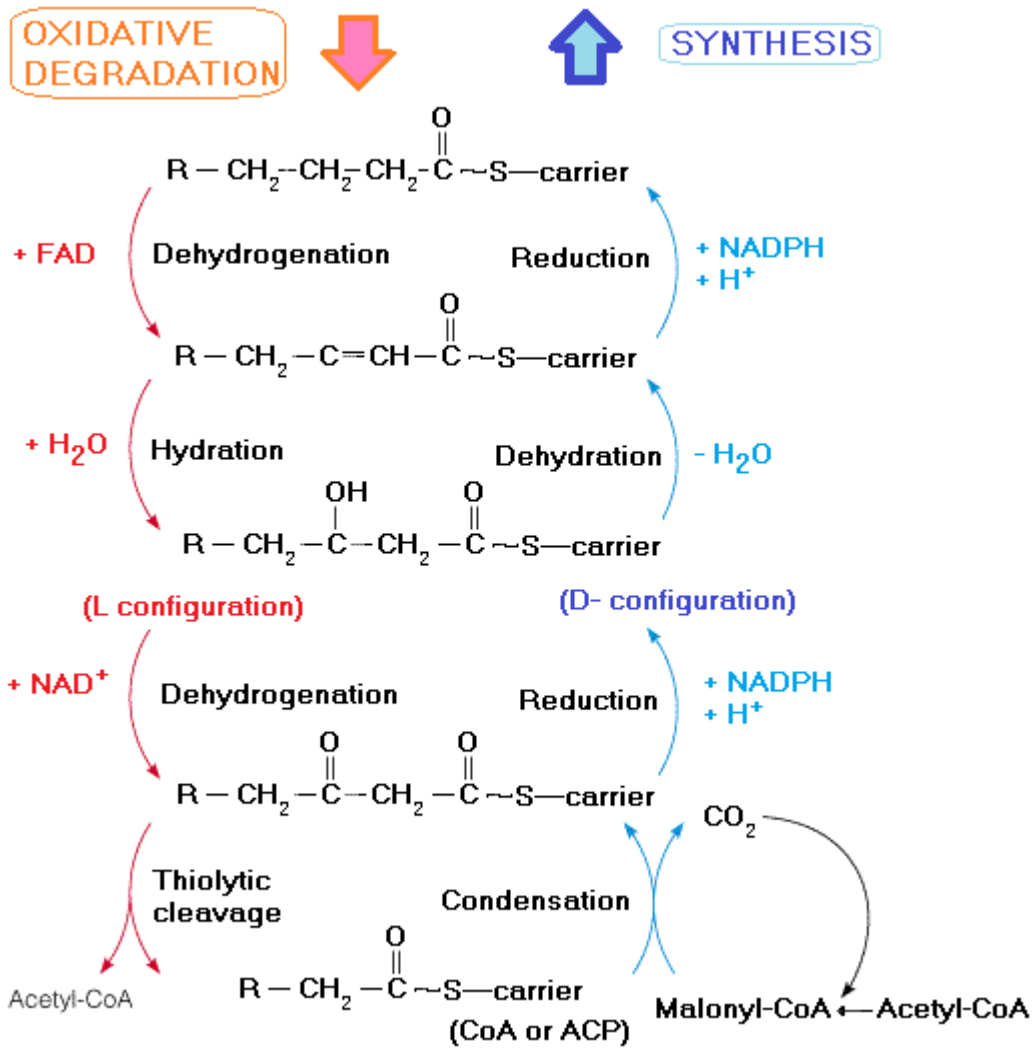
Fatty Acid Biosynthesis

Fatty acid biosynthesis occurs through steps similar to Beta-oxidation: acetyl groups are added to a growing chain just as they are cut off in the degradation. Nevertheless, the mechanism of the pathway is distinctly different from being simply the reverse of Beta-oxidation, as it is shown in the diagram below



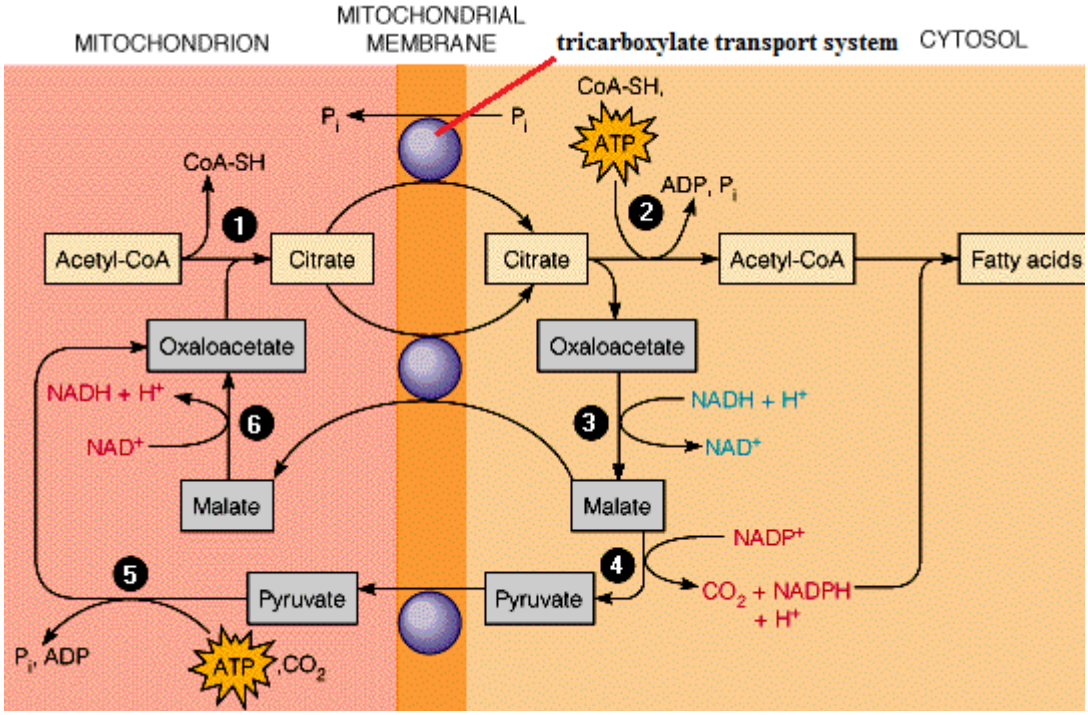
As we will see, fatty acid biosynthesis can be broken in to three separate pathways:
1. Synthesis of palmitate from acetyl-CoA
2. Elongation of palmitate
3. Desaturation

Transport of Mitochondrial Acetyl-CoA into the Cytosol

Acetyl-CoA is produced in two ways in the mitochondria:

- by Beta-oxidation of fatty acids,
- by combined action of **pyruvate dehydrogenase** (to decarboxylate pyruvate, producing acetate) and **dihydrolipoyl transacetylase** (to add the CoA to the acetate).

Acetyl CoA will accumulate when the Electron Transport Chain/Oxidative phosphorylation slows (why? - **a good question**). Under these conditions, acetyl-CoA is transported out of the mitochondrion to the cytosol where it can be used in fatty acid synthesis. This is accomplished using the **tricarboxylate transport system** in the inner mitochondrial membrane, which pumps citrate out.



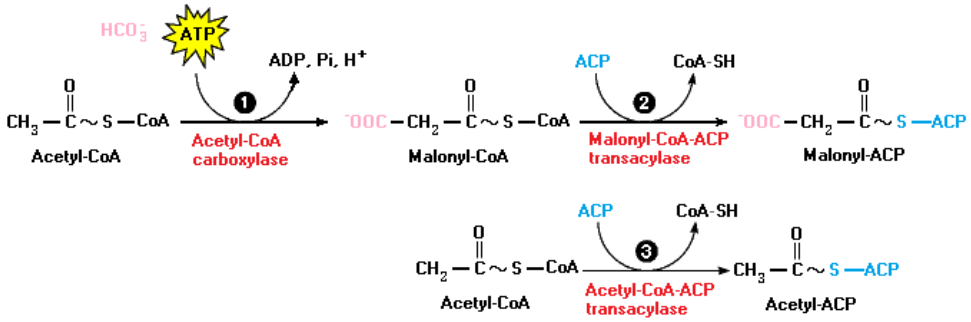
To summarize:

1- Acetyl-CoA can accomplish the synthesis of citrate when combined with oxaloacetate (inside the matrix).
2- Once transferred into the cytosol, citrate is broken back to oxaloacetate and acetyl-CoA by **ATP-citrate lyase** (using ATP and CoA).
3- Oxaloacetate can be reduced to malate by malate-dehydrogenase and NADH.
4- Malate can be converted to pyruvate by malic enzyme and NADP+.
5- The resulting pyruvate is permeable to the inner mitochondrial membrane and diffuses in. Inside the mitochondrion, pyruvate can be converted to oxaloacetate by pyruvate carboxylase (along with bicarbonate ion, and ATP), completing the cycle.
6- An alternative path is to transport malate across the inner membrane and convert it to oxaloacetate.

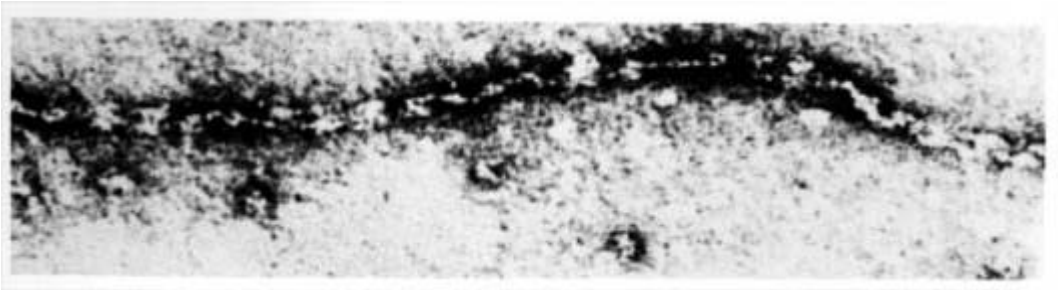
(Try to point out the steps in common with gluconeogenesis and TCA cycle)

Acetyl-CoA Carboxylase

The first committed step of fatty acid biosynthesis is catalyzed by Acetyl-CoA carboxylase. The enzyme contains biotin, and adds a CO_2 (resulting in a carboxyl group) to the methyl end of acetyl CoA (Reaction 1).



Note that this reaction is an energy requiring process (1 ATP per Malonyl-CoA formed). Acetyl-CoA carboxylase is an interesting enzyme. Studies of the enzyme from birds and mammals indicate that it forms long linear polymers.

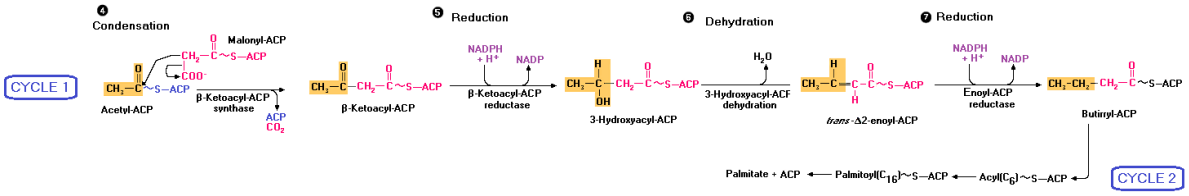


The polymer appears to be the active form of the enzyme. Monomeric units are inactive.
Citrate shifts the polymer - monomer equilibrium towards polymer formation.
Palmitoyl-CoA shifts the equilibrium towards monomer formation.

Another regulation of Acetyl-CoA carboxylase is by hormones.
Glucagon, epinephrine, and norepinephrine trigger a cAMP dependent phosphorylation (**remember the cascade system**) of the enzyme, that shifts the equilibrium towards monomer formation.
Insulin, conversely, stimulates desphosphorylation, favoring polymerization. The enzymes responsible for phosphorylating Acetyl-CoA carboxylase are **cAMP-dependent protein kinase** and **AMP-dependent protein kinase** (AMPK).

Fatty Acid Synthase

This multifunctional enzyme catalyzes the seven different reactions whereby two carbon units from malonyl-CoA are linked together, ultimately to form palmitoyl-CoA.
The dimeric form is the fully functional form of the enzyme. The overall synthesis of palmitate from acetyl-CoA requires 14 NADPHs, and 7ATPs.
Steps of fatty acid synthesis starting with Acetyl-CoA and Malonyl-CoA are as follows:



- Transfer of the malonyl group of malonyl-CoA to ACP (not in the above scheme - catalyzed by malonyl-CoA-ACP transacylase).
- Transfer of the acetyl group of Acetyl-CoA to ACP (not in the above scheme - catalyzed by acetyl-CoA-ACP transacylase).
- Addition of an acetyl group from malonyl-ACP between the thioester bond of the acetyl-ACP molecule (Reaction 4 - catalyzed by Beta-keto-ACP synthase - also called condensing enzyme).
- Reduction of the Beta-keto group to a Beta-hydroxyl group with NADPH (Reaction 5 - catalyzed by Beta-keto-ACP reductase).
- Dehydration between the alpha and Beta carbons (Reaction 6 - catalyzed by Beta-hydroxyacyl-ACP dehydrase).
- Reduction of the trans-double bond by NADPH (Reaction 7 - catalyzed by enoyl-ACP reductase).

All that is followed by the repetition of steps 2-6 six more times. The acetyl group of reaction 1 is replaced by the growing acyl-ACP molecule. (That is, new acetyl groups are added at the ACP end of the molecule).

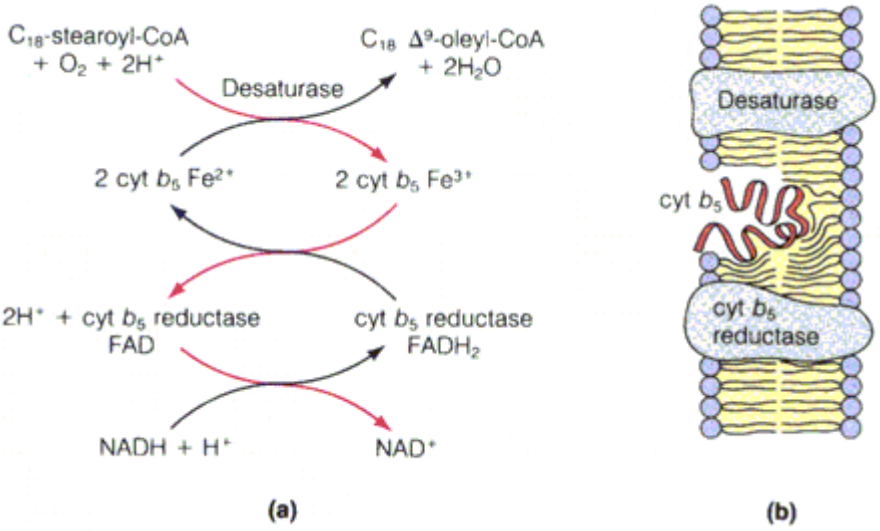
The product of this series of reactions, palmitoyl-ACP can be cleaved to palmitate and ACP by the enzyme palmitoyl thioesterase.

Elongation of Palmitate

The product of fatty acid synthase action, palmitate (C_{16}), is but one of many fatty acids synthesized by cells. Elongases are enzymes that act to lengthen palmitate to produce many of the other fatty acids. Elongases are present in mitochondria and the endoplasmic reticulum.
Elongation using elongase in the mitochondrion involves a mechanism that is essentially the reverse of Beta-oxidation except substitution of NADPH for $FADH_2$ in the last reaction.

Desaturation of Fatty Acids

Terminal desaturases produce unsaturated fatty acids. One such enzyme is fatty acyl-CoA desaturase. The reaction catalyzed by this enzyme on a stearoyl-CoA is shown below.



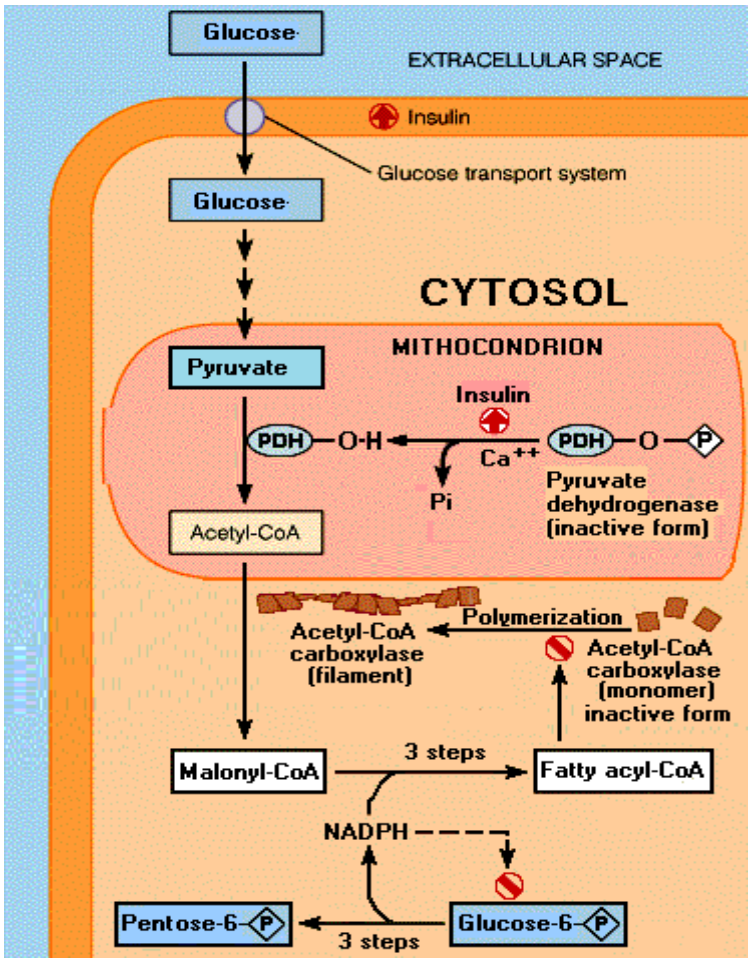
Electrons from NADH are ultimately passed to oxygen, forming water. The energy released in this process drives oxidation of stearoyl-CoA to oleyl-CoA.
From the free methyl end, mammals cannot make double bonds closer to the end than the Delta-9 position (Oleic acid is a Delta-9 fatty acid). Thus, linoleic acid (Delta 9,12 double bonds) and linolenic acid (Delta 9,12,15 double bonds) must be provided in the diet of mammals, and are called essential fatty acids.

Control of Fatty Acid Synthesis

Like all metabolic pathways, cells must have appropriate controls on fatty acid metabolism to be able to meet energy needs.
Precursors for energy generation - triacylglycerols, fatty acid/albumin complexes, ketone bodies, amino acids, lactate, and glucose - are all carried in the blood as needed for various tissues.

One mechanism of regulation involves hormone release.
Signals received in the pancreas (glucose concentration) trigger production of hormones.
Low blood sugar triggers glucagon release.
High blood sugar triggers insulin release.
Both of latter systems control glucose-related metabolism as well.
Fatty acid synthesis is controlled partly by short term regulation (mechanisms include substrate availability, allosterism, covalent modification of enzymes) but mostly by long term regulatory mechanisms.

Long term regulation involves controlling the quantity of enzyme by controlling the rate with which a protein is synthesized and/or degraded. A simple scheme depicting fatty acid biosynthesis regulation is shown in the scheme below



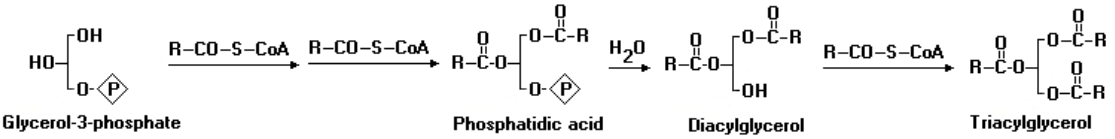
One of the reasons fats do not supply emergency energy is that control of their metabolism is largely by long term regulatory mechanisms whereas control of sugar metabolism is more prominent under short term regulatory mechanisms.
Insulin stimulates increased synthesis of acetyl-CoA carboxylase and fatty acid synthase (two critical enzymes for synthesizing fatty acids).
Starvation, conversely decreases synthesis of these enzymes.

Fatty acid oxidation is regulated by fatty acid concentration in the blood. This is controlled by the amount of hydrolysis of triacylglycerols in adipose tissue by hormone-sensitive **triacylglycerol lipase** (HSTL). This enzyme is phosphorylated in the hormonally-controlled cAMP-dependent phosphorylation cascade, which activates the lipase, stimulating release of fatty acids.
This cascade is turned on by the cell's binding of glucagon or epinephrine.
It should also be noted that the cAMP-dependent phosphorylation system also causes inactivation of acetyl-CoA carboxylase, an important control enzyme in fatty acid biosynthesis. Insulin opposes the effects produced by glucagon and epinephrine, stimulating glycogen formation and triacylglycerol synthesis, by favoring dephosphorylation of the enzymes phosphorylated as described above.

Synthesis of Triacylglycerols

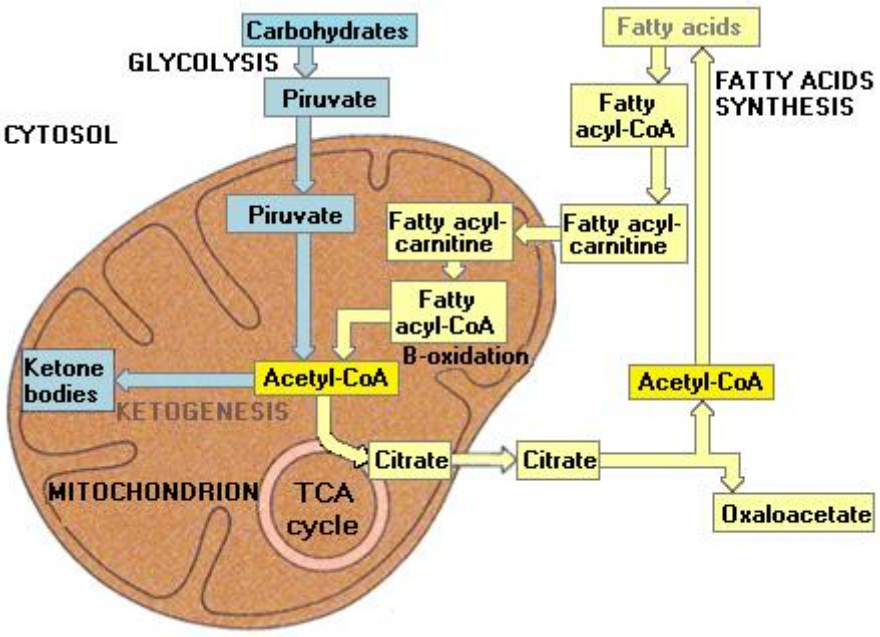
Synthesis of fatty acids is only half of the process of making triacylglycerols. In the first part of the process, a fatty acyl-CoA is linked to carbon #1 of dihydroxyacetone phosphate (DHAP) or glycerol-3-phosphate (Gly3P) by either dihydroxyacetone phosphate acyltransferase (for DHAP) or glycerol-3-phosphate

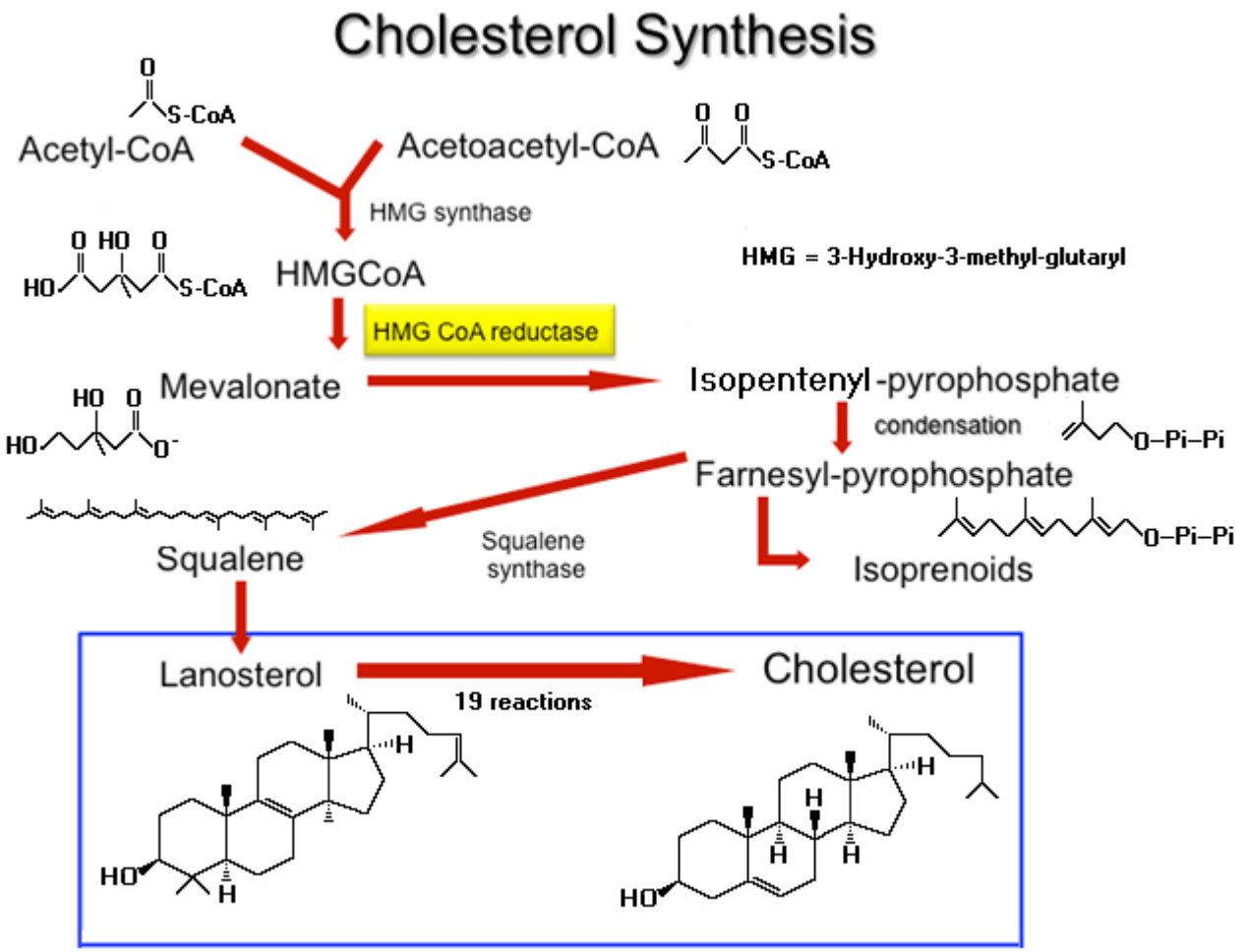
acyltransferase (for Gly3P) (The picture shows the reaction for Gly3P). The product of the reaction for Gly3P is lysophosphatidic acid.



The product of the DHAP reaction (acyl-dihydroxyacetone phosphate) can be converted to the product of the Gly3P reaction by NADPH and acyl-dihydroxyacetone phosphate reductase.
Lysophosphatidic Acid is acylated (with an acyl-CoA) at carbon #2 by 1-acylglycerol-3-phosphate acyltransferase to produce phosphatidic acid.
This intermediate can be converted to other phospholipids (such as phosphatidyl choline) or converted to triacylglycerols.
Phosphatidic acid is thus an important branch point between triacylglyerol biosynthesis and glycerophospholipid biosynthesis.
The phosphate of phosphatidic acid is removed by phosphatidic acid phosphatase, forming diacylglycerol.
Diacylglycerol is converted to triacylglycerol (with acyl-CoA, of course) by diacylglycerol transferase.

MAP OF LIPID MOLECULES PRODUCTION AND DEGRADATION





All animal cells manufacture cholesterol for their use, with relative production rates varying by cell type and organ function. About 20–25% of total daily cholesterol production occurs in the liver.
Synthesis within the body starts with one molecule of acetyl CoA and one molecule of acetoacetyl-CoA, which are hydrated to form 3-hydroxy-3-methylglutaryl CoA (HMG-CoA).
This molecule is then reduced to mevalonate by the enzyme HMG-CoA reductase. This is the regulated, rate-limiting and irreversible step in cholesterol synthesis and is the site of action for the statin drugs (HMG-CoA reductase competitive inhibitors).
Mevalonate is then converted to 3-isopentenyl pyrophosphate in three reactions that require ATP.
Mevalonate is decarboxylated to isopentenyl pyrophosphate, which is a key metabolite for various biological reactions.
Three molecules of isopentenyl pyrophosphate condense to form farnesyl pyrophosphate through the action of geranyl transferase.
Two molecules of farnesyl pyrophosphate then condense to form squalene by the action of squalene synthase in the endoplasmic reticulum.
Oxidosqualene cyclase then cyclizes squalene to form lanosterol.
Finally, lanosterol is converted to cholesterol through a 19-step process.

Biosynthesis of cholesterol is directly regulated by the cholesterol levels present, though the homeostatic mechanisms involved are only partly understood.
A higher intake from food leads to a net decrease in endogenous production, whereas lower intake from food has the opposite effect.