The TCA (tricarboxylic acid, citric acid and krebs) Cycle: Eight Enzymes
Purpose:

- Oxidation of Fuel
- Formation of:
  - Anabolic precursors
  - GTP (1)
  - NADH (3)
  - FADH2 (1)

Key reactions
Regulation

Three primary modes of regulation

- Availibility of substrate: increase in substrate will drive reactions forward
- Product inhibition
- Competitive feedback by downstream intermediates

Start and end with a four carbon molecule, oxaloacetate

- bring in two carbons in the form of an acetyl group to form a 6 carbon
- then, to oxidative decarboxylations
  - each will yield NADH and CO2
  - ending with 4 carbons
- then form GTP or ATP depending on the tissue
  - also FADH2 and NADH

The four carbon oxaloacetate brings in two carbons from acetyl group

- A condensation reaction that produces the six carbon molecule **citrate**

Citrate undergoes an isomerization

- Water is going to leave then come back again, forming **Isocitrate**

Next, the first oxidative decarboxylation

- Produces **alpha ketoglutarate**
- Redox reaction, so NAD+ is needed to accept electrons
- Yielding NADH and CO2 and a proton

The second oxidative decarboxylation

- Produces a four carbon **Succinyl CoA**
- NADH and a proton
- CO2

Substrate level phosphorylation occurs next

- ATP or GTP is produced and CoA
- Use GDP or ADP and inorganic phosphate
- Left with 4C **Succinate**

Oxidation/Reduction

- Oxidize succinate with FAD as the carrier, ending up with FADH2
- Left with 4C **Fumarate**

Hydration of Fumarate is next

- Add water to form 4C **Malate**

Oxidation/Reduction of Malate

- Use NAD+
- Left with 4c **Oxaloacetate**
- Produces NADH and proton

For every turn

- 3 NADh
- 1 FADH2
- 1 GTP or ATP
- reducing equivalents used in ETC to make ATP

Reaction 1:

- Enzyme: Citrate Synthase
- Condensation reaction forming 6C molecule
- Formation of **Citrate** from Oxaloacetate and Acetyl CoA
    - 4C Oxaloacetate + 2C Acetyl CoA yields Citryl CoA (intermediate) to 6C Citrate and CoA

Roles of Citrate in Metaboism:

- Mitochondria
    - Intermediate in the TCA cycle
        - When theses levels build up, goes to cyto
        - Signal that theres a lot of energy around
- Cytoplasm
    - Activator of Fatty Acid Synthesis
    - Precursor of Cytoplasmic AcetylCoA
        - Used to make fatty acids
    - Inhibiton of PFK1
        - No need for more AcetylCoA

Citrate Synthase

- Dimer: each monomer has a small and large domain
- Operates by sequential ordered reaction
- Oxaloacetate binds first, and causes the small subunit to rotate
    - Forms Acetyl CoA binding pocket
- Brings closer the residues that are necessary for the condensation reaction
- Only active when both substrates are available
    - Induced fit
- Regulation of enzyme:
    - Negative: ATP, NADH, Succinyl CoA

Reaction Two:

- Enzyme: Aconitase
- Isomerization in Two Steps
    - Dehydrations of citrates to yield a C=C, cis-Aconitate (intermediate)
    - Stereospecific rehydration of the double bond yields **Isocitrate**

- Go from a tertiary alc to a secondary alc

Reaction Three:

- Enzyme: Isocitrate Dehydrogenase
  - Oxidation – reduction reaction; NADH + H+ produced
    - Forms an unstable beta ketoacid intermediate, oxalosuccinate
  - Decarboxylation reaction, CO2 + alpha ketoglutarate produced
- First produced NADH and CO2
- Rate Limiting step
- Regulation:
  - Positive: ADP+ and NAD+
  - Neg: NADH (direct comp with NAD+) and ATP

Reaction Four:

- Enzyme: alpha ketoglutarate dehydrogenase complex
- A lot like pyruvate dehydrogenase complex
- Alpha keto glutarate + NAD+ + CoA yields succinyl CoA, CO2 and NADH
- From now on 4C

Alpha Ketoglutarate dehydrogenase

- A lot like PDC
- 3 enzymes: E1, E2, and E3
- 5 cofactors:
  - Tpp
  - Lipoamide
  - FAD (prosthetic group)
  - NAD+
  - CoA
- Products: Succinyl CoA, NADh and CO2
- Regulation:
  - Pos: AMP
  - Neg: Succinyl CoA, NADH

Reaction 5:

- Enzyme: succinyl CoA synthetase
- Named for reverse reaction
- Substrate level phosphorylation of GDP
- Production of GTP or ATP
- Succinyl Coa + Pi + GDP yield Succinate + CoA + GTP

Reaction 6:

- Oxidation-Reduction reaction
  - Using covalently attached FAD as cofactor
  - Electrons transferred to iron – sulfur clusters in enzyme and ultimately O2 by the way of other members of ETC
- FADH2 produced
- 4C succinate yields Fumarate
- Enzyme: succinate dehydrogenase
  - Flavin ring is covalently attached