ANABOLISM II: Biosynthesis of Fatty Acids & Lipids

ANABOLISM II: Biosynthesis of Fatty Acids & Lipids

1. Biosynthesis of fatty acids
2. Regulation of fatty acid degradation and synthesis
3. Assembly of fatty acids into triacylglycerol and phospholipids

Exam 4 ↑

4. Metabolism of isoprenes

Exam 5 ↓

   a. Isoprene biosynthesis and ketone bodies
   b. Isoprene polymerization
      i. Cholesterol
      ii. Steroids & other molecules
      iii. Regulation
      iv. Role of cholesterol in human disease

Fatty Acid Biosynthesis

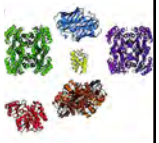
FAS I vs. FAS II

FAS I

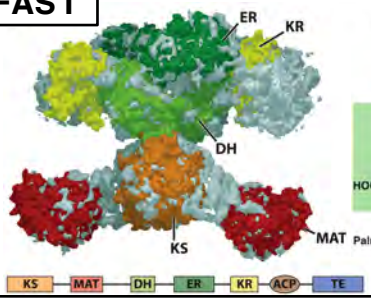
- Single polypeptide chain in vertebrates
- Leads to single product: palmitate 16:0
- C-15 and C-16 are from the acetyl-CoA used to prime the Rx
- FAS I in vertebrates and fungi

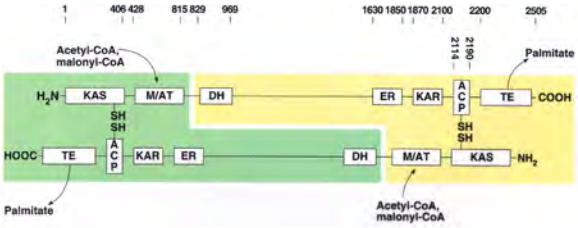
FAS II

- Made of separate, diffusible enzymes
- Makes many products (saturated, unsaturated, branched, many lengths, etc.)
- Mostly in plants and bacteria



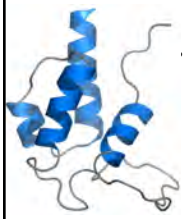
FAS I





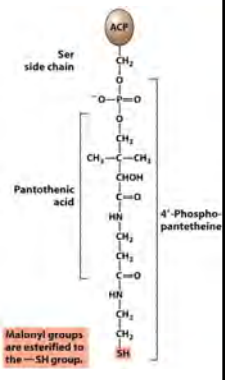
What you can't see is the ACP……

Fatty Acid Biosynthesis



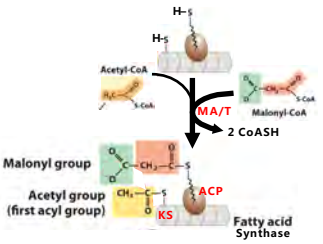
Acyl Carrier Protein (ACP)

- The *E. coli* ACP is a small 77-residue protein with a a covalently attached prosthetic group, 4'-phosphopantetheine, at a Ser residue.
  - In vertebrate FAS, it's a domain with a flexible arm to tether the growing acyl chain
- Delivers acetate (in the first step) or malonate (in all the next steps) to the fatty acid synthase enzymes
- Shuttles the growing chain from one active site to another during the four-step reaction



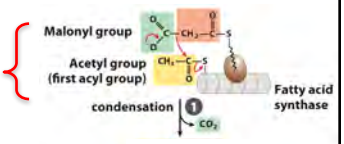
Priming FAS

- Two thiols must be **charged with the correct acyl groups** before the condensation reaction can begin.
  - thiol from 4-phosphopantethine in ACP
  - thiol from Cys β-ketoacyl-ACP synthase (KS)
- The acetyl group of acetyl-CoA is transferred to ACP.
  - catalyzed by malonyl/acetyl-CoA transferase (MAT)
  - ACP passes this acetate to the Cys of the KS domain of FAS 1.
  - ACP –SH group is recharged with malonate from malonyl-CoA again catalyzed by MAT



Fatty Acid Biosynthesis

Note that malonyl-CoA and acetyl-CoA have already been attached to complex via thioester linkages to enzyme and have shed their CoA attachments.



Step 1: Condensation reaction attaches two C from acetyl group (or longer fatty acyl chain) to two C from malonyl group.
- release of CO_2 activates malonyl group for attachment
- creates β-keto intermediate (acetoacetyl-ACP)
- Catalyzed by β-ketoacyl-ACP synthase (KS)

Step 2: First reduction: NADPH reduces the β-keto intermediate to an alcohol.
- carbonyl at C-3 reduced to form D-β-hydroxybutyryl-ACP
- NADPH is 2e^- donor
- catalyzed by β-ketoacyl-ACP reductase (KR)

Step 3: Dehydration: OH group from C-2 and H from neighboring CH_2 are eliminated, creating double bond (trans-alkene).
- OH and H removed from C-2 and C-3 of β-hydroxybutyryl-ACP to form *trans*-Δ^2-butenoyl-ACP
- catalyzed by β-hydroxyacyl-ACP dehydratase (DH)

Step 4: Second reduction: NADPH reduces double bond to yield saturated alkane.
- NADPH is the electron donor to reduce double bond of *trans*-Δ^2-butenoyl-ACP to form butyryl-ACP.
- catalyzed by enoyl-ACP reductase (ER)

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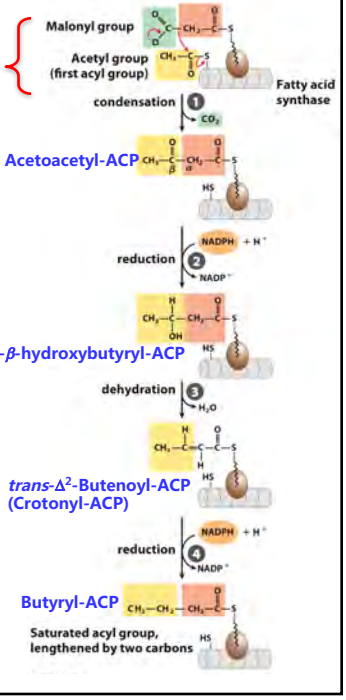

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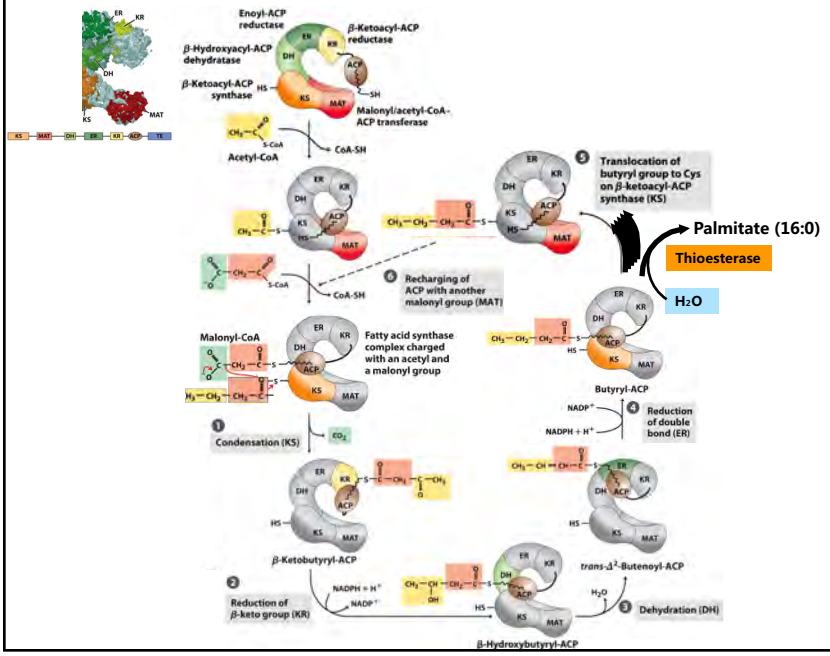
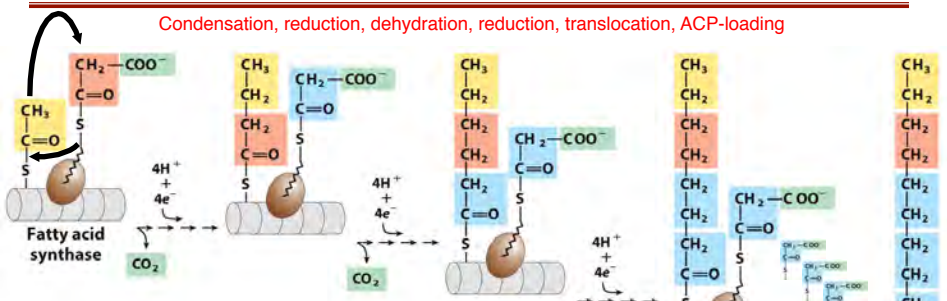
Fatty Acid Biosynthesis



Fatty Acid Biosynthesis

Condensation, reduction, dehydration, reduction, translocation, ACP-loading



Palmitate (16C) Synthesis

What is this like?

Protein synthesis; where the peptidyl-tRNA puts the growing peptide onto the incoming AA-tRNA

7 acetyl-CoA + 7 CO_2 + 7 ATP → 7 malonyl-CoA + 7 ADP + 7 P_i

acetyl-CoA + 7 malonyl-CoA + 14 NADPH + 14 H^+ → palmitate (16:0) + 7 CO_2 + 8 CoA + 14 $NADP^+$ + 6 H_2O

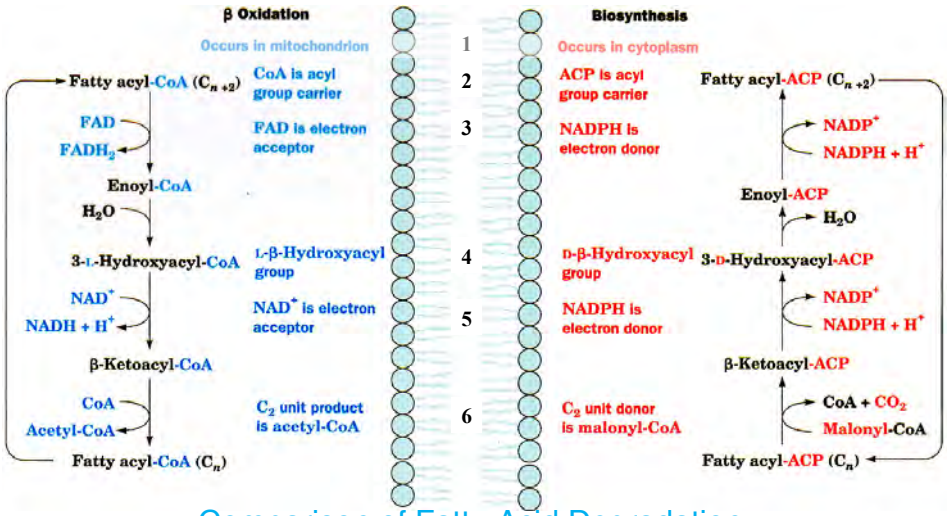
8 acetyl-CoA + 14 NADPH + 14 H^+ + 7 ATP → palmitate (16:0) + 8 CoA + 14 $NADP^+$ + 7 ADP + P_i + 6 H_2O

If you add in the transport (8x2=16): 23 ATP

Fatty Acid Biosynthesis

Comparison of Fatty-Acid Degradation
versus
Fatty-Acid Synthesis

Fatty Acid Biosynthesis



Comparison of Fatty-Acid Degradation
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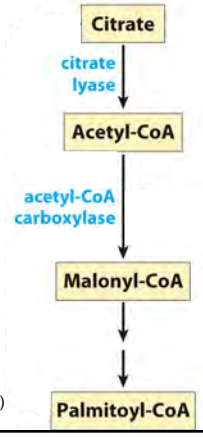
Fatty Acid Biosynthesis

Control of Fatty-Acid Synthesis

- **Acetyl CoA carboxylase (ACC) catalyzes the committal step.**
- **Allosteric Control**
  - Inhibited when energy is needed, fatty acids are acylated for degradation, and inhibit ACC.
    - ACC is feedback-inhibited by **palmitoyl-CoA**.
  - ACC is *activated* by **citrate**.
    - Citrate is made from acetyl-CoA in mitochondria (acetyl-CoA^{mt}).
    - Citrate signals excess energy to be converted to fat.
  - When [acetyl-CoA]^{mt} ↑ it is converted to citrate… citrate is exported to cytosol.
- **Hormonal Control**
  - Glucagon and epinephrine: leads to activation of AMP-dependent Protein Kinase (AMPK)
    - Phosphorylation inactivates ACC
    - Phosphorylation reverses the polymerization → monomers (inactive)
  - Insulin: leads to activation of Protein Phosphatase 2A
    - Dephosphorylation activates ACC
    - When dephosphorylated, ACC polymerizes into long filaments (active)
- **Changes in gene expression**
  - example: excess of certain polyunsaturated fatty acids (eicosanoids) bind to transcription factors called peroxisome proliferator-activated receptors (PPARS)



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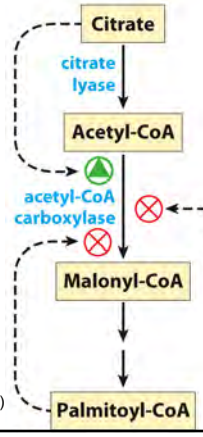
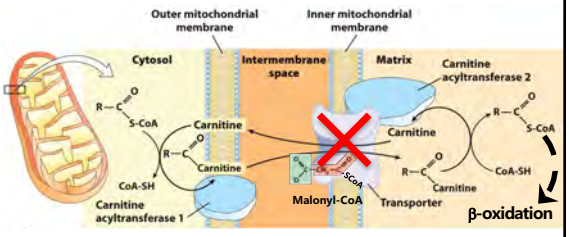
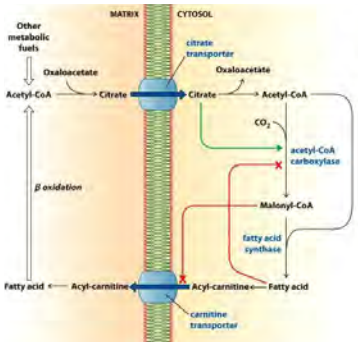


Fatty Acid Biosynthesis

Recall how β-oxidation starts: Acyl-Carnitine/Carnitine Transport:

- β oxidation of fatty acids occurs in mitochondria.
- If fatty acyl-CoAs are not transported in, they cannot be degraded
- Transport is via carnitine transporter.
- Blocking β oxidation with initial committed product of fatty-acid synthesis: malonyl-CoA





Control of Fatty Acid Metabolism

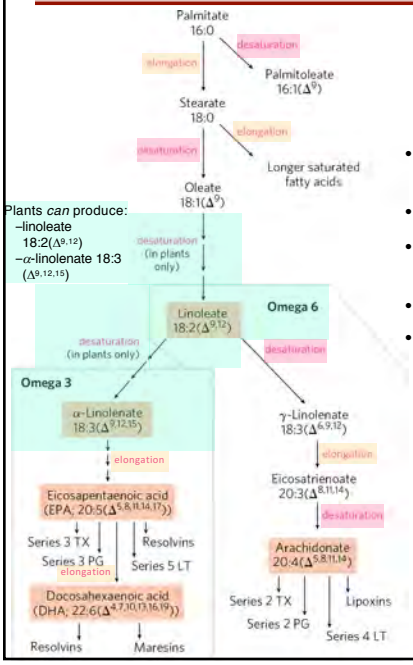
Inhibition

Activation

Reciprocal Control of Fatty-Acid Degradation *versus* Fatty-Acid Synthesis

ensures that fat synthesis and oxidation don't occur simultaneously

Fatty Acid Biosynthesis



Diversification of Palmitate: Elongation

Fatty Acid Elongation Systems

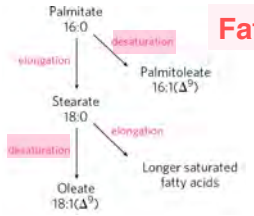
- Elongation systems in the endoplasmic reticulum and mitochondria create longer fatty acids.
- Use CoA derivatives
- As in palmitate synthesis, each step adds units of 2 C. Use malonyl-CoA.
- Stearate (18:0) is the most common product.
- Plants make linoleate (Ω6) and α-linolenate (Ω3), but animals can elongate these ingested essential fatty acids
  - These fatty acids are "essential" to humans.
    - –Polyunsaturated fatty acids (PUFAs) help control membrane fluidity.
    - –PUFAs are precursors to eicosanoids
  - The ratio of omega-6 to omega-3 fatty acids in the diet, if too high, can lead to cardiovascular disease

Eicosanoids

PG = Prostaglandins
TX = Thromboxanes
LT = Leukotrienes

Fatty Acid Biosynthesis

Diversification of Palmitate: Desaturation



Fatty Acyl-CoA Desaturase

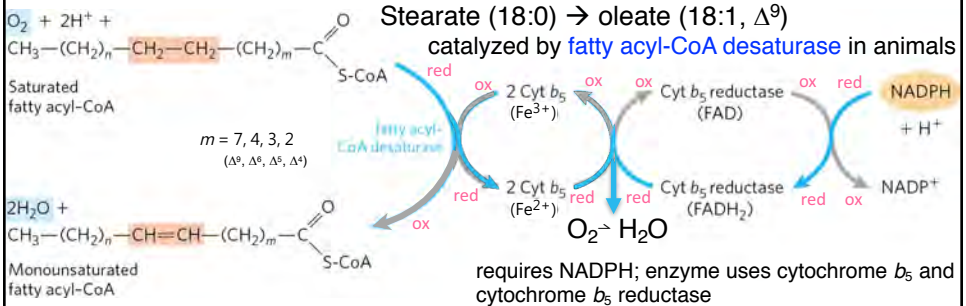
- Looks like a mixed-function oxidase: OXIDASE
- Humans have Δ^4, Δ^5, Δ^6, and Δ^9 desaturases but *cannot* desaturate beyond Δ^9.

FOR EXAMPLE: *Δ^9-desaturase*

Palmitate(16:0) → palmitoleate(16:1, Δ^9)

Stearate (18:0) → oleate (18:1, Δ^9)

catalyzed by fatty acyl-CoA desaturase in animals

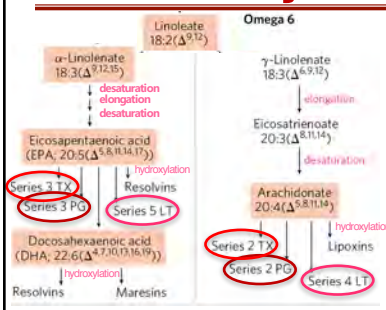


requires NADPH; enzyme uses cytochrome b_5 and cytochrome b_5 reductase

Both donate electrons to O_2 (total of 4)

In plants & bacteria the desaturases work on PL, not fatty-acyl CoA; needed for rapid changes in fluidity

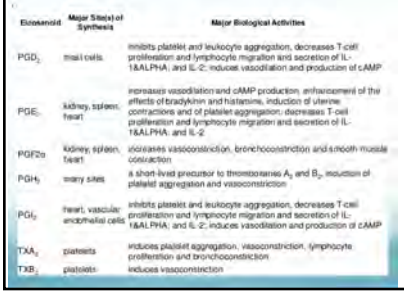
Fatty Acid Biosynthesis



Eicosanoids: Synthesis and Classes

- Linoleate (18:2($\Delta^{9,12}$) gets desaturated on both sides = γ-linolenate (18:3($\Delta^{6,9,12}$) & α-linolenate (18:3($\Delta^{9,12,15}$).
- Elongation to C20, and further desaturation of γ-linolenate (18:3($\Delta^{6,9,12}$) gives Arachidonate, 20:4($\Delta^{5,8,11,14}$)
- Elongation to C20, and TWO further desaturation of α-linolenate (18:3($\Delta^{9,12,15}$) gives EPA, 20:5($\Delta^{5,8,11,14,17}$). More of the same gets DHA, 22:6($\Delta^{4,7,10,13,16,19}$)
- These desaturations are all going closer to the carboxylate and can be accomplished by animals.

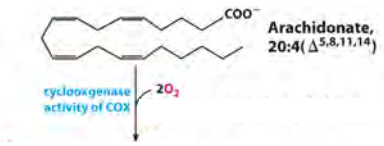
From these Eicosanoids (20C Polyunsaturated fatty acids (PUFAs)) bioactive paracrine signaling molecules are made:



PG = Prostaglandins
TX = Thromboxanes
LT = Leukotrienes

- Created from the arachidonate or EPA, which are incorporated into membranes phospholipids.
- In response to stimuli (hormone, etc.), phospholipase A_2 is activated
- The unsaturated C-2 fatty acid is released

Fatty Acid Biosynthesis



Eicosanoids: Cyclic Pathway

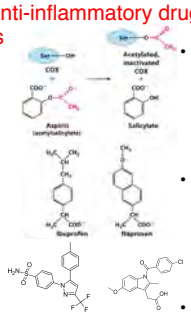
- PGH_2 synthase (cyclooxygenase, or COX) has isozymes
  - COX-1 is ubiquitous and catalyzes synthesis of prostaglandins that regulate *gastric mucin secretion and other smooth muscle functions*
  - COX-2 is inducible (except in brain) by immune response and acts at the sites of inflammation. It catalyzes synthesis of prostaglandins that mediate *pain, inflammation, and fever.*

Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit COX isozymes

Bengt Samuelsson
Nobel Prize 1982

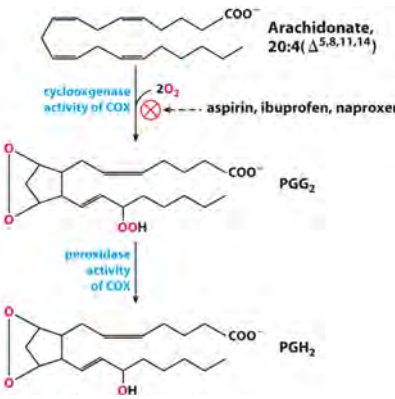
PGH_2 synthase is a cyclooxygenase/peroxidase enzyme that functions in the smooth ER.

- Step 1: PGH_2's cyclooxygenase activity adds 2 O_2 to form PGG_2.
- Step 2: PGH_2's peroxidase activity converts peroxide to alcohol, creates PGH_2.



- Aspirin (acetylsalicylate) is an irreversible inhibitor.
  - acetylates a Ser in active site
  - blocks active site in both COX isozymes
- Ibuprofen, naproxen, and indomethacin are competitive inhibitors.
  - resemble substrate; also block active site in both isozymes
- Celebrex and Vioxin are specific for COX-2

Fatty Acid Biosynthesis



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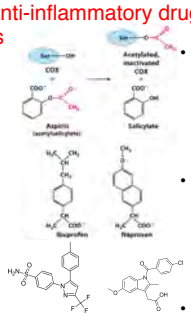

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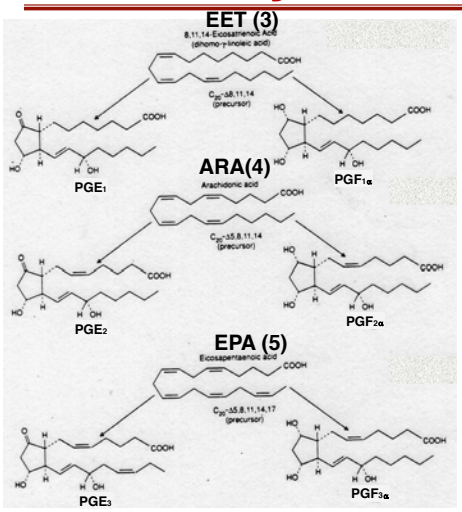
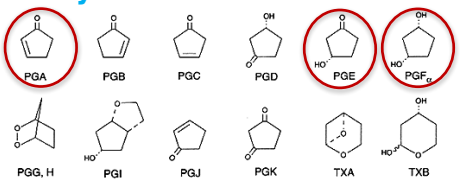
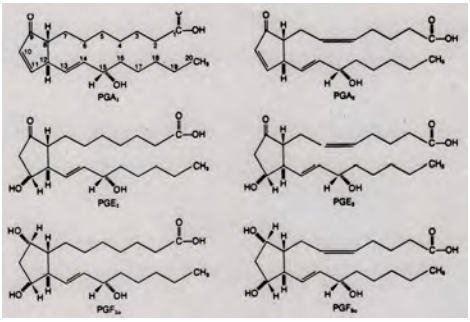


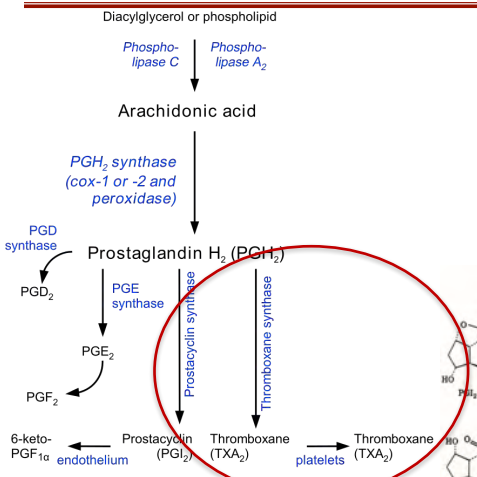
Prostaglandin & Thromboxane: Synthesis and Classes



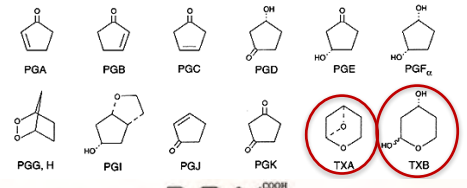


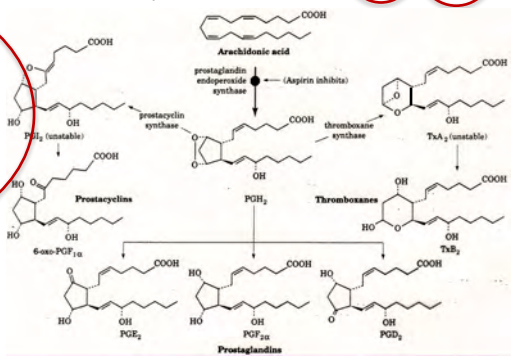
PG = Prostaglandins/**TB**=Thromboxane
–letters after are for number and oxidation state of oxygens and ring
–subscript is the number of double bonds

Fatty Acid Biosynthesis



Thromboxane: Synthesis and Classes





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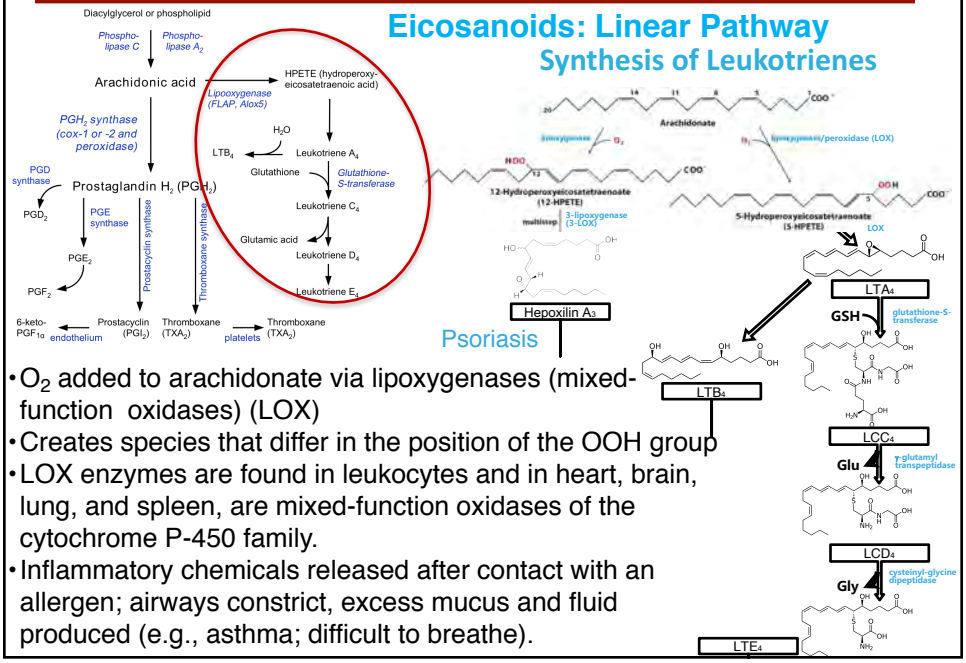
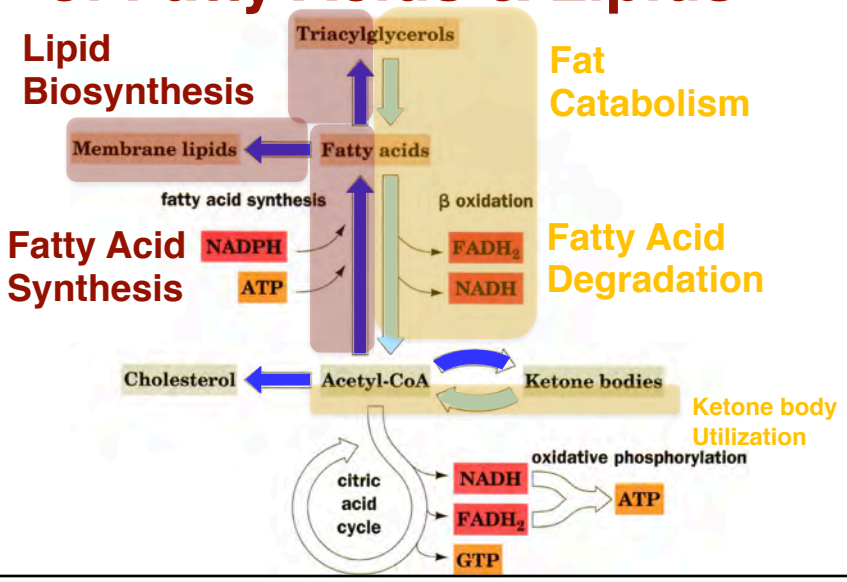
Platelet aggregation and wound healing

Fatty Acid Biosynthesis



- O_2 added to arachidonate via lipoxygenases (mixed-function oxidases) (LOX)
- Creates species that differ in the position of the OOH group
- LOX enzymes are found in leukocytes and in heart, brain, lung, and spleen, are mixed-function oxidases of the cytochrome P-450 family.
- Inflammatory chemicals released after contact with an allergen; airways constrict, excess mucus and fluid produced (e.g., asthma; difficult to breathe).

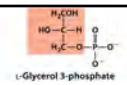
ANABOLISM II: Biosynthesis of Fatty Acids & Lipids



Lipid Biosynthesis

Biosynthesis of Triacylglycerol

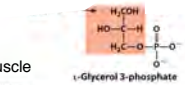
Fat (Triacylglycerol) and Phospholipids in Animals, Plants, and Bacteria have a common intermediate:
Phosphophatidic Acid



Usually saturated

Usually u

- Animals and plants store fat for fuel.
  - plants: in seeds, nuts
  - typical 70-kg human has ~15 kg fat
    - enough to last 12 weeks
    - compare with 12 hours worth of glycogen in liver and muscle
- All organisms make glycerophospholipids for membranes.
- Both molecules contain glycerol from Glycerol 3-P
- Most Glycerol 3-P comes from **dihydroxyacetone phosphate** (DHAP) in glycolysis.
  - via glycerol 3-phosphate dehydrogenase
- Minor amount from glycerol (liver and kidney).
  - via glycerol kinase
- **Phosphatidic acid** is the precursor to fat and glycerophospholipids.
  - fatty acids attached by acyl transferases
  - releases CoA



Memb.

signal molecule

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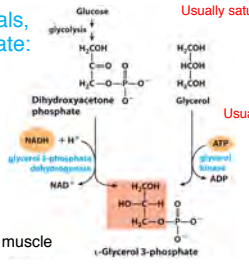
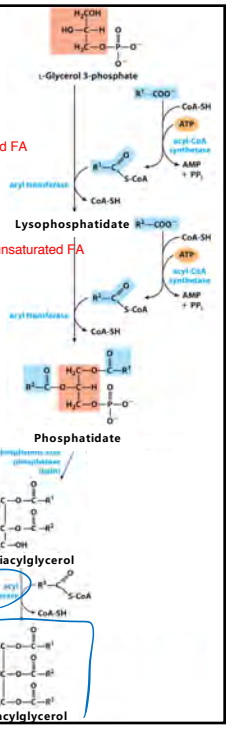


Usually saturated FA



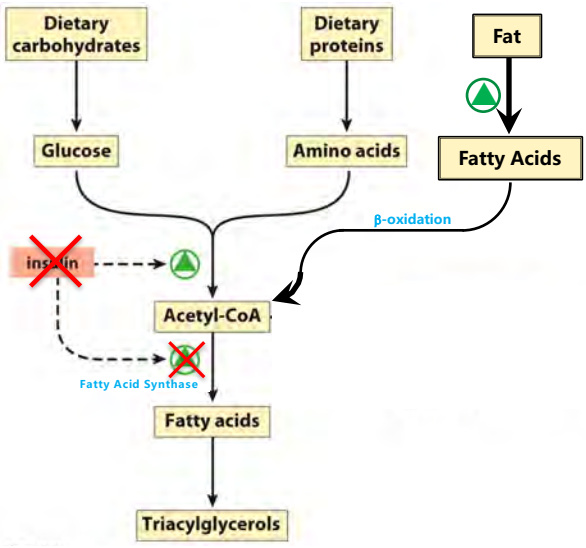
Usually unsaturated FA

Fat

Lipid Biosynthesis

Regulation of Triacylglycerol Synthesis by Insulin

- Secretion of Insulin results in stimulation of triacylglycerol synthesis.
- Lack of insulin results in:
  - –increased lipolysis
  - –increased fatty acid oxidation
    - And when citric acid cycle intermediates (oxaloacetate) are depleted (as in diabetes when glucose cannot get in), acetyl-CoA builds up and ketones bodies are made
  - –Repression of fatty acid synthase (PP2A)
  - –ACC is not active
  - –No malonyl-CoA means β-oxidation is active



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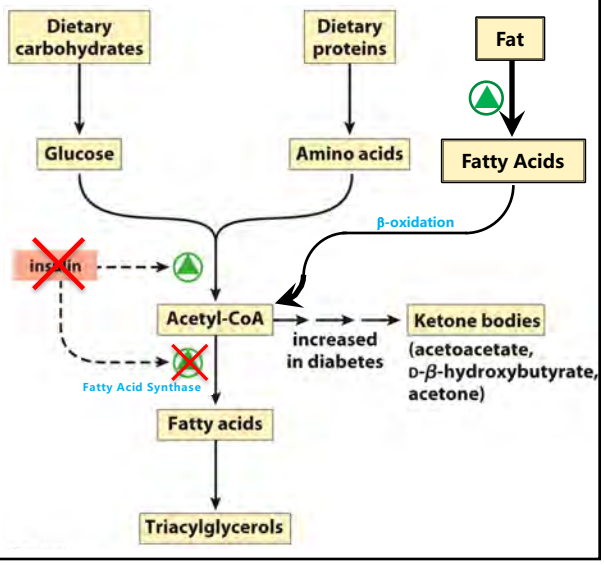

Lipid Biosynthesis

Triacylglycerol Cycle is a seemingly Futile Cycle

- Seventy-five percent of free fatty acids (FFAs) released by lipolysis are re-esterified to form TAGs, rather than be used for fuel.
  - Some recycling occurs in adipose tissue.
  - Some FFAs from adipose cells are transported to the liver, remade into TAG, and redeposited in adipose cells.
- Although the distribution between these two paths may vary, overall, the percentage of FFAs being esterified remains at ~75%.