Effect of apolipoprotein E phenotype on diet-induced lowering of plasma low density lipoprotein cholesterol[1]

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Abstract The National Cholesterol Education Program (NCEP) has recommended that dietary total fat, saturated fat, and cholesterol intake be reduced to ≤ 30% of calories, < 10% of calories, and < 300 mg/day, respectively (Step 1 diet) in the general population to reduce plasma low density lipoprotein (LDL) cholesterol levels and heart disease risk. We examined the LDL cholesterol-lowering response to such a diet (26% fat, 8% saturated fat, and 201 mg/day of cholesterol) as compared to an average American diet (39% fat, 15% saturated fat, and 435 mg/day of cholesterol) in 128 subjects using diet periods of 4–24 weeks for each diet phase. The mean LDL cholesterol reduction was 15% in males (n = 83) and 8% in post-menopausal females (n = 45). The effect of apolipoprotein (apo) E phenotype on responsiveness was examined. LDL cholesterol lowering in males was 14% for 60 apoE3/3 subjects, 23% for 10 apoE3/4 subjects, and 16% for 13 apoE3/2 subjects. Male apoE3/4 subjects had a significantly greater LDL cholesterol reduction (P = 0.006) and a greater decrease in the LDL/HDL ratio (P = 0.047) than apoE3/3 subjects. In females, 7% lowering in LDL cholesterol was observed in 34 apoE3/3 subjects and 11% lowering was observed in 7 apoE3/4 subjects (P = 0.12). A meta-analysis of data from published studies supports this conclusion. These data indicate that apoE phenotype modulates the LDL cholesterol-lowering response to a diet meeting NCEP Step 1 criteria, and that male subjects carrying the apoE4 allele are more responsive than other subjects.—**Lopez-Miranda, J., J. M. Ordovas, P. Mata, A. H. Lichtenstein, B. Clevidence, J. T. Judd, and E. J. Schaefer.** Effect of apolipoprotein E phenotype on diet-induced lowering of plasma low density lipoprotein cholesterol. *J. Lipid Res.* 1994. **35:** 1965–1975.

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In humans, apolipoprotein (apo) E is a 299 amino acid polypeptide synthesized primarily in liver. ApoE in serum is associated with chylomicrons, very low density lipoproteins (VLDL), and high density lipoproteins (HDL), and serves as a ligand for the low density lipoprotein (LDL) receptor and the LDL receptor-related protein (LRP) (1, 2). When apoE deficiency is present, there is marked accumulation of cholesterol-enriched lipoproteins of density < 1.006 g/ml containing apoB-48 and apoA-IV, as well as apoB-100. These data support the concept that apoE is important for the clearance of these lipoproteins (3). Genetic variation at the apoE locus results from three common alleles in the population, ϵ4, ϵ3, and ϵ2, with frequencies in Caucasian populations of approximately 0.150, 0.769, and 0.08, respectively (4). Population studies have shown that plasma cholesterol, LDL cholesterol, and apoB are highest in subjects carrying the apoE4 isoform, intermediate in those with the apoE3 isoform, and lowest in those with the apoE2 isoform (5–10). It has been suggested that apoE allelic variation may account for as much as 14% of the variation in total and LDL cholesterol levels in the general population (4, 11). In our own studies of participants in the Framingham Offspring Study, apoE isoforms accounted for 1% of LDL cholesterol variation in males and pre-menopausal females, and for 5% of variation in post-menopausal females (10). This relationship between LDL cholesterol levels and apoE genetic variation is not independent of environmental and ethnic factors. The association of the apoE4 isoform with elevated serum cholesterol levels is greater in populations consuming diets rich in saturated

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; LDL, low density lipoprotein; LRP, LDL receptor-related protein; BMI, body mass index; HFHC, high fat, high cholesterol; LFLC, low fat, low cholesterol; MRU, Metabolic Research Unit; NIDDM, non-insulin-dependent diabetes mellitus; FH, familial hypercholesterolemia; CETP, cholesteryl ester transfer protein.

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fat and cholesterol than in other populations (6, 12, 13). These data indicate that the higher LDL cholesterol levels observed in subjects carrying the apoE4 isoform are manifested primarily in the presence of an atherogenic diet characteristic of certain societies, and that the response to dietary saturated fat and cholesterol may differ among individuals with different apoE phenotypes.

In recent years, the interaction between lipoprotein responsiveness to dietary manipulation and apoE phenotype has been the subject of several investigations (14–24). Some studies report greater plasma lipid responses in subjects carrying the apoE4 allele (15, 17, 20, 23, 25), while others failed to do so (14, 16, 18, 21, 22, 24). None of the previous studies included subjects older than 55 years old. In the present study, we have examined the impact of apoE polymorphism on plasma lipid and lipoprotein response to a diet restricted in total fat, saturated fat, and cholesterol in young males and in older males and females. Our results confirm some previous studies showing that the presence of the apoE4 allele is associated with increased response of plasma LDL cholesterol to changes in dietary fat and cholesterol in men. In addition, the meta-analysis carried out using our data and previously published studies shows that apoE4 carriers are more responsive to dietary modification than those who are not apoE4 carriers.

METHODS

Subjects

A total of 133 subjects (86 males and 47 females) who participated in dietary intervention studies at three sites (USDA Human Nutrition Research Center on Aging at Tufts University, Beltsville Human Nutrition Research Center, and University of Texas Southwestern Medical Center in Dallas) were included in this study. The age, body mass index (BMI), and lipid levels of study subjects while consuming an average American diet are shown in **Table 1**, and they have been previously described in detail (26–30). Subjects at the Beltsville site were younger and their plasma lipid levels were lower than at the other two sites. Due to the significant differences observed between males and females for age and lipoprotein levels at baseline, data for males and females were analyzed separately.

Diets and experimental protocols

Subjects at the different sites followed a similar dietary protocol consisting of two diet periods, ranging from 4 to 24 weeks each: a high fat (mean ± SD: 39.3 ± 2.6% of total calories; range 35.4–40.7), high saturated (14.5%; range 12.9–15.2), high cholesterol (435 ± 97 mg/day; range 306–510) diet (HFHC) period; and a low fat

TABLE 1. Characteristics of the study population while consuming an average American diet (mean ± SD)

	Site	Males (86)	Females (47)	*P* Value Males vs. Females
Age (years)	All	45.4 ± 15.3	62.6 ± 7.7	<0.0001
	Boston	57.8 ± 11.7	66.9 ± 7.3	
	Beltsville	34.1 ± 9.0		
	Dallas	55.1 ± 12.4	59.5 ± 6.4	
BMI (kg/m^2)	All	25.6 ± 3.1	26.1 ± 4.5	0.3
	Boston	26.1 ± 1.9	26.6 ± 4.4	
	Beltsville	25.0 ± 3.4		
	Dallas	26.1 ± 3.3	25.3 ± 4.7	
Total cholesterol (mg/dl)	All	222 ± 41	247 ± 39	<0.0001
	Boston	210 ± 35	223 ± 31	
	Beltsville	197 ± 19		
	Dallas	267 ± 28	264 ± 35	
Triglyceride (mg/dl)	All	124 ± 62	135 ± 77	0.106
	Boston	112 ± 34	97 ± 30	
	Beltsville	101 ± 44		
	Dallas	168 ± 77	163 ± 88	
LDL cholesterol (mg/dl)	All	154 ± 40	169 ± 31	<0.01
	Boston	142 ± 30	151 ± 28	
	Beltsville	131 ± 26		
	Dallas	199 ± 27	181 ± 27	
HDL cholesterol (mmol/l)	All	47 ± 13	56 ± 10	<0.0001
	Boston	44 ± 6	54 ± 9	
	Beltsville	54 ± 12		
	Dallas	39 ± 13	57 ± 11	

(26.4 ± 5.1% of total calories; range 18.9–29.7), low saturated (7.6%; range 4.4–9.9), low cholesterol (201 ± 22 mg/day; range 178–230) diet (LFLC) period (**Table 2**).

At the Boston site, the experimental protocol was approved by the Human Investigation Review Committee of the New England Medical Center and Tufts University. In this protocol, all the food was provided to the subjects. The first phase was a 6-wk period during which subjects consumed a diet approximating that of the current American diet (baseline). The second phase was a 24-wk period during which subjects consumed a low fat, low cholesterol diet conforming to the National Cholesterol Education Program (NCEP) Step 2 recommendations. All diets were prepared in the Metabolic Research Unit (MRU) kitchen at the USDA-Human Nutrition Research Center on Aging at Tufts University and were composed entirely of natural foods consumed as three meals and one snack per day. Subjects were required to report to the MRU a minimum of five times per week and to eat at least one meal per visit at the unit. All food and drink was packaged for take-out. During each visit body weight and blood pressure were measured. Calorie levels were assigned so that the subjects neither gained nor lost weight. The Grand Forks database (GRAND, release 8606) was used to calculate nutrient composition of the diets and analytical data from Hazelton Laboratories (Madison, WI) was used as confirmation. The percent energy distribution of the baseline diet was as follows: 15 ± 1.2% protein, 49.4 ± 2.2% carbohydrate, 35.5 ± 2.3% fat (14.1 ± 2.2% saturated, 14.5 ± 1.0% monounsaturated, and 6.9 ± 1.2 polyunsaturated) and 147 ± 27 mg of cholesterol per 1000 kcal. During consumption of the NCEP Step 2 diet, the percent energy distribution was as follows: 17 ± 0.9% protein, 56.1 ± 2.9% carbohydrate, 25.9 ± 1.4% fat (4.2 ± 0.5% saturated, 11.2 ± 0.9% monounsaturated, and 10.4 ± 0.2% polyunsaturated) and 53 ± 13 mg of cholesterol per 1000 kcal. A total of 36 subjects, 18 females and 18 males, were studied at the Boston site with a mean age of 62 ± 11 years old.

TABLE 2. Characteristics of the diets and experimental protocols used at each site

	Boston	Beltsville	Dallas
High fat period			
Time (weeks)	6	10	4
% Protein[a]	15	14.8	17
% Carbohydrates	49.4	45.8	43
% Total fat	35.5	41	40
% Saturated fat	14.1	15	15
% Monounsaturated fat	14.5	14	15
% Polyunsaturated fat	6.9	8	9
Cholesterol (mg/4.184 kJ)	147	189	170
Low fat period			
Time (weeks)	24	10	12–16
% Protein	17	17.1	19
% Carbohydrates	56.1	67.3	51
% Total fat	25.9	19	30
% Saturated fat	4.2	4.4	10
% Monounsaturated fat	11.2	6.5	8
% Polyunsaturated fat	10.4	5.2	12
Cholesterol (mg/4.184 kJ)	53	76	120

[a] Percent energy

At the Beltsville site, the protocol was approved by the Institutional Review Boards of the National Cancer Institute, the National Institutes of Health, and Georgetown University School of Medicine. At this site, each diet period was 10 weeks in length. All meals were prepared in the MRU kitchen at the Beltsville Human Nutrition Research Center and were composed of foods commonly available to the public. On weekdays, breakfast and dinner were eaten in the facility and takeout lunches were provided. Weekend meals were prepackaged for home consumption. The high fat diet contained 41% of calories as fat (15% saturated fatty acids, 14% oleic acid, and 8% linoleic acid), 14.8% protein, 45.8% carbohydrates, and 189 mg of cholesterol per 1000 kcal. The low fat diet contained 19% of calories from fat (4.4% saturated fatty acids, 6.5 oleic acid, and 5.2% linoleic acid), 17.1% protein, 67.3% carbohydrate, and 76 mg of cholesterol per 1000 kcal. At this site 42 male subjects were studied, mean age 34 ± 9 years old.

The protocol at the Dallas site was approved by the Institutional Review Boards of both the University of Texas Southwestern Medical Center and the Veterans Administration Center at Dallas. The experimental approach was somewhat different than at the first two sites. The high fat diet was consumed for 1 month, whereas the low fat diet was consumed for 3–4 months. Diet counselling was provided for each phase with the intent of increasing or decreasing the fat and cholesterol content of the diets. After each initial counselling session, subjects were contacted by phone once during both the first and second week of each dietary period to answer questions and to verify compliance. Final assessment of the consumed diet was determined from subjects written, 7-day food records analyzed by Computrition computerized data base of the National Research Council's nutrient content of foods (Computrition, Inc., Chatsworth, CA). The high fat diet contained 40% of calories as fat (15% saturated fatty acids, 15% monounsaturated fatty acids, and 9% polyunsaturated fatty acids), 17% protein, 43% carbohydrates, and 170 mg of cholesterol per 1000 kcal. The low fat diet contained 30% of calories from fat (10% saturated fatty acids, 8% monounsaturated fatty acids, and 12% polyunsaturated fatty acids), 19% protein, 51% carbohydrates, and 120 mg of cholesterol per 1000 kcal. At this site 55 subjects were studied (26 males and 29 females), mean age 57 ± 9 years old.

TABLE 3. Age and BMI by apoE phenotype (mean ± SD)

Group	Number	Age	BMI
Males			
E3/4	10	43.0 ± 8.2	24.9 ± 3.6
E3/3	60	44.5 ± 15.5	25.7 ± 3.2
E2/3	13	46.6 ± 17.8	25.2 ± 3.1
Females			
E3/4	7	71.0 ± 4.0	29.0 ± 4.6
E3/3	34	62.0 ± 7.1	26.2 ± 4.5
E2/3	4	58.3 ± 6.5	24.0 ± 3.8

Lipid, lipoprotein analysis and apolipoprotein E phenotyping

Blood samples were collected after 12 h fast, in 0.1% EDTA during the last week of each study period. Plasma was isolated by centrifugation at 2500 rpm, 4°C, for 20 min. Total cholesterol and triglycerides were determined using enzymatic assays (31). Assays were standardized through participation in the Centers for Disease Control–National Heart, Lung, and Blood Institute Standardization program.

ApoE phenotyping was carried out at the Boston site by isoelectric focusing of whole plasma followed by immunoblotting as previously described (32) using a specific anti-human apoE antiserum.

Statistical analysis

The SAS statistical program (SAS Institute, Cary, NC) was used to perform statistical analyses. The *t* test was used for comparing continuous variables and ANCOVA for repeated measurements was used for discrete variables. Age and BMI were used as covariates. To account for differences in dietary protocols, plasma total cholesterol, LDL cholesterol, and HDL cholesterol at each site were adjusted to an average high fat diet (39% total fat, 15% saturated fat, 15% monounsaturated fat, 9% polyunsaturated fat, and 170 mg/day of cholesterol) and to an average low fat diet (26% total fat, 7% saturated fat; 9% monounsaturated fat, 10% polyunsaturated fat, and 92 mg/day of cholesterol) using the equations published by Hegsted et al. (33). Subjects with the less common phenotypes (E4/4, E2/2, and E2/4) were not included in the statistical analyses because of small sample size. Triglyceride and HDL-cholesterol/LDL-cholesterol ratio values were not normally distributed and were log transformed prior to the analysis. Z-score analysis of pooled data from previously published studies as well as our own data (15–22) was carried out as previously described (34).

TABLE 4. Plasma total cholesterol and triglycerides on each diet phase (mean ± SD)

Group	Number	High Fat	Low Fat	Change	% Change[a]
Total cholesterol (mg/dl)					
All males[b]	86	222 ± 41	191 ± 42	−31 ± 23*	−14
E3/4	10	234 ± 39	191 ± 33	−43 ± 32*	−17
		(236)	(198)	(−38)	(−16)
E3/3	60	222 ± 43	193 ± 44	−29 ± 22*	−13
		(222)	(193)	(−29)	(−13)
E2/3	13	212 ± 38	182 ± 45	−30 ± 23*	−14
		(209)	(182)	(−27)	(−13)
All females[c]	47	250 ± 39	232 ± 43	−18 ± 24*	−7
E3/4	7	262 ± 34	246 ± 39	−16 ± 16**	−6
		(273)	(253)	(−20)	(−7)
E3/3	34	249 ± 41	230 ± 46	−19 ± 26*	−8
		(244)	(227)	(−17)	(−7)
E2/3	4	233 ± 17	223 ± 27	−10 ± 17	−4
		(217)	(204)	(−13)	(−6)
Triglycerides (mg/dl)					
All males	86	124 ± 62	126 ± 42	2 ± 48	2
E3/4	10	126 ± 56	137 ± 70	11 ± 69	9
E3/3	60	127 ± 68	127 ± 55	0	0
E2/3	13	111 ± 35	118 ± 39	7 ± 21	6
All females	47	143 ± 79	148 ± 69	5 ± 57	3
E3/4	7	119 ± 34	153 ± 52	34 ± 34**	29
E3/3	34	150 ± 84	144 ± 70	−6 ± 61	−4
E2/3	4	164 ± 95	201 ± 61	37 ± 54	23

Numbers in parentheses represent adjusted values after correcting for study site, age, and BMI (see text for details).
[a] Percent change was calculated as: [High Fat] − [Low Fat]/[High Fat] × 100.
[b] Values for apoE4/4 (1), apoE2/2 (1), and apoE2/4 (1) subjects are not shown.
[c] Values for apoE4/4 (1) and apoE2/4 (1) subjects are not shown.
*$P < 0.005$; **$P < 0.05$, comparison between diet phases, paired *t*-test.

RESULTS

The distribution of the most common apoE phenotypes in our population is shown in **Table 3.** The relative frequencies of the apoE4, apoE3, and apoE2 isoforms (including the E4/4, E2/2, and E4/2 phenotypes) were 0.086, 0.837, and 0.077, respectively. No significant differences were noted among the three phenotypes (E4/3, E3/3, and E3/2) for age or BMI in either males or females. Plasma levels and changes in total cholesterol, triglycerides, LDL and HDL cholesterol in subjects with different apoE phenotypes are shown in **Table 4** and **Table 5.** The LFLC diet induced significant decreases ($P < 0.05$) in plasma total cholesterol, LDL cholesterol, and HDL cholesterol in both males and females. These decreases remained significant (with the exception of apoE2/3 females) after analyzing each of the phenotypes separately (see Tables 4 and 5). Plasma triglycerides showed a trend towards higher values during the LFLC diet; however, the increase was significant only in apoE3/4 females (Table 4). In males, no significant differences were noted among phenotypes for plasma total cholesterol, HDL cholesterol, and triglyceride levels regardless of the dietary treatment.

During the HFHC diet period, apoE3/4 males had significantly higher LDL cholesterol levels than subjects with the apoE3/3 phenotype ($P < 0.02$) (Table 5). The decrease in LDL cholesterol after the LFLC diet was significantly greater ($P = 0.006$) in male subjects with the apoE4/3 phenotype (−41 mg/dl) as compared to the changes observed in male subjects with the apoE3/3 phenotype (−21 mg/dl). This effect remained significant after adjusting for differences in the initial LDL cholesterol levels (percentage decrease 23% vs. 14%; $P < 0.05$). A similar trend was observed in females; with a 7% decrease in LDL cholesterol in apoE3/3 subjects and an 11% decrease in apoE3/4 subjects. These latter differences did not reach statistical significance.

LDL cholesterol levels for apoE3/4 and apoE3/3

TABLE 5. Plasma LDL and HDL cholesterol on each diet phase (mean ± SD)

Group	Number	High Fat	Low Fat	Change	% Change[a]
LDL Cholesterol (mg/dl)					
All males[b]	86	154 ± 40	130 ± 41	−23 ± 21*	−15
E3/4	10	168 ± 40	127 ± 33	−41 ± 34*, ***	−23***
		(171)	(132)	(−39)	(21)
E3/3	60	153 ± 41	132 ± 43	−21 ± 19*	−14
		(152)	(132)	(−20)	(−13)
E2/3	13	145 ± 38	122 ± 38	−23 ± 17*	−16
		(142)	(121)	(−21)	(−15)
All females[c]	47	171 ± 31	157 ± 35	−14 ± 21*	−8
E3/4	7	182 ± 28	162 ± 32	−20 ± 15**	−11
		(196)	(172)	(−24)	(−12)
E3/3	34	169 ± 33	158 ± 37	−11 ± 22**	−7
		(164)	(150)	(−14)	(−9)
E2/3	4	160 ± 14	143 ± 22	−17 ± 21	−11
		(150)	(129)	(−21)	(−14)
HDL cholesterol (mg/dl)					
All males	86	47 ± 13	40 ± 10*	−7 ± 8	−15
E3/4	10	44 ± 9	39 ± 8	−5 ± 7****	−11
		(43)	(39)	(−4)	(−9)
E3/3	60	48 ± 14	40 ± 10	−8 ± 8*	−12
		(48)	(42)	(−6)	(−13)
E2/3	13	48 ± 16	40 ± 15	−8 ± 5*	−17
		(49)	(42)	(−7)	(−14)
All females	47	55 ± 11	50 ± 11	−5 ± 7*	−9.00
E3/4	7	62 ± 14	55 ± 17	−7 ± 4**	−11
		(57)	(58)	(1)	(2)
E3/3	34	54 ± 10	49 ± 11	−5 ± 8*	−9
		(55)	(48)	(−7)	(−13)
E2/3	4	49 ± 3	47 ± 6	−2 ± 4	−4
		(49)	(44)	(−5)	(−10)

Numbers in parentheses represent adjusted values after correcting for study site, age, and BMI (see text for details).
[a] Percent change was calculated as: ([High Fat] − [Low Fat]/[High Fat]) × 100.
[b] Values on apoE4/4 (1), apoE2/2 (1), and apoE2/4 (1) subjects are not shown.
[c] Values on apoE4/4 (1) and apoE2/4 (1) subjects are not shown.
*$P < 0.005$; **$P < 0.05$; comparison between diet phases; paired t-test.
***$P < 0.001$; ****$P < 0.05$; comparison between phenotypes (E3/4 vs. E3/3 and E2/3 vs. E3/3), ANOVA for repeated measures with BMI and age as covariates.

TABLE 6. Site specific diet effects on LDL cholesterol in apoE3/4 and apoE3/3 subjects, mg/dl (mean ± SD)

Group	Number	High Fat	Low Fat	Change	%Change
Males					
Boston					
E4/3	3	149 ± 37	104 ± 17	−45	−29
E3/3	12	144 ± 33	128 ± 23	−16	−10
Beltsville					
E4/3	5	158 ± 31	125 ± 33	−33	−19
E3/3	29	127 ± 25	101 ± 24	−26	−20
Dallas					
E3/4	2	223 ± 1	167 ± 13	−56	−25
E3/3	19	198 ± 27	181 ± 28	−17	−8
Females					
Boston					
E3/4	4	174 ± 27	147 ± 31	−27	−16
E3/3	13	144 ± 27	124 ± 23	−20	−13
Dallas					
E3/4	2	201 ± 37	193 ± 20	−8	−3
E3/3	19	181 ± 27	178 ± 30	−3	−1

phenotypes at each site for men and women are given in **Table 6.** At both the Boston and Dallas sites, LDL cholesterol lowering was greater in apoE3/4 (−27%) than in apoE3/3 (−9%), while at Beltsville similar percent reductions were seen. In females, at both Boston and Dallas, LDL cholesterol reductions were greater in apoE3/4 than in apoE3/3 subjects, but differences were small.

To further assess whether the greater response to dietary treatment observed in apoE3/4 males was due to the slightly higher initial levels of LDL cholesterol in these subjects, we randomly pair-matched each apoE3/4 subject with one or two apoE3/3 subjects of similar age, sex, BMI, and LDL cholesterol level at baseline (HFHC diet) within the same study site (**Table 7**). For this baseline pairing, no statistical differences were noted for total cholesterol, HDL cholesterol, and triglycerides between groups. The decrease in LDL cholesterol levels after the LFLC diet remained significantly greater ($P = 0.003$) in subjects with the apoE3/4 phenotype (−41 mg/dl; −23%) as compared to apoE3/3 subjects (−9 mg/dl; −8%) after correcting for initial LDL cholesterol values. In this subset, no effects of the apoE phenotype were noted for plasma triglycerides and HDL cholesterol levels or changes.

For all males, the diet-induced decrease in HDL cholesterol in apoE3/4 subjects was less than in apoE3/3 males (−5 vs. −8 mg/dl, $P < 0.05$). The LDL/HDL

TABLE 7. Plasma lipid and lipoprotein levels during both diet phases in E3/4 males and in randomly pair-matched E3/3 males

Group	Number	High Fat	Low Fat	Change	% Change
Total Cholesterol (mg/dl)					
All	27	232 ± 39	205 ± 39	−27	−11
E4/3	10	234 ± 39	191 ± 33	−42	−17
E3/3	17	231 ± 40	213 ± 41	−18*	−8*
Triglycerides (mg/dl)					
All	27	129 ± 65	143 ± 66	15	−14
E4/3	10	126 ± 56	137 ± 70	11	−11
E3/3	17	130 ± 71	147 ± 66	17	−6
LDL cholesterol (mg/dl)					
All	27	164 ± 36	143 ± 38	−21	−12
E3/4	10	168 ± 40	127 ± 33	−41	−23
E3/3	17	162 ± 35	153 ± 39	−9**	−6**
HDL cholesterol (mg/dl)					
All	27	45 ± 13	38 ± 9	−7	−13
E3/4	10	44 ± 9	39 ± 8	−5	−11
E3/3	17	46 ± 14	38 ± 9	−8	−14

*$P < 0.03$; **$P < 0.003$; comparison of apoE3/3 vs. apoE3/4; ANOVA for repeated measures.

TABLE 8. LDL/HDL cholesterol ratio by E phenotype during both diet phases

Group	Number	High Fat	Low Fat	Change (H-L)
Males				
E4/3	10	4.01 ± 1.29	3.40 ± 1.28	0.61 ± 1.04[a,b]
E3/3	60	3.68 ± 1.91	3.54 ± 1.60	0.14 ± 0.82
E3/2	13	3.25 ± 1.34	3.22 ± 1.33	0.03 ± 0.42[c]
Females				
E4/3	7	3.05 ± 0.65	3.09 ± 0.79	-0.04 ± 0.37
E3/3	34	3.24 ± 0.93	3.30 ± 0.87	-0.15 ± 0.61
E3/2	4	3.26 ± 0.21	3.05 ± 0.51	0.22 ± 0.45

[a]Significantly different between diets ($P < 0.05$).
[b]Significantly different from E3/3 ($P < 0.05$).
[c]Significantly different from E4/3 ($P < 0.05$). ANOVA for repeated measures.

cholesterol ratio decreased significantly from 4.01 to 3.40 ($P = 0.013$) in apoE3/4 males but not in apoE3/3 males (3.68 to 3.54) (**Table 8**). These differences between phenotypes were also statistically significant ($P = 0.04$). No significant differences in HDL or LDL/HDL cholesterol changes were observed in females in different apoE phenotypic groups.

The results of the meta-analysis involving 9 studies and 612 subjects (**Fig. 1**) show that the presence of the E4 allele is associated with a significantly greater LDL cholesterol response to dietary manipulation than those subjects without the apoE4 allele (Z score = 0.36; 95% confidence interval: 0.17-0.54).

DISCUSSION

The extent of the response to changes in the amount and type of dietary fat and the amount of cholesterol varies among individuals (35-37). In some individuals the

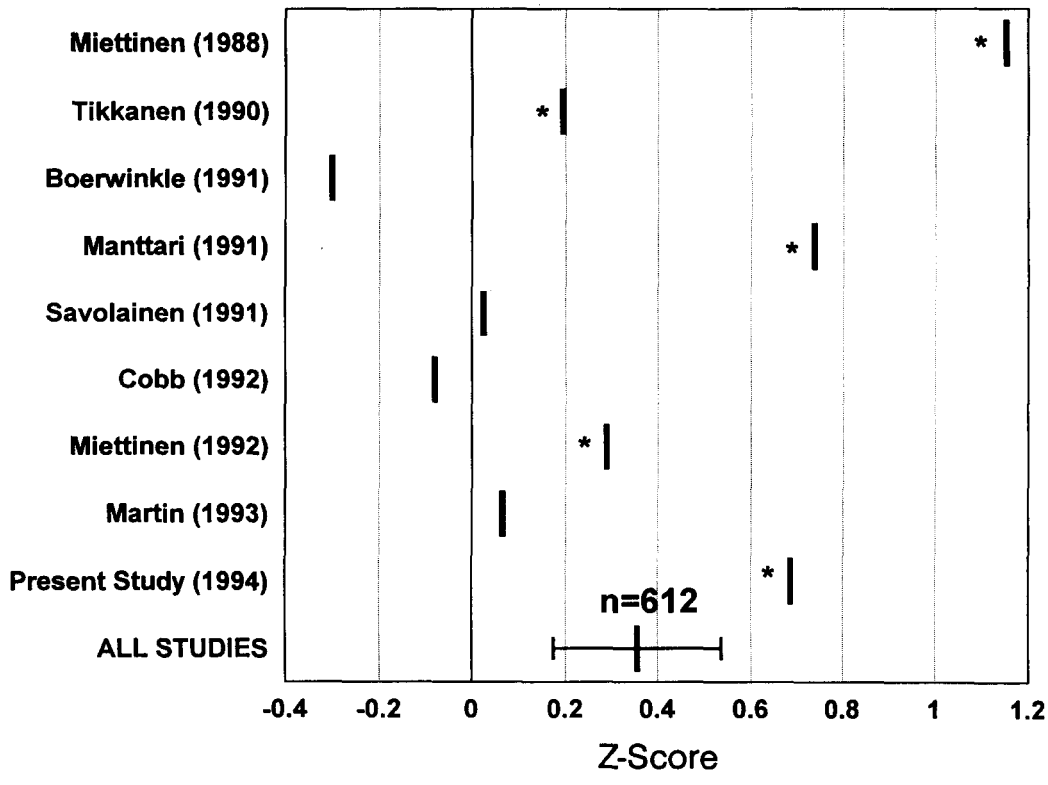

Fig. 1. Z-score values between diet-induced LDL cholesterol changes in apoE4 carriers versus non-carriers of each study analyzed in the metanalysis, and Z-score ± SD representing the 95% confidence interval for all studies. *Studies showing a significantly greater LDL cholesterol response associated with the apoE4 allele.

cholesterol level decreases considerably, after a low fat diet, while in others there is little change. It has been suggested that the serum lipoprotein response to dietary manipulation has a strong genetic component, although this is very difficult to prove in humans, and most of the evidence has been obtained in animals (38–42). Several studies have focused on the effect of the genetic variation at the apoE locus on the individual response of plasma lipid levels to different types of dietary manipulation. In six studies, the amounts of both dietary fat and cholesterol were modified in the experimental protocol (14–19); four of them concluded that apoE4 allele was associated with greater LDL cholesterol response to dietary modification (15, 17–19), while one of them did not find a significant difference (16). Another study did not have any subjects carrying the $\epsilon 4$ allele and found no significant differences in response between apoE3/3 and apoE2/3 subjects (14). In five studies, dietary cholesterol, but not the amount of fat, was modified (20–24). In two studies the presence of the apoE4 allele was associated with a greater response of plasma lipids to dietary cholesterol than the other alleles (20, 23), whereas in the others (21, 22, 24), no significant differences between phenotypes were noted. The data from those studies in which enough information was provided (15–22) were pooled with ours, and a meta-analysis was carried out. This analysis supports the concept that the apoE4 allele is associated with an increased LDL cholesterol response to dietary manipulation.

The interaction between dietary response, apoE phenotype, and diabetes has been also explored. Surprisingly, in non-insulin-dependent diabetics (NIDDM), the opposite effect was found. After consuming a "therapeutic" diet, diabetic subjects with the $\epsilon 2$ allele had the greatest response, whereas those with the $\epsilon 4$ had the least response (43). Other dietary modifications have also been explored. The use of vegetable protein induced a hypocholesterolemic response in apoE3/3 and apoE3/4 subjects with familial hypercholesterolemia (FH), but not in E2/3 patients with FH. However, it should be noted that in this study the removal of animal protein was also accompanied by a significant drop in the amount of dietary cholesterol and consequently the hypocholesterolemic effect attributed to the vegetable protein may, in part, have been due to the decrease in dietary cholesterol (44). Two other studies have reported that high fiber diets without fat modification induce the greatest hypocholesterolemic response in apoE3/3 subjects and in apoE2/3 subjects, suggesting that dietary fiber may reduce plasma cholesterol by a different mechanism than dietary fat and cholesterol (45, 46).

In our study, total, LDL and HDL cholesterol levels decreased significantly after consumption of a LFLC diet. The magnitude of LDL cholesterol lowering was twice as great in males as in females, which agrees with observations by other investigators (16, 47). In a large study of 4587 subjects placed on a low fat, low cholesterol diet for a 3-week period, the mean reduction in LDL cholesterol was 25% in males and 19% in females, also indicating a somewhat greater responsiveness in male than female subjects (47). In another study (48), an increase in dietary fat and cholesterol resulted in a greater LDL cholesterol rise in males than in females, whereas the response in HDL_2 cholesterol levels was greater in females. However, most of the females in our investigation were studied on an NCEP Step 1 diet, while males were studied on both NCEP Step 1 and Step 2 diets. At the Dallas site, mean LDL cholesterol reductions were 6% in women and 8% in men; at the Beltsville site only men were studied on an NCEP Step 2 diet and the mean LDL cholesterol reduction was 20%. At the Boston site, on an NCEP Step 2 diet, the LDL cholesterol reduction was 14% for both men and women. Therefore, when one does within-site data comparisons, similar reductions were observed in men and women.

When the effect of apoE phenotype on responsiveness was examined, our data show that the LDL cholesterol lowering observed in E3/4 males was 23%, which was significantly higher than that observed in E3/3 (14%) or E3/2 (13%) males. In females no differences between phenotypes were observed; this may be due to the lower dietary response in combination with the smaller number of female subjects in the study. At the Boston and Dallas sites, apoE3/4 males had LDL cholesterol reductions of 28 and 25%, respectively, versus 10% and 8% for apoE3/3 males, while at the Beltsville site reductions of 19% were noted in apoE3/4 males and 20% in apoE3/3 males. Whether these differences in response relate to the lower fat intake used in the therapeutic diets in Beltsville versus the other sites remains to be determined. With regard to females, at both the Boston and Dallas sites similar reductions in LDL cholesterol were observed for subjects with the apoE3/4 and the apoE3/3 phenotypes. These data indicate the need for further larger studies to determine the impact of apoE on diet responsiveness. The data do suggest that apoE3/4 males are more responsive in terms of LDL cholesterol lowering to diets restricted in saturated fat and cholesterol than apoE3/3 males.

Age and BMI have been previously shown to have an effect on the magnitude of the dietary response (48–50). In this study no differences were observed between apoE phenotypes for age and BMI and the addition of these variables to the analysis did not have any significant effect on the significance of the results. However, it should be noted that the greater response in LDL cholesterol levels associated with the apoE4 allele was due to the differences in older males (Boston and Dallas sites), but not in younger subjects (Beltsville site); however, at the Beltsville site the diet was high in fiber, which may be an additional confounder in this analysis (45, 46). In addition, older subjects also had higher LDL cholesterol levels making it

difficult to isolate the specific contribution of each of these variables to dietary response (e.g., age versus LDL cholesterol level).

The mechanisms involved in these effects have been partially elucidated, and they involve the differential removal of triglyceride-rich particles by the liver; however, the inconsistencies observed in the outcome of different studies suggest the existence of additional variables not accounted for in these studies. Additional mechanisms involving the plasma compartment or extrahepatic tissues may modulate the interaction between dietary response and apoE phenotype. It has been reported that a differential distribution of apoE isoforms exists among lipoprotein particles (51). ApoE4 preferentially associates with triglyceride-rich lipoproteins, whereas apoE2 has a greater affinity for HDL, with apoE3 being intermediate in this regard. Consequently, the increased presence of apoE4 in the triglyceride-rich lipoprotein fraction will enhance the affinity of lipoproteins for hepatic receptors and accelerate the clearance of these particles. In addition, apoE2 binds much less avidly to the LDL receptor than apoE3 or apoE4. Moreover, it has been shown that apoE isoforms alter the plasma cholesteryl ester transfer protein (CETP) response to cholesterol feeding (22). Increases in dietary cholesterol were accompanied by a 0%, 4%, and 12% increases in HDL cholesterol for apoE2/3, apoE3/3, and apoE3/4 subjects, respectively. The opposite effect was observed in terms of CETP activity for the three apoE phenotypes studied (E3/2, 37%; E3/3, 18%; and E4/3, 9%). Our data also show a different response of HDL cholesterol to changes in dietary fat and cholesterol, although in our case, for males, the apoE3/4 group was more resistant to diet-induced HDL cholesterol changes, whereas apoE3/3 and apoE2/3 subjects experienced a more significant decrease in HDL cholesterol after the low fat diet. More recently, it has been shown in vitro that the conformation of apoE in lipoproteins is affected by the lipid environment (52). In these experiments the differences were insignificant in terms of receptor-binding ability; however, it remains to be determined whether this may have a significant impact in vivo, especially in the context of dramatic changes in dietary fat and cholesterol.

Our data indicate that apoE phenotype may modulate the LDL cholesterol-lowering response to diet especially in males. Further large-scale studies in which subjects are selected based on their apoE phenotype are clearly needed to definitively address this issue and to determine the role of aging in this process.

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REFERENCES

1. Beisiegel, U., W. Weber, G. Ihrke, J. Herz, and K. K. Stanley. 1989. The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. *Nature.* **341:** 162–164.
2. Mahley, R. W. 1988. Apolipoprotein E:cholesterol transport protein with expanding role in cell biology. *Science.* **240:** 622–630.
3. Schaefer, E. J., R. E. Gregg, G. Ghiselli, T. M. Forte, J. M. Ordovas, L. A. Zech, F. T. Lindgren, and H. B. Brewer, Jr. 1986. Familial apolipoprotein E deficiency. *J. Clin. Invest.* **78:** 1206–1219.
4. Davignon, J., R. E. Gregg, and C. F. Sing. 1988. Apolipoprotein E polymorphism and atherosclerosis. *Arteriosclerosis.* **8:** 1–21.
5. Sing, C. F., and J. Davignon. 1985. Role of the apolipoprotein E polymorphism in determining normal plasma lipid and lipoprotein variation. *Am. J. Hum. Genet.* **37:** 268–285.
6. Ehnholm, C., M. Lukka, T. Kuusi, E. Nikkilä, and G. Utermann. 1986. Apolipoprotein E polymorphism in the Finnish population: gene frequencies and relation to lipoprotein concentrations. *J. Lipid Res.* **27:** 227–235.
7. Boerwinkle, E., S. Visvikis, D. Welsh, J. Steinmetz, S. M. Hanash, and C. F. Sing. 1987. The use of measured genotype information in the analysis of quantitative phenotypes in man. II. The role of the apolipoprotein E polymorphism in determining levels, variability and covariability of cholesterol, betalipoprotein and triglycerides in a sample of unrelated individuals. *Am. J. Med. Genet.* **27:** 567–582.
8. Kamboh, M. I., C. E. Aston, R. E. Ferrell, and R. F. Hamman. 1993. Impact of apolipoprotein E polymorphism in determining interindividual variation in total cholesterol and low density lipoprotein cholesterol in Hispanics and non-Hispanic whites. *Atherosclerosis.* **98:** 201–211.
9. Xhignesse, M., S. Lussier-Cacan, C. F. Sing, A. M. Kessling, and J. Davignon. 1991. Influences of common variants of apolipoprotein E on measures of lipid metabolism in a sample selected for health. *Arterioscler. Thromb.* **11:** 1100–1110.
10. Ordovas, J. M., L. Litwack-Klein, P. W. F. Wilson, M. M. Schaefer, and E. J. Schaefer. 1987. Apolipoprotein E isoform phenotyping methodology and population frequency with identification of apoE1 and apoE5 isoforms. *J. Lipid Res.* **28:** 371–380.
11. Demant, T., D. Bedford, C. J. Packard, and J. Shepherd. 1991. Influence of apolipoprotein E polymorphism on apolipoprotein B-100 metabolism in normolipemic subjects. *J. Clin. Invest.* **88:** 1490–1501.
12. Utermann, G. 1987. Apolipoprotein E polymorphism in health and disease. *Am. Heart J.* **113:** 433–440.
13. Boerwinkle, E., and G. Utermann. 1988. Simultaneous effects of the apolipoprotein E polymorphism on apolipoprotein E, apolipoprotein B, and cholesterol metabolism. *Am. J. Hum. Genet.* **42:** 104–112.
14. Fisher, E. A., C. B. Blum, V. I. Zannis, and J. L. Breslow. 1983. Independent effects of dietary saturated fat and cholesterol on plasma lipids, lipoproteins, and apolipoprotein E. *J. Lipid Res.* **24:** 1039–1048.

15. Tikkanen, M. J., J. K. Huttunen, C. Enholm, and P. Pietinen. 1990. Apolipoprotein E4 homozygosity predisposes to serum cholesterol elevation during high fat diet. *Arteriosclerosis.* **10:** 285–288.
16. Savolainen, M. J., M. Rantala, K. Kervinen, L. Jarvi, K. Suvanto, T. Rantala, and Y. A. Kesäniemi. 1991. Magnitude of dietary effects on plasma cholesterol concentration: role of sex and apolipoprotein E phenotype. *Atherosclerosis.* **86:** 145–152.
17. Manttari, M., P. Kosninen, C. Enholm, J. K. Huttunen, and V. Manninen. 1991. Apolipoprotein E polymorphism influences the serum cholesterol response to dietary intervention. *Metabolism.* **40:** 217–221.
18. Cobb, M. M., H. Teitlebaum, N. Risch, J. Jekel, and A. Ostfeld. 1992. Influence of dietary fat, apolipoprotein E phenotype, and sex on plasma lipoprotein levels. *Circulation.* **86:** 849–857.
19. Miettinen, T. A., H. Gylling, H. Vanhanen, and A. Ollus. 1992. Cholesterol absorption, elimination, and synthesis related to LDL kinetics during varying fat intake in men with different apoprotein E phenotypes. *Arterioscler. Thromb.* **12:** 1044–1052.
20. Miettinen, T. A., H. Gylling, and H. Vanhanen. 1988. Serum cholesterol response to dietary cholesterol and apoprotein E phenotype. *Lancet.* **2:** 1261.
21. Boerwinkle, E., S. A. Brown, K. Rohrbach, A. M. Gotto, Jr., and W. Patsch. 1991. Role of apolipoprotein E and B gene variation in determining response of lipid, lipoprotein, and apolipoprotein levels to increased dietary cholesterol. *Am. J. Hum. Genet.* **49:** 1145–1154.
22. Martin, L. J., P. W. Connelly, D. Nancoo, N. Wood, Z. J. Zhang, G. Maguire, E. Quinet, A. R. Tall, Y. L. Marcel, and R. McPherson. 1993. Cholesteryl ester transfer protein and high density lipoprotein responses to cholesterol feeding in men: relationship to apolipoprotein E genotype. *J. Lipid Res.* **34:** 437–446.
23. Lehtimäki, T., T. Moilanen, T. Solakivi, P. Laippala, and C. Ehnholm. 1992. Cholesterol-rich diet-induced changes in plasma lipids in relation to apolipoprotein E phenotype in healthy students. *Ann. Med.* **24:** 61–66.
24. Glatz, J. F. C., P. N. M. Demacker, P. R. Turner, and M. B. Katan. 1991. Response of serum cholesterol to dietary cholesterol in relation to apolipoprotein E phenotype. *Nutr. Metab. Cardiovasc. Dis.* **1:** 13–17.
25. Weissfeld, J. L., and J. J. Holloway. 1992. Precision of blood cholesterol measurement and high blood cholesterol case-finding and treatment. *J. Clin. Epidemiol.* **45:** 971–984.
26. Clevidence, B. A., J. T. Judd, A. Schatzkin, R. A. Muesing, W. S. Campbell, C. C. Brown, and P. R. Taylor. 1992. Plasma lipid and lipoprotein concentrations of men consuming a low-fat, high-fiber diet. *Am. J. Clin. Nutr.* **55:** 689–694.
27. Lichtenstein, A. H., L. M. Ausman, W. Carrasco, J. L. Jenner, L. J. Gualtieri, B. R. Goldin, J. M. Ordovas, and E. J. Schaefer. 1993. Effects of canola, corn, and olive oils on fasting and postprandial plasma lipoproteins in humans as part of a National Cholesterol Education Program step 2 diet. *Arterioscler. Thromb.* **13:** 1533–1542.
28. Meydani, S. N., A. H. Lichtenstein, S. Cornwall, M. Meydani, B. R. Goldin, H. Rasmussen, C. A. Dinarello, and E. J. Schaefer. 1993. Immunologic effects of National Cholesterol Education Panel step-2 diets with and without fish-derived n–3 fatty acid enrichment. *J. Clin. Invest.* **92:** 105–113.
29. Denke, M. A. 1994. Individual responsiveness to a cholesterol-lowering diet in postmenopausal women with moderate hypercholesterolemia. *Arch. Intern. Med.* **154:** 1977–1982.
30. Denke, M. A., and S. M. Grundy. 1994. Individual responses to a cholesterol-lowering diet in fifty men with moderate hypercholesterolemia. *Arch. Intern. Med.* **154:** 317–325.
31. McNamara, J. R., and E. J. Schaefer. 1987. Automated enzymatic standardized lipid analyses for plasma and apolipoprotein fractions. *Clin. Chim. Acta.* **166:** 1–8.
32. Havekes, L. M., P. de Knijff, U. Beisiegel, J. Havinga, M. Smit, and E. Klasen. 1987. A rapid micromethod for apolipoprotein E phenotyping directly in serum. *J. Lipid Res.* **28:** 455–463.
33. Hegsted, D. M., L. M. Ausman, J. A. Johnson, and G. E. Dallal. 1993. Dietary fat and serum lipids: an evaluation of the experimental data. *Am. J. Clin. Nutr.* **57:** 875–883.
34. Dallongeville, J., S. Lussier-Cacan, and J. Davignon. 1992. Modulation of plasma triglyceride levels by apoE phenotype: a meta-analysis. *J. Lipid Res.* **33:** 447–454.
35. Katan, M. B., A. C. Beynen, J. H. de Vries, and A. Nobels. 1986. Existence of consistent hypo- and hyperresponders to dietary cholesterol in man. *Am. J. Epidemiol.* **123:** 221–234.
36. Jacobs, D. R., J. T. Anderson, P. Hannan, A. Keys, and H. Blackburn. 1983. Variability in individual serum cholesterol response to change in diet. *Arteriosclerosis.* **3:** 349–356.
37. Beynen, A. C., and M. B. Katan. 1985. Reproducibility of the variations between humans in the response of serum cholesterol to cessation of egg consumption. *Atherosclerosis.* **57:** 19–31.
38. Eggen, D. A. 1976. Cholesterol metabolism in groups of rhesus monkeys with high or low response of serum cholesterol to an atherogenic diet. *J. Lipid Res.* **17:** 663–673.
39. West, C. E., and D. C. K. Roberts. 1974. Cholesterol metabolism in two strains of rabbits differing in their cholesterolaemic response to dietary cholesterol. *Biochem. Soc. Trans.* **2:** 1275–1277.
40. Imai, Y., and H. Matsumara. 1973. Genetic studies on induced and spontaneous hypercholesterolemia in rats. *Atherosclerosis.* **18:** 59–64.
41. Clarkson, T. B., and M. R. McMahan. 1980. Individual differences in the response of serum cholesterol to change in diet: animal studies. *In* Childhood Prevention of Atherosclerosis and Hypertension. R. M. Lauer and R. B. Shekelle, editors. Raven Press, New York. 127–135.
42. Rainwater, D. L.. 1994. Genetic effects on dietary response of Lp(a) concentrations in baboons. *Chem. Phys. Lipids.* **67:** 199–205.
43. Murakami, K., M. Shimizu, N. Yamada, S. Ishibashi, H. Shimano, Y. Yazaki, and Y. Akanuma. 1993. Apolipoprotein E polymorphism is associated with plasma cholesterol response in a 7-day hospitalization study for metabolic and dietary control in NIDDM. *Diabetes Care.* **16:** 564–569.
44. Gaddi, A., A. Ciarrocchi, A. Matteucci, S. Rimondi, G. Ravaglia, G. C. Descovich, and C. R. Sirtori. 1991. Dietary treatment for familial hypercholesterolemia—differential effects of dietary soy protein according to the apolipoprotein E phenotypes. *Am. J. Clin. Nutr.* **53:** 1191–1196.
45. Uusitupa, M. I. J., E. Ruuskanen, E. Mäkinen, J. Laitinen, E. Toskala, K. Kervinen, and Y. A. Kesäniemi. 1992. A controlled study on the effect of beta-glucan-rich oat bran on serum lipids in hypercholesterolemic subjects: relation to apolipoprotein E phenotype. *J. Am. Coll. Nutr.* **11:** 651–659.
46. Jenkins, D. J. A., R. A. Hegele, A. L. Jenkins, P. W. Connelly, K. Hallak, P. Bracci, H. Kashtan, P. Corey, M. Pintilia, H. Stern, and R. Bruce. 1993. The apolipoprotein E

gene and the serum low-density lipoprotein cholesterol response to dietary fiber. *Metabolism.* **42:** 585–593.
47. Barnard, R. J. 1991. Effects of life-style modification on serum lipids. *Arch. Intern. Med.* **151:** 1389–1394.
48. Clifton, P. M., and P. J. Nestel. 1992. Influence of gender, body mass index, and age on response of plasma lipids to dietary fat plus cholesterol. *Arterioscler. Thromb.* **12:** 955–962.
49. Pouliot, M-C., J-P. Després, S. Moorjani, P-J. Lupien, A. Tremblay, and C. Bouchard. 1990. Apolipoprotein E polymorphism alters the association between body fatness and plasma lipoproteins in women. *J. Lipid Res.* **31:** 1023–1029.
50. Cobb, M. M., and N. Risch. 1993. Low-density lipoprotein cholesterol responsiveness to diet in normolipidemic subjects. *Metabolism.* **42:** 7–13.
51. Steinmetz, A., C. Jakobs, S. Motzny, and H. Kaffarnik. 1989. Differential distribution of apolipoprotein E isoforms in human plasma lipoproteins. *Arteriosclerosis.* **9:** 405–411.
52. Lund-Katz, S., K. H. Weisgraber, R. W. Mahley, and M. C. Phillips. 1993. Conformation of apolipoprotein E in lipoproteins. *J. Biol. Chem.* **268:** 23008–23015.