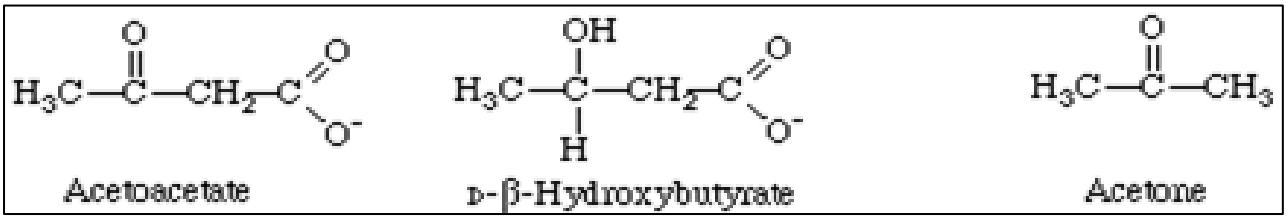
Ketone Body Metabolism & Fatty acid synthesis

Introduction to Ketone Body Metabolism

Ketone bodies are three chemicals that are produced when fatty acids are broken down in excess, and the Production of these compounds is called "Ketogenesis", and this is necessary in small amounts. The three ketone bodies are **Acetone**, **Acetoacetate** and **β-Hydroxy butyrate**.



The brain is an important organ. It is metabolically active and metabolically privileged. The brain generally uses 60-70% of total body glucose requirements, and always requires some glucose for normal functioning. Under most conditions, glucose is essentially the sole energy source of the brain. The brain cannot use fatty acids, which cannot cross the blood-brain barrier. Because animals cannot synthesize significant amounts of glucose from fatty acids, as glucose availability decreases, the brain is forced to use either amino acids or ketone bodies for fuel. Ketone bodies are produced from acetyl-CoA, mainly in the **mitochondrial matrix of liver cells**.

Ketone body synthesis

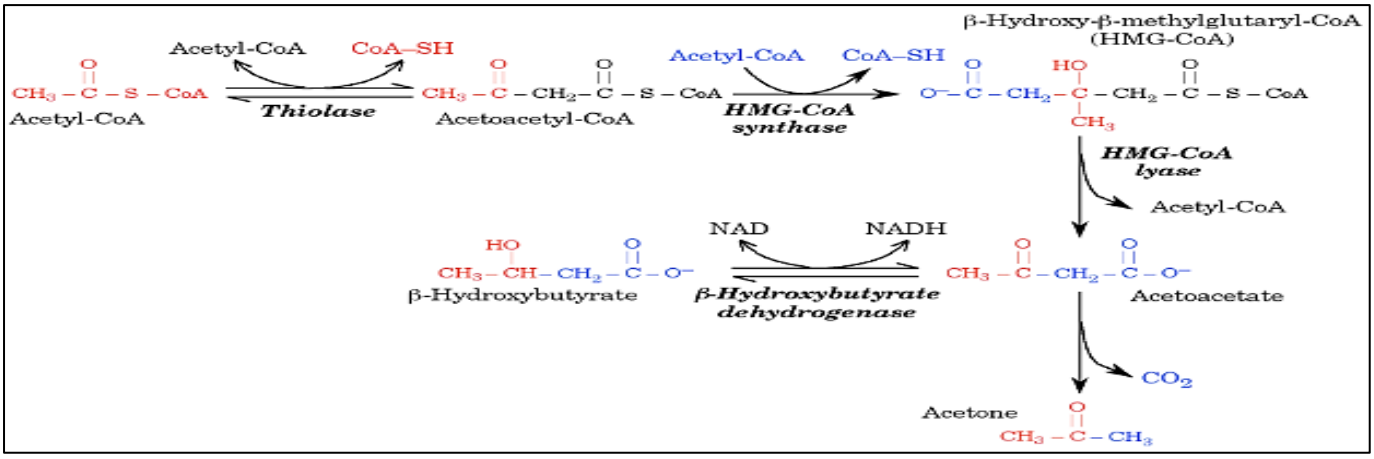
Ketone body synthesis occurs in Liver Mitochondria. Some of the Acetyl-CoA produced by fatty acid oxidation in liver mitochondria is converted to Acetone, Acetoacetate and β-hydroxybutyrate. Transport of fatty acids through the mitochondrial membrane is an important regulatory point.

Ketone body synthesis occurs normally under all conditions. However, their formation increases dramatically during starvation. This seems to be due to a combination of factors. Prolonged low levels of insulin result in both **increased fatty acid release from adipose tissue, and increased amounts of the enzymes required to synthesize and utilize ketone bodies.** In addition, in the liver, **increased demand for gluconeogenesis results in depletion of oxaloacetate, and therefore in decreased capacity for the TCA cycle.** This causes a rise in the levels of acetyl-CoA, the substrate for ketone body production. A person in starvation will not have oxaloacetate available for the conversion of acetyl CoA to citric acid.

Ketogenesis

1. First step – Reverse of Thiolase.
2. Second step – Synthesis of HMG CoA.
3. Third step – HMG CoA Lyase.

The first enzyme in the ketone body synthesis pathway is **Thiolase** (the same enzyme that is responsible for the cleavage step in β-oxidation). In ketone body biosynthesis, thiolase catalyzes the condensation of **two acetyl-CoA molecules to form acetoacetyl-CoA.** The next enzyme, **HMG-CoA synthase adds a third acetyl- CoA molecule**, to form **β-hydroxy-β-methylglutaryl-CoA (usually abbreviated HMG- CoA).** HMG-CoA is an important biosynthetic intermediate; however, in the mitochondria, it is only used for ketone body synthesis. The third enzyme, HMG- CoA lyase, **releases an acetyl-CoA from HMG-CoA to form acetoacetate.** The final enzyme in ketone body synthesis, **β** -hydroxybutyrate dehydrogenase, reduces acetoacetate to **β** -hydroxybutyrate.



Acetone is formed from spontaneous decarboxylation of acetoacetate. The levels of acetone are much lower than those of the other two types of ketone bodies. Acetone is produced in small quantities; highly volatile. Hence it is not used by the body. Acetone cannot be converted back to acetyl-CoA, so it is excreted in the urine and exhaled (it can be exhaled because it has a high vapor pressure and thus evaporates easily). The exhalation of acetone is responsible for the characteristic "fruity" odor of the breath of persons in ketotic states.

Acetoacetate is a β-ketoacid, and like many such compounds may spontaneously decarboxylate. Both β-hydroxybutyrate and acetoacetate are released into circulation. The ratio depends on the amount of NADH available in the liver mitochondria; if NADH concentration is high, the liver releases a higher proportion of β-hydroxybutyrate.

Both b-hydroxybutyrate and acetoacetate are organic acids. Both releases tend to lower the pH of the blood. Individuals with untreated Type I diabetes mellitus often release ketone bodies in such large quantities that the normal pH-buffering mechanisms are overloaded; the reduced pH, in combination with a number of other metabolic abnormalities associated with lack of insulin results in diabetic ketoacidosis, a life-threatening acute disorder of Type I diabetes.

Control of ketone body synthesis

1. Availability of the substrate (**Long Chain Fatty Acids**): from increased production by lipolysis with increased delivery of FA to the liver.
2. The level of **Malonyl Co A** in the liver, with its influence to inhibit the **Carnitine Palmitoyl Transferase I (CPT I)**
3. The **Glucagon / Insulin Ratio** : a high ratio increases lipolysis and activation of oxidative ketogenesis , a low ratio counteracts ketogenesis.

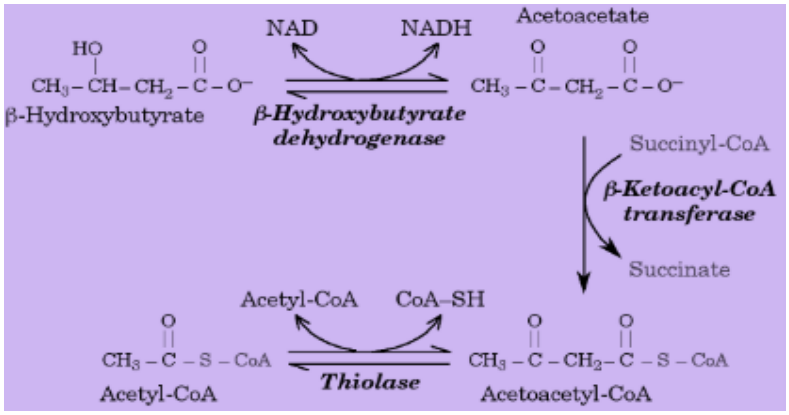
Use of ketone bodies by the peripheral tissues: ketolysis

Ketone Bodies are water soluble and can be transported across the inner mitochondrial membrane as well as across the blood-brain barrier and cell membranes.

- It represents a Source of fuel for brain, heart and muscle and the Major energy source (75%) for brain during starvation.
- Tissues that can use fatty acids can generally use ketone bodies in addition to other energy sources.
- The brain does not normally use fatty acids, which do not cross the blood-brain barrier; under ordinary circumstances. The metabolic rate of the brain is essentially constant. After a few days of fasting, the brain undergoes metabolic changes to adapt to the decreased availability of glucose. One major change is increased amounts of the enzymes necessary to metabolise ketone bodies.
- The liver synthesizes Ketone bodies, but lacks β-ketoacyl-CoA transferase, and therefore little ability to convert acetoacetate into acetyl-CoA. The utilization of ketone bodies requires one enzyme not present in the ketone body biosynthetic pathway**, β-ketoacyl-CoA transferase**, converts **acetoacetate** to **acetoacetyl-CoA**. As mentioned above, lack of this enzyme in the liver prevents use of ketone bodies by liver cells.
- Cells lacking mitochondria (for example, RBCs), cannot use ketone bodies.
- The β-ketoacyl-CoA transferase uses succinyl-CoA as the CoA donor, forming succinate and acetoacetyl-CoA. The acetoacetyl-CoA is actively removed by its convers ion to two acetyl CoAs. The other enzymes of the ketone body utilization pathway, β -hydroxybutyrate dehydrogenase and thiolase, are identical to the enzymes used for ketone body synthesis.

KB in blood & urine

- Normal KB in plasma: 0.2mmol/L
  - Starvation : 3-5 mmol/L
- Diabetic ketoacidosis: >12mmol/L
- KB of >12mmol/L, saturates all oxidative pathways.
- Normal KB in urine : <1mg/day



Metabolic Acidosis due to KBs

When excess ketone bodies accumulate, this abnormal (but not necessarily harmful) state is called **ketosis**. But when even larger amounts of ketone bodies accumulate such that the body's pH is lowered to dangerously acidic levels, this state is called **ketoacidosis**.
Both acetoacetate and beta-hydroxybutyrate are acidic, and, if levels of these ketone bodies are too high, the pH of the blood drops, resulting in **ketoacidosis**. This happens in untreated Type I diabetes, and also during prolonged starvation. Ketone bodies being acidic in nature, release H ions into blood. They are buffered by $HCO3^-$ Continuous production of ketone bodies depletes alkali reserve resulting in ketoacidosis.

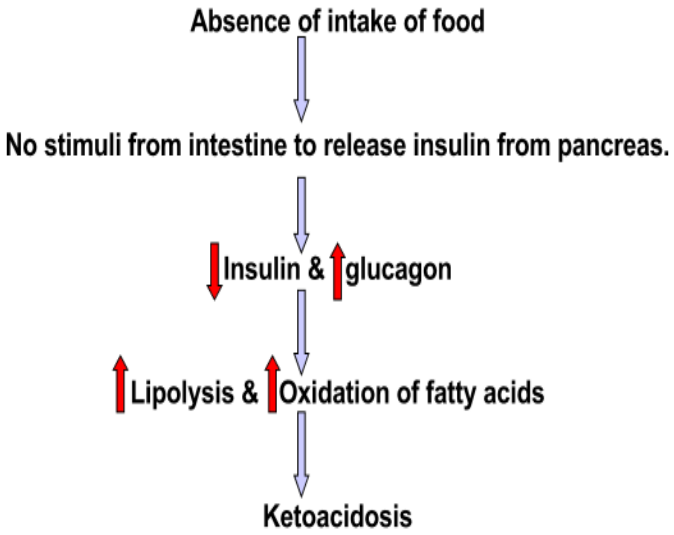
$$H^+ + HCO_3^- \xrightarrow[CA]{} H_2CO_3 \xrightarrow[CA]{} H_2O + CO_2 \uparrow$$

Excess of Ketogenesis

Liver catabolizes fatty acids to meet the energy demand by other tissues. Excess of Acetyl CoA is produced, which are destined to form ketone bodies. Ketone bodies are transported by blood to Muscle and Brain. Ketone body formation regenerates free CoA, which is required for β-oxidation.

Ketone bodies in Starvation

After the diet has been changed to lower blood glucose for 3 days, the brain gets 30% of its energy from ketone bodies. After 40 days, this goes up to 70% (during the initial stages the brain does not burn ketones, since they are an important substrate for lipid synthesis in the brain). The brain retains some need for glucose, because ketone bodies can be broken down for energy only in the mitochondria, and brain cells' long thin axons are too far from mitochondria.



Ketone Bodies and Diabetes; "Starvation of cells in the midst of plenty"

Glucose is abundant in blood, but uptake by cells in muscle, liver, and adipose cells is low due to absence of insulin. Cells, metabolically starved, turn to gluconeogenesis and fat/ protein catabolism. In type I diabetics, **oxaloacetate OAA** is low, due to excess gluconeogenesis, so Acetyl CoA from fat/protein catabolism does not go to TCA, but rather to ketone body production. Acetone can be detected on breath of type I diabetics.

Laboratory diagnosis of DKA; Blood glucose :> 250 mg/dl; Serum bicarbonate :< 15 mEq/L; pH: <7.3; Urine glucose: +++; Ketonuria: +++

Synthesis of fatty acids

A large proportion of the fatty acids used by the body are supplied by the diet. Carbohydrates and protein obtained from the diet in excess of the body's needs for these compounds can be converted to fatty acids, which are stored as TAGs. In adult humans, fatty acid synthesis occurs primarily in the liver and lactating mammary glands and, to a lesser extent, in adipose tissue. This cytosolic process incorporates carbons from **acetyl CoA** into the growing fatty acid chain, using energy in the form of (ATP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH).

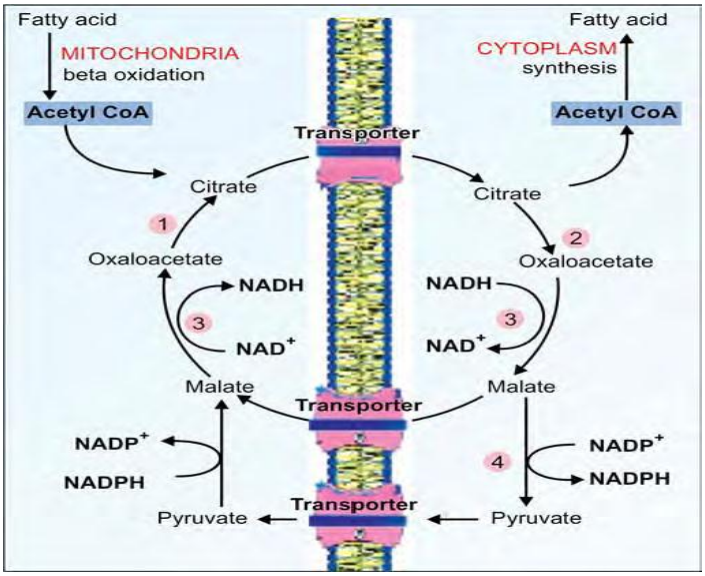
De novo synthesis of fatty acids

Fatty acids are synthesized mainly by a de novo synthetic pathway operating in the cytoplasm. So it is referred to as extra mitochondrial or cytoplasmic fatty acid synthase system. The major fatty acid synthesized de novo is palmitic acid, the 16C saturated fatty acid. The process occurs in liver, adipose tissue, kidney, brain, and mammary glands.

Stages of fatty acids synthesis

1. **Transport of Acetyl CoA and NADPH into cytoplasm.**
2. **Conversion of Acetyl CoA to Malonyl CoA.**
3. **Reactions of Fatty acid synthase complex.**

1- Transport of Acetyl CoA to Cytoplasm



The starting material for de novo synthesis is **acetyl CoA**. It is formed inside the mitochondria from pyruvate or as a result of beta oxidation. The inner membrane is not freely permeable to acetyl CoA. Hence the acetyl CoA units are **delivered to the cytoplasm as citrate** (Fig.). Citrate is transported from mitochondria by a **tricarboxylic acid transporter**. In the cytoplasm, citrate is cleaved to oxaloacetate and acetyl CoA in the cytoplasm. The enzyme is **ATP citrate lyase**. The oxaloacetate can return to the mitochondria as malate or pyruvate (Fig.).

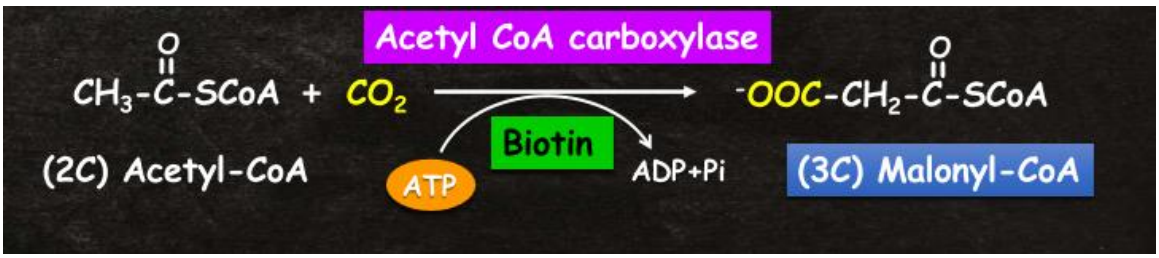
Note: The translocation of citrate to the cytosol occurs when the mitochondrial citrate concentration is high.

- The pathway of biosynthesis is not exactly reverse as β oxidation.
- Fatty acid breakdown and biosynthesis occur into different compartments of cells, utilized by different pathways & catalysed by different enzymes

Enzymes & co factors of fatty acids synthesis	
Two main enzymes;	**Co factors;**
1. acetyl CoA carboxylase (ACC) **2. fatty acid synthase**	**1-Biotin** **2-NADH** **3-Mg^{+}**

Step 1. Carboxylation of acetyl coenzyme A to malonyl coenzyme A

The carboxylation of acetyl CoA **(2C)** to form malonyl CoA **(3C)** is catalyzed by **acetyl CoA carboxylase (ACC)**, and requires CO_2 and ATP. **The coenzyme is the vitamin biotin**. **Acetyl CoA carboxylase ACC** is the rate-limiting enzyme.



- Biotin, a member of vitamins B complex, is necessary for this reaction.
- Prolonged consumption of a diet containing excess calories (particularly high-calorie, high- carbohydrate diets) causes an increase in ACC synthesis, thereby increasing fatty acid synthesis.
- Conversely, a low-calorie or a high-fat diet causes a reduction in fatty acid synthesis by decreasing ACC synthesis. The elongation of the fatty acid occurs by addition of 2 carbon atoms at a time. But the 2-carbon units are added as 3-carbon, malonyl units.

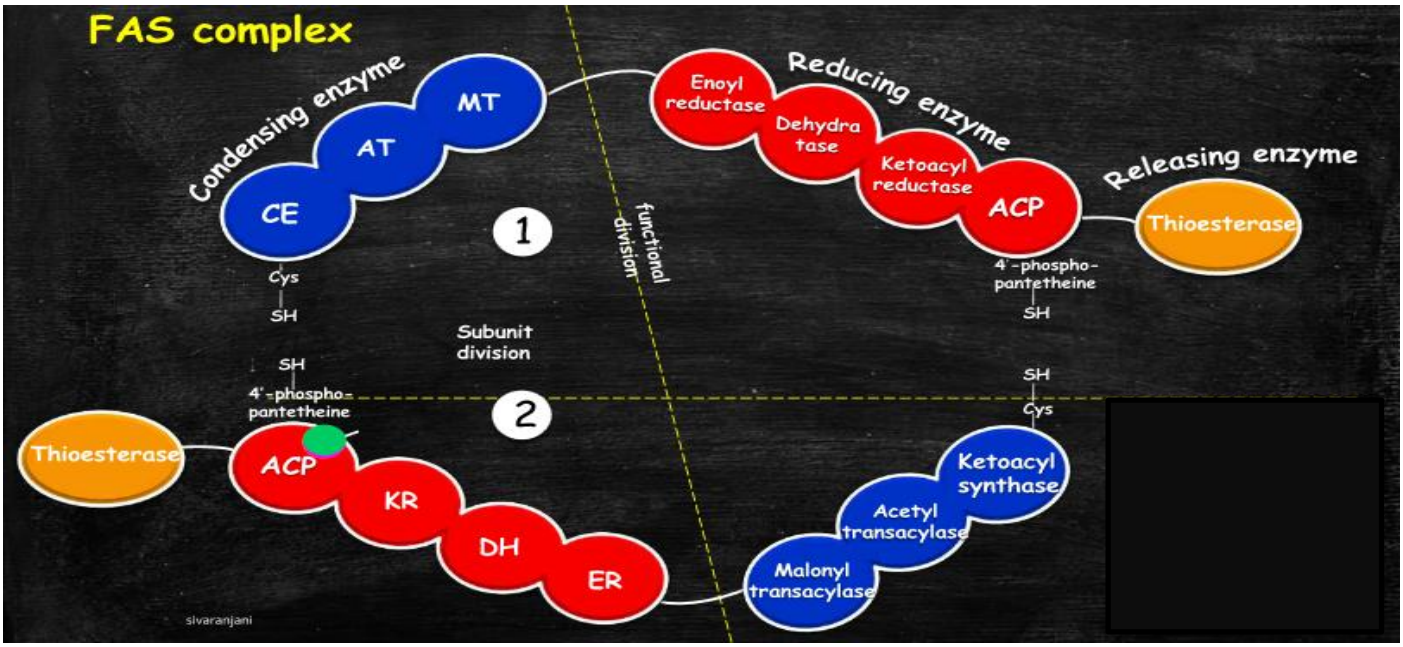
Reactions of Fatty acid synthase complex.

The enzyme exists as a multi-enzyme complex, and it is formed from a dimer with identical subunits. Each subunit is organized into 3 domains with 7 enzymes. The Subunits independently operate & both synthesize FA simultaneously. The enzyme subunits lie in Antiparallel (head to tail) orientation (fig. below).

1st Domain or Condensing Unit (initial substrate binding site); Beta-keto acyl synthase or Condensing enzyme (CE); Acetyl transferase (AT); and Malonyl trans acylase (MT).

2nd Domain or Reduction Unit - Dehydratase (DH); Enoyl reductase (ER); Beta-keto acyl reductase (KR) and Acyl carrier protein (ACP).

3rd Domain or Releasing Unit - release the FA synthesised. Thioesterase (TE) or Deacylase.

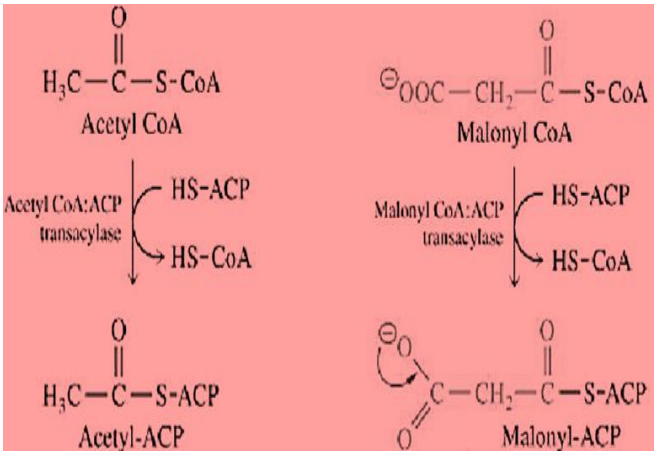


Activation

- Fatty acid synthesis starts with the formation of acetyl **ACP (Acetyl Carrier Protein)** and malonyl ACP.
- Acetyl transacylase and malonyl transacylase catalyze these reactions.

Acetyl CoA + ACP ↔ acetyl ACP + CoA

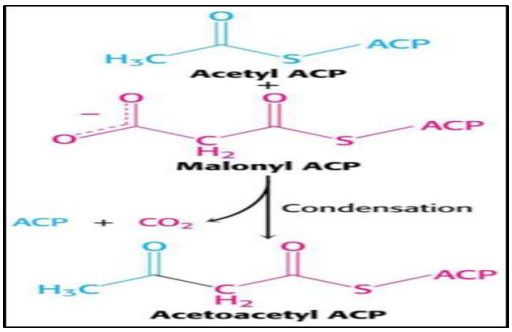
Malonyl CoA + ACP ↔ malonyl ACP + CoA



Reactions of fatty acid synthesis;

1. **Condensation reaction-**

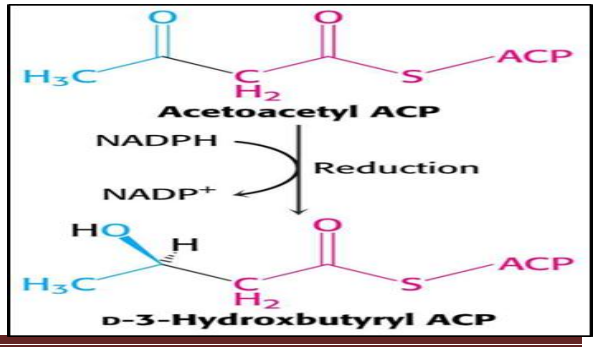
Acetyl ACP and Malonyl ACP reacts to form acetoacetyl ACP. Enzyme - acyl-malonyl **ACP condensing enzyme.**



2. **Reduction Reaction**

Acetoacetyl ACP is reduced to D-3-hydroxybutyryl ACP. NADPH is the reducing agent;

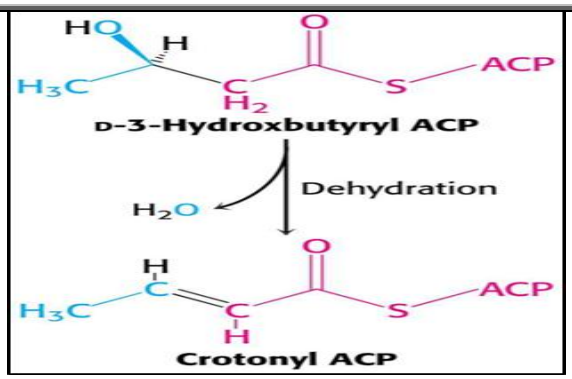
Enzyme: β-ketoacyl ACP reductase



3. **Dehydration Reaction**

D-3-hydroxybutyryl ACP is dehydrated to form crotonyl ACP (trans-Δ2-enoyl ACP).

Enzyme: 3-hydroxyacyl ACP dehydratase

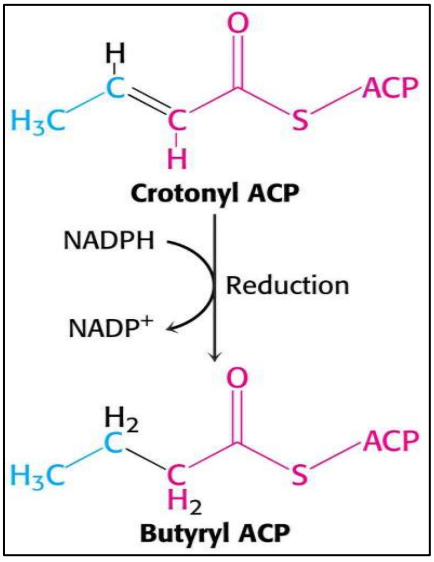


4. **Reduction Reaction**

The final step in the cycle reduces crotonyl ACP to butyryl ACP. NADPH is reductant. Enzyme - enoyl ACP reductase.

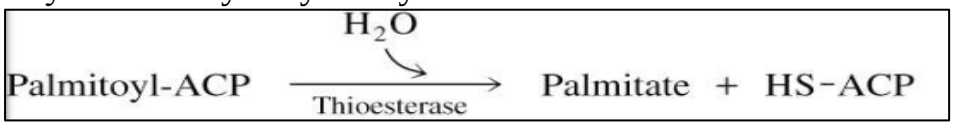
This is the end of first elongation cycle (first round).

In the second round butyryl ACP condenses with malonyl ACP to form a C6 -β-ketoacyl ACP. Reduction, dehydration, and a second reduction convert the C6 -β- ketoacyl ACP into a C6-acyl ACP, which is ready for a third round of elongation.



Termination

- Rounds of synthesis continue until a C16 palmitoyl group is formed
- Palmitoyl-ACP is hydrolyzed by **Thioesterase**

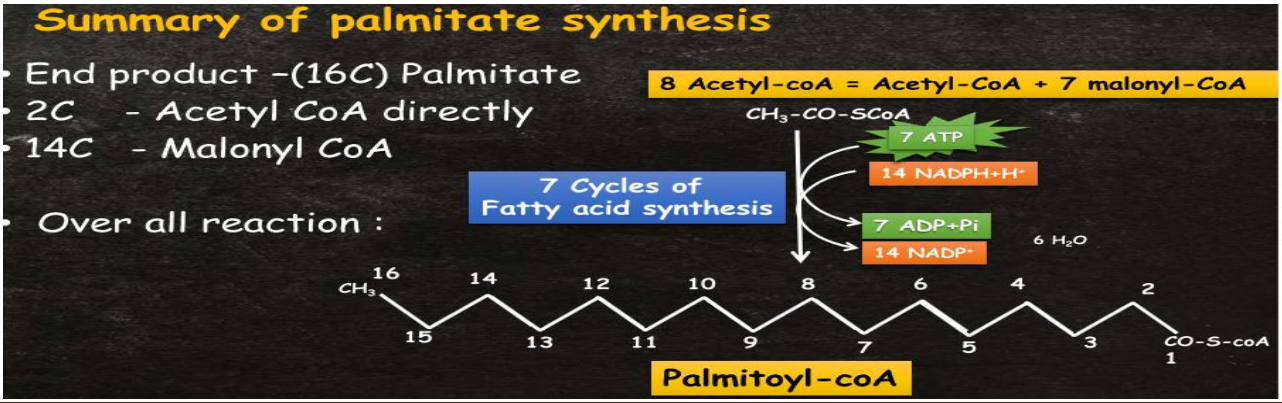


Net reaction;

8 acetyl-CoA + 14 NADPH + 7 ATP → palmitate + 14 NADP+ + 8 CoA + 7 ADP + 7 Pi

Over all Net Reaction-

acetyl-CoA + 7 malonyl-CoA + 14 NADPH → palmitate + 7 CO2 + 14 NADP+ + 8 CoA



Elongation of fatty acid chains

Although palmitate, a 16-carbon, fully saturated LCFA (16:0), is the primary end product of fatty acid synthase activity, it can be further elongated by the addition of two-carbon units to the carboxylate end in the smooth endoplasmic reticulum (SER).

Elongation requires a system of separate enzymes rather than a multifunctional enzyme. Malonyl CoA is the two-carbon donor, and NADPH supplies the electrons. The brain has additional elongation capabilities, allowing it to produce the very-long-chain fatty acids ([VLCFAs] over 22 carbons) that are required for synthesis of brain lipids.

Desaturation of fatty acid chains

Enzymes (desaturases) also present in the SER are responsible for desaturating LCFAs (that is, adding cis double bonds). The desaturation reactions require O_2, NADH, special reductase. The fatty acid and the NADH get oxidized as the O_2 gets reduced to H_2 O. The first double bond is typically inserted between carbons 9 and 10, producing primarily oleic acid, 18:1(9), and small amounts of palmitoleic acid, 16:1(9). A variety of polyunsaturated fatty acids can be made through additional desaturation combined with elongation.

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 the nutritional essentiality of the polyunsaturated acids ω-6 linoleic and ω-3 linolenic.

Synthesis of triacylglycerols

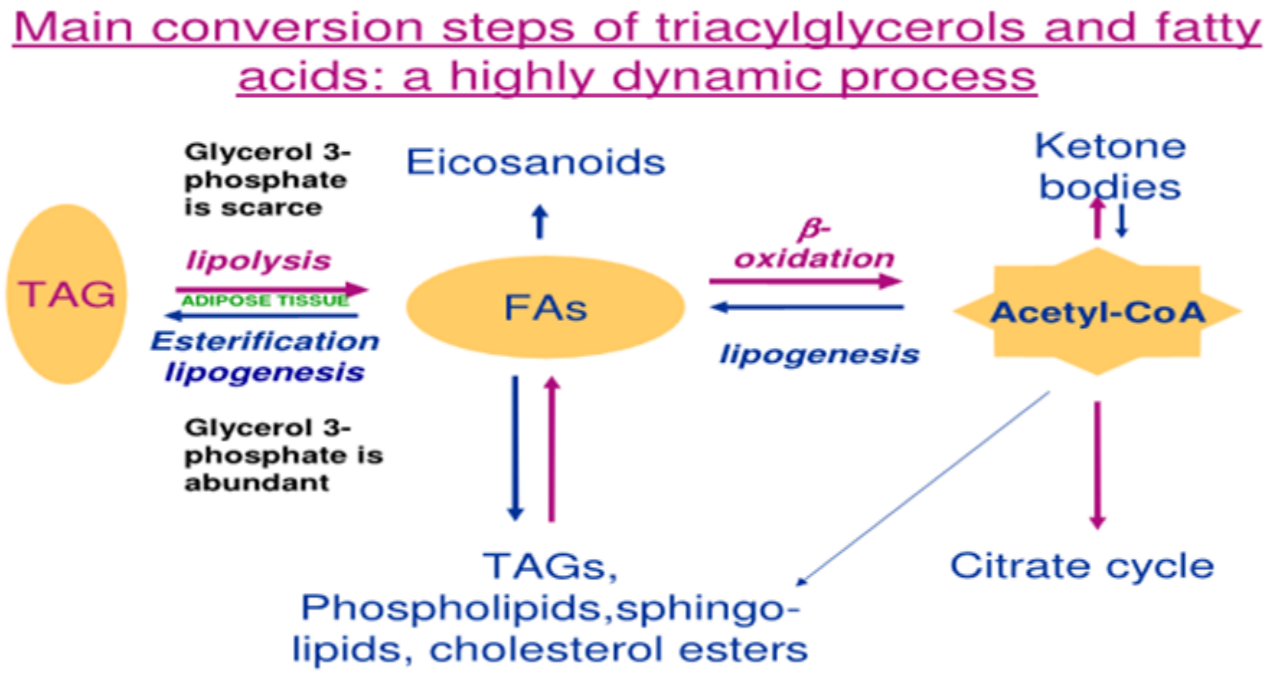
Triacylglycerol are esters of glycerol with fatty acids. Liver and adipose tissue are the major sites of triacylglycerol (TAG) synthesis. The TAG synthesis in adipose tissue is for storage of energy whereas in liver it is mainly secreted as VLDL and is transported. The TAG is synthesized by esterification of fatty acyl CoA with either glycerol-3-phosphate or dihydroxy acetone phosphates (DHAP) **both are products of glycolysis.**

Metabolism of adipose tissue

The adipose tissue serves as a storage site for excess calories ingested. The triglycerides stored in the adipose tissue are not inert. They undergo a daily turnover with new TGL molecules being synthesized and a definite fraction being broken down.

Under well-fed conditions, active lipogenesis occurs in the adipose tissue. The dietary triglycerides transported by chylomicrons and the endogenously synthesized triglycerides from liver brought by VLDL are both taken up by adipose tissue and esterified and stored as TAG. The lipoprotein molecules are broken down by the lipoprotein lipase present on the capillary wall. Insulin also causes inhibition of hormone sensitive lipase, and so lipolysis is decreased.

In fasting, TAG from the adipose tissue is mobilized under the effect of the hormones, glucagon and epinephrine. The cyclic AMP mediated activation cascade enhances the intracellular hormone sensitive lipase. The later enzyme acts on TAG and liberates fatty acids. Under conditions of starvation, a high glucagon, ACTH, glucocorticoids and thyroxin have lipolytic effect. The released free fatty acids (FFA) are taken up by peripheral tissues as a fuel.



The end