Lipid Metabolism

- The oxidation of long-chain fatty acids to acetyl-CoA is a central energy yielding pathway in many organisms and tissues.

- In mammalian heart and liver, for example, it provides as much as 80% of the energetic needs under all physiological circumstances.

- The electrons removed from fatty acids during oxidation pass through the respiratory chain, driving ATP synthesis; the acetyl-CoA produced from the fatty acids may be completely oxidized to CO2 in the citric acid cycle, resulting in further energy conservation.

- In some species and in some tissues, the acetyl-CoA has alternative fates. In liver, acetyl-CoA may be converted to ketone bodies— water-soluble fuels exported to the brain and other tissues when glucose is not available.

- In vascular plants, acetyl-CoA serves primarily as a biosynthetic precursor, only secondarily as fuel.

- Although the biological role of fatty acid oxidation differs from organism to organism, the mechanism is essentially the same; the repetitive four-step process by which fatty acids are converted into acetyl-CoA, called ***β* oxidation**.

- Triacylglycerols (triglycerides or neutral fats) are especially suitable for storage fuels.

- The long alkyl chains of their constituent fatty acids are essentially hydrocarbons, highly reduced structures with an energy of complete oxidation (~38 kJ/g) more than twice that for the same weight of carbohydrate or protein.

- This advantage is compounded by the extreme insolubility of lipids in water; cellular triacylglycerols aggregate in lipid droplets, which do not raise the osmolarity of the cytosol, and they are unsolvated.

- And because of their relative chemical inertness, triacylglycerols can be stored in large quantity in cells without the risk of undesired chemical reactions with other cellular constituents.

- The properties that make triacylglycerols good storage compounds, however, present problems in their role as fuels.

- Because they are insoluble in water, ingested triacylglycerols must be emulsified before they can be digested by water-soluble enzymes in the intestine, and triacylglycerols absorbed in the intestine or mobilized from storage tissues must be carried in the blood bound to proteins that counteract their insolubility.

- Also, to overcome the relative stability of the C—C bonds in a fatty acid, the carboxyl group at C-1 is activated by attachment to coenzyme A, which allows stepwise oxidation of the fatty acyl group at the C-3, or β, position—hence the name β oxidation.

- Cells can obtain fatty acid fuels from four sources: fats consumed in the diet, fats stored in cells as lipid droplets, fats synthesized in one organ for export to another, and fats obtained by autophagy (which degrades the cell's own organelles).

- Some species use all four sources under various circumstances, others use one or two.

- Vertebrates, for example, obtain fats in the diet, mobilize fats stored in specialized tissue (adipose tissue, consisting of cells called adipocytes), and, in the liver, convert excess dietary carbohydrates to fats for export to other tissues.

- During starvation, they can recycle lipids by autophagy. On average, 40% or more of the daily energy requirement of humans in highly industrialized countries is supplied by dietary triacylglycerols (although most nutritional guidelines recommend no more than 30% of daily caloric intake from fats).

- Triacylglycerols provide more than half the energy requirements of some organs, particularly the liver, heart, and resting skeletal muscle.

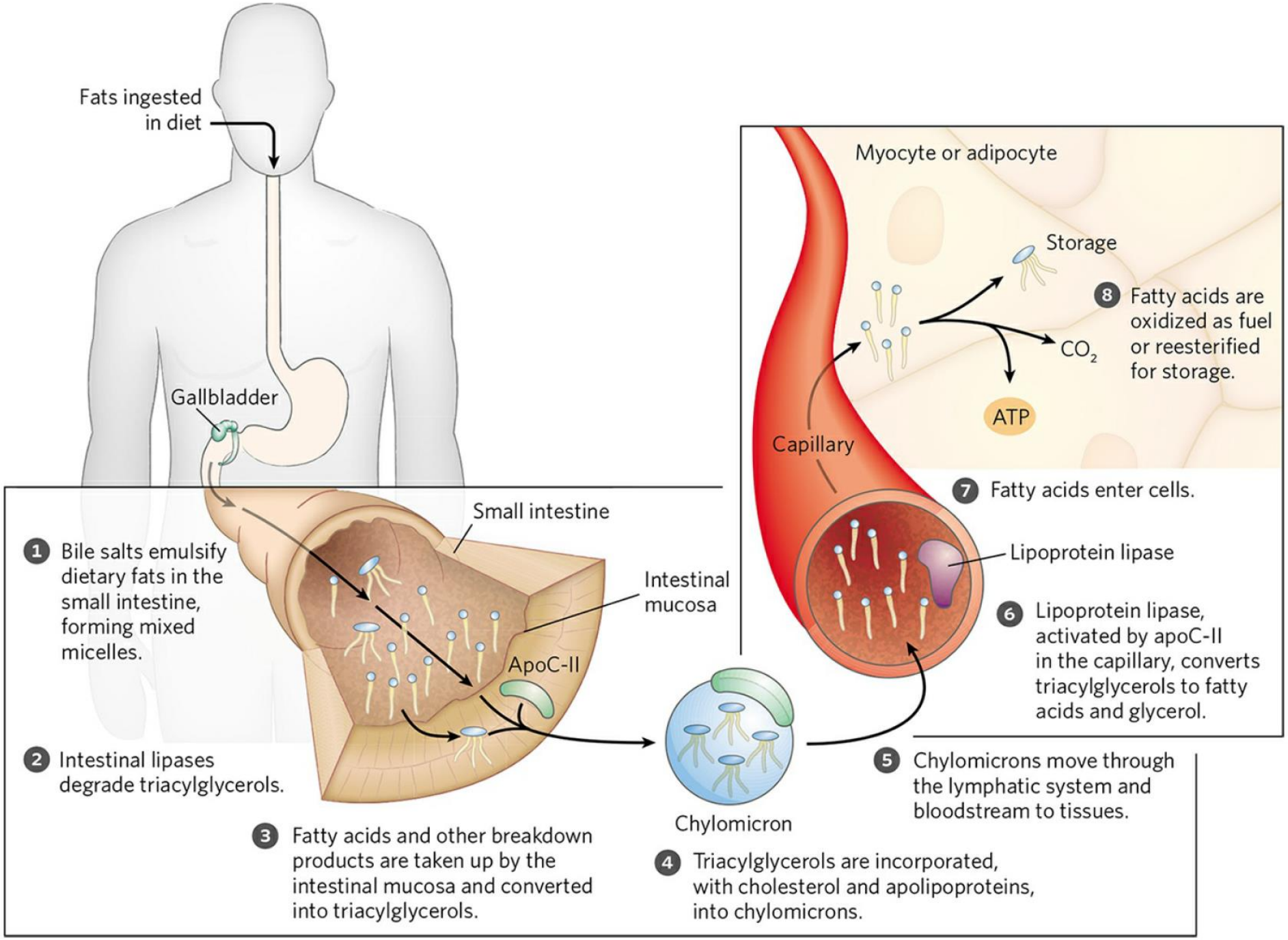
- Stored triacylglycerols are virtually the sole source of energy in hibernating animals and migrating birds.

- Protists obtain fats by consuming organisms lower in the food chain, and some also store fats as cytosolic lipid droplets.

- Vascular plants mobilize fats stored in seeds during germination, but do not otherwise depend on fats for energy.

- In vertebrates, before ingested triacylglycerols can be absorbed through the intestinal wall they must be converted from insoluble macroscopic fat particles to finely dispersed microscopic micelles.

- This solubilization is carried out by bile salts, such as taurocholic acid, which are synthesized from cholesterol in the liver, stored in the gallbladder, and released into the small intestine after ingestion of a fatty meal.

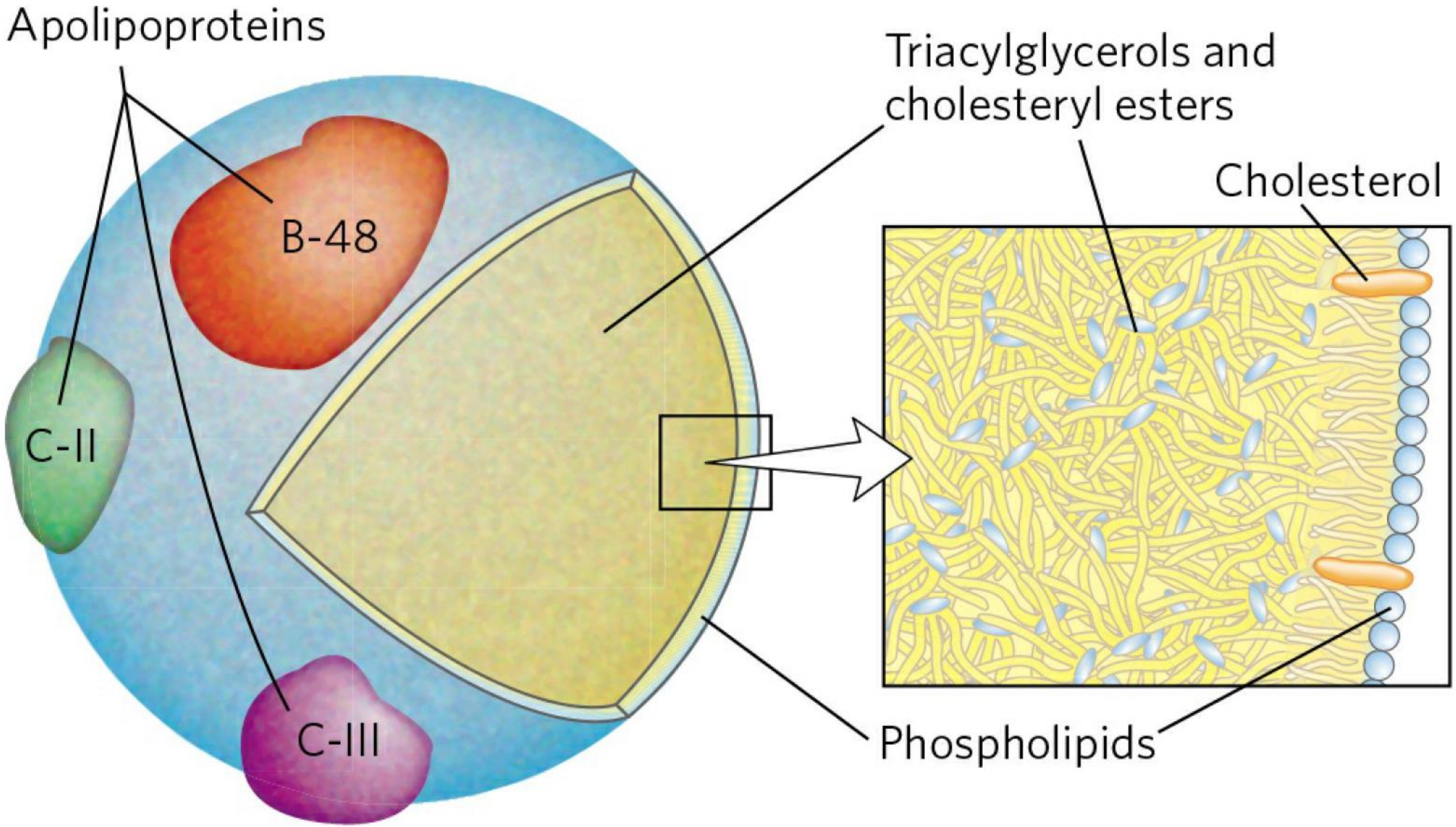

Fats ingested in diet
Gallbladder
Small intestine
Intestinal mucosa
ApoC-II
1 Bile salts emulsify dietary fats in the small intestine, forming mixed micelles.
2 Intestinal lipases degrade triacylglycerols.
3 Fatty acids and other breakdown products are taken up by the intestinal mucosa and converted into triacylglycerols.
4 Triacylglycerols are incorporated, with cholesterol and apolipoproteins, into chylomicrons.
Chylomicron
5 Chylomicrons move through the lymphatic system and bloodstream to tissues.
Myocyte or adipocyte
Capillary
6 Lipoprotein lipase, activated by apoC-II in the capillary, converts triacylglycerols to fatty acids and glycerol.
Lipoprotein lipase
7 Fatty acids enter cells.
Storage
8 Fatty acids are oxidized as fuel or reesterified for storage.
CO_2
ATP

- Apolipoproteins are lipid-binding proteins in the blood that are responsible for the transport of triacylglycerols, phospholipids, cholesterol, and cholesteryl esters between organs.

- Apolipoproteins combine with lipids to form several classes of lipoprotein particles, spherical aggregates with hydrophobic lipids at the core and hydrophilic protein side chains and lipid head groups at the surface.

- Various combinations of lipid and protein produce particles of different densities, ranging from chylomicrons and very-low-density lipoproteins (VLDL) to very-high-density lipoproteins (VHDL), which can be separated by ultracentrifugation.

- The protein moieties of lipoproteins are recognized by receptors on cell surfaces.

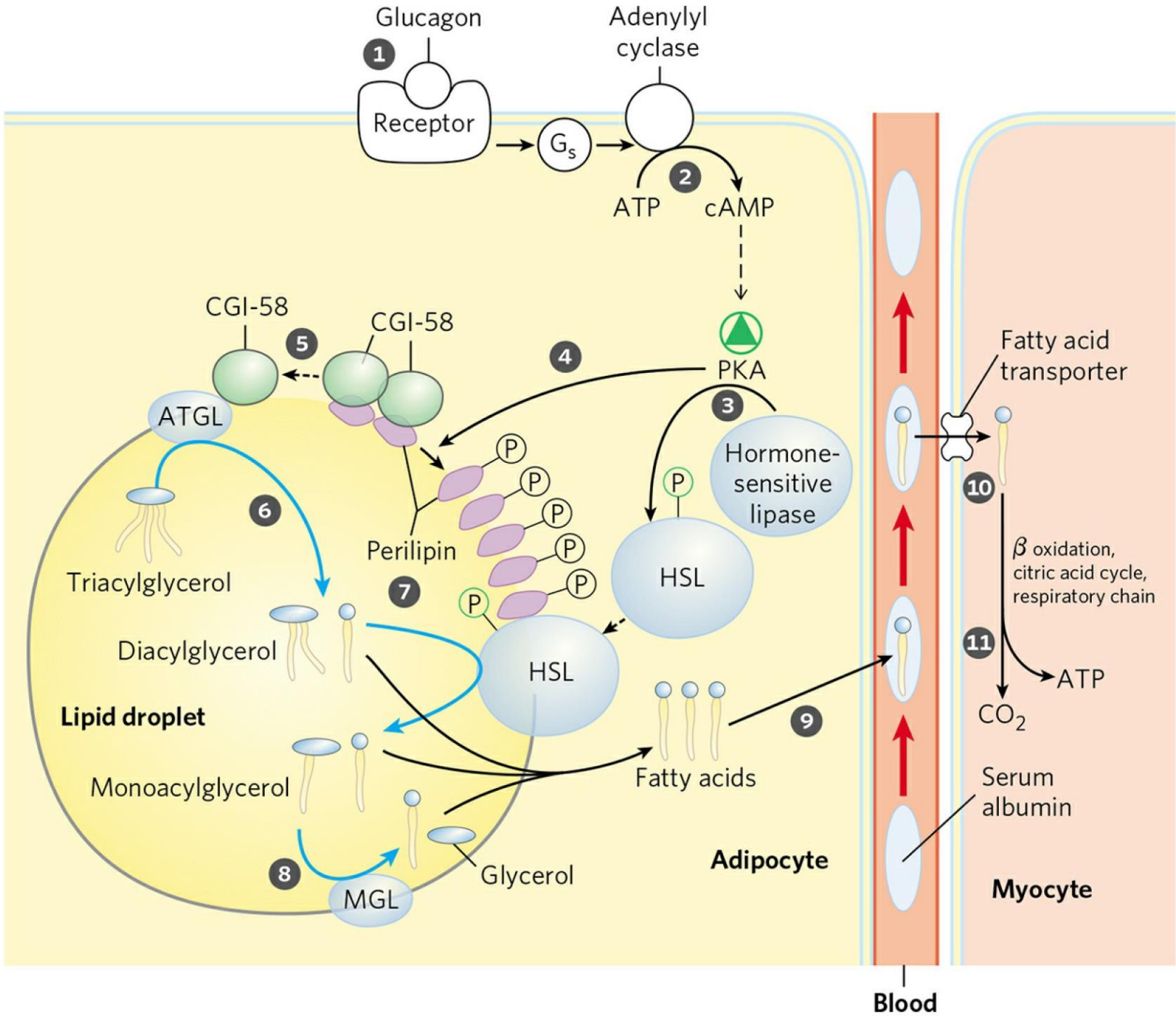

Apolipoproteins
B-48
C-II
C-III
Triacylglycerols and
cholesteryl esters
Cholesterol
Phospholipids

- Neutral lipids are stored in adipocytes (and in steroid-synthesizing cells of the adrenal cortex, ovary, and testis) in the form of lipid droplets, with a core of triacylglycerols and sterol esters surrounded by a monolayer of phospholipids.

- The surface of these droplets is coated with perilipins, a family of proteins that restrict access to lipid droplets, preventing untimely lipid mobilization.

- When hormones signal the need for metabolic energy, triacylglycerols stored in adipose tissue are mobilized (brought out of storage) and transported to tissues (skeletal muscle, heart, and renal cortex) in which fatty acids can be oxidized for energy production.

- The hormones epinephrine and glucagon, secreted in response to low blood glucose levels or a fight-or-flight situation, stimulate the enzyme adenylyl cyclase in the adipocyte plasma membrane, which produces the intracellular second messenger cyclic AMP (cAMP).


Glucagon
1
Receptor
G_s
Adenylyl cyclase
2
ATP
cAMP
PKA
4
3
Hormone-sensitive lipase
CGI-58
5
CGI-58
ATGL
6
Triacylglycerol
Perilipin
P
HSL
7
Diacylglycerol
HSL
Lipid droplet
Monoacylglycerol
8
MGL
Glycerol
Fatty acids
9
Adipocyte
Fatty acid transporter
10
β oxidation, citric acid cycle, respiratory chain
11
ATP
CO_2
Serum albumin
Myocyte
Blood

- Cyclic AMP–dependent protein kinase (PKA) triggers changes that open the lipid droplet to the action of three cytosolic lipases, which act on tri-, di-, and monoacylglycerols, releasing fatty acids and glycerol.

- The fatty acids thus released (free fatty acids, FFAs) pass from the adipocyte into the blood, where they bind to the blood protein serum albumin.

- This protein (Mr 66,000), which makes up about half of the total serum protein, noncovalently binds as many as 10 fatty acids per protein monomer.

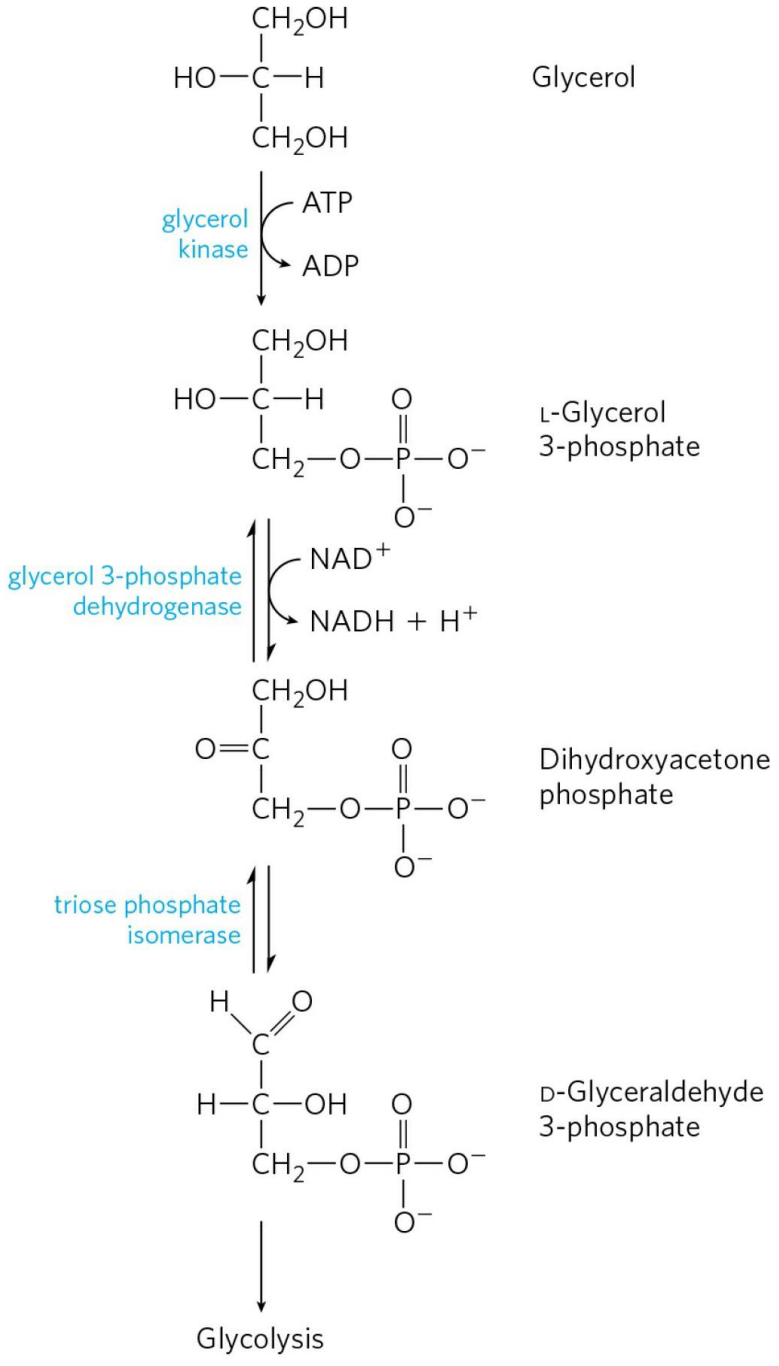
- Bound to this soluble protein, the otherwise insoluble fatty acids are carried to tissues such as skeletal muscle, heart, and renal cortex.

- In these target tissues, fatty acids dissociate from albumin and are moved by plasma membrane transporters into cells to serve as fuel.

- About 95% of the biologically available energy of triacylglycerols resides in their three long-chain fatty acids; only 5% is contributed by the glycerol moiety.

- The glycerol released by lipase action is phosphorylated by glycerol kinase, and the resulting glycerol 3-phosphate is oxidized to dihydroxyacetone phosphate.

- The glycolytic enzyme triose phosphate isomerase converts this compound to glyceraldehyde 3-phosphate, which is oxidized via glycolysis.



- The enzymes of fatty acid oxidation in animal cells are located in the mitochondrial matrix.

- The fatty acids with chain lengths of 12 or fewer carbons enter mitochondria without the help of membrane transporters.

- Those with 14 or more carbons, which constitute the majority of the FFAs obtained in the diet or released from adipose tissue, cannot pass directly through the mitochondrial membranes: they must first undergo the three enzymatic reactions of the carnitine shuttle.

- The first reaction is catalyzed by a family of isozymes of acyl-CoA synthetase, each specific for fatty acids having either short, intermediate, or long carbon chains.

- The isozymes are present in the outer mitochondrial membrane, where they promote the general reaction

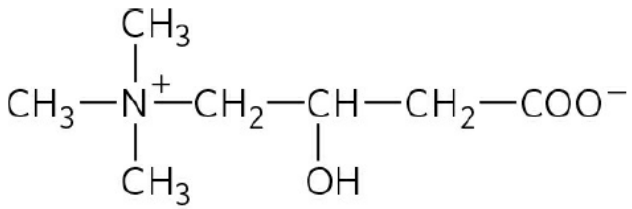
$$\text{Fatty acid} + \text{CoA} + \text{ATP} \rightleftharpoons \text{fatty acyl-CoA} + \text{AMP} + \text{PP}_\text{i}$$

- Thus, acyl–CoA synthetases catalyze the formation of a thioester linkage between the fatty acid carboxyl group and the thiol group of coenzyme A to yield a fatty acyl–CoA, coupled to the cleavage of ATP to AMP and PPi.

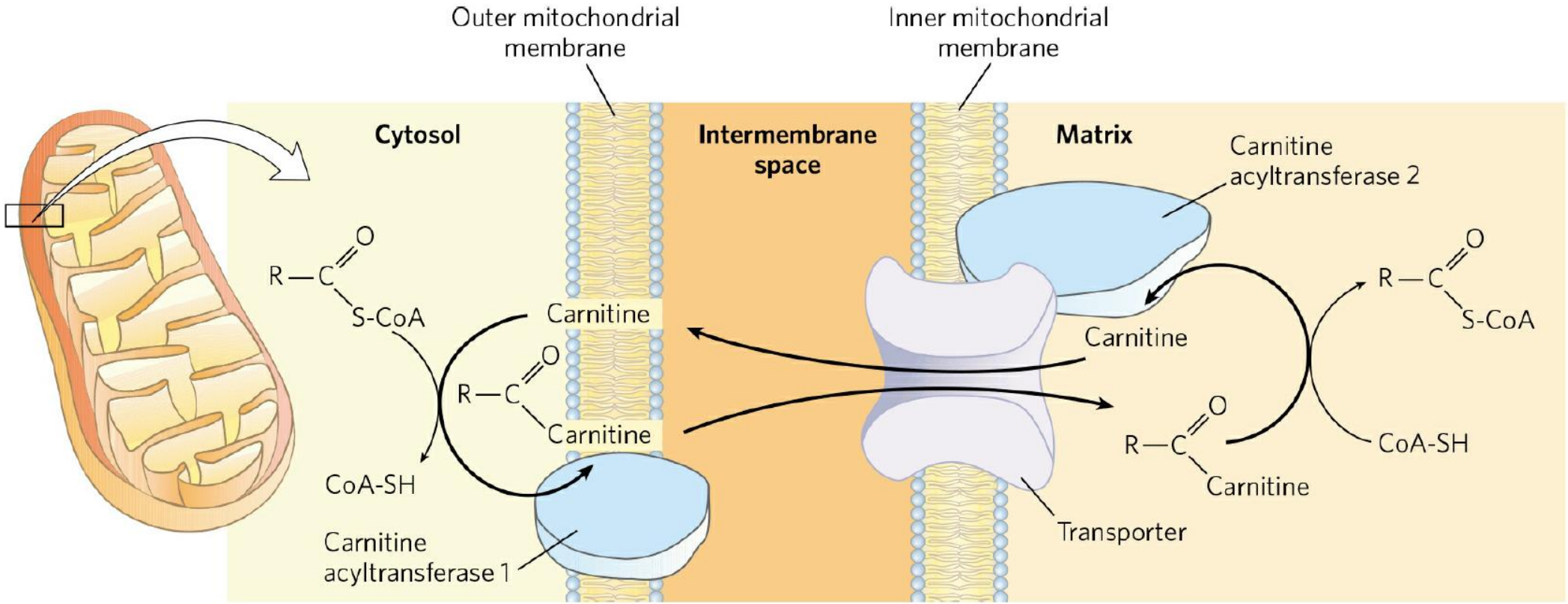
- Fatty acyl–CoAs, like acetyl-CoA, are high-energy compounds; their hydrolysis to FFAs and CoA has a large, negative standard free-energy change $\Delta G'^{\circ} = -31$ kJ/mol).

- Fatty acyl–CoA esters formed on the cytosolic side of the outer mitochondrial membrane can be transported into the mitochondrion and oxidized to produce ATP, or they can be used in the cytosol to synthesize membrane lipids.

- Fatty acids destined for mitochondrial oxidation are transiently attached to the hydroxyl group of carnitine to form fatty acyl–carnitine—the second reaction of the shuttle.



Carnitine



- In the third and final step of the carnitine shuttle, the fatty acyl group is transferred from carnitine to intramitochondrial coenzyme A by carnitine acyltransferase 2 (also called CPT2).

- This isozyme, located on the inner face of the inner mitochondrial membrane, regenerates fatty acyl–CoA and releases it, along with free carnitine, into the matrix

- This three-step process for transferring fatty acids into the mitochondrion — esterification to CoA, transesterification to carnitine followed by transport, and transesterification back to CoA—links two separate pools of coenzyme A and of fatty acyl–CoA, one in the cytosol, the other in mitochondria.

- These pools have different functions. Coenzyme A in the mitochondrial matrix is largely used in oxidative degradation of pyruvate, fatty acids, and some amino acids, whereas cytosolic coenzyme A is used in the biosynthesis of fatty acids.

- Fatty acyl–CoA in the cytosolic pool can be used there for membrane lipid synthesis or can be moved into the mitochondrial matrix for oxidation and ATP production.

- Conversion to the carnitine ester commits the fatty acyl moiety to the oxidative fate.

- The carnitine-mediated entry process is the rate-limiting step for oxidation of fatty acids in mitochondria and is a control point.

- In the first stage of β oxidation, fatty acids undergo oxidative removal of successive two-carbon units in the form of acetyl-CoA, starting from the carboxyl end of the fatty acyl chain.

- For example, the 16- carbon palmitic acid (palmitate at pH 7) undergoes seven passes through the oxidative sequence, in each pass losing two carbons as acetyl-CoA.

- At the end of seven cycles the last two carbons of palmitate (originally C-15 and C- 16) remain as acetyl-CoA.

- The overall result is the conversion of the 16- carbon chain of palmitate to eight two-carbon acetyl groups of acetyl-CoA molecules.

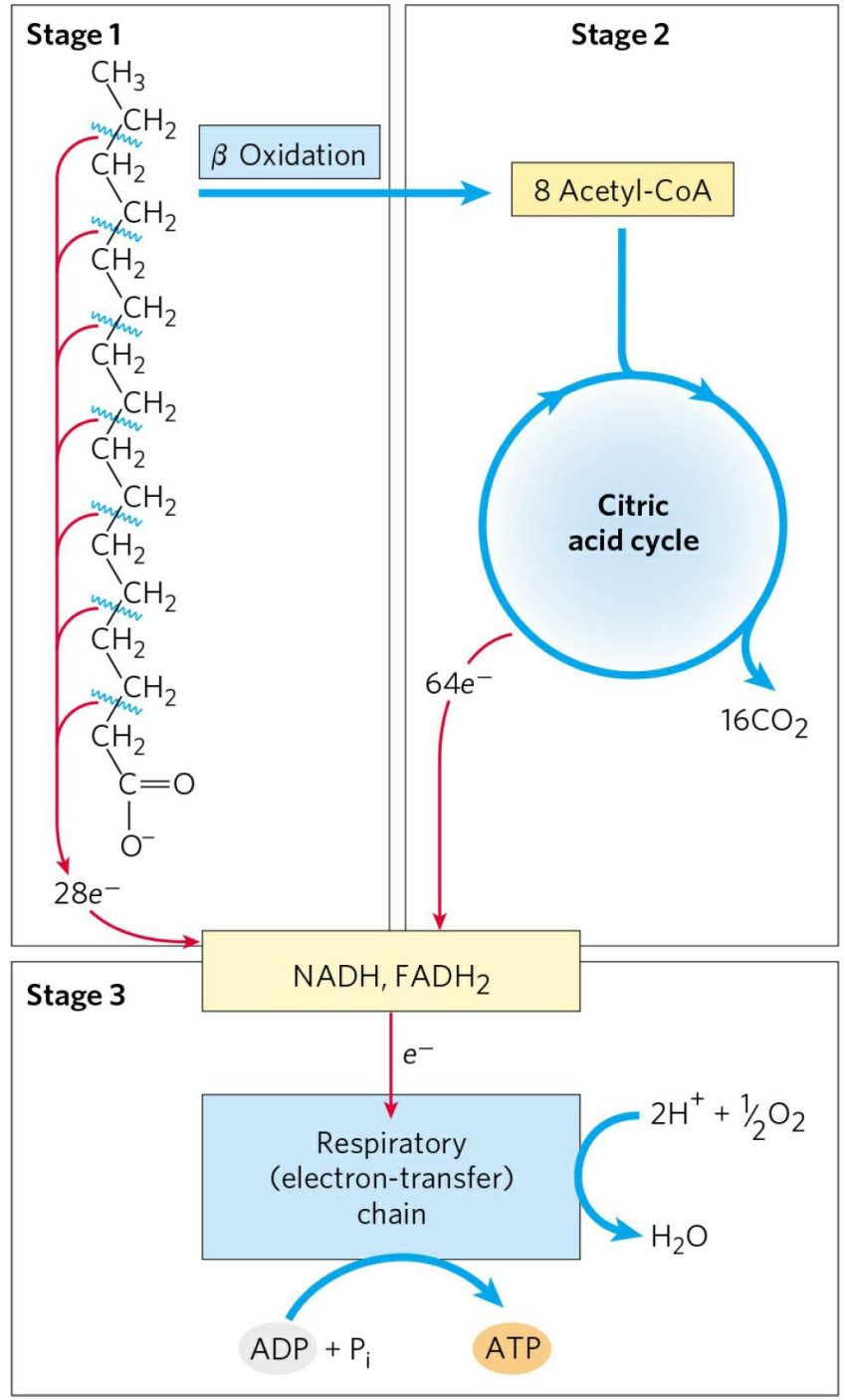
- Formation of each acetyl-CoA requires removal of four hydrogen atoms (two pairs of electrons and four H+) from the fatty acyl moiety by dehydrogenases.

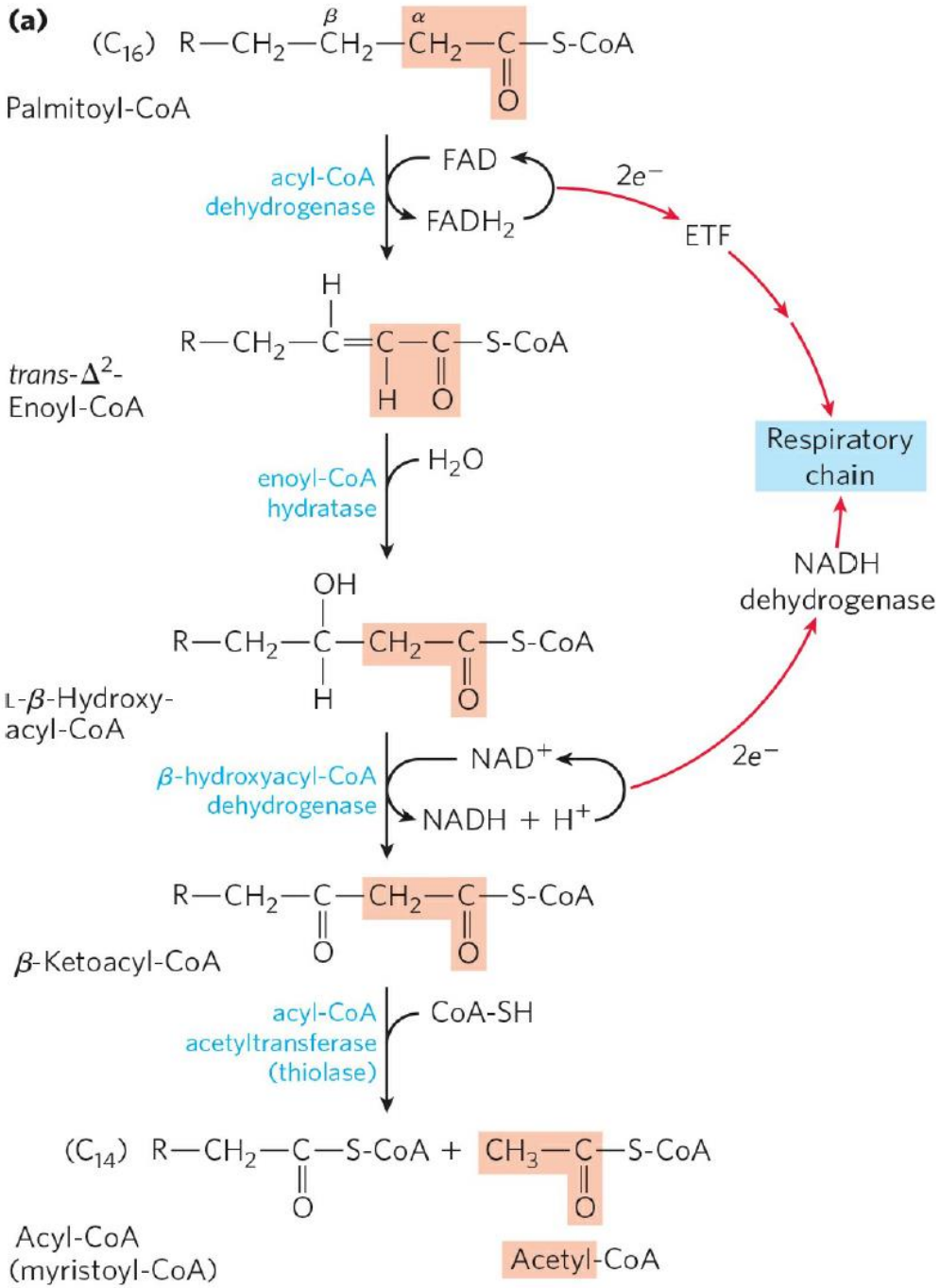
- In the second stage of fatty acid oxidation, the acetyl groups of acetyl- CoA are oxidized to CO_2 in the citric acid cycle, which also takes place in the mitochondrial matrix.

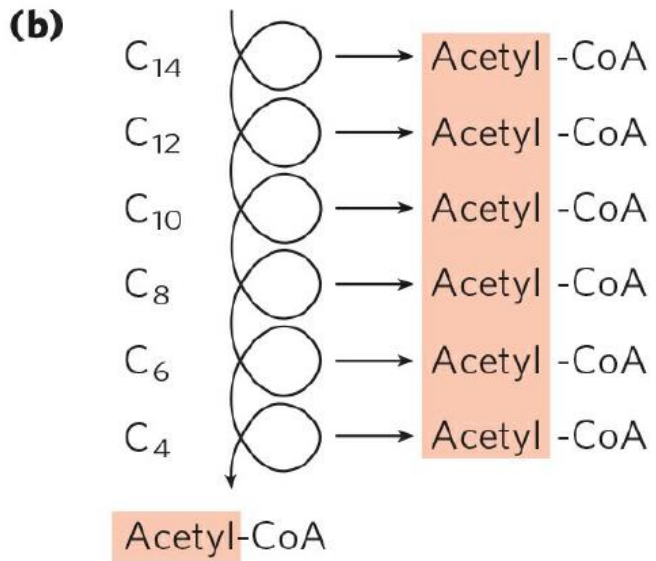
- Acetyl-CoA derived from fatty acids thus enters a final common pathway of oxidation with the acetyl-CoA derived from glucose via glycolysis and pyruvate oxidation.

- The first two stages of fatty acid oxidation produce the reduced electron carriers NADH and $FADH_2$ which in the third stage donate electrons to the mitochondrial respiratory chain, through which the electrons pass to oxygen with the concomitant phosphorylation of ADP to ATP.

- The energy released by fatty acid oxidation is thus conserved as ATP.


Stage 1
Stage 2
β Oxidation
8 Acetyl-CoA
Citric acid cycle
64e⁻
16CO2
28e⁻
NADH, FADH2
Stage 3
e⁻
Respiratory (electron-transfer) chain
2H+ + ½O2
H2O
ADP + Pi
ATP


(a)
(C16) R—CH2—CH2—CH2—C—S-CoA
Palmitoyl-CoA
acyl-CoA dehydrogenase
FAD
FADH2
2e−
ETF
Respiratory chain
NADH dehydrogenase
trans-Δ2-Enoyl-CoA
enoyl-CoA hydratase
H2O
L-β-Hydroxy-acyl-CoA
β-hydroxyacyl-CoA dehydrogenase
NAD+
NADH + H+
2e−
β-Ketoacyl-CoA
acyl-CoA acetyltransferase (thiolase)
CoA-SH
(C14) R—CH2—C—S-CoA + CH3—C—S-CoA
Acyl-CoA (myristoyl-CoA)
Acetyl-CoA


(b)
C14
C12
C10
C8
C6
C4
Acetyl -CoA
Acetyl -CoA
Acetyl -CoA
Acetyl -CoA
Acetyl -CoA
Acetyl -CoA
Acetyl-CoA

- Four enzyme-catalyzed reactions make up the first stage of fatty acid oxidation.

- First, dehydrogenation of fatty acyl–CoA produces a double bond between the α and β carbon atoms (C-2 and C-3), yielding a trans-Δ2-enoyl-CoA.

- This first step is catalyzed by three isozymes of acyl-CoA dehydrogenase, each specific for a range of fatty-acyl chain lengths: very long chain acyl-CoA dehydrogenase (VLCAD), acting on fatty acids of 12 to 18 carbons; medium-chain (MCAD), 4 to 14 carbons; and short-chain (SCAD), 4 to 8 carbons.

- VLCAD is in the inner mitochondrial membrane; MCAD and SCAD are in the matrix.

- All three isozymes are flavoproteins with tightly bound FAD as a prosthetic group.

- The electrons removed from the fatty acyl–CoA are transferred to FAD, and the reduced form of the dehydrogenase immediately donates its electrons to an electron carrier of the mitochondrial respiratory chain.

- The oxidation catalyzed by an acyl-CoA dehydrogenase is analogous to succinate dehydrogenation in the citric acid cycle.

- Both reactions the enzyme is bound to the inner membrane, a double bond is introduced into a carboxylic acid between the α and β carbons, FAD is the electron acceptor, and electrons from the reaction ultimately enter the respiratory chain and pass to O2, with the concomitant synthesis of about 1.5 ATP molecules per electron pair.

- In the second step of the β-oxidation cycle, water is added to the double bond of the trans-Δ2-enoyl-CoA to form the L stereoisomer of β-hydroxyacyl-CoA (3-hydroxyacyl-CoA).

- This reaction, catalyzed by enoyl-CoA hydratase, is formally analogous to the fumarase reaction in the citric acid cycle, in which H2O adds across an α-β double bond.

- In the third step, L-β-hydroxyacyl-CoA is dehydrogenated to form β- ketoacyl-CoA, by the action of β-hydroxyacyl-CoA dehydrogenase; NAD+ is the electron acceptor.

- This enzyme is absolutely specific for the L stereoisomer of hydroxyacyl-CoA. The NADH formed in the reaction donates its electrons to NADH dehydrogenase, an electron carrier of the respiratory chain, and ATP is formed from ADP as the electrons pass to O2.

- The reaction catalyzed by β-hydroxyacyl-CoA dehydrogenase is closely analogous to the malate dehydrogenase reaction of the citric acid cycle.

- The fourth and last step of the β-oxidation cycle is catalyzed by acyl-CoA acetyltransferase, more commonly called thiolase, which promotes reaction of β-ketoacyl-CoA with a molecule of free coenzyme A to split off the carboxyl-terminal two-carbon fragment of the original fatty acid as acetyl- CoA.

- The other product is the coenzyme A thioester of the fatty acid, now shortened by two carbon atoms.

- This reaction is called thiolysis, by analogy with the process of hydrolysis, because the β-ketoacyl-CoA is cleaved by reaction with the thiol group of coenzyme A.

- The thiolase reaction is a reverse Claisen condensation.

- The last three steps of this four-step sequence are catalyzed by either of two sets of enzymes, with the enzymes employed depending on the length of the fatty acyl chain.

- For fatty acyl chains of 12 or more carbons, the reactions are catalyzed by a multienzyme complex associated with the inner mitochondrial membrane, the trifunctional protein (TFP).

- TFP is a heterooctamer of α4β4 subunits. Each α subunit contains two activities, the enoyl-CoA hydratase and the β-hydroxyacyl-CoA dehydrogenase; the β subunits contain the thiolase activity.

- This tight association of three enzymes may allow efficient substrate channeling from one active site to the next, without diffusion of the intermediates away from the enzyme surface.

- When TFP has shortened the fatty acyl chain to 12 or fewer carbons, further oxidations are catalyzed by a set of four soluble enzymes in the matrix.

- In one pass through the β-oxidation sequence, one molecule of acetyl-CoA, two pairs of electrons, and four protons (H+) are removed from the long chain fatty acyl–CoA, shortening it by two carbon atoms.

- The equation for one pass, beginning with the coenzyme A ester of our example, palmitate, is;

$$\text{Palmityol-CoA} + \text{CoA} + \text{FAD} + \text{NAD}^+ + \text{H}_2\text{O} \rightarrow \text{myristoyl-CoA} + \text{acetyl-CoA} + \text{FADH}_2 + \text{NADH} + \text{H}^+$$

- Following removal of one acetyl-CoA unit from palmitoyl-CoA, the coenzyme A thioester of the shortened fatty acid (now the 14-carbon myristate) remains.

- The myristoyl-CoA can now go through another set of four β-oxidation reactions,

- Altogether, seven passes through the β-oxidation sequence are required to oxidize one molecule of palmitoyl-CoA to eight molecules of acetyl-CoA.

$$\text{Palmitoyl-CoA} + 7\text{CoA} + 7\text{FAD} + 7\text{NAD}^+ + 7\text{H}_2\text{O} \rightarrow 8\ \text{acetyl-CoA} + 7\text{FADH}_2 + 7\text{NADH} + 7\text{H}^+$$

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- Thus four molecules of ATP are formed for each two-carbon unit removed in one pass through the sequence (2.5 for NADH and 1.5 for FADH2).

- Note that water is also produced in this process. Each pair of electrons transferred from NADH or FADH2 to O2 yields one H2O, referred to as “metabolic water.”

- In hibernating animals, fatty acid oxidation provides metabolic energy, heat, and water—all essential for survival of an animal that neither eats nor drinks for long periods.

- Camels obtain water to supplement the meager supply available in their natural environment by oxidation of fats stored in their hump.

- The overall equation for the oxidation of palmitoyl-CoA to eight molecules of acetyl-CoA, including the electron transfers and oxidative phosphorylations, is

$$\text{Palmityol-CoA} + 7\text{CoA} + 7\text{O}_2 + 28\text{P}_\text{i} + 28\text{ADP} \rightarrow 8\ \text{acetyl-CoA} + 28\text{ATP} + 7\text{H}_2\text{O}$$

TABLE 17-1 Yield of ATP during Oxidation of One Molecule of Palmitoyl-CoA to CO_2 and H_2

Enzyme catalyzing the oxidation step	Number of NADH or $FADH_2$ formed	Number of ATP ultimately formed[a]
β Oxidation		
Acyl-CoA dehydrogenase	7 $FADH_2$	10.5
β-Hydroxyacyl-CoA dehydrogenase	7 NADH	17.5
Citric acid cycle		
Isocitrate dehydrogenase	8 NADH	20
α-Ketoglutarate dehydrogenase	8 NADH	20
Succinyl-CoA synthetase		8[b]
Succinate dehydrogenase	8 $FADH_2$	12
Malate dehydrogenase	8 NADH	20
Total		108

[a]These calculations assume that mitochondrial oxidative phosphorylation produces 1.5 ATP per $FADH_2$ oxidized and 2.5 ATP per NADH oxidized.

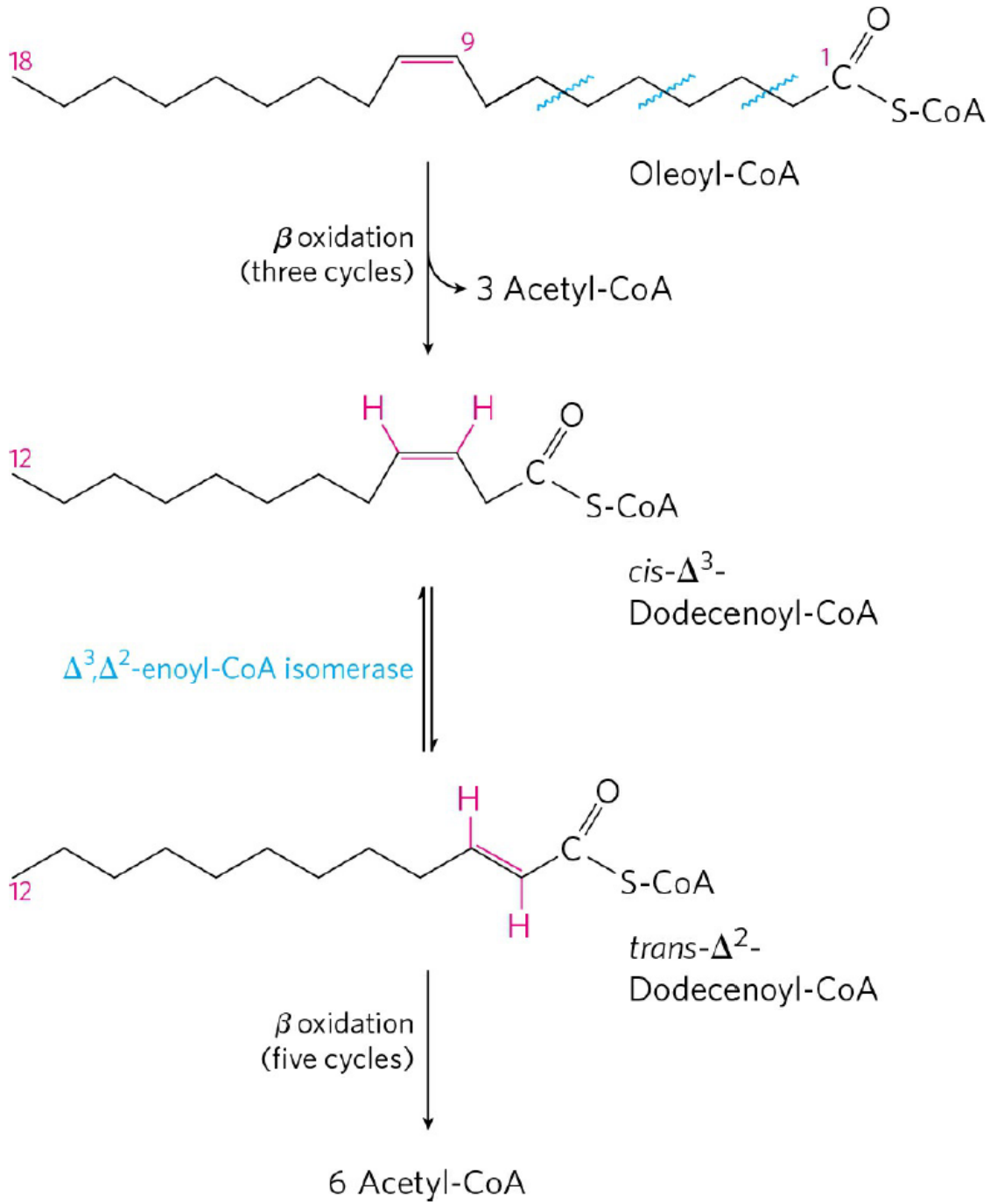
[b]GTP produced directly in this step yields ATP in the reaction catalyzed by nucleoside diphosphate kinase (p. 516).

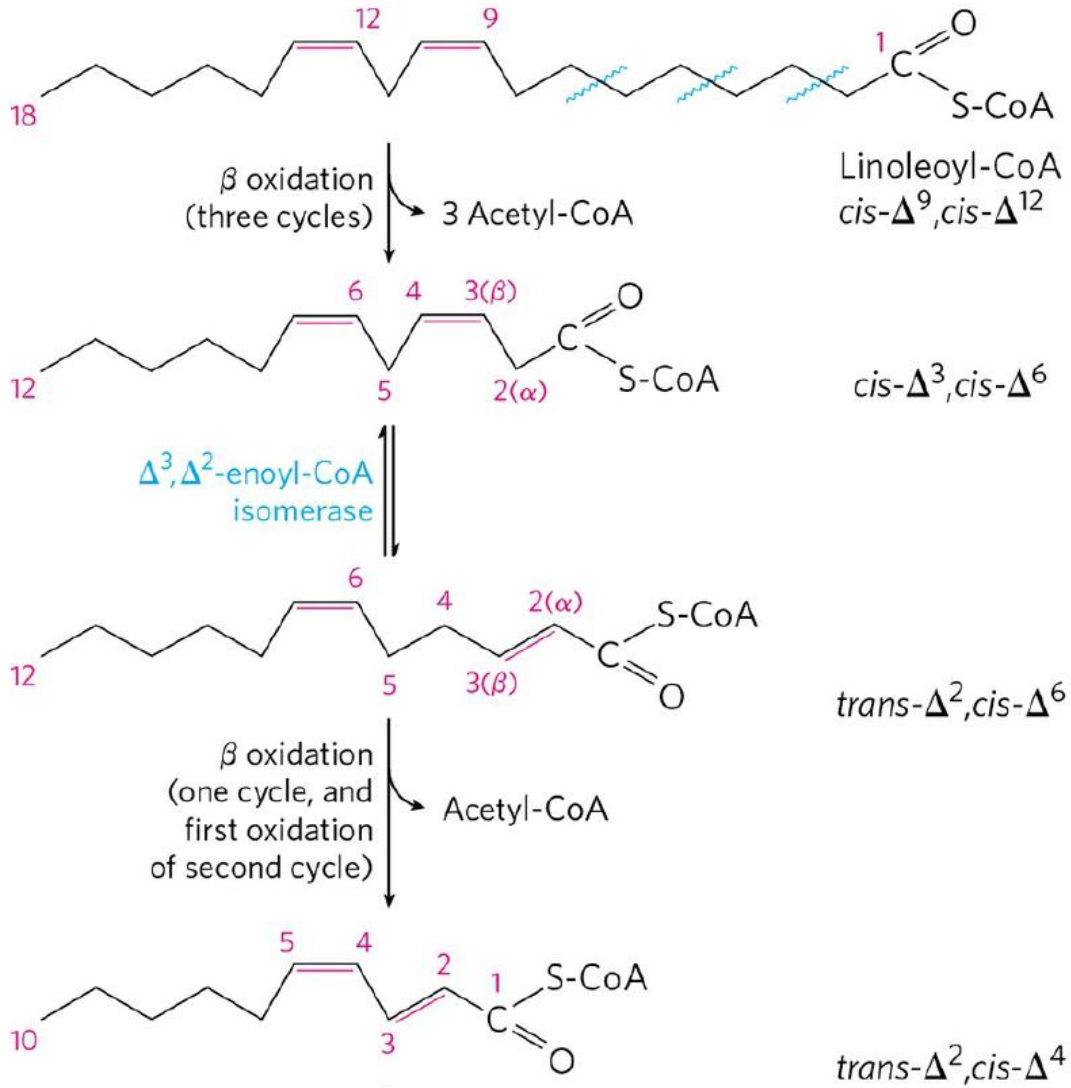
- The fatty acid oxidation sequence just described is typical when the incoming fatty acid is saturated (that is, has only single bonds in its carbon chain).

- However, most of the fatty acids in the triacylglycerols and phospholipids of animals and plants are unsaturated, having one or more double bonds.

- These bonds are in the cis configuration and cannot be acted upon by enoyl-CoA hydratase, the enzyme catalyzing the addition of H_2O to the trans double bond of the $\Delta 2$-enoyl-CoA generated during β oxidation.

- Two auxiliary enzymes are needed for β oxidation of the common unsaturated fatty acids: an isomerase and a reductase.


18
9
1
O
C
S-CoA
Oleoyl-CoA
β oxidation
(three cycles)
3 Acetyl-CoA
H
H
12
O
C
S-CoA
cis-Δ3-
Dodecenoyl-CoA
Δ3,Δ2-enoyl-CoA isomerase
H
12
O
C
S-CoA
H
trans-Δ2-
Dodecenoyl-CoA
β oxidation
(five cycles)
6 Acetyl-CoA


Linoleoyl-CoA
cis-Δ9,cis-Δ12
β oxidation
(three cycles)
3 Acetyl-CoA
cis-Δ3,cis-Δ6
Δ3,Δ2-enoyl-CoA
isomerase
trans-Δ2,cis-Δ6
β oxidation
(one cycle, and
first oxidation
of second cycle)
Acetyl-CoA
trans-Δ2,cis-Δ4
2,4-dienoyl-CoA
reductase
NADPH + H+
NADP+
trans-Δ3
enoyl-CoA
isomerase
trans-Δ2
β oxidation
(four cycles)
5 Acetyl-CoA

- Although most naturally occurring lipids contain fatty acids with an even number of carbon atoms, fatty acids with an odd number of carbons are common in the lipids of many plants and some marine organisms.

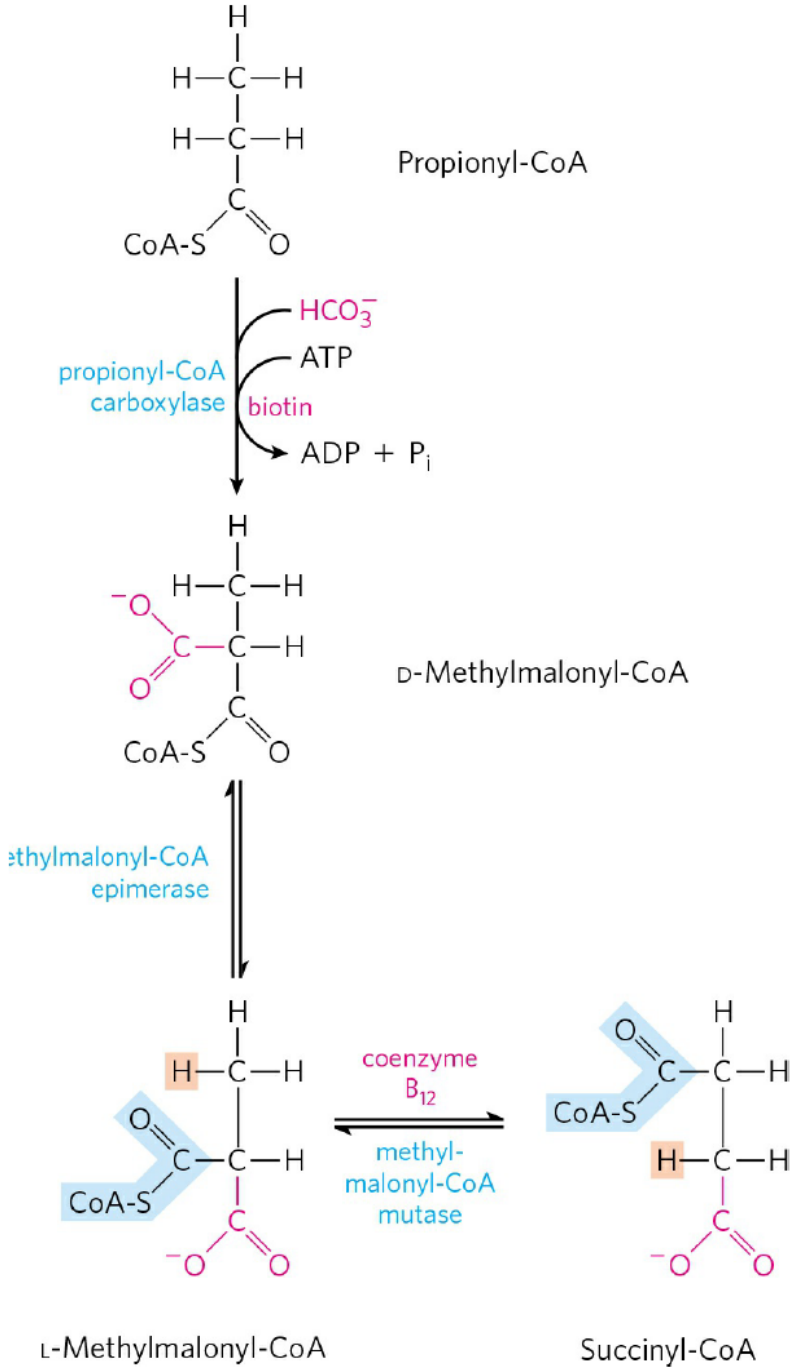
- Cattle and other ruminant animals form large amounts of the three-carbon propionate ($CH_3—CH_2—COO^-$) during fermentation of carbohydrates in the rumen.

- The propionate is absorbed into the blood and oxidized by the liver and other tissues.

- Long-chain odd-number fatty acids are oxidized in the same pathway as the even-number acids, beginning at the carboxyl end of the chain.

- However, the end products are acetyl-CoA's and propionyl-CoA.

- The acetyl-CoA can be oxidized in the citric acid cycle, of course, but propionyl-CoA enters a different pathway, having three enzymes.


Propionyl-CoA
HCO_3^-
ATP
propionyl-CoA carboxylase
biotin
ADP + P_i
D-Methylmalonyl-CoA
ethylmalonyl-CoA epimerase
coenzyme B_{12}
methyl-malonyl-CoA mutase
L-Methylmalonyl-CoA
Succinyl-CoA

- Oxidation of fatty acids consumes a precious fuel, and it is regulated so as to occur only when the organism's need for energy requires it.

- In the liver, fatty acyl–CoA formed in the cytosol has two major pathways open to it: (1) β oxidation by enzymes in mitochondria or (2) conversion into triacylglycerols and phospholipids by enzymes in the cytosol.

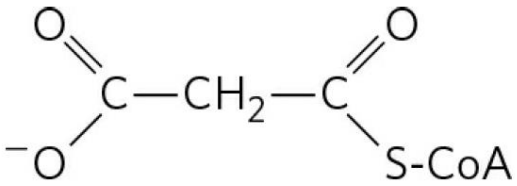
- The pathway taken depends on the rate of transfer of long-chain fatty acyl–CoA into mitochondria.

- The three-step process (carnitine shuttle) by which fatty acyl groups are carried from cytosolic fatty acyl–CoA into the mitochondrial matrix is rate-limiting for fatty acid oxidation and is an important point of regulation.

- Once fatty acyl groups have entered the mitochondrion, they are committed to oxidation to acetyl-CoA.

- Malonyl-CoA, the first intermediate in the cytosolic biosynthesis of longchain fatty acids from acetyl-CoA, increases in concentration whenever the animal is well supplied with carbohydrate; excess glucose that cannot be oxidized or stored as glycogen is converted in the cytosol into fatty acids for storage as triacylglycerol.

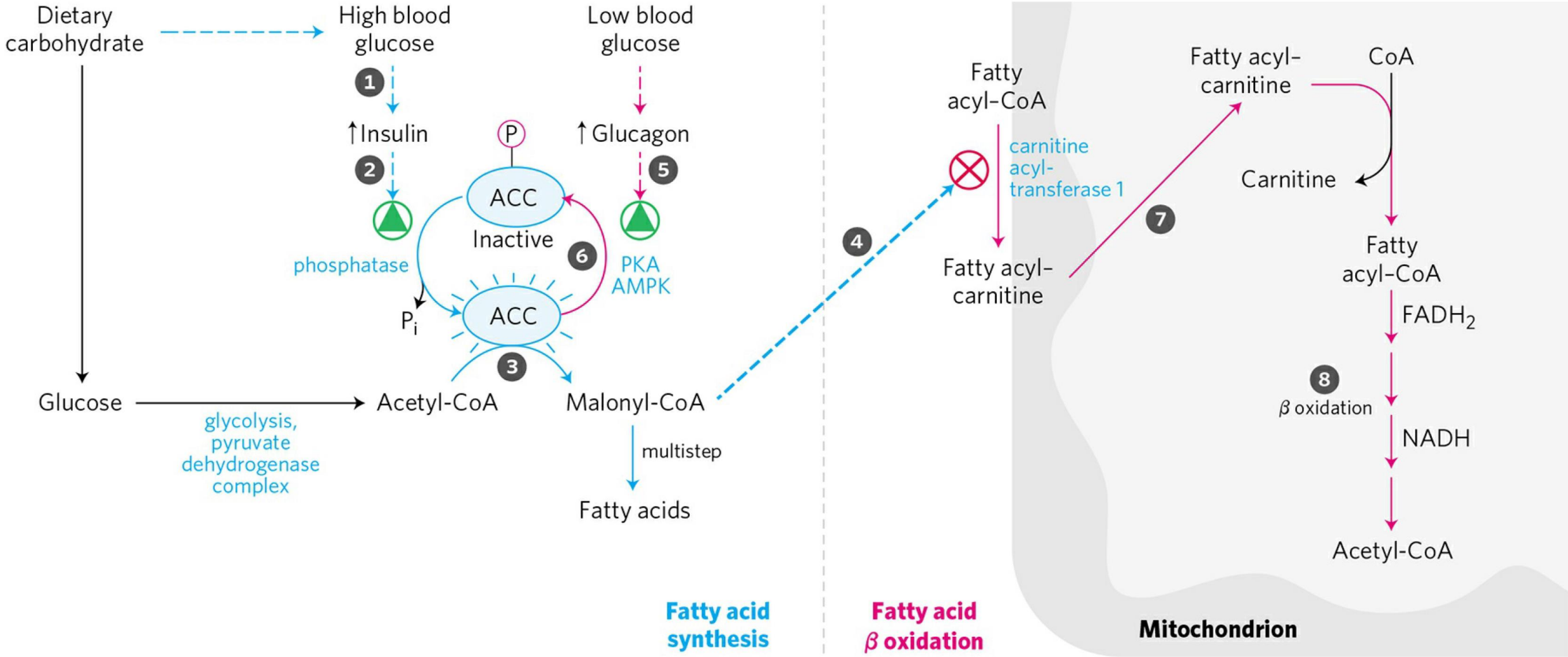
- The inhibition of carnitine acyltransferase 1 by malonyl-CoA ensures that the oxidation of fatty acids is inhibited whenever the liver is amply supplied with glucose as fuel and is actively making triacylglycerols from excess glucose.



Malonyl-CoA

- Two of the enzymes of β oxidation are also regulated by metabolites that signal energy sufficiency.

- When the [NADH]/[NAD+] ratio is high, β-hydroxyacyl-CoA dehydrogenase is inhibited; in addition, high concentrations of acetyl-CoA inhibit thiolase.


Dietary carbohydrate
High blood glucose
Low blood glucose
1
↑Insulin
↑Glucagon
2
5
P
ACC
Inactive
phosphatase
6
PKA
AMPK
P_i
ACC
3
Glucose
glycolysis, pyruvate dehydrogenase complex
Acetyl-CoA
Malonyl-CoA
multistep
Fatty acids
4
Fatty acyl-CoA
carnitine acyl-transferase 1
Fatty acyl-carnitine
7
Fatty acyl-carnitine
CoA
Carnitine
Fatty acyl-CoA
$FADH_2$
8
β oxidation
NADH
Acetyl-CoA
Fatty acid synthesis
Fatty acid β oxidation
Mitochondrion

- The mitochondrial matrix is the major site of fatty acid oxidation in animal cells, but in certain cells, other compartments also contain enzymes capable of oxidizing fatty acids to acetyl-CoA, by a pathway similar but not identical to that in mitochondria.

- In plant cells, the major site of β oxidation is not mitochondria but peroxisomes.

- In peroxisomes, organelles found in both animal and plant cells, the intermediates for β oxidation of fatty acids are coenzyme A derivatives, and the process consists of four steps, as in mitochondrial β oxidation: (1) dehydrogenation, (2) addition of water to the resulting double bond, (3) oxidation of the β-hydroxyacyl-CoA to a ketone, and (4) thiolytic cleavage by coenzyme A.

- The identical reactions also occur in glyoxysomes, organelles found only in germinating seeds.

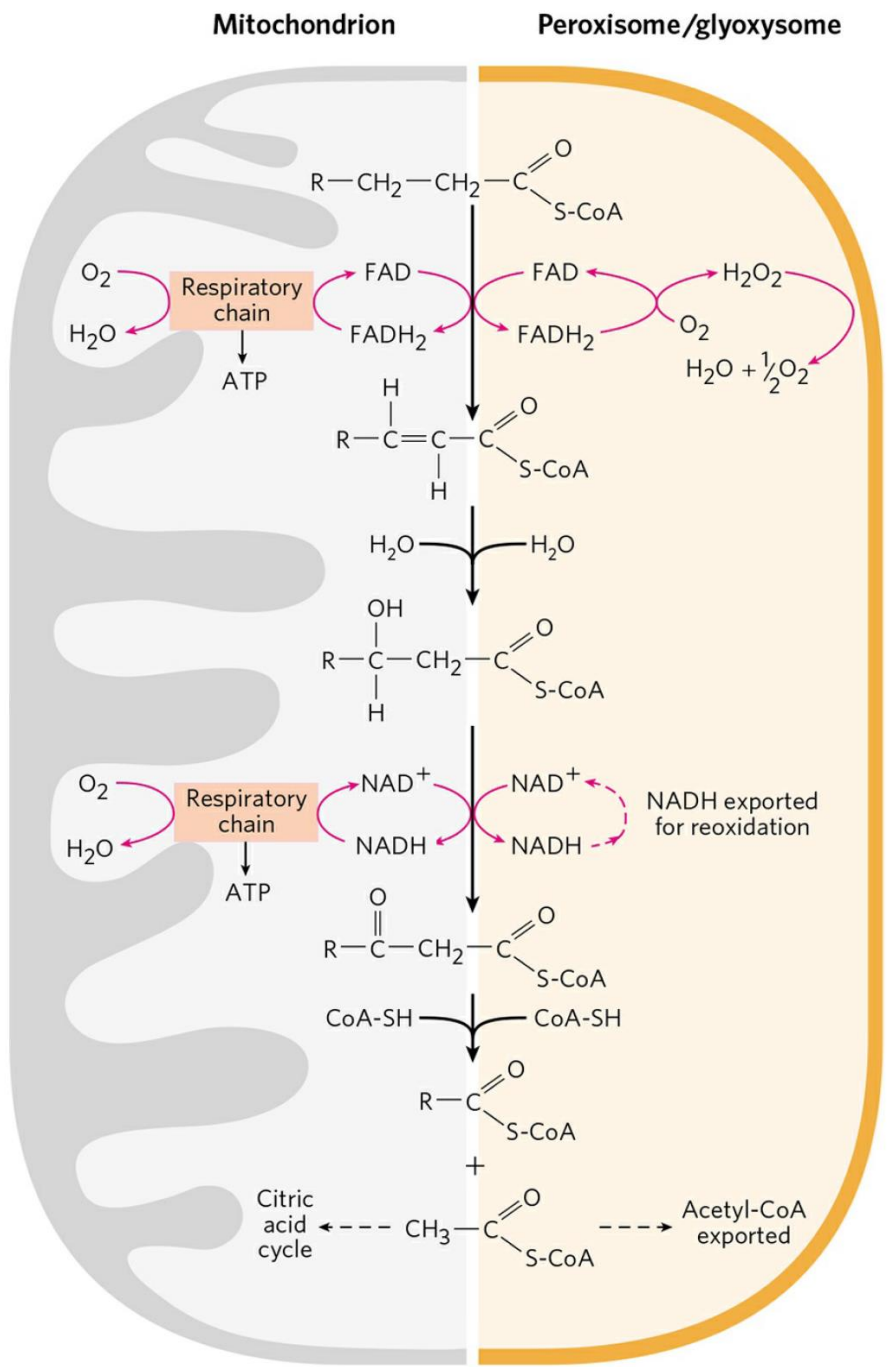
- One difference between the peroxisomal and mitochondrial pathways is in the chemistry of the first step.

- In peroxisomes, the flavoprotein acyl-CoA oxidase that introduces the double bond passes electrons directly to O2, producing H2O2 (thus the name “peroxisomes”).

- This strong and potentially damaging oxidant is immediately cleaved to H2O and O2 by catalase.

- In mitochondria, the electrons removed in the first oxidation step pass through the respiratory chain to O2 to produce H2O, and this process is accompanied by ATP synthesis.

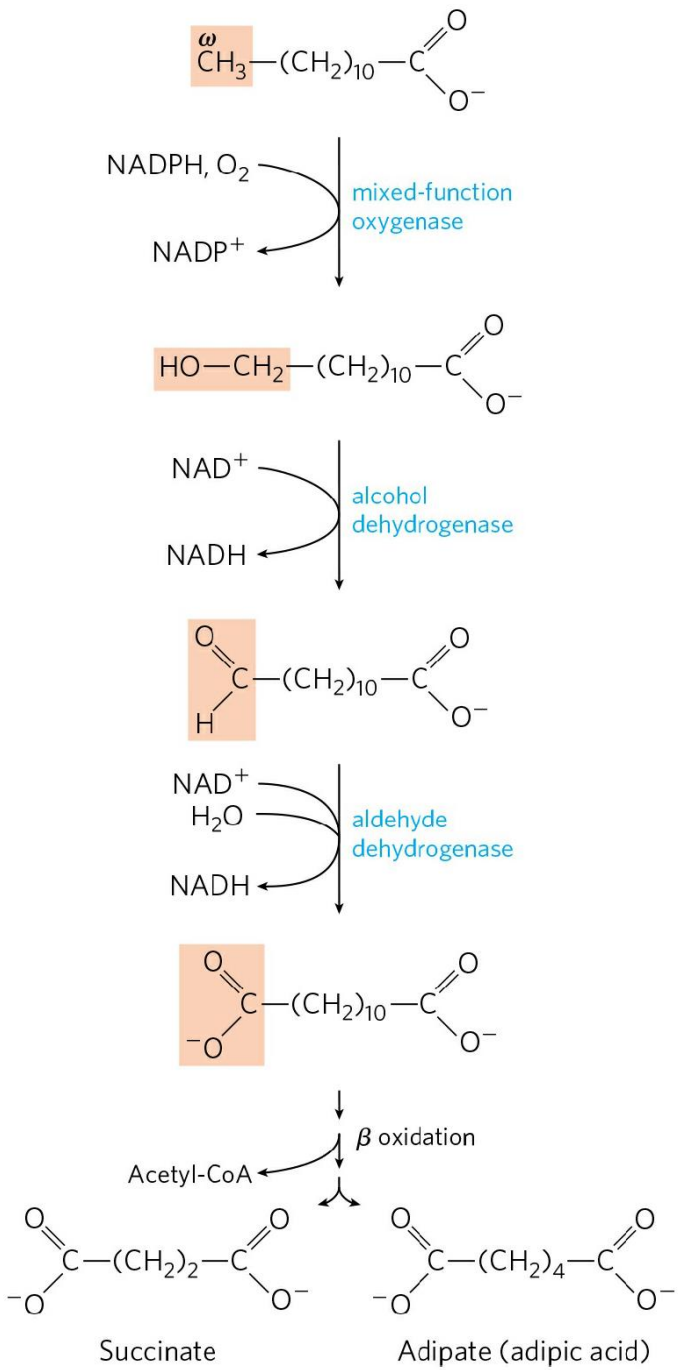
- In peroxisomes, the energy released in the first oxidative step of fatty acid breakdown is not conserved as ATP, but is dissipated as heat.

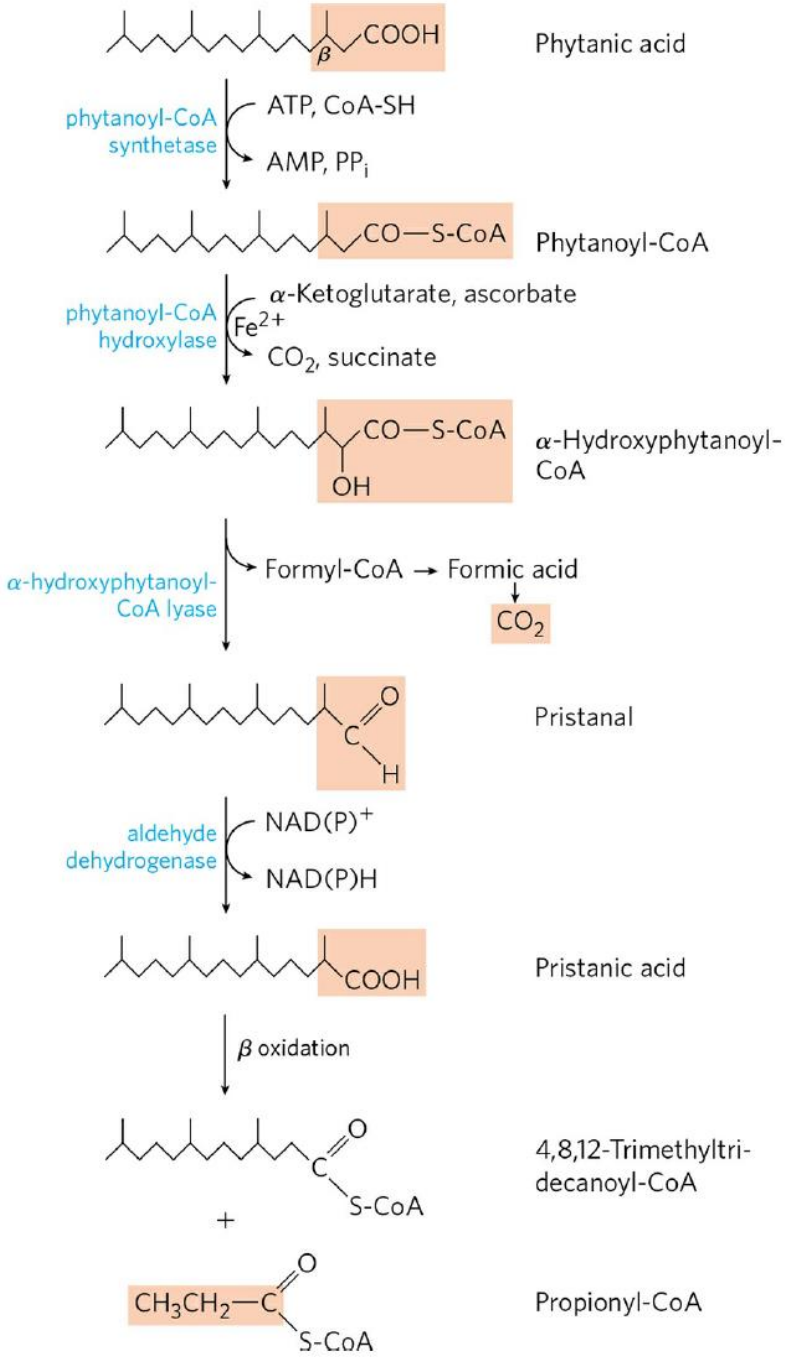

Mitochondrion
Peroxisome/glyoxysome
R—CH2—CH2—C(=O)—S-CoA
O2
H2O
Respiratory chain
ATP
FAD
FADH2
FAD
FADH2
H2O2
O2
H2O + ½O2
R—C(H)=C(H)—C(=O)—S-CoA
H2O
H2O
R—C(OH)(H)—CH2—C(=O)—S-CoA
O2
H2O
Respiratory chain
ATP
NAD+
NADH
NAD+
NADH
NADH exported for reoxidation
R—C(=O)—CH2—C(=O)—S-CoA
CoA-SH
CoA-SH
R—C(=O)—S-CoA
+
Citric acid cycle
CH3—C(=O)—S-CoA
Acetyl-CoA exported

- Although mitochondrial β oxidation, in which enzymes act at the carboxyl end of a fatty acid, is by far the most important catabolic fate for fatty acids in animal cells, there is another pathway in some species, including vertebrates, that involves oxidation of the ω (omega) carbon—the carbon most distant from the carboxyl group.

- The enzymes unique to ω oxidation are located in the endoplasmic reticulum of liver and kidney, and the preferred substrates are fatty acids of 10 or 12 carbon atoms.

- In mammals, ω oxidation is normally a minor pathway for fatty acid degradation, but when β oxidation is defective (because of mutation or a carnitine deficiency, for example) it becomes more important.


ω
CH3—(CH2)10—C
O
O−
NADPH, O2
NADP+
mixed-function oxygenase
HO—CH2—(CH2)10—C
NAD+
NADH
alcohol dehydrogenase
C
H
NAD+
H2O
NADH
aldehyde dehydrogenase
β oxidation
Acetyl-CoA
Succinate
Adipate (adipic acid)


COOH
β
Phytanic acid
phytanoyl-CoA synthetase
ATP, CoA-SH
AMP, PPi
CO—S-CoA
Phytanoyl-CoA
phytanoyl-CoA hydroxylase
α-Ketoglutarate, ascorbate
Fe2+
CO2, succinate
OH
α-Hydroxyphytanoyl-CoA
α-hydroxyphytanoyl-CoA lyase
Formyl-CoA → Formic acid
CO2
Pristanal
aldehyde dehydrogenase
NAD(P)+
NAD(P)H
Pristanic acid
β oxidation
S-CoA
4,8,12-Trimethyltridecanoyl-CoA
+
CH3CH2—C
Propionyl-CoA

- Phytanic acid, a long-chain fatty acid with methyl branches, is derived from the phytol side chain of chlorophyll.

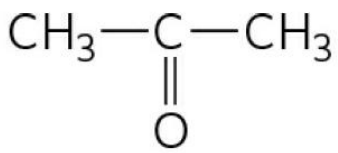
- The presence of a methyl group on the β carbon of this fatty acid prevents the formation of a β-keto intermediate, making its β oxidation impossible.

- Humans obtain phytanic acid in the diet, primarily from dairy products and from the fats of ruminant animals; microorganisms in the rumen of these animals produce phytanic acid as they digest plant chlorophyll.

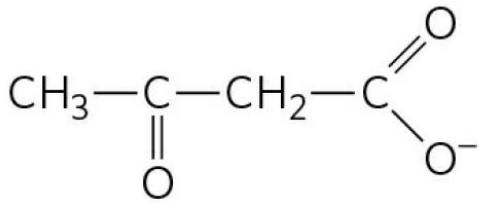
- The typical western diet includes 50 to 100 mg of phytanic acid per day.

- Phytanic acid is metabolized in peroxisomes by α oxidation, in which a single carbon is removed from the carboxyl end of the acid

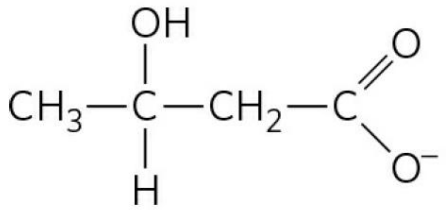
- In humans and most other mammals, acetyl-CoA formed in the liver during oxidation of fatty acids can either enter the citric acid cycle or undergo conversion to the “ketone bodies,” acetone, acetoacetate, and D-β-hydroxybutyrate, for export to other tissues.



Acetone



Acetoacetate



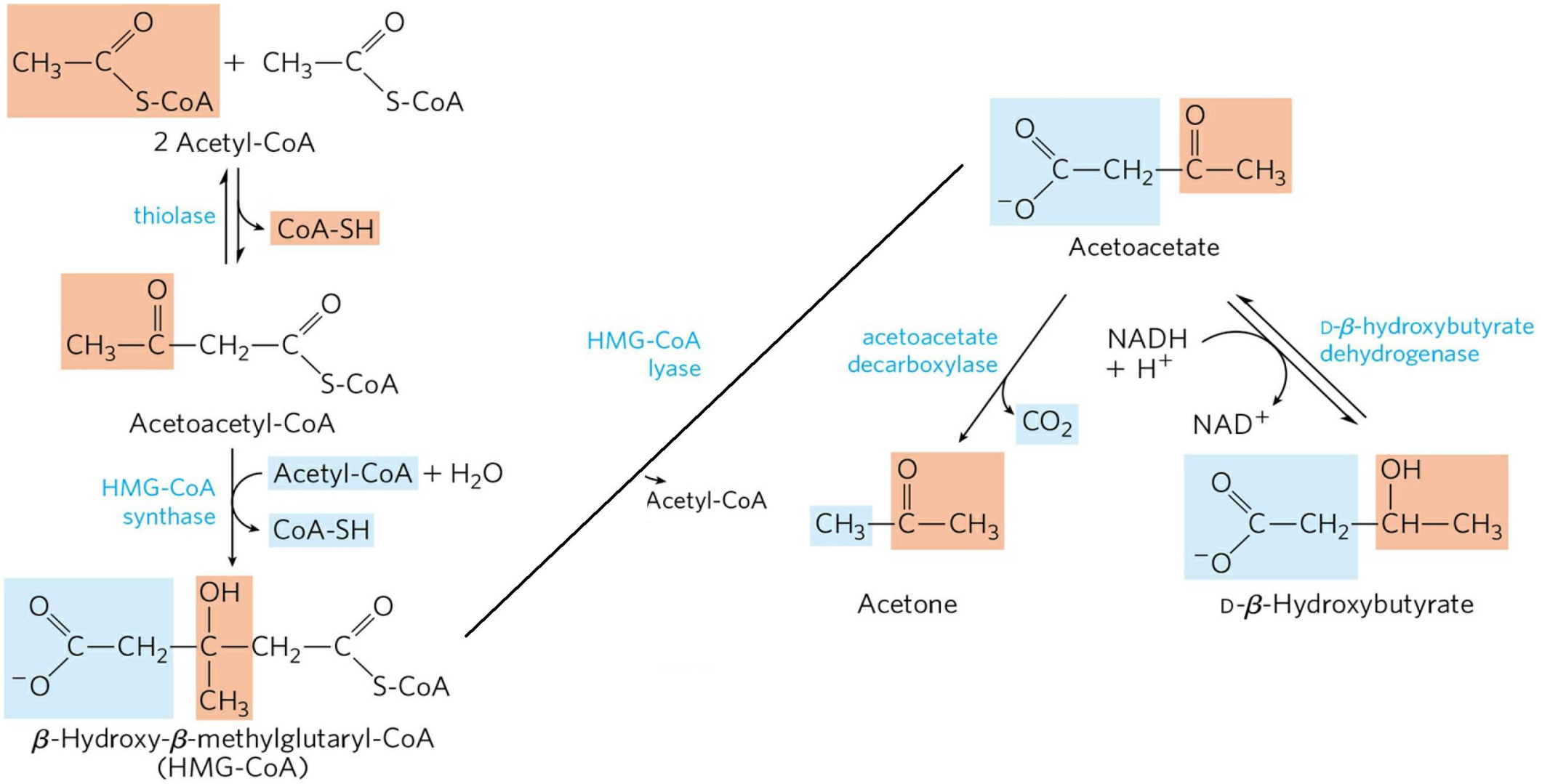
D-β-Hydroxybutyrate

- Acetone, produced in smaller quantities than the other ketone bodies, is exhaled.

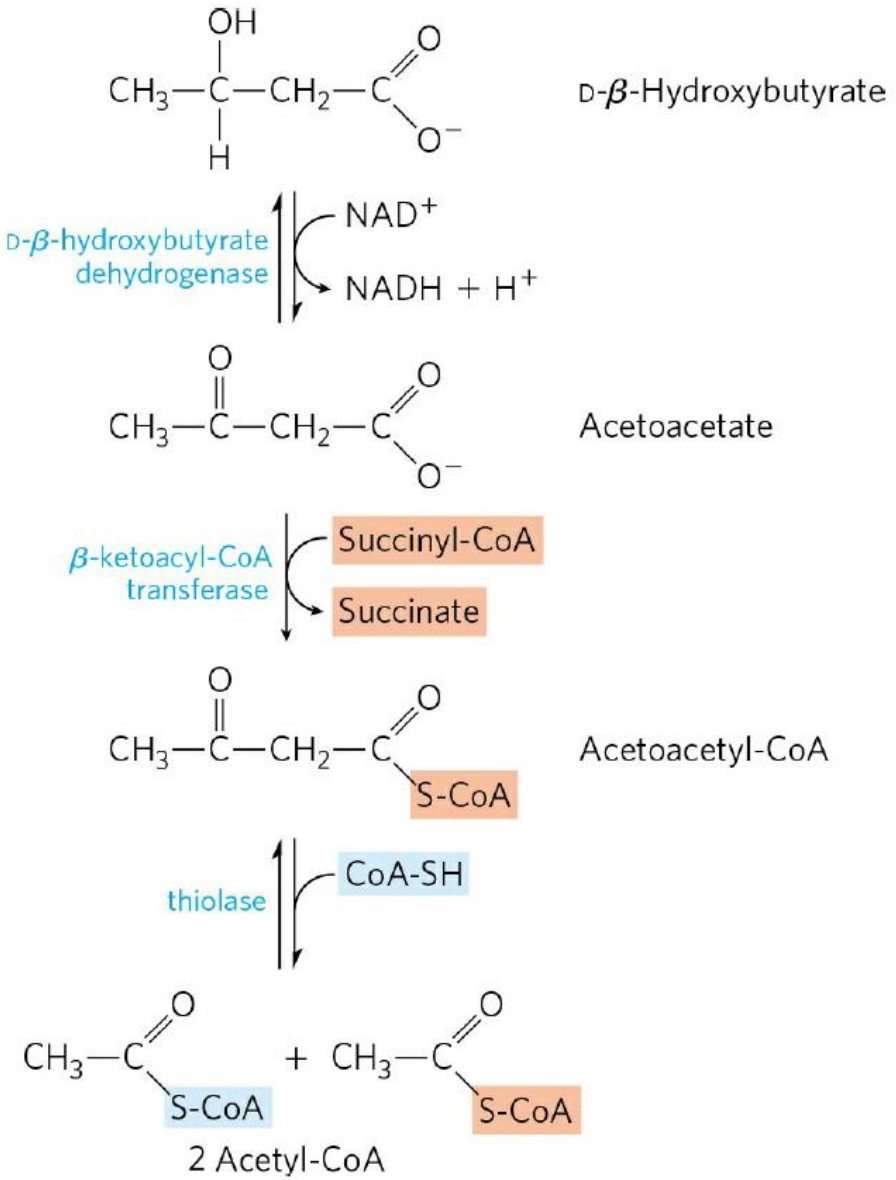
- Acetoacetate and D-β-hydroxybutyrate are transported by the blood to tissues other than the liver (extrahepatic tissues), where they are converted to acetyl-CoA and oxidized in the citric acid cycle, providing much of the energy required by tissues such as skeletal and heart muscle and the renal cortex.

- The brain, which preferentially uses glucose as fuel, can adapt to the use of acetoacetate and D-β-hydroxybutyrate under starvation conditions, when glucose is unavailable.

❖ In this situation, the brain cannot use fatty acids as fuel, because they do not cross the blood-brain barrier.



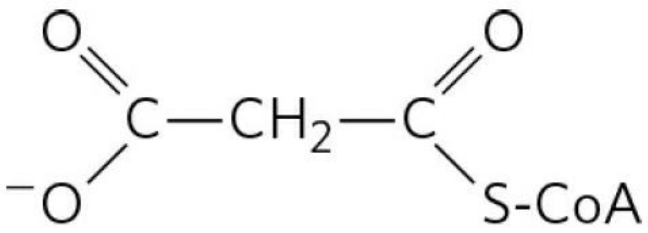
❖ D-β-Hydroxybutyrate, synthesized in the liver, passes into the blood and thus to other tissues, where it is converted in three steps to acetyl-CoA.



- After the discovery that fatty acid oxidation takes place by the oxidative removal of successive two- carbon (acetyl-CoA) units, biochemists thought the biosynthesis of fatty acids might proceed by a simple reversal of the same enzymatic steps.

- However, as they were to find out, fatty acid biosynthesis and breakdown occur by different pathways, are catalyzed by different sets of enzymes, and take place in different parts of the cell.

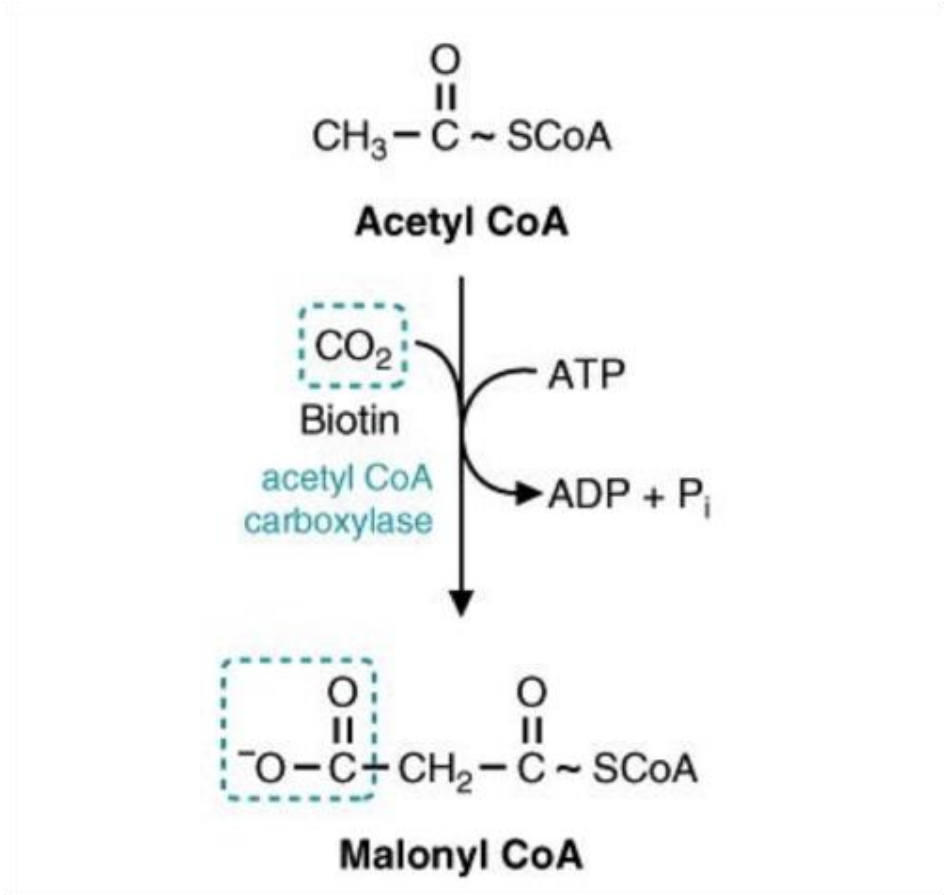
- Moreover, biosynthesis requires the participation of a three-carbon intermediate, malonyl-CoA, that does not appear in the path of fatty acid breakdown.



Malonyl-CoA

❖ The formation of malonyl-CoA from acetyl-CoA is an irreversible process, catalyzed by acetyl-CoA carboxylase.

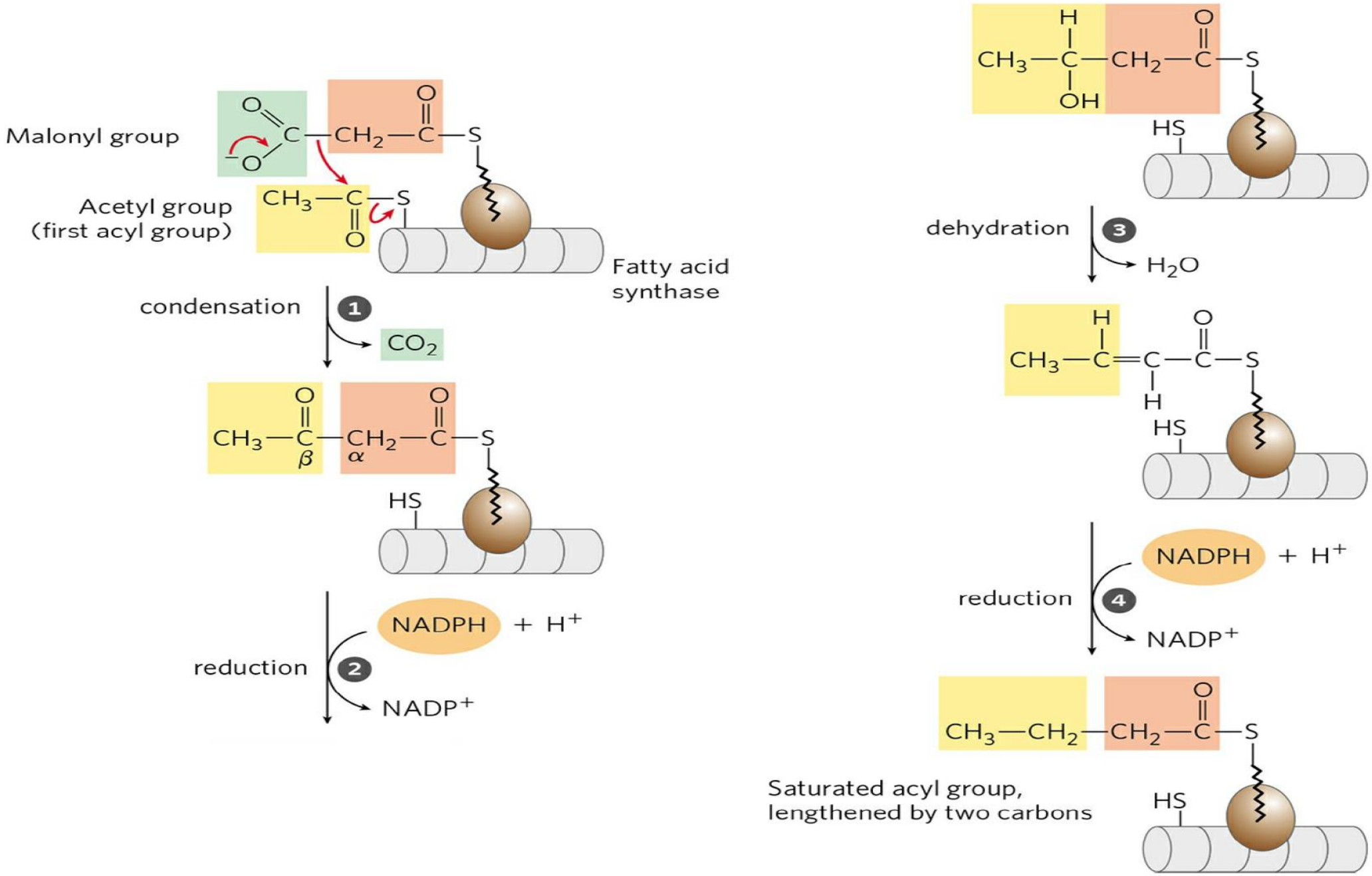
❖ The enzyme contains a biotin prosthetic group covalently bound in amide linkage to the ε-amino group of a Lys residue in one of the three polypeptides or domains of the enzyme molecule.

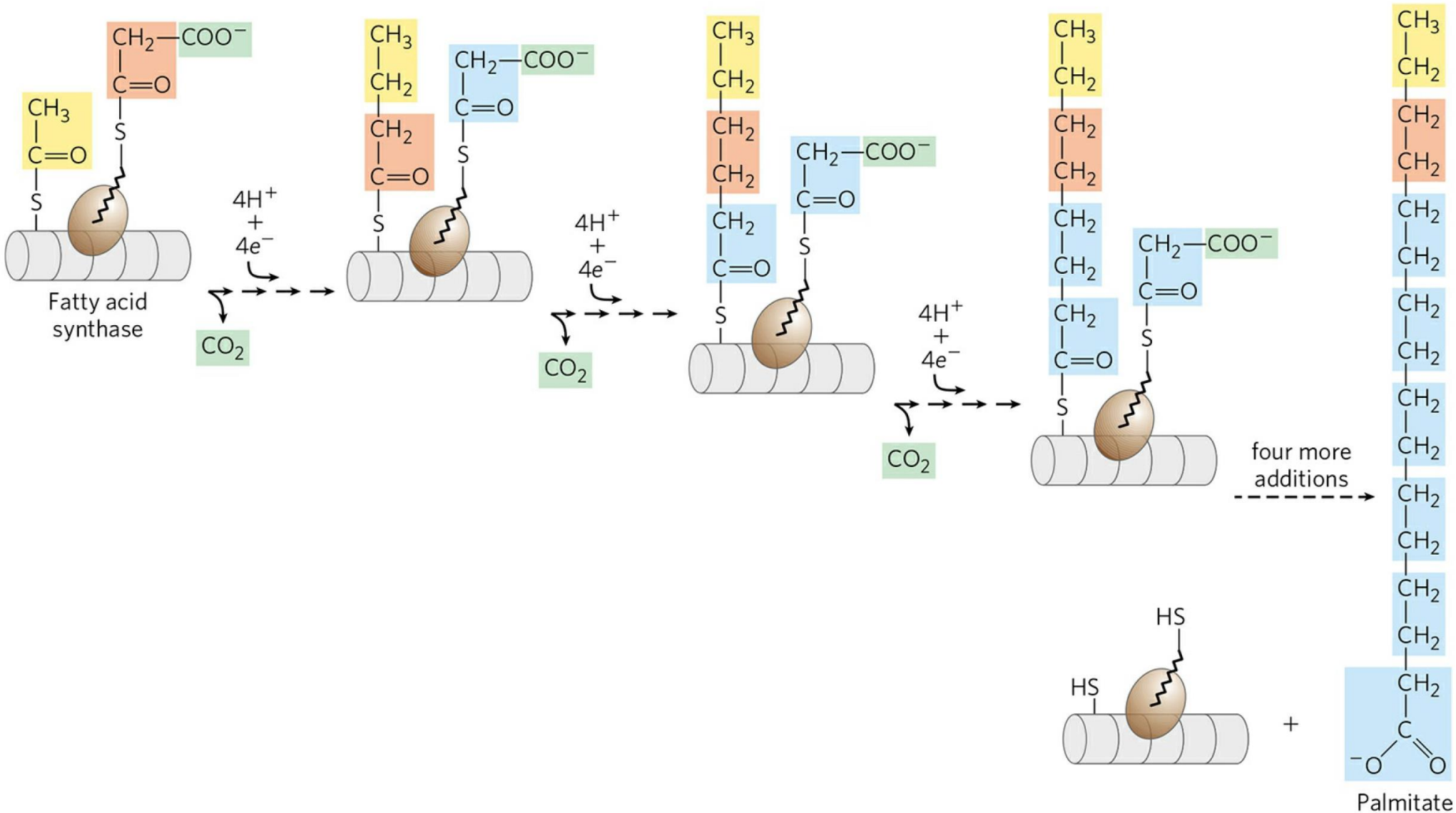


- In all organisms, the long carbon chains of fatty acids are assembled in a repeating four-step sequence, catalyzed by a system collectively referred to as fatty acid synthase.

- A saturated acyl group produced by each four-step series of reactions becomes the substrate for subsequent condensation with an activated malonyl group.

- With each passage through the cycle, the fatty acyl chain is extended by two carbons.


Malonyl group
Acetyl group
(first acyl group)
Fatty acid
synthase
condensation
1
CO_2
HS
reduction
2
NADPH + H^+
$NADP^+$
dehydration
3
H_2O
reduction
4
NADPH + H^+
$NADP^+$
Saturated acyl group,
lengthened by two carbons

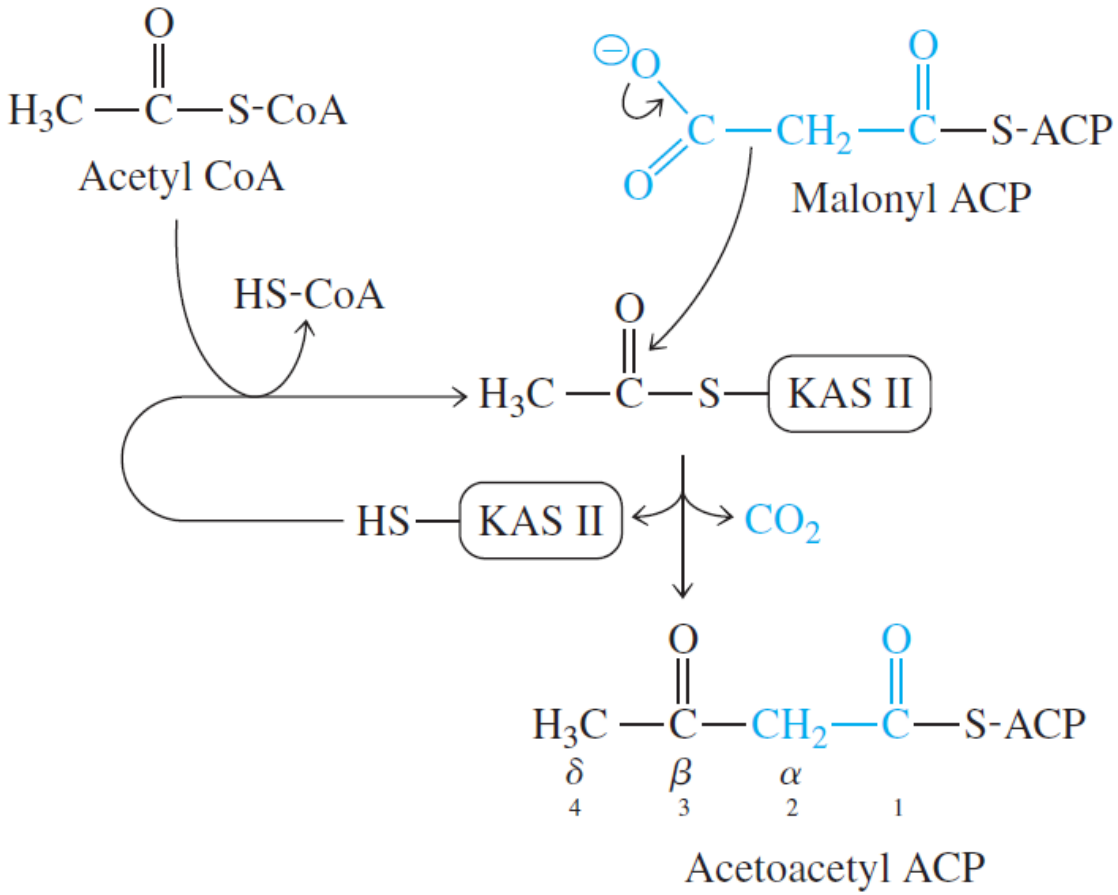

Fatty acid synthase
$4H^+ + 4e^-$
CO_2
$4H^+ + 4e^-$
CO_2
$4H^+ + 4e^-$
CO_2
four more additions
HS
HS
+
Palmitate

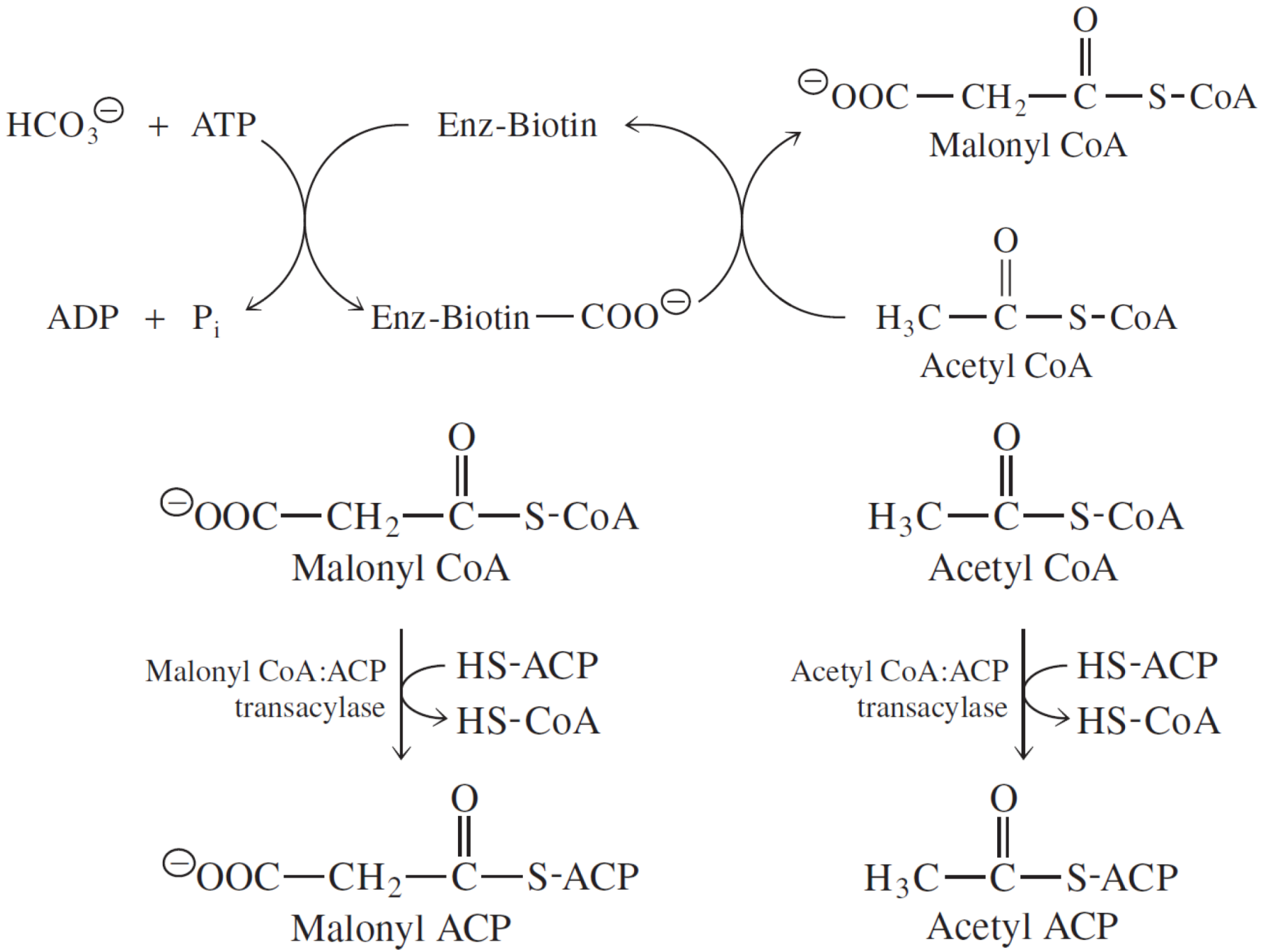
❖ Step 1 Condensation

❖ The first reaction in the formation of a fa[illegible] acid chain is a formal Claisen condensati of the activated acetyl and malonyl grou to form acetoacetyl-ACP,

❖ Simultaneously, a molecule of CO2 produced.

❖ In this reaction, catalyzed by β-ketoac ACP synthase, the acetyl group transferred from the Cys —SH group of t enzyme to the malonyl group on the —S of ACP, becoming the methyl-terminal t carbon unit of the new acetoacetyl group.




HCO3⊖ + ATP
ADP + Pi
Enz-Biotin
Enz-Biotin—COO⊖
⊖OOC—CH2—C(=O)—S-CoA
Malonyl CoA
H3C—C(=O)—S-CoA
Acetyl CoA
Malonyl CoA:ACP transacylase
HS-ACP
HS-CoA
⊖OOC—CH2—C(=O)—S-ACP
Malonyl ACP
Acetyl CoA:ACP transacylase
HS-ACP
HS-CoA
H3C—C(=O)—S-ACP
Acetyl ACP

- Step 2 Reduction of the Carbonyl Group

- The acetoacetyl-ACP formed in the condensation step now undergoes reduction of the carbonyl group at C-3 to form D-β-hydroxybutyryl-ACP.

- This reaction is catalyzed by β-ketoacyl-ACP reductase (KR), and the electron donor is NADPH.

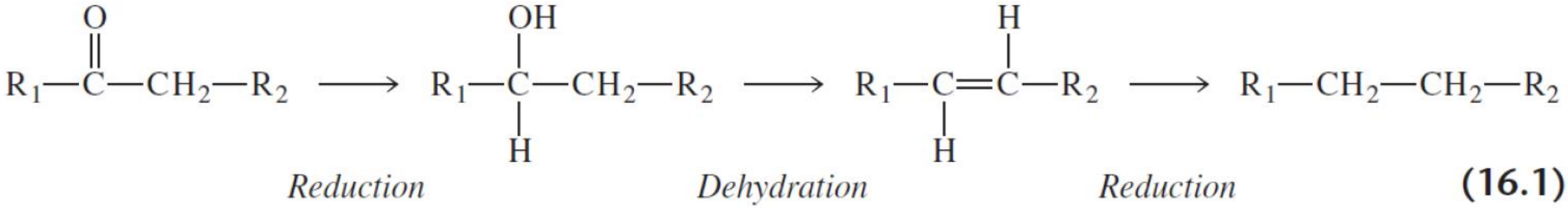
- Step 3 Dehydration

- The elements of water are now removed from C-2 and C-3 of D-β-hydroxybutyryl-ACP to yield a double bond in the product, trans- Δ2-butenoyl-ACP.

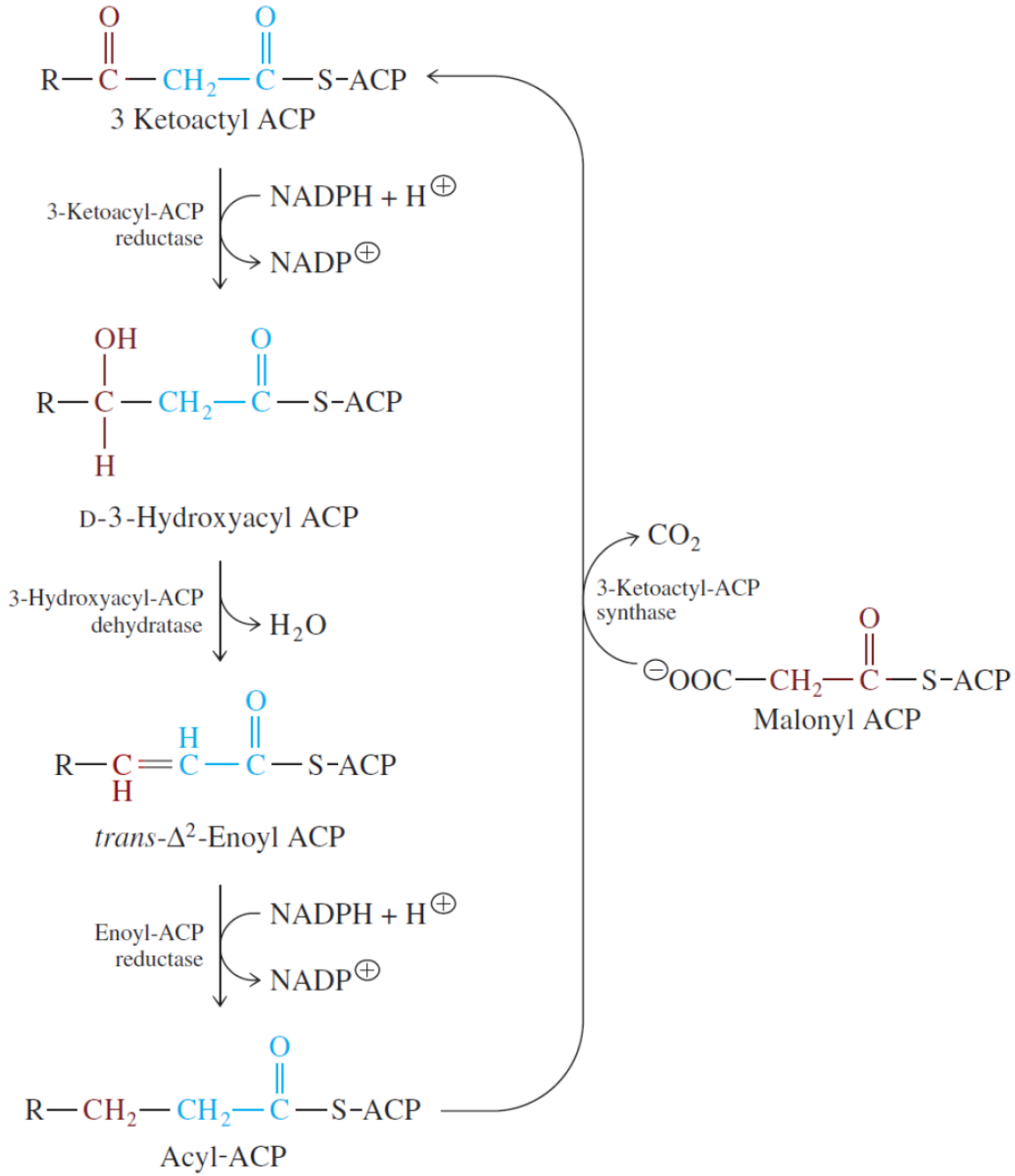
- The enzyme that catalyzes this dehydration is β-hydroxyacyl-ACP dehydratase (DH).

❖ Step 4 Reduction of the Double Bond

❖ Finally, the double bond of trans-Δ2-butenoyl-ACP is reduced (saturated) to form butyryl-ACP by the action of enoyl-ACP reductase (ER); again, NADPH is the electron donor.



(16.1)


R—C(=O)—CH2—C(=O)—S-ACP
3 Ketoactyl ACP
3-Ketoacyl-ACP reductase
NADPH + H⊕
NADP⊕
R—C(OH)(H)—CH2—C(=O)—S-ACP
D-3-Hydroxyacyl ACP
3-Hydroxyacyl-ACP dehydratase
H2O
R—C(H)=C(H)—C(=O)—S-ACP
trans-Δ2-Enoyl ACP
Enoyl-ACP reductase
NADPH + H⊕
NADP⊕
R—CH2—CH2—C(=O)—S-ACP
Acyl-ACP
CO2
3-Ketoactyl-ACP synthase
⊖OOC—CH2—C(=O)—S-ACP
Malonyl ACP

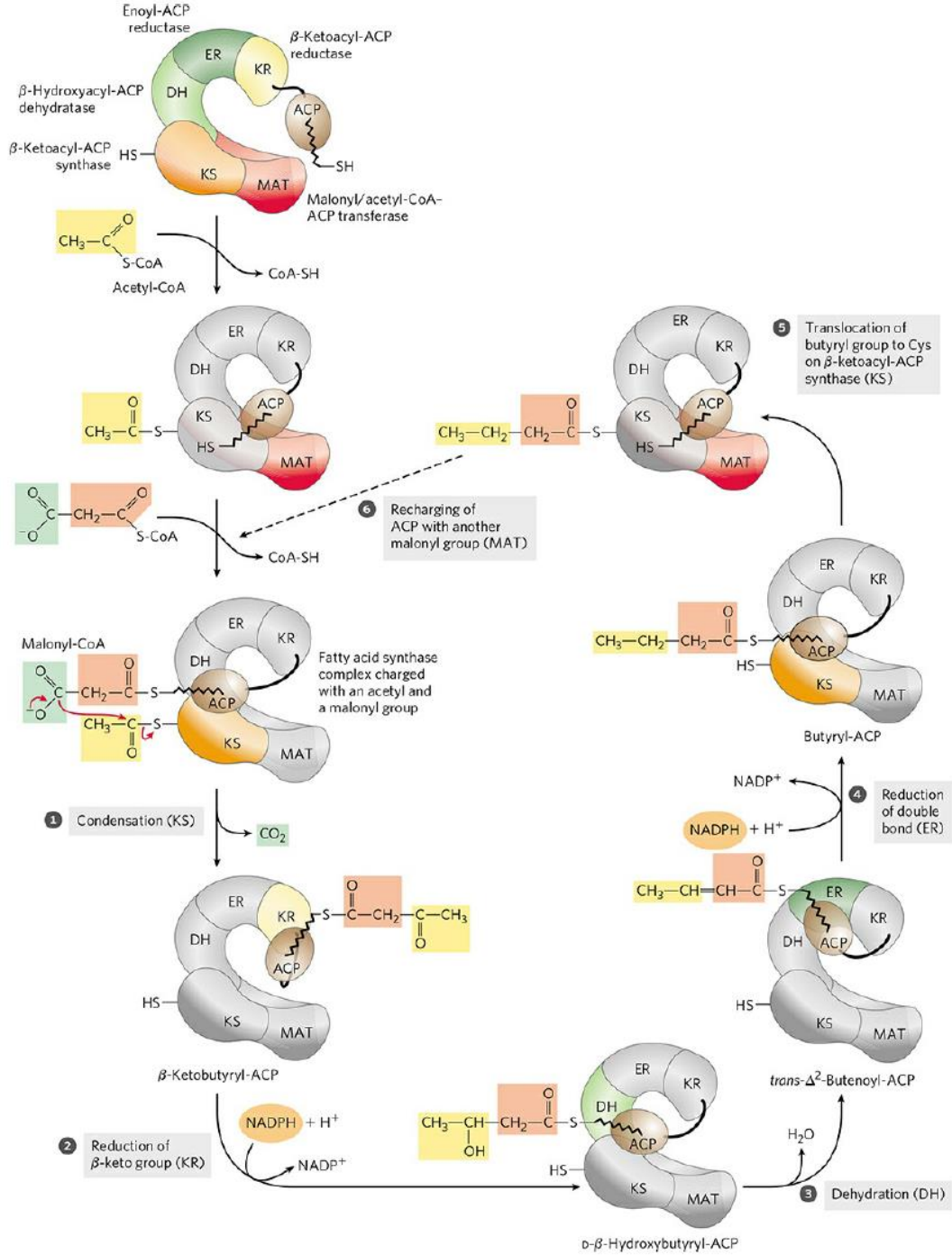
- Production of the four-carbon, saturated fatty acyl–ACP marks completion of one pass through the fatty acid synthase complex.

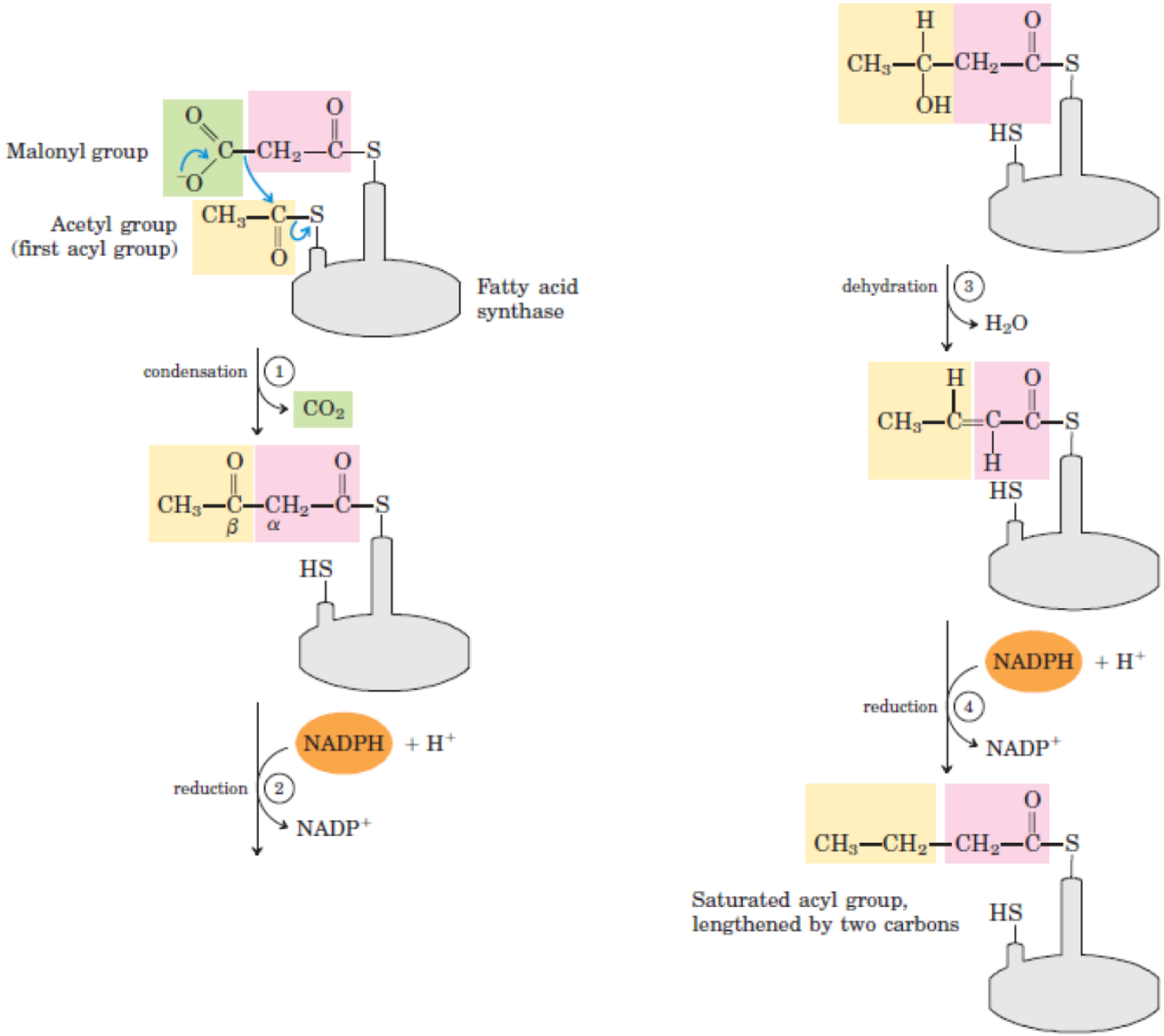
- In step 5 , the butyrylgroup is transferred from the phosphopantetheine —SH group of ACP to the Cys —SH group of β-ketoacyl-ACP synthase, which initially bore the acetyl group.

- To start the next cycle of four reactions that lengthens the chain by two more carbons (step 6), another malonyl group is linked to the now unoccupied phosphopantetheine —SH group of ACP.

- Condensation occurs as the butyryl group, acting like the acetyl group in the first cycle, is linked to two carbons of the malonyl-ACP group with concurrent loss of CO2.

- The product of this condensation is a six-carbon acyl group, covalently bound to the phosphopantetheine —SH group.


Enoyl-ACP reductase
β-Ketoacyl-ACP reductase
β-Hydroxyacyl-ACP dehydratase
β-Ketoacyl-ACP synthase
ER
KR
DH
ACP
HS
SH
KS
MAT
Malonyl/acetyl-CoA–ACP transferase
S-CoA
Acetyl-CoA
CoA-SH
Malonyl-CoA
Fatty acid synthase complex charged with an acetyl and a malonyl group
1 Condensation (KS)
CO2
β-Ketobutyryl-ACP
2 Reduction of β-keto group (KR)
NADPH + H+
NADP+
D-β-Hydroxybutyryl-ACP
3 Dehydration (DH)
H2O
trans-Δ2-Butenoyl-ACP
4 Reduction of double bond (ER)
Butyryl-ACP
5 Translocation of butyryl group to Cys on β-ketoacyl-ACP synthase (KS)
6 Recharging of ACP with another malonyl group (MAT)


Malonyl group
Acetyl group
(first acyl group)
Fatty acid
synthase
condensation
1
CO_2
HS
reduction
2
NADPH
+ H^+
$NADP^+$
dehydration
3
H_2O
reduction
4
NADPH
+ H^+
$NADP^+$
Saturated acyl group,
lengthened by two carbons
HS

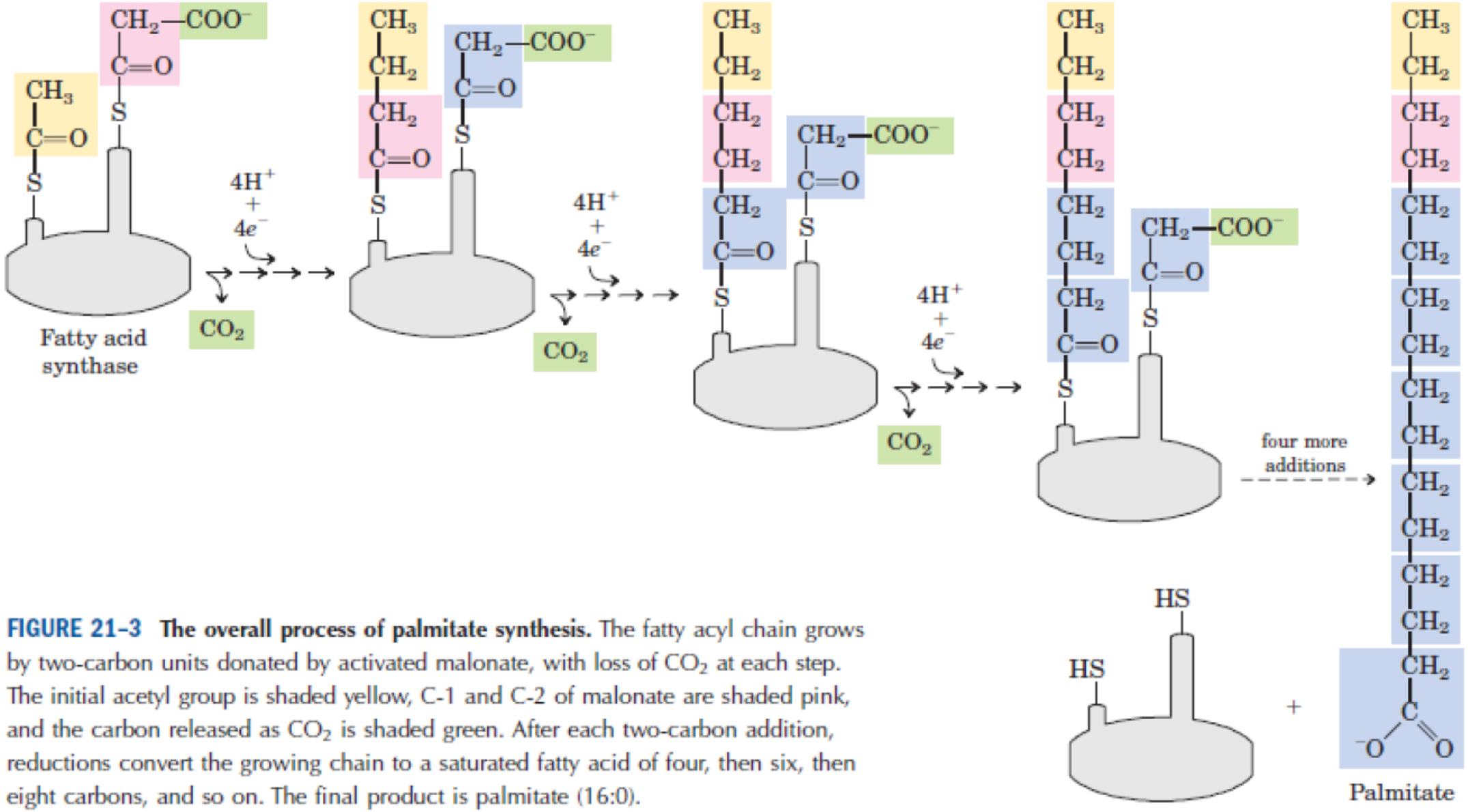


FIGURE 21–3 The overall process of palmitate synthesis. The fatty acyl chain grows by two-carbon units donated by activated malonate, with loss of CO_2 at each step. The initial acetyl group is shaded yellow, C-1 and C-2 of malonate are shaded pink, and the carbon released as CO_2 is shaded green. After each two-carbon addition, reductions convert the growing chain to a saturated fatty acid of four, then six, then eight carbons, and so on. The final product is palmitate (16:0).

- Seven cycles of condensation and reduction produce the 16-carbon saturated palmitoyl group, still bound to ACP.

- For reasons not well understood, chain elongation by the synthase complex generally stops at this point, and free palmitate is released from the ACP by a hydrolytic activity (thioesterase; TE) in the multifunctional protein.

- We can consider the overall reaction for the synthesis of palmitate from acetyl-CoA in two parts.

- First, the formation of seven malonyl-CoA molecules:

$$\mathbf{7Acetyl\text{-}CoA} + 7\mathrm{CO_2} + \mathbf{7ATP} \rightarrow \mathbf{7\ malonyl\text{-}CoA} + \mathbf{7ADP} + 7\mathrm{P_i}$$

- then seven cycles of condensation and reduction:

$$\mathbf{Acetyl\text{-}CoA} + \mathbf{7\ malonyl\text{-}CoA} + \mathbf{14NADPH} + 14\mathrm{H^+}$$
$$\rightarrow \mathbf{palmitate} + 7\mathrm{CO_2} + 8CoA + \mathbf{14NADP^+} + 6H_2O$$

❖ Notice that only six net water molecules are produced, because one is used to hydrolyze the thioester linking the palmitate product to the enzyme.

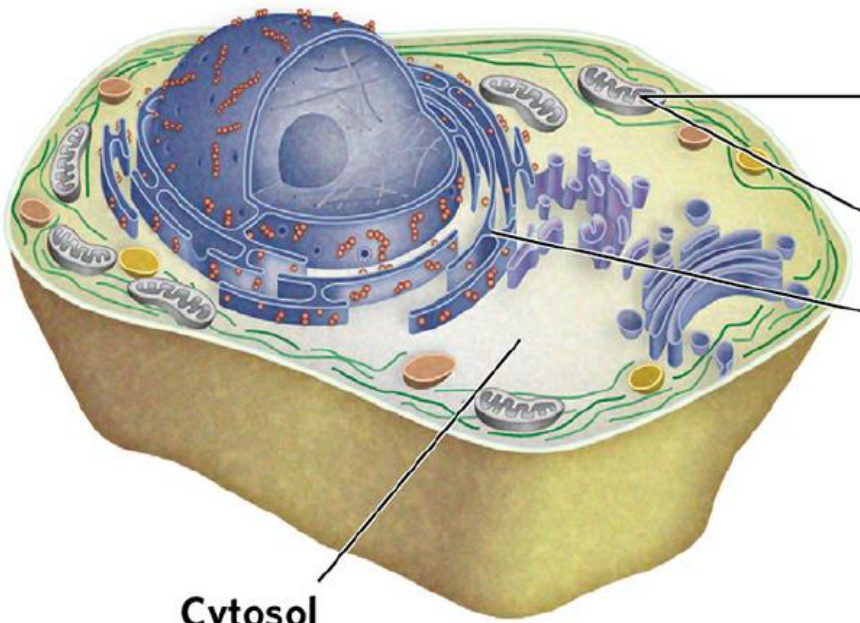
❖ The overall process

$$8\ \text{Acetyl-CoA} + 7\text{ATP} + 14\text{NADPH} + 14\text{H}^{+} \rightarrow \text{palmitate} + 8CoA + 7\text{ADP} + 7\text{P}_{\text{i}} + 14\text{NADP}^{+} + 6\text{H}_2\text{O} \qquad (21-3)$$

❖ In most higher eukaryotes, the fatty acid synthase complex is found exclusively in the cytosol, as are the biosynthetic enzymes for nucleotides, amino acids, and glucose.

❖ This location segregates synthetic processes from degradative reactions, many of which take place in the mitochondrial matrix.

Animal cells, yeast cells



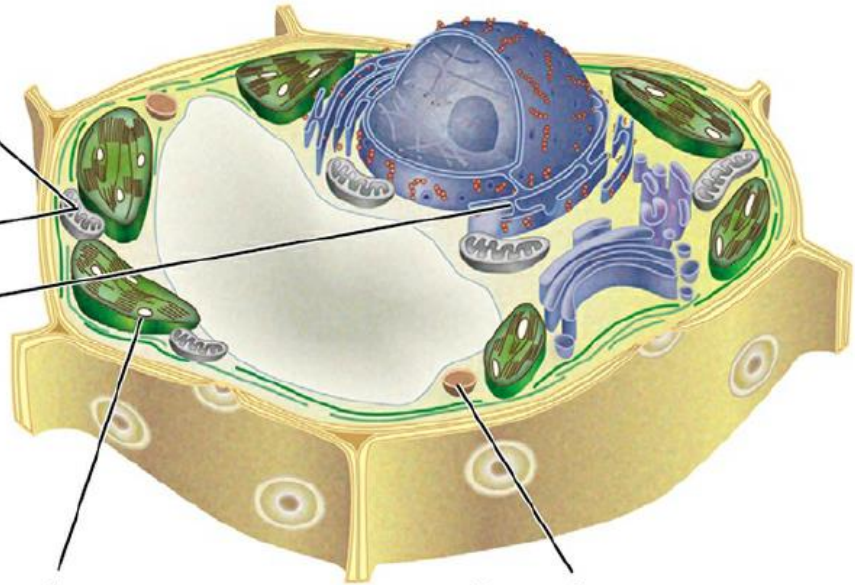
Mitochondria

- No fatty acid oxidation
- Fatty acid oxidation
- Ketone body synthesis
- Fatty acid elongation
- Acetyl-CoA production

Endoplasmic reticulum

- Phospholipid synthesis
- Sterol synthesis (late stages)
- Fatty acid elongation
- Fatty acid desaturation

Plant cells



Cytosol

- NADPH production (pentose phosphate pathway; malic enzyme)
- [NADPH]/[$NADP^+$] high
- Isoprenoid and sterol synthesis (early stages)
- Fatty acid synthesis

Chloroplasts

- NADPH, ATP production
- [NADPH]/[$NADP^+$] high
- Fatty acid synthesis

Peroxisomes

- Fatty acid oxidation (producing H_2O_2)
- Catalase, peroxidase: $H_2O_2 \longrightarrow H_2O$

- When a cell or organism has more than enough metabolic fuel to meet its energy needs, the excess is generally converted to fatty acids and stored as lipids such as triacylglycerols.

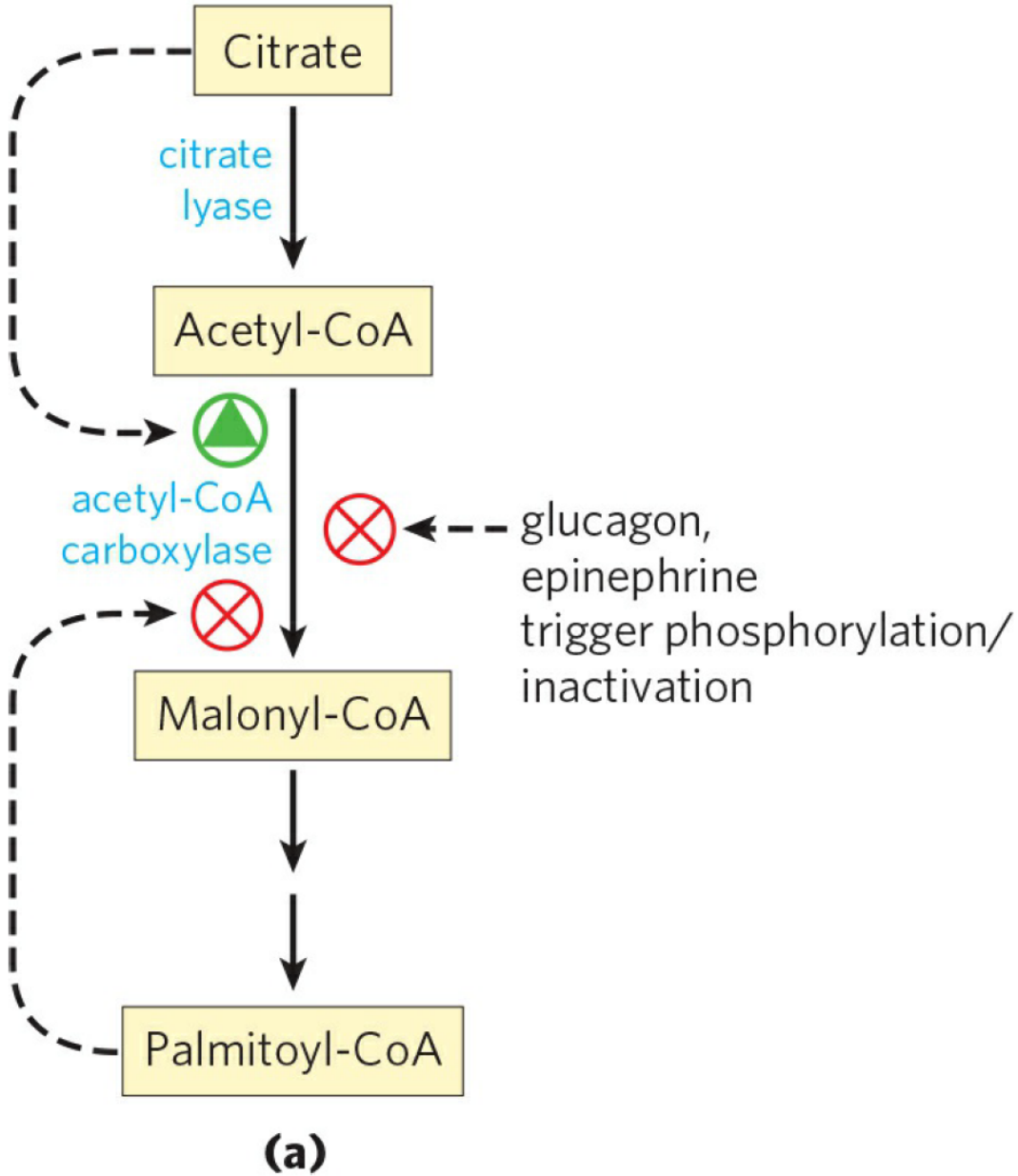
- The reaction catalyzed by acetyl-CoA carboxylase is the rate-limiting step in the biosynthesis of fatty acids, and this enzyme is an important site of regulation.

- In vertebrates, palmitoyl-CoA, the principal product of fatty acid synthesis, is a feedback inhibitor of the enzyme; citrate is an allosteric activator.

- Citrate plays a central role in diverting cellular metabolism from the consumption (oxidation) of metabolic fuel to the storage of fuel as fatty acids.

- When the concentrations of mitochondrial acetyl-CoA and ATP increase, citrate is transported out of mitochondria; it then becomes both the precursor of cytosolic acetyl-CoA and an allosteric signal for the activation of acetyl- CoA carboxylase.

- At the same time, citrate inhibits the activity of phosphofructokinase-1 reducing the flow of carbon through glycolysis.

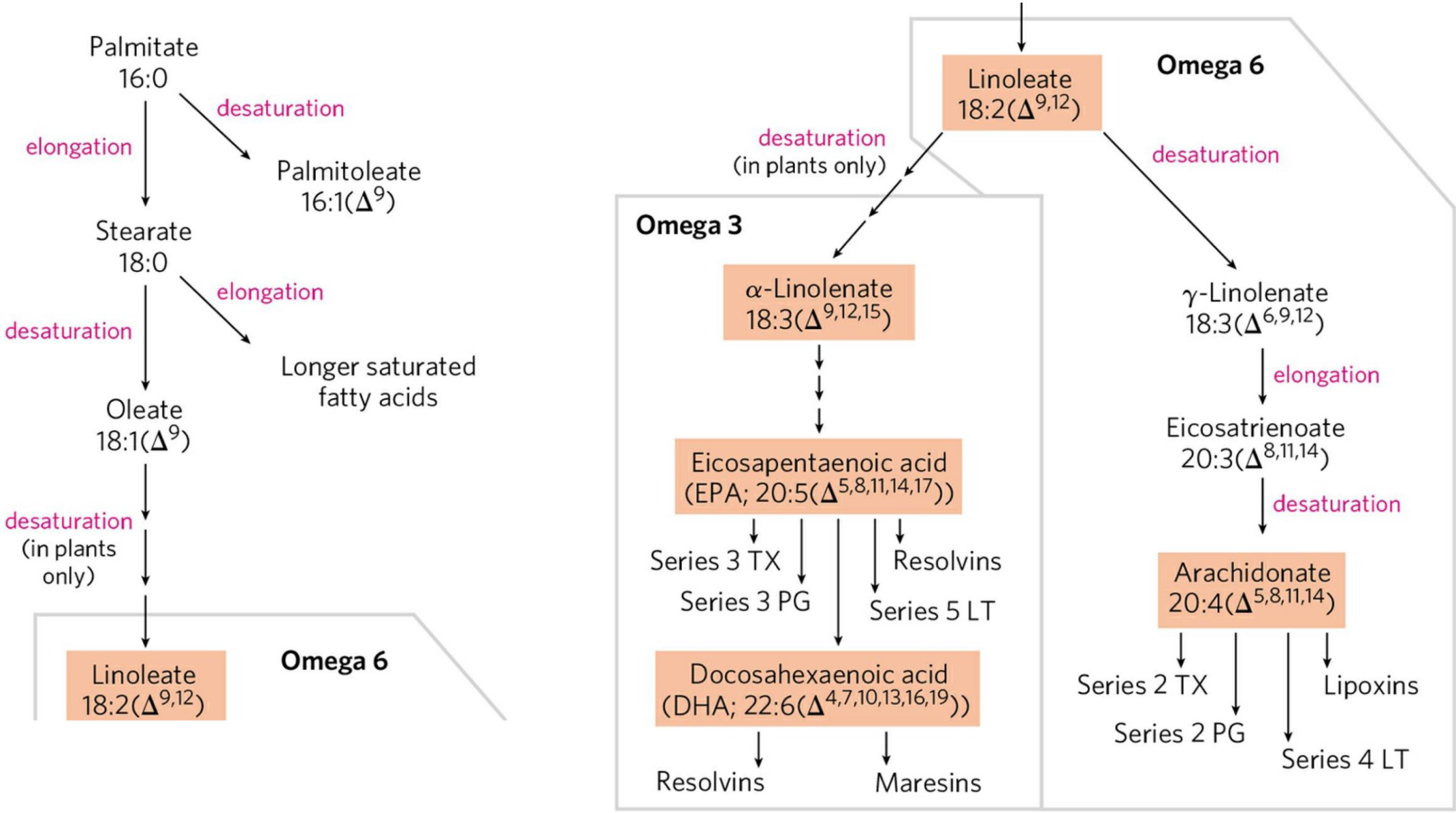

Citrate
citrate lyase
Acetyl-CoA
acetyl-CoA carboxylase
glucagon, epinephrine trigger phosphorylation/ inactivation
Malonyl-CoA
Palmitoyl-CoA
(a)

- Palmitate, the principal product of the fatty acid synthase system in animal cells, is the precursor of other long-chain fatty acids.

- It may be lengthened to form stearate (18:0) or even longer saturated fatty acids by further additions of acetyl groups, through the action of fatty acid elongation systems present in the smooth endoplasmic reticulum and in mitochondria.

- The more active elongation system of the ER extends the 16-carbon chain of palmitoyl-CoA by two carbons, forming stearoyl-CoA.

- Although different enzyme systems are used, and coenzyme A rather than ACP is the acyl carrier in the reaction, the mechanism of elongation in the ER is otherwise identical to that in palmitate synthesis: donation of two carbons by malonyl-CoA, followed by reduction, dehydration, and reduction to the saturated 18-carbon product, stearoyl-CoA.


Palmitate
16:0
desaturation
elongation
Palmitoleate
16:1(Δ9)
Stearate
18:0
elongation
desaturation
Longer saturated
fatty acids
Oleate
18:1(Δ9)
desaturation
(in plants
only)
Omega 6
Linoleate
18:2(Δ9,12)
Linoleate
18:2(Δ9,12)
Omega 6
desaturation
(in plants only)
desaturation
Omega 3
α-Linolenate
18:3(Δ9,12,15)
γ-Linolenate
18:3(Δ6,9,12)
elongation
Eicosapentaenoic acid
(EPA; 20:5(Δ5,8,11,14,17))
Eicosatrienoate
20:3(Δ8,11,14)
desaturation
Series 3 TX
Series 3 PG
Resolvins
Series 5 LT
Arachidonate
20:4(Δ5,8,11,14)
Docosahexaenoic acid
(DHA; 22:6(Δ4,7,10,13,16,19))
Series 2 TX
Series 2 PG
Lipoxins
Series 4 LT
Resolvins
Maresins

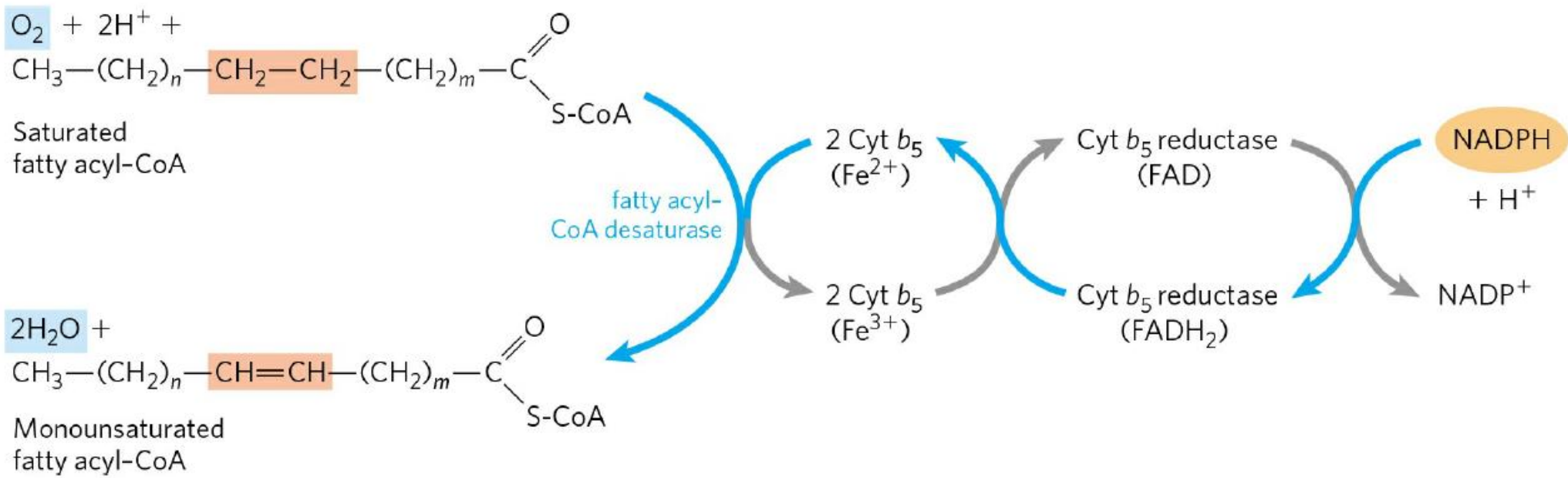
- Palmitate and stearate serve as precursors of the two most common monounsaturated fatty acids of animal tissues: palmitoleate, 16:1(Δ9), andoleate, 18:1(Δ9); both of these fatty acids have a single cis double bond between C-9 and C-10.

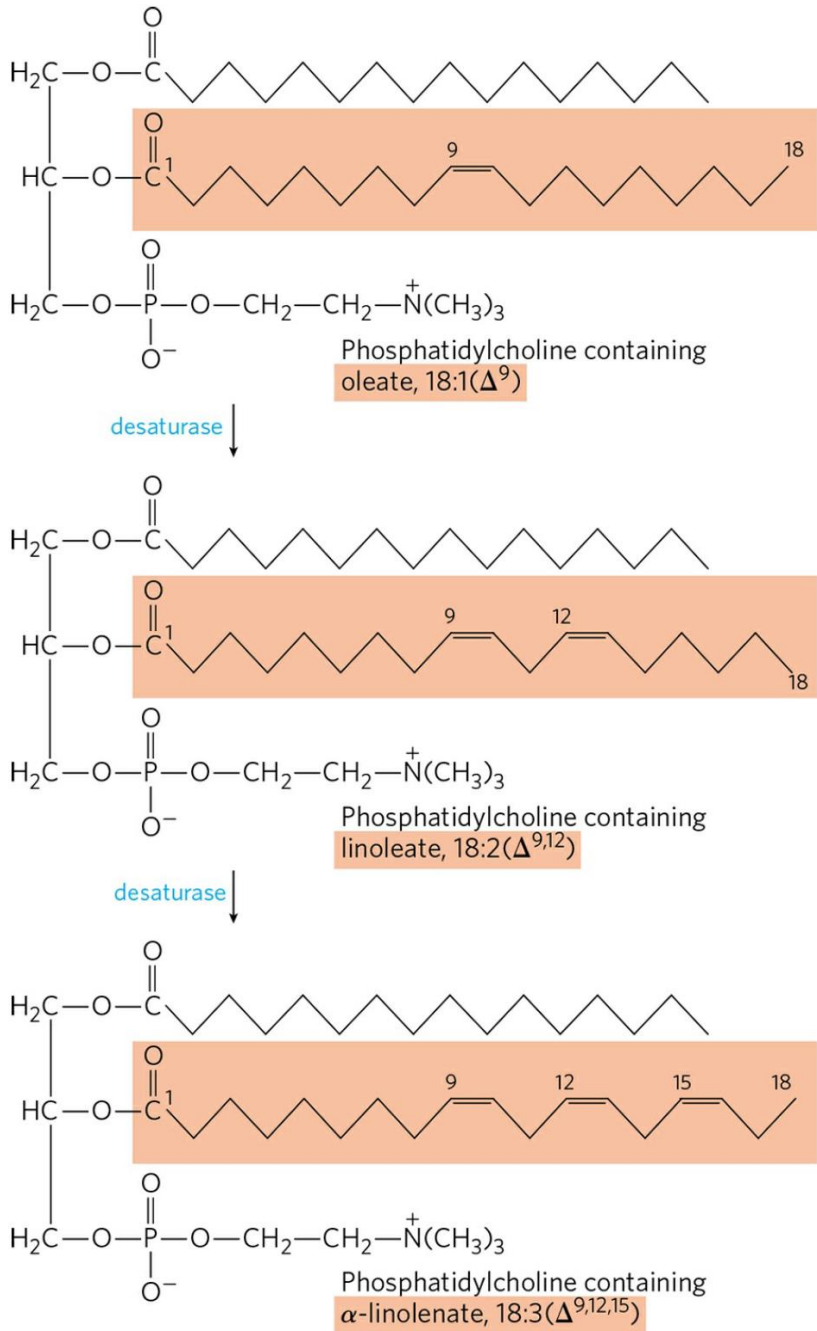
- The double bond is introduced intothe fatty acid chain by an oxidative reaction catalyzed by fatty acyl–CoA desaturase, a mixed-function oxidase.

- Two different substrates, the fatty acid and NADPH, simultaneously undergo two electron oxidations.

- The path of electron flow includes a cytochrome(cytochrome b5) and a flavoprotein (cytochrome b5 reductase), both of which, like fatty acyl–CoA desaturase, are in the smooth ER.

- In plants, oleate is produced by a stearoyl-ACP desaturase (SCD) that uses reduced ferredoxin as the electron donor in the chloroplast stroma.


O2 + 2H+ +
CH3—(CH2)n—CH2—CH2—(CH2)m—C
O
S-CoA
Saturated
fatty acyl-CoA
fatty acyl-
CoA desaturase
2 Cyt b5
(Fe2+)
2 Cyt b5
(Fe3+)
Cyt b5 reductase
(FAD)
Cyt b5 reductase
(FADH2)
NADPH
+ H+
NADP+
2H2O +
CH3—(CH2)n—CH=CH—(CH2)m—C
O
S-CoA
Monounsaturated
fatty acyl-CoA


Phosphatidylcholine containing
oleate, 18:1(Δ9)
desaturase
Phosphatidylcholine containing
linoleate, 18:2(Δ9,12)
desaturase
Phosphatidylcholine containing
α-linolenate, 18:3(Δ9,12,15)

- Eicosanoids are a family of very potent biological signaling molecules that act as short-range messengers, affecting tissues near the cells that produce them.

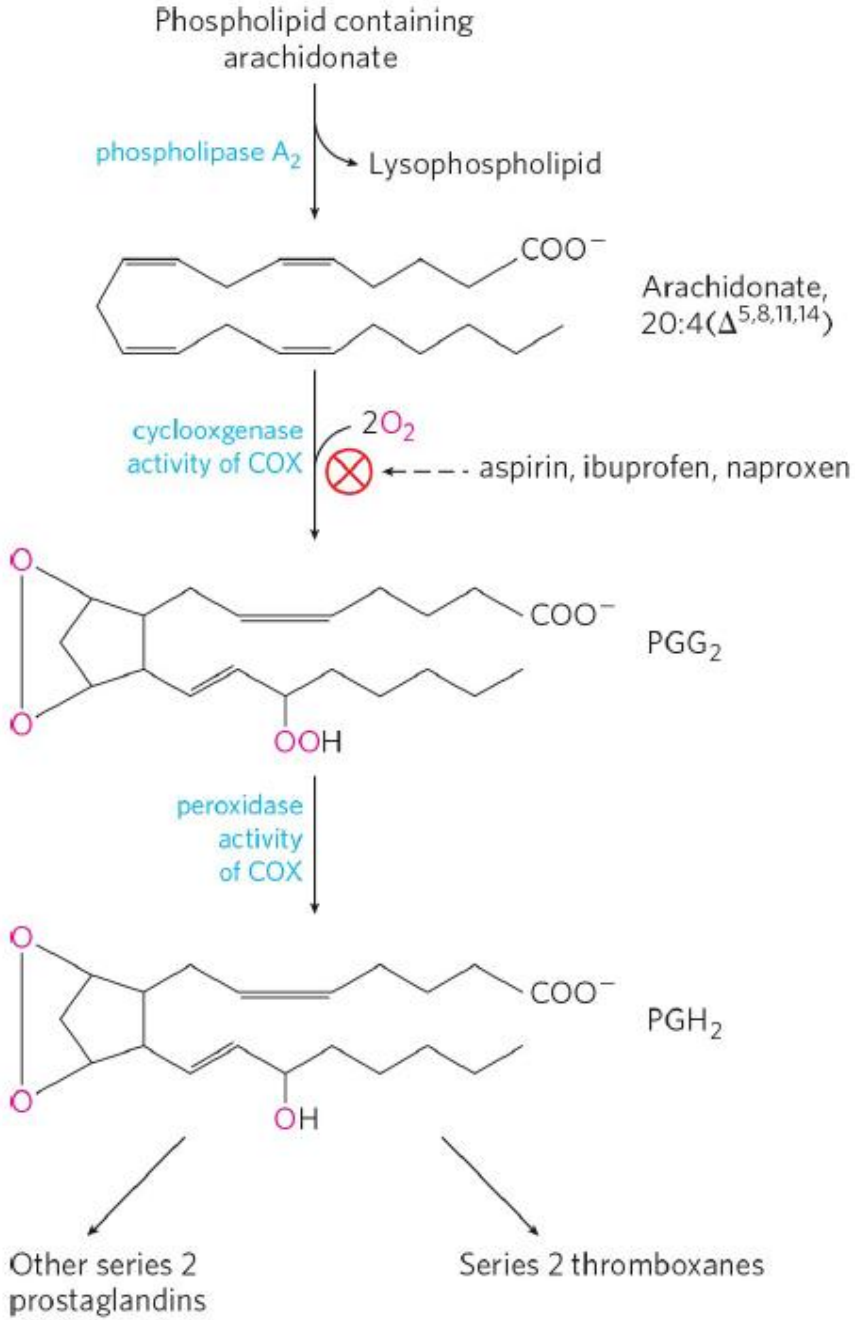
- In response to hormonal or other stimuli, phospholipase A2, present in most types of mammalian cells, attacks membrane phospholipids, releasing arachidonate from the middle carbon of glycerol.

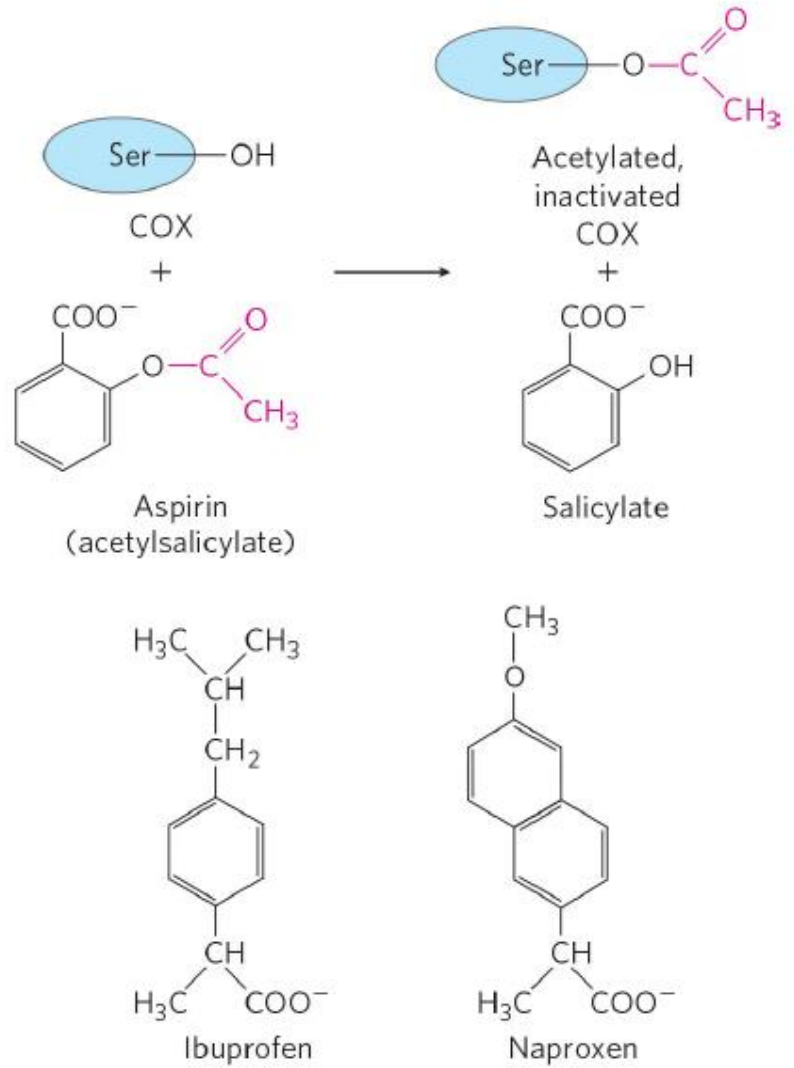
- Enzymes of the smooth ER then convert arachidonate to prostaglandins, beginning with the formation of prostaglandin H2 (PGH2), the immediate precursor of many other prostaglandins and thromboxanes.

- The two reactions that lead to PGH2 are catalyzed by a bifunctional enzyme, cyclooxygenase (COX), also called prostaglandin H2 synthase.

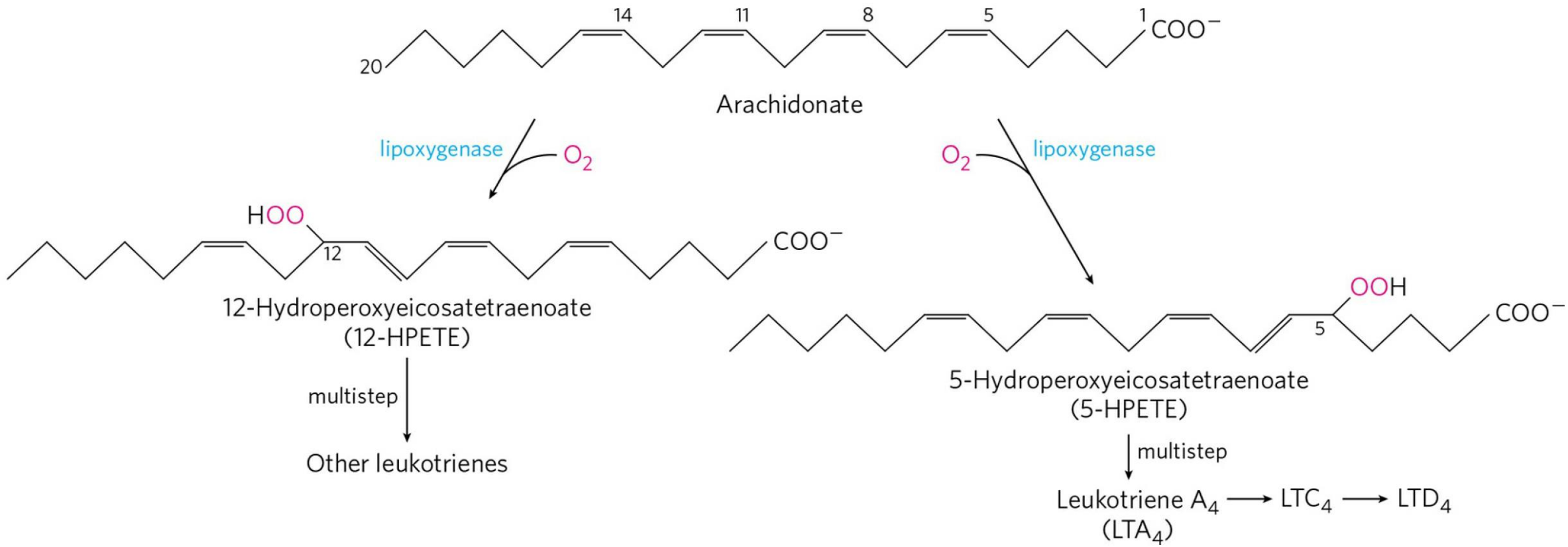
- In the first step, the cyclooxygenase activity introduces molecular oxygen to convert arachidonate to PGG2.

- The second step, catalyzed by the peroxidase activity of COX, converts PGG2 to PGH2.


Phospholipid containing arachidonate
phospholipase A_2
Lysophospholipid
COO⁻
Arachidonate, 20:4($\Delta^{5,8,11,14}$)
cyclooxgenase activity of COX
$2O_2$
aspirin, ibuprofen, naproxen
O
O
COO⁻
OOH
PGG_2
peroxidase activity of COX
O
O
COO⁻
OH
PGH_2
Other series 2 prostaglandins
Series 2 thromboxanes


Ser
OH
COX
+
COO⁻
O
O
C
CH₃
Aspirin (acetylsalicylate)
Ser
O
O
C
CH₃
Acetylated, inactivated COX
+
COO⁻
OH
Salicylate
H₃C
CH₃
CH
CH₂
CH
H₃C
COO⁻
Ibuprofen
CH₃
O
CH
H₃C
COO⁻
Naproxen

(b)


20
14
11
8
5
1
COO⁻
Arachidonate
lipoxygenase
O2
HOO
12
COO⁻
12-Hydroperoxyeicosatetraenoate
(12-HPETE)
multistep
Other leukotrienes
O2
lipoxygenase
OOH
5
COO⁻
5-Hydroperoxyeicosatetraenoate
(5-HPETE)
multistep
Leukotriene A4 → LTC4 → LTD4
(LTA4)

- In humans, the amount of body fat stays relatively constant over long periods, although there may be minor short-term changes as caloric intake fluctuates.

- Carbohydrate, fat, or protein ingested in excess of energy needs is stored in the form of triacylglycerols that can be drawn upon for energy, enabling the body to withstand periods of fasting.