

Review

Subfractions of Low-Density-Lipoprotein: Which is the Most Atherogenic?

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Abstract: Atherosclerosis is a widespread disease of the arteries, which manifests itself in the narrowing of the lumen of the vessel due to the formation of plaque on its walls. Despite an impressive history of study, atherosclerosis still leaves room for researchers. This is realized due to the complexity of pathogenesis, in which important roles are assigned to a variety of processes at all levels, from mitochondrial disorders to endothelial dysfunction. However, it is customary to pay the most attention to lipids, since, according to modern concepts, it is the modifications of lipid particles, namely LDL, that are a prerequisite for the development of atherosclerosis. In this review, we have collected data on the subfractions of this group of lipids, namely their atherogenicity. One of the central issues in the diagnosis and prevention of atherosclerosis is the measurement of atherogenicity.

Keywords: Atherosclerosis, Cardiovascular Disease, Atherogenicity, LDL, L5 LDL

Introduction

Atherosclerosis and subsequent Cardiovascular Disease (CVD) as well as its consequences including stroke and Myocardial Infarction (MI) are the main global cause of death. Multiple factors have been associated with elevated cardiovascular risk of which Low-Density Lipoprotein (LDL) has been researched most thoroughly (Frostedg ard, 2013). There has been provided ample evidence confirming the crucial role of cholesterol-containing LDL and other lipoproteins that include Apo Lipoprotein B (ApoB) (such as Lipoprotein (a) (Lp (a)), Intermediate Density Lipoproteins (IDL), Very Low-Density Lipoproteins (VLDL) and their remnants) in the pathophysiology of atherosclerotic CVD (Linton *et al.*, 2019).

However, some scientists are still doubting whether LDL levels are the main factor leading to CVD. With the development of novel and efficient medications aimed at decreasing lipid levels, including drugs with long-lasting effects, it has become essential to establish common guidelines on how to approve and apply new agents and

this requires a shared understanding of LDL's role in atherosclerotic CVD (Nelson, 2013).

Two Consensus Statements have been formulated. The first one assesses the role of LDL in the development of CVD. It is based on a number of trials, including epidemiologic, genetic, and clinical intervention trials. The other document examines the LDL influence on atherosclerosis based on the existing knowledge of the pathologic mechanisms behind CVD (FERENCE *et al.*, 2017). Although we will concentrate on LDL, the pathogenic effect of other particles that include ApoB should also be considered as well as the possible atherogenicity of particular lipidome and proteome elements apart from LDL ApoB and cholesterol (Behbodikhah *et al.*, 2021).

The majority of articles questioning the causal role of LDL in the initiation and progression of atherosclerosis refer to the results of individual trials or a small number of selected trials, missing the quantitative synthesis of the data. In order to avoid this pitfall, we have included data from several meta-analyses including various groups of more than 200 different trials that included more than 2 M

patients over a total follow-up period of over 20 years. More than 150 K cases of cardiovascular events have been evaluated in total. This pool of data provides exhaustive evidence of the causal role of LDL in atherogenesis (Borén *et al.*, 2020).

Low-Density Lipoprotein

LDL particles are quasi-spherical micelle-shaped complexes consisting of many molecules. Approximately 0.8 of the particle's weight falls upon its lipidome which includes over three hundred various lipid types. The proteome of LDL is presented by a single ApoB 100 molecule. Being one of the biggest proteins observed in mammals, ApoB 100 is responsible for supporting the structure of the particles. Unlike smaller apolipoproteins, it is preserved in the LDL during various stages of the particle's life (Davidsson *et al.*, 2010).

LDL is the main transporter of plasma cholesterol, each particle containing between 2-2.7 K cholesterol molecules, of which over 60% is esterified (at ~1 mmol/L). In addition, LDL is one of the main carriers of ubiquinol, E vitamin, and carotenoids. However, unlike HDL it contains few small non-coding RNAs and many microRNAs miR-155, the latter being atherogenic (Pirahanchi *et al.*, 2022).

LDL includes several types of particles with different properties and functions. For example, in (Table 1), we provided the classification of LDL particles based on density and size. Three main subclasses are usually identified in individuals with normal lipid profiles: Large LDL-I with a density between 1.0190-1.023 g/mL; medium-sized LDL-II (density 1.023-1.034 g/mL) and small LDL-III (density 1.034-1.044 g/mL) (Ivanova *et al.*, 2017). A 4th subclass of very small and dense LDL particles LDL-IV (density 1.044-1.063 g/mL) is observed in subjects with high TG levels. Common LDL-C measurements include the total cholesterol carried by the above-mentioned particle groups as well as by IDL and lipoprotein (a) (Liou and Kaptoge, 2020).

LDL Subfractions

Another way to divide LDL into subclasses is by the particles' charge. 5 subfractions are usually recognized, L1-L5, of which L1 is the biggest and the least negative class while L5 is the most negative and has been

demonstrated to have apoptotic properties. We summarized the differences between L1 and L5 in Fig. 1. L5 is almost impossible to observe in healthy people, however, its levels are markedly greater in subjects with CVD (King *et al.*, 2011). Studies have demonstrated that patients with thrombosis resulting in ST-Elevation Myocardial Infarction (STEMI) present with elevated L5. This condition may lead to death as a result of arrhythmia. L5 has also been established as a predictor of CVD and stroke. L5 has been shown to promote apoptosis induced by cytokines expressed in the endothelium. Furthermore, studies have reported that L5 leads to longer corrected QT intervals by affecting the flow in the dihydropyridine channel and KATP channel. ATP-sensitive potassium channels play an important role in protecting the heart during and after ischemia, as well as in moderating atrial and ventricular rhythm. Still, there is not enough research on the action of L5 on KATP (Ma *et al.*, 2020).

Factors Influencing the LDL Subclass Profile

VLDL-Triglyceride concentrations (VLDL-TG) largely determine the fraction's properties. Higher plasma TG concentrations are associated with the prevalence of Small Dense LDL (sdLDL) particles. Another important factor is sex: At the same TG rates, men tend to express more sdLDL than women due to greater lipase activity in the liver (Cho *et al.*, 2015). Metabolic models demonstrating the production of LDL-III and LDL-IV showed that TG from VLDL and/or chylomicrons are exchanged with cholesteryl esters and moved to the core of LDL particles, which is a crucial step in the formation of small LDL species. This process is moderated by cholesteryl ester transfer protein. Later, the LDL particle can undergo lipolysis by liver lipase and lose both the core triglycerides and surface phospholipid, forming a new particle that is smaller and denser (German *et al.*, 2006).

TG concentrations in plasma during the fasting state depend on hepatic VLDL expression, lipase, and lipoprotein activities in the liver, expression of VLDL-sized particles containing ApoB 48 in the intestine, and the degree of receptor-mediated uptake of the particles. Particles of different sizes can be produced in the liver: From LDL to medium-sized VLDL2 and larger VLDL1 particles, depending on the content of triglycerides in the liver (Choi and Ginsberg, 2011; Liu *et al.*, 2012).

Table 1: Classification of LDL based on density and size. Based on the data from Ivanova *et al.*, (2017)

Parameter	Large LDL-I	Medium LDL-II	Small LDL-III	Very small LDL-IV
density, g/mL	1,019-1,023	1,023-1,034	1,034-1,044	1,044-1,063
Ø, nm	26,4-28,5	25,5-26,4	24,2-25,5	22,0-24,1

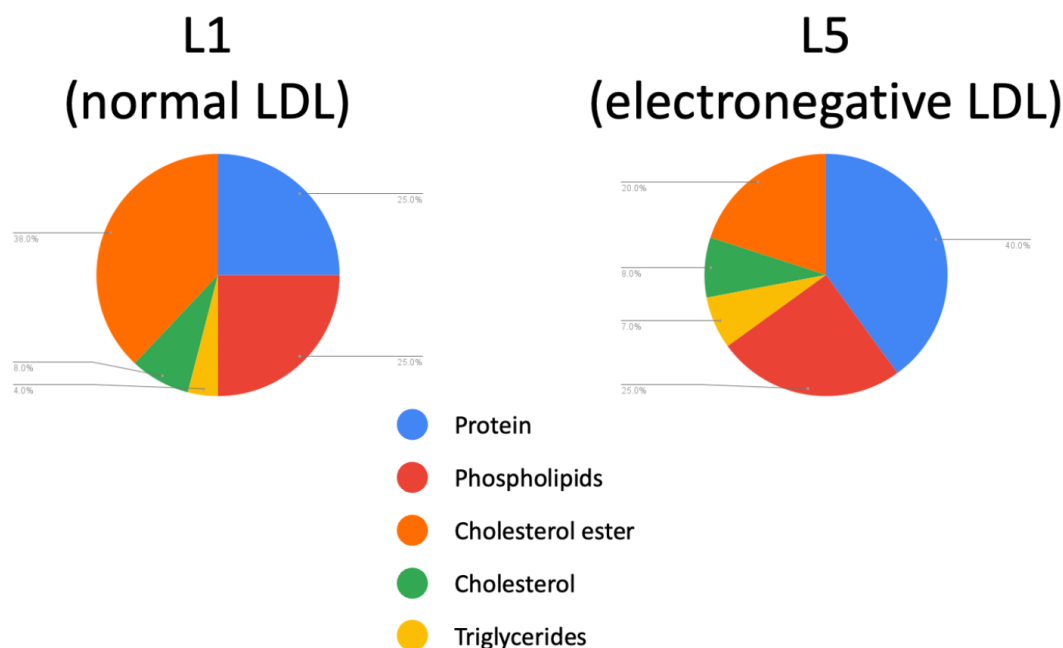


Fig. 1: Difference in the composition of L1 and L5 LDL particles. Based on the data from King *et al.*, (2011)

VLDL expression can also be affected by insulin resistance, while VLDL uptake and lipolysis are largely influenced by the activity of lipases and the presence of Angiotensin-like 3 and ApoC-III. The LDL subfraction profile is markedly affected by the properties of the expressed VLDL particles, their amount, activities of neutral lipid transfer proteins like CETP and lipases, tissue LDL Receptor (LDLr) function, and the affinity of the particles to bind to it which depends on the location of ApoB100 in the particle. These properties determine the LDL profile and distribution of different subfractions, as well as their lipid content (Packard *et al.*, 2022).

In subjects with plasma TG concentrations between 0.85-1.7 mmol/L (75-150 mg/dL) the LDL profile predominantly consists of LDL-II. Following hepatic expression of VLDL 1 and 2, they are quickly dilapidated to IDL and further to medium-sized LDL. If the TG plasma concentrations are below 0.85 mmol/L or 75 mg/dL, the lipolysis is more active and TG availability in the liver is usually lower (Simha, 2020). Thus, VLDL expressed in the liver is smaller, and sometimes the liver expresses IDL and LDL-sized particles directly. In this case, the LDL profile is dominated by large LDL-I particles. Such LDL profile is observed in healthy individuals. However, it is also typical for patients with familial hypercholesterolaemia when the LDL concentrations are elevated due to an overage of small VLDL and low LDL uptake as a result of an insufficient number of receptors. If plasma TG concentrations are

above 1.7 mmol/L (150 mg/dL), particularly over 2.23 mmol/L (200 mg/dL) as a result of elevated VLDL expression, it leads to the production of sdLDL (Feingold, 2017). This state is observed in patients with metabolic impairments, such as diabetes, insulin resistance, or metabolic syndrome as well as in individuals with impaired lipolysis as a result of elevated apoC-III levels. This LDL profile characterized by the prevalence of smaller particles is associated with atherogenic dyslipidemia characterized by an excess of remnant lipoproteins. Smaller particle sizes and different ApoB100 conformation decrease the particles' affinity for the LDLr, leading to a longer circulation time (Boren *et al.*, 2020).

Atherogenicity

At the moment, there is insufficient data on the correlation between the atherogenicity of different LDL subfraction profiles. Existing studies appear to be limited in one or another way. Firstly, mainly cross-sectional studies have been carried out so far. The few longitudinal studies assessing the results of a lifetime intervention still present only short-term results (Behbodikhah *et al.*, 2021). Secondly, there is no consensus on the atherogenicity of each subfraction. Thirdly, available studies did not measure atherogenicity inside the vessels or in the intima. Furthermore, only selected subclasses of lipoproteins have been evaluated. Finally, data from patients with metabolic syndrome is lacking (Dutheil *et al.*, 2014).

In order to provide more clarity on the matter, a long-term follow-up trial has been carried out. In this trial,

atherogenicity has been also measured inside the arteries. Carotid Intima-Media Thickness (CIMT) is an established way of measuring atherosclerosis-related cardiovascular risk. Framingham's risk score evaluates cardiovascular risk based on various factors such as age, hypertension, smoking, diabetes, and LDL/HDL-cholesterol levels (Kasliwal *et al.*, 2014).

A most significant reduction in Framingham score was observed three weeks into the trial and the value remained lower than the baseline throughout the whole 12-month follow-up period. CIMT reduction happened gradually until the 6th month of the trial, though the most remarkable change was also observed after the 3rd week of the trial (Makover *et al.*, 2022). While the Framingham score changed rapidly, LDL and HDL measurements mainly accounting for the decrease in its value, CIMT reduction happened more gradually, probably because it is not as dynamic as other markers as it takes longer time for the vascular wall to change its properties. Still, neither of the observed markers reached the same value as in the controls (Jia *et al.*, 2021).

The results of the trial were obtained by means of a generalized estimating equations model with multiple variables in order to adjust the data for confounding factors and account for variation in the correlation between the repeated measures. This allowed us to avoid false positive results. Furthermore, the Framingham score was modeled. There was observed a correlation between its value and ApoA1 as well as ApoB which are important structural proteins for HDL and LDL correspondingly (Tian *et al.*, 2019). The observed association between thicker intima and diabetes was also plausible. Still, there was observed no correlation between lipoprotein subfractions and intimal thickness which indicates a need for further research of this matter.

Atherogenic Modifications

The notion that cholesterol accumulation in the arterial wall as a result of high cholesterol concentrations in blood is the primary cause of atherosclerosis was suggested by Nikolai Anitschkow one century ago (Finking and Hanke, 1997). It was later observed that not all types of cholesterol are atherogenic and that the disease is primarily caused by LDL Cholesterol (LDL-C) which is prone to accumulating in the vessel walls. A positive association was established between blood atherogenicity and the content of modified lipids in plasma, which may be due to an imbalance between LDL and HDL, the latter being atheroprotective (Summerhill *et al.*, 2019).

According to the lipid theory of atherosclerosis, cholesterol is predominantly transported to the arterial cells by LDL. Thus, LDL levels are the main marker of lipid accumulation within the cells or lipodosis. It is worth mentioning that non-esterified cholesterol content is much greater in LDL compared to other

fractions (Huygen *et al.*, 2022). Still, not all types of LDL can induce atherosclerosis. For instance, native LDL-C is not susceptible to retention in the intima. Later it was observed that some LDL subfractions can be exposed for multiple modifications which increase the fraction's atherogenicity, desialylation being the first and the main of them (Orekhov *et al.*, 2014).

The Role of LDL Desialylation in Atherogenesis

There is extensive evidence confirming the role of desialylation in the process of cholesterol accumulation in the intima as the lack of sialic acid was observed in atherogenic LDL particles. A significant negative association was reported between sialic acid content within the LDL particles and cholesterol retention rates in the arterial walls (Mezentsev *et al.*, 2021). There was no observed significant association between the blood's atherogenic properties and other LDL modifications such as the oxidation degree of LDL, changes in the size and electrical charge of the fraction, the amount of lipid peroxidation end products, neutral lipids, phospholipids, and fat-soluble antioxidants in the particles as well as free lysine amino groups (Tertov *et al.*, 1996). Furthermore, recent studies have shown that desialylation is the main LDL modification taking place in the blood of individuals with atherosclerosis. Transcriptome analysis has revealed more than 30 signaling pathways responsible for cholesterol retention in macrophages which were cultivated *in vitro* involving naturally occurring LDL (Bobryshev *et al.*, 2016). The study demonstrated that 26 out of 33 pathways that regulate naturally occurring LDL are also regulating desialylated LDL. However, oxidized LDL is regulated only by two pathways in a similar way, while seven more pathways are regulated in an opposing manner (Orekhov *et al.*, 2020; Poznyak *et al.*, 2020). These data suggest that LDL desialylation accounts for the fraction's atherogenicity to a much greater extent than oxidation. Moreover, the ability of desialylated LDL to promote cholesterol accumulation inside vascular cells has been confirmed by numerous experiments.

LDL Subfraction Profile and Atherogenicity

In patients with diagnosed cardiometabolic disorders, certain LDL subfractions clearly demonstrate their atherogenic properties. Due to their biological characteristics, sLDL is sometimes associated with a higher risk of Coronary Heart Disease (CHD). True, that they penetrate the vascular intima more quickly than larger LDL, but it is difficult to assess their independent input into CHD aggravation because of their close metabolic connection with other atherogenic lipoproteins containing apolipoprotein B-100, like high concentrations of very low-density lipoprotein and remnant lipoproteins (Superko and Garrett, 2022). Still, according to large-

scale recent studies in human cohorts and a statin trial, sdLDL levels, unlike large LDL, allowed for the prediction of incident CHD regardless of LDL-C. The atherogenicity of LDL subfractions may also depend on their diverse lipidomic and proteomic profiles. For instance, sdLDLs, compared to larger LDLs, are mostly enriched with apolipoprotein C-III and glycated apolipoprotein B-100. Besides, electronegative LDL species causing endothelial disorder are also included in the sdLDL subfraction. Furthermore, under conditions of oxidative stress, sdLDLs containing unsaturated cholesteryl esters are highly prone to hydroperoxide modification (Hoogetveen *et al.*, 2014).

Genetic factors may impact the metabolism of LDL particles as well; it is reflected in their profile and may also increase the risk of CHD. For instance, a common non-coding DNA variant located on chromosome 1p 13, responsible for the production of sortilin and other proteins by the liver, may influence LDL-cholesterol levels and incident MI. The high-risk allele at this site correlates with elevated sdLDL levels, however, the mechanism of the association is still unclear (Strong and Rader, 2010; Khera and Kathiresan, 2017).

Atherogenic Mechanisms of LDL Subfractions

There exists a theory, not supported by enough evidence, though, that small LDL is more atherogenic than large and intermediate LDL. Compared to intermediate LDL, small and large LDL are not so much affined to the LDL receptor responsible for clearing low-density lipoproteins from plasma (Rajman, 1994; Galeano *et al.*, 1998). These LDL subfractions are to a lesser extent cleared by steroidogenic tissues and the liver and consequently their uptake by the vascular wall increases. Studies *in vivo* showed that plasma circulation time is longer for small LDL compared to large LDL. It may be explained by the fact that during LDL clearance from the circulation, small LDL is less exposed to the area of ApoB binding to the LDL receptor (Sacks and Campos, 2003; Levitan *et al.*, 2010). Animal studies showed that small LDL penetrated the arterial wall more often than other LDL because of longer circulation time and thus stimulating atherosclerosis development (Goel *et al.*, 2017). However, a study of LDL through the arterial wall in humans has not demonstrated any correlation with LDL size, which suggests that small and large LDLs are equally likely to penetrate the arterial wall in the same period of time. Large LDL particles carrying more cholesterol ester bring more of it into plaques (Zhang and Fernández-Hernando, 2020). Both small and large LDL attach to arterial proteoglycan, which is found on the endothelium and in the intima and they both have undesirable atherogenic features as binding to the proteoglycan on the endothelial surface facilitates penetration of lipoprotein into the arterial intima, while

binding to the proteoglycan in the intima may initiate or foster plaque formation (Lorey *et al.*, 2022).

OxLDL stimulates the formation of foam cells on vascular walls thus activating inflammation and promoting atherosclerosis development. Several studies demonstrated that circulating oxLDL increases the risk of CHD. An *in vitro* study discovered higher atherogenicity of small LDL due to its depletion of vitamin E and quicker oxidation *in vitro* a feature that could be reversed by supplementing vitamin E (Poznyak *et al.*, 2021). However, a study involving subjects with combined hyperlipidemia proved an absence of an independent association between LDL oxidizability and CIMT. Another trial, in healthy people, demonstrated that supplementing vitamin E could lower LDL oxidizability without reducing CIMT progression. Large-scale trials of different antioxidants including vitamin E could not prove their anti-CHD action, which means that atherogenic properties of LDL oxidizability are still a matter of further investigation (Mitu *et al.*, 2020). Unoxidized native LDL facilitates the production of TNF- α and IL-8 inflammation mediators by activated monocytes, thus directly stimulating atherosclerosis development. Large LDL is rich in cholesterol and is proven to cause early atherosclerosis in patients with FH being the primary LDL subfraction triggering the disease. Therefore, it is clear that both small and large LDLs are responsible for atherosclerosis development though it is impossible to say whether any of them is more pathogenic (Okoro, 2021).

L5

Many various trials studied the impact of the size of LDL particles in CVD patients. Almost all of them demonstrated that small LDL was strongly associated with an increased risk of Coronary Artery Disease (CAD); however, it can hardly be called the main and independent CAD risk factor, taking into account numerous other factors like, for example, triglycerides in plasma and concentration of HDL-C (Superko and Garrett, 2022). Thus, when a univariate analysis links a high CAD risk with small particle size, it may in fact result from a mixture of atherogenic factors, small dense LDL being one of them, and a direct causal connection between small, dense LDL and higher CVD risk has never been established. Therefore, the research focus turned to the atherogenic properties of L5, not a small LDL subfraction (Akyol *et al.*, 2020).

Evidence of L5's Atherogenicity In vitro

Multiple evidence confirms that depending on time and concentration L5 stimulates endothelial cell apoptosis in all stages of atherothrombosis (Chan *et al.*, 2013). Endothelial cell apoptosis, in its turn, promotes transendothelial permeability, while microparticles

increase the release of tissue factors promoting coagulation. Besides, L5 stimulates monocyte adhesion to endothelial cells, which is also an atherogenic factor in early atherosclerosis. Adhesion of cells is increased because endothelial cells produce IL-8, VCAM-1, CXC chemokines, and other secretion molecules, in accordance with the studies on LDL (Yang *et al.*, 2003).

Fibroblast growth factor 2 induces downstream effectors and kinases, like eNOS, Akt, Bad, Bax, Bcl-xL, and Bcl-2 which are responsible for the structural integrity and function of mitochondria (Sussman *et al.*, 2011). According to a recent study, L5 activates pro-apoptotic effectors TNF- α , Bad, and Bax in endothelial cells through LOX-1 and at the same time inhibits eNOS phosphorylation and expression of Bcl-2, Bcl-xL, and eNOS that contribute to mitochondria survival and stabilization (Lu *et al.*, 2009).

The L5 triggers signaling pathways that promote endothelial dysfunction and contribute to the development of atherosclerosis (Mineo, 2020).

Conclusion

The atherogenicity of lipids of various groups and subgroups is a big question, the answer to which will allow better prediction, prevention, and treatment of atherosclerosis, as well as its undesirable consequences. Our review presents data on LDL subfractions, which are distinguished depending on the charge, size, and density of the particle, as well as modifications of its structure. The LDL subfraction profile is influenced by a variety of factors, from the patient's gender to the health of their liver function. In patients with diagnosed cardiometabolic disorders, certain LDL subfractions clearly demonstrate their atherogenic properties. One of these subfractions, according to not fully confirmed theory, is sdLDL. However, according to numerous *in vitro* and *in vivo* evidence, particle size may not be the main determinant of atherogenicity. Therefore, modern data tend to separate subfractions based on the charge of the particle. The most atherogenic fraction is L5, which triggers signaling pathways that promote endothelial dysfunction and contribute to the development of atherosclerosis.

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Ethics

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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