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Small Dense LDL Particles: Clinically Relevant?

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Abstract

Purpose of review: Levels of small, dense LDL (sdLDL) particles determined by several analytic procedures have been associated with risk of atherosclerotic cardiovascular disease (ASCVD). This review focuses on the clinical significance of sdLDL measurement.

Recent findings: Results of multiple prospective studies have supported earlier evidence that higher levels of sdLDL are significantly associated with greater ASCVD risk, in many cases independent of other lipid and ASCVD risk factors as well as levels of larger LDL particles. A number of properties of sdLDL vs. larger LDL, including reduced LDL receptor affinity and prolonged plasma residence time as well as greater oxidative susceptibility and affinity for arterial proteoglycans, are consistent with their heightened atherogenic potential. Nevertheless, determination of the extent to which sdLDL can preferentially impact ASCVD risk compared with other apoB-containing lipoproteins has been confounded by their metabolic interrelationships and statistical collinearity, as well as differences in analytic procedures and definitions of sdLDL.

Summary: A growing body of data points to sdLDL concentration as a significant determinant of ASCVD risk. While future studies should be aimed at determining the clinical benefit of reducing sdLDL levels, there is sufficient evidence to warrant consideration of sdLDL measurement in assessing and managing risk of cardiovascular disease.

Keywords

Small dense LDL; cholesterol; cardiovascular disease

Introduction

Plasma low density lipoproteins (LDL) are defined as particles of density 1.019–1.063 g/ml. Within this spectrum, there are multiple subspecies varying in size, density, charge, and lipid and protein composition (1). Among these, as discussed below, small, dense, lipid depleted LDL particles (sdLDL) have been found to be particularly strongly associated with risk of atherosclerotic cardiovascular disease (ASCVD) (2). This review focuses on issues that have arisen as to the clinical significance of sdLDL measurement by addressing these questions: 1) how are sdLDL defined and measured?; 2) are sdLDL levels predictive of ASCVD risk independent of LDL-cholesterol (LDL-C) and beyond standard risk algorithms?; 3) is there

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evidence that sdLDL and large buoyant LDL (lLDL) differentially affect ASCVD risk?; and 4) is there a basis for selectively targeting sdLDL as a means of reducing ASCVD risk?

sdLDL Definition and Measurement

Multiple methodologies have been implemented for analysis of subclasses of LDL and other lipoprotein particle subspecies as a function of their density and/or diameter, including analytical and density gradient ultracentrifugation, non-denaturing gradient gel electrophoresis (GGE), nuclear magnetic resonance spectroscopy (NMR), and ion mobility (IM) (2–5). Table 1 describes properties of the LDL subspecies as a function of their analytic ultracentrifuge flotation rate (1), buoyant density (1), and particle size as assessed by IM (6). In this scheme, sdLDL can be considered to comprise the particle species spanning the range of small and very small LDL (vsLDL) from density 1.034 to 1.063 g/ml and size 214.1 to 180 Å. However, while there has been a report of correlations of LDL size subclass concentrations among several methodologies (4), their strengths varied considerably. Moreover, the subclass definitions have not been harmonized across the various analytic procedures, nor have their results been compared using common defined reference lipoprotein subfraction preparations. As has been pointed out (3, 5) these concerns have compromised the adoption of sdLDL concentration as a standard clinical laboratory measurement.

In addition to the methods for analyzing LDL particle concentrations noted above, a homogenous direct method for determination of plasma sdLDL-cholesterol (sdLDL-C) has been developed that has been shown to correlate well with levels of sdLDL defined as particles of $d = 1.044\text{--}1.063$ g/mL (7, 8). However, based on the LDL density ranges shown in Table 1, it is likely that this fraction corresponds with very small LDL-IV (vsLDL) rather than the small LDL-III subclass, as demonstrated by correlations of vsLDL-cholesterol determined by GGE with vsLDL mass concentrations measured by both analytical ultracentrifugation and IM (2). Thus, the potential ASCVD risk related to levels of LDL-III may not be identified by this procedure. Another consideration is that the 1.044–1.063 g/mL density range includes a small proportion of atherogenic lipoprotein(a) particles, and therefore it is possible that the sdLDL-C assay is also detecting this component. However the particle diameter of Lp(a) significantly exceeds that of sdLDL (9), and thus Lp(a) is not included in size-based measurements of sdLDL (GGE, NMR, and IM).

sdLDL in the Prediction of CVD Risk

The assessment of the relationship of sdLDL to ASCVD risk is confounded by the fact that this LDL category is a component of a lipoprotein phenotype that includes elevated levels of triglycerides and very low density lipoproteins (VLDLs) and reduced levels of high density lipoprotein (HDL)-cholesterol (10), and it is correlated with plasma apolipoprotein B and other ASCVD risk factors comprising metabolic syndrome (1). The multiple co-linearity among these measures creates challenges for assessing the specific impact of sdLDL using standard statistical approaches, which are subject to potential over-adjustment. Nevertheless, the question can be raised as to the extent to which sdLDL predicts ASCVD beyond standard risk measures.

Multiple prospective studies have assessed the relation of baseline sdLDL level to incidence of clinical cardiovascular events. A recent systematic review and meta-analysis assembled data from prospective studies (cohort, case-control, nested case-control, and randomized controlled trial) published through January, 2020 that utilized measurements derived from GGE, NMR, and the homogeneous sdLDL-cholesterol assay (11). The composite relative risk/hazard ratios in 14 studies with measurements of sdLDL other than sdLDL-C were 1.46, 1.72, and 1.54 (all but the last statistically significant) in analyses adjusted for co-variables (age and sex; demographics plus lifestyle risk factors; demographics plus lifestyle risk factors and lipid fractions, respectively). In a second group of 7 studies in which sdLDL-C was determined using GGE or the direct homogeneous assay (11), the values were 1.21, 1.92, and 1.75, respectively (all significant).

The question as to whether the relation of sdLDL-C to ASCVD risk is independent of total LDL and other risk factors was addressed in two of the prospective cohort studies included in the meta-analysis, as well as in several more recent reports. In the Atherosclerosis Risk in Communities study of 10,225 individuals followed for an average of 11 years, the hazard ratio for coronary heart disease (CHD) was 1.51 (95% confidence interval [CI] 1.21–1.88) for the highest versus the lowest quartile, respectively, in a model that included established risk factors, with similar values for individuals with total LDL-C greater or less than 100 mg/dL (12). Moreover, among 3,334 normoglycemic participants in the Multiethnic Study of Atherosclerosis (MESA), those in the top sdLDL-C quartile showed higher risk of incident CHD (hazard ratio, 2.41; $P=0.0037$) compared with those in the bottom quartile in multivariable adjusted models, with very similar values in those with total LDL-C above and below 100 mg/dL (13). In another analysis of MESA data, the estimated plasma level of sdLDL-C derived from an algorithm based on standard lipid measurements was the strongest lipid predictor of ASCVD after multivariate adjustment for other known ASCVD risk factors (14). Notably, in both ARIC and MESA (12, 13) there were no significant ASCVD associations with quartiles of lLDL-cholesterol, determined as the difference between total LDL-C and sdLDL-C.

Several recent studies not included in the meta-analysis also documented a relationship of sdLDL-C to ASCVD risk. Among 38,322 individuals participating in the Copenhagen General Population Study with a median 3.1 yr of follow-up, the hazard ratio for ASCVD per 1 mmol/l (39 mg/dl) higher sdLDL-C in a multivariable-adjusted Cox model was 1.62 (95% CI: 1.33–1.96) (15). In a nested prospective case-control study from the Women's Health Study, sdLDL-C was significantly associated with myocardial infarction (HR for Quartile 4 vs 1: 3.71 [95% CI: 1.59 to 8.63]; p for trend < 0.001) (16). Additionally, in 3094 individuals initially free of ASCVD in the Framingham Offspring Study, the ASCVD HR for sdLDL-C was 1.28 (CI 1.04–1.58, $p=0.021$) in a multivariable model including total cholesterol and HDL-C and other standard ASCVD risk factors (17). Moreover, elevated sdLDL-C was the best lipoprotein-related measure of incident ASCVD and added significant risk information to the ACC/AHA pooled cohort equation (17). In another recent study of patients with angiographically documented coronary artery disease (CAD), elevated level of sdLDL-C, but not total LDL-C or non-HDL-C, was predictive of ASCVD events, a finding driven by the presence of diabetes mellitus (18). Finally, in a trial of patients with stable CAD randomized to low dose (1 mg/d) vs. high dose (4 mg/d) pitavastatin,

there was a significant association of sdLDL-C quartiles with ASCVD events independent of LDL-C and other risk factors on the low dose, whereas there was no association on the high dose, suggested to reflect a significantly greater reduction of sdLDL-C in those with higher baseline levels (19).

A caveat to interpretation of the findings for sdLDL-C, as noted above, is that this may not represent the full spectrum of sdLDL particles (LDL-III + LDL-IV), as do, for example, the NMR and IM methods. Moreover, two studies employing detailed measurements of LDL size subfractions by IM have shown that levels of medium size LDL-II as well as small LDL-III (but not large LDL-I), are significantly predictive of ASCVD (20, 21).

Do sdLDL and lbLDL Differentially Impact ASCVD?

While the evidence reviewed above points to a significant association of sdLDL with ASCVD risk, in many cases independent of other risk factors, there has been controversy as to whether this represents differential atherogenicity of small and large LDL particles, and hence whether concentration of total LDL particles (LDL-Ps) or, as discussed below, apoprotein B (apoB), is sufficient as an LDL-related measure of ASCVD risk. A number of properties of sdLDL particles can be invoked to suggest heightened atherogenicity compared with larger LDL (22). These include prolonged plasma residence time (23) reflecting reduced LDL receptor binding (24) due to conformational changes in apoB (25), increased susceptibility to oxidative modification (26) and glycation (27), preferential enrichment in lipoprotein-associated phospholipase A2 (28) and the pro-inflammatory protein apoC-III (29), and content of specific components of the lipidome that can act to enhance the atherogenicity of sdLDL (30). On the other hand, it has been suggested that the higher cholesterol content of larger LDL particles should be considered as a feature that may promote atherogenesis to a similar degree as sdLDL (31). Nevertheless, despite these considerations, it cannot be assumed that the differing pathophysiologic properties of LDL subclasses translate into their impact on ASCVD outcomes.

Several additional arguments have been made in support of the premise that smaller and larger LDL particles are equally atherogenic, and hence that measurement of sdLDL level does not add to the risk information provided by total LDL-P concentrations. As described below, however, there are concerns with each of these arguments:

- When both peak LDL diameter and LDL-P concentration have been included in models assessing their relation to carotid atherosclerosis (32) or ASCVD (33, 34), only LDL-P has been found to be significantly predictive of risk. However, while peak LDL diameter can be used to identify larger and smaller LDL subclass phenotypes, as discussed below, it does not represent the more pathologically relevant absolute concentrations of the subclasses.
- Patients with familial hypercholesterolemia (FH) have a relative abundance of large LDL particles (35), and this has supported the case that large LDL-Ps as well as small LDL-Ps are atherogenic, and that thus the total number rather than the size of LDL-Ps is the key LDL determinant of CVD risk. This does not however consider that the total number of LDL-Ps is much greater in FH than

is typically seen in patients with atherogenic dyslipidemia, and also that due to LDL receptor deficiency, levels of small as well as large LDL are increased. Importantly however, if, as is likely, prolonged plasma residence time of LDL particles is a significant determinant of ASCVD risk, this mechanism may apply both to sdLDL by virtue of their lower LDL receptor affinity, as noted above, and to the more general impairment of plasma LDL-P clearance due to reduced LDL receptor number or function in FH.

- A related issue to consider is evidence that apoB, an established ASCVD index (36) which represents the total concentration of LDL, intermediate density lipoprotein, and VLDL particles, is strongly correlated with levels of small LDL-III [(12) and Figure 1], but not with large LDL-I (Figure 1). Hence it can be argued that apoB level may be sufficient to assess the impact of sdLDL, as well as other atherogenic lipoproteins, on ASCVD risk. The studies cited above indicating the relationship of sdLDL to ASCVD did not attempt to adjust for its collinearity with apoB. However, the variance in sdLDL explained by apoB [$r^2 \sim 40\%$, (12) and Figure 1] is not sufficient to consider apoB as a surrogate for sdLDL, nor do the relationships with apoB address the relative atherogenicity of small vs. large LDL particles.
- Since as noted above there is in general an inverse correlation between plasma levels of small and large LDL (4), it has been suggested that both subfractions should be included in regression models for assessing their independent relation to ASCVD risk. The application of such a model has indicated that while large LDL did not have a significant univariate relationship with risk, the association became significant when both fractions were included in the model (32, 33). On this basis, as well as observations in some studies that the association of sdLDL with ASCVD is reduced in significance with inclusion of total LDL-P in regression models, it has been concluded that large and small subfractions have similar atherogenic effects on a per-particle basis, and hence that total LDL-P is superior to sdLDL for risk stratification and lipid management (32, 33). This statistical method however obscures the confounding differential impact of LDL size phenotypes. It has been shown that peak LDL particle size and density generally follow a bimodal distribution in the population, with the modes representing individuals with predominance of either lbLDL (phenotype A) or sdLDL (phenotype B) (6, 37–39), and that these phenotypes have, in part, a genetic basis (40). Based on this bimodality, it would be considered appropriate to include both LDL size phenotype and LDL subfractions in multivariable regression models, as well as an interaction term for the phenotype. The results of including such an interaction in assessing the relationship between large and small LDL particle concentrations are illustrated in Figure 2, which is based on IM data from 158 overweight but otherwise healthy individuals [(41), (Krauss, R.M., personal communication)]. Figure 2A displays the modes in the peak LDL particle diameter distribution, and Figure 2B shows an inverse correlation between large and small LDL concentrations using a linear model. However, as shown in Figure 2C, a model employing a significant interaction term for

LDL size phenotype ($p < 0.001$) reveals that for phenotype B there is a significant positive association between large and small LDL concentrations ($p < 0.001$), whereas there is not a significant association for phenotype A. Thus, it would not be appropriate for ASCVD risk prediction to employ a model including both large and small LDL with the aim of adjusting for the relationship between them without including an LDL phenotype interaction term which could unmask the effect modification by these phenotypes. Differing relationships of large and small LDL concentrations between the two LDL subclass phenotypes are consistent with the significant differences in the physical and compositional properties of LDL subfractions between the phenotypes (42, 43).

In summary, these considerations, coupled with the results from the prospective studies reviewed above, do not provide strong support for the premise that all LDL particles are equally atherogenic. Multiple properties of sdLDL that differentiate them from larger LDL particles, as noted above, may contribute to a preferential impact on ASCVD risk.

Is there a basis for targeting sdLDL for reducing ASCVD risk?

Statins, the most commonly used lipid-lowering drugs, are effective in lowering levels of sdLDL, but the magnitude of reduction can differ between statin types (44) and doses (45), and may depend on a patient's dyslipidemic phenotype (45–47). In this regard, it has been shown that the reduction in sdLDL is strongly correlated with baseline triglyceride level (46), likely reflecting reduced LDL formation as a result of statin-induced catabolism of TG-rich precursors (47). Moreover, the measurement used to assess sdLDL may be a factor influencing the results, in that statins lower cholesteryl ester content of all apoB-containing lipoproteins (46), and hence assays that measure sdLDL-C may manifest a disproportionately greater reduction relative to sdLDL particle concentration. Finally, there is variation in the capability of differing analytic techniques to clearly distinguish sdLDL and vsLDL subspecies from other LDL subclasses (3,48), and in this regard there are several reports based on high resolution ion mobility measurements that statin-induced reductions in levels of vsLDL particles are much smaller than those for larger LDL (20, 49, 50) likely reflecting, at least in part, their reduced LDL receptor affinity. For this reason, and also to assess the therapeutic benefit of reducing levels of sdLDL, it would be desirable to develop therapeutic approaches for specifically targeting these particles. However, the strong metabolic interrelationships of sdLDL with other lipids and lipoproteins creates a challenge for devising such an intervention. Alternatively, the impact of lowering sdLDL on ASCVD risk could be predicted using a Mendelian randomization analysis. In this regard, it is of interest that a common ASCVD risk-raising allele at a locus that regulates expression of the *SORT1/CELSR2/PSRC1* gene cluster is associated with higher levels of sdLDL and vsLDL, but not with larger LDL particles (51). However, since this allele is also significantly associated with elevated levels of total LDL-C, it has not been feasible to conduct a Mendelian randomization analysis based on this genetic locus that specifically addresses the causality of the small and very small LDL subspecies.

Conclusion

There is a growing body of evidence for a strong relationship of sdLDLs to ASCVD risk, and this presents a case for their measurement in assessing and managing this risk. However there remain challenges for determining the utility of incorporating sdLDL concentration in standard clinical guidelines. These point to the need for standardization across sdLDL assays, as well as research aimed at testing the impact on ASCVD risk of selectively reducing plasma sdLDL concentrations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key points

* Recent prospective studies have supported a significant association of plasma sdLDL concentration with risk of ASCVD.

- There is evidence that this relationship is independent of plasma LDL cholesterol level and can add to the risk assessed by current algorithms.
- The strong metabolic and statistical interrelationships of sdLDL with other lipoprotein and ASCVD risk markers confound the ability to determine the specific contribution of sdLDL to disease risk.
- Several analytic methodologies are available for clinical measurement of sdLDL concentrations, but there remains the need to harmonize their results.
- Taken together with the pathophysiologic properties of sdLDL, the available data do not support the premise that all LDL particles are equally atherogenic and suggest that measurement of sdLDL has a role in the clinical assessment and monitoring of ASCVD risk.

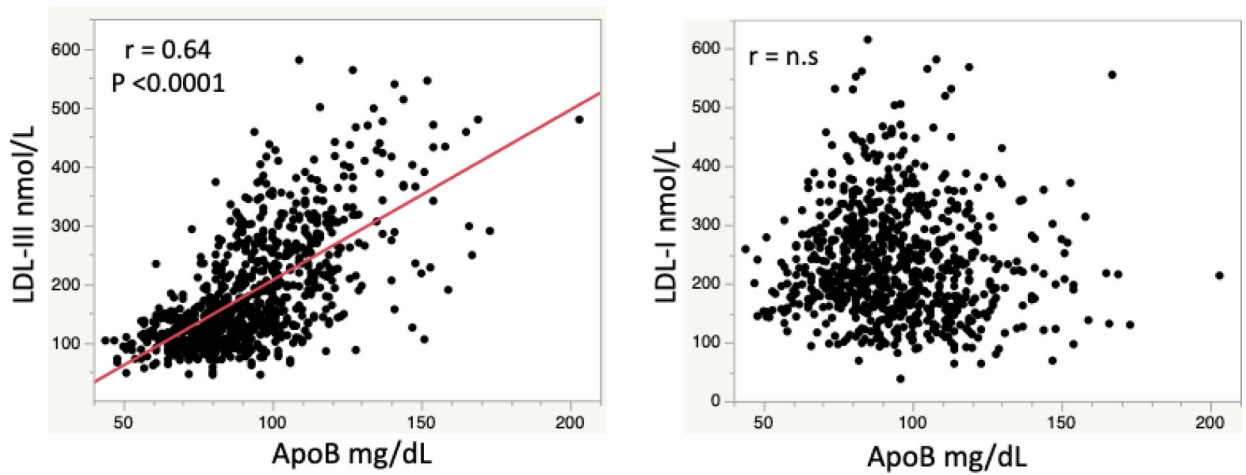


Figure 1. Correlations between plasma levels of immunochemically measured apoB and small LDL-III and large LDL-I.

LDL measurements were made by ion mobility in 804 healthy men and women using data derived from a previously published study (52). Similar results were obtained for small LDL defined as LDL-III plus LDL-IV. r: Pearson correlation coefficient. n.s.: not significant at $p < 0.05$.

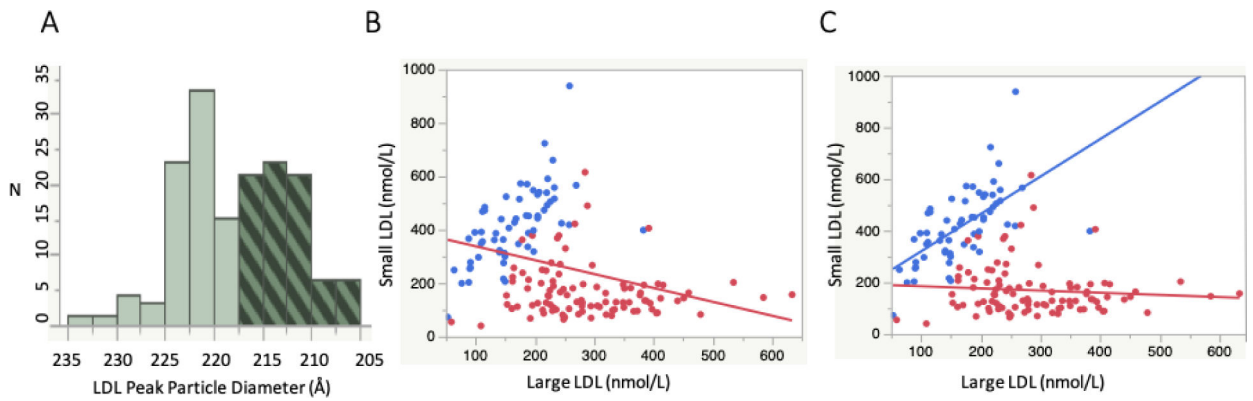


Figure 2. Statistical considerations in assessing interrelationships of large and small LDL.

2A: Bimodality of the distribution LDL peak particle diameter determined by ion mobility in 158 overweight but otherwise healthy participants in a previously published study (41). The clear bars represent phenotype A (peak diameter $>217.5 \text{ \AA}$) and the cross-hatched bars represent phenotype B. **2B:** Concentrations of lbLDL (LDL-I) vs. sdLDL (LDL-III). Red circles are individuals with phenotype B and blue circles are those with phenotype A. The line represents a significant inverse relationship ($p < 0.001$) using a linear regression model. **1C:** The same data as in Figure 1B with regression lines determined in a model incorporating an interaction for LDL size phenotype ($p < 0.001$). The positive slope for phenotype B is significant ($p < 0.001$), whereas the relationship for phenotype A is not.

Table.

Classification of LDL subclasses based on analytic ultracentrifugal flotation rate (1), density range (1), and particle size interval (4).

LDL Subclass Analytic ultracentrifugation flotation rate (Sf)	LDL Size Subspecies	Density Range Density gradient ultracentrifugation (g/ml)	Size Interval Ion mobility (Å)
Large LDL-I (Sf 7–12)	1	1.019 – 1.023	224.6 – 233.3
Medium LDL-II (Sf 5–7)	2a	1.023 – 1.028	220.0 – 224.6
	2b	1.028 – 1.034	214.1 – 220.0
Small LDL-III (Sf 3–5)	3a	1.034 – 1.041	208.2 – 214.1
	3b	1.041 – 1.044	204.9 – 208.2
Very small LDL-IV (Sf 0–3)	4a	1.044 – 1.051	199.0 – 204.9
	4b	1.051 – 1.063	190.0 – 199.0
	4c	Not determined	180.0 – 190.0