De Novo synthesis of fatty acids

An anabolic pathway, a reversal of fatty acid oxidation in some steps but not a total reversal.

It is sequential addition of a two carbon unit, existing temporarily as a three carbon unit.

Site : cytosol or extramitochondrial.

Starting material: acetyl-CoA , existing temporarily bound to an enzyme complex as malonyl-CoA.

Sources of cytoplasmic acetyl-CoA: mitochondrial acetyl-CoA from glycolysis and fatty acid oxidation.

Nutritional state of the cell: well fed state.

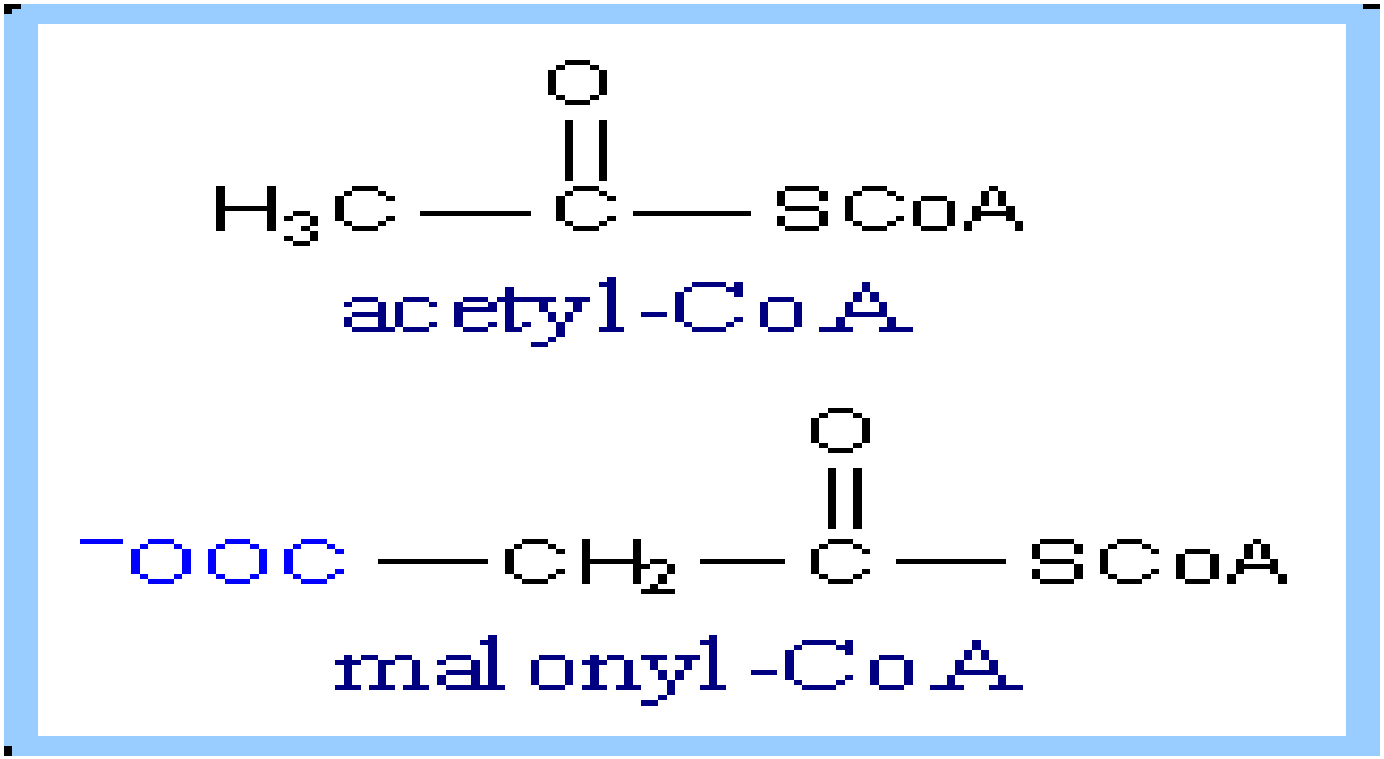
End product: fatty acid, e.g palmitate.

Enzymes: acetyl-CoA carboxylase
fatty acid synthase complex

Coenzymes: Biotin and NADPH.

	β oxidation	Fatty acid synthesis
site	mitochondria	cytosol
intermediates	Present as CoA derivatives	Covalently linked to SH group of ACP
enzymes	Independent proteins	Multienzyme complex
precursor	Fatty acyl-CoA	Malonayl-CoA (carboxylated acetly-CoA)
End product	Acetyl-CoA	Fatty acid
reactions	Sequential removal of a two carbon unit	Sequential addition of a two carbon unit, existing as a three carbon unit
Co-enzymes	FAD and NAD	Biotin, NADPH

- Both oxidation and synthesis of fats utilize an activated two carbon intermediate, acetyl-CoA. However, the acetyl-CoA in fat synthesis exists temporarily bound to the enzyme complex as malonyl-CoA.



- Acetyl-CoA is generated in the mitochondria primarily from two sources,

the pyruvate dehydrogenase (PDH) reaction and fatty acid oxidation.

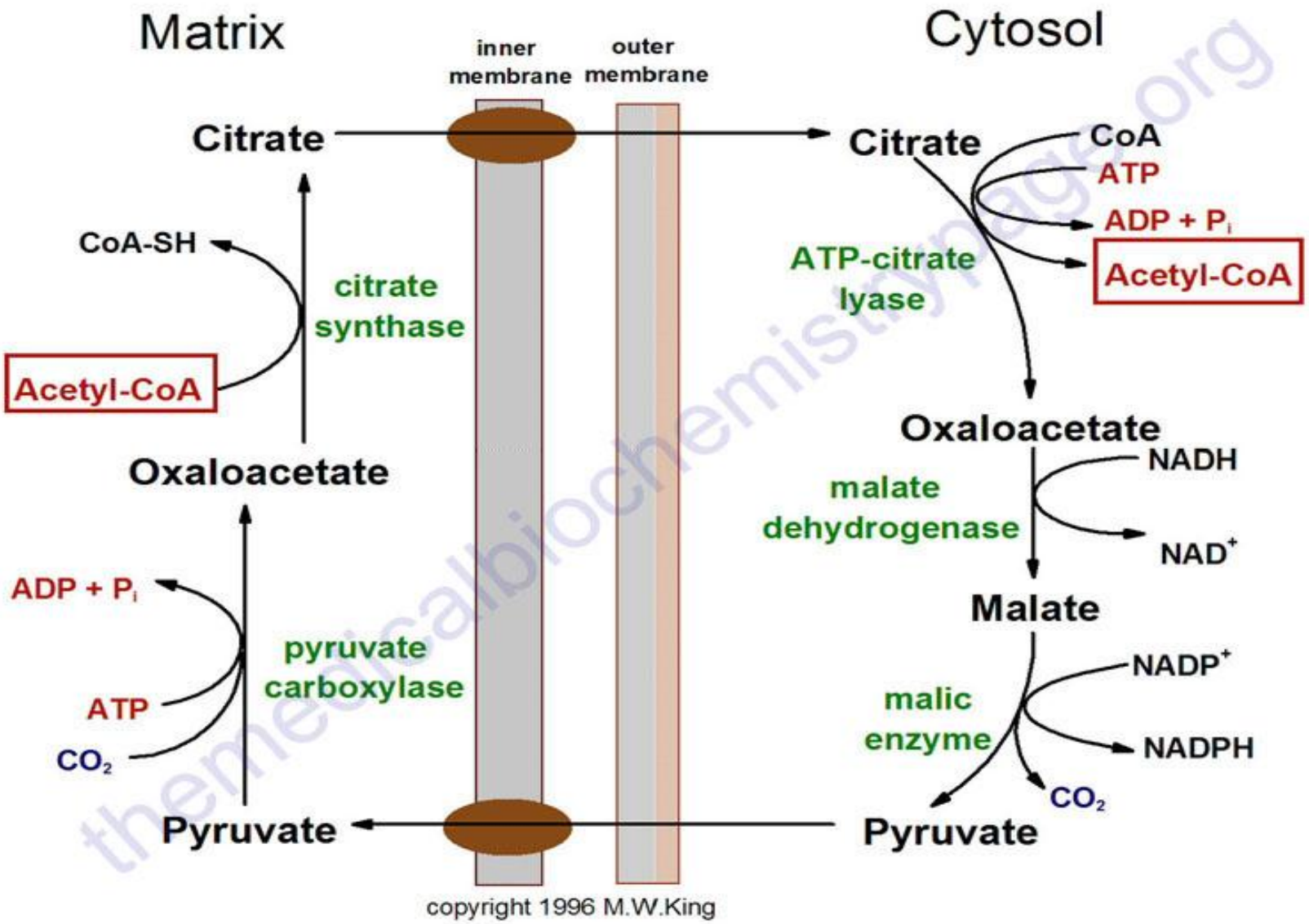
In order for these acetyl units to be utilized for fatty acid synthesis they must be present in the cytoplasm.

The shift from fatty acid oxidation and glycolytic oxidation occurs when the need for energy Diminishes,This results in reduced oxidation of acetyl-CoA in the TCA cycle and the oxidative phosphorylation pathway. Under these conditions the mitochondrial acetyl units can be stored as fat for future energy demands.

Transport of acetyl CoA from mitochondria to cytosol:

- Acetyl-CoA enters the cytoplasm in the form of **citrate** via the tricarboxylate transport system.
- In the cytoplasm, citrate is converted to **oxaloacetate** and **acetyl-CoA** by the ATP driven **ATP-citrate lyase reaction.** This reaction is essentially the reverse of that catalyzed by the TCA enzyme citrate synthase except it requires the energy of ATP hydrolysis to drive it forward.
- The resultant oxaloacetate is converted to **malate** by **malate dehydrogenase** (MDH).

- The malate produced by this pathway can undergo **oxidative decarboxylation** by **malic enzyme.**
- The co-enzyme for this reaction is $NADP^+$ generating NADPH. The advantage of this series of reactions for converting mitochondrial acetyl-CoA into cytoplasmic acetyl-CoA is that the NADPH produced by the malic enzyme reaction can be a major source of reducing co-factor for the fatty acid synthase activities.

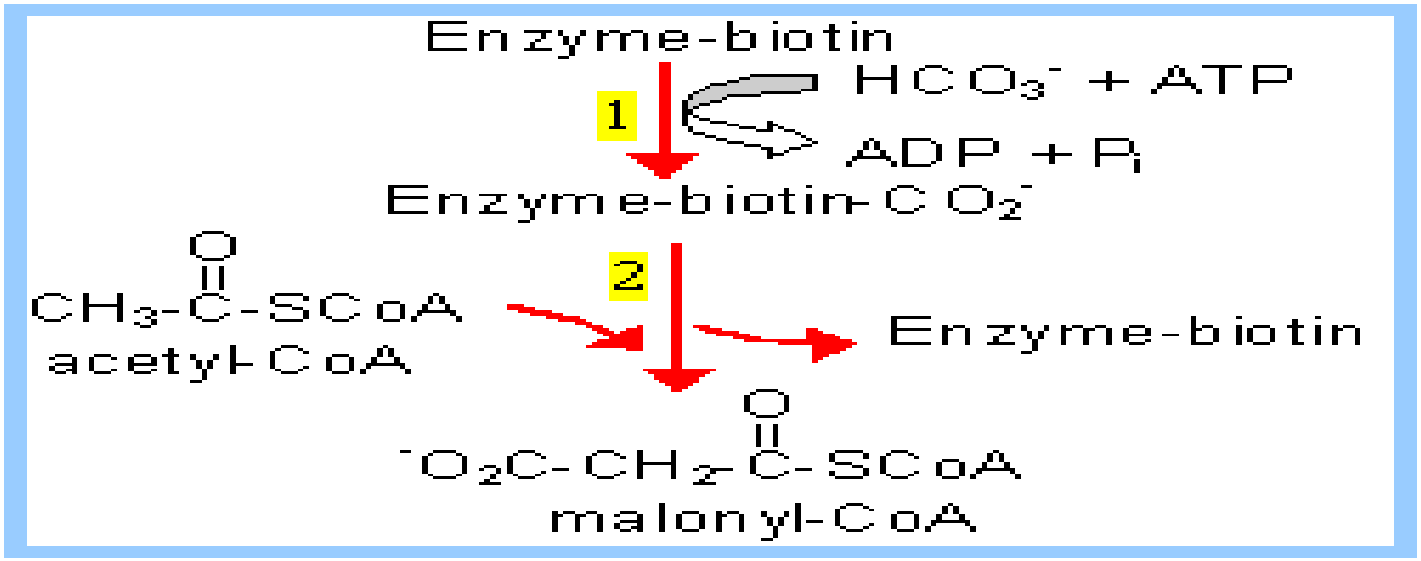


Steps of de novo synthesis of fatty acid:

Step 1: carboxylation of acetyl CoA to form malonyl CoA:

- The synthesis of malonyl-CoA is **the first committed step of fatty acid synthesis** and the enzyme that catalyzes this reaction, **acetyl-CoA carboxylase (ACC),** is the major site of regulation of fatty acid synthesis.
- Like other enzymes that transfer CO_2 to substrates, ACC requires a biotin co-factor.

- **Acetyl-CoA Carboxylase** catalyzes a 2-step reaction by which acetyl-CoA is carboxylated to form **malonyl-CoA**. As with other carboxylation reactions (e.g., Pyruvate Carboxylase), the enzyme prosthetic group is **biotin**.
- ATP-dependent carboxylation of the biotin, carried out at one active site (1), is followed by transfer of the carboxyl group to acetyl-CoA at a second active site (2).



- The overall reaction, which is **spontaneous**, may be summarized as:

HCO_3^- + ATP + acetyl-CoA ⟹ ADP + P_i + malonyl-CoA

Regulation of acetyl-CoA carboxylase

	active	inactive
Covalent modification	Dephosphorylated(protein phoaphatase)	Phosphorylated (AMP-activated kinase)
Energy sensor	ATP	AMP
Co-enzyme	NADPH	NADP
hormonal	insulin	Glucagon,epinephrine.
precursor	Increased malonyl-CoA	Decreased malonyl-CoA
Allosteric control	citrate	Palmitoyl CoA

Regulation of Acetyl-CoA Carboxylase:

- **Acetyl-CoA Carboxylase**, which converts acetyl-CoA to malonyl-CoA, is the **committed step** of the fatty acid synthesis pathway.

The enzyme is regulated by **phosphorylation/dephosphorylation**,

and there is **allosteric control** via local metabolites.

- **AMP** functions as an energy sensor and regulator of metabolism.

- When ATP production does not keep up with needs, a higher portion of a cell's adenine nucleotide pool is in the form of AMP.

- AMP promotes catabolic pathways that lead to synthesis of ATP, while inhibiting energy-utilizing synthetic pathways. For example, AMP regulates fatty acid synthesis and catabolism by controlling availability of **malonyl-CoA**.

- **AMP-Activated Kinase** catalyzes phosphorylation of **Acetyl-CoA Carboxylase** causing **inhibition** of the ATP-utilizing **production of malonyl-CoA .**

- Thus **acetyl-CoA Carboxylase** is **active** when **dephosphorylated** and **inactive** when **Phosphorylated.**

- Fatty acid synthesis is diminished by lack of the substrate malonyl-CoA.

- Fatty acid oxidation is stimulated due to decreased inhibition by malonyl-CoA of transfer of fatty acids into mitochondria

- A cyclic-AMP cascade, activated by the hormones glucagon and epinephrine when blood glucose is low, results inphosphorylation of Acetyl-CoA Carboxylase via **cAMP-Dependent Protein Kinase.**
- With Acetyl-CoA Carboxylase inhibited, acetyl-CoA remains available for synthesis of ketone bodies, the alternative metabolic fuel used when blood glucose is low.
- The antagonistic effect of **insulin**, produced when blood glucose is high, is attributed to activation of **Protein Phosphatase** which results in dephosphorylation of acetyl-CoA carboxylase and susbsequent activation.

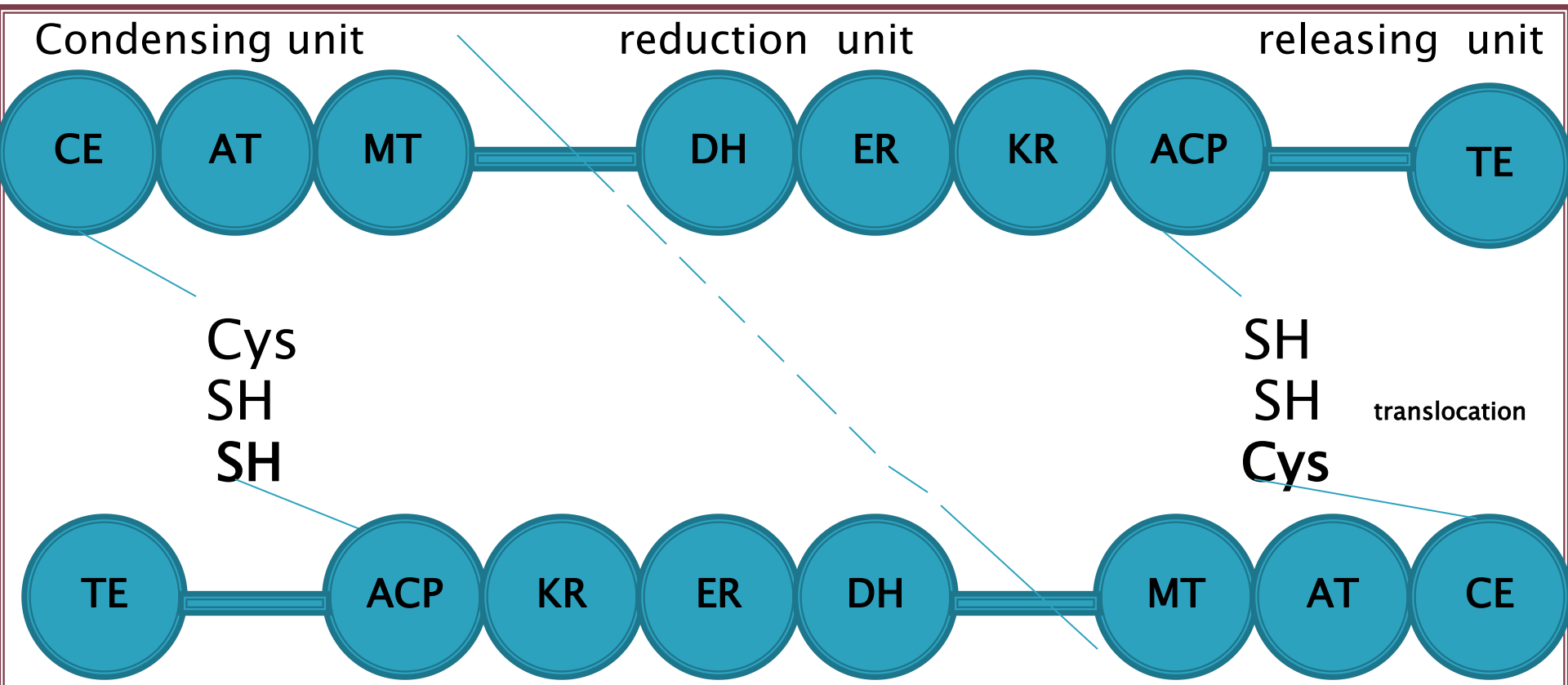
Regulation of Acetyl-CoA Carboxylase by local metabolites:

- **Palmitoyl-CoA**, the product of Fatty Acid Synthase, promotes the **inactive** conformation of Acetyl-CoA Carboxylase, diminishing production of malonyl-CoA, the precursor of fatty acid synthesis. This is an example of feedback inhibition.
- **Citrate** allosterically **activates** Acetyl-CoA Carboxylase. Citrate concentration is high when there is adequate acetyl-CoA entering Krebs Cycle. Excess acetyl-CoA is then converted via malonyl-CoA to fatty acids for storage.
- NADPH serves as electron donor in the two reactions involving substrate reduction. The NADPH is produced mainly by thePentose Phosphate Pathway.

Step 2-7: rest of the steps of de novo synthesis of fatty acids are catalyzed by a multienzyme complex called **fatty acid synthase complex.**

fatty acid synthase complex

- **A multienzyme complex .**
- **The enzymes form a dimer with identical subunits.**
- **Each subunit of the complex is organized into three domains joined by flexible regions.**
- **The multienzyme complex enhances the efficiency of the process because intermediates can easily interact with the active sites of the enzymes.**



CE: condensing enzyme.
MT: malonyl transacylase.
ER: enoyl reductase.
ACP: acyl carrier protein.

AT:acetyl transacylase
DH: dehydratase.
KR: ketoacyl reductase.
TE : thioesterase.

- **The first domain:**

it is called **condensing unit** and includes the initial substrate binding site.

Enzymes of the condensing unit:

β-ketoacyl synthase or condensing enzyme (CE)

Acetyl transacylase(AT)

Malonyl transacylase (MT).

- **The second domain:**

It is called **reduction unit.**

Enzymes of the reduction unit:

Dehydratase

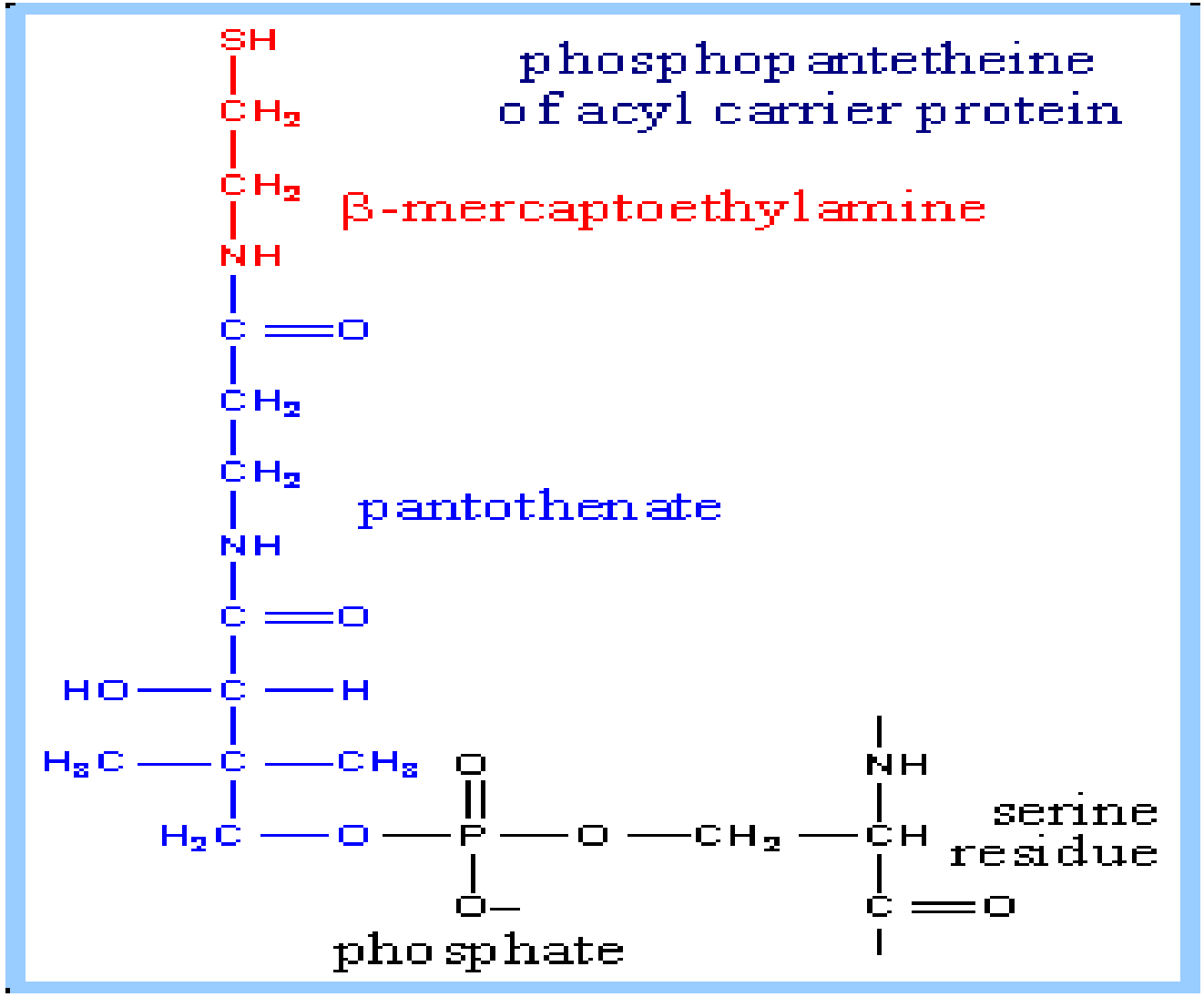
Enoyl reductase

Ketoacyl reductase

And a carrier protein called **acyl carrier protein(ACP)**

Acyl carrier protein:

- It's a polypeptide chain that has a **phospho-pantotheine group**, to which the intermediates are attached in a thioester linkage, as in the case of coenzyme A.
- The phosphopantothenyl group is **flexible and carries the substrate from one active site to another.**
- The whole reaction sequence catalyzed by the enzymes of fatty acid synthase complex occurs while the intermediates are bound to ACP(acyl carrier protein).


phosphopantetheine
of acyl carrier protein
β-mercaptoethylamine
pantothenate
serine
residue
phosphate

- **The third domain:**

It is called **releasing unit.**

It releases the fatty acid (palmitate) synthesized by the enzyme **thioesterase (TE).**

An outline of the steps catalyzed by fatty acid synthase complex in de novo fatty acid synthesis after the formation of malanoyl-CoA (the precursor), is as follows;

Step 2: **binding to active site of the multienzyme complex.**

Step3: **condensation reaction.**

Step4: **reduction reaction.**

Step 5: **dehydration reaction.**

Step6: **second reduction reaction.**

Step 7: **thiolysis to release the newly synthesized fatty acid.**

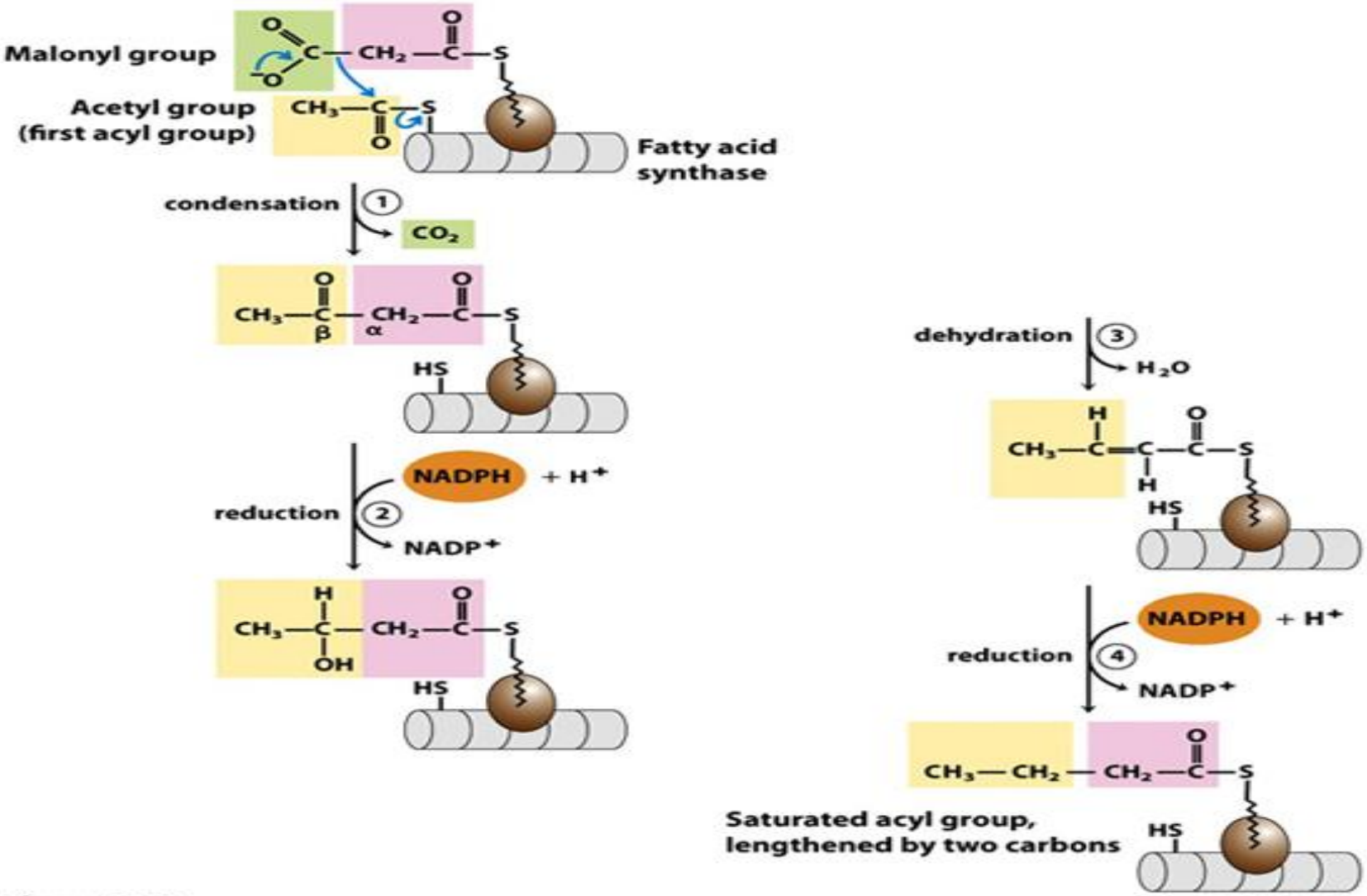
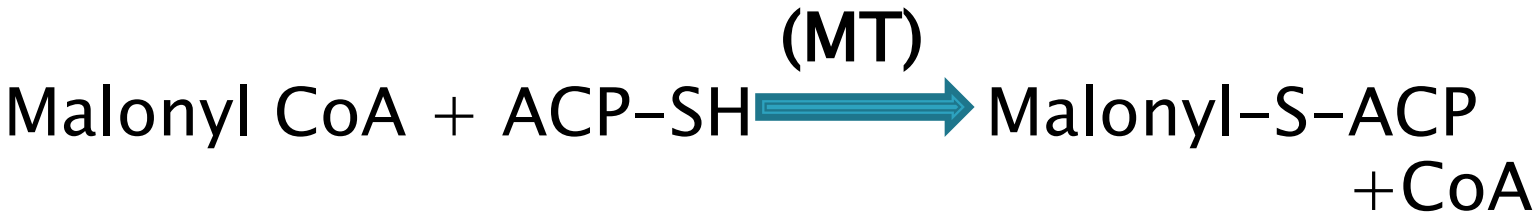


Figure 21-2
Lehninger Principles of Biochemistry, Fifth Edition

- The whole reaction sequence catalyzed by the enzymes of fatty acid synthase complex occurs while the intermediates are bound to ACP(acyl carrier protein).

Step 2: one molecule of acetyl CoA and one molecule of malonyl CoA bind to the multienzyme complex.

a, **malonyl transacylase (MT)** transfers the malonyl group to the **SH group of the ACP** of one monomer of the enzyme.



b, **acetyl transacylase (AT)** catalyzes the transfer of the acetyl group to the **cysteinyl SH group of the condensing enzyme (CE)** of the other monomer of the fatty acid synthase complex.

$$\text{Acetyl CoA} + \text{(CE)- SH} \xrightarrow{\text{(AT)}} \text{Acetyl-S-CE} + \text{CoA}$$

Step 3: condensation reaction:
Condensation between the acetyl and malonyl units to form a 4-C unit called β ketoacyl-S-ACP or acetoacetyl- ACP .

Substrate: acetyl and malonyl units.

Enzyme: condensing enzyme (CE) or ketoacyl synthase.

Product : β ketoacyl-S-ACP or acetoacetyl-ACP, with loss of one molecule of Co_2

Step 4: Reduction reaction:

The acetoacetyl ACP or β ketoacyl ACP is reduced by NADPH dependent β ketoacyl reductase (KR) to form β hydroxyacyl-ACP.

Substrate: acetoacetyl-ACP or β ketoacyl-ACP.

Enzyme: β ketoacylreductase.

Coenzyme : NADPH

Product: β hydroxyacyl-ACP.

Step 5: dehydration reaction:

The β hydroxy acyl –ACP is dehydrated by a dehydratase (DH) to form enoyl –ACP.

Substrate: β hdroxy acyl–ACP .

Enzyme: dehydratase.

Product: enoyl–ACP with removal of H_2O and unsaturation.

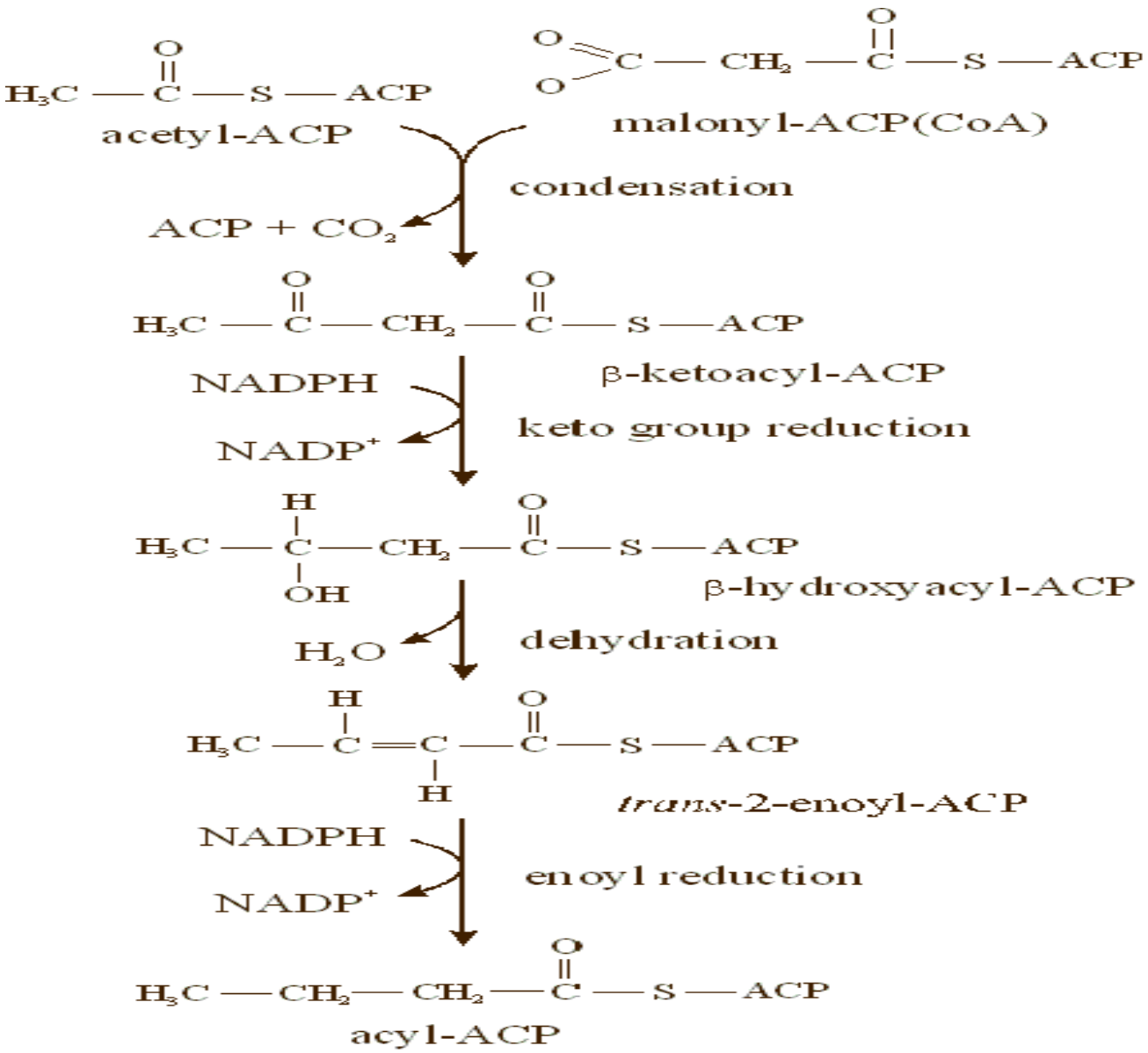
Step 6 : second reduction reaction:
the enoyl-ACP is again reduced by enoyl reductase (ER) utilising a 2nd molecule of NADPH to form acyl-ACP.

Substrate: enoyl-ACP.

Enzyme: enoyl reductase.

Coenzyme: NADPH.

Product: acyl-ACP (4 carbons) with removal of unsaturation.


acetyl-ACP
malonyl-ACP(CoA)
condensation
ACP + CO2
β-ketoacyl-ACP
NADPH
NADP+
keto group reduction
β-hydroxyacyl-ACP
H2O
dehydration
trans-2-enoyl-ACP
NADPH
NADP+
enoyl reduction
acyl-ACP

Recycling of reactions:

The acyl group (4 carbons) is now transferred to the SH group of the condensing enzyme on the other monomer and a 2nd malonyl-CoA molecule binds to the phosphopantothenyl-SH group of ACP.

The sequence of reactions(step 3,4,5,6) namely condensation, reduction, dehydration and reduction are repeated.

Step 7: releasing or thiolytic reaction:
the above cycle of reactions is repeated a total of (n/2 – 1) times to release a long chain saturated fatty acid. (n: number of carbon atoms of a fatty acid).

Most common is palmitic acid (16 carbons) the cycles are repeated a total of seven times , till palmitate is formed.

Thioesterase releases palmitate from the multienzyme complex.

Fatty acids formed in body tissues:

Liver and adipose tissue : palmitic acid (16 carbon).
Lactating mammary gland: capric acid (10 carbon) and lauric acid (12 carbon).

The net reaction of do novo synthesis of palmitic acid:

1 acetyl CoA + 7 malonyl CoA + 14 $NADPH^+$ + 14 H^+

→ 1 palmitate + 7 Co_2 +14 NADP + 8 CoA + 6 H_2O

Sources of NADPH for de novo synthesis of fatty acids:

1. Pentosephosphate pathway: the main source, tissues having active lipogenesis (liver, adipose tissue, lactating mammoray gland) have an active HMP shunt pathway.

2. Malic enzyme: the enzyme catalyzes oxidative decarboxylation of cytoplasmic malate:

$$\text{Malate} + \text{NADP} \xrightarrow{\textbf{malic enz.}} \text{Pyruvate} + Co_2 + \text{NADPH}+H^+$$

3. Cytoplasmic isocitrate dehydrogenase :

Isocitrate + NADP ⟹ αketoglutarate+ Co_2+ NADPH+H

The primary endproduct of fatty acid synthase activity is palmitic acid.

Palmitic acid can further undergo three processes:

1. chain enlongation.

2. desaturation to form unsaturated FA.

3. esterification to form triacyglycerols.

1. Elongation of fatty acid chain:
Palmitic acid can be further enlongated by the addition of two carbon units.

Site: the endoplasmic reticulum (ER) and the mitochondria.

Enzyme: a separate enzymic processes are used rather than multifunctional enzyme.

Coenzyme: NADPH.

Product : long chain fatty acid.

The brain can produce very long chain fatty acid (up to 24 carbons) that are required for synthesis of brain lipids.

2. Desaturation of fatty acid chain:

That is addition of cis double bonds .

Enzymes: mixed function oxidases (desaturases)

Site: the endoplasmic reticulum.

Coenzymes: NADH , O_2.

Product: polyunsaturated fatty acids.

humans have carbon 9,6,5 and 4 desaturases, but lack the ability to introduce double bonds from carbon 10 to the ω end of the chain , this is the basis for essentiality of polyunsaturated linoleic and linolenic acids.

Triacylglycerol (TAG) synthesis (lipogenesis)

An anabolic pathway

TAGs are the storage form of lipids and the major energy reserve of the body.

TAGs are only slightly soluble in water and can not form stable micelles by themselves, they coalesce within adipocytes to form oily droplets that are nearly anhydrous.

Three molecules of fatty acids are esterified to a molecule of glycerol, fatty acids are esterified through their carboxyl groups resulting in a loss of negative charge and formation of neutral fat.

The three fatty acids are usually not of the same type, the fatty acid on carbon 1 is typically saturated, that on carbon 2 is typically unsaturated and that on carbon 3 can be of either type.

Site : lipid storage organs; liver, adipose tissue, kidney, lactating mammary galnds.

Starting material (primer) :glycerol and fatty acyl CoA.

End product: triacyglycerol (TAG).

Enzymes:
fatty acyl CoA synthetase (for fatty acid Activation).

Glycerol kinase and/or glycerol phosphate dehdrogenase (to supply glycerol phosphate).

Acyltransferases.

Phosphatase.

Steps in TAGs synthesis

1. Synthesis of glycerol phosphate:

Glycerol phosphate is the initial acceptor of fatty acids during TAGs synthesis (primer).

There are two pathways for production of glycerol phosphate:

a. Dihydroxy acetone phosphate (DHAP), in the liver and adipose tissue produced during glycolysis. DHAP is reduced by glycerol phosphate dehydrogenase to glycerol Phosphate.

adipocytes must have glucose to oxidize in order to store fatty acids in the form of TAGs. DHAP can also serve as a backbone precursor for TAG synthesis in tissues other than adipose, but does so to a much lesser extent than glycerol.

b. Free glycerol is converted to glycerol phosphate by glycerol kinase in the liver.

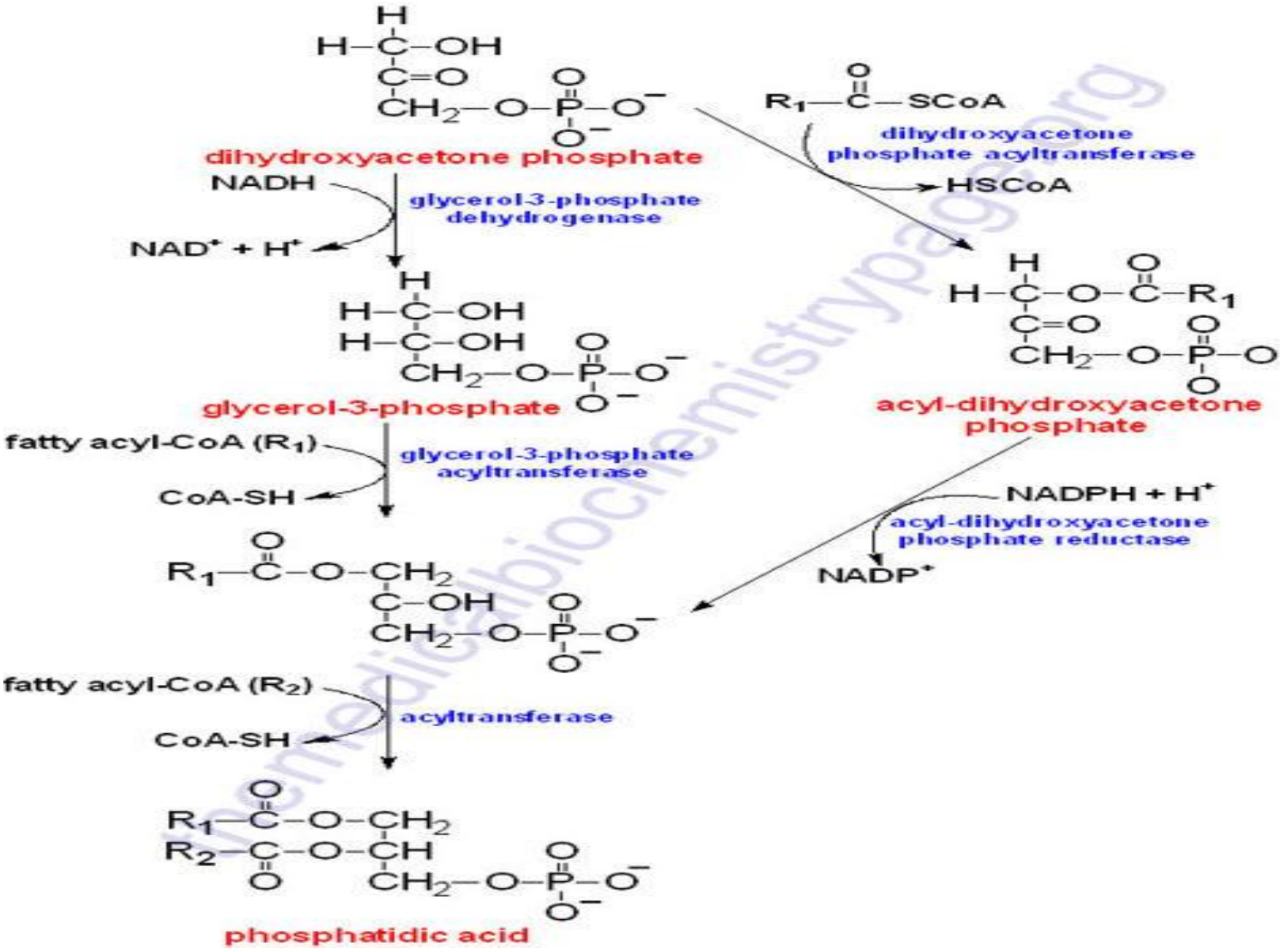
Adipocytes lack the enzyme glycerol kinase.

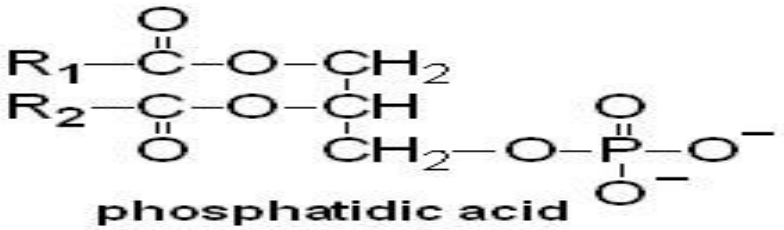
2. **Conversion of free fatty acid to its activated form:**

The fatty acids incorporated into TAGs are activated to acyl-CoAs through the action of acyl-CoA synthetases.

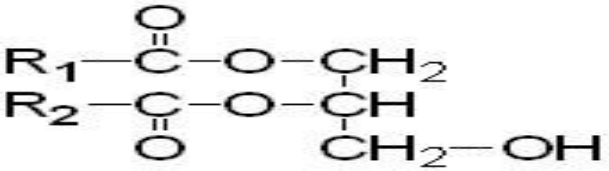
3. Synthesis of a molecule of TAG from glycerol phosphate and fatty acyl CoA:

Two molecules of acyl-CoA are esterified to glycerol3-phosphate to yield 1,2-diacylglycerol phosphate (commonly identified as phosphatidic acid).
The phosphate is then removed, by phosphatidic acid phosphatase (PAP1), to yield 1,2-diacylglycerol, the substrate for addition of the third fatty acid.
Intestinal monoacylglycerols, derived from the hydrolysis of dietary fats, can also serve as substrates for the synthesis of 1,2-diacylglycerols.

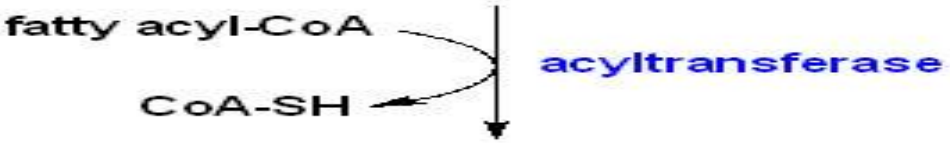

dihydroxyacetone phosphate
NADH
glycerol-3-phosphate dehydrogenase
NAD+ + H+
glycerol-3-phosphate
fatty acyl-CoA (R1)
glycerol-3-phosphate acyltransferase
CoA-SH
fatty acyl-CoA (R2)
acyltransferase
CoA-SH
phosphatidic acid
R1—C(=O)—SCoA
dihydroxyacetone phosphate acyltransferase
HSCoA
acyl-dihydroxyacetone phosphate
NADPH + H+
acyl-dihydroxyacetone phosphate reductase
NADP+

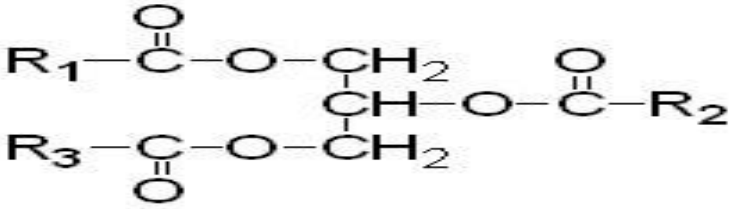


phosphatidic acid



1,2-diacylglycerol





triacylglycerol

Fates of TAG in the liver and adipose tissue

- In adipose tissue, TAG is stored in the cytosol of the cells in a nearly anhydrous form, ready for mobilization when the body requires it for fuel.
- In the liver, little TAG is stored, most is exported, packaged with cholesteryl esters, cholesterol, phospholipids and proteins such as apolipoprotein B-100 to form lipoprotein particles called very low density lipoprotein (VLDL). Nascent VLDL are secreted directly into the blood where they mature and function to deliver the endogenously derived lipids to the peripheral tissues.

Regulation of TAG synthesis

1. In well fed condition:

- Active lipogenesis occurs in adipose tissue.
- The dietay TAG transported by chylomicrons and the endogenously synthesized TAG from liver brought by VLDL are both taken up by adipose tissue and stored.
- Insulin and glucose levels are increased , insulin increases the activity of key glycolytic enzymes and glycerophosphate acyltransferase.
- Insulin inhibits hormone sensitive lipase and so lipolysis is decreased.

2. In fasting/starvation condition:

- TAG is mobilized from adipose tissue under the effect of hormones, glucagon and epinephrine.

- The cAMP mediated activation casecade enhances the intracellular hormone sensitive lipase . The phosphorylated form of the enzyme is active which acts on TAG and liberates fatty acids.

- Under conditions of starvation, high glucagon/insulin ratio favors lipolysis and inhibits lipogenesis,these fatty acids are taken up by peripheral tissues as a fuel.