METABOLISM

بسم الله الرحمن الرحيم

MID – Lecture 5

TCA Cycle (pt.2)

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

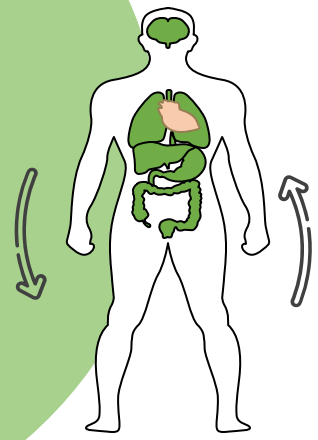
اللهم استعملنا ولا تستبدلنا

Written by:

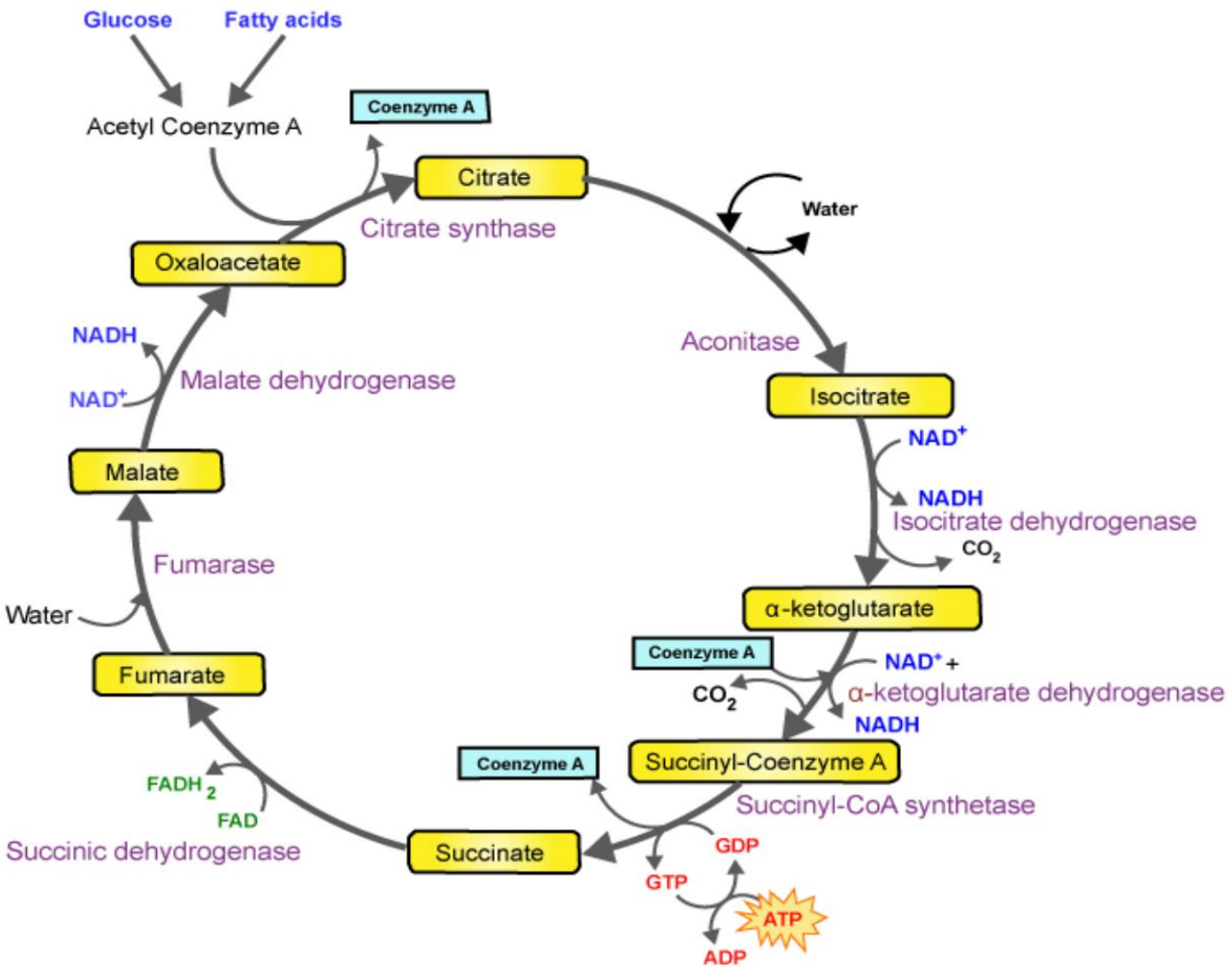
- Leen Mamoon
- Raghad Altiti

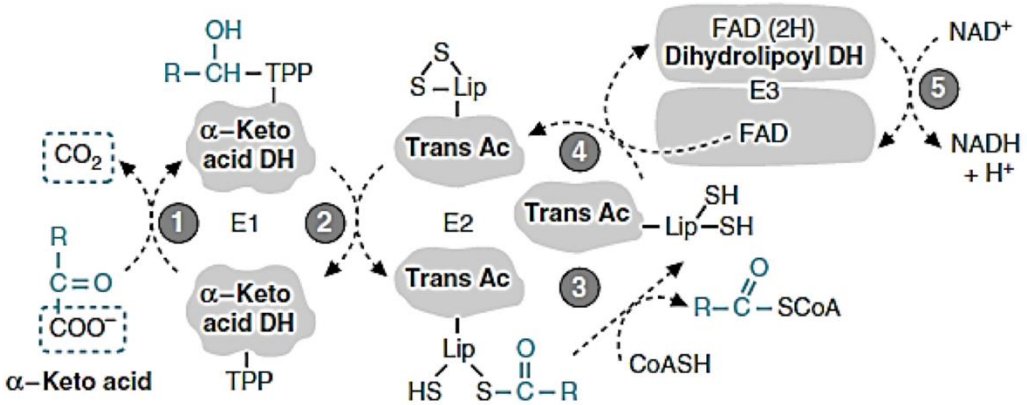
Reviewed by:

- Leen Mukahal



The doctor started with a short summary of the previous lecture. Nothing new was mentioned, so we will give some questions for review.





What is the function of E1 in the a-ketoacid dehydrogenase complex?

A)Decarboxylase
B) Transacylase
C) Dehydrogenase
D) Oxidase

What is a key advantage of the a-ketoacid dehydrogenase complex structure?

A) Higher reaction rate
B) Lower energy requirement
C) Increased substrate specificity
D) Reduced enzyme production

Before you start, make sure you have studied the previous lecture well:

The enzyme succinate dehydrogenase is unique in the Krebs cycle because:

A) It requires ATP as a cofactor
B) It is located in both the mitochondrial matrix and inner membrane
C) It produces NADH directly
D) It converts oxaloacetate to citrate

FAD is reduced in which of the reaction of the Kreb's cycle?

A) Isocitrate to oxaloacetate
B) Succinyl CoA to Succinate
C) Fumarate to malate
D) Succinate to fumarate

Which of the following enzymes is regulated by calcium ions (Ca^{2+}) to increase its activity in the Krebs cycle?

A) Citrate synthase
B) Isocitrate dehydrogenase
C) Fumarase
D) Succinate dehydrogenase

In Kreb's cycle, a molecule of GTP is produced during the conversion of:

A) Citrate into Ketoglutarate
B) Succinyl-CoA into succinate
C) Succinate into malate
D) Malate into oxaloacetate

How many CO_2 molecules are released per turn of the Krebs cycle?

A) 1 CO_2
B) 2 CO_2
C) 3 CO_2
D) 4 CO_2

What are the three main components of α-ketoacid dehydrogenase complexes?

A) Decarboxylase, transacylase, & dehydrogenase
B) TPP, lipoate, & FAD
c) a-ketoglutarate, pyruvate, & branched chain a-keto acid

Acetyl CoA is formed from pyruvate by_ reaction

A) Dehydration
B) Reduction
C) Oxidative decarboxylation
D) Dephosphorylation

Now you can start..

Bioenergetics of TCA Cycle

- Like all pathways, overall net -ΔG (-228 kcal/mole)
- 3NADH, FAD(H2), and GTP= (10ATP)
- Three reactions have large (-ve) values
- Physiologically irreversible, low products

✓ Favorable pathway
✓ Spontaneous

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
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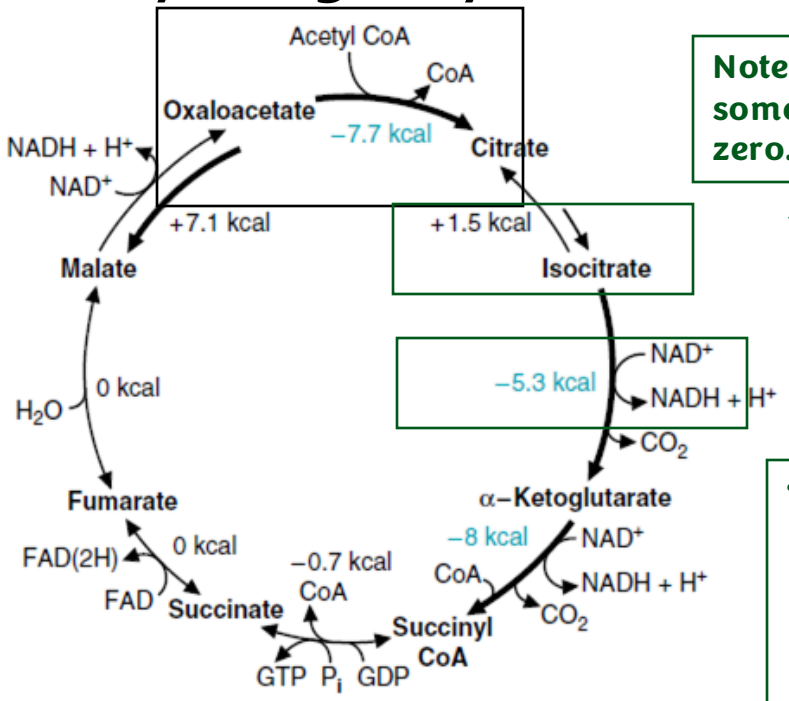
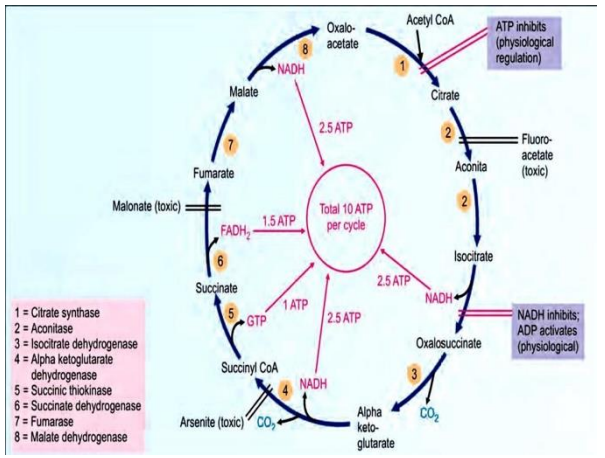


Note: The individual reactions of the TCA cycle have different values for ΔG; some have positive ΔG, some have negative values, and some have a ΔG of zero. However, the overall ΔG for the cycle is negative.

✓ In the first reaction of the Krebs cycle, acetyl-CoA reacts with oxaloacetate (OAA), releasing CoA. While CoA itself has energy, it is not directly used to make GTP in this step. Instead, the energy from acetyl-CoA is utilized to drive the reaction forward and to support other reactions with positive ΔG, making this step highly exergonic.

- Carboxylation is an energy-requiring process, as it involves the addition of a carboxyl group to a substrate, often using ATP or another energy source. In contrast, decarboxylation is typically an exergonic reaction, where a carboxyl group is removed from a substrate, releasing CO_2 and often resulting in the release of energy



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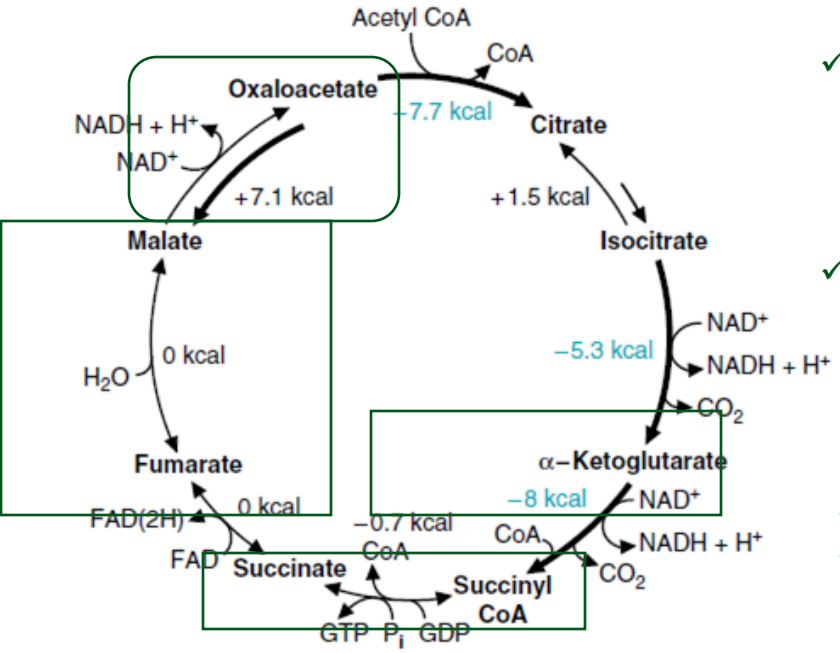
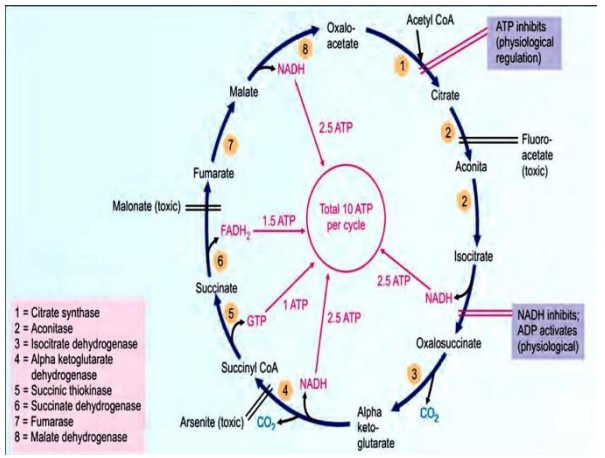
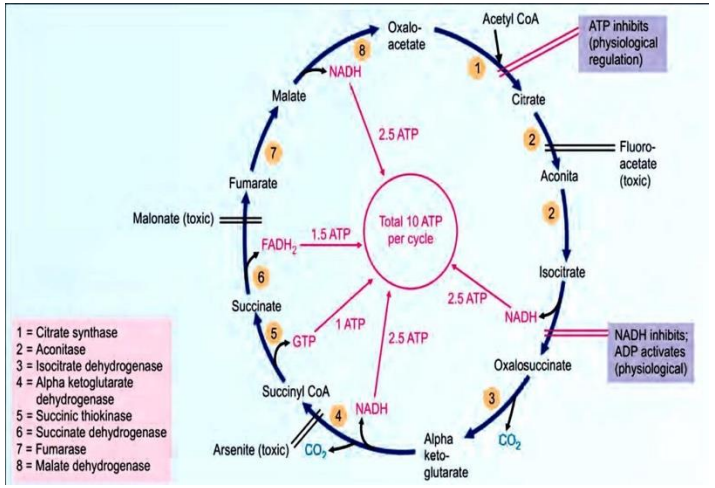


- ✓ Oxidative decarboxylation, particularly the reaction catalyzed by the α-ketoglutarate dehydrogenase complex, is one of the most exergonic reactions in the Krebs cycle with -8 Kcal
- ✓ The synthesis of GTP in the Krebs cycle is exergonic, with a ΔG of approximately -0.7 kcal/mol. The relatively small energy release occurs because the energy is used to phosphorylate GDP to GTP during the conversion of succinyl-CoA to succinate.
- ✓ Succinate oxidation to fumarate = 0 Kcal
- ✓ Fumarate hydration to malate = 0 Kcal
- ✓ Last step is highly Endergonic = +7.1 Kcal



Cycles like the Krebs cycle do not form large concentrations of intermediates. Instead, a steady supply of these intermediates is maintained to keep the cycle running efficiently. If the levels of intermediates, such as oxaloacetate (OAA), drop too low (not enough), the cycle cannot run . OAA is essential because it combines with acetyl-CoA to form citrate in the first step of the Krebs cycle. Without sufficient OAA, the entire cycle would be disrupted.



Remember

Physiological inhibitors/ activators are naturally occurring molecules within the body that regulate enzyme activity in metabolic pathways

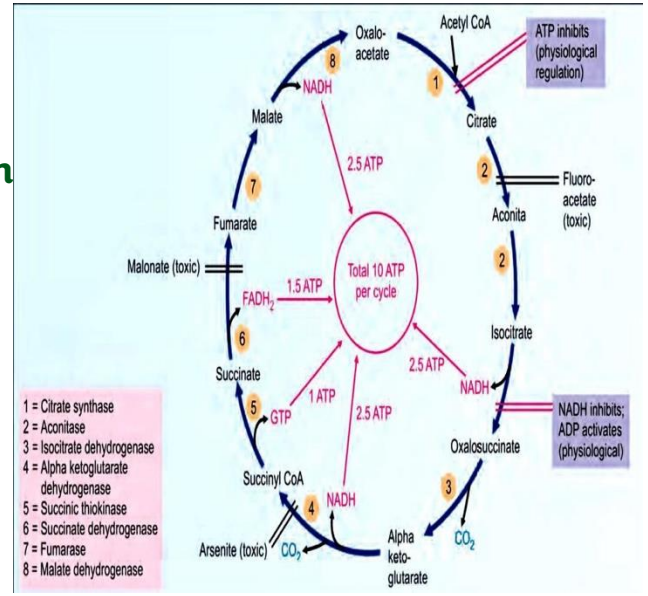
Unphysiological Inhibitors / activator :These refer to inhibitors that are not naturally found in the body under normal conditions and often result from external factors , Ex: toxins, drugs, Fluoroacetate is a toxic compound that acts as an inhibitor of aconitase (Children may be exposed to it.)

Recall : ATP acts as an inhibitor of citrate synthase, When ATP levels are high, it signals that the cell has sufficient energy, reducing the need for further energy production. As a result, ATP inhibits citrate synthase to prevent the cycle from starting, ensuring that energy and Acetal Co-A are not wasted when they are not needed.

اللهُم إنِّي أسالك خير ما أُمِرتْ بِه وأعوذُ بِك من شرِّ ما أُمِرَت بِه

Isocitrate dehydrogenase is an important enzyme in the Krebs cycle that is regulated by several factors:

1. **<u>Inhibition by ATP:</u> it's indicates a high-energy state in the cell. When ATP levels are elevated, there is less need for further energy production, so the enzyme activity is reduced.**
2. **<u>Activation by ADP:</u> it's signals a low-energy state. High ADP levels indicate that the cell needs to produce more ATP, prompting the cycle to run and generate energy.**
3. **<u>Inhibition by NADH:</u> because it is a product of the reaction catalyzed by the enzyme. High levels of NADH suggest that the cell has enough reducing equivalents and does not require more NADH for energy production. The Krebs cycle requires NAD+ to proceed with other oxidation reactions, so an excess of NADH means that the concentration of NAD+ is low. This balance is crucial, as multiple reactions in the cycle depend on NAD+ being available to accept electrons.**



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Each turn of the Krebs cycle produces a significant amount of energy in various forms:

3 NADH + GTP +FADH2

✓ It has often been suggested that each NADH molecule could generate up to 3 ATP during oxidative phosphorylation; however, the actual yield is approximately 2.5 ATP per NADH. Each run of the Krebs cycle (for each acetyl-CoA) will produce a total of about 10 ATP equivalents: 1 GTP (produced directly) and approximately 9 ATP (produced indirectly) through the electron transport chain via oxidative phosphorylation

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Net Result of TCA Cycle and its significance

It's amphibolic (Catabolic+Anabolic)

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 → A biochemical pathway, which involves both catabolism and anabolism
10. Anaplerotic role. → Chemical reactions that form intermediates of a metabolic pathway

Acetyl-CoA is derived from the degradation of various biomolecules, including carbohydrates, fats, and proteins. The oxidation of Acetyl-CoA in the Krebs cycle is important because it prevents the accumulation of Acetyl-CoA, allowing the cycle to function effectively and maintain energy production.

The intermediates of TCA cycle are connected with so many other metabolic pathways

Why?

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	

- ✓ **Fats are burned in the fire of carbohydrates,** the efficient burning of **fats** for energy depends on the presence of **carbohydrates** to provide the necessary oxaloacetate for the Krebs cycle
- ✓ **Fat cannot be converted to glucose because pyruvate dehydrogenase reaction is an absolutely irreversible step**

What happens in our bodies when we fast?

- ✓ During fasting, fatty acids are broken down , producing large amounts of Acetyl-CoA, which normally enters the Krebs cycle. However, for the Krebs cycle to proceed, there must be enough oxaloacetate available to combine with Acetyl-CoA. In fasting conditions, some of the available OAA is diverted for gluconeogenesis (the process of synthesizing glucose) This can limit the amount of OAA available for the Krebs cycle.As a result, the excess Acetyl-CoA that cannot enter the Krebs cycle is diverted into ketogenesis, leading to the production of ketone bodies.

- ✓ This is why people following a ketogenic diet, a high-protein diet, or intermittent fasting often reach a state of ketosis. In these states, fatty acids are broken down at a rate that exceeds the availability of OAA, leading to increased ketone body production.

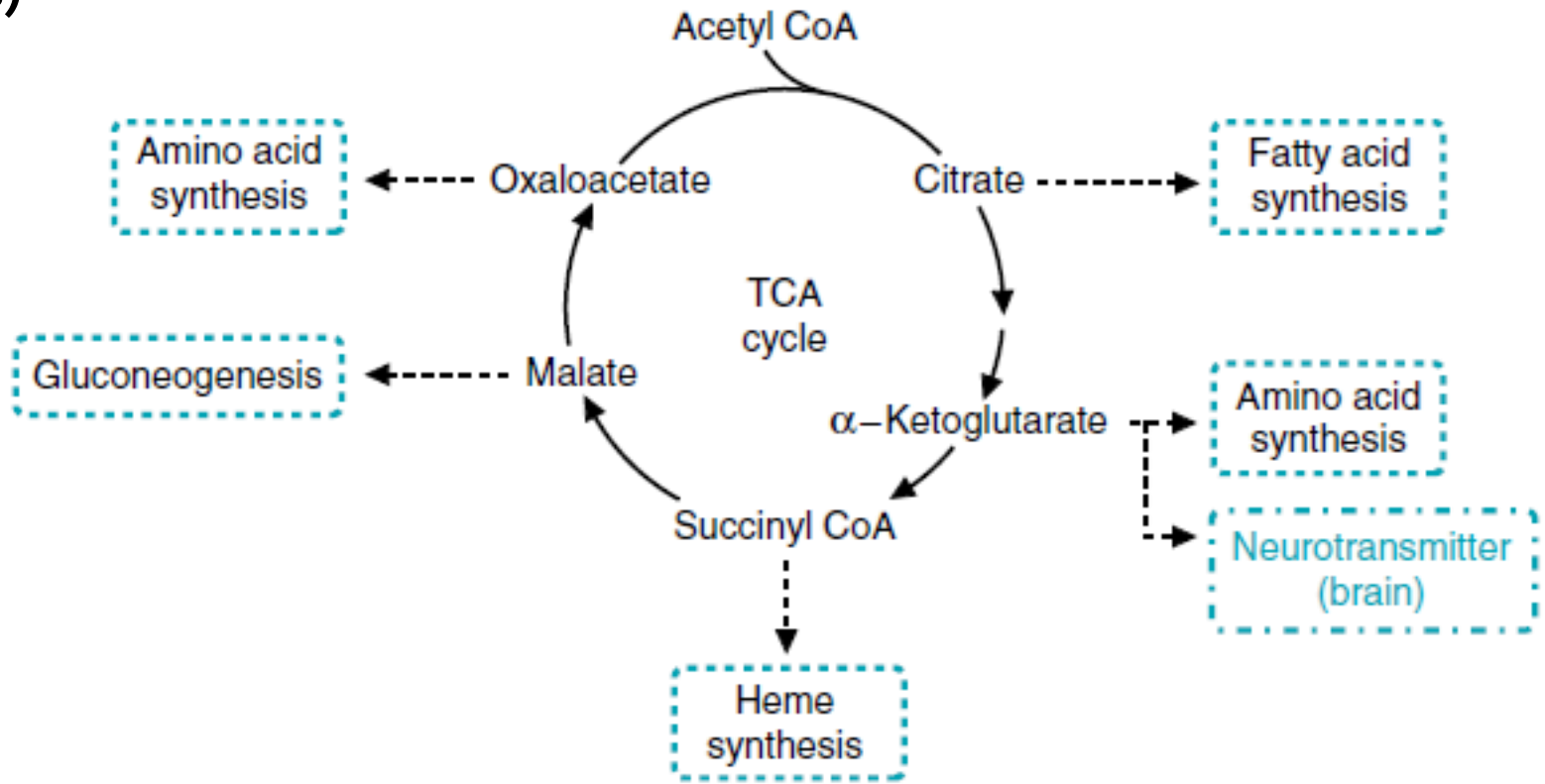
- ✓ The Acetyl-CoA that is released after the degradation of fatty acids cannot be converted back to pyruvate because this reaction is irreversible. Therefore, to run the Krebs cycle efficiently with the Acetyl-CoA from fatty acid degradation, there must be enough oxaloacetate (OAA) available. OAA is primarily derived from the metabolism of carbohydrates. This is why carbohydrates play an important role in supporting the Krebs cycle, as they provide the OAA needed to combine with Acetyl-CoA (as long as your cells have enough sugars (or carbohydrates), they will primarily use these for energy before turning to fatty acids or other sources)

Note: During fasting, some cells are completely dependent on glucose and cannot use fatty acids for energy. These include red blood cells (which lack mitochondria) and brain cells, which preferentially use glucose, though the brain can adapt to use ketone bodies during prolonged fasting, also I need to maintain the level of glucose in the blood within a certain range because if it decreases, it will affect osmotic pressure, as glucose is a solute, and this can impact water movement.". Other cells, such as muscle cells and liver cells, can oxidize fatty acids for energy, especially during fasting or low-carbohydrate conditions

- Glucose levels in the blood are not stable, as it is consumed by various tissues, such as the brain. This baseline level of glucose is referred to as fasting blood sugar, which is measured during routine checks in hospitals."

TCA Cycle Intermediates Interactions with Other Pathways

- **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)**



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The interaction with each pathway:

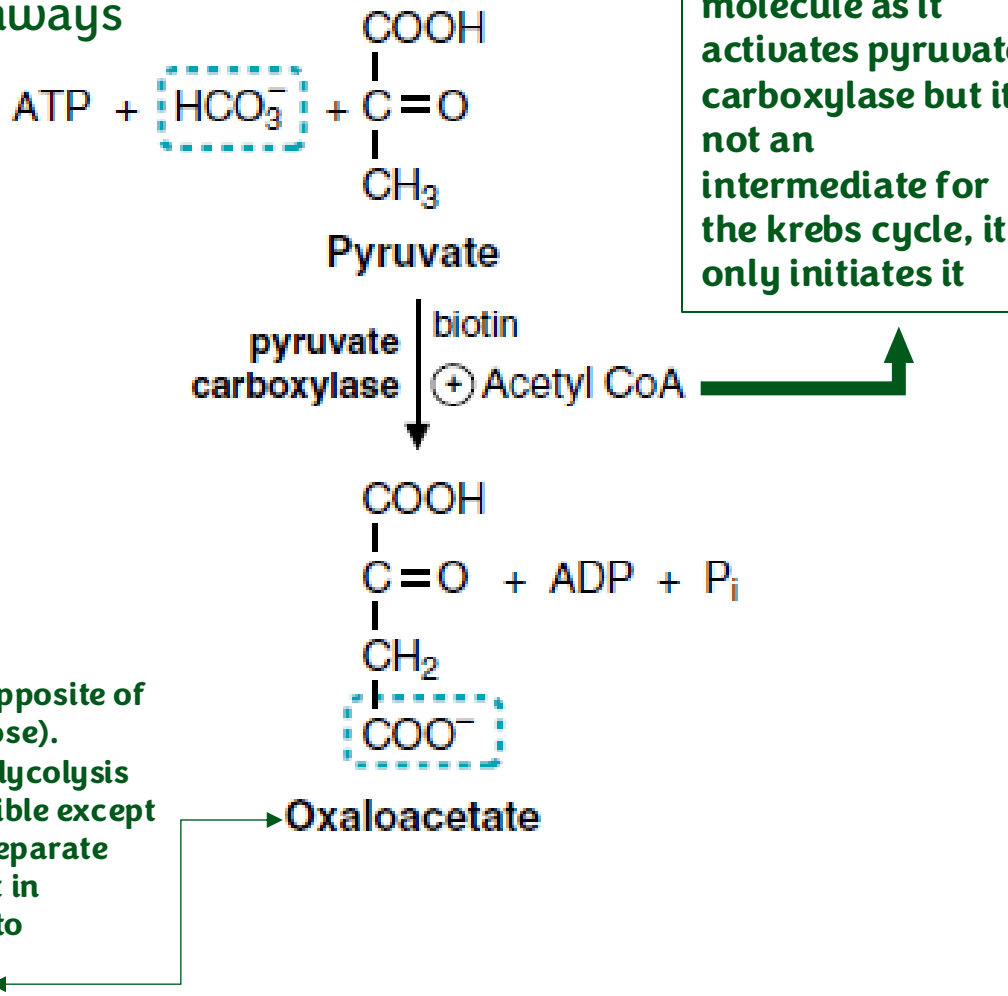
- The citrate: it can be used to make acetyl CoA to use it in fatty acids synthesis , it's also used as an inhibitor to regulate glycolysis
- Alpha ketoglutarate: participates in the synthesis of amino acids as it's the alpha keto acid of glutamate (it's used as an excitatory neurotransmitter(brain) and as an inhibitory neurotransmitter by forming GABA)
- Succinyl CoA: we use it with glycine to form the heme group
- Oxaloacetate:

Alpha ketoglutarate + amino group = glutamate

1. It's used in glycogenesis which is the formation of glucose but it can't be used directly as it's present inside the mitochondria and the sugar is formed in the cytosol, however we don't have transporters for oxaloacetate so we reduce it to malate that can leave to the cytosol and be oxidized again
2. It's also the alpha keto acid of aspartate so it makes aspartate amino acid that can be used to make asparagine

Anapleiotropic Routes

- **Pathways or reactions that replenish the intermediates of the TCA cycle** and other pathways
- **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)**



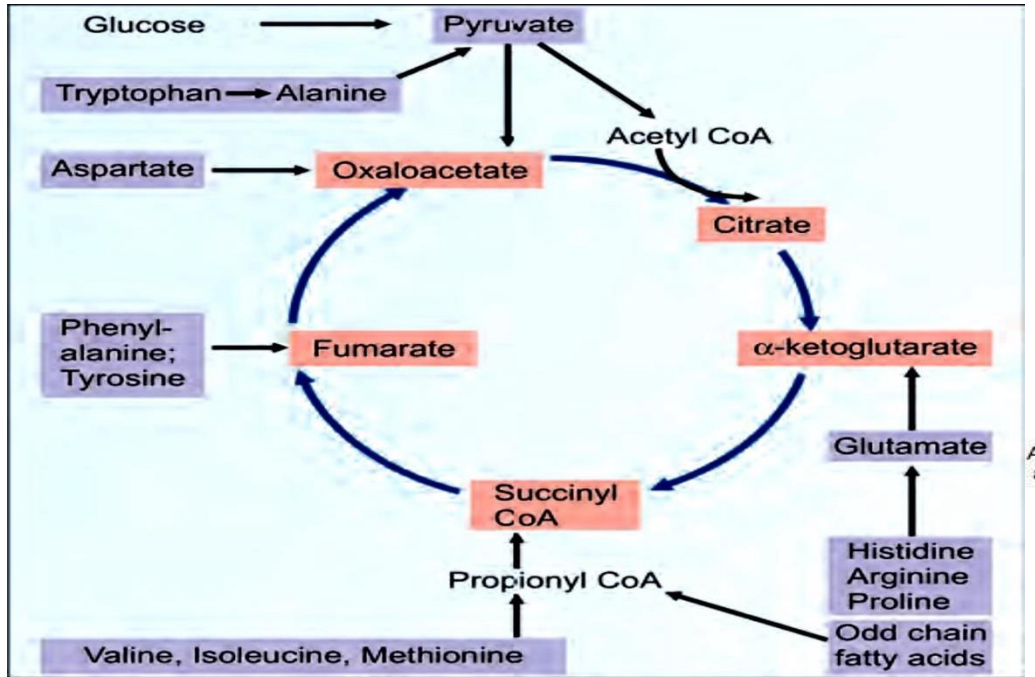
Regulatory molecule as it activates pyruvate carboxylase but it's not an intermediate for the krebs cycle, it only initiates it

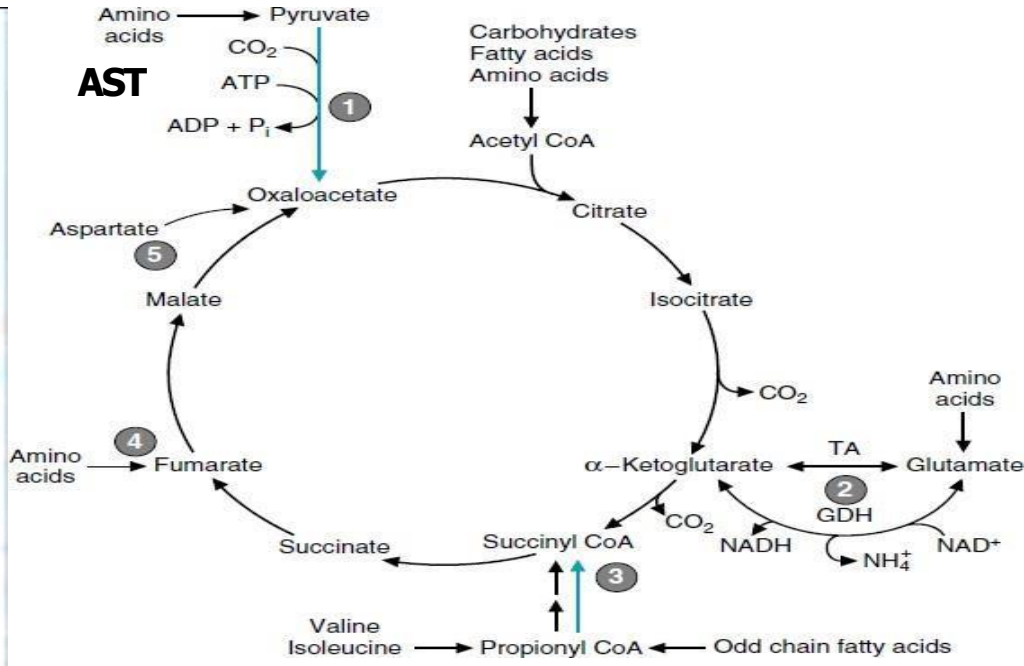
- ✓ Gluconeogenic pathway is the opposite of glycolysis (degradation of glucose).
- ✓ 90% of the reactions in both glycolysis and gluconeogenesis are reversible except one reaction which needs two separate steps instead of one to reverse it in gluconoeogensis (ex: pyruvate to Oxaloacetate)

Other Anapleiotropic Routes (amino Acid Degradation)

- All amino acids have alpha carbon, carboxyl group, hydrogen atom and amino group but they differ in the R group.
- In the metabolism, we will work on the amino group as it's a source of ammonia which is toxic so we need to get rid of it safely, so in their degradative pathway amino acids share the degradative pathway of the backbone groups especially the amino group, and they have different pathways for the R chains (carbon skeleton) which have a different final product.
- We can classify amino acids based on their final product which can be Krebs cycle intermediate, pyruvate, acetyl CoA or Acetoacetyl CoA (the last two are ketogenic amino acids)

Note: the doctor said some examples on amino acids degradation but they are not required for now so if you want to know them check the min 30 of the lecture https://youtu.be/xSCQjBGSq3U?si=Vm_ugnX7JL-AwLyM





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			
V1 → V2			

Additional Resources:

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اللهمَّ افتح علينا بالقرآن واهدنا بالقرآن وارزقنا بركته 💙