

Apolipoprotein B Particles and Cardiovascular Disease

A Narrative Review

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IMPORTANCE The conventional model of atherosclerosis presumes that the mass of cholesterol within very low-density lipoprotein particles, low-density lipoprotein particles, chylomicron, and lipoprotein (a) particles in plasma is the principal determinant of the mass of cholesterol that will be deposited within the arterial wall and will drive atherogenesis. However, each of these particles contains one molecule of apolipoprotein B (apoB) and there is now substantial evidence that apoB more accurately measures the atherogenic risk owing to the apoB lipoproteins than does low-density lipoprotein cholesterol or non-high-density lipoprotein cholesterol.

OBSERVATIONS Cholesterol can only enter the arterial wall within apoB particles. However, the mass of cholesterol per apoB particle is variable. Therefore, the mass of cholesterol that will be deposited within the arterial wall is determined by the number of apoB particles that are trapped within the arterial wall. The number of apoB particles that enter the arterial wall is determined primarily by the number of apoB particles within the arterial lumen. However, once within the arterial wall, smaller cholesterol-depleted apoB particles have a greater tendency to be trapped than larger cholesterol-enriched apoB particles because they bind more avidly to the glycosaminoglycans within the subintimal space of the arterial wall. Thus, a cholesterol-enriched particle would deposit more cholesterol than a cholesterol-depleted apoB particle whereas more, smaller apoB particles that enter the arterial wall will be trapped than larger apoB particles. The net result is, with the exceptions of the abnormal chylomicron remnants in type III hyperlipoproteinemia and lipoprotein (a), all apoB particles are equally atherogenic.

CONCLUSIONS AND RELEVANCE Apolipoprotein B unifies, amplifies, and simplifies the information from the conventional lipid markers as to the atherogenic risk attributable to the apoB lipoproteins.

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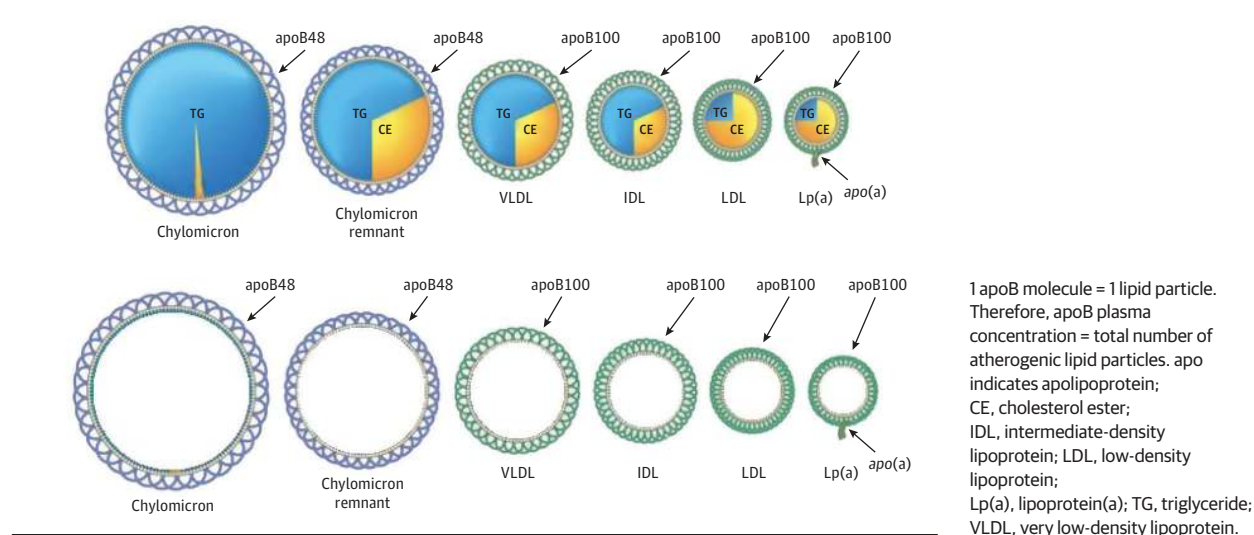
Trapping of apolipoprotein (apo) B particles within the arterial wall is the fundamental step that initiates and drives the atherosclerotic process from beginning to end, from the first appearance of fatty streaks to the ultimate development of the complex lesions that are vulnerable to the acute transformations, such as plaque rupture and endothelial erosion, that are the immediate precursors of clinical events.¹ The concentration of apoB particles within the arterial lumen is the primary determinant of the number of apoB particles that will be trapped within the arterial wall. However, the proportion of apoB particles that are trapped within the arterial wall vs the proportion that pass harmlessly through is also influenced by the size of the apoB particles and by the structure of the glycosaminoglycans within the subintimal space of the arterial wall. Trapping of apoB particles deposits atherogenic cholesterol within the arterial wall. However, because the cholesterol content, and therefore, the size of apoB particles, varies significantly² and because other components of apoB particles, such as phospholipids and apoB itself, if oxidized, are strong proatherogenic factors,^{3–5} neither low-density lipoprotein cholesterol (LDL-C) nor non-high-

density lipoprotein cholesterol (HDL-C) is as accurate as apoB as markers of cardiovascular risk. Moreover, recent data indicate that the risk from a very low-density lipoprotein (VLDL) particle approximates closely the risk from an LDL particle.⁶ Accordingly, apoB sums the atherogenic risk owing to the triglyceride (TG)-rich VLDL apoB particles and the cholesterol-rich LDL apoB particles and, in conjunction with the plasma lipids, could improve the clinical assessment and management of the atherogenic dyslipoproteinemias.⁷

Plasma Apolipoprotein B

Apolipoprotein B-containing lipoproteins are spherical particles (Figure 1). Each has a monolayer of phospholipids arranged around its circumference within which are small amounts of cholesterol and through which a single molecule of apoB48 or apoB100 encircles the lipoprotein particle.⁸ The apoB molecule provides structural stability and stays with the particle throughout its metabolic lifetime, whereas variable amounts of TG and cholesterol ester (CE) constitute the core of the particle (Figure 1).² The plasma concentrations of TG, non-HDL-C, and LDL-C are the sums of these lipids within the

Figure 1. Apolipoprotein B48 and B100 Lipoprotein Particles



apoB particles. Multiple other apolipoproteins, such as apoC and apoE, are present on the surface of chylomicrons and VLDL particles. These play important metabolic roles, particularly in modulating the rate at which TG-rich lipoproteins are cleared from plasma, but are not the focus of this review.⁷

Apolipoprotein B assays recognize both apoB48 and apoB100. Because there is a single molecule of either apoB48 or apoB100 per particle,⁸ plasma apoB equals the total number of apoB48, apoB100 particles, and lipoprotein(a) (Lp[a]) particles (Figure 1). However, because there are so few apoB48 particles at any time, even in postprandial samples, total apoB is simply the sum of VLDL, LDL, and Lp(a) particles. Thus, fasting is not necessary to measure apoB.

Figure 2 demonstrates the great differences in the relative numbers of the different apoB particles.² In individuals with normal TG (TG <133 mg/dL [to convert to mmol/L, multiply by 0.0113]), for every chylomicron and/or chylomicron remnant particle, there are approximately 10 VLDL particles. This is why, in general, VLDL particles are more important determinants of atherogenic risk than chylomicron remnant particles. Similarly, because VLDL particles have a short half-life in plasma whereas LDL particles have a longer half-life, there are many more LDL particles than VLDL particles in plasma.² Thus, in patients with normal TG levels, for every VLDL particle, there are approximately 9 LDL particles (Figure 2).² As plasma TG levels increase, the proportion of VLDL particles increases, but this relation is not exact and, with the exception of the uncommon disorder, type III hyperlipoproteinemia, there are always many more LDL particles than VLDL particles.²

Metabolic Bases for Variability in the Composition of VLDL and LDL Particles

The superiority of apoB over cholesterol and TG as a marker of cardiovascular risk is based on the variability in the lipid composition of the apoB lipoproteins. Much is known about the pathophysiological bases for the differences in cholesterol content in apoB particles.⁹⁻¹¹ Very low-density lipoprotein particles are grossly heterogeneous in composition and size. The liver may secrete larger TG-enriched VLDL particles, VLDL1 particles, or smaller VLDL2 particles, which contain less TG.¹² Moreover, the mass of TG within VLDL

particles diminishes as the TG is hydrolyzed by lipoprotein lipase. Although TG is the dominant core lipid, VLDL particles also contain substantial amounts of CE. Low-density lipoprotein particles can differ in the mass of CE within their core and consequently can differ in size.⁸ However, as will be demonstrated later in this article, all have the same atherogenic potential.

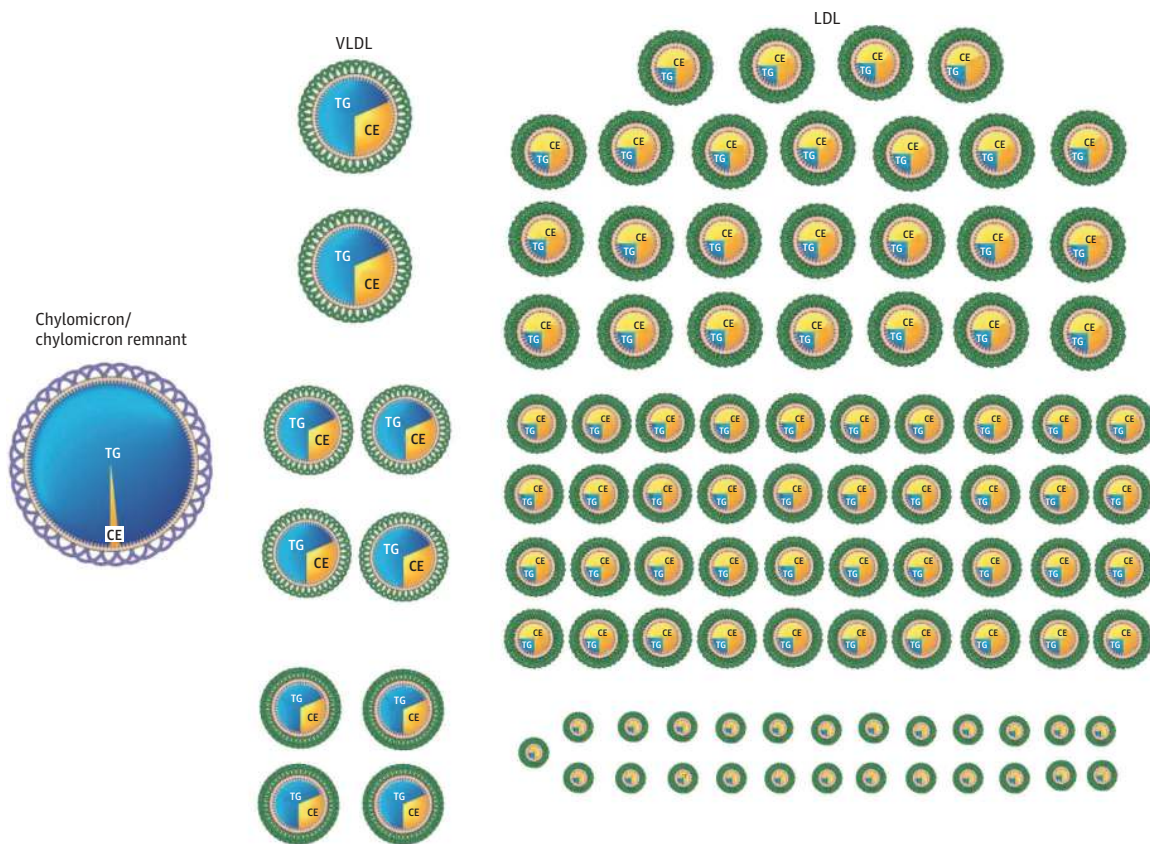
Variance in the composition of the apoB particles is based on CE transfer protein (CETP)-mediated exchange of the core lipids, CE and TG, among the plasma lipoproteins (Figure 3).^{12,13} If a TG from VLDL is exchanged for a CE molecule from LDL, the VLDL particle becomes relatively enriched with CE; the TG content of the LDL particle increases, but its CE content decreases. Subsequent hydrolysis of the TG within the LDL particle, probably by hepatic lipase, produces smaller, cholesterol-depleted particles.⁹ Once plasma TG are greater than 133 mg/dL, LDL particles, on average, contain less cholesterol than usual, are smaller than usual, and LDL-C will underestimate the number of LDL particles.¹⁴ The same sequence produces a low HDL-C in patients with hypertriglyceridemia explaining why this triad of lipid abnormalities—hypertriglyceridemia, low HDL-C, and small cholesterol-depleted LDL particles, the so-called atherogenic triad¹⁵—is so often so entwined and therefore why their relative pathophysiological significance is so difficult to disentangle.

Importantly, LDL particles can also be cholesterol enriched. However, the metabolic processes that lead to larger cholesterol-enriched particles are less well understood than the processes that lead to smaller cholesterol-depleted particles. In individuals with cholesterol-enriched apoB particles, TG levels are characteristically normal or even low, HDL-C are normal or high, and apoB are normal or high.¹⁶⁻²⁰

Epidemiological Basis for the Role of ApoB as a Marker of the Concentration of Proatherogenic Lipoproteins in Plasma

Modern epidemiological tools enable analyses that lead to conclusions consistent with the pathophysiological arguments outlined earlier. The original studies that suggested apoB to be a more accurate marker of cardiovascular risk than total cholesterol or LDL-C were simple cross-sectional analyses.^{21,22} Subsequently, prospective

Figure 2. Relative Numbers of Apolipoprotein B Particles in Plasma in the Postprandial Period



CE indicates cholesterol ester; LDL, low-density lipoprotein; TG, triglyceride; VLDL, very low-density lipoprotein.

observational studies²³ confirmed these initial findings. However, while most studies favored apoB over LDL-C, not all concluded that apoB was superior to non-HDL-C with some, such as the Emerging Risk Factor Study²⁴ and the Copenhagen Heart Study²⁵ reporting non-HDL-C and apoB were equivalent predictors. Great emphasis was placed on the C statistic to demonstrate whether a new marker significantly improved the prediction of risk. Unfortunately, while the C statistic does evaluate the overall performance of a risk model, it is not a reliable tool to judge which marker is responsible for risk.²⁶ Thus lay the balance of evidence until 2 new epidemiological analytical approaches—discordance analysis and mendelian randomization—were used to deal with the challenge of discriminating among highly correlated markers such as LDL-C, non-HDL-C, and apoB.

Correlation, Concordance, and Discordance Analysis

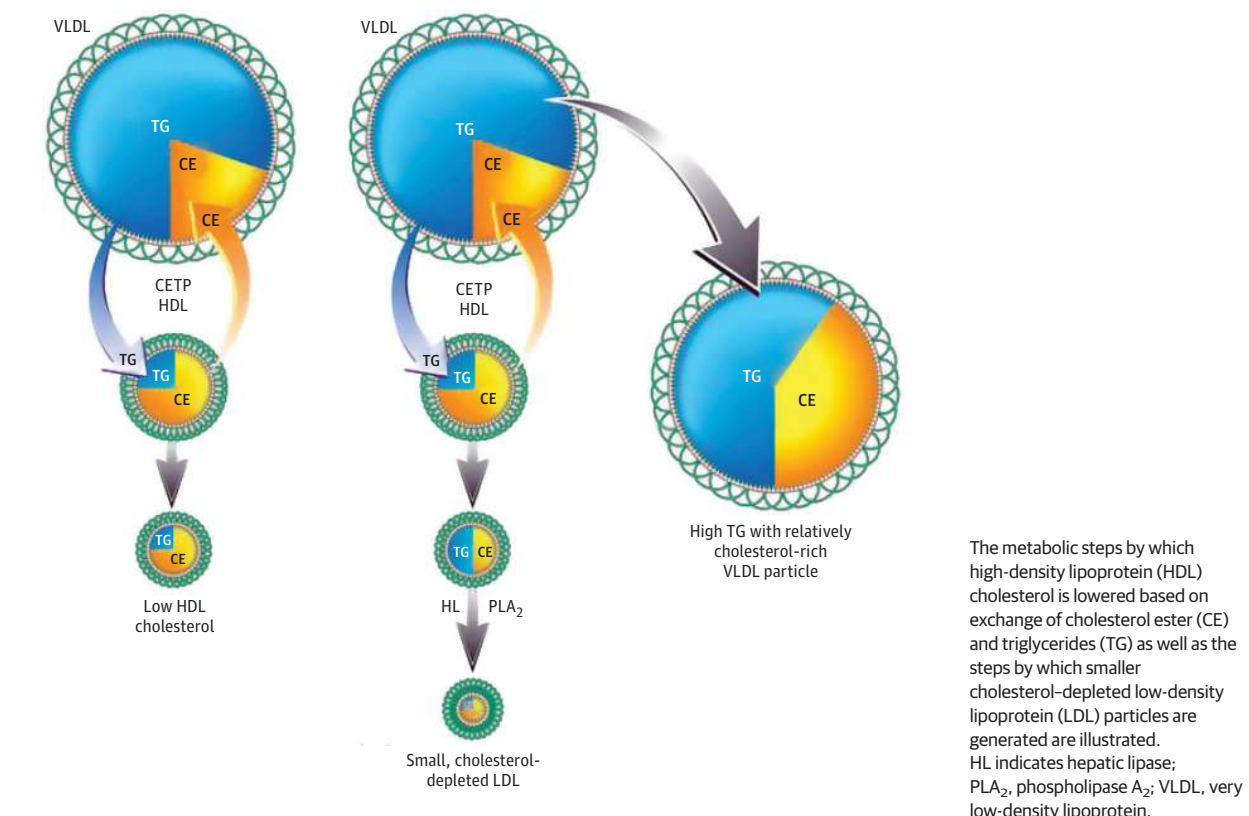
Correlation expresses the overall association between the changes in 2 variables. Neither the number of VLDL particles (VLDL apoB) nor the total apoB correlate well with plasma TG.² Accordingly, VLDL particle number cannot be reliably inferred from plasma TG. By contrast, because the variance in cholesterol mass per particle is less, LDL-C and apoB are highly correlated while non-HDL-C and apoB are even more highly correlated. These high correlations have been used to argue that LDL-C, and even more so, non-HDL-C, are clinically equivalent to apoB and therefore acceptable surrogates for apoB.²⁷

However, correlation at a population level does not establish clinical equivalence at an individual level. Figure 4A illustrates that as the number of apoB particles containing an average mass of cholesterol increases, the concentration of LDL-C/non-HDL-C and the concentration of apoB increase proportionately. The changes in LDL-C/non-HDL-C and apoB, in this instance, are concordant. However, Figure 4B illustrates that for a given value of apoB (the 50th percentile), the levels of LDL-C/non-HDL-C may range from the 25th to the 75th percentile. In these examples, the values of LDL-C/non-HDL-C and apoB are discordant and will predict risk differently. Figure 4C illustrates discordance when LDL-C/non-HDL-C are fixed but apoB varies. The accuracies of these differences in predictions can be tested in epidemiological studies.

Discordance analysis is constructed so that the markers, which are being compared, make diametrically different predictions: in the discordant groups with high LDL-C or high non-HDL-C but low apoB, cholesterol predicts high risk, apoB predicts low risk, whereas in the groups with low LDL-C or non-HDL-C but high apoB, the reverse is the case.²⁸ One marker will be right, and the other wrong. Multiple discordance analyses of major prospective observational studies have been published with either apoB or LDL, particle number compared with LDL-C and/or non-HDL-C.^{16,18-20,29-33}

Multiple methods have been used to create the discordant groups, ranging from division at the median of the markers to separation based on residuals. These different definitions have resulted

Figure 3. Cholesteryl Ester Transfer Protein (CETP) Exchange and the Atherogenic Lipoprotein Phenotype



in discordant groups that range from about 20% to about 60% of the total population. In all instances, the markers of particle number, apoB, or LDL particle number were stronger predictors of cardiovascular risk than LDL-C. In 5 major studies,^{16,18-20,32} apoB was shown to be a more accurate marker of risk than non-HDL-C. In the Women's Health Study and the UK Biobank study, a conventional analysis did not demonstrate that apoB was a more accurate marker of cardiovascular risk than non-HDL-C,^{31,32} whereas this was demonstrated by discordance analysis.^{20,32} Moreover, a discordance analysis based on mendelian randomization, which compared the benefit of lowering LDL-C vs the benefit of lowering apoB, confirmed these findings.³³ The results of discordance analysis are clear and consistent: apoB is a more accurate marker of cardiovascular risk attributable to the apoB lipoprotein particles than LDL-C and non-HDL-C and the size of the discordant groups is sufficiently large to make the phenomenon clinically relevant.

Evidence From Randomized Clinical Trials and Mendelian Randomization

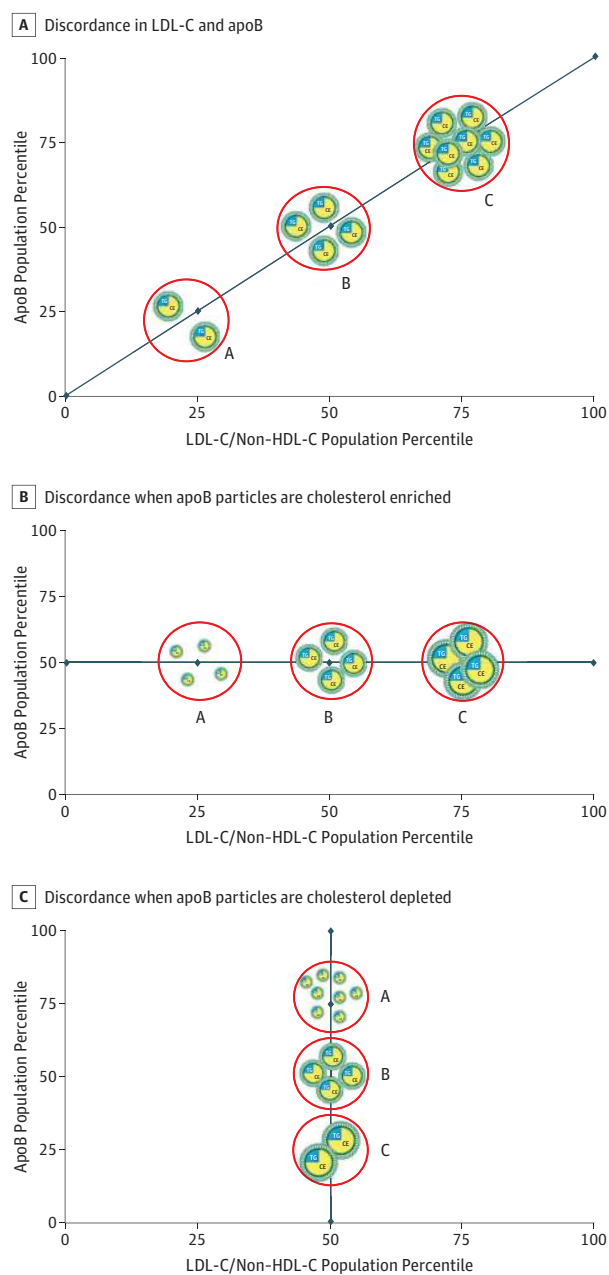
Randomized clinical trials have demonstrated that statins, statins plus ezetimibe, and statins plus PCSK9 inhibitors significantly lower cardiovascular risk.³⁴ All increase LDL receptor activity at the surface of hepatocytes, thereby increasing the rate at which apoB particles, primarily LDL apoB particles, are removed from plasma. Statins do so by reducing hepatic and intestinal cholesterol synthesis, ezetimibe by reducing cholesterol absorption and delivery to the liver, and PCSK9 inhibitors by reducing the degradation of LDL receptors within the

hepatocyte.³⁴ All lower LDL-C and non-HDL-C because all lower apoB particle number in plasma.

Statins lower LDL-C more than non-HDL-C and more than apoB³⁵ because larger cholesterol-rich LDL apoB particles interact more avidly with the LDL receptor than smaller cholesterol-depleted ones.³⁶ Therefore, their concentration will decrease more than the concentration of smaller cholesterol-depleted apoB particles. Conventional epidemiological analyses have yielded mixed results as to whether LDL-C, non-HDL-C, or apoB is the best marker of the effectiveness of 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.³⁷ A Bayesian analysis of clinical trials using multiple therapeutic agents did not demonstrate apoB to be a superior marker of benefit.³⁸ By contrast, a meta-analysis of 7 major statin trials, using both frequentist and Bayesian approaches, demonstrated that benefit was more closely associated with the decrease in apoB than to the decreases in LDL-C and non-HDL-C.³⁹

Mendelian randomization has added to the evidence that the number of apoB particles within the arterial lumen is the most direct measure of the atherogenic injury that the apoB particles will inflict over time on the arterial wall. Cholesterol ester transfer protein inhibitors were developed to test the hypothesis that raising HDL-C would reduce cardiovascular events. However, trials of statin-CETP inhibitor combination therapy, which demonstrated large decreases in LDL-C, did not produce significant clinical benefit,⁴⁰ a result that is inconsistent with a causal role for LDL-C in atherosclerotic cardiovascular disease.^{34,38}

Figure 4. Concordance and Discordance Between LDL-C/Non-HDL-C and ApoB



A, Discordance in LDL-C and apoB when the apoB particles contain an average mass of cholesterol. B, Discordance when apoB particles are cholesterol enriched. C, Discordance when apoB particles are cholesterol depleted. ApoB indicates apolipoprotein B; CE, cholesterol ester; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

To resolve the dilemma, Ference et al³³ combined variants in the *CETP* and *HMGCR* genes to create genetic scores that mimic the effects of CETP inhibitors and statins. A *CETP* score at or above the median was associated with higher levels of HDL-C, lower levels of LDL-C and apoB, and lower levels of cardiovascular risk. An *HMGCR* score 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. The attenuated reduction in apoB was associated with a nonsignificant decrease in cardiovascular risk, thus explaining the otherwise paradoxical finding of a significant decrease in LDL-C with combination statin-CETP inhibitor therapy without clinical benefit. Only in the REVEAL trial was the decrease in apoB large enough to produce significant clinical benefit.⁴¹ Thus, mendelian randomization indicates that the primary mechanism of benefit from lowering LDL-C is associated with the lowering of the number of LDL particles, ie, to the lowering of apoB. It follows that apoB is a more accurate index of the adequacy of LDL-lowering therapy than LDL-C. Indeed, Sathiyakumar et al⁴² demonstrated that in approximately one-third of patients who achieved a level of LDL-C less than 70 mg/dL (to convert to mmol/L, multiply by 0.0259), apoB was substantially higher, pointing to the potential for further benefit from LDL-lowering therapy.

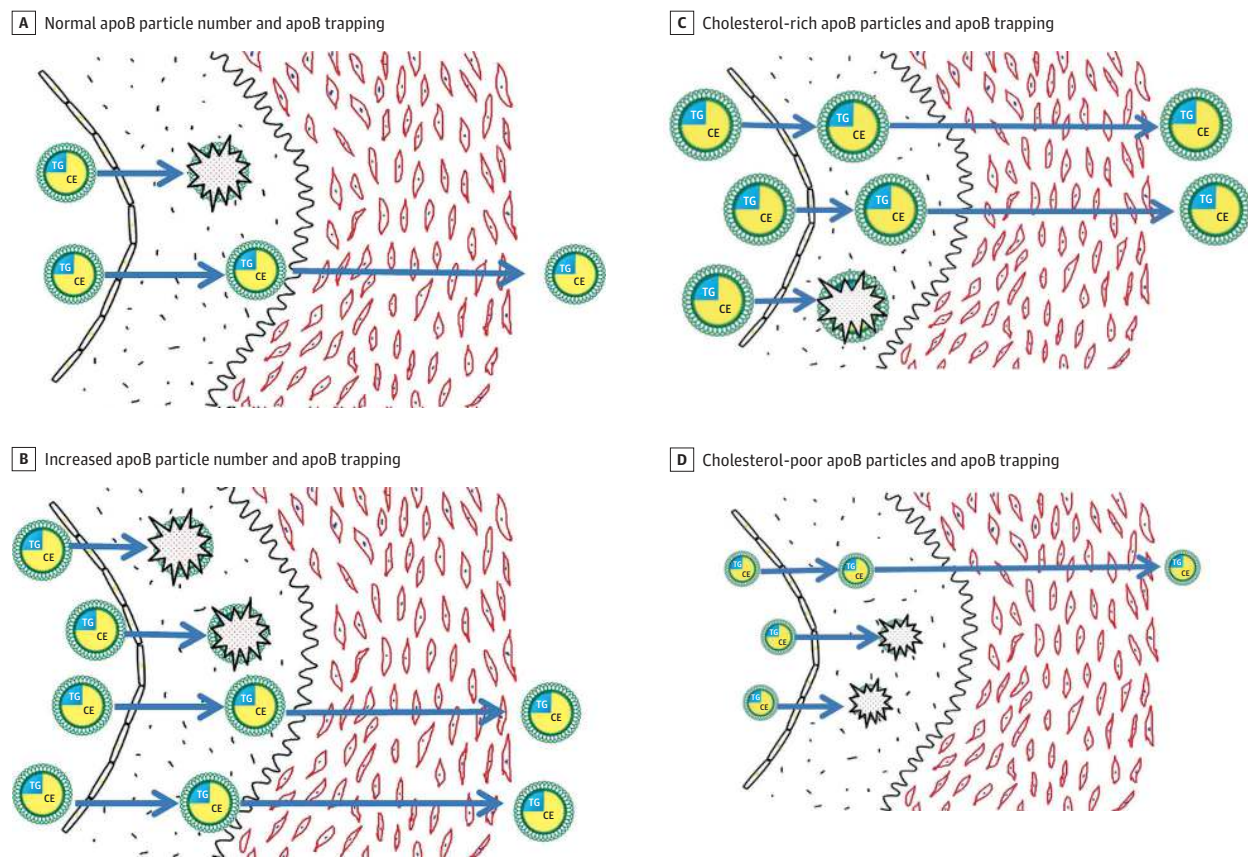
Ference and colleagues⁶ subsequently applied mendelian randomization to examine whether lowering TG reduces cardiovascular risk. Just as LDL-C has been accepted as the measure of LDL, so has TG been accepted as the measure of VLDL. Accordingly, Ference et al⁶ created a genetic score equivalent to a drug that would lower TG via increased lipoprotein lipase activity as well as the genetic score equivalent to a drug that would lower LDL-C via increased LDL receptor activity. The *LPL* genetic score was associated with a large decrease in TG but a very small, nonsignificant increase in LDL-C whereas the LDL receptor score was associated with a large decrease in LDL-C with only a very small decrease in TG. However, when these decreases in LDL-C and TG were normalized for the same decrease in apoB, the reduction in risk associated with the *LPL* genetic score and the reduction in risk associated with the LDL receptor score were very similar.

Taken together, these findings suggest that, except for the small minority too large to enter the arterial wall, most VLDL particles are as atherogenic as LDL particles. Because each VLDL and LDL particle has one molecule of apoB, plasma apoB represents the sum of the atherogenic risk attributable to VLDL plus LDL particles. Of interest, these findings are consistent with previous observational studies, which demonstrated the risk in patients with hypertriglyceridemia was determined by plasma apoB, not by plasma TG.⁴³⁻⁴⁸ They also explain why fibrates, which produce moderate to marked reductions in plasma TG and VLDL apoB, failed to consistently produce clinical benefit. Fibrates failed because although they produce large decreases in VLDL apoB, they produce only small decreases in LDL apoB, which make up most of the apoB particles in plasma. Consequently, in general, they produced only modest changes in total apoB.² However, in patients with hypertriglyceridemia in whom VLDL apoB rises to 25% to 30% of total apoB, the reduction in total apoB could reach clinical significance. This may explain the positive subgroup findings of benefit of fibrates in patients with hypertriglyceridemia and low HDL-C.⁴⁹ Therefore, the inconsistent effects of fibrates on clinical benefit are consistent with the model that benefit depends on reduction in apoB.

The ApoB Particle Model of Atherosclerosis

We now propose a model to explain why the atherogenic risk associated with the apoB lipoproteins relates more directly to their number than to the mass of cholesterol within them. Figure 5

Figure 5. Why All Apolipoprotein B (apoB) Particles Are Equally Atherogenic



The greater the number of apoB particles in the lumen of the artery, the greater the number of apoB particles that will enter and be trapped within the wall of the artery (A and B). The smaller, cholesterol-poor apoB particles that enter the

arterial wall are more likely to be trapped than larger, cholesterol-rich, apoB particles (C and D). CE indicates cholesterol ester; TG, triglycerides.

demonstrates that the number of apoB particles in the lumen of an artery is the primary determinant of the rate at which apoB particles enter the arterial wall and are trapped within the subintimal space of the arterial wall. The more apoB particles within the lumen of the artery, the more that will enter the arterial wall, and, all things being equal, the more apoB particles that will be trapped within the arterial wall. However, all things are not always equal: as illustrated in Figure 5, smaller apoB particles containing less cholesterol enter the arterial wall more easily⁵⁰ and bind more avidly to the glycosaminoglycans within the arterial wall than larger apoB particles containing more cholesterol.^{51,52} Thus, more smaller, cholesterol-depleted particles will be trapped than will a similar number of larger, cholesterol-enriched particles that have entered an arterial wall. On the other hand, the more cholesterol within an apoB particle that has been trapped within the arterial wall, the more cholesterol that will be released at that site to injure the wall. Therefore, there is an equivalence between greater injury per particle from trapping of cholesterol-richer particles but greater injury from trapping of more cholesterol-depleted particles. The net result is that all LDL particles pose, more or less, equal risk.

However, the unification and, therefore, the simplification offered by apoB have gone further: Ference et al⁶ have shown that VLDL particles pose equal atherogenic risk to LDL particles. Be-

cause the number of LDL particles is always many multiples of the number of VLDL particles,² the total risk from LDL particles is almost always much greater than the risk from VLDL particles, accounting for why cholesterol is much more closely linked to cardiovascular risk TG to cardiovascular risk, notwithstanding that hypertriglyceridemia is more common in patients with cardiovascular disease than hypercholesterolemia.^{53,54}

There are 2 exceptions to the rule that all apoB particles are equally atherogenic. The first is type III hyperlipoproteinemia, which is characterized by markedly increased numbers of abnormally cholesterol-enriched apoB48 and apoB100 remnant particles.^{55,56} The cholesterol content of these particles is so great as to make the damage per particle much greater than otherwise, and these abnormal particles are present in 20- to 40-fold excess of concentrations of remnants in patients without abnormality and patients with hypertriglyceridemia, who do not have type III.² Type III cannot be diagnosed from the conventional lipid panel, which is a significant limitation of current practice but can be accurately identified based on total cholesterol, TG, and apoB.⁵⁷

The second is Lp(a), given the strong evidence that elevated levels of Lp(a) independently add significantly to cardiovascular risk and are critically related to the pathophysiology of aortic stenosis.^{58,59} A large meta-analysis demonstrated that Lp(a) appears to confer

increased risk for cardiovascular risk despite statin therapy⁶⁰ and subanalyses from PCSK9 inhibitor trials have demonstrated that even among individuals with near-optimal LDL-C levels, Lp(a) remains a source of residual risk.⁶¹ Conversely, in individuals with familial hypercholesterolemia, characterized by a high burden of apoB particles, the presence of high Lp(a) further increases risk.^{62,63}

In addition, variations in glycosaminoglycan structure and perhaps other elements of the arterial wall might influence the avidity of binding of apoB and therefore increase fractional trapping of apoB particles.⁶⁴ Thus, the hypothesis that glycation of apoB particles promotes binding of apoB particles deserves further attention.⁶⁵ Finally, there is likely significant interindividual variation in the intensity of the innate and acquired immune responses, ie, B and T cell responses, to apoB particles trapped within the arterial wall and therefore significant variation in the inflammatory-mediated destruction of the arterial wall.⁶⁶

Accordingly, variance in the sequence of events after an apoB particle enters the arterial wall will account for much of the indi-

vidual variance of risk at the same apoB. Nevertheless, everything first depends on the entry of an apoB particle into the arterial wall, and this depends, most of all, on the concentration of apoB particles in the arterial lumen.

Conclusions

An apoB particle is the basic unit of injury to the arterial wall. The more apoB particles within the lumen of the artery, the greater the trapping of apoB particles within the arterial wall, the greater the injury to the arterial wall. The more apoB particles are reduced by therapy, the less the injury to the arterial wall, the greater the opportunity for healing. Moreover, nowadays apoB can be measured accurately and inexpensively.⁶⁷⁻⁶⁹ Thus, apoB integrates the information from the conventional lipid panel and, therefore, unifies, amplifies, and simplifies our understanding of the role of the apoB lipoprotein particles in atherogenesis.

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Acquisition, analysis, or interpretation of data: Sniderman, FERENCE.

Drafting of the manuscript: Sniderman, Glavinovic, FERENCE.

Critical revision of the manuscript for important intellectual content: Thanassoulis, Glavinovic, Navar, Pencina, Catapano, FERENCE.

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