LIPID METABOLISM

OXIDATION OF LONG-CHAIN FATTY ACIDS

Two Forms of Carrying Fatty Acids:

- **PLASMA ALBUMIN**: Can carry up to 10 molecules of fats in the blood serum.
    - Also carries varies drugs and pharmacological agents. The albumin capacity for carrying these drugs must be considered with polypharmacy.

ACTIVATION OF FATTY ACIDS: Fats are delivered to cells as free fats. They must be activated before they can be burned.

- ***Acyl-CoA Synthetase:*** **Free Fat ------> Acyl-CoA Thioester**, which has a high-energy bond.
    - ATP is required in the synthesis.
    - This step is fully reversible, as ATP and the Acyl-CoA Thioester product both have equivalent energy levels.
    - To prevent the reversibility, the reaction is coupled to ***Pyrophosphatase***, which catalyzes **Pyrophosphate ------> 2 Inorganic Phosphate**, which breaks a high energy bond to drive the reaction to the right.

TRANSLOCATION OF FATTY ACYL-CoA THIOESTER: The Acyl-CoA must get into the mitochondrial matrix.

- Once activated, the Acyl-CoA can get through the out mitochondrial membrane by traversing through a **Porin** protein.
- Carnitine Intermediate: *Only Long-chain fatty acids are converted to carnitine as an intermediate. Short-chain fats can traverse the inner membrane directly:*
    - INTERMEMBRANE SPACE: ***Carnitine Acyl Transferase I***: **Acyl-CoA ------> Acyl Carnitine**.
        - Carnitine is a simpler structure than Coenzyme-A. The fat is esterified to carnitine, temporarily, for the purpose of transport.
    - ***Translocase:*** *Only recognizes Acyl-Carnitine*. It translocated the carnitine structure through the inner membrane to the matrix.
    - MITOCHONDRIAL MATRIX: ***Carnitine Acyl Transferase II:*** **Acyl-Carnitine ------> Acyl-CoA**

    - In the matrix the fat is esterified back to Coenzyme-A.

beta-OXIDATION: A four-step process. Called beta-Oxidation because most of the chemistry occurs on the beta-Carbon (beta to the carbonyl) per turn of the cycle.

- The Four Steps: *Ultimately we are oxidizing the beta-Carbon from most reduced to most oxidized state*.
    - OXIDATION: ***Acyl-CoA Dehydrogenase*** catalyzes an elimination of hydrogens on the alpha-carbon, to create the alpha,beta-Unsaturated Acyl-CoA.
        - FAD ------> $FADH_2$ is the corresponding reduction.
    - HYDRATION: Add water across the double bond, creating an OH group on the beta-Carbon.
    - OXIDATION: Oxidize the OH group to a carbonyl function. Now we have a beta-keto-acid
        - NAD^+ ------> NADH is the corresponding reduction.
    - THIOLYSIS: Cut the end-acid off, and add CoA to the newly created keto-group.
        - An *additional mole* of **Coenzyme-A** is esterified to the beta-Keto function, leaving **Acetyl-CoA** and an **Acyl-CoA of two less carbons**
- The Ultimate Products: Every cycle of beta-Oxidation (1) reduces the fat-chain by two carbons and (2) yields a free Acetyl-CoA (which can then be further metabolized as directed).
    - **Acyl-CoA$_{(n-2)}$**
    - **Acetyl-CoA**
- **Similarity to TCA Cycle**: The final four steps of the TCA Cycle are nearly identical to these in their chemistry.
    - Succinate ------> Fumarate -- OXIDATION
    - Fumarate ------> Malate -- HYDRATION
    - Malate ------> Oxaloacetate -- OXIDATION
- Odd Chain Fats: Most fats are even-numbered. But beta-Oxidation can occur with odd chains, at which point the products are Acetyl-CoA (2C) and Propanoyl-CoA (3C). Propanoyl-CoA is then metabolized by a different mechanism.

ENERGETICS OF beta-OXIDATION:

- COST: **-2 ATP**, *but we only have to invest that once!*
    - -1 ATP for the Acyl-CoA Ligase
    - -1 ATP net for the Pyrophosphatase, since we actually end up with AMP.

- BENEFIT: Per turn of beta-Oxidation (i.e. per two carbons).
    - Acetyl-CoA +12 ATP
    - $FADH_2$ +2 ATP
    - NADH +3 ATP
    - Total +17 ATP per 2 carbon, or about **8 per carbon**
- By Comparison, Glucose gives us about +36 ATP per 6 carbons, or about **6 per carbon**

REGULATION OF beta-OXIDATION:

- Positive Effectors: Starvation and a general low-energy level
    - Low insulin and high glucagon (i.e. low insulin:glucagon ratio)
    - ADP
- *Malonyl-CoA* inhibits it because it is a reactant of fat-synthesis.
    - It inhibits the **Carnitine Acetyltransferase**, preventing the transport of fats into the mitochondria and thereby effectively slowing beta-oxidation.
    - This occurs in conjunction with fat-synthesis, so that newly synthesized fats are not immediately broken down again.
- Negative Effectors: General indicators of sufficient energy in the cell:
    - High insulin and low glucagon
    - High ATP

SYNTHESIS OF LONG-CHAIN FATTY ACIDS

ACETYL-CoA CARBOXYLASE. This is the enzyme we use to get Malonyl-CoA for *fat-synthesis.*

- **Acetyl-CoA + HCO_3^- + ATP ------> Malonyl-CoA**
- A simple carboxylation reaction -- adding a carboxylate group onto the alpha carbon.
- **Biotin** is an intermediate. First biotin is carboxylated, forming **Carboxybiotin** -- *This is the step that requires ATP*.
- Then Carboxybiotin transfers the carboxy group to the Acetyl-CoA, requiring no additional energy.

STEPS OF FATTY-ACID SYNTHESIS: Fatty-Acid Synthesis occurs in the cytosol.

- CONDENSATION:
  - **Acetyl Unit** (2C) **+ Malonyl Unit** (3C) **------> 4-carbon chain + CO_2**
  - *CO_2 is lost in order to provide the energy for the reaction. It is the same CO_2 that was just put on!!*
- REDUCTION:
  - The beta-Carbonyl from above is reduced to OH.
  - **NADPH ------> $NADP^+$** is concurrent oxidation. Remember NADPH is the most common biosynthetic cofactor.
- DEHYDRATION
  - The OH group is eliminated creating a double bond -- alpha,beta-unsaturated species.
  - Loss of H_2O
- REDUCTION
  - Add H across the double-bond, fully saturating it.
  - **NADPH ------> $NADP^+$** is concurrent oxidation.
- FINAL PRODUCT: **Butyryl Unit** -- a 4-carbon acid.

FATTY ACID SYNTHASE: A *single multi-functional enzyme* is used to synthesize fatty acids. It has multiple catalytic domains, similar to the ribosomal complex (A-Site and P-Site) in translation of proteins.

- Condensation Reaction:
  - **Cys** Residue binds Acetyl-CoA.
  - **Pantetheine** cofactor has sulfur groups that bind Malonyl-CoA.

    - These two parts of the big enzyme then bring the constituents close enough together to undergo condensation.
- Then the other reactions occur at distinct sites on the protein.
- Elongation:
    - The butyryl unit is then translocated back to the Cysteine site.
    - The now-free Pantetheine site can now accept another malonyl unit to continue elongation.
- Termination: The process stops by a hydrolysis reaction, always at 16-carbons, at **Palmitate** for some reason.

REGULATION OF FATTY ACID SYNTHESIS: Fat synthesis is an anabolic process, so it is promoted by *dephosphorylation*.

- Positive Effectors: General surplus of energy
    - High ATP
    - High insulin:glucagon ratio.
    - INSULIN -- STIMULATES **Acetyl-CoA Carboxylase** by **dephosphorylating** it.
        - Insulin activates a Phosphoprotein Phosphatase ------> Dephosphorylate the enzyme
    - CITRATE in the cytosol allosterically stimulates Acetyl-CoA Carboxylase.
        - Citrate can also break down to Oxaloacetate and Acetyl-CoA, and Acetyl-CoA can then be used as a fatty-acid building block.
- Negative Effectors: General lack of energy
    - High ADP
    - Low insulin:glucagon ratio
    - GLUCAGON, EPINEPHRINE -- INHIBITS **Acetyl-CoA Carboxylase** by **phosphorylating** it directly.
- Inhibited by *Long-Chain Acyl-CoA*, i.e. intermediates of beta-oxidation.

DESATURATION OF LONG-CHAINS FATTY ACIDS:

- ELONGATION: If we want more than 16 carbons, as in **Stearate**, we must further elongate the fat on the endoplasmic reticulum. This occurs two carbons at a time, utilizing a Coenzyme-A intermediate.
- DESATURASES: **Delta9-Desaturase** would put a double-bond between the 9th and 10th carbons. This would convert our Stearate ------> Oleate.
    - The unsaturation releases two hydrogens which are taken up with O_2 to form H_2O.

    - This is accomplished through an *electron-transport chain* of the following components: NADH, $FADH_2$, Cytochrome-b5, and two non-heme iron-sulfur components.
- Human beings have desaturases for **Delta9, Delta4, Delta5, and Delta6** positions.
    - Plants are different! They can put double-bonds at Delta9, Delta12, and Delta15.
- MODIFICATION OF ESSENTIAL FATS: **Linolenate --** We get this essential fat from plants. It comes in our diet intact with double-bonds at positions that we couldn't create ourselves. Then we can further modify it to create essential polyunsaturated fatty acids.
    - Elongation ***always occurs at the carboxyl end*** of the fat, so in effect what happens is that *the potential desaturation positions change* as we elongate the fat.
    - By alternating elongation with desaturation 4, 5, 6, and 9, we can get polyunsaturated fats of double-bonds three carbons apart.

METABOLISM OF CHOLESTEROL

BIOSYNTHESIS OF CHOLESTEROL:

- CONDENSATION: In three steps, we condense 3 acetyl-CoA to form the six carbon **Mevalonate**.
    - *Acetoacetyl-CoA Thiolase:* **2 Acetyl-CoA ------> Acetoacetyl-CoA**
    - *HMG-CoA Synthase:* **Acetoacetyl-CoA + Acetyl-CoA ------> 3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA)**
    - ***HMG-CoA Reductase:*** **HMG-CoA ------> Mevalonate**
        - This step *ONLY OCCURS IN THE CYTOSOL*. In the mitochondria, ketone bodies are made instead (see below).
        - This is a "double-reduction" of carboxylic-acid all the way to alcohol.
        - Concurrent Oxidation: **2 NADPH ------> 2 $NADP^+$**

- POLYMERIZATION: Mevalonate is polymerized to **Squalene** via polyisoprene intermediates.
    - **Mevalonate (C_6) ------> Isopentyl Pyrophosphate (C_5)**
        - This creates the C_5 isoprene building block for the reactions that follow.
    - **Isopentyl Pyrophosphate (C_5) ------> Geranyl Pyrophosphate (C_{10})**
        - Add another isopentyl pyrophosphate.
    - **Geranyl Pyrophosphate (C_{10}) ------> Farnesyl Pyrophosphate (C_{15})**
        - Add another isopentyl pyrophosphate
    - **Farnesyl Pyrophosphate + Farnesyl Pyrophosphate ------> SQUALENE (C_{30})**
        - Condense two Farnesyl Pyrophosphates with each other.
- CYCLIZATION -- Synthesis of **lanosterol**, and then modification to yield cholesterol.
    - **Squalene + O_2 ------> Squalene Epoxide**
    - **Squalene Epoxide ------> Lanosterol**
        - Cyclization occurs at this step.
    - **Lanosterol ------> Cholesterol:** Modifications to lanosterol at this step include the following:
        - C_{14} comes off as formic acid.
        - Lose two carbons as CO_2

SYNTHESIS OF KETONE BODIES: Ketone bodies originate from an intermediate of Cholesterol synthesis, **3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA)**

- ***HMG-CoA Lyase***: **HMG-CoA ------> Acetoacetate + Acetyl-CoA**
    - This is a simple cleavage of the 6-carbon HMG-CoA.
    - *THIS OCCURS IN THE MITOCHONDRIA ONLY.* In the cytosol, HMG-CoA is further synthesized to Cholesterol.

WHERE IS CHOLESTEROL SYNTHESIZED:

- THE LIVER is the primary site of synthesis.
- ADRENAL GLANDS (Corticosteroids) and GONADS (Sex Hormones) also synthesize significant amounts of cholesterol.

REGULATION OF CHOLESTEROL BIOSYNTHESIS: **HMG-CoA-Reductase** is the target enzyme!

- Short-Term Regulation
    - **INSULIN** causes DEPHOSPHORYLATION of the target enzyme (via a phosphatase) to INCREASE synthesis.

    - Conversely, Glucagon phosphorylates the target enzyme to decrease synthesis.
    - OXYSTEROLS (one of the products of synthesis) are negative effectors on the target enzyme.
- Long-Term Regulation
    - CHOLESTEROL itself (probably from diet) decreases gene-transcription of the target enzyme, probably through steroid-receptor interactions.
- Pharmacologic Regulation
    - MEVINOLIN (i.e. Simvastatin) decreases Cholesterol synthesis and is used therapeutically.

FORMATION OF CHOLESTEROL ESTERS: This is how we STORE cholesterol.

- Storage as **Lipid Droplets**: **ACAT -- Acyl-CoA Cholesterol Acetyltransferase**
    - **Cholesterol + Acyl-CoA ------> Cholesteryl Ester + Coenzyme-A**
        - This occurs in LIVER and some other steroid-tissues.
        - It is stored as a hydrophobic lipid droplet in the cytoplasm of these cells.
- Storage as **Lipoproteins**: **LCAT -- Lecithin Cholesterol Acetyltransferase**
    - **Cholesterol + Phosphatidylcholine ------> Cholesteryl Ester + Lysophosphatidylcholine**
        - This is the esterified form of Cholesterol found in the Bloodstream.

CYTOCHROME-P450: The common enzyme that is involved in **HYDROXYLATION OF CHOLESTEROL**. It is called a "mixed-function oxidase"

- Hydroxylation of cholesterol is the common step in modifying cholesterol to form steroid hormones.
- Two different types of Cytochrome-P450
    - *Mitochondrial Cyt-P450* -- Used to hydroxylate cholesterol to form sex-hormones
    - *Endoplasmic Reticulum Cyt-P450* -- used both for cholesterol hydroxylation and for metabolism of **xenobiotic (foreign)** substances such as **ethanol** in the liver!
- Mechanism:
    - **O_2** is used to hydroxylate on of the positions on the cholesterol.
        - $1/2O_2$ goes to hydroxylation cholesterol, and the other $1/2O_2$ eventually forms H_2O.

    - **Electron-Transport Chain:** A small ETC-Chain is used to reduce the O_2 to OH and to oxidize the carbon to an alcohol function. Order of events follows
        - Cytochrome-P450 (Fe II/III) ------> Cytochrome-P450 Reductase ------> NADPH.
        - Ultimate result: **O_2 is reduced while NADPH is Oxidized**

SYNTHESIS OF STEROID HORMONES:

- **CHOLESTEROL ------> PREGNENOLONE**: Removal of 6 of the side-chain carbons. *This step is common to all steroid hormone synthesis.*
    - This step occurs on the mitochondrial membrane.
- CORTICOSTEROIDS: Pregnenolone, plus:
    - Hydroxylation of C-21 (via Cyt-P450)
    - beta-Hydroxylation (OH pointing up) of C-11
- ALL SEX HORMONES: Pregnenolone, plus:
    - Further loss of *all carbons from the side chain.*
    - **beta17-Hydroxylation**
    - ESTRADIOL:
        - Loss of 19-methyl group
        - Aromatization of A-Ring
    - TESTOSTERONE:
        - Oxidation to 3-Keto Group.
- VITAMIN D3 (CHOLECALCIFEROL): Pregnenolone, plus:
    - B-Ring opens
    - 1-Hydroxylation
    - 25-Hydroxylation

OTHER IMPORTANT ISOPRENE DERIVATIVES:

- **Dimethylallyl-Phosphate** -- comes from Isopentyl Phosphate (C_5)
- **Dolichol Phosphate** -- 100 Carbons! Comes from condensations of **Farnesyl-Pyrophosphate** (C_{15}).
- **Geranylgeranyl Phosphate** -- also comes from Farnesyl pyrophosphate

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PULMONARY SURFACTANT

COMPOSITION OF SURFACTANT:

- It is about 90% lipid, of which 90% is phospholipid, of which about 2/3 is **1,2-Dipalmitoylphosphatidylcholine (DPPC)**.
    - DPPC has important mechanical properties that allow it to act as pulmonary surfactant. It has a melting temperature of 40C

L/S RATIO: Around the 32nd - 36th weeks, synthesis of phosphatidylcholine in the infant increases markedly. This is in indication that pulmonary surfactant is being synthesized.

- Phosphatidylcholine is also known as **Lecithin.**
- Synthesis of Sphingomyelin doesn't increase at all.
- Hence, one can measure the **L/S-Ratio** to determine whether the baby's lungs are yet mature enough to breathe. This is done clinically.

Respiratory Distress Syndrome (RDS): Any breathing problem in the newborn or adult.

- The most common cause of RDS in the newborn is a lack of surfactant, which isn't yet synthesized.

Type-II Alveolar Cells: They produce surfactant.

- Synthesis may be stimulated by glucocorticoids, cholinergic agents, and thyroxine. It may also be produced pharmaceutically by cholinergic agents.

Surfactant turns over very rapidly. Both Type-II lung cells and macrophages participate in the uptake of surfactant.

There are pharmacological agents available to emulate surfactant in the neonate until his/her lungs are mature enough to breathe on their own.

SURFACE TENSION: Surfactant decreases the surface tension in lungs, and therefore surface pressure. This prevents the coalescence of alveolar walls with each other.

DIGESTION, ABSORPTION, AND TRANSPORT OF LIPIDS

SYNTHESIS OF BILE ACIDS: Bile acids are synthesized from cholesterol in the liver, stored in the gall bladder, and secreted through the common bile duct.

- **alpha-Hydroxylation of 7-carbon** is the key step in bile-acid synthesis. This hydroxylation destines the product to become a bile-acid.
- **Lose 3 carbons of the alpha-side:** This is also common to all bile acids. Then the terminal carbon is oxidized to an acid (hence bile acid).
- **Ring Junction is reorganized:** The A-Ring reconnects with the B-Ring such that it is no longer in the plane of the molecule. This serves to *disrupt the membrane* by putting a kink in it, which aids bile acids in their detergent function.

CHOLATE: The major bile acid. It has three OH-groups in the rings, and a carboxylic acid at the end of the side chain.

DEOXYCHOLATE: The minor bile acid. It is missing one of the OH-groups (at the 12 carbon)

SYNTHESIS OF BILE SALTS: Bile salts are just bile acids with the carboxylate function modified by adding an amine to it via an amide linkage. Bile Salts are *even more polar* than their corresponding acids.

- **Glycocholate:** Cholate with glycine added as an amide function.
- **Taurocholate**: Cholate with **Taurine** added as an amide function. Taurinine is a modified cysteine.
- **Glycochenodeoxycholate** and **Taurochenodeoxycholate** are the minor bile salts.

SECONDARY BILE ACIDS AND SALTS: Bacteria in the intestine can modify bile salts by removing the 7alpha-carbon, to create a series of "secondary" bile salts.

BILE-ACID FUNCTIONS:

- **Emulsification** of triacylglycerols. Emulsification is a physical, not a chemical, process.
- Promote absorption of fat-soluble vitamins.
- Maintain cholesterol homeostasis by promoting excretion of cholesterol -- i.e. extra cholesterol in the system can be disposed of as bile.

COMPOSITION OF HUMAN BILE: It contains pure cholesterol, phospholipid, and bile acids.

- It is about 70% bile acids, consisting primarily of cholate (major bile acid), deoxycholate (secondary bile acid), and chenodeoxycholate (minor bile acid)
- Bile is normally about 8% cholesterol.

GALLSTONES: If the amount of cholesterol in bile acids get *above 15%*, then gallstones may result. If cholesterol gets too high, then they may crystallize out of solution in the gall bladder, where bile is concentrated tenfold before secretion.

- Cholecystectomy -- removal of gall bladder.
- Feeding of extra bile acids to dissolve the excess bile in gall bladder.
- Dissolve with organic solvent.
- Sonic disruption of the gallstone by a procedure known as **lithotripsy**.

ENTEROHEPATIC CIRCULATION: The circulation of bile between the intestine and liver.

- **New Synthesis:** A small of bile acids are newly synthesized every day. This is mixed in with bile acids that are recirculated.
- **Conjugation:** Adding the glycine or taurine to the bile acid to form a bile-salt, before secretion.
- **Secretion:** We secrete daily about 15-30 grams of bile acids into the GI tract.
- **Deconjugation:** Deconjugation and reduction of bile salts often occurs in the intestine.
- **Reabsorption:** 90% of the bile acids are reabsorbed in the intestinal tract -- in the ileum, after most nutrients have already been absorbed.
    - Reabsorption sends the acids through the **portal circulation** and ultimately back to the liver.
- **Excretion:** Some bile acids are excreted in the feces on a daily basis, about the same as the amount that is newly synthesized every day. This is one way to get rid of cholesterol.

ABSORPTION and DIGESTION of DIETARY FATS: Order of events that happens in digesting fats

- **EMULSIFICATION**: Physical (not chemical) breakdown of fats.
    - **MIXED MICELLES:** Emulsification of lipids in the GI-Tract leads to mixed micelles.
        - Mixed Micelles are *partially degraded lipids with detergent-like properties*. They contain bile acids, dietary lipids and phospholipids, fat-soluble vitamins, cholesterol, etc.
        - Mixed micelles are formed in the duodenum and the *nutrient-component* is absorbed in the Jejunum through microvilli in the intestinal wall.
        - The bile-acids themselves are not absorbed until later (approx. ileum).

- **CHEMICAL BREAKDOWN: Pancreatic Lipolytic enzymes** break down triacylglycerols to individual fatty acids.
    - **Lipase** Degrades triacylglycerols.
        - The proenzyme (zymogen) is activated by the presence of bile acids, phospholipids, or regulatory enzyme called co-lipase.
    - **Phospholipase A_2** Degrades phospholipids
        - The proenzyme (zymogen) is activated by trypsin and Ca^{+2}
    - **Cholesteryl Ester Hydrolase** Degrades dietary cholesterol, usually present in the form of cholesteryl esters.
        - The proenzyme is activated by bile salts.
- **ABSORPTION:** Individual fatty acids (not triacylglycerols) are absorbed through the brush border.
- **RE-ESTERIFICATION:** Once inside intestinal enterocytes, triacylglycerols and cholesteryl esters are reformed.
    - Form **Triacylglycerols** Using *Acyl-CoA-Synthetase* and *Acyltransferase*.
- **CHYLOMICRON ASSEMBLY**: The newly formed triacylglycerol droplets are then assembled into a chylomicron:
    - Chylomicron is a form of *lipoprotein*, consisting of lipid and protein.
    - This assembly occurs in the intestinal epithelial cells.
- **TRANSPORT** into the **lymphatic** system for the most part. Chylomicrons always travel in lymph to the extrahepatic tissues -- except short chained fats.
    - SHORT-CHAINED FATS (up to 10 carbons): The majority of these fats are carried through the **portal circulation** back to the liver -- not the lymph!
        - Here is another reason that fetuses utilize short-chains fats -- they are absorbed and sent directly to the infant's liver, rather than going through lymphatic system.
    - LONG-CHAINED FATS: (greater than 12 carbons): The majority of them are carried through the lymphatic system and thereby distributed throughout the body.
- **FATS TRAVEL to LIVER:** Fats make their way back to liver, from lymphatic or portal circulation. They then utilize **lipoproteins** (see below) to get distributed to target tissues.
- **UTILIZATION at TARGET TISSUE:** At the target tissue, fats are broken back down again, using **lipoprotein lipase.**
    - ***Lipoprotein Lipase:*** **Triacylglycerol ------> Monoacylglycerol + 2 Fatty Acids**.

GENERAL STRUCTURE OF LIPOPROTEINS:

- **Core** of non-polar lipids.

- **Monolayer** of phospholipids surrounding the core.
- Integral and peripheral **apoproteins** dispersed throughout.

CATEGORIES OF LIPOPROTEINS: Lipoproteins are often categorized according to density, which is dependent upon the relative amount of protein present. The more protein present, the higher the density of the lipoprotein.

- **Chylomicrons** -- these are the lowest density (and therefore) largest lipoproteins.
  - Half-life = about 30 minutes
  - This is why fasting overnight is sufficient to take a blood-test to measure triacylglycerols -- we get rid of the influence of chylomicrons in the bloodstream by doing this.
- **VLDL:** Very Low Density.
  - These are the *initial* lipoproteins to carry triacylglycerols from the liver to target tissues.
  - Half-life = a few hours
- **IDL**: Intermediate Density
  - Transient lipoprotein.
  - VLDL's become IDL's when they lose the triacylglycerol component.
- **LDL:** Low density
  - Half-life = a few days. These guys stick around the longest.
  - IDL's become LDL's when they lose the **Apo-E** protein.
- **HDL_2:** High Density
  - Half-life = a few days.
- **HDL_3:** High Density -- another variety of HDL.

KRINGLE DOMAINS: Lipoproteins have multiple kringle domains -- (a supersecondary protein-structure motif).

FUNCTION AND TRANSPORT OF LIPOPROTEINS:

- DIETARY LIPID TRANSPORT SYSTEM (INTESTINAL)
  - **Chylomicrons** are synthesized and secreted in the intestinal epithelium
  - They travel through lymph to target, where they "deliver" the lipids to the target tissues. (the lipids are the broken down).
  - **Chylomicron-Remnants** then go back to the liver where they are degraded.
    - Chylomicron-remnants contain apo-proteins as well as *cholesterol* -- this is one source of influx for cholesterol into the liver.

- TRIACYLGLYCEROL SECRETION SYSTEM (LIVER): Overall flow is VLDL ------> IDL ------> LDL
    - Liver synthesizes triacylglycerols and packages them into **VLDL's**.
    - VLDL'S travel to extra-hepatic tissues to deliver fats.
    - VLDL'S are then degraded to IDL's and then to LDL's. So we get **LDL'S from VLDL'S**.
- **REVERSE CHOLESTEROL** TRANSPORT SYSTEM (LIVER) -- this is a way of retrieving extra cholesterol from tissues.
    - Liver sends out an "empty" **HDL** particle which makes its way to target tissues, where it picks up cholesterol.
    - The HDL then carries the cholesterol back to the liver.

SUMMARY: Two ways of getting cholesterol from the tissues back to the liver -- Chylomicron-Remnants and Reverse-Transport HDL-Particles.

LIPOPROTEIN ENZYMES: Following are the enzymes that breakdown lipoproteins.

- **Lecithin-Cholesterol Acyltransferase: LCAT** -- it transfers acetyl groups from phosphatidylcholine to cholesterol, in order to form **cholesteryl esters**.
- **Lipoprotein Lipase --** breaks down chylomicrons to release the triacylglycerols at target tissues.
- Hepatic Lipase -- liver sinusoids
- Acid Lipase -- lysosomes

LIPIDS AS METABOLIC FUELS

FUEL RESERVES: Triacylglycerols are the major form of fuel reserve, found in adipose tissue. In muscle, fuel is stored primarily as protein. Liver stores some as protein, fat, and glycogen.

- Triacylglycerols are ideal storage form because: (1) they are hydrophobic and can thus by stored anhydrously, and (2) they store carbons in the most reduced form.

SYNTHESIS OF TRIACYLGLYCEROL: Triacylglycerol can be synthesized in multiple ways.

- From Phosphatidic Acid: Diacylglycerol can be taken to triacylglycerol by the action of *acyltransferase*.
    - **Phosphatidic Acid ------> Diacylglycerol ------> Triacylglycerol**.

- **GLYCEROL BACKBONE**: Adipose tissue requires that glycolysis is going on, in order to provide the glycerol backbone (3 carbon trialcohol) for triacylglycerols. Dihydroxyacetone Phosphate (DHAP), an intermediate in glycolysis, is the starting point for this backbone.
    - **DHAP ------> Glycerol-3-Phosphate ------>** "Glycerol Backbone"
- TRIACYLGLYCEROLS FROM LIPOPROTEINS:
    - ***Lipoprotein Lipase:*** Releases fatty acids from lipoproteins. **Fatty Acids ------> Acyl-CoA ------> Triacylglycerols**

REGULATION OF TRIACYLGLYCEROL SYNTHESIS: INSULIN turns it on.

- Insulin promotes **lipoprotein lipase** -- breakdown of fat-containing lipoproteins (such as chylomicrons) and subsequent release of the fats to adipose tissue.
- Insulin also promotes glucose transporters -- uptake of sugars by tissues, and hence lower blood glucose.

MOBILIZATION OF TRIACYLGLYCEROLS (LIPOLYSIS) -- GETTING RID OF THE FAT

- ***Hormone-Sensitive Triacylglycerol Lipase***: **Triacylglycerols ------> ------> ------> Fatty Acids + Glycerol**. This is just a matter of cutting each of three ester bonds to yield free fatty acids + glycerol. This is accomplished in three reactions by a series of lipases.
    - Glycerol then goes back to the liver.
    - Fatty Acids are taken up by **Albumin** and carried to target tissues in the bloodstream.

REGULATION OF LIPOLYSIS: Lipolysis generally is stimulated by catabolic hormones and inhibited by insulin.

- *Hormone-Sensitive Triacylglycerol Lipase* is activated by **Phosphokinase-A**, via the adrenergic pathway (i.e. via cAMP).
    - So, *Epinephrine and Glucagon* (generally catabolic) directly stimulate it.
    - Thyroxine and Cortisol *indirectly* stimulate it via regulation of protein synthesis.
    - The Lipase is PHOSPHORYLATED when ACTIVATED. Once again, it is activated by PKA, via the cAMP signal transduction pathway.
- **Phosphoprotein Phosphatase:** The standard regulatory enzyme which *turns off* fat-mobilization by dephosphorylating the Triacylglycerol Lipase.
- **Insulin:** Activates **phosphodiesterase** to get rid of the cAMP ------> turn off lipase activation.

BROWN ADIPOSE TISSUE: Most adipose is white. This is an exception.

- FUNCTION: **Thermogenic.** It would be found on the back of polar bears, to keep them warm in winter.
- Brown = it has lots of mitochondria. It mobilizes triacylglycerols and then uses them in the cells, in the mitochondria, to completely oxidize them and generate heat.
- Inner Mitochondrial Membrane does *not contain the standard ATPase (ATP-producing) channels.* Instead they contain different hydrogen channels which serve as an **uncoupler**, so that the electron-transport energy is not harnessed as ATP, but instead is dissipated as heat.

KETONE BODIES: Ketone Bodies are a ***fuel***. They are made primarily in the liver.

- **Acetoacetate**
- **3-hydroxybutyrate** -- a reduced derivative of acetoacetate.

SYNTHESIS OF KETONE BODIES: Synthesis occurs in the mitochondria only. **HMG-CoA ------> Acetoacetate + Acetyl CoA**.

- In the mitochondrion, **HMG-CoA Lyase** makes Acetoacetate from HMG. This was an alternative to synthesizing cholesterol via HMG-CoA Reductase.
    - So these are two different fates for the HMG-CoA intermediate. You can either make mevalonate in the cytosol or Acetoacetate in the mitochondria.

UTILIZATION OF KETONE BODIES: How we use them as energy

- ***CoA-Transferase***: Takes a Coenzyme-A from Succinyl-CoA: **Acetoacetate ----> Acetoacetyl-CoA**.
    - This then is an alternative use of Succinyl-CoA in the TCA cycle. The enzyme can divert here to generate more Acetyl-CoA.
- ***Thiolase:*** Forms Acetyl-CoA from it: **Acetoacetyl-CoA + Coenzyme-A ------> 2 Acetyl-CoA**
- Once we have Acetyl-CoA, we can further metabolize it as we please, in this case most likely through the TCA cycle.

KETONE BODY UTILIZATION: Tissue distribution. A *low Insulin:Glucagon ratio promotes the production of ketone-bodies.* That is, **Glucagon** promotes it.

- ADIPOSE TISSUE: Fatty Acids are mobilized in the adipose tissue, under the influence of Glucagon.

- LIVER: Ketone bodies are synthesized when Acetyl-CoA builds up in the liver.
    - It builds up under the influence of **Glucagon** because *Glucagon promotes beta-Oxidation in adipose tissue*, and beta-Oxidation leaves us with lots Acetyl-CoA. This acetyl-CoA makes its way to the liver and builds up.
    - Glucagon also promotes gluconeogenesis in the liver. Oxaloacetate is therefore diverted to work on making glucose and is hence unavailable for the TCA cycle. So the TCA cycle is actually depressed in the liver, under the influence of glucagon.
    - The result of (a) and (b) above is that Acetyl-CoA tends to build up in the liver, under the influence of high Glucagon levels.
    - Under these conditions, the liver will shunt the excess Acetyl-CoA to the production of ketone bodies.
- EXTRA-HEPATIC TISSUES: Receive the ketone bodies from the liver and utilize them to make Acetyl-CoA.

KETOACIDOSIS IN DIABETES: This is the buildup of ketone bodies with Diabetes, due to low Insulin and hence excessive GLucagon.

- The problem lies with ADIPOSE TISSUE. It mobilizes lots of fats in response to little insulin.
- Those fats make their way to the liver where they overload the Acetyl-CoA supply in the liver, and production of ketone bodies results.

ATHEROSCLEROSIS

ANATOMY OF A VESSEL: The layers, from innermost to outermost

- **Intima Layer** -- consists of endothelial cells on a basement membrane. Blood is exposed to this layer.
- **Internal Elastic Lamina and Membrane** -- expands and contracts as needed.
- **Medial Layer** -- the main layer of smooth muscle, which expands and contracts under regulation. This muscle is interspersed with connective tissue.
- **Adventitia Layer** -- outer layer of fibroblast cells, there to nourish and sustain the muscle cells.

DEGREES OF DAMAGE: Whether or not damage to an artery is reversible depends on how deep the damage is. It is only when there is damage to the endothelial, intimal, and medial layers that extreme damage may occur

CAUSES OF VASCULAR INJURY: Oxidized LDL-Cholesterol accumulating in the blood is the most common cause, but not the only cause.

- *Mechanical Injury* can occur with hypertension, especially at bifurcation points in the arterial tree. High blood pressure can slough off a layer of endothelial cells, exposing collagen ------> platelet aggregation.
- *Diabetes* -- excess glucose can be a source of chemical injury, if it glycates cells in the region (see small-group discussion)
- *Nicotine* -- may cause a chemical insult to vascular walls in a similar way.

LESIONS OF ATHEROSCLEROSIS: Different levels of injury

- **Fatty Streak:** COMPLETELY REVERSIBLE
  - These cells are rich in *cholesteryl esters* -- not unesterified (free) cholesteryl.
- **Intermediate Fibrofatty Lesion:** IRREVERSIBLE.
  - Layers of lipid-filled macrophages alternating with layers of smooth muscle cells.
  - These cells are rich in *free cholesterol*.
- **Advanced Fibrous Plaque:** IRREVERSIBLE DAMAGE
  - Contains *crystallized cholesterol.*
  - Core of necrotic material, including smooth muscle, macrophages, and lipid, surrounded by a dense fibrous sheath of connective tissue.
  - Increased tendency to **rupture** and causes thrombosis.

TYPES OF APO-PROTEINS:

- **Apo-A1**: Found in HDL and Chylomicrons. It *activates LCAT, which in turn promotes breakdown of cholesterol-containing lipoproteins.*
- **Apo-B100**: Very large. Found in LDL and VLDL and serves as a receptor for uptake of these particles by target cells. Also plays structural role.
- **Apo-B48:** Structural protein in chylomicrons.
- **Apo-CII:** Serves as a cofactor for lipoprotein lipase -- to aid in breakdown of lipoproteins. Found in HDL_2, VLDL, and Chylomicrons.
- **Apo-E:** Serves as a receptor-ligand for HDL particles and Chylomicrons.
  - Also found on **ApoE-Rich HDL**, a special form of HDL.
  - The presence of different alleles of this protein in brain cells has related to Alzheimer's Disease, in recent research.

THREE CELLULAR RECEPTORS INVOLVED IN LIPOPROTEIN METABOLISM: All of the following receptors recognize various Apo-Proteins and take up lipoprotein-particles into the cell via receptor-mediated endocytosis.

- **LDL-RECEPTOR** -- **"Apo-BE Receptor"** -- It recognizes Apo-BE particles -- i.e. **Apo-B100 or Apo-E**
    - It recognizes and takes up LDL-Particles; widely distributed throughout different tissues.
    - REGULATED -- The delivery of the cholesterol to the cell inhibits this receptor! That is, the product of the endocytosis gives negative feedback.
    - FUNCTIONS: Regulation of LDL-Levels in blood, redistribution and utilization of cholesterol.
    - STRUCTURE (Panel 87) -- The Apo-BE Receptor is a single-pass transmembrane protein, which sports the following domains... a defect in of the below can result in Hypercholesterolemia:
        - **Ligand-Binding domain**: It will bind both Apo-B100 and Apo-E.
        - O and N-Linked glycosylation regions.
        - EGF-Homologous domain
- **REMNANT RECEPTOR** -- **Apo-E**
    - Found in the liver only.
    - It recognizes *Chylomicron Remnants* and Apo-E Rich HDL receptors. (Remnants are the leftovers of the chylomicron particles, after they have already dumped off their fatty acids).
        - Remember -- Chylomicrons remnants still have lots of cholesterol in them!
    - FUNCTION: Uptake of cholesterol-loaded remnants and delivery of cholesterol to the liver.
    - NOT REGULATED
- **SCAVENGER RECEPTOR** -- Uptake of oxidized or chemically modified LDL-Particles by Monocytes in circulation or Macrophages in tissues.
    - Regulation is not understood.
    - FUNCTIONS: Degradation and uptake of modified ("damaged") lipoproteins, and uptake of bacteria to protect us from endotoxic shock.

EXPORT OF CHOLESTEROL BY LIVER:

- When the liver exports cholesterol along with triacylglycerols, it first packages it into **VLDL Particles**.
- Once the TAG-component gets metabolized (via lipoprotein lipases), the particle becomes and **IDL-Particle --** containing both Apo-E and Apo-B100.
- Then it becomes an **LDL-Particle --** having only Apo-B100.

VASCULAR INJURY -- HOW IT CAUSES A PLAQUE:

- Oxidized and unmodified LDL-Cholesterol in the blood causes endothelial cell injury.

- IMMUNE RESPONSE
    - Oxidized LDL in the arterial **sub-endothelial space** induces monocytes (circulating white blood cells) to come to the site of injury and become macrophages.
    - Macrophages then uptake the LDL and localized fibrosis occurs, such that the LDL gets **trapped in the ECM** of the blood vessel.
    - If injury spreads into the medial layer, *smooth muscle cells will migrate to the intimal layer, where they will lay down fibrous material to promote development of a plaque.*
- PLATELET AGGREGATION: In the meantime, the blood-clotting process is also affecting the artery.
    - Platelets aggregated around the site of injury.
    - They stimulate the release of **Platelet-Derived Growth Factor (PDGF)**.
    - This causes vascular smooth muscle cells to proliferate and secrete *matrix elements*, thus making them more fibrotic.
- **FOAM CELLS** (Panel 93)**:** These are the result of Macrophages taking up both oxidized and unmodified cholesterol particles.
    - These Macrophages contain the **Scavenger Receptors** that recognize Cholesterol in the absence of the Apo-BE Receptor.

STEPS IN ENDOCYTOSIS OF LDL-RECEPTOR: Various diseases are associated with defects in these steps.

- Formation of LDL-Particle in liver.
    - Defect: **Abetalipoproteinemia**, genetically low LDL-Levels.
- Binding of LDL-Particle to receptor.
    - Defect: **Familial Hypercholesterolemia.**
- Internalization of bound LDL-Receptor
    - Defect: **Familial Hypercholesterolemia.**
- Lysosomal Hydrolysis of cholesterol:
    - Defect **Cholesteryl Ester Storage Disease (Wolman's Disease)**.

FAMILIAL HYPERCHOLESTEROLEMIA (FH): Caused by a *defect in the LDL-Receptor*, such that it can no longer take up LDL-particles so cholesterol build up in the blood.

- CAUSES: There are multiple genetic defects that can occur in the synthesis and processing of the LDL-Receptor.
    - The receptor is not synthesized -- no function.
    - The receptor is synthesized, but it is transported slowly from ER to Golgi -- a defect in the glycosylation or targeting of the protein-product.

    - The receptor gets to the membrane just fine, but it fails to bind the ligand -- structural defect in ligand-binding site.
    - Receptor binds ligand just fine, but it fails to cluster in coated pits. This would result in failed or low endocytosis despite binding.
- CLEARANCE OF LDL-CHOLESTEROL IN BLOOD:
    - In normal individuals, the Apo-BE receptor clears most of the LDL-Cholesterol from the blood. A minority of particles are taken up by scavenger cells.
    - In FH individuals, **Scavenger Receptors** pick up the slack and uptake cholesterol that the faulty Apo-BE receptors leave behind.

NEWBORNS: Have **maximal Apo-B receptors and low LDL-Cholesterol.** Adults are in the middle, and people with familial hypercholesterolemia have way too much LDL-Cholesterol.

HIGH-DENSITY LIPOPROTEINS (HDL) -- WHY ARE THEY GOOD?

- **"Nascent HDL"**, secreted by the liver, absorbs cholesterol in many extra-hepatic tissues. It is like a cholesterol sponge.
- Once it is full, it is converted to **HDL_3**, and it then uses the **LCAT Enzyme** to convert its contents to cholesteryl esters.
- The HDL_3 then goes back to the liver (I think) and dumps off the extra cholesterol, which can then be synthesized into bile salts and disposed of.

ESTROGEN: Estrogen increases the levels of HDL. Hence pre-menopausal women are at lower rish for CHD than men. Other things (exercise and loss of body fat) also increase HDL.
