ANSC/NUTR 618
Lipids & Lipid Metabolism
Fatty Acid Synthesis

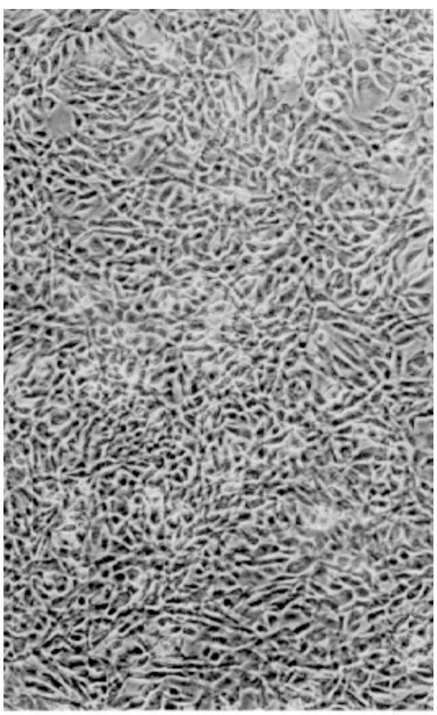
I. Overall concepts

A. Definitions

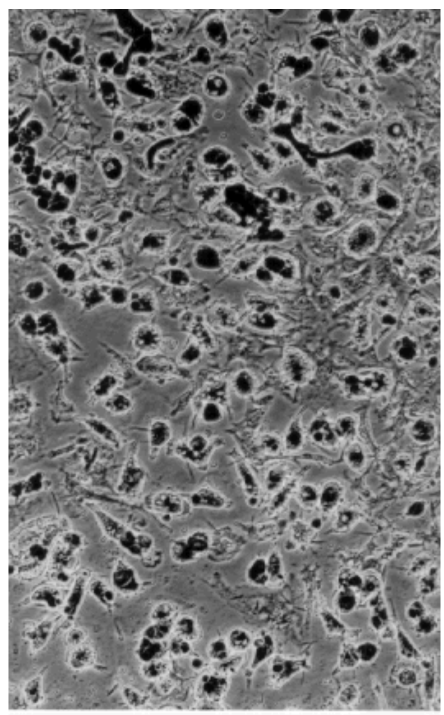
1. *De novo* synthesis = synthesis from non-fatty acid precursors
   a. Carbohydrate precursors (glucose, lactate, and pyruvate)
   b. Amino acid precursors (e.g., alanine, branched-chain amino acids)
   c. Short-chain organic acids (e.g., acetate, propionate)
2. *Lipogenesis* = fatty acid *or* triacylglycerol synthesis
   a. From preformed fatty acids (from diet or *de novo* fatty acid synthesis)
   b. Requires source of carbon for glycerol backbone

B. Tissue sites of *de novo* fatty acid biosynthesis

1. ***Liver.*** In birds, fish, humans, and rodents. In these species, lipids must be transported from the liver to the adipose tissue to increase fat mass.
2. ***Adipose tissue.*** All livestock species and *young* rodents.
3. ***Other tissues.*** Brain (and other nervous tissues) and the lungs.



3T3-L1 preadipocytes at confluence. No lipid filling has yet occurred.



3T3-L1 adipocytes after 6 d of differentiation. Dark spots are lipid droplets.

II. Substrates for fatty acid biosynthesis

A. ***General.*** Fatty acid biosynthesis requires a source of carbon (usually 2-carbon preursors) and reducing equivalents (i.e., NADPH).

B. ***Glucose.*** *All* species can utilize glucose to some extent.

1. *Nonruminants*. Glucose also is essential for lipogenesis from acetate (to provide G3P and NADPH via the pentose cycle).
2. *Ruminants.* Glucose is incorporated into fatty acids at about 1/10th the rate seen for acetate or lactate.

C. ***Acetate.*** *All* species can utilize acetate to some extent.

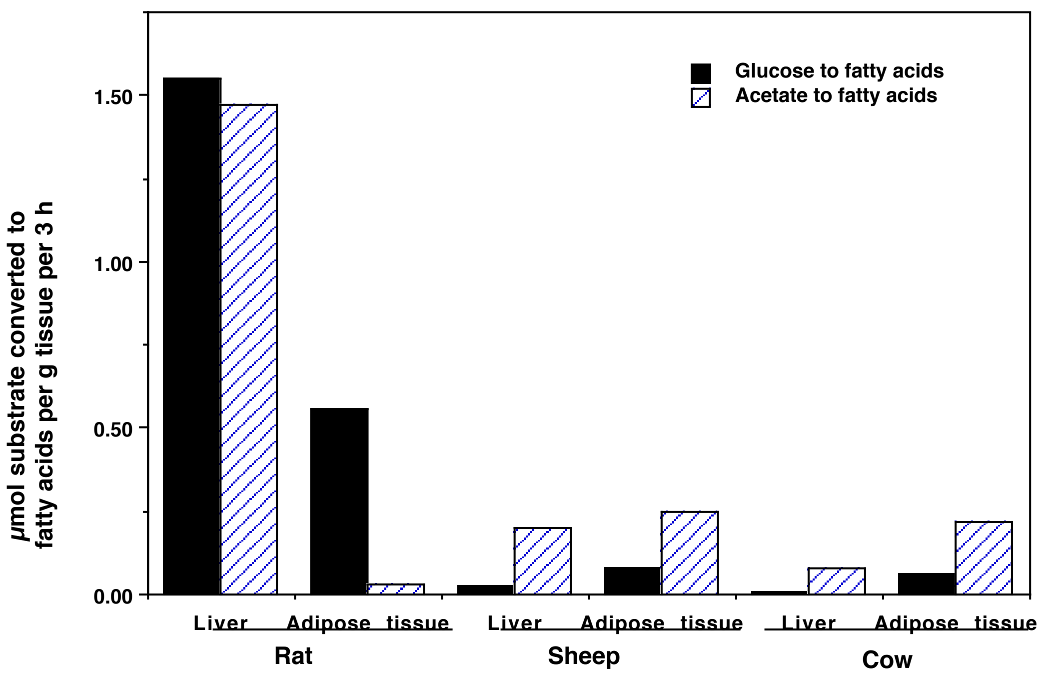
1. *Nonruminants*. If incubated in the presence of glucose, acetate is incorporated into fatty acids at high rates. Virtually no fatty acid synthesis occurs from acetate in the absence of glucose.
2. *Ruminants*. Ruminants have evolved to effectively utilize acetate.

D. ***Lactate.*** *All* species utilize lactate very effectively.

E. ***Propionate.*** This is important only in ruminants.

F. ***Acetate, lactate, and glucose in combination.***

1. Acetate inhibits lipogenesis from lactate and glucose.
2. Acetate provides > 80% carbons to lipogenesis, lactate 10-20% and glucose < 5%.



Rates of conversion of glucose and acetate to fatty acids in liver and adipose tissue of rat, sheep, and cows.

III. Pathways of fatty acid biosynthesis

A. ***Glucose.*** Most of the carbon from glucose enters fatty acid synthesis via glycolysis and the production of pyruvate.

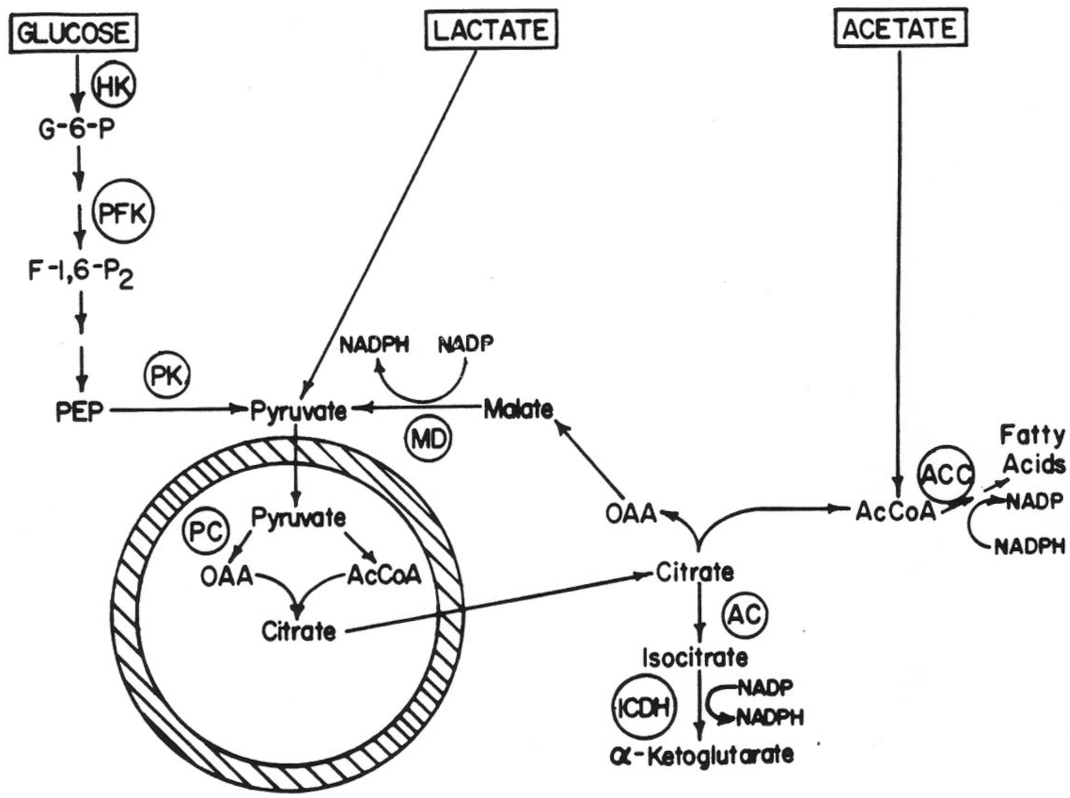
1. Pyruvate enters the mitochondria and is converted to both OAA and AcCoA, which form citrate.
2. The citrate exits the mitochondria and is hydrolyzed by ATP-citrate lyase.
3. The AcCoA is utilized for fatty acid synthesis.
4. The OAA is reduced to malate, when then is oxidatively decarboxylated (by NADP-malate dehydrogenase) back to pyruvate. This cycle can produce about 1/2 the NADPH required for fatty acid biosynthesis from glucose.

B. ***Acetate.*** Acetate is converted to AcCoA in the cytoplasm.

C. ***Lactate.*** Follows the same pathway as glucose; enters the pathway at pyruvate.

D. ***Propionate***

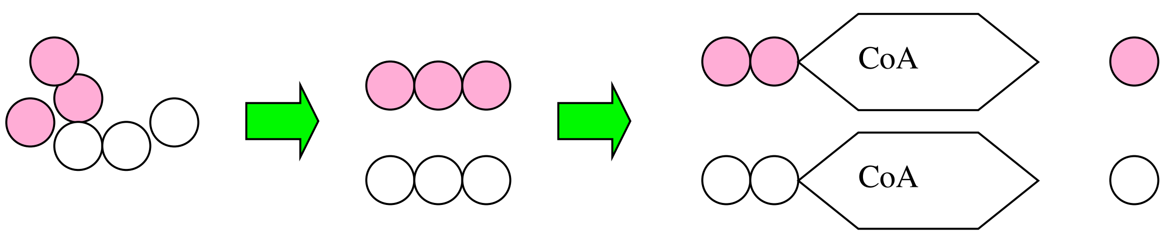
1. Propionate enters the fatty acid biosynthetic pathway after conversion to succinyl-CoA.
2. Fatty acid synthesis that incorporates propionate produces *odd-chained fatty acids.*



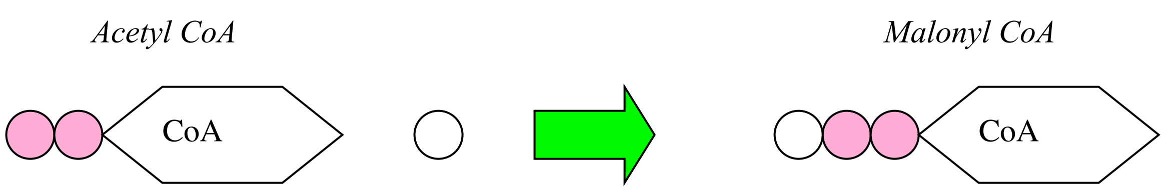
IV. The assembly of fatty acids

A. *Glycolysis and pyruvate dehydrogenase*

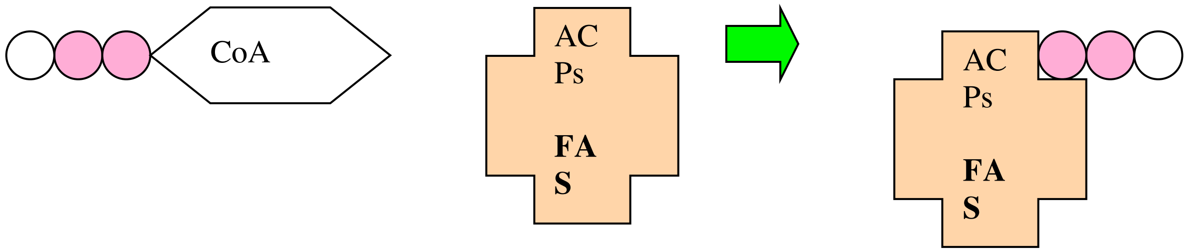
Glucose → 2 Pyruvate → 2 Acetyl CoA + 2CO_2



B. *AcCoA carboxylase*



C. *Fatty acid synthase*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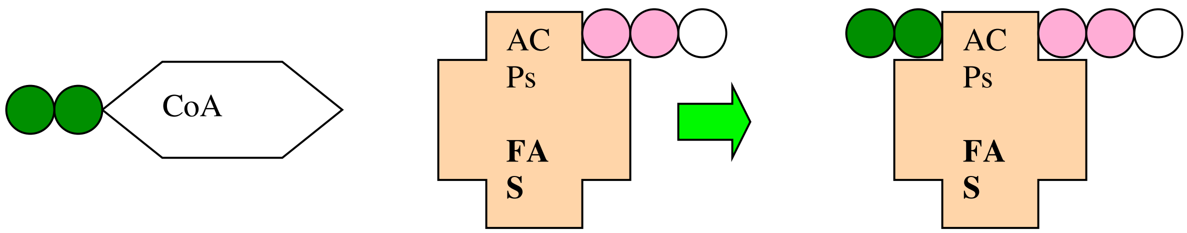
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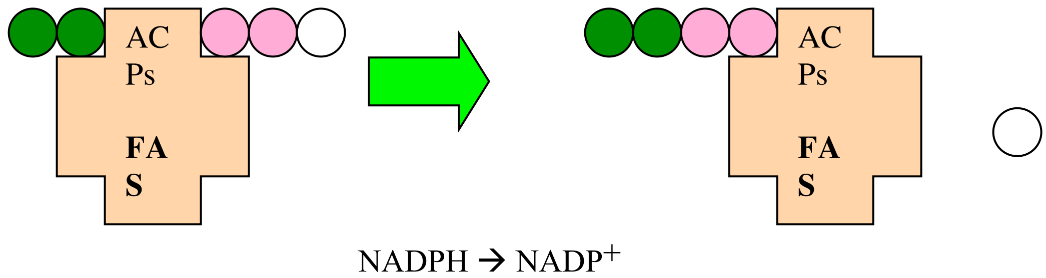
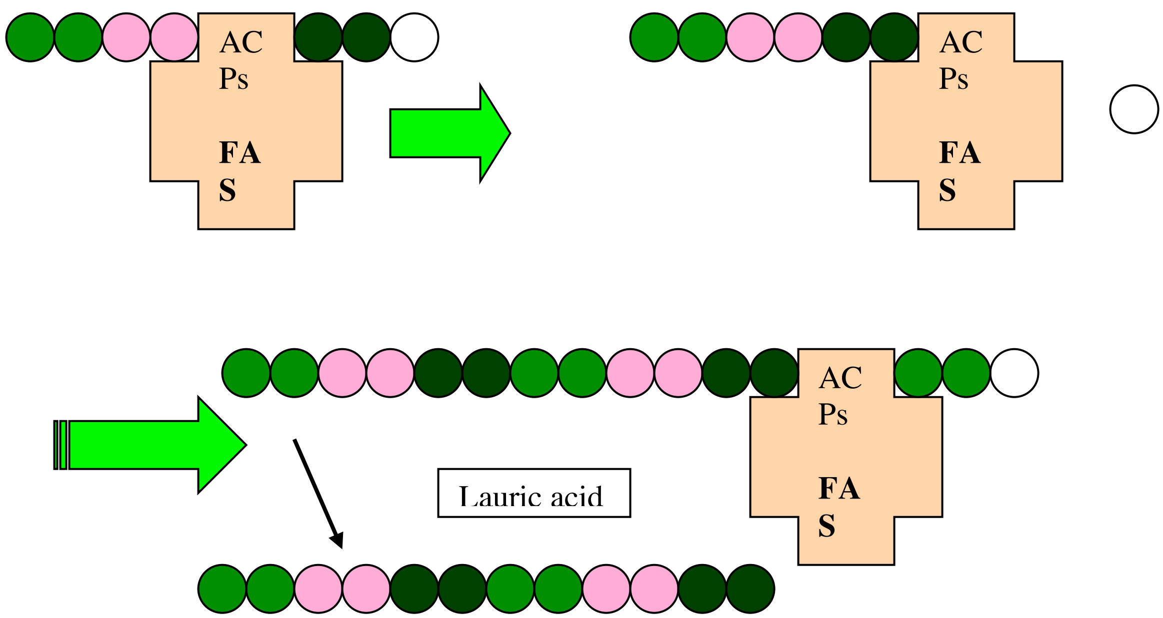
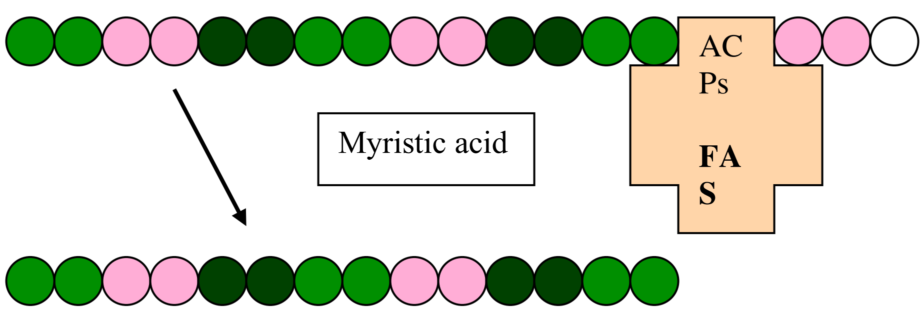


D. Elongation of fatty acids by fatty acid synthase

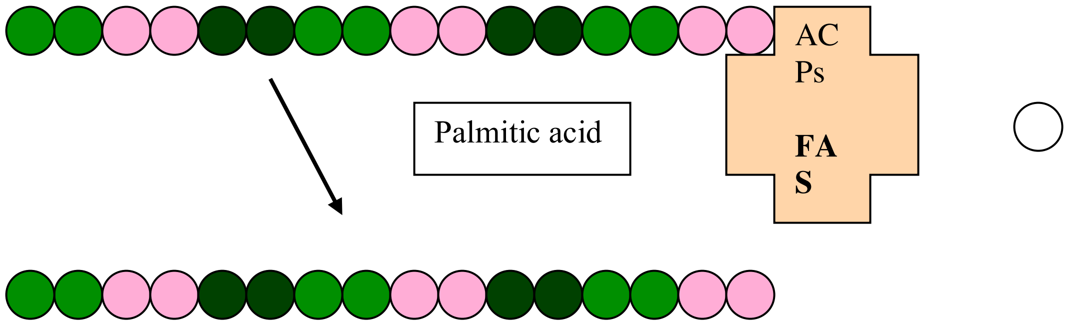
1. Lauric acid



2. Myristic acid



3. Palmitic acid (***final product of fatty acid synthase***)



V. Supporting pathways for fatty acid biosynthesis

A. ***Production of G3P.***

1. *Nonruminants.* G3P is provided by the metabolism of glucose (DHAP → G3P).

2. *Ruminants.* Glucose also is the primary source of G3P. However, to conserve glucose, ruminants very effectively convert lactate to G3P.

B. ***Production of NADPH.***

1. *Nonruminants.* Pentose cycle: 60% of the NADPH; malic enzyme: 40%.

2. *Ruminants*. Pentose cycle: 40-50% of the NADPH; malic enzyme: 10-20%; $NADP^+$-ICDH: 30-40%

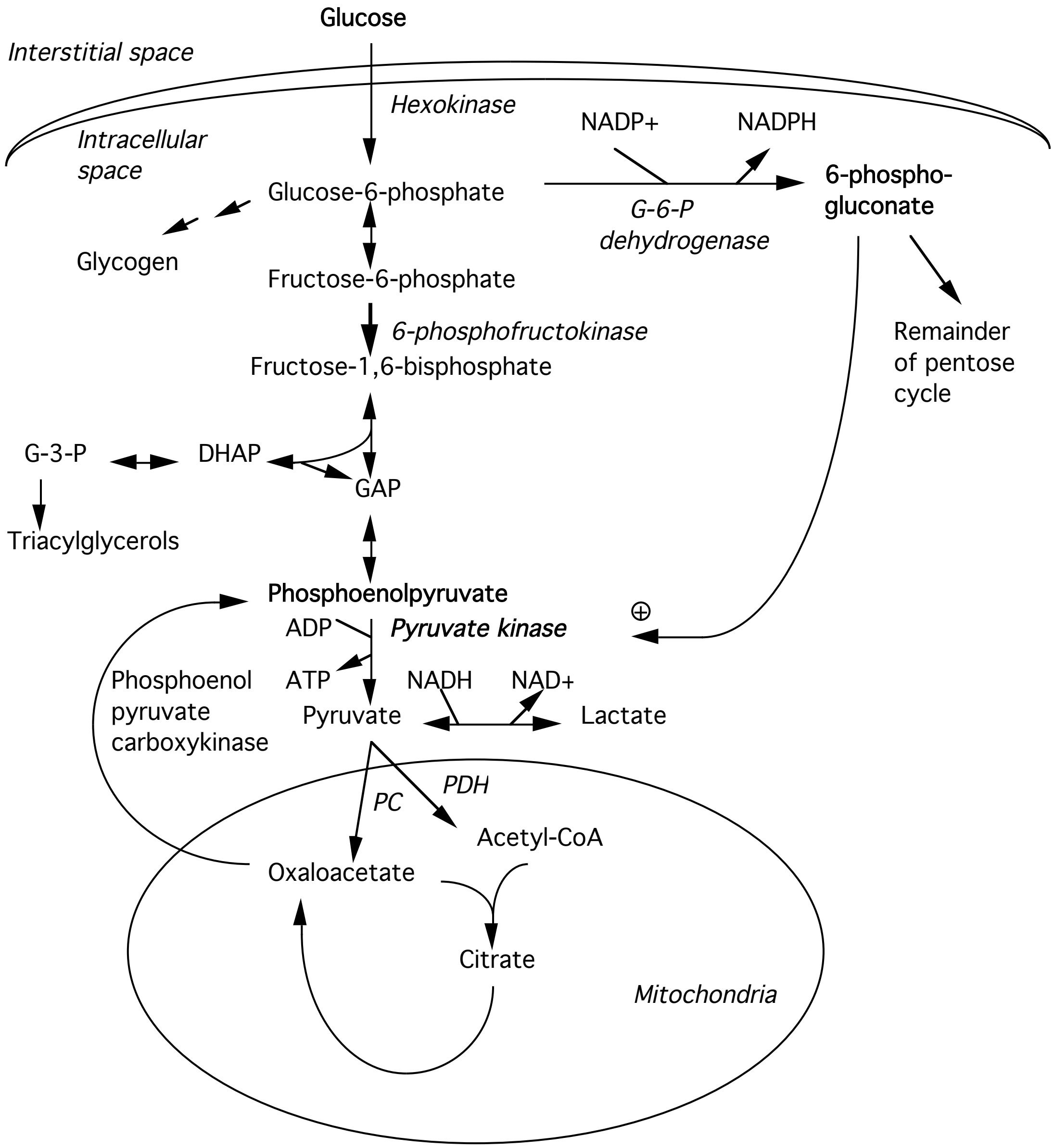
VI. What limits glucose use for fatty acid synthesis in ruminant adipose tissue?

A. ***Old theory:*** Low activities of ATP-citrate lyase and $NADP^+$-malate dehydrogenase

B. ***New theory:***

1. Competition between glycolysis and the pentose cycle.

2. Glycolysis is regulated at ***6-PFK***. Any glucose carbon that gets beyond 6-PFK is drawn off to lactate and G3P.



VII. Fatty acid elongation

A. ***General***

1. At least 60% of fatty acids in triacylglycerols are C18.
2. Free palmitic acid (16:0) synthesized in cytoplasm is elongated to stearic acid (18:0) by the addition of a C2 unit at the *carboxyl* terminal.
3. Virtually all cells contain one or more elongase isoenzymes.

B. ***Mitochondrial system***

1. Palmitic acid is activated to palmitoyl-CoA in the cytoplasm (*acyl-CoA synthase*).
2. Palmitoyl-CoA is transferred into the mitochondria via the carnitine acyltransferase system.

3. A C2 unit is added by what appears to be a reversal of ß-oxidation.
   a. Uses acetyl-CoA as carbon source.
   b. Uses NADH as source of reducing equivalents.
   c. FAD-dehydrogenase in the first step of ß-oxidation is replaced by an NAD^+-reductase.
4. Involved primarily in production of fatty acids for mitochondrial membranes; prefers unsaturated fatty acids as substrates.

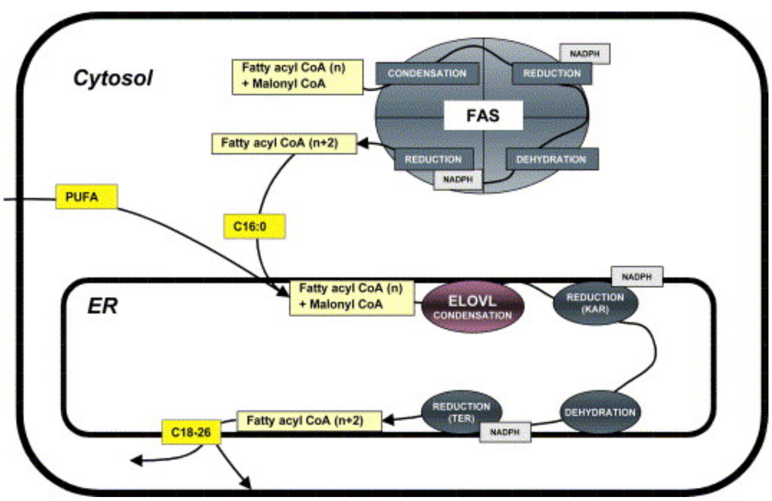
C. ***Microsomal system***

1. Palmitate is activated to palmitoyl-CoA in the cytoplasm.
2. Elongase enzymes are located in endoplasmic reticulum (microsomes) (*not cytoplasm*).
3. A C2 unit is added essentially as in the fatty acid biosynthetic pathway.
   a. Uses acyl-CoA (not acyl-ACP).
   b. Requires MalCoA (not AcCoA) as substrate.
   c. Can use NADH or NADPH as source of reducing equivalents.
   d. Pathway:

palmitoyl-CoA + malonyl-CoA + 2 NADPH + H^+ →

stearoyl-CoA + 2 $NADP^+$ + CoASH + CO_2

   e. *Virtually all fatty acids can be elongated (saturated, monounsaturated, and polyunsaturated).*



C. ***Elongase isozymes***

1. Saturated and monounsaturated fatty acids – ELOVL1, 3, and 6
   (ELOVL = Elongation of Very Long Chain Fatty Acids)

2. Polyunsaturated fatty acids – ELOVL2, 4, and 5

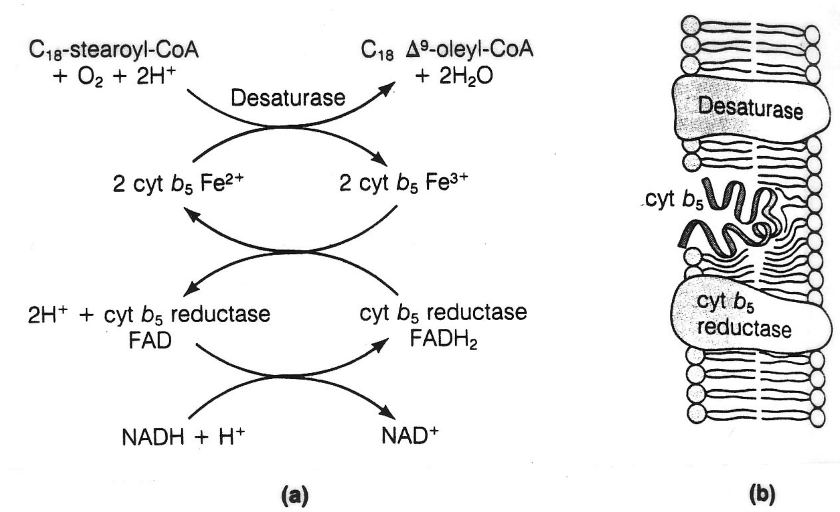
VIII. Fatty acid desaturation

A. ***General***

1. Usually alternates with fatty acid elongation.
2. Only three desaturases are present (Δ^9-, Δ^6-, and Δ^5-desaturases). There may be two independent Δ^6-desaturases.
3. If substrate fully saturated or is a trans-fatty acid, then first double bond is at C9 (e.g., stearic acid 18:0 to oleic acid 18:1Δ^9)
4. If substrate already unsaturated, then double bonds are inserted between the carboxyl group and the double bond nearest to the carboxyl group. (e.g., linoleic acid 18:2$\Delta^{9,12}$ to γ-linolenic acid 18: $\Delta^{6,9,12}$).
5. Desaturation maintains 1,4-diene composition of fatty acid.
6. Desaturation produces *cis*-double bonds.

B. ***Stearoyl-Coenzyme A desaturase (SCD)***

1. SCD is located on the endoplasmic reticulum (microsomes).
   a. SCD1 – liver
   b. SCD2 – adipose tissue of rodents (*only SCD1 in cattle and pigs*)
   c. As many as 5 SCD genes in mice and humans
2. SCD contains flavoprotein and cytochrome b_5 or cytochrome P-450.
3. Molecular oxygen is partially reduced by the NADH to produce an enzyme-bound superoxide radical, which oxidizes stearoyl-CoA.
4. *SCD can desaturate any saturated fatty acid and many trans-fatty acids.*



Overall reaction of stearoyl-CoA desaturase

C. ***Other desaturases***

1. ***Plants***

a. Starts with the *cis*-9 fatty acid (oleic acid) as substrate.

b. Oleic acid must be incorporated into phospholipids of plant membranes.

c. Desaturation is toward the ω-carbon.

d. There is no Δ^6 desaturase activity in most plants.

1) Arachidonic acid (20:4n-6) does not occur in most plants.

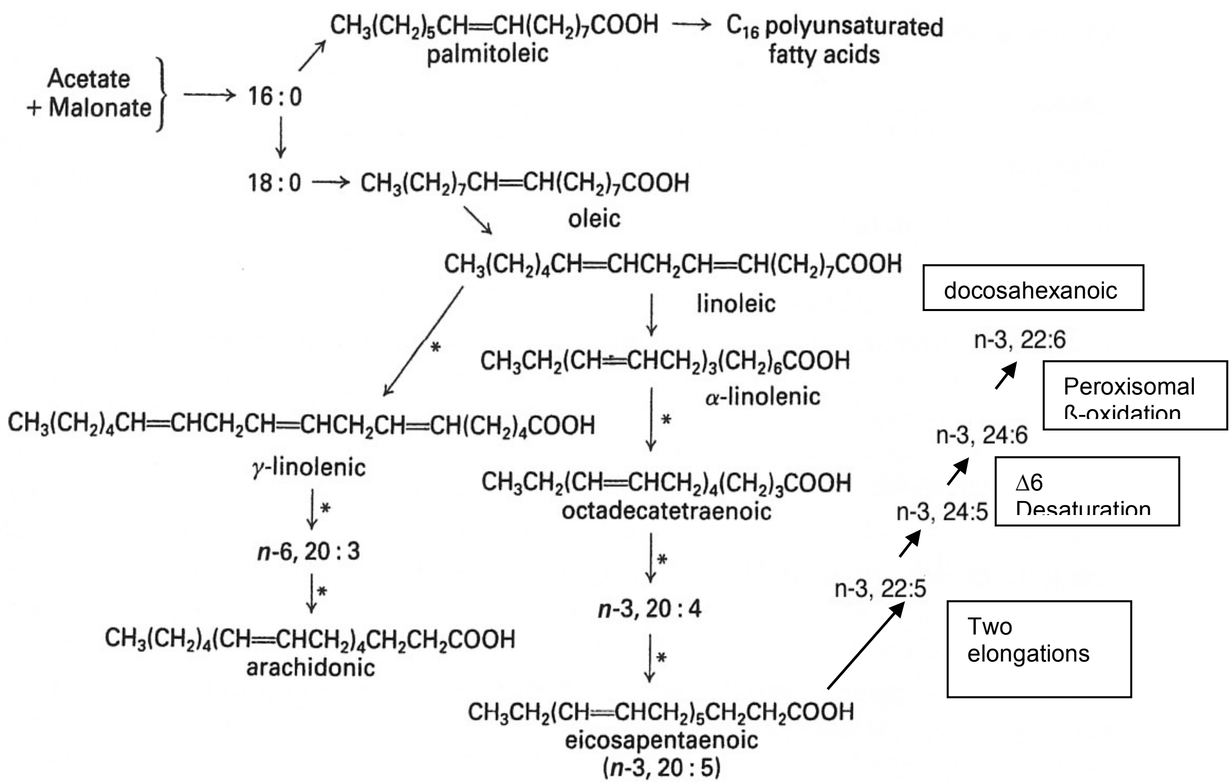
2) Fatty acid carbon is conserved for the production of α-linolenic acid (18:3n-3).

e. Most plants cannot elongate α-linolenic acid.

f. Most plants do not have a Δ^{15} desaturase.

1) Many terrestrial plants are enriched with α-linolenic acid.

2) Marine algae are the only organisms that can make large amounts of docosahexanoic acid.



Major pathways for polyunsaturated fatty acid synthesis in plants and algae. *Indicates a pathway found in high levels in marine algae and mosses, but less commonly in other algae or plants.

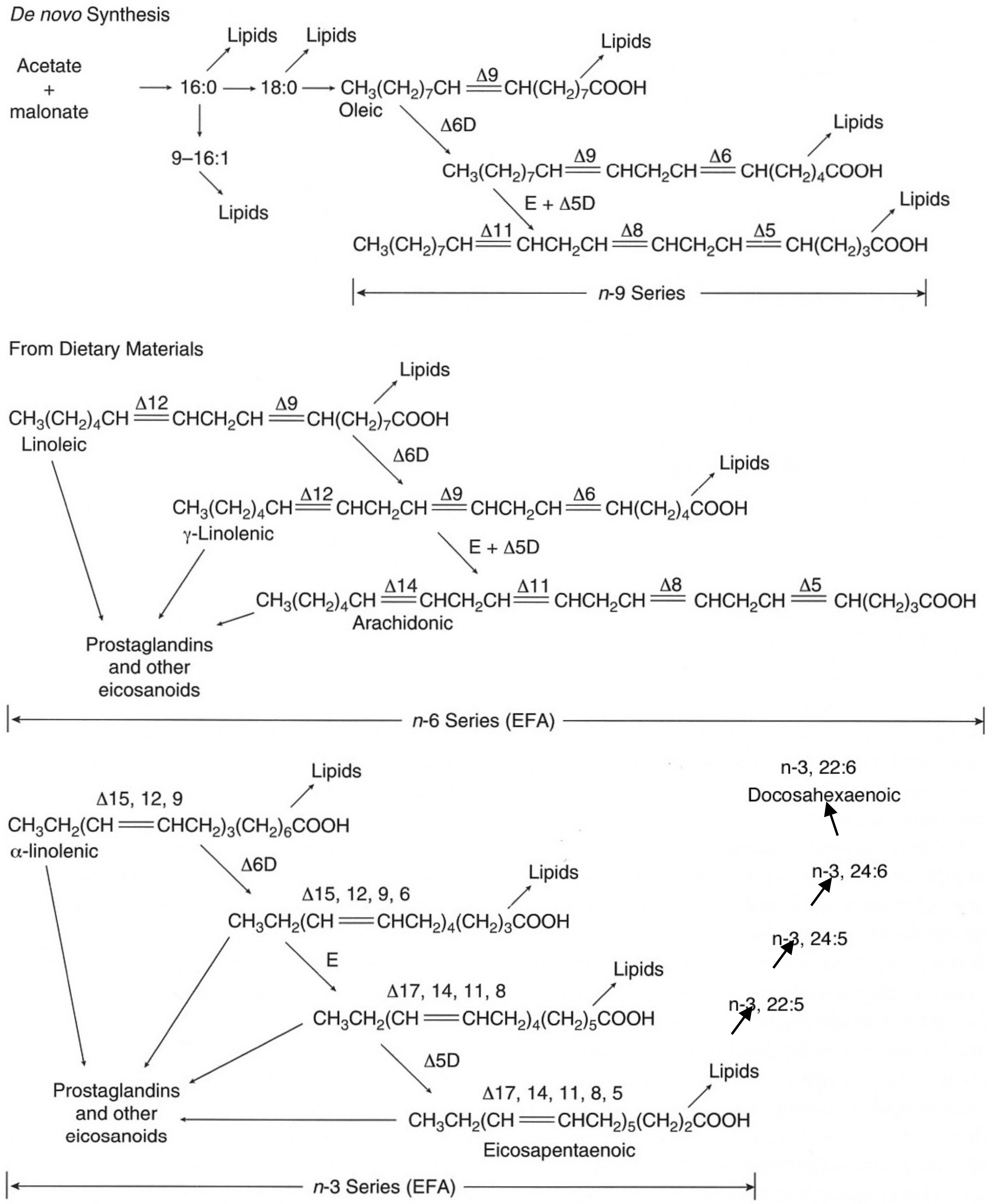
2. ***Animals***

a. Starts with a saturated fatty acid as substrate.

b. The fatty acid must be activated to its acyl-CoA thioester.

c. The first double bond is always at the Δ^9 position.

c. Desaturation is always toward the carboxyl-carbon.



Important pathways for unsaturated fatty acid formation in mammals. E = elongase; D = desaturase (positional specificity indicated).

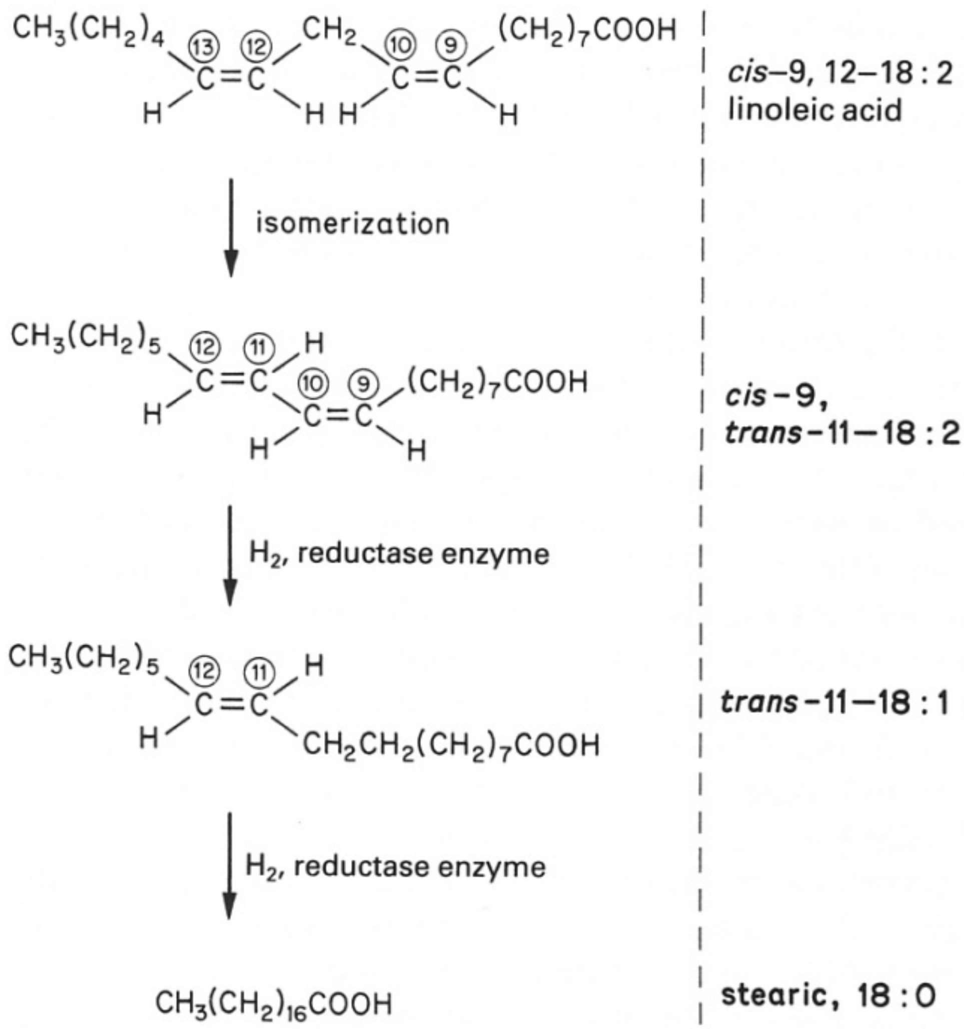
3. ***Fatty acid biohydrogenation***

   a. The double bond toward the methyl carbon is isomerized to a *trans*-double bond.

   b. The double bond nearest the #1 carbon is reduced (***hydrogenated***).

   c. The *trans*-double bond is reduced, usually producing stearic acid (18:0).

   d. Each reaction is carried out by a different microorganism.



Biohydrogenation.