Lesson 2: Lipoproteins

Lipoproteins: Overview

- Lipids vary in their water solubility. **Phospholipids and cholesterol are amphipathic.** Fatty acids have some solubility, depending on the size of the aliphatic chain. **Cholesteryl ester (CE) and triglycerides (TG) are completely insoluble.** So how are lipids moved around in circulation?
- **Fatty acids are usually bound to albumin,** although a small fraction of fatty acids freely circulates in plasma. Vitamins A and D are also bound to albumin or similar lipid-binding proteins.
- **Phospholipids, cholesterol, cholesteryl ester, and triglycerides are all part of lipoproteins.** Vitamins E and K are also carried by lipoproteins.
- Lipoproteins are **spherical macromolecules** that are made up of lipids and proteins. The proteins within lipoproteins are highly specialized and are known as **apoproteins.**
- In terms of structure, lipoproteins have two major parts. There is the **outer amphipathic layer or shell** that is made up of amphipathic lipids, which are phospholipids, free cholesterol, and apoproteins. Most lipoproteins also contain an **insoluble core that is made up of insoluble lipids, such as triglycerides and cholesteryl ester.**
- These are the major lipoproteins:
  - Chylomicrons (CM)
  - Very Low Density Lipoproteins (VLDL)
  - Intermediate Density Lipoproteins (IDL)
  - Low Density Lipoproteins (LDL)
  - High Density Lipoproteins (HDL)
- Apoprotein to lipids ratio determines lipoproteins density. Higher the ratio (i.e. greater the apoprotein content), greater the density.
• Lipoprotein density is inversely related to its size. Here, I have arranged lipoproteins according to their size. Chylomicron is by far the largest lipoprotein but is also the least dense of them all. In contrast, HDL is among the smallest but has the highest density.

• VLDL, IDL and LDL are related in a sense that VLDL is converted to IDL, which in turn is converted to LDL. As the lipid content is lost, these become smaller and more dense.

• Apoproteins are a critical component of lipoproteins. There are many different types of apoproteins. They not only provide density but also provide structural integrity to lipoproteins. Some apoproteins are important for lipoproteins to interact with specific tissues, while other apoproteins actually have enzymatic activity. In any case, the types of apoproteins within a lipoprotein determine the identity of that particular lipoprotein.

• Lipoproteins have life-cycles that are made up of certain stages that they go through. Typical stages include, assembly, maturation, metabolism, and clearance.

• CM and VLDL have similar lifecycles. Both carry mostly triglycerides. The ratio of TG to CE is 10:1.

• CM are assembled in enterocytes in the GI tract. They are responsible for supplying tissues in the body with dietary lipids, so they make up the Exogenous pathway. VLDL are assembled in the liver and are packaged with lipids that are already in the liver so they are part of the Endogenous Pathway. Both undergo similar steps in assembly and maturation. Both deliver half the triglycerides to muscles and adipose tissues. The remaining half of TG and all the CE
is delivered to the liver. The liver removes these particles by the process known as clearance.

- **HDL** is different from these two lipoproteins because it is responsible for removing cholesterol from the tissues and returning it to the liver, so it is a part of the **Reverse Cholesterol Transport**. HDL particles are mostly **formed in the liver** but are also produced in the GI tract as well within the plasma. When they are just assembled, HDL particles are pretty small and disk shaped when they are first small, but as they **take away cholesterol from tissues**, they fatten up and then finally deliver the cholesterol content to the liver.

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**CHYLOMICRONS**

- CM have short half-life (6–8 hrs), so they are not usually seen in plasma lipid profile of fasting patients.
- CM carry dietary lipids. Phospholipids and cholesterol are in the shell while TG and CE are in the core.
- However, dietary triglycerides (TG) and diglycerides (DG) need to be broken down to free fatty acids (FFA) and monoglycerides (MG) before they can be absorbed in the GI tract. This is catalyzed by **pancreatic lipase**.
- **Orlistat**, an anti-obesity drug, inhibits pancreatic lipase, thus depriving the patient of dietary calories derived from TG and DG.
- Bile salts combine with cholesterol and phospholipids to form **micelles**. Micelles are critical for absorption of these lipids. Micelles bring FFA, MG, and Cholesterol close to the enterocytes so that they can be absorbed.
- FFA and MG can enter enterocyte freely.
- Cholesterol [C] requires a specific sterol transporter known as **NPC1L1** in order to enter the enterocyte. The cholesterol lowering drug **Ezetimibe inhibits NPC1L1** and prevents cholesterol absorption.
Once inside the enterocyte, MG and FFA are converted to TG by **DGAT**. Cholesterol is esterified into cholesteryl ester (CE) by **ACAT**.

TG and CE are then incorporated into CM. How are CM made?

CM are assembled in the rough ER of enterocytes.

CM assembly begins with the synthesis of **apoB48**. When **apoB48** enters the ER lumen, it picks up phospholipids and free cholesterol from the ER membrane and forms the early version of CM. This is the **first step of the lipidation process**.

The second step of the lipidation process involves packaging of CM with TG and CE. This lipidation process is catalyzed by the enzyme known as **MTP**. The ratio of TG to CE in the newly formed CM is 10:1.

CM is then released from the enterocyte and enters lymph circulation where it undergoes maturation. Maturation involves an encounter with HDL, which donates **apoC-II** to CM. Now CM is fully mature and can undergo metabolism.

The process by which **CM transfers its lipid content to cells** is known as **metabolism**. Here I have shown an adipose cell because that's the most common tissue type that CM gives its lipids to. As with other target cells, adipose cells contain a multifunctional enzyme on its surface. This enzyme is known as **Lipoprotein Lipase or LPL**. It breaks down a molecule of TG from the CM into FFA and glycerol. The enzyme also acts as a transfer protein because it then
enables transfer of the newly formed FFA and glycerol into neighboring cells so that they can be used for energy and storage.

- LPL is normally inactive, but an interaction with apoC-II within CM causes activation.
- **Once 50% of the TG content has been delivered to the tissue**, the CM will detach from the cell and move back into circulation where it will become CM remnant (CMR). **CMR is formed when CM encounters HDL again.** This time, CM will **exchange apoC-II for apoE**. This exchange is critical as **apoE will be required for clearance by the liver.**
- **Clearance of CMR** by the liver is a **two step** process:
  1. First, the remaining half of the **TG content is hydrolyzed by hepatic lipase** and the resulting FFA are imported into hepatocytes.
  2. Once all the TG content is gone, the CMR is taken up by the hepatocytes by the process of **endocytosis**.
- Uptake by the liver involves one of two receptors: LDL Receptor (LDLR) or LRP.
- CM remnants bind to either of these receptor via the docking protein apoE. This interaction leads to the internalization of the CM remnant into the hepatocytes. Once inside the cell, the remnant will deliver the cholesterol content to the hepatocyte.

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**VLDL Assembly & Life-Cycle**

- VLDL assembly is similar to CM in many respects **except**:  
  - VLDL is assembled in **hepatocytes**  
  - VLDL assembly requires **apoB100**

  - ApoB100 and apoB48 are encoded from the **same gene** but in enterocytes there is an **editing complex (APOBEC1)** that introduces a **premature termination** codon into the mRNA, resulting in the truncated protein. apoB48 is 48% of the full length protein.
VLDL Lifecycle:

- Similar to CM
- Assembled in the **hepatocytes** and the maturation in circulation. Maturation involves receipt of apoC-II from HDL.
- Mature VLDL particles lose 50% of their TG content to muscles and adipose tissues via LPL, in a manner that is similar to CM.
- Once 50% of the TG is gone, the VLDL particle gives up apoC-II to HDL in exchange for apoE and becomes **IDL**.
- IDL gives up the remaining TG to the liver via hepatic lipase. It then has **two choices**:
  - Roughly 50% of IDL will be taken up by the hepatocytes.
  - The remaining 50% of IDL are **converted to LDL**. This conversion involves giving up apoE to HDL.
- Thus, IDL have very **short half-lives (30 min)**.
- **LDL lacks apoE**, so the only way it can be removed by the liver is through apoB100. **apoB100 can interact with only LDL receptor** and not LRP. Thus, LDL uptake is slow, resulting in **long half-life** of LDL particles (2-4 days).
- **LDL** is mostly cholesterol. In fact, it represents 70-75% of the total circulating cholesterol.
- LDL is also smaller than VLDL and IDL, because it has very little core left. As these particles become smaller, they also lose the surface lipids. These phospholipids are picked up by HDL during the aforementioned interactions.
**LDL Uptake & Cholesterol Homeostasis:**

- Uptake of LDL by hepatocytes begins when LDL docks with the LDL receptor at the surface of hepatocytes. This interaction is mediated by apoB100, the only apoprotein present in LDL.
- Subsequent to this interaction, the process of endocytosis begins with an invagination within the plasma membrane of the hepatocyte. **Endocytosis** is also critically dependent on two other proteins that are present within hepatocytes. Clathrin is a protein basically coats the invaginated intracellular surface. This distinct feature is recognized as **clathrin coated pit**. The other protein that is required for endocytosis is **ARH adapter protein**. Since this endocytic process is the only way to remove circulating LDL, any defect here will translate into higher LDL levels in plasma. In fact, that does happen from time to time. For example, there are patients who have a defect in the ARH adapter protein so that LDL endocytosis is very slow, resulting in higher plasma cholesterol.
- Once the endocytic vesicle has completely formed, it will pinch off from the cell membrane and move inward.
- The next step is the dismantling of the LDL particle. The endocytic vesicle will fuse with other vesicles that carry hydrolytic enzymes. These enzymes will **degrade apoB100** to amino acids, which can be used up by the cell in other metabolic pathways. The **cholesteryl ester is deposited** within the hepatocyte. **The LDL receptor is recycled back to the surface** where it is ready to bind to another LDL particle.
- LDL accounts for up to 75% of the circulating cholesterol. LDL endocytosis is the only significant means of removal of LDL from circulation, and this LDL uptake is critically dependent on LDL receptors. The liver regulates LDL uptake (and indirectly plasma LDL-C levels) by regulating the number of LDL receptors on hepatocytes.
Things that can increase LDLR expression:

- **Low fat diet:**
  - Low fat diet creates leads to low lipid levels in hepatocytes. This triggers an increase in LDLR expression.

- **Thyroid hormone:**
  - Patients who are hypothyroid also tend to have higher LDL levels because in the absence of adequate thyroid levels, LDLR expression declines significantly, raising LDL levels in blood.

- **Estrogen:**
  - Women undergoing menopause who previously had normal LDL levels suddenly begin to show higher LDL levels.

- **Genetic variability:**
  - There are multiple genetic factors that play a role here.

**LDLR Expression is regulated by free cholesterol levels in hepatocytes:**

- When the level of free cholesterol within the cell is very low, a particular sterol responsive regulatory protein known as SREBP is activated. This protein is normally anchored to the cell membrane is inactive. However, when the cholesterol levels are low, a particular protease is activated. The protease cleaves off the anchor that attaches the SREBP to the membrane. This way, SREBP is active in a sense that it can translocate to the nucleus and activate the gene encoding LDL receptor. So low cholesterol levels are a trigger for increasing LDL receptor expression in hepatocytes.

**Cholesterol homeostasis:**

- Free cholesterol can be converted to CE and vice versa.
- Free cholesterol is the usable form while CE is either stored or packed into VLDL particles for delivery to tissues. Cholesterol homeostasis is maintained
by interconversion of these two types of cholesterol. Enzymes that hydrolyze the ester form to free cholesterol are known as hydrolases, and the enzyme responsible for converting free cholesterol to the ester form is known as ACAT.

- The free cholesterol serves as the sensor for overall homeostasis of cholesterol.
- If there is low intracellular cholesterol, then the cell will:
  - Increase LDLR expression to import more CE
  - Hydrolyze CE to free cholesterol
  - Decrease storage
  - Increase biosynthesis of cholesterol
  - Decrease excretion of cholesterol through bile acid synthesis.
- If there is high intracellular free cholesterol, then the cell will:
  - Decrease LDLR expression
  - Convert cholesterol to CE for storage & VLDL packaging
  - Decrease biosynthesis
  - Increase bile acid synthesis (excretion)

**Bile Acid Synthesis:**
- Conversion of cholesterol to bile acid is dependent on the enzyme known as cholesterol 7-alpha hydroxylase. Once bile acids are formed, they are further converted to bile salts. These bile salts have the ability to emulsify lipids, so they combine with phospholipids and free cholesterol to form micelles. These micelles are then exported by the liver in bile and stored in gall bladder until needed.
**Enterohepatic Circulation**: Once released in the duodenum, 95% of the bile acids are reabsorbed and only 5% or less are excreted in feces. This is equivalent of cholesterol reabsorption, because bile acid are derived from cholesterol. If the fraction excreted is increased (e.g., sequestrants do this), then the liver will be forced to convert more cholesterol to bile acid.

### HDL Lifecycle

- HDL cholesterol (HDL-C) is considered to be “good” cholesterol, although there is no such thing as good or bad cholesterol. However, HDL is considered good for cardiovascular health in part because it is involved in **Reverse Cholesterol Transport**. Meaning that it works in the opposite direction of other lipoproteins by actually removing cholesterol from tissues.

- **HDL is assembled within hepatocytes** in the liver. Following this, it undergoes a maturation process while in circulation. Mature HDL is then able to remove cholesterol from tissues through an **efflux process**. After removing cholesterol from tissues, HDL also goes through another process where it interacts with mostly VLDL but also CM particles and **exchanges some of the cholesterol for triglyceride**. Finally, HDL delivers its lipid content to the liver and it is recycled to form new HDL particles.

- **HDL assembly** mainly occurs in the liver but can also occur within the GI tract. The assembly of HDL requires 3 minimal ingredients. These are apoprotein **apoA-1**, **phospholipids**, and **free cholesterol**. The assembly of these three is critically dependent on a transporter like protein known as **ATP Binding Cassette Protein**.
A1 or ABCA1. Apoproteins, cholesterol, and phospholipids are all amphipathic. As a result, all three will stay at the surface, so the initial HDL particle, which is known as Pre-beta-HDL is actually disk shaped because it doesn’t have a core which is normally made up of TG and CE. This Pre-beta-HDL is not very efficient at removing cholesterol, so it now needs to undergo a maturation process before it can remove cholesterol from tissues.

• The maturation process is not really distinct from cholesterol removal from tissues and both occur pretty much simultaneously. The goal of this process is to convert the disk shaped pre-beta-HDL into a spherical HDL particle. Free cholesterol on the surface of HDL is esterified to CE, which moves to the core. This esterification is catalyzed by the enzyme in plasma known as Lecithin-Cholesteryl Acyl transferase or LCAT. In this manner, the core begins to build up, giving HDL a more spherical shape and making it more efficient at removing cholesterol from other cells. This spherical HDL is of type HDL-3.

• As the free cholesterol from the surface of the HDL is esterified and moved to the core, HDL acquires a greater ability to accept free cholesterol from tissues. The free cholesterol from the surface of the cell moves to the surface of the HDL particle. The surface of the HDL gets bigger but at the same time LCAT continues to esterify this cholesterol, moving it to the core, so the HDL particle gets bigger as it continues to accumulate cholesterol. The transfer of free cholesterol from cells to HDL is facilitated by ABCA1. Remember, ABCA1 is also required for the actual assembly of the HDL particles even during the initial stages. This is an important detail, because people who carry mutations in the ABCA1 gene so that it doesn’t work efficiently, tend to have a condition known as Tangier's disease. They tend to have very low levels of circulating HDL particles. Consequently, they have cholesterol deposits in tissues throughout the body. They also have an early onset of cardiovascular diseases.
- Cholesterol efflux from macrophages involves another transporter, **ABCG1**, in addition to ABCA1. In absence of ABCG1, macrophages tend to accumulate cholesterol and this has an important impact when it comes to atherosclerosis.

- HDL Type 3 particles that have gather cholesterol from tissues then undergo an **exchange process** when they bump into other lipoproteins such as VLDL and CM. There are two parts of this exchange process. During the first part of this process, the **HDL particles give up some of their CE content to VLDL and CM in exchange for TG**. One consequence of this is that by getting rid of some of the CE, the HDL has increased its capacity to remove more cholesterol from other tissues. That's a good thing. However, the other consequence is that it **increases the amount of cholesterol that is circulating in form of VLDL or LDL**.

- This exchange is catalyzed by a protein called CETP, and there have been several inhibitors of this enzyme designed and tested. Some of them failed to give a clear benefit in experimental models, while some of them were actually thought to increase the cardiovascular risks. At least one of these inhibitors, known as **Anacetrapib** is currently in Phase III clinical trials. The goal of this inhibitor is to prevent this exchange and reduce the CE load of circulating VLDL (i.e. LDL) particles.

- In the **second part of this exchange**, phospholipids from other lipoproteins are transferred over to HDL, and this transfer is catalyzed by a protein known as **PLTP**. Remember that particles such as VLDL and CM tend to get smaller as they lose their core, so they need to shed some of their phospholipids. At the same time, HDL is building its core and needs to get bigger, so it needs some phospholipids. At the end of this exchange, HDL is now bigger and less dense. This form of HDL is identified as **HDL-type 2**. Now this HDL is ready to deliver its lipid content to the liver.
• Once the HDL particles are fully loaded with CE and TG, they are ready to deliver their lipid content to the liver. Again, this is very similar to what we saw with CM and VLDL remnants. This is a two step process. First, HDL discard their TG content. This is catalyzed by the hepatic lipase, which hydrolyzes the TG into FFA and then imports these FFA into the hepatocytes. Once the TG content is lost, the cholesterol-rich HDL particles dock with a receptor known as SR-B1 or Scrab1 and are internalized into the hepatocytes, just dumping their CE content. HDL particles are dismantled inside the hepatocytes, and apoA1 is recycled so that it can now form new HDL particles.

• Extra comments about HDL:
  o HDL plays an important role in maintaining overall lipid homeostasis by serving as a reservoir for apoproteins (apoC-II, apoE).
  o HDL itself undergoes many changes through apoprotein and lipid exchanges. There are different subtypes of HDL, and more than overall levels of HDL, it may be the relative abundance of certain subtypes that may be of greater relevance when it comes to cardiovascular health.
  o HDL contribute to better cardiovascular health not just because they remove cholesterol but also because they have other functions. For example, HDL particles are also associated with paraoxonases, which are enzymes that have antioxidant type of activity. These paraoxonases are thought to play a role in maintain good vascular health in part through responsive vasodilation.