Medical Committee
The University of Jordan

SLIDE SHEET

LECTURE#: 9

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Biochemistry

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(Kreb's, Citric Acid, TCA) Cycle

We are talking today about the third stage of energy production which is **Kreb's Cycle;** after degradation of the different macromolecules into smaller monomers in the second stage of energy production to give the common molecule which is named **Acetyl-CoA** and it will get into **Kreb's Cycle**.

*__Remember:__ Macromolecules in the first stage of energy production will be degraded within the GI tract, absorbed into the body stream then reach the cells. In the cells the second stage of energy production will start to different metabolic pathways; Carbohydrates, Proteins and lipids will be degraded to give the final common molecule ,which is the conversion point for different metabolic pathways, **Acetyl-CoA;** e.g: it could come from pyruvate or from beta-oxidation of fatty acids....etc.

- It has different names: **Kreb's** cycle, **Citric acid** cycle and **Tricarboxylic acid** cycle.

<u>Why is it named "Kreb's Cycle"</u>?

Sir Hans Adolf Krebs, a German biochemist, is who discovered it.

<u>Why is it called "Citric acid cycle"</u>?

Because the product of the first reaction is Citrate (citric acid).

- If you want to name it as "Citric acid cycle", then citric acid should be in the cycle. and if you want to name a cycle, logically you will name it according to the first molecule or the last molecule, not a molecule in the middle unless it was unique to the present.

<u>Why is it called "Tricarboxylic acid cycle" TCA</u>?

Because within the citric acid molecule there are three carboxylic groups.

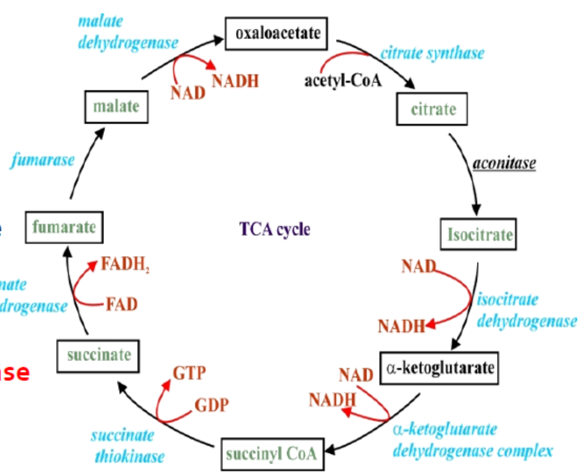
Electron (energy)-carrying molecules: NAD⁺ & FAD

Differences between NAD^+ and FAD:

NAD^+	FAD
- Accepts two electrons in a form of hydride ion H^- (one source).	- Accepts two electrons in a form of two hydrogen atoms (two sources).
- Accepts the two electrons together from one source as one unit; they are opposite to each other in the spin, so it won't get into the free radical state.	- Accepts the two electrons from two different sources in a sequential manner so it will get into the FADH before becoming $FADH_2$; and FADH is a free radical state.
- Can be found free in the solutions, come bound and released.	- Bound to proteins because it can be found in the free radical state. (Protection)
- Transfer of electrons from NADH to oxygen (forming water) will give a **higher** energy than from $FADH_2$.	- Transfer of electrons from $FADH_2$ to oxygen (forming water) will give a **lower** energy than from NADH.
- (NAD^+, NADH) redox couple has a fixed number of reduction potential that equals -320 millivolts.	- The reduction potential of Flavins is not known, it is known in reference to the protein to which the Flavin is bound. (FAD can be bound to different proteins), this is applicable to any coenzyme that is bound to different proteins.

Kreb's cycle enzymes: it has 8 enzymes, mostly named by the substrate name followed by the reaction type.

- Citrate cynthase
- Aconitase
- Isocitrate dehydrogenase
- α-ketoglutarate dehydrogenase
- Succinate thiokinase
- Succinate dehydrogenase
- Fumarase
- Malate dehydrogenase



What happens through Kreb's cycle?

The common conversion point for different metabolic pathways is **Acetyl-CoA** which is introduced into the **Kreb's cycle**. You have to know the **eight** compounds, the **eight** enzymes, reactants and products of the steps, effectors, activators and everything; we will take this in these two lectures. The food processes are oxidation processes, if you are breaking down molecules then you are oxidizing them to get off energy.

- A cyclic metabolic pathway means that at the end of the pathway the first material started with will be regenerated, which is **Oxaloacetate** (four-carbon molecule). At the beginning **Acetyl-CoA** (two-carbon molecule) is introduced to the cycle and it attacks **Oxaloacetate** to produce **Citrate** (six-carbon molecule) combining the two molecules together with the need of energy for the **ligation** process, this energy comes when CoA leaves (Any molecule attached to CoA has a high energy). The enzyme that catalyzes this step is named as **Citrate Synthase**.

*** In the first half of the cycle two carbon atoms will be detached to convert the six-carbon molecule to a four-carbon molecule. In the next half of the cycle, the four-carbon molecule will be reformulated to be similar to the beginning molecule which is Oxaloacetate. In the first half there are four reactions and in the second half there are four reactions.**

- In the citric acid cycle, acetate molecule is broken down to give off energy so it is an oxidation process. Can citrate be oxidized or not? **Citrate** is a tertiary alcohol so it can't be oxidized, so **citrate** is reformulated to another compound which is called **Isocitrate** by moving the hydroxyl group from carbon num **#3** to carbon num **#2** so it can be oxidized to a ketone. This is the second step of Kreb's cycle, it is catalyzed by an enzyme named as **aconitase** (it is the only enzyme name that is not related to its function), it is named so because there is an intermediate in the conversion from **citrate** to **isocitrate** called **aconitate**.

* Note:

Oxidation of primary alcohols → aldehydes

Oxidation of secondary alcohols → ketones

Tertiary alcohols can't be oxidized

There are two reactions left in the first half, everyone of each will detach one carbon unit to convert the six-carbon molecule to a four-carbon molecule. The carbons leave as CO_2 which represents the carboxylic groups.

- The next reaction is to convert **Isocitrate** to a five-carbon molecule, it is an oxidation process, because **Isocitrate** is a secondary alcohol, it is oxidized to a ketone with removal of two hydrogen atoms forming a double bond between the carbon and the Oxygen, also one carbon will leave so it is named **alpha-ketoglutarate** (ketone group with five carbon units). Hydrogens leave with their electrons so they are load on an electron-carrying molecule, which is **NAD^+** that will accept a hydride ion with two electrons and the remaining H^+ will leave to the solution, and here is where the first NADH molecule is produced. The enzyme that catalyzes the conversion of **Isocitrate** to alpha-**ketoglutarate** is named as the substrate and the type of the reaction **Isocitrate dehydrogenase**.

- The last reaction of the first half is the same story to the previous one, CO_2 will leave as a carbon unit, two electrons will be extracted out in the hydride ion form and the second hydrogen will leave as H^+ to produce **Succinyl CoA**, the two electrons will be loaded on NAD^+ to produce NADH and here is where the second NADH molecule is produced. The enzyme that catalyzes the conversion of **alpha-ketoglutarate** to the four-carbon molecule **Succinyl CoA** is known as **alpha-ketoglutarate dehydrogenase**.

* Anywhere you see a **dehydrogenase** enzyme, then there will be an electron-carrying molecule to take out the electrons with their hydrogens. When the carbon unit is removed from **alpha-ketoglutarate** to convert it to the four-carbon molecule, the carbon that it was attached to, will be reactive and it will get attached to Coenzyme A **(CoA)** so the whole compound will be named **Succinyl CoA**.

Now to make the four-carbon molecule that is attached to CoA similar to Oxaloacetate we will go through four different reactions

- The first one is the **removal of CoA,** removing CoA will result in energy as what happened in the first reaction (the energy was used to combine oxaloacetate with acetyl),but in this reaction the excess energy will be used to make **GTP** or you will find it in some textbooks as **ATP**; (When GTP is produced it is converted to ATP so it doesn't make a difference to say GTP or ATP; they have the same energy value), phosphate group is introduced on GDP directly, this reaction in the body involves production of high energy value molecules without the need of Oxygen and this type of reactions is called **Substrate level Phosphorylation**, because the high energy molecules are phosphorylated without the need of Oxygen, few reactions in the body belong to this, we will take three one of them, one is in Kreb's Cycle and the other two are in the carbohydrate metabolism.

* ATP is mostly produced by **oxidative phosphorylation** with the need of Oxygen in the electron transport chain at complex four that is converted to water to maintain a difference in the electronegativity of the membrane to generate ATP.

The product of this step of reaction is **Succinate** and the enzyme that catalyzes this step is named Succinate thiokinase; it is named thio because it deals with the thiol group of CoA and it is named Kinase because it transfers a phosphate group.

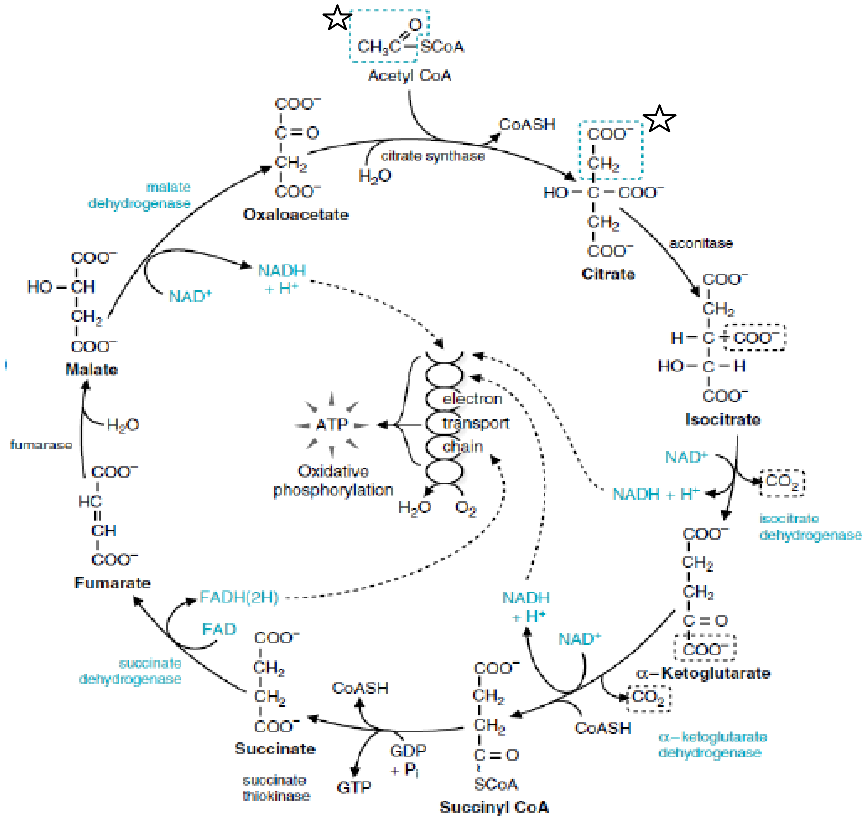
* *Kinases are a subgroup of transferases.*

Now we have a four-carbon molecule with same structures between the succinates; two carboxylic groups and two CH_2 groups. Oxaloacetate has two carboxylic groups, one CH_2 and one ketone group so it is called ketoacid. In order to convert **Succinate** to **Oxaloacetate**, CH_2 should be converted to a ketone group by converting it firstly to alcohol and this is done through:

- Oxidation of the carbon at the beginning where two hydrogens are removed and a double bond is formed between the two carbons; the product of this reaction is **Fumarate** and the enzyme that catalyzes it is called **Succinate dehydrogenase**. The two hydrogens removed with their electrons are used to make **$FADH_2$**.

- Now we have reached **Fumarate** which has a double bond between the two carbons, so water should be added to break down the double bond; OH is added to one carbon and hydrogen to the other one to produce alcohol which is called **Malate**, the type of enzyme that catalyzes this reaction is **hydratase** (adds water) and is named **Fumarase** in relation to the substrate.

- **Malate** has the hydroxyl group on carbon Num **#2**, it can be oxidized now by removing the hydrogen in the hydroxyl group and the hydrogen that is attached to the carbon to make a double bond between the carbon and the oxygen creating a ketone which is **Oxaloacetate**. The enzyme that catalyzes this reaction is called **malate dehydrogenase**. Again the two electrons are removed in the form of hydride ion and will be loaded on NAD^+ , the remaining H^+ will leave to the solution.



The two blue-dashed carbons come from **Acetyl-CoA**, we realize that the two carbons that leave the cycle as CO_2 are not the ones that come from Acetyl-CoA in the same cycle, the carbons that leave are from acetate, so the two added carbons by Acety-CoA will not leave in the same cycle but in the next one.

How many dehyrogenases are there in the citric acid cycle? Four.
How many electron-carrying molecules are produced in citric acid cycle? Four; three of them produce NADH; they are **Isocitrate dehydrogenase**, **alpha-ketoglutarate dehydrogenase** and **malate dehydrogenase**, while one produces $FADH_2$ which is **Succinate dehydrogenase**.

NAD^+ will bind to the first three enzymes and it will get the electrons and then it will be release to the solution to go to the electron transport chain. FAD when it accepts the electrons from the conversion of **succinate** to **Fumarate**, it will become $FADH_2$. Flavins are bound to proteins, when they take the electrons they will detach to the solutions and swim to reach the electron transport chain, but in this case FAD is bound to **succinate dehydrogenase** enzyme and $FADH_2$ is formed as a result of converting succinate into fumarate, but $FADH_2$ cannot detach the succinate dehydrogenase enzyme to reach the electron transport chain, so what will happen? The answer is: **succinate dehydrogenase** is a part of the electron transport chain and it is called **Complex 2** which is located in the inner membrane of mitochondria. So the electrons taken out from succinate to convert it so Fumarate, will be donated directly to the electron transport chain; imagine the Kreb's cycle hanged inside the mitochondria and the electron transport chain in the inner membrane, what hangs it? Succinate dehydrogenase.

How many NADH molecules are produced by the cycle?
Three, they have a fixed reduction value.
How many $FADH_2$ molecules will be produced by the cycle?
One, it has a fixed reduction value because it is bound to a known enzyme.
How many kilocalories we get out from NADH when its electrons move in the electron transport chain toward Oxygen? 53 kilocalories.
How many kilocalories are produced by $FADH_2$ when its electrons move in the electron transport chain toward Oxygen? 41 kilocalories.

Total amount of calories produced by Kreb's cycle can be calculated by

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

So we get 207 kilocaloris per each citrate molecule that begins the cycle. To know the efficiency of Kreb's cycle, we compare the real outcome with the expected one.
The real outcome of kreb's cycle is 207 kilocalories per mole of citrate while the expected outcome is 228, so the efficiency is around 90% ; citric acid cycle is one of the best machines in this world, there is no machine in the world that gives this high efficiency.

* The expected outcome is calculated by bringing the material and putting it in a combustion chamber then burning it while covering it -to prevent any loss of energy- then calculating how many calories were produced.

Wish you all the best :)