

E-content

M.Sc. Zoology (Semester II)
CC7- Biochemistry

Unit: 3.6

β -Oxidation of long chain fatty acids

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Beta oxidation of fatty acids

Fatty acids provide highly efficient energy storage, delivering more energy per gram than carbohydrates like glucose.

In tissues with high energy requirement, such as heart, up to 50–70% of energy, in the form of ATP production, comes from fatty acid (FA) beta-oxidation.

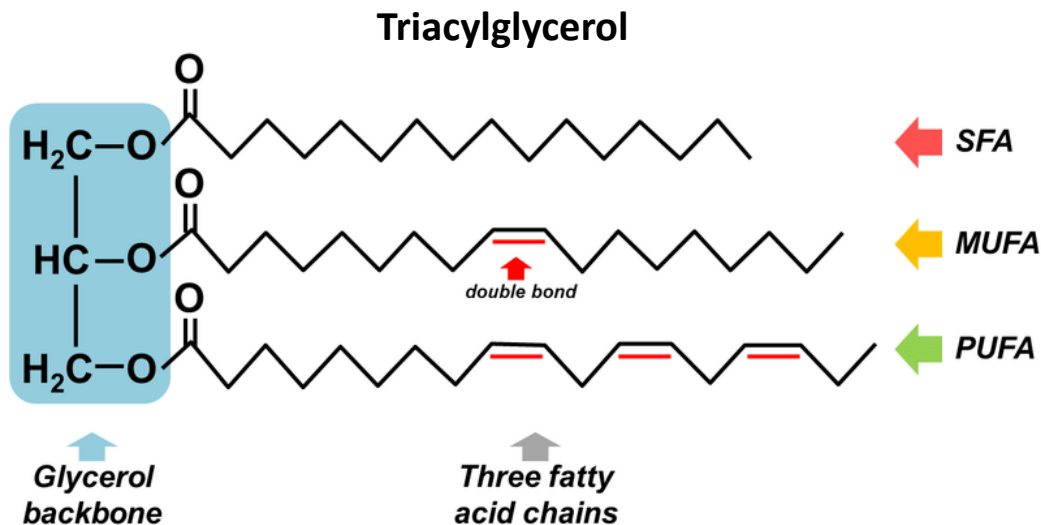
During fatty acid β -oxidation, long chain acyl-CoA molecules (the main components of FAs) – are broken to acetyl-CoA molecules.

It is referred as “beta oxidation” because the beta carbon of the fatty acid undergoes oxidation to a carbonyl group.

The Entry into Beta-oxidation

Most fats stored in eukaryotic organisms are stored as triglycerides.

In order to enter into beta-oxidation, bonds with glycerol must be hydrolysed usually with the use of a Lipase. The end result of these broken bonds are a glycerol molecule and three fatty acids in the case of triglycerides. Other lipids can be degraded as well.

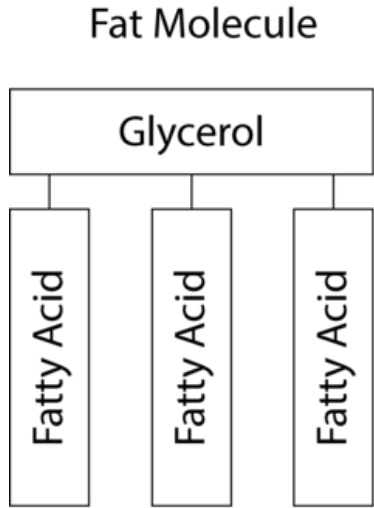


SFA: Saturated fatty acid

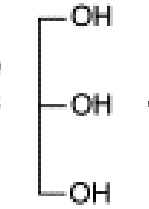
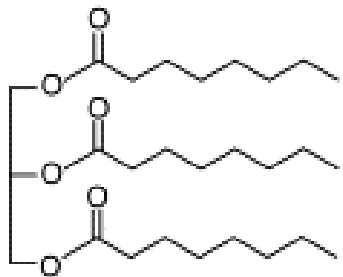
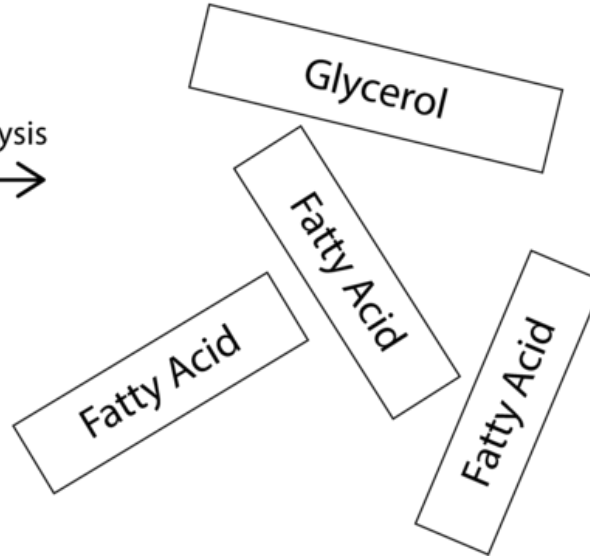
MUFA: Monounsaturated fatty acid

PUFA: Polyunsaturated fatty acid

Hydrolysis of Triglycerides



Hydrolysis
→



Triacylglycerol

Glycerol

Fatty acid

Free fatty acid are broken down by beta oxidation

Stages of Beta Oxidation of Fatty Acids

There are three Stages of beta oxidation

Stage I

Long Chain Fatty acid (Acyl Chain) must be activated: bond to coenzyme A
Occurs in Cytosol)

Stage II

Translocation of Activated Fatty acid From Cytosol into Mitochondrial Matrix: uses a shuttle mechanism known as **Carnitine Shuttle** (rate limiting step)

Stage III

Activated Fatty acid repeatedly oxidized (beta oxidation), cycling through 4 reactions: occurs In Mitochondrial Matrix

Stage I

Activation of fatty acid in cytosol is a **Preparative Phase**

Once the triglycerides are broken down into glycerol and fatty acids, the latter must be activated before they can enter into the mitochondria and proceed on with beta-oxidation.

This is done by Acyl-CoA synthetase to yield fatty acyl-CoA.

A long chain Fatty acid is termed as Acyl chain.

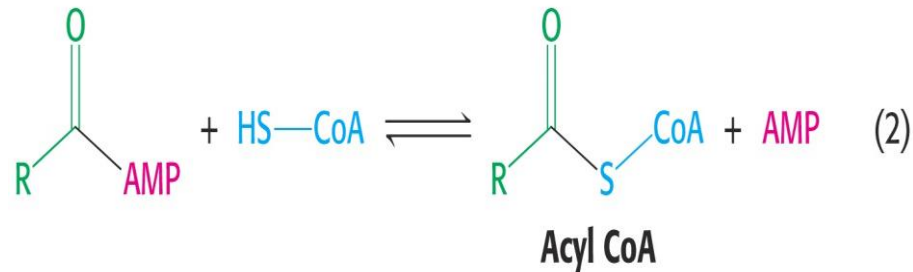
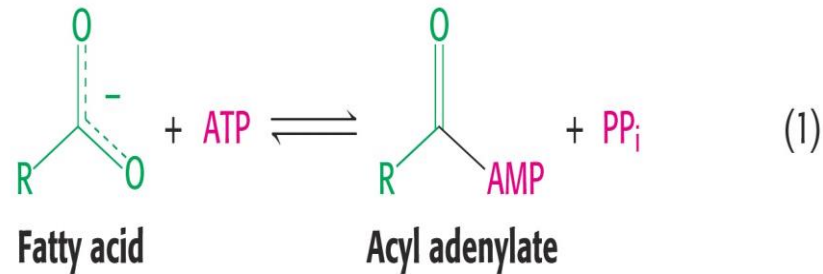
Site Of fatty acid activation: Fatty acid(Acyl Chain) is activated in Cytosol to Acyl-CoA .

Requirements of FA Activation

Enzyme: Thiokinase /Acyl CoA Synthetase

Coenzymes/Cofactors: CoA-SH, ATP, Magnesium ions (Mg^{++})

Activation Of a Fatty Acid is ATP Dependent



Acyl-CoA Synthetase/ Fatty Acid Thiokinase condenses Fatty acids with CoA, with simultaneous hydrolysis of ATP to AMP and PP_i

An Acyl-CoA is an activated energetic compound having high energy bond in it. Thus formation of Acyl-CoA is an expensive energetically

There are different Acyl-CoA Synthetase for fatty acids of different chain lengths.

Activated Fatty Acid (Acyl-CoA) is a high energy compound which facilitates second stage of Beta Oxidation of fatty acid

Stage II

Translocation of Acyl-CoA from cytosol into Mitochondrial Matrix with the help of Carnitine

β -oxidation of fatty acids occurs in Mitochondrial matrix.

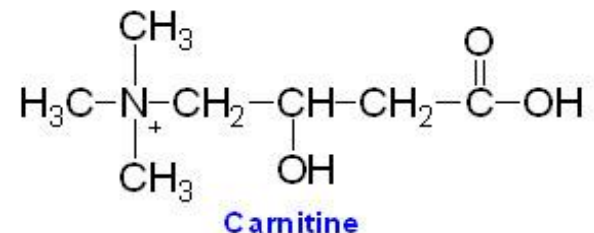
Long-chain Fatty acids more than 12 Carbon atoms cannot be directly translocated into the Mitochondrial matrix.

However, short chain Fatty acids are directly translocated into the Mitochondrial matrix

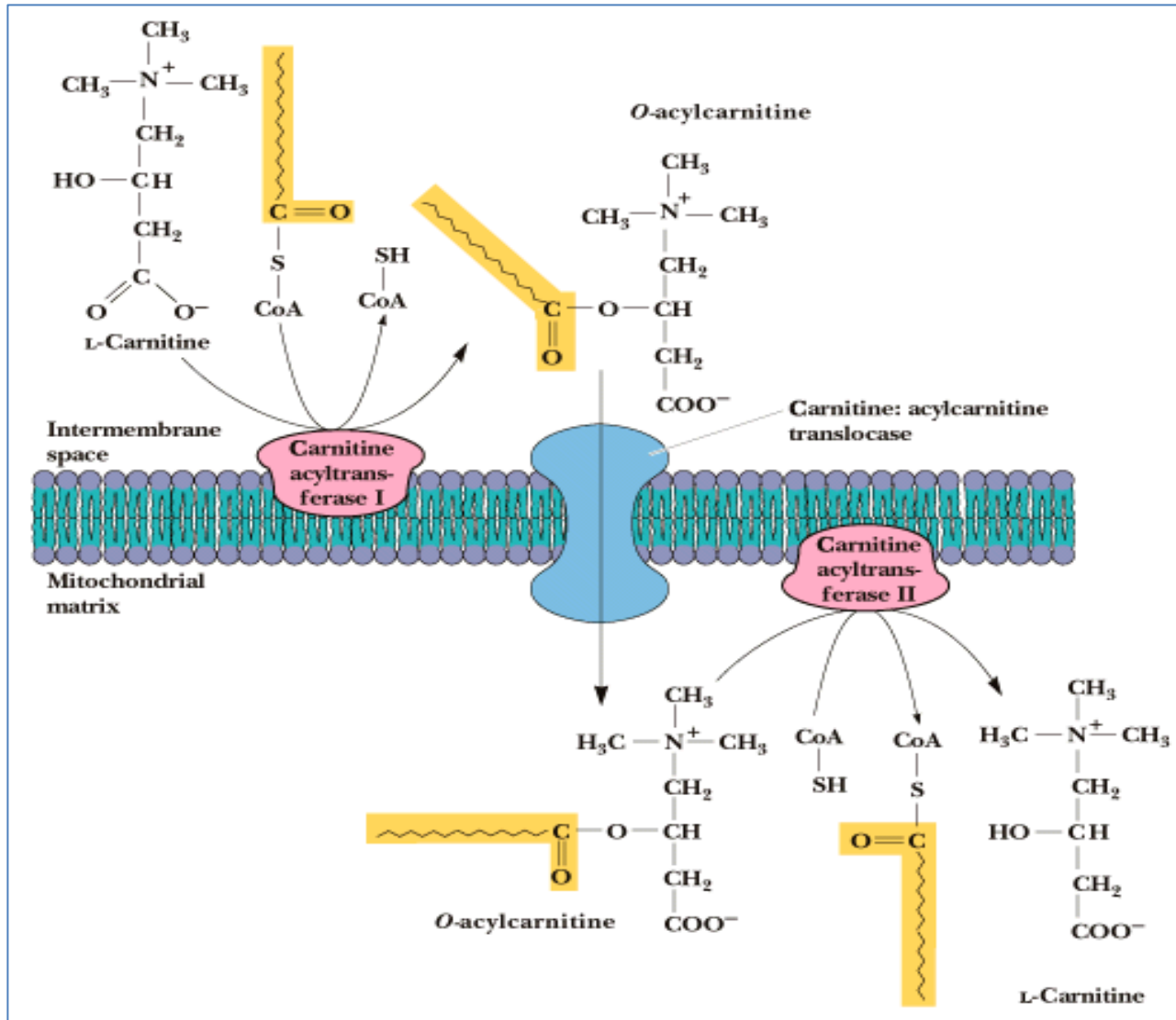
To translocate an activated long chain Fatty acid (Acyl-CoA) from cytosol to mitochondrial matrix there is a specialized Carnitine Carrier System.

Carnitine is a functional, Non Protein Nitrogenous (NPN) substance.

Carnitine is biosynthesized in the body by amino acids Lysine and Methionine.



Carnitine Carrier System



Long-chain FA are converted to Acyl Carnitine and are then transported. Acyl-CoA are reformed inside an inner membrane of mitochondrial matrix.

Acyl groups from Acyl-CoA is transferred to Carnitine to form Acyl- Carnitine catalyzed by Carnitine Acyl Transferase I (CAT I)

CAT I is present associated to outer mitochondrial membrane

Acylcarnitine is then shuttled across an inner mitochondrial membrane by a Translocase enzyme.

Acyl group is linked to CoA of Mitochondrial pool in mitochondrial matrix by Carnitine Acyl Transferase II (CAT II) to regenerate Acyl-CoA in mitochondrial matrix.

Finally, Carnitine is returned to cytosolic side by Protein Translocase, in exchange for an incoming Acyl Carnitine.

Cell maintains two separate pools of Coenzyme-A: Cytosolic pool of CoA and Mitochondrial pool of CoA

CoA is complex structure cannot transport across Mitochondrial membrane. CoA linked to Fatty acid in Mitochondria is different from that CoA used for Fatty acid activation.

Stage III

Beta oxidation of fatty acids includes four basic steps that are repeated over and over again until the entire molecule has been oxidized.

Steps of Beta Oxidation of fatty acids (Occurs in Mitochondrial Matrix)

Step1: Oxidation Reaction

Step2: Hydration Reaction

Step3: Oxidation Reaction

Step4: Cleavage Reaction

Step 1

To Bring Oxidation of the C_{α} - C_{β} bond of Fatty acid: catalysed by Acyl-CoA Dehydrogenase

Acyl CoA Dehydrogenase is a FAD linked Enzyme (Flavoprotein)

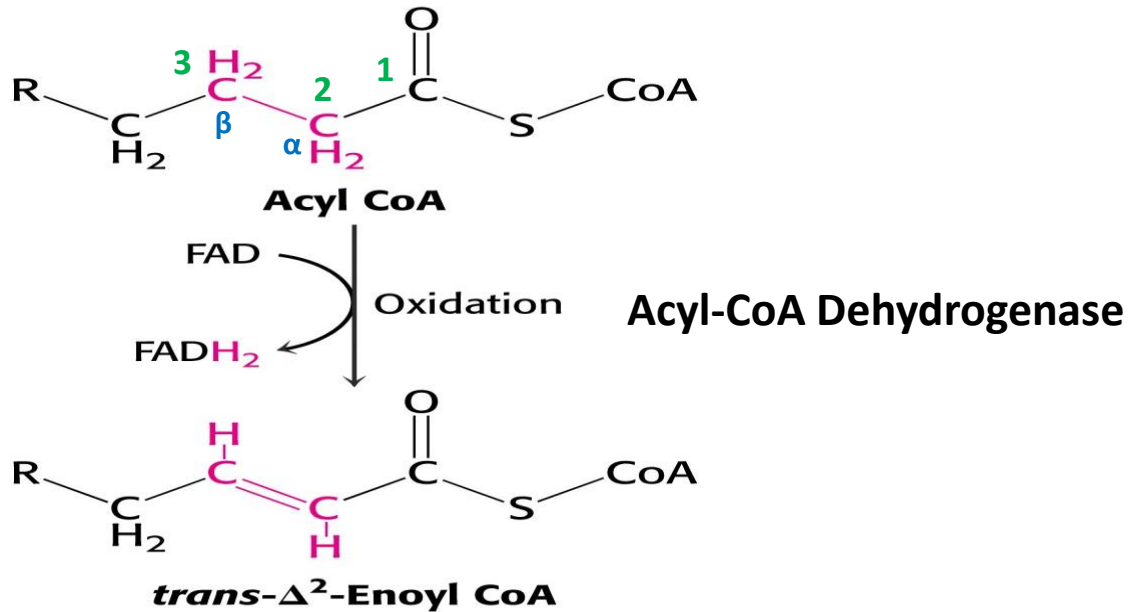
Acyl CoA Dehydrogenase catalyzes Oxidation reaction: In this reaction there is a removal of Hydrogen from alpha and beta carbon atoms of Acyl-CoA.

It forms a double bond between C_{α} - C_{β} / C2 and C3 of Fatty Acid. The product of this oxidation reaction is α - β Unsaturated Acyl CoA /Trans Enoyl CoA.

Coenzyme FAD is the temporary hydrogen acceptor in this oxidation reaction.

The reduced FADH₂ is generated by oxidation reaction of Acyl CoA Dehydrogenase.

FADH₂ is then reoxidized, after its enter into Electron Transport Chain.



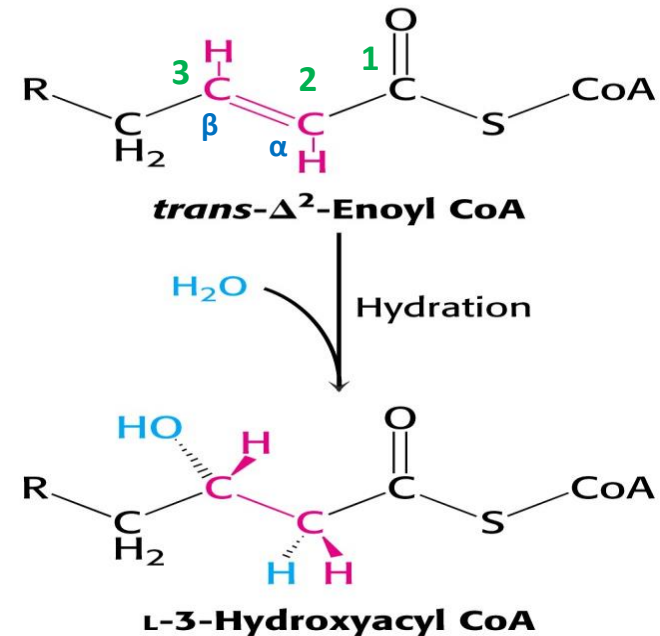
There are **different Acyl-CoA Dehydrogenases** :

- Short Chain Fatty acids (4-6 C),
- Medium Chain Fatty Acids (6-10 C),
- Long (12-18 C) and very long (22 and more) chain Fatty acids.

Step 2

Catalysed by Enoyl CoA Hydratase

- Enoyl-CoA Hydratase catalyzes stereospecific hydration of the trans double bond
- It adds water across the double bond at C2 and C3 of Trans Enoyl CoA
- This hydration reaction generates Hydroxyl (OH) group at beta carbon atom of FA
- Converts Trans-Enoyl-CoA to L β -Hydroxyacyl-CoA



Step 3

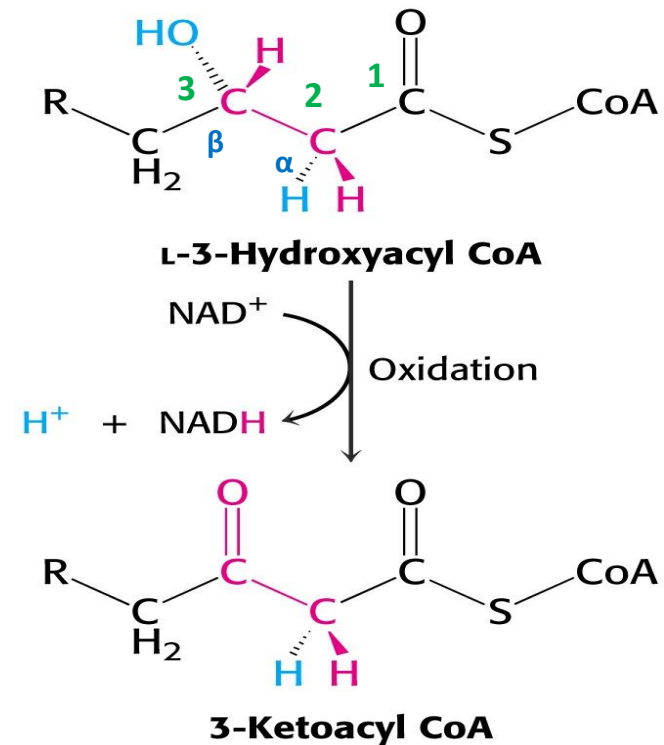
Catalysed by β -Hydroxyacyl-CoA Dehydrogenase

β -Hydroxyacyl-CoA Dehydrogenase is NAD^+ dependent enzyme

It catalyzes specific oxidation of the Hydroxyl group in the β position (C3) to form a ketone group.

NAD^+ is the temporary electron acceptor for this step which generates reduced form $\text{NADH} + \text{H}^+$

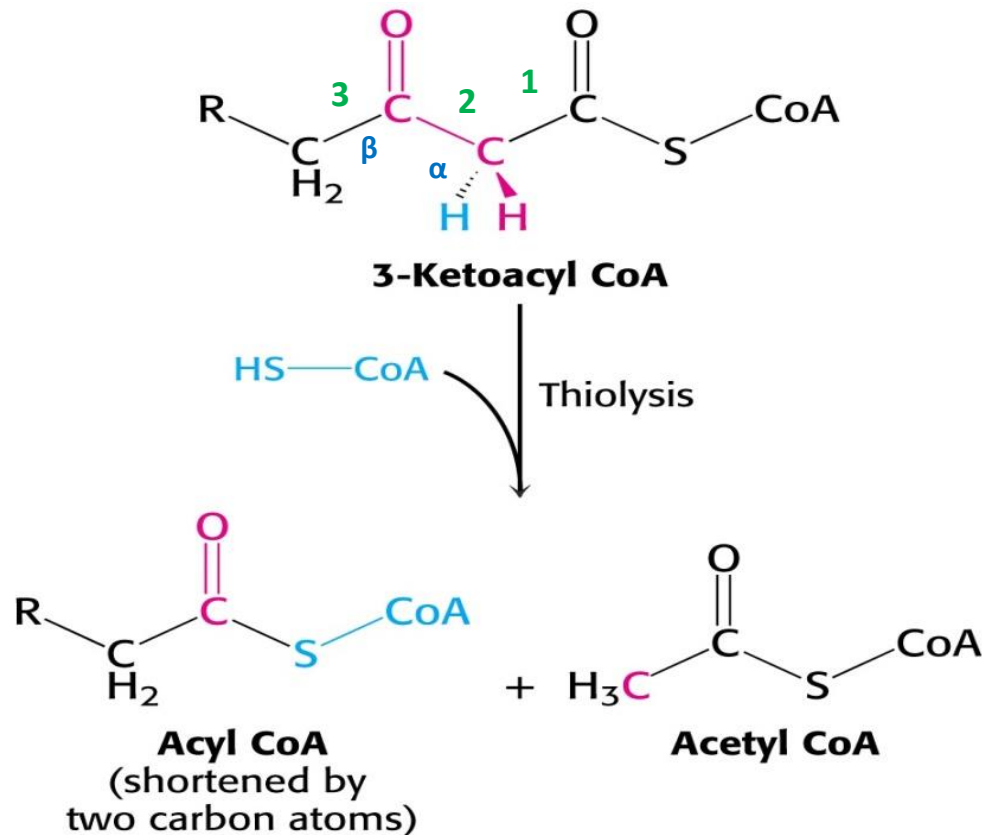
The oxidation of β -Hydroxyacyl CoA produces a product β - Ketoacyl-CoA.



Step 4

Catalysed by β -Ketothiolase /Thiolase. β -Keto Thiolase attacks the β -carbonyl group of β -Ketoacyl-CoA. This results in the cleavage of the C_{α} - C_{β} bond.

Releases Acetyl-CoA(2 Carbon) and an Acyl-CoA (-2 carbons shorter).



Repetitions of 4 Steps of Beta Oxidation

The β -oxidation pathway is cyclic. 4 Steps of Beta Oxidation are repeated till whole chain of Fatty acid is oxidized completely.

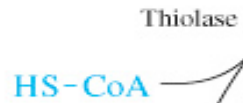
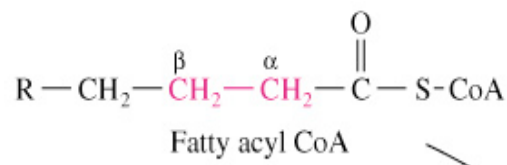
Product is 2 carbons shorter Acyl –CoA which acts as an input to another round/turn of the Beta Oxidation pathway.

Acyl CoA molecule released at end of Beta Oxidation that is the substrate for the next round of oxidation starting with Acyl CoA Dehydrogenase.

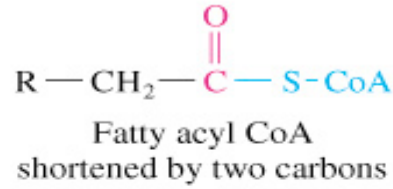
Repetition continues until all the carbon atoms of the original Fatty acyl CoA are converted to Acetyl CoA.

Each turn/cycle of β oxidation proper generates one molecule each of: FADH_2 , $\text{NADH}+\text{H}^+$, Acetyl CoA, Fatty Acyl CoA (with 2 carbons shorter each round)

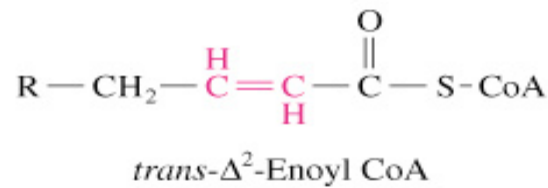
Summary of β -Oxidation of Saturated fatty acids



(4) **Thiolysis**



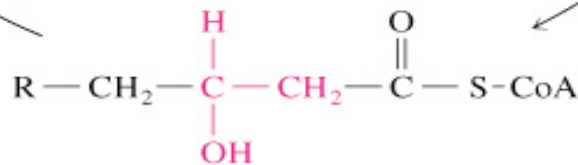
Oxidation (1)



Hydration (2)

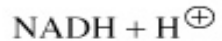


Enoyl-CoA
hydratase

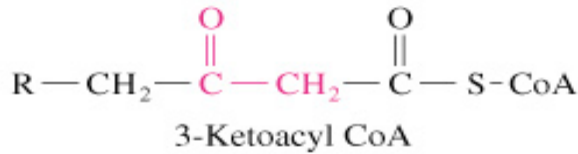
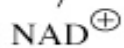


L-3-Hydroxyacyl CoA

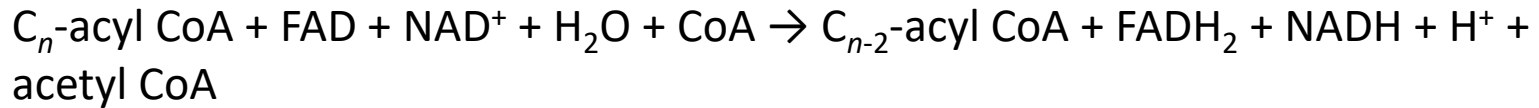
(3) **Oxidation**



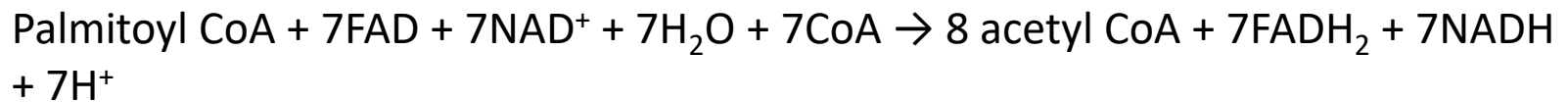
L-3-Hydroxyacyl-CoA
dehydrogenase



The overall reaction for one cycle of beta oxidation is:



We can now calculate energy yield from the complete oxidation of Palmitic acid (16 Carbon). The degradation of Palmitoyl CoA requires seven reaction cycles. In the seventh cycle, the C4-Ketoacyl CoA is thiolized to two molecules of acetyl CoA. Hence, the stoichiometry of the oxidation of palmitoyl CoA is:



- 1 NADH oxidation in respiratory chain yields approx 2.5 ATP molecules
- 1 FADH₂ oxidation in respiratory chain yields approx 1.5 ATP molecules
- 1 acetyl CoA oxidation in respiratory chain yields 10 ATP molecules

Therefore,

- 7 NADH: 7 X 2.5= 17.5 ATP
- 7 FADH₂: 7 X 1.5= 10.5 ATP
- 8 acetyl CoA: 8 X 10= 80 ATP

Total ATP produced = 108 ATP, 2 molecules of ATP is consumed in the activation of Palmitic acid. Thus, the complete oxidation of a molecule of palmitic acid yields 106 molecules of ATP

Beta Oxidation of Odd Chain Saturated Fatty Acids

Ingestion of Odd chain carbon Fatty acids are less common in human body.

Odd chain Fatty acids are formed by some bacteria in the stomachs of ruminants and the human colon.

β -oxidation of odd chain Saturated Fatty acid occurs same as even chain Fatty acid oxidation

Releasing Acetyl CoA (2C) in every turn.

Until the final Thiolase cleavage

Which results in a 3 Carbon Acyl-CoA /Propionyl-CoA in last cycle and last step of beta oxidation.

End Products Of Odd Chain Fatty Acid Oxidation Fatty acid is :

Acetyl-CoA(C2): Enter in TCA cycle and get completely oxidized.

Propionyl-CoA(C3): Is Converted into Succinyl CoA (4C), A TCA intermediate.

Beta Oxidation Of Unsaturated fatty acids (UFAs)

Unsaturated fatty acids have one or more double bonds between carbon atoms. The chain can occur in a cis or trans configuration. Cis-configuration are more common.

Double bonds of UFAs are cleaved by action of Extra Enzymes:

Isomerase (**Enoyl CoA Isomerase**)

(For odd numbered double bonds MUFAs)

Reductase (**2,4 Dienoyl CoA Reductase**)

(For even numbered double bonds PUFAs)

Beta Oxidation Of Odd numbered Unsaturated fatty acids

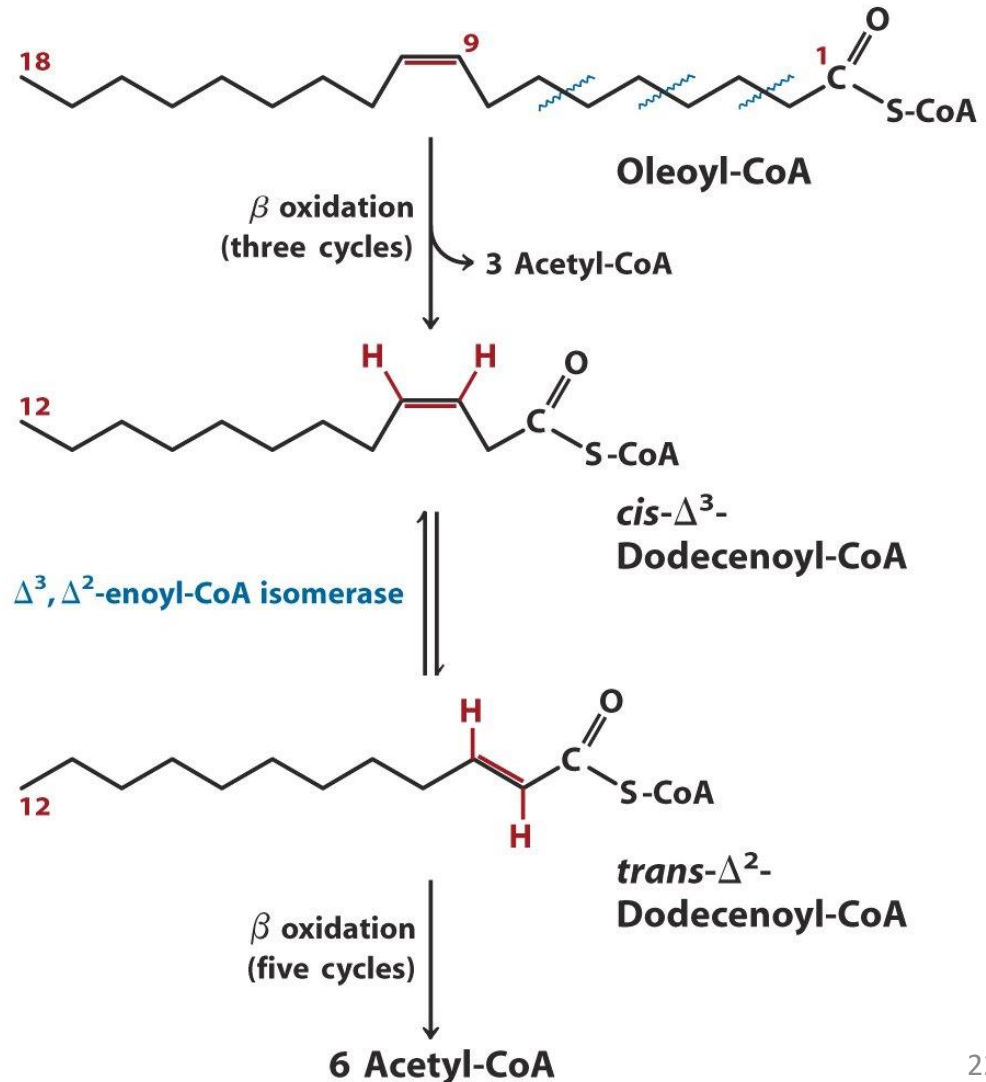
Example: Oleic Acid

oleic acid is classified as a monounsaturated omega-9 Fatty acid, abbreviated with a lipid number of 18:1 cis-9.

The steps of beta oxidation of oleic acid are mentioned in schematic

Oleic acid is first charged to form Oleoyl-CoA

Oleoyl-CoA enters into beta-oxidation cycle



Beta Oxidation Of even numbered Unsaturated fatty acids

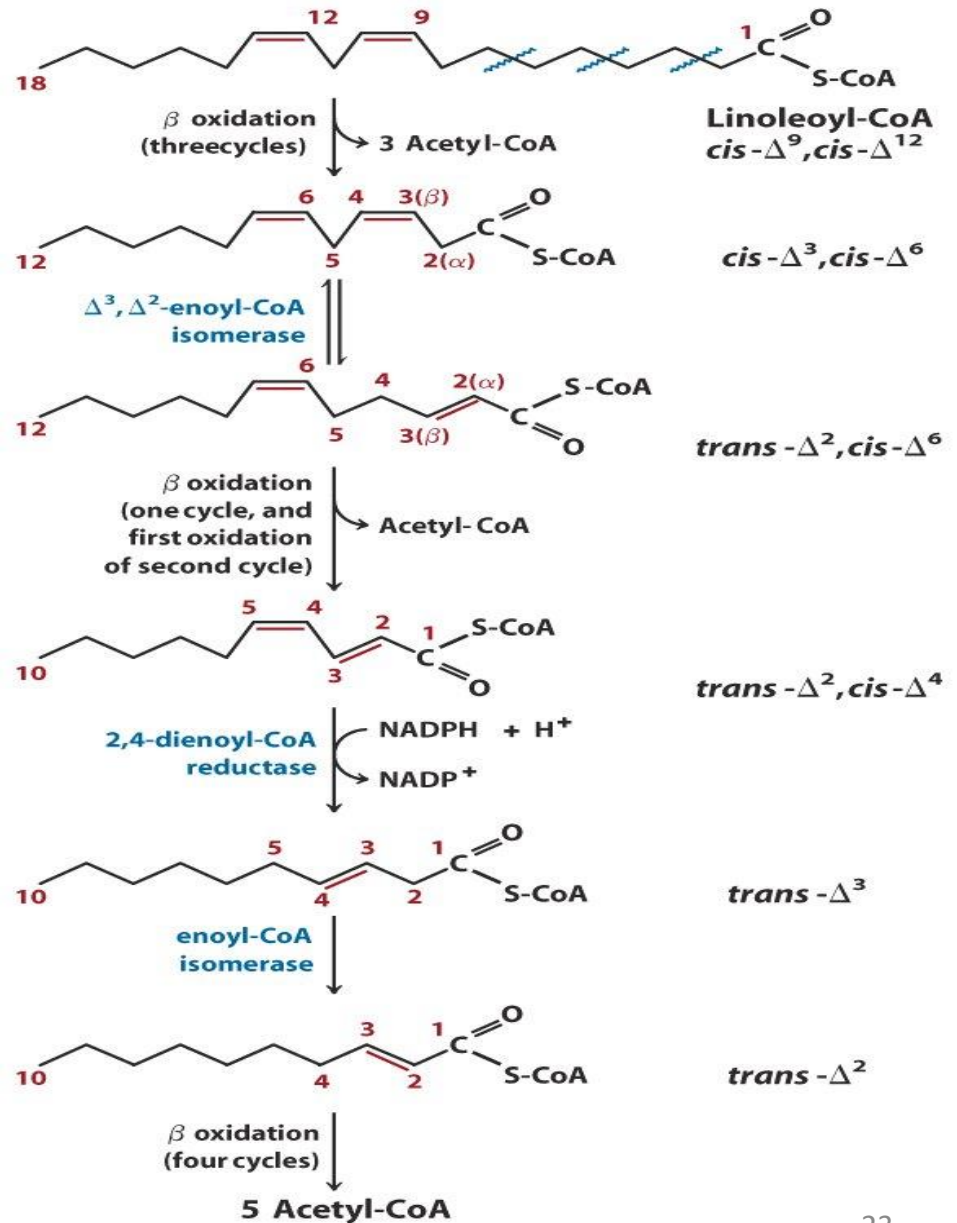
Example: Linoleic Acid

Linoleic acid is an 18-carbon chain with two double bonds in cis configuration, abbreviated as 18:2 *cis*-9,12. It belongs to Omega-6 fatty acids. This is because the number of carbons from the methyl end to the first double bond is six.

The steps of beta oxidation of Linoleic acid are mentioned in schematic

Linoleic acid is first charged to form Linoleoyl-CoA

Linoleoyl-CoA enters into beta-oxidation cycle



Regulation of Beta Oxidation of Fatty Acids

Lipolysis and β -Oxidation of Fatty acids are well regulated under Hormonal influence.

Insulin secretion occurs in well fed condition that inhibits Lipolysis of Fat (TAG) and mobilization of Free Fatty acids. Therefore, **insulin decreases β Oxidation of Fatty acids.**

Glucagon and Epinephrine secretion occurs in Emergency Condition: When Cellular or Blood Glucose lowers down there is secretion of these hormones. Glucagon and Epinephrine stimulates Lipolysis in emergency condition.

Glucagon and Epinephrine stimulates the Enzyme Hormone sensitive Lipase and hydrolyzes Fat (TAG). These hormones helps to mobilize Free fatty acids out into blood circulation and **Increases β Oxidation of Fatty acids.**

Regulation Of Beta Oxidation of Fatty Acid at Two Levels

- 1. Carnitine Shuttle**
- 2. Beta Oxidation Cycle**

1. Regulation at Carnitine Shuttle step

Transport of fatty acyl CoA from cytosol into Mitochondrial Matrix occurs via Carnitine Shuttle which is a rate- limiting step

Malonyl-CoA Regulates Beta Oxidation At Carnitine Transport Level. Malonyl-CoA is produced from Acetyl-CoA by the enzyme Acetyl-CoA Carboxylase during Fatty acid biosynthesis.

Malonyl-CoA an intermediate of Lipogenesis Is an Inhibitor of Carnitine Acyl Transferase I. This Control of Fatty acid oxidation is exerted mainly at the step of Fatty acid entry into mitochondria.

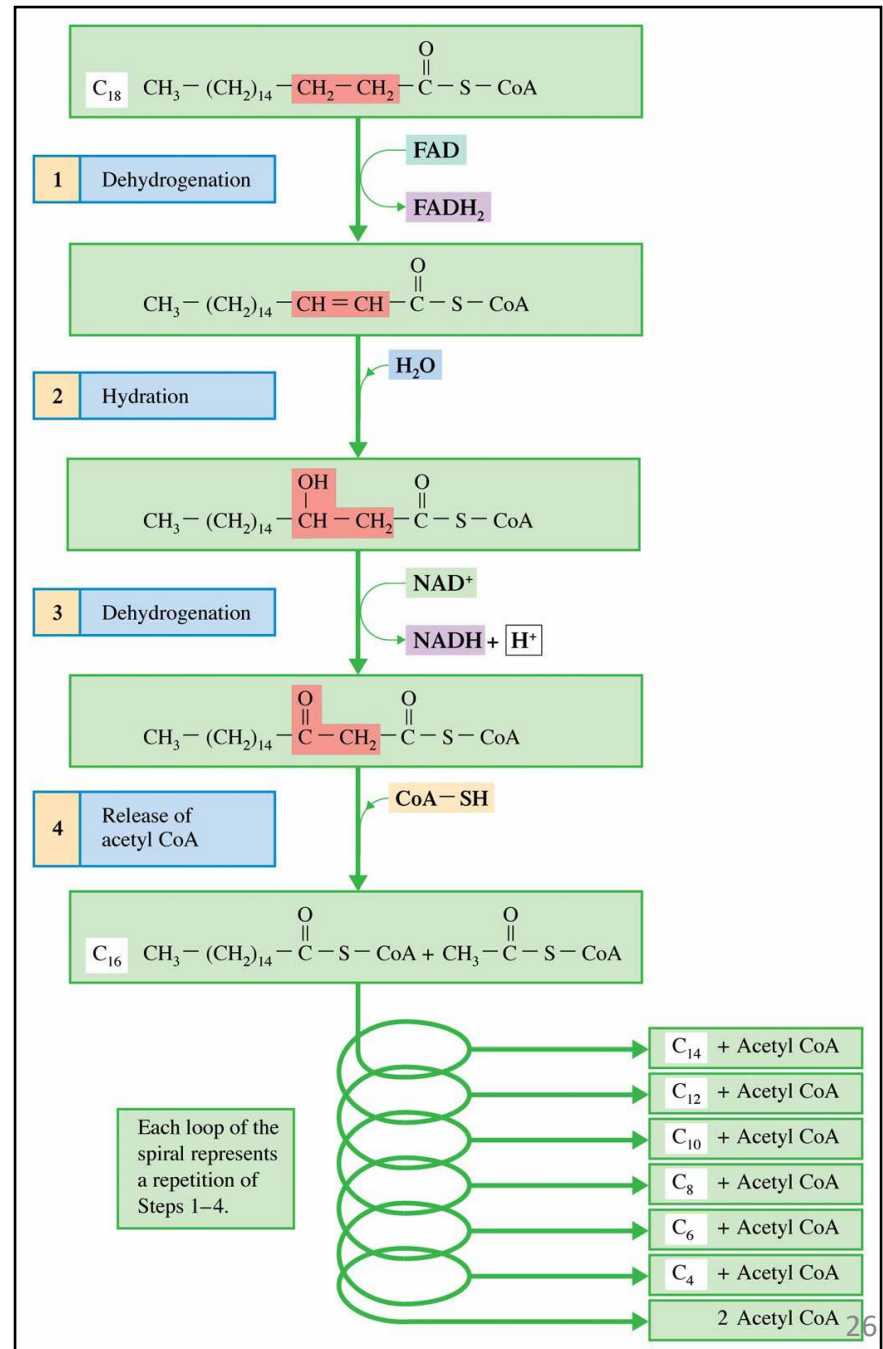
2. Regulation during Beta Oxidation Cycle

β -Hydroxyacyl-CoA Dehydrogenase is Regulatory or Key Enzyme of Beta Oxidation Of Fatty Acids regulated by energy sufficiency signals.

When the $[NADH]/[NAD]$ ratio is high, β -Hydroxyacyl-CoA Dehydrogenase is inhibited; in addition, high concentration of acetyl-CoA inhibits **Thiolase**.

Summary of Beta Oxidation of fatty acid

Example: Stearic acid



References

Lehninger Principles of biochemistry

Lubert Stryer Biochemistry

Voet and Voet Biochemistry

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