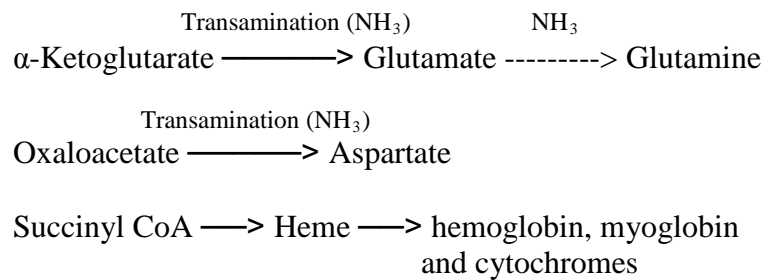


Tricarboxylic acid cycle

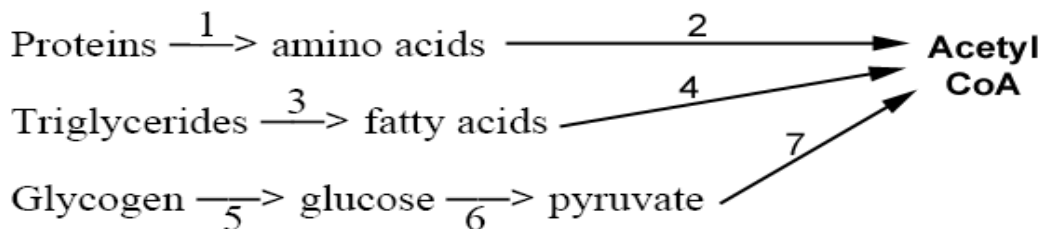
The TCA cycle, also known as the citric acid cycle or the Krebs cycle, is a cyclic series of enzymatically catalyzed reactions, carried out by a multienzyme system, consisting of eight enzymes. The cycle operates in the mitochondrial matrix. It serves to oxidize the acetyl group in acetyl CoA to CO₂ and to generate NADH and FADH₂.

TCA cycle is an amphibolic pathway because it is involved in both anabolic and catabolic processes. Anabolically, the cycle generates precursors for biosynthetic processes.



Catabolically, the cycle serves to oxidize the acetyl group. It provides a common pathway for the final oxidation of all metabolic fuels (carbohydrates, fatty acids, amino acids and ketone bodies).

Sources of acetyl CoA

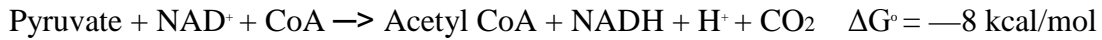


1, proteolysis; 2, amino acid metabolism; 3, lipolysis; 4, β -oxidation; 5, glycogenolysis; 6, glycolysis; 7, oxidative decarboxylation

Pyruvate arising from glycolysis has several fates. It can be transaminated to alanine, reduced to lactate, carboxylated to oxaloacetate or oxidatively decarboxylated to acetyl CoA.

Transamination to alanine requires pyridoxal phosphate; reduction to lactate requires NADH (niacin); conversion to oxaloacetate requires biotin; oxidative decarboxylation to acetyl CoA requires NAD⁺ (niacin), Coenzyme A (pantothenic acid), FAD (riboflavin), thiamine pyrophosphate, and lipoic acid.

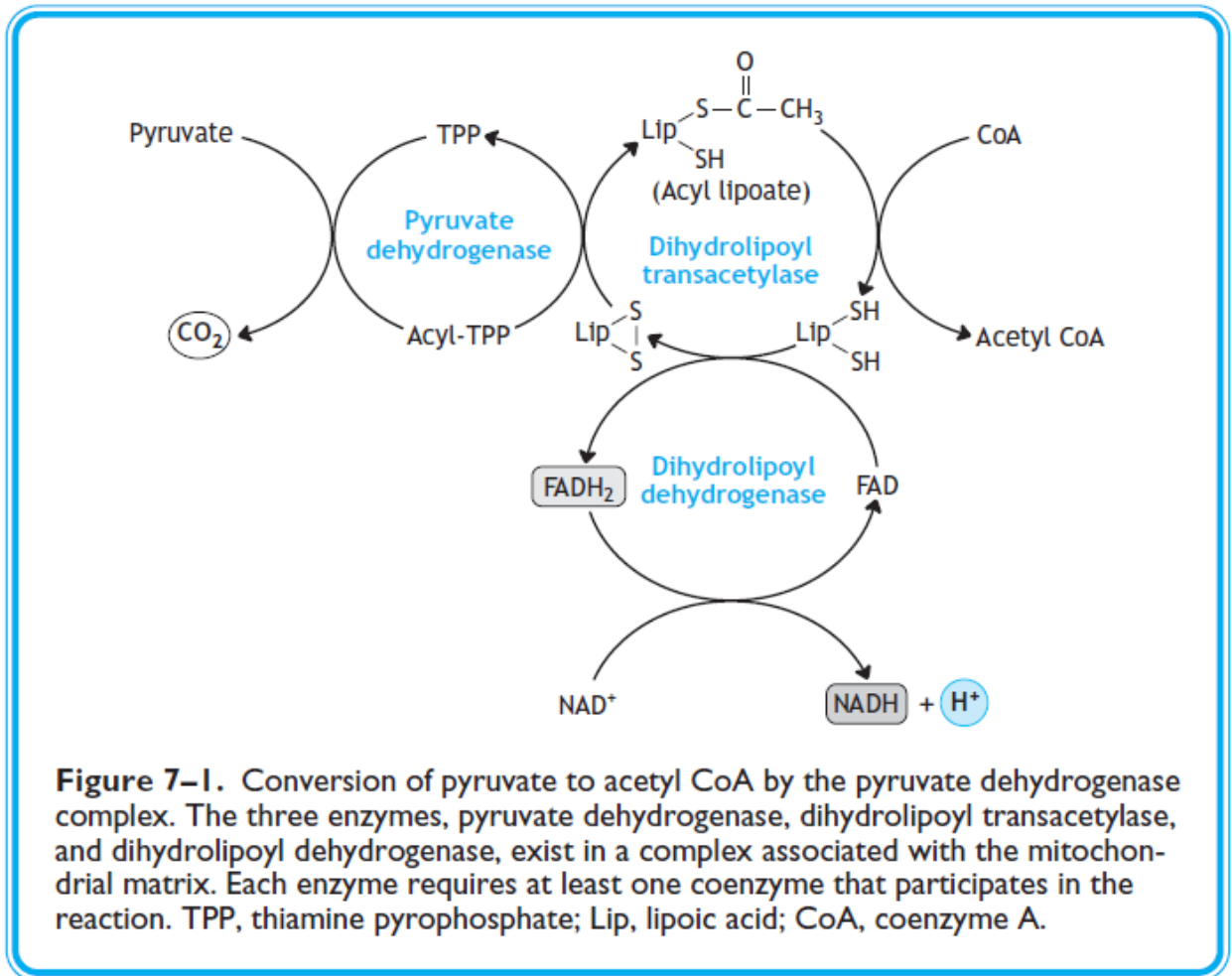
Formation of acetyl CoA from pyruvate is not a component of the TCA cycle. But, this is one of the major routes by which acetyl CoA is generated.



Because of the high exergonic nature of this reaction, it is essentially an irreversible reaction. Therefore, the reverse reaction, i.e. the conversion of acetyl CoA to pyruvate, does not occur.

Coenzyme A contains the vitamin pantothenic acid as a component. It has a thiol group (-SH) arising from β -mercaptoethylamine.

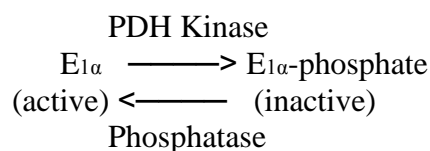
The enzyme catalyzing the reaction is called pyruvate dehydrogenase, a multienzyme complex. It consists of three different catalytic parts: pyruvate decarboxylase (E₁), dihydrolipoyl transacetylase (E₂), and dihydrolipoyl dehydrogenase (E₃). The multienzyme complex utilizes five different coenzymes: thiamine pyrophosphate, lipoic acid, coenzyme A, FAD and NAD⁺ (i.e. five vitamins: thiamine, lipoic acid, pantothenic acid, riboflavin and niacin). Coenzyme A and NAD⁺ are free in solution whereas the other three are bound to proteins. Thiamine pyrophosphate is bound to pyruvate dehydrogenase, lipoic acid to dihydrolipoyl transacetylase, and FAD to dihydrolipoyl dehydrogenase.



Regulation of pyruvate dehydrogenase complex

Product inhibition: Acetyl CoA and NADH inhibit the complex

Covalent modification: The pyruvate decarboxylase (E1) component of the multienzyme complex consists of two subunits, E1 α and E1 β . E1 α catalyzes the decarboxylation step and is regulated by covalent modification involving a pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase



NADH and acetyl CoA, the products of the pyruvate dehydrogenase complex, activate the PDH kinase which converts active E1 α to inactive E1 α -phosphate. The substrates of the

pyruvate dehydrogenase complex, namely pyruvate, CoA and NAD^+ , inhibit the PDH kinase, thereby keeping $\text{E}_{1\alpha}$ in the active form. The PDH kinase is cAMP-independent. However, **insulin activates the pyruvate dehydrogenase complex in adipose tissue and norepinephrine activates the complex in the heart by mechanisms, which do not involve cAMP. This hormonal regulation involves activation of phosphatase.**

Dichloroacetate stimulates pyruvate dehydrogenase (PDH) function by inhibiting PDH kinase, which phosphorylates and inactivates PDH. Hence, in conditions that result in accumulation of lactate (lactic acidosis), dichloroacetate activates PDH, enhances pyruvate oxidation, and facilitates conversion of lactate to pyruvate. Dichloroacetate has been shown to be beneficial in patients with lactic acidosis. This compound however may not be beneficial in genetic diseases involving PDH.

Acetyl CoA formed via the pyruvate dehydrogenase complex reaction can undergo three different routes of metabolism: enter TCA cycle and get oxidized, get converted to ketone bodies (acetoacetate and β -hydroxybutyrate) or enter metabolic pathway leading to synthesis of fatty acid and sterol.

Pyruvate dehydrogenase deficiency

The pyruvate dehydrogenase complex is involved in several pathological conditions. Defects in one or more of the component enzymes of the complex are a major cause of primary lactic acidosis in infants and young children. If PDH complex is not active, pyruvate cannot be converted into acetyl CoA; as consequence, pyruvate is converted into alanine by transamination and also into lactate by reduction. Thus, patients with PDH deficiency have elevated levels of alanine and lactate in circulation.

Patients have chronic acidosis and elevated levels of pyruvate, lactate and alanine. Brain tissue, with its absolute requirement for aerobic glucose oxidation, appears to be a primary site for these metabolic lesions, with concomitant neurological symptoms. $\text{E}_{1\alpha}$ is the major site of mutations. The clinical presentation of patients with $\text{E}_{1\alpha}$ deficiency is unusually heterogeneous. At one end of the spectrum is lethal lactic acidosis in the newborn period with few obvious neurological problems. At the other end of the spectrum are patients with little lactic acidosis but with severe brain dysfunction and structural brain abnormalities. An approximately equal number of males and females have been identified with $\text{E}_{1\alpha}$ deficiency, though the gene for $\text{E}_{1\alpha}$ is on the X chromosome. The X chromosome location of the gene has different consequences in males and females.

	<u>Males</u>	<u>Females</u>
Severe mutation	All cells affected Major neurological problems Not compatible with fetal survival	Not all cells affected Brain abnormalities Minimal lactic acidosis

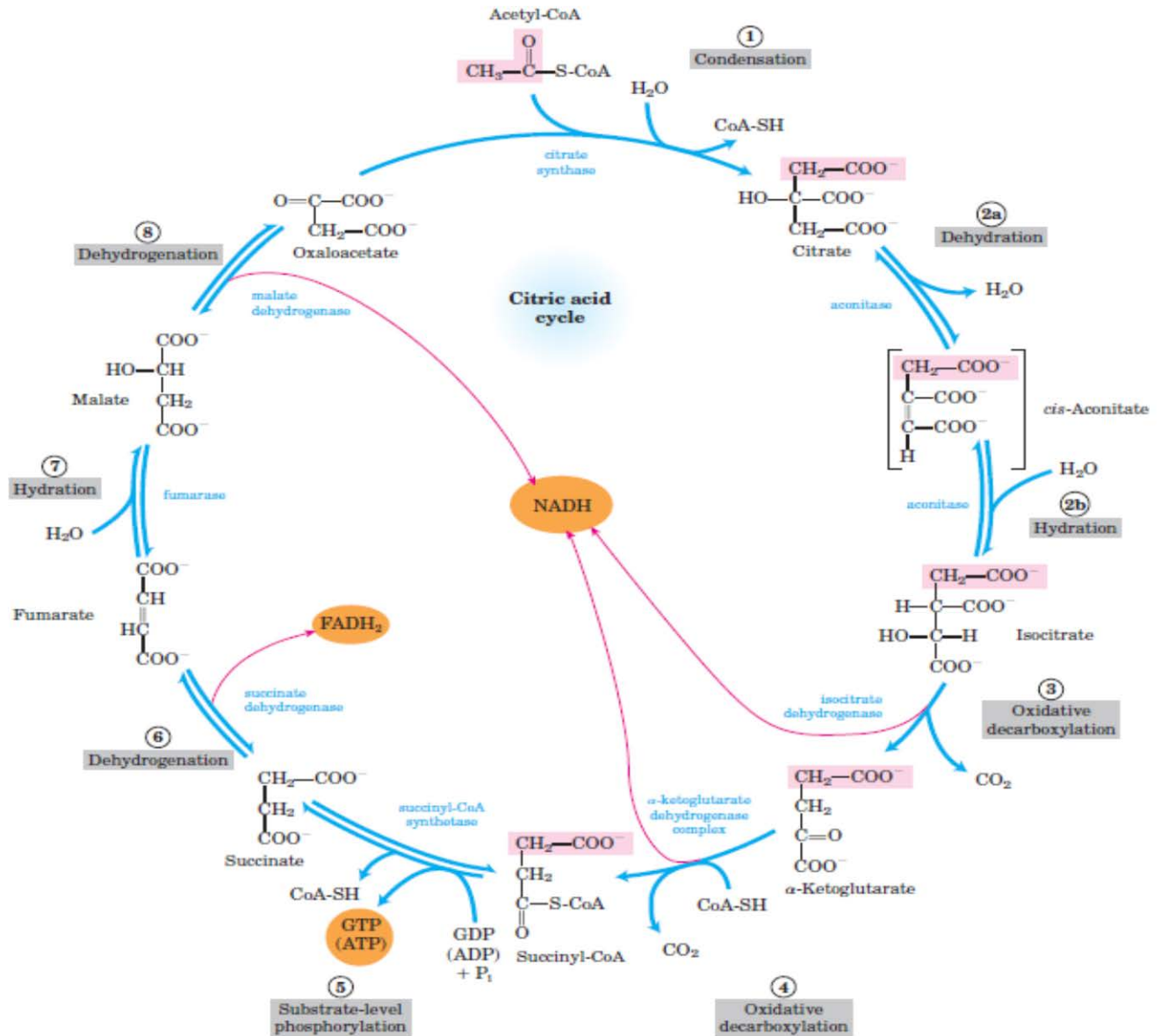
Mild mutation	All cells affected	Not all cells affected
	Lactic acidosis	No lactic acidosis
	Neurological problems	No or minor neurological problems
	Compatible with fetal survival	

In males, all cells are affected because there is only one X chromosome. In females, not all cells are affected because of the random inactivation of one of the two X chromosomes. Metabolites like lactic acid can be exchanged between cells, but this is not the case for ATP. Therefore in females, the cells with normal allele can metabolize the lactic acid released from the cells with the defective allele, but the normal cells cannot supply ATP to the defective cells. Even with severe mutation, the presence of normal cells ensures survival. This leads to a phenotype seen only in females, namely profound neurological symptoms and structural brain abnormalities, but with minimal lactic acidosis. Nonetheless, some females present with severe lactic acidosis, most likely caused by unfavorable X chromosome inactivation especially in the liver.

Primary biliary cirrhosis is an autoimmune disease characterized by the production of antibodies against components of the pyruvate dehydrogenase complex, most frequently against the E₂ subunit. The clinical picture includes progressive jaundice, fatigue, hepatosplenomegaly and pruritus. Liver biopsy findings include inflammatory destruction of interlobular bile ductules, ductular proliferation and fibrosis and cirrhosis. It is believed that in this disease biliary ductular cells aberrantly express on their luminal surface a molecule resembling mitochondrial pyruvate dehydrogenase E₂ subunit. This neoantigen triggers the autoimmune disease.

Defects in TPP-containing enzymes, including pyruvate dehydrogenase, have been reported in brain and peripheral tissues from patients suffering from Alzheimer's disease.

Reactions of the TCA cycle



Highlights of the TCA cycle

Two carbons, in the form of acetyl CoA, are introduced into the cycle at the level of citrate synthase and two carbons are eliminated as two molecules of CO_2 , one at the level of isocitrate dehydrogenase and the other at the level of α -ketoglutarate dehydrogenase. The carbon atoms in the eliminated CO_2 are not the carbon atoms introduced into the cycle as acetyl CoA. Oxaloacetate, which initiates the cycle, is regenerated at the end of the cycle.

Each cycle generates 3 molecules of NADH, 1 molecule of FADH₂, and 1 molecule of GTP. Since, in the electron transport chain, one molecule of NADH produces 2.5 molecules of ATP and one molecule of FADH₂ produces 1.5 molecules of ATP, the total number of ATP molecules generated during the oxidation of one molecule of acetate (as acetyl CoA) in the cycle is $(3 \times 2.5) + (1 \times 1.5) + 1 = 7.5 + 1.5 + 1 = 10$

If the carbon atoms in acetyl CoA are labeled, the label will appear in oxaloacetate at the end of the cycle, but there is no net synthesis of oxaloacetate. Since oxaloacetate can be converted to glucose via gluconeogenesis, the labeled carbon atoms in acetyl CoA will appear in glucose, but there is no net synthesis.

All enzymes of the cycle are either freely reversible or have negative ΔG° . The only exception is malate dehydrogenase ($\Delta G^\circ = +7$ kcal/mol). Even though endergonic, malate dehydrogenase reaction is normally pulled forward because oxaloacetate is efficiently removed by the citrate synthase reaction to form citrate.

Aconitase and succinate dehydrogenase contain non-heme iron atoms. Succinate dehydrogenase is also a part of the electron transport chain (Complex II), but is not coded by mitochondrial DNA.

Regulation of the cycle occurs mainly at the levels of isocitrate dehydrogenase and α -ketoglutarate dehydrogenase. Isocitrate dehydrogenase is the most important regulatory enzyme in TCA cycle. It is inhibited by NADH and ATP and activated by NAD⁺ and ADP. α -Ketoglutarate dehydrogenase is also inhibited by NADH. Citrate synthase is inhibited by ATP.

Fluoroacetate blocks TCA cycle because citrate synthase converts fluoroacetyl CoA to fluorocitrate which is a potent inhibitor of aconitase. Malonate blocks TCA cycle because it is a potent competitive inhibitor of succinate dehydrogenase.

The reaction catalyzed by α -ketoglutarate dehydrogenase is very similar to the reaction catalyzed by pyruvate dehydrogenase. α -Ketoglutarate dehydrogenase is also a multienzyme complex and the reaction involves cofactors derived from the following five vitamins: thiamine, lipoic acid, pantothenic acid (coenzyme A), niacin and riboflavin.

Phosphorylation of GDP to yield GTP in the reaction catalyzed by succinyl CoA synthetase is called substrate-level phosphorylation. Since the energy present in GTP can be used to synthesize ATP from ADP, generation of GTP in this reaction is equivalent to generation of ATP.

Acetyl CoA, generated from oxidation of fatty acids, cannot be converted to glucose via gluconeogenesis because i) pyruvate dehydrogenase is irreversible and ii) when acetyl CoA is taken through the TCA cycle once, two carbon atoms are eliminated as CO₂ (even though these two carbon atoms in the two molecules of CO₂ are not the same carbon atoms which were originally present in acetyl CoA) and there is no net synthesis of oxaloacetate which can serve as a precursor for gluconeogenesis.