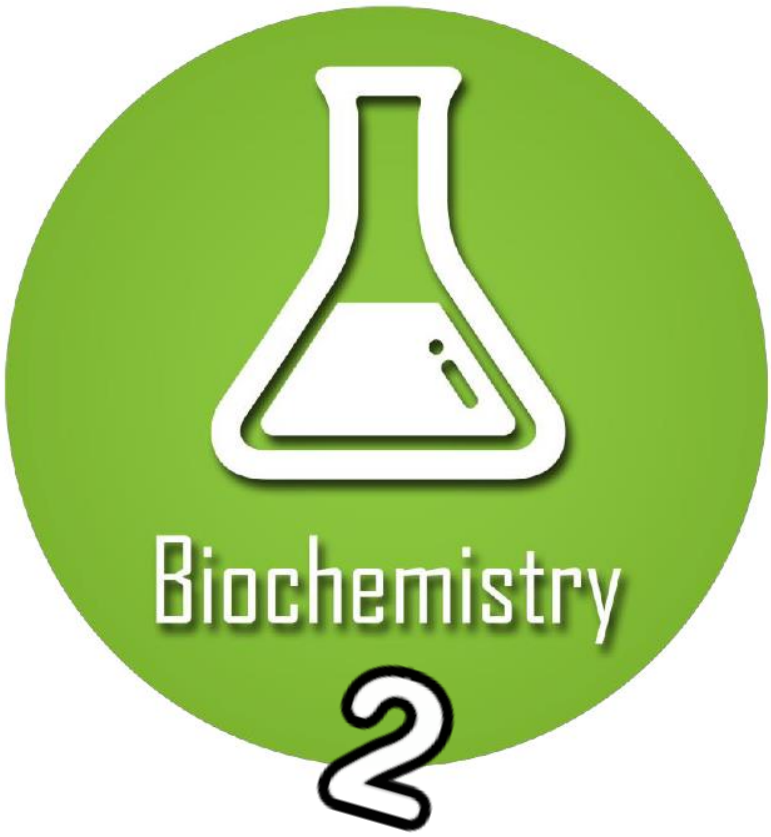

◉Sheet ○Slides

Number: 15

Done by: Laith Shlash

Corrected by: Yousef Al-As3d

Subject: Fatty acid synthesis

Doctor: Faisal Al-Khateeb

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In this sheet we will talk about synthesis of fatty acids and triacylglycerol.

Fatty acid synthesis requires 3 things:

1. Carbon source**: Acetyl CoA** is the only carbon source for fatty acid synthesis, this will lead to the formation of an even number of carbon atoms in the molecule because Acetyl CoA is a 2 carbon compound.
2. Reducing power: fatty acids contain a less proportion of oxygen than that of Acetyl CoA (Fatty acids are more reduced than Acetyl CoA) so we need a reducing power to remove the extra oxygen present in Acetyl CoA to synthesize fatty acids.
3. Energy input: energy is needed because we are joining units together, and the energy source is ATP.

Fatty acid synthesis mainly occurs in the liver then is transported to other tissues in the body.

Why is energy needed?

As we said before, to join the Acetyl groups together.

But let's take a look at the bioenergetics point of view:

Fatty acid oxidation: ΔG is negative (some of the energy is captured by NADH and FADH2 but part of energy is released as heat). And so we expect that if we go from Acetyl CoA to fatty acid (Fatty acid synthesis) ΔG will be positive –and it is. So the reaction will not proceed except if the concentration of Acetyl CoA is very very high. But after coupling this reaction with energy (ATP) , ΔG will be negative.

So, Energy is needed in the fatty acid synthesis because we are going in the opposite direction of a pathway that is usually exergonic. And we use energy to make it exergonic in the opposite direction so in other words energy will favour the opposite reaction, which is the fatty acid synthesis pathway.

Note: energy is always needed for synthesis such as in glycogen synthesis from glucose and protein synthesis from amino acids.

Fatty acid synthesis and degradation (oxidation) have some similarities in the chemical point of view.

In degradation (Oxidation):

Acyl CoA → oxidation → hydration → oxidation → thiolysis (cleavage)

These steps are repeated again and again until Acyl CoA is completely converted to Acetyl CoA.

In synthesis:

Condensation of Acyl CoA with Malonyl CoA → reduction → dehydration → reduction

(Opposite to thiolysis) (Opposite to oxidation) (Opposite to hydration) (Opposite to oxidation)

The synthesis and degradation pathways are complete opposites of each other in the chemical point of view except that Malonyl CoA is used in the synthesis pathway.

Note that reduction is the opposite of oxidation, dehydration is the opposite of hydration and condensation is the opposite of thiolysis.

What is Malonyl CoA?

It's a dicarboxylic acid with 3 carbons that comes from Acetyl CoA (we can say that the synthesis pathway occurs from Acetyl CoA and Acyl CoA through Malonyl CoA followed by the normal pathway).

Conversion of Acetyl CoA to Malonyl CoA:

- It is a Carboxylation reaction.
- Energy is needed to add CO_2.
- The reaction is catalyzed by **Acetyl CoA carboxylase**, which is very similar to carboxylation of pyruvate and carboxylation of propionyl CoA.
- Acetyl CoA carboxylase is a biotin containing enzyme

Note: Malyl CoA that is found in the oxidation of odd chain fatty acids is produced from a methyl group and Malonyl CoA.

All the remaining reactions (condensation, reduction, etc.) are catalyzed by the same enzyme complex called **fatty acid synthase** that has several catalytic sites.

Fatty acid synthase:

- Several active sites.
- Multifunctional enzyme complex.
- Dimer of 2 large identical polypeptide chains (dimer: active, monomer: inactive).
- Each monomer has Seven Catalytic Activities (we already mentioned 4). One activity is called condensing enzyme and it catalyzes the condensation reaction (the condensing enzyme has -SH).
- One domain is linked to phosphopantethiene (Has reactive –SH) (Acyl carrier protein).

Coenzyme A contains ADP, pantothenic acid and beta mercaptoethylamine which ends with an SH bond and it's the reactive group of coenzyme A.

Coenzyme A is the carrier of Acetyl and Acyl groups. (Naming came from **A**cylation).

Phosphopantethiene is part of the coenzyme A, its linked to a protein to carry the intermediates so instead of the presence of ADP a protein will be present which will lead to the formation of **Acyl carrier protein (ACP)** that will carry the intermediates during synthesis, unlike in degradation where the intermediates are carried by coenzyme A. Coenzyme A and Acyl carrier protein both have the same terminal (ending). So to sum up coenzyme A carries the intermediates in degradation while Acyl carrier protein carries the intermediates in synthesis.

Why is it called Acyl carrier protein as if it's a separate protein?

Because in bacteria fatty acid synthesis occurs in several enzymes, several proteins and each protein is alone but in humans they are together as a large multifunctional enzyme with several active sites.

Acyl carrier protein (ACP) in humans is part of the multifunctional enzyme that carries Acetyl and manolyl groups.

How will fatty acid synthesis proceed?

It will proceed in the opposite manner of the oxidation pathway. We start with Acetyl condensing enzyme and manolyl ACP then CO_2 is released. As if the Acetyl group isn't ready for condensation until it's activated by adding a carboxyl group producing manolyl CoA, and here it is released. (Same as gluconeogenesis when CO_2 is added to pyruvate then removed)

Condensation reaction will produce KetoAcyl ACP.

Acyl ACP will be formed from KetoAcyl ACP by the reduction, dehydration, reduction pathway.

Acyl ACP will condense with Malonyl group again (The Acyl will be transferred to the condensing enzyme and the ACP will carry a new Malonyl group) and the reaction can continue again and again until the fatty acid is completely synthesized.

What drives the formation of KetoAcyl ACP in the forward direction?

1. Cleavage of a high energy bond (Between the Acyl and the condensing enzyme)
2. Decarboxylation: it's the irreversible reaction that drives the reaction in the forward direction, and releases energy. So CO_2 was added at the beginning to make condensation irreversible

Note: We used ATP to make the carboxylation reaction. So the decarboxylation reaction must release energy.

Note: All decarboxylation reactions are irreversible.

In the reduction reaction instead of using NADH and FADH2, NADPH was used. NADPH is used for synthesis and differs from NADH by having one extra phosphate. NADPH is used for synthesis while NADH is used for oxidation (degradation). This variation in NADH and NADPH allows us to maintain a high level of NAD+ and NADPH at the same time. (They are not the same molecule and thus are not linked with a ratio).

Next steps are dehydration then the second reduction.

Acyl ACP is transferred to the condensing enzyme for one more cycle of synthesis. Fatty acid synthase contains ACP with SH bond and the condensing enzyme contains another SH bond. After Acetyl group enters it'll be transferred to condensing enzyme and ACP will be free, Malonyl will be added to ACP (entrance of substrates into two active sites). So the condensing enzyme has the Acetyl group and ACP has the Malonyl group. Condensation occurs and KetoAcyl ACP will be formed leaving the condensing enzyme free and the KetoAcyl ACP will continue in the synthesis pathway (Reduction → dehydration → reduction).

Next step a new Acyl group is transferred to the condensing enzyme to start another cycle with Malonyl CoA. This will continue and steps are repeated

until we have complete synthesis of fatty acid or palmitic acid connected to ACP. Then an enzyme called **Thioesterase** will remove palmitic acid and we will have a free enzyme with palmitate.

How many cycles of synthesis (condensation) do we need to synthesize a fatty acid with 16 carbons?

7 cycles, in the 7 cycles 8 Acetyl CoA are used 7 of which are Malonyl CoA and each cycle needs 2 NADPH. So the total NADPH used are 14.

Note: We used only 1 Acetyl CoA without carboxylation at the beginning, and we converted 7 molecules of Acetyl CoA to Malonyl CoA. So the total Acetyl CoA used are 8.

Production of Acetyl CoA for fatty acid synthesis:

What are the pathways that produce Acetyl CoA?

1. Glycolysis
2. Amino acid breakdown

Note: The fatty acid oxidation produces the most amount of Acetyl CoA in the body. But it is not used in the fatty acid synthesis because it's not logical to synthesis fatty acids from fatty acids breakdown.

Carbohydrate metabolism and lipid metabolism are linked together by pyruvate dehydrogenase (the fate of excess carbohydrates will be formed to glycogen and fatty acids). This happens in the inner mitochondrial membrane and it's impermeable to Acetyl CoA so it's transported out of the mitochondria by reacting it with oxaloacetate to produce citrate. If the energy level in the cell is low, citrate will continue the citric acid cycle. But if the energy level in the cell is high, citrate won't be converted to isocitrate because isocitrate dehydrogenase would be inhibited leading to the accumulation of citrate. Citrate then gets out of the mitochondria by citrate carrier into the cytosol, then citrate will be converted back to oxaloacetate and Acetyl CoA by **ATP-Citrate lyase** (ATP is required because of the presence of the high energy product Acetyl CoA). Note that this reaction is not the reverse of the joining reaction of Acetyl CoA and Oxaloacetate; the joining reaction is catalyzed by Citrate synthase.

Oxaloacetate has to enter the mitochondria again. But it can't enter the mitochondria so it must be first reduced to malate (NADH to NAD+) then

Malate enter oxidative decarboxylation producing Pyruvate (NADP+ to NADPH). Pyruvate can enter the mitochondria and be carboxylated to Oxaloacetate again.

What the purpose of this cycle? To get Acetyl CoA out of the mitochondria. Despite that this costs a lot of energy, we get NADPH that is needed in this conditions as we are seeking for fatty acid synthesis that requires NADPH.

To synthesize 16 carbon fatty acid, 8 NADPH will be produced by this way as a result of 8 Acetyl CoA molecules got out.

Note: All fatty acid synthesis happens in the cytosol

Regulation of fatty acid synthesis and oxidation:

Synthesis and oxidation should not occur at the same time because this will result in the loss of energy, so we have to regulate them.

- Regulation of Synthesis:

1) Regulation of Acetyl CoA carboxylase: it should be regulated since it's the 1st step of fatty acid synthesis and this will prevent the accumulation of intermediates:

   A) Allosteric mechanism: The enzyme is in inactive form; the conversion to the active form can be activated or inhibited.
      - Activation by **Citrate**: high level of citrate indicates abundance of building blocks and indicates for a high energy level.
      - Inhibition by high level of long **fatty Acyl chains** (negative feedback inhibition).

   B) Phosphorylation:
      - Phosphorylation inactivates the enzyme, and dephosphorylation activates the enzyme.
        - High level of **Glucagon** and epinephrine indicates the need of glucose and activation of gluconeogenesis, so it must inhibit fatty acid synthesis as it uses glucose, and it do this by activating **c-AMP-dependent protein kinase**, which phosphorylates Acetyl CoA carboxylase, and thus inhibiting the synthesis pathway.

- High **insulin** level indicates the well-fed state and activate the synthesis of fatty acids by activating **protein phosphatase** that dephosphorylates Acetyl CoA carboxylase.

Note: Phosphorylation of enzymes always favours the saving of glucose. And this is why phosphorylation inhibits Acetyl CoA carboxylase / glycogen synthase.

2) Regulating the amounts of the enzymes.

- Regulation of oxidation:

Activation: When fatty acids are abundant, they will be used for oxidation. Glucagon mobilize fatty acids from adipose tissue so it activate the oxidation.

Inhibition: - High levels of NADH (high level of ATP).

- Malonyl CoA: the first step in the oxidation of fatty acids is the entry of fatty acids into the mitochondria, Malonyl CoA inhibits this entry. So Malonyl CoA indicates active synthesis, so during synthesis there is no oxidation.