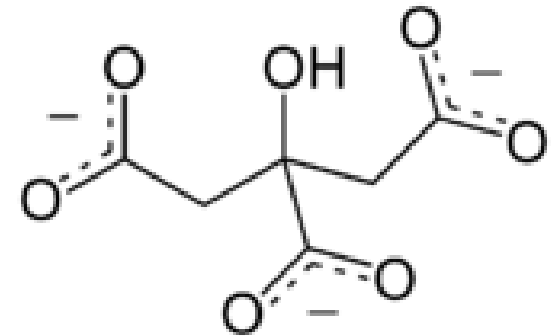


**Bryan Krantz: University of California, Berkeley**  
**MCB 102, Spring 2008, Metabolism Lecture 9**

**Reading: Ch. 16 & 17 of *Principles of Biochemistry*, “The Citric Acid Cycle” & “Fatty Acid Catabolism.”**

**Symmetric Citrate.** The left and right half are the same, having mirror image acetyl groups (-CH<sub>2</sub>COOH).

**Radio-label Experiment.** The Krebs Cycle was tested by <sup>14</sup>C **radio-labeling** experiments. In 1941, <sup>14</sup>C-Acetyl-CoA was used with normal oxaloacetate, labeling only the right side of drawing. But none of the label was released as CO<sub>2</sub>. Always the left carboxyl group is instead released as CO<sub>2</sub>, i.e., that from oxaloacetate. This was interpreted as proof that citrate is not in the cycle at all the labels would have been scrambled, and half of the CO<sub>2</sub> would have been <sup>14</sup>C.



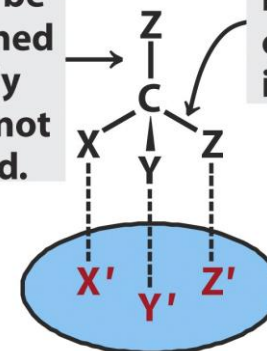
**Prochiral Citrate.** In a two-minute thought experiment, Alexander Ogston in 1948 (*Nature*, **162**: 963) argued that citrate has the potential to be treated as chiral. In chemistry, **prochiral** molecules can be converted from achiral to chiral in a single step. The trick is an asymmetric enzyme surface (*i.e.* aconitase) can act on citrate as through it were chiral. As a consequence the left and right acetyl groups are not treated equivalently.



*“On the contrary, it is possible that an asymmetric enzyme which attacks a symmetrical compound can distinguish between its identical groups.”*

This bond cannot be positioned correctly and is not attacked.

This bond can be positioned correctly and is attacked.

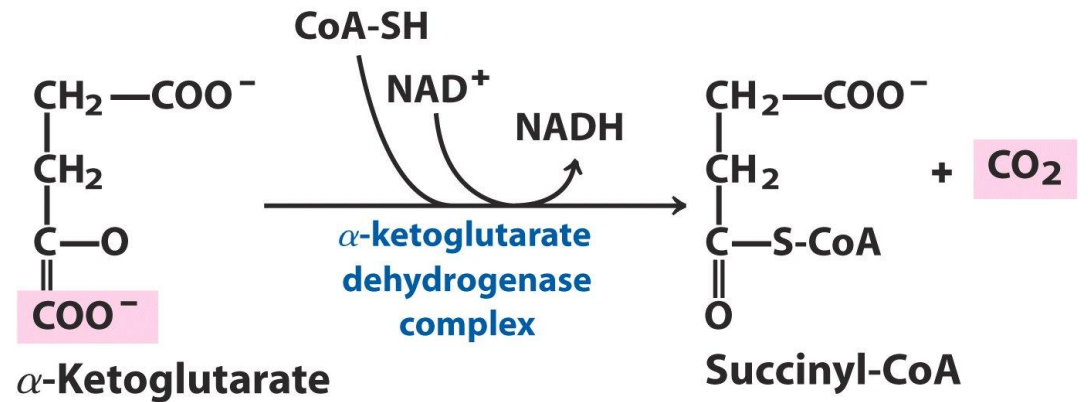


Active site has complementary binding points.

**[STEP 4]  $\alpha$ -Keto Glutarate Dehydrogenase.** This enzyme splits the carbon-carbon bond and is related to pyruvate dehydrogenase. E1 and E2 are similar, and E3 is identical in sequence!

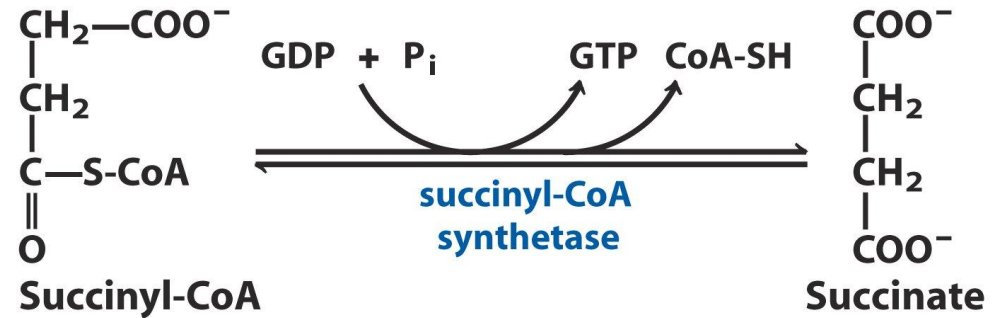
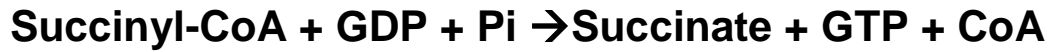


**Mechanism.**  $\alpha$ -keto glutarate dehydrogenase works exactly like pyruvate dehydrogenase. You have the five coenzymes: TPP, lipoyllysine, CoA, FAD and  $\text{NAD}^+$ . These are all used, and you get oxidation. The decarboxylated product occurs as a thioester. The product is succinyl-CoA. The thioester in the succinyl-CoA will be utilized later of course in an analogous manner.



$$\Delta G'^{\circ} = -33.5 \text{ kJ/mol}$$

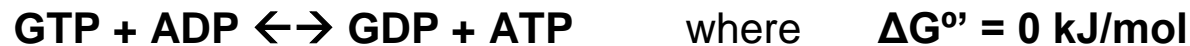
**[STEP 5] Succinyl-CoA Synthetase.**



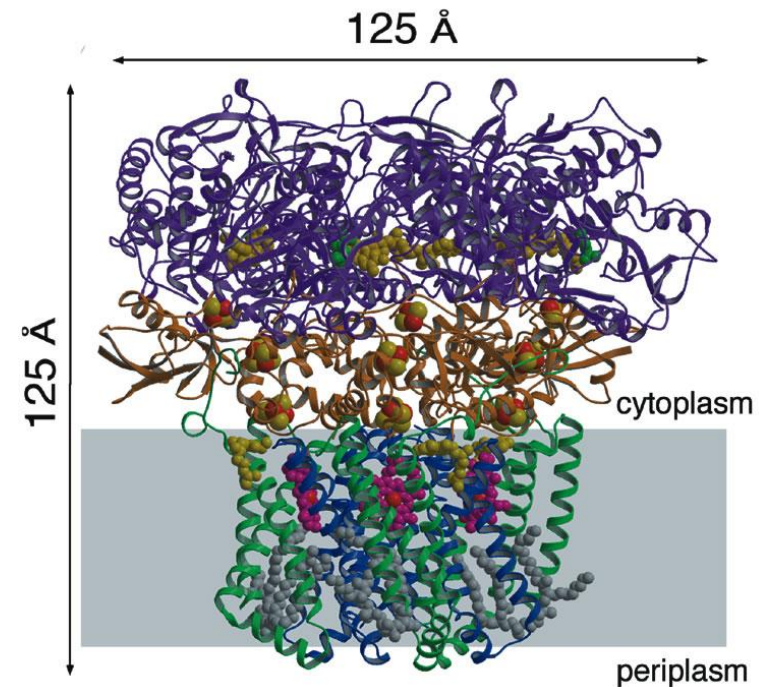
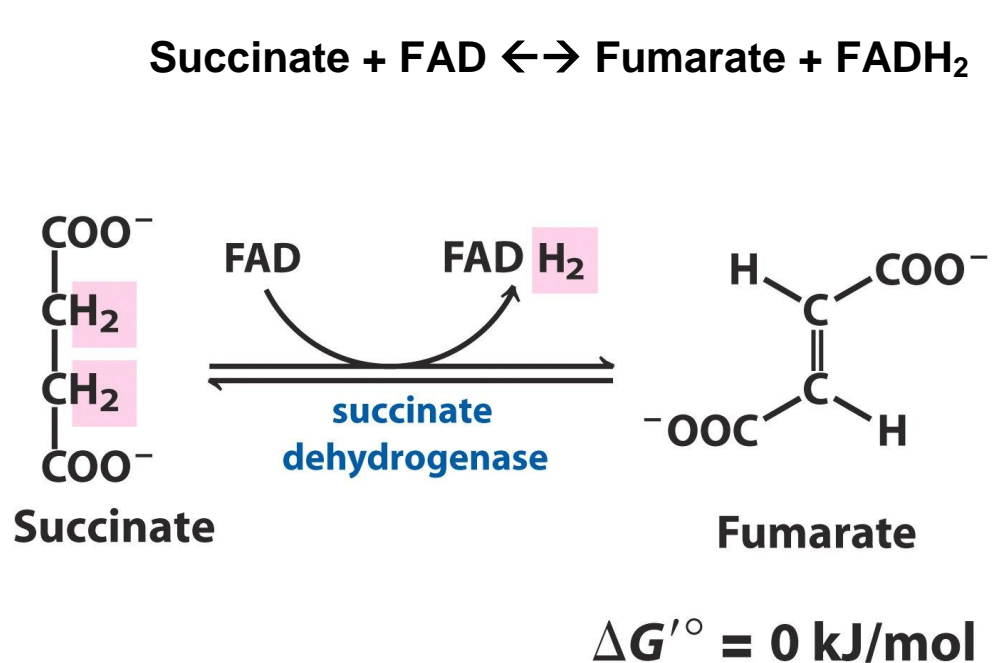
$\Delta G'^{\circ} = -2.9 \text{ kJ/mol}$

**Mechanism.** Phosphorylysis reaction is followed by phosphoryl transfer to GDP, producing succinate plus GTP. Note the phosphoryl group is transferred to the GDP via an intermediate that forms with a His residue on the enzyme's active site.

**Energetics.** Capitalized on the CoA thioester by capturing free energy as a GTP. GTP is as good as ATP, because there is a free conversion of nucleoside triphosphates in the cell (by the enzyme, **nucleoside diphosphate kinase**).

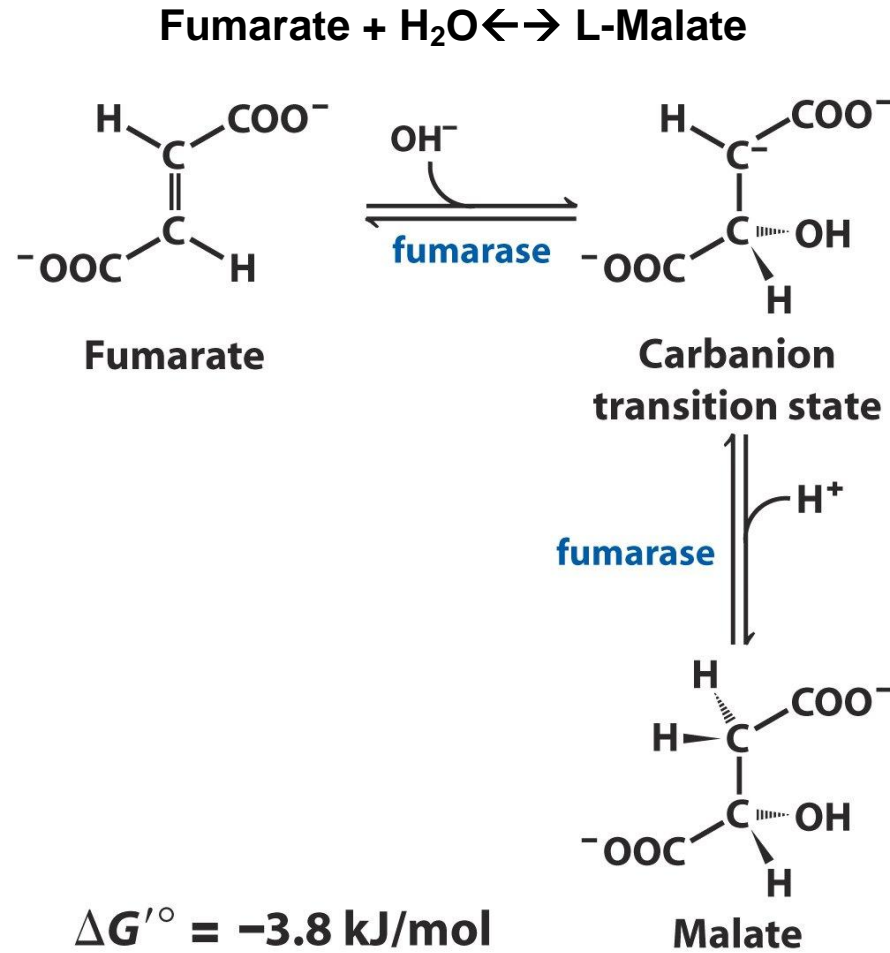


**[STEP 6] Succinate Dehydrogenase.** The next step is **succinate dehydrogenase**. Starting from succinate and take away two hydrogen atoms to make fumarate. FAD is reduced to form FADH<sub>2</sub>.



**Energetics.** The reason we use FAD in this reaction rather than NAD<sup>+</sup> is that the succinate is a rather poor electron donor. The reduction potential of the succinate/fumarate pair is +0.03 Volts. There is no way that you can use such a poor electron donor to reduce NAD<sup>+</sup>. The reduction potential of NAD<sup>+</sup>/NADH is -0.32 Volts. That is why you use the FAD/FADH<sub>2</sub> pair, which is much more oxidized.

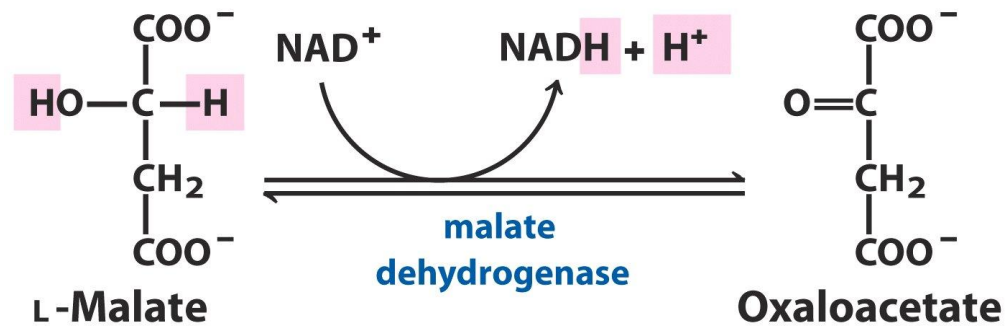
**[STEP 7] Fumarase.** The next reaction is called **fumarase**, which converts fumarate into a hydroxy-dicarboxylic acid called malate. This is an addition of water across the double bond—a recurring theme in the course.



**[STEP 8] Malate Dehydrogenase.** Finally, there is malate dehydrogenase, which uses  $\text{NAD}^+$  to oxidize this product malate to oxaloacetate. This process yields  $\text{NADH}$ .



- We talked about this reaction in gluconeogenesis as a way to get reducing equivalents into the cytosol.
- Here the reaction completes the CAC, remaking oxaloacetate.



$$\Delta G'^{\circ} = 29.7 \text{ kJ/mol}$$

**Overall Energetics.** The citric acid cycle is an oxidative pathway. In biology, oxidations are coupled to dehydrogenation and this is the theme in the citric acid cycle. For one acetyl-CoA, we generate 1 GTP (or ATP), 3 NADH, and 1 FADH<sub>2</sub>. Converting these e<sup>-</sup> carriers to ATP, a NADH is worth about 2.5 ATP, and an FADH<sub>2</sub> is worth about 1.5 ATP. (Some books have slightly larger numbers for this conversion.) So that's ~10 ATP (or ~12 ATP for the slightly higher numbers).

### *The pay-off is large but is this cycle possible?*

**TABLE 16-1** Stoichiometry of Coenzyme Reduction and ATP Formation in the Aerobic Oxidation of Glucose via Glycolysis, the Pyruvate Dehydrogenase Complex Reaction, the Citric Acid Cycle, and Oxidative Phosphorylation

<i>Reaction</i>	<i>Number of ATP or reduced coenzyme directly formed</i>	<i>Number of ATP ultimately formed*</i>
Glucose → glucose 6-phosphate	-1 ATP	-1
Fructose 6-phosphate → fructose 1,6-bisphosphate	-1 ATP	-1
2 Glyceraldehyde 3-phosphate → 2 1,3-bisphosphoglycerate	-2 NADH	3 or 5 <sup>†</sup>
2 1,3-Bisphosphoglycerate → 2 3-phosphoglycerate	-2 ATP	-2
2 Phosphoenolpyruvate → 2 pyruvate	-2 ATP	-2
2 Pyruvate → 2 acetyl-CoA	-2 NADH	-5
2 Isocitrate → 2 α-ketoglutarate	-2 NADH	-5
2 α-Ketoglutarate → 2 succinyl-CoA	-2 NADH	-5
2 Succinyl-CoA → 2 succinate	-2 ATP (or 2 GTP)	-2
2 Succinate → 2 fumarate	-2 FADH <sub>2</sub>	-3
2 Malate → 2 oxaloacetate	-2 NADH	-5
Total	—	30-32

\*This is calculated as 2.5 ATP per NADH and 1.5 ATP per FADH<sub>2</sub>. A negative value indicates consumption.

<sup>†</sup> This number is either 3 or 5, depending on the mechanism used to shuttle NADH equivalents from the cytosol to the mitochondrial matrix; see Figures 19-27 and 19-28.

**Reduction Potentials.** Consider  $\Delta G^{\circ} = -nF \Delta E^{\circ}$ . If the  $\Delta E^{\circ}$  is positive then the reaction is spontaneous (i.e.,  $\Delta G^{\circ} < 0$ ). The  $E^{\circ}$ 's for  $e^-$  carriers,  $NAD^+$  and FAD:



• **Isocitrate Dehydrogenase.** Here is the first half-reduction reaction in the cycle.



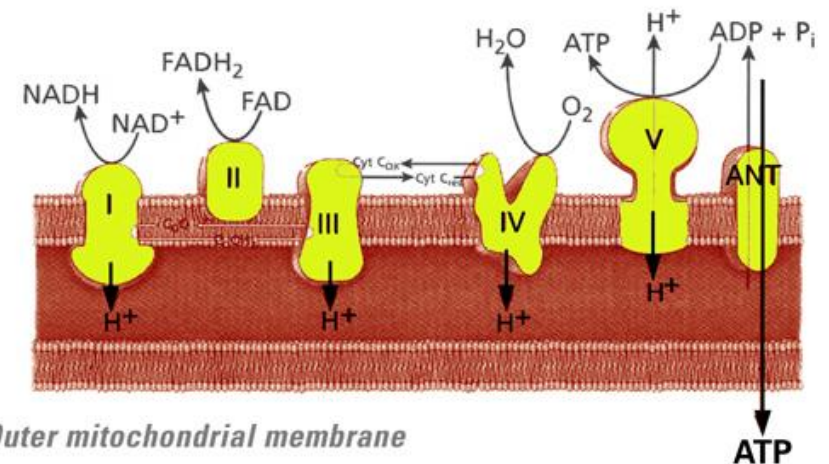
The  $\Delta E^{\circ}$  of **+0.06 V** is favorable and positive so this should go forward.

• **Succinate Dehydrogenase (SDH).**



*How can this occur? (1)* It uses FAD. Unlike  $NAD^+$ , FAD coenzymes are attached to the interior of the enzyme so the *correct* redox potential is not as depicted in the table. **(2)** SDH is a membrane protein and FAD may be reoxidized by the successive chain of electron carriers, eventually ending up in the reduction of oxygen to water—a favorable  $E^{\circ} = +0.816 \text{ V}$ . Downstream electron sink helps pull the reaction forward.

Inner mitochondrial membrane





- **Malate Dehydrogenase (MDH).**



The reaction is unlikely without some help:  $\Delta E^{\circ'} = -0.154 \text{ V}$ . Being the last step of the citric acid cycle, it can **thermodynamically couple** to the first step, citrate synthase.

Citrate synthase, because of the hydrolysis of the high-energy thioester bond in the original acetyl-CoA, has a very large negative standard free energy change of  $-30 \text{ kJ/mol}$ . Since the next reaction pulls the oxaloacetate very strongly, this reaction becomes possible.

So under standard conditions (and coupling these reactions), this MDH reaction can be driven to a slightly favorable  $\Delta G$  of  $-0.5 \text{ kJ/mol}$ . *That was close.*

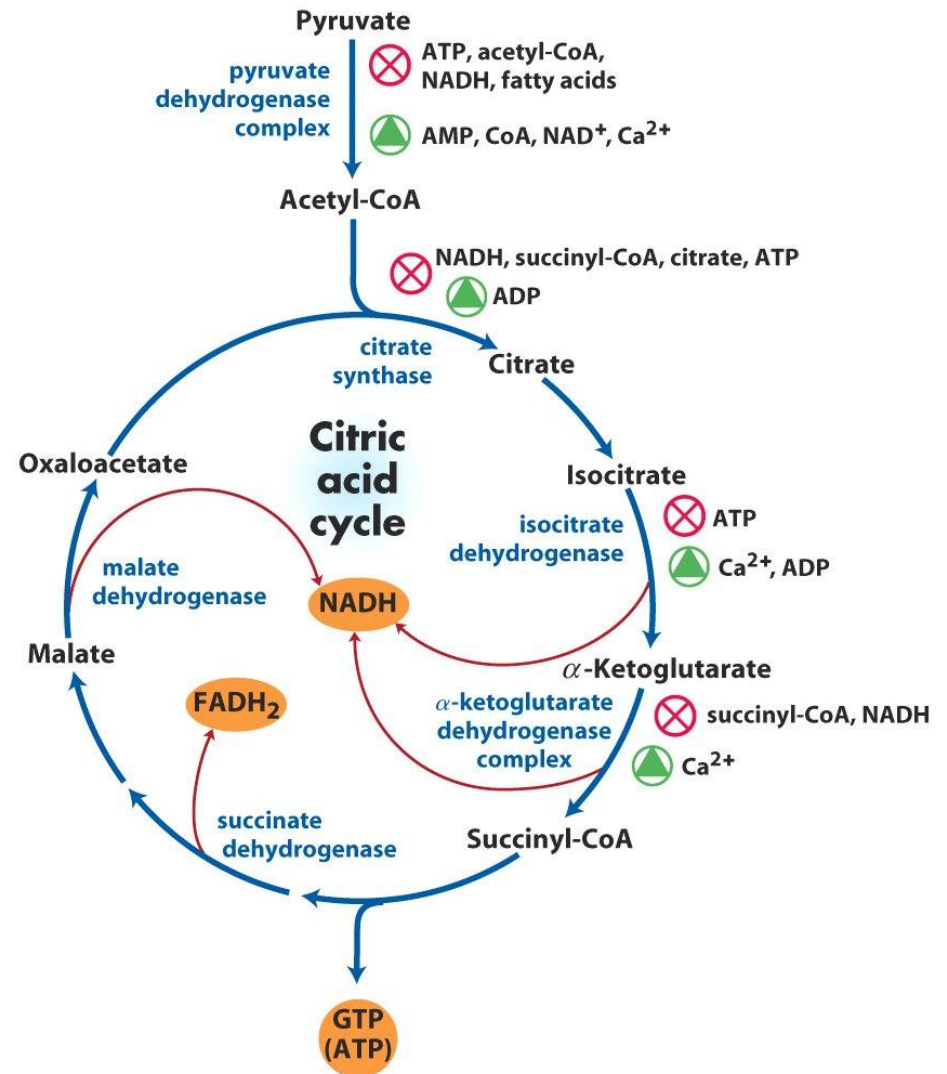
**Regulation.** *How do we regulate the citric acid cycle?*

What are the steps in the citric acid cycle with a large, negative  $\Delta G^0$ ?

- Pyruvate dehydrogenase (-33.4 kJ/mol)
- Citrate synthase (-32.2 kJ/mol)
- Isocitrate dehydrogenase (-8.4 kJ/mol)
- $\alpha$ -keto glutarate dehydrogenase (-33.5 kJ/mol)

In most cases, excess ATP, NADH or inhibit these enzymes. If the cell has enough energy molecules, the brakes are then applied.

*Why are there so many checkpoints on the cycle?*



**Biosynthesis Bonus.** Citric acid cycle intermediates are used in biosynthetic pathways:

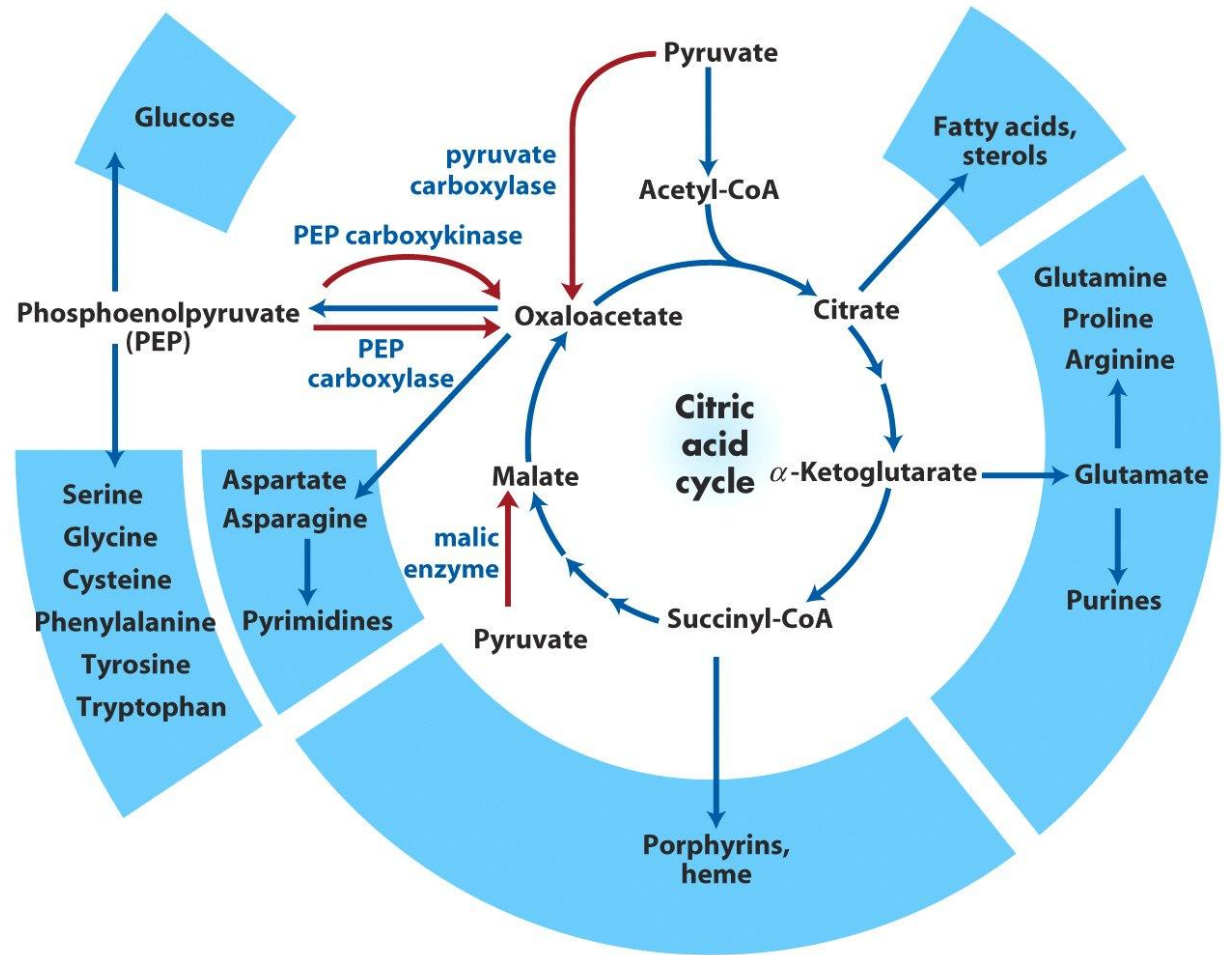
- The five-carbon and four-carbon compounds from the cycle to make amino acids.
- Other cofactors, heme, sterols, and nucleotides.

**Anaplerotic Reactions.**

Biosynthesis drains Citric Acid Cycle intermediates. Replenishing their supply occurs via **anaplerotic reactions**.

*E.g.*, pyruvate carboxylase makes oxaloacetate from pyruvate.

**The Two-carbon Limit.** Acetyl-CoAs cannot be assembled to make 4 carbon intermediates in humans. Basically, the Atkins diet cannot make all the building blocks. Bacteria & plants can do it, because they have a pathway called the **glyoxylate pathway**.

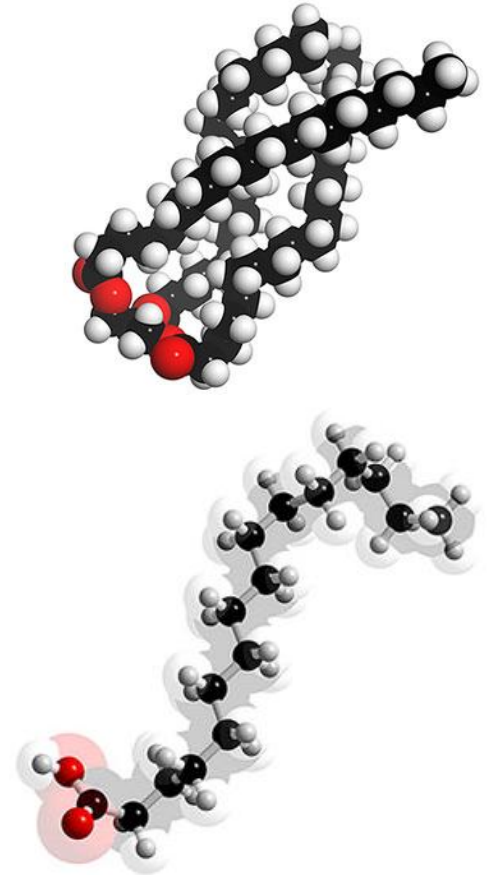


## LIPID CATABOLISM

We have a large amount of fat in our body, ~15 kg of fat, compared with only ~150 g of glycogen. Fat can sustain humans for weeks. Glycogen lasts hours or about a day. Fats (or **triglycerides**) are an important for long term energy storage.

**Triglycerides** (or **triacylglycerols**) contain three **fatty acid** acyl chains and one glycerol.

- Fats can come from diet and digestion in the intestine → fatty acids can enter the blood.
- Fat is stored in specialized cells that form adipose tissue.
- Hormonal signals cause the fat to be hydrolyzed, and then free fatty acids are released into the blood.
- Serum albumin binds fatty acids in the blood, acting as a carrier.
- Fatty acid catabolism occurs inside cells (mainly inside mitochondria).

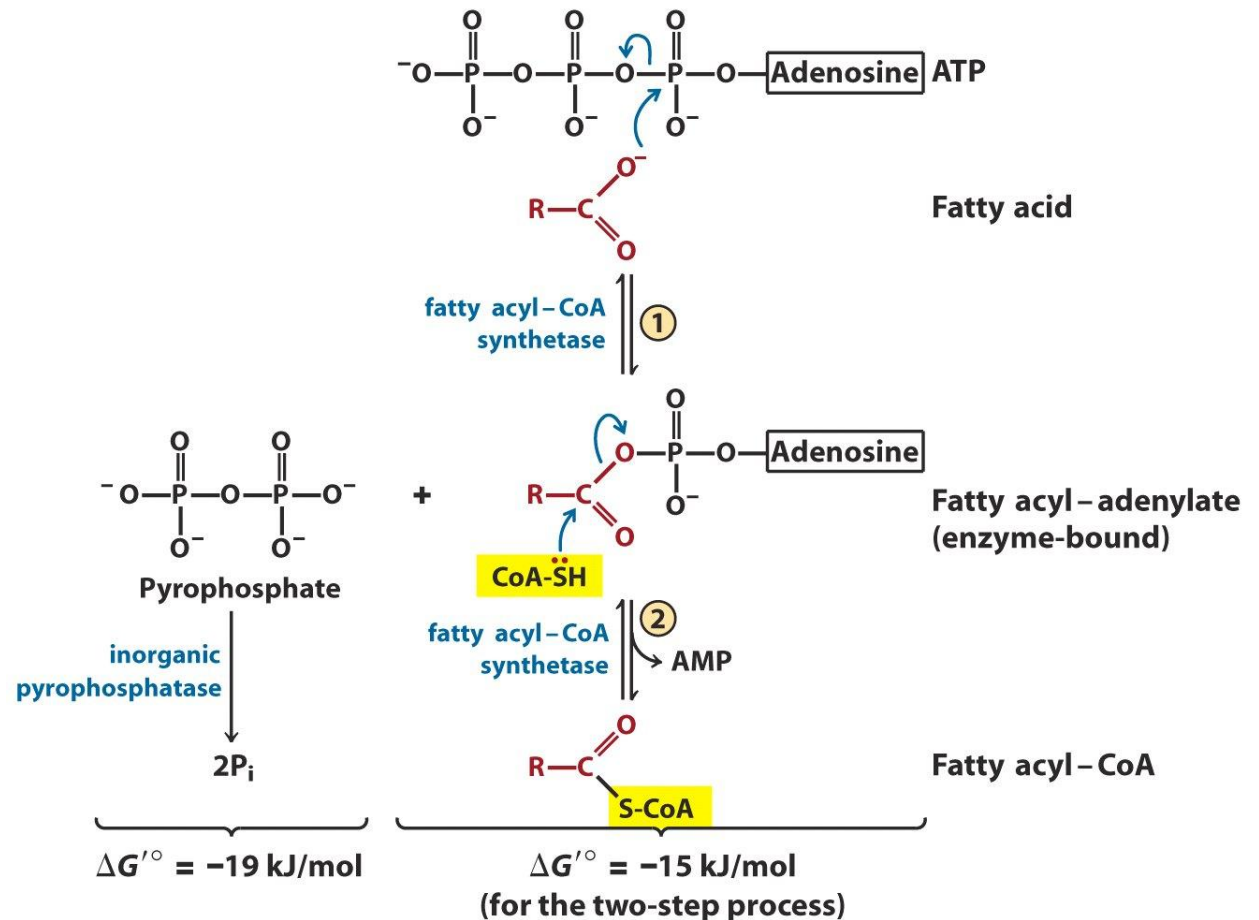


## Acyl-CoA Synthetase



**Mechanism.** **Acyl-OH** is notation for a fatty acid, which is converted to a thiolester with CoA. Also from the name, the process will involve ATP. You come in with the fatty acid and make a nucleophilic attack on one of the phosphorus atoms of ATP. The attack occurs not on the  $\gamma$ -phosphorus, but on the  $\alpha$ -phosphorus of ATP, generating a mixed acid anhydride, acyl-AMP, and  $\text{P}_i$ . Finally, the mixed acid anhydride is attacked by free coenzyme to get acyl-CoA.

**Energetics.** The initial reaction is exergonic and favorable. Also, inorganic pyrophosphate will be spontaneously hydrolyzed to two molecules of inorganic phosphate—a very downhill reaction.



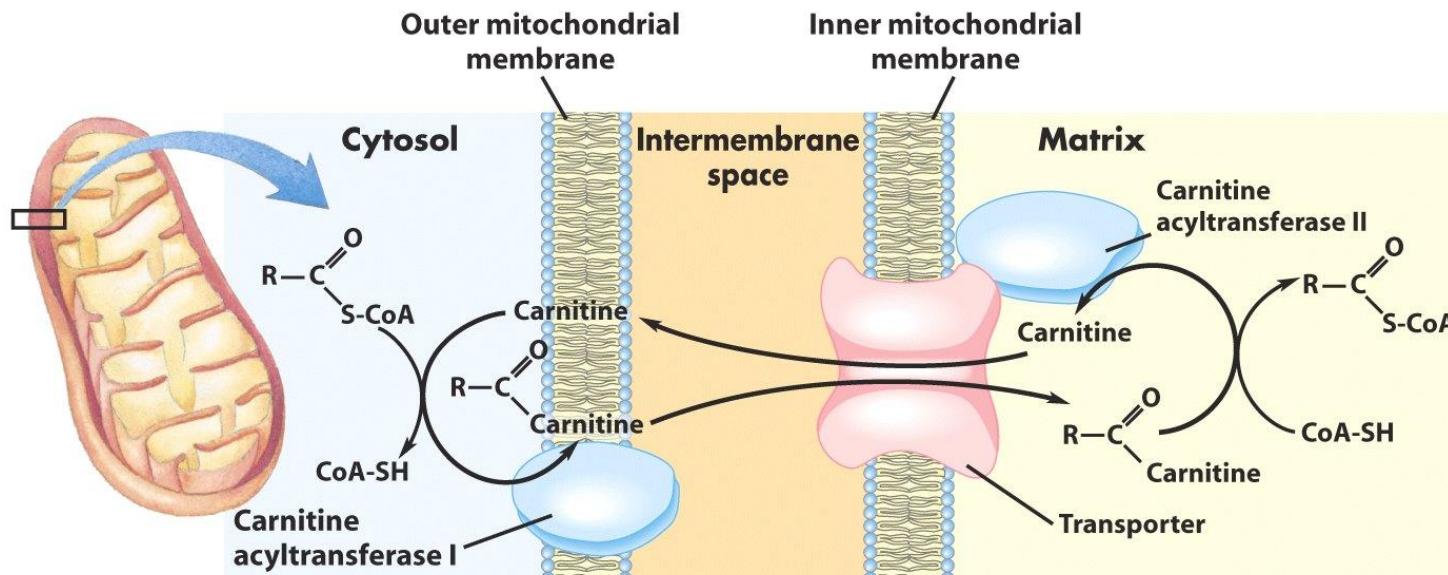
## Acyl-CoAs are transported into the mitochondria for oxidation

Acyl-CoA is made in the cytosol of the cell, but the actual degradation of fatty acids occurs in the interior of the mitochondria (one of the major discoveries of **Albert Lehninger**).



*How does it get there then?*

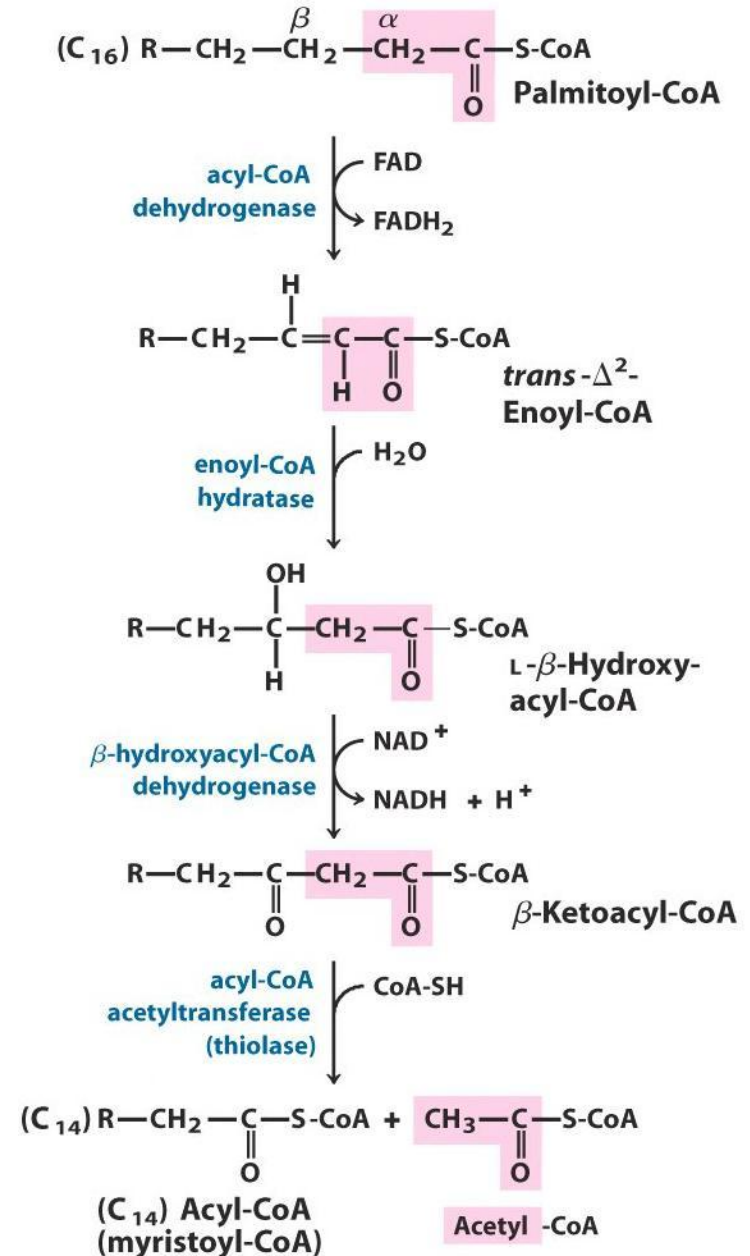
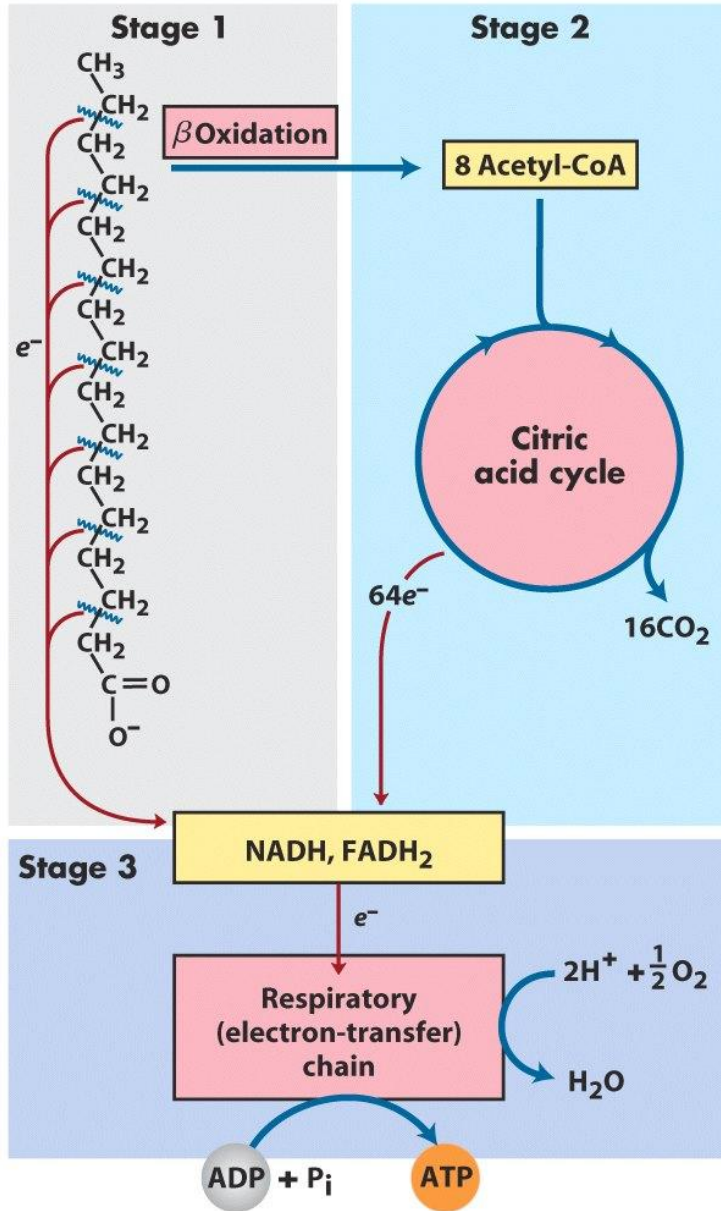
- The mitochondrial membrane does not contain a specific transporter for acyl-CoA.
- Instead, we use a carrier compound called **carnitine**. You make acyl-carnitine by transferring the acyl group from acyl-CoA.
- The mitochondrial membrane happens to have a transporter for acyl-carnitine.
- Acyl-carnitine → acyl-CoA via a complicated step.



*Why do this complicated mechanism?*

## Fatty Acid Catabolism Occurs via $\beta$ Oxidation

Analogous to the succinate  $\rightarrow$  oxaloacetate steps in Citric Acid Cycle.



## Acyl-CoA dehydrogenase.

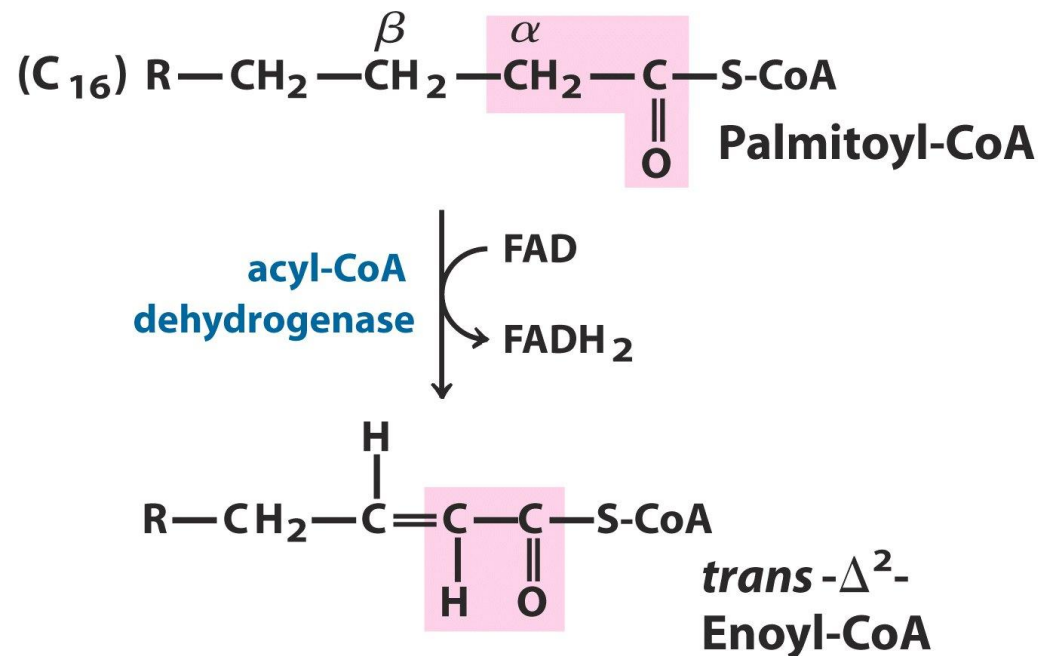


**Mechanism.** Hydrogen abstracted from the  $\beta$  carbon. If you take away hydrogen from this carbon, you end up producing trans- $\Delta^2$ -enoyl-CoA. You will notice that this reaction is pretty similar to what we have just seen in the citric acid cycle. FAD is the electron acceptor.

In the citric acid cycle, there is a very similar reaction. You start with succinate and end up with fumarate that has a trans double bond.

When we talked about succinate dehydrogenase, we said that the succinate/fumarate pair is not strongly reducing, so you cannot reduce  $\text{NAD}^+$  by using this reaction.

We use FAD, which is connected all the way to oxygen. This is precisely what happens here with acyl-CoA dehydrogenase. This enzyme is coupled to the reduction of FAD to  $\text{FADH}_2$ . A succession of electron carriers that are ultimately linked to  $\text{O}_2$ , which oxidizes  $\text{FADH}_2$ .



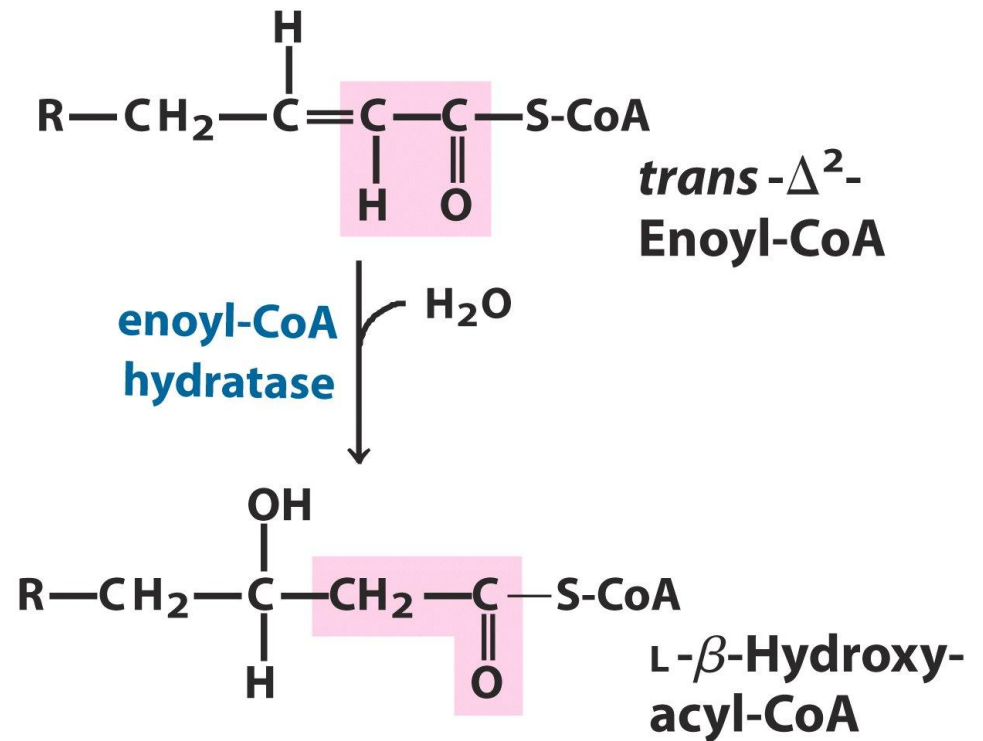


## Enoyl-CoA hydratase.

Remaining parallel to the citric acid cycle, we do a hydratase reaction and add H<sub>2</sub>O across the double bond.



In the citric acid cycle, what happened to *fumarate*? There was an addition of water across the double bond. Here hydroxy-fatty acid is produced. This is L-β-hydroxy-acyl-CoA. This enzyme is called **enoyl-CoA hydratase** for catalyzing the addition of water across the double bond.

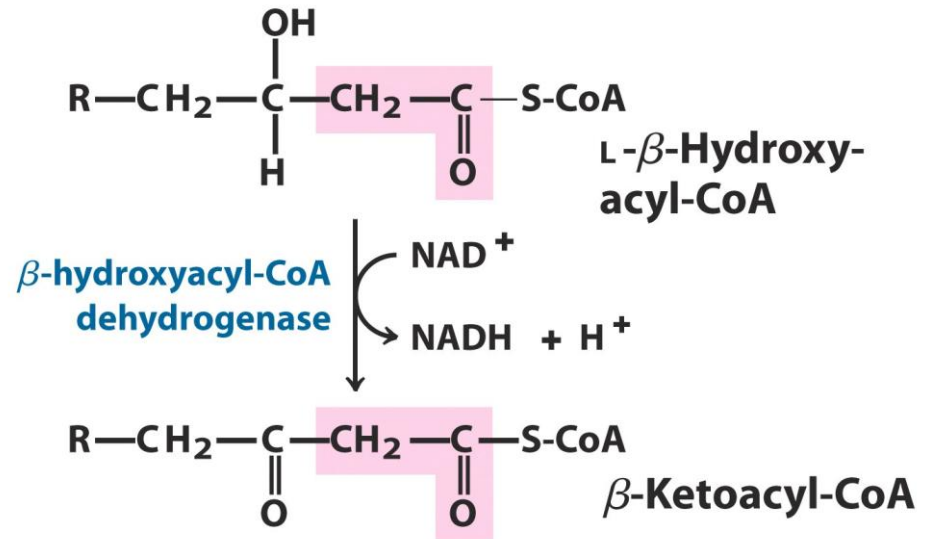


**β-hydroxy acyl-CoA dehydrogenase.**



*What happened to malate in the citric acid cycle?*

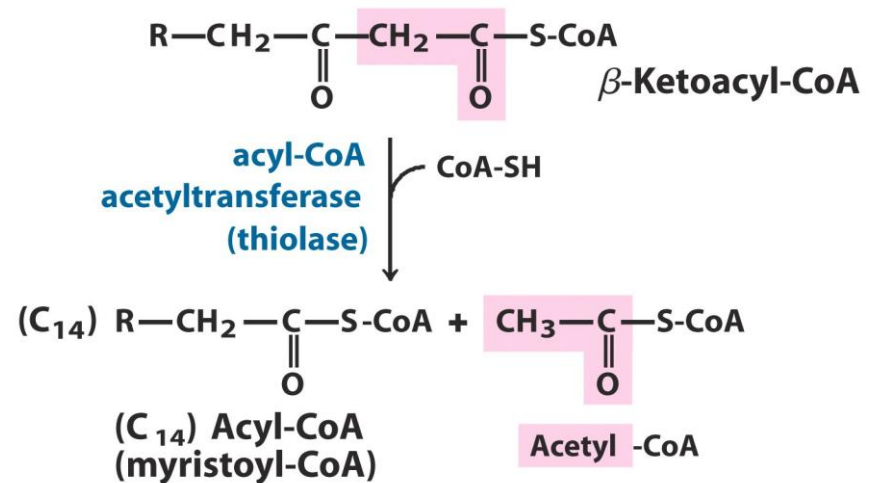
There was malate dehydrogenase and the same thing happens here. Oxidize malate to oxaloacetate by  $\text{NAD}^+$  to generate  $\text{NADH}$ . The final product is like oxaloacetate and has a keto group. You will generate a  $\beta$ -keto-acyl-CoA. This is the final product that we get through the cycle of the  $\beta$ -oxidation pathway.



## Thiolase



**Mechanism.** The ketone functional group at the  $\beta$ -carbon makes the acyl chain susceptible to attack by an incoming HS-CoA.



## Bookkeeping.

**TABLE 17-1** Yield of ATP during Oxidation of One Molecule of Palmitoyl-CoA to CO<sub>2</sub> and H<sub>2</sub>O

Enzyme catalyzing the oxidation step	Number of NADH or FADH <sub>2</sub> formed	Number of ATP ultimately formed*
Acyl-CoA dehydrogenase	7 FADH <sub>2</sub>	10.5
$\beta$ -Hydroxyacyl-CoA dehydrogenase	7 NADH	17.5
Isocitrate dehydrogenase	8 NADH	20
$\alpha$ -Ketoglutarate dehydrogenase	8 NADH	20
Succinyl-CoA synthetase		8 <sup>†</sup>
Succinate dehydrogenase	8 FADH <sub>2</sub>	12
Malate dehydrogenase	8 NADH	20
Total		108

## Saturated Fatty Acids are Broken Down in Pairs

Basically, the number of Acetyl-CoAs made is the chain length divided by two. This is different for branched and unsaturated fatty acids, where other steps are required. For unsaturated sites, the unsaturated bond is isomerized to fall in step with normal  $\beta$ -oxidation. However, branched fatty acids require  $\alpha$ -oxidation in the peroxisome.

