Lipids: de novo synthesis, oxidation, and hormonal regulation

Metabolismo y Enfermedades Metabólicas
Máster en Investigación Biomédica
Universidad de Valladolid

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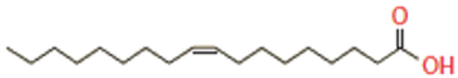
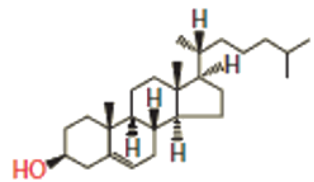
“Lipids may be broadly defined as hydrophobic or amphiphilic small molecules; the amphiphilic nature of some lipids allows them to form structures such as vesicles, multilamellar/unilamellar liposomes, or membranes in an aqueous environment.”

Albert Lehninger

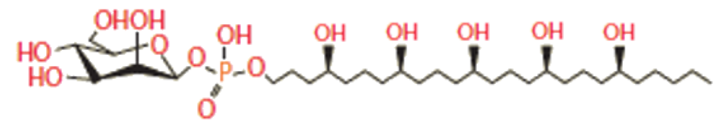
Lipid Categories



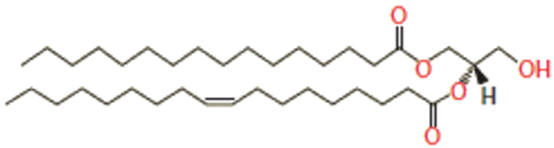
Fatty acyls
Fatty acids and conjugates
Eicosanoids
Docosanoids
Fatty Alcohols



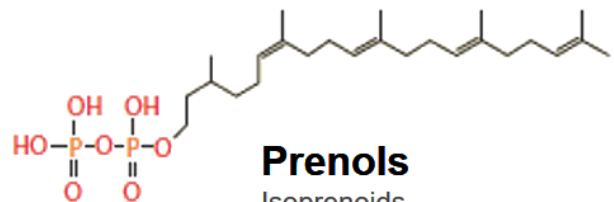
Sterols
Cholesterol and its esters
Steroid hormones



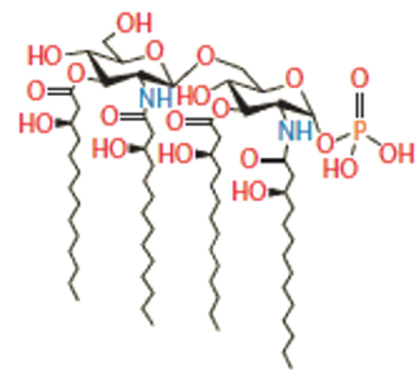
Polyketides
Linear Polyketides
Aflatoxins



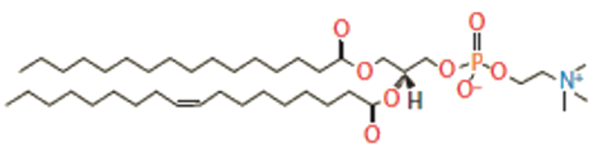
Glycerolipids
Monoradylglycerols
Diradylglycerols
Triradylglycerols



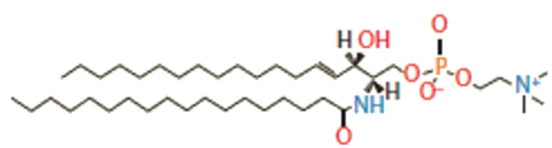
Prenols
Isoprenoids



Saccharolipids
Acylaminosugars

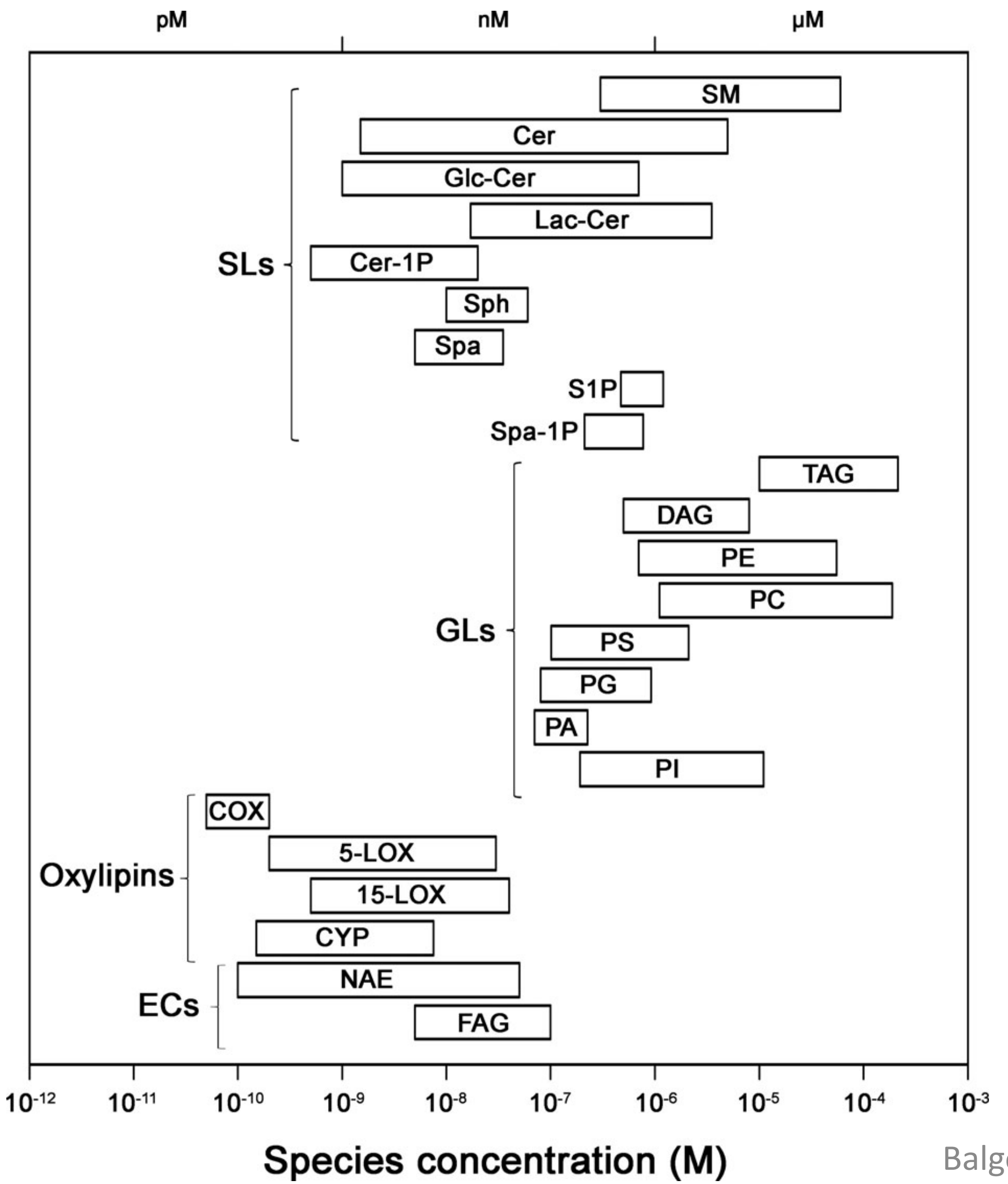


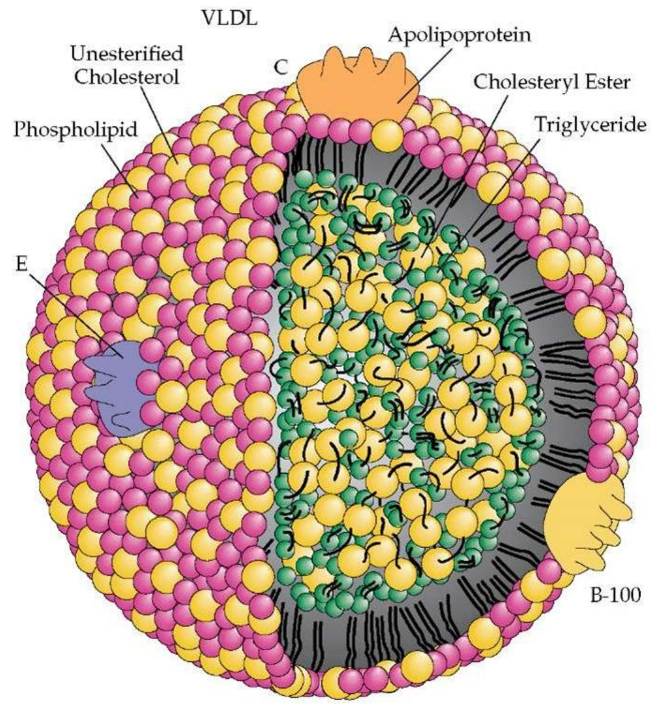
Glycerophospholipids
Glycerophosphocolines
Glycerophosphoethanolamines
Glycerophosphoinositols



Sphingolipids
Sphingoid bases
Ceramides
Phosphosphingolipids
Glycosphingolipids

Abundance Variability





Balgoma et al. *Mol. Nutr. Food Res.* 2013, *57*, 1359–1377

Lipids are hydrophobic or amphipathic small molecules that may originate entirely or in part by carbanion-based condensations of thioesters and/or by carbocation-based condensations of isoprene units

Lipids are fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds

What Is a “Fatty Acid”?

Palmitic acid

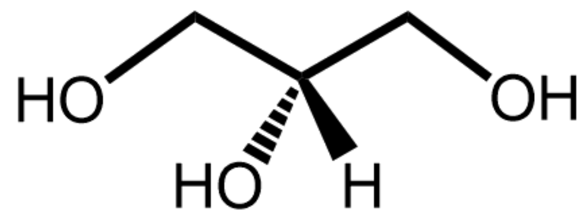
<u>Fatty acid</u>: a carboxylic acid with a long hydrocarbon chain. Usually it has an even number of carbons.

Ester group

<u>Fatty acid ester</u>: a fatty acid in which the carboxylic acid group has reacted with the alcohol group of another molecule (often glycerol)

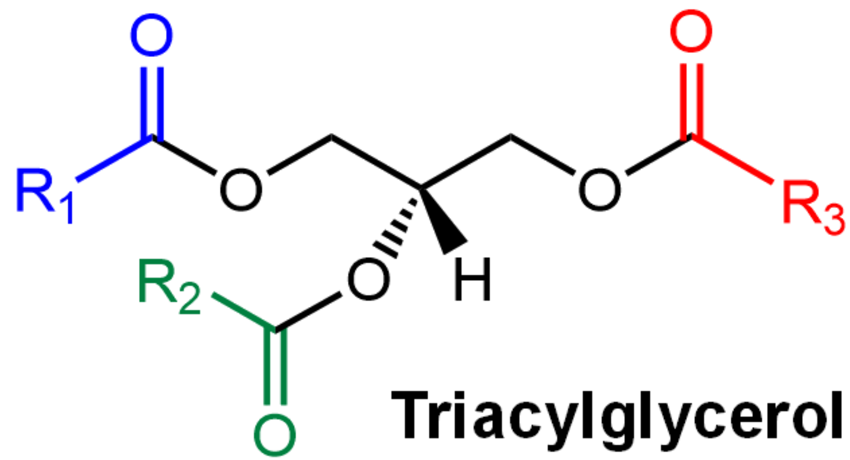
What Is a “Triglyceride”?

<u>Glycerol</u>: common name for 1,2,3-trihydroxy-propane.



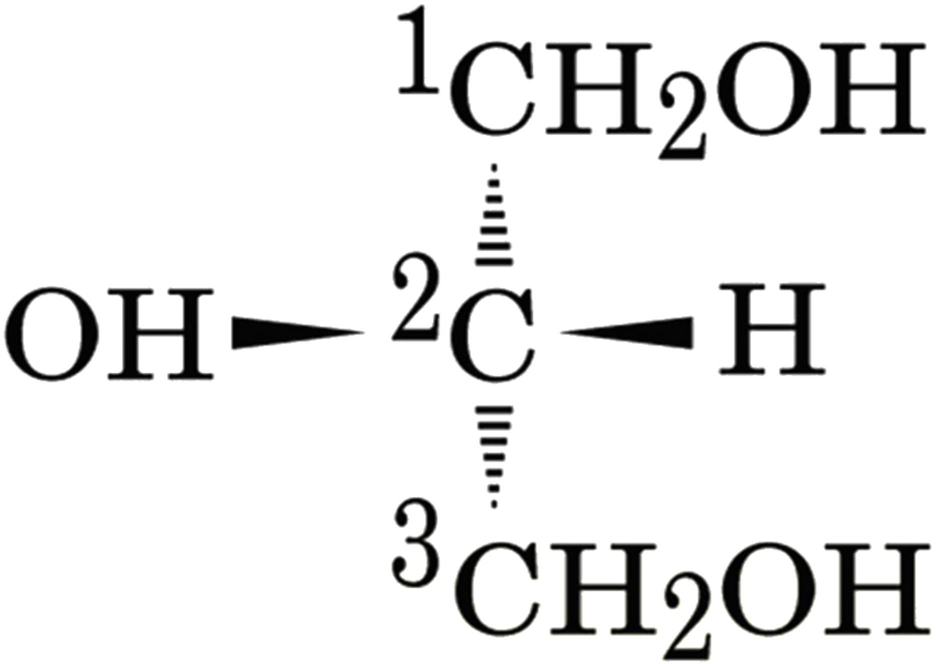
Glycerol

<u>Triglyceride</u>: a glycerol molecule with three esterfied fatty acid side chains. Also known more correctly as a “triacylglycerol”. Stable, non-polar, hydrophobic.



Triacylglycerol

Stereospecific Numbering



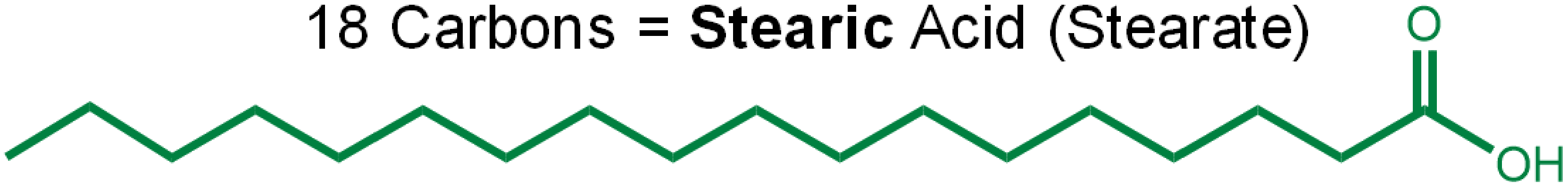
L-Glycerol

Common Saturated Fatty Acids

Saturated FA's have no double bonds

16 Carbons = **Palmitic** Acid (Palmitate)

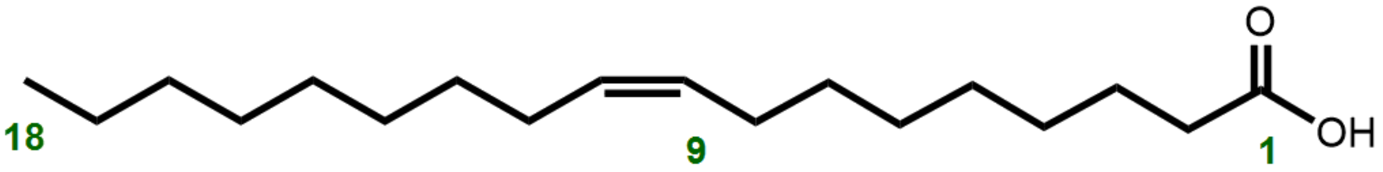
18 Carbons = **Stearic** Acid (Stearate)



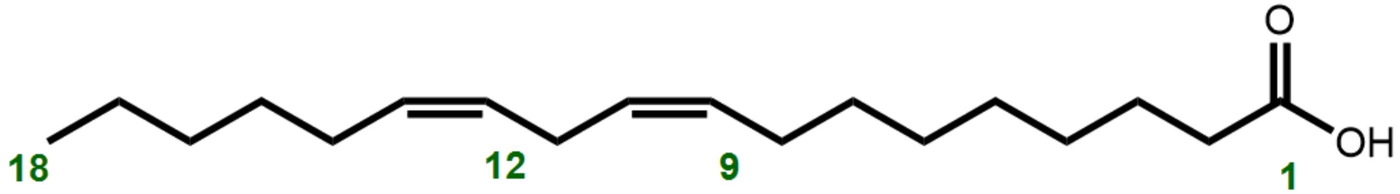
Common Unsaturated Fatty Acids

Unsaturated FA's have at least one double bond, usually in the Z (*cis*) conformation

18 Carbons, 1 double bond at c9 = **Oleic** Acid (Oleate)

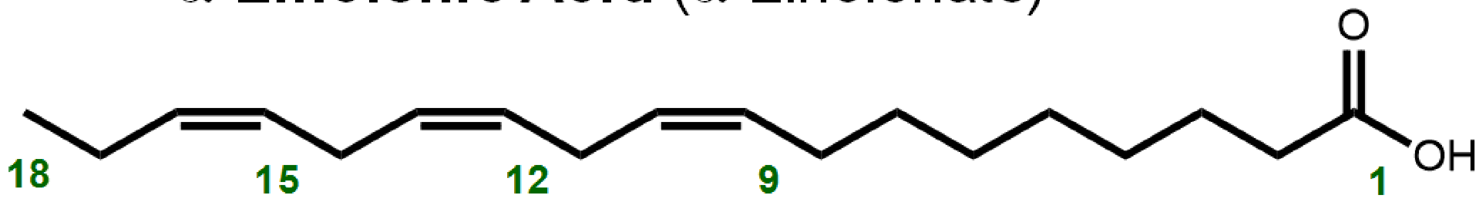


18 Carbons, 2 double bonds at c9 and c12 = **Linoleic** Acid (Linoleate)

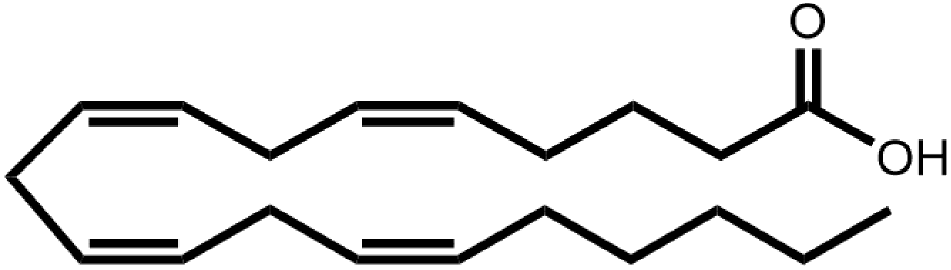


More Unsaturated Fatty Acids

18 Carbons, 3 cis double bonds at 9, 12 & 15 =
α-**Linolenic Acid** (α-Linolenate)

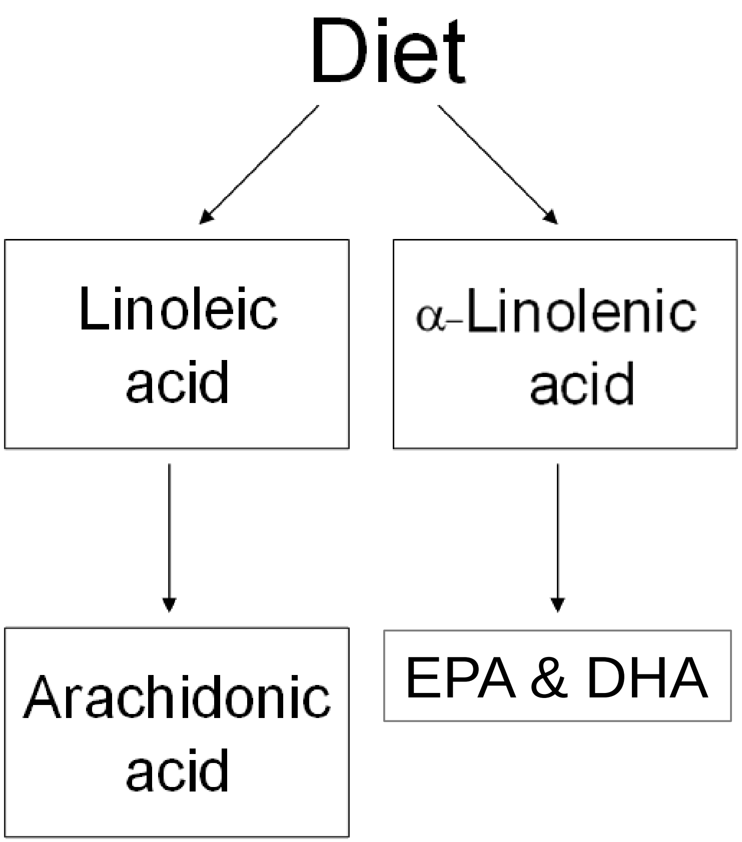


20 Carbons, 4 cis double bonds at 5,8,11 & 14
Arachidonic Acid (Arachidonate)



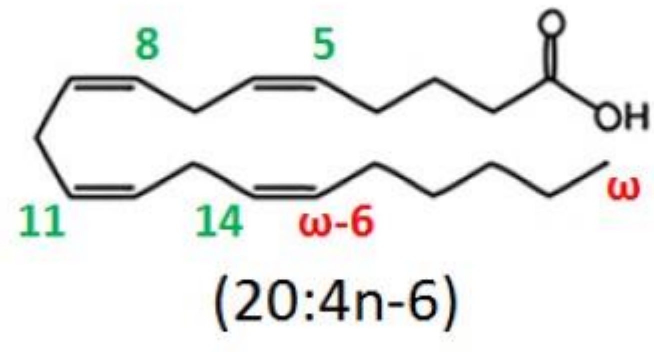
(5Z,8Z,11Z,14Z-Eicosatetraenoic Acid)

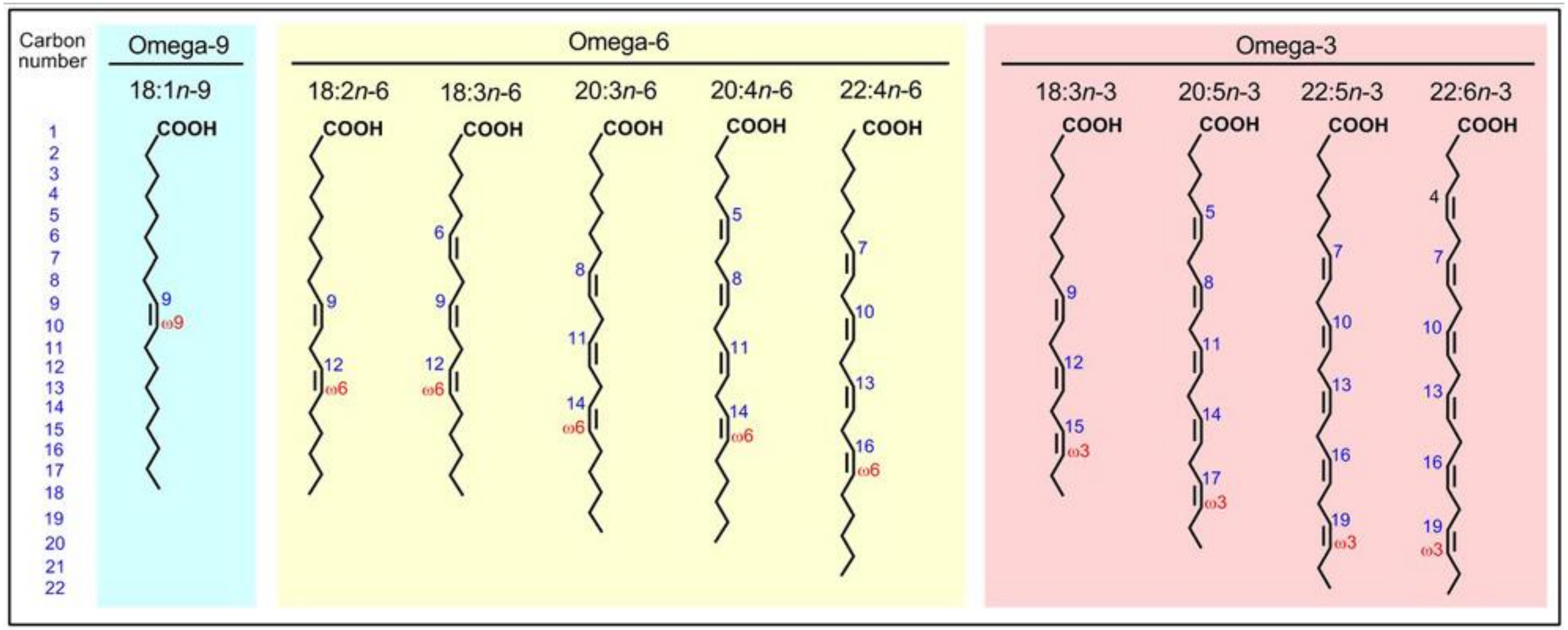
What Are Essential Fatty Acids?



- Two "Essential" FA's cannot be synthesized by humans
  - **Linoleic** acid **(ω-6)**
  - α-**Linolenic** acid **(ω-3)**
- Used in the biosynthesis of polyunsaturated fatty acid
- Must come from diet

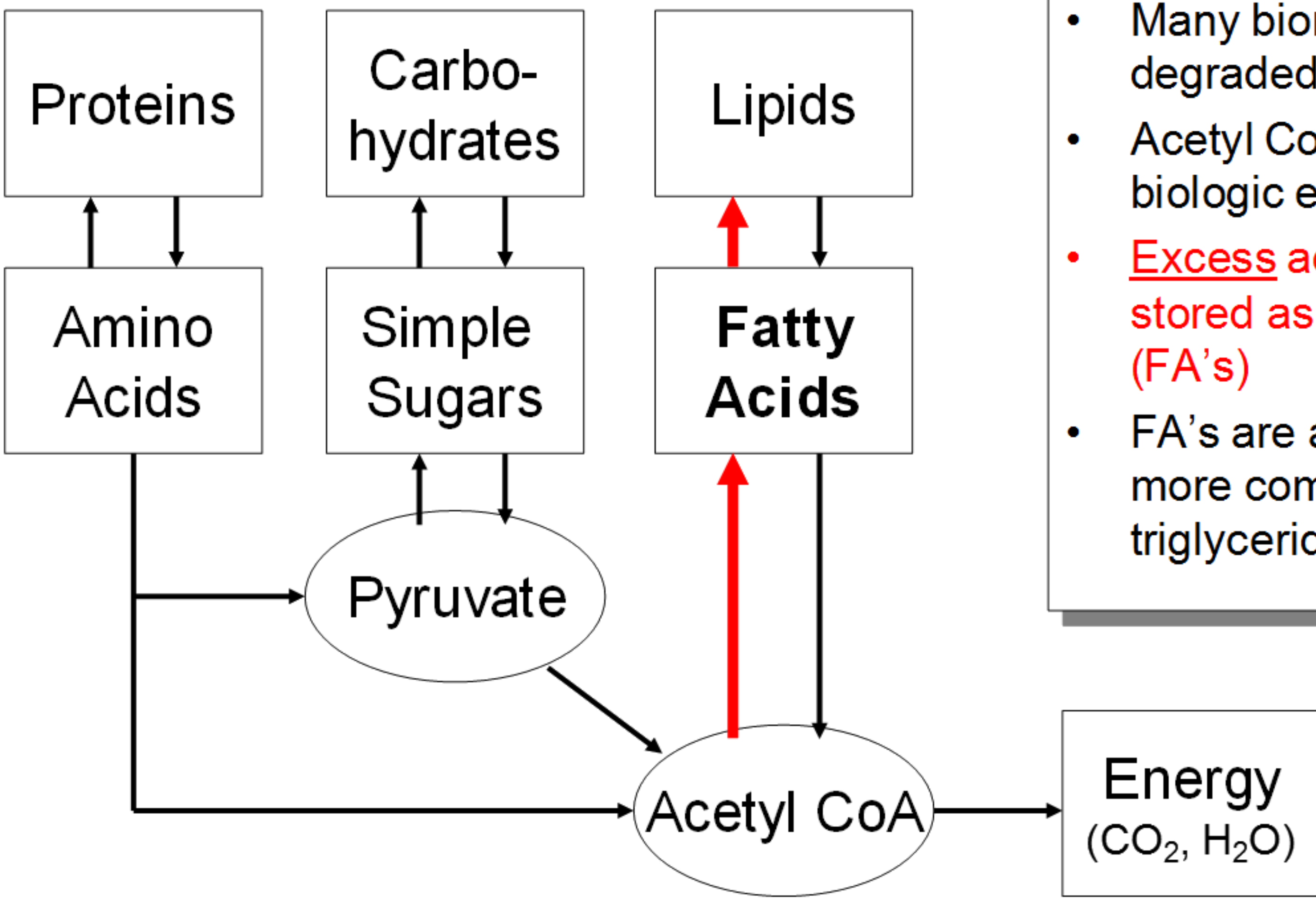
Common Unsaturated Fatty Acids





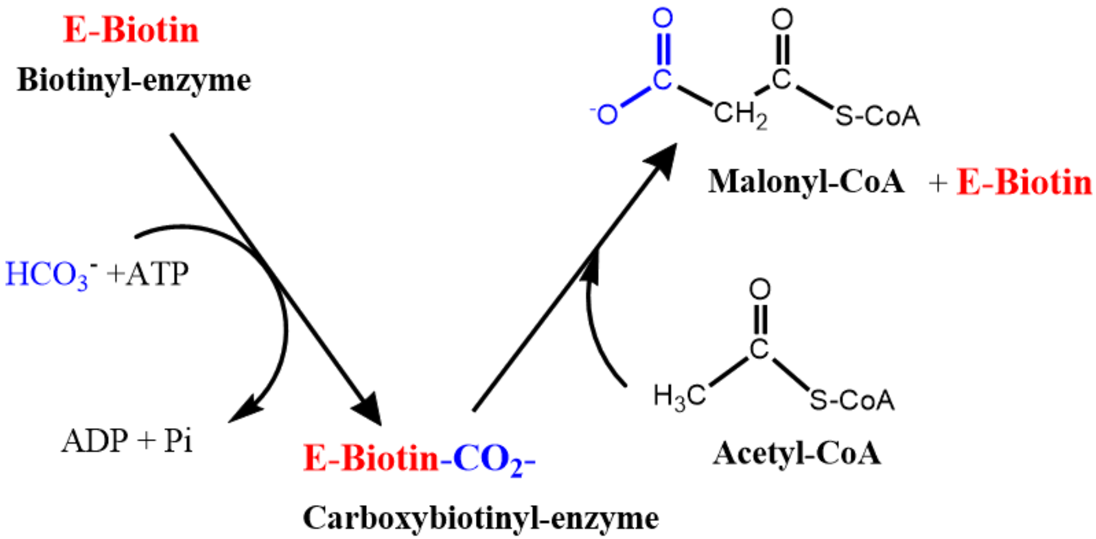
Fatty Acid Biosynthesis

Metabolism and Energy Overview



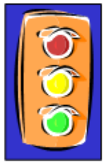
- Many biomolecules are degraded to Acetyl CoA
- Acetyl CoA provides biologic energy
- Excess acetyl CoA is stored as Fatty Acids (FA's)
- FA's are assembled into more complex lipids like triglycerides (TG's)

Key Enzyme: Acetyl-CoA Carboxylase



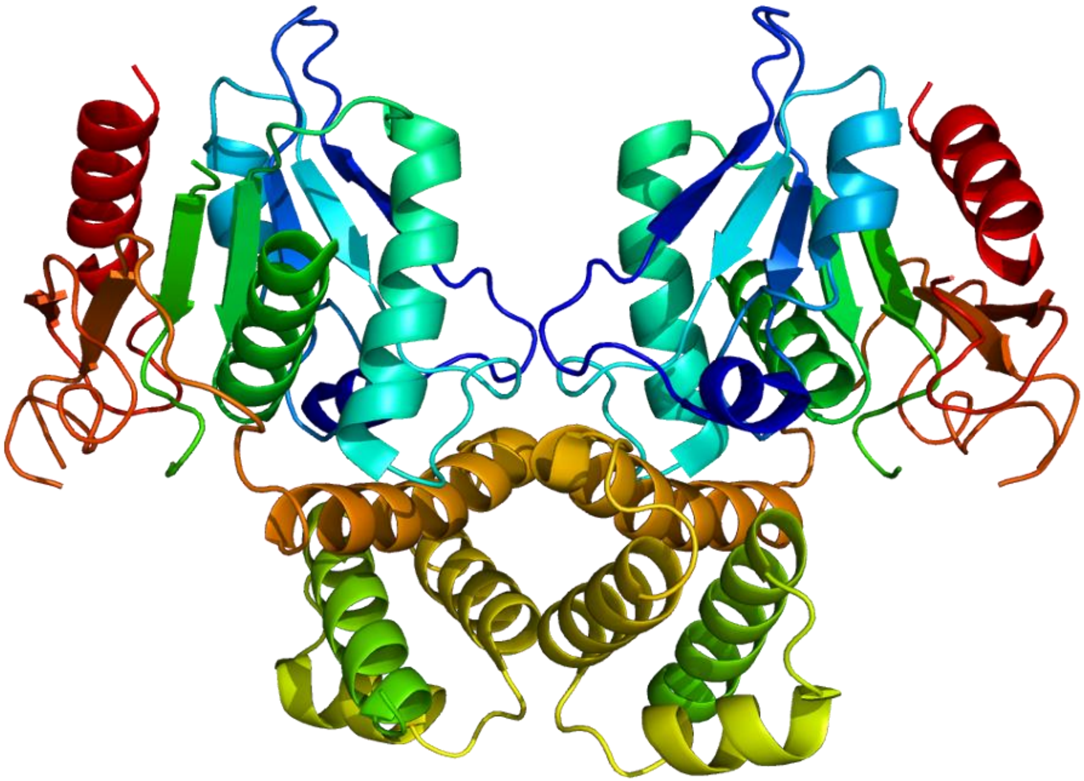
"E" above is the enzyme acetyl-CoA carboxylase, which is conjugated to biotin.

- Acetyl-CoA Carboxylase is a key enzyme
- Converts acetyl-CoA into malonyl-CoA
    - The CO_2 is released later
    - Biotin is a cofactor
- It is the "committed step" in FA synthesis
- It is the regulated, rate-limiting enzyme in FA synthesis



Key Enzyme Complex: FA Synthase

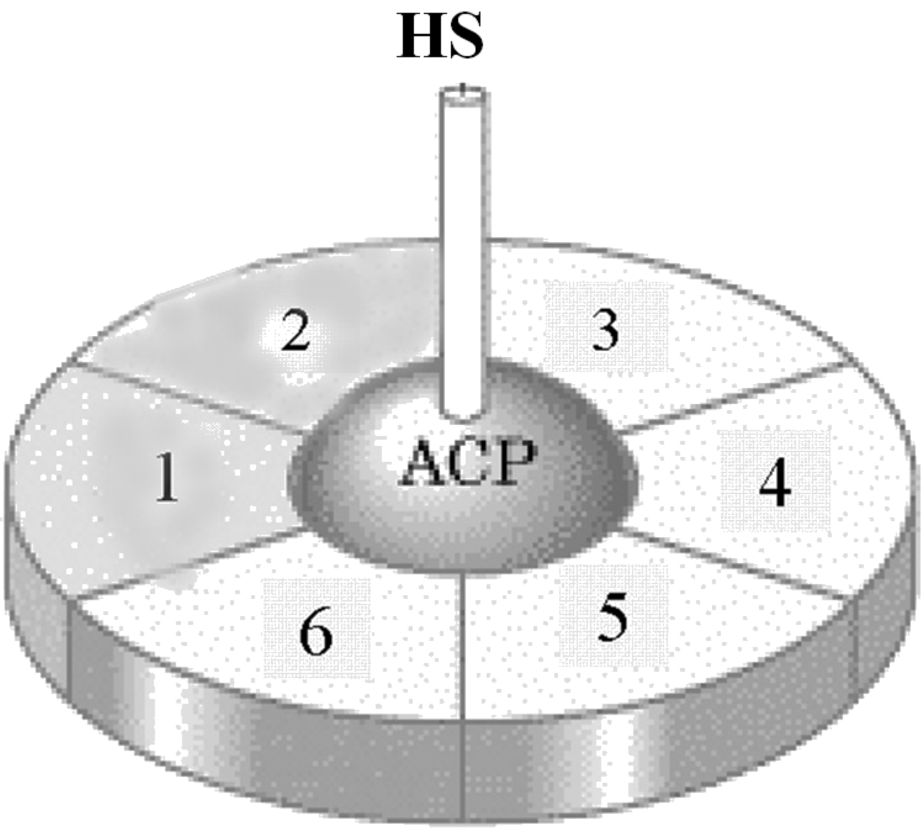
8 Acetyl CoA ⇌ Palmitic Acid + 8 CoASH



In animals, single large, multifunctional polypeptide.
The active form is a dimer

Key Enzyme Complex: FA Synthase

8 Acetyl CoA ⇌ Palmitic Acid + 8 CoASH



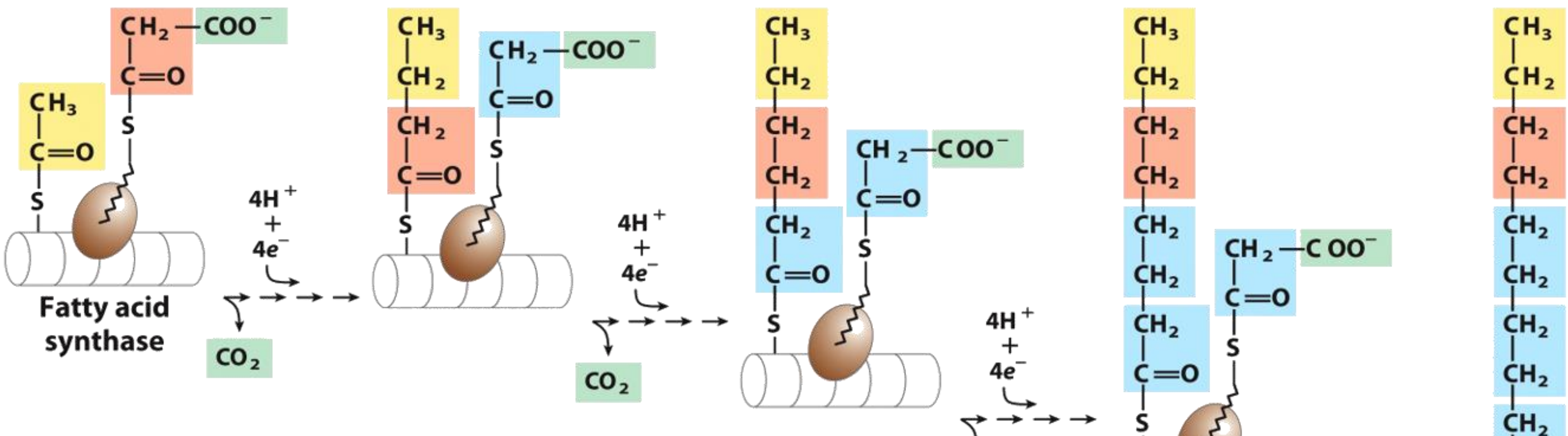
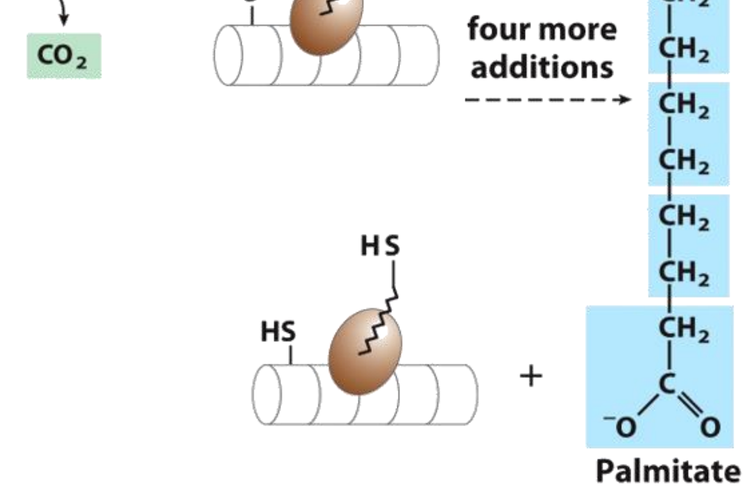
Cytosolic complex of enzymes.

Steps 1 - 6 happen iteratively in a co-located complex of enzymes.

ACP** is the carrier protein that holds the carbon backbone. Contains 4'phospho-pantetheine.

ACP stands for Acyl Carrier Protein. (10,000 kDa, Ser 36)

FA Synthesis

One iteration:

Step 1: Set up acetyl-ACP

Step 2: Set up malonyl-ACP

Step 3: Condense them, giving Acetoacetyl ACP

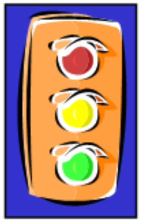
Step 4: Use NADPH to reduce distal carbonyl to a hydroxyl group

Step 5: Remove the hydroxyl group as H_2O leaving a double bond

Step 6: Use another NADPH to reduce the double bond

REPEAT: From step 2 using the new 4-carbon butyryl-ACP in place of acetyl-ACP in step 3

Regulation of FA Synthesis



Regulation occurs primarily at acetyl-CoA carboxylase, the rate limiting step

Feedback Mechanisms

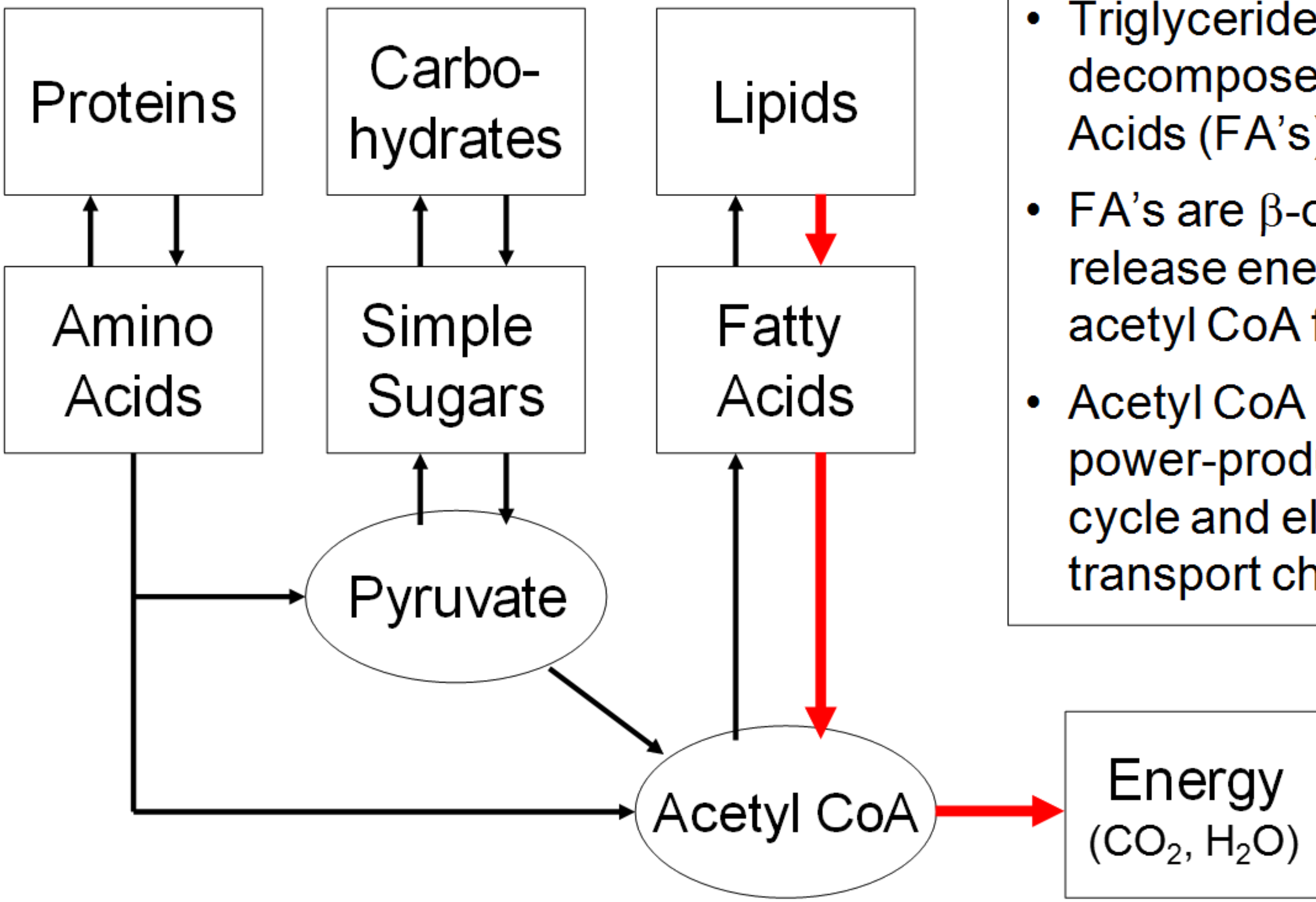
- **Citrate**, which builds up when acetyl-CoA is plentiful, accelerates FA synthesis.
- **Palmitoyl-CoA** weakly inhibits FA synthesis.

Hormonal Mechanisms

- **Insulin**, which signals a resting, energy rich state, dephosphoryl-ates and accelerates the enzyme.
- **Glucagon**, **epinephrine** and **norepinephrine**, which signal immediate energy needs, phos-phorylate and slow the enzyme [via AMP -dependent protein kinase and also via cAMP-dependent PKA].

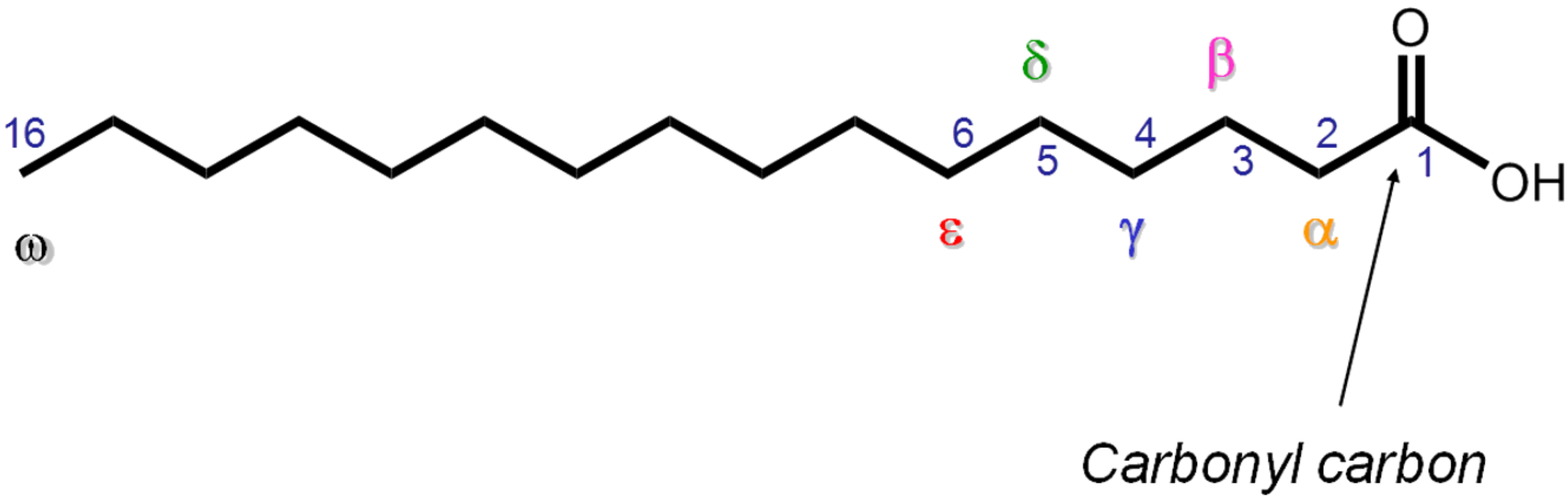
Fatty Acid Oxidation

Metabolism and Energy Overview



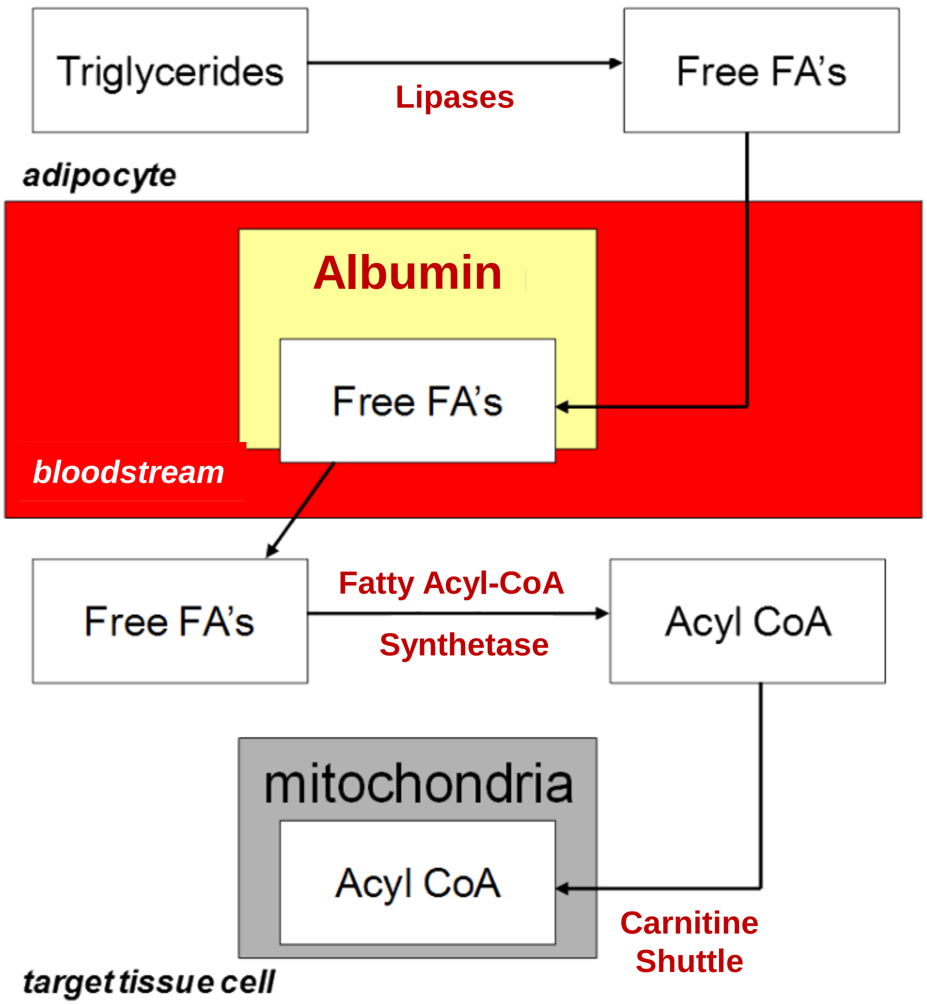
- Triglycerides (TG's) are decomposed into Fatty Acids (FA's).
- FA's are β-oxidized to release energy and create acetyl CoA fragments.
- Acetyl CoA enters the power-producing Krebs cycle and electron transport chain.

Naming Conventions: Palmitic Acid



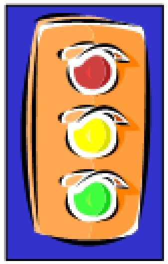
- ω omega, always the last alkyl carbon
- ε epsilon, fifth carbon after the carbonyl
- δ delta, fourth carbon after the carbonyl
- γ gamma, third carbon after the carbonyl
- β beta, second carbon after the carbonyl
- α alpha, first carbon after the carbonyl

Activate the Fatty Acid: Make Acyl-CoA



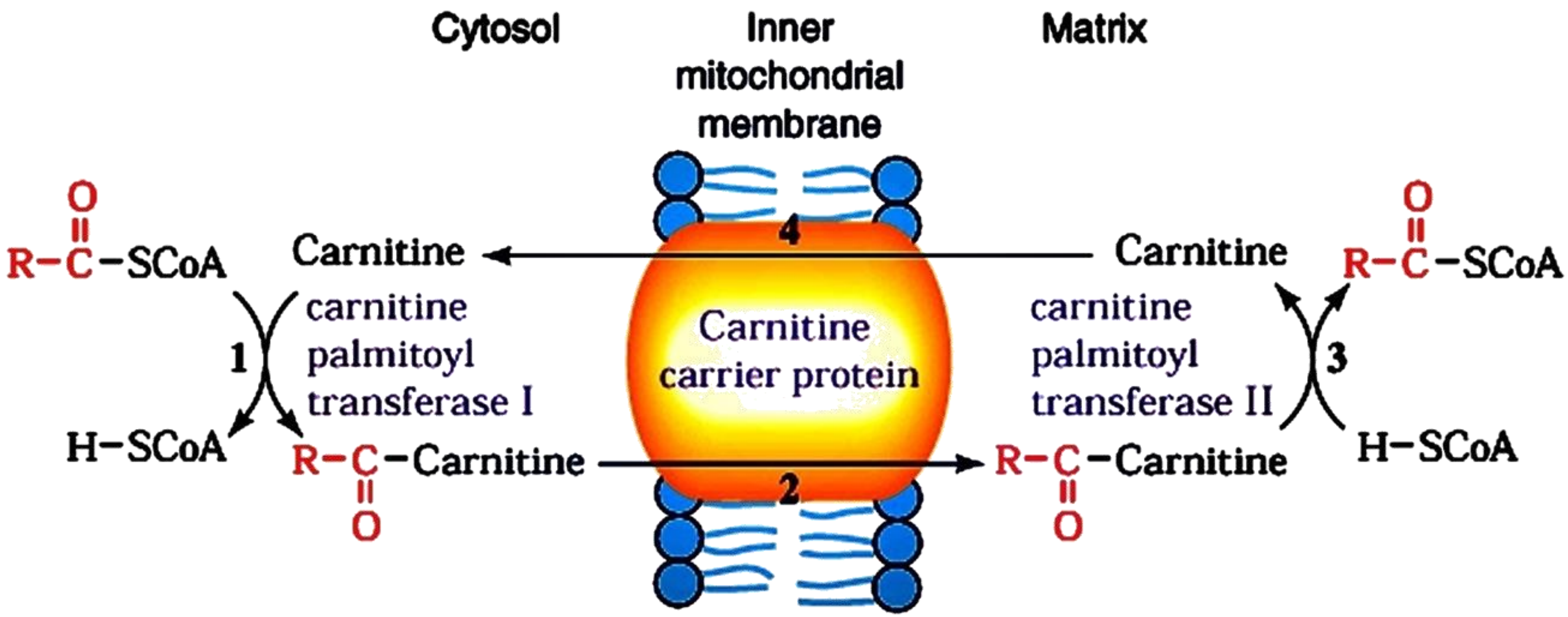
- **ATGL + HSL + MGL**
  - decomposes triglycerides
  - removes FA groups
  - free FA's diffuse through membrane
- **Albumin**
  - carries FA's to target tissue
  - FA's diffuse into tissue cells
- **Fatty acyl-CoA synthetase**
  - adds CoA to the free FA
- **Carnitine Shuttle**
  - moves fatty acyl CoA into the mitochondria

Regulation of FA Oxidation



- Hormone Sensitive Lipase is activated by cAMP
- Cyclic AMP also turns off acetyl CoA carboxylase, stopping FA synthesis
- Hormones like glucagon and epinephrine increase cAMP
  - FA synthesis slows
  - Triglycerides are broken down
  - FA's enter β-oxidation faster

Location, Location, Location

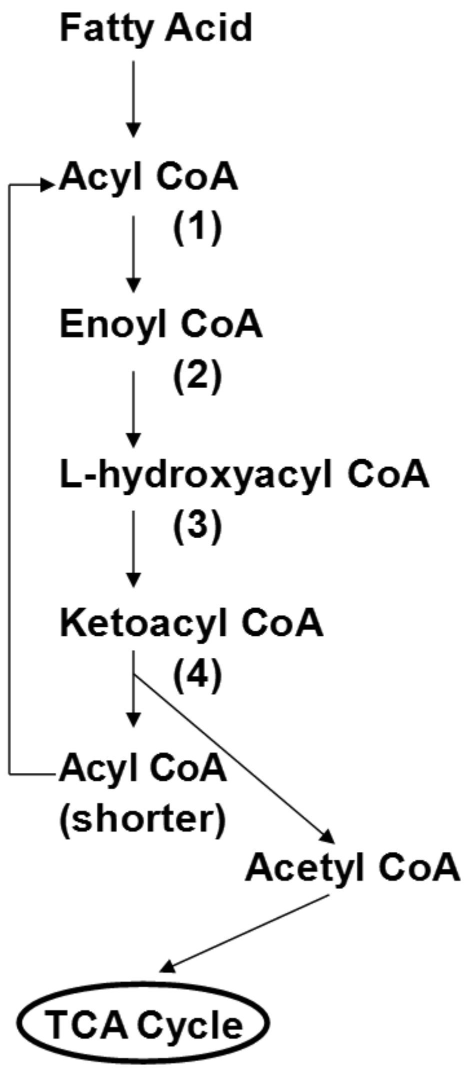


- Fatty Acyl CoA is oxidized inside the mitochondrial matrix
- The **carnitine shuttle** moves it into the matrix
  - Free carnitine is exchanged for acyl carnitine

- **Carnitine acyl transferases** catalyze reactions on both sides of membrane
  - driven by concentration gradient
- **CAT-1** is inhibited by high levels of malonyl CoA generated by acetyl CoA carboxylase in the pathway to Fatty Acid Synthesis.

Figure: Voet, D, Voet JG, Pratt CW (2006), Fundamentals of Biochemistry: Life at the Molecular Level, 2nd ed. Reprinted with permission of John Wiley & Sons, Inc.

β-Oxidation in a Nutshell…



One iteration of β-Oxidation:

Make fatty acyl CoA.

Step 1: Oxidize the β-carbon (C3)

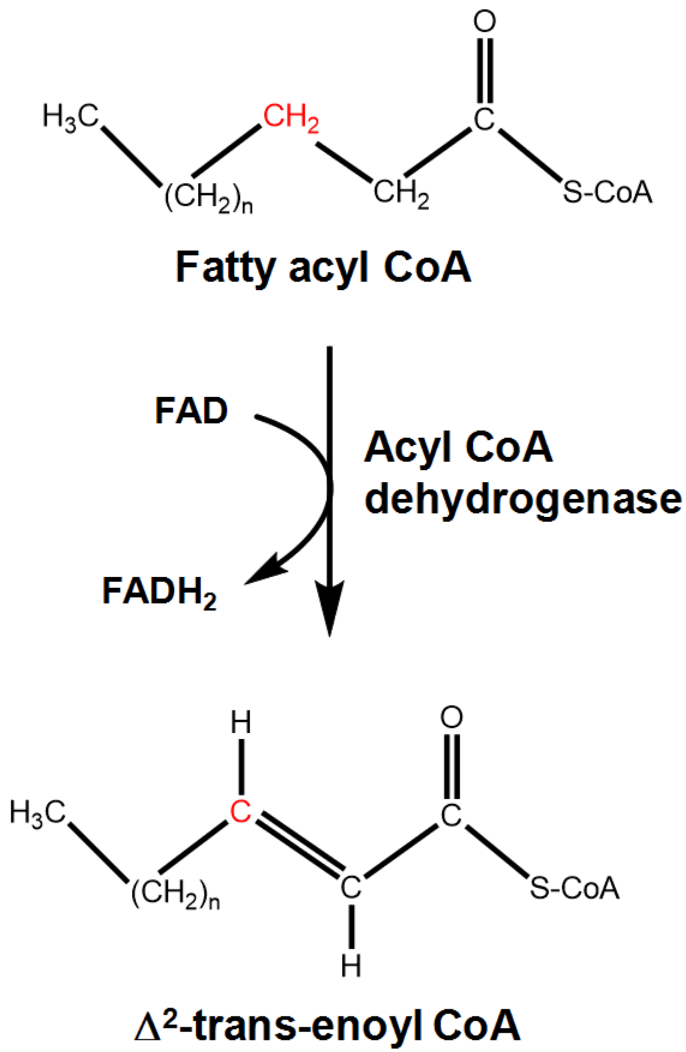
Step 2: Hydrate the β-carbon

Step 3: Oxidize the β-carbon, again!

Step 4: Thiolyze α–β bond, releasing acetyl CoA

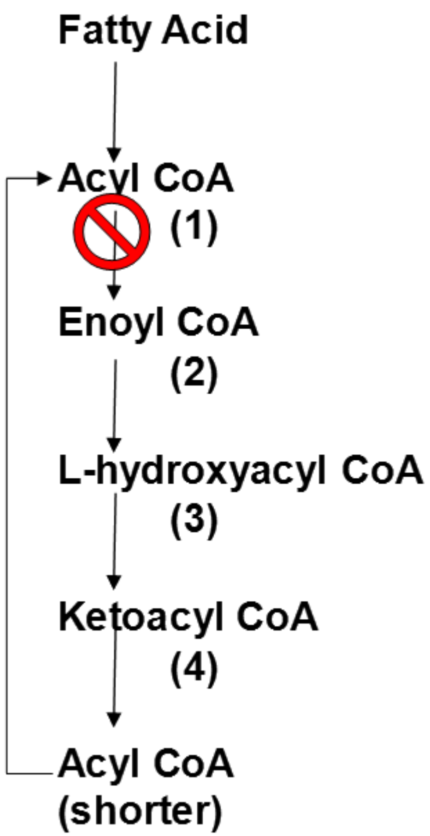
REPEAT from step 1, w/ 2 fewer carbons

Step 1: Oxidize the β-carbon



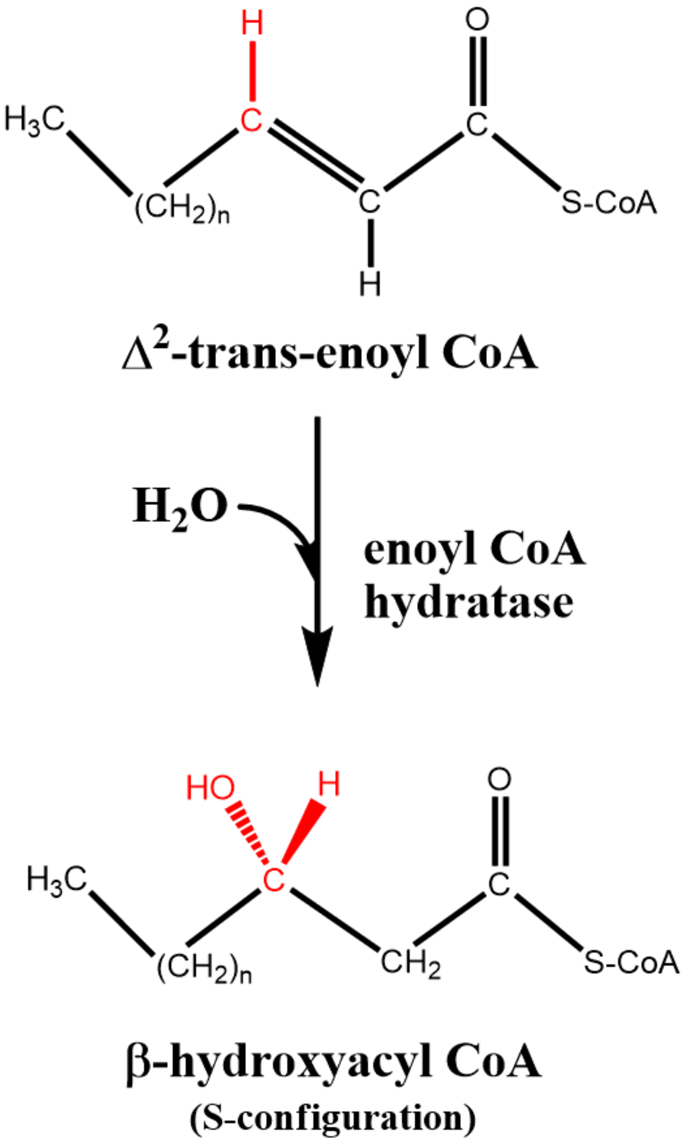
- Acyl CoA dehydrogenase oxidizes the β-carbon
  - 3 versions of the enzyme exist
  - Specific to short, medium and long chain substrates
- One $FADH_2$ is generated
  - makes 2 ATP's
- A *trans* double bond is created at the 2-carbon
- Product is an Enoyl CoA

Medium-chain Dehydrogenase Deficiency



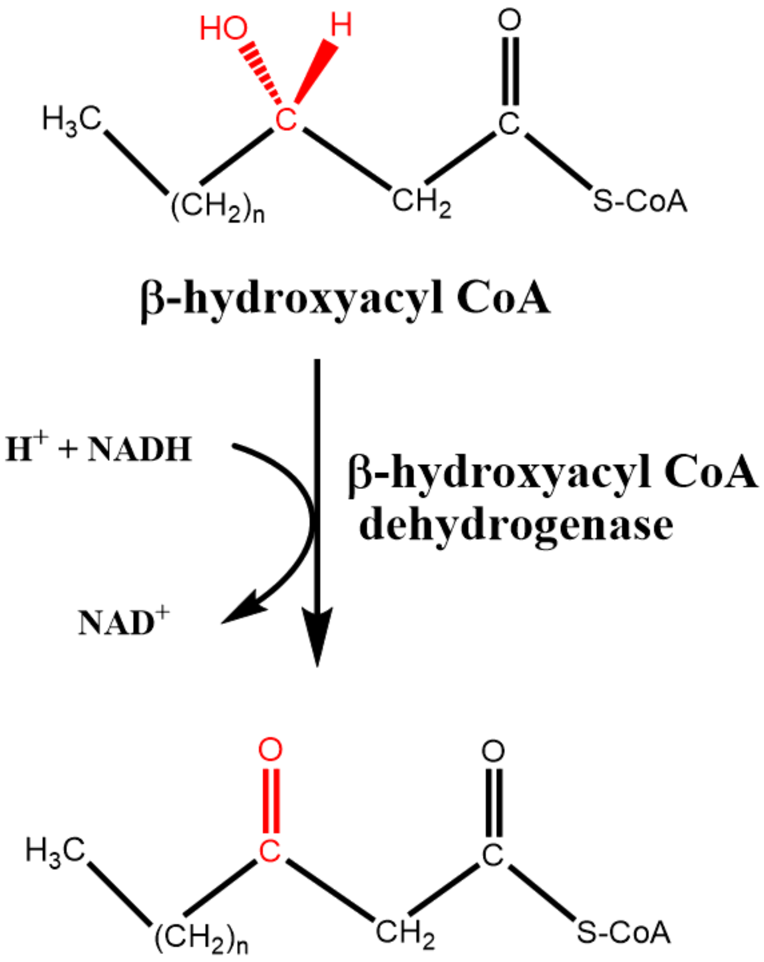
- **Incidence**: 1 in 10,000 live births
  - More common than phenylketonuria!
- **Symptoms**:
  - severe hypoglycemia >> lethargy, coma
    - little energy from FA's
    - glucose reserves are immediately burned
  - contributes to sudden infant death syndrome (10% of cases)
- **Mechanism**:
  - Normally, there are 3 separate fatty acyl CoA dehydrogenase enzymes for STEP 1 of β-oxidation
    - Specific for short, <u>medium</u> and long acyl chains, respectively
  - Autosomal recessive lack of medium chain enzyme
- **Treatment**: Special diet and supportive care

Step 2: Hydrate the β-carbon



- Enoyl CoA hydratase adds a water molecule across the double bond
- Product is β-hydroxyacyl CoA
  - S- stereoisomer

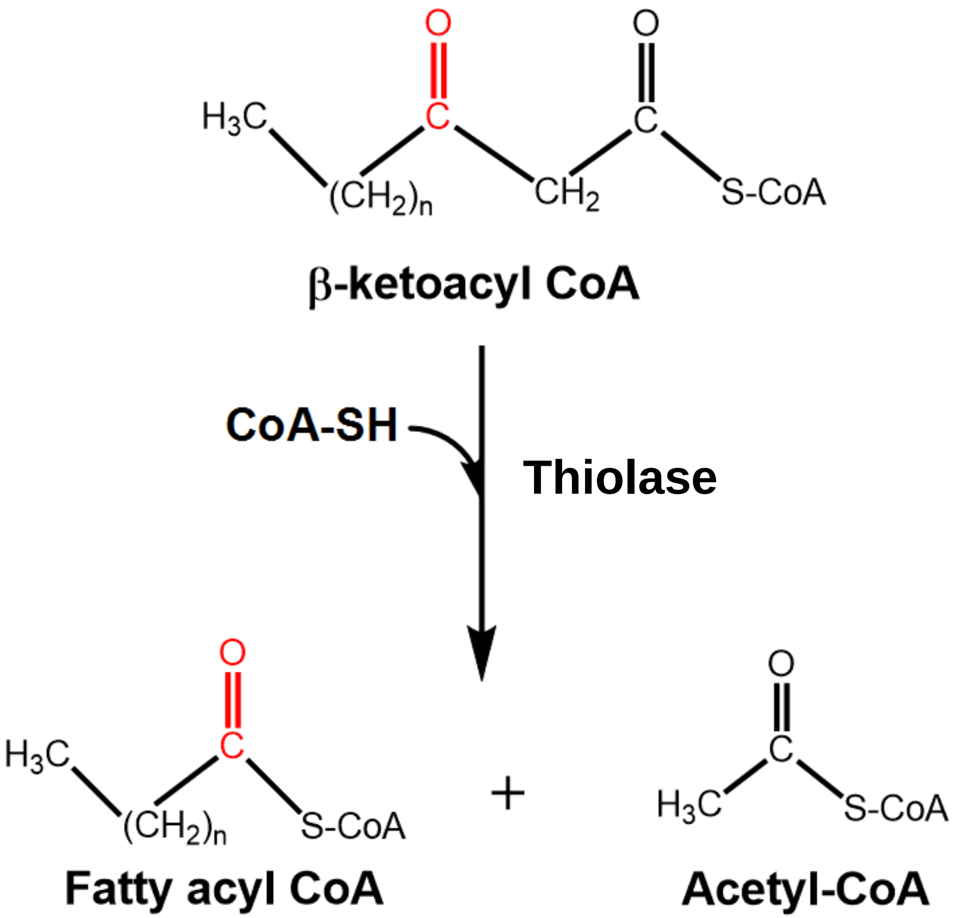
Step 3: Oxidize the β-Carbon, Again!



β-ketoacyl CoA

- Beta-hydroxyacyl CoA dehydrogenase oxidizes the β-carbon again
- One NADH is created
  - makes 3 ATP's
- A ketoacyl CoA is produced

Step 4: Thiolize off Acetyl-CoA



- **Thiolase**
  splits the ketoacyl
- A new CoASH is consumed
- Acetyl CoA is released
- A new, shorter Acyl CoA remains and re-enters the cycle

REPEAT with a Shorter Acyl-CoA

- Palmitoyl CoA (16 carbons) becomes myristoyl CoA (14 carbons).
- Each iteration releases another acetyl CoA.
- 7 iterations will release 8 acetyl CoA fragments from the original palmitoyl CoA.
- Net equation:

Palmitoyl CoA + 7CoASH + 7FAD$^+$ + 7NAD$^+$ + 7H$_2$0

yields: **8 Acetyl CoA + 7 FADH$_2$ + 7NADH + 7H$^+$**

How Much Energy?

Each palmitoyl CoA group released from a triglyceride:

- directly produces 7 NADH & 7 $FADH_2$
  - which generate 21 and 14 ATP's, respectively
- releases 8 acetyl CoA molecules for TCA Cycle
  - which each generate 12 ATP

Grand total is 131 ATP per fully oxidized palmitoyl CoA

** Recently, some have calculated energetically less ATP equivalents per NADH/FADH2 [2.5 and 1.5 instead of 3 and 2] which lowers the numbers somewhat (Berg).

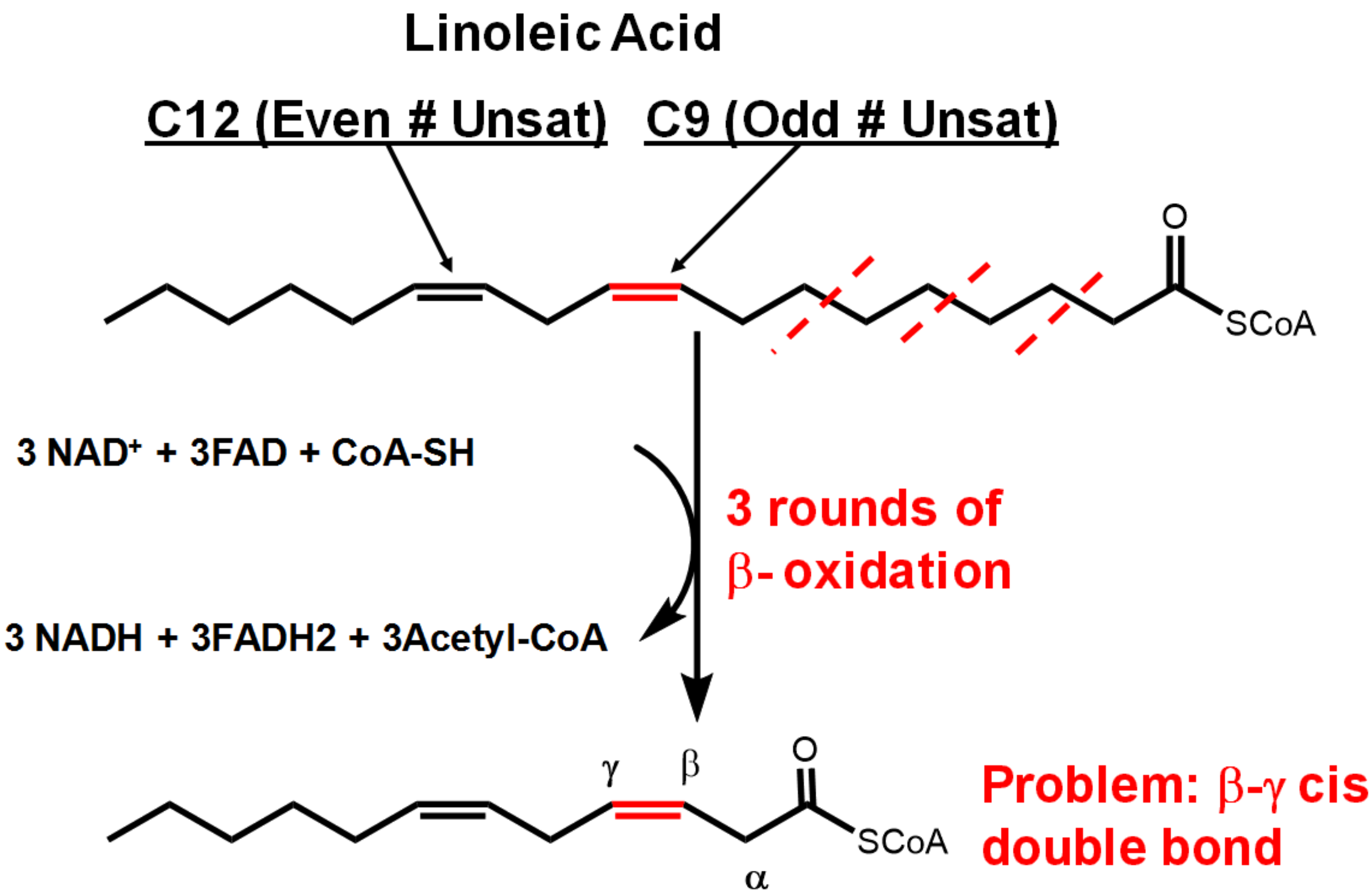
Efficiency of Fat Storage

Fat has 9 kcal/gram = 9 kcal/cc

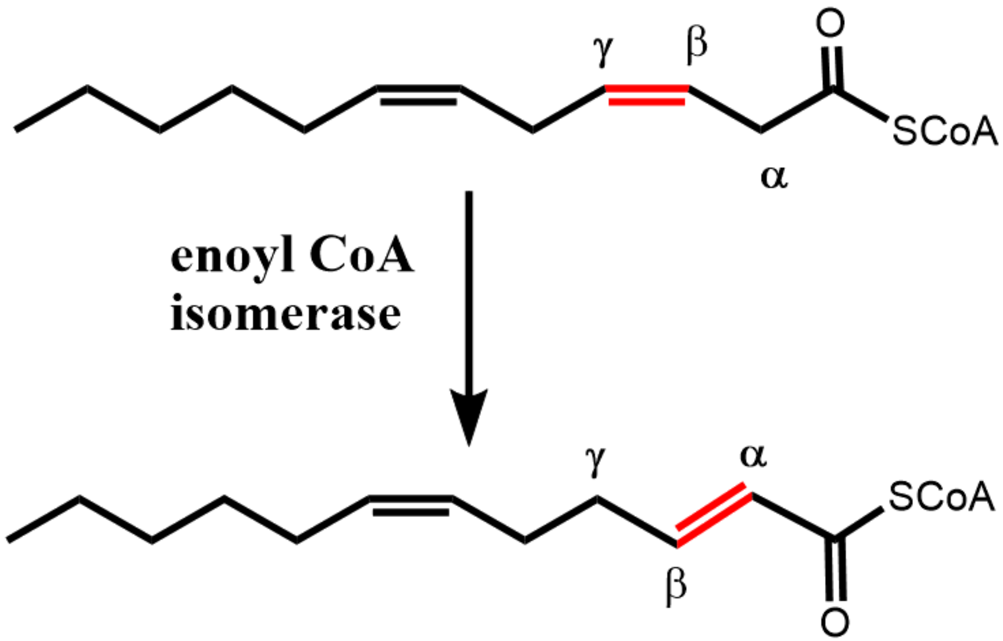
Both Carbohydrate and Protein have 4 kcal/gram = 4 kcal/3cc or 1.33 kcal/cc

Fat/(Carbohydrate or Protein) = 6x/cc!!!

Oxidizing Unsaturated FAs



Case 1: Odd Unsaturation



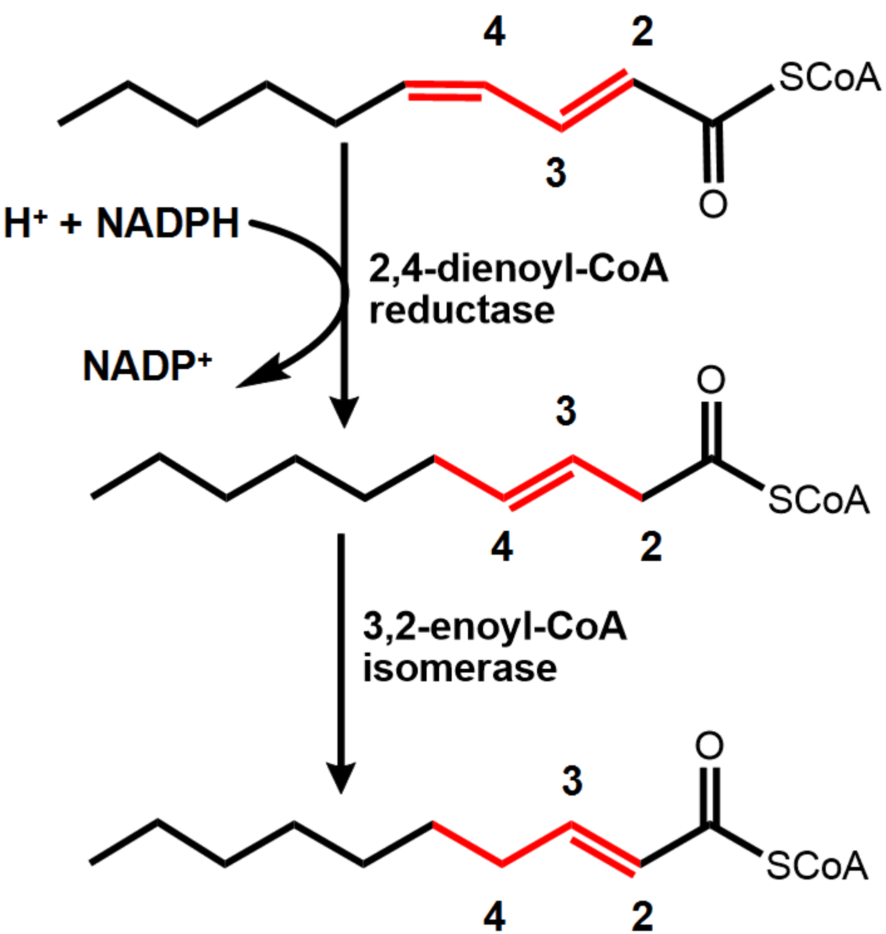
The GOOD News:

- Enzyme enoyl-CoA isomerase moves the double bond over 1 position.
- Oxidation process resumes

The BAD News:

- The first oxidation step is skipped
- One less $FADH_2$ is made
  - 2 fewer ATPs

Case 2: Even Unsaturation



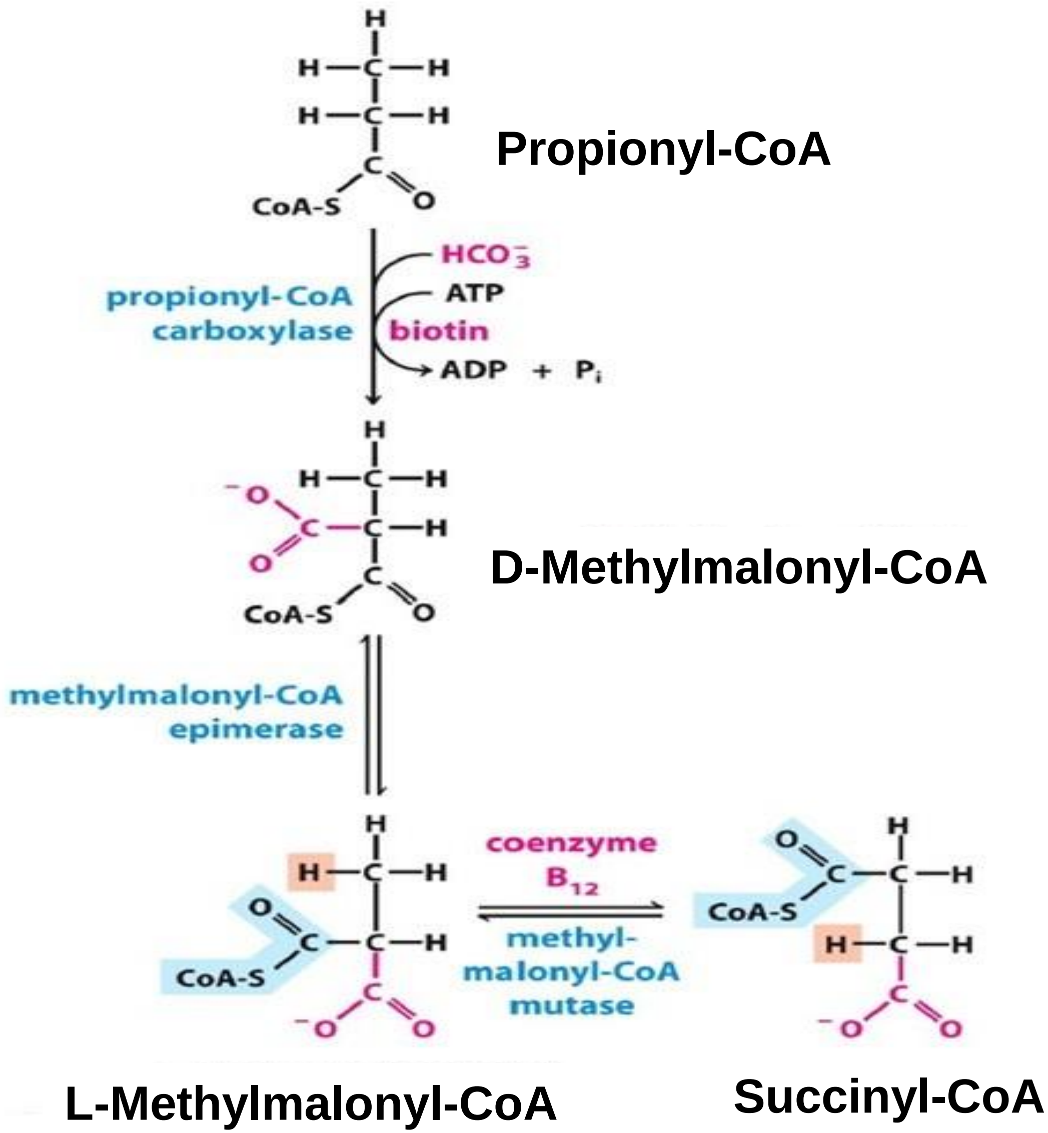
The GOOD News:

- Enzymes 2,4 dienoyl-CoA reductase and 3,2 dienoyl-CoA isomerase convert the $\gamma-\delta$ and $\alpha-\beta$ double bonds into a single $\alpha-\beta$ unsaturation.
- Oxidation process resumes

The BAD News:

- The conversion costs one NADPH directly.
- Net effect is the loss of one NADH
  - 3 fewer ATPs

Oxidizing Odd FAs



FA Synthesis *vs* FA Oxidation

	<u>Synthesis</u>	<u>Oxidation</u>
Cell Location	Cytosol	Mitochondria
Acyl carrier	ACP	CoA
2-Carbon Piece	Malonyl CoA	Acetyl CoA
β-hydroxyl acyl step	R-config	S-config
Electron carriers	NAD<u>P</u>H	NADH, FADH
Primary tissue site	Liver	Muscle, liver

FA Synthesis *vs* FA Oxidation

1. They are **not** the reverse of one another.
   - Different subcellular locations
   - R and S isomers of β-hydroxyacyl intermediates cannot easily jump to the other pathway
   - Electrons donated from oxidation (NADH, $FADH_2$) cannot directly enter synthesis, which uses NAD<u>P</u>H.

2. Separate, semi-independent pathways allow more sophisticated regulation.
   - Accelerating one pathway does not mean slowing the other.