

METABOLISM

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



MID – Lecture 4

TCA cycle (pt. 1)

﴿ وَإِن تَتَوَلَّوْا يَسْتَبَدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَلَكُمْ ﴾

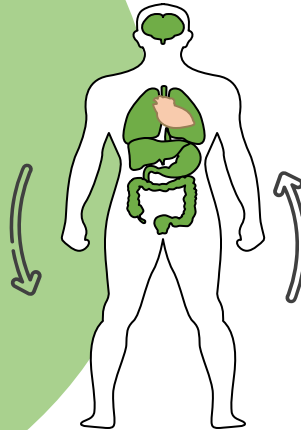
اللهم استعملنا ولا تستبدلنا

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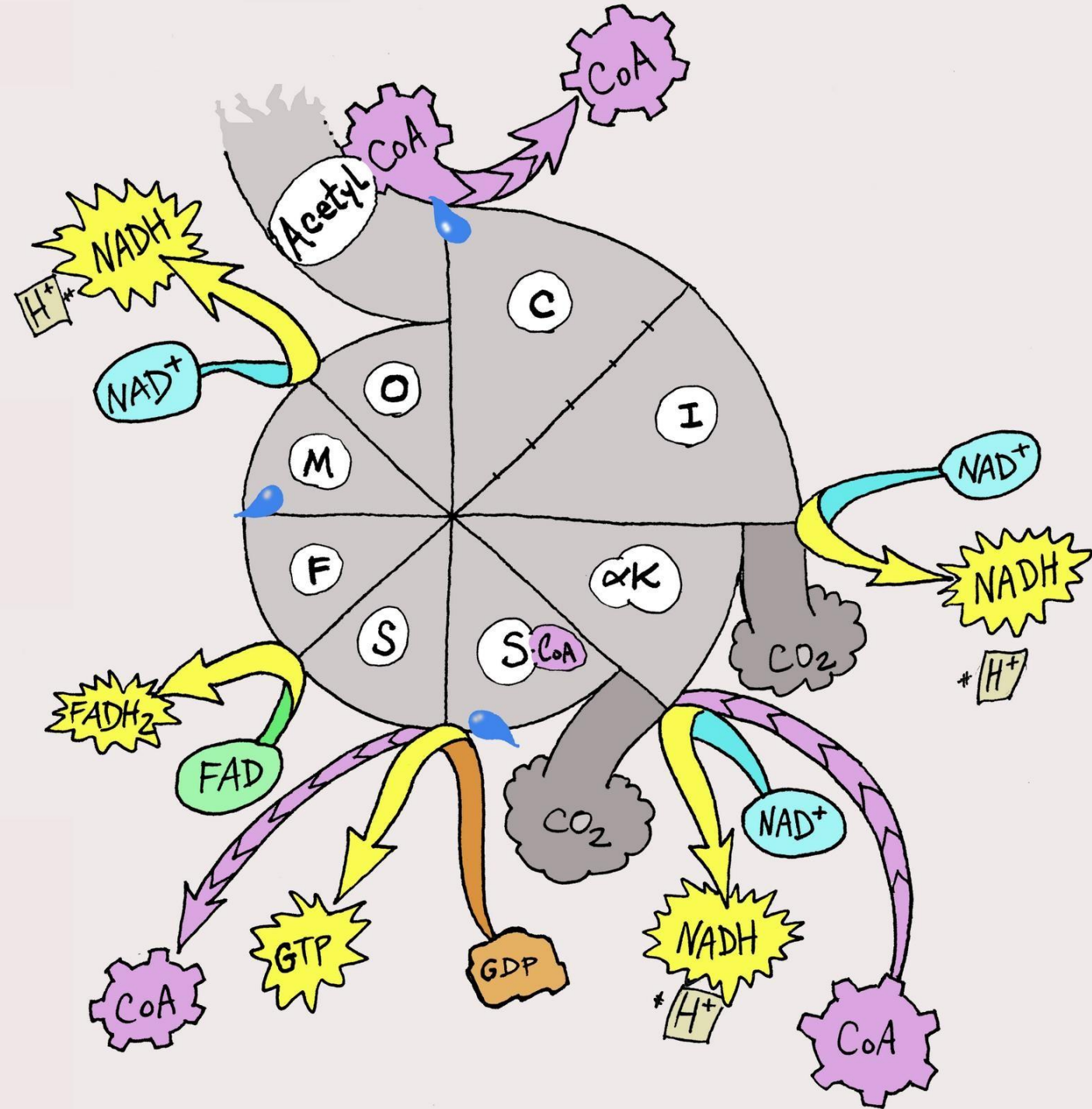
- Dana Hijeh



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

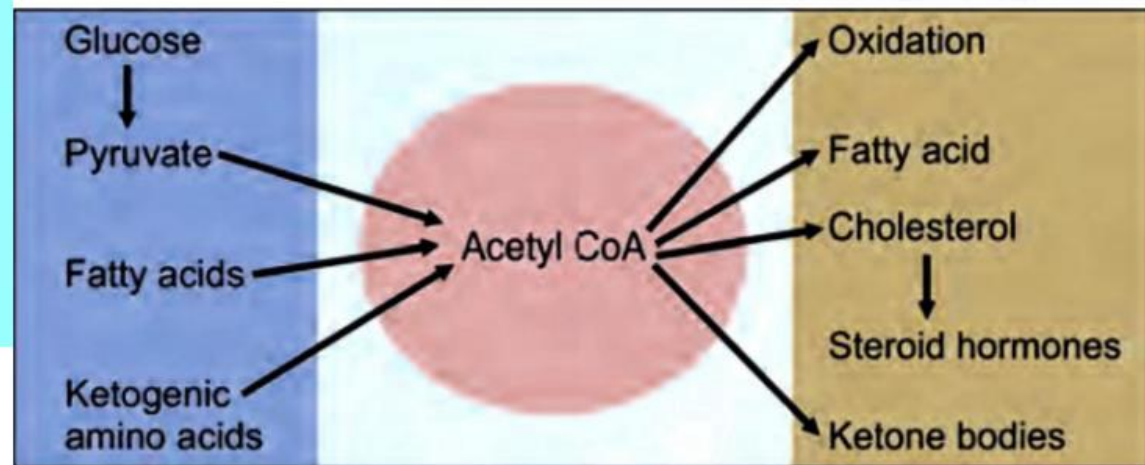
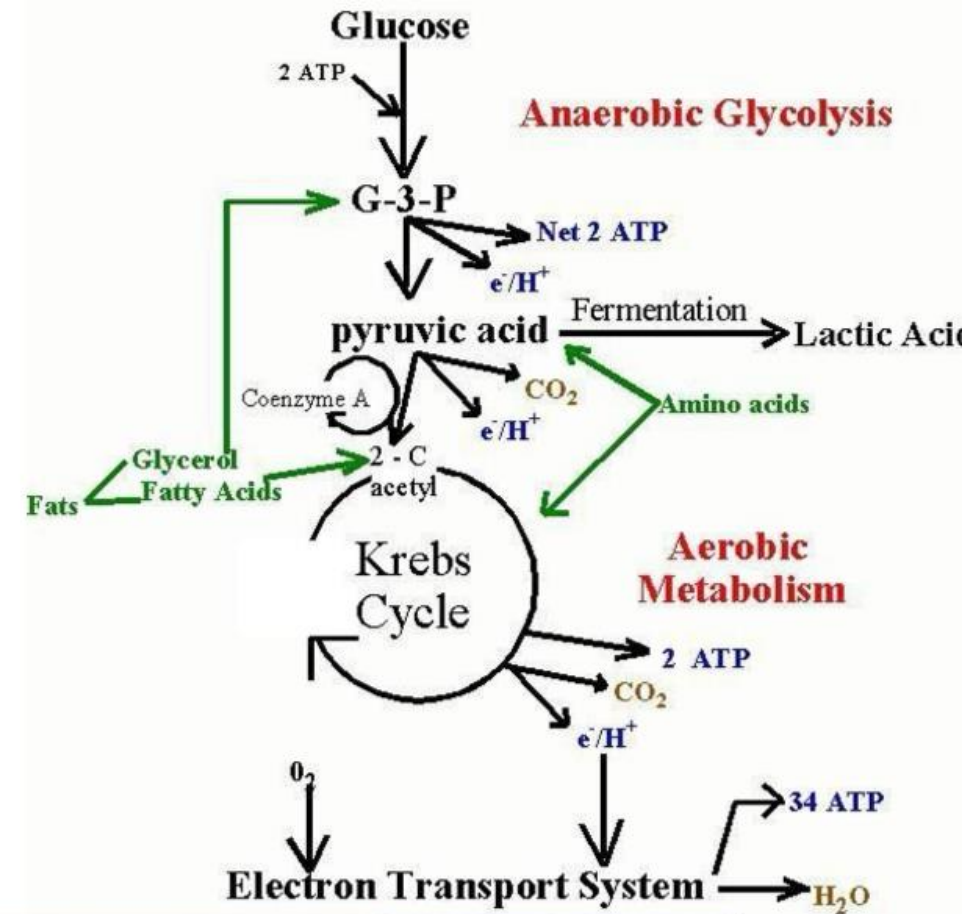
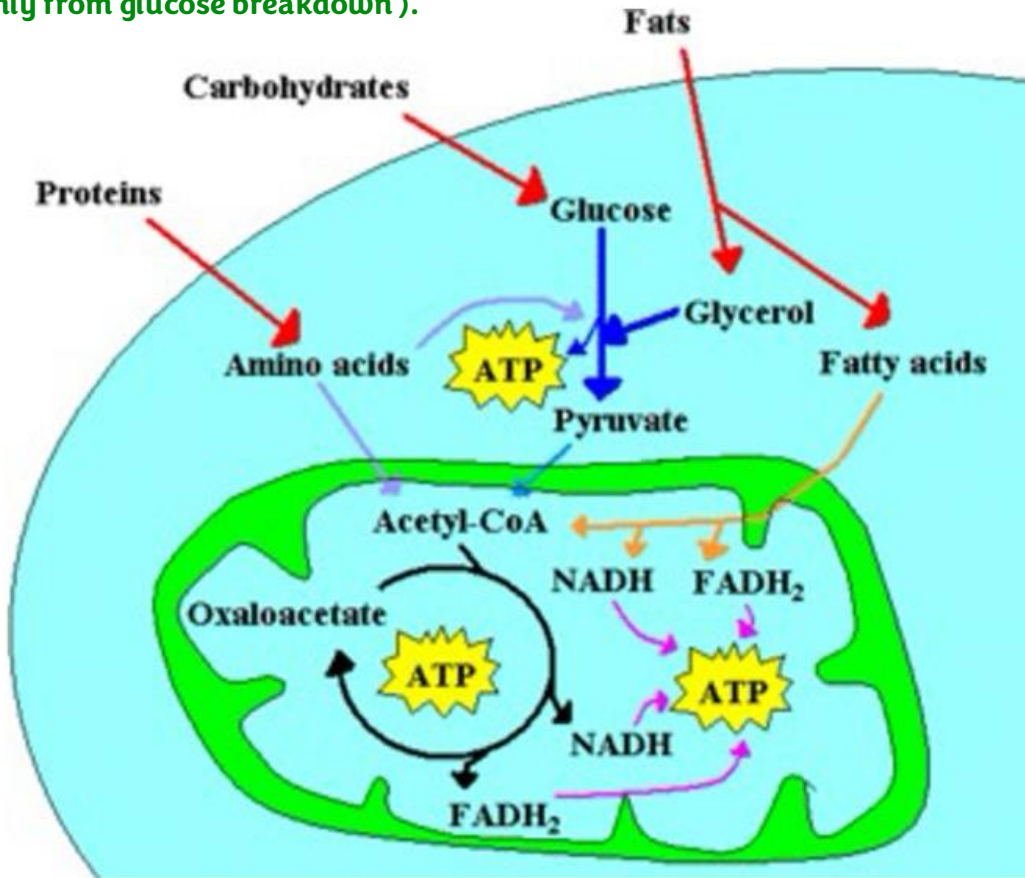
Produce citrate

TCA= citrate



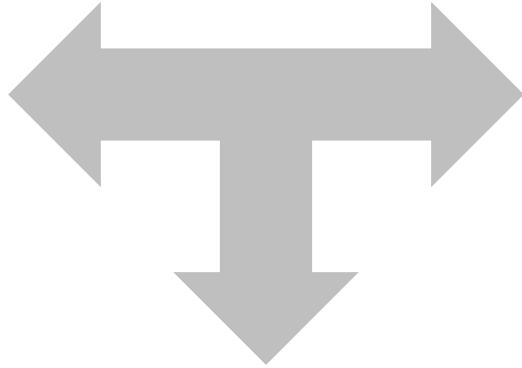
Sources and Uses of Acetyl-CoA

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 ?

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



Degradation of proteins gives amino acids. Some amino acids 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 needs **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, Acetyl-coA is used in a Krebs cycle, but sometimes when the Acetyl-coA is produced in higher concentrations than needed for Krebs cycle, the rest of Acetyl-coA will be used in manufacturing other compounds in order to avoid accumulation.

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

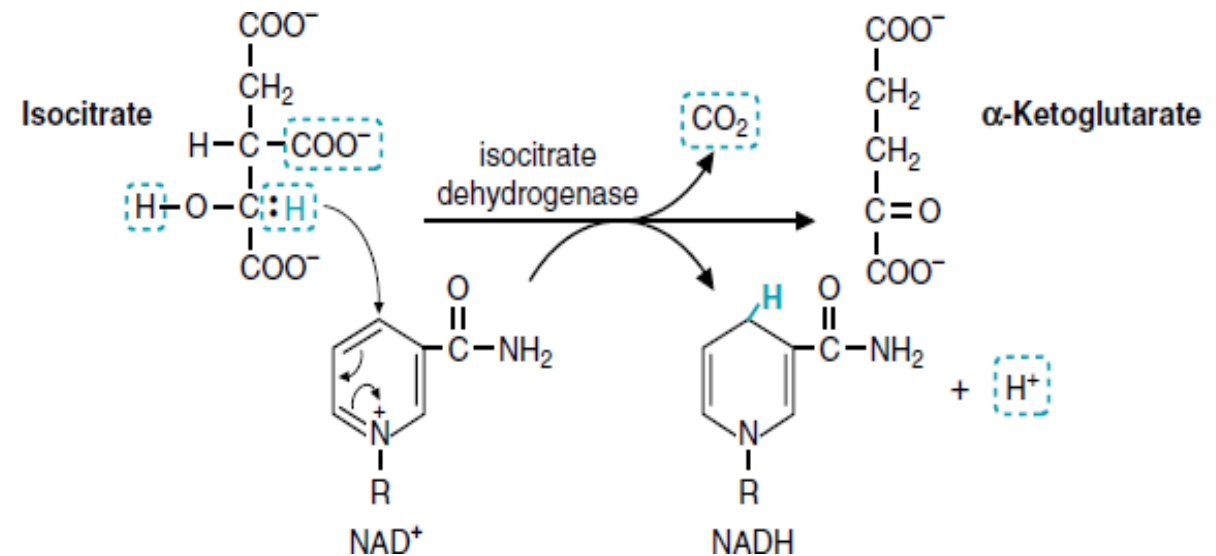
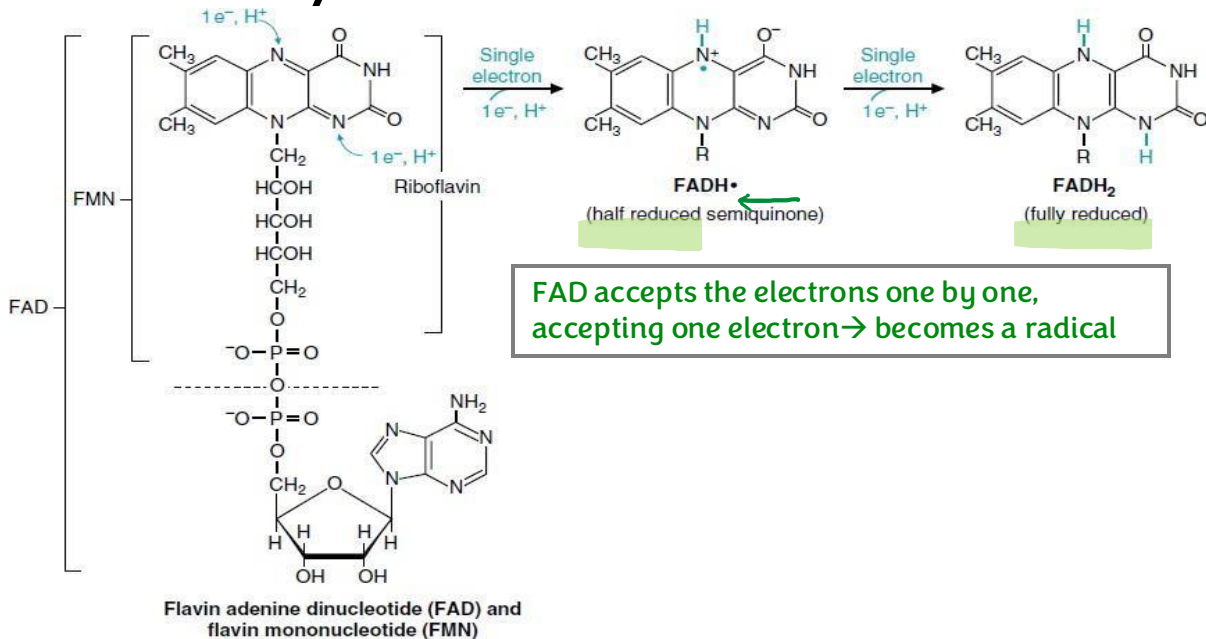
➤ FAD

➤ NAD

- Single electrons ($H\bullet$), 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° 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

Oxidation of Alcohol to Ketone accompanied by NAD⁺ reduction



Stepwise Reactions

اللهم أرشدني بك إليك ودلني بك عليك

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 O₂ introduced
- Two CO₂ exits

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

Oxidation of succinate accompanied with reduction to FAD

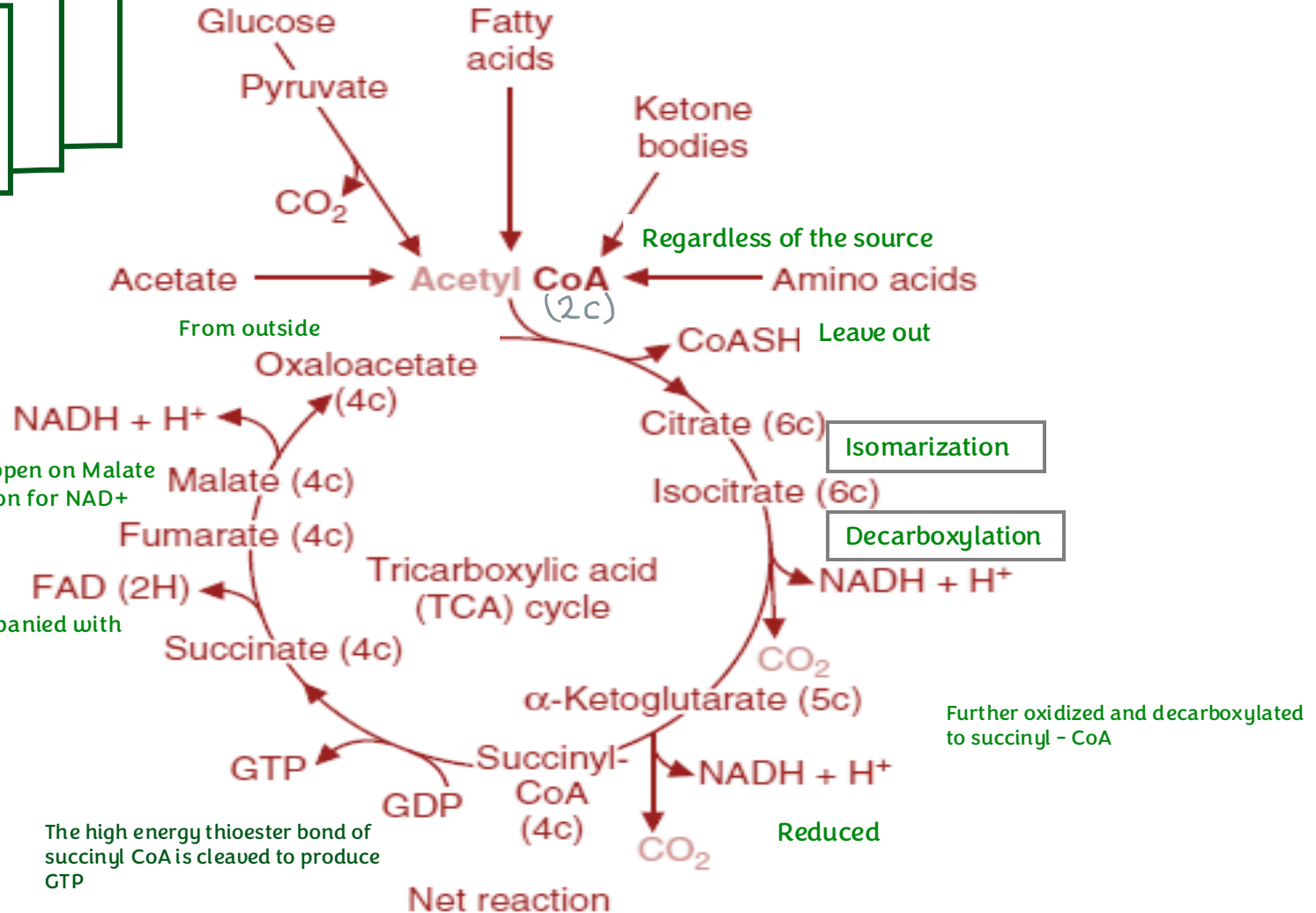
Succinyl coA is highly energy molecule due to presence of coA, this energy is used to phospholirate GDP to produce GTP

*Focus on number of Carbon for each compound :)

*What is the only energy molecule produced directly during Krebs cycle ? GTP

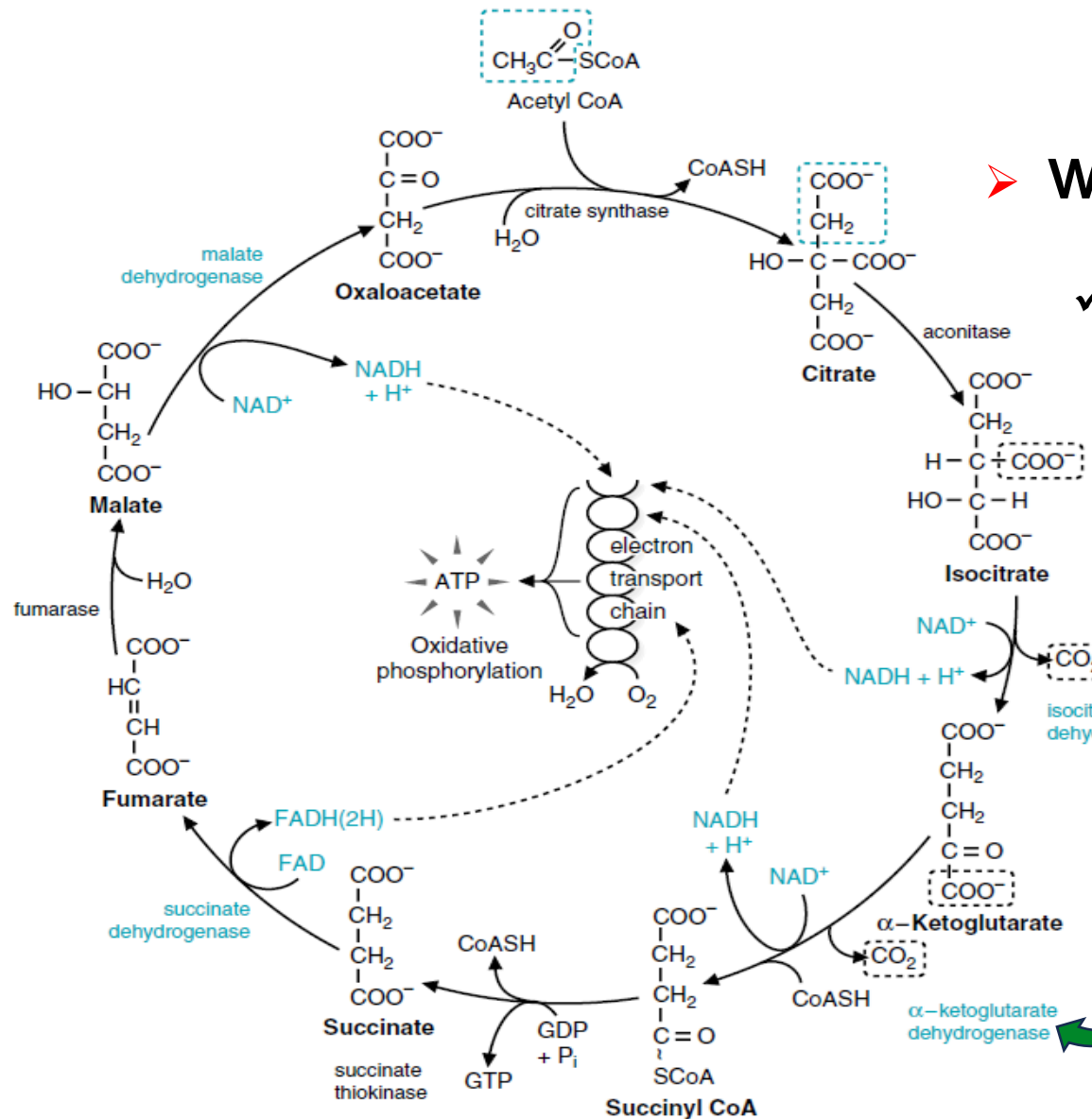
*2 carbons enter as Acetyl-coA and 2 carbons exit one by one as co2 (same carbons).

*krebs cycle considered as both anabolic and catabolic.



Does Acetyl-CoA exit as CO₂?

اللهم صل على سيدنا محمد عدد كمال الله وكما يليق بكماله .



➤ Why to make Isocitrate from citrate?

✓ Citrate is a tricarboxylic acid

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

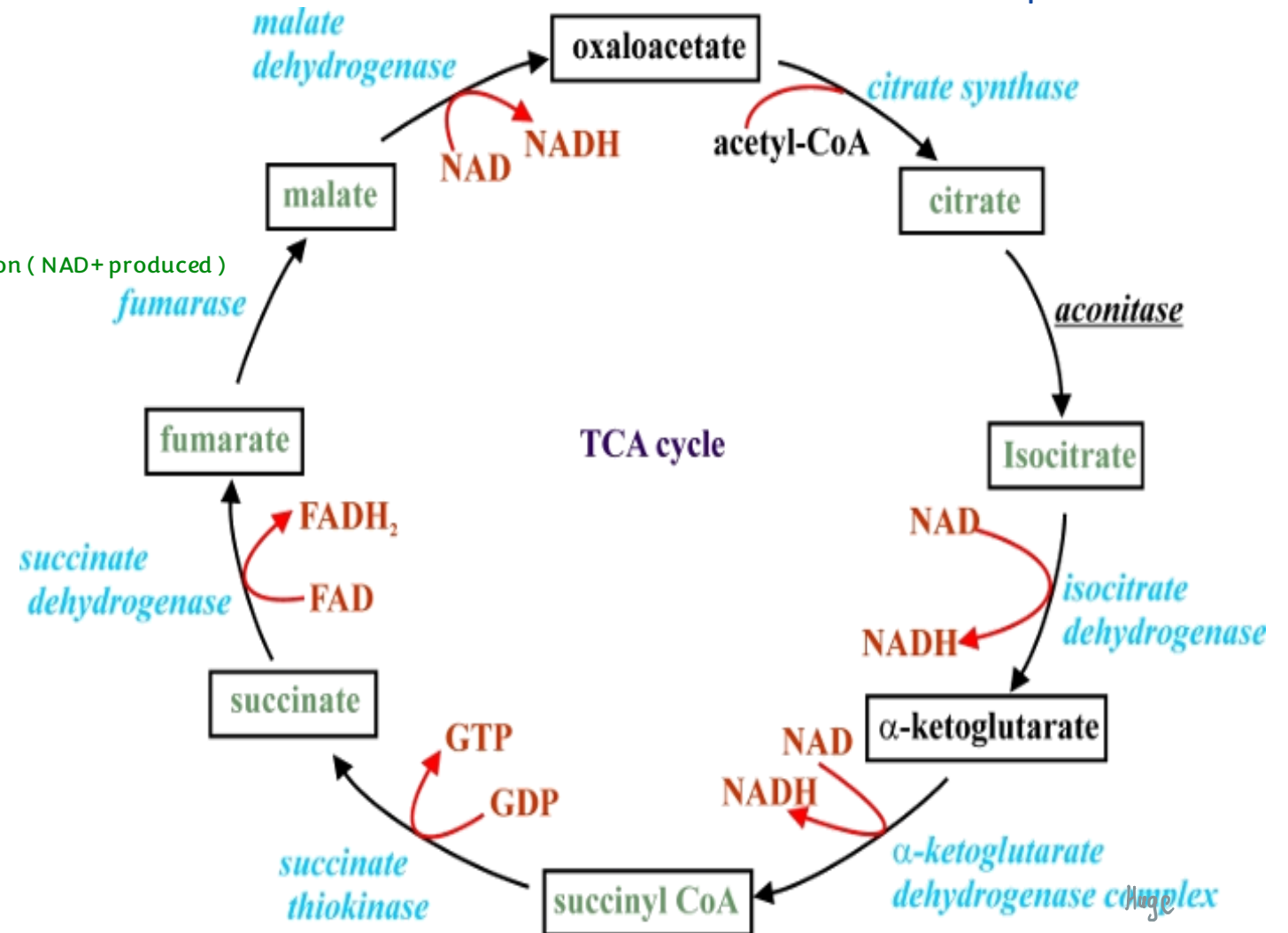
➤ Where does the CO₂ exit?

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⁺)

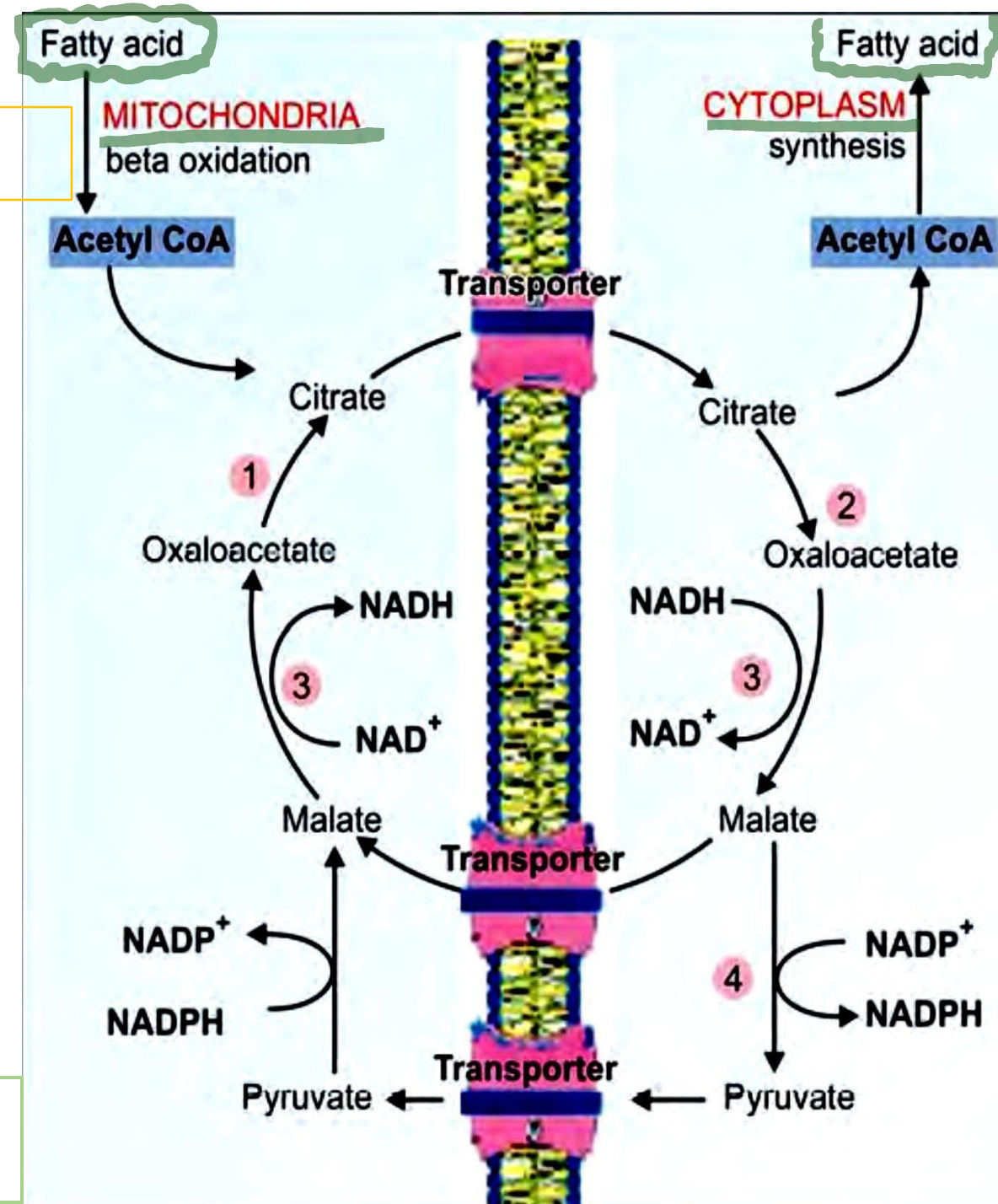
Which one is the only enzyme of krebs cycle enzymes that found in inner mitochondrial membrane protein ?
Succinate dehydrogenase. Responsible for catalyzing step six , the other enzymes are soluble in matrix .

Memorize compounds name



Step 1: Formation of Citrate

- ✓ Citrate synthase is inhibited by its product, citrate. (**Product inhibition**)
- ✓ 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 .

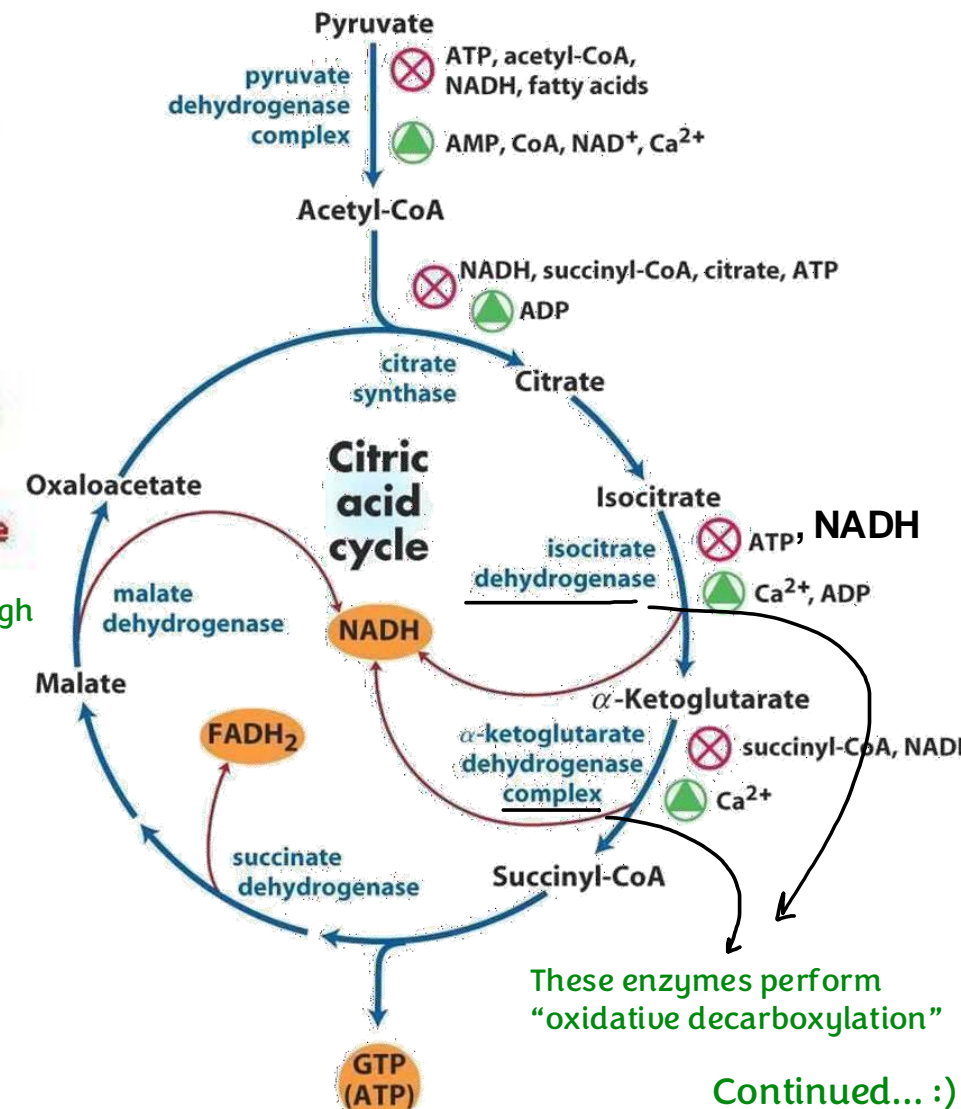
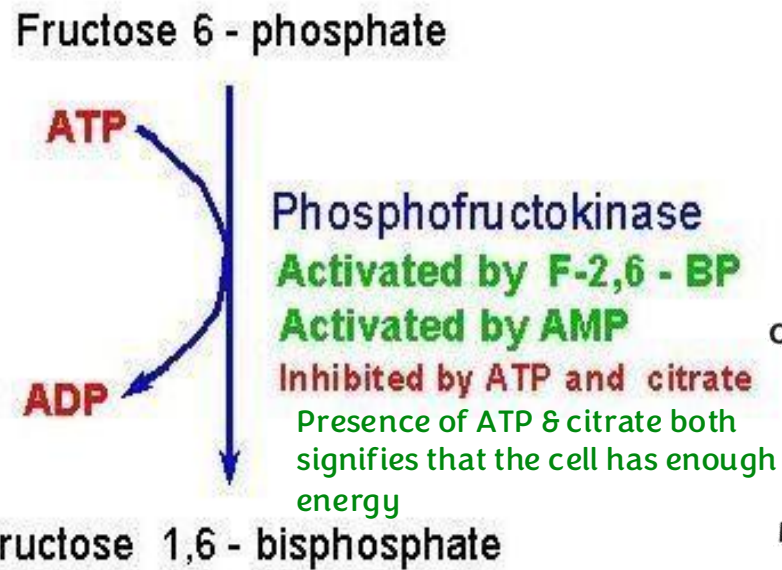
Citrate has transporter in inner mitochondrial membrane so it can exit to cytosol (excess citrate), in cytosol it can activate fatty acids synthesis by reproducing Acetyl coA (precursor molecule for synthesizing fatty acids)

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

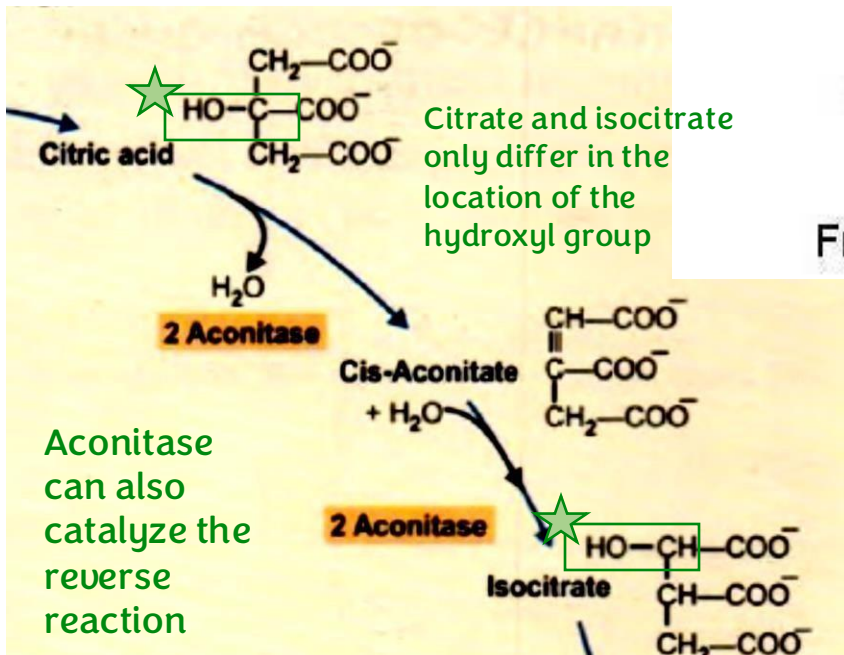
Step 2+3: Formation and Oxidation of Isocitrate

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

Control at the committed step of glycolysis



✓ Aconitase is an Fe-S protein



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

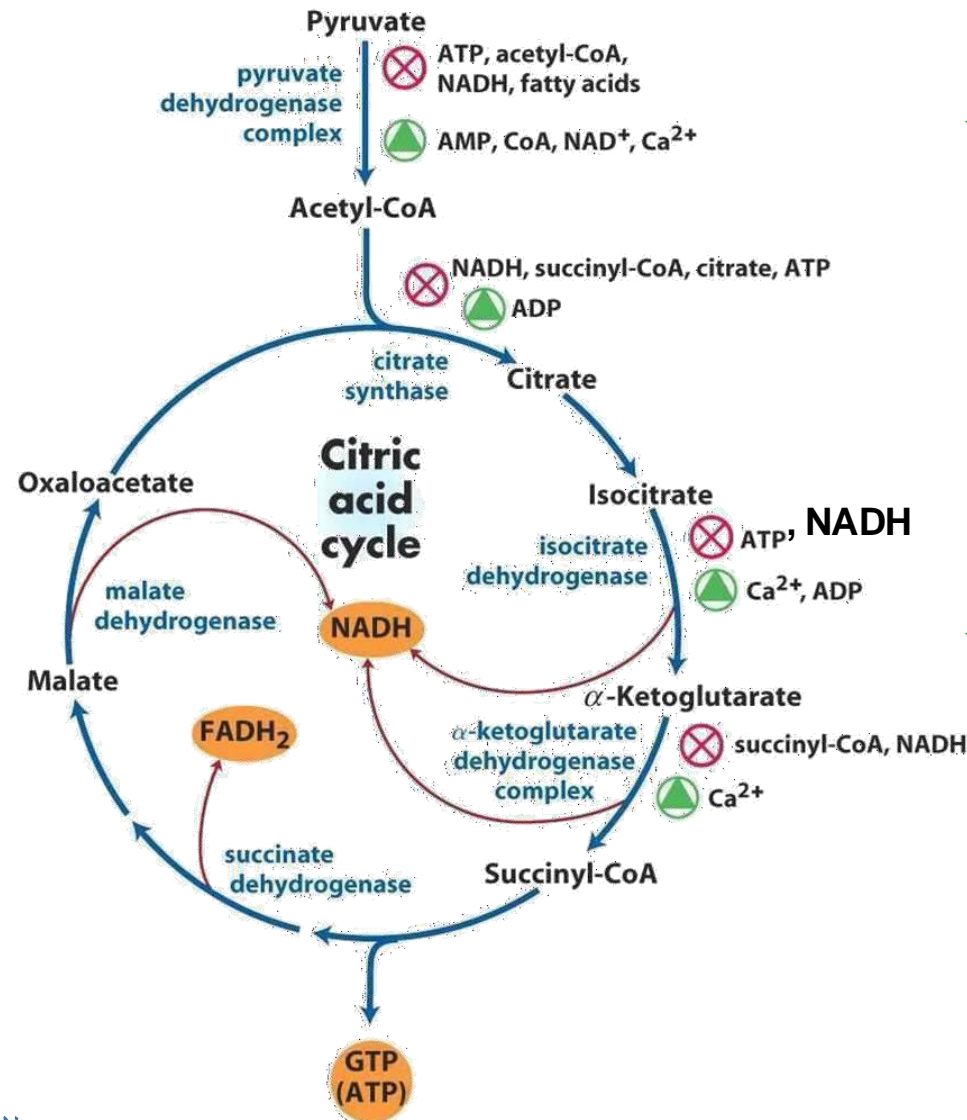
Continued... :)

Step 2+3: Formation and Oxidation of Isocitrate

و أنت تجعل الصعب إذا شئت سهلا

★ **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.

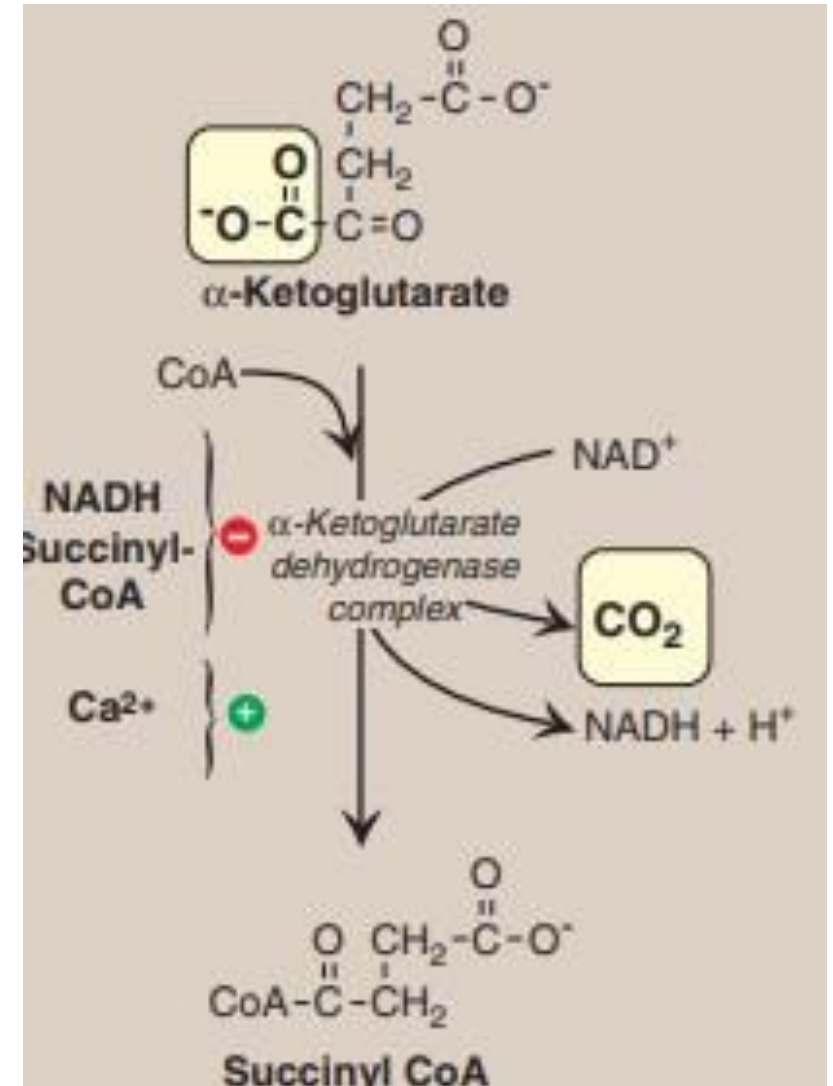
★ If [NADH] is high, this means we don't have the necessary coenzyme state (oxidized form) and the reaction is inhibited. **NAD⁺ is needed in the cell more than NADH.**

Remember: NADH and FADH₂ are not energy molecules, they are coenzymes.

Step 4: α -Ketoglutarate to Succinyl-CoA

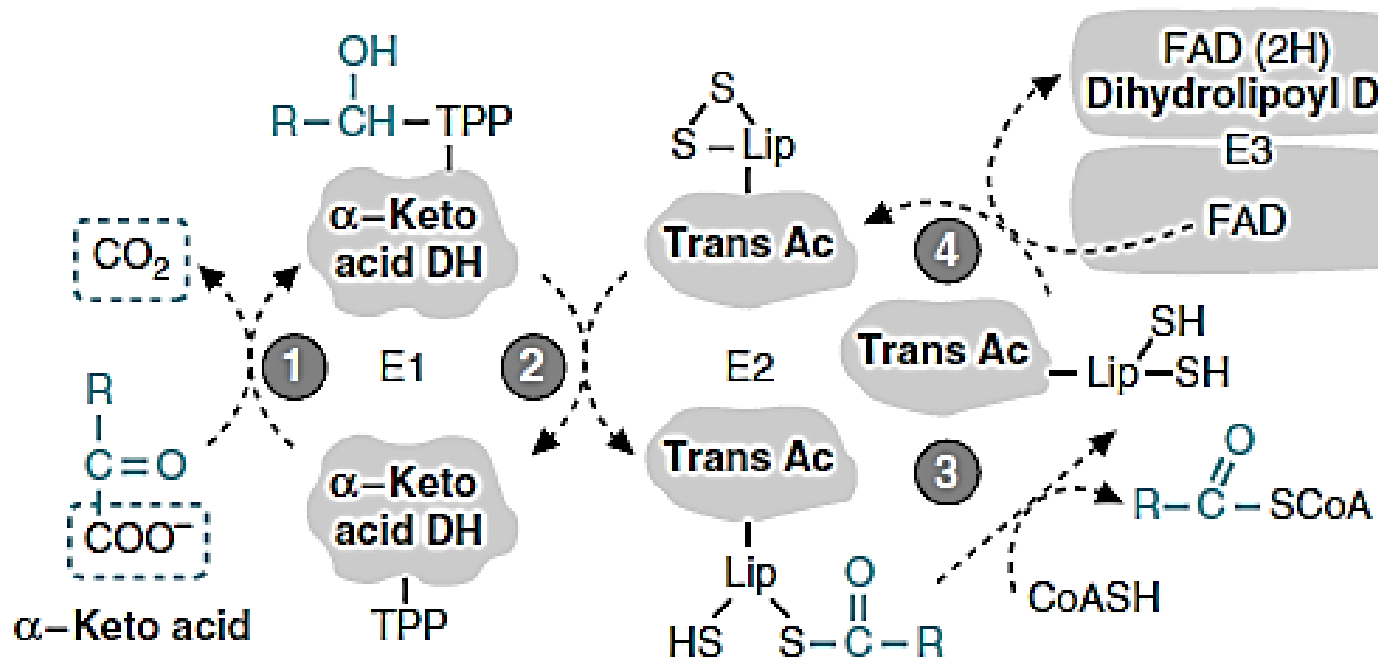
- Oxidative decarboxylation
- α -ketoglutarate dehydrogenase complex, a multimolecular aggregate of three enzymes
- Thiamine pyrophosphate, lipoic acid, FAD, NAD⁺, and CoA → All are examples of coenzymes
- Energy conserved as NADH, thioester bond

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

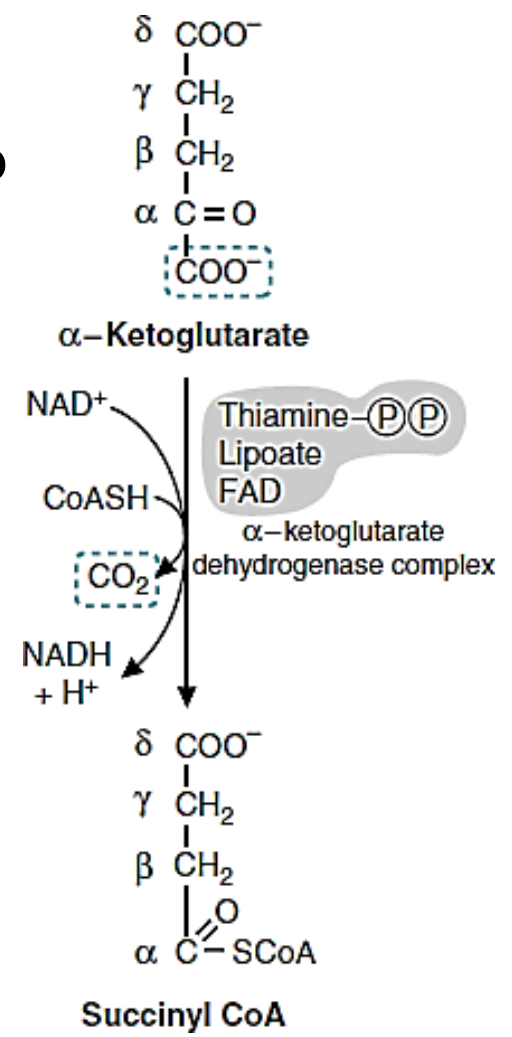


α -Ketoacid Dehydrogenase Complexes (TLCFN)

- (α -ketoglutarate, pyruvate, and branched chain α -keto acid) dehydrogenase complexes
 - Amino acid - NH_3 = keto acid
 - Alanine - NH_3 = pyruvate
- Huge enzyme complexes, multiple subunits of 3 different enzymes (no loss of energy, substrates for E2 and E3 remain bound \rightarrow higher rate)
- E1, E2, & E3 are a decarboxylase (TPP), a transacylase (lipoate), & a dehydrogenase (FAD)

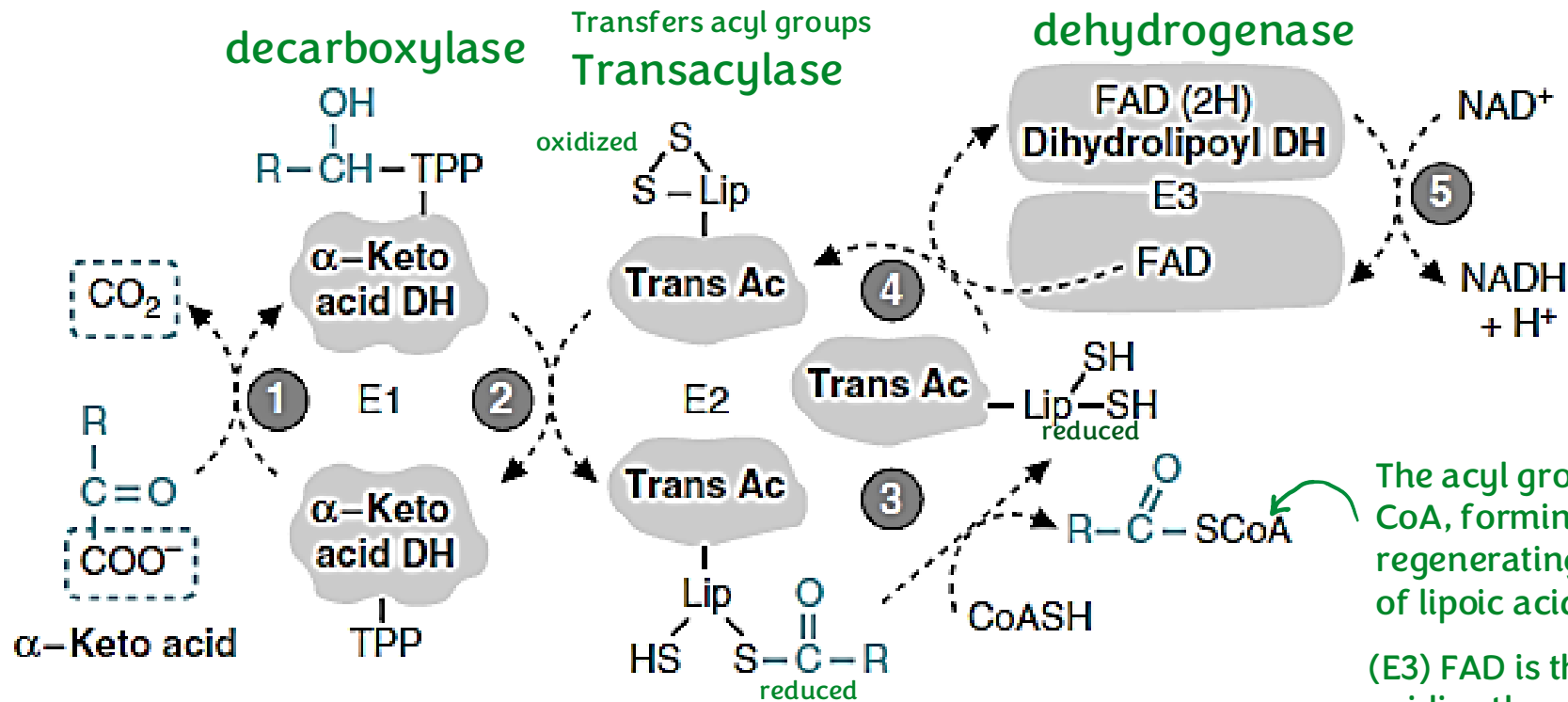


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α -Ketoacid Dehydrogenase Complexes (TLCFN)

TPP, lipoic acid, CoA, FAD, & NAD⁺



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

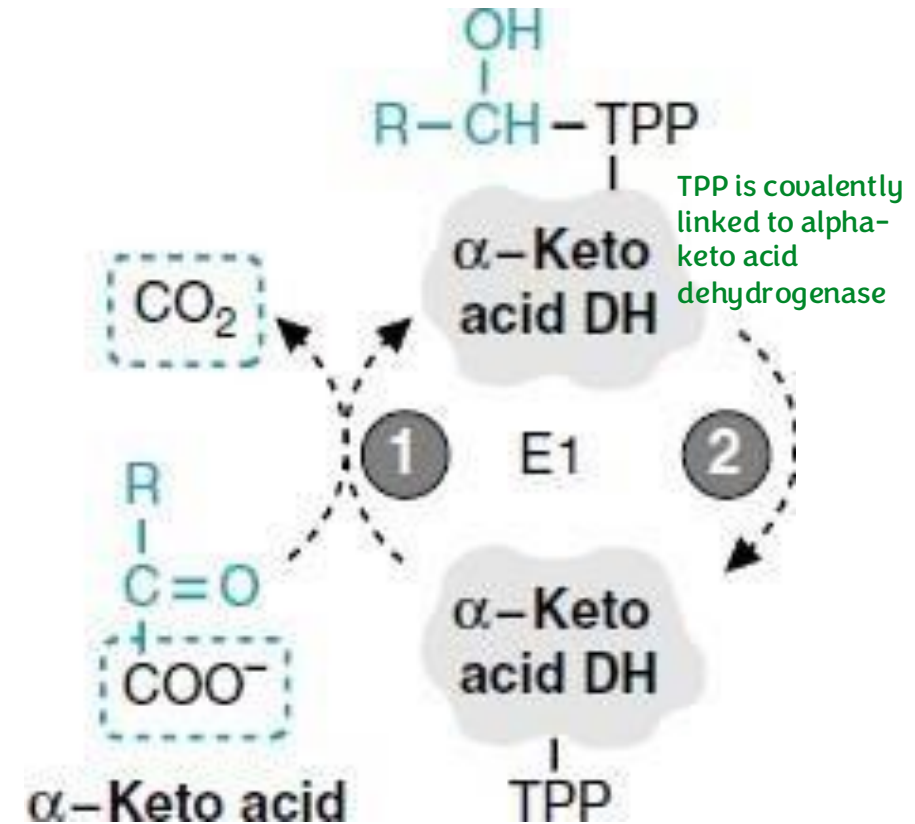
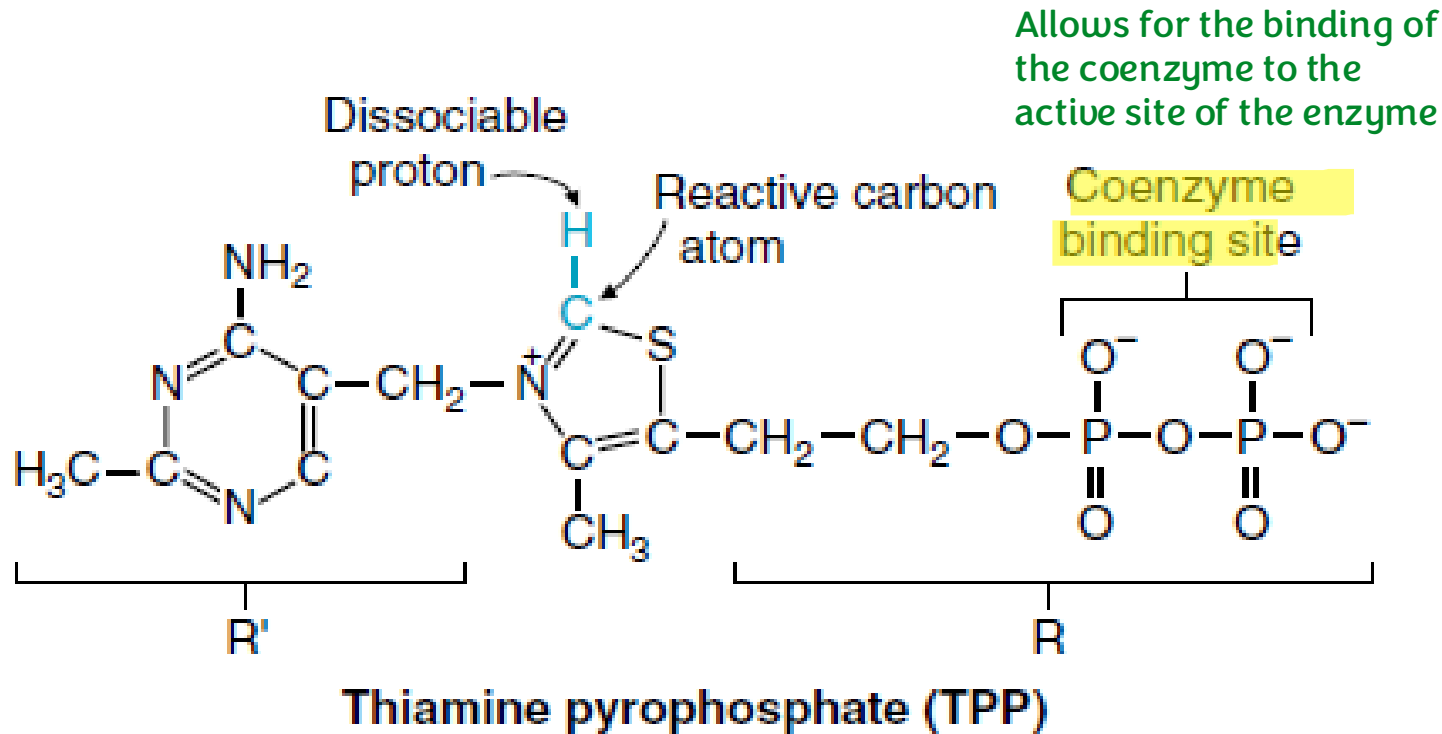
(E3) FAD is then reduced to oxidize the reduced lipoic acid (step 4)

NAD^+ is reduced to $\text{NADH} + \text{H}^+$ (step 5)

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.

Thiamine Pyrophosphate

- Thiamine (vitamin B₁) deficiency, α-ketoglutarate, pyruvate, & branched chain α-keto acids accumulate in the blood
Composed of 3 enzymes

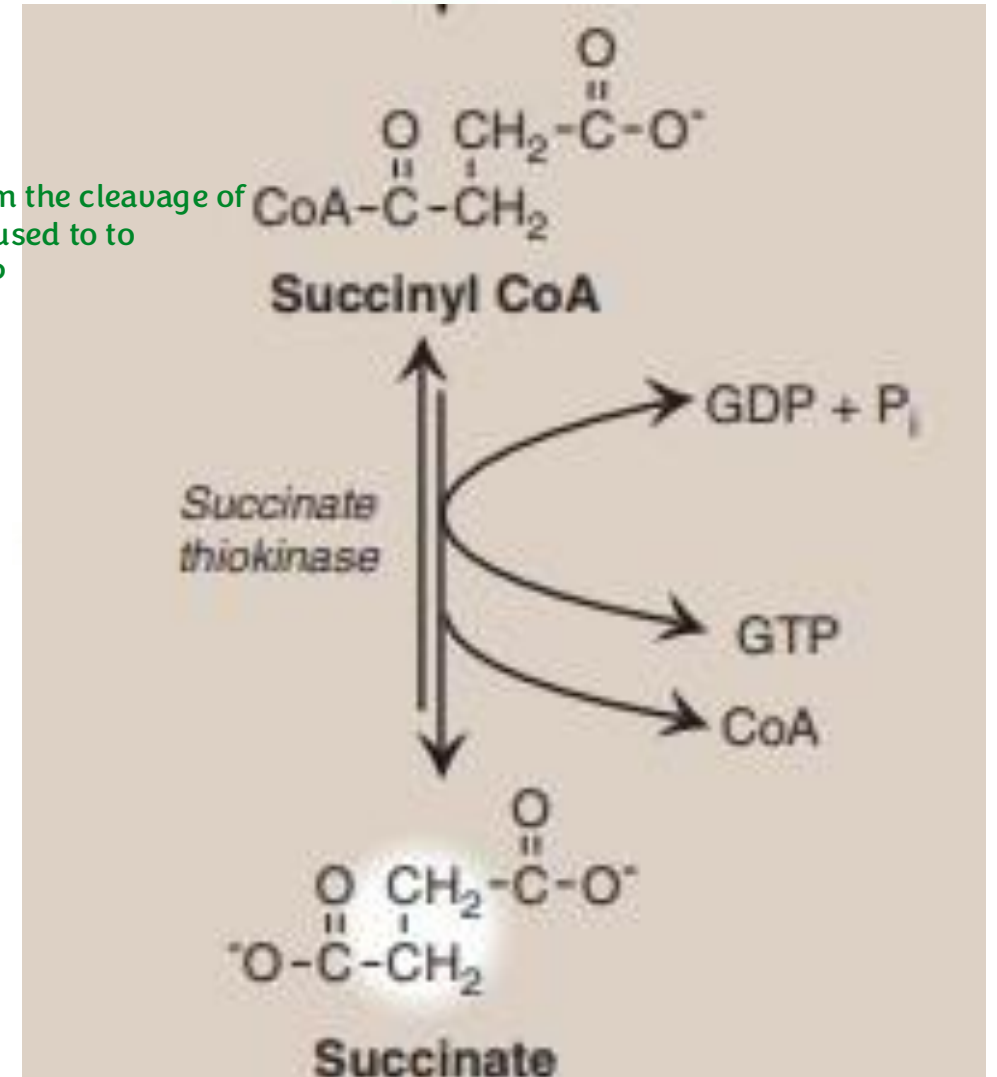


Step 5: Cleavage of Succinyl CoA & Generation of ATP

- Succinate thiokinase (succinyl CoA synthetase—named for the reverse reaction) cleaves the high-energy thioester bond of succinyl CoA
 - Succinyl CoA has a thioester bond (CoASH & an acyl group)
 - GTP is produced by substrate level phosphorylation
- ✓ GTP and ATP are energetically interconvertible by the nucleoside diphosphate kinase reaction



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



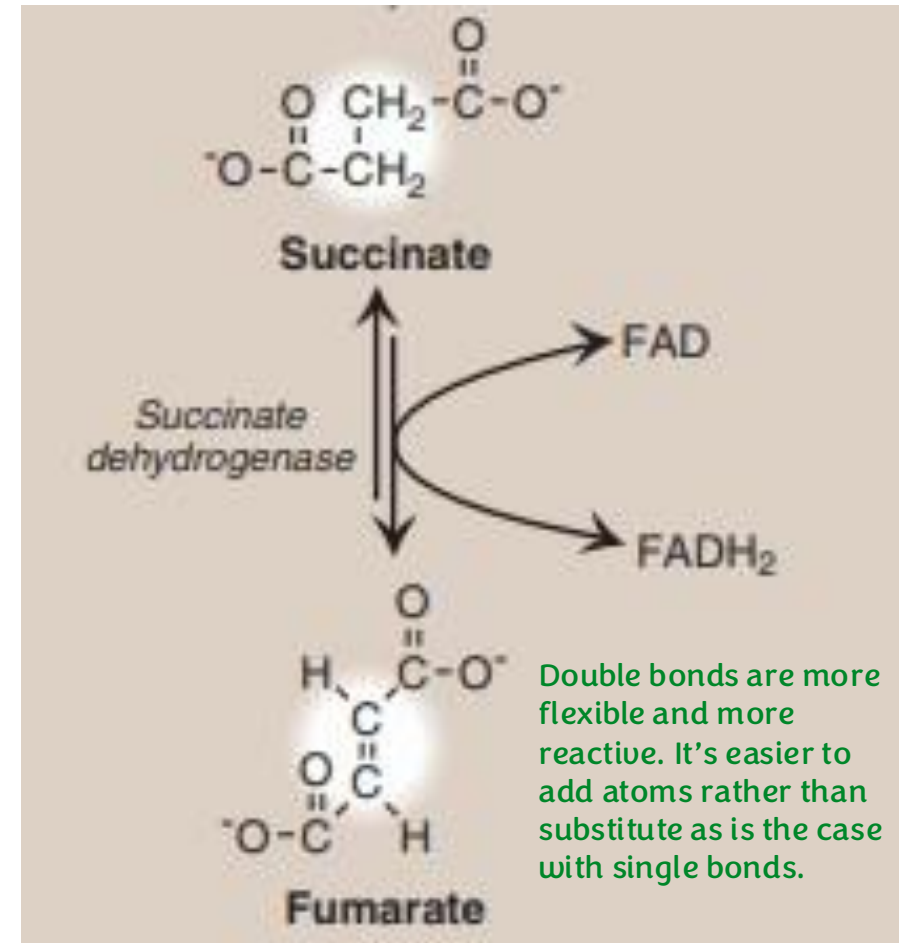
Step 6: Oxidation of succinate

Remember: this enzyme is the only enzyme present in the inner mitochondrial membrane

- 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

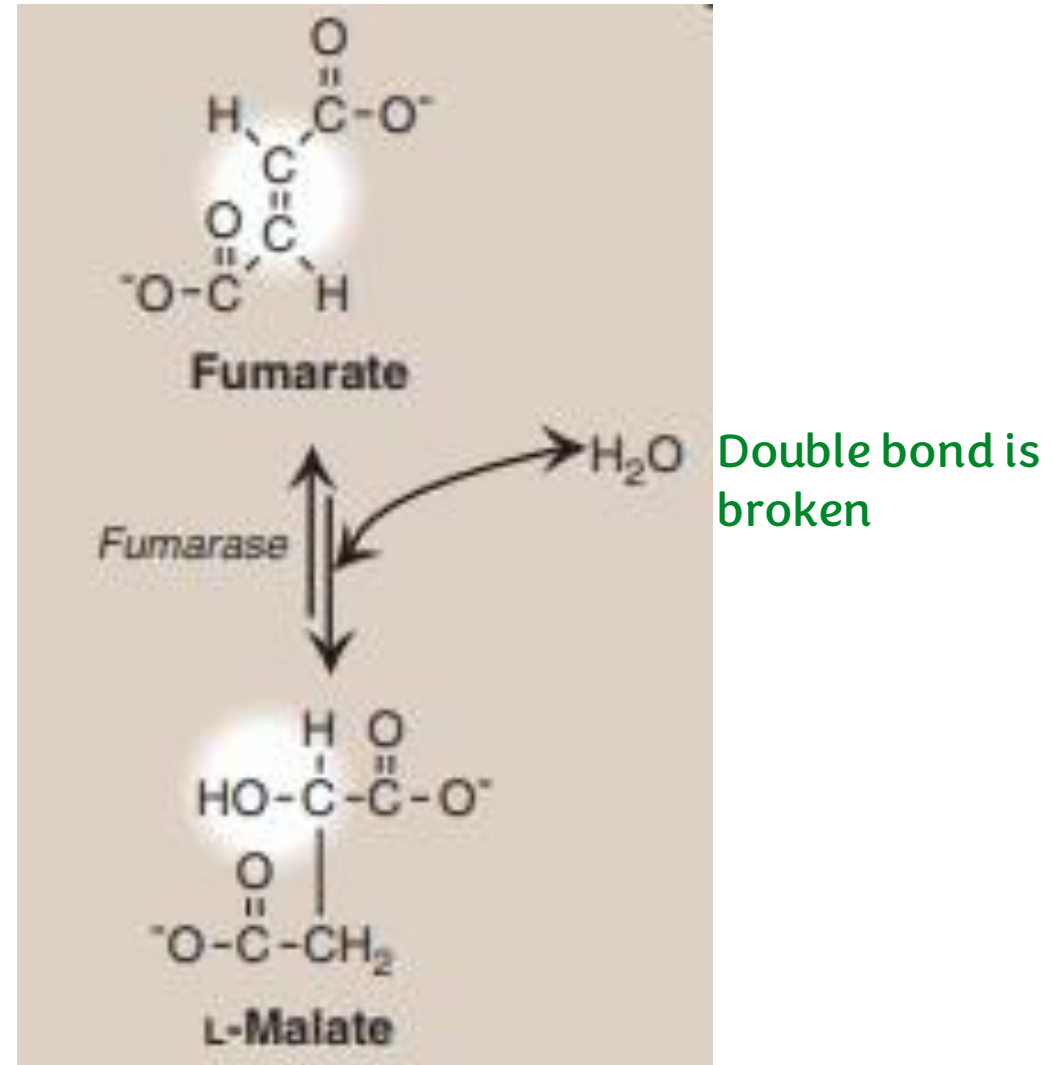
NAD⁺ has greater reducing potential than FAD & therefore gives off more energy.

This reaction is reversible



Step 7: Hydration of fumarate

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

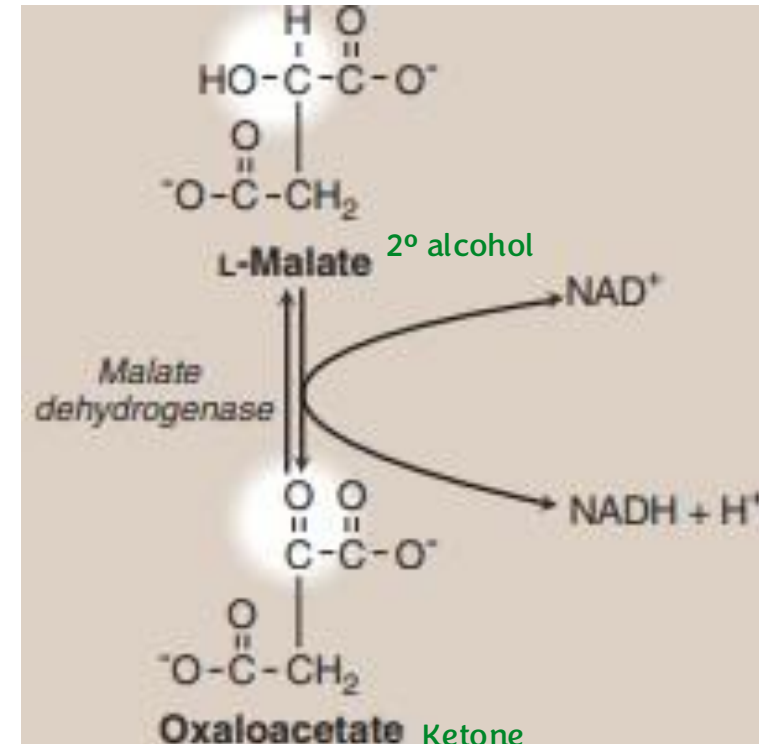


Last one fast one ;)

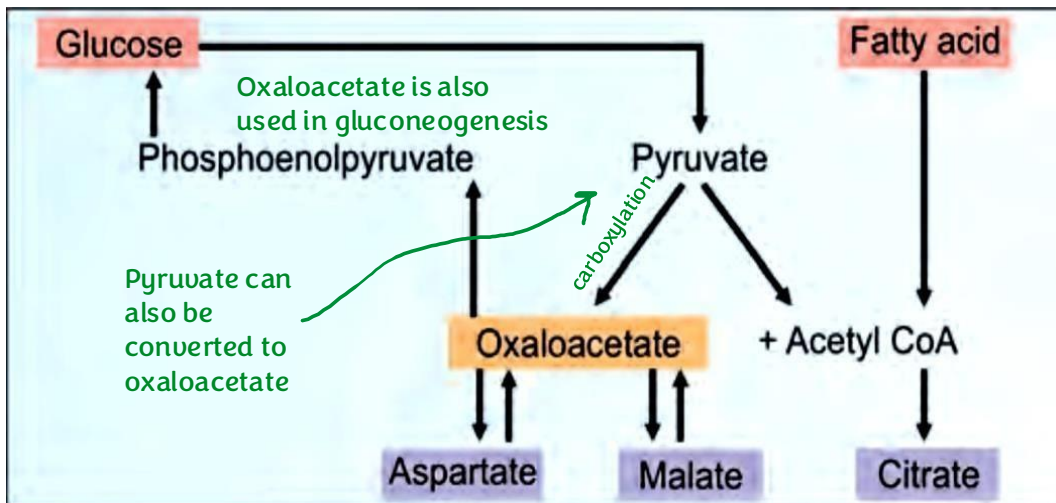
Step 8: Oxidation of malate

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 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 isn't a transporter for oxaloacetate. In the cytosol, malate converts back to oxaloacetate to synthesize glucose.

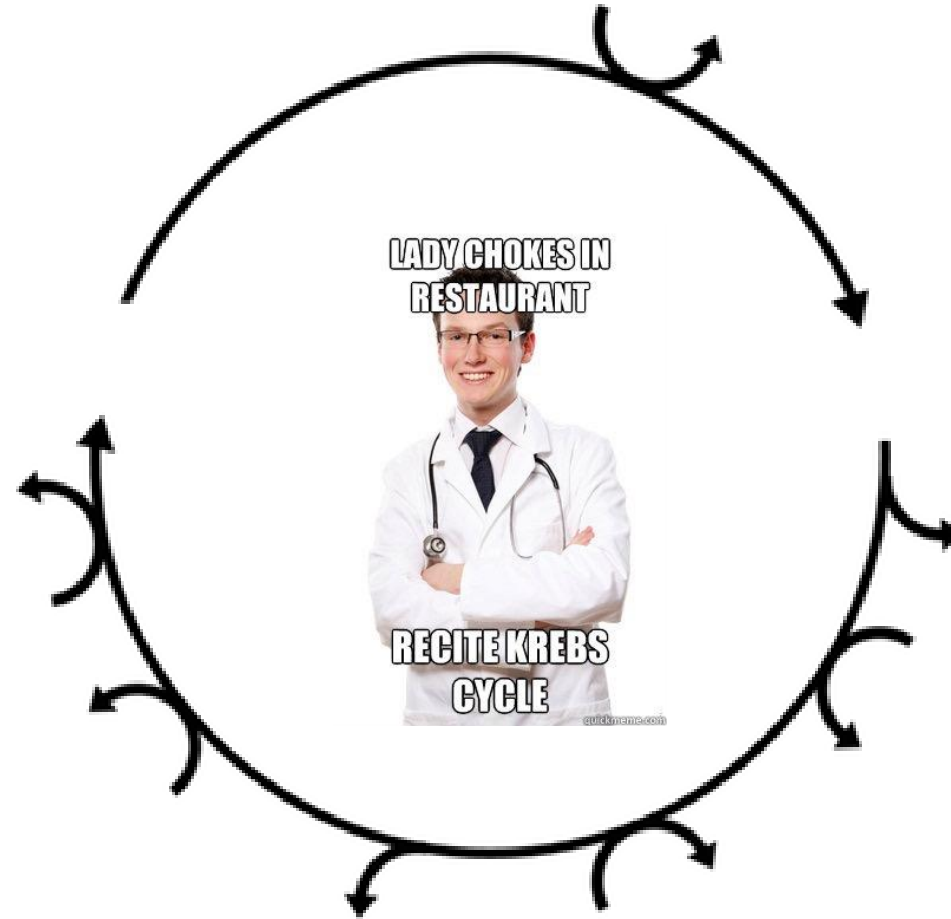
Oxaloacetate is the α -ketoacid of aspartate.

- 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



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	<ul style="list-style-type: none"> - “TPP is covalently linked...” (Slide #15) - Additional info prof. mentioned in a later lecture 	<ul style="list-style-type: none"> - “TPP is covalently linked to alpha-keto acids” - N/A 	<ul style="list-style-type: none"> - “TPP is covalently linked to alpha-keto acid dehydrogenase” - “NAD+ & FAD are not energy molecules...” (Slide #11). - General info on slide #17. - “Ketone” & “2° alcohol” (Slide #19).
V1 → V2	<ul style="list-style-type: none"> - Slide 4 (in the right bottom box) - Slide 9 (next to first enzyme) 	<ul style="list-style-type: none"> - Fatty acids are used in Krebs.. - Inhibition feedback by same enzyme 	<ul style="list-style-type: none"> - Acetyl-CoA instead of fatty acid - product inhibition
V2 → V3	Slide #19	“Oxaloacetate is also used in glycogenesis ”	“Oxaloacetate is also used in gluconeogenesis ”
V3 → v4	Slide 6		
V4 → v5	Slides 16-19 were missed	Further oxidized and carboxylated to succinyl – CoA- was on the left side of the cycle	Further oxidized and decarboxylated to succinyl – CoA – on the right side - This sentence is added on the left side : The high energy thioester bond of succinyl CoA is cleaved to produce GTP

Additional Resources:

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](#)

رسالة من الفريق العلمي:

قَالَ رَسُولُ اللَّهِ ﷺ: كَانَ مِنْ دُعَاءِ دَاوُدَ صَلَّى اللَّهُ عَلَيْهِ وَسَلَّمَ: "اللَّهُمَّ إِنِّي أَسْأَلُكَ حُبَّكَ، وَحُبَّ مَنْ يُحِبُّكَ، وَالْعَمَلَ الَّذِي يُبَلِّغُنِي حُبَّكَ، اللَّهُمَّ اجْعَلْ حُبَّكَ أَحَبَّ إِلَيَّ مِنْ نَفْسِي، وَأَهْلِي، وَمِنَ الْمَاءِ الْبَارِدِ

اللهم نستودعك أهالي غزة 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 وثبت أقدامنا وانصرنا على القوم
الكافرين، اذكروهم في ركعتي قيام .
المجازر مستمرة ..