A very-low-fat diet is not associated with improved lipoprotein profiles in men with a predominance of large, low-density lipoproteins[1–3]

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ABSTRACT

Background: We found previously that men with a predominance of large LDL particles (phenotype A) consuming high-fat diets (40–46% fat) show less lipoprotein benefits of low-fat diets (20–24% fat) than do men with a high-risk lipoprotein profile characterized by a predominance of small LDL (phenotype B). Furthermore, one-third of men with phenotype A consuming a high-fat diet converted to phenotype B with a low-fat diet.

Objective: We investigated effects of further reduction in dietary fat in men with persistence of LDL subclass phenotype A during both high- and low-fat diets.

Design: Thirty-eight men who had shown phenotype A after 4–6 wk of both high- and low-fat diets consumed for 10 d a 10%-fat diet (2.7% saturates) with replacement of fat with carbohydrate and no change in cholesterol content or ratio of polyunsaturates to saturates.

Results: In 26 men, phenotype A persisted (stable A group) whereas 12 converted to phenotype B (change group). LDL cholesterol did not differ from previous values for 20–24%-fat diets in either group, whereas in the change group there were higher concentrations of triacylglycerol and apolipoprotein B; greater mass of HDL, large LDL-I, small LDL-III and LDL-IV, and HDL_3; lower concentrations of HDL cholesterol, apolipoprotein A-I; and lower mass of large LDL-I and HDL_2.

Conclusions: There is no apparent lipoprotein benefit of reduction in dietary fat from 20–24% to 10% in men with large LDL particles: LDL-cholesterol concentration was not reduced, and in a subset of subjects there was a shift to small LDL along with increased triacylglycerol and reduced HDL-cholesterol concentrations. *Am J Clin Nutr* 1999;69:411–8.

KEY WORDS Lipoproteins, low-fat diet, LDL subclasses, HDL, men, coronary artery disease, LDL phenotype

INTRODUCTION

LDLs comprise a spectrum of particles that differ in physical and chemical properties (1–3) and in metabolic characteristics (4). Studies have shown that risk of coronary artery disease is significantly greater in individuals with a predominance of small, dense LDL (LDL subclass phenotype B) than in those with larger LDL particles (phenotype A) (5–8).

We previously carried out 2 studies of effects of high-fat (40–46% fat) and low-fat (20–24% fat) diets on plasma lipoproteins in healthy, normolipidemic men (9, 10). In both studies, the reduction in LDL cholesterol with the low-fat diet was significantly less in men with phenotype A during the high-fat diet than in those with phenotype B. Moreover, in men with phenotype A, there was a shift in LDL particle mass from larger, lipid-enriched LDL to smaller, lipid-depleted LDL subfractions, indicative of a change in LDL composition with minimal change in particle number and consistent with the observation of reduced plasma LDL cholesterol without reduced apolipoprotein (apo) B (11). In one-third of the men with phenotype A, this shift resulted in conversion to phenotype B with the low-fat diet (9–11).

The present study was designed to investigate effects on concentrations and distributions of LDL particles of further short-term reductions in dietary fat (to 10% of energy) in 38 men who were previously determined to have LDL subclass phenotype A with both high-fat (40–46%) and low-fat (20–24%) diets. In particular, we wished to test the extent to which both phenotype A and relative resistance of LDL-cholesterol reduction to reduced dietary fat persisted in these men after the very-low-fat diet.

SUBJECTS AND METHODS

Subjects

Subjects were a subset of 238 healthy, nonsmoking men aged >20 y who had participated in 1 of our 2 previous dietary intervention protocols (9–11). In the first study, 105 men consumed diets containing 46% and 24% of energy as fat for 6 wk each in a randomized crossover design (9, 11). In the second study, 133 men consumed a 40%-fat diet for 3 wk followed by

40–46%-Fat diets

Phenotype A ($n = 180$) Phenotype B ($n = 58$)

20–24%-Fat diets

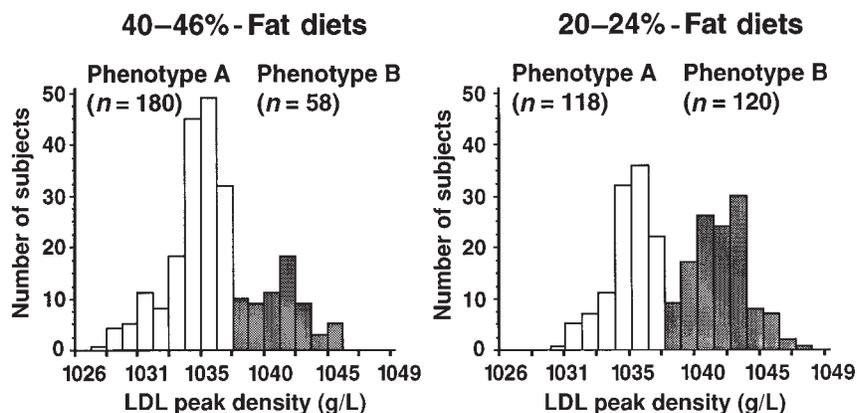Phenotype A ($n = 118$) Phenotype B ($n = 120$)

FIGURE 1. Distribution of LDL peak particle densities determined by analytic ultracentrifugation in 238 men consuming high-fat (40–46% fat) and low-fat (20–24% fat) diets. White bars represent subjects with LDL subclass phenotype A and shaded bars subjects with LDL subclass phenotype B.

a 20%-fat for 4 wk (10). All subjects had been free of chronic disease during the previous 5 y and were not taking medication likely to interfere with lipid metabolism. In addition, they were required to have plasma total cholesterol concentrations <6.74 mmol/L (260 mg/dL), triacylglycerol <5.65 mmol/L (500 mg/dL), resting blood pressure <160/105 mm Hg, and body weight <130% of ideal (12). Each participant signed a consent form approved by the Committee for the Protection of Human Subjects at EO Lawrence Berkeley National Laboratory, University of California, Berkeley, and participated in a medical interview.

The distribution of LDL peak density in all 238 men, as determined by analytic ultracentrifugation for both high-fat and low-fat diets (13, 14), is shown in **Figure 1**. For each diet, subjects were grouped on the basis of 2 modes, with one grouping comprising subjects with "buoyant-mode" profiles [density (d) ≤ 1038 g/L], designated phenotype A, and the other those subjects with "dense-mode" profiles (d > 1038 g/L), designated phenotype B (14). Of 180 men with phenotype A after consuming the high-fat diet, 62 shifted to phenotype B after consuming the low-fat diet (change group), whereas 118 men remained phenotype A (stable A group). The 58 men with phenotype B during the high-fat diet did not change phenotype during the low-fat diet (stable B group).

The 118 men with the stable A phenotype were eligible for the present study. Of these, 41 volunteered and 3 were excluded because on repeat screening they were found to have phenotype B with their usual diet. Mean (±SD) age and body mass index (BMI; in kg/m^2) of the 38 men included in this study were 52.5 ± 12.1 y (range: 32.0–71.0 y) and 25.4 ± 3.0 (range: 20.7–31.6), respectively. The length of time since the previous study ranged from 1 to 5 y (mean: 2.6 ± 1.3 y). The mean BMI of the subjects had not changed significantly during this interval.

Experimental design

In a crossover design, subjects were randomly assigned to follow their usual diets (with an average of ≈35% of energy as fat) or a very-low-fat outpatient diet providing 10% of energy as fat for 10 d each. Subjects then switched to the alternate diet for an additional 10 d, an interval that we found to be sufficient to ensure maximal expression of LDL subclass phenotype B (DM Dreon, RM Krauss, unpublished observations, 1997). Detailed measurements of lipids, lipoproteins, and lipoprotein subclasses were carried out after the usual diet and at the completion of the

experimental diet. BMI was calculated from weight and height measurements taken after each diet period.

Experimental 10%-fat diet

The nutrient composition of the experimental diet was based on a 10-d menu and was calculated by using the Minnesota Nutrition Data System (NDS) software developed by the Nutrition Coordinating Center (version 2.1; University of Minnesota, Minneapolis) (15, 16). The nutrient contents of the usual and 10%-fat diets are shown in **Table 1**. The usual diets were composed of a mean of 31.8% of energy from fat (10.8% saturated, 11.8% monounsaturated, and 6.9% polyunsaturated), 52.1% from carbohydrate, and 14% from protein. The very-low-fat, high-carbohydrate experimental diet was designed to supply <10% of energy from fat (2.7% saturated, 3.7% monounsaturated, and 2.6% polyunsaturated), with 75% from carbohydrate (with equal amounts of naturally occurring and added simple and complex carbohydrate) and 15% from protein. The experimental diet provided 35.7 mg cholesterol/1000 kJ, 1.2–1.4 g dietary fiber/1000 kJ, and the ratio of polyunsaturated to saturated fat (P:S) was 1.0. The diet was designed to meet age- and sex-specific recommended dietary allowances for energy, protein, and micronutrients. The subjects were allowed ad libitum consumption of non-energy-containing beverages and they were instructed to maintain their customary level of physical activity.

Registered dietitians supplied the participants with personalized menus showing the number and size of servings for the experimental 10%-fat diet. The staff contacted the subjects during the study to encourage them. Compliance with the experimental diet was assessed with a daily checklist of foods eaten from the menu. An average daily diet deviation of >5% of total energy would have excluded the subject from data analyses. However, no subjects were eliminated from the present study for noncompliance. The subjects weighed themselves daily at home and the staff adjusted energy intake from the menu if necessary to maintain body weight.

Clinical and laboratory measurements

Lipids, lipoproteins, and apolipoproteins

Subjects reported to our clinic in the morning, having abstained for from all food and vigorous activity for 12–14 h. Blood pressure, body weight, and height were measured at the end of each diet

TABLE 1
Mean daily nutrient contents of usual and 10%-fat diets

	Diet	
	Usual	10%-Fat
Energy (kJ)	11 054	12 600
Fat (% of energy)	31.8	10.4
Saturated	10.8	2.7
Monounsaturated	11.8	3.7
Polyunsaturated	6.9	2.6
Carbohydrate half simple sugars (% of energy)	52.1	75.7
Protein (% of energy)	14.0	14.5
Cholesterol (mg)	294.7	301.0
P:S[1]	0.74	0.96
Dietary fiber (g)	25.9	17.4

[1] Ratio of polyunsaturated to saturated fatty acids.

period. Plasma samples were prepared within 2 h of collection from venous blood collected in tubes containing Na_2EDTA (1.4 g/L), and blood and plasma were kept at 4°C until processed. Plasma total cholesterol and triacylglycerol concentrations were determined by enzymatic procedures on a Gilford Impact 400E analyzer (Gilford, Oberlin, OH). These measurements were consistently controlled with monitoring by the Centers for Disease Control and Prevention standardization program. HDL cholesterol was measured after heparin-manganese precipitation of plasma (17). LDL-cholesterol concentration was calculated with the formula of Friedewald et al (18), unless triacylglycerol concentrations were >400 mg/dL (4.52 mmol/L), in which case, LDL cholesterol was measured by direct quantitation of the ultracentrifugal plasma fraction with $d > 1006$ g/L (19). Apo A-I and apo B concentrations in plasma were determined by an immunoturbidometric assay described previously (20). LDL peak particle diameter was determined by nondenaturing gradient gel electrophoresis of plasma (2, 5–7).

Analytic ultracentrifugation

Lipoproteins were analyzed by analytic ultracentrifugation, a procedure that provides measurements of lipoprotein mass as a function of Svedberg peak flotation rate (S_f^0 for lipoproteins with $d < 1063$ g/L, and $F_{1.20}$ for lipoproteins with $d < 1210$ g/L) (13). Mass concentrations were determined for large, very-low-density lipoproteins (VLDLs) (S_f^0 100–400), intermediate VLDLs (S_f^0 60–100), small VLDLs (S_f^0 20–60), large intermediate-density lipoproteins (IDLs) (S_f^0 14–20), small IDLs (S_f^0 10–14), and of 4 major LDL subclasses, LDL-I (S_f^0 7–10), LDL-II (S_f^0 5–7), LDL-III (S_f^0 3–5), and LDL-IV (S_f^0 0–3) (21). For LDL, this procedure provides a measurement of peak flotation rate, as well as density (g/L) and diameter (nm) of the peak LDL for each subject (13). In addition, mass was determined for concentrations of 2 major HDL subclasses, HDL_2 ($F_{1.20}$ 3.5–9) and HDL_3 ($F_{1.20}$ 0–3.5) (13).

Statistics

Mean lipoprotein measurements are reported separately for the usual and 10%-fat diets and for the previous 20–24%-fat diets. Univariate analyses were by the Kruskal-Wallis test when 2 groups were being compared, and by the Wilcoxon signed-rank test for paired-difference analyses. Correlations were tested by using Spearman's method. Results with P values ≤0.05 were considered significant. Group averages are always reported as means ± SEs. STATVIEW 4.1 software (Abacus Concepts, Inc, Berkeley, CA) was used to perform all statistical analyses, with two-sided tests of significance.

RESULTS

Effects of usual and 10%-fat diets on plasma lipids

The plasma lipoprotein concentrations of all subjects while consuming their usual diets and the 10%-fat diet are shown in

TABLE 2
Plasma lipid and lipoprotein concentrations in all subjects[1]

	Usual diet	10%-Fat diet	Difference
Triacylglycerol (mmol/L)	0.95 ± 0.06	1.48 ± 0.11[2]	0.53 ± 0.10
VLDL mass (mg/L)			
Large (S_f^0 100–400)	100 ± 20	289 ± 43[2]	189 ± 41
Intermediate (S_f^0 60–100)	130 ± 18	316 ± 35[2]	186 ± 32
Small (S_f^0 20–60)	354 ± 35	527 ± 36[2]	172 ± 36
LDL cholesterol (mmol/L)	3.48 ± 0.15	3.05 ± 0.16[3]	−0.44 ± 0.10
IDL mass (mg/L)			
Large (S_f^0 14–20)	170 ± 16	157 ± 18	−13 ± 15
Small (S_f^0 10–14)	319 ± 20	273 ± 24[4]	−47 ± 15
LDL mass (mg/L)			
LDL-I (S_f^0 7–10)	1224 ± 72	866 ± 72[2]	−358 ± 72
LDL-II (S_f^0 5–7)	1100 ± 59	1009 ± 50	−91 ± 39
LDL-III (S_f^0 3–5)	371 ± 25	538 ± 52[4]	167 ± 50
LDL-IV (S_f^0 0–3)	82 ± 11	11.5 ± 14[5]	33 ± 14
HDL cholesterol (mmol/L)	1.47 ± 0.08	1.23 ± 0.06[2]	−0.24 ± 0.04
HDL mass (mg/L)			
HDL_2 ($F_{1.20}^0$ 3.5–9)	593 ± 90	430 ± 65[4]	−163 ± 50
HDL_3 ($F_{1.20}^0$ 0–3.5)	1997 ± 68	1854 ± 64[5]	−143 ± 55
Apolipoprotein A-I (μmol/L)	50.9 ± 1.8	45.2 ± 3.5[2]	−5.7 ± 1.0
Apolipoprotein B (μmol/L)	1.9 ± 0.06	2.0 ± 0.07[5]	0.08 ± 0.03
LDL peak diameter (nm)	26.62 ± 0.068	26.05 ± 0.13[3]	−0.57 ± 0.12

[1] $\bar{x}$ ± SE; n = 38. IDL, intermediate-density lipoprotein; S_f^0 and $F_{1.20}^0$, Svedberg flotation rate for lipoproteins of different densities.
[2–5] Significantly different from usual diet: [2] $P < 0.0001$, [3] $P < 0.001$, [4] $P < 0.01$, [5] $P < 0.05$.

TABLE 3
Plasma lipid and lipoprotein concentrations during the usual diet in the stable LDL subclass phenotype A and change groups[1]

	Stable A group (n = 26)	Change group (n = 12)
Triacylglycerol (mmol/L)	0.91 ± 0.07	1.03 ± 0.10
VLDL mass (mg/L)		
Large (S_f^0 100–400)	82 ± 20	137 ± 44
Intermediate (S_f^0 60–100)	109 ± 20	176 ± 36
Small (S_f^0 20–60)	347 ± 46	370 ± 54
LDL cholesterol (mmol/L)	3.42 ± 0.17	3.60 ± 0.31
IDL mass (mg/L)		
Large (S_f^0 14–20)	158 ± 20	195 ± 26
Small (S_f^0 10–14)	309 ± 24	343 ± 34
LDL mass (mg/L)		
LDL-I (S_f^0 7–10)	1247 ± 80	1174 ± 152
LDL-II (S_f^0 5–7)	1051 ± 70	1207 ± 107
LDL-III (S_f^0 3–5)	353 ± 30	411 ± 46
LDL-IV (S_f^0 0–3)	78 ± 12	89 ± 23
HDL cholesterol (mmol/L)	1.55 ± 0.06	1.29 ± 0.06
HDL mass (mg/L)		
HDL_2 ($F^0_{1.20}$ 3.5–9)	724 ± 120	308 ± 75[2]
HDL_3 ($F^0_{1.20}$ 0–3.5)	2052 ± 83	1879 ± 115
Apolipoprotein A-I (μmol/L)	53.4 ± 2.3	45.4 ± 1.5
Apolipoprotein B (μmol/L)	1.9 ± 0.07	2.0 ± 0.13
LDL peak diameter (nm)	26.70 ± 0.09	26.47 ± 0.10

[1] $\bar{x}$ ± SE. IDL, intermediate-density lipoprotein; S_f^0 and $F^0_{1.20}$, Svedberg flotation rate for lipoproteins of different densities.

[2,3] Significantly different from stable A group: [2] $P < 0.02$, [3] $P < 0.03$

Table 2. After the 10%-fat diet, there were significant increases in concentrations of triacylglycerol, mass of all VLDL subfractions, and apo B, and decreases in mass of small IDL, LDL cholesterol, HDL cholesterol, mass of HDL_2 and HDL_3, and apo A-I. There were also decreases in the mass of larger LDL-I and increases in the mass of the smaller LDL-III and LDL-IV subfractions, along with decreases in LDL particle size.

The distribution of LDL peak density of the subjects consuming their usual diet and the 10%-fat diet is shown in **Figure 2**. The majority of subjects (n = 26) remained in the phenotype A mode, whereas 12 of the subjects changed into the denser phenotype B mode with LDL peak densities >1038 g/L.

Plasma lipids in phenotype subgroups

Lipoprotein concentrations during the usual diet of the group of 26 subjects with stable phenotype A and the group of 12 subjects who changed to phenotype B with the 10%-fat diet are shown in **Table 3**. With the usual diet, there were few significant group differences for concentrations of plasma lipoproteins. The change group had lower HDL_2 mass and apo A-I than did the stable A group.

The lipoprotein concentrations during the 10%-fat diet of the stable phenotype A and change groups are shown in **Table 4**. During the 10%-fat diet, the change group had higher concentrations of triacylglycerol and greater mass of all VLDL subfractions than did the stable A group. Mean LDL-cholesterol concentrations were not significantly different between the groups. During the 10%-fat diet, the other significant differences between groups were what would be expected from the LDL subclass phenotypes. The change group had lower concentrations of larger LDL-I and higher concentrations of smaller LDL-III and LDL-IV subfractions, along with smaller LDL peak particle size compared with the stable A group. In addition, the change group had significantly lower concentrations of HDL cholesterol and mass of HDL_2. Concentrations of apo B were marginally higher in the change group than in the stable A group.

Mass concentrations with the 10%-fat diet compared with the 20–24%-fat diets from previous studies

The measurements of plasma lipid and lipoprotein concentrations during 20–24%-fat diets made during previous studies (9–11) were not significantly different between the men who remained stable A and those who changed when usual fat intake was reduced to 10% (**Table 5**). Comparison of Tables 4 and 5 shows that for the change group in the present study, the 10%-fat diet resulted in 2-fold higher triacylglycerol concentrations [2.16 ± 0.18 compared with 0.99 ± 0.07 mmol/L (191.0 ± 15.7 compared with 87.6 ± 6.2 mg/dL), $P < 0.0001$] and lower HDL-cholesterol concentrations [1.02 ± 0.01 compared with 1.18 ± 0.06 mmol/L (39.4 ± 0.2 compared with 45.5 ± 2.4 mg/dL), $P < 0.01$] and HDL_2 mass than the previous 20–24%-fat diets. Both the stable phenotype A and change groups had higher plasma VLDL, apo B, and HDL_3 mass concentrations after the 10%-fat diet than during the previous 20–24%-fat diet. The 10%-fat diet did not result in lower LDL cholesterol than the 20–24%-fat diets in either group. However, in the change group, the 10%-
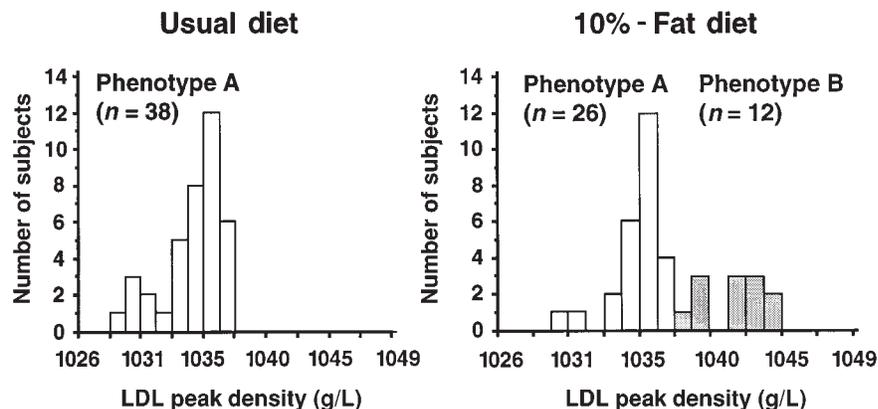

FIGURE 2. Distribution of LDL peak particle densities determined by analytic ultracentrifugation in 38 men consuming their usual diet (<32% fat) and a 10%-fat diet. White bars represent subjects with LDL subclass phenotype A and shaded bars subjects with LDL subclass phenotype B.

TABLE 4
Plasma lipid and lipoprotein concentrations after a 10%-fat diet in the stable LDL subclass phenotype A and change groups[1]

	Stable A group (n = 26)	Change group (n = 12)	Group difference (P)
Triacylglycerol (mmol/L)	1.16 ± 0.09	2.16 ± 0.18^2	<0.0001
VLDL mass (mg/L)			
Large (S_f^0 100–400)	162 ± 27^3	562 ± 76^4	<0.001
Intermediate (S_f^0 60–100)	212 ± 26^3	540 ± 54^2	<0.0001
Small (S_f^0 20–60)	471 ± 41^3	647 ± 60^4	<0.01
LDL cholesterol (mmol/L)	3.14 ± 0.18	2.84 ± 0.29	0.23
IDL mass (mg/L)			
Large (S_f^0 14–20)	150 ± 23	173 ± 31^3	0.33
Small (S_f^0 10–14)	278 ± 32	260 ± 33	0.89
LDL mass (mg/L)			
LDL-I (S_f^0 7–10)	1031 ± 83	508 ± 60^2	<0.0001
LDL-II (S_f^0 5–7)	1037 ± 53	948 ± 114	0.41
LDL-III (S_f^0 3–5)	378 ± 33	885 ± 85^2	<0.0001
LDL-IV (S_f^0 0–3)	90 ± 14	169 ± 26^5	0.01
HDL cholesterol (mmol/L)	1.33 ± 0.07	1.02 ± 0.05^5	<0.01
HDL mass (mg/L)			
HDL$_2$ ($F^0_{1.20}$ 3.5–9)	564 ± 82	141 ± 23^3	<0.001
HDL$_3$ ($F^0_{1.20}$ 0–3.5)	1841 ± 84^3	1881 ± 93^5	0.62
Apolipoprotein A-I (μmol/L)	46.4 ± 1.8	42.5 ± 1.5	0.22
Apolipoprotein B (μmol/L)	1.9 ± 0.05^2	2.2 ± 0.14^2	0.06
LDL peak diameter (nm)	26.48 ± 0.08	25.12 ± 0.16^2	<0.0001

[1] $\bar{x} \pm$ SE. IDL, intermediate-density lipoprotein; S_f^0 and $F^0_{1.20}$, Svedberg flotation rate for lipoproteins of different densities.
[2–5] Significantly different from 20–24%-fat diet (see Table 5 and references 9–11): [2] $P < 0.0001$, [3] $P \leq 0.005$, [4] $P < 0.001$, [5] $P < 0.01$.

fat diet resulted in higher mass of large IDL, and as expected from the change in phenotype, lower mass of large LDL-I, higher mass of small LDL-III and LDL-IV subfractions, and smaller LDL peak particle size.

Correlations of diet-induced differences in lipoprotein subfractions

The interrelated differences (10%-fat diet results minus 20–24%-fat diet results) among VLDL, IDL, and LDL mass, as well as apo B concentration, in the stable A and change groups are shown in **Table 6**. Higher VLDL mass concentrations were correlated with higher LDL-II and LDL-III and lower LDL-I concentrations in the stable A group, and with higher LDL-IV and lower LDL-I concentrations in the change group. Differences in LDL-III were positively correlated with differences in IDL and LDL-II in both groups and inversely correlated with LDL-I in the stable A group. In the change group, higher concentrations of IDL were correlated with higher LDL-II. Finally, higher concentrations of apo B were correlated with higher LDL-II in the stable A group and with higher LDL-II, LDL-III, and IDL in the change group.

Differences (10%-fat diet results minus 20–24%-fat diet results) in triacylglycerol were significantly related to differences in LDL peak particle size as measured by gradient gel electrophoresis in the group of men who remained phenotype A and in the men who changed subclass phenotype during the 10%-fat diet (combined $r_s = -0.65$, $P < 0.0001$) (**Figure 3**). Differences in triacylglycerol concentration were also positively correlated with differences in apo B in both groups combined ($r_s = 0.57$, $P < 0.001$). In addition, differences in apo B and LDL size were negatively correlated in the change group ($r_s = -0.61$, $P < 0.05$).

FIGURE 3. Spearman's correlation coefficient of difference in triacylglycerol and LDL size for 10%- and 20–24%-fat diets in 38 men by LDL subclass phenotype. $R^2 = -0.65$, $P < 0.001$. Data for 20–24%-fat diets from references 9–11.

DISCUSSION

In previous reports (9, 10) and in Figure 1 we showed that approximately one-third of men who manifested LDL subclass phenotype A (large, buoyant LDL) while consuming a high-fat diet converted to phenotype B (small, dense LDL) with a reduction in fat to 20–24% of energy. In the present study, we found that an additional 32% of men who had stable phenotype A during a 20–24%-fat diet converted to phenotype B after short-term consumption of a very-low-fat (10% fat), high-carbohydrate diet. Thus, it appears that with progressive reduction in dietary fat and a corresponding increase in carbohydrate, an increasing propor-
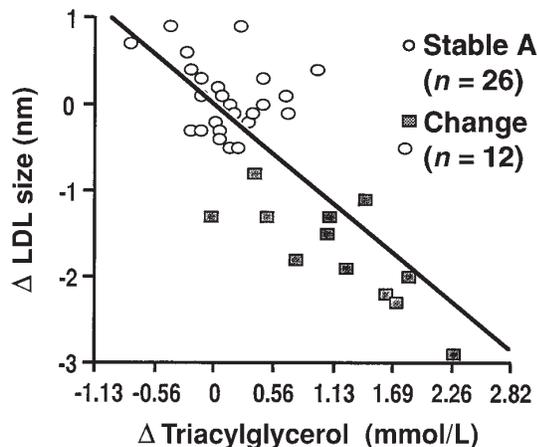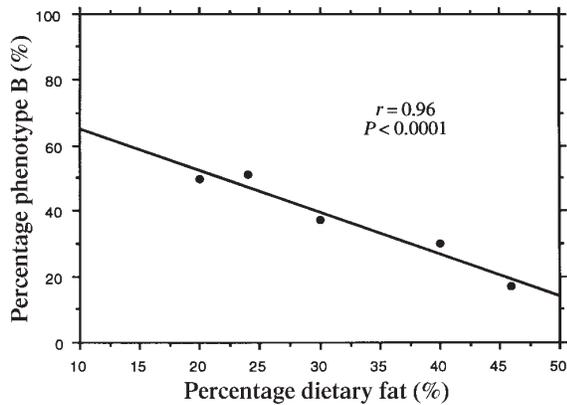
FIGURE 4. Relation of percentage dietary fat with prevalence of LDL subclass phenotype B in groups of healthy men. Data for diets containing 46% and 24% fat are for 105 men from reference 9; data for diets containing 20% and 40% fat are for 133 men from reference 10; data for diet containing 30% fat are from a subgroup of 45 men from reference 9.

tion of men show conversion of LDL subclass distributions from phenotype A to phenotype B. This is illustrated in **Figure 4**, in which data from this and 2 previous studies (9, 10) were combined, showing a significant inverse relation in men between the prevalence of LDL subclass phenotype B and percentage of energy from fat in isoenergetic diets (ranging from 20% to 46% fat). The regression predicts that with a diet with 10% of energy as fat, approximately two-thirds of men would express phenotype B. This prediction is consistent with the current finding that of the population of 238 men from whom the study subjects were drawn, 120 (50%) expressed phenotype B while consuming diets with 20% or 24% of energy as fat, and an additional 34% of the remaining 50% of subjects (17%) converted to phenotype B with further reduction in dietary fat to 10% of energy (total: 67%).

The metabolic basis for a shift in the predominant LDL species from large to small in subsets of men who reduce their fat intake

TABLE 5
Plasma lipid and lipoprotein concentrations of men consuming 20-24%-fat diets in previous studies in the stable LDL subclass phenotype A and change groups[1]

	Stable A group (n = 26)	Change group (n = 12)
Triacylglycerol (mmol/L)	0.098 ± 0.04	0.99 ± 0.07
VLDL mass (mg/L)		
Large (S$_f^0$ 100–400)	97 ± 12	88 ± 21
Intermediate (S$_f^0$ 60–100)	147 ± 16	150 ± 22
Small (S$_f^0$ 20–60)	381 ± 30	356 ± 28
LDL cholesterol (mmol/L)	3.08 ± 0.16	2.77 ± 0.25
IDL mass (mg/L)		
Large (S$_f^0$ 14–20)	132 ± 1.8	99 ± 20
Small (S$_f^0$ 10–14)	260 ± 2.0	220 ± 24
LDL mass (mg/L)		
LDL-I (S$_f^0$ 7–10)	988 ± 67	843 ± 85
LDL-II (S$_f^0$ 5–7)	1026 ± 63	965 ± 91
LDL-III (S$_f^0$ 3–5)	381 ± 39	380 ± 38
LDL-IV (S$_f^0$ 0–3)	83 ± 12	68 ± 11
HDL cholesterol (mmol/L)	1.26 ± 0.05	1.18 ± 0.06
HDL mass (mg/L)		
HDL$_2$ (F$^0_{1.20}$ 3.5–9)	491 ± 79	325 ± 82
HDL$_3$ (F$^0_{1.20}$ 0–3.5)	1680 ± 57	1665 ± 70
Apolipoprotein A-I (μmol/L)	44.5 ± 1.2	42.0 ± 1.1
Apolipoprotein B (μmol/L)	1.6 ± 0.07	1.5 ± 0.09
LDL peak diameter (nm)	26.42 ± 0.09	26.82 ± 0.19

[1] $\bar{x}$ ± SE. IDL, intermediate-density lipoprotein; S$_f^0$ and F$^0_{1.20}$, Svedberg flotation rate for lipoproteins of different densities. Data from references 9–11.

is not known. As in previous studies (11, 22), increases in mass of dense LDL-III and reductions in buoyant LDL-I were inversely correlated with increases in triacylglycerol concentrations and VLDL mass. Other studies have shown that changes in LDL peak particle size were inversely correlated with changes in triacylglycerol (22, 23). It is possible that diet-induced increases in secretion or reductions in clearance of triacylglycerol-rich lipoproteins, with concomitant triacylglycerol enrichment of apo B–containing lipoproteins by cholesteryl ester transfer protein, result in the for-

TABLE 6
Spearman's correlation coefficients (r$_s$) of diet-induced changes (10%-fat diet results minus 20-24%-fat diet results) in lipoproteins in the stable phenotype A and change groups[1]

Group	VLDL (S$_f^0$ 20–400)	IDL (S$_f^0$ 10–20)	LDL-I (S$_f^0$ 7–10)	LDL-II (S$_f^0$ 5–7)	LDL-III (S$_f^0$ 3–5)	LDL-IV (S$_f^0$ 0–3)
Stable A (n = 26)						
IDL (S$_f^0$ 10–20)	0.38					
LDL-I (S$_f^0$ 7–10)	−0.43[2]	0.16				
LDL-II (S$_f^0$ 5–7)	0.50[2]	0.35	0.14			
LDL-III (S$_f^0$ 3–5)	0.67[3]	0.40[2]	−0.53[4]	0.51[2]		
LDL-IV (S$_f^0$ 0–3)	0.32	0.07	−0.11	0.21	0.33	
Apolipoprotein B	0.19	0.01	0.20	0.64[4]	0.28	−0.22
Change (n = 12)						
IDL (S$_f^0$ 10–20)	0.32					
LDL-I (S$_f^0$ 7–10)	−0.61[2]	−0.22				
LDL-II (S$_f^0$ 5–7)	0.00	0.78[2]	0.02			
LDL-III (S$_f^0$ 3–5)	0.49	0.84[4]	−0.31	0.67[2]		
LDL-IV (S$_f^0$ 0–3)	0.71[2]	0.29	−0.43	−0.11	0.48	
Apolipoprotein B	0.39	0.86[4]	−0.42	0.64[2]	0.87[4]	0.50

[1] IDL, intermediate-density lipoprotein; S$_f^0$, Svedberg flotation rate. 20-24%-fat diet results from references 9–11.
[2] P < 0.05.
[3] P < 0.001.
[4] P < 0.01.

mation of IDLs or large LDLs that are capable of being converted to small LDLs by lipolysis. Although there is evidence that hepatic lipase contributes to this lipolytic transformation, we reported previously that hepatic lipase activity is reduced by a low-fat diet and that this change in enzyme activity is correlated with reductions in small VLDL and IDL, but not with changes in small LDL (24). It may be that there is sufficient activity of hepatic lipase, even with a low-fat diet, to mediate the production of small LDL from triacylglycerol-enriched precursors.

The dietary factors responsible for LDL subclass phenotype changes are not understood. High-carbohydrate, low-fat diets are known to result in increased plasma concentrations of larger, more triacylglycerol-rich VLDL particles (11, 25). It may be that those subjects whose LDL phenotype changed from A to B were more responsive to this effect because with the low-fat diet, this group had 2-fold higher concentrations of triacylglycerol and higher concentrations of all VLDL fractions, particularly the largest VLDL particles, compared with the stable A group. Although it is thought that increased intake of carbohydrates, particularly simple sugars, is a major factor responsible for increased hepatic triacylglycerol production, it is not known whether reduced dietary fat intake contributes to these lipoprotein changes as well, perhaps as a result of reduced triacylglycerol clearance (24).

Despite the relations of triacylglycerol and VLDL changes to changes in LDL subclass profiles, we have not found baseline triacylglycerol concentration to be a significant predictor of low-fat diet–induced change in LDL subclass phenotype (11). Given the evidence for major gene effects on LDL particle distributions (26–28), it is possible that genetically influenced metabolic variations not related to plasma triacylglycerol concentrations determine susceptibility to conversion from phenotype A to phenotype B during low-fat diets, and that different genetic or metabolic traits operate to promote this conversion at differing fat intakes (Figure 4).

The present study was also designed to test the LDL-cholesterol response to a very-low-fat diet in men who had previously shown relatively small changes in total LDL with manipulation of dietary fat content. Consistent with our previous results in subjects with phenotype A consuming high-energy, low-fat diets (9, 10), reduced concentrations of LDL cholesterol with the 10%-fat diet were not accompanied by reductions in plasma apo B. Although this reflects in part small increases in VLDL and IDL-apo B, it is principally indicative of a shift from larger, cholesterol-rich to smaller, cholesterol-poor particles, without a significant reduction in LDL particle number. As noted above, the conversion from phenotype A to B in a subset of men is a more pronounced manifestation of this phenomenon. It is possible that other compositional differences in LDL particles, such as exchange of triacylglycerol for cholesteryl ester (29, 30), also could have contributed to the reduction in LDL cholesterol with consumption of the 10%-fat diet.

Because the present study involved short-term administration of diets with extreme variations in fat and carbohydrate content, it is not possible to draw conclusions as to possible long-term metabolic or clinical consequences of the lipoprotein subclass responses to the very-low-fat diets observed here. In addition, potential differences in metabolic effects of complex compared with simple sugars (31) and the tendency for ad libitum consumption of low-fat diets to promote weight loss need to be considered. The present results suggest, however, that in healthy normolipidemic men with LDL subclass phenotype A consuming average American diets, lipoprotein changes induced by further restriction of dietary fat and isoenergetic substitution of carbohydrates are not indicative of reduced risk of coronary artery disease; in a subset of men who convert to phenotype B, the changes are suggestive of an increase in coronary disease risk.

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