Dietary and Genetic Probes of Atherogenic Dyslipidemia

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Abstract—A goal of dietary management of cardiovascular disease risk in patients with obesity and metabolic syndrome is improvement in the atherogenic dyslipidemia comprising elevated triglyceride, reduced high-density lipoprotein (HDL) cholesterol, and increased numbers of small, dense low-density lipoprotein (LDL) particles. Individuals with a genetically influenced trait characterized by a high proportion of small, dense LDL (phenotype B) respond to a low-fat, high-carbohydrate diet with greater reduction of LDL cholesterol, apoprotein B, and mid-sized LDL2 particles than unaffected subjects (phenotype A). In contrast, in phenotype A subjects there is a reciprocal shift from large LDL1 to small LDL3 such that a high proportion convert to phenotype B. There is evidence for heritable effects on these diet-induced subclass changes and for the involvement of specific genes. For example, a haplotype of the APOA5 gene associated with increased plasma triglyceride and small, dense LDL predicts greater diet-induced reduction of LDL2, a haplotype-specific effect that is strongly correlated with both increased VLDL precursors and LDL4 products. Understanding of such diet-genotype interactions may help to elucidate mechanisms that are responsible for phenotype B and for its differential dietary responsiveness. This information may also ultimately help in identifying those individuals who are most likely to achieve cardiovascular risk benefit from specific dietary interventions. (Arterioscler Thromb Vasc Biol. 2005;25:0-0.)

Key Words: TO □ COME

The dyslipidemia associated with obesity, metabolic syndrome, insulin resistance, and type 2 diabetes consists of increased triglyceride, reduced high-density lipoprotein (HDL) cholesterol, and increased numbers of small, dense low-density lipoprotein (LDL) particles.1,2 The strong interrelationships of these changes and their associations with increased risk for atherosclerotic cardiovascular disease have led to their collective designation as atherogenic dyslipidemia.3 This dyslipidemia results from complex interactions among predisposing genetic traits and modifying factors including age, gender, and adiposity. Current recommendations for managing the cardiovascular disease risk associated with atherogenic dyslipidemia focus on lifestyle modifications that have been shown to be of benefit, particularly in reduction of excess adiposity and increase in physical activity.4

The guidelines from the Adult Treatment Panel III of the National Cholesterol Education Program state that the principal lipid target for patients with metabolic syndrome should be LDL cholesterol, or in the case of individuals with triglyceride levels >200 mg/dL, non-HDL cholesterol.5 Diet remains the mainstay for LDL cholesterol management in this syndrome, but optimal dietary treatment requires consideration of factors that influence the spectrum of atherogenic lipoprotein changes, including those that lead to the increased concentrations of small, dense LDL particles found in this syndrome. As reviewed, dietary change can operate on the background of genetic factors that influence the generation of differing LDL particle profiles, including that seen in atherogenic dyslipidemia. Understanding the interactions of diet and genetic predisposition can help to elucidate metabolic pathways affecting atherosclerosis risk and the basis for interindividual differences in dietary effects on these pathways.

Clinical Significance of LDL Heterogeneity
LDL particles comprise multiple distinct subclasses that differ in size, density and lipid content.6 These can be grouped into four broad categories ranging from the largest, most buoyant, and lipid-enriched LDL1, to the smallest, most dense, and lipid-depleted LDL4.7 In addition to having reduced lipid content, the smaller LDL species have been shown to be of benefit, particularly in reduction of excess adiposity and increase in physical activity.4

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tein (VLDL) and triglyceride. Moreover, based on measurement of peak LDL diameter or ultracentrifugal density, individuals generally cluster into 2 broad subgroups, the majority with a predominance of larger or medium sized LDL, designated phenotype A, and a substantial minority with a higher proportion of smaller LDL particles, phenotype B. Phenotype B is also associated with lower HDL and the cluster of abnormalities that characterize the metabolic syndrome, including insulin resistance. There is prospective evidence from most but not all studies that small LDL particle diameter and/or increased levels of small dense LDL predict increased risk of cardiovascular disease. Because this increased risk can also be attributed to other metabolic changes associated with phenotype B, particularly elevated triglyceride, reduced HDL, and insulin resistance, it has been designated an atherogenic lipoprotein phenotype. Evidence regarding the extent to which small dense LDL may contribute to cardiovascular disease risk independently of associated metabolic abnormalities has been discussed extensively elsewhere.

Metabolic Influences on LDL Heterogeneity

A number of efforts have been made to develop metabolic models that account for the production of individual LDL subclasses and the regulation of their plasma levels. Whereas earlier models considered the production of LDL to result from sequential delipidation and remodeling of triglyceride-rich apoB-containing lipoprotein precursors, subsequent studies established that individual LDL subclasses can be formed from particles that enter plasma through independent metabolic pathways originating in the liver with secretion of apoB-containing lipoprotein particles of differing composition. The contribution of differing hepatic secretory pathways to LDL heterogeneity is also supported by the existence of marked differences in apoB glycosylation among LDL subclasses, ranging from 80 mg/g protein in the more buoyant fractions to ≤ 12 mg/g protein in the most dense fractions. Glycosylation of apoB is not known to be modified by intravascular processes, with the exception of the relatively small amount of terminal sialylation. Hence, it is likely that the differences in carbohydrate content of the major LDL subclasses result primarily from hepatic secretion of precursors with differing extent of apoB glycosylation.

Candidates for metabolic precursors of specific LDL subtypes exist within the spectrum of liver-derived triglyceride-rich apoB-containing lipoprotein particles. As is the case for LDL and HDL, VLDL and intermediate-density lipoproteins (IDL) include distinct subclasses of differing size and density distribution. Figure 1 displays a simplified hypothetical model for the origins of the major human LDL subclasses based on a variety of lines of evidence, including in vivo metabolic studies and analyses of lipoprotein subclass interrelationships. The scheme includes both parallel production pathways and remodeling of lipolytic products in plasma. Although the specific precursor-product relationships in this model have not been validated by in vivo kinetic analyses, it is consistent with findings from perfused livers in a primate model, and with the hypothesis that differences in hepatic lipid availability influence properties of apoB-secretory products. For example, when particles secreted by the liver are relatively triglyceride poor, we have hypothesized that they are primarily in the IDL size and density range and include species that give rise to larger LDL. A second pathway is suggested to originate with the secretion of small VLDL, designated VLDL2 in Figure 1. Although there is a broad spectrum of size and density of these particles, they are generally defined by flotation rate (Sf) of 20 to 60 and overlap with larger IDL of Sf 12 to 20 creating a broad single distribution. It is supposed that the lipolytic products of these particles are predominantly mid-sized LDL. This subclass has been shown to have higher LDL receptor affinity than other LDL particles, suggesting the likelihood of shorter plasma residence time.

Previous studies from our laboratory, based primarily on size distribution as assessed by nondenaturing gradient gel electrophoresis, have indicated that there is a discrete transition between VLDL2 and the more triglyceride-enriched VLDL1. In Figure 1, we have proposed that this represents differing hepatic VLDL production pathways for VLDL1 and VLDL2. Although these particles can be cleared as remnants by virtue of acquiring apoE, they can also be enriched in apoC3, a property that retards their intravascular lipolysis and receptor-mediated clearance, resulting in the accumulation of remnant products that overlap in size and density with IDL. These can be further metabolized to smaller LDL (eg, LDL3) through a series of lipolytic steps and transfer of lipids by cholesterol ester transfer protein, and finally through the action of hepatic lipase. As noted, the smaller LDL are cleared relatively less efficiently by the LDL receptor than are medium sized LDL. Hence particles throughout this pathway may have overall slower turnover, with a greater plasma residence time and potential for arterial uptake.

Finally, the largest VLDL particles (VLDL1 in Figure 1) that are secreted under conditions of high hepatic triglyceride secretion yield larger remnants that can be cleared to a great extent without transformation to LDL. We have hypothesized, however, that small amounts of lipid-poor LDL 4 can be formed from triglyceride-enriched subspecies of VLDL1 particles. Particles in this size and density range can also be formed from extensive remodeling of larger LDL. Overall, the scheme in Figure 1 provides a possible metabolic framework for the origins of the particles that are
characteristic of the A and B LDL subclass phenotypes. In this model, the B phenotype is a relatively discrete marker for a metabolic shift to increased lipid transport through the VLDL1 pathway, and for the broader range of lipoprotein changes associated with obesity and insulin resistance. As discussed above, although it has been difficult to determine the relative atherogenic potential of specific particles in this pathway, there is evidence that levels of both small IDL36 and the smallest LDL species37 are particularly strong indicators of coronary artery disease progression.

**Genetic Influences on LDL Heterogeneity**

The existence of modality in the LDL particle size distribution suggested the possibility of underlying major gene effects. Family studies have confirmed this hypothesis, although the mode of transmission, and the specific genes involved, have not been firmly established.38–42 Genetic linkage and association studies have, however, identified several candidate loci related to variation in LDL particle size profiles.43 Among genes for which significant genetic linkage or association have been reported in at least 2 studies are those for cholesteryl ester transfer protein (CEPT),44-50 LDL receptor (LDLR),44,51 manganese superoxide dismutase (MNSOD),44,45 lipoprotein lipase,47,52 and apoA5.53,54 Other studies have failed to confirm linkage with the LDLR locus.45,55 Of interest, a nonsynonymous polymorphism of CEP, I405V, was found to be associated with larger LDL and HDL particles and longevity in Ashkenazi Jews.56 Polymorphisms in the gene for hepatic lipase have been linked to peak LDL size and other features of the atherogenic lipoprotein phenotype,57 but despite a report of an association of LDL density with a common promoter polymorphism of the hepatic lipase gene,57 this polymorphism was not associated with peak LDL size.58 Other genes for which polymorphisms have been associated or genetically linked with LDL particle profiles include apolipoprotein E (apoE), microsomal triglyceride transfer protein, apoC3,54 and apoB.61 In addition, genome-wide linkage studies have reported a number of chromosomal loci linked to LDL particle size phenotypes, although none has yet been confirmed.57-66 Moreover, no specific genetic polymorphism has been firmly implicated in the cause of LDL subclass phenotype B.

Family and twin studies have provided estimates of heritability of LDL size phenotypes ranging from ≈40% to 60%.66–69 Among other factors influencing phenotype B expression are age, gender, adiposity, and dietary macronutrient composition, as described further.

**Effects of Dietary Fat and Carbohydrate on LDL Subclass Phenotypes**

Given the influence of dietary fat and carbohydrate composition on metabolism of LDL and other apoB-containing lipoproteins, we hypothesized that these dietary effects would differ in subjects with LDL subclass phenotypes A and B. In an initial study, high-fat (46% energy) and low-fat (24% energy) diets were consumed for 6 weeks each by 105 healthy, middle-aged, nonobese men in a randomized crossover design on an outpatient basis.70,71 Change in total fat content was achieved by varying saturated and polyunsatu-rated fat in equal proportion (ratio ≈1.0), and substituting a 50:50 mixture of complex and simple carbohydrates. Intake of total energy, protein (15% energy), cholesterol, and fiber were not changed. Unexpectedly, we found that the LDL cholesterol reduction with the lower fat diet was significantly greater in the 18 men with phenotype B than in the 87 men with phenotype A on the high-fat diet, and that this difference was independent of other baseline differences between these 2 groups.70,71

An additional 510 men and 72 premenopausal women have undergone 3- to 6-week dietary interventions using diets similar to those in the first study, except that the lower fat diet contained 20% fat, and the higher fat diet contained 40% fat for the men and 35% for the women72,73 (Krauss et al, unpublished 2005). These additional studies confirmed the initial findings: pooled data from the total of 687 subjects show that LDL cholesterol reduction was substantially greater in the 165 subjects classified as LDL phenotype B on the high-fat diet (−19.1 ± 22.3 [SD] mg/dL versus −6.6 ± 17.9, P<0.0001), and that this differential response was independent of age, gender, or baseline body mass index, LDL cholesterol, triglyceride, and HDL cholesterol level. Moreover, the ratio of LDL cholesterol/HDL cholesterol decreased from 3.39±0.74 to 3.25±0.74 in phenotype B (P<0.0007) but increased from 2.47±0.89 to 2.72±0.92 in phenotype A subjects (P<0.0001; change versus phenotype B <0.0001). Thus, the predicted benefit of LDL cholesterol lowering by this type of dietary intervention in healthy subjects is much greater in the minority of higher risk subjects with LDL subclass phenotype B than in those within phenotype A.

Further analyses of the diet-induced changes in LDL subclasses were performed using analytical ultracentrifuge measurements of total mass of LDL particles.71 The pooled results in the 615 men are shown in Figure 2. In phenotype A, there was a reduction in a larger LDL1 and an increase in LDL3, changes that were strongly reciprocally related, as described previously.7,8,71,74 In phenotype B subjects, in
contrast, there was reduction in LDL1 and 2, with no significant increase in LDL3 and a modest increase in LDL4. In these subjects there was a strong inverse relationship between reduction in LDL2 and increase in LDL4. All diet-induced changes in LDL subclass concentrations differed significantly between the phenotypes (P<0.0001).

Overall, the results in Figure 2 indicate that the greater reduction in LDL cholesterol in phenotype B versus A is primarily caused by a greater reduction in LDL2 and a lesser increase in small dense LDL3 plus LDL4. Whereas the specific metabolic causes for these differences are not known, they can be considered in the context of the metabolic scheme described in Figure 1. For subjects with phenotype A we propose, as described previously, that there is a shift from the particle pathway resulting in larger LDL1 to that associated with higher hepatic triglyceride output which results in the production of smaller LDL3. The findings in Figure 2 also indicate that in the phenotype A subjects there is relatively little net effect of the diet on the pathway responsible for regulation of levels of LDL2. Because diets with reduced saturated fat are thought to reduce plasma LDL primarily by increasing receptor-mediated uptake, it is possible that increased LDL2 production is balanced by increased clearance in phenotype A, whereas there is an excess of production over clearance for LDL3.

In subjects with phenotype B, who have a “constitutive” increase in the LDL3 pathway, the lower fat, higher carbohydrate diet results in a shift from the VLDL2-LDL2 pathway to that resulting in increased hepatic production of larger VLDL1.71 Because many of the particles in this pathway are cleared without conversion to LDL, this shift, together with increased receptor-mediated LDL 2 clearance, may be responsible for the for the overall LDL reduction in phenotype B subjects. An additional factor contributing to the greater LDL reduction in these individuals is the very low cholesterol content of LDL4 particles that are produced in response to the dietary change.

The changes in LDL subclass levels in response to the lower-fat, higher-carbohydrate diet are also reflected in changes in the expression of the A and B phenotypes (Figure 3). The reciprocal shift from LDL1 to LDL3 in phenotype A subjects resulted in a shift to phenotype B in 35% of the phenotype A men, whereas only 6% changed from phenotype B to A. These changes resulted in an overall increase in the prevalence of phenotype B from 26% to 51%. As reported in other cohorts,39,75,76 the prevalence of phenotype B in premenopausal women was much lower than in men: 4% on diets with an average fat content of 35%.72 However, the reciprocal changes in LDL subclasses in women with change to a 20% fat diet were similar to those in the men.

In studies using a wider range of dietary fat and carbohydrate intakes, without change in protein or total calories, we have shown that the prevalence of phenotype B in both men and premenopausal women is strongly related to the percent of dietary carbohydrate (positive) and fat (negative)77 (Figure 4). We have recently extended these observations in a study of 178 overweight men in whom the prevalence of phenotype B was reduced by lowering dietary carbohydrate content from 54% to 39% and increasing protein intake from 15% to 29% without significantly changing fat, fat composition, or calories.73 This indicates that carbohydrate rather than fat is a major dietary determinant of expression of phenotype B in susceptible individuals.

Although these were short-term intervention studies, it is notable, as shown by the shaded areas in Figure 4, that a carbohydrate intake corresponding to the average in the United States (∼50% of total calories) would predict a prevalence of phenotype B in men and premenopausal women of ∼30% to 35% and 5% respectively, values consistent with those observed in studies of individuals in the general population.38,75 This suggests that the relationships shown in Figure 4 also operate over the long term. It should, however, be recognized that particularly in the upper range of carbohydrate intakes used for these studies, there is a high content of sugars as well as starches that can amplify the triglyceridemic response,79 and that diets that are higher in plant-based versus simple carbohydrates can, in conjunction with weight loss, improve features of atherogenic dyslipidemia.80

Figure 3. Distribution of peak LDL ultracentrifugal flotation rates (SF) in 687 subjects on high-fat and low-fat diets (see text for details). Shaded bars represent subjects with LDL subclass phenotype B as determined by analytical ultracentrifugation71,87; white bars are subjects with phenotype A.

Figure 4. Relation of prevalence of LDL subclass phenotype B to percent dietary fat/carbohydrate in short-term feeding studies (3 to 6 weeks). Shaded circles: men (n=615), open circles: women (n=72). Shaded bars indicate the prevalence of pattern B predicted for individuals consuming diets with average US carbohydrate and fat intakes.

Genetic Influences on LDL Subclass Response to Change in Dietary Carbohydrate and Fat
Given our observations that phenotype B is genetically influenced, we sought to determine whether there is also a
genetic component to susceptibility to the dietary induction of phenotype B. To address this question, we have performed studies in families, initially in 50 offspring of 29 parental pairs that were characterized as a function of their LDL phenotypes.\textsuperscript{81} This design was based on the inference that offspring of two phenotype B parents were more likely to carry a predisposing allele for this phenotype B than offspring of A by A or A by B parents. A very high carbohydrate (75%), short-term (10 day) intervention was used to test for conversion from phenotype A to B in the offspring. All 6 cases of this conversion were in offspring of 2 phenotype B parents. In addition, LDL particle diameter decreased significantly more in offspring of B by B than A by A matings. These observations are consistent with heritability of susceptibility to dietary induction of phenotype B.

We have tested the genetic basis for heritability of LDL subclass responses to change in dietary fat and carbohydrate by seeking associations of these responses with genotypes in candidate genes that have been associated with LDL subclass phenotypes. The apoA5 gene has been particularly informative in this regard. As noted, single nucleotide polymorphisms in the apoA5 gene have been significantly associated with variations in both plasma triglyceride and LDL particle size.\textsuperscript{53,54} The Table extends plasma lipid measurements previously reported from the Berkeley Lipid Study Population in heterozygote carriers of the \textit{APOA5}*1/*3 haplotype.\textsuperscript{82} \textit{APOA5}*3 results from a C-to-G substitution (c.56C>G) that changes codon 19 from serine to tryptophan and has an allele frequency of 6% in whites.\textsuperscript{82} As shown previously, compared with unaffected individuals (\textit{APOA5}*1/*1), subjects with \textit{APOA5}*1/*3 had higher plasma triglyceride levels on both high-fat and low-fat diets. However, there was no difference between the groups in the triglyceride increase induced by the low-fat, high-carbohydrate diet. The Table also shows that LDL cholesterol levels were significantly higher in \textit{APOA5}*1/*3 subjects on the high fat diet, but not on the low-fat diet, and that diet-induced changes in LDL cholesterol were not significantly different between the haplotype groups. On both diets, subjects with \textit{APOA5}*1/*3 versus *1/*1 had significantly higher concentrations of small dense LDL subclasses 3 and 4, lower peak LDL Sf rate, indicative of smaller size and higher density of the LDL modal peak, and higher prevalence of LDL subclass phenotype. These relationships were independent of gender, age, and body mass index. After adjustment for plasma triglyceride, group differences in LDL 3 and peak Sf became nonsignificant, but the difference in LDL 4 remained significant on both diets ($P=0.011$ for high-fat and 0.0012 for low-fat).

Notably, in \textit{APOA5}*1/*3 subjects, the diet-induced reduction in levels of LDL 2 was nearly 3-fold greater than in unaffected subjects, and this was the only statistically significant response among the LDL subclass measurements ($P=0.006$). Moreover, this difference remained significant after adjustment for LDL phenotype, age, BMI, gender and change in triglyceride level. However, the association of high fat LDL phenotype with diet-induced LDL2 change demonstrated in Figure 2 remained significant after adjusting for \textit{APOA5} haplotype, indicating that the greater reduction in phenotype B subjects is not fully explained by the association of \textit{APOA5}*3 with phenotype B. Rather, the significant association of the \textit{APOA5} haplotype with reduction in LDL2 was restricted to the subjects with phenotype A on the high fat diet who converted to phenotype B on the low fat, high carbohydrate diet, an interaction confirmed by 2-way analysis of variance (data not shown). This interaction in turn was independent of plasma triglyceride concentration.

These findings indicate that although variation of the \textit{APOA5} gene does not appear to be responsible for dietary induction of phenotype B, the presence of the \textit{APOA5}*3 haplotype in individuals in whom phenotype B is induced is a significant determinant of the reduction in LDL2 that is seen on the low-fat diet in subjects with this phenotype. The results lend further support to the hypothesis that genetic variation underlies susceptibility to the changes in LDL subclass levels induced by low-fat, high-carbohydrate diets. In this regard, we have also observed associations of polymorphisms in \textit{CETP} and \textit{LDLR}, which as described above have been associated with LDL size phenotypes, with diet-induced LDL 2 reduction in the Berkeley Lipid Study Population (Krauss et al, unpublished 2005). It may therefore be that...
genes underlying susceptibility to phenotype B also lead to the low-fat diet-induced lowering of LDL2 that occurs in conjunction with phenotype B expression.

Associations of specific genotypes with dietary effects on LDL subclasses may also be used to probe for genetic influences on metabolic pathways that give rise to these subclasses. This possibility is illustrated by the further analysis of the relation of APOA5*1/*3 to changes in metabolically related lipoprotein subclasses (Figure 5). Specifically, changes in VLDL2 (Sf 20 to 60) were inversely related to changes in LDL2, consistent with precursor–product relationships indicated in Figure 1, but this was significant only for subjects with APOA5*3. Significant APOA5 haplotype differences were also seen for the inverse relationship between changes in total VLDL (Sf 20 to 400) and LDL2 (Figure 5A). This observation is consistent with the possibility that the APOA5 variant impairs the rate and/or amount of formation of LDL2 from VLDL. This effect could in turn reflect recent evidence for a role of apoA5 in promoting lipolysis of triglyceride-rich lipoproteins.

Figure 5B shows that the inverse relationship between changes in LDL2 and LDL4 was also significantly stronger for subjects with APOA5*1/*3 than *1/*1. The APOA5 haplotype difference was also significant if restricted to subjects with phenotype A on the higher-fat diet (data not shown).

Hence, the greater diet-induced reduction of LDL2 in APOA5*1/*3 carriers could result from both impaired production from VLDL precursors and increased shunting to the pathway resulting in production of LDL4 (Figures 1 and 2). This hypothesis is subject to direct experimental testing. APOA5 is, however, only one component of the multigenic system that can modulate pathways involved in LDL subclass metabolism. Therefore, more comprehensive analyses of genetic determinants of dietary response of specific lipoprotein subclasses will be required to gain a more complete understanding of the basis for the wide interindividual variability in the LDL response. This information can in turn lead to more individualized dietary recommendations for reduction of risk for cardiovascular disease.

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