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Apolipoprotein B compared with low-density lipoprotein cholesterol in the atherosclerotic cardiovascular diseases risk assessment

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ABSTRACT

The subendothelial retention of apolipoprotein B (apoB)-containing lipoproteins is a critical step in the initiation of pro-atherosclerotic processes. Recent genetic and clinical evidence strongly supports the concept that the lipid content of the particles is secondary to the number of circulating atherogenic particles that are trapped within the arterial lumen. Since each low-density lipoproteins (LDL) particle contains one apoB molecule, as do intermediate density lipoprotein (IDL) and very low-density lipoprotein (VLDL) particles, apoB level represents the total number of atherogenic lipoproteins, which is independent of particle density, and not affected by the heterogeneity of particle cholesterol content (clinically evaluated by LDL-cholesterol level). From this perspective, apoB is proposed as a better proxy to LDL-cholesterol for assessing atherosclerotic cardiovascular disease risk, especially in specific subgroups of patients, including subjects with diabetes mellitus, with multiple cardiometabolic risk factors (obesity, metabolic syndrome, insulin resistance, and hypertension) and with high triglyceride levels and very low LDL-cholesterol levels. Therefore, given the causal role of LDL-cholesterol in atherosclerotic cardiovascular disease (ASCVD) development, routine measurement of both LDL-cholesterol and apoB is of utmost importance to properly estimate global cardiovascular risk and to determine the 'residual' risk of ASCVD in patients receiving therapy, as well as to monitor therapeutic effectiveness.

Assessment of low-density lipoprotein cholesterol (LDL-C) is a key component of the management of the risk of atherosclerotic cardiovascular disease (ASCVD) [1,2]. However, despite overwhelming evidence that LDL-C-targeted therapies effectively reduce ASCVD risk, many individuals with a normal or low concentration of LDL-C still experience ASCVD-related events. Although the view of cardiovascular risk has always been lipid-centric, evidence has suggested that several factors contribute to the residual risk, such as the systemic burden of inflammation or metabolic impairment. However, there is no doubt that the residual risk of lipid origin is based on atherogenic dyslipidemia, characterized by an increase in triglycerides (TG) and triglyceride-rich lipoproteins and qualitative alterations in low-density lipoprotein (LDL) particles [3]. This suggests that a focus solely on the measurement of LDL-C is not an optimal strategy for all patients. There is now a large body of evidence to support the hypothesis that the key initiating event in atherogenesis is the retention of cholesterol-rich apolipoprotein B (apoB)-containing lipoproteins within the arterial wall [4]. As a consequence, several guidelines propose using apoB to stratify cardiovascular risk and to define therapeutic goals [5–7]. This review aims to evaluate the relevant literature and critically discuss the role of apoB measurement in the management of patients for cardiovascular prevention.

1. From biological insights to the pathogenesis of atherosclerotic cardiovascular disease

Apolipoproteins are structural components of plasma lipoproteins (Table 1). The major apolipoproteins involved in the regulation of lipoprotein metabolism are apolipoprotein B-100 (apoB-100), apolipoprotein B-48 (apoB-48), apolipoprotein A-I (apoA-I), apolipoprotein C-II (apoC-II), apolipoprotein C-III (apoC-II), apolipoprotein E (apoE), and apolipoprotein(a) (apo(a)). ApoB-100 is the major structural component of very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and lipoprotein(a) (Lp(a)). ApoB-48 is a truncated isoform of apoB-100; it is the only specific marker for intestinal chylomicrons and cannot be exchanged between lipoproteins, like apoB-100 [8]. ApoA-I is the major component of high-density lipoprotein (HDL) particles and plays an important role in reverse cholesterol transport. ApoC-II, apoC-III, and apoE are involved

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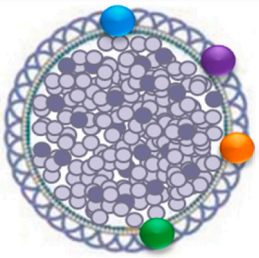
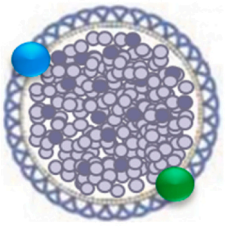
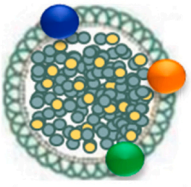
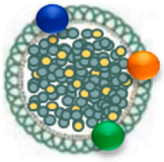
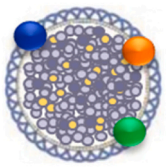
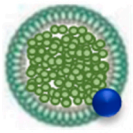
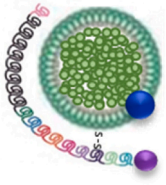
Table 1
Main characteristics of plasma lipoproteins.

	Major apo-proteins	Density (g/ml)	Diameter (nm)	Major lipids	Structure
Chylomicrons	ApoB-48 ApoC ApoE ApoA-I ApoA-II ApoA-IV	≤ 0.950	75-1200	TG	
Chylomicrons remnants	ApoB-48 ApoE	0.930–1.006	30-80	TG, CE	
VLDL	ApoB-100 ApoE ApoC	0.930–1.006	30-80	TG	
VLDL remnants	ApoB-100 ApoE ApoC	< 1.006	30-60	TG	
IDL	ApoB-100 ApoE ApoC	1.006–1.019	25-35	TG, CE	
LDL	ApoB-100	1.019-1.063	18-25	CE	
Lp(a)	ApoB-100 Apo(a)	1.040–1.130	~25	CE	
HDL	ApoA-I ApoA-II ApoC ApoE	1.063-1.210	7-13	CE, PH	

CE: cholesterol ester, HDL: high-density lipoprotein, IDL: intermediate-density lipoprotein, LDL: low-density lipoprotein, Lp(a): lipoprotein(a), PH: phospholipid, TG: triglyceride, VLDL: very-low-density lipoprotein.

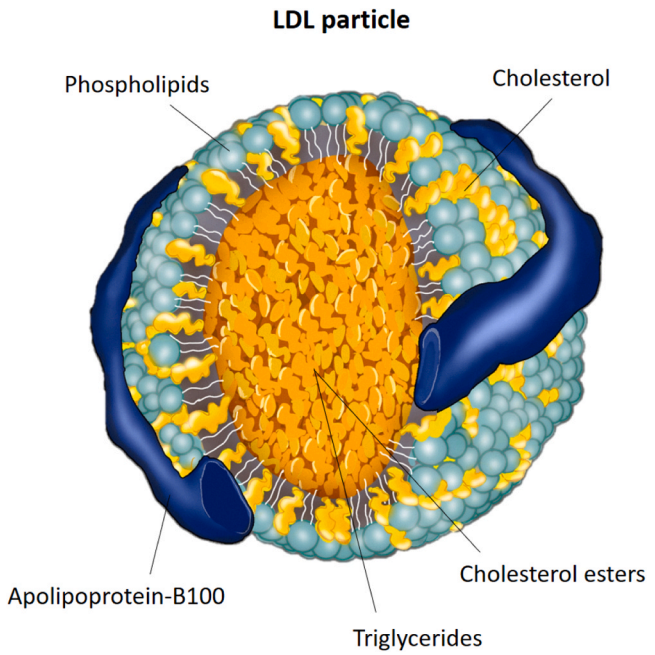


Fig. 1. Structure of LDL (low-density lipoprotein), the main apolipoprotein B-containing lipoprotein.

in the metabolism of triglyceride-rich lipoprotein. Finally, apo(a) binds covalently to apoB-100 in the Lp(a) particle [9,10].

Apolipoprotein B assays recognize both apoB-48 and apoB-100. Because there are few apoB-48 particles (even in the postprandial phase), total apoB simply represents the sum of VLDL, LDL, and Lp(a) particles [11].

ApoB–containing lipoproteins are quasi-spherical particles (Fig. 1). Each has a monolayer of phospholipids arranged around its circumference within which there are small amounts of cholesterol and through which a single molecule of apoB-100 encircles the lipoprotein particle [12]. The apoB molecule provides structural stability and integrity, acts as lipoprotein receptor ligand, and solubilizes neutral lipids in the plasma. It stays with the particle throughout its metabolic lifetime, whereas variable amounts of TG and cholesteryl esters (CE) constitute the core of the particle [13].

Ingested dietary fatty acids are absorbed in the intestinal lumen (Fig. 2) and are combined with apoB-48 in enterocytes, forming chylomicrons. These particles are secreted in circulation where they are rapidly hydrolysed by lipoprotein lipase (LPL), releasing free fatty acids that can be used by muscle cells as a source of energy or resynthesized into triglycerides and stored in the adipose tissue. Chylomicron remnants, rich in TGs, are removed from circulation by hepatocytes. TGs are also produced in the liver and, combining with apoB-100, form VLDL, which enter blood circulation as VLDL particles. VLDL-triglycerides are a substrate for LPL and are hydrolysed at the luminal surface of adipose tissue and muscle capillaries. The removal of TGs from VLDL results in the formation of VLDL remnants and intermediate density lipoproteins (IDL). IDL particles, relatively enriched in CE, can be further processed into LDL particles through lipolysis by hepatic lipase (HL). These LDL particles contain a core of CE and a smaller amount of TG. LDL are in charge of the transport of endogenous cholesterol. Their catabolism goes mainly through the binding to hepatic LDL receptors (LDLR), thanks to the specific interaction with apoB-100. Internalization causes the membrane at the junction to sink in for endocytosis. In the vesicles, LDL is separated from the receptor and fused with the lysosome. ApoB-100 is decomposed into amino acids by a lysosomal proteolytic enzyme, and cholesteryl esters are hydrolysed into free cholesterol and fatty acids by cholesterol esterase for cell utilization.

In individuals with normal TG, there are approximately 10 VLDL particles for each chylomicron and/or chylomicron remnant particle. Similarly, since VLDL particles have a short half-life (4–6 h) in plasma while LDL particles have a longer half-life (2–4 days), there are many more circulating LDL particles than VLDL particles. As plasma TG levels increase, the proportion of VLDL particles increases [13]. VLDL particles are grossly heterogeneous in composition and size. The liver may secrete larger TG-enriched VLDL particles or smaller VLDL particles, which contain less TGs [14]. LDL particles can differ in the mass of cholesteryl esters within their core, and consequently can differ in size. Variance in the composition of these particles is based on CE transfer protein (CETP)–mediated exchange of the core lipids, CE and TG, between the plasma lipoproteins [11].

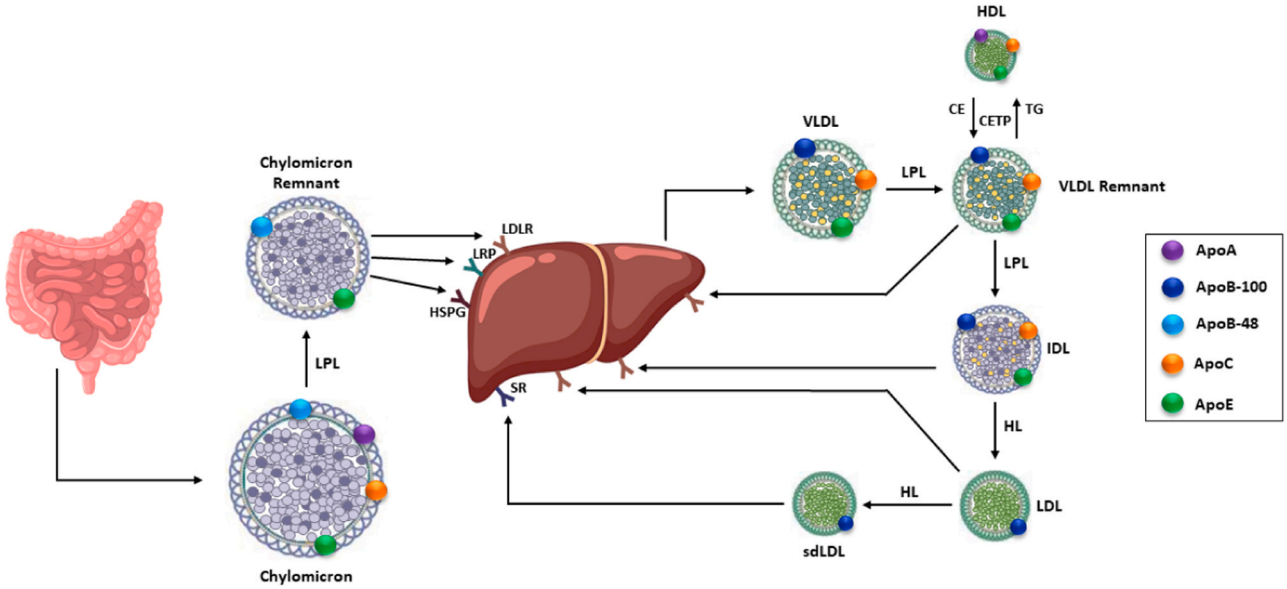


Fig. 2. Lipoprotein metabolism and lipid exchange mechanism. ApoA: apolipoprotein(a), ApoB-100: apolipoprotein B-100, ApoB-48: apolipoprotein B-48, ApoC: apolipoprotein C, ApoE: apolipoprotein E, CE: cholesteryl ester, CETP: cholesteryl ester transfer protein, HDL: high-density lipoprotein, HL: hepatic lipase, sdLDL: small dense LDL, HSPG: heparan sulfate proteoglycans, IDL: intermediate-density lipoprotein, LDLR: low-density lipoprotein (LDL) receptor, LPL: lipoprotein lipase, LRP: LDL receptor–like protein, SR: scavenger receptor, TG: triglyceride, VLDL: very-low-density lipoprotein.

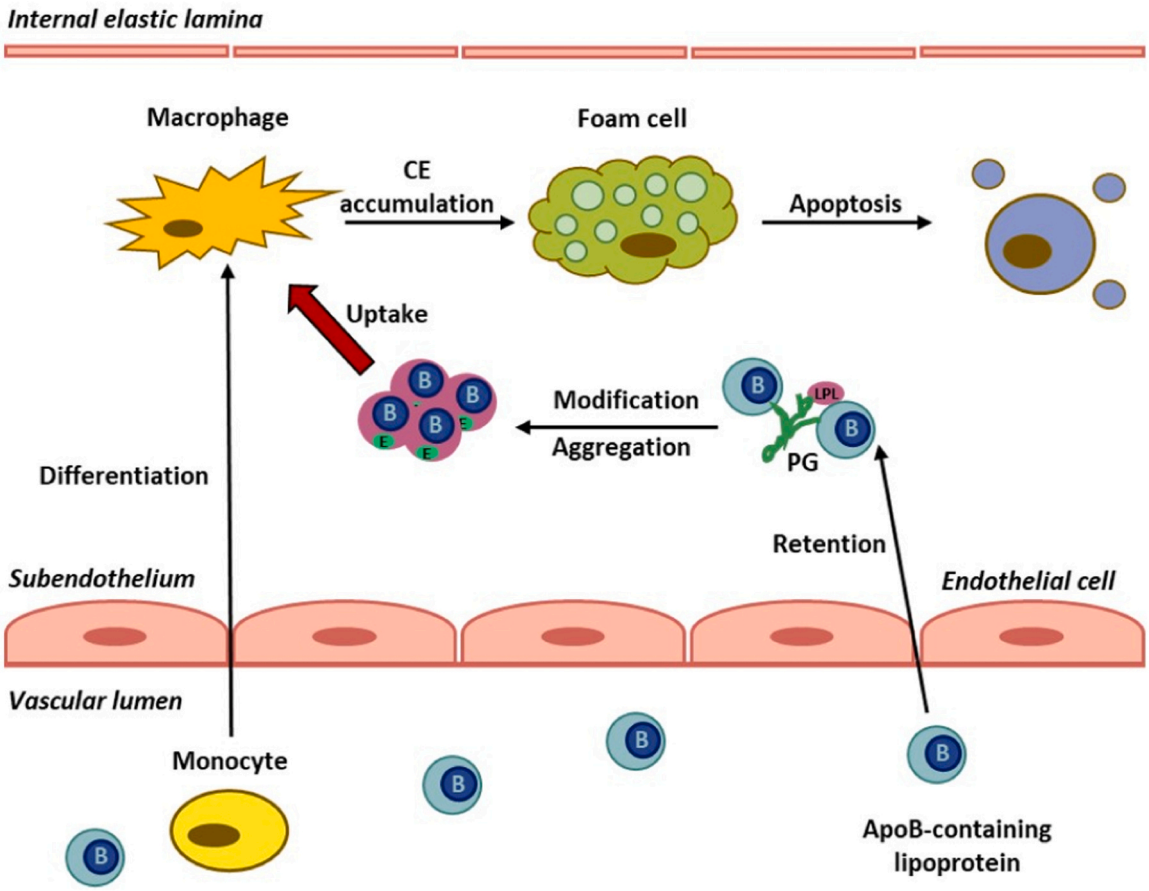


Fig. 3. Development of atherosclerosis associated with modified lipoproteins. ApoB-100: apolipoprotein B-100, CE: cholesteryl ester, LPL: lipoprotein lipase, PG: proteoglycan.

All apoB-100-containing lipoproteins less than 70 nm in diameter, including TG-rich VLDL remnants and LDL, freely flux across the endothelial barrier and can be retained in the artery wall. As the first step, the cluster of basic amino acid residues on apoB-100 binds to proteoglycans in the arterial intima, leading to lipoprotein retention [15]. The retained particles are modified by oxidation of phospholipids and of apoB-100 lysyl residues [16]. These oxidized particles induce a local inflammatory and immune response, such as increased expression of leukocyte chemoattractants on endothelial cells [17]. Peripheral blood monocytes enter the intimal space where they differentiate into macrophages expressing scavenger receptors; following an uncontrolled uptake of lipids, they become foam cells, a major hallmark of early-stage atherosclerotic lesions [4]. The inflammatory cascade, together with smooth muscle cell mobilization from the media to the intima [18], are eventually the key events leading to the formation of the atherosclerotic plaque (Fig. 3).

LDL particles are not the only lipoproteins involved in the development of atherosclerosis. This is the main reason why, especially in some patients, measuring LDL-cholesterol alone is an imperfect tool to assess lipoprotein-associated cardiovascular risk.

An approach suggested by several guidelines is the calculation of non-HDL cholesterol (non-HDL-C), which provides the cholesterol content in all apoB-containing lipoproteins (LDL, VLDL, VLDL remnants, and Lp(a)), all carrying only one apoB molecule per particle. This allows apoB measurement to serve as a particle number for these lipoproteins. Because of its extended plasma residence time, more than 90% of apoB-containing particles in plasma are LDL, which means that apoB measurement mostly represents LDL particle number [19]. However, this could be not the case in post-prandial state, or in patients with hypertriglyceridemia or diabetes. Indeed, if the mass of cholesterol per apoB particle were invariant, non-HDL-C and apoB would be identical predictors of the risk of cardiovascular disease. In reality, the cholesterol mass per apoB can vary substantially. Small lipid-depleted LDL subfractions contain less cholesterol than larger ones. These small LDL particles are typically observed in patients with elevated TG concentrations or conditions such as diabetes or metabolic syndrome [20]. Moreover, in a given patient, the cholesterol composition of LDL can also change in response to lifestyle modifications or to lipid-altering treatments. As an example, kinetic studies have shown that weight loss reduces VLDL-apoB secretion in viscerally obese patients, and significantly increase the catabolism of LDL apoB-100 [21,22].

As risk management decision-making relies so heavily on LDL-C measurement, it is important to know which marker is more strongly related to cardiovascular disease (CVD).

2. Biomarkers for atherosclerotic cardiovascular disease risk assessment

Early observations that cholesterol is a key component of arterial plaques gave rise to the cholesterol hypothesis for the pathogenesis of atherosclerosis, the underlying cause of heart attack, stroke, and peripheral vascular disease [23]. Population studies have demonstrated that elevated levels of LDL-C, non-HDL-C, and apoB are directly associate with the risk for ASCVD [24–26].

Despite apoB, non-HDL-C, and LDL-C being highly correlated, they are not identical. Indeed, both apoB and non-HDL-C comprise all apoB–containing lipoproteins (not only LDL); however, non-HDL-C measures the cholesterol content of these lipoproteins, whereas apoB provides an estimate of the total number of circulating particles.

Studies that include these 3 lipid traits as independent variables for

ASCVD risk have found diverging results because high intercorrelation can mask the additive influence of apoB and non-HDL-C in addition to LDL-C [25,27]. In discordance analyses, ASCVD risk tracks better with apoB and non-HDL-C than with LDL-C [28].

Cromwell et al. performed an analysis on the Framingham Offspring Cohort, showing that the number of LDL particles, measured by nuclear magnetic resonance (NMR), was related more strongly to future CVD than LDL-C or non–HDL-C in multivariable models adjusting for non-lipid CVD risk factors. As individuals with low LDL-C concentrations also have cholesterol-poor particles, irrespective of TG level and LDL size, the findings of low LDL-C levels, even when resulting from LDL-lowering therapy, can contribute to the underestimation of both LDL-C and CVD risk by measured levels of LDL-C [29]. Interestingly, they also observed that non-HDL-C weaklier related to incident CVD than LDL particle numbers. This finding should be read considering that VLDL constitute only a small fraction (about 5%) of the total number of atherogenic particles. This is true also when TG are significantly elevated, as the excess TG is carried predominantly by large VLDL, which are relatively few in number [30]. Another clear demonstration of the discordance between atherogenic particle number and cholesterol mass comes from the MESA study [31]. In a community-based cohort of 6814 persons free of clinical CVD at entry and followed for CVD events, LDL-C and LDL particle number (measured by NMR spectroscopy) were overall associated with incident CVD, but for those with discordant levels, only LDL particle number (i.e. apoB) was associated with incident CVD. Sniderman et al. conducted a meta-analysis of all the published studies reporting estimates of the relative risks of fatal and nonfatal ischemic vascular events with different lipid parameters, observing that apoB was the most potent marker of CVD risk compared to LDL-C and non-HDL-C, whether analyzed individually or head to head [32].

The Apoprotein-related Mortality Risk Study (AMORIS) is probably the largest and the first study that definitively confirms the superiority of apoB versus LDL-C in predicting the risk of fatal myocardial infarction among 175,553 Swedes. ApoB was superior to LDL-C at every level of cholesterol, the difference being particularly marked in those in the lower half of the distribution [33]. Thus, in about half the population with normal to low concentrations of LDL-C, apoB should be preferred over LDL-C to indicate the risk of fatal myocardial infarction. In a large Mendelian randomization (MR) study on more than 440,000 participants from the UK Biobank, independent genetic variants associated with LDL-C, apoB, TG, HDL-C, and apo A-I were tested for the potential causal role in coronary heart disease (CHD) incidence [34]. In a multivariable model, apoB was shown to be strongly associated with the risk of CHD and to attenuate to the null the effect estimates for all other entities. The robustness of these findings is granted by the analytical approach, as multivariable MR allows to simultaneously account for genetic associations with lipids and apolipoproteins. This was confirmed in another MR study evaluating lipoprotein measures as mediators between lipid-associated genetic variants and coronary artery disease, in which the top combination of 30 lipoprotein measures ranked by the model score contains apoB only [35]. More recently, Marston et al. [36] conducted a prospective cohort study that included 389,529 participants in the primary prevention group and 40,430 participants in the secondary prevention group. In the primary prevention cohort, apoB, non–HDL-C, and TG each individually were associated with incident myocardial infarction. When the three lipid markers were evaluated together, only apoB was associated with myocardial infarction in both the primary prevention (adjusted Hazard Ratio [aHR]: 1.27; 95% CI, 1.15–1.40) and secondary prevention (aHR: 1.17; 95% CI, 1.00–1.36) groups, suggesting that the amount of lipid (cholesterol or TG) carried on the apoB-containing lipoprotein particles did not confer additional risk beyond apoB concentration. Yun et al. [37] conducted a prospective cohort study on the Korean Genome and Epidemiology Study and showed that apoB had the highest aHR per 1-SD of 1.26 (95% CI, 1.11–1.43), followed by non-HDL-C with an aHR of 1.25 (95% CI, 1.11–1.41), and LDL-C with the lowest aHR of 1.20 (95% CI, 1.06–1.37) after adjusting for sex, age, hypertension, diabetes mellitus, current smoking, and family history of premature ASCVD and chronic kidney disease. Finally, Lim et al. analysed 912 patients with type 2 diabetes mellitus (T2DM) and found that apoB had a significant relationship with metabolic syndrome regardless of LDL-C, suggesting that apoB could be an effective risk factor for predicting CVD in patients with T2DM [38].

3. The evaluation of drug efficacy and goal attainment

Randomized clinical trials (RCTs) have demonstrated that lipid-lowering drugs such as statins, ezetimibe, and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors significantly lower ASCVD risk [1]. All these drugs increase LDLR activity, thus increasing the rate at which apoB particles are removed from the plasma. In 2015, a large individual-level Mendelian randomization study found that lowering LDL-C with statins, ezetimibe, or combination therapy with both ezetimibe and a statin reduces the risk of CHD by approximately the same amount per unit lower LDL-C, and the magnitude of the observed clinical benefit is proportional to the absolute magnitude of the reduction in LDL-C, regardless of which treatment is used [39]. However, several studies clearly showed that, despite achieving target LDL-C levels with lipid-lowering treatment, there is still a high residual risk of coronary artery disease-related events [40]. The different effect of various hypolipidemic drugs on LDL-C, non-HDL-C, and apoB may likely explain much of the sometimes-unexpected evidence derived from RCTs [11]. Therefore, a key question is whether on-treatment apoB or on-treatment non-HDL-C is a more informative indicator of lipoprotein-attributable residual risk.

Analyses so far have yielded heterogeneous results as to whether LDL-C, non-HDL-C or apoB is the best marker of the effectiveness of cardiovascular therapy. A participant level meta-analysis of 8 major statin trials demonstrated that non-HDL-C was a marginally more accurate marker of residual risk than apoB or LDL-C, showing a stronger association with the risk of major cardiovascular events than LDL-C or apoB [41]. A Bayesian random-effects meta-analysis of 12 statin trials did not demonstrate apoB to be a superior marker of benefit [42]. By contrast, a meta-analysis of 7 major placebo-controlled statin trials, using both frequentist and Bayesian approaches, demonstrated that the relative CHD risk reduction was more closely related to reductions in apoB than to reductions in either non-HDL-C or LDL-C [43]. This evidence has also been confirmed by a more recent analysis of non-HDL-C versus apoB [44]. Johannesen et al. followed 13,015 individuals treated with a statin for 8 years; discordant subjects with apoB above the median with LDL-C below presented a HR of 1.49 (95% confidence interval [CI]: 1.15–1.92) for myocardial infarction, compared with concordant apoB and LDL-C below the medians. In contrast, discordant subjects with LDL-C above the median with apoB below were not associated with increased risk of all-cause mortality [45].

The MR approach has played a key role also in explaining some contradictory findings from trials of statin-CETP inhibitor (developed to test the hypothesis that raising HDL-C would reduce cardiovascular events) combination therapy, which demonstrated that large decreases in LDL-C did not produce significant clinical benefit [46]. In an MR analysis, a CETP score (a genetic score that mimics the effect of CETP-inhibitors) at or above the median was associated with higher levels of HDL-C, lower levels of LDL-C and apoB, and lower cardiovascular risk. A genetic score mimicking the effect of statins at or above the median was not associated with significant changes in HDL-C but was associated with lower levels of LDL-C, apoB, and cardiovascular risk. For participants with both scores above the median, which is analogous to combination therapy with a CETP inhibitor and a statin, the reduction in LDL-C was additive, but the reduction in apoB was attenuated and associated with a non-significant decrease in cardiovascular risk, thus explaining the otherwise paradoxical finding [47]. Only in the REVEAL trial, the decrease in apoB was large enough to produce significant clinical benefit [48].

Another MR study [49] evaluating the effect of an LPL genetic score (mimicking a TG-lowering therapy) and an LDLR score (mimicking an LDL-C-lowering therapy) on ASCVD risk showed that the risk reduction associated with the LPL genetic score and that associated with the LDLR score was similar per unit decrease in apoB. This led the authors to conclude that the clinical benefit of lowering TG and LDL-C is proportional to the absolute change in apoB. In turn, this supports the hypothesis that the risk of ASCVD is determined by the total concentration of circulating apoB particles regardless of their lipid content, and, therefore, the clinical benefit of any lipid-lowering therapy is proportional to the absolute reduction in apoB concentration regardless of the corresponding changes in LDL-C and TG.

These findings also explain why fibrates, which produce moderate to marked reductions in plasma TG and VLDL, failed to consistently produce clinical benefit. Indeed, although they produce large decreases in VLDL, they produce only small decreases in LDL, which make up most of the apoB particles in plasma. Consequently, fibrates produce only modest changes if any in total apoB. However, in hypertriglyceridemic patients in whom VLDL rises to 25–30% of total apoB, the reduction in total apoB could reach clinical significance [50], confirming once again that the benefit depends on apoB reduction. This evidence on fibrates might also explain the recent result obtained by the PROMINENT trial, which failed to show that pemafibrate improved cardiovascular outcomes [51]. The goal of this randomized, double-blind trial was to evaluate the effects of placebo or pemafibrate, a selective peroxisome proliferator–activated receptor α (PPARα) modulator, in patients with T2DM and hypertriglyceridemia treated with statin therapy. The authors showed that, despite being associated with a significant reduction in TG levels, pemafibrate therapy was not associated with a reduction in cardiovascular events; of note, an increase in apoB levels was reported compared with placebo. These results are also consistent with the FIELD trial [52].

Analysis of the effects of omega-3 long-chain polyunsaturated fatty acids (n-3 PUFA) can also provide interesting insights. Supplementation of n-3 PUFA has been associated with a significant risk reduction of myocardial infarction and cardiac mortality [53], despite a marginal effect on LDL-C levels. The indirect metabolic effect of n-3 PUFAs on lipid metabolism is the decrease of plasma triglycerides mediated through modifying free fatty acid availability to hepatocytes, subsequent changes of VLDL metabolism, and the reduction of atherogenic apoB-carrying lipoproteins. Skulas-Ray et al. showed beneficial effects of 3.4 g/d EPA (eicosapentaenoic acid) plus DHA (docosahexaenoic acid) supplementation on apo B, in addition to corroborating its effectiveness for reducing plasma VLDL-C [54]. Chan et al. [55] observed that 4 g daily of n-3 PUFA in dyslipidemic subjects decreased hepatic production of VLDL-apoB by 29% more than corn oil, with an increased conversion of VLDL-apoB to IDL-apoB, and IDL-apoB to LDL-apoB, possibly due to the lower number of VLDL particles per LPL enzyme. More recently, in successful REDUCE-IT trial [56], icosapent ethyl treatment significantly and favourably affected non-HDL, LDL-C, and apoB levels (−8.6%, −7.4%, and −6.7%) compared to placebo.

Finally, it is also interesting to discuss the effect of the angiopoietin-like 3 protein (ANGPTL3) inhibitors, recently made available on the market. ANGPTL3 plays a major role in promoting uptake of circulating triglycerides into white adipose tissue in the fed state, through the inhibition of postprandial LPL and endothelial lipase [57]. Evinacumab, a monoclonal antibody that binds to and pharmacologically inhibits ANGPTL3, lowers LDL-C predominantly by increasing apoB-containing lipoprotein clearance from the circulation, increasing IDL-apoB and LDL-apoB fractional catabolic rates, and reducing VLDL-apoB production rate [58], resulting in a decrease of − 25% of LDL-C, - 31% of apoB, and − 46% of non-HDL-C with 20 mg/kg every 4 weeks (Q4W). Another approach targeting ANGPTL3, the antisense oligonucleotides (ASOs) vupanorsen, was associated with modest effect on these lipid parameters, as it was shown to reduce LDL-C, apoB, and non-HDL-C by up to 18%, 12%, and 9%, respectively, as compared with placebo, in patients with elevated fasting plasma TG levels, T2DM, and hepatic steatosis [59].

4. Current guidelines and recommendations

For several years, literature reported compelling evidence that plasma apoB level is a better index of CHD risk than LDL-C [60]. Early, 2011 European Society of Cardiology (ESC)/European Atherosclerosis Society (EAS) Guidelines for the management of dyslipidaemias recommended to assess non-HDL-C and apoB to estimate the number of circulating atherogenic particles [61]. However, notwithstanding evidence supports the superiority of apoB over LDL-C and non-HDL-C, LDL-C still remains the primary goal recommended for clinical care [62].

Presumably, this is because guideline recommendations that guide physicians' clinical practice are based on results from trials and LDL-C is the primary metric reported in RCTs of statins, ezetimibe, and PCSK9 inhibitors [63]. In addition, the hesitancy by clinicians to welcome changes [64] and the need of pedagogical interventions (education of physicians and patients) have to be acknowledged. Indeed, the apoB measurement offers several advantages: standardized, automated, and accurate methods to measure ApoB are available [65], fasting is not required, and the analytical performances of ApoB measurement methods are superior to the measurement or calculation of LDL-C and non-HDL-C [66]. The actual cost of producing an apoB result on a modern chemistry analyzer is just a fraction of the price typically charged (e.g. 2021 Centers for Medicare and Medicaid Services reimbursement rate for apoB was $21.09) [67]. Moreover, plasma LDL-C in clinical medicine is most often calculated using the Friedewald formula [68]. Although convenient, the Friedewald calculated value of LDL-C has several well-established limitations: it could not be used with high TG values (>400 mg/dL), for patients with type III hyperlipoproteinemia or chylomicronemia, or in non-fasting status. To overcome these problems, a number of modifications for the calculation of LDL-C have been suggested [69], and direct enzymatic methods for the measurement of LDL-C have been developed [70]. These latter, however, also have limitations, including systematic bias and inaccuracy in patients with dyslipidaemia, especially for high TG levels [71]. Nonetheless, many clinical laboratories do not offer an apoB assay. One path to improving clinical practice is for laboratories to make apoB routinely available as part of a comprehensive lipid panel. Although this is unlikely to overcome the practical barrier presented by a decades-long framing of residual risk and lipid-lowering treatment solely in terms of LDL-C, clinical laboratories could play an important role in gradually broadening the clinical adoption of apoB [67].

The 2018 American College of Cardiology (ACC)/American Heart Association (AHA) guideline for the management of blood cholesterol has recommended apoB as a risk assessment enhancer for individuals with intermediate ASCVD risk when evaluated with traditional risk factors [6]. The most recent 2019 ESC/ EAS Guidelines [72] for the management of dyslipidaemias state that, given the central causal role of apoB-containing lipoproteins in the initiation and progression of atherosclerosis, direct measurement of plasma apoB for risk estimation and therapy selection would be ideal. Moreover, considering the potential inaccuracy of LDL-C in patients with diabetes mellitus, high TG levels, obesity, or metabolic syndrome, as well as in patients with very low LDL-C levels, measuring apoB is recommended as part of routine lipid analysis. These observations do not deny the clinical utility of LDL-C and non-HDL-C: as they correlate with apoB in a large proportion of patients and are more easily understood by patients themselves, these parameters can still be used to approximate lipoprotein particle concentration and estimate CVD risk when apoB is not available. Moreover, there is also value in the traditional lipid panel in understanding what is driving a high concentration of apoB-containing lipoproteins, for example in guiding the diagnosis of a familial form of hypercholesterolemia or hypertriglyceridemia.

Table 2
Recommendations by international guidelines on LDL-C and apoB measurements.

Guidelines	Version	Recommendations for LDL-C measurement	Recommendations for apoB measurement
American College of Cardiology (ACC)/American Heart Association (AHA)[73]	2018	Measurement of direct LDL-C is always reasonable to estimate atherosclerotic cardiovascular disease risk	A relative indication for apoB measurement would be TG $\geq$ 200 mg/dL. An apoB level $\geq$ 130 mg/dL corresponds to an LDL-C $\geq$ 160 mg/dL and constitutes a risk-enhancing factor.
Canadian Cardiovascular Society (CCS) [74]	2021	LDL-C should be considered the primary laboratory measurement for considering initiation of statin treatment and as a treatment target in low, intermediate and high-risk individuals.	For any patient with TG > 130 mg/dL, non-HDL-C or ApoB should be used instead of LDL-C as the preferred lipid parameter.
European Society of Cardiology (ESC)/ European Atherosclerosis Society (EAS)[7]	2019	LDL-C analysis is recommended for risk assessment and for considering initiation of statin treatment.	ApoB analysis is recommended for risk assessment, particularly in people with high TG levels, diabetes mellitus, obesity, metabolic syndrome, and very low LDL-C. It can be used as an alternative to LDL-C, if available, as the primary measurement for screening, diagnosis, and management, and may be preferred over non-HDL-C in people with high TG levels, diabetes mellitus, obesity, metabolic syndrome, and very low LDL-C.
National Lipid Association (NLA)[75]	2015	Periodic monitoring of LDL-C and non-HDL-C is extremely recommended as important tools in the implementation of a successful treatment strategy.	Non-HDL-C should be used as the first index of cardiovascular risk associated with apoB lipoproteins. ApoB measurement is recommended only as an optional secondary target after LDL-C and non–HDL-C targets are achieved.
Japan Atherosclerosis Society (JAS) [76]	2018	LDL-C is considered the major risk factor for the atherosclerotic cardiovascular disease.	A high apo B level is a risk factor for atherosclerotic cardiovascular disease.

ApoB: apolipoprotein B, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol.

A summary of recommendations by international guidelines is reported in Table 2.

5. Conclusions

In summary, the number of LDL particles (i.e. apoB level) has been shown to be more strongly causally related to ASCVD than the cholesterol content (i.e. LDL-C level). As cholesterol content in the particles can vary widely between individuals, LDL-C or non-HDL-C measurement does not always reflect the number of atherogenic particles. Available evidence suggests that apoB should be preferred over LDL-C and non-HDL-C as a marker of cardiovascular risk in specific subgroups of patients, including patients with diabetes mellitus, high TG levels, obesity or metabolic syndrome, and patients with very low LDL-C levels. This evidence and the availability of fully automated tests that can be implemented in clinical laboratories, support the recommendation of measuring apoB routinely for better assessment of ASCVD risk. Moreover, if the primary target (LDL-C) is at goal, but non-HDL-C or apoB is still high, attainment of the secondary goals will require intensified lifestyle intervention or the addition of pharmacological options.

CRediT authorship contribution statement

Federica Galimberti: Writing – original draft, Writing – review & editing. **Manuela Casula:** Writing – original draft, Writing – review & editing. **Elena Olmastroni:** Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: FG, EO, and MC report no disclosures.

Data Availability

No data was used for the research described in the article.

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