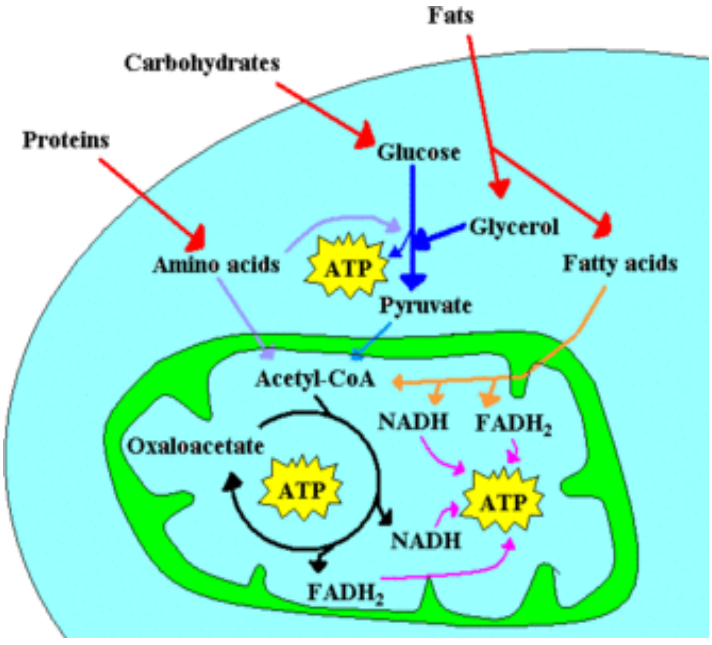
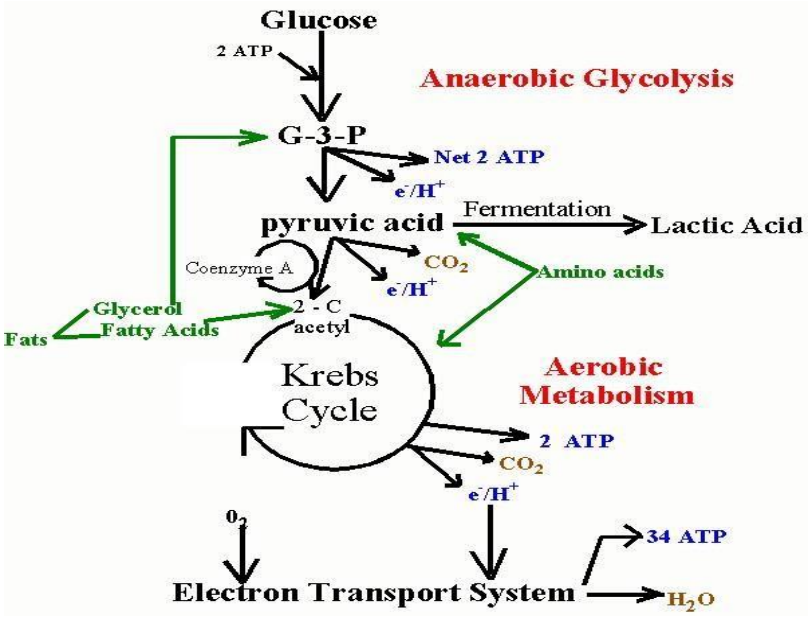
(Kreb's, Citric Acid, TCA) Cycle

Krebs cycle is the third stage in energy production, it was named Krebs cycle after the German scientist Hans Krebs who had discovered it, it is also called citric acid cycle because citric acid is the first molecule in that cycle, and tricarboxylic acid cycle because it happens that citric acid which is the first molecule produced (the first intermediate) in that cycle has three carboxylic groups in its a structure.

How does it fit?

What was mentioned in the slide:





What was mentioned in the lecture:

where is it located? as the third stage in energy metabolism, after all molecules produce a common molecule called acetyl-CoA, this acetyl-CoA well enter Krebs cycle inside the matrix of the mitochondria where Krebs cycle is active.

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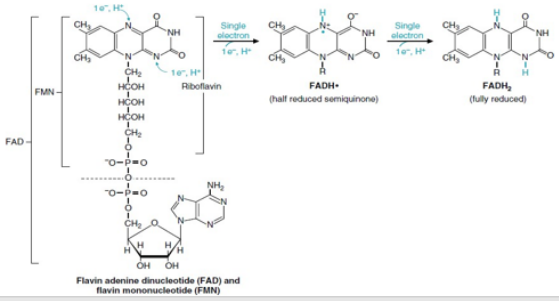
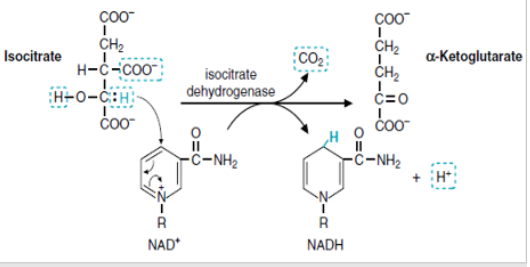
Electron (energy) Carrying Molecules (NAD+, FAD)

What was mentioned in the slide:

- **FAD**
  - Single electrons (H•), different sources
  - Succinate to fumarate, lipoate to lipoate disulfide in α-KG
  - FAD must remain tightly, sometimes covalently, attached to its enzyme
  - *E*° for enzyme-bound FAD varies
- **NAD**
  - Pair of electrons (H-), same source
  - Alcohols to ketones by malate dehydrogenase & isocitrate dehydrogenase
  - NADH plays a regulatory role in balancing energy metabolism





What was mentioned in the lecture:

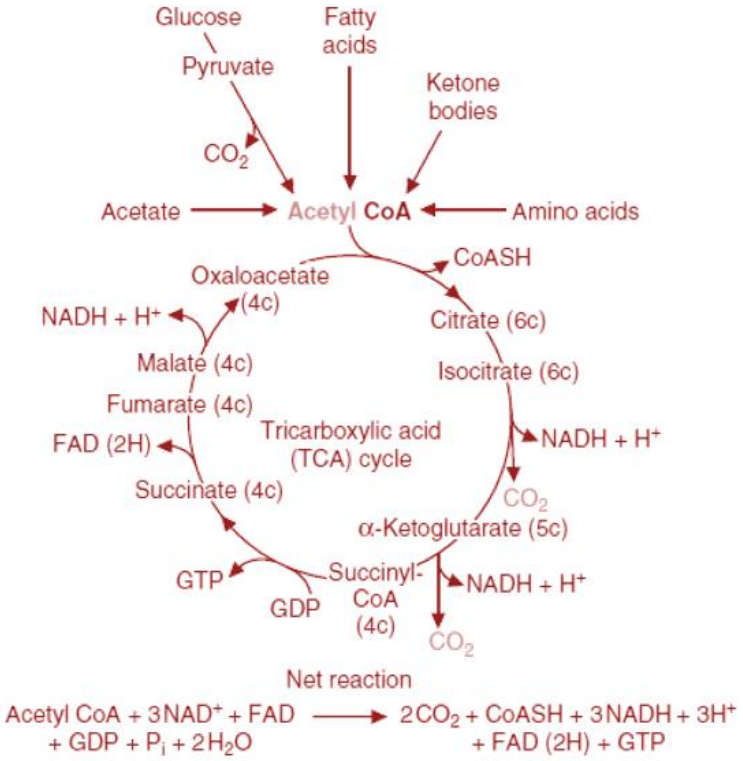
Those are the differences in between FAD and NAD^+ , we've discussed that last semester, we've discussed that in the previous lecture, please know the differences in between them, for FAD there is a companion molecule called FMN, in the case of NAD^+ there is a companion molecule called $NADP^+$ FAD and FMN are doing the same job, and NAD^+ and $NADP^+$ are doing the same job, but each one in a place in the cell for organization purposes.

Components & stepwise reactions

What was mentioned in the slide:

CIA Sent Soldiers For My Office

- **No O_2 introduced, two CO_2 exits**

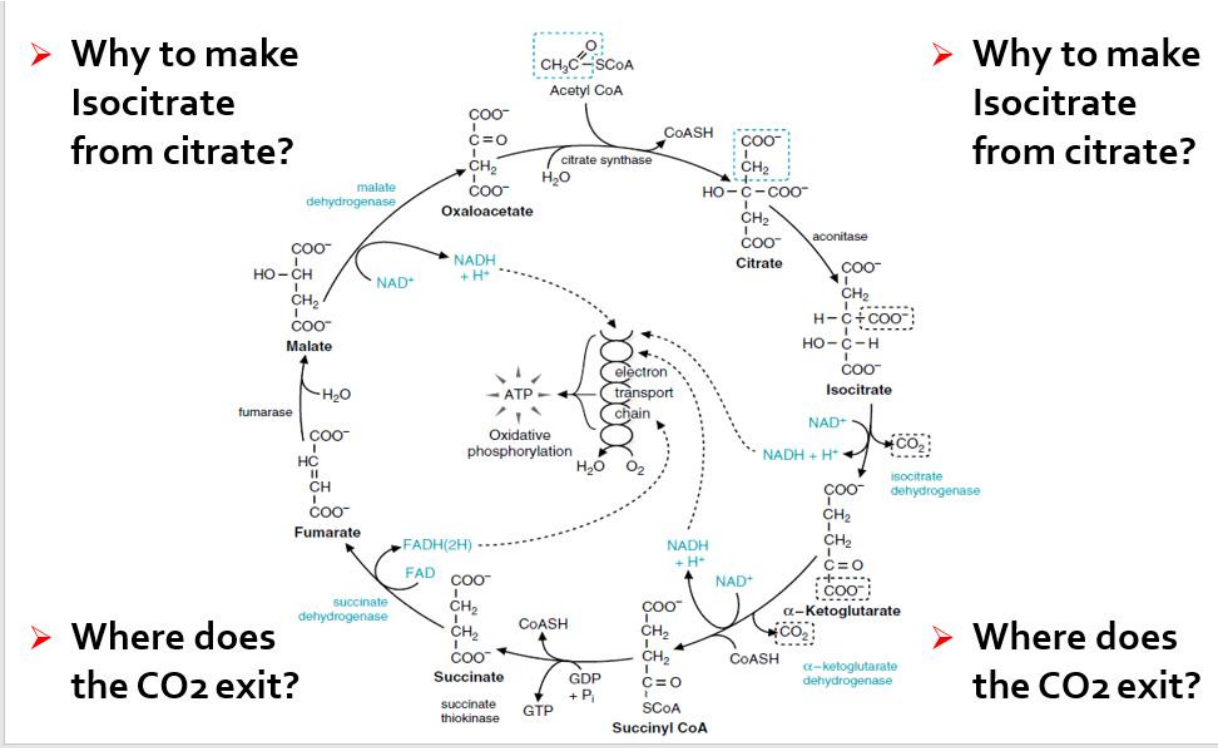


What was mentioned in the lecture:

it starts as all molecules are giving you a common molecule, this molecule is acetyl CoA, acetyl CoA enters the cycle, it is the outside molecule that enters the cycle, and by definition, Krebs cycle is a cyclic pathway, meaning that the end product must me the same as the molecule we've started with, that means logically, as long as you are introducing two carbon atoms as you can see, acetyl-CoA is introduced to the cycle where CoA does not get to the cycle, it leaves, and the acetate part (the two - carbon unit) gets to the cycle, so logically there should be elimination of those two carbons outside of the cycle, otherwise it cannot be named a cycle.

Does Acetyl-CoA exit as 2 CO_2?

What was mentioned in the slide:



What was mentioned in the lecture:

Krebs cycle is an eight-reaction pathway, with eight steps, eight enzymes and eight intermediates within the cycle, you must know the names of the intermediates, the chemical structure of the Intermediates, the enzymes for each step and the products for each step, so everything is required from you.

the thing that is introduced to the cycle is acetate, it's a two carbon unit molecule, it will join oxaloacetate which is a 4-carbon molecule with two carboxyl groups and a ketone group, therefore it is a ketoacid, this joining will result in a 6-carbon molecules which is the first intermediate in the cycle which is citrate.

Krebs cycle is a degradative exergonic pathway, because acetate that enters it leaves as two CO_2 molecules, (remember: oxidative pathways are degradative (catabolic) pathways, and reductive pathways are building up (anabolic) pathways) .

when you look at any molecule to know if it can be oxidized or not, you should look at the functional groups, if we look at the functional groups of citrate, the functional groups in the molecule are either carboxylic groups which are the highest oxidation state and cannot be oxidized and it has an alcoholic group, the alcoholic group can be oxidized but look at this one in citrate, it's bound to a carbon which is bound to three carbons, so it's a tertiary alcohol which cannot be oxidized (tawjihi), and because the concept of the citric acid cycle is an oxidation process (it's a degradative process), then citrate has to be modified, and the simple modification is to make an isomerization reaction.

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so in the first reaction we built up acetate with oxaloacetate to make citrate, the enzyme employed is citrate synthase and its name simply implies the action, it is the enzyme which synthesizing citrate and citrate cannot be oxidized so it has to be converted to another molecule called isocitrate (an isomer for citrate, the number of carbons is not different but it is a secondary alcohol), the name of the enzyme which converts citrate to isocitrate does not imply the action of that enzyme, it's called aconitase, it is called like that because when citrate is converted to isocitrate it produces an intermediate on the way called aconitate, this is why the enzyme was named aconitase.

To be able as to understand the citric acid cycle, you have to split it into two halves: the first half of the cycle engages itself in making the six carbon unit molecule into the four carbon unit molecule again where the second half of the cycle engages itself in reformulation of the four carbon unit molecule to get it back to its original shape.

in the first half, we have four reactions, the first reaction produces citrate, the second one is the isomerization process, we have two reactions left, each reaction should give us one carbon dioxide molecules, which was on the chemical structure as carboxylic group.
In this consecutive reaction CO_2 will leave, the carboxylic group will leave as CO_2, and why did we convert citrate to isocitrate? To oxidize it, we want to oxidize this carbon (the one bearing -OH group in isocitrate) how to oxidize it? we will remove a hydrogen from it and a hydrogen form the hydroxyl group and a double bond will be in between the carbon and the oxygen, so far, one carbon left, so we converted the six carbon unit molecule into a five carbon unit molecule explained by the name “gluta” and it has a keto group so we call it ketoglutarate ‘alpha ketoglutarate’ , then electrons will be loaded on NAD^+ and becomes NADH molecule, it is the first NADH molecule to be produced, regarding the enzyme, this reaction impulse decarboxylation and dehydrogenation, so the enzyme is called isocitrate dehydrogenase and because it has more than one function we call isocitrate dehydrogenase complex.

the same exact reaction occurs on the following step which is oxidative decarboxylation reaction, and the enzyme name is alpha ketoglutarate dehydrogenase complex where another NADH will be produced and another CO_2 will leave. because the carboxylic group that leaves as CO_2 in this reaction is peripheral that results in a carbonyl group that is peripheral, we leave the carbonyl group as a peripheral group and it's very reactive in that case, it cannot stay that way, so it will join coenzyme A to make it stable and for coenzyme A -as we've studied previously- it will take it from a place to a place, now coenzyme will join the molecule producing a four carbon unit molecule called succinate, so succinate joining coenzyme, so succinyl CoA, this is the first half of the cycle.

these are the first four reactions, we’ll will continue with the other four reactions, the molecule that I have until now is succinyl-CoA, the first thing that succinyl CoA must do to mimic oxaloacetate is to lose the CoA, when coenzyme a will leave it will produce energy, and that energy will be utilized to couple inorganic phosphate with GDP to produce GTP as an energy molecule , you will find it in some textbooks as ATP because the amount of energy in ATP is exact to GTP, CTP, UTP etc. all of these molecules have a similar amount of energy due to the loss or the gain of the first phosphate, so you'll find it in some textbooks as ATP, but the exact molecule which is produced is GTP, so that energy will be utilized to produce GTP without the need for oxygen, we call these reactions substrate level phosphorylation, we are phosphorylating the substrate directly without the need for oxygen and outside of the oxidative phosphorylation process, there are three reactions inside the body that adopts this mechanism: two in the glycolysis process this is the third one.
so coenzyme a will leave, GTP will be produced and now the four carbon units are succinate, the enzyme which converts succinyl CoA to succinate has more than one name in different textbooks, the name which we adopt is succinate thiokinase because it involves the substrate and the product which is succinate , “thio” it tells you that there is coenzyme A, and kinase it makes you understand that there is a transfer of phosphate, (addition of phosphate to GDP to become GTP).

Comparing the structure of succinate with that of oxaloacetate, the difference is that oxaloacetate bears a ketone group. how to produce a keto group from an alkane in the middle ? we have to lose the two hydrogens and double bond a carbon to an oxygen, so we will lose these two hydrogens (one from each atom) then a double bond will be generated so succinate will be fumarate, and the enzyme which catalyzes this process is called succinate “as the substrate” and what it does it is that it takes hydrogens out and because they are from different sources and because the energy limits, electrons will move to FAD not NAD^+ and will become $FADH_2$, FAD is not swimming inside the solution, it is hidden inside the enzyme which is called succinate dehydrogenase.

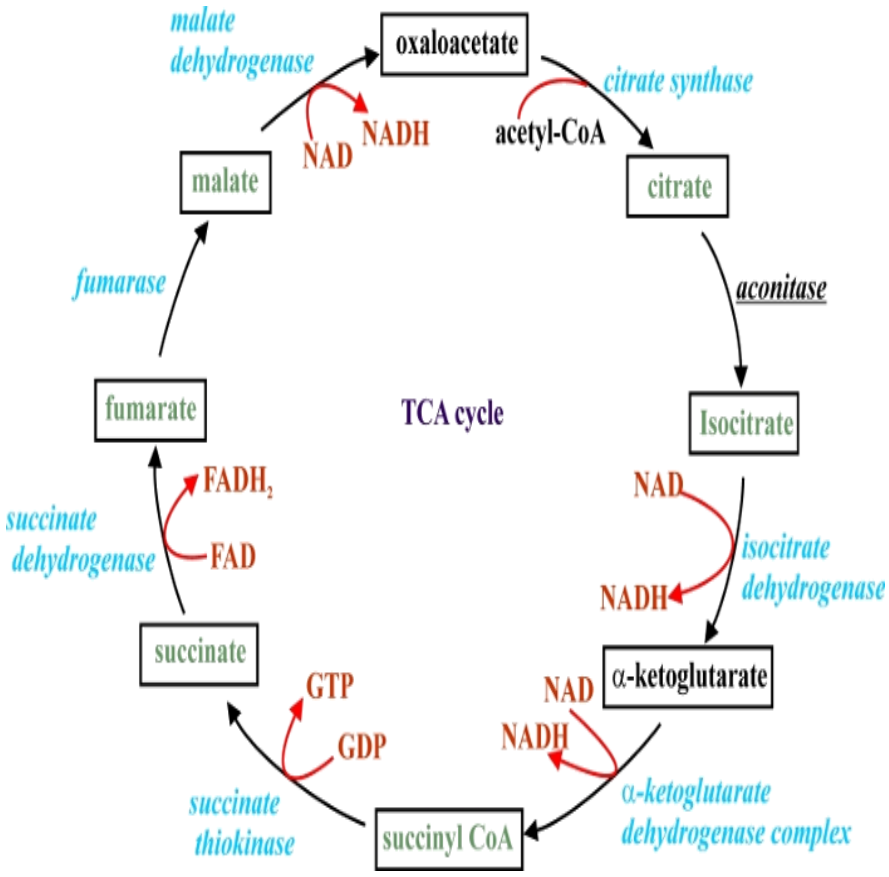
Now we have a molecule that has a double bond (alkene) in the middle, now what happens is an hydration reaction, we add in water now the enzyme fumarase which is a hydratase enzyme adds water so one of the carbons will take the hydrogen and the other carbon will take the hydroxyl group becoming a secondary alcohol which is called malate, now what should happen with malate? it should lose the hydrogens, what would we define this reaction? It is a dehydrogenation which is an oxidation reaction, so the enzyme which is called malate “as the substrate” and the type of reaction “dehydrogenation”, so malate dehydrogenase will take out these hydrogens and generates a double bond and the electrons will be loaded in NAD^+ and NADH will be produced and this is the third NADH that is getting produced Inside this Krebs cycle producing again the oxaloacetate that we started with

how to memorize exactly the chemical structures of all the eight compounds? look it's as easy as is: you'll have three types of structures: six carbon unit molecules, five carbon unit molecules and four carbon uniy molecules. you'll have two structures with the six carbon unit molecule: citrate and isocitrate, citrate is a tertiary alcohol while isocitrate is a secondary alcohol so it's easy to recognize, the five carbon unit molecule it's only one molecule in the citric acid cycle which is alpha ketoglutarate, now the four carbon unit molecule, we have five molecules starting with one bonded to coenzyme A which is easy to recognize: succinyl CoA, if in the middle it's an alkane so it is succinate, if it has a double bond (alkene) then it is a fumarate, if it is a secondary alcohol (in the middle it has -OH group) then it is malate and if it is a keto acid then it is oxaloacetate.
The two carbons that leave a round of TCA cycle are not the same exact atoms that had entered the same round by acetyl CoA, rather, the two carbons that leaves the cycle are from oxaloacetate, meaning that, the carbons that leave are derived from acetyl CoA that had entered in previous cycles, it’s like in each cycle we take two carbons from oxaloacetate as two carbon dioxide molecules but we give oxaloacetate two carbons back in the form of acetyl CoA.
The final products of this cycle is: three NADH molecules, one $FADH_2$ molecule, one GTP molecule, and two carbon dioxide molecules, that’s something worth remembering

Enzymes of the TCA Cycle

What was mentioned in the slide:

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



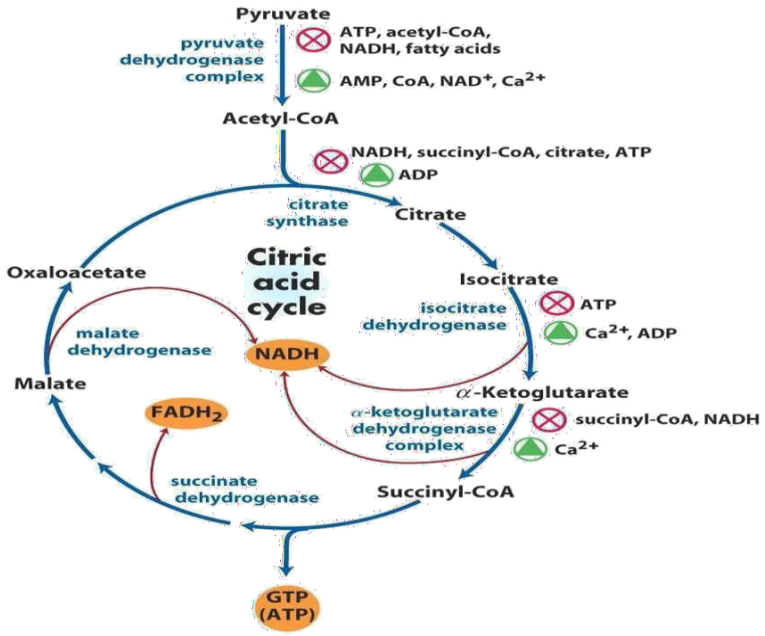
What was mentioned in the lecture:

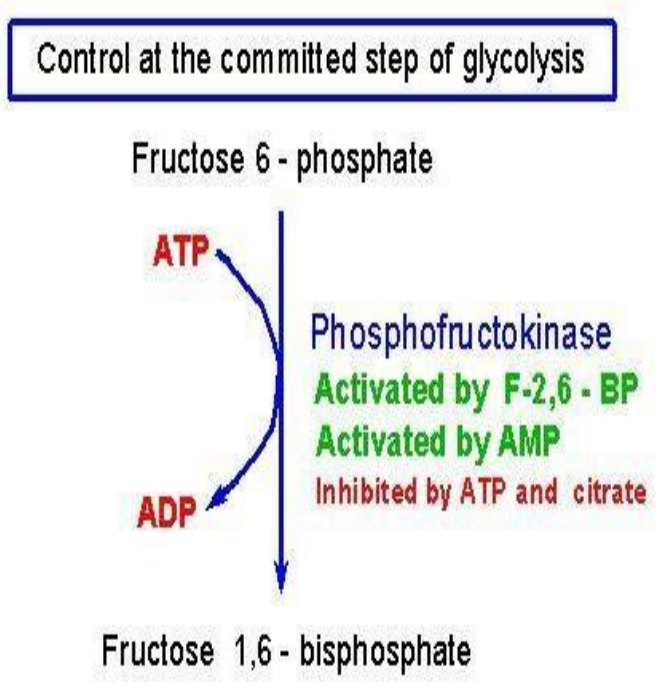
those are the names of the enzymes (the eight enzymes)

Formation and Oxidation of Isocitrate

What was mentioned in the slide:

- Oxidative decarboxylation, CO_2
- 3^{o} to 2^{o} alcohol





What was mentioned in the lecture:

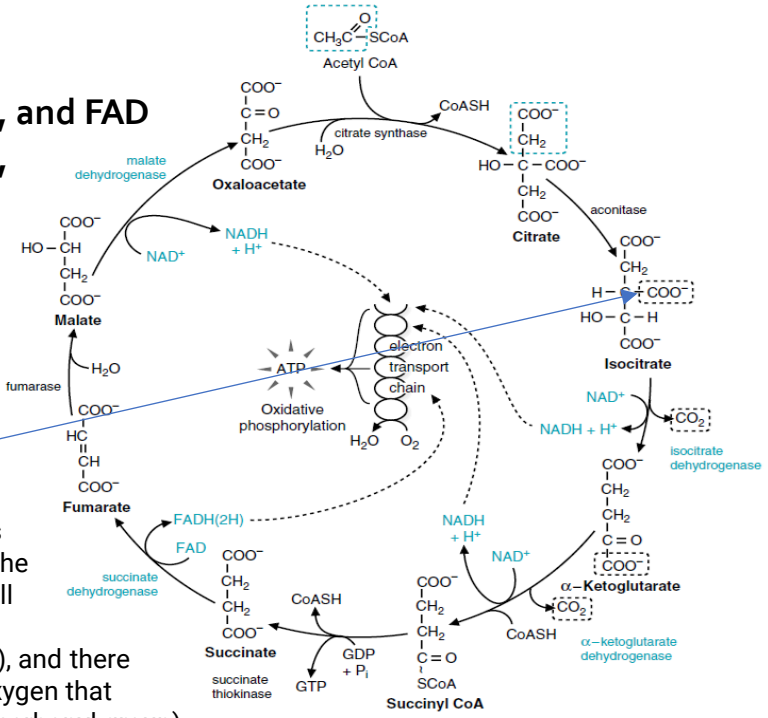
everybody of you knows the glycolytic process, (we're gonna take glycolysis with Dr. Fisal, glycolysis is the process of converting glucose to, pyruvate, pyruvate then will be converted to acetyl-CoA which will enter the TCA cycle). the rate limiting step of the glycolytic ten-step pathway is the convertion of fructose 6-phosphate into fructose 1,6-bisphosphate, which is catalyzed by PFK enzyme (Phosphofructokinase), This enzyme catalyzes the slowest step in the glycolytic pathway and as it is the slowest step, it has the highest regulation, This enzyme is activated by fructose 2,6-bisphosphate and AMP, AMP sends a message that I have low ATP which means that glycolysis has to be activated then pyruvate will be produced and then acetyl-CoA and citric acid cycle will go through, PFK is also inhibited by ATP, ATP sends a message that we have ATP in high amounts so we do not need the glucose to be broken down, but we need to store glucose, PFK is also inhibited by citrate, citrate present inside the mitochondria, but When the concentration of citrate increases up to a certain limit, it exits outside of the mitochondria, go to the cytosol and affect this enzyme, that sends a message that I have a lot of enough citrate , do not produce more Acetyl-CoA from pyruvate, which means stop the glycolytic process, This is why citrate inhibits this enzyme (PFK), This is how the different metabolism pathways like carbohydrate metabolism with energy metabolism are all interconnected to each other.
This is how allosteric enzymes (like PKF) function, they have many binding sites for many regulators that come to the enzyme and tell it how to work.

α-Ketoglutarate to Succinyl CoA

What was mentioned in the slide:

- Oxidative decarboxylation
- Thiamine pyrophosphate, lipoic acid, and FAD
- Keto group oxidized to acid, CoA-SH, succinyl CoA
- Energy conserved as NADH, thioester bond

What was mentioned in the lecture:

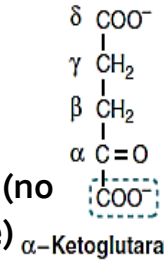


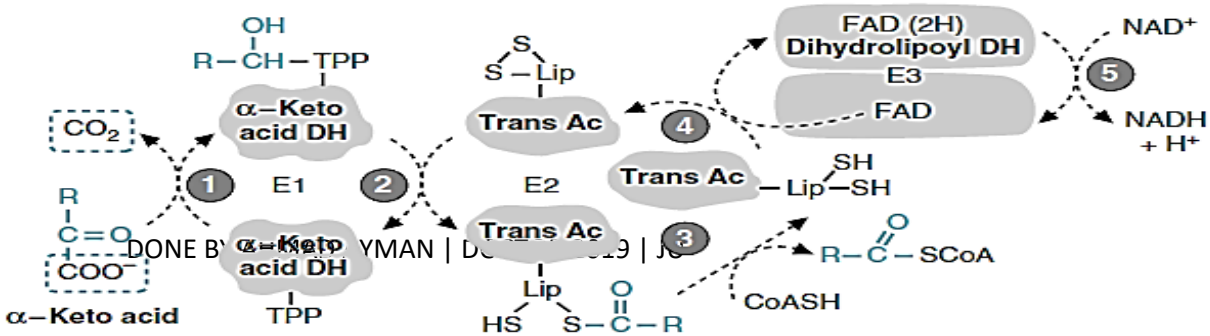
When isocitrate goes into alpha-ketoglutarate, it's easy to understand the mechanism of the reaction from the structures, this is the carboxyl group that goes out as CO_2, it will be replaced by a hydrogen atom, and the carbon atom that bears the secondary alcohol group will be dehydrogenated (a hydrogen from it and a hydrogen from the secondary alcohol group on it will be removed), and there will be a double bond in between this carbon and the oxygen that remained from the -OH group, forming a keto group, (a carbonyl group), converting the isocitrate into alpha-ketoglutarate, So the mechanism is easy to understand, but when alpha-ketoglutarate is getting converted to succinyl-CoA, the mechanism cannot be understood from the structures, this is why we gonna explain it by itself. (to be continued)

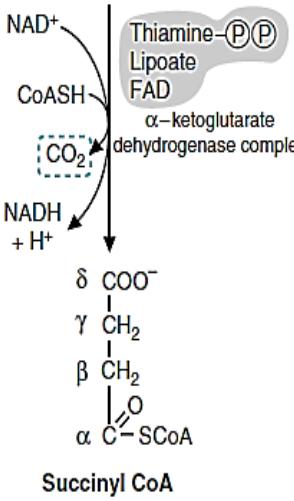
α-Ketoacid Dehydrogenase Complexes (TLCFN)

What was mentioned in the slide:

- (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 E2and E3 remain bound → higher rate)
- E1, E2, & E3 are a decarboxylase (TPP), a transacylase (lipoate), & a dehydrogenase (FAD)







What was mentioned in the lecture:

the enzymes that catalyzes the reaction of converting α-ketoglutarate to succinyl CoA is called α-ketoglutarate dehydrogenase complex, what does it mean if we followed the name of the enzyme by complex? It means the reaction is catalyzed by a cluster of enzymes that are complexed together, there are three complexes in the body which work exactly with the same mechanism for three different complexes, Those complexes have different amino acid sequence, which makes them able to react with different substrates, However, the same mechanism applies to all three complexes, one of these complexes is called α-ketoglutarate dehydrogenase complex, meaning that the substrate for this complex is α-ketoglutarate, we have another complex, which is called pyruvate dehydrogenase complex And the substitute for this enzyme is pyruvate, the third one is called - as you can see in the slide-, α- ketoacid dehydrogenase complex, and the substitute for this complex are the α-ketoacids, ketoacids are generated by transamination of certain amino acids (removing the amine group from them and substitute it with a keto group), we will talk about this when we talk about amino acid metabolism at the end of the semester and we'll talk then about α-ketoacid dehydrogenase complex. We'll talk about the pyruvate dehydrogenase complex with dr. Fisal, and now we're going to talk about the α-ketoglutarate dehydrogenase complex, all them work by same mechanism. (in the figure in the slide, for generalization, we are connecting the substrate to what to R to indicate that the three complex function with same mechanism regardless of the substrate for example, pyruvate has three carbons, depending on the amino acid from which the ketoacid was derived the number of carbon might vary, for example α-ketoglutarate has five carbons),

Now, because it is a complex, it actually composed of a group of enzymes, α-ketoglutarate dehydrogenase complex has three enzymes connected to each other, they are called E1, E2 and E3. (we know that the reaction of conversion of α-ketoglutarate to succinyl CoA is an oxidative decarboxylation reaction, So it must involve an oxidation reaction, there should be a decarboxylation reaction, and there should be joining to coenzyme A), E1 works as a decarboxylase , E2 works as a transacylase (it takes the carbons and joins them to coenzyme A), while E3 works as a dehydrogenase, as long as we want to do decarboxylation, there should be thiamine pyrophosphate (remember from summer semester: thiamine pyrophosphate is a coenzyme derived from vitamin B_1), the E1 enzyme is attached to TPP, it will attack the substrate with its TPP attacking the Carbonyl group , it makes an external bonding with it, making the C-COO^- bond weaker and easier to be broken off, that causes the -COO^- group to leave as CO_2 and the rest of the carbons regardless of their number will be connected to TPP, then thiamine pyrophosphate has to detach from these carbons, so they'll be activated (remember from the organic chemistry course: a carbon must have four bonds and in our case, a lateral carbon will have three bonds), so thiamine will get back to its original form and the activated carbons will be donated to the second enzyme which is the transacylase, transacylase is attached to a coenzyme called lipoic acid (lipoate, it is abbreviated lip in the figure), the structure of lipoate contains a disulfide group (R-S-S-R), the activated carbons will attach to one of the sulfurs in this group, causing the break down of the disulfide group, when the disulfide bond gets broken by attaching of the carbon to one sulfur atom, the other sulfur atom will be activated abstracting a hydrogen from the solution and making this sulfur as a thiol group, now coenzyme will come in the second enzyme and take the rest of the carbons and gets attached to it, So there will be an Acyl-CoA regardless of the number of the carbons (it might be acetyl CoA, malonyl CoA, etc.), but in the case of α-ketoglutarate dehydrogenase complex, this molecule will be called succinyl CoA, so the product of the complex will leave at the level of E2.

Now when these carbons will leave the lipoate and get attached to CoA, another sulfur of lipoate is activated, so it will abstract another hydrogen from the solution and we'll have another thiol group, two thiol groups are not involved in the original structure of lipoate and we need to conserve its original from to be able to get involve into another reactions, what has to be done to make it again in its original form

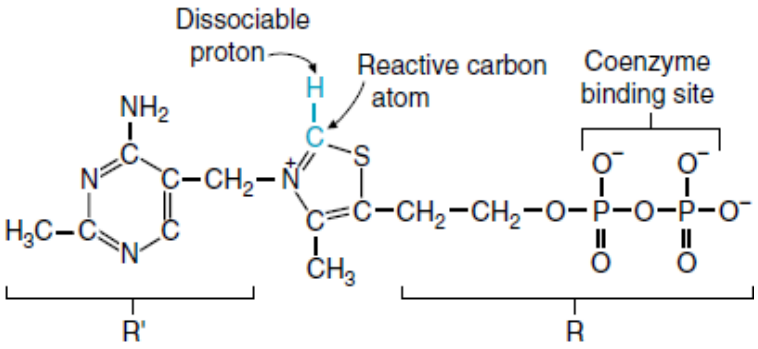
is to remove to hydrogens, The hydrogens should leave and because they are from two different sources (atoms), they should be loaded on FAD, so by the E3, which works as a dehydrogenase, both hydrogens will be loaded on FAD which will become $FADH_2$, then The lipoate will gain back its disulfide group in between the two Sulfurs, but now we have another problem, in the original form of the E3 enzymes, the FAD doesn't have two hydrogens, and we must restore the original form so the enzyme can do its function again, so there should be something external that comes and takes the electrons with their hydrogens out, what that will be in that case is NAD^+, NAD^+ will come and take the two electrons and become NADH, getting back the $FADH_2$ to its original situation as FAD.

now we've understood the mechanism of how α-ketoglutarate is converted to succinyl-CoA, How many coenzymes are involved in the process? We have thiamine pyrophosphate, lipoic acid, FAD, coenzyme A and NAD^+ there are five, but how many coenzymes that constitute a part of the complex? There are three: FAD, lipoate, and TPP. NAD^+ and coenzyme A are involved in the process but there are not attached to the enzymes of the complex and so they are not part of it?

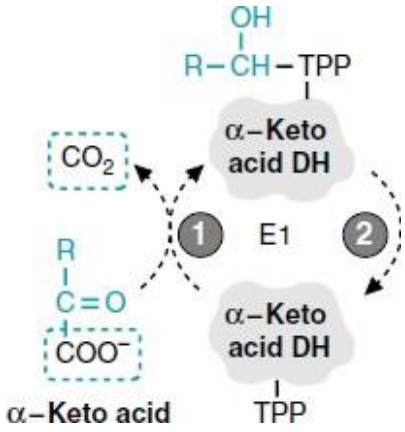
Thiamine Pyrophosphate

What was mentioned in the slide:

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



Thiamine pyrophosphate (TPP)



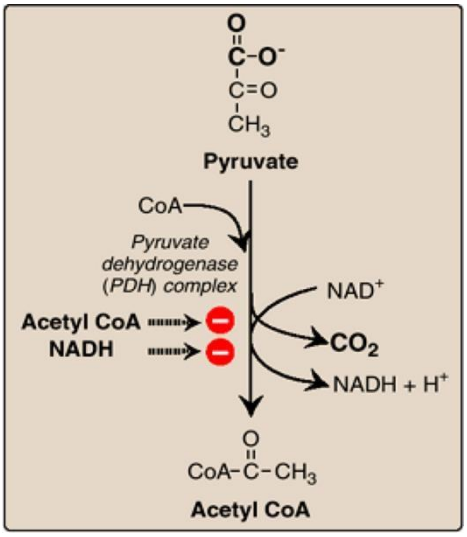
What was mentioned in the lecture:

now as the case with any coenzyme, TPP is derived from vitamins, vitamins can be in short supply inside the body because they are essential, we cannot synthesize them, now is someone has thiamine deficiency what do you expect as a clinician? Decarboxylation reactions will be defect, resulting in accumulation of ketoacids like pyruvate and α-ketoglutarate in blood, So if you take a blood sample, you'll see high levels of α-ketoglutarate and pyruvate.

What was mentioned in the textbook:

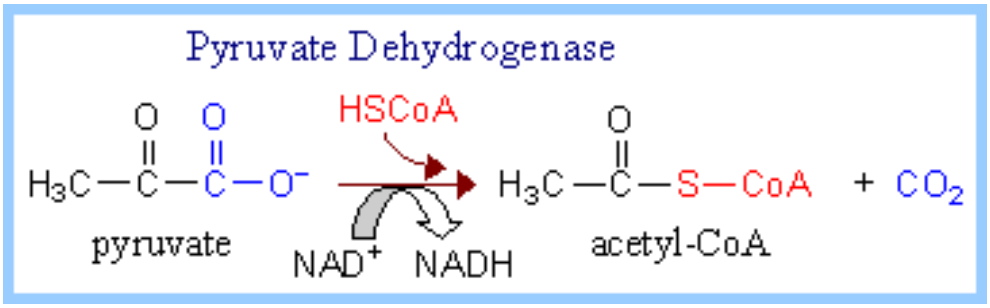
Deficiencies of thiamine or niacin can cause serious central nervous system problems. This is because brain cells are unable to produce sufficient ATP (via the TCA cycle) if the PDH complex is inactive. Wernicke-Korsakoff, an encephalopathy-psychosis syndrome due to thiamine deficiency, may be seen with alcohol abuse.

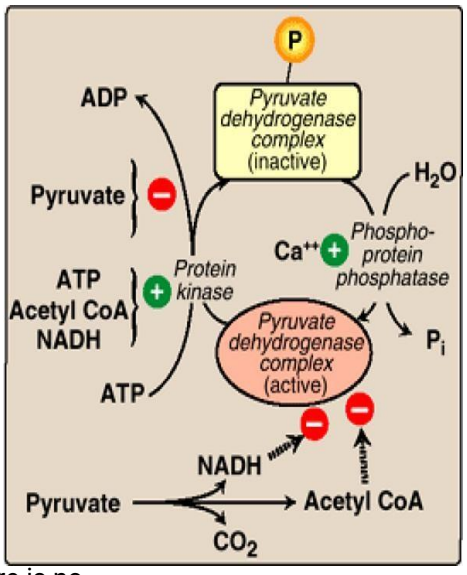
Oxidative decarboxylation of pyruvate



What was mentioned in the slide:

- **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**





What was mentioned in the lecture:

So far, we've discussed the the enzymes and complexes involved in oxidative carboxylation reactions, what we'll say now in applied to pyruvate dehydrogenase deficiency and α-ketoglutarate deficiency, etc. a deficiency or a mutation that occurs inside this big complex is reported as follows: E1 component is the most common one reported (which is the decarboxylase) to be deficient or to have a mutation, which affects its action resulting, in higher levels of pyruvate or α-ketoglutarate, this mutation is an X-linked mutation and there is no treatment it, and it causes congenital lactic acidosis, Why congenital lactic acidosis? a because pyruvate is usually getting transformed to acetyl-CoA in the aerobic respiration and then Acetyl CoA will enter TCA cycle and then ATP will be generated, Now, if there is a problem in conversion of pyruvate to acetyl CoA, most of it will get converted to lactic acid, if lactic acid increases in its concentration, it gets outside to the bloodstream causing lactic acidosis.

Another thing to know about is the arsenic poisoning, arsenic is a chemical element whose atomic number is 33, it is very poisonous to the human beings, And there are lots of murder cases that have been related to arsenic poisoning, so what does arsenic do exactly? lipoic acid has a disulfide group

Now when the, when the lipoic acid is connected to a disulfide group, it normally gets broken down to thiol groups, arsenic binds to those sulfurs inside the lipoate in a ligation mechanism where it cannot be broken down to two thiol groups, so the lipoate will not be able to bind the carbon units and donate them to the coenzyme a, accordingly, there will be no product, and depending on the concentration provided, arsenic will be killing because if the citric acid cycle is stopped, then there will be

X- liked means that the gene that codes for E1 is carried on the X sexual chromosome, meaning that the disease is sex-related like color blindness, remember for tawjihi.

no electric carrying molecules and no oxidative phosphorylation, and no ATP generation.

What was mentioned in the textbook:

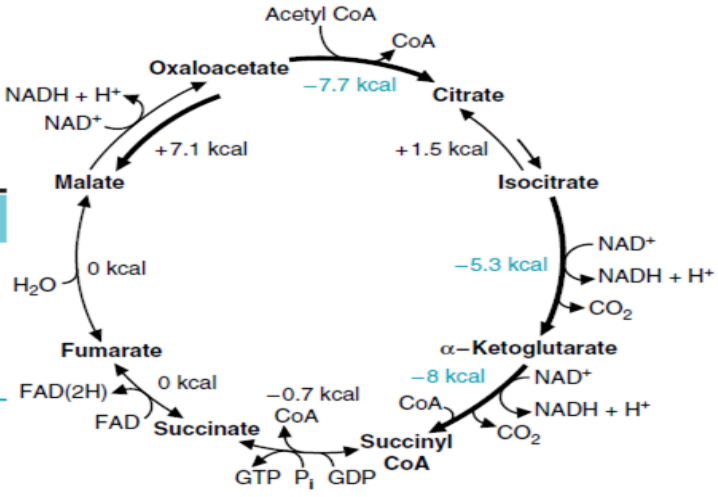
- . Pyruvate dehydrogenase complex deficiency: A deficiency in the activity of the α subunit of the dimeric E1 component of the PDH complex, although rare, is the most common biochemical cause of congenital lactic acidosis. This enzyme deficiency results in an inability to convert pyruvate to acetyl CoA, causing pyruvate to be shunted to lactate via lactate dehydrogenase This creates particular problems for the brain, which relies on the TCA cycle for most of its energy and is particularly sensitive to acidosis. Symptoms are variable and include neurodegeneration; muscle spasticity; and, in the neonatal onset form, early death. The gene for the α subunit is X linked, and, because both males and females may be affected, the deficiency is classified as X-linked dominant. Although there is no proven treatment for PDH complex deficiency, dietary restriction of carbohydrate and supplementation with thiamine may reduce symptoms in select patients.
- Leigh syndrome (subacute necrotizing encephalomyelopathy) is a rare, progressive, neurodegenerative disorder caused by defects in mitochondrial ATP production, primarily as a result of mutations in genes that code for proteins of the PDH complex, the electron transport chain, or ATP synthase. Both nuclear and mitochondrial DNA can be affected.
- Mechanism of arsenic poisoning: pentavalent arsenic (arsenate) can interfere with glycolysis at the glyceraldehyde 3- phosphate step, thereby decreasing ATP production. “Arsenic poisoning” is, however, due primarily to inhibition of enzymes that require lipoic acid as a coenzyme, including E2 of the PDH complex, α-ketoglutarate dehydrogenase and branched-chain α-keto acid dehydrogenase. Arsenite (the trivalent form of arsenic) forms a stable complex with the thiol (–SH) groups of lipoic acid, making that compound unavailable to serve as a coenzyme. When it binds to lipoic acid in the PDH complex, pyruvate (and, consequently, lactate) accumulates. As with PDH complex deficiency, this particularly affects the brain, causing neurologic disturbances and death

Bioenergitics of the TCA Cycle

What was mentioned in the slide:

- **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



What was mentioned in the lecture:

Regarding Bioenergetics of the cycle, any reaction or pathway in this world is theoretically reversible, However, there are pathways and reactions in this life which are essentially irreversible because there is a huge difference in energy, between the reactants and the products, regarding the citric acid cycle, it always goes in the forward direction (from citrate to oxaloacetate), It doesn't go in the backward direction. Why? Because there is a huge difference in energy, As you can see, from oxaloacetate to citrate, from isocitrate to alpha-ketoglutarate and from alpha-ketoglutarate to succinyl-CoA, Those three reactions have very large negative ΔG, which makes the reaction essentially goes into one direction only.
how much is the amount of energy produced inside the citric acid cycle?
The energy within the GTP is exactly the same as ATP upon the release of one phosphate, it is 7 Kcal/mole. Regarding NADH, upon the release of its two electrons eventually to oxygen in the electron transport chain, The difference in energy is 53 kilocalories per mole and we have three molecules, each produces 53 Kcal/mole, 3*53 equals 159 Kilocalories/mole, considering the $FADH_2$, which is another energy molecule, We have one molecule of $FADH_2$ that it produces 41 kilocalories/mole upon release of its electrons to oxygen. so in total we have 207 kilocalories/mole generate by Krebs cycle, is that good or bad in terms of efficiency? Efficiency of any machine in this world is defined as the ratio of the experimentally and actually detected amount of energy that has not wasted to the theoretically calculated amount of energy, يعني مثلا كفاءة السيارة تساوي كمية الطاقة الي استهلكتها في المشي تقسيم كمية الطاقة الموجودة في البنزين، في جزء من الطاقة في البنزين بضيع على شكل حرارة. It is the actual output divided by the theoretical output. We can calculate the theoretical output of Krebs cycle by burning a mole of acetate in a pressure pot and measuring the energy released by a calorimeter, because all molecules will keep turning in the cycle but not acetyl CoA, acetyl CoA is the molecules that will be degraded in the cycle, we must know the energy that is stored is acetate and the energy that has left with the CO_2, one mole of acetate store energy of 228 kcal, Krebs cycle provide 207 kcal/mole, so by dividing these two numbers we see that the efficiency of Krebs cycle is nearly 90%, you'll never find the machine in this world or any other biochemical pathway within the human body that has this great efficiency, Krebs cycle is the best machine in the world. The rest 10% is converted to heat or other purposes.

Regulation of the TCA Cycle

What was mentioned in the slide:

- 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

Citrate Synthase

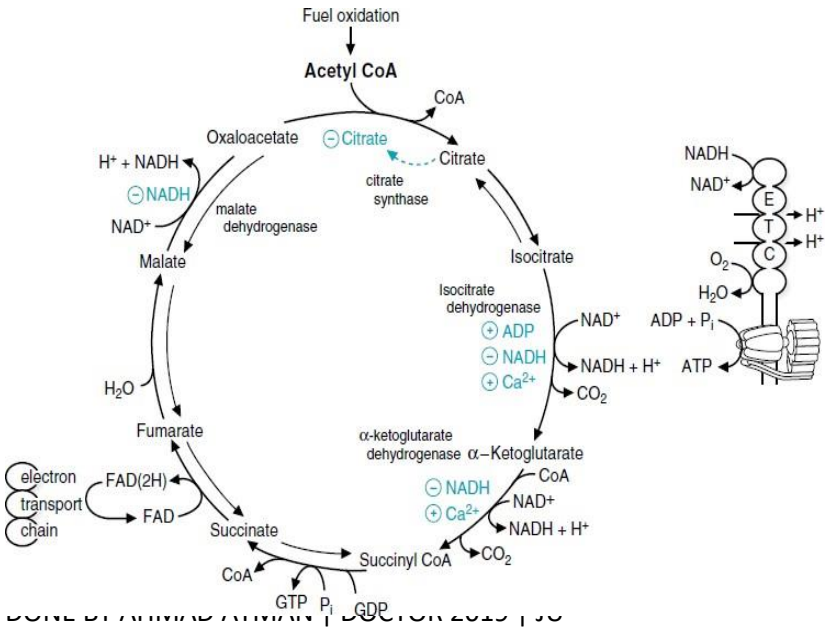
- The first step, no allosteric regulation
- Rate regulated by oxaloacetate & citrate (inhibitor)

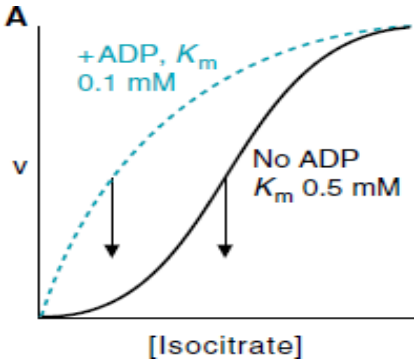
Isocitrate DH

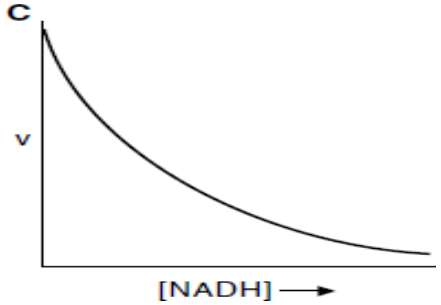
- Best regulation at rate- limiting step (Isocitrate DH)
- Allosterically: activated (ADP, Ca^{+2})
- Inhibition (NADH)
- No ADP vs. ADP (K_m less), a small change in ADP, great effect

α-Ketoglutarate DH

- Inhibited by NADH and succinyl CoA, GTP
- Activated by Ca^{+2}, muscle contraction







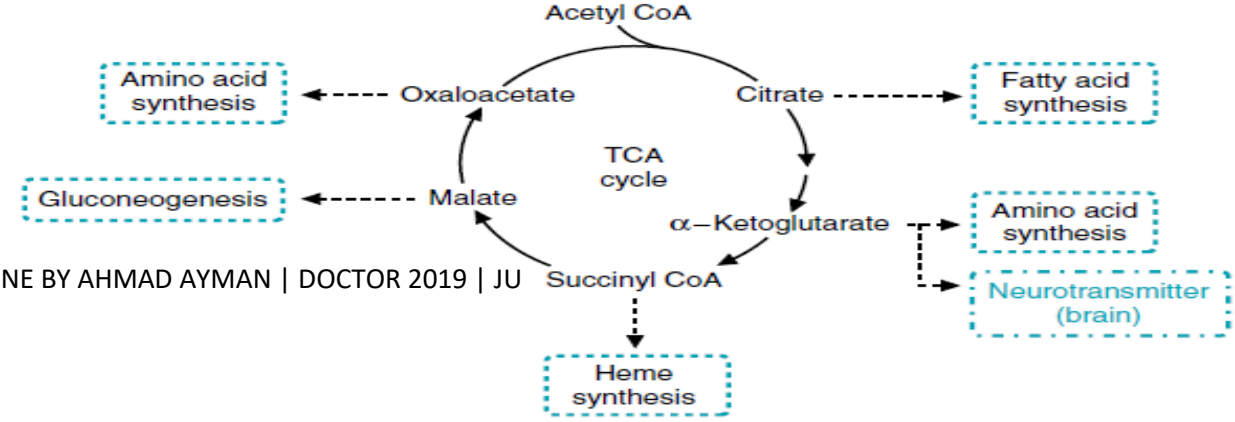
What was mentioned in the textbook:

In contrast to glycolysis, which is regulated primarily by PFK-1, the TCA cycle is controlled by the regulation of several enzymes The most important of these regulated enzymes are those that catalyze reactions with highly negative ΔG^0, those include: citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase complex. Reducing equivalents (like NADH and $FADH_2$) needed for oxidative phosphorylation are generated by the PDH complex and the TCA cycle, and both processes are upregulated in response to a decrease in the ratio of ATP to ADP. (meaning that these two process are activated by APD but inhibited by ATP, remember that ADP sends a message that there is not enough ATP so we have to activate these processes to produce more ATP, but excess ATP indicates that there more than enough ATP so we don't need these two processes) .
In humans, citrate synthase is not an allosteric enzyme (meaning that it is not controlled from away, but just from its substrate and product, not like PFK which is activated by fructose 2,6-bisphosphate which is not a substrate or a product of PFK). It is inhibited by its product, citrate. Substrate availability is another means of regulation for citrate synthase. (meaning that it is activated by oxaloacetate and acetyl CoA)
Isocitrate dehydrogenation is one of the rate-limiting steps of the TCA cycle. The enzyme isocitrate dehydrogenase is allosterically activated by ADP (a low-energy signal, ADP tells that there is no energy) and Ca^{2+} and is inhibited by ATP and NADH, levels of which are elevated when the cell has abundant energy stores.
ADP induces synergistic activation on isocitrate dehydrogenase, citrate also activates isocitrate dehydrogenase, but when ADP is present also, citrate and ADP have a synergistic effect, meaning that the activation by both of these together is more than the sum of the activation By both of these separately (pharmacology), ATP also induces conformational changes that enhance the binding of isocitrate to isocitrate, therefore decreasing K_m (remember from the summer semester: K_m equals the rate of disassociation of the substrate from the enzyme divided by the rate of binding of it)
α-Ketoglutarate dehydrogenase complex is inhibited by its products, NADH and succinyl CoA, and activated by Ca^{2+}. However, it is not regulated by phosphorylation/dephosphorylation reactions as described for PDH complex.
Calcium is also an important regulator of the citric acid cycle; an increase in concentrations of both ADP and calcium ions (Ca^{2+}) are a consequence of changes in cellular activity. Therefore, the signal that stimulates muscle contraction is also activating the production of the ATP which sustains it.

TCA Cycle Intermediates

What was mentioned in the slide:

- **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)**



What was mentioned in the lecture:

Is TCA cycle exclusive for the sake of making energy or for the sake of extracting electrons and passing them to the electron transport chain, or it is connected to other biochemical pathways? this leads us to sense how greatly the different biochemical pathways are interconnected to each other.
TCA cycle Intermediates are connected to other biochemical pathways in the human body, They are not exclusively being used just in the citric acid cycle, For example, citrate present in the fatty acid synthesis. Alpha-ketoglutarate can be transaminated to glutamate acid which is an amino acid, so these intermediates also participate in amino acid synthesis, GABA (gamma aminobutyric acid), the inhibitory neurotransmitter can be also synthesized out of glutamate, also glutamate by itself is an excitatory neurotransmitter, (fun fact: anti-seizure drugs used in epilepsy are usually GABA agonists), Considering the succinyl-CoA, it can be converted to propionyl CoA which participates in heme synthesis, heme is required for hemoglobin, myoglobin, and many other proteins that uses heme, malate is a key molecule in the process of gluconeogenesis (carbohydrate metabolism), Oxaloacetate can be transaminated to aspartic acid, so it participates in amino acid synthesis, So they are not exclusively being used in the citric acid cycle but If you will have extra amounts of them, they can leave the cycle and, participates in building up of other molecules in the cytosol, degradation of molecules and energy production occur inside the mitochondria, but building up of molecules and use of energy happens outside of the mitochondria in the cytosol.

Anaplerotic Reactions

What was mentioned in the slide:

- Pathways or reactions that replenish the intermediates of the TCAcycle
- 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)

What was mentioned in the lecture:

anaplerotic reactions is another concept where other reactions might generate (replenish) citric acid cycle intermediates, meaning that, when I have deficiency in citric acid cycle intermediates, what are the reactions that compensate this deficiency? We are not talking about the intermediates consumed by other reactions, but we are talking about the other reactions making citric acid cycle intermediates, which is the opposite to what we were discussing just now, acetyl CoA can be generated from amino acids, fatty acids, carbohydrates, glutamate can be converted to alpha-ketoglutarate, Aspartate can give us oxaloacetate, a fumarate can be generated from different amino acids in the urea cycle, succinyl-CoA can be produced from propionyl CoA, the same things that we were discussing but in the opposite direction, the main reaction in anaplerotic reactions is the one where pyruvate can be converted to oxaloacetate. pyruvate is a three carbon unit molecule while oxaloacetate is a four carbon unit molecule, this reaction is catalyzed by pyruvate carboxylase where we add a carboxyl group in a form of CO_2, if you want to add one carbon unit (we want to make the molecule bigger), then we need energy, this is why this reaction requires an ATP molecule to aid it, So if there is a decrease in the concentration of oxaloacetate, who's going to replenish this oxaloacetate? It is pyruvate Carboxylase through carboxylation of pyruvate, This

reaction is carboxylase so it needs the coenzyme biotin which is derived from vitamin B_7 (fun fact: biotin can be synthesized in our body by our gut flora so its deficiency is rare, but can happen in cases of long time using of antibiotics and excessive eating of raw eggs), this reaction is also activated by acetyl-CoA when acetyl CoA is high in concentration, it needs oxaloacetate to bind it,