On the atherogenicity of triglyceride-rich lipoproteins and novel markers for the assessment of their atherogenic potentials

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Summary : It is generally accepted that cholesterol-rich low density lipoproteins are more significantly associated with atherosclerotic disease than triglyceride-rich very low density lipoproteins. This paper addresses two aspects of the atherogenic potentials of apoB-containing lipoproteins, the first of which relates to the differential atherogenicity of apoB-containing lipoproteins and the second to the adequacy of plasma triglycerides as a marker for atherogenic capacity of triglyceride-rich lipoproteins. These topics are explored and discussed on the basis of the lipoprotein family concept that classifies plasma lipoproteins on the basis of their unique apolipoprotein composition rather than density properties. A brief presentation of the lipoprotein family concept is followed by a review of recent epidemiologic and clinical studies from this and other laboratories showing that some intact or partially delipidized triglyceride-rich lipoproteins possess atherogenic capacities equal, to if not greater than, those of cholesterol-rich lipoproteins. Furthermore, the concept of differential atherogenicity of apoB-containing lipoprotein families is introduced and the evidence is presented that the apoC-III levels associated with apoB-containing lipoproteins are a more consistent independent marker for the atherogenicity of triglyceride-rich lipoproteins than plasma triglycerides.

Keywords : lipids, apolipoproteins, lipoprotein families, atherogenicity.

ARTICLE

Prospective epidemiologic studies have demonstrated that hypercholesterolemia is more significantly associated with atherosclerotic disease than hypertriglyceridemia and that cholesterol (CHOL)-rich low density lipoprotein (LDL) particles have a greater atherogenic potential than triglyceride (TG)-rich lipoproteins [1-3]. Furthermore, a number of prospective clinical trials have established that the lowering of LDL-C levels has a beneficial effect on the progression and stabilization of atherosclerotic lesions and reduction of coronary events [4, 5]. However, despite aggressive LDL-C reduction, 20-50% of patients continue to show progression of atherosclerosis [6]. Clearly, factors in addition to CHOL-rich LDL particles contribute to the progression of atherosclerosis in these patients. Since hypertriglyceridemia with or without hypercholesterolemia occurs more frequently in patients with premature coronary artery disease (CAD) [7], one of these possible factors may be increased levels of intact or modified TG-rich lipoproteins [8-10].

Although most case-control studies have shown a strong univariate association between plasma TG levels and CAD, prospective studies have provided little evidence that TG levels represent an independent risk factor for CAD after adjustments are made for other traditional risk factors such as

total cholesterol (TC), high density lipoprotein (HDL)-C and hypertension [11-13]. Thus, in contrast to elevated levels of TC or LDL-C, the independent contribution of plasma TG concentrations to atherogenic disease has remained a controversial issue. However, it has been shown in several epidemiologic and clinical studies that in some subsets of patient populations (women, elderly subjects, diabetes mellitus type II, etc.) increased levels of plasma TG represent an independent risk factor for CAD [14-21]. These findings have been supported by results of a meta-analysis of population-based prospective studies clearly demonstrating that plasma TG levels are, indeed, a risk factor for CAD independent of HDL-C levels [22]. Results of the recently reported Copenhagen Male Study [23] and the Prospective Cardiovascular Münster (PROCAM) study [24, 25] have confirmed that hypertriglyceridemia is an independent risk factor for coronary events in middle-aged and elderly men even after adjustments for a number of lipid and clinical confounding factors; when stratified for HDL-C levels, TG concentrations were found to form a gradient of risk of coronary events with increasing TG levels within each HDL-C level. In both studies, subgroups of patients with high TG levels and either low (<35 mg/dL) or high (>55 mg/dL) HDL-C concentrations were associated with a higher risk of coronary events than patients with intermediate HDL-C levels. It may be concluded from these two studies that high TG levels and low HDL-C levels are a high risk for fatal and non-fatal coronary events and that increased concentrations of TG represent a risk factor for CAD regardless of HDL-C levels. In addition, several clinical and metabolic studies have indicated that modified TG-rich lipoproteins ("remnant lipoproteins") may have atherogenic potential similar, if not equal, to that of CHOL-rich LDL particles [6, 9, 10, 26-34].

It has been suggested that triglyceride molecules should not be equated with intact and especially modified TG-rich lipoproteins when interpreting findings of epidemiologic studies by statistical rather than biomedical modeling [10, 32, 33]. There are obvious differences between LDL-C as a marker of CHOL-rich lipoproteins and plasma TG as a marker of TG-rich lipoproteins. Whereas LDL-C encompasses a relatively narrow density segment of apolipoprotein (apo) B-containing lipoproteins with the majority of particles characterized by apoB as the sole protein, plasma TG encompass all lipoproteins including non-atherogenic chylomicrons and large VLDL, atherogenic IDL and LDL and anti-atherogenic HDL. Thus, when estimating atherogenic potential of apoB-containing lipoproteins, the use of plasma TG as a marker of TG-rich lipoproteins introduces an *a priori* bias in statistical modeling and evaluation. The other weaknesses of plasma TG as a marker of atherogenic potential of TG-rich lipoproteins are its relatively wide intra- and inter-individual variations in comparison with other lipid variables, a significant correlation between TG and other lipid and apolipoprotein constituents such as the most characteristic inverse correlation between TG and HDL-C, and the chemical, metabolic and atherogenic heterogeneity of TG-rich lipoproteins [6, 10, 32-37]. Based on these considerations, it appears that the use of plasma TG as a marker of potentially atherogenic TG-rich lipoproteins is not equivalent to the use of LDL-C as a marker of CHOL-rich lipoproteins. If, indeed, the controversy regarding the independent role of plasma TG or TG-rich lipoproteins in the genesis and development of atherosclerosis is due, to a great extent, to the selection of TG as an inadequate marker, is it possible to identify another constituent or a lipoprotein particle that may qualify as a more specific and consistent indicator of the potential pathophysiologic capacity of TG-rich lipoproteins?

There are two aspects of apoB-containing lipoproteins to be addressed in this article. The first one pertains to the differential atherogenicity of individual apoB-containing lipoproteins and the second one to the identification of an alternate marker for TG-rich lipoproteins. These topics will be explored

and discussed on the basis of lipoprotein family concept that defines plasma lipoproteins by their apolipoprotein composition rather than physical properties.

Lipoprotein family concept

Plasma lipoproteins may be classified on the basis of their physical properties (density, size or electric charge) or on the basis of their apolipoprotein composition. Due to a discontinuous distribution along a density gradient from 0.92 to 1.25 g/mL, plasma lipoproteins may be subdivided into several major lipoprotein density classes including chylomicrons (0.92-0.94 g/mL), VLDL (0.94-1.006 g/mL), IDL (1.006-1.019 g/mL), LDL (1.019-1.063 g/mL), HDL (1.063-1.21 g/mL) and very high density lipoproteins (VHDL > 1.25 g/mL) [38]. Studies on the macromolecular distributions showed that each major lipoprotein density class is a polydisperse system of particles differing from one another with respect to density, size and lipid/protein ratios. However, despite recognized structural and compositional heterogeneity, major lipoprotein density classes have been accepted as the fundamental physical-chemical and metabolic entities of the plasma lipoprotein system. This conceptual view was supported by its emphasis on lipids as potentially injurious agents in the genesis and development of atherosclerosis, disclosure of metabolic relationship between major lipoprotein density classes, and their clinical usefulness for characterizing and classifying hyperlipoproteinemias [38, 39]. However, the discovery of a number of apolipoproteins constituting the protein moiety of lipoproteins [36, 40, 41] had a deep effect on the conceptual aspects of plasma lipoproteins, because chemical, immunologic and genetic studies have shown that protein constituents play pivotal roles in the structural integrity and metabolic specificity of lipoproteins. Studies on the distribution and localization of major (apoA-I, apoA-II, apoB) and minor (apoC-I, apoC-II, apoC-III, apoE, apoD, apoF, apoH, etc.) apolipoproteins in main lipoprotein density classes (VLDL, IDL, LDL, HDL) have shown that each density class contains a major and several minor apolipoproteins [36]. Quantitative analyses of apolipoproteins along the density gradient have indicated an uneven localization and distribution of apolipoproteins suggesting that lipoprotein density classes contain several types of lipoprotein species of similar density properties, but different and characteristic apolipoprotein composition [36]. Thus, major lipoprotein density classes are heterogeneous not only with respect to densities, sizes and lipid/protein composition but also with respect to qualitative and quantitative composition of apolipoprotein-defined lipoprotein species. Apolipoproteins have emerged from these studies as the most useful markers for the identification and differentiation of lipoprotein particles and for studying their interactions during all phases of lipid transport process [36, 41].

To emphasize the chemical and immunochemical uniqueness of apolipoproteins as ideal markers for identifying lipoproteins regardless of densities or other non-specific physical properties, we have suggested that apolipoproteins be used as a criterion for the classification of plasma lipoproteins [36, 41]. In this classification system, plasma lipoproteins are viewed as a mixture of discrete lipoprotein families of particles defined solely on the basis of their specific qualitative apolipoprotein composition; lipoprotein families are named according to their apolipoprotein composition. For example, a lipoprotein family which only contains apoA-I as its protein component is named lipoprotein A-I (Lp-A-I), while a lipoprotein family with apolipoproteins B, C and E as its protein constituents is named lipoprotein B:C:E (Lp-B:C:E).

There are two major classes of lipid-protein complexes, one of which is characterized by apoB as the major protein moiety (d 0.92-1.063 g/mL) and the other by apoA (apoA-I+apoA-II) as the major

protein constituent (d 1.063-1.25 g/mL). As shown in *Figure 1*, the apoB-containing lipoproteins of densities lower than 1.063 g/mL may be further subdivided into five major lipoprotein families, each of which is characterized by a specific apolipoprotein composition. These include cholesterol-rich lipoprotein B (Lp-B), cholesterol-enriched Lp-B:E and triglyceride-rich Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E [36, 41]. The apoA-containing lipoproteins of densities higher than 1.063 g/mL consist of three major lipoprotein families including Lp-A-I, Lp-A-I:A-II and Lp-A-II [36, 41]. The fractionation of individual apoA- and apoB-containing lipoprotein families has been achieved both by the sequential immunoprecipitation [42] and immunoaffinity chromatography with affinity-purified polyclonal antisera to individual apolipoproteins [43]. These two immunological procedures have been used not only for the isolation but also for the quantification of individual lipoprotein families.

All apoA- and apoB-containing lipoprotein families represent polydisperse systems of particles differing from one another with respect to size, density and lipid/protein composition but maintaining the same qualitative apolipoprotein composition [36, 41]. As shown in *Figure*, apoA-containing lipoprotein families overlap within the HDL density segment and apoB-containing lipoprotein families within the VLDL, IDL and LDL density ranges. Lines under each of lipoprotein families indicate their actual (solid lines) and potential (broken lines) distributions along the lipoprotein density gradient. In the case of apoB-containing lipoproteins, the extent of polydispersity and distribution of individual lipoprotein families depends on the amounts of neutral lipids to be transported and processes responsible for their degradation and removal. For example, Lp-B:C particles with their usual load of TG have density characteristics of VLDL particles; however, lipolytic degradation results in the formation of Lp-B:C particles of IDL and even LDL density properties characterized by decreasing lipid/protein ratios and smaller particle sizes [36]. Similarly, the polydispersity of apoA-containing lipoprotein families depends most probably on changes in lipid/protein ratios which, in turn, are influenced by processes regulating the metabolism of apolipoproteins, phospholipids and neutral lipids.

It has already been established in a number of studies that apolipoprotein-defined apoA- and apoB-containing lipoprotein families are characterized not only by specific apolipoprotein composition but also by specific metabolic and functional properties [26, 41].

The turnover rate of apoA-I in Lp-A-I has been found to be faster than that of apoA-I in Lp-A-I:A-II [44]. The Lp-A-I particles, but not Lp-A-I:A-II particles, seem to function as the acceptors of peripheral cholesterol [45] and, in association with lecithin:cholesterol acyltransferase and cholesterol ester transfer protein, as templates for esterifying and transferring cholesterol in plasma [46]. Moreover, Lp-A-I particles bind to various cell membranes with greater affinity than Lp-A-I:A-II particles [47]. On the other hand, it appears that one of the main functions of Lp-A-I:A-II particles is to accept apoC-peptides and apoE released during the degradation of TG-rich lipoproteins, and to provide these minor apolipoproteins for the extracellular completion of formation of TG-rich lipoproteins in Space of Disse [48].

Studies in this laboratory have shown that HepG2 cells secrete mainly, if not exclusively, TG-rich Lp-B and Lp-B:E particles, suggesting that these lipoprotein families may act as precursors for the extracellular formation of Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E particles [41]. Furthermore, it has been demonstrated that the three major TG-rich lipoprotein families have different affinities for lipoprotein lipase (LPL) despite almost identical TG and apoC-III contents; thus, the Lp-B:C:E particles

have been found to be the most efficient substrate for LPL followed by decreasing efficiency of Lp-B:C and low efficiency of Lp-A-II:B:C:D:E particles [49]. The Lp-B:E particles bind to LDL receptors on human fibroblasts, HepG2 and HeLa cells with greater affinity than Lp-B particles [50, 51]; in contrast, the binding of Lp-B:C and Lp-B:C:E particles with phenotype E2/E2 to LDL receptors of HeLa cells has been found to be negligible, suggesting that these lipoprotein families have very little effect on the regulation of HMG-CoA reductase activity [51]. All these findings provide strong evidence for the crucial role of minor apolipoproteins in modifying and determining the metabolic properties of individual apoA- and apoB-containing lipoproteins.

Atherogenicity of TG-rich lipoproteins

As presented and discussed in the introductory remarks, there is now compelling evidence that plasma TG and especially modified TG-rich lipoproteins play a significant role in atherogenesis. However, there is still a paucity of information regarding the atherogenic potential of TG-rich lipoproteins in comparison with that of CHOL-rich lipoproteins as well as the possibility of replacing plasma TG with a more adequate marker for the atherogenic potential of TG-rich lipoproteins.

There is a general consensus that the atherogenic potential of plasma lipoproteins resides in apoB-containing and antiatherogenic capacity in apoA-containing lipoproteins. It is clinically useful to subdivide apoB-containing lipoproteins into TG-rich and CHOL-rich lipoproteins. According to the lipoprotein family concept, lipoprotein families Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E are considered to constitute the TG-rich and Lp-B and Lp-B:E the CHOL-rich lipoproteins. The sum of three TG-rich lipoprotein families is referred to as Lp-$B_{complex}$ or Lp-B_c, while the sum of two CHOL-rich lipoprotein families is simply called Lp-B. Because each of Lp-B_c families contains apoC-III, they can be separated from Lp-B (Lp-B+Lp-B:E) families by immunoaffinity chromatography on an anti-apoC-III immunosorber [43, 52]. As mentioned before, we have also developed immunologic procedures for separating and quantifying all five apoB-containing lipoprotein families [42, 43]. As a means for determining the efficacy of catabolic processes responsible for the lipolytic degradation of TG-rich lipoprotein families, we have developed a procedure based on the measurement of apoC-III distribution between HDL or apoA-containing and VLDL+LDL or apoB-containing lipoproteins: the higher the ratio of apoC-III-HDL/apoC-III-VLDL+LDL, the greater the degradation of TG-rich lipoproteins and vice versa [48].

The relationship of apoB-containing lipoproteins to atherosclerosis and the search for an alternate marker for atherogenicity of TG-rich lipoproteins have been studied on the basis of lipoprotein family concept in three prospective clinical trials. Two of these studies, the Cholesterol Lowering Atherosclerosis Study (CLAS) [53] and Monitored Atherosclerosis Regression Study (MARS) [54], were randomized, placebo-controlled coronary angiographic trials, the purpose of which was to determine the effect of LDL lowering on coronary angiographic findings in patients with documented coronary artery disease and moderate hypercholesterolemia. The 2-year treatment with niacin-colestipol in the CLAS and with lovastatin in the MARS resulted in significantly reduced progression of atherosclerotic lesions due to lowering effects of drugs on the constituents of CHOL-rich lipoproteins (apoB, LDL-C) or Lp-B particles in terms of lipoprotein family concept. However, it was also shown that, despite highly reduced levels of LDL-C (97±27 mg/dL in the CLAS and 82±17 mg/dL in the MARS), the continuing progression of atherosclerotic disease in 35-40% of patients was associated with increased levels of the constituents of TG-rich lipoproteins (apoC-III in VLDL+LDL, TG). In the

CLAS, multivariate analysis revealed non-HDL-C as a significant independent predictor of progression in the placebo group and that of apoC-III in HDL in the drug group, suggesting a potentially important role of TG-rich lipoproteins in the progression of atherosclerotic lesions in both placebo and drug groups [53]. The reason for this outcome was shown in a *post hoc* study in which the treatment with the niacin-colestipol combination significantly lowered the levels of CHOL-rich Lp-B particles, but had little or no reducing effect on the levels of TG-rich Lp-B:C and Lp-B:C:E particles [55]. In the MARS, the CHOL-rich Lp-B, TG-rich Lp-A-II:B:C:D:E and the sum of Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E (Lp-B_c) particles, were directly measured for the first time in a prospective, angiographic trial [56]. The above mentioned beneficial effect of lovastatin on the progression of atherosclerotic lesions (31% of progressors in the lovastatin group *vs* 58% progressors in the placebo group) was attributed to its lowering effect on LDL-C or CHOL-rich Lp-B particles. However, lovastatin had very little effect on the sum of TG-rich lipoproteins (Lp-B_c) or Lp-A-II:B:C:D:E particles as one of the individual TG-rich lipoproteins. In fact, it was found that the higher the concentration of Lp-B_c particles, the higher the relative risk of coronary artery lesion progression. Another unexpected finding of the MARS was that increased levels of CHOL-rich Lp-B particles were associated with the progression of large lesions (>50% diameter stenosis), whereas increased levels of TG-rich Lp-B_c particles were associated with the progression of small to moderate lesions (<50% diameter stenosis) [57]. The progression of mild to moderate lesions was also one of the most significant predictors of clinical coronary events.

Results of CLAS and MARS trials have demonstrated that some intact or partially delipidized TG-rich or complex apoB-containing lipoprotein families (Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E) have equal, if not greater, atherogenic capacities than CHOL-rich lipoprotein families (Lp-B and Lp-B:E). The findings of the MARS trial have also shown that lipoprotein family profiling of apoB-containing lipoproteins is a better predictor of progressive coronary artery disease than the measurement of lipid variables.

It has generally been accepted that the atherogenic capacity of apoB-containing lipoproteins diminishes with increasing size of lipoprotein particles [58]. However, in addition to particle size as an essential atherogenic determinant, recent *in vitro* and *in vivo* studies have suggested that minor apolipoproteins such as apoA-II, apoC-peptides, apoE and apoD may also play an important role in modifying the *relative* atherogenic potentials of individual apoB-containing lipoproteins [53, 56, 59]. As mentioned before, the results of the CLAS and MARS studies have shown that the progression of coronary artery atherosclerosis is associated with increased levels of intact or partially delipidized triglyceride-rich lipoprotein families, each of which is characterized by the presence of minor apolipoproteins. In contrast, the CHOL-rich Lp-B particles, constituting the bulk of LDL and containing apoB as the sole protein constituent, contributed only marginally to the progression of coronary artery atherosclerosis [56]. These findings do not necessarily argue against the atherogenicity of Lp-B particles but merely suggest that its potential may be of a lesser degree than previously considered. In a separate study of a group of normotensive, non-obese, non-diabetic subjects undergoing coronary angiography, the severity of coronary artery disease was found to be significantly correlated with TG-rich Lp-B_c, but not CHOL-rich Lp-B particles [60]. Measurements of apoB-containing lipoprotein families have shown that the levels of Lp-B:C, but not Lp-B or Lp-B:C:E, were significantly higher in type II diabetic subjects than in non-diabetics, and that diabetics with clinically verified vascular disease had two-fold higher levels of Lp-B:C particles than diabetics without vascular disease [61]. The concentrations of Lp-B:C, but not Lp-B or Lp-B:C:E, particles were also found to be significantly higher in predialytic subjects with chronic renal disease than in corresponding controls

[62]. Both hyperlipidemia phenotypes IV and V are characterized by very high levels of Lp-B:C and Lp-B:C:E and relatively low levels of Lp-B particles [55, 63]. Although the high concentration of Lp-B particles represents the characteristic feature of lipoprotein particle profile in familial hypercholesterolemia, the levels of Lp-B:C:E particles are similar to those of type IV and V phenotypes [63]. Recent studies in patients with type II diabetes have provided additional evidence for the atherogenic potential of Lp-B:C particles. In the first of these two studies, the levels of Lp-B:C particles were shown to be independently associated with macrovascular complications of type II diabetes displaying the highest odds ratio among independent variables [20]. The second study was an ancillary, prospective nested case-control CARE trial conducted with 242 diabetic subjects who had had myocardial infarction [64]. During 5 years, 121 of diabetics had a recurrent coronary event and 121 diabetic subjects had not. The levels of Lp-B, Lp-B:C and Lp-B:C:E particles were measured in VLDL and IDL+LDL density ranges. In a multivariate model consisting of Lp-B, Lp-B:C and Lp-B:C:E measured in VLDL and IDL+LDL, only the levels of Lp-B:C particles in IDL+LDL were significantly associated with recurrent cardiovascular events. Remarkably, it was determined that only 5 mg/dL of Lp-B:C particles were associated with a similar 3-fold increase in risk of recurrent events as 50 mg/dL of Lp-B particles.

Results of an *in vitro* study have shown that the isolated Lp-B, Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E particles incubated with human THP1 macrophages had been taken up at different rates and by different mechanisms [59]. The rates of cholesterol ester and apoB accumulation in macrophages were significantly higher for Lp-B:C and Lp-B:C:E than for Lp-A-II:B:C:D:E and Lp-B particles. It appeared that the former lipoprotein families had been taken up by a low-affinity, unsaturable mechanism and the latter lipoprotein families by a high-affinity saturable mechanism. These *in vitro* and *in vivo* studies have suggested that all apoB-containing lipoprotein families possess atherogenic potentials with size and modifying effect of minor apolipoproteins as main determinants. However, if the size requirement is taken into consideration, the composition of minor apolipoproteins appears to be the main factor responsible for differences in the atherogenic capacities of individual apoB-containing lipoproteins. Thus, the relative atherogenic capacity of Lp-B:C and Lp-A-II:B:C:D:E particles seems to be greater than those of Lp-B, Lp-B:E and Lp-B:C:E particles. Although the concept of relative atherogenicity of apoB-containing lipoproteins necessitates additional experimental evidence, it is possible to speculate how different metabolic properties of individual lipoprotein families might possibly be related to their atherogenic capacities. For example, how does the marked atherogenicity of Lp-B:C particles and lesser atherogenic capacities of other lipoprotein families reflect their metabolic properties? Under normal metabolic conditions, the efficient lipolytic degradation of Lp-B:C particles results in low plasma concentrations of this lipoprotein family [49, 63]. However, even a slight impairment in the formation and/or degradation of Lp-B:C particles, further exacerbated by the lack of binding affinity to LDL receptors [51], results not only in increased plasma levels and residence time but also in an enhanced uptake of these lipoprotein particles by macrophages [59]. Due to a low affinity for lipoprotein lipase, Lp-A-II:B:C:D:E particles have a prolonged residence time in the circulation [49]; although this lipoprotein family may be taken up by LDL receptors, their rate of uptake by macrophages seems to be higher than that of Lp-B particles [59]. In contrast, the relative atherogenic capacities of Lp-B:C:E and Lp-B:E particles may be less pronounced than that of Lp-B particles. This might be due to high rates of the lipolytic degradation of Lp-B:C:E particles on one hand [49] and high affinity binding and uptake of Lp-B:E particles by LDL receptors on the other [50]. Under normal metabolic conditions, plasma levels of Lp-B:C:E and Lp-B:E

particles are relatively low except in hypercholesterolemic and hypertriglyceridemic states [63]. It has recently been shown that the quantity rather than the qualitative composition of LDL or Lp-B particles is related to the severity and premature atherosclerosis in patients with familial hypercholesterolemia [65]. This interesting observation is confirmed and illustrated by the finding that a 3-fold increase in coronary events in type II diabetic patients required a 10-fold greater molar concentration of Lp-B than Lp-B:C particles [64].

Markers for the atherogenicity of TG-rich lipoproteins

Recent studies on the apolipoprotein-defined TG-rich lipoprotein families have revealed not only the characteristic differences in chemical and metabolic properties but also in relative atherogenic capacities of this group of lipoproteins. In fact, it has been shown that some of these lipoprotein families, notably Lp-B:C and Lp-A-II:B:C:D:E, may have atherogenic potentials equal, to if not greater than, that of CHOL-rich Lp-B, the major lipoprotein of LDL density range. In view of these findings, it has been puzzling that in several prospective studies on the atherogenicity of apoB-containing lipoproteins plasma TG levels have been an inconsistent independent risk factor for CAD. We have explored this puzzling phenomenon in an ancillary Cholesterol and Recurrent Events (CARE) study, the purpose of which was to investigate the relationship between plasma TG, VLDL-lipids, VLDL-apoB, apoC-III and apoE in VLDL+LDL and HDL, LDL-C and HDL-C and recurrent coronary events in 418 subjects who experienced either a non-fatal myocardial infarction or coronary death during a 5-year interval [66]; age- and gender-matched 370 event-free subjects served as controls. Analyses were performed on baseline plasma samples. VLDL-C, VLDL-TG, VLDL-apoB and apoC-III and apoE in VLDL+LDL were interrelated, and each was found to be an univariate predictor of subsequent coronary events. The VLDL variables were studied together in multiple logistic regression analysis which showed that VLDL-apoB levels (RR=3.2 for the fifth compared to the first quintile, $p<0.03$) and the levels of apoC-III in VLDL+LDL (RR=2.25, $p<0.001$) were the only significant independent predictors of recurrent coronary events in a model including plasma TG, LDL-C and HDL-C. The amount of apoC-III per VLDL and LDL particles (apoC-III/apoB ratio) was a significant predictor, whereas the apoE/apoB ratio had no relationship to coronary events. Adding LDL-C, HDL-C and plasma TG to apoC-III in VLDL+LDL had no effect on the relative risk of this latter variable. However, the predictive value of plasma TG, significant in univariate analysis (RR=1.58), was eliminated (RR=0.95). On the basis of these results we have concluded that levels of apoC-III in VLDL+LDL and VLDL-apoB are independent predictors of recurrent coronary events and explain the weaker relationship between plasma TG and coronary events. Results of this study are consistent with the known metabolic properties of apoC- and apoB-containing lipoprotein families (Lp-B:C, Lp-A-II:B:C:D:E and Lp-B:C:E) which link them to atherosclerosis, and strongly suggest that the levels of VLDL-apoB and apoC-III associated with VLDL+LDL or apoB-containing lipoprotein families are better markers than plasma TG for assessing the atherogenic potential of intact or partially delipidized TG-rich lipoproteins.

Abbreviations: Chylos: chylomicrons, VLDL: very low density lipoproteins, IDL: intermediate density lipoproteins, LDL: low density lipoproteins, HDL: high density lipoproteins, VHDL: very high density lipoproteins, HDL2: high density lipoprotein subfraction with d = 1.064 1.25 g/mL, HDL3: high density subfraction with d = 1.25 1.21 g/= mL, Apo: apolipoprotein, Lp-B: lipoprotein B characterized by apo

B as the sole protein constituent, Lp-B:C: lipoprotein B:C characterized by apoB and apoC as protein constituents, etc.

CONCLUSION

The recently accumulated experimental evidence from a number of prospective epidemiologic and clinical studies, based either on measurements of individual lipid and apolipoprotein constituents or integral lipoprotein particles, has clearly established that increased levels of TG-rich lipoproteins are an independent risk factor for CAD. Specifically, results of these studies have led to the following conclusions:

1. All apoB-containing lipoproteins are potentially atherogenic with size of lipoprotein particles as the major limiting factor.

2. Some intact or partially delipidized TG-rich apoB-containing lipoprotein families (Lp-B:C, Lp-A-II:B:C:D:E and Lp-B:C:E) have atherogenic potentials similar, to if not greater than, the CHOL-rich lipoprotein families (Lp-B and Lp-B:E). It appears, however, that individual apoB-containing lipoproteins may have different relative atherogenic potentials possibly related to their specific metabolic properties.

3. The number of VLDL-apoB particles and the levels of apoC-III associated with apoB-containing lipoproteins are significant independent predictors of recurrent coronary events superior in their predictive potential to LDL-C, HDL-C and plasma TG.

4. VLDL-apoB and apoC-III associated with VLDL+LDL or apoB-containing lipoproteins are markers superior to plasma TG for assessing the atherogenicity of TG-rich lipoproteins.

5. The traditional assessment of plasma TG, LDL-C and HDL-C levels may not provide the optimal measure of CAD risk.

In view of a significant contribution of TG-rich lipoproteins to the increased risk of CAD, it is recommended that an improved, but still simple, assessment of CAD be based on measurements of LDL-C, HDL-C and the distribution of apoC-III in apoA- and apoB-containing lipoproteins (apoC-III-ratio) as a predictive marker superior to plasma TG. It is anticipated, however, that the measurement of individual apoB-containing lipoprotein families will provide a physiologically more meaningful means than the determination of single lipid or apolipoprotein constituents for evaluating lipid transport processes in health and disease.

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Illustrations

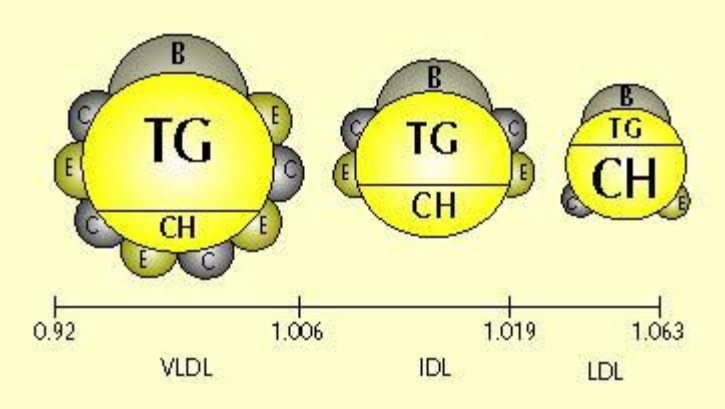

Figure. *Polydispersity.*

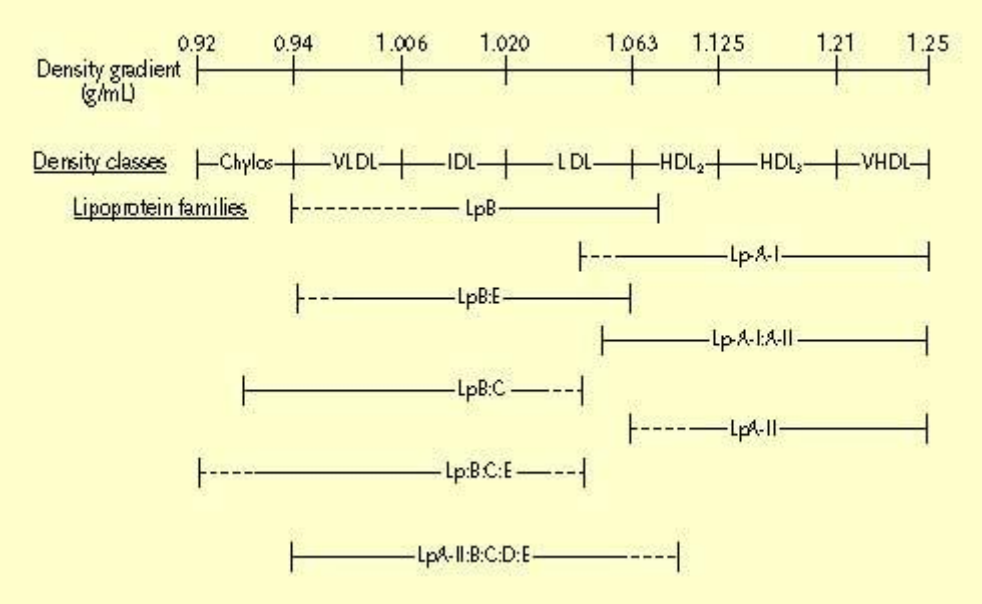

Figure 1. *The relationship of individual apoA- and apoB-containing lipoprotein families defined by their unique apolipoprotein composition to major lipoprotein density classes against the density gradient background (d 0.92 d 1.25 g/mL). The lines under lipoprotein families designate the approximate density boundaries with solid lines depicting the actual and with broken lines the possible localization of each lipoprotein family. Each of the lipoprotein families represents polydisperse systems of particles, each of which has a different lipid/protein ratio but the same qualitative apolipoprotein composition. The polydisperse character of each lipoprotein family is the main reason for their overlap within certain density segments.*