THE SMALL, DENSE LDL PHENOTYPE AND THE RISK OF CORONARY HEART DISEASE: EPIDEMIOLOGY, PATHO-PHYSIOLOGY AND THERAPEUTIC ASPECTS

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SUMMARY - More than decade ago, several cross-sectional studies have reported differences in LDL particle size, density and composition between coronary heart disease (CHD) patients and healthy controls. Three recent prospective, nested case-control studies have since confirmed that the presence of small, dense LDL particles was associated with more than a three-fold increase in the risk of CHD. The small, dense LDL phenotype rarely occurs as an isolated disorder. It is most frequently accompanied by hypertriglyceridemia, reduced HDL cholesterol levels, abdominal obesity, insulin resistance and by a series of other metabolic alterations predictive of an impaired endothelial function and increased susceptibility to thrombosis. Whether or not the small, dense LDL phenotype should be considered an independent CHD risk factor remains to be clearly established.

The cluster of metabolic abnormalities associated with small, dense LDL particles has been referred to as the insulin resistance-dyslipidemic phenotype of abdominal obesity. Results from the Québec Cardiovascular Study have indicated that individuals displaying three of the numerous features of insulin resistance (elevated plasma insulin and apolipoprotein B concentrations and small, dense LDL particles) showed a remarkable increase in CHD risk. Our data suggest that the increased risk of CHD associated with having small, dense LDL particles may be modulated to a significant extent by the presence/absence of insulin resistance, abdominal obesity and increased LDL particle concentration. We suggest that the complex interactions among the metabolic alterations of the insulin resistance syndrome should be considered when evaluating the risk of CHD associated with the small, dense LDL phenotype. From a therapeutic standpoint, the treatment of this condition should not only aim at reducing plasma triglyceride levels, but also at improving all features of the insulin resistance syndrome, for which body weight loss and mobilization of abdominal fat appear as key elements. Finally, interventions leading to reduction in fasting triglyceride levels will increase LDL particle size and contribute to reduce CHD risk, particularly if plasma apolipoprotein B concentration (as a surrogate of the number of atherogenic particles) is also reduced.

Key-words: Coronary heart disease, insulin resistance, LDL, small and dense LDL, triglycerides.

RESUME - Phénotype LDL petites et denses et risque coronarien: épidémiologie, pathophysiologie et aspects thérapeutiques.

Depuis plus de dix ans, plusieurs études transversales ont rapporté des différences de taille, de densité et de composition des particules LDL entre les sujets atteints de maladie coronarienne (MC) et les témoins sains. Trois études prospectives récentes, ont confirmé que la présence de telles particules était associée à un risque de MC multiplié par trois ou plus. Le phénotype LDL petites et denses n’est que très rarement retrouvé isolé, puisqu’il est accompagné le plus souvent d’hypertriglycéridémie, d’HDL cholestérol abaissé, d’obésité abdominale, d’insulino-résistance et d’anomalies métaboliques prédisant une altération de la fonction endothéiale et une susceptibilité accrue à la thrombose. Il reste à démontrer que ce phénotype LDL petites et denses soit un facteur de risque de MC indépendant. Le regroupement des anomalies métaboliques associées à ce phénotype LDL a été considéré comme le profil lipoprotéique caractéristique du syndrome d’insulino-résistance et de l’obésité abdominale. Les résultats de l’Etude Cardiovasculaire de Québec ont indiqué que les sujets porteurs des trois signes d’insulino-résistance suivants: insulinémie élevée, apo B élevée et LDL petites et denses, présentent un risque de MC remarquablement élevé. Nos données indiquent que le risque coronarien accru associé aux particules LDL petites et denses paraît modulé par la présence ou l’absence d’insulino-résistance d’obésité abdominale et de concentrations élevées de LDL. Il est ainsi suggéré que soient prises en considération les interactions complexes entre les composantes du syndrome d’insulino-résistance dans l’évaluation du risque coronarien lié au phénotype LDL petites et denses.

Mots-clés : Risque coronarien, insulino-résistance, LDL, LDL petites et denses, triglycérides, fibrates, statines.
The relationship between plasma LDL cholesterol and coronary heart disease (CHD)-related mortality has been substantiated by recent large primary and secondary prevention studies [1-5]. Although the atherogenic risk attributed to elevated plasma LDL cholesterol concentrations is well beyond dispute, there is considerable overlap in the distribution of plasma cholesterol and LDL cholesterol levels between healthy subjects and patients with documented CHD [6-9]. This suggests that all individuals with elevated cholesterol levels may not subsequently develop premature CHD. Treating elevated LDL cholesterol levels also does not guarantee protection against CHD. For example, despite the fact that cholesterol-lowering therapy yielded a remarkable 30 % reduction in the number of CHD-related events in the 4S study [1], approximately one out of 5 (19 %, N = 431) of the treated patients who did achieve significant reduction in plasma LDL cholesterol levels had recurrent CHD [10]. There is accumulating evidence to support the fact that a large proportion of patients with CHD may be characterized by a constellation of additional metabolic deteriorations, which may each contribute to their disease state.

LDL comprises a heterogeneous spectrum of particles that differ in size, density and composition and the cholesterol concentration within the LDL subclass reflects only one aspect of the particle. There is accumulating evidence to suggest that other characteristics of LDL, particularly particle size and density, may also impact on the risk of CHD. The objective of the present review is to discuss the role of small, dense LDL particles in the etiology of premature CHD. Methodological issues will be briefly reviewed with emphasis on the gradient gel electrophoretic method. The relationship of the small, dense LDL phenotype to the risk of CHD will be critically discussed, with consideration for the concomitant variation in other markers of a disturbed lipoprotein/lipid metabolism. A number of mechanisms whereby small, dense LDL may directly impact on the atherosclerotic process have been proposed and they will be briefly reviewed. Finally, therapeutic aspects and their clinical implications will be discussed.

PHYSICAL CHARACTERIZATION OF LDL PARTICLES

It is beyond the scope of the present paper to provide an extensive review of methods used to characterize LDL particle size, density or composition. These have been the topic of several excellent published reviews [11, 12]. Nevertheless, a number of technical issues must be addressed.

The heterogeneity of LDL particles has been documented more than 30 years ago in studies using analytical ultracentrifugation to characterize lipoprotein flotation rate [13]. The Svedberg flotation (Sf) rates of human LDL (density 1.019-1.063 g/ml) range from 0 to 12. Higher Sf rates describe particles of lower density, larger size, and increased lipid to protein ratio. A number of early studies have reported multiple peaks within the LDL subfractions [13-15] while other reports have revealed that the polydisperse (multiple peak) LDL patterns were more likely to be found in subjects with hypertriglyceridemia or diabetes mellitus than in euglycemic and normotriglyceridemic subjects [16, 17].

Analysis by density – Several preparative ultracentrifugation methods can be used to identify and characterize subfractions along the LDL spectrum. Among others, density gradient ultracentrifugation (DGU) of LDL samples has revealed the existence of up to four discrete LDL bands (LDL-I to LDL-IV) in normal subjects [18]. Each of these bands was shown to have characteristic buoyant densities and flotation rates as determined by analytical ultracentrifugation. The LDL-II subpopulation is generally the most abundant species of LDL particles among healthy normolipidemic subjects, while LDL-I (larger and less dense) and LDL-III (smaller and denser) particles may be found in lesser but varying concentrations [18]. The LDL-IV subfraction is generally hardly detectable, its concentration being frequently confounded by the presence of HDL or Lp(a) [19].

Analysis by size – Polyacrylamide gradient gel electrophoresis (PAGGE) in non-denaturing conditions is a technique that has been used widely to characterize LDL particles according to size [11, 12]. Small amounts of whole plasma or ultracentrifuged LDL are first subjected to electrophoresis on a 2 % to 16 % polyacrylamide gradient gel. The discrete LDL bands can be resolved by staining the gel for lipids or proteins. LDL subspecies are subsequently identified by densitometric scan at the appropriate wavelength and the diameter of each LDL band within an individual is computed based on the migration distance of high molecular weight standards [12]. Up to 7 LDL peaks can be identified by PAGGE but most individuals display only two or three LDL subclasses on the densitometric scan [18, 20]. The determination of LDL particle size by PAGGE can be performed using several approaches. The simplest method is to identify the diameter of the most abundant subspecies of LDL within one individual [21]. This measure has been defined as the LDL peak particle size and has been used widely in epidemiological studies. Another approach consists in computing a mean LDL particle size based on the relative abundance of each of the LDL subclasses within one individual [20, 22]. This latter approach provides a more accurate description of the whole distribution of LDL particle size compared to the information derived from the “peak particle
diameter” method. For example, an individual with two major subclasses of LDL particles having a diameter of 255 Å and 260 Å respectively, each contributing 25% and 75% to the whole LDL distribution, would have a peak particle size of 260 Å and a mean LDL particle size of 258.75 Å. A dichotomous classification of LDL particle size has also been defined based on peak particle size and pattern of distribution of LDL subclasses [23, 24]. Thus, individuals with predominant large LDL particles (diameter > 255 Å) and skewing of the densitometric scan to the right have been characterized as having LDL phenotype A whereas LDL phenotype B has been defined by a predominant of small LDL particles (diameter < 255 Å) and skewing of the densitometric scan to the left. Data suggested that 85-90% of individuals may be characterized by either LDL phenotype A or B, while the remainder may have an intermediate phenotype [23]. Austin et al. have suggested that about 30% of the population could be defined as having the phenotype B whereas 70% of the population could be classified into phenotype A or into the intermediate phenotype [25].

### SMALL, DENSE LDL PARTICLES AND THE RISK OF CHD: EPIDEMIOLOGICAL EVIDENCE

Several cross-sectional studies have confirmed the observation of Fisher et al. [16] who, more than 15 years ago, were the first to suggest that “polysdisperse” LDL may be more prevalent among CHD cases with premature atherosclerosis compared with controls. Subsequent cross-sectional studies have reported that the odds of finding CHD among individuals with small, dense LDL particles was increased by 2-5 folds compared with individuals having larger, more buoyant LDL particles [24, 26-29]. It is interesting to note that despite the use of a variety of CHD end-points and different laboratory measures of LDL heterogeneity (PAGGÈ or density gradient ultracentrifugation), most of the cross-sectional studies consistently reported that small, dense LDL particles were more prevalent among CHD cases than among controls. The majority of these studies were performed in Caucasian men. Only two studies were performed in women but results tend to support the notion that small, dense LDL particles may be a risk factor in both genders [24, 30]. Additional studies are clearly warranted in order to determine whether the magnitude of the association between LDL particle size and the risk of CHD is similar across various ethnic groups and populations.

It must be underscored that studies referenced above were cross-sectional investigations comparing the prevalence of small, dense LDL particles among CHD cases and controls. Three studies using a prospective, nested case-control design have recently examined the relationship between LDL particle size as determined by PAGGÈ and the risk of CHD in men. In the Physicians’ Health Study [31], patients with documented myocardial infarction (MI) over a 7 years follow-up had significantly smaller LDL peak particle diameter than did controls matched for age and smoking (256 ± 9 Å vs. 259 ± 8 Å). Each increase of 8 Å in LDL peak particle size was associated with a significant 38% increase in the 7-year risk of MI after adjustment for age and smoking (95% confidence interval 18%-62%). In the Stanford Five-City Project [32], incident coronary artery disease cases had significantly smaller LDL peak particle size compared with controls matched for age, sex and ethnicity (262 ± 10 Å vs. 267 ± 9 Å). Among all physiologic risk factors, LDL peak particle size was the best predictor of the coronary artery disease status. Finally, in the Quebec Cardiovascular Study [33], the proportion of men with small, dense LDL particles (LDL peak particle size < 256 Å) who develop ischemic heart disease over a 5-year follow-up period was significantly higher than that in controls matched for age, body mass index, cigarette smoking and alcohol consumption (50% vs. 34%). Small, dense LDL was associated with a significant 3.6 fold increase in the risk of ischemic heart disease (95% confidence interval 1.5-8.8) [33].

### ARE SMALL, DENSE LDL PARTICLES ONLY PARTNERS IN CRIME?

**Evidence from cross-sectional studies** – In general, the increased risk of CHD associated with small, dense LDL has been shown to be independent of traditional risk factors such as age, obesity, cigarette smoking, gender and hypertension [24, 33]. Also, LDL particle size or density generally shows no association with plasma LDL cholesterol levels (Fig. 1). Consistent with this observation, multivariate adjustment for variations in plasma LDL cholesterol levels (Fig. 1) is interesting to note that despite the use of a variety of CHD end-points and different laboratory measures of LDL heterogeneity (PAGGÈ or density gradient ultracentrifugation), most of the cross-sectional studies consistently reported that small, dense LDL particles were more prevalent among CHD cases than among controls. The majority of these studies were performed in Caucasian men. Only two studies were performed in women but results tend to support the notion that small, dense LDL particles may be a risk factor in both genders [24, 30]. Additional studies are clearly warranted in order to determine whether the magnitude of the association between LDL particle size and the risk of CHD is similar across various ethnic groups and populations.

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**Evidence from prospective studies** – Results from the recent prospective studies tend to support the notion that the increased CHD risk associated with
small, dense LDL particles could be attributed to some extent to the simultaneous presence of an atherogenic metabolic profile. In the Physicians’ Health Study, LDL particle size was no longer a significant predictor of CHD risk after multivariate adjustment for plasma triglyceride levels [31]. Of note are the facts that only 15% of blood samples in the Physicians’ Health Study were taken following a 12-hour fasting period and that plasma triglyceride concentrations were the best predictor of risk in this cohort. In the Stanford Five-City Project, the baseline difference in LDL peak particle size between cases and controls remained significant after adjustment for concomitant variations in plasma triglyceride and HDL cholesterol levels, but was reduced to insignificance by the statistical adjustment for the total/HDL cholesterol ratio [32]. Blood samples in the Stanford Five-City Project were not taken in the fasting state. Finally, in the Québec Cardiovascular Study, small, dense LDL particles remained a significant predictor of the risk of CHD after control for plasma LDL cholesterol, triglyceride and HDL cholesterol [33]. However, adjustment for apolipoprotein B levels and for the cholesterol/HDL cholesterol ratio substantially attenuated the risk of CHD attributed to the presence of small, dense LDL particles. Results from the Stanford Five-City Project and the Québec Cardiovascular Study suggest that the “prospective” association between LDL particle size and the risk of CHD may be more (yet not completely) dependent on variations in other risk factors than what was initially hypothesized based on results from cross-sectional studies.

**LDL particle size vs. particle number** – It must be emphasized that assessment of LDL particle size by PAGGE does not provide information about the concentration or “number” of small, dense LDL particles. It appears that the concept of LDL “particle number” may be critical in the proper assessment of CHD risk attributable to LDL “particle size”. There has been accumulating evidence to suggest that the number of LDL particles in the circulation (as opposed to their cholesterol content) should be considered as an important factor when assessing the LDL-related risk of CHD [27, 35-37]. Apolipoprotein B is the major protein moiety of LDL particles. Unlike cholesterol, which concentration can vary substantially within one LDL particle, there is systematically only one apolipoprotein B molecule per LDL particle. Thus, assessing apolipoprotein B levels within the LDL subfraction provides a direct measure of LDL particle number in the circulation. Because 80-90% of apolipoprotein B in the circulation is found within the LDL density, it is assumed that plasma apolipoprotein B concentrations are roughly equivalent to LDL apolipoprotein B levels [38]. Results from the Québec Cardiovascular Study have indicated that the increased CHD risk attributed to the presence of small, dense LDL particles was significant only among men with high concentrations of these particles, as reflected by elevated apolipoprotein B concentrations [33]. Indeed, among men with small, dense LDL, those with relatively low apolipoprotein B levels (below the median of plasma apolipoprotein B levels) were not at increased risk for CHD, whereas those with elevated plasma apolipoprotein B concentrations (increased LDL particle number) had a 6-fold increase in the risk of CHD compared with men having both large LDL and reduced plasma apolipoprotein B levels (Fig. 2). These results emphasize the critical importance of obtaining information on LDL particle number in order to adequately assess the risk of CHD associated with the presence of small, dense LDL particles.

**PROPOSED MECHANISMS WHEREBY REDUCED LDL SIZE MAY DIRECTLY CAUSE CHD**

Whether or not small, dense LDL particles should be considered an independent CHD risk factor is a complex question to address because of the close interrelationships among metabolic processes leading to atherosclerosis and CHD. There is, however, evidence suggesting that small, dense LDL particles have potentially atherogenic properties per se. Smaller and denser LDL particles are more susceptible to in vitro oxidation [39-41], a mechanism that may contribute to the formation of foam cells in vivo, thereby enhancing the atherosclerotic process. Small, dense LDL particles have also been shown to be degraded less rapidly than particles of intermediate densities [42]. This process has been attributed, among other factors, to the reduced binding affinity of small, dense LDL particles
to the LDL receptor [42, 43]. Small LDL particles also
display an increased potential for interaction with pro-
eteoglycans of the arterial wall [44-46]. These proces-
ses could contribute to accelerate the formation of the
atherosclerotic plaque and could explain, at least
partly, the relationship between LDL particle size and
density and the risk of CHD risk.

THE SMALL, DENSE LDL
PHENOTYPE: AN INCREASING LIST
OF PARTNERS IN CRIME

This section will review the evidence suggesting
that the small, dense LDL phenotype, beyond its own
atherogenic properties, could also be an additional
marker of an athero-thrombotic profile associated with
hypertriglyceridemia, low HDL cholesterol levels, ins-
ulin resistance, abdominal obesity and other features
of the insulin resistance syndrome (Table 1).

In their first description of the atherogenic lipopro-
tein phenotype, Austin et al. [23] reported that the
presence of the phenotype B was accompanied by
hypertriglyceridemia and by low HDL cholesterol
concentrations. Reaven and colleagues later identified
the small, dense LDL phenotype as a common feature
of the insulin resistance syndrome [47]. The typical
dyslipidemia of the insulin resistance syndrome (high
triglycerides, low HDL cholesterol) is also a feature of
type 2 diabetes mellitus, suggesting a greater likeli-
hood of finding the LDL phenotype B in these patients
[48-50]. There is data to suggest that the diabetic
status per se may contribute to reductions in LDL
particle size in patients with type 2 diabetes [49]. How-
ever, there is a highly significant inverse correla-
tion between fasting triglyceride levels and LDL par-
ticle size and the former is generally the best meta-
bolic correlate of the latter both in non diabetic [22, 51]
and diabetic populations [52].

Abdominal obesity has not been systematically as-
associated with marked elevations in plasma LDL chole-
sterol levels [53, 54]. Our data suggest that the major-
ity of insulin resistant but non-diabetic abdomi-
 nal obese patients with high triglyceride levels, redu-
ced HDL cholesterol concentrations are likely to dis-
play the small, dense LDL phenotype [22]. We have
reported that abdominal obese patients with relatively
“normal” LDL cholesterol levels but with hypertrigly-
 ceridemia and reduced plasma HDL cholesterol
concentration were characterized by a 20-25 % in-
crease in total plasma apolipoprotein B and LDL-
apolipoprotein B levels [55]. These observations em-
phasize the notion that the relatively normal plasma
LDL cholesterol levels frequently observed among
abdominal obese, insulin resistant patients (with or
without type 2 diabetes) could be misleading in the
assessment of LDL particle concentrations since
small, cholesterol-depleted particles may be more
abundant in these individuals. In keeping with results
from numerous previous investigations, fasting trigly-
 ceride concentration was the best correlate of LDL
size in our studies [22]. Variations in abdominal adi-
pose tissue levels were no longer associated with
changes in LDL particle size after control for fasting
triglyceride levels, suggesting that the presence of
small, dense LDL particles in abdominal obese pa-
tients may be largely attributed to the underlying hy-
pertriglyceridemic state [22].

Hypertriglyceridemia from a variety of causes is
associated with an increased exchange of triglycerides

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TABLE I. Metabolic abnormalities commonly found among subjects with small, dense LDL particles.

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<tr>
<th>Abdominal obesity</th>
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<tr>
<td>Hypertriglyceridemia</td>
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<td>Low HDL cholesterol</td>
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<td>Increased cholesterol/HDL chol. ratio</td>
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<td>Normal or marginally elevated LDL cholesterol</td>
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<tr>
<th>Insulin resistance</th>
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<tr>
<td>Hyperinsulinemia</td>
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<tr>
<td>Glucose intolerance and type 2 diabetes</td>
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<td>Elevated fibrinogen and PAI-1 levels</td>
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<tr>
<td>Impaired tolerance to dietary fat</td>
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<td>Altered endothelial reactivity</td>
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Fig. 2. Combined contribution of LDL particle size and particle num-
ber (as estimated by plasma apolipoprotein B concentrations) in deter-
mining the 5-year risk of ischemic heart disease (IHD) in men. Adapted
from [33].
from triglyceride-rich lipoproteins to LDL and HDL particles in exchange of cholesteryl esters through the action of the cholesteryl ester transfer protein (CETP) [56, 57]. This phenomenon results in the generation of VLDL particles enriched in cholesteryl esters and to smaller, cholesteryl ester-depleted LDL and HDL particles. This deteriorated lipoprotein profile is common among abdominal obese, insulin resistant and hypertriglyceridemic patients [58]. The reduced plasma HDL cholesterol concentration noted in these patients has been attributed to a marked reduction in the concentration of large, cholesterol rich HDL2-like particles [53]. Thus, the HDL fraction is generally characterized by a preponderance of small HDL3-like particles.

The reduced plasma HDL cholesterol concentration characterizing hypertriglyceridemic individuals with small, dense LDL particles may also be resulting from altered intravascular lipase activities. We have previously shown that abdominal obesity was associated with a reduced plasma post-heparin lipoprotein lipase activity and with an increased hepatic lipase activity [53, 59, 60] and these metabolic alterations may contribute to the hypertriglyceridemic-low HDL cholesterol dyslipidemia noted among subjects with small, dense LDL particles. We have recently reported that subjects with small, dense LDL particles have a deteriorated tolerance to dietary lipids and are characterized by a higher concentration of small, triglyceride-rich lipoproteins eight hours after an oral fat tolerance test [61]. These alterations in post-prandial metabolism among men with small, dense LDL particles were observed even among normotriglyceridemic subjects, suggesting that LDL particle size may represent an early marker of an impaired capacity to clear dietary fat in apparently normotriglyceridemic individuals. We would like to suggest that although the small, dense LDL phenotype is most frequently found among hypertriglyceridemic individuals, the concentration of small, dense LDL particles may be determined by factors other than plasma triglyceride levels such as insulin resistance, abdominal obesity and unknown genetic factors. Additional studies in this area are clearly warranted.

There is considerable literature suggesting that the metabolic profile found among carriers of the dense LDL phenotype may be quite atherogenic. Indeed, studies have suggested that fasting hyperinsulinemia, as a crude marker of insulin resistance in non-diabetic patients, was associated with an increased risk of CHD, particularly in middle-aged men [62, 63]. Results from the Framingham Heart Study [64, 65], the PROCAM study [66], the Helsinki Heart Study [67, 68] and from the Copenhagen Male Study [69] have all provided support for the notion that the high triglyceride-low HDL cholesterol dyslipidemic state characterizing abdominally obese, insulin resistant individuals with small, dense LDL particles was associated with a substantial increase in CHD risk. As mentioned above, we have reported that elevated apolipoprotein B concentrations were an independent predictor of CHD risk in middle-aged men [70, 71] and that there was a further increase in risk when it was accompanied by small, dense LDL particles [33]. Furthermore, the combination of hyperinsulinemia, elevated apolipoprotein B levels and of the small, dense LDL phenotype was associated with a 20-fold increase in CHD risk [72]. We believe that this cluster of abnormalities (which we refer to as the atherogenic metabolic triad of nontraditional risk factors) may represent a very prevalent and atherogenic combination of metabolic risk factors among CHD patients.

In addition to the factors described above, there are additional alterations that may contribute to the proatherothrombotic profile of subjects with the small, dense LDL phenotype. These complications are also features of the increasing list of components of the insulin resistance syndrome. First, an impaired fibrinolysis and an increased susceptibility to thrombosis have been reported among subjects with small, dense LDL particles and insulin resistance [73]. Plasminogen activator inhibitor-1 levels may be increased among subjects with the small, dense LDL phenotype. As mentioned above, small, dense LDL particles may be associated with an impaired endothelial function and with an increased susceptibility of the plasma to oxidative stress. Whether small, dense LDL particles are themselves involved in these processes or whether they are a marker of additional metabolic aberrations of the insulin resistance syndrome will require further studies. At this stage, however, there is considerable evidence suggesting that small, dense LDL particles are seldom observed as an isolated disorder. Rather, it is most likely that the small, dense LDL phenotype is an important component of a “minestrone soup” of pro-atherothrombotic abnormalities and that smaller and cholesterol-depleted LDL particles exacerbate the risk of CHD when accompanied by other components of the atherogenic dyslipidemia of insulin resistance. For instance, we do not know whether the fairly high prevalence of individuals with small, dense LDL particles found among populations on a low fat intake but on high carbohydrate diets [74] causes prejudice to their cardiovascular health as opposed to affluent populations on a higher fat intake. From the substantial difference in the prevalence of CHD between populations consuming low fat/high complex carbohydrate diet vs. those on high sucrose/high fat diets and with a high rate of obesity, it is hypothesized that small, dense LDL particles, in the absence of elevated LDL particle concentration may not cause major harm to CHD risk. This hypothesis will, however, require rigorous testing.
THERAPEUTIC APPROACHES

The following section describes the effects of various diets, exercise training programs and pharmacological interventions on LDL particle heterogeneity.

Diet

Dreon and colleagues [74] have investigated the lipid and lipoprotein response to reduced dietary fat intake in relation to baseline LDL phenotype in 105 men subjected to a high fat (46%) and a low fat (24%) solid food diet in random order. Reduction in plasma LDL cholesterol concentrations induced by the low-fat diet were two-fold greater in men exhibiting LDL pattern B compared with pattern A subjects and apolipoprotein B levels decreased only in the former group of men. A significant proportion of men (41%) with LDL pattern A changed to LDL pattern B following the low fat diet [75]. These changes could be attributed to a significant shift in LDL particle mass from larger, lipid-enriched LDL-I and LDL-II to smaller, lipid-depleted LDL-III and LDL-IV while there was no change in LDL particle number as reflected by apolipoprotein B concentrations [75]. None of the study participants with LDL pattern B converted to pattern A following the low fat diet. The magnitude of the increase in plasma triglyceride concentrations induced by the low fat diet was greater in men with LDL pattern B compared with LDL pattern A subjects while the decrease in plasma HDL cholesterol was similar between both groups [75]. The magnitude of the LDL cholesterol reduction following the low fat diet appeared to be related to apolipoprotein E phenotype, with greater reductions in levels of large LDL from apolipoprotein E 2/3 to E 3/3 to E 4/3 [76]. Finally, changes in intake of total saturated fatty acids, as well as myristic (14:0) and palmitic (16:0) acids, were positively associated with increases in the mass of large LDL particles, LDL peak particle diameter and flotation rate, but with no change in plasma LDL cholesterol concentration [77]. Taken altogether, these results suggest 1- that LDL phenotype can be significantly altered through nutritional intervention and 2- that baseline LDL phenotype may be an important factor to consider when investigating the diet-induced changes in the lipoprotein-lipid risk profile.

The identification of the most appropriate dietary approach for the treatment of the atherogenic dyslipidemia remains a matter of great controversy. The dietary studies outlined above have been conducted under isocaloric conditions, that is, when caloric intake is imposed to maintain weight stability. There is abundant evidence supporting the fact that low fat/high carbohydrate diets, when consumed ad libitum, are frequently associated with a spontaneous reduction in caloric intake and subsequent weight loss [78]. There is also evidence to suggest that reduction in body weight can have a significant impact on LDL particle size [79, 80]. It has been argued that when individuals are not followed under ad libitum feeding conditions, arguments pertaining to the potentially deleterious effects of low fat/carbohydrate rich diets may be more relevant to the metabolic ward than to the “real world” [81]. Future studies are clearly warranted to investigate and document the effects of low fat diets consumed ad libitum and subsequent body weight reduction on the atherogenic LDL phenotype and their impact on the risk of CHD.

Exercise

Endurance exercise training is known to generally increase HDL cholesterol concentrations and to decrease plasma triglyceride and LDL cholesterol levels [82, 83]. Endurance exercise training may also have beneficial effects on LDL particle size and density. Williams et al. [84] have reported that men engaged in regular endurance exercise for several years had reduced concentration of the smaller, denser LDL particles compared with sedentary individuals. A similar comparative study was conducted in trained and sedentary hypercholesterolemic men [85]. Although the concentration of LDL particles in both groups was similar, trained hypercholesterolemic subjects had lower levels of small, dense LDL particles and had a greater proportion of their LDL as large, light LDL particles compared with sedentary hypercholesterolemic men. A limited number of studies have examined the effects of an exercise training intervention protocol on the LDL subfraction profile. One year of intensive endurance training induced a significant reduction in the concentration of small LDL concentrations and the magnitude of this reduction was closely associated with the degree of weight loss and also with distance run [86]. LDL peak particle diameter has been shown to increase significantly following a one-year exercise training program [80]. Houmard et al. [87] reported that, despite no change in plasma LDL concentration, endurance exercise resulted in favorable changes in the composition of LDL. The increased molecular weight, particle size and total lipid content of LDL particles was associated with exercise training-induced reduction in body fat mass, plasma triglycerides and fasting glucose concentrations [87]. Studies are clearly warranted to document the effects of exercise training on LDL particle distribution in women.

A single exercise session could potentially affect LDL particle size if the associated energy expenditure is large enough. A thirty-km cross-country run has been associated with a significant reduction in plasma triglyceride levels but did not alter LDL concentrations in 13 healthy men [88]. However, changes in the concentration of small, dense LDL particles were correlated with exercise-induced changes in plasma triglyceride levels. Thus, the largest decrease in the
concentration of small LDL particles occurred among subjects in whom the single bout of endurance exercise produced the largest decrease in plasma triglyceride levels [88]. In addition, the triglyceride content of all LDL subfractions (large and small) declined significantly immediately after the 30-km acute bout of exercise [88]. Finally, LDL particle size increased in 21% of the men who participated in an endurance triathlon (2.4-mile swim, 112-mile bicycle ride, 26.2-mile run, in succession) whereas no change occurred in women, even though apolipoprotein B levels decreased significantly in both genders [89]. Participants who’s LDL particle size increased with exercise were those who showed the greatest reduction in plasma triglyceride levels [89].

There is also data to suggest that exercise training combined with dietary intervention may significantly reduce the propensity for LDL particles to oxidation. Parks et al. [90] have reported that a treatment program that combined intensive exercise therapy, stress management and consumption of a diet containing 10% fat significantly reduced the oxidative potential of LDL particle. The principal determinants of LDL oxidative susceptibility were the increased antioxidant content of LDL. Beard et al. [91] examined the effects of low (10% of calories) fat/high (70% of calories) unrefined carbohydrates combined with daily aerobic exercise on LDL particle size and susceptibility to oxidation. They reported a significant increase in LDL particle diameter, which correlated with the parallel decrease in plasma triglyceride concentrations [91]. Initial levels of LDL oxidation fell by 21% while the lag time before copper-induced oxidation increased by 13% [91]. These changes in LDL properties could explain, at least partly, the beneficial effects of dieting and exercise training on the risk of CHD.

Pharmacotherapy

Statins – Statins are potent inhibitors of hydroxymethylglutaryl-coenzyme A (HMG CoA) reductase, the rate-limiting enzyme in hepatic cholesterol synthesis and are the primary drug of choice for the treatment of elevated plasma LDL cholesterol concentrations [92]. Statins also decrease to a certain extent plasma triglyceride concentrations and slightly increase HDL cholesterol levels [93, 94].

Several studies have examined the effects of statins on LDL subclass distribution. The effects of lovastatin were investigated in hypercholesterolemic subjects with severe peripheral vascular disease [95]. No mean change in the LDL density distribution was observed in the treated group but results indicated that the change in plasma triglyceride levels (decrease or increase) determined the qualitative changes in LDL observed during lovastatin treatment [95]. The effects of fluvastatin on the distribution and composition of LDL subspecies was investigated in 26 patients with baseline LDL cholesterol > 4.1 mmol/L [96]. Approximately 40% of treated individuals showed slight and subtle shift in electrophoretic mobility towards larger, less dense LDL particles whereas the other 60% showed either no change or changes towards smaller particles following lipid-lowering therapy with fluvastatin [96]. Simvastatin has been shown to increase LDL particle diameter significantly in patients IIb hyperlipoproteinemia [93]. On the other hand, simvastatin may reduce the plasma concentration of both small and large LDL particles, while having no effect on the mean distribution of LDL particle diameter. Treatment with simvastatin has also been shown to lower the concentration of large LDL particles in hypercholesterolemic patients with no effect on smaller LDL subfractions [97]. Finally, treatment with pravastatin has been shown to favorably alter plasma lipoprotein-lipid levels in patients with familial combined hyperlipidemia without affecting the LDL particle size distribution and composition [98]. A study by Cheung et al. [99] revealed that no major changes in LDL particle diameter were seen in patients with primary hypercholesterolemia following treatment with pravastatin. Taken as a whole, the literature on statins suggests that this class of drugs may reduce CHD risk mainly through their major effects on LDL particle concentration rather than by modifying LDL particle size.

Fibrates – Fibrates have a major impact on triglyceride metabolism. The main effect of fibrates is mediated by the nuclear receptors PPARGs: which then act on responsive elements of genes regulating the metabolism of triglyceride-rich lipoproteins [100].

Treatment of type 2 diabetic patients with gemfibrozil, a fibric-acid derivative, resulted in a significant increase in LDL particle size and decrease in density [101]. Patients in whom gemfibrozil induced an increase in LDL peak particle diameter also showed reductions in plasma triglycerides [101]. Similar results were observed in hypertriglyceridemic men following treatment with gemfibrozil [102]. The significant impact of lipid-lowering therapy with gemfibrozil in hypercholesterolemic individuals on LDL subclass distribution [103] and lack thereof [102] has been attributed to initial plasma triglyceride concentrations, the greatest impact being noted among individuals with elevated baseline triglyceride levels. Ciprofibrate in patients with combined hyperlipidemia has been shown to produce marked reductions in plasma triglycerides and apolipoprotein B concentrations and to normalize LDL particle diameter mainly by increasing the diameter of the smaller and denser LDL subspecies since no change in the diameter of larger subclasses were observed [104, 105]. The susceptibility of small, dense LDL particles to in vitro oxidation was also reduced significantly after treatment with clofibrate in hypertriglyceridemic patients [40]. Bezafibrate treatment in hyperlipoproteinemic patients had no effect on the cholesterol and triglyceride content of
large buoyant LDL subclasses while these two parameters were significantly reduced in small, dense LDL subclasses [106]. LDL diameter has also been shown to increase significantly in hypertriglyceridemic patients following bezafibrate therapy, along with significant reductions in total plasma cholesterol and triglyceride concentrations and elevations in plasma HDL cholesterol levels [107]. Finally, the dense LDL subfraction pattern was replaced by a light LDL subfraction pattern following a 2-month treatment with clofibrate in moderately hypertriglyceridemic subjects [40].

In summary, fibrates appear to have a powerful impact on modifying LDL subclass distribution. These agents represent the most potent drug currently available to reduce plasma triglyceride levels, which may explain to a large extent their significant impact on LDL particle size and density.

Other classes of hypolipidemic drugs – Fibrates and statins are currently the most widely used lipid-lowering agents. Other classes of lipid-lowering drugs have been used in the past and this section will briefly summarize their effects on LDL heterogeneity.

Probucol is an antioxidant and a potent hypocholesterolemic agent [108, 109]. Treatment with probucol generally yields significant reductions in plasma cholesterol and HDL cholesterol levels with marginal effects on plasma triglyceride concentrations [108-111]. Probucol treatment in hypercholesterolemic subjects led to a significant reduction in the cholesterol and apolipoprotein B content of large LDL subclasses with no effect in smaller and denser subpopulations [112]. Colestipol, a bile acid sequestering resin, lowers plasma LDL cholesterol levels by induction of the hepatic LDL receptor activity [113]. Colestipol treatment has been shown to produce a disproportionate decrease in the cholesterol content of LDL compared with LDL apolipoprotein B levels, leading to a substantial reduction in the LDL cholesterol/LDL apolipoprotein B ratio as well as in LDL particle size and to a specific decrease in the proportion of large, more buoyant LDL particles [113]. Cholestyramine, an another ion exchange resin, has been shown to decrease total LDL mass in normolipidemic subjects by reducing selectively the larger, less dense LDL subfraction [114]. Nicotinic acid and its analogue acipimox lower triglyceride and LDL cholesterol levels mainly by inhibiting the mobilization of fatty acids from adipose tissue, thus suppressing the hepatic synthesis of VLDL [115]. Results suggest that treatment with nicotinic acid produced a more significant increase in LDL particle size in individuals with LDL pattern B compared with LDL pattern A individuals [46]. Moreover, all patients with pattern B in whom nicotinic acid reduced plasma triglyceride concentrations below 1.58 mmol/L converted to pattern A [46]. Franceschini et al. [116] reported that LDL particles in type IV hyperlipidemic men were larger and more buoyant following treatment with acipimox. This elevation in LDL particle size could be attributed to the 25% increase in the cholesteryl ester content of LDL and the 46% reduction of triglycerides within the LDL fraction.

In summary, any dietary treatment, exercise training program or pharmacological intervention affecting plasma triglyceride concentrations is most likely to have an effect on LDL particle size or density. Thus, the most potent strategies aiming at reducing plasma triglyceride levels may produce significant clinical benefits not only on plasma HDL cholesterol concentrations but also on the size and possibly concentrations of LDL particles.

### CLINICAL CONSIDERATIONS

Should the measurement of LDL particle size (or density) be implemented in the current clinical practice in an attempt to refine the assessment of CHD risk? Although there is now sufficient information to recognize that small, dense LDL particles play a primary role in the etiology of CHD, additional unresolved issues challenge the use of LDL particle size or density on a clinical, population basis. First, larger, population-based studies will have to document the "true" independent impact of small, dense LDL particles on the risk of CHD since the data available to date is based on cross-sectional reports and nested, case-control prospective studies. Another important remaining issue pertains to the approach that should be used to optimally quantify LDL heterogeneity. Should LDL particle size as a risk factor be dichotomized into two patterns (A or B) or more simply into large or small using an arbitrary, clinically relevant cut-point? Should it rather be used as a continuous variable (like, for example, cholesterol)? Should the heterogeneity of LDL particles be described on the basis of density rather than size? These questions will have to be examined in future studies. The method ultimately selected to characterize LDL particles will have to display a favorable cost-effectiveness ratio and be fairly simple to be applied on a large-scale, population basis. In that regard, all methods that have been described and used so far to characterize LDL heterogeneity are time-consuming, tedious and not likely to be used in a clinical context. Based on all of the above considerations, it is obvious that the measurement of LDL particle size or density will not be included as part of the lipoprotein/lipid profile currently used in clinics to assess the risk of CHD. Another aspect that we believe deserves greater scrutiny in future studies is the combined importance of LDL particle size (or density) vs. particle number. Combining a measure of LDL particle number may indeed prove to be the most...
critical approach to optimize the interpretation of LDL particle size regarding its use in the estimation of CHD risk.

**CONCLUSIONS**

Although the measurement of plasma LDL cholesterol concentrations is highly relevant in the estimation of CHD risk, this disease has a complex multifactorial etiology and a cluster of atherogenic alterations may exacerbate the patients' risk. Small, dense LDL particles are most frequently part of a complex pluri-metabolic syndrome, which may represent the most prevalent cause of CHD in affluent populations. The legitimacy of using monotherapy aimed at reducing plasma LDL cholesterol levels and the extent to which this approach represents the optimal pharmacological treatment of this common atherogenic dyslipidemia is not known [10]. Answering this question will represent a considerable challenge, but additional major developments in this area may represent significant leaps in preventive cardiology.

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