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

See online here

Fats or fatty acids represent an important energy source for the human body. Thus, their intake, storage, and degradation have to be strictly regulated. Fatty acid synthesis is also possible, which is of little significance in industrial countries where foods with fatty acids are abundant 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 increased need for energy, i.e., during sport. For fatty acids, the storage form is **triacylglycerol (TAG)**.

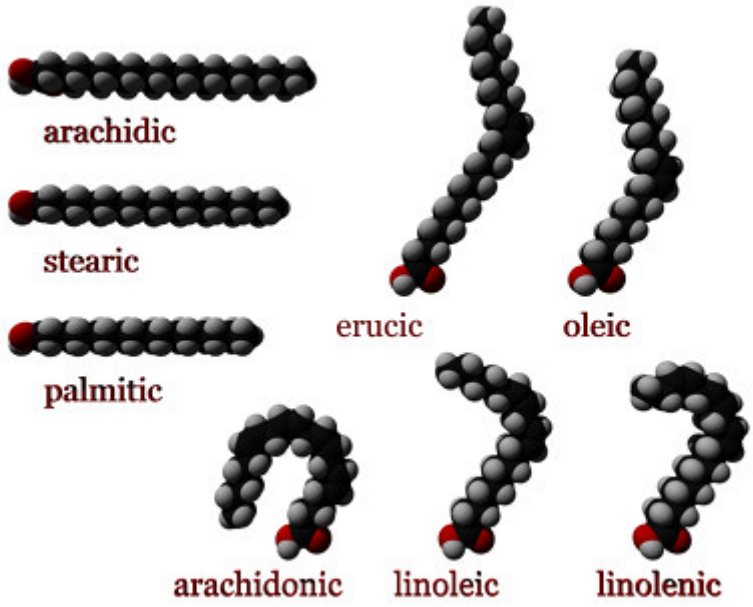


Image: 'Three-dimensional representations of several fatty acids' by Lojban. License: CC BY-SA 3.0

Storage degradation

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

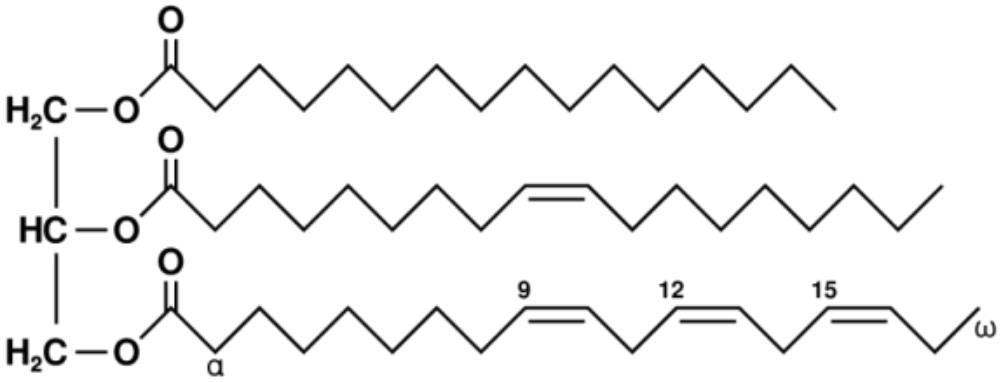


Image: 'Line of a fat triglyceride' by Wolfgang Schaefer. License: Public Domain.

Fatty Acid Degradation and β-oxidation

After TAGs are degraded to fatty acids and glycerol, 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 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 **coenzyme A (CoA),** thus, forming Acyl-CoA. This reaction is catalyzed by **acyl-CoA-synthetase** and consumes ATP.

Step 2

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

Step 3

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

Step 4

In the matrix, there is a 2nd transferase, **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

Enoyl-CoA is then hydrated to **hydroxyacyl-CoA** via **enoyl-CoA-hydratase**.

Step 3

At this point, the 2nd 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 2 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 4 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 4 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 propionyl-CoA-carboxylase, **propionyl-CoA** is carboxylated to **methylmalonyl-CoA** in the form of the D-isomer.

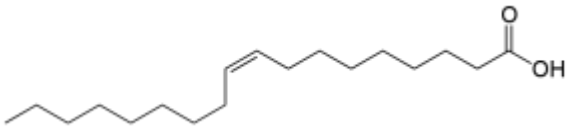
Step 2

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

Step 3

L-methylmalonyl-CoA is converted to **succinyl-CoA** via a **mutase**. This last step is vitamin B12-dependent 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 unsaturated fatty acids 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 2 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+ are formed 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+ are formed. Acetyl-CoA is further processed in the citric acid cycle, while NADH is further utilized in the respiratory chain. One mole of palmitic acid eventually yields 106 moles ATP.

Ketone Body Metabolism

The acetyl-CoA resulting from the degradation of 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 is 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 industrial countries – the body can cover its need for fats via food intake. Still, the synthesis of fatty acids from acetyl-CoA is possible. In contrast to fatty acid degradation, the synthesis occurs in the cytosol, so acetyl-CoA has to be transported from the mitochondria 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, fatty acids are synthesized by an enzymatic complex, **fatty acid synthase**. It is a homodimer enzyme with 2 identical subunits, each having several domains with catalytic centers.

Each subunit has 2 exposed SH-groups that have a central and peripheral location, respectively, with regard to the U-shaped protein complex. The central SH-group is a functional group of phosphopantetheine. It is anchored to the acyl-carrier-protein (ACP) and serves in 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, biotin-enzyme forms, whose activated CO_2 group is transferred to acetyl-CoA, so that malonyl-CoA is formed. This reaction is irreversible and serves as a 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** are formed. 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** is formed.

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-hydroxy acyl-dehydratase**. **Crotonyl-ACP** is formed 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 is a reduction reaction that uses NADPH as a 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, and reduction**. **Butyryl-ACP** is condensed with malonyl-CoA to ketoacyl-ACP. Eventually, it is provided for the next elongation as acyl-ACP with 6 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 that 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 that are located at the endoplasmatic reticulum.

This reaction requires NADH and O2. The complex of catalyzing enzymes contains a **cytochrome-reductase**, the heme-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 O2 molecule binds to the fatty acid chain, a double bond forms in the fatty acid, whereby water is split off. Mammals cannot insert a double bond further distally than the 9th carbon atom since they lack the appropriate enzymes.

Energy balance

The synthesis of 1 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 are formed.

Regulatory mechanisms

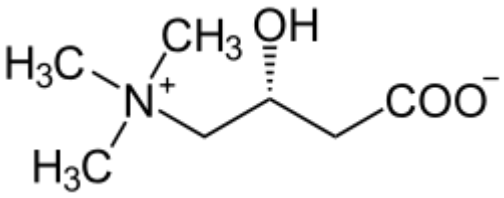
Fatty acid synthesis is regulated by influencing the key enzyme of the synthesis, acetyl-CoA-carboxylase. This enzyme is activated by citrate (substrate) and inhibited by acyl-CoA compounds (products).

Malonyl-CoA inhibits carnitine-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 epinephrine, glucagon, and insulin have an effect on fatty acid synthesis. Epinephrine and glucagon lead to the phosphorylation of acetyl-CoA-ß-carboxylase, which inhibits the enzyme. Insulin activates the enzyme since it mediates dephosphorylation. Thus, insulin has an antagonistic effect with respect to epinephrine and glucagon.

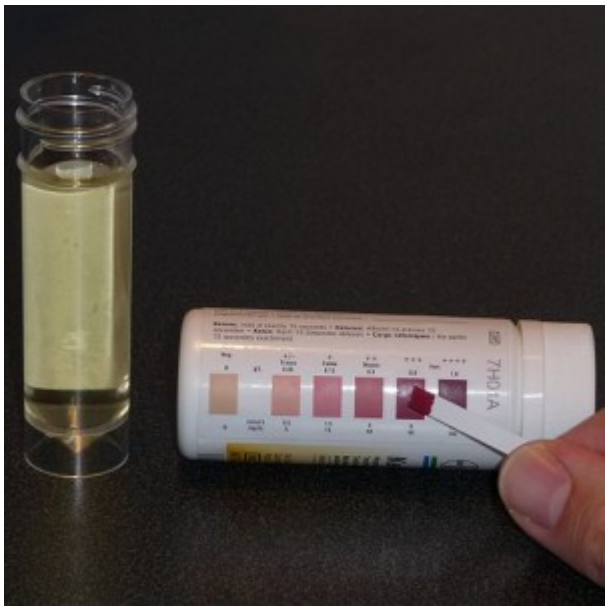
Increased occurrence of AMP, which is considered a hunger signal, leads to phosphorylation via an AMP-activated protein kinase and, thus, to the inactivation of acetyl-CoA carboxylase.

Pathophysiology



Carnitine deficiency

Depending on the severity, carnitine deficiency presents itself as muscle cramps or states of weakness and can even lead to death. One sign of carnitine deficiency is muscle weakness after long periods of 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 undergoing prolonged catabolic situations. Examples are long fasting, fever, or continuous physical exertion. Generally, metabolic diseases like diabetes mellitus have to be excluded.

References

Fukao, T., & Henry Stewart Talks (Firm). (2007). *Inborn Errors of Ketone Body Metabolism*. London: Henry Stewart Talks.

MOBILIZATION OF DEPOT FAT. (2009). *Nutrition Reviews*, *13*(7), 207-209. doi:10.1111/j.1753-4887.1955.tb03484.x

Pathways of nutritional biochemistry: Fatty acid oxidation. (1991). *The Journal of Nutritional Biochemistry*, *2*(4), 185. doi:10.1016/0955-2863(91)90014-v

Ward, C. (2015). Ketone body metabolism (revision number 19). *Diapedia*. doi:10.14496/dia.51040851169.19

Notes