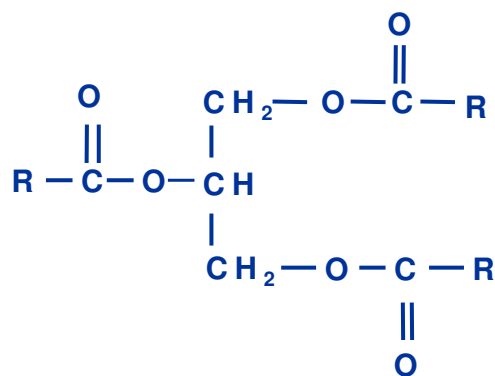


Metabolism (degradation) of triacylglycerols and fatty acids

Jiří Jonák and Lenka Fialová

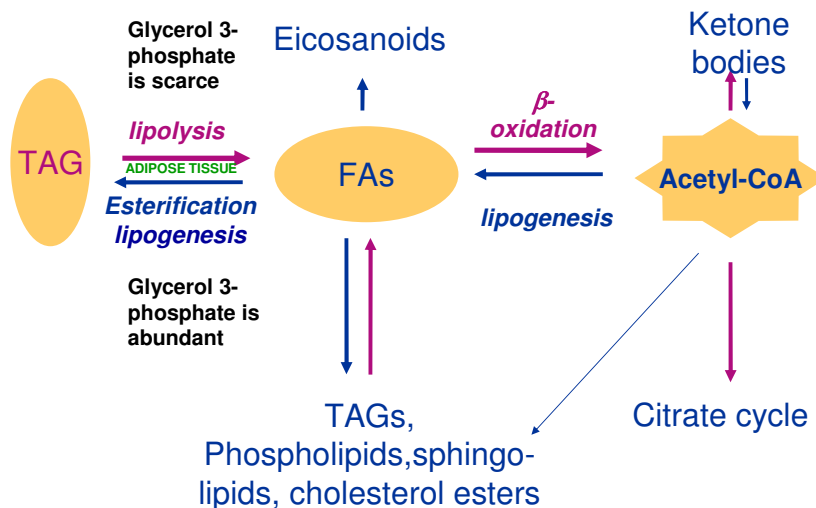
Institute of Medical Biochemistry,
1st Medical Faculty of the Charles University, Prague

Triacylglycerols (TAGs)



TAGs in adipose cells are **continually** being hydrolyzed and resynthesized. Adipose tissue is specialized for the esterification of FAs (**lipogenesis**) and their release from TAGs (**lipolysis**).

Main conversion steps of triacylglycerols and fatty acids: a highly dynamic process



Utilization of fatty acids

1. Mobilization of FAs by hydrolysis of stored TAGs in the adipose tissue: FAs in the circulation →

(FAs are taken up by cells and used for energy production)



2. Activation of FAs – formation of fatty acyl~CoAs (by enzymes in ER and OMM, release to the cytosol)
(Acyl-CoA Synthetase)
3. Transport of fatty acyl~CoAs into mitochondria via fatty acylcarnitine as a carrier; fatty acyl~CoA regeneration
4. Degradation of fatty acyl~CoAs in the matrix of mitochondria: oxidation at β -carbon; production of acetyl~CoA, NADH and FADH₂: **ENERGY PRODUCTION** (mainly in cardiac and skeletal muscle tissues)

Ad 1)

a.) Mobilization of fatty acids from triacylglycerols

- A) During FASTING, during the intensive physical labor and in response to stress condition: release of free FAs from adipose tissue is an adaptation process to provide energy for skeletal and cardiac muscle and also indirectly to the brain via ketone bodies. (Level of insulin falls during fasting; rate of lipolysis=TAG hydrolysis increases; glucose utilization is inhibited)

Controlled by hormones (similarly as the degradation of glycogen)

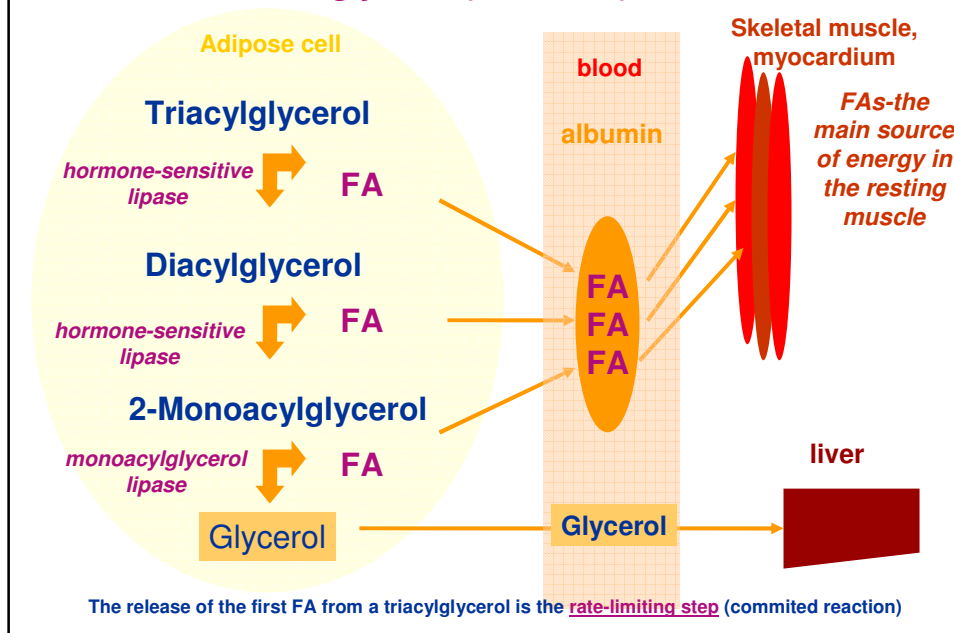
- *FAs mobilization is catalyzed by hormone-sensitive lipases – in adipocytes*
 - *active form* – phosphorylated: LIPOLYSIS
 - Low level of glucose in the blood: *adrenalin (epinephrine), ACTH secretion (stress) and glucagon secretion (fasting, starvation)* → cAMP level increases in adipose cells → protein kinase A activation → lipase phosphorylation
 - *inactive form* – dephosphorylated: LIPOGENESIS
 - High level of glucose in the blood: *insulin secretion* – antilipolytic effect
 - » Inhibition of cAMP synthesis
 - » Stimulation of phosphodiesterase (cAMP breakdown)
 - » Stimulation of protein phosphatase (lipase dephosphorylation)

Ad 1

b.) Mobilization of fatty acids from triacylglycerols

- B) In the FED STATE: lipoprotein lipase on the surface of capillary endothelial cells in adipose tissue and cardiac and skeletal muscles. It hydrolyses TAGs in circulating chylomicrons (a TAG-rich plasma lipoprotein; the TAG originating from food) + VLDL (another TAG-rich lipoprotein, assembled in liver from excess of carbohydrates and amino acids)

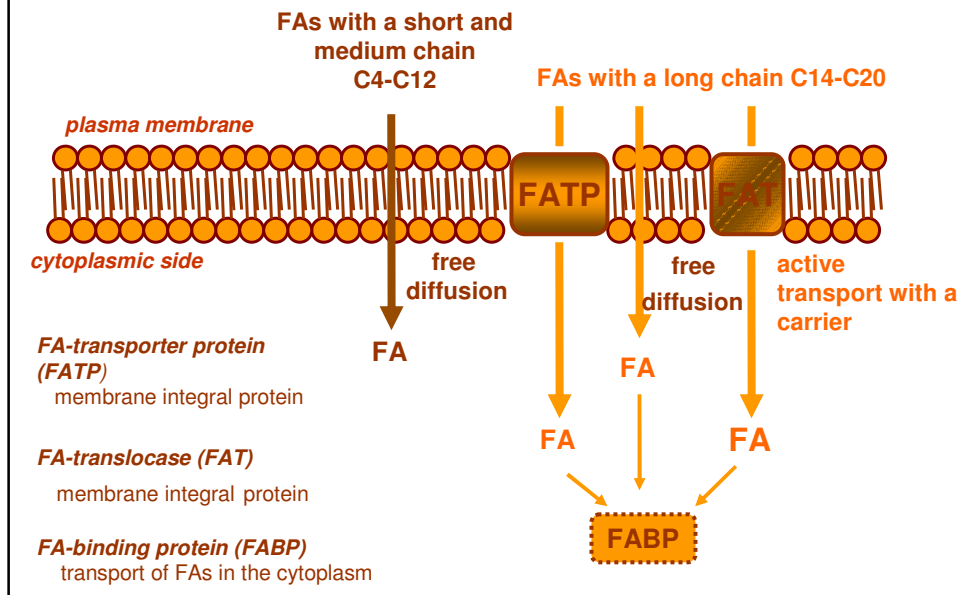
Ad1) Degradation of triacylglycerols to fatty acids and glycerol (a scheme)



Further fate of the mobilized fatty acids and glycerol

- **Products of the lipolysis are released to blood**
 - **glycerol** is transported to liver, phosphorylated by glycerol-kinase to 3-phosphoglycerol and can be utilized for gluconeogenesis and/or lipid synthesis
 - **fatty acids**, complexed to **albumin** ("**free fatty acids**", **10:1**), are transported by blood to tissues - **unesterified, short half-life (minutes)**
 - **ketone bodies** (acetoacetate, **β -hydroxybutyrate**) made from acetyl CoA in liver and kidney mt during fasting are excellent fuels for **many nonhepatic tissues, incl. myocardium, resting skeletal muscles, and brain** (**glucose in short supply-prolonged 2-3 days fasting; insulin deficiency**)
Muscle retains glucose, its preferred fuel for bursts of activity
- **Utilization - Degradation of FAs takes place preferentially in mitochondria, and partially in peroxisomes**

Entry of fatty acids into the cell via the plasma membrane

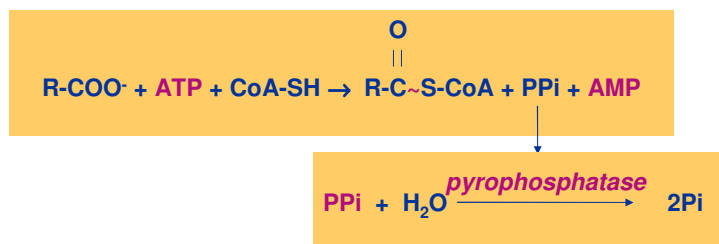


2. Activation of fatty acids

- Conversion of a FA into a fatty acyl CoA thioester (the thioester bond has a high energy transfer potential) - *fatty~acyl CoA*
- Requirements:
 - ATP
 - CoA
 - enzyme *acyl CoA-synthetase (fatty acid thiokinase)*

Acyl-CoA Synthetase

- It catalyses the formation of a *thioester bond* between the carboxyl group of a FA and the -SH group of CoA. This endergonic reaction is driven forward by the consumption of two high energy (phospho-anhydride) bonds of ATP: ATP → AMP, whereas only one high energy bond is formed



- The reaction proceeds in two steps with an intermediate *acyl adenylate*, R-CO-O-AMP ($\text{C}_n\text{H}_{2n+1}\text{-CO-O-5'PO(OH)-O-Adenosine}$)

Acyl CoA Synthetases

<i>substrate specificity</i>	<i>classes</i>	<i>localization</i>
<ul style="list-style-type: none"> Specificity for the length of the fatty chain <ul style="list-style-type: none"> for FAs with a short chain, less than 6 carbon atoms for FAs with a medium chain, 6-12 carbon atoms for FAs with a long chain, 14-20 carbon atoms for FAs with a very long chain, more than 20 carbon atoms 	<ul style="list-style-type: none"> For FAs with <i>a long chain (14-20 carbon atoms)</i> <ul style="list-style-type: none"> In the outer mitochondrial membrane in membranes of the endoplasmic reticulum For FAs with <i>a short and medium chain</i> <ul style="list-style-type: none"> In the matrix of mitochondria 	

3. Transport of fatty acids across the mitochondrial membrane

Fatty acids are activated on the outer mitochondrial membrane, whereas they are oxidized in the mitochondrial matrix

- Activated FAs cross the outer mt membrane through pores
- The inner mt membrane is not freely permeable for activated FAs (for CoA) with a long (more than 12 c.a.) chain:
a special transport mechanism is needed
- They are carried across the inner mt membrane by *carnitine*

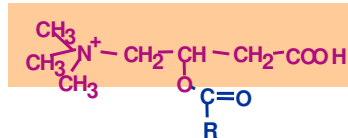
Carnitine

- 3-hydroxy-4-trimethylaminobutyrate, a compound with a quaternary nitrogen
 $(\text{CH}_3)_3\text{N}^+-\text{CH}_2-\text{CH}(\text{OH})-\text{CH}_2-\text{COO}^-$
- only L-isomeric form of carnitine is active
- Sources of carnitine:
 - exogenous – meat and dairy products
 - endogenous – synthesized from lysine and methionine, mainly in brain and kidneys, the synthesis covers the demands
- Carnitine is essential particularly for *myocardium and skeletal muscles*, because one of their main sources of energy are long chain FAs. Carnitine is not required for the permeation of medium chain acyl CoA into mt matrix
- Carnitine is transported into cells by a specific transporter

- **Carnitine deficiency**
 - origin
 - Low-carnitine diet
 - Drugs stimulating excretion of carnitine by kidneys
 - Long-term hemodialysis
 - Inherited – impaired membrane transporter for carnitine
 - effects - phenotype
 - A decreased flow of acyl CoA derivatives of FAs to mitochondria
 - lipid accumulation
 - aching and feeble muscles
 - heart muscle cell impairment
 - Increased utilization of glucose - hypoglykemia
 - therapy – high carnitine diet
- **Carnitine palmitoyltransferase I or II deficiency – a phenotype similar to that of carnitine deficiency**

Carnitine-mediated transport of activated fatty acids across the inner mitochondrial membrane

acyl-CoA – carnitine transesterification (from the sulphur atom of CoA to the 3-hydroxyl group of carnitine) catalysed by a *carnitine palmitoyltransferase I* in the outer mitochondrial membrane

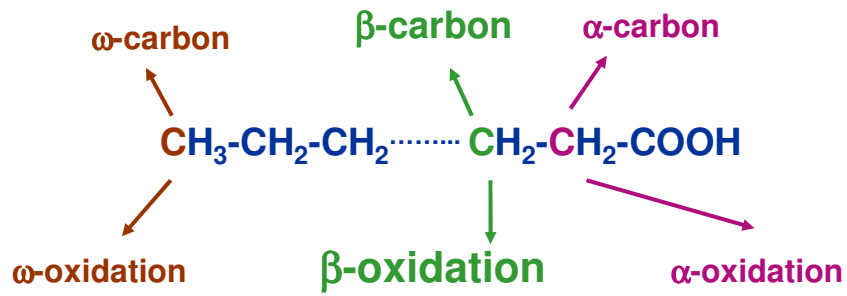


–formation of acyl carnitine is the rate-limiting step of β -oxidation of FAs in mitochondria- inhibition by malonyl CoA (the first intermediate in the biosynthetic pathway of FAs)

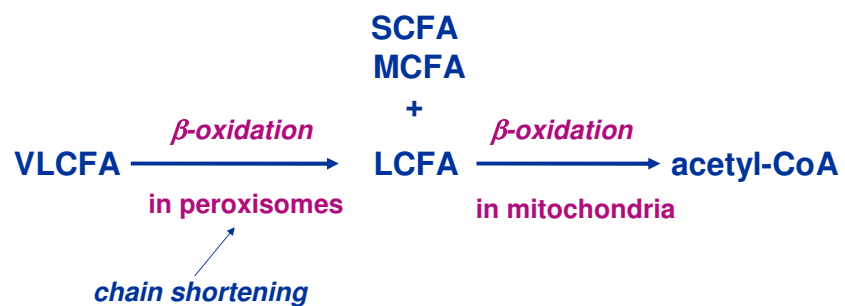
- Acyl carnitine is delivered into the mt matrix by a translocase situated in the inner mt membrane
- On the matrix side of the inner mt membrane the acyl group is transferred back to CoA (acyl CoA regeneration) by a *carnitine acyltransferase II*

4. Degradation of fatty acids: oxidation

Mechanisms



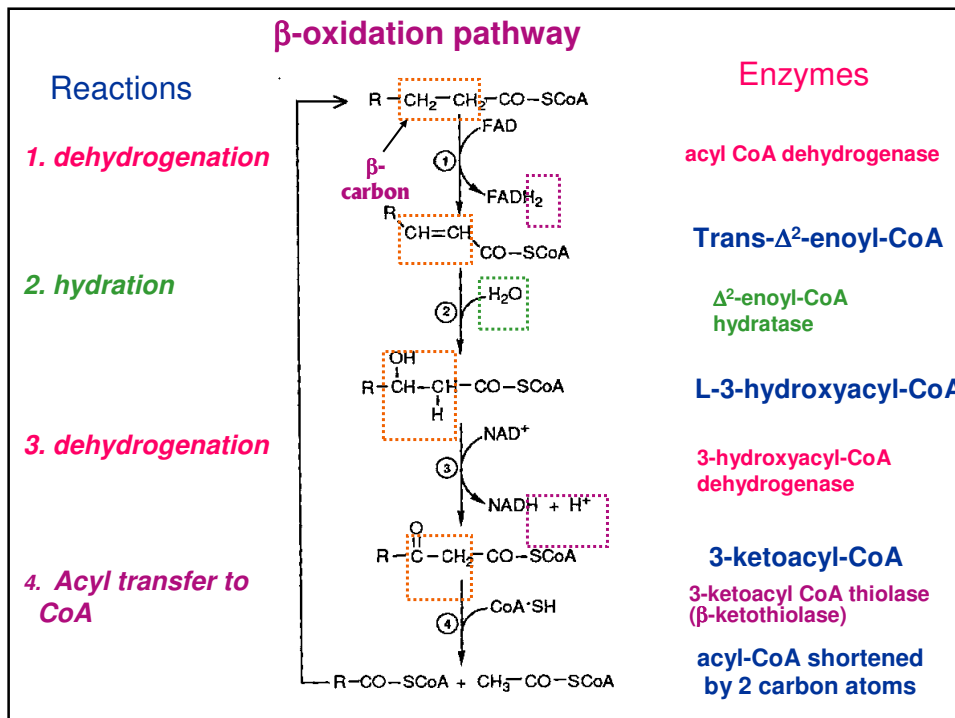
Degradation of fatty acids: β -oxidation



VLCFA- very-long chain fatty acid: > 20C
LCFA-long-chain fatty acid: 14-20C
MCFA -medium-chain fatty acid: 6-12C
SCFA-short-chain fatty acid: < 6C

β -oxidation in mitochondria

- The main degradation pathway of fatty acids (in the form of fatty acyl~CoA)
- aerobic process
 - Direct coupling with the citric acid cycle and the respiratory chain
 - A recurrent sequence of four reactions in which the fatty chain is shortened by two carbon atoms in the form of acetyl CoA:
 - dehydrogenation
 - hydration
 - dehydrogenation
 - thiolysis



β -oxidation

- Oxidation of methylene group at C-3 ($C\beta$) of the FA to a keto group + the cleavage of the 3-ketoacyl CoA by the thiol group of a second molecule of CoA
- The shortened acyl CoA then undergoes another cycle of oxidation starting with acyl CoA dehydrogenation
- Acetyl CoA, NADH, and $FADH_2$ are generated in each round of FA oxidation

Some enzymes of the β -oxidation pathway

Acyl CoA dehydrogenases – *homotetramers* (in the mt matrix) *difference in substrate specificity*

- for (C_4 - C_6) acyl CoAs
- for (C_4 - C_{12}) acyl CoAs
- for (C_8 - C_{20}) acyl CoAs

Trifunctional enzyme – bound in the mt membrane

- Substrate: acyl CoA with a long fatty acid chain
- Activities: Δ^2 -enoyl-CoA hydratase
3-hydroxyacyl-CoA dehydrogenase
3-ketoacyl-CoA thiolase

β-oxidation – resemblance to the last steps of the citric acid cycle

∇ β-oxidation

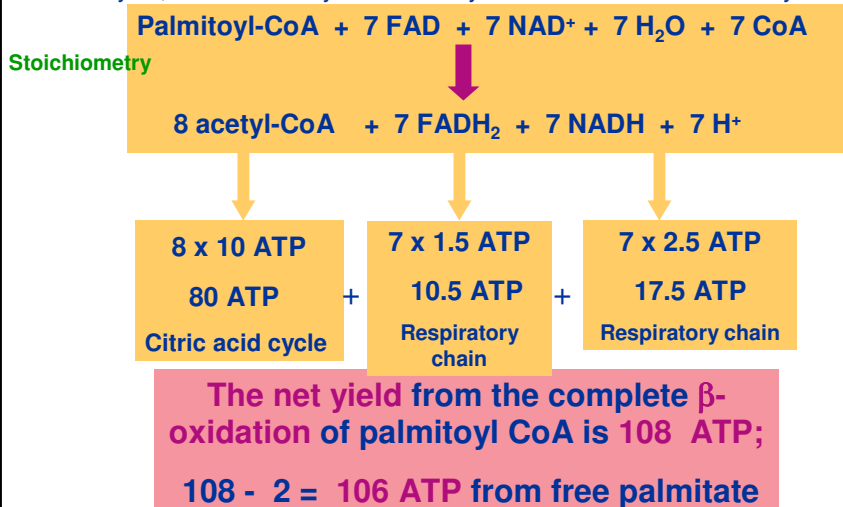
- acyl-CoA
- $R-CH_2-CH_2-CO \approx SCoA$
- **acyl-CoA dehydrogenase**
- unsaturated acyl CoA
- $R-CH=CH-CO \approx SCoA$
- !! Δ^2 **enoyl-CoA hydratase**
- 3-hydroxyacyl CoA
- OH
- |
- $R-CH-CH_2-CO \approx SCoA$
- **3-hydroxyacyl-CoA dehydrogenase**
- 3-ketoacyl-CoA
- O
- ||
- $R-C-CH_2-CO \approx SCoA$

• Citric acid cycle

- succinate
- $HOOC-CH_2-CH_2-COOH$
- **Succinate dehydrogenase**
- fumarate
- $HOOC-CH=CH-COOH$
- **Fumarate hydratase**
- malate
- OH
- |
- $HOOC-CH-CH_2-COOH$
- **Malate dehydrogenase**
- oxalacetate
- O
- ||
- $HOOC-C-CH_2-COOH$

Energy yield derived from the β-oxidation of saturated fatty acids

Oxidation of **palmitoyl CoA** (C16:0 CoA) requires seven reaction cycles. In the seventh cycle, the C4-ketoacyl CoA is thiolysed to two molecules of acetyl CoA



Energy conservation in the fatty acid β -oxidation and complete degradation of glucose – a comparison

- The net yield from the complete β -oxidation of one molecule of palmitate is **106 ATP molecules**
 - The yield per one atom of carbon is:
 $106 : 16 = \mathbf{6.6 \text{ ATP}}$
- The net yield from the complete oxidation of one molecule of glucose is about **31 ATP molecules**
 - The yield per one atom of carbon is:
 $31 : 6 = \mathbf{5.2 \text{ ATP}}$

Conclusion: The net yield of energy per one carbon atom obtained from the oxidation of palmitate is higher than that from the oxidation of glucose

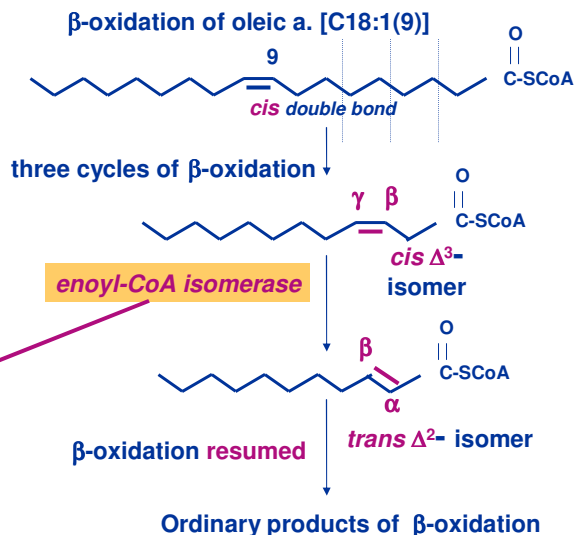
(fatty acids are less oxidized than glucose)

An isomerase is required for the oxidation of unsaturated FAs

- Unsaturated FAs in our body are usually **cis isomers**

cis Δ^3 -isomers are neither a substrate for acyl CoA dehydrogenase nor for Δ^2 -enoyl-CoA hydratase

- Converts **cis-isomer** into a **trans-isomer** and shifts the double bond from the **β - γ** into the **α - β** position to resume the β -oxidation process



Oxidation of unsaturated fatty acids

- The **net yield** from the oxidation of unsaturated fatty acids is always **lower** than that from saturated ones: the acyl CoA dehydrogenation step at the double bond is not required; therefore, no FADH₂ is formed
- A second accessory enzyme **epimerase** is needed for the oxidation of **polyunsaturated FAs** [e.g. C18:2(cis- Δ^6 and cis- Δ^9)]. In the course of the degradation process it catalyses the inversion of the hydroxyl group at C-3 from the D-isomer to the L-isomer.

FAOD-Fatty Acid β -Oxidation Disorders

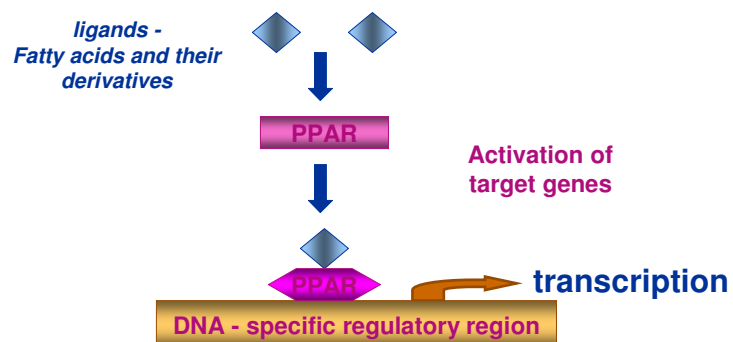
- Triggered off by a catabolic stress (starvation)
- **Biochemical features**
 - Low level production of energetic substrates - acetyl-CoA and ketone bodies
 - Accumulation of free FAs (and toxic intermediates of the acyl-CoA degradation pathway)
 - Formation of abnormal metabolites
 - dicarboxylic and hydroxy dicarboxylic FAs resulting from the ω -oxidation process
 - conversion of acyl-CoA to respective acylglycines
 - Hypoglycemia and a relatively low level of ketone bodies
- **Clinical features**
 - myopathy
 - cardiomyopathy
 - liver dysfunction
 - metabolic coma
 - **SIDS - sudden infant death syndrome**

- *The FAOD disorder is the most frequently caused by the defective acyl CoA dehydrogenase for C₄-C₁₂ acyl CoA*
- *therapy*
 - prevention of catabolic situations, e.g. starvation
 - increased intake of saccharides
 - low level natural fats diet + an adequate intake of essential fatty acids
 - carnitine substitution

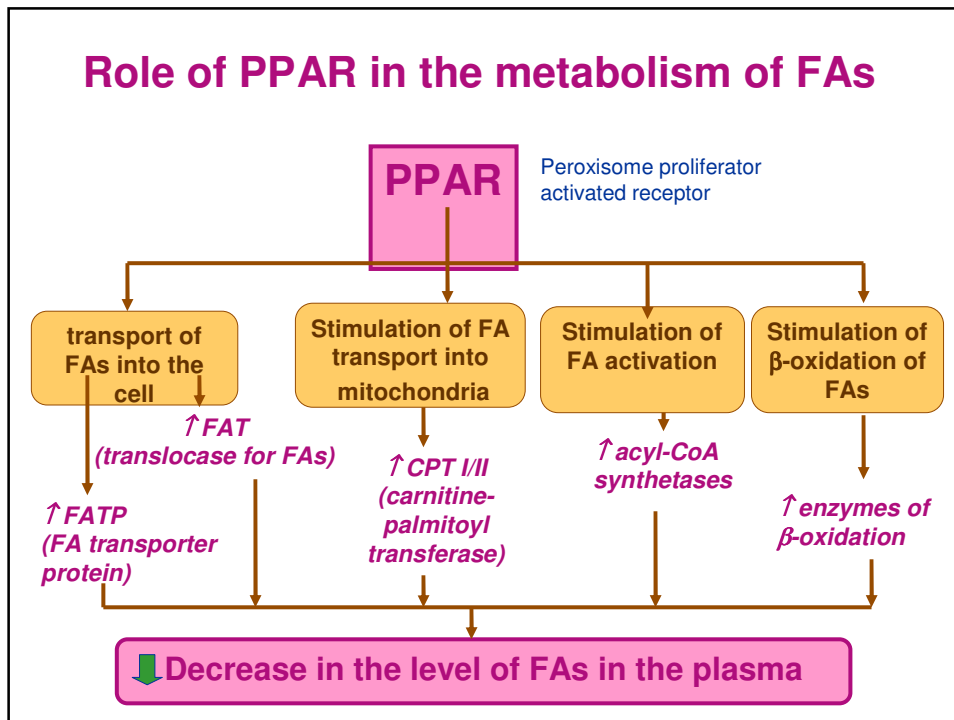
Control of lipid metabolism by peroxisome proliferator activated receptor (PPAR)

PPAR - receptors activated by compounds inducing peroxisome multiplication

- Belong to the family of hormonal nuclear receptors
- The receptors are proteins activated by ligands that change their conformation and then influence DNA transcription in the nucleus



Role of PPAR in the metabolism of FAs



The role of peroxisomes in the oxidation of fatty acids

- Oxidation of FAs with a very long chain (VLCFAs, > 20 carbon atoms) in mitochondria is not efficient
- FA degradation pathways in peroxisomes:
 - !! β -oxidation of VLCFAs (> 18 C – a specific acyl CoA synthetase)
 - !! β -oxidation of some unsaturated FAs
 - Degradation of unusual FAs
 - e.g. C22:1(13) - erukoic acid
 - very slow: deposits of TAGs in the myocardium
 - α -oxidation of FAs with a branched chain
 - Phytanic acid

FA oxidation in mitochondria and peroxisomes: differences in biochemistry

Peroxisomes:

FADH₂, produced in the first oxidation step (dehydrogenation) of the β -oxidation pathway **is directly oxidized by molecular oxygen**, H₂O_{2k} is formed and cleaved into H₂O + O₂ by a *catalase*

- energy loss of 1,5 ATP

NADH, produced in the third oxidation step (dehydrogenation II) of the β -oxidation pathway **is exported into the cytosol**

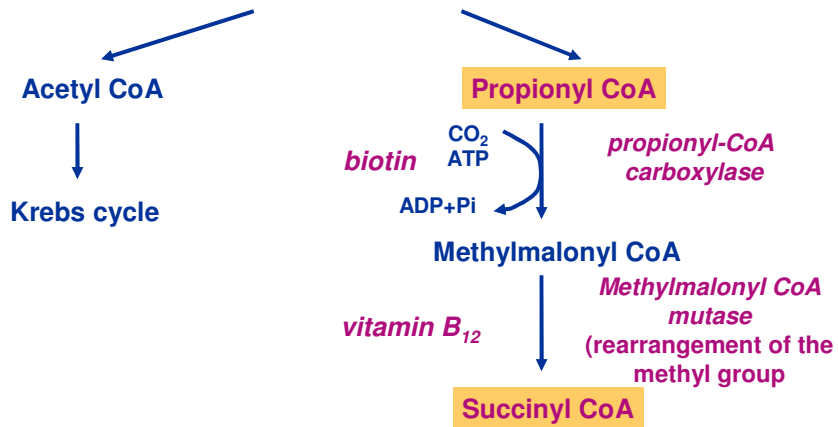
- **enzymes of the citrate cycle are not present in peroxisomes**, generated acetyl CoA can be utilized:
 - for the synthesis of cholesterol and bile acids
 - exported to cytosol/mitochondria and oxidized
- **Acyl CoAs shortened to octanoyl CoA are transported into mt and degraded by β -oxidation**
- **A high-fat diet increases oxidation level in peroxisomes**

Zellweger syndrome (Cerebrohepatorenal syndrome)

- Inherited disease
- **Absence of functional peroxisomes**
 - peroxisomal enzymes deficiency
 - low rate of degradation of VLCFAs + fytanic acid – they accumulate in tissues
- psychomotoric retardation, hepatomegaly, hypotony, dysmorfia craniofacialis
- **70 % of affected children die in the first 6 months of life**

Oxidation of odd-chain fatty acids

β -oxidation pathway is followed up to the last thiolitic cleavage

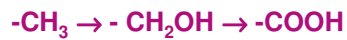


Vitamin B₁₂ deficit – increased excretion of propionic and methylmalonic acids in the urine

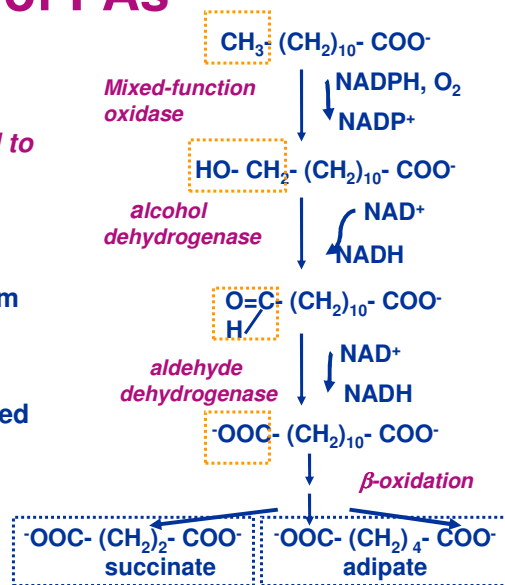
ω -oxidation of FAs

A minor degradation pathway

The ω -carbon atom is oxidized to $-\text{COOH}$



- In the endoplasmic reticulum of *liver and kidneys*
- C10 and C12 fatty acids oxidized preferentially
- **Dicarboxylic acids** are formed (C4 succinate, C6 adipate)
- Ends in β -oxidation



Excreted in the urine

α -oxidation of fatty acids

- A minor degradation pathway for **free (non activated) FAs**
 - COOH is removed as CO₂ (decarboxylation)
 - Not favourable as an energy source
- Degradation of **branched FAs**
 - β -oxidation is blocked by the presence of methyl group on C-3
 - Oxidation of phytanic acid
- Degradation of **odd-chain FAs**
 - oxidized in the same way as FAs having an even number of carbon atoms, except that propionyl CoA and acetyl CoA, rather than two molecules of acetyl CoA, are produced in the final round of degradation
- Degradation of **FAs with a very long chain (VLCFAs)**

Formation of ketone bodies - ketogenesis

Ketone bodies (acetone, acetoacetate and hydroxybutyrate) are formed, mainly **by the liver mt**, from acetyl CoA if fat, fatty acid breakdown predominates and/or the fat and carbohydrate degradation are not appropriately balanced and **oxalacetate becomes depleted (glucose oxidation is suppressed, fat catabolism is accelerated)**.
Overnight fasting: 0.05 mM, 2 day fasting: 2 mM, 40 day fasting: 7 mM

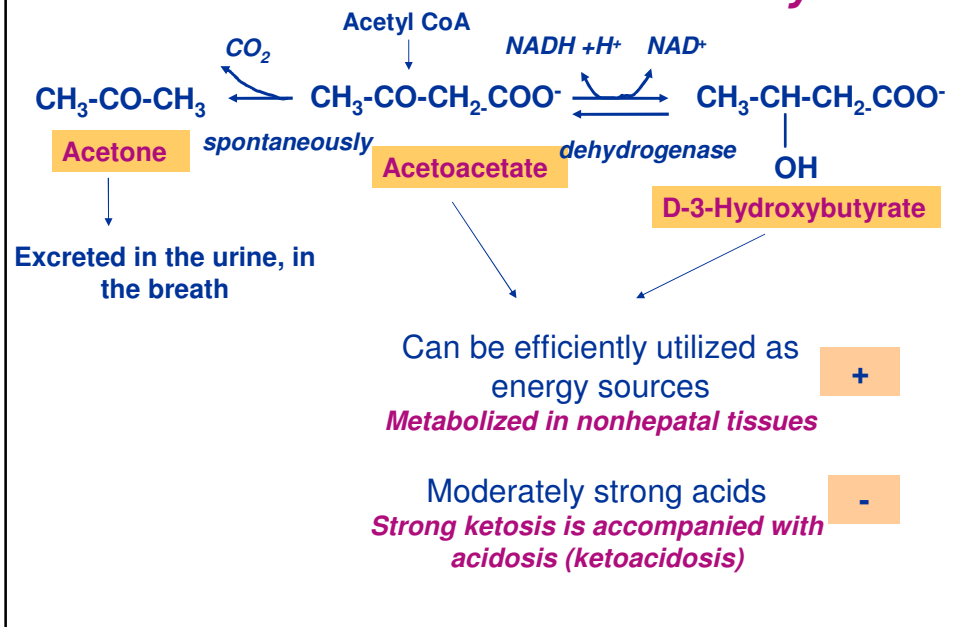
- **This may happen:**
 - **fasting** – oxalacetate is primarily used to form glucose to provide fuel for the brain and other tissues absolutely dependent on this fuel (red blood cells)
 - **diabetes mellitus**
 - **high fat, low carbohydrate diet** (The Atkin's Diet)
- **Physiology of ketone bodies**
 - Ketone bodies (except acetone) are a water soluble “equivalent of FAs” utilizable as an **universal fuel by all cells except hepatocytes**
 - **Saving of glucose** (for neural cells)
 - **Saving of muscle proteins** – gluconeogenesis from amino acids is reduced
 - **Ketonemia, ketonuria**

THESE CHANGES IN FUEL USAGE ARE REFERRED TO AS **KETOSIS:**

a) from fasting (normal ketosis)

b) pathological hyperketonemia of diabetic ketoacidosis

Ketone bodies biochemistry



Ketone bodies formation

matrix of hepatocyte mitochondria

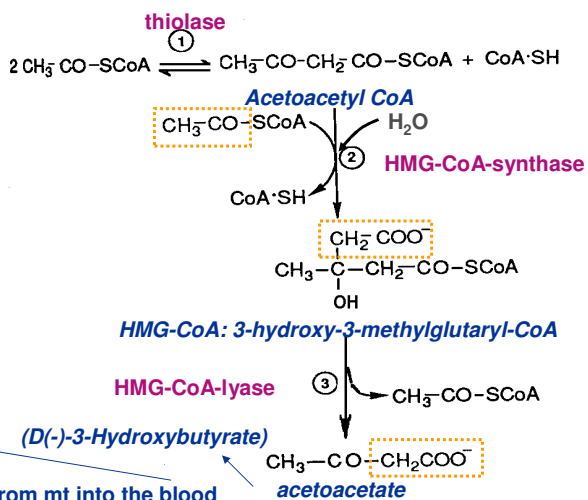
HMG CoA-synthase in mitochondria:

- induced during fasting
- expressed mainly in liver
- controls the rate of ketone bodies formation

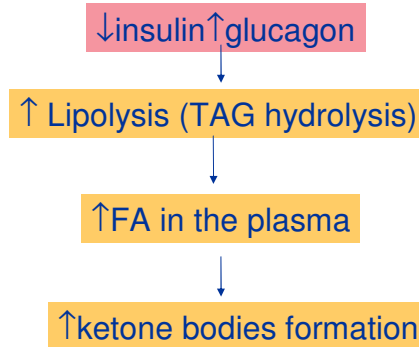
HMG CoA-synthase in the cytosol:

- HMG CoA pool in the cytosol gives rise to mevalonate for the synthesis of cholesterol
- $\text{OHCH}_2\text{-CH}_2\text{-C(CH}_3\text{)OH-CH}_2\text{-COO}^-$

Transported from the liver to other tissues by the blood



Regulation of ketone bodies formation



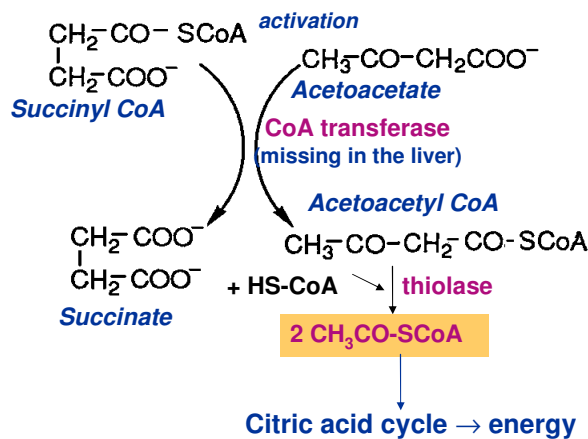
- **Insulin** signals the fed state: **suppressory effect**
- **Glucagon** signals a low glucose in the blood: **stimulatory effect**

Regulation of ketone bodies formation

- **Insulin** signals the fed state: suppresses the formation of ketone bodies and stimulates the formation of glycogen, TAGs and proteins (anabolic effect)
- **Glucagon** signals a low level of glucose in the blood: stimulates the formation of ketone bodies, glycogen breakdown, gluconeogenesis by the liver and TAG hydrolysis (lipolysis) by adipose tissue to mobilize FAs
- **Epinephrine (adrenalin)** and **norepinephrine (noradrenalin)** have effects like glucagon, except that muscle rather than liver is their primary target

Utilization of ketone bodies as an energy source

Utilized by
all tissues
except
hepatocytes



Ketone bodies as fuels

- **Heart muscle and renal cortex** use acetoacetate in preference to glucose
- **Brain:** glucose is the major fuel in well-nourished persons on a balanced diet. However, 75% of the fuel needs are met by acetoacetate and 3-hydroxybutyrate during prolonged starvation (>2-3 days) and in diabetes. FAs do not serve as fuels for the brain because they are bound to albumin and so they do not traverse the blood-brain barrier. Here, ketone bodies are transportable equivalent of FAs.
- **Skeletal muscles:** fuels are glucose (3/4 glycogen body stores), FAs (in resting muscle), ketone bodies