

LDL PARTICLE SIZE: DOES IT MATTER?

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Introduction

While both small and large LDL particles may be atherogenic, it is currently widely believed that small LDL particles are more atherogenic than large particles due to the greater oxidation potential of small particles and their relationship to other metabolic abnormalities, particularly high levels of triglyceride-rich lipoproteins and low serum concentration of HDL cholesterol. This view is supported by findings from epidemiological studies which have shown that individuals with predominantly small LDL particles (pattern B) have greater cardiovascular disease (CVD) risk than those with predominantly large LDL (pattern A) [1-3], although a few studies found that large LDL size was associated with CVD [4,5].

An important limitation of these studies is that LDL size was measured using gradient gel electrophoresis, which determines only the distribution of LDL subclasses or average LDL size phenotype (large or small) but does not quantify the number of small versus large particles. In particular, a decrease in average LDL size as measured by gradient gel electrophoresis does not necessarily translate into greater number of small LDL particles, since it could also be due to fewer large LDL particles (Figure 1).

Prior studies that used gradient gel electrophoresis also could not directly compare the risk associated with small versus large LDL particles on a *per particle* basis. This is important because small LDL particles contain substantially less cholesterol than large ones, such that at the same serum concentration of LDL cholesterol (LDL-c), individuals with predominantly small LDL have greater total concentration of LDL particles than those with predominantly large LDL (Figure 2) [6]. In addition, prior studies did not adequately control for the inverse correlation between small and large LDL particle concentrations (LDL-p) and potential confounding due to their differing associations with other lipoproteins, lipids, and traditional cardiovascular risk factors [7-9].

In comparison with gradient gel electrophoresis, nuclear magnetic resonance (NMR) spectroscopy enables quantification of the concentrations (number) of lipoprotein particles of varying size and composition [10], unlike traditional methods of quantifying cholesterol in lipoproteins, classified by density. Subclasses of different sizes and composition are individually

detected and quantified by NMR on the basis of the distinct lipid methyl group NMR signal that each subclass emits [7].

LDL Size and Subclinical Atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA)

In a recent report from a multi-ethnic cohort of 5,538 asymptomatic individuals, aged 45-84 years and free from CVD at the time of enrollment (2000-2002), we sought to directly compare the associations of small and large LDL particles with carotid intima-media thickness (IMT), a direct and non-invasive measure of subclinical atherosclerosis [11]. We hypothesized that: 1) NMR-measured small and large LDL particles were both associated with IMT and 2) the concentration of LDL particles, but not LDL particle size, was associated with IMT.

In this large multi-ethnic cohort of asymptomatic individuals (38% white Americans, 28% African Americans, 22% Hispanic, and 12% Chinese, with 53% women), small and large LDL particle concentrations were inversely correlated with each other (Spearman correlation coefficient - 0.64) and they correlated in opposite directions with LDL size. Without accounting for LDL subclass correlation, LDL size and small LDL-p separately were associated with IMT (-20.9 and 31.7 micron change in IMT per 1-SD, respectively, both $P < 0.001$), but large LDL-p was not (4.9 microns, $P = 0.27$). However, after accounting for their inverse correlation, both LDL subclasses showed highly significant and independent associations with IMT (36.6 and 52.2 micron higher IMT per 1-SD of large and small LDL-p respectively, both $P < 0.001$). Interestingly, there was a greater difference in IMT per large LDL particle compared with small LDL when compared on a *per particle* basis (17.7 and 11.6 microns per 100 nmol/L of large and small LDL-p respectively, both $P < 0.001$). Smaller LDL size was no longer significant after taking into account the particle concentrations of the two LDL subclasses and risk factors.

Thus, small LDL was a strong confounder of the association of large LDL with carotid atherosclerosis and adjusting for small LDL unmasked the true association of large LDL with IMT. Similarly, we found that small LDL size is a strong confounder for the association of LDL particle number with IMT. A confounding variable is both associated with the risk factor and is also causally associated with the outcome. As shown in Figure 3, a confounder (e.g. small LDL) is a variable that masks the relationship between the risk factor (e.g. large LDL) and the outcome (e.g. atherosclerosis). This is where the statistical technique of multiple regression can be very helpful in parsing out the independent effects of each of the correlated variables, especially when the two variables are at least moderately correlated. As shown in Figure 4, a study population may be comprised of individuals with predominantly large LDL (i.e. familial hypercholesterolemia) and those with predominantly small LDL (i.e. insulin resistance or metabolic syndrome). Confounding occurs when the study population has both types of individuals. In order to unmask the association of large LDL with IMT, we divided participants into those with low ($<$ sex-specific median) and high (\geq sex-specific median) levels of small LDL-p. In these stratified analyses, large LDL-p was now significantly associated with IMT in participants with low or high levels of small LDL-p (26.5 and 25.4 micron higher IMT per 1-SD increment in large LDL-p, $P < 0.001$ and $P = 0.001$, respectively).

Correlations among lipoproteins are well understood. Small LDL predominate in a triglyceride-rich environment, because smaller precursors are secreted by the liver and larger particles are transformed into smaller particles by cholesterol ester transfer protein (CETP)-mediated cholesterol-triglyceride transfer. In analyses that did not adjust for small LDL, we

found that large LDL was only weakly associated with atherosclerosis, consistent with prior reports [7-9,12]. After accounting for particle correlations, we demonstrated that the magnitude of association between small LDL and carotid atherosclerosis became equal to large LDL (on a per 1-SD basis) or less than large LDL (on a per particle basis). Failure to account for the strong negative correlation between small and large LDL and their different associations with other lipoproteins may underlie the belief that small LDL particles are a more potent atherogenic subclass than large LDL.

There are several mechanisms that may underlie the atherosclerotic effect of both large and small LDL [13]. At both extremes of LDL size, there is decreased receptor-binding affinity for LDL receptors [14]. Small LDL may be oxidized more rapidly and have been associated with endothelial dysfunction and metabolic dyslipidemia [15]. In comparison, large LDL predominate in patients with familial hypercholesterolemia and those consuming high saturated fat diets [16]. Large LDL have higher core cholesterol ester content, potentially delivering more cholesterol per particle to arterial walls [17], a speculation supported by our finding a greater IMT difference for large compared to small LDL on a per particle basis.

Summary

Small LDL confounded the association of large LDL with IMT because of its strong inverse correlation with large LDL, which may underlie the widespread belief that large LDL confers less cardiovascular risk than small LDL. Contrary to current opinion, both small and large LDL were significantly associated with subclinical atherosclerosis independent of each other, traditional lipids, and established risk factors, with no association between LDL size and atherosclerosis after accounting for the concentrations of the two subclasses. This knowledge may contribute to our understanding of atherogenesis, and future studies examining LDL size and atherosclerosis should account for the significant inverse correlation between small and large LDL.

References

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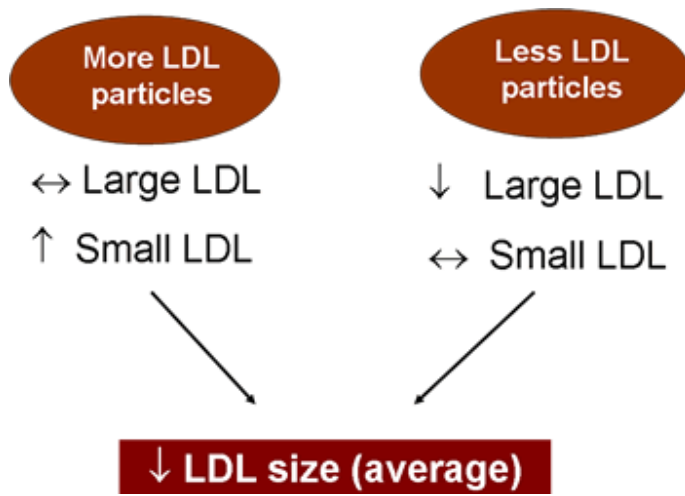
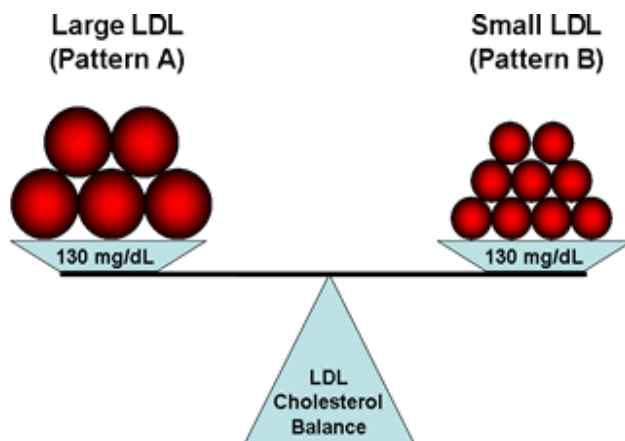
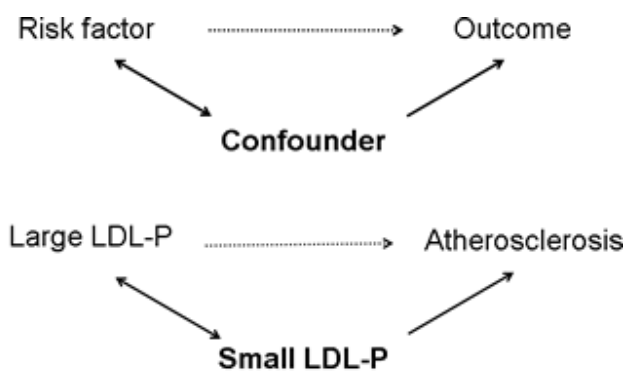


Figure 1. A decrease in average LDL particle size, as measured by gradient gel electrophoresis, may be due to an increase in the number of small LDL particles (left panel) or a decrease in the number of large LDL particles (right panel). Note that the number of total LDL particles is greater in the left compared with the right panel. This difference in LDL particle number is not captured by the measured average LDL size, which is lower in both cases.



Modified from J Otvos

Figure 2. Individuals with predominantly small LDL particles (shown on the right) are believed to have greater atherosclerotic risk than those with large LDL (shown on the left). However, individuals with smaller LDL size also have more LDL particles compared with individuals with larger LDL size, at any given level of LDL-C (in this example, LDL-C of 130 mg/dL).



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Figure 3. A confounder (e.g. small LDL) is a variable that masks the relationship between the risk factor (e.g. large LDL) and the outcome (e.g. atherosclerosis). The confounder is both associated with the risk factor and is also causally associated with the outcome.

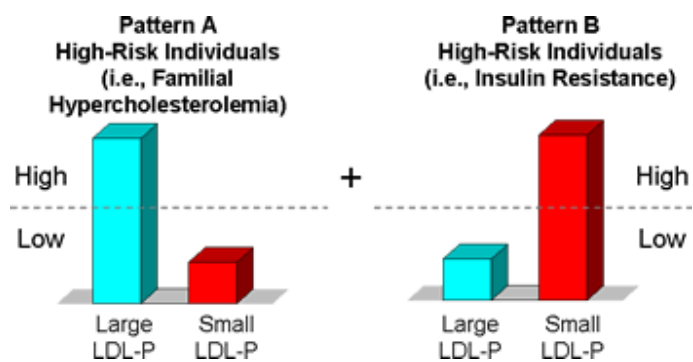


Figure 4. Large and small LDL confound each other in relation to cardiovascular disease.