Atherogenic Lipoprotein Phenotype and Diet-Gene Interactions\textsuperscript{1,2}

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ABSTRACT Studies employing analysis of LDL subclasses have demonstrated heterogeneity of the LDL response to low fat, high carbohydrate diets in healthy nonobese subjects. In individuals with a genetically influenced atherogenic lipoprotein phenotype, characterized by a predominance of small dense LDL (LDL subclass pattern B), lowering of plasma LDL cholesterol levels by diets with ≤24% fat has been found to represent a reduction in numbers of circulating mid-sized and small LDL particles, and hence an expected lowering of cardiovascular disease risk. In contrast, in the majority of healthy individuals with larger LDL (pattern A, found in ~70% of men and a larger percentage of women), a significant proportion of the low fat diet-induced reduction in plasma LDL cholesterol is made by depletion of the cholesterol content of LDL particles. This change in LDL composition is accompanied by a shift from larger to smaller LDL particle diameters. Moreover, with progressive reduction of dietary fat and isocaloric substitution of carbohydrate, an increasing number of subjects with pattern Aconvert to the pattern B phenotype. Studies in families have indicated that susceptibility to induction of pattern B by low fat diets is under genetic influence. Thus, diet-gene interactions affecting LDL subclass patterns may contribute to substantial interindividual variability in the effects of low fat diets on coronary heart disease risk. J. Nutr. 131: 340S–343S, 2001.

KEY WORDS: cholesterol • LDL • diet • fat • carbohydrate • lipoprotein subclasses

Current dietary guidelines for the prevention and treatment of atherosclerotic cardiovascular disease include a strong emphasis on maintaining optimal LDL cholesterol levels, primarily by limiting intake of saturated fat and cholesterol in the context of diets that are also limited in total fat (<30% of energy). On average, such diets do achieve significant reductions of total and LDL cholesterol; however, the magnitude of change varies substantially among individuals (Katan and Beynen 1987, Schaefer et al. 1997), with many showing either little change or even increases in these parameters. Moreover, low fat, high carbohydrate diets can also result in reduced plasma HDL cholesterol and/or increased triglyceride, raising the possibility that these changes may offset to varying degrees the expected benefit of LDL cholesterol lowering. The wide interindividual variations in lipoprotein response to altered dietary composition have been attributed at least in part to genetic differences among individuals (Dreon and Krauss 1997, Ordovas 1999, Schaefer et al., 1997). Although a number of studies have demonstrated associations of these variations with polymorphisms in candidate genes (Dreon and Krauss 1997, Ordovas 1999), the magnitude of these effects has generally been small, and it is likely that multiple factors, many as yet unrecognized, are involved.

Atherogenic lipoprotein phenotype

In recent years, identification of distinct LDL subclasses that differ in particle size and density has led to recognition that these subclasses differ in their metabolic and pathologic properties, as well as their dietary and genetic determinants (Krauss 1997). Moreover, there is variation in the distribution of these subclasses among individuals. In the majority of healthy subjects, the major forms of LDL are large and buoyant, but in a substantial subset of the population, there is a predominance of small, dense LDL particles (Austin et al. 1990). The small, dense LDL profile, designated LDL subclass pattern B, is associated with relative increases in plasma triglyceride and other proatherogenic metabolic changes, including increased intermediate density lipoproteins, reduced HDL...
choleroster (Austin et al. 1990) and reduced insulin sensitivity (Reaven et al. 1993). Moreover, small LDL particles appear to have greater atherogenic potential than large LDL by virtue of reduced receptor-mediated clearance (Campos et al. 1996) and higher endothelial transport (Nielsen 1996), proteoglycan binding (Anber et al. 1997) and oxidative susceptibility (Tribbe et al. 1992). Overall, this profile can result in approximately a threefold increase in risk for coronary artery disease (Austin et al. 1988, Gardner et al. 1996, Lamarche et al. 1997, Stampfer et al. 1996), an observation that supports its designation as an atherogenic lipoprotein phenotype. The magnitude of the risk, however, is strongly dependent on overall plasma concentrations of apolipoprotein (apo)B-containing lipoproteins, suggesting that the quantity as well as the quality of LDL subfractions should be considered in assessing atherosclerosis risk.

**Genetic influences on LDL subclasses**

Studies in families have indicated that LDL subclass patterns are influenced by major genes (Austin et al. 1988, Austin 1994); linkages of LDL particle size phenotypes to several candidate gene loci have been reported (Allayee et al. 2000, Austin et al. 1998, Nishina et al. 1992, Rotter et al. 1996, Talmud et al. 2000). To date, the most consistent evidence for linkage has been found for a locus in the vicinity of the LDL receptor gene on chromosome 19p (Nishina et al. 1992, Rotter et al. 1996), the apoCIII gene locus on chromosome 11 (Allayee et al. 1998, Rotter et al. 1996) and the cholesteryl ester transfer protein gene on chromosome 16 (Rotter et al. 1996, Talmud et al. 2000). Thus, major genes may act singly or in combination to influence LDL particle size. Although a number of the linked genes have a plausible basis for contributing to variation in LDL subclass profiles, in the case of the LDL receptor gene, analyses of DNA sequences in the coding region (Naggett et al. 1997) as well as immediately upstream (Naggett, J.K., Nishina, P.M., Krauss, R.M., personal communication) showed no significant differences in pattern B vs. pattern A subjects from informative families. Coupled with the observation of normal LDL receptor function in fibroblasts of pattern B subjects (Campos et al. 1996), these findings rule out an LDL receptor structural variant in the etiology of pattern B, but do not rule out an abnormality related to regulation of this gene.

**Gene-diet interactions involving small dense LDL**

Despite the evidence for major gene effects on LDL subclass patterns, studies in twins have indicated that heritability of LDL peak particle size as a quantitative trait is < 50% (Austin et al. 1993, Lamon-Fava et al. 1991). This is consistent with the strong influence of modifying factors on the expression of LDL subclass pattern B. Age and gender are major determinants, with a prevalence of ~30% in men, 15–20% in postmenopausal women and 5–10% in younger individuals (Austin et al. 1990 and 1993, Campos et al. 1992). In addition, LDL size is influenced by metabolic factors affecting plasma triglyceride (Krauss et al. 1988, McNamara et al. 1987), including abdominal adiposity (Terry et al. 1989) and insulin resistance (Reaven et al. 1993). Given the evidence for differences in the metabolic and pathologic behavior among LDL subclasses and for both genetic and environmental influences on LDL particle profiles, we have sought to determine whether diets designed to lower LDL cholesterol have different effects on LDL subclasses and whether genetic factors underlying susceptibility to the atherogenic lipoprotein phenotype contribute to interindividual variability in lipoprotein response to such diets.

Initial observations were made in a cohort of 105 healthy nonobese normolipidemic men who consumed high (46% of energy) and low fat (24% of energy) diets for 6 wk each in a randomized, crossover design (Dreon et al. 1994, Krauss and Dreon 1995). Differences in composition of the diets involved proportional changes in both saturated and polyunsaturated fat with reciprocal changes in carbohydrate (equally distributed between sugar and starch); no change in content of monounsaturated fat, protein, cholesterol or fiber; and adjustment of energy to maintain constant body weight. Compared with the high fat diet, the low fat diet resulted in a significant mean reduction in LDL cholesterol of 11%, consistent with the difference predicted from equations described previously (Hegsted et al. 1993, Keys 1957). However, the distribution of values was broad (Fig. 1), with a range from −49 to +51%.

Subjects were categorized on the basis of LDL subclass patterns during consumption of the high fat diet, i.e., 87% pattern A or intermediate pattern and 18% pattern B. Men with a predominance of small, dense LDL (pattern B) consuming the high fat diet (n = 18) exhibited a twofold greater reduction in LDL cholesterol than men within pattern A design (Dreon et al. 1994, Krauss and Dreon 1995). This was associated with significantly greater reductions in mass of mid-sized (LDL 2) and small (LDL 3) LDL subfractions measured by analytic ultracentrifugation (Krauss and Dreon 1995). Furthermore, only pattern B subjects showed significant reductions in plasma apoB, and in LDL relative to HDL cholesterol levels (Dreon et al. 1994).

Of the 87 men with pattern A consuming the high fat diet, 36 converted to pattern B when consuming the low fat diet (Dreon et al. 1994). In these men, there was a shift in LDL particle mass from larger, lipid-enriched (LDL1 and 2) to smaller, lipid-depleted (LDL 3 and 4) subfractions (Krauss and Dreon 1995), suggestive of change in LDL composition with minimal change in particle number, and consistent with the observation of reduced plasma LDL cholesterol without reduced plasma apoB. The group differences in LDL and apoB response could not be attributed to differences in plasma lipid levels or body mass indices or to apoE phenotypes. Increases in plasma triglyceride and reductions in HDL cholesterol with the low fat, high carbohydrate diet were comparable in pattern

![FIGURE 1 Distribution of changes in LDL cholesterol between high fat (46% energy) and low fat (24% energy) diets in 105 healthy men; [data from Dreon et al. (1994)]](image-url)
A and B subjects (Dreon et al. 1994). Taken together, these results indicate that in the majority of men, the reduction in LDL cholesterol seen during consumption of 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 consuming a high fat diet.

These results, which have been confirmed in a second study in 133 men (Dreon and Krauss 1995), indicate that reduction in dietary fat and increase in carbohydrate can elicit the expression of LDL subclass pattern B in a subset of healthy men. Moreover, a short-term (10 d) dietary challenge of a 10% fat diet in 38 healthy men with pattern A consuming diets containing 20 –24% fat resulted in a conversion to pattern B in 12 men (32%) (Dreon et al. 1999). There were no significant reductions in LDL cholesterol levels in the group as a whole, but those who converted to pattern B had significantly greater increases in levels of triglyceride and apoB and reductions in HDL cholesterol than those who remained pattern A (Dreon et al. 1999).

Overall, in a series of such studies employing diets with varying fat content and reciprocal variation in carbohydrate content, there is a strong linear relationship of decreased fat and increased carbohydrate intake with prevalence of LDL subclass pattern B in healthy men (Fig. 2). These results indicate that the prevalence of pattern B in men consuming 30% fat is ~30–35%, a figure that is consistent with the prevalence of pattern B in men in the general population (Austin et al. 1990, Campos et al. 1992). Hence, the results suggest that the short-term effects of variation in diet composition on LDL subclass phenotypes are indicative of the effects of long-term diet consumption.

**Genetic influences on response of LDL subclass patterns to low fat diet**

The results in Figure 2 indicate that dietary fat and/or carbohydrate intake are strong determinants of subclass pattern B, and may act to induce expression of this trait in susceptible individuals. Moreover, given the evidence for genetic effects on LDL subclass patterns, the results raise the possibility that induction of pattern B by a low fat, high carbohydrate diet is also under genetic influence. Heritability of this diet response has been demonstrated in two family studies involving premenopausal women (Dreon et al. 1997) and children (Dreon et al. 2000), groups with low expression of pattern B in whom genetic susceptibility to this trait was inferred by its presence in one or both parents. In both studies, prevalence of pattern B after consumption of a low fat diet was greatest in offspring of two pattern B parents.

On the basis of the evidence for heritability of induction of pattern B by a low fat diet, we hypothesized that one or more of the genes linked to variation in LDL particle size may be responsible for this diet effect. To test this hypothesis, we recently studied the effects of reduction in dietary fat from 40 to 20% of energy in a cohort of 298 brothers from 135 families in whom linkage to a polymorphism in the LDL receptor was tested by nonparametric sibpair linkage analysis (Krauss et al. 1999). Significant linkage was observed during consumption of both high and low fat diets, confirming earlier results in families in whom diet was not controlled. No genetic linkage was found for other lipid or lipoprotein variables, except for HDL cholesterol, which showed weak linkage to the LDL receptor gene (P < 0.05) for both diets. Interestingly, linkage of LDL subclass pattern (qualitative phenotype) was stronger with consumption of the high fat diet, whereas linkages of quantitative measures (LDL density and size) were stronger with consumption of the low fat diet. Most notably, the tendency for a low fat diet to induce expression of LDL subclass pattern B was also linked to the LDL receptor gene. It therefore appears that the genetic locus on chromosome 19q that influences LDL subclass pattern with consumption of a high fat diet also contributes to susceptibility for reduced size and increased density of LDL particles, and induction of the pattern B phenotype during consumption of a low fat diet. Thus, it is likely that one or more genes at this locus underlie diet-gene interactions affecting LDL subclass phenotypes.

**SUMMARY**

There is increasing awareness of the potential for genetic variation among individuals to influence nutrient requirements and biological responses to nutrient intake (Simopoulos 1999). In the case of genes influencing LDL subclass patterns, gene-diet interactions contribute to wide interindividual differences in the effects of low fat, high carbohydrate diets on risk for coronary heart disease. The recognition of such differences in metabolic response is prompting greater attention to the potential for individualization of nutritional approaches for prevention of heart disease (Krauss et al. 1996). Once specific genes responsible for these effects are identified, it will be possible to use this information to target low fat dietary interventions more effectively to those individuals most likely to achieve a benefit for cardiovascular disease risk (Krauss 2000).

**LITERATURE CITED**


