University of Jordan
Faculty of Medicine

Medical Committee
The University of Jordan

Biochemistry

☑ Sheet ○ Slides

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Title: TCA Cycle Regulation

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CAC: citric acid cycle DH: dehydrogenase (refer to slides)

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BIOENERGETICS OF CAC

Efficiency OF CAC : is the ratio of how many calories we actually get out of CAC to the theoretical amount that we get due to complete consumption in a closed chamber.

If we have citrate in a closed chamber we will get 228 kcal/mol & then if we calculate how much energy we are getting actually out of CAC (look to the table) the total sum of all molecules is 207 kcal/mol

Efficiency = 207 / 228 = 90%

It is very hard to find a machine in this efficiency.

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

* What drives the CAC is three large negative ΔG (exergonic) reactions.
* Theoretically all CAC reactions are reversible reactions (they can go in either directions), essentially they are irreversible **WHY** ??

Because there are three large -ΔG reactions which makes the reaction goes forward all the time.

*There is two +ΔG reactions; the 1st one which catalyzed by Aconitase & another reaction which catalyzed by Malate DH.

***Aconitase** catalyzes the isomerization reaction (the convergence of citrate to Isocitrate) this reaction is a +ΔG reaction which means that this reaction prefers the reverse direction (the reaction goes backward more).

→ This creates more citrate than Isocietrate at equilibrium & the ratio between their concentrations is 20:1

*Why do we have citrate more than Isocitrate ?

The physiological relevance to this is that citrate can be used by other pathways.

→ When citrate accumulates:

a- It inhibits the glycolysis by inhibiting phosphofructokinase (catalyzes the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate)
b- Leaves the mitochondrial matrix to the cytosol where it will be cleaved and then used for synthesis of cholesterol & fatty acids

*Same story with **Malate DH** which catalyzes convergence of Malate to Oxaloacetate, also has a +ΔG

PS: these +ΔG reactions proceed in the forward direction in the body because the body deals with the pathways as 1 reaction, and we have to remember that the ΔG is additive.

* The physiological relevance behind having high ratio of [malate]/[oxaloacetate] is that Malate is being used for other pathways in the body mainly the **Gluconeogenesis** which is a very important pathway that produces glucose from other non-carbohydrate sources, which occurs during fasting, between meals if your glucose level gets low…etc.

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Regulation of CAC

How the CAC is regulated?

*Feed back inhibition has a great role in CAC

* CAC gives two kinds of products:
  - A- immediate products (NADH, FADH2, GTP, CO2)
  - b- Eventual product (ATP)

*Which of the immediate products regulates the CAC

→ we have two main regulators of CAC:

a- [ATP:ADP]

* If the [ATP] gets high, this affects the CAC by making it slower, and If the [ADP/ATP] gets high then the CAC will be activated

*Q: Does [ADP/ATP] affect all enzymes?

→ No because not all enzymes have specific site to bind with ADP or ATP

b- [NAD^+]:[NADH] . If **[NAD^+]** gets higher, the cycle will be activated, and if **[NADH]** gets high → feedback inhibition, the cycle will slow down

* We do not consider [FAD]/[FADH] as a regulator because it is bounded to enzyme, so it is not free in the solution & can't go & affect other enzymes, it just regulates the enzyme that it is bounded to .

*** Q in the exam: how the CAC is regulated ?**

→ we should care about these notes:

- high[NADH] → inhibit
- high[NAD^+] → activates
- high[ATP] →inhibit
- high[ADP] → activates

Which enzymes regulate the CAC?

Citrate synthase

- **It catalyzes the first step -also considered the rate limiting and the committed step- in CAC**: it is always logical to control the first step of the reaction to save energy since we don't need to synthesize the intermediates.
- **Not an allosteric enzyme** (so the regulation isn't allosteric)
- **This type of regulation depends on substrate (oxaloacetate) & products (citrate)**

*Citrate works as a feedback inhibitor for citrate synthase

*High [citrate] will inhibit the action of citrate synthase by binding on the active site of the enzyme, since it is not an allosteric enzyme.

*As we said Aconitase reaction has a +ΔG so it usually provides high [citrate], logically, the CAC should not be activated all the time and then inhibited when it is not needed, since it produced energy and we need that energy. Instead, it works all the time but at a low speed.

*CAC works all the time

1- at low speed because of higher [citrate] which is facilitated by Aconitase

2- low [oxaloacetate], which is facilitated by Malate DH **WHY?**

→ Because Malate DH catalyze a reaction with a +ΔG which prefers higher [Malate] over [oxaloacetate]

How this happens:
1st: when we need CAC →the citrate reaction will go forward → then the [citrate] gets low → the inhibition of citrate synthase will be reduced, operates more, equilibrium tampered → [Malate] increase → produces more oxaloacetate & this is how the reaction gets more efficient

2nd: Malate DH provides an equilibrium at which [malate] is greater than [oxaloacetate], however its concentration is less than the K_m of the enzyme, so the reaction isn't that efficient and doesn't go → when the reaction goes forward → [Malate] get higher than the K_m → then the reaction will move toward the oxaloacetate.
PS: K_m: is the concentration of the substrate to achieve 50% of V_{max}
*What makes Malate DH get affected by this:
→ It depends on energy "NADH:NAD^+" (if I have energy this means high [NADH] → inhibition of Malate DH → leads to (and maintains) low [oxaloacetate] → inefficient action of citrate synthase (because the substrate is low). (Vise versa)

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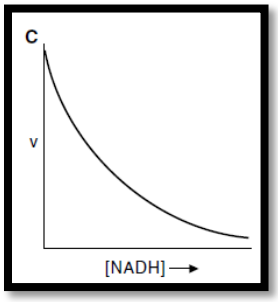
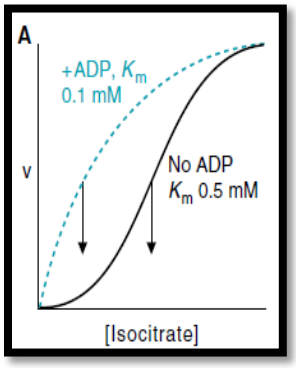
Isocitrate DH

- **The most important enzyme in controlling the CAC**
- **It is a rate limiting step in the CAC**
- **It is allosterically regulated by ADP (the only regulated DH by ADP)** – activates enzyme
- **It is allosterically regulated by Ca** – activates enzyme
- **It is regulated non-allosterically by the NADH it produces** (NADH inhibits the DH through a non-allosteric manner by binding to the active site)



*Allosteric enzymes adopt a sigmoidal fit for their velocity
*Why sigmoidal fit??
→Because of the cooperative property. Initially the velocity will be low; then as the first substrate binds to one of the subunits it will increase, this enhances the binding of the next substrate so velocity increases further, until the enzyme is saturated.
* Adding activator (for example ADP) will shift the fit to the left to be more hyperbolic than sigmoidal
* Adding inhibitor will shift the fit to the right to be more sigmoidal and less hyperbolic



α- ketoglutarate DH

- Produces NADH and Succinyl CoA which work as an inhibitor for the reaction
- GTP works also as an inhibitor for the reaction
- Ca works as an activator for the reaction (allosterically)

Why Ca activates both enzymes (α- ketoglutarate DH, Isocitrate DH)?
Ca is a product of muscle contraction, so increasing workload of a muscle increases the Ca levels, which then send massage to CAC to produce more ATP

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Intermediates of CAC

→ Compounds getting produced & consumed again through the reactions in the cycle (8 compounds); these intermediates have functions in other pathways.

§ Citrate
If the concentration of citrate get high in the mitochondria then it can leave the mitochondria to cytosol & then broken down to be used in fatty acid synthesis & cholesterol synthesis

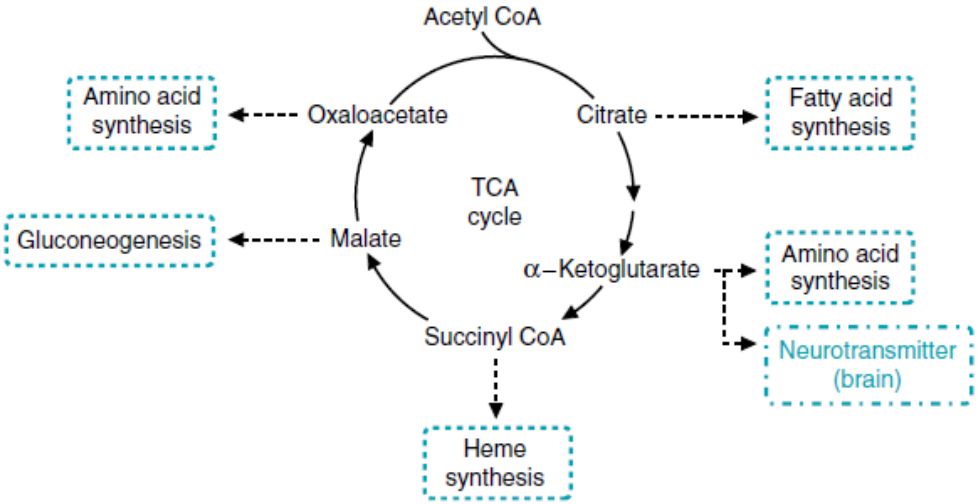
§ α-ketoglutarate
It can be converted to glutamate (amino acid synthesis) & glutamine (most important carrier for the ammonia in the blood)
* Glutamate in nervous system can give GABA (gamma amino butaric acid) which is a neurotransmitter in the brain.

§ Succynil CoA
It can be used threw a long pathway in Heme synthesis

§ Malate
It is crucial for the process of gluconeogenesis, because of that Malate is kept in high concentrations

§ Oxaloacetate
It can be converted to aspartate through aspartate transaminase enzyme → so it can participate in amino acid synthesis

*If the concentrations of the intermediates get low and we need more of these intermediates there are other reactions in the body which can replenish these intermediates, we call these reactions (Anaplerotic reactions) – pyruvate carboxylase is the most important one.



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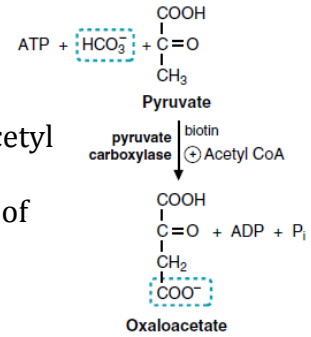
* Pyruvate has 3 carbon atoms, it get carboxylated by <u>pyruvate carboxylase</u> to produce oxaloacetate and replenish it.

→This enzyme is aided/catalyzed by Biotin (vitamin B7)

→This enzyme can be (+) regulated by Acetyl CoA **why?**

- Because when [acetyl CoA] gets high then we need more oxaloacetate to join this acetyl CoA for the cycle to start, so [acetyl CoA] gets high → activates pyruvate carboxylase, then we get more oxaloacetate to join Acetyl CoA which leads to synthesis of citrate.

*This enzyme has high concentration in tissues



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α-ketoglutarate, can be replenished through amino acids such as glutamate, which can be converted to α-ketoglutarate in two main pathways:-

a- Transaminase, transamination reaction by moving the amine group from glutamate to other amino acid, then it will be α-ketoglutarate .

b- Removing of the amino group totally from glutamate by the action of Glutamate DH (GDH) and then the result will be α-ketoglutarate and a free amino group

→ Any of these two pathways we can convert amino acids to α-ketoglutarate.

Valine & isolucien can be converted to propenyl CoA that can in turn be converted to succinyl CoA (replenish it)

6 Amino acids in metabolism can replenish Fumarate

Aspartate transaminase can convert aspartate to oxaloacetate.

Sorry for any mistakes