SHEET NO.

Lipid 2

METABOLISM

DOCTOR 2019 | MEDICINE | JU

DONE BY : Doctor 2018

SCIENTIFIC CORRECTION :

GRAMMATICAL CORRECTION :

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➢ Mobilization of Stored Fat

- Fat is stored in adipose tissues in the form of triacylglycerol. 90% of the volume of adipocytes is made up of fat.
- It serves as the body's major fuel storage reserve.
- Adipose tissues store TAG to release fatty acids when energy is required by other tissues.

How?

A hormonal signal reaches the adipocytes, informing them that energy is required by other tissues.

❖ Fatty acid release from fat

- After the hormonal signal is reached, fatty acids are released by the complete hydrolysis of TAG to fatty acids.
- The hydrolysis of TAGs is the first step in the release of fatty acids to other tissues. This reaction occurs in the cytoplasm.
- The hydrolysis reaction is catalyzed by an enzyme called lipase.
- Remember that triacylglycerol is composed of a glycerol molecule bonded with 3 fatty acids. In order to break these bonds, 3 water molecules are added.

Lipases found in the liver are hormone sensitive since they get activated by a hormone that reaches adipocytes.

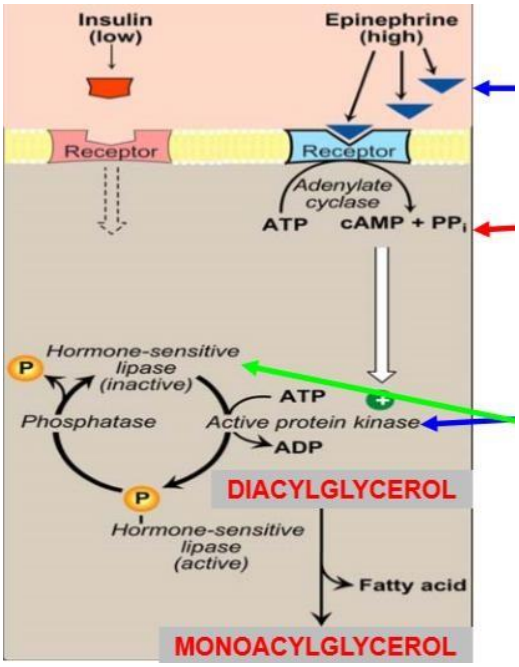
$$TAG + 3H_2O \rightarrow Glycerol + 3\ Fatty\ Acids$$

❖ Hormone-sensitive lipase regulation

- Lipases are activated by certain hormones, such as:
  a) **Glucagon**: it is secreted when blood glucose is low. It stimulates the release of fatty acids from adipose tissues so they can serve as an alternative fuel to glucose.
  b) **Epinephrine/Norepinephrine**: they are secreted during stress when there's an increased demand for energy.
  c) **ACTH (adrenocorticotropic hormone)**: it is a pituitary hormone that activates the adrenal cortex. It is secreted during stress when there's an increased demand for energy.
- In conclusion, lipases are activated by hormones in two cases:
  ✓ When glucose level in the blood is low so an alternative fuel is needed. **(Glucagon)**
  ✓ When there's an increased\ demand for energy. **(Epi/Nor/ACTH)**
- When phosphorylated, hormone-sensitive-lipases are in their active form.

❖ Hormones mechanism of action

➲ Epinephrine:



- When epinephrine is secreted, it binds to receptors found on adipocytes.
- The binding of epinephrine to these receptors leads to the activation of Adenylate Cyclase which catalyzes the production of cAMP.
- cAMP activates protein kinase A.
- Protein kinase A phosphorylates (adds a phosphate group to) lipase, converting it to its active form.

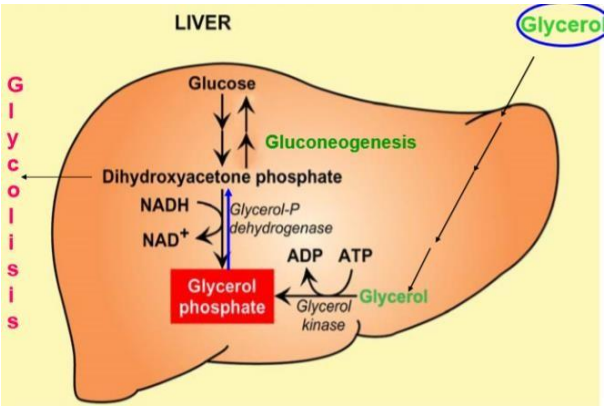
Note! Phosphorylation can be thought of as a mean to decrease the dependence of the body on the utilization of glucose. In another words, it decreases the demand on glucose.

- Lipase catalyzes the hydrolysis of triacylglycerol to diacylglycerol then to monoacylglycerol, releasing a fatty acid each time.
- When hydrolysis is completed, a glycerol molecule and 3 fatty acids are released.

➲ Insulin:

- In the case of high blood glucose, insulin is secreted. Insulin stimulates phosphatase to remove the phosphate group on lipase converting it to its inactive form.
- This is similar to the regulation of degradation of glucose.

❖ Fate of glycerol



- Glycerol is very soluble in water.
- It's transported from adipocytes, through the blood, to the liver to be metabolized there.
- The first step of metabolism of glycerol is **phosphorylation**.
- A phosphate group is added to C3 of glycerol by an enzyme called **glycerol kinase** consuming 1 ATP. The resulting product is called glycerol 3-phosphate.

- Glycerol 3-phosphate gets oxidized at C2 by **glycerol 3-phosphate dehydrogenase** producing dihydroxyacetone phosphate and NADH.
- Dihydroxyacetone phosphate (DHAP) is an intermediate of both glycolysis and gluconeogenesis.

➲ Now the question is, which pathway will DHAP proceed in?

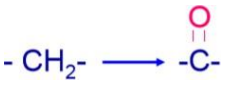
✓ Under the condition of low blood glucose, glucagon is active and DHAP participates in gluconeogenesis to produces glucose.

❖ Fate of fatty acids

- Fatty acids are insoluble in water. They are transported to different tissues while bound to Albumin.
- The major pathway for catabolism of fatty acids is a pathway called β-oxidation which occurs in the mitochondria.
- In β-oxidation, degradation of fatty acids is achieved by oxidation of β-carbon (Carbon 3) followed by cleavage of two-carbon units.

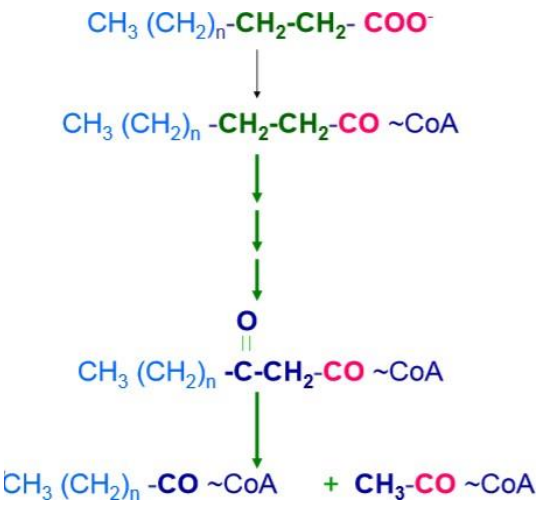
❖ β-oxidation (Overview)

✓ Oxidation of β-carbon involves removal of two hydrogen atoms and introduction of one oxygen atom.



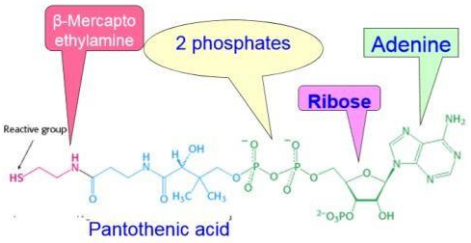
✓ The two-carbon units that are removed after oxidation are C1 and C2.

- Prior to β-oxidation, the fatty acid needs to be activated. This activation is achieved by the joining of the fatty acid to coenzyme A (CoA) by a high energy bond producing acyl CoA.
- This is followed by 3 reactions that convert -CH2- at β-carbon to a ketone group.
- Once -CH2- is oxidized to a ketone group, two-carbon units (carboxyl carbon + α-carbon) are released from the acyl CoA in the form of acetyl CoA.
- After the release of acetyl CoA, a new acyl CoA (shorter by 2 carbons) is produced.
- This is the first cycle of β-oxidation. It consists of 4 reactions involving the β-carbon that results in the shortening of the fatty acid by two carbons at the carboxylate end.
- The cycle is repeated over and over again until the fatty acid is completely degraded to acetyl CoA.



❖ Structure of Coenzyme A (CoA)

- ✓ The reactive group (functional group) is SH.
- ✓ SH can form a thioester bond which is a high energy bond.



❖ Activation of fatty acids

- Activation of fatty acids involves joining them to CoA by a thioester bond which is a high energy bond; as in the **cleavage** of this bond by the **addition of water** will release high amount of energy.
- The activation step is catalyzed by an enzyme called thiokinase or Acyl CoA Synthetase.

$$FA + HSCoA + ATP \rightleftharpoons FA\sim CoA + AMP + PP_i$$

- This reaction is **reversible** because:
  - ✓ We are breaking a high energy bond between P1 and P2 in ATP (releases 7 kcal) and forming another high energy bond between CoA and the fatty acid (requires 7 kcal).
  - ✓ I.e. the amount of energy that's released due to breaking the bond between the two phosphates is equal to the amount of energy required to form the bond between CoA and the fatty acid.
- This reaction is made irreversible by the continuous hydrolysis of one of the products (pyrophosphate) which is carried out by an abundant enzyme called pyrophosphatase. This prevents the shifting of the reaction to the left.

$$PP_i + H_2O \longrightarrow 2\ P_i$$

- If we add the two reactions together after the hydrolysis of pyrophosphate, we'll find that the activation of each fatty acid requires 1 ATP molecule.

$$FA + HSCoA + ATP \longrightarrow FA\sim CoA + AMP + 2\ P_i$$

➲ This activation step requires the hydrolysis of ATP to AMP which is equivalent to the hydrolysis of 2 ATP to 2 ADP. Therefore, **the activation of each fatty acid actually costs the cell 2 ATP.**

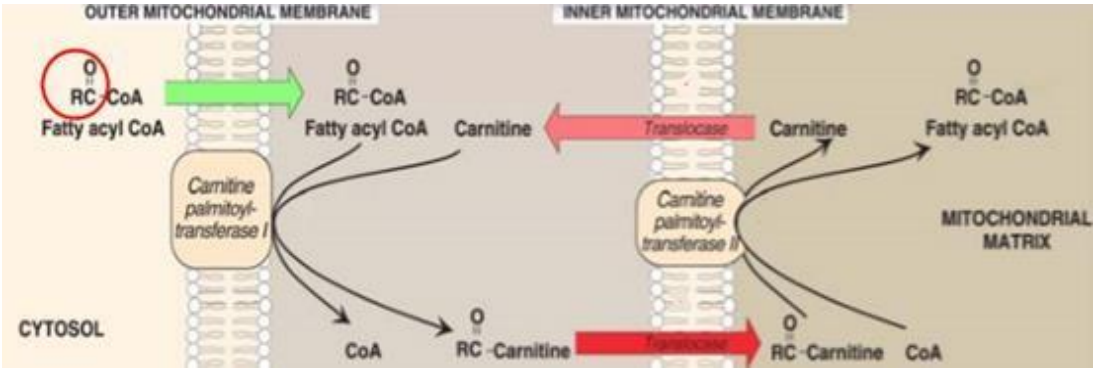
$$AMP + ATP \rightleftharpoons ADP + ADP$$
$$FA + HSCoA + 2ATP \longrightarrow FA\sim CoA + 2ADP + 2\ P_i$$

- Activation of fatty acids occur in two different locations depending on the **length** of the fatty acid:
  - ✓ Activation of long-chain fatty acids occurs in the outer mitochondrial membrane.
  - ✓ Activation of short/medium-chain fatty acids occurs in the mitochondrial matrix.

❖ Long-chain fatty acid transport into the mitochondria

- Once activated, long-chain fatty acids become ionized so they can no longer cross the inner mitochondrial membrane since it's impermeable to most ions. Therefore, a transport protein is needed.
- The transport protein responsible for the transport of acyl CoA is a carrier system called **carnitine shuttle**. This carrier system is inhibited during fatty acids synthesis.
- Carnitine shuttle consists of:
  - ✓ A carrier molecule (carnitine).
  - ✓ Two enzymes.
  - ✓ Membrane transport protein.
- Short and medium chain fatty acids **can cross** the inner mitochondrial membrane since their activation occurs in the mitochondrial matrix.

- Translocation goes in the following steps:
  1. The long-chain fatty acid is joined to CoA by Acyl CoA synthetase (thiokinase) in the outer mitochondrial membrane. The produced Acyl CoA can cross the outer mitochondrial membrane.
  2. In the intermembrane space there's a molecule called carnitine. The acyl group is transferred from CoA to carnitine by *carnitine palmitoyltransferase I*, an enzyme of the outer mitochondrial membrane. This reaction forms an acylcarnitine and regenerates free CoA.
  3. The acylcarnitine is transported into the mitochondrial matrix by translocase.
  4. *carnitine palmitoyltransferase II,* an enzyme of the inner mitochondrial membrane, catalyzes the transfer of the acyl group from carnitine to CoA in the mitochondrial matrix, regenerating free carnitine. Free carnitine can cross back to the intermembrane space by a transporter called translocase.



- Carnitine is like a car; it carries the passenger (the acyl group) across the inner mitochondrial membrane down its concentration gradient.

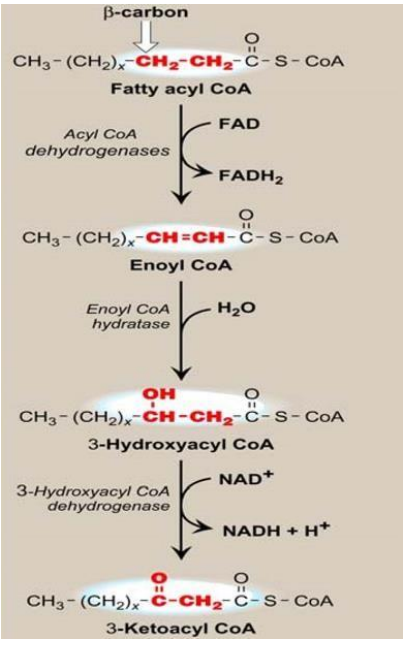
❖ β-oxidation reactions

∝ Each cycle of β-oxidation consists of 4 reactions involving the β-carbon:

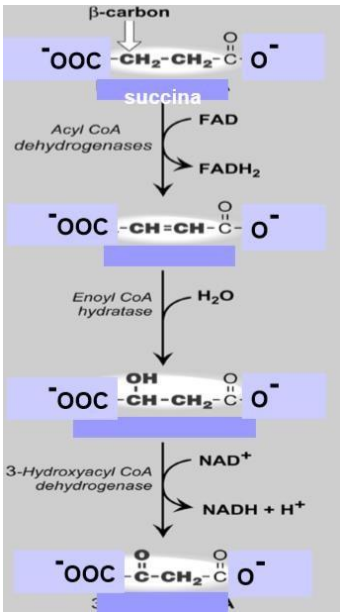
1. Oxidation that produces $FADH_2$
   - ✓ The first reaction involves oxidation of β-carbon by removal of 2 hydrogen atoms and transferring them to FAD resulting in its reduction to $FADH_2$.
   - ✓ The removal of the two hydrogen atoms leads to the formation of a double bond between the β-carbon and the α-carbon.
   - ✓ The resulting molecule is called **En**oyl CoA.
   - ✓ This reaction is catalyzed by Acyl CoA dehydrogenase. This enzyme has many isoforms depending on the length of the fatty acid chain. There are different isoforms for long, medium and short chain fatty acids.

2. Hydration (addition of H_2O)
   - ✓ The second reaction involves the addition of H_2O to the double bond between Cα and Cβ.
   - ✓ The enzyme that catalyzes this reaction is called Enoyl CoA hydratase. It's called hydratase and not hydrolase because it catalyzes the addition of H_2O without lysis of the molecule. It's a specific enzyme; it adds the (OH) to Cβ and the (H) to Cα resulting in the production of a secondary alcohol.
   - ✓ Since the hydroxyl group is added to Cβ (C3), the resulting molecule is called 3-Hydroxyacyl CoA.

3. A second oxidation that produces NADH
   - ✓ The third reaction involves oxidation of the hydroxyl group to a ketone group.
   - ✓ The resulting molecule is called 3-Ketoacyl CoA.
   - ✓ In this reaction, 2 hydrogen atoms are removed; one from the hydroxyl group and the other one from the β-carbon.
   - ✓ Since NAD^+ accepts 2 electrons, one of the two hydrogen atoms will be accepted by NAD^+ and the other one will be released as a proton (H^+).
   - ✓ This reaction is catalyzed by 3-Hydroxyacyl CoA dehydrogenase.

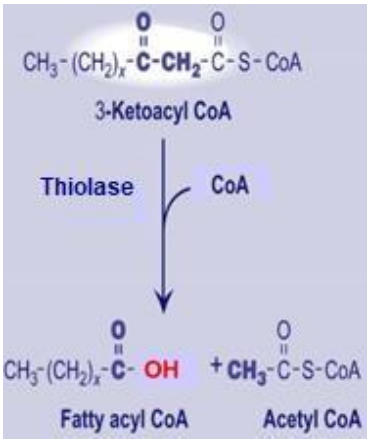


➲ The 3 reactions of β-oxidation are similar to the last 3 reactions of the citric acid cycle.



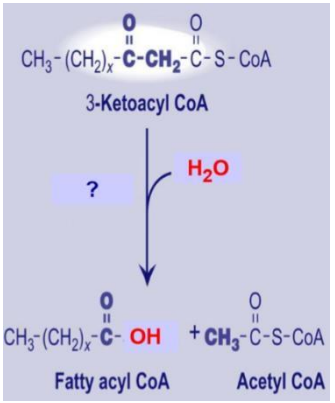
- We can see the resemblance by replacing S-CoA with O^- and the side chain with COO^-. Now we have succinate.
- Remember that in the citric acid cycle, succinate is oxidized by FAD dependent enzyme producing fumarate.
- Addition of H_2O to fumarate gives malate.
- Oxidation of malate by an NAD^+ dependent enzyme gives oxaloacetate.

4. A CoA-dependent thiolytic cleavage that releases a molecule of acetyl CoA
   - ✓ The presence of the ketone group facilitates the cleavage of two-carbon units as an acetyl CoA molecule from 3-Ketoacyl CoA.
   - ✓ The cleavage occurs by the addition of another CoA molecule (**not H2O**).
   - ✓ CoA attacks the β-carbon leading to the cleavage of the 3-Ketoacyl CoA to:
     - Acetyl CoA **(contains the original CoA)**
     - Fatty acyl CoA **(contains the CoA added in this step)**
   - ✓ It's called thiolytic cleavage because it's achieved by a sulfur containing group (CoA).
   - ✓ The enzyme that catalyzes the cleavage is called thiolase.



∝ What is the advantage of cleaving 3-ketoacyl CoA by adding CoA instead of H_2O?

- Remember that prior to β-oxidation we need to activate the fatty acid by joining it to CoA. This activation process costs the cell 2 ATP molecule. When we cleave 3-ketoacyl CoA by adding CoA, we get an already activated fatty acid (fatty acyl CoA), which means that we don't need to activate the fatty acid again and consume another 2 ATP molecules. Adding H_2O instead of CoA would cost the cell another 2 ATP molecules in order to activate the fatty acid again.

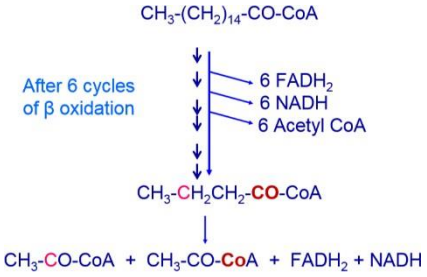


To sum it up, in β-oxidation two-carbon fragments are successively removed from the carboxyl end of the fatty acyl CoA, producing acetyl CoA, NADH and $FADH_2$.

❖ Energy yield from fatty acid oxidation

- Each cycle of β-oxidation produces:
  - ✓ 1 Acetyl CoA → 12 ATP.
  - ✓ 1 NADH → 3 ATP.
  - ✓ 1 $FADH_2$ → 2 ATP.
  - ✓ Fatty acyl CoA (shorter by 2 carbons)

- Suppose that we have a 16 carbon long fatty acid. After 6 cycles of β-oxidation the products are:
  - ✓ 6 Acetyl CoA
  - ✓ 6 NADH
  - ✓ 6 $FADH_2$
  - ✓ Fatty acyl CoA (shorter by 12 carbons → butyric acyl CoA)



- In the seventh cycle, butyric acyl CoA (4 carbons) will be cleaved to 2 identical Acetyl CoA molecules.

- The complete oxidation of a fatty acid that is 16 carbon long to 8 Acetyl CoA molecules requires 7 cycles of β-oxidation.
  The total energy yield is:
  - ✓ 7 NADH → 7 x **3** ATP → 21 ATP
  - ✓ 7 $FADH_2$ → 7 x **2** ATP → 14 ATP
  - ✓ 8 Acetyl CoA → 8 x **12** ATP → 96 ATP
  - ✓ Activation process (only done once) → consumes 2 ATP

From the citric acid cycle

➲ Net ATP molecules produced = 131 – 2 = 129.

- We can calculate the number of cycles needed to completely oxidize a fatty acid that contains (n) number of carbons using the following equation:

Number of cycles = (n/2)-1

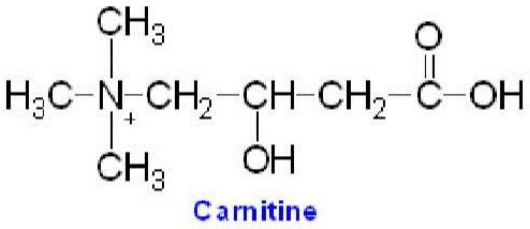
Number of $FADH_2$/NADH = number of cycles

Number of Acetyl CoA produced= (n/2)

- Oxidation of 1 mole of a 16 carbon long fatty acid yields 129 ATP while oxidation of 1 mole of glucose yields (36-38) ATP. The amount of ATP produced due to the oxidation of 1 mole of a fatty acid is much higher than the amount produced due to the oxidation of 1 mole of glucose.

❖ Carnitine

- Carnitine is a small molecule that's obtained from the diet.
  - ✓ It contains a carboxyl group and a quaternary amine.
  - ✓ It cannot cross the inner mitochondrial membrane without a transporter (translocase).
  - ✓ Its synthesis requires amino acids and occurs in the liver and the kidney.



Carnitine

- Functions of carnitine:
  - Export of branched chain acyl groups from mitochondria.
  - Excretion of acyl groups that cannot be metabolized in the body.

❖ Carnitine deficiencies

a) Congenital deficiency (from the time of birth):
   - ✓ Decrease in the synthesis of the enzyme that synthesizes carnitine.
   - ✓ Decrease in the uptake of carnitine by cells.
   - ✓ Decreased tubular reabsorption of carnitine by the kidney.

b) Secondary deficiency
   - ✓ Acquired throughout the person's life due to suffering from malnutrition or a liver disease.

- Carnitine deficiency leads to:
  - ✓ Decrease in the ability to use fatty acids as fuel.
  - ✓ Muscles that depend on fatty acids become fatigue.
  - ✓ Accumulation of fatty acids and branched acyl groups in cells.

- Diet manipulation:
  - ✓ Avoid long chain fatty acids and take short and medium chain fatty acids such as butyric acid (found in butter and dairy products).

- Carnitine deficiencies are not very rare.

- Some people use carnitine to build their muscles.