METABOLISM

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ARKAN ACADEMY

❖ TCA cycle

- It is called Kreps cycle, Citric acid cycle and Tricarboxylic acid cycle (TCA)
  - ➢ It represents the oxidation of acetyl group of acetyl CoA to CO_2
  - ➢ It involves the release of energy in the form of GTP and stored in NADH and $FADH_2$
  - ➢ No O_2 is required (***anaerobic*** pathway)

- All of the major nutrients can be converted to acetyl CoA in the second stage of metabolism

- TCA cycle occurs in the *mitochondrial matrix* except succinate dehydrogenase (**step 6**) occurs in the *inner mitochondrial membrane*

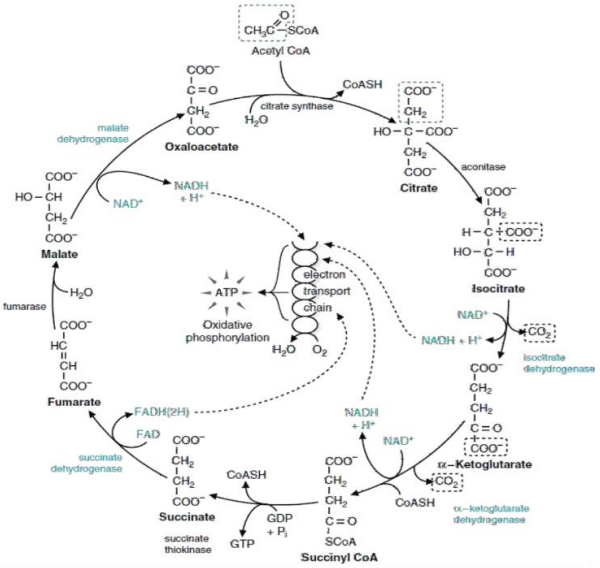
- Oxaloacetate (4C) is the final intermediate of the TCA and it has no net synthesis or degradation

- **Each turn** of the TCA cycle involves:
  - ➢ Release of ***2 CO_2***
    - ✓ The source of carbons released is Acetyl CoA
  - ➢ Transfer of 3 pairs of electrons in the form of hydride ions to NAD^+ to form ***3 NADH***
  - ➢ Transfer of 1 electron in the form of hydrogen atom to reduce FAD to ***$FADH_2$***
  - ➢ Substrate level phosphorylation which results in the formation of ***GTP*** from GDP and P_i

♦ Steps and Enzymes of TCA cycle

1) Citrate synthase
   - ➢ It produces ***citrate*** by the condensation of acetyl with oxaloacetate (OAA)
   - ➢ This step is inhibited by citrate, ATP and succinyl CoA and activated by substrates
   - ➢ Citrate activates acetyl CoA carboxylase (Fatty acid synthesis), and also citrate represents a source of Acetyl CoA which is used in the synthesis of FAs
   - ➢ Citrate inhibits phosphofructokinase (glycolysis)
   - ➢ Citrate Stimulates fructose-1,6-bisphosphatase (gluconeogenesis)
   - ➢ ΔG^o = - 9 kcal/mol

2) Aconitase
   - ➢ It isomerizes citrate (3° alcohol) into ***isocitrate*** (2° alcohol) by forming an intermediate called aconitate
   - ➢ Dehydration followed by hydration
   - ➢ Fluoroacetate (rat poison, a non-competitive inhibitor), succinyl CoA and NADH inhibits aconitase
   - ➢ Activated by ADP and Ca^{+2}
   - ➢ ΔG^o = + 1.5 kcal/mol



3) Isocitrate dehydrogenase complex

- It undergoes oxidative decarboxylation of isocitrate into ***α-ketoglutarate***
- Release ***CO_2*** and reduces NAD^+ into ***NADH*** + ***H^+***
- It is considered as the **rate limiting step**
- Activated by ADP and Ca^{+2}
  - ✓ ADP lowers K_m 10 folds which increases the affinity of the enzyme to its substrate
- Inhibited by ATP and NADH
- ΔG^o = - 5 kcal/mol

4) α-Ketoglutarate dehydrogenase complex

- It undergoes oxidative decarboxylation of α-Ketoglutarate into ***Succinyl CoA***
- Release ***CO_2*** and reduces NAD^+ into ***NADH*** + ***H^+***
- This complex is a multimolecular aggregate of 3 enzymes
  - ✓ E1 (decarboxylase) requires thiamine pyrophosphate (***TPP***) as a cofactor
  - ✓ E2 (dihydrolipoyl transacylase) requires ***lipoic*** acid and ***CoA*** as cofactors
  - ✓ E3 (dihydrolipoyl Dehydrogenase) requires ***FAD*** and ***NAD^+*** as cofactors

TPP is a Vitamin B1 derivative

Lipoic acid and FAD are covalently attached to E2 and E3

- This complex is one of α-Ketoacid dehydrogenase complexes which act in α-Ketoglutarate, pyruvate and branched chain α-Ketoacids
- Inhibited by ATP, GTP, NADH, and succinyl CoA, and activated by AMP and Ca^{+2}
- Inhibited by ***Arsenite*** (non-competitive inhibitor)
- ΔG^o = - 8 kcal/mol

5) Succinyl thiokinase

- Succinyl CoA is converted into ***Succinate***, by the cleavage of thioester bond (high energy bond)
- It is coupled to phosphorylation of GDP to ***GTP*** (substrate level phosphorylation)
  - ✓ GTP and ATP are energetically interconvertible by the nucleoside diphosphate kinase reaction
- ΔG^o = -8 kcal/mol

6) Succinate dehydrogenase

- Oxidation of succinate into ***fumarate***
- The only dehydrogenation in TCA cycle that is not NAD-linked, but FAD to form **$FADH_2$**
  - ✓ Uses FAD instead of NAD^+, because succinate reducing power is not sufficient to reduce NAD^+
- Placed in the **inner mitochondrial membrane** not the matrix
- Succinate dehydrogenase functions as Complex II of the electron transport chain
- **Malonate** is a competitive inhibitor
- ΔG^o = 0 kcal/mol

7) Fumarase (fumarate hydratase)

- **Reversible** hydration of fumarate to **L-malate**
- ΔG^o = 0.9 kcal/mol

8) Malate dehydrogenase

- Completes the cycle by regenerating ***OAA*** (a regenerating substrate)
- It is the final of three reactions in which ***NADH + H^+*** is produced
- ΔG^o = + 7.1 kcal/mol (highly positive), but it is driven by the very low concentration of OAA due to the highly exergonic citrate synthase reaction.

- The net reaction of TCA cycle is:
  Acetyl CoA + 3NAD + FAD + GDP + P_i + $2H_2O$ → $2CO_2$ + 3NADH + $2H^+$ + $FADH_2$ + GTP + CoA-SH

- The Overall net ΔG of TCA cycle is negative (***favorable***, -228 Kcal/mole)
  - It produces GTP, 3 NADH, $FADH_2$ which energetically equals 10 ATP molecules
  - Most steps are ***irreversible*** steps

- Efficient burning depends mainly on the presence of carbohydrates to provide OAA
- *Fats can't be converted into glucose*, because pyruvate dehydrogenase reaction is an absolutely **irreversible** step
  - Pyruvate dehydrogenase is the enzyme that oxidizes pyruvate into Acetyl CoA

- It is a central pathway because it is **amphibolic** (contributes in ***catabolism*** and ***anabolism***)
  - Citrate is important in the synthesis of FAs (in the liver)
  - Malate is important in the gluconeogenesis during fasting (in the liver)
  - Succinyl CoA is important in the **heme** biosynthesis in the bone marrow
  - α-Ketoglutarate is important in the production of GABA (an inhibitory neurotransmitter in the brain) and can be converted into glutamine which is an amino acid used in protein synthesis
  - OAA is important in the production of asparagine (protein synthesis)

- TCA is **Anapleiotropic** pathway where its intermediates must be maintained
  - OAA is replenished by pyruvate carboxylase, which:
    - ✓ Found in the liver, kidney, brain, adipocytes and fibroblasts (mainly liver and kidney)
    - ✓ Activated by **Acetyl CoA**
    - ✓ It requires biotin (vitamin B7) as a cofactor

Past Papers

1. All of the following enzymatic reactions happen during TCA cycle EXCEPT:

   A. Phosphoryl transfer
   B. Intramolecular phosphoryl transfer
   C. Oxidation reduction
   D. Dehydration
   E. Decarboxylation

2. How many high-energy phosphate molecules are produced in the conversion of Citrate to Succinate?

   A. 0
   B. 3
   C. 6
   D. 7

3. What is the maximal amount of ATP produced from the oxidation of isocitrate to alpha-ketoglutarate?

   A. 0
   B. 2
   C. 3
   D. 1

4. One of these is not an intermediate in Krebs cycle:

   A. Citrate
   B. Alpha ketoglutarate
   C. Acetyl CoA
   D. Fumarate
   E. Oxaloacetate

5. Which of the following is the coenzyme for dihydrolipoyl transacetylase:

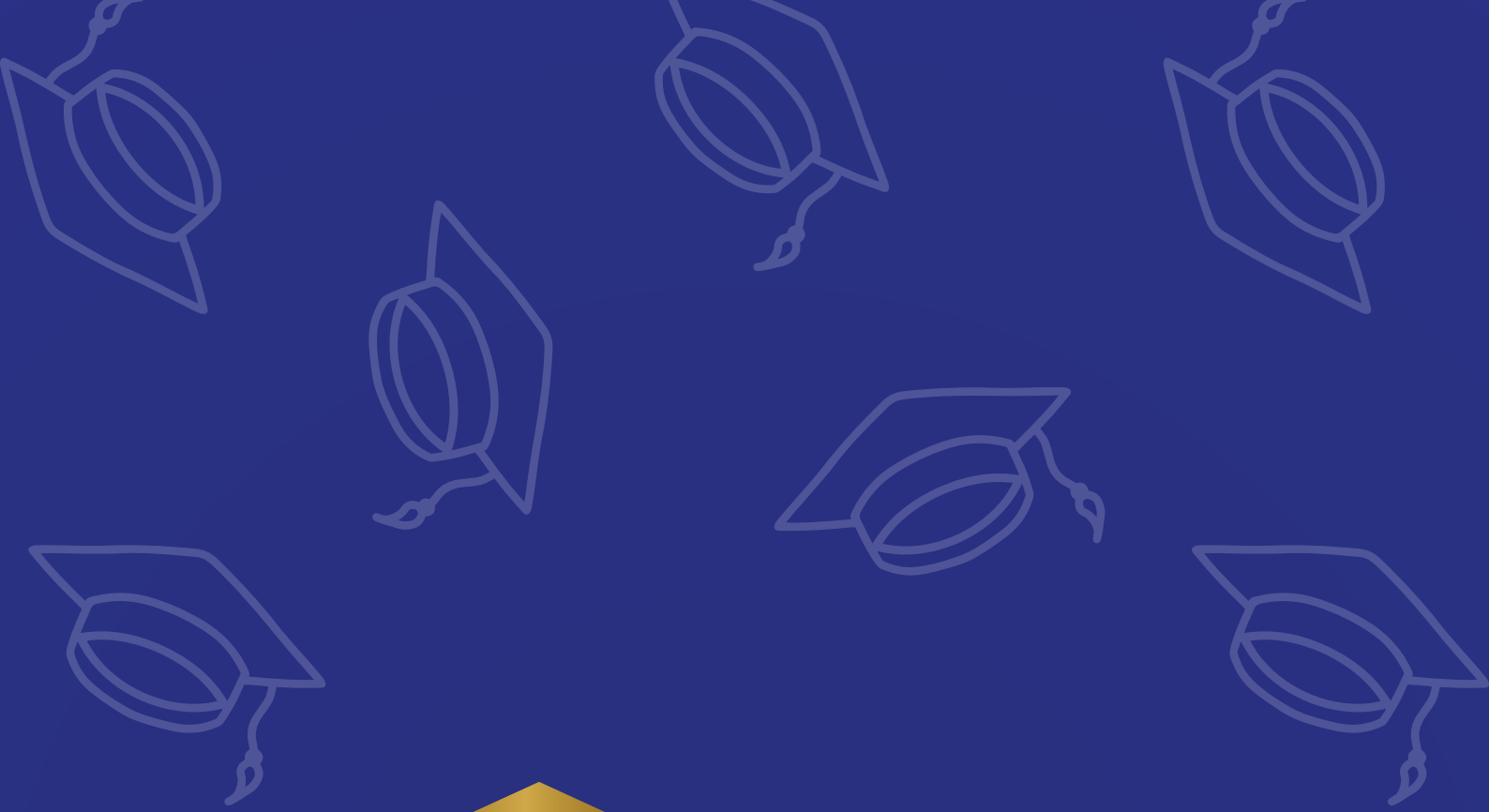
   A. NAD + FAD
   B. CoA + NAD
   C. Lipoic acid
   D. TPP
   E. Lipoic acid + CoA

6. The reaction which results in the reduction of FAD into $FADH_2$ is:

   A. Citrate to isocitrate
   B. Malate to oxaloacetate
   C. Succinate into fumarate
   D. Fumarate to malate

7. Which of the following is not a coenzyme of alpha ketoglutarate dehydrogenase?

   A. NAD
   B. FAD
   C. Lipoic acid
   D. ATP



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