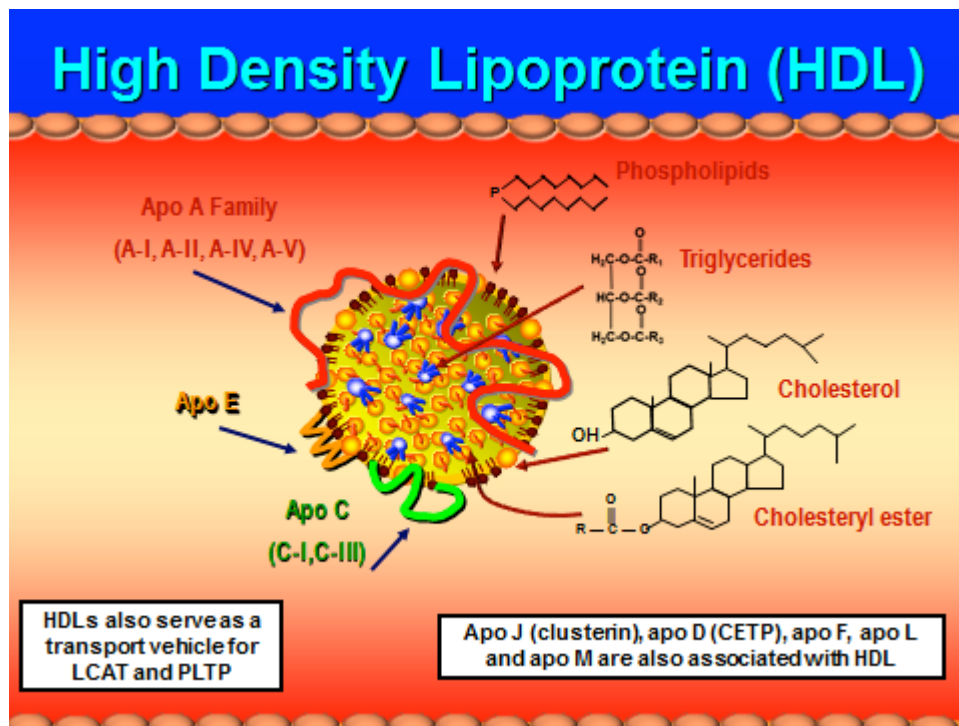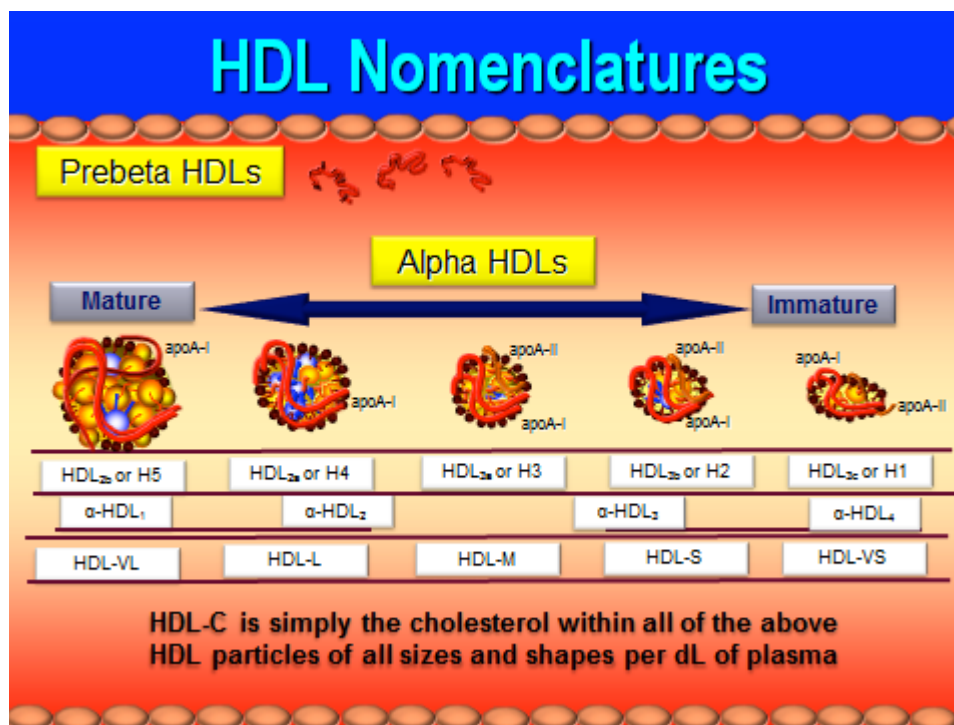High density lipoprotein (HDL) is the smallest of the lipoproteins that transport lipids in the plasma. HDL particles apart from their participation in peripheral cholesterol transport also have distinct immunologic and antiatherogenic properties which are now referred to as the functionality of the HDL particle. There seems to be little relationship between HDL particle functionality and the cholesterol content of HDLs, namely HDL-cholesterol (HDL-C) or HDL sizes. There are many genetic, lifestyle, hormonal, metabolic and inflammatory influences on HDL's makeup and function and many of these may be manipulated by pharmacologic and lifestyle interventions. Structurally HDLs are similar to all lipoproteins with a one molecule thick surface of phospholipids (PL) and free or unesterified cholesterol (UC) and a core of mostly cholesteryl ester (CE) but also some triglycerides (TG). On the surface are numerous proteins including the main structural protein which is 1 to 4 copies of apolipoprotein A-I.

HDL NOMENCLATURE

HDL nomenclature can be very confusing and some terms are technology dependent. There is a numerical ultracentrifuge classification where super large HDLs (not always present) are called HDL_1. As the particles shrink in size the names change to: HDL2b, HDL2a (both large with b being larger than a) and HDL_{3a}, HDL_{3b} and HDL_{3c} (with 3a being the largest and 3c the smallest). These terms are also used by labs utilizing gradient gel electrophoretic fractionation such as Berkeley Heart Lab.
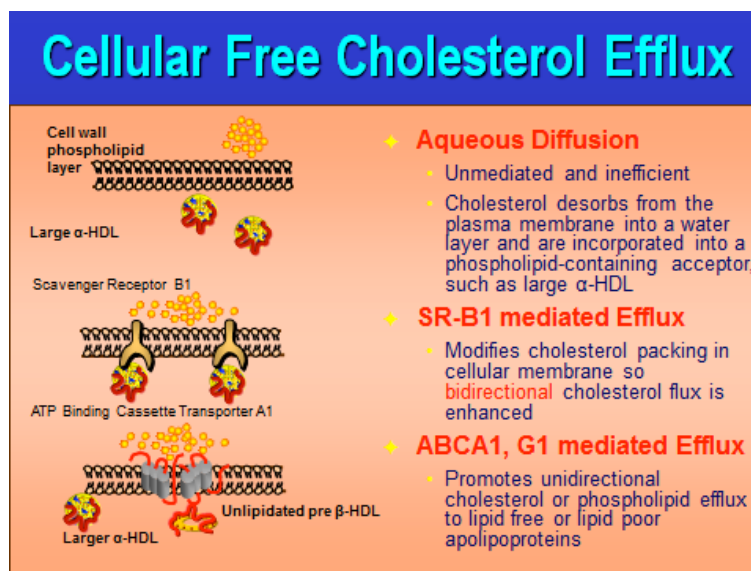
NMR spectroscopic classification of HDLs (LipoScience) uses the terms: H1 through 5 (with 5 being largest). However on their real-world report forms, LipoScience simply reports Total HDL-P, Large HDL-P. Small HDL-P can be calculated by subtracting large HDL-P from total HDL-P. Note that neither Berkeley nor LipoScience reports prebeta HDL or unlipidated apoA-I measurements. Boston Heart Lab using 2D electrophoresis with apoA-I staining reports apoA-I, prebeta-HDL and the α-HDL subspecies. Some labs report how much cholesterol is in various HDL subspecies (i.e. HDL_2-C, which in reality has little clinical usefulness). An expert opinion paper (Clinical Chem 2011;57:392-410) suggested we abandon the above nomenclature and simply use the terms very small (VS-HDL), small (S-HDL), medium (M-HDL), large (L=HDL) and very large HDLs (VL-HDL).

HDL OVERVIEW: Although much of what an HDL does is related to its surface proteome, HDLs in conjunction with the apoB-containing lipoproteins traffics hydrophobic lipids in plasma. This lipid transport (trafficking) process at its most simplistic analysis involves both forward (from the liver and intestine to the periphery) and reverse (back from the periphery to the liver or intestine) cholesterol transport (RCT). The HDL part of lipid trafficking (apoA-I mediated lipid trafficking) is a multifaceted, dynamic pathway by which unlipidated apoA-I secreted by hepatocytes or enterocytes enters the plasma, enters tissue vascular beds, gathers UC (lipidation) via the action of membrane sterol efflux transporters, leaves the tissue, rapidly esterifies the cholesterol using the enzyme called lecithin-cholesterol acyltransferase (LCAT), returns to cells for further efflux transporter lipidation, swaps core lipids with apoB particles (heterotypic exchange) or with other apoA-I particles (homotypic exchange) using a lipid transfer protein termed cholesteryl ester transfer protein or CETP (pronounced CEE TEE PEE), delivers CE to steroidogenic tissues (adrenal cortex and gonads) and adipocytes or returns

CE and UC via delipidation or internalization at the liver or delipidation at the intestine (a process called Trans Intestinal Cholesterol Efflux or TICE) or internalization. The UC and CE once returned to the liver is handled by several potential pathways: bile acid formation, hepatocyte cell membranes, direct transfer to the bile, apoA-I lipidation or VLDL lipidation. Each step is an intricate process regulated by many factors (genes, nuclear transcription factors, membrane sterol transporter proteins, lipid transfer proteins, lipase enzymes, etc.). Reverse cholesterol transport, one aspect of PCT, is a complex, dynamic flux process performed by apoA-I particles (direct) and apoB particles (indirect): the cholesterol involved can originate in the liver, gut, peripheral tissues or arterial wall sterol-laden macrophages (foam cells). Delipidation of the arterial wall foam cells (one likely critical aspect of what an HDL does) is now termed macrophage reverse cholesterol transport (MRCT). MRCT, which has no influence on plasma HDL-C concentration, is likely the only cholesterol -related antiatherogenic process mediated by HDLs. In summary HDL begins as a secreted protein that lipidates (vast majority of lipidation is hepatic or enterocytic) delivers CE to steroidogenic tissues and returns UC and CE by itself (direct RCT) to the liver and gut or by CETP-mediated transfer to apoB particles (mostly LDLs) which under nonpathologic conditions returns the cholesterol to the liver (indirect RCT). Total RCT = direct RCT plus indirect RCT: direct is HDL-mediated and indirect is apoB-mediated. The only part of RCT that is cardioprotective is the clinically unmeasureable MRCT.

HDLs constantly acquire (lipidate) and give up (delipidate) UC and CE. Entry and exit of cholesterol into a cell is accomplished by passive diffusion, cell membrane influx and efflux proteins and lipid transfer proteins (specifically CETP, also known as apolipoprotein D and its inhibitor apolipoprotein F or Lipid Inhibitory Transfer Protein). The cellular membrane efflux transporters are ATP binding cassette transporters A1 (ABCA1), which lipidate apoA-I or prebeta HDLs and G1 (ABCG1) which lipidates somewhat more mature alpha HDLs with UC. The scavenger receptor B1 (SRB1) can also serve as either a major cellular CE influx or efflux transporter, i.e. it is a bidirectional transporter. The SRB1 can both lipidate and delipidate HDLs.

Cellular Free Cholesterol Efflux

Cell wall phospholipid layer

Large α-HDL

Scavenger Receptor B1

ATP Binding Cassette Transporter A1

Unlipidated pre β-HDL

Larger α-HDL

★ Aqueous Diffusion
  · Unmediated and inefficient
  · Cholesterol desorbs from the plasma membrane into a water layer and are incorporated into a phospholipid-containing acceptor, such as large α-HDL

★ SR-B1 mediated Efflux
  · Modifies cholesterol packing in cellular membrane so bidirectional cholesterol flux is enhanced

★ ABCA1, G1 mediated Efflux
  · Promotes unidirectional cholesterol or phospholipid efflux to lipid free or lipid poor apolipoproteins

When lipids (cholesterol, phytosterols, fatty acids, monoacylglycerols) are absorbed into the small intestine epithelial cells or enterocytes they are handled in complex fashion. Fatty acids are rapid are rapidly esterified to glycerol lipids forming TG or PL. Only unesterified sterols can be absorbed into enterocytes utilizing the Niemann Pick C1 like 1 protein. Most UC is immediately esterified to CE utilizing the enzyme acyl-cholesterol acyl transferase 2 (ACAT2). Phytosterols such as sitosterol and campesterol are poor substrates for ACAT and unlike cholesterol few get esterified. Most unesterified sterols are rapidly returned to the gut lumen utilizing the sterol efflux proteins called ATP binding cassette transporters G5 and G8 (ABCG5, ABCG8). However some UC or phytosterols can be effluxed via ABCA1 out of the enterocyte into unlipidated apoA-I or prebeta HDLs. CE, TG and PL, interact with apolipoprotein B$_{48}$ and are incorporated into a very large TG-rich lipoprotein called a chylomicron. Both UC and any remaining phytosterols (amphipathic or partially polar molecules) which have not been effluxed out of the enterocyte intermix with PL on the chylomicron surface. CE and TG, being nonpolar seek the core of the chylomicrons.

ApoA-I can be synthesized and excreted from intestinal cells or be attached to the surface of a chylomicron. The mature chylomicron enters the intestinal lymphatics and eventually enters the plasma at the thoracic duct. While in the lymph, and ultimately upon entering the plasma at the thoracic duct, the chylomicron begins to acquire other apolipoproteins (C family, E, A-V, additional apoA-I, etc.) from already existing HDL and VLDL particles. As the chylomicron enters the plasma it is subject to lipolysis (hydrolysis of TG to fatty acids and monoacylglycerol) via the actions of lipoprotein lipase (LPL). As the core TGs are hydrolyzed the chylomicron particle shrinks and parts of its surface content (phospholipids and apolipoproteins) are released into the plasma where the PL bind to phospholipid transfer protein. In this way the apoA-I on a chylomicron is released and is free to begin functioning on its own. PLTP ultimately releases its PL to cells or to maturing HDL particles which require PL for their surface. Thus PLTP is a key player in HDL maturation.

Upon activation of peroxisome proliferator-activated receptor alpha (PPAR-α) apoA-I genes produce messenger RNA that will generate apoA-I (hepatocyte and enterocyte) and also apoA-II synthesis (hepatocyte). The hepatic secreted free apoA-I joins with enterocytic released apoA-I, and chylomicron released apoA-I and either attaches to existing HDL particles (enhancing their maturation) or binds to hepatic, intestinal, tissue or macrophage membrane ABCA1 with are in part regulated by liver X receptors (LXR). ApoA-I is lipidated with PL (phospholipidation) and UC (or phytosterols if present) and becomes a small, transient, discoidal, nascent, pre-beta HDL. As you can see cholesterol absorption from the intestine is complex and occurs not only via chylomicrons but also via lipidation of HDL particles. Many people who hyperabsorb cholesterol have elevated HDL-C levels. Note that the cholesterol put into the core of chylomicrons is esterified (CE) whereas UC and a few phytosterols resides on the surface, but all of the cholesterol (or phytosterols) effluxed from enterocytes to apoA-I or prebeta HDL is unesterified.

The first phase of HDL particle genesis is apoA-I lipidation resulting in the production of prebeta HDL which does not dramatically increase total HDL-C or total HDL-P levels (as measured by NMR spectroscopy). Although the vast majority of cholesterol lipidation occurs at the liver and gut, any cell with extra cholesterol can upregulate ABCA1 and transfer PL and UC to unlipidated apoA-I and pre-beta HDL. Through continued ABCA1 lipidation, prebeta HDL continues to accumulate UC. ApoA-I and apoA-II associates with an enzyme called lecithin acylcholesterol transferase (LCAT). This enzyme transfers fatty acids (acyl groups) from the PL called lecithin (phosphatidylcholine) to cholesterol in effect replacing the -OH group at the #3 position on the cholesterol molecule with the acyl group, forming CE. Due to its hydrophobic (nonpolar) properties, the CE moves to the center of the particle causing the discoidal HDL to evolve into a larger and somewhat spherical particle termed an alpha HDL. These maturing alpha HDLs will continue to be lipidated with UC via a different membrane efflux transporter called ABCG1. Although apoA-I and prebeta HDLs can be lipidated by any cell in the body that has extra cholesterol, the vast majority of the cholesterol within HDL originates in the liver (70-80%) and small intestine (20-30%) with only a tiny amount from peripheral cells (5%). The majority of cholesterol and CE trafficked in HDL (HDL-C) is carried by the larger HDL species (HDL$_2$ using electrophoresis nomenclature or H4, H5 using NMR nomenclature). Since these larger HDL particles carry more CE, the HDL-C level rises dramatically during this phase (maturation of alpha HDLs) of apoA-I mediated peripheral cholesterol transport.

The HDL particles participate in lipid trafficking as follows:

APOA-I MEDIATED "FORWARD" CHOLESTEROL TRANSPORT

A major function of HDL is to transport the hepatic and enteric cholesterol to steroidogenic tissues and adipocytes. Gonads and adrenals of course need cholesterol to make steroids hormones. Adipocytes consume large amounts of cholesterol in their cell wall synthesis. The glands and adipocytes upregulate membrane SR-B1 receptors which delipidate CE from the large HDLs. Adipocytes can also delipidate HDLs using adipocyte-produced CETP. The HDL becomes smaller and returns to the liver or intestine to relipidate or if small enough is excreted by the kidneys. HDLs have a half life of 4-6 days, ensuring there are enough of them in circulation to supply cholesterol during periods of heavy demand for the adrenal in a time of physiologic crisis.

APOA-I MEDIATED MACROPHAGE RCT

Of great importance is the ability of HDL particles to enter the subendothelial space of the arterial intima and participate in a critical process termed (by Dan Rader and colleagues) macrophage reverse cholesterol transport (MRCT). The HDL attaches to the surface of foam cells (lipid laden macrophages) and via cholesterol efflux transporters: ABCA1, ABCG1, or the SR-B1 accepts UC and CE respectively from the cell. The HDL particle can also accept cholesterol by the process of free or passive diffusion. These surface transporters efflux cholesterol from (delipidate) the foam cell. UC is transferred

to a lipid acceptor such as discoidal (prebeta) or small (alpha) HDL via ABCA1 and large (alpha) HDL particles via ABCG1 or CE to SR-B1. The amount of cholesterol acquired by HDL particles from foam cells is minor with respect to the amount acquired from hepatocytes and enterocytes so macrophage MRCT does not increase plasma HDL-C levels, but the delipidation may be critical in stabilizing plaque. MRCT is one aspect of HDL functionality. There are no readily available lab tests including HDL-C that would confirm successful therapeutic induction of macrophage RCT.

Think about it: this antiatherogenic process, MRCT has no impact on the plasma HDL-C level. Previously most physicians have assumed when HDL-C is elevated by therapeutic intervention, the HDL-C increase is from cholesterol exiting the artery. That belief is erroneous. Therapeutically, most of the HDL-C increase that occurs is a result of lipidation of HDL by hepatic or intestinal cell ABCA1. Interestingly in mice increasing HDL-C via upregulation of hepatic ABCA1 offers no cardioprotection but raises HDL-C, but upregulating macrophage ABCA1 is cardioprotective but does not increase HDL-C.

The mature lipidated alpha HDL particles in the plasma have several options other than delivering sterols to steroidogenic tissues:

APOA-I MEDIATED RCT

Direct RCT

1) Large HDL can also be delipidated by hepatic, adrenal, gonadal or adipocyte SR-B1. After delipidation by SR-B1 the now smaller HDL can resume the flux process and be relipidated. Very small HDL particles are also vulnerable to catabolism and renal excretion of apoA-I. The SR-B1 delipidation process will reduce HDL-C concentrations and paradoxically SR-B1 induced decrease of HDL-C is cardioprotective in mice. The CE that leaves the HDL and enters the hepatocyte after de-esterification is delivered to the biliary system for excretion via ABCG5/G8 transporters or used in other ways.

2) There is also limited evidence that hepatic apoA-I beta-chain synthase or "holoparticle or catabolism receptors" can endocytose and catabolize the "whole" HDL particle facilitating apoA-I mediated RCT. In this process the particle apoA-I is degraded and lost, so it is not as efficient aspect of RCT as is delipidation of HDL by SR-B1 which preserves apoA-I.

3) Large HDL particles enriched in apoE can be endocytosed by hepatocyte LDL receptors.

4) Enterocytes can express SR-B1 and delipidate large HDL particles. The cholesterol is de-esterified and then effluxed into the gut lumen via ABCG5/G8 transporters. The UC can then be excreted in the stool. Delipidation of HDLs at the gut as previously mentioned is termed TICE.
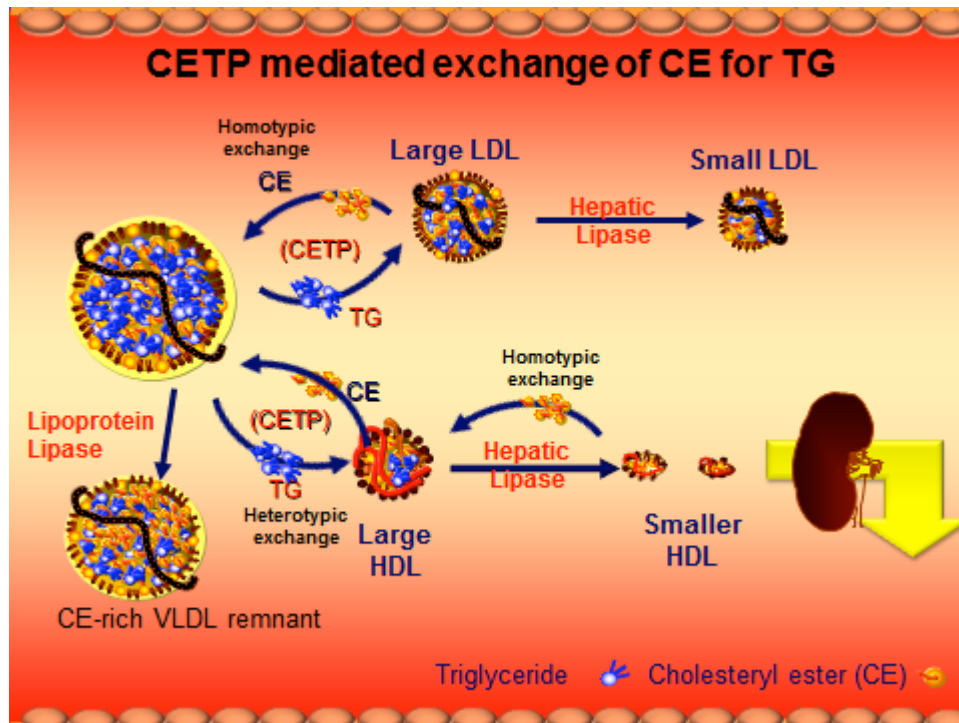
5) As mentioned previously, adipocytes can delipidate HDLs through SR-B1 or using adipocyte synthesized CETP. Some have termed this "lateral cholesterol transport" but that term has not caught on.

6) HDLs can give up their core CE by transferring it to another lipoprotein specifically to an apoB (heterotypic exchange) or another apoA-I particle (homotypic exchange). Utilizing CETP, the CE in an HDL can be swapped for TG within apoB particles (most of which are LDLs). The CE goes from HDL primarily to LDL and VLDL. HDLs can also swap lipids among themselves (large to small HDL species): this homotypic exchange and is a crucial part of HDL remodeling.

Indirect RCT

1) The most intriguing aspect of RCT is that mediated by CETP. ApoB particles that carry even a minimal excess of TG, exchange their TG for the CE in HDL. CETP ferries the lipids back and forth between the apoA-I and apoB particles (termed a heterotypic exchange). This process makes the large alpha HDL particle TG-rich, and CE poor, in effect reducing the cholesterol mass within HDL particles. In the process the apoB particles, VLDL, IDL and LDL give up TG and become more cholesterol rich (keep in mind 90-95% of apoB particles are LDLs so most of the CE leaving an HDL goes over to an LDL). Indeed much of the cholesterol within an LDL core originates in HDLs. Such cholesterol enriched apoB particles are now available for removal by hepatic LDL receptors. This process in which LDL acquire CE from HDL and bring it to the liver is termed "indirect RCT." The now TG-rich, cholesterol-poor HDL on exposure to hepatic and perhaps endothelial lipase undergoes lipolysis and becomes a very small alpha HDL, which because of its very small size, may be catabolized and apoA-I is subject to renal excretion via megalin and cubilin membrane proteins in the renal tubules or the prebeta HDL becomes available for relipidation. In this manner, even subtle hypertriglyceridemia in a patient with functional CETP is often associated with reduced HDL-C and apoA-I (HDL-P) levels. NMR analysis of HDLs reveals a significant reduction in large HDL particle (H4, H5) concentration in such patients. Gel fractionation tests would note an absence of larger HDL_2 and an increased presence of smaller HDL_3. Interestingly because the TG originated in LDLs, almost all insulin resistant persons with low HDL-C have a very high total LDL-P.
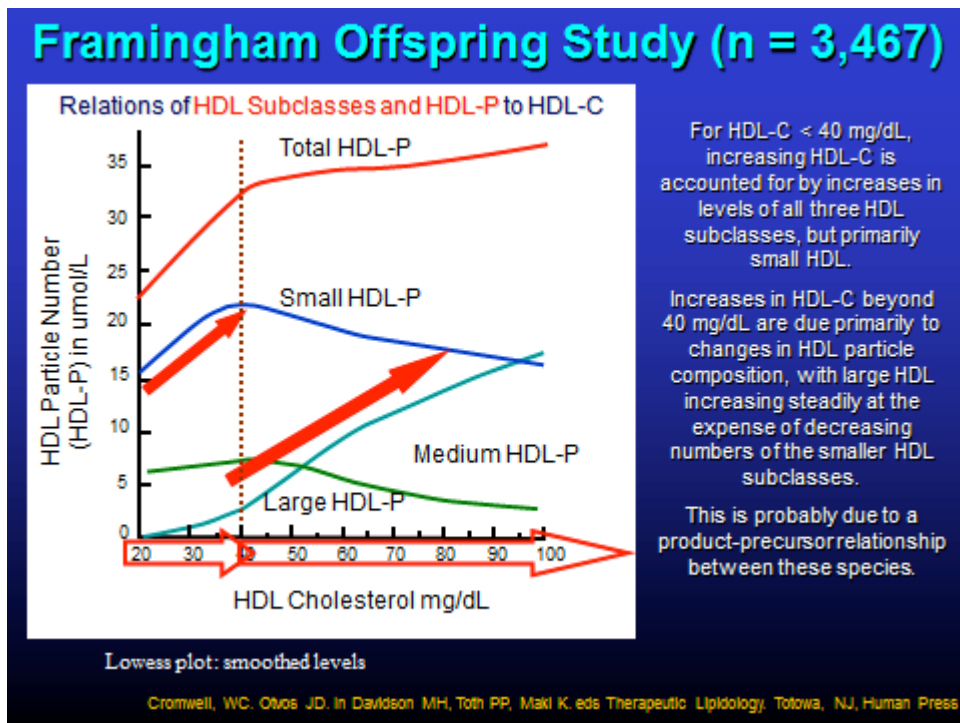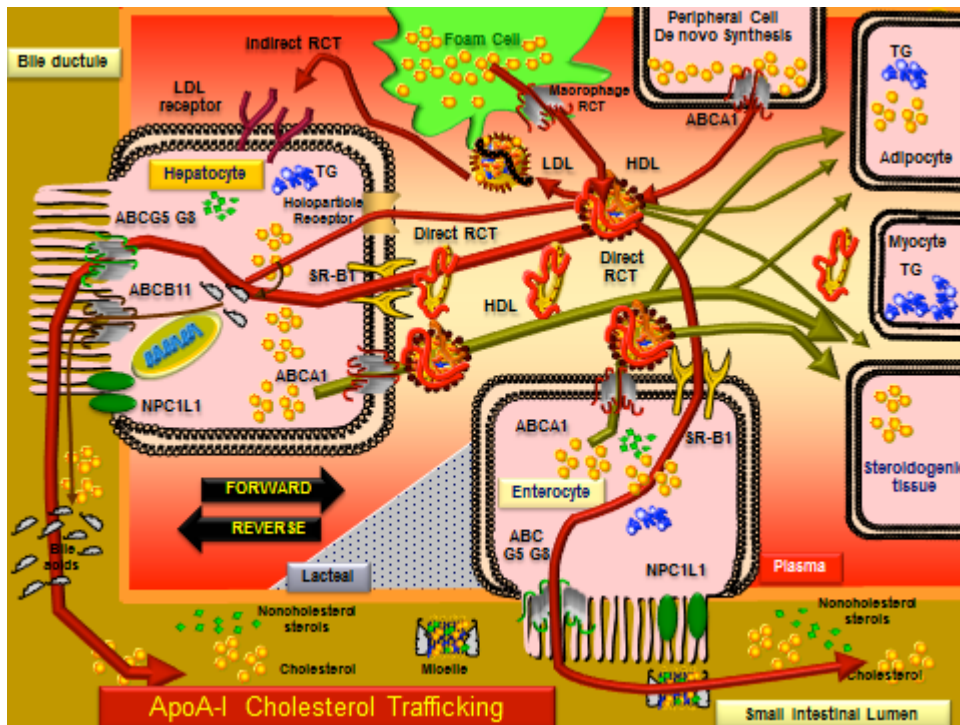
This association of low HDL-C (lack of larger alpha-HDL) and atherogenic apoB particles explains much of the CV risk in such patients. These lipoprotein changes are very characteristic of insulin resistance. Of course, a reduction in HDL-P likely reduces the delipidation rate of foam cells and reduces the number of functional HDL particles that can participate in other anti-atherogenic functions.

The CE accumulated by the liver from direct or indirect RCT after de-esterification to UC can now be excreted into the biliary system, where it ultimately enters the gall bladder and duodenum. It can also be converted to a bile acid (BA), and excreted into the bile. From there the UC and BA enters the jejunum where at least 50% of the UC is reabsorbed at the brush border of the intestinal epithelium and the rest is excreted in the stool. The BA are reabsorbed at the ileum or excreted. It is easy to forget that the last phase of RCT is biliary and then fecal excretion of UC and BA.

We have to redefine our HDL terminology. As with all lipoproteins, HDL particles are involved with vascular cholesterol transport (moving it in several directions). HDL acquires UC mostly from the liver and small intestine but also from peripheral tissues and esterifies it. The CE is trafficked to the periphery (steroidogenic tissues, adipocytes) or directly back to the liver or intestine (TICE) or transfers it to apoB particles (LDLs). HDL participates in both direct and indirect RCT, although only a minor amount of the CE in HDL derives from what is termed MRCT (delipidation arterial wall macrophages). Thus HDL particles participate in both forward and reverse cholesterol transport. If one looks at the overall efficient flux process of peripheral cholesterol transport, where HDL particles are formed, lipidated, exchange lipids, and then delipidated one can understand that the overall process will not affect the total HDL-C level in a predictable fashion, as there is constant remodeling or shifting of HDL particle size and cholesterol content. Despite what has been taught for decades, a serum HDL-C has no relationship to the apoA-I lipid trafficking process (review the next graphic). Indeed there is no available blood test (e.g. HDL sizes, HDL-P, HDL subfraction-cholesterol) that a clinician can use to assay exactly what HDLs are doing in a given individual.

Total HDL-C levels represent the sum of the cholesterol in all of the HDL particles (big and small) at a given moment of time: this is called a static HDL-C level. It is obvious that such static levels of HDL-C might be very poor predictors of the efficiency of the

RCT process. What if one had very large HDL particles that were not being delipidated? HDL-C would be high but RCT would be impaired and CV risk could increase as seen in some of those with hepatic lipase and some CETP deficiency disorders. What if one had very small HDL particles that were very efficient in RCT (lipidation/delipidation)? The HDL-C level would be low but RCT would be fine and CV risk would not be elevated despite the low HDL-C (this is seen with ApoA-I Milano).

In the graphic above, note that as an HDL-C of 20 rises to 40 mg/dL there is a very significant increase in total HDL-P and almost all of that increase is due to an increase in the small HDL species. As the HDL-C starts to go above 40-45 mg/dL there is very little further increase in total HDL-P, but not the disappearance of the small HDLs (small HDL-P) and the increase in the larger HDLs (large HDL-P). Clearly rises in HDL-C above 40 mg/dL are explained by enlargement (maturation) of the HDL particle, not by an increase in HDL number.