SHEET 7

METABOLISM

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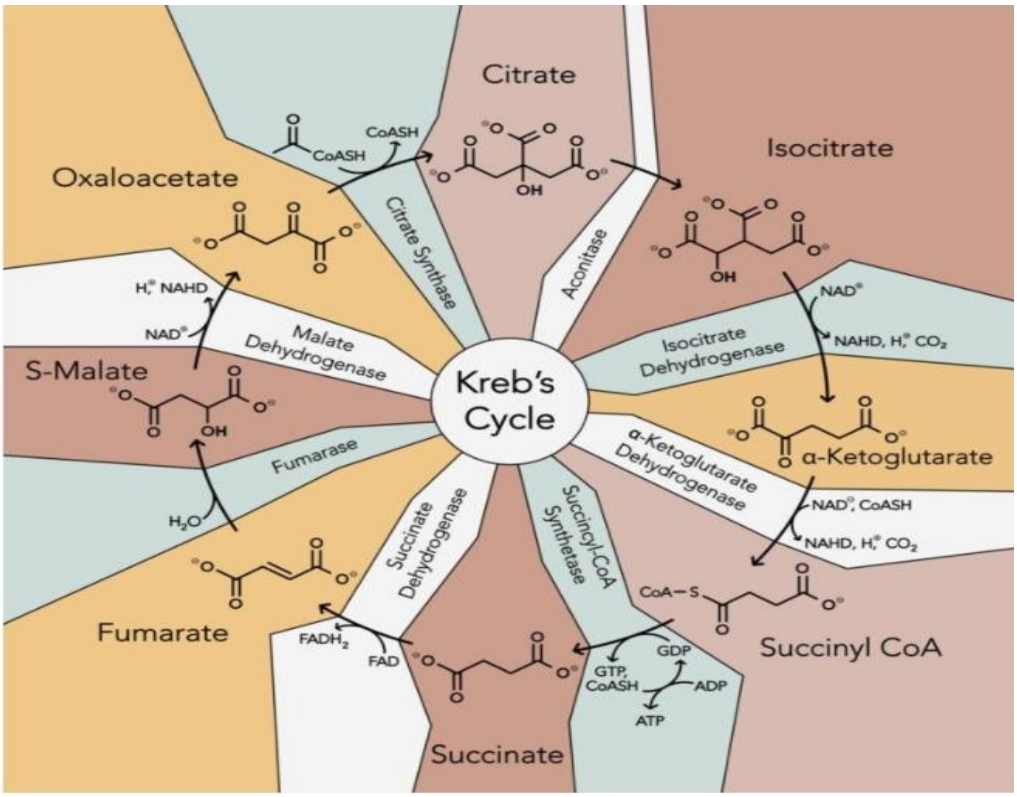
PREVIOUSLY IN METABOLISMS LECTURES:

- **8 Steps.**
- **8 Rxns.**
- **8 Enzymes.**

→ **KREB'S/ TCA/ CITRIC ACID CYCLE.**



-**AcetyleCoA** comes to the cycle from external sources.

-**CoA** leaves the **Acetyle**, producing energy which is used to couple the **Acetyle**(2C) with the **Oxaloacetate**(6C), forming our important first intermediate **CITRATE**(6C).

-The Citric acid CAN'T be oxidized due to the tertiary Alcohol; thus it's transformed into **Isocitrate**(6C).

-**Isocitrate** then will undergo an oxidative decarboxylation rxn, to become **Alpha-ketoglutarate**(5C).

-Another oxidative decarboxylation rxn happens to the **Alpha-ketoglutarate** so it becomes **Succinyl CoA** (4C). (BOTH RXNs ARE COUPLED WITH PRODUCING CO2 AND NADH).

-The second half journey to reform the **OXALOACETATE** starts with the **Succinyl CoA**, firstly with the removal of the **CoA** (THE ENERGY IS PRODUCED TO FORM GTP), then leaving the molecule as a **Succinate**(4C).

-**Succinate** will be also oxidized to form the **Fumarate**(4C)(remember it's formed to be an alkene, and it's coupled with reducing FAD to FADH2), then **Fumarate** to **Malate**(4C).

-At the end we have our molecule that we were spinning for! **Malate** is oxidized to form the **Oxaloacetate**(4C), which is coupled with reducing NAD+ to NADH/H+.

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*From here we can start our lecture!

We kept talking about enzymes, electron carriers, intermediate molecules and so on in the cycle..

Now we are going to get deeper in the main reason why kreb's cycle occurs, which is ENERGY!

-I'll start with telling you that you have the best machine in the whole world with a 90% efficiency (سبحان الله).

*$e = \frac{\text{Outcome E.}}{\text{Income E.}} \times 100 = \frac{207}{228} \times 100 =$ ~

-The rxns are going in one way, although some of them are REVERSIBLE but it's due to the overall high negative charge in △G.

-What determines how much energy has entered the cycle is Acetate and it's nearly 228 kcal/mole.

-The amount of energy that I'm truly getting from the cycle:

3 NADH = -159 Kcal/mol
1 FADH2 = -41 Kcal/mol
1 GTP/ATP = 7 Kcal/mol
207 Kcal/mol

*NADH donates 2e⁻ to the final e⁻ acceptor O_2 so the ΔG=-53Kcal and we have 3//.

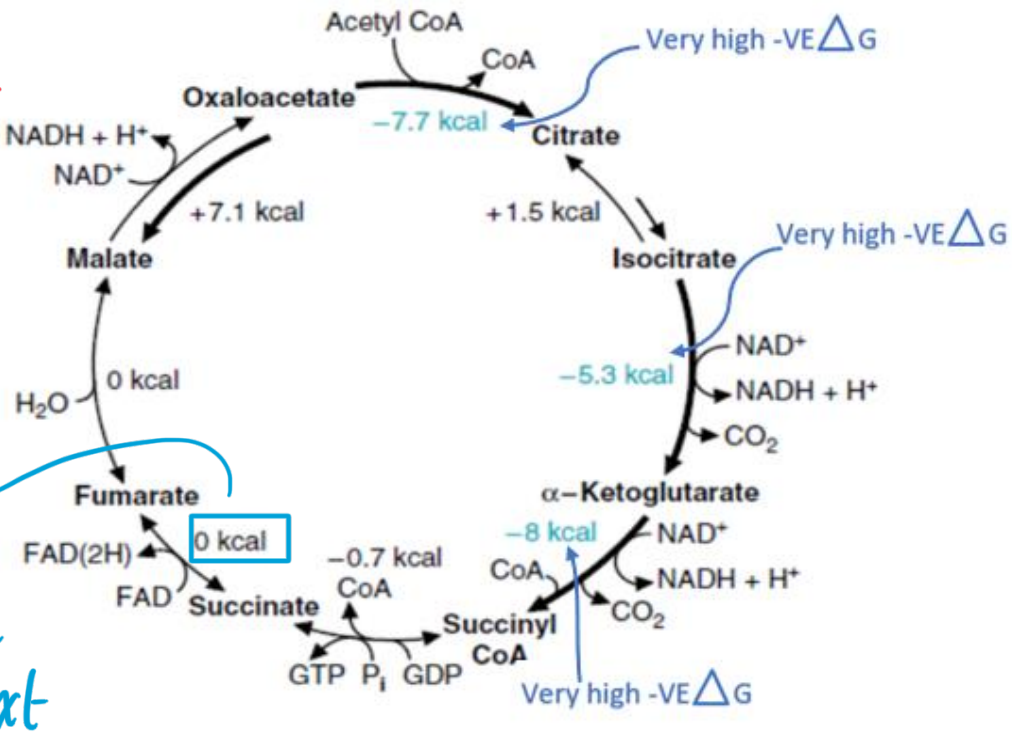
*ΔG for $FADH_2$ is variable BUT remember it was in the Succinyl dehydrogenase which is -41Kcal.

*This is the energy outcome.

THE EFFICIENCY OF KREB'S CYCLE

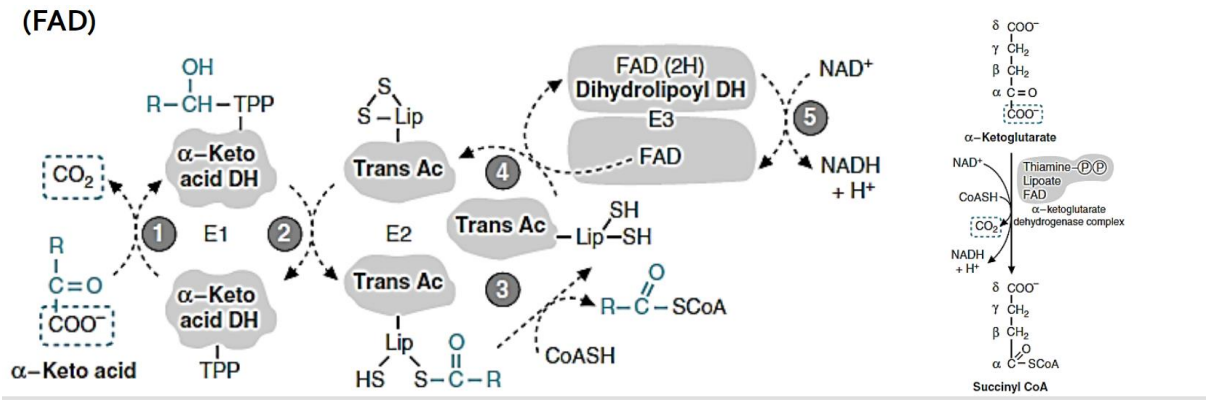
هو قديييييش الدورة استغلت الطاقة اللي فايتة عليها .

How much energy (from what entered the cycle) does the cycle took advantage of.



Remember this one very well, it'll be mentioned next lecture.

A- KETOACID DEHYDROGENASE COMPLEXES :



We will discuss the conversion of a-ketoglutarate to Succinyl CoA :

TP L C FAD NAD lipoat CoA

SOME NOTES :

- a-Ketoglutarate is oxidatively decarboxylated to form Succinyl CoA by the a- ketoglutarate dehydrogenase.

- The enzyme a-Ketoglutarate dehydrogenase is a multi enzyme complex having 3 enzyme proteins and 5 co-enzymes.
It's called : a-Ketoglutarate dehydrogenase complex .

- We have similar complexes that work in the same mechanism:
*Pyruvate dehydrogenase complex.
*a-Ketoacid dehydrogenase complex.

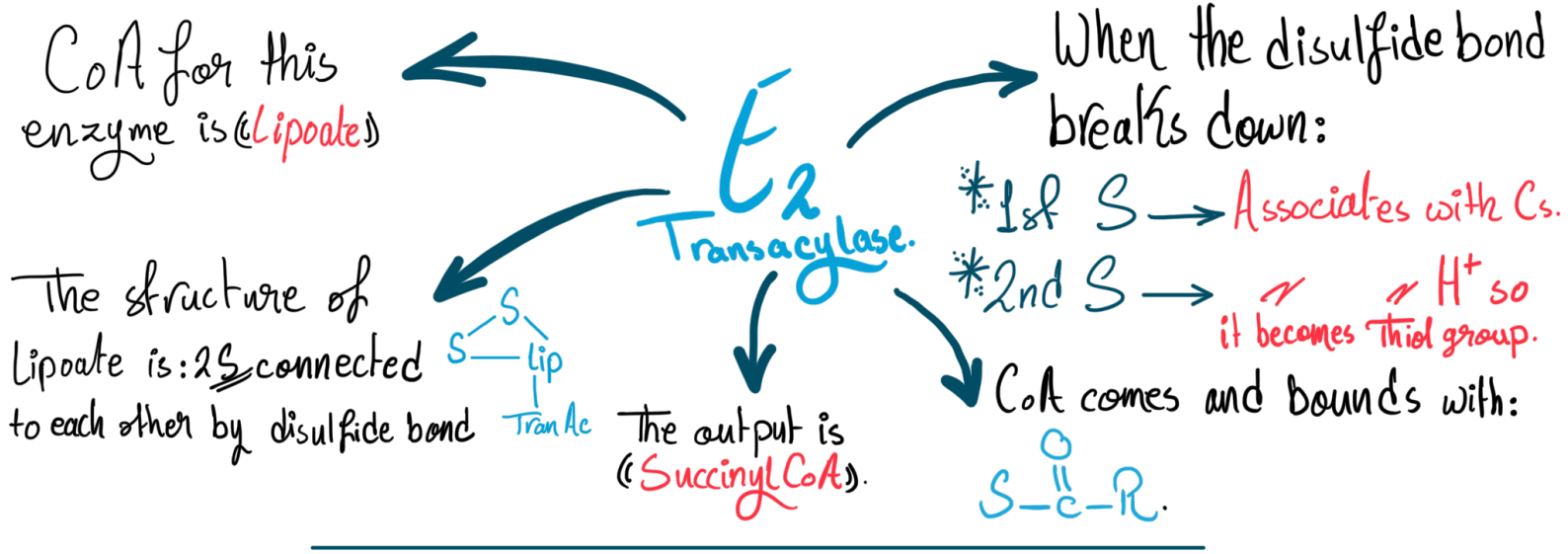
CO2 molecule is removed in this step.

Mechanism of action:

There are three enzymes connected to each other:

E1 Decarboxylase

1. Releases CO_2
2. CoA is attached to it called « TPP ».
3. It's connected to the 2nd C from the end. * The other Carbons are attached to the CoA (TPP).
4. The CoA (TPP) has to release the Cs. * To return into normal.
5. The released Cs are transfered to another enzyme called Trans Acylase



SOME CUTE INFORMATION:

In vitamin B1 deficiency

Decarboxylation reactions will stop. This leads to the accumulation of the substrates (alpha ketoglutarate, pyruvate and alpha keto acids) of the E1 component in the blood.

-Deficiency of vitamins B2, B3, B5 will also lead to substrate accumulation in the blood.

Arsenic poisoning:

is due to inhibition of enzymes that require lipoic acid as a coenzyme, including E2 of the PDH complex, α-ketoglutarate dehydrogenase and branched-chain α-keto acid dehydrogenase.

. Arsenic **attacks the disulfide bond in lipoic acid** forms a stable complex with the 2 sulfur atoms of lipoic acid.

Making that compound **unavailable to serve as a coenzyme** (because the sulfurs are now unable to form any bonds with the carbons).

When it binds to lipoic acid in the PDH complex → **pyruvate accumulates.**
Depending on the levels of toxicity and the conc. of As present it might prevent the entire reaction from proceeding and the entire cycle will be stopped which is why it can be fatal (**no energy metabolism**).

Pay attention:

Calcium ions activate many enzymes involved in metabolism since it causes muscle contraction .

This means that more energy is needed so more ATP is produced.

- When pyruvate is in the mitochondrial matrix, it is converted to acetyl CoA by the pyruvate dehydrogenase complex (PDH complex) which is a multi-enzyme complex.

-Component enzymes: The PDH complex is a protein aggregate of multiple copies of three enzymes, E1, E2, E3

. -Coenzymes: E1 requires thiamine pyrophosphate (TPP), E2 requires lipoic acid and CoA, and E3 requires FAD and NAD+.

Just a small note:

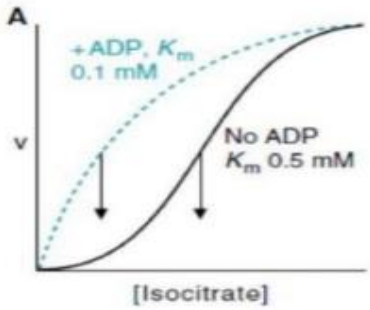
• regulating the **glycolysis** process: -

The rate limiting step in this pathway is the conversion of Fructose 6- phosphate into Fructose 1,6- bisphosphate which is catalyzed by phosphofructokinase enzyme.

- This the slowest step in glycolysis and the highest regulation step.

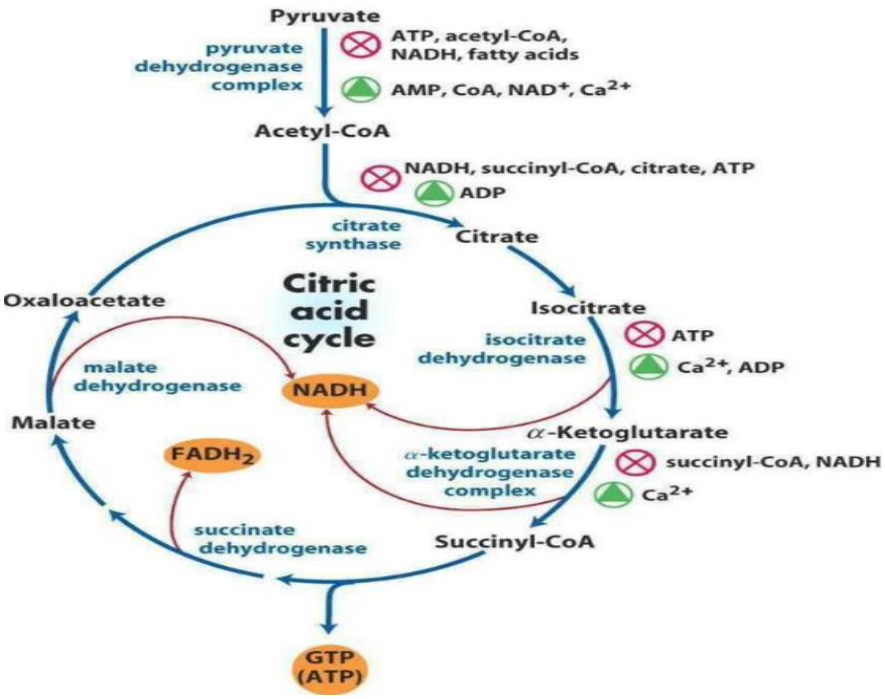
- This enzyme (phosphofructokinase) is **activated** by Fructose 2,6- bisphosphate and by AMP (it sends a message that we don't have enough ATP) .
It is **inhibited** by ATP and citrate.

REGULATION OF THE CITRIC ACID CYCLE:

Citrate synthase	-It is the first enzyme in the cycle. It is a simple enzyme (not allosteric). -Excess amounts of citrate will inhibit the activity of this enzyme.
Isocitrate dehydrogenase 	-It facilitates the rate limiting step (isocitrate → alpha ketoglutarate). This step is highly regulated. -It is inhibited by NADH and ATP. Activated by ADP and Ca ions. -It is the only enzyme in the cycle that is activated by ADP. **(ADP is an allosteric activator for isocitrate DH)** **-km** for this enzyme with the presence of ADP decreases. (affinity for substrates increases). -A small change in ADP concentration will affect the enzyme's activity greatly.
α-ketoglutarate dehydrogenase	-Inhibited by its products NADH and succinyl CoA (feedback inhibition). -Activated by Ca ions.

- ADP/ATP and NAD+/NADH ratios control the rate of Krebs cycle.

- High levels of ADP and NAD+ activate the cycle. On the other hand, high levels of ATP and NADH inhibit the cycle.



Why FAD can't regulate the cycle like NAD^+?

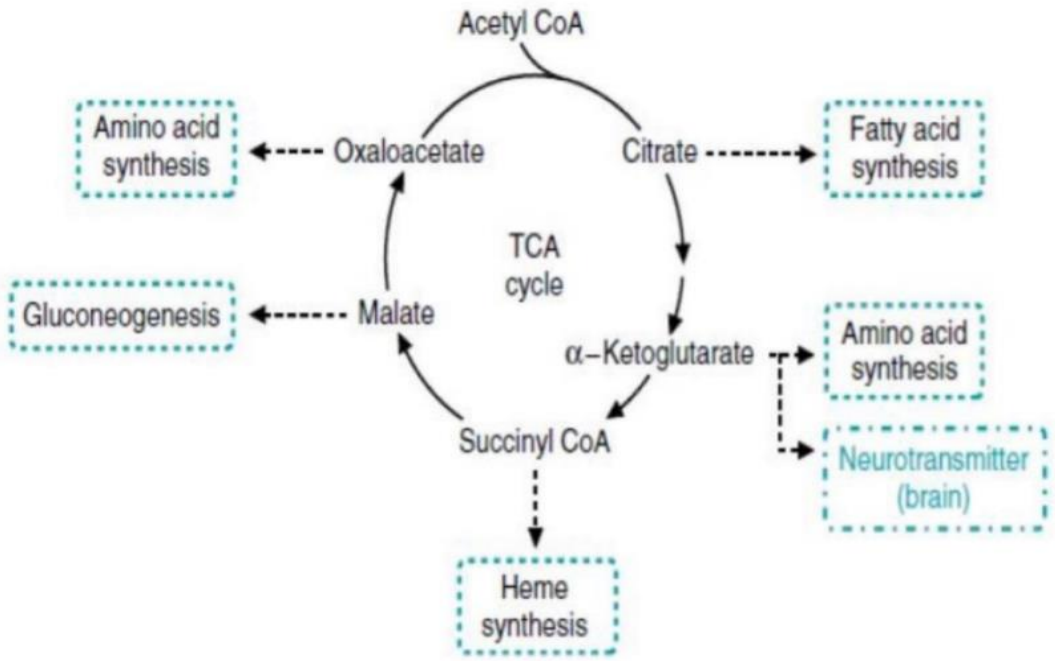
Because it is already bounded to enzyme

(NOT ACTIVE)

CITRIC ACID CYCLE INTERMEDIATES:

• The citric acid cycle provides precursors for many biosynthetic pathways.

Oxaloacetate (and other keto acids)	-Can be used in amino acid synthesis. -Example: oxaloacetate →aspartate
Citrate	Can leave the mitochondria and be used in **fatty acid** synthesis.
α-Ketoglutarate	-Can be turned to the amino acid glutamate. -Glutamate can function as a **neurotransmitter.** -The inhibitory neurotransmitter **GABA** can be synthesized using glutamate. -Glutamine is used to synthesize amino acids. It is synthesized in skeletal muscles then transported to other tissues, so they can synthesize amino acids and proteins.
Succinyl CoA	Used in **heme** biosynthesis in bone marrow.
Malate	-A key molecule in gluconeogenesis. -**Gluconeogenesis** is the process of generating glucose from **non-carbohydrate** sources when the person is fasting. It mostly occurs in the liver and kidneys.



ANAPLEROTIC REACTIONS:

Reactions that replenish the intermediates of the citric acid cycle.

Anaplerotic pathways:

Amino acid degradation:

-Aspartate can provide oxaloacetate.
-Glutamate provides α-Ketoglutarate.
-Propionyl CoA provides succinyl CoA.
-Many amino acids can provide fumarate.

One important example is the **conversion of carbon dioxide and pyruvate to oxaloacetate** which is catalyzed by Pyruvate carboxylase.

- Pyruvate carboxylase is a mitochondrial matrix protein (that requires biotin).

-The pyruvate carboxylase reaction occurs in the mitochondria of liver, kidney, brain, fibroblasts and adipocyte cells and has **two purposes:**

1- To provide an important substrate for gluconeogenesis.

2-And to provide oxaloacetate that can replenish the TCA cycle intermediates that may become depleted.

-Pyruvate carboxylase is activated by acetyl CoA. That makes sense because acetyl CoA enters the cycle by reacting with oxaloacetate.

-High levels of acetyl CoA in mitochondria signal a metabolic state in which the increased synthesis of oxaloacetate is required.

-Gluconeogenesis is very active in the liver and kidneys. This process consumes malate.

This decreases the concentration of malate and oxaloacetate.

-Oxaloacetate has the lowest concentration in kidneys and liver (where the concentration of pyruvate carboxylase is highest).