

Unsaturated and Odd-Chain Fatty Acid Catabolism

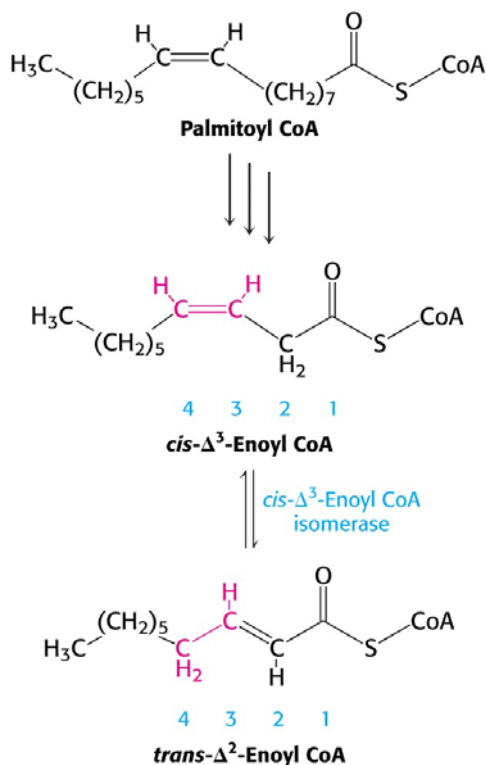
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Bryant Miles

The complete oxidation of saturated fatty acids containing an even number of carbon atoms is accomplished by the β -oxidation pathway. Not all fatty acids are saturated or contain an even number of carbon atoms. The oxidation of these fatty acids requires additional steps and enzymes.

I. Unsaturated Fatty Acids

Unsaturated fatty acids are found in our high fat diets. The complete oxidation of unsaturated fatty acids presents some difficulties. Happily most of the reactions involved in the oxidation of these unsaturated fatty acids are the same as β -oxidation. In addition, we need two additional enzymes, an isomerase and a reductase.

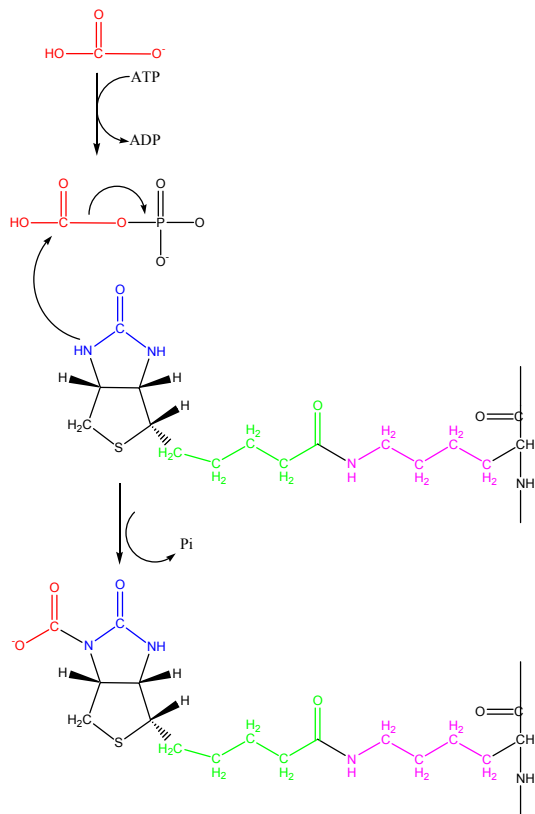


Do you remember from BICH-410 palmitoleic acid? This is a 16 carbon unsaturated fatty acid with a double bond between the C9 and the C10 carbons. This fatty acid is activated by acyl-CoA synthetase, transported across the mitochondrial inner membrane by carnitine and transferred back to CoA to form palmitoyl CoA. Palmitoyl CoA undergoes 3 cycles of β -oxidation to produce a cis- Δ^3 -enoyl CoA.

This cis- Δ^3 -enoyl CoA is not a substrate for acyl CoA dehydrogenase. The presence of the cis double bond between the C3 and C4 carbons prevents the formation of a double bond between the C2 and C3 carbons.

Cis- Δ^3 -enoyl CoA isomerase converts this double bond into a trans- Δ^2 -double bond. This trans- Δ^2 -enoyl CoA is a substrate for acyl CoA dehydrogenase and proceeds through the β -oxidation pathway.

For our next example let us consider the oxidation of a polyunsaturated fatty acid such as linoleate, a C18 polyunsaturated fatty acid with cis- Δ^9 -double bond and a cis- Δ^{12} double bond. After three rounds of normal β -oxidation, a cis- Δ^3 double bond is formed which can be converted by Cis- Δ^3 -enoyl CoA isomerase to convert the double bond to the trans- Δ^2 -double bond. This compound can now undergo another round of β -oxidation to form a cis- Δ^4 -double bond. Dehydrogenation of this compound forms a 2, 4-dienoyl intermediate. This 2, 4-dienoyl intermediate is not a substrate for enoyl-CoA hydratase. 2, 4-dienoyl intermediates is a substrate for **2, 4-dienoyl CoA reductase**. This enzyme uses NADPH to reduce the 2, 4-dienoyl intermediate into trans- Δ^3 -enoyl CoA. Cis- Δ^3 -enoyl CoA isomerase then converts the trans- Δ^3 -enoyl CoA to the trans- Δ^2 -enoyl CoA which is a metabolite for β -oxidation.

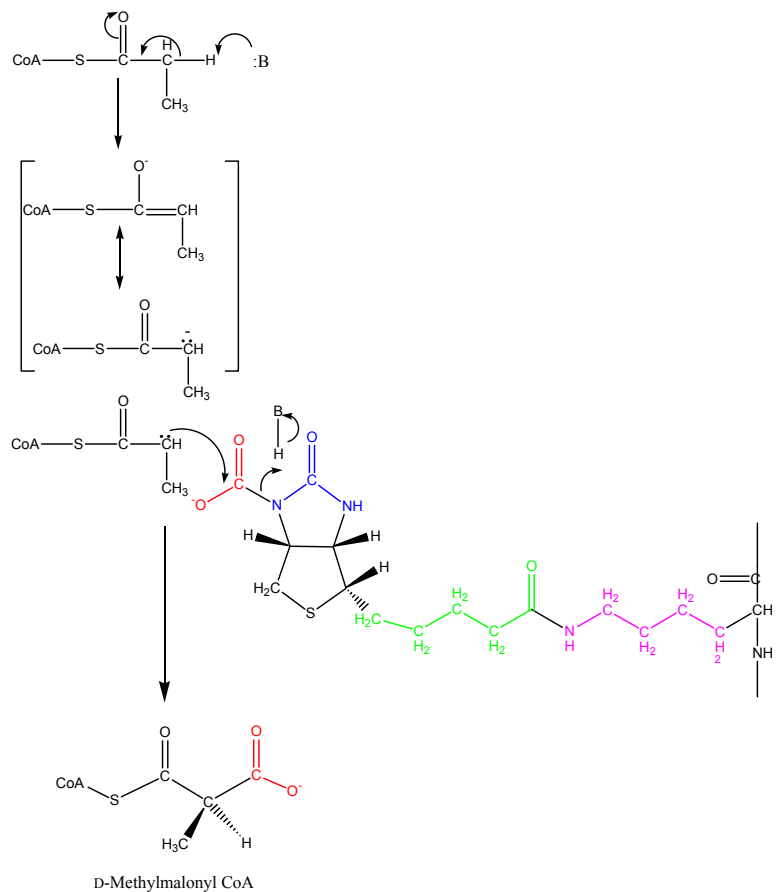


In the first step, propionyl CoA is carboxylated by a biotin containing enzyme, **propionyl CoA carboxylase**. Recall biotin from pyruvate carboxylase of gluconeogenesis.

The first step of biotin containing enzymes is the activation of bicarbonate by ATP to form the high energy intermediate carboxyphosphate.

Carboxyphosphate then reacts with the biotin cofactor which is covalently bound by an amide bond with a lysine residue.

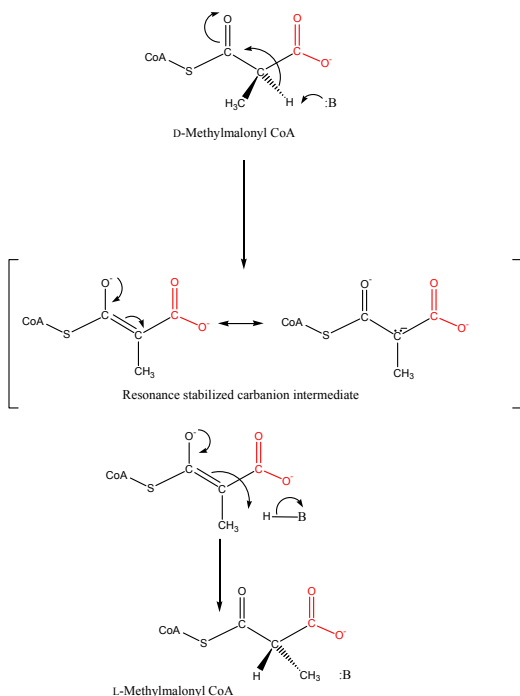
The result is carboxylated biotin, ready to be transferred to an enolate nucleophile.



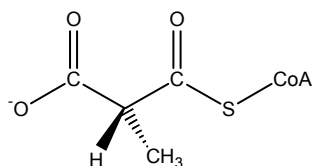
The enolate of propionyl CoA is generated in the active site of this enzyme.

The enolate is an excellent nucleophile that attacks our activated CO₂ group to regenerate biotin and form D-methylmalonyl CoA.

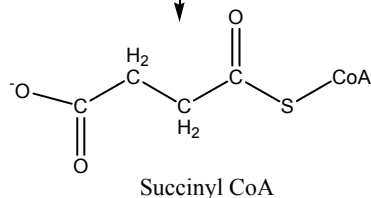
Methylmalonyl CoA Epimerase



The next step is the epimerization of D-methylmalonyl CoA into L-methylmalonyl CoA. The enzyme that catalyzes this reaction is methylmalonyl CoA epimerase. The mechanism of this enzyme is shown to the left. An active site general base abstracts the proton to generate the resonance stabilized enolate intermediate which is re-protonated on the opposite face to epimerize the C2 carbon. This reaction is fully reversible meaning this enzyme will take L-methylmalonyl CoA and epimerize it into D-methylmalonyl CoA.



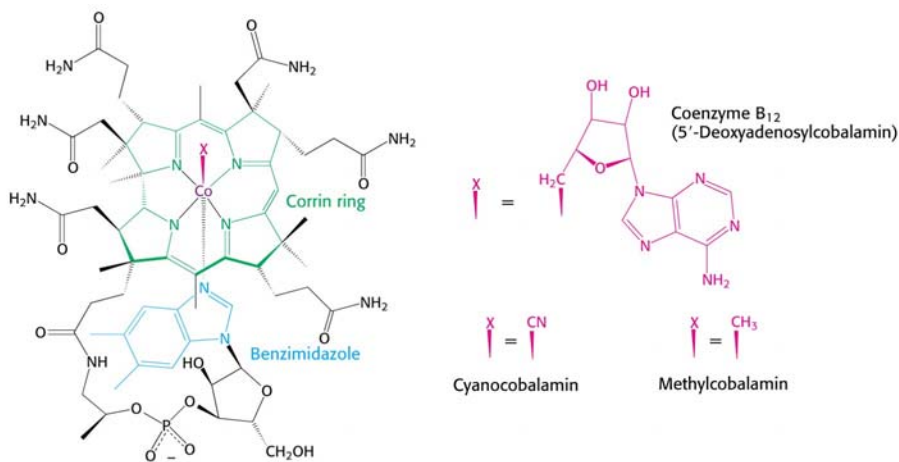
L-Methylmalonyl CoA



Succinyl CoA

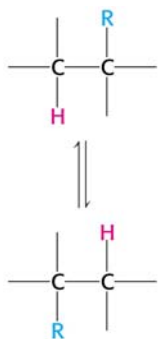
The third reaction is a vitamin B₁₂ catalyzed rearrangement which converts methylmalonyl CoA into succinyl CoA. The enzyme is called methylmalonyl CoA mutase.

This is a difficult reaction because it involves the migration of the carbonyl-CoA group from one carbon to its neighbor. The migration requires a cobalamin or vitamin B₁₂ cofactor.



Cobalamin containing enzymes are found in almost every organism. Cobalamin containing enzymes catalyze alkyl rearrangements, methylations and the reduction of ribonucleotides to deoxyribonucleotides. The core of cobalamin is the corrin ring which is coordinated to a cobalt atom. The corrin ring is similar to porphyrin rings with four pyrrole rings. The corrin ring is more reduced than porphyrin rings and has different constituents. The cobalt atom is coordinated to the 4 planar nitrogens of the pyrroles. The cobalt atom is axially coordinated to one of the nitrogens of a methylbenzimidazole as shown above in blue. In vitamin B₁₂, the axial 6th ligand coordinated to the cobalt atom is 5'-deoxy adenosine. This 6th position can also be occupied by a cyano group, a methyl group or other ligands. In all of these compounds the cobalt is the +3 oxidation state.

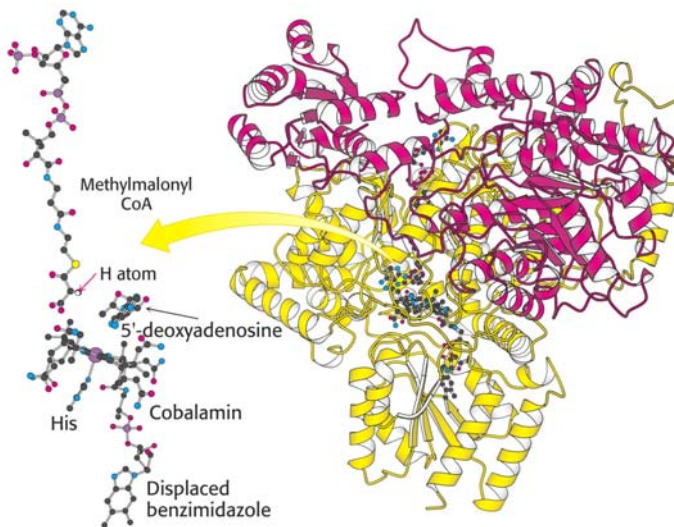
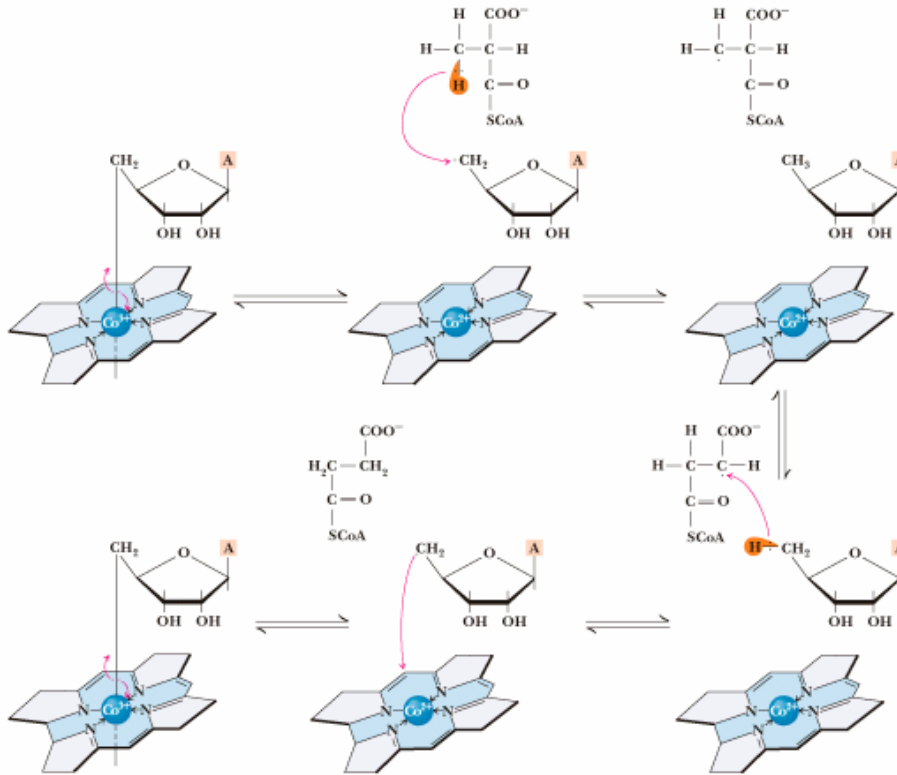
Vitamin B₁₂ is not synthesized by animals or plants. Only a few species of bacteria synthesize this complex coenzyme. Carnivorous animals easily obtain sufficient amounts of vitamin B₁₂ from meat. Herbivorous animals depend on their intestinal bacteria to synthesize vitamin B₁₂ for them. Vitamin B₁₂ is one the most potent vitamins. Of all the vitamins, Vitamin B₁₂ is needed in the smallest amount. A Vitamin B₁₂ deficiency results in pernicious anemia.



The rearrangement reactions catalyzed by coenzyme B₁₂ exchange two groups attached to adjacent carbon atoms as shown to the left. The R group can be an amino group, hydroxyl group or a substituted carbon. In the case of methylmalonyl mutase a hydrogen atom and the carbonyl attached of CoA concomitantly move in opposite directions.

Methylmalonyl CoA mutase is specifically binds L-methylmalonyl CoA. The methylmalonyl CoA mutase catalyzed reaction begins with the homolytic cleavage of the Co³⁺--C—bond in cobalamin as shown below. The homolytic bond cleavage produces Co²⁺ and a

radical on the 5'-carbon of 5'-deoxy adenosine. The $\text{CH}_2\cdot$ radical is highly reactive. This radical abstracts an hydrogen from the methyl group of methylmalonyl CoA generating a methylmalonyl radical that undergoes rearrangement to form a succinyl CoA radical. The succinyl CoA radical abstracts the hydrogen back from the 5'-carbon of the deoxy adenosine to yield succinyl CoA. The radical deoxyadenosine recombines with the Co^{2+} cobalt to regenerate the cofactor.



The role of the cobalamin cofactor is to serve a source of free radicals for the abstraction of hydrogen atoms to promote intramolecular migrations. The cobalt carbon is weak and readily undergoes homolytic cleavage to generate a radical.

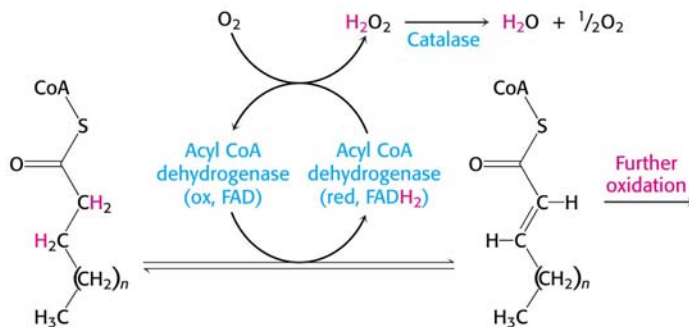
The active site of methylmalonyl mutase is shown to the left.

The succinyl CoA derived from propionyl CoA enters into the citric acid cycle where it is converted to oxaloacetate. Oxaloacetate can be converted into glucose by the gluconeogenic pathway. Thus odd chain fatty acids **can** be used for glucose synthesis. Alternatively succinyl

CoA can be converted into malate, transported into the cytosol, where it is oxidatively decarboxylated into pyruvate, which is then transported back into the matrix of the mitochondria where it is converted into acetyl CoA and oxidized by the TCA into CO_2 .

III. Peroxisome β -Oxidation

The principle pathway for the β -oxidation of fatty acids occurs in the matrix of the mitochondria. There are several other minor pathways that play important physiological roles in fatty acid catabolism. Other organelles can carry out β -oxidation such as the peroxisomes and the glyoxysomes. Peroxisomes are organelles that carry out flavin dependent oxidation reactions that produce highly reactive oxygen species such as hydrogen peroxide. Peroxisomes are abundant in liver and kidney tissues. Each liver cell contains about 1,000 peroxisomes. The peroxisomes contain high concentrations of catalase which convert hydrogen peroxide into two water molecules and molecular oxygen.



Peroxisomal β -oxidation of fatty acids is similar to the mitochondrial β -oxidation except that the initial double bond formation is catalyzed by an FAD-dependent acyl-CoA oxidase. This enzyme transfers the electrons from FADH₂ to O₂ to generate hydrogen peroxide.

In the mitochondria, Acyl CoA dehydrogenase transfers the electrons from FADH₂ to an electron transport chain that ultimately produces 1.5 ATP molecules per FADH₂. As a result peroxisomal β -oxidation produces fewer ATP molecules.

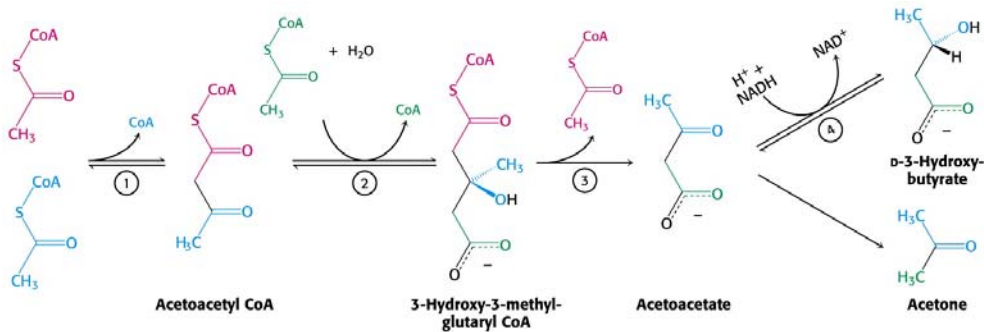
The peroxisomal β -oxidation halts at octanoyl CoA formation. Fatty acyl CoA molecules with carbon chains shorter than 8 carbons are not substrates for the peroxisomal enzymes of β -oxidation. The octanoyl CoA molecules produced in the peroxisome are transported to the mitochondria for further degradation.

IV. Ketone Bodies

Most of the acetyl CoA produced by β -oxidation undergoes complete oxidation through the citric acid cycle. The entry of acetyl CoA into the citric acid cycle depends on the availability of oxaloacetate. Under fasting conditions or diabetes, gluconeogenesis depletes the concentration of oxaloacetate. Oxaloacetate becomes unavailable to condense with acetyl CoA. Under these conditions the acetyl CoA produced by β -oxidation is diverted into three important metabolites: **acetone**, **acetoacetate** and **β -hydroxybutyrate**. These molecules are called ketone bodies or ketobodies. The process that produces these molecules is called **ketogenesis**.

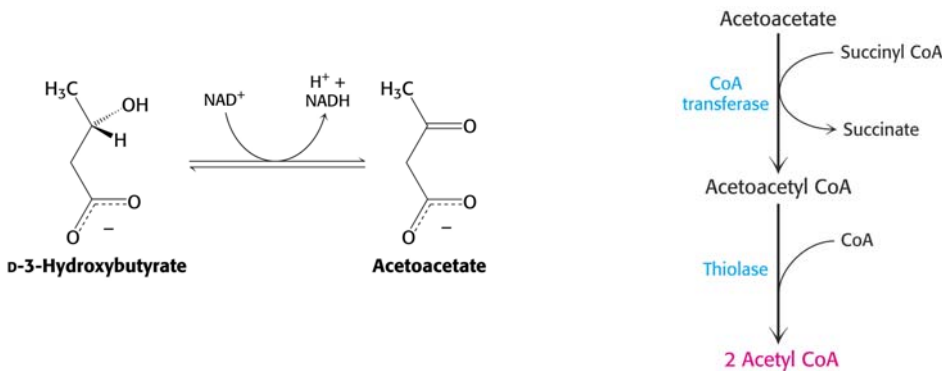
Ketogenesis occurs primarily in the liver. The ketone bodies produced are used by many tissues as fuel, including muscles, the heart and under starvation conditions the brain. Ketone bodies are the preferred energy source for the kidney cortex and heart muscle.

Ketogenesis occurs in the matrix of the mitochondria. The first step is the condensation of two acetyl CoA molecules in a reaction catalyzed by thiolase^① to form acetoacetyl CoA. Acetoacetyl CoA then reacts with a third acetyl CoA molecule to form β -hydroxy- β -methyl glutaryl CoA. This reaction is catalyzed by hydroxymethyl glutaryl CoA synthase (HMG-CoA synthase) ^②. The hydroxymethyl glutaryl CoA is then cleaved into acetyl CoA and acetoacetate by the HMG-CoA lyase ^③.



The NADH dependent reduction of acetoacetate by D-β-hydroxybutyrate dehydrogenase produces D-β-hydroxybutyrate. The ratio of acetoacetate to hydroxybutyrate depends on the NADH/NAD⁺ ratio in the matrix of the mitochondria. Acetoacetate is a β-ketoacid which spontaneously undergoes a slow decarboxylation to form acetone which is why people with a high concentration of acetoacetate in their blood have the odor of acetone on their breath.

The ketone bodies produced in the mitochondria diffuse from the matrix into the blood where they are carried to the tissues. Ketone bodies are an important source of metabolic energy. In the tissues that absorb the keto bodies, D-β-hydroxybutyrate is reoxidized into acetoacetate by β-hydroxybutyrate dehydrogenase. Acetoacetate is activated by the transfer of CoA from succinyl CoA by CoA transferase to form acetoacetyl CoA. Acetoacetyl CoA is then cleaved by thiolase into 2 molecules of acetyl CoA which can then enter the citric acid cycle.



The liver does not contain the CoA transferase.
Ketone bodies are water soluble acetyl group carriers.

Diabetes mellitus is the most common endocrine disease and the third leading cause of death in the United States. Diabetes is characterized by abnormally high concentrations of glucose in the blood. Type I diabetes, the elevated glucose concentrations are caused by inadequate production of insulin. Type II diabetes, the elevated glucose concentrations caused by insensitivity to insulin because of the lack of insulin receptors. Type II diabetics have normal or elevated blood concentrations of insulin. Insulin is the hormone signals the body that the blood concentrations of glucose are high. Insulin turns off gluconeogenesis as well as fat and protein catabolism. Insulin also activates the transport of glucose into muscle, liver and adipose tissue. In diabetes the transport of glucose is not activated. The tissues are glucose starved despite high concentrations of glucose in the blood. As a result gluconeogenesis is turned on as is the catabolism of protein and fat. Gluconeogenesis consumes all of the available oxaloacetate. This results in the formation of ketone bodies. A symptom of diabetes is acetone in the breath which results from the high concentrations of ketone bodies in the blood.