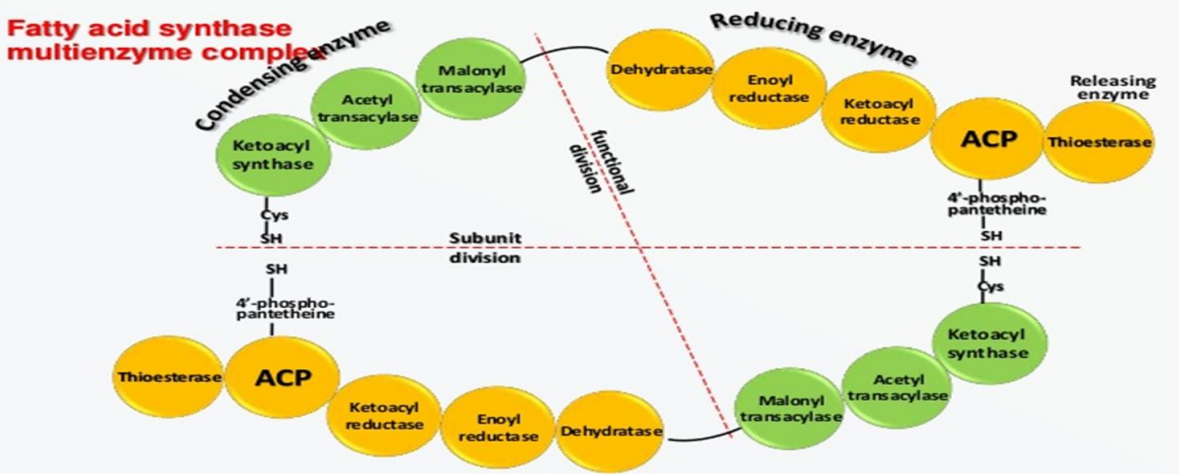
Fatty acids metabolism



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Biosynthesis of F.A.s

- F.A.s biosynthesis starts with **acetyl CoA**.
- The enzyme systems are involved in this process.
- 1- Extra-mitochondrial (cytosolic) system. 2- Microsomal system.

Requirements; acetyl CoA, NADPH+H+ and group of enzymes called collectively fatty acid synthase complex.

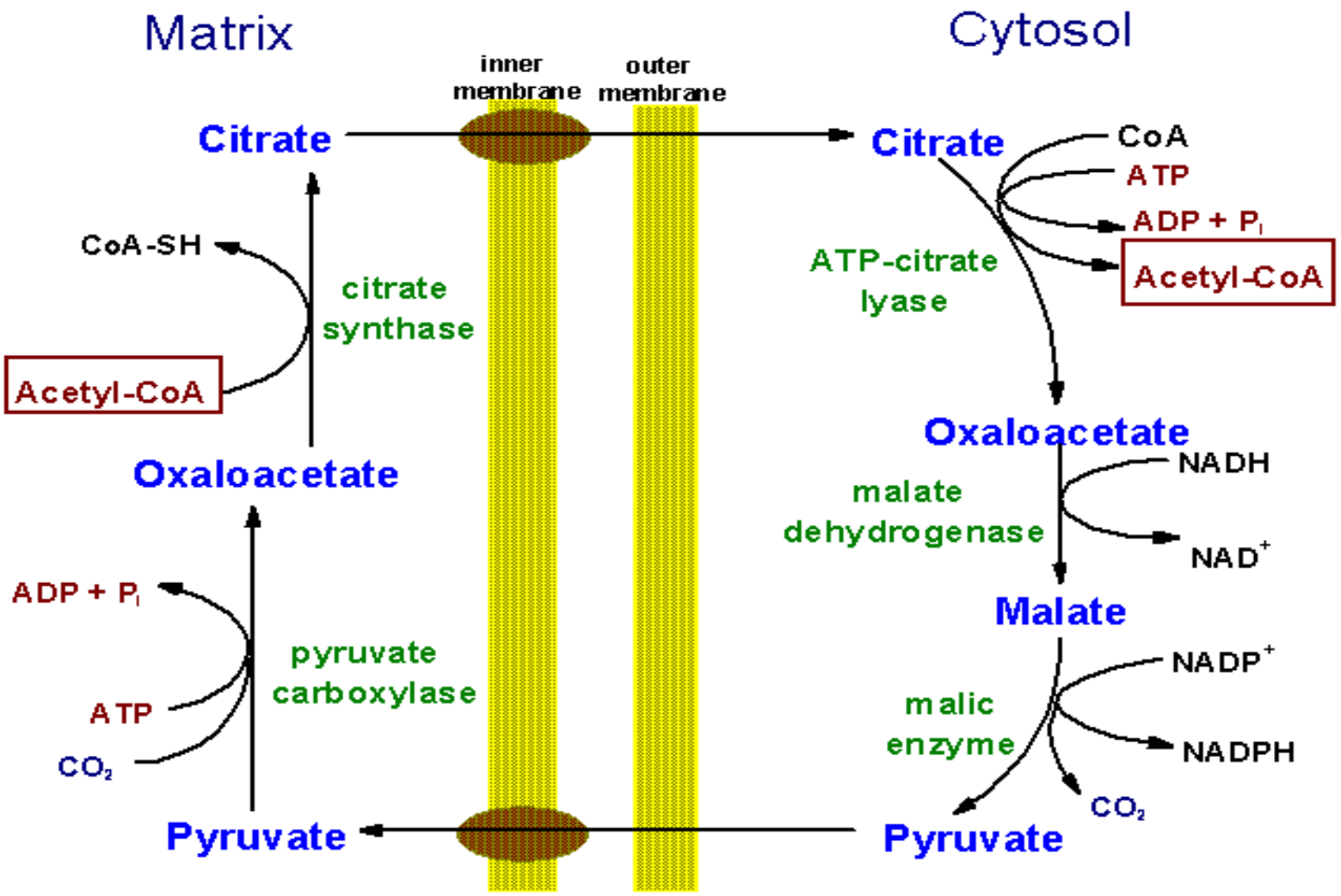
Acetyl CoA formed from pyruvate within the mitochondria doesn't diffuse readily to the cytosol, the principal site of F.A. synthesis.

-Translocation to cytosol involves condensation with oxaloacetate to form citrate. (CITRATE SHUTTLE)

- When citrate is formed in excess of TCA cycle requirement, it diffuses into cytosol.
- By the aid of ATP citrate lyase, citrate is splitted down into acetyl CoA and oxaloacetate.

-**Acetyl CoA** may also pass through mitochondrial membrane into cytosol in the form of acetyl carnitine by carnitine acetyl transferase

Translocation of acetyl CoA to cytosol



Note: Acetyl CoA used for fatty acid synthesis always derived from glucose and never from fatty acids. This is because insulin hormone secreted after meal stimulates both glucose oxidation (+acetyl CoA) and lipogenesis (=Fatty acid synthesis) and Inhibits lipolysis (+Fatty acid oxidation+Acetyl CoA).

NADPH+H+: It is provided by 3 sources:

a) Pentose phosphate pathway.

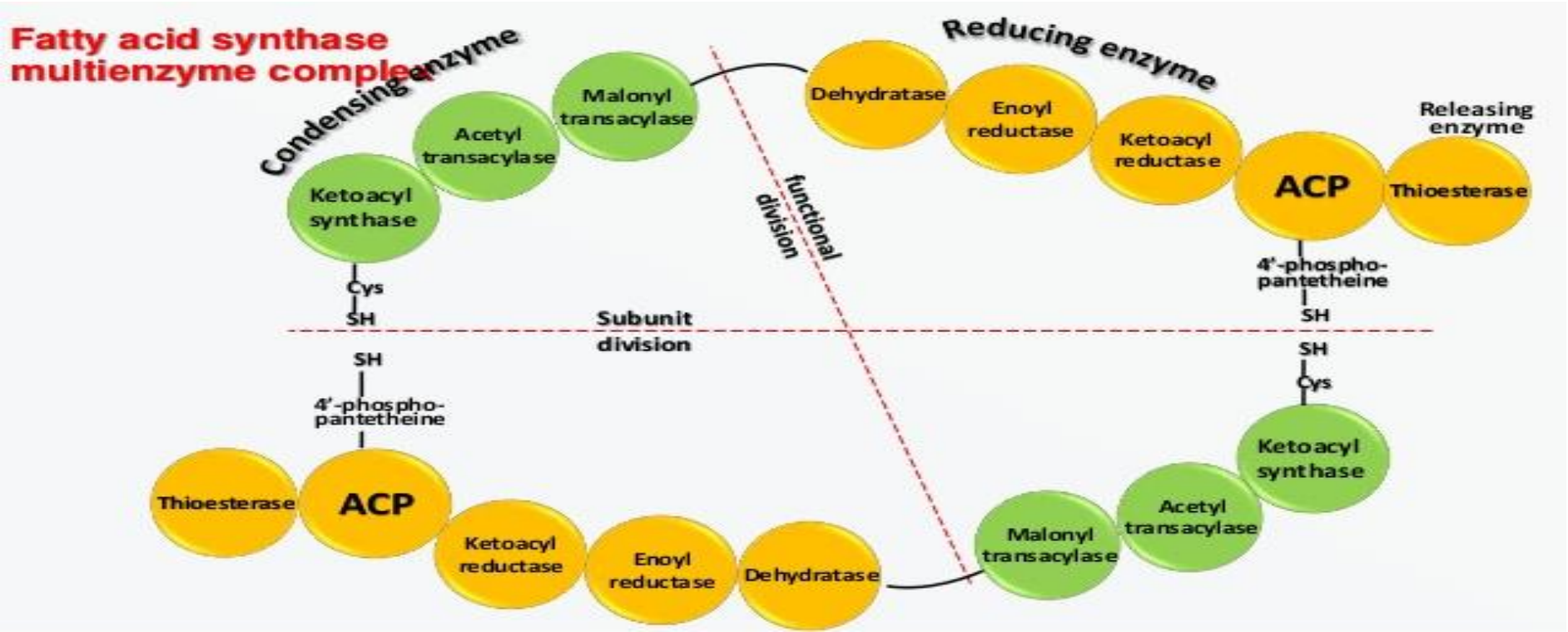
b) Action of cytoplasmic isocitrate dehydrogenase on isocitrate. It is similar to mitochondrial one but it uses NADP+ as hydrogen carrier.

c) Action of malic enzyme on malate to produce pyruvate:

Fatty acid synthase complex:

- This enzyme is a dimer i.e. formed of 2 subunits.
- Each unit, which is called monomer, contains 7 enzymes and a terminal protein called acyl carrier protein (ACP).

- ACP is a protein contains the vitamin pantothenic acid in the form of phosphopantotheine. ACP is the part that carries the acyl group.
- Each monomer contains 2 –SH groups, one provided by phosphopantotheine and attached to ACP. The other is provided by cysteine attached to the enzyme 3- ketoacyl synthase.
- The 2 monomers are arranged head to tail, so the -SH group of ACP of one monomer is very close to the -SH group provided by 3- ketoacyl synthase of the other monomer.



Extra-mitochondrial system

- It is the only system responsible for de novo synthesis of F.A.s from acetyl CoA, free palmitate is the main product.

Site:

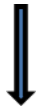
Intracellular location: Cytosol.

8 molecules of acetyl CoA (C2) are used in the formation of palmitate (C16)

-7 acetyl CoA are converted into malonyl CoA by the enzyme acetyl CoA carboxylase.

-The process occurs in 7 repeated cycles, each requires 2 mol of $NADPH+H^+$, and liberates 1 mol of CO_2 and 1 mol of H_2O.

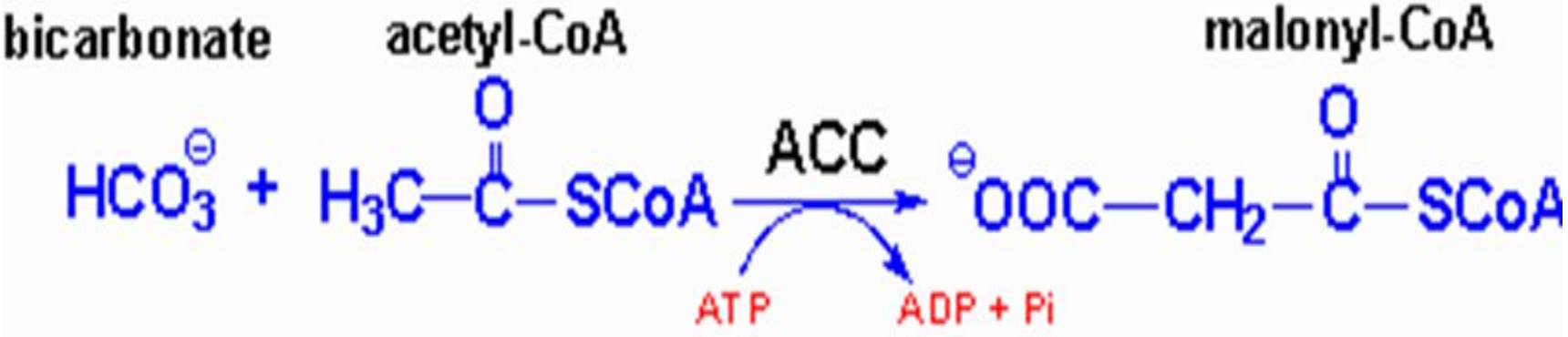
Acetyl CoA + 7 Malonyl CoA + 14 $NADPH+H^+$

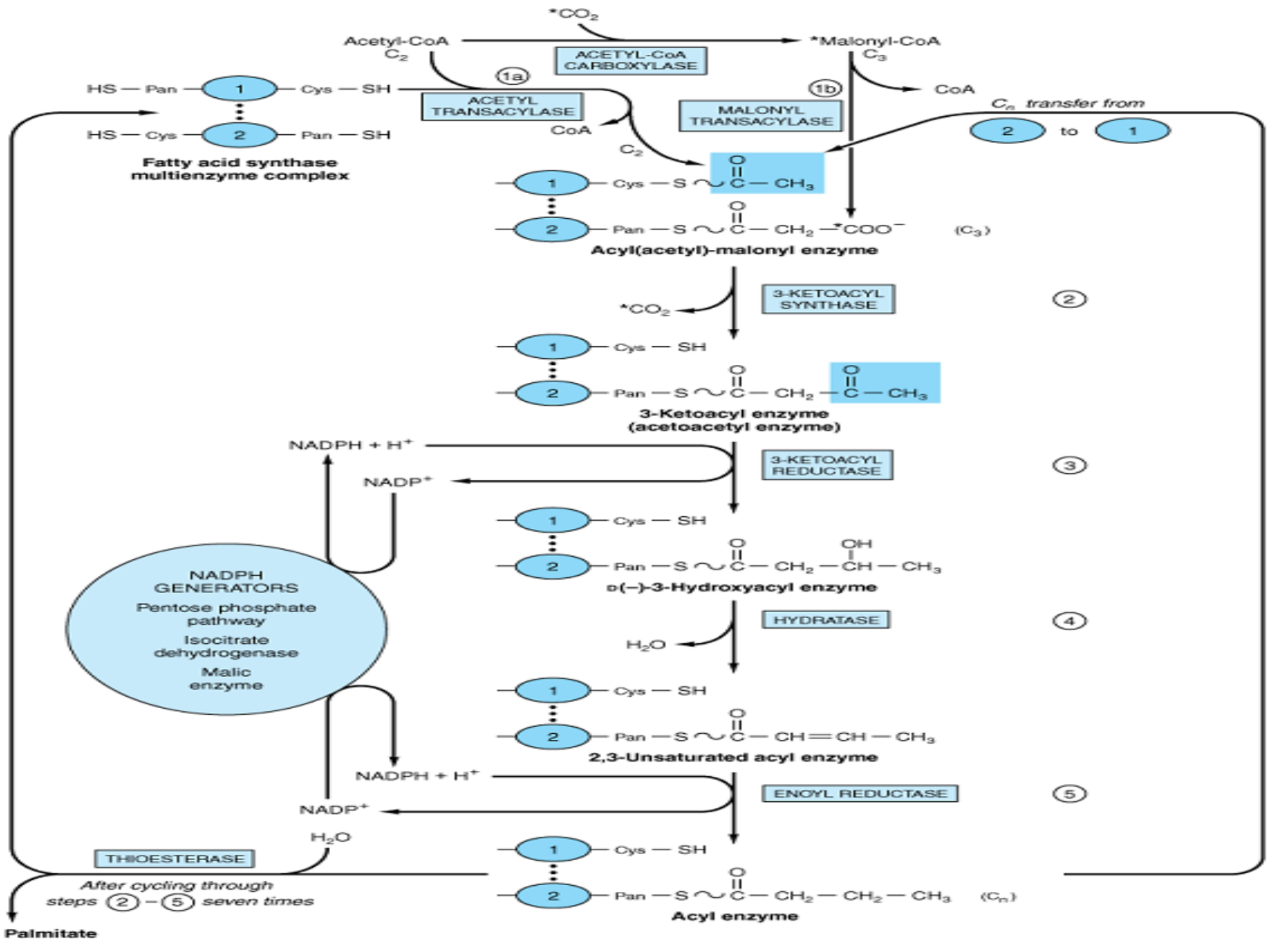


Palmitic acid+ 8 COA+ 14 NADP+ 7 CO_2+ 6 H_2O ??

-The synthesis of malonyl-CoA is the first committed step of fatty acid synthesis and the enzyme that catalyzes this reaction, acetyl-CoA carboxylase (ACC), is the major site of regulation of fatty acid synthesis.

- Like other enzymes that transfer CO_2 to substrates, ACC requires a biotin co-factor.




*CO2
Acetyl-CoA
C2
ACETYL-CoA CARBOXYLASE
*Malonyl-CoA
C3
1a
1b
ACETYL TRANSACYLASE
MALONYL TRANSACYLASE
CoA
HS — Pan — 1 — Cys — SH
HS — Cys — 2 — Pan — SH
Fatty acid synthase multienzyme complex
Cn transfer from 2 to 1
Acyl(acetyl)-malonyl enzyme
(C3)
3-KETOACYL SYNTHASE
2
3-Ketoacyl enzyme (acetoacetyl enzyme)
NADPH + H+
NADP+
3-KETOACYL REDUCTASE
3
D(−)-3-Hydroxyacyl enzyme
NADPH GENERATORS
Pentose phosphate pathway
Isocitrate dehydrogenase
Malic enzyme
HYDRATASE
4
H2O
2,3-Unsaturated acyl enzyme
ENOYL REDUCTASE
5
THIOESTERASE
After cycling through steps 2 – 5 seven times
Acyl enzyme
(Cn)
Palmitate

Regulation of F.A.s synthesis

1- Acetyl CoA carboxylase: Allosterically:

- Activated by citrate.
- Inhibited by long chain acyl-CoA.

2- Acyl CoA:

- Inhibits the transport of citrate from mitochondria to cytosol.
- Inhibits PDH and citrate synthase.

3- Insulin:

- After a carbohydrate rich meal, insulin is secreted, it increases glycolysis, acetyl CoA, oxaloacetate (needed for acetyl radical transport and citrate production step) for FA synthesis.
- It inhibits lipolysis through cAMP inhibition which reduces the concentration of long chain acyl CoA (inhibitor of lipogenesis).
- It activates PDH, acetyl CoA carboxylase to the active dephosphorylated form.
- During starvation: The reverse occurs
- Citrate activates acetyl CoA carboxylase (the rate limiting step in fatty acids biosynthesis).

Fate of palmitate:

1. Esterification:

Palmitate esterifieid with glycerol to from acylglycerols or with cholesterol to form cholesteryl ester.

2. Chain elongation:

Palmitate may be elongated to form a longer fatty acid.

3. Desaturation: i.e. synthesis of unsaturated fatty acid: palmitate may undergo desaturation to form palmitoleic acid.

4. Sphingosine formation:

It is formed by condensation of palmityl CoA and the amino acid serine

MICROSOMAL PATHWA Y FOR FA TTY ACID SYNTHESIS:

This is probably the main site for the elongation of existing long chain fatty acid molecules i.e. production of fatty acids longer than 16 carbon atoms.

A. The elongated molecules are derived from :

1. Palmitate : by cytoplasmic pathway .
2. fatty acids of diet.

B. The microsomal pathway needs **malonyl CoA** as acetyl donor and NADPH+H+ as coenzyme .

C. Function : This system becomes active during myelination of nerves in order to provide C22 and C24 fatty acids which are present in sphingolipids

Synthesis Of Unsaturated Fatty Acids:

A. Nonessential unsaturat ed fatty acids:

1. **These are fatty acids which contain one double bond e.g. palmitoleic acid (16: 1) and oleic acid (18:1).**

2. **Synthesis of oleic acid (oleyl CoA) : It is synthesized - in the microsomes - from stearyl CoA (active stearic acid)**

B. Essential fatty acid:

These are unsaturated fatty acids which contain more than one double bond.

1. They are essential because they cannot be formed in the body and should be taken in the diet

2. Examples: linoleic acid (18:2) linolenic (18:3) and arachidonic acid (20:4).

3. Sources: Vegetable oils as corn oil and cotton seed oil.

4. <u>Functions:</u>

a- They are Important for normal growth.

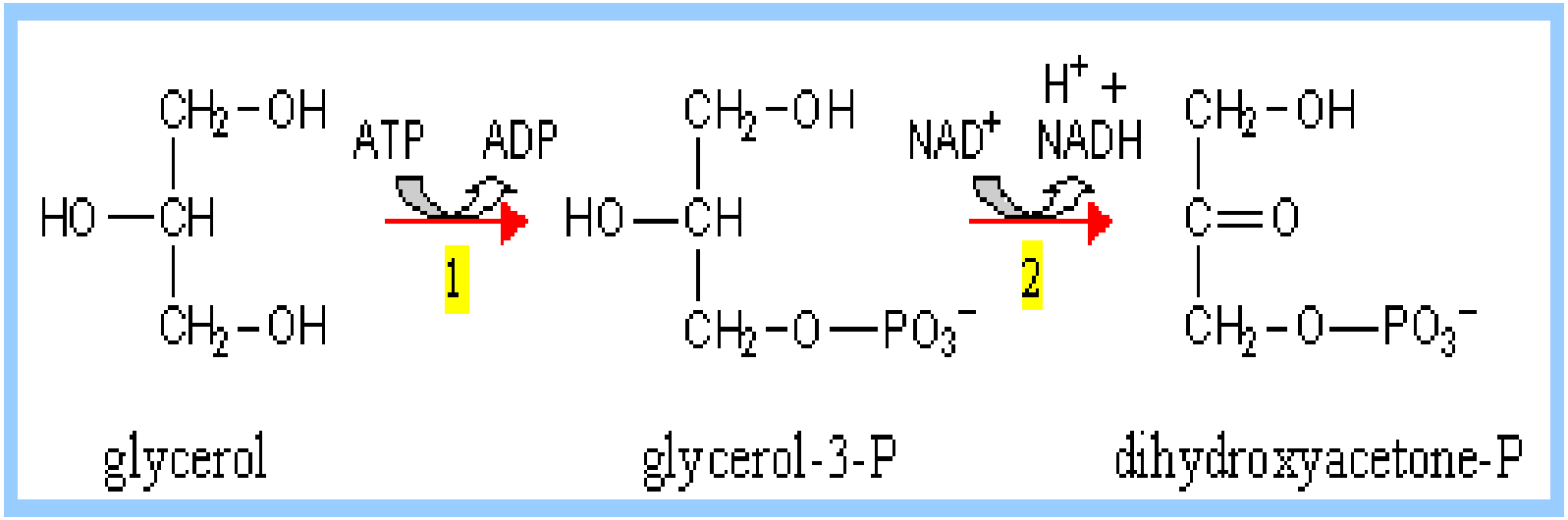
b- Synthesis of phospholipids:

c- Prevention of atherosclerosis: Essential fatty acids combine with cholesterol forming esters which are rapidly metabolized by the liver. This prevents precipitation of free cholesterol along the endothelium of blood vessels ~ prevents atherosclerosis.

d· Synthesis of eicosanoid.

Synthesis of TAG (Lipogenesis):

- Fatty acids are stored for future use as triacylglycerols in all cells, but primarily in adipocytes of adipose tissue.
- Triacylglycerols constitute molecules of glycerol to which three fatty acids have been esterified.
- The major building block for the synthesis of triacylglycerols, in tissues other than adipose tissues is **glycerol-3-phosphate** which is formed from glycerol by glycerokinase or from glycolysis.
- Adipocytes and muscles lack **glycerol kinase,** therefore, dihydroxyacetone phosphate (**DHAP**), produced during glycolysis, is the precursor for triacylglycerol synthesis in adipose tissues.



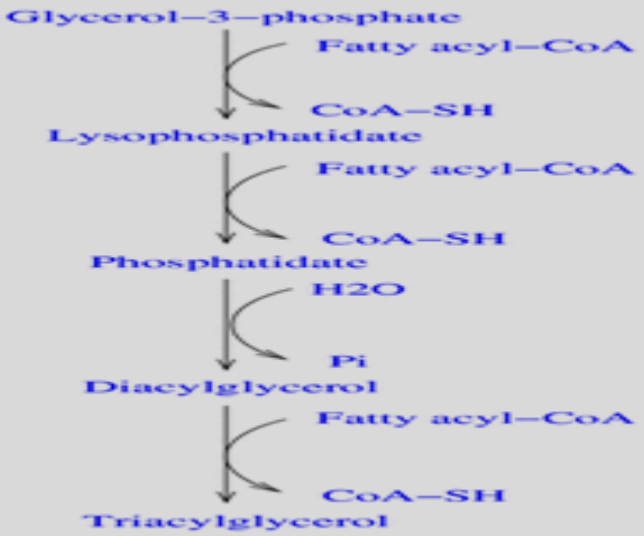
The fatty acids incorporated into triacylglycerols are activated to *acyl-CoA* through the action of acyl-CoA synthetases.

-Two molecules of acyl-CoA are esterified to glycerol-3-phosphate to yield 1,2-diacylglycerol phosphate (commonly identified as phosphatidic acid).

-The phosphate is then removed, by phosphatidic acid phosphatase, to yield 1,2-diacylglycerol, the substrate for addition of the third fatty acid.

-**N.B.** After meal, insulin is secreted which stimulate glycolysis which supplies **DHAP** that converted to glycerol phosphate in adipose tissue so **lipogenesis is stimulated.**

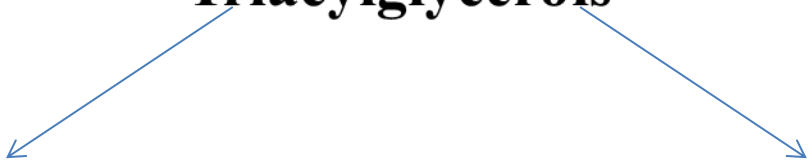
Triacylglycerol Synthesis



Catabolism of TAG (Lipolysis):

- Lipolysis is carried out by three enzymes present in adipose tissue :
  1. Hormone sensitive triacylglycerol lipase:
  2. Diacylglycerol lipase.
  3. Monoacylglycerol lipase.

Triacylglycerols



Glycerol

- Glucose by gluconeogenesis.
- Pyruvate by glycolysis.
- Triacylglycerol by lipogenesis

Fatty acids

- Oxidation to give energy.
- May remain in adipose tissue to be re-esterified into TAG

- **N.B**. Glycerol in adipose tissue cannot be used in re-esterification of fatty acids to form triacylglycerol due to deficiency of **glycerokinase enzyme.**

Regulation of lipolysis

The key enzyme controlling lipolysis is **Hormone sensitive triacylglycerol lipase (HSL):**

- This enzyme is **activated when phosphorylated** by 3′ 5′-cyclic AMP-dependent protein kinase.
- 3′ 5′-cyclic AMP is produced in adipocyte when one of several hormones (mainly epinephrine) binds to receptors on cell membrane and activates adenylate cyclase. In the presence of high plasma level of insulin and glucose, HSL is dephosphorylated, and become inactive. So during fasting → stimulation of lipolysis.
- Coffee contains caffeine and tea contains theophylline. Both inhibit phosphodiesterase enzyme → stimulation of lipolysis.

Causes of excessive lipolysis: where there is a need for energy;

starvation, diabetes mellitus, low carbohydrate diet, and in certain infectious disease as in tuberculosis (due to high catabolic state).

Types of fatty acid oxidation

- Fatty acids can be oxidized by:

1- β- oxidation- major mechanism, occurs in the mitochondrial matrix. 2-C units are released as acetyl CoA per cycle.

2- α- oxidation- predominantly takes place in brain and liver, one carbon is lost in the form of CO_2 per cycle.

3- ω- oxidation- minor mechanism, but becomes important in conditions of impaired β-oxidation

4- Peroxisomal oxidation- mainly for the trimming of very long chain fatty acids.

Fatty acid Oxidation

Oxidation of fatty acids occurs in the mitochondria.

-The CoA derivatives of long chain F.A. can not penetrate the inner mitochondrial membrane (short chain F.A.s & their acyl CoA can penetrate).

- The transport of fatty acyl-CoA into the mitochondria is accomplished via an acyl – carnitine intermediate

$$\text{Fatty acid} + \text{ATP} + \text{CoA} \xrightarrow{\text{thiokinase}} \text{Acyl-CoA} + PP_i + \text{AMP}$$

- Fatty acids results from TAG hydrolysis in adipose tissue are taken up by most tissues and must be activated in the cytoplasm before being oxidized in the mitochondria.

- Activation is catalyzed by fatty acyl-CoA synthetase or (thiokinase).

Long chain F.A.s transport

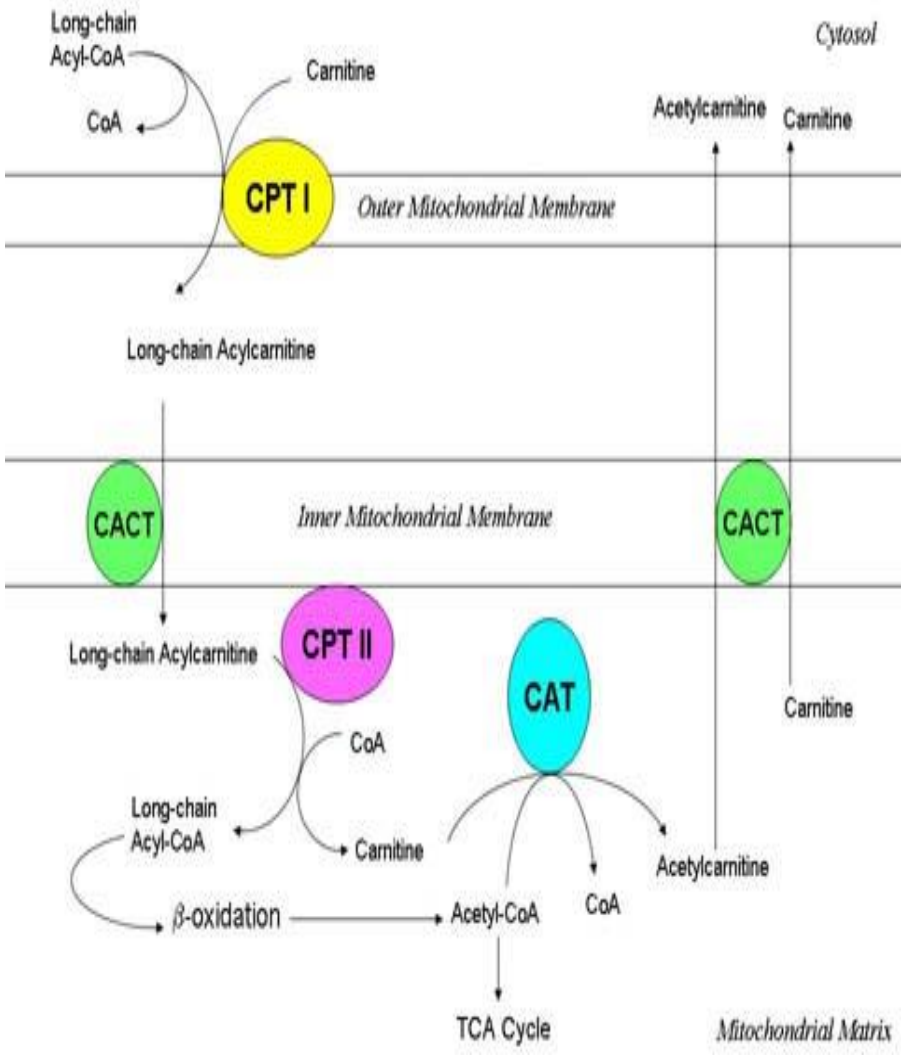
Carnitine:

It is β- hydroxy - γ- trimethylamino butyric acid

-The enzyme carnitine-palmitoyl transferase I transfers the acyl radical from the acyl-CoA to the hydroxyl group of carnitine, forming acylcarnitine

- It can cross the inner mitochondrial membrane in exchange with carnitine.
- Acyl carnitine transported to the inner mitochondrial membrane is accomplished by carnitine acyl-carnitine translocase.
- In mitochondria carnitine is regenerated by carnitine palmitoyl transferase II enzyme, and the active acyl CoA is now ready for oxidation and energy production.

Carnitine Shuttle



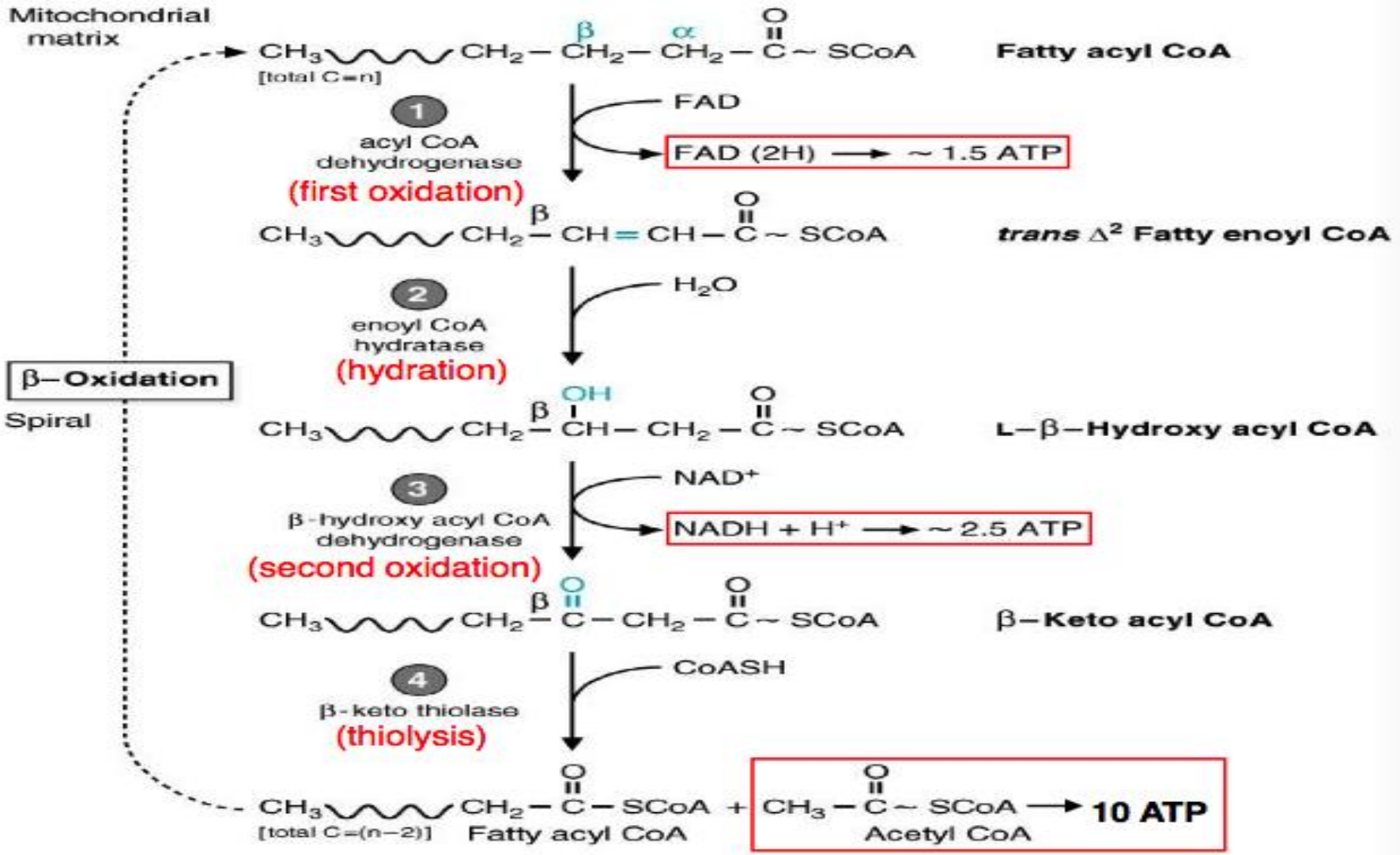
β –Oxidation

-In the mitochondrial matrix there is a group of enzymes called **F.A.s oxidase** that are responsible for F.A.s oxidation.

- The process is multi-cyclic, in each cycle 2 carbons as active acetate are removed.
- Also, two reduced coenzymes are produced ($FADH_2$ and $NADH+H^+$).
- Active acetate is oxidized in TCA cycle, producing also, reduced coenzymes.

-The net reduced coenzymes are oxidized via ETC for ATP production.

- β-oxidation occurs in many tissues except brain where F.A.s can not be uptaken by brain tissues and RBCs (because no mitochondria).
- It is termed β -oxidation since, it occurs through the sequential removal of 2-carbon units by oxidation at the β -carbon position of the fatty acyl-CoA molecule.



Energy production of β-oxidation

-Calculation formula of energy production for fatty acid oxidation :

$= \{(N/2 - 1) \times 4 \text{ ATP}\} + (N/2 \times 10 \text{ ATP}) - 2 \text{ ATP}$

where N represents the number of carbon atoms of fatty acid.

Regulation of fatty acid oxidation:

Through energy production: ↑ATP → inhibit ETC → inhibit β-oxidation.

Importance of β-oxidation:

1- Energy production.

2- Production of acetyl CoA which enters in many pathways

3- Ketone body formation : Acetoacetyl CoA is the last 4 carbon atoms in the course of β-oxidation, it may be converted into acetoacetate; one of ketone bodies.

Disorders associated with impaired β-oxidation

1- Carnitine deficiency: It leads to accumulation of toxic amounts of free fatty acids and branched-chain acyl groups

- It occurs in patients with:

1- liver disease.

2- malnutrition.

3- In those with increased requirement of carnitine as sever infection and burns.

4- During hemodialysis which removes carnitine from blood.

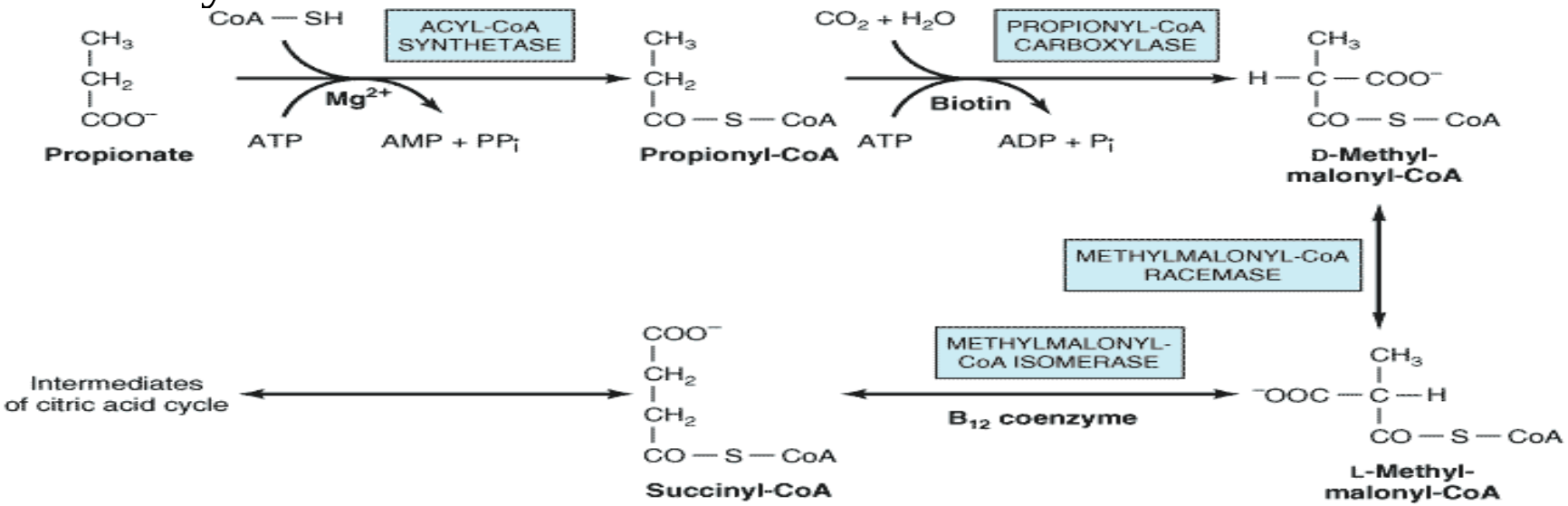
-Symptoms include muscle cramps during exercise, severe weakness

- Muscle weakness related to importance of fatty acids as long term energy source
- Hypoglycemia and hypo ketosis are common findings
- Diet containing medium chain fatty acids is recommended since they do not require carnitine shuttle to enter mitochondria.

β- oxidation of odd chain fatty acids

-Fatty acids with an odd number of carbon atoms are oxidized by the pathway of β-oxidation, producing acetyl-CoA, until a three-carbon (propionyl - CoA) residue remains.

-This compound is converted to Succinyl-CoA, a constituent of the citric acid cycle



-The propionyl residue from an odd-chain fatty acid is the only part of a fatty acid that is glucogenic.

α -Oxidation

-Takes place in the microsomes of brain and liver

-Involves decarboxylation process for the removal of single carbon atom at one time with the resultant production of an odd chain fatty acid that can be subsequently oxidized by beta oxidation for energy production.

- It is strictly an aerobic process.

- No prior activation of the fatty acid is required.

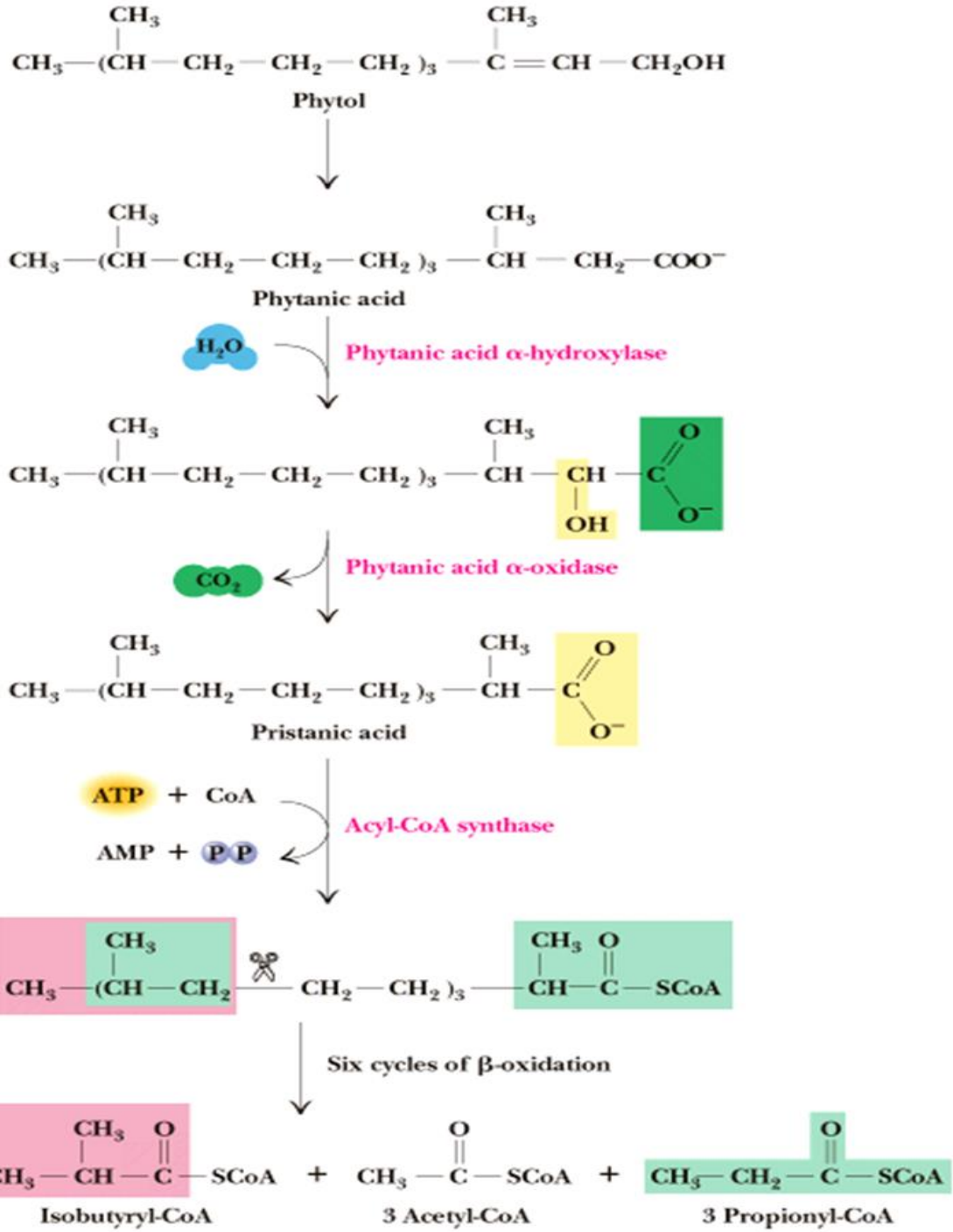
-The process involves hydroxylation of the α-carbon with a specific α-hydroxylase that requires Fe^{++} and vitamin C/FH4 as cofactors.

Biological significance of α- oxidation

-α- Oxidation is most suited for the oxidation of phytanic acid, produced from dietary phytol, a constituent of chlorophyll of plants.

- **Phytanic acid** is a significant constituent of milk lipids and animal fats.

- Normally it is metabolized by an initial **α- hydroxylation followed by dehydrogenation and decarboxylation.**


Phytol
Phytanic acid
H_2O
Phytanic acid α-hydroxylase
CO_2
Phytanic acid α-oxidase
Pristanic acid
ATP + CoA
AMP + PP
Acyl-CoA synthase
Six cycles of β-oxidation
Isobutyryl-CoA
3 Acetyl-CoA
3 Propionyl-CoA

- α-oxidation can not occur initially because of the presence of 3- methyl groups, but it can proceed after decarboxylation.
-The whole reaction produces three molecules of propionyl CoA, three molecules of Acetyl co A, and one molecule of iso butyryl Co A .

-Phytanic acid is oxidized by phytanic acid α oxidase (α- hydroxylase enzyme) to yield CO2 and odd chain fatty acid

Clinical significance of α- oxidation

-**Refsum's disease (RD)-**is a neurocutaneous syndrome that is characterized biochemically by the accumulation of phytanic acid in plasma and tissues.
- Patients with **Refsum's** disease are unable to degrade phytanic acid because of a deficient activity of phytanic acid oxidase enzyme catalyzing the first step of phytanic acid α-oxidation.
- **Peripheral polyneuropathy, cerebellar ataxia, retinitis pigmentosa, and Ichthyosis (rough, dry and scaly skin) are the major clinical components**.

Zellweger's (cerebrohepatorenal) syndrome: Due to rare inherited absence of peroxisomes in all tissues.

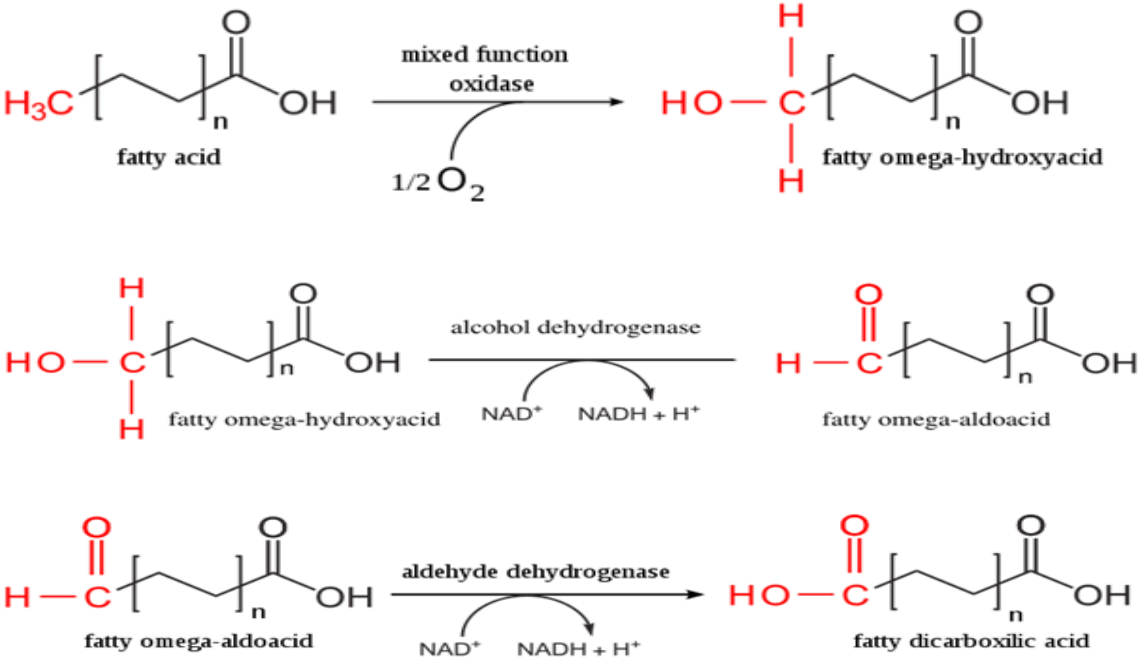
-They accumulate C26-C38 polynoic acids in brain tissue owing to inability to oxidize long-chain fatty acids in peroxisomes.

ω -Oxidation

- Involves **hydroxylation** and occurs in the endoplasmic reticulum of many tissues.
- **Hydroxylation takes place on the methyl carbon** at the other end of the molecule from the carboxyl group or on the carbon next to the methyl end.
- It uses **the "mixed function oxidase" type of reaction requiring Cytochrome P450, O2 and NADPH,** as well as the necessary enzymes.
- Hydroxy fatty acids can be further oxidized to a dicarboxylic acid via **sequential reactions of Alcohol dehydrogenase and aldehyde dehydrogenases.**
- The process occurs **primarily with medium chain fatty acids.**

-Dicarboxylic acids so formed can undergo β- oxidation to produce shorter chain dicarboxylic acids such as adipic acids (C6) and succinic acid (C4).

- The microsomal (endoplasmic reticulum, ER) pathway of fatty acid ω-oxidation represents a minor pathway of overall fatty acid oxidation.
- However, in certain pathophysiological states, such as **diabetes, chronic alcohol consumption, and starvation,** the ω-oxidation pathway may provide an effective means for the elimination of toxic levels of free fatty acids.



Fatty acids are synthesized and degraded by different pathways

Degradation (β-Oxidation)	Synthesis
1- In the mitochondria matrix	- In the cytosol
2- Intermediates are linked to CoA	- Intermediates are linked to an acyl carrier protein (ACP) complex
3- No linkage of the enzymes involved	- Enzymes are joined in one polypeptide chain -> FA synthase
4- The oxidants are NAD^+ and FAD	- The reductant is NADPH
5- Degradation by C_2 units -> Acetyl-CoA	- Elongation by addition of malonyl ACP + release of CO_2
	- Synthesis stops at palmitate (C16), additional enzymes necessary for further elongation