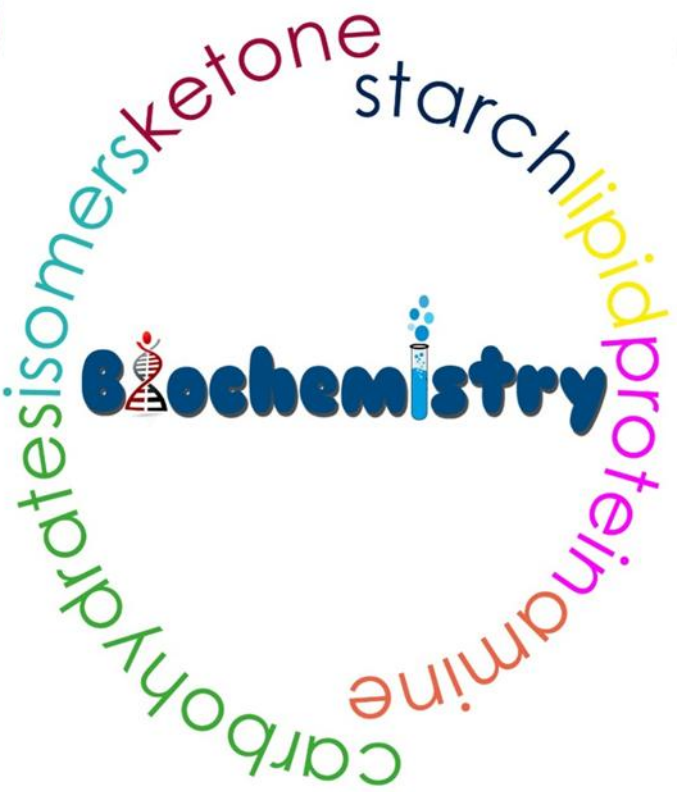


Subject:	TCA cycle
Done by:	Nada Al-Antary
Corrected by:	Sufian Alhafez
Number:	6

The Citric Acid Cycle

Tricarboxylic Acid (TCA) cycle or the Krebs cycle (3rd stage in energy production):

Citric acid cycle→ Referring to the first intermediate produced in the cycle.

TCA cycle → It has an acid that has three carboxyl groups (Citric acid).

Krebs cycle → Named after the scientist who was responsible for elucidating this pathway, Hans Krebs.

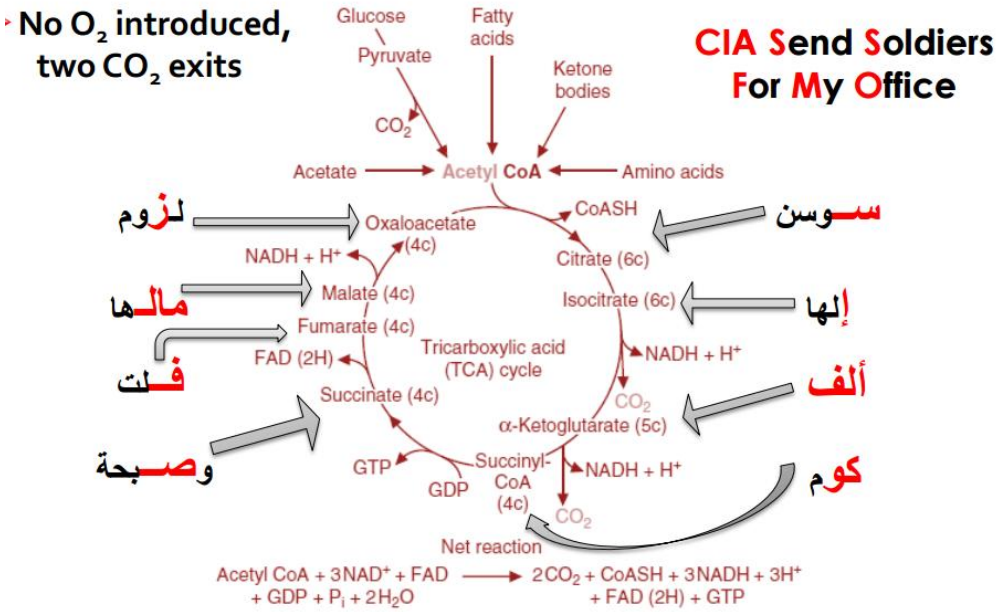
- TCA cycle is needed to extract electrons that are carried by NAD+ and FAD

FAD	NAD+
- Single electrons (H•), different sources. - Succinate to fumarate, lipoate to lipoate disulfide in a-KG. - FAD must remain tightly, sometimes covalently, attached to its enzyme. - E^0 for enzyme-bound FAD varies.	- Pair of electrons (H-), same source. - Alcohols to ketones by malate dehydrogenase & isocitrate dehydrogenase - NADH plays a regulatory¬ role in balancing energy metabolism

Remember that the **two** Pyruvate molecules that are produced from glycolysis, are later converted into **two** molecules of acetyl CoA by a multi-enzyme complex, which then enters the Krebs cycle

- The cycle has eight steps, each catalyzed by a specific enzyme (which you should know, you can use the following figure (1) for easier memorization)

Figure(1)



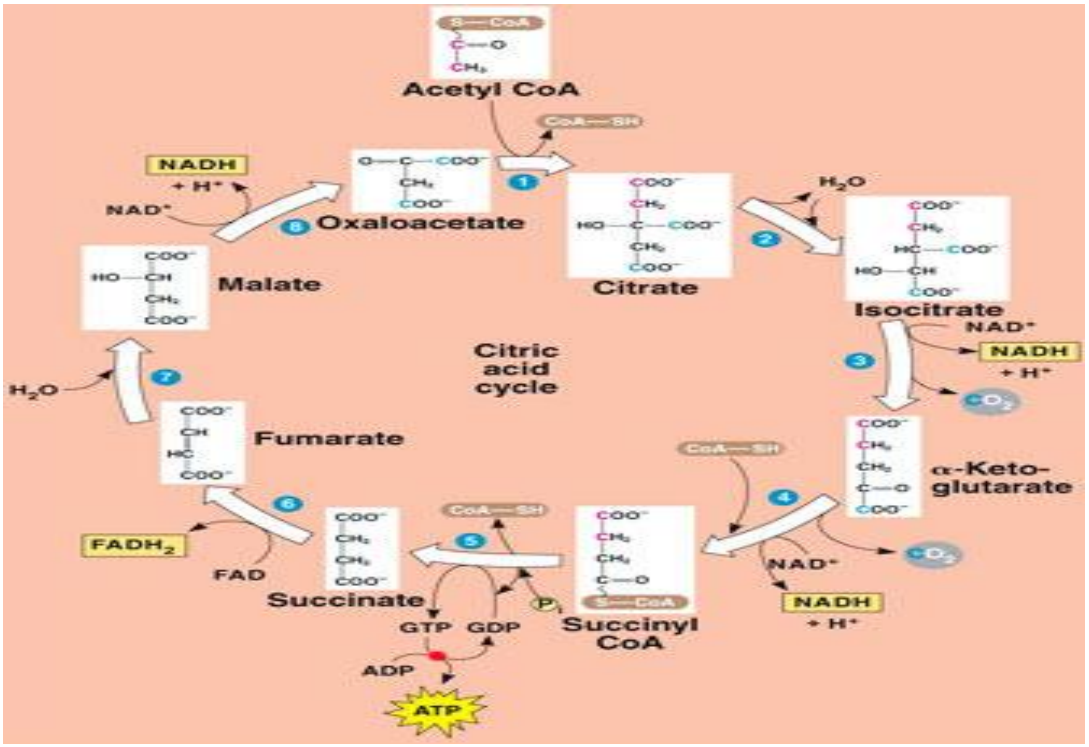


Figure (2)

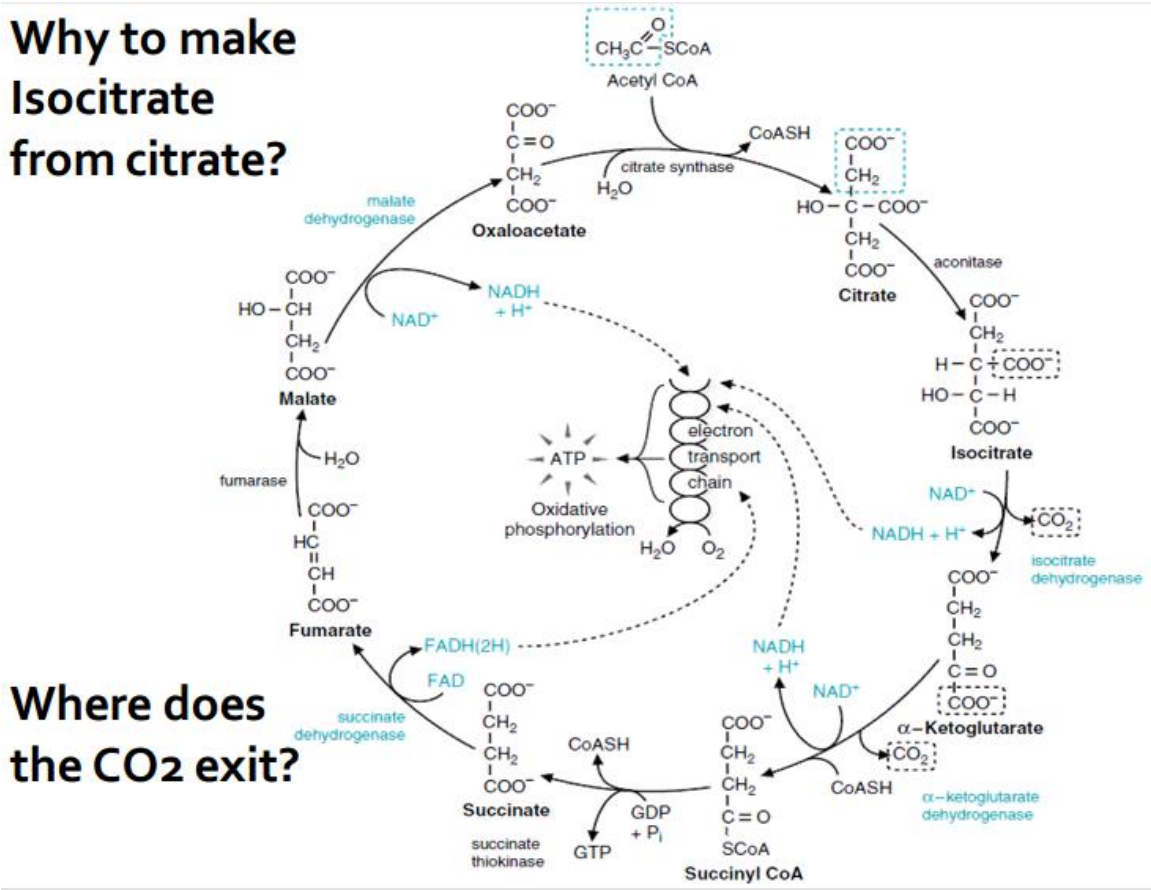


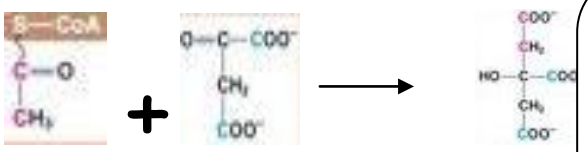
Figure (3)

Step 1:

Acetyl CoA (that has entered the cycle after conversion of pyruvate) adds its two-carbon acetyl group in the form of **acetate** (after breaking CoA-SH) to **oxaloacetate** (a Keto acid that has four carbon molecules), producing **citrate** (six - carbon molecule).

- The enzyme that catalyzes this step is **citrate synthase**.

Acetyl-CoA + **oxaloacetate** + H2O → **citrate** + CoA-SH



Notice that, we can divide the cycle into two halves, each includes four reactions, in which the first half starts with a six-carbon molecule and works to give a four- carbon molecule, by losing two carbons in the form of CO2, in order to go back to the first molecule, we started the cycle with (Oxaloacetate). And the second half includes rearrangements of the atoms in the four carbon molecule (from the first half) to get Oxaloacetate again.

- This reaction is an anabolic step, which means it requires energy. So, the energy used in this reaction is the one produced from breaking Co enzyme (A) from acetyl CoA.

Minute: 00:00-10:00

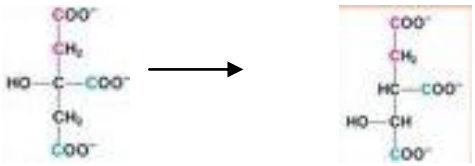
Why to make isocitrate from citrate?

An oxidation reaction has to occur next, in order to get ATP and other molecules, but citrate can't be further oxidized since it is a tertiary alcohol and it already has three carboxyl groups, which represents the highest oxidation state this molecule can establish. Therefore, citrate has to be converted to another isomer that can be oxidized, which's isocitrate. So we move on to step 2.

Step 2:

Citrate is converted to its isomer **isocitrate**, by removal of one water molecule and addition of another, which results in rearrangement of the hydroxyl group (removed from carbon number 3 and added to carbon number 2 to give a secondary alcohol):

- In citrate → -OH is attached to a carbon that's attached to three other carbons (tertiary alcohol that can't be oxidized)
  In isocitrate → -OH is attached to a carbon that's attached to two other carbons (secondary alcohol) which can be oxidized to a ketone group.



- The enzyme that catalyzes this step is **aconitase**:
  The name of this enzyme refers to the intermediate aconitate that the reaction passes through when converting citrate into isocitrate.

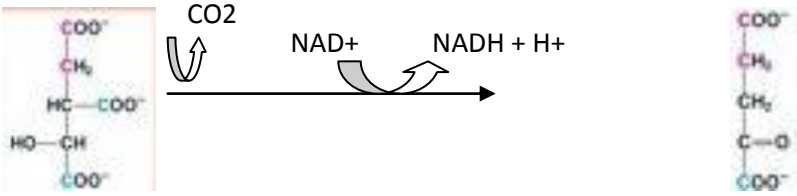
- The equilibrium in this reaction favors citrate over isocitrate (20:1). This is considered as a key point of regulation of Krebs cycle, which means the following:

  1- If the concentration ratio was less than 20:1 (Citrate: Isocitrate), formation of isocitrate (step 2) doesn't happen. There the reaction is reversed, where isocitrate is converted into citrate. (Equilibrium favors the backwards reaction).
  2- The concentration of citrate has to be very high in order for step 2 to occur and for the cycle to go on.
  3- When citrate reaches high concentrations it goes out of the mitochondria into the cytosol, and there it inhibits glycolysis, because since there is high concentration of citrate, there's no need to form Acetyl CoA.
  4- Also, when citrate enters the cytosol it participates in lipid biosynthetic pathway. (When the body has enough energy, it works to inhibit glycolysis, where glucose is stored as glycogen, and citrate is used in fat making).
  5-When citrate is high in concentration it inhibits citrate synthase (competitive inhibitor) in a feedback mechanism.
  As we've seen citrate participates in many metabolic pathways, that's why it is important for regulation.

So far, we have six-carbon molecule (via the first two steps). So, in the next two steps we need to lose 2 carbon molecules in order to get a four – carbon molecule.

Step 3:

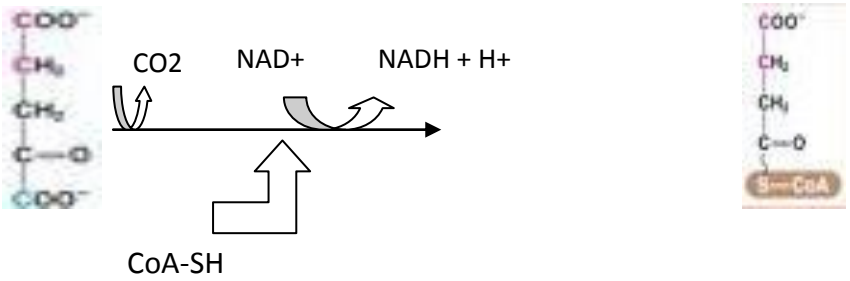
Isocitrate loses a carbon in the form of ***CO2*** molecule (decarboxylation reaction) and the resulting compound is oxidized to form **α-Ketoglutarate** (Five-carbon molecule) while reducing NAD+ to **NADH** (The first Oxidative-decarboxylation reaction).



- The enzyme that catalyzes this reaction is **isocitrate dehydrogenase**.

Step 4:

α- Ketoglutarate loses another **CO2** (decarboxylation) and the resulting compound is oxidized, while reducing NAD+ to **NADH**. The remaining molecule is immediately attached to Coenzyme A, forming **Succinyl CoA**. (The second Oxidative-decarboxylation reaction).

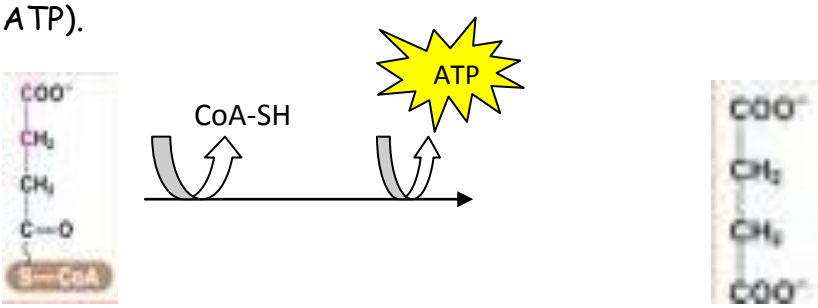


- The enzyme that catalyzes this reaction is **α- Ketoglutarate dehydrogenase**.

Now that we have a four-carbon molecule, the following four reactions work to rearrange the atoms to form the starting molecule, **Oxaloacetate**.

Step 5:

CoA is broken from **Succinyl CoA**, forming **Succinate**, this bond breaking results in production of energy, which is used to join an inorganic phosphate group to GDP forming **GTP** or to ADP forming **ATP** (by **substrate – level phosphorylation**, where no oxygen is used to produce ATP).



- The enzyme that catalyzes this reaction is **Succinate thiokinase.**

Minute: 10:00-20:00

Step (6-8) Succinate differs from oxaloacetate in the fact that it's missing an oxygen (of the carbonyl group on C2). So, in order to convert the CH2 of succinate to a carbonyl group of the oxaloacetate:

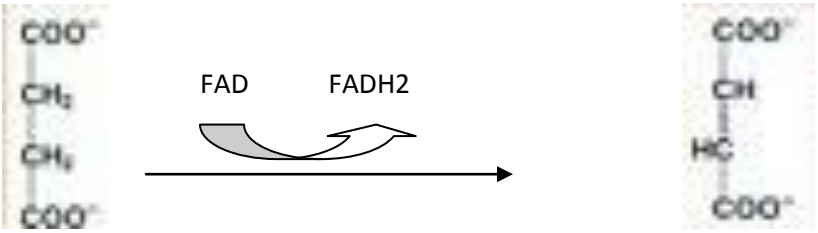
1- Forming a double bond (between C2 and C3) by an **oxidation** reaction (dehydrogenation). Step **6**

2- Converting the double bond to a hydroxyl group by addition of H2O (**Hydration** reaction), where the –OH binds to C2 and the –H binds to C3. Step **7**

3- Converting the hydroxyl group on C2 to a carbonyl group by an **oxidation** reaction (dehydrogenation). Step **8**

Step 6:

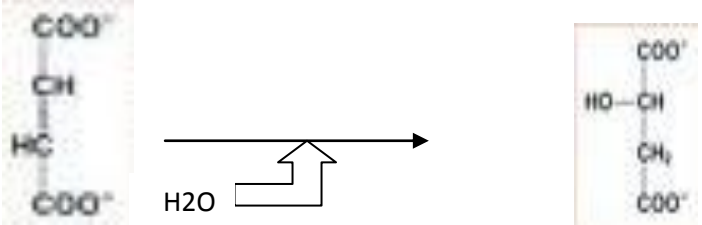
Succinate is **oxidized** to form **Fumarate**, by transferring two hydrogens to FAD, (Reduction) to form FADH2



- The enzyme that catalyzes this reaction is **Succinate dehydrogenase**.

Step 7:

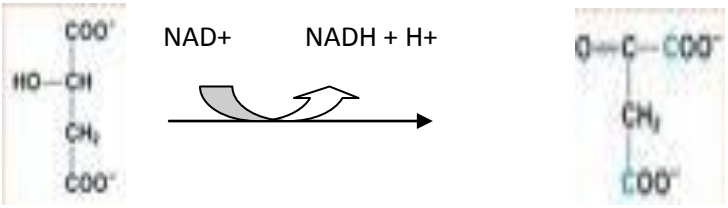
Addition of a water molecule to **Fumarate** (hydration), which rearranging the bonds in the substrate to form **Malate**



- The enzyme that catalyzes this reaction is **fumarase**.

Step 8:

Malate is oxidized to regenerate **oxaloacetate**, while reducing NAD+ to **NADH**



- The enzyme that catalyzes this reaction is **Malate dehydrogenase**.

So we have by **one turn** of the cycle:

- 3 NADH
- 1 FADH2
- 1 GTP (or ATP)
- 2 CO2

Notice that,

The two carbon molecules of acetate that enter the cycle (colored in red in Fig 2) are **not** the same as the two carbon molecules that exit the cycle in the form of 2 CO2 in step 3 and 4 (colored in blue in Fig 2), but in subsequent cycles they will leave.

- No O2 is introduced to the cycle.
- Theoretically, this pathway is reversible. However, practically Krebs cycle goes in one way only (irreversible) due to the highly negative free energy (ΔG).

General information related to the cycle:

- **Aconitase Inhibition:**

Aconitase is inhibited by fluoroacetate (rat poison):

Fluoroacetate (acetate with F atom) is attached to CoA to form **fluoroacetyl CoA**, which then reacts with **Oxaloacetate** to form

fluorocitrate. Then, fluorocitrate is attached to the active site of **Aconitase**, resulting in inhibition of the function of Aconitase (to convert **Citrate** to its isomer **isocitrate**), so with no isocitrate, Krebs cycle will not continue, no energy produced.

fluoroacetyl CoA + Oxaloacetate →fluorocitrate (citrate accumulation).

Minute: 20:00-30:00

- **Conversion of Isocitrate to α- Ketoglutarate (step 3) is the rate limiting step**

It's the key reaction in Krebs cycle, in which **isocitrate dehydrogenase** (the enzyme that catalyzes this reaction) is the **only** enzyme that's getting allosteric regulation by **ADP** (it is activated by ADP)

(*ATP: ADP ratio is an indication of the energy level in the cell, which's important for regulation. We will take further details regarding this topic in upcoming lectures)

- **α- Ketoglutarate to Succinyl CoA (Step 4)**

  ➔ catalyzed by **α- Ketoglutarate dehydrogenase complex**

  Complex means: Multiple subunits of more than one enzyme connected together, used in enzyme regulation.

  ➔ Changes that occur when converting **α- Ketoglutarate to Succinyl CoA:**

  - Decarboxylation, so there's a need for a decarboxylase.
  - Oxidation, so there's a need for a dehydrogenase.
  - Attachment of CoA-SH, so there's a need for an enzyme to catalyze this change (reaction). Remember that CoA-SH works as an acyl – transfer group.

Further illustration,

As shown in Figure (4),(next page):

- **a- Ketoglutarate** is the substrate in RXN 1; E1 is the first enzyme in the complex and it works as a **decarboxylase**, in which there's a removal of carbon from **a- Ketoglutarate** in the form of CO_2, resulting in a four – carbon molecule with a very reactive carbonyl group.

- **The enzyme that works as a decarboxylase in the a-Ketoglutarate dehydrogenase** needs a coenzyme; which is Thiamine pyrophosphate (TPP, the active form of vitamin B1).

- **So,** immediately after losing CO_2, the previously mentioned reactive carbonyl (part of the four- carbon molecule) binds with **TPP**. As you know, the enzyme (along with its coenzyme) seeks to go back to its original form after changes (where there's no carbonyl attached), therefore, the four –carbon molecule detaches from E1 moving to the next enzyme E2 in the complex.

- E2 is a transacylase (transfers the acyl group).E2 works with a coenzyme named **lipoate**. lipoate has a disulfide bond in its structure. So, after releasing the four –carbon molecule from TPP, it attaches to one of the sulfur groups (after breaking the disulfide bond) while the other sulfur of the bond is reduced (H is added) to form a thiol group, and since this is not the original form of lipoate, it releases the four –carbon molecule.

- Again, the reactive carbonyl group of the molecule immediately binds to CoA-SH to form **Succinyl CoA**.

- After E2 releases the carbons, it still has two thiol groups, so in order to regain the original form and reform the disulfide bond, E2 loses two hydrogens to **E3** which is a dehydrogenase.

- E3 (dehydrogenase) uses **FAD** as a coenzyme. FAD is reduced into $FADH_2$ (two hydrogens are added from E2). Since this is not the original form of the enzyme, it loses two electrons to **NAD^+** (found in the surrounding solution) in the form of hydride to give NADH.

Notice that NAD+ can't take the two hydrogens lost from the thiol groups, so the hydrogens are transferred to FAD first then to NAD+ (hydride ion).

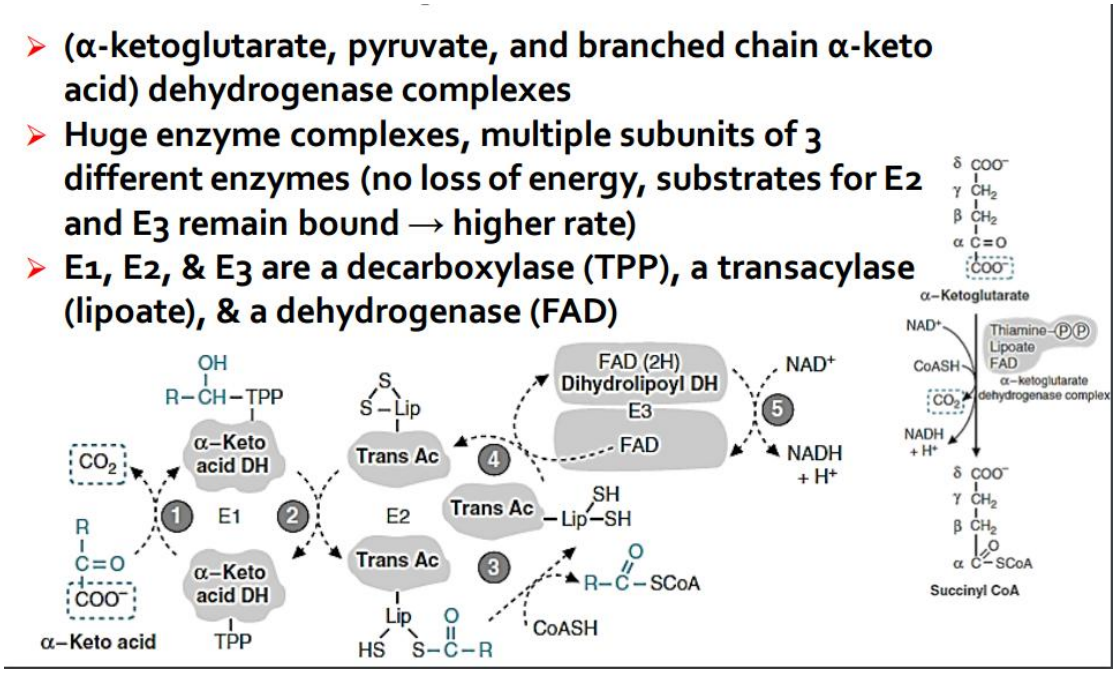


Fig. (4)

Common questions on Citric Acid Cycle:

Q1- How many coenzymes are **involved** in the process of conversion of **α- Ketoglutarate** into **Succinyl CoA**??

A: five → TPP, llpoate, Coenzyme A, FAD, NAD+

Q2- How many coenzymes are **contained** within the enzyme complex (**α- Ketoglutarate dehydrogenase complex**) responsible for conversion of **α-Ketoglutarate** into **Succinyl CoA**??

A: three →TPP, lipoate, FAD (they are part of the enzyme)

*** α- Ketoglutarate dehydrogenase complex** is similar to other enzyme complexes such as:

- Pyruvate dehydrogenase: converts pyruvate, which is a three-carbon molecule, to acetyl Co-A, which is a two – carbon molecule. During this reaction; acetyl Co-A, NADH and CO2 are produced. On the other hand, **α- Ketoglutarate**

dehydrogenase converts **α- Ketoglutarate** (five carbons) to Succinyl CoA (four carbons). During this reaction; Succinyl Co-A, NADH and CO2 are produced.

- Branched chain α- Keto acid dehydrogenase: Involved in amino acid metabolism, produces NADH, CO2 and a product with Co-A. (will be taken later in details)

- **Thiamine Pyrophosphate deficiency**
  - ❖ Vitamin B1 (or the active form TPP) deficiency affects all the reactions and enzymes where TPP is involved. Example, E1 in **α- Ketoglutarate dehydrogenase complex** that works as a decarboxylase.

As a result, the substrate consumption decreases, leading to accumulation of the substrate (high concentration in blood if a sample was taken).

➔ In the given example, **α- Ketoglutarate** (the substrate) will accumulate. Also, Pyruvate dehydrogenase will be affected leading to accumulation of pyruvate (the substrate). In addition to the effect on the branched chain α- Keto acid dehydrogenase, leading to accumulation of branched chain α-Keto acids.

- ❖ TPP deficiency might result in psychosis, including hallucinations and several disorders in the brain (encephalopathy). Because it might affect the main reactions that give energy in the CNS, thus, leading to brain dysfunction and psychosis.

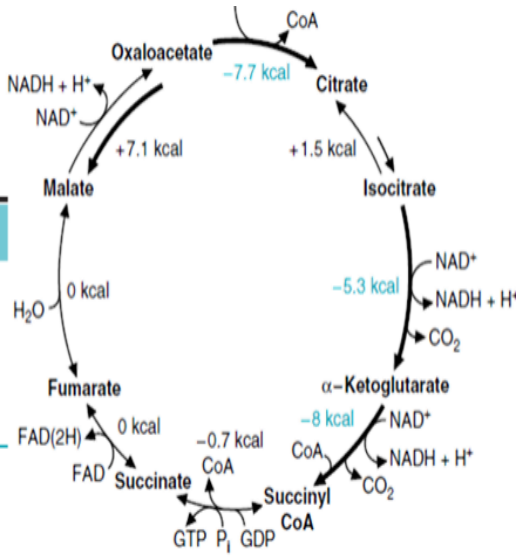
Example

Wernicke-Korsakoff syndrome (an encephalopathy –psychosis syndrome), which could also be a result of alcohol abuse, alcohol consumption affects the absorption of vitamin B1 through the

GI tract, and it affects the conversion of thiamine into its active form TPP.

- **Bioenergetics of the Krebs cycle**

kcal/mole	
3 NADH: 3 × 53	= 159
1 FAD(2H)	= 41
1 GTP	= 7
Sum	= 207



Generally, to find the efficacy:

The actual output / the expected output

- ❖ Citric acid cycle has an efficacy of 90%, better than almost all machines we use in our lives (the best machine has an efficacy of maximum 35%)

To find the efficacy of citric acid cycle, we compare the inputs and outputs:

Input: Acetate → (-228 kcal/mol)

Output: 3 NADH→ (one molecule: 53 Kcal/Mole, three molecules: 53*3=159) 1 FAD(H2)→ 41 Kcal/Mol

GTP →7 Kcal/Mol

Sum: 207 (The actual energy, 90% of the expected energy)

- ❖ Three reactions in the cycle have large (-ve) values (Regulation)
- ❖ Physiologically irreversible, low products.