"Tricarboxylic Acid Cycle and Pyruvate Dehydrogenase Complex"

I. Cycle Overview

- The tricarboxylic acid cycle (TCA cycle) can also be referred to as:
    - Citric acid cycle
    - Krebs cycle
- It plays several roles in metabolism.
- It is the final pathway where the oxidative catabolism of the following converge:
    - Carbohydrates
    - Amino acids
    - Fatty acids
- The carbon skeletons of these macronutrients are converted to carbon dioxide (CO_2).
- This oxidation provides energy for the production of the majority of ATP in most animals, including humans.
- The TCA cycle occurs totally in mitochondria, placing it in close proximity to the electron transport chain (ETC).

- The ETC oxidizes the following reduced coenzymes which are produced by the cycle:
    - Nicotinamide adenine dinucleotide (NADH)
    - Flavin adenine dinucleotide ($FADH_2$)
- The TCA cycle is an aerobic pathway, because oxygen (O_2) is required as the final electron acceptor.
- Reactions such as the catabolism of some amino acids generate intermediates of the cycle.
- These reactions are called anaplerotic (from the Greek for "filling up") reactions.
- The TCA cycle also provides intermediates for a number of important anabolic reactions, such as:
    - Glucose formation from the carbon skeletons of some amino acids
    - Synthesis of some amino acids
    - Synthesis of heme
- Therefore, this cycle should not be viewed as a closed system but, instead, as an open one with compounds entering and leaving as required.

II. Cycle Reactions

Overview of TCA Cycle Progression

- In the TCA cycle:
    - Oxaloacetate (OAA) is first condensed with an acetyl group from acetyl coenzyme A (CoA).
    - OAA is regenerated as the cycle is completed.

- Two carbons enter the cycle as acetyl CoA, and two leave as CO_2.

- Therefore, the entry of one acetyl CoA into one round of the TCA cycle does not lead to the net production or consumption of intermediates.

A. Acetyl CoA Production

- The major source of acetyl CoA for the TCA cycle is the oxidative decarboxylation of pyruvate by the multienzyme pyruvate dehydrogenase complex (PDH complex, or PDHC).

- However, the PDHC is not a component of the TCA cycle.

- Pyruvate, the end product of glycolysis, is transported from the cytosol into the mitochondrial matrix by the pyruvate mitochondrial carrier of the inner mitochondrial membrane.

- In the mitochondrial matrix, the PDHC converts pyruvate to acetyl CoA.

- (Note: Fatty acid oxidation is another source of acetyl CoA.

1. PDHC Component Enzymes

- The PDHC is a protein aggregate of multiple copies of three enzymes:

    - Pyruvate decarboxylase ([E1] sometimes called PDH)
    - Dihydrolipoyl transacetylase (E2)
    - Dihydrolipoyl dehydrogenase (E3)

- Each enzyme catalyzes a part of the overall reaction.

- Their physical association links the reactions in proper sequence without the release of intermediates.

- In addition to the enzymes participating in the conversion of pyruvate to acetyl CoA, the PDHC also contains two regulatory enzymes:
    - Pyruvate dehydrogenase kinase (PDH kinase)
    - Pyruvate dehydrogenase phosphatase (PDH phosphatase)

2. Coenzymes

- The PDHC contains five coenzymes that act as carriers or oxidants for the intermediates of the reactions.
- Coenzyme requirements by PDHC enzymes:
    - E1 requires: Thiamine pyrophosphate (TPP)
    - E2 requires: Lipoic acid and CoA
    - E3 requires: FAD and NAD^+
- (Note: TPP, lipoic acid, and FAD are tightly bound to the enzymes and function as coenzymes–prosthetic groups.
- Deficiencies of thiamine or niacin can cause serious central nervous system problems.

- This is because brain cells are unable to produce sufficient ATP via the TCA cycle if the PDHC is inactive.

- Wernicke-Korsakoff, an encephalopathy-psychosis syndrome due to thiamine deficiency, may be seen in persons with alcohol use disorder.

3. Regulation

Covalent Modification of E1

- Covalent modifications by the two regulatory enzymes of the PDHC alternately activate and inactivate E1.

- PDH kinase:
    - Phosphorylates and inactivates E1.

- PDH phosphatase:
    - Dephosphorylates and activates E1.

Allosteric Regulation of PDH Kinase

- The kinase is allosterically activated by:
    - ATP
    - Acetyl CoA
    - NADH
- Therefore, in the presence of these high-energy products, the PDHC is turned off.
- (Note: It is actually the rise in the ratios of the following that affects enzymic activity.) :
    - ATP/ADP (adenosine diphosphate)
    - NADH/NAD^+
    - Acetyl CoA/CoA

Inhibition and Activation of PDH Kinase and Phosphatase

- Pyruvate is a potent inhibitor of PDH kinase.
- Therefore, if pyruvate concentrations are elevated, E1 will be maximally active.

- Calcium (Ca^{2+}) is a strong activator of PDH phosphatase, stimulating E1 activity.

- This is particularly important in skeletal muscle, where Ca^{2+} release during contraction:

    - Stimulates the PDHC
    - Enhances energy production

- (Note: Although covalent regulation by the kinase and phosphatase is primary, the PDHC is also subject to product inhibition by:

    - NADH
    - Acetyl CoA)

4. Deficiency

PDHC E1 α-Subunit Deficiency

- A deficiency of the α subunits of the tetrameric E1 component of the PDHC, although very rare, is the most common biochemical cause of congenital lactic acidosis.

- This deficiency leads to:
    - A decreased ability to convert pyruvate to acetyl CoA.
    - Shunting of pyruvate to lactate via lactate dehydrogenase.
- Consequences:
    - Particularly problematic for the brain, which:
        - Relies on the TCA cycle for most of its energy.
        - Is particularly sensitive to acidosis.
- Symptoms are variable, and may include:
    - Neurodegeneration
    - Muscle spasticity
    - In neonatal-onset form: early death

Genetic Basis

- The gene for the α-subunit is located on the X chromosome.

- Inheritance pattern: X-linked dominant
    - Both males and females are affected.
    - Inheritance of just one X chromosome with the mutation results in disease.

Management

- No proven treatment for PDHC deficiency.
- Possible symptomatic relief in select patients with:
    - Dietary restriction of carbohydrate
    - Supplementation with thiamine

Leigh Syndrome

- Leigh syndrome (also known as subacute necrotizing encephalomyelopathy) is a:
    - Rare
    - Progressive
    - Neurodegenerative disorder

- Caused by:
    - Defects in mitochondrial ATP production, primarily due to mutations in genes encoding:
        - PDHC proteins
        - Electron Transport Chain (ETC) proteins
        - ATP synthase
- Both nuclear DNA and mitochondrial DNA can be affected.

5. Arsenic Poisoning

Mechanism of Action

- Pentavalent arsenic (arsenate) interferes with glycolysis at the glyceraldehyde 3-phosphate step → decreases ATP production.
- However, arsenic poisoning primarily results from:
    - Inhibition of enzyme complexes that require lipoic acid as a coenzyme.

Enzymes Inhibited by Arsenite

- Pyruvate dehydrogenase complex (PDH)
- α-Ketoglutarate dehydrogenase
- Branched-chain α-keto acid dehydrogenase

Mechanism of Inhibition

- Arsenite (trivalent form of arsenic):
  - Forms a stable complex with thiol (-SH) groups of lipoic acid.
  - Makes lipoic acid unavailable to function as a coenzyme.
- Result:
  - Inhibition of PDHC → accumulation of pyruvate and lactate.
  - Neurologic disturbances and death, particularly affecting the brain (similar to PDHC deficiency).

B. Citrate Synthesis

Reaction

- Irreversible condensation of:
    - Acetyl CoA + Oxaloacetate (OAA) → Citrate (a tricarboxylic acid)
- Enzyme: Citrate synthase (initiating enzyme of TCA cycle)
- Reaction Type: Aldol condensation
    - Highly negative ΔG° → strongly favors citrate formation

Regulation of Citrate Synthase

- Product inhibition: Inhibited by citrate.
- Substrate availability:
    - OAA binding greatly increases the enzyme's affinity for acetyl CoA.

Metabolic Roles of Citrate (Note)

- Intermediate in the TCA cycle
- Source of acetyl CoA for:
    - Fatty acid synthesis (cytosolic)
    - Cholesterol synthesis
- Regulates metabolism:
    - Inhibits PFK-1 (rate-limiting enzyme of glycolysis)
    - Activates acetyl CoA carboxylase (rate-limiting enzyme of fatty acid synthesis)

C. Citrate Isomerization

Reaction

- Citrate → Isocitrate via hydroxyl group migration
- Enzyme: Aconitase (also called aconitate hydratase)
    - Type: Iron-sulfur protein

Inhibition

- Fluoroacetate:
    - A plant toxin used as a pesticide
    - Converted in vivo to fluoroacetyl CoA
    - Fluoroacetyl CoA condenses with OAA → forms fluorocitrate
    - Fluorocitrate is a potent inhibitor of aconitase

D. Oxidative Decarboxylation of Isocitrate

Reaction

- Isocitrate → α-Ketoglutarate
- Enzyme: Isocitrate dehydrogenase
- Reaction type: Irreversible oxidative decarboxylation

Products

- 1st NADH of the TCA cycle (total of 3 NADH per cycle)
- 1st CO_2 released

Regulation

- Rate-limiting step of the TCA cycle
- Allosterically activated by:
  - ADP (signals low-energy state)
  - Ca^{2+}
- Inhibited by:
  - ATP
  - NADH (both elevated in energy-rich conditions)

E. Oxidative Decarboxylation of α-Ketoglutarate

Reaction

- α-Ketoglutarate → Succinyl CoA
- Enzyme: α-Ketoglutarate dehydrogenase complex

Enzyme Complex Details

- Multienzyme complex (like PDHC):
    - Contains multiple copies of 3 enzymes
- Mechanism similar to PDH complex (Pyruvate → Acetyl CoA)

Products

- 2nd CO_2 released
- 2nd NADH of the cycle

Required Coenzymes (Same as PDHC)

- Thiamine pyrophosphate (TPP)
- Lipoic acid
- FAD
- NAD^+
- CoA

Thermodynamics

- Large negative ΔG° → strongly favors succinyl CoA formation
- Succinyl CoA is a high-energy thioester (similar to acetyl CoA)

Regulation

- Inhibited by:
  - NADH
  - Succinyl CoA (product inhibition)
- Activated by:
  - Ca^{2+}
- Not regulated by phosphorylation/dephosphorylation, unlike PDHC

Additional Note

- α-Ketoglutarate can also be produced by:
    - Oxidative deamination
    - Transamination of glutamate

F. Succinyl CoA Cleavage

Reaction

- Succinyl CoA → Succinate
- Enzyme: Succinate thiokinase (also called succinyl CoA synthetase)

Mechanism

- Cleaves high-energy thioester bond of succinyl CoA
- Coupled to:
    - GDP → GTP (substrate-level phosphorylation)
    - Enzyme uses energy from thioester bond to phosphorylate GDP

GTP to ATP Conversion

- Catalyzed by nucleoside diphosphate kinase:
    - $GTP + ADP \rightleftharpoons GDP + ATP$

Significance

- Another example of substrate-level phosphorylation (direct ATP/GTP generation without ETC involvement)

Additional Sources & Fate of Succinyl CoA

- Also formed from:
    - Propionyl CoA (from odd-chain fatty acids)
    - Several amino acids
- Can be:
    - Converted to pyruvate for gluconeogenesis
    - Used in heme synthesis

G. Succinate Oxidation

Reaction

- Succinate → Fumarate
- Enzyme: Succinate dehydrogenase
- Coenzyme: FAD → $FADH_2$

Key Features

- Only TCA enzyme embedded in inner mitochondrial membrane
- Also functions as Complex II of the electron transport chain

Why FAD instead of NAD^+?

- Succinate's reducing power is insufficient to reduce NAD^+
- Hence, FAD is used as the electron acceptor

H. Fumarate Hydration

Reaction

- Fumarate ⇌ Malate
- Enzyme: Fumarase (also called fumarate hydratase)
- Freely reversible hydration reaction

Other Sources of Fumarate

- Produced in:
    - Urea cycle
    - Purine synthesis
    - Catabolism of phenylalanine and tyrosine

I. Malate Oxidation

Reaction

- Malate → Oxaloacetate (OAA)
- Enzyme: Malate dehydrogenase
- Produces: 3rd and final NADH of TCA cycle

Thermodynamics

- ΔG° is positive (unfavorable)
- Driven forward by:
    - Highly exergonic citrate synthase reaction (removal of OAA pulls the reaction forward)

Other Sources of OAA

- Also formed by transamination of aspartate

III. Energy Produced by the TCA Cycle

Electron Transfers

- Total: 4 pairs of electrons transferred per turn
    - 3 $NAD^+ \rightarrow$ 3 NADH
    - 1 FAD $\rightarrow$ 1 $FADH_2$

ATP Yield from Electron Transport Chain

- 1 NADH $\rightarrow$ 3 ATP
- 1 $FADH_2 \rightarrow$ 2 ATP

Total ATP Yield from One Acetyl CoA

Product	Quantity	ATP Yield
3 NADH	3	9 ATP
1 $FADH_2$	1	2 ATP
1 GTP ($\rightleftharpoons$ ATP)	1	1 ATP
Total	—	12 ATP

Carbon Balance

- 2 carbon atoms enter as acetyl CoA
- 2 CO_2 released — no net gain or loss of carbon skeletons

IV. Regulation of the TCA Cycle

Key Differences from Glycolysis

- Glycolysis mainly regulated by: PFK-1
- TCA cycle is regulated at multiple enzymatic steps

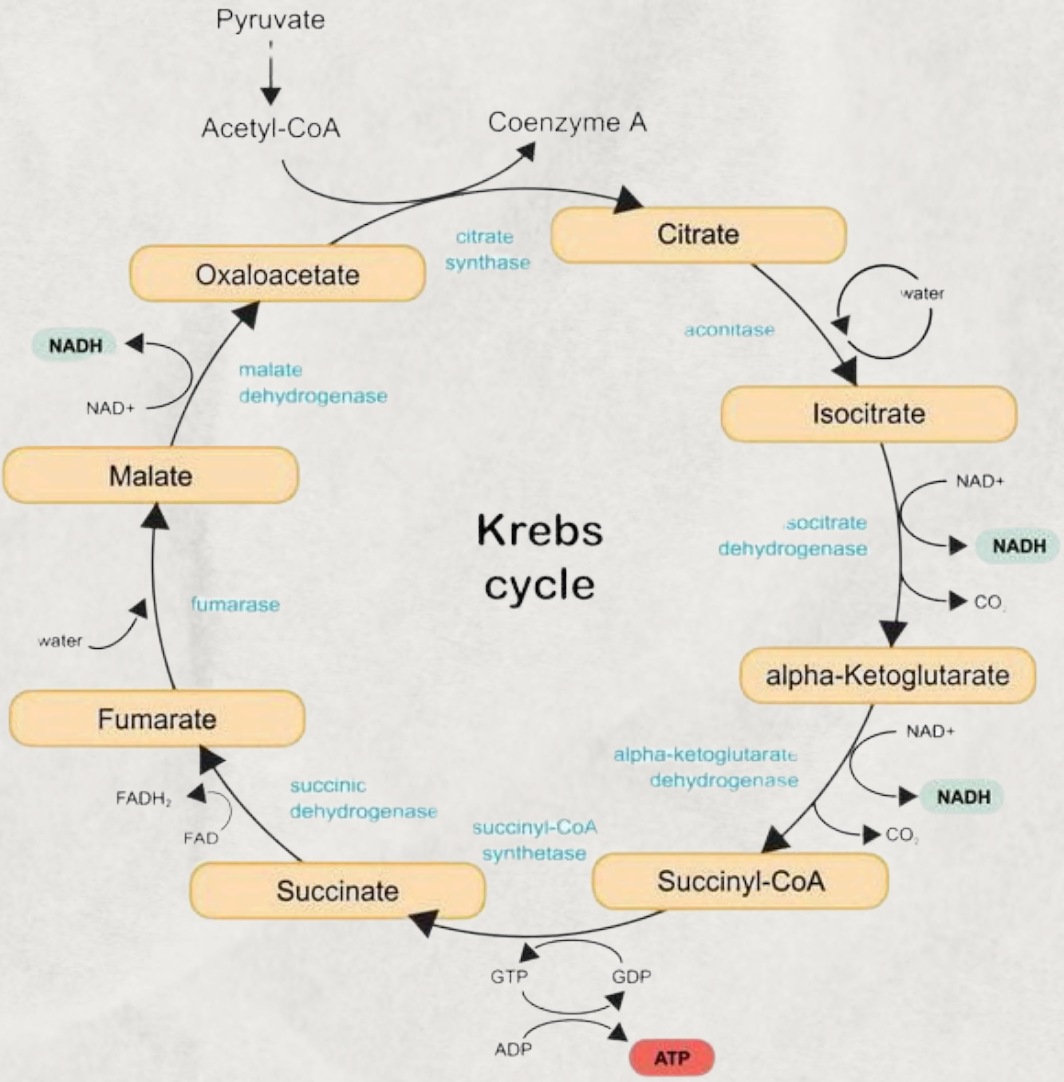
Key Regulatory Enzymes (High $-\Delta G^{\circ}$)

1. Citrate synthase
2. Isocitrate dehydrogenase
3. α-Ketoglutarate dehydrogenase complex

Regulatory Triggers

- Low ATP/ADP ratio (i.e., low energy state):
    - Upregulates PDHC and TCA cycle
    - Increases reducing equivalents for oxidative phosphorylation

TCA Cycle Flowchart



Products per cycle

- 2 molecules of CO_2
- 3 molecules of NADH
- 1 molecules of $FADH_2$
- 1 molecules of ATP