Chapter 17
Homework Assignment

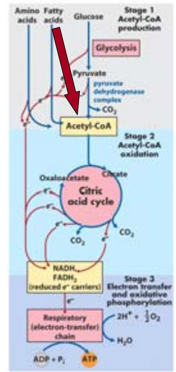
- The following problems will be due once we finish the chapter:

2, 6, 12, 13, 16, 17

- Additional Problems:
  - Write out the four reaction steps of β-oxidation, using structures to describe the intermediates. Use the correct stoichiometry to show the final products derived from one saturated fatty acid molecule. Identify the enzyme and any required cofactors for each step. Use arrows to show which reactions are irreversible and which are reversible.
  - In addition, know the two additional steps required to run an unsaturated fatty acid through β-oxidation

Chapter 17
Fatty Acid Catabolism

Cellular Respiration



Overview

<u>Stage 1</u>: Acetyl-CoA Production from:
- Glucose (CHP 14)
- Fatty Acids
- Amino Acids (CHP 18; not for us!)

<u>Stage 2:</u> Acetyl-CoA Oxidation (TCA cycle; CHP 16)

<u>Stage 3:</u> Electron Transfer & Oxidative Phosphorylation (CHP 19)

Fats as Fuel
Advantages

- Fats
  - At 38 kJ/gm (or 9.1 kcal/gm), they have twice the energy of sugars (since fats are almost fully reduced)
  - No water of hydration (saves weight)
  - Lipid droplets don't affect osmolarity
  - Nearly insoluble in water
  - Chemically inert

Fats as Fuel

Disadvantages

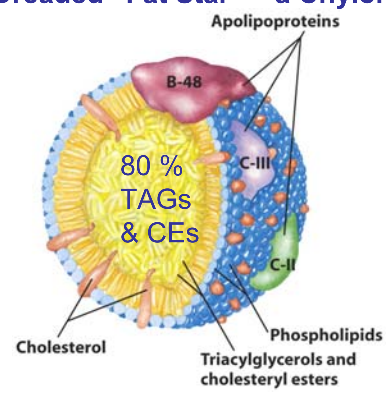
- **Fats**
  - Nearly insoluble in water
  - Chemically inert
  - Difficult to disperse and transport

To be useful as energy sources, fats must be:

- Solubilized
- Have their stable bonds activated
- Transported to the mitochondria

Digestion, Mobilization & Transport of Fats

The Dreaded "Fat Star" – a Chylomicron



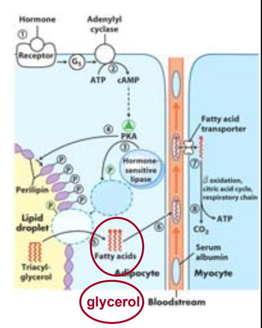
Digestion, Mobilization & Transport of Fats

Path of Fat from the Diet

Digestion, Mobilization & Transport of Fats

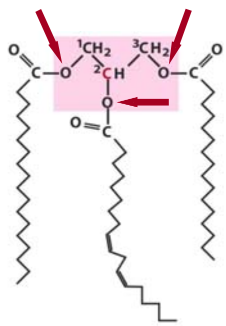
Path of Fat from "Fat"

- Adipose tissue (aka "fat") harbors very concentrated stored triacylglycerols (TAG's)
- Hormones such as ***glucagon*** and ***epinephrine***, which are secreted in response to low blood glucose, stimulate adenylyl cyclase to produce cAMP
- Resulting in phosphorylation of the ***Perilipins*** by a cAMP-stimulated kinase
- This phosphorylation allows for Lipase access to the interior of the lipid droplet.



Digestion, Mobilization & Transport of Fats
TAG Lipase

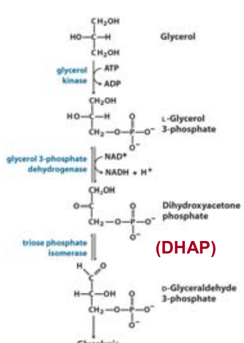
- PKA also phosphorylates TAG Lipase
- After it becomes phosphorylated, the hormone-activated ***TAG lipase*** moves into the lipid droplet and cleaves three bonds to yield one molecule of glycerol and three fatty acids.
- The free fatty acids leave the adipocyte and bind to serum albumin in the blood for transport



Digestion, Mobilization & Transport of Fats
The Glycerol Moves into Glycolysis

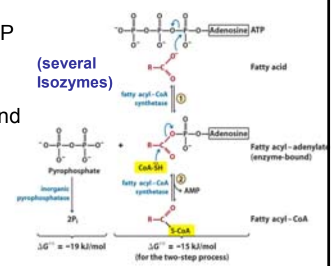
- By two simple steps:
  - Activation by ***glycerol kinase*** (group transfer from ATP), then
  - Oxidation by ***glycerol-3-P dehydrogenase***
- The product, DHAP, is an intermediate in the glycolytic pathway, and

you should know what happens next…

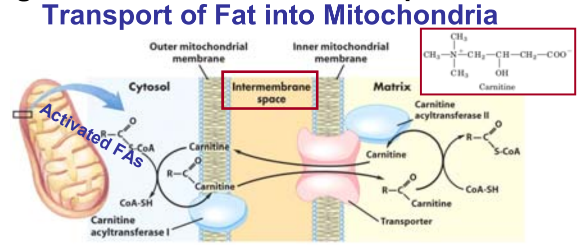


Digestion, Mobilization & Transport of Fats
And the Fatty Acids Get Activated…

- In the outer membrane of mitochondria by ***acyl-CoA synthetases***, "driven" by ATP hydrolysis
- Isozymes of this family are specific for short, medium, and long chain fatty acids
- Curiously, neither CoA nor these fatty acid-CoA esters can cross the inner mito-chondrial membrane…



Digestion, Mobilization & Transport of Fats
Transport of Fat into Mitochondria



- ***Carnitine acyltransferase I*** transfers the fatty acid from CoA to carnitine
- An acyl-carnitine/carnitine antiport transporter brings the FA-carnitine ester across the inner membrane by **facilitated diffusion**, after which
- On the other side ***Carnitine acyltransferase II*** transfers the fatty acyl group back to CoA, releasing free carnitine and FA-CoA into the **mitochondrial matrix**

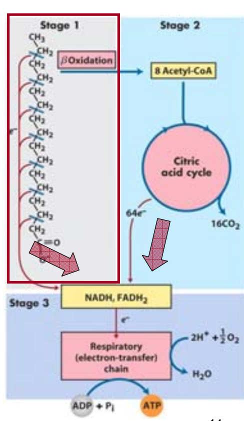
The net result is…voila!

Digestion, Mobilization & Transport of Fats
A Few Points to Note

- The cytosolic and mitochondrial (MT) pools of CoA do not intermingle, and have separate functions:
  - – Cytosolic CoA is used in fatty acid biosynthesis
  - – MT CoA is used in oxidative degradation of fatty acids, pyruvate, and some amino acids
- Carnitine-mediated transport is the rate-limiting step for fatty acid oxidation in the mitochondria
- Once inside, the activated fatty acids rapidly undergo β-oxidation in two-carbon units, with minor variations seen between fatty acids that are:
  - – Saturated, unsaturated and/or odd-chain length

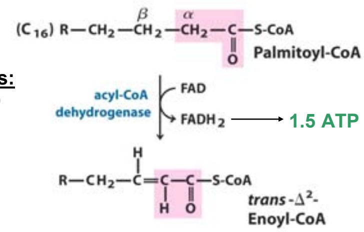
Oxidation of Fatty Acids

- **Stage 1** has four basic steps
- Each cycle of removing an acetyl-CoA 2-carbon unit (and passing it to **Stage 2 – TCA cycle**)
- Stage 1 itself generates one molecule of **$FADH_2$** and one of **NADH** (?? ATP) During oxidation of the β-carbon
- These equivalents plus those generates from Stage 2 are donated to the respiratory chain in the inner mitochondrial membrane



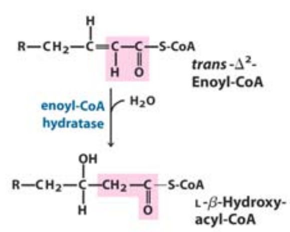
Oxidation of Fatty Acids
The First Step of β-Oxidation

Isozymes:
VLCAD
MCAD
SCAD



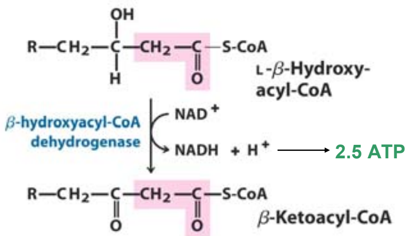
- Short, medium, and long chain ***Acyl-CoA-dehydrogenase*** isozymes catalyze the formation of a double bond between the C_α and C_β of the fatty acid chain

Oxidation of Fatty Acids
The Second Step of β-Oxidation



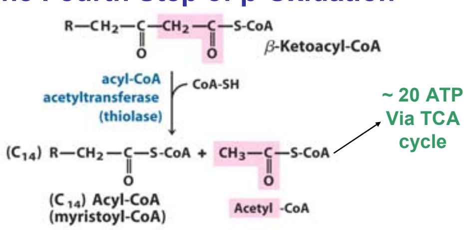
- ***Enoyl-CoA hydratase*** catalyzes the addition of water across the double bond to form the β-derivative alcohol

Oxidation of Fatty Acids
The Third Step of β-Oxidation



- A second ***dehydrogenase*** step forms β-ketoacyl-CoA
- This reaction is specific fo the L stereoisomer of hydroxyacyl-CoA

Oxidation of Fatty Acids
The Fourth Step of β-Oxidation



- The $C_{(n-2)}$ chain from β-ketoacyl-CoA can be transferred to a free CoA by ***thiolase***, releasing acetyl-CoA and sending the rest of the fatty acid back for another round of oxidation

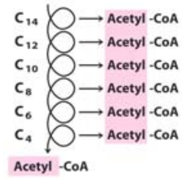
Oxidation of Fatty Acids
β-Oxidation of a C_{16} Fatty Acid

The overall equation for this oxidation is:

Palmitoyl-CoA + 7 CoA + 7O_2 + 28 P_i + 28 ADP

↓

8 Acetyl-CoA + 28 ATP + 7H_2O



Through the TCA cycle, the 8 acetyl-CoAs generate another 80 ATPs + 16H_2O + 16CO_2

Oxidation of Fatty Acids
Summary of Energy Yield

TABLE 17–1 Yield of ATP during Oxidation of One Molecule of Palmitoyl-CoA to CO_2 and H_2O

Enzyme catalyzing the oxidation step	*Number of NADH or $FADH_2$ formed*	*Number of ATP ultimately formed**
Acyl-CoA dehydrogenase	7 $FADH_2$	10.5
β-Hydroxyacyl-CoA dehydrogenase	7 NADH	17.5
Isocitrate dehydrogenase	8 NADH	20
α-Ketoglutarate dehydrogenase	8 NADH	20
Succinyl-CoA synthetase		8†
Succinate dehydrogenase	8 $FADH_2$	12
Malate dehydrogenase	8 NADH	20
Total		108
	Cost of fatty acid activation	-2

*Assumption: mitochondrial oxidative phosphorylation produces 1.5 ATP per $FADH_2$ and 2.5 ATP per NADH oxidized

Net gain: 106 ATP

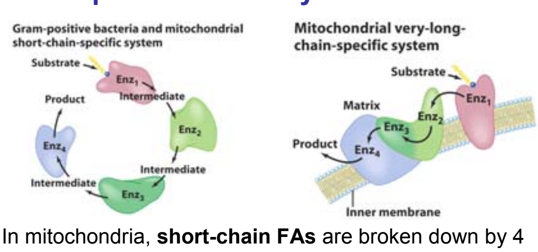
Oxidation of Fatty Acids

Comments on Energetics

- From a triacylglycerol, only about 5% of the energy is derived from the glycerol, and 95% from the three fatty acids
- The standard free energy for the complete oxidation of palmitate is 9800 kJ/mol; under prevailing cellular conditions the efficiency of energy recovery in the form of ATP is > 60% (remarkable!)
- As well as metabolic energy, fatty acid oxidation provides heat and water (23 molecules/palmitate molecule, for example) – of great benefit to both bears and camels… (see Box 17-1)

Oxidation of Fatty Acids

Not All β-oxidation Enzymes are the Same

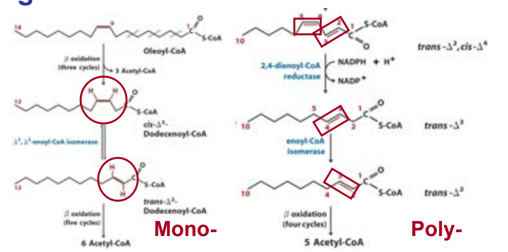


- In mitochondria, **short-chain FAs** are broken down by 4 separate soluble enzymes
- **Very-long-chain FAs** are metabolized by membrane-bound Enz_1 plus the dimeric "TFP" (trifunctional protein – $Enz_{2\text{-}4}$)

Can you name the 4 activities?

Oxidation of Fatty Acids

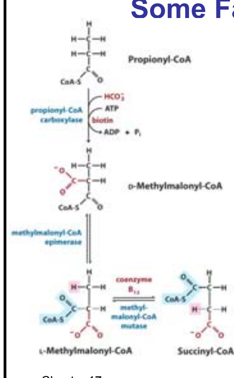
What About MUFAs and PUFAs?
Bring on an Isomerase and a Reductase!



- Problem: enoyl-CoA hydratase cannot act on *cis*-double bonds; with multiples thereof a reductase is also needed to reposition them

Oxidation of Fatty Acids

Some Fatty Acids are Odd…

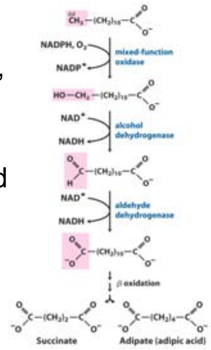


- With odd-numbered carbon chains, the final product of β-oxidation will be propionyl-CoA – **what to do?**
- The cellular answer is:
  1. Carboxylate (needs biotin)
  2. Epimerize (D- to L-)
  3. Rearrange (which needs coenzyme B_{12})
- Ending up with succinyl-CoA, and you know where *that* can go…

Does cost 1 ATP!!!

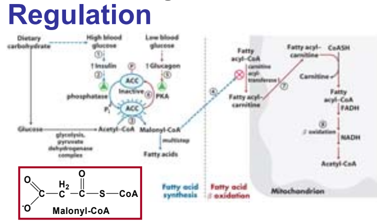
Oxidation of Fatty Acids
Another Alternative: ω-oxidation

- In the ER (endoplasmic reticulum) of liver and kidney, fatty acids may be degraded from the other end
- After 3 steps, a double-ended (dicarboxylic) FA is produced
- Which can be attached to CoA at either end, and proceed through β-oxidation



Oxidation of Fatty Acids
Regulation

- When carbohydrates are readily available, β-oxidation is not necessary and fatty acids are synthesized
- In this case, Acetyl-CoA is converted to Malonyl-CoA, the first intermediate in biosynthesis of fatty acids



- This conversion is catalyzed by ***Acetyl-CoA carboxylase*** (ACC)
- When blood glucose is high, insulin is released and, through its cascade, triggers a protein phophatase to dephosphorylate ACC, turning it on.
- ACC catalyzes Malonyl-CoA formation, which them moves into fatty acid biosynthesis **AND** acts an an inhibitor of the ***Carnitine acyltransferase I***
- This inhibition prevents the movement of fatty acids into the mitochondrial matrix so no β-oxidation!

What does Glucagon do?

Oxidation of Fatty Acids
Genetic Defects and Disease

- Stored TAGs are typically the chief source of energy for muscle contractions
- An inability to oxidize fatty acids from TAGs has serious consequences for health.
- The most common defect is due to mutations in the gene encoding ***Medium chain acyl-CoA dehydrogenase (MCAD)*** which leads to inadequate levels of the enzyme in the body
  - This enzyme catalyzes the first step of β-oxidation for fatty acids with chain lengths of 4 - 14 carbons
- This disorder is recessive, meaning you must be homogenous with two defective copies for the disease to present.
  - If you are heterogeneous, you are a carrier for the disease, but will not exhibit the symptoms
- The disease is characterized by recurring episodes that include fat accumulation in the liver, high blood levels of octanoic acid, low blood glucose (hypoglycemia), sleepiness, vomiting and coma
- If detected early, the disorder can be controlled by diet modification and avoiding periods of fasting.

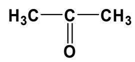
What type of diet would you suggest?

Oxidation of Fatty Acids
Summary of β-Oxidation

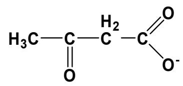
- Stored or ingested TAGs can provide up to 80% of the energy needs of heart and liver by FA oxidation
- After activation via a thioester linkage to coenzyme A, a cyclic 4-step process cleaves off 2-carbon units as acetyl-CoA
  - In what two different metabolic directions can this go next?
- Specialized steps act on MUFAs, PUFAs, and odd-chain length fatty acids
- The rate-limiting step for β-oxidation is entry into the mitochondrion, which can be controlled by malonyl-CoA (**WHY?**)

Ketone Bodies

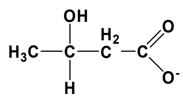
- In humans and most mammals, acetyl-CoA formed in the liver during oxidation of fatty acids can either enter Stage 2 (TCA cycle) of undergo conversion to “ketone bodies”
- **Ketone bodies** include ***Acetone, acetoacetone*** and ***D-β-hydroxybutyrate***
- Acetone, produced in smaller quantities than the others, is exhaled
- Acetoacetone and D-β-hydroxybutyrate are transported by the blood to tissues other than the liver where they are converted to acetyl-CoA and oxidized in the TCA cycle
- This provides much of the energy used by tissues such as skeletal and heart muscle and the renal cortex,
- The brain can adapt to the use of ketone bodies under starvation conditions when glucose is unavailable.



Acetone



Acetoacetate

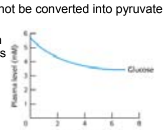


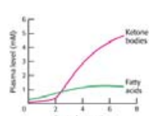
D-β-Hydroxybutyrate

Ketone Bodies

Overproduction in Starvation

- Glucose is the metabolite of choice of both the brain and working muscle, yet the body only stores ~ a day’s supply of carbohydrate.
- The low blood sugar resulting for even an overnight fast results in an increase in glucagon secretion and a decrease in insulin secrection.
  - These hormone levels cause an increase in mobilization of fatty acids from adipose tissue and their oxidation
  - The decrease in insulin levels also inhibits the uptake of glucose by the muscle tissues (remember the GLUT4 transporter?!)
- Together, starvation leads to a shift in metabolism from glucose breakdown to fatty acid oxidation, however, some organs (like the BRAIN!!) require glucose for energy.
- So, we need to make some glucose.
  - Fatty acids can’t help because Acetyl-CoA cannot be converted into pyruvate or oxaloacetate.
  - Could use the amino acids derived from the breakdown of proteins, a major source of which is muscle tissues (a futile cycle if ever there was one!)
  - Our best bet is adaptation to Ketone Bodies (produced by the liver due to the depletion of the TCA cycle intermediates)





Ketone Bodies

Overproduction in Diabetes

- In diabetes mellitus, insulin is either not secreted in sufficient amounts or does not efficiently stimulate its target.
- As a consequence, blood glucose levels become so high that it “spills over” into the urine (a good place for a diagnostic test!)
- Despite the large amount of glucose in the blood, the cells still “starve” because the insulin-stimulated movement of glucose into the cells (remember the GLUT4 transporter?!) is impaired.
- This causes the levels of Malonyl-CoA to drop and its inhibition of Carnitine Acyltransferase I to cease, allowing fatty acids to be converted to Acetyl-CoA.
- The lack of TCA cycle intermediates forces the Acetyl-CoA into ketone body formation, beyond the capacity of the extrahepatic tissues to convert them into energy.
- Ketone bodies in the blood of untreated diabetics can reach 90 mg / 100 mL (compared to < 3 mg / 100 mL for non-diabetics) resulting in ***ketosis***
- This buildup of ketone bodies (they are acids remember?) decreases the pH straining the buffering capacity of the blood leading to ***acidosis***