Session 4
Lecture 1
Oxidative Phosphorylation

Dr. Khawla A. Shemran

Oxidative Phosphorylation (Stage 4 of Metabolism)

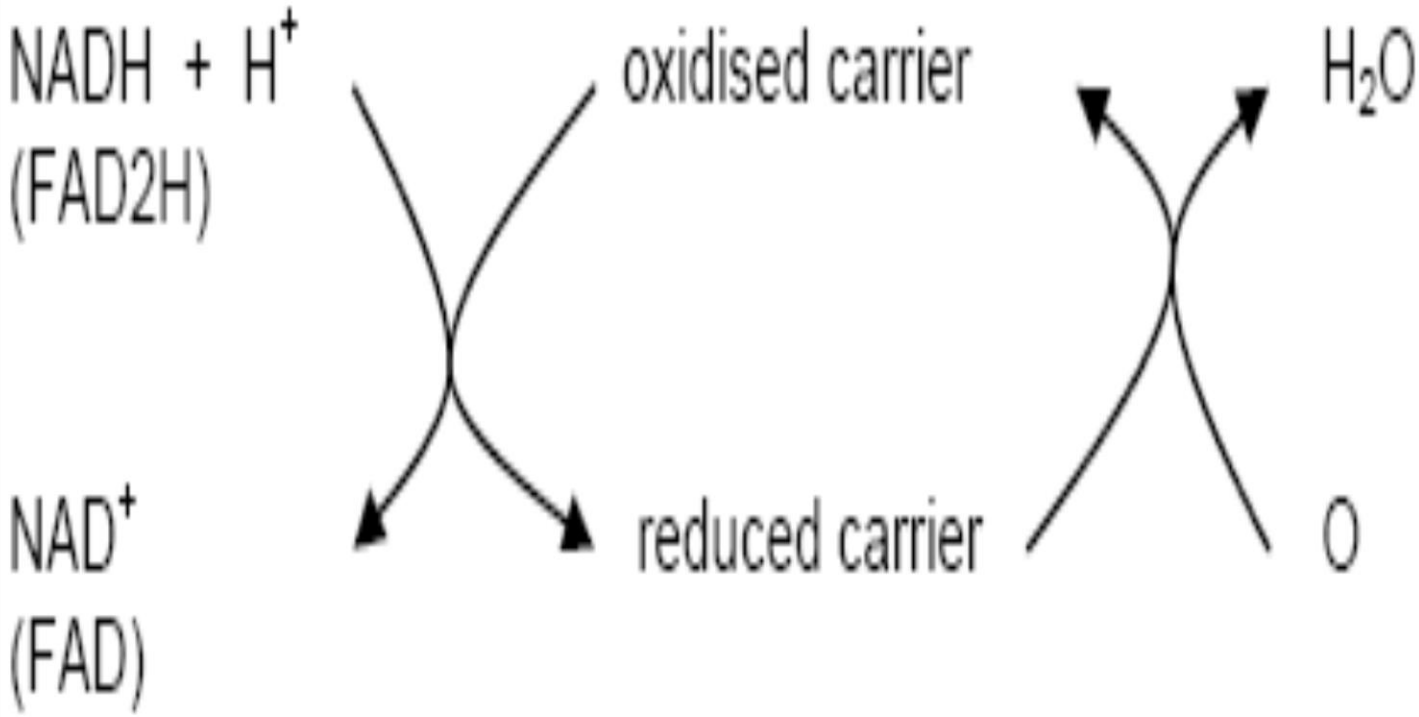
- The complete oxidation of glucose can be represented by the following equation:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$$

$$\Delta Go = -\ 2{,}870\ kJ/mole$$

- Till now only 2ATP and 2GTP are formed from oxidation of glucose which equal to 124 kJ/mole
- The question is Where has all the energy gone?
- The answer is that energy is still carried by the carriers of the reducing equivalents (NADH & FADH2)

Consider the energy liberated from oxidation of these carriers:



$NADH + H^+ + O \rightarrow NAD^+ + H_2O$ $\Delta G^\circ = -220$ kJ/mole

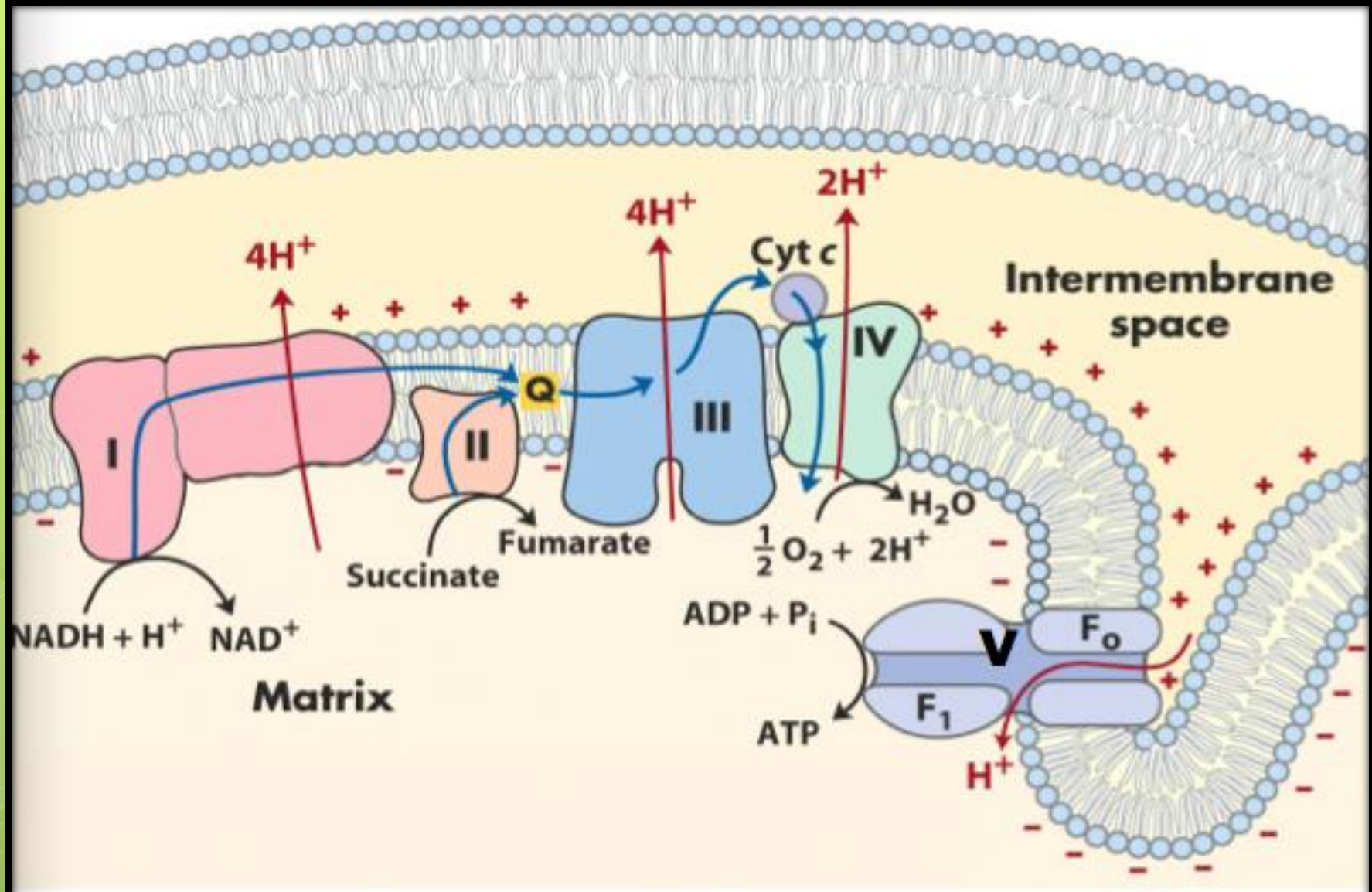
$FAD2H + O \rightarrow FAD + H_2O$ $\Delta G^\circ = -152$ kJ/mole

Oxidation of NADH and FADH2

- It is carried out in a pathway called oxidative phosphorylation (OP)
- OP represents 2 processes, the first is the oxidation of NADH and FADH2 in the electron transport chain and the second phosphorylation of ADP to produce ATP
- The energy liberated from the oxidation of the carriers of the reducing equivalents was used for phosphorylation of ADP to produce ATP

Electron Transport Chain (ET)

- **complex I** (NADH-Q oxidoreductase): oxidizes NADH and transfers electrons to coenzyme Q
- **coenzyme Q** (Ubiquinone)
- **complex II** (Succinate-Q reductase): oxidizes $FADH_2$ and transfers electrons to coenzyme Q
- **complex III** (Q-cytochrome c oxidoreductase): passes the electrons to cytochrome c
- **complex IV** (Cytochrome c oxidase): passes the electrons to O_2
- **Comlex V** (ATP synthase): production of ATP.



Note: Complex II contains succinate dehydrogenase (citric acid cycle)

- Three of the complexes (I, III and IV) in addition to transferring electrons, also act as **proton translocating complexes**.

- They use the **free energy** to move protons from the **inside** to the **outside** of the inner mitochondrial membrane. The membrane itself is impermeable to protons

- The proton translocating complexes transform the chemical bond energy of the electrons into an electro-chemical potential difference of protons. **This is known as the *proton motive force* (p.m.f).**

How does ATP synthesize after oxidation of NADH and FADH2?

- ATP hydrolysis results in the release of energy:

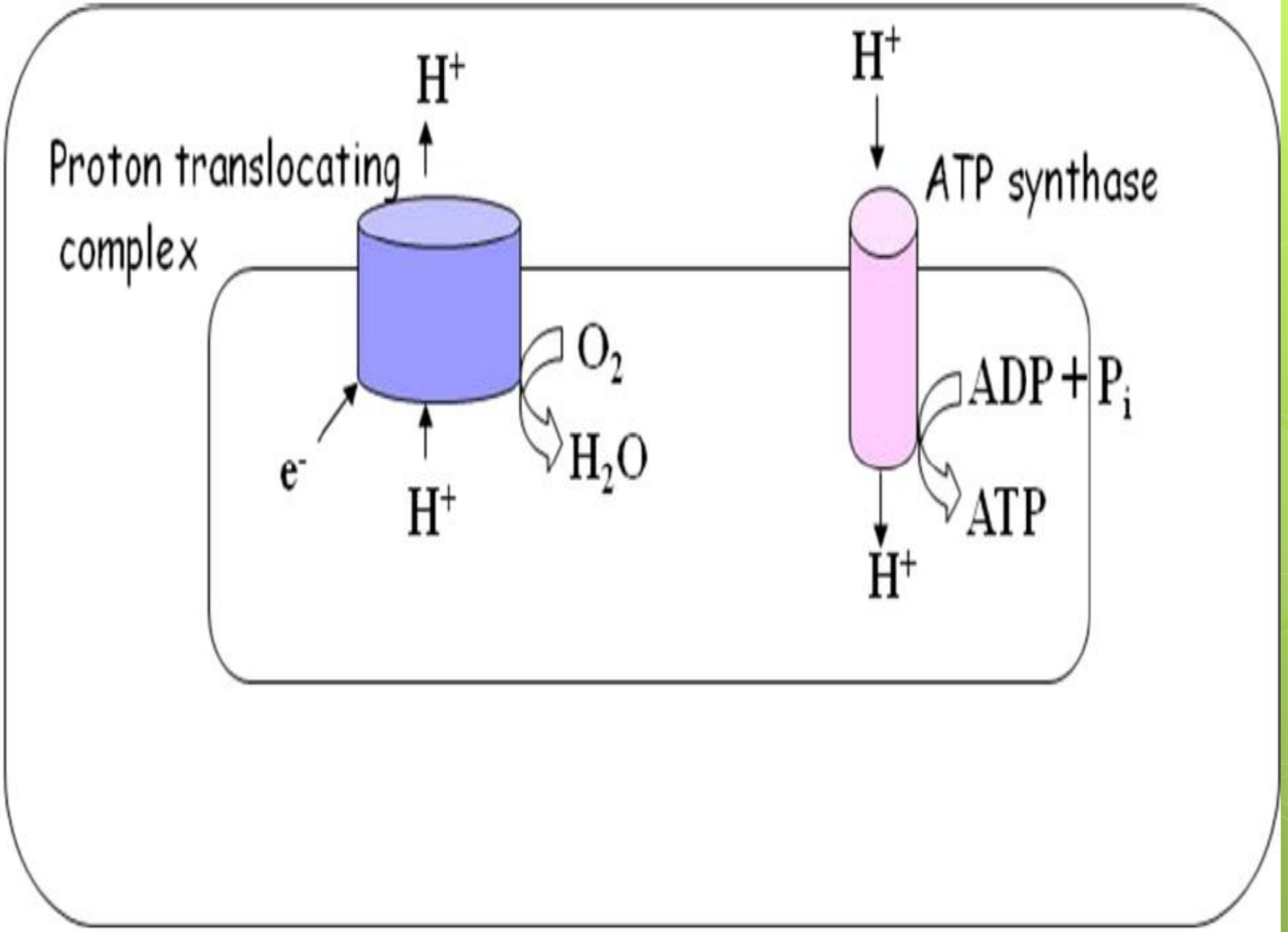
$$ATP + H_2O \rightarrow ADP + Pi \quad \Delta Go = -31 \text{ kJ/mole}$$

- This means that ATP synthesis from ADP and Pi requires +31 kJ/mole to drive the reaction

- This amount is derived from the p.m.f that has been produced across the inner mitochondrial membrane .

- Protons can normally only re-enter the mitochondrial matrix via the ATP synthase complex, driving the synthesis of ATP from ADP and Pi as in the following:

$$2H^+_{outside} + ADP + Pi \rightarrow 2\ H^+_{inside} + ATP$$


H+
Proton translocating complex
H+
ATP synthase
O2
ADP + Pi
e-
H2O
H+
ATP
H+

Coupling between electron transport (ET) and ATP synthesis

Normally ET and ATP synthesis are tightly coupled.

The mitochondrial concentration of ATP **regulates** both processes. **When [ATP] is high**:

- • The [ADP] is **low** and the ATP synthase stops (lack of substrate).
- • This prevents the transport of protons back into the mitochondria.
- • The [H^+] outside increases to a level that prevents more protons being pumped.
- • In the absence of proton pumping, the electron transport stops.
- The reverse occurs **when [ATP] is low.**

Inhibitors of OP

- Uncouplers
- Inhibitors of electron transport
- Inhibitors of ATP synthase

Uncouplers

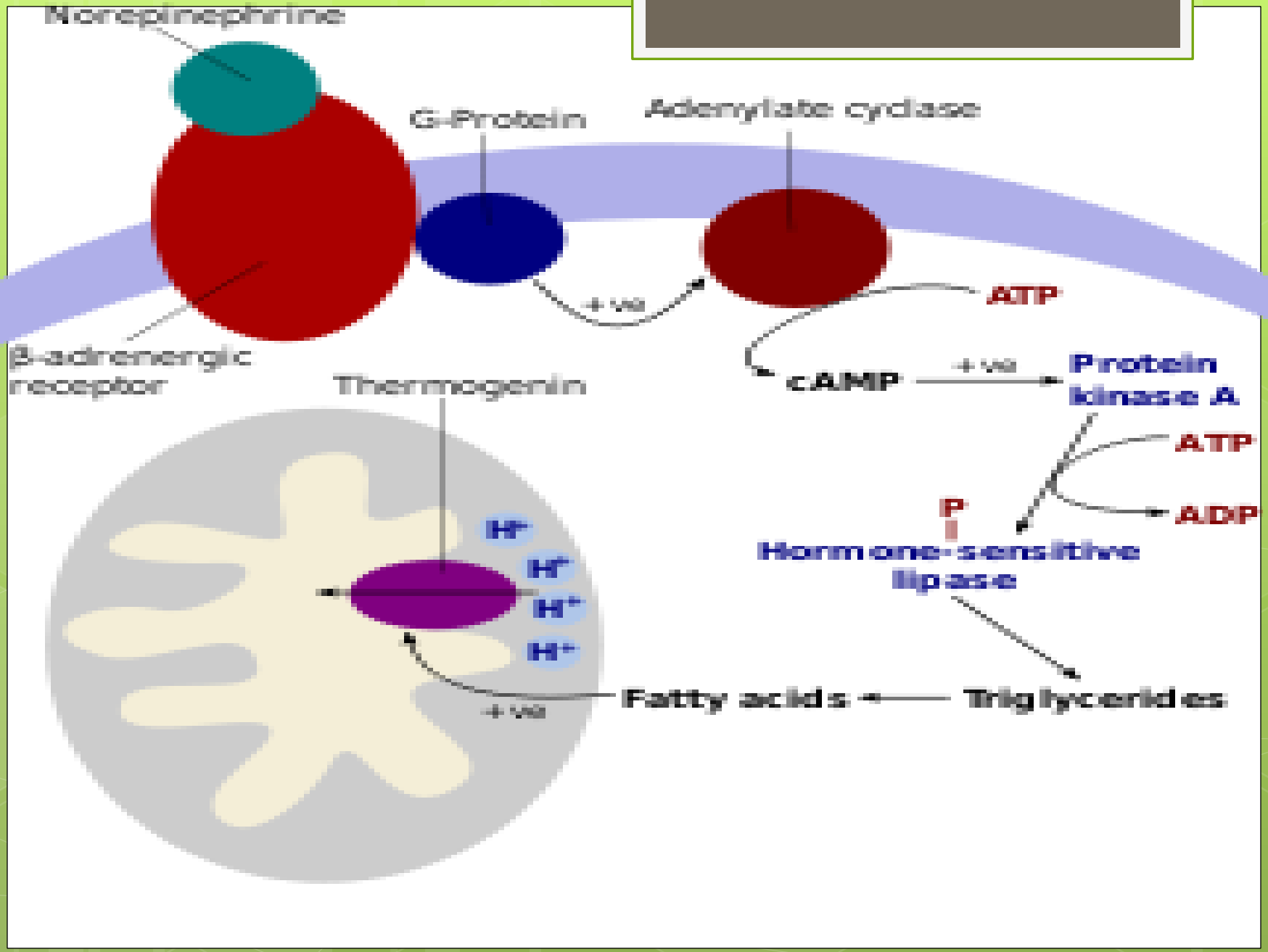
- Some synthetic substances like **dinitrophenol** and **dinitrocresol** increase the permeability of the inner mitochondrial membrane to protons
- ET continues, ATP synthesis stops and energy is dissipated as heat (**thermogenesis**).
- **Uncoupling proteins** (UCP1 to UCP5) are identified in tissues to produce heat.

Uncoupling proteins

- UCP1-3 are important to induce a leak of protons across the membrane, reducing the p.m.f and inhibiting ATP synthesis.
- Proton leak is physiologically important and accounts for 20-25% of the basal metabolic rate.
- UCP1 (previously known as thermogenin) is expressed in brown adipose tissue and is involved in non-shivering thermogenesis, which enables mammals to survive in cold environments.

Uncoupler Proteins

- In response to cold, noradrenaline is released and stimulates lipolysis releasing fatty acids to provide fuel for oxidation in brown adipose tissue
- Fatty acids breakdown produce FADH2 and NADH, driving ET and increasing the p.m.f. Noradrenaline also activates UCP1 to inhibit ATP synthesis, which dissipates the p.m.f as heat.
- UCP3 has been found in skeletal muscle, brown adipose tissue and the heart and it appears to be involved in modifying fatty acid metabolism


Norepinephrine
G-Protein
Adenylate cydase
β-adrenergic receptor
+ Vd
ATP
cAMP
+ Vd
Protein kinase A
ATP
ADP
P
Hormone-sensitive lipase
Thermogenin
H+
H+
H+
H+
+ Vd
Fatty acids
Triglycerides

Inhibitors of Electron Transport

- Carbon monoxide, cyanide, rotenone and antimycin are inhibitors for components of ET.
- Without the p.m.f, ATP cannot be synthesized and no heat is generated. Irreversible cell damage rapidly occurs

Oxidative stress

- During oxidative phosphorylation, **reactive oxygen species** are produced as a by-product.
- Mitochondria produce superoxide radicals ($O2^{-}$ $^{\bullet}$) which may be converted to hydrogen peroxide (H_2O_2) .
- Some superoxide radicals react with nitric oxide to form peroxynitrite (ONOO-) and hydroxyl radicals (•OH).
- These highly reactive agents can cause **damage to cells**, particularly to membranes.
- Some cells, such as **neutrophils** release large amounts of ROS to destroy bacteria

FUEL STORAGE AND LIPID METABOLISM

SESSION 4
LECTURE 2

DR. KHAWLA A. SHEMRAN

Glucose storage (glycogen metabolism)

- some tissues require a continuous supply of glucose. Initially this is met by storing glucose (as glycogen).
- If the period between meals is long to deplete the stored glycogen (8-12 hr.) then glucose has to be synthesized by the process of **gluconeogenesis**.
- Glycogen is a highly branched polymer of glucose linked together by **glycosidic** bonds of two types, **a-1-4** and **a-1-6** . The **a-1-6** bonds are the branch points.
- Glycogen is a large molecule that is stored in liver and skeletal muscle

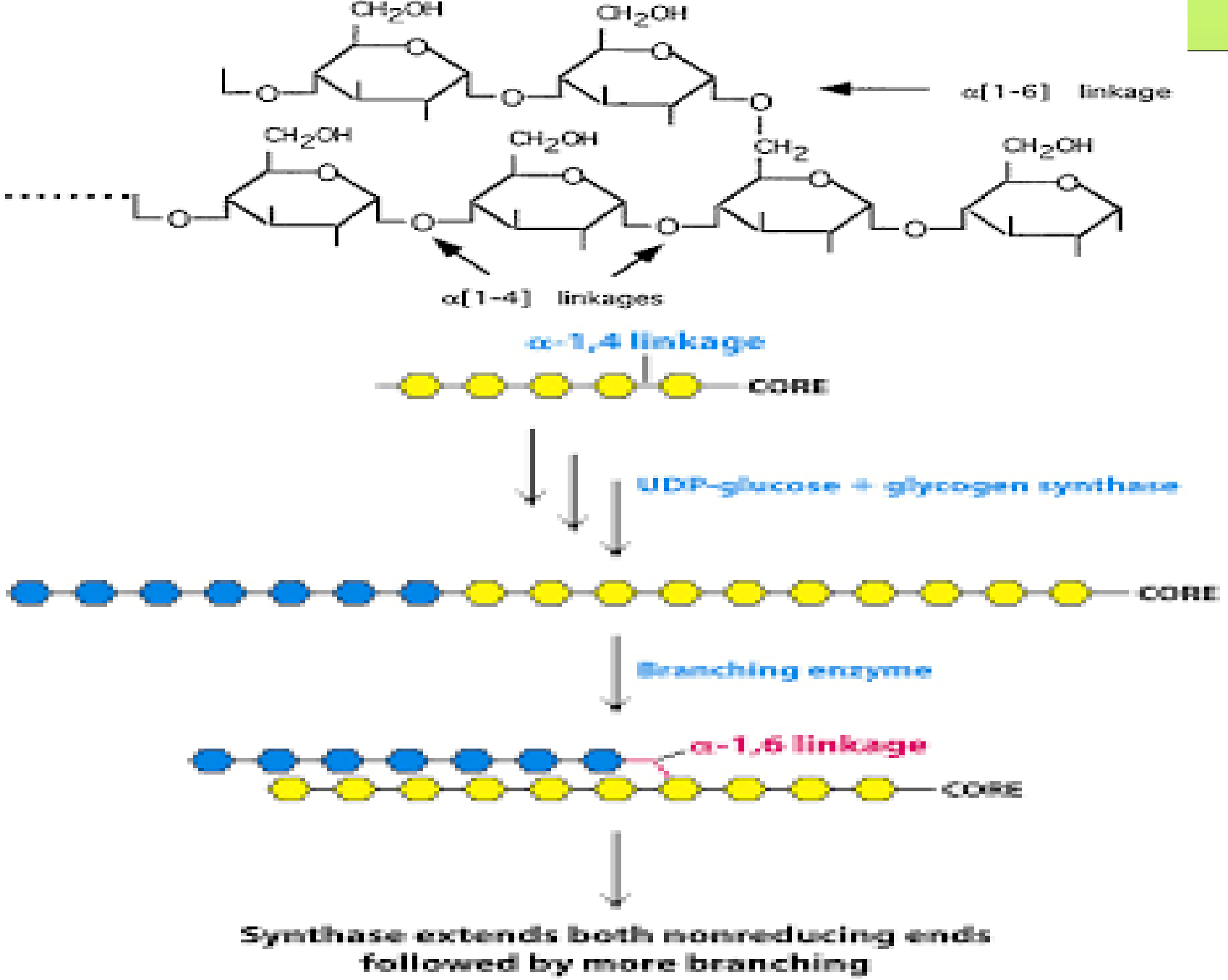

α[1–6] linkage
α[1–4] linkages
α-1,4 linkage
CORE
UDP–glucose + glycogen synthase
CORE
Branching enzyme
α-1,6 linkage
CORE
Synthase extends both nonreducing ends
followed by more branching

Figure 21.17

Glycogen synthesis (glycogenesis)

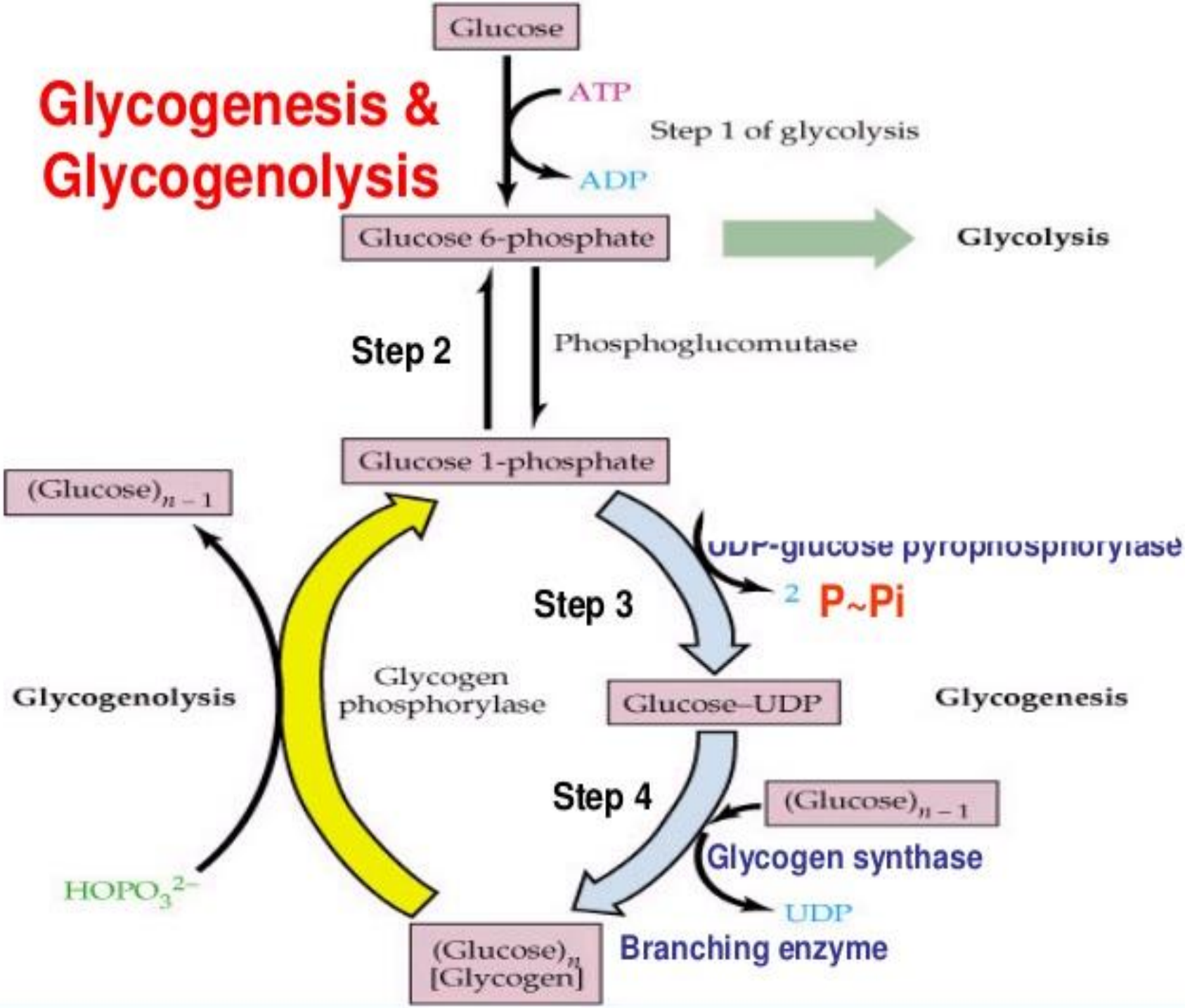
1-Glucose + ATP → glucose 6-P + ADP catalyzed by *hexokinase* (*glucokinase in liver*)

2. Glucose 6-P ↔ Glucose 1-P catalysed by *phosphoglucomutase*

3. Glucose 1-P + UTP + H2O → UDP-glucose + 2Pi

4. Glycogen (n residues) + UDP-glucose → glycogen (n + 1 residues) + UDP

This irreversible reaction is catalyzed by two enzymes, *glycogen synthase and branching enzyme*.

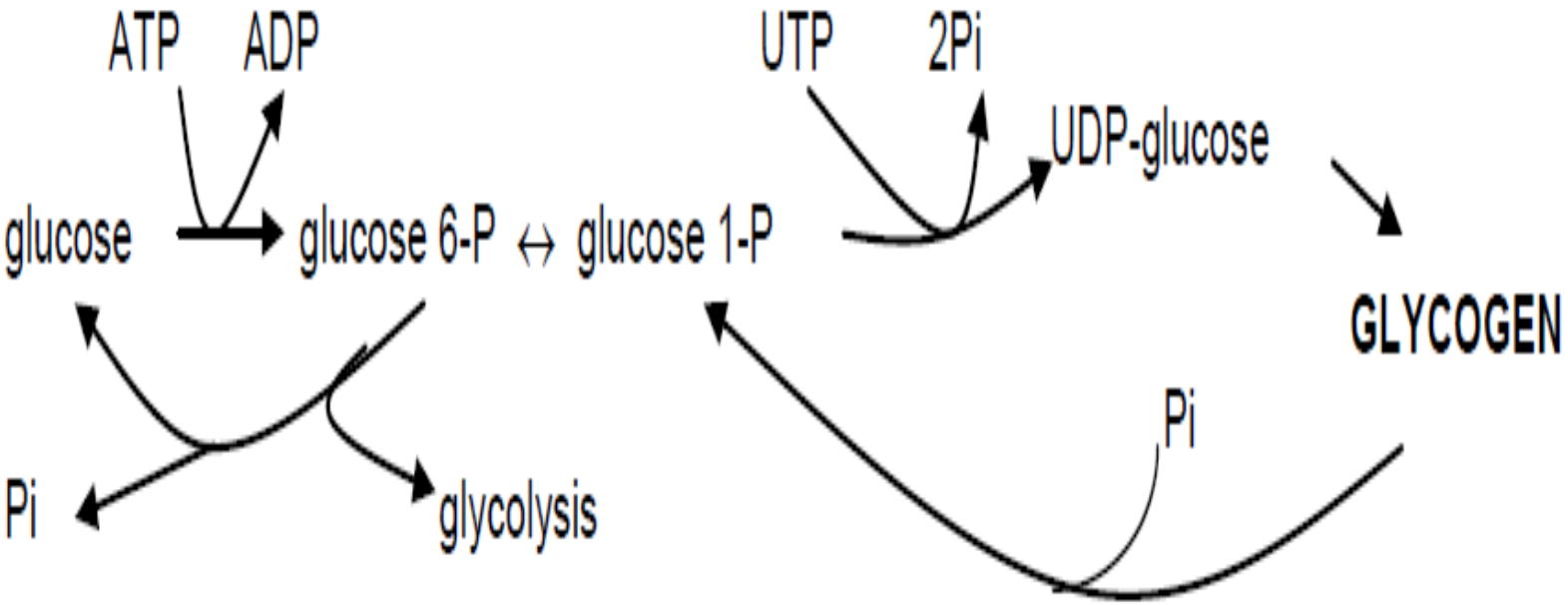

Glycogenesis &
Glycogenolysis
Glucose
ATP
Step 1 of glycolysis
ADP
Glucose 6-phosphate
Glycolysis
Step 2
Phosphoglucomutase
Glucose 1-phosphate
(Glucose)n – 1
UDP-glucose pyrophosphorylase
2 P~Pi
Step 3
Glycogenolysis
Glycogen
phosphorylase
Glucose–UDP
Glycogenesis
Step 4
(Glucose)n – 1
Glycogen synthase
UDP
HOPO3 2–
(Glucose)n
[Glycogen]
Branching enzyme

Glycogen degradation (glycogenolysis)

- Glycogen is degraded in skeletal muscle in response to exercise and in the liver in response to fasting or as part of the stress response.
- The complete degradation of glycogen can be represented by the equation:

Glycogen (n residues) + nPi → 0.9n glucose 6-phosphate + 0.1n glucose

Overview of glycogen metabolism



Regulation of glycogen metabolism

Glycogen synthase is inhibited by phosphorylation and .activated by de-phosphorylation

while *glycogen phosphorylase is activated by phosphorylation and inhibited by de-phosphorylation*.

Glucagon and adrenaline increase phosphorylation of both enzymes while insulin promotes their dephosphorylation.

Glycogen storage diseases

- increased or decreased amounts of glycogen which may cause:
- - tissue damage if excessive storage.
- -fasting hypoglycaemia (low blood glucose).
- - poor exercise tolerance.
- -glycogen structure may be abnormal.
- - usually liver and/or muscle are affected.

Classes of lipids

Are of 3 types

1. Fatty acid derivatives:

- Fatty acids – fuel molecules.
- Triacylglycerols – fuel storage
- Phospholipids – components of membranes and plasma lipoproteins
- Eicosanoids – local mediators

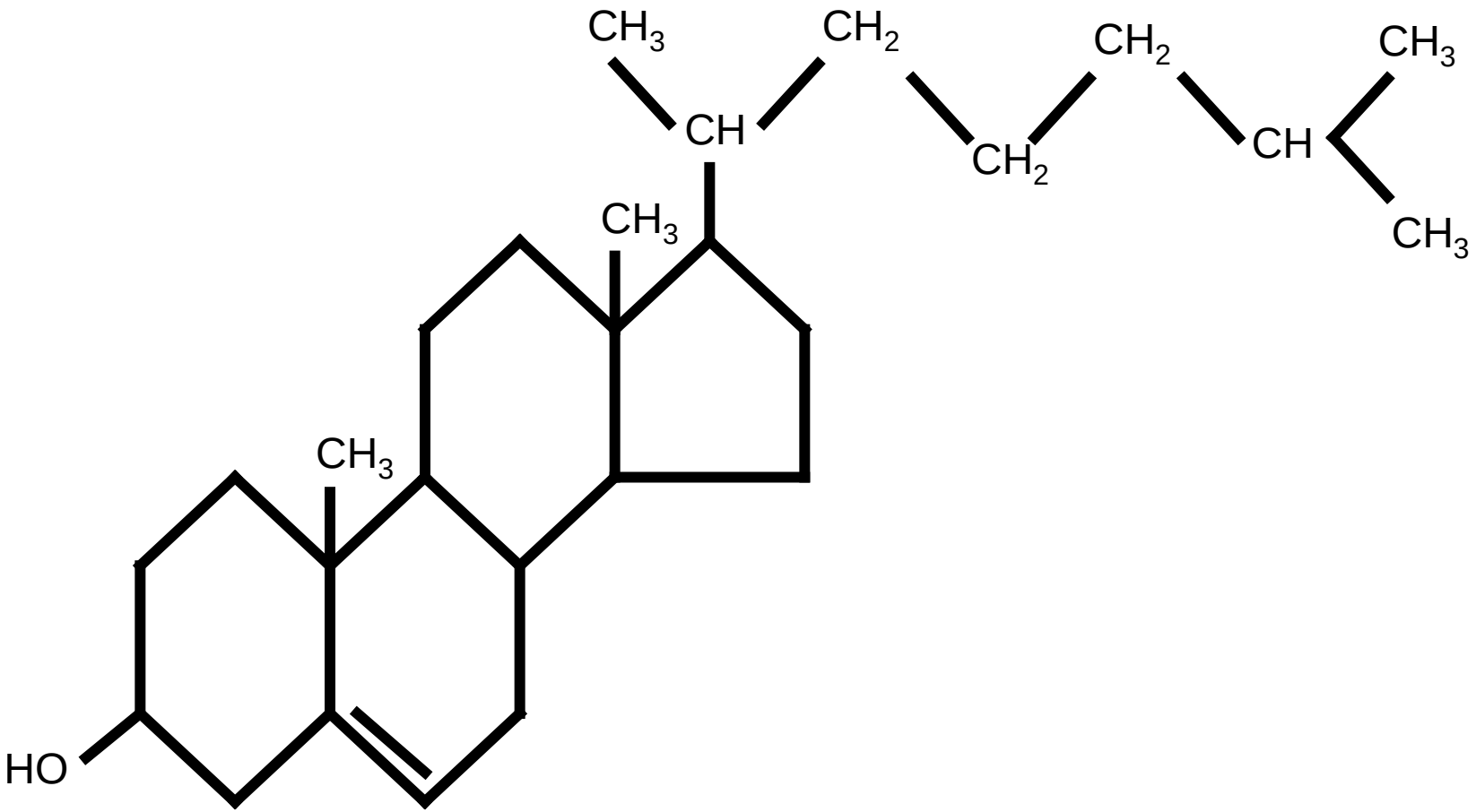
2. Hydroxy-methyl-glutaric acid derivatives (C6 compound):

- Ketone bodies (C4) – **water soluble** fuel molecules
- Cholesterol (C27) – membranes and steroid hormone synthesis
- Cholesterol esters – cholesterol storage
- Bile acids and salts (C24) – lipid digestion

3. Vitamins

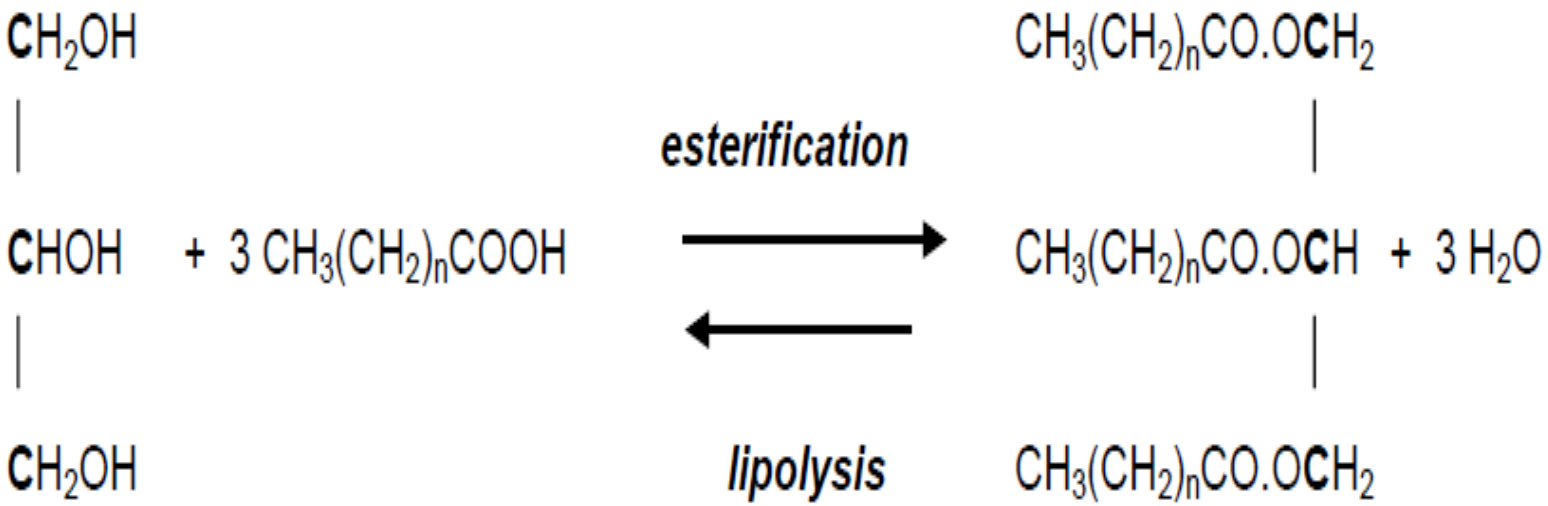
- A, D, E and K.

Cholesterol



Triacylglycerols

Triacylglycerols are the major dietary and storage lipid in the body. They consist of three fatty acids (usually long chain n=16) esterified to glycerol:

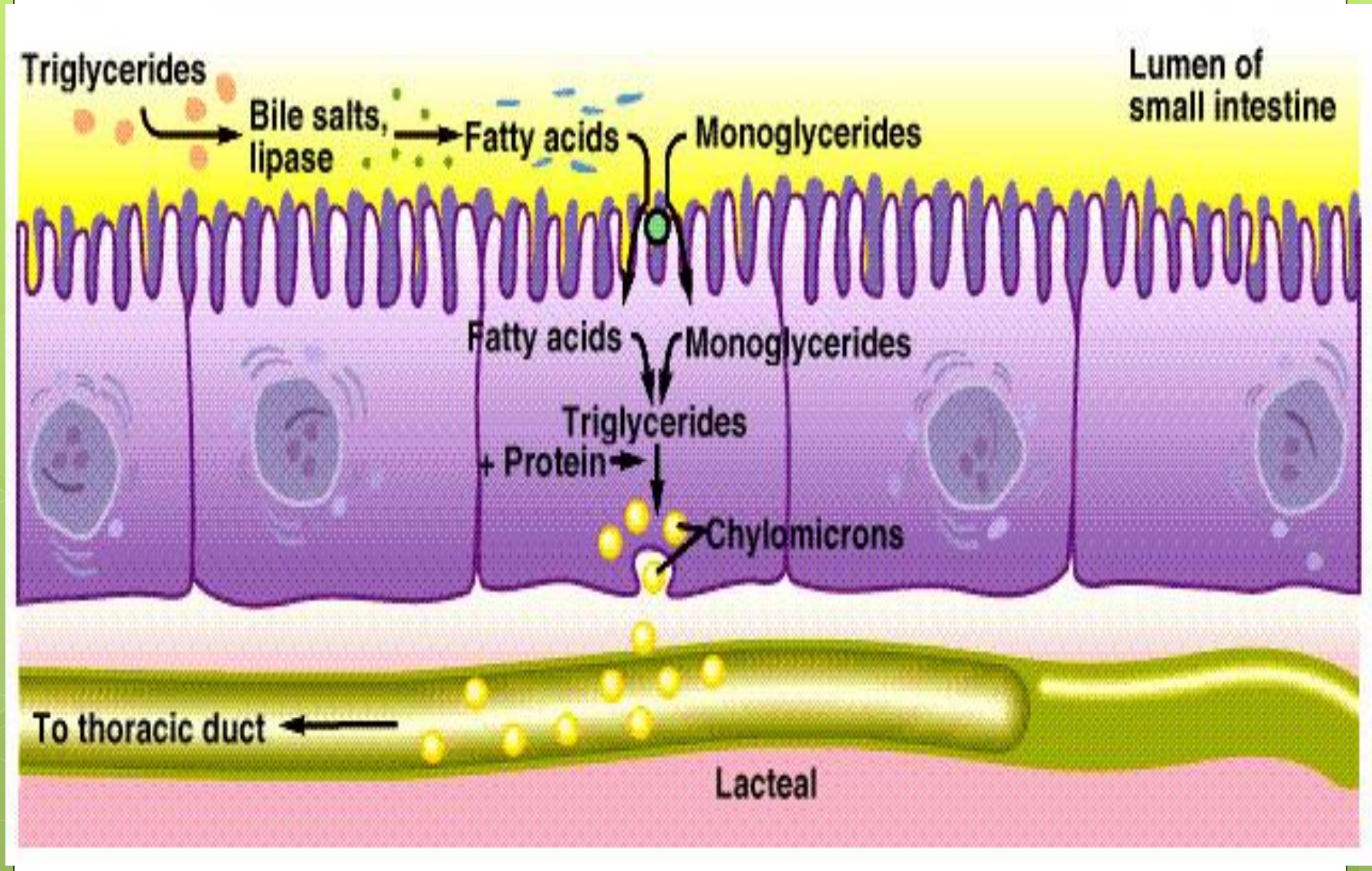


Stage:1 metabolism of triacylglycerols

-The major dietary lipids are triacylglycerols (butter, ghee, margarine, vegetable oils).

-These are hydrolyzed by **pancreatic lipase** in the small intestine to release glycerol and fatty acids.

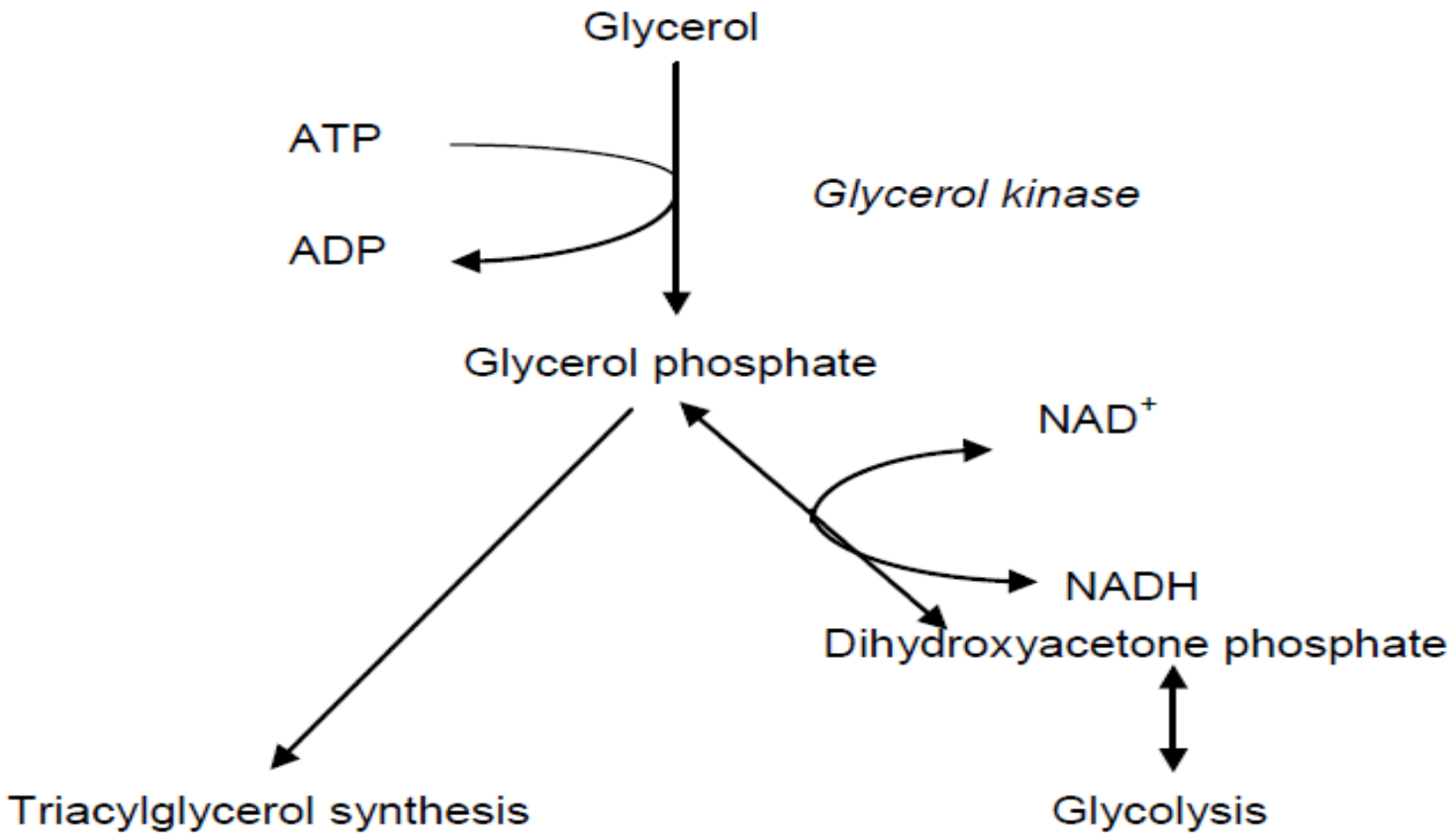
-This is a complex process that requires bile salts and a protein factor called **colipase.**


Triglycerides
Bile salts, lipase
Fatty acids
Monoglycerides
Lumen of small intestine
Fatty acids
Monoglycerides
Triglycerides + Protein
Chylomicrons
To thoracic duct
Lacteal

Glycerol metabolism

Triacylglycerols hydrolysis → Glycerol

And enters the blood stream and is transported to the liver where it is metabolized:



Fatty acids

- The most common fatty acids in the body are long-chain molecules that contain an even number of C atoms: $CH_3(CH_2)_nCOOH$ (n = 14 to18).

- They may be saturated or unsaturated (contain C=C double bonds). The saturated fatty acids are non-essential components of the diet .

- Certain polyunsaturated fatty acids (>1 double bond) are essential components of the diet . Arachidonic acid (C20:4) is an important polyunsaturated fatty acid as it is the starting point for the synthesis of the eicosanoids (prostoglandins).

Stage 2 catabolism of fatty acids

When the body is subjected to stress situations (aerobic exercise, starvation, lactation) adipose tissue triacylglycerols are hydrolysed by the enzyme ***hormone-sensitive lipase*** to release fatty acids and glycerol that diffuse from the tissue. This process is known as **lipolysis**.

It is activated by adrenaline, glucagon, growth hormone, cortisol and thyroxine and inhibited by insulin. Why?

- The fatty acids are carried to tissues via the blood stream bound non-covalently to albumin. The albumin-bound fatty acids are variously called free fatty acids (FFA).

- The glycerol is transported in the blood to the liver where it may be oxidised, converted to glucose or used in the synthesis of triacylglycerols.

- Many tissues including liver, heart muscle and skeletal muscle can use fatty acids as a source of energy.

- The process by which fatty acids are oxidised to release energy is known as β-oxidation and it occurs in mitochondria. Thus, cells such as red blood cells, central nervous system (brain and spinal cord) , cannot oxidise fatty acids.

Fatty acid activation

In order for fatty acids to be oxidised they have to be activated.

-by linking to coenzyme A (See Marks p109-111).

-This reaction requires ATP

- and is catalysed by ***fatty acyl CoA synthase***:

$$CH_3(CH_2)_nCOOH + ATP + CoA \rightarrow CH_3(CH_2)_nCO\sim CoA + AMP + 2\,Pi$$

fatty acid — fatty acyl~CoA

$$CH_3COOH + ATP + CoA \rightarrow CH_3CO\sim CoA + AMP + 2\,Pi$$

acetic acid — acetyl~CoA

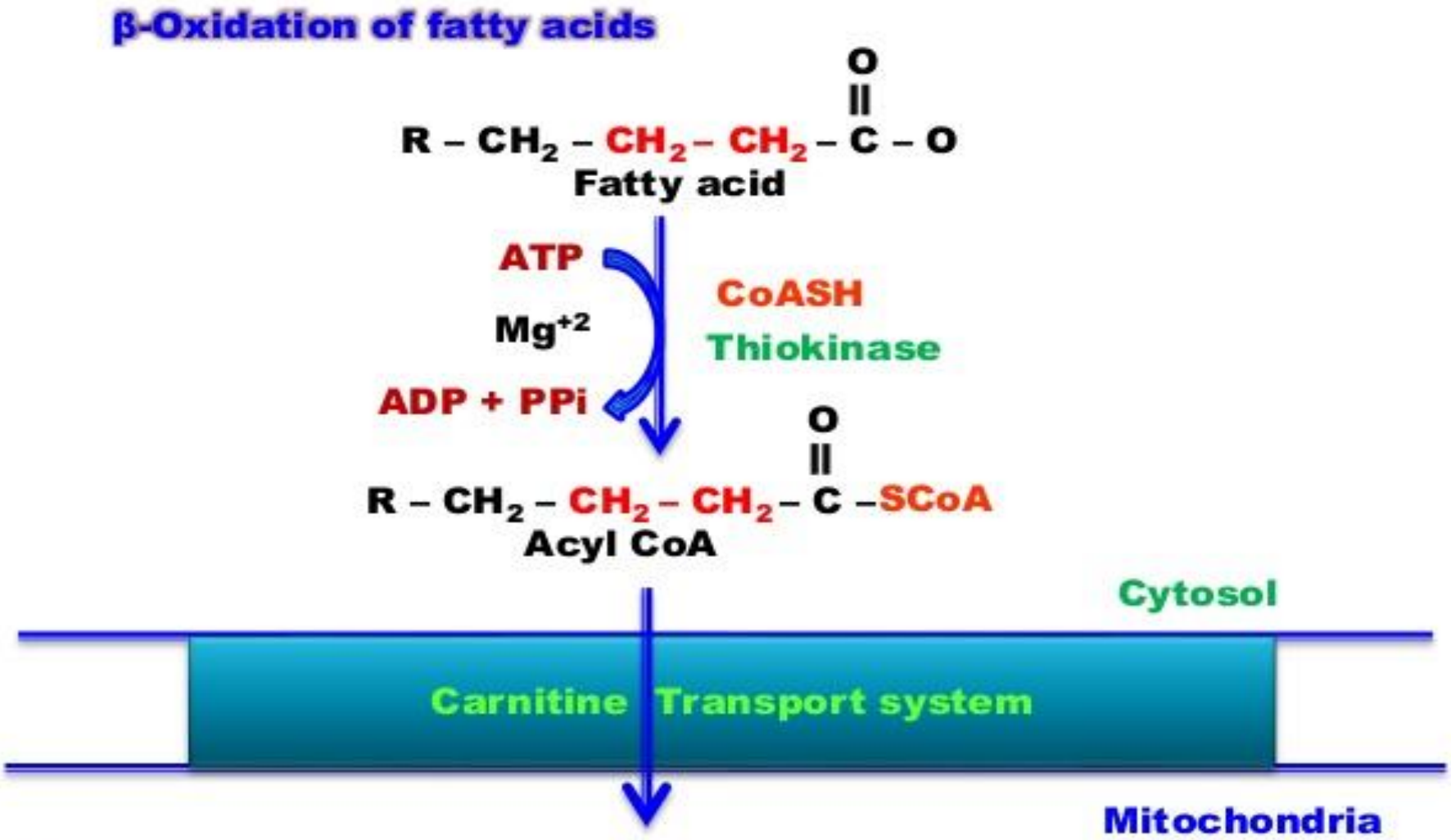
Stages of fatty acid oxidation

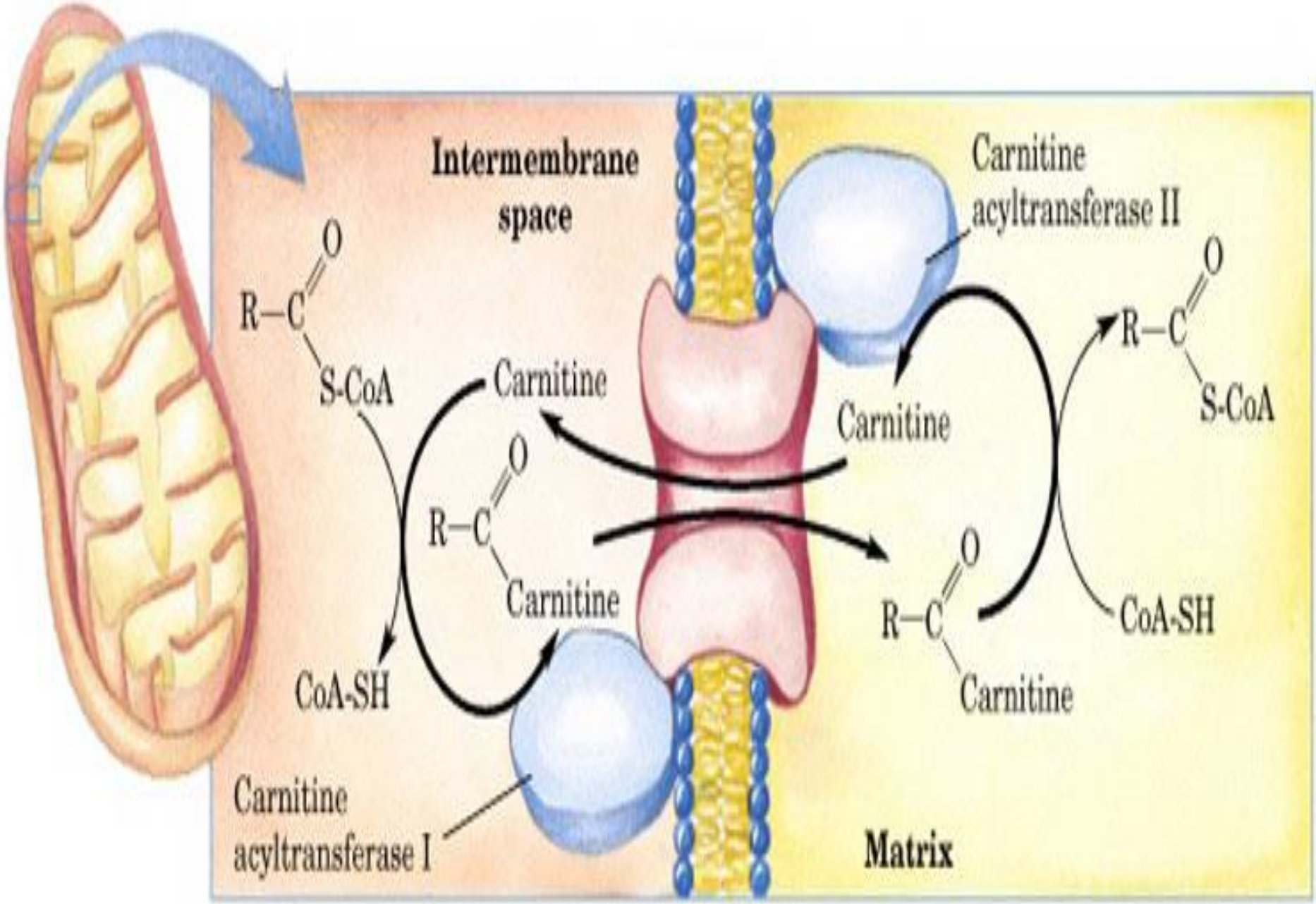
(1) Activation of fatty acids takes place **on the outer mitochondrial membrane**

(2) Transport into the mitochondria

(3) Degradation to two-carbon fragments (as acetyl CoA) **in the mitochondrial matrix (B-oxidation pathway)**

(1) Activation




Intermembrane space
Carnitine acyltransferase II
R—C
O
S-CoA
Carnitine
Carnitine
R—C
O
S-CoA
R—C
O
Carnitine
R—C
O
Carnitine
CoA-SH
CoA-SH
Carnitine acyltransferase I
Matrix

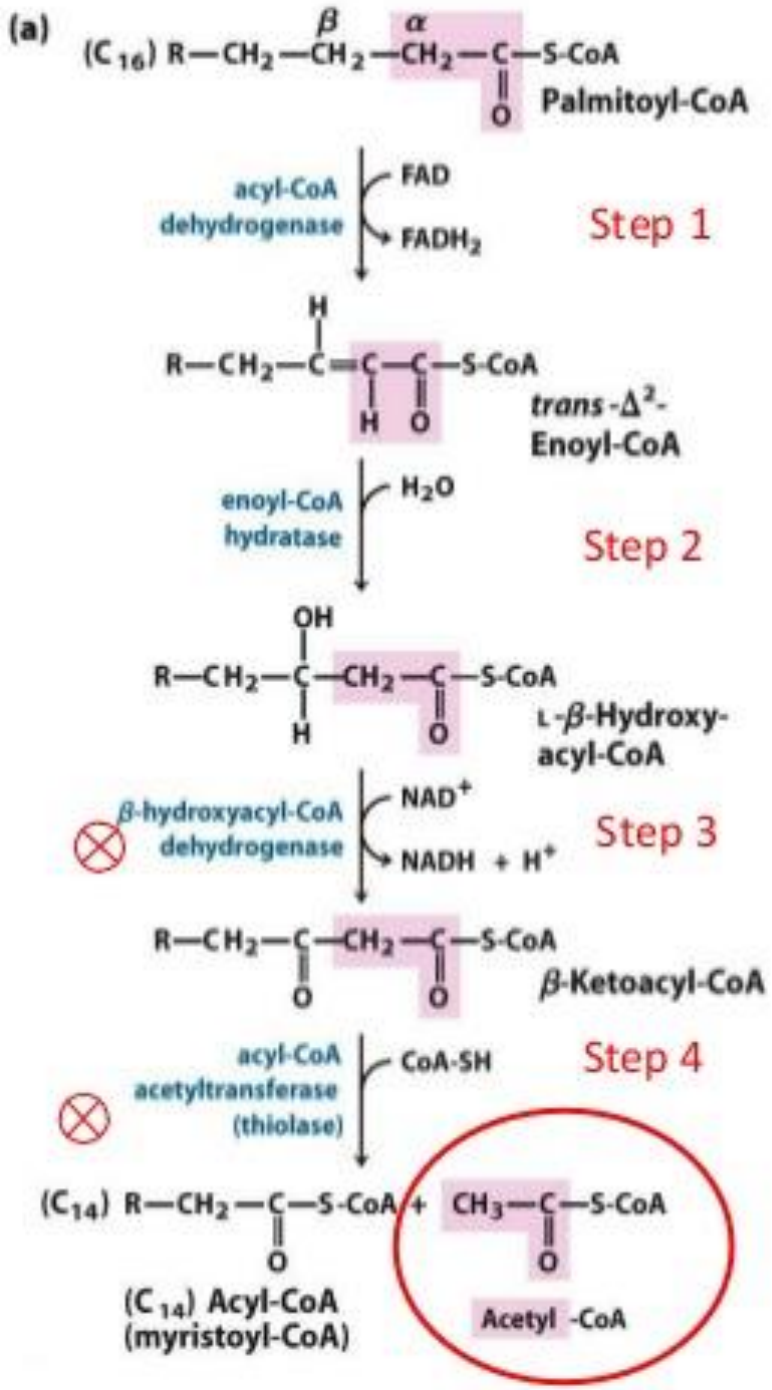
4 Steps of β-oxidation

1. Dehydrogenation of the fatty acyl-CoA to make a trans double bond between α and β carbon.
   - Short, medium, and long chain acyl-CoAdehydrogenases
   - e^- removed transferred to FAD
2. Hydration of the double bond

1. Dehydrogenation of the β-hydroxyl group to a ketone

   - e^- removed transferred to NAD^+

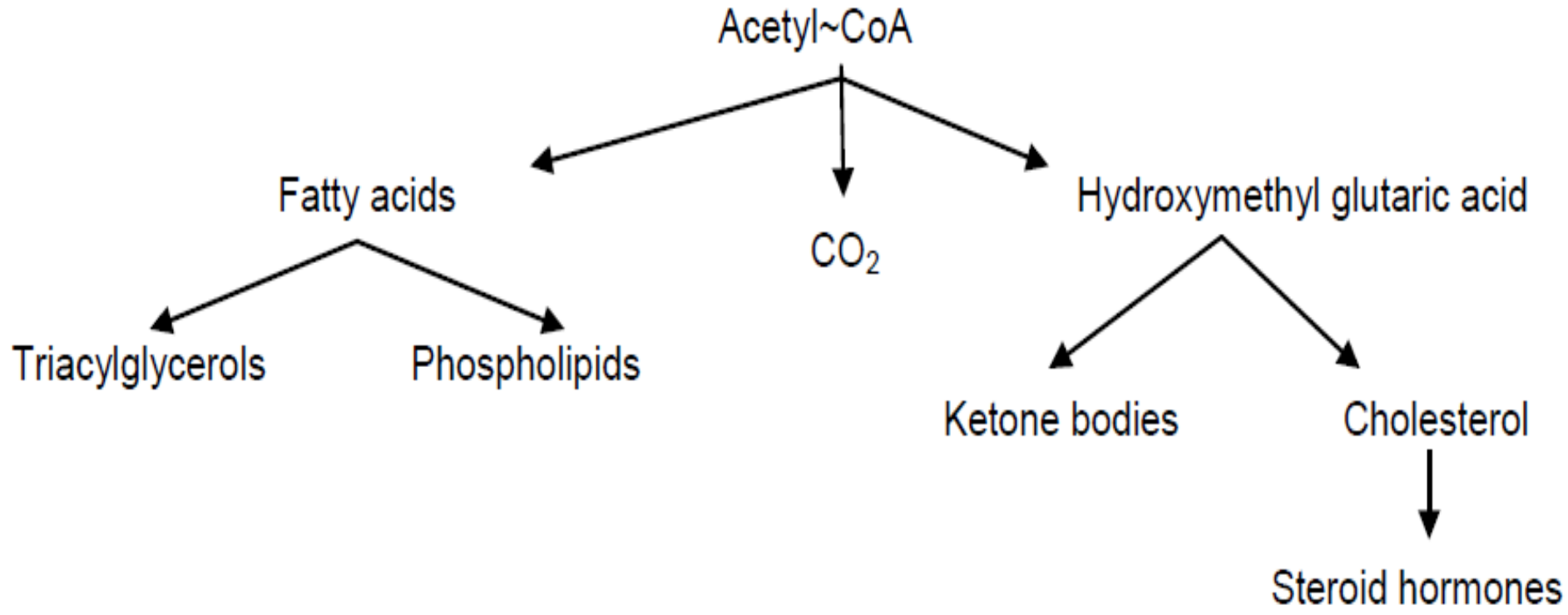
1. Acylation – addition of CoA and production of acetyl-CoA



Acetyl~CoA

Acetyl~CoA is produced by the catabolism of fatty acids, sugars, alcohol and certain amino acids .

It is also an important intermediate in lipid biosynthesis. The major site of lipid synthesis in the body is the liver .



Fatty acid synthesis (lipogenesis)

Fatty acids (e.g. palmitic acid, $CH_3(CH_2)_{16}COOH$) are synthesised from acetyl~ CoA (derived from the catabolism of carbohydrate, amino acids) at the expense of ATP and NADPH. The pathway occurs in the **cytoplasm** and can be represented by the overall equation:

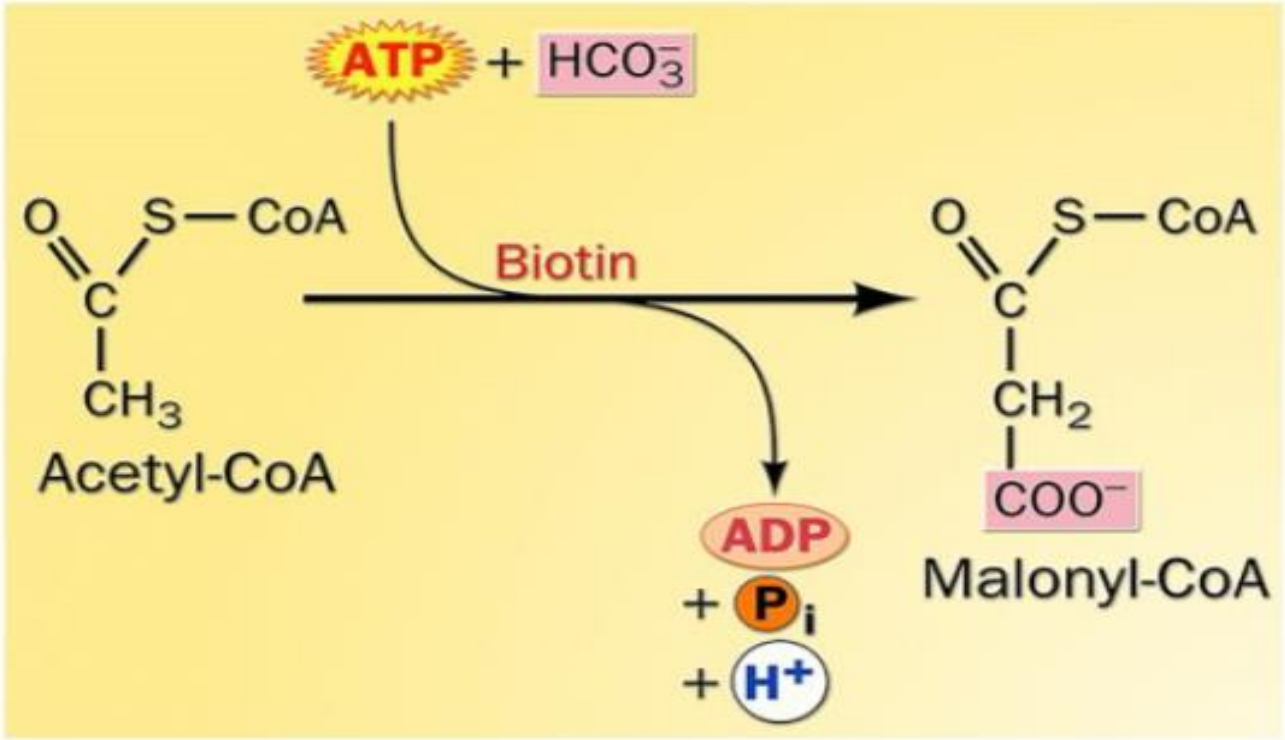
$$8\ CH_3CO\sim CoA + 7\ ATP + 14\ NADPH + 6\ H^+ \downarrow$$

$$CH_3(CH_2)_{14}COOH + 14\ NADP^+ + 8\ CoA + 7\ ADP + 7\ Pi + 6\ H_2O$$

B. Acetyl CoA Carboxylase makes the malonyl CoA for fatty acid synthesis

ATP + biotin + HCO_3^- ⟶ ADP + Pi + biotin-CO_2^-

biotin-CO_2^- + acetyl-CoA ⟶ biotin + malonyl-CoA.



Activation of acetate : Acetyl-CoA to malonyl CoA

Malonyl~CoA is produced from acetyl~CoA by the enzyme *acetyl~CoA carboxylase* in a reaction that requires biotin:

$CH_3CO\sim CoA + CO_2 + ATP \rightarrow CH_2(COOH)CO\sim CoA + ADP + P_i$

Acetyl~CoA carboxylase is plays an important role in controlling the rate of fatty acid synthesis.

Insulin **activates** the enzyme by promoting its dephosphorylation while glucagon and adrenaline **inhibit** the enzyme by promoting its phosphorylation.

- most of the dietary carbohydrates and proteins in excess of requirement convert to fatty acids and esterified to triacylglycerols to be stored in adipose tissue.

- These processes are important clinically as excessive lipid synthesis and storage is the cause of:

1. **obesity**
2. **type 2 diabetes**
3. **Atherosclerosis** (CVD)

The process is stimulated by insulin and inhibited by the anti-

insulin hormones glucagon and adrenaline.

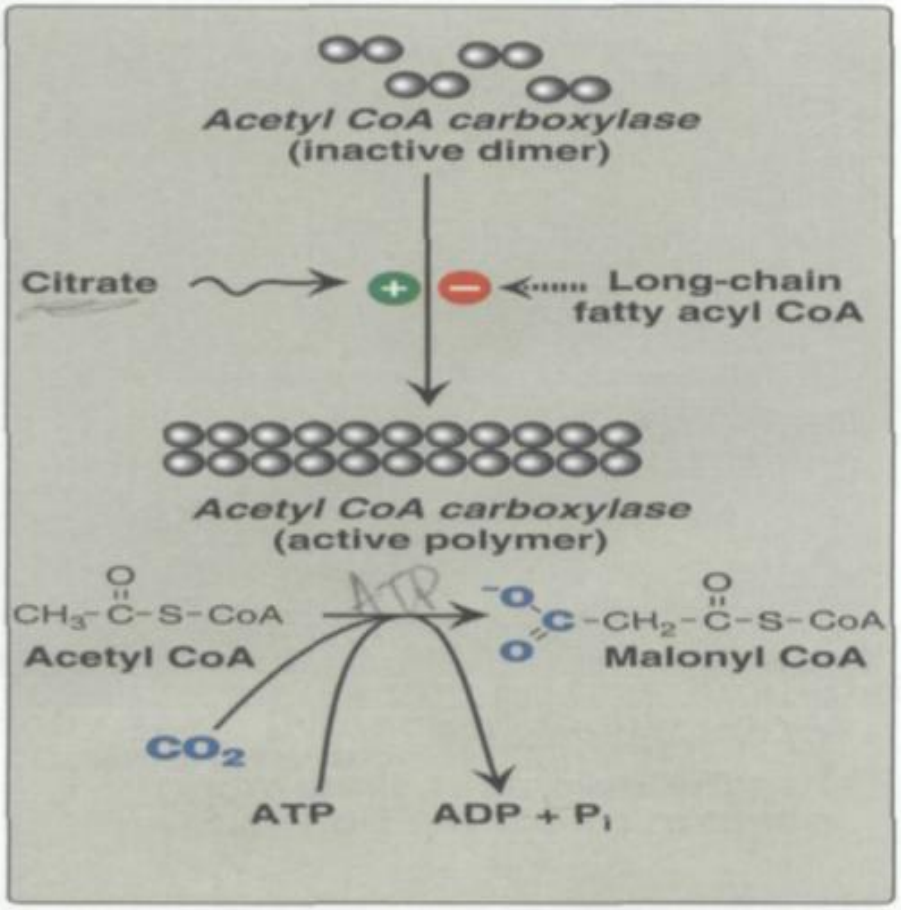
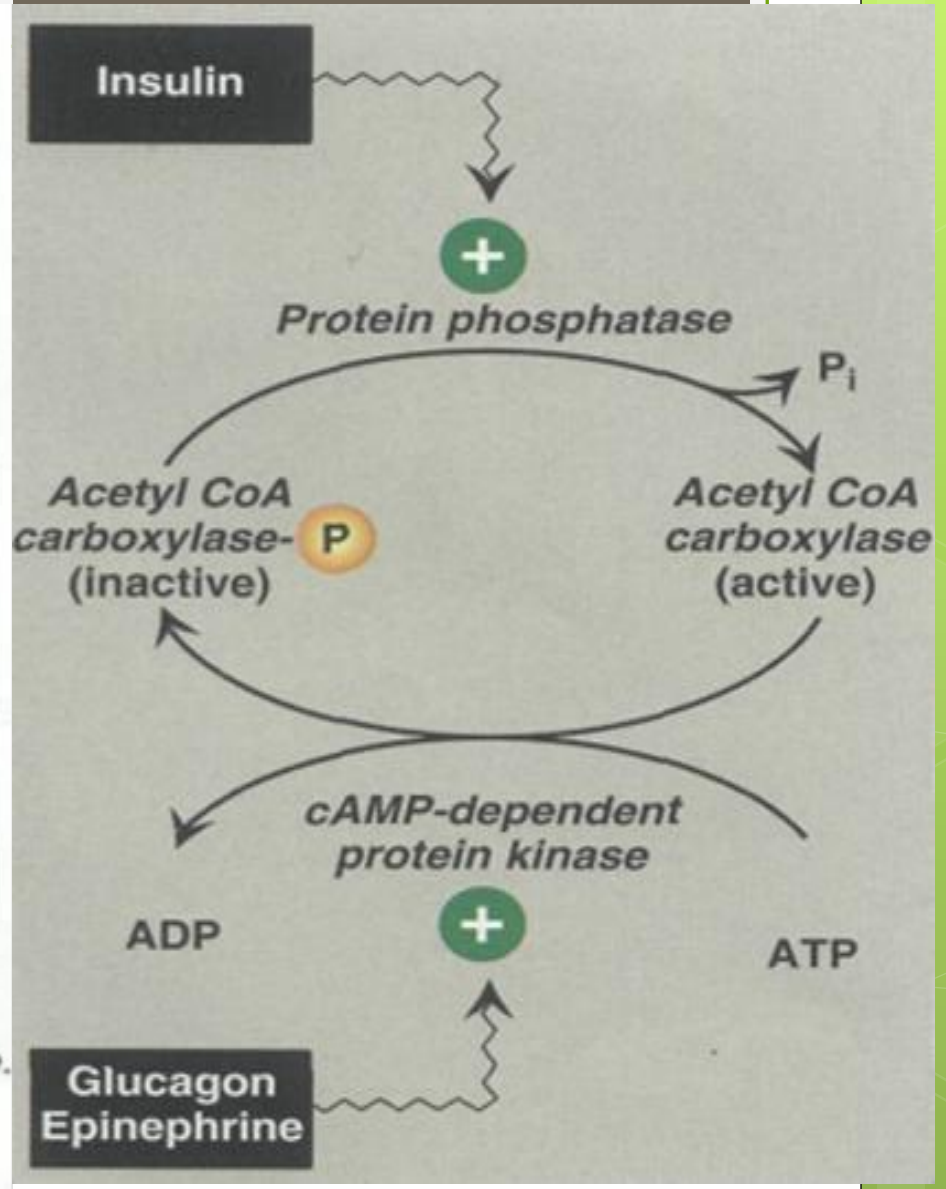


Figure 16.7
Allosteric regulation of malonyl CoA synthesis by *acetyl CoA carboxylase*. The carboxyl group contributed by dissolved CO_2 is shown in blue.



Thank you