LIPOPROTEINS

Many important molecules in the body are lipids. But transporting these molecules around the body through the blood presents an obvious problem, because, by definition, lipids are nonpolar and thus not very soluble in water. Small amounts of fatty acids are transported in the blood bound to blood proteins. These are called **free fatty acids** (despite the binding). Beyond this, however, other lipids are transported in special particles called **lipoproteins**.

To emphasis, lipoproteins are not molecules, but rather **particles** comprised of several thousand molecules. These particles solve the problem of lipid/water incompatibility via the **amphipathic** nature of **phospholipids**.

One end of these molecules is polar and the other end nonpolar.

Lipoproteins have a **single layer** of phospholipid molecules on their outside, surrounding a central core. (By contrast, plasma membranes are comprised of a BILAYER.) Since the polar part of each phospholipid faces out, the outside of the phospholipid molecule is polar and thus compatible with the surrounding water environment. On the other hand, the nonpolar portion of each phospholipid faces inward, and thus is compatible with the very nonpolar ingredients of the core of the lipoprotein. In addition, some **cholesterol** is found in the outer layer of phospholipid.

As shown in the figure, the outer layer of the lipoprotein also has a protein molecule called an **apolipoprotein**. Like phospholipids, this protein is amphipathic and helps stabilize the particle. But even more important, the protein serves to identify the specific lipoprotein. For example, some lipoproteins transport dietary lipids from the small intestine to adipocytes and the liver. Other lipoproteins transport cholesterol between different part of the body. Each type of lipoprotein can be identified because it has a different apolipoprotein.

At the target cell, in some cases, the apolipoprotein binds to a **receptor** and then the lipoprotein is then taken up by **receptor mediated endocytosis**. In other cases, an enzyme on the capillary wall, termed **lipoprotein lipase**, unloads triacylglycerol from the lipoprotein by breaking triacylglycerol into fatty acids and glycerol.

(The terminology here often confuses students. Remember the lipoprotein is a **particle**, the apolipoprotein is a **protein** that is part of the particle. Also,

In the figure above, the arrangement of apolipoprotein on the outside of the lipoprotein is based purely on my imagination.)

The core of the lipoprotein contains the most nonpolar substances. A lipoprotein formed in the small intestine, for example, would have much **triacylglycerol** (triglyceride) derived from the diet. **Esterified cholesterol** is also found in the core. ("Esterified" means that a fatty acid is combined with the cholesterol at its one vaguely polar spot to make an even more nonpolar molecule.) A small amount of ordinary cholesterol is also found in the phospholipid outer layer.

In order for the body to make use of dietary lipids, they must first be absorbed from the small intestine. The predominant form of dietary lipid in the human diet is triglyceride. Since these molecules are oils, they are essentially insoluble in the aqueous environment of the intestine. The solubilization (or emulsification) of dietary lipids is accomplished principally in the small intestine by means of the bile acids. Bile acids are synthesized from cholesterol in the liver and then stored in the gallbladder. Following the ingestion of food, bile acids are released and secreted into the gut. Some lipid emulsification occurs in the stomach due to the churning action in this organ which renders some of the lipid accessible to gastric lipase.

The emulsification of dietary fats renders them accessible to various pancreatic lipases in the small intestine. These lipases, pancreatic lipase and pancreatic phospholipase A_2 (PLA_2) generate free fatty acids and a mixture of mono- and diglycerides from dietary triglycerides. Pancreatic lipase degrades triglyceride at the *sn*-1 and *sn*-3 positions sequentially to generate 1,2-diglycerides and 2-acylglycerols. Phospholipids are degraded at the *sn*-2 position by pancreatic PLA_2 releasing a free fatty acid and the lysophospholipid. The products of pancreatic lipases then enter the intestinal epithelial cells via the action of various transporters as well as by simple diffusion. Within the enterocyte the lipids are used for re-synthesis of triglycerides.

Dietary triglyceride and cholesterol, as well as triglyceride and cholesterol synthesized by the liver, are solubilized in lipid-protein complexes. These complexes contain triglyceride lipid droplets and cholesteryl esters surrounded by the polar phospholipids and proteins identified as apolipoproteins. These lipid-protein complexes vary in their content of lipid and protein.

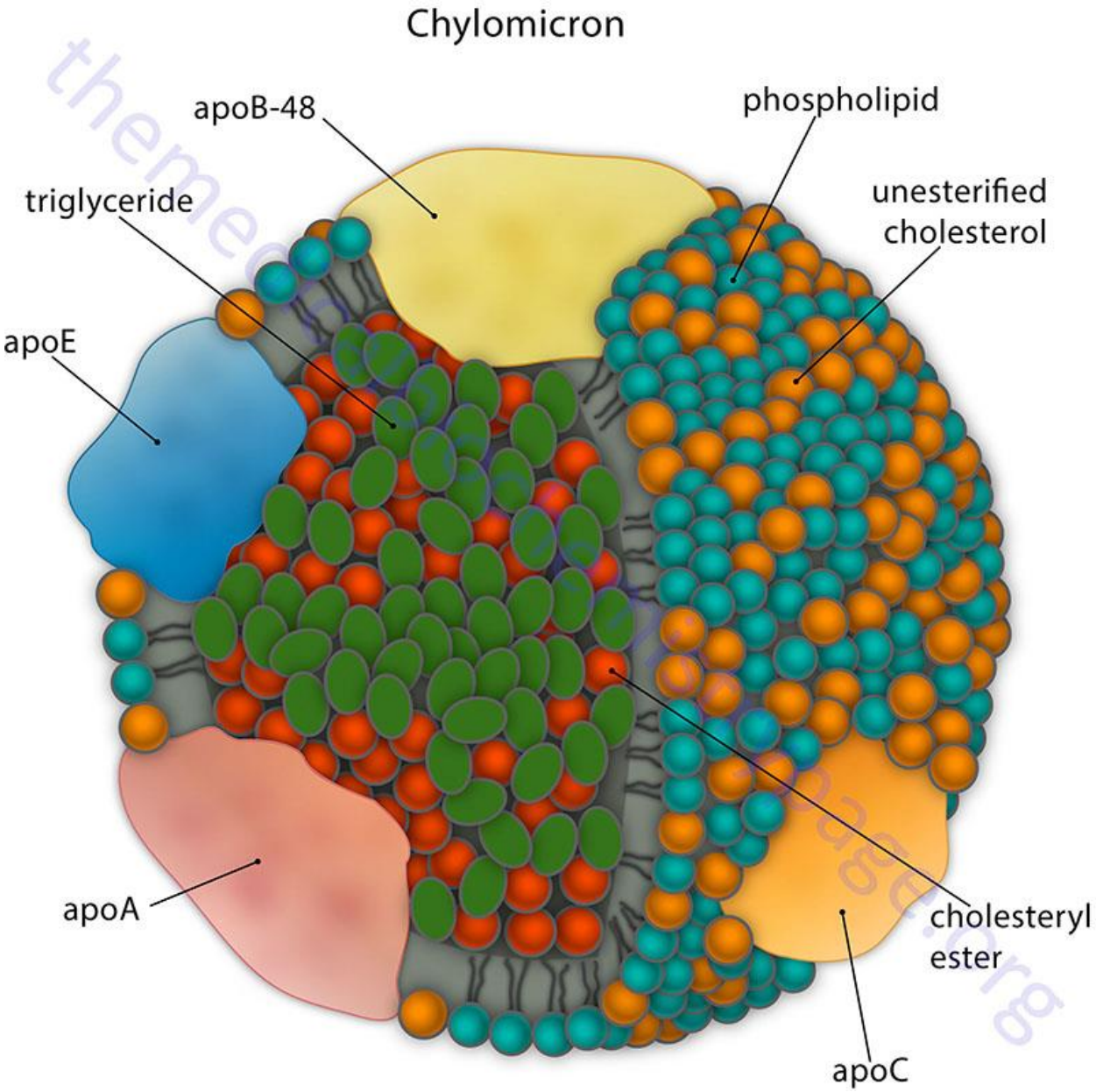

FIGURE 1. Structure of a chylomicron as a representative structure of a typical lipoprotein particle. Image demonstrates the phospholipid and free cholesterol outer layer with primarily triglycerides and cholesteryl esters internally. Each lipoprotein type, chylomicron, LDL, and HDL, contain apolipoproteins. Apolipoprotein B-48 (apoB-48) is specific for chylomicrons just as apoB-100 is specific for LDL. (SOURCE: Michael W King, PhD; http://themedicalbiochemistrypage.org/lipoproteins.php)

Table 1. Composition of the Major Lipoprotein Complexes

Complex	Source	Density (g/ml)	%Protein	%TG[a]	%PL[b]	%CE[c]	%C[d]	%FFA[e]
Chylomicron	Intestine	<0.95	1-2	85-88	8	3	1	0
VLDL	Liver	0.95-1.006	7-10	50-55	18-20	12-15	8-10	1
IDL	VLDL	1.006-1.019	10-12	25-30	25-27	32-35	8-10	1
LDL	VLDL	1.019-1.063	20-22	10-15	20-28	37-48	8-10	1
*HDL2	Intestine, liver (chylomicrons and VLDLs)	1.063-1.125	33-35	5-15	32-43	20-30	5-10	0
*HDL3	Intestine, liver (chylomicrons and VLDLs)	1.125-1.21	55-57	3-13	26-46	15-30	2-6	6
Albumin-FFA	Adipose tissue	>1.281	99	0	0	0	0	100

[a]Triglycerides, [b]Phospholipids, [c]Cholesteryl esters, [d]Free cholesterol, [e]Free fatty acids
*HDL2 (HDL_3) and HDL3 (HDL_2) are derived from nascent HDL as a result of the acquisition of apoproteins and cholesteryl esters

Lipid Profile Values

Standard fasting blood tests for cholesterol and lipid profiles will include values for total cholesterol, HDL cholesterol (so-called "good" cholesterol), LDL cholesterol (so-called "bad" cholesterol) and triglycerides. Family history and life style, including factors such as blood pressure and whether or not one smokes, affect what would be considered ideal versus non-ideal values for fasting blood lipid profiles. Included here are the values for various lipids that indicate low to high risk for coronary artery disease.

Total Serum Cholesterol

<200mg/dL = desired values

200–239mg/dL = borderline to high risk

240mg/dL and above = high risk

HDL Cholesterol

With HDL cholesterol the higher the better.

<40mg/dL for men and <50mg/dL for women = higher risk

40–50mg/dL for men and 50–60mg/dL for woman = normal values

>60mg/dL is associated with some level of protection against heart disease

LDL Cholesterol

With LDL cholesterol the lower the better.

<100mg/dL = optimal values

100mg/dL–129mg/dL = optimal to near optimal

130mg/dL–159mg/dL = borderline high risk

160mg/dL–189mg/dL = high risk

190mg/dL and higher = very high risk

Triglycerides

With triglycerides the lower the better.

<150mg/dL = normal

150mg/dL–199mg/dL = borderline to high risk

200mg/dL–499mg/dL = high risk

>500mg/dL = very high risk

Apolipoprotein Classifications

Apoprotein - MW (Da)	Gene	Lipoprotein Association	Function and Comments
apoA-I - 29,016	APOA1 11q23–q24	Chylomicrons, HDL	major protein of HDL, binds ABCA1 on macrophages, critical anti-oxidant protein of HDL, activates lecithin:cholesterol acyltransferase, LCAT
apoA-II - 17,400	APOA2 1q23.3	Chylomicrons, HDL	primarily in HDL, enhances hepatic lipase activity
apoA-IV - 46,000	APOA4 11q23	Chylomicrons and HDL	present in triglyceride rich lipoproteins; synthesized in small intestine, synthesis activated by PYY, acts in central nervous system to inhibit food intake
apoB-48 - 241,000	APOB 2p24–p23	Chylomicrons	exclusively found in chylomicrons, derived from apoB-100 gene by RNA editing in intestinal epithelium; lacks the LDL receptor-binding domain of apoB-100
apoB-100 - 513,000	APOB 2p24–p23	VLDL, IDL and LDL	major protein of LDL, binds to LDL receptor; one of the longest known proteins in humans
apoC-I - 7,600	APOC1 19q13.2	Chylomicrons, VLDL, IDL and HDL	may also activate LCAT; clustered with APOC2 and APOE genes on chromsome 19

apoC-II - 8, 916	APOC2 19q13.2	Chylomicrons, VLDL, IDL and HDL	activates lipoprotein lipase; clustered with APOC1 and APOE genes on chromsome 19
apoC-III - 8,750	APOC3 11q23.3	Chylomicrons, VLDL, IDL and HDL	inhibits lipoprotein lipase, interferes with hepatic uptake and catabolism of apoB-containing lipoproteins, appears to enhance the catabolism of HDL particles, enhances monocyte adhesion to vascular endothelial cells, activates inflammatory signaling pathways
apoD, 33,000	APOD 3q29	HDL	closely associated with LCAT
cholesterol ester transfer protein, CETP	CETP 16q21	HDL	plasma glycoprotein secreted primarily from the liver and is associated with cholesteryl ester transfer from HDLs to LDLs and VLDLs in exchange for triglycerides
apoE - 34,000 (at least 3 alleles E2, E3, E4); the apoE2 allele has Cys at amino acids 112 and 158; apoE3 has Cys and Arg at these two positions, respectively; apoE4 has Arg at both positions	APOE 19q13.2	Chylomicron remnants, VLDL, IDL and HDL	binds to LDL receptor; clustered with APOC1 and APOC2 genes on chromsome 19; apoEε4 allele amplification associated with late-onset Alzheimer's disease
apoH - 50,000 (also known as β_2-glycoprotein I)	APOH 17q24.2	negatively charged surfaces	inhibits serotonin release from platelets, alters ADP-mediated platelet aggregation

apo(a) - protein ranges in size from 300,000–800,000 as a result of from 2–43 copies of the Kringle-type domain; Kringle domains contain around 80 amino acids which form the domain via three intrachain disulfide bonds	LPA 6q26	LDL	disulfide bonded to apoB-100, forms a complex with LDL identified as lipoprotein(a), Lp(a); strongly resembles plasminogen; may deliver cholesterol to sites of vascular injury, high risk association with premature coronary artery disease and stroke

Apolipoprotein A-IV and the Control of Feeding Behaviors

Apolipoprotein A-IV (apoA-IV) is synthesized exclusively in the small intestine and the hypothalamus. The apoA-IV gene (gene symbol = APOA4) is located on chromosome 11q23 and is closely linked to the apoA-I and apoC-III genes. The gene is composed of only two exons and encodes a protein of 46 kDa. Utilizing isoelectric focusing it has been determined that two isoforms of apoA-IV, designated A-IV-1 and A-IV-2, can be identified in plasma. Intestinal synthesis of apoA-IV increases in response to ingestion and absorption of fat and it is subsequently incorporated into chylomicrons and delivered to the circulation via the lymphatic system. Systemic apoA-IV has been shown to have effects in the CNS involving the sensation of satiety.

Intestinal apoA-IV

Following the consumption of fat, the intestinal absorption of the lipid content stimulates the synthesis and secretion of apoA-IV. The increased production of apoA-IV by the small intestine in response to lipid absorption is the result of enhanced transcription of the apoA-IV gene in intestinal enterocytes. The precise signal for this increase in intestinal transcription is the formation and secretion of chylomicrons. It has been shown that neither digestion, uptake, or the re-esterification of absorbed monoglycerides and fatty acids to form triglyceride is the inducing signal for apoA-IV transcription. This was conclusively demonstrated in experiments showing that the intestinal absorption of only myristic acid or long-chain fatty acids is sufficient to stimulate the lymphatic transport of both chylomicrons and apoA-IV. However, it is still unclear whether different types of triglyceride (those containing either saturated, monounsaturated, or polyunsaturated fatty acids) are equally effective in stimulating the secretion of apoA-IV. Although it is known that chylomicrons serve as the inducing signal for apoA-IV transcription and secretion, the precise mechanism by which the

transcriptional enhancement is effected is currently undetermined. What is known is that an intact vagal innervation from the CNS to the gut is not necessary since vagotomy does not affect intestinal apoA-IV synthesis in response to lipid absorption.

Leptin is a peptide synthesized and secreted by adipocytes whose principle effects result in decreased food intake and increased energy expenditure. The levels of circulating leptin increase in response to the consumption of a high-fat diet and are directly correlated to the amount of fat stored in adipose tissue. The level of apoA-IV transcription has been shown to be reduced within 90 minutes of ingesting a high fat meal and this reduction is a result of increased leptin secretion. Although numerous studies have demonstrated a negative correlation between leptin levels and apoA-IV expression, the mechanism by which this effect is exerted is not fully understood. There are leptin receptors in the gut and, therefore, leptin binding to these receptors could lead to direct effects on intestinal enterocytes. Alternatively, leptin could exert indirect effects on intestinal cells by increasing fatty acid oxidation through the induction of enzymes that shift fuel metabolism to favor β-oxidation of fatty acids. Given that circulating leptin levels increase as an individual becomes more obese it is likely that leptin is involved in the attenuation of the intestinal apoA-IV response to lipid ingestion. Although the initial response to consumption of a high-fat diet is increased plasma apoA-IV levels, this increase disappears over time. This finding makes it tempting to speculate that the autoregulation of apo AIV in response to chronic high fat feeding is related to the elevation of circulating leptin.

Direct infusion of lipid into the ileum results in increased expression of ileal and jejunal apoA-IV, whereas, infusion of lipid into the duodenum only results in increased jejunal apoA-IV expression. These results strongly suggest that a signal is released by the distal gut during active lipid absorption which is capable of stimulating apoA-IV synthesis in the proximal gut. A strong candidate for this signal is the ileal peptide PYY. To determine if PYY is indeed involved in increased apoA-IV expression experiments were performed in rats involving intravenous injections of physiological doses of PYY. These experiments showed that PYY infusion does indeed result in significant stimulation of jejunal apoA-IV synthesis and lymphatic transport in fasting animals. Further experiments demonstrated that the stimulation of jejunal apoA-IV synthesis by PYY is the result of effects on translation of the mRNA as opposed to increased transcription of the gene since the levels of the mRNA were unaltered but synthesis of the protein was markedly stimulated. Whereas fat absorption-mediated increases in apoA-IV expression do not require vagal innervation, the responses to PYY do involve the vagal nerve.

Hypothalamic apoA-IV and satiety

Only recently was it determined that both the mRNA and apoA-IV protein are present in the hypothalamus, primarily in the ARC. The presence of apoA-IV in the hypothalamus, a site intimately involved in regulating energy homeostasis, suggests that the effects exerted on appetite by apoA-IV may be due to direct hypothalamic synthesis and secretion. Experiments in rodents, aimed at determining the role of apoA-IV in hypothalamic functions, clearly demonstrated a role for this apolipoprotein in feeding behaviors. Blocking apoA-IV actions by central injection of antibodies to the protein results in increased food consumption, even during the light phase when rodents normally do not eat. Additional studies have shown that apoA-IV is involved in inhibiting food intake following the ingestion of fat. Infusion of lymph fluid that contains chylomicrons results in markedly suppressed food intake during the first 30 min of administration. However, it is not the lipid content of the chylomicrons that is responsible for the suppression of food intake since infusion of a mixture of triglycerides and phospholipids does not exert the same effect. If apoA-IV is removed from chylomicrons prior to infusion, via the use of specific antibodies, there is no observed effect on food intake. If apoA-IV itself is infused, the level of suppression of food intake is the same as that seen with infusion of fatty lymph fluid containing chylomicrons.

The plasma levels of apoA-IV in humans adapt in response to prolonged consumption of fat. Chronic consumption of a high-fat diet initially results in significantly elevated plasma apoA-IV levels. However, the increased level disappears over time. Conversely, on a low-fat diet, intestinal apoA-IV gene expression is sensitive to fasting and lipid feeding, being low during fasting and high during lipid absorption. Consumption of a high-fat diet results in a slow and progressive reduction in hypothalamic apoA-IV mRNA over time. The response of hypothalamic apoA-IV gene expression to chronic consumption of a high-fat diet is only partially similar to the response seen in the small intestine. In animals that are chronically fed a high-fat diet there is no observable increase in hypothalamic apoA-IV expression in response to intragastric infusion of lipid following a period of fasting. In contrast, intragastric infusion of lipid into fasted animals that have been consuming normal chow, results in significant stimulation of hypothalamic apoA-IV mRNA levels. These results demonstrate that chronic consumption of a high-fat diet significantly reduces apoA-IV mRNA levels and the response of hypothalamic apoA-IV gene expression to dietary lipids. Therefore, it is highly likely that dysregulation of hypothalamic apoA-IV could contribute to diet-induced obesity.

Chylomicrons

Chylomicrons are assembled in the intestinal mucosa as a means to transport dietary cholesterol and triglycerides to the rest of the body. Chylomicrons are, therefore, the molecules formed to mobilize dietary (exogenous) lipids. The predominant lipids of chylomicrons are triglycerides (see Table above). The apolipoproteins that predominate before the chylomicrons enter the circulation include apoB-48 and apoA-I, apoA-II and apoA-IV. ApoB-48 combines only with chylomicrons.

Chylomicrons leave the intestine via the lymphatic system and enter the circulation at the left subclavian vein. In the bloodstream, chylomicrons acquire apoC-II and apoE from plasma HDLs. In the capillaries of adipose tissue and muscle, the fatty acids of chylomicrons are removed from the triglycerides by the action of lipoprotein lipase (LPL), which is found on the surface of the endothelial cells of the capillaries. The apoC-II in the chylomicrons activates LPL in the presence of phospholipid. The free fatty acids are then absorbed by the tissues and the glycerol backbone of the triglycerides is returned, via the blood, to the liver and kidneys. Glycerol is converted to the glycolytic intermediate dihydroxyacetone phosphate (DHAP). During the removal of fatty acids, a substantial portion of phospholipid, apoA and apoC is transferred to HDLs. The loss of apoC-II prevents LPL from further degrading the chylomicron remnants.

Chylomicron remnants, containing primarily cholesteryl esters, apoE and apoB-48, are then delivered to, and taken up by, the liver. The remnant particle must be of a sufficiently small size such that can pass through the fenestrated endothelial cells lining the hepatic sinusoids and enter into the space of Disse. Chylomicron remnants can then be taken up by hepatocytes via interaction with the LDL receptor which requires apoE. In addition, while in the space of Disse chylomicron remnants can accumulate additional apoE that is secreted free into the space. This latter process allows the remnant to be taken up via the chylomicron remnant receptor, which is a member of the LDL receptor-related protein (LRP) family. The recognition of chylomicron remnants by the hepatic remnant receptor also requires apoE. Chylomicron remnants can also remain sequestered in the space of Disse by binding of apoE to heparan sulfate proteoglycans and/or binding of apoB-48 to hepatic lipase. While sequestered, chylomicron remnants may be further metabolized which increases apoE and lysophospholipid content allowing for transfer to LDL receptors or LRP for hepatic uptake.

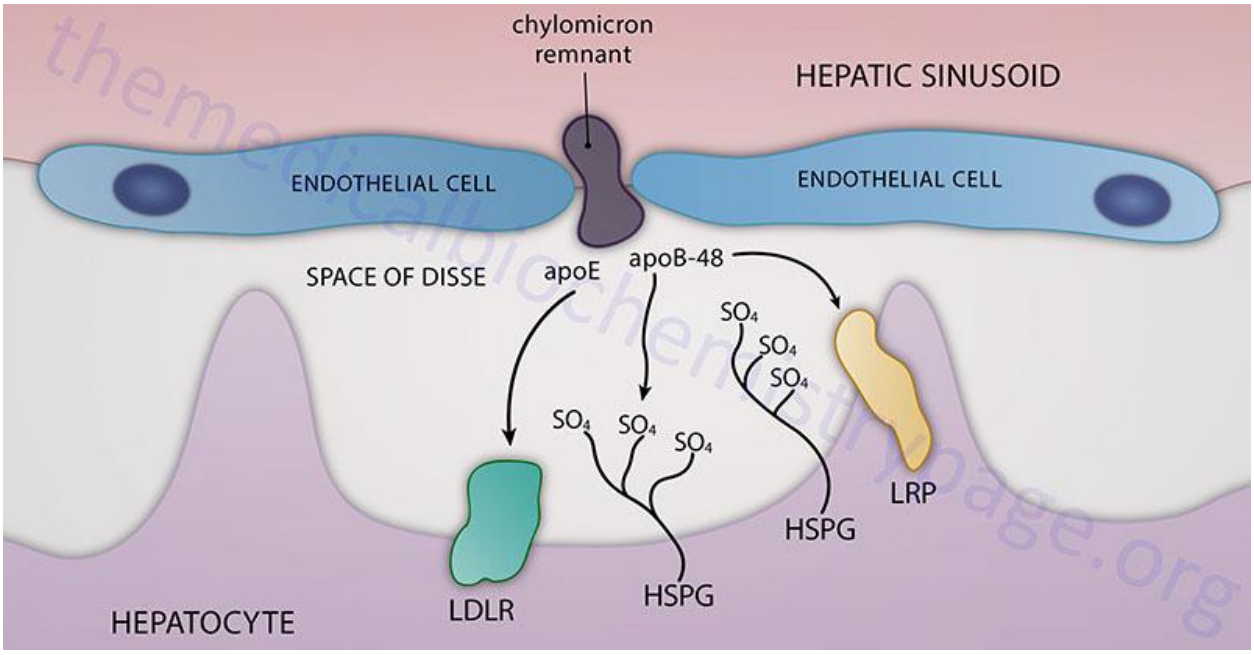

FIGURE 2. Detail of the uptake of chylomicron remnants by the liver. Diagram depicts the interaction of the vasculature of hepatic sinusoids with hepatocytes. The space between hepatic sinusoidal endothelium and hepatocytes is called the space of Disse. Chylomicron remnants containing primarily cholesterol esters, apoE, and apoB-48 are rapidly taken up by the liver. The remnants pass through the endothelial lining of the hepatic sinusoid and in the space of Disse interact with specific receptors as well as heparin sulfated proteoglycans (HSPG). Hepatocyte uptake of remnants is initiated by sequestration of the particles on HSPG followed by receptor-mediated endocytosis of the remnants. The receptor-mediated endocytic process may be mediated by LDL receptors (LDLR) and/or LDL receptor-related protein (LRP). The interaction of remnants with HSPG involves apoB-48 and the interaction with LDLR or LRP involves apoE.

VLDLs, IDLs, and LDLs

The dietary intake of both fat and carbohydrate, in excess of the needs of the body, leads to their conversion into triglycerides in the liver. These triglycerides are packaged into VLDLs and released into the circulation for delivery to the various tissues (primarily muscle and adipose tissue) for storage or production of energy through oxidation. VLDLs are, therefore, the molecules formed to transport endogenously derived triglycerides to extra-hepatic tissues. In addition to triglycerides, VLDLs contain some cholesterol and cholesteryl esters and the apoproteins, apoB-100 (a single copy), apoC-

I, apoC-II, apoC-III and apoE. Like nascent chylomicrons, newly released VLDLs acquire apoCs and apoE from circulating HDLs.

The fatty acid portion of VLDLs is released to adipose tissue and muscle in the same way as for chylomicrons, through the action of lipoprotein lipase. The action of lipoprotein lipase coupled to a loss of certain apoproteins (the apoCs) converts VLDLs to intermediate density lipoproteins (IDLs), also termed VLDL remnants. IDLs contain multiple copies of apoE and a single copy of apoB-100. The presence of the multiple copies of apoE enable these lipoprotein particles to have very high affinity for the LDL receptor on cells such as hepatocytes and adrenal cortex cells. Conversion of VLDL to IDL is also associated with loss of apoCs by transfer back to HDLs. Further loss of triglycerides, as well as transfer of apoE back to HDL converts IDLs to LDLs. The presence of the apoB-100 protein allows LDL to be recognized by the LDL receptor but the lack of apoE makes the affinity much lower than that of IDL.

The liver takes up IDLs after they have interacted with the LDL receptor to form a complex, which is endocytosed by the cell. For LDL receptors in the liver to recognize IDLs requires the presence of apoB-100 and is enhanced in the presence of apoE. The LDL receptor is also sometimes referred to as the apoB-100/apoE receptor. The importance of apoE in cholesterol uptake by LDL receptors has been demonstrated in transgenic mice lacking functional apoE genes. These mice develop severe atherosclerotic lesions at 10 weeks of age.

The cellular requirement for cholesterol as a membrane component is satisfied in one of two ways: either it is synthesized *de novo* within the cell, or it is supplied from extra-cellular sources, namely, chylomicrons and IDL/LDL. As indicated above, the dietary cholesterol that goes into chylomicrons is supplied to the liver by the interaction of chylomicron remnants with the remnant receptor. In addition, cholesterol synthesized by the liver can be transported to extra-hepatic tissues if packaged in VLDLs. In the circulation VLDLs are converted to IDLs and LDLs through the action of lipoprotein lipase. IDLs and LDLs are the primary plasma carriers of cholesterol for delivery to all tissues.

The exclusive apolipoprotein of LDLs is apoB-100. LDLs are taken up by cells via LDL receptor-mediated endocytosis, as described above for IDL uptake. The uptake of LDLs occurs predominantly in liver (75%), adrenals and adipose tissue. As with IDLs, the interaction of LDLs with LDL receptors requires the presence of apoB-100. The endocytosed membrane vesicles (endosomes) fuse with lysosomes, in which the apoproteins are degraded and the cholesterol esters are hydrolyzed to yield free cholesterol. The

cholesterol is then incorporated into the plasma membranes as necessary. Excess intracellular cholesterol is re-esterified by sterol *O*-acyltransferase 2 (SOAT2), for intracellular storage. The activity of SOAT2 is enhanced by the presence of intracellular cholesterol. The original name given to SOAT2 was acyl-CoA: cholesterol acyltransferase 2 (ACAT2). This designation conflicts with that for the official ACAT2 enzyme (a thiolase), acetyl-CoA acetyltransferase 2. The SOAT2 gene is located on chromosome 12q13.13 and is composed of 16 exons that encode a 522 amino acid protein. Another SOAT gene, SOAT1, is also involved in the regulation of intracellular cholesterol concentrations. The SOAT1 gene is located on chromosome 1q25 and is composed of 17 exons that generate three alternatively spliced mRNAs.

Insulin and tri-iodothyronine (T3) increase the binding of LDLs to liver cells, whereas glucocorticoids (e.g., dexamethasone) have the opposite effect. The precise mechanism for these effects is unclear but may be mediated through the regulation of apoB degradation. The effects of insulin and T3 on hepatic LDL binding may explain the hypercholesterolemia and increased risk of atherosclerosis that have been shown to be associated with uncontrolled diabetes or hypothyroidism.

The consumption of alcohol is associated with either a protective or a negative effect on the level of circulating LDL. Low level alcohol consumption, particularly red wines which contain the antioxidant resveratrol, appear to be beneficial with respect to cardiovascular health. Resveratrol consumption is associated with a reduced risk of cardiovascular, cerebrovascular, and peripheral vascular disease. One major effect of resveratrol in the blood is the prevention of oxidation of LDLs, (forming oxLDL). Oxidized LDLs contribute significantly to the development of atherosclerosis. Conversely excess alcohol consumption is associated with the development of fatty liver which in turn impairs the ability of the liver to take up LDL via the LDL receptor resulting in increased LDL in the circulation. Clearly a reduction in alcohol consumption will have a significant impact on overall cardiovascularr and hepatic function.

An abnormal form of LDL, identified as lipoprotein-X (**Lp-X**), predominates in the circulation of patients suffering from lecithin-cholesterol acyl transferase (**LCAT**, see HDL discussion for LCAT function) deficiency or cholestatic liver disease. In both cases there is an elevation in the level of circulating free cholesterol and phospholipids.

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High Density Lipoproteins, HDLs

HDLs represent a heterogeneous population of lipoproteins in that they exist as functionally distinct particles possessing different sizes, protein content, and lipid composition. One of the major functions of HDLs is to acquire cholesterol from peripheral tissues and transport this cholesterol back to the liver where it can ultimately be excreted following conversion to bile acids. This function is referred to as reverse cholesterol transport (RCT). The role of HDLs in RCT represents the major atheroprotective (prevention of the development of atherosclerotic lesions in the vasculature) function of this class of lipoprotein. In addition to RCT, HDLs exert anti-inflammatory, antioxidant, and vasodilatory effects that together represent addition atheroprotective functions of HDLs. Evidence has also been generated that demonstrates that HDLs possess anti-apoptotic, anti-thrombotic, and anti-infectious properties. With respect to these various atheroprotective functions of HDLs, it is the small dense particles (referred to as HDL_3) that are the most beneficial.

HDLs are synthesized *de novo* in the liver and small intestine, as primarily protein-rich disc-shaped particles. These newly formed HDLs are nearly devoid of any cholesterol and cholesteryl esters. The primary apoproteins of HDLs are apoA-I, apoC-I, apoC-II and apoE. In fact, a major function of HDLs is to act as circulating stores of apoC-I, apoC-II and apoE. ApoA-I is the most abundant protein in HDLs constituting over 70% of the total protein mass. In addition to apoproteins, HDLs carry numerous enzymes that participate in the anti-oxidant activities. These enzymes include glutathione peroxidase 1 (GPx), paraoxonase 1 (PON1) and platelet activating factor acetylhydrolase (PAF-AH, also called lipoprotein-associated phospholipase A_2, Lp-PLA_2: see below for functions of Lp-PLA_2). Two additional functionally important enzymes found associated with HDLs are lecithin:cholesterol acyltransferase (LCAT, see next paragraph) and cholesterol ester transfer protein (CETP, see below and the next section). Another important HDL component is the compound sphingosine-1-phosphate (S1P; details of S1P activities can be found in the Sphingolipids page). Depending upon the HDL subclass characterized, as many as 75 different proteins have been shown to be associated with circulating HDLs.

The primary mechanism by which HDLs acquire peripheral tissue cholesterol is via an interaction with monocyte-derived macrophages in the subendothelial spaces of the tissues. Macrophages bind nascent HDLs, that contain primarily apoA-I, through interaction with the ATP-binding cassette transport protein A1 (ABCA1). The transfer of cholesterol from macrophages via the action of ABCA1, involves apoA-I and results in the formation of

nascent discoidal lipoprotein particles termed pre-β HDLs. The free cholesterol transferred in this way is esterified by HDL-associated LCAT. LCAT is synthesized in the liver and so named because it transfers a fatty acid from the C-2 position of lecithin to the C-3-OH of cholesterol, generating a cholesteryl ester and lysolecithin. The activity of LCAT requires interaction with apoA-I, which is found on the surface of HDLs. The cholesteryl esters formed via LCAT activity are internalized into the hydrophobic core of the pre-β HDL particle. As pre-β HDL particles enlarge with progressive uptake of cholesterol they become larger and spherical generating the HDL_2 and HDL_3 particles as indicated above. The importance of ABCA1 in reverse cholesterol transport is evident in individuals harboring defects in ABCA1 gene. These individuals suffer from a disorder called Tangier disease which is characterized by two clinical hallmarks; enlarged lipid-laden tonsils and low serum HDL.

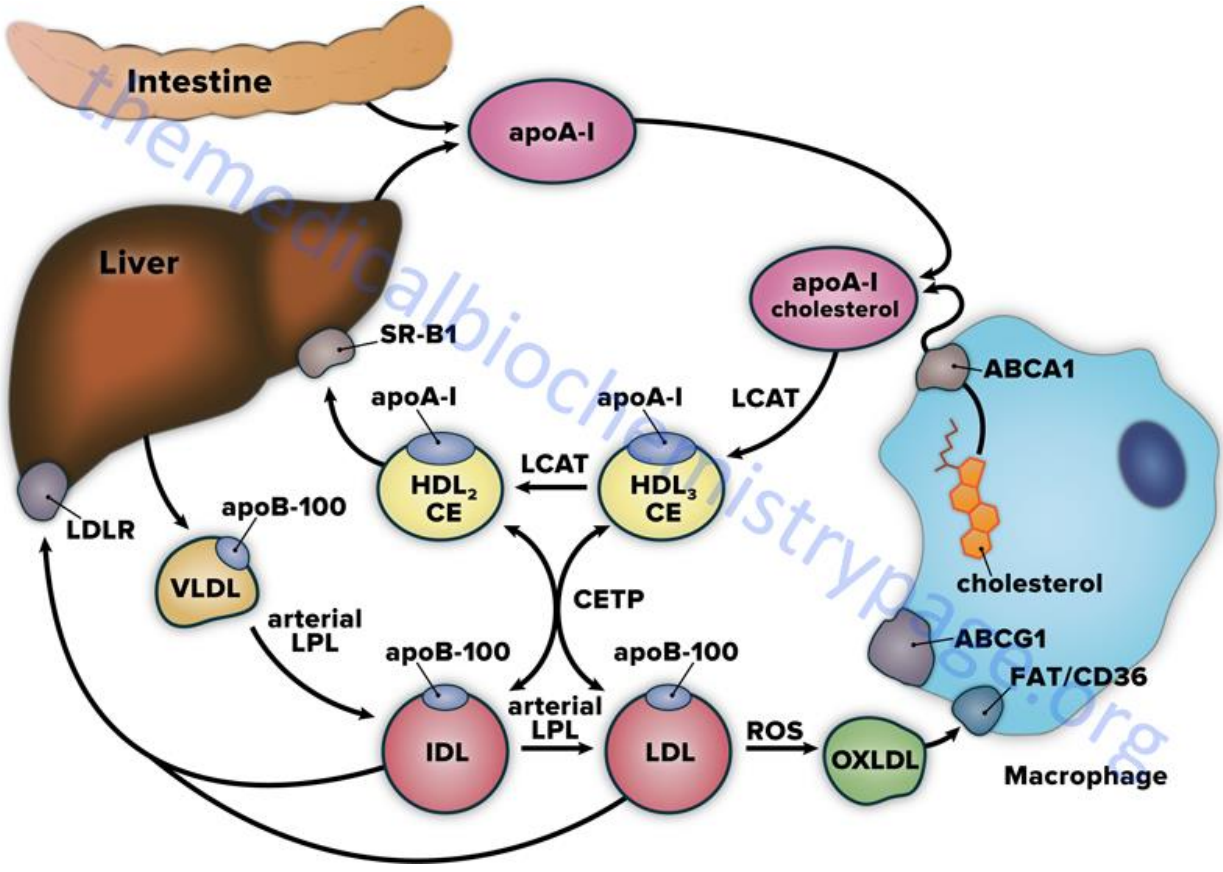

FIGURE 3. Detail of the interactions between HDL and LDL within the vasculature. As indicated in the text HDL begins as protein-rich discoidal structures, composed primarily of apoA-I, produced by the liver and intestines. Within the vasculature apoA-I interacts with the ATP-binding cassette transporter, ABCA1 (such as is diagrammed for interaction with

macrophages) and extracts cholesterol from cells. Through the action of LCAT the apoA-I-associated cholesterol is esterified forming cholesterol esters. This process results in the generation of HDL_3 particles. As the HDL_3 particles continue through the circulation they pick up more cholesterol and through the action of LCAT, generate more cholesterol esters. As HDL migrates through the vasculature there is an interaction between them and IDL and LDL. This interaction occurs through the action of CETP which exchanges the cholesterol esters in the HDL for triglycerides from LDL. This interaction results in the conversion of HDL_3 particles to HDL_2. The differences between these two types of HDL particle are detailed in the Table at the start of this page. HDL can also remove cholesterol from cells via interaction with the ATP-binding cassette transporter ABCG1. Approximately 20% of HDL uptake of cellular cholesterol occurs via ABCG1. HDL is then removed from the circulation by the liver through binding of the HDL to hepatic scavanger receptor SR-B1. Cholesterol ester-rich IDL and LDL can return to the liver and be taken up through interaction with the LDL receptor (LDLR). Within the vasculature the generation of ROS results in oxidation of lipid components of LDL generating oxidized LDL (oxLDL) which is taken up by macrophages via the scavenger receptor, FAT/CD36.

HDLs also acquire cholesterol by extracting it from cell surface membranes. This process has the effect of lowering the level of intracellular cholesterol, since the cholesterol stored within cells as cholesteryl esters will be mobilized to replace the cholesterol removed from the plasma membrane. The transfer of cholesterol from peripheral tissue cells to HDLs in this way involves the action of the ATP-binding cassette protein G1 (ABCG1). Approximately 20% of HDL uptake of peripheral tissue cholesterol occurs via the ABCG1-mediated pathway.

Cholesterol-rich HDLs return to the liver, where they bind to a receptor that is a member of the scavenger receptor family, specifically the scavenger receptor BI: SR-BI (see below). When HDL binds to SR-BI it is not internalized as is the case for LDLs following their binding to the LDL receptor but the cholesteryl esters of HDLs are taken up by the hepatocytes through caveolae while the HDL and SR-BI remain on the plasma membrane. Caveolae (Latin for little caves) are specialized "lipid rafts" present in flask-shaped indentations in the plasma membranes of many cells types that perform a number of signaling functions.

HDL particles exhibit complex, and sometimes contradictory rolls in vascular biology. Depending upon the vascular context, as well as the make-up of HDL particle, these lipoproteins can serve antiatherogenic or proatherogenic functions. In the absence of systemic inflammation many of the enzymes and apolipoproteins associated with HDLs play important roles in reducing the amount of oxidized lipid to which peripheral tissues are exposed. Some of these important proteins are apoA-I, PON1, GPx (an important anti-oxidant enzyme), and PAF-AH (see section below for the discussion of this important activity). However, when an individual has an ongoing systemic inflammatory state, these anti-oxidant proteins can be dissociated from the

HDL or become inactivated resulting in the increased generation of oxidized and peroxidized lipids which are proatherogenic. Atherosclerotic plaques also produce myeloperoxidase which chemically modifies HDL-associated apoA-I rendering it less capable of interacting with cell surfaces such as macrophages. This latter effect results in a reduced capacity for removal of cholesterol from lipid-laden macrophages (foam cells) leaving the foam cells in a more pro-inflammatory state.

Reverse cholesterol transport can also involve the transfer of cholesterol esters from HDLs to VLDLs and LDLs. This transfer requires the activity of the plasma glycoprotein cholesterol ester transfer protein (**CETP**). The transfer of cholesteryl esters from HDLs to VLDLs via CETP activity also involves an exchange of triglycerides from the VLDLs to the HDLs. VLDLs are eventually converted to LDLs and the cholesterol acquired from HDLs can be returned to the liver via the interaction of LDL with the hepatic LDL receptor. This action of HDL CETP has the added effect of allowing the excess cellular cholesterol to be returned to the liver through the LDL receptor. However, some of the LDL is oxidized in the periphery (generating oxLDL) where it can participate in atherogenesis. Additionally, when HDL particles become enriched with triglycerides they are better targets for the action of hepatic lipase. As hepatic lipase acts on the triglyceride-rich HDLs they become progressively smaller and unstable which results in the release of apoA-I. The loss of apoA-I renders the HDL particle unable to participate in reverse cholesterol transport. Blocking the activity of CETP keeps HDL particles less triglyceride-enriched while also reducing cholesterol transfer to VLDLs resulting in reduced circulating levels of proatherogenic oxLDL. This latter observation suggests that CETP inhibition may be a viable therapeutic approach for elevating the circulating levels of HDLs. This is discussed in the section below.

Anti-oxidant & Anti-inflammatory Activities of HDLs

Using a range of both *in vitro* and *in vivo* assays it has been possible to quantify the anti- and pro-inflammatory properties, as well as the anti-oxidant functions of HDLs. Cell-free assays have been used to measure the ability of HDLs to prevent the formation of oxidized phospholipids in LDLs as well as to determine the ability of HDLs to degrade oxidized phospholipids that are already formed. In cell culture assays HDLs have been shown to

inhibit monocyte chemotaxis in response to oxidized LDL or to prevent the upregulation of cell adhesion molecules on endothelial cells. Both of these latter effects are strongly anti-inflammatory since monocytes need to migrate to a site of inflammation via a chemotactic gradient and then adhere to the endothelium at the site of injury or inflammatory event. The role of HDLs in promoting cholesterol efflux from cells, especially from macrophages, (the process of reverse cholesterol transport) reduces the activation of inflammatory responses in these cells. The analysis of HDL functions in oxidative and inflammatory events has identified the role of various apolipoproteins associated with HDLs in these processes which are outlined in the following sections.

Apolipoprotein A-I: Numerous lines of evidence demonstrate that apoA-I is a major anti-atherogenic and anti-oxidant factor in HDL due to its critical role in the HDL-mediated process of reverse cholesterol transport. In addition to reverse cholesterol transport, apoA-I can remove oxidized phospholipids from oxidized LDLs (oxLDLs) and from cells. Specific methionine residues (Met112 and Met148) of apoA-I have been shown to directly reduce cholesterol ester hydroperoxides and phosphatidylcholine hydroperoxides.

Apolipoprotein A-II: Experiments in transgenic mice have demonstrated that human apoA-II-enriched HDLs served to protect VLDLs from oxidation more efficiently than HDLs from control animals. The human apoA-II-enriched HDLs support highly effective reverse cholesterol transport from macrophages. Although there is a demonstrated benefit of apoA-II in reverse cholesterol transport and in reduced LDL oxidation, these transgenic mice exhibited increased displacement of PON1 and PAF-AH from HDLs. The displacement of these two beneficial HDL-associated proteins (see below) likely explains the increased atherosclerosis seen in dyslipidemic mice that overexpress either human or murine apoA-II. However, recent clinical studies in human patients show that the higher the plasma apoA-II concentration the lower is the risk of developing coronary artery disease (CAD).

Apolipoprotein A-IV: Apolipoprotein A-IV has multiple activities related to lipid and lipoprotein metabolism as well as the control of feeding behaviors (see earlier section related to this protein). ApoA-IV participates in reverse cholesterol transport by promoting cholesterol efflux as well as through by activation of LCAT. ApoA-IV has also been shown to have anti-oxidant, anti-inflammatory and anti-atherosclerotic actions. ApoA-IV is secreted only by the small intestine in humans (although it is expressed in the hypothalamus) and its synthesis in the gut is stimulated by active lipid absorption. Intestinal apoA-IV synthesis is enhanced by peptide tyrosine-tyrosine (PYY) secreted

from the ileum. Intestinal apoA-IV, present in the circulation following ingestion of fat, as well as hypothalamic apoA-IV is an anorexigenic peptide which mediates, in part, the appetite suppressing effects of a lipid-rich meal.

Apolipoprotein E: The anti-atherosclerotic activity associated with apoE is well known. This beneficial effect of apoE is due primarily to its role in the process of receptor-mediated uptake of LDLs by the liver. Although apoE-mediated hepatic uptake of LDLs results in a reduction in hypercholesterolemia, apoE has also been shown to inhibit atherosclerosis without any significant effect on hypercholesterolaemia. In addition, different apoE alleles have demonstrated activities. For example apoE2 stimulates endothelial nitric oxide (NO) release and has anti-inflammatory activities, whereas, apoE4 is pro-inflammatory.

Paraoxonases 1 and 3: Paraoxonases are a family of enzymes that hydrolyze organophosphates. Paraoxonase 1 (PON1) is synthesized in the liver and is carried in the serum by HDL. PON1 possesses anti-oxidant properties, in particular it prevents the oxidation of LDLs. Evidence suggests that the direct anti-oxidant effect of HDLs, on LDL oxidation, is mediated by PON1. PON1 has been shown to enhance cholesterol efflux from macrophages by promoting HDL binding mediated by ABCA1, which in turn results in a reduction of pro-inflammatory signaling. This anti-inflammatory action of PON1 serves an anti-atherosclerotic function of the protein. That PON1 is indeed important in preventing atherosclerosis has been demonstrated in mice deficient in the protein. Atherosclerotic lesions that develop in these mice when fed a high-fat diet are twice the size that develop in similarly fed control mice. In human clinical studies, a higher level of PON1 activity is associated with a lower incidence of major cardiovascular events. Other pathological conditions in humans that are associated with oxidative stress, such as rheumatoid arthritis and Alzheimer disease, are frequently associated with reduced activity of PON1.

PON3, which is another HDL-associated paraoxonase, has also been shown to prevent the oxidation of LDL. Transgenic mice expressing human PON3 have been shown to be protected from the development of atherosclerosis, without any significant changes in plasma lipoprotein cholesterol, triglyceride or glucose levels.

Platelet-activating factor acetylhydrolase (PAF-AH): There are two major forms of PAF-AH, cytosolic and plasma lipoprotein-associated. The plasma form of PAF-AH circulates bound to HDLs. Given that PAF-AH is a member of the PLA_2 family and that it also circulates bound to lipoprotein it is more commonly referred to as the lipoprotein-associated PLA_2 (Lp-PLA_2 section below). Experimental data suggests that Lp-PLA_2, rather than PON1,

is the major HDL-associated hydrolase that is responsible for the hydrolysis of oxidized phospholipids. Lipoproteins that are isolated from transgenic mice expressing human Lp-PLA_2 are more resistant to oxidative stress. In addition, these mice have been shown to have reduced levels of foam cell (lipid-rich macrophages) formation and enhanced rates of cholesterol efflux from macrophages. In experimental atherosclerosis models, gene transfer of LP-PLA_2 inhibits atherosclerotic lesion formation in apoE-deficient mice. In humans, Lp-PLA_2 deficiency is associated with increases in cardiovascular disease, while conversely circulating levels of Lp-PLA_2 serve as an independent marker of the risk for developing coronary artery disease.

Glutathione peroxidase 1: Glutathione peroxidase 1 (GPx1) functions primarily to reduce hydrogen peroxide to water, but it has been shown to also reduce lipid hydroperoxides to corresponding hydroxides effectively detoxifying these types of abnormally modified lipids. Numerous human clinical studies indicated that GPx1 provides a protective role against the development of atherosclerosis. These effects of GPx1 have also been shown in mice deficient in apoE where concomitant loss of the peroxidase results in increased rates of atherosclerotic plaque formation. The role of GPx1 in the protection from development of atherosclerosis is most pronounced under conditions of significant oxidative stress.

Sphingosine-1-phosphate (S1P): S1P is a bioactive lysophospholipid involved in a number of physiologically important pathways. For more detailed information of S1P activities visit the Sphingolipids page. Within the blood, HDLs are known to be the most prominent carriers of S1P. Indeed, many of the biological effects of HDL are mediated, in part, via S1P binding to its cell surface receptors. Effects of HDL on endothelial cells, such as migration, proliferation, and angiogenesis, are mediated, in part, by S1P associated with HDLs. HDL-associated S1P inhibits pro-inflammatory responses, such as the generation of reactive oxygen species, activation of NAD(P)H oxidase and the production of monocyte chemoattractant protein-1. While the HDL-associated forms of S1P exhibit these anti-inflammatory effects, free plasma S1P can activate inflammatory events dependent upon the receptor sub-type to which it binds.

Therapeutic Benefits of Elevating HDLs

Numerous epidemiological and clinical studies over the past 10 years have demonstrated a direct correlation between the circulating levels of HDL

cholesterol (most often abbreviated HDL-c) and a reduction in the potential for atherosclerosis and coronary heart disease (CHD). Individuals with levels of HDL above 50mg/dL are several time less likely to experience CHD than individuals with levels below 40mg/dL. In addition, clinical studies in which apoA-I, (the predominant protein component of HDL-c) or reconstituted HDLs are infused into patients, raises circulating HDL levels and reduces the incidence of CHD. Thus, there is precedence for therapies aimed at raising HDL levels in the treatment and prevention of atherosclerosis and CHD. Unfortunately current therapies only modestly elevate HDL levels. Both the statins and the fibrates have only been shown to increase HDL levels between 5%–20% and niacin is poorly tolerated in many patients. Therefore, alternative strategies aimed at increasing HDL levels are being tested.

Cholesterol ester transfer protein (CETP) is plasma glycoprotein secreted primarily from the liver and plays a critical role in HDL metabolism by facilitating the exchange of cholesteryl esters (CE) from HDL for triglycerides (TG) in apoB containing lipoproteins, such as LDL and VLDL. The activity of CETP directly lowers the cholesterol levels of HDLs and enhances HDL catabolism by providing HDLs with the TG substrate of hepatic lipase. Thus, CETP plays a critical role in the regulation of circulating levels of HDL, LDL, and apoA-I. It has also been shown that in mice naturally lacking CETP most of their cholesterol is found in HDL and these mice are relatively resistant to atherosclerosis. The potential for the therapeutic use of CETP inhibitors in humans was first suggested when it was discovered in 1985 that a small population of Japanese had an inborn error in the CETP gene leading to hyperalphalipoproteinemia and very high HDL levels. To date three CETP inhibitors have been used in clinical trials. These compounds are anacetrapib, torcetrapib, and dalcetrapib. Although torcetrapib is a potent inhibitor of CETP, its' use has been discontinued due to increased negative cardiovascular events and death rates in test subjects. Treatment with dalcetrapib results in increases in HDL (19–37%) and a modest decrease (≈6%) in LDL levels. Treatment with anacetrapib results in a significant increase in both HDL (≈130%) and LDL (≈40%). Anacetrapib is currently in phase III clinical studies.

As described in the section below on therapeutic intervention in hyperlipidemias/hypercholesterolemias, the fibrates (e.g. fenofibrate) are a class of drugs that has been shown to result in small increases in HDL levels. The fibrates function by activation of the peroxisome proliferator-activated receptor-α (PPARα) class of transcription co-activators. However, the level of HDL increase with the current PPARα agonists is minimal at best primarily due to lack of specificity for PPARα. Therefore, current research is focused on subtype-specific PPARα agonists that have increased potency. One

compound currently being tested, GFT505, is a selective PPARα agonist with a potency 100-fold greater than fenofibrate.

The liver X receptors (LXRα and LXRβ) are transcription co-activators that are involved in the regulation of lipid metabolism and have also been associated with regulation of inflammation. LXR agonists have been shown to inhibit the progression of atherosclerosis in mouse models of the disorder. Although the precise mechanism by which these LXR agonists effect a reduction in the progression of atherosclerosis is not clear, it is known that the genes encoding ABCA1 and ABCG1 contain LXR-binding sites. In fact, LXR agonists up-regulate the expression of both ABCA1 and ABCG1 in macrophages which leads to increased reverse cholesterol transport. Less cholesterol in macrophages leads to a reduced inflammatory activity of the macrophage which in turn likely contributes to the reduced atherosclerosis. However, there is a limitation to the utility of LXR agonists as shown by the first generation synthetic LXR ligands which activate both LXRs and lead to marked increases in hepatic lipogenesis and plasma triglyceride levels. These effects are due to the role of LXRs in activation of hepatic SREBP-1c and the resultant activation of each of its target genes as described above. Although it could be theoretically possible to enhance the reverse cholesterol effects of LXRs without targeting hepatic lipogenesis with the use of LXRβ-specific ligands since most of the hepatic responses are due to activation of LXRα, this will be a difficult challenge as the ligand binding pocket in both isoforms has been shown to be nearly identical. In addition, there are species-specific differences in overall LXR responses that need to be carefully considered meaning the use of animal models that more closely resemble humans in their metabolic pathways.

Lipoprotein Receptors

LDL Receptors

LDLs are the principal plasma carriers of cholesterol delivering cholesterol from the liver (via hepatic synthesis of VLDLs) to peripheral tissues, primarily the adrenals and adipose tissue. LDLs also return cholesterol to the liver. The cellular uptake of cholesterol from LDLs occurs following the interaction of LDLs with the LDL receptor (also called the apoB-100/apoE receptor). The sole apoprotein present in LDLs is apoB-100, which is required for interaction with the LDL receptor.

The LDL receptor is a polypeptide of 839 amino acids that spans the plasma membrane. An extracellular domain is responsible for apoB-100/apoE binding. The intracellular domain is responsible for the clustering of LDL receptors into regions of the plasma membrane termed coated pits. Once LDL binds the receptor, the complexes are rapidly internalized (endocytosed). ATP-dependent proton pumps lower the pH in the endosomes, which results in dissociation of the LDL from the receptor. The portion of the endosomal membranes harboring the receptor are then recycled to the plasma membrane and the LDL-containing endosomes fuse with lysosomes. Acid hydrolases of the lysosomes degrade the apoproteins and release free fatty acids and cholesterol. As indicated above, the free cholesterol is either incorporated into plasma membranes or esterified (by SOAT2) and stored within the cell.

The level of intracellular cholesterol is regulated through cholesterol-induced suppression of LDL receptor synthesis and cholesterol-induced inhibition of cholesterol synthesis. The increased level of intracellular cholesterol that results from LDL uptake has the additional effect of activating SOAT2, thereby allowing the storage of excess cholesterol within cells. However, the effect of cholesterol-induced suppression of LDL receptor synthesis is a decrease in the rate at which LDLs and IDLs are removed from the serum. This can lead to excess circulating levels of cholesterol and cholesteryl esters when the dietary intake of fat and cholesterol exceeds the needs of the body. The excess cholesterol tends to be deposited in the skin, tendons and (more gravely) within the arteries, leading to atherosclerosis.

LDL Receptor-Related Proteins (LRPs)

The LDL receptor-related protein family represents a group of structurally related transmembrane proteins involved in a diverse range of biological activities including lipid metabolism, nutrient transport, protection against atherosclerosis, as well as numerous developmental processes. The LDL receptor (LDLR) described above represents the founding member of this family of proteins. The LRPs include LRP1, LRP1b, LRP2 (also called megalin), LRP4 (also called MEGF7 for multiple epidermal growth factor-like domains protein 7), LRP5/6, LRP8 (also called apolipoprotein E receptor 2), the VLDL receptor (VLDLR), and LR11/SorLA1 (LDL receptor relative with 11 ligand binding repeats/sorting protein related receptor containing LDLR class A repeats).

LRP1 is also known as CD91 or α_2-macroglobulin receptor. This receptor is expressed in numerous tissues and is known to be involved in diverse activities that include lipoprotein transport, modulation of platelet derived growth factor receptor-β (PDGFRβ) signaling, regulation of cell-surface

protease activity, and the control of cellular entry of bacteria and viruses. Regulation of PDFGRβ activity mediates the protective effects of LRP1 in development of atherosclerosis. LRP1 is synthesized as a 600kDa precursor that is proteolytically processed into a 85kDa transmembrane protein and a 515kDa extracellular protein. The extracellular protein non-covalently associates with the transmembrane protein. LRP1 has been shown to bind more than 40 different ligands that include lipoproteins, extracellular matrix proteins, cytokines and growth factors, protease and protease inhibitor complexes, and viruses. This diverse array of ligands clearly demonstrates that LRP1 is involved in numerous biological and physiological processes.

LRP2 was originally identified as an autoantigen in a rat model of autoimmune kidney disease called Heymann nephritis. LRP2 is expressed in numerous tissues and is found in the apical surfaces of epithelial borders as well as intracellularly in endosomes. In the proximal convoluted tubule of the kidney LRP2 is involved in the reabsorption of numerous molecules. LRP2 binds lipoproteins, hormones, vitamins, vitamin-binding proteins, proteases and, protease inhibitor complexes.

The LRP5/6 proteins serve as co-receptors in Wnt signaling (see the Wnts, TGFs, and BMPs page for more details).

Scavenger Receptors

The founding member of the scavenger receptor family was identified in studies that were attempting to determine the mechanism by which LDL accumulated in macrophages in atherosclerotic plaques. Macrophages ingest a variety of negatively charged macromolecules that includes modified LDLs such as oxidized LDLs (oxLDLs). These studies led to the characterization of two types of macrophage scavenger receptors identified as type I and type II. Subsequent research determined that the scavenger receptor family consists of several families that are identified as class A receptors, class B receptors, mucin-like receptors, and endothelial receptors. After binding ligand the scavenger receptors can either be internalized, similar to the process of internalization of LDL receptors, or they can remain on the cell surface and transfer lipid into the cell through caveolae or they can mediate adhesion.

The class A receptors include the type I and II macrophage scavenger receptors as well as an additional macrophage receptor called MARCO (**ma**crophage **r**eceptor with **co**llagenous structure).

The class B receptors include CD36 and scavenger receptor class B type I (SR-BI). The CD36 receptor is also known as fatty acid translocase (FAT;

thus often designated FAT/CD36) and it is one of the receptors responsible for the cellular uptake of fatty acids as well as for the uptake of oxidized LDL (oxLDL) by macrophages. FAT/CD36 and SR-BI are closely related multi-ligand receptors and are most recognized for their roles in lipid and lipoprotein metabolism. The role of these receptors in platelet function has recently been the focus of numerous studies. Several of the identified ligands for FAT/CD36 include the gut hormone ghrelin, phospatidylserine (PS), β-amyloid, serum amyloid A, bacterial lipopeptides, and specific forms of oxidized phospholipids (oxPLs) either associated with LDLs (referred to a oxLDL) or free that contain an oxidized polyunsaturated fatty acid at the *sn*-2 position. These latter oxPLs are referred to as $oxPC_{CD36}$ because they are predominantly phosphatidylcholine PLs and they bind FAT/CD36. The endothelial receptors that bind oxLDL and are called the LOX-1 receptors. LOX-1 is a member of the C-type lectin superfamily of carbohydrate recognition proteins. The receptor is also called the oxidized LDL receptor 1 (OLR1) and as such the LOX-1 protein is encoded by the OLR1 gene. The mucin-like receptors include CD68/macrosialin and the fruit fly scavenger receptor; dSR-CI.

The SR-BI protein has been shown to be the endogenous receptor for HDLs in the liver. Additionally, the HDL-SR-BI interaction in the adrenal glands is the mechanism for the delivery of cholesterol to the steroid hormone synthesizing cells of this tissue. HDLs first bind to SR-BI and then the cholesteryl esters present in the HDLs are transferred to the membrane for uptake via caveolae. The importance of the fact that the HDL-SR-BI complex remains at the cell surface is evident from the observation that this ligand-receptor interaction is also involved in the removal of cholesterol from cells by HDLs in the process of reverse cholesterol transport.

Lipoprotein-Associated Phospholipase A_2: Lp-PLA_2

Platelet activating factor (PAF) is a lipid compound of the plasmalogen family of phospholipids (ether-linked glycerophospholipid) that is involved in numerous proinflammatory activities. Inactivation of PAF was originally ascribed to an activity called PAF-acetylhydrolase (PAF-AH). Subsequent to its initial characterization, PAF-AH was shown to be a member of a large family of enzymes that all hydrolyze the *sn*-2 position of glycerophospholipids. This family of enzymes is the PLA_2 family. A detailed

discussion of the PLA_2 family of enzymes can be found on the Bioactive Lipids page. There are two major forms PAF-AH, one that is cytosolic and one that is secreted and found in the plasma. The plasma form of PAF-AH circulates bound to lipoproteins. Given that PAF-AH is a member of the PLA_2 family and that it also circulates bound to lipoprotein it is more commonly referred to as the lipoprotein-associated PLA_2 (Lp-PLA_2). Lp-PLA_2 is found in the plasma bound primarily to LDLs but is also found associated with HDLs and lipoprotein(a) [Lp(a)]. Of clinical significance is the fact that Lp-PLA_2 has been implicated in atherosclerosis and cardiovascular disease but its precise role in these pathophysiological processes is not completely understood.

The human Lp-PLA_2 protein is encoded by the PLA2G7 gene and is composed of 441 amino acids following cleavage of the signal peptide. The protein contains two sites of *N*-glycosylation. The enzymatic activity of Lp-PLA_2 is specific for short chain acyl groups (up to 9 methylene groups) at the *sn*-2 position of phospholipids. When PAF is the substrate for Lp-PLA_2 the products are lyso-PAF and acetate. When phospholipids of the phosphatidylcholine (PC) family are oxidized by free radical activity (referred to as oxPL) they can be a substrate for Lp-PLA_2 even if the unsaturated fatty acid at the *sn*-2 position is longer than 9 carbon atoms. The ability of Lp-PLA_2 to recognize oxPL as substrates is due to the presence of aldehydic or carboxlic moieties at the omega (ω) end of the *sn*-2 peroxidized fatty acyl residues. The products of Lp-PLA_2 activity on oxPL are oxidized free fatty acids (oxFFA) and lyso-PC. Numerous types of oxPL have been identified in oxidized LDL (oxLDL) particles and many of them exhibit biological activity and exert key effects in atherogenesis. Lp-PLA_2 can also hydrolyze long chain fatty acyl phospholipid hydroperoxides, phospholipids containing isoprostanes esterified at the *sn*-2 position and other lipid esters such as short-chain diglycerides, triglycerides, and acetylated alkanols. In addition to its hydrolytic activity Lp-PLA_2 exhibits transacetylase activity. The transacetylase function transfers acetate and short-chain fatty acids from PAF to ether- and ester-linked lysophospholipids. The transacetylase function is evident when Lp-PLA_2 is associated with LDL.

In humans with normal levels of circulating lipids and no detectable Lp(a), essentially all of the Lp-PLA_2 in the plasma is bound to LDL. The interaction of Lp-PLA_2 with LDL occurs through apolipoprotein B-100 (apoB-100). When plasma levels of Lp(a) rise in excess of 30mg/dL there is an enrichment in the association of Lp-PLA_2 with this abnormal lipoprotein particle. When expressed as enzyme mass, Lp(a) carries 1.5–2 times more Lp-PLA_2 than does LDL. As in its association with LDL, Lp-PLA_2 interacts with apoB-100 in Lp(a) particles. Abnormalities in lipoprotein metabolism, such as those resulting in Lp(a) production, significantly affect the plasma levels of Lp-PLA_2. For example in familial hypercholesterolemia the level of LDL-Lp-PLA_2

activity increases in parallel with the severity of the hypercholesterolemia. The level of plasma Lp-PLA_2 can be positively affected by low-calorie diets associated with weight loss or after drug treatment with the various classes of hypolipidemic drugs discussed below in Pharmacologic Intervention. In the context of atherosclerosis and cardiovascular disease numerous epidemiological studies have shown that increased levels of plasma Lp-PLA_2 approximately double the risk for primary and secondary cardiovascular events. In fact it is suggested that measurement of Lp-PLA_2 levels is useful as a cardiovascular risk marker independent of and additive to traditional risk factors. However, whether Lp-PLA_2 is a novel biomarker or is causal in the development of atherosclerotic diseases remains controversial. This is because there are both anti- or proatherogenic activities associated with Lp-PLA_2. The antiatherogenic functions of Lp-PLA_2 are attributed to its role in hydrolyzing and inactivating the powerful proinflammatory lipid, PAF. Additionally, by hydrolyzing oxPLs Lp-PLA_2 effectively lowers the circulating levels of this class of inflammatory mediators. On the other hand the proatherogenic and proinflammatory actions associated with Lp-PLA_2 are in fact due to its hydrolysis of oxPLs. The hydrolysis of oxPLs releases both lyso-PC and oxFFA both of which have been shown to have proatherogenic effects.