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ARTICLES

Apolipoprotein E Isoform Phenotype and LDL Subclass Response to a Reduced-Fat Diet

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Abstract

Abstract We investigated the association of apolipoprotein (apo) E isoform phenotype with lipoprotein response to reduced dietary fat intake in 103 healthy men (apoE3/2, n=10; apoE3/3, n=65; and apoE4/3, 4/4, n=28). In a randomized, crossover design, subjects consumed high-fat (46%) and low-fat (24%) diets for 6 weeks each. High-fat LDL cholesterol differed among phenotypes, with apoE4/3, 4/4>apoE3/3>apoE3/2. Reduction of LDL cholesterol on the low-fat diet was greater for apoE4/3, 4/4 than apoE3/3 ($P<.05$). There was no significant change in plasma apoB level within any of the apoE phenotype groups on the low-fat diet. This result, together with measurements of LDL subfraction mass by analytical ultracentrifugation, indicated that the primary basis for the diet-induced reduction in LDL cholesterol was not reduced LDL particle number but rather a shift from large, buoyant, cholesterol-rich LDL

particles (flotation rate, 7 to 12) to smaller, denser LDL particles (flotation rate, 0 to 7). The magnitude of this effect was related to apoE phenotype, with progressively greater reductions in levels of large LDL ($P<.01$) from apoE3/2 to apoE3/3 to apoE4/3, 4/4. These results indicate that reduced dietary fat lowers levels of large, buoyant LDL particles by an apoE-dependent mechanism.

LDL cholesterol apolipoprotein E LDL mass diet fatty acids

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Apolipoprotein (apo) E is a protein associated with triglyceride-rich lipoproteins and HDL. A major physiological role of lipoprotein-bound apoE is as a ligand for LDL and remnant receptors[1] and the LDL receptor–related protein.[2] Three alleles of the apoE gene, ε2, ε3, and ε4, are responsible for the main apoE isoforms: apoE2, apoE3, and apoE4, with relative allele frequencies of approximately 10%, 75%, and 15%, respectively.[3] The three alleles result in three homozygous phenotypes, E2/2, E3/3, and E4/4, and three heterozygous phenotypes, E3/2, E4/3, and E4/2. ApoE phenotype accounts for up to 7% of the interindividual variation in total serum cholesterol in the general population.[3] The ε4 allele is associated with higher and the ε2 with lower LDL cholesterol (LDL-C) and apoB than is the ε3 allele.[3 4 5 6 7 8] The effects of these alleles on apoB, however, vary in different populations.[9] Recently, the ε4 allele has been associated with elevations in plasma triglycerides and low concentrations of HDL cholesterol (HDL-C).[10] Some reports have indicated that the ε4 allele is also associated with increased risk of coronary artery disease (CAD).[3 11 12]

ApoE phenotype has been reported to influence changes in total cholesterol and LDL-C induced by low-fat diets, with greater responses in subjects with the apoE4 isoform.[13 14] However, the magnitude of this effect is variable,[13 14 15 16 17] and the biological mechanism has not been established. Additionally, LDLs in plasma comprise multiple distinct subclasses differing in size, density, and chemical composition,[18] and the relation of apoE isoforms to concentrations and dietary responsiveness of LDL subclasses has not been investigated.

In the present study, we tested whether measurements of LDL subclasses may define more clearly the role of the apoE phenotype in influencing LDL-C response to alteration in dietary fat intake.

Methods

Subjects

Healthy, nonsmoking, male volunteers over age 20 years[19] 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 <6.72 mmol/L (260 mg/dL) and triglyceride concentration <5.65 mmol/L (500 mg/dL); (3) resting blood pressure <160/105 mm Hg; (4) body weight not greater than 130% of ideal according to the 1985 Metropolitan Life Insurance Co Tables; (5) no use of medication likely to interfere with lipid metabolism; and (6) no apoE2/2 phenotype. Each participant signed a consent form approved by the Committee for the Protection of Human Subjects at Lawrence Berkeley Laboratory, University of California, and participated in a medical interview. One hundred fifty men volunteered for the study. Twenty-one individuals were ineligible and twenty-four were unavailable for follow-up. There was no differential dropout rate with respect to a particular diet regimen or apoE phenotype. Data from two individuals with E4/2 phenotype were not included in the present analysis. Mean±SD values for age and body mass index (BMI) of the 103 men in the study were 48.8±11.1 years (range, 28.0 to 79.0) and 25.5±3.0 kg/m^2 (range, 17.1 to 35.1), respectively.

Experimental Design

The participants were first randomly assigned (irrespective of apoE phenotype) to outpatient treatment with either a high-fat (46% of energy) or a low-fat (24% of energy) diet for 6 consecutive weeks each. The subjects then switched to the alternate diet for an additional 6 weeks. A 6-week diet was chosen based on the experience in previous longer term diet studies that lipoprotein changes appear to stabilize between 4 and 6 weeks after subjects begin a diet.[20 21] Registered dietitians instructed the subjects on the experimental diets by giving them 2-week cycle menus demonstrating number and size of servings. Nutrient compositions for the experimental diets (Table 1⇓) were calculated by using MINNESOTA NUTRITION DATA SYSTEM (version 2.1) software developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis.[22 23] The change from the 46% high-fat diet to the 24% low-fat diet was achieved primarily by reducing percentage of calories from saturated fat (18% to 5%) and polyunsaturated fat (13% to 4%). The fat was replaced with carbohydrate (which increased from 38% to 60% of energy), and the carbohydrate calories remained equally distributed between simple and complex sources. There were no significant differences between the diets in total calories, percentage of energy from monounsaturated fat (12%) and total protein (16%), cholesterol (0.030 to 0.036 g/1000 kJ), ratio of polyunsaturated fat to saturated fat (0.7), and dietary fiber (0.96 to 1.20 g/1000 kJ). Registered dietitians instructed the subjects to refrain from alcohol during the study and to keep exercise and body weight constant between the two diets.

Table 1.

Nutrient Content of Experimental Diets

	High Fat	Low Fat
Energy, kJ	12 017	12 000
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, g/1000 kJ	0.034	0.030
Polyunsaturated fat/saturated fat	0.69	0.74
Dietary fiber, g/1000 kJ	1.17	1.20

Values are the mean of 2-week cycle menu for a 12 000-kJ energy level.

The staff contacted the subjects weekly to encourage motivation. Subjects measured their body weights daily at home, and the staff adjusted energy intake if necessary to minimize weight variability. The subjects were surveyed for dietary intake (4-day food records of Thursday through Sunday),[22 23 24] body

weight, and plasma lipids and lipoproteins once at screening and once during the last week of each experimental diet. Daily diet deviation records were also used as a measure of dietary compliance. Although half the subjects had the low-fat diet first, we use the expression “diet-induced change,” for every variable, to mean “low-fat value minus high-fat value,” regardless of the actual order of the diets.

Laboratory Analyses

Lipids, Lipoproteins, and Apolipoproteins

Venous blood samples were collected in tubes containing Na_2-EDTA, 1.4 mg/mL, after the subjects had fasted for 12 to 14 hours. Plasma was prepared within 2 hours of collection, and blood and plasma were kept at 4°C until processed. Plasma total cholesterol and triglycerides were determined by enzymatic procedures on a Gilford Impact 400E analyzer. HDL-C was measured after heparin-manganese precipitation of plasma.[25] These measurements were consistently in control as monitored by the Centers for Disease Control and Prevention standardization program. LDL-C was calculated from the formula of Friedewald et al[26] unless triglycerides were >4.52 mmol/L (400 mg/dL), in which case LDL-C was measured in the density >1.006 g/mL ultracentrifugal plasma fraction.[27] ApoA-I and apoB concentrations in plasma were determined by maximal radial immunodiffusion.[28 29]

Analytical Ultracentrifugation

Lipoproteins were analyzed by analytical ultracentrifugation, a procedure that provides measurements of lipoprotein mass as a function of Svedberg flotation rate (S_f^o for d<1.063 g/mL lipoproteins and $F^o_{1.20}$ for d<1.21 g/mL lipoproteins). Mass concentrations were determined for total LDL (S_f^o 0 to 12) and for concentrations of four major LDL subclasses: LDL-I (S_f^o 7 to 12), LDL-II (S_f^o 5 to 7), LDL-III (S_f^o 3 to 5), and LDL-IV (S_f^o 0 to 3).[18] Mass concentrations were also determined for IDL (S_f^o 12 to 20) and VLDL (S_f^o 20 to 400). For LDL, this procedure provides a measurement of peak flotation rate as well as density and diameter of the peak LDL for each subject.[30] In addition, mass was determined for total HDL ($F^o_{1.20}$ 0 to 9) and for concentrations of two major HDL subclasses, HDL_2 ($F^o_{1.20}$ 3.5 to 9) and HDL_3 ($F^o_{1.20}$ 0 to 3.5).[30]

ApoE Phenotype

ApoE isoforms were determined by isoelectric focusing of VLDL apolipoproteins.[31 32] Isoform phenotypes were designated according to recommended nomenclature.[32]

For all laboratory analyses, personnel were blinded as to the subjects’ identity and high- or low-fat diet treatment.

Statistics

Lipid, lipoprotein, apolipoprotein, and dietary data are presented for three apoE phenotype groups, E3/2 (n=10), E3/3 (n=65), and E4/3 (n=28). Results are expressed as mean±SEM. Three apoE4/4 homozygotes were combined with the E4/3 group for all calculations since removing these three subjects from the analyses did not substantially change the results. Univariate analyses were by the Mann-Whitney two-sample test and the Kruskal-Wallis test for three-group comparisons. The Wilcoxon signed rank test was used for paired difference analyses. These analyses showed no diet treatment order effect, ie, the changes (low-fat value minus high-fat value) were not significantly related to the actual order of the diets (high fat to low fat, n=48 versus low fat to high fat, n=55) for any of the lipid, lipoprotein, or apolipoprotein variables. Multivariate analyses were by multiple regression. SAS software[33 34] was used to perform the statistical analyses, and two-sided tests of statistical significance were employed.

Results

Mean age and BMI were not significantly different among apoE phenotype groups (data not shown). There were no significant mean diet-induced changes in body weight within or among the groups throughout the experimental period (data not shown).

Nutrient intake as estimated from the 4-day food records indicated good compliance with the experimental diets. Table 2⇓ shows reported dietary intake on the high-fat and low-fat diets for 102 subjects (one participant in the apoE3/3 group did not provide diet records during the study). During the high-fat diet, the percentage fat intakes for the apoE3/2, 3/3, and 4/3 groups were 45%, 45%, and 46%, respectively, with no significant differences among phenotypes. Similarly, the groups achieved a fat intake on the low-fat diet of 24%, 25%, and 24%, respectively, with no significant group differences. For intakes of other major nutrients, there were no significant differences among apoE phenotypes on either the high- or low-fat diets.

Table 2.

Reported Dietary Intake (4-Day Record) on the High-Fat and Low-Fat Diets by ApoE Phenotype

	ApoE3/2 (n=10)		ApoE3/3 (n=64)		ApoE4/3 (n=28)	
	High Fat	Low Fat	High Fat	Low Fat	High Fat	Low Fat
Energy, kJ	11 975±525	11 838 ±508	12 188±179	11 721±229	11 429±358	11 375 ±413

	ApoE3/2 (n=10)		ApoE3/3 (n=64)		ApoE4/3 (n=28)	
	High Fat	**Low Fat**	**High Fat**	**Low Fat**	**High Fat**	**Low Fat**
Fat, %	45.2±0.7	24.2±0.6	45.3±0.3	24.6 ±0.5	45.6±0.4	24.0±0.5
Saturated, %	18.7 ±0.3	5.9±0.2	18.2±0.2	6.1±0.2	18.5±0.2	5.9 ±0.1
Monounsaturated, %	12.2±0.2	12.1±0.4	12.6 ±0.1	11.7±0.2	12.4±0.2	11.7±0.2
Polyunsaturated, %	11.5±0.7	3.9±0.2	11.6±0.2	4.4±0.1	11.9 ±0.3	4.0±0.1
Carbohydrate, %	39.2±0.7	59.7 ±0.7	39.0±0.4	58.5±0.4	38.5±0.4	59.3±0.5
Protein, %	16.2±0.1	16.0±0.2	16.3±0.1	16.7 ±0.2	16.5±0.1	16.5±0.5
Cholesterol, g/1000 kJ	0.038 ±0.002	0.031±0.003	0.037±0.001	0.033±0.001	0.037 ±0.001	0.033±0.001
Polyunsaturated fat/ saturated fat	0.62±0.04	0.67±0.02	0.64±0.01	0.73±0.02	0.64 ±0.02	0.69±0.02
Dietary fiber, g/1000 kJ	1.09 ±0.07	1.16±0.06	1.12±0.02	1.18±0.03	1.10 ±0.05	1.21±0.04

Apo indicates apolipoprotein. There were no significant between-group differences.

Effects of Diet and ApoE Phenotype on Plasma Lipid, Lipoprotein Cholesterol, and Apolipoprotein Concentrations

Table 3⇓ shows plasma concentration of lipids, lipoprotein cholesterol, and apolipoproteins on the high- and low-fat diets by apoE phenotype group. After the high-fat diet, there were no significant differences in triglycerides, total cholesterol, HDL-C, or apoA-I among phenotypes. However, LDL-C (P<.01) and apoB (P<.05) were significantly different among phenotypes, with progressive increases from apoE3/2 to apoE3/3 to apoE4/3. On the low-fat diet, differences in LDL-C and apoB among apoE phenotypes showed a similar trend to that on the high-fat diet, with progressive increases from apoE3/2 to apoE3/3 to apoE4/3 (P<.05).

Table 3.

Plasma Lipid, Lipoprotein Cholesterol, and Apolipoprotein Concentrations by ApoE Phenotype on High-Fat and Low-Fat Diets After Each Diet Sequence

	ApoE3/2 Phenotype (n=10)			ApoE3/3 Phenotype (n=65)			ApoE4/3, 4/4 Phenotype (n=28)		
	High Fat	Low Fat	Difference	High Fat	Low Fat	Difference	High Fat	Low Fat	Difference
Triglycerides, mmol/L	1.47 ±0.25	2.23±0.47	0.76 ±0.41	1.12±0.07	1.58 ±0.10	0.46 ±0.08[4]	1.04 ±0.08	1.37±0.12	0.33 ±0.09
Cholesterol, mmol/L									
Total	5.09 ±0.28	4.94±0.31	–0.15±0.16	5.40±0.12	5.06 ±0.12	–0.34±0.07[4]	5.78±0.18	5.15±0.16	–0.63 ±0.10[4]
LDL	3.06±0.18	2.75±0.21	–0.31 ±0.13[2]	3.65±0.11	3.26±0.12	–0.39±0.06[4]	4.04 ±0.16	3.46±0.14	–0.58±0.1

	ApoE3/2 Phenotype (n=10)			ApoE3/3 Phenotype (n=65)			ApoE4/3, 4/4 Phenotype (n=28)		
	High Fat	Low Fat	Difference	High Fat	Low Fat	Difference	High Fat	Low Fat	Difference
HDL	1.36 ±0.12	1.18±0.07	–0.18±0.10	1.25±0.03	1.07 ±0.03	–0.17±0.02[4]	1.26±0.05	1.07±0.04	–0.20 ±0.03[4]
ApoB, µmol/L	1.69±0.12	1.67 ±0.10	–0.02±0.05	1.99±0.06	1.97±0.05	–0.01 ±0.03	2.15±0.08	2.11±0.09	–0.04±0.0
ApoA-I, µmol/L	46.0±2.57	44.5±2.07	–1.50 ±1.50	44.8±0.75	40.6±0.54	–4.14±0.54[4]	44.8 ±1.11	40.5±1.32	–4.29±0.9

Apo indicates apolipoprotein. To convert values to milligrams per deciliter, multiply by 88.57 for triglycerides, 38.67 for cholesterol, 55.0 for apoB, and 2.80 for apoA-I. Values are mean±SEM. Significance of differences between apoE phenotype groups on both high- and low-fat diets are indicated in text.

1 Comparison of diet-induced differences among phenotype groups by Kruskal-Wallis test.

2 *P*<.05,

3 *P*<.001,

4 *P*<.0001 for low- vs high-fat diet difference.

Table 3⇑ also shows changes in plasma lipids, lipoprotein cholesterol, and apolipoproteins B and A-I on low-fat minus high-fat diets. Triglycerides were increased on the low-fat diet, and total cholesterol, HDL-C, and apoA-I were decreased in all apoE phenotype groups; these changes reached significance only for the apoE3/3 and apoE4/3 groups. ApoB levels did not change significantly in any group after changing to the low-fat diet. Diet-induced decreases in total cholesterol were significantly (*P*=.02) different between phenotypes, with progressively greater reductions from apoE3/2 (–0.15±0.16 mmol/L) to

apoE3/3 (–0.34±0.07 mmol/L) to apoE4/3 (–0.63±0.10 mmol/L). Diet-induced decreases in LDL-C were significant in all groups, but the differences among phenotypes for three-group comparisons did not reach significance (*P*=.11). Two-group comparisons showed that the decrease in LDL-C in apoE4/3 (–0.58±0.10 mmol/L) was significantly (*P*=.05) greater than in apoE3/3 (–0.39±0.06 mmol/L). However, after adjustment for group differences in high-fat LDL-C or LDL total mass (S_f^o 0 to 20), apoE4/3 no longer significantly predicted change in LDL-C. There were no significant differences in diet-induced changes in triglycerides, HDL-C, apoB, or apoA-I among the apoE phenotypes.

Dietary Changes in Lipoprotein Mass Concentrations by ApoE Phenotype

Table 4⇓ shows plasma lipoprotein mass concentrations after the high- and low-fat diets by apoE phenotype group. After the high-fat diet, there were no significant differences between the groups for mass of VLDL, IDL, LDL-III, LDL-IV, HDL_2, or HDL_3. There were significant (*P*<.05) differences among phenotypes, however, for mass of LDL-I and LDL-II, with progressive increases from apoE3/2 to apoE3/3 to apoE4/3 for both LDL-I and LDL-II. After the low-fat diet, the differences among the groups remained significant (*P*<.05) for LDL-II.

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Table 4.

Plasma Lipoprotein Mass Concentrations by ApoE Phenotype on High-Fat and Low-Fat Diets

	ApoE3/2 (n=10)			ApoE3/3 (n=65)			ApoE4/3 (n=28)		
	High Fat	Low Fat	Difference	High Fat	Low Fat	Difference	High Fat	Low Fat	Difference
VLDL, S_f^o 20-400	119.5±30.0	203.2±50.2	83.7 ±7.0	77.1±7.3	125.1±10.1	48.0±8.3[5]	60.9 ±9.0	101.7±11.0	40.8±10.5[4]

	ApoE3/2 (n=10)			ApoE3/3 (n=65)			ApoE4/3 (n=28)		
	High Fat	**Low Fat**	**Difference**	**High Fat**	**Low Fat**	**Difference**	**High Fat**	**Low Fat**	**Difference**
IDL, S_f^o 12-20	39.1±4.9	39.3±4.3	0.2 ±3.4	33.0±2.0	33.2±2.1	0.2±1.8	31.4±3.3	32.2 ±3.1	0.8±2.6
LDL-I, S_f^o 7-12	97.8±7.8	77.3±7.5	–20.6±7.0[2]	133.4 ±5.9	99.8±5.7	–33.6±4.3[5]	138.4±8.5	82.8 ±4.2	–55.6±6.8[5]
LDL-II, S_f^o 5-7	86.0±10.4	81.0±11.1	–5.1±9.7	123.3 ±4.5	108.7±4.4	–14.5±3.9[4]	137.1±6.8	114.7 ±6.1	–22.4±6.5[4]
LDL-III, S_f^o 3-5	55.9±13.0	66.8±10.8	10.9±7.8	60.3±4.9	78.6 ±5.0	18.3±4.1[5]	62.4±6.5	91.1±7.5	28.7 ±6.6[4]
LDL-IV, S_f^o 0-3	10.7 ±3.3	18.2±5.1	7.5±5.1	10.9±1.4	17.4±2.0	6.4 ±1.4[5]	11.8±1.5	19.1±2.7	7.3±2.4[3]
HDL_2, $F^o_{1.20}$ 3.5-9	43.5 ±13.6	27.1±8.0	–16.5±12.9	36.0±4.2	23.6 ±2.8	–12.4±2.5[5]	35.3±5.2	24.4±4.6	–10.9 ±3.4[3]

	ApoE3/2 (n=10)			ApoE3/3 (n=65)			ApoE4/3 (n=28)		
	High Fat	Low Fat	Difference	High Fat	Low Fat	Difference	High Fat	Low Fat	Difference
HDL_3, $F^o_{1.20}$ 0-3.5	207.8±13.6	204.4±14.3	–3.3±7.7	190.7 ±4.2	179.8±3.4	–10.8±3.4[4]	186.1±5.1	179.4 ±5.9	–6.7±5.6

Apo indicates apolipoprotein; S_f^o and $F^o_{1.20}$, peak flotation rate. Values are mean±SEM and are given in milligrams per deciliter. Significance of differences between apoE phenotype groups on both high- and low-fat diets are indicated in the text.

1 Comparison of diet-induced differences among phenotype groups by Kruskal-Wallis test.

2 *P*<.05,

3 *P*<.01,

4 *P*<.001,

5 *P*<.0001 for low- vs high-fat diet difference.

Table 4⇑ also shows diet-induced changes in plasma lipoprotein mass concentrations by apoE phenotype. VLDL mass increased in all groups but reached significance only for the apoE3/3 and apoE4/3 groups. There were no diet-induced differences in IDL within phenotypes. Decreases in mass of LDL-I were significant in all groups and were significantly (*P*=.01) related to apoE phenotype, with progressively greater reductions from apoE3/2 (–20.6±7.0 mg/dL) to apoE3/3 (–33.6±4.3 mg/dL) to apoE4/3 (–55.6±6.8 mg/dL). Two-group comparisons showed that the reduction in LDL-I in apoE4/3 was significantly (*P*=.02) greater than apoE3/3 and significantly (*P*<.01) greater than apoE3/2. Within the LDL-I flotation range, the greatest differences in response between apoE4/3 and apoE3/3 was found for S_f^o 7 to 8 (–27.1±3.1 versus –15.4±2.1 mg/dL, respectively) (*P*<.01). Baseline LDL-C level predicts diet-induced changes in LDL-C,[35] and after adjustment for differences in high-fat LDL-C among apoE phenotypes, apoE4/3 was still significantly (*P*<.05) associated with greater

reductions in mass of larger LDL after the low-fat diet (data not shown). Mass of LDL-II, HDL_2, and HDL_3 decreased, and LDL-III and LDL-IV increased in all groups, with significant changes in the apoE3/3 and apoE4/3 groups. There was a trend toward greater decreases in LDL-II and increases in LDL-III from apoE3/2 to apoE3/3 to apoE4/3, but these group differences did not reach significance. Within the LDL-II flotation range, the differences in response between apoE4/3 and apoE3/3 for S_f^o 6 to 7 (–21.8±3.6 versus –13.7±2.4 mg/dL, respectively) were marginally significant (*P*=.08). There were no significant group differences for changes in mass of IDL, LDL-IV, HDL_2, or HDL_3.

Discussion

Differences in dietary lipoprotein response have frequently been examined in relation to apoE genetic polymorphism.[13 14 15 17 36 37 38 39] Some reports have indicated that the apoE4 isoform is associated with higher LDL-C and a greater response of LDL-C to dietary lipids[13 14 15 36 37] than are the other isoforms. The effect of apoE phenotype on the variation of LDL-C that has been observed in association with a high-fat diet,[6 7 40] however, has been reported to disappear with a low-fat diet,[16] a result that was not confirmed in the present study. Other studies report that apoE phenotype is not associated with lipid response to dietary intervention.[17 38 39 41] There are important differences among these studies that could account for such discrepant results. First, study groups differed in gender, age, and baseline lipid levels. Second, the dietary intervention protocols were not consistent among studies. Finally, most of the studies had relatively small numbers of subjects, and the nonsignificant effect of apoE phenotype on dietary lipid response may be due to lack of statistical power.[10] Those studies that showed a significant apoE effect evaluated comparatively larger sample sizes.[13 14 15]

Using analyses of LDL heterogeneity, the present study identified a strong relation between apoE phenotype and LDL response to reduced dietary fat intake. ApoE4/3 was significantly related to greater decreases in mass of larger, more buoyant LDL particles (S_f^o >7) but not to changes in smaller, more dense LDL or IDL. Previous discrepant results[13 14 15 16 17 36 37 38 39 41] on the association of apoE phenotype with differences in dietary LDL-C response may depend on whether cholesterol is transported predominantly in larger or smaller LDL particles.

The mechanism by which apoE polymorphism influences LDL levels is still uncertain but may involve effects of apoE on the catabolism of triglyceride-rich particles.[42 43 44 45 46] There is also evidence for differential distribution of apoE isoforms among lipoprotein particles. ApoE4 has a greater association with triglyceride-rich lipoproteins than apoE3 and apoE2.[47 48] Clearance of chylomicron remnants is more rapid in subjects with apoE4 than apoE3 isoforms.[42] This, coupled with enhanced cholesterol absorption in subjects with apoE4,[49] may increase the intrahepatic pool of cholesterol, downregulate hepatic cholesterol synthesis and LDL receptor activity, and consequently elevate plasma LDL levels.[47] ApoE3 and apoE4 bind normally to the LDL receptor, but the reduced binding affinity of apoE2[50] may upregulate LDL receptor activity and increase LDL clearance. Thus, enhanced uptake of apoE4-containing remnant particles along with the reduced receptor binding of apoE2 could contribute to receptor–mediated differences in the effect of apoE alleles on LDL response to reduced dietary lipid.

It is also possible that differential apoE content of larger LDL or its precursors is responsible for the differential effects on LDL particles.[51 52 53] ApoE-enriched larger LDL particles could have a greater affinity for binding to LDL receptors.[54] Low-fat diets are known to increase LDL receptor activity, and apoE-containing lipoproteins have a greater affinity for the LDL receptor than do apoB-100–containing lipoproteins.[54] Thus, larger LDL particles containing apoE may be more rapidly catabolized.[55] Since clearance rates are faster for apoE4- than apoE3-containing particles,[42] this would result in a greater decrease in larger LDL particles in subjects with apoE4 phenotypes.

In the present study, reduction in dietary fat resulted in decreases in LDL-C levels without a change in plasma apoB. Since plasma apoB in normotriglyceridemic subjects primarily reflects the number of LDL particles, the results indicate that the predominant mechanism of the reduction in LDL-C with reduced fat intake does not involve a reduction in the number of LDL particles but rather a shift from larger, cholesterol-rich to smaller, cholesterol-poor LDL particles. This shift in LDL particle size was demonstrated by decreases in mass of larger LDL-I and LDL-II particles and concomitant increases in mass of smaller LDL-III and LDL-IV particles, an effect that decreased progressively from apoE4 to apoE3 to apoE2. It is not known whether this change in LDL particle distribution results from enhanced conversion of larger to smaller LDL or from changes in triglyceride-rich lipoprotein precursors that lead to the preferential production of smaller, lipid-depleted LDL. In either case, it is possible that differences in lipid composition of the smaller particles result in decreased apoE binding and consequently a slower clearance rate, with a weaker relation between clearance of these particles and apoE phenotype.[56]

Several studies have shown that LDL heterogeneity is associated with CAD.[57 58 59 60 61] Either large, buoyant LDL-I[57 58] or small, dense LDL-III[59 60 61] particles are frequently found in patients with CAD. However, recent studies have shown that small, dense LDL is potentially more atherogenic than larger LDL by virtue of its increased susceptibility to oxidative modification[62 63] and its increased promotion of intracellular cholesterol ester accumulation.[61] Reductions in small LDL have been associated with decreased progression of CAD,[64 65] but the effects of decreases in larger LDL particles on CAD have not been documented. Substantial therapeutic reductions in levels of LDL-I and LDL-II in patients with CAD are not associated with reduced angiographic progression.[64] Thus, the therapeutic implications of reduction of larger LDL are unclear. In this regard, it is notable that the association of apoE4 as a risk factor for CAD[3 11] may be independent of LDL-C levels.[12]

Our finding that reductions in HDL-C and apoA-I accompany reductions in LDL-C in all groups of subjects on the low-fat diet is consistent with others' results.[66 67 68] Decreases in HDL-C seen on low-fat diets may be an adaptive mechanism reflecting decreased flux of HDL cholesteryl ester transport through the HDL metabolic pathway.[69]

The results of this study demonstrate that apoE phenotypes influence the magnitude of LDL-C reduction on low-fat diets by mechanisms that promote a shift from larger, cholesterol-rich to smaller, cholesterol-depleted LDL particles. Our results apply only to reduction in total fat intake, and it is possible that apoE isoforms operate differently in influencing the response to other dietary manipulations designed to lower LDL-C, such as substitution of monounsaturated or polyunsaturated fat for saturated fat or reduction in cholesterol intake. The present results indicate that the relative magnitude of LDL-C reductions induced by a low-fat diet in subjects with differing apoE phenotypes may depend on whether cholesterol is transported predominantly in

larger or smaller LDL particles.

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