

# METABOLISM

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



MID – Lecture 6

## TCA CYCLE (pt.3)

﴿ وَإِن تَتَوَلَّوْا يَسْتَبَدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَلَكُمْ ﴾

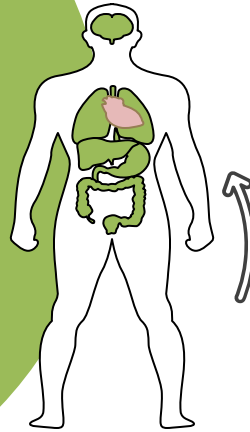
اللهم استعملنا ولا تستبدلنا

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# Regulation of the TCA Cycle

Remember that Krebs cycle is connected to the subsequent Electron Transport Chain that produces ATP.

➤ **Correspond to ETC (ATP/ADP)**

➤ **Two major messengers (feedback):**

**(a) phosphorylation state of adenines**

✓ ATP and ADP levels in the cell are related together because they are from the same type of molecules but different phosphorylation states, and they have a relatively constant pool, so they alternate between these different states and this will affect the activity of the enzymes of TCA Cycle.

**(b) the reduction state of NAD**

✓ Another molecule that is NAD that alternate between two states, the oxidized and the reduced states. It also has a relatively constant pool, but we have to have NAD<sup>+</sup> available in this format for the Krebs Cycle to proceed.

# Regulation of the TCA Cycle

## ➤ Adenine nucleotides pool and NAD pool are relatively constant

ATP and ADP

✓ **Constant pool means:** even though these molecules (adenine nucleotides and NAD) are constantly used, the cell can quickly recycle and regenerate them so their overall amount don't change much.

- Let's discuss the different locations where these molecules and other molecules as well can affect the activity of Krebs Cycle enzymes.

# Regulation-Citrate and Citrate Synthase

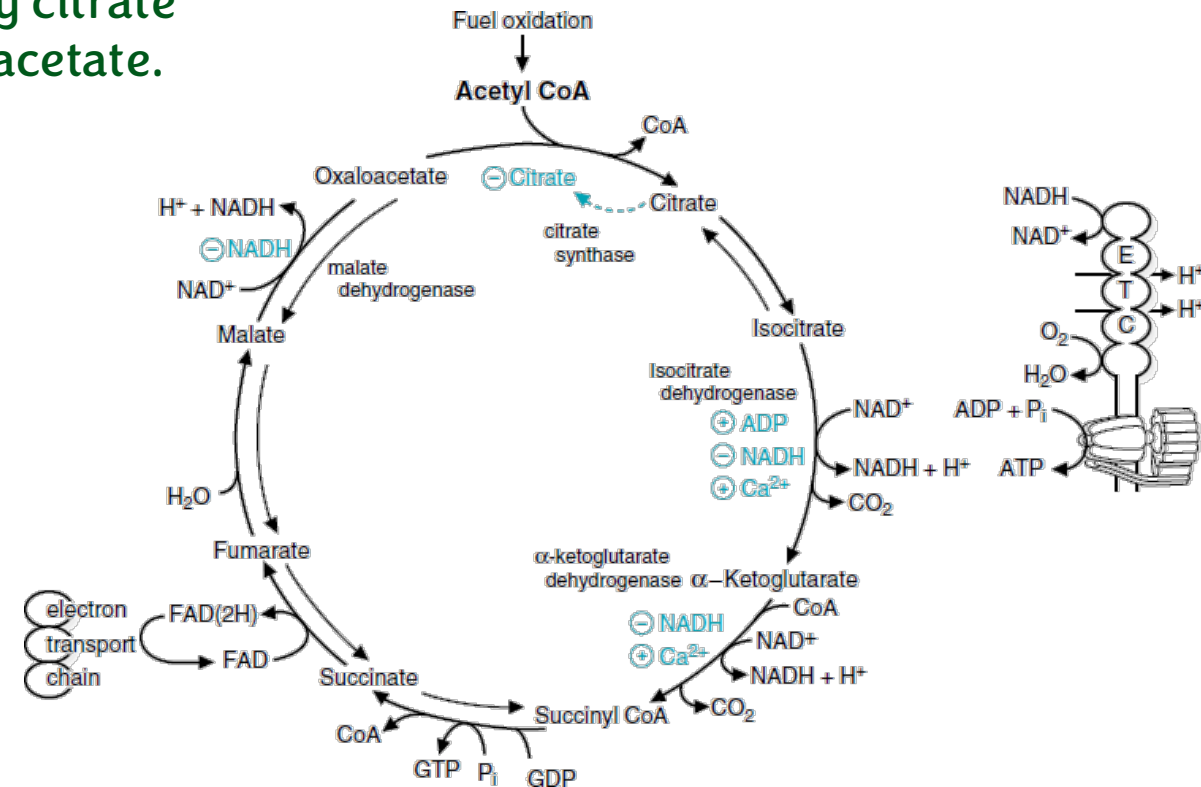
The first regulation occurs in the reaction catalyzed by citrate synthase producing citrate from acetyl CoA and oxaloacetate.

## ➤ Rate regulated by oxaloacetate & citrate (inhibitor)

- ✓ Oxaloacetate regulates this reaction and pushes it forward, whereas the citrate (the product of this reaction) will have a feedback inhibition mechanism that inhibits the same enzyme (citrate synthase) if the concentration of citrate is high.

## ➤ ATP acts as an allosteric inhibitor of citrate synthase

- ✓ High concentrations of ATP molecules act as an allosteric inhibitor for citrate synthase enzyme, and this is logical because if we have high ATP levels, this indicates that the energy state in the cell is high, so there is no need to activate Krebs Cycle to make more energy and it is logical to start this inhibition as early as the first step of the cycle.



# Regulation-Citrate and Citrate Synthase

More as Interactions rather than regulations

Other interactions of citrate molecule with other pathways

## ➤ Effect of citrate:

➤ Allosterically inhibits PFK, the key enzyme of glycolysis

✓ Citrate allosterically inhibits PFK-1 (phosphofructokinase), the enzyme that is involved in glycolysis, Or activates glycolysis → it actually catalyzes the rate limiting step of glycolysis.

➤ Stimulates fructose-1,6-bisphosphatase, a key enzyme of gluconeogenesis

✓ Citrate stimulates the enzyme fructose-1,6-bisphosphatase, this enzyme is going to remove the phosphate of fructose-1,6-bisphosphate and make it fructose-6-phosphate resulting in inhibition of glycolysis and activation of gluconeogenesis (the opposite pathway of glycolysis).

➤ Activates acetyl CoA carboxylase, a key enzyme of fatty acid synthesis

✓ Also, high concentration of citrate can be transported through the transporter of it in the inner mitochondrial membrane to the cytosol where it can be used to generate acetyl CoA, and this acetyl CoA can be used to make fatty acids by activation of acetyl CoA carboxylase that is an important and a key enzyme in synthesis of fatty acids.

# Isocitrate DH

Isocitrate dehydrogenase is the other enzymes responsible for regulating Krebs Cycle

## ➤ Best regulation (rate-limiting)

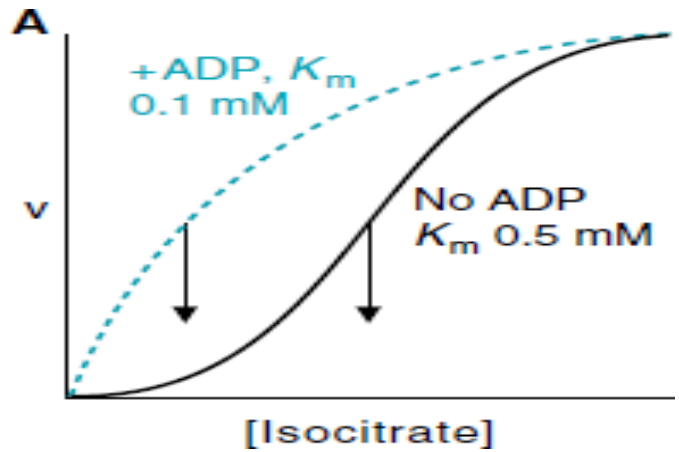
✓ Isocitrate dehydrogenase catalyses an irreversible step (from isocitrate to  $\alpha$ -ketoglutarate) which is actually the rate limiting step, so it provides the best regulation on this step

## ➤ Allosterically activated by (ADP, $\text{Ca}^{+2}$ ) :

- ✓ High level of ADP indicates low energy state in the cell, so Krebs Cycle should be activated by the activation of isocitrate dehydrogenase
- ✓ High level of Calcium ions indicates high activity and high need for energy in the cell, that's why Krebs Cycle needs to be activated to provide this energy needed

## ➤ Inhibition (NADH)

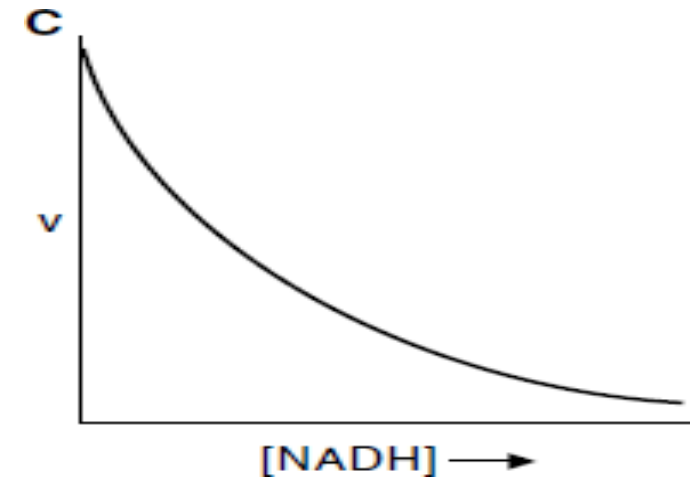
- ✓ High level of NADH acts as an inhibitor of this enzyme (isocitrate dehydrogenase)
- ✓ This step (from isocitrate to  $\alpha$ -ketoglutarate) as well as other steps in which redox reactions occur, need the molecules of Nicotinamide adenine dinucleotide to be in the oxidized form ( $\text{NAD}^+$ ), that's why high level of the reduced form (NADH) causes inhibitory effect -See figure 2 in the next slide-



No ADP vs. ADP ( $K_M$ ), a small change in ADP, great effect

- ✓ When ADP is present, the  $K_m$  is 0.1 mM, suggesting a higher affinity for isocitrate (the substrate)
- ✓ Without ADP, the  $K_m$  is 0.5 mM, meaning the enzyme has a lower affinity for isocitrate.
- ✓ This indicates that ADP enhances the enzyme's affinity (activates it) for its substrate, likely reflecting the enzyme's regulation in response to energy demand
- ✓ High ADP  $\rightarrow$  reduced  $K_m$   $\rightarrow$  less concentration of isocitrate-substrate needed to activate enzyme or reach higher speed

- ✓  $K_m$  represents the Michaelis constant.  $K_m$  is a measure of the substrate concentration at which the reaction velocity ( $v$ ) is at half its maximum ( $V_{max}$ )



Effect of NADH on the speed of reaction

- ✓ NADH again is needed in those reactions as a Coenzyme, in oxidation - reduction reactions that occur in TCA Cycle, that means they are needed in the oxidized form ( $NAD^+$ ), so high level of NADH causes inhibition for dehydrogenases in the TCA Cycle
- ✓ As we can see from the figure, any increase in the concentration of NADH in the cell will cause decrease in the speed of the enzyme .

- ✓ The inhibitory effect of NADH on malate and  $\alpha$ -ketoglutarate DH will be discussed in the next slides

# a-ketogluterate DH

The other regulated step in the TCA Cycle is an irreversible step catalyzed by a-ketogluterate DH

## ➤ Inhibited: NADH, succinyl CoA, GTP

- ✓ NADH will act as an inhibitor for this reaction, as it is needed in the oxidized form as a coenzyme NAD<sup>+</sup> in this reaction
- ✓ GTP as well acts as an inhibitor because it indicates high energy state, so it will inhibit Krebs Cycle overall and this reaction specifically
- ✓ Succinyl CoA is the product of this step that also acts as an inhibitor for a-ketogluterate dehydrogenase

## ➤ Activated: Ca<sup>+2</sup>

- ✓ High level of Calcium ions in the cytosol indicates high activity and high need for energy in the cell that's why Krebs Cycle needs to be activated to provide this energy needed



- ✓ **One of the steps that is also regulated by NADH is the one catalyzed by malate DH. However, because it is a reversible step, the regulation in this step is a little bit less than the previous steps although catalyzed by a DH**
  - ✓ **The previous steps are irreversible and the step catalyzed by ISOCITRATE DH is the rate limiting step, so it provides better regulation**
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- ✓ **ALL the mentioned factors are physiological activators or inhibitors because they are originally present in our cells**

# Inhibitors of TCA Cycle (Physiological?)

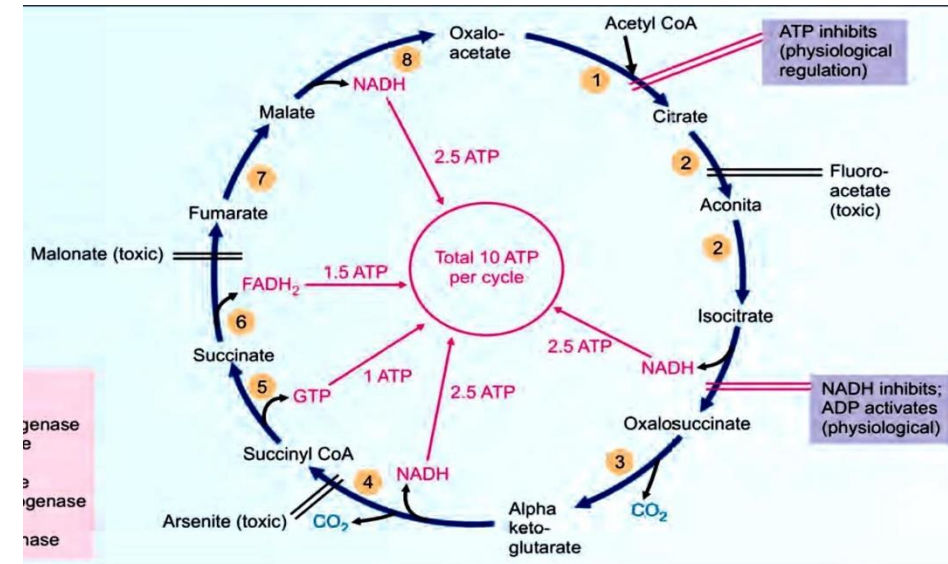
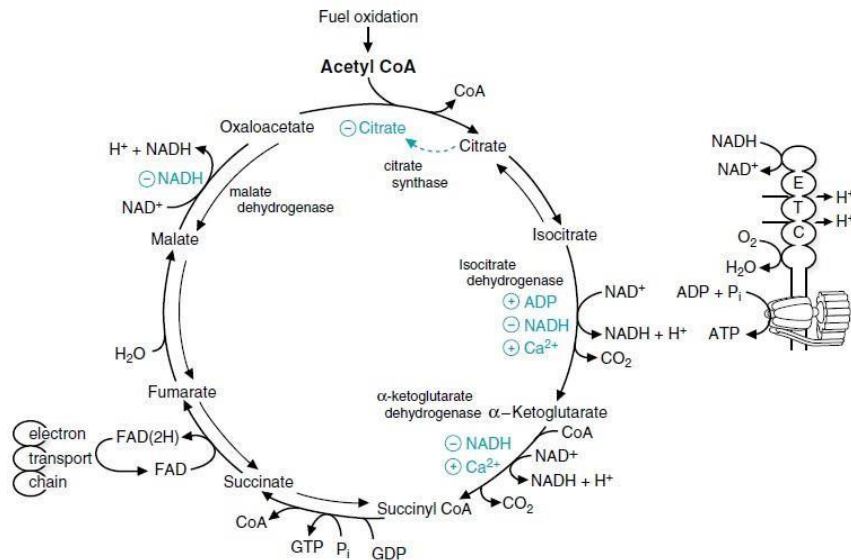
We may have other factors or regulatory molecules that come from outside sources , so they are non-physiological regulators as the examples mentioned

- ✓ **Aconitase** (citrate to aconitate) is inhibited by fluoroacetate (non- competitive inhibition)
- ✓ **Alpha ketoglutarate dehydrogenase** (alpha ketoglutarate to succinyl CoA) is inhibited by Arsenite (non- competitive inhibition)

Arsenite is a trivalent As that might be obtained from different sources like mining, volcanoes, some geological resources, sometimes from water contaminated with arsenic, different components of the food chains, so it can reach the human body and affect TCA Cycle by inhibiting  $\alpha$ -ketoglutarate dehydrogenase which is a highly regulated step in this cycle making this inhibitor have high toxicity.

✓ **Succinate dehydrogenase** (succinate to fumarate) is inhibited by malonate (competitive inhibition)

- ✓ **Malonate is important (as a pretreatment) to reduce the size of infarct after heart attacks**
- ✓ **Myocardial infarction leaves infarct which is a site where the cells are going to die because of ischemia or low blood supply, so when we reperfuse or reactivate these regions, malonate can be used to reduce the infarct size in this case. Although it is an inhibitor, it can be used as a treatment.**
- ✓ **Malonate is not a source of hazard like other mentioned examples.**



✓ **These two pictures will be summarized in the following table**

<b>Step of TCA Cycle or enzyme</b>	<b>Regulator</b>	<b>Effect</b>	<b>Physiological/non-physiological factor</b>
<b>Citrate synthase</b>	Citrate	Inhibition	Physiological
	Oxaloacetate	Activation	Physiological
	ATP	Inhibition	Physiological
<b>Citrate isomerase (Citrate to aconitate specifically)</b>	Fluoroacetate (toxic)	Inhibition (non-competitive)	Non- physiological
<b>Isocitrate dehydrogenase</b>	ADP	Activation	Physiological
	NADH	Inhibition	Physiological
	Ca+2	Activation	Physiological
<b>a-ketoglutarate dehydrogenase</b>	NADH	Inhibition	Physiological
	Ca+2	Activation	Physiological
	Succinyl CoA	Inhibition	Physiological
	GTP	Inhibition	Physiological
	Arsenite (toxic)	Inhibition (non-competitive)	Non-Physiological
<b>Malate dehydrogenase</b>	NADH	Inhibition	Physiological
<b>Succinate dehydrogenase</b>	Malonate (toxic)	Inhibition (competitive)	Non-physiological

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	12	Oxaloacetate and ATP affect isocitrate	Oxaloacetate and ATP affect citrate synthase
	12	Added information	Malonate is a competitive inhibitor
V1 → V2	10	The doctor said by mistake that "Aresnite is a trivalent Ar"	Aresnite is a trivalent As

## Additional Resources:

## رسالة من الفريق العلمي:

"اللهم اكسر بنا شوكتهم، اللهم نكس بنا رايتهم، اللهم أذل بنا قاداتهم، اللهم حطم بنا هيبتهم، اللهم أزل بنا دولتهم، اللهم أنفذ بنا قدرك فيهم بالزوال والتدمير والتتبير يا رب العالمين، حقق بنا آمال شعبنا بالعودة والتحرير".

- يحيى السنوار.