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## Apolipoprotein B Particles and Cardiovascular Disease: A Narrative Review.

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### Abstract

**Background:** Trapping of apoB lipoprotein particles within the arterial wall initiates and drives the atherosclerotic process from beginning to end. Very low-density lipoprotein particles (VLDL) contain most of the triglyceride in plasma whereas low-density lipoprotein particles (LDL) particles contain most of the cholesterol. Smaller numbers of chylomicron and Lp(a) particles are also present in plasma. All these particles have one molecule of apoB. Therefore, plasma apoB equals the total number of apoB particles. Because the lipid content of apoB particles is variable, plasma triglyceride and cholesterol are not always accurate-measures of the number of apoB particles.

**The Cholesterol Model of Atherosclerosis**—The conventional model of atherosclerosis presumes that the mass of cholesterol within VLDL and LDL particles is the principal determinant of the mass of cholesterol that will be deposited within the arterial wall and will drive atherogenesis. But cholesterol can only enter the arterial wall within apoB particles and the mass of cholesterol that will be deposited is determined by the rate at which apoB particles are trapped within the arterial wall rather than passing harmless through.

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**Tweet:** #apoB clarifies and unifies -and therefore simplifies- the role of the atherogenic lipoproteins in causing CV disease.

Originality of Content

All the material including the Figures is original and has not been published elsewhere.

**The ApoB Particle Model of Atherogenesis**—The number of apoB particles that enter and are trapped within 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. If so, a cholesterol-enriched particle would deposit more cholesterol than a cholesterol-depleted apoB particle. By contrast, 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 Lp(a), all apoB particles are equally atherogenic.

ApoB, therefore, unifies, amplifies, and simplifies the information from the conventional lipid markers as to the atherogenic risk attributable to the apoB lipoproteins.

## Introduction

Trapping of apoB lipoprotein 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.<sup>1</sup> 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. But the proportion of apoB particles that are trapped within the arterial wall versus 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 significantly<sup>2</sup> and because other components of apoB particles, such as phospholipids and apoB itself, if oxidized, are strong proatherogenic factors,<sup>3-5</sup> neither LDL-C nor non-HDL-C are as accurate as apoB as markers of cardiovascular risk. Moreover, recent data indicate that the risk from a VLDL particle approximates closely the risk from an LDL particle.<sup>6</sup> Accordingly, apoB sums the atherogenic risk due to the 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.<sup>7</sup>

## Plasma apoB

ApoB-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.<sup>8</sup> 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.<sup>2</sup> (Figure 1) The plasma concentrations of TG, non-HDL-C and LDL-C are the sums of these lipids within the apoB particles. Multiple other apolipoproteins, such as the apoC apolipoproteins and apoE, are present on the surface of chylomicrons and VLDL particles. These play important metabolic roles, particularly in

modulating the rate at the TG-rich lipoproteins are cleared from plasma, but are not the focus of this review.<sup>7</sup>

ApoB assays recognize both apoB48 and apoB100. Because there is a single molecule of either apoB48 or apoB100 per particle,<sup>8</sup> plasma apoB equals the total number of apoB48, apoB100 particles and 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.<sup>2</sup> In subjects with normal TG (TG < 1.5 mmol/L), 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.<sup>2</sup> Thus, in patients with normal TG levels, for every VLDL particle, there are approximately 9 LDL particles.<sup>2</sup> (Figure 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.<sup>2</sup>

#### **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.<sup>9-11</sup> VLDL particles are grossly heterogeneous in composition and size. The liver may secrete larger triglyceride-enriched VLDL particles, VLDL1 particles, or smaller VLDL2 particles, which contain less TG.<sup>12</sup> 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. LDL particles can differ in the mass of CE within their core and, consequently, can differ in size.<sup>8</sup> But, as will be demonstrated beneath, all have the same atherogenic potential.

Variance in the composition of the apoB particles is based on CETP-mediated exchange of the core lipids- CE and TG- amongst the plasma lipoproteins.<sup>12,13</sup> (Figure 3) 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.<sup>9</sup> Once plasma TG are greater than 1.5 mmol/L, LDL particles, on average, contain less cholesterol than usual, are smaller than usual, and LDL-C will underestimate the number of LDL particles.<sup>14</sup> 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 triad-<sup>15</sup> are 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 are characteristically normal or even low, HDL-C normal or high, and apoB normal or high.<sup>16-20</sup>

### **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 above. The original studies that suggested apoB to be a more accurate marker of cardiovascular risk than TC or LDL-C were simple cross-sectional analyses.<sup>21,22</sup> Subsequently, prospective observational studies<sup>23</sup> confirmed these initial findings. However, while the majority of studies favoured apoB over LDL-C, not all concluded that apoB was superior to non-HDL-C with some, such as the Emerging Risk Factor Study<sup>24</sup> and the Copenhagen Heart Study<sup>25</sup> 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.<sup>26</sup> Thus lay the balance of evidence until two new epidemiological analytical approaches-discordance analysis and Mendelian randomization-were employed to deal with the challenge of discriminating amongst highly correlated markers such as LDL-C, non-HDL-C and apoB.

### **Correlation, Concordance, and Discordance Analysis**

Correlation expresses the overall relation between the changes in two variables. Neither the number of VLDL particles (VLDL apoB) nor the total apoB correlate well with plasma TG.<sup>2</sup> 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.<sup>27</sup>

However, correlation at a population level does not establish clinical equivalence at an individual level. Figure 4, Panel A 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, panel B illustrates that for a given value of apoB (the 50<sup>th</sup> percentile), the levels of LDL-C/non-HDL-C may range from the 25<sup>th</sup> the 75<sup>th</sup> percentile. In these examples, the values of LDL-C/non-HDL-C and apoB are discordant and will predict risk differently. Panel C 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 low risk whereas in the groups with low-LDL-C or non-HDL-C but high apoB, the reverse is the case.<sup>28</sup> One marker will be right, the other wrong. Multiple discordance analyses of major prospective observational studies have been published with either apoB or LDL, particle number compared to LDL-C and/or non-HDL-C<sup>16,18-20,29-33</sup>.

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 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 five major studies<sup>16,18-20,32</sup> 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<sup>31,32</sup> whereas this was demonstrated by discordance analysis.<sup>20,32</sup> Moreover, a discordance analysis based on Mendelian randomization, which compared the benefit of lowering LDL-C versus the benefit of lowering apoB, confirmed these findings.<sup>33</sup> 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 (RCTs) and Mendelian Randomization**

RCTs have demonstrated that statins, statins plus ezetimibe, and statins plus PCSK9 inhibitors significantly lower cardiovascular risk.<sup>34</sup> 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.<sup>34</sup> 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 more than apoB<sup>35</sup> because larger cholesterol-rich LDL apoB particles interact more avidly with the LDL receptor than smaller cholesterol-depleted ones.<sup>36</sup> 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.<sup>37</sup> A Bayesian analysis of clinical trials using multiple therapeutic agents did not demonstrate apoB to be a superior marker of benefit.<sup>38</sup> By contrast, a meta-analysis of 7 major statin trials, using both frequentist and Bayesian approaches, demonstrated that benefit was more closely related to the decrease in apoB than to the decreases in LDL-C and non-HDL-C.<sup>39</sup>

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. CETP inhibitors (CETPI) 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,<sup>40</sup> a result, which is inconsistent with a causal role for LDL-C in ASCVD.<sup>34,38</sup>

To resolve the dilemma, Ference et al<sup>33</sup> 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 non-significant 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.<sup>41</sup> Thus, Mendelian randomization indicates that the primary mechanism of benefit from lowering LDL-C relates to the lowering of the number of LDL particles- that is, 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, Martin et al demonstrated that in approximately one third of patients who achieved a level of LDL C <70mg/dl, apoB was substantially higher, pointing to the potential for further benefit from LDL lowering therapy.<sup>42</sup>

Ference and his colleagues subsequently applied Mendelian randomization to examine whether lowering TG reduces cardiovascular risk.<sup>6</sup> Just as LDL-C has been accepted as the measure of LDL, so TG has been accepted as the measure of VLDL. Accordingly, Ference et al created a genetic score equivalent to a drug that would lower TG via increased LPL activity as well as the genetic score equivalent to a drug that would lower LDL-C via increased LDL receptor (LDLR) activity.<sup>6</sup> The *LPL* genetic score was associated with a large decrease in TG but a very small, non-significant, increase in LDL-C whereas the *LDLR* 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 *LDLR* score were very similar.

Taken together, these findings suggest that, except for the small minority too large to enter the arterial wall, the great majority of VLDL particles are as atherogenic as LDL particles. Since 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.<sup>43-48</sup> 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.<sup>2</sup> However, in those hypertriglyceridemic patients 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 hyperTG patients with low HDL-C.<sup>49</sup> The inconsistent effects of fibrates on clinical benefit are, therefore, 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 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 easily<sup>50</sup> and bind more avidly to the glycosaminoglycans within the arterial wall than larger apoB particles containing more cholesterol.<sup>51,52</sup> 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. There is, therefore, 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.

But the unification and, therefore, the simplification offered by apoB have gone further: Ference et al have shown that VLDL particles pose equal atherogenic risk to LDL particles.<sup>6</sup> Since the number of LDL particles is always many multiples of the number of VLDL particles,<sup>2</sup> 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 triglycerides to cardiovascular risk, notwithstanding that hypertriglyceridemia is more common in patients with cardiovascular disease than hypercholesterolemia.<sup>53,54</sup>

There are two 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.<sup>55,56</sup> The cholesterol content of these particle 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 normals and hypertriglyceridemic patients, who do not have type III.<sup>2</sup> 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 TC, TG and apoB.<sup>57</sup>

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.<sup>58,59</sup> A large meta-analysis demonstrated that Lp(a) appears to confer increased risk for cardiovascular risk despite statin therapy<sup>60</sup> 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.<sup>61</sup> Conversely, in individuals with familial hypercholesterolemia, characterized by a high burden of apoB particles, the presence of high Lp(a) further increases risk.<sup>62,63</sup>

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.<sup>64</sup> Thus, the hypothesis that glycation of apoB particles promotes binding of apoB particles deserves further attention.<sup>65</sup> 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.<sup>68</sup>

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

## Summary

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.<sup>67-69</sup> 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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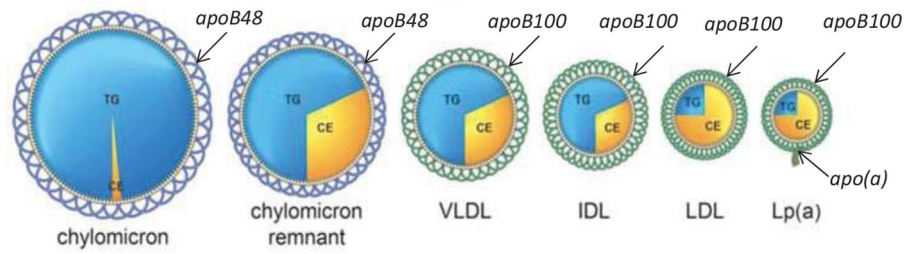
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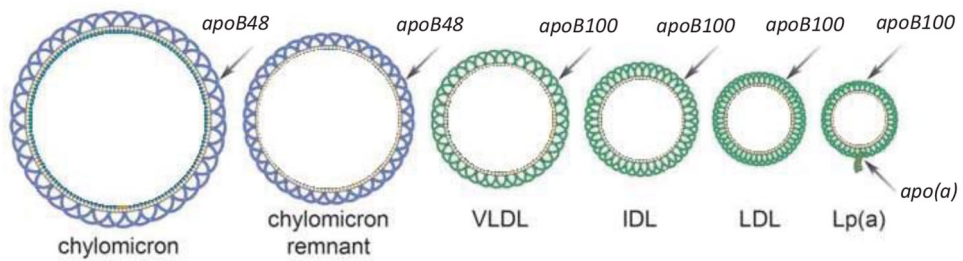
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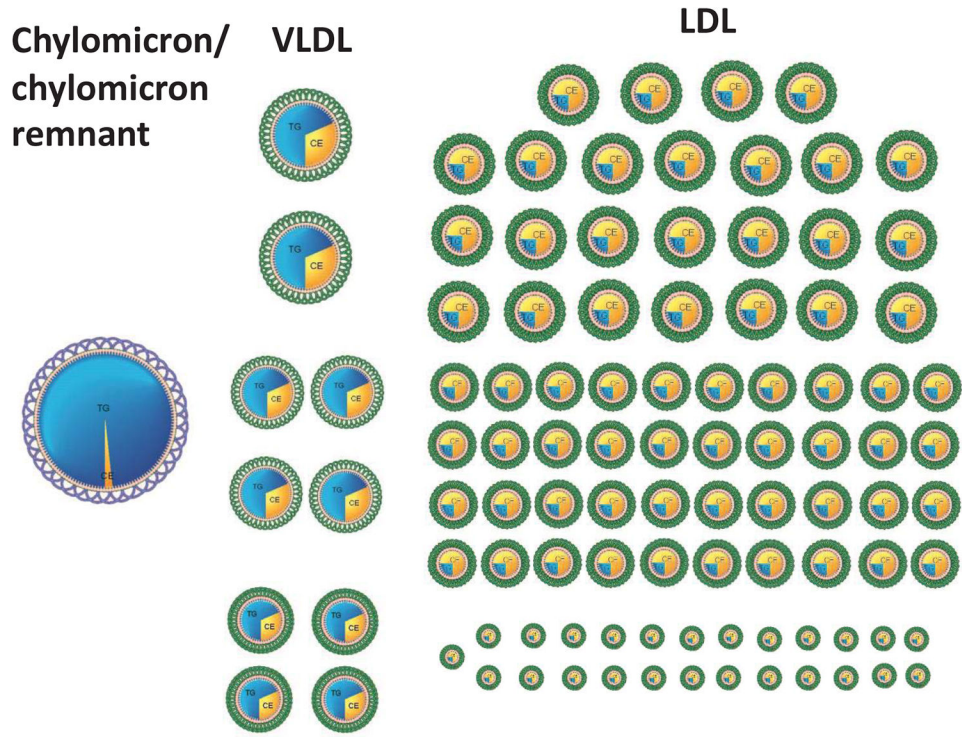
**apoB lipoproteins**



**apoB lipoproteins**

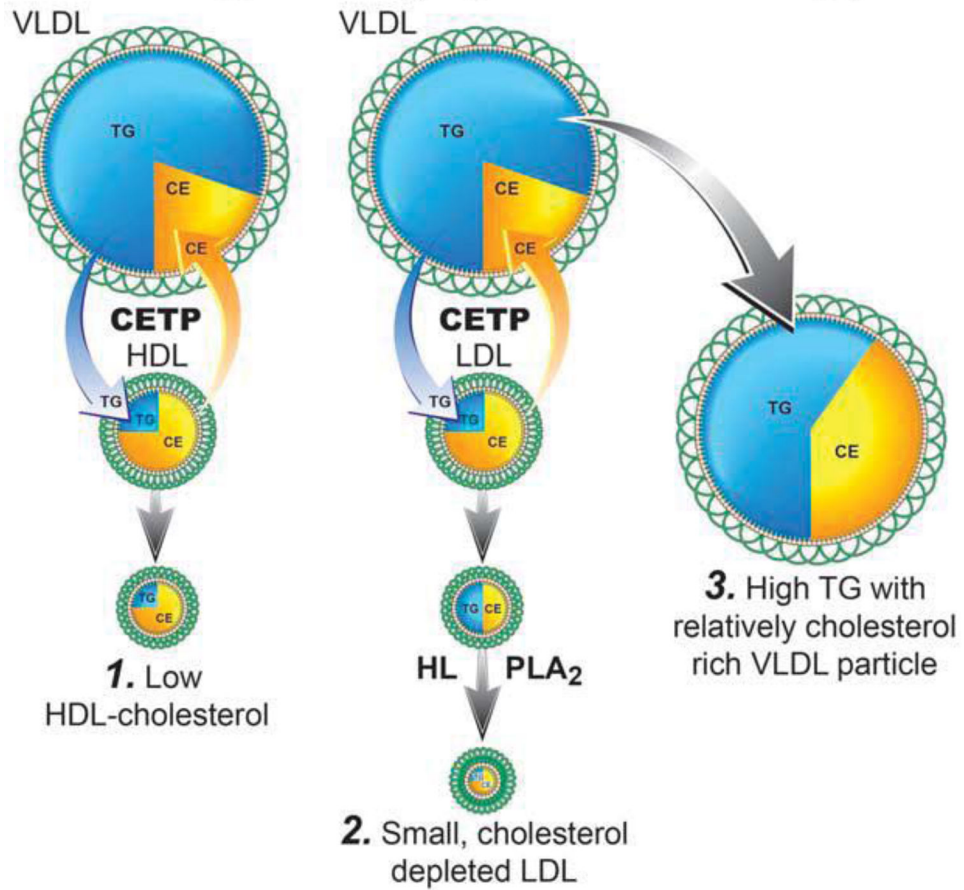


**Figure 1.** illustrates schematically the apoB48 and apoB100 lipoprotein particles. 1 apoB molecule = 1 lipid particle. Therefore, apoB plasma concentration = total number of atherogenic lipid particles



**Figure 2.** illustrates the relative numbers of apoB particles in plasma in the postprandial period.

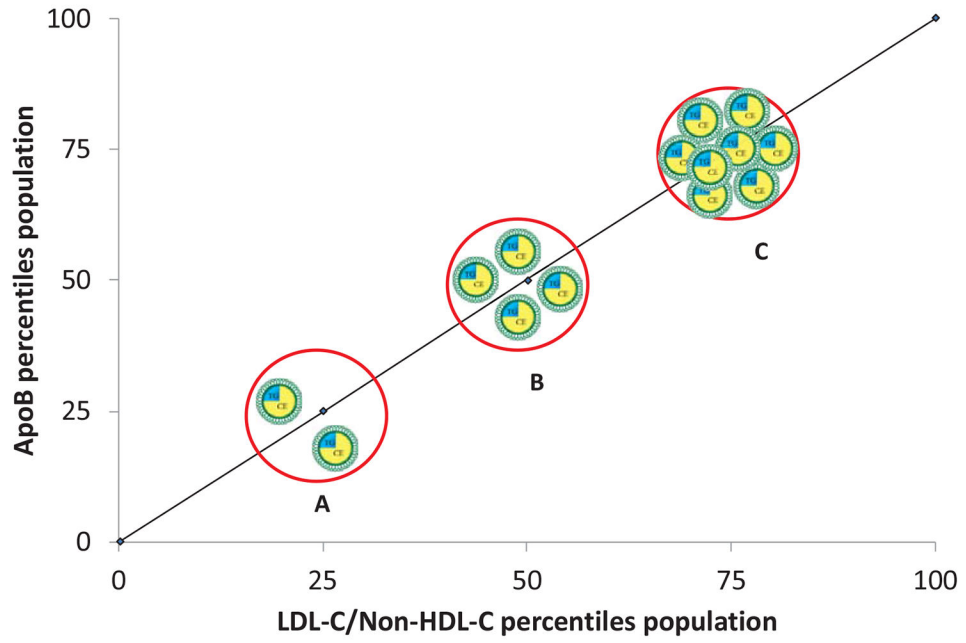
## CETP exchange and the Atherogenic Lipoprotein Phenotype



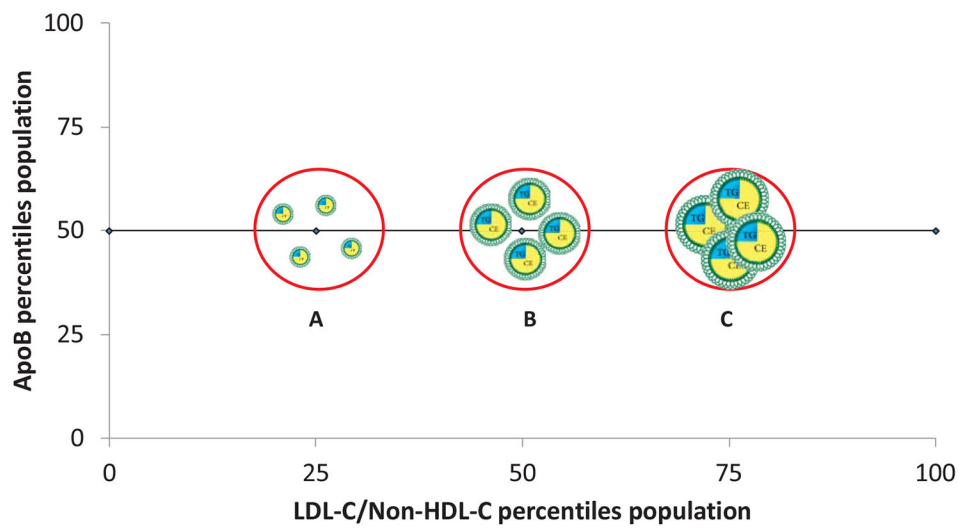
**Figure 3.** illustrates the metabolic steps by which HDL-C is lowered based on exchange of CE and triglycerides as well as the steps by which smaller cholesterol—depleted LDL particles are generated.



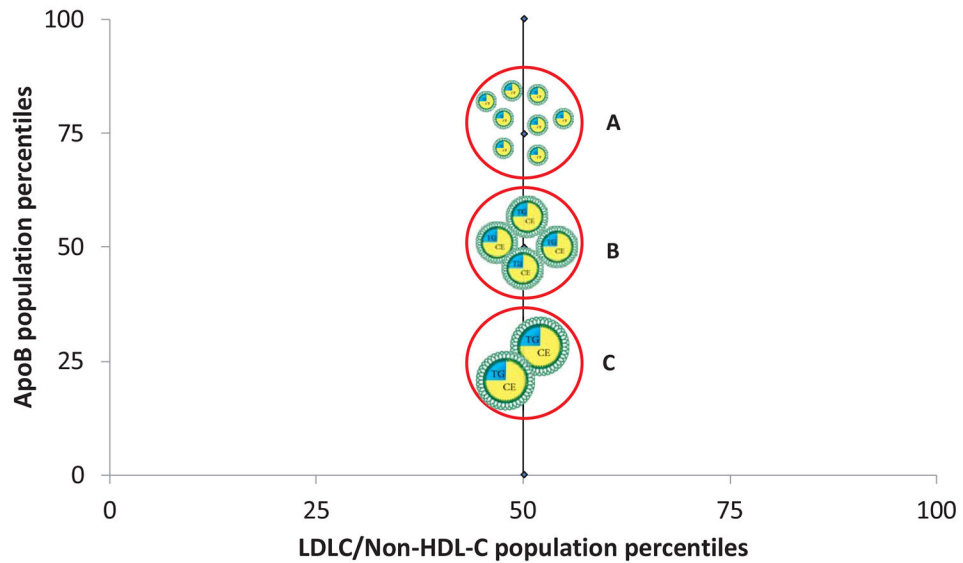
### Panel A



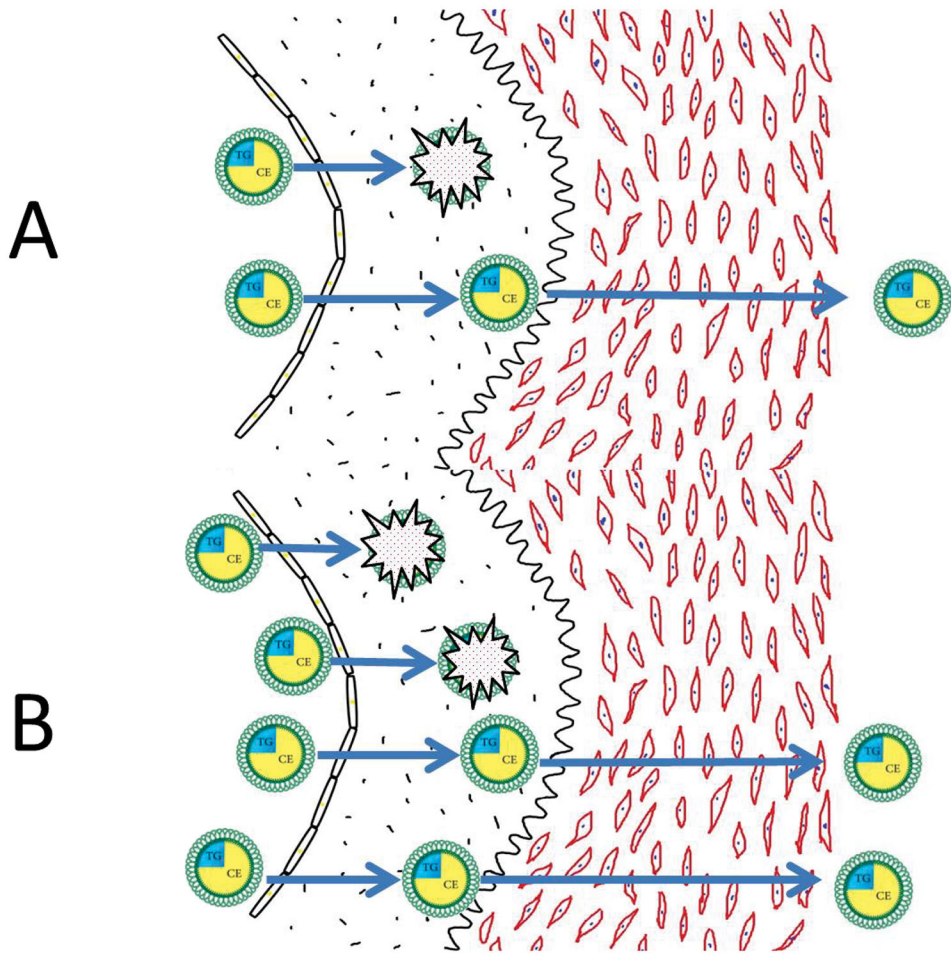
### Panel B

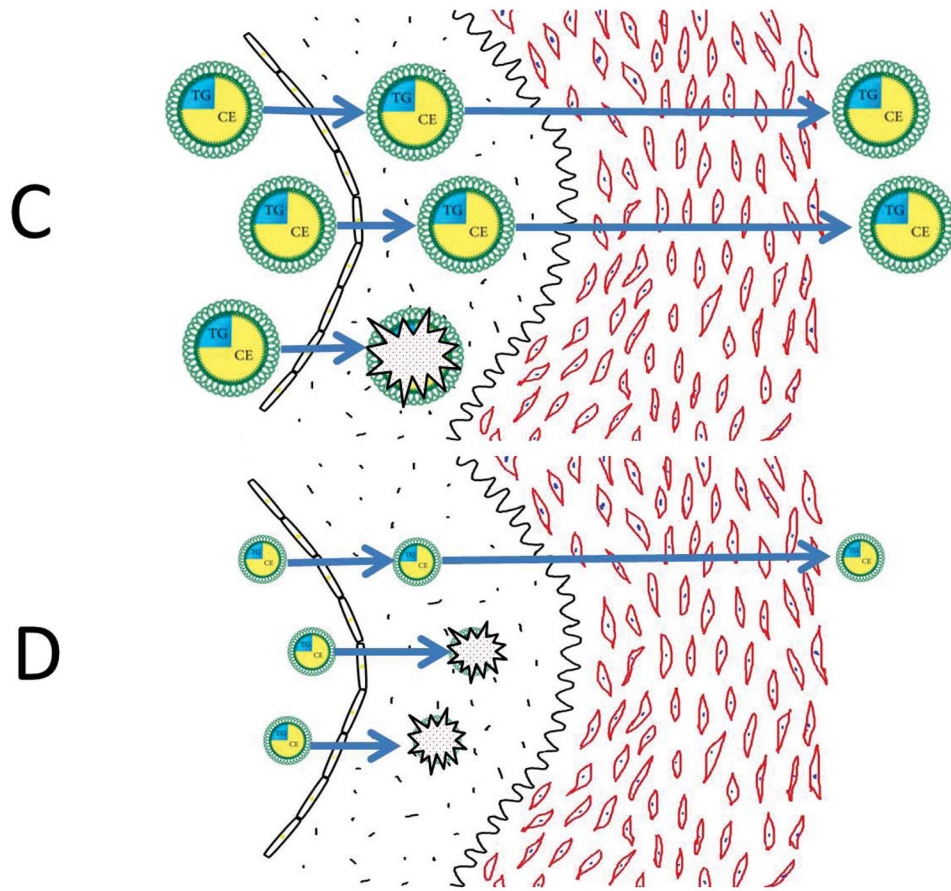


## Panel C



**Figure 4.** Panel A illustrates discordance in LDL-C and apoB when the apoB particles contain an average mass of cholesterol. Panel B illustrates discordance when apoB particles are cholesterol-enriched whereas Panel C illustrates discordance when apoB particles are cholesterol-depleted.





**Figure 5.**

Panel A illustrates the number of apoB particles within the lumen of an artery is the prime determinant of the number of apoB particles trapped within the arterial wall. ApoB particles trapped within the arterial wall initiate and sustain the inflammatory, destructive processes that ultimately produce advanced atherosclerosis within that segment of the arterial wall.