Lipid Drugs and HDL Subfractions

Thomas Dayspring MD, FACP

Does it really matter what a drug does to HDL subparticles? HDL particle subpopulations and their physiologic flux (dynamic remodeling) is a continuous and complex process dependent on many variables. Since many practitioners are now doing advanced lipoprotein testing, they are obtaining and observing HDL particle baseline and follow up data. Many do not know how to interpret such data properly. Please review the diagrams at the end of the article

Quick review: Since HDLs lipidate and then traffic sterols in several directions, including forward and reverse cholesterol transport (RCT) where they delipidate, a serum HDL-C has little relationship with what HDL actually does. HDL or apoA-I mediated trafficking of cholesterol involves lipidation (acquiring lipids) and delipidation (giving up lipids) of HDL particles. Much of what an HDL does, after it acquires cholesterol can be considered as forward cholesterol transport (FCT). Any unwanted sterols can be returned in the RCT process to the liver or small intestine for further processing. RCT occurs via 3 pathways:

1) Unlipidated apoA1 or very small prebeta HDL particles are lipidated at the liver or small intestine or peripheral cells including arterial wall macrophages via ABCA1 transporters and then delipidated at steroid producing glands (adrenal or gonads) or adipocytes in the forward cholesterol transport pathway or at the liver via upregulated Scavenger Receptors B1 (SR-B1). This part of the cholesterol flux process is termed direct RCT or apoA1 mediated RCT. The vast majority of the cholesterol trafficked within HDLs was acquired at the liver and jejunum.

2) Cholesteryl ester (CE) can be transferred in exchange for TG from apoB particles (LDL, IDL, and VLDL). The apoB particle, with the cholesterol just acquired from HDLs, can then be endocytosed by hepatic LDL receptors and deliver its cholesterol contents to the hepatocyte. This is termed indirect RCT or apoB mediated RCT.

3) Large or small HDL can attach to arterial wall foam cells (macrophages) and delipidate the foam cell: small HDLs attach to ABCA1 and the more mature HDLs attach to ABCG1,G4 or SR-B1. This process termed “macrophage RCT” is crucial to stabilizing or delipidating plaque but contributes little to a plasma HDL-C level. This process has no effect on the total HDL-C concentrations as the amount of cholesterol delipidated from arterial wall foam cells is trivial compared to the amount of cholesterol HDLs acquire from hepatocytes and enterocytes.

The overwhelming majority of the cholesterol within HDL particle originates in the liver. Thus, 80-90% of the HDL-C level originates from the liver: not from peripheral cells or artery walls.

Niacin does increase Total HDL-P (apoA-I) as well as the numbers of large HDL particles. Neither LipoScience nor any governing body nor the FDA recommends HDL particle concentration or size be a goal of therapy. The LipoScience report form properly states (based on good epidemiological data) that at baseline (<u>in patients not on lipid</u>

modulating drugs) the lack of large HDL particles is a risk factor highly correlated with insulin resistance, small LDL and VLDL remnants. What explains the relationship between a lack of large HDL particles and atherogenic apoB particles? Insulin resistant patients have increased TG carried in hepatic produced VLDL. Through cholesteryl ester transfer protein (CETP) mediated exchange (between HDL and VLDL) of TG for cholesteryl ester, the large cholesterol-rich HDL particles become TG-rich and cholesterol poor. The lipolytic action of hepatic lipase hydrolyzes (removes) TG and rapidly changes these large alpha HDL particles to much smaller alpha HDL which are subject to renal excretion due to their tiny diameter (5-7 nm): this is reflected by a reduced amount of both total and large HDL on the NMR LipoProfile test and decreased HDL-C on conventional lipid panels. When one treats at-risk patients with low HDL-C, the NCEP suggested target of therapy is not necessarily (although it is encouraged) to raise HDL-C or HDL subparticles to some magical level, but rather, to reduce the concentration of atherogenic apoB (LDL-P) or their NCEP surrogates (LDL-C and Non HDL-C). NCEP makes no recommendations that one should attempt to make HDL particles large!

NCEP and most experts will tell you that fractionation of HDL is not recommended at this time and has little role in current clinical practice as far as determining the efficacy of a drug. That being said, different drugs do different things to HDL-C and HDL subparticles. If a clinician is following HDL particles on follow up testing (NMR) the clinician had better do some serious reading and develop a thorough understanding of the topic. The process of reverse cholesterol transport is a very complicated, dynamic flux, particle remodeling process that cannot be predicted from HDL-C levels or subparticle determination. Clinical trials demonstrate one can lower TG, lower apoB and reduce outcomes and in the process make HDL either large or small. It will depend on the particular therapy used. The bottom line is that CV risk reduction can occur independent of what happens to HDL particle size. Drugs that have outcome data in patients with low HDL-C include statins, fibrates and niacin. The latter raises HDL-C more than the others but outcome data using any as a monotherapy is quite similar.

To give you an idea on the complexity of HDL subparticles, please read on. The process of reverse cholesterol transport (RCT) is a flux process that involves many "players" some of which (to name just a few) include PPAR alpha, gamma and delta, hepatic, lipoprotein and endothelial lipase, LCAT, cholesteryl ester transfer protein, phospholipid transfer protein, scavenger receptors B1, CD-36, ABCA1 transporters, Liver X receptors, farnesol X receptors (FXR)sterol regulatory element binding proteins, hepatic and intestinal apoA-I, other apolipoproteins, cubilin and megalin, and LDL receptors.

If assembled and functioning properly, **all** HDL particles (big and small) are beneficial. There is this absurd belief being promulgated that only big HDL particles are beneficial. Patients with apoA-I Milano have very small HDL particles, very low HDL-C and yet do not have CHD risk. They have very efficient RCT. Some Japanese patients with CETP disorders have very large HDL particles and very high HDL-C levels, yet the particles are dysfunctional, impairing RCT and the elevated HDL-C may at times be associated with CV risk. Recent data from the EPIC Norfolk epidemiological trials

and the IDEAL study (a statin outcome trial) suggested that HDL-P or apoA-I is more important than the HDL-C level.

Maybe we would be better off looking at apoA-I or HDL-P measurements as the best way to judge therapy. ApoA-I is the surface apoprotein on HDL particles and its measurement is an indicator of total HDL mass or particle concentration. As apoA-I levels increase, HDL particle numbers increase. However there are 2,3 or 4 apoA-I molecules per HDL particle. There are several studies (epidemiological and clinical trial) that have determined the usefulness of apoA-I levels at baseline and as a predictor of therapy (See AFCAPS, AMORIS trials).

There are several ways to increase HDL-P or apoA-I: Note that one can raise apoA-I without dramatically raising HDL-C levels: (see gemfibrozil in VA-HIT). Also one can raise HDL-C without elevating apoA-I as in the case of resins: they increase HDL-C via an FXR/liver X receptor (LXR) induction of ABCA1 which will facilitate the transfer of hepatic cholesterol to apoA-I (small or pre-beta HDL) particles. There is no major increase in apoA-I.

1) Induce hepatic production of apoA-I: Fibrates do this through PPAR alpha agonism. Estrogen does it through estrogen receptor agonism. Statins may do it through an associated PPAR alpha effect. Other drugs can also have an effect: Dilantin, TZDs.

2) Induce lipolysis (TG removal) of chylomicrons containing apoA-I. ApoA-I and phospholipids will break away from the chylomicron and be used by newly forming HDL. Anything that increases lipoprotein lipase (LPL) will do that. Fibrates are the best agents for increasing LPL (another PPAR alpha effect). Statins and N3 FA can also induce LPL to variable degrees.

3) Downregulate hepatic SR-B1 (HDL receptor) preventing hepatic delipidation of large HDL particles. Estrogen does this. The HDL particle will deliver less cholesterol to the liver.

4) Downregulate the hepatic HDL "holo-particle" or catabolism receptor. Niacin is the only drug that does this. Without that receptor, large HDL particles cannot undergo hepatic endocytosis and thus they stay in the plasma. In effect this prevents catabolism of apoA-I HDL particles and increases HDL plasma residence time (raising apoA-I and HDL-C). However if large particles are no longer endocytosed, reduced amounts of cholesterol is getting to the liver through this direct RCT mechanism (apoA-I mediated RCT).

What HDL subparticle data do we have from trials?

In the VA-HIT, with gemfibrozil there was in increase in apoA-I, total HDL-P and small HDL particles which was statistically significantly related to event reduction. There was a reduction in the number of large HDL particles. If one has knowledge of how fibrates work, one would realize that the number of small HDL particles has to increase. Fibrates

increase apoA-I (from liver production and chylomicron lipolysis): Fibrates help upregulate ABCA1 transporters in tissues and arterial wall macrophages (foam cells), causing lipidation of apoA-I, creating the transient existence of large HDL particles. Such large HDL particles are rapidly delipidated (and made small) by steroid producing endocrine glands or liver (upregulated by fibrates) SRBI. Although large HDL can also be made small by the action of plasma CETP, which transfers cholesteryl ester from large HDL to apoB particles (LDL and VLDL) in return for TG, fibrates decrease CETP activity as do most drugs that lower TG.

So although fibrates are efficiently enhancing both direct (apoA-I mediated) and indirect (through CETP transfer to apoB particles), small HDL will be the predominant species and predominant particle found in plasma in patients on fibrates. This is highly desirable as small HDL (lipid free or lipid-poor apoA-I) is the HDL particle that can attach to hepatic ABCA1 and lipidate the particle. However, the delipidated HDL can also attach to arterial wall foams cells and participate in "macrophage RCT." Macrophage RCT does not markedly contribute to the HDL-C level but is critical in plaque delipidation and likely event reduction. This HDL flux process was an important MOA of the fibrate in the VA-HIT. Fibrates thus efficiently effect HDL lipidation and delipidation: The cholesterol returned via SR-B1 to the liver is excreted in bile: too much cholesterol enhances formation of lithogenic bile. Fenofibrate also downregulates the jejuna Niemann Pick C1 Like 1 protein and reduces jejunal reabsorption of hepatic secreted biliary cholesterol

In HATS (a successful, angiographic trial using simvastatin and high dose Slo-Niacin and IR niacin) the niacin and the statin caused an increase in large HDL and a reduction in small HDL. This is exactly what should happen. Niacin does not significantly increase apoA-I production. Statins have an ability to do increase apoA-I (their PPAR alpha effect is due to inhibited prenylation of rho and ras). Rosuvastatin (Crestor) is by far the most powerful statin increasing apoA-I and HDL-C). There is evidence that niacin, acting through a metabolite that increases prostaglandin D2 has a PPAR gamma effect of upregulating hepatic ABCA1 lipidation of apoA-I (creating large HDL). Once the HDL particles are lipidated (acquire cholesterol) and are made large (mature), the HDL particles will tend to stay large on statins and niacin as neither drug increases SR-B1 delipidation of HDL and both reduce CETP activity. However, large HDL particles can enter the arterial intima and help delipidate foam cells via the ABCG1 transporter which transfers cholesteryl ester to large HDL. Large HDLs can also undergo lipolysis by macrophage secreted lipoprotein lipase, hepatic lipase and phospholipid transfer proteins which by shrinking the large HDL, release unlipidated apoA-I from the HDL surface. The unlipidated apoA-I then attaches to macrophage ABCA1. This inducement "macrophage RCT" contributes little to HDL-C levels but is crucial in plaque stabilization.

1) Statins have some variable inhibitory effect on CETP, which will prevent transfer of cholesteryl ester from large HDL to apoB particles. Statins also can have an inhibitory effect on hepatic lipase (an enzyme that enhances HDL particle lipolysis). Both

of these effects will keep HDL particles large. Statins can also increase apoA-I production through PPAR alpha agonism effects.

2) Niacin inhibits hepatic lipase. This also will prevent lipolysis (TG removal) of large HDL and thus slow the creation of small HDL. However, there is evidence that if large HDL does not undergo some modification from hepatic lipase, the HDL particle cannot attach as efficiently to hepatic SRB1 which would impair hepatic delipidation of the HDL particle (in effect inhibiting delivery of HDL cholesteryl ester to the liver). Niacin also downregulates the hepatic HDL "holo-particle" or catabolism receptor (a receptor that performs endocytosis of large HDL particles). The effect is that direct RCT (apoA-I mediated) is lessened. It is possible RCT could occur if the large HDL particles created by niacin transfer the cholesteryl ester/TG exchange to apoB using CETP (indirect RCT). It is also likely that the delayed catabolism of large HDL allows the HDL particle to stay in the plasma and perform many other antiatherogenic functions (anti-inflammatory). Note that if HDL particles stay large, they are less likely to initiate further direct (apoA-I mediated) RCT as large HDL do not readily attach to hepatic or other tissue ABCA1. Only small lipid-poor HDL particles can do that. However, as mentioned, large HDL can shrink at the macrophage surface releasing apoA-I and the larger HDLs can also participate in the all important “macrophage RCT” via the macrophage ABCG1 transporter. In effect niacin increases HDL-C and apoA-I by increasing hepatic lipidation of HDLs, inhibiting HDL lipolysis via hepatic lipase inhibition and delaying the catabolism of large HDL particles. There is no decrease in RCT on niacin, because of the CETP mechanism of HDL delipidation.

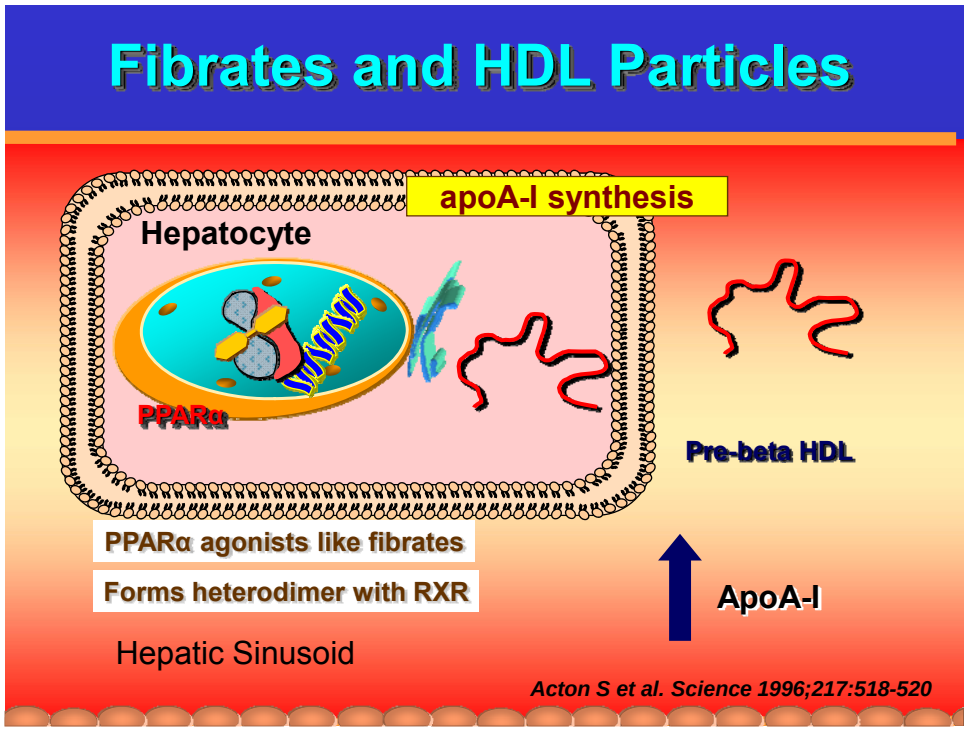
Bottom line: If you use a fibrate expect small HDL particles and apoA-I to increase. The HDL-C increase is very variable and will mostly be related to the baseline TG and HDL-C level: (the higher TG level and the lower the HDL-C at baseline, the more will be the fibrate induced HDL-C rise). Multiple trials have demonstrated that fibrates reduce clinical events. If one prescribes a fibrate and is doing NMR lipoprotein followup, <u>never stop the fibrate because small HDL-P is increasing and large HDL-P is not increasing</u>. Total HDL-P increases on fibrates. The apoB particles (LDL and VLDL) will reduce in number and the HDL particle is fluxing (remodeling) properly. Small HDL particles created by fibrates participate in macrophage RCT by attaching to ABCA1.

If you use niacin, there will be an increase in large HDL particles, apoA-I and HDL-C. One prospective, empowered trial showed that niacin by itself reduces clinical events: the Coronary Drug project from which there is very little HDL data and no HDL particle data. In HATS, niacin was associated with an increase in large HDL. Niacin through its inhibitory effect on TG synthesis lowers apoB (VLDL production). Like fibrates, niacin lowers atherogenic apoB concentrations. Niacin decreases the catabolism of large HDL allowing the HDL particles to perform antiatherogenic functions as well as release apoA-I at the foam cell surface and participate in macrophage RCT.

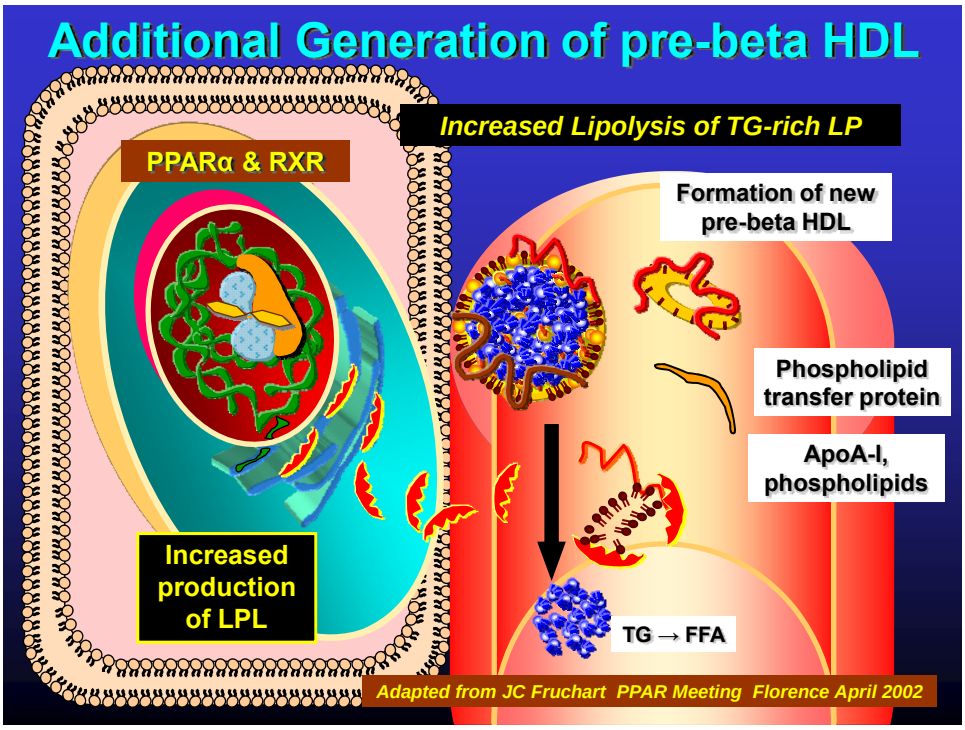
Statins are quite efficacious and as monotherapy they do variably increase HDL-C, apoA-I. Outcome reduction is not statistically related to what statins do to HDL-C or apoA-I levels. Statins mostly work because they significantly lower apoB.

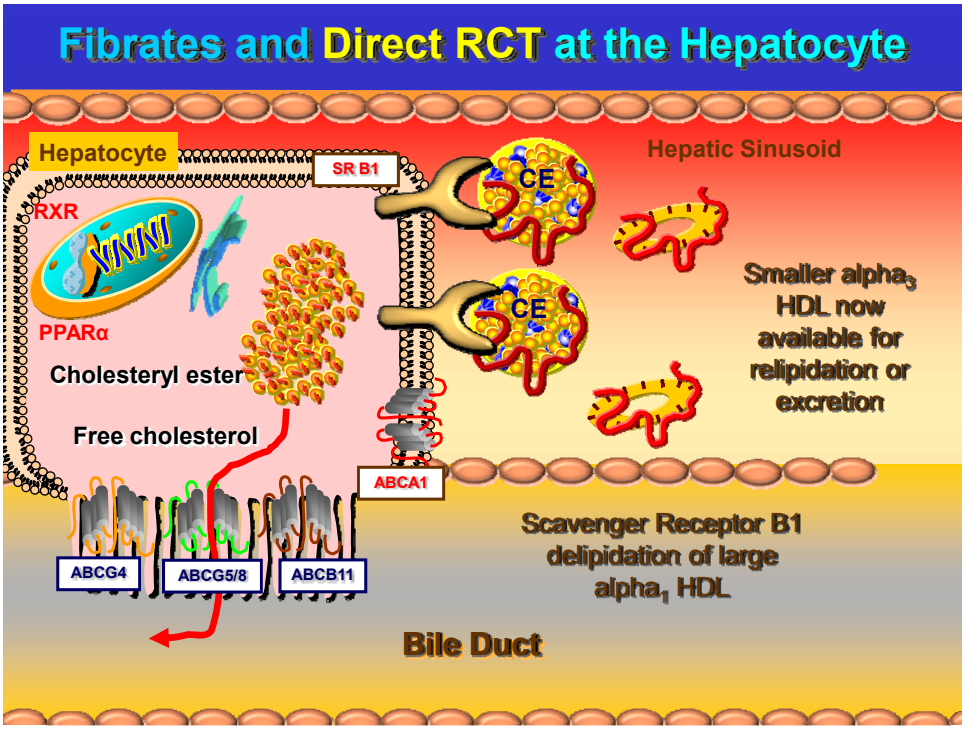
Lipid Drugs and HDL Subfractions
Thomas Dayspring MD, FACP

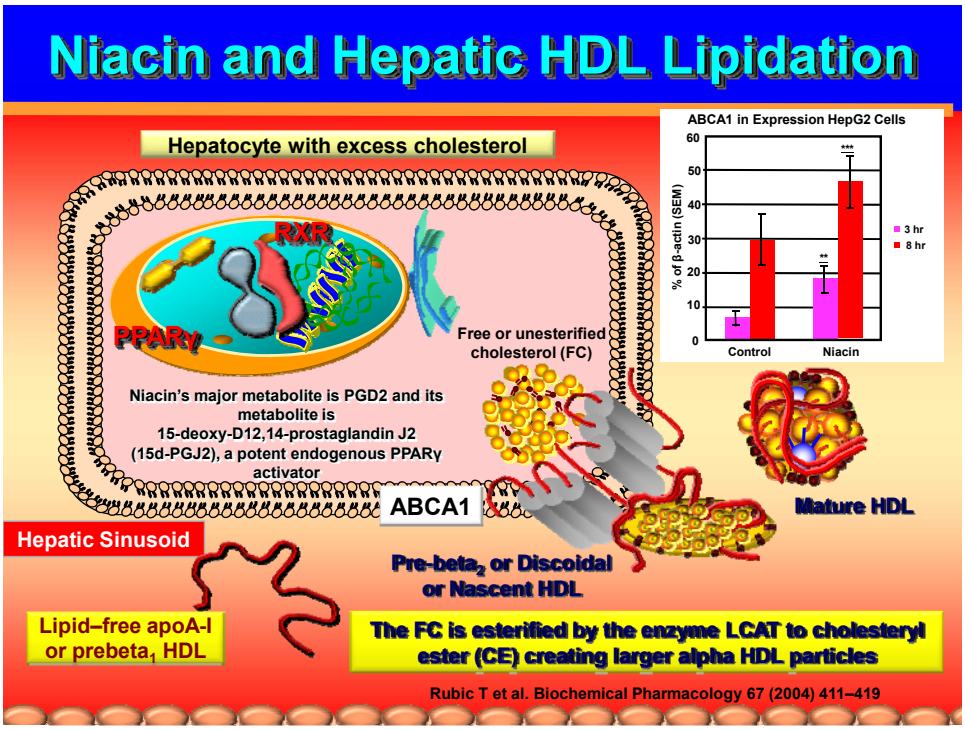
Guess what: Statins, fibrates and niacin work very well in reducing CV events and have similar outcome reductions. So it should not be important to the average clinician what those drugs do to HDL subparticles. But please do not be hoodwinked by anyone on this topic of how important it is to affect HDL size. It has no comparative meaning that niacin increases HDL size and fibrates do not. Be happy if a patient on niacin develops large HDL and be very happy if on a fibrate the number of small HDL increases. Never stop a fibrate because the number of large HDL decreases and the number of small HDL increases. THIS IS A VERY COMPLEX TOPIC and most reps and many speakers are in way over their heads on this topic.

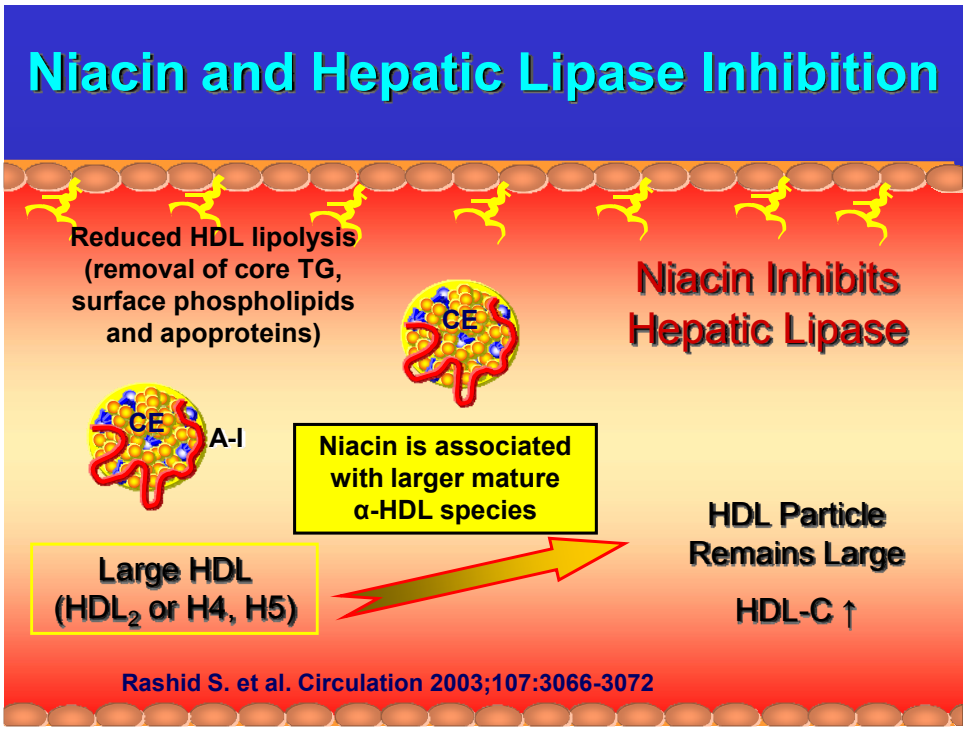


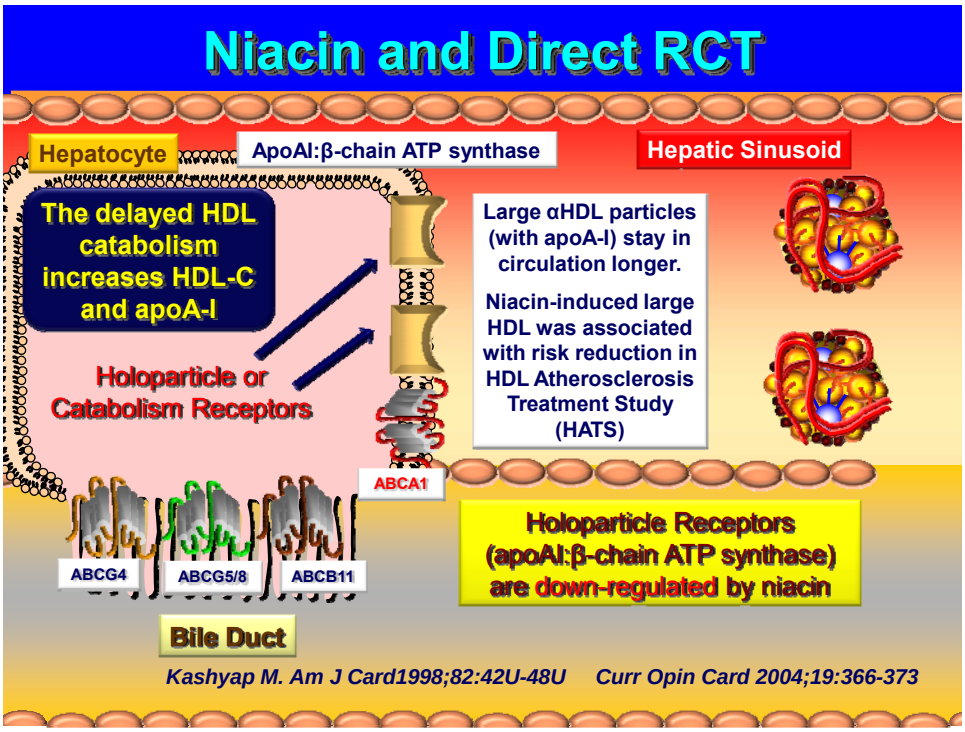
Lipid Drugs and HDL Subfractions
Thomas Dayspring MD, FACP

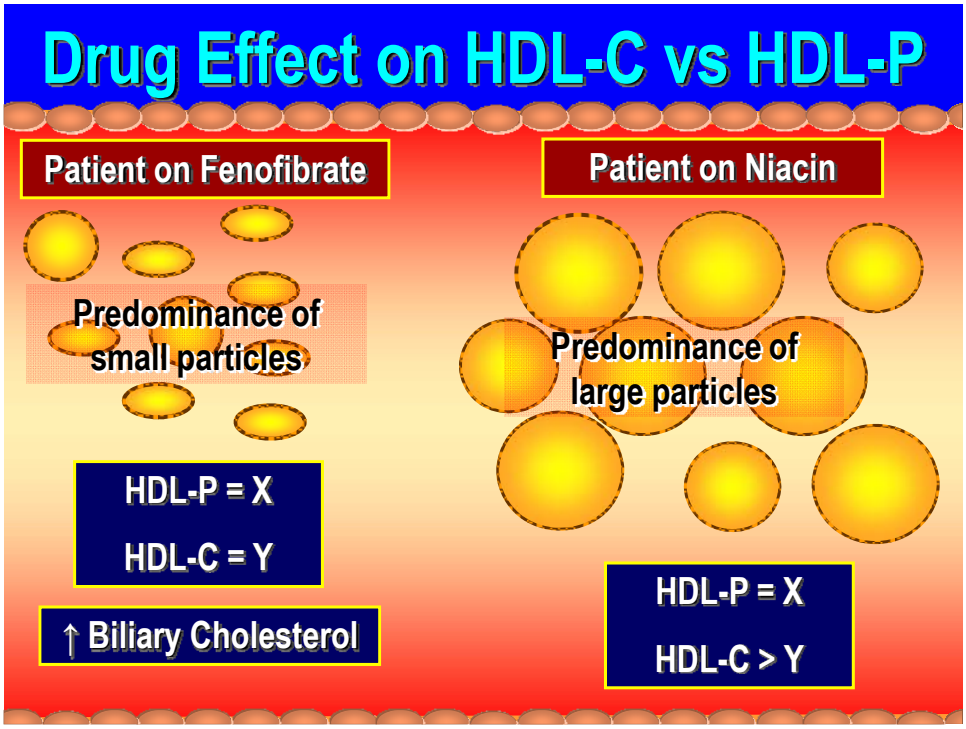





Niacin and Hepatic HDL Lipidation
Hepatocyte with excess cholesterol
RXR
PPARγ
Niacin's major metabolite is PGD2 and its metabolite is 15-deoxy-D12,14-prostaglandin J2 (15d-PGJ2), a potent endogenous PPARγ activator
Free or unesterified cholesterol (FC)
ABCA1
Hepatic Sinusoid
Lipid–free apoA-I or prebeta1 HDL
Pre-beta2 or Discoidal or Nascent HDL
Mature HDL
The FC is esterified by the enzyme LCAT to cholesteryl ester (CE) creating larger alpha HDL particles
ABCA1 in Expression HepG2 Cells
% of β-actin (SEM)
Control
Niacin
3 hr
8 hr
Rubic T et al. Biochemical Pharmacology 67 (2004) 411–419


Niacin and Hepatic Lipase Inhibition
Reduced HDL lipolysis (removal of core TG, surface phospholipids and apoproteins)
CE
A-I
Niacin Inhibits Hepatic Lipase
Niacin is associated with larger mature α-HDL species
Large HDL (HDL2 or H4, H5)
HDL Particle Remains Large
HDL-C ↑
Rashid S. et al. Circulation 2003;107:3066-3072


Niacin and Direct RCT
Hepatocyte
ApoAI:β-chain ATP synthase
Hepatic Sinusoid
The delayed HDL catabolism increases HDL-C and apoA-I
Large αHDL particles (with apoA-I) stay in circulation longer.
Niacin-induced large HDL was associated with risk reduction in HDL Atherosclerosis Treatment Study (HATS)
Holoparticle or Catabolism Receptors
ABCA1
ABCG4
ABCG5/8
ABCB11
Holoparticle Receptors (apoAI:β-chain ATP synthase) are down-regulated by niacin
Bile Duct
Kashyap M. Am J Card1998;82:42U-48U
Curr Opin Card 2004;19:366-373


Drug Effect on HDL-C vs HDL-P
Patient on Fenofibrate
Predominance of small particles
HDL-P = X
HDL-C = Y
↑ Biliary Cholesterol
Patient on Niacin
Predominance of large particles
HDL-P = X
HDL-C > Y