Lipid metabolism lecture 2 of 3

Lipolysis, fatty acid oxidation and ketone bodies

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Lipids metabolism	1. Fatty acids metabolism a. Fatty acid synthesis b. Fatty acid catabolism 2. Cholesterol synthesis 3. Eicosanoids synthesis from fatty acids

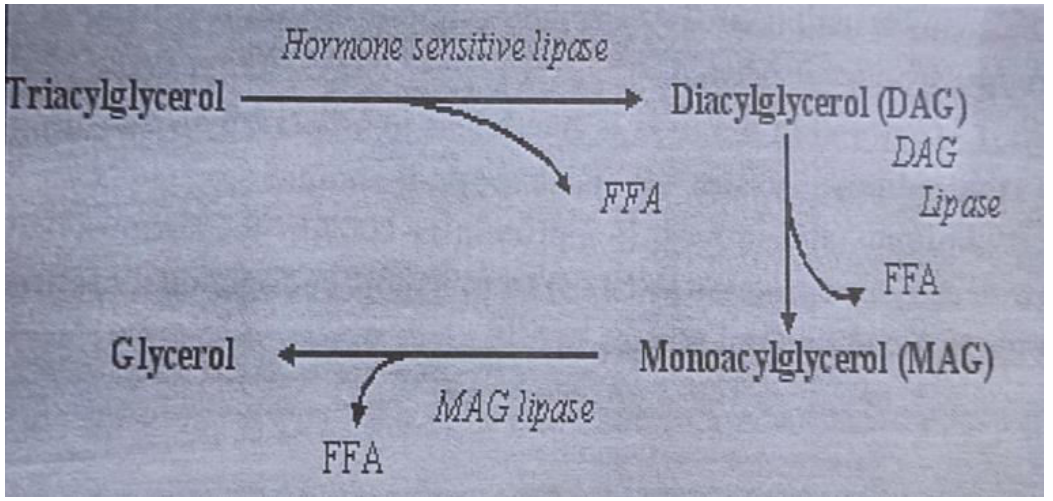
Adipose tissues and energy stores

- Types of adipose tissue
    - *White adipose tissue:* mainly concerned with energy storage
        - Has very few mitochondria
        - TAG makes 80% of it
    - *Brown adipose tissue:* involved in thermogenesis
        - Numerous mitochondria, cytochromes → brown colour
        - Important in new-borns and hibernating animals

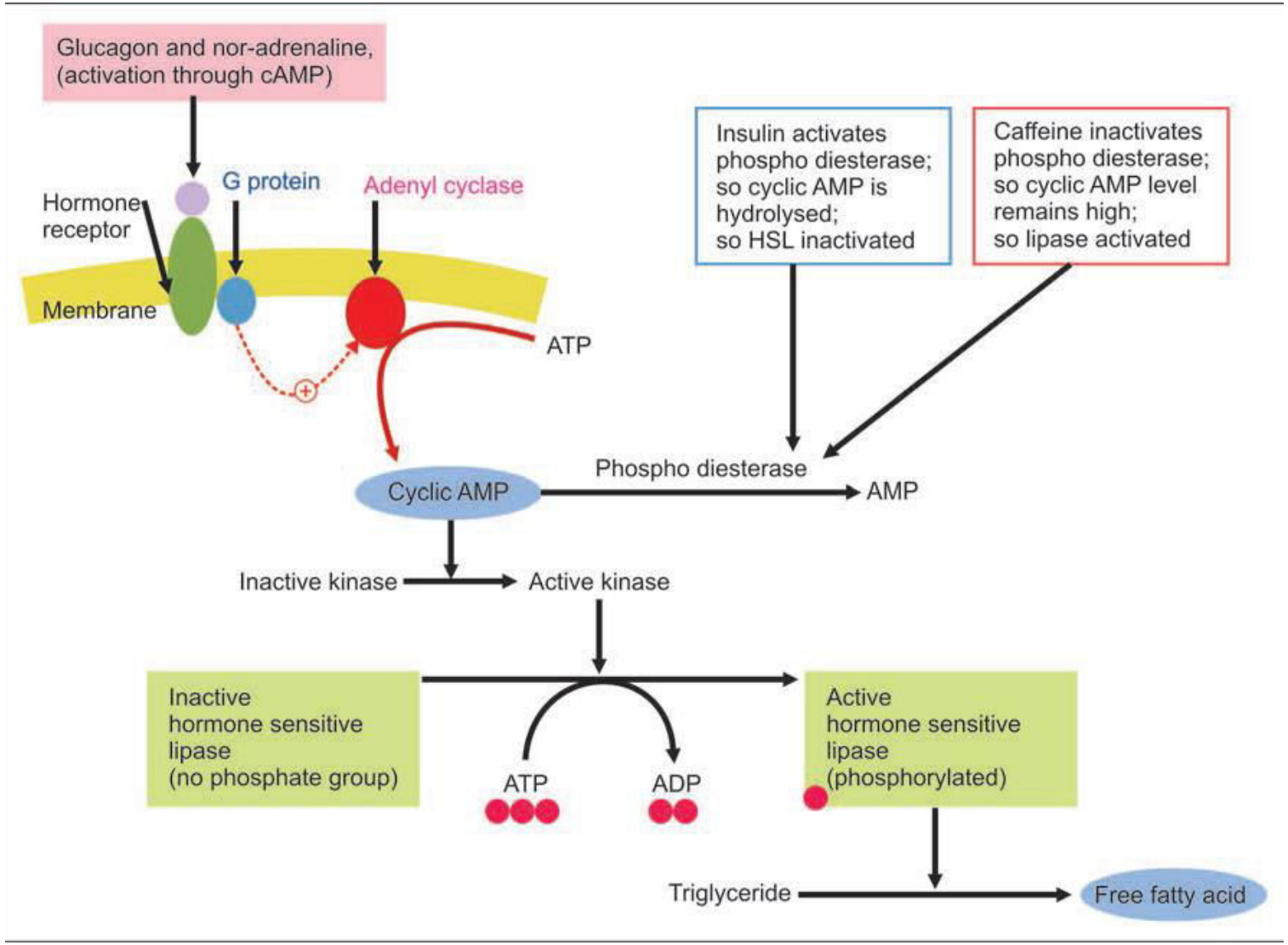
- Thermogenesis
    - Process in which heat is liberated by uncoupling oxidation from phosphorylation → energy is released as heat
        - Occurs due to presences of uncoupling protein (thermogenin)

Process of mobilization of stored fats

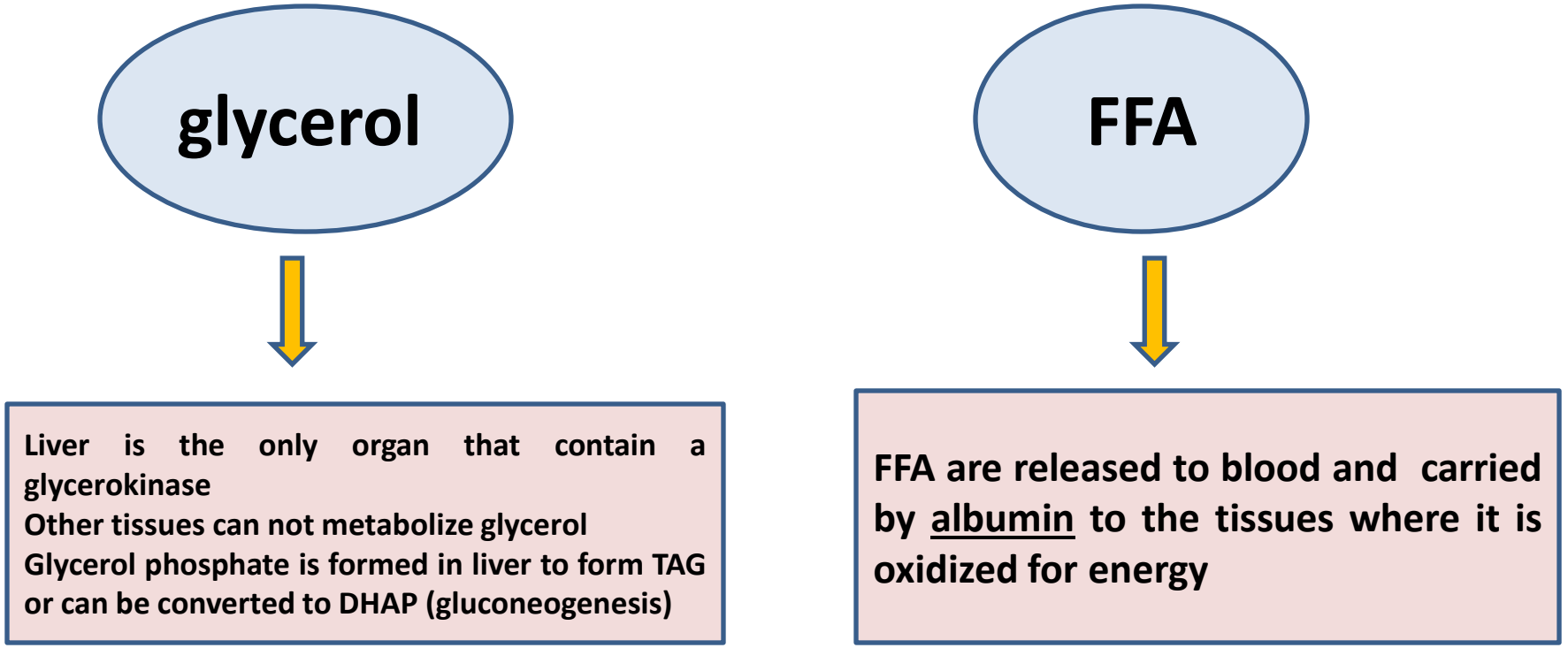
- **Lipolysis:** process of appearance of FAs in blood during fasting is due to mobilization of fat stores
- This is via hydrolytic release of FAs from glycerol in TAG
- Initiated by hormone sensitive lipase (removes FAs from carbon 1 and/or carbon 3 of TAG)
- Additional lipases remove the remaining FAs from diacylglycerol or monoacylglycerol



Regulation of hormone sensitive lipase



- Fatty acids are stored in adipose tissue as TAG
- TAG are the major fuel storage reserve.
- Lipolysis is the hydrolysis of stored TAG in adipose tissue into glycerol and FA



Fatty acids oxidation

- The main pathway for FA oxidation is present in the **mitochondria** and known as **β-oxidation**

- **Other specified pathways are:**
  - α-oxidation of FA
  - ω-oxidation

β-oxidation of fatty acids

- **Site:**

All cells containing mitochondria

- **Steps:**

  Several enzymes, known collectively as **“FA oxidase”** are found in the mitochondrial matrix adjacent to citric acid cycle

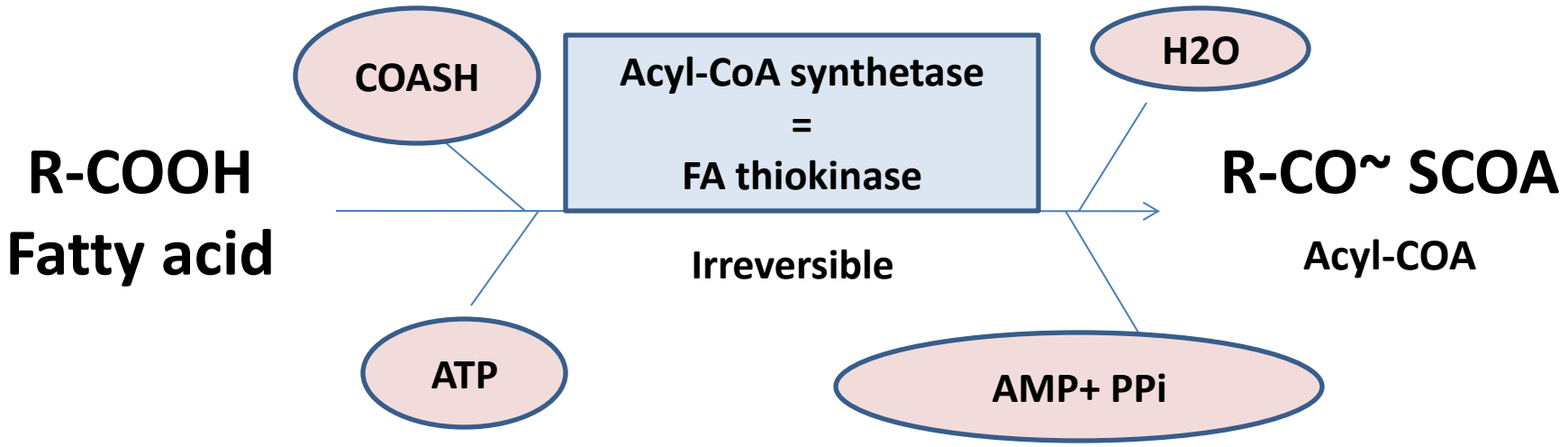
Steps:

1- Activation of FA to acyl-CoA

2-Transport of acyl-CoA through mitochondrial membrane by the **carnitine shuttle**

3-Oxidation of acyl-CoA inside the mitochondrial matrix

1- Activation of FA



Coenzyme required: CoASH

Energy required:

ATP which converted into AMP & PPi (pyrophosphate)

The PPi is hydrolyzed by inorganic pyrophosphatase with the loss of further high-energy phosphates

So, **the total loss, two "high" energy phosphates**.

Fate of activated FAs

- If energy charge of cell is low
    - Activated acyl coA will be moved to mitochondrial matrix by carnitine shuttle → **FA oxidation**

- If energy charge of cell is high
    - FA synthesis is favoured → movement of activated acyl coA is inhibited and it is used for TAG or membrane lipid synthesis in cytosol

2- Transport of acyl-CoA through the inner mitochondrial membrane

- After activation of FA to fatty acyl-CoA,:
  - short & medium chain FA (shorted then 12C) can penetrate the inner mitochondrial membrane for oxidation
  - Transport of long chain acyl-CoA **requires** the presence of **carnitine**. They are transported through the membrane as **acyl-carnitine**.

Carnitine (β-hydroxy-γ-trimethylammoniumbutyrate), $\{(CH_3)_3N^+-CH_2-CH\ (OH) - CH2-COO\}$ - is present in all tissues & in excess in muscle

- **Carnitine acyl (palmitoyl)transferase-1 (CAT-1 or CPT-1),** present in the outer mitochondrial membrane, converts the long chain acyl-CoA to **acylcarnitine**

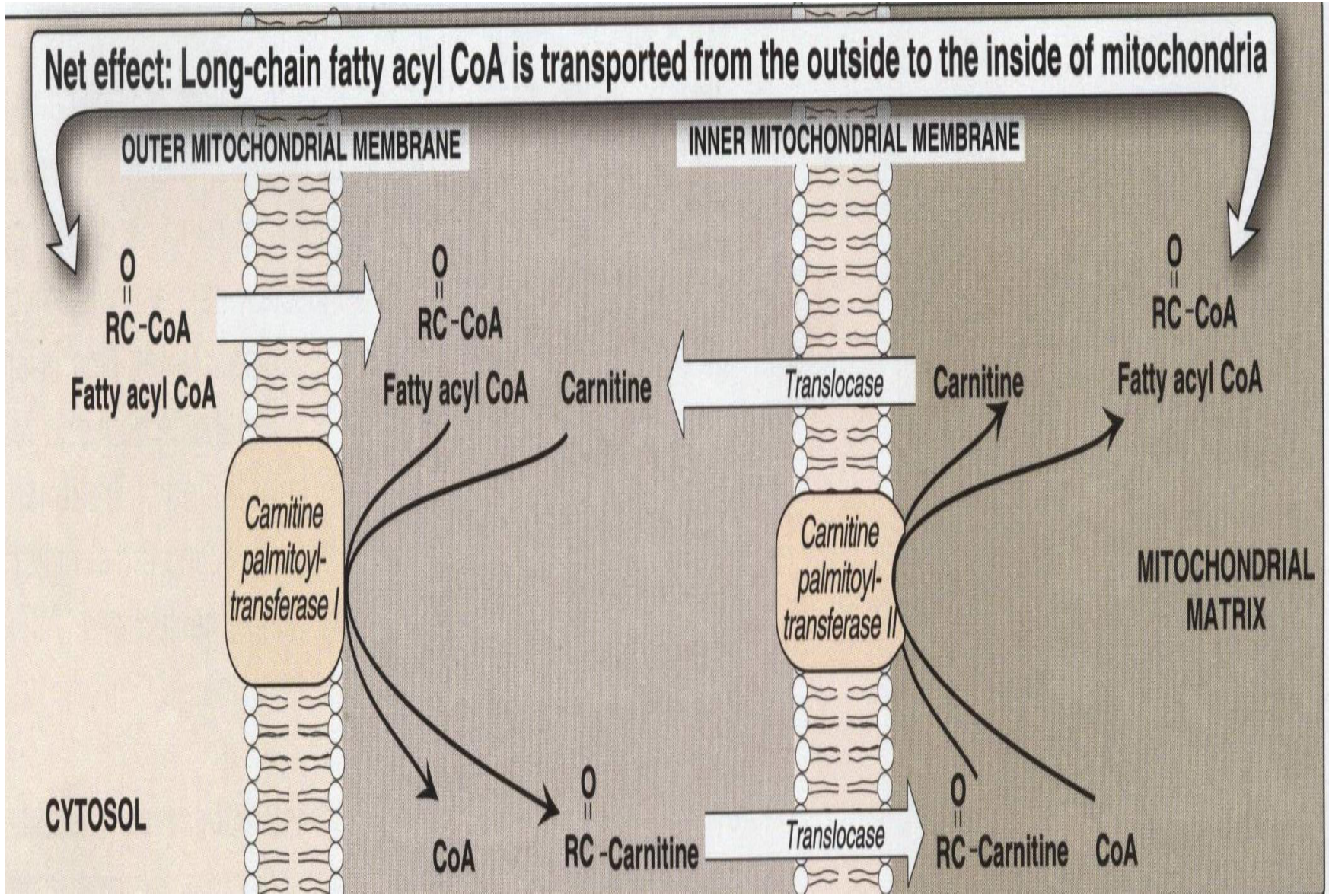
- Acylcarnitine is able to penetrate the inner membrane and gain access to the **β** –oxidation

- Carnitine-acylcarnitine translocase acts as an inner membrane exchange transporter

- Acylcarnitine is transported in exchange with Carnitine

- Acylcarnitine then reacts with CoA, catalyzed by **carnitine acyl (palmitoyl)transferase-2 (CAT-2 or CPT-2),** and located on the inside of the inner membrane

- Acyl-CoA is reformed in the mitochondrial matrix (mitosome) and carnitine is liberated


Net effect: Long-chain fatty acyl CoA is transported from the outside to the inside of mitochondria
OUTER MITOCHONDRIAL MEMBRANE
INNER MITOCHONDRIAL MEMBRANE
O
RC-CoA
Fatty acyl CoA
O
RC-CoA
Fatty acyl CoA
Carnitine
Translocase
Carnitine
O
RC-CoA
Fatty acyl CoA
Carnitine palmitoyl-transferase I
Carnitine palmitoyl-transferase II
MITOCHONDRIAL MATRIX
CYTOSOL
CoA
O
RC-Carnitine
Translocase
O
RC-Carnitine
CoA

Info about carnitine shuttle

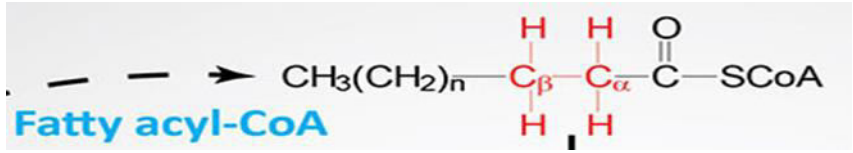
- Carnitine is primarily found in meat
- It can also be synthesized from amino acids lysine and methionine
  - Happens in liver and kidney
  - Does not happen in skeletal muscles or heart (totally dependent on exogenous carnitine or that distributed in blood)
- **Malonyl coA** inhibits CAT-1 preventing entry of long chain acyl groups from entering inner mitochondrial membrane → turn FA oxidation off
- Short and medium chain FA can cross inner mitochondrial membrane **without** shuttle
  - Their oxidation is not dependent on carnitine or inhibited by malonyl coA

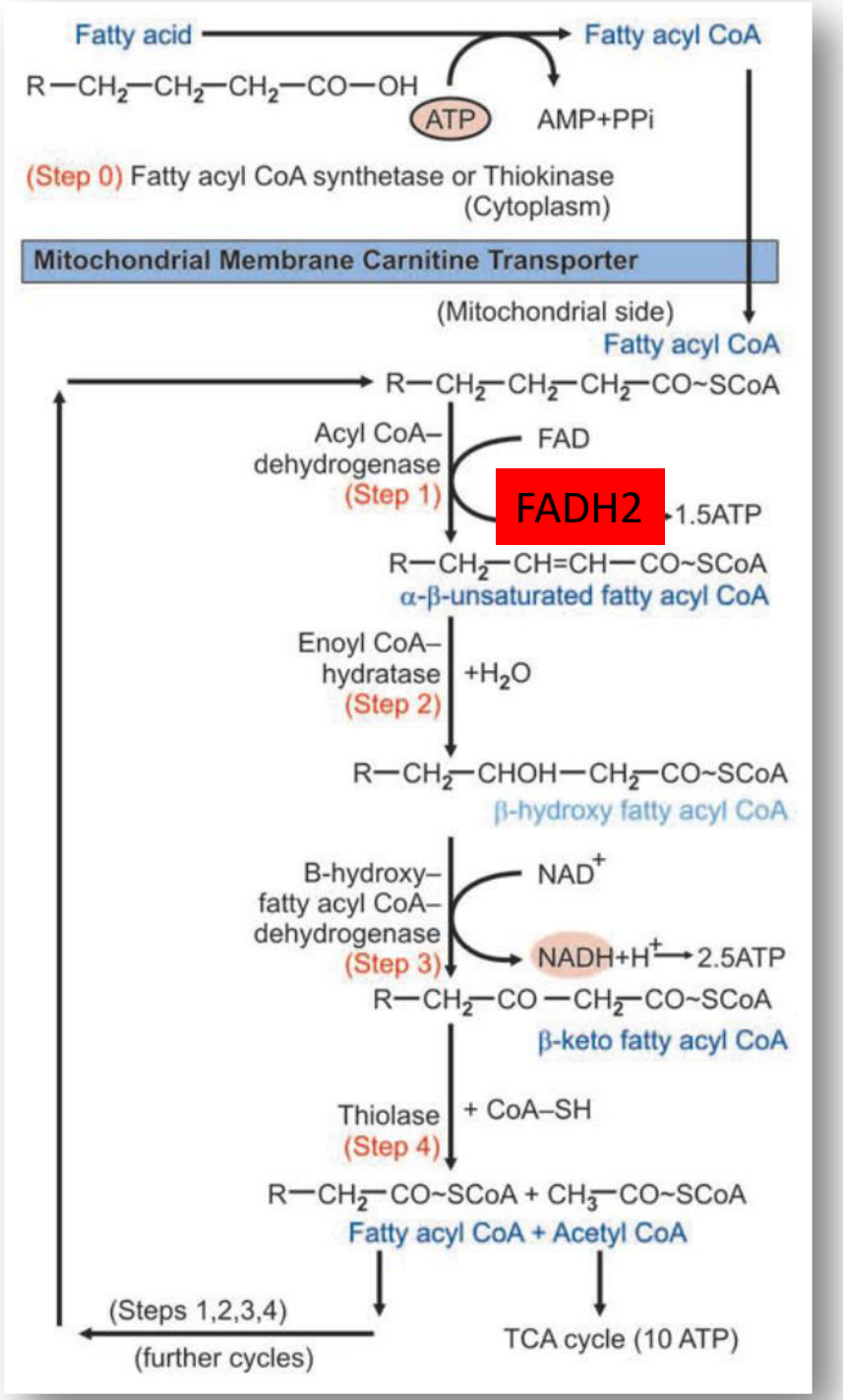
3-Oxidation of acyl-CoA

- The process is multi-cyclic
  - each cycle catalyzes removal of two carbons (from carboxyl end of acyl coA) as **active acetate (acetyl coA)**
    - & two reduced coenzymes are formed (**FADH2 & $NADH+H^+$**)

- Active acetate are oxidized in citric acid cycle to 2 CO2

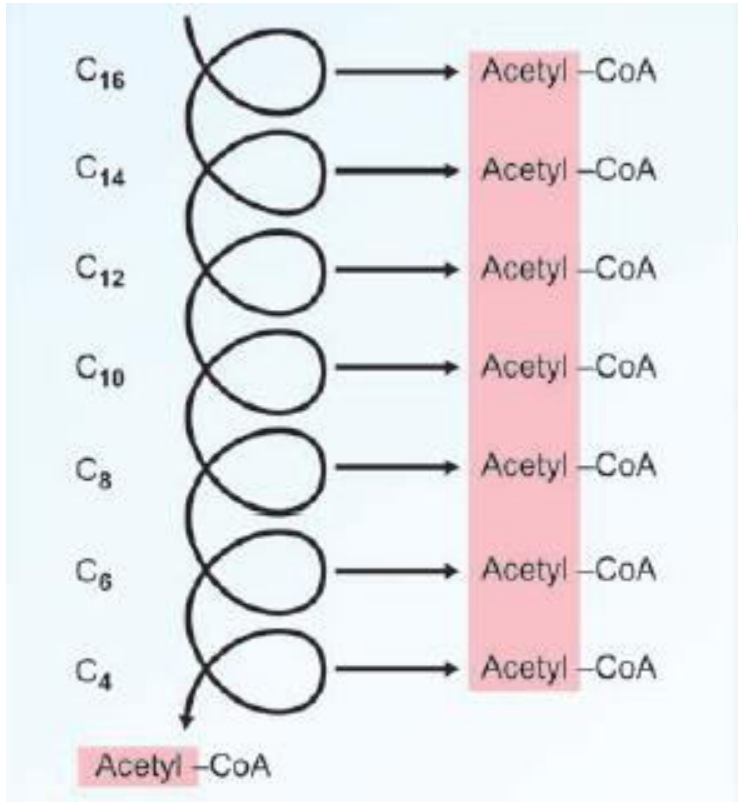
- Reduced coenzymes produced by **β-oxidation and citric acid cycle are oxidized by electron transport chain (ETC) for synthesis of ATP**


CH3(CH2)n
Cβ
Cα
SCoA
Fatty acyl-CoA


Fatty acid
Fatty acyl CoA
R—CH2—CH2—CH2—CO—OH
ATP
AMP+PPi
(Step 0) Fatty acyl CoA synthetase or Thiokinase
(Cytoplasm)
Mitochondrial Membrane Carnitine Transporter
(Mitochondrial side)
Fatty acyl CoA
R—CH2—CH2—CH2—CO~SCoA
Acyl CoA–
dehydrogenase
(Step 1)
FAD
FADH2
1.5ATP
R—CH2—CH=CH—CO~SCoA
α-β-unsaturated fatty acyl CoA
Enoyl CoA–
hydratase
(Step 2)
+H2O
R—CH2—CHOH—CH2—CO~SCoA
β-hydroxy fatty acyl CoA
B-hydroxy–
fatty acyl CoA–
dehydrogenase
(Step 3)
NAD+
NADH+H+
2.5ATP
R—CH2—CO—CH2—CO~SCoA
β-keto fatty acyl CoA
Thiolase
(Step 4)
+ CoA–SH
R—CH2—CO~SCoA + CH3—CO~SCoA
Fatty acyl CoA + Acetyl CoA
(Steps 1,2,3,4)
(further cycles)
TCA cycle (10 ATP)

When one molecule of palmitate undergoes beta-oxidation, the net reaction is:

Palmitoyl CoA		8 Acetyl CoA
+ 7FAD		+ $7FADH_2$
+ $7NAD^+$	→	+ 7NADH
+ $7H_2O$		+ $7H^+$
+ 7HSCoA		



Calculation of energy yielded from complete oxidation of FA (e.g. palmitic acid):

- Palmitic acid is C16, saturated FA
- Palmitic acid is activated to palmityl-CoA = (-2 ATP).
- Complete oxidation of palmityl-CoA gives **8** mol of acetyl-CoA (16/2 = 8) through **7** β oxidation cycles.
- Each turn of β oxidation gives FADH2 & NADH+H+ which by respiratory chain give 5 ATP (old system), 4 (new system)
- So 7 cycles x 5 ATP = 35 ATP (old) or
- 7 cycles x 4 ATP = 28 ATP (new)

- Each acetyl-CoA by citric acid cycle gives **12** ATP (old system),
- so 8 acetyl-CoA x 12 ATP= **96** ATP (old system)
- The total gain : 96 + 35 = 131 ATP (old system)
- The net gain : 131- 2 = **129** ATP (old system)

Exam question: what is total net energy of complete oxidation of a fatty acid with 18 carbons (for example)

New system

8 acetyl CoA × 10	=	80 ATP
7 $FADH_2$ × 1.5	=	10.5 ATP
7 NADH × 2.5	=	17.5 ATP
Gross total	=	108 ATP
Net yield	**= 108–2 = 106 ATP**	

Importance of β oxidation:

- 1- source of energy during fasting
- 2- source of acetyl-CoA which can be converted to other important compounds as cholesterol and acetyl choline

Regulation of β oxidation:

(1) feeding status:

- Fasting increases lipolysis →release of FFA from adipose tissue → ↑↑ FA in tissues → stimulation of β oxidation

- CHO feeding → ↑ insulin → inhibition of lipolysis in adipose tissue → ↓↓ FFA in tissues → inhibition of β oxidation

Regulation of beta oxidation

- **Rate limiting step** of beta oxidation is formation of fatty acyl carnitine (catalysed by CAT1)
- Malonyl coA (1st intermediate of synthesis of FA) allosterically inhibits CAT1
- **In fed state:**
  - ↑ insulin/glucagon ratio → fatty acid synthesis is promoted in liver (insulin activates acetyl coA carboxylase) → ↑ malonyl coA → inhibition of CAT1 → ↓ beta oxidation
- **In starvation:**
  - ↓ insulin/glucagon ratio → glucagon inhibits acetyl coA carboxylase → ↓ malonyl coA → release inhibition of CAT1 → ↑ beta oxidation
- Hormone sensitive lipase is activated by phosphorylation (glucagon)
  - Its activity is low when insulin levels are high

(2) Energy needs by cells:

- ↑↑ ATP → ↓↓ respiratory chain → FADH2 and NADH+H+ remain reduced → inhibition of DH “dehydrogenases” of β oxidation

- ↓↓ ATP & ↑ ADP and Pi → ↑↑ respiratory chain so, FAD& NAD+ are oxidized → stimulation of DH of β oxidation

Notes:

- Oxidation of FA supplies **NADH** and **ATP** **required for gluconeogenisis** and supplies **excess acetyl CoA.**

- Acetyl CoA allosterically activates pyruvate carboxylase and inhibits pyruvate dehydrogenase. **This directs pyruvate towards gluconeogenesis rather than oxidation.**

- **If FA oxidation is inhibited, gluconeogenesis is inhibited.**

Oxidation of FA with an odd number of C atoms:

- Odd chain FA are oxidized by β oxidation producing acetyl-CoA but only at the last step one **propionyl-CoA** is produced

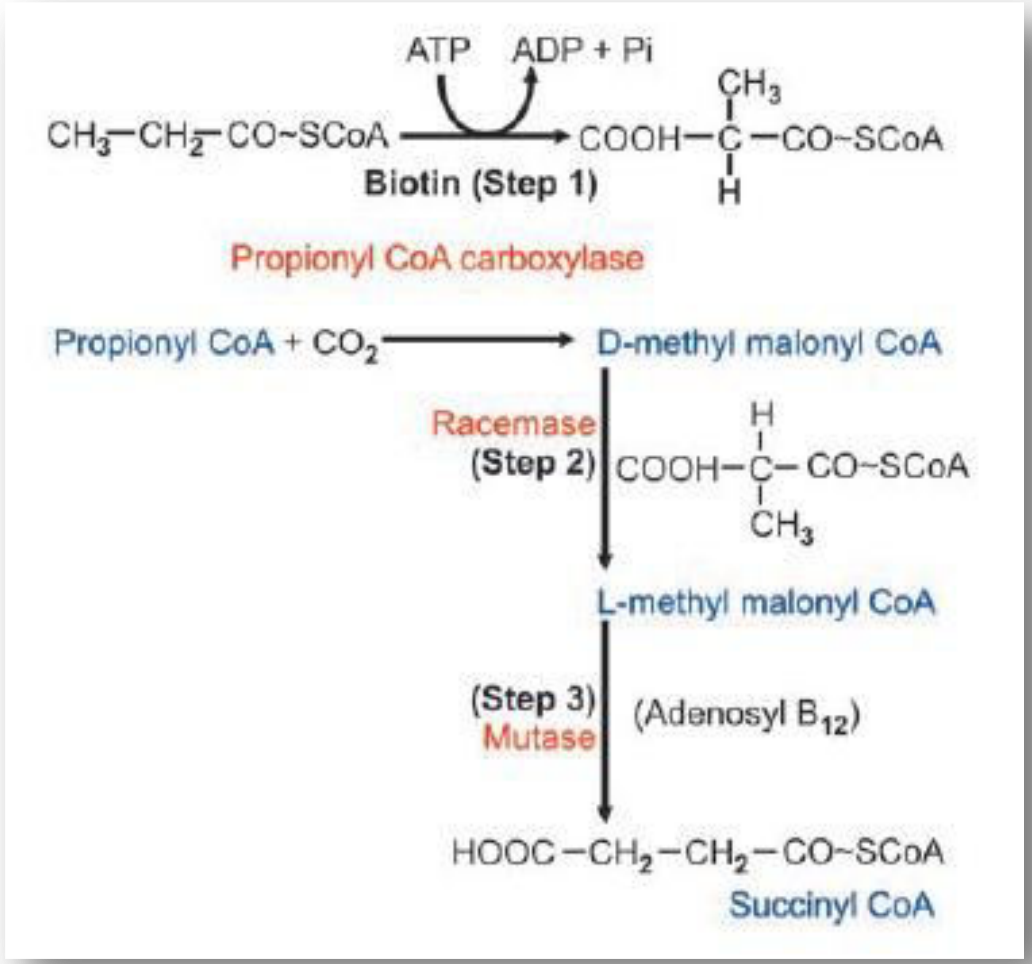
- Propionyl CoA can be converted to methyl malonyl CoA which is converted to succinyl-CoA → citric acid cycle → oxaloacetate → glucose

This is the only mechanism by which Fatty acids are converted to glucose

- 3 C units from odd chain FA are glucogenic

- Cow's milk contains significant quantity of odd chain FAs

Metabolism of propionyl-CoA



Metabolic disorders of FA oxidation:

- These include deficiency of carnitine, CPT1, CPT2 and acyl CoA dehydrogenase
  - → impairment of FA oxidation, fasting hypoglycemia (due to decreased gluconeogenesis as well as increased uptake of glucose by muscles and heart), muscle weakness , and fatty liver, finally produce coma and death

- Patients with deficiency of carnitine, CPT1, or CPT2 should avoid prolonged fasting & may benefit from the ingestion of fats rich in medium chain fatty acids

α-oxidation of FA

- It is a **minor** pathway for the oxidation of FA that have methyl group in the β carbon , e.g. phytanic acid (found in animal and milk fats)
- The site of oxidation is the peroxisome of brain and liver mainly
- **α-oxidation occurs in the α- position because the** β carbon is occupied by a methyl group
- **The α carbon is oxidized and removed as CO2, now the methyl group is at the α position (no energy produced, no coA needed) and the** β carbon is free to undergo β oxidation **forming propionyl-CoA in the last turn**

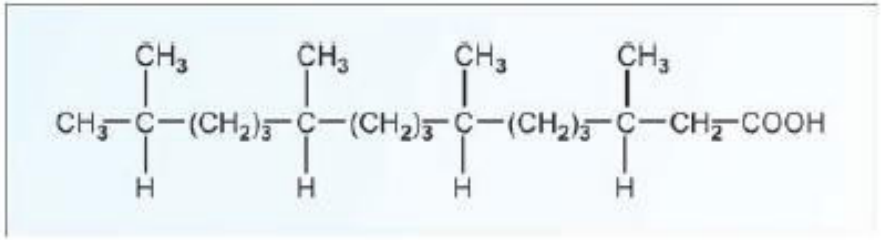
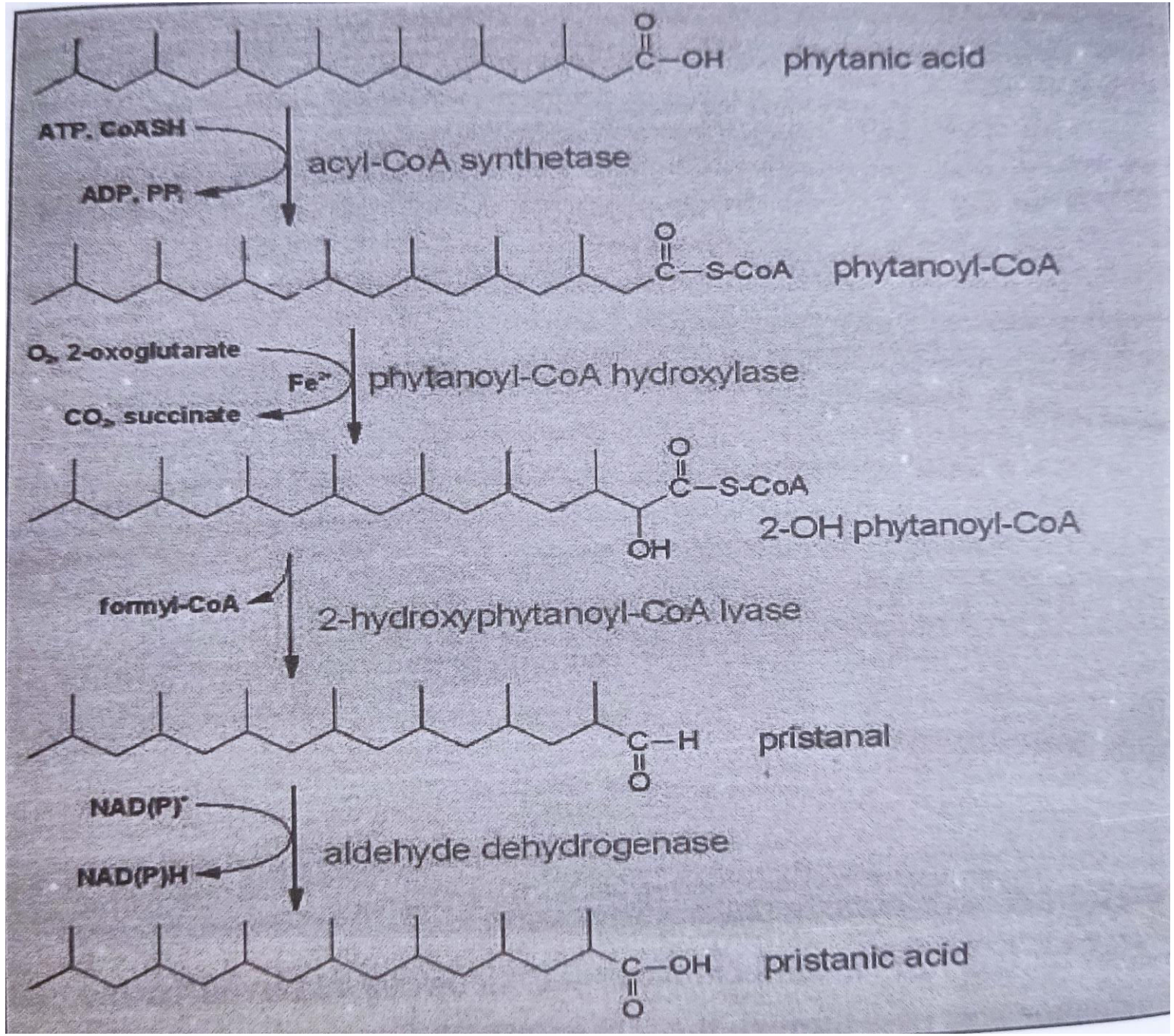


Fig. 11.12. Phytanic acid


phytanic acid
ATP, CoASH
ADP, PPi
acyl-CoA synthetase
phytanoyl-CoA
O2, 2-oxoglutarate
Fe2+
CO2, succinate
phytanoyl-CoA hydroxylase
2-OH phytanoyl-CoA
formyl-CoA
2-hydroxyphytanoyl-CoA lyase
pristanal
NAD(P)+
NAD(P)H
aldehyde dehydrogenase
pristanic acid

*Refsum's disease:

- Rare autosomal recessive disorder
- Defect in alpha oxidation
- Due to congenital deficiency of enzyme system of **α-oxidation leading to accumulation of large amounts of phytanic acid in the brain, liver and blood**
  - **Polyneuropathy, cerebellar ataxia, deafness and blindness occur at young age**

Treatment: dietary restriction to halt disease progression

- ***Ataxia*** *is a neurological sign consisting of lack of voluntary coordination of muscle movements that can include gait abnormality, speech changes, and abnormalities in eye movements. Ataxia is a clinical manifestation indicating dysfunction of the parts of the nervous system that coordinate movement, such as the cerebellum.*

ω-oxidation

- It is a minor pathway for FA oxidation
- Site : in the liver endoplasmic reticulum (involves cytochrome p-450)

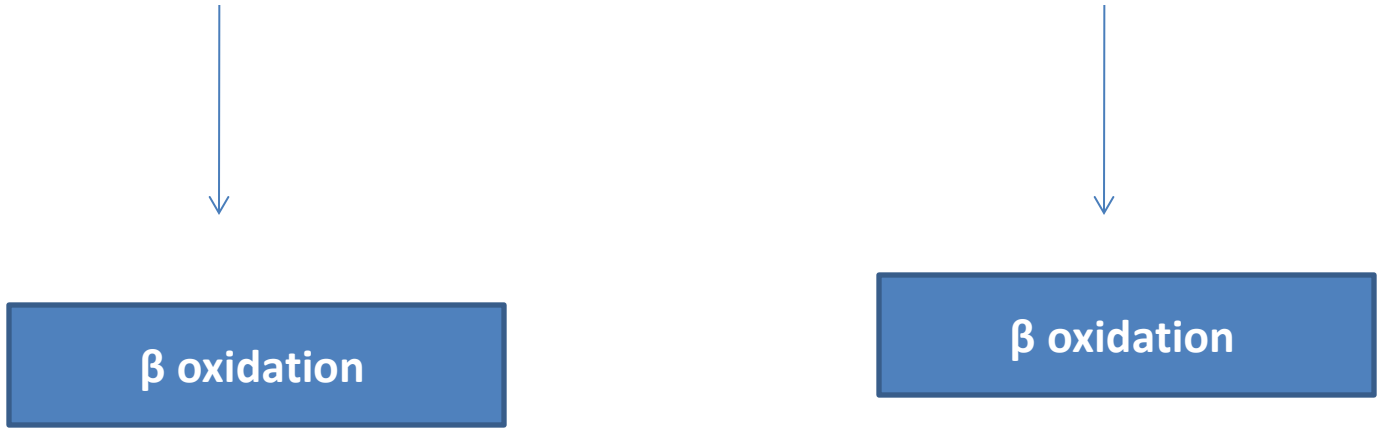
- The oxidation occurs at the terminal methyl group (**ω carbon)→ formation of a dicarboxylic acid**

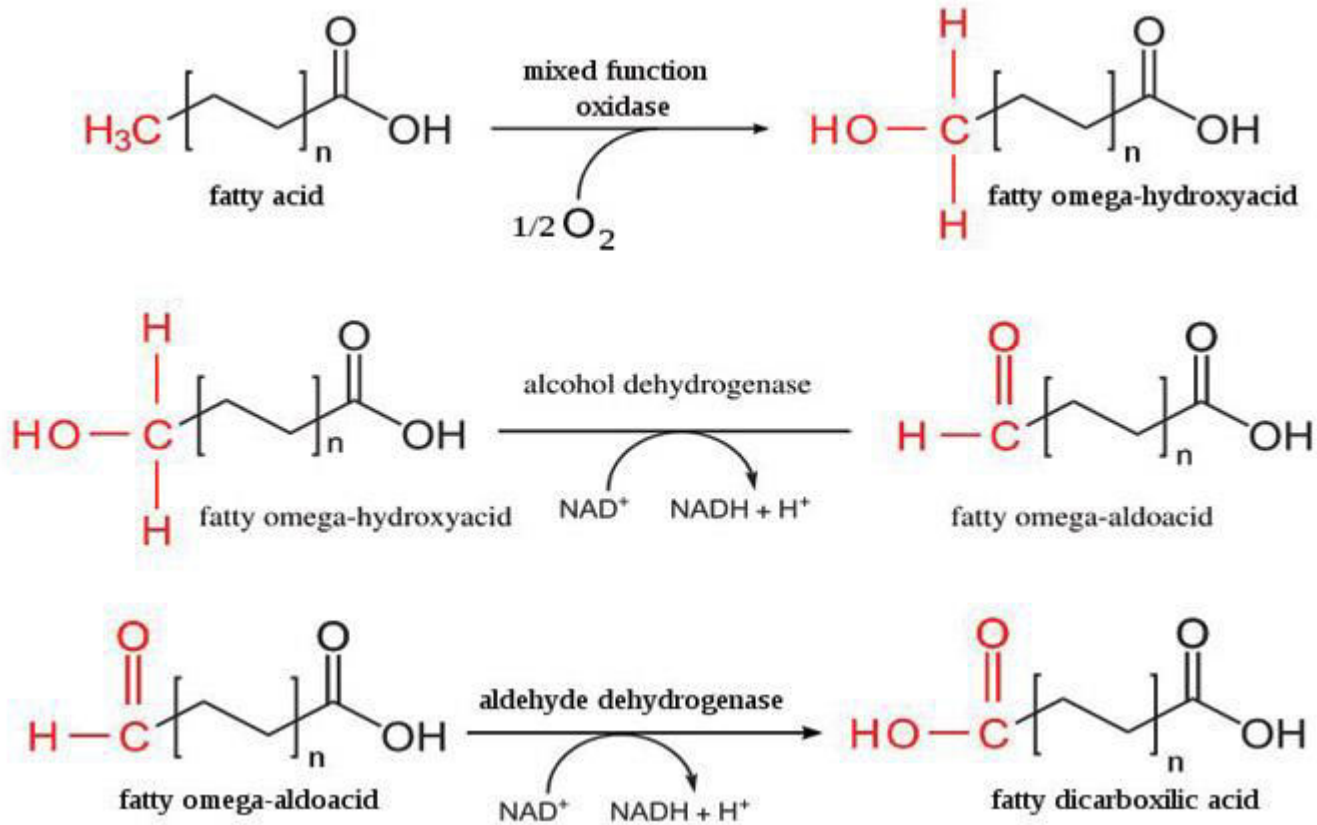
- **The dicarboxylic acid is oxidized from both ends by β oxidation liberating acetyl-CoA**

- **It ends with the formation of adipic acid (C6) which is excreted in urine.**

- It occurs to average chain length FA (10-14 C).
- It produces acetyl-CoA faster.

HOOC-CH2-CH2----------CH2-CH2-COOH





Omega oxidation is upregulated when beta oxidation is defective as is seen with medium-chain acyl-CoA dehydrogenase (MCAD) deficiency

Introduction- ketone bodies

- Acetoacetate, β-hydroxyl butyrate & acetone are collectively called ketone bodies
- **Ketogenesis**: formation of ketone bodies (occurs in liver)
- **Ketolysis**: utilization of ketone bodies as fuel (occurs in extrahepatic tissues)
- Under normal conditions, production of ketone bodies is at relatively low rate
- Increased ketone bodies is known as **ketosis** while high blood level is known as **ketonemia**

Metabolism of ketone bodies

- Fats are burned in the fire of carbohydrates
    - Acetyl coA formed from FAs enters Krebs → oxidised only when oxaloacetate is present (oxaloacetate comes mainly from CHO)

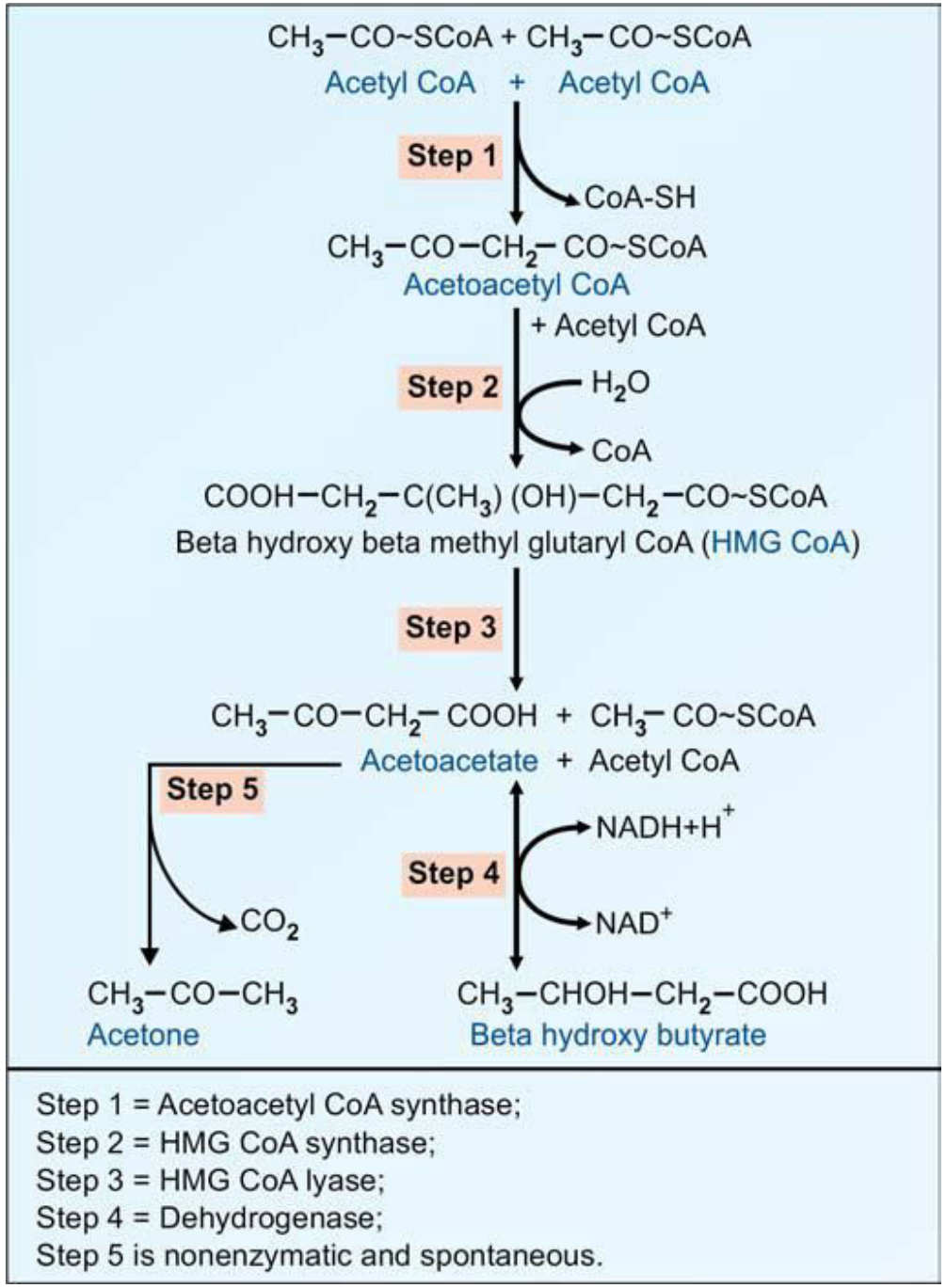
- During starvation and DM, acetyl coA takes the alternate fate of formation of ketone bodies (ketogenesis)
    - This allows **heart** and skeletal muscle (to some extent, increased use in fasting) to use ketone bodies (ketolysis) as major source of energy → preserving limited glucose supply for brain

- Ketone bodies are water soluble
    - Transported across inner mitochondrial membrane, blood brain barrier and cell membranes
        - Used as fuel for a variety of tissues including CNS
        - Preferred substrates for aerobic heart and muscles (to some extent, increased use in fasting)

Ketogenesis

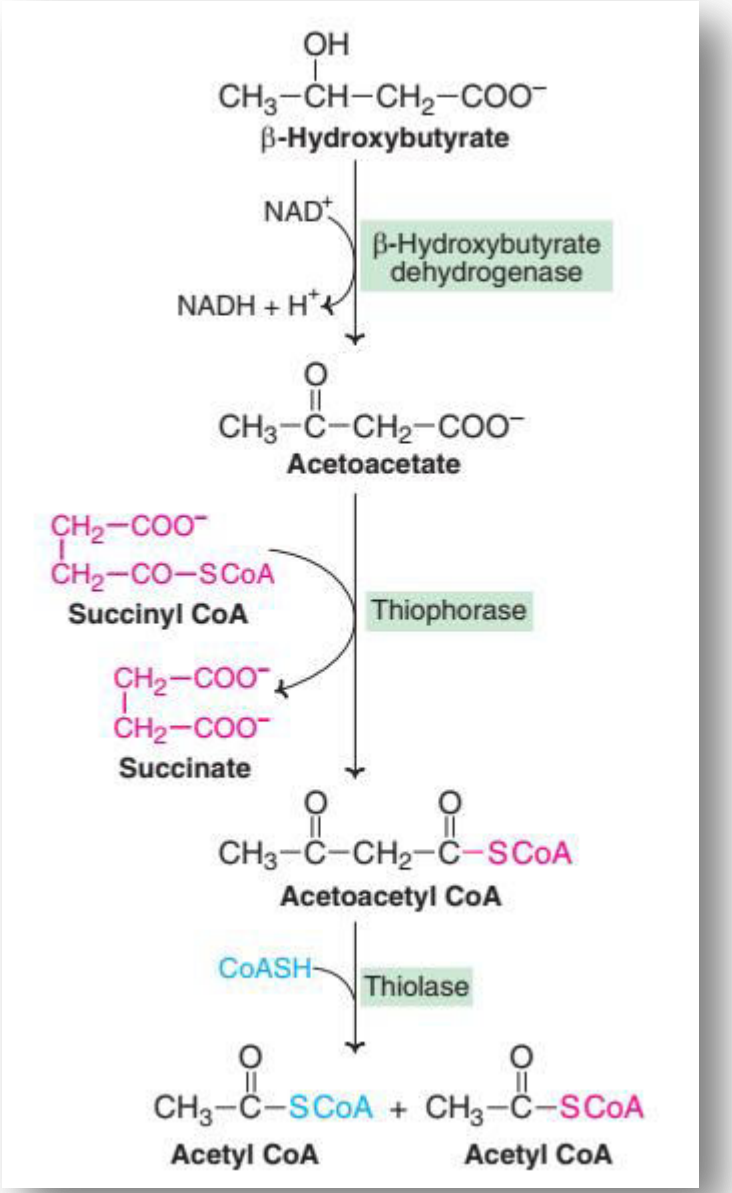
- Acetoacetate is primary ketone body
- Synthesised **exclusively** in liver **mitochondria**
- 4 Steps:
  - Condensation
  - Production of HMG coA
  - Lysis
  - Reduction
  - Spontaneous decarboxylation

HMG coA synthase is rate limiting step in synthesis of ketone bodies and is present in significant quantities only in liver

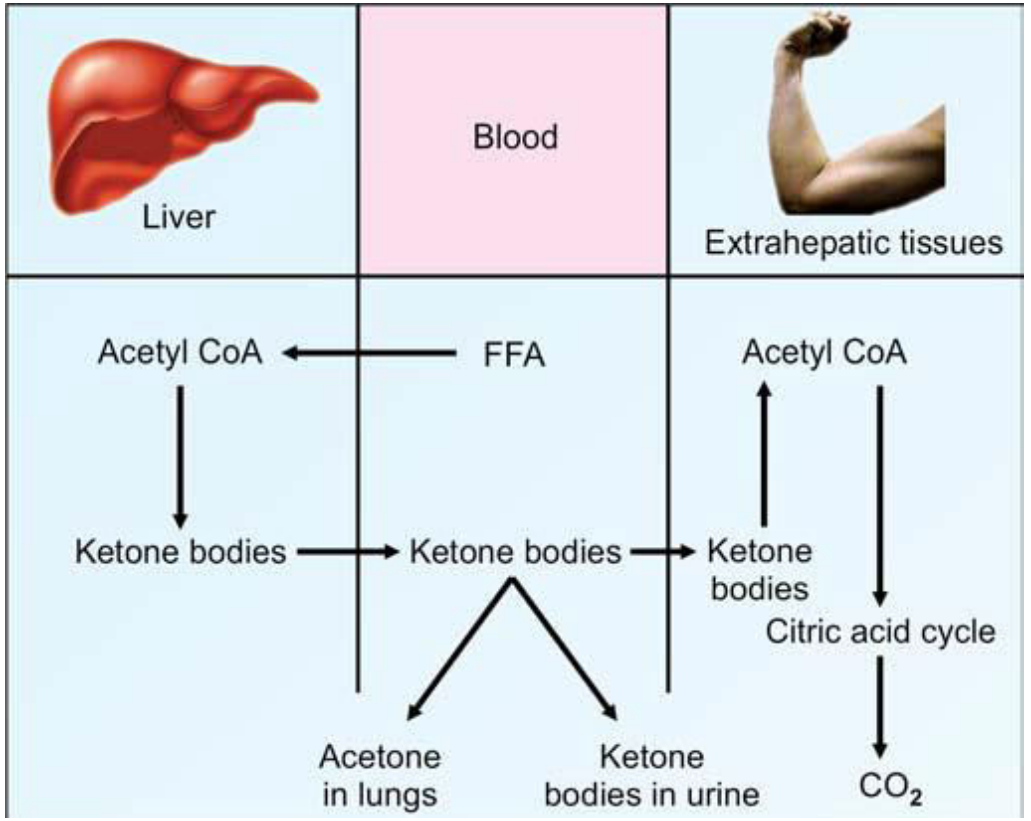


Ketolysis

- Ketone bodies are formed in liver but utilized in extrahepatic tissues
- Heart muscle, renal cortex sometimes prefer ketone bodies to glucose as fuel
- Muscle can also utilize ketone bodies



Fate of ketone bodies



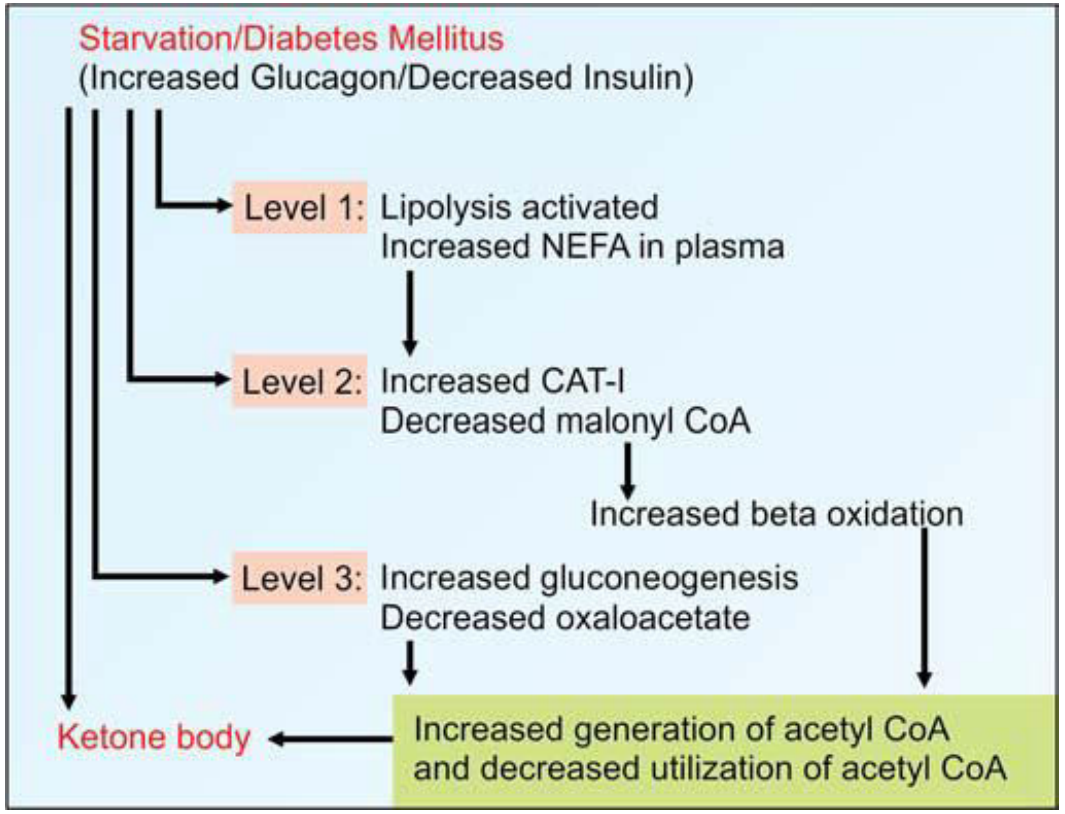
Ketosis

- Causes
  - **Uncontrolled DM:** most common cause of ketosis
    - » Glucose is plenty but **deficiency of insulin** → accelerated lipolysis → increased acetyl coA
      - Enhanced gluconeogenesis restricts oxidation of acetyl coA in TCA as there is less oxaloacetate
  - **Starvation:** dietary supply of glucose reduced → oxaloacetate channelled to gluconeogenesis, increased lipolysis to provide fuel, excess acetyl coA converted to ketone bodies
    - Hyperemesis in pregnancy may also lead to starvation like condition → ketosis

- Explanation of ketogenesis
  - Starvation and DM: glucagon is increased →
    - Inhibits glycolysis
    - Activates gluconeogenesis
    - Activates lipolysis
    - Decreases malonyl coA
    - Stimulates ketogensis (high glucagon/ insulin ratio is ketogenic)

Insulin has opposite effect

Ketosis



Salient Features of Ketosis

1. **Metabolic acidosis.** Acetoacetate and beta-hydroxy butyrate are acids. When they accumulate, metabolic acidosis results. (see Chapter 29).
2. **Reduced buffers.** The plasma bicarbonate is used up for buffering of these acids.
3. **Kussmaul's respiration.** Patients will have typical acidotic breathing (see Chapter 24) due to compensatory hyperventilation.
4. **Smell of acetone** in patient's breath.
5. **Osmotic diuresis** induced by ketonuria may lead to dehydration.
6. **Sodium loss.** The ketone bodies are excreted in urine as their sodium salt, leading to loss of cations from the body.
7. **Dehydration.** The sodium loss further aggravates the dehydration.
8. **Coma.** Hypokalemia, dehydration and acidosis are contributing for the lethal effect of ketosis.