Clinical Lipidology Roundtable Discussion

HDL as a treatment target†

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Opening/Introductions

W. Virgil Brown, MD: I have taken advantage of having some of the most noted experts in lipoprotein metabolism gathered during the 2009 American Heart Association Scientific Sessions to have a roundtable discussion of a most difficult topic in clinical lipidology. Our topic is “high-density lipoprotein (HDL) as a potential target of treatment.” The major issues are whether HDL truly offers an opportunity...
for therapeutic intervention, what aspect of this complex system do we modify, and how do we do it. I would like to begin by discussing our current state of knowledge about HDL metabolism and its biochemical and physiological role in vascular disease. Second, we should discuss the effectiveness of current methods of modifying HDL. Finally, I hope to hear your opinions as to the research efforts that hold promise of giving clinicians a better set of tools to change HDL metabolism with resulting beneficial effect on arteriosclerosis.

My first question is: What is the most important function of HDL particles in human metabolism? Why is it important? Dr. Rader, what is HDL doing in the body that is useful?

Daniel Rader, MD: Well, HDL clearly evolved a long time ago, so it probably does things that we don’t fully understand. I think the most clear-cut thing is that it does promote efflux of excess cholesterol from cells and returns that cholesterol to the liver in reverse cholesterol transport. This is a physiologic function that has to happen, and HDL serves that role. This probably contributes to its ability to protect against atherosclerosis, but I have to say I do think HDL does other things. It participates in innate immunity. It serves as a platform for the assembly of protein-protein complexes that serve different functions. Frankly, this is an area in which there is a lot of research that still needs to be done.

Ernst Schaefer, MD: I think Dr. Rader has made a very important point. At the present time it is clear that an important function of HDL is to remove cholesterol from cells, but HDL has many other functions as well, such as being an anti-inflammatory and antithrombotic agent.

Dr. Brown: HDL is a complex group of molecules performing these several functions, and I know that Dr. Schaefer has spent a great deal of time defining the structure and function of different particles that comprise HDL. I wonder if you would comment on how you see the flow of cholesterol-changing particle size and composition and what the specific role of some of these discrete particle groupings might be.

Dr. Schaefer: People are always asking the question about whether the small or the large HDL particles are good or bad for you. The first thing I would like to say is that all of the particles are important, and they are all important in the overall HDL metabolic cycle. The methodology that we have used was originally developed in Paul Roheim’s laboratory in New Orleans and is done by my colleague, Bela Asztalos.

This two-dimensional gel electrophoresis methodology separates HDL particles by size (large to small, 12 nm to 6 nm in diameter) and by charge (pre-beta and alpha), and the particles are visualized by immunoblotting with specific apolipoprotein (apo) A1 antibody. apoA1 is made in the liver and the intestine and combines with a small amount of phospholipid to form very small discoidal pre-beta 1 HDL. This particle interacts with ATP binding cassette transporter (ABC) A1 on the surface of cells to pick up free cholesterol and phospholipid. During this process it is converted to very small discoidal alpha 4 HDL. This particle is acted upon by lecithin:cholesterol acyl transferase (LCAT) to place a fatty acid from lecithin or phosphatidylcholine onto free cholesterol to form cholesteryl ester, which moves into the core of the particle. This particle also picks up apoA2 and surface component shed from triglyceride-rich lipoprotein (TRL) during lipolysis. During these processes, a small spherical alpha 3 HDL is formed. This particle then interacts with another ABC transporter, ABCG1, to pick up more cellular free cholesterol, which also gets esterified by LCAT, to form medium-sized spherical alpha 2 HDL. The cholesteryl ester on this particle can be exchanged for triglyceride from TRL by the action of cholesterol ester transfer protein (CETP) to form large spherical alpha 1 HDLs that are enriched in phospholipid and triglyceride. Both alpha 2 and alpha 1 HDL particles can interact with the liver scavenger receptor SR-B1 to donate or pick up cholesterol. Large alpha-1 HDL is acted upon by hepatic lipase and endothelial lipase and the apoA1 on these particles can recycle back to pre-beta 1 HDL or alpha 4 HDL, and the cycle can repeat itself multiple times. Most of the catabolism of apoA1 occurs via these very small discoidal particles being taken up by cubulin in the kidney. Coronary heart disease (CHD) patients tend to have an HDL pattern with increased very small discoidal HDL and a marked decrease in large alpha 1 HDL.

Dr. Brown: Let’s focus on the cholesterol-flow issue for the moment. Clearly, ABCA1 is very important in loading the particles. We learned from Tangier disease that if you don’t have that function, then you never make larger particles and those that remain small get cleared very rapidly. Then, the second step is esterifying the cholesterol that is absorbed into the HDL structures? So we need LCAT. Is LCAT really an essential function here? How does it factor into the whole picture?

Dr. Rader: LCAT is absolutely essential for maintaining normal HDL metabolism. We know from experiments that people who lack LCAT have very, very low levels of HDL, and rapid turnover. What is less clear—and we may have some debate about this—is how critical LCAT is for actual maintenance of reverse cholesterol transport and protection against atherosclerosis. Frankly, the LCAT-deficient patients, although they are low in number, aren’t obviously at major increased risk. Dr. Brewer can speak to the subject of LCAT-deficient mice, but that is a complicated story as well. We recently showed using a model (that may or may not reflect humans) that LCAT wasn’t all that critical
for maintaining reverse cholesterol transport. My own personal bias is that LCAT is clearly necessary for maintaining normal HDL metabolism and the normal half life of HDL, but in terms of reverse cholesterol transport per se I am just not convinced it is that critical. This is in contrast to the initial conception of LCAT as generating a gradient of free cholesterol from cells to the HDL particle and therefore critical in promoting cholesterol efflux.

H. Bryan Brewer, MD: I agree. You will still have effective cholesterol efflux in the absence of LCAT into pre-beta HDL. The metabolic problem in LCAT deficiency is that you then get rapid catabolism of HDL. I think, as you have mentioned, that the patients who have LCAT deficiency don’t have a significant degree of cardiovascular disease, which really reflects that they probably have effective cholesterol efflux and the pathway to get it out of the cell is okay. It is after it gets out of the cell that you get a significant rapid catabolism of your protein moiety that ends up giving you kidney disease, but it doesn’t give you a problem associated with cardiovascular disease. I think it isn’t necessary to maintain the cholesterol homeostasis within the macrophage because you can do it with the pre-beta HDL and the ABCA1 transporter, but to maintain normal plasma levels of HDL you clearly need LCAT for normal formation and function of HDL. The functions of HDL that may be required independent of efflux could be significantly decreased because of the lack of the circulating alpha-HDL and some of the other functions, such as the HDL acting as a transport vehicle, and as an anti-inflammatory agent changing the adhesion molecules on endothelial cells. Those may be other functions of HDL that are important, and which may contribute to changes in the pathophysiology of patients with LCAT deficiency, but I think per se in terms of reverse cholesterol transport it probably is not a major problem, because once again the cholesterol that comes out is free cholesterol. It is also important to note that the free cholesterol can exchange between the plasma lipoproteins.

Dr. Rader: And free cholesterol can be directly taken up by the liver as well via SR-BI.

Dr. Schaefer: If patients do not make apoA1, they do have any apoA1-containing HDL in plasma, and they have marked HDL deficiency, normal LDL levels, and usually develop strikingly premature CHD, usually before age 40. If they have Tangier disease with no functional ABCA1 transporters, they only have pre-beta 1 HDL in their plasma, which is rapidly cleared. Because very little cholesterol gets onto HDL particles in Tangier patients, they have very little cholesteryl ester to transfer to TRL via CETP, so their LDL cholesterol levels are about 50% of normal. Despite this, they develop premature CHD in their 50s and 60s. LCAT-deficient patients have both pre-beta 1 and alpha 4 HDL, which is rapidly cleared from plasma, and they have very abnormal LDL particles with almost no cholesteryl ester. They do not appear to get premature CHD.

Dr. Brown: Of course, the strangest particles are those that are never develop a core filled with cholesteryl ester because in LCAT deficiency you wind up with a tremendous amount of free cholesterol, which combines with phospholipid and forms these membranous structures. Are those damaging? We see that appear in liver disease where LCAT deficiency is also a part of the picture. Could some of the problems in patients with LCAT deficiency be caused by these abnormal lipoprotein forms?

Dr. Schaefer: Dr. Brewer and I at the National Institutes of Health many years ago studied patients with liver disease. We studied patients with primary biliary cirrhosis. Early on in this disease patients have high HDL as a result of hepatic lipase deficiency probably from elevated bile acids in plasma. In contrast, in late-stage disease patients develop LCAT deficiency and very low HDL levels, at that point, they may develop cirrhosis and ultimately become candidates for liver transplantation. At this point they are strong candidates for a liver transplant. Such patients benefit from resins to decrease their pruritis. In my view they should not be treated with statins.

Dr. Brown: So the essential components here are making apoA1, getting it then to interact with ABCA1, picking up that cholesterol initially, esterifying the cholesterol and that point, we begin to form spherical particles.

Dr. Schaefer: Right—you do then form spherical particles.

Dr. Brown: I believe the evidence is that the spherical particles do not have an effective interaction with ABCA1. Does that make it essential to have this ABCG1 transporter, and what role is it playing in transport?

Dr. Brewer: I think, as you have mentioned, there really are two major pathways to efflux cholesterol from the cell. One pathway involves pre-beta as the ligand and the ABCA1 transporter. The other major pathway involves alpha-HDL and the ABCG1 transporter. There are a number of studies that suggest that both of these pathways are very important in modulating the intracellular cholesterol concentration. The problem is, if you don’t have ABCA1, you can’t use your ABCG1 transporter because you never make the alpha particle. So in terms of the development of the system, you need to have the ABCA1 to make the alpha-HDL particle, which can then interact with the ABCG1 transporter. I think there are several studies trying to assess what the degree of functionality is of one versus the other pathway. I think the current data suggest that both of them appear to be important. I think the bottom line for the clinician is that there are two major pathways and that both of them may function to remove cholesterol from the cell. It is also important to point out that cholesterol efflux may also occur by interaction of the alpha-HDL with SRB1 and by passive diffusion.
Dr. Brown: We have a complete particle after the cholesterol transport by the ABCG1 and SRB1 with esterification of the cholesterol by LCAT?

Dr. Schaefer: You also need lipolysis. In the setting of lipoprotein lipase (LPL) deficiency and severe hypertriglyceridemia you also do not form normal HDL particles, and you only have very small discoidal pre-beta 1 HDL and alpha 4 HDL particles. It appears that humans need normal apoA1 synthesis and normal ABCA1, LCAT, and LPL function to get spherical medium-sized alpha HDL particles containing both apoA1 and apoA2.

Dr. Brown: Very-low-density lipoprotein (VLDL) and chylomicrons are being metabolized along with the HDL. Some of the components of HDL are actually derived from those two sources. What role do these other lipoproteins play in HDL metabolism? HDL acquires the C-proteins, apoE, and several others including apoD. How essential are they to normal HDL function in terms of cholesterol transport? What do we know about that?

Dr. Schaefer: Many years ago, we studied chylomicron metabolism isolated from a patient collecting lymph in her pleural space. We were able to label her chylomicrons and inject them into her plasma space. The chylomicron apoB was rapidly cleared and was not converted to LDL, whereas the apoA1 was almost all rapidly found in the HDL density region. At the time we hypothesized that chylomicron apoA1 may be a major source of HDL apoA1. However, since that time with the use of stable isotope studies we have been convinced that apoA1 enters the plasma in very small discoidal pre-beta 1 HDL and then matures into larger spherical particles. The apoA1 on chylomicrons is donated to them via HDLs that have crossed into the lymphatic space. We believe this is the case for apoA4, and the C apolipoproteins as well. Once chylomicrons enter the plasma they undergo lipolysis and these proteins and phospholipids (surface components) are rapidly transferred to HDL, and the particles pick up apoE and cholesteryl ester from HDL. The acquisition of apoE allows for the efficient clearance of chylomicron remnants by the liver.

Dr. Brown: There are very old data that if you collect chylomicrons directly out of the lymph they have a slow mobility, but if you incubate them with plasma, even very briefly, they take on a much higher charge and move more quickly through a gel electrophoresis system, suggesting that they enter the lymph essentially naked except for apoB and perhaps a few other proteins. There is much space on the surface to acquire other proteins, and perhaps that is what is going on.

Dr. Brewer: I think all the data would certainly support that view. I think it is a separate question if you say how much A1 is made in the intestine versus the liver versus how much is A1 is exchanged by the laws of mass action between the plasma lipoproteins. The current data on the basis of mouse knockout and selective tissue expression of the ABCA1 transporter suggest that approximately 70% of HDL-cholesterol is coming from the liver, another 5% from the periphery, and another 20% or so coming from the intestines. The apoA1 is synthesized in both the liver and intestine. Thus the trafficking of the cholesterol in terms of metabolism, as Dr. Schaefer says, is really a very dynamic process.

Dr. Schaefer: I think a lot of the A1 that is made by the intestine is also coming in as very small particles.

Dr. Brown: Many of our metabolic concepts have come from animal studies, but I think we should keep in mind is that the animal data on flux can be quite different from human data.

Dr. Brewer: Right, and that is why it is important to stress that these data are on the basis of mouse studies using knockout mice and overexpression of specific genes. Whether humans do the same thing we don’t know.

Dr. Schaefer: We still do not understand HDL cholesterol metabolism in humans very well. The work of Charles Schwartz and colleagues suggests that HDL-free cholesterol but not cholesteryl ester is a major source of biliary cholesterol, whereas the cholesteryl ester in the bile is mainly coming from apoB-containing lipoproteins. More research needs to be done in this area.

Dr. Brown: We have talked a lot about the assembly of HDL. Now I want to ask you about an enzyme or a protein called CETP. Why did this system evolve? Some seem to believe that this was an evolutionary error. I would like to hear your opinions as to the purpose of this protein in terms of the physiology, its functionality in humans and whether it is a good thing or a bad thing under all circumstances. If it is a bad thing, do we need to be doing something about it?

Dr. Rader: Why we evolved CETP is anybody’s guess. I think you have to go back to what the primordial roles of HDL were related to certain innate, immune, or anti-inflammatory functions. Presumably CETP evolved to modulate HDL lipid composition in a way that provided a selective advantage; its effects on the metabolism of apoB-containing lipoproteins may have also had something to do with its original role. It is anybody’s guess, but it does clearly have a major impact on HDL and, frankly, lipoprotein metabolism. It is probably the single most powerful gene in the entire genome that influences a variation in HDL cholesterol in humans, and obviously humans who lack it have very high levels of HDL. It is very important for lipoprotein metabolism, but it is a parlor game to say what it actually is doing and why.

Dr. Brown: Do you think it might be playing a major role in moving cholesterol from HDL into a particle that is readily taken up by the liver under normal circumstances, namely the VLDL remnant? In that role, might it be facilitating reverse cholesterol transport?

Dr. Brewer: I think the data would suggest that is the case. A significant amount of HDL cholesterol ester is going back to the liver, probably through transfer into the B-containing lipoproteins and a major portion of free cholesterol is transported back to the liver by the interaction of HDL with the hepatic SRB1 receptor.

Dr. Brown: Is there any way to get a handle on the relative flux through those two pathways? It is so complex that
we have a hard time understanding how much flows through ABCA1 and how much flows through ABCG1. Don’t we have the same circumstance here? How much of it flows from HDL directly into the liver and how much of it flows through the pathway involving VLDL and perhaps chylomicron remnant clearance into the liver?

Dr. Schaefer: Studying the metabolism of free cholesterol in humans is problematic because of free cholesterol exchange. Cholesterol ester transfer protein may have evolved in higher mammals to get us through times of starvation. CETP allows cholesterol ester to be reused. Cholesterol is very important for the production of bile acids, cortisol, and sex hormones, as well as for brain function.

Dr. Brown: So it may be an efficiency gene to maintain a complex molecule that is synthesized in the body?

Dr. Rader: I would like to comment on the question asked about how we sort out these pathways. It strikes me that all four of us have done studies over the years in individuals with defined genetic disorders to try to use them as tools to understand lipoprotein metabolism. Certainly the CETP-deficient patients have taught us something about the role of CETP in lipoprotein metabolism. Other deficient patients, coupled to sophisticated ways to actually trace cholesterol metabolism through the pathway, will help us ultimately define which of these pathways are important and quantitatively which ones play certain roles.

Dr. Brown: We are now considering several drugs that inhibit this enzyme, CETP. That is being done in part because we feel that loading VLDL with cholesterol as it becomes a remnant of lipolysis action it also becomes an atherogenic lipoprotein. More cholesterol would seem to make it more atherogenic. And furthermore the alteration in the HDL that has been loaded with triglyceride and reduced in cholesterol may be less functional HDL in reverse cholesterol transport. Is this a viable hypothesis? Is it well documented that that cholesterol depleted and triglyceride-rich HDL as well as more cholesterol rich VLDL are indeed major risk factors?

Dr. Schaefer: It is clear that chylomicron and VLDL remnants are atherogenic. These particles are enriched in cholesteryl ester. Preventing cholesteryl ester from getting to these particles via CETP inhibition should provide protection from atherosclerosis. It must also be said that in the postprandial state after a fat-rich meal, there is an increase in cholesteryl ester in TRL, accompanied by decreases of cholesteryl ester in LDL and HDL. Maintaining CETP for unloading cholesteryl ester from LDL may be a beneficial effect, and inhibiting this process may not be a good thing.

Dr. Brown: And you believe that is a function of CETP as well?

Dr. Schaefer: Is it a function of CETP? I think it is. Yes.

Dr. Brewer: I think there is no doubt that there is change between the B-containing lipoproteins by CETP as well as the exchange between the HDL system and the B-containing lipoprotein, so it is modulating the cholesterol content in all of those lipoprotein particles.

Dr. Brown: I want to come back to that and talk about targeting HDL and the blood concentrations of HDL cholesterol. What are the pros and the cons of doing it by different mechanisms? I would like to leave that toward the end of this roundtable. Let’s talk a little bit about the other functionalities. We mentioned immune functions, and I think it would be useful for the readers to understand some of the things that seem to be well-documented in terms of the protection offered by HDL in dealing with various pathogenic organisms.

Dr. Rader: Yes, I can speak to this briefly: I think one of the best-documented and fascinating examples of HDL serving as a platform that defends against pathogens is the situation with a certain species of trypanosome. There is a trypanosome lytic factor that was discovered in human plasma but is missing in all other species. It turns out there is a certain type of HDL that has two proteins that only exist in humans but not in nonhuman primates and other species. Humans have the ability to lyse these species of trypanosomes, and fascinatingly some humans deficient in one of these proteins actually are then susceptible to infection with this particular trypanosome. I think this is more broadly an example of how HDL can serve as a platform to assemble proteins that then together work to engage in host defense. I suspect it is not going to be the only example of that.

Dr. Brewer: It is also known that HDL has been very effective in decreasing gram-negative sepsis because the circulating endotoxin is bound to HDL, and years ago HDL infusions were used for this function. An additional way in which HDL can modulate inflammation is related to its ability to modulate adhesion such as VCAM-1 and ICAM-1 molecules on the endothelial cell. Thus HDL can decrease atherosclerosis by increasing cholesterol efflux and decreasing inflammation.

Dr. Schaefer: I would like to add there are lots of complement proteins on HDL, and they are not all on the same particles—but they seem to be coming on and off large HDL particles. There are also a lot of proteins involved in thrombosis on HDL particles. We also know that with a severe infection, including a viral infection, HDL cholesterol levels can markedly decrease, and then return back to normal within several weeks. The serum amyloid A protein is involved in this process.

Dr. Brown: Perhaps related to the inflammatory role of HDL are certain antioxidant properties. Would you like to comment on that issue? A lot of research has been done on this. It seems to be a changeable property; different people have different capacities to have antioxidant activity in the HDL fraction.

Dr. Schaefer: HDL does have antioxidant properties. It must be said that antioxidants, including probucol and probucol analogs, have not been shown to decrease heart disease risk. Moreover antioxidants have been shown to blunt the HDL-raising effects of niacin, as well as the clinical benefit of niacin in the HDL Atherosclerosis Treatment Study (HATS) done by Dr. B. Greg Brown of the University of Washington.
Dr. Brown: By this you mean the interventional studies?

Dr. Schaefer: The interventions with antioxidants.

Dr. Brown: The failure to demonstrate reduction in vascular event when we have used agents which deliver antioxidants into the plasma space?

Dr. Schaefer: Yes, exactly. Having said that, I think that clearly HDL does have antioxidant functions.

Dr. Brown: Dr. Rader, how important are the antioxidant properties of HDL?

Dr. Rader: The term “antioxidant” is, I think, overused and one has to define it. One can describe functions of HDL in a test tube that could be described as antioxidant functions. I suppose a classic example of an activity in HDL that might be considered antioxidant is the paraoxonase activity. Paraoxonase is an interesting enzyme. There is some epidemiology that it is related to coronary disease, and I suppose it could be argued that HDL, by carrying paraoxonase, which serves as sort of an endogenous enzyme that cleaves oxidized molecules, maybe protects against coronary disease. At least that is the conventional wisdom right now. It is probably not that simple, though.

Dr. Brewer: There are a lot of people who are looking at the potential role that HDL has as an antioxidant. Some laboratories have really looked at that very carefully, and I have concluded that we just don’t know the answer yet. Whether it is clinically going to be relevant from the extrapolation of the studies in vitro, or not, you can say that HDL will clearly modulate the oxidation of LDL. How we extrapolate that to the final clinical patient is really very difficult at this point in time with the techniques we have to work with.

Dr. Brown: Providing antioxidant activity is one aspect of interest but another and related issue is the ability to clean up the damage that oxidation produces. One of the enzymes that seem to do that is lipoprotein-related or lipoprotein-bound phospholipase A2 (Lp-PLA2). I wonder Dr. Brewer, if you would like to comment on the role of that enzyme. Is it a good actor or a bad actor? I know that some in the pharmaceutical industry are thinking of this as a target for treatment. They have developed inhibitors for Lp-PLA2 and, in fact, have them in clinical trials at the moment.

Dr. Brewer: It is hard to know what the bottom line is in terms of the role of the Lp-PLA2 system in the development of atherosclerosis in humans. The products of the enzyme activity are lysolecithin and an oxidized fatty acid, which could increase atherosclerosis. Therefore, you have oxidized phospholipids on the lipoprotein particle and if you inhibit the enzyme, the oxidized phospholipids will remain on the lipoprotein. The question remains as to whether that is still going to be a good thing. Some of the animal studies have suggested that the inhibition of Lp-PLA2 is, in fact, associated with decreased atherosclerosis, particularly in the pigs by Wilensky and his colleagues at the University of Penn. I think that the final data to determine whether inhibiting the enzyme will be associated with decreased atherosclerosis is going to require the completion of a morbidity and mortality clinical trial. Is it good to cleave those phospholipids that are oxidized? Is that going to cause more of a problem or less of a problem? I think we won’t know until the morbidity and mortality trial is completed.

Dr. Brown: In the plasma, albumin has high binding capacity for these products. Some have suggested that the real role for this is to deliver the inhibitor into the subendothelial layer and have it function there. These oxidized molecules might be more damaging when released by the phospholipase with immediate access to cells involved in atherogenesis. When released in the blood stream, they are probably swept out of the plasma as a component of albumin.

Dr. Brewer: But it is hard to know in terms of whether both the oxidized phospholipids as well as lysolecithin and oxidized fatty acids are bad if you want to look in that way. That is, you have oxidized phospholipids that are on lipoproteins. If you cleave them you would release lysolecithin and the oxidized fatty acids. Neither one of these situations appear to be ideal for the prevention of atherosclerosis in our patients. I think it very hard to know if LpPLA2 is a good target or not, and I think the data is really, at this point in time, very hard to make a definitive conclusion of the efficacy of this approach other than waiting for the clinical event trial to see what the bottom line is.

Dr. Schaefer: Until recently we have not had a readily available automated assay for LpPLA2; however, such an assay is now available. Elevated levels of LpPLA2 are associated with an increased risk of heart disease and stroke. LpPLA2 is made by macrophages and may be a marker of unstable plaque. LpPLA2 causes the formation of oxidized phospholipids in LDL, and lipoprotein(a) or Lp(a) serves as an acceptor of these oxidized phospholipids.

Dr. Brown: Is it possible that Lp PL-PLA2 (and C-reactive protein [CRP] for that matter) are just manifestations of ongoing atherosclerosis, and they are signals that the process is underway, rather than being causative elements in atherogenesis?

Dr. Schaefer: CRP is made in the liver. Lp-PLA2 is made in monocytes and macrophages, so they are markers for different cell types. Any thoughts about this, Dr. Rader?

Dr. Rader: I am pretty much persuaded that CRP is a good marker of cardiovascular risk, but I don’t really believe that CRP is directly promoting atherogenesis.

Dr. Brown: The question is wouldn’t that same reasoning apply to phospholipase A2 at this point in time without the evidence that inhibiting it actually makes a difference?

Dr. Brewer: Well, I think you have to be careful, because I think I would agree with Dr. Rader that CRP is not a player. It is clearly a marker, whereas actually Lp-PLA2 can be a marker and a player. Inhibiting it really has the potential if you think that it is better to have oxidized phospholipids in the plasma lipoproteins rather than the lysolecithin and the oxidized fatty acids. I think you do have the data that has been generated in the pigs that clearly inhibiting the enzyme resulted in decreased atherosclerosis in that animal model. The intravascular ultrasound (IVUS) study was very confusing because the primary end
points were not positive. The question is whether data on the plaque composition will turn out to be relevant in terms of being associated with decreasing the vulnerable plaque and therefore decreasing clinical events will only be known after the clinical trial is completed. I do think it is different from CRP because Lp-PLA2 can be a player as well as a marker.

**Dr. Schaefer:** There is some data that CRP, even though it is made in the liver, may have deleterious effects on the artery wall. In both the PROVE-IT Study and in JUPITER, those who got their LDL cholesterol below 70 mg/dL and their CRP to less than 1 mg/L had very favorable outcomes versus those who did achieve this goal.

**Dr. Rader:** I didn’t say there is no evidence, but I think if you look at the totality of evidence, including Mendelian randomization in the genetic studies, I think it suggests that CRP is unlikely to be a direct player. I would just like to add I agree with Dr. Brewer that I think Lp-PLA2 is a different story and that, in fact, targeting it does make some sense. It is going to be a fascinating experiment to see if inhibition of Lp-PLA2 actually reduces cardiovascular events.

**Dr. Brown:** We talked about generating HDL and some of its functions. Are there any other major functions you think we should discuss before we leave that topic?

**Dr. Rader:** I think we should at least mention the provocative data that HDL can promote the synthesis of nitric oxide in endothelial cells. This is clearly a function of HDL. It might require efflux from the endothelial cells. There is some in vivo data suggesting HDL is related to endothelial function. This is one of the properties that doesn’t get talked about as much, but I am somewhat persuaded that this could be one of the functions by which HDL is directly protecting against atherosclerosis. I think we need to keep our eye on it and be thinking about it as another important function.

**Dr. Brown:** Tetrahydrobiopterin is not carried by HDL is it? The availability of that compound seems to be one of the rate-limiting issues, not in terms of activity of endothelial nitric oxide synthase but in terms of the actual product of endothelial nitric oxide synthase whether it produces superoxide or produces nitric oxide. Without tetrahydrobiopterin you don’t get nitric oxide production.

**Dr. Rader:** I am not aware that it is carried by HDL.

**Dr. Brown:** Let’s shift to the issue of clearing HDL. It is leaving the plasma space, and so what are the mechanisms by which components of HDL or the entire molecule is removed from the plasma? Dr. Brewer maybe you should take this one?

**Dr. Brewer:** I think that there are two facets in terms of HDL metabolism. One is cholesterol removal, and the other is the catabolism of the protein. Clearly, one of the facets of the protein degradation is the size of the lipoprotein particle. The major sites of catabolism of the protein are the liver and the kidney by the cubulin system. The kidney may be one of the major pathways for just the clearance of proteins if the lipoproteins are small and filtered by the kidney. This is very relevant for the disease states where you don’t lipidate the HDL and you get increased HDL catabolism. In the liver, the receptor(s) for the uptake of the holoparticle or for the protein is still, I think, a matter of a great deal of interest but has not been definitively established. As we previously discussed, the hepatic uptake of the cholesterol moiety of HDL is by direct interaction of HDL with SRB1 as well as by the transfer into the B-containing lipoproteins which are cleared by the LDL receptor.

**Dr. Schaefer:** In patients with Tangier disease, they only have pre-beta 1 HDL with no cholesterol, and this particle is rapidly removed from their plasma. These data indicate that the more cholesterol on the HDL particle the more delayed is its clearance. The fractional catabolism of HDL apoA1 is a major determinant of HDL cholesterol levels, and clearance is enhanced in patients with hypertriglyceridemia where more cholesteryl ester is transferred in exchange for triglyceride. However, the well-known difference in HDL cholesterol and apoA1 levels relates to greater production rates in women, which appears to be estrogen mediated.

**Dr. Rader:** I will add that in the remodeling of the HDL that contributes to the turnover, lipases play a key role. I think hepatic lipase—we have brought it up before—clearly is important and is also one of the top genes in the genome in terms of its influence on variation in HDL among people. It hydrolyzes the triglyceride in HDL that gets there via CETP, and it probably hydrolyzes some of the phospholipid as well. Then, as mentioned, endothelial lipase, a related member of the family that also hydrolyzes HDL phospholipid and plays a role in the turnover. I think for some reason these enzymes evolved to remodel the HDL, perhaps to reconstitute it, to make it better at doing what it needs to do, to regenerate it, to keep that cycle going that Dr. Schaefer referred to. One of the downsides, if you will, of these enzymes is that they do drive catabolism and, therefore, tend to lower HDL levels. Again, trying to figure out what that means from a clinical standpoint is one of our challenges.

**Dr. Brown:** Is endothelial lipase important only in terms of the interaction with HDL or does it have other roles?

**Dr. Rader:** I think the body of work would suggest that endothelial lipase is very important for HDL and that physiologically is largely involved in HDL metabolism. One example would be the genetics that suggest that a loss of function of endothelial lipase pretty much affects HDL but doesn’t really affect apoB-containing lipoproteins. In animal models, when you overexpress it at high levels, you can affect apoB lipoproteins, but I think its physiological role is largely limited to HDL.

**Dr. Brown:** Do you believe that removing some of that phospholipid through the action of endothelial lipase improves the functionality of HDL?

**Dr. Rader:** Well, like most of the proteins we are talking about, questions of why they evolved and what is their purpose are fascinating but largely unknown. I think endothelial lipase may generate HDL that is better at doing what
it is doing like promoting efflux. It may generate signals from HDL phospholipid that go into cells and actually activate nuclear receptors. It may facilitate the uptake of the HDL particle in certain cell types, but again these are largely speculative issues.

**Dr. Brewer:** But without those enzymes we couldn’t have the long half-life of the proteins and the repetitive function, so I think you are really right in the sense it is clearly the remodeling that is keeping the system rolling.

**Dr. Schaefer:** Estrogens increase the production of apoA1 but at greater doses also increase triglycerides, CRP, and fibrinogen. These latter effects are probably deleterious in terms of heart disease.

**Dr. Brown:** Don’t you think, though, that most of the trouble that we have gotten into with estrogen therapy has to do with the thrombosis system rather than with the lipoprotein system?

**Dr. Schaefer:** Yes, and increased inflammation as well.

**Dr. Brown:** One other question about the clearance system. Henry Ginsberg published data from our laboratory correlating the C3 content of HDL apoC3/A1 ratios with the lifespan of HDL in the plasma. Do you think that is real or do you think it might just be that larger HDL will correlate with longer lifespan and that C3 rides around on larger HDL? Is it a functional component in prolonging the residence time of the particle or simply a marker of having larger particles? Those data did not have particle size as part of the presentation.

**Dr. Schaefer:** ApoC3 is an important player in lipoprotein metabolism. It inhibits lipolysis and uptake of remnant particles. It may interfere with the ability of liver receptors to recognize apoE. ApoC3 cycles back and forth between TRL and HDL. Cohn and Davignon have documented that the residence times in HDL of apoA1 is about 5 days, of apoC3 is about 3.5 days, and of apoE is about 1 day. ApoC3 is found on large spherical HDL as well as in the free form, whereas apoE is mainly found on its own large HDL particle, which has pre-beta mobility independent of apoA1.

**Dr. Brown:** So it may be preventing interaction with uptake sites because of its negative charge perhaps? Some mechanism related to the protein that interferes with its clearance from plasma?

**Dr. Schaefer:** Yes. Atherogenic small dense LDL is triglyceride-rich, can contain apoC-III, and has a prolonged residence time. Moreover, Sacks and colleagues have documented that LDL containing apoC-III is associated with an increased risk of CHD.

**Dr. Brown:** That looks very interesting. Well, before we leave that what about apoE? Does apoE have a role in HDL metabolism, or is it simply riding around waiting to jump on another chylomicron or VLDL as they leave their tissues of origin?

**Dr. Rader:** Well, I guess I am gradually becoming persuaded that most of these proteins on HDL are probably there for a reason. On the other hand, I have never been completely persuaded that, physiologically under normal circumstances, apoE is playing a key role in mediating catabolism of a fraction of HDL.

**Dr. Brewer:** I would agree. I think that it is a very small percent of the HDL particles have apoE. Whether HDL particles containing apoE have a specific function is unclear. However, apoE HDL does have the ability to facilitate macrophage cholesterol efflux. In Alan Tall’s study, he showed that if you have E and LCAT on your alpha-HDL it may be more effective in effluxing from the ABCG1 transporter. All these results are interesting observations on the role of E in lipoprotein metabolism, but the E-deficient patients have had relatively normal lipoprotein metabolism and not significant cardiovascular disease until they are pretty old.

**Dr. Schaefer:** My view of apoE in HDL is that it is almost entirely on a separate particle. We can see that on the 2-dimensional gels. It is mainly on a large HDL particle with pre-beta mobility. It is what Robert Mahley called HDL-C that he saw in cholesterol feeding with dogs. The major role of apoE is to facilitate the clearance of remnant lipoproteins. In familial apoE deficiency there is accumulation of remnant lipoproteins with normal HDL cholesterol levels. In contrast, in apoA1 deficiency there is almost no HDL, and they remainder of the lipoproteins are reasonably normal. The only time we have seen large amounts of apoA1, apoA2, and apoE on very large HDL particles is in human CETP deficiency.

**Dr. Brewer:** I think that the clinical significance is not clear.

**Dr. Schaefer:** Proteomic studies do indicate the presence of some apoE on apoA1-containing lipoproteins.

**Dr. Brown:** I would like to turn to the topic of what we might do about low HDL. Is low HDL-cholesterol an appropriate target for treatment at this time? And if not, what should we be doing to create an intervention that will, in fact, change HDL so that it becomes more functional and performs better these desirable functions that we have described here? What are the most timely ways of tackling HDL from a clinical point of view to improve it?

**Dr. Brewer:** There are two different approaches in terms of raising HDL: One is acute HDL therapy, and the other is chronic HDL therapy. Clearly, with acute HDL therapy we have the infusions of the A1/A1 Milano type, which are giving us changes in atherosclerosis as assessed by IVUS, but we still don’t have the data that will give us data on changes in clinical endpoints. For the patient with acute coronary syndrome I think that is a potential way in which we could significantly change their atherosclerosis. In terms of the mechanism, what we do with infusions is dramatically increase the pre-beta HDL that binds to the ABCA1 transporter. That is why we get greater changes in atherosclerosis by the IVUS technique in 5 to 7 weeks that you get with statin therapy for a year and a half. In the infusion studies the lipid poor apoA1 is an excellent ligand for the ABCA1 transporter, as well as having a very significant potential anti-inflammatory effect on the vessel wall as assessed by Phil Barter and colleagues. One of the ideal
approaches for chronic HDL therapy would be to turn on the A1 gene. On the basis of all the animal studies that we have, to turn on the A1 gene would give you significant protection against cardiovascular disease. Resverlogix has hoped that they have been able to turn on the A1 gene and that is clearly being developed as a potential way to raise HDL. They can increase HDL in animals and primates, and their clinical trials are beginning to be formulated to look at the effect on plasma lipoproteins and atherosclerosis. To increase HDL you could also increase LCAT activity, and there is a company that is trying to do that now. Those approaches are the major ones that are more actively being pursued right now, except obviously for the CETP inhibitor would increase HDL and lower LDL. At present to both increase HDL and decrease LDL we use combination therapy. An additional approach to increase HDL is to develop a niacin formulation that is more effectively tolerated by our patients.

**Dr. Brown:** Let’s come back to the niacin issue. You first mentioned means of simply delivering more HDL, containing little lipid, into the plasma either by having an endogenous system, through genetic manipulation, make more apo-A1 to generate more small particles. The second mechanism was to remove the lipid physically from the HDL and reinfuse the residual particles, which are presumably, like the beta1 HDL particles. Do others of you agree that is the best way to change HDL? Is that the way that is best documented from current experimentation?

**Dr. Schaefer:** Lifestyle is an important player in modulating HDL metabolism. Weight loss can be a very effective modality to markedly increase HDL and large HDL particles. This is especially evident in morbidly obese patients studied before and 1 year after gastric bypass. High polyunsaturated fat diets may lower HDL levels somewhat but may up-regulate the SR-B1 receptor, allowing for more efficient reverse cholesterol transport.

**Dr. Brown:** There does seem to be a very good correlation with body-fat mass and HDL and even with normal weight people if they lose fat mass, as runners perhaps, you see the HDL rise.

**Dr. Schaefer:** Yes, but it takes some time to change the body composition. It must also be said that often to really raise HDL in our patients with heart disease, we must rely on niacin. In our view niacin may work differently that people have suggested. Rather than decreasing VLDL apoB synthesis, we found that extended release niacin at 2 g/day enhanced VLDL apoB fractional catabolism. Moreover niacin did not delay HDL apoA-I clearance but increased its production. Recent data in monkeys and hamsters indicate to us that extended release niacin increases liver apoA-I mRNA 40% and hepatic ABCA1 mRNA 100%.

**Dr. Brown:** So those are both at least theoretically very good things, increase A1 synthesis and increase the flux by ABCA1 transporter. More research is needed about the specific mechanisms of action of niacin.

**Dr. Brown:** After 55 years?

**Dr. Schaefer:** After 55 years, yes.

**Dr. Brewer:** That is because it is the only game in town right now. I think that the ability for the patient to have a niacin-type of preparation and have decreased side effects would be extremely useful for the clinician. From all of the data we have available, niacin has the ability to lower LDL, raise HDL, and decrease inflammation. It would be a fantastic drug if we could make compliance a little easier.

**Dr. Rader:** To go back a bit, I do agree with Bryan. I think the concept of acute apo-A1 based infusions, whether it is full length or apo-A1 mimetic peptides, is extraordinarily conceptually attractive. It is one of the few areas we have data for in humans, and I think that I would very much like to see that continuing. I for one believe that there is no question that if it can be shown to be effective, say in an acute coronary syndrome-type setting, there is going to be a market for it.

I do want to mention one other category that hasn’t been mentioned, and that is promoting reverse cholesterol transport through LXR agonism. This is an extraordinarily attractive concept. We have a tremendous amount of animal data suggesting that when you up-regulate LXR pharmacologically or genetically, you promote efflux; you have anti-inflammatory effects; you regress atherosclerosis. This field was stalled and almost died because of issues of LXR agonists in the liver increasing the expression of SREBP-1c, thus promoting lipogenesis and steatosis and in some cases hypertriglyceridemia and even elevated LDL. But, hopefully there will be a new generation of LXR agonists that potentially have the ability to do what we hoped they could do without having these hepatic effects. Again, if that turns out to be the case, I view this as another extremely exciting area in developing HDL therapeutics, so we should stay tuned.

**Dr. Brown:** Is there any evidence that LXR is altered with niacin therapy?

**Dr. Schaefer:** We haven’t looked at that. So, Dr. Rader, do you have any thoughts about whether niacin could be affecting LXR?

**Dr. Rader:** I would be very surprised if it is a direct ligand for LXR, but perhaps it could somehow be indirectly turning on LXR?

**Dr. Brown:** Some other ligand for the LXR system in the liver might be secondary.

**Dr. Brewer:** What you would really like to have, because still you have the problem with the LXR selectivity, is the development of a transcription factor for modulation of the ABCA1 transporter. It would really be great if you could turn on ABCA1selectively.

**Dr. Schaefer:** I think that is what niacin does, but that remains to be determined by other labs as well.

**Dr. Rader:** I agree with Dr. Brewer. I think one of the reasons for really continuing to work on the basic cell biology of ABCA1—not just its synthesis but its turnover and
its subcellular localization—is maybe there could be a very specific therapeutic intervention that would inhibit the turnover of ABCA1 in such a way that it would keep more of it on the cell surface active and doing what it is doing.

**Dr. Schaefer:** If niacin does not work through the niacin receptor other than the flushing, then that provides hope for pharmaceutical companies to make niacin analogues that don’t cause flushing but have beneficial effects on lipoprotein metabolism.

**Dr. Brown:** So far they have not been able to come up with a ligand that binds to the so-called niacin receptor on the Langerhans cells that is better and more effective than niacin, but specificity may be another issue. As you say, it is another binding site almost certainly. Any other thoughts about niacin, before we leave that? I want to talk a little bit about peroxisome proliferator-activated receptor (PPAR)-alpha activation and its potential role in HDL metabolism. Then, I want to close with pharmacological inhibition of CETP.

**Dr. Rader:** Well, you have heard a lot of enthusiasm for niacin. We very recently heard a study presented and published on the effect of niacin in regressing carotid intima media thickness. Quite a bit of circumstantial evidence suggests niacin really does have beneficial effects on atherosclerotic plaque, so we await two big outcome trials with niacin added to a statin. I think most of us expect and hope those are going to be positive as a way of affirming that niacin use really should be done more than it is done now. I think, certainly in my practice, a high-risk patient who is on a statin and who has a low HDL, I am going to offer them niacin as adjunct to their therapy.

**Dr. Brown:** Would you say at this point, though, that we know definitely that it is the HDL effect of niacin versus its VLDL or LDL effect?

**Dr. Rader:** Absolutely not, I didn’t really say it was definitely the HDL increasing. Obviously, niacin is doing a lot of things.

**Dr. Brown:** I just want to clarify that point because I think it still leaves us with the question of changing HDL with current therapy and getting a benefit directly from that.

**Dr. Schaefer:** The focus always has been on LDL, and that is understandable. However, patients with dyslipidemia or high triglycerides/low HDL are at very high risk. To look at the system and just focus on LDL is very foolish. In addition to insist that you have to show improvement for just for one lipoprotein and not others is not feasible. There isn’t a single agent that only affects one lipoprotein class.

**Dr. Brown:** PPAR-alpha activation has turned out to be most effective in the very setting that you just mentioned, where LDL cholesterol is not a big risk factor—at least in terms of its mass—but where triglycerides are elevated and HDL cholesterol is low. Do you think it is through the alteration of HDL metabolism that PPAR-alpha activation provides for reduced atherogenesis?

**Dr. Brewer:** Well, particularly with the VA-HIT trial it is clear that the changes in HDL were only a part of the story and that its ability to change transcription factors in one sense is good and also bad because it is a transcription factor modulator that can have several effects on numerous different genes, some of which can be beneficial and some of which may not be in terms of the toxicity of the compounds. The patient who appears to get the most dramatic response from these types of compounds is the one who does have high triglycerides and low HDL. That patient seems to profit from a PPAR-alpha ideally compared with the other patients. I think part of the problem is that the investigators who are interested in developing PPAR-alphas and the gammas and the combinations are still having a great deal of difficulty with the co-repressors/co-activators, and getting the right combination for the toxicity and turning on selective genes with the transcription-factor type of modulators. I think that is why it is a challenge to get the right PPAR-alpha with the minimum amount of side effects to be able to use it in what appears to be the ideal patient, the insulin-resistant metabolic syndrome patient.

**Dr. Rader:** I think PPAR-alpha is a potentially interesting example of the mass versus flux issue. PPAR-alpha agonists don’t really raise HDL cholesterol or apoA1 that much, but they have been shown to promote cholesterol efflux from macrophages in vitro. We showed that they do so in vivo. They do increase apoA1 production. Actually, we studied a very potent PPAR-alpha agonist that had very little effect on apoA1 levels, but it quite convincingly raised apoA1 production by about 30% and also increased catabolism concurrently. That’s the turnover of apoA1, not HDL cholesterol, but at least it suggests that the flux through the system in a situation like that, even where steady state levels aren’t changing, might actually be substantial and potentially anti-atherogenic. I think this is an area that really does need some more attention.

**Dr. Schaefer:** Niacin increases very large alpha 1 HDL, and we have documented that this increase is associated with benefit in terms of coronary artery plaque regression. Fibrates, on the other hand, increase intermediate-sized HDL, which contain both apoA1 and apoA2. Fibrates increase the secretion of both apoA1 and A2 but also significantly enhance apoA1 catabolism. Fibrates are especially useful in patients with elevated triglycerides and low HDL and in subjects with high insulin levels. Moreover, they appear to be very useful for the prevention of retinopathy and amputations in diabetic patients.

**Dr. Brown:** We have the potential for raising HDL by blocking the transfer of cholesteryl ester from HDL into the VLDL in exchange for triglyceride. We know that this group of drugs that inhibit CETP raise HDL more dramatically than any drugs or any other mechanism that we know of, but we have seen a failure of benefit in one large clinical end-point trial and two trials with surrogate end points. We now have additional drugs that are in tests that seem hopeful. What do you believe is the potential for these CETP inhibitors? Do you think that these newer drugs have properties that might make them successful, whereas the previous drug, torcetrapib, failed?
Dr. Brewer: Well, I think that the unfortunate off-target toxicity that was associated with torcetrapib generated a great deal of interest in looking at a second generation of CETP inhibitors that don’t have the properties that are characteristic of torcetrapib toxicity. I think in vitro studies have clearly identified some newer second-generation CETP inhibitors that, on the basis of current information, don’t appear to have the off-target toxicity related to torcetrapib. Torcetrapib really didn’t have an opportunity to test the hypothesis that inhibiting CETP was a good mechanism to decrease atherosclerosis because it had problems associated with the off-target toxicity. I think with the data that has been generated for the two other drugs that are currently in clinical development, we will be able to test the hypothesis. Obviously, the question is whether that will turn out to be an effective way to raise HDL. It is very important to realize that with some of the drugs, you can both raise HDL and lower LDL, whereas dalcetrapib has only the ability to raise HDL. The advantage of the two is that we will be able to look directly at raising HDL without significant change in LDL with dalcetrapib, which will allow us to see whether by raising the HDL alone with this mechanism we will decrease atherosclerosis. Whereas the other CETP inhibitors that are available will both raise HDL and lower LDL, so it will be a combination effect that may be useful in terms of decreasing atherosclerosis. I think at this point we can speculate a great deal on whether this will be a good mechanism. With regard to the question that we were creating a dysfunctional HDL, this has been pretty effectively addressed by several different laboratories. There are very few data at this point with the tests that have been done to suggest that the HDL is significantly dysfunctional on the basis of efflux, but there are a number of studies that haven’t been done as yet on some of the other HDL functions to see whether these particles are functioning as effectively as a “normal” HDL particle. The bottom line is that we will only really be able to definitively answer the question of whether CETP inhibition is an effective mechanism to increase HDL and decrease atherosclerosis with the completion of the clinical trials that are underway.

Dr. Brown: In respect to its functionality, what about the HDL particles that are generated by CETP inhibitors? Do we know how they interact with SRB1? Does it function normally with that transporter and does it function with the ABCG1 transporter as efficiently as native HDL?

Dr. Brewer: Current data do not suggest that cholesterol efflux to HDL isolated from patients with reduced CETP activity is impaired. In fact, Alan Tall did some very nice studies demonstrating that even the HDL particle isolated from patients with complete CETP deficiency were effective in cholesterol efflux. What Alan found was the E-containing HDL particles that have both LCAT and E on the LpA1/A2 particles from the completely CETP deficient patients were very effective in effluxing by the ABCG1 transporter. These results were very helpful in addressing the concept that if we increased CETP inhibition and increased the HDL to a certain point and passed a magic line in the sand we would create a dysfunctional HDL with a decreased capacity to efflux cholesterol. I think the data at least at this point suggests that the HDL that is generated from CETP inhibition, even to the stage of getting the patients who have no CETP, still is able to efflux very effectively. Is it going to be totally effective? At least as tested in the laboratory we don’t have data to support that it is a dysfunctional HDL. Now the question is whether it delivers cholesterol to the liver as effectively by SRB1. We just don’t really have direct data to support whether there is a decrease in the clearance of the cholesterol from those lipoprotein particles into the liver; and secondly, even if there is decreased clearance is there decreased flux because the pool size is significantly increased? Even with decreased cholesterol clearance the net cholesterol flux to the liver could be basically okay. At the present time I think we don’t know the answer to this question.

Dr. Brown: Do we know how dalcetrapib and anacetrapib, the new drugs that are in clinical trials, have an impact on the distribution of particles in the two-dimensional electrophoresis system?

Dr. Schaefer: We have not studied those products, but their effects on HDL particles would be predicted to be similar to what we have observed for torcetrapib (increases in large alpha 1 HDL). Animal studies indicate that CETP inhibitors will significantly decrease CHD risk. However torcetrapib had off-target effects such as raising aldosterone and cortisol, and lowering potassium, which may have been the cause of the deleterious outcomes in the large ILLUMINATE Trial. These effects are not shared by dalcetrapib or anacetrapib. Dalcetrapib is less potent as a CETP inhibitor than torcetrapib or anacetrapib. Rabbit studies indicate that inhibiting CETP decreases diet-induced atherosclerosis. Animals that lack CETP are very resistant to diet-induced atherosclerosis. The rabbit has the highest levels of CETP and is the most sensitive. Human genetic studies suggest that CETP is a major player in regulating HDL levels, so if low CETP levels were deleterious, then we shouldn’t see this inverse relationship between HDL and heart disease. We still don’t know exactly what the excess mortality was caused by, but it was not only cardiovascular issues but also infections. From the lipoprotein metabolism viewpoint, everything looked good. We collaborated with Dr. Rader and documented that torcetrapib decreased apoA1 clearance, and enhanced the clearance of apoB100, apoB48, and apoE in TRL, as compared with placebo.

Dr. Brown: I want to hear Dr. Rader’s opinion about the combination of a statin-enhancing LDL and VLDL remnant clearance and the superimposition on that of the CETP inhibition. What are the implications there? It would seem that have a drug that is enhancing the removal of VLDL remnants, it might be a good idea to put cholesterol in that pathway rather than preventing it from entering that pathway.

Dr. Rader: This really gets to the key issue of what CETP is doing in terms of net reverse cholesterol transport. Even to the issue of once cholesterol is effluxed from macrophages of the arterial wall, is that enough or does it really
matter where it goes after that, that it ultimately gets back to the liver? There are different points of view on this by very smart people, so I don’t think we know the answer. I think it is safe to say that CETP facilitates the return of HDL cholesterol ester to the liver faster. One could argue that this promotes reverse cholesterol transport and that up-regulation of the LDL receptor speeds that up even further. One hypothesis that has been put forth is that maybe CETP inhibition would be most effective in people who have perhaps more sluggish LDL receptor-mediated uptake. A great example would be a person who is homozygous for familial hypercholesterolemia; they would be very likely to benefit from a CETP inhibitor. At the end of the day, I have to agree with my colleagues, that one of the great experiments of lipid metabolism in atherosclerosis is the question of whether CETP inhibition is going to reduce cardiovascular risk. We didn’t get the answer with torcetrapib for reasons we have talked about, and studies with additional CETP inhibitors are going to be absolutely fascinating for the field.

Dr. Brown: Clinically, a very important issue. It could provide a totally new means of improving lipoprotein metabolism that has major clinical benefit. However, this concept has also stimulated experiments that may give some fundamental physiologic information about basic lipoprotein metabolism.

Dr. Schaefer: The lipoprotein field has long been divided about whether CETP inhibition is a good or a bad thing. Only future clinical trials will allow us to adequately answer that question. At the present time we do have a considerable amount of trial evidence indicating a beneficial effect of niacin on heart disease risk reduction.

Dr. Brown: There have been several studies suggesting that there is channeling of cholesterol flow in the liver from HDL directly to the biliary system. Do we know what happens to bile content under the influence of CETP inhibitors? Have we looked at that? Do you know of any information on the biliary content of cholesterol phospholipid?

Dr. Schaefer: We could not document any effect of torcetrapib on fecal cholesterol excretion in humans. It would probably be worthwhile doing such studies carefully in a nonhuman primates.

Dr. Brewer: But maybe with Resverlogix selectively turning on the apoA1 gene we could get some useful information on the flux of cholesterol and bile acids into the GI tract. This will clearly be much more complicated in terms of other mechanisms that increase HDL such as CETP inhibition.

Dr. Rader: There also are data that in people with elevated triglycerides, CETP is worse for you if you have high triglycerides. I am suggesting we do a trial in which the non-HDL is over a certain desirable level, such as 100 mg/dL.

Dr. Brown: Dalcetrapib versus anacetrapib: What are the differences in what we know already published to date?

Dr. Rader: We have a really great opportunity in that we have two CETP inhibitors in late-stage clinical development, one of which raises HDL-C modestly and has relatively small effects on apoB-lipoproteins, and the other with a more potent effect on raising HDL-C and also lowers LDL. Essentially, we are testing the CETP hypothesis with two different compounds that have fundamental differences in terms of their effects on lipoproteins. It will provide even more information ultimately on the impact of CETP inhibition on cardiovascular risk.

Dr. Brown: I think we would all agree that HDL is a very complex group of molecules. We know a lot about it but still have a lot to learn, and it is a very exciting time because we now have some drugs and mechanisms of action that will actually change HDL and that offer significant hope that we will, in fact, see a relationship between vascular disease reduction and the changes that we make in HDL. Gentlemen, thank you very much for joining me today, and for sharing what you know about the outlook for HDL as a target of therapy. Our readers will undoubtedly find this discussion of interest.