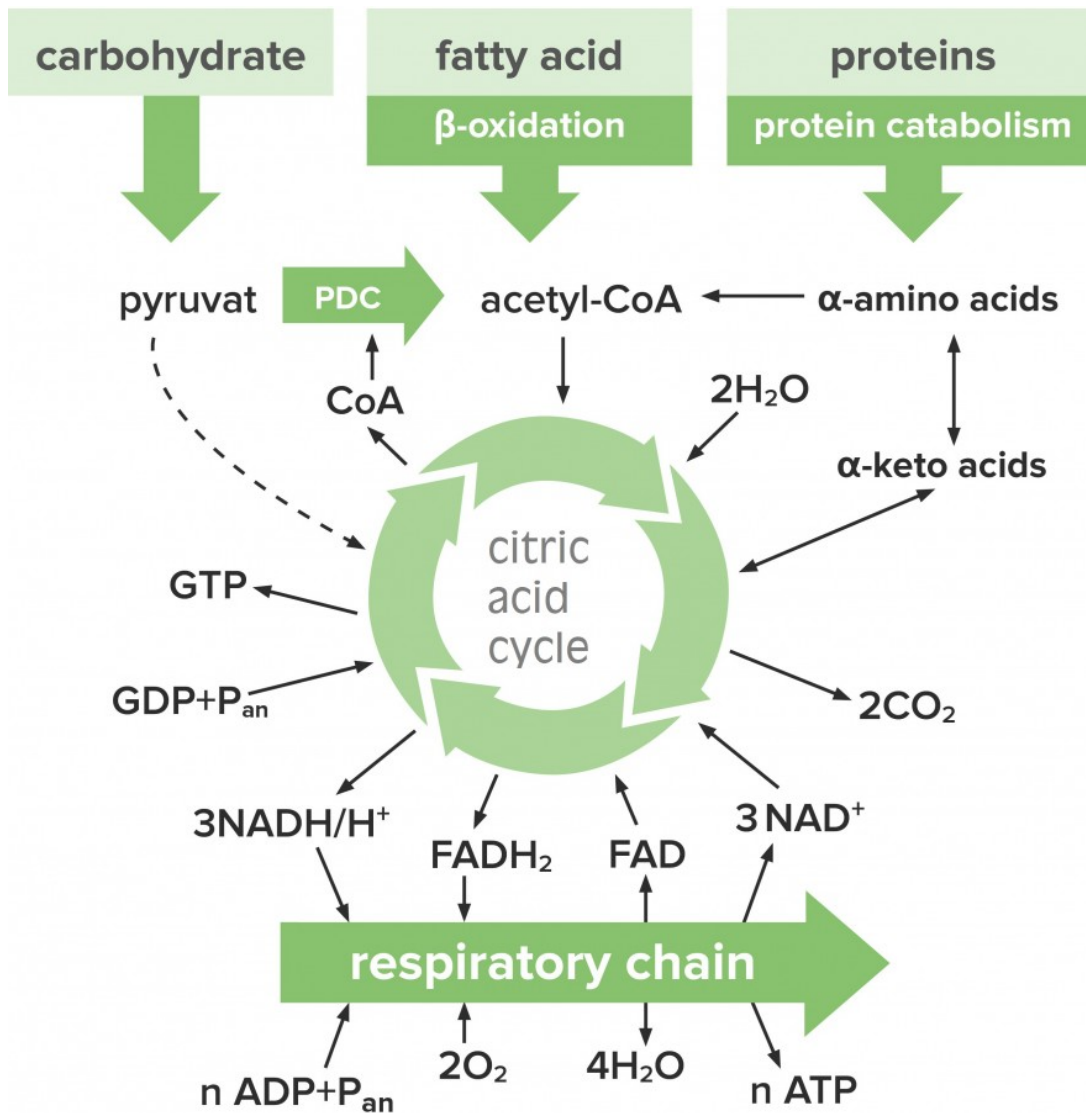


Glyoxylate cycle summary

- 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 must 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 of alpha-ketoglutarate and **aspartate via transamination** of oxaloacetate.

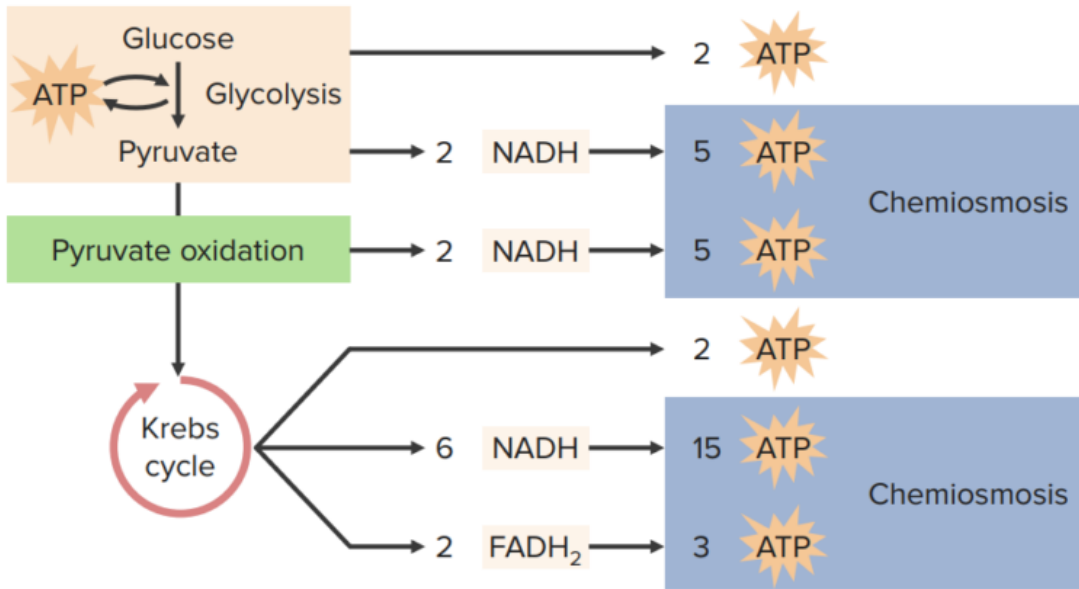
Carbohydrate metabolism: On one hand, glycolysis leads to the citric acid cycle via the link of the pyruvate dehydrogenase. On the other, oxaloacetate is a substrate for gluconeogenesis. Here, one should never forget that, due to the irreversibility of the **pyruvate dehydrogenase reaction**, acetyl-CoA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



ATP yields / glucose is contentious

- 2.5 ATP per NADH
- 1.5 ATP per FADH
- 2 ATP per glycolysis

= 28 ATP (Red box)

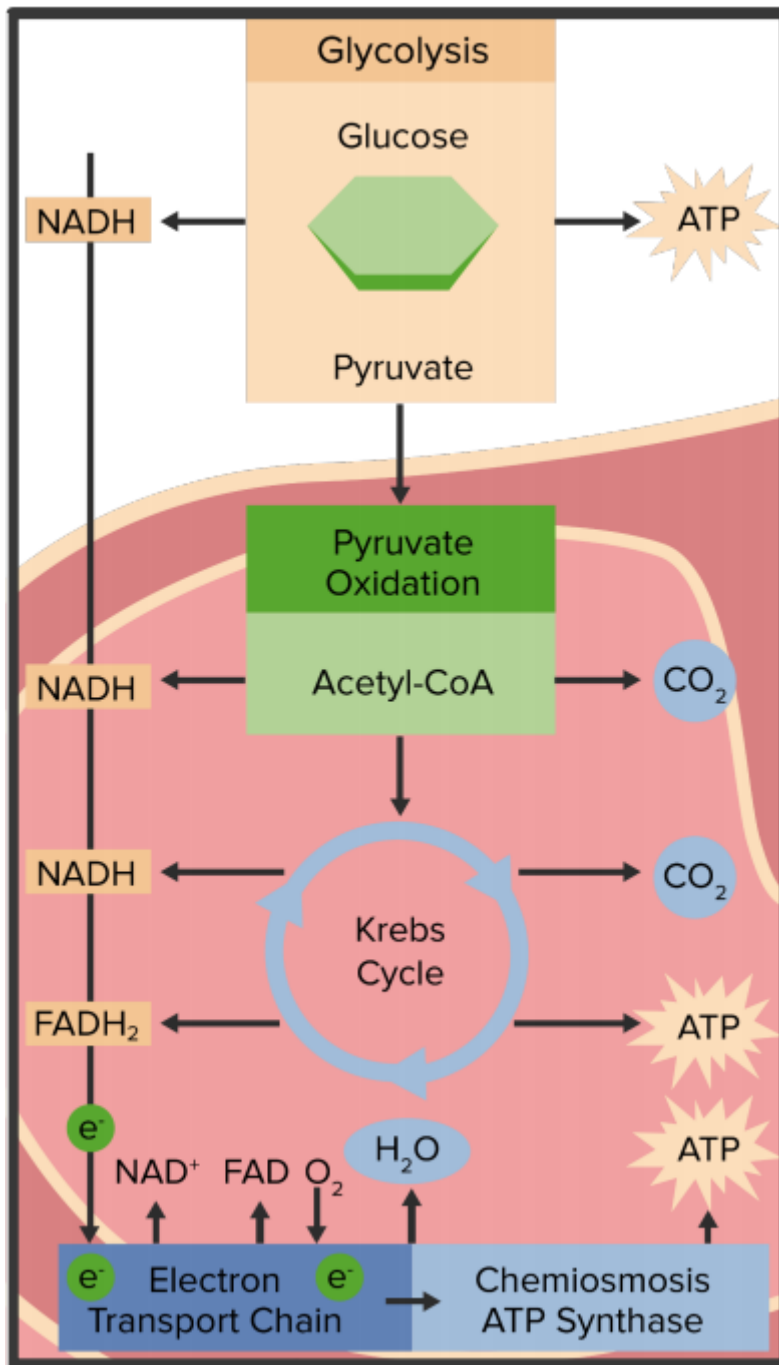
= 30 ATP (Green box)

Efficiency

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

$$\frac{7.3 \frac{\text{kcal}}{\text{mol}} (30\text{ATP})}{686 \text{ kcal/mol}} = 32\%$$

Cellular respiration occurs in 4 phases



Mitochondria are considered the power plant of the cell.

Review Questions

The correct answers can be found below the references.

1. Some enzymes of the citric acid cycle use NAD⁺ as coenzymes. Which of the following 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.



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Lloyd, A. J. (1990). *Citric acid cycle enzymes of Methylophilus methylotrophus*. University of Bath.

Lowenstein, J. M. (1969). *Citric Acid Cycle*. New York [usw.: Dekker.]

Sandberg, N. M., & Ward's Natural Science Establishment, inc. (1971). *Introduction to the krebs cycle*. Rochester, NY: Ward's Natural Science Establishment.

Correct Answers: 1B, 2D, 3B

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Notes