MACQUARIE University

Lipid metabolism - 2

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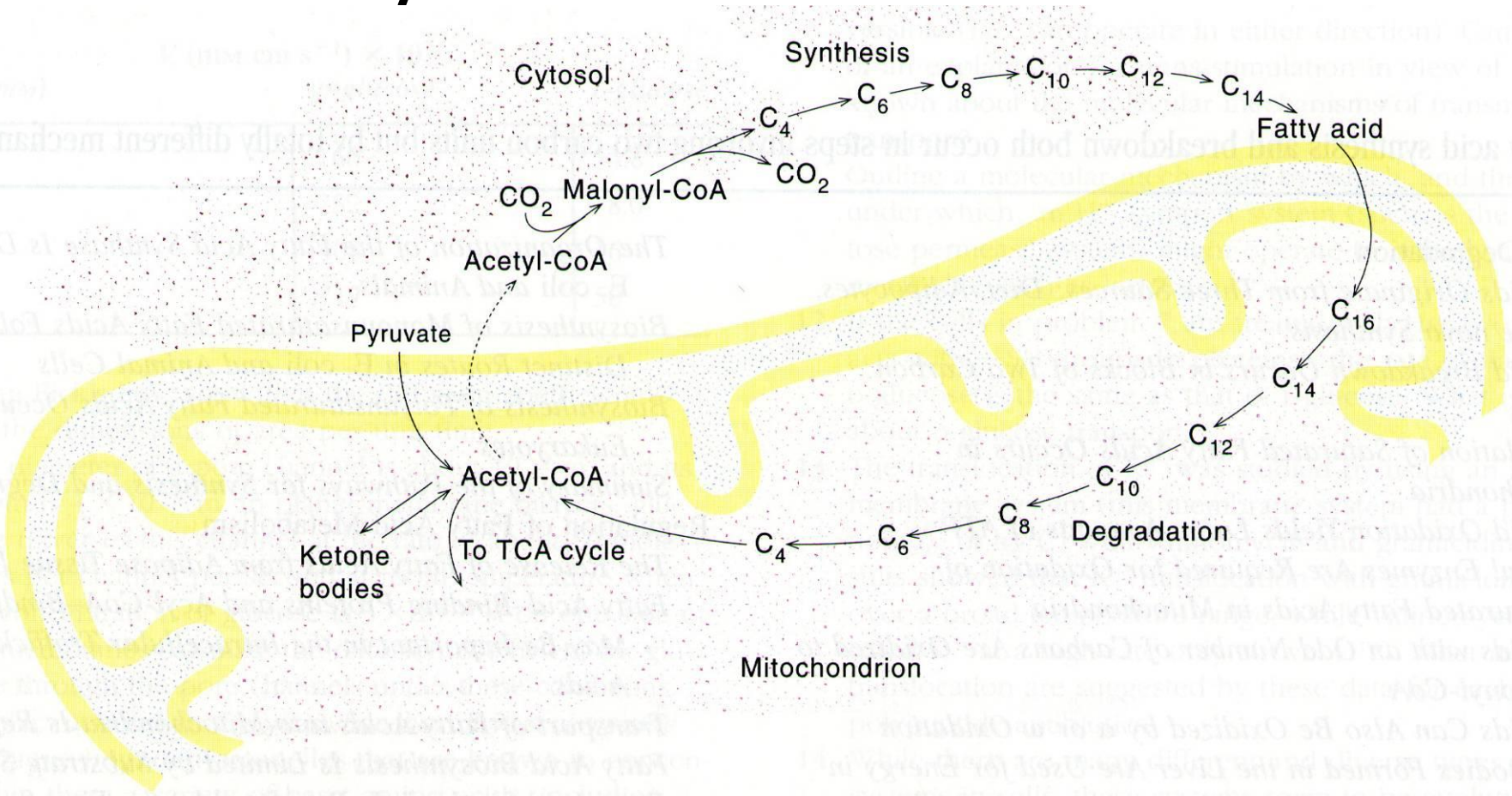
Objectives

- Lipid synthesis
  - Making the substrates: acetyl-CoA and malonyl-CoA
  - Enzyme: fatty acid synthase: also needs NADPH!

We need lipids (recap)

- Major source of energy
- Three sources of fatty acids
  - diet, adipose tissue and **biosynthesized fatty acids**.
- Major source is diet (around 40%)
- The role of fatty acid oxidation varies with the organism or tissue
  - Mammals - fuel, excess can be stored in adipose tissue
  - Plants - biosynthetic precursors and as a fuel
- Regardless of the organism, the reactions are the same.

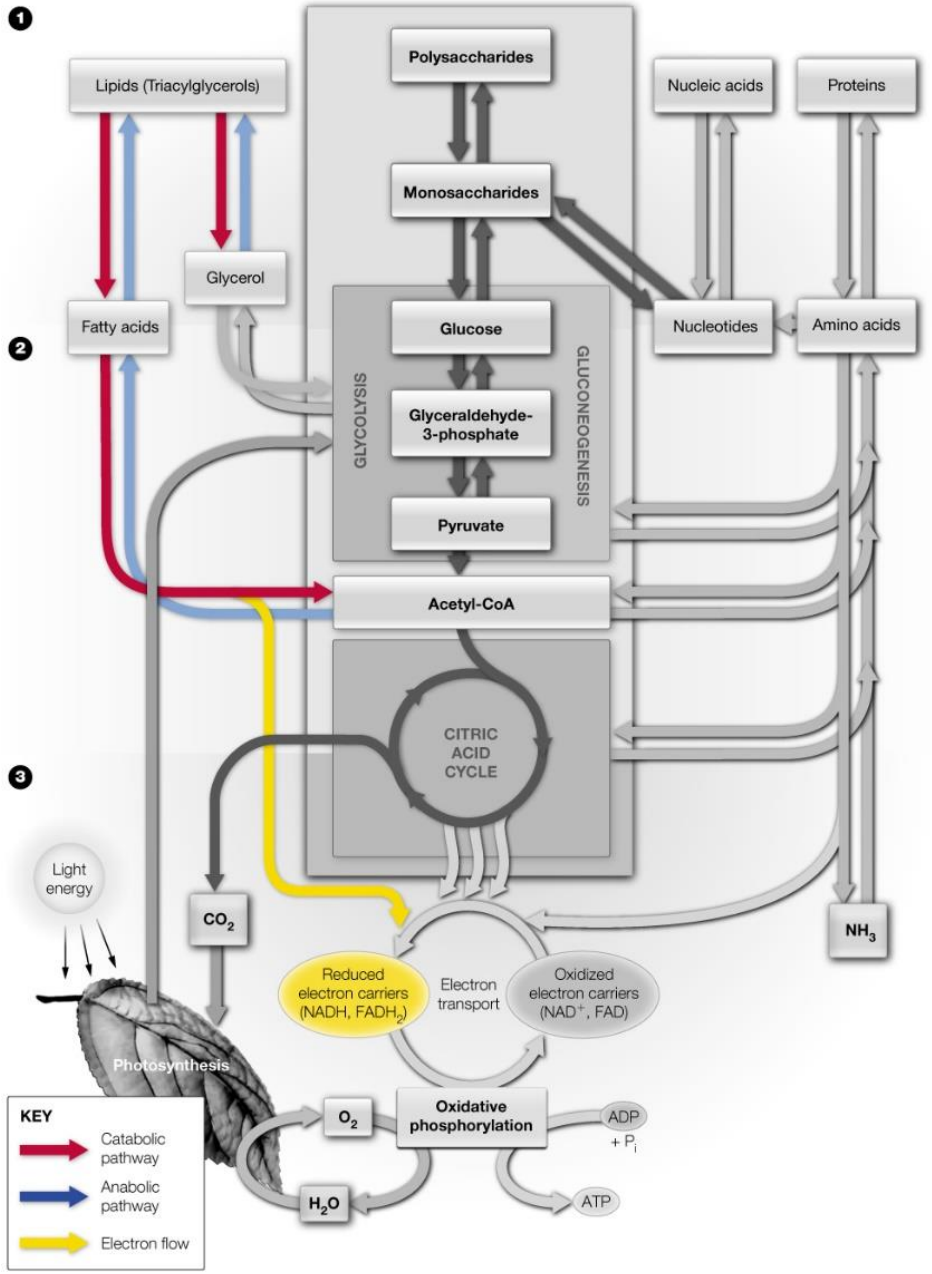
Degradation – mitochondria;
Synthesis – cytosol



Fatty Acid Biosynthesis

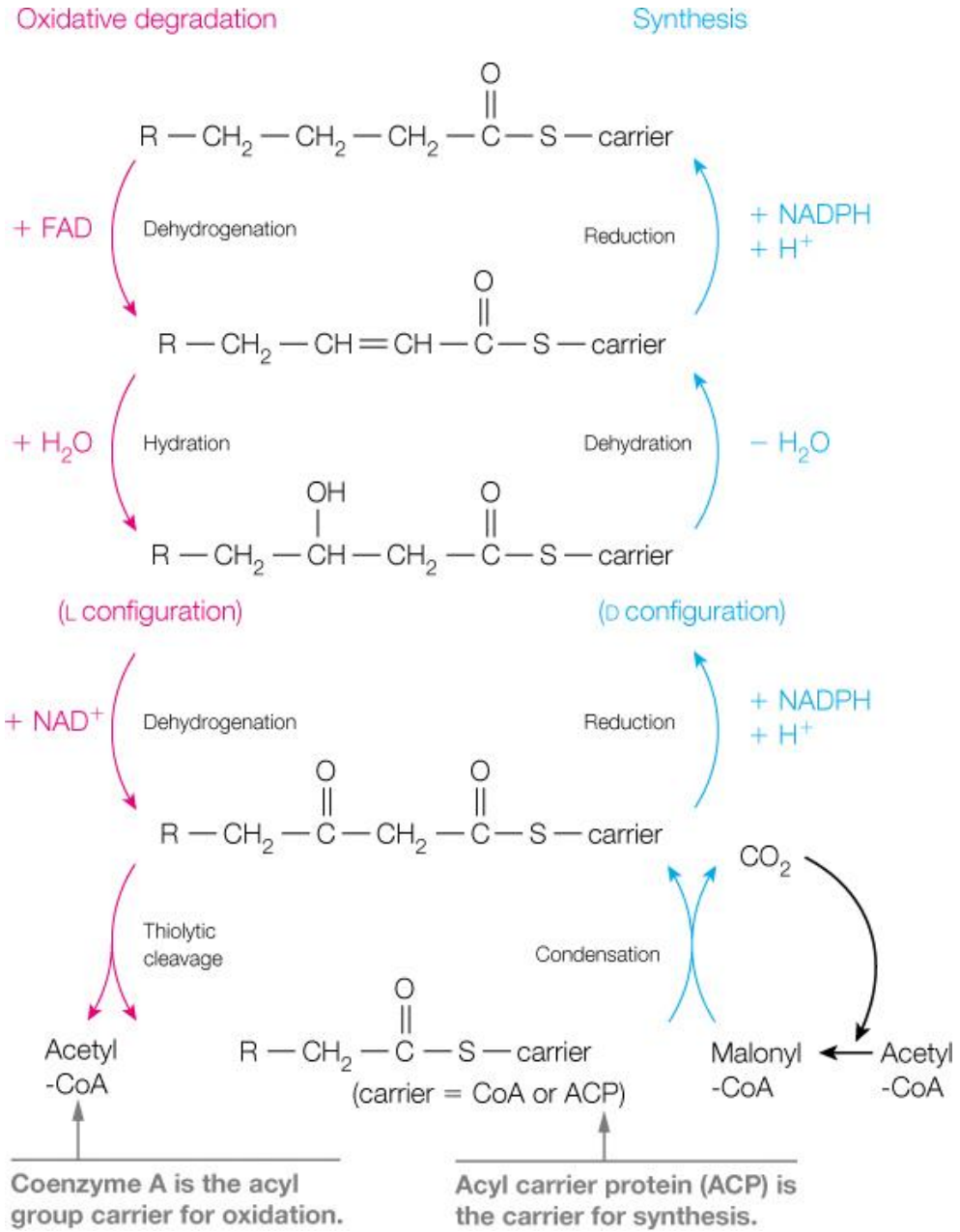
- The tricarboxylate transport system transfers **acetyl-CoA** into the cytosol for fatty acid synthesis.
- Fatty acid synthesis begins with the carboxylation of acetyl-CoA to generate **malonyl-CoA**.
- ***Fatty acid synthase*** carries out seven reactions and lengthens a fatty acid **two carbons** at a time.
- Elongases and desaturases may modify fatty acids.
- Triacylglycerols are synthesized from glycerol and fatty acids.

Chapter 16



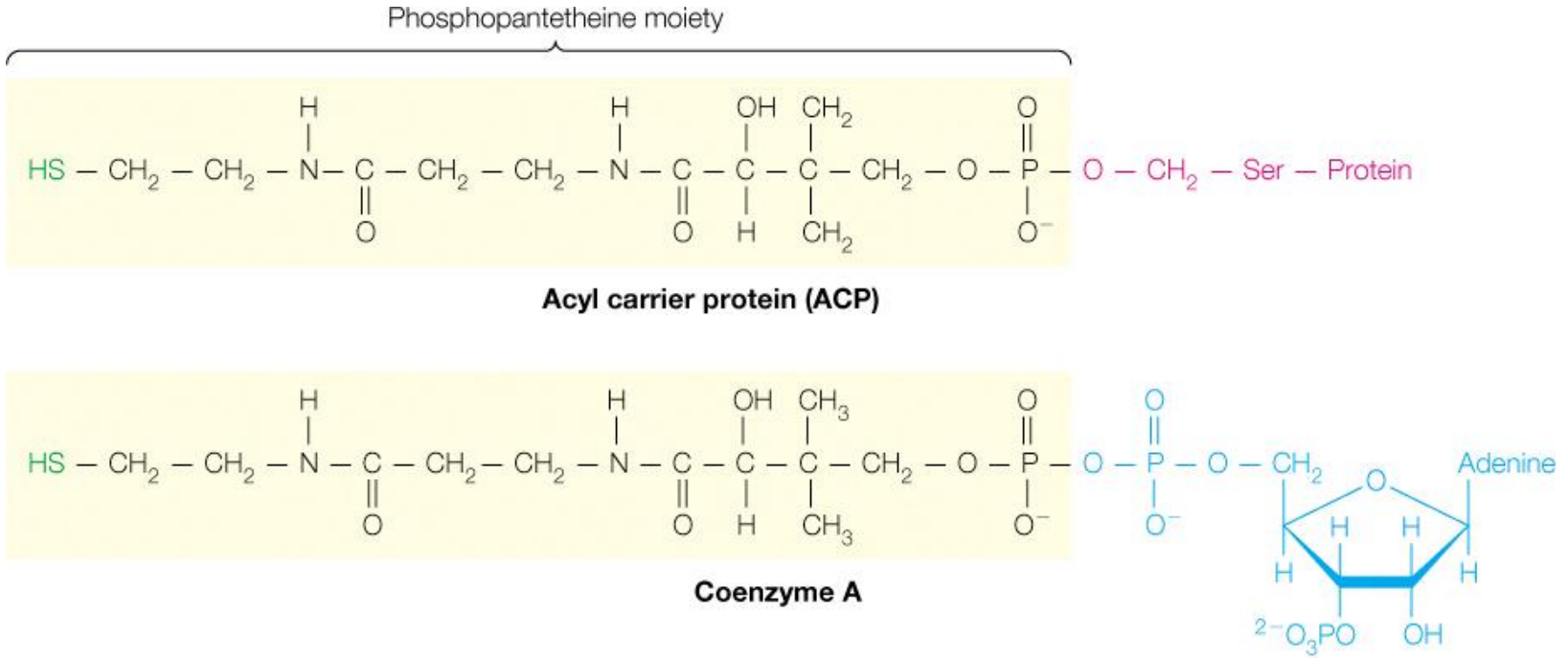
Fatty Acid Oxidation and Biosynthesis Compared

- Both occur via the same intermediates, but along different pathways
- Malonyl-CoA interacts with another acyl group carrier, that is, acyl carrier protein (ACP)



ACP: acyl carrier protein

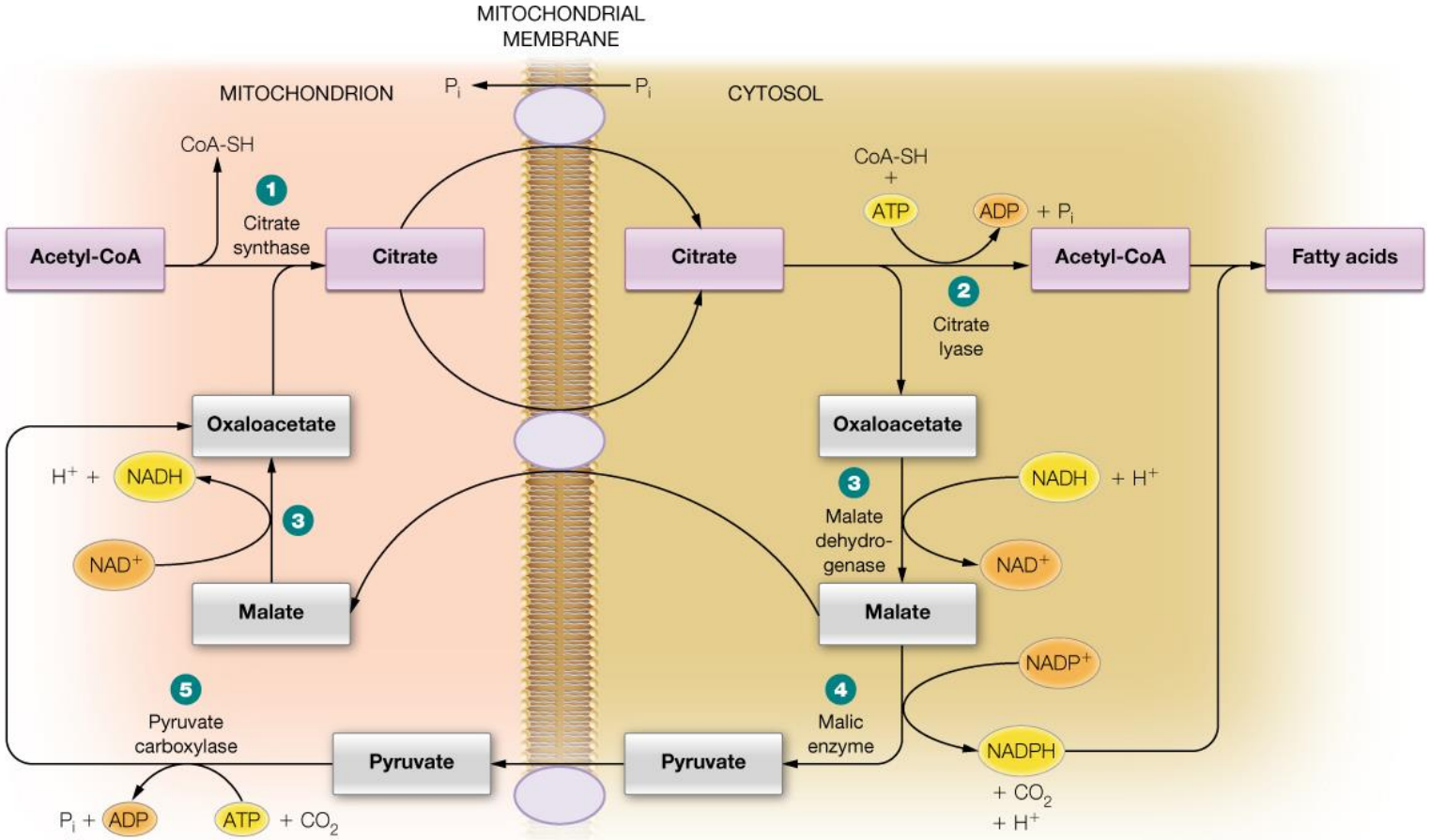
- ACP is a small (77 residues in *E. coli*) protein that binds the acyl group via a phosphopantetheine moiety (derived from **Vit. B_5**) with a reactive sulfhydryl (as seen in conzyme A)



Fatty acid Biosynthesis

- Condensation of C2 units in the form of **acetyl-CoA**: "reverse" of β-oxidation
- The **intermediate** required however is **C3**: **malonyl-CoA**
- Biosynthesis occurs in the cytosol
    1. So **acetyl-CoA** must be *transported* out of the mitochondria
    2. **acetyl-CoA carboxylation** to **malonyl-CoA**
    3. Enzyme **Fatty acid synthase** (FAS) can make upto C16 (i.e. seven cycles): **palmitate (or palmitic acid)**
        - other enzymes diversify the fatty acid product
        - Extensions beyond C16 in the mitochondria and the ER.

Generation and Transport of Acetyl-CoA



Mitochondria

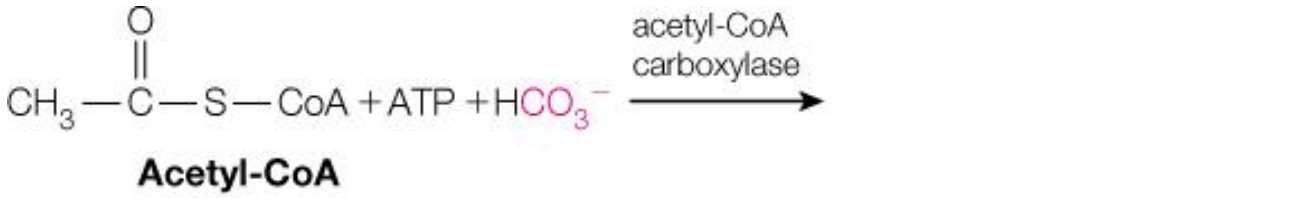
- Acetyl-CoA is here from *pyruvate*
  - ➢ Needs ATP!
- When [ATP] is high, no need to oxidise acetyl-CoA
  - ❖ Converted to citrate (CAC) and transported to the cytosol for FA synthesis

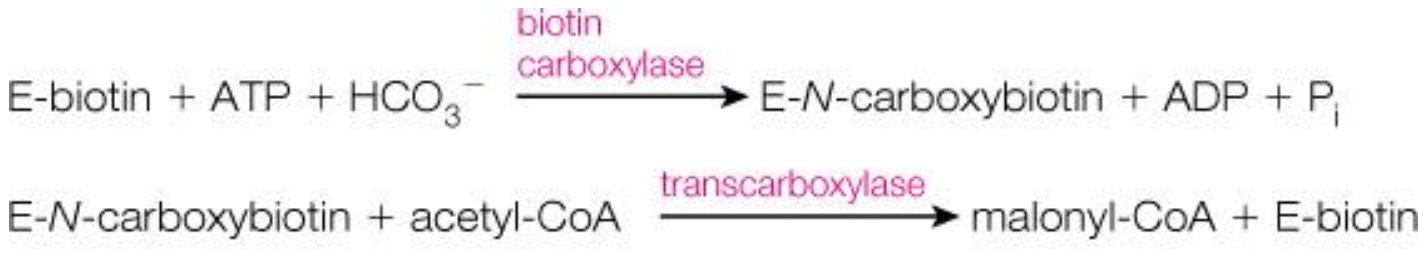
Cytosol

- ATP-citrate lyase regenerates acetyl-CoA
  - ➢ Needs ATP!
- End product pyruvate sent back to mitochondria

Synthesis of Malonyl-CoA

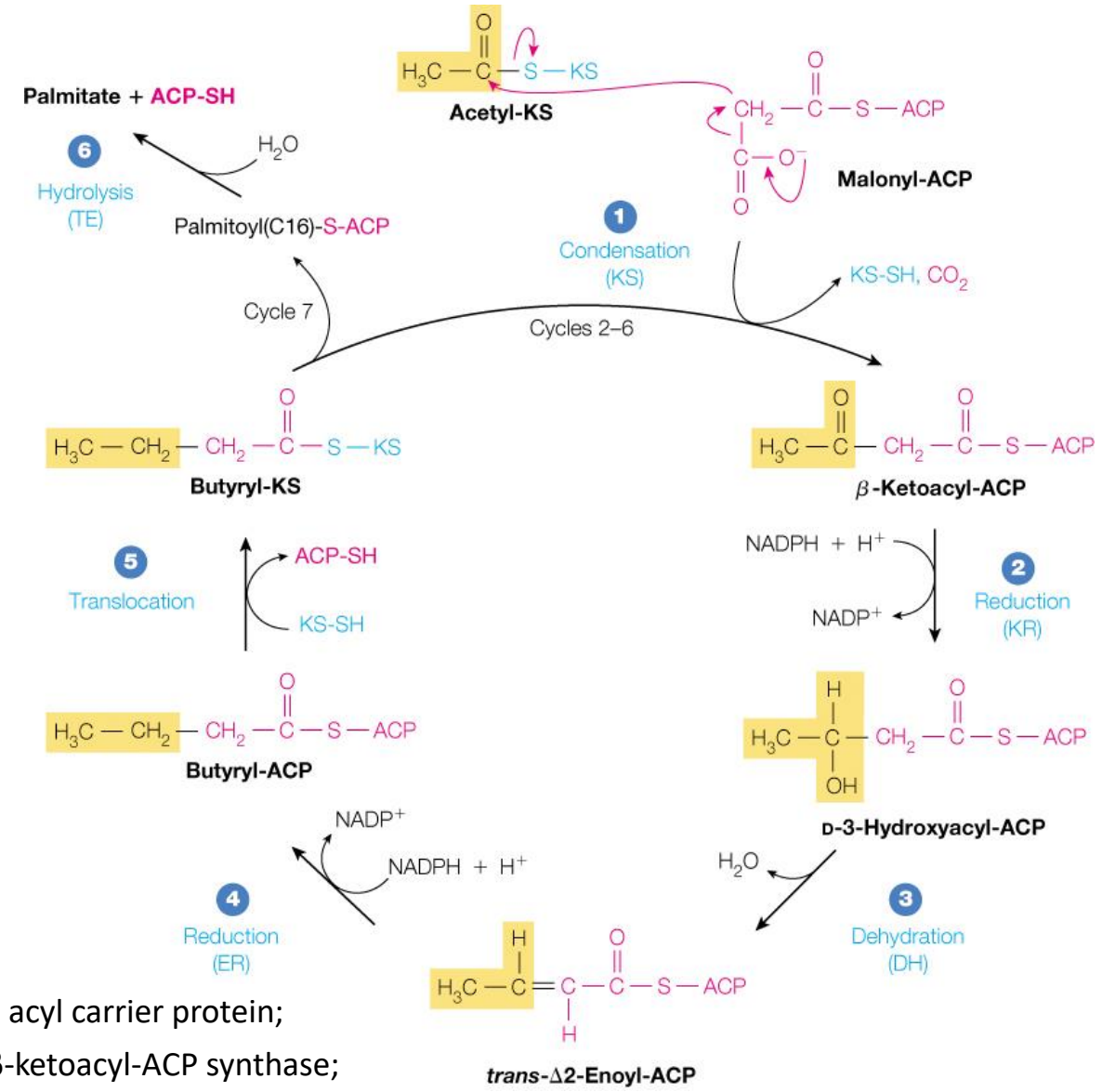
- Malonyl-CoA is synthesized from acetyl-CoA and bicarbonate by acetyl-CoA carboxylase (ACC): biotin is the required cofactor





- First committed step of FA synthesis
  - ACC – **needs ATP!**
  - major regulation site
  - **Biotin-dependent enzyme**
  - Product is a 3C group: malonyl-CoA – this is essential for FA synthesis.
- Two isoforms in mammals: **ACC1** in the liver is in the lipid synthesis pathway while **ACC2** is in muscle with the product **malonyl-CoA** inhibiting lipid import into mitochondria for β-oxidation

Synthesis of Palmitate (C16) from Malonyl-ACP Information only



1. **Condensation:** 2C unit added from malonyl-CoA, making acetoacetate derivative and CO_2.
2. **Reduction:** needs **NADPH**
3. **Dehydration**: to make C=C
4. **Reduction**: needs **NADPH**
5. **Translocation**: ACP released with 4C chain entering 1.

Repeat 6 times

6. **Hydrolysis**: When chain is 16C: hydrolyse ACP part and release palmitic acid as **palmitate**

ACP: acyl carrier protein;
KS: β-ketoacyl-ACP synthase;
KR: β-ketoacyl-ACP reductase;
DH: β-ketoacyl-ACP dehydrase;
ER: enoyl-ACP reductase;
TE: thioesterase

Requirement for Reducing Equivalent and Energy

- Reducing power (NADPH) and energy (ATP) are required to drive the biosynthesis of lipid:

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

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

- The net equation describes the process:

$$8\ \text{Acetyl-CoA} + 7\ \text{ATP} + 14\ \text{NADPH} + 7\ H^+ \rightarrow$$
$$\text{palmitate} + 14\ NADP^+ + 8\ \text{CoA-SH} + 7\ \text{ADP} + 7\ P_i + 6\ H_2O$$

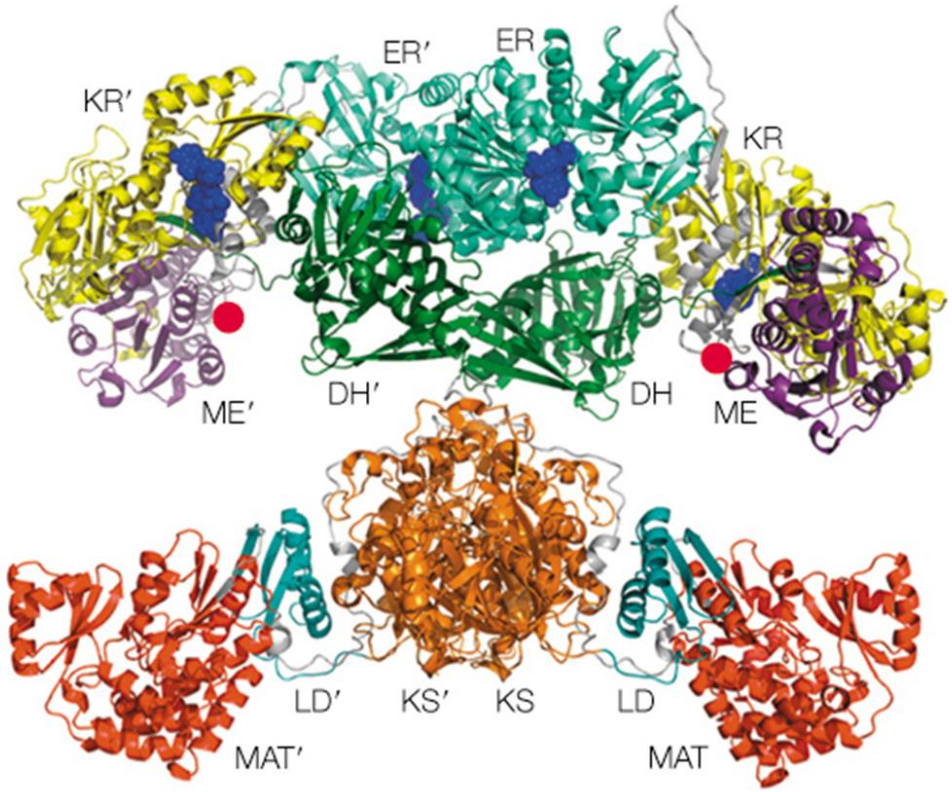
However, 1 ATP was needed to make acetyl-CoA from citrate (catalysed by citrate lyase) (slide 7): hence total 8 ATPs!

Type I and II Fatty Acid Synthesis

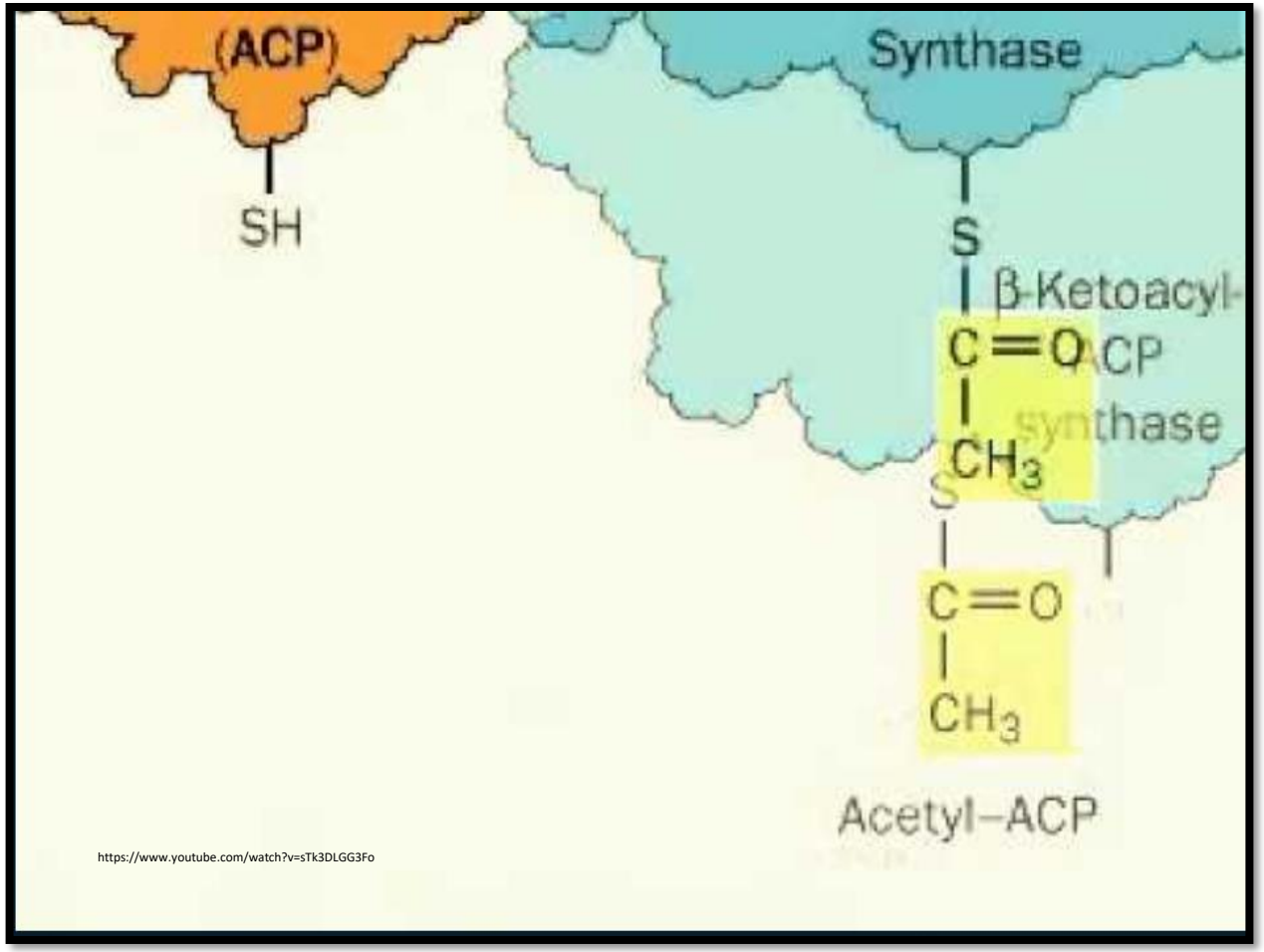
- In all plants and most bacteria, the enzymes catalyzing this pathway are separate protein molecules (type II fatty acid synthesis)
- In most nonplant eukaryotes and some bacteria, the enzymes needed for fatty acid synthesis are linked in a giant multienzyme protein (megasynthase or type I **fatty acid synthase**)
- In animals, the complex is a homodimer of 273 kDa subunits, each of which has all seven active sites

Structure of mammalian FAS: makes two FAs simultaneously

- Example of a multi-reaction enzyme complex
- Entire pathway in this mega complex
- In animals, the complex is a homodimer of 273 kDa subunits, each of which has all seven active sites



Short video: Biosynthesis of Fatty acids from malonyl-CoA precursors



Overall reaction to make 1 palmitate

$$\text{Acetyl-CoA} + 7\,\text{malonyl-CoA} + 14\,\text{NADPH} + 7\,\text{H}^+ \rightarrow \text{palmitate} + 7\,\text{CO}_2 + 14\,\text{NADP}^+ + 8\,\text{CoA} + 6\,\text{H}_2\text{O}$$

Since the 7 malonyl-CoA are derived from acetyl-CoA as follows:

$$7\,\text{Acetyl-CoA} + 7\,\text{CO}_2 + 7\,\text{ATP} \rightarrow 7\,\text{malonyl-CoA} + 7\,\text{ADP} + 7\,\text{P}_i + 7\,\text{H}^+$$

the overall stoichiometry for palmitate biosynthesis is

$$8\,\text{Acetyl-CoA} + 14\,\text{NADPH} + 7\,\text{ATP} \rightarrow \text{palmitate} + 14\,\text{NADP}^+ + 8\,\text{CoA} + 6\,\text{H}_2\text{O} + 7\,\text{ADP} + 7\,\text{P}_i$$

{Note: 1 ATP was needed to make acetyl-CoA from citrate (catalysed by citrate lyase) (slide 9)}

Fatty Acid β Oxidation & Biosynthesis
Differences

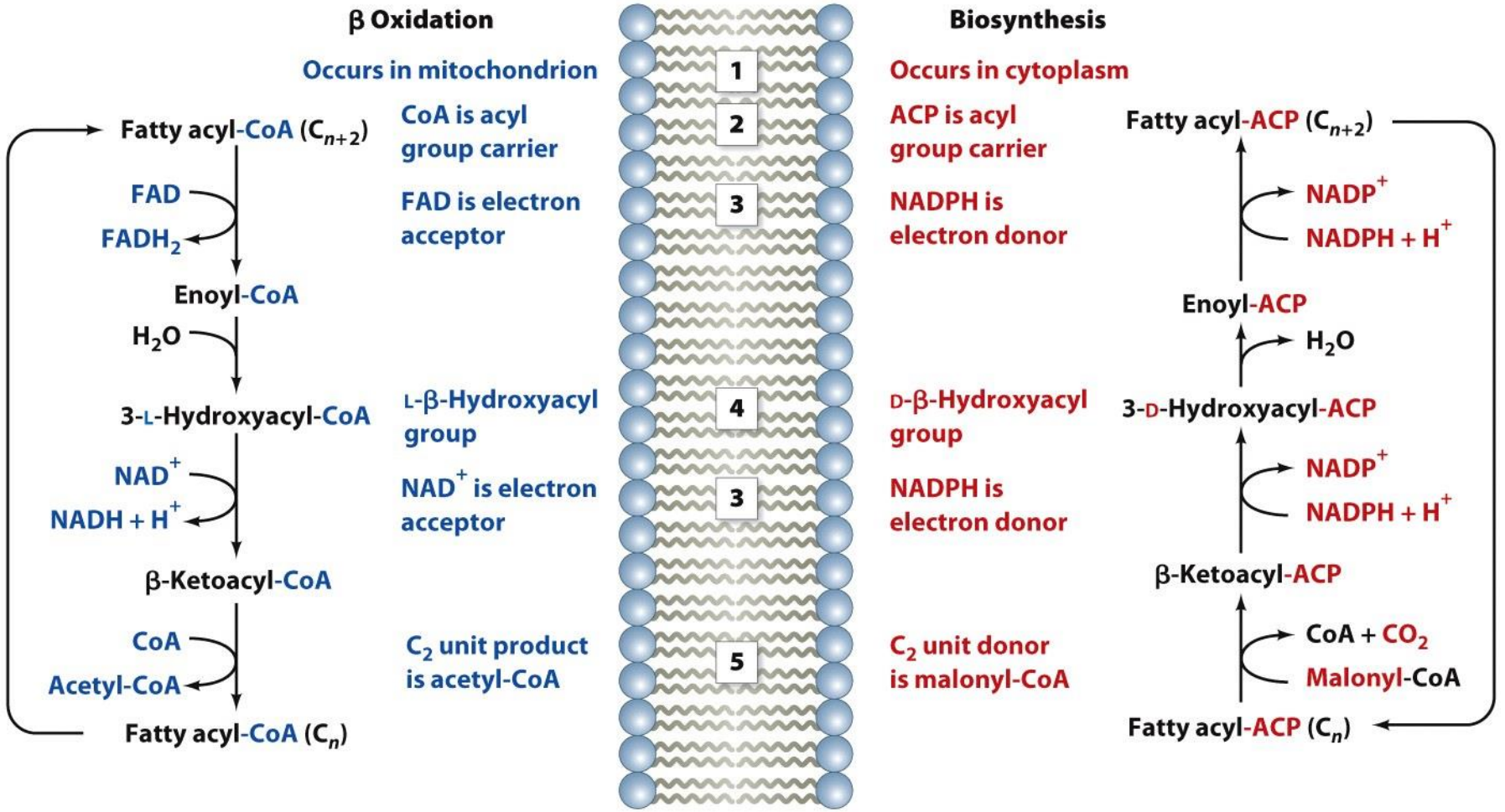


Figure 20-23

Differences between Fatty Acid Biosynthesis and Fatty Acid Breakdown in Eukaryotes

1. Intermediates in synthesis are linked to -SH groups of **acyl carrier proteins** (as compared to -SH groups of CoA)
2. Synthesis in **cytosol**; breakdown in mitochondria
3. Enzymes of synthesis are one polypeptide, the **fatty acid synthase**
4. Biosynthesis uses **NADPH/NADP$^+$**; breakdown uses FAD/$FADH_2$ and NADH/NAD^+

Fatty Acid Elongation and Desaturation

- Elongation occurs in the **mitochondria** and the **endoplasmic reticulum** and involves same reactions as those leading to palmitate, but using separate enzymes and acyl-CoAs instead of acyl-ACPs
- For example:

  palmitoyl-CoA (C16) + malonyl-CoA ⟶ stearoyl-CoA (C18)
- Desaturation takes place in a membrane-bound enzyme system in the **endoplasmic reticulum**; the first reaction is between C9 and C10
- Animals cannot desaturate beyond C9–10; therefore, linoleic (18: 2Δ9,12) and linolenic (18: 3Δ9,12,15) acids must be provided in the diet (they are essential fatty acids)

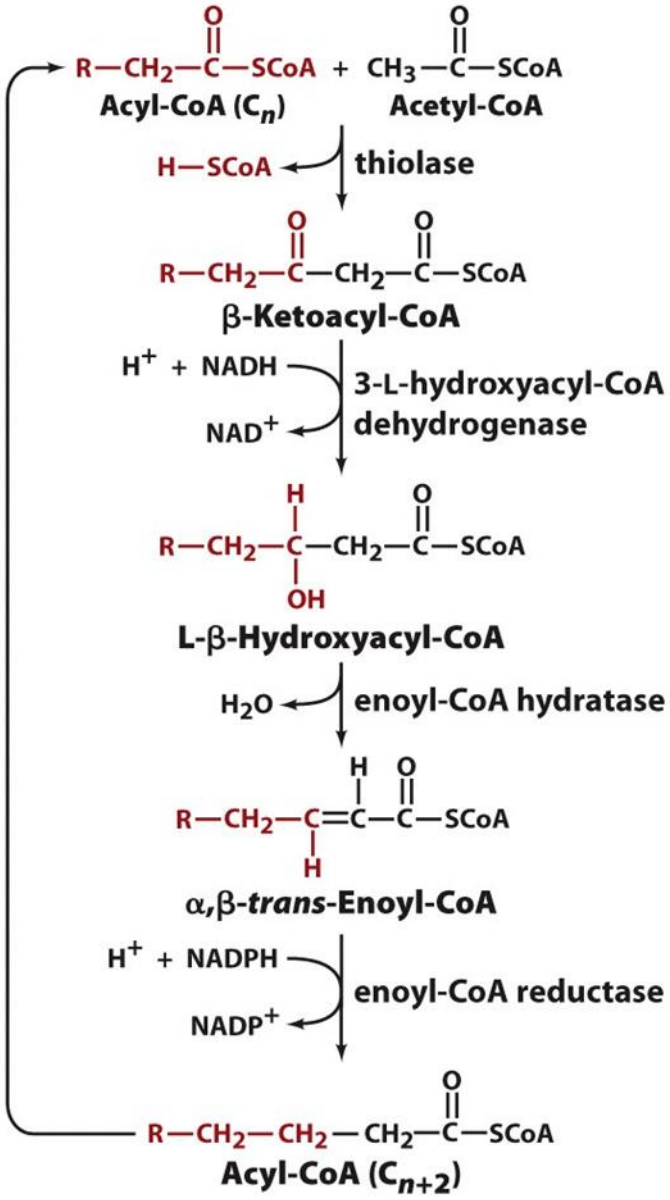


Figure 20-28

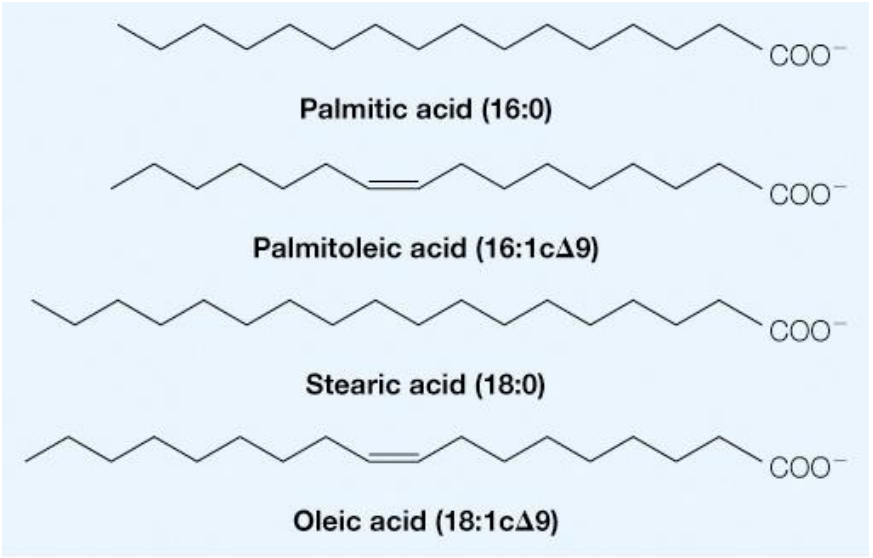
Fatty Acid Desaturation

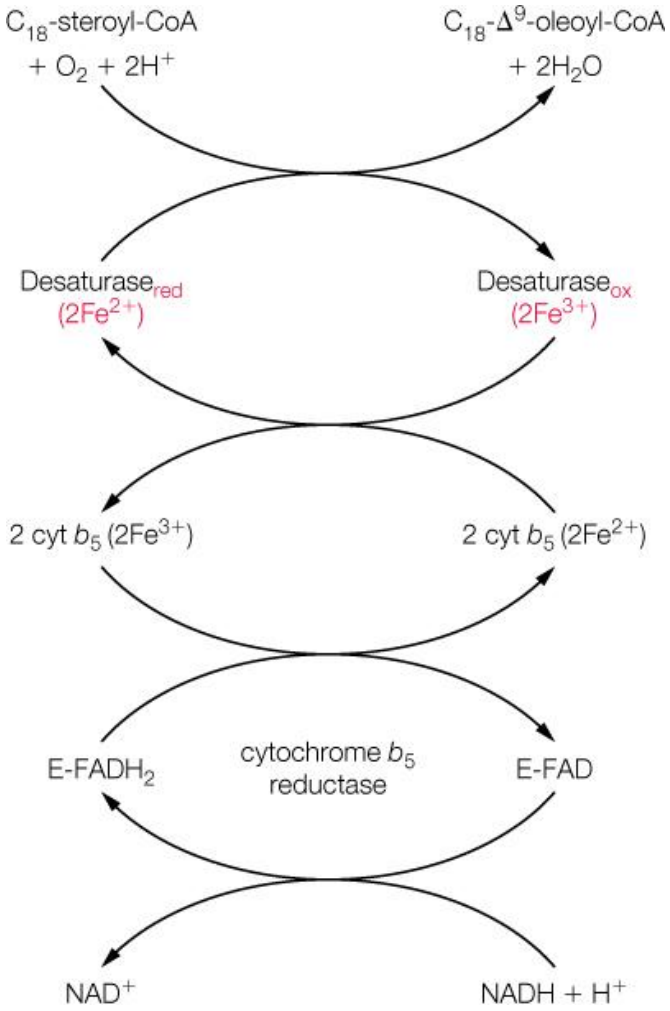
The fatty acid Δ9 desaturation system – conversion of steroyl-CoA to oleoyl-CoA

Needs NADH.

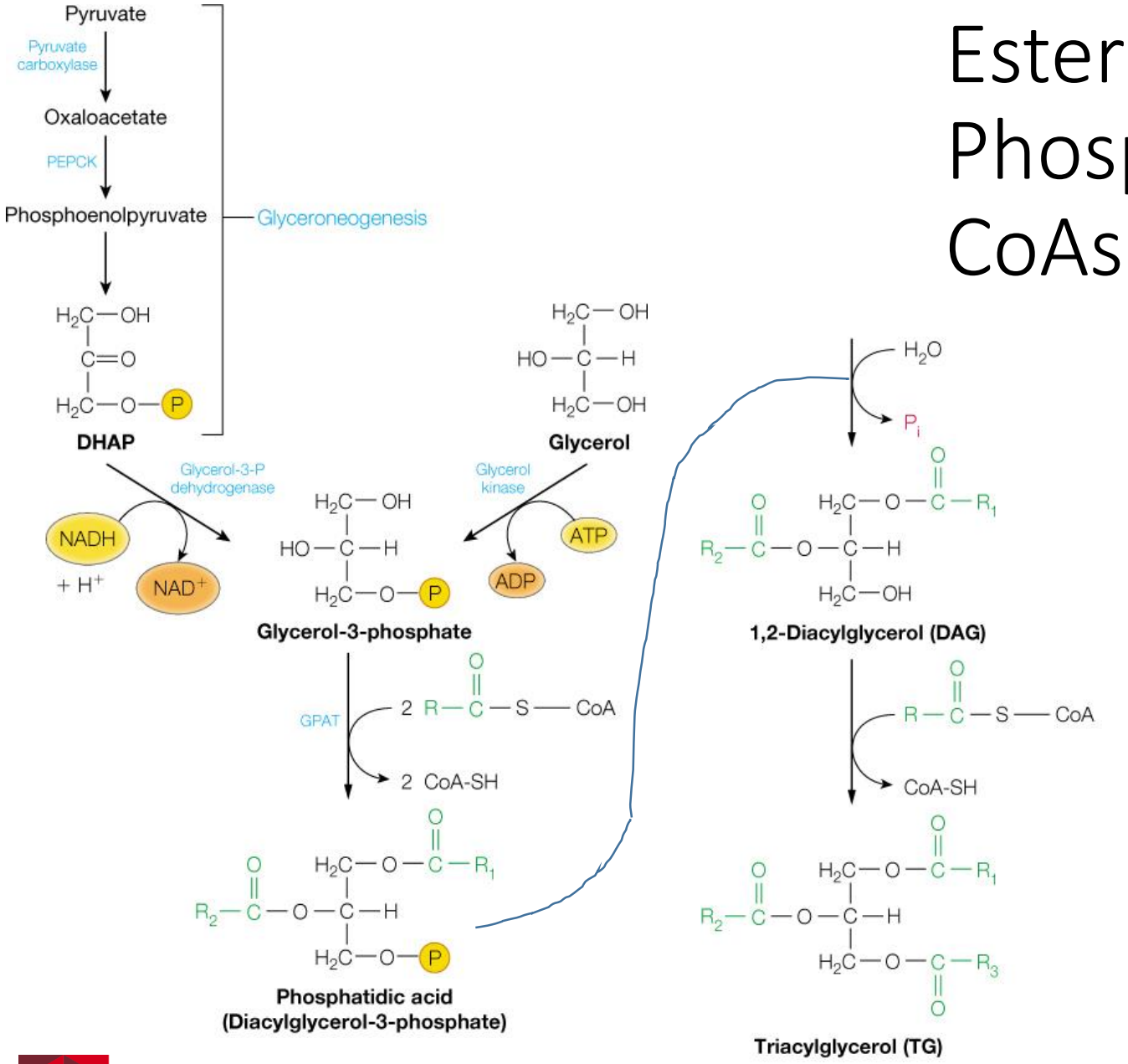
$$\text{Stearoyl-CoA (18:0)} + \text{NADH} + \text{H}^+ + \text{O}_2 \rightarrow$$

$$\text{oleoyl-CoA (18:1cΔ9)} + \text{NAD}^+ + 2\ \text{H}_2\text{O}$$



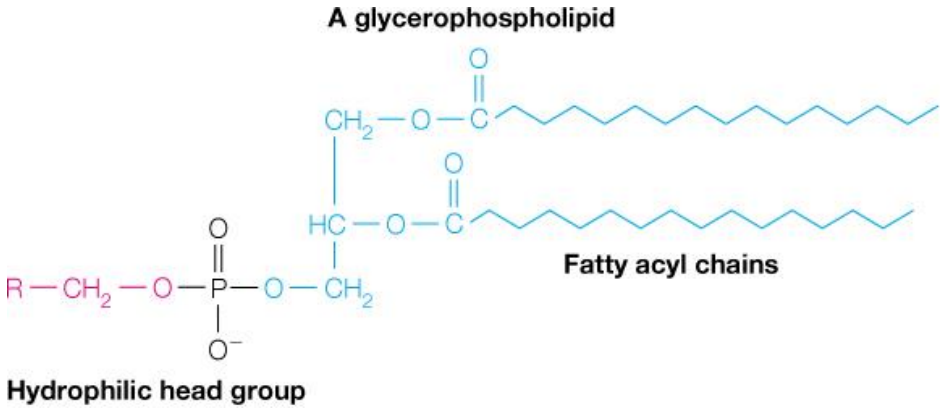


Esterification of Glycerol 3-Phosphate with Fatty Acyl-CoAs

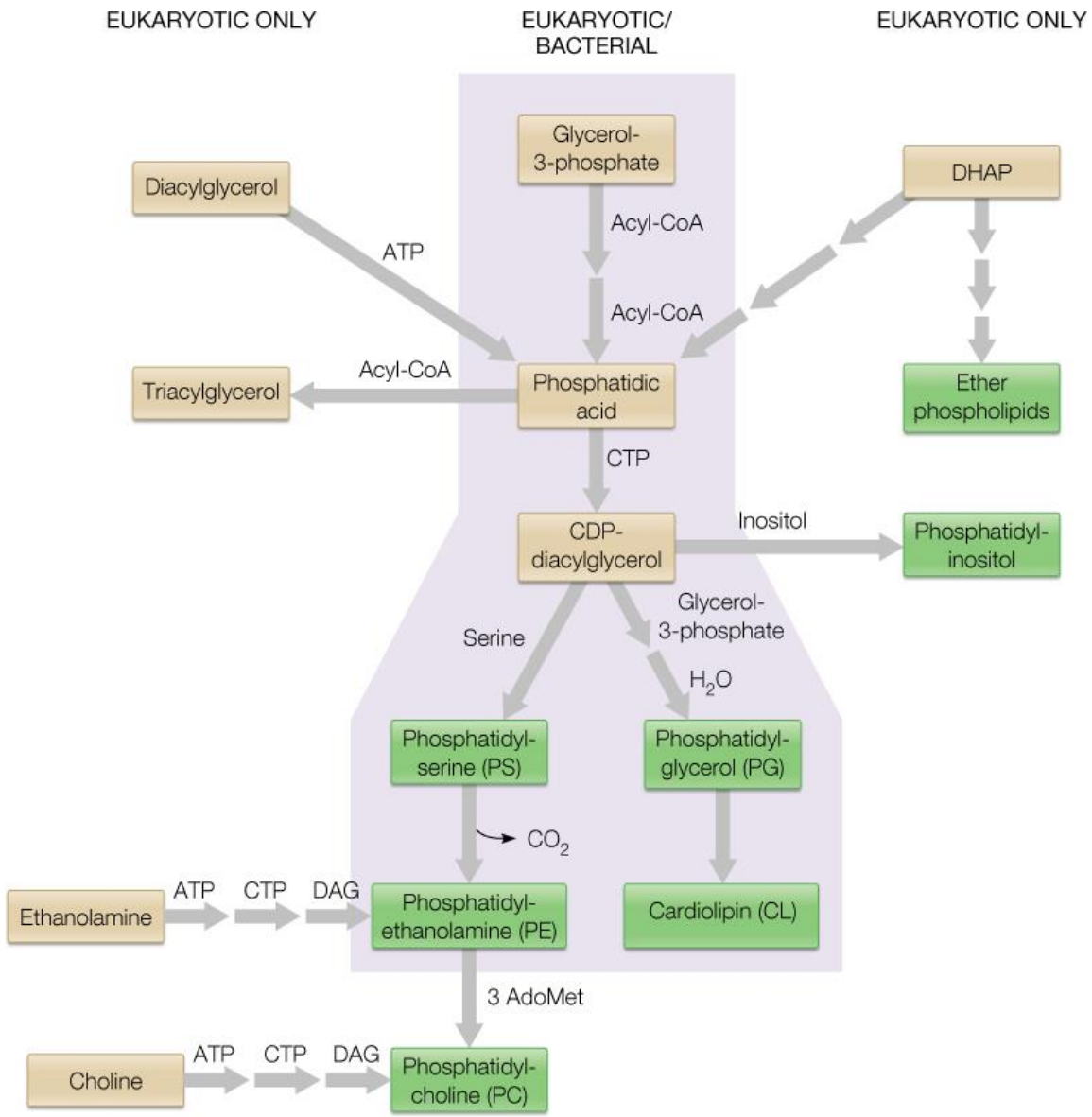


- Made from FA and glycerol-3-phosphate or dihydroxyacetone phosphate
- In the ER or peroxisomes
- Final product: triacylglycerol: called **glyceroneogenesis**
  - During starvation, 30% of FA entering the liver are re-esterified to triacylglycerol and exported as VLDL
- Intermediate phosphatidic acid can be diverted to phospholipids.

Glycerophospholipid Synthesis



Phosphatidic acid is the major precursor to all glycerophospholipids



Mammals cannot synthesize most polyunsaturated FAs

- **Essential fatty acids:** Linoleic acid and linolenic acid
  - **Linoleic acid** (18:2) is the precursor of arachidonic acid (20:4) and both these are termed **ω6 fatty acids**
    - Mammals can make arachidonic acid from linoleic acid, which then leads to eicosanoids…..
    - ω6 fatty acids are precursors of hormones: prostaglandins, thromboxanes, and leukotrienes.
  - **Linolenic acid** (18:3) is the precursor of eicosapentaenoic acid and docosahexaenoic acid and these three are termed **ω3 fatty acids**
    - ω3 fatty acids are cardioprotective, anti-inflammatory, and anticarcinogenic.

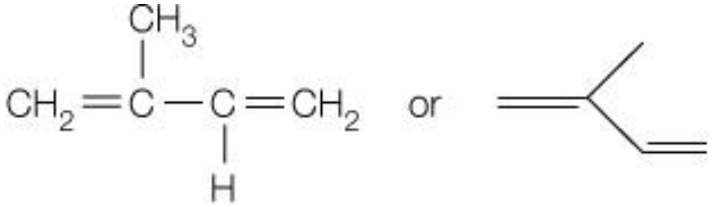
Essential unsaturated dietary FAs

- Linoleic acid is an essential fatty acid!

18:2*n*–6	**Linoleic acid**	**9,12-Octadecadienoic acid**

- It is a precursor for prostaglandins and other related compounds (called eicosanoids)
- On a fat-free diet,
  - Initally: poor growth, poor wound healing and dermatitis – also increased thirst!
  - Eventually, fatal!

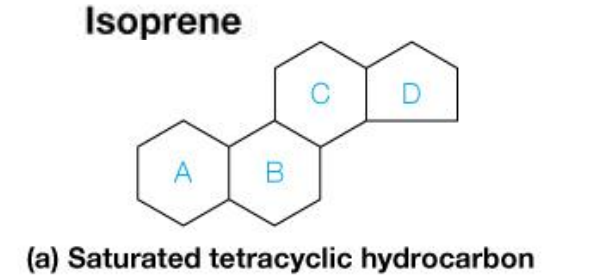
Actetyl-CoA also makes Steroids



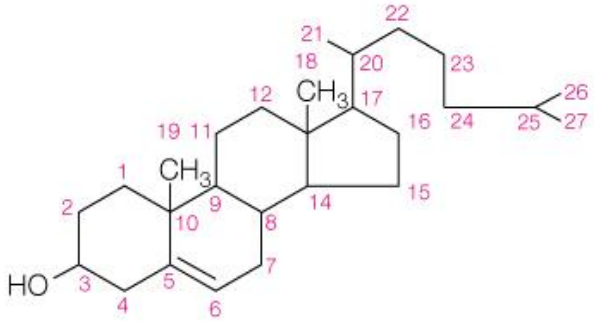
- Steroids are lipids based on a four ring structure
- Steroids are the best known of the isoprenoids (built of one or more isoprene molecules)
- Steroid hormones are made from cholesterol

Biosynthesis of cholesterol is a long process

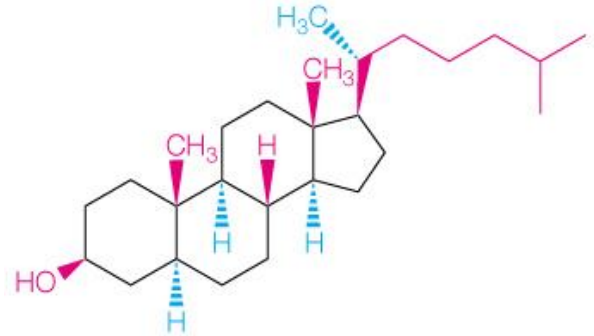
1. Mevalonate is synthesized from acetoacetyl-CoA and acetyl-CoA and in two catalytic steps via a 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) intermediate
2. Isopentenyl pyrophosphate (or its isomer dimethylallyl pyrophosphate) are synthesized using four (or five) different enzymes
3. Isopentenyl pyrophosphate reacts with its isomer, dimethylallyl pyrophosphate to form squalene in three catalytic steps
4. A cyclization (three steps) and a series of 19 additional reactions are required to produce cholesterol



(a) Saturated tetracyclic hydrocarbon



(b) Cholesterol (a steroid alcohol, or sterol)



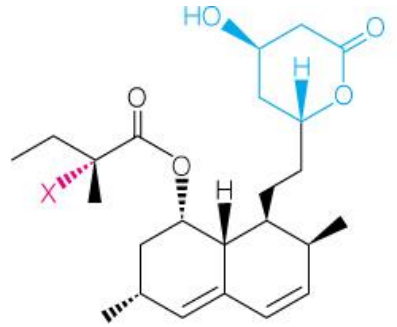
(c) Stereochemistry of cholestanol

Lipid-Soluble Vitamins

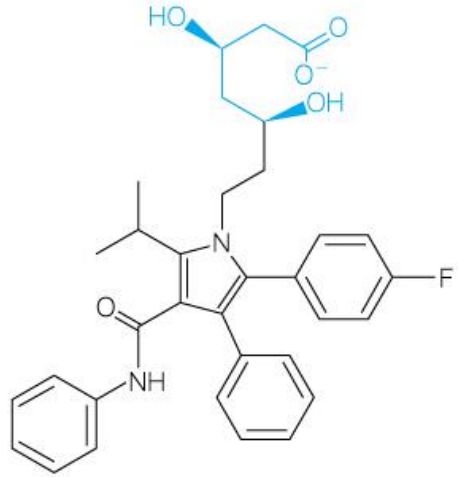
- Vitamins are organic compounds that are essential for normal growth and nutrition and are required in small quantities in the diet because they cannot be synthesized by the body
- Vitamins D, A, E, and K are **lipid-soluble isoprenoid compounds**
- Of these, however, only Vitamin D is a derivative of cholesterol, acts analogously to a steroid hormone and is involved in the regulation of calcium and phosphorus metabolism

Bringing cholesterol down

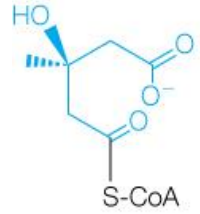
- Competitive inhibitors of cholesterol synthesis are approved drugs
- Lipitor is one the best-selling pharmaceutical compounds known.



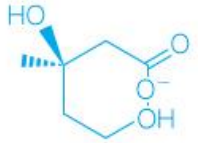
X = H Lovastatin (Mevacor)
X = CH_3 Simvastatin (Zocor)



Atorvastatin (Lipitor)



HMG-CoA



Mevalonate

Summary of Lipid Biosynthesis

https://www.youtube.com/watch?v=8OSgfu9SzJA

1

Essential Biochemistry

Third Edition

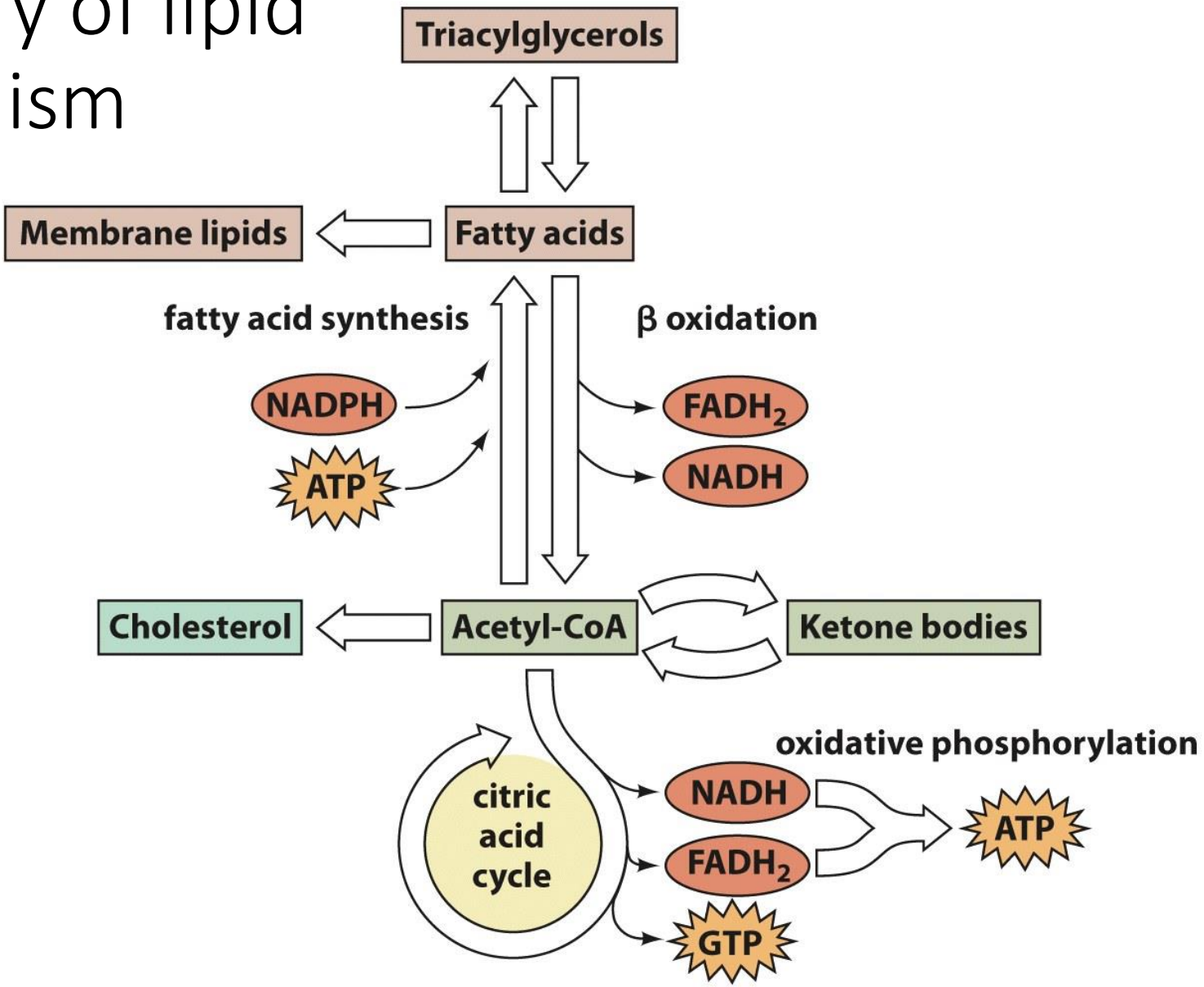
Charlotte W. Pratt | Kathleen Cornely

Chapter 17

Lipid Metabolism

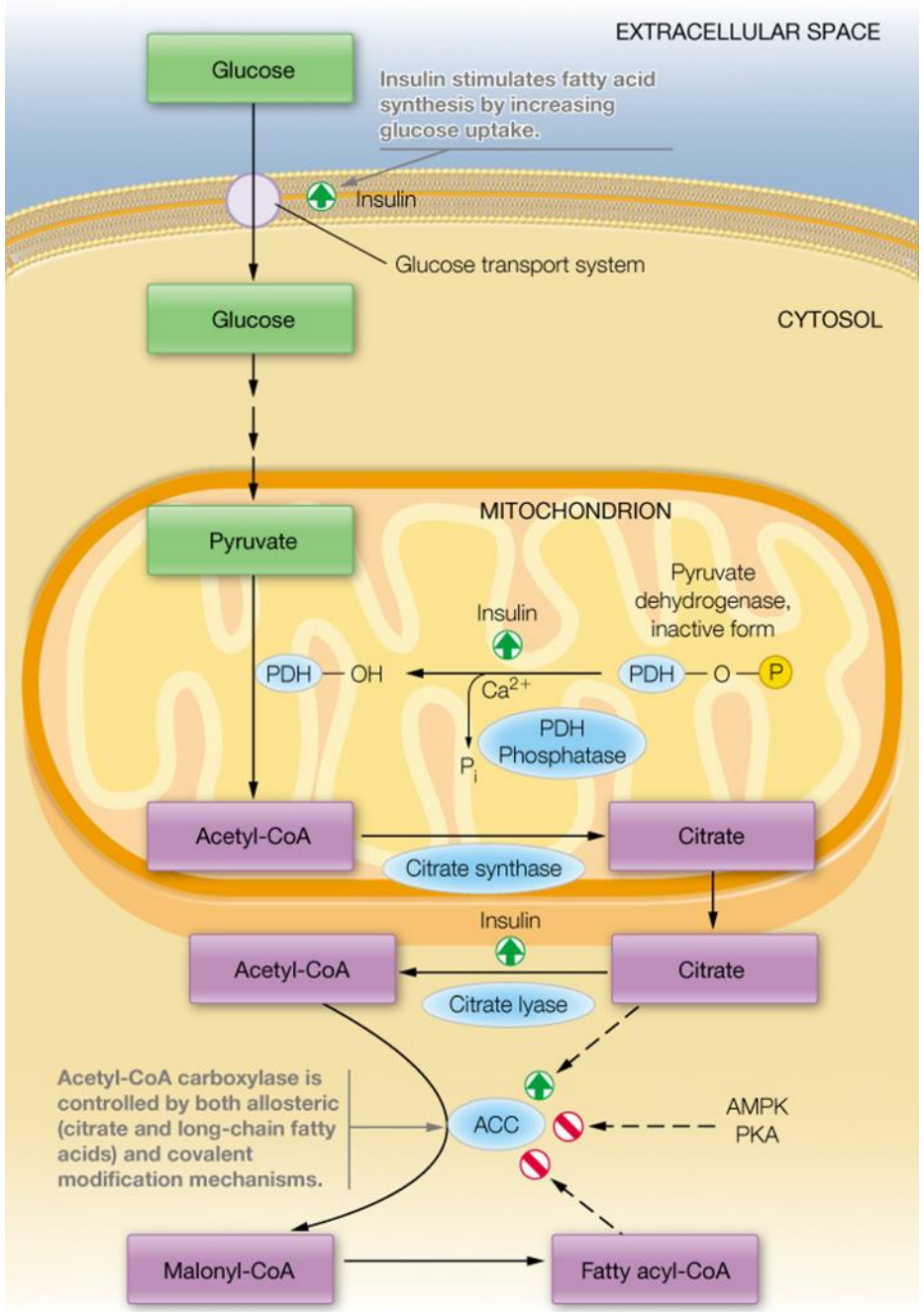
Summary of Lipid Metabolism

Summary of lipid metabolism



Control of Fatty Acid Synthesis

- **Insulin** promotes glucose uptake, promotes phosphorylation (activation) of pyruvate dehydrogenase, citrate lyase, acetyl-CoA carboxylase (ACC)
- AMP-activated protein kinase promotes phosphorylation of acetyl-CoA carboxylase (inhibition) under low energy charge conditions
- Citrate (activation) and long-chain fatty acids (inhibition) are also allosteric modulators of ACC
- **Glucagon** and **epinephrine** activate protein kinase A (PKA), which phosphorylates and inhibits the enzyme.



Regulation of fatty acid biosynthesis and degradation

1. Control of fatty acid biosynthesis

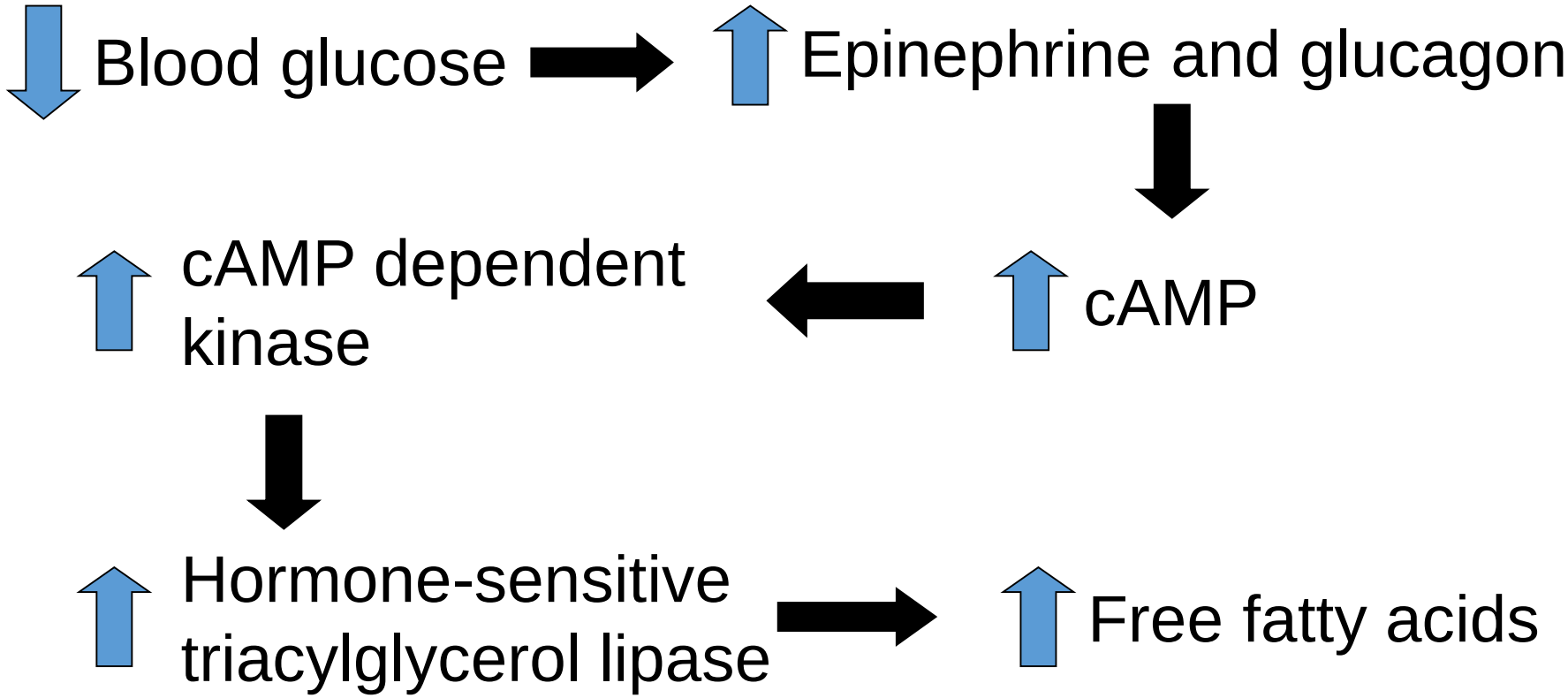
- **citrate** activates **ACC** (**acetyl-CoA carboxylase**) leading to increase malonyl-CoA formation through protein polymerisation (vertebrates only)
- Plants ACC activated by increase in pH and Mg^{2+}
- ACC is inhibited by C16:0 (palmitate)
- ACC is regulated by glucagon and epinephrine through protein phosphorylation-inactivation
  - **Glucagon** activates lipases and inhibits ACC
- ACC levels decrease during fasting
  - However, **insulin** inhibits lipases and activates ACC

Regulation of fatty acid biosynthesis and degradation

2. **β-oxidation is regulated by**
   - substrate supply - triacylglycerol hydrolysis-hormone sensitive
     - **Glucagon** activates lipases
   - malonyl-CoA through inhibition of carnitine palmitolyl transferase I: promotes lipid synthesis
   - increase in the NADH/NAD^+ ratio inhibits β-hydroxylacyl-CoA dehydrogenase
   - high concentrations of acetyl-CoA inhibit thiolase

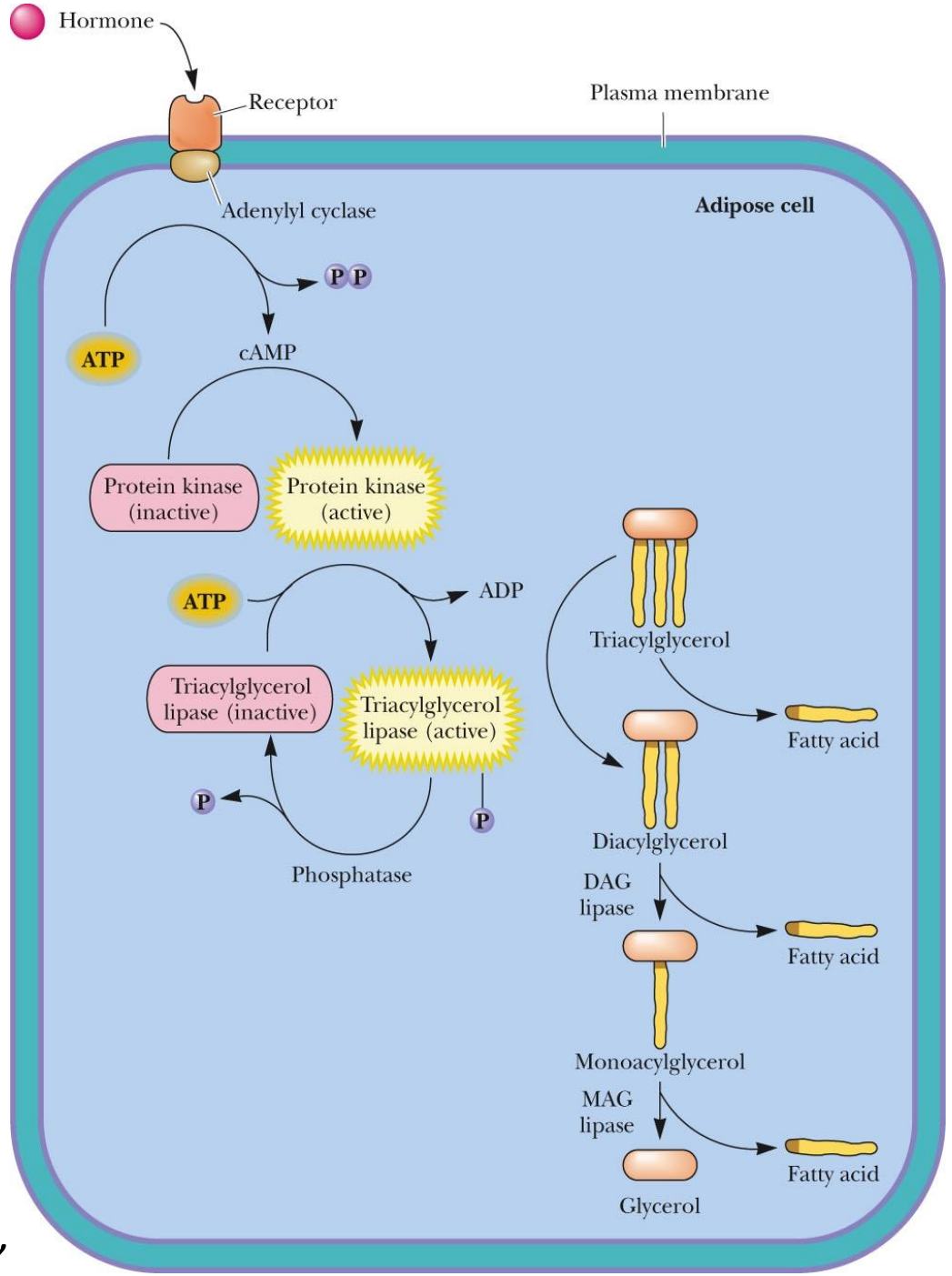
Mobilization of fat stores (recap)

- Hormones signal the need for fuel by tissues
  - skeletal muscle, heart, renal cortex



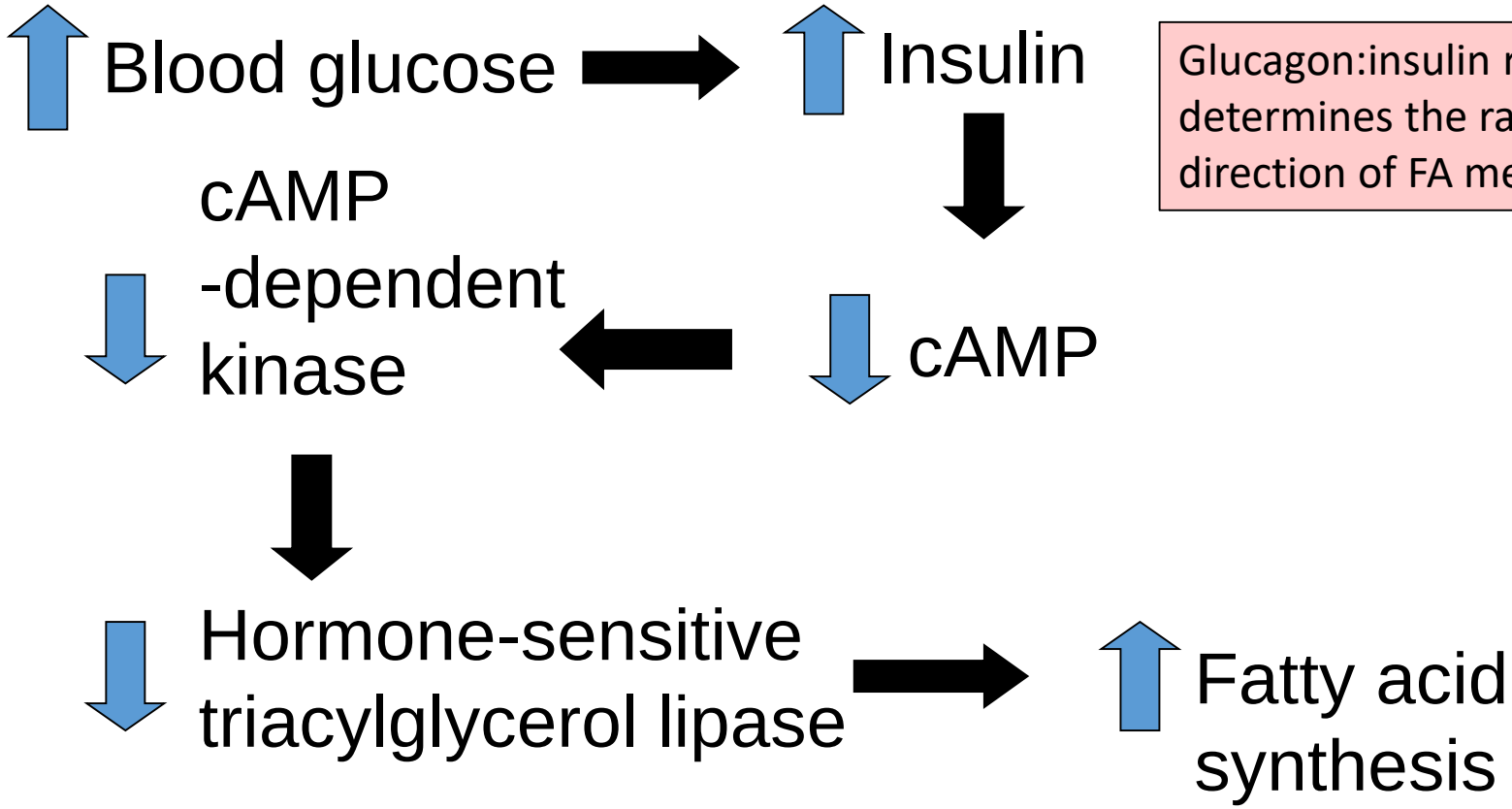
Liberation of fatty acids from diet and adipose tissue (recap)

- 2 ATPs required for lipase activation
- Fatty acid release from triacylglycerols



Insulin has the opposite effect of *glucagon*

- Hormones signal the need for fuel by tissues
  - skeletal muscle, heart, renal cortex



Glucagon:insulin ratio determines the rate and direction of FA metabolism

Sites of regulation of fatty acid metabolism

- Organs and tissues networked by the blood stream

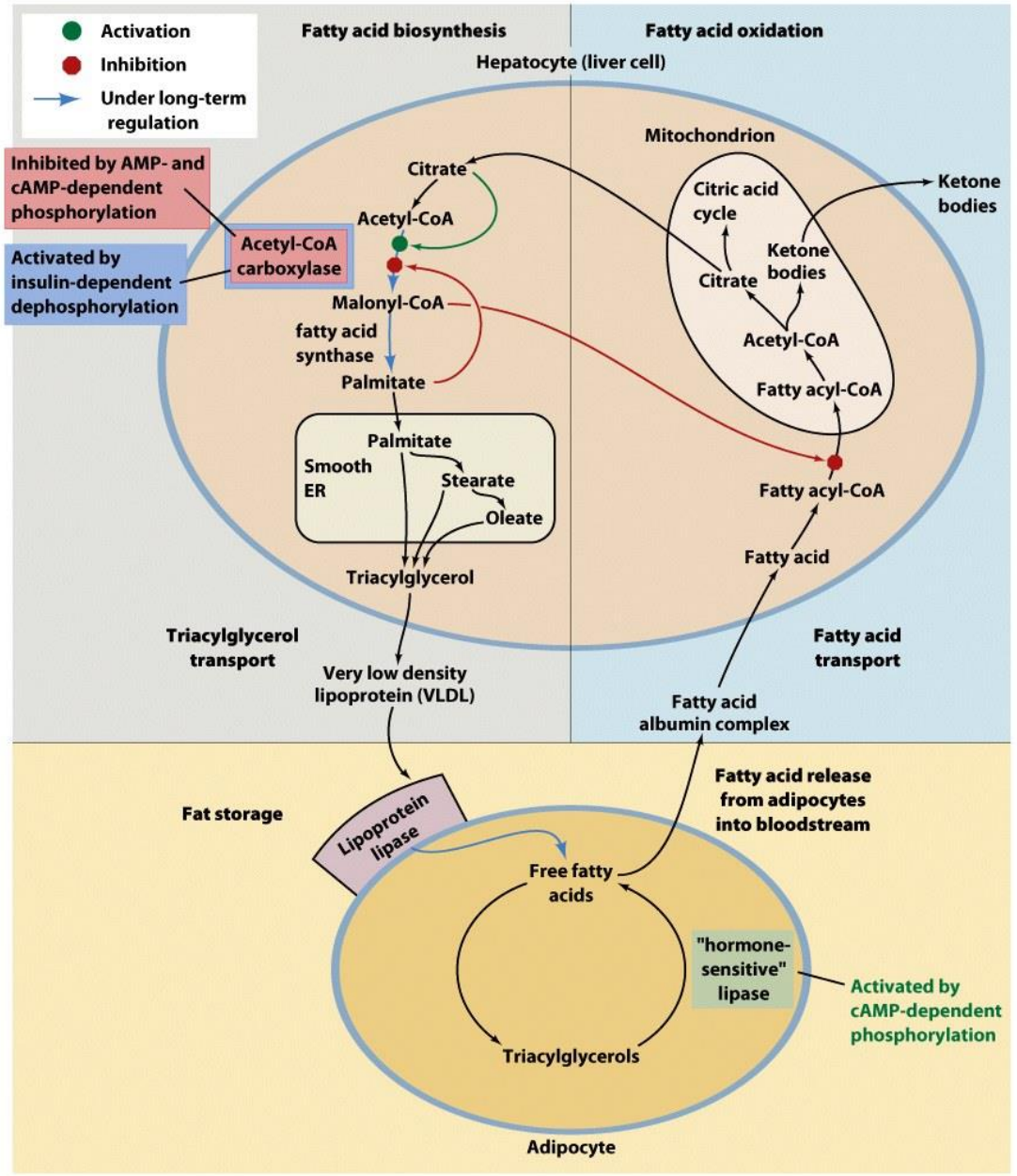


Figure 20-31

Summary

- Fatty acids from either the diet or adipose tissue are transported via the blood stream to the heart, muscle or other tissues for fuel
- Once inside the cell as CoA esters are translocated into the mitochondrial matrix by a carnitine shuttle
- Four reactions are required for β-oxidation resulting in the formation of $FADH_2$, NADH and acetyl-CoA
- $FADH_2$ and NADH are converted to ATP by the respiratory chain

Summary contd.

- acetyl-CoA can be
  - oxidized via the citric acid cycle to produce more reducing equivalents for ATP production
  - used as biosynthetic precursors
  - used to make **ketone bodies** as alternative fuels
- Fatty acid biosynthesis and degradation are tightly controlled

Chapter 16 Summary (1 of 5)

- Triacylglycerols (neutral fats) are high-energy fuel molecules that derive from three different sources:
  1) the diet
  2) *de novo* biosynthesis (liver)
  3) storage depots in adipocytes
- Dietary triacylglycerols are emulsified in the small intestine and broken down into free fatty acids and other products, which are both taken up by the intestinal mucosa and then reconverted into triacylglycerols

Chapter 16 Summary (2 of 5)

- Triacylglycerols combine with apolipoproteins and other lipids to form chylomicrons, which travel through the lymphatic system and bloodstream to tissues, where the triacylglycerols in chylomicrons are broken down into fatty acids and glycerol, before fatty acids are transported into tissue cells
- Triacylglycerols stored in adipose tissues are hydrolyzed in three steps to fatty acids and glycerol in a process tightly regulated by hormones, before fatty acids can be taken by tissue cells from the bloodstream

Chapter 16 Summary (3 of 5)

- Fatty acids entering the cytosol of tissue cells are converted to fatty acyl-CoA and diffuse or are transported into the mitochondria
- The β-oxidation pathway in the mitochondria breaks down most fatty acids in four enzymatic steps to acetyl-CoA units
- Under conditions, in which further oxidation of acetyl-CoA through the citric acid cycle is limited, acetyl-CoA is used to synthesize ketone bodies

Chapter 16 Summary (4 of 5)

- Fatty acid synthesis occurs via the stepwise addition of two-carbon fragments originated from malonyl-CoA using seven different catalytic activities, which are in eukaryotic cells performed by a multienzyme complex (megasynthase)
- Triacylglycerols are formed by transferring the fatty acyl groups to the hydroxyl groups of glycerol 3-phosphate and diacylglycerol
- Steroids, including cholesterol, are synthesized from acetoacetyl-CoA and acetyl-CoA via C_5, C_{10}, C_{15}, and C_{30} (squalene) intermediates

Chapter 16 Summary (5 of 5)

- Cyclization of squalene results in the formation of cholesterol, the precursor for all steroid hormones
- Orchestrated by the liver, cholesterol (and other lipids) is combined with apolipoproteins into various forms of packages (HDL, LDL, IDL, VLDL, etc.)
- LDLs (low-density lipoproteins) are the major form of cholesterol transport to peripheral tissues
- Specific receptors in cellular membranes bind LDLs and take them up via endocytosis, before LDLs are degraded inside the cell and the cholesterol cargo is released