BIOCHEMISTRY

Lec:3 : 2nd stage **Dr. shaimaa S.Mutlak**

METABOLISM OF LIPIDS

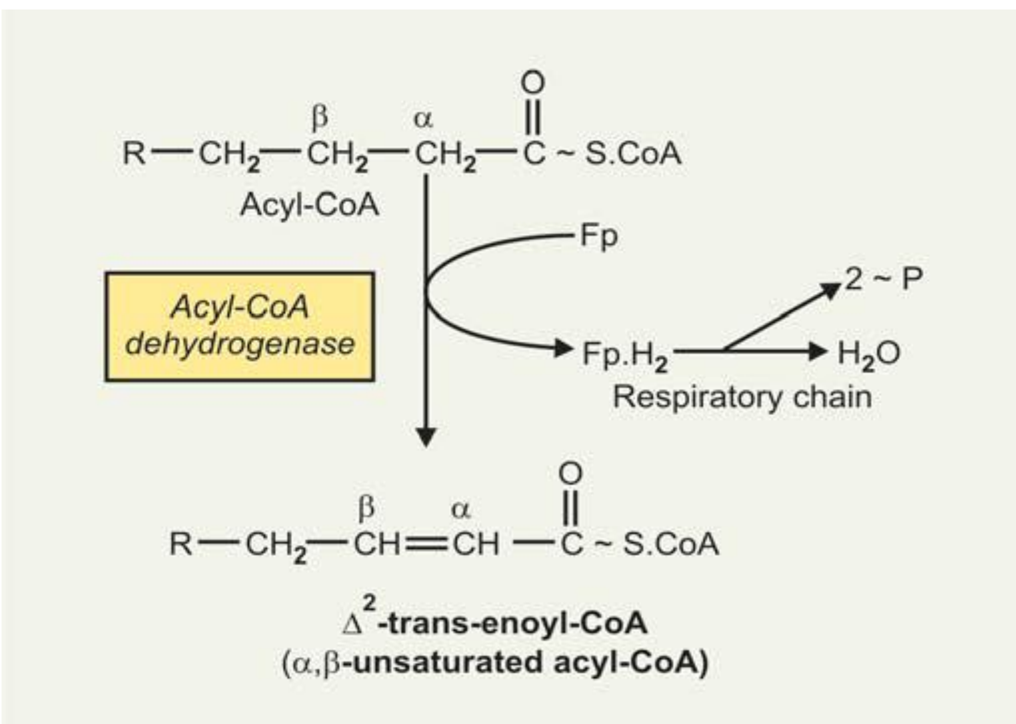
STEPS OF β-OXIDATION

Once acyl-CoA is transported by carnitine in the mitochondrial matrix, it undergoes β-oxidation by Fatty acid oxidase complex. The successive steps are as follows:

1. Dehydrogenation: Removal of 2 H atoms:

Removal of two hydrogen atoms from the 2 (α) and 3 (β) carbon atoms is catalysed by the enzyme **acyl-CoA dehydrogenase,** resulting in formation of Δ2 -transenoyl-CoA (also called α, β-unsaturated acyl-CoA).

Hydrogen acceptor, i.e the co-enzyme for this dehydrogenase is a **flavo-protein,** containing FAD as prosthetic group, whose re-oxidation in the respiratory chain requires the mediation of another flavoprotein, called **electron transferring flavoprotein (ETF) + 2 ATP**



Types of acyl-CoA dehydrogenases: At least three acyl-CoA dehydrogenases have been described:

1. **"G"-green-coloured,** cu-containing, catalyses oxidation of fatty acids having chain length–C4 to C8.
2. **'Y' or 'y1'-Yellow** flavo protein, catalyses oxidation of FA having chain length –C4 to C18, more specific for C6 "hexonyl-CoA dehydrogenase"
3. **'y' or 'Y2'**-more active on FA having chain-length C6 to C18. Maximum activity on FA having chain-length C16 "hexa-decanoyl dehydrogenase".

2. Hydration: Addition of one molecule of H2O:

One molecule of water is added to saturate the double bond to form 3-OH acyl-CoA (called also as β-OH acyl-CoA), the reaction is catalysed by the enzyme "Δ 2 - enoyl -CoA hydratase" (also called as Enoyl hydrolase).

3. Dehydrogenation: Removal of 2 hydrogen atoms:

The 3 – OH – Acyl-CoA undergoes further dehydrogenation on the 3 carbon, catalysed by the enzyme **3–OH–acyl-CoA dehydrogenase,** to form the corresponding 3–ketoacyl-CoA (β-ketoacyl-CoA).

Hydrogen acceptor, i.e. coenzyme of this dehydrogenase is NAD+. Reduced NAD when oxidized in respiratory chain produces 3 ATP.

4. Thiolytic cleavage:

Finally, 3-keto-acyl-CoA is split at the 2,3 position by **thiolase** ("3 – keto acyl thiolase" or "acetyl–CoA acyl transferase"), which catalyses a thiolytic cleavage involving another molecule of CoA.

End-products of this reaction: The thiolytic cleavage results in formation of:

1. One molecule of acetyl-CoA .
2. An acyl-CoA molecule containing 2-carbons less than the original acyl-CoA molecule, which enters for oxidation by the enzyme acyl-CoA dehydrogenase (reenters at step 1).

In this way, a long-chain FA may be degraded completely to "acetyl-CoA" (C-2 units). Acetyl-CoA can be oxidized to CO2 and H2O and thus complete oxidation of FA is achieved. Thus, end-product of β-oxidation of a

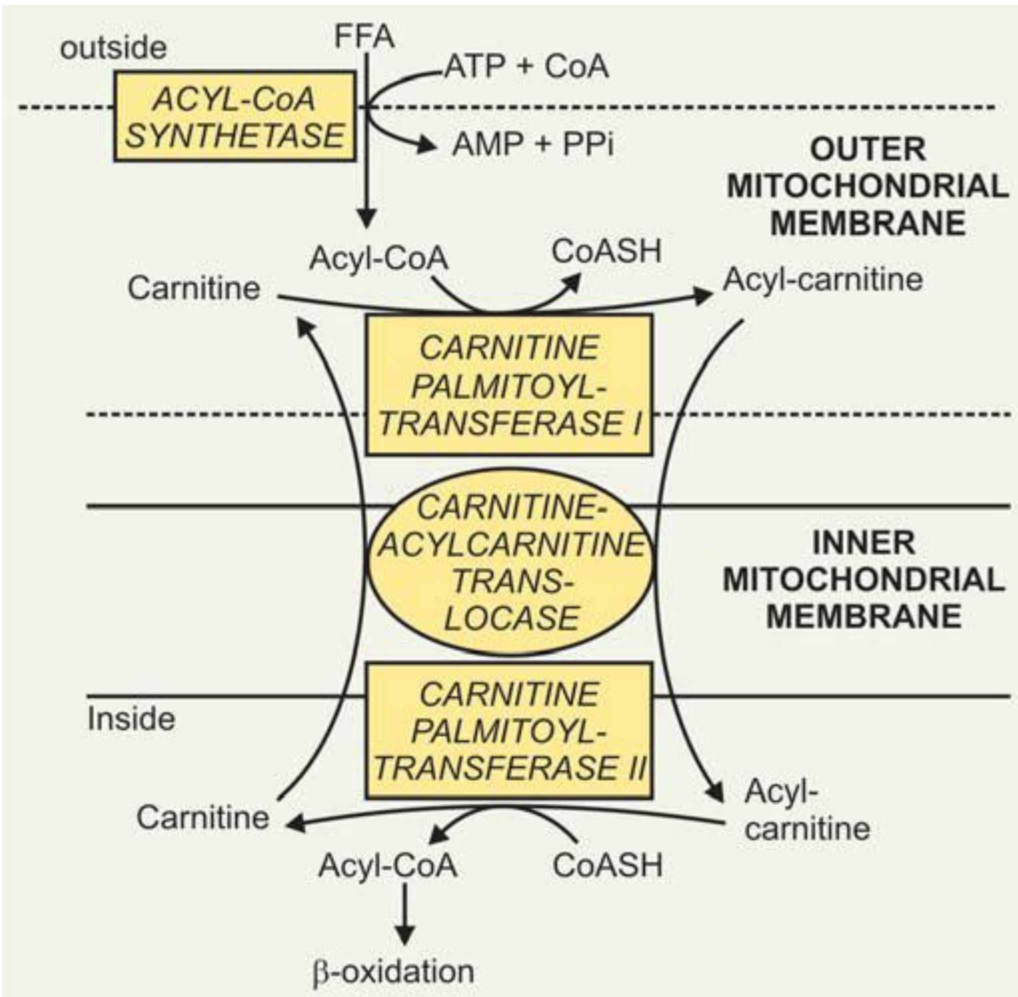
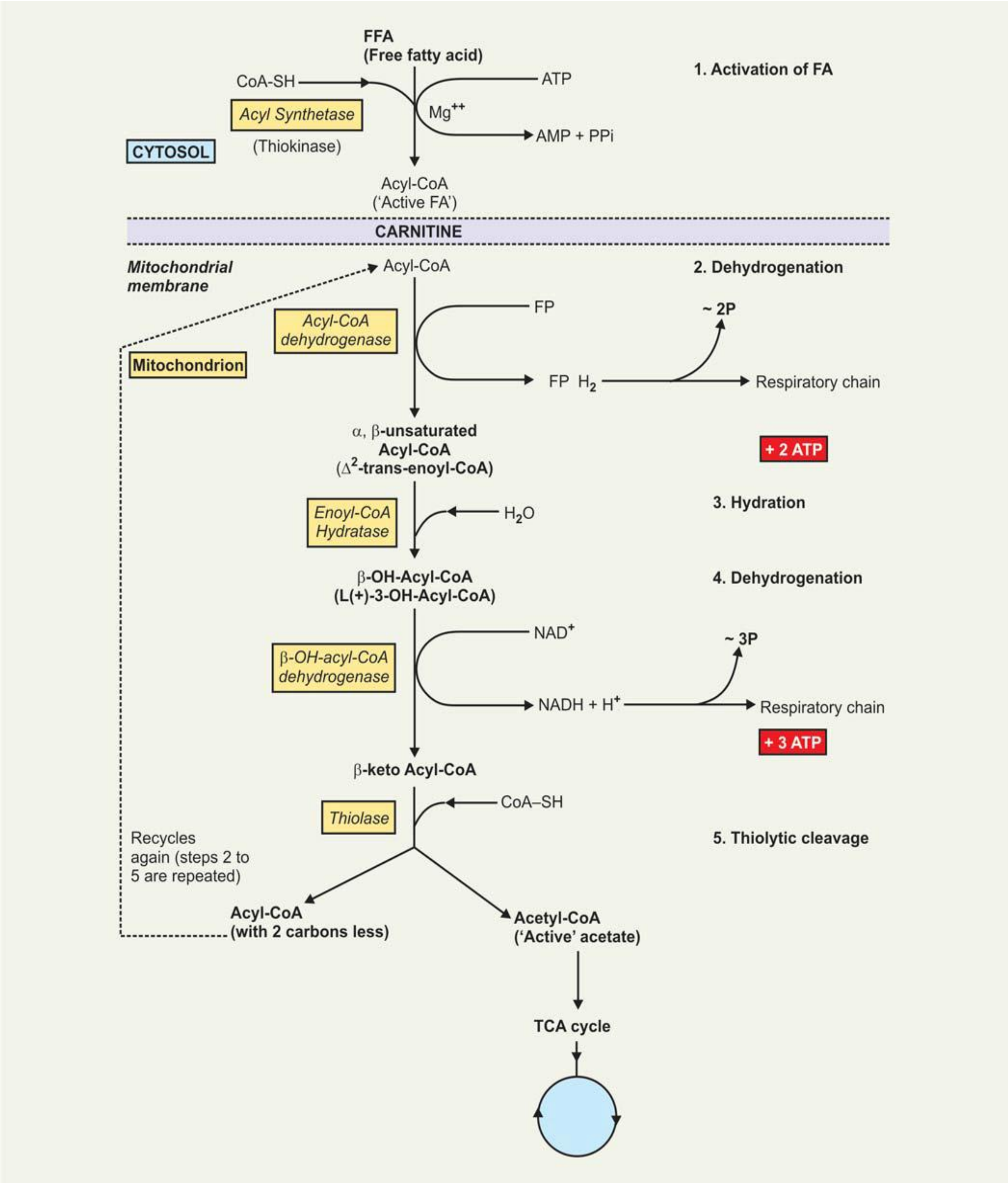
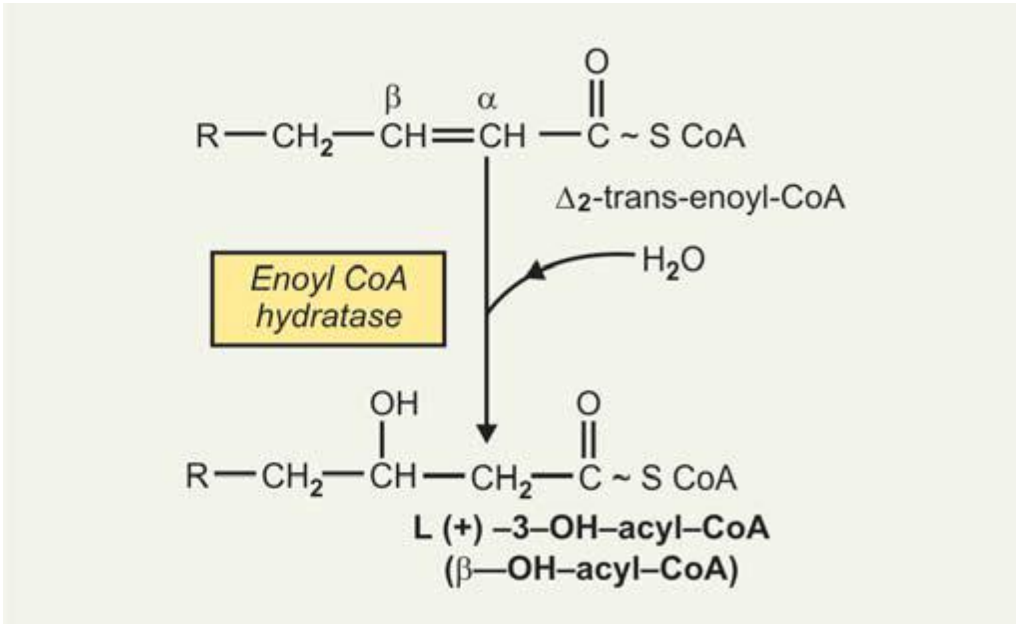
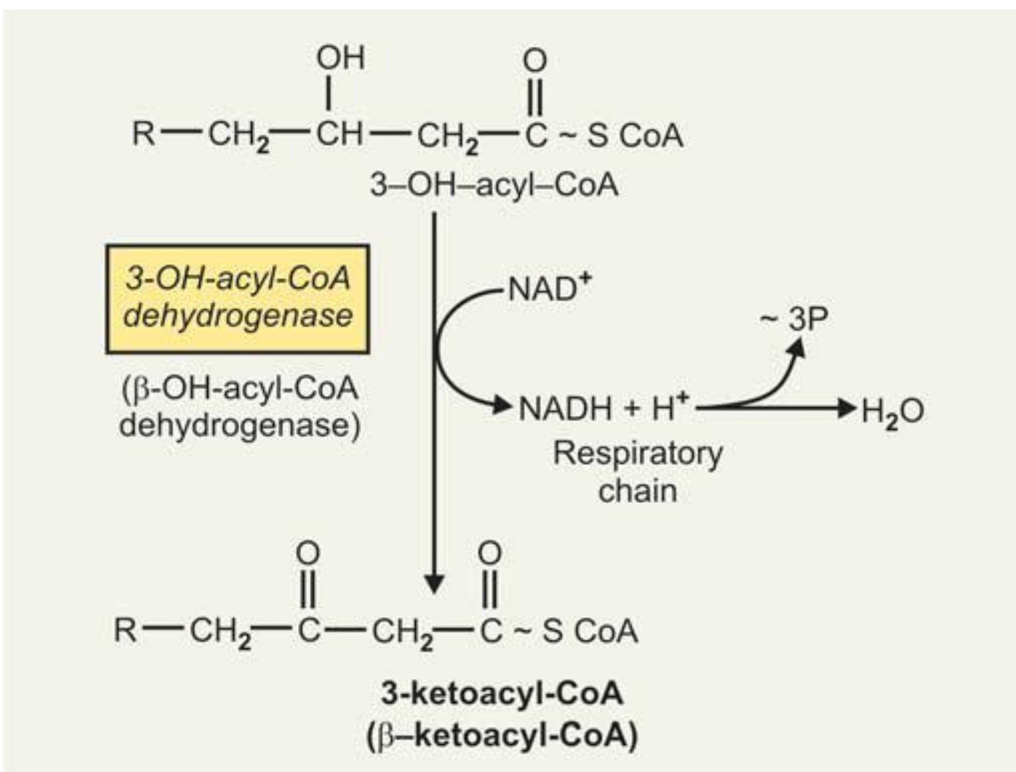


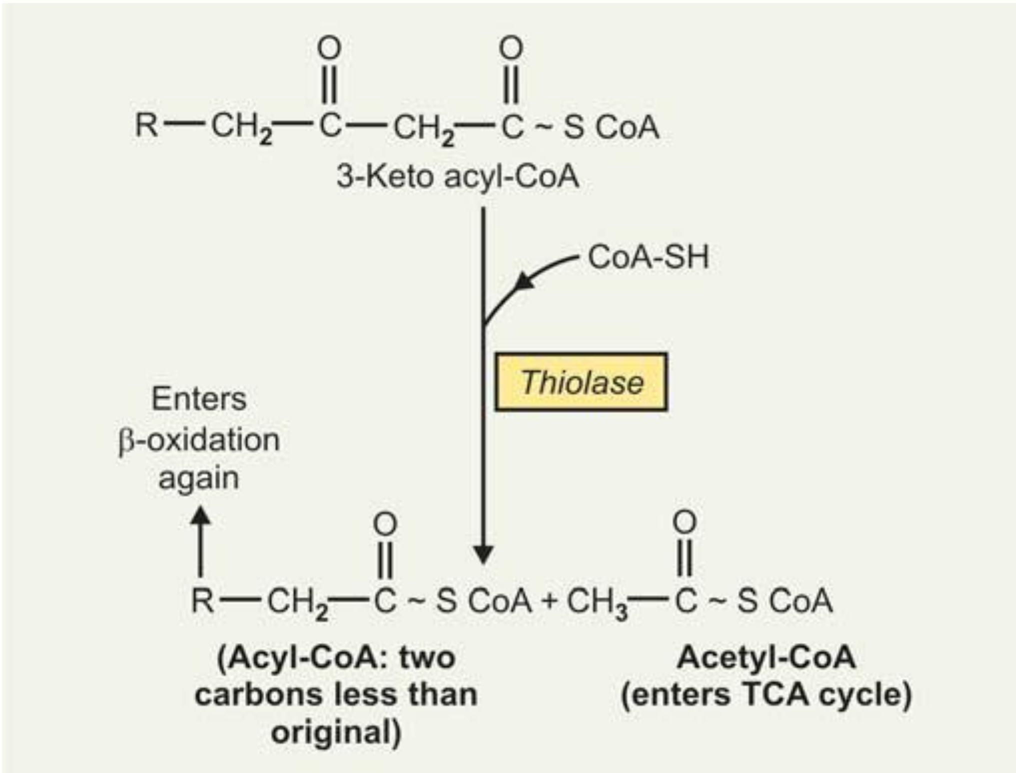
Fig: Role of carnitine in the transport of long-chain FA



FFA
(Free fatty acid)
1. Activation of FA
CoA-SH
ATP
Acyl Synthetase
Mg++
CYTOSOL
(Thiokinase)
AMP + PPi
Acyl-CoA
('Active FA')
CARNITINE
Mitochondrial membrane
Acyl-CoA
2. Dehydrogenation
Acyl-CoA dehydrogenase
FP
~ 2P
Mitochondrion
FP H2
Respiratory chain
α, β-unsaturated Acyl-CoA
(Δ2-trans-enoyl-CoA)
+ 2 ATP
Enoyl-CoA Hydratase
H2O
3. Hydration
β-OH-Acyl-CoA
(L(+)-3-OH-Acyl-CoA)
4. Dehydrogenation
β-OH-acyl-CoA dehydrogenase
NAD+
~ 3P
NADH + H+
Respiratory chain
+ 3 ATP
β-keto Acyl-CoA
Thiolase
CoA–SH
Recycles again (steps 2 to 5 are repeated)
5. Thiolytic cleavage
Acyl-CoA
(with 2 carbons less)
Acetyl-CoA
('Active' acetate)
TCA cycle


β
α
O
R—CH2—CH=CH—C ~ S CoA
Δ2-trans-enoyl-CoA
H2O
Enoyl CoA hydratase
OH
O
R—CH2—CH—CH2—C ~ S CoA
L (+) –3–OH–acyl–CoA
(β—OH–acyl–CoA)


OH
O
R—CH2—CH—CH2—C ~ S CoA
3–OH–acyl–CoA
3-OH-acyl-CoA dehydrogenase
(β-OH-acyl-CoA dehydrogenase)
NAD+
~ 3P
NADH + H+
H2O
Respiratory chain
O
O
R—CH2—C—CH2—C ~ S CoA
3-ketoacyl-CoA
(β–ketoacyl-CoA)



How many acetyl-CoA are produced from β-oxidation of palmitic acid?

Palmitic acid is C15 H31 COOH. In β-oxidation, it will **undergo 7 (seven) cycles**, producing 7 acetyl-CoA (in 7 cycles) + 1 acetyl-CoA (last cycle-one extra).

∴ **Total acetyl-CoA produced by β-oxidation of one molecule of palmitic acid = 8 acetyl-CoA.**

β-Oxidation of FA with an odd number of carbon Atoms: Fatty acids with an odd number of carbon atoms are oxidized by β-oxidation pathway to produce acetyl-CoA until a 3-carbon residue **propionyl-CoA** is left.

Propionyl-CoA is metabolised to succinyl-CoA through methyl malonyl-CoA.

Bioenergetics of β-Oxidation and its Efficiency

Palmitic acid, C15H31COOH, on complete oxidation (β-oxidation) **produces 8 acetyl-CoA** (Refer discussion above). Transport of electrons in respiratory chain from reduced Fp and NAD in each cycle produces 5 (five) high energy phosphate bonds.

Hence, 7 cycles (7 × 5) = 35 ~ P
Total 8 molecules of acetyl-CoA,
When oxidised in TCA cycle will
produce = 12 × 8 = 96 ~ P
Total high energy phosphate bonds
produced = 131 ~ P
Total = 131 ~ P
In initial activation of
FA~ P bond utilised = –2 ~ P
∴ **Total gain = 129 ~ P**
∴ Energy Production = 129 × 7.6 = 980 Kc
(or 129 × 30.5 = 3935 Kj)
Caloric value of Palmitic acid
(Bomb calorimeter) = 2340 Kc/mol
Hence, **efficiency = 980/2340 × 100 = 41% of the total energy of combustion of FA.**

B. α-OXIDATION

α-oxidation is another alternative pathway for oxidation of FA which involves decarboxylation of the COOH group after hydroxylation and the formation of a FA containing an "odd" number of carbon atoms, which subsequently undergoes repeated β-oxidation. **No initial activation of FA is necessary in this process.**

C. ω-OXIDATION (VERKADE)

In ω-oxidation, Fatty acids undergo oxidation at the carbon atom farthest removed from the carboxyl group (ω-carbon) producing a ***dicarboxylic acid,*** which is then subjected to β-oxidation and cleavage to form successively smaller dicarboxylic acids. Both processes occur principally in brain microsomes but are negligible in extent as compared to β-oxidation.

Essential differences and similarities between α-oxidation and ω-oxidation are shown in **Table**.

Table 1: Differences in α-and ω-oxidation

α-**oxidation**	ω-**oxidation**
1. **Substrate:** Even carbon long-chain FA (some of them)	1. Some medium- and long-chain FA
2. **Presence of O2**—oxidised aerobically in presence of O2	2. Occurs aerobically in presence of O2
3. **Sites:** Microsomes of brain and liver	3. Liver microsomes
4. **Enzyme:**α-hydroxylase-a monooxygenase	4. FA ω-hydroxylase also a onooxygenase
5. **Cofactors required**—Fe++, Vitamin C/FH4	5. Cytochrome P450, flavoprotein reductase and NADP+
6. **No initial activation of FA is required**	6. **No initial activation of FA is necessary.**
7. **Steps: (i) Step-1:** Formation of α-OH-FA R – CH2 – CH2 – CH2 – COOH α – hydroxylase ↓ Fe++ Vit C/or FH4 OH | R – CH2 – CH2 – CH – COOH α – OH – FA	7. **Steps: (i) Step-1:** Hydroxylation of ω carbon. CH3 – CH2 – (CH2)n – CH2 – COOH Even C – FA ω-hydroxylase ↓ O2, Cyt P 450 → H2O OH | CH2 – CH2 – (CH2)n – CH2 – COOH ω-OH-FA

(ii) Step-2: Decarboxylation to produce an OH | R – CH2 – CH2 – CH – COOH NAD+ Dehydrogenase NADH + H+ O ‖ R – CH2 – CH2 –C – COOH α—keto acid CO2 R – CH2 – CH2 – COOH Odd chain FA with one carbon less	**(ii) Step-2:** ω-OH FA is oxidised with the help of an NADPdependent enzyme to produce- α-ω **dicarboxylic acid** OH CH2 – CH2 –(CH2)n – CH2 – COOH ω – OH FA NADP+ Enz NADPH + H+ H | C – CH2 – (CH2)n – CH2 – COOH ‖ O Aldehyde Enz O2 HOOC – CH2 – (CH2)n – CH2 – COOH α, ω—dicarboxylic acid
(iii) Step-3: Odd chain FA undergoes repeated β-oxidation to produce (Acetyl-CoA)n + Propionyl-CoA" ↓ Succinyl-CoA ↓ TCA cycle	**(iii) Step-3:** Even carbon dicarboxylic acid then undergoes-repeated β-oxidation to yield (Acetyl-CoA)n and one molecule of succinyl-CoA (intermediate in TCA Cycle). ↓ TCA cycle