Biochemistry

Title = TCA
Lec no = 12
Done By = Baraa Safi

وقل رب زدني علما

Krebs cycle (tricarboxylic acid cycle; TCA)

Ahmed Salem, MD, MSc, PhD, FRCR

Introduction

- **Name:** tri-carboxylic acid cycle (TAC), Krebs cycle, or citric acid cycle

أهمية

- It is the major common pathway of oxidation of CHO, lipids & protein (they all yield acetyl coA)
  - Generates 12 ATP (old) or 10 ATP (new) + 2 CO_2

- **Site:** totally in mitochondria & generates high amount of energy → ***mitochondria is power house of cell***

- ***To start, you need one molecule of acetyl coA & one molecule of oxaloacetate***

Location of reactions:

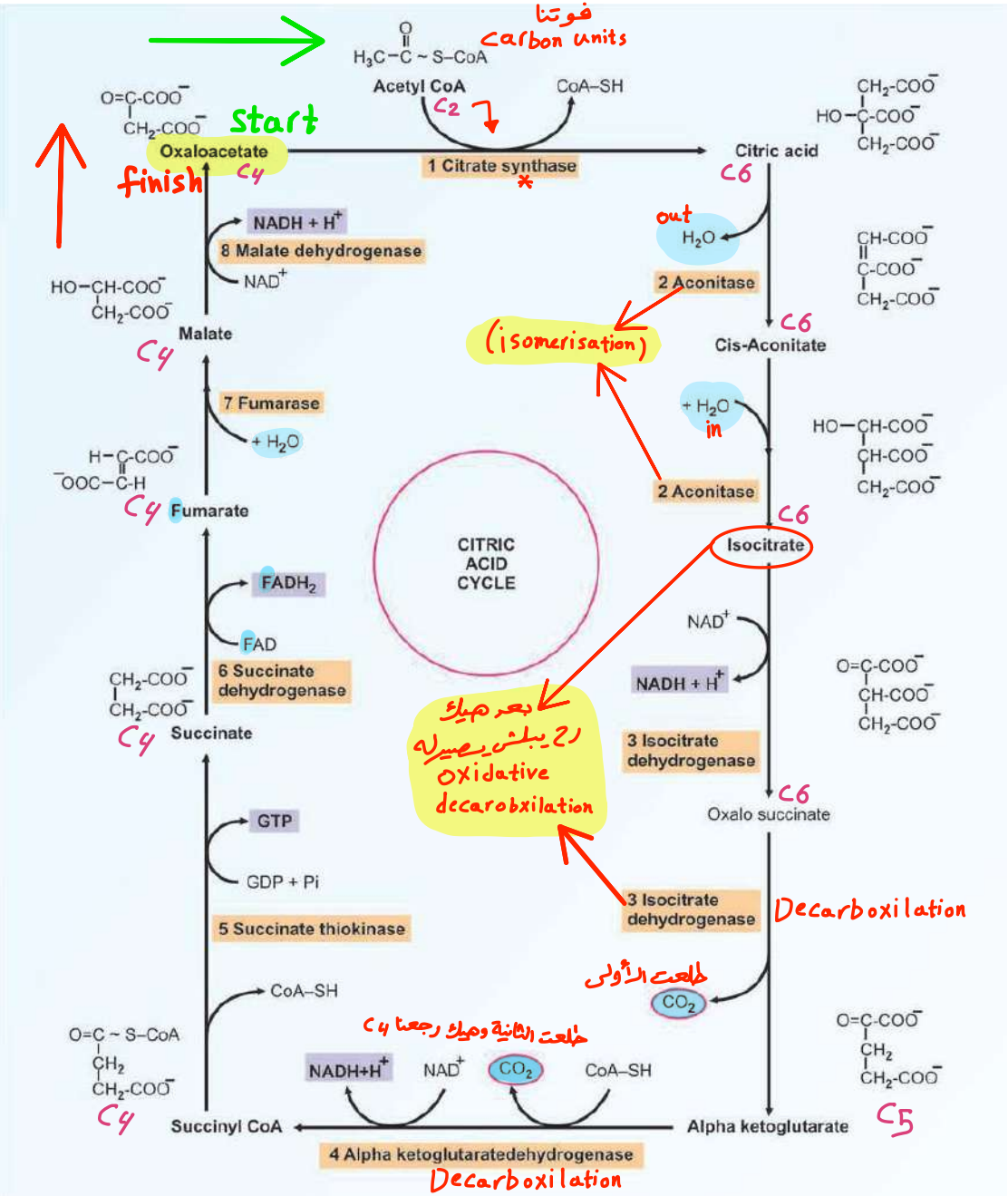
-Glycolysis occurs in cytoplasm

Pyrovate enters the mitochondria by specific carrier, and here it turns to Acetyl CoA.

- Acetyl CoA that comes from beta oxidation, it also be synthesised in mitochondria

— it is cycle, and not Pathway

كثير شغلا تتعلقه بال (Krebs cycle) تحرشغي بال (mitochondria)



Acetyl CoA (2 carbon), enters the cycle. These are released as CO_2 in steps 3 and 4. So Acetyl CoA is completely oxidised by the time cycle reaches alpha ketoglutarate.

All reactions are readily reversible; except 1st and 4th steps

End result:

2 carbons from acetyl coA leave as 2 x CO2 3/4

Energy captured:

1 x GTP 5

3 x NADH+H 3/4/8

1 x FADH2 6

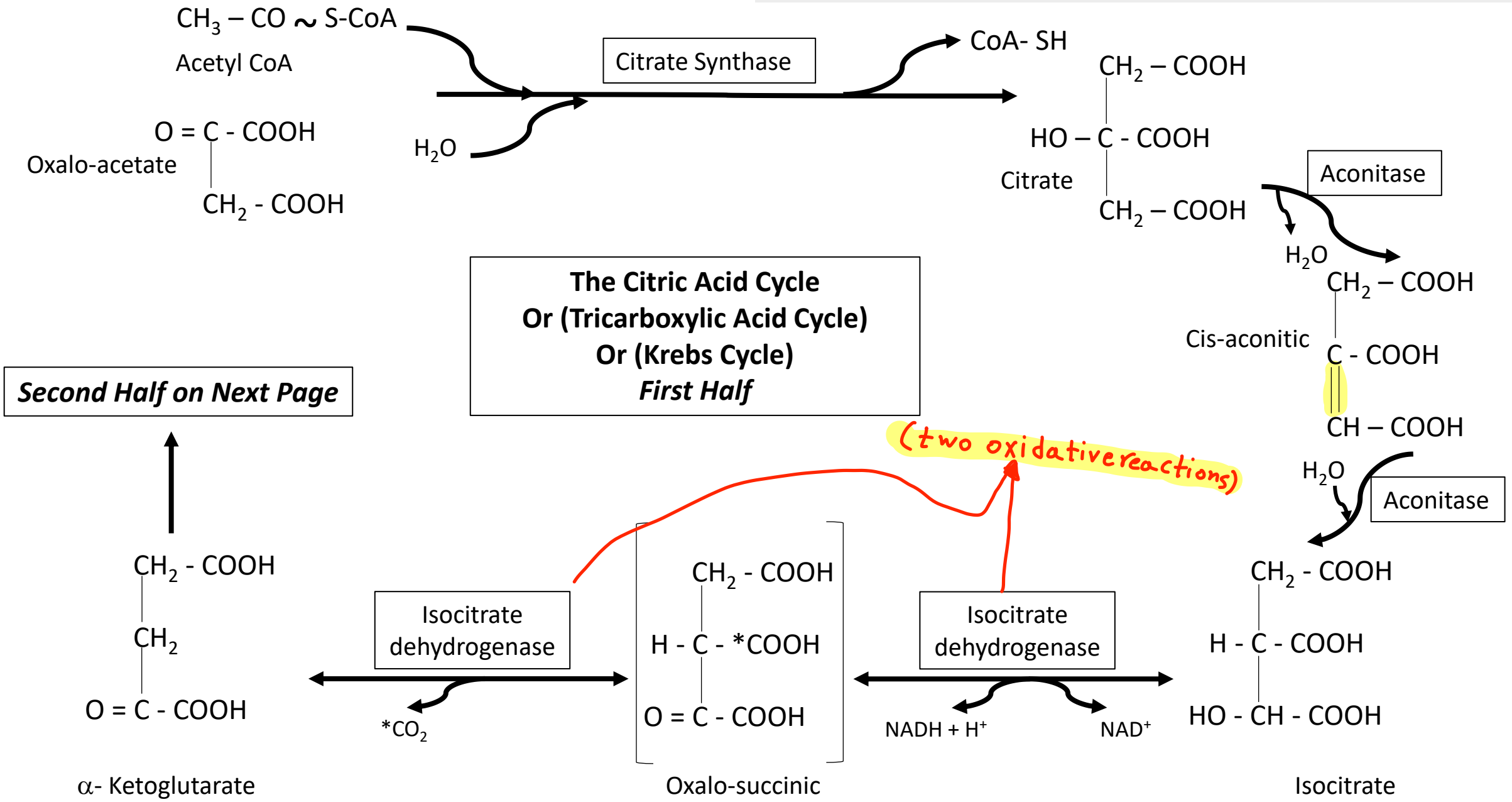
*كل (C) بتطلع أثناء الـ(cycle) بتطلع على شكل (CO_2)

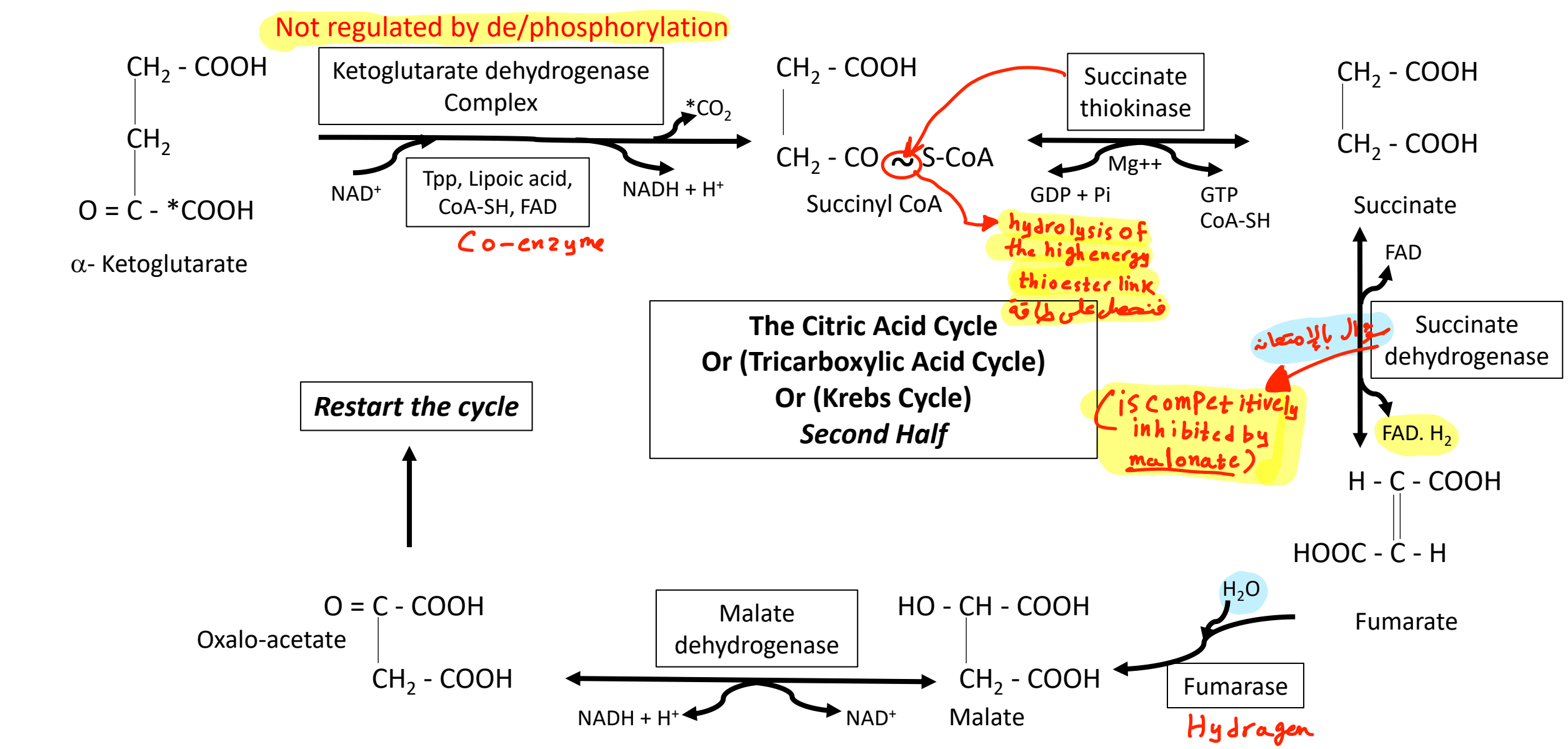
(4) (Ketolysis) / (3) (Ketogenic aminoacid) / (2) (Fatty acid oxydation) / (1) (Glycolysis) الـ أي تكملة (Pyrovate) منه (Acetyl CoA) ممكنة تكونه -

* ليش مش (synthatase) ؟ لأنه مافي حاجة للـ (ATP)

- the reaction from α-Ketoglutarate to Succinyl CoA by Ketoglutarate dehydrogenase complex is the important step in the Krebs cycle.

Reversible vs irreversible reactions: look in upcoming slides





Reversible vs irreversible reactions: look in upcoming slides

* لأنه احنا بنفوت (2 Acetyl CoA) لل (Krebs cycle) فبصفي الي إنتاج (20 ATP)

Pathway	Step	Enzyme	Source	Method of ATP formation	No of ATPs gained per glucose (new calculation)		No of ATPs as per old calculation
Glycolysis	1	Hexokinase	-		Minus	1	Minus 1
Do	3	Phospho-fructokinase	-		Minus	1	Minus 1
Do	5	Glyceralde-hyde-3-P DH	NADH	Respiratory chain	2.5 x 2 =	5	3 x 2 = 6
Do	6	1,3-BPG kinase	ATP	Substrate level	1 x 2 =	2	1 x 2 = 2
Do	9	Pyruvate kinase	ATP	Substrate level	1 x 2 =	2	1 x 2 = 2
Pyruvate to Acetyl CoA	-	Pyruvate dehydrogenase	NADH	Respiratory chain	2.5 x 2 =	5	3 x 2 = 6
TCA cycle	3	Isocitrate DH	NADH	Respiratory chain	2.5 x 2 =	5	3 x 2 = 6
Do	4	alpha keto glutarate DH	NADH	Respiratory chain	2.5 x 2 =	5	3 x 2 = 6
Do	5	Succinate thiokinase	GTP	Substrate level	I x 2 =	2	I x 2 = 2
Do	6	Succinate DH	$FADH_2$	Respiratory chain	1.5 x 2 =	3	2 x 2 = 4
Do	8	Malate DH	NADH	Respiratory chain	2.5 x 2 =	5	3 x 2 = 6
Net generation in glycolytic pathway					9 minus 2=	7	10 minus 2= 8
Generation in pyruvate dehydrogenase reaction					=	5	= 6
Generation in citric acid cycle					=	20	= 24
Net generation of ATP from one glucose mol					=	32	= 38

1.5 x 2 for FADH2 if glycerol phosphate shuttle

سؤال ممكن ييجي بالإمتحان بعتمد فهمه على محاضرة 10/11/12

اذا بدأت من (lactate) وبعد هيك صنعت (Glucose) وبعد هيك حرقته بال(krebs cycle)

بدنا نفكر منه(4) منطلقات :

الأول أنّي بدي أحول منه(Lactate)إلى(Pyrovate)والتي ستنتج (NADH)وكما أعلم أنني أحتاج (2) عشانه يحول (3C) والجلوكوز(6C) ، إذا أنا سأنتج (2 NADH) يعني (5ATP).

الثاني أنه بدي أحول من(Pyrovate) إلى (Glucose) عن طريق ال (gluconeogenesis) والتي ستستلك منه (pyrovate) وستستهلك (6ATP) وتستهلك 2NADH

الثالث أنه أنا عشانه ادخله بـ (krebs cycle) بدي (Acetyl CoA) وأنا عارف أنه بال (Glycolysis) منه (Glucose) إلى (Pyrovate) بنتج عندي (2NADH)+(2ATP) يعني (7ATP) ، ولما أحول منه (Pyrovate) إلى (Acetyl CoA) ينتج عندنا (5ATP)

الرابع وهو (krebs cycle) لكل (1Acetyl coA) ينتج عندنا (10ATP) ← X2 ← 20 ATP

∴ الحصلة : استهلكنا 11ATP ، وأنتجنا (7 + 5 + 20 + 5)

37 − 11 = 26

Important facts of Krebs cycle

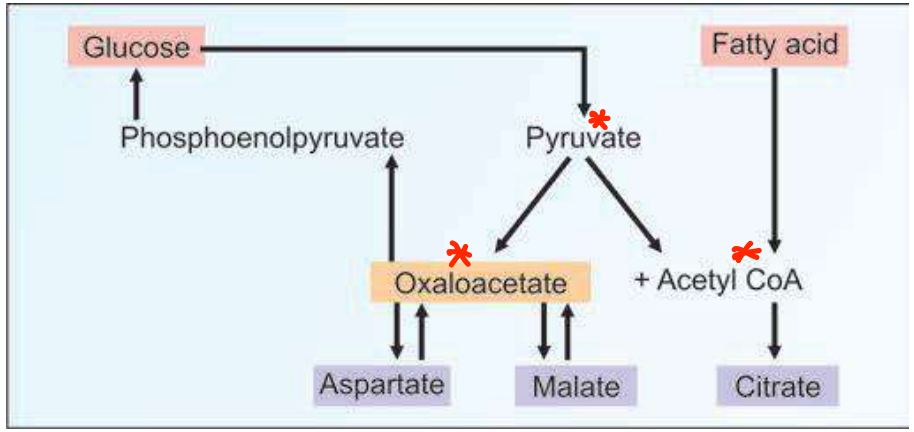
- على عكس Contrary to glycolysis, Krebs cycle can only happen under aerobic conditions
- Enzymes of TCA are found in the mitochondrial matrix, in close proximity to the enzymes of the respiratory chain
- Different isocitrate dehydrogenases are seen (isoenzymes)
  - NAD+ specific in mitochondria
  - NADP+ specific in cytoplasm

  → سؤال بالإمتحان
- **Alpha ketoglutarate dehydrogenase is irreversible step** (the only always irreversible)
  - Citrate synthase is irreversible but body can reverse it via ATP-citrate lyase
  - IDH step of the citric acid cycle is often (**but not always**) an irreversible reaction due to its large negative change in free energy

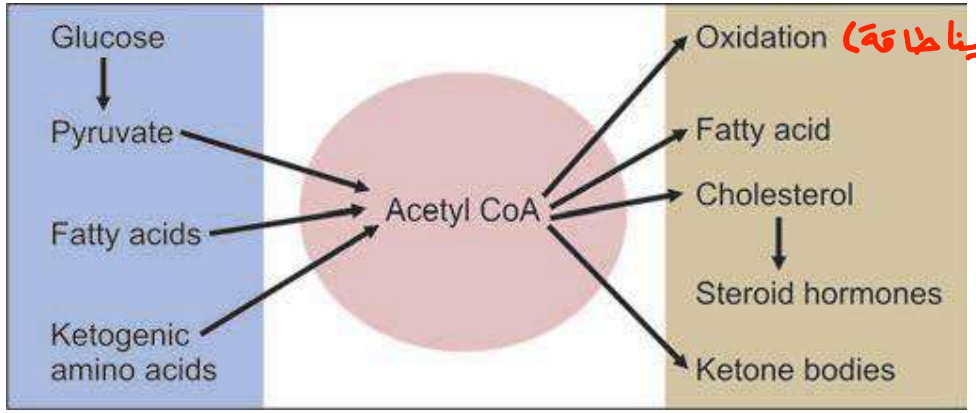
(isocitrate dehydrogenases)

I D H

Oxaloacetate as a "catalyst" and junction point of metabolism



حفظ الـ (structure) تاعهم (***)



Sources and utilization of acetyl coA

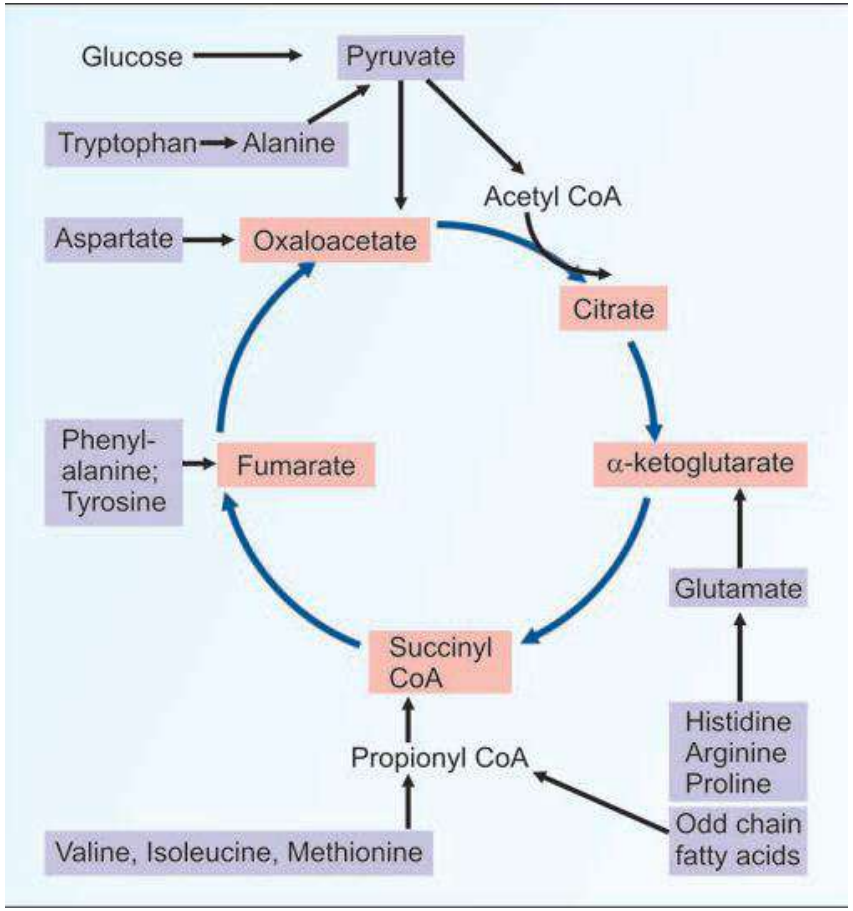
Significance/ importance of Krebs cycle (1 of 2)

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

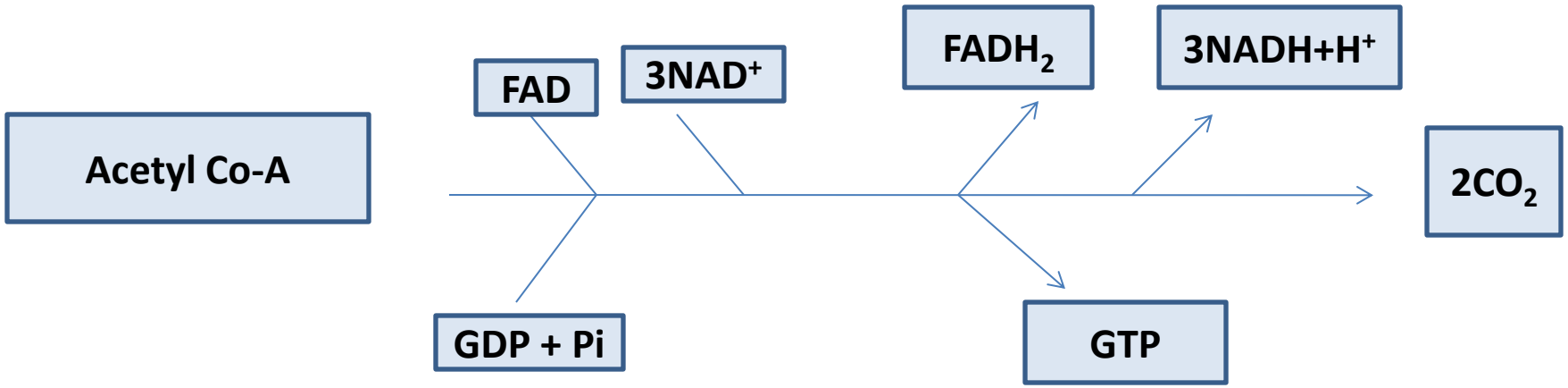
fire / فتيل

(carbohydrates are required for fat oxidation)

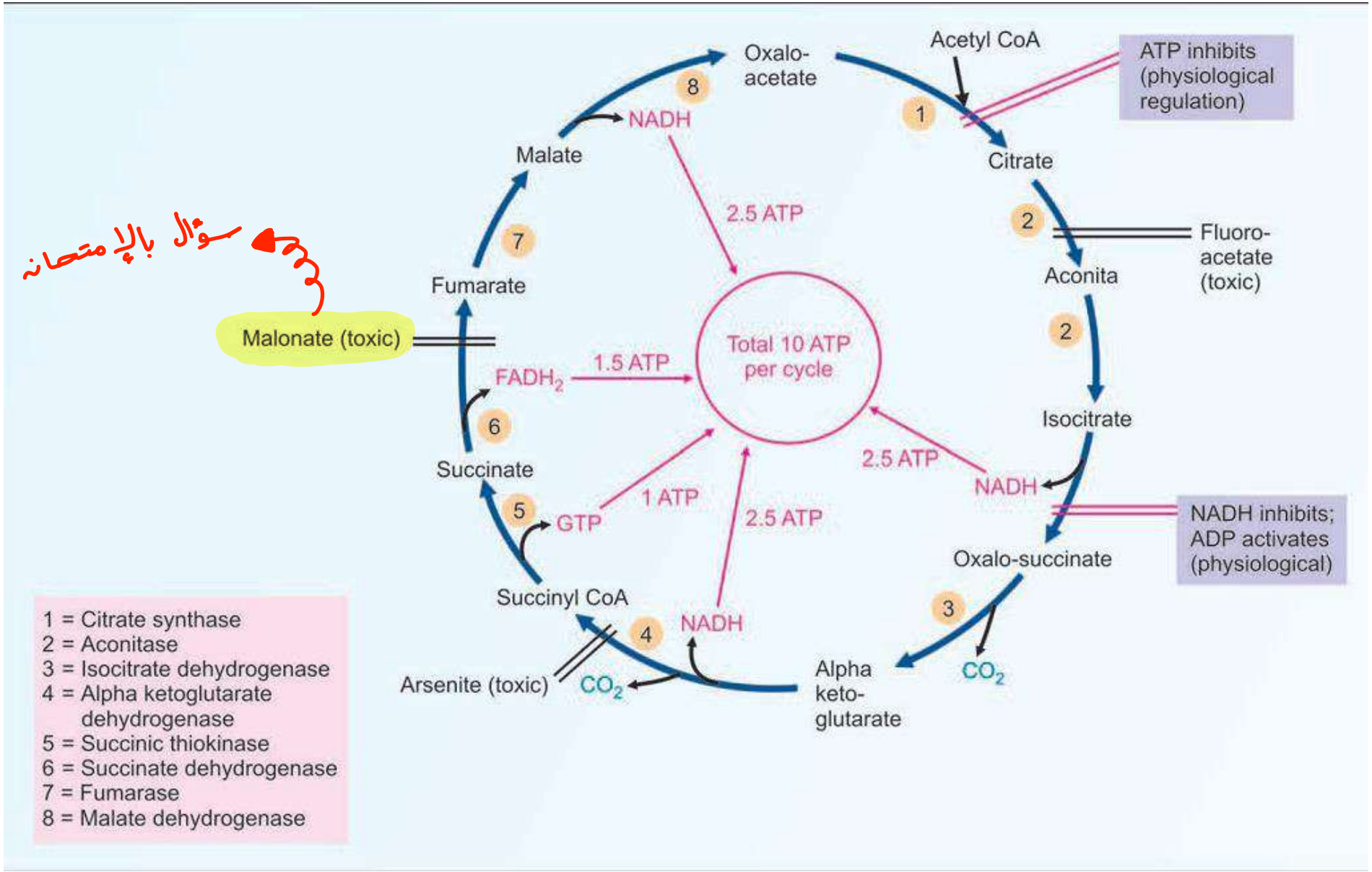
((carbohydrate) احنا بنحرق الـ (fat) عن طريق وجود الـ)



- The overall reaction for one turn of the TCA cycle is:



ENERGY YIELD OF KREBS CYCLE (NEW system):



سؤال بالإمتحانه

- **ENERGY YIELD OF KREBS CYCLE (old system):**
- 3 ATP in step 4 from oxidation of NADH.
- 3 ATP in step 6 from oxidation of NADH.
- 1 GTP in step 7 substrate level.
- 2 ATP in step 8 from oxidation of FADH2.
- 3 ATP in step 10 from oxidation of NADH.
- So, total yield **12 ATPs**: 11 ATPs from respiratory chain & one ATP from substrate level.

Table 18.1. ATP generation steps

Step no.	Reactions	Co-enzyme	ATPs (old-calcu-lation)	ATPs (new calcu-lation)
3	Isocitrate → alpha ketoglutarate	NADH	3	2.5
4	Alpha ketoglutarate → succinyl CoA	NADH	3	2.5
5	Succinyl CoA → Succinate	GTP	1	1
6	Succinate → Fumarate	$FADH_2$	2	1.5
8	Malate → Oxaloacetate	NADH	3	2.5
		Total	12	10

Don't worry about numbering of reactions as numbers vary from author to author; imp thing is to know the NAME of reaction where NADH+H… is produced

Significance/ importance of Krebs cycle (2 of 2)

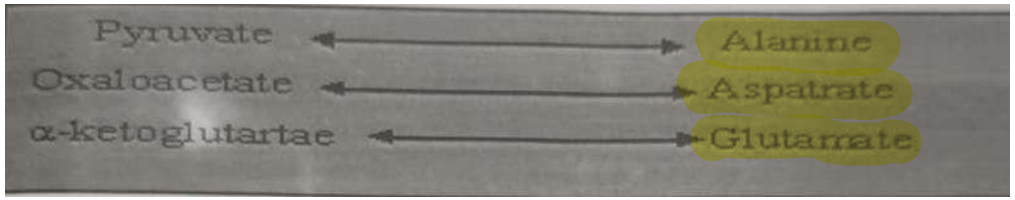
6. Excess carbohydrates are converted as neutral fat (Glucose → pyrovate → Acetyl co A → fat)
7. No net synthesis of carbohydrates from fat
8. Carbon skeletons of amino acids finally enter the citric acid cycle
9. Amphibolic pathway
10. Anaplerotic role

Amphibolic aspects of Krebs cycle (serve as catabolic and anabolic pathway)

Catabolic role

Anabolic role: Synthesis of some important intermediate

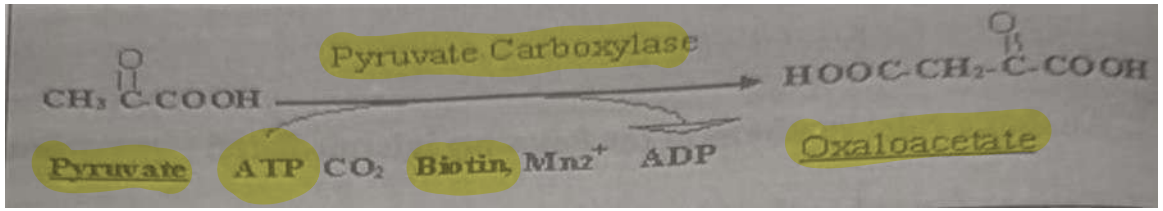
- Citrate → ***acetyl coA*** + oxaloacetate (**citrate lyase** in cytoplasm) → (FA synthesis) → (fat يصنع (Acetyl coA) أنه (معلومة انتفنا
- Non essential amino acids



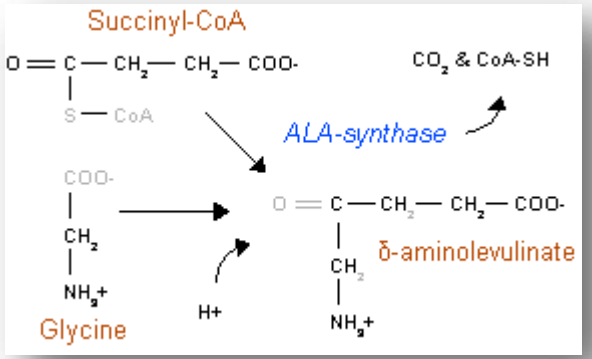
- Aminolevulinate required in heme synthesis
- Gluconeogenesis

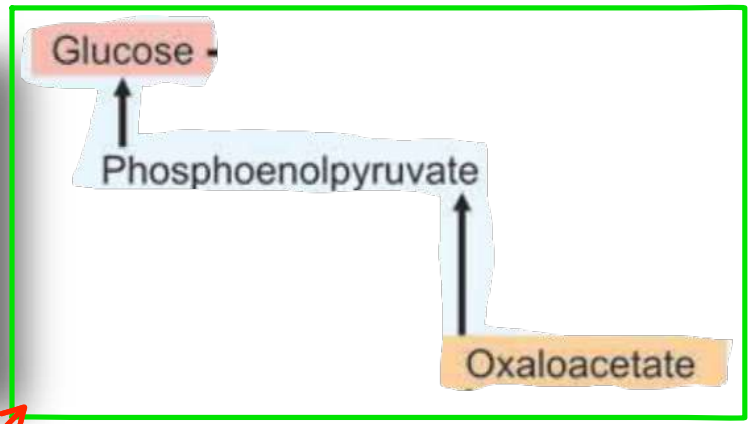
Anaploretic (إعادة تجديد) **reactions to replenish Krebs cycle intermediates:**

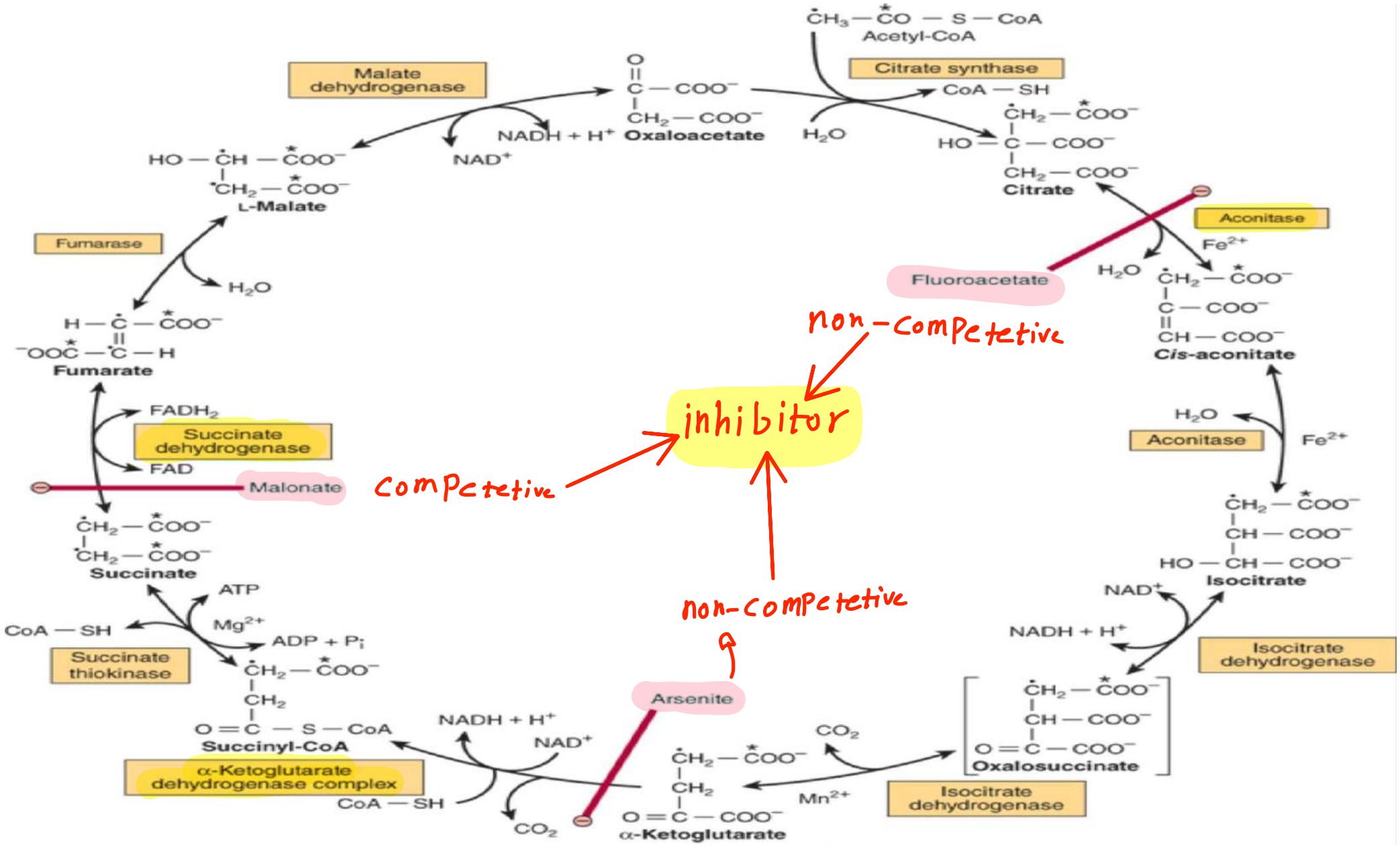
- From Amino acid like the table at the bottom left of this page
- Carboxylation of pyruvate to oxaloacetate



- Transamination reactions





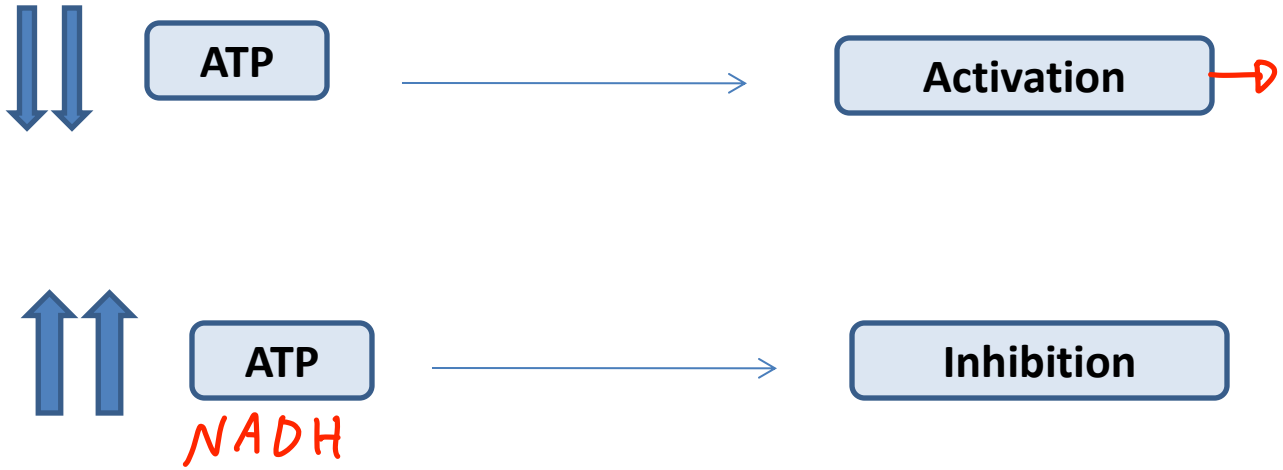


Source: V. W. Rodwell, D. A. Bender, K. M. Botham, P. J. Kennelly, P. A. Weil: Harper's Illustrated Biochemistry, 13th Edition
www.accessmedicine.com

Regulation of Krebs cycle :

- The main function of Krebs cycle is ATP production so:

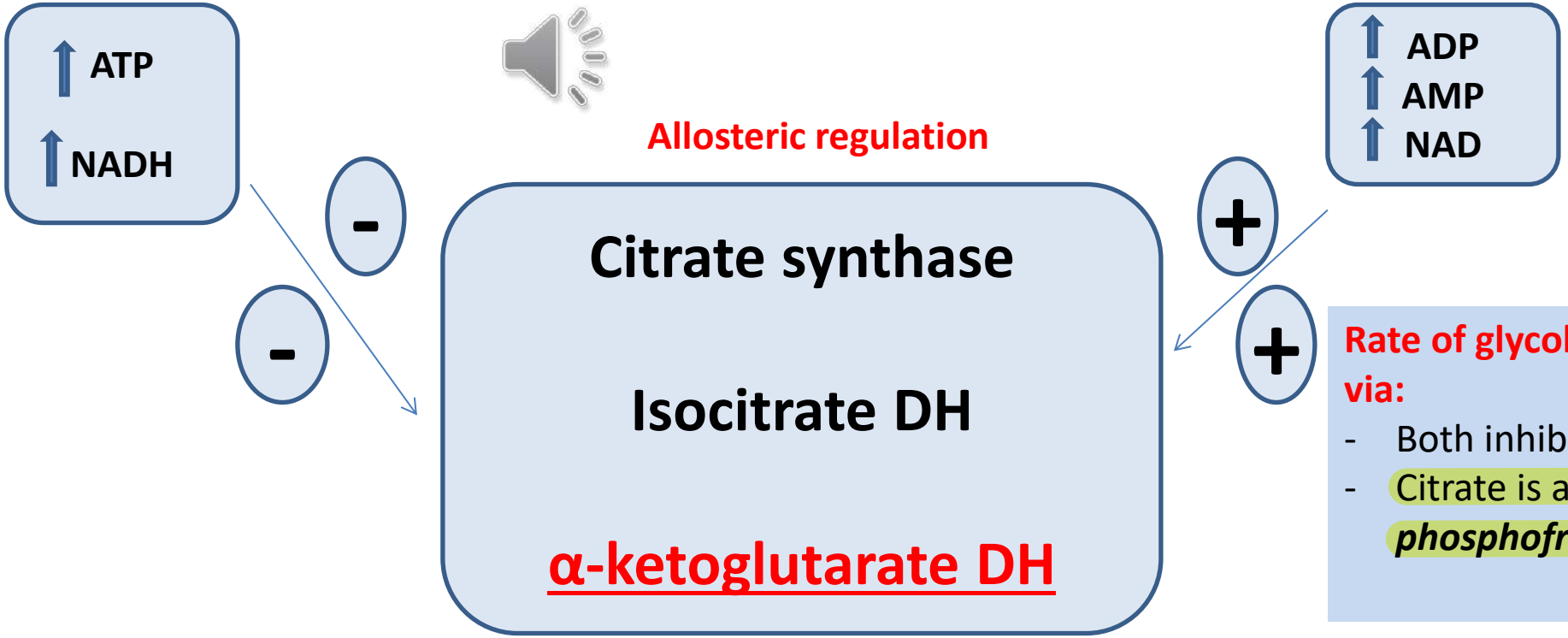
Allosteric regulation



عشانه نعوضه

Activation ↑ (NAD+, AMP/ADP) والعكس لوكانه

inhibition ↓ FAD



Rate of glycolysis and Krebs cycle are matched via:

- Both inhibited by high levels of ATP & NADH
- Citrate is an important allosteric inhibitor of ***phosphofructokinase 1***

Substrate availability is required for Krebs cycle:

- **NAD and FAD:** NADH+H and FADH2 must be re-oxidised via active respiratory chain
  - Active respiratory chain needs high oxygen and high ADP concentrations
- **Acetyl coA** (via glucose oxidation, FA & KB oxidation and catabolism of ketogenic AA)
- **Oxaloacetate** (from malate, aspartate and pyruvate)

- ال (Krebs cycle) رح تشتغل بشكل أسرع لما تكون ال (energy) تاعت الخلا يا قليلة.
- ال (cycle) كتير (regulated and coupled) مع ال (respiratory chain)
- (Krebs cycle) أكبر ممول لبطاريات منه قبلها ال (ATP) بتطلع بال (respiratory chain)
- بوجود (hypoxia) (نقص oxygen) صاد بعمل (inhibition) لل (Krebs cycle) عنه طريقة أنه بترجع (FADH2/NADH)

Table 18.2. Metabolic defects of oxidative metabolism

Enzymes	Reactions catalyzed	Abnormalities
Pyruvate dehydro-genase	Pyruvate → acetyl CoA (irreversible)	Lactic acidosis → (lactate) فنزيد الـ (Pyrovate) بزيد الـ Neurological disorders
Acyl CoA-dehydro-genase	Fatty acyl CoA → alpha, beta-unsaturated fatty acyl CoA	Organic aciduria, glu-taric aciduria, acido-sis, hypoglycemia Electron flow from FAD → CoQ affected
Pyruvate carboxylase	Pyruvate → Oxaloacetate	Oxaloacetate needed for sparking (إثارة) TCA cycle is deficient. Lactic acidosis, hyperammonemia and hyperalaninemia