Medical Committee
The University of Jordan

SLIDE SHEET

LECTURE#: 16

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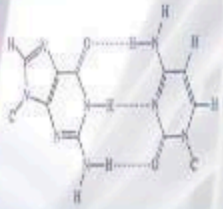
Biochemistry



Majida Al-Foqaraa'

# Ketone Bodies & Fatty Acid Synthesis

Note: this sheet was done according to section 3 recording.

This sheet covers two topics: 1) Ketone bodies 2) Fatty acid synthesis.

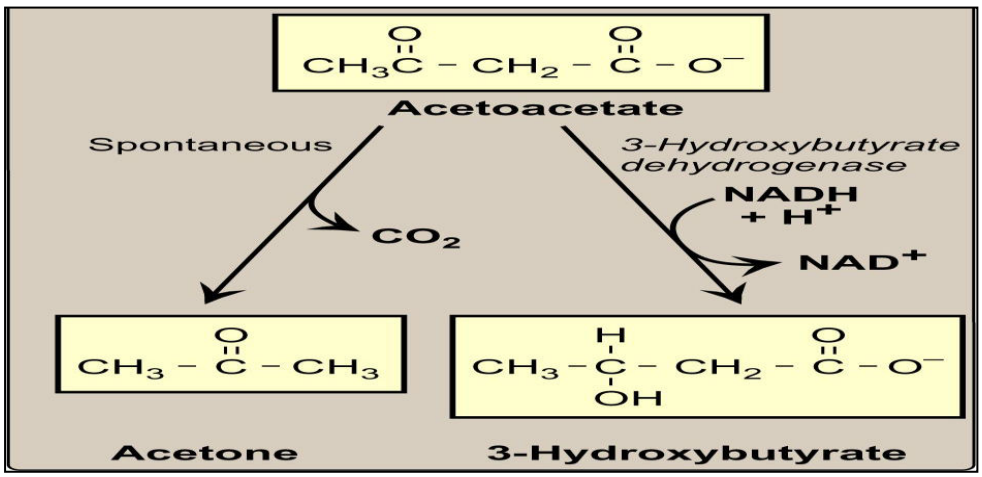
## Ketone bodies:

As we know, the end product of β oxidation is acetyl CoA, which can be oxidized completely by TCA (Creb's cycle) or it can be converted into ketone bodies.

-What are ketone bodies?

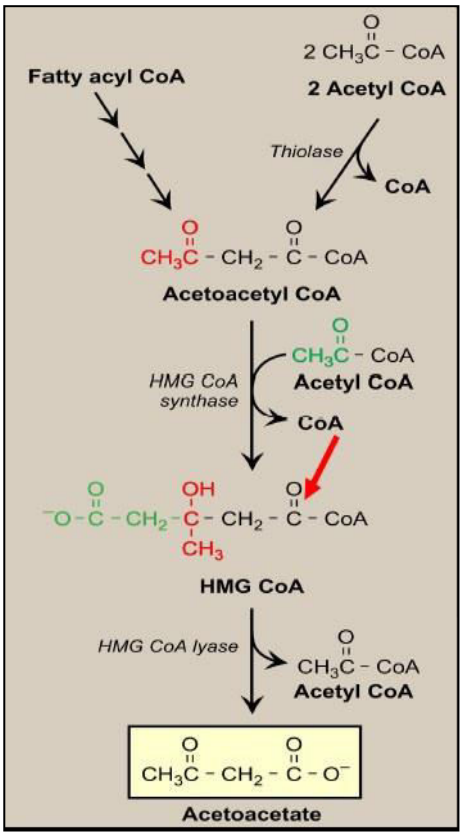
They're 3 compounds; notice that the first structure (acetoacetate) is a derivative of butyric acid. The left half of the compound ($CH_3CO$-) is acetic acid, and the right half is acetate, acetic group and acetic group linked together, so it's named acetoacetate, it's one of the ketone bodies, and it can be reduced by NADH. The ketone group at β carbon is reduced to hydroxyl group, or it undergoes spontaneous decarboxylation (the carboxyl group to the right is lost as $CO_2$). This reaction is spontaneous and does not require any enzyme because the compound has a ketone group near the carboxyl group and this facilitate its removal as $CO_2$ without enzymes - non-enzymatic reaction- (if we have butyric acid for example, it may not lose its carboxyl group, it will be stable)

Notice the product for the reduction reaction, the carboxyl group is at β carbon, its name is β-Hydroxybutyrate or 3- Hydroxybutyrate (NADH is needed for the reduction). Acetone is the product of the decarboxylation reaction (an organic solvent), together they are ketone bodies.



Ketone bodies are synthesized in the liver starting from acetyl CoA (the precursor) which is produced by β oxidation of fatty acids, the synthesis occurs all the time but at high rates during fasting and uncontrolled diabetes mellitus, during these situations the synthesis of ketone bodies increases.

The pathway of the synthesis has 3 steps; the first step is a condensation of 2 acetyl CoA molecules which produces acetoacetyl CoA, this product is the last intermediate in β oxidation, the last step in β oxidation is the cleavage of acetoacetyl CoA to acetyl CoA. Therefore, acetyl CoA whether it's coming from β oxidation or from anywhere else (like amino acids), it gives acetoacetyl CoA (from 2 acetyl CoA).




The second step is a condensation reaction; a third acetyl CoA condenses with acetoacetyl CoA to give a product with the name HMG CoA, so named because if you look at its structure you'll notice a 5-carbon chain with 2 carboxyl groups at each end, it's similar to glutaric acid which is a 5-carbon dicarboxylic acid, so HMG CoA is derivative of glutaric acid, notice that it has methyl and hydroxyl groups at β carbon, so the name becomes 3-hydroxy-3-methylglutaryl CoA (or β-hydroxy- β-methylglutaryl CoA) abbreviated HMG CoA.

The third and last step is cleavage of HMG CoA by HMG CoA lyase, acetyl CoA is removed and what remains is acetoacetate. Notice that the second uses acetyl CoA, and the last (third) step produces (removes) acetyl CoA (the third acetyl CoA enters the pathway at step 2 and it's produced at step 3), as this acetyl CoA is an intermediate and this step (the entry and removal of acetyl CoA) is done to give a different product and maybe because of the regulation, but what is important is the end product which is acetoacetate.

Note: the doctor didn't mention all enzymes which run this pathway, but to be in the safe side take a look at them in the figure above.

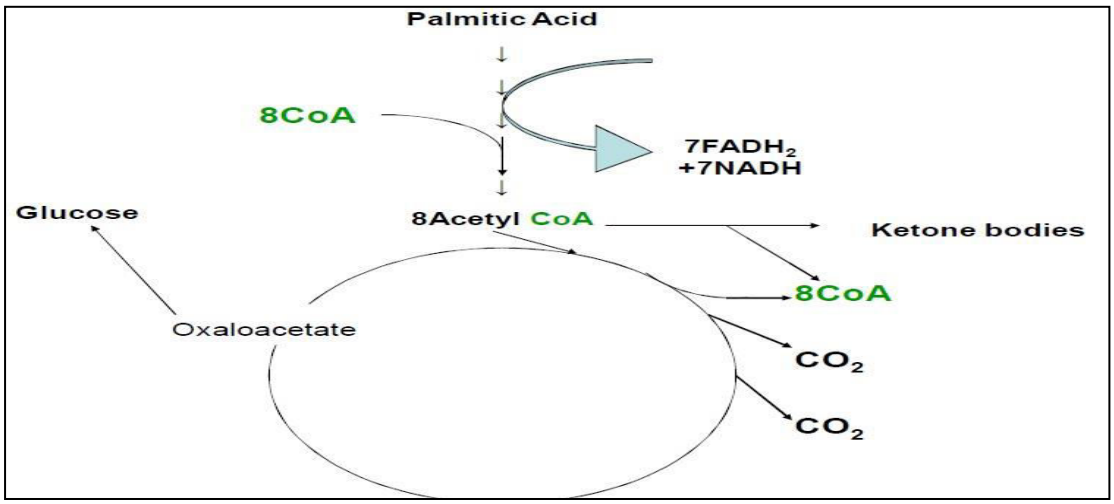
What is the net of this pathway?

Any intermediate in the pathway is not mentioned (any compound that is used in one step and produced or removed in another step is not considered from the net of the reaction), such as acetyl CoA (the third one) and HMG CoA which are intermediates so they're not mentioned. Therefore 2 acetyl CoA produce acetoacetate and 2 CoA so the net reaction is:

**2 Acetyl CoA → Acetoacetate + 2 CoA**

From the net you can tell the purpose of the pathway for these ketone bodies. The aim is to produce (regenerate) **CoA** which is important in the liver, acetoacetate is also produced in the net but for the liver it's considered as a waste product (acetoacetate in other tissues may be used but in the liver it's not used).

Notice the pathway in the figure below; palmitic acid (16-carbon fatty acid) is oxidized by β oxidation to give 8 acetyl CoA. Under normal conditions acetyl CoA enters citric acid cycle and condenses with oxaloacetate and after 8 cycles acetyl CoA are completely degraded to give CO2, NADH, $FADH_2$, and coenzyme A is regenerated and released in the first reaction.



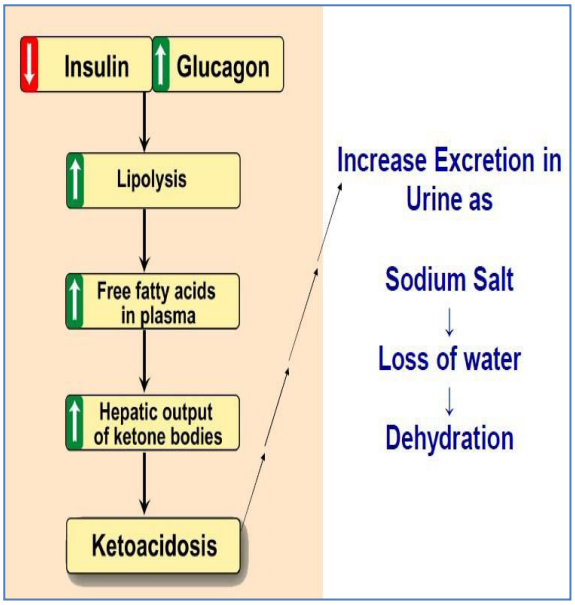

As you know, citric acid cycle uses intermediates, and for oxaloacetate there's no net production or consumption, it's required in catalytic amounts (it's needed with no net), it's used in the first reaction and produced in the last one so it does not increase or decrease during the pathway but without it the cycle cannot run, so catalytic amounts (small amounts) of oxaloacetate are required.

During fasting, the rate of fatty acid oxidation in adipose tissues is high (adipose tissue is releasing large amounts of fatty acids, they reach the liver and liver during fasting uses oxaloacetate to produce glucose (gluconeogenesis), as a result the level of oxaloacetate decreases as it's being converted into glucose, therefore the cycle cannot continue and it runs at very slow rate, so this is the purpose of β oxidation. Notice that β oxidation requires 8 CoA for palmitic acid, and the end product is 8 acetyl CoA, that means that CoA is locked or trapped as acetyl CoA. In citric acid cycle CoA is regenerated as it's explained above (in the first step of the cycle), but here CoA is regenerated by ketone bodies synthesis. Ketone bodies synthesis allows acetyl CoA to be converted to ketone bodies to regenerate CoA that will be used in β oxidation.

So the aim for ketone bodies synthesis is not to produce ketone bodies for the liver, but to allow CoA to be available for β oxidation to go on.

β oxidation produces NADH and $FADH_2$ to participate in the electron transport chain, therefore the amount of energy produced is relatively small (28-35 ATP) as there's no complete oxidation, and as you remember in glycolysis, the end product is pyruvate, to convert glucose into pyruvate, $NAD^+$ is converted to NADH, and if there's no enough oxygen, NADH cannot be reoxidized to $NAD^+$. In this situation NADH will reduce pyruvate to make lactate which is an end product in muscles and cannot be used for energy generation and lactate here is similar to ketone bodies which are end product for the liver, they cannot be used for energy production but it can be used by other tissues.

During fasting, rate of fatty acid production is high as well as gluconeogenesis. In diabetic patients, blood glucose is very high (maybe 3 or 4 times the level normal glucose) so the liver is expected to lower the production of glucose, however the liver is actively making glucose even the blood glucose level is high, because the liver does not sense the level of glucose in blood, it senses hormones; insulin and glucagon. Insulin is low, glucagon is high, and they tell the liver to do gluconeogenesis, liver continues to do gluconeogenesis even though the blood glucose is high because it responds to insulin and glucagon but not the glucose level in blood.

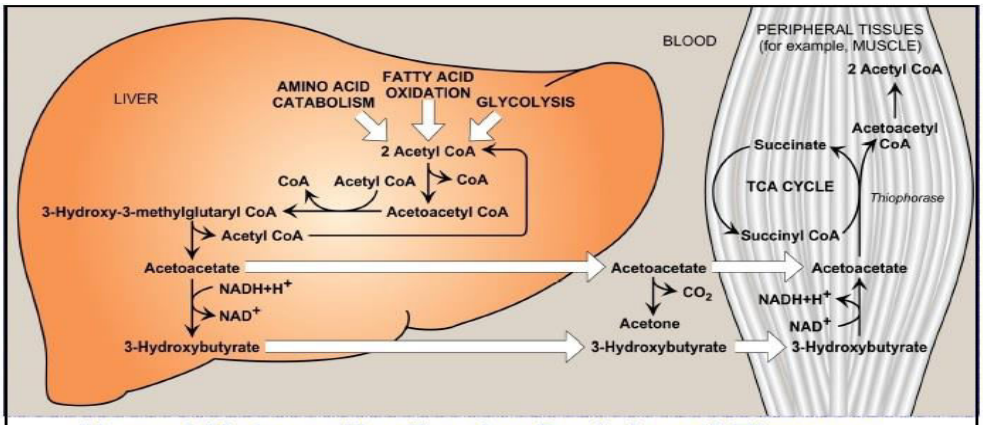


Active gluconeogenesis in diabetes, active lipolysis, free fatty acids in the plasma are high, hepatic output of ketone bodies is high, this all results in ketoacidosis. Acidosis means drop in the pH. Ketone bodies like acetoacetate or β-Hydroxybutyrate are acids; therefore they lose their protons and that leads to an increase in proton concentrations ($H^+$ concentrations) which is acidosis, and this acidosis which results from ketone bodies production is known as ketoacidosis.

In uncontrolled diabetes, ketoacidosis occurs, and ketoacidosis results in increase excretion of ketone bodies in urine (the level of acetoacetate and β-Hydroxybutyrate is very high in the plasma so they will be excreted in the urine, they are anions, negatively charged, so they are excreted as sodium salts, and loss of sodium occurs as the Cation accompanying acetoacetate, and loss of sodium ions results in loss of water (sodium and water go together; loss of sodium results in loss of water and retention of sodium results in retention of water). As a result dehydration occurs (emergency case).

Before using insulin for diabetic patients, death occurred in many diabetic patients who had acidosis, but nowadays the rate of deaths by ketoacidosis in diabetic patients is low due to the use of insulin. This condition is known as DKA (diabetic ketoacidosis), and if it continues it leads to loss of consciousness and coma and if persist it may lead to death.

In other tissues, like muscle tissue, it takes ketone bodies which are released from the liver into the circulation, they are taken by muscles. Hydroxybutyrate can be oxidized to acetoacetate, then acetoacetate can be consumed but it requires CoA, it should be linked to CoA to produce acetoacetyl CoA, succinyl CoA is the donor of CoA, it's an intermediate in TCA that provides CoA for acetoacetate to be converted into acetoacetyl CoA which can be cleaved into 2 acetyl CoA to be used in the citric acid cycle in the muscles or extrahepatic tissues (tissues outside the liver).

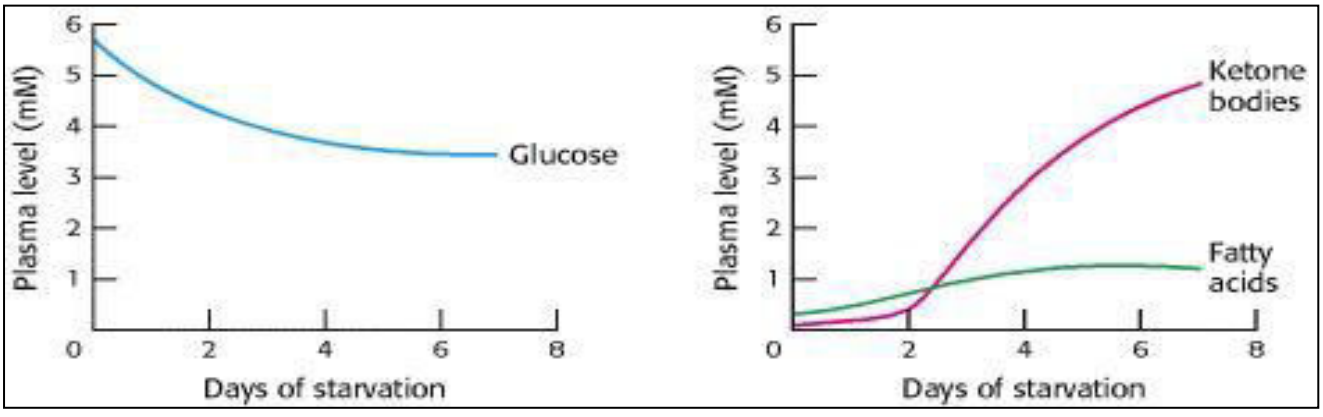


Use of Ketone Bodies by Peripheral Tissues: Skeletal Muscle, Cardiac Muscle and In Brain During Prolonged Fasting (Starvation)

Muscles prefer ketone bodies; cardiac muscle prefers ketone bodies (acetoacetate) as a source of energy (as if they are derived from fatty acid and soluble fatty acids, they are preferred fuel).

In liver as you know, oxaloacetate is not available to run citric acid cycle, ketone bodies are considered as waste products in liver, but in muscles oxaloacetate is high because gluconeogenesis is not active in the muscles so oxaloacetate level is sufficient to keep the cycle running, so ketone bodies are used as a source of energy in skeletal and cardiac muscles. Even the brain during prolonged fasting can use ketone bodies as a source of energy. Normally brain completely depends on glucose, fatty acids are not a source of energy for the brain, but if there is fasting and the brain continues to use only glucose as source of energy, brain can use glucose generated by amino acids and proteins. Amino acids and proteins are the source of gluconeogenesis to generate glucose. Proteins have very important functions; albumin, immunoglobulins, contractile proteins or myoglobin, they all have important functions, they can be used to produce glucose if needed in the brain. If brain continues to use glucose at the same level/rate, then protein degradation will occur at high rate and protein mass will be used. So this is the purpose that the brain can use ketone bodies, brain saves glucose that is coming from protein degradation and as a result saving proteins, it stops using proteins in muscles and stops their degradation to be used as a source of glucose, it uses ketone bodies during prolonged starvation.

Notice the figure below that represents what happens during starvation: concentration of fatty acids in the second or third day increases almost 3 times, fatty acids in high concentrations in the circulation. Notice the level of ketone bodies, it increases 10-20 times, so they're available and can be used as a source of energy. Glucose is maintained at 80%



Fuel metabolism in starvation:

Glucose used by the brain is 100 grams/day after 3 days whereas 40g/day after 40 days of starvation, glucose consumption is reduced from almost to the half (60%).

For ketone bodies the consumption was 50 to 100 g/day by the brain alone.

Fat mobilization is the same (180g/day)

Muscle protein degradation from 75 to 20 g/day (notice how much muscle-protein we have saved by using ketone bodies as a source of energy; an important adaptation during starvation).

Glucose output in the liver from 150 to 80 g/day (it has been reduced to the half), liver is still doing gluconeogenesis using lesser amount of protein (saving proteins by using ketone bodies instead).

**Fuel metabolism in starvation**

| | Amount formed or consumed in 24 hours (grams) | |
|---|---|---|
| **Fuel exchanges and consumption** | **3rd day** | **40th day** |
| Fuel use by the brain | | |
| Glucose | 100 | 40 |
| Ketone bodies | 50 | 100 |
| All other use of glucose | 50 | 40 |
| Fuel mobilization | | |
| Adipose-tissue lipolysis | 180 | 180 |
| Muscle-protein degradation | 75 | 20 |
| Fuel output of the liver | | |
| Glucose | 150 | 80 |
| Ketone bodies | 150 | 150 |

## Fatty Acid Synthesis:

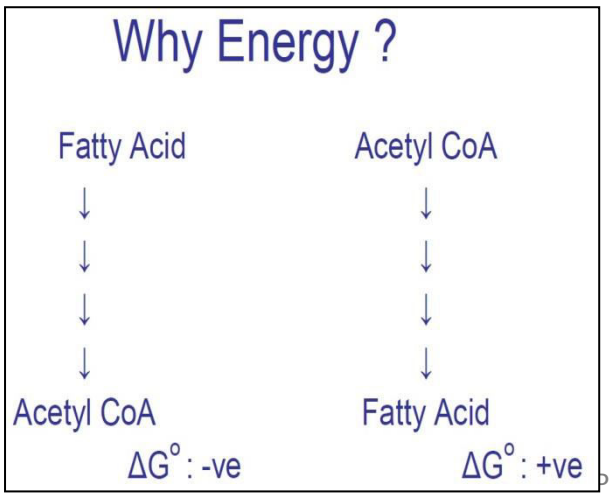
To make fatty acids carbons are needed, acetyl CoA is the source from which carbon is used to synthesize fatty acids.

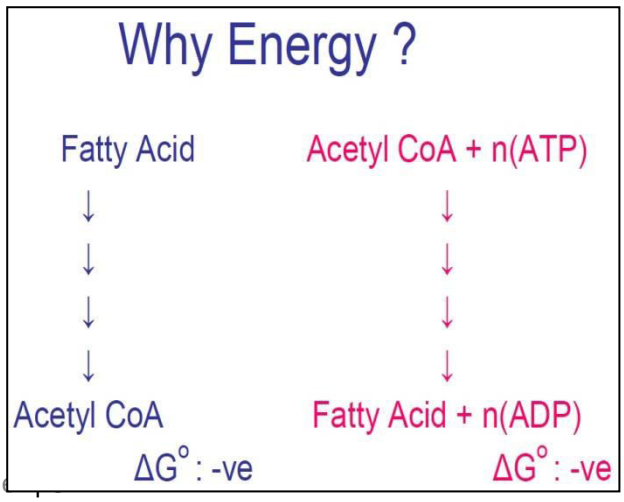
β oxidation is not the source of acetyl CoA which is needed for fatty acid synthesis, in β oxidation the end product is acetyl CoA and fatty acid synthesis requires acetyl CoA, so it's not logical to degrade fatty acids in order to build fatty acids, fatty acid synthesis does not occur while β oxidation is active and vice versa.

Notice that fatty acid β oxidation proceeds with overall negative ΔG˚ to produce acetyl CoA, it means that the sequence of the reaction is irreversible and it's exergonic pathway.

To convert acetyl CoA and synthesize fatty acids, ΔG˚ is positive so energy is needed for this pathway. If fatty acid synthesis is combined with ATP hydrolysis the overall ΔG˚ becomes negative (coupling the synthesis with ATP hydrolysis).

In glycolysis under anaerobic condition produce 2 ATP (from glucose to lactate) whereas in gluconeogenesis (from lactate to glucose) 6 ATP are needed, so building (synthesis) requires energy and it's not logical for the synthesis and degradation to occur at the same time.



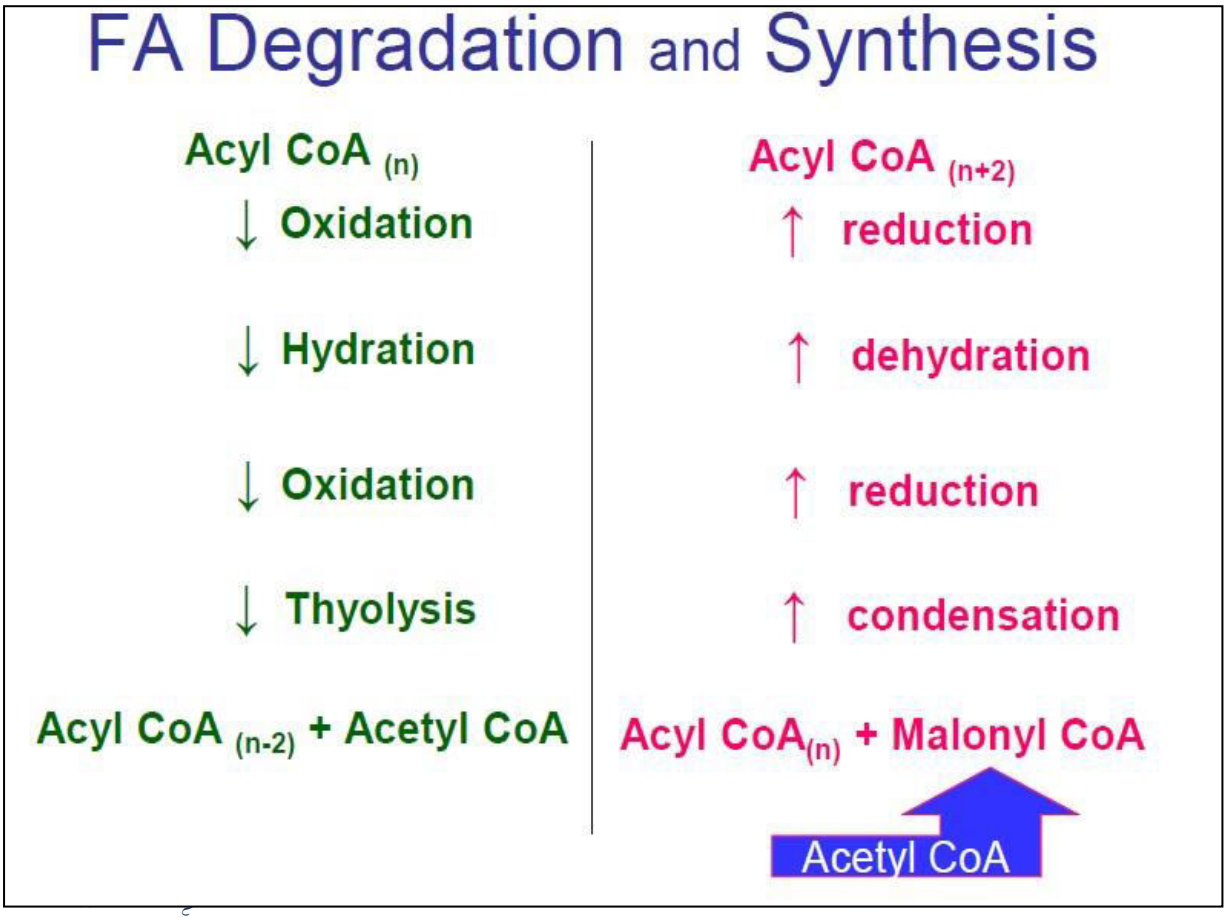
Fatty acid degradation and synthesis:

Although fatty acid degradation and synthesis are two different pathways, there's something common between them chemically.

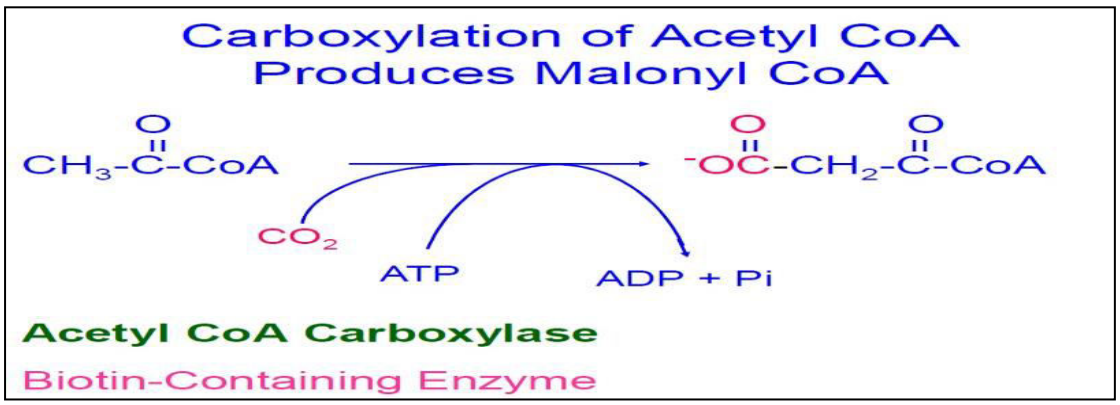
Fatty acid oxidation proceeds as oxidation, hydration, oxidation and thiolysis to give acyl CoA and acetyl CoA, and acyl CoA repeats the cycle again and again until the fatty acid is completely degraded

Fatty acid synthesis starts with condensation acyl CoA and acetyl CoA, followed by reduction, dehydration and reduction and the cycle is repeated again and again (every time 2 carbons are added as acetyl CoA). Usually fatty acids are composed of even number of carbons because they're formed from condensation of 2 carbons each cycle.

During synthesis, acetyl group is activated to malonyl group (malonyl CoA is activated acetyl CoA), then condensation occurs (acetyl CoA undergoes condensation in the form of malonyl CoA).



Malonic acid is a dicarboxylic acid with 3 carbons. For acetyl CoA to be converted into malonyl CoA a carboxyl group is added by carboxylation reaction, this reaction requires energy (ATP). This reaction is similar to pyruvate carboxylation and propionyl CoA carboxylation, it requires energy and the name of the enzyme in the reaction is acetyl CoA carboxylase and usually the carboxylation reaction requires the activation of a carboxyl group (coenzyme is required which is biotin). This step is the rate limiting and the regulating step.

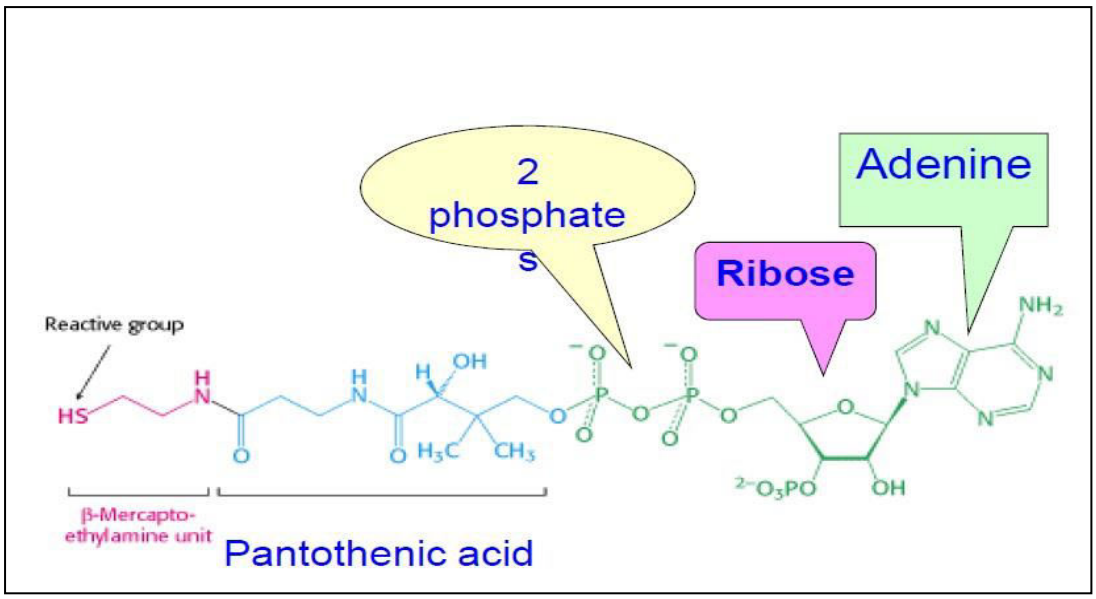



After formation of malonyl CoA, fatty acid synthase catalyzes the remaining steps, it has multiple functions (multifunctional enzyme complex), it's formed from dimer (two identical polypeptide chains), when they are dimer the enzyme is active (dimerization is important for the function of the enzyme even though the two monomers are identical).

Fatty acid synthase has 7 catalytic activities. One activity is known as condensing enzyme that has –SH group so it can attach to the intermediate by thioester bond.

One domain of the enzyme is linked to phosphopantetheine with reactive –SH, this domain which carries phosphopantetheine carries the intermediates during synthesis. The intermediates are acyl (any fatty acid with any number of carbon atoms more than 2), acetyl (2-carbon compound) and malonyl groups. These intermediates are carried by acyl carrier protein (ACP) and it contains phosphopantetheine. Note that CoA carries the fatty acid, so it's required as carrier for acyl groups.

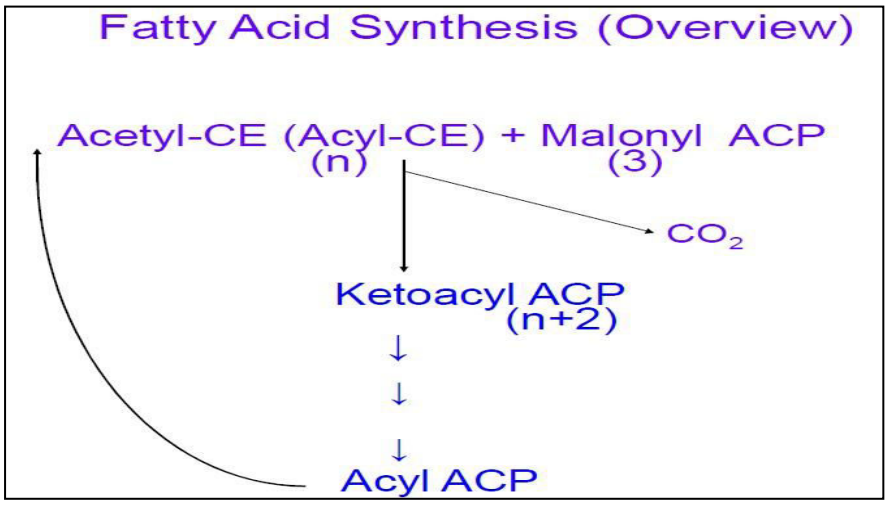
Intermediates during degradation are linked to CoA and during synthesis are linked to acyl carrier protein.

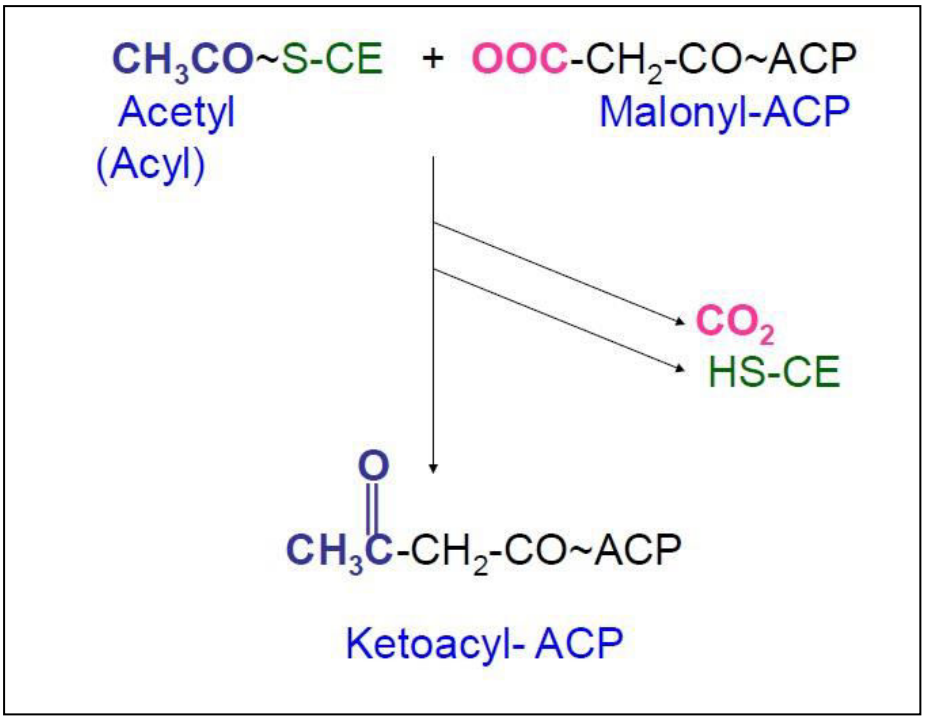



## Fatty Acid Synthesis (Overview):

In each cycle acetyl group is linked to condensing enzyme (Acetyl-CE (Acyl-CE)) condenses with Malonyl ACP (malonyl acyl carrier protein). After condensation, $CO_2$ is removed Ketoacyl ACP is produced. $CO_2$ is the same group that was added to the acetyl group to convert acetyl CoA into malonyl CoA, it's now released, what it does to reaction that it pushes it in the forward direction (to activate acetyl CoA), it's similar to what happens during gluconeogenesis when $CO_2$ is added.

After 3 steps of reduction, dehydration and reduction, acyl ACP is produced and it'll be used again and again until the fatty acid is completely synthesized with 16 carbons.






بهدي هاي الشيت لقرايبي رند و صوفيا حدادين

أحلى تحية للكوركشن تيم