METABOLISM

بسم الله الرحمن الرحيم

MID – Lecture 4

TCA cycle (pt.1)

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا ولا تستبدلنا

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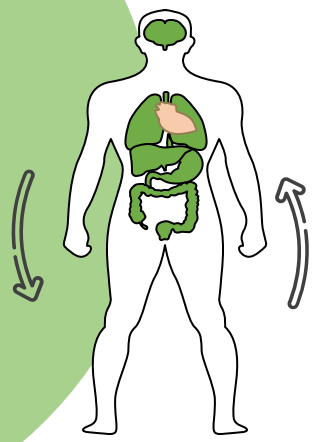


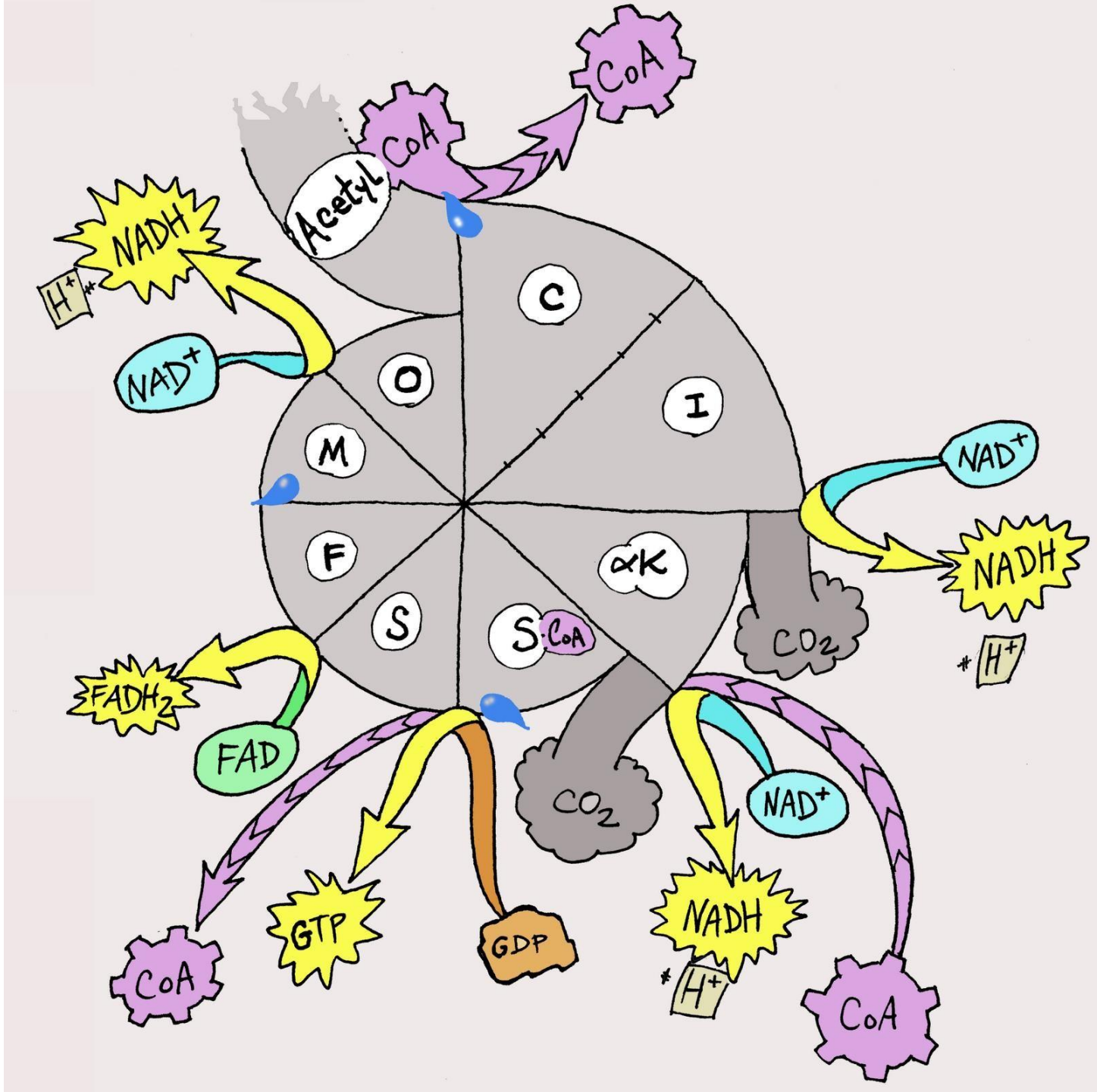
ومن للأمة إلا شبابها ! بسم الله

Produce citrate

(Kreb's, Citric Acid, Tricarboxylic Acid) Cycle

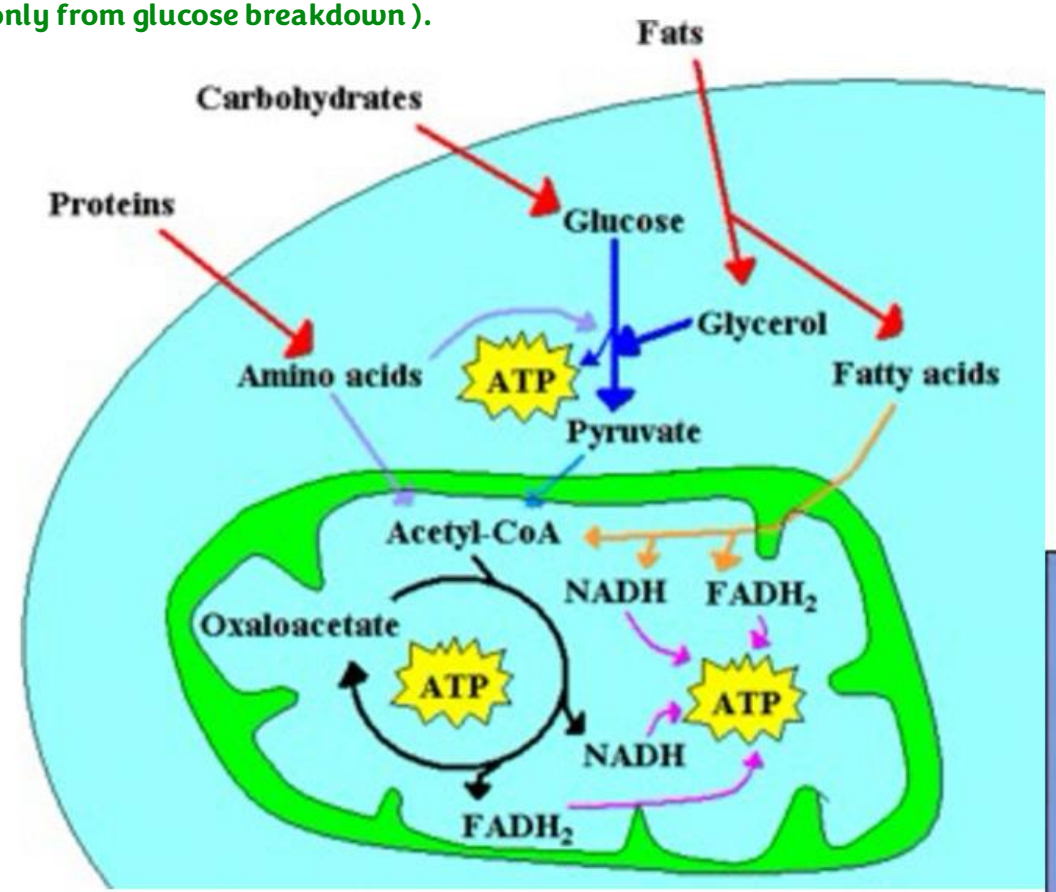
TCA= citrate

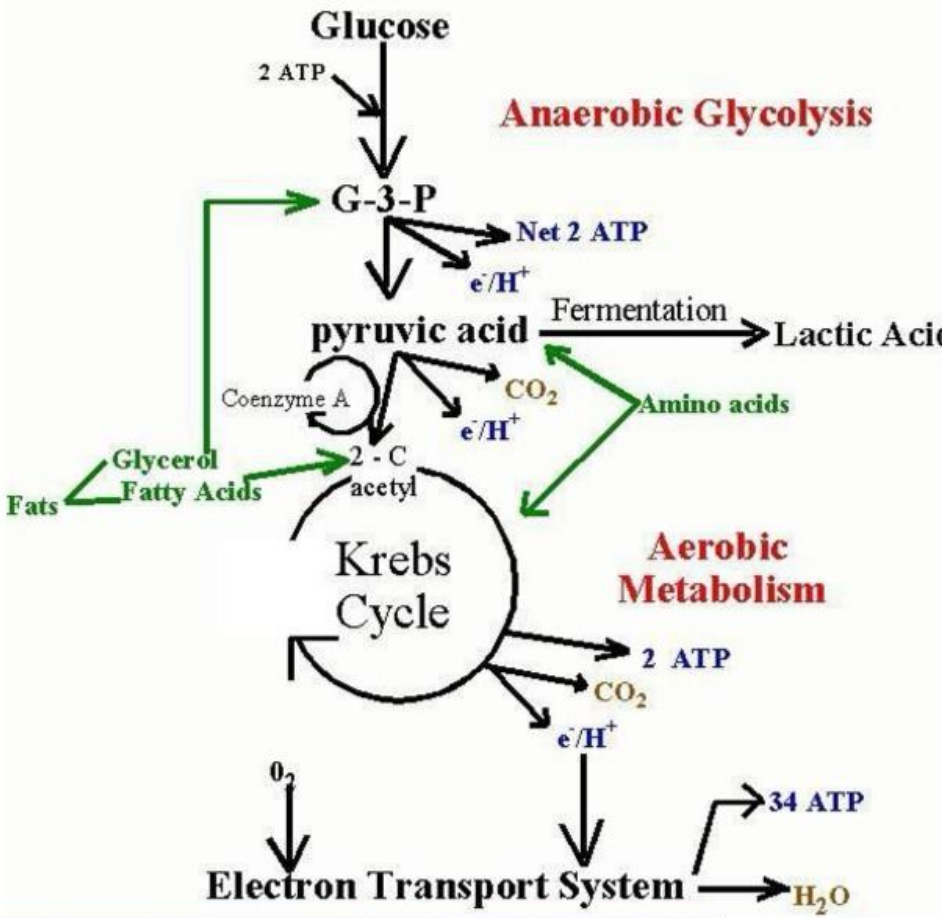
Dr. Diala Abu-Hassan, DDS, PhD

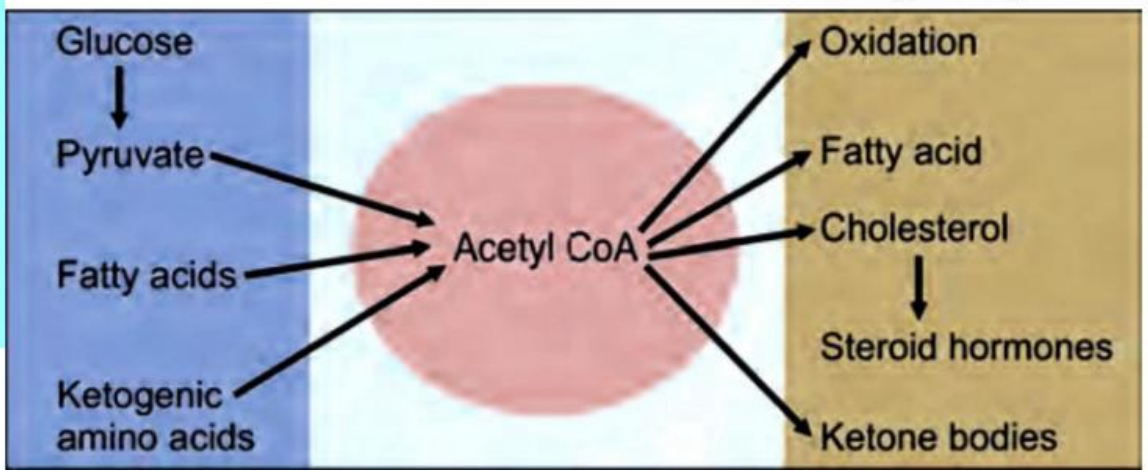


Sources and Uses of Acetyl-CoA

This cycle starts with Acetyl-CoA that react with last intermediate (Oxaloacetate) .
What is the importance of Krebs cycle ? It's a universal pathway which means it happen in all cells,
Krebs cycle occurs inside each cell that contains mitochondria.
There is no direct relation between the source of Acetyl-CoA and the cycle (Acetyl-CoA doesn't have to be only from glucose breakdown).



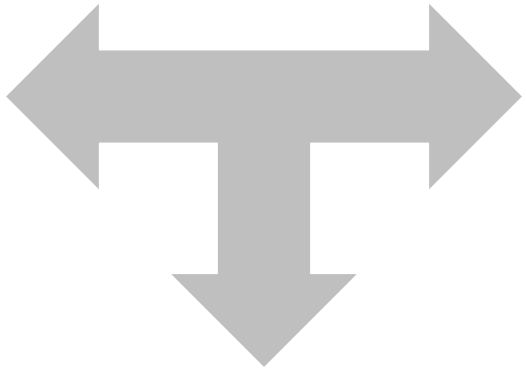




What is the sources of Acetyl-CoA ?

[: قولوا يارب .. معلش

Oxidation of carbohydrates produces pyruvate , and pyruvate becomes Acetyl-CoA by pyruvate dehydrogenase complex.



Degradation of proteins gives amino acids, some of amino acids once they are degraded they produce Acetyl-CoA. These amino acids are called ketogenic.
(Explanation: Breakdown of specific amino acids gives acetyl-CoA , which are then used to produce ketone bodies).

Fatty acids oxidation : fatty acids stored in adipose tissue as Triacylglycerol , in certain conditions when we need to breakdown Triacylglycerol and use it to produce energy , ester bonds between glycerol and fatty acids will be hydrolyzed so fatty acids go to bloodstream, then albumin brings them to the tissue and oxidation of fatty acids will begin to produce Acetyl-CoA
(The main substrate's oxidation means degradation of it to produce energy—> catabolism.
Reduction of the main substrate happens in anabolism).

Remember: oxidation of substrate accompanied with reduction of co -enzyme .
Note : An 18-carbon fatty acid will produce 9 acetyl-CoA molecules (2 carbon for each)during oxidation.

Krebs cycle need **oxygen** to be activated (Explanation : the Krebs cycle itself does not need oxygen and doesn't produce enough energy directly, mostly indirectly by electrons carrier , it is dependent on oxygen for the overall process of aerobic respiration , we need oxygen for electron transport chain since the last acceptor for electrons is oxygen (if there is no oxygen for electrons transport chain, Krebs cycle stops)).

ways for using Acetyl-CoA:

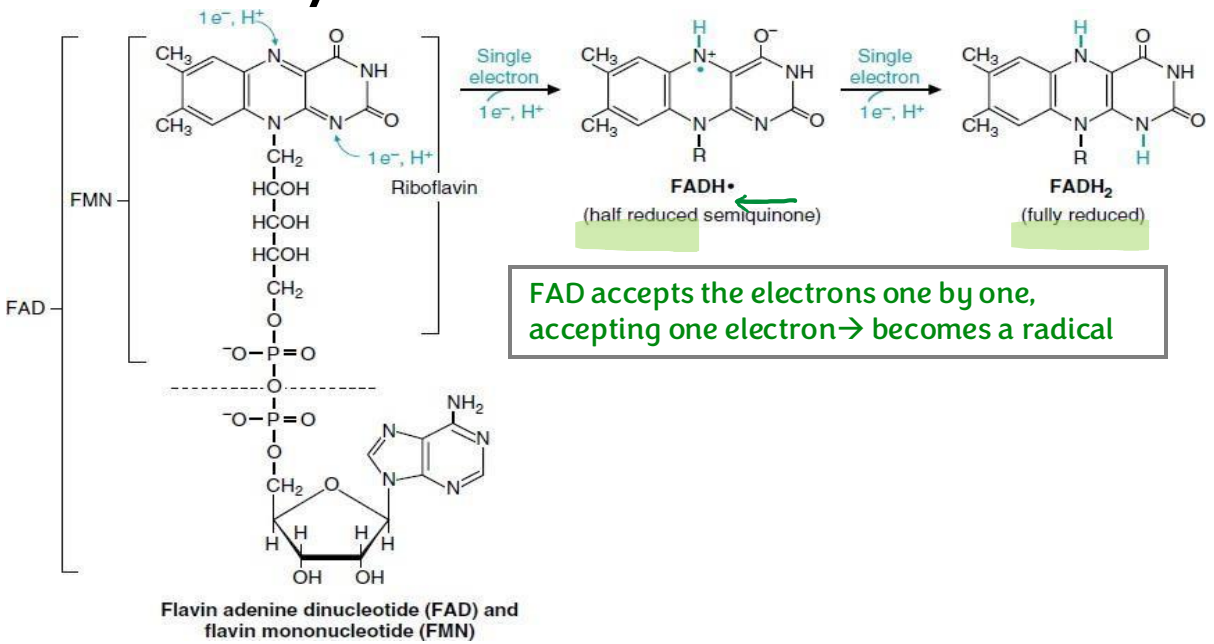
- **it could be used to produce fatty acid (so Acetyl-CoA come from degradation of fatty acids and vice versa but not simultaneously and at totally different conditions) .**
- **oxidation in Krebs cycle .**
- **in Cholesterol manufacturing (steroids compound in general).**
- **In ketone bodies manufacturing.**

Basically, fatty acids are used in a Krebs cycle, but sometimes when the fatty acids are produced in higher concentrations than needed for Krebs cycle, the rest of fatty acids will be used in manufacturing other compounds in order to avoid accumulation.

Electron (energy) carrying Molecules (NAD+, FAD)

FAD

- Single electrons (H•), different sources
- Succinate to fumarate during Krebs cycle , lipoate to lipoate disulfide
  in α-KG
- FAD must remain tightly, sometimes covalently, attached to its enzyme
- E^o for enzyme-bound FAD varies



Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

FAD accepts the electrons one by one, accepting one electron→ becomes a radical

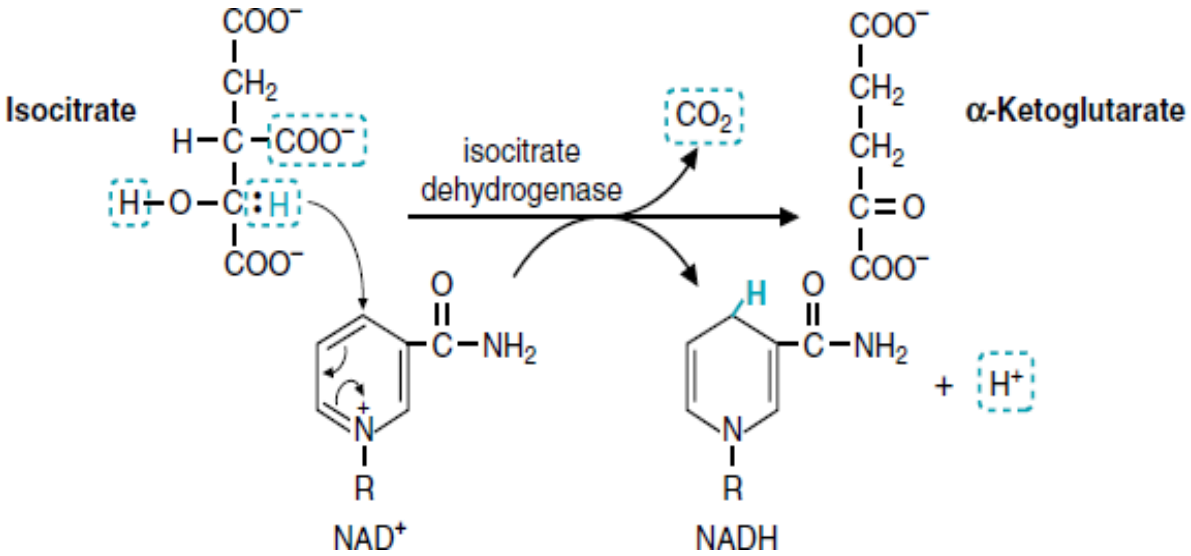
NAD

Hydride

- Pair of electrons (H-), same source
  Alcohols to ketones by malate dehydrogenase & isocitrate dehydrogenase

Oxidation of Alcohol to Ketone accompanied by NAD+ reduction

- NADH plays a regulatory role in balancing energy metabolism



Stepwise Reactions

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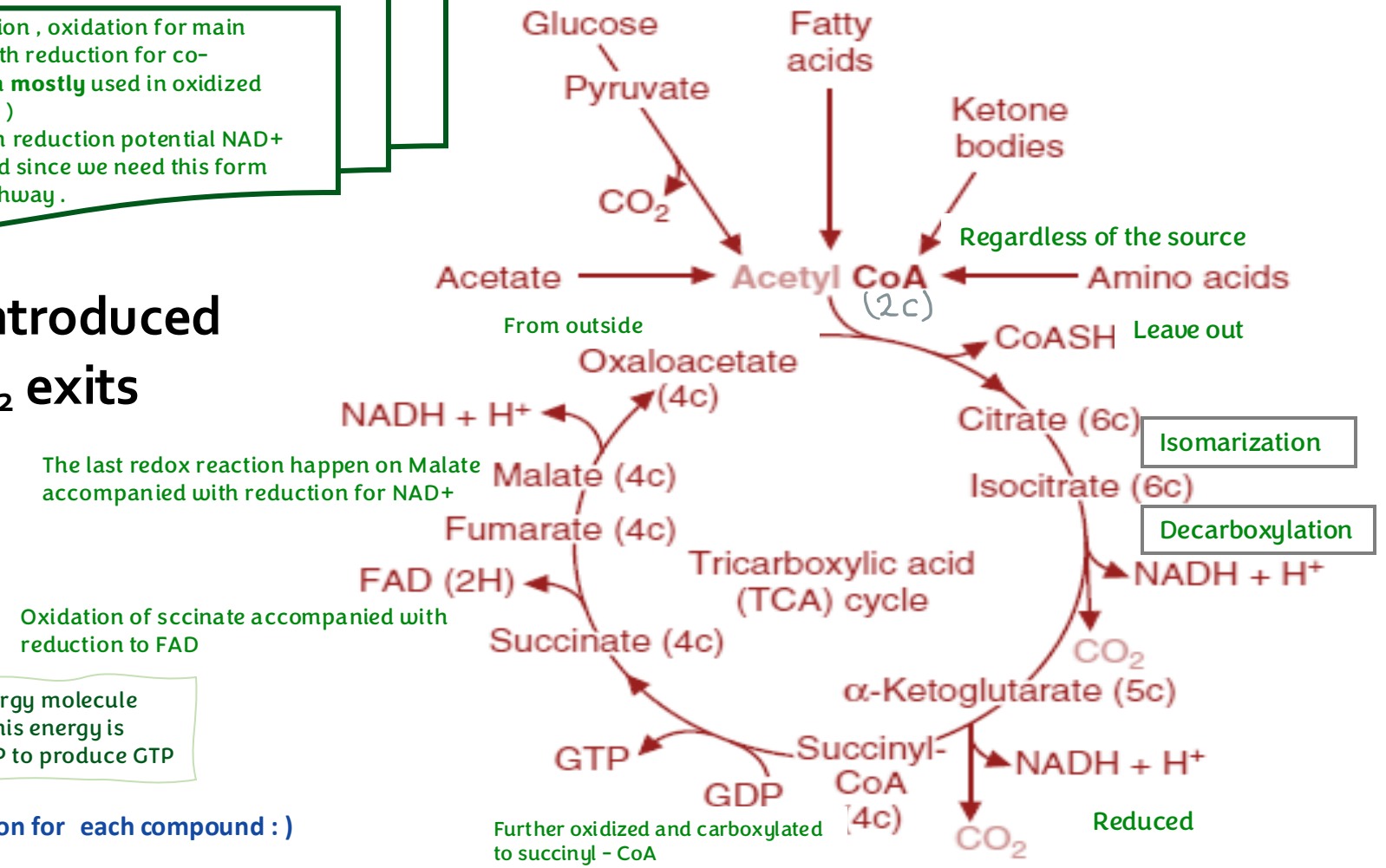
Through Krebs cycle reaction , oxidation for main substrate accompanied with reduction for co-enzyme (FAD \ NAD which **mostly** used in oxidized form and become reduced)
REMEMBER : depending on reduction potential NAD+ favors to become produced since we need this form of it in most metabolic pathway .

- No O_2 introduced
- Two CO_2 exits



The last redox reaction happen on Malate accompanied with reduction for NAD+

Oxidation of sccinate accompanied with reduction to FAD

Succinyl coA is highly energy molecule due to presence of coA , this energy is used to phospholirate GDP to produce GTP

Further oxidized and carboxylated to succinyl – CoA

***Focus on number of Carbon for each compound :)**

*What is the only energy molecule produced directly during Krebs cycle ? GTP
*2 carbons enter as Acetyl-coA and 2 carbons exit one by one as co2 (same carbons).
*krebs cycle considered as both anabolic and catabolic.

Net reaction

$$\text{Acetyl CoA} + 3NAD^+ + FAD + GDP + P_i + 2H_2O \longrightarrow 2CO_2 + CoASH + 3NADH + 3H^+ + FAD\,(2H) + GTP$$

Does Acetyl-CoA exit as CO2?

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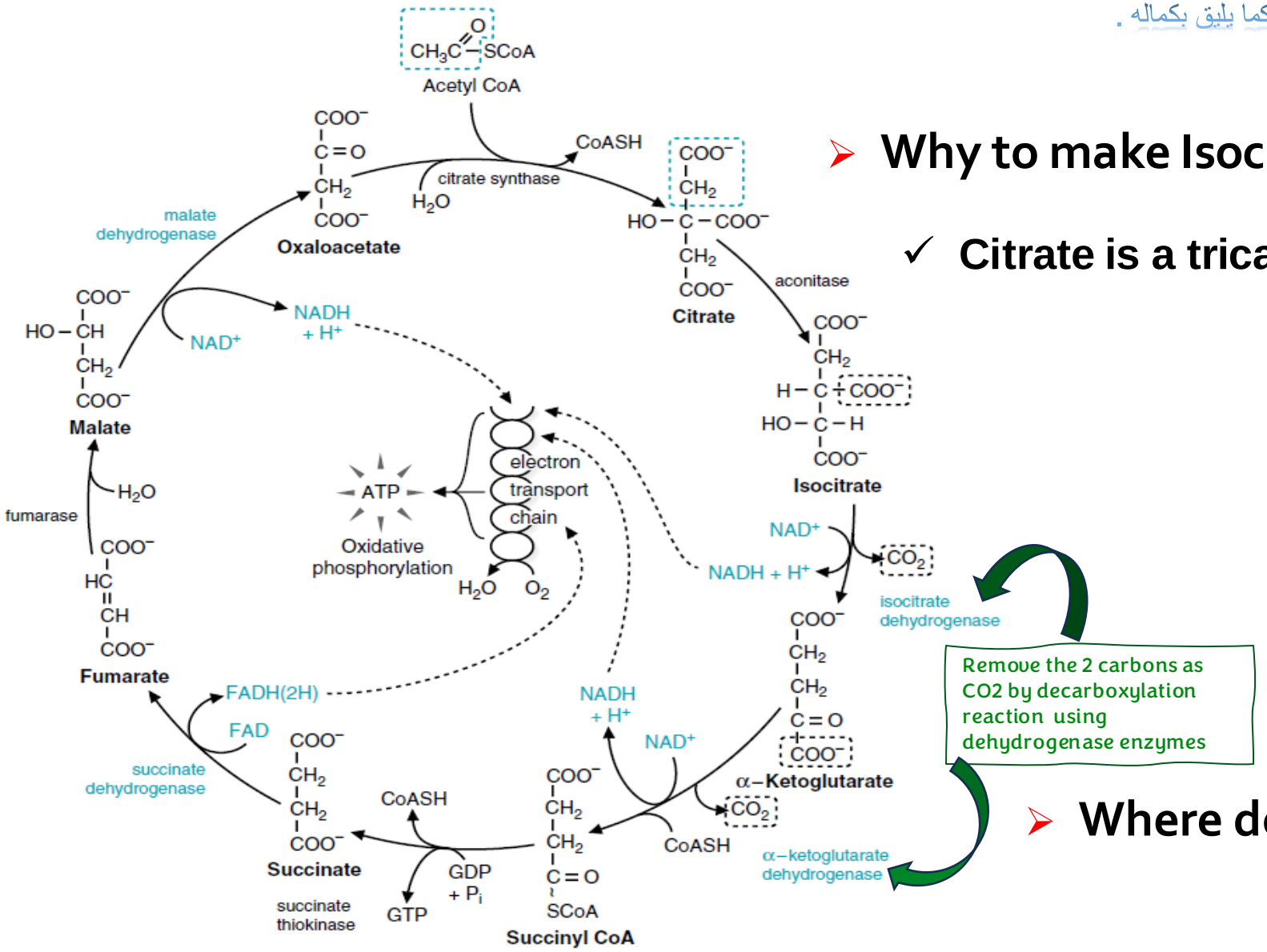


- **Why to make Isocitrate from citrate?**
  - ✓ **Citrate is a tricarboxylic acid**

Remove the 2 carbons as CO2 by decarboxylation reaction using dehydrogenase enzymes

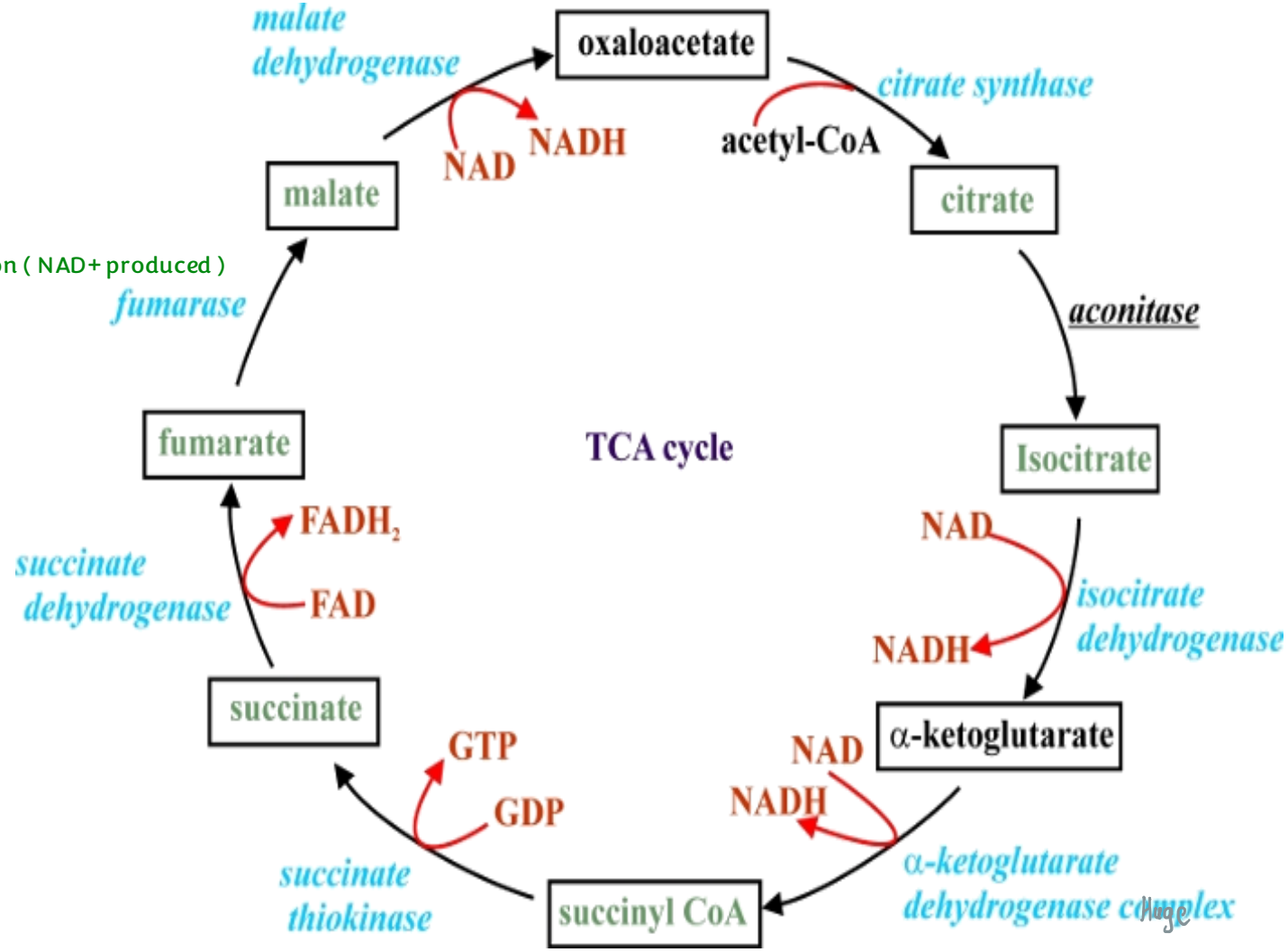
- **Where does the CO2 exit?**

Enzymes of the TCA Cycle

- Citrate synthase (not an allosteric enzyme)
- Aconitase Isomerase (catalyze reversible reaction)
- Isocitrate dehydrogenase For redox reaction (NAD+ produced)
- α-ketoglutarate dehydrogenase
- Succinate thiokinase
- Succinate dehydrogenase. redox reaction but different co-enzyme (FAD)
- Fumarase not for redox reactions
- Malate dehydrogenase for redox reaction (NAD+)

Which one is the only enzyme of krebs cycle enzymes that found in inner mitochondrial membrane protein ?
Succinate dehydrogenase. Responsible for catalyzing step six , the other enzymes are soluble in matrix .

Memorize compounds name



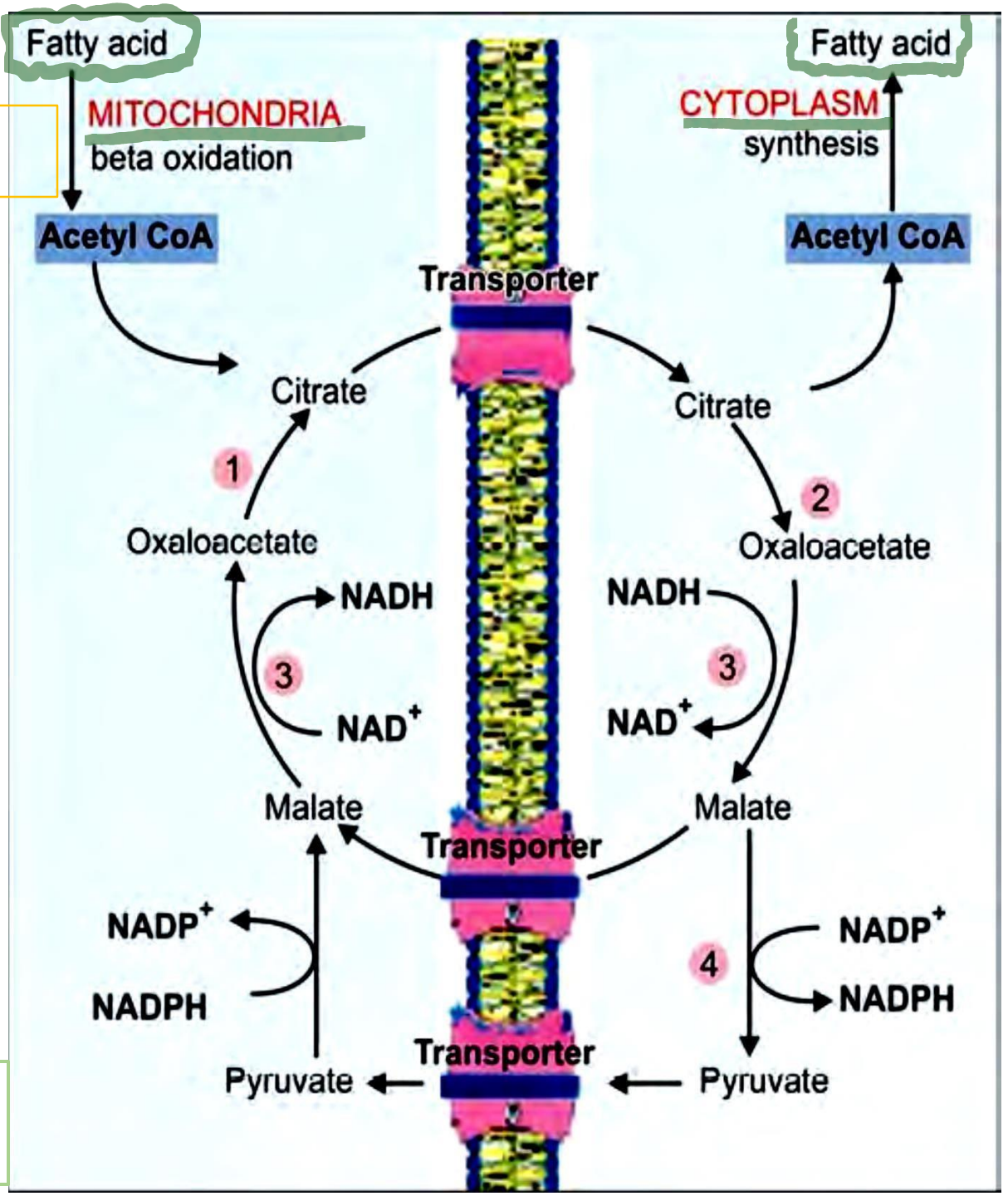
Step 1: Formation of Citrate

- ✓ Citrate synthase is inhibited by its product, citrate. **(inhibition feedback by same enzyme)**
- ✓ Substrate availability is another way of regulation for citrate synthase.
- ✓ The binding of oxaloacetate causes a conformational change in the enzyme that generates a binding site for acetyl CoA.
- ✓ Citrate provides a source of acetyl CoA for synthesis of fatty acids and activates their synthesis
- ✓ Citrate inhibits phosphofructokinase (glycolysis)

*The cycle Neither increase nor decrease the intermediate compounds . Presence of oxaloacetate to start the cycle is essential, oxaloacetate interact with many metabolic pathway. Citrate act as regulator for glycolysis (binding with glycolysis enzyme to inhibit it)
* A lot of citrate → inhibition of glucose degradation (why ? High concentration of citrate means The Krebs cycle is operating well so we have a lot of ATP , there is no need for glucose degradation so inhibit it .

Citrate has transporter in inner mitochondrial membrane so it can exit to cytosol (excess citrate), in cytosol it can activate fatty acids synthesis by reproducing Acetyl coA (precursor molecule for synthesizing fatty acids)

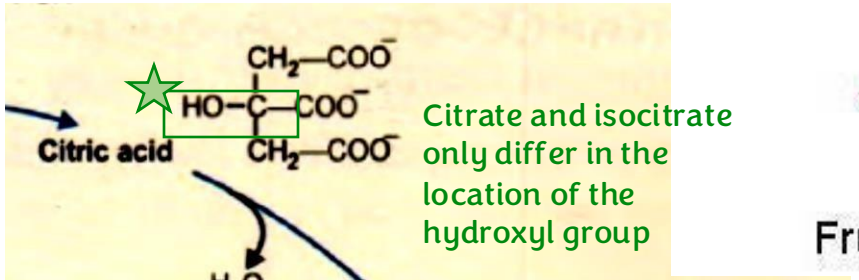
separate the process(degradation and production of fatty acids)because of compartmentalization (cytoplasm vs mitochondria)



Step 2+3: Formation and Oxidation of Isocitrate

- **Oxidative decarboxylation (irreversible)**
- **3° to 2° alcohol**

✓ **Aconitase is an Fe-S protein**



Citrate and isocitrate only differ in the location of the hydroxyl group

Aconitase can also catalyze the reverse reaction

Control at the committed step of glycolysis

Fructose 6 - phosphate

ATP

ADP

Phosphofructokinase

Activated by F-2,6 - BP

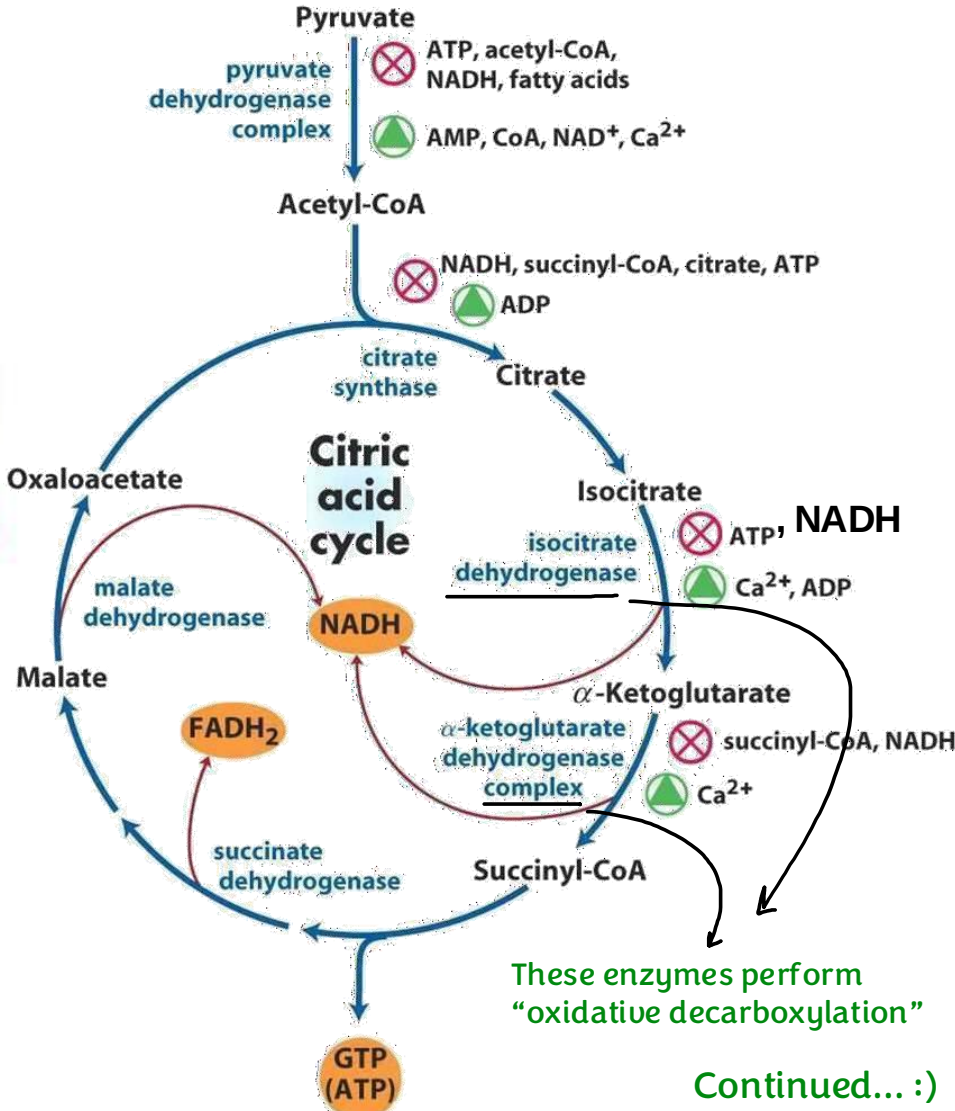
Activated by AMP

Inhibited by ATP and citrate

Presence of both ATP & citrate both signify that the cell has enough energy

Fructose 1,6 - bisphosphate

Application: Aconitase is inhibited by fluoroacetate that is used as a rat poison



These enzymes perform "oxidative decarboxylation"

Continued... :)

Step 2+3: Formation and Oxidation of Isocitrate

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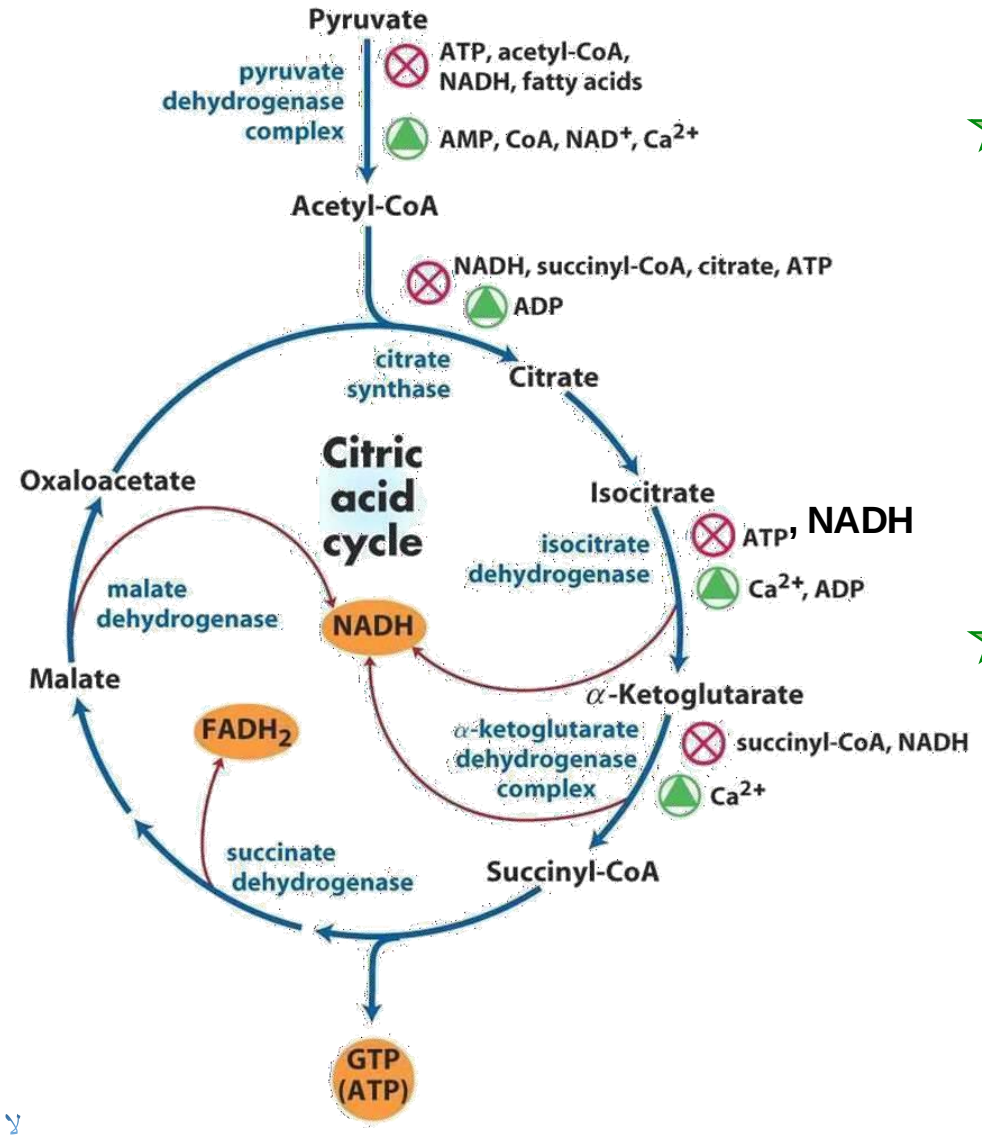
Isocitrate dehydrogenase is regulated because it's an irreversible enzyme (proceeds in one direction only). Irreversible steps tend to be more regulated than reversible steps in pathways.

Low levels of ATP also serve as **activators** of isocitrate dehydrogenase. "Low energy state" → the cell need more energy → activation of the Krebs cycle, & vice versa.



Calcium ions are **activators** of isocitrate dehydrogenase. High [Ca2+] signify that a muscle cell is in a state of contraction. This means that the cell needs energy, which activates the Krebs cycle.

If [NADH] is high, this means we don't have the necessary coenzyme state (oxidized form) and the reaction is inhibited. **NAD+ is needed in the cell more than NADH.**

لا إله إلا الله، وحده لا شريك له، له الملك، وله الحمد، وهو على كل شيء قدير

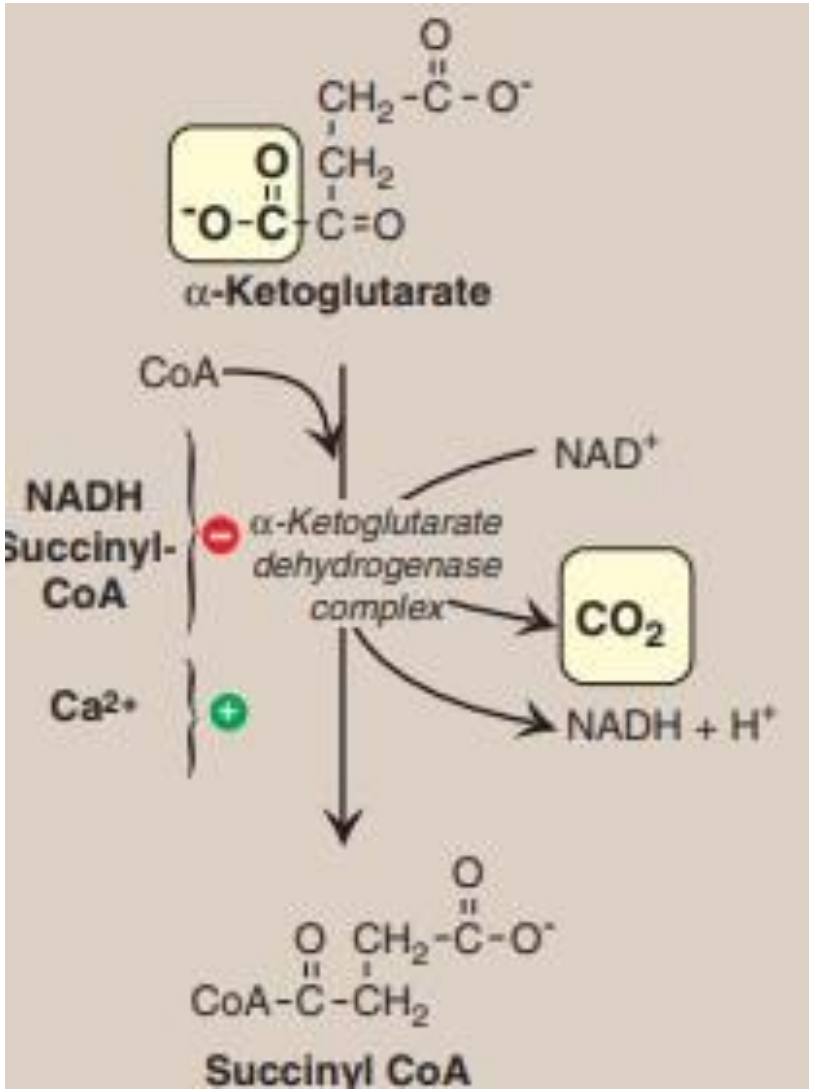
Step 4: α-Ketoglutarate to Succinyl-CoA

- **Oxidative decarboxylation**
- **α-ketoglutarate dehydrogenase complex, a multimolecular aggregate of three enzymes**
- **Thiamine pyrophosphate, lipoic acid, FAD, NAD+, and CoA**
- **Energy conserved as NADH, thioester bond**

Big enzyme made up of multiple enzymes all working together. Enzymes hand off to each other which factors into the increased efficiency of the complex

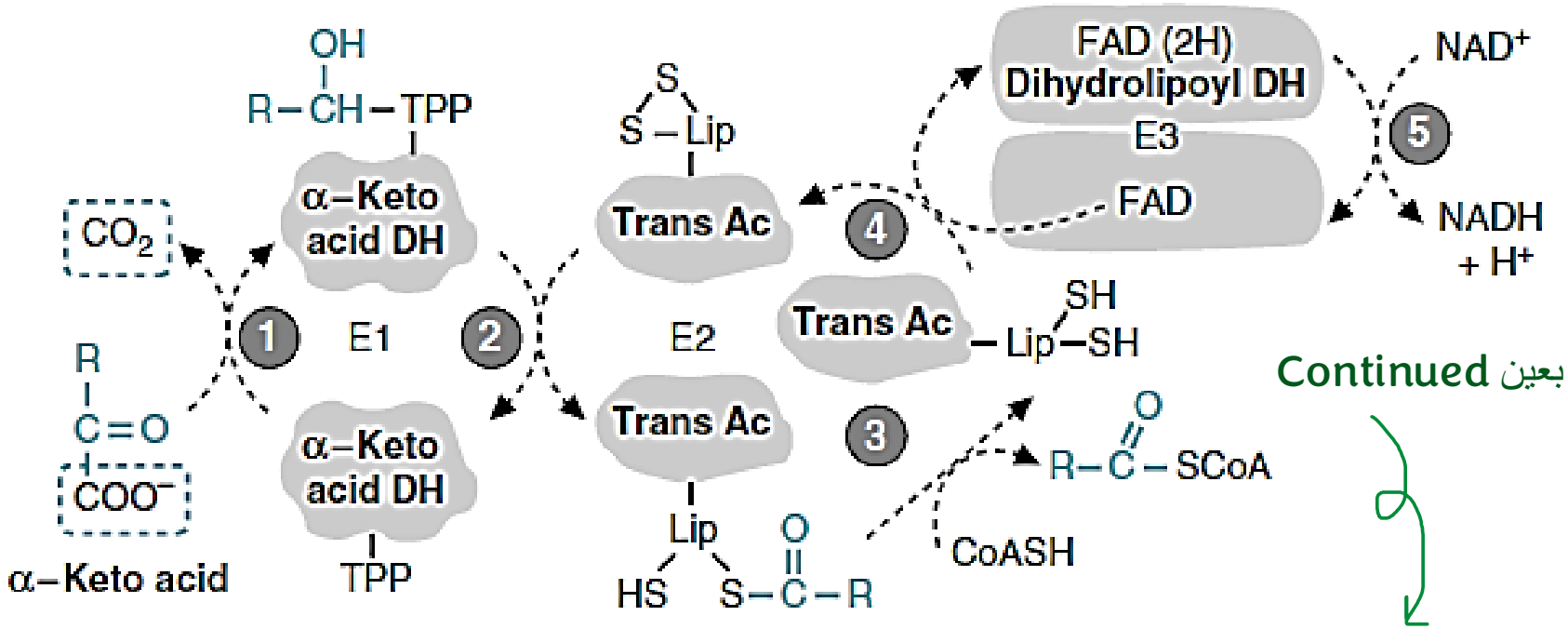
Decarboxylation

→ All are examples of coenzymes



α-Ketoacid Dehydrogenase Complexes (TLCFN)

- (α-ketoglutarate, pyruvate, and branched chain α-keto acid) dehydrogenase complexes
  Amino acid – NH3 = keto acid
  Alanine – NH3 = pyruvate
- Huge enzyme complexes, multiple subunits of 3 different enzymes (no loss of energy, substrates for E2 and E3 remain bound → higher rate)
- E1, E2, & E3 are a decarboxylase (TPP), a transacylase (lipoate), & a dehydrogenase (FAD)



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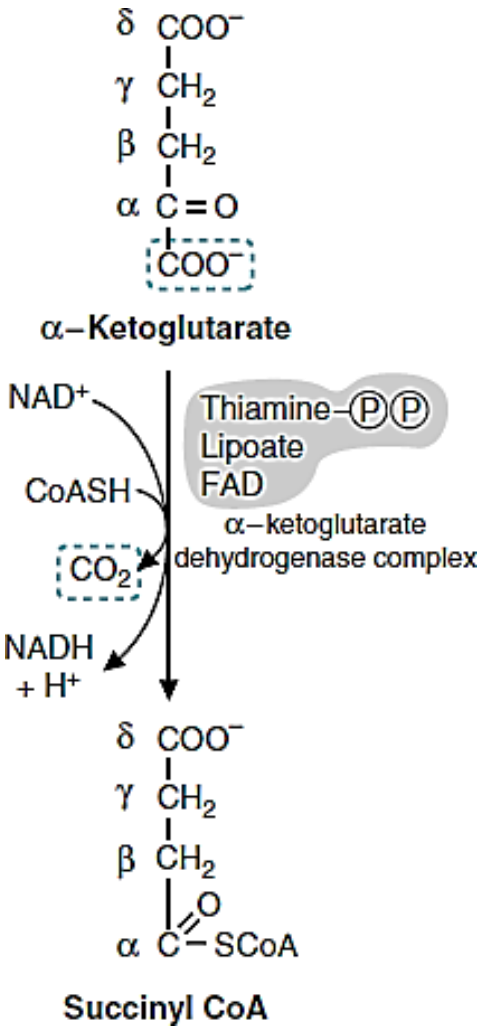
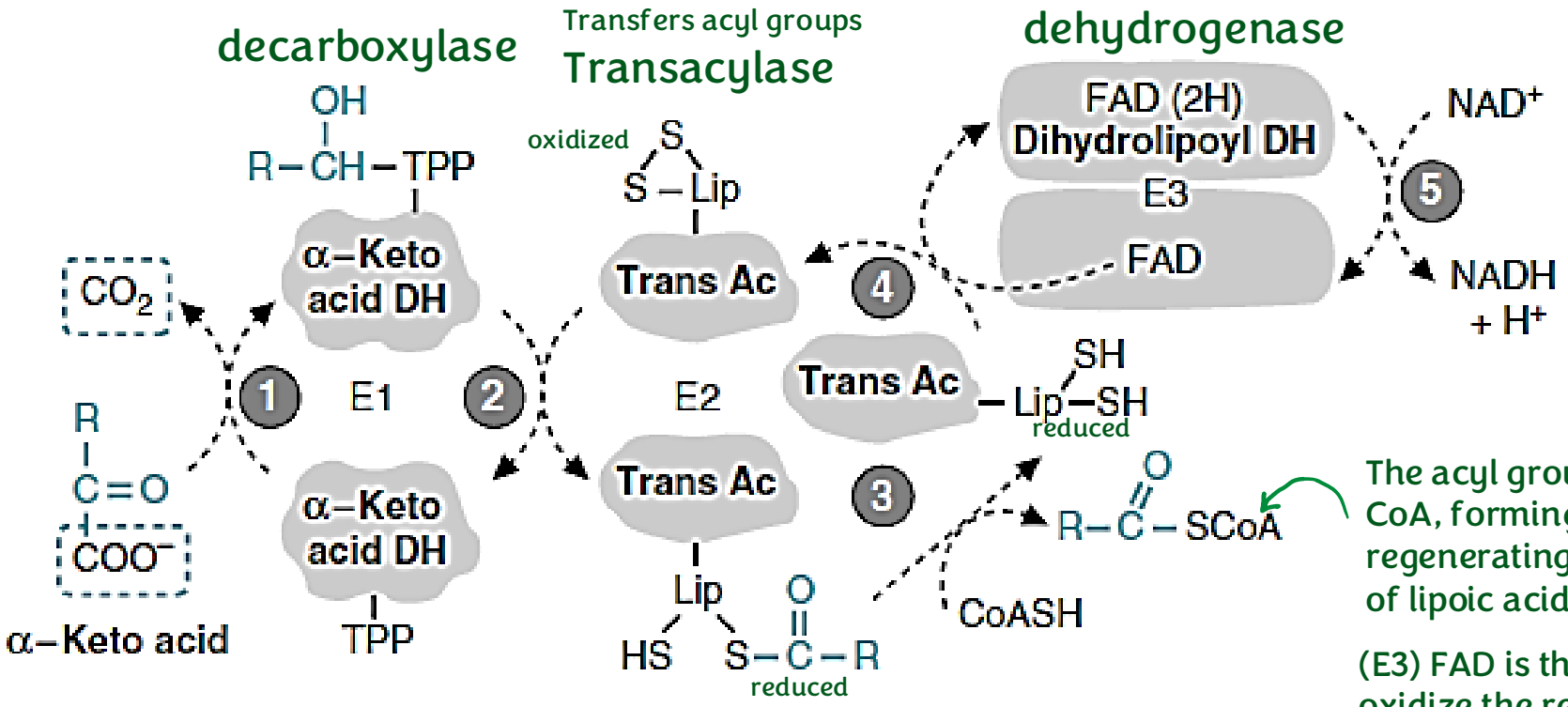


α-Ketoacid Dehydrogenase Complexes (TLCFN)

TPP, lipoic acid, CoA, FAD, & NAD+



Lipoic acid is the coenzyme that aids in the transfer of the acyl groups. It has 2 sulfurs, so It can form disulfide bridges & alternate between oxidized & reduced states.

The acyl group is transferred to CoA, forming succinyl CoA and regenerating the reduced form of lipoic acid

(E3) FAD is then reduced to oxidize the reduced lipoic acid (step 4)
NAD+ is reduced to NADH (step 5)

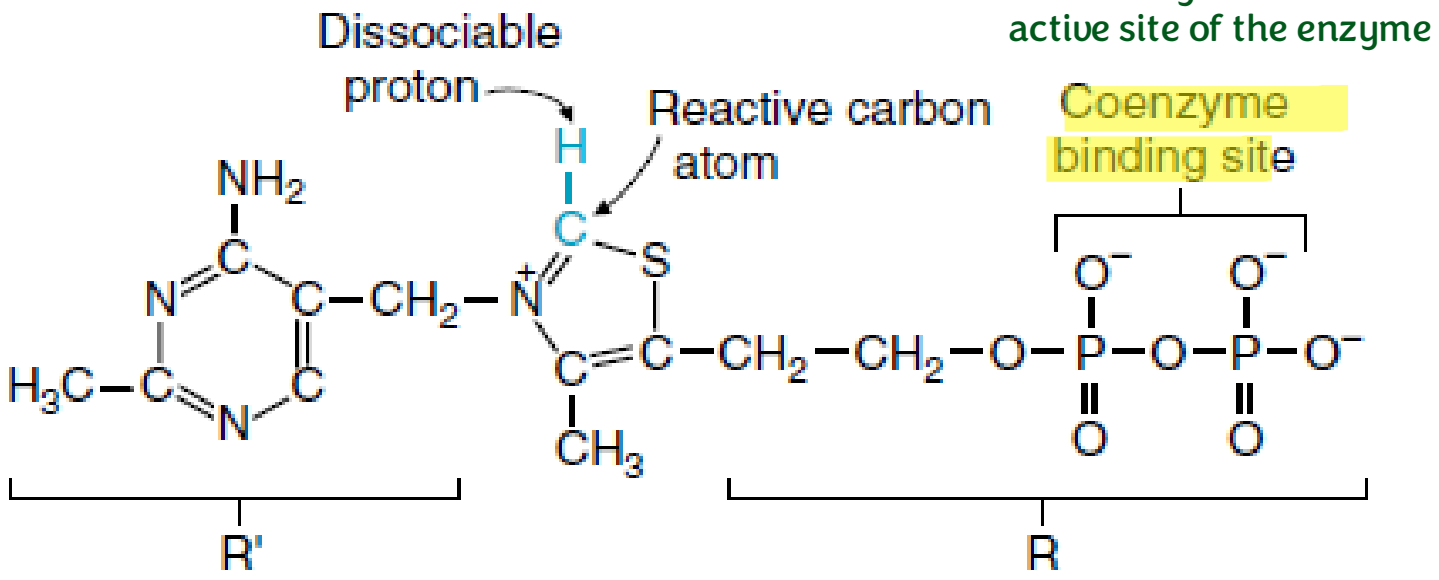
سبحان الله، والحمد لله، ولا إله إلا الله، والله أكبر، ولا حول ولا قوة إلا بالله

Thiamine Pyrophosphate

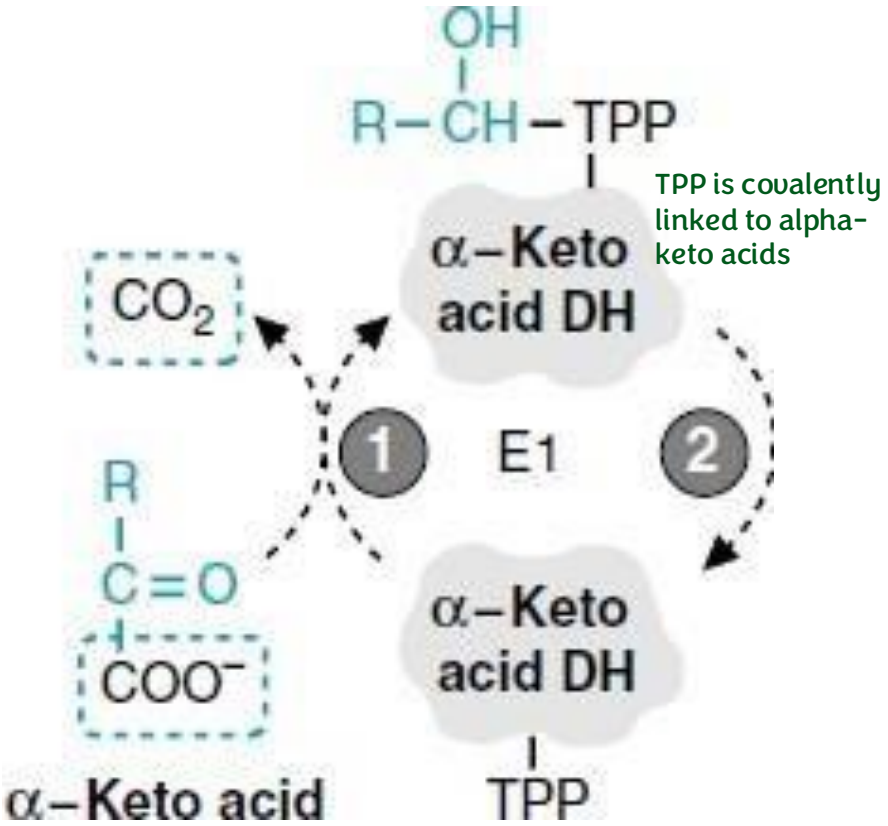
- **Thiamine (vitamin B1) deficiency, α-ketoglutarate, pyruvate, & branched chain α-keto acids accumulate in the blood**

Composed of 3 enzymes

Allows for the binding of the coenzyme to the active site of the enzyme



Thiamine pyrophosphate (TPP)



Step 5: Cleavage of Succinyl CoA & Generation of ATP

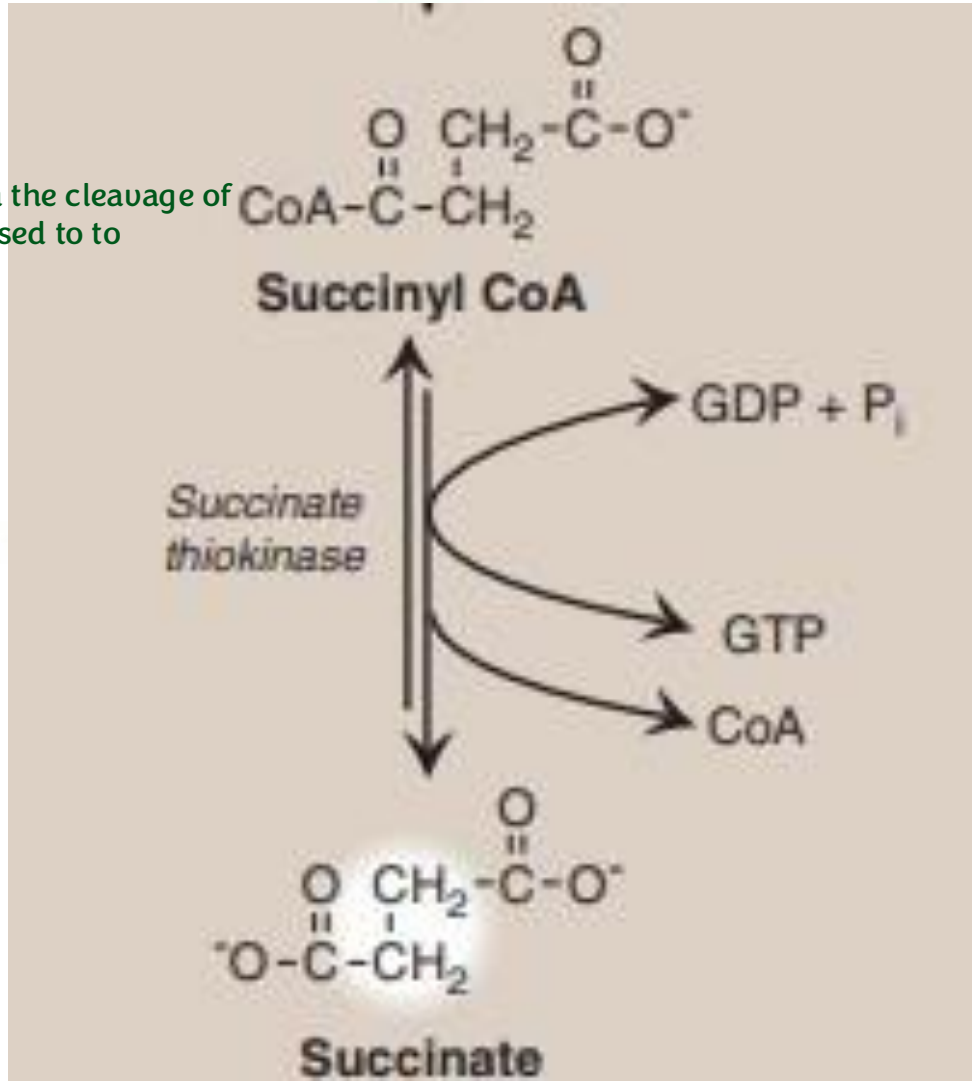
- Succinate thiokinase (succinyl CoA synthetase—named for the reverse reaction) cleaves the high-energy thioester bond of succinyl CoA

The energy produced from the cleavage of this high-energy bond is used to to phosphorylate GDP→ GTP

- Succinyl CoA has a thioester bond (CoASH & an acyl group)
- GTP is produced by <u>substrate level phosphorylation</u>

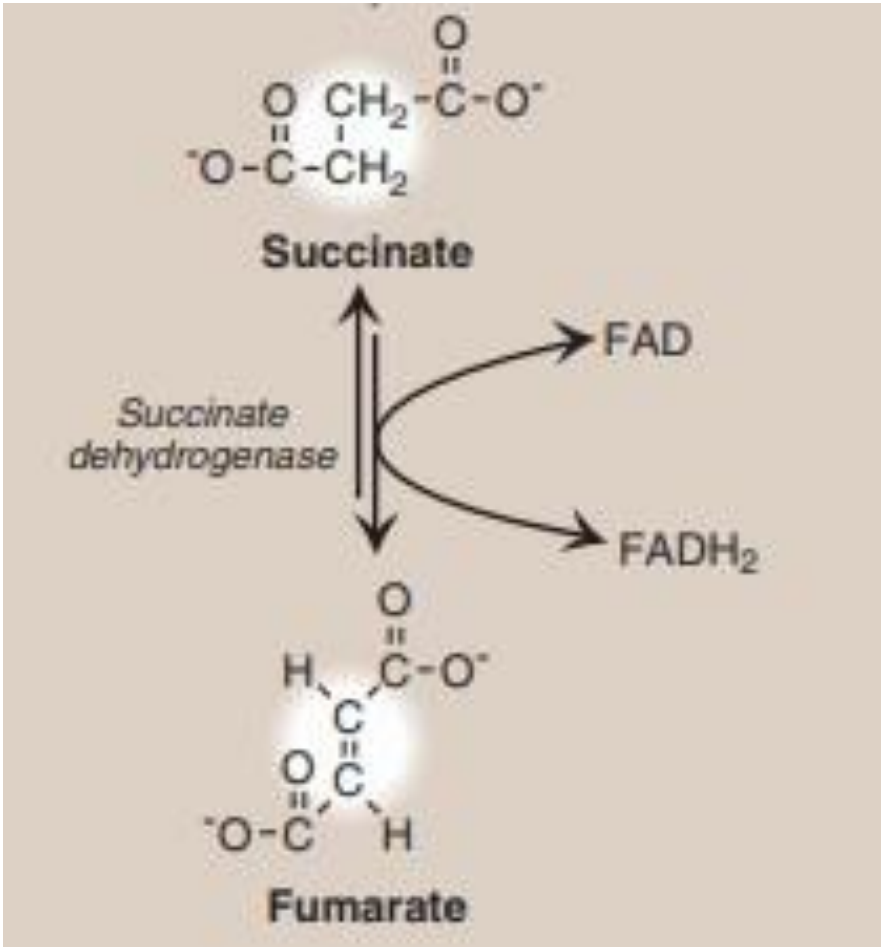
✓ GTP and ATP are energetically interconvertible by the nucleoside diphosphate kinase reaction

GTP +ADP ⟷ GDP + ATP



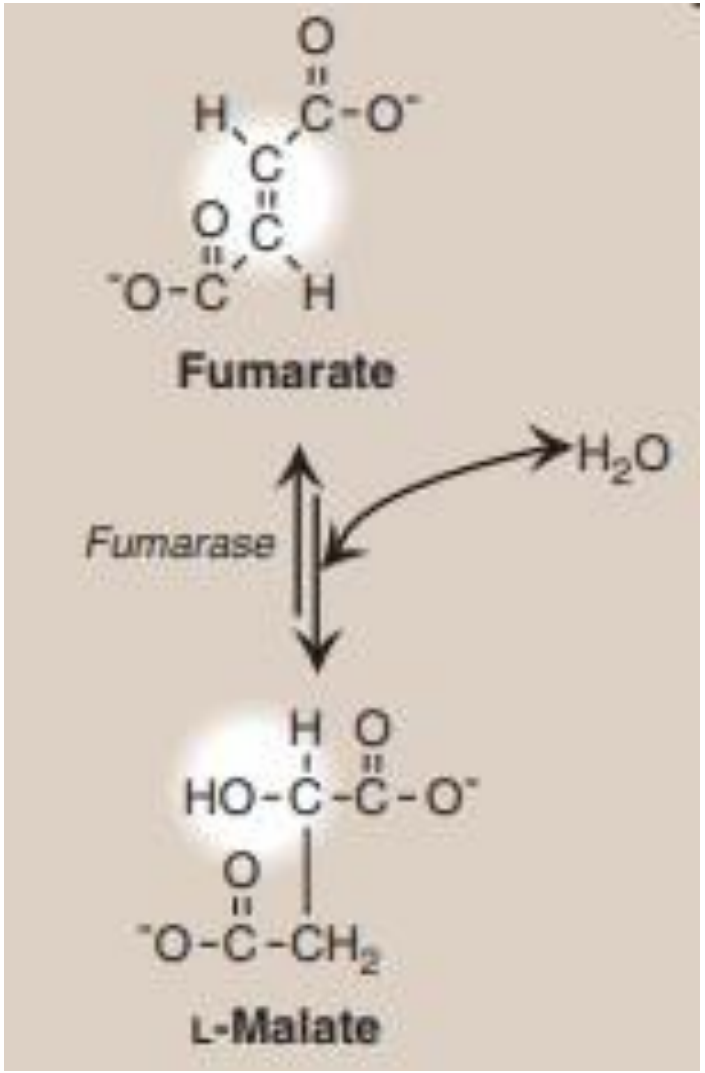
Step 6: Oxidation of succinate

- Succinate is oxidized to fumarate by succinate dehydrogenase Loss of H2 → double bond
- FAD (its coenzyme) is reduced to FADH2
- FAD, rather than NAD+, is the electron acceptor because the reducing power of succinate is not sufficient to reduce NAD+
- Succinate dehydrogenase is the only enzyme of the TCA cycle that is embedded in the inner mitochondrial membrane.
- Succinate dehydrogenase functions as Complex II of the electron transport chain



Step 7: Hydration of fumarate

- Fumarate is hydrated to malate by fumarase (fumarate hydratase)
- A reversible reaction



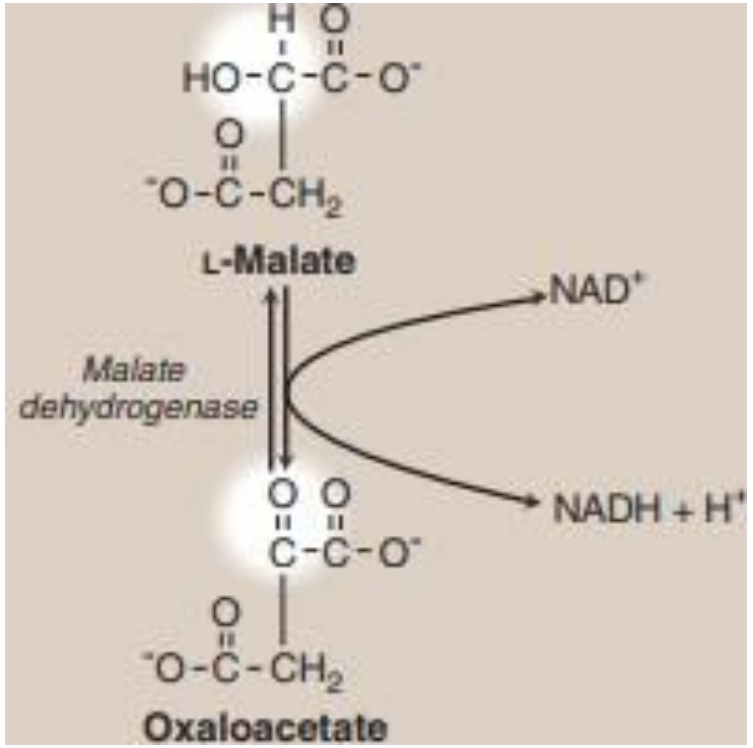
Double bond is broken

Last one fast one ;)

Step 8: Oxidation of malate

Reversible reaction

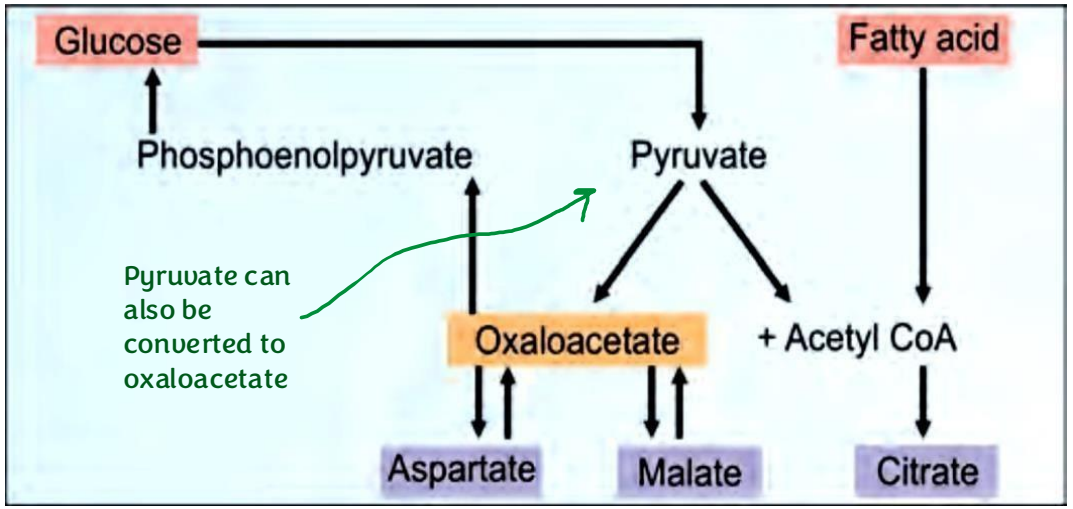
- Malate is oxidized to oxaloacetate by malate dehydrogenase
- The Alcohol group of malate oxidized to a keto group
- This reaction produces the third and final NADH of the cycle.
- The ΔG^0 of the reaction is positive, but the reaction is driven in the direction of oxaloacetate by the highly exergonic citrate synthase reaction.



Oxaloacetate is the α-ketoacid of aspartate.

Oxaloacetate as a junction point

Or a connecting point between the Krebs cycle pathway & other pathways



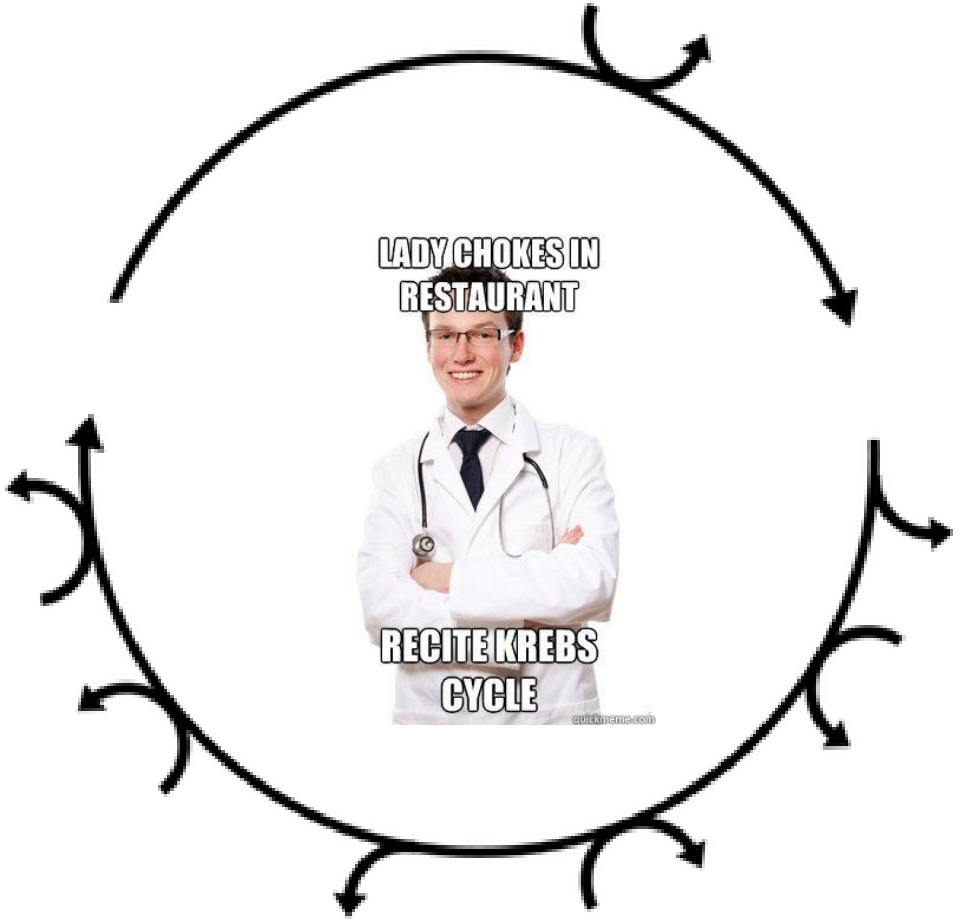
Oxaloacetate → malate is an efficient way to export oxaloacetate from the mitochondria, because there's exists no transporter for oxaloacetate. In the cytosol, malate converts back to oxaloacetate to synthesize glucose.

- **An important junction point in metabolism**

Now it's your turn!

Fill the cycle in, and don't forget to include the enzymes for each step!

Hint: Citrate Is Krebs Starting Substrate For Making Oxaloacetate



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For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:

Reference Used:
(numbered in order as cited in the text)

Lippincott’s Biochemistry 8th Edition- pages 331-344

Extra References for the Reader to Use:

Metabolism | The Krebs Cycle- Ninja Nerd

قَالَ رَسولُ اللَّهِ ﷺ: كانَ مِن دُعاءِ دَاوُدَ صَلّى اللهُ عَلَيْهِ وسَلَّم: "اللَّهمَّ إنِّي أَسْأَلُكَ حُبَّكَ، وَحُبَّ مَنْ يُحِبُّكَ، وَالعمَل الَّذِي يُبَلِّغُني حُبَّكَ، اللَّهُمَّ اجْعل حُبَّكَ أَحَبَّ إِلَيَّ مِن نَفسي، وأَهْلي، ومِن الماءِ البارِدِ

اللهم نستودعك أهالي غزّة وفلسطين ولبنان والسودان فانصرهم واحفظهم بعينك التي لا تنام، واربط على قلوبهم وأمدهم بجُندك وأنزل عليهم سكينتك وسخر لهم الأرض ومن عليها ربنا افرغ علينا صبرا و ثبت أقدامنا وانصرنا على القوم الكافرين، اذكروهم في ركعتي قيام .
المجازر مستمرة ..