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Dense LDL and Coronary Artery Disease

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Running head: Dense LDL and coronary artery disease

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Abstract

A common, genetically-influenced subclass profile characterized by a predominance of small, dense LDL particles is associated with relative increases in plasma triglyceride and apoB-100, and reduced levels of HDL-cholesterol and apoAI. Recently, this phenotype has also been associated with features of the insulin resistance syndrome as well as familial combined hyperlipidemia. Case-control studies of patients with myocardial infarction and angiographically documented coronary artery disease (CAD) have demonstrated that up to 40-50% of patients have the small, dense LDL phenotype, and that this is associated with a 2-3 fold increase in disease risk. However, because of strong statistical interrelationships among the multiple features of this phenotype, it has been difficult to determine whether one or more of its metabolic alterations are primarily responsible for increased CAD susceptibility. More direct evidence for enhanced atherogenicity of lipoproteins in this trait derives from a recent report that LDL cholesterol lowering by diet and drug treatment resulted in reduced coronary angiographic progression in CAD subjects with predominantly dense LDL, but that an equivalent lowering of LDL cholesterol in subjects with more buoyant LDL was associated with no angiographic benefit. Furthermore, *in vitro* findings have indicated increased susceptibility of small, dense LDL to oxidative modification, and relatively greater binding of these particles to arterial wall proteoglycans. Thus, the small, dense LDL trait may underlie familial predisposition to coronary artery disease in a large proportion of the population, and its presence may indicate the potential for benefit from specific therapeutic interventions.

Key words: Low density lipoproteins, coronary artery disease, atherosclerosis, insulin resistance, oxidation.

LDL subclasses and atherogenic lipoprotein phenotype

LDL comprise multiple distinct subclasses with differing physical, chemical, and metabolic properties [1,2]. The density, size, and ultracentrifugal flotation rates of the major LDL subclasses have been defined in our laboratory using density gradient ultracentrifugation, pore-gradient gel electrophoresis, and analytical ultracentrifugation, respectively [1,2]. The plasma distribution of these subclasses varies among age and sex categories, and also in relation to levels of other lipoproteins [3,4]. Levels of the largest and most buoyant LDL (LDL-I) are higher in women than in men, are reciprocally correlated with levels of smaller, more dense LDL-III, and are positively correlated with levels of HDL, specifically, HDL_2 [3]. Size and buoyancy of the major LDL species are also strongly inversely correlated with levels of VLDL [3] and plasma triglyceride [4]. Additionally, it has been shown recently that peak LDL particle diameter is strongly inversely related to plasma insulin and features of the insulin resistance syndrome, including elevated blood pressure [5].

Earlier studies indicated that in most normolipidemic individuals, there is a predominance of larger and more buoyant LDL particles (LDL-I or LDL-II) but some subjects have predominantly smaller, more dense LDL (specifically LDL-III) [6]. Generally, the smallest and most dense LDL particles (LDL-IV) are found in relatively low concentrations in plasma, except in patients with severe hypertriglyceridemia. The profile characterized by a predominance of LDL-III was originally identified on the basis of LDL subclass patterns discerned by gradient gel electrophoresis, and has been designated LDL subclass pattern B [7,8]. However, the subgroup of individuals with pattern B is also identifiable as a discrete mode in the continuous distribution of LDL peak particle size as determined by gradient gel electrophoresis [9], and an even more clearly defined

mode in the distribution of LDL peak density as measured by analytical ultracentrifugation (Figure 1).

Population studies of LDL subclass profiles have generally utilized gradient gel electrophoresis [8,10,11] which is considerably more amenable to large scale application than is ultracentrifugal methodology, and can clearly assign patterns in 80-85% of subjects (the remainder have intermediate or mixed patterns) [7,8]. Based on studies in largely Caucasian American populations, the prevalence of pattern B is approximately 10-15% in males younger than age 20 and in premenopausal females, and increases to 30-35% in adult men and 25-30% in postmenopausal women [8,10,11]. An earlier study had indicated that the prevalence in postmenopausal women was greater [8], but the population studied was enriched in pattern B families.

In accord with the metabolic relationships described above, individuals with subclass pattern B have been shown to have higher levels of triglyceride-rich lipoproteins and remnants, reduced levels of HDL_2 [8], and a greater degree of resistance to insulin-stimulated glucose uptake than subjects with predominantly larger LDL (pattern A)[5]. Levels of plasma and LDL apoB are relatively higher in pattern B than pattern A subjects, indicative of increased numbers of LDL particles, but total LDL-cholesterol is minimally if at all higher [8], consistent with the shift toward denser, more lipid-depleted LDL particles.

The evidence that individuals with small, dense LDL have a number of interrelated metabolic features associated with increased risk of coronary artery disease led to the designation of pattern B as an atherogenic lipoprotein phenotype (ALP) [8]. Case-control studies of subjects with acute myocardial infarction [7] and angiographically documented coronary artery disease [12;13] have shown up to a three-fold increase in risk associated with a predominance of small, dense LDL. However, the high degree of intercorrelation among the metabolic features

of ALP have made it difficult to determine the extent to which individual components of the syndrome contribute to disease risk. While in all studies to date inclusion of plasma triglyceride levels in regression models has eliminated the significance of the association of LDL particle size with coronary risk, the strength of the relationship between LDL size and triglyceride level makes it impossible to draw causal inferences from these analyses.

More direct evidence for enhanced atherogenic properties of lipoproteins in subjects with subclass pattern B was recently obtained from analyses performed in conjunction with the Stanford Coronary Risk Intervention Program (SCRIP), a four-year randomized multifactorial risk reduction trial carried out in 300 patients with angiographically documented coronary artery disease [14]. Specific therapeutic regimens varied among patients in this study, but most received one of several hypolipidemic drugs in conjunction with an AHA Step 2 diet. Despite similar extent of disease at baseline, and comparable diet- and drug-induced reductions in levels of total LDL cholesterol, patients with subclass pattern B, but not those with pattern A, demonstrated reduced progression of atherosclerosis as determined by quantitative coronary angiography [15]. This differential benefit was associated with greater reductions in levels of both plasma triglyceride and LDL-III in pattern B subjects, although it was not possible to determine whether one or both of these changes were directly related to the outcome. It is also of interest that substantial therapeutic reductions in levels of LDL-I and LDL-II in patients with pattern A were not associated with reduced angiographic progression when compared with subjects in the control group who did not receive risk reduction therapy.

In vitro studies have provided further information suggesting that LDL subclasses differ in properties which may affect the development or progression of atherosclerosis. Several reports have documented that susceptibility to copper-

induced oxidation, as assessed by lag time prior to initiation of the propagation phase of free radical generation, is greater for small, dense LDL (LDL-III) than for larger, more buoyant LDL particles (cf. [16]). Another property of small, dense LDL that might be of importance in atherogenesis is a greater affinity for arterial wall proteoglycans. There is evidence that sialic acid declines as a function of increasing density and decreasing size of LDL particles [17], that reduced LDL sialic acid is found more commonly in patients with coronary disease than in healthy subjects [18], and that reduced LDL sialic acid results in increased binding to arterial proteoglycans [19]. If such increased binding occurs *in vivo*, it would be expected to increase residence time and hence the likelihood of oxidative modification of LDL in the arterial wall. This might be further enhanced by an increased oxidative susceptibility of the bound LDL [20].

Genetic influences on LDL subclass patterns

Complex segregation analyses in healthy families [21], and in families of probands with familial combined hyperlipidemia [22], have indicated that LDL subclass pattern B, as identified by gradient gel electrophoresis, is under the influence of a major gene or genes, each with transmission consistent with autosomal dominant inheritance, and an additional polygenic or additive component. More recent studies, in which the small, dense LDL phenotype was assessed by density gradient ultracentrifugation [23] and by measurement of LDL particle size as a continuous parameter [24], have confirmed a major gene effect. The frequency of the allele(s) responsible for pattern B in these studies, which involved four different study populations, ranged from 0.1 to 0.3, consistent with the prevalence of the trait as assessed by population studies.

There is also evidence that the pattern B phenotype is a feature of, and may predispose to the development of, familial combined hyperlipidemia, one of the most prevalent monogenic disorders associated with increased risk of premature

coronary artery disease. In a study of 231 subjects in seven large kindreds with familial combined hyperlipidemia, there was evidence for a major gene underlying LDL subclass pattern B with a similar mode of inheritance as described previously in other populations [22]. Moreover, subjects with this trait accounted for the majority of individuals with elevated levels of plasma apoB-100, a hallmark of this form of hyperlipidemia. Of particular note is that the distribution of apoB levels in pattern B, but not pattern A subjects, was bimodal [25], raising the possibility of a second major gene responsible for apoB elevations that is fully expressed only in the genetic background of pattern B.

Other studies have focused on identifying the genetic loci underlying the pattern B phenotype by determining linkage of LDL subclass patterns and particle size to candidate genes. Significant linkage was detected to markers on chromosome 19p13.3, with the highest LOD score (an indication of the likelihood of linkage) at the LDL receptor gene locus [26]. The gene mapped to this locus has been designated ATHS, for atherosclerosis susceptibility (lipoprotein-related). Two studies have been carried out recently to test for linkage of pattern B to the LDL receptor gene in other populations. There was no evidence for linkage in a group of families of probands with familial combined hyperlipidemia, but most of this effect was due to non-linkage in one large kindred [27]. More recently, using quantitative sib pair analysis, Drs. Jerome Rotter, Aldons Lusis, and colleagues, in collaboration with our laboratory, have confirmed a significant linkage (p=0.007) of LDL particle size, as a continuous variable, with the LDL receptor gene among 264 members of 24 kindreds ascertained by a proband with coronary artery disease [28]. In this same group of families, linkage of LDL size was also demonstrated to three other loci: the apoAI/CIII/AIV gene cluster on chromosome 11 (p=0.004), the manganese superoxide dismutase (MnSOD) gene on chromosome 6 (p=0.002),

and the cholesteryl ester transfer protein (CETP) gene on chromosome 16 (p=0.0001) [28].

These findings have led to the hypothesis that several different genetic loci underlie the expression of the small, dense LDL phenotype, that these genes cumulatively account for the prevalence of the trait in the general population, and that in any given family one or more of the loci are responsible for the major gene and additive effects identified by complex segregation analyses. Moreover, the results suggest that different genetically-determined metabolic mechanisms may give rise to ALP, and that these differences, as well as gene-gene interactions, may result in variability of metabolic and pathologic manifestations among affected individuals.

Studies in twins have afforded another approach to examining genetic as well as non-genetic influences on the LDL particle distribution. Heritability of LDL particle size, as assessed by relative concordance in monozygotic vs. dizygotic twins, has indicated that genetic factors account for approximately 40-50% of the variation in LDL particle size in both men [29] and women [10], with the remainder due to non-genetic or environmental influences. A number of such influences have been identified, including abdominal adiposity [30] presence of diabetes mellitus [31-33], and use of progestin-containing oral contraceptives [23].

In addition, recent studies in our laboratory [34] have indicated that low-fat high-carbohydrate diets are capable of inducing expression of pattern B in susceptible individuals. These studies additionally showed that in subjects with LDL subclass pattern B on a high fat diet, a reduced-fat, high-carbohydrate diet does not lead to conversion to pattern A. However the diet-induced reductions in LDL cholesterol and apoB in the pattern B subjects are substantially greater than subjects with pattern A, suggesting a greater relative improvement in

coronary disease risk. These findings raise the possibility that the genetic and metabolic factors underlying the pattern B trait may lead to differential responsiveness to other hypolipidemic therapies aimed at reducing coronary disease risk.

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This work was supported by the National Institutes of Health Program Project Grant HL 18574 from the National Heart, Lung, and Blood Institute, a grant from the National Dairy Promotion and Research Board administered in cooperation with the National Dairy Council, and was conducted at the Lawrence Berkeley Laboratory through the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

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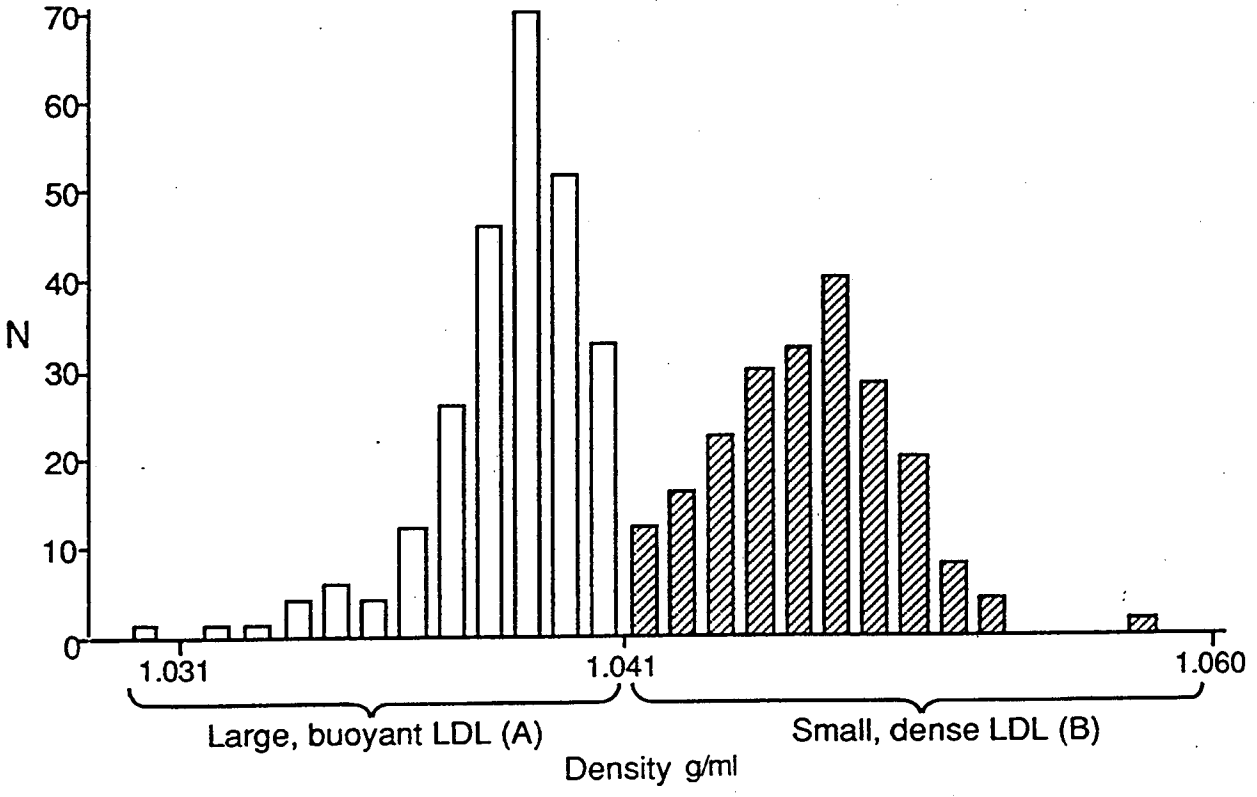
Figure Legend

Distribution of peak LDL buoyant density in 460 men with coronary artery disease. Measurements were carried out by analytical ultracentrifugation with adjustment of calculated densities to those based on preparative ultracentrifugation [Lindgren, 1972 #2216]. Samples were obtained at baseline in 252 men who participated in the Stanford Coronary Artery Intervention Project [SCRIP, 14] and 208 participants in the Monitored Atherosclerosis Regression Study (MARS, samples kindly provided by Dr. David Blankenhorn).


70
60
50
40
N
30
20
10
0
1.031
1.041
1.060
Large, buoyant LDL (A)
Small, dense LDL (B)
Density g/ml

Dense LDL and Coronary Artery Disease

Ronald M. Krauss

Miniabstract

A common, genetically-influenced subclass profile characterized by a predominance of small, dense LDL particles is associated with relative increases in plasma triglyceride and apoB-100, and reductions of HDL-cholesterol and apoAI. Recently, this phenotype also has been associated with the insulin resistance syndrome as well as familial combined hyperlipidemia. Case-control studies of patients with acute myocardial infarction and angiographically defined coronary artery disease have demonstrated increased risk in subjects with this trait. However, because of strong interrelationships among the metabolic characteristics of this trait, it has been difficult to determine whether one or more of its features are primarily responsible for coronary disease susceptibility. Results of a recent coronary disease intervention trial, together with *in vitro* evidence that small, dense LDL have both increased oxidative susceptibility and affinity for arterial wall proteoglycans, have supported the hypothesis that these lipoproteins, and possibly their metabolic precursors, have enhanced atherogenic properties.

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