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

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MASTER

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INTRODUCTION

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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.

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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).

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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

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

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

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

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METHODS

24 Subjects

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

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

12

13 Experimental Design

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

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

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

13

14 **Laboratory Analyses**

15 *Lipids, Lipoproteins, and Apolipoproteins.* Venous blood samples were
16 collected in tubes containing Na₂EDTA, 1.4 mg/ml, after the subjects had fasted for
17 12-14 hours. Plasma was prepared within two hours of collection, and blood and
18 plasma were kept at 4°C until processed. Plasma total cholesterol and triglyceride
19 levels were determined by enzymatic procedures on a Gilford Impact 400E analyzer.
20 HDL-C was measured after heparin sulfate and magnesium chloride precipitation of
21 plasma (27), and LDL-C was calculated from the formula of Friedewald et al. (28).
22 Apo A-I and apo B concentrations in plasma were determined by maximal radial
23 immunodiffusion (29,10).

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

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

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

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

1 Statistics

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

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RESULTS

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15 Effects of high-fat and low-fat diets on levels of plasma lipids, mass of lipoprotein 16 fractions, and LDL particle distribution

17 In Table 2 are presented plasma levels of lipids, lipoproteins, and major
18 lipoprotein fractions in all subjects on the two diets. Increased plasma triglyceride
19 levels on the low-fat, high-carbohydrate diet was associated with increases in VLDL
20 mass of comparable magnitude in large, intermediate, and small VLDL particles.
21 Reduced LDL cholesterol resulted from reductions in lipoproteins of S_f^0 5-14,
22 including small IDL and larger LDL I and LDL II particles. These decreases were
23 partially offset by increased levels of smaller, denser LDL III and LDL IV. Finally,
24 reductions in HDL cholesterol were found in conjunction with reduced HDL₂ and a
25 smaller reduction in HDL₃.

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

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

15 Table 3 presents plasma lipid and lipoprotein values for the 51 subjects with
16 pattern A or an intermediate pattern on both diets (stable pattern A group). In
17 general the significant changes on the low-fat vs. high-fat diet paralleled those
18 described in Table 2 for the group as a whole, with the exception that there was no
19 significant reduction in mass of LDL II or HDL₃. Table 3 also shows that the low-fat
20 diet induced significant reduction in LDL peak flotation rate and particle diameter in
21 this group, although the mean values remained well within the pattern A range.

22 Lipid and lipoprotein measurements for the subjects with pattern B on both
23 diets (stable pattern B group) are given in Table 4. On each of the diets, compared
24 with the stable pattern A group, stable pattern B subjects had significantly ($p < 0.001$)
25 higher levels of triglyceride, and masses of all VLDL fractions, large IDL, LDL III and
26 LDL IV. In addition the stable B group had significantly ($p < 0.001$) lower levels of
27 LDL I mass, HDL-cholesterol, and mass of the HDL₂ subfraction, and, as expected,

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

7

8 Diet-induced changes in plasma lipids and lipoprotein fractions

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

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

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

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

3 Finally, Tables 3 and 4 show that while there were similar reductions in HDL-
4 cholesterol for the stable A and stable B subgroups on the low-fat diet, the reduction
5 in HDL mass for the stable A group was almost exclusively in HDL₂, while the
6 reduction in the stable B group was primarily in HDL₃ ($p < 0.05$ for both group
7 differences).

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

22 HDL-cholesterol levels were similar in the change and pattern A groups on
23 the high-fat diet (Table 5), but were lower on on the low-fat diet, and higher than
24 levels for stable B subjects on both diets. On both diets mass of HDL₂ was lower
25 than in the stable A group, and mass of HDL₃ was higher on the high-fat diet only.

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

1 A group, but not significantly different from the changes in the stable B group (data
2 not shown). Decreases in LDL-cholesterol were intermediate, while as in the stable
3 A group, there was no reduction in plasma apoB. The magnitude of both the
4 reduction in LDL I and the increase in LDL III in the change group significantly
5 exceeded that in the other two categories. Finally, the reduction in HDL-cholesterol
6 on the low-fat diet was greatest in the change group, with a greater reduction of
7 HDL₃ compared with the stable A group, and a greater reduction of HDL₂ compared
8 with the stable B group.

9

10 **Interrelated diet-induced changes in lipoprotein subfractions**

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

21

22 **Multiple regression models for prediction of LDL changes**

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

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

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

20

DISCUSSION

1
2 The present study was designed to determine whether differences in LDL
3 particle distribution contribute to interindividual variation in response to reduced
4 fat, high carbohydrate diets. In a previous report based on this study (10), it was
5 shown that the group of 18 individuals with predominantly smaller LDL particles
6 (subclass pattern B) on a 46% fat diet exhibited a two-fold greater reduction in LDL-
7 cholesterol after consuming a 24% fat diet than did the 87 subjects with a
8 predominance of larger LDL (pattern A). Moreover, reductions in plasma levels of
9 apoB, a measure of the number of potentially atherogenic particles, were observed
10 in the group with pattern B on the high-fat diet, but not in subjects with pattern A.
11 Finally, 44% of the pattern A subjects converted to pattern B on the low-fat diet.
12 These findings suggested that a substantial portion of the LDL cholesterol reduction
13 in pattern A subjects might be explained by a shift from larger, cholesterol-enriched
14 to smaller, cholesterol-depleted particles, without a change in LDL particle number.
15 In contrast, it was suggested that a reduced number of LDL particles contributed to
16 the greater LDL cholesterol reduction observed in pattern B subjects.

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

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

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

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

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

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

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

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

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

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

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

1 increased risk of myocardial infarction (21,50) and angiographically assessed CAD
2 (22,23,50). Because of the strong interrelationships of other metabolic variables with
3 LDL particle distribution, in most of these studies it has not been possible to
4 determine whether small LDL contribute directly to CAD risk. However, recent
5 studies have shown that small, dense LDL are potentially more atherogenic than
6 larger LDL by virtue of increased susceptibility to oxidative modification (51,52) and
7 increased promotion of intracellular cholesterol ester accumulation (53). The
8 results from the present study suggest that while individuals with LDL subclass
9 pattern B are at higher CAD risk than pattern A subjects on a high-fat diet, when
10 placed on a low-fat diet they may experience a greater relative improvement in risk
11 by virtue of significant reductions in the numbers of smaller, dense LDL particles
12 (both LDL II and LDL III). This inference is consistent with results recently reported
13 from the St. Thomas Atherosclerosis Regression Study, in which a diet-induced
14 reduction in LDL III was the strongest lipoprotein predictor of the benefit of
15 intervention on CAD progression assessed angiographically (54).

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

1 similar magnitude of HDL-cholesterol reduction in pattern A was confined to HDL₂.
2 However, the relative importance of these subfractions with regard to CAD risk has
3 not been clearly established (59,60).

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

13

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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)
	<u>mg/dl, mean \pm SEM</u>		
Triglycerides	100.0 \pm 4.8	140.4 \pm 7.6	40.4 \pm 5.8**
VLDL Mass:			
Large S_f^o 100-400	15.4 \pm 1.8	31.0 \pm 3.3	15.6 \pm 2.8**
Intermed. S_f^o 60-100	19.4 \pm 1.8	36.3 \pm 2.7	16.9 \pm 2.3**
Small S_f^o 20-60	41.6 \pm 2.9	59.0 \pm 3.2	17.4 \pm 2.5**
LDL Cholesterol	142.8 \pm 3.3	126.2 \pm 3.1	-16.6 \pm 1.9**
IDL Mass:			
Large S_f^o 14-20	20.0 \pm 1.2	21.0 \pm 1.2	1.0 \pm 1.0
Small S_f^o 10-14	35.0 \pm 1.1	31.0 \pm 1.0	-3.9 \pm 0.9**
LDL Mass:			
LDL I S_f^o 7-10	109.4 \pm 4.2	74.4 \pm 3.6	-35.0 \pm 3.1**
LDL II S_f^o 5-7	123.0 \pm 3.8	107.3 \pm 3.4	-15.6 \pm 3.2**
LDL III S_f^o 3-5	60.0 \pm 3.7	80.7 \pm 3.9	20.8 \pm 3.2**
LDL IV S_f^o 0-3	11.0 \pm 1.0	17.9 \pm 1.5	6.9 \pm 1.2**
HDL Cholesterol	49.0 \pm 1.0	42.0 \pm 0.8	-7.0 \pm 0.6**
HDL Mass:			
HDL ₂ $F_{1.20}^o$	37.1 \pm 3.3	24.7 \pm 2.4	-12.4 \pm 2.2**
HDL ₃ $F_{1.20}^o$	190.8 \pm 3.2	181.9 \pm 3.6	-8.8 \pm 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)
	<u>mg/dl, mean \pm SEM</u>		
Triglycerides	76.6 \pm 4.7	96.8 \pm 4.6	20.2 \pm 5.0**
VLDL Mass:			
Large S _f ^o 100-400	10.0 \pm 2.0	15.5 \pm 2.0	5.5 \pm 2.3 [†]
Intermed. S _f ^o 60-100	12.1 \pm 2.2	22.3 \pm 2.0	10.2 \pm 2.4***
Small S _f ^o 20-60	28.4 \pm 2.9	41.0 \pm 2.4	12.6 \pm 3.0***
LDL Cholesterol	131.8 \pm 4.9	121.6 \pm 4.9	-10.3 \pm 2.3***
IDL Mass:			
Large S _f ^o 14-20	14.1 \pm 1.4	14.3 \pm 1.5	0.1 \pm 1.5
Small S _f ^o 10-14	31.6 \pm 1.5	28.1 \pm 1.5	-3.6 \pm 1.2*
LDL Mass:			
LDL I S _f ^o 7-10	125.6 \pm 6.4	94.2 \pm 5.9	-31.4 \pm 5.1***
LDL II S _f ^o 5-7	113.4 \pm 5.5	115.6 \pm 5.0	2.2 \pm 3.3
LDL III S _f ^o 3-5	38.6 \pm 2.6	51.1 \pm 3.8	12.5 \pm 3.4**
LDL IV S _f ^o 0-3	7.7 \pm 0.9	9.1 \pm 0.9	1.3 \pm 1.0
HDL Cholesterol	51.4 \pm 1.4	45.4 \pm 1.2	-6.0 \pm 1.0***
HDL Mass:			
HDL ₂ F _{1,20} ^o 3.5-9	48.7 \pm 5.4	37.4 \pm 3.9	-11.3 \pm 3.3*
HDL ₃ F _{1,20} ^o 0-3.5	183.8 \pm 4.1	182.3 \pm 4.1	-1.5 \pm 3.8
Apo B	98.3 \pm 3.2	98.4 \pm 3.3	0.1 \pm 1.8
Peak S _f ^o	6.7 \pm 0.1	6.2 \pm 0.1	-0.6 \pm 0.1***
Peak Diameter (Å)	268.1 \pm 0.6	265.4 \pm 0.6	-2.7 \pm 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
	<u>mg/dl Mean \pm SEM</u>			
Triglycerides	166.1 \pm 12.3	225.4 \pm 19.0	59.3 \pm 17.2*	0.04
VLDL Mass:				
Large S _f ^o 100-400	34.8 \pm 5.8	66.5 \pm 10.9	31.8 \pm 9.0*	0.01
Intermed. S _f ^o 60-100	40.1 \pm 4.3	64.7 \pm 6.5	24.6 \pm 5.6**	0.01
Small S _f ^o 20-60	78.1 \pm 8.3	91.2 \pm 6.2	13.0 \pm 7.0	0.95
LDL Cholesterol	150.8 \pm 7.5 [†]	120.7 \pm 7.6	-30.1 \pm 4.6	0.0001
IDL Mass:				
Large S _f ^o 14-20	31.2 \pm 3.0	29.8 \pm 1.7	-1.4 \pm 2.2	0.61
Small S _f ^o 10-14	36.5 \pm 2.4	33.8 \pm 1.8	-2.7 \pm 1.8	0.70
LDL Mass:				
LDL I S _f ^o 7-10	62.5 \pm 4.1	49.9 \pm 4.0	-12.6 \pm 3.6*	0.003
LDL II S _f ^o 5-7	114.3 \pm 7.9	81.4 \pm 9.4	-32.9 \pm 6.7***	0.0001
LDL III S _f ^o 3-5	118.3 \pm 8.7	105.6 \pm 7.4	-12.6 \pm 6.3	0.0004
LDL IV S _f ^o 0-3	23.5 \pm 3.7**	37.1 \pm 4.3	13.6 \pm 3.8*	0.005
HDL Cholesterol	41.4 \pm 1.8**	36.3 \pm 1.8	-5.2 \pm 0.9***	0.52
HDL Mass:				
HDL ₂ F _{1.20} ^o 3.5-9	14.2 \pm 3.8	11.2 \pm 2.9	-3.0 \pm 2.0	0.03
HDL ₃ F _{1.20} ^o 0-3.5	188.7 \pm 9.1	170.6 \pm 6.8	-18.1 \pm 6.5 [†]	0.03
Apo B	126.3 \pm 5.9	114.7 \pm 4.6	-11.6 \pm 2.7**	0.001
Peak S _f ^o	4.6 \pm 0.1	4.2 \pm 0.1	-0.4 \pm 0.1*	0.24
Peak Diameter (Å)	252.9 \pm 0.7	250.8 \pm 1.0	-2.0 \pm 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)
	<u>mg/dl, mean \pm SEM</u>		
Triglycerides	99.9 \pm 5.4	159.5 \pm 13.3	59.5 \pm 11.6***
VLDL Mass:			
Large S _f ^o 100-400	13.2 \pm 2.0	35.1 \pm 5.3	21.8 \pm 5.3**
Intermed. S _f ^o 60-100	19.5 \pm 2.1	42.0 \pm 5.1	22.5 \pm 44.6***
Small S _f ^o 20-60	42.1 \pm 3.2	68.4 \pm 6.0	26.3 \pm 4.7***
LDL Cholesterol	154.2 \pm 4.7	135.5 \pm 4.3	-18.7 \pm 3.5***
IDL Mass:			
Large S _f ^o 14-20	22.8 \pm 1.6	26.2 \pm 1.8	3.4 \pm 1.6 [†]
Small S _f ^o 10-14	38.9 \pm 1.7	33.9 \pm 1.5	-5.1 \pm 1.6*
LDL Mass:			
LDL I S _f ^o 7-10	109.9 \pm 4.5	58.6 \pm 2.3	-51.3 \pm 3.9***
LDL II S _f ^o 5-7	140.9 \pm 5.6	108.6 \pm 4.2	-32.3 \pm 5.3***
LDL III S _f ^o 3-5	61.2 \pm 4.4	110.3 \pm 4.7	49.1 \pm 4.0***
LDL IV S _f ^o 0-3	9.4 \pm 1.1	20.7 \pm 2.1	11.3 \pm 2.0***
HDL Cholesterol	49.4 \pm 1.7	40.0 \pm 1.1	-9.4 \pm 1.0***
HDL Mass:			
HDL ₂ F _{1,20} ^o 3.5-9	32.1 \pm 4.4	13.4 \pm 1.7	-18.7 \pm 3.9***
HDL ₃ F _{1,20} ^o 0-3.5	201.7 \pm 5.5	187.1 \pm 5.6	-14.6 \pm 4.5*
Apo B	118.6 \pm 3.8	121.1 \pm 3.7	2.5 \pm 2.3
Peak S _f ^o	6.0 \pm 0.1	4.7 \pm 0.1	-1.3 \pm 0.1***
Peak Diameter (Å)	266.4 \pm 0.7	253.0 \pm 0.5	-13.4 \pm 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 ^o ₇₋₁₀	LDL II S _f ^o ₅₋₇	LDL III S _f ^o ₃₋₅	LDL IV S _f ^o ₀₋₃
Independent variables (high-fat diet):		<u>Coefficient</u>					
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 electrophoresis. 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).

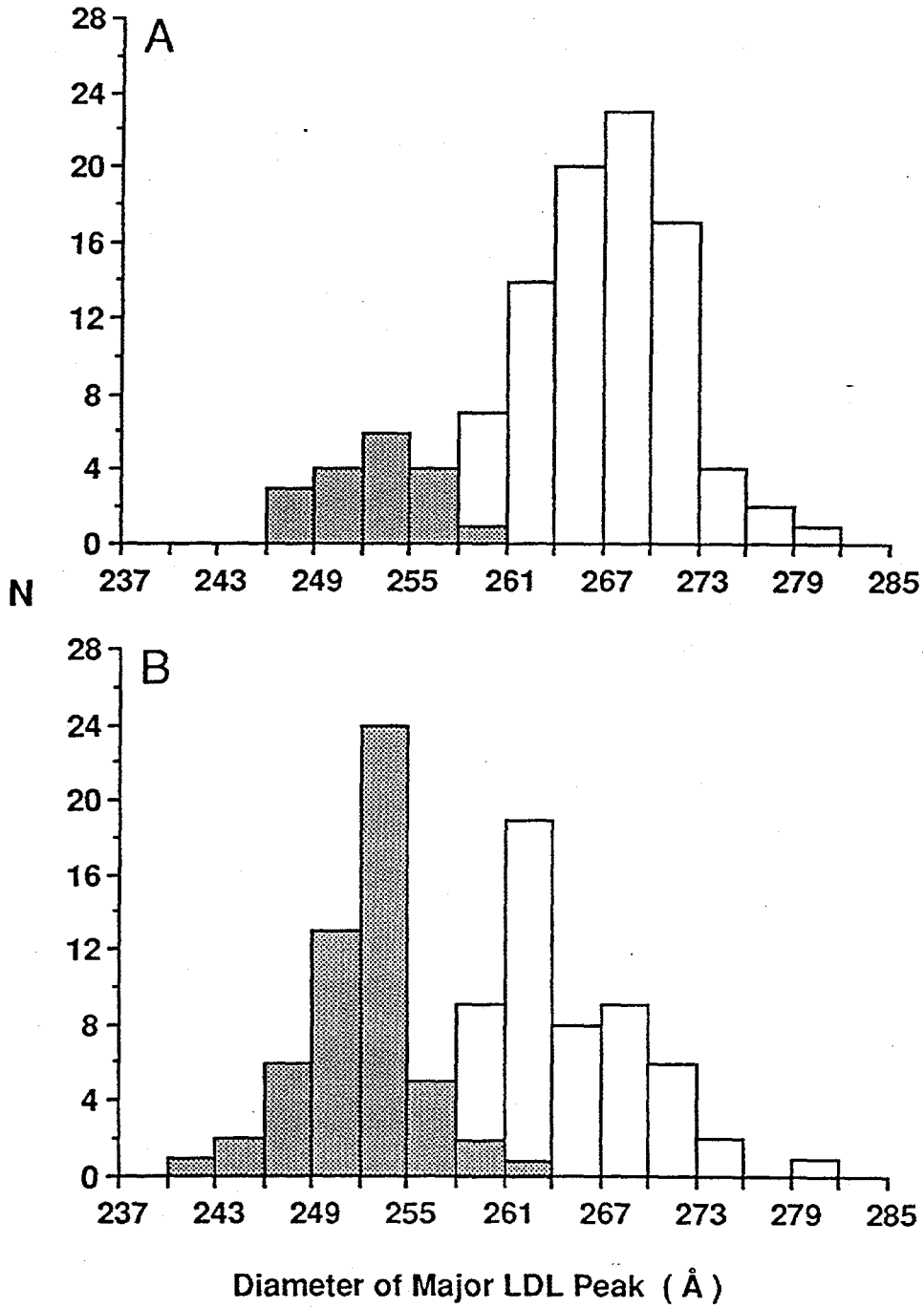


Figure 1
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