Medical Committee
The University of Jordan

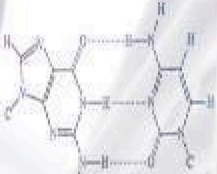
○ SLIDE ● SHEET

LECTURE#: 14

DR.NAME: Dr. Faisal

DONE BY: Bana AlMikhi

Biochemistry



Majida Al-Foqaraa'

Lipid Metabolism

Fatty acids and Triacylglycerol metabolism

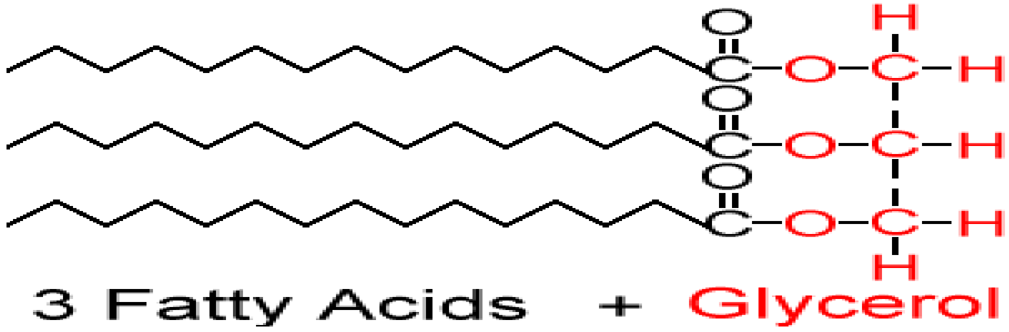
This lecture is from chapter 16, and not in the same order of the book. The Dr. mentions that we'll be taking chapter 15 later on.

-Note: the Dr. says that Lippincott is only good for revision and not for understanding the material. He suggests that you listen to the lecture, then scan the slides, and after that read the book to fill in the gaps of necessary information, since every lecture won't be more than 4-5 pages. Also, pay attention that some subjects we are not required to know from the book.

To begin with, we'll take a revision of what we have studied previously in the summer course:

- What is triacylglycerol?
Glycerol is an alcohol formed of 3 carbons with 3 hydroxyl groups; each hydroxyl group is esterified to a fatty acid. When the fatty acid is esterified, the fatty acid group participating in this bond is known as **acyl group**. So, triacylglycerol means that it's a glycerol that is esterified to 3 fatty acids and each ester bond can be hydrolyzed in a reverse reaction to make the 3 fatty acids and glycerol once again.

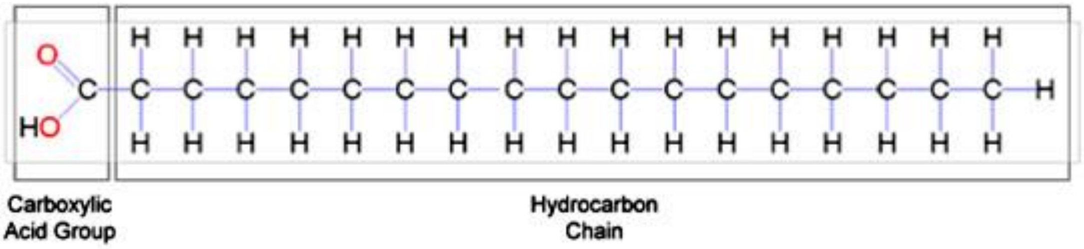
- the 3 fatty acids can be the same but usually they are different forming a mixture of fatty acids, some of them may have double bonds (unsaturated) and the others might be saturated.



-What are fatty acids?
Fatty acids are molecules of a long hydrocarbon chain that ends with a carboxyl group.

- Fatty acids differ according to the length of the hydrocarbon chain, but they all must end with a carboxyl group no matter what is the number of carbons it contains. The pKa of the carboxyl group is nearly 5. When the PH is higher than the PKa, the carboxyl group will always lose its proton (hydrogen ion) leaving a negative charge at the end forming an anion.

So, at physiological PH (which is almost 7) pH >pKa, the fatty acid loses its proton and is found in the anionic form (COO-). The anionic form can react much better with water, and can improve solubility. **BUT** generally speaking, fatty acid is **<u>insoluble</u>** in water due to the long hydrocarbon chain that can't interact and form hydrogen bonding with water.



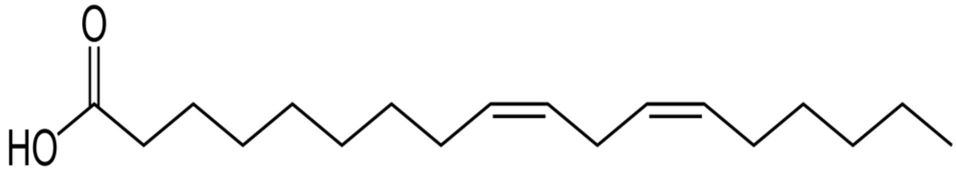
We can simply write the structure of a fatty acid by using the following method :
CH3 (CH2)n COO-
Whereas n = the number of carbons.

$$CH_3\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}COO^-$$

Or

$$CH_3\ (CH_2)_n\ COO^-$$

-Fatty acids can have one or more double bond in the chain:
If it's only ONE double bond → Mono-unsaturated fatty acid.
If there are TWO or MORE double bonds → Poly-unsaturated fatty acid.



** Looking at the above figure (Linoleic acid), you'll see that the fatty acid contains two double bonds, one is located on carbon number 9 (starting with the carboxylic carbon as number one), and the second one is located on carbon number 12. Notice that the double bonds are separated by 3 carbons, supposing it had one more double bond it'll definitely be on carbon number 15.

- The double bonds in the fatty acids are **NOT conjugated**, meaning that they're not alternating between double and single bonds. (We have a double bond then two single bonds until the next double one)

** Another method to write the formula is by using the Greek letters (alpha, beta,...etc) The alpha carbon is number 2; the beta is number 3, the gamma is number 4, and so on But normally we name it by the last carbon which is the omega one (whether you have 14, 15, 16 or whatsoever, the last carbon will always be named omega, because it is the last Greek letter).

-A more simple way of writing the structure is 18:2Δ(9,12) and from this formula you can extract that:
The fatty acid in the figure is made up of 18 carbons with 2 double bonds located on the 9th and 12th carbons.
Or you can just write it as 18: (9, 12)

-Another way to write down the location of the double bonds is by starting from the omega carbon, so linoleic acid will be omega 6 (ω6) (and no need to write the number of the next double bond).

The omega classification of fatty acids can result in omega6, omega 9, omega 3, omega 7 (families of fatty acids), how to know what is the omega classification of a fatty acid?
Simply by subtracting the carbon number of the last double bond from the total carbon number.

*Some fatty acids of physiological importance:

Most fatty acids are of even number of carbon atoms, we don't use the systemic naming method (for example hexanomic acids, or decanomic acids), we usually use the common name.

We are required to memorize the structures of some common fatty acids and they are illustrated in the slides by Arrows.

The **common names** come from the source of which the fatty acids are derived from, for example:
Butyric acid is derived from butter, Palmitic acid is derived from palm tree, Stearic acid is derived from wax, Oleic acid is derived from olive oil, Linoleic and Linolenic acids are derived from linen seed.

Oleic acid is composed of 18 carbons, with one double bond on carbon number 9, so what is its omega classification? ⟶ omega-9.

Arachidonic acids are composed of 20 carbons with 4 double bonds, located on carbon 5, 8, 11, 14.

- The **triacylglycerol** or **fat** is the major energy source of the body for different processes. They are stored in adipose tissue and later on the adipose tissue will release the fat to be used as an energy source in other tissues.

-It is **MORE** efficient to store energy in the form of triacylglycerols in comparison with carbohydrates, why?
(1) Fatty acids are more reduced, they have less oxygen, therefore they can be better oxidized and we get energy by oxidation reactions.
(2) Fatty acids upon full oxidation they produce around **9Kcal/g**, whereas carbohydrates provide 4Kcal/g.
(3) They are hydrophobic (the common property among all lipids) and non-polar (insoluble in water); therefore they could be found in the cell as such *without* attached water. The carbohydrates in an aqueous environment will absorb water; whereas fat is stored in adipose tissue nearly *without* any water making 90% of the volume of adipocyte is ONLY triacylglycerols.
But each stored gram of carbohydrates will store with it 2 grams of water.

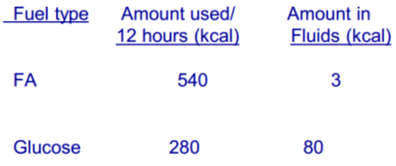
- An average adult of 70 kilograms has around 10kgs of fat, how much energy could be produced by complete oxidation of the 10kgs of fat?
10kgs = 10,000 grams
10,000 x 9 = 90,000 kcal
The average daily need of calories for such a person per day is about 2,000 kcal, so this amount of energy (90,000 kcal) is sufficient for 45 days if you don't eat at all.
** If this amount of energy is to be stored as carbohydrates, how many kilograms of carbohydrates do we need?
90,000 / 4 = 22.5 kgs

- As you can see from the above example, upon using fats we've saved about 12.5kgs and also 45kgs of water (since each gram of carbohydrates need 2 grams of water, the 22.5 grams would need 45kgs of water). In total 10kgs of fat is almost equal to 70kgs of both carbohydrates and water needed for its storage.

In conclusion, it's way more efficient for animals to store energy in the form of fats rather than carbohydrates.

- Fats are not **only** used for storage of energy when needed, in fact the adipose tissue is always active, there is always metabolic activity in it resulting in constant turnover between fat degradation while fasting and triacylglycerols formation when there is a sufficient food supply.

- Fatty acids are the major fuel used by tissues, while glucose is the major circulating food. Therefore the amount of fuel in the form of fatty acids in our plasma (extracellular fluid) is very little.
But, what is being used more? The answer is fatty acids, and this means that they are undergoing turnover in the plasma (they are found in little amounts and are constantly being renewed)



Fuel type	Amount used/ 12 hours (kcal)	Amount in Fluids (kcal)
FA	540	3
Glucose	280	80

- The dr. explained a slide in which there is a table for fatty acids that shows how many kilocalories have been used for a specific period of time.
For example, in 12 hours the body uses 60 grams (540kcal ÷9kcal per g) of fatty acids are used, but if measured in plasma you'll only find 0.5 grams (3kcal÷9 kcal per g), meaning that there is constant turnover.

Fatty acids are continuously (Produced, released into plasma, then taken by adipose tissue, and adipose tissue will become metabolically active)
On the other hand, in 12 hours the amount of glucose needed is 70 grams (280÷ 4) and at any time the amount is plasma (fluid) is approximately 20grams (80÷4).

- Mobilization of stored fats:

As we have previously mentioned, adipose tissue is used for fat storage, so how can the adipose tissue tell whether the other tissues such as liver, cardiac muscles, etc need fatty acids?
There should be **communication** between tissues and adipose, that means there must be a **hormonal signal**.
Hormonal signal reaches the adipose tissue to tell the adipose tissue that *fuel is needed*, and that hormonal signal leads to activation of enzyme named **hormone sensitive lipases,** this enzyme would cause hydrolysis of triacylglycerol into glycerol and 3 fatty acids to be released. Triacylglycerol doesn’t leave the cell or tissue as such; it must undergo hyrolysis.

*-Note: In order for triacylglycerols to leave the adipose tissue they must be degraded by lipases, and since this enzyme is sensitive to hormonal stimulation it is known as **Hormone Sensitive Lipase** to differentiate it from other lipases that are not sensitive to hormones.*

- What are the hormones that stimulate lipases?

1-glucagon.
2-epinephrine.
3-norepinephrine.
4-ACTH.

Now, let's explain each one separately

1-Glucagon:

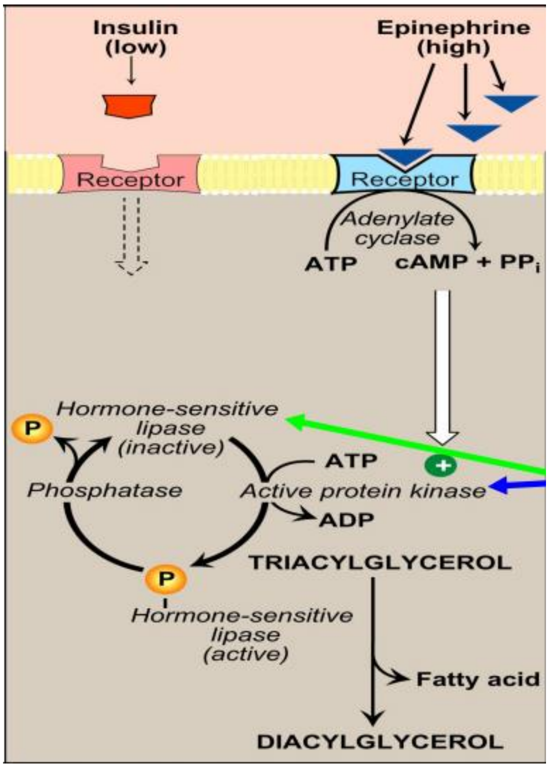
Hormone that indicates low blood sugar (if glucagon is high → blood sugar is low), meaning that there is a need for alternative fuel. When blood glucose is low, it will not be used by muscles and other organs, so fatty acids would serve as an alternative fuel.

2- Epinephrine and norepinephrine:

They indicate high demands for energy. So they stimulate hormone sensitive lipase to produce fatty acids that reach different tissues.
The dr. is now explaining the figure in the slides, he says the following:

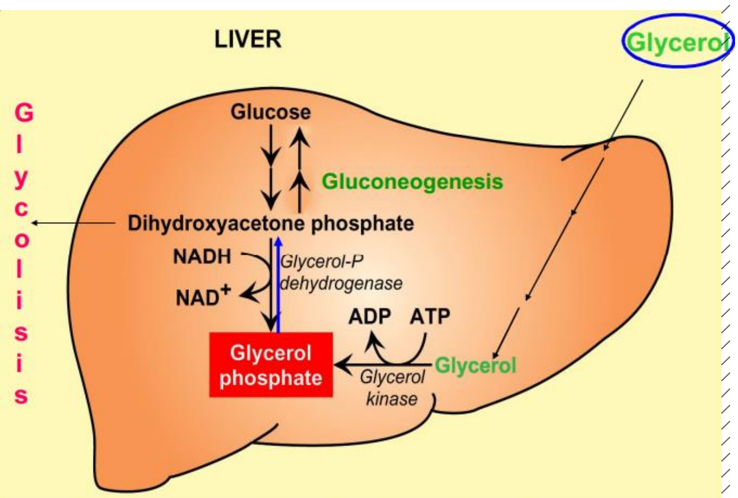
Notice that epinephrine is indicated by a triangle, and when it's concentration is high, it can bind to receptors found in the plasma membrane that is specific for norepinephrine (β adrenergic receptor). This binding will stimulate the enzyme adenylate cyclase, its activation converts ATP into cAMP. Also notice that the hormone did NOT enter the cell, the cAMP indicates the presence of the hormone outside.

cAMP (second messenger) activates an enzyme called protein kinase A (adds a phosphate group to proteins) one of these proteins is the hormone sensitive lipase.
Hormone sensitive lipase upon phosphorylation becomes in its active form (phosphorylated form), and then it can degrade triacylglycerols into diacylglycerol.



Whether the hormone sensitive lipase acts directly on triacylglycerol or diacylglycerol, this point is controversial (some say that triacylglycerol is converted into diacylglycerol by a removal of a fatty acid and catalyzed by hormone sensitive lipase, while others say that diacylglycerol is the substrate for lipase). In both cases, they ultimate result is 3 fatty acids and glycerol.

-What is the fate of glycerol?
Glycerol that is formed in the adipose tissue, is transported to the blood (it is water soluble) reaches the liver and then phosphorylated into **glycerol-phosphate** by glycerol kinase. After that, glycerol phosphate can undergo an oxidizing reversible reaction that converts it into dihydroxyacetone phosphate.
Dihydroxyacetone phosphate is an intermediate in:
1- Glycolysis and is converted to pyruvate.
2- Gluconeogenesis and is converted into glucose.



-Now, which of the above pathways is **efficient (preferred)** in these conditions (demand of energy)?
Of course the gluconeogenesis, because triacylglycerol is hydrolyzed and glycerol is reaching the liver and the blood glucose is low.

- β oxidation of fatty acids :

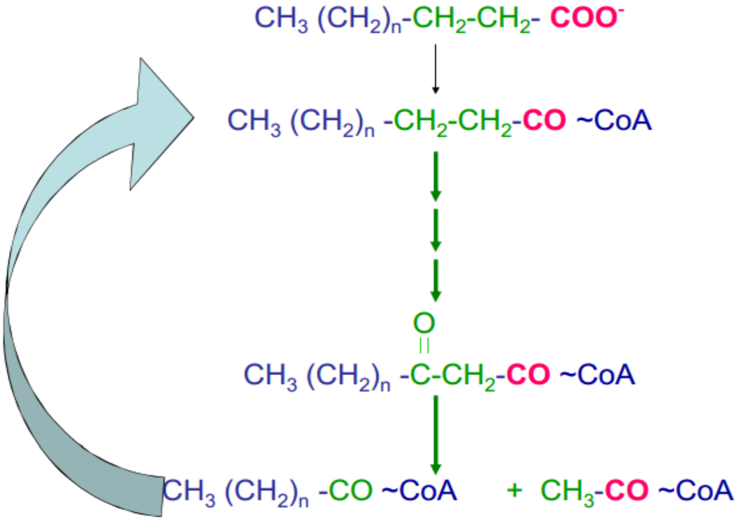
Fatty acids that are formed in the adipose tissue are diffused to plasma; in plasma they are **insoluble** in water, so how can they be transported?

⟶ By binding to albumin, they bind precisely to albumin in plasma (why albumin?) because it has some hydrophobic parts that allows hydrophobic substances like fatty acids' binding.

Firstly, fatty acid is activated by binding to CoA (this step is followed by 3 steps) then the degradation of fatty acids is done by oxidizing the beta carbon (carbon number 3) followed by a cleavage of two carbon units (the cleavage happens between carbon 2&3). For more detailing:

The beta carbon loses two hydrogen atoms and accepts one oxygen and now it is a ketone group (on carbon number 3), and after that, carbon number 1 and 2 are cleaved as acetyl CoA, and the rest is a new fatty acid attached to coenzyme A.

What's the difference between the fatty acid that we started with and the final one? The final one is shorter by two carbons. Then, this process starts all over again.



-So, how many times do we need the activation?

Only once, because every time we get an activated fatty acid.

How many times is this process done?

......we will see it later on.

- β oxidation is done in the mitochondria, the most important tissues are cardiac muscles, skeletal muscles, and liver.

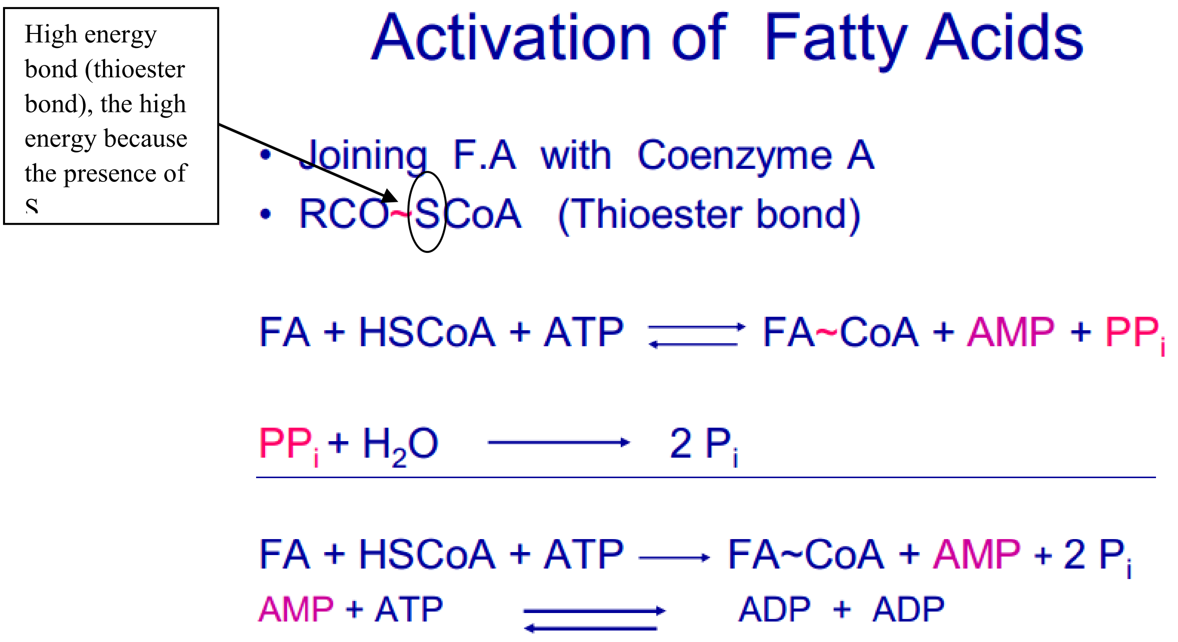
What about?

The brain does NOT use fatty acids as an energy source.

The RBCs (no β oxidation) they only depend on glycolysis since they don't have a mitochondria (no citric acid cycle, and no oxidative phosphorylation).

- The first step in β-oxidation is the **activation of fatty acids** which is a *reversible* step and is done by their joining to coenzyme A, this bond is a high energy bond (a **thio**ester bond), this step requires alot of energy.

Fatty acids + coenzyme A + **ATP** ⟶ Acyl-coA + AMP + PPi



High energy bond (thioester bond), the high energy because the presence of S

Activation of Fatty Acids

- Joining F.A with Coenzyme A
- RCO~SCoA (Thioester bond)

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

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

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

$$AMP + ATP \rightleftharpoons ADP + ADP$$

As you can see ATP is converted into AMP, which means **two** phosphate groups are removed as pyrophosphate in a reversible reaction.

Why is it a *reversible* reaction?

Because we are breaking one high energy bond and making one high energy bond as well, the net = 0 making the reaction reversible.

But how can we make it irreversible?

Pyrophosphate (PPi) is rapidly removed by an enzyme known as pyrophosphatase, and when you are continuously removing one of the products, the reversible reaction becomes irreversible.

- Notice that the fatty acids consumes an equivalent of two **high energy bonds** (because ATP is converted into AMP and pyrophosphate), BUT only **one ATP** directly participated in the reaction.

How many ATP equivalents? The answer is **TWO**,

When AMP is turned back into ATP, the first step is AMP + ATP → ADP + ADP

So if we add the two reactions together there are 2 ATP used for the activation so the two reactions together (ATP conversion to AMP + 2 Pi, then converting AMP to ADP) are equivalent to using 2 ATP to get 2 ADP.

$$\mathrm{FA + HSCoA + ATP \rightarrow FA{\sim}CoA + AMP + 2\,Pi}$$
$$\underline{\mathrm{AMP + ATP \rightarrow ADP + ADP}}$$
$$\mathrm{FA + HSCoA + 2\,ATP \rightarrow FA{\sim}CoA + 2\,ADP + 2\,Pi}$$

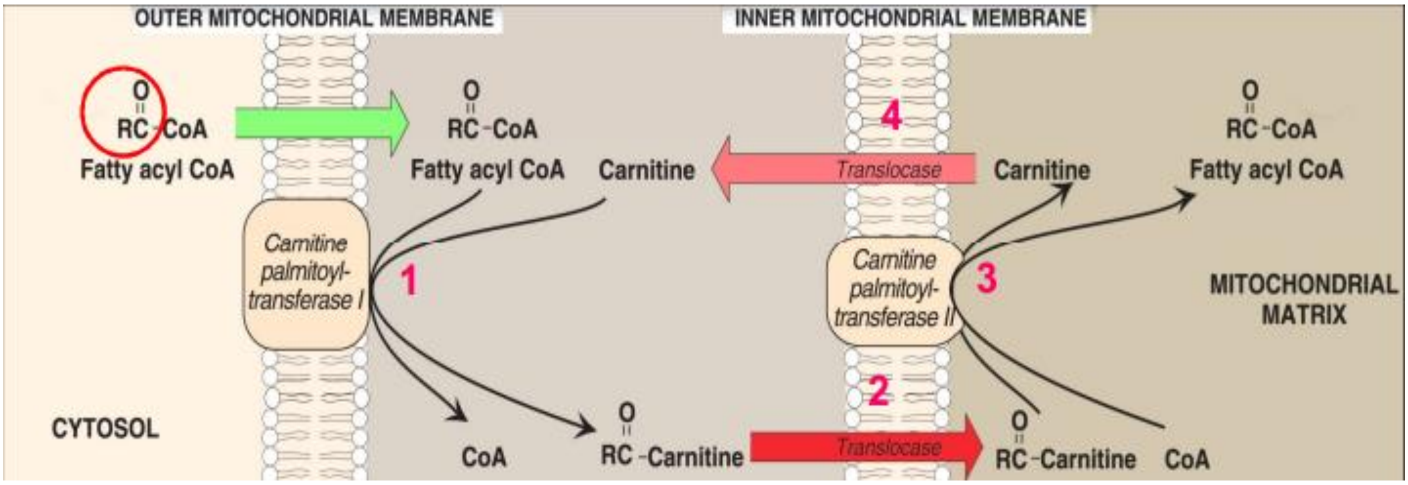
In conclusion, when ATP is converted into AMP + Pi (here the dr. said Pi but it must be 2Pi) is equivalent to hydrolysis of 2ATP

Enzyme used in activation of fatty acid is named **thiokinase** or **acyl co-A synthetase**.

** The reaction can occur at the following locations:
1- Outer mitochondrial membrane.
2- In the matrix of the mitochondria. (Only for short and medium chain fatty acids [~10 carbons], those can be directly activated inside the mitochondria)

Transport of long chain fatty acids can't occur in the mitochondria because the inner mitochondrial membrane is impermeable to acyl-CoA. In order to get the long chains inside the mitochondrial matrix, they need carrier system named **carnitine shuttle** (which takes the fatty acids across the mitochondrial membrane, enters as acyl carnitine and leaves as carnitine, also we require 2 enzymes)

- The dr. is explaining a slide that has the events and enzymes involved in the transport, he says the following:



Looking at the slide you'll see the acyl-CoA that is produced from the first reaction , Acyl-CoA can enter through the outer mitochondrial membrane , and in the intermembranous space, acyl-CoA is transported to the matrix by carnitine shuttle and this results in forming of acyl-carnitine and coenzyme A in a **transfer** reaction (the acyl group is transferred from the CoA to carnitine) by the transferase enzyme (**carnitine palmitoyl transferase** also known as **carnitine acyltransferase I**).

Now, the acyl carnitine CAN enter in the inner mitochondrial membrane because there is translocation, inside the mitochondria the acyl group is transferred to coenzyme A making Acyl-CoA and carnitine by an enzyme known as **carnitine acyltransferaseII**.

Carnitine exits the matrix and brings in another acyl group. Because of that it's called shuttle = the bus that transport.