بسم الله الرحمٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Metabolism | Lecture #5

TCA Cycle

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The Central Metabolic Hub

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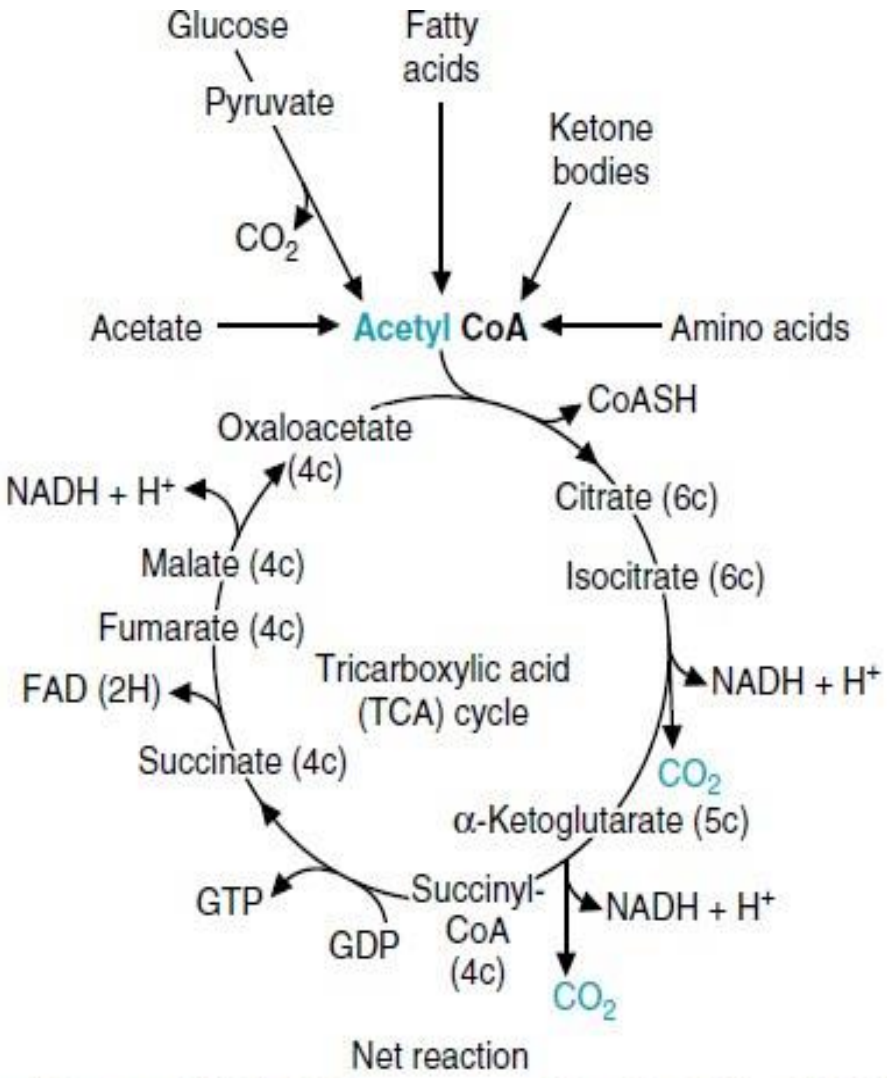
- The **Krebs Cycle** (refers to the scientist : Hans Krebs) , also known as the **Citric Acid** Cycle or the **TCA** (tricarboxylic acid) Cycle , all these names refer to the same metabolic pathway. It is a central metabolic hub inside the cells. It is considered the electron extraction machinery within cells, inside the mitochondria.

- Why is it called the Citric Acid Cycle?
  Because one of its **intermediates** is **citric acid**, which is the first product of the cycle.
- And why is it called the TCA Cycle or Tricarboxylic Acid Cycle?
  Because citric acid has **three carboxylic groups** in its structure. That's why it's referred to as tricarboxylic acid.
- Citric acid – or tricarboxylic acid – is also the acid found in lemon juice & lemon salt (ملح الليمون)



Net reaction

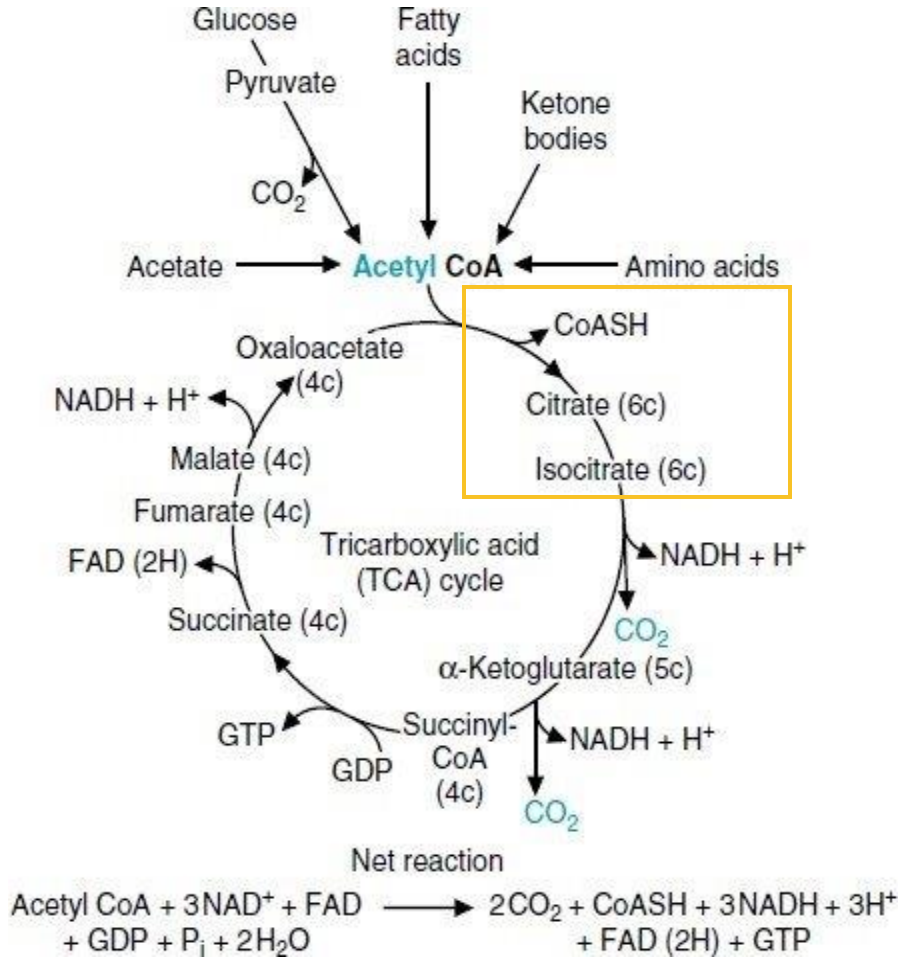
$$\text{Acetyl CoA} + 3\text{NAD}^+ + \text{FAD} + \text{GDP} + P_i + 2H_2O \longrightarrow 2CO_2 + \text{CoASH} + 3\text{NADH} + 3H^+ + \text{FAD (2H)} + \text{GTP}$$

This cycle constitutes 8 steps, 8 intermediates, 8 enzymes all these intermediates are required by name & structure with their catalyzing enzymes .

COMPONENTS & STEPWISE REACTIONS

- **CIA Sent Soldiers For My Office**

After the second stage of energy metabolism, various molecules – including fatty acids, ketone bodies, carbohydrates, acetate, and certain carbon backbones of amino acids – produce a compound known as **acetyl-CoA**.
This molecule consists of a **two-carbon unit** attached to **coenzyme A** and is considered a **high-energy compound**, because breaking the bond between acetyl and coenzyme A releases **energy** that can be used in subsequent metabolic reactions.

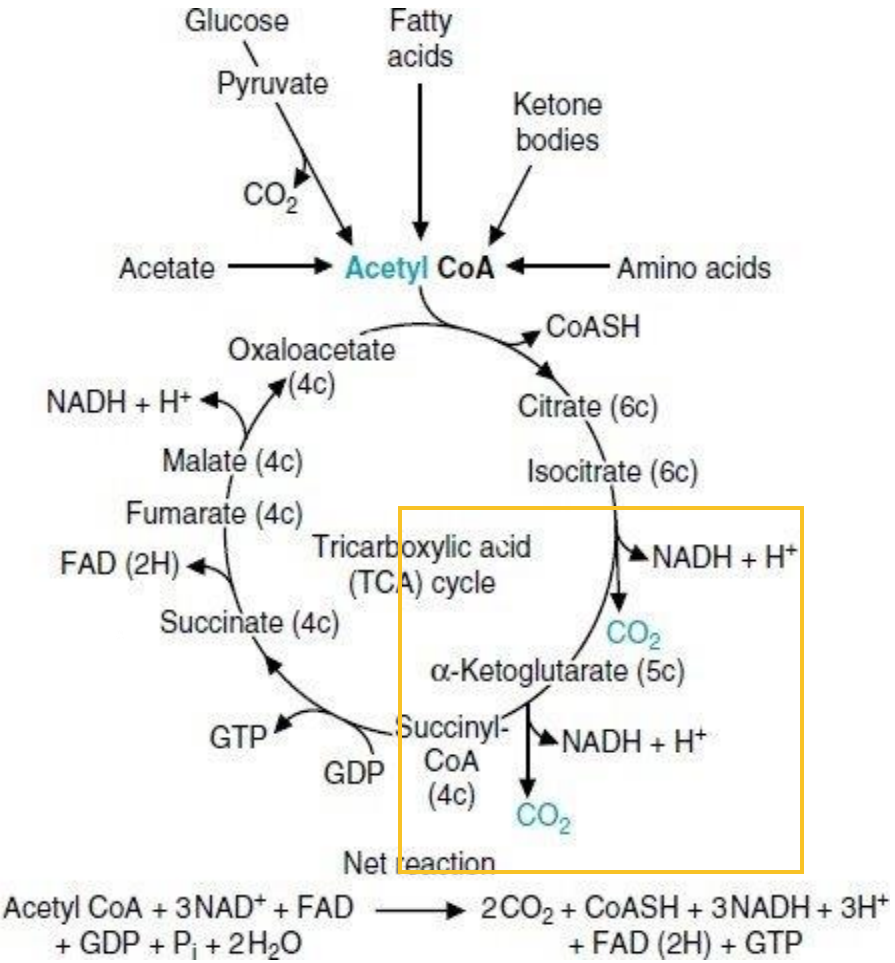


1.Formation of Citrate

The acetate molecule, which contains two carbon atoms, combines with a four-carbon molecule called **oxaloacetate**.
When these two molecules are joined together, they form a six-carbon compound known as citrate (**citric acid**) – the first product of the Krebs cycle.
This reaction is catalyzed by the enzyme citrate synthase.
The process requires energy, which is provided by the release of coenzyme A (CoA) from the structure

2.Conversion of Citrate to Isocitrate

Citrate is converted into **isocitrate**, an isomer that has the same number of carbons but a different structural arrangement.
This rearrangement prepares the molecule for subsequent oxidation steps.
The enzyme responsible for this conversion is **aconitase**, named after the intermediate **cis-aconitate** formed during the reaction.



- **Succinate** is oxidized to **fumarate**,
- **Fumarate** is hydrated to **malate**,
- **Malate** is oxidized to regenerate **oxaloacetate**, completing the cycle.

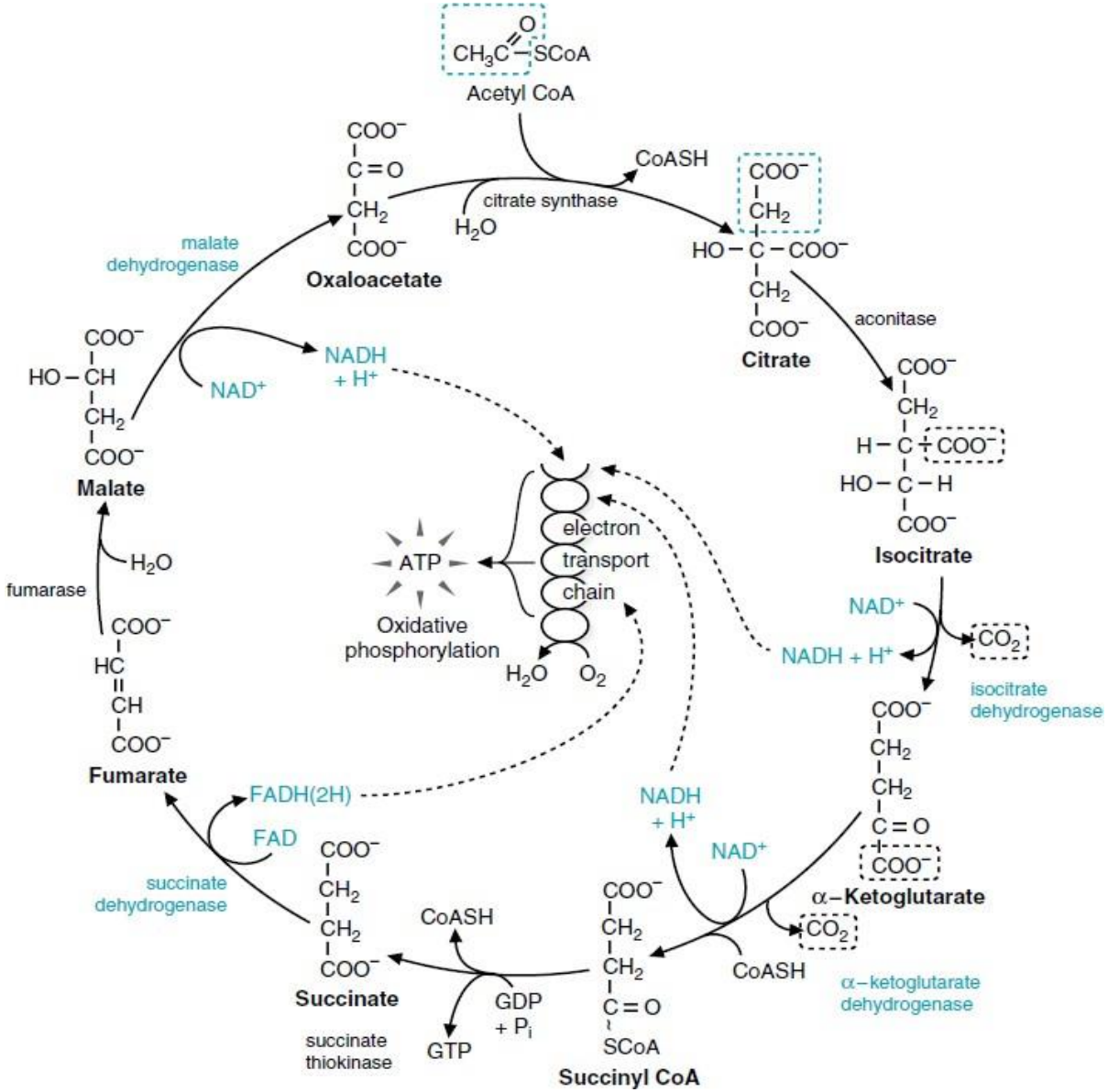
3. Decarboxylation Steps

Then **isocitrate** would release one carbon unit as **CO_2**, producing a five-carbon unit molecule which is called **alpha-ketoglutarate**.
In the subsequent reaction, a four-carbon unit molecule which is called **succinate**, coupled to coenzyme A – this is why it is named **succinyl-CoA** – and one carbon unit is released as **CO_2**.
So the two carbon units provided by acetate are released in this half cycle
Now we are with a four-carbon unit molecule coupled to its coenzyme A, and now we should make it **oxaloacetate** again.
As we define cyclic pathways, we must regenerate the first molecule that we started with.

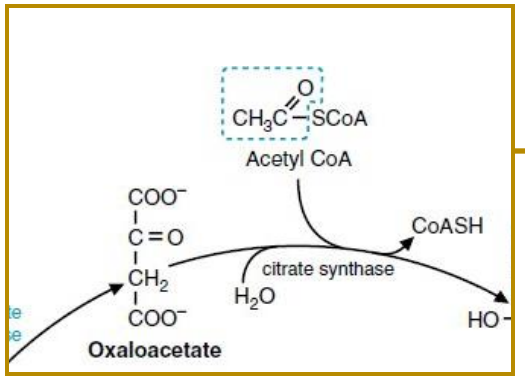
4.Regeneration of Oxaloacetate

So, the first step is to remove coenzyme A, and when coenzyme A is released, it will release its energy.
This energy is used to couple inorganic phosphate with GDP to produce GTP as a high-energy molecule, leaving succinate behind – as you can see with a four-carbon unit molecule.
Succinate is oxidized to become fumarate, fumarate is hydrated to become malate, and malate is oxidized to become oxaloacetate again.
This regeneration step is crucial because it allows the cycle to continue repeatedly.

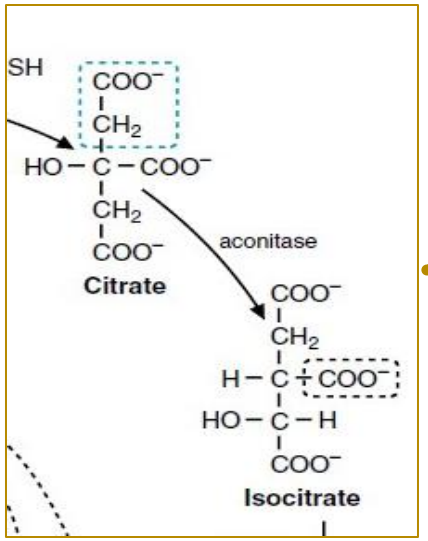
COMPONENTS & STEPWISE REACTIONS



COMPONENTS & STEPWISE REACTIONS

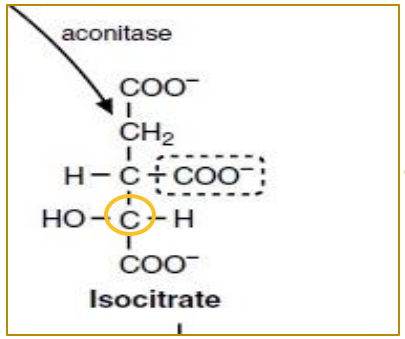


- Structure-wise, as you can see, the acetate molecule is a two-carbon unit molecule that joins oxaloacetate.
- Oxaloacetate is a four-carbon unit molecule, where it has two carboxylates on its terminals, and the two carbons in the middle – one of them is CH2 and the other is a carbonyl group.
- It will join these two carbon units to become citrate by the action of citrate synthase.

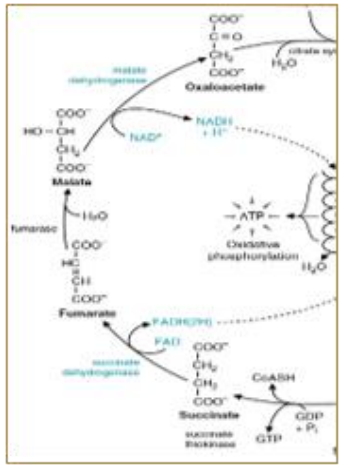


- This citrate carries, as the name implies, TCA – tricarboxylic acid – one carboxylic group, second carboxylic group, and this is the third carboxylic group.
- If you look at the structure of citrate – and since energy production is a process of oxidation – if you look at the carbon units, none of them can be oxidized.
- You will have three carboxylic groups, which are the highest state of oxidation out of these functional groups, and we have a hydroxyl group representing an alcoholic group, which is in a tertiary form – tertiary alcohol.
- So this one also cannot be oxidized since the carbon is connected to three carbon units.

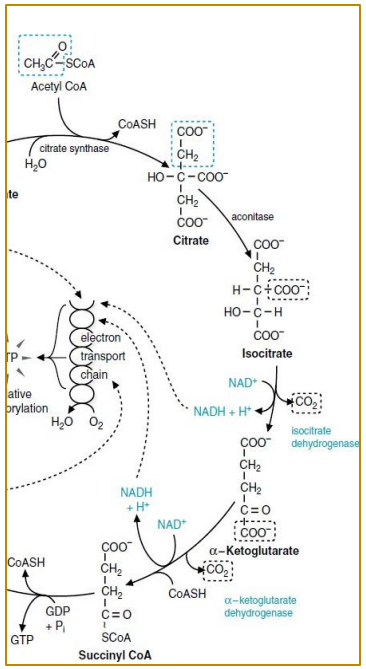
And this is why citrate is converted to isocitrate – the isomer form of citrate – where this hydroxyl group is translocated to another carbon.



- The isomer form of citrate where this hydroxyl group is transformed to is replaced to join this carbon unit translocated.
- This isocitrate is the product, and the enzyme that converts citrate to isocitrate, catalyzing this isomerization reaction, is called aconitase, which does not imply the action of the enzyme.
- It is named after an intermediate in the process of conversion of citrate to isocitrate, which is called cis-aconitate.
- So the enzyme is named after the intermediate in between citrate and isocitrate.

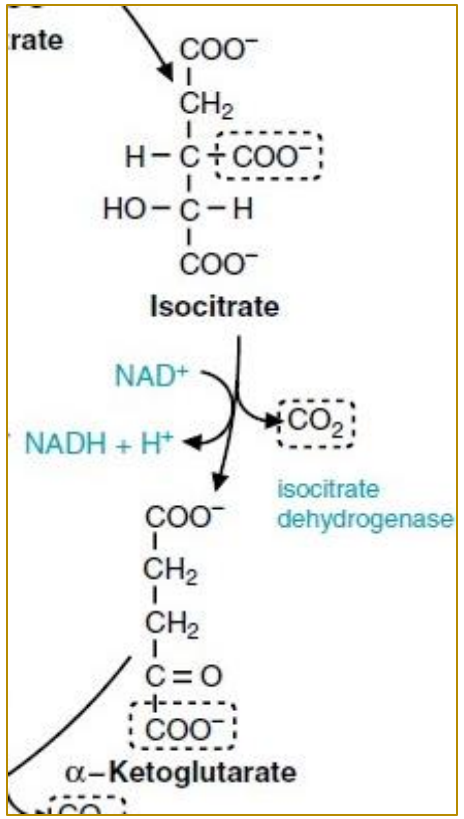


In the other half, citric acid cycle is engaged in reformulating this four-carbonyl molecule to become **oxaloacetate** again.

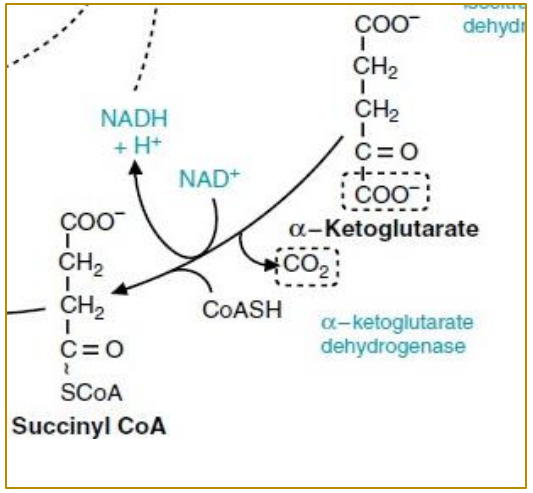


<u>we have to split the citric acid cycle into two halves.</u>

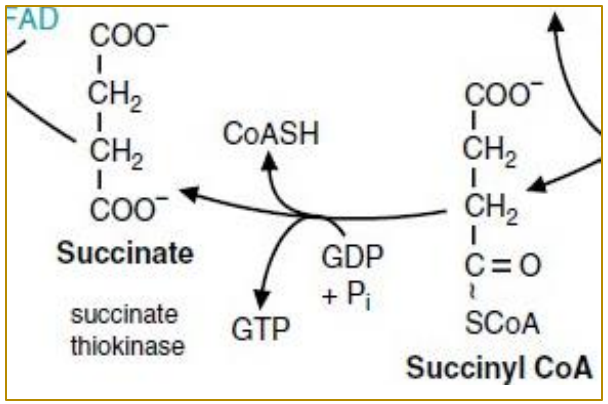
- These are eight-step reactions, so we will take the first four as one unit and the other four as another unit.
- In the first half of the cycle, the citric acid cycle is engaged in converting the six-carbon unit molecule into a four-carbon unit molecule.
- So in the first half of the cycle we have four reactions.
  In the first one, we made **citrate**.
  In the second one, we **isomerized citrate into isocitrate**.
  We are left with two other reactions.
  These two reactions should release two carbon units – because we got to the cycle with two carbon units, so we have to remove them t preserve the nature of a cycle.
  Otherwise, molecules will keep enlarging in their structure.



•So, in this reaction, **CO_2 should leave**, and it is dashed representing the carboxylic groups that are leaving the structure.
So this carbon, this carboxylic group, is leaving as **CO_2** – and always carboxylic groups leave the structures as CO_2.
And CO_2 gets to the structures as carboxylic groups.
•So this carboxylic group leaves as CO_2.
And why did we convert citrate to isocitrate?
For the purpose of oxidation.
•So, at this level, this proton leaves, producing the **first NADH molecule** inside the cycle.
After the hydrogens leave, there will be a double bond between the carbon and the oxygen – a keto group.
•And this is why the molecule is named "**keto**."
It's a **five-carbon unit** molecule, represented by the word "glutarate," and "-ate" represents the acidic nature of the molecule.
•So **alpha-ketoglutarate** is the molecule produced by the oxidation of isocitrate.
•And the enzyme that catalyzes this conversion is an oxido-reductase called **isocitrate dehydrogenase**.

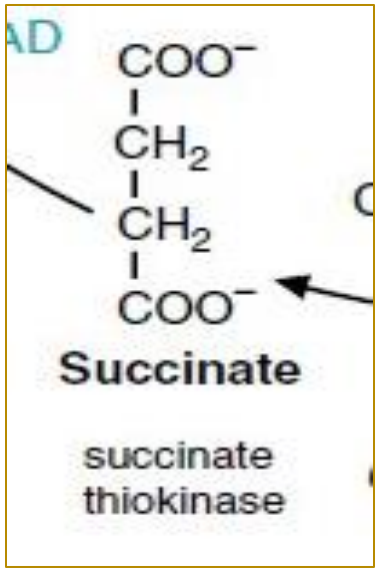


After that, **α-ketoglutarate** undergoes another decarboxylation and oxidation, producing **succinyl CoA**, a four-carbon compound coupled with coenzyme A.
The carbon lost here is the second CO_2 – meaning the two carbons that entered the cycle as acetyl CoA are now released.
And here we finish the first half of the reaction , four reactions are done



Here, coenzyme A is released, and when it's released, it gives off its energy.
This energy is used to couple inorganic phosphate with **GDP** to form **GTP**, a high-energy molecule. GTP has the same amount of energy loaded on ATP and This is why you see it sometimes in text box here as ATP.

So, from this step, we produce a high-energy molecule – **without the need for oxygen** or oxidative phosphorylation.
This process is called **substrate-level phosphorylation** – we produce energy directly from the substrate.
There are three substrate-level phosphorylation reactions in our body.
This is one of them, and the other two occur in glycolysis, which you'll take in carbohydrate metabolism.



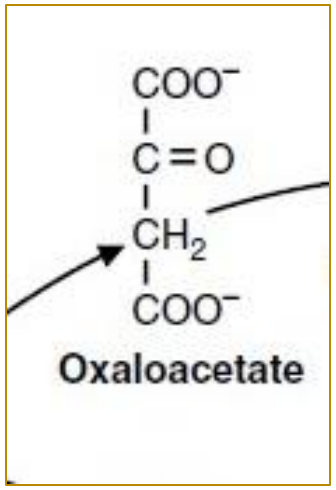
So, leaving this four-carbon unit molecule which is the succinate with some reformulation this succinate molecule has a very nice structure two carboxylic groups on terminals and two CH2 groups in the middle (you can read it from the top you can read it from the bottom the same way).

The enzyme that catalyses this step (the conversion of succinyl co A into succinate) it has a three names :

- **Succinyl-CoA synthetase**
- **Succinyl-CoA thiokinase**
- **Succinate thiokinase**

the enzyme that the doctor required is **succinate thiokinase** and this enzyme represents the reaction happening. Where succinate represents either succinate in reactants or in the products Thio represents the presence of a coenzyme in the structure, because the active group is the thiol group

kinase because it phosphorylates GDP so it tells me what's going on inside the reaction and we are now ended up by succinate



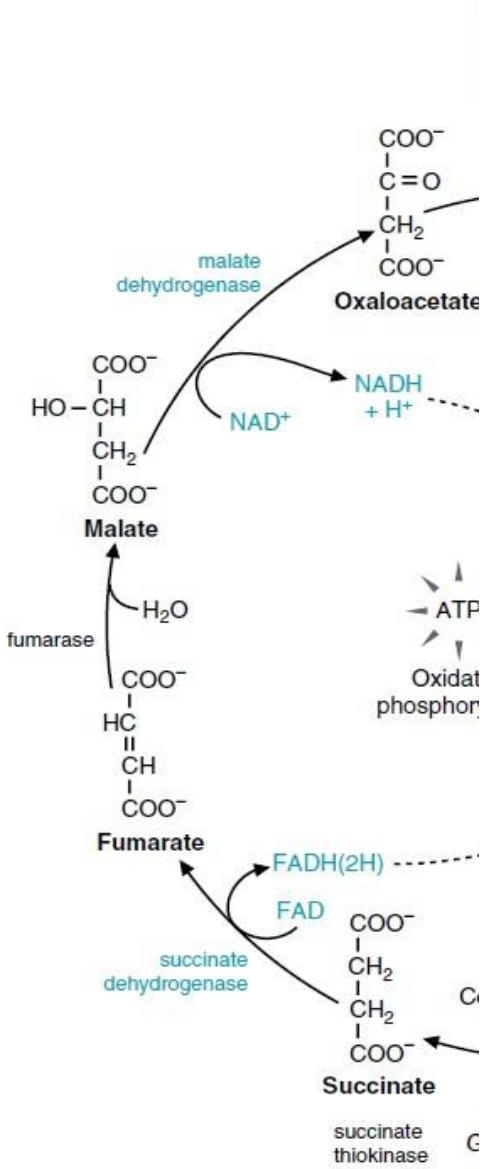
If you look at the structure of succinate and compare it to oxaloacetate. So we have two carboxylates on the terminals the same. We have CH2 in both .

So, what's different is that we have a carbonyl group on one of the carbons while it is here as CH2.

Both are 4-carbon dicarboxylic acids.

The difference: oxaloacetate has a carbonyl (C=O) instead of CH2.

This means oxaloacetate is the oxidized form of succinate.



If you take it backward, it will make understanding better… How can I make a carbonyl group? From a secondary alcohol."

A carbonyl is formed by oxidizing a secondary alcohol.
So to form oxaloacetate, we oxidize malate.
Reaction: Malate → Oxaloacetate + NADH + H^+
Enzyme: Malate dehydrogenase

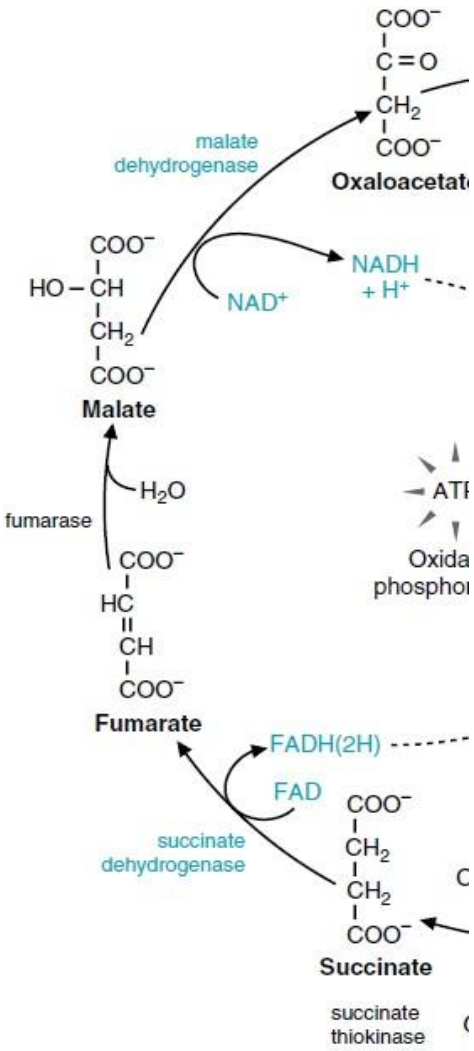
– Hydration Step
"How do I produce this alcoholic group? By hydration of an alkene… this one will take a hydrogen and this one a hydroxyl group."

Fumarate has a double bond (C=C).
By adding water across it, we form malate.
Reaction: Fumarate + H_2O → Malate
Enzyme: Fumarase

– Oxidation Step
"If I remove hydrogens, I'm making oxidation… The enzyme succinate dehydrogenase."

Removing two hydrogens from succinate forms fumarate.
Reaction: Succinate + FAD → Fumarate + $FADH_2$
Enzyme: Succinate dehydrogenase
Cofactor: FAD (not NAD^+)

Fumarate will be hydrated to become malate, and malate will be oxidized to become oxaloacetate



Step	Substrate	Product	Enzyme	Cofactor	Reaction Name
1	Succinate	Fumarate	Succinate dehydrogenase	FAD → $FADH_2$	Oxidation reaction
2	Fumarate	Malate	Fumarase	H_2O	Hydration reaction
3	Malate	Oxaloacetate	Malate dehydrogenase	NAD^+ → NADH	Oxidation reaction

By this the cycle is finished: three NADH, one $FADH_2$, and one ATP (or GTP) molecule are produced.
Per one acetyl-CoA molecule:

3 NADH → 7.5 ATP

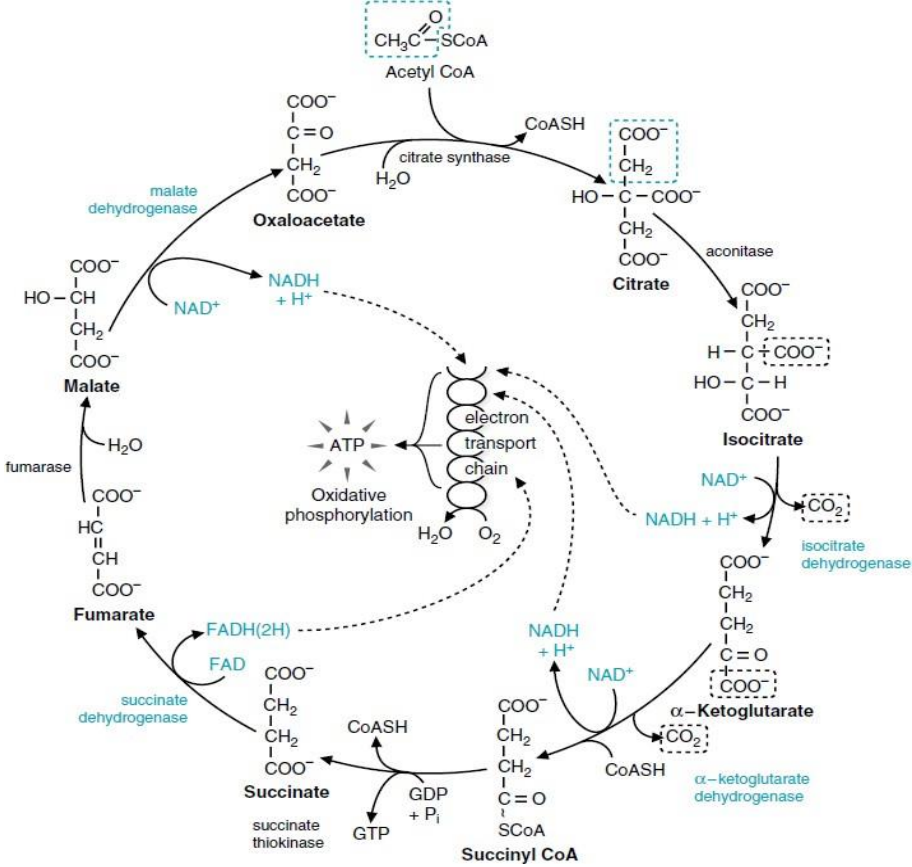
1 $FADH_2$ → 1.5 ATP

1 GTP → 1 ATP

Total = 10 ATP

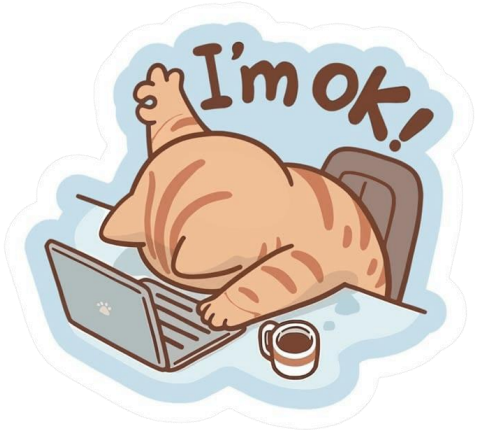
Four Dehydrogenases:

1. Isocitrate dehydrogenase
2. α-Ketoglutarate dehydrogenase
3. Succinate dehydrogenase
4. Malate dehydrogenase



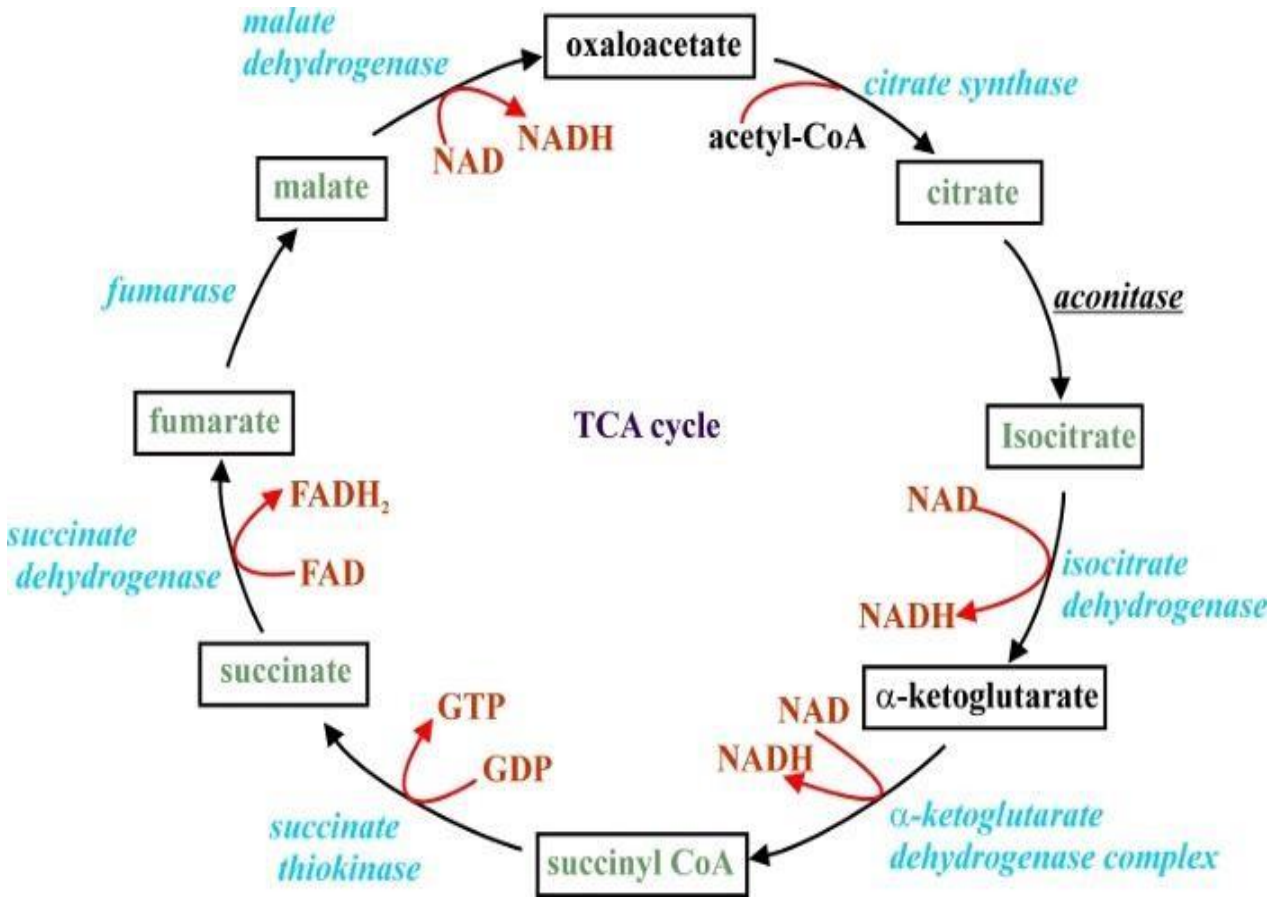
A high-energy molecule (ATP) without the need for oxygen, without going through oxidative phosphorylation... This is called substrate-level phosphorylation."

Occurs at the **succinyl-CoA → succinate** step.
Enzyme: Succinyl-CoA synthetase (succinate thiokinase)
Energy: GTP (or ATP) formed directly from GDP + Pi.



ENZYMES OF THE TCA CYCLE

- Citrate synthase
- Aconitase
- Isocitrate DH
- α-ketoglutarate DH
- Succinate thio-kinase
- Succinate DH
- Fumarase
- Malate DH



FORMATION OF CITRATE

What drives the reaction forward?

Is it reversible or irreversible?

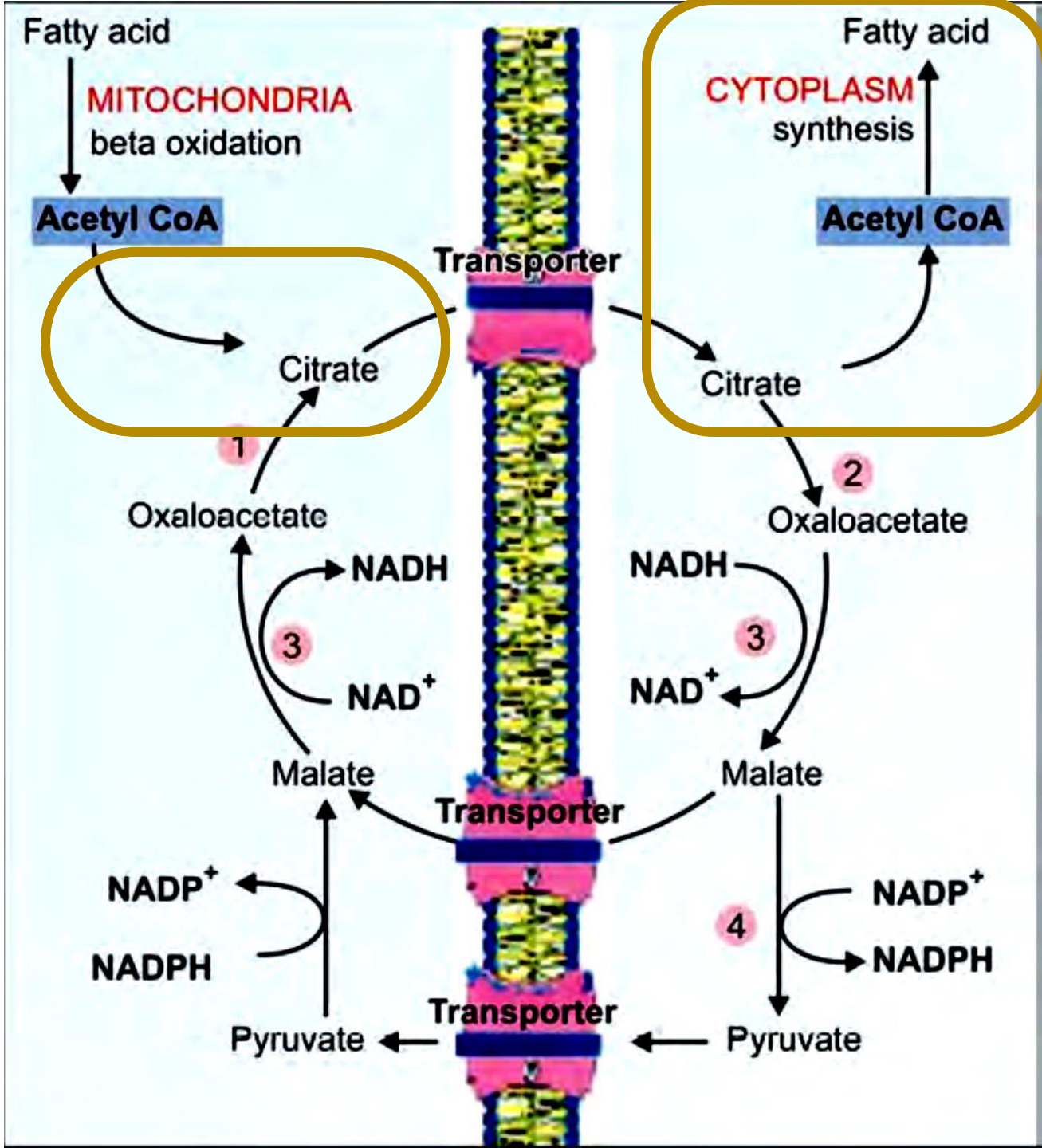
Can it be reversed?

ATP-Citrate lyase or ATP-Citratase

Activated by insulin and is highly expressed in lipogenic tissues like the liver and adipose tissue

Link to cancer metabolism

Let's Answer every question



citrate synthase:
this reaction is very special because it's considered the **Commitment step** for Krebs cycle.
Once it's crossed, I'm committed to make oxaloacetate again-(I'm in the cycle)

What drives the reaction forward?	The breaking down of **acetyl co-A** that produces energy
Can it be reversed?	Citrate can exist outside the mitochondria through a special transporter **if the concentration of citrate is high** , and it became high when I have **good energy state** (good import of food), regardless if the food was fat -(if it is fat I'll have more acetyl co-A) , or carbs (if I have a lot of carbs, I will have a lot of oxaloacetate) . If you have a positive energy state (enough ATP inside your body) then citrate will be high in concentration, then it will leave the mitochondria out to the cytosol, and there , the reaction can be reversed by the action of another enzyme called ATP Citrate-lyase (or ATP citratase) it breaks down the citrate into acetyl co-A and oxaloacetate.

Activated by insulin and is highly expressed in lipogenic tissues like the liver and adipose tissue

Then when you have good concentration of acetyl co-A in cytoplasm you can make fatty acetyl. That's why when you have good energy state you will start building up fats inside your body. And this is why this enzyme is presented in high concentrations in adipose tissue.
(these tissues' function is to **store up fat**)

Insulin is produced in good energy state when you have high glucose concentration; which represents good energy state

Is it reversible or irreversible?

Irreversible. If you make citrate you cannot go back to make acetyl co-A again and oxaloacetate

ATP-Citrate lyase or ATP-Citratase!

Citrate can be broken down (but outside the mitochondria in the cytoplasm)

Link to cancer metabolism

Cancer is proliferating cells; and proliferating cells needs membranes and membranes needs phospho-lipids that are fatty acids are attached to glycerol molecules; so I need to synthesize what we call fatty acids. so this enzyme is enhanced in cancer states

الله بيعين...

FORMATION AND OXIDATION OF ISOCITRATE

Control at the committed step of glycolysis

Fructose 6 - phosphate

ATP

ADP

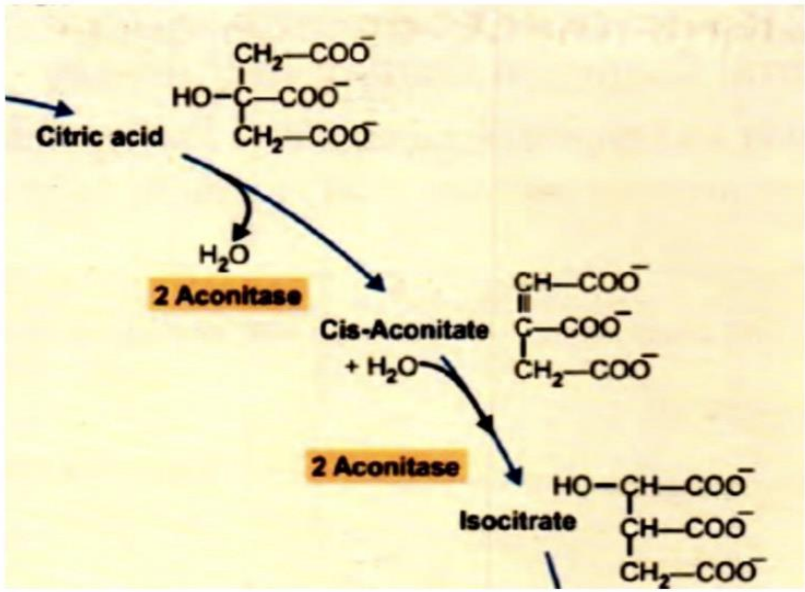
Phosphofructokinase

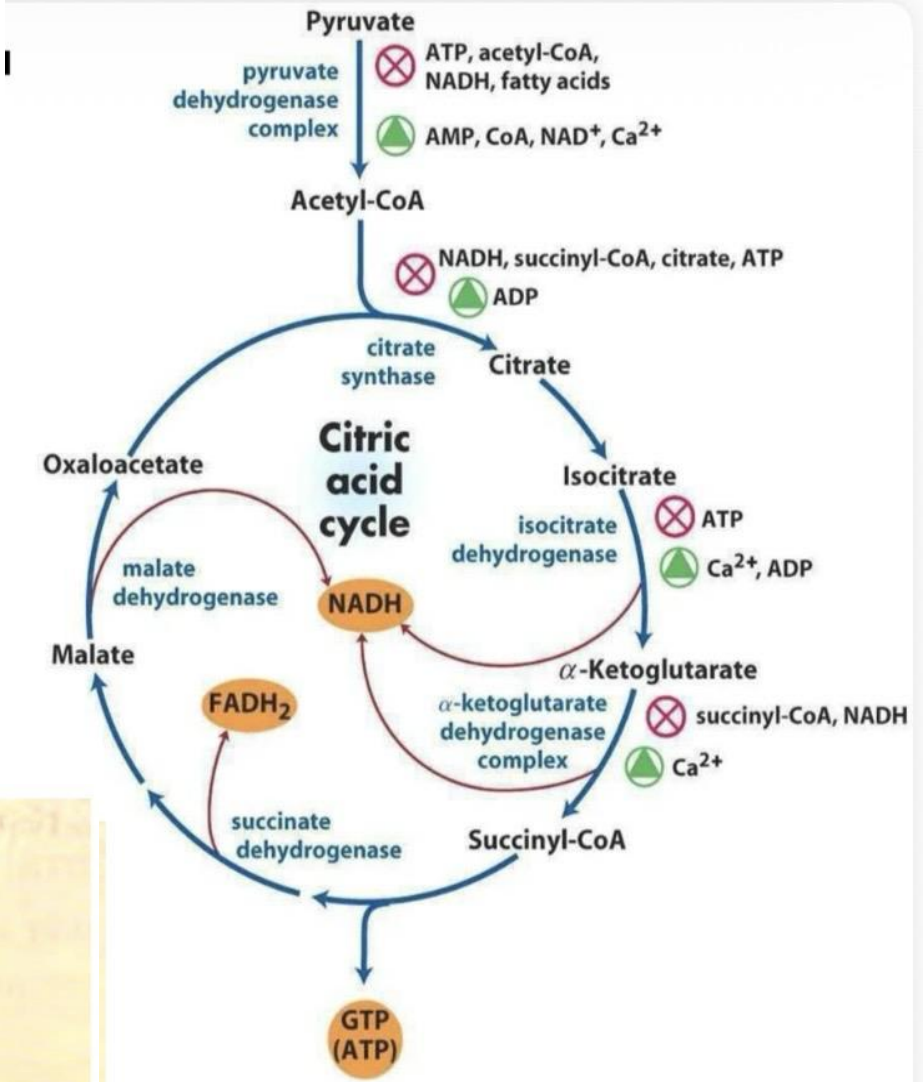
Activated by F-2,6 - BP

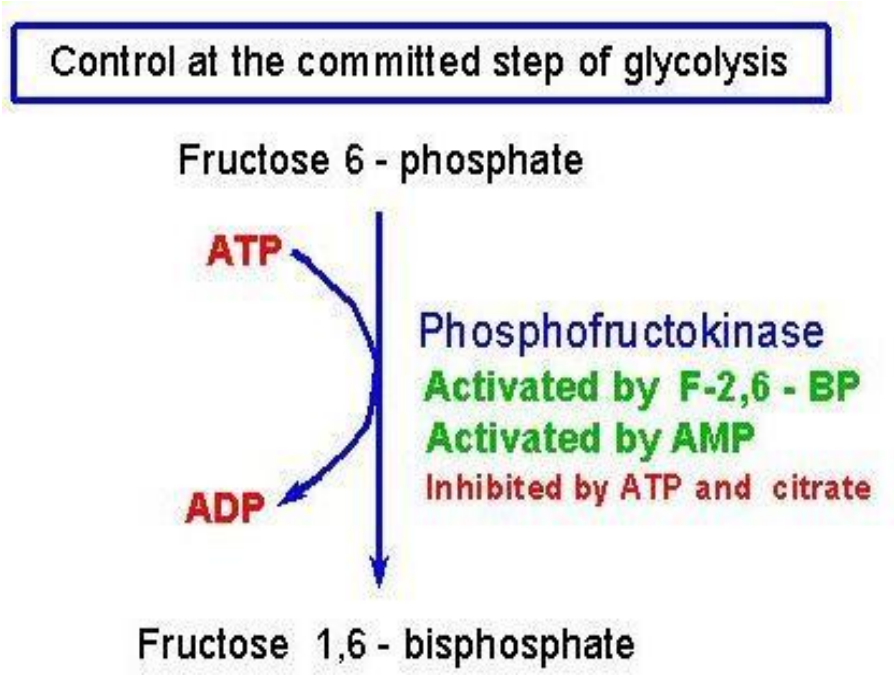
Activated by AMP

Inhibited by ATP and citrate

Fructose 1,6 - bisphosphate





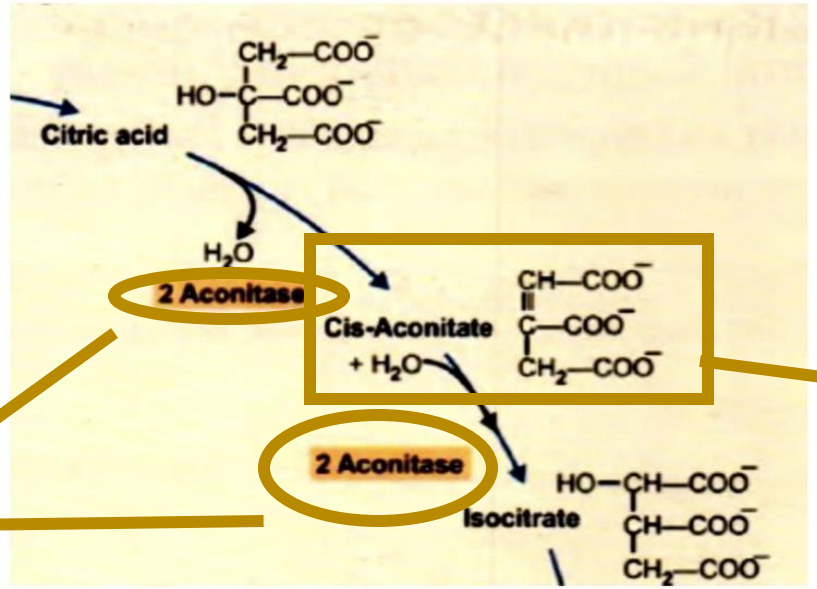


The conversion of fructose 6-phosphate to fructose 1,6- bisphosphate -> the committed step and the rate limiting step in the common pathway- glycolysis. Things that affect glycolysis:

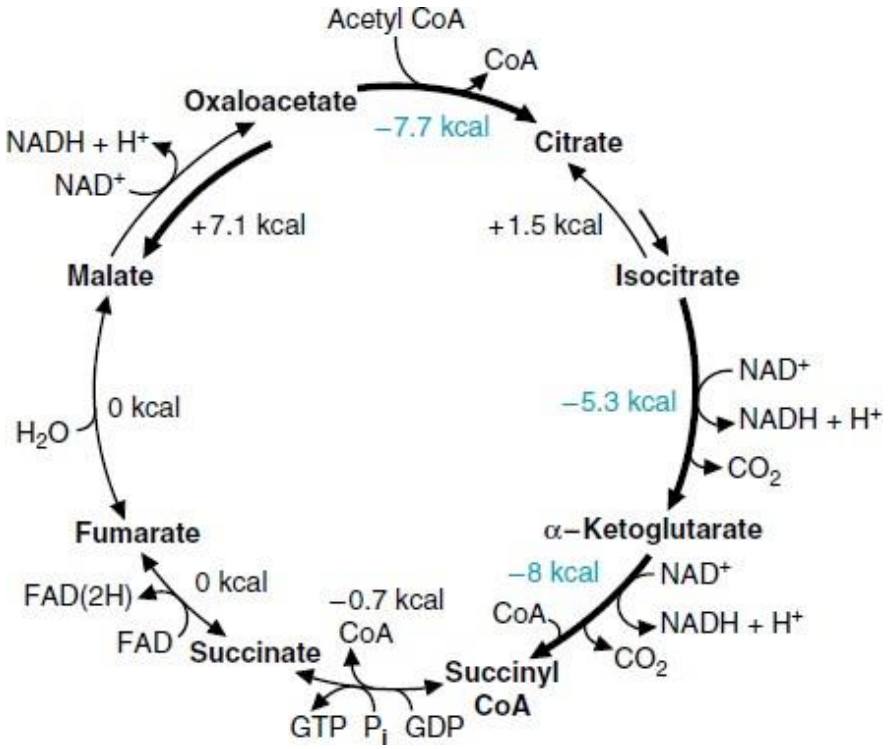
-> Citrate: when citrate is high in concentration it exits outside mitochondria to the cytosol, it can affect the enzyme which is called phosphofructokinase that catalyzes this step and inhibits it.

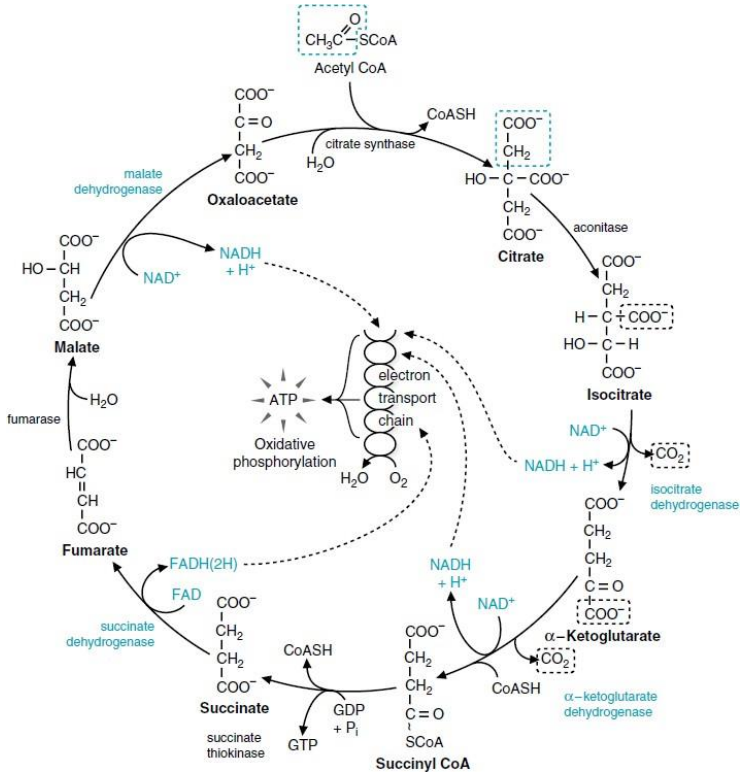
(it makes sense for this enzyme to be inhibited by citrate, because it is already high in concentration).

The enzyme takes its name after the molecule



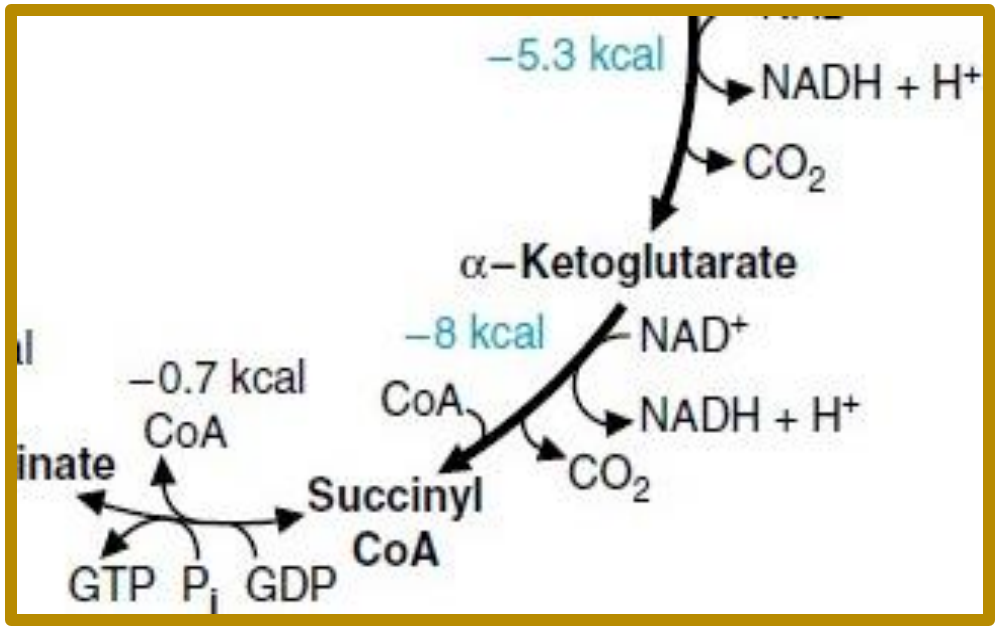
Represents the intermediate of conversion of citric acid to isocitrate

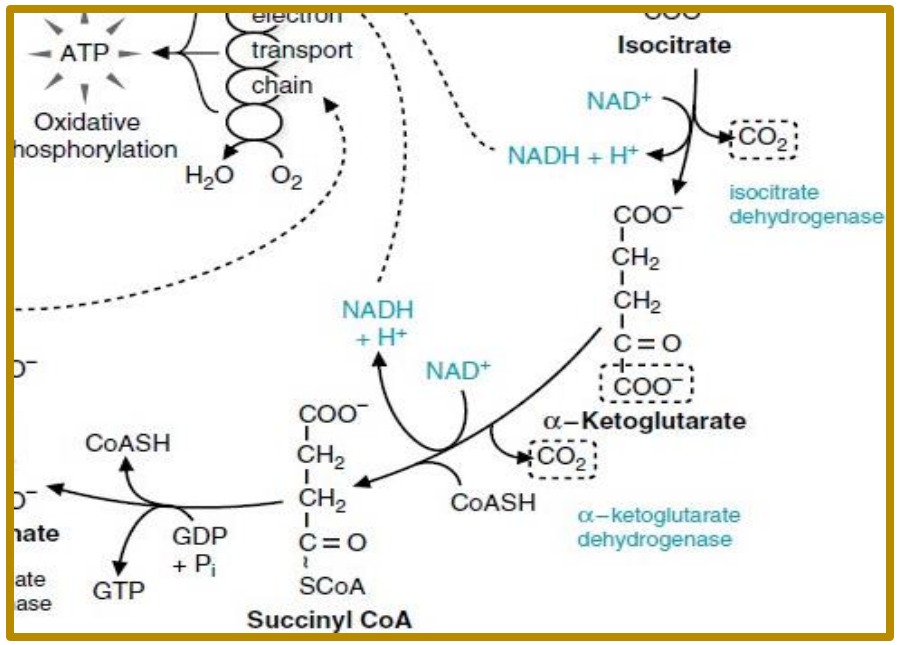




α-KETOGLUTARATE TO SUCCINYL COA

- **Oxidative decarboxylation**
- **Thiamine pyrophosphate, lipoic acid, and FAD**
- **Keto group oxidized to acid, CoA-SH, succinyl CoA**
- **Energy conserved as NADH, thioester bond**
- **The highest energy yield provided!**





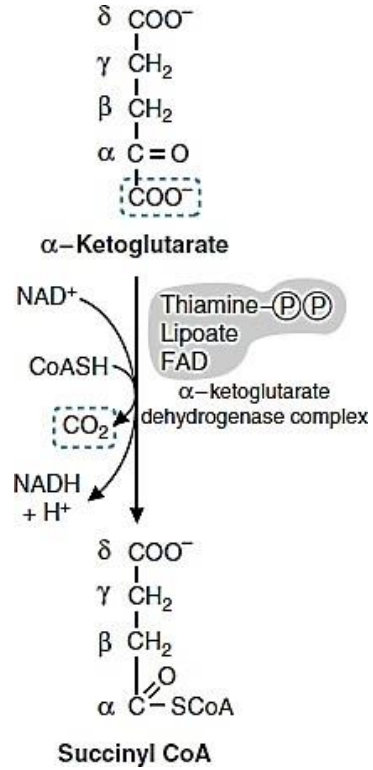
Enzyme complexes :

Group of enzymes that are grouped together so you'll have more than one enzyme from more than one class. However, the product of the first enzyme can't be used except from the second enzyme also the product of the second one is given to the third enzyme to use it. Now, to decrease the effect of diffusion and to make the reactions go faster we will couple both enzymes together.

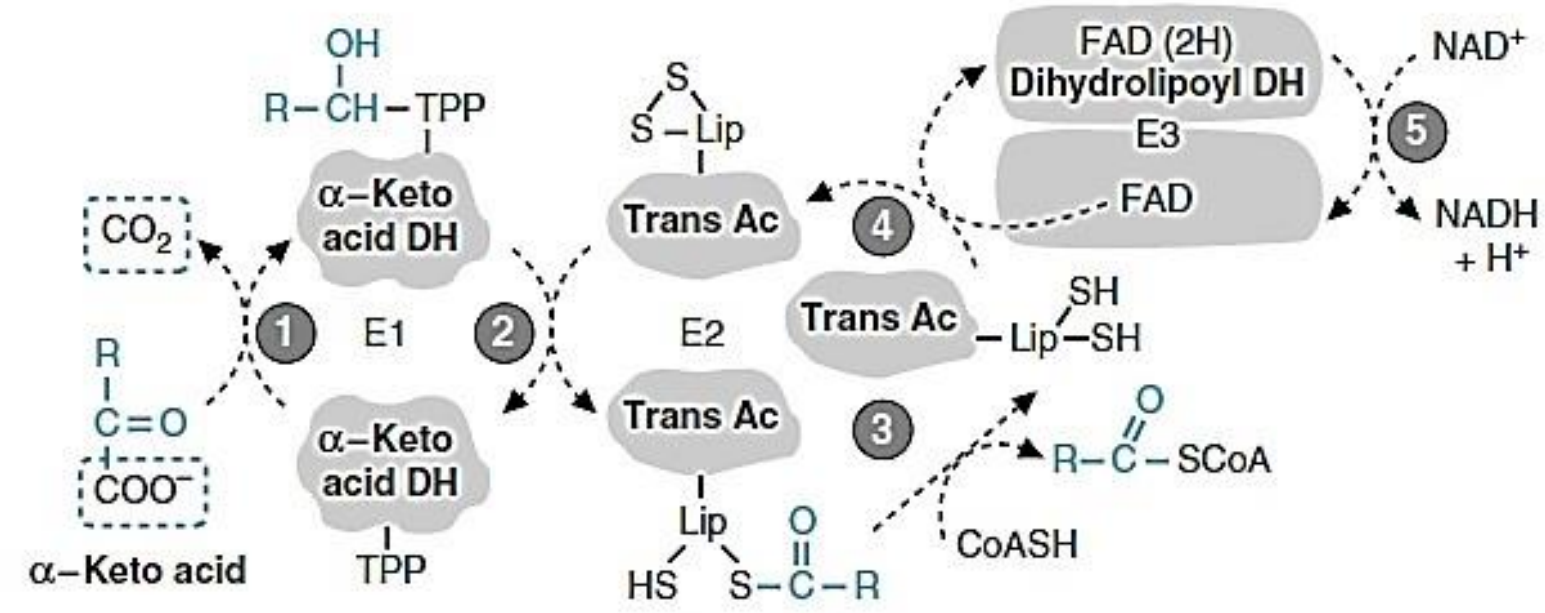
α-KETOACID DEHYDROGENASE COMPLEXES (TLCFN)

(α-ketoglutarate, pyruvate, and branched chain α-keto acid) dehydrogenase complexes

Huge enzyme complexes , multiple subunits of 3 different enzymes (no loss of energy, substrates for E2 and E3 remain bound → higher rate)



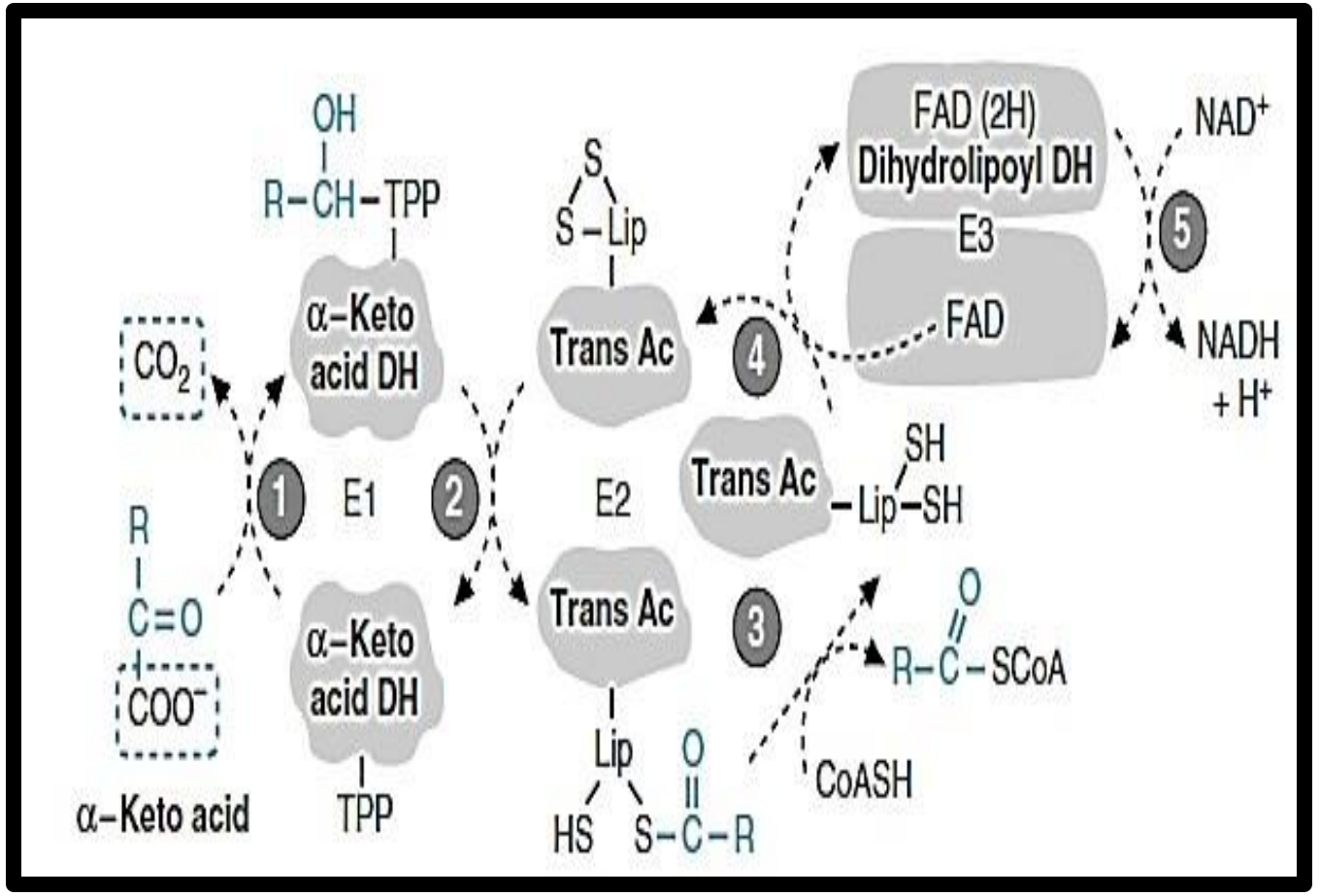
- E1, E2, & E3 are a decarboxylase (TPP), a transacylase (lipoate), & a dehydrogenase (FAD)



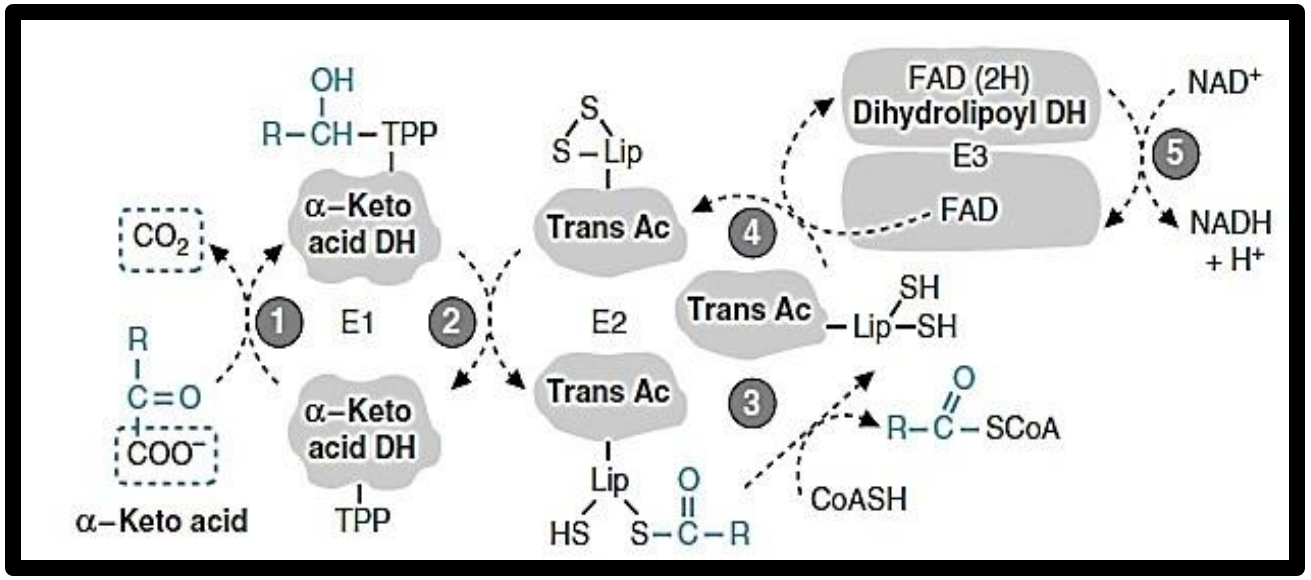
1) TPP attacks the carbonyl group in the structure of alpha-ketoglutarate , once it attacks the carbonyl group it weakens the bond between carbonyl and carboxylate , making co2 to leave the structure.

2)The TPP becomes attached to the carbonyl group This is the first product of the enzyme.

3)Here TPP is attached to the structure and this is not the original form of the enzyme (to have Tpp alone) so we have to hand this this structure to the second enzyme which is a transacyclase , this enzyme has a lipoid acid.



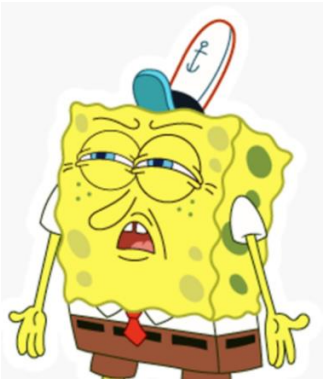
E1 ->decarboxylase ->TPP
E2 -> transacylase -> lipoate
E3 -> dehydrogenase -> FAD

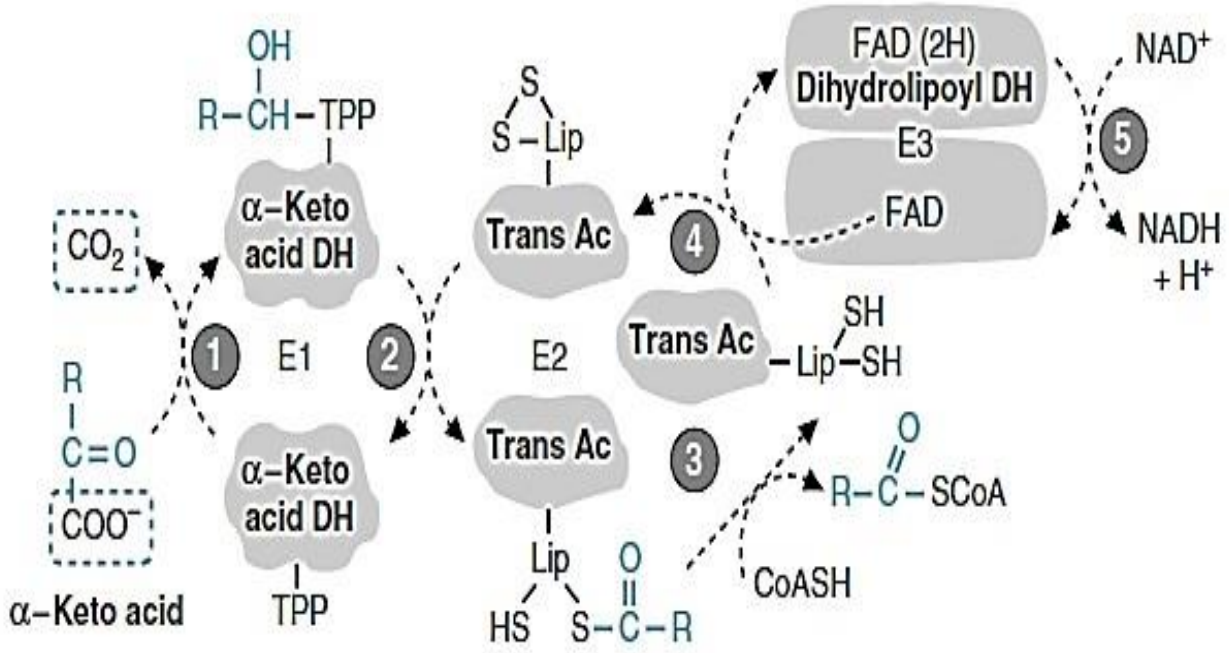


4) The structure of lipoid acid involves a disulfide bridge , so the. Carbon unit will be attached to one of the surfers, causing the breakdown of the disulfide bridge.

5) One surfer is attached to the carbon, and the other one is in the solution by itself with a negative sign , so it will abstract proton from the solution to become a thiol group. Also, this is not the original form of the enzyme .

So those carbons will leave the sulfur coupled with coenzyme A and this is the second product of the molecule which is Succinyl CoA.





Co2 left the reaction at the level of E1. SCoA left the reaction at the level of E2 , leaving the sulfur behind . Sulfur will abstract proton from the solution to become a second thick group and ScoA left . Now we have 2 thiol groups . And this is not the original form of the enzyme , so we have to remove the hydrogens

Removing the hydrogens is a function for dehydrogenase, so to return to the original form , E3 which is a dehydrogenase will take out these hydrogens and the coenzyme which is involved in this process is FAD.
So, FAD takes out these hydrogens causing the disulfide bridge again and E2 and FAD will be reduced to become FADH2 .

Now NAD will come to the picture to pick up these two electrons from E3 to become NADH

There are 3 coenzymes only in the structure of the alpha-ketoglutarate dehydrogenase complex

There are 5 coenzymes are involved in the process of conversion of alphaketoglutarate to succinylCoA

Why did we put an R group here not alpha ketogutarate ?

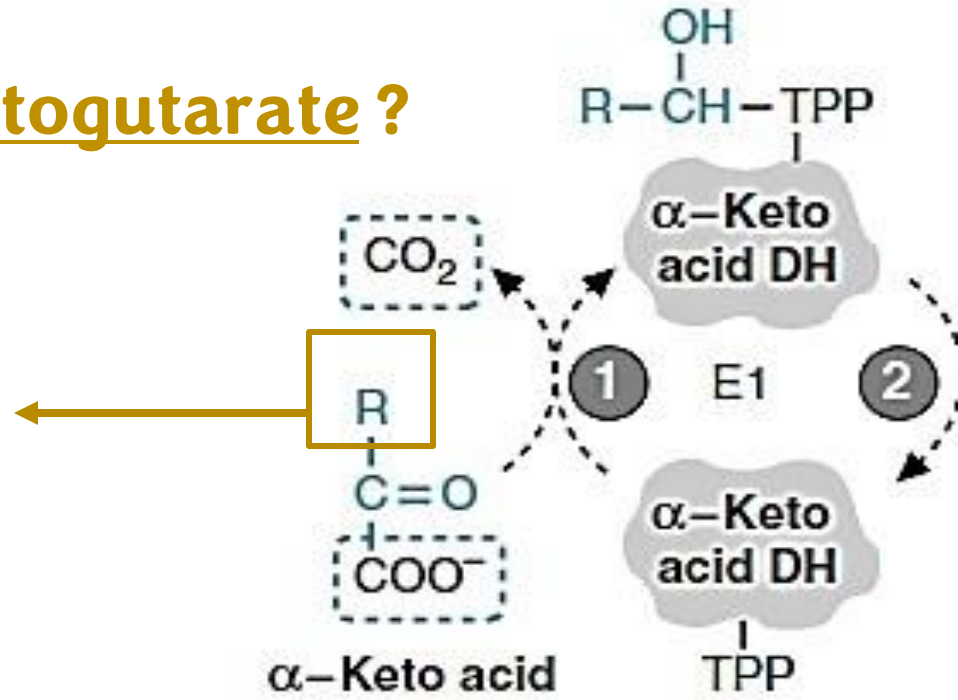
The enzyme is specific and it uses alpha ketoglutarate only. However, we have two other enzyme complexes in our body that uses different substrates but they adopt the same the general mechanism.

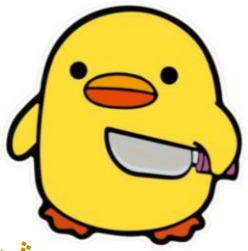
(α-ketoglutarate, pyruvate, and branched chain α- keto acid) **dehydrogenase complexes**

In the case of **pyruvate**, we should place CH3 instead of R .

in case if **alpha-ketoglutarate** we will place CH2COO- instead of R .

The third enzyme which functions in the same way is present in the amino acid metabolism that p's called the alpha-keto acid dehydrogenase that works on certain amino acids

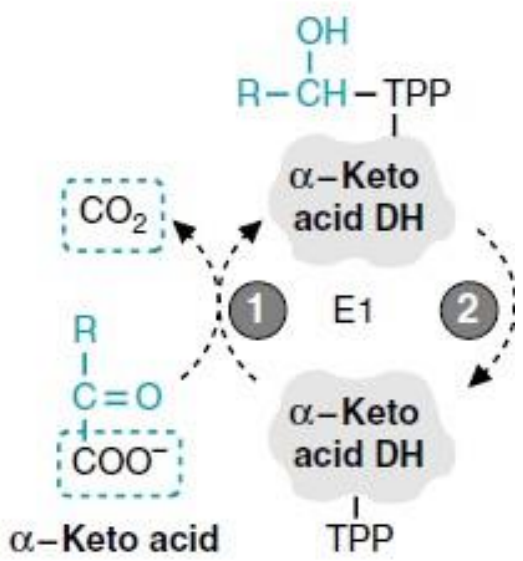


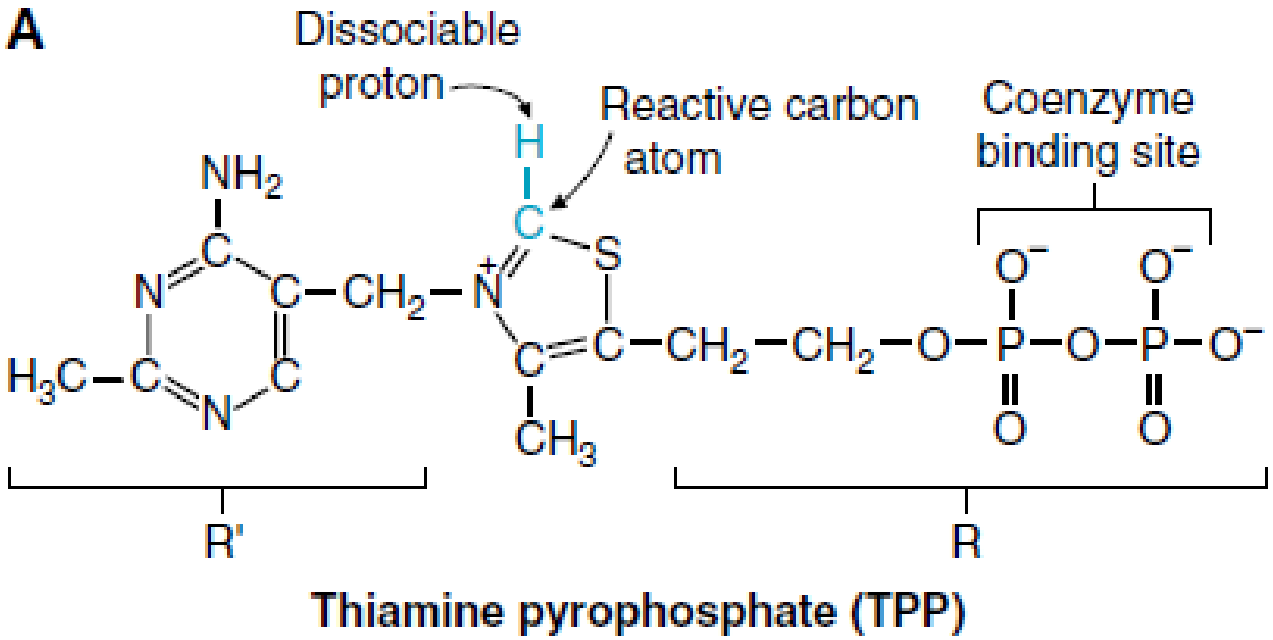


شد حيلك!

THIAMINE PYROPHOSPHATE

- Thiamine deficiency, α-ketoglutarate, pyruvate, & branched chain α-keto acids accumulate in the blood





Thiamine deficiency

Thiamine can be deficient since it's a vitamin which affects the level of thiamine pyrophosphate , which affects the function of this enzyme.

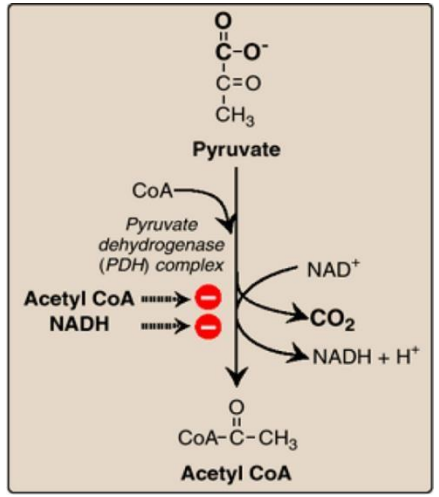
Also, this enzyme can have a genetic problem; not to be efficient in its function.

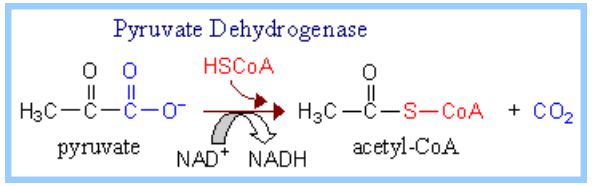
if there is a thiamine deficiency this enzyme won't work properly, and accordingly there will be high concentrations the substrate of the enzyme, which is either alpha-pyroglutarate or pyruvate in the blood.

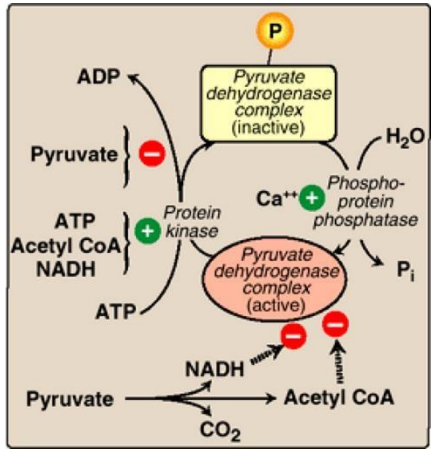
If the pyruvate is high; **lactic acid** will also be high. And this is why if there is a deficiency in E1 enzyme, it is the most common biochemical cause of congenital lactic acidosis.
It is an x-linked disease and there is no treatment for it.(see in the next slide)

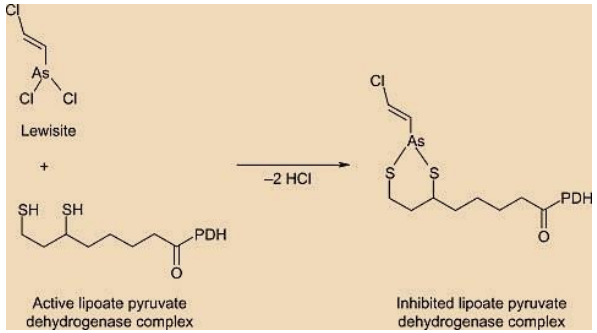
OXIDATIVE DECARBOXYLATION OF PYRUVATE

- Component enzymes
- Coenzymes
- Regulation of the pyruvate dehydrogenase complex
  - Pyruvate dehydrogenase deficiency: A deficiency in E_1 component is the most common biochemical cause of congenital lactic acidosis (X-linked, no treatment)
- Mechanism of arsenic poisoning





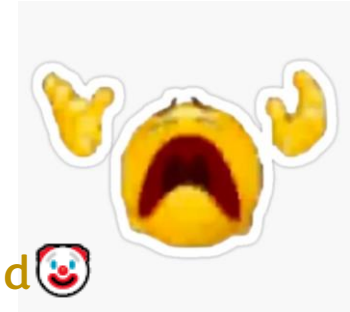




The structure of lipoic acid
That is present in E2 at the level of pyruvate dehydrogenase and alpha keto acid dehydrogenase and alpha ketoglutarate dehydrogenase.

What does As does?
Arsenic (As) poisoning:

Arsenic breaks down the disulfide bridge in between the two sulfurs inside the lipoic acid , and it's connected to them in an irreversible manner; so the substrate from E1 cannot be handed to E2 ; blocking this enzyme action , so accordingly there will be accumulation of the substrates of these reactions including pyruvate , with subsequent lactic acidosis and alpha-ketoglutarate. They will block the citric acid cycle and it will not function properly, and the conversion of pyruvate into acetyl-coA will not function properly, and there will be no production of energy inside the human .
Accordingly there will be death .
So depending on the concentrations of As provided it is fatal easily .

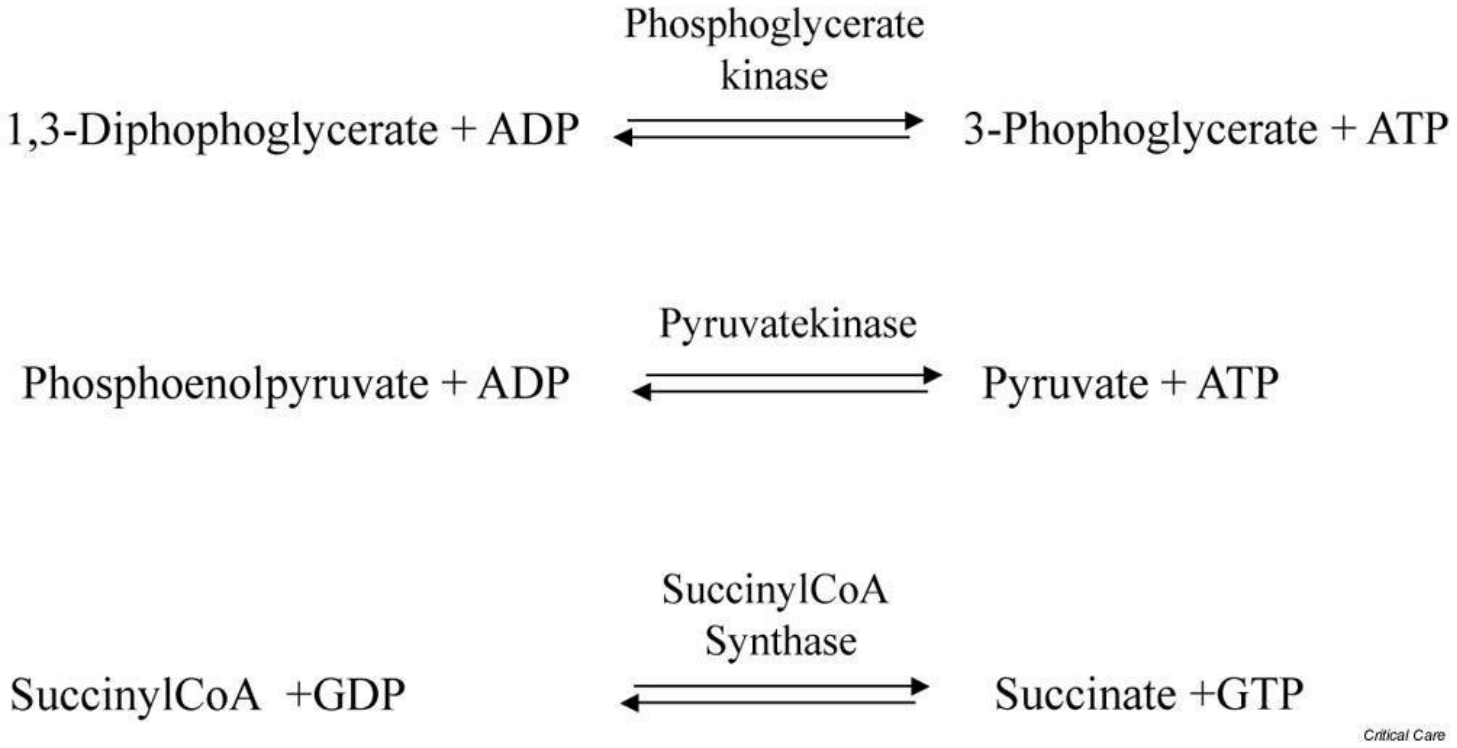


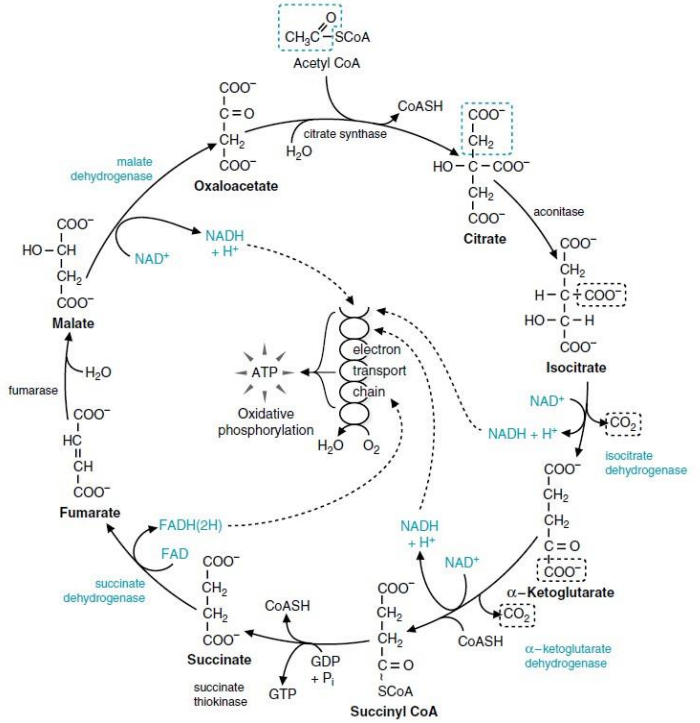
You're not even halfway through this modified🤡

GENERATION OF GTP

- **Succinyl CoA thioester bond, succinate thiokinase, substrate level phosphorylation**

GTP +ADP ↔ GDP + ATP



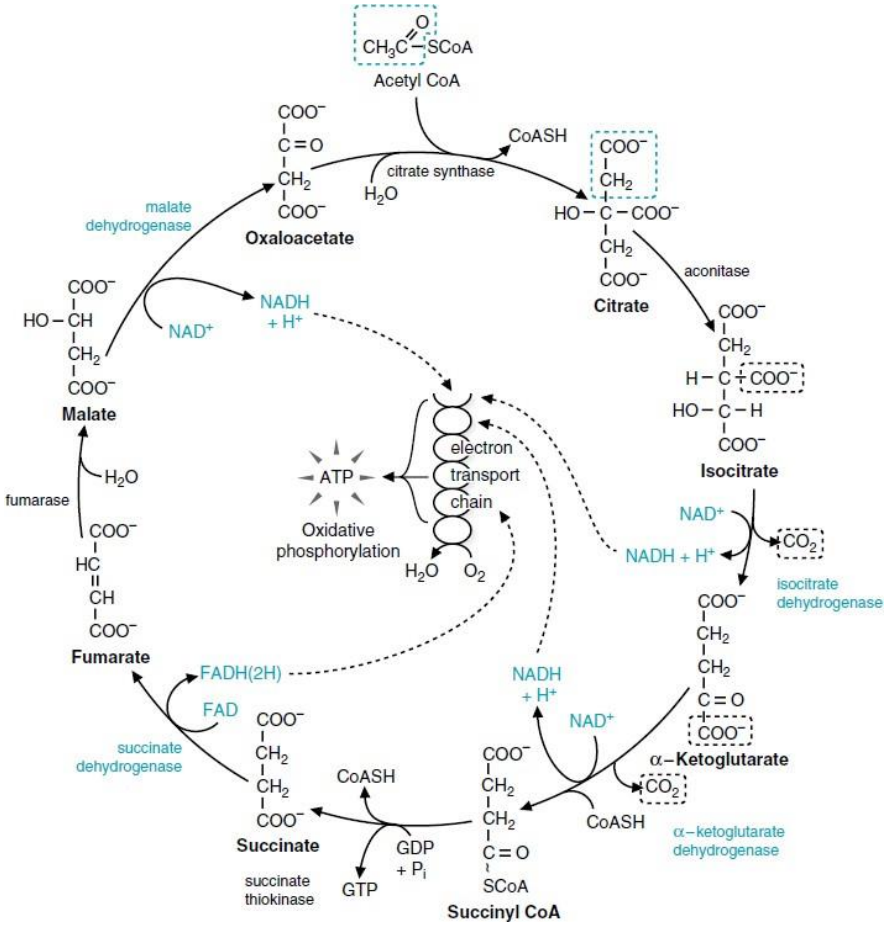


OXIDATION OF SUCCINATE TO OXALOACETATE

Oxidation of succinate to fumarate, succinate dehydrogenase, FAD

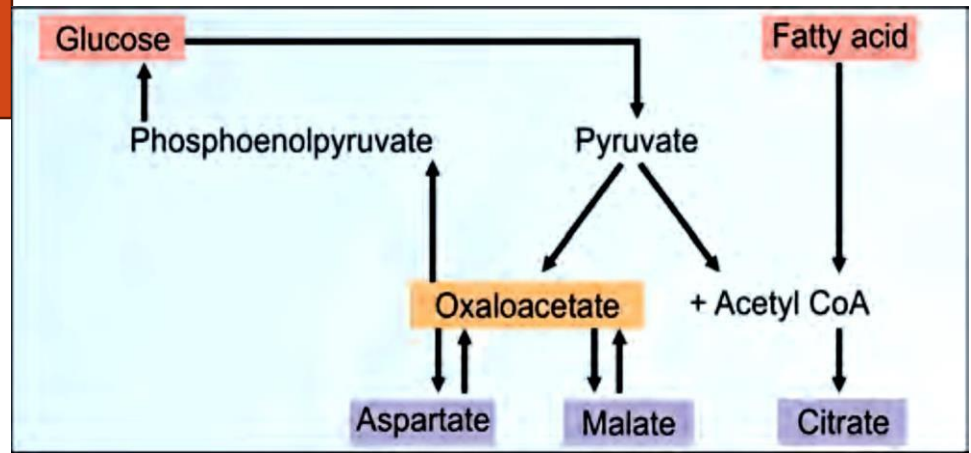
Fumarase, OH + H^+ from water, fumarate to malate

Alcohol group of malate oxidized to a keto group, NADH



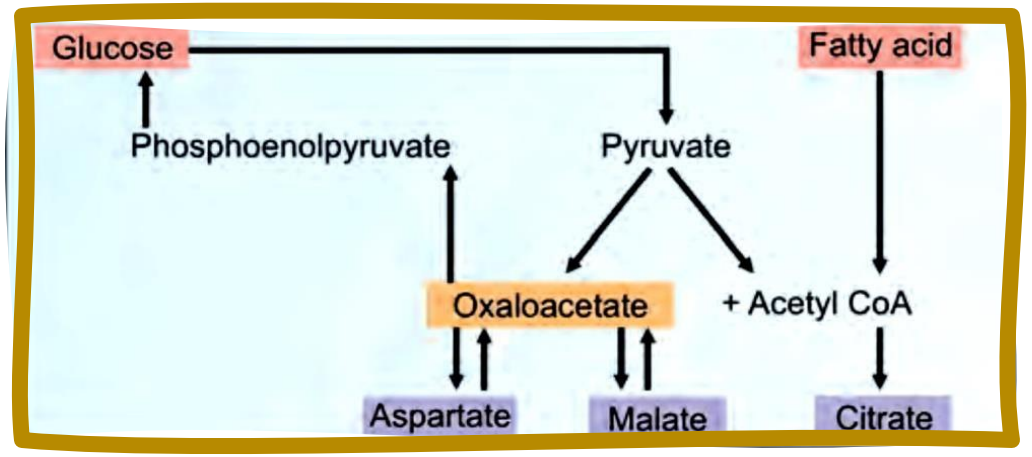
Oxaloacetate as a Junction Point

- Viewed as a **catalyst**
- An important junction point in metabolism



Oxaloacetate

- although it's an intermediate, they view it as a **catalyst** because it's very important in metabolism. It can exit the mitochondria to become malate, which can then be transformed into phosphoenolpyruvate and then into glucose through a very important pathway in our body called **gluconeogenesis** (the formation of glucose from non-carbohydrate sources. It can also be converted to amino acids like aspartate.



BIOENERGETICS OF TCA CYCLE

- **Like all pathways, overall net -ΔG (-228 kcal/mole), not 100%**
- **NADH, FAD(H2), and GTP (10ATP), 207 Kcal, 90%**
- **Three reactions have large (-ve) values**
- **Physiologically irreversible, low products**

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

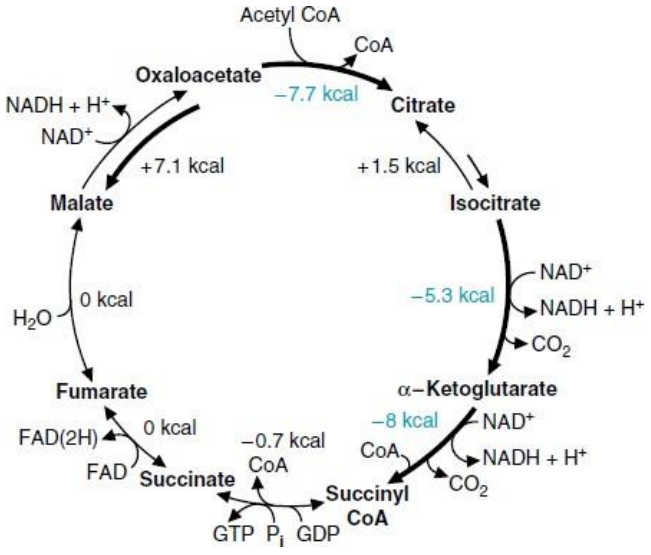
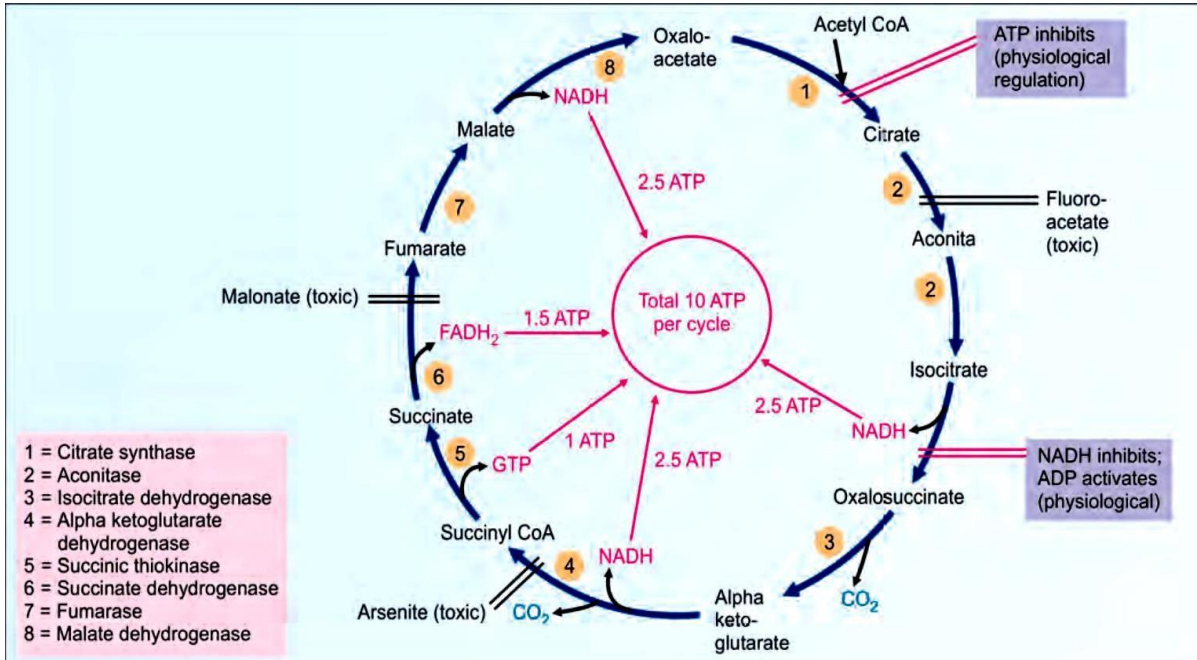


TABLE 19.1: ATP generation steps

Step No	Reactions	Co-enzyme	ATPs (old-calculation)	ATPs (new calculation)
3	Isocitrate → alpha keto glutarate	NADH	3	2.5
4	Alpha keto glutarate → succinyl CoA	NADH	3	2.5
5	Succinyl CoA→Succinate	GTP	1	1
6	Succinate → Fumarate	$FADH_2$	2	1.5
8	Malate → Oxalo acetate	NADH	3	2.5
		Total	12	10



What is efficiency?

Efficiency is the output divided by the input. It is a measure of how much you actually get on how much you should get. In other words, it tells you, from the input energy, how much output energy you get .

The efficiency of machines in general, never reaches 100%. There is always a loss of energy, and you have to look for where this loss of energy goes. Most machinery in the world, whether physiological or man-made operates at approximately 25-40% efficiency.

Krebs cycle is the best efficiency machinery in the world, whether physiological or man-made.

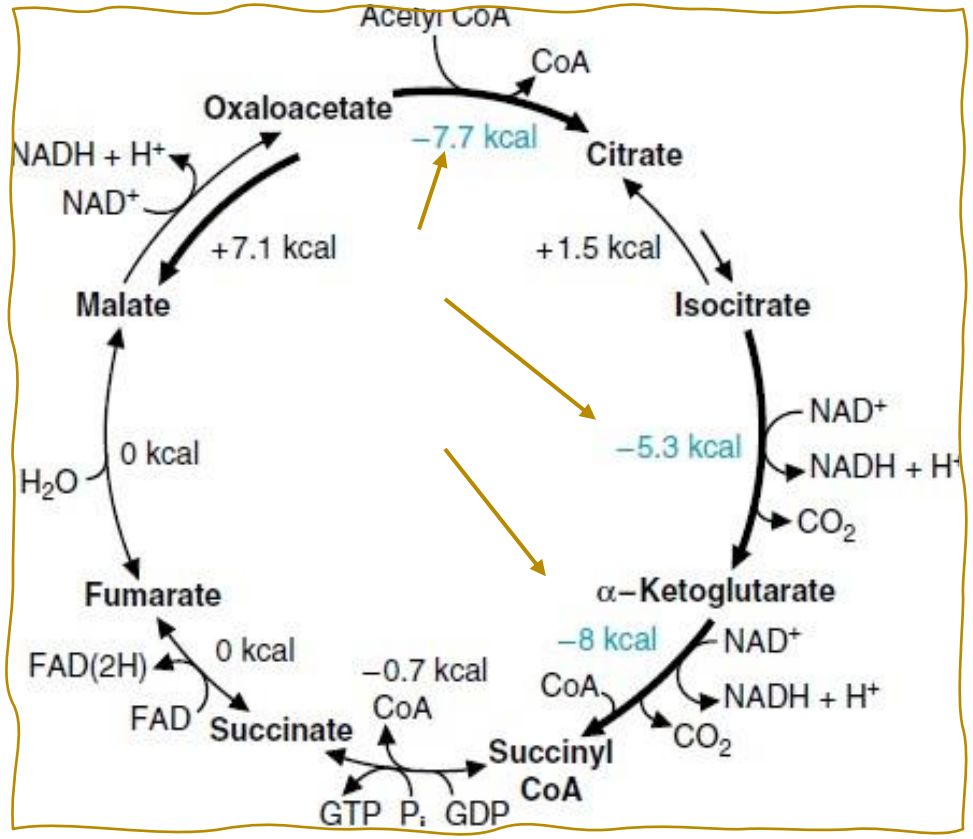
- The TCA cycle produces 3 NADH molecules, each one of them produces 53 kcal per mole also 1 $FADH_2$ produces 41 kcal per mole, and 1 GTP produces 7 kcal per mole. To sum up, they all produce 207 kcal per mole.

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

- If we burn out, the acetate molecule that enters the cycle and use calorie meter, 1 mole of it will give **-228** kcal per mole. Since we are getting 207 kcal, dividing them gives about 90% efficiency.

cycle is physiologically **irreversible**. Although some reactions in the cycle are reversible.

The reaction in which α-ketoglutarate becomes succinyl co-A has a highly negative ΔG, so it proceeds in only one direction. Also, the reactions highlighted in blue have a highly negative ΔG, so they proceed only in one direction.

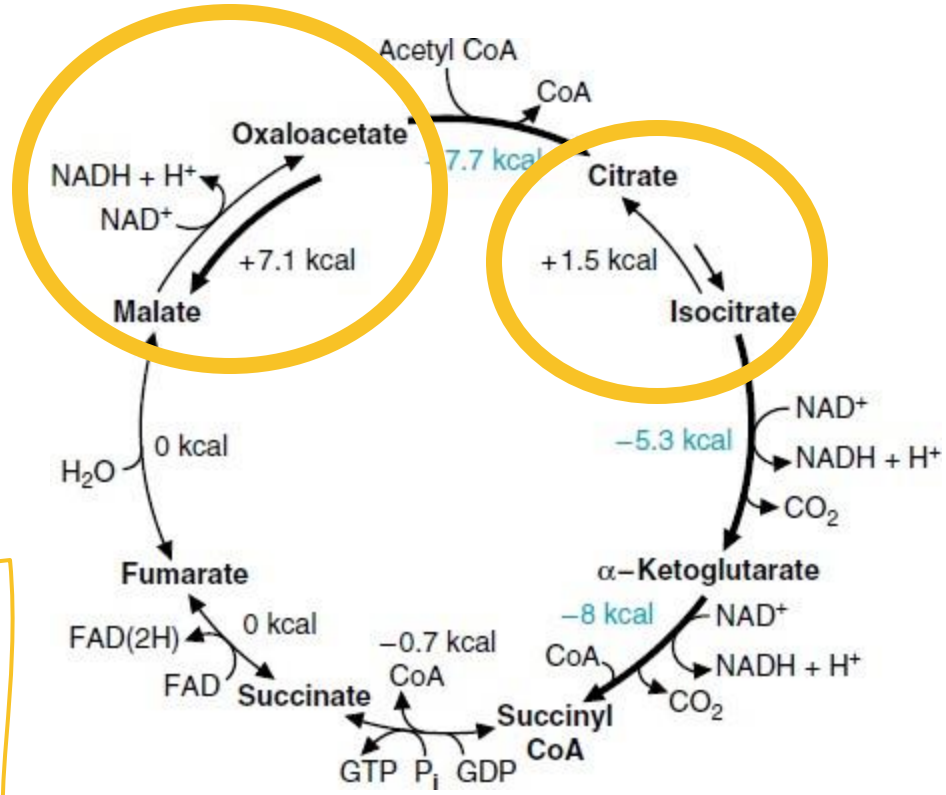


Meaning of the arrows

As you see, here we have a positive ΔG. What does it mean? It means that at equilibrium, we will have much more citrate compared to isocitrate and much more malate compared to oxaloacetate.. , I need a high concentration of malate compared to oxaloacetate to overcome the energy barrier and I need much more citrate than isocitrate to overcome the energy barrier .

(The reaction favors citrate). And this is a control mechanism so the cycle won't complete if we don't have enough citrate. So, there is no loss of energy.

If I have a small amount of oxaloacetate, it will rapidly become citrate , because the acetyl-CoA is present, and the ΔG -7.7 that is why I should have small amounts of Oxaloacetate to limit the reaction



NET RESULT OF THE CYCLE & ITS SIGNIFICANCE

TABLE 19.2: Stoichiometry of the TCA cycle

Acetyl CoA Oxaloacetate FAD 3NAD$^+$ GDP + PI GTP	→	2 CO_2 +CoA-SH Oxaloacetate $FADH_2$ 3 NADH

Why?

Box 19.1: Significance of citric acid cycle

1. Complete oxidation of acetyl CoA
2. ATP generation
3. Final common oxidative pathway
4. Integration of major metabolic pathways
5. Fat is burned on the wick of carbohydrates
6. Excess carbohydrates are converted as neutral fat
7. No net synthesis of carbohydrates from fat
8. Carbon skeletons of amino acids finally enter the citric acid cycle
9. Amphibolic pathway
10. Anaplerotic role.

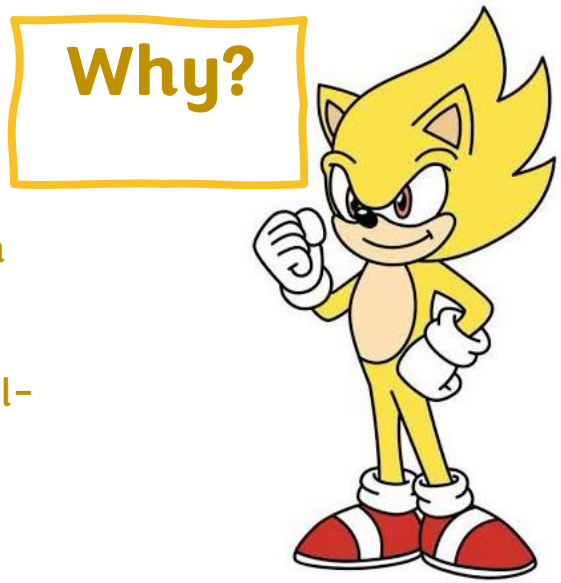
- ✓ **Fats are burned in the fire of carbohydrates**
- ✓ **Fat cannot be converted to glucose because pyruvate dehydrogenase reaction is an irreversible step**

1. Complete oxidation of acetyl CoA

The two carbons leave as CO_2.

6. Excess carbohydrates are converted as neutral fat
7. No net synthesis of carbohydrates from fat

So, if we have excess carbohydrate, it's going to become pyruvate. And pyruvate can be converted into acetyl-CoA or oxaloacetate. So more citrate will be produced, and citrate will synthesize fat.
But the other way around, breaking down fatty acids results in only producing acetyl-CoA. This is why I can't synthesize carbohydrate from fats.



9. Amphibolic pathway

The amphibolic pathway is both anabolic and catabolic. The TCA cycle is an amphibolic pathway because it provides certain molecules to run other pathways, and it breaks down other molecules.

FAT IS BURNED ON THE WICK OF CARBOHYDRATES

- What Happens in a Low-Carbohydrate State? (The "Fire Goes Out")
  - **OAA is Drained and Pyruvate is Diverted**
- **Krebs Cycle Stall!!!**
- **The Pathological Consequences: Ketogenesis**
- **Clinical Correlations**
  - **Diabetic Ketoacidosis (DKA), Starvation, Weight Loss Diets**

fat is burned on the wick of carbohydrates

In a low-carbohydrate state, there is no pyruvate and therefore no acetyl-CoA or oxaloacetate. So, the TCA cycle will not be efficient. The body will start to breakdown fats There will be an accumulation of acetyl-CoA from the breakdown of fat. This will lead to ketogenesis, which produces ketone bodies that supply cells with energy.

In diabetes, the problem is that glucose is in the blood but can't reach the cells. The cells "understand" that there is no glucose, so they start synthesizing ketone bodies, which can cause diabetic ketoacidosis.

This is the goal of a keto diet for weight loss. It involves the breakdown of fat.

Cataplerosis

means that these intermediates in the cycle are not solely present for the function of the citric acid cycle. They can be used in other metabolic pathways either by modifications or by themselves. In other words, they broke down to produce other molecules, following the same concept as catabolic reactions.

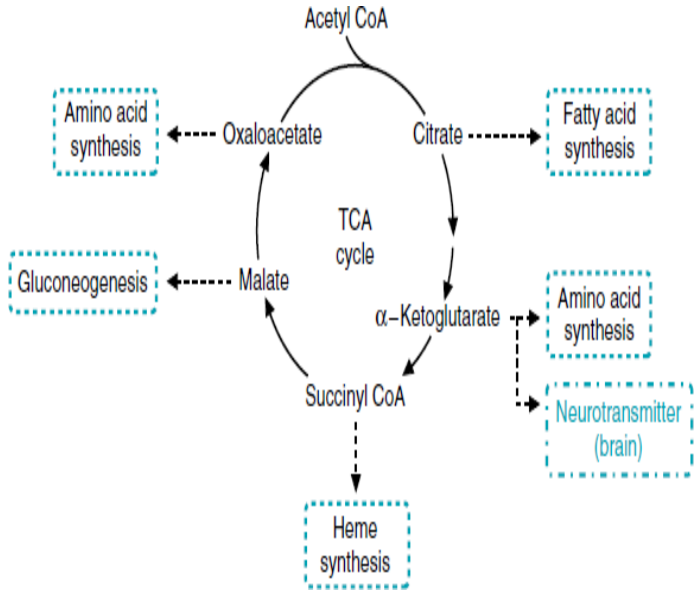
Citrate can be used in fatty acid synthesis by an enzyme called atp citratase as mentioned previously, producing acetyl-CoA, which can then be used in fatty acid synthesis.

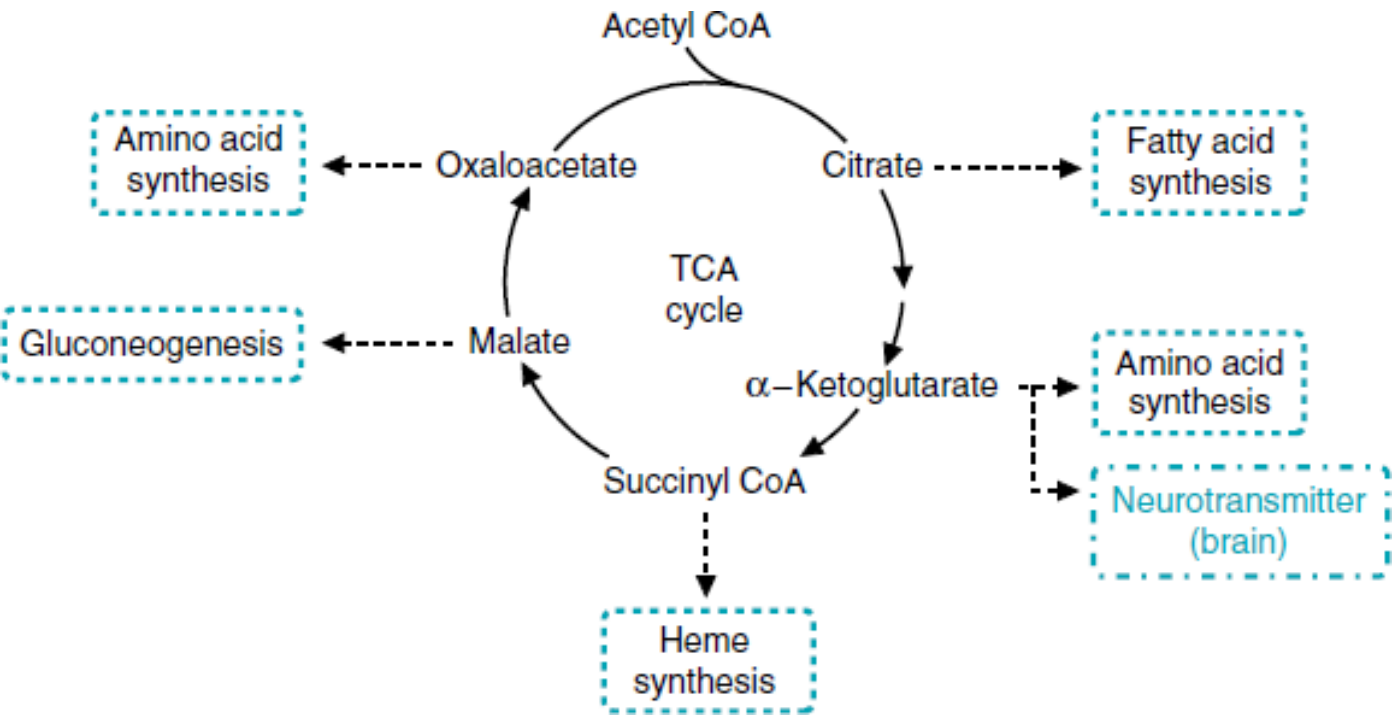
Alpha-ketoglutarate is used to synthesize the common amino acid glutamate, an excitatory neurotransmitter, by itself. From glutamate, we can synthesize glutamine and GABA, an inhibitory neurotransmitter.

Succinyl-CoA can participate in heme synthesis.

Malate participates in gluconeogenesis, which is the synthesis of new glucose from non-carbohydrate sources in cases of fasting or starvation.

Oxaloacetate can be converted to malate and participate in gluconeogenesis, or it can be converted to the common amino acid aspartic acid, which can then be converted to asparagine



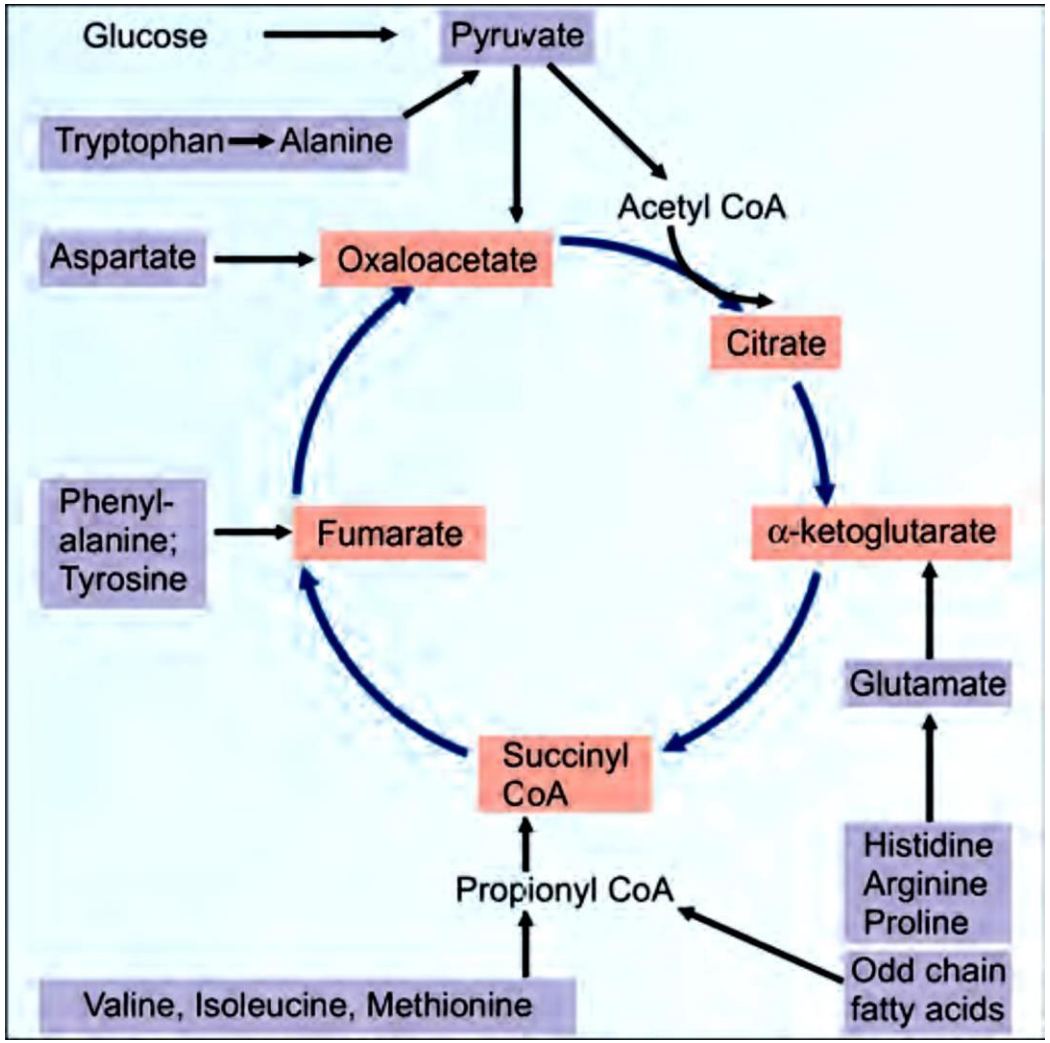


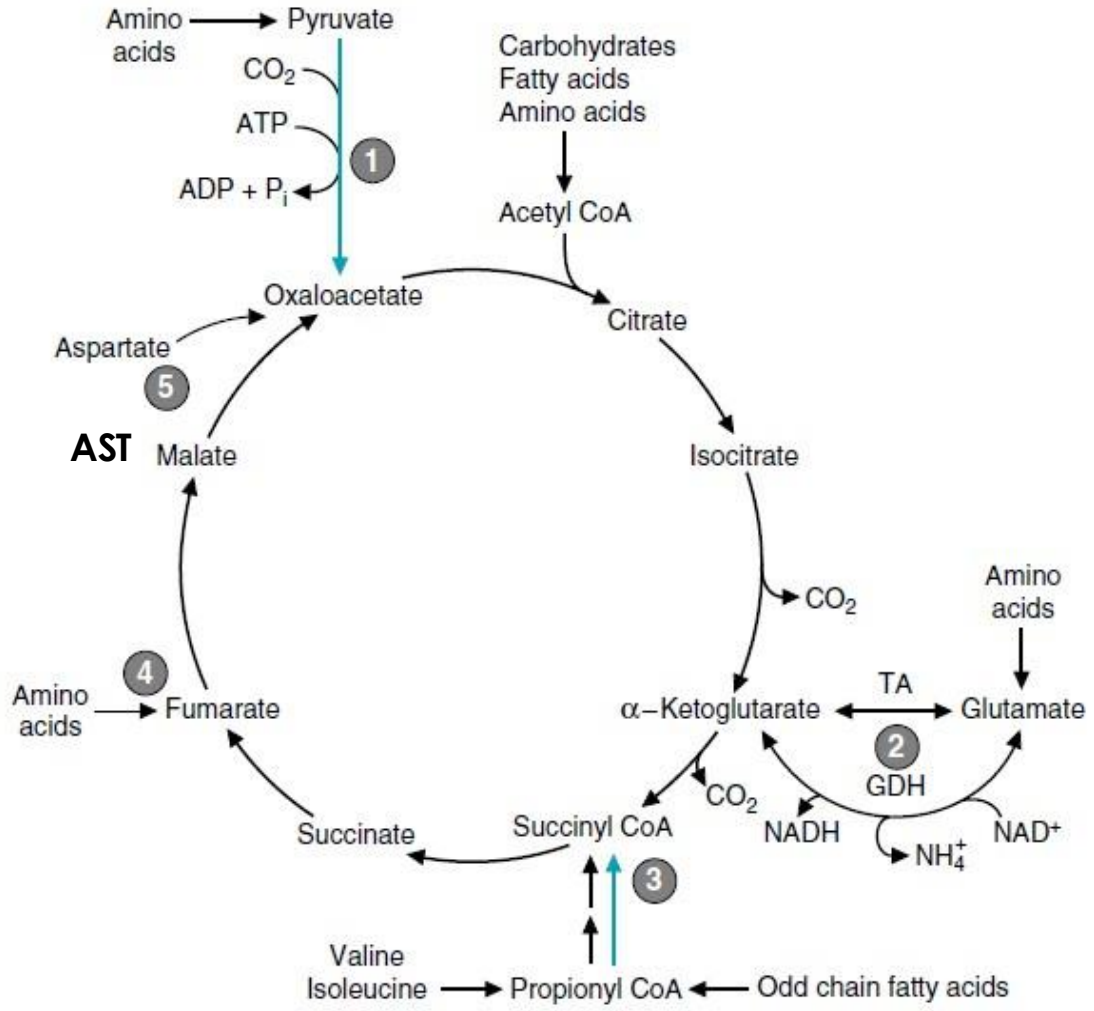
CATAPLEROSIS

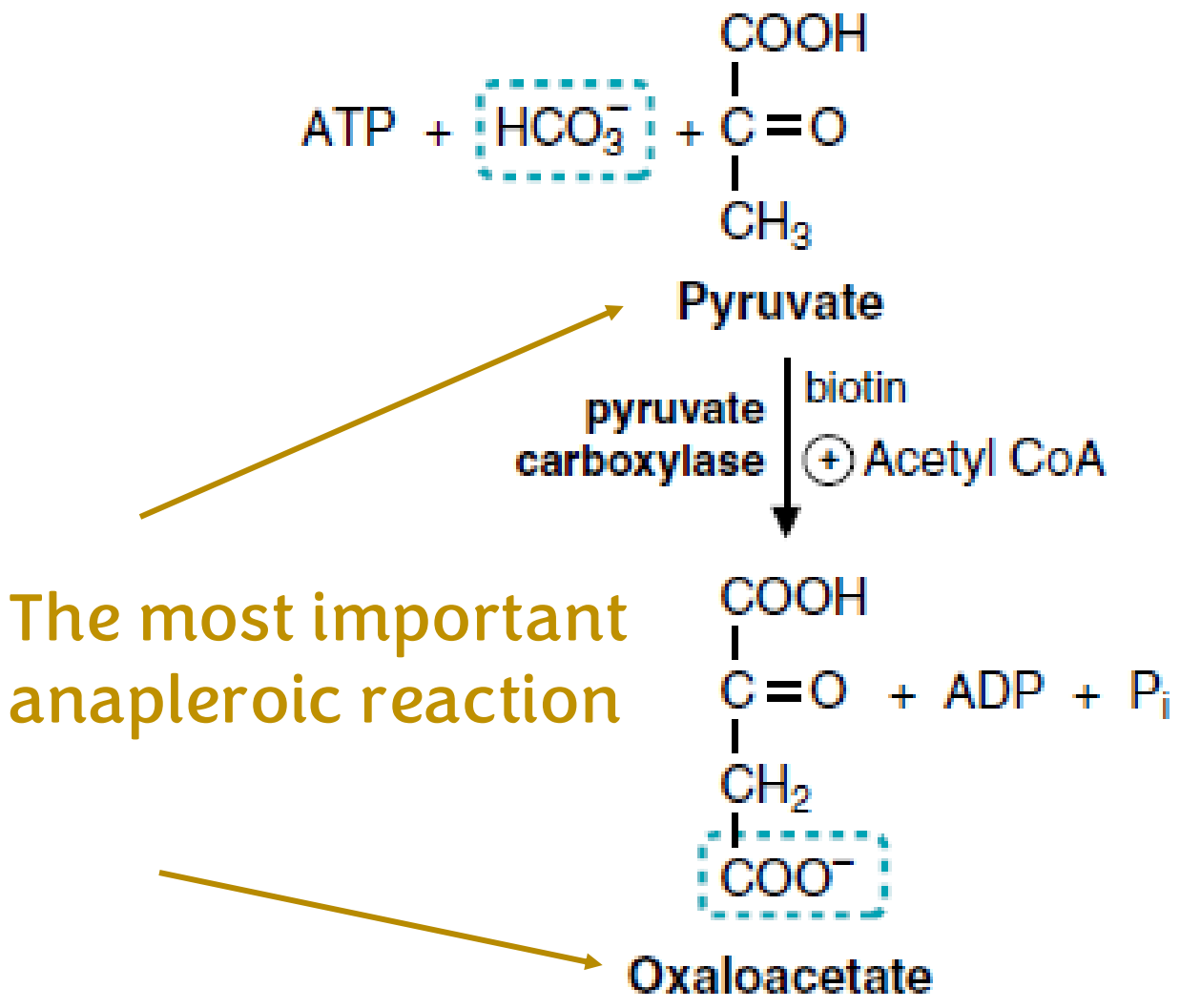
- Intermediates are Precursors for Biosynthetic Pathways
  - (citrate, acetyl CoA, fatty acid synthesis, liver)
  - (fasting, malate, gluconeogenesis, liver)
  - (Succinyl CoA, heme biosynthesis, bone marrow)
  - (α-ketoglutarate, glutamate, GABA, a neurotransmitter, brain)
  - (α-ketoglutarate, glutamine, skeletal muscle to other tissues for protein synthesis)
  - (OAA, Aspartate, Asparagine, Gluconeogenesis, liver)

ANAPLEROTIC ROUTES (AMINO ACID DEGRADATION)

Anaplerosis is the other way around. If we have a deficiency in these intermediates, other reactions can supply us with them.







The most important anapleroic reaction

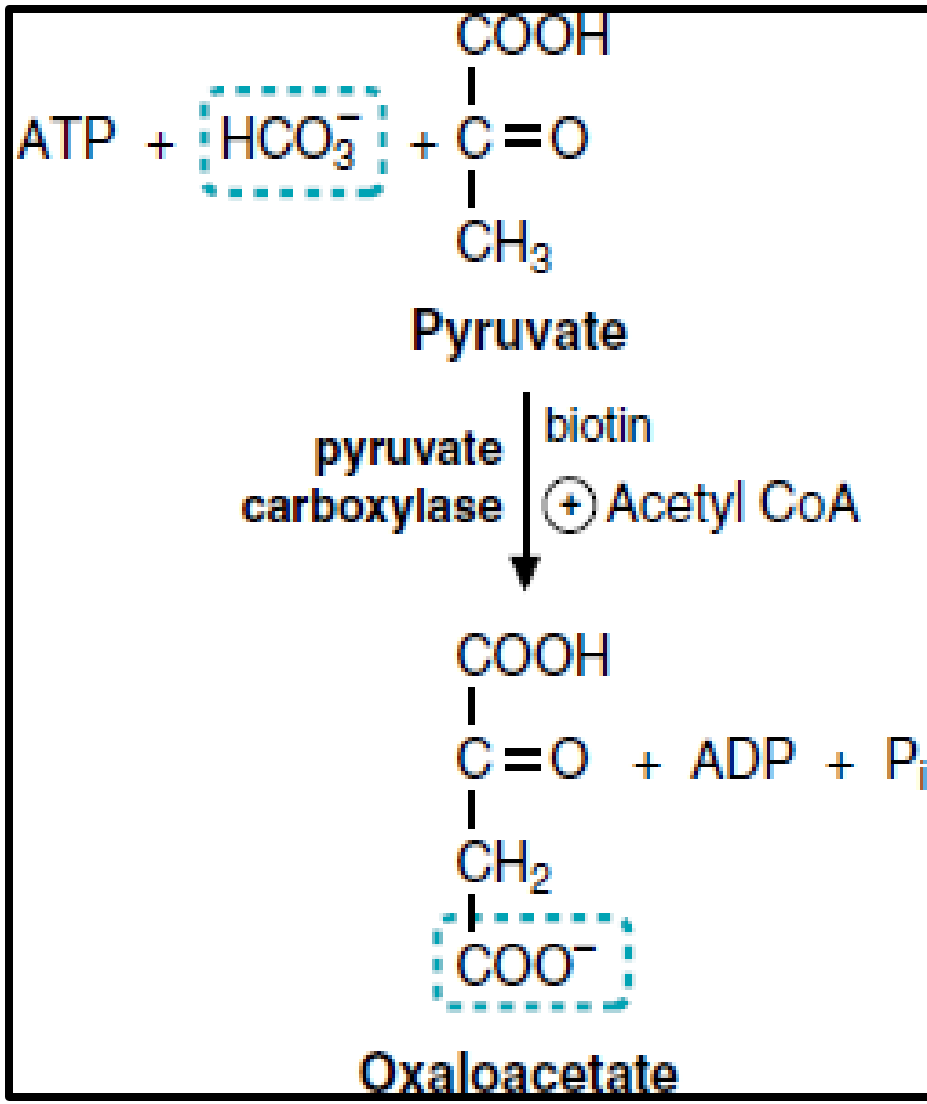
ANAPLEROTIC REACTIONS

- Pathways or reactions that replenish the intermediates of the TCA cycle
- Pyruvate Carboxylase is a major anaplerotic enzyme (requires biotin)
- Found in many tissues, liver, kidneys, brain, adipocytes, and fibroblasts
- Very high conc. In liver and kidney (gluconeogenic pathway)
- Activated (acetyl CoA)

The difference between pyruvate and oxaloacetate is an additional carboxylic group on oxaloacetate. By a carboxylation reaction, pyruvate ends up as oxaloacetate. The enzyme in this reaction requires the coenzyme biotin, which is vitamin B7. It also requires energy in the form of ATP as we are increasing the carbon skeleton. This reaction is activated by acetyl-CoA. Note that none of the materials can act as an activators or inhibitors unless they are in excess amounts. So, if acetyl-CoA is in excess, it implies that we need more oxaloacetate to couple with it and keep the TCA cycle running.

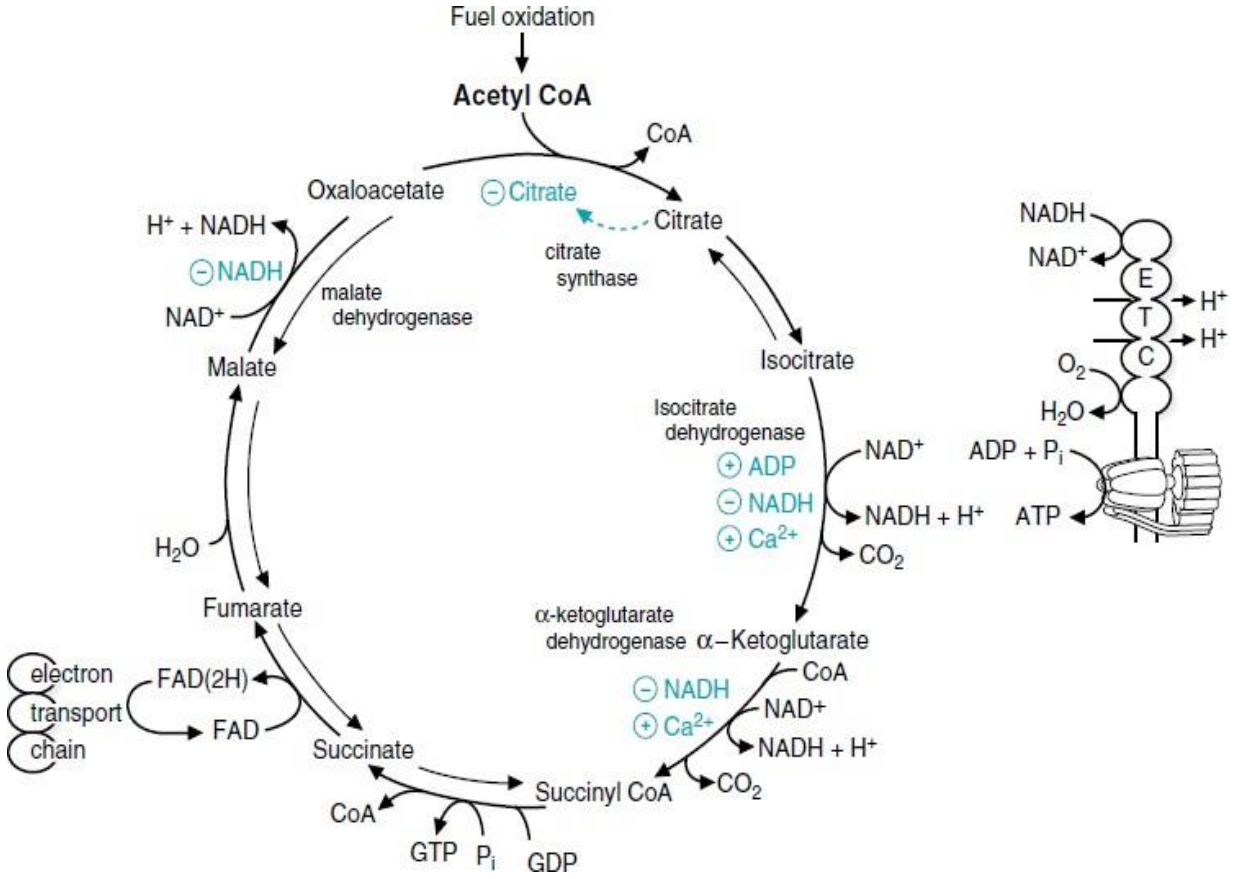
Very high conc. In liver and kidney (gluconeogenic pathway)

The enzyme is in high concentration in these tissues because malate exits out in this process, and oxaloacetate becomes low in concentration. Therefore, we need the enzyme to replace that deficiency



NADH is a product of **isocitrate dehydrogenase, alpha ketoglutarate dehydrogenase** and **malate dehydrogenase.**

NADH acts as an allosteric inhibitor for these enzymes. If NADH is high in concentration, comes back to the Enzyme in a feedback mechanism and inhibits these enzymes,

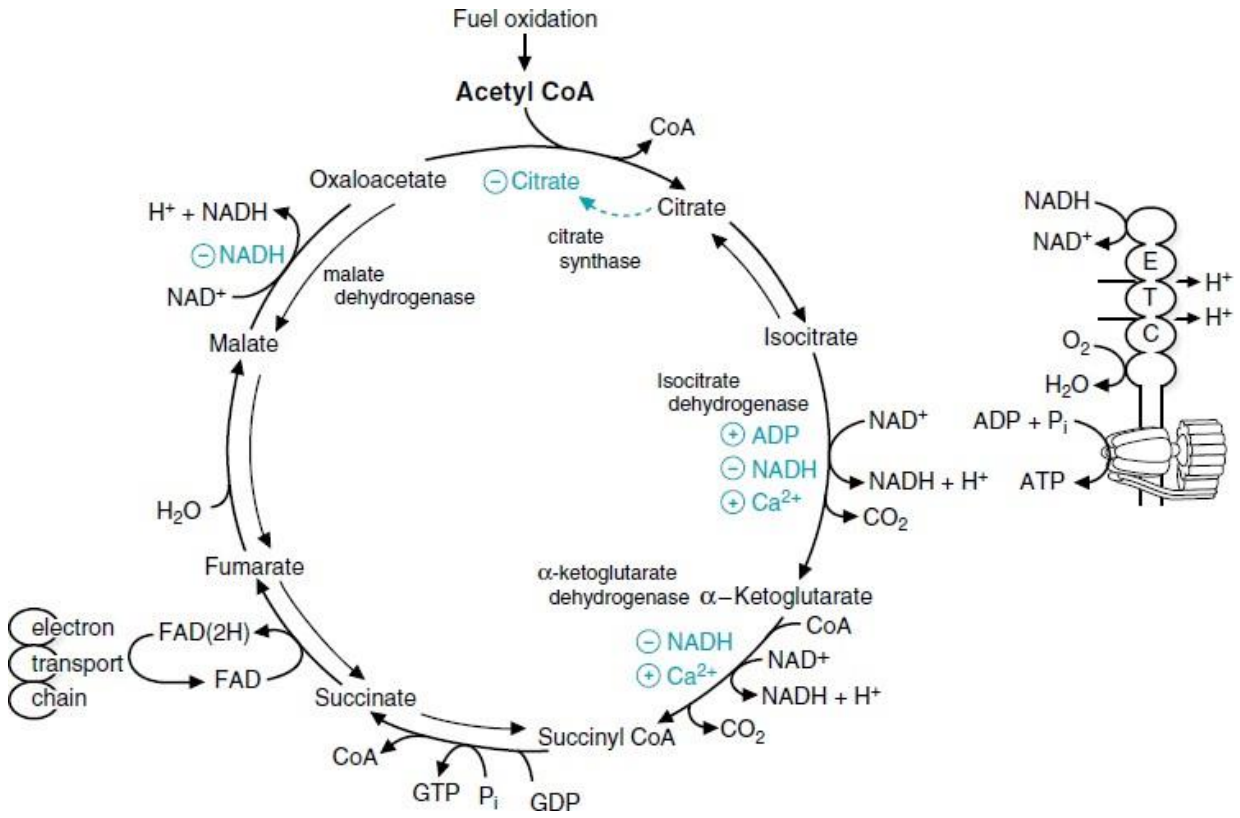


REGULATION OF THE TCA CYCLE

- Correspond to ETC (ATP/ADP)
- Two major messengers (feedback): (a) phosphorylation state of adenines, (b) the reduction state of NAD
- Adenine nucleotides pool and NAD pool are relatively constant

How much is the concentration of NAD plus compared to NADH, which are a redox couples So if one of them is increasing, the other is decreasing.

REGULATION – CITRATE & CITRATE SYNTHASE



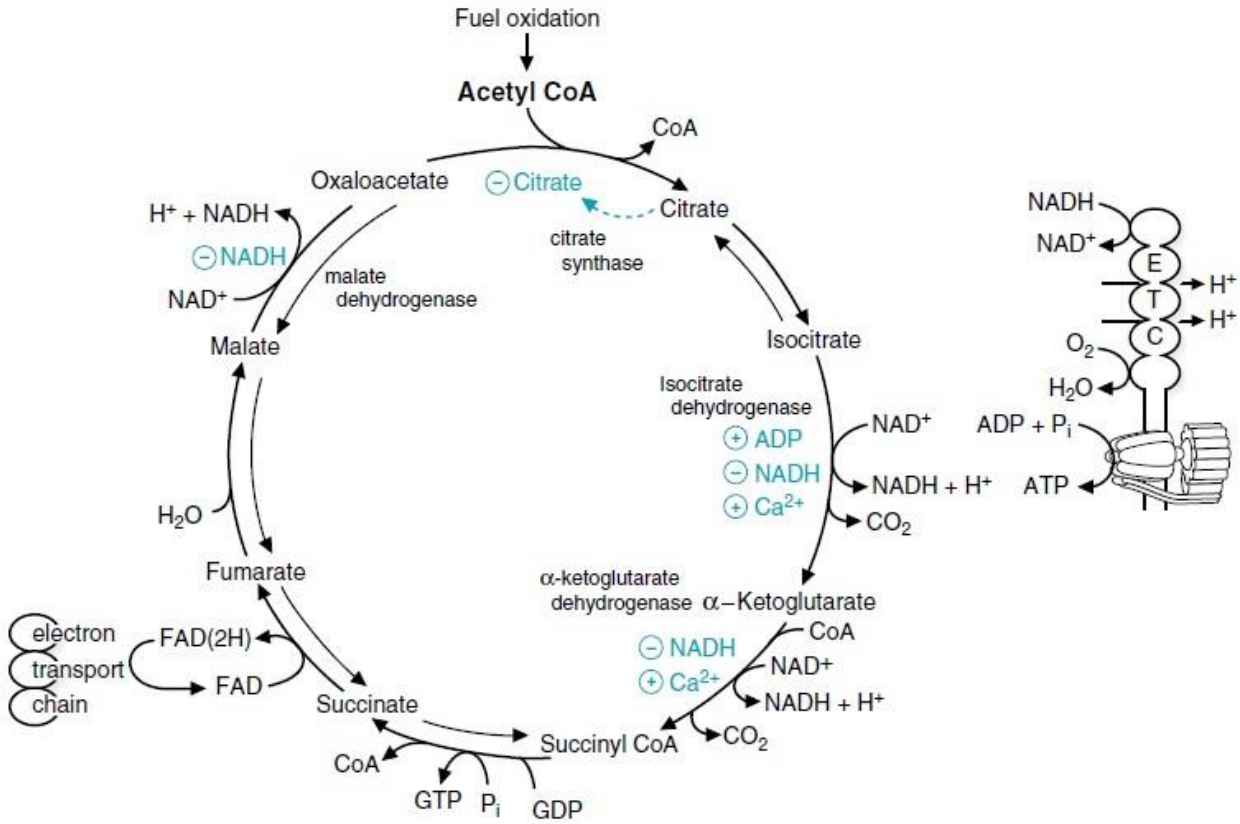
- Rate regulated by oxaloacetate & citrate (inhibitor)
- ATP: allosteric inhibitor
- Effect of citrate:
  - Allosterically inhibits PFK, the key enzyme of glycolysis
  - Stimulates fructose-1,6-bisphosphatase, a key enzyme of gluconeogenesis
  - Activates acetyl CoA carboxylase, a key enzyme of fatty acid synthesis

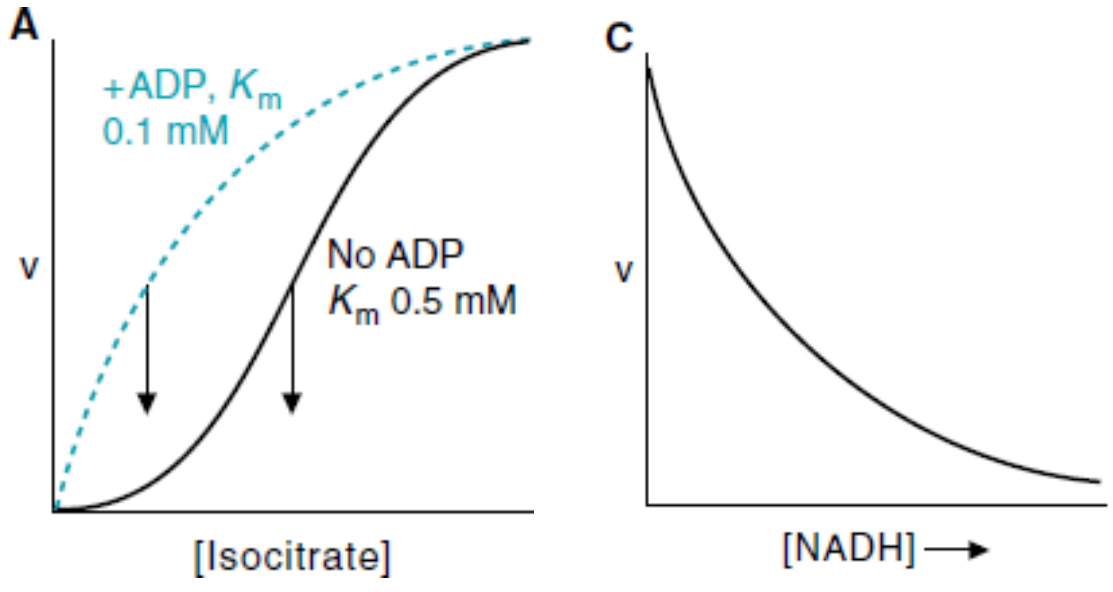
ISOCITRATE DH

- Best regulation (rate-limiting)
- Allosterically: activated (ADP, Ca^{+2})
- Inhibition (NADH)
- No ADP vs. ADP (K_M), a small change in ADP, great effect

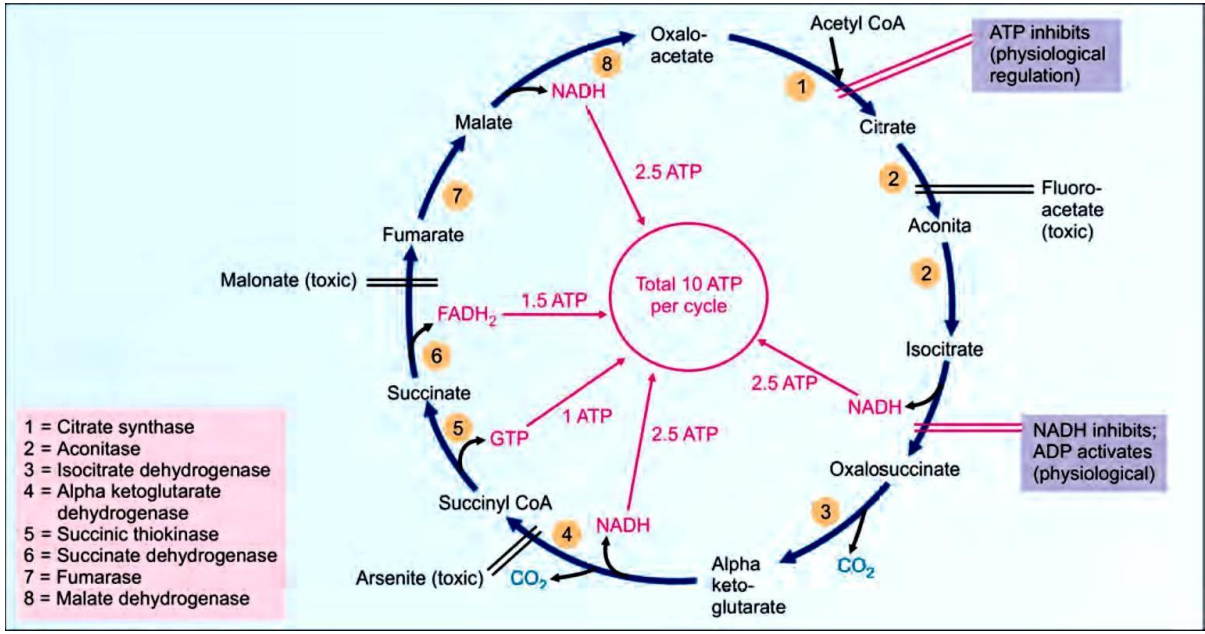
α-Ketoglutarate DH

- Inhibited: NADH, succinyl CoA, GTP
- Activated: Ca^{+2}





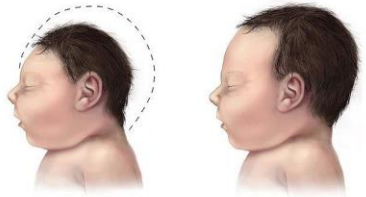
INHIBITORS OF TCA CYCLE (PHYSIOLOGICAL?)



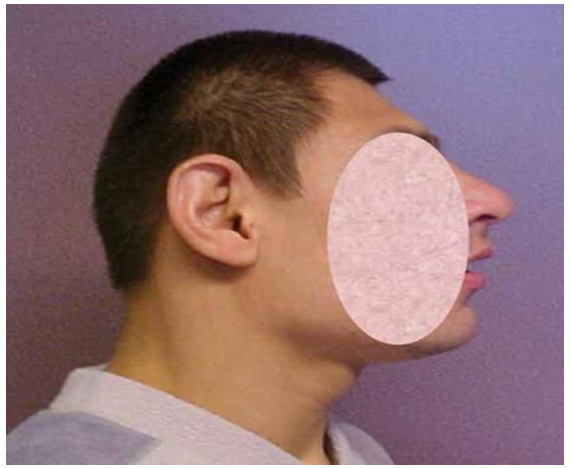
- A. Aconitase (citrate to aconitate) is inhibited by fluoroacetate (non-competitive inhibition)
- B. Alpha ketoglutarate dehydrogenase (alpha keto-glutarate to succinyl CoA) is inhibited by Arsenite (non-competitive inhibition)
- C. Succinate dehydrogenase (succinate to fumarate) is inhibited by malonate (competitive inhibition)

INBORN ERRORS OF METABOLISM

- **PDC Deficiency:**
  - Most common enzymatic cause of primary lactic acidosis
- Often presenting in neonates with severe neurological impairment
- Treatment involves a ketogenic diet to provide an alternative fuel source for the brain.



- **Fumarase Deficiency:** Causes severe encephalopathy, seizures, and developmental delay due to the accumulation the accumulation of fumarate.



The brain size loss

ONCOMETABOLITES AND CANCER:

- **IDH Mutations:**
  - Common in gliomas and acute myeloid leukemia (AML)
  - Confer a neomorphic activity that produces 2-hydroxyglutarate (2-HG): a competitive inhibitor of α-KG-dependent dioxygenases, leading to a hypermethylation of DNA and histones
  - This epigenetic dysregulation blocks cellular differentiation and promotes tumorigenesis.
- **SDH and FH Mutations:**
  - Cause hereditary paragangliomas and leiomyomatosis, respectively.
  - Accumulating succinate or fumarate inhibits prolyl hydroxylases (PHDs), leading to the stabilization of HIF-1α under normal oxygen conditions (pseudohypoxia).
  - This activates angiogenic and glycolytic programs, driving the Warburg effect and tumor growth.

NEURODEGENERATION

- Impaired mitochondrial function is the hallmark of neurodegenerative diseases like Parkinson's and Alzheimer's.
- The resulting energy deficit, oxidative stress, and disrupted calcium buffering contribute to excitotoxicity and neuronal death.

- An important point behind mentioning these diseases is not to know the symptoms of these diseases is to know that the effect of energy is high, However, cases of reported in citric acid cycle is very low because the mutation is highly affecting the energy production resulting in death. some people can live yet their life will be disastrous, because Krebs cycle is a central metabolic hub. It is there in every cell it is needed for the process of energy production. So if it is affected The whole energy machinery will be affected and accordingly you need the energy to build up your cells, like neurone or others. But if the neurone are affected ,the control mechanism over your body will be affected. This is why the krebs cycle involved in every system of your body.

Additional Resources:

رسالة من الفريق العلمي :

External Sources :

Youtube :

Ninja Nerd

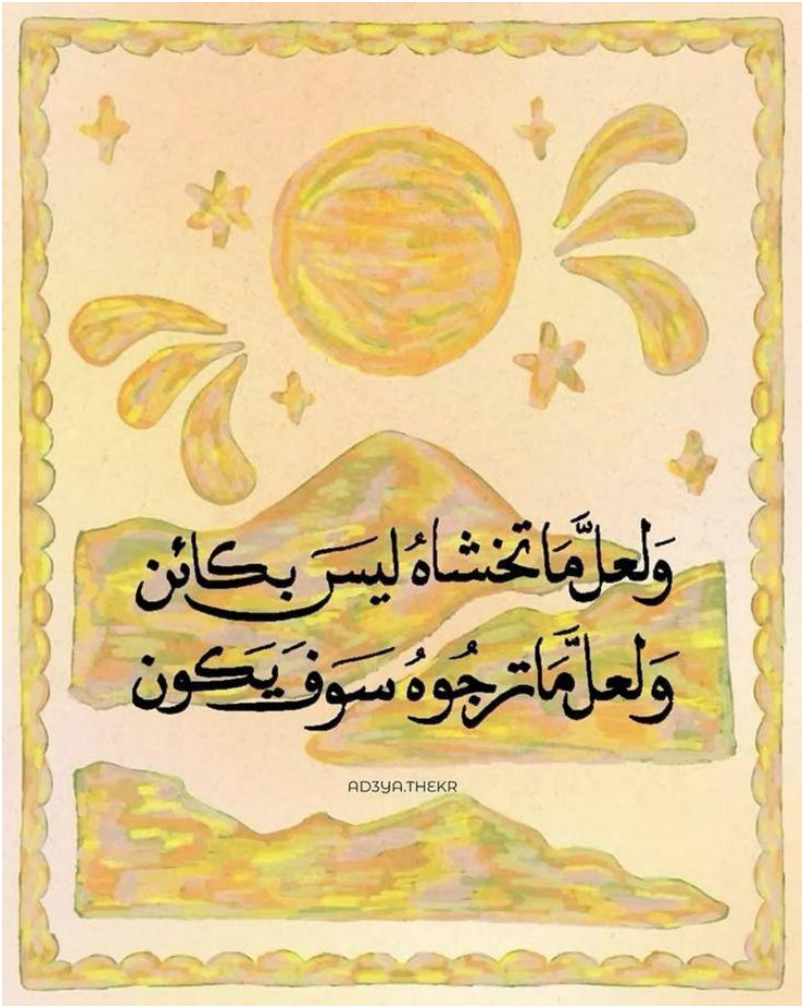
Medicine with Jafar (3 parts)

ChatGPT :

Use it to generate MCQ's to test yourself

Legan Aldofat App :

Past papers



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	Slide 28 4) The structure of lipoid involves a disulfide bridge so the arbor unit	Arbor	Carbon
V1 → V2			