

Preclinical Biochemistry: Fat Mobilization, β -Oxidation and Ketone Body Metabolism

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Fats or fatty acids represent an important energy source for the human body. Thus, intake, storage and degradation have to be strictly regulated. Fatty acid synthesis is also possible, which is of little significance in industrial countries, but is vital for a body with too little fat intake.



Fat Mobilization

For the human body to function in the long term, it is important that excessive energy sources (like fatty acids) are stored after food intake in order to be degraded in phases of hunger or at increased need for energy, i.e., during sport. For fatty acids, the storage form is **triacylglycerol (TAG)**.

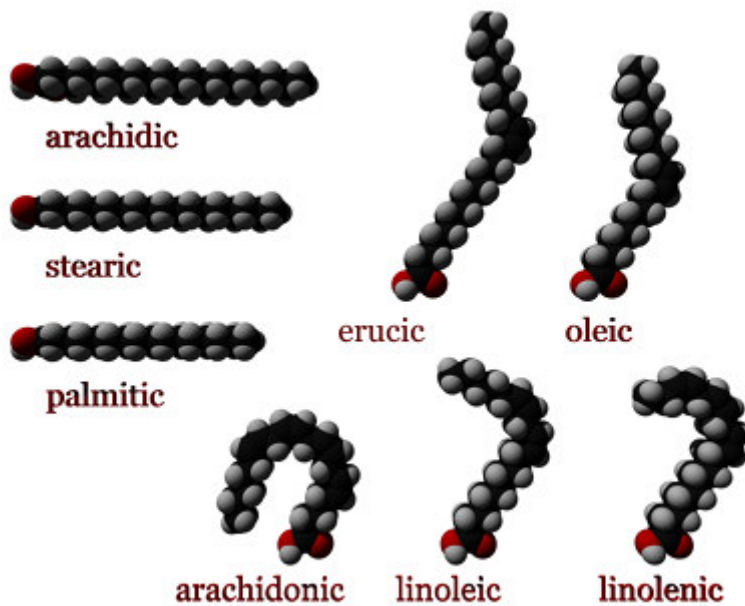


Image: "Three-dimensional representations of several fatty acids" by Lojban. License: [CC BY-SA 3.0](#)

Storage degradation

The fatty acids are stored in the adipocytes as triglycerides and can be degraded at the same location, if needed.

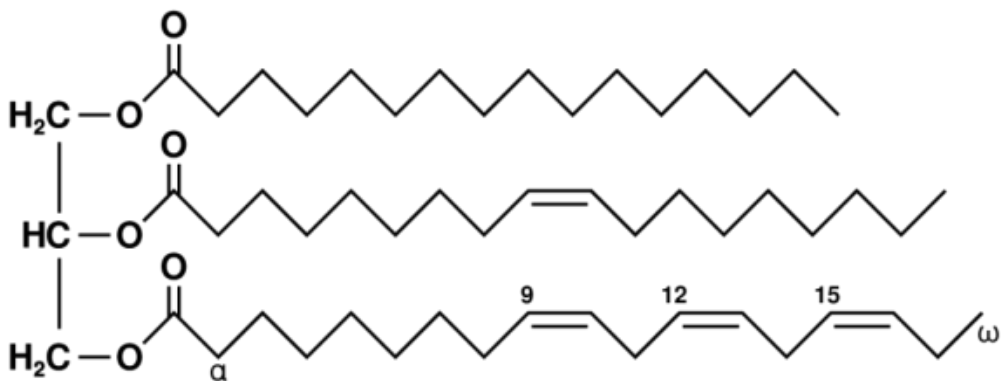


Image: "Line of a fat triglyceride (Strichformel eines Fett-Triglycerides)" by Wolfgang Schaefer. License: Public Domain.

Fatty Acid Degradation and β -oxidation

After triacylglycerides are degraded to fatty acids and glycerol, the free fatty acids can further be used for energy extraction. While the TAGs are degraded in the cytosol of the cells, fatty acid degradation and oxidative energy extraction occur in the mitochondria, the so-called powerhouses of the cells.

Before the process begins, however, the fatty acids have to be imported into the

mitochondria. Small fatty acids with less than 10 C atoms can probably diffuse freely through the mitochondrial membrane, whereas larger fatty acids need transport enzymes.

Fatty acid import into the mitochondria

Step 1

Before fatty acids can pass the mitochondrial membrane, they are activated with the **coenzyme A (CoA)**, thus forming Acyl-CoA. This reaction is catalyzed by **Acyl-CoA-synthetase**, consuming ATP.

Step 2

Carnitine acyltransferase I (or carnitine palmitoyl transferase I) transfers the acyl group to carnitine. **Acylcarnitine** forms, releasing CoA.

Step 3

Translocase can transport the resulting acylcarnitine into the mitochondrion matrix via the mitochondrial membrane, in exchange for carnitine.

Step 4

In the matrix, there is a second transferase, the **carnitine acyltransferase II**, which again transfers the acyl group to CoA. Thus, carnitine is free and can be used for another exchange with acylcarnitine for the translocase.

β -oxidation

In the mitochondrial matrix, acyl-CoA can be further broken down to **acetyl-CoA**. This degradation is performed via a metabolic cycle called **β -oxidation**, since the oxidation occurs at a β -carbon atom.

Step 1

Acyl-CoA is oxidized to **enoyl-CoA** under the conversion of **FAD (flavin adenine dinucleotide)** to **FADH₂**. This reaction is catalyzed by **acyl-CoA-dehydrogenase**.

Step 2

Then, enoyl-CoA is hydrated to **hydroxyacyl-CoA** via **enoyl-CoA-hydratase**.

Step 3

At this point, the second oxidation reaction occurs: **Hydroxyacyl-CoA** is oxidized to **ketoacyl-CoA** via hydroxyacyl-CoA-dehydrogenase. During this process, **NAD⁺** is converted to **NADH** and **H⁺**.

Step 4

A **ketothiolase** catalyzes the next reaction. This happens as the thiol group of another CoA molecule is transferred to the ketoacyl-CoA. Thus, acetyl-CoA is split off and an acyl-CoA that is now shorter by two carbon atoms remains. The latter can enter another oxidation cycle.

Even and saturated fatty acids

Even and saturated fatty acids can be completely degraded to acetyl-CoA via the four steps of β -oxidation mentioned above. This is the simplest form of degradation. The same scheme also occurs in the degradation of uneven or unsaturated fatty acids. However, further intermediate steps and enzymes are necessary.

Uneven fatty acids

At first, uneven fatty acids are oxidized just like the even fatty acids, according to the four steps mentioned above. This continues until the point where **propionyl-CoA** remains after acetyl-CoA has been split off in the last step. Via several additional steps, it can be converted into **succinyl-CoA**, which then enters the citric acid cycle.

Step 1

Under ATP consumption and via the propionyl-CoA-carboxylase, **propionyl-CoA** is carboxylated to **methylmalonyl-CoA** in the form of the D-isomer.

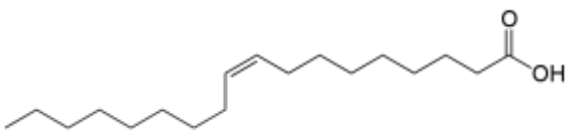
Step 2

The D-isomer of methylmalonyl-CoA is epimerized to its L-isomer.

Step 3

The L-methylmalonyl-CoA is converted to **succinyl-CoA** via a **mutase**. This last step is vitamin B₁₂-dependant since it is an intramolecular rearrangement.

Unsaturated fatty acids



Unsaturated fatty acids have double bonds, which frequently occur in the cis-configuration. They need special enzymes to be degraded. β -oxidation of an unsaturated fatty acid begins just like it does with even fatty acids until the cycle reaches the double bond. An isomerase converts the cis-double bond into a trans-double bond, so that the enoyl-CoA hydratase can continue the normal cycle of β -oxidation.

For the degradation of multiple unsaturated fatty acids, reductases are also needed, since molecules with two double bonds cannot simply be degraded. These reductases are able to form single bonds under NADPH consumption, so that multiple unsaturated fatty acids can be degraded via β -oxidation.

Energy balance

In one oxidation cycle, acetyl-CoA, FADH₂, and NADH and H⁺ form as mentioned above. However, 2 ATPs are used to regenerate AMP to ATP for the activation of the fatty acids in the cytosol.

For example, the degradation of C16 palmitic acid results in 7 oxidation cycles. This means that 8 acetyl-CoA, 7 FADH₂, and 7 NADH and H⁺ form. Acetyl-CoA is further processed in the citric acid cycle, while NADH is further utilized in the respiratory chain.

One mole palmitic acid eventually leads to 106 moles ATP.

Ketone Body Metabolism

The acetyl-CoA resulting from the degradation of the fatty acids, would, at one point, enter the citric acid cycle. In order for this to happen, acetyl-CoA has to be bound to oxaloacetate. Oxaloacetate forms as a product of glycolysis. If the number of carbohydrates that are taken in is insufficient, or if oxaloacetate is used for gluconeogenesis in the state of hunger, an imbalance of acetyl-CoA and oxaloacetate develops.

As a result, acetyl-CoA cannot enter the citric acid cycle. Thus, in this situation, it is converted into the so-called ketone bodies, which consist of acetoacetate, D-3-hydroxybutyrate and acetone. You can read how [ketone body synthesis](#) proceeds, and which tissues rely on it.

Fatty Acid Synthesis

In most situations – at least in the industrial countries – the body can cover its need for fats via food intake. Still, the synthesis of fatty acids out of acetyl-CoA is possible. In contrast to the fatty acid degradation, the synthesis occurs in the cytosol, so Acetyl-CoA has to be transported from the mitochondrion to the cytosol.

This takes place via the citrate-malate-shuttle: Acetyl-CoA is transferred to oxaloacetate, resulting in citrate. In exchange with a molecule of malate, it is transported into the cytosol via the mitochondrial membrane.

Fatty acid synthase

In the cytosol, the fatty acids are synthesized by an enzymatic complex, the **fatty acid synthase**. It is a homodimer enzyme with two identical subunits, each of which has several domains with catalytic centers.

Each subunit has two exposed SH-groups, which are located centrally and peripherally of the U-shaped protein complex. The central SH-group is a functional group of phosphopantetheine. It is anchored in the acyl-carrier-protein (ACP) and serves the temporary fixation of intermediate products and the malonyl groups during fatty acid synthesis.

The peripheral SH-group is at the amino terminal domain of the subunits. It accommodates the intermediate products of fatty acid synthesis, while the central SH-group binds a new malonyl group.

Steps of synthesis

Step 1

Under ATP consumption, **acetyl-CoA** is converted to **malonyl-CoA** via **acetyl-CoA-carboxylase**, which contains biotin. In an intermediate step, a biotin-enzyme forms, whose activated CO₂ group is transferred to acetyl-CoA, so that malonyl-CoA forms. This reaction is irreversible and serves as pacemaker reaction of fatty acid synthesis.

Step 2

For the elongation of the chain, **acetyl-CoA** and **malonyl-CoA** are bound to the **acyl-carrier-protein (ACP)**. Thus, **acetyl-ACP** and **malonyl-ACP** form. This step is catalyzed by **acetyl-transacylase** and **malonyl-transacylase**.

Step 3

Acetyl-ACP and malonyl-ACP are then condensed in a reaction that is catalyzed by **β -ketoacylsynthase**. Thus, **acetoacetyl-ACP** forms.

Step 4

With the help of NADPH as a reduction equivalent, acetoacetyl-ACP is reduced to **D-3-hydroxybutyryl-ACP**.

Step 5

The next reaction is catalyzed by **3-hydroxyacyl-dehydratase**. **Crotonyl-ACP** forms as water is split off from D-3-hydroxybutyryl-ACP. Thus, this step realizes the process of dehydration.

Step 6

With the help of **enoyl-reductase**, crotonyl-ACP is converted to **butyryl-ACP**. This reaction is a reduction which uses NADPH as reduction equivalent. Hereby, the first round of the elongation is terminated.

Step 7

For further elongation of the chain, steps 3-6 are repeated: **condensation, reduction, dehydration, reduction**. **Butyryl-ACP** is condensed with malonyl-CoA to ketoacyl-ACP. Eventually, it is provided for the next elongation as acyl-ACP with six carbon atoms.

Step 8

A frequent result of fatty acid synthesis is a chain of 16 carbon atoms. A **thioesterase** can split the ACP off from **C₁₆-acyl-ACP**, resulting in **palmitate** and **ACP**. In order to elongate the chains to more than 16 carbon atoms, further C₂-units of malonyl-CoA molecules are attached to the chain. This reaction is catalyzed in the cytosol by enzymes, which are located in the **endoplasmatic reticulum**.

Synthesis of unsaturated fatty acids

The above-mentioned steps of fatty acid synthesis only lead to the elongation of the chain. The insertion of double bonds depends on other enzymes, which are located at the endoplasmatic reticulum.

This reaction requires NADH and O₂. The complex of catalyzing enzymes contains a **cytochrome-reductase**, the hem-iron **cytochrome b₅** and a **desaturase**. The latter accepts O₂ and transfers it to the fatty acid and H⁺. NADH provides electrons for the O₂-binding to the fatty acid. At first, these electrons are transferred to the cytochrome-reductase.

If the oxygen molecule binds to the fatty acid chain, a double bond forms in the fatty acid, whereby water is split off. Mammals cannot insert double bond further distally than the ninth carbon atom since they lack the appropriate enzymes.

Energy balance

The synthesis of one palmitate requires 8 molecules of acetyl-CoA, 7 ATP, 14 NADPH and 6 H⁺ ions. Besides palmitate, 14 NADP⁺, 8 CoA, 6 water molecules, 7 ADP and 7 Pi form.

Regulation mechanisms

Fatty acid synthesis is regulated via influencing the key enzyme of the synthesis, acetyl-CoA-carboxylase. This enzyme is activated by citrate (substrate) and inhibited by acyl-CoA compounds (products).

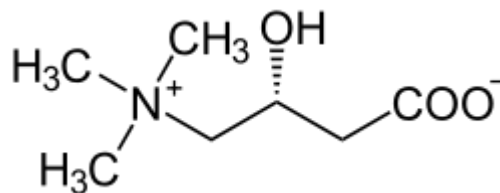
Malonyl-CoA inhibits carnitin-acyltransferase I, which catalyzes the step determining the speed of fatty acid degradation. This prevents the simultaneous synthesis and degradation of fatty acids.

The hormones adrenalin, glucagon and insulin have an effect over the fatty acid synthesis. Adrenalin and glucagon lead to phosphorylation of acetyl-CoA-β-carboxylase, which inhibits it. Insulin activates the enzyme since it mediates dephosphorylation. Thus, insulin has an antagonistic effect concerning adrenalin and glucagon.

Increased occurrence of AMP, which is considered a hunger signal, leads to phosphorylation via an AMP-activated proteinkinase and, thus, to inactivation of acetyl-CoA carboxylase.

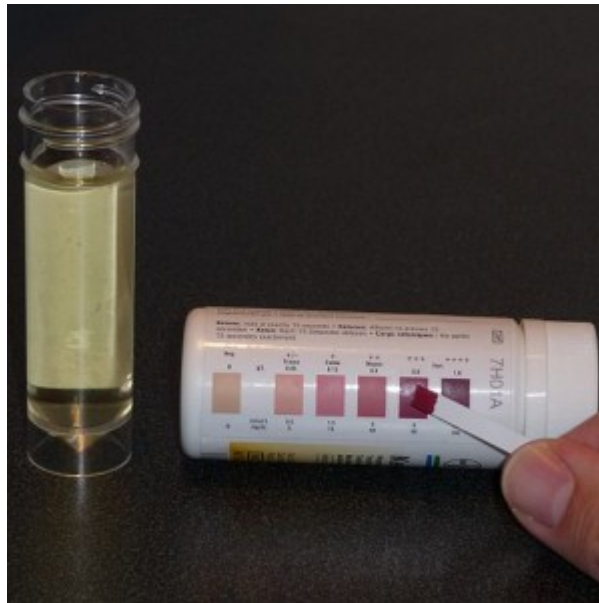
Pathophysiology

Carnitine deficiency



Depending on the severity, a carnitine deficiency presents itself as muscle cramps or states of weakness and can even lead to death. One sign is muscle weakness after long strain, when the muscle relies on long-chain fatty acids as an energy source. These fatty acids, however, cannot be transported into the mitochondria for utilization due to the carnitine deficiency.

Ketonuria



The term ketonuria or acetonuria means the presence of ketone bodies in the urine. This can be an expression of several causes, which can also occur in healthy people in longer catabolic situations. Examples are long fasting, fever or continuous physical strain. Generally, metabolic diseases like diabetes mellitus have to be excluded.

Popular Questions in Exams Concerning Fat Mobilization, β -oxidation and Ketone Body Metabolism

1. Carnitine deficiency most likely leads to...

- A. ...decreased degradation of short- and medium-chain fatty acids.
- B. ...increased degradation of long-chain fatty acids.
- C. ...increased degradation of short- and medium-chain fatty acids.
- D. ...decreased degradation of long-chain fatty acids.
- E. ...weakness at the slightest kind of movement.

2. If a lot of malonyl-CoA is present...

- A. ...degradation of fatty acids is increased.
- B. ...carnitine-acyl-transferase I is stimulated.
- C. ...acetyl-CoA carboxylase is inhibited.
- D. ...fatty acid degradation is inhibited.
- E. ...fatty acid synthesis is inhibited.

3. Which sequence concerning fatty acid synthesis is correct?

- A. Condensation, reduction, dehydration, reduction
- B. Condensation, reduction, reduction, dehydration
- C. Condensation, dehydration, reduction, reduction
- D. Reduction, dehydration, condensation, reduction
- E. Dehydration, condensation, reduction, reduction

References

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Correct answers: 1D, 2D, 3A

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