Formation of fatty acids and triacylglycerols

We perceive the formation of fatty acids and triacylglycerols as a highly energy-demanding process localized mainly in the cells of the liver, adipose tissue, CNS or lactating mammary gland. It takes place mainly in the postprandial period.

The process of fatty acid formation is in many ways the reverse of β-oxidation – instead of oxidation, reduction takes place, similarly, hydration is replaced by dehydration. However, it is not an exact reversal of events, the two processes differ in many significant ways. We will show these differences before describing the individual reactions.

Differences between the breakdown and synthesis of fatty acids

- Synthesis of FA takes place **in the cytoplasm** , degradation in the mitochondrial matrix.
- Intermediate products of FA synthesis are **bound** to the so-called *acyl carrier protein* (ACP, acyl-carrying protein), intermediate products of degradation to the coenzyme A.
- Enzymes of FA synthesis are combined into a **multi-enzymec omplex** called *FA synthase*, degradation enzymes are stored freely in the matrix.
- The fatty acid chain is **always lengthened by two carbon atoms** the initial substrate is AcCoA (the activated donor is malonyl~CoA).
- The reducing agent of synthesis is **NADPH**, the oxidizing agents of degradation are FAD and NAD^+.
- Chain elongation at FA synthase ends with the formation of palmitate (C_{16}), further chain elongation and the formation of unsaturated acids takes place by the action of other enzymes in the ER and in the mitochondria.

Now we will look at the individual reactions of fatty acid synthesis.

Formation of malonyl~CoA

1. The input substance for the synthesis of fatty acids is AcCoA.

In the first step, when ATP is consumed, it is **carboxylated to malonyl-CoA** :
$AcCoA + ATP + HCO_3^- \rightarrow malonyl\sim CoA + ADP + P_i + H^+$
This reaction is catalyzed by the regulatory enzyme **AcCoA-carboxylase**, whose cofactor is **biotin** – vitamin H, or B 7 (in general, it is a cofactor of carboxylases).

The formation of malonyl~CoA occurs in two steps. First, the ATP-dependent **carboxylation of biotin** takes place:

$$\text{Biotin-enzyme} + ATP + HCO_3^- \rightarrow CO_2\text{-biotin-enzyme} + ADP + P_i$$

2. Subsequently, the carboxyl is transferred to acetyl-CoA

$$CO_2\text{-biotin-enzyme} + AcCoA \rightarrow \text{malonyl-CoA} + \text{biotin-enzyme}$$

Biotin is linked to the enzyme by an amide bond between the carboxyl of biotin and the ε-amino group of lysine. CO_2 is removed from the molecule again during condensation with the growing fatty acid chain.

Fatty acid synthase

Mammalian fatty acid synthase has a homodimer structure composed of two identical subunits (260 kDa). Each subunit consists of **three domains** connected by mobile regions:

1. **Domain 1** – substrate entry and condensation unit – both transferases (acetyltransferase and malonyltransferase) and β-ketoacyl synthase (condensation enzyme – CE).
2. **Domain 2** – the reducing unit – contains ACP, β-ketoacyl reductase, dehydratase and enoyl reductase.
3. **Domain 3** – palmitate-cleaving thioesterase.

The **intermediate binding sites** for FA synthase are:

- **Thiol group of cysteine CE**
- **The thiol group of phosphopantetheine**, which binds to the serin in ACP. The phosphopantetheine arm can also be found in the coenzyme A molecule. This flexible arm enables the transfer of intermediates between the individual catalytic sites of the synthase.

Individual steps of fatty acid synthesis

1. Synthesis of malonyl-CoA

- catalyzed by acetyl-CoA carboxylase – does not take place on FA synthase

2. Binding of AcCoA to CE

- acetyl transacylase

3. Binding of malonyl-CoA to ACP

- malonyl transacylase

4. Condensation reaction

- condensing enzyme

5. FA synthase functions as a dimer.

In this step, condensation occurs between the malonyl suspended on the ACP of one subunit and the acetyl on the condensing enzyme of the other subunit. The new acyl remains attached to the ACP:

Acetyl-CE + malonyl-ACP → acetoacetyl-ACP + CE + CO_2

6. First reduction

- β-ketoacyl reductase

Acetoacetyl-ACP + NADPH + H^+ → D-3-hydroxybutyryl-ACP + $NADP^+$

7. Dehydration

- 3-hydroxyacyl dehydratase

D-3-Hydroxybutyryl-ACP → krotonyl-ACP + H_2O

8. Second reduction

- enoyl reductase

Krotonyl-ACP + NADPH + H^+ → butyryl-ACP + $NADP^+$

9. Chain transfer from ACP to the SH group of the condensing enzyme of the same subunit.

10. The new malonyl binds to the ACP of the second subunit.

Subsequently, condensation occurs on the opposite subunit of the dimer than it was during the first condensation. Thus, the subunits alternate regularly during synthesis.

Another FA synthesis procedure

The newly synthesized **fatty acid chain** is gradually lengthened. Termination occurs at the length of C_{16} the end product of FA synthase is therefore **palmitate**. Thioesterase cleaves it from the bond to ACP (hydrolysis of the thioester bond to phosphopantetheine).

In total, the **formation of palmitate** requires 8 molecules of AcCoA, 14 molecules of NADPH and 7 molecules of ATP:

$$8\ AcCoA + 7\ ATP + 14\ NADPH + 14\ H^+ \rightarrow palmitate + 14\ NADP^+ + 8\ CoA + 6\ H_2O + 7\ ADP + 7\ P_i$$

AcCoA is produced in the **mitochondrial matrix**, FA synthesis takes place **in the cytoplasm**. However, the inner mitochondrial membrane is impermeable to AcCoA, so it is transported to the cytoplasm **in the form of citrate** (see below). 8 molecules of NADPH are obtained by transporting citrate into the cytoplasm, the remaining 6 in the pentose cycle.

Citrate as a carrier of acetyls from the mitochondrial matrix to the cytosol

If there is enough AcCoA in the mitochondrial matrix, it reacts with OAA to form citrate (catalyzes citrate synthase).

It is transported to the cytoplasm, where it is cleaved by ATP-citrate lyase (ATP consumption):

$$Citrate + ATP + HSCoA + H_2O \rightarrow AcCoA + ADP + P_i + OAA$$

Thus, AcCoA and OAA enter the cytosol together. AcCoA is used in the cytoplasm, whereas OAA must be returned to the matrix. What is his fate?

The inner membrane of the mitochondrion means an **impermeable dam** for him. OAA is therefore reduced with the participation of NADH to malate by **cytosolic malate dehydrogenase** :

$$OAA + NADH + H+ \rightarrow malate + NAD+$$

Malate is then **oxidatively decarboxylated** by the $NADP^+$-malate enzyme (the so-called **malic enzyme**) to pyruvate:

$$Malate + NADP+ \rightarrow Pyr + CO2 + NADPH$$

Pyruvate can enter the mitochondrion, where it is carboxylated by pyruvate carboxylase::

$$Pyruvate + CO_2 + ATP + H_2O \rightarrow OAA + ADP + P_i + 2\ H^+$$

Summary transport equation:

$$NADP^+ + NADH + ATP + H_2O \rightarrow NADPH + NAD^+ + ADP + P_i + H^+$$

Regulation of FA formation

The synthesis of fatty acids takes place in a situation where the body has **enough substrates and enough energy**. AcCoA-carboxylase plays a key regulatory role:

- Insulin stimulates FA synthesis by activating carboxylase.
- Citrate activates it - it means enough building units and energy.
- Glucagon and adrenaline have the opposite effect - they **inhibit carboxylase** (through its phosphorylation).
- Palmitoyl-CoA inhibits it - it is a product of FA synthesis, and if it is not removed, there is no need to create another - **feedback inhibition**.
- AMP inhibits it.

Insulin signals the body to get rid of glucose at all costs. When glycolysis and glycogen formation are insufficient, excess glucose is converted to pyruvate, which is irreversibly changed to AcCoA by the pyruvate dehydrogenase reaction. Fatty acids are formed from this. Insulin also strengthens the activity of the pyruvate dehydrogenase complex.

Elongation and desaturation of fatty acids

FA synthase can **only synthesize palmitate**. Other FAs are synthesized by other enzymes. Chain lengthening (elongation) and the formation of unsaturated FAs (desaturation) takes place on the side of the **ER membrane** facing the cytosol and **in the mitochondria**.

A description of the exact course of elongation exceeds the scope of this text. We limit ourselves to noting that it is catalyzed by elongases.

Desaturases introduce **double bonds** into the FA chain (in the cis configuration). Mammals lack the enzymes catalyzing the entry of the double bond beyond C9 fatty acids. New double bonds are always introduced between an already existing double bond and a carboxyl group. Thus, mammals can **not synthesize linoleic acid** (18:2 cis Δ9, Δ12, belongs to ω-6 FA) or **α-linolenic acid** (18:3 cis Δ9, Δ12, Δ15, belongs to ω-3 FA) – both are essential. On

the contrary, we can synthesize arachidonic acid (20:4 cis Δ5, Δ8, Δ11 a Δ14, ω-6 acid – it is formed by desaturation and elongation of linoleic acid), eicosapentaenoic acid (20:5 cis Δ5, Δ8, Δ11, Δ14a Δ17, ω-3 – arises from linolenic acid) či kyselinu dokosahexaenovou or docosahexaenoic acid (22:6 cis Δ4, Δ7, Δ10, Δ13, Δ16a Δ19, ω-3 – again arises from linolenic acid).

As an example, we can cite the formation of oleoyl-CoA (cis Δ9) from stearoyl-CoA:

$$\text{Stearoyl-CoA} + \text{NADH} + H^+ + O_2 \rightarrow \text{oleoyl-CoA} + NAD^+ + 2\ H_2O$$

The description of the exact course is again beyond the scope of this text.

Synthesis of triacylglycerols

In order for the newly synthesized fatty acids to fulfill the role of energy reserves, they must first be converted into **triacylglycerols**. Similar to fatty acids, most TAG is formed in **liver cells and adipocytes**. Their synthesis requires **activated glycerol – glycerol-3-P**, and activated fatty acids. Formation takes place **on the endoplasmic reticulum** .

Glycerol-3-P is produced mainly by reduction of dihydroxyacetone phosphate, the reaction is catalyzed by glyceraldehyde-3-P-dehydrogenase. A second source may be glycerol released by lipolysis. The latter is activated by phosphorylation catalyzed by glycerol kinase. Glycerol-3-P is then gradually combined with two acyl-CoA molecules (catalyzed by acyltransferases), phosphatidic acid is formed. A phosphate group is cleaved from it to form 1,2-diacylglycerol, which is esterified with the help of the last molecule of acyl-CoA – triacylglycerol is formed. TAGs formed in the liver are transported to adipose tissue wrapped in VLDL lipoprotein particles.