

Lipoprotein Pathophysiology

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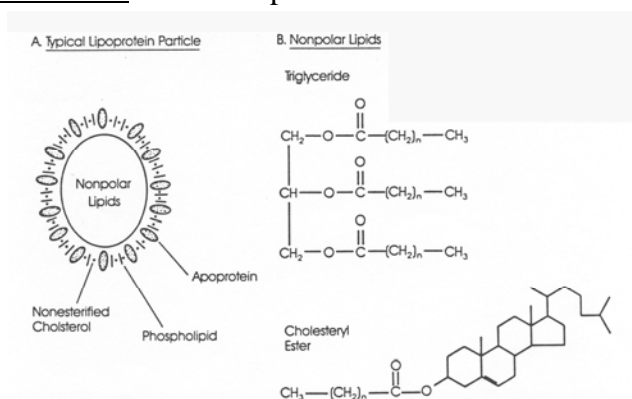
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Objectives:

1. To understand the physiology of lipoproteins and their various components.
2. To understand the pathophysiology of lipoproteins and how these abnormalities result in various lipid disorders.

Introduction:

Lipoprotein physiology is the study of fat transport through the aqueous environment of the body. Fats serve primarily two functions in the body. First, phospholipids and cholesterol are the major structural components of all cell membranes. Second, triglycerides and free fatty acids are the major energy sources of the body. It appears that the preferred direction of transport is: (1) for triglycerides to be transported from the gut and liver (where they are absorbed or manufactured) to the muscle (where they are utilized for energy) and fat tissue (where they are stored); and (2) for cholesterol to be transported from all the other tissues back to the liver. It should be remembered



that even though no tissue in the body can degrade cholesterol, essentially all tissues make enough cholesterol to be self-sufficient.

Therefore, the major problem that most tissues face is an excess of cholesterol, not a deficiency. In fact, it is difficult to find a unique physiological function for LDL. It appears to be the end-product of VLDL metabolism, which must be removed from the plasma before it causes any serious damage to the only tissue with which it has intimate contact, the vascular wall.

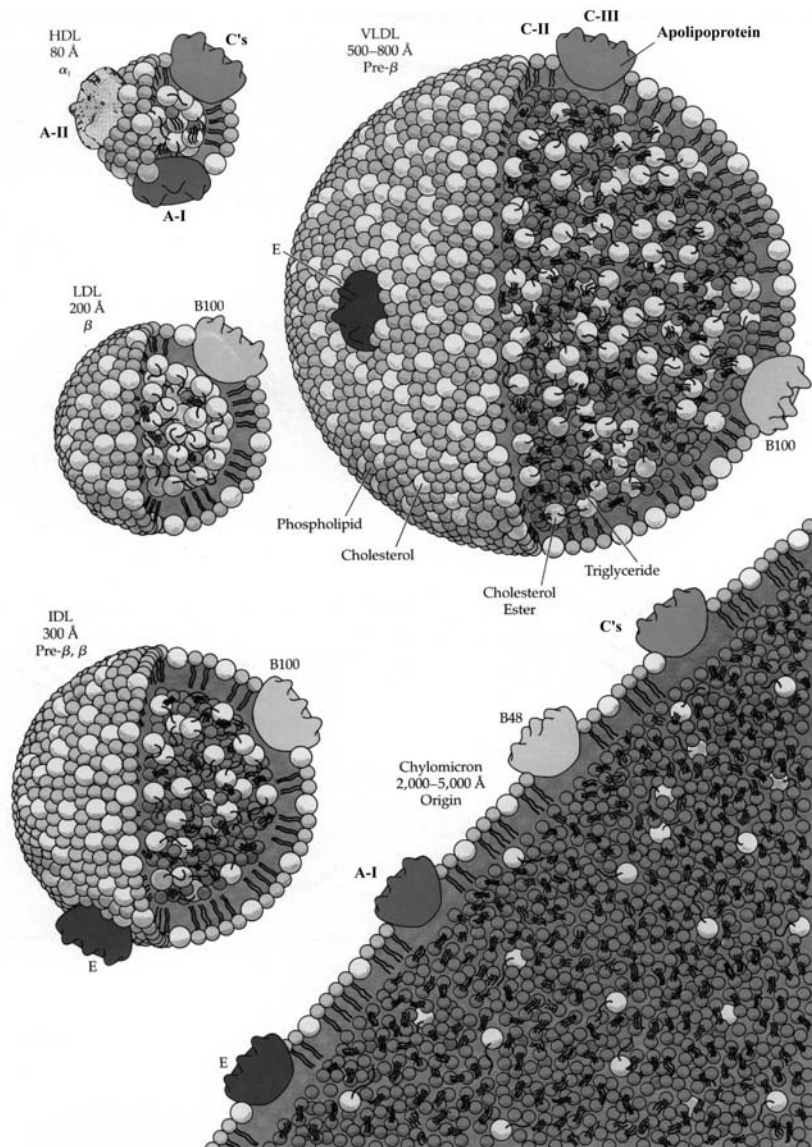
Cholesterol and polyunsaturated fatty acids also perform a variety of other functions (such as serving as substrates for the production of hormones and second messengers), which will not be discussed in any detail in this chapter. Phospholipids, free cholesterol, cholesterol esters, and triglycerides are carried through the blood in lipoprotein particles. These particles allow these fats to be dispersed into small enough lipid droplets so that they can easily circulate through the vascular system. Without lipoproteins, these fats would coalesce into grease balls that would clog even the largest blood vessels. The other major components of lipoproteins are the apolipoproteins. These proteins serve three functions. First, some apolipoproteins influence the structural characteristics of the lipoprotein particle (e.g. size, hydrophobicity, etc). Second, some act as enzyme cofactors, which activate or inhibit the various enzymes that control lipoprotein metabolism. Finally, some apolipoproteins are ligands for cellular receptors, thus dictating their receptor-mediated cellular interactions.

Lipoprotein pathophysiology is the study of the genetic or environmental alterations in the various steps of lipoprotein metabolism. A prerequisite for understanding lipoprotein pathophysiology is a thorough understanding of normal lipoprotein physiology. An appropriate

review should be made if needed. All of the major Internal Medicine or cardiology textbooks have excellent chapters on this subject. Most of the genetic lipoprotein abnormalities are due to mutations of the apolipoproteins, their receptors, or the intracellular (especially liver, intestinal, and adipose tissue) and extracellular (LPL, Hepatic Lipase, LCAT, CETP) enzymes, which control lipid metabolism. Most of the environmental and secondary causes of lipoprotein pathology are the result of over or under production of one or more of the lipoprotein components. Of course, there are many situations in which the genetic and environmental factors interact. For example, an individual may have a subtle genetic defect in LPL, which only becomes clinically evident (as severe hypertriglyceridemia) when he/she becomes diabetic.

When conceptualizing lipoprotein pathophysiology, it is very important to remember that it is a system in motion with the players constantly changing their characteristics and personalities. Thus, even a mild defect at a critical point in the system can be amplified into a major lipid aberration. Furthermore, the inability of one of the players to exit the system will generally produce more dramatic alterations in the static measurements of lipids than will the over-production of one of the lipid components.

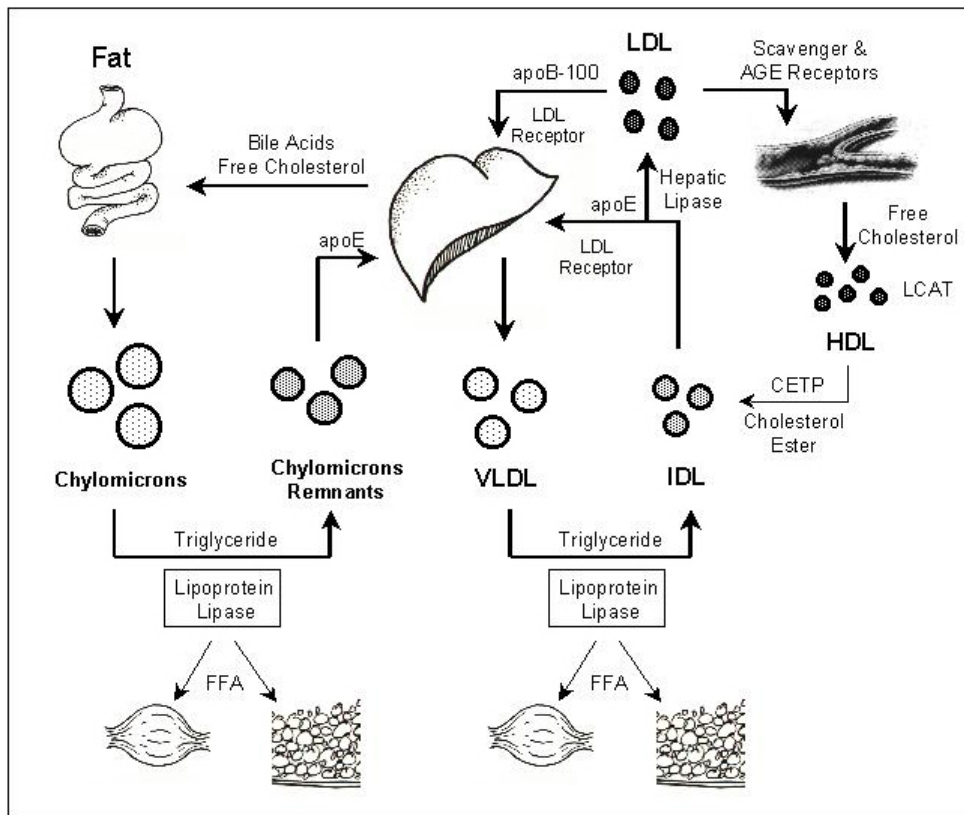
Each step of lipoprotein metabolism will be discussed in these notes and possibly in the lectures. Relatively common examples of pathological defects will be given. These discussions will not be an exhaustive chronicle of all known abnormalities of lipoprotein metabolism, but should provide you with an understanding of how a variety of etiologies can produce lipid disorders. The suggested reading assignment will provide additional details that may help you to understand the particular diseases that are discussed. It will be very important to understand the mechanism of



The differences among the five types of lipoproteins reflect their varying roles in lipid transport. For example, chylomicrons transport fat that is absorbed through the intestine and therefore contain predominantly triglyceride, whereas LDL, the major carrier of cholesterol used for synthesis of membranes, bile acids, and steroid hormones, contains high levels of this lipid.

FROM SCIENTIFIC AMERICAN Medicine, 9 Metabolism, II Hyperlipoproteinemias

disease if appropriate and specific therapy is to be rendered to patients. All test questions will come from these notes.



Suggested Reading:
Harrison's Principles of Internal Medicine, Latest Edition. The first few pages can be skipped if you already have a thorough understanding of lipoprotein metabolism. This text is available in the Reserved Section in the library.

Pathophysiology: (Outline)

I) Chylomicron (CM) or VLDL Production:

- Reduced ApoB Production
- Excess ApoB Production
- Excess VLDL Triglyceride Production

II) Delipidation of CM or VLDL to CM-remnants and IDL:

- Reduced Lipoprotein Lipase activity or apoC-II levels
- Excess apoC-III

III) Decreased clearance of IDL or conversion of IDL to LDL:

- Mutations of apoE (Dysbetalipoproteinemia or Type III HLP)
- B,E receptor defects: (elevated IDL and LDL cholesterol)
- Hepatic Lipase deficiency: (elevated IDL and HDL)

IV) Clearance of LDL (elevated LDL-cholesterol):

- B,E receptor (LDL) defects
- Mutations of apoB

V) HDL Metabolism:

- Reverse Cholesterol Transport
- ApoA-I production
- Reduced conversion of HDL₃ to HDL₂ (reduced HDL-cholesterol)
- Increased conversion of HDL₂ to HDL₃ and clearance from the plasma
- Reduced conversion of HDL₂ to HDL₃ (high HDL₂ cholesterol)

Pathophysiology:

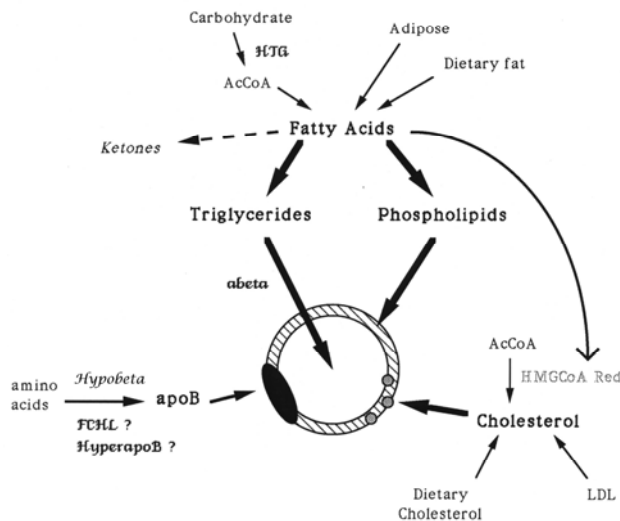
I) Chylomicron (CM) or VLDL Production:

Reduced ApoB Production

CMs and VLDL (very low-density lipoprotein) are triglyceride-rich lipoproteins (75%-95% of their core lipid is triglyceride) made in the small intestine and liver, respectively. Their primary structural protein is apoB. However, the apoB made in the intestine is smaller (B-48, i.e. 48% the size of B-100), approximately half the size of liver apoB (B-100, MW approximately 550K). The difference between these two forms of apoB is that one end of the B-100 has been clipped off during manufacture in the intestine (mRNA editing). The piece that has been deleted from apoB-48 contains the LDL receptor-binding region. Therefore, neither CMs nor their metabolic products can bind to the LDL receptor via apoB. We will later see that CM remnants are cleared via a receptor that recognizes apoE. Each CM and VLDL particle contains one and only one molecule of apoB,

and this molecule never leaves its original particle. In other words, no exchange takes place.

VLDL Production:



The assembly of CMs and VLDL begins with the synthesis of apoB in the rough ER. ApoB is so hydrophobic that it remains integrated into the membrane of the ER until it accumulates enough phospholipids and triglycerides to produce its own particle. It then sequesters additional TG and non-esterified (free) cholesterol as it grows into a mature particle in the rough ER and smooth ER. The particles are ultimately secreted via secretory vesicles after passage through the Golgi. The apoE and apoC's apolipoproteins that are vital for the

subsequent metabolism of both CMs and VLDL do not appear to attach to these particles to any great extent until after they have been secreted into the blood. They, then, rapidly acquire these apolipoproteins from HDL, which acts as an intravascular reservoir for these apolipoproteins.

Disorders of apoB synthesis have a great potential for producing lipoprotein disorders. Neither the intestine nor the liver can secrete triglyceride without synthesizing apoB. Genetic truncations of apoB synthesis lead to hypobetalipoproteinemia (which is a relatively common abnormality). These truncations are caused by mutations, which cause premature termination of apoB transcription such that the mature apoB is shorter than normal. Some common truncations are: apoB-89, apoB-75, apoB-54.8, and apoB-31. These specific numbers are not important except that they represent the percentage of the native molecule that is actually transcribed. Approximately 50 specific mutation-truncations have been identified. Truncated apoB particles are typically secreted at a reduced rate while their clearance is usually faster (but rarely slower) than normal. Heterozygotes typically have LDL cholesterol levels 25-50% of normal (half of their VLDL apoB will be apoB-100) and are asymptomatic. Most of these patients will have increased fat in the liver

and some may develop steato-hepatitis (fatty liver) due to their inability to secrete triglycerides from the liver. Homozygotes have extremely low levels of apoB-containing lipoproteins and will frequently present with fat malabsorption (causing fat-soluble vitamin deficiency), acanthocytosis (due to linoleate deficiency), prothrombin deficiency (due to vitamin K deficiency), retinitis pigmentosa (due to deficiency of vitamin A and E), and neuromuscular degeneration (also caused by deficiency of vitamin E) similar to abetalipoproteinemia (discussed below). Of course, the severity of these abnormalities depends upon the particular truncation and the level of lipoprotein dysfunction that this truncation produces. The renal tubule rapidly clears the VLDL containing truncated apoB, so there is a relative deficiency of truncated apoB vs apoB-100 in LDL. However, the truncated particles that are metabolized to remnants are usually found in the dense LDL or HDL fractions and may, therefore, be mistaken for Lp(a) if analyzed only by density patterns.

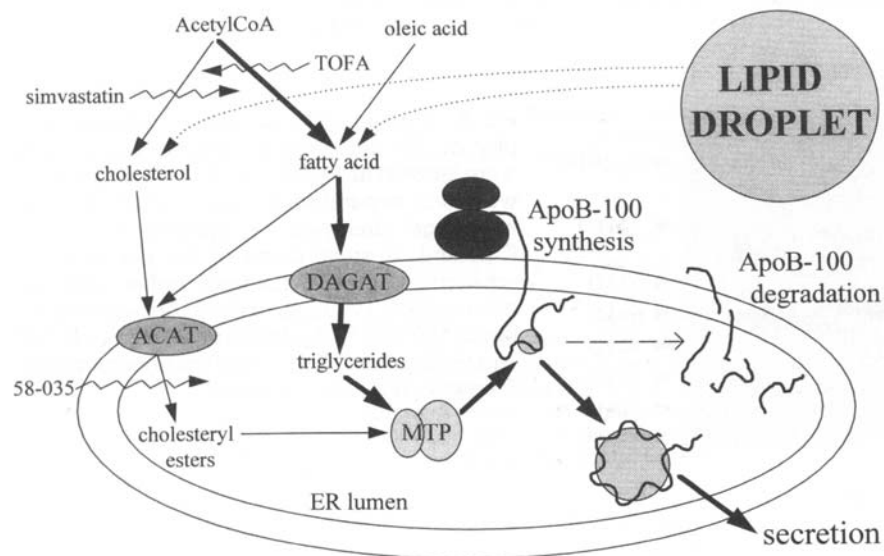
Table 1. Major apolipoproteins

Apolipoprotein	Lipoprotein association	Primary source	Functions
ApoA-I	HDL	Intestine, Liver	Structural protein for HDL, activator of LCAT binds ABCA1, SR-B1
ApoA-II	HDL	Liver	Inhibits reverse chol transport
ApoA-IV	HDL, CM, VLDL	Intestine	Unknown (satiety?)
ApoB-48	CM	Intestine	Chylomicron synthesis and secretion
ApoB-100	VLDL, IDL, LDL, LP(a)	Liver	VLDL synthesis and secretion; ligand for binding to LDL-receptor
ApoC-I	CM, VLDL, HDL	Liver	Inhibits CETP & particle removal
ApoC-II	CM, VLDL, HDL	Liver	Cofactor for LPL
ApoC-III	CM, VLDL, HDL	Liver	Inhibits LPL & particle removal
ApoE	CM, IDL, HDL	Liver Macrophages	Ligand for binding to LDL recept and LRP
Apo(a)	Lp(a)	Liver	Unknown

ApoA-I indicates apolipoprotein A-I; HDL, high-density lipoprotein; LCAT, lecithin cholesterol acyltransferase; LDL, low-density lipoprotein; CM, chylomicron; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LPL, lipoprotein lipase; and LRP, LDL-receptor-related protein.

Abetalipoproteinemia is caused by an absolute deficiency of "Microsomal Triglyceride Transfer Protein" (MTP). This protein is normally found in the endoplasmic reticulum of enterocytes and hepatocytes and is responsible for transporting triglycerides and cholesterol esters between phospholipid surfaces (membranes and lipoproteins) and controls the formation of a lipid droplet to which apoB can attach. MTP function appears to be physiologically controlled since its mRNA levels are up-regulated by high fat diets. Heterozygotes appear to make enough MTP to transport adequate quantities of lipid for chylomicron or VLDL synthesis. Therefore, this partial deficiency results in no apparent abnormalities of lipoprotein concentrations or metabolism. Abetalipoproteinemia is, therefore, a recessive trait. However, homozygous individuals have no functional MTP and, therefore, cannot assemble chylomicron or VLDL particles. This severe

deficiency results in an inability to transport triglycerides out of enterocytes or hepatocytes and, therefore, the clinical presentation and treatment of homozygous abetalipoproteinemia is very similar to homozygous hypobetalipoproteinemia except these symptoms are typically worse with abeta. The only way to clinically differentiate these two syndromes is to examine the family tree to see if the heterozygotes have low levels of apoB-containing lipoproteins.



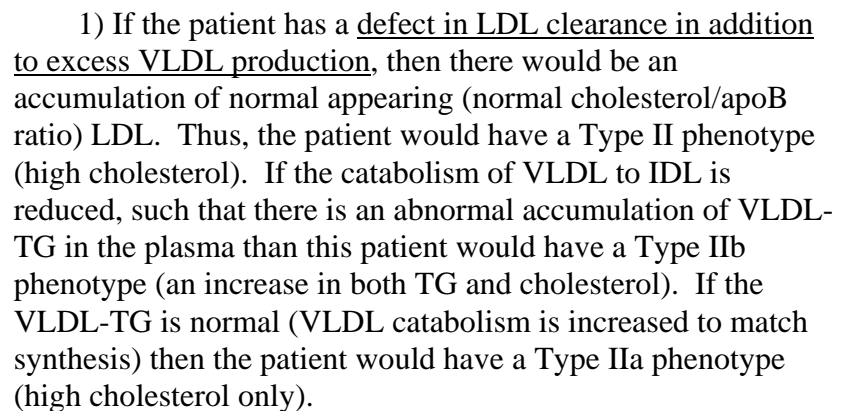
Excess ApoB Production

Two, VERY IMPORTANT genetic conditions that appear to result in excessive apoB synthesis in the liver are: Familial Combined Hyperlipidemia (FCHL) and Hyperapobetalipoproteinemia (Table 2: at end of syllabus). Both are currently defined clinically and, as such, are not mutually exclusive. In fact, it appears that patients with FCHL frequently have Hyperapobeta (but not always). Three gene clusters have been associated with FCHL, one of which is involved in apoB/LDL metabolism, thus explaining the frequent coexistence of these two disorders. It is important to understand these disorders because they are relatively common and both are associated with a high risk of developing cardiovascular disease.

FCHL is defined as a family that manifests a variety of lipid phenotypes. Some members have elevated TG (Type IV, TG = 160-800 mg/dl), some have elevated total cholesterol (Type IIa, TC > 240 mg/dl), and others have both (Type IIb). In addition, if the same individual is tested repeatedly, he/she can frequently change phenotypes. These patients have an increased number of VLDL particles but the VLDL has a relatively normal composition.

Hyperapobeta is characterized by an increased concentration of apoB in the LDL density range with a normal or low content of cholesterol, thus a “cholesterol depleted” apoB-100 LDL particle. This particle is “heavier” (more dense) and smaller than normal LDL, but can have an increased triglyceride content. It is easier to diagnose Hyperapobeta because it can be done with a single blood test (be it a very sophisticated blood test), while FCHL requires family studies for an accurate diagnosis. For example, you may see a patient with an increased number of VLDL particles, each with a normal lipid composition, and an increase in LDL apoB levels without an increase in LDL cholesterol. This patient definitely has Hyperapobeta but you cannot diagnose FCHL (even though

The key metabolic characteristic of both of these disorders is an increase in apoB synthesis (and, thus, VLDL particles) by the liver with essentially normal lipid composition of the VLDL (normal amounts of TG and cholesterol are added to each apoB molecule as the particle is constructed). The VLDL appears to be normally metabolized to LDL through IDL in this disorder (that is, no more than the usual number of VLDL particles are removed from the plasma before being converted to LDL). Thus, there are greater numbers of LDL particles being made. Three LDL conditions could result from this situation:



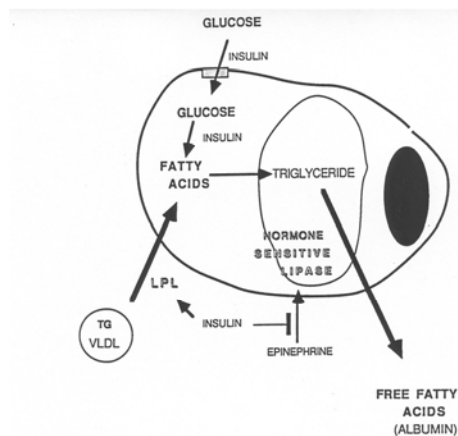
3) Finally, if there were an increase in LDL clearance that matches or exceeds LDL production than there would be normal or low concentrations of LDL apoB and cholesterol. If

FCHL and Hyperapobetalipoproteinemia are probably two of the most difficult lipid disorders to understand at this time. As we learn more about them, we will probably find that we can subdivide them into multiple, but more clearly defined, disorders that will be easier to understand and differentiate. For example, as noted above, a subset of patients with FCHL is actually heterozygous for Lipoprotein Lipase deficiency (Type I Hyperlipidemia).

Normally, apoB synthesis appears to be relatively constant. Therefore, if there is a need to

transport more lipids, the usual solution is to form larger particles. This is easily demonstrated in the gut where B-48 is secreted at a relatively constant rate but, when fat is ingested, much larger CM particles are secreted than during the fasting condition. One genetic disorder that is characterized by TG over-production is Familial Hypertriglyceridemia (FHT). In this disorder, the VLDL apoB production by the liver and plasma concentration are normal (or only slightly increased) while the liver TG production, TG plasma concentration, and TG/apoB ratio are high (TG typically 150-400 mg/dl). The VLDL particles have been shown to be very large. These individuals typically have low concentrations of LDL and HDL (much of the VLDL is cleared directly from the plasma and is not converted to LDL) and, therefore, have a Type IV phenotype (high TG only).

The susceptibility of these individuals to cardiovascular disease is variable. If a patient comes from a family with a high incidence of cardiovascular disease (CVD) he/she appears to be prone to develop CVD. In fact, this is one of the most common genetic abnormalities identified in patients



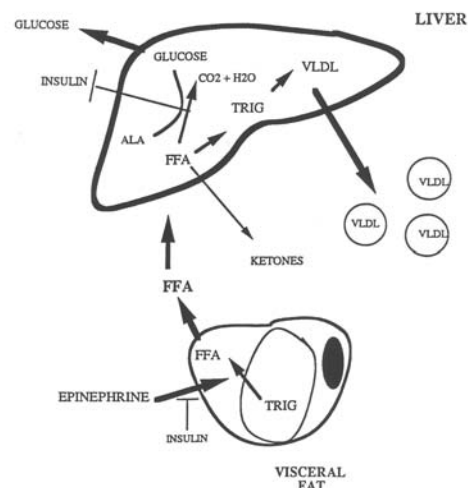
who have had a heart attack. However, if the individual comes from a family with little CVD, then he/she appears to be at no increased risk of CVD. It is currently unknown why this disease heterogeneity exists, but it may be due to different populations of VLDL particles or differences in the way that these particles are metabolized. The HDL-C plasma concentration itself does not differentiate FHT patients who will develop CVD from those who will not, since it is low in both groups. However, post-prandial HDL metabolism appears to be different in these two groups even though their plasma concentrations are similar. In addition, the patients

with CAD may be more likely to have insulin resistance.

Secondary (environmental) causes of excess VLDL-TG production are a much more common cause of hypertriglyceridemia than are the familial disorders. Anything that increases the amount of fatty acids or triglycerides being delivered to the liver will increase the synthesis and secretion of VLDL. Examples of this mechanism are:

1) Dietary fat is delivered to the liver via chylomicron remnants and stimulates VLDL-TG secretion. For this reason, post-prandial VLDL appears to be almost as large as CMs.

2) Visceral adipocytes (intra-abdominal fat cells - omental and mesenteric) release large amounts of free fatty acids that are taken up by the liver and secreted as VLDL-TG. An increased number of visceral fat cells are usually seen in hyperandrogenism in women, hypoandrogenism in men, and hypercortisolism, as well as other insulin resistant states. Therefore, visceraally obese



individuals typically have elevated VLDL production and plasma VLDL-TG levels.

3) Glucose can be a substrate for triglyceride synthesis in the liver, but at normal plasma concentrations (70 – 110 mg/dl) very little glucose is actually taken up by the liver. However, as the plasma concentration increases above 150 mg/dl, more glucose is transported into the liver and a significant amount is converted to triglycerides. Therefore, the high glucose concentrations in Type 2 DM (Diabetes Mellitus) or inadequately treated Type 1 DM will lead to an increase in acetyl-CoA synthesis, and subsequently to an increase in "de novo" fatty acid synthesis and VLDL-TG secretion. Some insulin is required for TG synthesis, therefore, TG synthesis is very low in diabetic ketoacidosis. However, there is plenty of insulin for this purpose in Type 2 or partially treated Type 1 DM. Since visceral obesity is very common in Type 2 DM, these individuals have two reasons to have an increase in VLDL-TG secretion. Some diabetic patients appear to have an increase in VLDL apoB synthesis as well as triglyceride synthesis. This combination leads to an increase in the number of VLDL particles, most of which are enlarged by triglycerides. The mechanism for the increase in apoB synthesis is not known. Reducing the blood glucose by diet, exercise, sulfonylurea, metformin, pioglitazone, or insulin therapy reduces VLDL-TG and apoB production as well as their plasma levels.

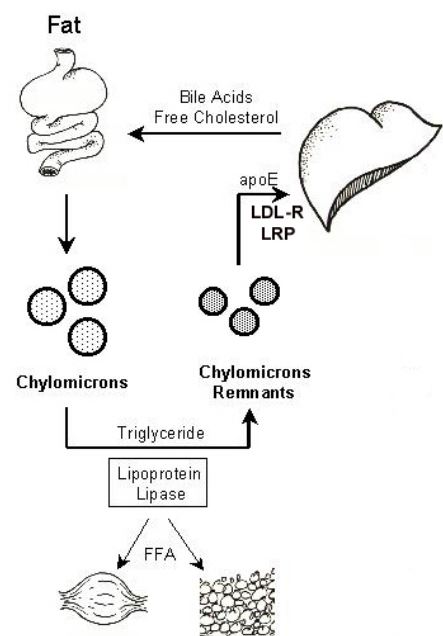
4) Fructose and alcohol are very good substrates for TG synthesis and are rapidly taken up by the liver at all concentrations. Therefore, diets high in sucrose or fructose (i.e. “table” sugar or fruit) will increase VLDL-TG secretion.

A common cause of excess VLDL cholesterol is the ingestion of certain saturated fatty acids (lauric [C-12] > myristic [C-14] > palmitic [C-16]), which appear to stimulate cholesterol synthesis in the liver. These dietary saturated fats also down-regulate LDL receptors in the liver thus inhibiting LDL clearance and increasing LDL-C in the plasma. Both an increase in cholesterol synthesis and a decrease in cholesterol clearance, therefore, contribute to the hypercholesterolemia seen with these diets.

As is obvious from this discussion, there are a number of factors that impact VLDL production. Many of these factors can coexist in the same individual and thus amplify the problem. For example, a visceroally obese diabetic with poor blood glucose control who eats a high saturated fat, high sucrose diet is very likely to have excess VLDL production. However, this does not guarantee that he/she will have high triglyceride or cholesterol plasma levels because he/she may have inherited a super catabolic mechanism to clear this excess VLDL. It is very likely that people with excessive VLDL production are at an increased risk of developing CVD.

II) Delipidation of CM or VLDL to CM-remnants and IDL:

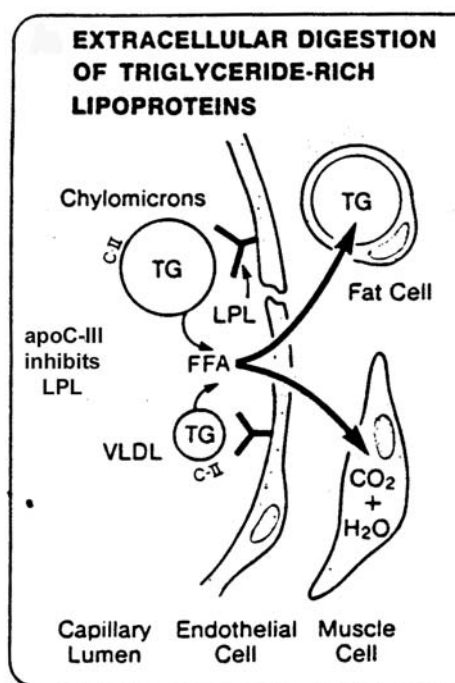
Once chylomicrons and VLDL are released into the circulation, the triglyceride in their core is removed by the action of Lipoprotein Lipase (LPL). This is an enzyme that is



synthesized by adipose cells, muscle cells, and macrophages (and probably other cells). It is secreted and transported to the plasma surface of the endothelial cells where it is bound and where it exerts its action. This enzyme specifically hydrolyses triglycerides in CM and VLDL to free fatty acids and monoglyceride. The fatty acids are transported into the fat or muscle cells where they are stored as TG or utilized for energy. Two of the smaller apolipoproteins modulate LPL. ApoC-II is required for normal activity of this enzyme while apoC-III inhibits its activity.

Genetic deficiencies of either LPL or apoC-II will substantially reduce the clearance of TG from the circulation. Homozygous deficiencies lead to severe hypertriglyceridemia (>1000 mg/dl, Type I Hyperlipidemia) in infants. LPL biosynthesis requires some minimal amount of insulin so that severe insulin deficiency (diabetic ketoacidosis) is associated with very low LPL activity and a high TG concentration (can be >10,000 mg/dl). Since VLDL-TG production should be low in severe insulin deficiency, this massive amount of TG appears to come from dietary fat and is present mostly in chylomicrons. It is generally assumed that when the plasma TG is >800 mg/dl, there is a substantial accumulation of chylomicrons in the plasma. VLDL-TG, by itself, rarely accounts for this level of hypertriglyceridemia.

Usually, patients with Type 2 DM have plasma concentrations of insulin adequate to maintain normal LPL synthesis however, in some cases, there appears to be a functional abnormality (possibly excess apoC-III) of the LPL leading to slow clearance of TG. Since patients with Type 2 DM usually have a very high production of VLDL-TG (as discussed above), even a slightly abnormal clearance of TG will usually lead to moderate hypertriglyceridemia (150 to 800 mg/dl, Type IV Hyperlipidemia). A further increase in VLDL production (due to worsening of blood glucose control, for example) combined with this relatively subtle defect in LPL can overwhelm the LPL such that it can no longer adequately metabolize chylomicron-TG. This can lead to severe hypertriglyceridemia with increased VLDL and chylomicrons (>800 mg/dl, Type V HLP).



The difference between Type I and V HLP is that Type V HLP has both chylomicrons and large VLDL (visible as a white suspension) in the plasma while Type I HLP does not have large VLDL, only CM. The practical way to make this differential diagnosis is to leave the plasma in the refrigerator overnight. Type I HLP plasma will form a chylomicron layer (looks like thick cream) at the top of the tube but the plasma below will be clear yellow (no large VLDL). Type V plasma, on the other hand, will have the chylomicron layer but there will also be a large amount of large VLDL left in the plasma (since it is not as large as chylomicrons) making the plasma cloudy. Type I HLP is a rare condition and is seen only when there is a genetic absence of LPL or apoC-II. It is almost always diagnosed in infants because of fat malabsorption (diarrhea) and recurrent pancreatitis. Type V HLP is a much more common abnormality and is seen in patients with a combination of VLDL over-production and a partial LPL abnormality. Type V HLP usually manifests

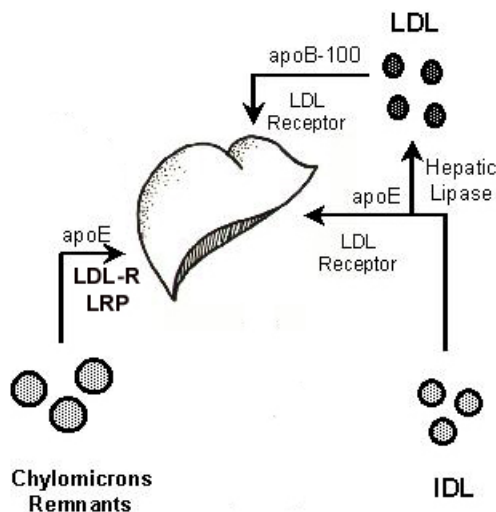
itself in adulthood. Therefore, it is not usually difficult to clinically differentiate these conditions.

The final etiology of reduced TG clearance that we will discuss is an increase in apoC-III, an inhibitor of LPL. It is difficult to know the true incidence of this condition because most laboratories do not measure apoC-III. However, hepatic apoC-III synthesis is increased and its plasma levels elevated in patients with insulin resistance (Type 2 DM and chronic renal insufficiency), contributing to the hypertriglyceridemia frequently seen in these patients. It is, also, likely that excess apoC-III is one factor causing the apparent functional abnormality of LPL in Type 2 DM discussed above. However, many will also have subtle genetic abnormalities of LPL, which contribute to this defect. Most of these patients also have an increase in apoC-II but the excess apoC-III is apparently a stronger inhibitor of LPL than the apoC-II is a stimulator of LPL. The plasma TG levels appear to correlate better with apoC-III than with apoC-II. Transgenic mice manipulated to have only an elevated level of apoC-III develop hypertriglyceridemia with reduced LPL activity and a reduced clearance of VLDL and IDL, typical findings of several human clinical disorders that result in secondary hypertriglyceridemia.

III) Decreased clearance of IDL and/or conversion of IDL to LDL:

Mutations of apoE (Dysbetalipoproteinemia or Type III HLP)

ApoE is a medium sized protein (MW = 36,000 D) that is synthesized in liver, macrophages, and central nervous system (CNS). It may be one of the most primitive lipid transport proteins in humans, and, as such, appears to frequently function on its own (apart from the apoB and apoA-I containing lipoproteins). This is certainly the case in the CNS where it is secreted by cells in response to ischemic injury and is very important in cleaning up (solubilizing) the cell membrane and myelin sheath debris. It is also secreted by cholesterol-loaded macrophages along with the cholesterol that these cells are exporting. Since macrophages are one of the primary cells involved in atherosclerosis, substantial work is currently focusing on how apoE secretion may be involved in this process.



The more mainstream function of apoE seems to be its role in clearing the remnants of TG-rich lipoproteins (CM, VLDL) from the circulation. ApoE is secreted from the liver either with VLDL or with HDL-sized particles. However, not every VLDL particle has an apoE molecule attached to it and no chylomicron is secreted from the intestine with apoE (since apoE is not made in the intestine). ApoE is also secreted from macrophages (foam cells) in the arterial wall as part of reverse cholesterol transport. It is unclear what percentage of plasma apoE is derived from each source. Like the apoC's, a plasma reservoir of apoE is maintained in HDL. When new VLDL or CM particles enter the plasma, some of this apoE is transferred to

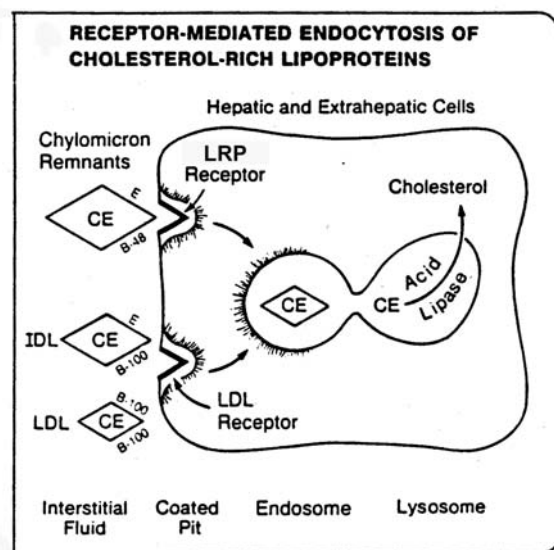
them from HDL. The amount of apoE transferred may depend on the length of time the remnant particle remains in the circulation and the degree of lipolysis (TG removal) that these particles have undergone. When CMs or VLDL have undergone adequate lipolysis (removal of TG), this apoE

can then bind to liver receptors (both B-E receptors and other less specific receptors).

An additional function of apoC-III (besides its inhibition of LPL), appears to be the prevention of premature removal of CMs and VLDL from the plasma before they have had most of their TG removed. ApoC-III is lost during lipolysis, so that eventually these remnant particles become depleted of apoC-III and are allowed to bind to their appropriate receptors via apoE. The apoC-III is recycled to HDL. Of course, if there is a primary excess of apoC-III, then this excess will inhibit the removal of these very atherogenic remnant particles.

VLDL remnants (IDL, intermediate density lipoproteins) are bound by LDL receptors on the liver (also called B-E receptors because they bind both apoB and apoE). Even though the LDL receptor binds to apoB in LDL, it can only bind to apoE in IDL. This is because the apoB on the larger IDL particle is configured in such a way as to hide the LDL receptor-binding site. This

binding site is exposed when IDL is converted to the smaller LDL particle by hepatic lipase (to be discussed later).

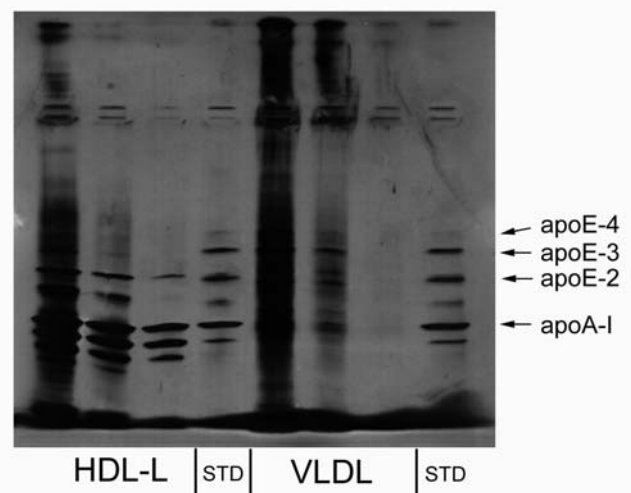


The binding of apoE and apoB to the LDL receptor is a charge interaction. There are a series of positive charges on apoE between amino acids 140-160. These interact with a series of negative charges on the LDL receptor. If one of these positively charged amino acids mutates to a neutral amino acid, then IDL binding can be disrupted. The usual way to identify this abnormality is to run the apoE on an isoelectric focusing gel. This technique separates proteins by their overall charge (adding all of the positive and negative charges together).

If a protein has one additional positive charge, it will focus one step closer to the negative pole of the gel and vis versa. ApoE-3 is considered to have the "normal" number of charges and is produced by the most common allele for apoE found in people (75%). ApoE-2 is deficient in one positive charge (or has an extra negative charge) while apoE-4 has one extra positive charge. Each makes up about 12% of the apoE alleles. If the positive charge lost or the negative charge added is in the LDL receptor binding area (amino acids: 140-160), then IDL binding is likely to be reduced. Since everyone has two genes for apoE, an individual can be either a homozygote (E2/E2, E3/E3, E4/E4) or heterozygote (E2/E3, E2/E4, E3/E4).

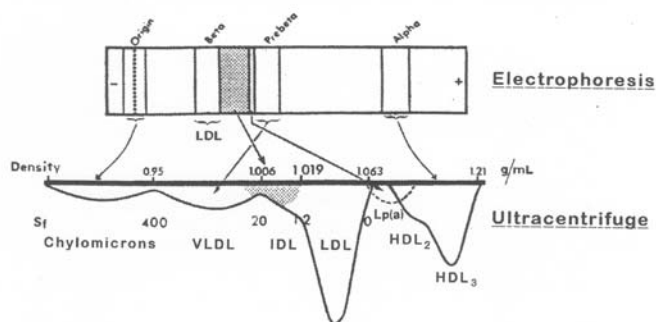
There is a significant amount of phenotypic variability within each of these genotypes. The most dramatic disease

IEF Gel for apoE Isotypes



associated with apoE disorders is Type III Hyperlipidemia, also called Familial Dysbetalipoproteinemia. This disorder almost always occurs in patients that are E2/E2. The population frequency of the E2/E2 genotype is about 1% but the frequency of Type III HLP is only one in 10,000 individuals (a rare disease). Clearly, many individuals with E2/E2 do not develop this disease. This may be because the mutation occurred at a site outside of the LDL receptor-binding region, or the patients are able to compensate for this abnormal binding in some way. A more common problem is the more subtle effects that the presence of an E2 or E4 allele has on a patient's lipoprotein distribution. Subjects with one or two apoE-2 alleles tend to have low LDL levels and high triglycerides even if they do not have Type III HLP. This is probably because of poor conversion of IDL to LDL. On the other hand, subjects with apoE-4 tend to have high LDL levels due to more efficient conversion of IDL to LDL. These effects are independent of other lipid disorders. For example, if a patient has Type 2 DM and E3/E4, he/she will probably have high VLDL production (due to the Type 2 DM) and an increase in IDL to LDL conversion (due to the apoE4) giving rise to increases in both VLDL and LDL (a Type IIb phenotype). Whereas, if the same individual is E2/E3, he/she would probably have lower LDL levels and possibly very high VLDL and/or IDL because of the poor conversion to LDL. This would produce a Type IV phenotype. ApoE4 is currently the best genetic predictor of Alzheimer's disease, especially with homozygous apoE4/E4, while subjects with apoE2 appear to be protected from Alzheimer's disease. ApoE is very important in the formation or inhibition of beta-amyloid, an important component of the senile plaques typically seen in this disorder. It appears that apoE phenotypes may be involved in many neurological diseases.

Familial Dysbetalipoproteinemia is a specific genetic disorder found almost exclusively in subjects with an E2/E2 genotype and frequently exacerbated by some environmental or other factor such as obesity, diabetes, hypothyroidism, etc. It is characterized by the presence of beta-VLDL in the plasma. This is a large, cholesterol-rich particle that has the electrophoretic mobility of LDL (which used to be called beta lipoprotein) on agarose electrophoresis. Another name for this disorder is "broad-beta" disease because a broad band stretching from the beta position (LDL) to the pre-beta position (VLDL) is frequently seen on these agarose gels. This broad band is due to the heterogeneous nature of these beta-VLDL particles. Beta-VLDL starts out as normal, triglyceride-rich VLDL but with an apoE2 molecule attached. VLDL particles undergo normal delipidation by LPL but cannot be removed from the circulation because its apoE2 cannot bind to the LDL receptor on the liver. The longer this particle stays in the plasma, the more cholesterol ester (CE) it accumulates from HDL (via CE transfer protein activity, to be discussed later). Therefore, it begins



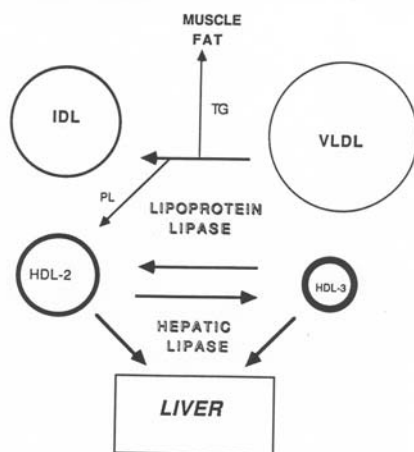
to grow until, frequently, it achieves a size similar to VLDL. If the plasma is ultracentrifuged, beta-VLDL will float at the same hydrated density as VLDL, thus the name "beta-VLDL". The difference is that now its core lipid has a high concentration of cholesterol esters rather than triglycerides. One criteria used to identify this disease is a VLDL cholesterol to total triglyceride (mg/dl) ratio of greater than 0.3.

The reason that this is an important lipoprotein disease is that it is associated with a high

incidence of atherosclerosis. Beta-VLDL is taken up by scavenger receptors on macrophages without having to be oxidized first (LDL has to be oxidized before it can be taken up by macrophages). Therefore, at high concentrations, beta-VLDL can produce foam cells in arterial walls. Chylomicron remnants are also unable to be removed from the plasma, because they depend on the same apoE. Therefore, they also accumulate in the plasma. These patients usually present with both elevated plasma TG and cholesterol. Documenting the presence of beta-VLDL (cholesterol-rich VLDL) with little or no true LDL (by ultracentrifugation) is needed to suggest the diagnosis of Type III HLP. The diagnosis is then made by isoelectric focusing of apoE to demonstrate that only apoE2 is present. It is important to note that in clinical practice, LDL is calculated after measuring the total cholesterol, triglycerides, and HDL cholesterol. The VLDL cholesterol is calculated by assuming that it is 20% of the total plasma triglyceride. The VLDL and HDL cholesterol are then added together and subtracted from the total to obtain the LDL cholesterol. Obviously, these assumptions are not accurate in this disorder and, therefore, the calculated LDL is not correct. A very useful physical finding that is only seen in this disorder is the presence of orange fat deposits in the creases of the hand. Unfortunately, not all patients with Type III HLP present with such an obvious physical finding.

Along with abnormalities of apoE, LDL receptor defects can result in reductions in the conversion of IDL to LDL and accumulation of IDL. The most common causes of LDL receptor abnormalities are Familial Hypercholesterolemia and hypothyroidism. These will be discussed in detail in the next section. However, these disorders are characterized by elevated LDL concentrations while dysbetalipoproteinemia is characterized by low LDL concentrations. High saturated fat diets may also reduce LDL receptor activity and can, therefore, lead to an increase in IDL (and LDL).

Hepatic Lipase is an enzyme that is made in the liver and resides on the external surface of these cells as well as steroid hormone producing cells (adrenals, gonads) and perhaps other cells as well. It has both "phospholipase" and "triglyceride lipase" activities and appears to have two major



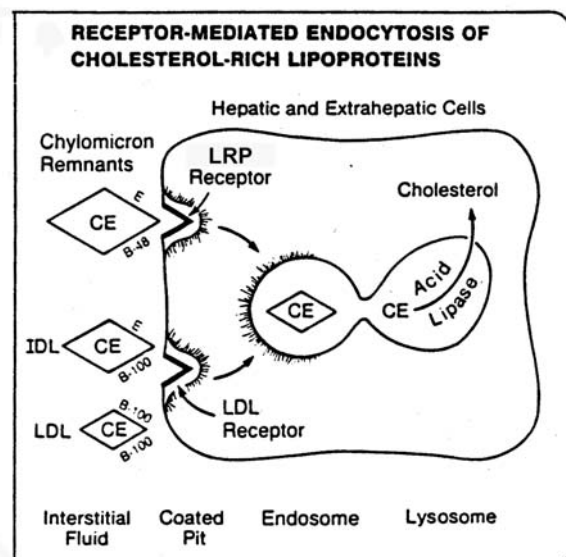
hyperthyroidism typically have low concentrations of IDL and HDL, while women without hyperandrogenism and individuals with hypothyroidism have high concentrations of IDL and HDL. There are also very rare genetic deficiencies (autosomal recessive) of this enzyme, which will increase triglycerides (200-900 mg/dl), LDL (150-200 mg/dl), and HDL₂ with triglyceride enrichment of the LDL and HDL particles.

IV) Clearance of LDL (elevated LDL-cholesterol):

B,E (LDL) receptor defects

One of the most clearly defined and famous lipoprotein disorders is Familial Hypercholesterolemia (FH). This disorder is usually caused by a variety of abnormalities of the LDL receptor that prevents the normal processing of LDL by cells (primarily hepatocytes). Many of these abnormalities were identified by Dr.'s Brown and Goldstein in Dallas for which they have been awarded the Nobel Prize. The LDL receptor is a complex protein that spans the plasma membrane, but has multiple binding sites for LDL on the external side of the membrane. These receptors are clustered in "coated pits" in the plasma membrane, which are internalized into the cell when these "pits" invaginate into cytoplasmic vesicles. The LDL is released from the receptor and degraded, while the vesicle is then recycled back into the plasma membrane. Any number of mutations can occur that reduce the synthesis of the receptor, prevent its migration to the cell surface, prevent the formation of coated pits, reduce LDL binding, or prevent internalization of the receptor-LDL complex.

FH heterozygotes (autosomal dominant) have one allele for a particular LDL receptor abnormality and present with plasma cholesterol levels of 250 to 600 mg/dl without an increase in triglyceride (Type IIa). The genetic frequency of this disorder in our population is 1 in ~500. These individuals can be diagnosed at birth by measuring their plasma cholesterol level. Affected men begin having myocardial infarctions in their twenties and 85% have had an MI by the age of 60. Women are generally protected from heart disease until after menopause but they then develop heart disease at a rate that parallels that of men (it is only 10-15 years delayed). It should be noted that only 5% of all Myocardial Infarctions (MI) are caused by FH so that most patients with heart disease do not have this disorder. Tendon Xanthomas are diagnostic of FH but their presence is age



dependent. Up to 75% of people with FH will eventually develop tendon xanthomas. An individual can inherit two different LDL receptor defects (double heterozygote), which will generally produce a plasma cholesterol in the 500 to 800 mg/dl range, but not make them a homozygote. Homozygotes (frequency: 1 in a million) frequently have cholesterol levels over 1000 mg/dl and begin to have MIs before the age of 10 years. The only treatment at this time for the homozygous condition is LDL plasmapheresis or liver transplantation.

As discussed above, the LDL receptor is required for removal of IDL by the liver. In

humans, approximately 50% of the IDL are directly removed by the liver and are, therefore, never converted to LDL. If there is a LDL receptor abnormality, this removal of IDL is dramatically reduced and LDL production drastically increased (a very bad situation in these patients with poor LDL clearance). Even the normal IDL clearance rate of humans is actually relatively poor since rats, dogs, and many other species typically clear up to 90% of the IDL from the plasma. These animals, therefore, normally have relatively low levels of LDL cholesterol (<50 mg/dl) and almost never develop atherosclerosis.

It should be stressed that most patients with a Type IIa phenotype do not have FH but have polygenic hypercholesterolemia; that is, they have a cholesterol level that places them at one end of the bell-shaped curve for the general population. This is probably due to a number of subtle changes in LDL production and/or degradation that add up to a net increase in LDL cholesterol. Secondary causes of a high LDL-C are a high saturated fat diet (reduces LDL receptor activity) and hypothyroidism. Thyroid hormone is required for LDL receptor production and patients with hypothyroidism can have cholesterol levels of >500 mg/dl and are at high risk for the development of atherosclerosis. Thyroid studies should be obtained on every person with hypercholesterolemia.

Mutations of apoB

ApoB binds to the LDL receptor by an ionic bond similar to apoE. Therefore, mutations that affect the positively charged amino acids in the LDL receptor binding region of apoB will reduce LDL binding and increase LDL concentrations to a degree similar to LDL receptor defects (Type IIa phenotype). Several families have now been identified with these abnormalities and would be clinically diagnosed as Familial Hypercholesterolemia. They would be indistinguishable from patients with LDL receptor defects (very high LDL levels) except they would be able to clear IDL from the plasma normally since this process is mediated through apoE.

V) HDL Metabolism:

Reverse Cholesterol Transport

High Density Lipoprotein is metabolized very differently than the apoB-containing lipoproteins (CM, VLDL, IDL, and LDL). HDL is best thought of as a truck that continually loads

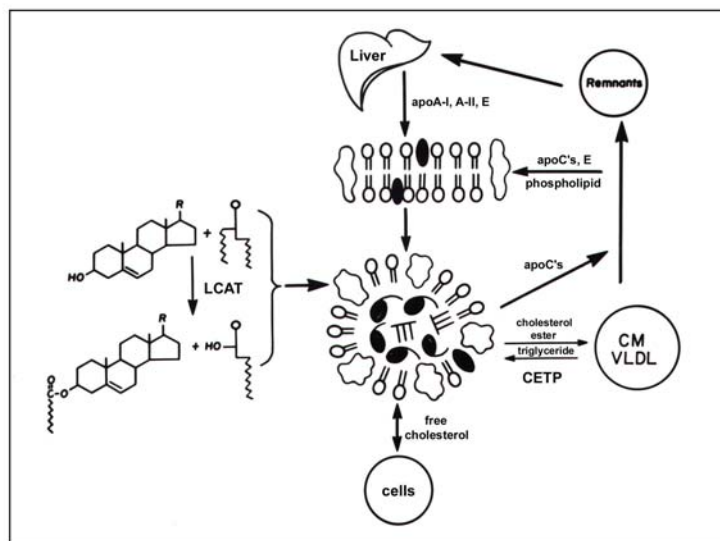


Figure 28-2. HDL metabolism.

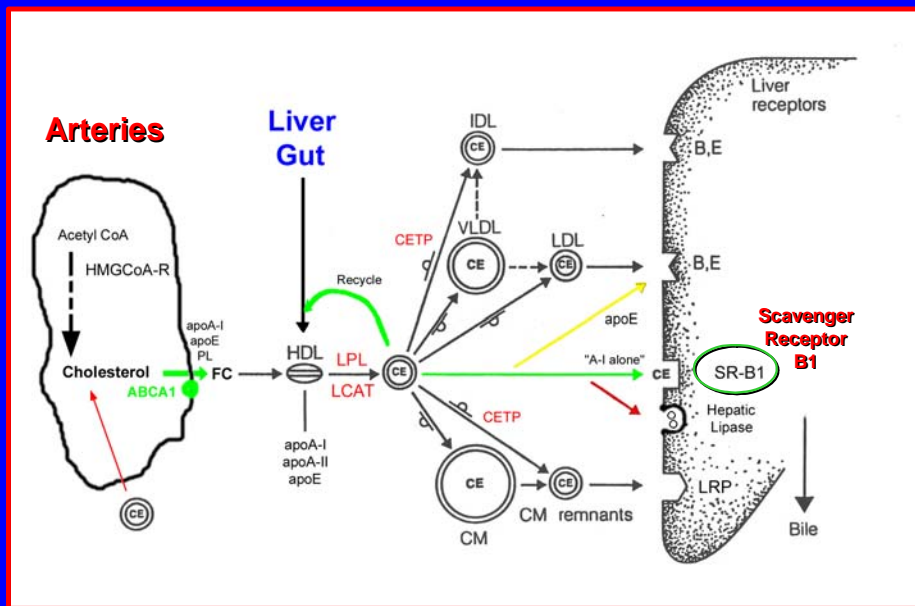
and unloads its cargo without being taken apart at each stop. The VLDL→IDL→LDL pathway basically is a one-way trip for apoB starting with VLDL and finally delivering cholesterol to the liver (or arteries) via LDL. ApoB is destroyed in the final step of the process. The apoB lipoproteins, therefore, have relatively short plasma half-lives; from hours (VLDL) to a few days (LDL). HDL, on the other hand, is not secreted in a form at all like the mature plasma particle. In addition, its protein

moiety (apoA-I continually exchanges lipid with other lipoproteins and cells without it being consumed). This gives apoA-I a half-life of over five days while HDL lipid half-life is a matter of hours.

ApoA-I (MW = 28,400) is synthesized in both the liver and small intestine while apoA-II (MW = 17,400) is synthesized only in the liver. Almost all HDL particles contain apoA-I (LpA-I) while some contain both apoA-I and apoA-II (LpA-I:A-II). There are essentially no particles that contain only apoA-II. LpA-I and LpA-I:A-II are metabolized quite differently and LpA-I appears to be the most important in preventing atherosclerosis because it is more efficient in “reverse cholesterol transport”. However, we currently have only a limited understanding of the metabolic differences between these two classes of HDL. A very small population of HDL particles contains only apoE (no apoA-I) and probably originate in vascular macrophages. The function of these particles is currently unknown, but there is some interesting speculation that they are a significant mechanism by which HDL cholesterol is delivered directly to the liver. You do not need to be concerned with apoE-HDL at this time.

ApoA-I is secreted from the liver attached to disc-shaped particles (called “nascent pre-beta HDL”) made up of a bilayer of phospholipid (PL) with a small amount of free cholesterol (FC) interdigitated between the PL molecules. Similar particles can be secreted from the small intestine but apoA-I is usually secreted from the gut attached to chylomicrons (especially following a high-fat meal). Gut apoA-I is rapidly released from CMs with phospholipid when the surface monolayer of PL becomes redundant during lipolysis and pinches off from the main particle. Again a disc-shaped HDL particle is formed.

Reverse Cholesterol Transport



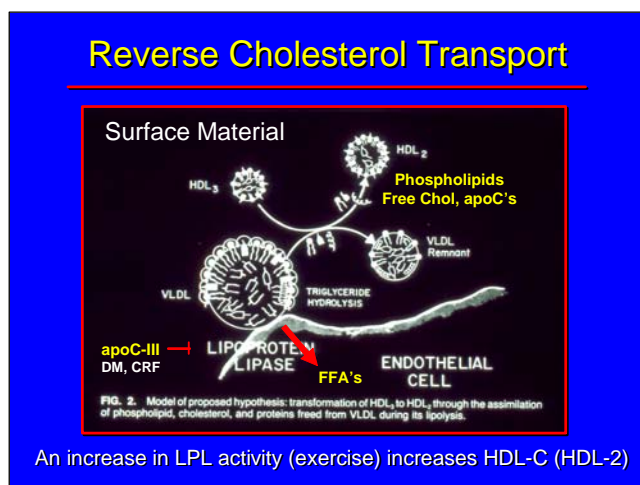
Multiple potential pathways for cholesterol transport

A four-step process then takes place, which constitutes "reverse cholesterol transport":

(1) Cholesterol efflux from cells (multiple pathways): "Lipid-Poor" apoA-I (nascent pre-beta HDL containing 2 apoA-I molecules secreted from liver or gut) binds to ABCA1 (a cholesterol unidirectional transporter - efflux only) and rapidly accrues additional free cholesterol and phospholipid generating larger disc-like particles. This process will continue until "free cholesterol-rich pre-beta HDL" has been formed (discs that are saturated with free cholesterol; generally one molecule of FC can be carried by two molecules of PL). By combining with lipid, renal excretion of apoA-I is prevented. The bulk of plasma "FC-rich pre-beta HDL" is actually formed at the surface of the liver. Therefore, the plasma concentration of pre-beta HDL does not reflect arterial cholesterol, even though a similar process is occurring in the artery wall, simply because arterial cholesterol efflux makes up only a small percentage of total ABCA1 activity.

When any cell (hepatocyte or "peripheral [non-liver] cell") accumulates excess cholesterol, it will up-regulate its ABCA1 transporters and secrete more cholesterol and phospholipid. ABCA1 is also up-regulated by cAMP, PPAR γ , and niacin but down-regulated by interferon-gamma (inflammation). Plaque macrophages will also secrete apoE with free cholesterol and phospholipid via ABCA1 and these particles can be combined with pre-beta HDL or small spherical HDL. When the surface phospholipid of the HDL particle becomes saturated with free cholesterol, cholesterol transfer will stop. Tangier's Disease is caused by a genetic absence or severe dysfunction of the ABCA1 transporter, which leads to cholesterol accumulation in various tissues, including arteries, and very low HDL levels. There are probably more subtle abnormalities of ABCA1 that produce less dramatic reductions in HDL but can still be very detrimental clinically.

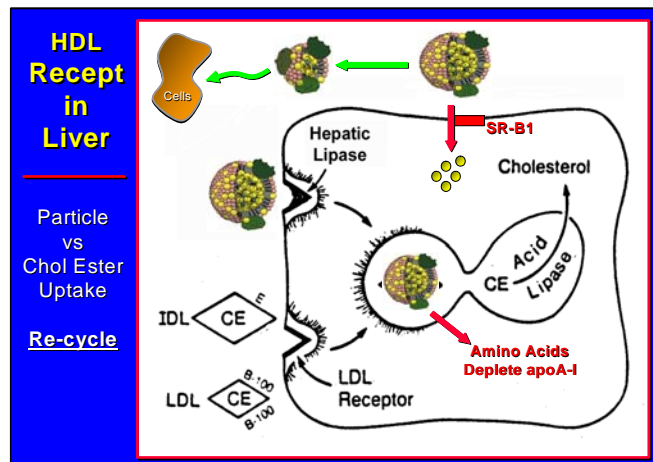
(2) In order to prevent the accumulation of free cholesterol on the surface of HDL particles, the FC is esterified by LCAT (lecithin-cholesterol acyltransferase). This is an enzyme found only in plasma and transfers a fatty acid from phospholipid to free cholesterol to form a cholesterol ester (CE). This reaction occurs almost entirely on HDL since apoA-I is the primary activator of LCAT. Because CE is insoluble in water and cannot even remain interdigitated between the PL molecules, it must move into the interior of the HDL disc or sphere. This enlarges the core of the particle



much like blowing up a balloon. This process is fostered by the action of lipoprotein lipase because, as VLDL or CM undergoes lipolysis, excess surface material (PL, free cholesterol and apoC's) is transferred to HDL. This additional surface material enlarges the HDL particle and improves its cholesterol carrying capacity. These reactions gradually enlarge the HDL from small discs (pre-beta HDL) to small spheres (HDL₃) and, finally, to larger spheres (HDL₂). ABCG1 and ABCG4 are additional unidirectional cholesterol transporters found in many cells that can add free cholesterol to the larger spherical HDL and contribute to further

enlargement of these particles. In addition, most cells also have a bidirectional (influx and efflux) transporter called: SR-B1 (scavenger receptor – B1). These transporters interact with large, spherical HDL particles to transfer cholesterol-esters (the particle core lipid) in both directions such

that the net transfer will depend on the specific concentration gradient. For example, the net transfer in liver (discussed below) and adrenals, which utilize substantial amounts of cholesterol, will be into the cell. On the other hand, most other cells will export cholesterol ester via this route because they synthesize or collect more cholesterol than they utilize. Because the critical final delivery of cholesterol esters to the liver from HDL is mediated by SR-B1, the enlargement of the HDL particles by LPL is vital to efficient reverse cholesterol transfer.



(3) HDL cholesterol is delivered directly to the liver by one of two pathways. First, HDL can bind to Hepatic Lipase and the whole particle is taken into the liver to be destroyed. This is not the preferred pathway because the apoA-I cannot be recycled and therefore the HDL concentration falls. The second pathway is via the SR-B1 receptor (described above), which appears to be one of the most important HDL receptors. When large HDL binds to the SR-B1 receptor, only the cholesterol ester is removed and the HDL remnant containing apoA-I can be released to circulate and gather

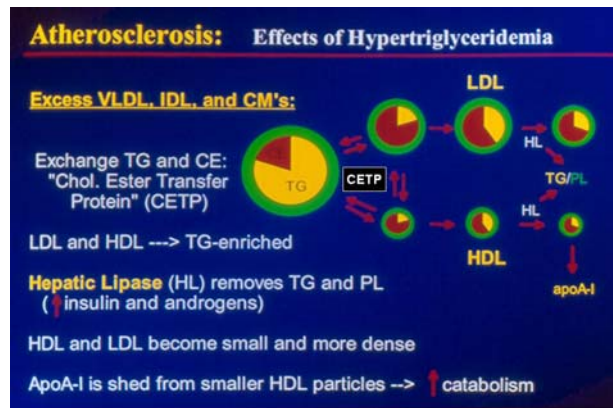
more cholesterol. This recycling may occur many times unless there is an increase in Hepatic Lipase activity (due to hyperandrogenism, estrogen deficiency, hyperinsulinemia, hyperthyroidism, or adiponectin deficiency in obesity, etc.), which will short-circuit this recycling. The large HDL particles containing apoA-I without apoA-II ("LpA-I" HDL) are the most efficient in cholesterol delivery via SR-B1 (as it is with almost every step of reverse cholesterol transport) and are, therefore, the best marker for cardio-protective HDL. SR-B1 is up-regulated by fibrates and polyunsaturated fats (thus tend to lower HDL) and down-regulated by cellular cholesterol and estradiol.

(4) An alternative pathway for cholesterol delivery to the liver is via chylomicron remnants, IDL, and LDL. CETP (cholesterol ester transfer protein) is a plasma protein that transfers CE and TG (the very hydrophobic lipids) between the various lipoproteins. If this protein interacts with LDL and HDL, there is probably little net effect since both have lipid cores rich in CE (there is an exchange of one CE for another). However, if there is an interaction of CETP with HDL (a CE-rich particle) and VLDL (a TG-rich particle), then there will be a net transfer of TG from VLDL to HDL and CE from HDL to VLDL. The end result of this process is movement of cholesterol from cells to HDL to VLDL and its metabolites (IDL, LDL). A similar result would occur if CMs (and CM remnants) were involved. It has recently been discovered that humans and many other species have a "CETP inhibitor" that is actually primarily responsible for determining the overall activity of CETP in most individuals. Therefore, it is the CETP inhibitor concentration, not the CETP level itself that determines the TG--CE transfer rate in most people. Finally, CM-R, IDL, or LDL binds to LDL receptors or LRP (LDL receptor-related protein) and is taken up as a whole particle into hepatocytes, thus delivering their cholesterol to the liver. This cholesterol can then be secreted into the bile and exit the body, thus completing reverse cholesterol transport (movement of cholesterol out of the body).

This alternative pathway could provide efficient delivery of cholesterol to the liver because the

liver contains about 90% of all LDL receptors that are typically expressed at any given time. Therefore, most of the cholesterol that is in CM-R, IDL, and LDL will be delivered to the liver until these receptors are saturated. Unfortunately, LDL receptors are saturated at a LDL cholesterol concentration of about 25 mg/dl (similar to the human level at birth). Above this level, these lipoproteins begin to be deposited in other tissues, primarily arteries, which lead to atherosclerosis. The average LDL concentration in adults in this country is ~130 mg/dl, obviously a very atherogenic level.

Hypertriglyceridemia (either excess CM or VLDL) is usually associated with low HDL cholesterol concentrations. A major mechanism by which hypertriglyceridemia reduces HDL-C is the CETP-mediated exchange of triglycerides and cholesterol esters with subsequent removal of the triglycerides and phospholipids by Hepatic Lipase. This removal of lipid will allow the apoA-I to detach from the particles and the kidneys will rapidly excrete this free apoA-I. This loss of apoA-I will prevent the usual recycling of HDL and aggravate the reduction in total HDL cholesterol already initiated by the CETP mediated lipid exchange. A similar pathway involving LDL leads to the generation of the small, dense LDL particles usually seen in patients with hypertriglyceridemia.



CETP is a critical factor in determining the total HDL cholesterol concentration and has been implicated in a number of familial disorders. Some families with genetic dysfunctions of CETP may have very high HDL concentrations but also have a very high incidence of atherosclerosis. However, other families with probably different mutations also have high HDL concentrations but are protected from atherosclerosis. Therefore, some transfer of cholesterol from HDL to apoB-particles seems to be important.

ApoA-I production

Changes in apoA-I synthesis or the production of abnormal apoA-I will clearly alter HDL levels. There have been a number of mutations of apoA-I identified, which reduce apoA-I levels and lower HDL concentrations. Most of these disorders are associated with an increased risk of atherosclerosis, but not all. For example, apoA-I Milano has been shown to provide very rapid reverse cholesterol transport so that even though the HDL cholesterol is very low (<10 mg/dl), there is no atherosclerosis. In fact, an infusion of apoA-I Milano has been demonstrated to shrink coronary plaques within four weeks in patients with severe atherosclerosis. These severe genetic disorders are relatively rare. However, more subtle (and perhaps more common) alterations in apoA-I production or structure are probably responsible for some of the cases of hyperalphalipoproteinemia (high HDL) and hypoalphalipoproteinemia (low HDL) that you will see. The mechanisms for most of these disorders have not yet been characterized.

Reduced conversion of HDL₃ (small) to HDL₂ (large) (reduced HDL-cholesterol)

LCAT deficiency is a very rare disease characterized by low concentrations of cholesterol esters in the plasma and low levels of HDL (Table 3, Complete LCAT Deficiency vs Fish-eye Disease). When the plasma HDL particles have been examined, they have been found to be disc-

shaped; very similar to those identified from liver perfusion experiments. Without LCAT to generate cholesterol esters, the HDL particles never develop into spheres because there is no core material. These individuals have elevated VLDL levels, but all lipoprotein particles are deficient in cholesterol esters and have a very abnormal composition. Reverse cholesterol transport may be very poor in this condition but not necessarily so. For your information (will not be on test), these individuals are at increased risk for cataracts, hemolytic anemia, proteinuria, and renal failure but have a variable risk for atherosclerosis. The partial deficiency (“Fish-Eye Disease”) is usually caused by a molecular defect that prevents LCAT binding to HDL but allows binding to VLDL and LDL. Therefore, HDL cholesterol ester is very low but LDL and VLDL can esterify cholesterol probably activated by apoC-I.

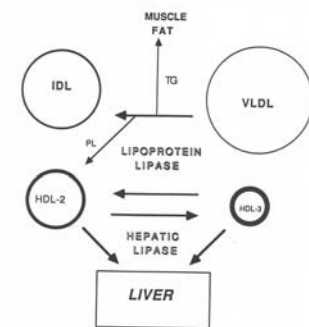
A much more common cause for low HDL cholesterol is a reduction in Lipoprotein Lipase activity. This will reduce the transfer of phospholipid to HDL₃, which is required for its enlargement to HDL₂. We have already discussed many of the conditions that reduce LPL activity, including genetic defects of LPL or apoC-II, diabetic ketoacidosis, excess apoC-III, and possibly Type 2 DM (typically associated with excess TNF α which inhibits LPL).

Increased conversion of HDL₂ to HDL₃ and clearance from the plasma

The most common cause currently identified for an increase in HDL clearance is an increase in Hepatic Lipase activity. As we have previously discussed, hyperinsulinemia,

hyperthyroidism, and hyperandrogenism are three common causes of an increase in Hepatic Lipase activity while estradiol and adiponectin (a cytokine released from small fat cells, i.e. thin people) inhibit Hepatic Lipase. There are also likely to be genetic variations in enzyme activity that could lead to significant alterations in activity. Hepatic Lipase is a less selective lipase than is LPL in that it cleaves fatty acids from both phospholipids and triglycerides. LPL, on the other hand,

greatly prefers triglycerides as a substrate. HL also prefers to work on smaller particles such as HDL and LDL rather than VLDL. HL appears to remove phospholipid from all HDL subfractions but especially from HDL₂. It also removes most of the triglyceride that the HDL has accumulated due to CETP activity. The removal of PL appears to encourage the movement of free cholesterol into the liver without destruction of the entire particle. This is one mechanism by which HDL can directly deliver cholesterol to the liver (this is probably not the major pathway, however). What remains is an HDL₃-like particle that can recycle back to the peripheral tissues to pick up more cholesterol. Unfortunately, as noted above, HL is not only a lipase but also functions as a receptor much like the LDL receptor. Therefore, when HDL binds to it, the entire particle can be taken into the cell and destroyed, thus preventing the HDL from recycling. As a result, a high activity of HL is usually associated with an increased risk of atherosclerosis (hyperandrogenism, hyperinsulinemia, obesity).



Reduced conversion of HDL₂ to HDL₃ (high HDL₂ cholesterol)

Several families with a genetic deficiency of CETP have been identified (as mentioned above). The homozygotes are characterized by a 6-fold increase in HDL₂ cholesterol but less than a 2-fold increase in apoA-I. This leads to a 50% increase in average HDL size. These patients also have a 4-fold increase in apoE-containing HDL. ApoE-HDL usually makes up a very small fraction of the

total number of HDL particles in humans but contributes to a much greater extent to the HDL of rats and dogs. Interestingly, these species normally have very low CETP activities. Heterozygotes have only a 2-fold increase in HDL₂ cholesterol and a slight increase in apoA-I. Identifying the heterozygotes is difficult because their HDL cholesterol concentrations are frequently in the high normal range, therefore, not clearly different than many other individuals in our population.

As noted above, the incidence of atherosclerosis is variable in subjects with CETP deficiency. CETP is a two-edged sword. On the one hand, it delivers cholesterol esters to CM and VLDL remnants, which can be efficiently removed by the liver. On the other hand, if these remnants are not efficiently cleared from the plasma, the CE is passed through to LDL (or the CE is delivered directly to LDL), and this increase in LDL cholesterol can induce atherosclerosis. In addition, if the CM and VLDL remnants are not rapidly removed from the plasma, they are themselves very atherogenic particles. Of course, the cholesterol esters in HDL must eventually be delivered to the liver if reverse cholesterol transport is going to proceed. It is likely that the high concentrations of apoE-HDL (via the LDL receptor) or HDL₂ (via the SR-B1 receptor) serve this purpose. The reduced CETP activity allows the HDL particles to become very large which may or may not improve their interaction with SR-B1, thus cholesterol ester delivery into the liver may be increased or reduced.

As mentioned above, it has recently been discovered that there is a lipid transfer inhibitor protein (LTIP or "CETP inhibitor") in human plasma (MW = 29,000). It is carried primarily on HDL particles with both apoA-I and apoA-II and is a potent inhibitor of CETP activity. Therefore, increased concentrations of this protein are associated with higher HDL₂ cholesterol concentrations. It appears that much of the species variability in CETP activity is actually the result of differences in LTIP. For example, rats and swine appear to have almost no CETP activity. However, when LTIP is removed from their plasma, they both have substantial increases in CETP activity. Specifically, swine have a change in CETP activity from essentially undetectable activity to a level 4-fold greater than normal human plasma, when the inhibitor is removed. Human plasma CETP activity also increases over 100% when this inhibitor is removed. It is felt that a substantial amount of the variability of CETP activity in humans is due to differences in this inhibitor.

This concludes our review of lipoprotein metabolism. The lectures will not necessarily cover all of the material in this syllabus, therefore, it is important that you understand all of the material presented in this handout. Once again, all test questions will come from this syllabus and the answers are underlined.

Table 2. Primary lipid disorders

Genetic disorder	Molecular defect	Lipoproteins elevated	Lipoprotein phenotype	Clinical findings	Genetic transmission	Estimated incidence
Familial Chylomicronemia Syndrome	LPL deficiency, apoC-II deficiency	Chylomicrons	Type I hepatosplenomegaly, pancreatitis	Eruptive xanthomas, recessive	Autosomal	Rare
Familial Dysbetalipoproteinemia	Abnormal apoE (i.e., apoE-2/2)	Chylomicrons, VLDL remnants beta-VLDL	Type III	Palmar and tubero-eruptive xanthomas, premature atherosclerosis	Autosomal recessive or autosomal co-dominant	1/5000
Familial Combined Hyperlipidemia	Unknown	VLDL, IDL, LDL	Type IIb, sometimes IIa or IV, rarely V	Premature atherosclerosis	Autosomal dominant	1/200
Familial Hypertriglyceridemia	Unknown	VLDL, occasionally chylomicrons	Type IV, occasionally Type V	Usually none	Autosomal dominant	1/500
Familial Hepatic Lipase Deficiency	Hepatic lipase Deficiency	VLDL remnants	Type IIb	Premature atherosclerosis	Autosomal recessive	Rare
Familial Hypercholesterolemia	LDL-receptor deficiency	LDL	Type IIa premature Atherosclerosis	Tendon xanthomas	Autosomal co-dominant	1/500
Familial defective ApoB-100	Abnormal apoB-100 (i.e., Arg3500 Gln)	LDL	Type IIa premature atherosclerosis	Tendon xanthomas	Autosomal co-dominant	1/600

LPL, lipoprotein lipase; apoC-II, apolipoprotein C-II; apoE, apolipoprotein E; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; apoB-100, apolipoprotein B-100;

Table 3. Genetic disorders associated with low—HDL-cholesterol levels

Genetic disorder	Molecular defect	Metabolic defect	Lipoprotein abnormalities	Clinical findings	Premature atherosclerosis	Genetic transmission
Familial apoA-I deficiency	apoA-I deficiency	Absent apoA-I biosynthesis	HDL <5 mg/dl TG normal	Planar xanthomas, Corneal opacities	+ +	Autosomal codominant
Familial apoA-I structural mutants	Abnormal apoA-I	Rapid apoA-I catabolism	HDL 15–30 TG increased	Often none; sometimes corneal opacities	No	Autosomal dominant
Familial LCAT deficiency	LCAT deficiency (complete)	Rapid HDL catabolism	HDL <10 mg/dl TG increased	Corneal opacities, anemia, proteinuria, renal insufficiency	No	Autosomal recessive
Fish-eye disease	LCAT deficiency (partial)	Rapid HDL catabolism	HDL <10 mg/dl TG increased	Corneal opacities	No	Autosomal recessive
Tangier disease	ABCA1 deficiency	Unable to efflux cholesterol to HDL	HDL <5 mg/dl; TG usually increased	Corneal opacities, enlarged, orange tonsils, hepatosplenomegaly, peripheral neuropathy	+ +	Autosomal recessive
Familial hypoalpha-lipoproteinemia	Unknown	Usually rapid HDL catabolism	HDL 15–35 TG normal	Often none, sometimes corneal opacities	No to + +	Autosomal dominant

+ +, commonly occurs; +, sometimes occurs; apoA-I indicates apolipoprotein A-I; HDL, high-density lipoprotein; TG, triglycerides; and LCAT, lecithin cholesterol acyltransferase.