فريق طوفان الاقصى

METABOLISM

Modifide N. **6**

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PYRUVATE METABOLISM , TRICARBOXYLIC ACID CYCLE, AND ELECTRON TRANSPORT CHAIN

اللهم ارزق إخواننا في فلسطين الصمود والقوة في وجه الطغيان وانصرهم.
اللَّهُمَّ نجِّ المُسْتَضْعَفِينَ مِنَ المُؤْمِنِينَ.
اللهم انصر ضعفهم وَرُد إلينا المسجد الأقصى ردًا جميلًا.
اللهم إنا لا نملك لأهلنا في فلسطين إلا الدعاء.
اللهم احفظهم بحفظك وانصرهم واخذل كل من خذلهم.

METABOLISM

1st SEMESTER, 2023

DR. NABIL BASHIR

اللهم لا تخيب رجاءنا وأنت أرحم الراحمين نسألك لأهل فلسطين النّصر على من عاداهم، عاجلًا غير آجل يا رب العالمين.
اللهم احفظ أرواح المجاهدين في فلسطين، وردهم إلى أهلهم مردًا كريمًا آمنًا.

- ***All* details within this lecture's slides are required for memorization (good luck)**

PYRUVATE METABOLISM

Aim: to explain the mechanism and control of pyruvate dehydrogenase, the multienzyme system responsible for the conversion of pyruvate to acetyl-CoA.

Content:

1. The reaction **mechanism** involved in the conversion of pyruvate to acetyl-CoA.
2. The organization of the **3 enzymes-E1, E2, E3-** of the multienzyme complex.
3. The **5 coenzymes** involved in the reaction and the 5 B vitamins from which they derived.
4. The **allosteric and covalent modification** of the kinase and phosphatase controlling E1.

OBJECTIVES

1. Write out the reactions involved in the conversion of pyruvate to acetyl-CoA catalyzed by pyruvate dehydrogenase
2. Explain the functions of TTP, lipoate, coenzyme A, FAD, and NAD in the pyruvate dehydrogenase-catalyzed reaction.
3. Demonstrate that you understand how the activity of the enzyme is influenced by insulin and fed state.
4. Demonstrate that you understand how the liver enzyme is controlled in the fasted state when that organ is a glucose producer
5. Explain the **central role of pyruvate** and acetyl-CoA in metabolism.

- We will see how TCA cycle is used to oxidize acetyl co-A into 2 molecules of CO_2 + the production of some reducing powers that are important for energy production.

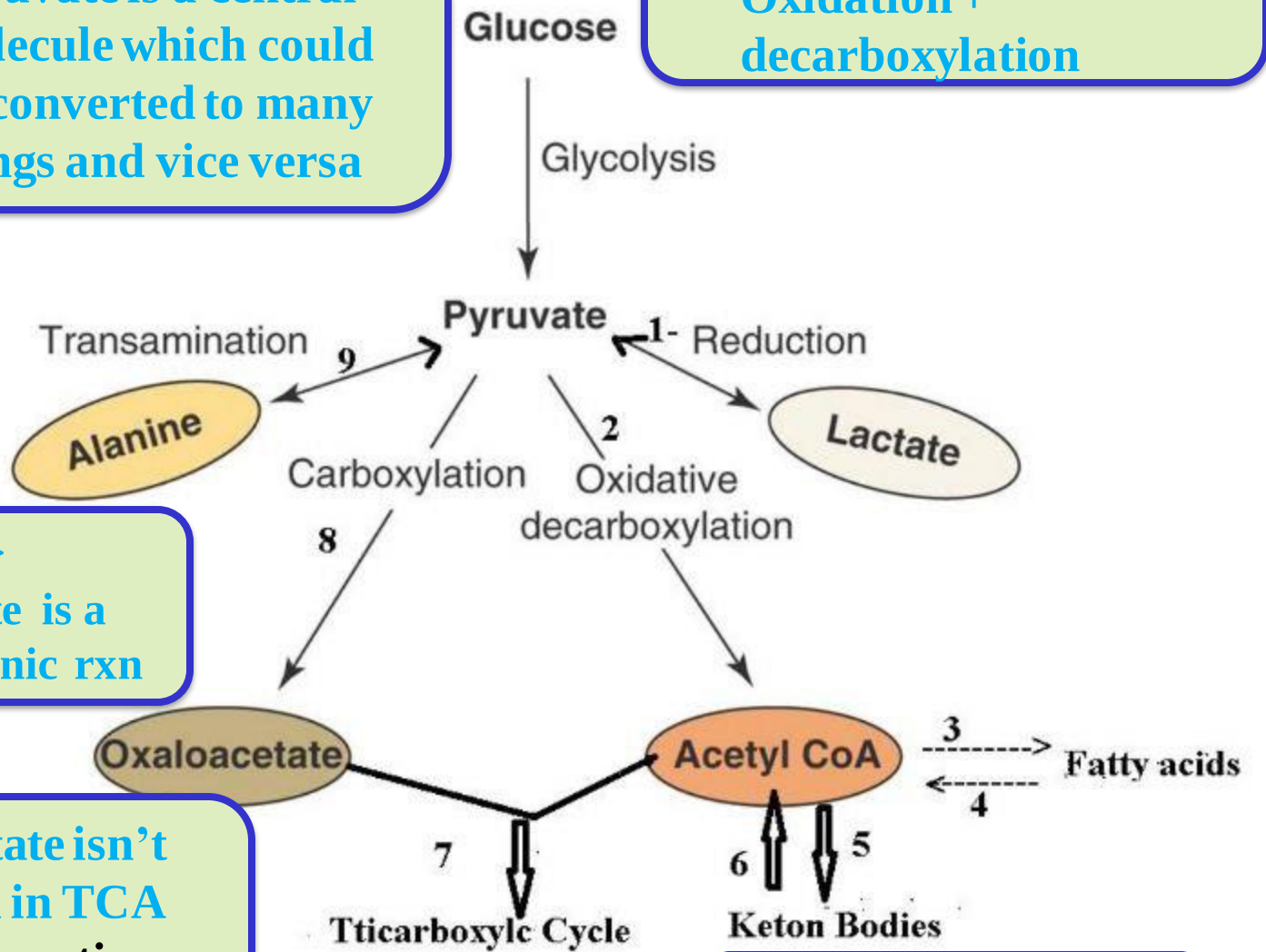
- As we have mentioned in the stages of catabolism, stage 2 involved the production of 2 pyruvate molecules from glucose. Also, pyruvate can be produced from Lactate using anaerobic glycolysis.

Pyruvate is at important metabolic crossroads

1. Lactate dehydrogenase
2. Pyruvate dehydrogenase
3. Fatty acid synthesis
4. Fatty acid beta- oxidation
5. Ketone body synthesis
6. Ketone body utilization
7. Citrate synthase
8. Pyruvate carboxylase
9. transamination

- As you could see, Pyruvate is a central molecule which could be converted to many things and vice versa

- Oxidative decarboxylation : Oxidation + decarboxylation



- Pyruvate -> Oxaloacetate is a Gluconeogenic rxn

- Oxaloacetate isn't consumed in TCA cycle(explanation later)

- And Cholesterol

PYRUVATE DEHYDROGENASE:

Oxidative decarboxylation of pyruvate to acetyl CoA

The reaction occurs in mitochondrial matrix

- This enzyme has 3 enzymatic activities

- Which is a highly-regulated enzyme and used in the metabolism of many biomolecules

3 enzymes, 5 coenzymes-thiamin pyrophosphate(B1), lipoamide, Flavin adenine dinucleotide (B2), coenzyme A (contain B3), and NAD (niacin)-are required.

E1 : Pyruvate dehydrogenase decarboxylase

E2 : Dihydrolipoyl transacetylase

E3 : Dihydrolipoyl dehydrogenase

- These 3 enzymes will require 5 co-enzymes (which are mostly vitamins) to carry out Pyruvate oxidation, any deficiency in these vitamins would affect the entire metabolic activity of the body (because it affects the core enzyme of this activity)

In addition , there are two enzymes, a kinase and a phosphatase, which have key role to play in the control of pyruvate dehydrogenase complex.

- Kinase —> adds phosphate groups
- Phosphatase—> removes phosphate groups

Phosphatase action on E1 **activates** it, **phosphorylation** of E1 by the kinase causes **inactivation**.

Several key metabolites such as CoASH, acetyl-CoA, NADH affect the activity of the kinase and phosphatase

It is important to emphasize the irreversible nature of the reaction catalyzed by the PDH complex. Thus acetyl CoA CANNOT be converted to pyruvate by any known enzyme or pathway: this is the reason that a net conversion of acetyl CoA from fatty acid catabolism to carbohydrate cannot occur in mammals.

- **E1: Pyruvate Dehydrogenase Carboxylase uses TPP**

  E2: Dihydrolipoyl transacetylase uses Lipoamide (transfers the acetyl group from TPP to it) and Co-Enzyme A (by transferring the acetyl group from the Lipoamide to it)

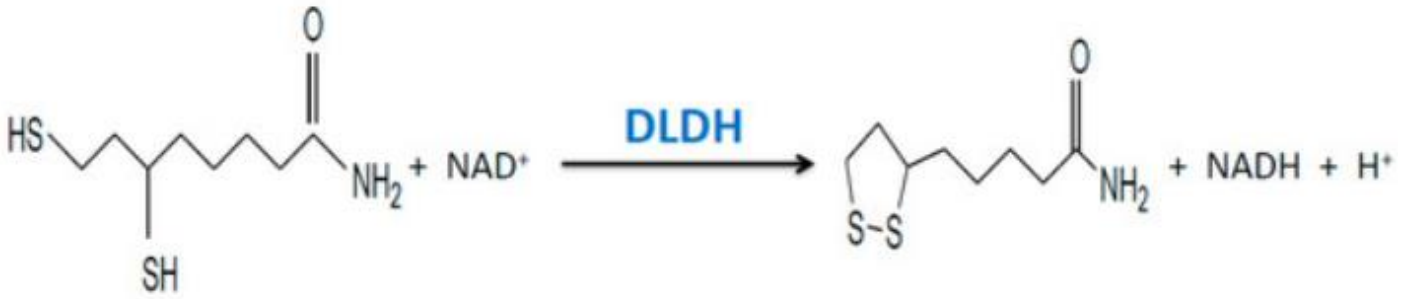
  E3: Dihydrolipoyl Dehydrogenase uses the energy conducted from the transfer of acetyl group from Lipoamide to Co-Enzyme A to Reduce FAD to FADH2 and then FADH2 reduces NAD+ to NADH + H+

- **Conversion of Acetyl-CoA back to pyruvate?**

  The professor emphasised on the point that Acetyl-CoA CANNOT be converted back to pyruvate, so glucose cannot be synthesized, otherwise, acetyl co-A can be used to make fatty acids; as there isn't any known enzyme in mammals to convert Acetyl-CoA back to pyruvate/glucose.That's why we can't synthesize glucose from fats, but fats can be synthesised from glucose.

  This irreversible nature of the Pyruvate Dehydrogenase Enzyme is why people who consume a lot of carbohydrates on a daily basis have more fats within their body.

- **The professor uses the terms Lipoic acid, Lipoamide, and Dihydrolipoyl (Dihydrolipoamide) very interchangeably. The reason (as shown below) is because the CoEnzyme used changes between Lipoamide and Dihydrolipoamide to carry out the reduction of FAD which is used to reduce NAD+ in turn. Refer to the next slide for a better understanding on this reaction.**



Dihydrolipoamide lipoamide

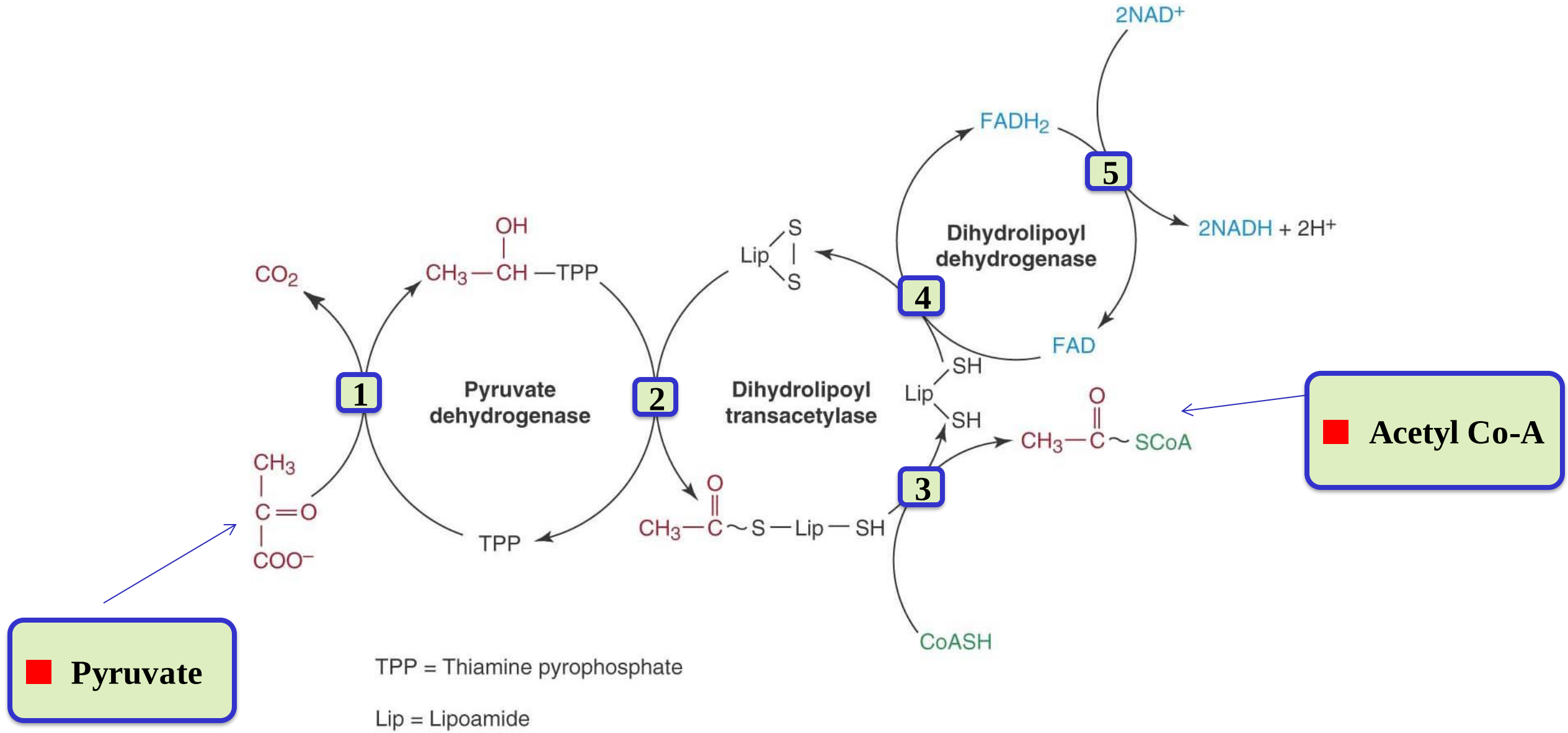


Figure 14.14 Mechanism of the pyruvate dehydrogenase multienzyme complex.

- **Rxn #1: Pyruvate Dehydrogenase Decarboxylase uses Thymine Pyrophosphate (TPP) as a coenzyme by binding to the Acetyl group (-CH3COO) of the pyruvate , releasing carbon dioxide as a product of decarboxylation.**

- **Rxn #2: Following the first rxn, the Acetyl-bound TPP will transfer its acetyl group to a Lipoamide group using the Dihydrolipoyl Transacetylase Enzyme which by opening the cyclic group in the Lipoamide (that is shown in slide 8) and binding the transferred acetyl to one of the sulfur groups of the lipoic acid. TPP is released to be reused in the first rxn.**

- **Rxn #3: Dihydrolipoyl Transacetylase carries out a second reaction by transferring the acetyl group bound to Dihydrolipoamide to the sulfur end of CoEnzyme-A (read this slowly and you should understand), this reaction produces the Acetyl Co-A needed for the next step of Cellular Respiration, and also produces the reduced form of Dihydrolipoamide (where both sulfur ends have hydrogen) which will be used in the next rxn (in the oxidized form) .**

- **Rxn #4: The reduced form of Dihydrolipoamide will be used by the third enzyme in this complex, Dihydrolipoyl Dehydrogenase, by oxidizing it into its Lipoamide form (the closed cyclic group) and using the two hydrogens released from the oxidation to reduce the FAD CoEnzyme into FADH2.**

- Rxn #5: The produced FADH2 in Rxn #4 will be used to reduce NAD+ into NADH + H, which releases FAD (coenzyme) back to the enzyme complex to oxidize another Dihydrolipoamide.

- So to sum up. Pyruvate was decarboxylated (producing CO2) into an acetyl group which was transferred into Acetyl-CoA. And these reactions were used to reduce NAD+ into a NADH + Hydrogen.

  It is good to note that FADH2 is NOT a product of this reaction, since it is recycled back into the enzyme as FAD.

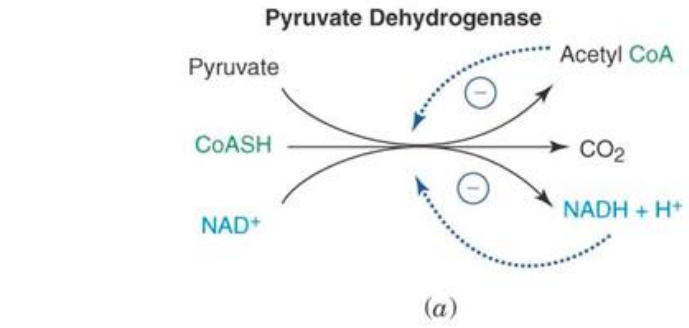
The final products of the Pyruvate Dehydrogenase Enzyme are :

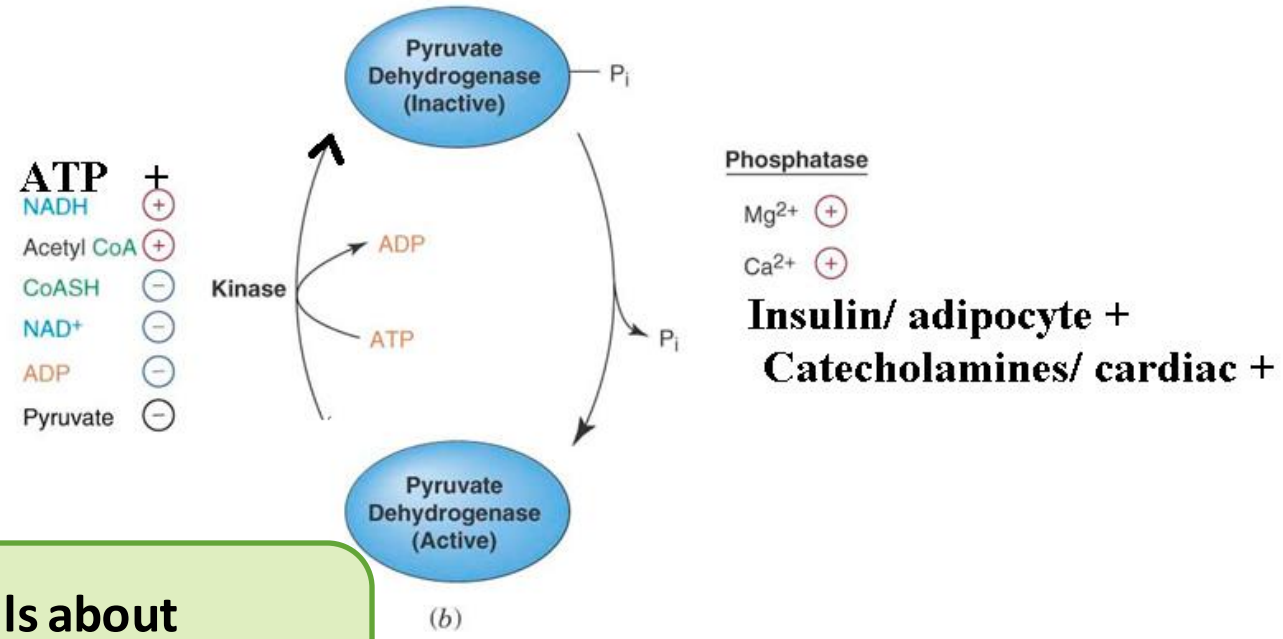
Acetyl-CoA + CO2 + NADH + H+

Control of Pyruvate Dehydrogenase;

1. In the **fed state** in the liver this rex should be **turned on**" in order that glucose can be efficiently converted to fatty acids.
2. During the **fasted state** this rex should be **turned off** in order that pyruvate will all be driven into gluconeogenic pathway.
3. NADH/NAD, ACETYL COA/COA effects
4. Mg and Ca++
5. Insulin & catecholamines.

Ref: Molecular biology and biochemistry of pyruvate dehydrogenase complexes, Mulchand Patel and Thomas Roche, The FASEB Journal 4: 3224-3233, 1990





- These products will regulate PDH via feedback inhibition.

- **Details about Kinase/Phosphatase in the next slide**

- You might remember the reversible covalent modification we've taken in Biochemistry as a regulation mechanism of enzymes. The same happens to Pyruvate Dehydrogenase through the use of PDH Kinase and PDH Phosphatase

- The PDH kinase, which phosphorylates the E1 component, is activated by High Energy Charged molecules such as ATP, NADH and Acetyl-CoA. Which causes the PDH to be INHIBITED. While Low Energy Charged molecules such as CoASH, NAD+, ADP and Pyruvate would inhibit the kinase, which means no more phosphorylation, causing the PDH to be ACTIVATED). This makes sense because while there are high amounts of high energy molecules, there will be no significant need for the TCA cycle and the enzyme will be inhibited (feedback inhibition).

- The PDH Phosphatase, which removes the phosphate group from the E1 component (causing the PDH to be ACTIVATED), is activated by Mg+2, Ca+2, Insulin in adipocyte tissue and Catecholamines in Cardiac tissue. This is a type of covalent modification, the phosphorylated form —> inactive, the dephosphorylated form —> active

TCA(TRICARBOXYLIC ACID) CYCLE, KREB'S CYCLE, CITRIC ACID CYCLE, AND ELECTRON TRANSPORT CHAIN AND OXIDATIVE PHOSPHORYLATION

- **Aim:** To explain the reactions of krebs tricarboxylic acid cycle and the associated electron transport chain and oxidative-phosphorylation.

Contents:

- The reactions of TCA.
- The fate of carbons from OAA and acetyl CoA in the TCA cycle.
- NADH, FADH2 and GTP production.
- Substrate level formation of GTP.
- Succinate dehydrogenase and FAD.
- The control of TCA cycle.
- Shuttles of cytosolic NADH.
- The organization of electron transport chain.
- Iron sulfur proteins, ubiquinone and cytochromes. Cytochrome c oxidase.
- Inhibitors of electron transport chain-action of rotenone, antimycine A, carbon monoxide and cyanide.
- Theories of oxidative phosphorylation.
- ATP synthase.
- Uncoupling of oxidation and phosphorylation.
- Action of oligomycin. ATP yield from aerobic metabolism of glucose.

Objectives

1. Write the reactions of TCA and follow the fate of the 2-carbon unit in acetyl-CoA.
2. Identify the reactions in which NADH is formed
3. Recognize the reactions of TCA where GTP and FADH2 are generated.
4. Define those reactions of TCA where energy charge and NADH/NAD controls the rate.
5. Demonstrate an understanding of the 5 complexes in the ETC.
6. Identify those reactions in ETC where protons may be generated
7. Demonstrate knowledge of the sites of action of inhibitors of ETC.
8. Explain how proton gradient is generated and its anatomical relationship of ATP synthase.
9. Understand how uncoupler of OXPHOS works and the consequences of its action on respiratory control in mitochondria.
10. Be able to calculate high energy phosphate production associated with aerobic and anaerobic metabolism of carbohydrates and fatty acids

IMPORTANT FEATURES OF TCA CYCLE

- **Amphibolic means that it's used for both catabolism (energy generation) and anabolism (biosynthesis)**

All of the major **nutrients** can be converted to **acetyl CoA** in the first 2 stages of metabolism.

The complete **oxidation of acetyl group** of acetyl CoA to CO2 and water is accomplished by the enzymes of TCA cycle –stage 3.

It is a vital pathway for metabolism in all aerobics and occupies of a central position in metabolism because it is the **common pathway** for the oxidation of all major nutrients-carbohydrates, lipids, and proteins.

It provides **intermediates for the synthesis** of biomolecules- it is **amphibolic**.

The oxidation of acetyl unit **(and production of CO2)** results in the reduction of NAD & FAD to NADH+H and FADH2.

The hydrogens or electrons of these reduced cofactors, are transferred to oxygen to form water via ETC

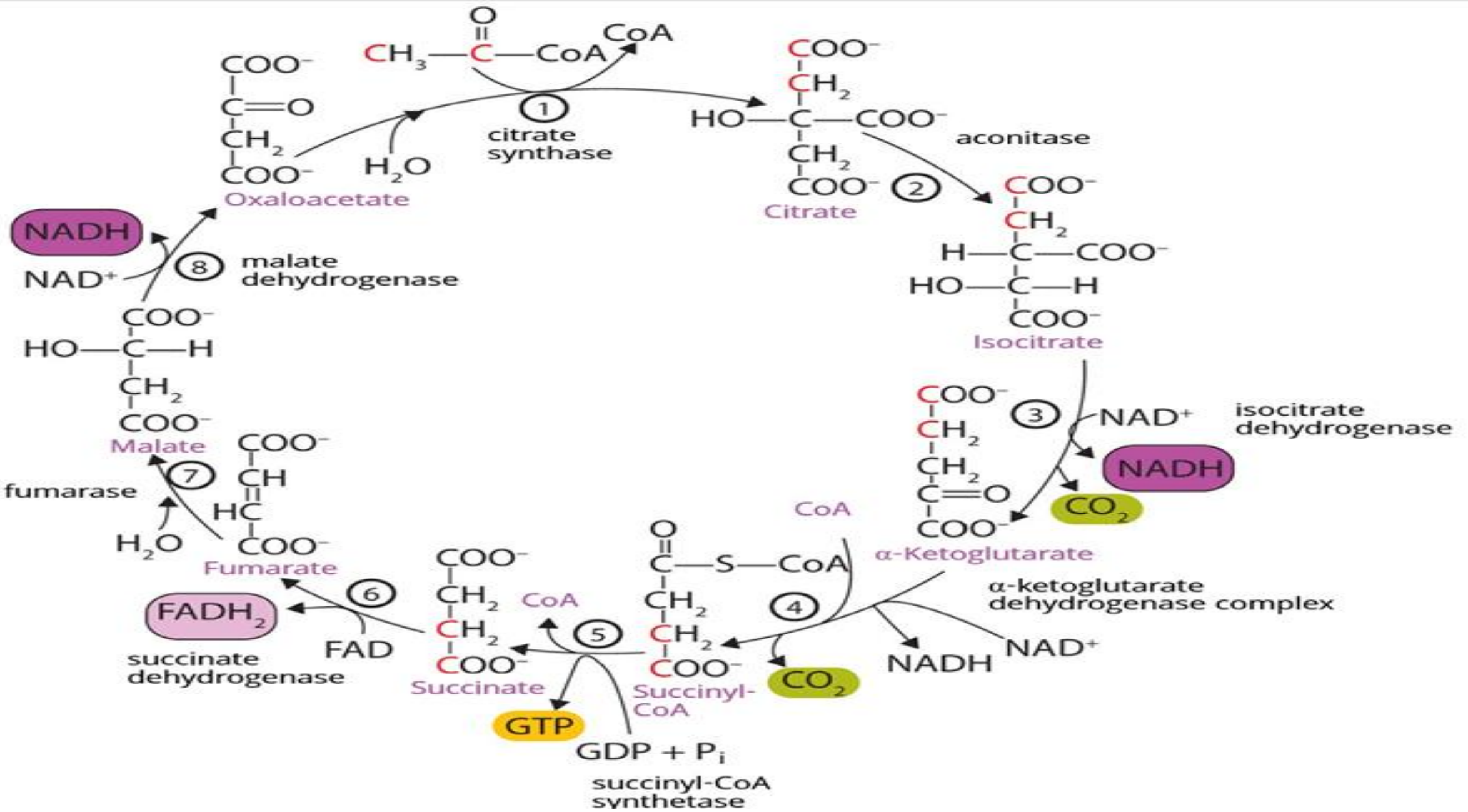
ATP is generated as electrons are transferred to oxygen.

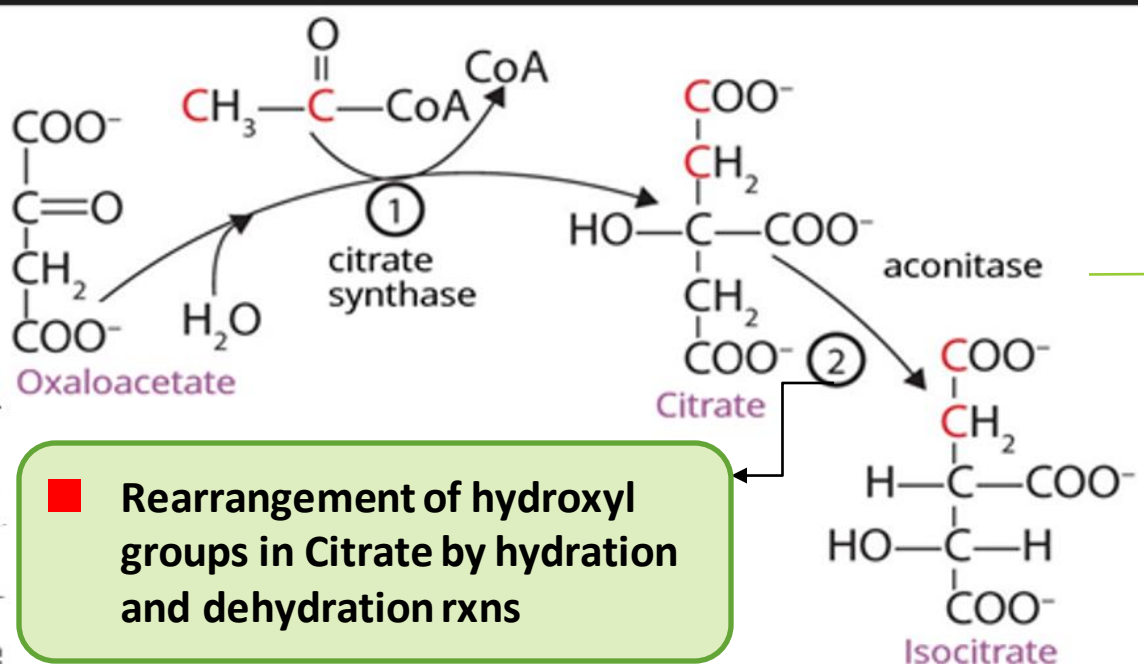
The reactions of TCA occur in the mitochondrial matrix except succinate dehydrogenase

- TCA cycle is very important and a central pathway in metabolism that oxidizes acetyl coA that comes from any metabolic pathway, to fullfil the purpose of metabolism which is extracting energy.

- "Anaplerosis" is one of the importances of TCA cycle, which is the use of some intermediates for synthesis of other molecules.

- Oxaloacetate will be regenerated during reaction in order to receive another Acetyl CoA molecule and to oxidize it.
- Oxaloacetate is synthesised in a very low amount that is called "catalytic amount" which means an amount that ONLY suffices to react with available Acetyl CoA molecules and to undergo the reaction- > and that's the "Normal physiological condition".
- If TCA cycle requires oxaloacetate, it will be produce by a "Glucogenic Pathway (carboxylation of pyruvate)".

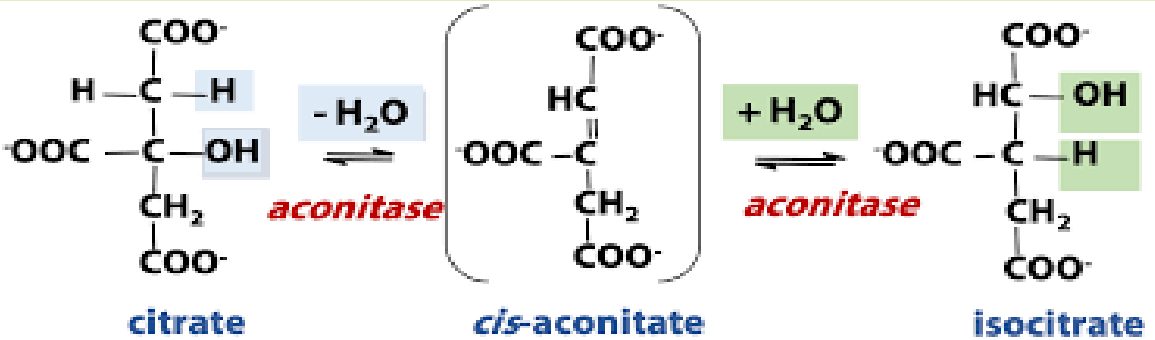

Oxaloacetate
citrate synthase
Citrate
aconitase
Isocitrate
isocitrate dehydrogenase
NADH
CO2
α-Ketoglutarate
α-ketoglutarate dehydrogenase complex
NADH
NAD+
CO2
Succinyl-CoA
GTP
GDP + Pi
succinyl-CoA synthetase
Succinate
succinate dehydrogenase
FADH2
FAD
Fumarate
fumarase
Malate
malate dehydrogenase
NADH
NAD+



- Rearrangement of hydroxyl groups in Citrate by hydration and dehydration rxns

- **EXTRA:**

Citrate TO Isocitrate:

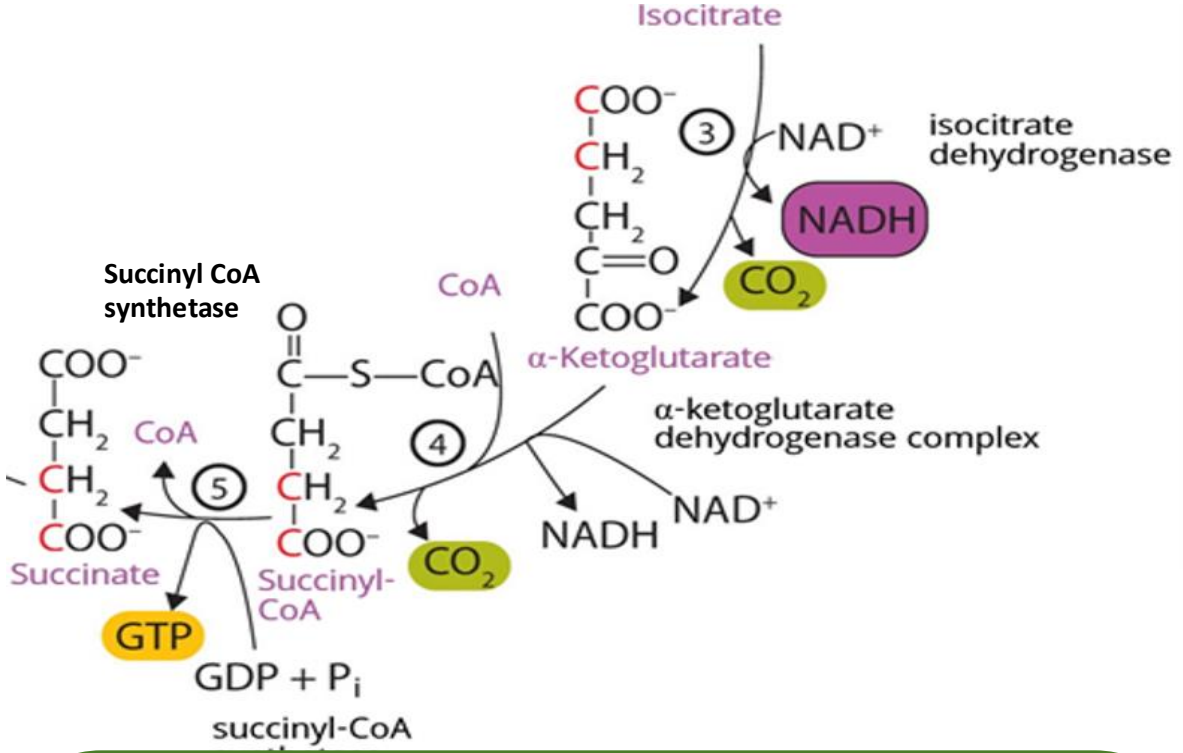


- All cells perform the TCA cycle
- Red carbons are from Acetyl CoA.
- The changes are done on Acetyl CoA's carbons ONLY, Oxaloacetate's carbons will not change.
- Citrate is a tricarboxylic acid (has three carboxylic group).
- It is synthesised by the condensation of Oxaloacetate with acetyl Co-A

- Citrate is important in TCA cycle and FA biosynthesis because citrate carries Acetyl CoA.
- FA biosynthesis requires Acetyl CoA as a precursor (building block) .
- Cistrate synthase is one of the site of regulation of TCA cycle -> It is inhibited by succinyl CoA
- Citrate inhibits glycolysis.

3-Isocitrate(6C)To alpha KG(5C)(oxidative decarboxylation):

- The first site of production of NADH(oxidation) and releasing CO2(decarboxylation).
- The carbon in the "CO2" released is equivalent to one of the 2 carbons that were in *Acetyl Co-A*
- This step is a "RATE LIMITING STEP"
- Step 3 is energetically favorable "ΔG is negative", it is activated by low energy charge "ex: AMP,ADP", and inhibited by high energy charge "ex: NADH, ATP"

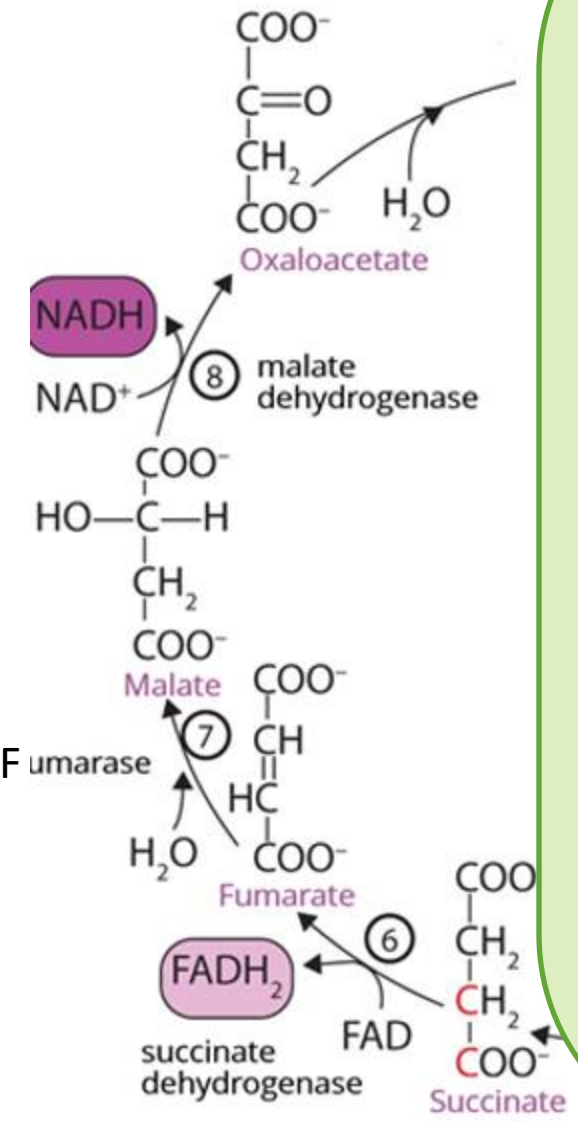


4-Alpha KG(5C)To Succinyl CoA(4C) (oxidative decarboxylation):

- Many amino acids could be converted to Glutamate then to alpha KG "anabolic pathway of alpha KG",
- Alpha KG to Succinyl CoA is the "catabolic pathway"
- Alpha KG could be converted to many amino acids.
- Second CO2 is released (also equivalent to one of the carbons in Acetyl CoA) and second NADH is produced.
- Alpha ketoglutarate dehydrogenase is regulated as isocitrate dehydrogenase & citrate synthase.
- Alpha ketoglutarate dehydrogenase resembles pyruvate dehydrogenase complex as it has "three enzymes, and 5 coenzymes".

5-Succinyl CoA(4C) To Succinate(4C):

- Succinyl CoA is a high energy compound because of thioester bond.
- The importance of this step is the phosphorylation of GDP to produce GTP and this energy comes from the hydrolysis of "thioester bond". "It is the ONLY step that produces GTP".
- The "S-CoA bond" is of high energy amount.
- Step 5 is the ONLY substrate level phosphorylation step in TCA cycle



6-Succinate To Fumarate (Redox):

- **Succinate dehydrogenase is a "Flavoenzyme: means that the enzyme use FAD (coenzyme) and converts it to FADH2, ONLY one in TCA cycle".**
- **Succinate dehydrogenase is found in the inner mitochondrial membrane NOT in the matrix as the other dehydrogenases.**

7-Fumarate To Malate (Hydration)

- **By adding a hydroxyl group**

8-Malate To Oxaloacetate (Redox):

- **Unfavorable reaction BUT it is required to regenerate oxaloacetate which is needed for another TCA cycle.**
- **Requires NAD+**
- **This rxn happens by 2 factors: 1. coupling reactions 2. The amount of oxaloacetate is very low "catalytic amount". So,Malate will be always converted to Oxaloacetate.**

THE Results of ONE TCA CYCLE:

3 NADH + 1 FADH2 ->ETC

1 GTP -> Substrate level phosphorylation

IMPORTANT FEATURES OF TCA CYCLE

All the enzymes of TCA are associated with mitochondria (aerobic). Glycolysis is anaerobic and occurs in the cytoplasm.

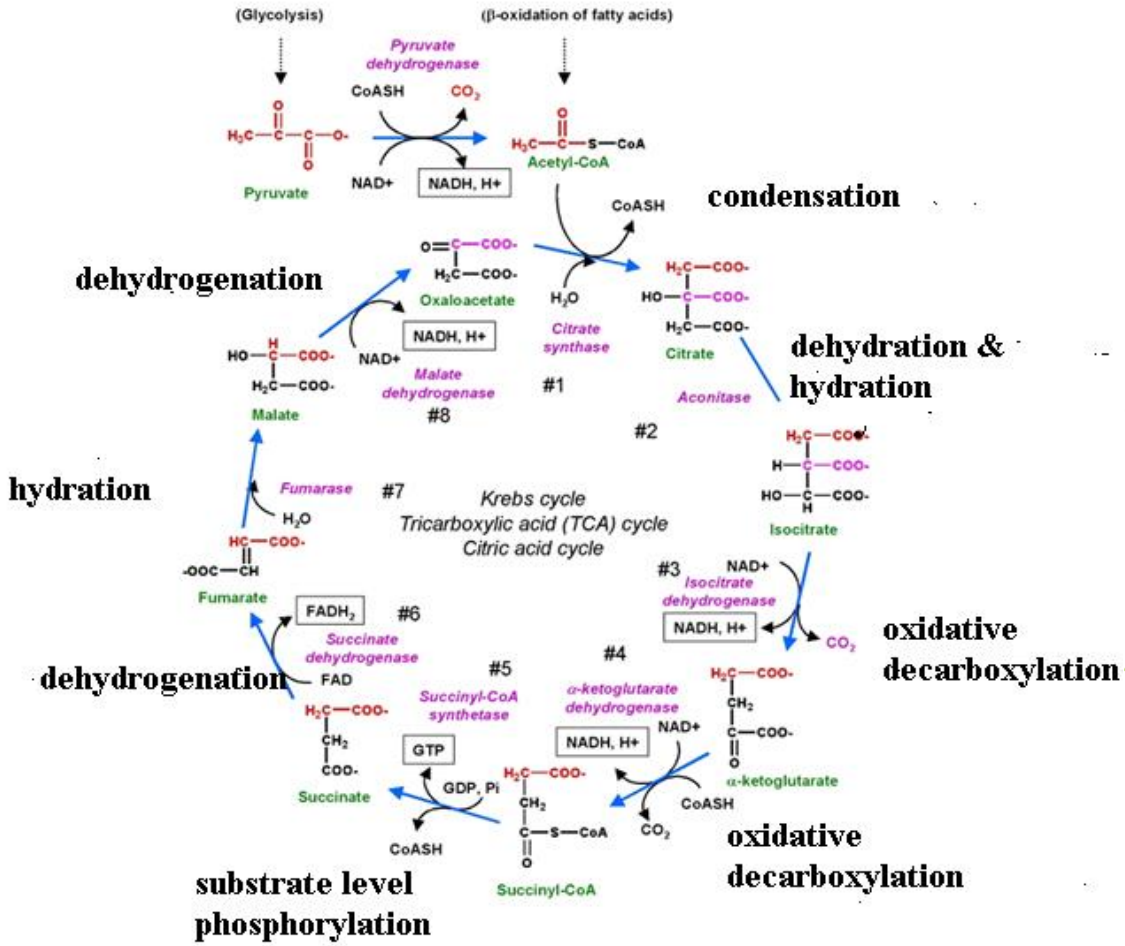
OAA acts CATALYTICALLY. There is no net synthesis or degradation of the four carbon intermediates.

Each turn of the TCA cycle involves **the uptake of 2 carbon atoms** in the form of acetyl CoA and the release of **2 carbon atoms as CO2** but not the same carbons that were taken upon condensation.

Each turn of the cycle results in the transfer of 3 pairs of electrons in the form of hydride ions to NAD to form NADH; transfer of 1 pair of electrons in the form of 2 hydrogen atoms to reduce FAD to FADH2.

There is a substrate level phosphorylation which results in the formation of GTP from GDP and Pi

REACTIONS OF THE TCA CYCLE

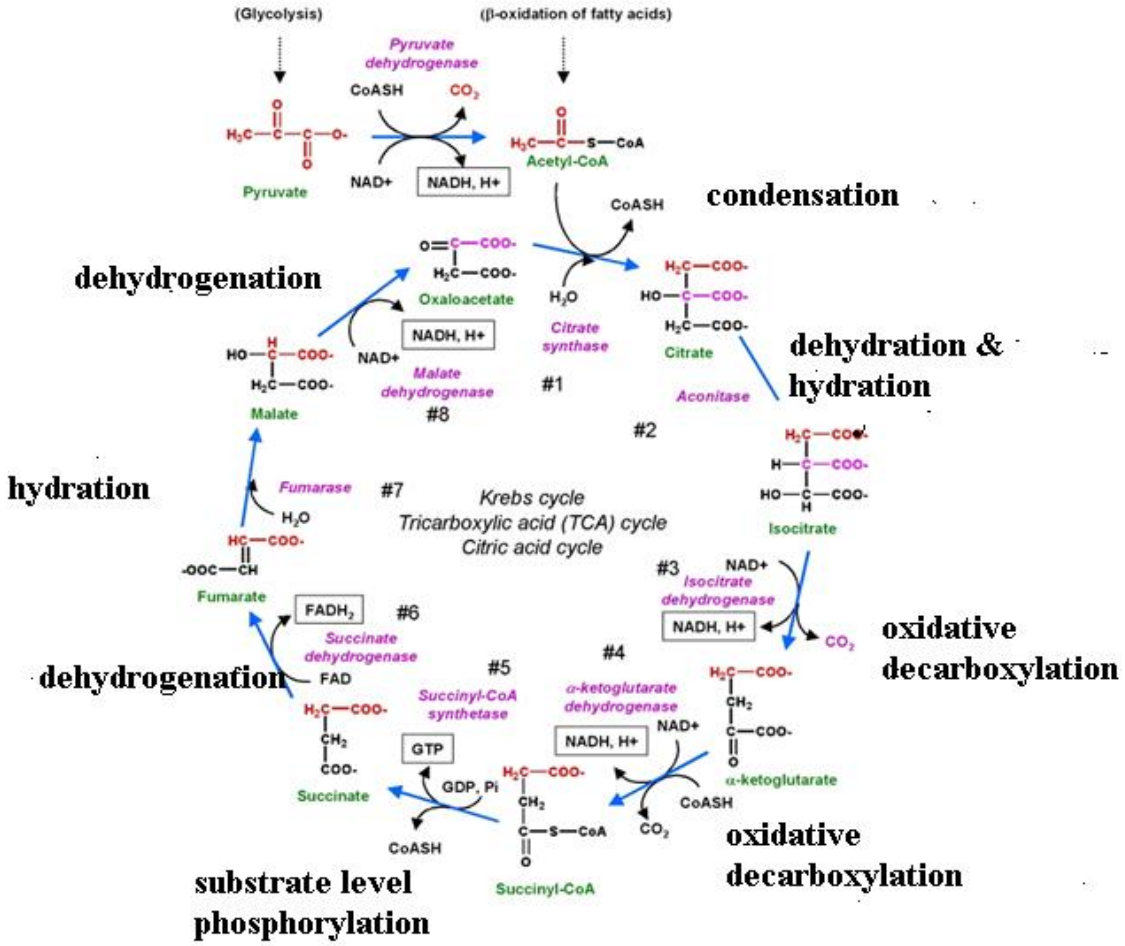


1. CITRATE SYNTHASE: Candidate for regulation, Citrate synthesis is necessary for fatty acid synthesis, ΔG0`= -9kcal/mol

2. ACONITASE: dehydration followed by hydration ΔG0`= +1.5 kcal/mol

3. ISOCITRATE DEHYDROGENASE: ΔG0`= -5 kcal/mol, oxidative decarboxylation of isocitrate to alpha-ketoglutarate; 1st of four dehydrogenases in the cycle, NADH+H+ formation. AMP& ADP stimulate by lowering km 10 folds. ATP&NADH inhibit the enzyme .inhibition of this enzyme will result in an increase in citrate which can be transported out of mit as substrate for fatty aci synthesis

4. α-KETOGLUTARATE DEHYDROGENASE COMPLEX : ΔG0`= -8 kcal/mol 2nd molecule of CO2, and the 2nd NADH+H formation; TPP, lipoic acid, CoAsh, FAD, and NAD are involved. ATP, GTP, NADH, and succinyl CoA inhibit the complex, AMP is a positive effector, calcium is positive effector. The complex consists of α-ketoglutarate dehydrogenase, dihydrolipoyl transsuccinylase and dihydrolipoyldehydrogenase. α-ketoglutarate represents a significant point of convergence in metabolism. Several aa are converted to glutamate which if transaminated or oxidatively deaminated yields alpha ketoglutarate



REACTIONS OF THE TCA CYCLE

5**. SUCCINYL THIOKINASE ,ΔG0`**= -8 kcal/mol: cleavage of thioester bond is coupled to phosphorylation of GDP to GTP- **substrate level phosphorylation .**

Nucleoside diphosphate kinase:

GTP + ADP→GDP+ ATP (1)

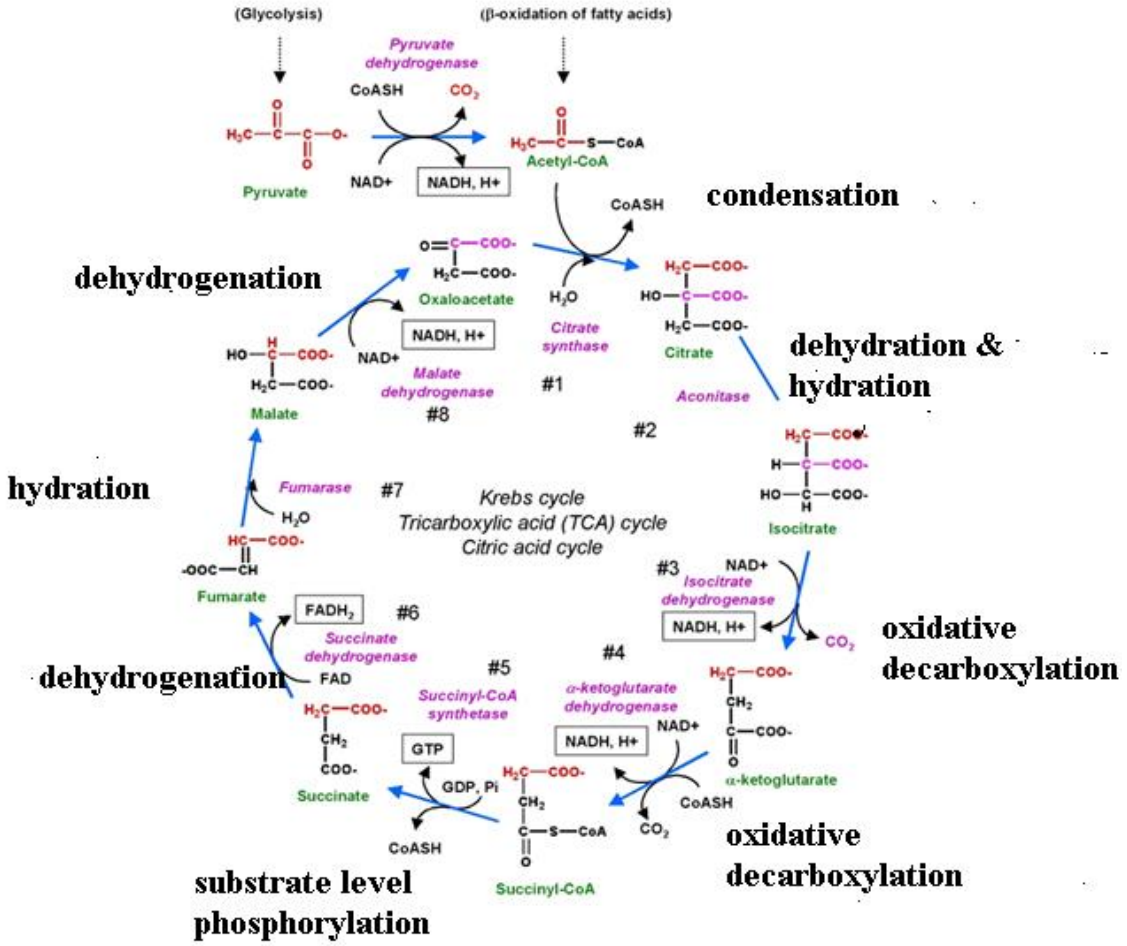
Adenylate kinase: AMP+ATP→2ADP…(2)

SUM: GTP+AMP→GDP+ADP

AMP promotes the formation of GDP for the continuation of the cycle.

6. **SUCCINATE DEHYDROGENASE ΔG0`**=0 : the only dehydrogenation in TCA cycle that is not NAD-linked, but FAD to form FADH2.malonate is a competitive inhibitor

7. **FUMARASE ΔG0`**=0.9 : reversible hydration of fumarate to L-malate, this enzyme is specific for the trans and L-isomers of the unsaturated and hydroxy acids, respectively.



REACTIONS OF THE TCA CYCLE

8. MALATE DEHYDROGENASE: **ΔG0`**=+7.1. completes the cycle by regenerating OAA-A REGENERATING SUBSTRATE. It is the final of three reactions in which NADH+H is produced.

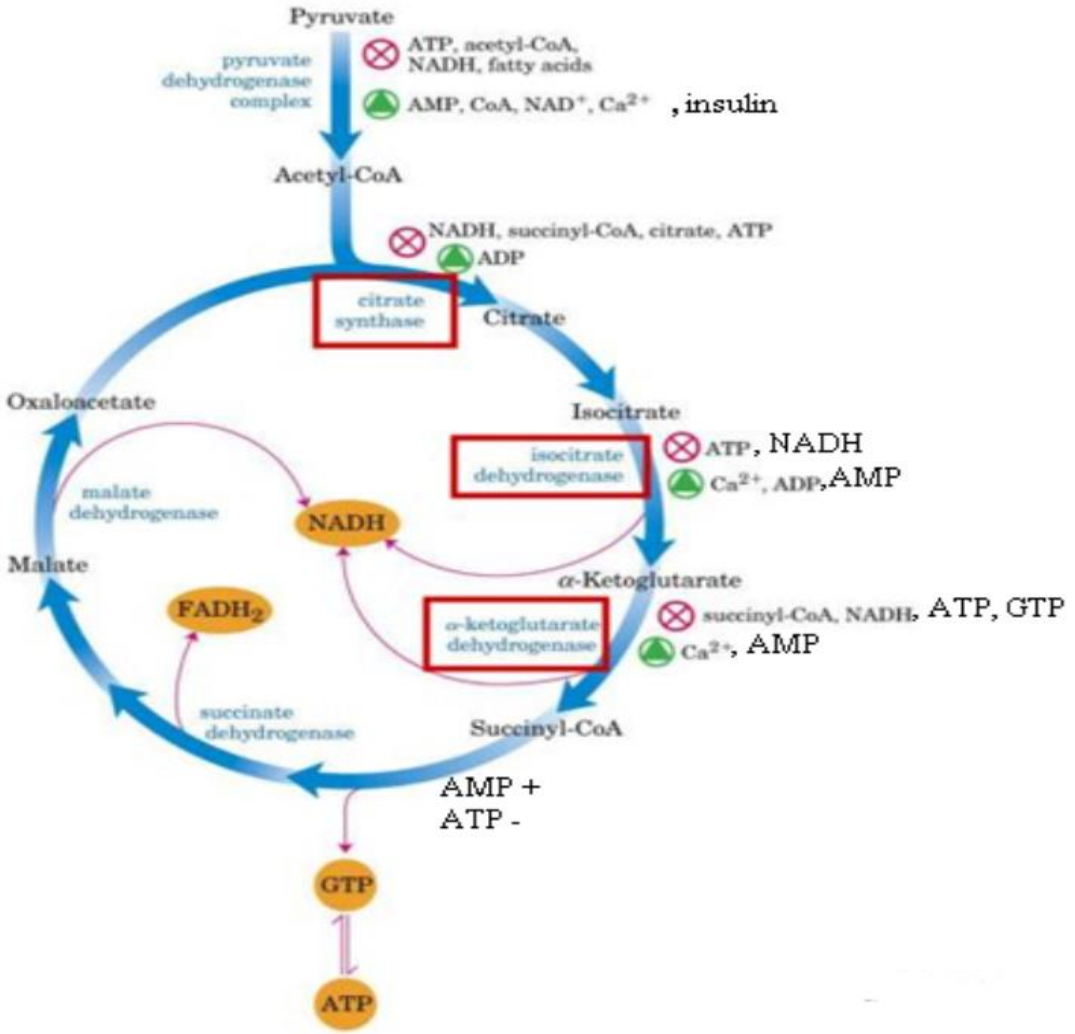
The equilibrium greatly favors the reverse reaction , the reduction of OAA. However, citrate synthesis is closely associated with the dehydrogenase and removal of OAA assists in pulling the malate dehydrogenase reaction towards the formation of OAA. OAA can be reversibly transaminated to aspartate

SUM:

AcetylCoA+3NAD+FAD+GDP+Pi+2H2O→

2CO2+3NADH+2H+FADH2+GTP+CoASH

Regulation of Citric Acid Cycle



The citric acid cycle is regulated at its three exergonic steps

ELECTRON TRANSPORT, SHUTTLES, AND OXIDATIVE PHOSPHORYLATION

Products of TCA cycle include NADH+H+ and FADH2 which are energy rich molecules because they contain a pair of electrons of high transfer potential.

Transfer of these electrons to oxygen thru a series of carriers results in the release of a large amount of energy which can be used to generate ATP.

oxidative phosphorylation is the process in which ATP is formed as electrons are transferred by this series of carriers from NADH+H+ and FADH2 to O2.