At times when the diet supplies more carbohydrate and protein than is required for routine maintenance of body protein and glycogen stores, the excess is converted first to acetyl-CoA, then to fatty acids, and finally to triglyceride (fat). The acetyl-CoA produced in catabolism arises within the mitochondrion, but *de novo* synthesis of fatty acids (from acetyl-CoA) occurs in the cytosol. Again, since the mitochondrial membrane is impermeable to CoA and CoA derivatives, a shuttle system must be used to transport the substrate; in this case the carrier used is citrate, the intermediate in the citric acid cycle.

1. **Generation of cytosolic acetyl-CoA** from mitochondrial acetyl-CoA (Fig. 11-14, p164).

2. **Formation of malonyl-CoA** from acetyl-CoA by acetyl-CoA carboxylase (see Fig. 11-16).

Acetyl-CoA carboxylase catalyzes the synthesis of malonyl-CoA from Acetyl-CoA, ATP & HCO$_3^-$.

This is the rate-limiting reaction in fatty acid biosynthesis.

This step represents the principal control point in regulation of the rate of fatty acid synthesis. The enzyme is active when in the form of a filamentous polymer of many subunits (each a protomer). Citrate leads to aggregation of protomers and thus to activation. Palmitoyl-CoA (an end product), leads to dissaggregation and thus inactivation. Phosphorylation (resulting from glucagon binding to the plasma membrane) also leads to inactivation of the enzyme. Long-term regulation involves an increase in enzyme concentration in response to insulin.
3. Palmitate synthesis by the fatty acid synthase complex:

Note that in higher animals, the active enzyme exists as a **dimer**, each subunit of which contains all the enzyme activities necessary for palmitate synthesis from malonyl-CoA and one molecule of acetyl-CoA (acts as a "primer"). Each enzyme subunit is "polyfunctional", with separate domains that perform several different enzymatic functions. The dimer structure is required because during synthesis, the growing fatty acyl chain is "handed off" periodically to the opposing subunit for "temporary parking" while the original subunit "reloads" with another malonyl group for addition to the growing carbon chain.

Acyl groups are carried on 2 protein-bound thiol groups: a "central" –SH on the phosphopantetheine group of the **acyl carrier protein** (ACP) and a "peripheral" cysteiny1-SH group which is part of the β-ketoacyl synthase activity (KS). For the purpose of this discussion, assume that everything could take place on one polypeptide (other than carboxylation of acetyl-CoA).
4. Elongation and desaturation - (see Figs 11-18 & 11-19, p. 168)

In the ER (one of the most important sites for the reaction), elongation also requires malonyl-CoA as substrate. Two-carbon units are sequentially added to the carboxyl end of the acyl-CoA (not an acyl-ACP). NADPH is the reductant.

Some elongation also takes place within the mitochondrion, but acetyl-CoA is used as donor and NADH is one reductant, with NADPH (in place of FADH₂) as the other in what is nearly, but not quite, a reversal of β-oxidation.

Desaturation occurs in the ER, requires molecular oxygen, NADPH, cytochromes, and several proteins. Double bonds may be added at Δ9 or closer to the carboxyl, but not beyond C9.

5. Energy cost: Acetyl-CoA to palmitate

ATP is required (one for each 2-carbon fragment).

One ATP is used for each cytosolic citrate cleaved by ATP citrate lyase to acetyl-CoA & oxaloacetate, i.e., 8 total ATP required (one for each 2-carbon fragment).

One ATP is used for formation of each of the seven malonyl-CoA needed, i.e., 7 more ATP.

Seven "reduction spirals" are required, each of which uses 2 NADPH, i.e., 14 NADPH. Assuming each NADPH might otherwise be used to produce 2.5 ATP, the total cost here would be 14 x 2.5 + 35 ATP, or a grand total of 8 + 7 + 35 = 50 ATP per palmitate!! How could anybody afford to get fat???

Glucose conversion to palmitate:

(4.5) Glucose (27 C) + 15.5 ATP => (1) palmitate (16 C) + 11 CO₂ + 8 NADH (=> 20 ATP)

i.e. a "profit" of 4.5 ATP per palmitate formed from glucose

or 4.5 ATP/4.5 glucose = one extra ATP formed per glucose converted to palmitate.

*italics* = input and *Bold* = output
6. Triglyceride Biosynthesis: (see Fig. 11-20, p. 169 & 11-21, p. 170)

Triglyceride Synthesis

There are 2 pathways for triglyceride biosynthesis

1. The **general pathway** operates in the liver and other organs where fatty acid biosynthesis occurs.

2. The **intestinal pathway** is responsible for the resynthesis of triglyceride following digestion and absorption of dietary triglyceride.

![Diagram of Triglyceride Biosynthesis](image-url)