Lipoproteins and Apoproteins

Lipids are a group of fatty substances that includes triglycerides (fat), phospholipids and sterols (e.g. cholesterol). They constitute an important source of energy, serve as precursors for a number of essential compounds, and are key components of cells and tissues. Cholesterol, for example, is an indispensable constituent of cellular membranes (1), as well as the precursor for both steroid hormones and bile acids. On average, the body utilizes approximately 1000 milligrams of cholesterol per day, 30% of which comes directly from foods of animal origin, and the rest is synthesized in the liver. Due to the insolubility of cholesterol and other fatty compounds in the blood, their redistribution in the body requires specialized carriers capable of solubilzing, ferrying, and unloading them at specific target sites. Miscarriage of lipids while in circulation may lead to atherosclerosis; a clinical condition marked by fatty deposits in the inner walls of arteries, and the leading cause of death and disability in Western countries.

Most lipids are transported in the blood as part of soluble complexes called lipoproteins (LPs). Plasma LPs are spherical particles composed of a hydrophobic lipid core surrounded by a hydrophilic layer, which renders the particles soluble. The lipid core contains primarily triglycerides (TG) and cholesteryl esters (CE), as well as small amounts of other fatty compounds, such as sphingolipids and fat-soluble vitamins (e.g. vitamins A, D, E, and K). The external layer is made of phospholipids, unesterified cholesterol, and specialized proteins, called apolipoproteins or apoproteins. These proteins facilitate lipid solubilization and help to maintain the structural integrity of LPs. They also serve as ligands for LP receptors and regulate the activity of LP metabolic enzymes. As depicted in Figure 1, the amphipathic molecules that compose the outer layer of LPs are arranged so that their hydrophobic parts face the central core, and their hydrophilic regions face the surrounding aqueous environment.
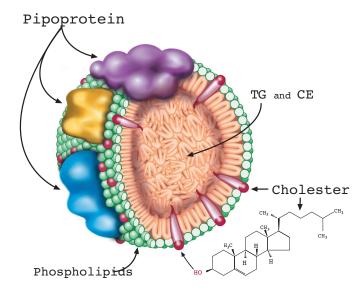
Cholesteryl esters, which do not contain a free hydroxyl group (-OH) are more hydrophobic than cholesterol, and better accommodated in the core of LPs. The conversion of cholesterol to CE is catalyzed by a LP-associated enzyme called lecithin-cholesterol acyltransferase (LCAT). This enzyme, which promotes packaging of cholesteryl molecules in LPs, is critical for normal cholesterol metabolism. Deficiency of LCAT activity leads to accumulation of unesterified cholesterol in tissues, and is associated with a number of clinical conditions including corneal opacity, hemolytic anemia, and premature atherosclerosis.

During ordinary metabolism, plasma LPs lose, acquire, and exchange their lipid and protein constituents. Normally, fat-rich LPs lose most of their fat within a few hours of food ingestion, and become smaller and denser particles with higher relative cholesterol content. The depletion of fat from LPs is catalyzed by lipoprotein lipase (LPL). This lipolytic enzyme is located on the surface of endothelial capillaries, and degrades triglycerides to free fatty acids (FFAs) and glycerol. The released FFAs may stay in circulation bound to albumin, or be taken-up by muscle and fat cells for usage and storage, respectively.

Lipids of dietary origin are processed by intestinal epithelial cells, and then secreted into the bloodstream as part of large, fat-rich LPs called chylomicrons (chylo = milky, micron= indicates particle size). En route to the liver, chylomicrons (CM) pass through endothelial capillaries, lose some fat, and their remnants are taken-up by liver cells. In the liver, the lipids obtained from CM remnants are re-processed and then secreted back into the bloodstream as part of very low-density LPs (VLDL). Depletion of fat from VLDL transforms the particle into an intermediate density lipoprotein (IDL), which upon further degradation of its fat is converted into a relatively stable particle, called low density

Figure 1- Schematic Illustration of a Lipoprotein Particle

lipoprotein (LDL). Because of its high cholesterol content, LDL is also called LDL-cholesterol. Of the total blood cholesterol, 60-75% is found in LDL and the rest primarily in high-density lipoprotein (HDL) particles. The main characteristics of plasma LPs and their associated apoproteins are summarized in Tables I and II, respectively.

All peripheral cells express the LDL-receptor (LDLR), and recycle it to the cell surface upon need for cholesterol. Cholesterol is delivered to these cells through binding of LDL to LDLR, which triggers endocytosis (internalization) of both species. When the need for cholesterol is satisfied, the recycling of LDLR is discontinued. Normally, an LDL particle stays in circulation for no more than a few days before being consumed by a cholesterol needing cell. However, under conditions of sustained cholesterol excess, the particle stays in circulation for longer periods of time, and becomes more vulnerable to undesired modifications (e.g. oxidation). As high levels of oxidized LDL are commonly found in atherosclerotic plaques, they are thought to be the major inducer of atherosclerotic lesions. Hence, LDL became known as bad cholesterol. However, today we know that not all LDL particles are bad, and that some LDL particles, especially very large ones (with diameter >21.3nm), may even provide protection against atherosclerosis (2). LDL and HDL particle sizes are largely determined by a LP-associated protein, called CETP (cholesteryl ester transfer protein). This protein enhances exchange of non-polar lipids, primarily CE and TG, and facilitates tight packaging of CE within the core of the particles. The end result of prolonged and/or efficient CETP action is smaller LDL and HDL particles. [The LP-anchored CETP can be envisioned as having a hand that rotates between the interior and exterior of the particle and capable of holding only one lipid molecule at a time. Grasping of one molecule releases another and vise versa.]

Genetic variation at the human CETP gene generates proteins with varying degrees of activity. For example, a single codon variation, from isoleucine to valine at position 405, generates a mutant protein, designated I405V, which manifests significantly reduced CETP activity (3, 4). In a new observational study, Barzilai, N. et al. (2) found that people with homozygosity for the I405V allele have larger HDL and LDL particles, and that this genotype is associated with exceptional longevity and a markedly reduced risk of coronary artery disease (CAD). Of the 213 centenarians enrolled in the study, 80% had a high proportion of large LDL particles, compared to just 8% of the subjects in the control group (256 people in their 60's and 70's) (2). Interestingly, HDL and LDL particle sizes are significantly larger in women than in men, which may account, at least in part, for the longer life expectancies of women.

Unlike LDL, HDL is not recognized by LDLR, and cannot deliver cholesterol to tissue cells. Instead, it has the ability to remove excess peripheral cholesterol and return it to the liver for recycling and excretion. This process, called reverse cholesterol transport, is thought to protect against atherosclerosis. Observational studies over the last 2 decades have consistently shown strong correlation between elevated HDL levels and low incidents of coronary heart disease (CHD). Hence HDL has been dubbed "good" cholesterol.

HDL is synthesized in the liver and intestine as a nascent, discoid-shaped particle that contains predominantly apoA-I, and some phospholipids. Upon maturation, HDL assumes a spherical shape, and the composition of its core lipids becomes very similar to that of LDL. However, the relative higher protein content in HDL renders the particle denser and more resistant to undesired modifications. Unlike the case of LDL, the clearance of HDL from circulation is not negatively affected by excess cholesterol, which may be another reason why HDL, despite being much smaller particle than LDL (10nm versus 20nm), is not found in atherosclerotic plaques. It's worth noting, that the potential of LPs to become harmful is also influenced by the character of their lipid constituents. For example, vitamin E and lipids containing omega-3 fatty acid moieties appear to protect the particles from harmful oxidation and from getting stuck on the walls of blood vessels.

The functional difference between LDL and HDL results primarily from the different character of their major apoproteins, apoB-100 and apoA-I, respectively.

Table I- General Characteristics of Plasma Lipoproteins.

LP Particle	Size	Density (g/ml)*	TG/CE Ratio*	L/P Ratio*	Associated Apoproteins
CM	1000nm	<0.95	28.83	65.66	**apoB-48,** apoA, apoC, apoE, apoH
VLDL	70nm	0.98	3.89	10.76	**apoE,** apoB-100, apoC
IDL	40nm	1.01	0.82	8.09	**apoE,** apoB-100, apoC
LDL	20nm	1.04	0.18	3.76	**apoB-100,** apoC, apoE, apo(a)
HDL	10nm	1.13	0.16	1.22	**apoA-1,** apoC, apoD, apoE

LEGEND: * Average values, TG-triglycerides, CE-cholesteryl esters, L/P- lipid/protein, **Bold** represents the major apoprotein

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ApoB-100, which is found in VLDL, IDL, and LDL, but not in HDL, serves as a ligand for LDLR, and provides LDL with the means to deliver cholesterol to tissue cells. On the other hand, apoA-I, which is found exclusively in HDL, has a unique ability to capture and solubilze free cholesterol. This apoA-I ability enables HDL to act as a cholesterol scavenger.

A mutant apoA-I protein, called apoA-I Milano (apoA-Im), has been identified in a group of people that live in a small village in northern Italy (5). Carriers of this protein, all heterozygous for the mutation, had very low levels of HDL (7-14 mg/dl) but showed no clinical signs of atherosclerosis (5-7). HDL particles in these subjects were markedly larger than control (12nm versus 9.4nm), which may account for their immunity against premature atherosclerosis. ApoA-Im differs from natural apoA-I by having a cysteine residue at position 173 instead of arginine. This cysteine residue forms disulfide bridges with other apoA-I molecules or with apoA-II (6, 7), which apparently lead to larger HDL particles. It also renders apoA-I more susceptible to catabolism (8), accounting for the low HDL levels in apoA-Im carriers.

The therapeutic potential of apoA-I has been recently assessed in patients with acute coronary syndromes (9). Of the 47 patients that participated in a randomized controlled trial, 36 received 5 weekly infusions of recombinant apoA-Im/phospholipid complexes, and 11 received only saline infusions. The results showed significant regression in coronary atherosclerotic volume in the apoA-Im treated group, and virtually no change in the control group (9). These results, if reproduced in larger clinical trials, may constitute a revolutionary breakthrough in the non-invasive treatment of cardiovascular disease. They should also encourage further exploration into the therapeutic usefulness of apoA-Im and normal apoA-I in managing atherosclerotic vascular diseases.

Table II- Classification of Apoproteins.

Apoprotein	(MW)	Function and Comments
ApoA-I	(29kDa)	Major protein of HDL, activates LCAT, high levels of apoAI are associated with a reduced risk of CHD.
ApoA-II	(17.4kda)	Primarily in HDL, enhanced hepatic lipase activity.
ApoA-IV	(46kDa)	Present in fat rich LPs.
ApoB-48	(24kDa)	Derived from ApoB-100 gene by RNA editing, found exclusively in CMs, Lack the LDLR binding domain of ApoB-100.
ApoB-100	(513kDa)	Major protein of LDL, binds to LDLR, high levels of apoB- 100 are associated with an increased risk of CAD.
ApoC-I	(7.6kDa)	Appears to be involved in activation of LCAT.
ApoC-II	(8.9kDa)	Activates LPL, deficiency of ApoC-II results in accumulation of CMs and high TG levels.
ApoC-III	(8.75kDa)	Inhibits LPL.
ApoD	(33kDa)	Found only in HDL, closely associated with LCAT.
ApoE	(34kDa)	Three known apoE alleles (E2, E3, E4). Binds to LDLR, inhibits development of atherosclerosis, apoE4 is associated with late-onset Alzheimer's disease.

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