Lipid metabolism I

Triacylglycerols

Biochemistry I
Lecture 8

2008 (J.S.)

Major classes of lipids

Simple lipids **Triacylglycerols**

serve as energy-providing nutrients, the turnover about 100 g per day in an adult person

(Waxes, ceramides)

Complex lipids **Phospholipids**
Glycolipids

- both types are mainly structural components of biomembranes, the turnover about 2 g / d

Derived "lipids" (rather isoprenoid compounds)

Cholesterol and other steroids
Eicosanoids
Carotenoids

Triacylglycerols (as well as free fatty acids and both free and esterified cholesterol) are **very hydrophobic**. They are not soluble in water unless they are emulsified or included in micelles in the presence of tensides.

In the intestine

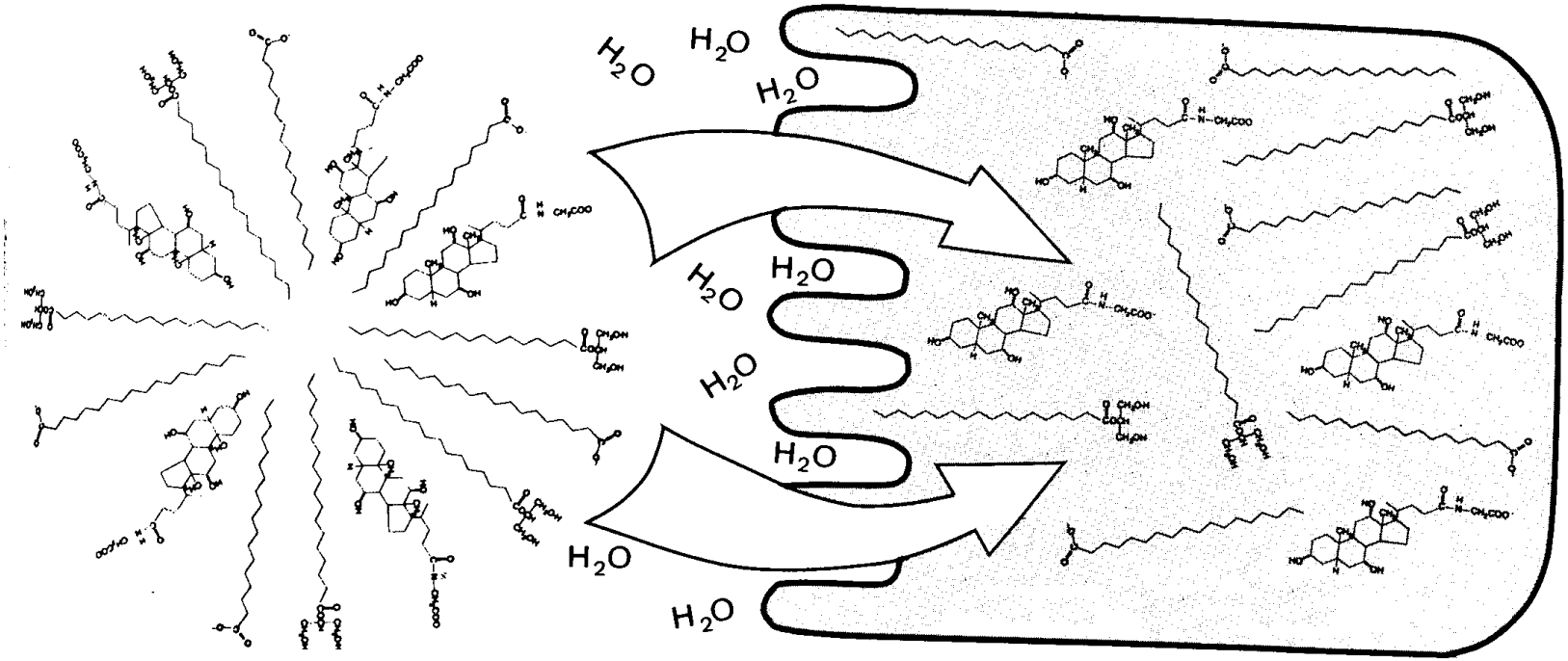
fat droplets are emulsified in the presence of **bile salts** and form **mixed micelles** from the products of digestion catalysed by the **pancreatic lipase**.
Lipid absorption is preceded by dissociation of the micelles and the components are separately absorbed through the brush border microvilli of the epithelial cells (enterocytes) lining the lumen.

In the extracellular fluids

hydrophobic lipids are transported in the form of **lipoprotein particles**

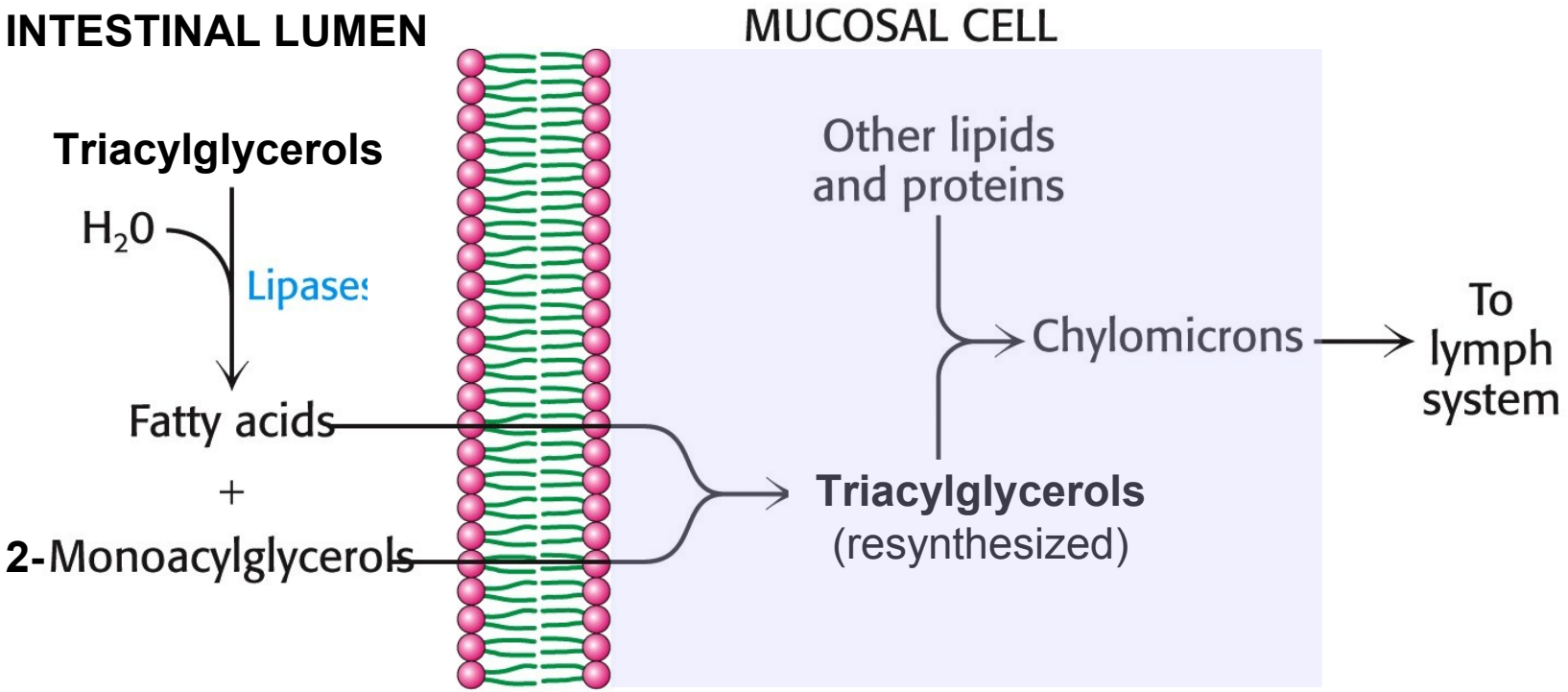
The mixed micelles

in the chyme are composed, in varying proportions, of the fatty acids (FFA), mono- and diacylglycerols (MG and DG), perharps some unhydrolysed triacylglycerol (TG), and anions of bile acids, together with minor components of the diet such as phospholipids and fat-soluble vitamins.



Intestinal lumen **Mucosal cell (enterocyte)**

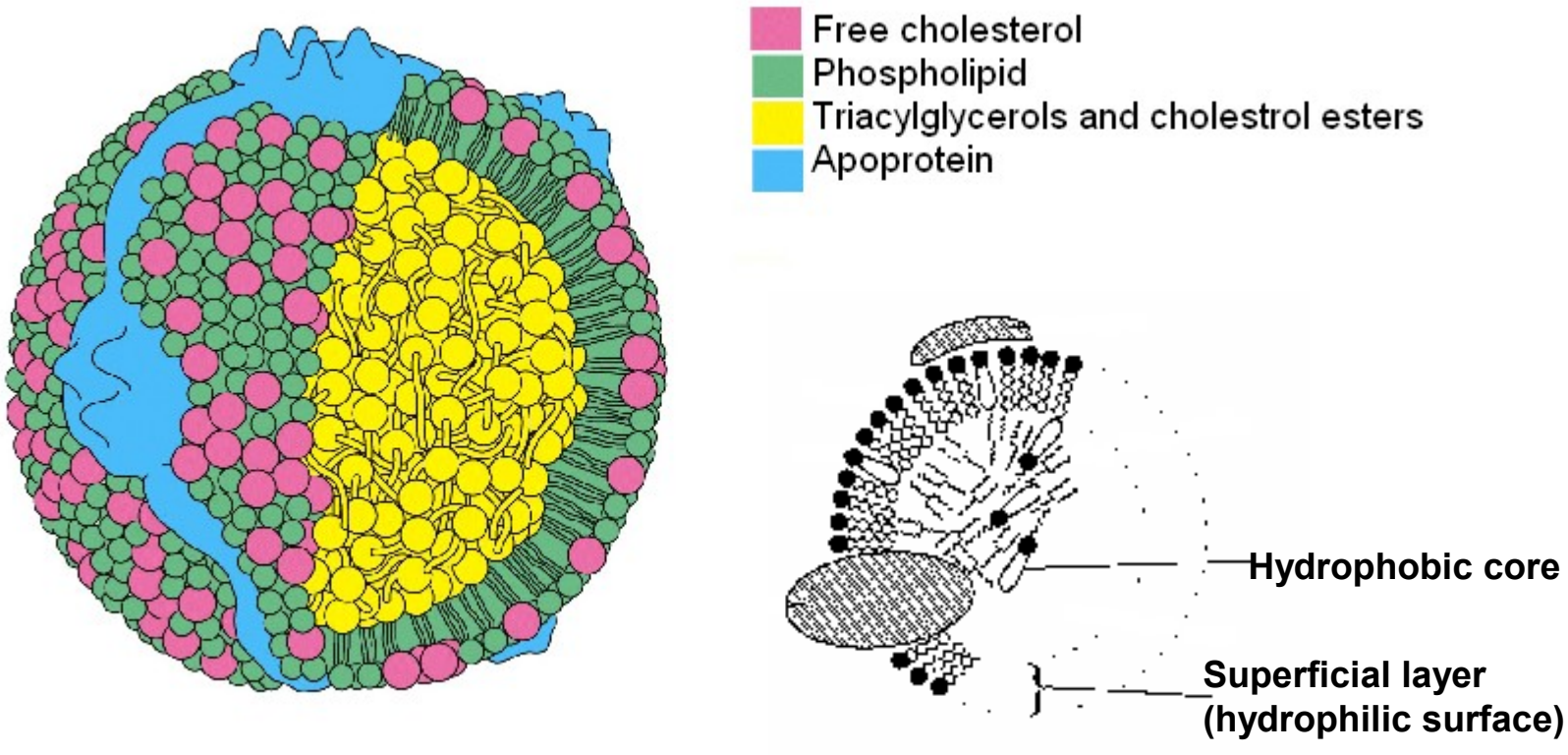
Within the mucosal cells, triacylglycerols are resynthesized (the details are given in the part Synthesis of triacylglycerols) and embodied into chylomicrons - a class of lipoprotein particles.



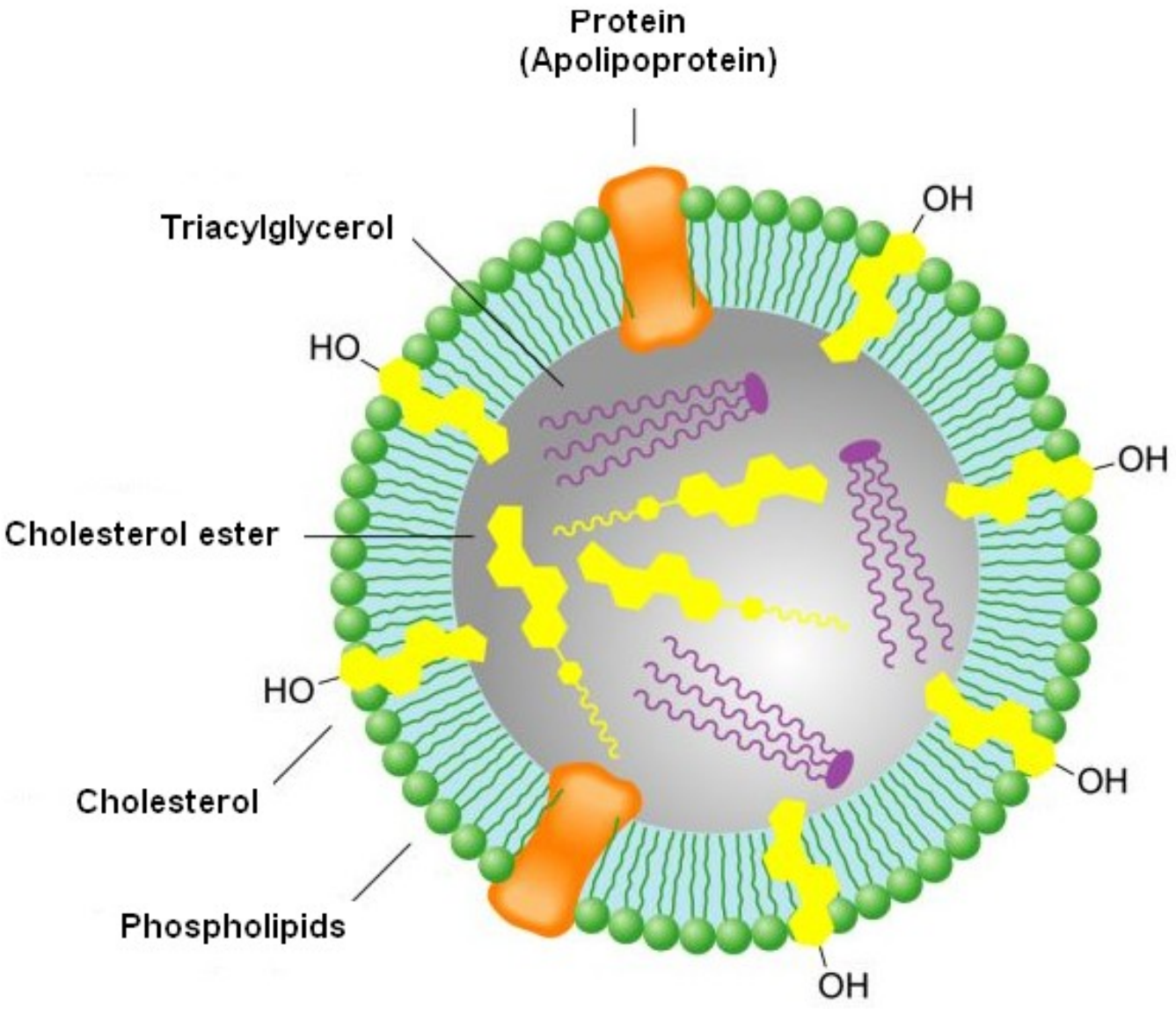
Chylomicrons secreted from the mucosal cells enter the chyle of the lymphatic lacteals. Thoracic duct delivers chylomicrons into the blood.

Lipoprotein particles transport triacylglycerols and cholesterol in body fluids

Common structure of lipoprotein particles:



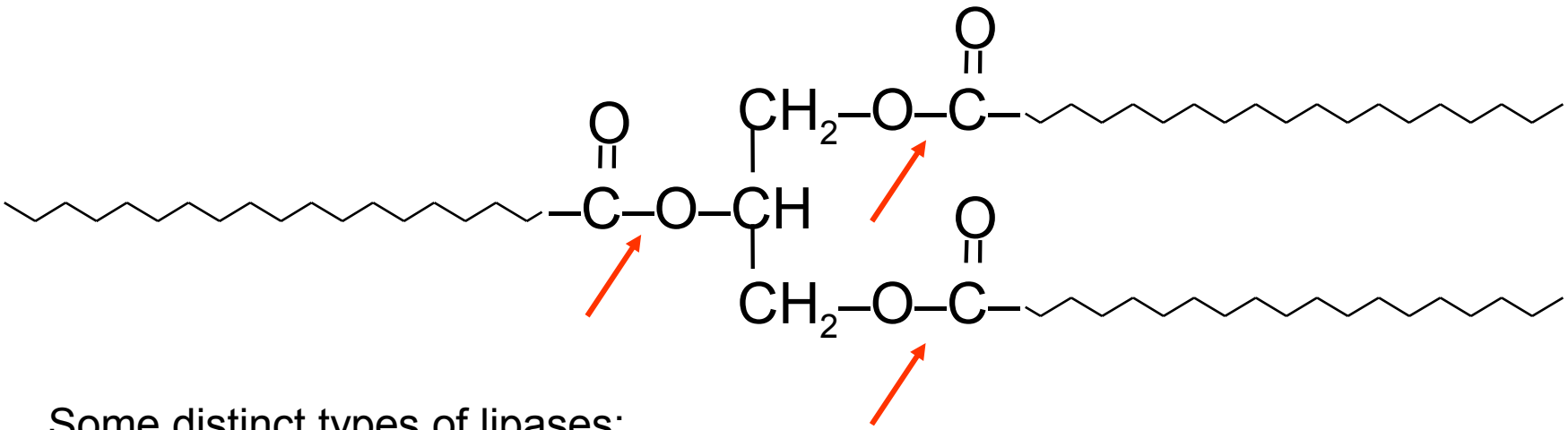
E.g. the diameter of a low-density lipoprotein (LDL) particle is about 30 nm and it consists of about 50 % cholesterol (both free and esterified), 20 % phospholipids, 20 % apoprotein B-100 and 10 % triacylglycerols.


Protein
(Apolipoprotein)
Triacylglycerol
HO
Cholesterol ester
HO
Cholesterol
Phospholipids
OH
OH
OH
OH
OH

Metabolism of triacylglycerols

Lipases

is the group name for enzymes that catalyse **hydrolysis of ester bonds of triacylglycerols** releasing so free fatty acids.



Some distinct types of lipases:

Extracellular lipases

- **Pancreatic lipase** secreted into the duodenum,
- **Lipoprotein lipase** on the surface of the endothelium lining the capillaries

Intracellular lipases

- **„Hormone sensitive" lipase in adipocytes** mobilizing fat stores
- **Lysosomal lipase**

Hormone-sensitive lipase in adipocytes

is an **intracellular lipase** that through **hydrolysis of triacylglycerols** **mobilizes the fat energy reserves** stored in adipose tissue.

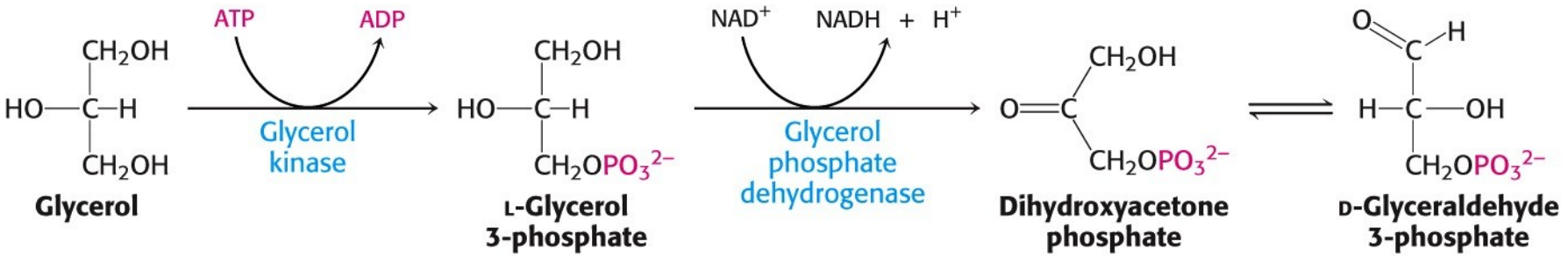
The activity of this lipase is controlled by hormones:.

Glucagon (at low blood glucose) and **adrenaline/noradrenaline** (in stress) cause an increase in lipase activity by its phosphorylation.

Both free fatty acids and glycerol are released into the blood.

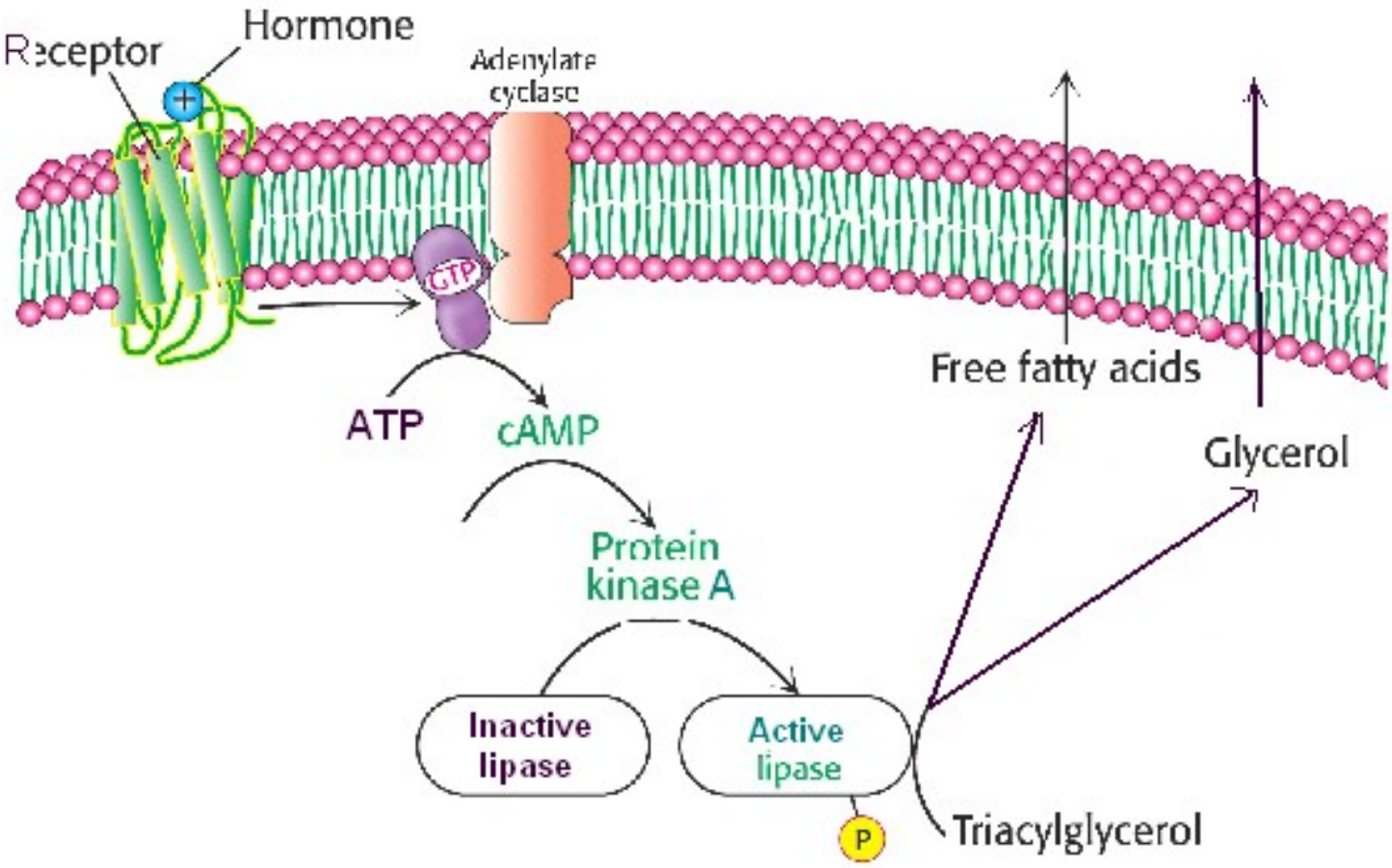
Fatty acids are taken up promptly from the blood plasma by tissues that require nutrients (fatty acids are transported bound to albumin).

Glycerol cannot be utilized in adipocytes (they are lacking in glycerol kinase) and serves as the substrate for gluconeogenesis in the liver:



Insulin exhibits opposite effect: it support dephosphorylation of the lipase in adipocytes and initiates the **synthesis of triacylglycerols.**

Mechanism of the hormone-sensitive lipase activation



Degradation of fatty acids – the β-oxidation pathway

Fatty acids serve as an **energy source** for most of the cells (not for the nervous system and for red blood cells).

The tissues gain fatty acids
- either from lipoprotein particles after the triacylglycerols have been hydrolysed by lipoprotein lipase,
- or as fatty acids mobilized by the action of hormones on the fat stores in adipose tissue and supplied bound onto albumin.

The utilization of fatty acids in the cells requires **three stages of processing**

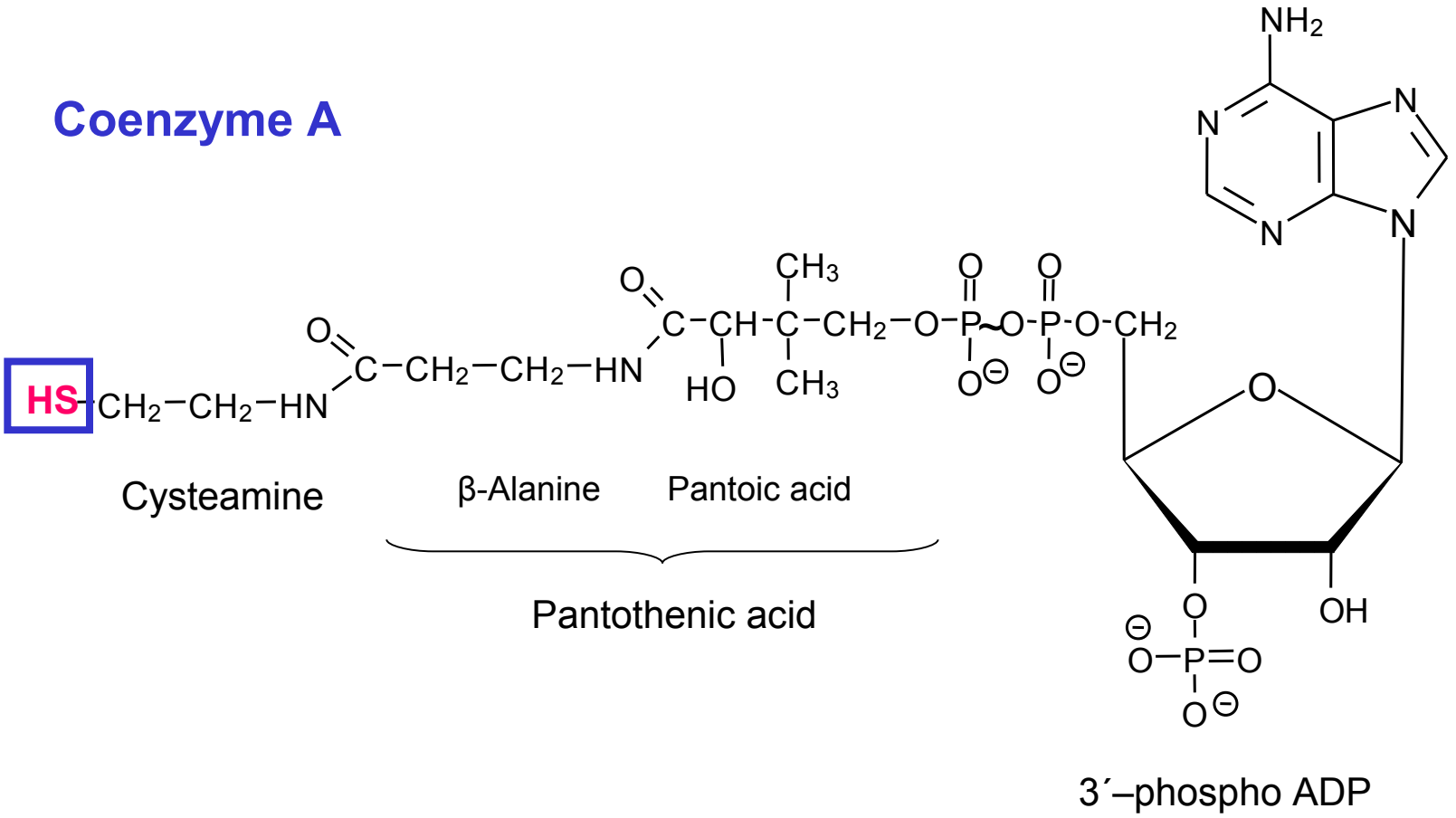
1 **Activation by linking to coenzyme A**,

2 **transport of acyl CoA into the mitochondrial matrix** by conjugating it to carnitine,

3 **β-oxidation of acyl CoA** in the mitochondrial matrix to acetyl CoA that enters the citrate cycle.

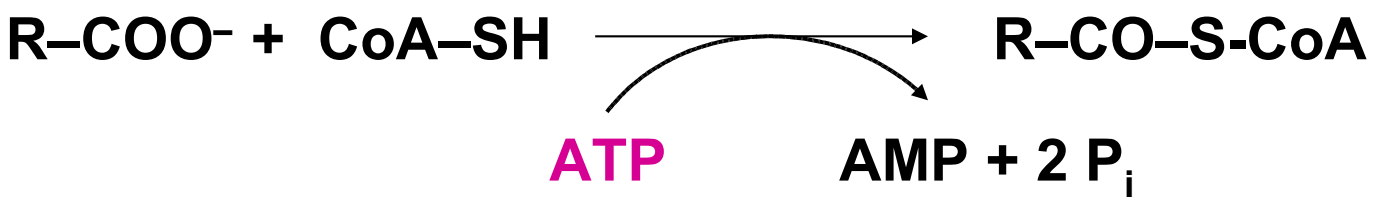
1 Activation of a fatty acid – synthesis of acyl coenzyme A

Coenzyme A



Acyls can be attached to the sulfanyl group by means of a **thioester bond**.

The synthesis of the high-energy acyl-CoA thioester is catalysed by ***acyl-CoA synthetases*:**



Acyl-CoA synthetases are located **on the outer mitochondrial membrane.**

There is a **loss of energy** equivalent to 2 molecules of ATP, because the reaction is made irreversible by the hydrolysis of inorganic diphosphate..

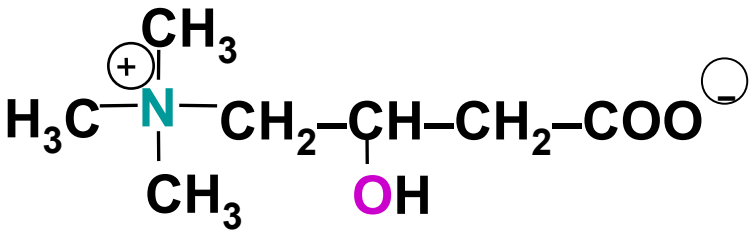
In fact, the activation is accomplished in two steps.
First, the fatty acid reacts with ATP to form an *acyl adenylate.* In this mixed anhydride, the acyl is bonded to the phosphoryl group of AMP.
The sulfanyl group of CoA then attacks the acyl adenylate, which is tightly bound to the enzyme, to form acyl-CoA and AMP.

2 Carnitine carries long-chain activated fatty acids into the mitochondrial matrix

Acyl-CoA itself cannot cross the inner mitochondrial membrane; instead, acyl groups are transferred to **carnitine**, transported across the membrane as **acylcarnitine**, and transferred back to CoA within the mitochondrial matrix.

Short-chain fatty acids (4 – 10 carbon atoms) **do not** require the carnitine shuttle, they can cross the inner mitochondrial membrane..

Carnitine



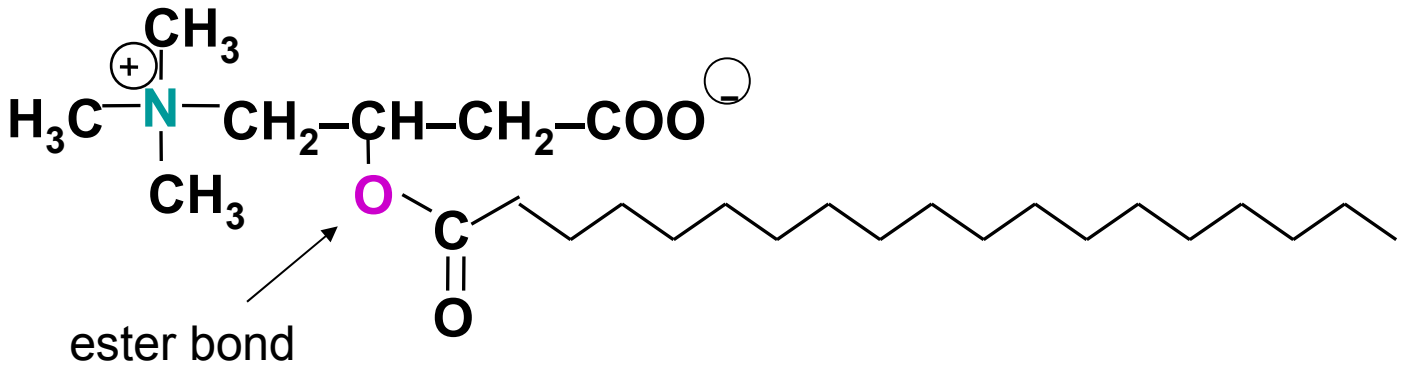
Trimethyl(2-hydroxy-3-carboxypropyl)ammonium

(Carnitine may be also seen as β-hydroxybutyric acid, to which trimethylammonium group was attached.)

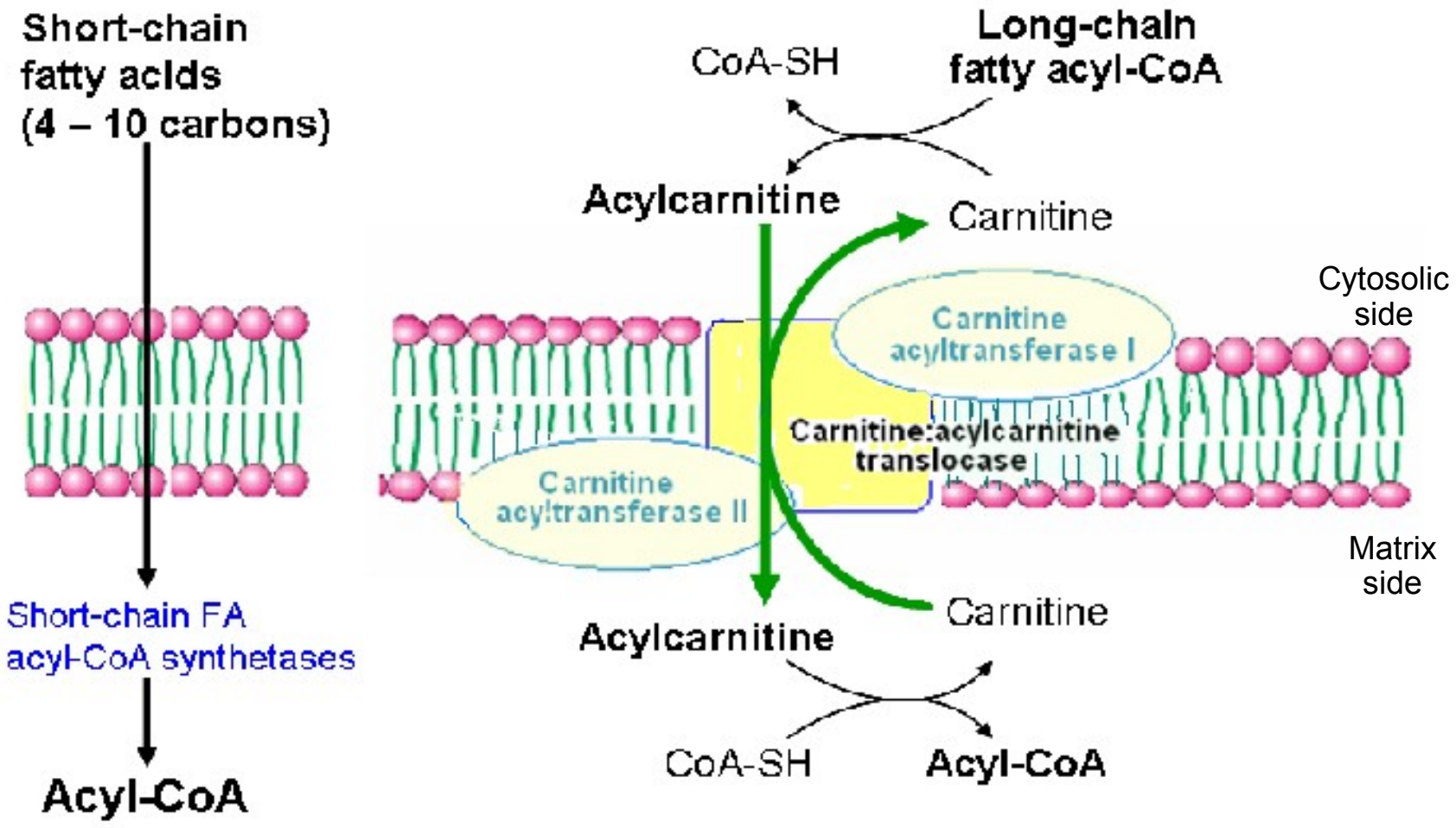
Carnitine is **synthesized from lysine** bound in body proteins.
Daily intake in the food is about 100 mg / d (meat, milk and other foodstuffs of animal origin).
There is no reliable evidence that supplementation of food with carnitine increases muscle strength.

The transfers of acyls from acyl-CoA to carnitine and from acylcarnitines to CoA are catalysed by ***carnitine acyltransferases I and II***.

O-Acylcarnitine



Carnitine shuttle
of the inner mitochondrial membrane



3 The β-Oxidation of acyl CoA

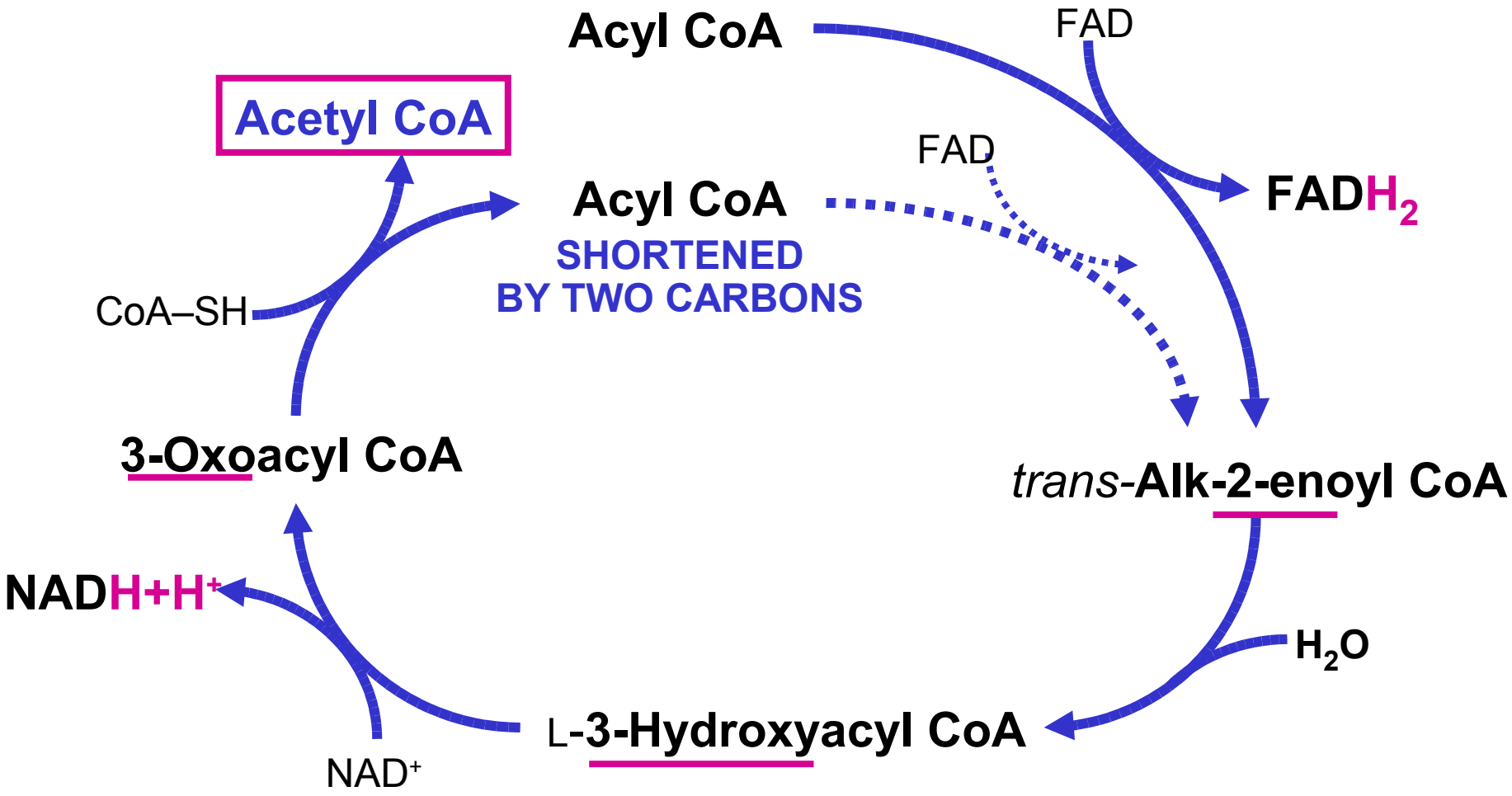
Fatty acyl CoAs are degraded in the mitochondrial matrix by the repetition of a **recurring sequence of four reactions**:

- **dehydrogenation by FAD,**
- **hydration,**
- **the second dehydrogenation by NAD^+,** and by
- **thiolysis by CoA.**

As a result of these reactions, the fatty acyl chain is

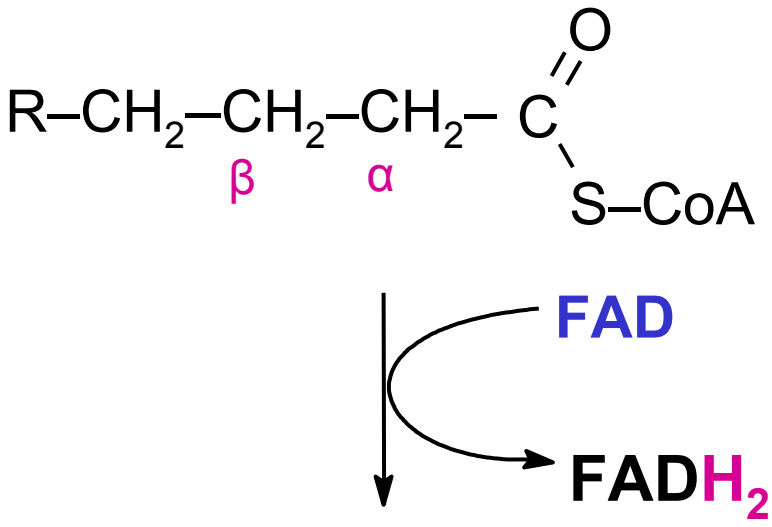
- **shortened by two carbon atoms, and**
- **$FADH_2$, NADH + H^+, and acetyl CoA are generated**.

This series of reactions is called the β-oxidation pathway, because oxidation is on the **β carbon**.


Acyl CoA
FAD
FADH2
Acetyl CoA
FAD
Acyl CoA
SHORTENED
BY TWO CARBONS
CoA–SH
3-Oxoacyl CoA
trans-Alk-2-enoyl CoA
NADH+H+
H2O
L-3-Hydroxyacyl CoA
NAD+

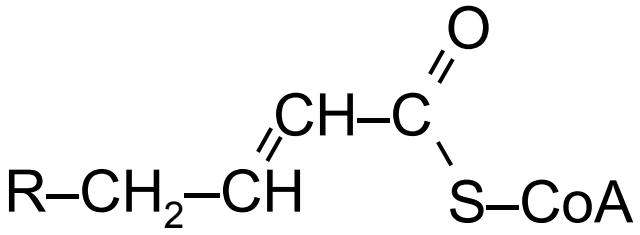
The first dehydrogenation

Saturated acyl CoA



α,β-Unsaturated acyl CoA

(2,3-unsaturated)

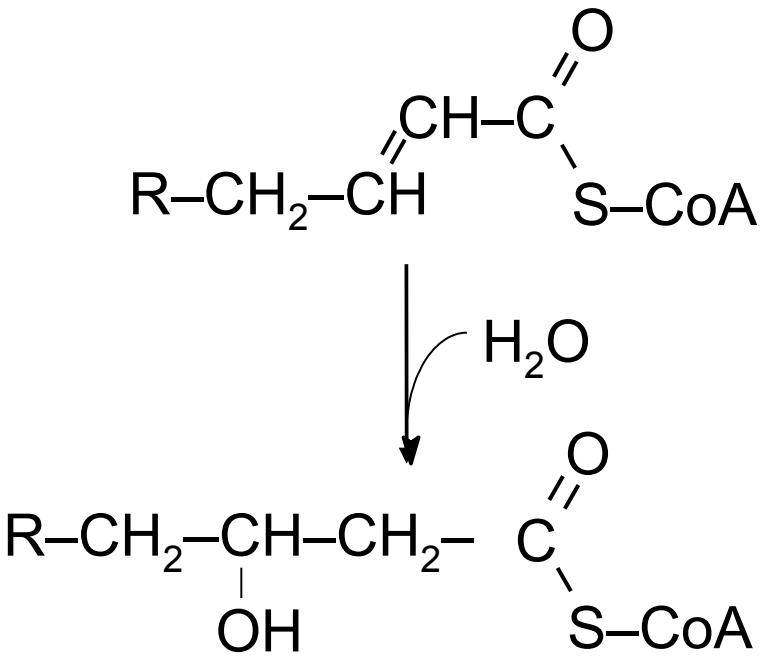


Configuration *trans*

The reaction is catalysed by ***acyl CoA dehydrogenase*** that is the component of the complex II of the terminal respiratory chain.

Hydration of the double bond between C-2 and C-3

α,β-Unsaturated acyl CoA



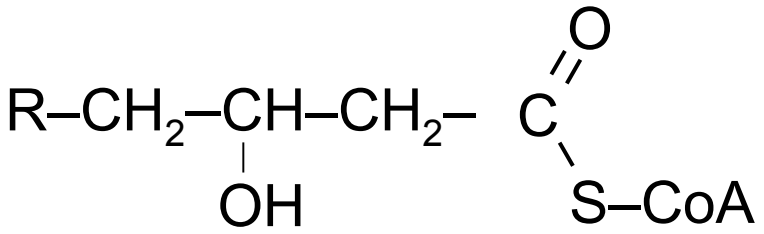
β-Hydroxyacyl CoA
(L-3-Hydroxy)

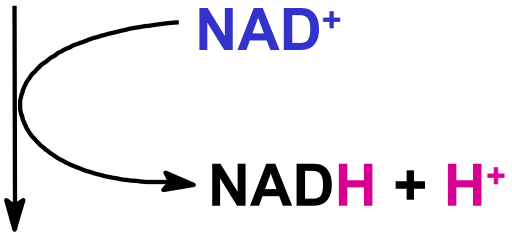
The reaction is catalysed stereospecifically by ***enoyl CoA hydratase***. The enzyme also hydrates a *cis*-double bond, but the product is then the D isomer.

Hydration is **not a redox reaction**, by addition of water to a double bond the sum of the oxidation numbers of both carbon atoms remain the same.

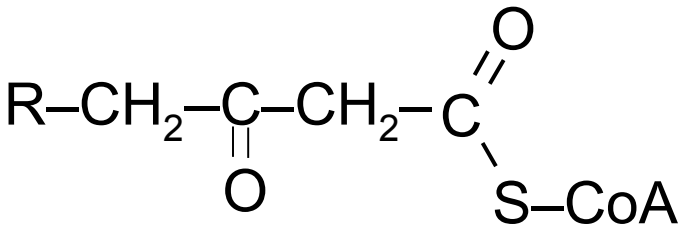
The second oxidative step (dehydrogenation)

β-Hydroxyacyl CoA
(L-3-Hydroxy)





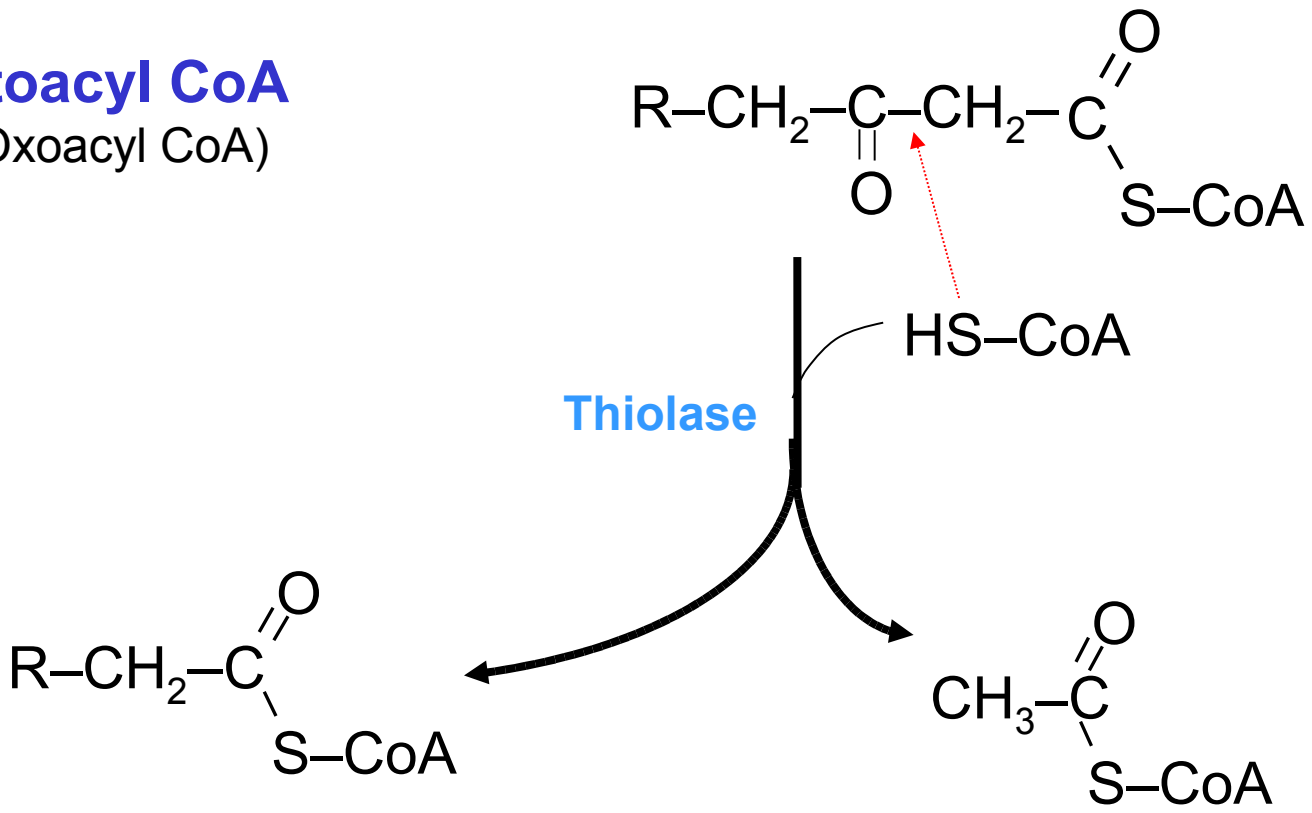
β-Ketoacyl CoA
(3-Oxoacyl CoA)



The reaction is catalysed by ***L-3-hydroxyacyl CoA dehydrogenase***, which is stereospecific for the L isomer of the hydroxyacyl CoA.

The final step of a recurring sequence – the thiolysis of 3-oxoacyl CoA by a molecule of CoA-SH:

β-Ketoacyl CoA
(3-Oxoacyl CoA)



ACYL CoA
SHORTENED BY TWO CARBONS

Acetyl CoA
Substrate for the citrate cycle

Principal reactions in fatty acid oxidation

Step	Reaction	Enzyme
1	Fatty acid + CoA + ATP $\rightleftharpoons$ acyl CoA + AMP + PP_i	Acyl CoA synthetase [also called fatty acid thiokinase and fatty acid:CoA ligase (AMP)]
2	Carnitine + acyl CoA $\rightleftharpoons$ acyl carnitine + CoA	Carnitine acyltransferase (also called carnitine palmitoyl transferase)
3	Acyl CoA + E-FAD $\longrightarrow$ *trans*-Δ^2-enoyl CoA + E-$FADH_2$	Acyl CoA dehydrogenases (several isozymes having different chain-length specificity)
4	*trans*-Δ^2-Enoyl CoA + H_2O $\rightleftharpoons$ L-3-hydroxyacyl CoA	Enoyl CoA hydratase (also called crotonase or 3-hydroxyacyl CoA hydrolyase)
5	L-3-Hydroxyacyl CoA + NAD^+ $\rightleftharpoons$ 3-ketoacyl CoA + NADH + H^+	L-3-Hydroxyacyl CoA dehydrogenase
6	3-Ketoacyl CoA + CoA $\rightleftharpoons$ acetyl CoA + acyl CoA (shortened by C_2)	β-Ketothiolase (also called thiolase)

The energetic yield of β-oxidation of palmitate

– to eight acetyl coenzymes A

Palmitoyl CoA + 7 FAD + 7 NAD^+ + 7 H_2O + 7 CoA ⟶

⟶ **8 acetyl CoA + 7 $FADH_2$ + 7 NADH + 7 H^+**

14 ATP + 21 ATP – 2 ATP (activation of palmitate)

– and eight acetyl CoA in the citrate cycle

8×12 ATP = 96 ATP

Net yield of complete palmitate oxidation to CO_2

14 ATP + 21 ATP – 2 ATP + 96 ATP = **129 ATP**

Net yield of the aerobic breakdown of glucose is

38 mol ATP / mol glucose (M = 180 g / mol; 6 mol C),

i.e. **0.21 mol ATP / g glucose**, or

6.3 mol ATP / mol C.

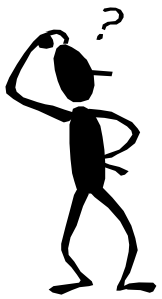
Net yield of complete oxidation of palmitate is

129 mol ATP / mol palmitate (M = 256 g / mol; 16 mol C),

i.e. **0.50 mol ATP / g palmitate**, or

8.1 mol ATP / mol C.

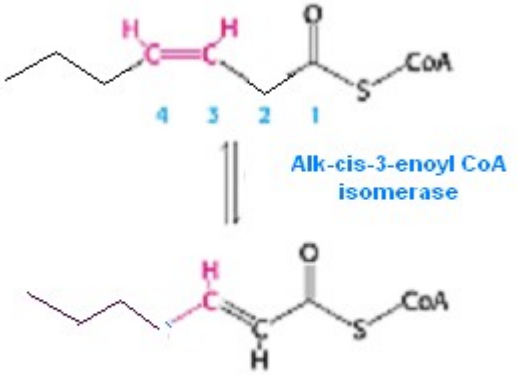
Why there is a difference in energetic yields per gram or per carbon atom? Explain.



Certain fatty acids require additional steps

Unsaturated fatty acids

Oleoyl CoA (octadec-*cis*-9-enoyl CoA) → dodec-*cis*-3-enoyl CoA



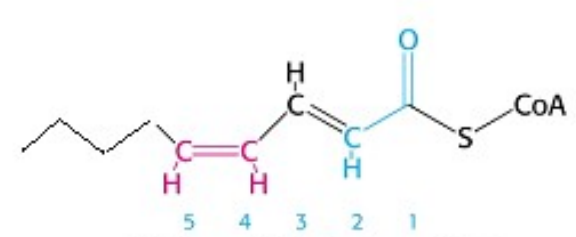
dodec-*trans*-2-enoyl CoA

Linoleoyl CoA (octadec-cis,cis-9,12-dienoyl CoA) →

→ dec-*trans*-2-*cis*-4-dienoyl CoA (inhibits hydratase) that must be reduced (NADPH) to *trans*-3-enoyl CoA and then isomerized to *trans-2-enoyl CoA*.

Odd-chain fatty acids

are uncommon in lipids. If present, the product of β-oxidation will be **propionyl CoA** that is carboxylated to methylmalonyl CoA and isomerized (B_{12} coenzyme) **to succinyl CoA**, similarly as in catabolism of valine, isoleucine and methionine.

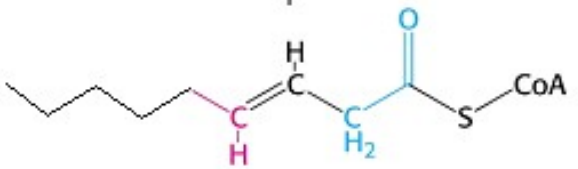


Alk-trans-2-cis-4-dienoyl CoA

2,4 Dienoyl CoA reductase

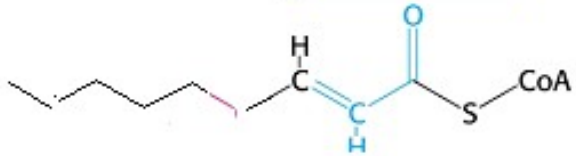
NADPH + H^+

$NADP^+$

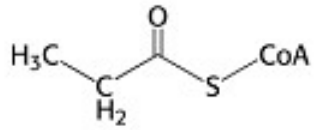


Alk-trans-3-enoyl CoA

cis-Δ^3-Enoyl CoA isomerase



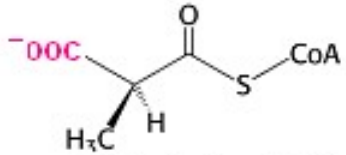
Alk-trans-2.enoyl CoA



Propionyl CoA

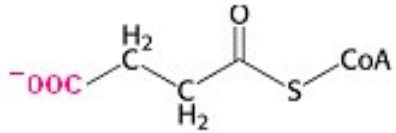
HCO_3^- + ATP

ADP + P_i



D-Methylmalonyl CoA

L-Methylmalonyl CoA



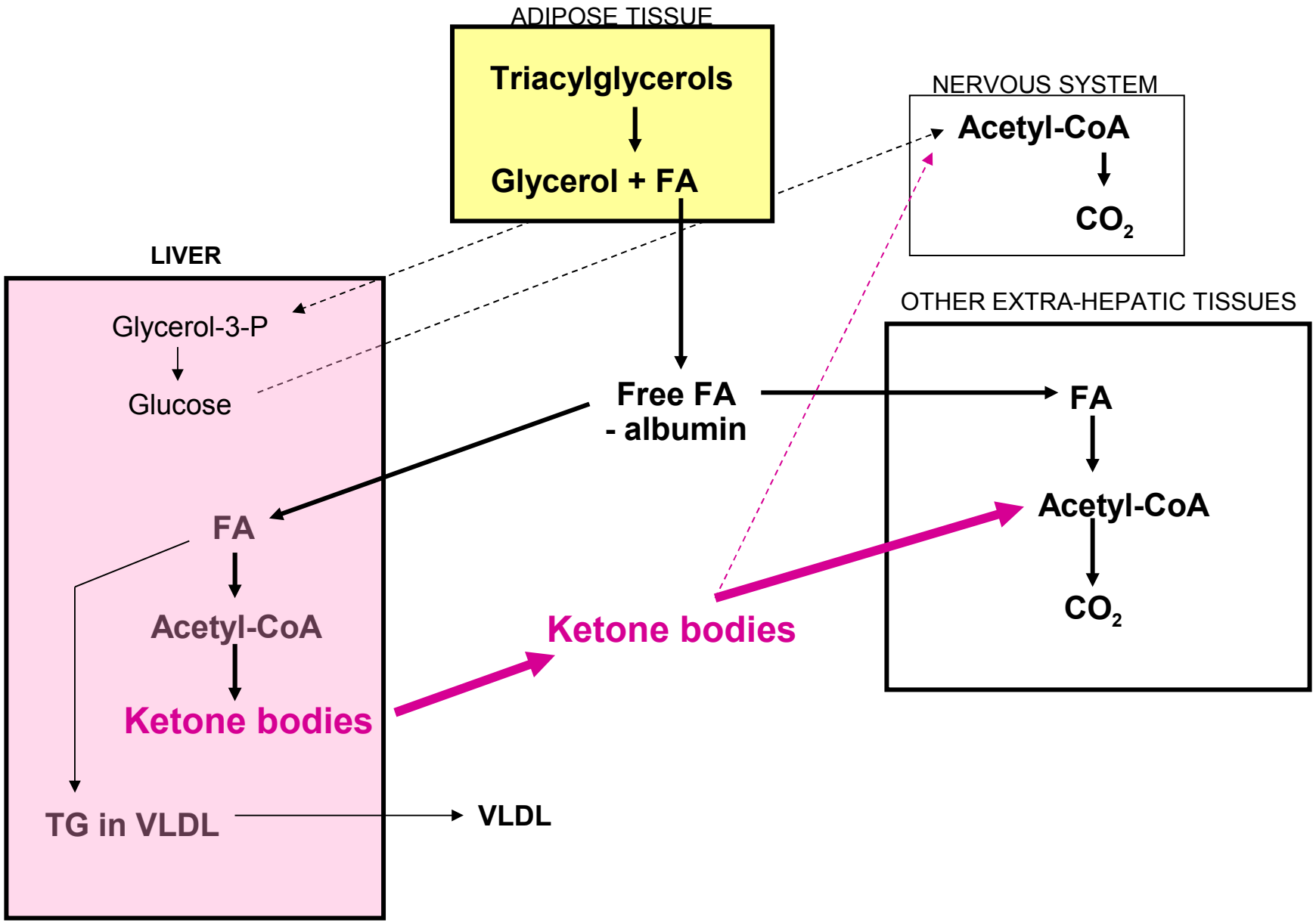
Succinyl CoA

β-Oxidation of fatty acids is a powerfull source of energy. **It occurs if the cells require energy and the access to glucose is not sufficient,** i.e.

in the post-absorptive phase, during fasting, and in stress respectively.

Mobilization of fat stores due to the action of **glucagon** (or **adrenaline**) on adipose tissue increases the plasma level of free fatty acids, which are taken up by the liver and other peripheral tissues (esp. muscle, myocard and kidney) at the rates proportional to the plasma concentration.

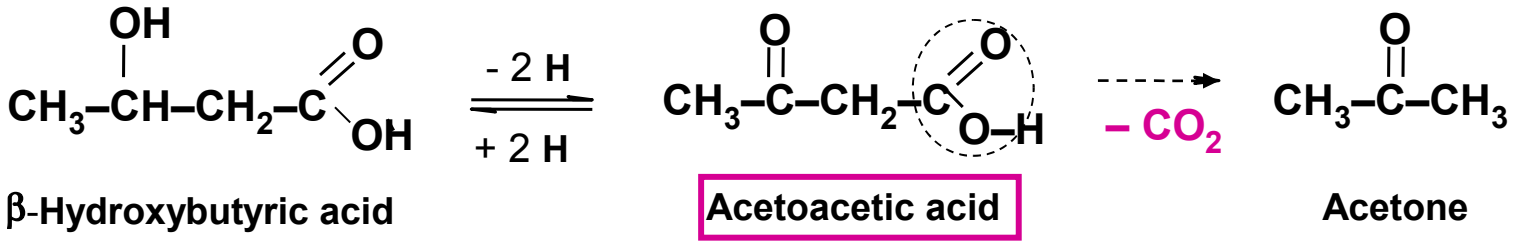
The special role is appointed to **the liver**:
Uptake of plasma FFA is in excess of requirements for complete FA oxidation to CO_2 and synthesis of triacylglycerols is depressed. The liver cells then cover the energy requirements mostly from β-oxidation of fatty acids to acetyl-CoA. A great part of acetyl-CoA is diverted to the **production of ketone bodies**, which are released in to the circulation and serve as an excellent nutrient for extra-hepatic tissues.


ADIPOSE TISSUE
Triacylglycerols
Glycerol + FA
NERVOUS SYSTEM
Acetyl-CoA
CO_2
LIVER
Glycerol-3-P
Glucose
Free FA
- albumin
OTHER EXTRA-HEPATIC TISSUES
FA
Acetyl-CoA
CO_2
FA
Acetyl-CoA
Ketone bodies
Ketone bodies
TG in VLDL
VLDL

Formation of ketone bodies - ketogenesis

The term "ketone bodies" may be used only for the three compounds:

acetoacetic acid (3-oxobutanoic or β-ketobutyric acid), its reduction product **β-hydroxybutyric acid** (3-hydroxybutanoic acid), and the product of non-catalysed decarboxylation of acetoacetate **acetone** (propanone).



Ketone bodies are formed in the **liver mitochondria** and released into blood plasma. The two acids are detectable in plasma at any time, the usual ratio β-hydroxybutyrate to acetoacetate is 3 – 6 (it reflects the intramitochondrial NADH/NAD^+ ratio). There are always traces of ketone bodies in urine, since there is no renal threshold for the two acids.

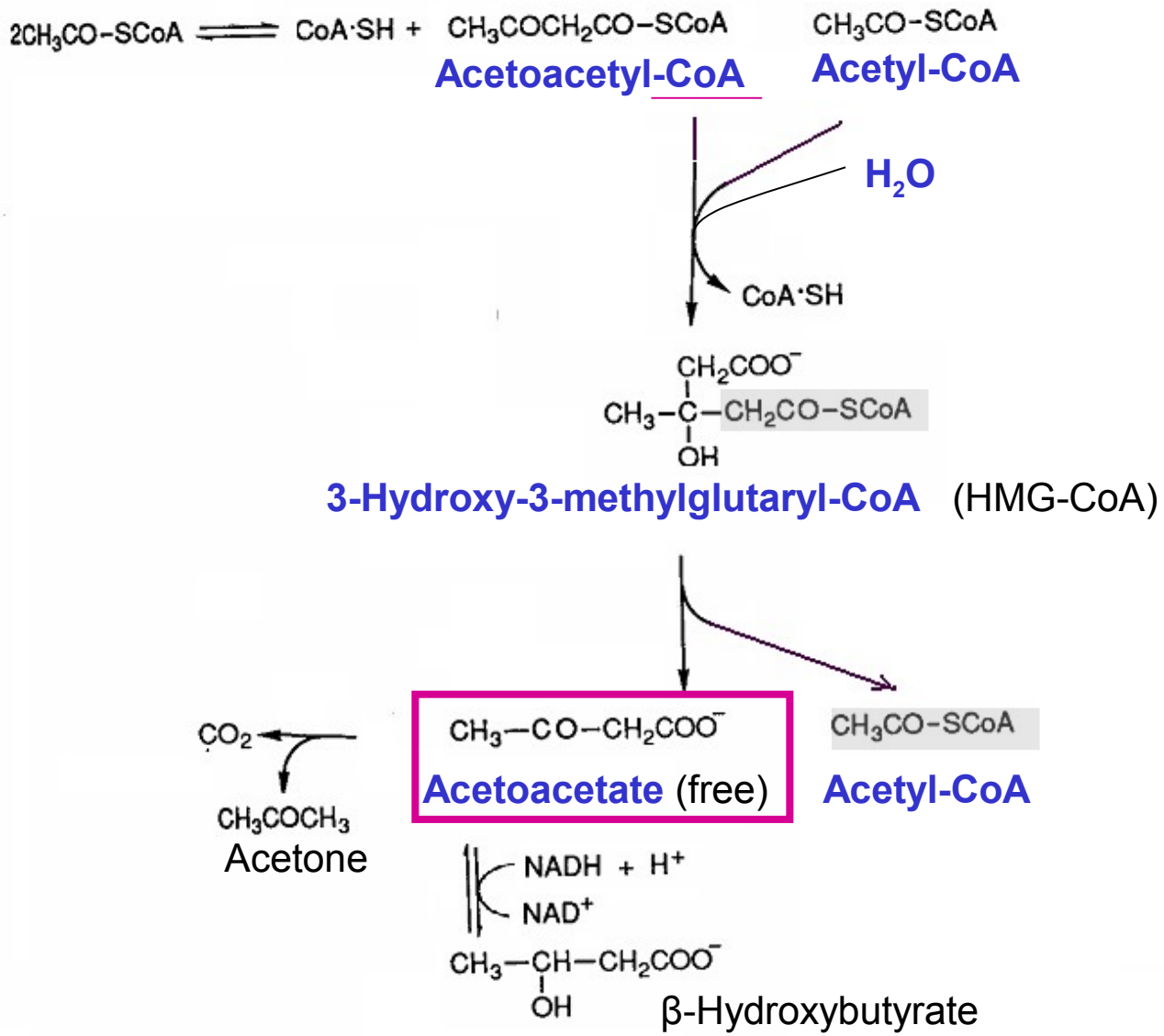
Ketone bodies are readily metabolised in **non-hepatic tissues.**

The production of ketone bodies increases at high ratios glucagon / insulin, when fat stores are mobilized (prolonged fasting, starvation, uncontrolled diabetes mellitus type I).

An extreme production of ketone bodies (**ketosis**) is very dangerous, because ketogenesis is a proton-producing process that disturbs acid-base balance (evoking **ketoacidosis**) and, through excretion of the two acids into urine, is a cause of serious loss of cations.

Acetoacetic acid	$pK_a = 3.52$
β-Hydroxybutyric acid	$pK_a = 4.70$

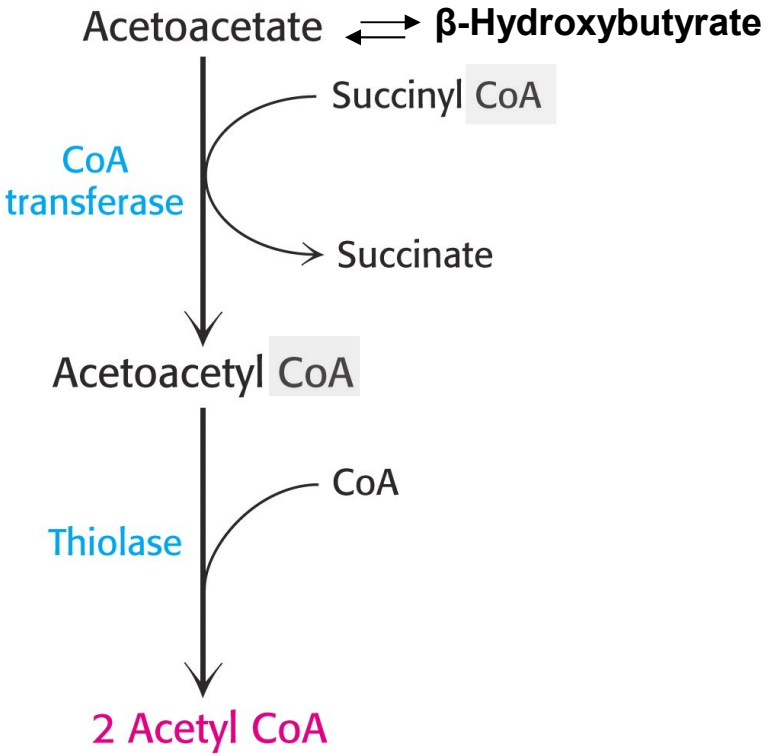
Ketogenesis in liver mitochondria



Utilization of ketone bodies in non-hepatic tissues

β-Hydroxybutyrate and **acetoacetate** are important in providing energy for peripheral tissues.
Acetoacetate is reactivated to acetoacetyl-CoA not directly in the reaction with ATP and CoA-SH, but through the transfer of CoA from succinyl-CoA.

Acetone is a waste product, eliminated by the kidney or expired, it can be smelt on the breath.



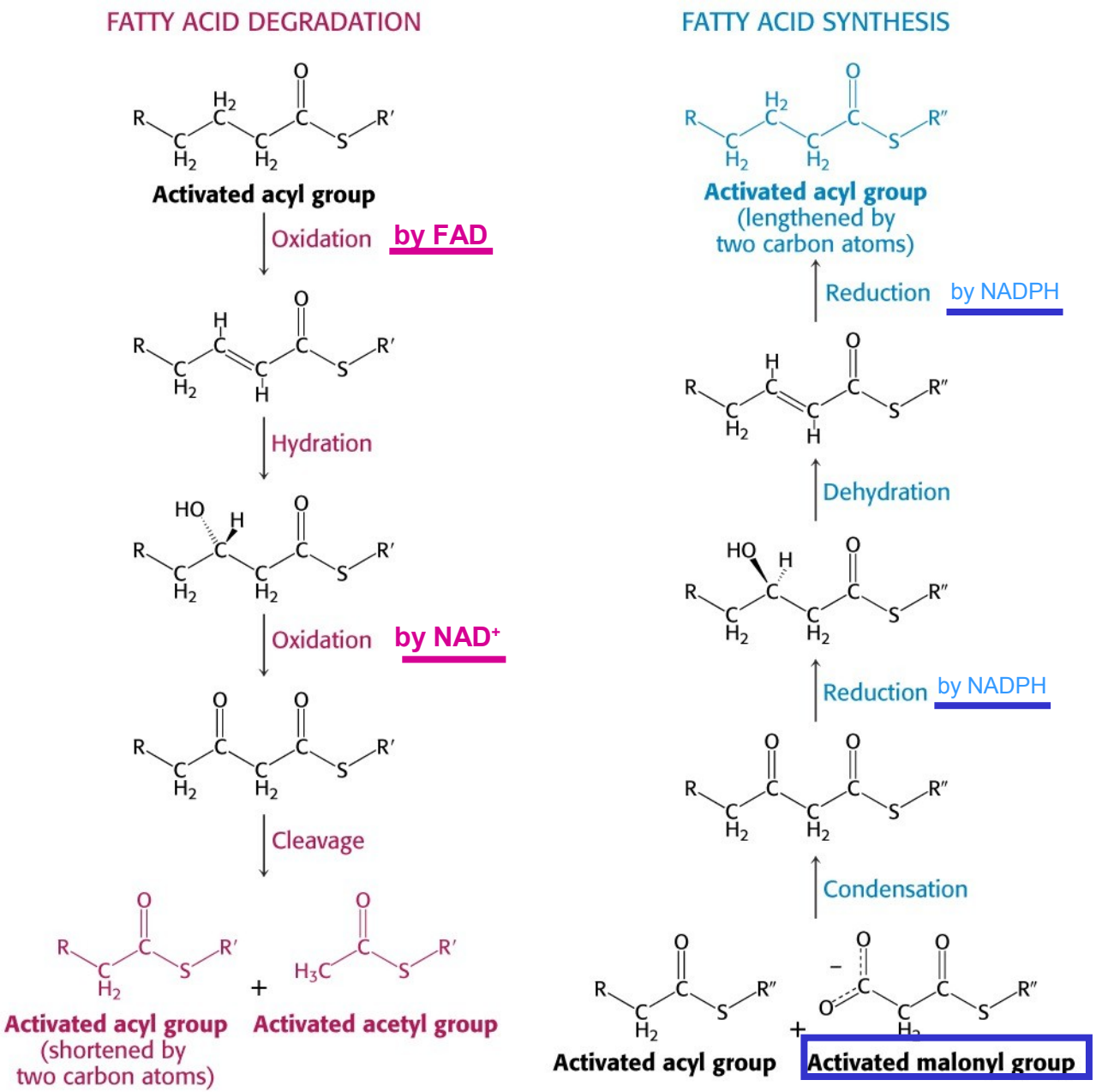
are broken down in the **citrate cycle**

Fatty acid synthesis

Long-chain fatty acids are synthesized by the sequential addition of two-carbon units derived from acetyl CoA.
Fatty acid synthesis is not a reversal of the degradative pathway.

There are some **important differences** between the pathways:

- Synthesis is located **in the cytosol**.
- Intermediates in fatty acid synthesis are covalently linked to the -SH groups of **phosphopantethein of an acyl carrier protein** (ACP), not to coenzyme A.
- The activated donor of two-carbon units is **malonyl CoA**, the elongation reaction is driven by the release of CO_2.
- The reductant in fatty acid synthesis is **NADPH**, whereas the oxidants in fatty acid degradation are NAD^+ and FAD.


FATTY ACID DEGRADATION
Activated acyl group
Oxidation
by FAD
Hydration
Oxidation
by NAD+
Cleavage
Activated acyl group
(shortened by
two carbon atoms)
Activated acetyl group
FATTY ACID SYNTHESIS
Activated acyl group
(lengthened by
two carbon atoms)
Reduction
by NADPH
Dehydration
Reduction
by NADPH
Condensation
Activated acyl group
Activated malonyl group

A very intensive synthesis of fatty acids takes place in the cytosol of **liver, adipose tissue, and lactating mammary gland**.

Conditions favourable to synthesis of fatty acids:

– **the fed state** – sufficient amounts of glucose are available producing **acetyl CoA**,

– **low energy expenditure** – high **ATP** concentrations within the cells inhibit decomposition of acetyl CoA in the citrate cycle,

– absence of stress that activates mobilization of fat stores, free fatty acids released through the action of catecholamines inhibit fatty acid synthesis.

Fat synthesis and storage are essential components of fuel metabolism in the body, but excess accumulation of fat leads to **obesity** which is becoming a growing problem.

The control of energy balance depends on many factors, some of which are

– **genetically linked** (appetite control involves a number of recently discovered protein messengers and receptors),

– **environmental** (e.g. the relative abundance of food and the type of food, esp. the energy-dense foods currently in vogue).

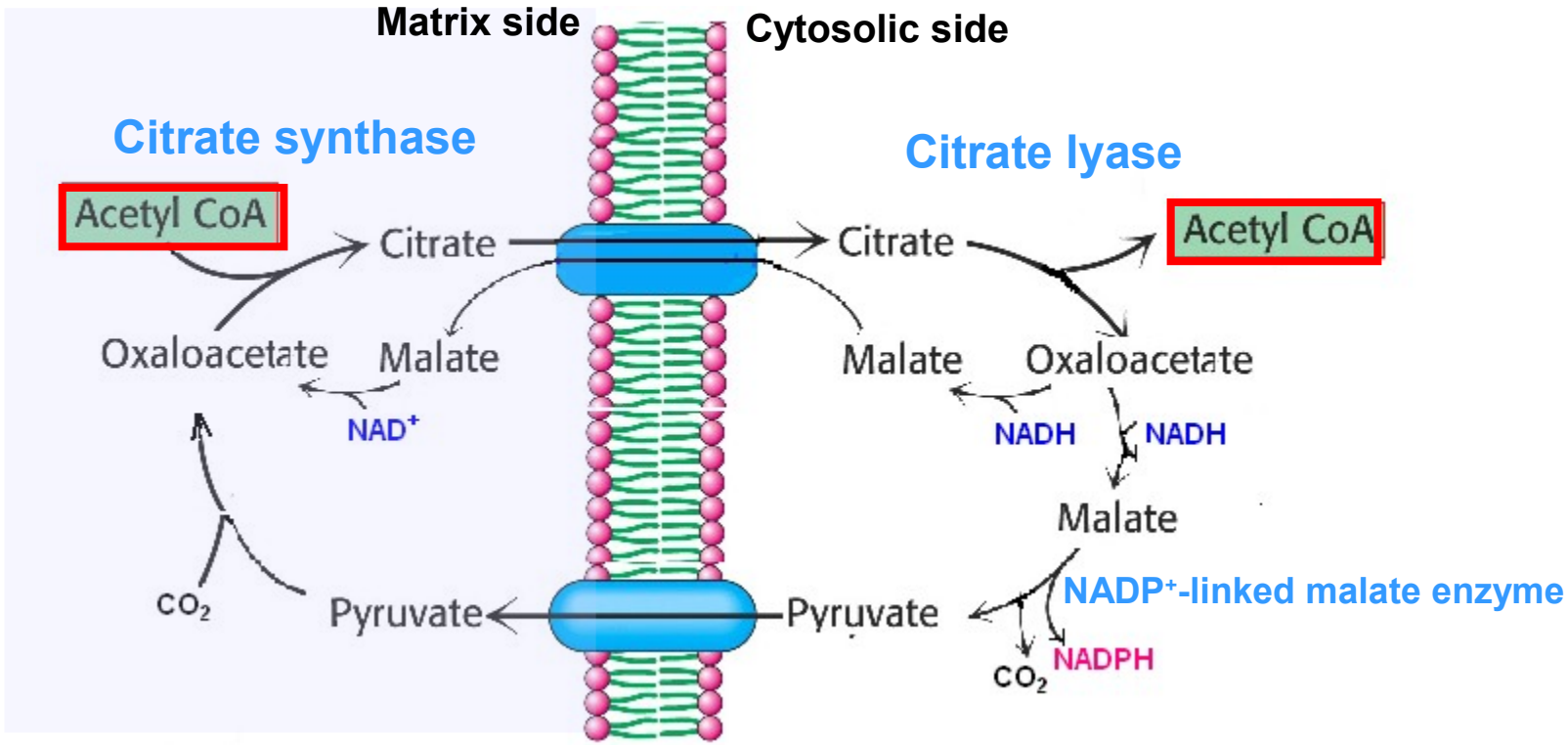
Supposing that there is enough ATP in the cell and sufficient quantity of acetyl CoA produced from glucose (by oxidative decarboxylation of pyruvate) or amino acids in mitochondria,

acetyl CoA has to be transported from mitochondria into cytosol.

Then fatty acid synthesis can be considered as a two-stage process:

- stage 1 – **synthesis of malonyl CoA**,
- stage 2 – **reactions catalysed by the fatty acid synthase complex**.

Transfer of acetyl CoA to the cytosol



Citrate lyase catalyses the reaction

Citrate + ATP + CoA-SH + H_2O → acetyl-CoA + ADP + P_i + oxaloacetate

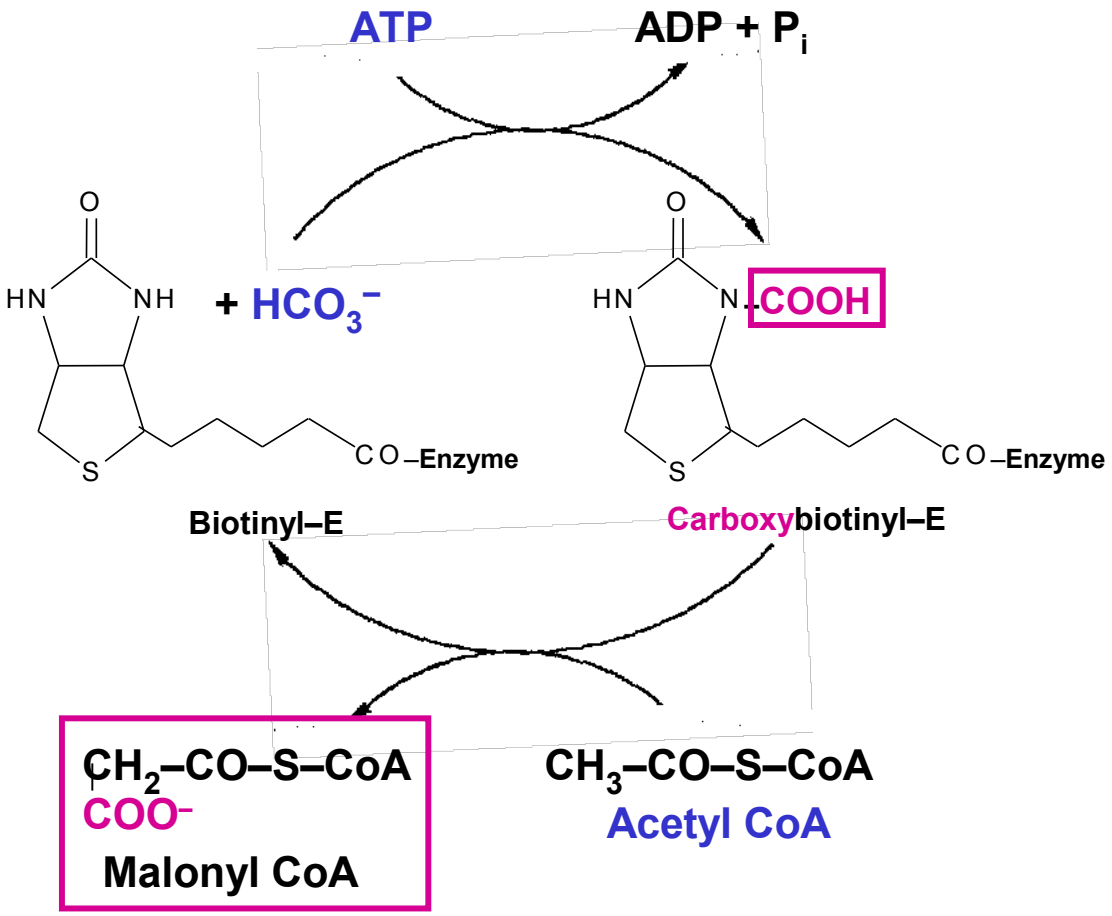
Synthesis of malonyl CoA

is the rate-limiting step in fatty acid synthesis, catalysed by **acetyl-CoA carboxylase**:

The enzyme complex consists of several identical subunits, each containing
biotin,
biotin carboxylase,
biotin carboxyl carrier protein (BCCP), and transcarboxylase.

The enzyme is **inhibited by phosphorylation** catalysed by AMP-dependent protein kinase. It is inhibited also **by palmitoyl-CoA** (due to dissociation of the active fibrous enzyme polymer to inactive octamers).

Enzyme **activation by citrate** (polymerizing is promoted) and by **dephosphorylation** (dependent on insulin).



The fatty acyl synthase complex

In mammals, the complex is a **homodimer**. Each monomer is arranged in three domains carrying the seven catalytic activities. One domain in both monomers includes the "acyl carrier protein (ACP)" area to which the phosphopantethein "arm" is attached. Both monomers cooperate so that each of them takes part on the synthesis of two fatty acids processed simultaneously,

Seven enzyme activities:

AT
Acetyl/acyl-CoA transacylase

MT
Malonyl transacylase

CE
Condensing enzyme (Oxoacyl-PPt synthase)

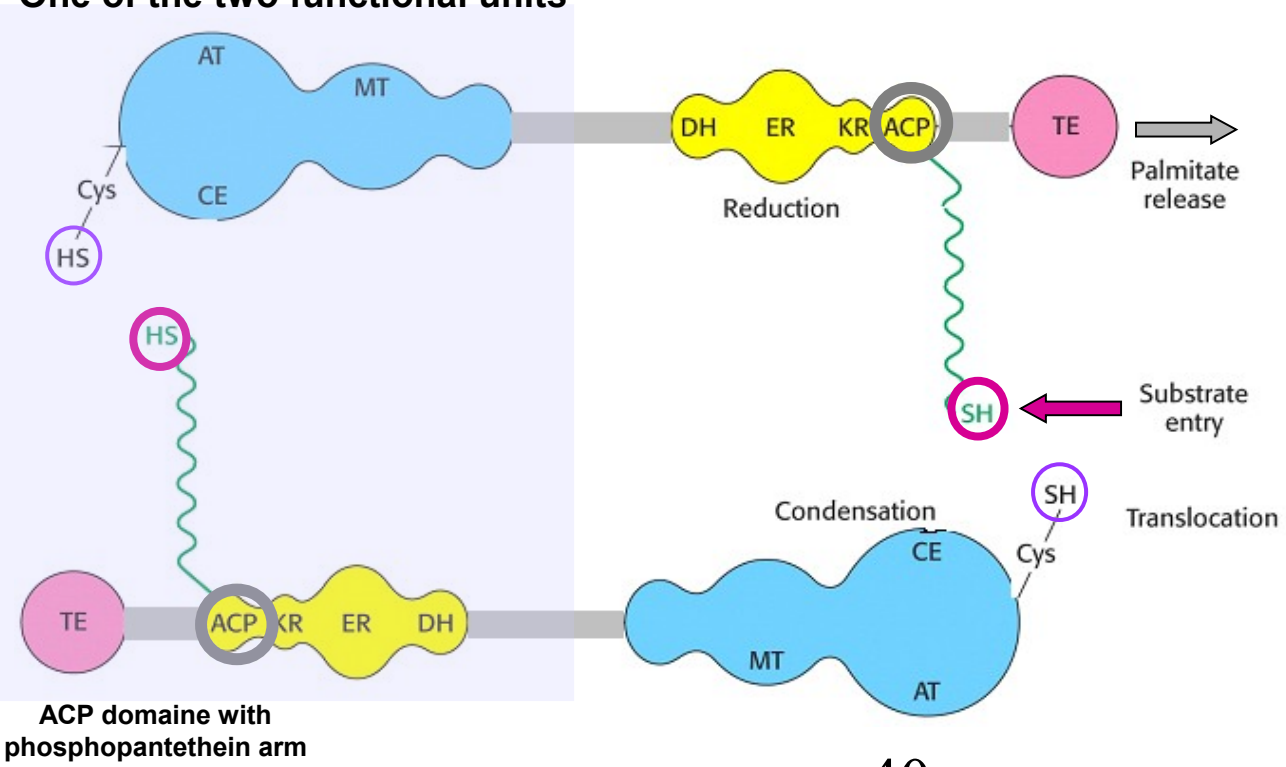
KR
Oxoacyl reductase

DH
Hydroxyacyl dehydratase

ER
Enoyl reductase

TE
Palmitoyl thioesterase

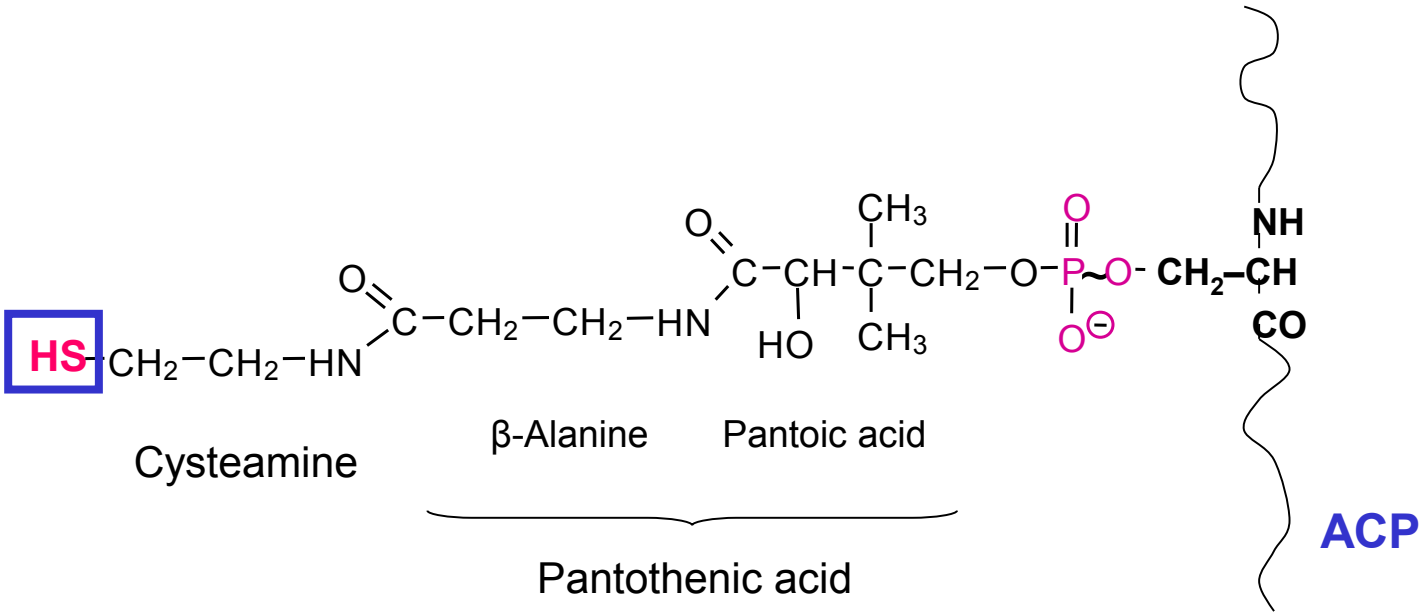
One of the two functional units



ACP domaine with phosphopantethein arm

The flexible phosphopantethein "arm" of the synthase

linked to a serine residue of acyl carrier protein ACP is found also in coenzyme A (as just one half of the coenzyme A molecule):



The processed acyls attached to the sulfanyl group are carried from one active site of the synthase complex to another.

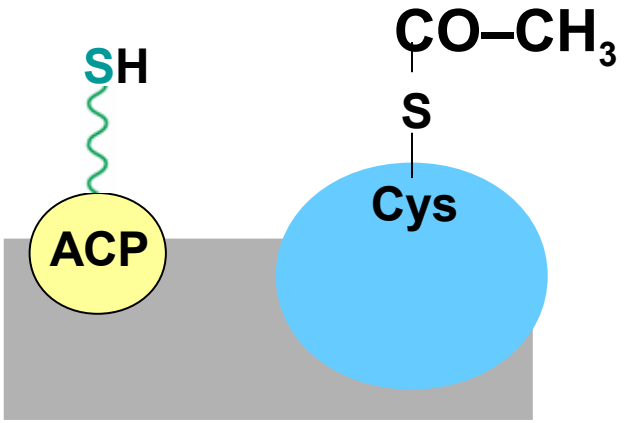
Principal reactions in fatty acid synthesis

Step	Reaction	Enzyme
1	$\text{Acetyl CoA} + HCO_3^- + \text{ATP} \longrightarrow \text{malonyl CoA} + \text{ADP} + P_i + H^+$	Acetyl CoA carboxylase
2	$\text{Acetyl CoA} + \text{ACP} \rightleftharpoons \text{acetyl ACP} + \text{CoA}$	Acetyl transacylase
3	$\text{Malonyl CoA} + \text{ACP} \rightleftharpoons \text{malonyl ACP} + \text{CoA}$	Malonyl transacylase
4	$\text{Acetyl ACP} + \text{malonyl ACP} \longrightarrow \text{acetoacetyl ACP} + \text{ACP} + CO_2$	Acyl-malonyl ACP condensing enzyme
5	$\text{Acetoacetyl ACP} + \text{NADPH} + H^+ \rightleftharpoons \text{D-3-hydroxybutyryl ACP} + \text{NADP}^+$	β-Ketoacyl ACP reductase
6	$\text{D-3-Hydroxybutyryl ACP} \rightleftharpoons \text{crotonyl ACP} + H_2O$	3-Hydroxyacyl ACP dehydratase
7	$\text{Crotonyl ACP} + \text{NADPH} + H^+ \longrightarrow \text{butyryl ACP} + \text{NADP}^+$	Enoyl ACP reductase

Reactions of fatty acid synthesis

1

The synthesis begins with the transfer of the **acetyl group** of acetyl CoA **to the sulfur of a cystein residue** of the condensing enzyme. The reaction is catalysed by **acetyl transacylase.**

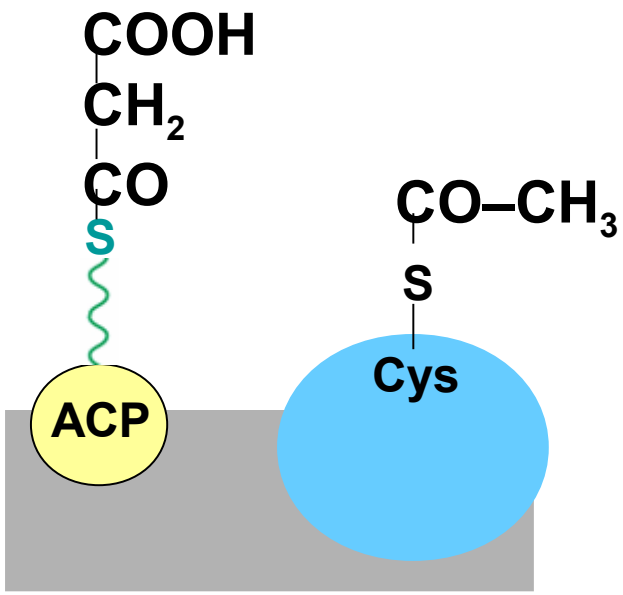


"Priming“

2

Similarly, the **malonyl group** is transferred to the **sulphur atom of the phosphopantetheine** attached to ACP.

The reaction is catalysed by **malonyl transacylase**.



"Loading“

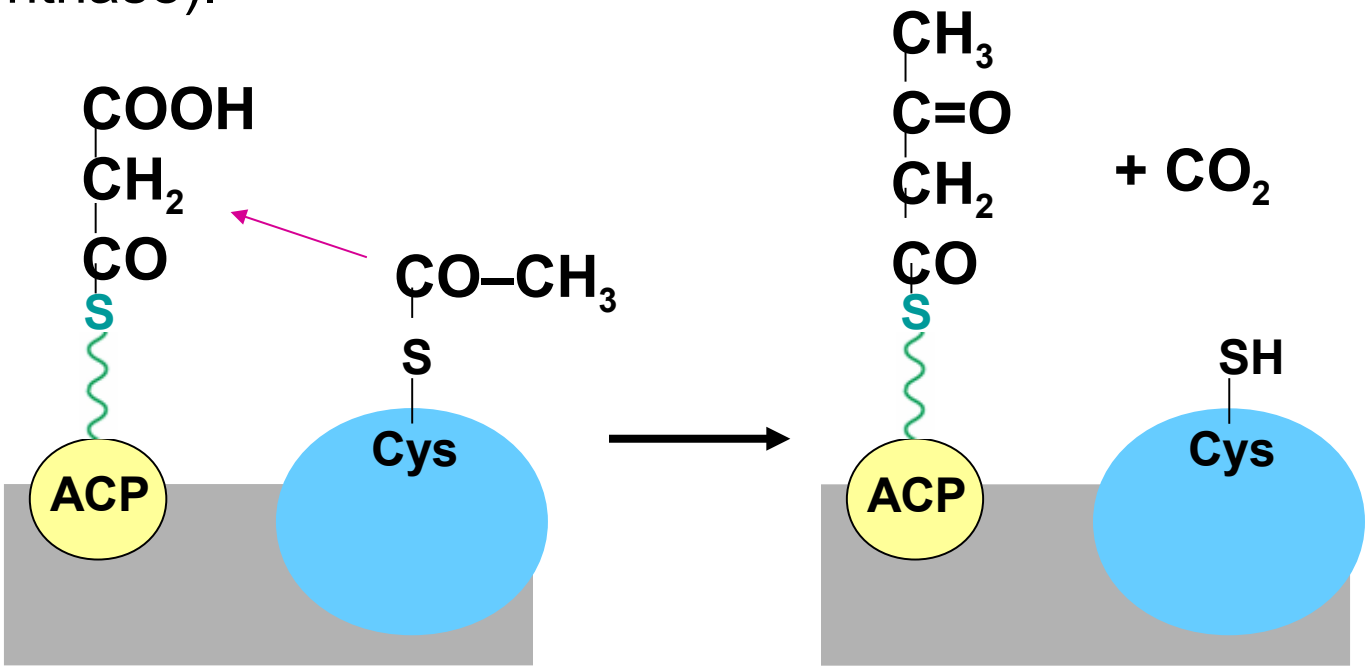
3

Condensation

The beginning of elongation: The **joining of the acetyl unit to** a two-carbon part of the **malonyl unit** on phosphopantetheine. **CO_2 is released.**

An **acetoacetyl unit** is formed of PPt.

The reaction is catalysed by **condensing enzyme** (3-oxoacyl synthase).

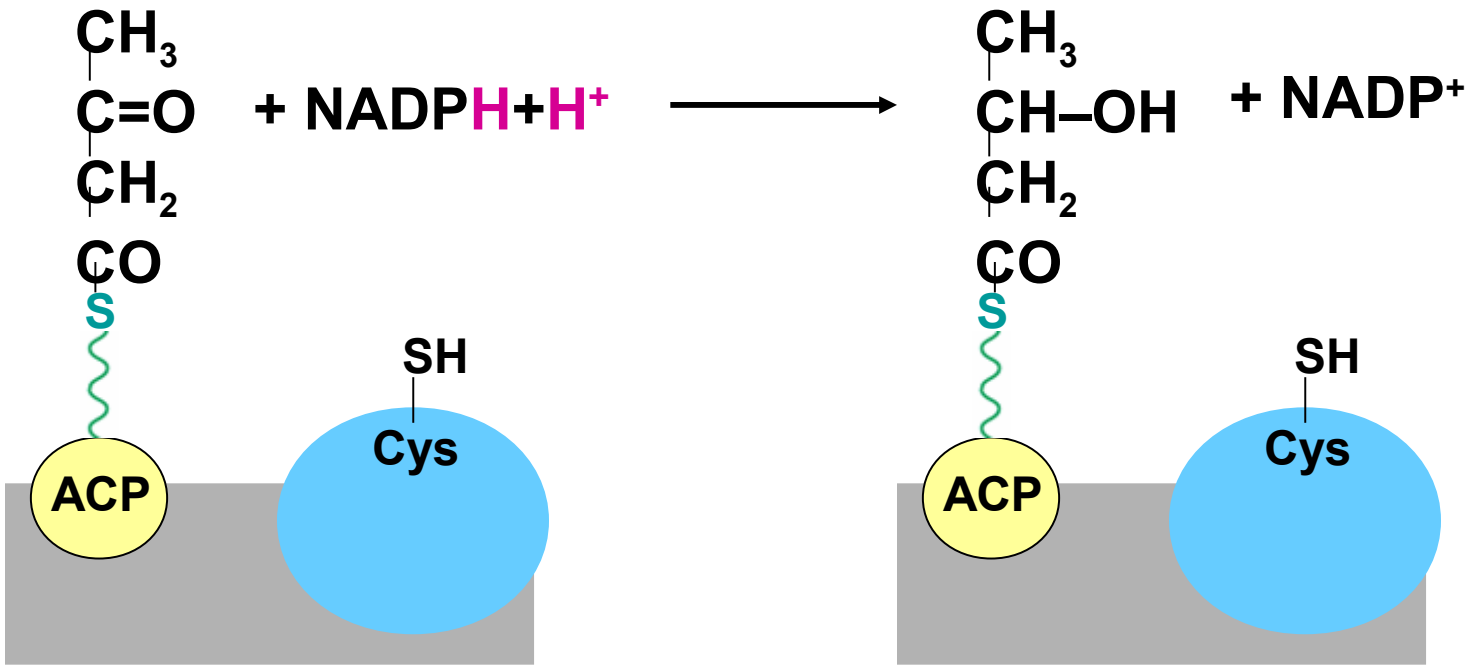


4

The first reduction

catalysed by **β–ketoacyl reductase** with **NADPH**.

The product is **3–hydroxyacyl** unit.

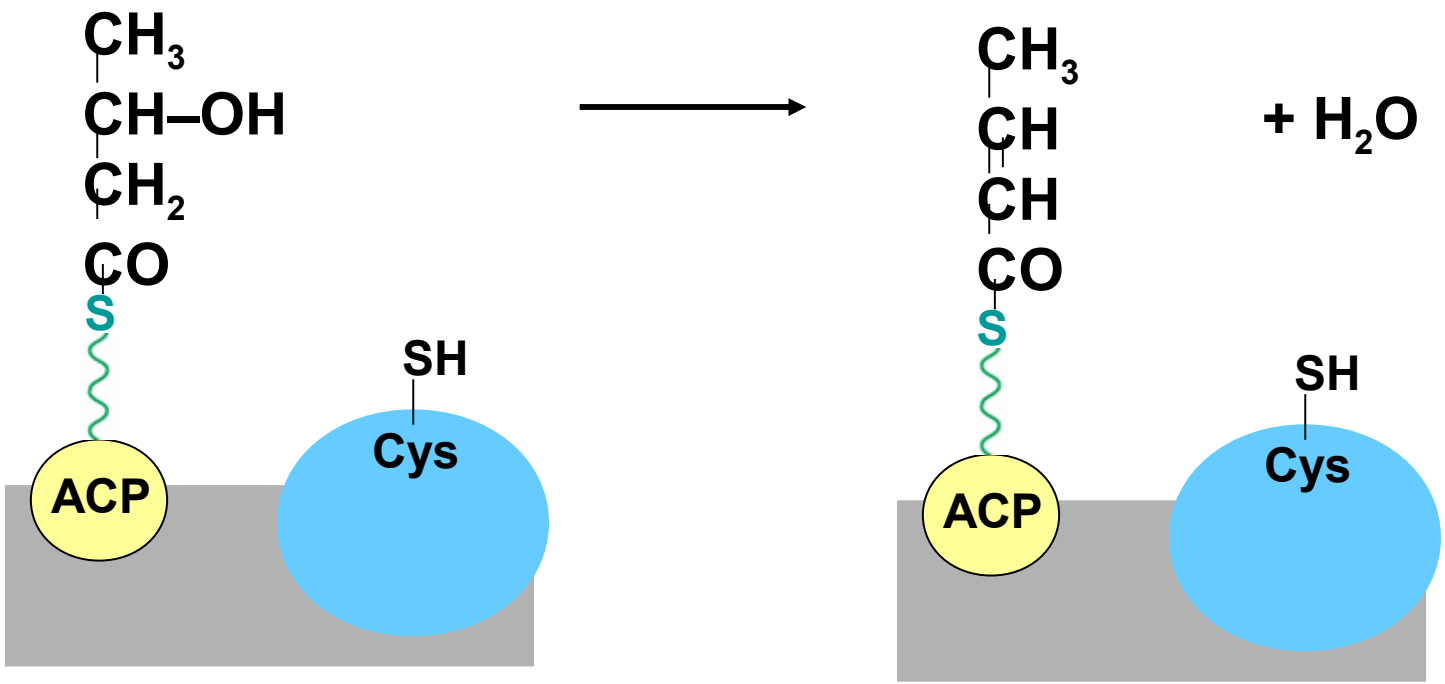


5

Dehydration

catalysed by **3-hydroxyacyl dehydratase**.

The product is ***trans–2–enoyl*** (named crotonyl) unit.



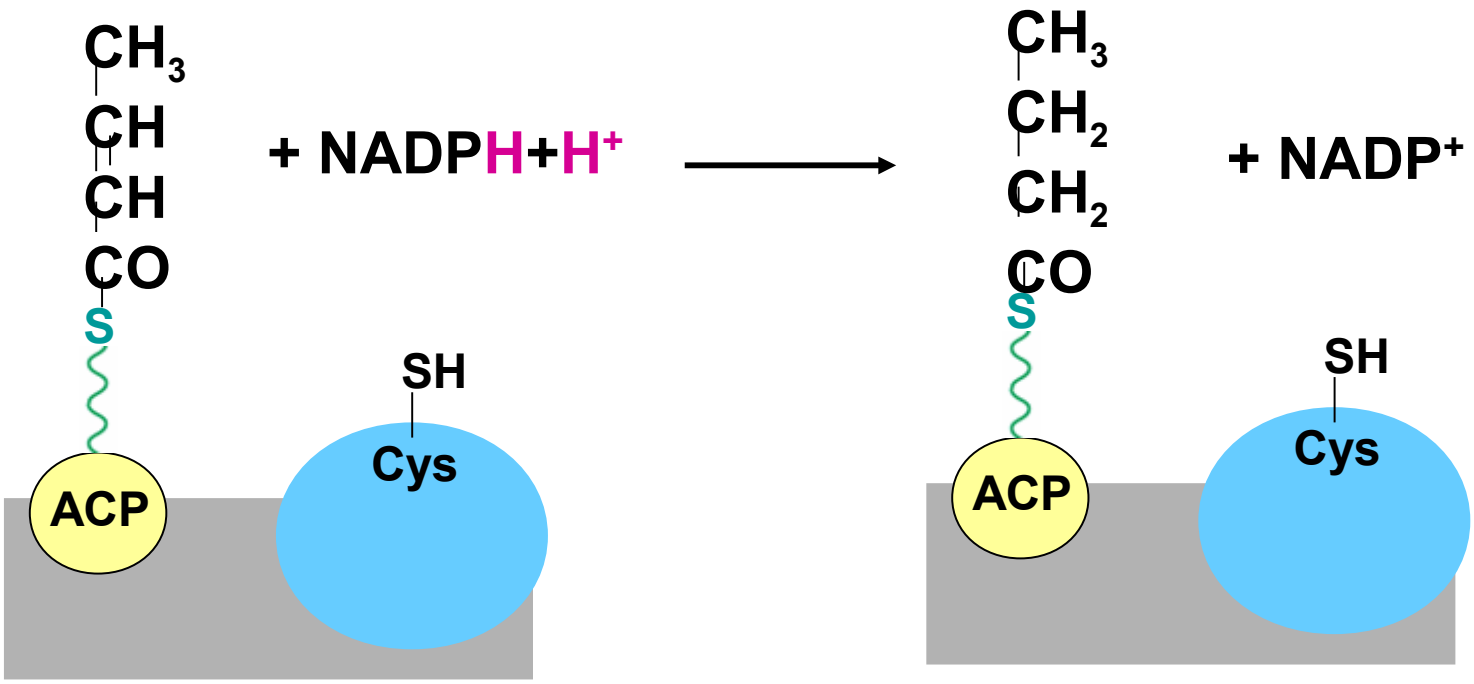
6

The second reduction

catalysed by **enoyl reductase** with **NADPH**.

The product is **saturated acyl** (now butyryl) unit.

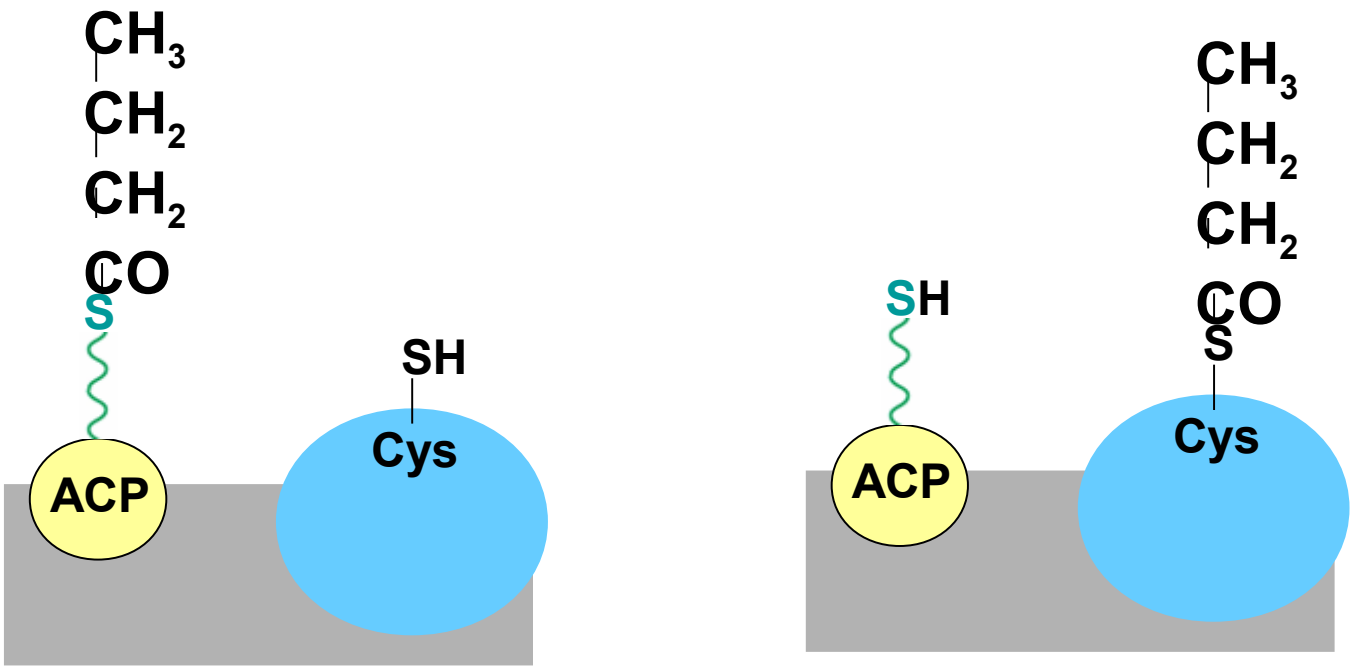
Initial acetyl was elongated by two carbon atoms.



7

The saturated **acyl is transferred to the cysteine sulfur** atom on the condensing enzyme.

The synthase is now ready for another round of elongation



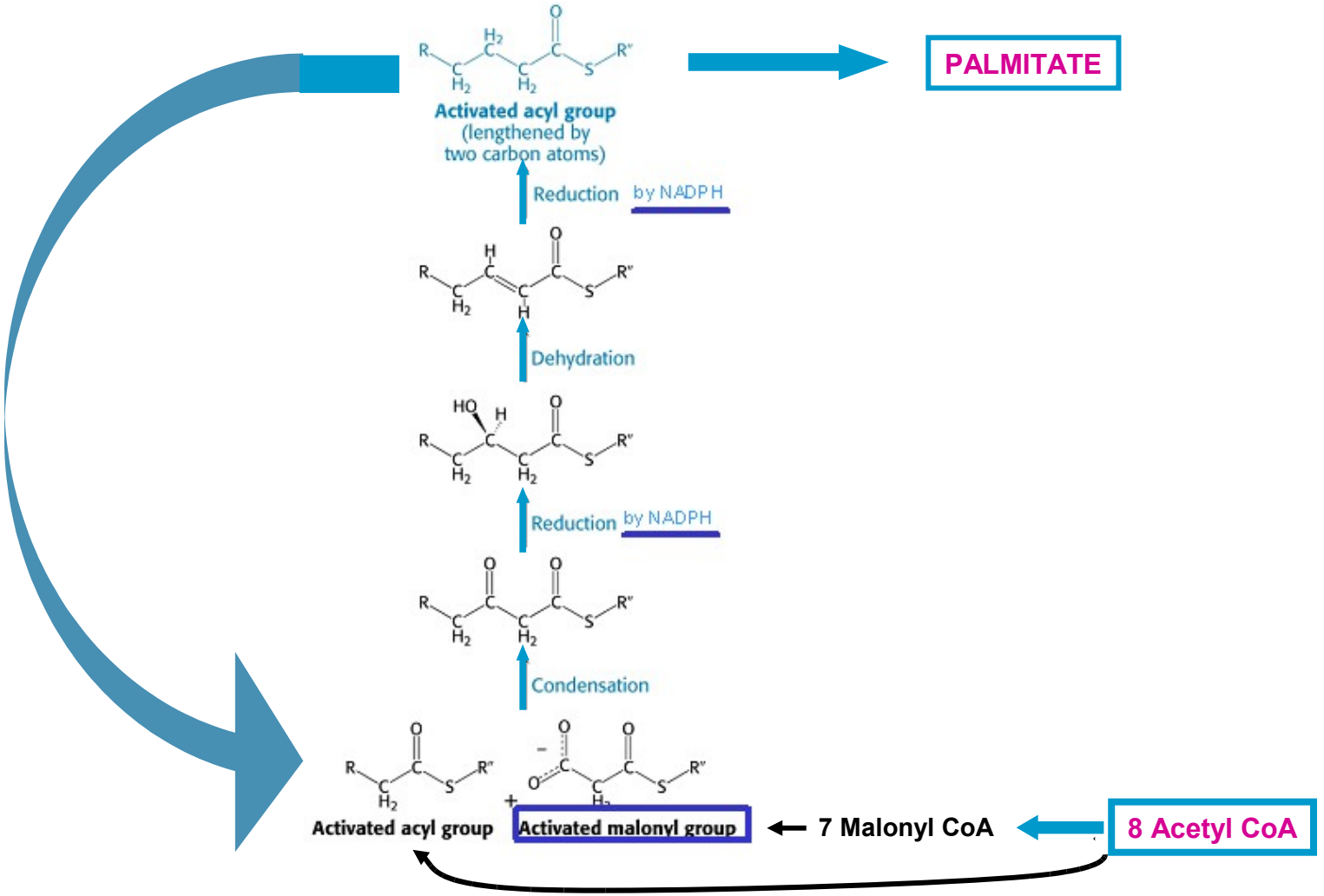
After the completion of the first elongating cycle, **new malonyl is "loaded“** on the sulfanyl group of PPt.
In the second round of fatty acid synthesis, butyryl unit condenses with malonyl to form a C_6-acyl, ……

The elongation cycles continue until C_{16}-acyl unit (palmitoyl) is formed.
Palmitoyl unit is a good substrate for **thioesterase** that hydrolyses palmitoyl-PPt to yield **palmitate** (16:0).

In mammals, palmitate is the major product of FA synthesis.
A minor saturated product is stearate (18:0).

Further elongation of fatty acids is provided by similar mechanisms, but the elongating system is located on the membranes of endoplasmic reticulum.

The fatty acid synthesis



NADPH is required in the reductive steps of FA synthesis

The main source of NADPH is the **pentose phosphate pathway**.

A certain part of NADPH is supplied by the reaction catalysed by **$NADP^+$–linked malate enzyme** ("malic enzyme“):

$$\text{Malate} + NADP^+ \rightarrow \text{pyruvate} + CO_2 + NADPH$$

The reaction takes part on the transport of acetyl-CoA (in the form of citrate) across the inner mitochondrial membrane.

The stoichiometry of fatty acid synthesis

The synthesis of palmitate (C_{16}):

The synthesis of malonyl CoA

7 Acetyl CoA + 7 CO_2 + 7 ATP $\rightarrow$ 7 malonyl CoA + 7 ADP + 7 P_i + 14 H^+

The synthesis catalysed by the fatty acid synthase complex

Acetyl CoA + 7 malonyl CoA + 14 NADPH + 20 H^+ $\rightarrow$

$\rightarrow$ palmitate + 7 CO_2 + 14 $NADP^+$ + 8 CoA + 6 H_2O

The overall stoichiometry for the synthesis of palmitate is

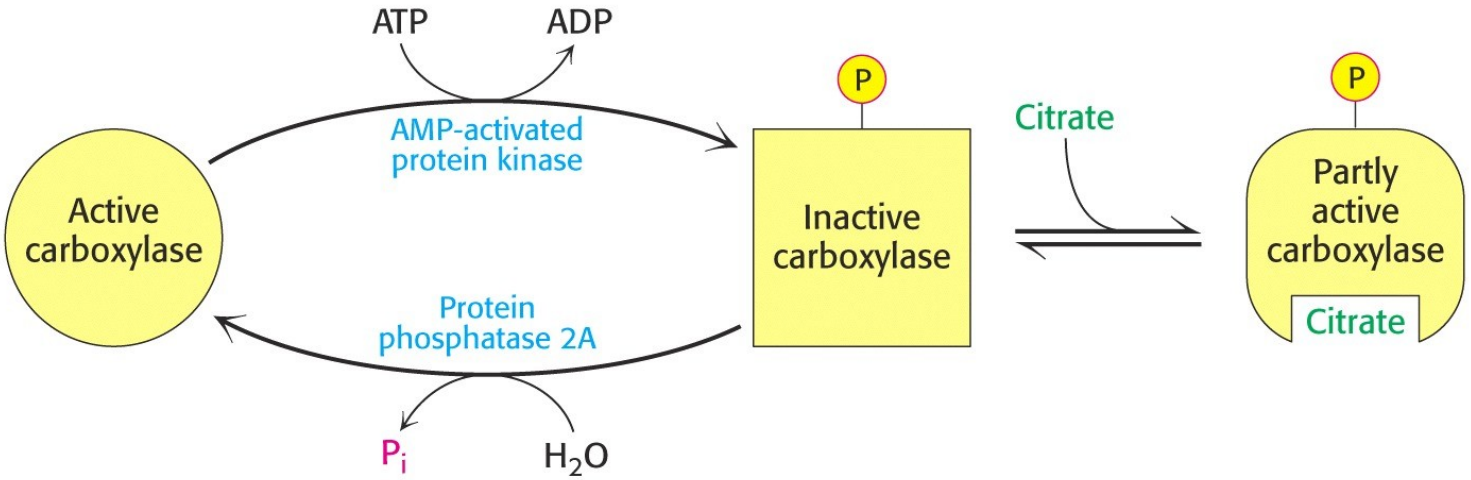
8 Acetyl CoA + 7 ATP + 14 NADPH + 6 H^+ $\rightarrow$

$\rightarrow$ palmitate + 14 $NADP^+$ + 8 CoA + 6 H_2O + 7 ADP + 7 P_i

Control of fatty acid synthesis

Regulation is carried out by means of **reversible phosphorylation of acetyl-CoA carboxylase.**
This enzyme phosphorylated by AMP-dependent protein kinase is inhibited, dephosphorylation – dependent on insulin – activates the carboxylase.

Local regulation is provided by **citrate** that activates the carboxylase, **palmitoyl-CoA** inactivates this key enzyme.



Elongation of fatty acids

Although **palmitate (C_{16}) is the major product** of the fatty acid synthase complex, and is the chief saturated fatty acid in human fat,

stearate and oleate (C_{18}) are common and longer-chain fatty acids,

arachidate (C_{20}),

behenate (C_{22}) and

lignocerate (C_{24}) occur in phospholipids.

Elongation by enzymes bound to the endoplasmic reticulum:

– Activation of palmitate by conversion to palmitoyl CoA,
– activation of acetyl CoA by its carboxylation to malonyl CoA,
– elongation *similar* to synthesis catalysed by FA synthase complex, but the **intermediates are CoA-thioesters**, not enzyme-bound acyls. The reductant is also NADPH.

Elongation process in mitochondria (for the synthesis of fatty acids incorporated into mitochondrial lipids):

– Reversal of the β-oxidation.

Desaturation of fatty acids

A large proportion (> 50 %) of acyl groups in human triacylglycerols contain double bonds. Such fatty acids are formed by desaturation of long-chain fatty acyl-CoA.

E.g. **Palmitoyl-CoA + NADH + H^+ + O_2** ⟶

⟶ **Palmitoleoyl-CoA + 2 H_2O + NAD^+**

In **higher animals**, only the desaturases are known which generate double bonds **at carbons 9, 6, 5, and 4** in the fatty acid chain**.** Mammals lack the enzymes to introduce double bonds at carbon atoms beyond C-9.

Fatty acids containing double bonds beyond C-9 are synthesized by **plants,** they contain also **12- and 15-desaturase**.

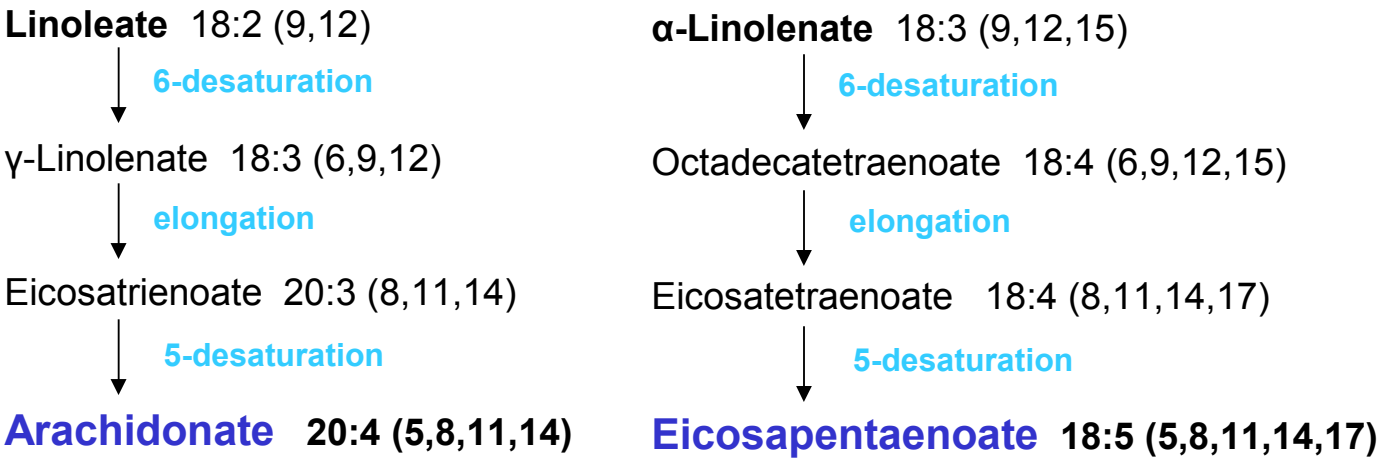
Unsaturated fatty acids of the series *n-6* are comprised in all plant oils (olive oil, sunflower oil etc.).

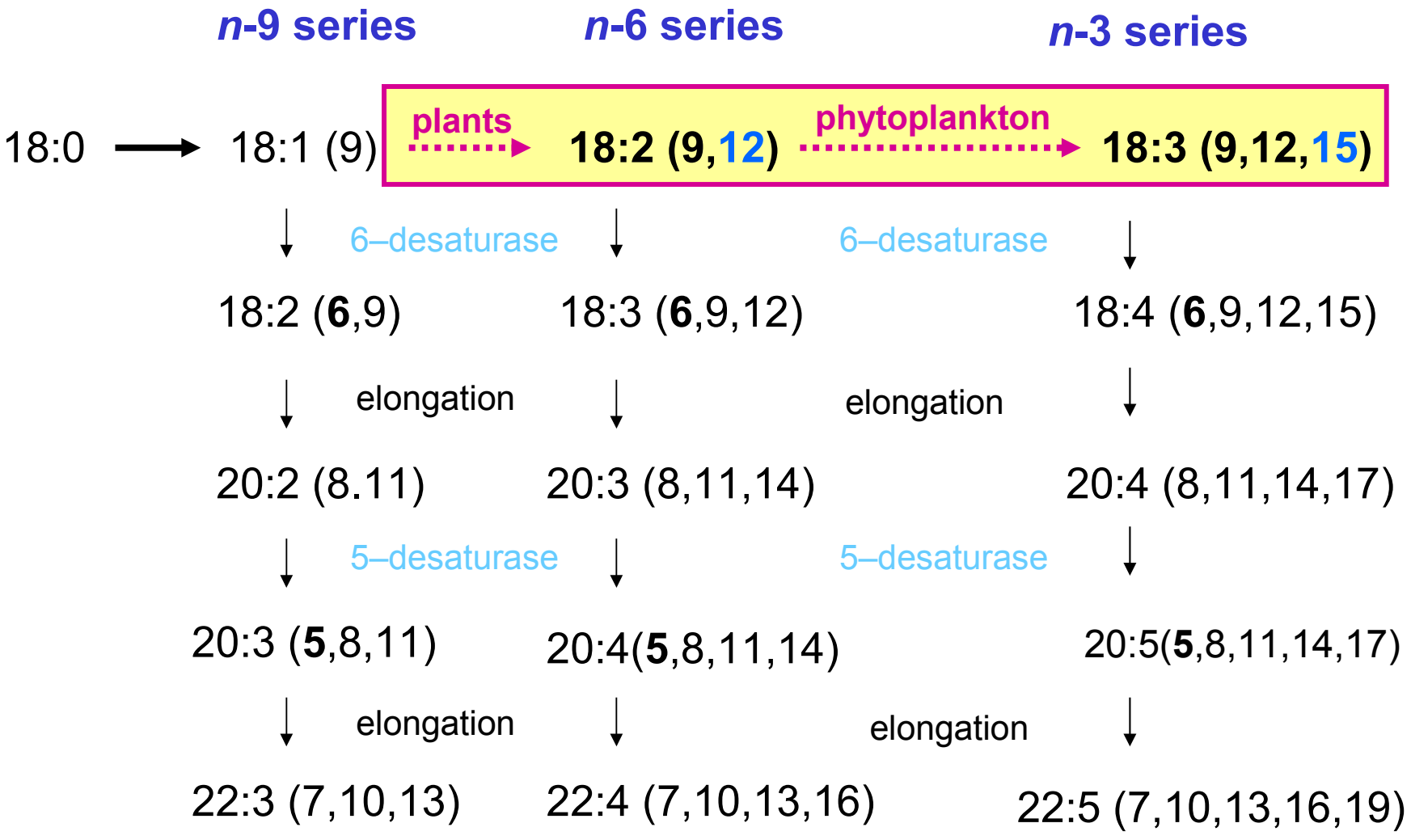
15-Desaturase is present predominantly in plants growing in cold water (algae, phytoplankton), then a high concentration of polyunsaturated fatty acyls of the series *n*-3 is in fish oils (fish feeds phytoplankton).

Polyunsaturated fatty acids (*n*-6 and *n*-3) **are essential for animals**

Fatty acids *n*-6 and *n*-3 are essential dietary constituents for animals and serve as **precursors of eicosanoids** (prostanoids and leukotrienes).

Providing the dietary intake is sufficient (vegetable seed oils, resp. fish), **linoleate** and **α-linolenate** can act as precursors of other polyenoic acids such as **arachidonate** (n-6) and **eicosapentaenoate** *(n*-3), from which eicosanoids are formed.

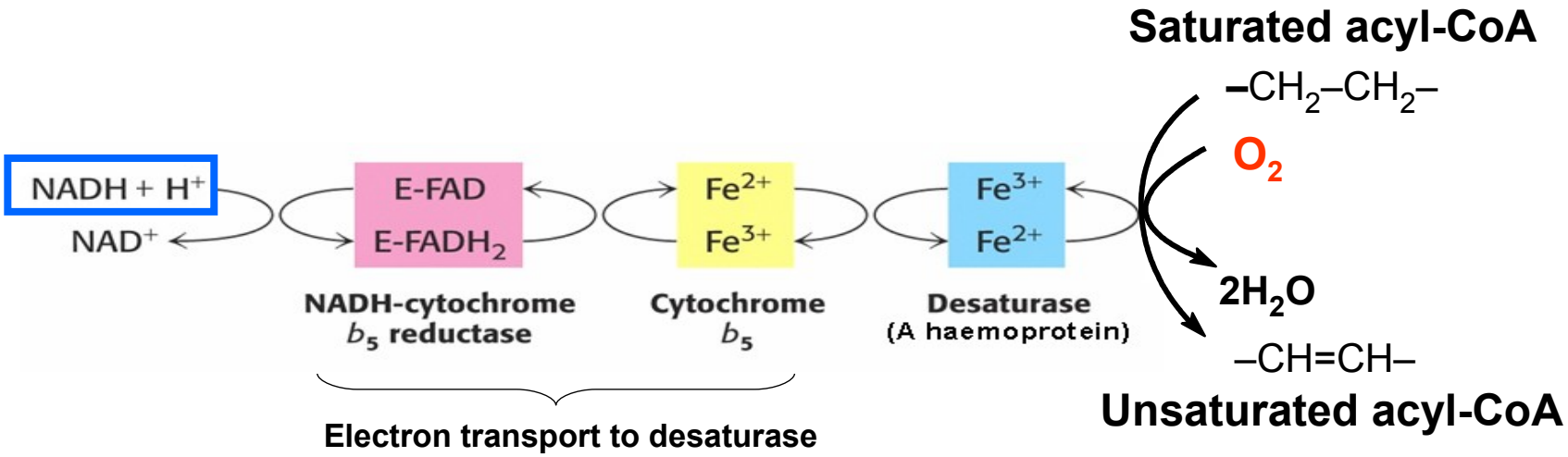



n-9 series
n-6 series
n-3 series
18:0
18:1 (9)
plants
18:2 (9,12)
phytoplankton
18:3 (9,12,15)
6–desaturase
6–desaturase
18:2 (6,9)
18:3 (6,9,12)
18:4 (6,9,12,15)
elongation
elongation
20:2 (8.11)
20:3 (8,11,14)
20:4 (8,11,14,17)
5–desaturase
5–desaturase
20:3 (5,8,11)
20:4(5,8,11,14)
20:5(5,8,11,14,17)
elongation
elongation
22:3 (7,10,13)
22:4 (7,10,13,16)
22:5 (7,10,13,16,19)

Mechanism of long-chain fatty acyl-CoAs desaturation

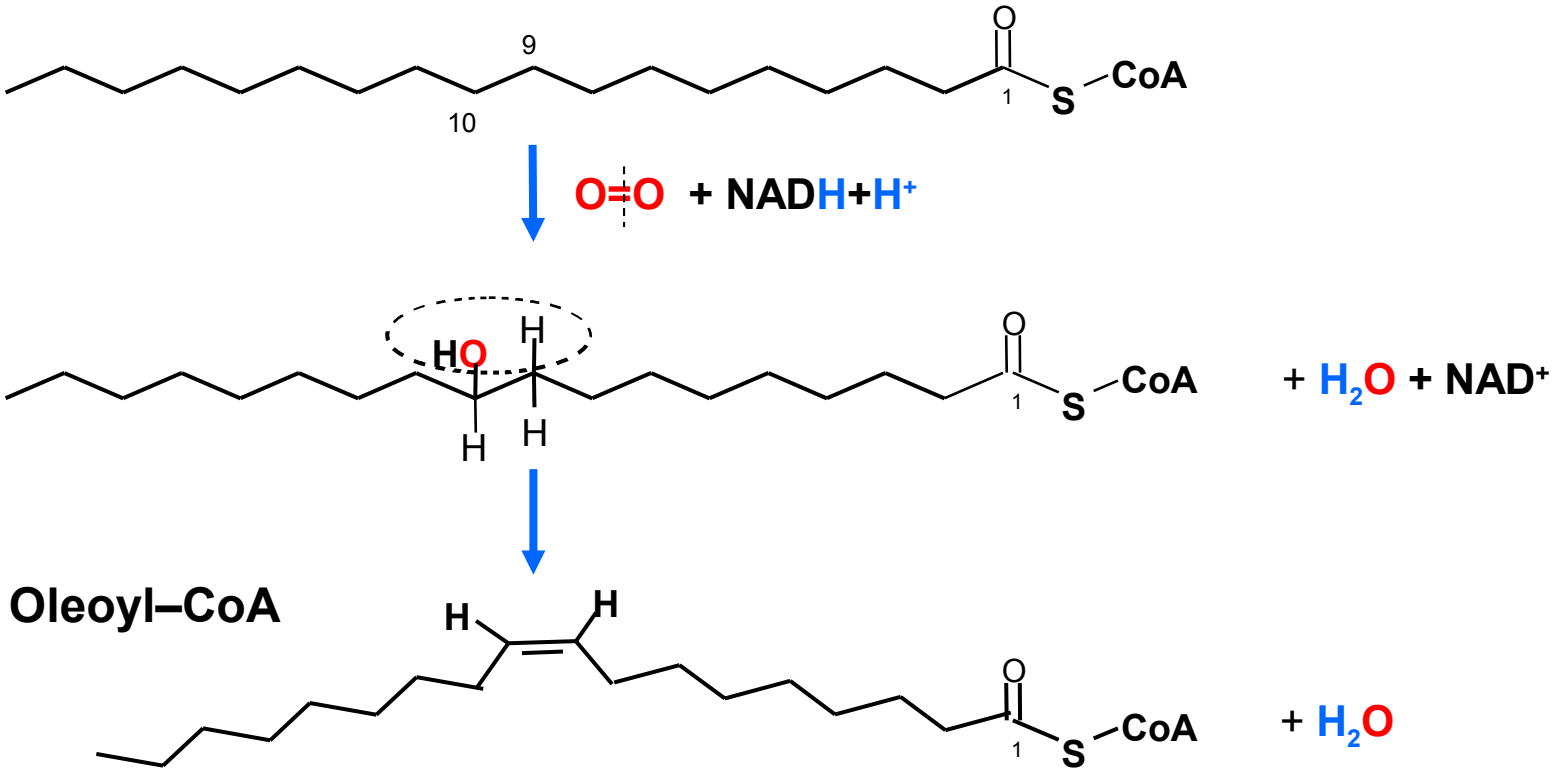
The enzymatic systems that catalyse desaturation are located in the smooth **endoplasmic reticulum** of liver cells.

Desaturases are **hydroxylating monooxygenases**, although water is eliminated from the hydroxylated product in the formation of the double bond. The reductant is **NADH+H$^+$**, from which the electrons are carried by the flavine enzyme and the cytochrome b_5 to a desaturase.



Example:

Stearoyl–CoA



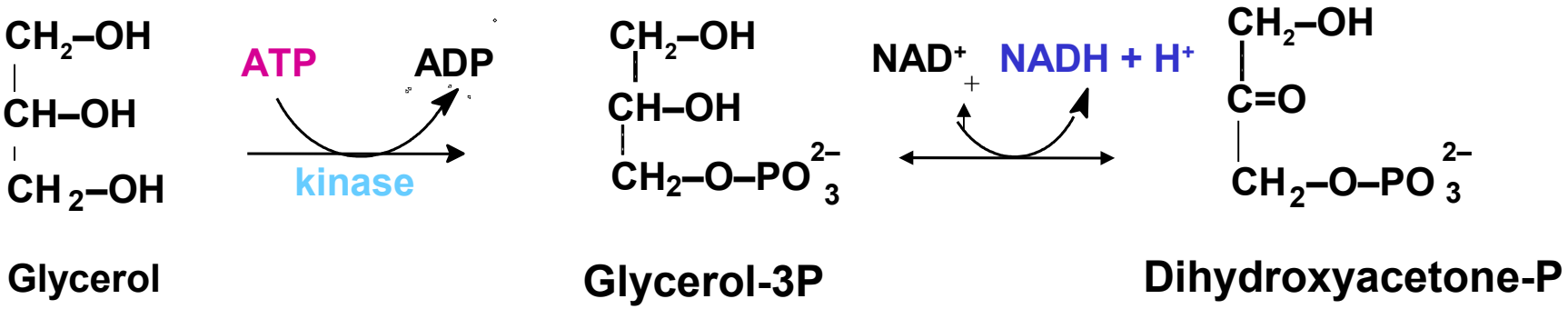
Synthesis of triacylglycerols

is provided by esterification of **glycerol 3-phosphate** (or dihydroxy-acetone phosphate) by activated fatty acids - **acylcoenzymes A**.

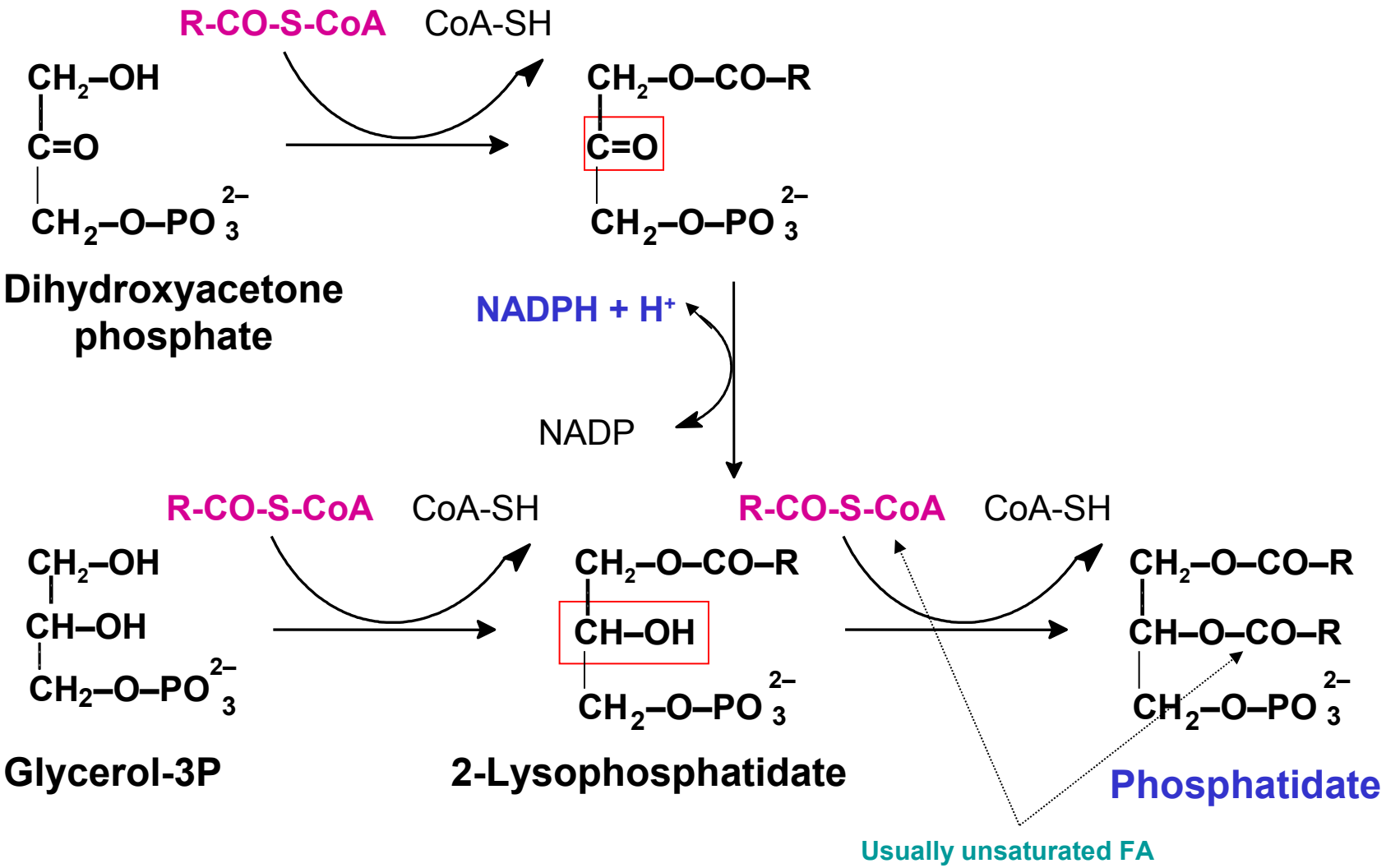
There are two possible sources of glycerol phosphate:

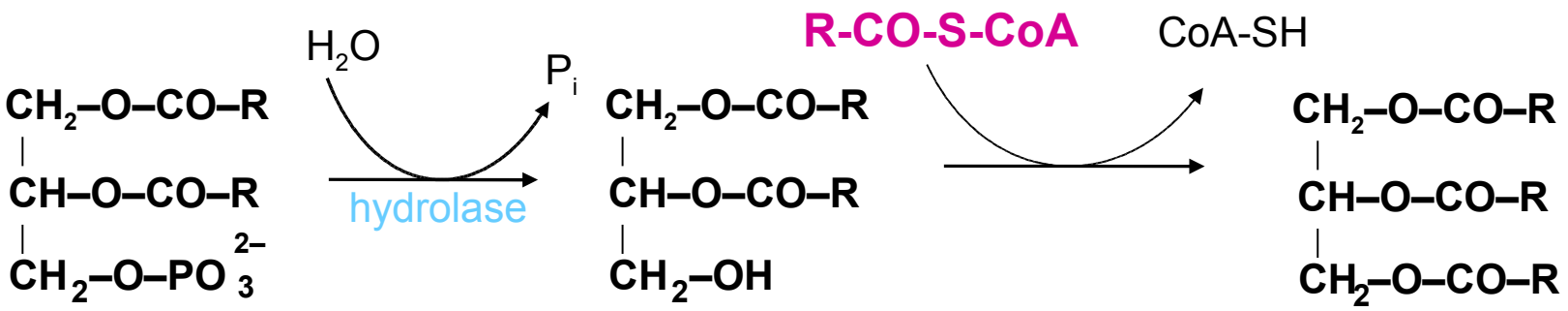
In **liver and small intestine** (but **not in adipose tissue**) is glycerol phosphorylated by **glycerol kinase.**

In **most other tissues** glycerol phosphate originates by reduction of dihydroxyacetone phosphate, an intermediate of glycolysis, by the action of **glycerol phosphate dehydrogenase**



Phosphatidate **is an intermediate** in the synthesis of triacylglycerols and glycerophospholipids in the endoplasmic reticulum:





Phosphatidate **1,2-Diacylglycerol** **TRIACYLGLYCEROLS**

Small intestine → **Chylomicrons**
Liver cells → **VLDL**
Adipocytes → **Reserve fat**

Glycerophospholipids

Phosphatidylserine
Phosphatidylinositol
Cardiolipin
Phosphatidylcholine
Phosphatidylethanolamine