LIPID CASE 243: Pseudohypertriglyceridemia

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Lipidaholics: Welcome! What a case I have for you this week. It is a disease that until you know it exists you will miss it every time. However once you are taught it or have a case yourself, you will never miss it again. I've presented this to a bunch of smart lipid buddies and all struck out. Only one colleague got it and he is not an MD but rather a PhD. Let's see how you do. Please read the history carefully and scrutinize the laboratory findings for some obvious clues.

I received this case from a clinician in San Antonio who did make the diagnosis and sent it my way to see if I could also do so: fortune was with me and I correctly nailed it. Here we go: a 58 year old white male, hypertensive, frequent blood donor with a long history of elevated TG. He uses alcohol 2-3 times a week. Weight 164, Height 66 inches, BMI is 26.5. Only current medications are Crestor 10mg daily, lisinopril 10mg daily and aspirin 81 mg. This patient has been treated for years by cardiologists for "high triglycerides." Statins did not help. Niacin, fibrates, Omega-3s, etc also failed to reduce the high TG. Even on a low carbohydrate diet his TG actually went up. None of his triglyceride treatments had any effect on his triglyceride levels! Over the last several years only once did the TG ever come back as perfect with a reading of 55 mg/dL: that assay was done by Berkeley Heart Labs. That excellent level was looked at very skeptically as multiple tests from other conventional labs and LipoScience always revealed a very high TG.

Lab analysis:

(Fasting) TC = 127  LDL-C = 10  TG = 381  HDL-C = 41  Non-HDL-C = 86

Total LDL-P = 1064 nmol/L (perfect – at the 20th percentile population cutpoint)
Small LDL-P = 927 nmol/L (~ 60th percentile)
Large LDL-P = 132 nmol/L (low)
Large VLDL-P = 0 nmol/L (perfect)
Medium VLDL-P = 3.3 nmol/L (low)
Small VLDL-P = 29.1 nmol/L (low)
IDL-P = 5 nmol/L (normal)
Total HDL-P = 41 umol/L (excellent)
Large HDL-P = 5.0 (slightly low 30th percentile)
Medium HDL-P = 3.8 (50th percentile)
Small HDL-P = 32.2 (high 95th percentile)

glucose 90, A1c 5.7  TSH 2.27  CPK 117 Urine Micro albumin negative
DAYSPRING ANALYSIS

Pretty amazing case: With the information provided I’ll assume he has moderate CHD risk and is seemingly well below his LDL-C and non-HDL-C goal. Likely his LDL-P was significantly higher prior to the Crestor Rx. He is also below his LDL-P goal for a moderate risk person (~1300 nmol/L). We cannot do Framingham Risk scoring as he is on medication and FRS is only a validated tool in drug naive patients. So what conclusions can one draw from the high TG level? Also it is interesting that he has never been able to find a physician using the standard TG-lowering therapies capable of reducing his TG.

First let’s review a little TG biochemistry. Triglycerides (TG) should actually be called triacylglycerols (TAG). TG or TAG are molecules with a glycerol (a carbohydrate) backbone to which are attached three acyl groups. Phospholipids (PL) are also derived from glycerol. If glycerol is not used to synthesize TG or PL it enters gluconeogenesis or glycolysis pathways. It does that by being converted into glycerol-3-phosphate using an enzyme called glycerol kinase. Acyl groups are derived from hydrolyzed fatty acids (which are carboxylic acids or COOH). When an acyl group is attached to an -OH on a glycerol, the process is called esterification. Esterification of glycerol will produce TG or PL. Glycerol with one acyl group is a monoacylglycerol (MAG), those with 2 acyl groups a diacylglycerol (DAG) and of course those with 3 a triacylglycerol or triglyceride molecule. There are very specific enzymes involved in each of the three esterification steps. The most well known is the enzyme that converts DAG to TAG and it is called diacyl-glycerol transferase (DGAT). Drugs that inhibit DGAT would reduce TG assembly (fibrates, niacin, N-3 fatty acids). Three FA acyl groups supply considerable energy and thus TG serve as an energy supplier for muscle or energy storage molecules in adipocytes. Enzymes capable of de-esterifying glycerol esters (TAG, DAG) are called lipases. The most potent triglyceridases (a lipase that hydrolyzes TG) that humans have are lipoprotein lipase (LPL) primarily expressed in adipocyte and muscular vascular beds and hormone sensitive lipase (now called triglyceride lipase) expressed in adipocytes. The lipases ultimately convert TG or TAG to FA and MAG. This de-esterification of the molecule is required as TG as a whole molecule cannot be absorbed into the enterocyte cell membranes or those of other cells throughout the body: of course FA can pass through membranes using fatty acid binding proteins. TG present in food are hydrolyzed almost immediately by salivary, gastric and ultimately pancreatic lipases. In the plasma LPL hydrolyzes the TG carried in the TG-rich lipoproteins (chylomicra and VLDLs). Please refer to the figure below showing TG structure. Each of the carbons in the glycerol molecule are numbered using the “stereospecific numbering (sn) system.” Thus one FA acyl group is attached to the -sn1 position, the second (middle carbon) to the -sn2 position and the third to the -sn3 position. Believe it or not the positioning of the acyl groups to the various sn positions has great biologic importance but that is beyond this discussion. Providers are not taught to consider which FA acyl groups are in a given patients TG. Do your TG carry 3 saturated fats (hope not), monounsaturated?, polyunsaturated? N-3FA? or combinations of all. As one might imagine there are thus multiple types of possible TG molecules. A TAG mixture with just five different fatty acids can therefore exist as 105 different TAG molecular species (TAG-MS) according to differences in positional composition. What would you call a TG that consists of a saturated fat (say palmitic acid: an 16 carbon fat with no double bonds), a monounsaturated fat (say oleic acid: an 18 carbon fat with one double bond at the n9 position) and a polyunsaturated fat (say linolenic acid: an 18 carbon fat with three double bonds, the first of which is at the 3
position)? That mouthful would be: 1-palmitoyl 2-oleoyl 3-linolenoylglcerol or in short-hand POL (where P is palmitic acid, O is oleic acid and L is linolenic acid).

Conventionally when describing at which carbon the first double bond exists we count backwards from the terminal methyl group (end) of the FA acyl chain - so if the first double bond is at the third carbon from the end it is called an omega-3 FA (omega being the last letter in the Greek alphabet) or as is now more correct an n3 FA. Omega-3 does not mean the FA has three double bonds (although it might - it means the first double bond is at the 3rd carbon). Oleic acid has its first double bond at the 9th carbon and is an omega-9 or N9 FA. Linoleic acid (not to be confused with the n3 FA linolenic acid mentioned above) is an omega-6 or n6 FA. EVERYONE GOT ALL THAT???? (Nutrition Research Reviews 2009;22:3–17).

![Diagram of FA acyl chain position](image)

One may quickly assume because of the high TG, we may be dealing with insulin resistance, but he does not meet all of the metabolic syndrome criteria (has hypertension and seems to have high TG - but no other criteria. Other parameters that suggest IR is a mild reduction of large HDL-P and a slight increase in small LDL-P. VLDLs look great and usually with IR there is increased large VLDL-P. Glucose is 90 and A1C is above 5.

Note the LDL-P is perfect but then why is the LDL-C so very low? Is it possible to have ~1000 nmol/L of LDL-P with an LDL-C of 10 mg/dL. At first glance, the obvious answer seems to be that his LDLS are not only small they have an abnormal core composition and are carrying TGs. If LDLs are cholesterol-depleted it will take more LDLs to traffic a given level of LDL-C compared to LDL particles are not cholesterol depleted. The conditions that cause LDL particles to be cholesterol-depleted are small size, abnormal core composition with a preponderance of TG (normally an LDL has 80% cholesteryl ester and 20% TG) an we are also now finding out that statin therapy can in some folks deplete the LDL core of its CE creating a cholesterol depleted particle. One might surmise that all three may be at play in this patient. The patients with the
most cholesterol depleted particles are those with small particles and increased TG in the core of those particles. However, the LDLs in this man would have to be EXTREMELY cholesterol-depleted LDL particles. I am becoming a “doubting Thomas.”

Let’s take a close look at the LDL-C. What if the reported (calculated) LDL-C is a lab error? How could one make an error with LDL-C when using the classic Friedewald formula?

\[
\text{LDL-C} = \text{TC} - [\text{HDL-C} + \text{VLDL-C}] \text{ where VLDL-C is also a calculation: } \text{TG}/5.
\]

In this case
\[
\text{LDL-C} = 127 - [41 - 381/5] = 127 - [41 + 76] = 121 - 117] = 10 \text{ mg/dL}
\]

Thus is LDL-C has to be 10 mg/dL!!!! Everyone agree? Direct LDL-C assays are totally non-standardized with values all over the place and in general should never be relied on but in this case I suspect the direct LDL-C would not be 10 mg/dL. The LDL-C of 10 mg/dL (the 2nd percentile population cutpoint is discordant (does not agree with) with the LDL-P of 1000 nmol/L (approaching the 20th percentile population cutpoint).

The non-HDL-C of 86 (2nd percentile) is also discordant the LDL-P (20th percentile). Maybe in this case we should discard the LDL-C and the non-HDL-C or should we ignore the LDL-P. How do we explain this disconnect? However in a patient with this degree of risk both the lipid and lipoproteins concentrations are at goal so who really cares? One of the nice things about non-HDL-C is that TG do not figure into the calculation (TC – HDL-C) as they do with LDL-C.

Another warning signal that something bizarre is going on is why is the HDL-C normal in the face of such a high TG? Typically with such high TG, cholesteryl ester transfer protein (CETP) moves TG for cholesteryl ester (CE) from TG-rich lipoproteins like VLDLs to the typically CE-rich HDLs and LDLs. As HDLs lose their CE and instead fill with TG, HDL-C usually drops. One could theorize in this case the HDL-C originally was low but maybe the Crestor helped raise it -- yet with a TG of 381 mg/dL, HDL-C should still be low. So lipidologists should be getting suspicious that something very strange is at play: a totally not believable LDL-C and an unusually normal HDL-C in the face of very high TG. When labs tests do not make sense, maybe, just maybe the results are not correct.

When I first saw the case my first train of thought was the patient has TG-induced cholesterol depleted particles which can help explain high LDL-P in the face of a normal or even low LDL-P. Then, as always I carefully looked at every single NMR parameter. I always stress that you should look at VLDLs especially when TG are abnormal. As soon as I saw that the VLDL-P in this patient were extremely low I knew the hypertriglyceridemia was a false positive or as it is called pseudohypertriglyceridemia. As almost always coronary risk revolves around the lipoproteins that traffic the lipids. You must ask yourself, what particles are carrying the large amount of TG???? - Normally in someone with a TG of 381 mg/dL, we see either lots of VLDL particles or increased numbers of large VLDL-P. Yet in this case all of the VLDL-P, IDL-P and LDL-P numbers are very good and indeed with respect to the VLDLs the numbers are on the “very low” side -- so you have to figure out where the heck are the TG hiding???? They have to be somewhere? Yet, there are very few IDLs and there is no way normal numbers of LDLs and HDLs are trafficking 381 mg/dL of TG. Could this be a false positive TG level? If so what causes that? The answer is indeed a condition called
Pseudohypertriglyceridemia -- in other words the TG is reported by the lab as very high but in reality it is not. It seems like only a few lipidologists have heard of PSEUDOHYPERTRIGLYCERIDEMIA. (Clin Chem 1995;41:619-620 and Postgrad. Med. J. 2008;84:552-554 as well as the paper cited below).

Lipidologists should have some basic knowledge of how labs assay various lipid and lipoprotein concentrations. So when you send serum off to the lab how are TG analyzed? Lipoprotein lipase is added to the serum and the de-esterification process begins. Before you know it the TG are changed into collections of FA and glycerol molecules. The lab runs an assay on glycerol concentration and report the glycerol level as TG levels. Obviously the more TG molecules a patient has the more glycerol will be generated in the lab analysis. In essence labs measure the glycerol component not the FA of the TG molecule. Unfortunately there are a few patients out there who have very high glycerol levels. If you take their serum and analyze it for TG, the reported level will be extremely high and have no correlation with what their actual TG level is. If you have a patient with high glycerol levels who have perfect amounts of actual TG molecules in their plasma, the reported TG level will be very high and it is in effect a false positive TG level or pseudohypertriglyceridemia. Wow: The lab test we use to measure TG is in fact a test that measures glycerol. Here are the actual TG assay steps performed by Atherotech (almost all other labs do something similar). The glycerol is phosphorylated by ATP with glycerol kinase to produce glycerol-3-phosphate and ADP. Glycerol-3-phosphate is oxidized to dihydroxyacetone phosphate by glycerol phosphate oxidase producing hydrogen peroxide. In a color reaction catalyzed by peroxidase, the H2O2 reacts with 4-aminoantipyrine to produce a red colored dye. The absorbance of this dye is proportional to the concentration of TG in the serum.

Guess what: there is a human condition resulting in high plasma glycerol levels and it is called glycerol kinase deficiency (GKD) which is an X-linked recessive disorder. There are two types, an isolated form and a complex form. The clinical and biochemical phenotype of isolated GKD may vary from a life-threatening childhood metabolic crisis to asymptomatic adult "pseudohypertriglyceridemia", resulting from hyperglycerolemia. The clinical manifestations such as an altered consciousness and seizures in isolated GKD patients can be classified as glucose deprivation symptoms precipitated by catabolic situations such as poor oral intake, intercurrent illness or exercise. (italics are from J. Inherit. Metab. Dis. 23 (2000) 529-547). Normally as a substrate for gluconeogenesis, glycerol is converted into glycerol-3-phosphate by an enzyme called glycerol kinase. Patients who have a glycerol kinase deficiency have very high plasma glycerol levels and of course falsely reported high TG levels. To make the diagnosis glycerol can be assayed in the urine (glyceroluria) and it would be quite high.

The following labs do standard triglyceride assays (reporting total triglycerides, not using blanked triglycerides) using lipoprotein lipase as the first step to generate glycerol and free fatty acids: Quest, LabCorp, all office labs, and LipoScience and Atherotech (VAP). Interestingly, Berkeley does a "blank" in their assay, which controls for any free glycerol which is present before lipolysis. Remember in this case the patient's Berkeley Panel reported a TG level of only 55 mg/dl.
Let's go back and recalculate the LDL-C using the correct TG level reported by Berkeley Labs of 55 mg/dL instead of the one using the erroneous TG to calculate VLDL-C.

\[ \text{LDL-C} = \text{TC} - [\text{HDL-C} + \text{VLDL-C}] \]

Now let’s plug in a TG of 55 instead of 381 mg/dL. Wow the VLDL-C changes from 76 to 11. Look what happens to LDL-C!

LDL-C = 127 - [41 - 55/5] = 127 - [41 + 11] = 127 - 52 = 75 mg/dL.

Thus what seemed to be at first glance (LDL-C of 10) a case of hypobetalipoproteinemia is not because the LDL-C is actually 75, not 10 mg/dL. Of course the LDL-P was never at hypobetalipoproteinemia levels. The LDL-C and LDL-P are in reality are a lot less discordant than they were at first glance (but they are not perfectly concordant showing why we should always rely of LDL-P). Once you realize the TG in this case are in fact a lab error, which can be ignored, proper management returns to normalizing LDL-P. Well, the LDL-P is at goal on Crestor so nothing else is needed. Think of all the wasted time and effort over years in repeating lipid and lipoprotein levels and subjecting this man to every lipid drug on the planet likely in high doses. Talk about potential toxicity, cost and no benefit!!

So with respect to LDL-C and TG, not everything is as it may seem. IT IS THE PARTICLES!