



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
الَّذِي عَلَّمَ بِالْقَلَمِ ۖ عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ ۝

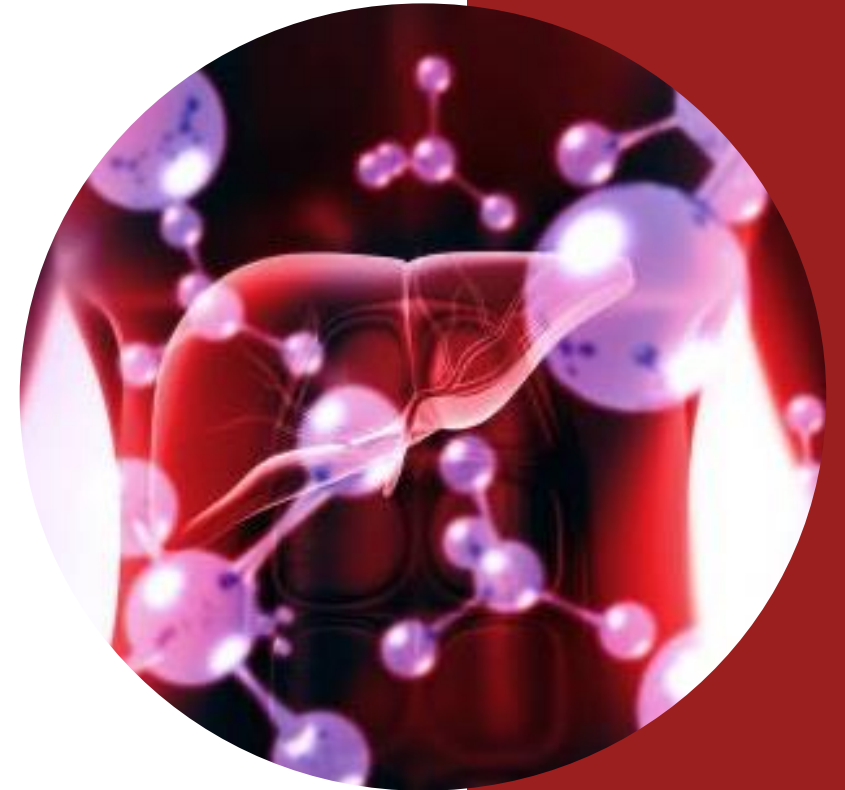
⚙ METABOLISM

MID | Lecture 6

TCA Cycle Pt.2

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Color coding used in the modified:



Black: the original slides



Maroon: the doctor's explanation/words



Gray: additional information and explanation



Red: important information

By this the cycle is finished: three NADH, one FADH₂, and one ATP (or GTP) molecule are produced.
Per one acetyl-CoA molecule:

3 NADH → 7.5 ATP

1 FADH₂ → 1.5 ATP

1 GTP → 1 ATP (substrate-level)

Total = 10 ATP

Usually Oxidative phosphorylation produces ATP using NADH and FADH₂ through the electron transport chain,

Four Dehydrogenases:

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

most dehydrogenases use NAD⁺, except succinate dehydrogenase, which uses FAD.

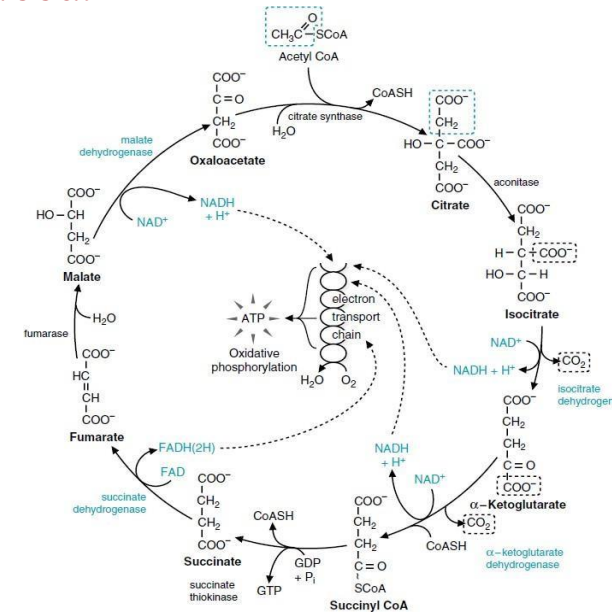
But A high-energy molecule (ATP) without the need for oxygen, without going through oxidative phosphorylation... This is called **substrate-level phosphorylation**.”

We have 3 substrate-level phosphorylation reactions in our body, one in TCA cycle, and 2 in Glycolysis (phosphoglycerate kinase, pyruvate kinase)

In TCA cycle: at the **succinyl-CoA → succinate** step.

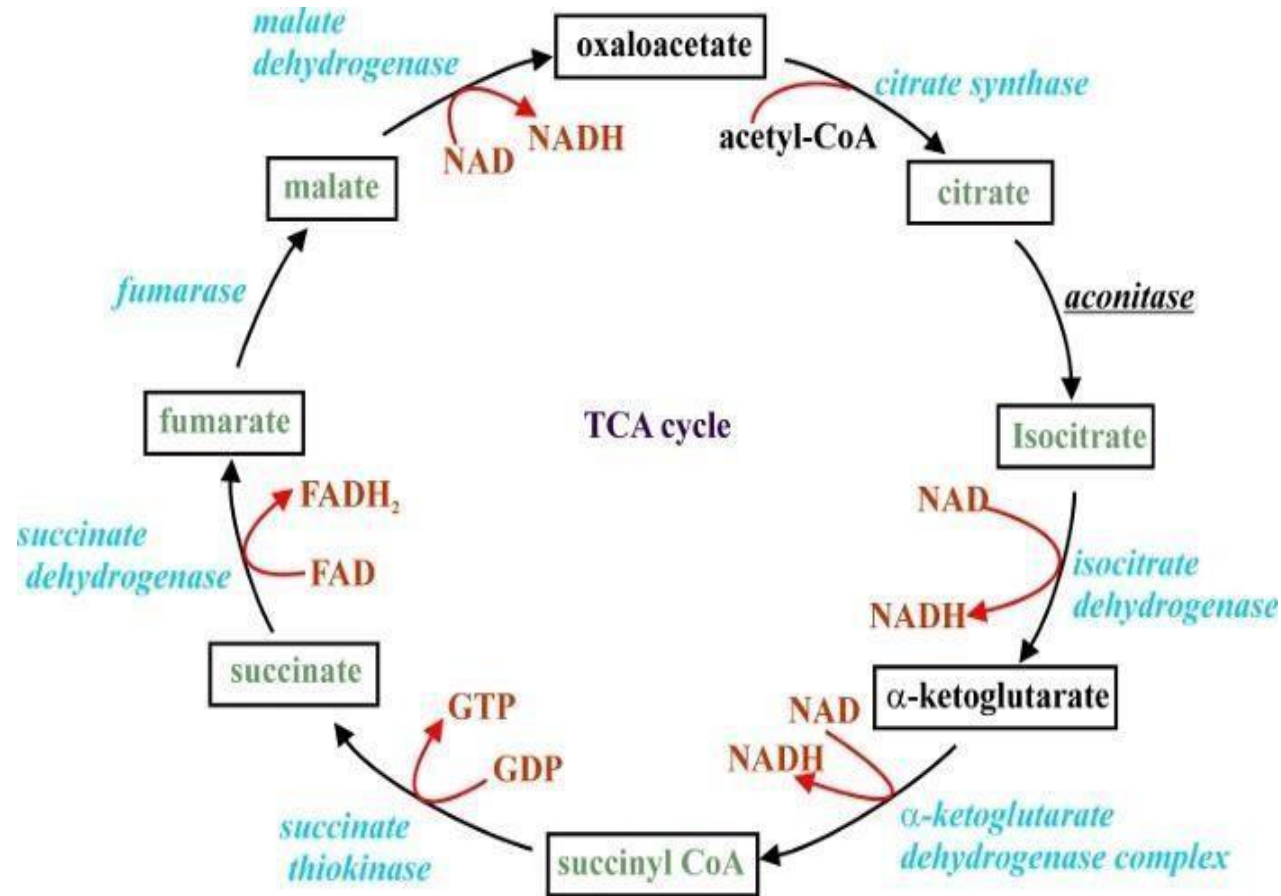
Enzyme: Succinyl-CoA synthetase (succinate thiokinase)

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



After studying the sequence of the Krebs cycle,
can the cycle proceed in the reverse direction?

Theoretically, many biochemical reactions are reversible. However, physiologically, reversing a reaction may require a huge amount of energy because the reverse reaction is not energetically favorable. This makes the reverse reaction very unlikely under normal cellular conditions. The same principle applies to the Krebs cycle. There are **three reactions** with highly negative delta G values, which makes them physiologically irreversible. These reactions are catalyzed by citrate synthase, isocitrate dehydrogenase, and α -ketoglutarate dehydrogenase. (Take a look at slide 27)

The first irreversible reaction is the conversion of oxaloacetate and acetyl-CoA into citrate, catalyzed by citrate synthase. This reaction cannot easily be reversed under physiological conditions because it is energetically unfavorable. In addition, the enzyme **ATP citrate lyase**, which converts citrate into acetyl-CoA and oxaloacetate using ATP, is **not** located in mitochondria, but available in the cytosol.

In this process, acetyl-CoA is used to synthesize fatty acids, which can then be combined with other molecules to make fats.

That's why enzyme is linked to cancer metabolism. cancer cells have a high amount of this enzyme. It breaks down citrate into acetyl-CoA, which is used to make fats that cancer cells need to create new membranes in order to proliferate.

This detail will be discussed later when we study fat.

FORMATION OF CITRATE

Let's Answer the question to organize the idea

What drives the reaction forward?

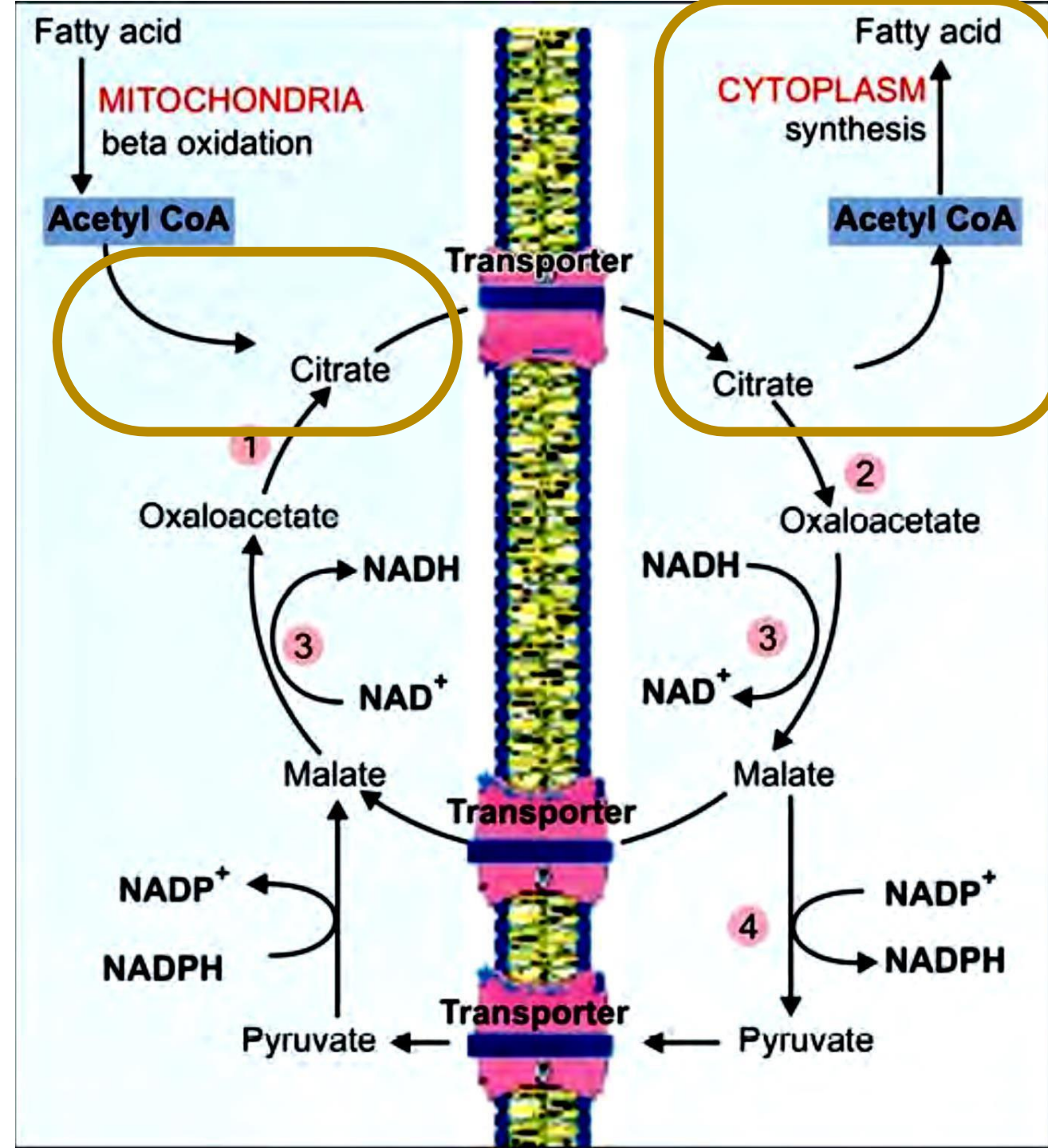
Is it reversible or irreversible?

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



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, and by the highly negative ΔG of its irreversible steps.

Can it be reversed?

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

Citrate can leave the mitochondria and enter the cytosol through a special transporter **when** its concentration in the mitochondria becomes high, which usually occurs when the cell is in a good energy state with sufficient ATP and an adequate supply of nutrients. These nutrients can come from fats, whose breakdown provides acetyl-CoA, or carbohydrates, whose metabolism produces pyruvate that is converted into acetyl-CoA and can also contribute to oxaloacetate production. Once citrate enters the cytosol, an enzyme called ATP citrate lyase (or ATP citratase) uses ATP to convert it into acetyl-CoA and oxaloacetate. The acetyl-CoA can then be used to synthesize fatty acids, which are subsequently used to produce fats for storage. This explains why excess nutrients and a good energy state promote fat synthesis and storage, and why ATP citrate lyase is important in adipose tissue, whose main function is to store fat. Insulin is released by the pancreas when blood glucose levels rise, such as after a carbohydrate-rich meal, signaling that nutrients are available and promoting processes such as fatty acid synthesis and fat storage.

Is it reversible or irreversible?

Irreversible in mitochondria. 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)

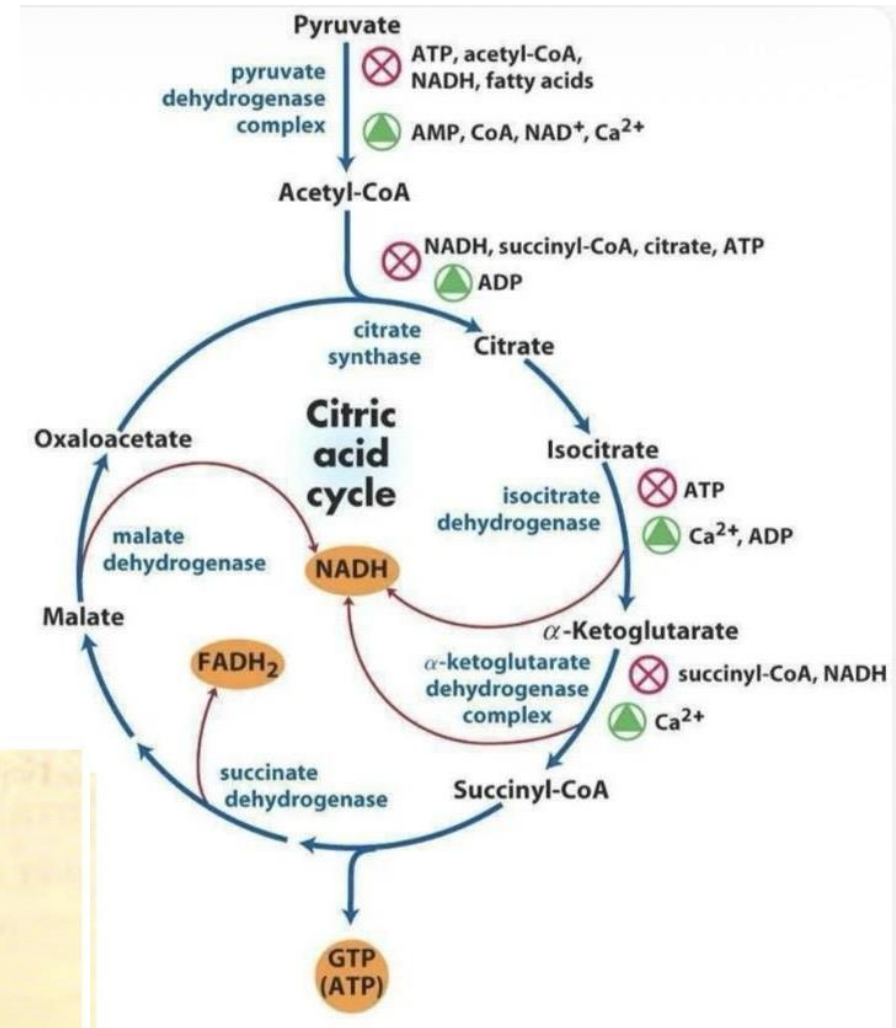
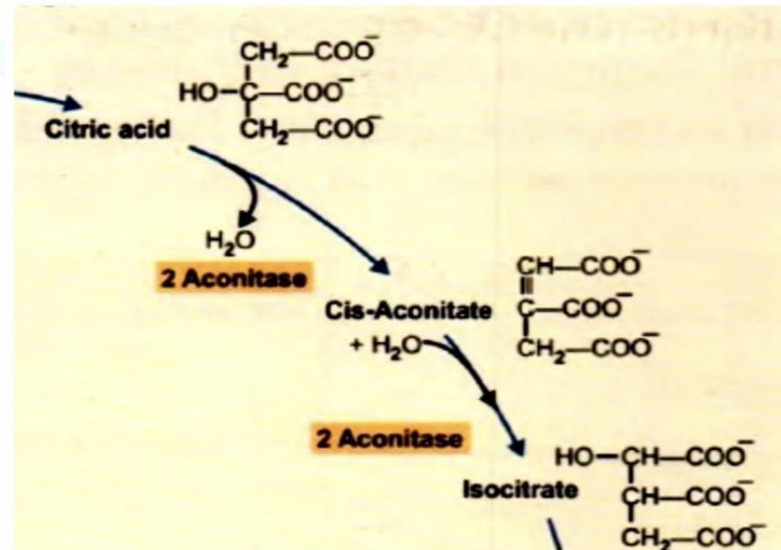
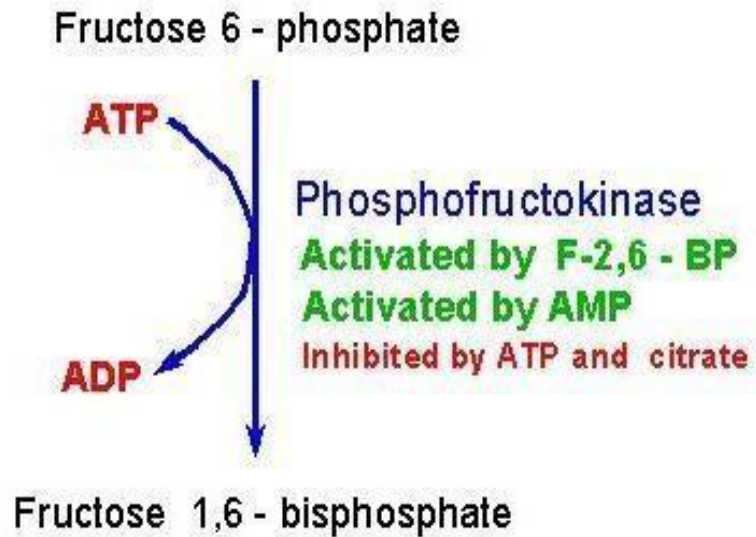
Link to cancer metabolism

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

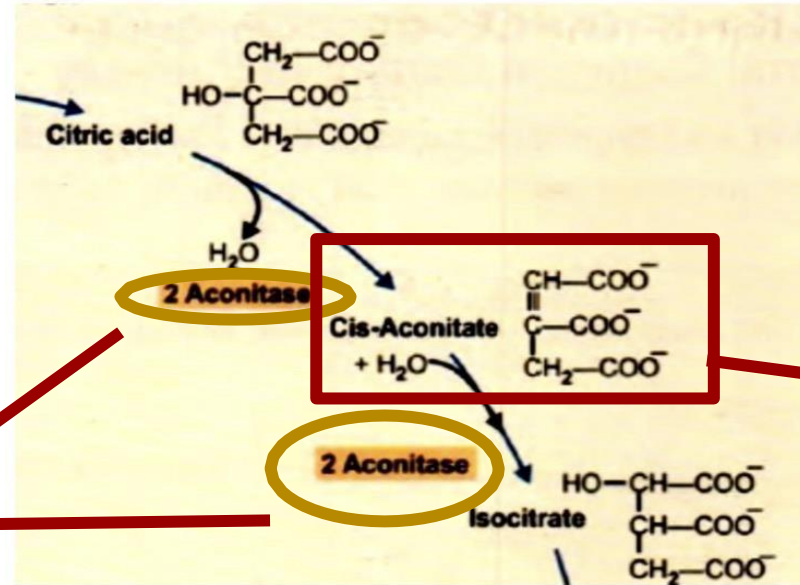


FORMATION AND OXIDATION OF ISOCITRATE

Control at the committed step of glycolysis



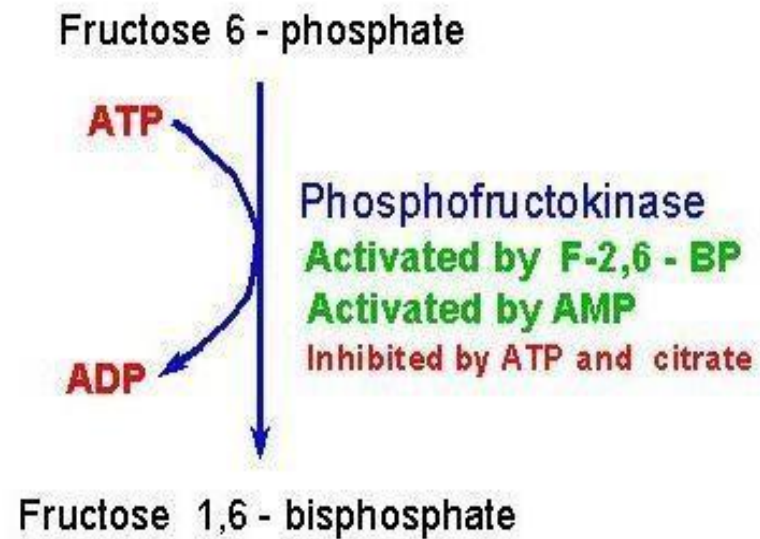
THE FORMATION:



The enzyme takes its name after the molecule

Represents the intermediate of conversion of citric acid to isocitrate

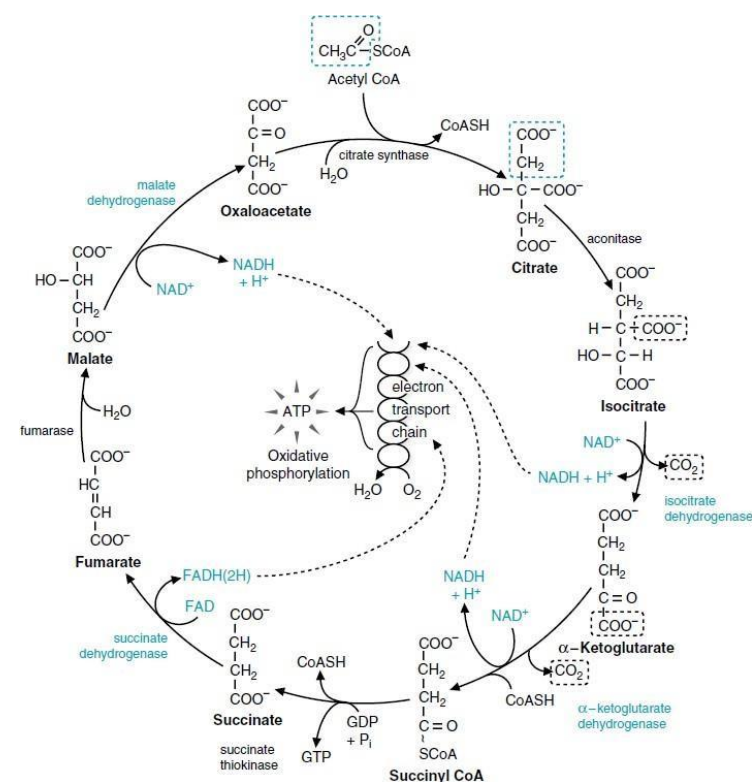
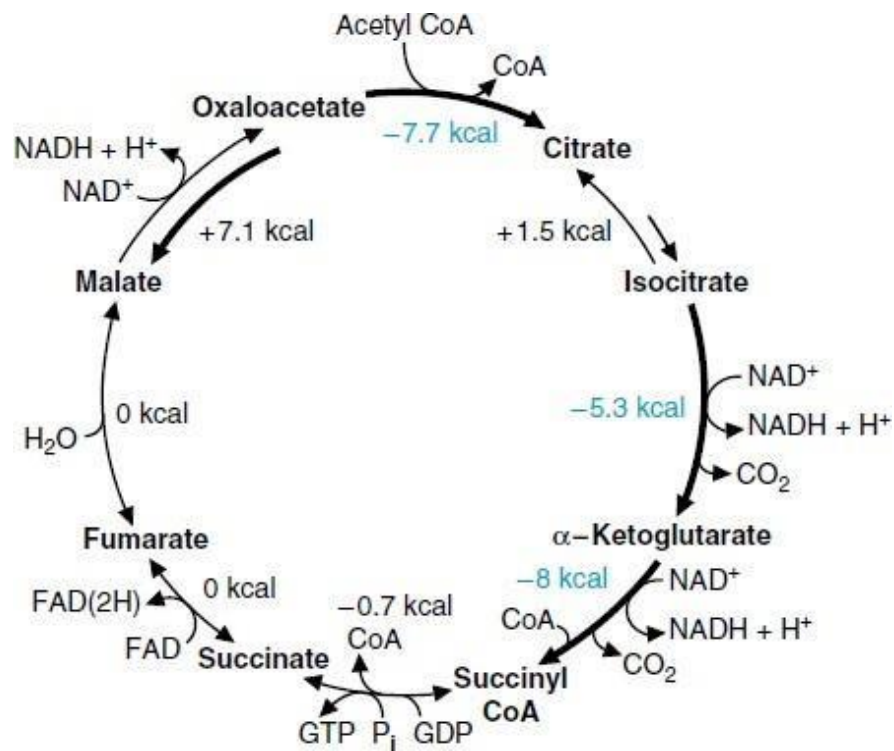
Control at the committed step of glycolysis



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 to regulate glycolysis, it can affect the enzyme which is called phosphofructokinase that catalyzes this step and inhibits it.

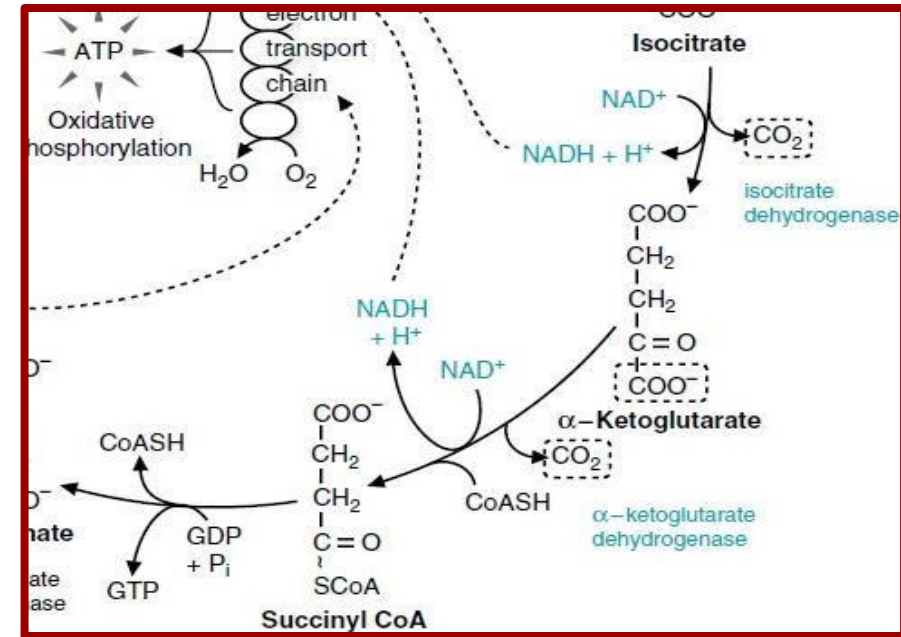
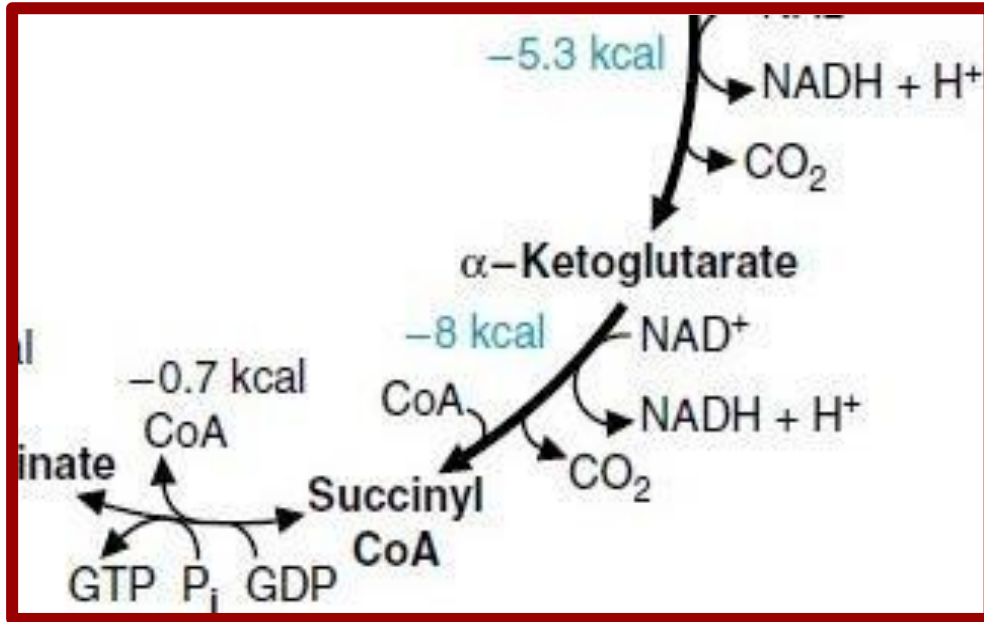
(it makes sense for this enzyme to be inhibited by citrate, because it is already high in concentration, there is less need to break down more glucose to produce ATP).



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!





It's easy to visualize the decarboxylation process catalyzed by isocitrate dehydrogenase because we can clearly identify the compound before and after the removal of CO_2 . However, in the α -ketoglutarate dehydrogenase reaction, the process is less straightforward to visualize because it is not carried out by a single enzyme, but by a complex of multiple enzymes.

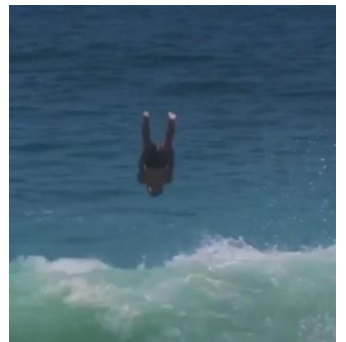
Enzyme complexes :

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)

I need you to focus here,
Open your eyes, ears and brain then dive in!

p.s. Looking back, maybe not ears



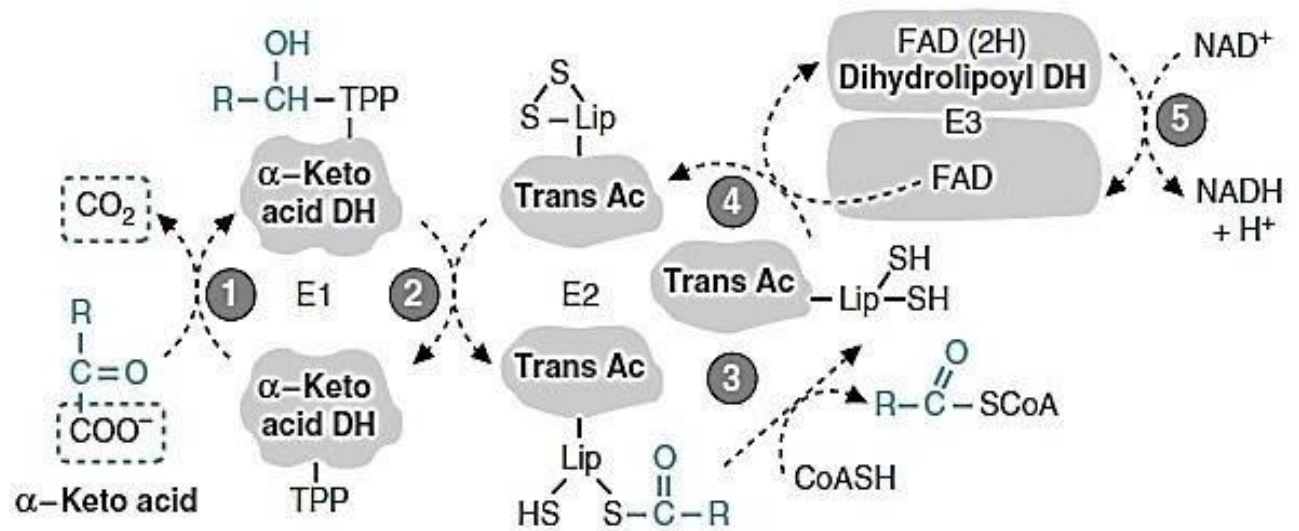
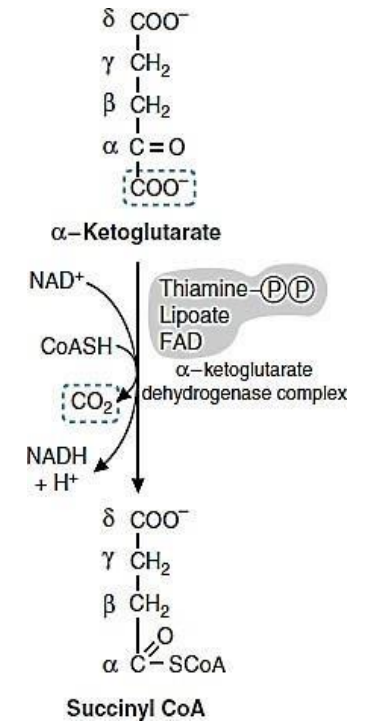
α -KETOACID DEHYDROGENASE COMPLEXES (TLCFN)

(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 $\rightarrow$ higher rate)

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

As each enzyme has a different category, each one should use a different co enzyme



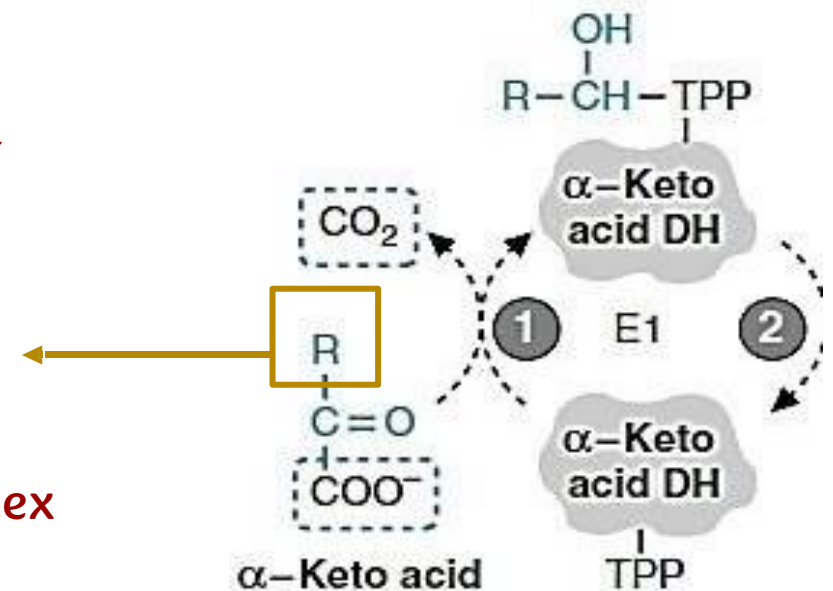
Why did we put an R group here not alpha ketogutarate ?

The enzyme is specific and it uses alpha ketoglutarate only. However, we have two other enzyme complexes in our body (α -ketoglutarate, pyruvate, and branched chain α - keto acid dehydrogenases) that uses different substrates (different R only) but they adopt the same the general mechanism, same product (CO_2 and NADH) -the third product differs- and the same corresponding molecule Co-A.

In case of pyruvate, we should place CH_3 instead of R, the complex turned it to acetyl CoA, CO_2 , and NADH .

In case of alpha-ketoglutarate we will place CH_2COO^- instead of R .

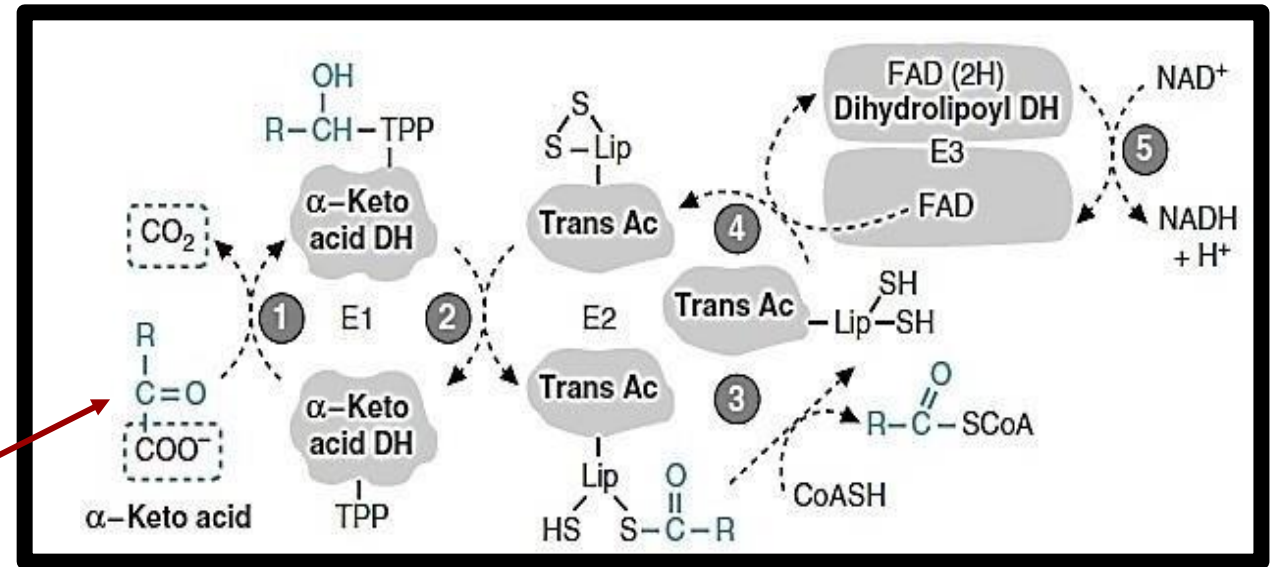
The third enzyme which functions in the same way is present in the amino acid metabolism that p's called the alpha-keto acid dehydrogenase that works on certain amino acids



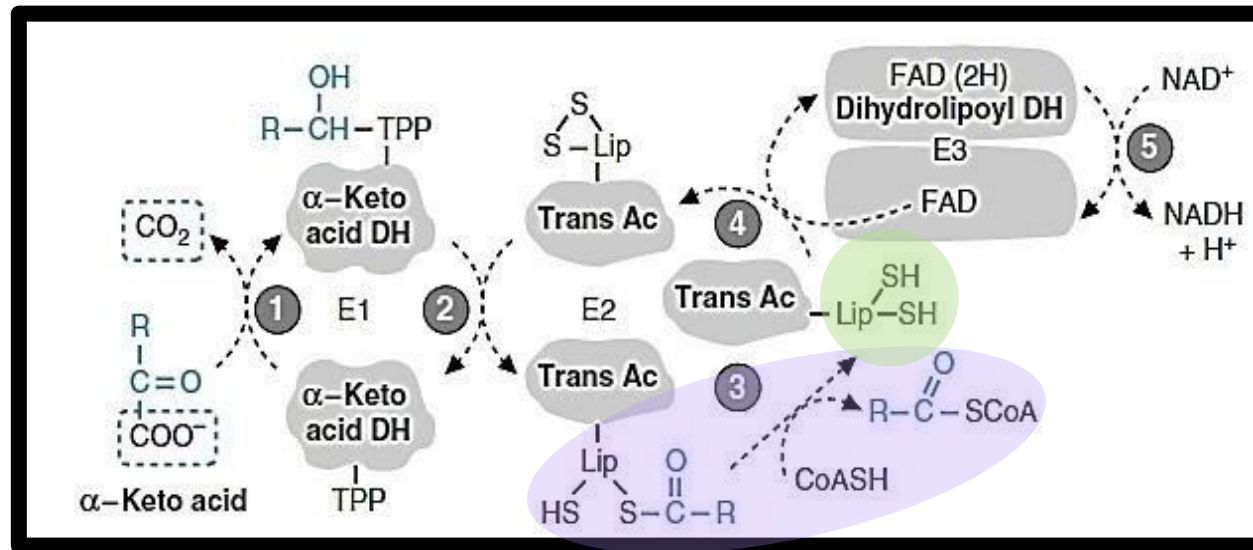
1) TPP (connected to the enzyme) attacks the carbonyl group in the structure of alpha-ketoglutarate, once it attacks the carbonyl group it weakens the bond between carbonyl and carboxylate

2) **CO₂** leaves the structure, and the TPP becomes attached to this carbon

Here TPP is attached to the structure and this is not the original form of the enzyme (it is 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**.



Here are the enzymes and their co-enzymes!
E1 -> decarboxylase -> TPP
E2 -> transacylase -> lipoate
E3 -> dehydrogenase -> FAD
We'll see their products after a few slides



3) 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.

4) One surfer is attached to the carbon (same one that had an arrow last slide) and the other one will abstract proton from the solution to become a thiol group (-SH). And still, this is not the original form of the enzyme.

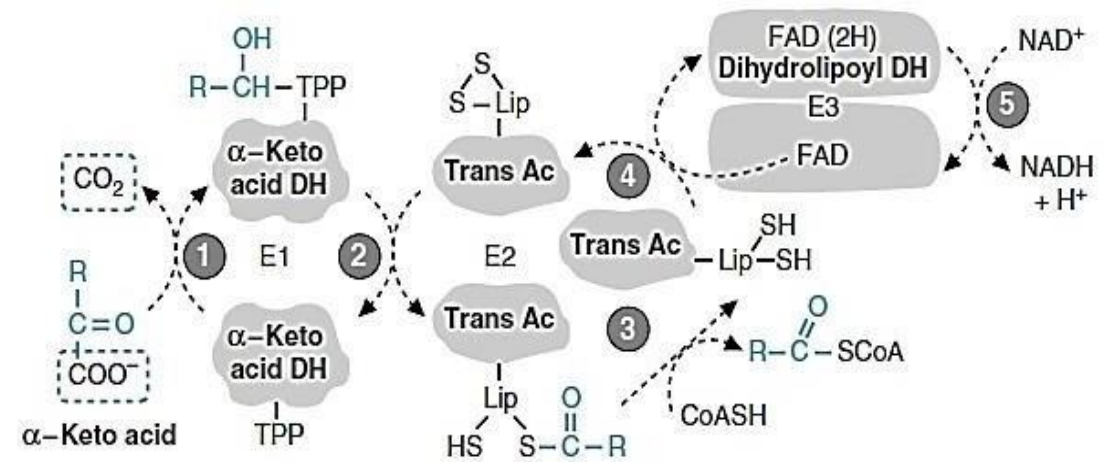
5) Those carbons will leave the sulfur to combine with coenzyme A and this is the **second product, which is Succinyl CoA**. (Step highlighted)

Notice, the enzyme isn't in its original shape still, the sulfur has hydrogens (Highlighted)

CO₂ left the reaction at the level of E1. SCoA left the reaction at the level of E2, leaving the sulfur behind. As we said Sulfur will abstract protons. Now we have 2 thiol groups. And this is not the original form of the enzyme, so we have to remove the hydrogens 👍

Removing hydrogen is a function of a dehydrogenase. Therefore, to restore the original oxidized form of the enzyme, E3 (dihydrolipoyl dehydrogenase) catalyzes the removal of hydrogen equivalents from the reduced E2-bound lipoamide.

The coenzyme FAD accepts the electrons and hydrogen, becoming FADH₂. This allows the two sulfur atoms in lipoamide to form their original disulfide bond (S-S) again.

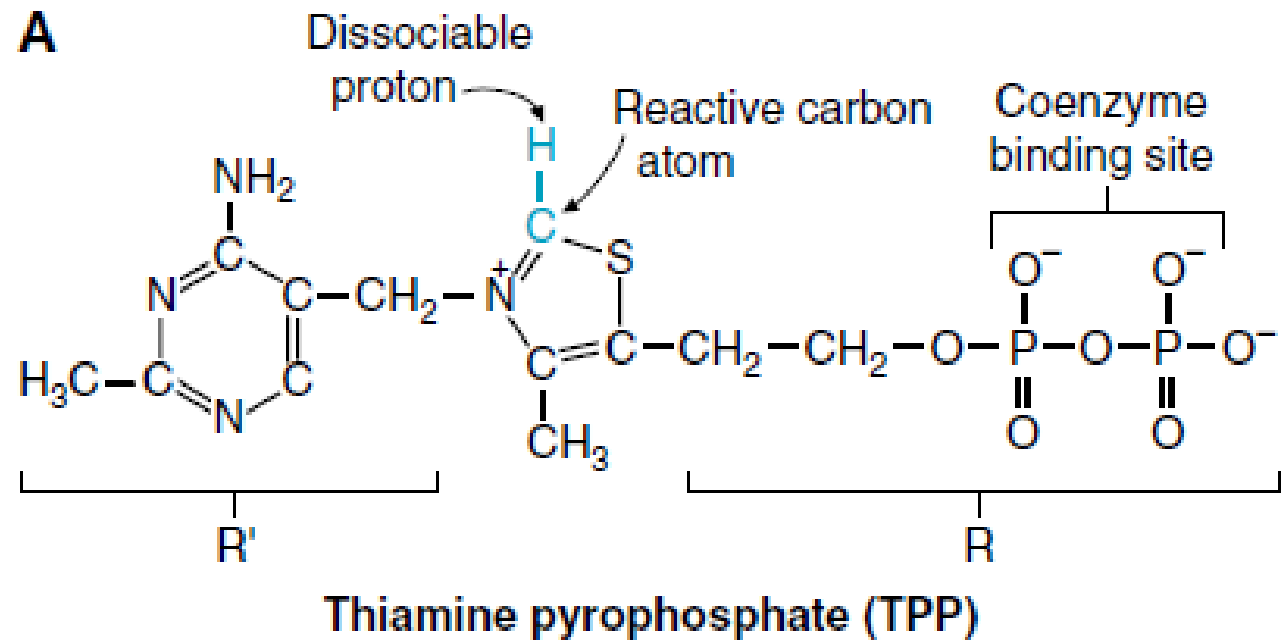
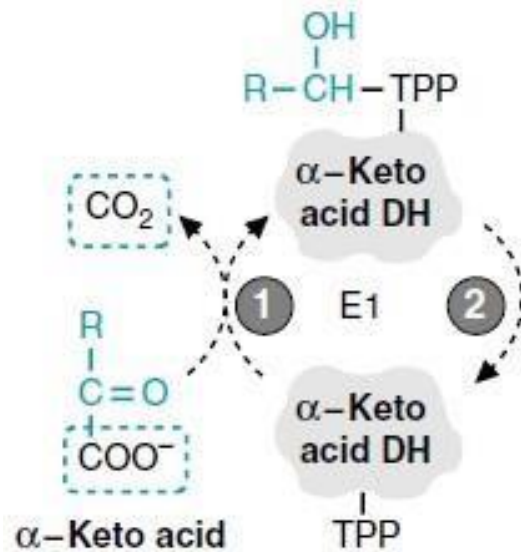


Now NAD will come to the picture to pick up these two electrons from and become NADH
Why not stay as FAD you ask?
Because FAD is kind of bound to E3, while NAD⁺ can enter and leave the enzyme.

There are 3 enzymes and 5 coenzymes involved in conversion of α -ketoglutarate to succinyl-CoA
E1: CO₂ is released.
E2: Succinyl-CoA is formed.
E3: NADH + H⁺ is produced.

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, that 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.

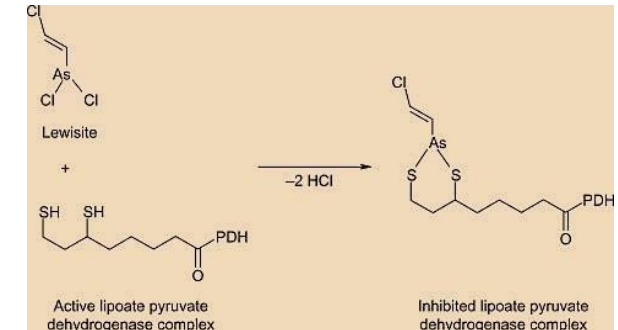
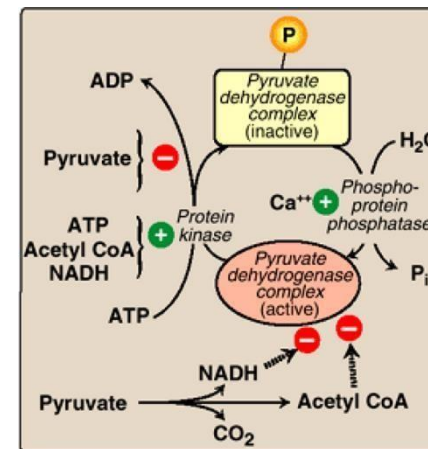
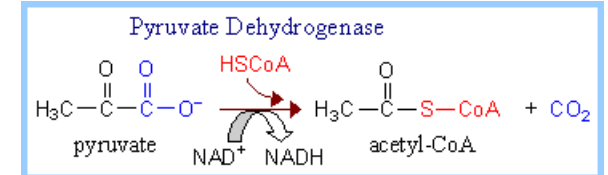
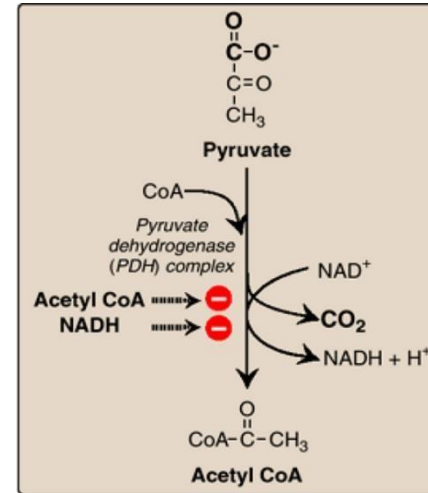
If there is a thiamine deficiency this enzyme won't work properly, and accordingly there will be high concentrations the substrate of the enzyme, which is either alpha-pyrogutarate or pyruvate in the blood.

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 E₁ 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 do? Arsenic (As) poisoning:

Arsenic breaks down the disulfide bridge in between the two sulfurs inside the lipoic acid, and it's bound irreversibly; 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.

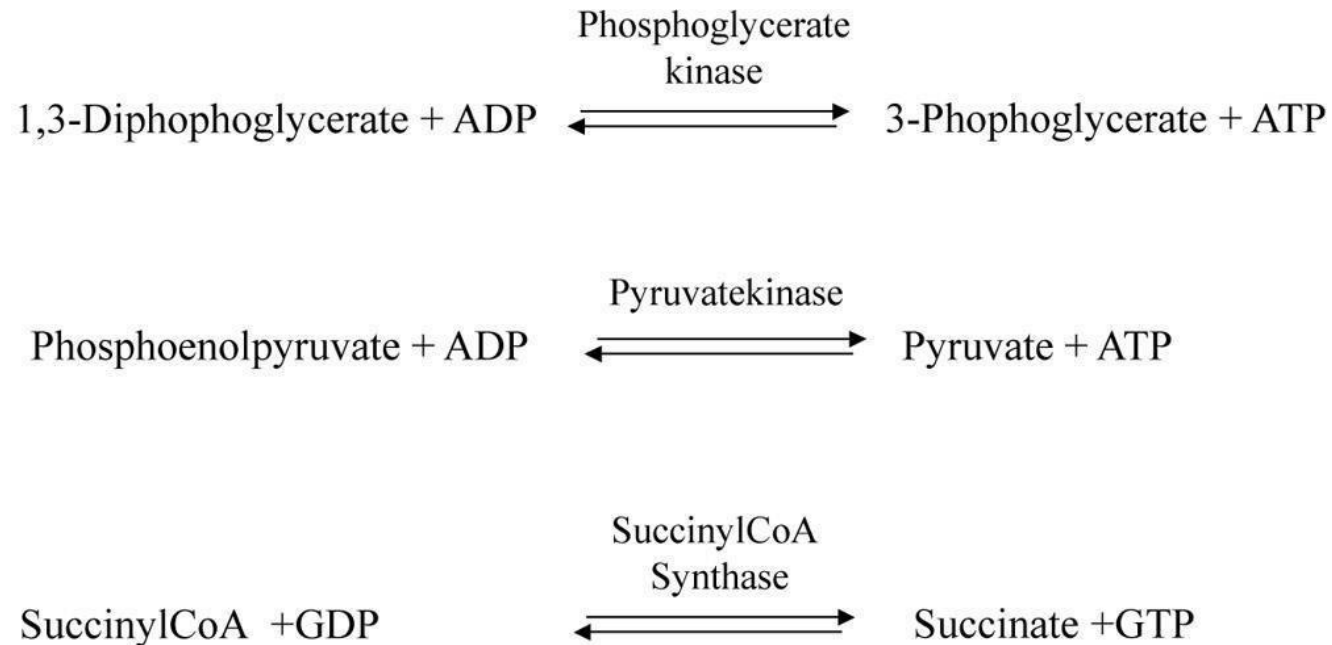
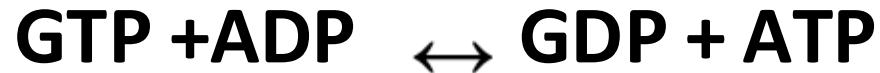
So depending on the concentrations of As provided it is fatal easily.

You rn

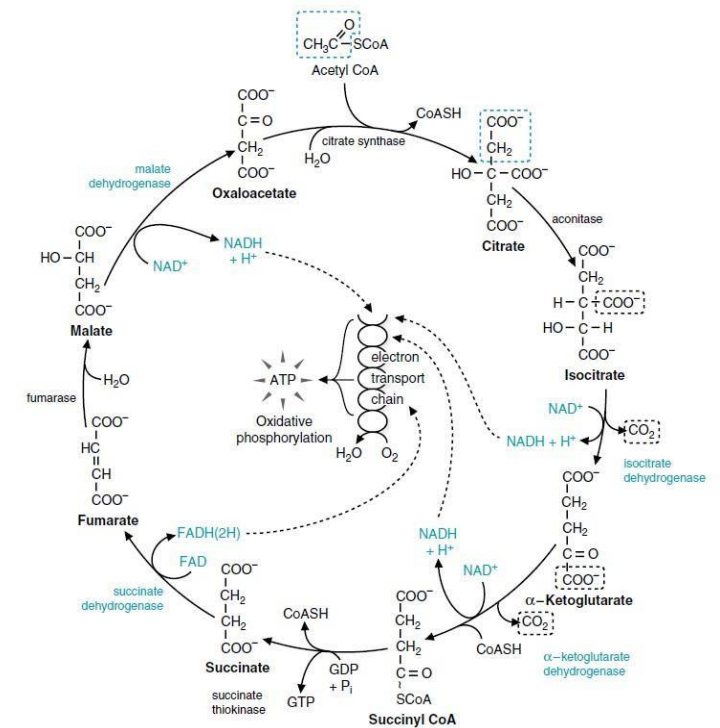


GENERATION OF GTP

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



The formation of ATP without the need of oxygen is called substrate level phosphorylation, We have three reactions that does this, one of which we have learned and two by glycolysis!

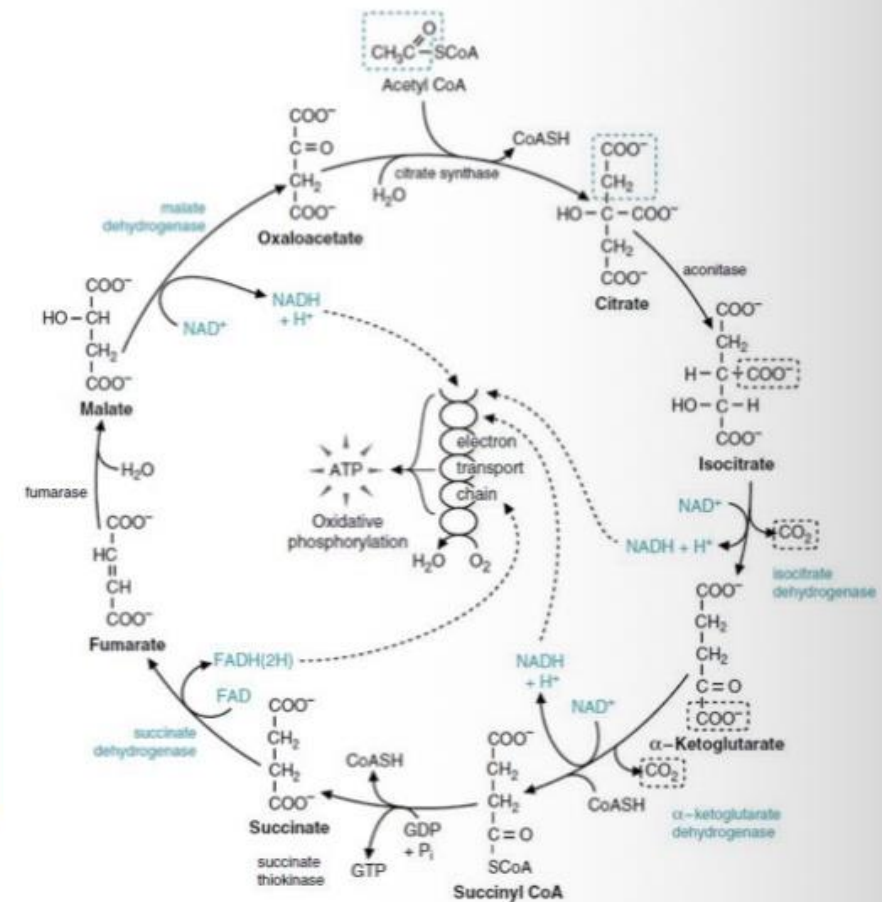


OXIDATION OF SUCCINATE TO OXALOACETATE

Oxidation of succinate to fumarate, succinate dehydrogenase, FAD

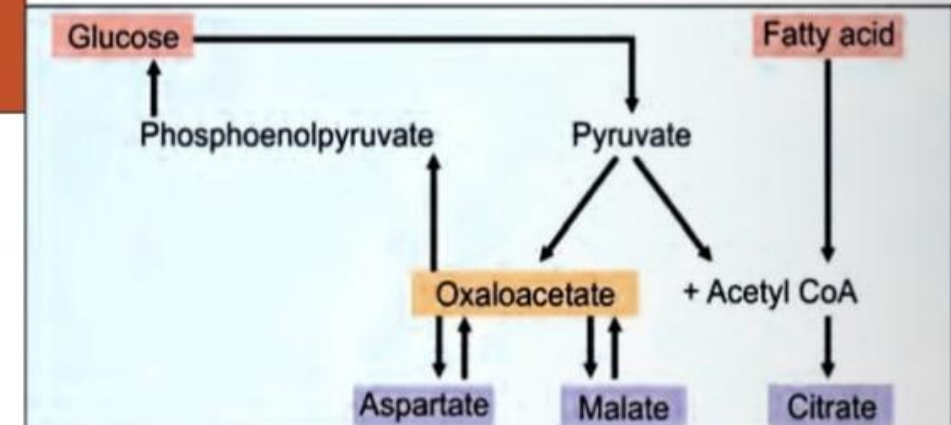
Fumarase, $\text{OH} + \text{H}^+$ from water, fumarate to malate

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

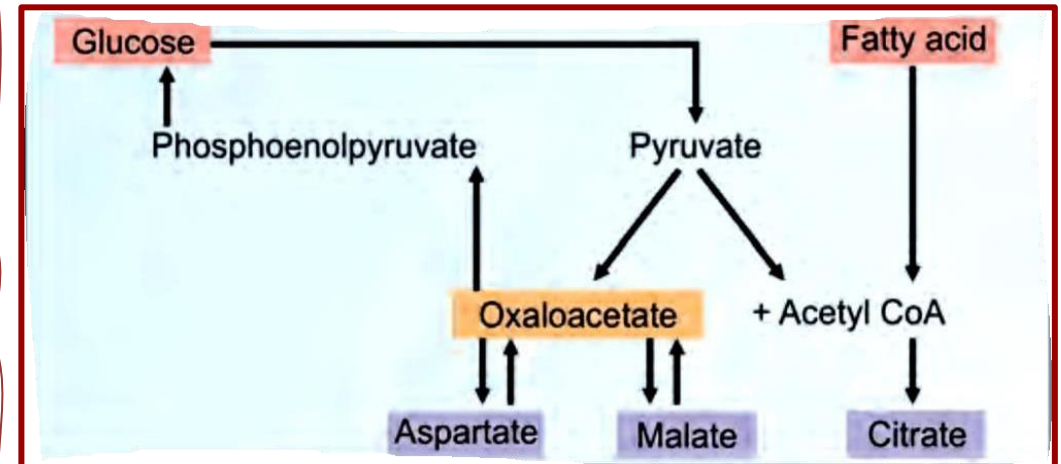


Oxaloacetate

- Although oxaloacetate is an intermediate, it plays an important role in metabolism, especially gluconeogenesis at low glucose state (while fasting or starvation).
- It cannot cross the inner mitochondrial membrane directly, so what we do is to convert it into malate, which can exit the mitochondria and be converted back into oxaloacetate in the cytoplasm. Oxaloacetate can then be converted into phosphoenolpyruvate (PEP) and eventually into glucose through gluconeogenesis (formation of glucose from non-carbohydrate sources).
- And by carboxylation of pyruvate (it'll become a four-carbon molecule = oxaloacetate)
- It can also be converted into amino acids such as aspartate.

Glucose is very essential. Why ?

Red blood cells depend entirely on glucose for energy because they do not have mitochondria. Therefore, they cannot use fatty acids to produce energy and rely on anaerobic glycolysis to generate ATP.



BIOENERGETICS OF TCA CYCLE

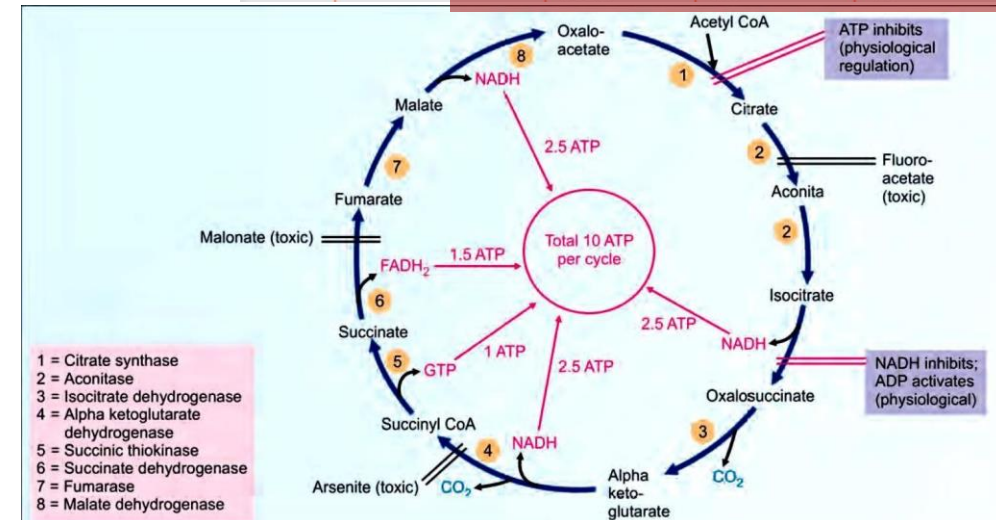
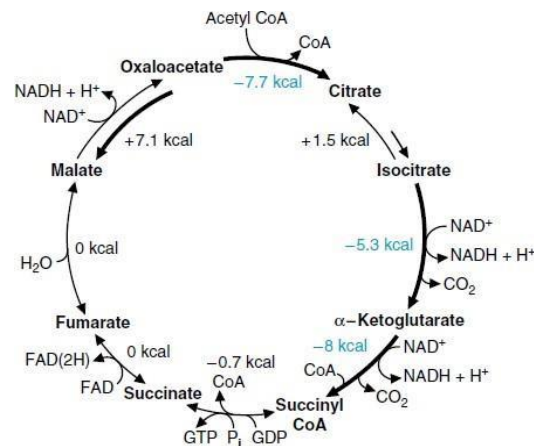
- Like all pathways, overall net $-\Delta G$ (-228 kcal/mole), not 100%
- NADH, FAD(H₂), and GTP (10ATP), 207 Kcal, 90%
- Three reactions have large (-ve) values
- Physiologically irreversible, low products

- O₂ is the last electron acceptor will turn to H₂O
- For every O₂ we have 4 hydrogen molecules and 4 electrons

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

kcal/mole

$$\begin{aligned}
 3 \text{ NADH: } & 3 \times 53 = 159 \\
 1 \text{ FAD(2H)} & = 41 \\
 1 \text{ GTP} & = 7 \\
 \text{Sum} & = 207
 \end{aligned}$$



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's machinery has the best efficiency in the world, nothing is compared to it neither physiological nor man-made

- The TCA cycle produces 3 NADH molecules, each one of them produces 53 kcal/mol also 1 FADH₂ produces 41 kcal/mol, and 1 GTP produces 7 kcal/mol. To sum up, they all produce 207 kcal/mol.
- If we burn out the acetate molecule that enters the cycle (not acetyl-coA) and use a calorie meter, 1 mole of it will give -228 kcal/mol. Since we are getting 207 kcal, dividing them gives about 90% efficiency !

سبحان الله

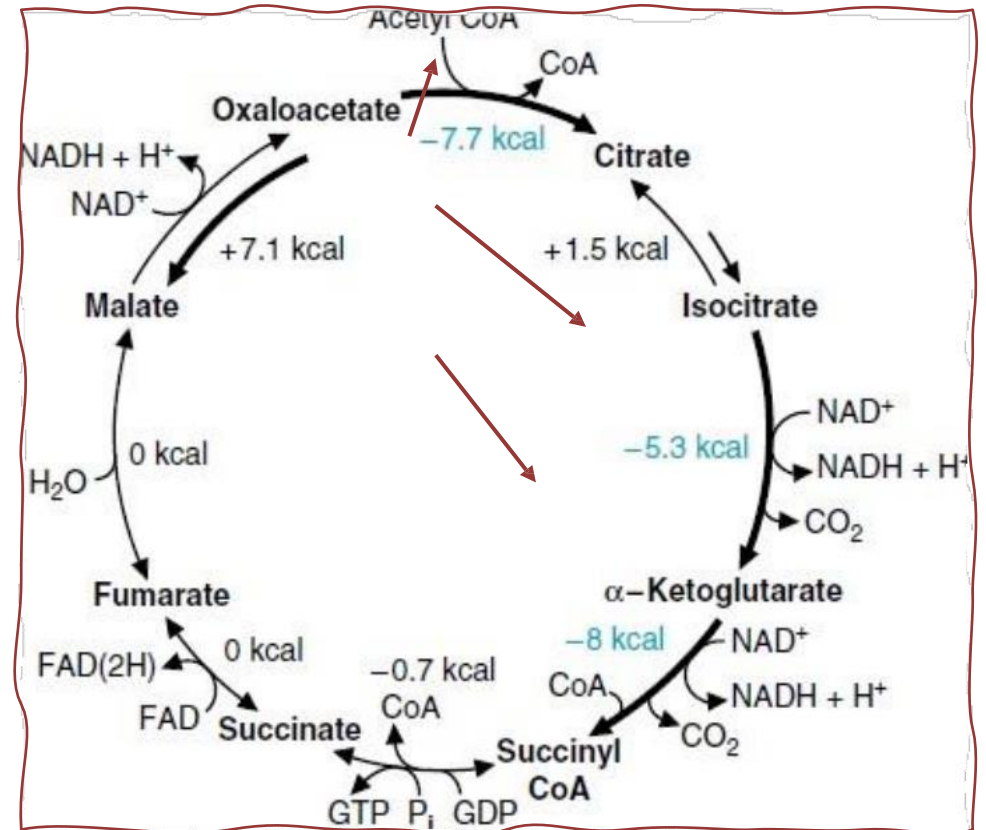
Values Already Calculated
from equation in the
previous chapter

kcal/mole

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



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.

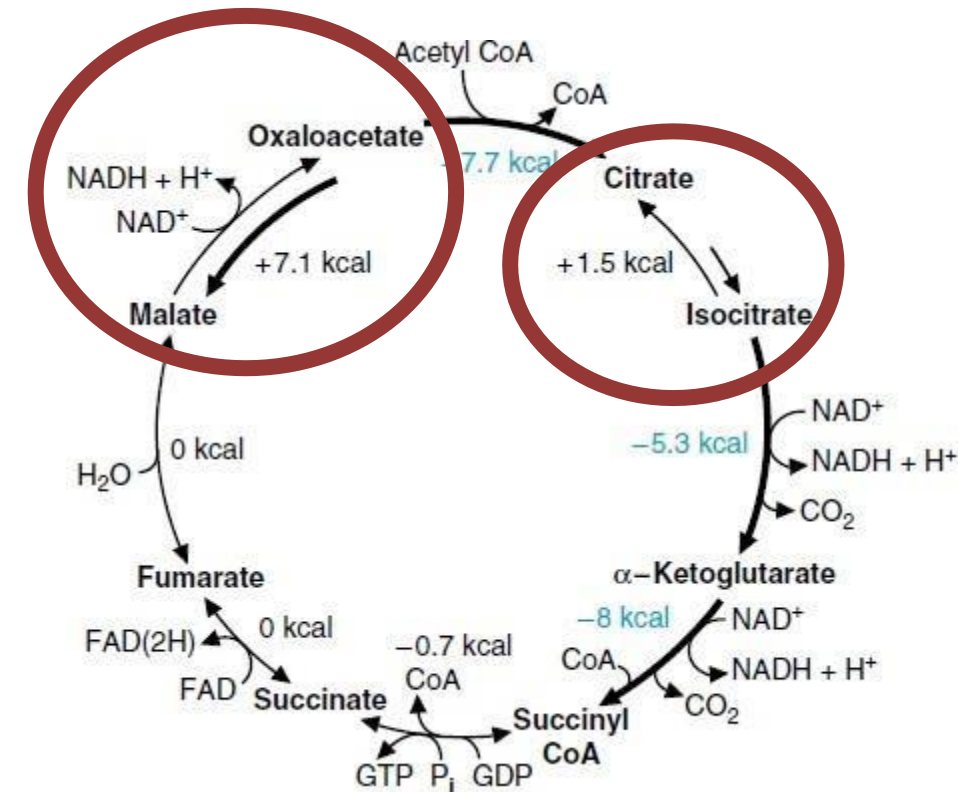


Meaning of the arrows

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	}	→	}	2 CO ₂ + CoA-SH
Oxaloacetate				Oxaloacetate
FAD				FADH ₂
3NAD ⁺				3 NADH
GDP + PI				
GTP				



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 CO₂.

- 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. But I can't synthesize carbohydrate from fats, because the energy difference between acetyl-CoA & pyruvate is very high.

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.

I can't burn fat, so when I'm out of oxaloacetate, acetyl-CoA won't have it nearby, so it will go towards the synthesis of ketone bodies (we will dive in this mechanism in lipid metabolism) and my main dependence of energy will be on ketone bodies, & the work of TCA cycle will decline. This mechanism takes place in the bodies of diabetic patients whom their cells don't have enough sugar, and the ones whom are on keto diet (they cut sugar out of their food intake)

- Low sugar → low insulin → high glucagon

Because dietary glucose is scarce, the liver must rely on gluconeogenesis (making glucose from non-carbohydrate sources) and ketogenesis (making ketones from fat) to survive



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.

Additional resources:

Reference Used:

Prof . Nafez Abutarbosh's recorded lecture



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Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	Slide 19	Why not stay as NAD	Why not stay as FAD
V1 → V2			