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Low Density Lipoprotein Subclasses and Response to a Low-Fat Diet in Healthy Men

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ABSTRACT

Lipid and lipoprotein response to reduced dietary fat intake was investigated in relation to differences in distribution of LDL subclasses among 105 healthy men consuming high-fat (46%) and low-fat (24%) diets in random order for six weeks each. On high-fat, 87 subjects had predominantly large, buoyant LDL as measured by gradient gel electrophoresis and confirmed by analytic ultracentrifugation (pattern A), while the remainder had primarily smaller, denser LDL (pattern B). On low-fat, 36 men changed from pattern A to B. Compared with the 51 men in the stable A group, men in the stable B group (n=18) had a three-fold greater reduction in LDL cholesterol and significantly greater reductions in plasma apoB and mass of intermediate (LDL II) and small (LDL III) LDL subfractions measured by analytic ultracentrifugation. In both stable A and change groups, reductions in LDL-cholesterol were not accompanied by reduced plasma apoB, consistent with the observation of a shift in LDL particle mass from larger, lipid-enriched (LDL I and II) to smaller, lipid-depleted (LDL III and IV) subfractions, without significant change in particle number. Genetic and environmental factors influencing LDL subclass distributions thus may also contribute substantially to interindividual variation in response to a low-fat diet.

Key Words: LDL cholesterol; lipoproteins; LDL subclasses; VLDL; HDL; dietary fat

INTRODUCTION

Lipid response to variation in dietary fat and cholesterol intake varies widely among individuals (1-3), and it has been hypothesized that a significant proportion of this variability is attributable to genetic factors (4). Polymorphisms at several genetic loci have been reported to be associated with variation in dietary fat and cholesterol responsiveness, notably apoE (5), apo AIV(6), apoB (7), and apoAI (8). However these effects have not been demonstrated consistently, (e.g., (9)), and their magnitude is relatively small.

Recently, we have investigated the relationships of plasma LDL subclass patterns to the lipid and lipoprotein response to reduced total fat intake (10). Although non-genetic factors are known to affect LDL subclasses (11,12), there is also evidence for the existence of genetic determinants of the LDL particle distribution (13), as assessed by particle size (14,15) and density (16). In particular, complex segregation analyses have indicated that a phenotype characterized by a predominance of small, dense LDL, designated LDL subclass pattern B, is influenced by a major gene or genes, with a prevalence in the American population estimated to be as high as 0.25 (14). The specific gene(s) responsible for this trait have not been identified, but linkage to polymorphic markers near the LDL receptor gene on chromosome 19p has been reported (17).

In our previous analyses, we showed that among 105 men studied on a high-fat (46% of energy) diet, the 18 with LDL subclass pattern B were found to have a greater lowering of LDL-cholesterol on a low-fat (24%), high-carbohydrate (60%) diet than the 87 men with a predominance of larger LDL (subclass pattern A) (10). Moreover, a significant reduction in plasma of apoB was observed only in the pattern B group. Finally, in a subset of 36 men with pattern A, the low-fat diet induced conversion to pattern B. Thus, the genetic and environmental

determinants of LDL subclass patterns may also have important effects on the lipoprotein response to reduced dietary fat intake.

Differential responsiveness of subjects with larger and smaller LDL to low-fat diets may be of particular significance with regard to the impact of dietary fat reduction on risk of coronary artery disease. Subclass pattern B is associated with a number of potentially atherogenic metabolic aberrations, including elevated triglyceride and apoB (18), reduced HDL (18), and features of the insulin resistance syndrome (19,20). Furthermore, in case-control studies, up to a three-fold increased risk of acute myocardial infarction (21), and a similar increase in risk for coronary atherosclerosis (22,23) has been found for subjects with pattern B, leading to its designation as an atherogenic lipoprotein phenotype. Thus, diet-induced changes in LDL, in particular smaller LDL particles, may be of major importance with regard to the development of coronary artery disease.

The effect of a low-fat diet on levels and distributions of larger and smaller LDL particles has been further investigated in the present report, using measurements of mass of lipoprotein subfractions by analytic ultracentrifugation. In particular, we wished to test: (1) whether the LDL cholesterol reduction in subjects with pattern B represented a significant reduction in levels of small, dense LDL; and (2) whether the smaller reduction in LDL cholesterol in pattern A subjects, without a concomitant reduction in plasma apoB level, represented a shift from larger, lipid-enriched to smaller, lipid-depleted LDL particles.

METHODS

Subjects

Healthy, non-smoking male volunteers over age 20 were recruited through newspaper and radio announcements, flyers, and direct mail contact. Eligibility criteria for acceptance into the study were as follows: (1) no cardiovascular disease,

acute illness, or active chronic disease in the past 5 years, (2) plasma total cholesterol concentration less than 260 mg/dL (6.72 mmol/L) and triglyceride concentration less than 500 mg/dL (5.65 mmol/L), (3) resting blood pressure less than 160/105 mm Hg, (4) body weight not greater than 130% of ideal (Metropolitan Life Insurance Company Tables, 1985), (5) no use of medication likely to interfere with lipid metabolism, and (6) no apo E2/2 phenotype. Each participant signed a consent form approved by the Committee for the Protection of Human Subjects at Lawrence Berkeley Laboratory, University of California, Berkeley, and participated in a medical interview. The age and body mass index (BMI) (wt {kg}/ht $\{m\}^2$) (mean ± SD) of the 105 men who completed the study were 48.9 ± 11.1 years (range 28.0-79.0) and 25.5 ± 3.0 kg/m^2 (range 17.4-35.1), respectively.

Experimental Design

As described previously (10), the subjects were randomly assigned to outpatient treatment with a high-fat (46%) or low-fat (24%) diet (Table 1) for six weeks each in a double crossover design. Although half the subjects had the low-fat diet first, we use the expression "change from high-fat to low-fat diet", for every variable, to mean "low-fat value minus high-fat value", regardless of the actual order of the diets. The participants were instructed on the experimental diets by registered dietitians and were given two-week cycle menus demonstrating number and size of servings. Diet composition was calculated from the average of the two-week menus using the Minnesota Nutrition Data System (NDS) software, developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN, Version 2.1 (24,25). Subjects were instructed to refrain from alcohol during the study and to keep exercise and body weight constant between the two diets. The staff contacted the subjects weekly to encourage motivation.

Subjects were surveyed for body weight, dietary intake (4-day food records of Thursday to Sunday) (24-26), and plasma lipids and lipoproteins at screening and during the last week of each experimental diet. Body weights were measured daily at home and caloric intake was adjusted to minimize weight variability. BMI was calculated at screening and after each experimental diet.

As reported previously (10), mean nutrient intake as estimated from the reported four-day food records indicated good compliance to the experimental diets, and there were no significant differences in reported nutrient intake between the subjects by LDL subclass pattern. Mean BMI was not significantly different for the LDL subclass groups and there were no significant changes in mean body weight between any of the subgroups throughout the experimental period (data not shown).

Laboratory Analyses

Lipids, Lipoproteins, and Apolipoproteins. Venous blood samples were collected in tubes containing Na2EDTA, 1.4 mg/ml, after the subjects had fasted for 12-14 hours. Plasma was prepared within two hours of collection, and blood and plasma were kept at 4°C until processed. Plasma total cholesterol and triglyceride levels were determined by enzymatic procedures on a Gilford Impact 400E analyzer. HDL-C was measured after heparin sulfate and magnesium chloride precipitation of plasma (27), and LDL-C was calculated from the formula of Friedewald et al. (28). Apo A-I and apo B concentrations in plasma were determined by maximal radial immunodiffusion (29,10).

LDL Subclass Patterns. Non-denaturing polyacrylamide gradient gel electrophoresis, which separates LDL particles by size and shape, was used to identify subpopulations of LDL particles (30). Electrophoresis of whole plasma was performed using Pharmacia PPA 2/16% gradient gels as described previously (30,31).

Stained gels were scanned with a Transidyne RFT Scanning Densitometer and peak particle diameters were calculated from calibration curves using standards of known size. The coefficient of variation of the calculated particle diameters has been estimated to be <3% by this procedure (30).

On the basis of the resulting scans, LDL subclass patterns were identified as described previously (32). Pattern B is characterized by a major peak of smaller, denser LDL particles (LDL III, diameter 255Å or less), often with skewing to larger particle diameters. Pattern A is characterized by a predominance of larger, more buoyant LDL particles (LDL I or II, diameter 264Å or greater), often with skewing to smaller particle diameters. Some individuals have an intermediate LDL subclass pattern with a single or double peak of LDL in the size range of 256-263Å (LDL II). LDL subclass patterns were determined for all study subjects at the end of the standardized high-fat and low-fat dietary periods by three readers who were blinded as to the subjects' identity and high- or low-fat diet treatment. For the analyses presented below, intermediate patterns were grouped with A patterns ("narrow" definition of pattern B (14,21,18). The results did not differ substantially when men who exhibit intermediate patterns were excluded from the analyses.

Analytical Ultracentrifugation. Lipoproteins were analyzed by analytical ultracentrifugation which measures mass of lipoproteins as a S_f^o function of Svedberg flotation rate (S_f^o d<1.063; and $F_{1.20}^o$ d<1.21) (33). Mass concentrations were determined for total LDL (S_f^o 0-12) and levels of four major LDL subclasses, LDL I (S_f^o 7-12), LDL II (S_f^o 5-7), LDL III (S_f^o 3-5), and LDL IV (S_f^o 0-3) (30); IDL (S_f^o 12-20); and VLDL S_f^o 20-400). For LDL, this procedure provides a measurement of peak S_f^o, as well as density (g/mL), and size (Å) of the peak LDL for each subject (33). In addition, mass was determined for total HDL ($F_{1.20}^o$), and levels of two major HDL subclasses, HDL2 ($F_{1.20}^o$ 3.5-9) and HDL3 ($F_{1.20}^o$ 0-3.5) (33).

Statistics

Mean levels of lipoprotein measurements are reported separately for the high-fat and low-fat diets and for differences between the two diets by LDL subclass pattern. Univariate analyses were by the Kruskal-Wallis test, when three groups were being compared, and by Wilcoxon Signed Rank test, for paired difference analyses. The changes reported herein were not related to the actual order of the diets. Multivariate analyses were performed by multiple regression. Analysis of variance was used to estimate each of the effects in the multivariate model, with the intercept set to zero so that the main effect of each group could be estimated. SAS software (34,35) was used to perform all data analyses.

RESULTS

Effects of high-fat and low-fat diets on levels of plasma lipids, mass of lipoprotein fractions, and LDL particle distribution

In Table 2 are presented plasma levels of lipids, lipoproteins, and major lipoprotein fractions in all subjects on the two diets. Increased plasma triglyceride levels on the low-fat, high-carbohydrate diet was associated with increases in VLDL mass of comparable magnitude in large, intermediate, and small VLDL particles. Reduced LDL cholesterol resulted from reductions in lipoproteins of S_f^o 5-14, including small IDL and larger LDL I and LDL II particles. These decreases were partially offset by increased levels of smaller, denser LDL III and LDL IV. Finally, reductions in HDL cholesterol were found in conjunction with reduced HDL_2 and a smaller reduction in HDL_3.

On the high-fat diet, the majority of subjects exhibited LDL subclass pattern A (n=72) or an intermediate phenotype (n=15), while 18 subjects were found to have

pattern B. Figure 1A displays the distribution of the particle diameters of the major LDL peaks as determined by gradient gel electrophoresis in all subjects on the high-fat diet. A bimodal particle size distribution was observed. The larger grouping defined by this modality comprised peak particle diameters of ≥ 258Å, and included values for all 87 subjects with the A or intermediate patterns, and for only one of the subjects with pattern B. On the low-fat diet, 36 subjects (26 with pattern A and 10 with an intermediate pattern on the high-fat diet) converted to pattern B on the low-fat diet, while all subjects with pattern B on the high-fat diet retained this classification on the low-fat diet. The particle size distribution on the low-fat diet (Figure 1B) again revealed two modes. As on the high-fat diet, the grouping with peak particle diameter ≥ 258Å on the low-fat diet comprised all subjects with pattern A and an intermediate phenotype (n=51), and only 3 of the 54 subjects with pattern B. Results for the subjects with an intermediate phenotype were included with those for the pattern A group, as described previously (10).

Table 3 presents plasma lipid and lipoprotein values for the 51 subjects with pattern A or an intermediate pattern on both diets (stable pattern A group). In general the significant changes on the low-fat vs. high-fat diet paralled those described in Table 2 for the group as a whole, with the exception that there was no significant reduction in mass of LDL II or HDL_3. Table 3 also shows that the low-fat diet induced significant reduction in LDL peak flotation rate and particle diameter in this group, although the mean values remained well within the pattern A range.

Lipid and lipoprotein measurements for the subjects with pattern B on both diets (stable pattern B group) are given in Table 4. On each of the diets, compared with the stable pattern A group, stable pattern B subjects had significantly ($p<0.001$) higher levels of triglyceride, and masses of all VLDL fractions, large IDL, LDL III and LDL IV. In addition the stable B group had significantly ($p<0.001$) lower levels of LDL I mass, HDL-cholesterol, and mass of the HDL_2 subfraction, and, as expected,

lower LDL peak flotation rates and particle diameters. Mean LDL-cholesterol was higher than that in the stable A group on the high-fat diet ($p<0.05$), but not on the low-fat diet, while mean plasma apoB level was substantially higher on the high-fat diet ($p<0.0001$), and less so on the low-fat diet ($p<0.05$). Finally, on the low-fat diet only, mass of small IDL was higher ($p<0.05$) and mass of LDL II was lower ($p<0.01$) in the stable B than the stable A subjects.

Diet-induced changes in plasma lipids and lipoprotein fractions

Table 4 also presents the significance of the differences between the stable pattern A and B subgroups in the magnitude of diet-induced changes in plasma lipids and lipoproteins. The most striking difference was a three-fold greater reduction in LDL-cholesterol ($p<0.0001$) in the stable pattern B group, due primarily to a greater reduction in mass of LDL II ($p<0.01$), and LDL III ($p<0.001$). On the other hand, compared with the stable A group, there was a smaller reduction in LDL I, and a greater increase in LDL IV (both $p<0.01$). LDL peak flotation rate and particle diameter decreased somewhat less in stable pattern B compared with stable A subjects, but the group differences were not significant at $p<0.05$.

Notably, as reported previously (10), there was a significant reduction in plasma apoB in the stable pattern B subjects ($p<0.01$), while as described for Table 2, there was no mean change for stable A (group difference significant at $p<0.001$).

The stable pattern B group also exhibited a three-fold greater increase in plasma triglyceride on the low-fat diet than did the stable pattern A subjects ($p<0.05$). Interestingly, among the VLDL subfractions, the increase in large VLDL was greatest for the stable B group and lowest for the stable A group ($p=0.011$ for the group difference). Pattern B subjects also had a greater increase in intermediate sized VLDL (group difference $p<0.01$), while the mean increases in small VLDL were very

similar for the two groups, although the increase was not significant at $p<0.05$ for the stable B group.

Finally, Tables 3 and 4 show that while there were similar reductions in HDL-cholesterol for the stable A and stable B subgroups on the low-fat diet, the reduction in HDL mass for the stable A group was almost exclusively in HDL_2, while the reduction in the stable B group was primarily in HDL_3 ($p <0.05$ for both group differences).

Table 5 presents the lipid and lipoprotein results for the 36 subjects who changed to pattern B on the low-fat diet. On both diets, analysis of variance indicated that levels of plasma triglyceride as well as the VLDL and large IDL fractions were intermediate between the stable pattern A and B groups (not significant for large VLDL on the high-fat diet only). Levels of LDL cholesterol, small IDL mass, and plasma apoB were higher in the change group than in the stable pattern A group on both diets, but were not significantly different from values for subjects with stable pattern B. Among the LDL fractions, the change in phenotype on the low-fat diet was associated with reductions in mass of LDL I and LDL II, and increases in mass of LDL III and LDL IV. On the high fat diet (where pattern A was manifest in the change group), levels of LDL II and LDL III were significantly higher than in the stable A subjects, while on the low-fat diet (where pattern B was expressed), levels of LDL II were higher, and LDL IV lower than in the stable B group.

HDL-cholesterol levels were similar in the change and pattern A groups on the high-fat diet (Table 5), but were lower on on the low-fat diet, and higher than levels for stable B subjects on both diets. On both diets mass of HDL_2 was lower than in the stable A group, and mass of HDL_3 was higher on the high-fat diet only.

Analysis of variance indicated that diet-induced increases in triglyceride and all VLDL fractions were significantly greater in the change group than in the stable

A group, but not significantly different from the changes in the stable B group (data not shown). Decreases in LDL-cholesterol were intermediate, while as in the stable A group, there was no reduction in plasma apoB. The magnitude of both the reduction in LDL I and the increase in LDL III in the change group significantly exceeded that in the other two categories. Finally, the reduction in HDL-cholesterol on the low-fat diet was greatest in the change group, with a greater reduction of HDL_3 compared with the stable A group, and a greater reduction of HDL_2 compared with the stable B group.

Interrelated diet-induced changes in lipoprotein subfractions

Table 6 shows that changes among LDL subfractions and VLDL on the low-fat diet were intercorrelated and that the relationships differed among the three phenotypic categories. Increases in total VLDL mass were correlated with increases increases in LDL IV in all groups and with increased LDL III in the change group. Reductions in LDL I were strongly inversely correlated with increases in LDL III in the atable pattern A and change groups, while reductions in LDL II were correlated with increases in LDL IV in the stable B group. Finally, changes in LDL III were positively correlated with changes in both LDL II and LDL IV in the stable A group, while decreases in LDL I and LDL II were strongly intercorrelated in the stable A group.

Multiple regression models for prediction of LDL changes

Determinants of the magnitude of reductions in LDL cholesterol, apoB, and mass of LDL subfractions on the low-fat diet were evaluated in multiple regression models (Table 7). Independent variables examined included LDL cholesterol, triglyceride, and LDL subclass pattern on the high-fat diet. Age and BMI were not significant predictors of LDL response. Both LDL subclass pattern and high-fat LDL-

cholesterol independently predicted reduction in LDL cholesterol and apoB, explaining a total of 22% and 15% of the interindividual variance, respectively. Inclusion of fasting triglyceride level caused no significant change in these regression parameters. Among LDL subfractions, reduction in mass of LDL I and increase in mass of LDL III was related to both high-fat LDL-cholesterol (positive) and triglyceride (negative) with a significant triglyceride-LDL subclass pattern interaction. On the other hand, reductions in LDL II mass and increases in LDL IV were related to LDL subclass pattern (greater for pattern B) and a triglyceride-subclass pattern interaction, with these factors explaining 28% of the variance of change in LDL II and 17% of the variance in LDL IV. For pattern B subjects, higher triglyceride levels predicted increases (or smaller decreases) in LDL II and decreases (or smaller increases) in LDL IV, while for pattern A, higher triglyceride predicted decreases (or smaller increases) in LDL II.

The results in Table 7 were not significantly different when high-fat peak LDL particle diameter, as a continuous variable, was substituted for the dichotomous LDL subclass pattern groupings (data not shown). In addition, for prediction of LDL cholesterol change, high-fat LDL mass was used as a surrogate variable for high-fat LDL cholesterol to minimize any effect of regression to the mean (36), and again the results were not significantly altered (data not shown).

DISCUSSION

The present study was designed to determine whether differences in LDL particle distribution contribute to interindividual variation in response to reduced fat, high carbohydrate diets. In a previous report based on this study (10), it was shown that the group of 18 individuals with predominantly smaller LDL particles (subclass pattern B) on a 46% fat diet exhibited a two-fold greater reduction in LDL-cholesterol after consuming a 24% fat diet than did the 87 subjects with a predominance of larger LDL (pattern A). Moreover, reductions in plasma levels of apoB, a measure of the number of potentially atherogenic particles, were observed in the group with pattern B on the high-fat diet, but not in subjects with pattern A. Finally, 44% of the pattern A subjects converted to pattern B on the low-fat diet. These findings suggested that a substantial portion of the LDL cholesterol reduction in pattern A subjects might be explained by a shift from larger, cholesterol-enriched to smaller, cholesterol-depleted particles, without a change in LDL particle number. In contrast, it was suggested that a reduced number of LDL particles contributed to the greater LDL cholesterol reduction observed in pattern B subjects.

The analyses in the present report addressed these hypotheses using measurements of LDL subfractions by analytical ultracentrifugation. We found that the reduction in LDL-cholesterol in subjects with pattern A on both the high-fat and low-fat diets was primarily due to a reduction in mass of the largest, most buoyant LDL subfractions, corresponding to LDL I, while there was a reciprocal increase in mass of smaller, denser LDL fractions, corresponding to LDL III. In contrast, in subjects with pattern B on the high-fat diet, the greatest reductions were observed for subfractions of intermediate size and density (LDL II), with reciprocal increases in the smallest, most dense LDL IV fractions. Compared with the stable pattern A group, there were greater reductions in both LDL II and LDL III, presumably accounting for reduced plasma apoB, and hence, reduced particle number.

The findings in the group who changed LDL subclass pattern confirmed the suggestion that this change was due to reductions in mass of larger LDL (both LDL I and LDL II), with reciprocal increases in smaller, denser LDL (LDL III and LDL IV). Thus in both the stable pattern A and change groups, it is likely that a substantial portion of the reduction in LDL-cholesterol resulted from a shift from more buoyant, lipid-enriched to more dense, lipid-depleted LDL particles. It is also possible that other compositional differences in LDL particles, such as exchange of triglyceride for cholesteryl ester (37,38), could have contributed to the reduction in LDL-cholesterol on the low-fat diet. Such triglyceride enrichment could be promoted by the increases in levels of VLDL triglyceride observed on the low-fat, high-carbohydrate diet in all subjects. However, in preliminary studies we have not detected significant differences in LDL cholesteryl ester/triglyceride ratios in fractions of similar density from normolipidemic subjects on the high- and low-fat diets studied here (Tribble, D.L., Krauss, R.M., unpublished)

The low-fat high-carbohydrate studied here also induced differential changes in VLDL subfractions in the LDL subclass groups. Stable pattern A subjects showed a predominant increase in small and intermediate sized VLDL, while the greatest increase in pattern B subjects was in the largest VLDL fractions. Thus, while high carbohydrate diets are reported to increase plasma levels of larger, more triglyceride-rich VLDL particles (39), this effect appears to be most pronounced for subjects with LDL subclass pattern B.

The only consistent relationship between VLDL levels and LDL subclass changes was a positive correlation with the minor LDL IV fraction in all subclass groups. Thus it is not clear whether diet-induced changes in triglyceride-rich lipoproteins are directly connected with the changes in LDL subfraction profile reported here. It is possible that some other parameter of triglyceride-rich lipoprotein metabolism would be more indicative of such a connection than the

mass measurements reported here. It is also possible that changes in VLDL and LDL fractions were affected by different components of the dietary intervention. For example, the increase in triglyceride and VLDL may represent primarily the well-known effects of increased carbohydrate and simple sugar intake (39), while the effects on the LDL fractions may have been more strongly influenced by increased LDL receptor activity induced by the low-fat diet (40). The finding of only small and inconsistent changes in IDL mass among subjects in all groups is unexpected, and raises the possibility of metabolic regulation of IDL levels in response to dietary change.

Multivariate analyses (Table 7) indicated that LDL subclass patterns on the high-fat diet were independent predictors of diet-induced changes in LDL-cholesterol, and apoB, and along with a significant triglyceride interaction, were also predictors of LDL II and LDL IV response, accounting for 8-13% of the interindividual variance of these parameters. As reported by others (36), high-fat LDL-cholesterol level strongly predicted the magnitude of LDL-cholesterol reduction on the low-fat diet. Among the LDL subfractions, this was particularly striking for LDL I, where high-fat LDL-cholesterol accounted for 32% of the variance in the response, but effects on the other LDL fractions were much smaller.

We have previously reported that pattern B is characterized by interrelated metabolic differences from pattern A including increased triglycerides, reduced HDL-C (18), and recently, insulin resistance (19,20). There is evidence that alterations in triglyceride metabolism may be of fundamental importance in pathways resulting in production of small, dense LDL (41-45). However, our previous analyses (10), as well as the multiple regression models presented here (Figure 7), indicate that the association of LDL particle size with triglyceride level did not influence the LDL-C or apo B responses to the low-fat diet. On the other hand, for the LDL subfractions, the regression results suggested that dietary response was

weakly influenced by factors related to plasma triglyceride level. For pattern A subjects, lower triglyceride levels predicted greater decreases in LDL I and increases in LDL II. For pattern B subjects, lower triglyceride levels predicted greater decreases in both LDL I and LDL II, and increases (or smaller decreases) in LDL III and LDL IV. The possible metabolic basis for these interactions is not apparent; they do, however, suggest that some aspects of diet-induced changes in the LDL subfraction profile in both pattern A and B subjects may be amplified by factors which decrease plasma triglyceride levels.

Family studies have suggested heritability of LDL subclass patterns, with a major gene influencing the inheritance of pattern B (14-16,35,37). Recent studies have identified several potential loci for pattern B (17,46); however, the responsible genetic defect(s) are unknown. Heritability analyses suggest that approximately one third to one half of the variation in LDL size could be attributed to genetic factors (11,12) with the remainder due to environmental effects, such as adiposity (47), hormonal factors (48), and, as shown here, diet composition. The present findings suggest that the gene(s) influencing LDL particle size distribution may also be contributing to the variation in dietary responsiveness reported here.

In a separate analysis, we have found that apoE isoforms in the subjects studied here also influenced dietary LDL response (49). The apoE4 isoform was associated with the largest LDL response to reduced dietary fat, and this effect was limited to larger, more buoyant LDL subfractions. We have also found that, in contrast to the LDL subclass effect, apoE isoform phenotypes accounted for only a small portion (<5%) of the variance in LDL response to diet (Dreon, D.M., Krauss, R.M., unpublished).

The present findings may have implications regarding the potential impact of low-fat, high carbohydrate diets on coronary disease risk. A number of case-control studies have established that a predominance of small dense LDL is associated with

increased risk of myocardial infarction (21,50) and angiographically assessed CAD (22,23,50). Because of the strong interrelationships of other metabolic variables with LDL particle distribution, in most of these studies it has not been possible to determine whether small LDL contribute directly to CAD risk. However, recent studies have shown that small, dense LDL are potentially more atherogenic than larger LDL by virtue of increased susceptibility to oxidative modification (51,52) and increased promotion of intracellular cholesterol ester accumulation (53). The results from the present study suggest that while individuals with LDL subclass pattern B are at higher CAD risk than pattern A subjects on a high-fat diet, when placed on a low-fat diet they may experience a greater relative improvement in risk by virtue of significant reductions in the numbers of smaller, dense LDL particles (both LDL II and LDL III). This inference is consistent with results recently reported from the St. Thomas Atherosclerosis Regression Study, in which a diet-induced reduction in LDL III was the strongest lipoprotein predictor of the benefit of intervention on CAD progression assessed angiographically (54).

It is not known to what extent concomitant increases in triglycerides and reductions in HDL-cholesterol might offset the benefits that could be attributed to diet-induced reductions of smaller LDL particles in pattern B subjects. The present studies indicate that the increase in triglyceride level in these subjects was associated primarily with large VLDL particles. Although it has been pointed out that large VLDL particles in hypertriglyceridemic subjects may have atherogenic properties (55), it has also been suggested that increases in large VLDL induced by estrogen treatment may not be of pathologic significance (56). Further, prospective studies of patients with angiographically defined CAD have suggested that triglyceride-rich lipoprotein lipolytic remnants and IDL may have a more direct role in atherosclerosis progression than larger VLDL particles (57,58). With regard to HDL, the reduction in pattern B subjects was limited to the HDL3 subfraction, whereas a

similar magnitude of HDL-cholesterol reduction in pattern A was confined to HDL_2. However, the relative importance of these subfractions with regard to CAD risk has not been clearly established (59,60).

In summary, the present study suggests that a low-fat, high carbohydrate diet may preferentially benefit the minority of subjects with a high-risk lipoprotein profile characterized by increased levels of small, dense LDL particles. Genetic as well as environmental factors that have been shown to influence this phenotype may also contribute to interindividual variation in dietary response and promote beneficial LDL reduction on a low-fat diet. However, the smaller LDL-cholesterol reductions associated with a shift from larger to smaller LDL may have less favorable effects on CAD risk in the majority of the healthy population with predominantly larger LDL particles.

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Table 1

Nutrient Content of Experimental Diets*

	High-Fat	Low-Fat
Calories	2884	2880
% Fat	46.0	23.9
% Saturated	18.3	5.4
% Monounsaturated	12.4	12.3
% Polyunsaturated	12.5	4.0
% Carbohydrate	38.6	60.0
% Protein	16.2	16.1
Cholesterol (mg)	411.5	360.2
P/S ‡	0.69	0.74
Dietary Fiber (gm)	14.0	14.4

* Mean of 2-week cycle menu for 2880 calorie level

‡ The ratio of polyunsaturated to saturated fat.

Table 2

Plasma Lipoprotein Concentrations in All Subjects

	High-Fat Diet	Low-Fat Diet	Difference (low-fat minus high-fat)
		mg/dl, mean ± SEM	
Triglycerides	100.0 ± 4.8	140.4 ± 7.6	40.4 ± 5.8**
VLDL Mass:			
Large S_f^o 100-400	15.4 ± 1.8	31.0 ± 3.3	15.6 ± 2.8**
Intermed. S_f^o 60-100	19.4 ± 1.8	36.3 ± 2.7	16.9 ± 2.3**
Small S_f^o 20-60	41.6 ± 2.9	59.0 ± 3.2	17.4 ± 2.5**
LDL Cholesterol	142.8 ± 3.3	126.2 ± 3.1	-16.6 ± 1.9**
IDL Mass:			
Large S_f^o 14-20	20.0 ± 1.2	21.0 ± 1.2	1.0 ± 1.0
Small S_f^o 10-14	35.0 ± 1.1	31.0 ± 1.0	-3.9 ± 0.9**
LDL Mass:			
LDL I S_f^o 7-10	109.4 ± 4.2	74.4 ± 3.6	-35.0 ± 3.1**
LDL II S_f^o 5-7	123.0 ± 3.8	107.3 ± 3.4	-15.6 ± 3.2**
LDL III S_f^o 3-5	60.0 ± 3.7	80.7 ± 3.9	20.8 ± 3.2**
LDL IV S_f^o 0-3	11.0 ± 1.0	17.9 ± 1.5	6.9 ± 1.2**
HDL Cholesterol	49.0 ± 1.0	42.0 ± 0.8	-7.0 ± 0.6**
HDL Mass:			
HDL_2 $F_{1.20}^o$	37.1 ± 3.3	24.7 ± 2.4	-12.4 ± 2.2**
HDL_3 $F_{1.20}^o$	190.8 ± 3.2	181.9 ± 3.6	-8.8 ± 2.7*

*$p < 0.01$; **$p < 0.0001$

Table 3

Plasma Lipoprotein Concentrations in Stable LDL Subclass Pattern A Group

	High-Fat Diet	Low-Fat Diet	Difference (low-fat minus high-fat)
		mg/dl, mean ± SEM	
Triglycerides	76.6 ± 4.7	96.8 ± 4.6	20.2 ± 5.0**
VLDL Mass:			
Large S_f^o 100-400	10.0 ± 2.0	15.5 ± 2.0	5.5 ± 2.3†
Intermed. S_f^o 60-100	12.1 ± 2.2	22.3 ± 2.0	10.2 ± 2.4***
Small S_f^o 20-60	28.4 ± 2.9	41.0 ± 2.4	12.6 ± 3.0***
LDL Cholesterol	131.8 ± 4.9	121.6 ± 4.9	-10.3 ± 2.3***
IDL Mass:			
Large S_f^o 14-20	14.1 ± 1.4	14.3 ± 1.5	0.1 ± 1.5
Small S_f^o 10-14	31.6 ± 1.5	28.1 ± 1.5	-3.6 ± 1.2*
LDL Mass:			
LDL I S_f^o 7-10	125.6 ± 6.4	94.2 ± 5.9	-31.4 ± 5.1***
LDL II S_f^o 5-7	113.4 ± 5.5	115.6 ± 5.0	2.2 ± 3.3
LDL III S_f^o 3-5	38.6 ± 2.6	51.1 ± 3.8	12.5 ± 3.4**
LDL IV S_f^o 0-3	7.7 ± 0.9	9.1 ± 0.9	1.3 ± 1.0
HDL Cholesterol	51.4 ± 1.4	45.4 ± 1.2	-6.0 ± 1.0***
HDL Mass:			
HDL_2 $F_{1.20}^o$ 3.5-9	48.7 ± 5.4	37.4 ± 3.9	-11.3 ± 3.3*
HDL_3 $F_{1.20}^o$ 0-3.5	183.8 ± 4.1	182.3 ± 4.1	-1.5 ± 3.8
Apo B	98.3 ± 3.2	98.4 ± 3.3	0.1 ± 1.8
Peak S_f^o	6.7 ± 0.1	6.2 ± 0.1	-0.6 ± 0.1***
Peak Diameter (Å)	268.1 ± 0.6	265.4 ± 0.6	-2.7 ± 0.8**

†$p < 0.05$; *$p < 0.01$; ** $p < 0.001$; ***$p < 0.0001$

Table 4

Plasma Lipoprotein Concentrations in Stable LDL Subclass Pattern B Group

	High-Fat Diet	Low-Fat Diet	Difference (low-fat minus high-fat)	Significance of difference (p) vs. pattern A
	mg/dl Mean ± SEM			
Triglycerides	166.1 ± 12.3	225.4 ± 19.0	59.3 ± 17.2*	0.04
VLDL Mass:				
Large S_f^o 100-400	34.8 ± 5.8	66.5 ± 10.9	31.8 ± 9.0*	0.01
Intermed. S_f^o 60-100	40.1 ± 4.3	64.7 ± 6.5	24.6 ± 5.6**	0.01
Small S_f^o 20-60	78.1 ± 8.3	91.2 ± 6.2	13.0 ± 7.0	0.95
LDL Cholesterol	150.8 ± 7.5†	120.7 ± 7.6	-30.1 ± 4.6	0.0001
IDL Mass:				
Large S_f^o 14-20	31.2 ± 3.0	29.8 ± 1.7	-1.4 ± 2.2	0.61
Small S_f^o 10-14	36.5 ± 2.4	33.8 ± 1.8	-2.7 ± 1.8	0.70
LDL Mass:				
LDL I S_f^o 7-10	62.5 ± 4.1	49.9 ± 4.0	-12.6 ± 3.6*	0.003
LDL II S_f^o 5-7	114.3 ± 7.9	81.4 ± 9.4	-32.9 ± 6.7***	0.0001
LDL III S_f^o 3-5	118.3 ± 8.7	105.6 ± 7.4	-12.6 ± 6.3	0.0004
LDL IV S_f^o 0-3	23.5 ± 3.7**	37.1 ± 4.3	13.6 ± 3.8*	0.005
HDL Cholesterol	41.4 ± 1.8**	36.3 ± 1.8	-5.2 ± 0.9***	0.52
HDL Mass:				
HDL_2 $F_{1.20}^o$ 3.5-9	14.2 ± 3.8	11.2 ± 2.9	-3.0 ± 2.0	0.03
HDL_3 $F_{1.20}^o$ 0-3.5	188.7 ± 9.1	170.6 ± 6.8	-18.1 ± 6.5†	0.03
Apo B	126.3 ± 5.9	114.7 ± 4.6	-11.6 ± 2.7**	0.001
Peak S_f^o	4.6 ± 0.1	4.2 ± 0.1	-0.4 ± 0.1*	0.24
Peak Diameter (Å)	252.9 ± 0.7	250.8 ± 1.0	-2.0 ± 1.0	0.63

†$p < 0.05$; *$p < 0.01$; ** $p < 0.001$; ***$p < 0.0001$

Table 5

Plasma Lipoprotein Concentrations in Changed LDL Subclass Pattern Group

	High-Fat Diet	Low-Fat Diet	Difference (low-fat minus high-fat)
		mg/dl, mean ± SEM	
Triglycerides	99.9 ± 5.4	159.5 ± 13.3	59.5 ± 11.6***
VLDL Mass:			
Large S_f^o 100-400	13.2 ± 2.0	35.1 ± 5.3	21.8 ± 5.3**
Intermed. S_f^o 60-100	19.5 ± 2.1	42.0 ± 5.1	22.5 ± 44.6***
Small S_f^o 20-60	42.1 ± 3.2	68.4 ± 6.0	26.3 ± 4.7***
LDL Cholesterol	154.2 ± 4.7	135.5 ± 4.3	-18.7 ± 3.5***
IDL Mass:			
Large S_f^o 14-20	22.8 ± 1.6	26.2 ± 1.8	3.4 ± 1.6†
Small S_f^o 10-14	38.9 ± 1.7	33.9 ± 1.5	-5.1 ± 1.6*
LDL Mass:			
LDL I S_f^o 7-10	109.9 ± 4.5	58.6 ± 2.3	-51.3 ± 3.9***
LDL II S_f^o 5-7	140.9 ± 5.6	108.6 ± 4.2	-32.3 ± 5.3***
LDL III S_f^o 3-5	61.2 ± 4.4	110.3 ± 4.7	49.1 ± 4.0***
LDL IV S_f^o 0-3	9.4 ± 1.1	20.7 ± 2.1	11.3 ± 2.0***
HDL Cholesterol	49.4 ± 1.7	40.0 ± 1.1	-9.4 ± 1.0***
HDL Mass:			
HDL_2 $F_{1.20}^o$ 3.5-9	32.1 ± 4.4	13.4 ± 1.7	-18.7 ± 3.9***
HDL_3 $F_{1.20}^o$ 0-3.5	201.7 ± 5.5	187.1 ± 5.6	-14.6 ± 4.5*
Apo B	118.6 ± 3.8	121.1 ± 3.7	2.5 ± 2.3
Peak S_f^o	6.0 ± 0.1	4.7 ± 0.1	-1.3 ± 0.1***
Peak Diameter (Å)	266.4 ± 0.7	253.0 ± 0.5	-13.4 ± 0.7***

†$p < 0.05$; *$p < 0.01$; **$p < 0.001$; ***$p < 0.0001$

Table 6

Correlation Coefficients Among Major Diet-Induced Changes in Lipoprotein Variables

Group	VLDL S_f^o 20-400	LDL I S_f^o 7-10	LDL II S_f^o 5-7	LDL III S_f^o 3-5
Stable A (n = 51)				
LDL I S_f^o 7-10	-0.35†	--	-0.17	-0.75***
LDL II S_f^o 5-7	0.11	-0.17	--	0.36*
LDL III S_f^o 3-5	0.38*	-0.75***	0.36*	--
LDL IV S_f^o 0-3	0.32†	-0.21	0.04	0.45**
Stable B (n = 18)				
LDL I S_f^o 7-10	-0.22	--	0.78**	-0.44
LDL II S_f^o 5-7	-0.45	0.78**	--	-0.11
LDL III S_f^o 3-5	0.10	-0.44	-0.11	--
LDL IV S_f^o 0-3	0.75**	-0.59†	-0.77**	0.05
Change (n = 36)				
LDL I S_f^o 7-10	-0.02	--	0.21	-0.43*
LDL II S_f^o 5-7	-0.14	0.21	--	0.05
LDL III S_f^o 3-5	0.09	-0.43*	0.05	--
LDL IV S_f^o 0-3	0.40†	-0.21	-0.42†	0.46*

†$p < 0.05$; *$p < 0.01$; **$p < 0.001$; ***$p < 0.0001$

Table 7

Regression Models for Diet-Induced Changes (Low-Fat Minus High-Fat) in LDL Components

	LDL Chol.	apo B	LDL I S_f^o7-10	LDL II S_f^o5-7	LDL III S_f^o3-5	LDL IV S_f^o0-3
Independent variables (high-fat diet):				Coefficient		
Subclass A	14.90†	11.10†	-7.70	-9.70	47.10**	-12.60
Pattern B	0.55	-0.92	25.70†	10.40	-50.20†	4.40
LDL Chol	-0.20***	-0.07	0.05	0.26*	-0.22†	-0.39***
Trig. Pattern A	--	--	0.70	0.10	-0.33**	0.33**
Pattern B	--	--	-0.12†	-0.38*	0.30†	0.25†
R^2	-0.22	-0.15	-0.17	-0.33	-0.28	-0.33
R^2 without subclass pattern	-0.14	-0.05	-0.06	-0.20	-0.15	-0.32

†$p < 0.05$; *$p < 0.01$; **$p < 0.001$; ***$p < 0.0001$

Figure Legend

Distribution in 105 men on the high-fat (A) and low-fat (B) diets of particle diameter of the major LDL peak (determined by peak height) as analysed by gradient gel elecytrophoresis. Open bars and portions of bars represent subjects with LDL subclass pattern A; shaded bars and portions of bars represent subjects with LDL subclass pattern B (see methods).

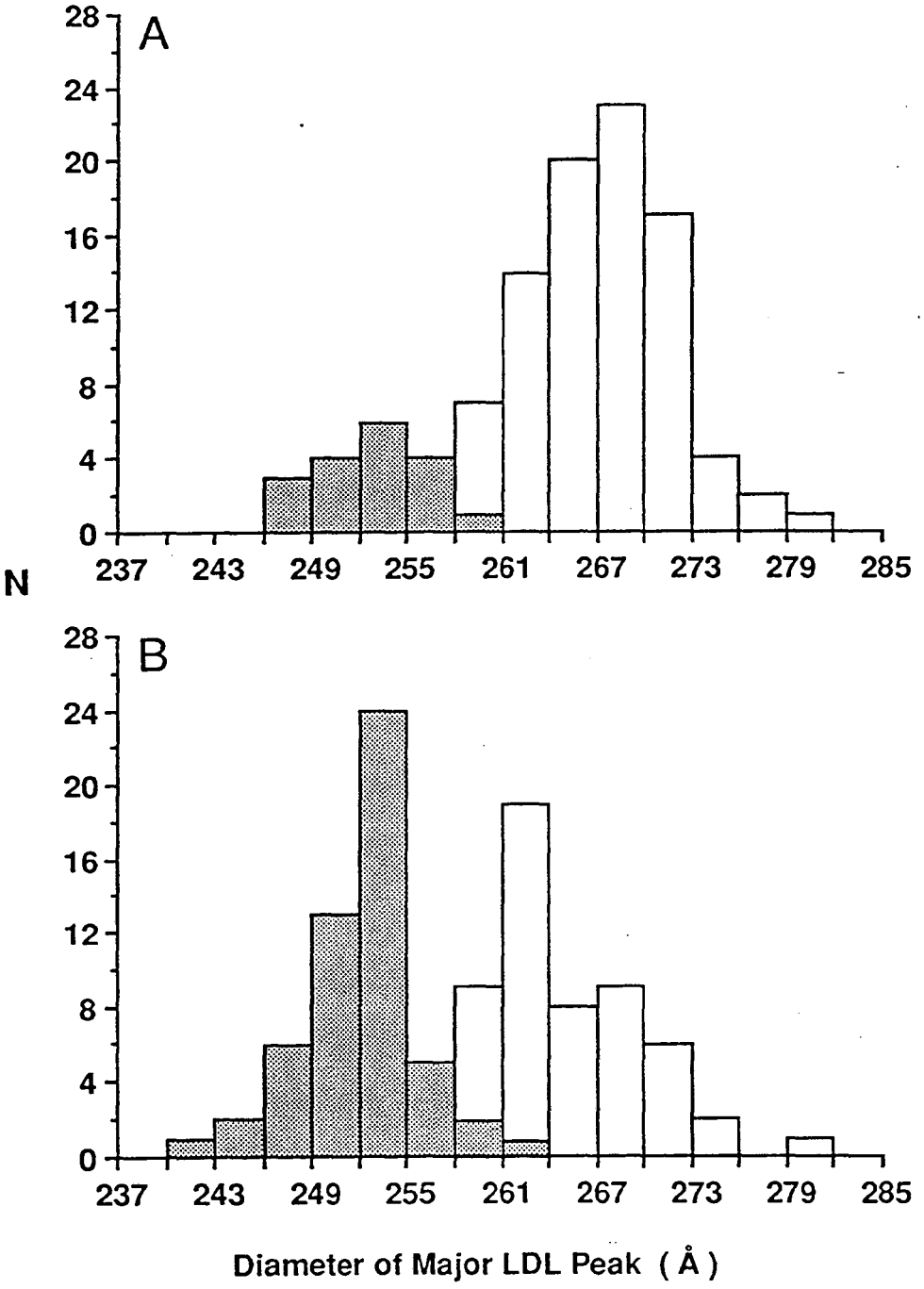

Figure 1
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