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JCL Roundtable: Hypertriglyceridemia due to defects in lipoprotein lipase function

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Abstract

In this Roundtable, our intent is to discuss those rare genetic disorders that impair the function of lipoprotein lipase. These cause severe hypertriglyceridemia that appears in early childhood with Mendelian inheritance and usually with full penetrance in a recessive pattern. Dr Ira Goldberg from New York University School of Medicine and Dr Stephen Young from the University of California, Los Angeles have agreed to answer my questions about this topic. Both have done fundamental work in recent years that has markedly altered our views on lipoprotein lipase function. I am going to start by asking them to give us a brief history of this enzyme system as a clinical entity.

Keywords

Lipoprotein; Lipase; Hypertriglyceridemia; LPL; Genetic disorders

Dr Brown: In this Roundtable, our intent is to discuss those rare genetic disorders that impair the function of lipoprotein lipase (LPL). These cause severe hypertriglyceridemia that appears in early childhood with Mendelian inheritance and usually with full penetrance in a recessive pattern. Dr Ira Goldberg from New York University School of Medicine and Dr Stephen Young from the University of California, Los Angeles have agreed to answer my questions about this topic. Both have done fundamental work in recent years that has markedly altered our views on LPL function. I am going to start by asking them to give us a brief history of this enzyme system as a clinical entity.

Dr Goldberg: I think people had known for a long time that there was a syndrome of severe hypertriglyceridemia that caused pancreatitis in young children. But the lipase enzyme itself

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Hypertriglyceridemia due to defects in lipoprotein lipase function: Recommended reading

was first uncovered in 1943. Hahn at the University of Rochester was studying methods of measuring red cell volume in dogs. To prevent clotting of his catheters, he injected heparin during the studies. He observed that in dogs that had eaten a fat containing meal and by chance had lipemic plasma, a rapid clearing of the lipemia occurred. Hahn wrote a short paper in *Science* in 1943 describing what he called a lipemia clearing factor that was induced by heparin. This activity was subsequently attributed to an enzyme and renamed LPL. So the discovery of LPL starts in 1943.

The linking of this enzyme to a human disorder was in 1960 when Richard Havel and Robert Gordon made the observation that the children who had severe hyperchylomicronemia and pancreatitis had a defect in LPL action. These investigators obtained postheparin plasma from patients with fasting hyperchylomicronemia and showed that, as opposed to normal, postheparin plasma from these children failed to degrade chylomicrons, ie, hydrolyze the chylomicron triglyceride into free fatty acids. Thus, Havel and Gordon connected the human disease that was termed type I hyperlipoproteinemia or familial chylomicronemia syndrome with defective LPL.

Dr Young: Dr Goldberg properly attributes the discovery of postheparin clearing factor to Paul Hahn. He not only showed that the lipemia was cleared by heparin in the dog but that it continued to clear after the plasma was removed from the animal and placed on the laboratory bench. Later, Ed Korn showed that the responsible enzyme, LPL, was present in the heart and that it was released from the surface of blood vessels by heparin. He proposed that LPL cleaved triglycerides into molecules (glycerol and free fatty acids) that were not opalescent (explaining the “clearing” effect of heparin). He also proposed that, in the absence of heparin, LPL-mediated triglyceride hydrolysis occurs in the heart along the surface of blood vessels.

Dr Goldberg: The other piece of the story that is interesting is a couple of years after the Havel paper, others found that postheparin plasma from children who appeared to be LpL deficient was able to hydrolyze triglyceride when it was contained in emulsion particles. This was a surprise and at first seemed to contradict the original observation that hyperchylomicronemia syndrome was due to LPL deficiency. But the key difference in the assays was the use of chylomicrons rather than lipid emulsions, and these seemingly contradictory findings led to the discovery of the second major postheparin lipase enzyme, hepatic triglyceride lipase. Chylomicrons, but not smaller triglyceride-rich emulsions, are a poor substrate for hepatic lipase whose primary in vivo substrates are remnants, intermediate density lipoproteins, and high-density lipoprotein (HDL) phospholipids. We now know that there is a third member of this lipase family in the postheparin plasma, and that is endothelial lipase.

Dr Brown: Well, we now know that the system is involved in removal of triglycerides from lipoproteins produced in the intestine as we absorb fat (chylomicrons) and in the liver as it unloads excess energy delivered from the diet and body stores in the form of very low–density lipoproteins (VLDLs). What are the tissues of origin of the LPL and where is it located in the body?

Dr Young: LPL is synthesized mainly in white and brown adipose tissue and striated muscle (heart and skeletal muscle). However, depending on the sensitivity of the technique used to detect LPL expression, you can find LPL in most tissues. For example, by northern blot or real time - polymerase chain reaction, it is possible to find LPL transcripts in the mouse liver (where LPL is often assumed to be absent). LPL is also expressed at high levels in certain regions of the brain. Why LPL is produced in neurons of the brain remains an enigma, at least as far as I am concerned. Of note, GPIHBP1 is not present in brain capillaries at appreciable levels. For that reason, I strongly suspect that LPL in the brain is not involved in *intravascular* triglyceride hydrolysis and instead plays some other function.

Dr Goldberg: LpL expression appears to be very specific to certain cells. In the brain, the highest level expression is in hippocampal neurons, but LpL messenger RNA is also found in other cortical neurons and Purkinje cells of the cerebellum 4. In the kidney, LpL expression is greatest in the proximal tubules.

For a number of years, there was a hypothesis that fatty acids were a brain satiety signal. Some animal studies in which areas of the brain were exposed to fatty acids seemed to support this idea. However, from the perspective of in vivo physiology, this really made no sense. High blood levels of fatty acids are found during starvation, when you certainly would not wish to curb appetite. Eckel had suggested that triglycerides, that are increased postprandially, rather than free fatty acids were an appetite regulator. He went on to knockout LpL in the hippocampus, and reported that this led to increased weight gain in mice 5.

Dr Brown: So the adult human liver is devoid of LPL?

Dr Young: We rarely work with human liver samples, but LPL transcripts are easily detectable in the mouse liver.

Dr Brown: There is also work independently from that of Dr. Daniel Steinberg's laboratory showing that LPL is made by macrophages in tissue culture, and I believe the data from human arteries is confirmatory.

Dr Young: Yes, indeed, LPL is produced by macrophages.

Dr Goldberg: Macrophages are the only white blood cells that express LpL, and these cells are the major source of LpL within atherosclerotic plaques.

Dr Brown: Since there are macrophages in the liver, this might be one hepatic source of LPL?

Dr Goldberg: Yes. But under some conditions such as fatty liver, LpL expression increases within hepatocytes. It is also synthesized by the vitamin A storing stellate cells.

Dr Brown: There are mysteries in the reasons for certain tissues to contain the lipase but we know that tissues that need or store a source of energy, namely muscle, or that store energy, such as adipose tissue, are the major sources of this enzyme system. However, we also know most of the function is not in the cells of origin. The enzyme must move from the

parenchymal cells to the lumenal surface of the capillaries and there set up a complex interface with chylomicrons and VLDL. Dr Young has done groundbreaking studies on this complex transport and activating system for LPL.

Dr Young: There are several important differences in the expression patterns of LPL and GPIHBP1 (the endothelial cell LPL transporter). For most tissues, the pattern of GPIHBP1 expression mirrors that of LPL, with high levels of expression in white adipose tissue, brown adipose tissue, heart, and skeletal muscle. However, there are several noteworthy differences. First, unlike LPL, GPIHBP1 is not expressed at appreciable levels in the brain. The other site where LPL and GPIHBP1 expression differs is the lung. GPIHBP1 is expressed at very high levels in capillaries of the lung, whereas the levels of LPL expression in the lung are very low. We have proposed that GPIHBP1 in the lung scavenges LPL that reaches the bloodstream.

Dr Brown: Are there any new molecules that are important for LPL biology?

Dr Young: Yes, there are several new molecules, elucidated over the past 10 to 15 y, which are important for LPL biology. An important new molecule, studied in detail by Mark Doolittle and coworkers at UCLA, is lipase maturation factor 1 (LMF-1). LMF-1 is found in the endoplasmic reticulum and is important for the secretion of LPL, likely because it is required for LPL dimerization. To be catalytically active and to be secreted from cells, LPL monomers need to assemble into homodimers. LMF-1 is crucial for this process. LMF-1 is also required for the dimerization of 2 other lipase family members, endothelial lipase and hepatic lipase.

A complete deficiency of LMF-1 results in very low levels of LPL and hepatic lipase secretion, and that leads to severe hypertriglyceridemia. There have been only a handful of human patients with well-documented LMF-1 deficiency. A patient homozygous for a nonsense mutation in LMF-1 (Y439X) had severe hypertriglyceridemia; the levels of LPL and hepatic lipase in the postheparin plasma were reduced by 91% and ~50%, respectively. Another patient with a homozygous nonsense mutation in LMF-1 (W464X) also had severe hypertriglyceridemia; the levels of LPL activity in the postheparin plasma of that patient were reduced by ~75%.

Dr Goldberg: The initial observation leading to the discovery of LMF-1 was made in Dr Brown's laboratory. He and his colleagues reported in Science in 1983 that a severely hypertriglyceridemic mice that had a defect in tail formation also had a defect in both LpL and hepatic lipase activity. The defective tail was not the reason for the lipolytic defect. I then took more than 20 y, to go from the mouse observation to figuring out what protein was defective; this search occupied Drs Schotz and Peterfy for many years.

Dr Brown: We initially called this combined lipase deficiency (CLD), but of course these animals did not have a lipase deficiency at the cellular level. Fortunately, I knew of Dr Robert Scow's work on lipid transport across the endothelium and of his elegant studies with the electron microscope. We were able to collaborate with him in some studies that clearly indicated that both LPL and hepatic lipase were synthesized but were not able to leave the cells of origin. It was later that the key protein, the LMF-1 was discovered as a required

factor in delivering the lipases to the cell membrane and secretion. It was the gene for this protein that was defective in these animals.

Dr Goldberg: Like the CLD mice, LpL knockout mice created by Weinstock et al in the Breslow lab died within 1 d after birth. LpL deficiency is not a neonatal lethality in humans. We then overexpressed LpL in the liver and found that this prevented the neonatal death7. Why is this? When you overexpress the LPL hepatocytes, the lipid comes back into the liver and gets resecreted, so the triglyceride levels don't go down that much. I remember sitting with Jan Breslow who kept asking, “Why did these mice not die? And how come the liver rescued them?”

And so then, we went back and discovered that LpL knockout mice became severely hypoglycemic. Their glucose levels went below 20 and that they were dying of hypoglycemia. Mouse milk, which is 90% fat, was their sole source of calories and with LpL deficiency they were unable to utilize this source of calories. And they had almost no carbohydrates in their diets. The CLD mice, I suspect, die for the same reason. Also, I recollect that Jim Paterniti, the first author of the Science paper, was able to keep them alive by giving them glucose by an eyedropper.

So why is the clinical presentation in humans different? Probably the neonatal lethality is prevented by the greater carbohydrate content of human milk. Or perhaps the human milk–derived chylomicrons are better substrates for hepatic lipids than are the mouse chylomicrons. I am not sure how human LMF-1 deficiency presents.

Dr Brown: And do we know if the few patients reported were producing totally dysfunctional LMF-1? Was it a partial defect in the functionality of LMF-1?

Dr Young: Two of the LMF-1 homozygotes described in the literature were homozygous for nonsense mutations and presumably had a complete deficiency in LMF-1 function. Those patients were severely affected. Both had had very severe hypertriglyceridemia associated with multiple bouts of pancreatitis. Both of these patients responded well to a low-fat diet.

Dr Brown: So, we now know a lot more about the enzyme being delivered to the cell surface. However, we need to bring the lipase to the lumenal surface of the endothelial cells. That means it needs to go around or through the cells in that endothelial layer. How does that happen?

Dr Young: How LPL, an enzyme secreted by myocytes and adipocytes, managed to reach the capillary lumen was a mystery for decades. In fact, it was a mystery for so long that many in the field had actually forgotten that it was a mystery and were not concerned with this gap in our knowledge. In recent years, however, this mystery was solved. The story starts with a 2003 *JBC* paper by Ioka and coworkers from Japan. They had used expression cloning to search for complementary DNAs (cDNAs) that would confer upon chinese hampster ovary cells the ability to bind HDL. They came up with the cDNA for SRB-1, which was expected, and they also came up with a novel cDNA that encoded a protein that they called glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1

(GPIHBP1). They recognized that this protein was a glycosylphosphatidylinositol (GPI)-anchored protein of the lymphocyte antigen 6 (Ly6) family. The Ly6 family was well known in medicine. Other family members include CD59, a complement-modulating protein that is central to the pathogenesis of paroxysmal nocturnal hemoglobin. Another well-known LY6 protein is the urokinase-type plasminogen activator receptor. Another Ly6 family member is SLURP1, a secreted protein of keratinocytes that has been implicated in the pathogenesis of a rare palmoplantar keratoderma (*mal de Meleda*).

The hallmark of all Ly6 proteins, including GPIHBP1, is an ~80–amino acid domain containing 10 cysteines, all arranged in a characteristic spacing pattern and all disulfide bonded so as to form a 3-fingered structural motif. This structural motif was initially identified in protein toxins in cobra and viper venom. Those snake toxins bind avidly to crucial mammalian proteins such as acetylcholine receptors and blood clotting factors (so as to disable prey).

GPIHBP1 has a typical Ly6 domain, but unlike other members of the Ly6 protein family, GPIHBP1 has a striking acidic domain at its amino terminus. In human GPIHBP1, 21 of 26 consecutive amino acids are aspartic acid or glutamic acid. Plus, GPIHBP1's acidic domain is predicted to contain a sulfated tyrosine.

Ioka and coworkers found that GPIHBP1 bound HDL, and for that reason, they speculated that the protein played a role in reverse cholesterol transport. A few years later, our group, in association with collaborators at Genentech, generated and characterized GPIHBP1 knockout mouse. These mice had a striking phenotype: creamy plasma and severe hypertriglyceridemia. Even when the mice were maintained on a low-fat chow diet, the plasma triglyceride concentrations were 2000 to 4000 mg/dL, about 100-times normal. When the mice were placed on a high-fat diet, the plasma triglyceride levels were often greater than 30,000 mg/dL. The only phenotype that we observed in the knockout mice was severe hypertriglyceridemia.

The finding of hypertriglyceridemia put a new spin on GPIHBP1 and shifted thinking away from a possible role in reverse cholesterol transport. We speculated that GPIHBP1 somehow affected LPL function. In early experiments, we expressed GPIHBP1 in chinese hampster ovary cells and found that the GPIHBP1 on the cell surface bound LPL avidly; the binding of LPL by GPIHBP1 could be blocked by heparin. The other important finding in our early work was that GPIHBP1 is expressed exclusively in capillary endothelial cells. In our initial paper, we proposed that GPIHBP1 was a binding site for LPL and could constitute a “platform for lipolysis” in capillaries.

Interestingly, GPIHBP1 is found only in the smallest capillaries. As soon as a capillary increases in size by 50% to become the smallest venule, the expression of GPIHBP1 disappears. GPIHBP1 is not found in endothelial cells of veins or arteries.

Subsequent studies revealed that GPIHBP1 is expressed on both the luminal and basolateral face of capillary endothelial cells. The next step for our research group was to test whether GPIHBP1 might be the “long-lost” molecule that transported LPL across endothelial cells to the capillary lumen. To make a long story short, GPIHBP1 is the LPL transporter. GPIHBP1

efficiently transports LPL across cultured endothelial cells; it also transports a GPIHBP1-specific antibody across endothelial cells. Transport was also evident in in vivo studies; GPIHBP1 transported a GPIHBP1-specific antibody across capillary endothelial cells in skeletal muscle and elsewhere. Interestingly, GPIHBP1-mediated transport across capillaries is bidirectional.

The transporter function of GPIHBP1 was also quite evident when we examined LPL localization in wild-type mice and GPIHBP1 knockout mice. In wild-type mice, nearly all of the LPL in tissue sections was located on capillary endothelial cells (mirroring the localization of GPIHBP1). LPL was not present on the endothelium of arteries or veins. In GPIHBP1 knockout mice, Dr Loren Fong, Dr Anne Beigneux, and coworkers showed LPL was mislocalized to the interstitial spaces, and none was present along the luminal surface of capillaries.

For decades, textbook dogma held that LPL is bound to heparin sulfate proteoglycans (HSPGs) on the glycocalyx coating of capillaries. At the current time, that dogma is, at least as far as I am concerned, “out the window.” LPL is bound to GPIHBP1 on capillaries, not HSPGs. However, it is still possible that HSPGs could play a role in the initial binding of LPL within the interstitial spaces. The LPL that is found within the interstitial spaces of GPIHBP1 knockout mice is tightly bound, presumably attached to HSPGs, and can be released by heparin. In wild-type mice (and humans), we suspect that the LPL secreted by myocytes and adipocytes is initially bound to HSPGs surrounding the parenchymal cells but is quickly transferred to GPIHBP1 on endothelial cells. We believe that GPIHBP1 has a very high affinity for LPL, higher than the affinity of HSPGs for LPL, enabling capillary endothelial cells to “sweep up” all of the LPL secreted by parenchymal cells.

GPIHBP1 binds LPL avidly, but it does not bind endothelial lipase or hepatic lipase. Interestingly, although GPIHBP1 was initially identified as an HDL-binding protein, we found that it has little ability to bind to HDL. We don't fully understand the discrepancy. We know that GPIHBP1 binds to apo-AV and LPL. We speculate that the HDL used by Ioka and coworkers might have contained small amounts of LPL or apolipoprotein AV.

Dr Brown: Is there LPL on HDL?

Dr Goldberg: We didn't find much LPL on HDL when we assessed postheparin LpL from humans. By gel filtration, we found that most LpL was associated with large LDL, the product of VLDL lipolysis. But, if you provide a subject with a large oral triglyceride bolus (we used ice cream) you would find it on the triglyceride particles also.

Dr Brown: Is there a good theory as to how GPIHBP1 manages the endothelial barrier? Does it carry the lipase through the gaps between endothelium or could it actually go through the cytoplasm?

Dr Young: No, we don't think that GPIHBP1 and LPL move past gaps between endothelial cells. We have published some pretty good electron micrographs showing that GPIHBP1 and LPL move across capillary endothelial cells in vesicles. One of the interesting things about GPI-anchored proteins is that they are clustered on the surface of the cells by

multivalent ligands, for example, antibodies. And when they cluster, they tend to move to caveolar-like invaginations on the surface of cells. I suspect that chylomicrons have the capacity to cluster LPL–GPIHBP1 complexes in caveolar-like invaginations on endothelial cells. Such a phenomenon might improve the efficiency of lipolysis.

Dr Brown: Is GPIHBP1 important in causing chylomicrons to stop along the capillary endothelium, so that lipolysis can proceed?

Dr Young: Yes, GPIHBP1 is crucial for lipoprotein margination in capillaries. In recent studies, Dr Loren Fong and coworkers found that GPIHBP1, and more specifically the LPL–GPIHBP1 complex, is responsible for the margination of triglyceride-rich lipoproteins. In the absence of GPIHBP1, triglyceride-rich lipoproteins never stop in capillaries and instead simply “flow on by” in the bloodstream. The involvement of the LPL–GPIHBP1 complex in the margination of triglyceride-rich lipoproteins is a new concept for the field. In the past, many had suggested that lipoproteins marginate along capillaries because of electrostatic interactions between the glycocalyx and positively charged apolipoproteins on lipoprotein particles.

Dr Brown: What do we know about mutations in GPIHBP1 causing hypertriglyceridemia in humans?

Dr Young: Yes. Over the past few years, several groups have shown that GPIHBP1 mutations cause severe hypertriglyceridemia. In most cases, the responsible mutations have been missense mutations involving conserved amino acids in GPIHBP1's Ly6 domain, but there have been several exceptions. One GPIHBP1 missense mutation was located in the carboxyl terminus of the protein and likely interfered with the addition of the GPI anchor. Also, several groups described large genomic DNA deletions encompassing the entire GPIHBP1 gene. Thus far, no one has described a clinically significant mutation in GPIHBP1's acidic domain.

Dr Anne Beigneux and coworkers have shown that the Ly6 domain missense mutations causing hypertriglyceridemia abolish the ability of GPIHBP1 to bind LPL. Interestingly, many of the mutations in the Ly6 domain have involved conserved cysteines that are crucial for establishing the 3-fingered structure of the Ly6 domain. To our surprise, most of the cysteine mutations have little effect on the trafficking of GPIHBP1 to the cell surface. However, the cysteine mutations leave an unpaired cysteine and lead to inappropriate intermolecular disulfide bonds, resulting in GPIHPB1 dimers and multimers. That is important for disease pathogenesis because only GPIHBP1 monomers, and not GPIHBP1 dimers and multimers, are capable of binding LPL.

Dr Brown: Are there any LPL mutations that block the ability of LPL to bind to wild-type GPIHBP1?

Dr Young: Yes. We have studied 2 LPL missense mutations, both identified initially in hypertriglyceridemia patients, which abolish LPL's ability to bind to wild-type GPIHBP1. One was a C418Y mutation; the other was an E421K mutation. Both are located in the

carboxyl terminal portion of LPL; neither affects LPL catalytic activity. Thus, rare LPL mutations cause hypertriglyceridemia by abolishing LPL's capacity to bind to GPIHBP1.

Dr Brown: So far, we have discussed the 2 major functional proteins that are essential for full function of LPL action as it exists on the endothelium awaiting the lipoprotein substrate. There are at least 2 proteins that arrive from the liver and intestine with the triglyceride-rich lipoproteins, namely apoC2 and apoA5. And I guess the simplest of these is APOC2, which seems to directly interact with both lipase and substrate. What do we know about that functionality?

Dr Young: How apo-CII activates LPL is not clear. There are discrepant findings in the literature about the region of the LPL molecule that binds apo-CII (or is relevant to apo-CII activation). I don't think that this issue has been settled. In contrast, more is known about the portions of the apo-CII molecule that are required to activate LPL.

Dr Brown: I agree. The interesting part is that when it is sufficiently pure, it activates at very low concentrations in vitro but with very large amounts in artificial substrates, it can inhibit. From transgenic mouse models, it also appears to inhibit lipase actions but again at very high plasma concentrations.

Dr Goldberg: If you review the apoC transgenic model, CI, CII, and CIII overexpression will cause hypertriglyceridemia. I suspect that this occurs because the additional apoCs coated the lipoprotein surface and prevent the movement of some core triglyceride to the lipoprotein surface.

Dr Brown: With apoC2, the activation is maximal and stable across very wide boundaries of concentration. Its inhibition is probably nonspecific, spacial interference at high surface concentrations on the lipoprotein. The important issue for all mammals is that apoCII appears to be essential for lipase action, correct? Without that protein, we see severe hypertriglyceridemia that presents with evident deficiency of lipase activity. We seem to have a mystery remaining around apoA5. What does it do, why is it so important?

Dr Goldberg: There is not a whole lot of apoA5 in the circulation; less than 1 A5 on each lipoprotein. How could this possibly modulate triglyceride clearance? Steve's observation is that the A5 helps to anchor the particles to the heparin, LpL, and perhaps it bounces off and goes onto another lipoprotein afterward.

Dr Young: We have reported that apo-AV binds to GPIHBP1, which is not a huge surprise because apo-AV, like LPL, has a strong heparin-binding domain rich in positively charged amino acid residues. GPIHBP1 has this strong acidic domain, so it makes sense that GPIHBP1 would interact with apo-AV. The interaction of apo-AV with GPIHBP1 appears to involve only GPIHBP1's acidic domain and not the Ly6 domain. A mutation in the Ly6 domain that abolished LPL binding had little impact on apo-AV binding. At this point, we don't know whether the ability of apo-AV to bind to GPIHBP1 is physiologically important.

More work is needed to understand the role of apo-AV in lipolysis. Some data have suggested that apo-AV increases LPL activity. For example, when LPL is bound to HSPGs

on 96-well plates, adding apo-AV has been reported to accelerate triglyceride hydrolysis. So apo-AV may have a direct effect in “speeding up” lipolysis.

Dr Brown: This seems to be evidence for its residing as a component of the lipase complex on the endothelium?

Dr Young: That is possible. In my opinion, much more work needs to be performed to understand the importance of apo-AV (and other regulatory apolipoproteins) on the efficiency of lipolysis. Thus far, most of what we know about the activity of LPL is based on biochemical studies of purified LPL preparations. However, we know from immunohistochemistry studies that virtually all of the LPL in tissues is tightly bound to GPIHBP1 on the surface of capillaries. In the future, more work is needed to understand the impact of GPIHBP1 binding on LPL activity. And after that, the field needs to carefully assess the impact of apo-AV and apo-CIII on the activity of the LPL–GPIHBP1 complex.

Dr Brown: Have you tried attaching GPIHBP1 with bound lipase to a solid matrix and testing other potential activators or inhibitors in such a system?

Dr Young: We have often talked about performing those sorts of studies. We know that soluble forms of GPIHBP1, when immobilized on agarose beads, bind LPL avidly. The next step is to use these immobilized LPL–GPIHBP1 complexes to assess the roles of other key proteins in lipolysis (apo-AV, apo-CIII, and ANGPTL4) in modulating the activity of GPIHBP1-bound LPL.

Dr Goldberg: I'm not sure I agree with where the lipolysis occurs. More than 20 y ago, Olivecrona and colleagues showed that more LpL is present in the blood postprandially. And so, actually there was a controversy for a while as to how much of the lipolysis occurred at the vessel wall and how much of it occurred after the LPL came off the blood vessel wall.

The release of LpL led to the idea that it could work not only as an enzyme but also as a receptor ligand that helped to clear chylomicrons via interaction with receptors such as LRP1. I wonder whether fatty acids also disrupt the LpL GPIHBP1 interaction.

Dr Young: We have not directly tested the effect of free fatty acids on LPL–GPIHBP1 binding. We have proposed those sorts of studies in grant applications, but we have not yet performed these studies.

Dr Brown: We have covered the molecular basics that are currently understood. Let's go to the clinic. What can we tell about the biochemistry from looking at the patient? Are there differences in clinical syndromes in patients who present with hypertriglyceridemia with genetic dysfunction of LMF-1, or GPIHBP1 or the LPL itself?

Dr Young: Homozygous deficiency of LPL, GPIHBP1, and LMF-1 lead to severe hypertriglyceridemia in humans. All can be associated with the usual clinical findings of familial chylomicronemia (eruptive xanthomas, lipemia retinalis, and pancreatitis). Heterozygous deficiency in GPIHBP1 does not appear to cause increased plasma

triglyceride levels. In contrast, heterozgyous LPL deficiency clearly leads to mild–moderate increases in plasma triglyceride levels.

Dr Goldberg: You're not getting much of an answer because we're not sure of the difference in these rare forms of genetic hyperchylomicronemia. The heterozygous LpL deficiency more often is associated with low HDL; the triglyceride levels tend to increase later in life. The heterozygotes have a risk of chylomicronemia and pancreatitis with stresses such as diabetes and pregnancy.

Dr Brown: What are the usual physical findings on the initial examination of a patient with severe LPL dysfunction?

Dr Young: Patients with severe hypertriglyceridemia often present with eruptive xanthomas, lipemia retinalis, hepatosplenomegaly, and abdominal pain from pancreatitis. Occasionally, there are signs of dysfunction in the central and/or peripheral nervous systems. Hepatosplenomegaly is common. The pancreatitis can be life threatening. For that reason, lowering plasma triglyceride levels with a very low–fat diet is very important.

Dr Goldberg: In the acute pancreatitis situation, most endocrinologists would recommend the patients to remain without food or fluids by mouth and reduce hepatic triglyceride production by using low-dose insulin to block adipose lipolysis.

Dr Brown: What are the key diagnostic tests that might clarify the diagnosis as lipase functional deficiency?

Dr Young: The diagnosis of LPL deficiency is generally made by documenting low levels of LPL activity or mass in the postheparin plasma. GPIHBP1 deficiency also leads to very low levels of LPL, both in the preheparin and postheparin plasma. However, over the past few years, these sorts of measurements have been performed infrequently. Most often, these disorders are now diagnosed with genetic testing. The data in the literature suggest that homozygous LPL deficiency is substantially more frequent than GPIHBP1 deficiency.

Dr Goldberg: I agree except that insurance coverage of genetic testing is uncertain.

Dr Brown: What types of genetic testing might make the disorder more easily managed?

Dr Goldberg: There are many mutations in LpL and unless the patient is from a well-established genetic pool such as French Canadians, without sequencing, the defect might be missed. I suspect that there will also be multiple defects yet to be discovered in GPIHBP1.

Dr Brown: Is genetic testing helpful in managing any of the specific molecular defects we have discussed? In the clinical management of these disorders, what are the fundamental prescriptions for diet or medications?

Dr Goldberg: The diet and lifestyle prescriptions are similar for most forms of severe hypertriglyceridemia. The most severe patients are also given medium chain triglyceride oils for cooking. I try to get patients who are less than diligent with their diet to also take orlistat to block pancreatic lipase. This is like a forced low-fat diet.

Dr Brown: There is available in Europe, a genetic therapy called Glybera, which is designed to provide the lipase gene in a vector. Is this successful in LPL deficiency?

Dr Goldberg: Yes, it appears to be at least in the short term. However, longer term effectiveness still needs to be studied.

I want to thank you both for bringing us up to date on this fascinating topic of LPL function. We thought we fully understood this enzyme system many years ago. You have provided the new story of biological complexity that has explained some of the clinical and physiological disconnects in the genetic “hyperchylomicronemic syndromes” and has pointed the way to further questions that deserve much study.

Biography

Dr Brown

Dr Goldberg

Dr Young

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