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DIETARY AND GENETIC EFFECTS ON LOW-DENSITY LIPOPROTEIN HETEROGENEITY

Ronald M Krauss
Lawrence Berkeley National Laboratory, Department of Molecular Medicine, University of California, Berkeley, California 94720; e-mail: Rmkrauss@lbl.gov

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■ **Abstract** We have tested whether differences in distribution and dietary responsiveness of low-density lipoprotein (LDL) subclasses contribute to the variability in the magnitude of LDL-cholesterol reduction induced by diets low in total and saturated fat and high in carbohydrate. Our studies have focused on a common, genetically influenced metabolic profile, characterized by a predominance of small, dense LDL particles (subclass pattern B), that is associated with a two- to threefold increase in risk for coronary artery disease. We have found that healthy normolipidemic individuals with this trait show a greater reduction in LDL cholesterol and particle number in response to low-fat, high-carbohydrate diets than do unaffected individuals (subclass pattern A). Moreover, such diets result in reduced LDL particle size, with induction of pattern B in a substantial proportion of pattern A men. Recent studies have indicated that this response is under genetic influence. Future identification of the specific genes involved may lead to improved targeting of dietary therapies aimed at reducing cardiovascular disease risk.

CONTENTS

INTRODUCTION 283
LDL HETEROGENEITY 284
SMALL, DENSE LDL SUBCLASS PHENOTYPE 285
LDL HETEROGENEITY AND RISK OF CORONARY ARTERY DISEASE 286
DIETARY EFFECTS ON LDL SUBCLASSES 287
CONCLUSION 290

INTRODUCTION

Reduction of LDL cholesterol by limitation of saturated fat intake is one of the keystones of dietary regimens aimed at reducing risk for coronary heart disease (43, 45). In conjunction with this goal, current dietary guidelines also advocate limitation of total fat intake, with substitution of carbohydrate. The principal

mechanism by which reduced saturated fat intake can lower LDL cholesterol is via increased LDL receptor activity, which in turn leads to increased hepatic clearance and excretion of cholesterol (63). However, the metabolic effects of low-fat, high-carbohydrate diets are complex and may include increased triglycerides and very-low-density lipoproteins (VLDLs), reduced high-density lipoproteins (HDLs), and increased insulin (23), a constellation of factors associated with increased atherosclerosis risk (57). Moreover, because VLDLs are the metabolic precursors of LDLs, increased LDL production from VLDL may attenuate the reductions of LDL cholesterol achieved by saturated fat restriction.

Another factor to be considered in population-wide dietary approaches to coronary risk reduction is the considerable interindividual variability that has been demonstrated in the lipoprotein response to modification of dietary fat intake (14, 61). A portion of this variability has been attributed to genetic factors, but only a small number of specific genes have been reported to be related to lipoprotein responsiveness to dietary fat change (31, 55). Among common genetic variants, the strongest effects on plasma LDL cholesterol, and to a lesser extent on LDL response to dietary fat and cholesterol change, have been observed for the E4 variant of the apolipoprotein (apo) E gene, found in approximately 14% of the population (27, 31, 55, 60, 67).

LDL is known to be heterogeneous, comprising a number of distinct subpopulations (41). This review describes recent evidence that improved understanding of dietary and genetic influences on LDL may result from consideration of the macromolecular heterogeneity of this class of lipoproteins.

LDL HETEROGENEITY

LDL subpopulations have been defined on the basis of a number of characteristics, including particle density, size, charge, and chemical composition (41). The distribution of mass among LDL subclasses in plasma is reflected by the particle diameter and buoyant density of the predominant LDL species. Particle diameter is most often assessed by nondenaturing gradient gel electrophoresis, which can identify as many as seven distinct electrophoretic components based on variations in particle size and shape. Density gradient and analytical ultracentrifugation are used to assess LDL buoyant density and flotation rate, respectively (41). Plasma triglyceride and VLDL levels are strongly correlated with increasing density and decreasing size of the predominant LDL species (46, 47). These LDL characteristics in turn are inversely related to levels of plasma HDL, particularly the HDL_2 subclass species (46, 47).

Although there is not yet a detailed understanding of the metabolic bases for these relationships, evidence indicates that heparin-releasable lipase activities may be intimately involved. Postheparin plasma lipoprotein lipase activity is associated with levels of both larger LDL (40) and HDL (39), and this may be due, at least in part, to transfer of surface lipids and apos in the course of chylomicron and

VLDL triglyceride hydrolysis. In a group of 43 healthy normolipidemic men, we found significant inverse relationships of postheparin lipoprotein lipase activity with plasma levels of triglyceride, apoB, large VLDL mass, and small, dense LDL (LDL3) (18).

Hepatic lipase, an enzyme that catalyzes degradation of HDL lipids, is also involved in the clearance of intermediate-density lipoproteins and the production of small, dense LDL from more buoyant precursors (56). Heparin-released hepatic lipase activity has been correlated with levels of LDL3 (18), and absence of small, dense LDL has been reported in humans (12) and mice (56) with genetic deficiency of hepatic lipase.

SMALL, DENSE LDL SUBCLASS PHENOTYPE

A distinct LDL subclass pattern characterized by a predominance of small, dense LDL particles (principally LDL3) has been identified using both nondenaturing gradient gel electrophoresis (7, 41) and analytic ultracentrifugation (41, 52). The prevalence of this trait, which has been designated LDL subclass pattern B, is 30%–35% in adult men, but it is much lower in males <20 years of age and in premenopausal women (5%–10%) (7) and is intermediate (15%–25%) in postmenopausal women (17, 62). Evidence from several studies for major gene determinants of this phenotype (8) has been summarized previously (2). More recently, two additional studies using complex segregation analysis have confirmed major gene effects on LDL diameter as a quantitative trait (6, 16). The data have been most consistent with an autosomal dominant or codominant model for inheritance of the pattern B phenotype with varying additive and polygenic effects. Linkage of pattern B to the region of the LDL receptor gene locus on chromosome 19p (54) has been confirmed using quantitative sibling-pair linkage analysis of LDL particle size in 25 kindreds ascertained on the basis of two affected members with coronary artery disease (59). In these families, preliminary evidence was also obtained for linkage to regions near three other genetic loci: the apoAI/CIII/AIV cluster on chromosome 11, the CETP locus on chromosome 16, and the manganese superoxide dismutase gene on chromosome 6. Recent analyses, however, have failed to demonstrate linkage to these candidate loci in hyperlipidemic kindreds (9). Finally, in a group of dizogytic female twins, there was significant linkage of peak LDL particle size to the apoB gene locus (10), although earlier studies had indicated significant nonlinkage of LDL subclass B to this locus in healthy families (48) and in families with familial combined hyperlipidemia (11). Taken together, these studies suggest that multiple genes may contribute to determination of particle size of the major LDL subclass in plasma, and that the responsible genetic mechanisms may differ among affected families.

Estimates of heritability of LDL particle size based on twin studies have ranged from approximately 30% to 50% (2), indicating the importance of nongenetic and environmental influences. In view of the close relationship between change in

plasma triglyceride levels and change in LDL particle size (47, 51), and in view of the metabolic relationships described above, it is likely that both genetic and non-genetic determinants of pattern B involve coordinate effects on plasma triglyceride and LDL subclass metabolism. In addition to effects from age and gender, LDL particle size and density distribution have been shown to be affected by abdominal adiposity (66), oral contraceptive use (25), and the hypertriglyceridemia of AIDS (33). As described below, variation in dietary fat and carbohydrate can also strongly influence expression of the small, dense LDL phenotype and can contribute to the variations in LDL particle size distribution that are observed among individuals and population groups.

Another important metabolic correlate of the pattern B lipoprotein profile is insulin resistance, manifest as relative elevations in fasting and postglucose insulin levels (58, 62), fasting glucose levels (58), and increased steady state plasma glucose levels (58). The metabolic syndrome associated with insulin resistance has been shown to include raised triglyceride and reduced HDL levels, and the relationships of these variables to LDL particle size appear to account for correlations of smaller LDL diameter with parameters of insulin resistance (58). Insulin resistance has also been related to a tendency toward increased blood pressure, which has also been demonstrated in pattern B subjects with this syndrome (58, 62). A high prevalence of the small, dense LDL trait has been demonstrated in patients with type 2 diabetes mellitus (13, 32), and it is likely that this reflects, in large measure, the insulin resistance found in the majority of patients with this disease. A relative increase in LDL3 has also been reported in well-controlled patients with insulin-dependent diabetes mellitus in conjunction with increased levels of intermediate density lipoproteins (IDL) (50).

Thus, the occurrence of the small, dense LDL phenotype may result from interaction of multiple genetic and environmental determinants, and the trait can be viewed as a marker for the mechanisms underlying these effects. In particular, the prevalence of pattern B characteristically denotes triglyceride levels greater than 140–160 mg/dl and is uncommonly found in association with triglyceride levels below 100–110 mg/dl (7, 52). Candidate mechanisms thus include those that result in overproduction and/or impaired clearance of triglyceride-rich lipoproteins.

LDL HETEROGENEITY AND RISK OF CORONARY ARTERY DISEASE

The plasma lipoprotein profile accompanying a predominance of small, dense LDL particles (specifically LDL3) is associated with a two- to threefold increased risk of coronary artery disease. This has been demonstrated in case-control studies of myocardial infarction (3), and of angiographically documented coronary disease (19, 22, 24). More recently, nested case-control analyses in prospective studies of three population cohorts have all demonstrated that reduced LDL particle size at baseline was a significant predictor for the development of coronary heart disease (34, 49, 64). In most, but not all, studies to date, the disease risk associated

with small, dense LDL was no longer significant after adjusting for triglyceride (3, 22, 24, 64) or other risk factors (19, 34). The high degree of intercorrelation between LDL size, triglyceride, and HDL cholesterol, and the resulting multiple colinearity, confound efforts to determine the extent to which small, dense LDL is independently related to coronary disease risk. In this regard, it is likely that multiple features of the phenotype contribute to atherosclerosis and its cardiovascular manifestations. However, enhanced atherogenicity of smaller vs larger LDL is suggested by evidence of that smaller LDLs are taken up less readily by LDL receptors (53), penetrate more readily into arterial tissue (15), bind more tightly to arterial proteoglycans (1), and are oxidized more rapidly than larger LDL particles (21).

Further evidence for clinically important atherogenicity of small, dense LDL derives from analyses of the effects of lipid-altering therapy on progression of coronary artery disease, as assessed by quantitative coronary angiography. In one study involving a multiple risk factor intervention protocol including lipid-lowering drugs, reduced coronary angiographic progression with treatment was observed only in the subgroup of subjects with a predominance of small, dense LDL (45% of the total) but not in those with more buoyant LDL, despite comparable LDL cholesterol lowering in the two groups (52). More recently, in subjects with a familial history of atherosclerosis and elevated plasma apoB levels, change in coronary artery stenosis with lipid-lowering drugs was found to be most strongly related to change in LDL density, independent of changes in other risk factors (70).

It is noteworthy that a predominance of small, dense LDL is commonly found in conjunction with familial disorders of lipoprotein metabolism that are associated with increased risk of premature coronary artery disease. These include familial combined hyperlipidemia (4, 5, 36–38), as well as hyperapobetalipoproteinemia (65) and hypoalphalipoproteinemia (35). There is evidence that the inheritance of familial combined hyperlipidemia involves at least two major gene loci: one responsible for increased plasma apoB levels and one for LDL subclass pattern B (38). Such interactions of the genes underlying pattern B with other genes or environmental factors, such as diet, may contribute to related dyslipidemic syndromes, and it is likely that a portion of coronary disease risk in these syndromes is mediated by the metabolic changes responsible for the small, dense LDL phenotype.

DIETARY EFFECTS ON LDL SUBCLASSES

Cross-sectional population analyses have suggested an association of reduced LDL particle size with relatively reduced dietary animal fat intake, and increased consumption of carbohydrates (20). Dietary effects on levels of LDL were evaluated directly in a study of 105 healthy, middle-aged men who consumed a high-fat diet (46% fat, 34% carbohydrate) for 6 weeks and a low-fat diet (24% fat, 56% carbohydrate) for 6 weeks in a randomized crossover design (26, 44). Saturated and polyunsaturated fat diets were reduced in parallel to achieve a constant polyunsaturated/saturated ratio (0.7), and intake of monounsaturated fat, cholesterol, protein, and dietary fiber were similar on the two diets.

Men with a predominance of small, dense LDL (pattern B) on the high-fat diet ($n = 18$) exhibited a twofold greater reduction in LDL cholesterol than did pattern A men. This was associated with significantly greater reductions in mass of midsized (LDL2) and small (LDL3) LDL subfractions measured by analytic ultracentrifugation. Furthermore, only pattern B subjects showed significant reductions in plasma apoB, and in LDL relative to HDL cholesterol levels. Of the 87 men with pattern A on the high-fat diet, 36 converted to pattern B on the low-fat diet. In these men, there was a shift in LDL particle mass from larger, lipid-enriched (LDL1 and -2) to smaller, lipid-depleted (LDL3 and -4) subfractions, which suggests a change in LDL composition with minimal change in particle number, and which is consistent with the observation of reduced plasma LDL cholesterol without reduced apoB (44). The group differences in LDL and apoB response could not be attributed to differences in plasma lipid levels, body mass index (which was held constant), or apoE phenotypes. Taken together, these results indicate that in the majority of healthy normolipidemic men, the reduction in LDL cholesterol seen on a low-fat, high-carbohydrate diet is due in large measure to a shift from larger, more cholesterol-enriched LDL to smaller, cholesterol-depleted LDL, whereas much greater reductions in LDL cholesterol and a reduction in the number of smaller LDL particles are achieved in individuals with a predominance of small, dense LDL on a high-fat diet (Figure 1). It should be noted that these studies were performed using isocaloric diets designed to maintain stable body weight. Because weight loss has been shown to result in reduction of small dense LDL and a shift to larger, more buoyant particles, this could attenuate the effects of low-fat, high-carbohydrate diets described here (68, 69).

Recent studies have been extended to examine the effects on LDL subclass phenotypes of further restriction in dietary fat to levels as low as 10% of calories

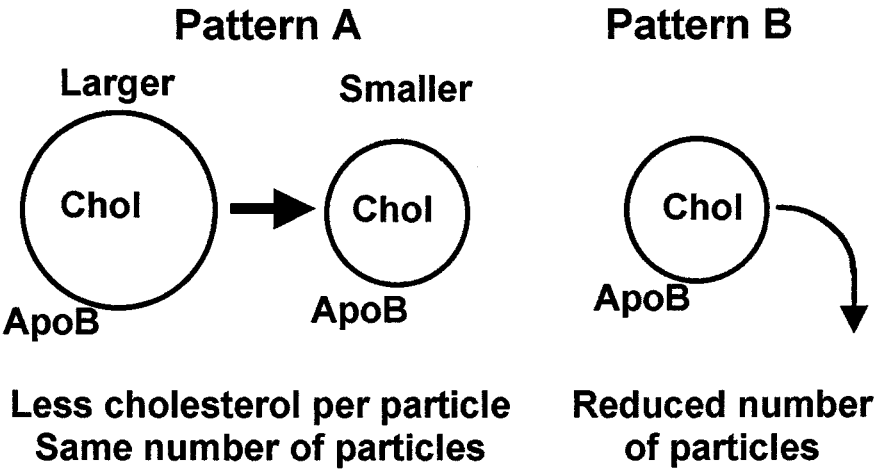

Figure 1 Different mechanisms underlying reductions in low-density lipoprotein (LDL) cholesterol (chol) in men with LDL subclass patterns A and B. Apo, apolipoprotein.

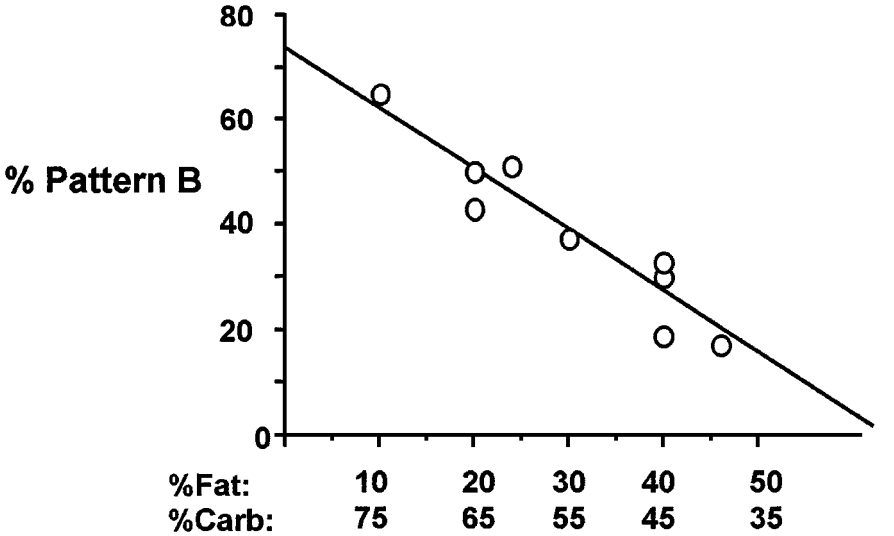

Figure 2 Prevalence of low-density lipoprotein subclass pattern B as a function of dietary fat and carbohydrate content. Data are derived from five studies involving a total of 596 healthy, nonobese, normolipidemic men (updated from Reference 29). The data points fit the relationship: % pattern B $= -1.16$ (% dietary fat) $+74$ ($r = -0.94$, $p < 0.0001$).

(29). The results have indicated that the prevalence of LDL subclass phenotype B in men increases in direct proportion to the degree to which dietary fat is replaced by carbohydrate in the diet (Figure 2). With 30% of calories as fat, approximately one third of men express the B trait, a figure that approximates the prevalence of this trait in the general population, whereas with short-term challenge of a 10% fat diet, the estimated prevalence of phenotype B in men increases to an estimated two thirds of the population. A hypothesis that has been put forward to explain these results is that genetic predisposition to phenotype is expressed with greater penetrance as dietary fat is reduced to lower levels and replaced by carbohydrate.

A possible genetic basis for the differential effects of low-fat diets on LDL levels in pattern B subjects was examined in 72 premenopausal women who were switched from their basal diet (mean 35% fat) to an outpatient low-fat (20%) diet for 8 weeks (28). Because of the low expression of pattern B in premenopausal women, genetic predisposition for pattern B was assessed by parental LDL subclass pattern. In the daughters, decreases in plasma levels of LDL cholesterol and IDL mass, as well as increases in plasma triglyceride, were significantly related to the number of pattern B parents and could not be explained by the daughters' adherence to the diet or baseline characteristics, including initial lipoprotein profile. Thus, in premenopausal women, genetic and metabolic factors responsible for LDL subclass pattern B may result in enhanced LDL responsiveness to reductions in dietary fat even though the trait is largely unexpressed because of low penetrance.

We also tested for a heritable basis for differences in LDL subclass response to low-fat diets by studying responses to a short-term challenge with a very-low-fat diet in a group of 50 children (mean age 14) according to parental LDL subclass patterns on a low-fat diet (30). Offspring of two pattern B parents had smaller LDL peak particle diameter, greater prevalence of pattern B, and higher LDL cholesterol and triglycerides than did offspring of two pattern A parents. Diet-induced reductions in LDL size and the proportion of subjects shifting from pattern A to pattern B were also greater in children of two pattern B parents. These findings suggest that parental LDL subclass patterns are informative in determining which children may be genetically susceptible to expression of subclass pattern B and related metabolic changes with consumption of a low-fat, high-carbohydrate challenge diet. Whether parental subclass pattern affects the response to longer-term consumption of less extreme diets remains to be determined.

These studies suggest that genetic factors underlying predisposition to LDL subclass phenotype B influence the lipoprotein response to low-fat diets and, in particular, determine the propensity to express phenotype B on a low-fat diet. This hypothesis is currently under investigation in studies employing pairs of siblings to test whether LDL subclass response to low-fat diets is linked to candidate genes that have been linked to LDL particle profiles in previous studies. Preliminary results of these studies have indicated that induction of pattern B by a low-fat, high-carbohydrate diet is linked to the LDL receptor gene locus on chromosome 19p (42).

Earlier studies had tested whether variants of the apoE gene influenced LDL subclass response to dietary fat restriction. Although apoE variants have significant effects on LDL levels, a strong relationship of the apoE gene to LDL subclass phenotypes has not been demonstrated. We have, however, demonstrated a significant effect of the E4 allele on LDL subclass response to reduced fat intake in men (27). This did not involve a significant difference in response of small LDL, which increased modestly in all apoE isoform groups (apoE, 2/3; apoE, 3/3; and apoE, 4/3 + 4/4). Rather, the 14% of men who were either heterozygous or homozygous for apoE4 demonstrated a significantly greater reduction in mass of large LDL than did men with apoE 3/3 (the most common genotype). Although the mechanism for this finding is not known, it may relate to the observation that larger LDL particles have greater content of apoE, and it is thus possible that the LDL elevations associated with the apoE4 allele on high-fat diets are primarily related to an effect on these larger LDL.

CONCLUSION

Genetic factors underlying susceptibility to an increased proportion of small, dense LDL particles in plasma (subclass phenotype B) may also influence the LDL response to replacement of dietary fat by carbohydrate. The minority of healthy individuals who express this trait on a high-fat diet achieve a significantly greater

LDL cholesterol reduction with dietary fat reduction than do subjects with larger LDL (phenotype A). The reduction of small, dense LDL particles in these individuals would be predicted to achieve a reduction in the increased coronary disease risk associated with this trait. In contrast, a substantial subset of healthy, nonobese men with phenotype A exhibit a metabolic response to dietary fat restriction that results in a shift from larger to smaller LDL and expression of phenotype B with minimal change in particle number. In these men, LDL cholesterol reductions largely represent reduced cholesterol content of LDL particles and, hence, would not be expected to result in reduced risk of coronary heart disease. A genetic basis for the LDL subclass response, and the conversion from phenotype A to B, is suggested by recent studies with families. The apoE gene, which is not linked to LDL subclass phenotype B, appears to influence levels of large, but not small, LDL. Improved understanding of genetic factors influencing LDL subclass levels may provide further information regarding the basis for the marked interindividual variability in LDL response to variation in dietary fat intake.

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