Medical Committee
The University of Jordan

SLIDE SHEET

LECTURE#: 15

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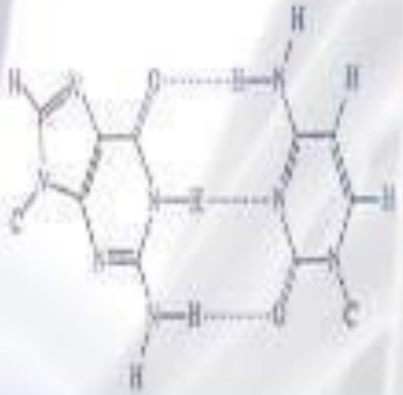
Biochemistry



Majida Al-Foqaraa'

Fatty Acid Oxidation

- β – Oxidation of fatty acids is the major catabolic mitochondrial pathway of fatty acids, in which two-carbon fragments are successively removed from the carboxyl end of the fatty acyl CoA, producing acetyl CoA, NADH and $FADH_2$.

1. Transport of long-chain fatty acids into the mitochondria:

As mentioned previously, long chain fatty acids are transported to the mitochondrial matrix through "carnitine shuttle" after their cytosolic activation by thiokinase (acyl CoA synthetase). The inner mitochondrial membrane is impermeable to CoA; therefore a specialized carrier is needed – carnitine – that transports the acyl group to the mitochondrial matrix by the rate limiting transport process "carnitine shuttle".

- **Carnitine shuttle is inhibited by fatty acid synthesis (discussed later).**
- **Fatty acids shorter than 12 carbons can cross the inner mitochondrial membrane without the aid of carnitine or the CPT system. Once inside the mitochondria they are activated by matrix enzymes and are oxidized.**

2. Reactions of β- oxidation:

It consists of a sequence of four reactions (one cycle) involving the β- carbon that results in shortening the fatty acid chain by two carbons at the carboxylate end. The first 3 reactions convert the fatty acyl CoA into a ketoacyl CoA and the last reaction results in cleavage.

- These four steps include:

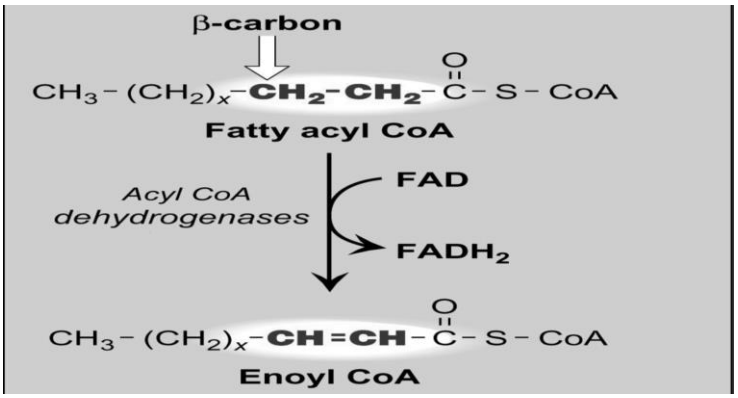
1. Oxidation that produces $FADH_2$.
2. Hydration step.
3. Second oxidation that produces NADH.
4. Thiolytic cleavage that releases a molecule of acetyl CoA.

-Step 1: Oxidation that produces $FADH_2$: (Acyl CoA dehydrogenase)

Two hydrogen atoms are removed from β-carbon (carbon 3) and α-carbon (carbon 2) resulting in the formation of a **trans** double bond. The two hydrogen atoms are then transferred to the protein bound cofactor FAD thereby reducing it to $FADH_2$ by the enzyme Acyl CoA dehydrogenase.

- Normally occurring double bonds in fatty acid chains usually have *cis-configuration* whereas the bond formed in β-oxidation has trans-

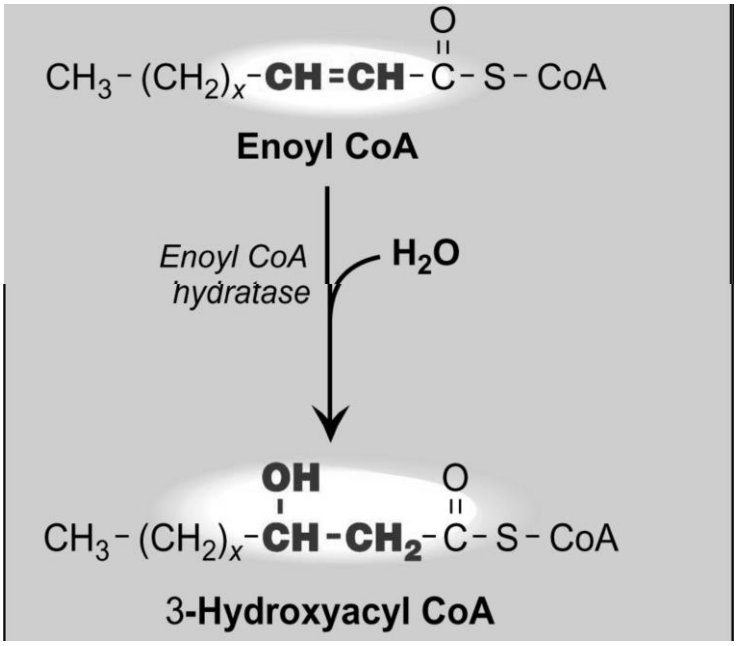
configuration due to the specificity of the upcoming enzyme – Enoyl CoA hydratase which can only hydrate trans double bonds. Bearing in mind that enzymes are highly specific; they produce and react with either cis or trans configurations and not both of them.



-Step 2: Hydration: (Enoyl CoA hydratase)

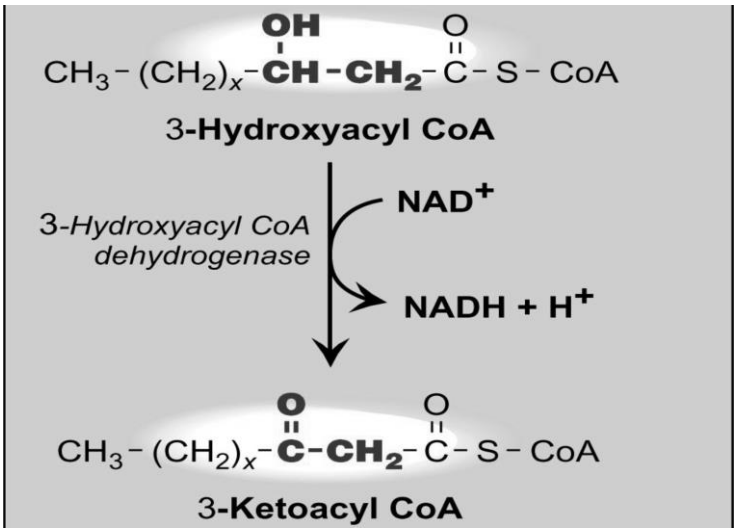
Enoyl CoA is hydrated by the addition of water molecule to the trans double bond and **3-Hydroxyacyl CoA** is produced. Note that, the addition of water across the alkenes double bonds yields alcohols and here it's achieved by Enoyl CoA hydratase. Enoyl CoA hydratase adds a hydroxyl group to β-carbon (carbon- 3) only and this further emphasizes its high specificity.

Enoyl indicates the presence of double bond or alkene.



-Step 3: Second oxidation that produces NADH: (3-Hydroxyacyl CoA DH)

Characterized by the oxidation of secondary alcohol (3-Hydroxyacyl CoA) to ketone (3-Ketoacyl CoA) and this involves the transfer of electrons to NAD^+ and the formation of NADH.



❖ Remarks :

- Hydroxyacyl CoA, Ketoacyl CoA are derivatives of Acyl CoA which are a group of temporary compounds formed when coenzyme A attaches to the long chain fatty acid inside living cells.
- Oxidases catalyze oxidation-reduction reactions involving **molecular oxygen (O_2)** as an electron acceptor and it is reduced to either water or hydrogen peroxide. On the other hand, dehydrogenases catalyze oxidation-reduction reactions involving usually **NAD^+/$NADP^+$/FMN/FAD** as an electron acceptor (Not O_2). Both of them belong to oxidoreductases.
- Acyl CoA dehydrogenase has FAD as a coenzyme instead of NAD^+ and that's because hydrogen atoms are removed from two adjacent carbons (two sources) and not in the form of hydride ion (single source).
- **Hydrolysis** is the cleavage of chemical bonds by the addition of water → **Hydrolase**.
- **Hydration** is the addition of water to unsaturated substrate → **Hydratase**.
- FAD is considered as a prosthetic group (tightly bound coenzyme) due to its consistent binding to the enzyme and that the reduced $FADH_2$ can't dissociate from the enzyme complex. But NAD is considered as a second substrate because NADH eventually dissociates from the enzyme. Both coenzymes are oxidized by the electron transport chain.

❖ Studying Tips:

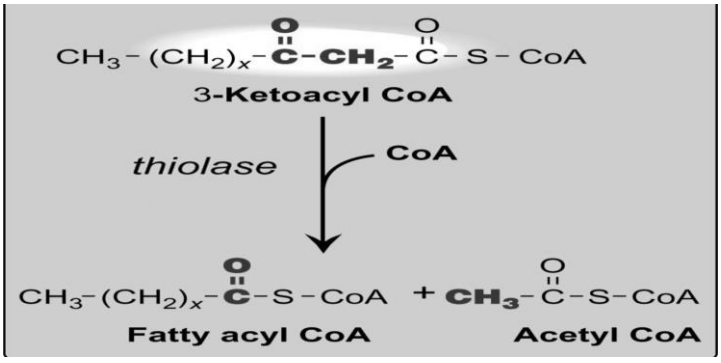
I. Enzymes are named according to their substrate and the type of reaction they catalyze. For example, phosphorlysis →phosphorylase. So it's very beneficial to note down the steps in order to know what happened in each step and how the structures change.

II. There is a significant degree of similarity between these three reactions of β-oxidation and the last three reactions in the TCA cycle. If we kept the α and β carbon and substituted both sides by a carboxyl group (COO^-) , we will form firstly succinate → $^-OOC-CH_2-CH_2-COO^-$ and the same sequence of reactions will follow :

   a. Oxidation to Fumarate → $^-OOC-CH_2=CH_2-COO^-$ by succinate DH and FAD.
   b. Hydration to Malate → $^-OOC-CH(OH)-CH_2-COO^-$ by fumarase.
   c. Oxidation to Oxaloacetate → $^-OOC-CH(O)-CH_2-COO^-$ by Malate DH and NAD^+.

-Step 4: Thiolytic cleavage and release of acetyl CoA: (Thiolase)

Thiolysis is a reaction with a thiol (R-SH) that cleaves one compound into two. It is similar to hydrolysis which involves water instead of a thiol. But here the catalyzing enzyme-Thiolase- prevents hydrolysis by preventing the attack of H_2O molecules to the C-2/C-3 bond and thus preventing reactivation of fatty acids. In other words, if hydrolysis occurs instead of thiolysis, the hydrogen will bind to the α carbon and acetyl CoA will be formed, and –OH will bind to the β carbon and an inactivated fatty acid is regenerated rather than fatty acyl CoA which is already activated. Note that, activation occurs only once in the cytosol and consumes 2ATP and by preventing hydrolysis there is no ATP consumption in each β-oxidation cycle, consequently preserving energy and that's the main advantage of thiolysis.



❖ Remark:

Acetyl CoA is a positive allosteric effector of pyruvate carboxylase thus linking fatty acid oxidation and gluconeogenesis.

3. Energy yield from fatty acid oxidation:

-The energy yield from the β-oxidation pathway is high. For example, the complete oxidation of a molecule of palmitoyl CoA (16-carbon) produces 8 acetyl CoA (Each cycle the acyl CoA is two carbons shorter) and to achieve this, 7 cycles are needed and thus, 7 NADH and 7 $FADH_2$ are produced.

-The amount of ATP that is produced by palmitoyl CoA **β-oxidation only** is 35 ATP in which:

a. 7 $FADH_2$, each of which provides 2ATP when oxidized by CoQ of the ETC, yield = 14 ATP.
b. 7 NADH, each of which provides 3 ATP when oxidized by Complex 1of the ETC, yield = 21 ATP.

-**Complete oxidation** of palmitoyl CoA by the TCA cycle generates 131 ATP in which each acetyl CoA provides 12 ATP when converted to CO_2 and H_2O. (8 acetyl CoA→ 96 ATP)

-So the total energy yield is 14+21+96= 131 ATP. However, activation of the fatty acid requires 2 ATP. Therefore, the net yield from plamitate is 129 ATP.

- This high energy yield is nearly equivalent to that of three glucose molecules and that emphasizes the fact of fatty acids being more reduced and thus yielding more energy by being more oxidized.

❖ Carnitine:

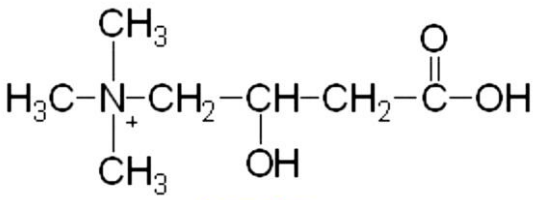
-Carnitine is needed in the long-chain acyl group transport from the cytosol into the mitochondrial matrix.

-It is a quaternary ammonium compound with a carboxylic group. (Structure not required).

- **Sources of carnitine:**

a. Carnitine can be obtained from the diet, where it is found primarily in meat products (muscles).
b. Carnitine can also be synthesized from essential amino acids (lysine and methionine) in the liver and the kidney (where fatty acid oxidation mainly takes place).

-Skeletal or heart muscles are totally dependent on uptake of carnitine provided by endogenous synthesis or the diet and distributed by the blood. Note that skeletal muscles contain about 97% of all carnitine in the body.



Carnitine

-Other functions of carnitine:

a. Export of branched chain acyl group from the mitochondria.
b. Renal excretion of acyl groups that can't be metabolized in the body.

-Carnitine deficiencies:

-Such deficiencies result in decreased ability of tissues to use long chain fatty acids as fuel which in turn leads to fatty acids and branched acyl groups' accumulation in cells.

1. Primary carnitine deficiency (inborn error): It is caused by:
   a. Defects in enzymes involved in carnitine biosynthesis pathway.
   b. Defects in membrane transporter that prevent uptake of carnitine by cardiac and skeletal muscles.
   c. Decreased tubular reabsorption which leads to increased carnitine secretion.
2. Secondary acquired carnitine deficiency: It can be seen in:
   a. Patients with liver disease (decreased carnitine synthesis).
   b. Malnutrition (lack of essential amino acids-Lysine and methionine- to synthesize carnitine)
   c. Increased carnitine requirements.

-As a result, increased utilization of glucose will take place culminating in hypoglycemia and in severe cases coma and death can occur.

-Treatment includes carnitine supplementation (sometimes used to build up muscles) and adopting a diet high in carbohydrates and low in fat beside avoidance of fasting.

❖ Oxidation of unsaturated fatty acids:

1. Monounsaturated fatty acids: Oleic acid → 18:1(9)

Oxidation of monounsaturated fatty acids requires one additional enzyme, **enoyl CoA isomerase**, which converts the 3-cis derivative obtained after three cycles of β-oxidation to the 2-trans derivative required as a substrate by enoyl CoA hydratase.

Firstly oleic acid has a double bond on carbon-9, after the third cycle of β-oxidation the double bond becomes on carbon-3 (β carbon) and the formed fatty acid is 12: cisΔ^3 → **CH3 – (CH2)7-CH = CH CH2-CO~CoA .** This compound can't be used as a substrate by enoyl CoA hydratase or by acyl CoA DH so it undergoes an isomerisation reaction by the enzyme isomerase to form 12:transΔ^2 → **CH3 (CH2)7 CH2-CH=CH-CO~CoA** and the successive reactions precede as usual.

- The number of the carbon atom that has the double bond from the methyl end remains the same which is omega-9 because the cleavage occurs at the carboxylic end.

2. Polyunsaturated fatty acids: (linolenic acid) → 18:2(9,12)

Oxidation of polyunsaturated fatty acids requires an NADPH-dependent dienoyl CoA reductase in addition to isomerase.

After three cycles of β-oxidation and the formation of three acetyl CoA, the resulting fatty acid is 12:2 (3, 6) that undergoes isomerisation so it can be acted upon by enoyl hydratase and becomes 12:2 (2,6). This fatty acid enters the β-oxidation cycle and results in 10:1 (4) →

CH3-(CH2)4-CH=CH-CH2-CH2-CO-CoA

This compound can be used as a substrate by **acyl CoA dehydrogenase** and another double bond is formed between α and β carbons: 10:2 (2, 4) →

CH3-(CH2)4-CH=CH-CH=CH-CO-CoA

Then, both double bonds are reduced to a single double bond by **dienoyl CoA reductase**: 10:1 (3) →

CH3-(CH2)4-CH_2-CH=CH-CH_2-CO-CoA

And this product must undergo another isomerisation reaction in order to displace the double bond to α carbon and complete the β-oxidation reactions.

Q: Why doesn't the product (**10:2(2,4**)) undergo a hydration reaction directly by enoyl CoA hydratase instead of undergoing a reductase reaction then hydratase ?

Because the double bond between carbons number 4 and 5 produce a kink which makes the molecule not available to be used by the enzyme hydratase (lock and key theory).

-Note: The oxidation of unsaturated fatty acids provides less energy than that of saturated fatty acids because unsaturated fatty acids are less highly reduced so they are less oxidized therefore producing less energy.

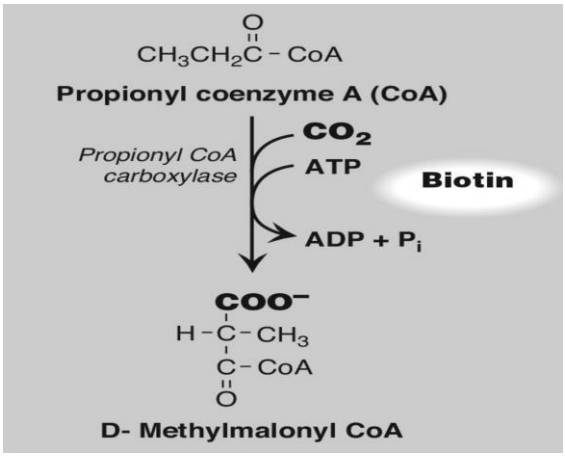
❖ Oxidation of fatty acids with an odd number of carbons:

Fatty acids with an odd number of carbons are found mainly in animal fat as 13 and 15 carbons fatty acids. Their oxidation process proceeds by the same reaction steps as that of fatty acids with an even number of carbons, until the final three carbons are reached. For example, a 15-carbon fatty acid, after six cycles of β-oxidation, instead of having 7 acetyl CoA, 6 acetyl CoA and one **propionyl CoA** will be formed. Propionyl CoA → **CH3-CH2-CO~CoA** is a derivative of propionic acid and is metabolized by a three step pathway:

1. Synthesis of D-methylmalonyl CoA.
2. Formation of L-methylmalonyl CoA.
3. Synthesis of succinyl CoA.

1-Synthesis of D-methylmalonyl CoA:

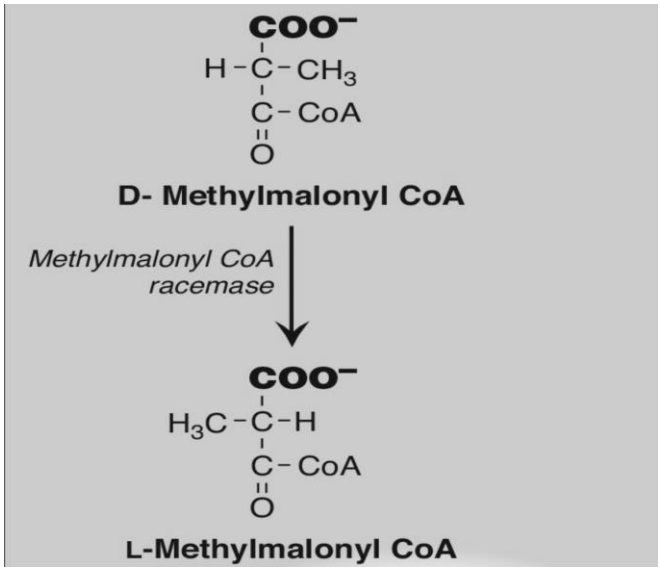
Propionyl CoA is carboxylated to D-methylmalonyl CoA which is an acyl CoA derivative of malonic acid (a three carbon dicarboxylic acid) attached to it a methyl group. The catalyzing enzyme, **propionyl CoA carboxylase** requires the coenzyme biotin as do most other carboxylases such as pyruvate carboxylase.



2-Formation of L-methylmalonyl CoA:

D-methylmalonyl CoA is converted to the L-form by the enzyme, **methylmalonyl racemase.**

-D/L indicates the arrangement of the different groups of atoms around the asymmetric carbon (chiral carbon).



3-Synthesis of succinyl CoA:

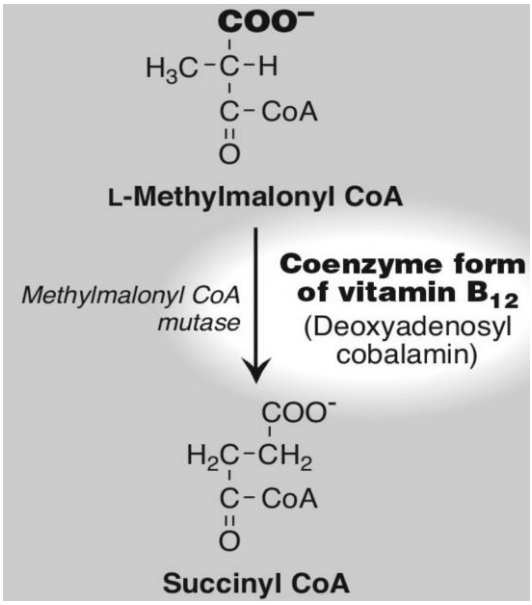
The carbons of L-methylmalonyl CoA are rearranged in which the carboxyl group is shifted to the methyl group and the chiral carbon becomes achiral. As a result succinyl CoA forms which is a derivative of succinic acid (four carbon dicarboxylic acid). Succinyl CoA can enter the TCA cycle making it the only glucogenic precursor generated from fatty acid oxidation means it can be used to form glucose during gluconeogenesis.

This reaction is catalyzed by the enzyme methylmalonyl CoA mutase that requires a coenzyme form of vitamin B12 (cobalamin).Furthermore, the

mutase reaction is one of only two reactions in the body that require vitamin B12.

-Vitamin B12 deficiency: leads to accumulation of methylmalonyl CoA (methylmalonate) and propionate and both are excreted in urine. Previously, B12 levels couldn't be measured directly as today so they used to measure methylmalonyl levels as it was also excreted in the urine forming methylmalonyl-uria.

*<u>**General rule**</u> >> any enzyme deficiency will causes the accumulation of its substrate.



❖ <u>β – Oxidation in the peroxisome:</u>

-Very long chain fatty acids of more than 22 carbons undergo a preliminary β-oxidation in peroxisomes because their synthetase enzyme is located in peroxisomes so they can't be oxidized directly in the mitochondria. Then, the shortened fatty acid (14-16 carbons) diffuses to a mitochondrion for further oxidation.

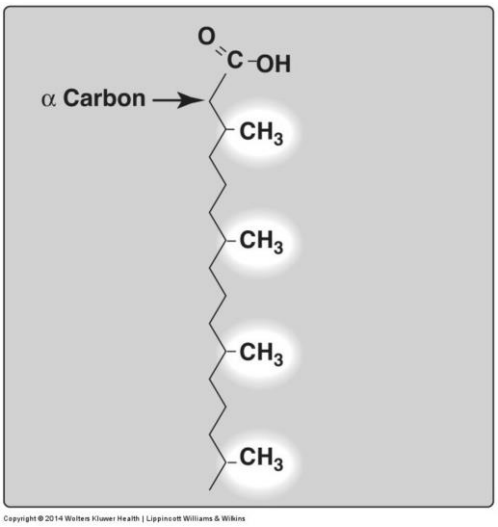
-The initial dehydrogenation in peroxisomes of very long chain fatty acids is catalyzed by FAD-containing acyl CoA oxidase (O_2 is a substrate). The $FADH_2$ produced is oxidized by molecular oxygen, which is reduced to H_2O_2 (therefore the organelle is called peroxisome).

<u>Notes:</u>

a. No ATP is generated by this step.
b. The H_2O_2 is reduced to H_2O by catalase.

❖ α – Oxidation of fatty acids:

Branched-chain fatty acids that have several methyl groups can't be a substrate for acyl CoA dehydrogenase because of the methyl group located on its β-carbon.



In order to oxidize this branched-chain fatty acid, it is hydroxylated at the α-carbon then carbon-1 is released as CO_2 in oxidative decarboxylation reaction. After that, the added hydroxyl group is further oxidized to carboxyl group and the resulting end product will have a methyl group on its α-carbon instead of its β-carbon so it can proceed as usual.

CH3 (CH **(CH₃)**- CH2-CH2-CH2)3-CH**(CH₃)**-CH2-COO-

↓

CH3(CH**(CH₃)**- CH2-CH2-CH2)3-CH**(CH₃)**- CH(**OH**)-COO-

↓

CH3(CH**(CH₃)**- CH2-CH2-CH2)3- CH**(CH₃)**-COO- + CO2

-These fatty acids are products of chlorophyll metabolism so they are found abundantly in plants.

-Enzyme deficiency in the enzyme catalyzing hydroxylation is rare but it's found as an autosomal recessive disorder that leads to the accumulation of branched-chain fatty acids resulting in neurologic symptoms such as brain damage. Early diagnosis is very important in order to detect the possibility of having diseased brothers and start early treatment that involves dietary restriction. ☺

"I didn't come to medical school just to survive it- I came to medical school because I believed caring for patients was my calling"

Best Wishes ☺